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# DOCTOR OF PHILOSOPHY

# Extraction of Frankincense Resins

Mitchell, Joe

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DOCTORAL THESIS

# Extraction of Frankincense Resins

*Author: Joseph William Mitchell* *2021* *Supervisor: Dr. Patrick J Murphy*

## *Acknowledgements*

Firstly, I would like to thank the KESS 2 scheme for the funding of this project, who without none of this would have been possible. Equally, I would like to thank my supervisor Dr Patrick J Murphy for his knowledge, experience and patience throughout the project. I was also fortunate enough to have access to the excellent facilities at Suprex Ltd, where I carried out my $CO_2$ extraction experiments under the technical expertise of Professor Ray Marriot and Dr Gee-Sian Leung and have Frankincense sourced to thanks to Andrew Beggin. I would also like to express my sincere gratitude to Professor Luay Rashan at Dhofar University, for kindly supplying me with my *Boswellia sacra* resin samples and for inviting me to the University to take part in the 'First International Conference on Frankincense and Medicinal Plants' back in 2018.

In addition, I would like to thank everyone at Phytovation and Bangor University who I was lucky enough to be in contact with over the course of this project. I am also deeply grateful to everyone I worked with in the 6$^{th}$ floor lab in the Chemistry tower over the years, especially enjoying the insight and company of Jamie Tibble, Dr Dan Evans and Dr Zahraa Al-Taie who I worked alongside for the full length of the project. I'd also like to give huge thanks to Professor Christopher Rayner and Dr Martina Lahmann for their detailed feedback on my thesis

Finally, I would like to thank my family for their love and support throughout everything in my life and for believing in me.

# Contents

## *Abstract*

Detailed compositional experiments of *Boswellia carterii, sacra, papyrifera* and *occulta* frankincense, including the first discussion of the contents of grades 1-4 of *Boswellia papyrifera* resin were carried out via hydrodistillation and maceration in common laboratory solvents. Hydrodistillation of these resins found the major compounds to be α-pinene for *Boswellia carterii* and *Boswellia sacra* (62-69% and 63-80% respectively), octyl acetate for *Boswellia papyrifera* (54-90%) and methoxydecane for *Boswellia occulta* (49-73%). Maceration experiments found high triterpene content in all the resins, made up mostly of boswellic acids, but high value compounds incensole and incensole acetate were found in large content in *Boswellia papyrifera* and *Boswellia occulta* extracts, with cumulatively between 9-15% of the resin mass made up of them after isolation via column chromatography.

Investigations into extractions using Supercritical $CO_2$ were performed on *Boswellia carterii* and *Boswellia occulta* resins. Extracting with a flow rate of 10 g/min $CO_2$ in a 100 mL extraction vessel at 40$^{o}$C and 100 bar pressure afforded α-pinene rich (24-49%) essential oil fractions from *Boswellia carterii* resin (7.7-9.1% mass recovery based on the resin mass). Using the same flow rate and extraction vessel separately on successive *Boswellia occulta* resin at 40-50$^{o}$C and 90-300 bar pressure resulted in the isolation of the high value compound incensole (18% mass recovery based on the resin mass, 80% purity determined by GC/MS)

Furthermore, following a series of simple, cost-effective steps such as maceration in petroleum (40-60), low quantity stationary phase column chromatography experiments and vacuum distillation using a Kugelrohr, high incensole acetate content fractions were isolated from extracts of *Boswellia occulta* and grades 1-4 of *Boswellia papyrifera* (up to 20% mass recovery based on the resin mass, 83% purity determined by HPLC)

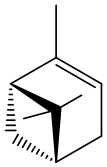
# *Abbreviations*

| | |
|---|---|
| AI | Arithmetic index |
| $^{13}C$ | Carbon-13 |
| $^{1}H$ | Proton |
| 5-LO | 5-Lipoxygenase |
| AKBA | 3-*O*-Acetyl-11-keto-β-boswellic acid |
| ATP | Adenosine triphosphate |
| *B.* | *Boswellia* |
| cc | Coiled coil |
| CDP | cytidine 5'diphospho |
| CoA | Coenzyme A |
| conc. | Concentrated |
| DEPTQ | Distortionless Enhancement by Polarisation Transfer with retention of Quaternaries |
| DMAP | Dimethylaminopyridine |
| DMAPP | Dimethylallyl pyrophosphate |
| DNA | Deoxyribonucleic acid |
| DXP | Deoxyxylulose phosphate |
| equiv. | Molecular equivalents |
| GC | Gas Chromatography |
| GC/MS | Gas Chromatography/Mass Spectrometry |
| hlh | helix-loop-helix |
| HMBDP | 1-Hydroxy- 2-methyl-2-(*E*)-butenyl 4-diphosphate |
| HMG | 3-Hydroxy-3-methylglutaryl |
| HPLC | High-Performance Liquid Chromatography |
| HSQC | Heteronuclear Single Quantum Coherence |
| $IC_{50}$ | Inhibitory Concentration to inhibit by 50% |
| IkBα | Inhibitor of kB kinase |
| IKK | IkB kinase |
| IMS | Industrial methylated spirits |
| In | Incensole |
| InAc | Incensole acetate |
| IPA | Isopropyl alcohol |
| IPP | Isopentyl pyrophosphate |
| IR | Infrared Spectroscopy |
| KBA | 11-Keto-β-boswellic acid |
| KI | Kovats Index |
| LPS | Lipopolysaccharide |
| LTA4 | Leukotriene A4 |
| LTB4 | Leukotriene B4 |
| LTC4 | Leukotriene C4 |
| LTD4 | Leukotriene D4 |
| LTE4 | Leukotriene E4 |
| lz | Leucine zipper |
| m/z | Mass to charge ratio |

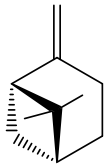
| | |
|---|---|
| MEP | Methyl erythritol phosphate |
| $M_L$ | Molecular weight of the stationary phase |
| MR | Mass recovery |
| mRNA | Messenger RNA |
| MS | Mass Spectroscopy |
| MW | Molecular weight |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NBD | NEMO Binding Domain |
| NEMO | NF-κB Essential Modulator |
| NF-κB | Nuclear factor-kB |
| NMR | Nuclear Magnetic Resonance |
| $P^o$ | Saturated vapour pressure |
| PP | Pyrophosphate |
| R | Gas constant |
| $R_f$ | Retention factor |
| RHD | Rel homology domain |
| RP-HPLC-DAD | Reversed-Phase High-Performance Liquid Chromatography with a Diode Array Detector |
| RT | Retention time |
| Sat. | Saturated |
| ss | Serine residues |
| TAD | Transcriptional activation domains |
| TLC | Thin-Layer Chromatography |
| TNF-α | Tumor Necrosis Factor alpha |
| TRPV3 | Transient receptor potential cation channel, subfamily V, member 3 |
| *V* | Specific Retention Volume |
| zf | Zinc finger |

## *Key Compounds*

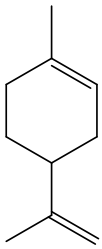
The following compounds were commonly referred to throughout the thesis as major compounds in various frankincense extracts. They were detected in various contents with regards to the mass of the resin extracted and were added here as a reference for some of the more frequently mentioned components.



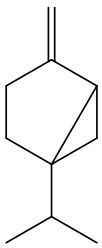
*alpha*-Pinene **25**



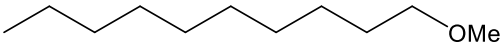
*beta*-Pinene **28**



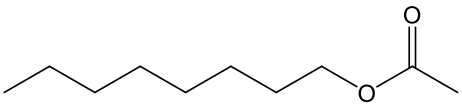
Limonene **33**



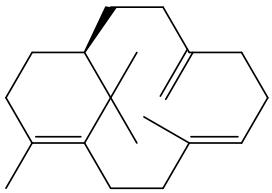
Sabinene **27**



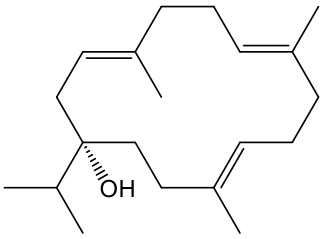

Methoxydecane **71**



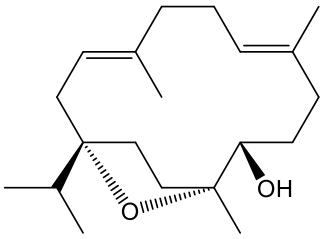

Octyl acetate **50**



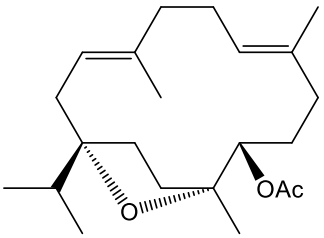
Verticilla-4(20),7,11-triene **58**



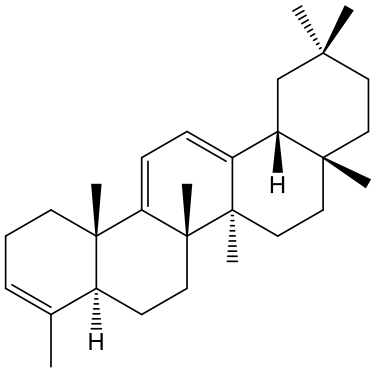

Serratol **48**



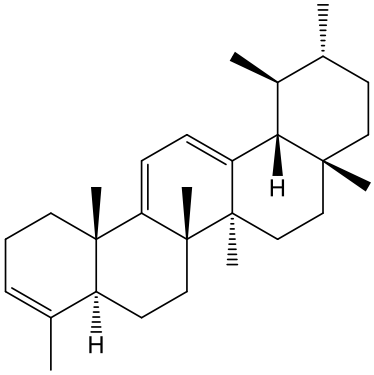

Incensole **49**



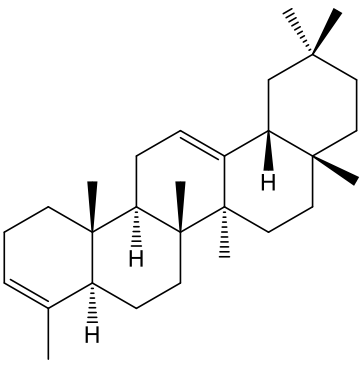

Incensole acetate **53**



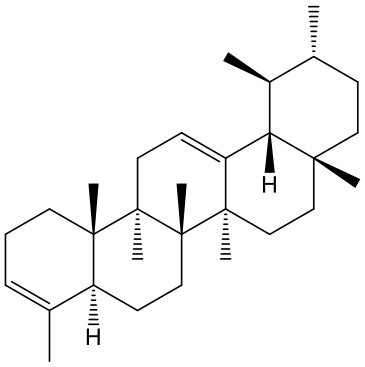

24-Noroleana-3,9(11),12-triene **124**



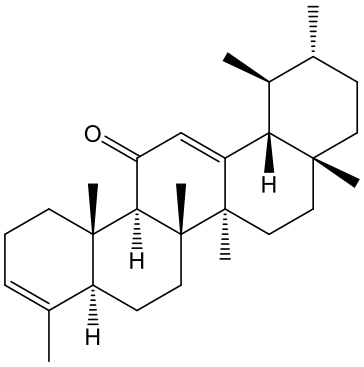

24-Norursa-3,9(11),12-triene **125**



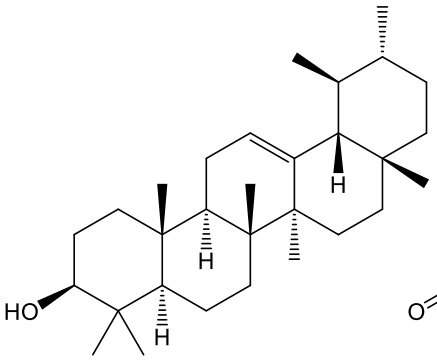
24-Noroleana-3,12-diene **126**



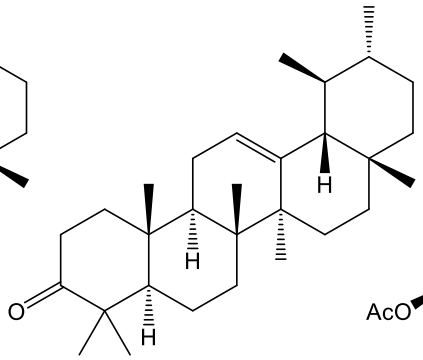
24-Norursa-3,12-diene **127**



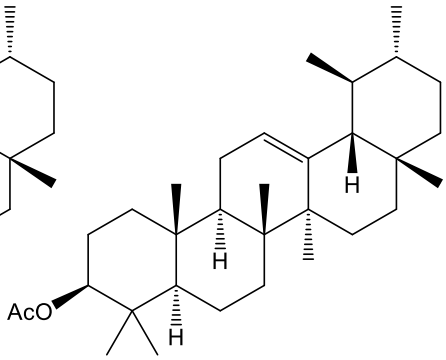
24-Norursa-3,12-dien-11-one **128**



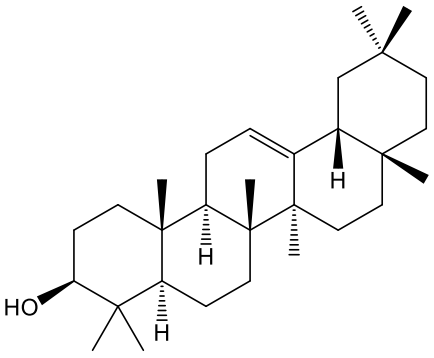
*alpha*-Amryin **89**



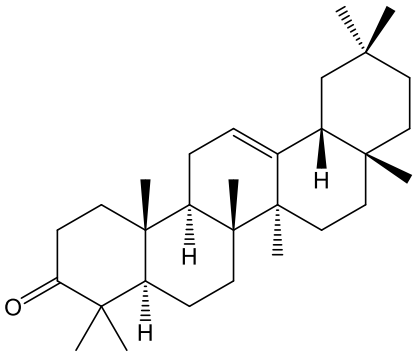
*alpha*-Amyrenone **90**



*alpha*-Amyrin acetate **129**



*beta*-Amyrin **99**



*beta*-Amyrenone **100**

# Chapter 1. The Significance of Secondary Metabolites

Since the early stages of history, plants have been used for a wide variety of things such as food, shelter, medicine, fragrances and clothing. The resins in these plants have been used substantially in these areas, often being utilized for their incense in many cultural ceremonies since ancient times.[1] It was believed that the burning of these incenses would please the Gods and soothe their souls.[2] As time progressed, more applications for these resins and their oils were discovered, with medicine and natural remedies being of the most frequent uses.[3]

The Burseraceae family has about 700 species and 19 genera and plants from this family can be found in all tropical regions and many subtropical areas. The bark of these plants possess resin ducts which produce complex oils which can have potent anti-inflammatory, anti-bacterial, anti-fungal, anti-viral activity and many other properties. These oils are retrieved by 'tapping' which is the process of making an incision in the bark of the plant, resulting in a resin being exuded.[4] The resin is exuded as a natural defence for the wound made in the bark mainly for repelling certain herbivores which are a threat through consuming the organism, but can also attract certain animals which may spread its pollen or seeds.[5,6]

Essential oils can also be found in buds, flowers, stems, seeds, twigs, leaves, fruits, roots, wood and sap in largely varying contents and relative masses. The chemical composition of such oils can also strongly vary with species, climate, age, soil composition and the stage of the organism's vegetative cycle. These oils are made up of monoterpenes, sesquiterpenes, esters and aromatics and are commonly used in perfumes, cosmetics, food preservers, natural remedies and food additives. They are often extracted by steam distillation or hydrodistillation due to their volatility, yielding only these lower terpenoids in the oil while leaving the higher molecular weight diterpenes, triterpenes and anything else such as polysaccharides mostly behind.[5]

## 1.1 *Boswellia* Genus

One genus of Burseraceae is *Boswellia Roxb*. Ex Colber, which has around 30 species and its resin is known as Frankincense (figure 1.01). Frankincense is a French word that means 'pure incense' and is collected by the scraping of the dried resin after a *Boswellia* species has been cut to exude the resin. The drips of the resin are usually collected separately sometimes up

to weeks later after it has hardened. Harvesting usually starts in December and hits its peak during the March to May period.[6]

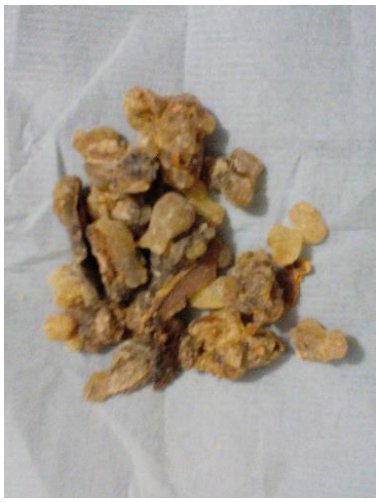


Figure 1.01. Frankincense resin collected from *Boswellia sacra.*

Frankincense has been used as early as the second century as Celsus suggested and it could treat wounds, bleeding, internal bleeding, superficial bruising and hemlock. During the eleventh century, Avicenna suggested that it was effective as an anti-inflammatory against urinary tract infections and could be used to combat amentia and amnesia. More recently, it is used to treat a wide range of inflammatory conditions such as Crohn's disease, asthma and a variety of arthritic ailments. In addition to its medical uses, it is often used in religious ceremonies, food supplements, perfumes and toiletries.[6,7]

## 1.2 Biosynthesis of Terpenes

### 1.2.1 The Isoprene Rule

The diverse healing ability of the resin and its oils is due to the wide range of secondary metabolites that are produced by the plant. These are mainly monoterpenes, sesquiterpenes, diterpenes and triterpenes in the case of frankincense resins, which are compounds that appear to have their carbon skeletons derived from isoprene units. This observation was called "the isoprene rule". The rule is essentially that terpenes are formed from units of isoprene **1** added end on end giving structures usually with carbon numbers in multiples of 5. These units can add head to tail **2** or tail to tail **3** and undergo functionality changes and skeletal rearrangements to give rise to a huge number of difference possible structures (figure 1.02).[8]

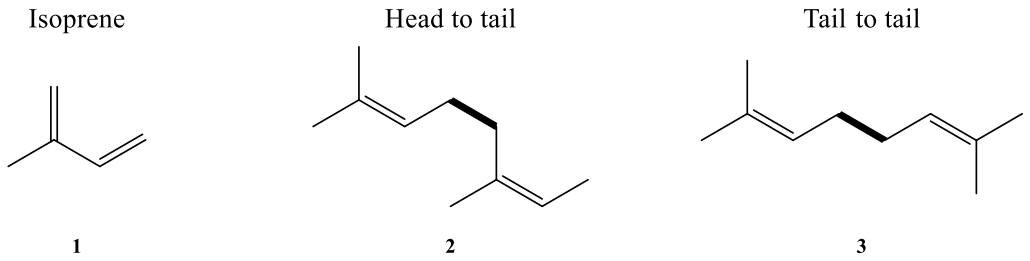
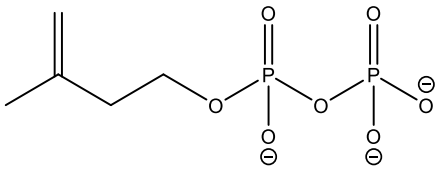


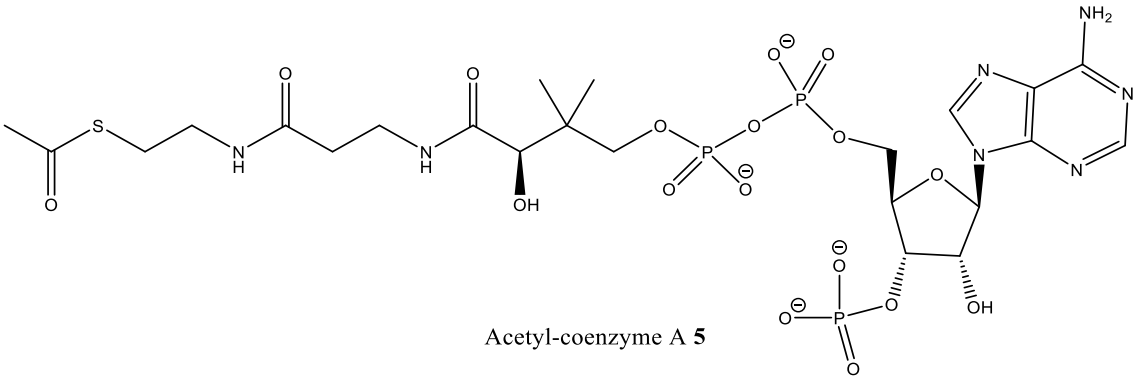

Figure 1.02. Isoprene and orientations for the isoprene rule.

The biological equivalent of isoprene is isopentyl pyrophosphate **4** (figure 1.03) which is the precursor for all terpenes. IPP is formed from acetyl-coenzyme A **5** (figure 1.04) over several steps but has two mainly accepted pathways: the mevalonate pathway and the deoxyxylulose phosphate pathway.



Isopentyl pyrophosphate **4**

Figure 1.03. Structure of isopentyl pyrophosphate, the biological equivalent of isoprene.



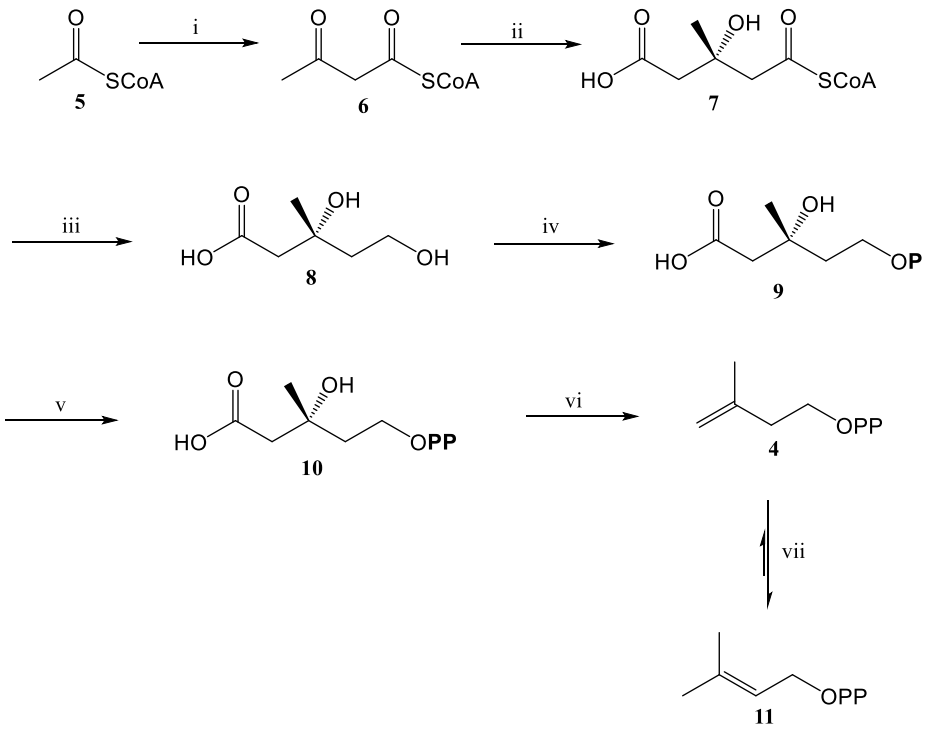
Acetyl-coenzyme A **5**

Figure 1.04. Structure of acetyl coenzyme A.

## 1.2.2 The Mevalonate Pathway

This pathway is present in most eukaryotes, eubacteria and archaea. In the mevalonate pathway, acetyl-CoA **5** first reacts with another acetyl-CoA unit in a Claisen condensation reaction to make acetoacetyl-CoA **6**, followed by an aldol addition with a further acetyl-CoA unit to form **7**. The next step is a NADPH dependent double reduction at the acetyl-CoA functional group to give the alcohol moiety on **8** through HMG-CoA reductase, which is a key enzyme in the biosynthesis of cholesterol. Therefore, this enzyme is a key target of statins, a

class of compounds known as HMG-CoA reductase inhibitors. Stepwise phosphorylation with ATP follows to give **9** and then **10**. IPP **4**, is yielded through ATP assisted decarboxylation and DMAPP **11**, is formed through isomerisation of IPP in an equilibrium that favours the DMAPP product (scheme 1.1). The formation of **6**, **9**, and **10** are enzymatically catalysed by acetoacetyl-CoA thiolase, HMG-CoA synthase and mevalonate kinase respectively, which have been related to the presence of inherited human diseases. Furthermore, some pathogenic gram-positive bacteria also produce these enzymes, although disruption of the genes responsible for encoding these enzymes have been demonstrated to reduce proliferation of these.[9,10]

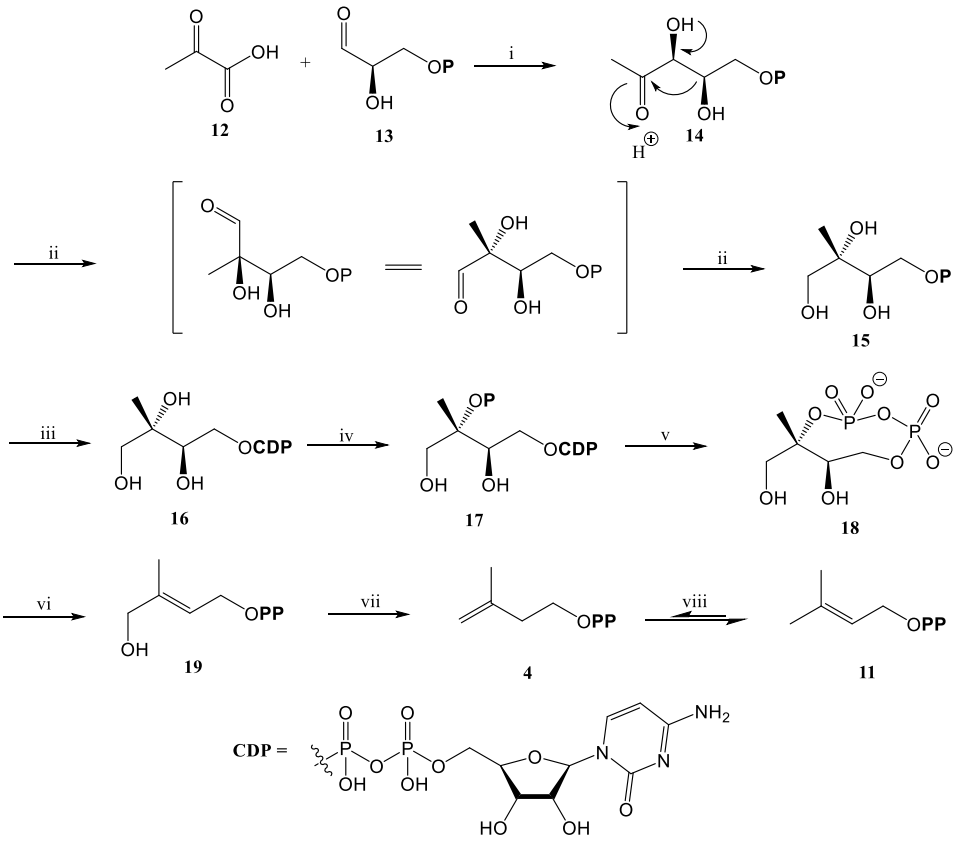



Scheme 1.1 - Biosynthesis of IPP and DMAPP via the mevalonate pathway. Enzymes: i) acetoacetyl-CoA thiolase; ii) HMG-CoA synthase; iii) HMG-CoA reductase; iv) mevalonate kinase; v) phosphomevalonate kinase; vi) mevalonate 5-diphosphate decarboxylase; vii) isopentyl-diphosphate δ-isomerase.

## 1.2.3 The Deoxyxylulose Pathway

The other biosynthetic pathway towards DMAPP and IPP is the deoxyxylulose phosphate (DXP) route. A 2-carbon unit is derived from pyruvate **12** and 3 carbons from glyceraldehyde 3-phosphate **13** to form the 5-carbon skeleton of DXP **14** in a thiamine diphosphate dependent reaction. This is followed by an intramolecular rearrangement and reduction, catalysed by 1-deoxy-D-xylulose 5-phosphate reductoisomerase to form an intermediate, as

shown, which remains held by the enzyme, which is simultaneously reduced by NADPH to make MEP **15**. At this point, the 5-carbon skeleton shape of IPP and DMAPP can be seen. A subsequent condensation reaction between MEP and cytidine 5-diphosphate, followed by phosphorylation of the tertiary alcohol and a condensation cyclization gives **18**. HMBDP **19** is formed via a reductive ring opening reaction and IPP and DMAPP are formed through several further reactions (scheme 1.2).[11] It should be noted that there is evidence of DMAPP being formed independently of IPP and not through isomerization.[12]



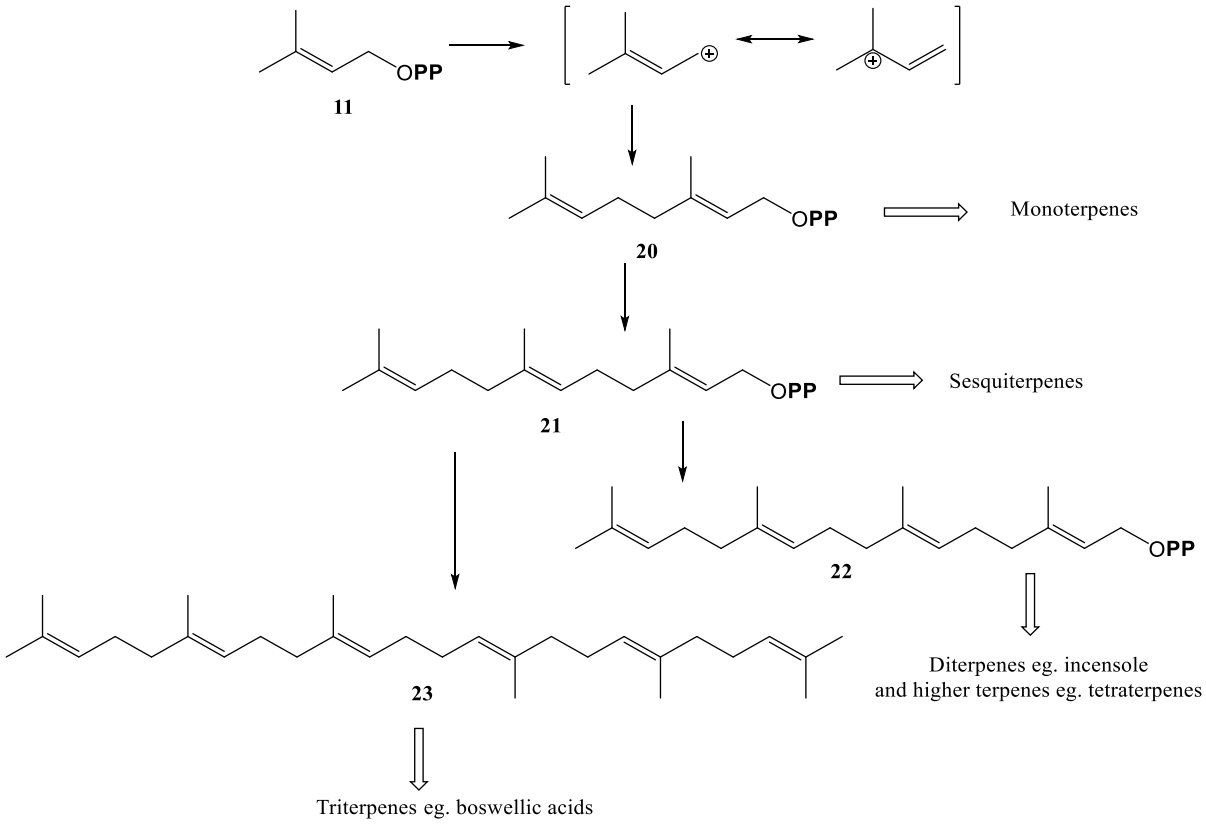

Scheme 1.2 - Biosynthesis of IPP and DMAPP via the deoxyxylulose phosphate pathway. Enzymes: i) 1-deoxy-D-xylulose 5-phosphate synthase; ii) 1-deoxy-D-xylulose 5-phosphate reductoisomerase; iii) 4-diphosphocyticyl-2C-methyl-D-erythritol synthase; iv) 4-diphosphocyticyl-2C-methylerythritol kinase; v) 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; vi) 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; vii) 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase; viii) isopentenyl diphosphate isomerase.

It is unclear which the main pathway for constructing the isoprene unit is. Animals and fungi seem to lack the deoxyxylulose phosphate pathway, so go through the mevalonate route only. However, many organisms, such as bacteria and plants seem to utilise both pathways in tandem. In the mevalonate case, the main terpenoids formed are sterols, brassinosteroids, sesquiterpenes, triterpenes, polyterpenes, polyprenols, dolichols, ubiquinones and prenyl moieties. However, with deoxyxylulose diphosphate, monoterpenes, diterpenes, isoprene,

carotenoids, abscisic acid, gibberellins and photosynthesis related compounds are the main types of terpenoids synthesized.[12,13]

### 1.2.4 Biosynthesis of $(C_5)_n$ Terpenes

Terpenoid nomenclature depends on the number of 5-carbon ($C_5$) units there are in the compound. Monoterpenes are $C_{10}$, sesquiterpenes are $C_{15}$, diterpenes are $C_{20}$, and triterpenes are $C_{30}$ (scheme 1.3). Monoterpenes, sesquiterpenes and diterpenes are formed by sequential head to tail condensation reactions and the transformations from sesquiterpenes to triterpenes and diterpenes to tetraterpenes are via the tail to tail condensation reaction.[9,14]




Scheme 1.3. Biosynthesis of different size terpenes from IPP precursor.

## 1.3 Isolation and Characterization of Oils

The complex chemistry that exists in frankincense resins results in a very wide range of products that are isolated through extraction. Even when using methods that only take a fraction of the resin can produce oils with 5-10 major products, with possibly hundreds more detectable compounds in very low concentrations.[4,15] Techniques such as distillation, solvent

extraction and chromatography are all common ways to isolate oils from a resin and often, more than one of these will be used to extract and eventually isolate the desired compound.

The analysis of these oils can depend on how they are isolated. For example, volatile mixtures can often be separated easily in a GC column. When coupled with a Mass Spectrometer, the method is called GC/MS and this is a powerful technique for separating and then characterising the products by the time they take to reach the detector and their fragmentation pattern, which will be discussed further in **Section 1.3.1**.

Oils that are a high purity of one compound, will often be analysed by techniques such as NMR spectroscopy, IR and various Chromatography methods such as TLC, MS and HPLC. Complex oils of different volatilities can be crudely analysed by techniques such as distillation, to estimate the size of the volatile fraction compared to the heavy oils. After such fractionation, the separate oils can be analysed separately using the appropriate methods.

### 1.3.1 Characterization Using GC/MS

The majority of the classification of the oils isolated in this work was through GC/MS analysis using the methods in **Section 6.02**. Using a combination of the fragmentation pattern, the Kovats index (discussed in **Section 1.3.2**) and literature sources, compounds were tentatively assigned. The confidence of the assignment for each compound was stated in the chemical profile tables throughout **Chapter 2** and were stated to either be high, medium or low in certainty. Fragmentation patterns were compared carefully with the available literature. Firstly, since there was literature available for each species studied in this work, general ideas of the extraction profiles were known prior to discussing here. These common compounds were generally assigned at high in assignment confidence. Compounds that were less familiar with regards to the literature were assigned at lower confidence. All the chromatograms and fragmentation patterns discussed herein were from experiments carried out for this work and were analysed using the AMDIS software.[16] It should be noted that all fragmentation mechanisms were based on literature suggestions.

Separation of compounds in the GC column depends on their volatility and their adsorption with the solid stationary phase, which in this case interacts more with the polar compounds meaning the non-polar compounds elute faster. The resulting chromatogram can be used to give a chemical profile of the mixture, with the content of each compound corresponding to

its relative peak area. An inert carrier gas acts as the mobile phase for the gaseous mixture of oil and solvent which lead to an electron ionization detector in this work directly after the column. Here, the analytes are shot with an electron beam which causes compounds to become molecular ions, by losing an electron ($M^{+\cdot}$). There is a surplus energy from the ion source and these molecular ions are less stable than their non-charged equivalents so fragmentation often occurs. All the charged fragments, including the parent ion are detected along with their corresponding mass to charge ratios (m/z), which gives a reproducible fragmentation pattern, meaning the pattern can be compared with literature data for identification of the compound. The size of the peak for a particular m/z corresponds to how much has been detected, meaning the larger peaks mean more of a certain ion has been formed, which can be used to suggest fragmentation mechanisms. This combination of elution time, which is also reproducible when converted to its Kovats Index, and fragmentation pattern is very useful for confident classification of an oil mixture.[17]

### 1.3.2 Volatile Oils

Hydrodistillation (figure 1.05) and steam distillation can be used to extract volatile compounds from a mixture of volatiles and non-volatiles. The setup has the extractable material mixed with water in a round bottom flask in contact with a heat source. When the water is boiled with resin, an azeotropic mixture of oil and water is formed and they boil off together. A condenser allows these immiscible phases to re-enter the liquid phase and the Clevenger apparatus feeds water back into the heated flask which allows for continuous distillation until exhaustion of the material. The layers are then easily separable by liquid-liquid extraction or simply by tapping off the water separately from the oil.[18] Changing the length of the distillation can affect the resultant oil as some compounds will distil very quickly and others will very slowly distil due to lower volatility. In addition, the temperature of distillation has a large effect as higher temperatures allow lower volatility compounds to distil in the mixture. Even small amounts of low volatility compounds such as Boswellic acids can be found in the distillate if the temperature is relatively high.[19]

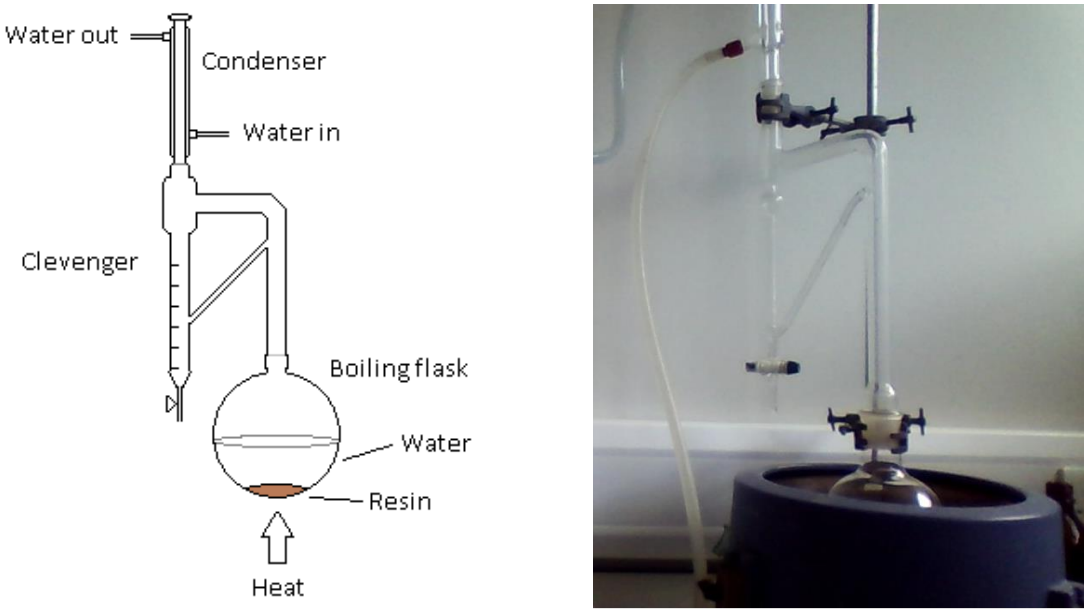



Figure 1.05. Hydrodistillation setup using a mantle to heat the water and resin mixture in the round bottom flask connected to a Clevenger with the condenser on top.

The oil is then analysed by GC/MS and by using a calibration standard, their Kovats index (KI) can be determined. The KI is a unitless retention factor that can account for the differences in the equipment used for analysis and a gives a system independent retention factor for an isothermal system (equation 1.1). To calculate this index, a calibration standard is needed which gives reference points before and after the compounds in question. The calibration standard may typically be a mixture of known alkanes often ranging in size from octane ($C_8H_{18}$) to triacontane ($C_{30}H_{62}$).[20]

$$KI_x = 100n + 100((\log V_x - \log V_n)/(\log V_N - \log V_n))$$

Equation 1.1. Kovats index ($KI_x$) calculated using the number of carbons in the closest reference alkane before the analyte (n) and the specific retention volumes of each the analyte ($V_x$), the closest reference alkane before the analyte ($V_n$) and the closest reference alkane after the analyte ($V_N$).

The specific retention volume describes the volume of eluent required to elute each compound from the column. The problem with this approach is that calculation of the specific retention volume relies on the accurate determination of the saturated vapour pressure (equation 1.2). In most GC systems, the experiment will have a temperature gradient as time elapses in the analysis which results in changes of pressure throughout the column. This is difficult to get reproducible data for as many GC systems do not have the proper gauges for the measurement of the column inlet pressure.[20]

$$V = RT/M_L P^o$$

Equation 1.2. The specific retention volume (*V*), calculated using the gas constant (R), the temperature (T), the molecular weight of the stationary phase ($M_L$) and the saturated vapour pressure ($P^o$).

It was later found that the retention factor could be generalised in the case of a linear temperature gradient by adjusting equation 1.1. This results in the determination of the arithmetic index, a dimensionless constant that can be used to identify the retention factor independent of the system given by equation 1.3.[20]

$$AI_x = 100n + 100((RT_x - RT_n)/(RT_N - RT_n)$$

Equation 1.3 - Arithmetic index ($AI_x$) calculation used in temperature dependent systems for the identification of the components of a mixture. $RT_x$ = the retention time of the unknown analyte, $RT_n$ = the retention time of the closest alkane which has a lower retention time than x, $RT_N$ = the retention time of the closest alkane which has a higher retention time than x, n = the number of carbons in the known smaller alkane.

This index gives a general retention factor that can be compared against the literature which is useful when fragmentation patterns look similar for 2 different compounds. The fragmentation patterns are then compared to literature patterns of volatile compounds which are well documented. Although it should be noted that typically, the calculated Arithmetic index can range up to around +/-10, with larger ranges often being found for less volatile compounds.[21,20] The content of each compound can be found using the relative area under each peak on the Chromatogram. This is calculated by taking the ratio of the area under each individual peak compared to the area under all the peaks combined (equation 1.4).

$$C_x = (A_x/A_{total})*100$$

Equation 1.4 – The content of a specific peak ($C_x$) calculated using the area under that peak ($A_x$) and its ratio with the combined area under all peaks ($A_{total}$), then multiplying by 100 to give a percentage.

For volatile mixtures, the content calculated is fairly representative, although to achieve a more certain quantification, a pure standard of the main component could be used and the content could be calculated relative to the pure sample's area, given that the injected sample contained the same oil mass. Although, in this work, the peaks in each chromatogram were merely compared to each other to give a general chemical profile. For less volatile mixtures, compounds enter the gas phase less easily and therefore result in a less accurate content for the resultant peaks, as some compounds are not being represented fully in the chromatogram

with regards to their relative peak area. This can also lead to broader peaks, which are more affected by noise in the baseline, therefore giving less accurate data. As a rule of thumb, the sharper peaks are more reliable as they are less affected by noise in the baseline. In these cases, the content can still be useful as a guide, but a pure standard for comparison would give a much better representation of the analyte's chemical profile. In addition, liquid chromatography techniques such as HPLC can be useful for these less volatile compounds, although a pure standard of the compound again gives a better representation of the content of the compound in the mixture by comparison of the two.

### 1.3.3 Extraction of Heavy Oil Fraction

Solvent extraction can be utilized to extract a large range of lipids including monoterpenes, sesquiterpenes, diterpenes, triterpenes and other resin compounds. This means that the solvent extracted material of frankincense resins have a much higher mass recovery than the distilled oils due to the large amount of heavy oils in the resin. Methods can include maceration, reflux extraction or Soxhlet. Maceration is the mixing of the plant material with solvent and mixing thoroughly before filtering off the insoluble material before removing the solvent by evaporation.[18] Reflux extraction involves higher temperature to increase the solvent power of the solvent and solubility of the material, which is cooled and filtered before removing the solvent. Soxhlet extraction (figure 1.06) involves boiling solvent and allowing it to condense over a porous container that holds the plant material. This allows for extraction of the material with the warm solvent without having to filter later. The solution of solvent and oil then siphons back to the round bottom flask where solvent can boil off again to collect more while the oils stays in solution in the flask. This means over time the oil is increased cumulatively in the round bottom flask. After exhaustion of the material, the mixture is cooled, and the solvent is evaporated. The main things affecting the resulting oil are the temperature, the length of extraction and the solvent used. Increasing the temperature and the extraction time increases the mass recovery as the oils are more soluble in higher temperatures and increasing the extraction time gives better contact time with the solvent so the material can be fully exhausted. The solvent used has a very large effect, as less polar compounds are less likely to dissolve in a polar solvent such as methanol, so essential oils are often left behind. On the other hand, using a non-polar solvent such as hexane dissolves the essential oils very well, but leaves behind some of the more polar compounds. Furthermore,

the choice of solvent also affects the temperature you can extract at, as solvents such as diethyl ether have a very low boiling point, meaning a reflux extraction is not as hot an extraction compared with a solvent like methanol, which has a much higher boiling point.

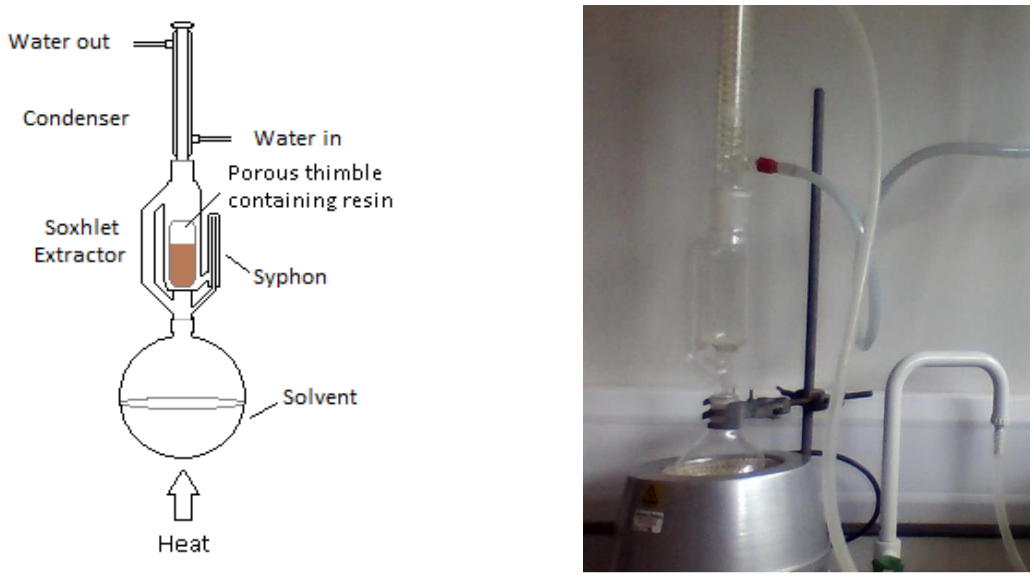



Figure 1.06. Soxhlet extraction setup with a round bottom flask containing solvent in to be heated by a mantle (bottom) attached to a Soxhlet extractor which is topped with a condenser. Photograph taken at Suprex Ltd of a Soxhlet extraction.

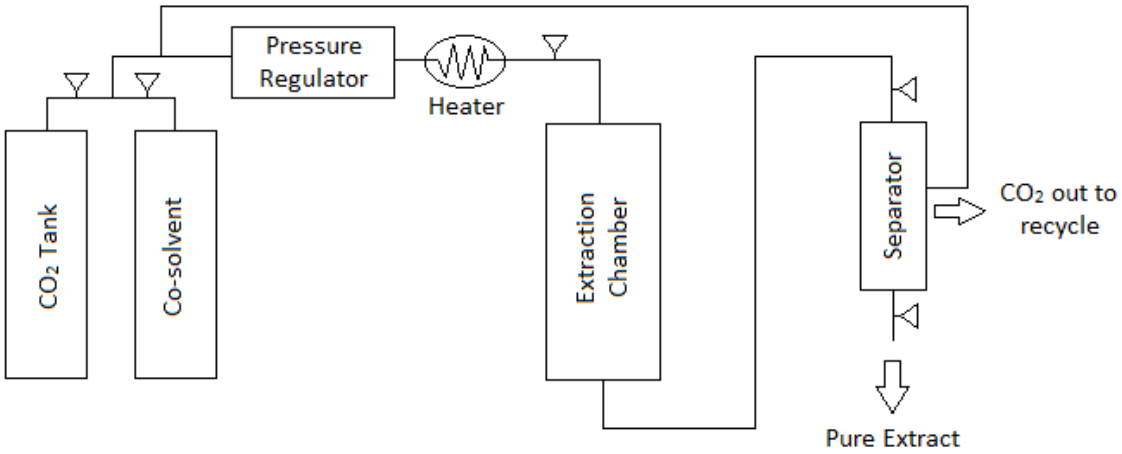
### 1.3.4 Non-Specific Extraction Methods

Some methods of extraction and purification are not specific to the type of compound. For example, $CO_2$ can be used to extract a complex mixture, but the experiment can result in several separate fractions based on the relative polarity of the mixture. Distillation can be performed with different fractions collecting mixtures that boil at different temperatures. In its simplest form, this can result in a volatile and a non-volatile fraction, but fractional distillation methods can result in several semi-volatile fractions in between. Column Chromatography can be used to purify mixtures of compounds based on their relative polarity and the method can vary largely, by using different mobile and stationary phases, along with using different amounts of these things to achieve different results. Each of these will be discussed further throughout **Section 1.3.4.1-1.3.4.3**.

### 1.3.4.1 Extraction by Supercritical $CO_2$

Extraction by supercritical $CO_2$ (figure 1.07) offers many advantages over typical distillation and solvent extraction. Due to its low viscosity, it can move through solids and have less

friction when flowing. The high density of a supercritical fluid also allows for good solvent power. A supercritical fluid is a substance that can no longer be forced back into the liquid phase by increasing the pressure or back into the gas phase by an increase in temperature as it has passed its critical point. Firstly, $CO_2$ offers good selectivity and since it disperses into the air after extraction by a large drop in pressure once the mixture has left the extraction vessel. This means the oil can just be tapped off as a pure extract with no solvents to remove. $CO_2$ is also non-toxic like the trace solvent left in the solvent extracts and can be done at mild temperatures, so does not cause thermal degradation the way a typical distillation can. The technology can be expensive, but the $CO_2$ gas is relatively inexpensive and has a mild critical point of 31.06°C and 73.825 bar (figure 1.08). [22,23]



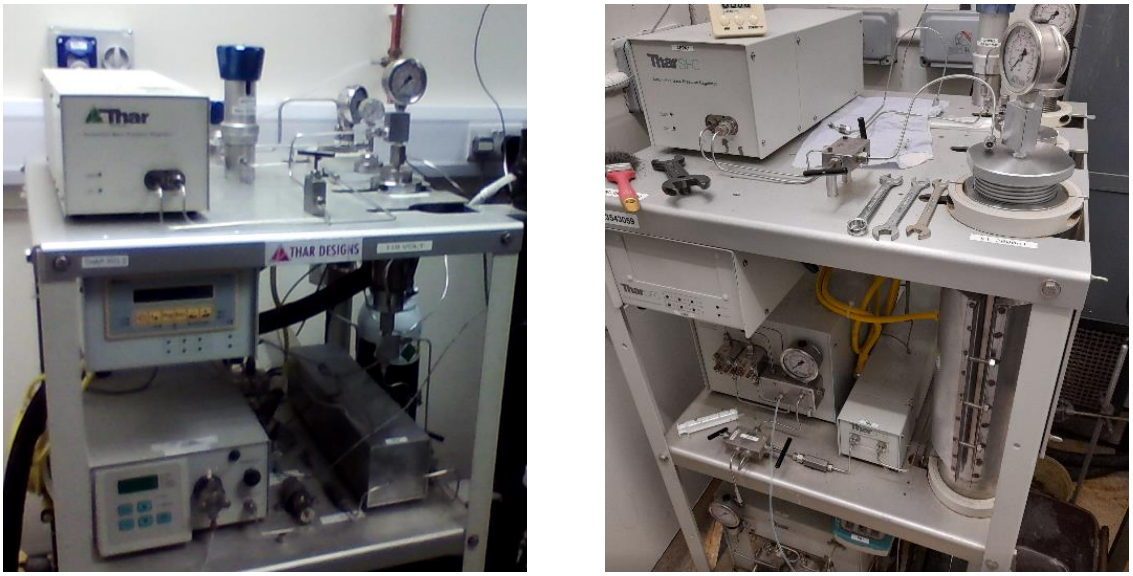




Figure 1.07. Schematic (top) of a general $CO_2$ rig including a $CO_2$ pump, pressure regulators, extraction chambers and separators where the pressure is dropped, and $CO_2$ returns to a gas leaving only the extract. Photographs are of the 100 mL rig (left) and the 2 L rig (right).

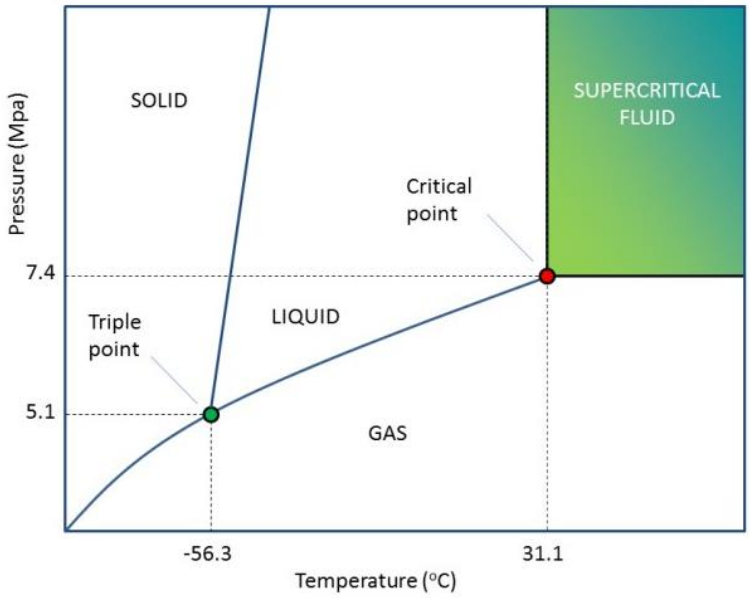



Figure 1.08. Phase diagram for $CO_2$ which has a mild critical point (picture from source).[24]

Small changes in temperature and pressure around the critical point causes large changes in $CO_2$ density, allowing for good selectivity in the extractions. Around the critical temperature, increasing the pressure sharply increases the $CO_2$ density due to the larger force on the particles in the same area, allowing for higher solvent power (figure 1.09). In contrast, around the critical pressure, increasing the temperature results in a sharp drop in $CO_2$ density, due to the increased kinetic energy of the particles, which significantly reduces the solvent power of the system (figure 1.10). In each case, as the pressure and temperature move further away from the critical point, the corresponding effect on the density is less significant.

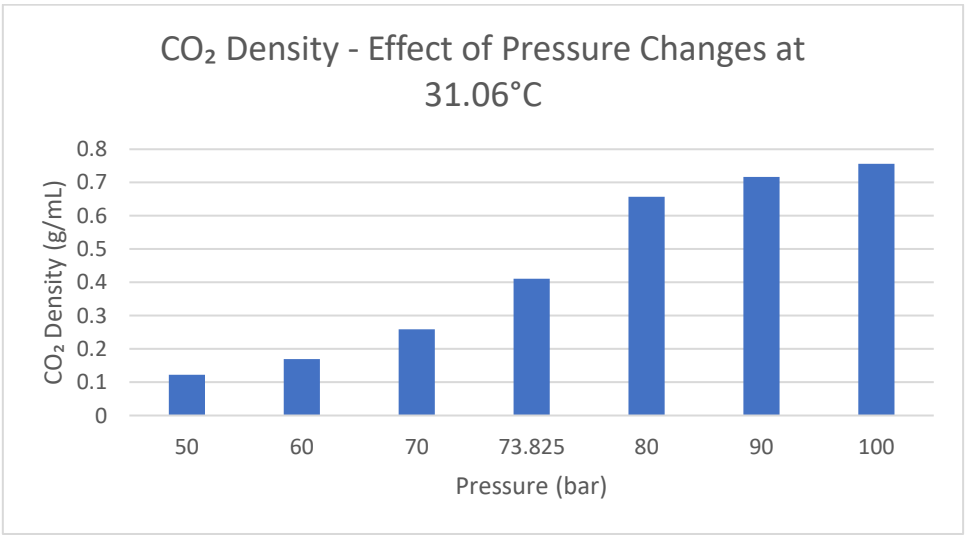



Figure 1.09. $CO_2$ density profile at the critical temperature with varying pressures (critical pressure is 73.825 bar). Data calculated using an online source.[25]

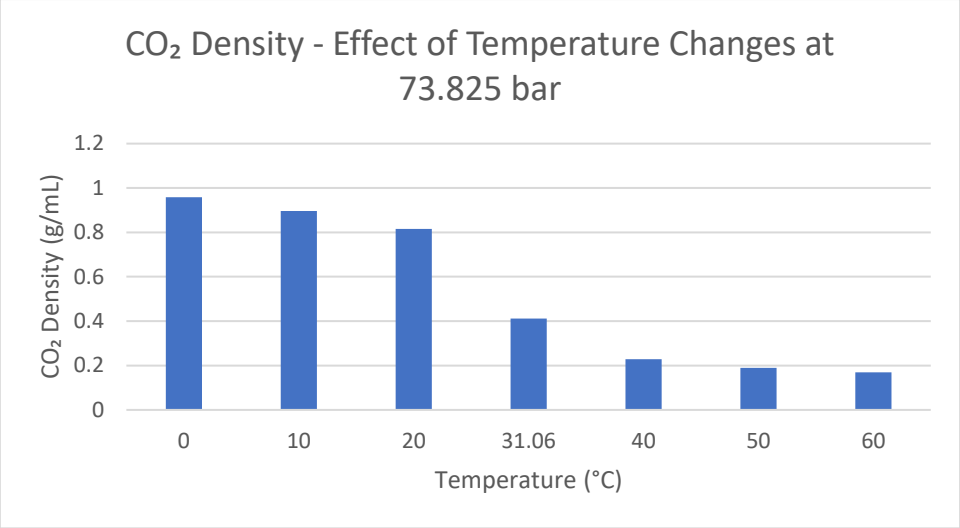



Figure 1.10. $CO_2$ density profile at the critical pressure with varying temperatures (critical temperature is 31.06°C. Data calculated using an online source.[25]

Increasing the pressure significantly to 300 bar, as was commonly done in this work, causes a significant increase in $CO_2$ density. At this high pressure, increasing the temperature has less of an effect on the solvent power as the $CO_2$ density is not affected as much (figure 1.11). Therefore, increasing the temperature will increase the solvent power due to the higher kinetic energy of the system separating the solute particles more easily.

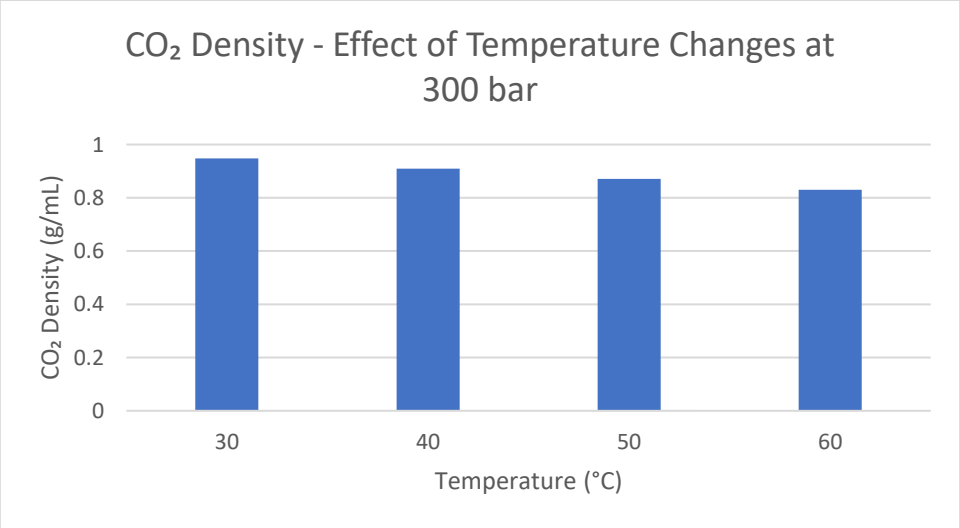



Figure 1.11 - $CO_2$ density profile at 300 bar with varying temperatures. Data calculated using an online source.[25]

This phenomenom can be utilised to separate complex mixtures into separate fractions based on their relative polarity. At a lower $CO_2$ density, less polar compounds will elute more easily, meaning the volatile fractions of the plant matter can be removed first before increasing the

solvent power by applying these temperature and pressure alterations. This can reduce the cost and time of isolation as fewer further purification steps will be necessary. $CO_2$ extraction also has the option to introduce a co-solvent at a later point, such as IMS to extract the remaining material, but in this case the solvent would have to be removed by evaporation under vacuum. This would be adding extra steps so the goal would be to avoid this if possible and extract all the desired oil just using $CO_2$.[22,23]

### 1.3.4.2 Vacuum Distillation Methods

As mentioned in **Section 1.3.2**, volatile oils can be separated from a complex resin by methods such as hydrodistillation. This is not limited to the resin, as a mixture could be extracted by solvent or $CO_2$ and then distilled to give a residue fraction and a distillate. Furthermore, fractional distillation by using a Kugelrohr, which carries out distillation under vacuum can be used to give a series of fractions, all with different general compositions and sizes (figure 1.12). This could potentially be increased in scale to a thin film evaporator.

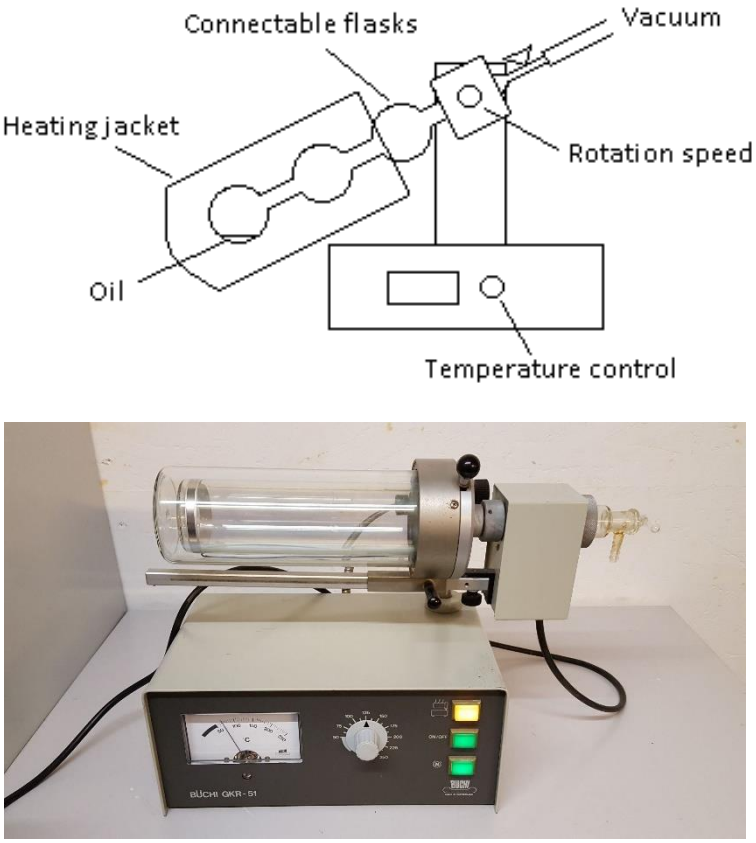



Figure 1.12. Kugelrohr setup for the vacuum distillation of an oil to give several fractions based on their volatility. Photograph from source.[26]

These are both types of vacuum distillation, which means that the temperature of the experiment can be reduced below the boiling point observed at atmospheric pressure for the target compounds. Lower temperatures are often easier to achieve in a system and can help protect the target compound from thermal degradation. A Kugelrohr is a very useful piece of equipment, especially on the smaller scale, as mixtures can be distilled in as short as around 20 minutes. One problem with the Kugelrohr however, is that a large amount of the oil is in the bulk of the mixture and the phase change is more efficient at the surface. Therefore, thin film distillation should theoretically give much purer products, with less overlap of volatile compounds being left in the residue and vice versa.

The relationship between the pressure and temperature applied throughout a distillation is determined using a pressure temperature nomograph (figure 1.13).[27]

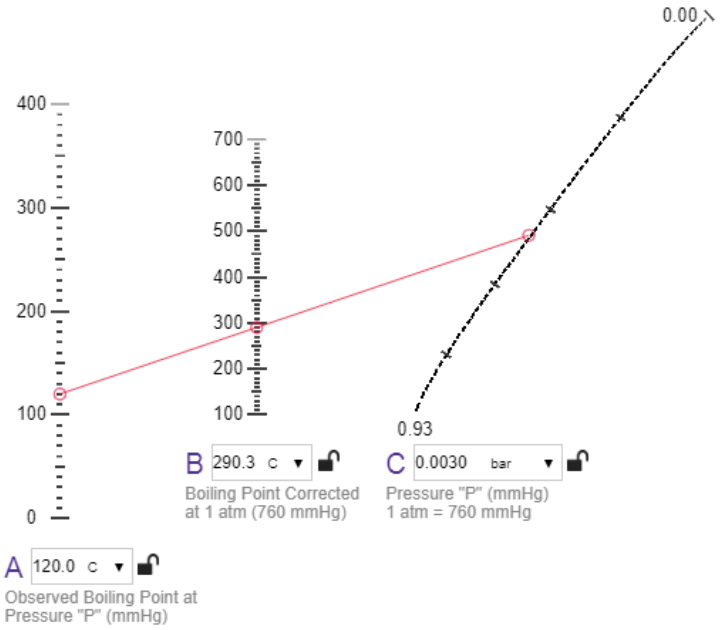



Figure 1.13. Pressure-Temperature Nomograph used to approximate the conditions to use in a distillation of a mixture (picture from source).[27]

As shown, at 3 mBar, if nothing distils until 120°C in the system, the boiling point of the same components at atmospheric pressure will be around 290°C. This is a large drop in the temperature required, as a compound with such a high boiling point at atmospheric pressure may thermally degrade significantly before distilling. This shows the importance of techniques such as vacuum distillation.

### 1.3.4.3 Column Chromatography

Column Chromatography is a very common technique for the purification of organic compounds. Since its invention in the early 1900's, in which the method was shown to separate compounds based on their relative polarity, Column Chromatography has been used extremely widely on a large variety of compounds.[28] It is an efficient way to isolate a high mass yield of a compound, often over 90% based on the compound's content in the mixture while minimising the loss of the compound. Depending on the system, a very polar or non-polar compound can be simply and quickly isolated in high purity.[29]

Using a non-polar solvent, such as petroleum spirits (40-60), the non-polar compounds in a mixture can be collected, with silica gel as the stationary phase. Silica gel is quite polar, and therefore interacts with polar compounds more strongly than non-polar compounds. This means that polar compounds travel through the column more slowly. As the mobile phase is increased in polarity, the polar compounds travel more freely, and there is less interaction with the stationary phase. The polarity of the mobile phase can be increased by mixing the non-polar solvent with increasing amounts of a more polar solvent such as $Et_2O$ or EtOAc.[30] Using this concept, non-polar compounds can be collected first, before gradually increasing the relative polarity of the system to collect the more polar compounds separately (figure 1.14). Furthermore, the column can often be re-used, if the silica packed in the column is thoroughly washed with a solvent that will strip any slow running compounds from it, before resetting the column by flushing with a non-polar solvent. It is important to note that the sequential solvents used for flushing and carrying out the purification must be miscible, otherwise separation will be poor, and the column may not be consistent throughout. Therefore, if the column was flushed with MeOH to remove the baseline compounds, using a solvent such as $Et_2O$ or EtOAc followed by petroleum spirits (40-60) is sufficient to set the polarity back to the relative zero. This can often be cheaper than replacing the stationary phase if not too much solvent is required and can save time.

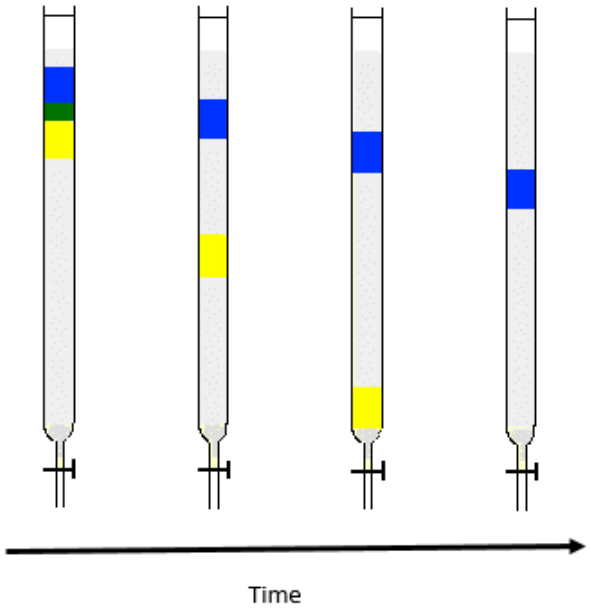



Figure 1.14. Column Chromatography as time elapses (from left to right) which in this case means more mobile phase has been added. 1 sample has 2 compounds (blue and yellow) which travel at different speeds on the stationary phase, silica. The yellow sample is a less polar compound in this case. Picture edited from source.[30]

## 1.4 Chemical Composition of Frankincense

The chemical compositions of these resins depend on many factors, such as the species of *Boswellia* the frankincense came from, the geographical location, the harvesting method and factors such as storage conditions. The extraction procedure can also have a large effect on the observed composition as some compounds may not be extracted and some will have changed chemically if the extraction procedure was too harsh.[31] Furthermore, within a species, there are often grades of frankincense and this usually will have large effects on the composition.[32] Generally speaking, frankincense contains 60-85% resins, 6-30% gums and 5-9% essential oil by weight. The resin fraction is composed of triterpenes such as the boswellic acids, which the name of the genus *Boswellia* comes from. The gums are primarily polysaccharides including some oxidizing and digestive enzymes. The essential oil fraction is usually a diverse mixture of monoterpenes, sesquiterpenes and diterpenes.[7]

### 1.4.1 Volatile Oil Composition

The most famous types of frankincense come from the bark of *Boswellia carterii*, *sacra*, *serrata*, *papyrifera* and *frereana* which spread across Africa, Arabia and India. There are 30

accepted species of *Boswellia* with many not having had much attention to their compositions. There are many known chemotypes of frankincense essential oils which are characteristic for the species (table 1.1). These chemotypes have large abundances of a specific volatile oil and usually have a similar set of compounds that would be found throughout different samples of the same species. However, some species can produce oils from conflicting chemotypes meaning that two resins from *Boswellia frereana* for example may have both α-pinene and thujene dominated oils respectively.[2,33] This can be due to several reasons, such as different locations geographically of the plants, the general environment and changes in the environment. The harvesting method, frequency of harvesting and storage/transport conditions can all affect the composition as well due to the volatility of the compounds in the essential oil fraction.[4] Furthermore, there are often issues with authenticity even when the resin has organic certification.[34]

Table 1.1. Summary of the literature on known *Boswellia* species that produce Frankincense.

| ***Boswellia* species** | **Location** | **Distilled Mass Recovery (%)** | **Essential Oil Chemotypes Reported** |
|---|---|---|---|
| ***B. ameero*** | Socotra Island | 1.8%[35] | Thujene,[36,37] cosmene[35] |
| ***B. bullata*** | Socotra Island | - | Caryophyllene/*trans*-β-farnesene/δ-cadinene/guaiol/α-cadinol/unknown MW = 204/unknown MW = 264[36] |
| ***B. carterii*** | Somalia, Somaliland, Aden, Ethiopia, India | 2.1-8.2[23,38–41] | α-Pinene,[33,34,41,42] thujene,[33,34,43] α-pinene/limonene,[15,33,34,44] octyl acetate,[23,39,40,45] caryophyllene/caryophyllene oxide,[15] *p*-menth-2-en-1-ol[46] |
| ***B. dalzielii*** | West/central Africa | 1.1-16.0[47–49] | α-Pinene,[47,49] α-pinene/myrcene,[47] 3-carene[48] |
| ***B. dioscoridos*** | Socotra Island | 0.3%[50] | Thujene/ α-pinene,[50] thujene[36] |
| ***B. elongata*** | Socotra Island | 0.4-2.3%[35,50] | Thujene/myrcene/caryophyllene,[37] verticiol/caryophyllene,[35] incensole/incensole acetate/verticilla-4(20),7,11-triene,[50] thujene/α-pinene/cembrene/unknown diterpene MW = 306 possibly incensole[36] |

| | | | |
|---|---|---|---|
| ***B. frereana*** | Somalia | - | α-Pinene,[2,33] thujene,[33,43] α-pinene/thujene,[33] thujene/*p*-cymene,[4] phellandrene dimer[15] |
| ***B. nana*** | Socotra Island | - | Thujene[36] |
| ***B. neglecta*** | Uganda, Kenya, Tanzania, Ethiopia, Sudan, Somalia | 5.0%[51] | α-Pinene,[33] α-pinene/thujene,[2,37,51] α-pinene/thujene/4-terpineol[52] |
| ***B. occulta*** | Somaliland | 1.0-1.5[53] | Methoxydecane[34,53] |
| ***B. ovalifoliolata*** | India | 0.2-5%[54] | β-Pinene, α-terpineol, caryophyllene[55] |
| ***B. papyrifera*** | Ethiopia, Eritrea, Sudan | 0.8%[56] | Octyl acetate[4,15,44,52,56,57] |
| ***B. pirottae*** | Ethiopia | 5.0%[51] | 4-Terpineol/*trans*-verbenol[51] |
| ***B. popoviana*** | Socotra Island | - | Caryophyllene oxide,[37] thujene[36] |
| ***B. rivae*** | Somalia, Ethiopia, Kenya | 4.0%[51] | α-Pinene,[37] limonene/3-carene/ α-pinene,[2] α-pinene/limonene/*p*-cymene,[52] limonene[44,51,57] |
| ***B. sacra*** | Oman, Somalia, Yemen | 5.0-8.5[32,58] | α-Pinene,[4,15,19,32,33,42] (*E*)-β-ocimene/limonene,[58] β-pinene/ α-terpinene[59] |
| ***B. serrata*** | India | 5.0-11.6[60–62] | Thujene,[44,60,61] myrcene,[2] α-pinene,[63] thujene/methylchavicol/myrcene[4,15] |
| ***B. socotrana*** | Socotra Island | 0.2-1.2[35,50] | (*E*)-2,3-Epoxycarene/*cis*-2-thujen-4-ol,[35] 2-hydroxy-5-methoxy-acetophenone,[50] α-pinene,[36] thujene[36] |
| ***B. thurifera*** | India | - | α-Pinene[33,64] |

### 1.4.1.1 *Boswellia sacra* Essential Oil

*Boswellia sacra* is historically significant as it was the main source of frankincense back in classical times.[65] It originates from Oman and is mainly cultivated across the Dhofar region. Resin from this species is commercially available in 4 grades which are named after the geographical location the resin was harvested from and the highest grades are typically lighter coloured and larger size. The 4 grades in order of highest grade to lowest are Hoojri, Najdi, Shathari and Sha'abi.[32] Distillation of this resin gives a range of around 5-8.5% mass recovery. Most of the literature that discuss *Boswellia sacra* essential oils show high α-pinene **25** content (figure 1.15) and is usually mostly made up of monoterpenes with only low

sesquiterpene and diterpene content. In fact, the highest α-pinene content comes from *Boswellia sacra* plants, giving essential oils of around 78.5% α-pinene **25**. There is one report of (*E*)-β-ocimene **46** (32.3%) and limonene **33** (33.5%) as the major constituents of a *Boswellia sacra* essential oil.[58] If the sample is indeed authentic, it could be due to other factors such as overharvesting or due to a change in the habitat. Either way, during the biosynthesis, this sample has clearly favoured not making the second cyclisation to α-pinene, but instead remaining completely acyclic for (*E*)-β-ocimene and cyclising just once to make limonene. One recent study reported β-pinene **28** (25.6%) and α-terpinene **47** (18.6%) to be the major compounds in the essential oil.[59] Once again, something enzyme related in the biosynthesis would be the likely cause if the sample is authentic. It should be noted that β-pinene and limonene are commonly found in lower concentrations in a wide range of *Boswellia sacra* samples, but α-terpinene and (*E*)-β-ocimene are rare and except in the one case where they are part of the chemotypes, are only detected in small concentrations (figure 1.16).[4,15,19,32,33,42]

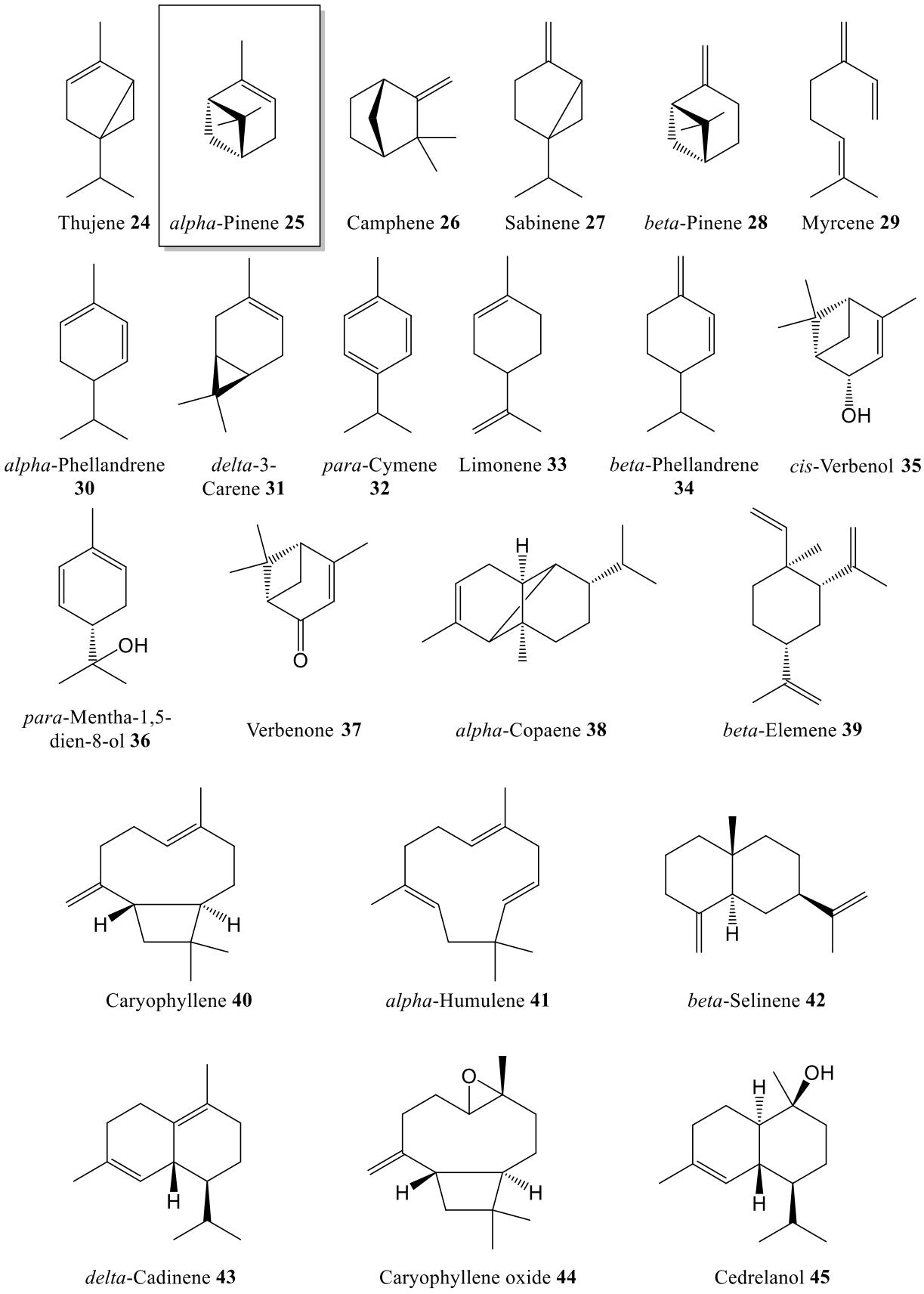



Figure 1.15. Commonly reported major compounds from *Boswellia sacra* resin of the α-pinene chemotype.

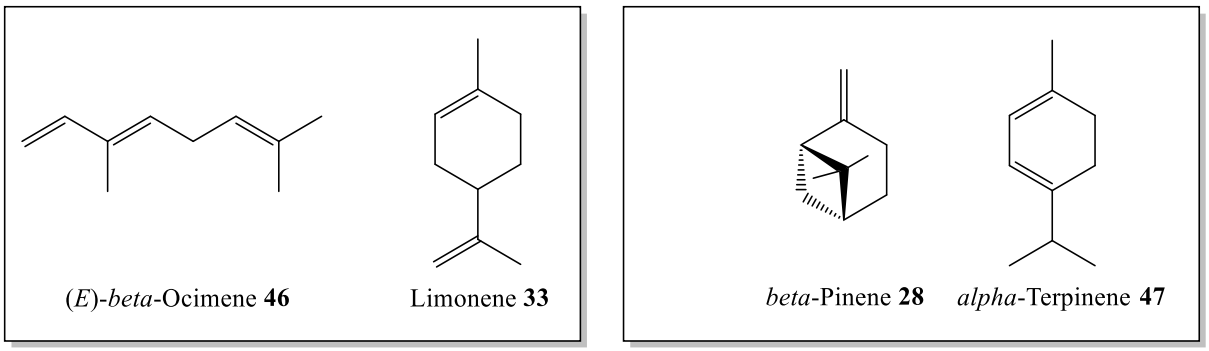



Figure 1.16. Alternate native chemotypes for B*oswellia sacra* volatile oil fractions.

### 1.4.1.2 *Boswellia carterii* Essential Oil

*Boswellia carterii*, which is often referred to as synonymous with *Boswellia sacra*, is another popular source of frankincense and its essential oil is the most widely studied. It is mainly traded throughout Somalia and most commonly gives high α-pinene **25** essential oils at around 40% of the content in its essential oil being α-pinene. However, the α-pinene levels are not generally as high as in *Boswellia sacra* essential oil and has a wide range of chemotypes (α-pinene content 4.8-37.3%). This similarity to *Boswellia sacra* oils, along with sharing many other lower concentration compounds led to the confusion about whether it is a separate species. However, there are geographical, growth and differences in the physical appearance that make the two species still mainly considered separate. They are still traded as distinct species and *Boswellia sacra* is generally seen as the Arabian counterpart to *Boswellia carterii* being the African species. A noticeable difference in *Boswellia sacra* and *carterii's* α-pinene chemotypes is the lack of major sesquiterpenes in *carterii*. *Boswellia carterii* has a large monoterpene content as well and tends to have large limonene concentration, which comes at a cost of α-pinene levels. Furthermore, *carterii* commonly produces the diterpenes incensole **49** and serratol **48**, whereas *sacra* is known to have very little to no diterpene fraction as mentioned before (figure 1.17).[33,34,41,42]

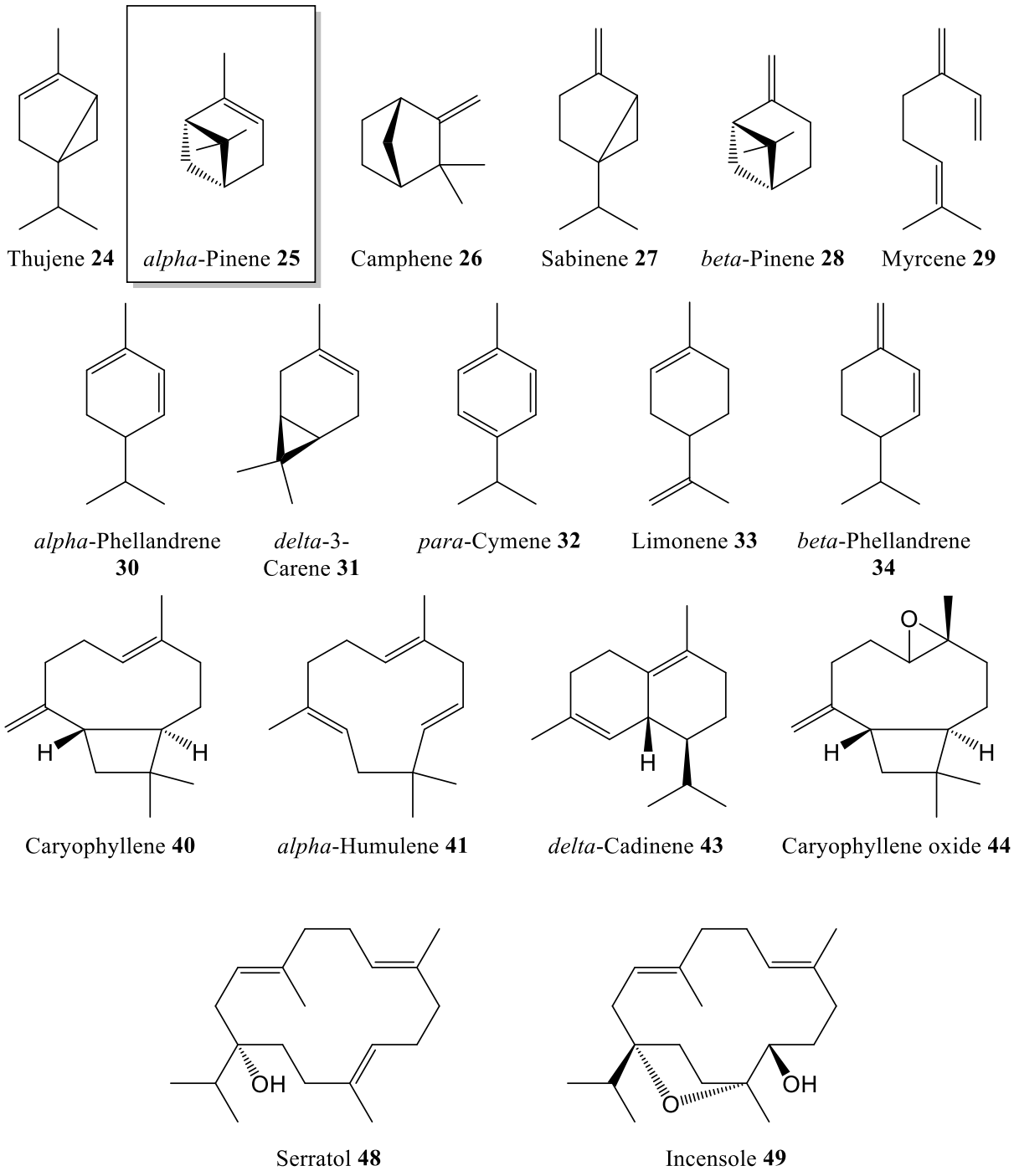



Figure 1.17 Common major compounds detected in *Boswellia carterii* essential oil of the α-pinene chemotype.

There are several different chemotypes (table 1.2) that have been found for *Boswellia carterii* essential oil. These include α-pinene/limonene,[15,33,34,44] thujene,[33,34,43] octyl acetate **50**,[23,38–40] caryophyllene **40**/caryophyllene oxide **44**[15] and *p*-menth-2-en-1-ol **51**[46] dominated oils (figure 1.18).

Table 1.2. The GC/MS determined content of alternative chemotypes for different reported oils in the essential oil fraction of *Boswellia carterii.*

| Chemotype | Content (%) |
|---|---|
| **α-Pinene/limonene** | 23.2-32.3 and 22.4-44.8 |
| **Thujene** | 32.9-50.6 |
| **Octyl acetate** | 34.7-60.0 |
| **Caryophyllene/caryophyllene oxide** | 16.9 and 13.1 |
| ***p*-Menth-2-en-1-ol** | 34.5 |

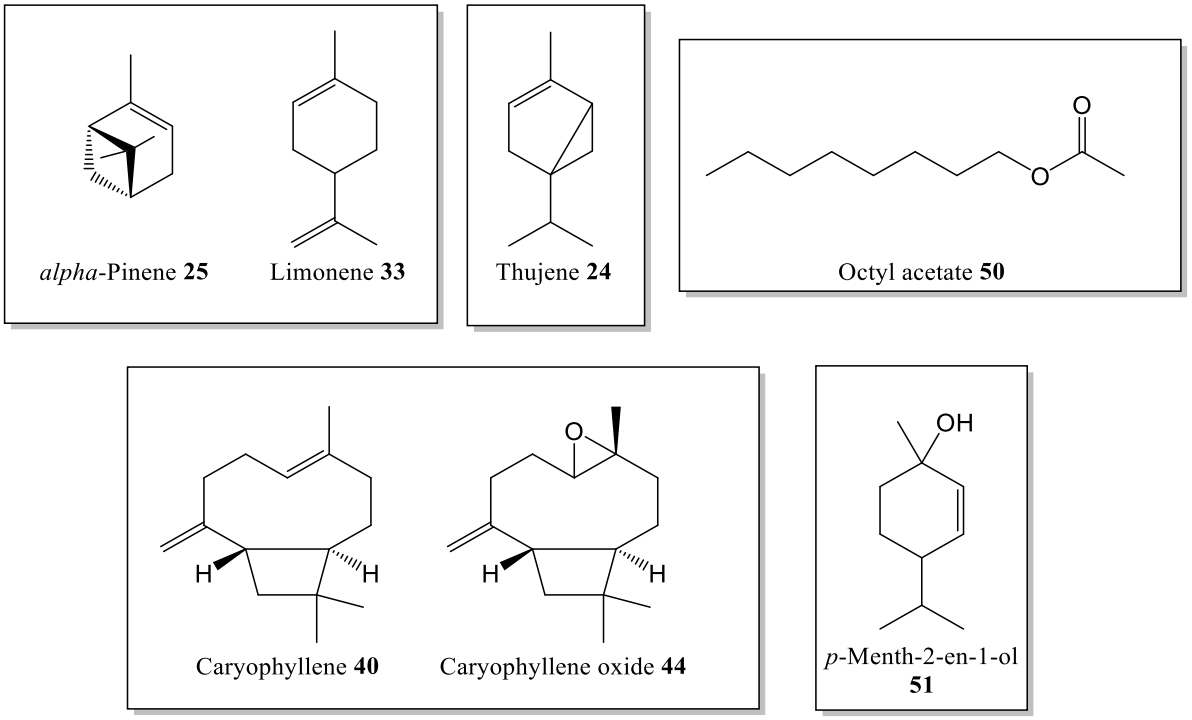



Figure 1.18. Alternative native chemotypes for *Boswellia carterii's* essential oil fraction.

The α-pinene/limonene chemotype is a fairly common alternative to the α-pinene based oils. Chemically this is similar to the α-pinene chemotype discussed above but with limonene levels getting close to or even rising above the α-pinene concentrations so is generally considered different. Furthermore, levels of caryophyllene **40** are often higher in this chemotype, with levels reaching 10.5%.[44]

The thujene **24** chemotype is fairly common and shares many of the monoterpenes present for the α-pinene dominated oils. However, from sample to sample various monoterpenes increase quite largely in content with α-pinene **25** reaching 14.8%, sabinene **27** 10.9%, *p*-cymene **32,** 9.7% and thuja-2,4(10)-diene **52** 8.4% in its essential oil. Furthermore, there is a much weaker sesquiterpene fraction and very little to no diterpene fraction.[33,41]

An octyl acetate **50** chemotype often containing diterpenes such as incensole **49** (2.7-6.1%) and incensole acetate **53** (13.0%) has also been reported. This has a lot in common with *Boswellia papyrifera* resin which seems to exclusively give this chemotype with large diterpene fractions so it is possible that there could have been confusion regarding the taxonomic identification unless it is truly a different chemotype of *Boswellia carterii*.[23,38,40]

Both the caryophyllene **40**/caryophyllene oxide **44** and *p*-menth-2-en-1-ol **51** chemotypes have only been reported once for this resin but are interesting in their own ways. The caryophyllene type is unusual because the oil is usually dominated vastly by monoterpenes and *p*-menth-2-en-1-ol was not noted in any other *Boswellia carterii* essential oil.[15,46]

### 1.4.1.3 *Boswellia papyrifera* Essential Oil

*Boswellia papyrifera* is said to have been the main source of frankincense in antiquity.[65] Throughout the literature, it seems to produce the octyl acetate **50** chemotype in diterpene rich volatile oils (figure 1.19). The octyl acetate content in the distillates are reported to range from 51.3-65.7% and octanol **54** is usually found as another major compound at 2.9-17.8%. As mentioned, this resin produces a large diterpene fraction, made up of the cembrene skeleton and commonly give high concentrations of the interesting diterpenes, incensole **49** (0.7-3.2%) and incensole acetate **53** (1.7-10.8%) in the essential oil.[4,15,44,52,56] This resin is interesting as it does not seem to produce any other chemotypes except for octyl acetate. This is unusual for frankincense resins and other species which have been discussed produce a wide range of chemotypes. This could be due to a lack of understanding on what each species really is based upon or could just be an interesting trait of *Boswellia papyrifera*.

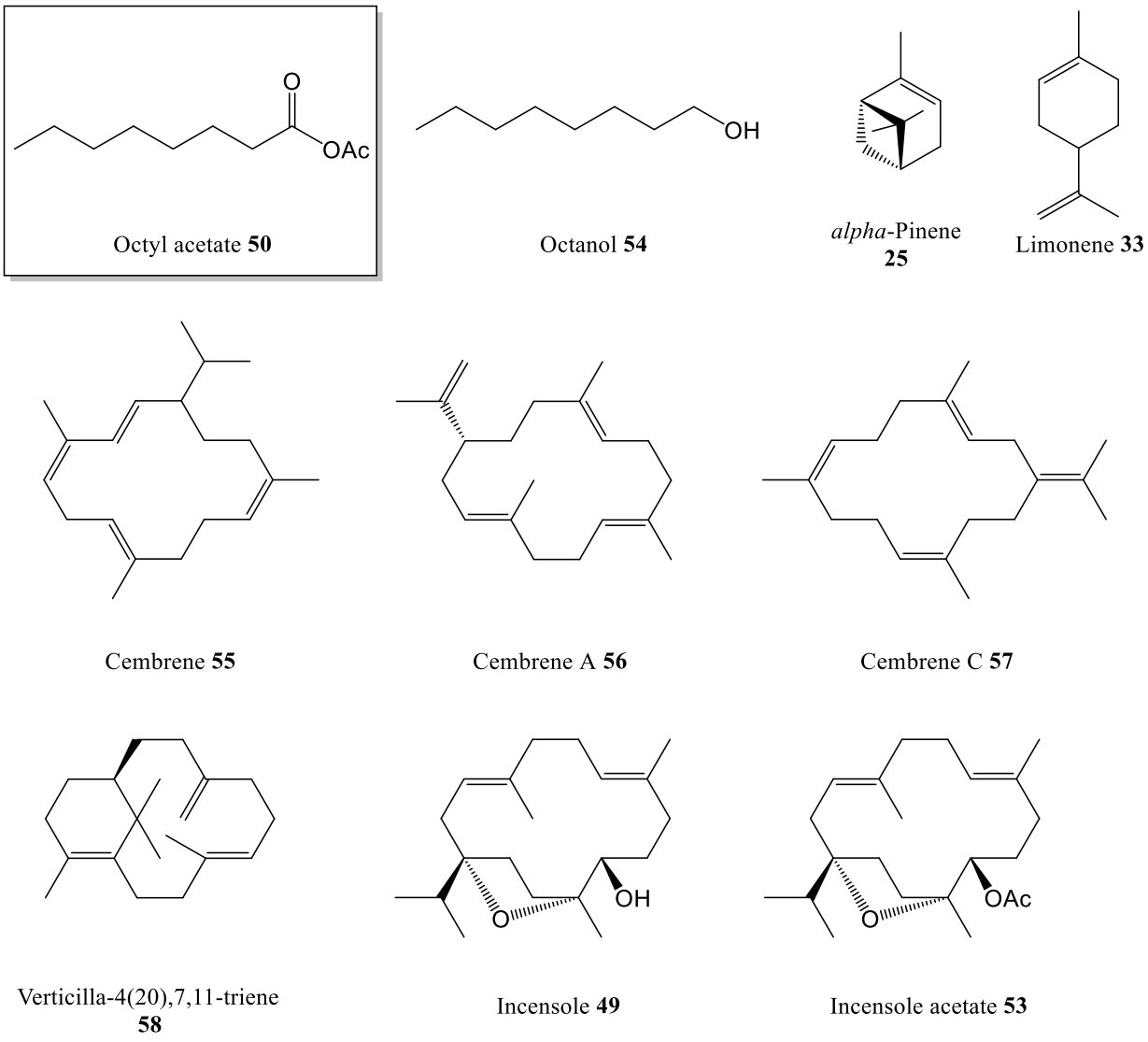
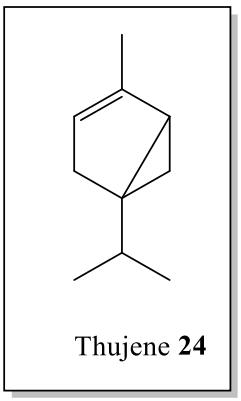


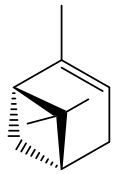

Figure 1.19. Commonly reported compounds for *Boswellia papyrifera* essential oil.

### 1.4.1.4 *Boswellia serrata* Essential Oil

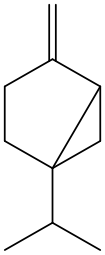
*Boswellia serrata*, also commonly referred to as Indian frankincense, is a common source of the thujene **24** chemotype (22.5-69.8%) in its essential oil. These oils have a wide variety of 'terpinene' shaped essential oils. However, there are several phenolic compounds as major constituents found in some *Boswellia serrata* volatile fractions, but not others, such as methylchavicol **59** (0-6.7%), benzyl tiglate **60** (0-5.5%) and methyl isoeugenol **68** (0-3.1%) which could be used as biomarkers for different chemotypes of this species (figure 1.20).[44,60–62]



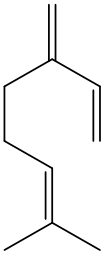
Thujene **24**



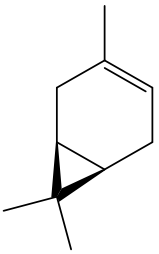
*alpha*-Pinene **25**



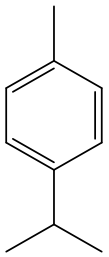
Sabinene **27**



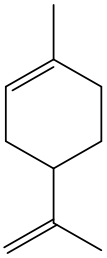
Myrcene **29**



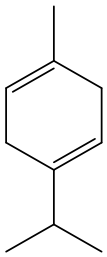
*delta*-3-Carene **31**



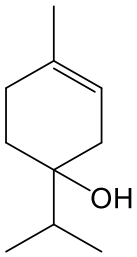
*para*-Cymene **32**



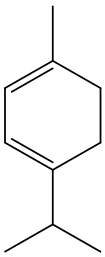
Limonene **33**



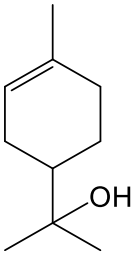
*gamma*-Terpinene **62**



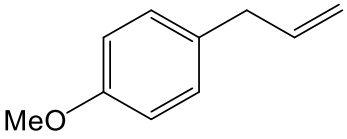

4-Terpineol **63**



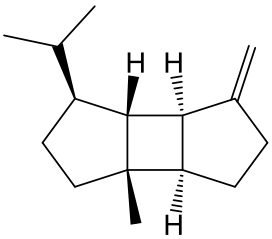
*alpha*-Terpinene **47**



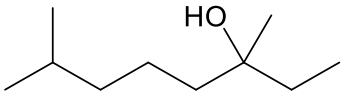

*alpha*-Terpineol **64**



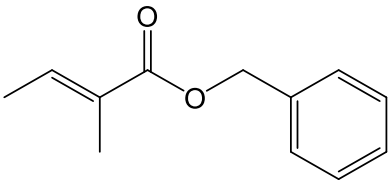

Methylchavicol **59**



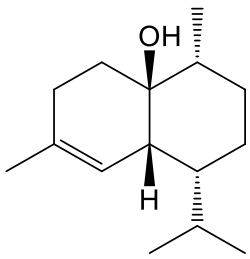

*beta*-Bourbonene **65**



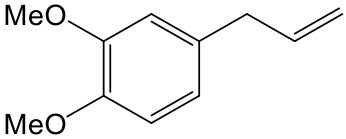

Tetrahydrolinalool **66**




Benzyl tigilate **60**




*epi*-Cubenol **67**




Methyl isoeugenol **68**

Figure 1.20. *Boswellia serrata's* commonly reported volatile oils in Thujene chemotypes.

A similar chemotype is the thujene/myrcene/methylchavicol type which mainly differs in thujene content. Studies with this chemotype showed thujene concentrations to be much lower (11.7-15.2%) due to the presence of myrcene (4.1-7.0%) and methylchavicol (8.9-12.3%) in much higher concentrations than seen in the thujene dominated oils. Myrcene and

methylchavicol are often found in the thujene rich oils, however they are typically in lower concentrations so therefore it is likely a separate chemotype. Furthermore, these oils both had slightly more diterpene content with small concentrations of incensole, serratol and some cembrenes.[4,15] Myrcene and α-pinene chemotypes have also been reported for this species.[2,63]

### 1.4.1.5 *Boswellia Occulta* Essential Oil

A new species of frankincense producing tree has recently been discovered which is named *Boswellia occulta*.[66] Distillation of this resin results in the isolation of a less common chemotype which has methoxydecane **71** in large concentrations (26.7-54.9%). Serratol **48** and incensole **49** also are produced in this chemotype (figure 1.21). This resin could be useful in the isolation of significant diterpenes such as incensole through simple extraction methods, as the methoxydecane fraction can be removed simply by distillation and the diterpene fraction does not seem overly complex. Previously the occurrence of this new oil was thought to be a result of overharvesting of *Boswellia carterii* plants. However, it seems more likely that it was due to the increase in demand of frankincense, meaning trees in previously undisturbed areas are now being harvested. Furthermore, oils with lower methoxydecane content could be due to mixed resin samples of *Boswellia carterii* and *occulta*.[34,53]

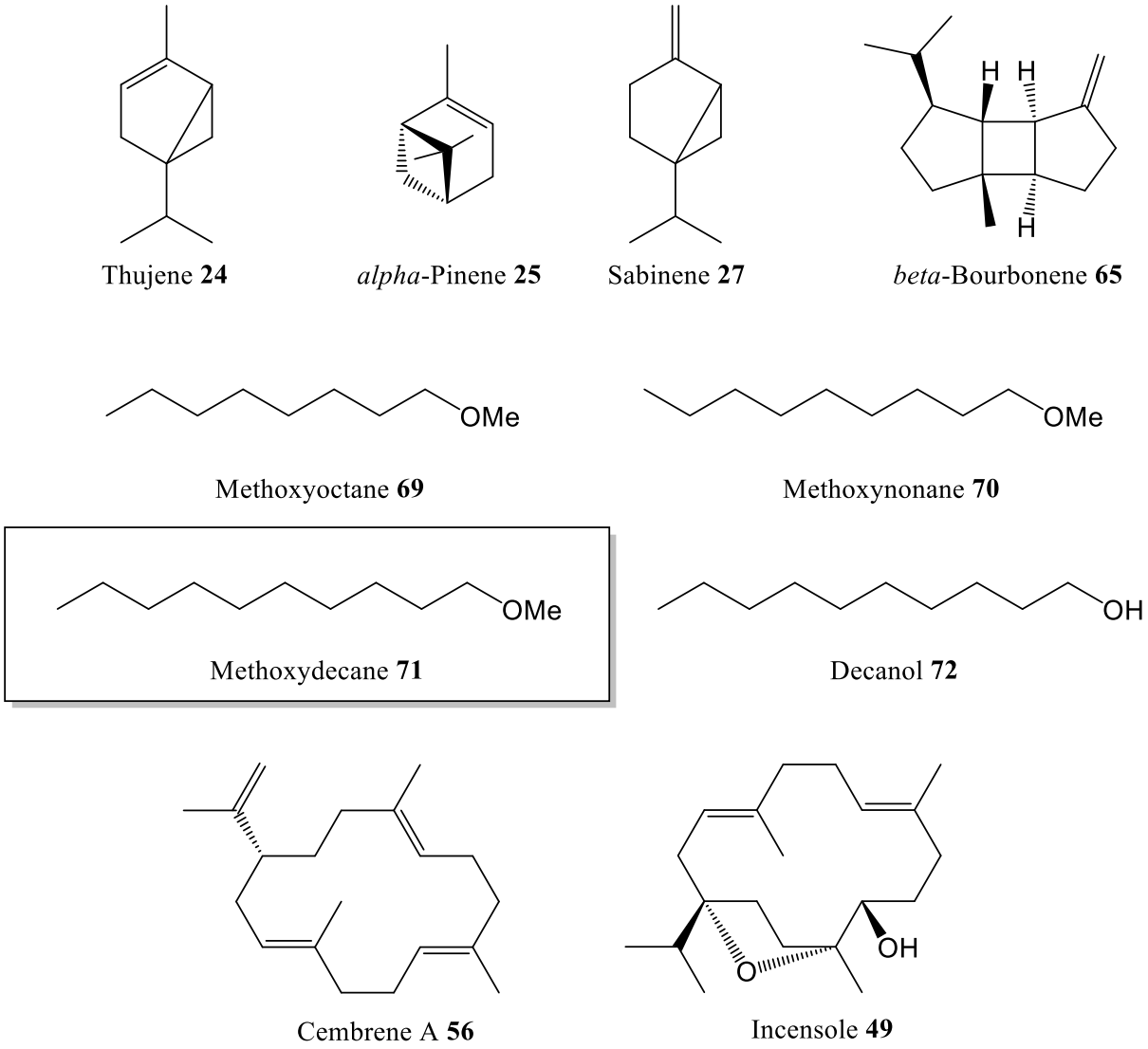



Figure 1.21. *Boswellia occulta's* common compounds in the methoxydecane chemotype.

## 1.4.2 Non-Volatile Composition

Some common volatile fractions of frankincense resins have been discussed, however, this fraction only accounts for a small amount of the total mass of the resin. As mentioned earlier, around 60-85% of the resin is alcohol soluble compounds such as the boswellic acids and many other compounds that will not distil or will only distil in small amounts. Many compounds have been found in the heavy oil fraction of frankincense resins which are known to include boswellic acids, tirucallic acids, lupeols/lupeolic acids, roburic acids, nyctanthic acids, canaric acids and amyrins. Most of the heavy oil composition in frankincense comes from triterpenes of the ursane **73**, oleanane **74**, lanostane **75** and lupine **76** structures (figure 1.22).

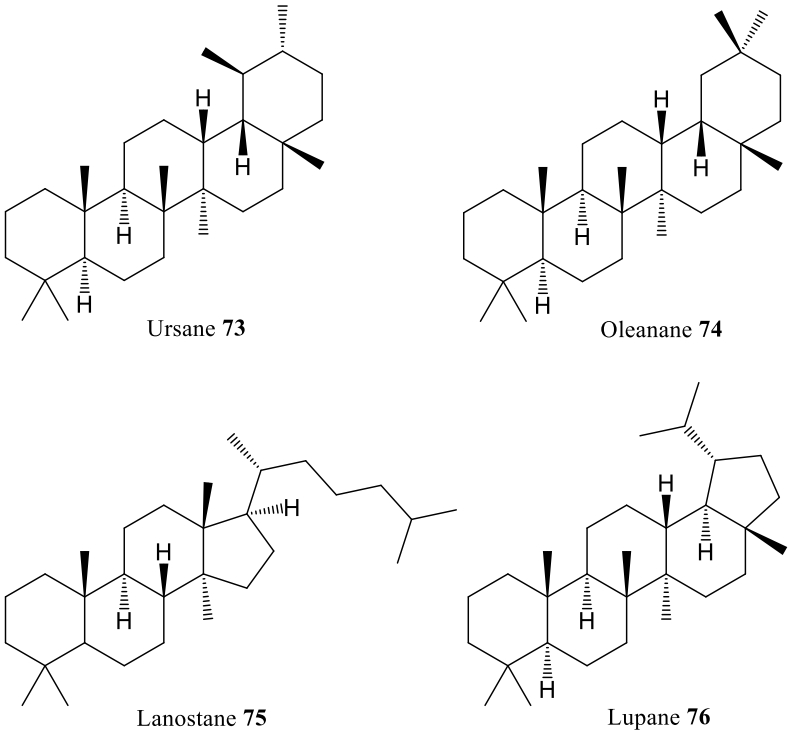
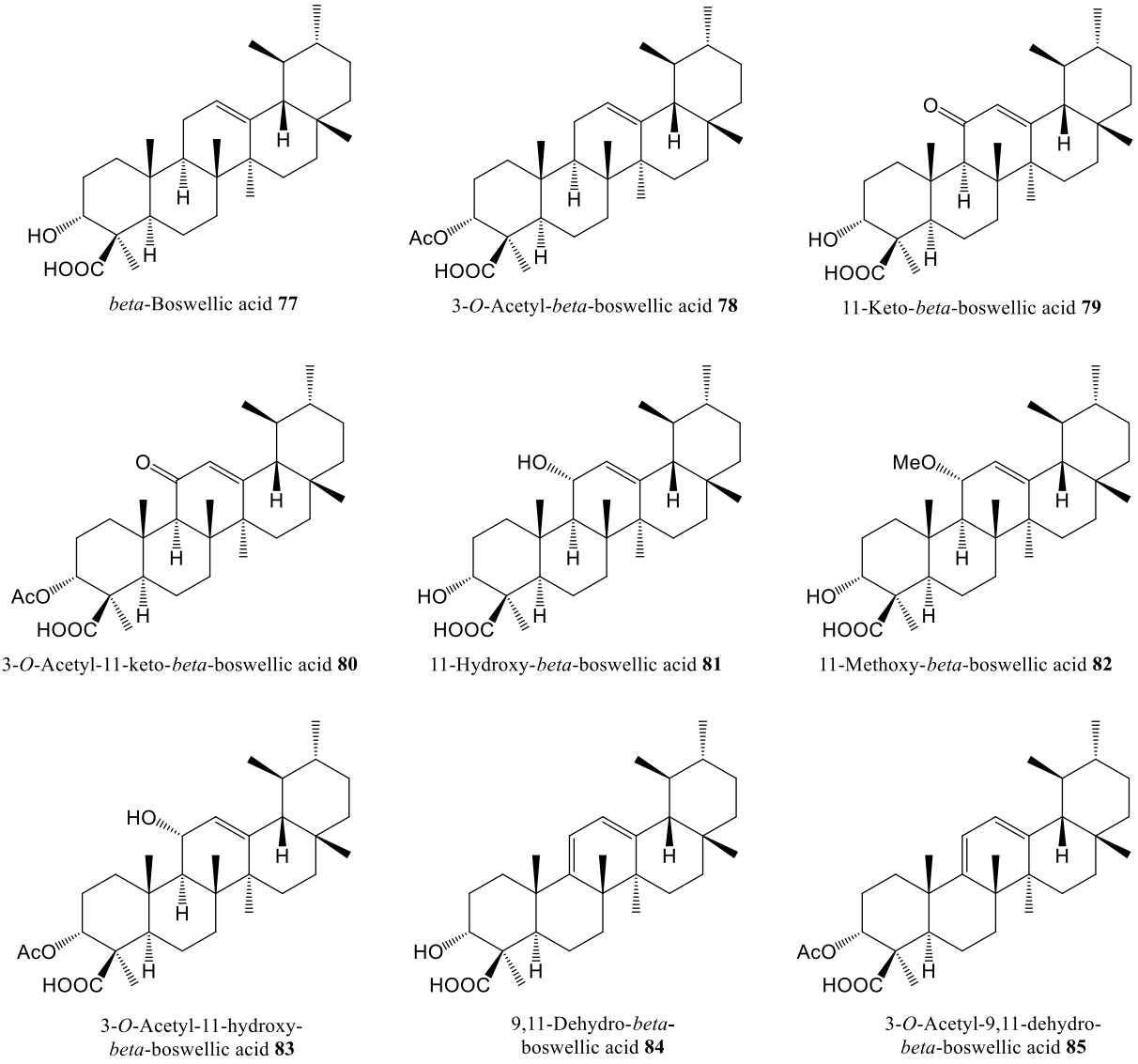



Figure 1.22. General structures of triterpenes in frankincense.

The ursane type structure gives rise to β-boswellic acids, roburic acids and α-amyrins (figures 1.23-1.25). This type is very common and often covers the highest concentration in the resin, with compounds like the β-keto-boswellic acids often being the most abundant.[2,67,68] The oleanane structure covers very similar compounds, with the change of position of one methyl group being the difference in the core structure. Compounds that are α-boswellic acids, β-amyrins and nyctanthic acids fit in to this group (figures 1.26-1.28). Boswellic acids which have this shape are less abundant than their β-counterparts, but are still found extremely commonly in many species of the resin.[2,69] Lanostane is the general structure for tirucallic acids which are also quite abundant in these resins and are tetracyclic triterpenes instead (figure 1.29). Lupane type compounds are also common in frankincense and cover compounds such as lupeols, lupeolic acids and canaric acids (figure 1.30). *Boswellia frereana* is reported to be very high in lupeol and lupeolic acid.[2,70]




*beta*-Boswellic acid **77**

3-*O*-Acetyl-*beta*-boswellic acid **78**

11-Keto-*beta*-boswellic acid **79**

3-*O*-Acetyl-11-keto-*beta*-boswellic acid **80**

11-Hydroxy-*beta*-boswellic acid **81**

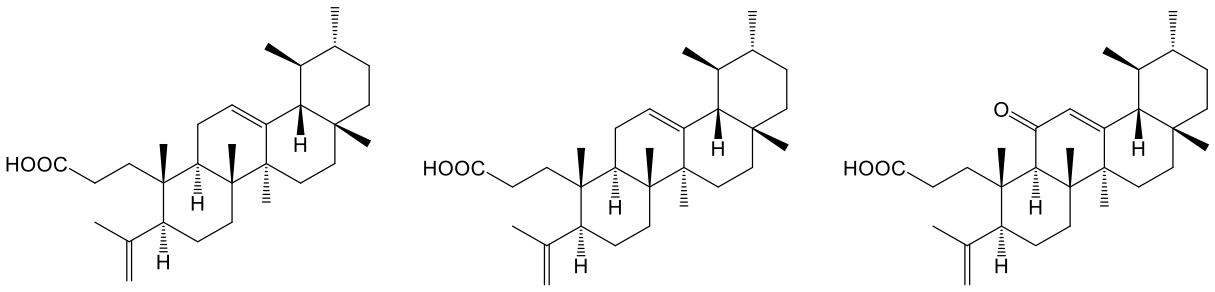
11-Methoxy-*beta*-boswellic acid **82**

3-*O*-Acetyl-11-hydroxy-*beta*-boswellic acid **83**

9,11-Dehydro-*beta*-boswellic acid **84**

3-*O*-Acetyl-9,11-dehydro-*beta*-boswellic acid **85**

Figure 1.23. β-Boswellic acids from the ursane structure found in frankincense.

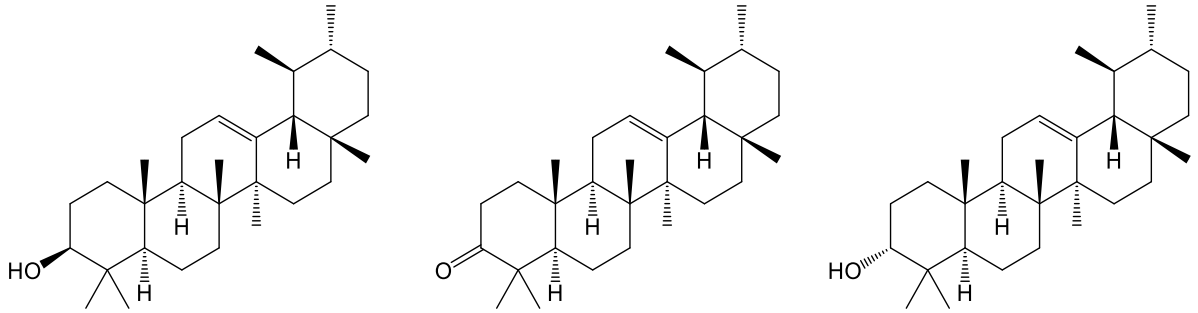



Roburic acid **86**

4(23)-Dihydroroburic acid **87**

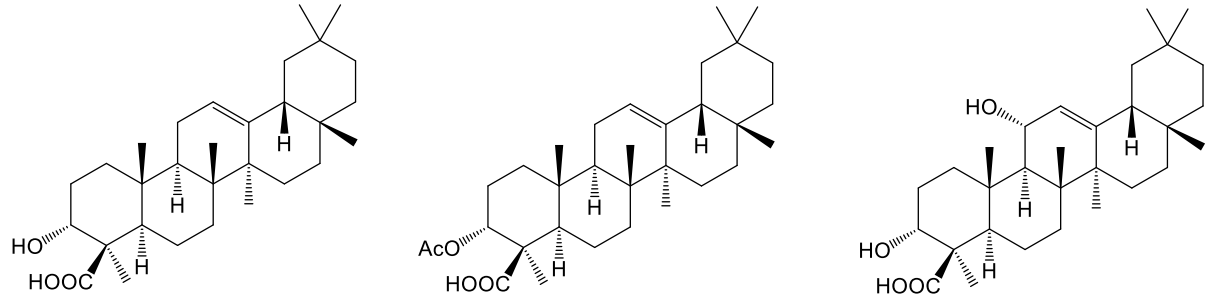
4(23)-Dihydro-11-keto-roburic acid **88**

Figure 1.24. Roburic acids from the ursane structure found in frankincense.

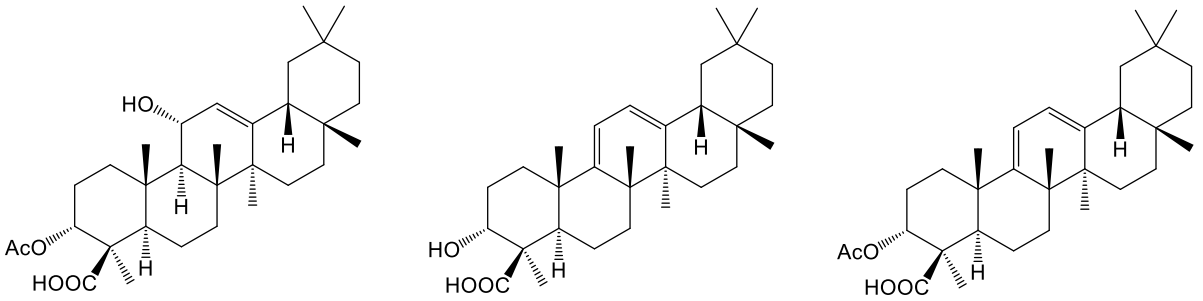



*alpha*-Amyrin **89** | *alpha*-Amyrenone **90** | 3-*epi*-*alpha*-Amyrin **91**

Figure 1.25. Amyrins from the ursane structure found in frankincense.

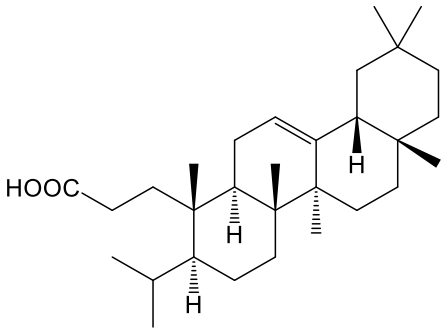



*alpha*-Boswellic acid **92** | 3-*O*-Acetyl-*alpha*-boswellic acid **93** | 11-Hydroxy-*alpha*-boswellic acid **94**



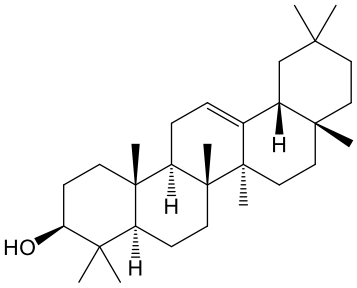

3-*O*-Acetyl-11-hydroxy-*alpha*-boswellic acid **95** | 9,11-Dehydro-*alpha*-boswellic acid **96** | 3-*O*-Acetyl-9,11-dehydro-*alpha*-boswellic acid **97**

Figure 1.26. Boswellic acids from the oleanane structure found in frankincense.

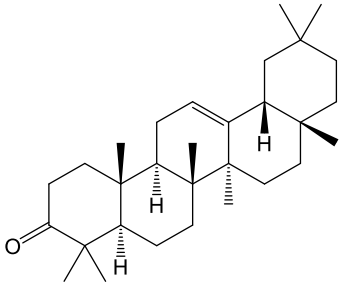



4(23)-Dihydronyctanthic acid **98**

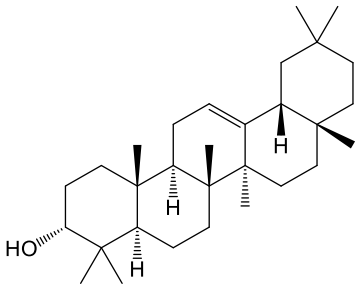
Figure 1.27. 4(23)-Dihydronyctanthic acid from the oleanane structure found in frankincense.




*beta*-Amyrin **99**

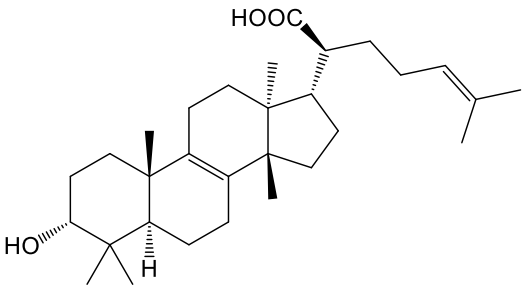



*beta*-Amyrenone **100**

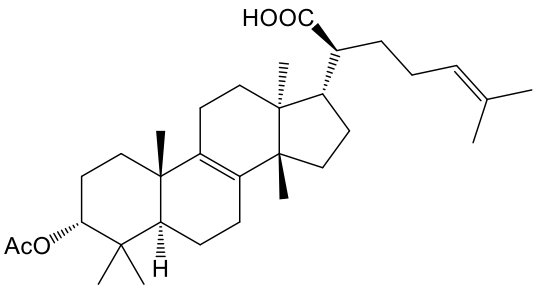



3-*epi*-*beta*-Amyrin **101**

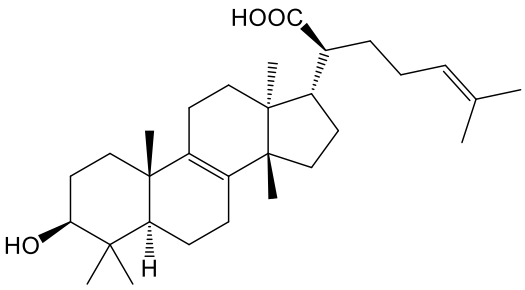
Figure 1.28. Amyrins from the oleanane structure found in frankincense.



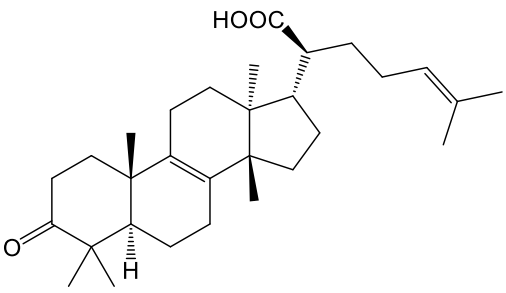

3-*alpha*-hydroxy-8,24-diene-tirucallic acid **102**



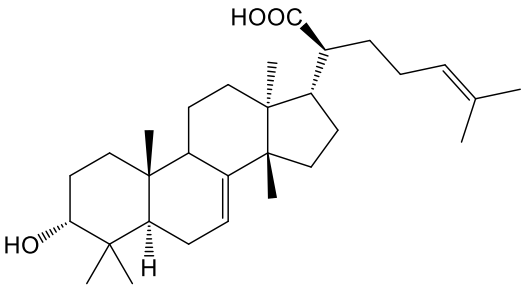

3-*alpha*-*O*-acetyl-8,24-diene-tirucallic acid **103**



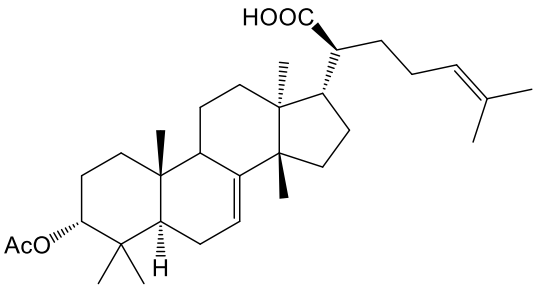

3-*beta*-hydroxy-8,24-diene-tirucallic acid **104**




3-oxo-8,24-diene-tirucallic acid **105**




3-*alpha*-Hydroxy-7,24-diene-tirucallic acid **106**




3-*alpha*-*O*-acetyl-7,24-diene-tirucallic acid **107**

Figure 1.29. Tirucallic acids from the lanostane structure found in frankincense.

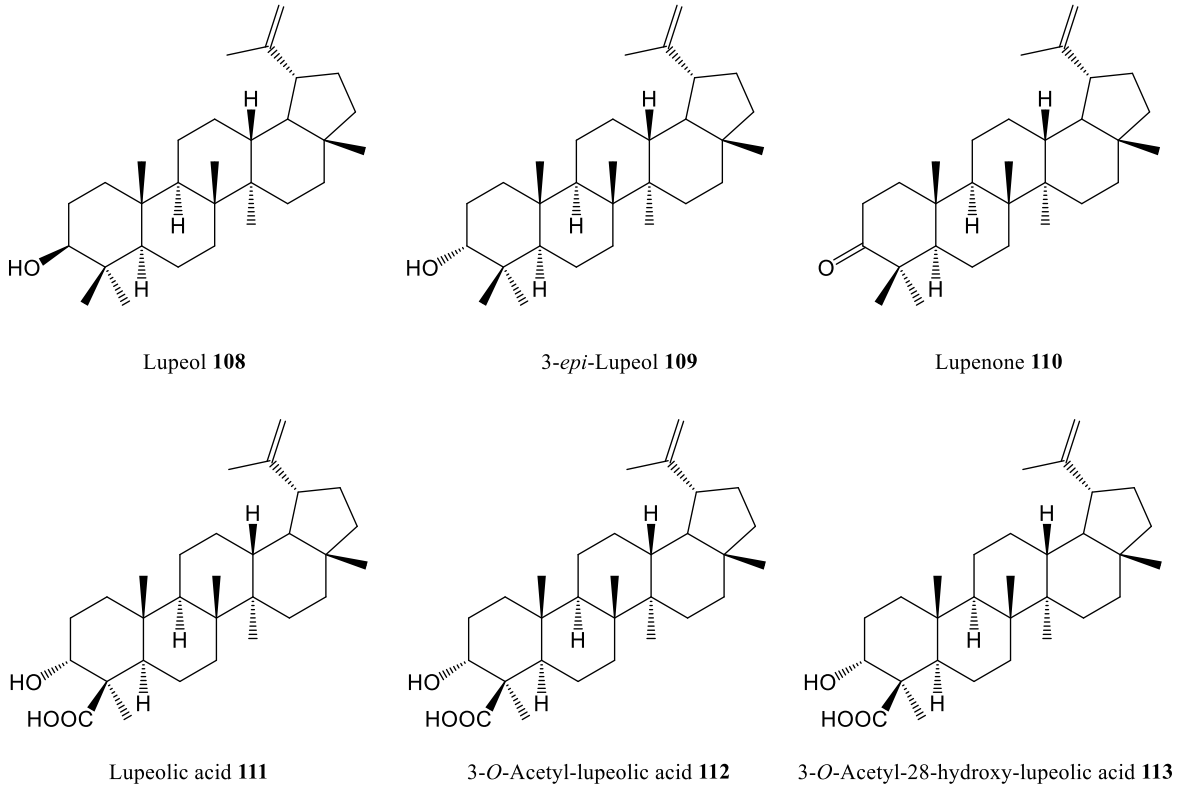



Figure 1.30. Lupeols and lupeolic acids from the lupane structure found in frankincense.

### 1.4.3 Major Products of From the Solvent Extracts of Various *Boswellia* Species

A study which compared resins from *Boswellia papyrifera*, *serrata*, *sacra* and *carterii* gave some interesting biomarkers when the heavy organic constituents of the diethyl ether extract were analysed (figure 1.31). *Boswellia papyrifera* showed a rich diterpene fraction as discussed before, with incensole **49**, incensole acetate **53** and verticilla-4(20),7,11-triene **58** being in high concentration along with the acids 3-*O*-acetyl-11-keto-β-boswellic acid **80** and 3-oxo-8,24-diene-tirucallic acid **105**. *Boswellia serrata* was high in both α-boswellic acid **92** and β-boswellic acid **77** as well as **105**, iso-serratol **122** and serratol **48**. This, along with about a 1:1 ratio of 11-keto-β-boswellic acid **79** to **80** made *Boswellia serrata* quite unique in this instance. *Boswellia sacra* and *carterii* had very similar profiles, which why there has been such confusion about whether they are the same species or not which just grows in a different location. As well as sharing high concentrations of 3β-hydroxy-lanostane **123**, they both have comparable concentrations of lupeolic acid **111** and 3-*O*-acetyl-lupeolic acid **112**. There was a small difference in **77**, 3-*O*-acetyl-β-boswellic acid **78**, **79** and **80** but even they are very similar, with *sacra* having slightly higher levels of **77** and **78** and *papyrifera* having the higher levels of **79** and **80**. This slight rise in the none keto acids corresponding with a fall in the keto

boswellic acids was suggested to be a biosynthetic trade off, where they will directly affect the concentration of the other pair of acids.[70]

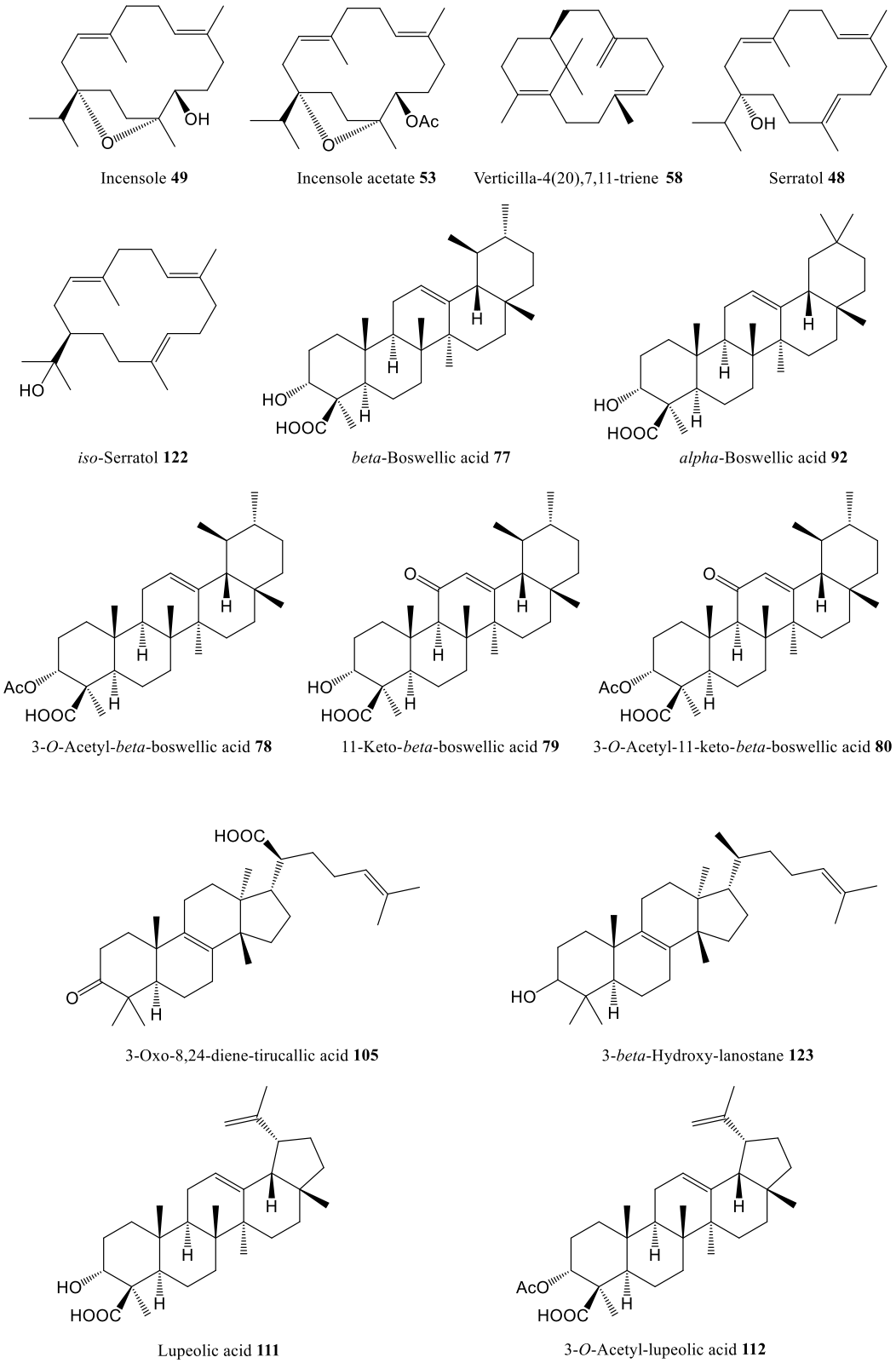



Figure 1.31. Reported major compounds found in the $Et_2O$ extracts of *Boswellia papyrifera*, *serrata*, *sacra* and/or *carterii.*

## 1.4.4 GC/MS Characterization of Boswellic Acids

It is challenging to identify and quantify some of these compounds as they can be difficult to separate and cannot be simply analysed by GC/MS like the volatile fraction as they are not volatile enough to enter the gas phase. Instead, the boswellic acids thermally decompose and these decarboxylated compounds are detected. The problem with this is several boswellic acids will decompose into the same compound, so these fingerprint compounds are just a guide to the type of boswellic acids present (figure 1.32). The fragmentation patterns for many of the observed triterpenes have been previously reported so were used as a guide for the classification carried out herein.[2,71]

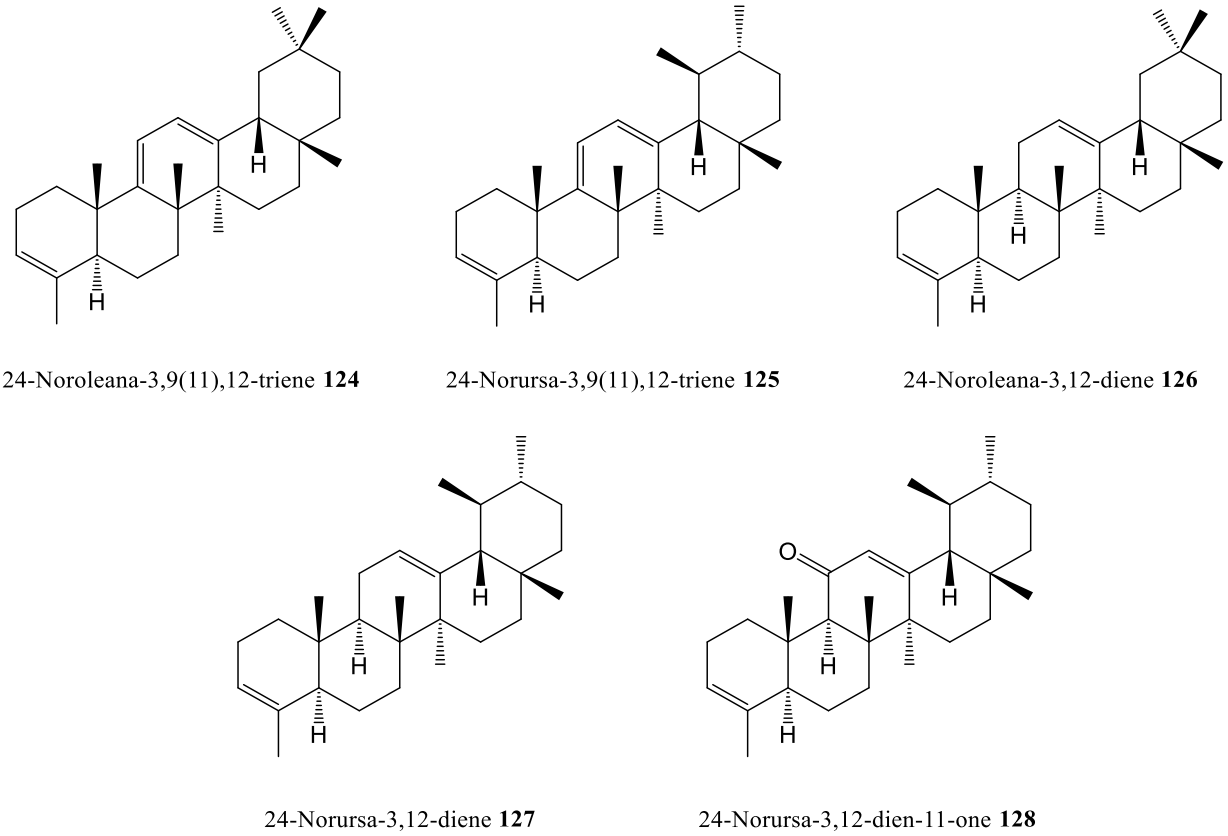



Figure 1.32. Triterpenes that represent boswellic acids as detected by GC/MS.

The most abundant nortriterpene found in frankincense is usually 24-norursa-3,12-dien-11-one **128**, which is formed by β-keto-boswellic acids thermally degrading resulting in the loss of $H_2O$ or $CH_3COOH$ and $CO_2$ (figure 1.33).

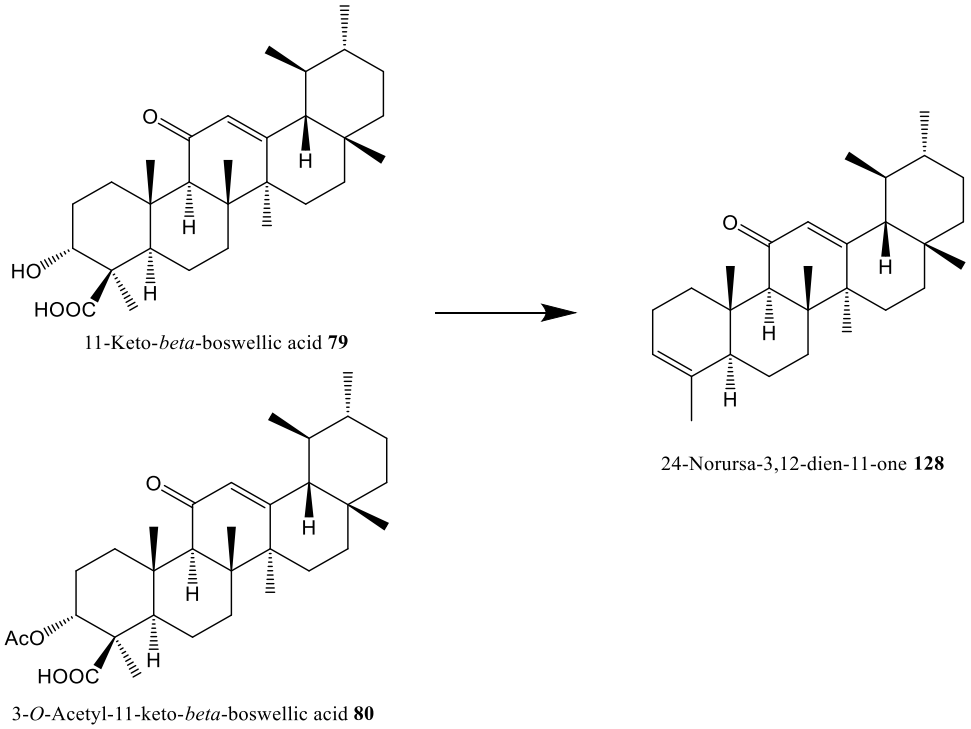



Figure 1.33. Keto-β-boswellic acids degrading to 24-norursa-3,12-dien-11-one by thermally decomposing

Both β-keto-boswellic acids lose their carboxylic acid groups as well as a molecule of $H_2O$ for **79** and $CH_3COOH$ for **80**. This results in **128**, which has a molecular weight of 408, which can be seen on the fragmentation pattern (figure 1.34).

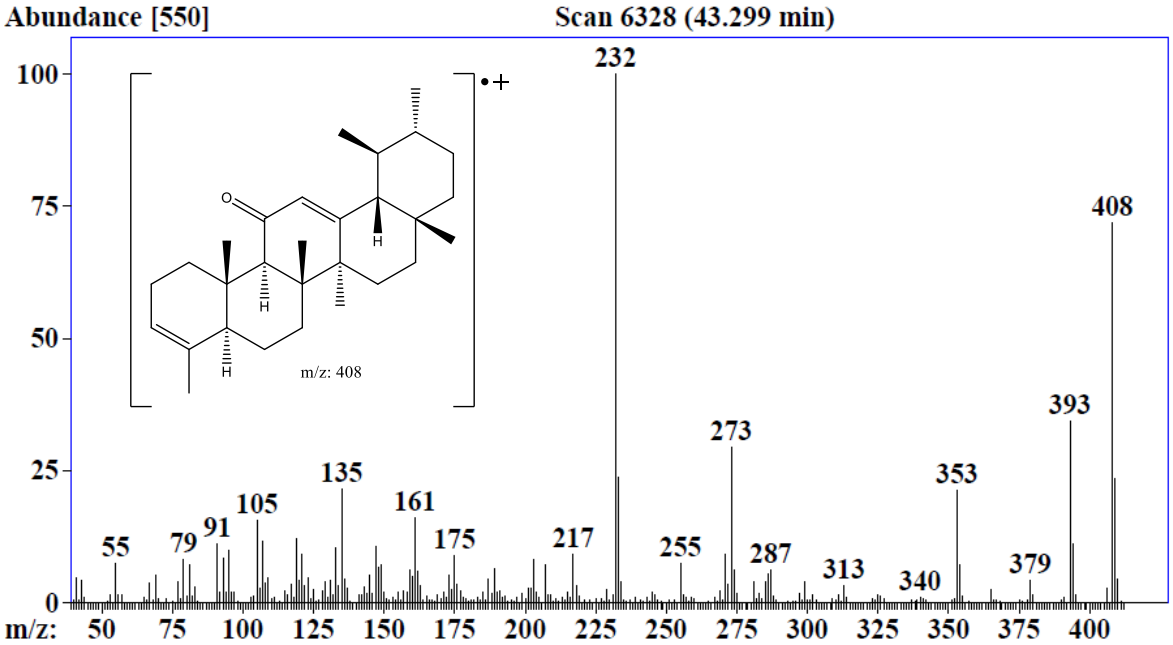



Figure 1.34. Fragmentation pattern for 24-Norursa-3,12-dien-11-one **128**, AI = 3309 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with methanol following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[2]

The main mechanisms of fragmentation are through a retro-Diels-Alder pathway and a McLafferty rearrangement (figures 1.35 and 1.36). The retro-Diels-Alder pathway results in the large peak at m/z = 232, which occurs from the middle ring of the compound, splitting it into two halves. The McLafferty rearrangement gives rise to the peak at m/z = 273, which happens due to the ketone group's interaction with a hydrogen atom on the left most ring. Due to the larger size of the peak at m/z = 232 when compared to the peak at m/z = 273, it has been suggested that the retro-Diels-Alder pathway is the favoured mechanism in this case.[2]

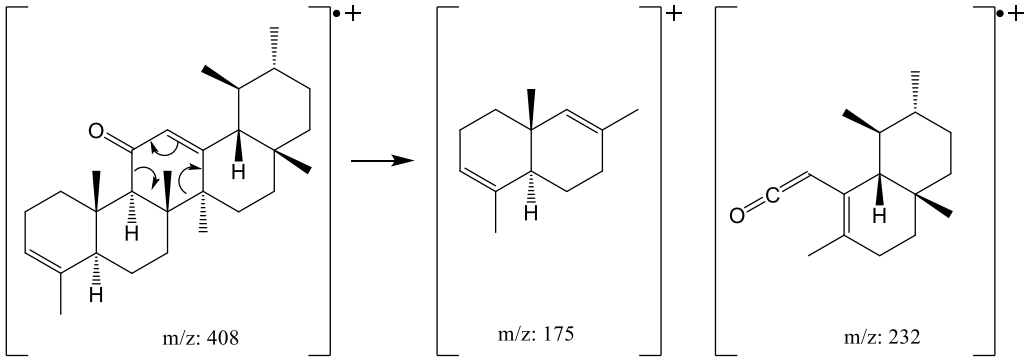



Figure 1.35. Retro Diels-Alder fragmentation suggested by the literature in the β-*keto*-boswellic acids.[2]

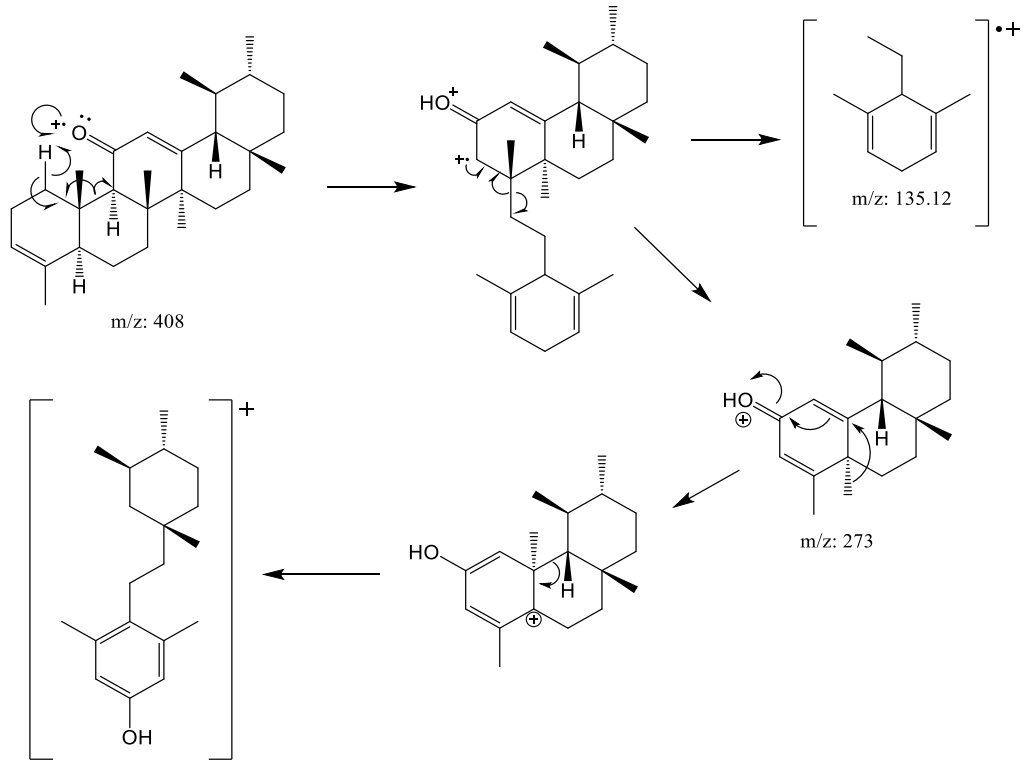



Figure 1.36. McLafferty rearrangement based on a literature suggestion in the fragmentation of β-*keto*-boswellic acids.[2]

Another abundant nortriterpene that is seen via GC/MS is **127**, which is formed through the thermal decomposition of β-boswellic acids that are not substituted at the 11 position such as **77** and **78** (figure 1.37).

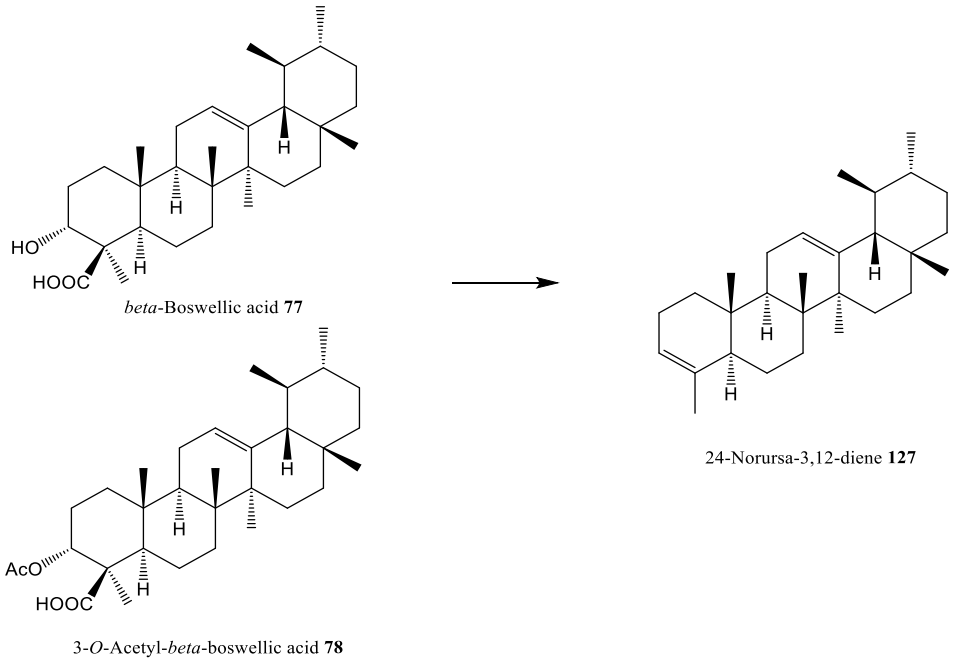



Figure 1.37. Thermal decomposition of β-boswellic acids that are not substituted at the 11 position.

Compound **77** degrades by losing $CO_2$ and $H_2O$ whereas **78** loses $CO_2$ and $CH_3COOH$ in its degradation mechanism. This results in the formation of **127**, which has a molecular weight of 394, which can be seen in the fragmentation pattern (figure 1.38).

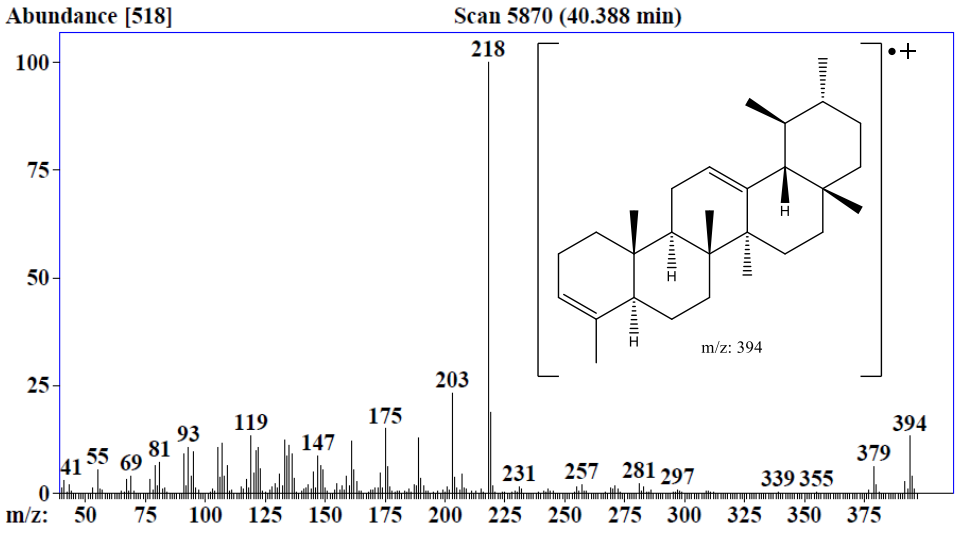



Figure 1.38. Fragmentation pattern for 24-norursa-3,12-diene **127**, AI = 3062 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with methanol following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[2]

The retro-Diels-Alder mechanism (figure 1.39) also takes places for this nortriterpene to form a fragment of m/z = 218 and is very favourable judging by the how large the peak is. Subsequently, the loss of a methyl from this fragment causes the peak at m/z = 203. This peak at m/z = 203 is quite low, due to the fact the methyl group is not on a fully substituted carbon. Therefore, the α-boswellic acids are expected to have a larger peak at m/z = 203, as losing a methyl in that case is more favourable.

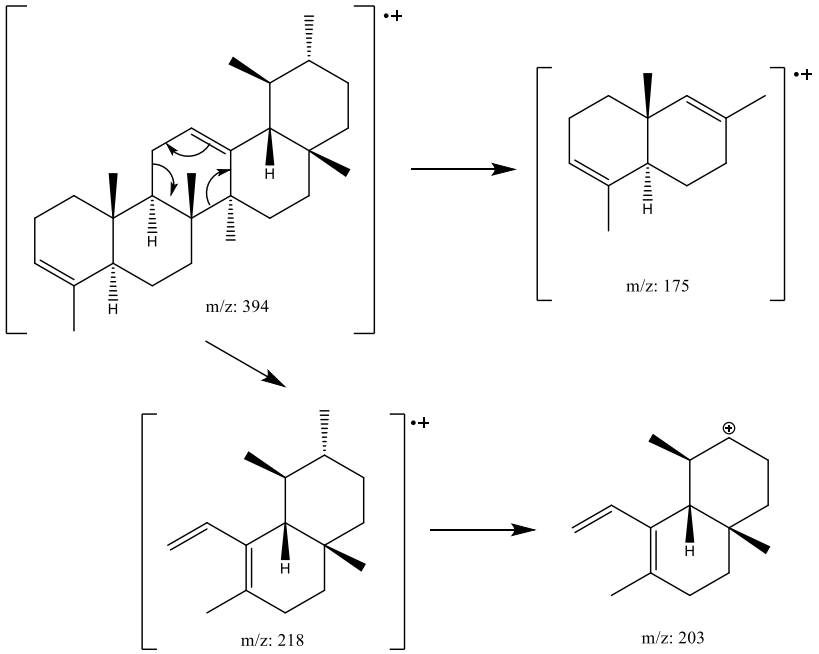



Figure 1.39. Literature suggested fragmentation of **127** via the retro-Diels-Alder route.[2]

Boswellic acids that form the oleanane type structure of **126**, come from thermal degradation of α-boswellic acids that are not substituted at the 11 position, such as **92** and **93**. These are similar to the ursane structures described previously, however the position of the methyl on the far right ring causes a recognisable difference in the fragmentation pattern (figures 1.40 and 1.41).

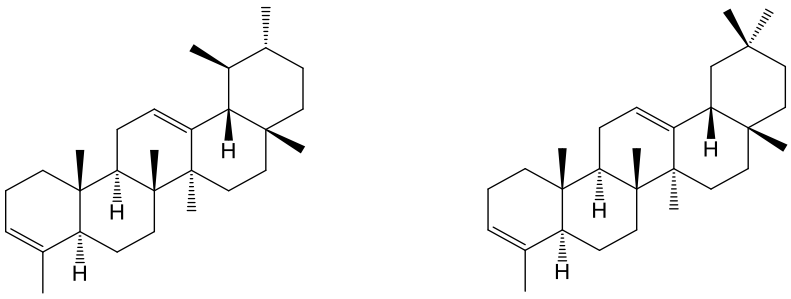



Figure 1.40. Ursane type nortriterpene (left) and oleanane type nortriterpene (right).

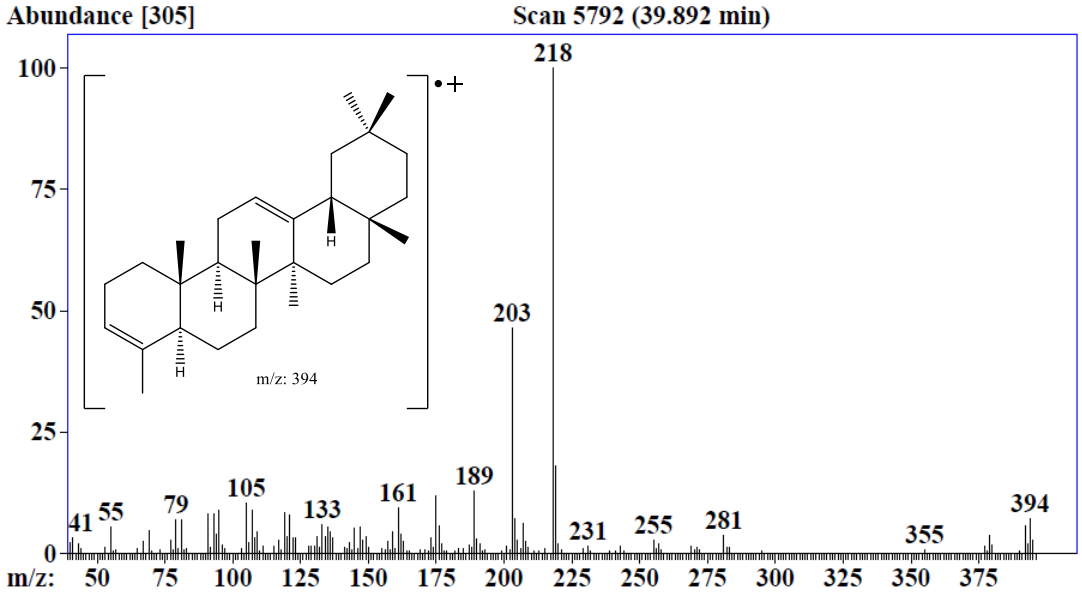



Figure 1.41. Fragmentation pattern for 24-noroleana-3,12-diene **126**, AI = 3015 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with methanol following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[2]

Since this oleanane type nortriterpene undergoes the same fragmentation of the retro-Diels-Alder mechanism, so has a large peak at m/z = 218, it looks similar to the ursane shape that was discussed above. However, the large peak at m/z = 203 represents a more favourable loss of a methyl group, which is the loss of one of the methyls on the right most ring, which is more highly substituted and therefore can lose the methyl more easily (figure 1.42).

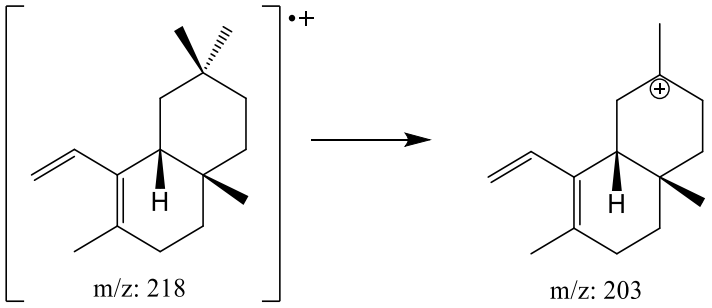
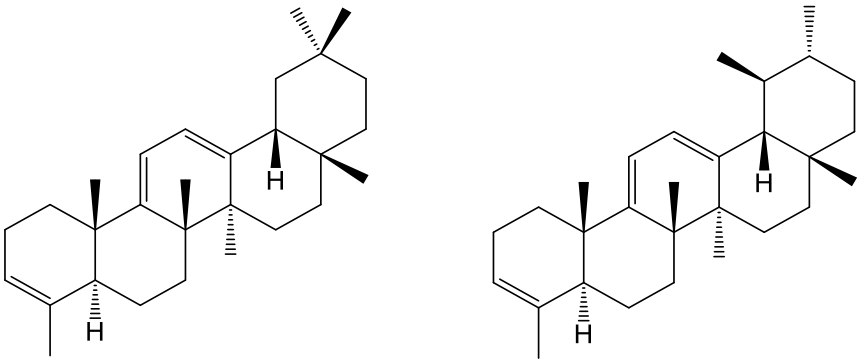



Figure 1.42. Loss of a methyl group from the right-hand side of **126** after retro-Diels-Alder step, suggested in the literature.[2]

Compounds **124** and **125** are produced by the thermal decomposition of 11-substituted α and β-boswellic acids respectively such as 11-methoxy, 11-hydroxy and even 9,11-dehydro boswellic acids. Both **124** and **125** have similar fragmentation patterns to each other, but do not undergo the retro-Diels-Alder mechanism due to the there being 2 sets of carbon-carbon double bonds in the middle ring (figure 1.43).



24-Noroleana-3,9(11),12-triene **124** 24-Norursa-3,9(11),12-triene **125**

Figure 1.43. Structures of 24-noroleana-3,9(11),12-triene **124** and 24-norursa-3,9(11),12-triene **125**.

The fragmentation patterns of these compounds both have characteristic peaks at m/z = 392 (figure 1.44 and 1.45). In addition, they both have peaks at m/z = 255, which has been reported to be a characteristic trait of these nortriterpenes.[2] To distinguish between the 2 compounds, the order they elute in was considered. The oleanane type generally elute first and seem to occur in lower concentration, so this information is used to determine the which isomer it is. Furthermore, the peak at m/z = 377 most likely represents the cleavage of one of the methyls on the E ring in the same way that **126** and **127** had a methyl cleavage in their fragmentations. Therefore, the larger peak at m/z = 377 represents the oleanane structure, due to it being able to lose its more substituted methyl more easily.

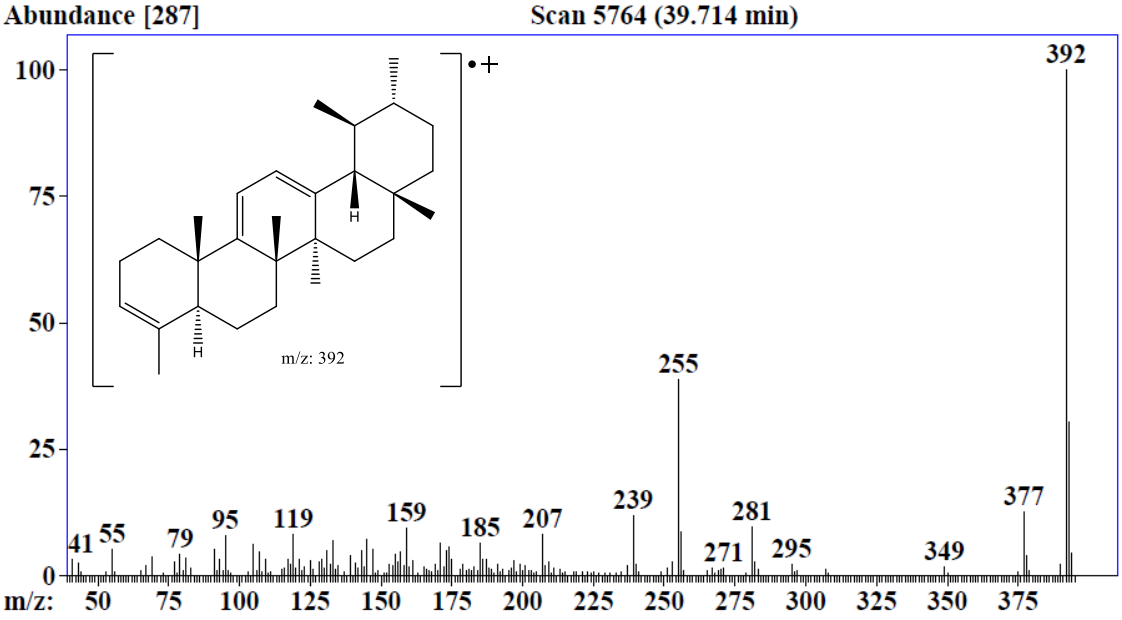



Figure 1.44. Fragmentation pattern for norursa-3,9(11),12-triene **125**, AI = 2999 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with methanol following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[2]

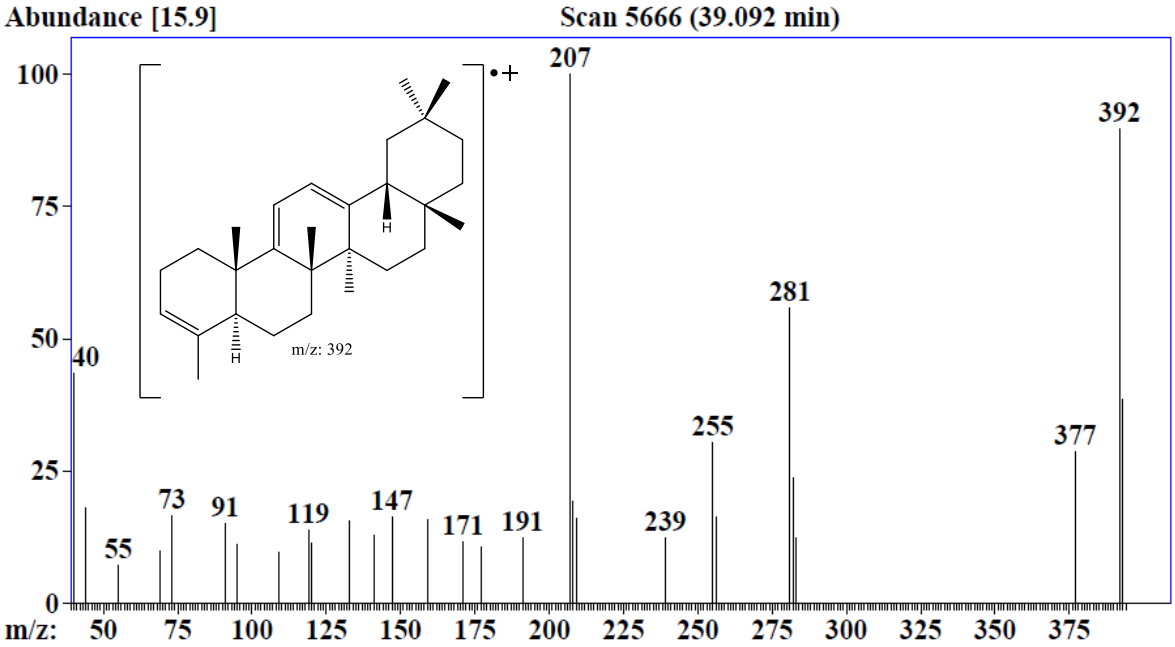



Figure 1.45. Fragmentation pattern for noroleana-3,9(11),12-triene **124**, AI = 2944 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with methanol following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[2]

## 1.5 Anti-inflammatory Properties of Boswellic Acids

The Boswellic acids are where the frankincense producing plants get their name *Boswellia* from. They are quite thoroughly studied due to their potent anti-inflammatory properties on conditions such as asthma, arthritis and irritable bowel diseases.[72] Leukotrienes have been known as mediators for inflammatory and allergic reactions for some time now, so the inhibition of Leukotriene biosynthesis has drawn interest. They are naturally formed by the conversion of arachidonic acid **114** to Leukotriene A4 **117** (LTA4), an unstable epoxide intermediate. This is then either hydrolysed to Leukotriene B4 **118** (LTB4) or converted to Leukotriene C4 **119** (LTC4) by glutathione S-transferase. Successive reactions in the pathway forming LTC4 gives Leukotrienes D4 **120** and E4 **121** and all 3 of these are cysteine containing leukotrienes which are bronchoconstrictors which can lead to asthmatic attacks.[73] The mechanism of action is increased vascular permeability which leads to an increased reactivity in the airways. It also allows eosinophils into the lung, which increases the formation of cysteine leukotrienes and stimulates mucous secretion. Cysteine leukotrienes have been reported to be between 100-1000 times more potent than histamine in triggering allergic reactions in the airways.[74] The conversion of arichodonic acid is catalysed by arachidonate 5-

lipoxygenase (5-LO), meaning that the inhibition of 5-LO has been targeted for investigation in various studies (figure 1.46).[75] 5-LO is sensitive to anti-oxidants, redox cyclers and radical scavengers, which led to redox type inhibitors being most commonly explored. The issue with this option though is that they are not selective which can cause side effects when administered on a living organism. Boswellic acids have shown great selectivity and caused inhibition of 5-LO, thus reducing the Leukotriene concentration at inflammation areas. The most potent 5-LO inhibitor is AKBA **80**, with $IC_{50}$ values of 1.5 µM and 7.0 µM with *in vivo* and *in vitro* systems respectively. KBA **79**, the non-acetylated version shows a slight decrease in potency with $IC_{50}$ values of 3 and 15 µM for *in vivo* and *in vitro*. Without the ketone group, β-boswellic acid **77**, caused partial inhibition of about 60% for both *in vivo* and *in vitro* and further reductions diminished any inhibitory action. Furthermore, a synthetic pentacyclic triterpene with the ketone group on C-11 did not show any inhibition of 5-LO, although selective binding still occurred. These observations imply that a pentacyclic triterpene is required for the highly selective binding along with 11-keto and C-4 hydrophilic groups.[76]

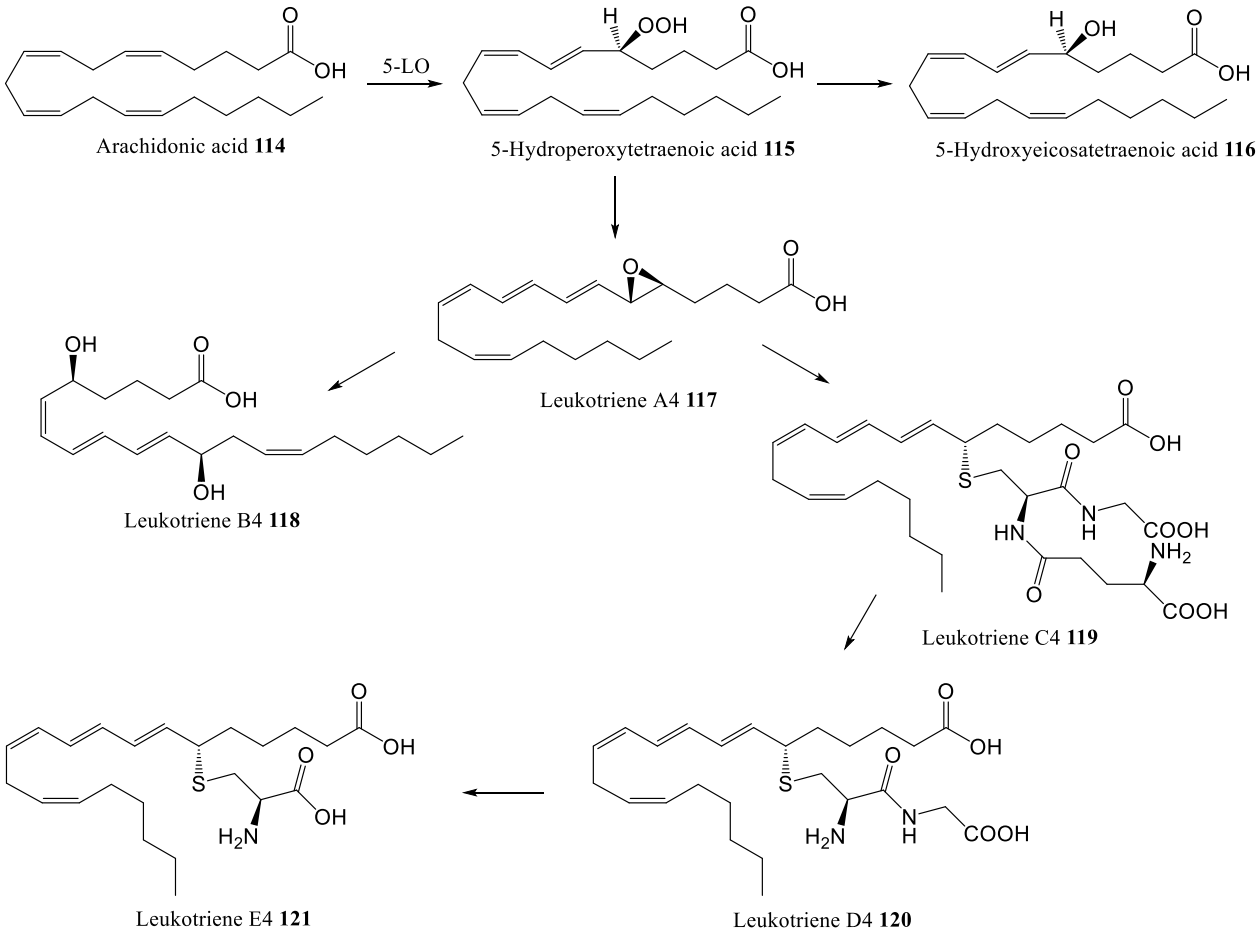



Figure 1.46. Biosynthesis of Leukotrienes from Arachidonic acid catalysed by 5-LO.

The Nuclear Factor, NF-κB pathway has long been known as a major part of regulating chronic inflammatory diseases. Inhibition of the NF-κB pathway reduces the inflammatory response which can decrease the intensity of diseases like rheumatoid arthritis, asthma and irritable bowel disease. As inflammatory responses are a part of protecting the body, these processes naturally occur and are regulated through a complex system of proteins (figures 1.47).[77] IκBα is an inhibition protein involved in the regulation of this activity and is phosphorylated by activated IKK phosphorylates, which leads it to degradation. IKK phosphorylates are activated by stimuli such as cytokines such as tumour necrosis factor (TNF-α) and stresses. This degradation of IκBα results in the translocation of the Rel family of DNA binding proteins, such as RelA (p65) to the nucleus, where transcription takes place. This results in the activation of NF-κB signalling so therefore, this pathway is often studied when testing the anti-inflammatory properties of both natural and synthetic compounds.[76,78,79]

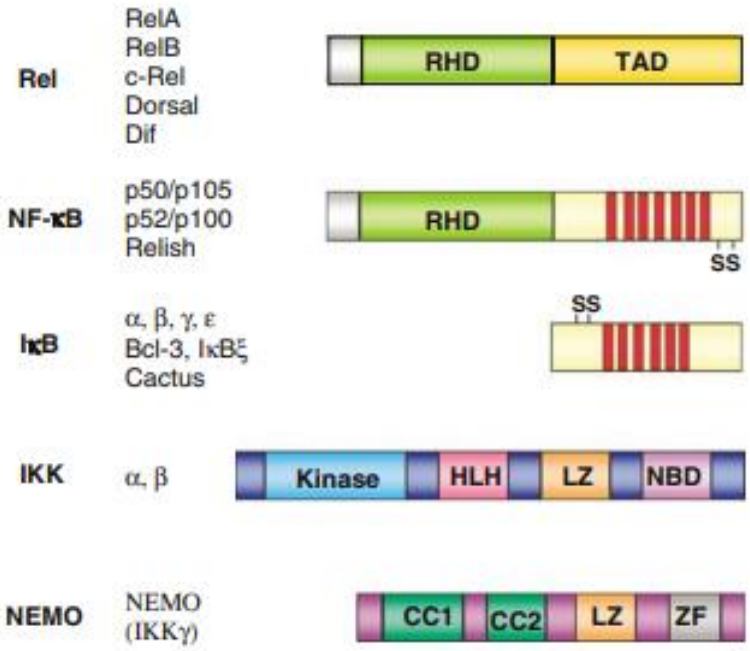



Figure 1.47. Simplified structures of the key NF-κB signalling proteins. The Rel homology domain (RHD) is for DNA-binding and dimerization. The transcriptional activation domains (TAD) are a part of the C-terminal Rel proteins. The red bars represent inhibition domains which can be removed by proteolysis. The serine residues (SS) are phosphorylation sites, subject to a stimulus. The generalised structures of IKK and NEMO proteins are also shown: HLH) helix-loop-helix; LZ) leucine zipper; NBD) NEMO binding domain; CC) coiled coil; ZF) zinc finger (picture from source).[77]

There have been a number of studies on the impact of boswellic acids on NF-κB, which have found reasonable inhibition, mainly from AKBA. One study found lesion size in LPS-injected

mice to be reduced by around 50% in size when pretreated with 10 µM AKBA. In addition, under a microscope, dark nuclear staining shows significant nuclear translocation of p65 proteins, which was much lighter in the AKBA treated mice compared to the control group.[80] Another study found concentration dependent downregulation of TNF-α from AKBA and 3-*O*-acetyl-α-boswellic acid, which was almost a 10 fold reduction of TNF-α in the case of 10 µmol/L AKBA. This resulted in the inhibition of NF-κB activity by 41% and 77% for 3-*O*-acetyl-α-boswellic acid and AKBA respectively.[79] The major part of *Boswellia* species' resins contain boswellic acids, which are mainly main up of *keto*-boswellic acids, so these findings are quite significant with regards to the importance of these resins.

## 1.6 Aims of Project

There are several aims of this project which include isolation of significant compounds in high purity through a repeatable method and studying the compositions of these resins. The main compound of interest is incensole acetate **53**, a diterpene with considerable value due to its various potential effects medicinally. Several other diterpenes found in frankincense have interest as well including serratol **48** and incensole **49**.

Incensole acetate has been found to exhibit anti-depressive, anxiolytic, anti-inflammatory and neuro-protective properties. It was found to be a potent activator of transient receptor potential vanilloid 3 (TRPV3) ion channels which are associated with warmth in the skin. However, TRPV3 mRNA has been found in the brain. The same study found wild-type mice to exhibit considerable anti-depressive and anxiolytic behaviour after 50mg/kg incensole acetate was injected.[81] Another study found incensole acetate to dose dependently reduce submissive behaviour in selectively bred submissive mice at 1, 5 and 10mg/kg dose levels. Concomitantly, corticosterone levels were lowered as a result of the dosed mice, which is a hormone that often gets released by stress.[82] β-Amyloid peptide is a neurotoxin which is strongly associated with Alzheimer's disease. Activity was reduced significantly with a 100uM injection of incensole acetate. Furthermore, there was a significant proliferative effect on human olfactory bulb neural stem cells observed.[83] Further neuroprotective effects were observed against cerebral ischemic injury. At doses of 1, 10 and 50mg/kg incensole acetate reduced lesion sizes by 22%, 58% and 71% respectively. As a result, cytokynes tumor necrosis factor-α, interleukin-1β and transforming growth factor-β were reduced by 88%, 77% and

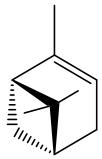
80% respectively at 50mg/kg which are all associated with inflammation. Furthermore, NF-κB, a transcription factor that is key in inflammatory responses, was inhibited by up to 84% at the same dose.[84] An earlier study showed that incensole acetate acted as an anti-inflammatory agent through NF-κB *in vitro* and then found that in the mouse paw edema model inflammation was reduced. Pain was also assumed to be reduced by a decrease in paw licking.[3]

Another aim of the project was to explore the compositions of some different species of frankincense and to draw trends and differences between them. As discussed earlier, different species of resin produce largely varying oils and can do in very different mass recoveries as well. However, this can also be true within a species and can sometimes be due to the grade of resin as well as the geographic location and many other varying factors such as the specific habitat, the storge conditions, the harvesting conditions and the frequency of harvesting. To explore this, three resins from Somaliland were supplied, three from Oman and four from Sudan. Their species are discussed herein, however the geographical location is not enough to define the species. The resins were compared to the literature and were fractionated in various ways for comparison to identify them. Extraction methods varied from distillation, maceration, Soxhlet extraction and supercritical $CO_2$ extraction, which generally gave different contents in the oils isolated. The fractionation was achieved by exploiting solubility effects of different solvents at different temperatures/pressures and for isolation of higher purity compounds, column chromatography was generally used. Identification of compounds was mainly through GC/MS analysis with NMR Spectroscopy used on pure compounds. This method separates volatile compounds in the gas phase and then produces a fragmentation pattern for each detected species. As discussed earlier, both of these pieces of information are used to identify the unknown compounds, as they separate according to their volatility, therefore the time in which it is detected can be compared against a data library. The fragmentation pattern then helped to tentatively identify the compound as it can be compared to a library of known compounds which have a general retention index and fragmentation pattern associated with them. Furthermore, each of the *Boswellia* species studied herein have previously been reported, meaning there are certain compounds to be expected in large concentrations. Therefore, careful attention was paid to the literature when making assignments.[21]

# Chapter 2. Compositions of *Boswellia sacra, papyrifera, carterii and occulta*

Four different species of frankincense resins were experimented with throughout this project. This resulted in a total of ten resins, of which three sourced from Oman, three from Somaliland and four from Sudan.

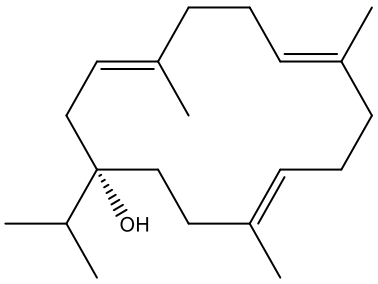
The Omani resins, to be discussed in detail in **Section 2.1,** consisted of three different grades, harvested from *Boswellia sacra* plants. These contained relatively large essential oil fractions, obtained through hydrodistillation with mass yields of 4.2-7.2% compared to the extracted resin's mass. The resultant oils were all high in the monoterpene, α-pinene **25** (figure 2.01), which was consistent with the literature.[32,42] These resins were very low in diterpenes, but did contain a large triterpene fraction, due to the presence of boswellic acids.



*alpha*-Pinene **24**

Figure 2.01. α-Pinene: a common monoterpene from *Boswellia sacra* resin.

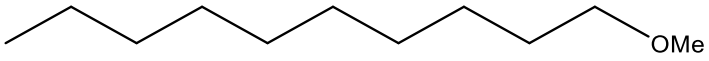
Out of the three Somaliland based resins, which will be discussed further in **Section 2.2,** two were harvested from *Boswellia carterii* plants. When hydrodistilled, they both gave oils with high concentrations of α-pinene, similarly to the *Boswellia sacra* samples. Furthermore, these volatile fractions were obtained in similar mass yields between 5.4-8.3% when compared to the resin mass distilled. However, when extracted by solvent or $CO_2$, the *carterii* resins were found to have diterpene fractions mainly made up of serratol **48** (figure 2.02). This is unlike the *Boswellia sacra* resins, which are often considered to be synonymous with resins from *Boswellia carterii.*[4,34] This observation means that the diterpene fraction could potentially be used as a distinguishing factor between the two species.




Serratol **48**

Figure 2.02. Serratol: found in the diterpene fraction of *Boswellia carterii* resin.

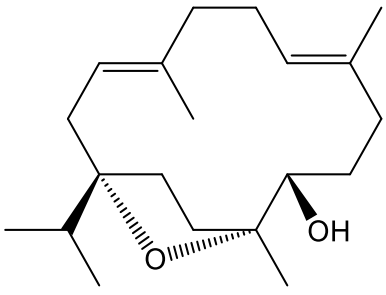
The third resin from Somaliland was chemically quite different to the other two and is most likely a *Boswellia occulta* sample, which is a newly discovered species of frankincense producing plant.[66] When hydrodistilled, its resin produced only small amounts of oil, giving mass recoveries of 2.1-2.7% when compared to the resin's mass. This was primarily made up of methoxydecane **71** (figure 2.03), which has only been found in *Boswellia occulta* resin.[34]




Methoxydecane **71**

Figure 2.03. Methoxydecane: Uniquely found in *Boswellia occulta* resin.

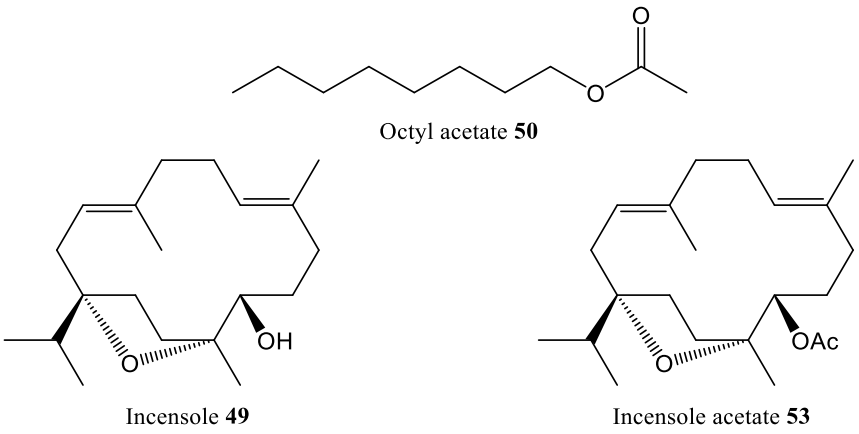
In addition to its interesting volatile fraction, *Boswellia occulta* resin also contains large concentrations of the high value compound incensole **49** (figure 2.04), which can be isolated simply by solvent extraction and subsequently purified. This finding makes the *Boswellia occulta* resin very useful with regards to the aims of this project, which include isolating this compound through simple methods.




Incensole **49**

Figure 2.04. Incensole: found in several resins including *Boswellia occulta.*

The Sudanese resins, which will be discussed in detail in **Section 2.3**, were from the species *Boswellia papyrifera*. When hydrodistilled, they gave mass recoveries similar to the *Boswellia occulta* resin, isolating volatile fractions between 1.2-2.0% when compared with the resin's mass. These volatile fractions were made primarily up of octyl acetate **50** which is typical for this species of resin, although the diterpene fraction is more complex, consisting of incensole, incensole acetate an various other cembrene type compounds (figure 2.05).[15,44,56]




Octyl acetate **50**

Incensole **49**

Incensole acetate **53**

Figure 2.05. Octyl acetate, incensole and incensole acetate: Commonly found in *Boswellia papyrifera* extracts.

This interesting diterpene fraction containing incensole **49** and incensole acetate **53** were found in higher concentrations from the solvent extracts of this resin alongside several other 'cembrene' type compounds. The difficulty with this oil is to isolate the two named diterpenes in high purity with quite an abundance of less desirable cembrenes with regards to this project's aims.

## 2.1 Omani Frankincense Composition

There were three resin samples obtained from Oman, which consisted of three different grades of *Boswellia sacra* resin (figure 2.06). The first sample was the Hoojri type, which is the highest grade of *Boswellia sacra's* resin. It was mostly pale yellow in colour and generally had larger resin pieces. A second sample was of the Najdi type resin which is the second highest grade and is typically smaller resin pieces than Hoojri with a slightly darker colour. Finally, a Sha'abi sample was supplied which is the fourth grade of the resin and is much darker with various sized resin pieces, which could be an indicator for different contents of these resins. As will be discussed in more detail in **Section 2.1.3,** this grade of *Boswellia* sacra, did contain a larger sesquiterpene fraction (up to 11%), whereas grades one and two are almost all

monoterpenes (92-95%) in their essential oil. The third grade of *Boswellia sacra* resin, known as Shathari, looks similar to Sha'abi resin, but was not sourced for this work.

Figure 2.06. Three grades *Boswellia sacra* Frankincense; Hoojri (left), Najdi (middle) and Sha'abi (right).

All the distilled oils for this species of resin gave a pale-yellow oil with a strong 'pine' like smell. The Hoojri type resin gave the highest mass recovery of oil giving 6.0-7.2% compared to the distilled resin mass. The Najdi type gave a narrow range of 4.4-4.7% and Sha'abi gave 4.2-5.9%. In addition to this, the general chemical profile was assessed through extractions with various solvents and then analysed by GC/MS.

## 2.1.1 Hoojri Resin

Duplicate hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** Along with giving the highest amount of oil distilled from the *Boswellia sacra* samples, the Hoojri resin also had the highest concentration of α-pinene **25** as determined by GC/MS (table 2.01 & figure 2.07). The major compounds detected in this oil were α-pinene **25** (76.6-80.1%), sabinene **27** (3.3-3.9%) and limonene **33** (2.9-3.0%). This lines up with previous findings on this grade except for the absence of sabinene in the literature. The remaining composition also lines up quite well, with limonene **33** and β-elemene **39** also being in similar concentrations. As can be seen in the chromatogram, the Hoojri grade has a strong monoterpene fraction, which runs up to around 19 minutes, with little in the sesquiterpene region, which was around28 minutes. There were no diterpenes detected in the distillate and these compositional characteristics are all supported by the literature.[32] However, since the oil was so highly dominated with α-pinene, the remaining composition did not really have many major compounds. Although, the mass recovery was higher for this resin, so per gram, it is possible that Hoojri resin produces more of the compounds that have higher concentrations in the other two resins.

Table 2.01. Chemical profile of Hoojri grade frankincense essential oil, isolated through hydrodistillation. Carried out in duplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[32] |
|---|---|---|---|---|---|
| **25** | **930** | **α-Pinene** | **High** | **76.6-80.1** | **76.0** |
| **27** | **979** | **Sabinene** | **High** | **3.3-3.9** | **-** |
| **31** | 1010 | δ-3-Carene | High | 0.3-2.3 | - |
| **33** | **1030** | **Limonene** | **High** | **2.9-3.0** | **2.6** |
| **132** | 1125 | α-Campholenal | High | 0.4-0.6 | 0.4 |
| **133** | 1136 | *trans*-Pinocarveol | Medium | 0.9 | 0.5 |
| **35** | 1138 | *cis*-Verbenol | High | 0.3-0.5 | - |
| **130** | 1141 | *trans*-Verbenol | High | 1.6-2.7 | - |
| **36** | 1180 | *p*-Mentha-1,5-dien-8-ol | Medium | 1.5 | 1.2 |
| **63** | 1200 | 4-Terpineol | High | 0.3-0.5 | - |
| **134** | 1201 | Myrtenol | Medium | 0.4-0.5 | - |
| **37** | 1205 | Verbenone | High | 0.7-0.8 | 0.4 |
| **39** | 1392 | β-Elemene | High | 1.6-1.7 | 2.1 |
| **42** | 1495 | β-Selinene | High | 0.9-1.1 | 1.5 |
| **135** | 1500 | α-Selinene | High | 0.8 | 1.0 |
| **Mass Yield (%)[c]** | | | | **6.0-7.2** | **8.5** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled

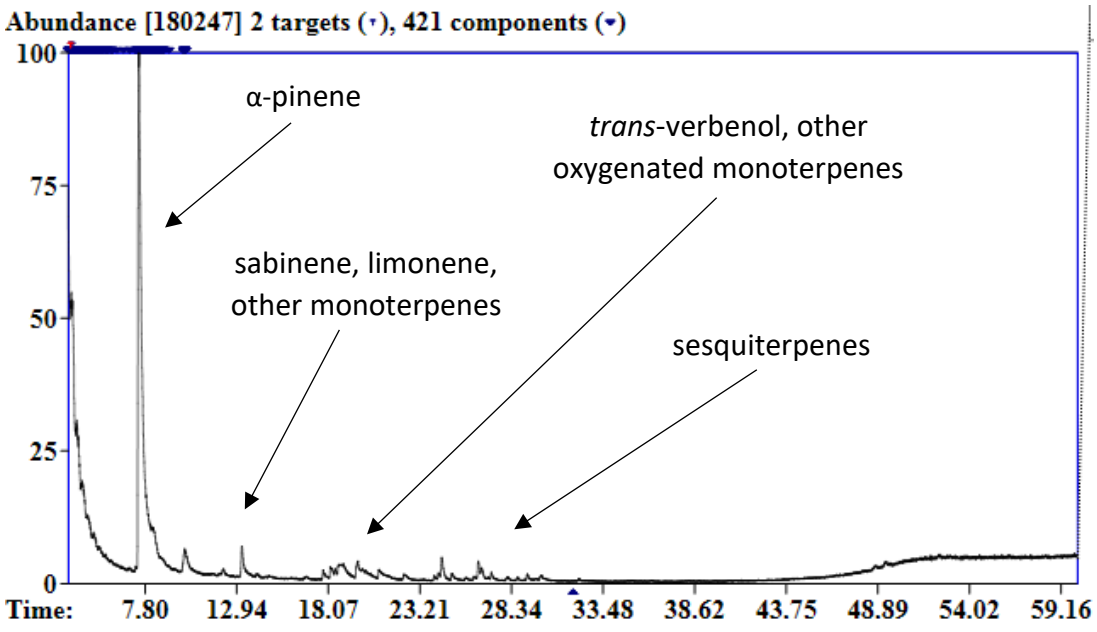



Figure 2.07. Chromatogram of the essential oil isolated via hydrodistillation of the Hoojri grade resin following the method in **Section 6.05**.

The fragmentation pattern indicates that the largest peak on the GC for this oil is caused by the detection of α-pinene (figure 2.08). Along with its short elution time, which gave an arithmetic index of 930 which is in range of this compound (literature = 932), the fragmentation pattern also aligns with the literature. The characteristic peak at m/z = 136 is common for monoterpenes, so more subtle differences such as how big other fragment peaks are compared to each other, such as m/z = 121, 105, 93 and 77 are used to characterise the compound. The major peak, m/z = 93 is caused by the loss of $CH(CH_3)_2$ which makes the difference of 43 lost from the original m/z = 136 of the unfragmented compound.

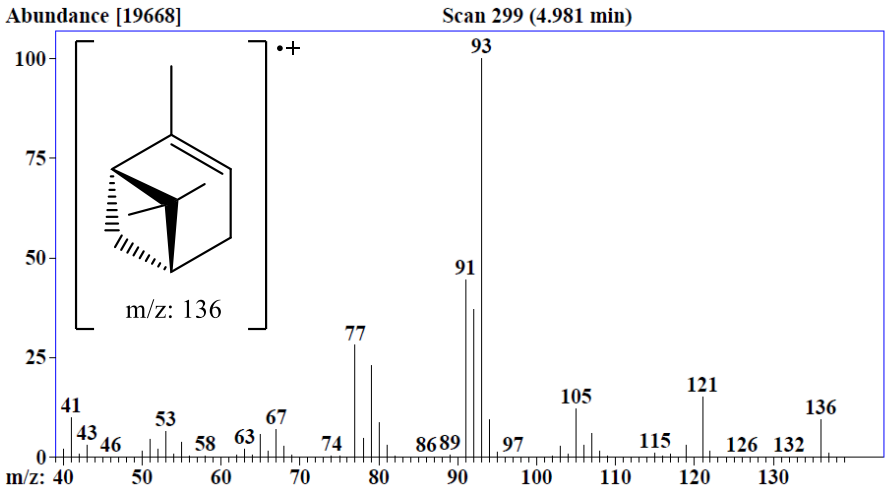



Figure 2.08. Fragmentation pattern of α-pinene **25**, AI = 930 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

The solvent extractions that were carried out to assess the bulk composition of the resin included an acetone maceration extraction followed by filtration, a Soxhlet extraction using acetone and duplicate IMS macerations followed by filtration (table 2.02). The maceration and Soxhlet extractions followed the methods in **Section 6.06** and **Section 6.07** respectively. These extractions gave mass recoveries of 62%, 66% and 57-77% respectively and were mainly comprised of triterpenes such as the boswellic acids and amyrins as the chromatogram shows (figure 2.09). The broad range in the IMS extraction mass recoveries once again highlights the inconsistency in the resin. However, the major components of the extracts remained similar throughout and were primarily made up of triterpenes. The largest area peak was found to be 24-norursa-3,12-dien-11-one **128** (18.7-24.1%), which is common for these resins. Other major compounds detected were 24-norursa-9,12-diene **127** (5.3-22.0%), 24-norursa-

3,9(11),12-triene **125** (5.9-10.8%), α-amyrin **89** (2.8-10.2%), α-amyrin acetate **129** (3.8-10.1%) and 24-noroleana-3,12-diene **126** (2.2-8.5%). There was low essential oil content in all the extractions except for the Soxhlet, which indicates that the warm acetone that condenses over the resin has a strong enough solvent effect to dissolve the essential oil, whereas when at room temperature, it fails to extract much of the volatile material.

Table 2.02. Chemical profiles of three maceration extraction experiments of the Hoojri grade of frankincense. 1) Acetone maceration extract; 2) Acetone Soxhlet extract; 3) Range of duplicate IMS maceration extracts. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignement Confidence[b] | Content (%) | | |
|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 |
| **25** | **934** | **α-Pinene** | **High** | **1.6** | **9.3** | **0.6** |
| **27** | 973 | Sabinene | High | trace | 0.9 | 0.2-0.4 |
| **-** | 1112 | Unknown monoterpene | - | trace | 0.5 | 0.5-0.7 |
| **130** | 1148 | *trans*-Verbenol | High | trace | 0.5 | 0.4-0.6 |
| **48** | 2154 | Serratol | High | 2.7 | - | trace-1.3 |
| **124** | 2956 | 24-Noroleana-3,9(11),12-triene | High | 0.4 | 0.2 | 0.4 |
| **125** | **3012** | **24-Norursa-3,9(11),12-triene** | **High** | **10.3** | **5.9** | **8.9-10.8** |
| **126** | **3027** | **24-Noroleana-3,12-diene** | **High** | **8.5** | **2.2** | **5.1-6.0** |
| **-** | 3047 | Unknown triterpene | - | 0.3 | - | 0.4-0.5 |
| **127** | **3074** | **24-Norursa-3,12-diene** | **High** | **22.0** | **5.3** | **13.2-17.6** |
| **-** | 3272 | Unknown triterpene | - | 0.5 | 1.5 | trace |
| **136** | 3283 | Ursa-9(11),12-dien-3-one | Low | 1.1 | 1.6 | 1.6-1.7 |
| **-** | 3299 | Unknown triterpene | - | 1.9 | 1.4 | trace-1.9 |
| **137** | 3310 | Ursa-9(11),12-dien-3-yl acetate | Low | 1.9 | 2.6 | trace-2.3 |
| **128** | **3320** | **24-Norursa-3,12-dien-11-one** | **High** | **18.7** | **20.3** | **22.6-24.1** |
| **99** | **3329** | **β-Amyrin** | **High** | **5.6** | **6.2** | **5.6-5.9** |
| **138** | 3351 | β-Amyrin acetate | Medium | trace | 1.3 | 1.9-2.9 |
| **-** | 3361 | Unknown triterpene | - | - | 3.0 | - |
| **90** | 3364 | α-Amyrenone | High | 1.6 | 4.9 | 3.6-7.9 |
| **89** | **3374** | **α-Amyrin** | **High** | **2.8** | **10.2** | **8.8-9.0** |
| **129** | **3386** | **α-Amyrin acetate** | **Medium** | **10.1** | **3.8** | **4.1-4.8** |
| **-** | 3397 | Unknown triterpene | - | 2.2 | 5.0 | 3.5-4.2 |
| **-** | 3477 | Unknown triterpene | - | - | 1.2 | 0.7-1.3 |
| **-** | 3543 | Unknown triterpene | - | - | 1.8 | 0.7-1.0 |
| **-** | 3556 | Unknown triterpene | - | 0.5 | 0.9 | 0.7 |
| **-** | 3587 | Unknown triterpene | - | 0.6 | 2.6 | 0.6-1.3 |
| **Mass Yield (%)[c]** | | | | **61.5** | **66.4** | **56.6-76.5** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted

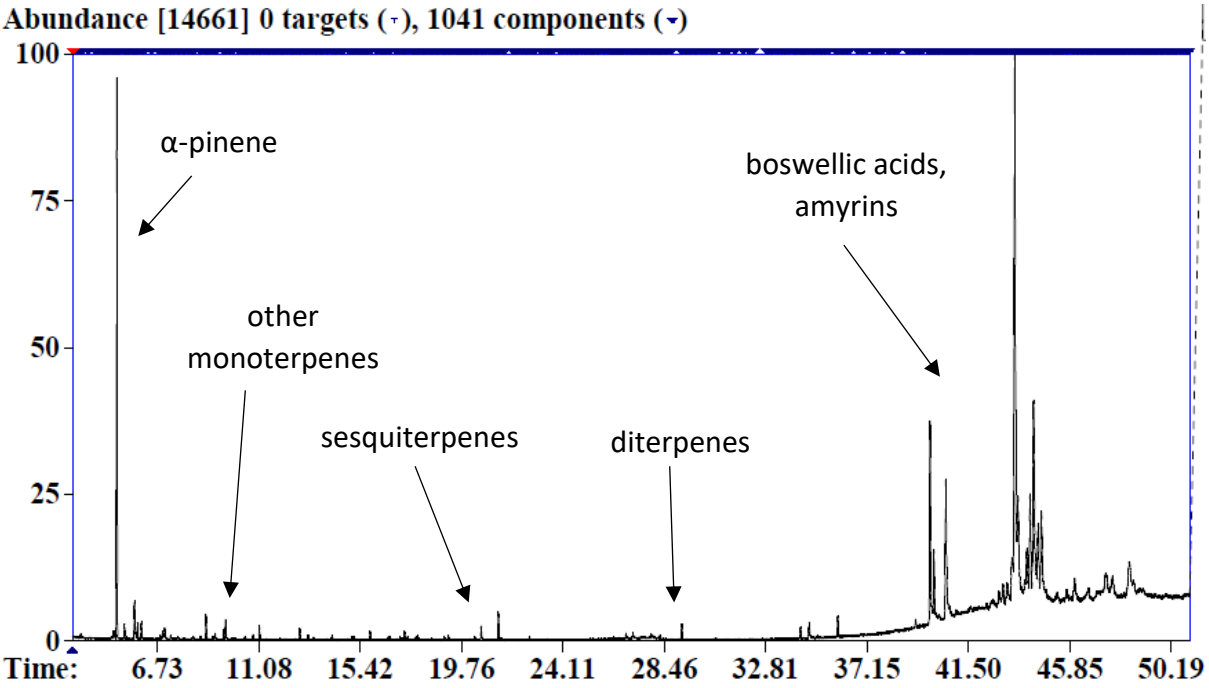



Figure 2.09. Chromatogram of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**.

Amyrins seem to not break down during the GC/MS experiment, meaning they are able to enter the gas phase. That being said, the amyrins could be thermally degraded compounds themselves. An α-amyrin follows the same skeletal shape structure as β-boswellic acids, which are ursane type structures (figure 2.10). Due to this structural similarity, the oleanane type amyrins, which are the β-amyrins, elute before the ursane type, which is also true for the boswellic acids.

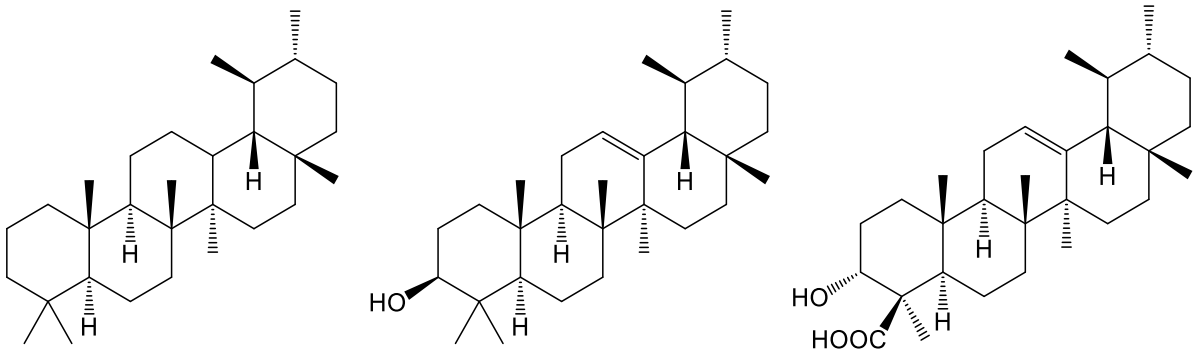



Figure 2.10. Ursane, α-amyrin and β-boswellic acid all share the same general skeletal structure.

The 2 key differences between boswellic acids and amyrins are the stereochemistry of the hydroxy moiety and the fact the boswellic acids have a carboxylic acid group where the

amyrins have just the unoxidised methyl group. Due to this, the fragmentation patterns are quite similar (figure 2.11). Firstly, for α-amyrin, there is a medium intensity peak which represents the unfragmented compound at m/z = 426. Both α-amyrin and β-boswellic acid undergo retro-Diels-Alder to give a large peak at m/z = 218 and the peak at m/z = 203 is the loss of a methyl from this fragment. This peak is smaller for the ursane type amyrin as well as the ursane type boswellic acid, so this, along with the slower elution time can be used to identify this compound with reasonable confidence.

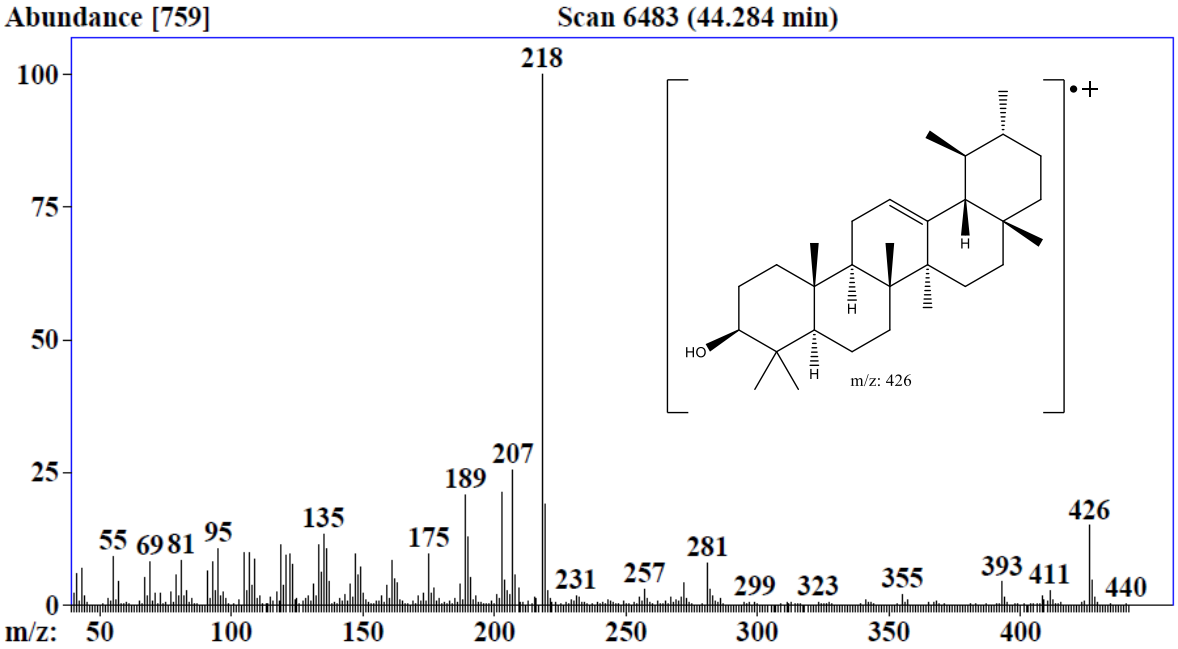



Figure 2.11. Fragmentation pattern of α-amyrin **89**, AI = 3374 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. Assignment confidence = high. Literature comparison (in the supplementary information).[85]

The peak at 44.45 minutes was tentatively assigned at α-amyrin acetate **129**. This was due to its similarity with the fragmentation pattern of α-amyrin **89**, along with the peak at m/z = 468, which represents the unfragmented compound (figure 2.12). Furthermore, the acetate pathway is very common in these resins, as the acetylated boswellic acids are commonly found. Since it is just the acetylated version of α-amyrin, the fragmentation pattern also shows a peak at m/z = 218, which once again shows the retro-Diels-Alder process.

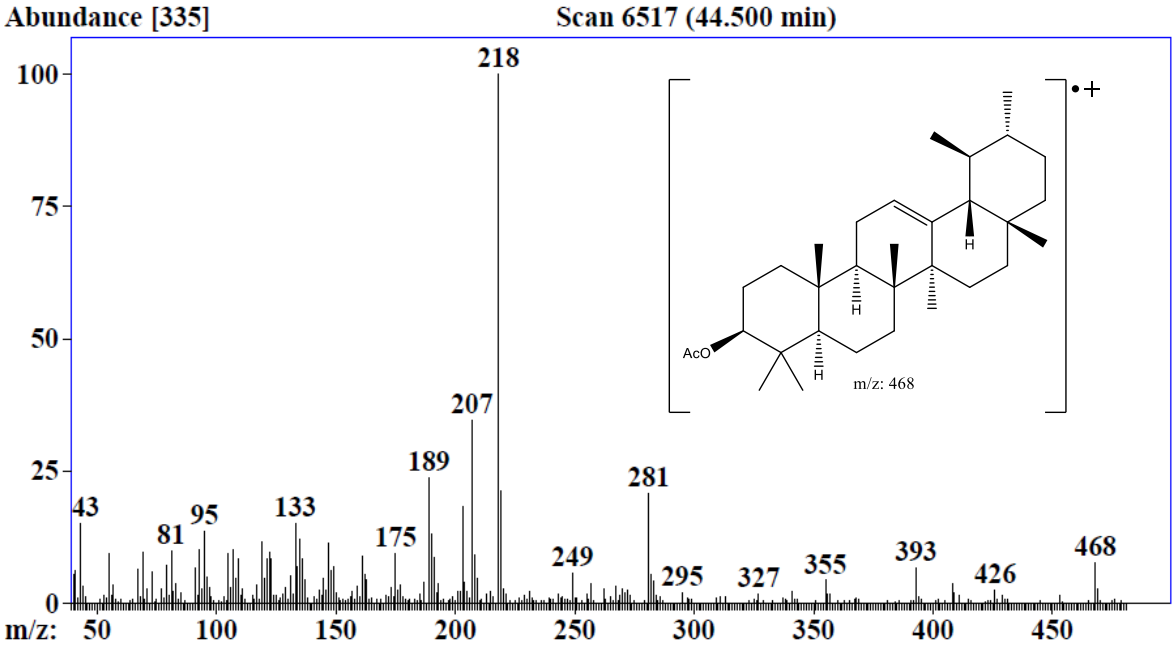



Figure 2.12. Fragmentation pattern of α-amyrin acetate **129**, AI = 3386 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. Assignment confidence = medium. Literature comparison (in the supplementary information) only includes β-amyrin acetate, but the peak size at m/z = 203 and its similarity to α-amyrin **89** allowed for tentative assignment of this compound.[85]

## 2.1.2 Najdi Resin

Two hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** The highest detected compound in the Najdi resin's essential oil was also α-pinene **25** which was detected in concentrations of 63.4-67.7% (table 2.03). The major compounds detected in these distillation experiments were α-pinene **25** (63.4-67.7%), sabinene **27** (5.4-5.8%), limonene **33** (4.2-4.7%), *trans*-verbenol **130** (4.4-5.4%) and *p*-mentha-1,5-dien-8-ol **36** (3.4-4.0%). As the α-pinene content was not as high as the Hoojri resin, several other compounds were found in higher concentrations such as limonene, *trans*-verbenol and *p*-mentha-1,5-dien-8-ol. However, only trace δ-3-carene was detected in this oil whereas it was found in both the Hoojri and Sha'abi oils. This sample was quite different to the literature's on the same grade. It was reported that the limonene content was much higher than what was found in this study. In addition, sabinene was only found in very low levels in the literature (0.8%) whereas here it was found to the in the highest levels of all the Omani oils (5.4-5.8%) and *trans*-verbenol was only found in 0.2% concentration.[32]

Table 2.03. Chemical profile of Najdi grade frankincense essential oil, isolated through hydrodistillation. Carried out in duplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[32] |
|---|---|---|---|---|---|
| **25** | **930** | **α-Pinene** | **High** | **63.4-67.7** | **46.8** |
| **27** | **979** | **Sabinene** | **High** | **5.4-5.8** | **0.8** |
| **33** | **1030** | **Limonene** | **High** | **4.2-4.7** | **15.9** |
| **132** | 1125 | α-Campholenal | High | 1.3-1.4 | 0.4 |
| **133** | 1136 | *trans*-Pinocarveol | Medium | 1.8-1.9 | 1.4 |
| **35** | 1138 | *cis*-Verbenol | High | 0.9-1.0 | 0.2 |
| **130** | **1141** | ***trans*-Verbenol** | **High** | **4.4-5.4** | **0.2** |
| **36** | **1180** | ***p*-Mentha-1,5-dien-8-ol** | **Medium** | **3.4-4.0** | **2.4** |
| **-** | 1200 | Unknown monoterpene | Medium | 0.5-1.2 | - |
| **37** | 1205 | Verbenone | High | 1.1-1.6 | 0.9 |
| **140** | 1218 | *trans*-Carveol | Medium | 0.5-0.7 | 0.3 |
| **141** | 1287 | Bornyl acetate | High | 0.6-0.7 | 0.4 |
| **65** | 1390 | β-Bourbonene | High | 0.4-0.8 | 0.3 |
| **39** | 1392 | β-Elemene | High | 0.8-1.8 | 0.9 |
| **40** | 1438 | Caryophyllene | High | 0.4-0.7 | 1.5 |
| **42** | 1495 | β-Selinene | High | 0.7-1.2 | 1.2 |
| **135** | 1500 | α-Selinene | High | 0.4-0.8 | 0.5 |
| **Mass Yield (%)[c]** | | | | **4.4-4.7** | **5.5** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled

This grade also produced an oil vastly dominated by monoterpene content and lacked sesquiterpenes and diterpenes (figure 2.13). The chromatogram shows a small response in the sesquiterpene fraction between around 28 minutes, which was mainly β-elemene and a trace response in the diterpene region at around 38 minutes.

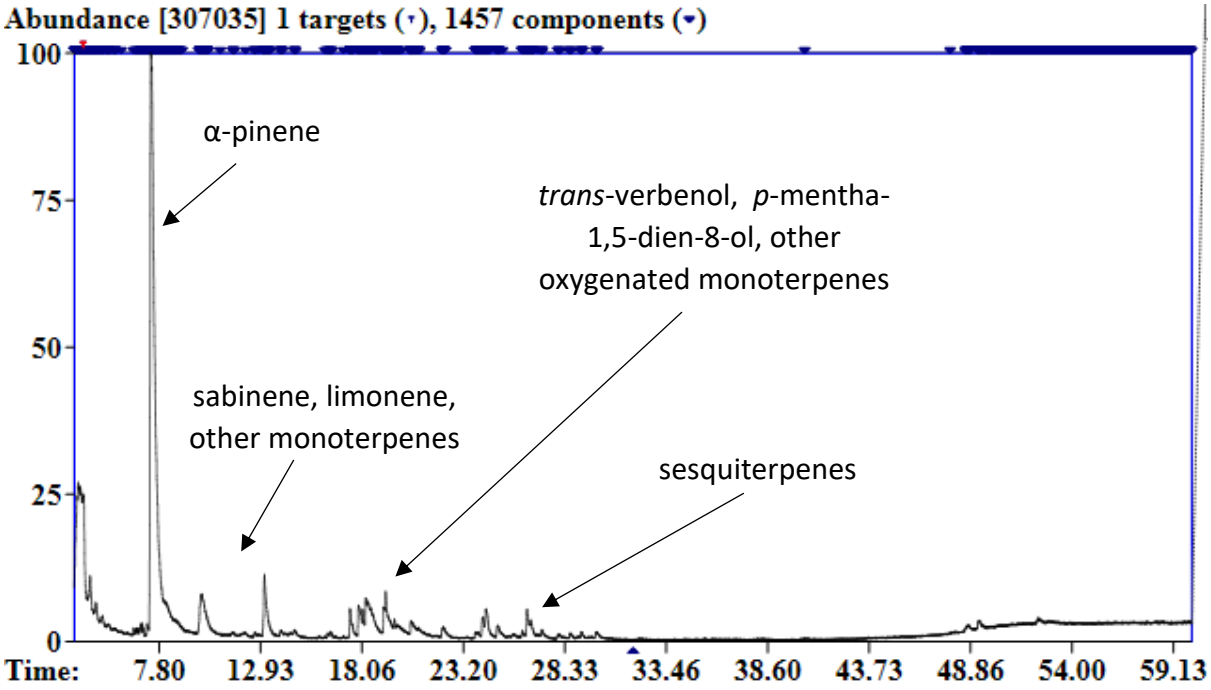



Figure 2.13. Chromatogram of the essential oil isolated via hydrodistillation of the Najdi grade resin following the method in **Section 6.05**.

Sabinene is quite easily identified by its fragmentation pattern. Along with the calculated arithmetic index being close to the reported index of 969, the upwards sloping of the peak sizes from m/z = 105, 121 and 136 was not observed for any other oil at this retention time. The large peak at m/z = 93 is due to the isopropyl group fragmenting leaving the ring system (figure 2.14).

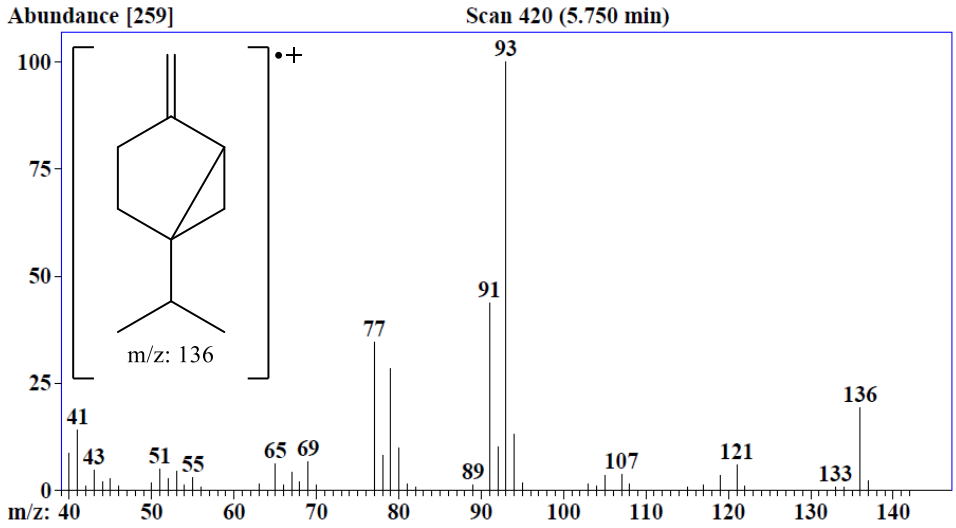



Figure 2.14. Fragmentation pattern of sabinene **27**, AI = 979 from the GC/MS analysis of an oil isolated through hydrodistillation of the Najdi grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

In addition to the distillations, an acetone maceration extraction, an acetone Soxhlet extraction and an IMS maceration extraction was carried out on the Najdi resin. The maceration and Soxhlet extractions followed the methods in **Section 6.06** and **Section 6.07** respectively. These extractions gave mass recoveries of 68%, 69% and 61% respectively (table 2.04). The major compounds in these extractions were similar to the Hoojri resin, with 24-norursa-3,12-dien-11-one **128** (18.7-28.2%), 24-norursa-3,12-diene **127** (4.0-19.8%), α-amyrin **89** (6.0-15.7%), 24-norursa-3,9(11),12-triene **125** (3.9-9.8%) and 24-noroleana-3,12-diene **126** (1.5-6.9%). Once again, low essential oil content was detected in the solvent extractions except for the acetone Soxhlet, which had α-pinene level of 11.2%, slightly higher than the Hoojri resin. Although the distillations did show the Hoojri resin to contain more α-pinene so a larger sample size may contradict this.

Table 2.04. Chemical profiles of three separate extracts from the Najdi grade of frankincense. 1) Acetone maceration extract; 2) Acetone Soxhlet extract; 3) IMS maceration extract. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | |
|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 |
| **25** | **935** | **α-Pinene** | **High** | **1.6** | **11.2** | **0.8** |
| **33** | 1030 | Limonene | High | trace | 2.1 | trace |
| **-** | 1113 | Unknown monoterpene | - | 0.5 | 0.7 | 0.9 |
| **130** | 1149 | *trans*-Verbenol | High | 0.4 | 0.6 | 1.0 |
| **37** | 1211 | Verbenone | High | 0.3 | 0.4 | 0.6 |
| **65** | 1390 | β-Bourbonene | High | 0.1 | 1.7 | 0.6 |
| **42** | 1494 | β-Selinene | High | 0.3 | 1.6 | 0.6 |
| **135** | 1499 | α-Selinene | High | trace | 1.1 | trace |
| **142** | 1659 | β-Eudesmol | High | - | 0.8 | 0.3 |
| **48** | 2152 | Serratol | High | trace | - | 1.0 |
| **124** | 2958 | 24-Noroleana-3,9(11),12-triene | High | 0.5 | 0.2 | 0.5 |
| **125** | **3014** | **24-Norursa-3,9(11),12-triene** | **High** | **9.8** | **3.9** | **7.9** |
| **126** | **3030** | **24-Noroleana-3,12-diene** | **High** | **6.9** | **1.5** | **4.8** |
| **127** | **3077** | **24-Norursa-3,12-diene** | **High** | **19.8** | **4.0** | **13.4** |
| **136** | 3288 | Ursa-9(11),12-dien-3-one | Low | 2.2 | 1.2 | 2.0 |
| **-** | 3305 | Unknown triterpene | - | 3.6 | 2.4 | - |
| **137** | 3315 | Ursa-9(11),12-dien-3-yl acetate | Low | 2.3 | 2.4 | 2.4 |
| **128** | **3323** | **24-Norursa-3,12-dien-11-one** | **High** | **20.4** | **18.7** | **28.2** |
| **99** | **3335** | **β-Amyrin** | **High** | **6.4** | **8.1** | **5.7** |
| **-** | 3345 | Unknown triterpene | - | 1.7 | trace | - |
| **138** | 3353 | β-Amyrin acetate | Medium | trace | 1.9 | 3.0 |
| **90** | **3368** | **α-Amyrenone** | **High** | **4.1** | **4.6** | **6.3** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **89** | **3380** | **α-Amyrin** | **High** | **11.2** | **15.7** | **6.0** |
| **129** | **3391** | **α-Amyrin acetate** | **Medium** | **2.5** | **3.0** | **7.6** |
| - | 3403 | Unknown triterpene | - | 3.4 | 3.9 | trace |
| **Mass Yield (%)[c]** | | | | **65.1** | **70.3** | **63.6-65.1** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass extracted.

The chromatogram again shows a large boswellic acid and amyrin content, a reasonable amount in the essential oil region and a small amount in the diterpene region (figure 2.15). There is a stronger sesquiterpene response here when compared to the extracts of the Hoojri resin, which was almost completely characterised by α-pinene in the essential oil fraction.

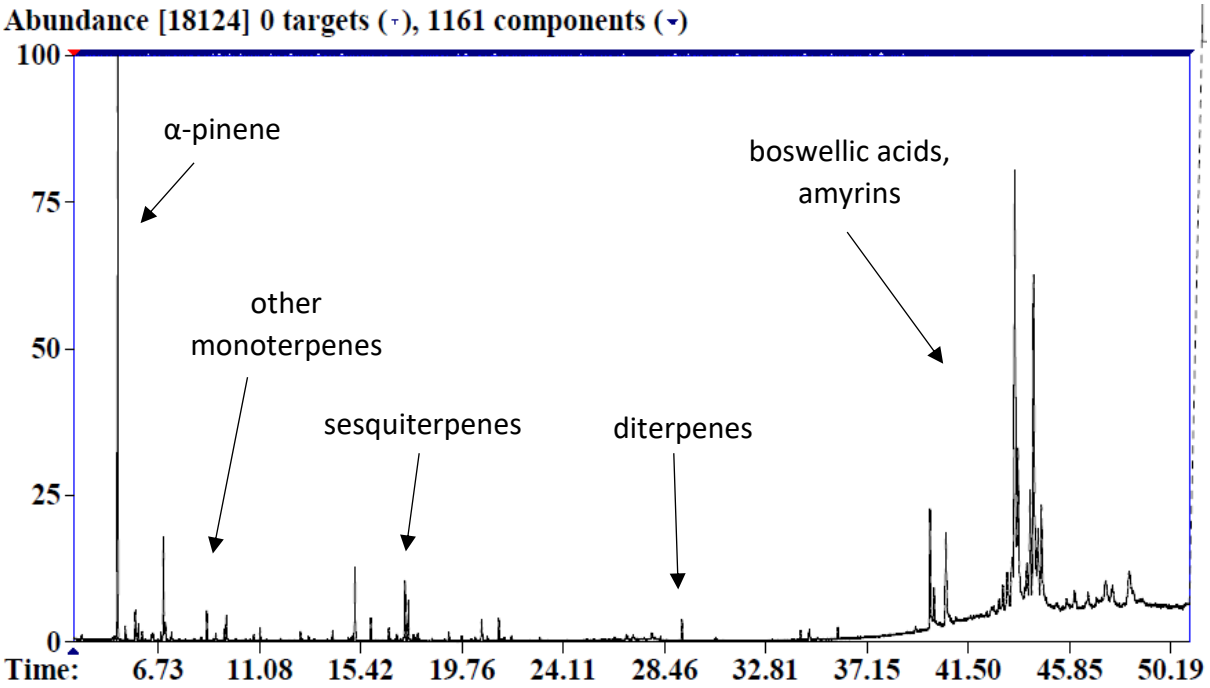



Figure 2.15. Chromatogram of an extract isolated via the acetone Soxhlet extraction of the Najdi grade resin following the method in **Section 6.07**.

β-Amyrin **99** was also detected in reasonable levels (5.0-6.2%) which was identified by its larger peak at m/z = 203 on its fragmentation pattern, which as discussed earlier, is representative of the oleanane type structure (figure 2.16).

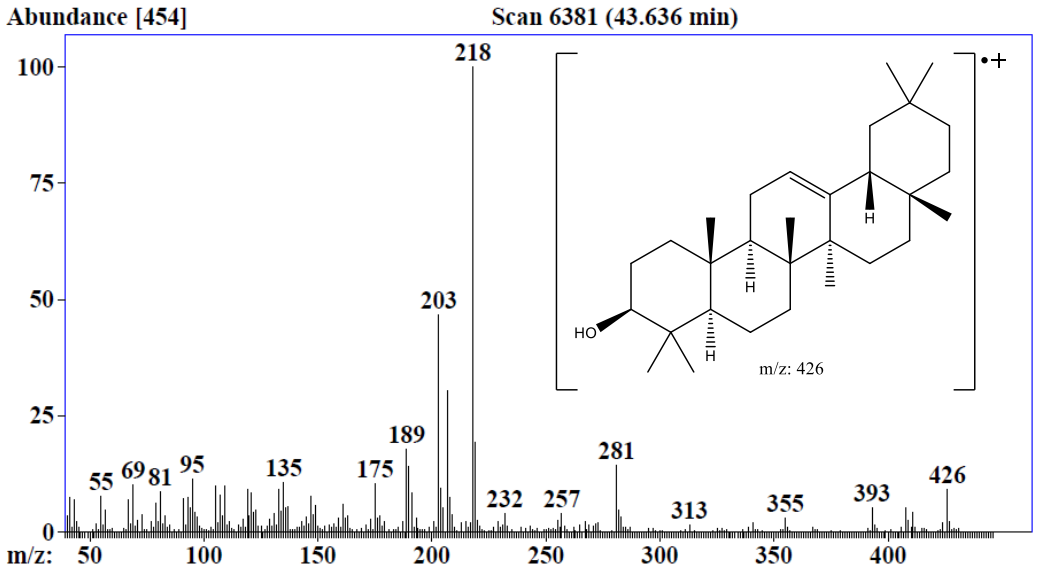



Figure 2.16. Fragmentation pattern of β-amyrin **99**, at AI = 3323 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. Assignment confidence = high. Literature comparison (in the supplementary information).[85]

## 2.1.3 Sha'abi Resin

Two hydrodistillation experiments were carried out for this resin following the method in **Section 6.05**. The Sha'abi resin had similar α-pinene **25** content to the Najdi, as well as sharing a similar mass recovery of essential oil at 4.2-5.9%. The distillate also had a similar composition of the other major compounds, with limonene **33** and *trans*-verbenol **130** also both being quite high for this resin (table 2.05). However, the literature's oil of this grade yielded a higher mass recovery and had some differences in content, with the limonene content being quite different.[32]

Table 2.05. Chemical profile of Sha'abi grade frankincense essential oil, isolated through hydrodistillation. Carried out in duplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[32] |
|---|---|---|---|---|---|
| **25** | **930** | **α-Pinene** | **High** | **64.0-67.8** | **68.5** |
| **27** | 979 | Sabinene | High | 1.3-1.7 | 0.3 |
| **28** | 980 | β-Pinene | High | 0.6-1.2 | 1.8 |
| **30** | 1007 | α-Phellandrene | High | 0.5-2.5 | 3.2 |
| **31** | 1012 | δ-3-Carene | High | 0.8-2.2 | - |
| **33** | **1030** | **Limonene** | **High** | **3.4-7.0** | **1.7** |
| **132** | 1125 | α-Campholenal | High | 0.9-1.0 | 0.3 |
| **133** | 1136 | *trans*-Pinocarveol | Medium | 1.2-1.6 | 0.6 |
| **35** | 1138 | *cis*-Verbenol | High | trace-0.7 | - |

| | | | | | |
|---|---|---|---|---|---|
| **130** | **1141** | ***trans*-Verbenol** | **High** | **3.6-4.0** | **-** |
| **36** | 1182 | *p*-Mentha-1,5-dien-8-ol | Medium | 1.9-2.1 | 1.1 |
| **134** | 1201 | Myrtenol | Medium | 1.5 | - |
| **37** | 1205 | Verbenone | High | 0.6-0.8 | 0.4 |
| **140** | 1218 | *trans*-Carveol | Medium | 0.3-0.5 | - |
| **141** | 1287 | Bornyl acetate | High | 0.9-1.0 | 0.4 |
| **38** | 1382 | α-Copaene | High | 0.4 | 0.2 |
| **65** | 1390 | β-Bourbonene | High | trace-0.5 | 0.3 |
| **39** | 1392 | β-Elemene | High | 2.0-2.7 | 2.6 |
| **40** | 1420 | Caryophyllene | High | 0.4-0.5 | 1.5 |
| **143** | 1480 | γ-Muurolene | Medium | 0.4-0.5 | - |
| **42** | 1495 | β-Selinene | High | 1.2-1.5 | 1.8 |
| **135** | 1500 | α-Selinene | High | 1.5-1.8 | 1.1 |
| **43** | 1520 | δ-Cadinene | High | 0.6-0.7 | 0.2 |
| **144** | 1654 | α-Eudesmol | High | 0.1-0.6 | - |
| **131** | 1815 | Phellandrene dimer | Low | 0.4-1.1 | - |
| **Mass Yield (%)[c]** | | | | **4.2-5.9** | **7.0** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass distilled.

Although this resin did not have as high α-pinene content as the Hoojri grade, the chromatogram shows the vast majority of the essential oil was composed of monoterpenes, with very little sesquiterpenes and only a trace amount of diterpenes (figure 2.17).

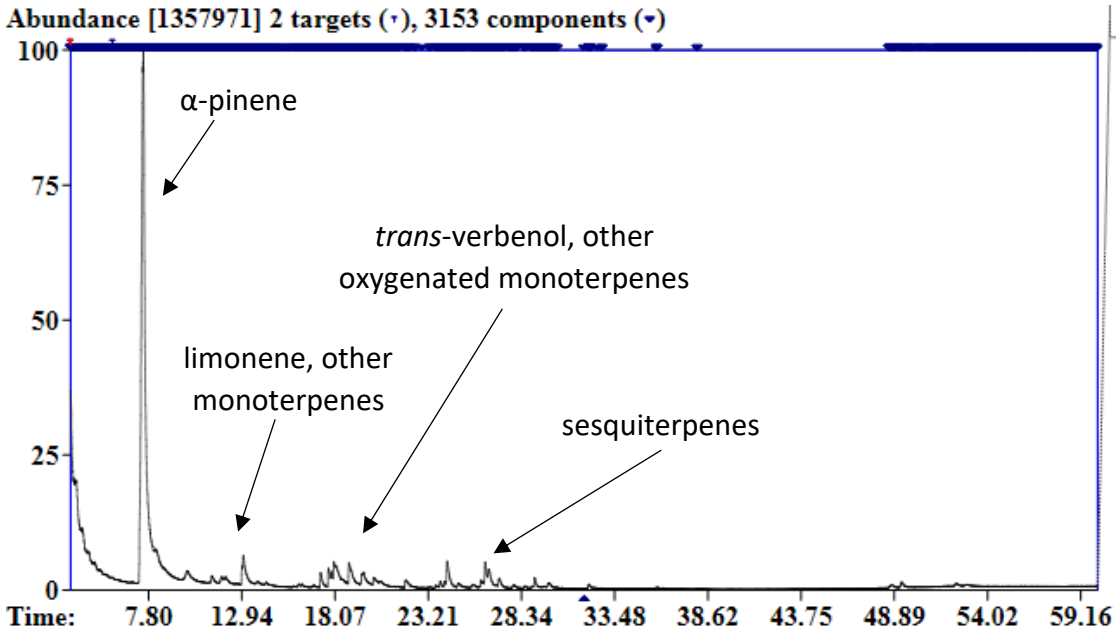



Figure 2.17. Chromatogram of the essential oil isolated via hydrodistillation of the Sha'abi grade resin following the method in **Section 6.05**.

Several samples of Sha'abi resin were also extracted by maceration in acetone, by Soxhlet in acetone and 2 separate extractions were done by maceration in IMS (table 2.06). The maceration and Soxhlet extractions followed the methods in **Section 6.06** and **Section 6.07** respectively. This gave oils with mass recoveries of 65%, 70% and 64-65% respectively. The largest mass recovery was for the Soxhlet extract which makes sense considering the higher temperature of the solvent used, which increases its ability to extract the compounds. Once again, the major compounds were dominated by boswellic acids and also amyrins to a lesser extent, with 24-norursa-3,12-dien-11-one **128** (13.5-30%), 24-norursa-3,12-diene **127** (4.6-22.5%), α-amyrin **89** (4.3-15.6%), 24-norursa-3,9(11),12-triene **125** (3.4-11.0%), 24-noroleana-3,12-diene **126** (1.4-9.2%) and β-amyrin **99** (3.8-8.1%). Larger essential oil content were detected in the acetone Soxhlet again (α-pinene = 9.4%) but interestingly, this extract had significantly lower concentrations of boswellic acids compared to the other oils. It could be argued that it is because of the essential oil content, but larger quantities of amyrins were detected in the Soxhlet extract. This indicates that they may only be partially soluble in IMS and acetone at room temperature.

Table 2.06. Chemical profiles of three maceration extraction experiments of the Sha'abi grade of frankincense. 1) Acetone maceration extract; 2) Acetone Soxhlet extract; 3) Range for duplicate IMS maceration extracts. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| **Compound Number** | **AI[a]** | **Compound** | **Assignment Confidence[b]** | **Content (%)** | | |
|---|---|---|---|---|---|---|
| | | | | **1** | **2** | **3** |
| **25** | 934 | α-Pinene | High | **1.7** | **9.4** | **0.1-0.5** |
| **-** | 1011 | Unknown monoterpene | - | 0.5 | 0.9 | trace |
| **-** | 1014 | Unknown monoterpene | - | 2.0 | - | trace |
| **39** | 1390 | β-Elemene | High | trace | 0.7 | 0.3 |
| **42** | 1491 | β-Selinene | High | 0.7 | 0.8 | 0.2-0.3 |
| **135** | 1499 | α-Selinene | High | - | 0.6 | 0.2-0.3 |
| **-** | 2018 | Unknown diterpene | - | trace | 0.9 | trace-0.3 |
| **-** | 2173 | Unknown diterpene | - | trace | 0.8 | 0.2-0.3 |
| **124** | 2954 | 24-Noroleana-3,9(11),12-triene | High | - | 0.2 | 0.6-0.7 |
| **125** | 3014 | 24-Norursa-3,9(11),12-triene | High | **10.8** | **3.4** | **7.5-14.5** |
| **126** | 3030 | 24-Noroleana-3,12-diene | High | **7.9** | **1.4** | **5.8-12.6** |
| **-** | 3048 | Unknown triterpene | - | trace | - | trace-0.8 |
| **127** | 3077 | 24-Norursa-3,12-diene | High | **20.7** | **4.6** | **16.0-22.5** |
| **-** | 3274 | Unknown triterpene | - | - | 0.8 | trace |
| **136** | 3286 | Ursa-9(11),12-dien-3-one | Low | 3.2 | 1.6 | 1.2-3.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| - | 3303 | Unknown triterpene | - | 3.4 | 3.6 | trace-3.4 |
| **137** | 3313 | Ursa-9(11),12-dien-3-yl acetate | Low | trace | 3.5 | trace-2.8 |
| **128** | 3323 | 24-Norursa-3,12-dien-11-one | High | **23.4** | **13.5** | **20.9-30.4** |
| **99** | 3334 | β-Amyrin | High | **5.8** | **8.1** | **3.7-3.9** |
| **138** | 3357 | β-Amyrin acetate | Medium | 1.8 | 2.8 | 1.0-4.7 |
| **90** | 3367 | α-Amyrenone | High | 3.8 | 4.6 | 3.9-7.3 |
| **89** | 3378 | α-Amyrin | High | **11.2** | **15.6** | **4.3-9.0** |
| **129** | 3390 | α-Amyrin acetate | Medium | 2.8 | 5.4 | 2.5-7.1 |
| - | 3401 | Unknown triterpene | - | 0.4 | 4.1 | trace-2.6 |
| - | 3547 | Unknown triterpene | - | - | 0.9 | trace |
| - | 3596 | Unknown triterpene | - | - | 2.5 | trace |
| **Mass Yield (%)[c]** | | | | **65.1** | **70.3** | **63.6-65.1** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted.

The chromatogram of these extracts shows a large triterpene fraction, including a strong response from the amyrins at around 44 minutes. In addition to the boswellic acid fingerprint compounds, this makes a fairly complex triterpene fraction (figure 2.18)

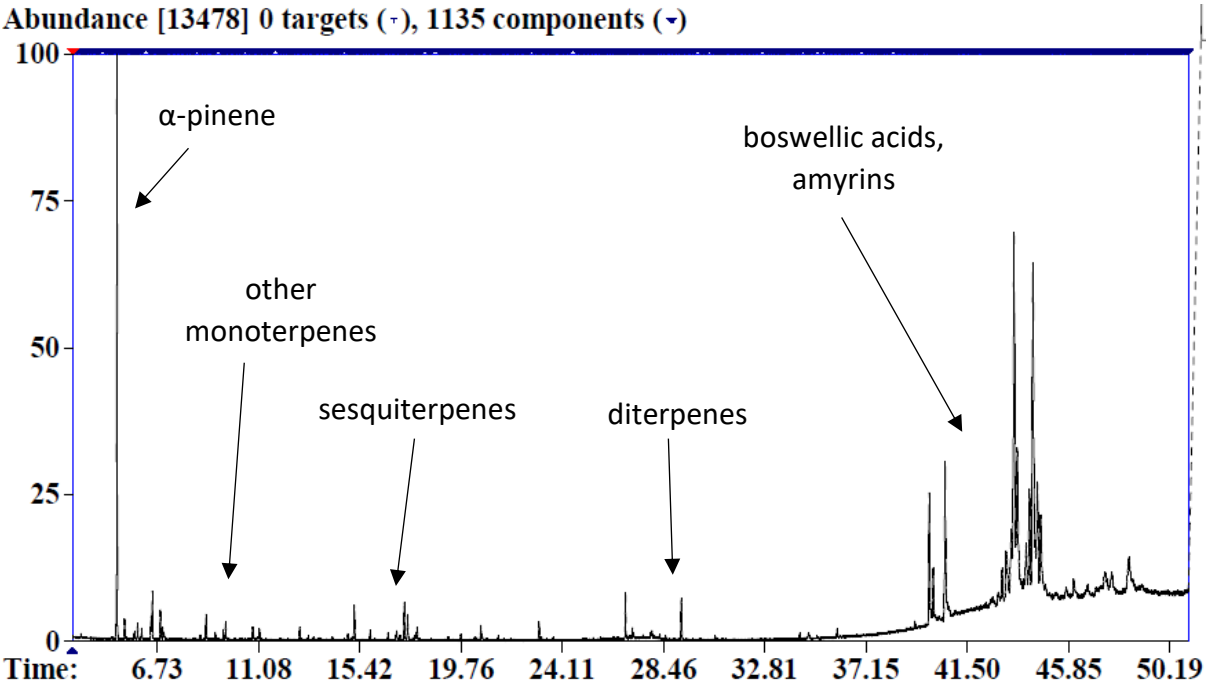
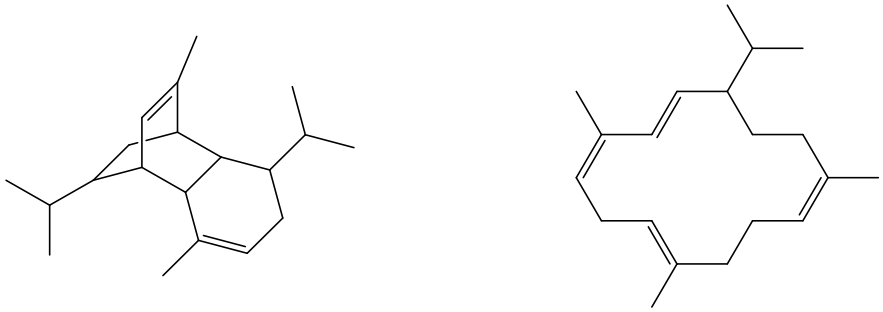



Figure 2.18. Chromatogram of an extract isolated via the acetone Soxhlet extraction of the Najdi grade resin following the method in **Section 6.07**.

### 2.1.4 *Boswellia sacra* Resin

As stated above, hydrodisatillation of the Hoojri resin produced an oil with the highest mass recovery compared to the resin mass (6.0-7.2%) along with having the largest concentration of α-pinene **25** (76.6-80.1%). As the Hoojri grade is considered the highest grade of *Boswellia sacra's* frankincense, it makes sense that it would produce an oil with the largest α-pinene content because this would increase the strong, pine fragrance it possesses. Furthermore, a more complex mixture of major compounds could darken the colour of the resin, which it is largely graded on. The Najdi resin produced less oil through hydrodistillation, but in a consistent range (4.4-4.7%). This was also a less pure oil, with the α-pinene levels being lower, although they remained the major compound (63.4-67.7%). That being said, the general composition of the distillates of the Hoojri and Najdi resins were very similar, being made up of 92-95% monoterpenes. The Sha'abi resin produced distillates of similar α-pinene content to the Najdi resin (64.0-67.8% α-pinene content), but had a wider range for the mass recovery (4.2-5.9%). This lower consistency in the Sha'abi resin may contribute to the darker colour and therefore the overall lower grade of the resin, since it is the fourth grade of this species. The monoterpene content in the essential oil was noticeably lower for this resin (88-91%) and contained more sesquiterpenes (7-11%). Furthermore, there was some diterpene content (1%), mainly comprising of a phellandrene dimer **131**, which was only seen as very low and trace values in the other oils. This is interesting as many other resins of *Boswellia* species produce cembrene type diterpenes which were not observed for these resins and the phellandrene dimers are generally seen as a biomarker for *Boswellia frereana's* frankincense[15] (figure 2.19).



**131** **55**

Figure 2.19. Phellandrene dimer type diterpene **131** found in Omani and *Boswellia frereana* frankincense and cembrene type diterpenes **55** commonly found in most frankincense resins.

For each of these Omani resins, the solvent extraction method did not affect the mass recovery of oil too much, as the solvent choices were quite similar for these resins. The bulk of the resin is made up of polar triterpenes, which are easily extracted using polar solvents and these resins all contained similar triterpene content. However, the acetone Soxhlet extractions yielded oils that had significant essential oil fractions of up to 26% concentration by GC/MS. These Soxhlets also gave a larger ratio of amyrins, whereas the triterpene fractions were mostly made up of boswellic acids for IMS and acetone extractions at room temperature.

## 2.2 Somaliland Frankincense Composition

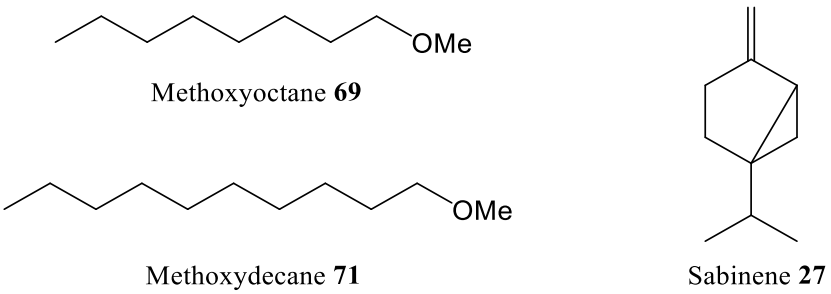
Three resins were studied from Somaliland that were all harvested within about thirty miles of each other and the genus was uncharacterised. They were from the North Coast, Erigavo and Gudmo Biyo Cas and are likely to be either to be *Boswellia carterii*, *occulta*, *frereana* or *rivae* based on where they were collected (figure 2.20). The North Coast resin was of the methoxydecane chemotype which has only been found in the newly discovered species, *Boswellia occulta* and the Gudmo Biyo Cas and Erigavo resins were the α-pinene type.

Figure 2.20. Frankincense harvested from *Boswellia* plants in Somaliland at the north coast (left), Gudmo Biyo Cas (middle) and Erigavo (right).

### 2.2.1 North Coast Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05** and the resultant oil gave a clear, very pale yellow coloured oil. Mass recoveries were fairly consistent giving a range of 2.1-2.7%. The main components of this essential oil (figure 2.21) were methoxydecane **71** (48.8-73.0%), methoxyoctane **69** (9.5-11.2%) and

sabinene **27** (4.0-6.0%). Incensole **49** was also found which, alongside **71** is characteristic for *Boswellia occulta's* resin so it is likely the newly discovered type of frankincense (table 2.07).[34]




Methoxyoctane **69**

Methoxydecane **71**

Sabinene **27**

Figure 2.21 Major compounds in the essential oil of *Boswellia occulta* resin

Table 2.07. Chemical profile of *Boswellia occulta* frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[86] |
|---|---|---|---|---|---|
| **25** | 927 | α-Pinene | High | 1.3-2.7 | 0.3 |
| **27** | **976** | **Sabinene** | **High** | **4.0-6.0** | **1.8** |
| **-** | 1011 | Unknown Monoterpene | - | trace-1.4 | - |
| **69** | **1023** | **Methoxyoctane** | **High** | **9.5-11.2** | **7.0** |
| **62** | 1060 | γ-Terpinene | High | 0.2-0.8 | 0.2 |
| **130** | 1137 | *trans*-Verbenol | High | 0.2-0.5 | - |
| **70** | 1140 | Methoxynonane | High | 1.8-2.4 | 1.9 |
| **63** | 1201 | 4-Terpineol | High | 0.5-0.8 | 0.4 |
| **71** | **1225** | **Methoxydecane** | **High** | **48.8-73.0** | **35.1** |
| **-** | 1234 | Unknown Monoterpene | - | trace-2.6 | - |
| **72** | 1270 | Decanol | High | 0.5-2.4 | 0.8 |
| **38** | 1379 | α-Copaene | High | 0.2-1.4 | 0.8 |
| **65** | 1390 | β-Bourbonene | High | 0.6-5.7 | 0.3 |
| **39** | 1392 | β-Elemene | High | trace-1.1 | trace |
| **40** | 1420 | Caryophyllene | High | 0.4-1.0 | 0.2 |
| **-** | 1426 | Unknown Sesquiterpene | - | 0.3-0.6 | - |
| **145** | 1496 | Germacrene D | Medium | 0.2-2.6 | - |
| **43** | 1520 | δ-Cadinene | High | 1.0-1.7 | - |
| **146** | 1555 | Elemol | Medium | 1.6-1.7 | 2.1 |
| **147** | 1605 | Guiaol | Medium | 0.6-1.9 | trace |
| **49** | 2150 | Incensole | High | trace-3.3 | 6.7 |
| **Mass Yield (%)[c]** | | | | **2.1-2.7** | **2.7-5.5** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass distilled.

The chromatogram shows some small monoterpene and sesquiterpene composition across with only trace amounts in the diterpene region (figure 2.22). However, it should be noted that the main response found in the chromatogram was for the alkane methoxy ethers such as methoxydecane **71**.

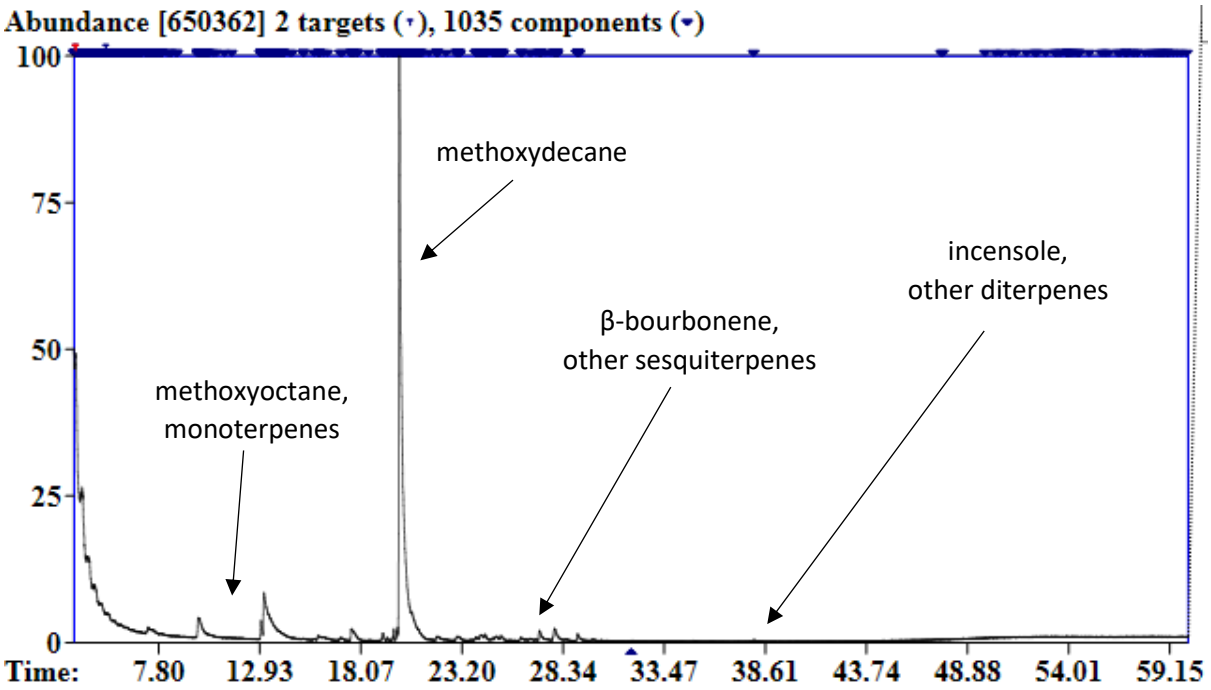



Figure 2.22. Chromatogram of the essential oil isolated via hydrodistillation of the *Boswellia occulta* resin following the method in **Section 6.05**.

The fragmentation pattern of the main constituent of the essential oil represents methoxydecane (figure 2.23). The parent ion has an m/z of 172 which shows almost no response due to the facile cleavage of the methyl ether upon electron ionization, which results in the characteristic peak at m/z = 140. The remaining peaks represent the sequential losses of terminal alkyl groups.

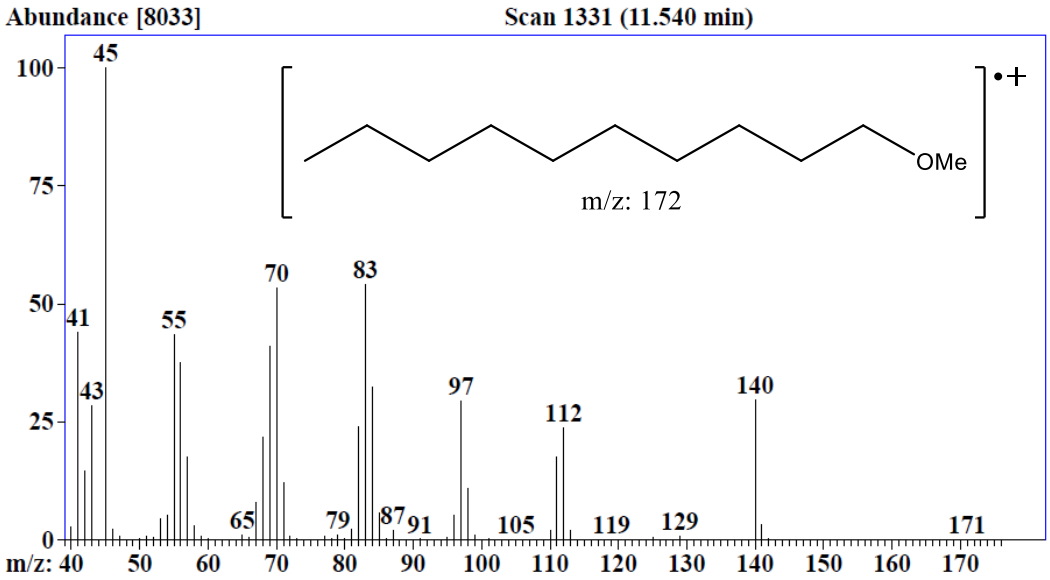



Figure 2.23. Fragmentation pattern for methoxydecane **71**, AI = 1225 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[87]

This resin was also extracted by maceration, using methanol, chloroform, diethyl ether and hexane following the method in **Section 6.06**. These extractions were filtered, and the solvent was evaporated to give yellowish, viscous oils with mass recoveries of 51-53%, 50-57%, 46-52% and 29-34% respectively. The hexane extracts were much lower in mass recovery than the others, which all resulted in quite similar oil masses. However, the GC/MS profile did not show a great deal of difference for the hexane extracts, albeit having the largest diterpene concentration at 53% and lowest triterpene concentration at 42% on average (table 2.08). The main components of these solvent extracts were serratol **48** (3.5-4.6%), incensole **49** (35.7-45.6%), 24-norursa-3,9(11),12-triene **125** (4.2-7.5%), 24-norursa-3,12-diene **127** (3.4-6.7%) and 24-norursa-3,12-dien-11-one **128** (28.6-37.2%). The Chromatograms generally showed a small essential oil fraction, which supports the low mass recovery of the essential oil from distillation. The diterpene fraction, between 25-31 minutes, has a large peak for incensole at about 29 minutes, surrounded by several small concentration peaks of other cembrene type diterpenes. Finally, the triterpene fraction, between 39-45 minutes, was almost fully composed of boswellic acids, with only small concentrations of amyrins detected (figure 2.24). A small concentration of a phellandrene dimer was detected in this resin (trace-0.3%). However, in contrast to the *Boswellia sacra* resins, there is a large concentration of cembrene type diterpenes as well, including incensole, serratol, verticilla-4(20),7,11-triene

and (*3E*)-cembrene A. Although incensole was detected in concentrations up to 45% by GC/MS, this is likely not the true concentration in the oil. This is because not everything in the mixture is detectable by this method, especially for the less volatile compounds. Furthermore, it is likely that the detectable non-volatile compounds, such as the boswellic acids and amyrins, are present in larger concentrations than shown due to their poorer detection. To get a more accurate representation of the concentration of various species in the mixture, purification techniques can be used to isolate either a fraction of similar compounds or even pure compounds. Techniques such as acid/base extraction, distillation and column chromatography can be utilised to achieve this.

Table 2.08. Chemical profiles of four separate maceration extraction experiments of the *Boswellia occulta* resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **71** | 1223 | Methoxydecane | High | 0.6-1.4 | 1.7-1.9 | 1.6-1.8 | 1.6-2.0 |
| **-** | 1562 | Unknown sesquiterpene | - | 0.3-0.4 | 0.5 | 0.4-0.5 | 0.5 |
| **147** | 1613 | Guaiol | Medium | 1.8-2.4 | 2.3-2.4 | 2.4-2.6 | 2.5-2.9 |
| **144** | 1653 | α-Eudesmol | High | 0.2-0.7 | trace-0.2 | trace-0.9 | 0.3-1.0 |
| **56** | 1952 | (*3E*)-Cembrene A | High | 0.7-0.9 | 0.9 | 0.9 | 1.0-1.1 |
| **-** | 1996 | Unknown Diterpene | - | trace-0.2 | trace | trace | trace-0.3 |
| **58** | 2012 | Verticilla-4(20),7,11-triene | Medium | 0.6-1.0 | 0.9-1.0 | 0.8-0.9 | 1.0-1.1 |
| **48** | **2135** | **Serratol** | **High** | **3.0-4.3** | **4.1-4.2** | **4.1-4.2** | **4.4-4.7** |
| **49** | **2148** | **Incensole** | **High** | **31.7-43.3** | **42.5-43.3** | **35.6-39.6** | **44.2-47.7** |
| **-** | 2266 | Unknown diterpene | - | 0.4-0.6 | 0.6-0.7 | 0.4-0.5 | trace-0.4 |
| **125** | **3002** | **24-Norursa-3,9(11),12-triene** | **High** | **4.4-9.6** | **4.1-4.2** | **4.7-5.9** | **4.0-4.8** |
| **126** | 3018 | 24-Noroleana-3,12-diene | High | 2.1-5.2 | 1.4-1.9 | 2.0-2.9 | 2.1-2.5 |
| **127** | **3065** | **24-Norursa-3,12-diene** | **High** | **3.1-9.2** | **3.2-3.6** | **4.1-4.8** | **5.0-5.9** |
| **128** | **3313** | **24-Norursa-3,12-dien-11-one** | **High** | **35-1-38.3** | **34.4-35.3** | **35.6-38.5** | **27.3-30.1** |
| **138** | 3345 | β-Amyrin acetate | Medium | trace | trace-1.3 | trace-1.1 | trace-0.8 |
| **129** | 3378 | α-Amyrin acetate | Medium | trace-0.8 | 0.7-0.9 | trace-0.8 | 0.8-0.9 |
| **Mass Yield (%)[c]** | | | | **51.0-52.5** | **49.5-57.4** | **45.9-51.5** | **29.3-34.3** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted.

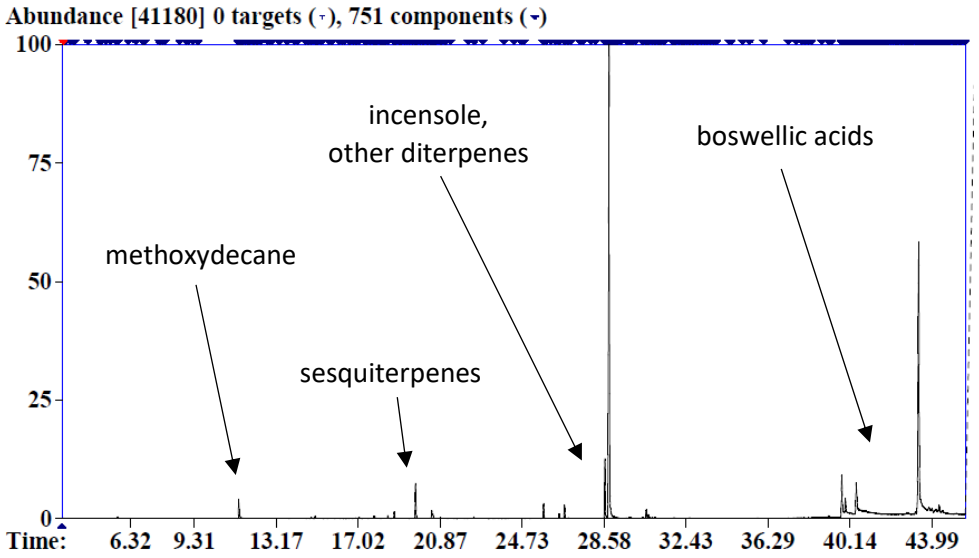



Figure 2.24. Chromatogram of an extract isolated via the chloroform maceration extraction of the *Boswellia occulta* resin following the method in **Section 6.06**.

Another interesting difference for these extracts when compared to many other frankincense resins is the triterpene fraction. GC/MS analysis shows that this fraction is almost completely composed of boswellic acids, with some very minor amyrin content.

Incensole **49** was found as one of the major products of this resin, which was confirmed by GC/MS and NMR. The fragmentation pattern showed a strong molecular ion peak for the unfragmented compound at m/z = 306. A peak a m/z = 263 shows the loss of a fragment of m/z = 43 which is likely the isopropyl group (figure 2.25). [52]

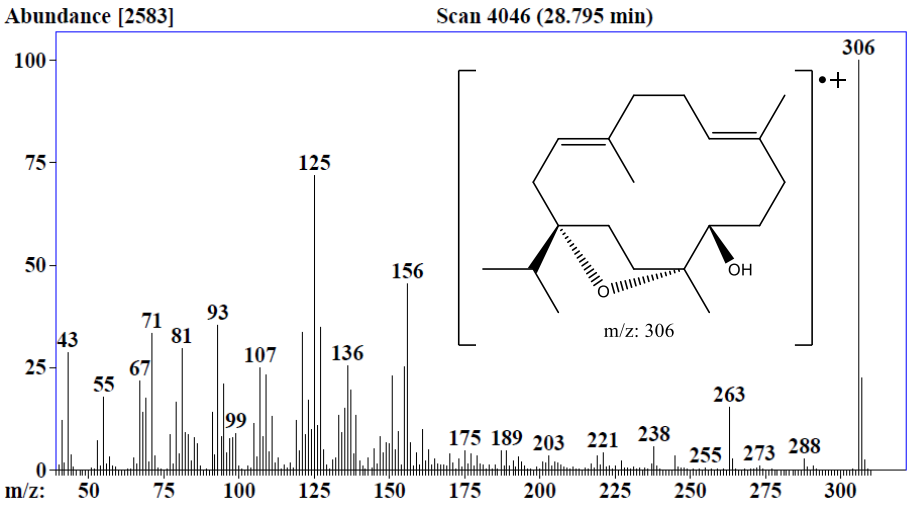



Figure 2.25. Fragmentation pattern for incensole **49**, AI = 2148 from the GC/MS analysis of an extract of *Boswellia occulta* resin, isolated through maceration with chloroform following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[21]

For confirmation of this compound's structure, a small amount of incensole was purified from one of the extracts via column chromatography using 10-30% $Et_2O$ in petroleum spirits (40-60) as an eluent. This oil was submitted for $^1H$, $^{13}C$ and HSQC NMR spectroscopy (figures 2.26-2.28). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.91 (s, 3H), 0.93 (s, 3H), 1.09 (s, 3H), 1.34 (dddd, 1H, *J* = 13.9, 10.3, 5.4, 1.9 Hz), 1.52 (s, 3H) 1.61 (ddd, 1H, *J* = 11.3, 7.8, 4.0 Hz), 1.64 (s, 3H), 1.77 (m, 1H), 1.86 (m, 1H), 1.90 (br s, 1H), 1.93 (m, 1H), 1.98 (s, 1H), 2.06 (m, 1H), 2.06 (m, 1H), 2.13 (br dd, 1H, *J* = 8.2, 4.1 Hz), 2.13-2.19 (m, 2H), 2.13-2.19 (m, 2H), 2.19 (m, 1H), 3.32 (d, 1H, *J* = 10.2 Hz), 5.09 (m, 1H, *J* = 6.5 Hz), 5.13 (m, 1H, *J* = 7.0 Hz). $^{13}C$ NMR ($CDCl_3$, 100MHz): $\delta_C$ 16.1, 18.0, 18.1, 18.2, 20.7, 24.8, 30.6, 30.7, 32.4, 33.7, 34.8, 36.4, 38.6, 75.6, 84.2, 88.6, 121.8, 125.1, 134.2, 134.2.

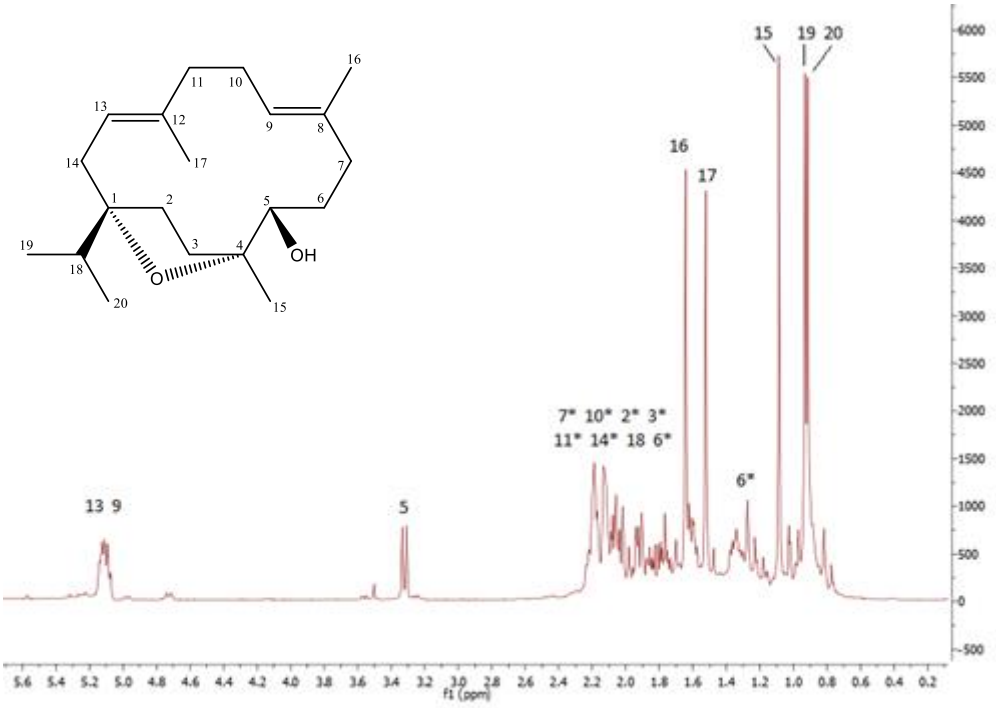



Figure 2.26. $^1H$ NMR (400 MHz, $CDCl_3$) of incensole which corresponds to the literature. Peak numbers correspond to their position on the compound. *Protons from $CH_2$ groups where each CH produces a separate signal.

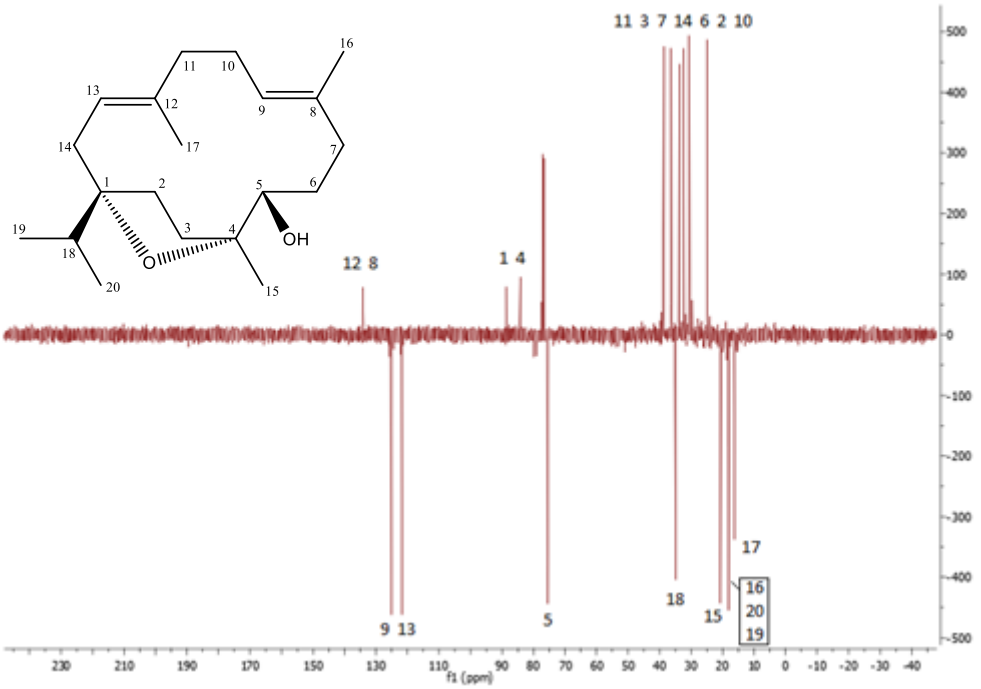



Figure 2.27. $^{13}C$ NMR (100 MHz, $CDCl_3$) of incensole which corresponds to the literature. Peak numbers correspond to their position on the compound.

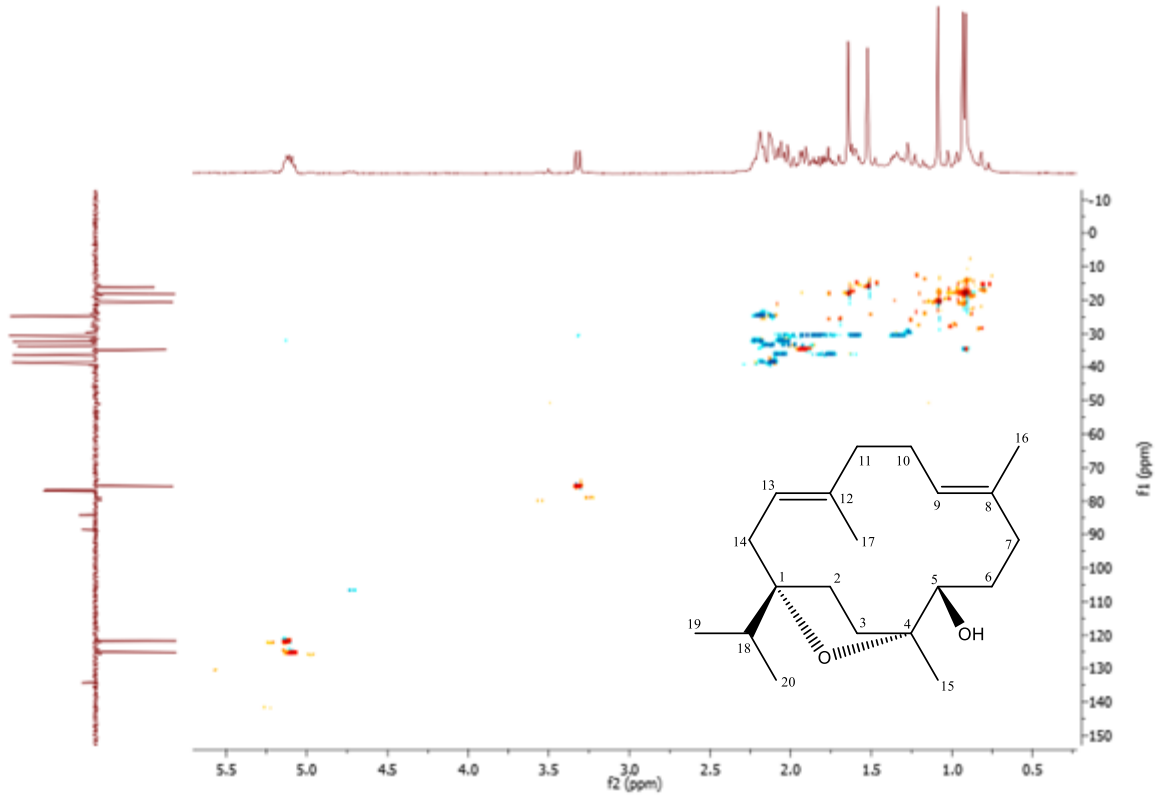



Figure 2.28. HSQC ($CDCl_3$) NMR of incensole confirming the $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) peak overlaps.

Along with the GC/MS data and the literature NMR data as a comparison to the NMR experiments ran in this work, the compound isolated was confirmed to be incensole.[70] There were twenty carbon atoms detected using $^{13}C$ NMR, with the phase separation of DEPTQ

allowing for the observation of eleven carbons substituted with an even number of protons and nine carbons substituted with an odd number of protons. A multiplet detected at around 5.3 ppm in the $^{1}$H NMR was due to two merging triplets, which the unsaturated CH groups on carbons 9 and 13 were responsible for. The doublet at around 3.3 ppm was caused by the proton on the 5 carbon, due to it being beside a $CH_2$ group and a quaternary carbon. At around 1.3 ppm, there is dddd splitting, which is characteristic for the βH on carbon 6 (figure 2.29). As described in previous work, this complex splitting occurs due to the H6β having interactions with its four neighbours (the H5, H6α and both H7 protons). The splitting for the H6α proton (1.9 ppm) was not classified in this case, due to its overlap with other peaks in the same region. Each proton on $CH_2$ groups in the 14 membered ring are diastereomic, so are locked in separate magnetic environments resulting in separate signals for each.[70]

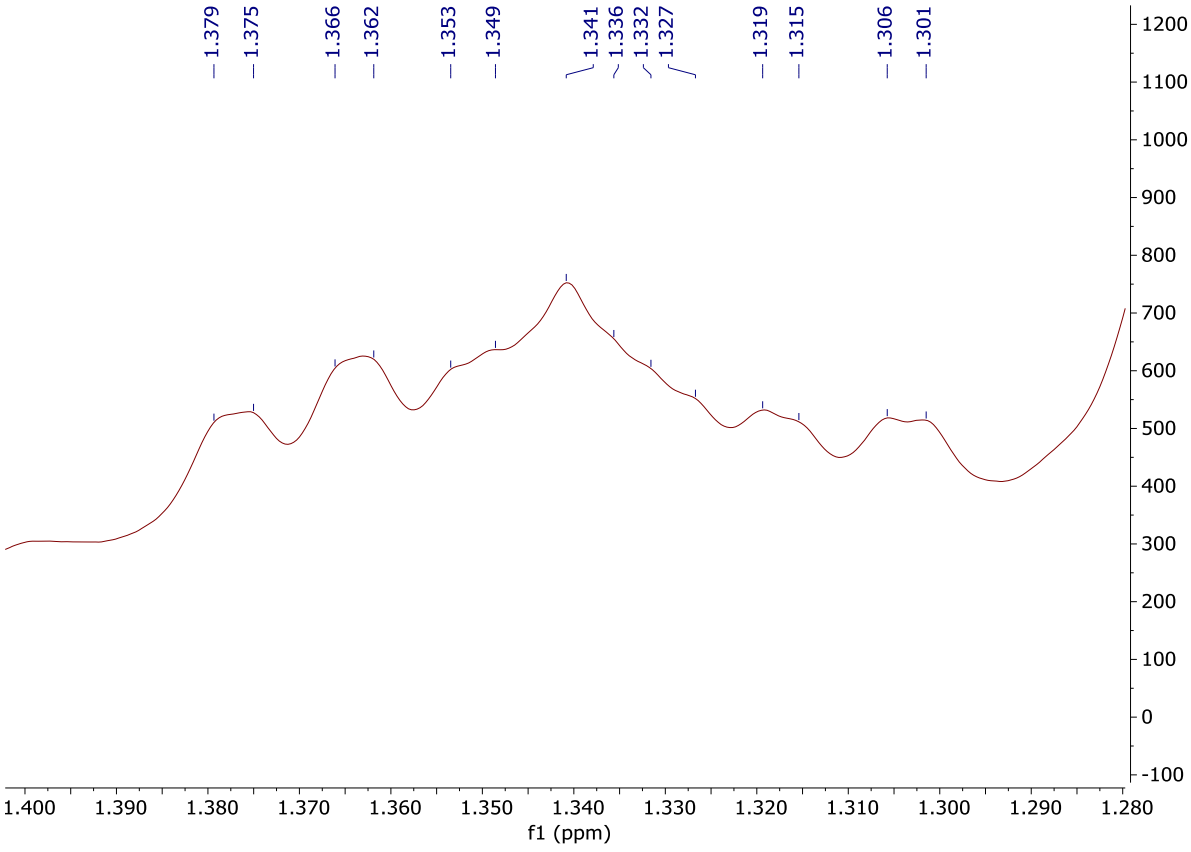
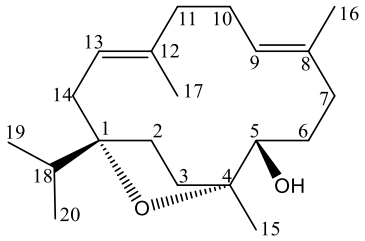



Figure 2.29. Characteristic dddd splitting for the H6β on incensole found using $^{1}$H NMR (400 MHz, $CDCl_3$) spectroscopy.

This structure follows the general shape of cembrene, which produces similar NMR data for many of the groups in incensole, including the $CH_3$, $CH_2$ and unsaturated carbon groups (table 2.09).[88]

Table 2.09. Structural assignment of incensole via $^{1}H$, $^{13}C$ and HSQC NMR spectroscopy compared to a literature source. N.s – Not specified.




| Carbon No. | $^{13}C$ NMR | | $CH_n$ | $^{1}H$ NMR | | Multiplicity/J-Coupling (Hz) | |
|---|---|---|---|---|---|---|---|
| | Literature[70] | Observed | | Literature[70] | Observed | Literature[70] | Observed |
| **1** | 88.56 | 88.56 | C | - | - | - | - |
| **2** | 30.66 | 30.63 | $CH_2$ | 1.84<br>1.59 | 1.86<br>1.61 | N.s<br>ddd, *J* = 11.6, 7.6, 4.0 | m<br>ddd, *J* = 11.3, 7.8, 4.0 |
| **3** | 36.36 | 36.36 | $CH_2$ | 2.06<br>1.74 | 2.06<br>1.77 | N.s<br>N.s | m<br>m |
| **4** | 84.14 | 84.15 | C | - | - | - | - |
| **5** | 75.57 | 75.55 | CH | 3.30 | 3.32 | dd, *J* = 10.0, 0.8 | d, *J* = 10.2 |
| **6** | 30.72 | 30.72 | $CH_2$ | 1.89<br>1.32 | 1.90<br>1.34 | m<br>dddd, *J* = 14.0, 10.0, 5.6, 1.6 | br s,<br>dddd, *J* = 13.9, 10.3, 5.4, 1.9 |
| **7** | 33.65 | 33.66 | $CH_2$ | 2.12<br>1.99 | 2.13<br>1.98 | dd, *J* =14.0, 5.6<br>m | br dd, *J* = 8.2, 4.1<br>s |
| **8** | 134.18 | 134.18 | C | - | - | - | - |
| **9** | 125.13 | 125.12 | CH | 5.08 | 5.09 | m | m, *J* = 6.5 |
| **10** | 24.83 | 24.84 | $CH_2$ | 2.11-2.18 | 2.13-2.19 | m | m |
| **11** | 38.62 | 38.62 | $CH_2$ | 2.11-2.16 | 2.13-2.19 | m | m |
| **12** | 134.23 | 134.24 | C | - | - | - | - |
| **13** | 121.79 | 121.77 | CH | 5.12 | 5.13 | m | m, *J* = 7.0 |
| **14** | 32.35 | 32.35 | $CH_2$ | 2.04<br>2.17 | 2.06<br>2.19 | N.s<br>N.s | m<br>m |
| **15** | 20.64 | 20.66 | $CH_3$ | 1.07 | 1.09 | s | s |
| **16** | 18.15 | 18.17 | $CH_3$ | 1.62 | 1.64 | m | s |
| **17** | 16.10 | 16.13 | $CH_3$ | 1.50 | 1.52 | m | s |
| **18** | 34.85 | 34.83 | CH | 1.90 | 1.93 | sep, *J* = 6.8 | m |
| **19** | 17.98 | 18.00 | $CH_3$ | 0.89 | 0.91 | d, *J* = 6.8 | br s |
| **20** | 18.05 | 18.07 | $CH_3$ | 0.91 | 0.93 | d, *J* = 6.8 | br s |

## 2.2.2 Gudmo Biyo Cas Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** The *Boswellia carterii* resin's essential oil from Gudmo Biyo Cas was of the α-

pinene chemotype and was a relatively large fraction. The hydrodistilled oil yields ranged from 7.3-7.9% compared to the resin mass and had a rich pine like smell (table 2.10).

Table 2.10. Chemical profile of the Gudmo biyo cas frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Content estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[42] |
|---|---|---|---|---|---|
| **148** | 921 | Tricyclene | High | 0.3-0.6 | trace |
| **24** | 924 | Thujene | High | 0.4-0.8 | 7.9 |
| **25** | **932** | **α-Pinene** | **High** | **62.7-66.6** | **37.3** |
| **26** | 946 | Camphene | High | 0.8 | 0.8 |
| **27** | **969** | **Sabinene** | **High** | **4.5-9.0** | **4.9** |
| **28** | **974** | **β-Pinene** | **High** | **1.6-5.8** | **1.8** |
| **30** | 1002 | α-Phellandrene | High | 0.8-1.8 | 3.3 |
| **31** | 1008 | δ-3-Carene | High | 0.2-1.1 | 0.9 |
| **32** | 1020 | *p*-Cymene | High | 0.8-1.4 | 4.0 |
| **33** | **1024** | **Limonene** | **High** | **1.3-6.9** | **14.4** |
| **149** | 1032 | (*Z*)-β-Ocimene | High | 0.2-0.6 | 0.1 |
| **46** | 1044 | (*E*)-β-Ocimene | High | 0.3-3.3 | - |
| **133** | 1135 | *trans*-Pinocarveol | Medium | 0.1-0.6 | 0.4 |
| **35** | 1137 | *cis*-Verbenol | High | 0.3-1.3 | 0.4 |
| **-** | 1170 | Unknown monoterpene | - | 0.4-1.4 | - |
| **-** | 1181 | Unknown monoterpene | - | 0.4-0.5 | - |
| **64** | 1190 | α-Terpineol | High | 0.2-0.9 | 0.4 |
| **37** | 1204 | Verbenone | High | 0.1-1.0 | 0.3 |
| **-** | 1214 | Unknown monoterpene | - | 0.8-0.9 | - |
| **141** | 1284 | Bornyl acetate | High | 0.2-0.6 | 0.3 |
| **65** | 1387 | β-Bourbonene | High | 0.4-0.6 | 0.7 |
| **40** | 1417 | Caryophyllene | High | 0.5-1.2 | 2.8 |
| **150** | 1431 | 6,9-Guaiadiene | Medium | 0.5-2.8 | - |
| **-** | 1441 | Unknown sesquiterpene | - | 0.3-1.1 | - |
| **43** | 1522 | δ-Cadinene | High | 0.3-0.6 | 0.5 |
| **-** | >3600 | Unknown triterpene | - | 0.3-0.8 | - |
| **Mass Yield (%)[c]** | | | | 7.3-7.9 | Not specified |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass distilled.

As expected, the main component of the oil was α-pinene **25** (62.7%-66.6%), with other major compounds including sabinene **27** (4.5-9.0%), β-pinene **28** (1.6%-5.8%) and limonene **33** (1.3%-6.9%).

When compared with the literature it seems like either *Boswellia carterii*, *frereana*, *neglecta* or *rivae*. All 4 of these in the literature show similar essential oil fractions when they produce the α-pinene chemotype and are found in Somaliland. However, there are differences found between these resins as *rivae* and *neglecta* are known to have strong monoterpene fractions, but little to no diterpenes and sesquiterpenes. A key difference between *frereana* and *carterii* is in the diterpene fraction. *Frereana* is known to frequently produce a dimer of phellandrene which was also detected in the *Boswellia occulta* and *Boswellia sacra* extracts, whereas *carterii* often produces cembrene type diterpenes, such as incensole.[2,7,89,90] Although this sample was considered to be *Boswellia carterii*, there were still some differences found when compared to the literature, such as having higher α-pinene content and lower limonene content than in the literature. The Chromatograms for these distillates all showed a strong monoterpene fraction and a small, but varied sesquiterpene fraction (figure 2.30). Some hydrodistillations may find diterpenes in the resultant oils, but this can be because of the temperature of the experiment, so the maceration extractions in solvent are likely to show a diterpene fraction more often than a distillation.

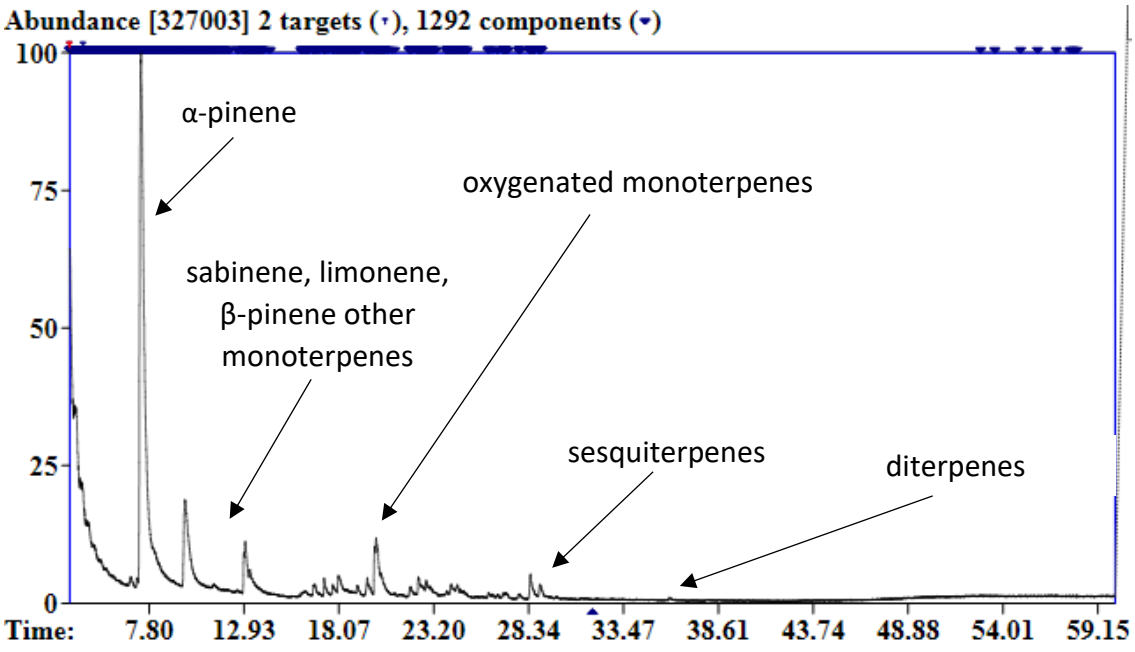



Figure 2.30. Chromatogram of the essential oil isolated via hydrodistillation of the Gudmo biyo cas resin following the method in **Section 6.05**.

Extractions were carried out on this resin by maceration, followed by filtration and evaporating the solvent following the method in **Section 6.06**. The resultant oils depend largely on the solvent used and gave mass recoveries between 53-61%, 63-67%, 60-69% and 38-44% when using methanol, chloroform, diethyl ether and hexane respectively. Generally, these extracts had quite similar extraction profiles, with a wide variety of compounds detected throughout the whole chromatogram (table 2.11 & figure 2.31).

Table 2.11. Chemical profiles of four separate maceration extraction experiments of the Gudmo biyo cas resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **25** | **931** | **α-Pinene** | **High** | **trace** | **5.6-7.7** | **6.5-9.2** | **4.5-7.3** |
| **27** | 971 | Sabinene | High | trace | 0.7-1.0 | 0.8-1.1 | 0.5-0.7 |
| **28** | 977 | β-Pinene | High | trace | 0.8-1.1 | 0.8-1.4 | 0.8-1.0 |
| **33** | 1028 | Limonene | High | trace | 0.5-0.8 | 0.5-0.7 | 0.7-0.9 |
| **-** | 1145 | Unknown monoterpene | - | 0.6-1.0 | 0.9-1.1 | 0.7-1.0 | 1.0-1.1 |
| **152** | 1641 | *epi*-α-Cadinol | Medium | 0.4-0.6 | 0.6-0.8 | 0.6 | 0.6-0.7 |
| **-** | 2135 | Unknown Diterpene | - | 0.9-1.0 | 1.1 | 0.9-1.0 | 1.1-1.2 |
| **48** | **2148** | **Serratol** | **High** | **11.4-14.8** | **15.3-15.8** | **11.6-13.4** | **14.2-16.1** |
| **125** | 3002 | 24-Norursa-3,9(11),12-triene | High | 5.4-7.2 | 2.2-2.4 | 3.3-4.0 | 2.9-3.1 |
| **126** | 3011 | 24-Noroleana-3,12-diene | High | 3.3-5.2 | 1.1-1.2 | 2.4-2.5 | 2.7 |
| **127** | 3070 | 24-Norursa-3,12-diene | High | 7.8-9.5 | 2.1-2.5 | 4.1-5.2 | 4.5-6.8 |
| **-** | 3275 | Unknown Triterpene | - | 0.8-1.1 | 1.1-1.2 | 1.2-1.4 | 1.1-1.5 |
| **-** | 3291 | Unknown Triterpene | - | 1.4-1.7 | 2.1-2.2 | 2.0-2.3 | 1.8-2.2 |
| **-** | 3303 | Unknown Triterpene | - | 0.9-1.0 | 1.4-1.5 | 1.1-1.3 | 1.1-1.3 |
| **128** | **3313** | **24-Norursa-3,12-dien-11-one** | **High** | **31.8-33.7** | **21.3-23.5** | **23.9-26.6** | **21.4-22.8** |
| **99** | **3323** | **β-Amyrin** | **High** | **5.3-6.0** | **8.2-8.3** | **6.9-7.1** | **5.5-7.0** |
| **138** | 3347 | β-Amyrin acetate | Medium | 1.7-2.2 | 3.0-3.2 | 2.5-2.9 | 1.7-2.7 |
| **90** | 3356 | α-Amyrenone | High | 3.8 | 5.1-5.4 | 4.2-4.4 | 4.5-5.3 |
| **89** | **3367** | **α-Amyrin** | **High** | **7.4-8.2** | **11.0-11.6** | **9.7-10.1** | **10.3-12.1** |
| **129** | **3378** | **α-Amyrin acetate** | **Medium** | **5.3-5.7** | **7.4-8.3** | **5.5-6.1** | **6.6-7.9** |
| **-** | 3388 | Unknown Triterpene | - | 1.5-2.0 | 2.7-2.9 | 2.3-2.5 | 2.5-2.8 |
| **Mass Yield (%)[c]** | | | | **53.0-60.6** | **63.4-69.4** | **59.6-69.3** | **38.2-43.9** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted.

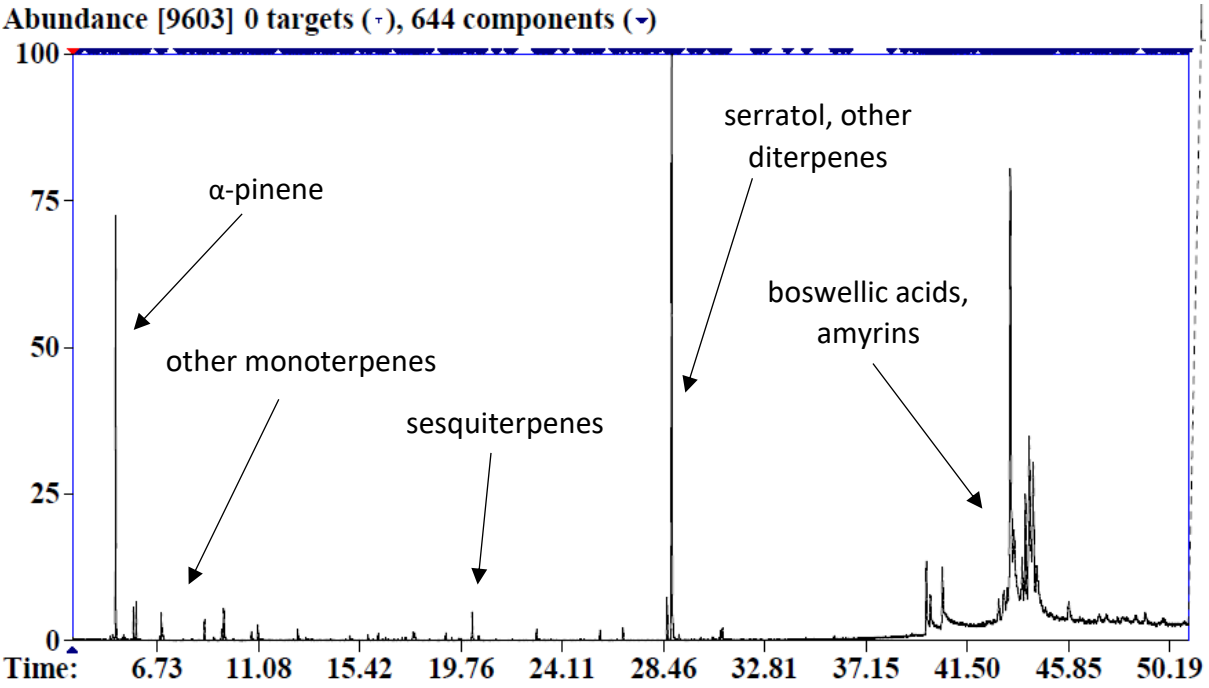



Figure 2.31. Chromatogram of an extract isolated via the chloroform maceration extraction of the Gudmo biyo cas resin following the method in **Section 6.06**.

The chloroform, diethyl ether and hexane extracts all had quite large essential oil fractions of 10.8-11.9%, 12.6-13.3% and 9.5-12.7% respectively. When applied to the mass recovery of the oil compared to the resin mass, these essential oil fractions give estimations of 7.2-8.2% for chloroform, 8.0-8.7% for diethyl ether and 3.6-5.1% for hexane which are all quite high considering the distillations gave mass recoveries between 7.3-7.9%. The methanol extracts however only recorded trace α-pinene content, which is the dominating volatile compound produced in this resin. This could be useful for fractionation, as the polar solvents such as methanol could be used to strip the heavier oils off before simply extracting the residue in a non-polar solvent to retain just the non-polar volatiles in high purity. This would also negate the need for high temperature distillations which can cause decomposition of volatile compounds. The major compounds found overall in these solvent extractions were serratol **48** (11.4-16.1%), 24-norursa-3,12-dien-11-one **128** (21.3-33.7%), β-amyrin **99** (5.3-8.3%), α-amyrin **89** (7.4-12.1%) and α-amyrin acetate **129** (5.3-8.3%).

The main composition of the maceration extractions was found to be triterpenes, with quite high content of non-boswellic acid triterpenes, which were mainly amyrins (figure 2.32). 24-norursa-3,12-dien-11-one, the fingerprint compound of the β-keto-boswellic acids, was the main component. In the *Boswellia sacra* samples, 24-norursa-3,12-diene, the fingerprint

compound of the 11-unsubstituted β-boswellic acids were found in much higher levels compared to this resin. The trade off in this resin seems to be the increase in amyrin content and a reasonable diterpene fraction, as all other boswellic acid marker compounds were found in relatively low concentrations for this resin (0.4-9.5%).

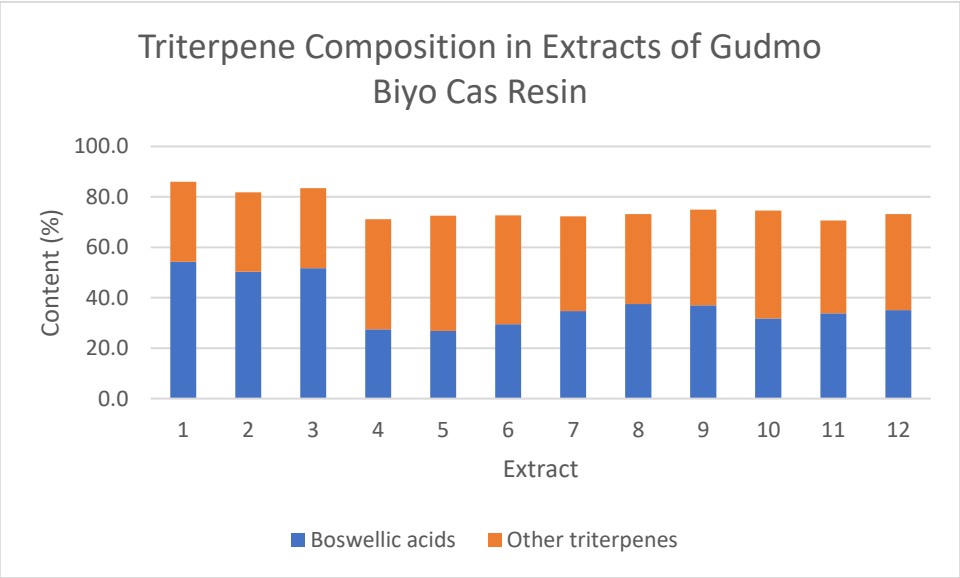



Figure 2.32. Triterpenes in Gudmo Biyo Cas extracts, high in amyrins and boswellic acids. Content determined using GC/MS.

The main amyrins that were detected in this resin were α-amyrin **89** (7.4-12.1%), β-amyrin **99** (5.3-8.3%) and α-amyrin acetate **129** (5.3-8.3%). Another amyrin type triterpene found in this resin in reasonable concentration is α-amyrenone **90** (3.8-5.4%) which is of the ursane type skeletal structure. The fragmentation pattern for this compound shows a parent ion peak at m/z = 424, notably m/z = 2 less than α-amyrin, which accounts for the ketone group as opposed to the alcohol group that α-amyrin has. The peak just below this is at m/z = 409 which represents the loss of a methyl group. This compound has a carbon-carbon double bond in a ring system like the boswellic acids and other amyrins, meaning that it can undergo the retro-Diels-Alder mechanism upon fragmentation, which results in the large peak at m/z = 218. Once again, the later elution of this compound and the smaller peak at m/z = 203 represents the ursane type structure, meaning this compound can be characterised as α-amyrenone **90** and not β-amyrenone **100** (figure 2.33).[2]

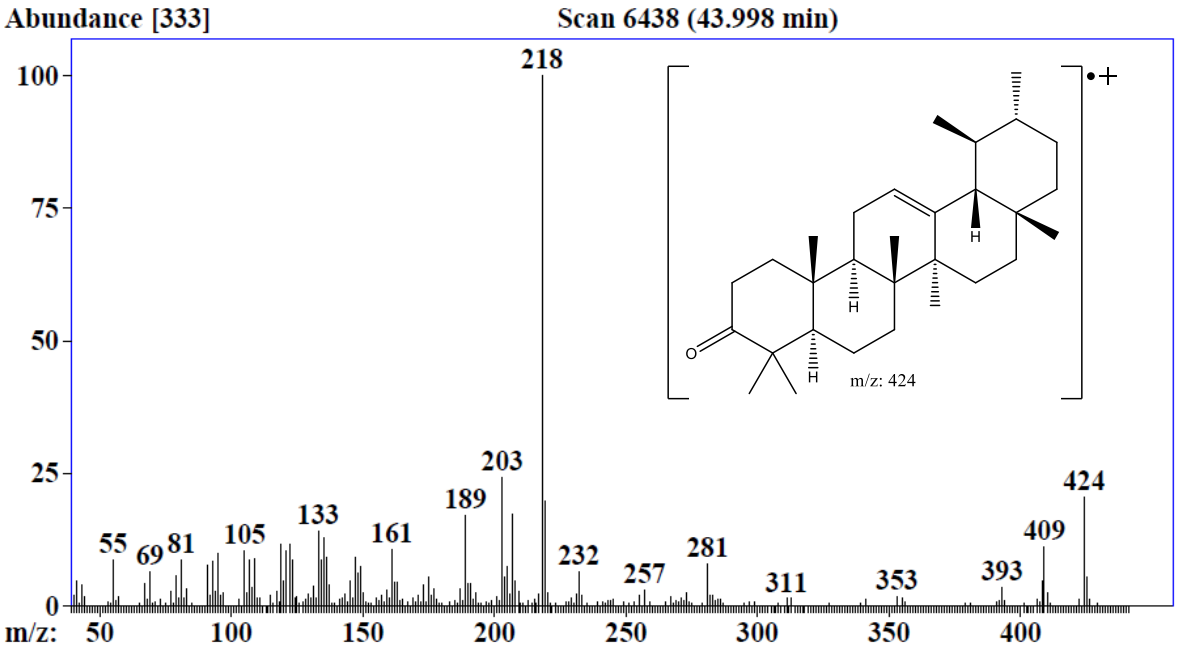



Figure 2.33. Fragmentation pattern of α-amyrenone **90**, AI = 3356 from the GC/MS analysis of an extract of Gudmo biyo cas resin, isolated through maceration with chloroform following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison (in the supplementary information).[85]

The diterpene fraction is almost completely defined by the serratol content in this resin (11.4-16.1%). This compound was identified by its fragmentation pattern from the GC/MS analysis, followed by purification via column chromatography to analyse by NMR. The fragmentation pattern (figure 2.34) shows the parent ion peak at m/z = 290. There is a small peak at m/z = 306 which is likely a small concentration of incensole which elutes at the same time. Incensole gives a very strong molecular ion peak as discussed above for the *Boswellia occulta* resin meaning a small peak here likely represents a small concentration of the compound. The literature suggests that the large peak at m/z = 272 shows the facile loss of a $H_2O$ group and the peak at m/z = 229 is the subsequent loss of $C_3H_7$. At m/z = 247, there is another peak which is when the parent ion loses the $C_3H_7$ first, which can then lose $H_2O$ to produce the fragment at m/z = 229 again.[91]

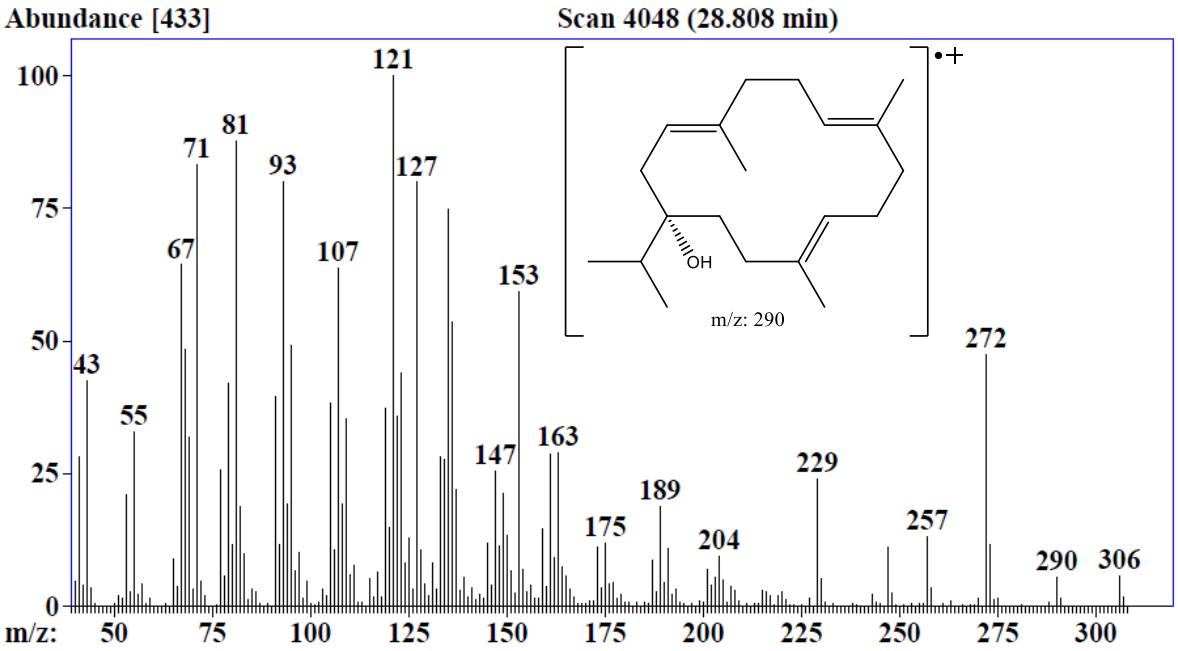



Figure 2.34. Fragmentation pattern of serratol **48**, AI = 2148 from the GC/MS analysis of an extract of Gudmo biyo cas resin, isolated through maceration with chloroform following the procedure in **Section 6.06**. Slight co-elution with incensole likely causes the m/z = 306 peak as described in previous literature.[4] Assignment confidence = high. Literature comparison.[91]

The solvent made less of an impact on the content of serratol in the oil. The presence of this compound is usually only found in *Boswellia serrata* in high concentrations, so this could either be a sample of resin that has not previously been studied or could show that *Boswellia serrata* grows in Somaliland as well as India. Furthermore, the essential oil content is relatively high in the literature (5.0-11.6%) and its bark has been found to produce an α-pinene type oil.[63,92]

The isolation of serratol was carried out using the method in **Section 6.12** to attempt to quantify it. Methanol was used to extract the resin by maceration. After filtration and removal of the solvent, the crude extract was isolated (55% mass recovery compared to the resin mass). The acidic constituents were removed by extraction with 2% KOH solution to give a neutral fraction (29% mass recovery compared to the resin mass). Next, this neutral fraction was purified by column chromatography using $Et_2O$ in petroleum spirits (40-60) as the mobile phase (5%, 10%, 15% eluent gradient). This resulted in a 4.3% mass recovery of serratol compared to the resin mass as a clear, colourless oil, which was analysed by NMR following the method in **Section 6.04**. This yield of serratol is slightly lower than the amount estimated by GC which is to be expected as the acid fraction separation step and column

chromatography purification both result in small losses. Furthermore, GC can give inaccurate concentrations when there are non-volatile compounds in the mixture as they do not enter the gas phase as easily and therefore are not detected as easily resulting in oil contents that are not completely representative. NMR data was gathered on this oil to fully characterise it as serratol (figures 2.35-2.37). $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.95 (t, 6H, *J* = 6.9 Hz), 1.57 (s, 3H), 1.59 (s, 3H), 1.61 (s, 3H), 1.65 (t, 2H, *J* = 7.6 Hz), 1.72 (m, 1H, *J* = 6.8 Hz), 1.79 (t, 1H, *J* = 7.2 Hz), 1.93 (t, 1H, 7.5 Hz), 1.97 (t, 1H, *J* = 7.0 Hz), 2.11 (s, 1H), 2.12 (m, 2H), 2.14 (br s, 1H), 2.14 (t, 1H, *J* = 7.8 Hz), 2.20 (br s, 1H), 2.22-2.23 (m, 2H), 2.34 (t, 1H, *J* = 7.1 Hz), 4.91 (br t, 1H, *J* = 6.1 Hz), 5.02 (br t, 1H, *J* = 6.8 Hz), 5.27 (br t, 1H, *J* = 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta_C$ 15.1, 15.2, 16.4, 16.6, 16.8, 23.8, 24.8, 33.5, 34.6, 34.7, 34.9, 39.5, 39.9, 76.9, 120.9, 123.2, 125.9, 133.3, 135.6, 136.7.

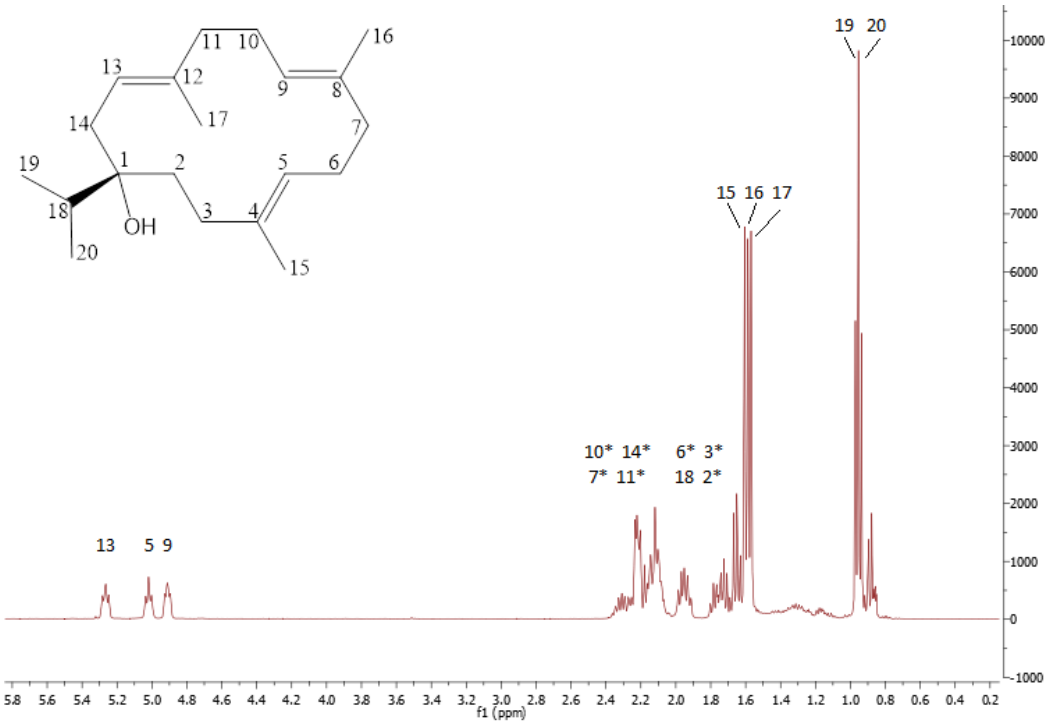



Figure 2.35. $^{1}$H NMR (400 MHz, $CDCl_3$) of serratol which is concordant with the literature. Peak numbers correspond to the atom numbering. * Protons from $CH_2$ groups where each CH produces a separate signal.

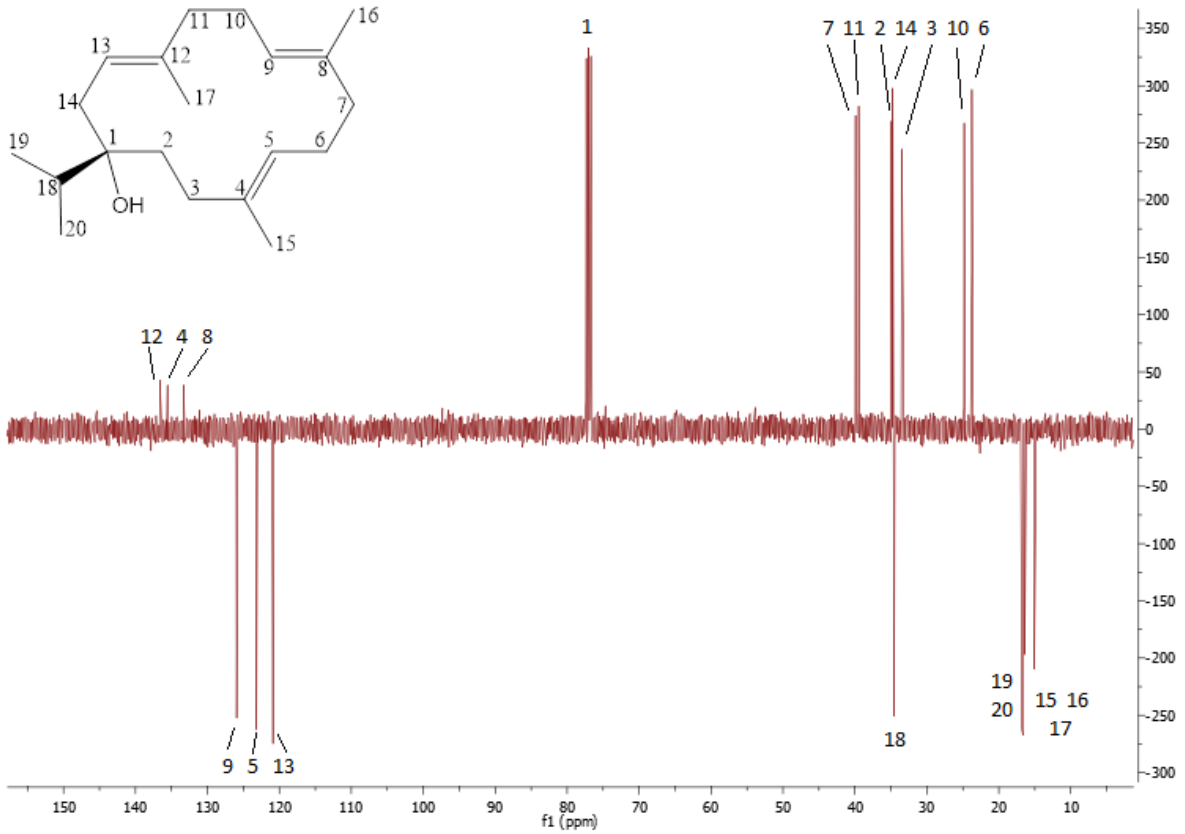



Figure 2.36. $^{13}C$ NMR (100 MHz, $CDCl_3$) of serratol which is concordant with the literature. Peak numbers correspond to the atom numbering.

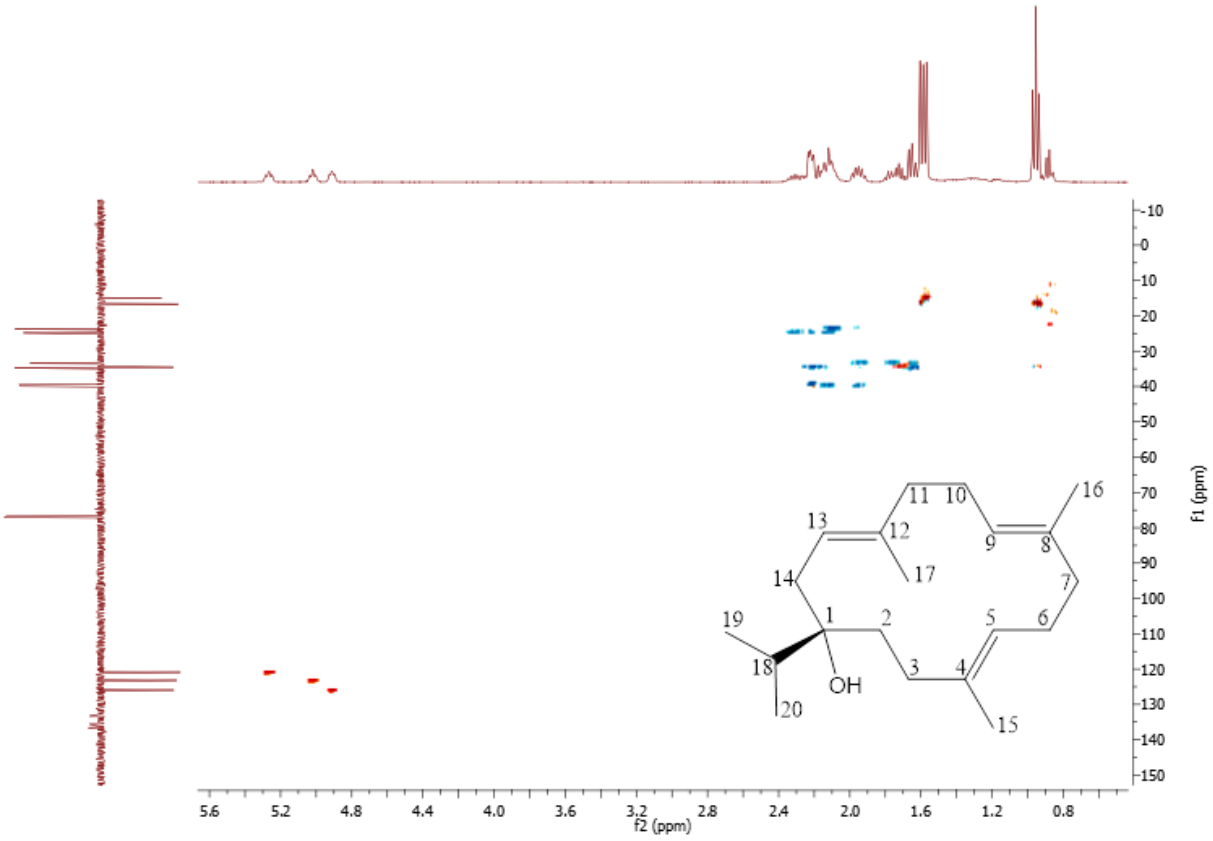
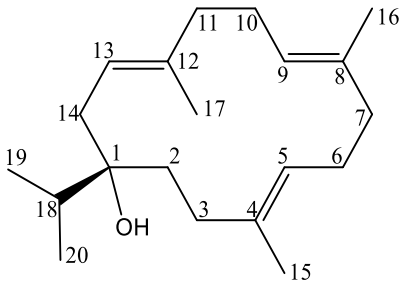



Figure 2.37. HSQC NMR ($CDCl_3$) of serratol confirming the $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) peak overlaps.

Using a combination of the $^{1}H$, $^{13}C$ and HSQC NMR data in addition to GC/MS data and the literature confirmed the yielded oil to be serratol.[70] This compound follows the typical 'cembrene' type structure and as a result, produced similar NMR spectra to it.[88] Five clear methyl peaks were detected in the $^{1}H$ NMR spectrum, 2 of which overlapped as the isopropyl $CH_3$ groups, were are interchangeable in the structural assignment. Unlike incensole which has 2 unsaturated CH groups, serratol has three due to the fact the cyclisation has not occurred from the OH group to carbon 4. This extra unsaturated CH group is illustrated in both the $^{13}C$ and $^{1}H$ NMR spectra by having an additional peak at around 120 ppm and 5 ppm respectively. Due to this structure being a 14-membered ring, the $CH_2$ protons are unable to interact equally with neighbouring protons, meaning they are magnetically non-equivalent. This results in separate signals for each proton on $CH_2$ groups in this compound in the $^{1}H$ NMR spectra. Including four quaternary carbons, the $^{13}C$ spectrum detected twenty carbon atoms, with the phase separation of DEPTQ showing eleven with an even number of protons and nine with an odd number of protons (table 2.12).

Table 2.12 Structural assignment of serratol via $^{1}H$, $^{13}C$ and HSQC NMR spectroscopy compared to a literature source. N.s – Not specified.




| Carbon No. | $^{13}C$ NMR (ppm) | | $CH_n$ | $^{1}H$ NMR (ppm) | | Multiplicity/J-Coupling (Hz) | |
|---|---|---|---|---|---|---|---|
| | Literature[70] | Observed | | Literature[70] | Observed | Literature[70] | Observed |
| **1** | 76.83 | 76.85 | C | - | - | - | - |
| **2** | 34.90 | 34.93 | $CH_2$ | 1.65<br>1.65 | 1.65 | N.s<br>N.s | t, *J* = 7.6 |
| **3** | 33.45 | 33.46 | $CH_2$ | 1.93<br>1.75 | 1.79<br>1.93 | N.s<br>N.s | t, *J* = 7.2<br>t, *J* = 7.5 |
| **4** | 135.52 | 135.55 | C | - | - | - | |
| **5** | 123.15 | 123.18 | CH | 4.99 | 5.02 | br dd, t | br t, *J* = 6.8 |
| **6** | 23.73 | 23.75 | $CH_2$ | 2.09<br>2.09 | 2.12 | N.s<br>N.s | m |
| **7** | 39.89 | 39.91 | $CH_2$ | 1.95<br>2.11 | 1.97<br>2.14 | N.s<br>N.s | t, *J* = 7.0<br>t, *J* = 7.8 |
| **8** | 133.27 | 133.30 | C | - | - | - | - |
| **9** | 125.91 | 125.93 | CH | 4.88 | 4.91 | br dd, t | br t, *J* = 6.1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **10** | 24.79 | 24.81 | $CH_2$ | 2.11<br>2.30 | 2.11<br>2.34 | N.s<br>N.s | s<br>t, *J* = 7.1 |
| **11** | 39.45 | 39.48 | $CH_2$ | 2.21<br>2.21 | 2.22-2.23 | N.s<br>N.s | m |
| **12** | 136.57 | 136.65 | C | - | - | - | - |
| **13** | 120.87 | 120.90 | CH | 5.24 | 5.27 | br dd, t | br t, *J* = 7.0 |
| **14** | 34.72 | 34.74 | $CH_2$ | 2.14<br>2.20 | 2.14<br>2.20 | N.s<br>N.s | br s<br>br s |
| **15** | 16.36 | 16.38 | $CH_3$ | 1.58 | 1.61 | br s | s |
| **16** | 15.16 | 15.18 | $CH_3$ | 1.56 | 1.59 | br s | s |
| **17** | 15.04 | 15.06 | $CH_3$ | 1.54 | 1.57 | br s | s |
| **18** | 34.51 | 34.55 | CH | 1.71 | 1.72 | N.s | m, *J* = 6.8 |
| **19** | 16.78 | 16.81 | $CH_3$ | 0.92 | 0.95 | d, *J* = 6.8 | t, *J* = 6.9 |
| **20** | 16.62 | 16.63 | $CH_3$ | 0.94 | | d, *J* = 6.8 | |

## 2.2.3 Erigavo Resin

The Erigavo resin was another resin that was collected from Somaliland and was suggested to be *Boswellia carterii* from the supplier. Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** These experiments produced a rich pine like smelling oil in mass recoveries of 5.6-6.9%. This essential oil fraction was very similar to the oil distilled from the Gudmo Biyo Cas resin (table 2.13 & figure 2.38).

Table 2.13. Chemical profile of the Erigavo frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[42] |
|---|---|---|---|---|---|
| **148** | 921 | Tricyclene | High | 0.9-1.3 | trace |
| **24** | 924 | Thujene | High | 0.3-0.4 | 7.9 |
| **25** | **932** | **α-Pinene** | **High** | **61.5-69.2** | **37.3** |
| **26** | 946 | Camphene | High | 0.9-1.0 | 0.8 |
| **153** | 953 | Thuja-2,4(10)-diene | Medium | 0.4-0.5 | - |
| **27** | **969** | **Sabinene** | **High** | **3.3-6.7** | **4.9** |
| **28** | **974** | **β-Pinene** | **High** | **3.4-4.6** | **1.8** |
| **30** | 1002 | α-Phellandrene | High | 0.8-3.6 | 3.3 |
| **32** | 1020 | *p*-Cymene | High | 1.3 | 4.0 |
| **33** | **1024** | **Limonene** | **High** | **3.8-5.7** | **14.39** |
| **46** | 1044 | (*E*)-β-Ocimene | High | 0.1-0.9 | - |
| **133** | 1135 | *trans*-Pinocarveol | Medium | 0.5-0.6 | 0.4 |
| **35** | 1137 | *cis*-Verbenol | High | 1.0-1.1 | 0.4 |
| **130** | 1140 | *trans*-Verbenol | High | 0.4-0.5 | - |

| | | | | | |
|---|---|---|---|---|---|
| - | 1174 | Unknown monoterpene | - | 1.4-1.6 | - |
| **64** | 1190 | α-Terpineol | High | 0.6-0.8 | 0.4 |
| **37** | 1204 | Verbenone | High | 0.8-0.9 | 0.3 |
| - | 1213 | Unknown monoterpene | - | 0.6-0.8 | - |
| **141** | 1284 | Bornyl acetate | High | 0.6 | 0.3 |
| **65** | 1387 | β-Bourbonene | High | 0.5-0.7 | 0.7 |
| **40** | 1417 | Caryophyllene | High | 0.3-0.9 | 2.8 |
| - | 1431 | Unknown sesquiterpene | - | 0.4-1.4 | - |
| **43** | 1522 | δ-Cadinene | High | 0.3-0.7 | 0.5 |
| **Mass Yield (%)[c]** | | | | **5.6-6.9** | **Not specified** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass distilled.

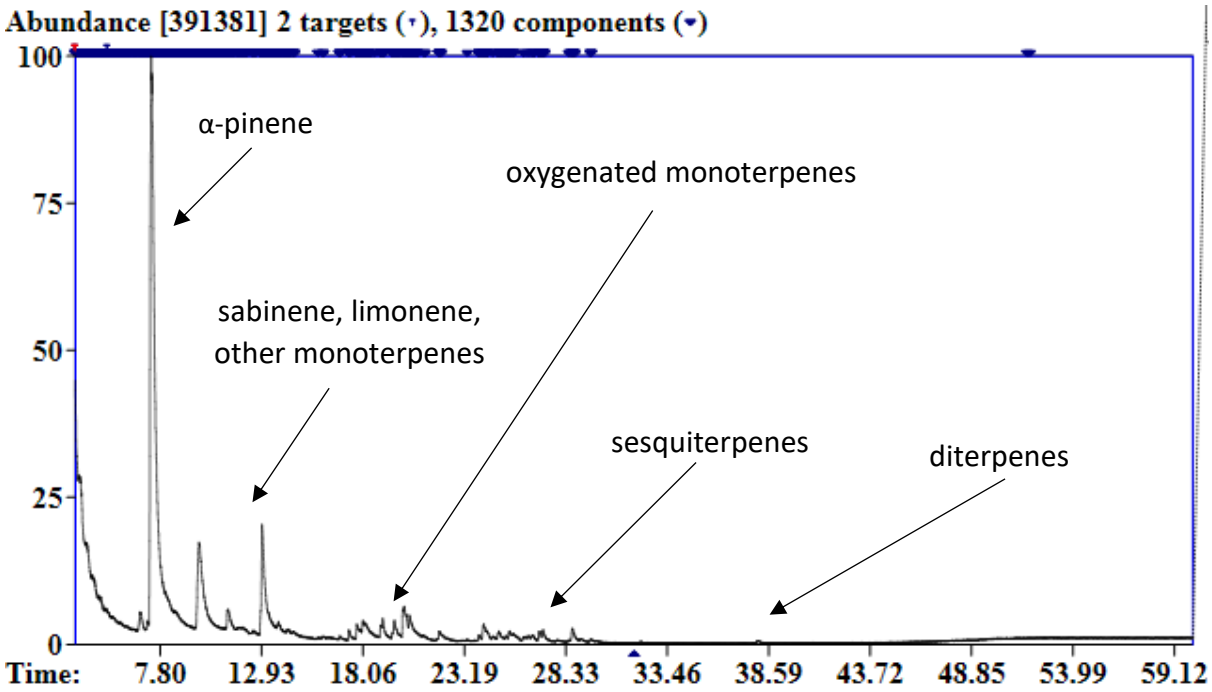



Figure 2.38. Chromatogram of the essential oil isolated via hydrodistillation of the Erigavo resin following the method in **Section 6.05**.

The most significant similarity between the Gudmo and Erigavo essential oil is the chemotype, which is α-pinene **25**, found in contents of 62.7-66.6% and 61.5-69.2% respectively. Both resins produced oils with similar concentrations of this compound. The other major compounds of this resin were sabinene **27** (3.3-6.7%), β-pinene **28** (3.4-4.6%) and limonene **33** (3.8-5.7%). This finding also makes this resin seem to belong to *Boswellia carterii*, but

shares some components with *Boswellia frereana*, *neglecta* and *rivae*. Although, similarly to the Gudmo biyo cas essential oil, it differed in α-pinene and limonene levels to the literature. However, the solvent extract could contain a diterpene fraction which would be more telling of its origin.

The fragmentation pattern of β-pinene is characterised by its parent ion having an m/z = 136, which is common for these monoterpenes. The fragmentation is also similar to α-pinene, having peaks at m/z = 121, 107 and 93. Therefore, the Kovats index was used in addition to the fragmentation pattern to characterize this as β-pinene (figure 2.39).

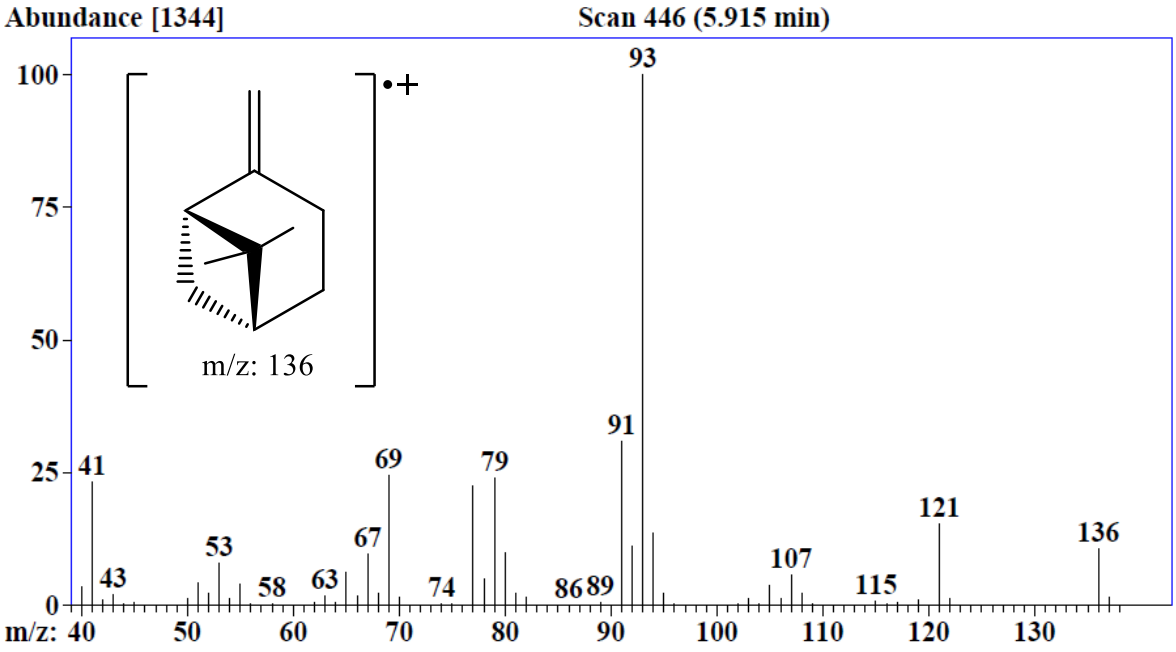



Figure 2.39. Fragmentation pattern of β-pinene **28**, AI = 974 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

Extraction by maceration, followed by filtration and evaporation of the solvent was carried out and GC/MS analysis followed to study the heavy oil composition of the resin (table 2.14 & figure 2.40). These extractions followed the method in **Section 6.06** and yielded oils in the range of 60-65%, 65-78%, 62-65% and 44-47% for methanol, chloroform, diethyl ether and hexane extracts respectively compared to the resin mass. Similarly to the Gudmo Biyo Cas resin, these extracts all gave strong essential oil fractions apart from the methanol extracts, which failed to take any more than trace α-pinene into solution. If these concentrations are applied to the mass recoveries, estimations on the essential oil content are 0-2.9%, 0-9.9%,

4.3-12.2% and 4.8-7.0% for the methanol, chloroform, diethyl ether and hexane extracts respectively. These ranges are quite broad and highlight the inconsistency in this resin. The Gudmo Biyo Cas resin, which belongs to the same chemotype, gave much more consistent chemical profiles for each solvent's extracts making it more reliable in the long term with regards to larger scale extractions.

Table 2.14. Chemical profiles of four separate maceration extraction experiments of the Erigavo resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **25** | **932** | **α-Pinene** | **High** | **trace** | **6.8-11.7** | **11.0-16.8** | **10.9-12.1** |
| **147** | 1596 | Guaiol | Medium | trace-4.6 | trace-3.4 | trace-3.8 | trace |
| **-** | 2135 | Unknown Diterpene | - | trace | trace-1.6 | trace-1.7 | trace |
| **48** | **2148** | **Serratol** | **High** | **39.7-50.9** | **27.0-39.2** | **26.6-36.0** | **32.4-35.4** |
| **125** | 3003 | 24-Norursa-3,9(11),12-triene | High | 5.3-15.6 | 3.1-7.2 | 4.0-5.0 | 3.4-4.7 |
| **126** | 3019 | 24-Noroleana-3,12-diene | High | trace-6.9 | 2.2-3.0 | trace-1.7 | 2.3-2.6 |
| **127** | **3066** | **24-Norursa-3,12-diene** | **High** | **8.2-12.5** | **6.0-6.8** | **4.5-5.8** | **7.1-9.7** |
| **128** | **3313** | **24-Norursa-3,12-dien-11-one** | **High** | **12.6-25.3** | **16.8-27.2** | **17.8-20.1** | **15.1-18.3** |
| **138** | 3347 | β-Amyrin acetate | Medium | trace-4.3 | trace-3.5 | trace-3.6 | trace-3.9 |
| **90** | 3356 | α-Amyrenone | High | trace | trace-4.3 | 3.4-4.0 | 2.4-4.3 |
| **89** | 3369 | α-Amyrin | High | trace | trace-6.7 | 5.5-6.1 | trace-5.6 |
| **129** | **3379** | **α-Amyrin acetate** | **Medium** | **trace-18.7** | **9.7-13.0** | **10.4-12.5** | **14.4-14.9** |
| **Mass Yield (%)[c]** | | | | **60.4-65.3** | **65.3-77.8** | **61.6-65.0** | **44.0-47.1** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted.

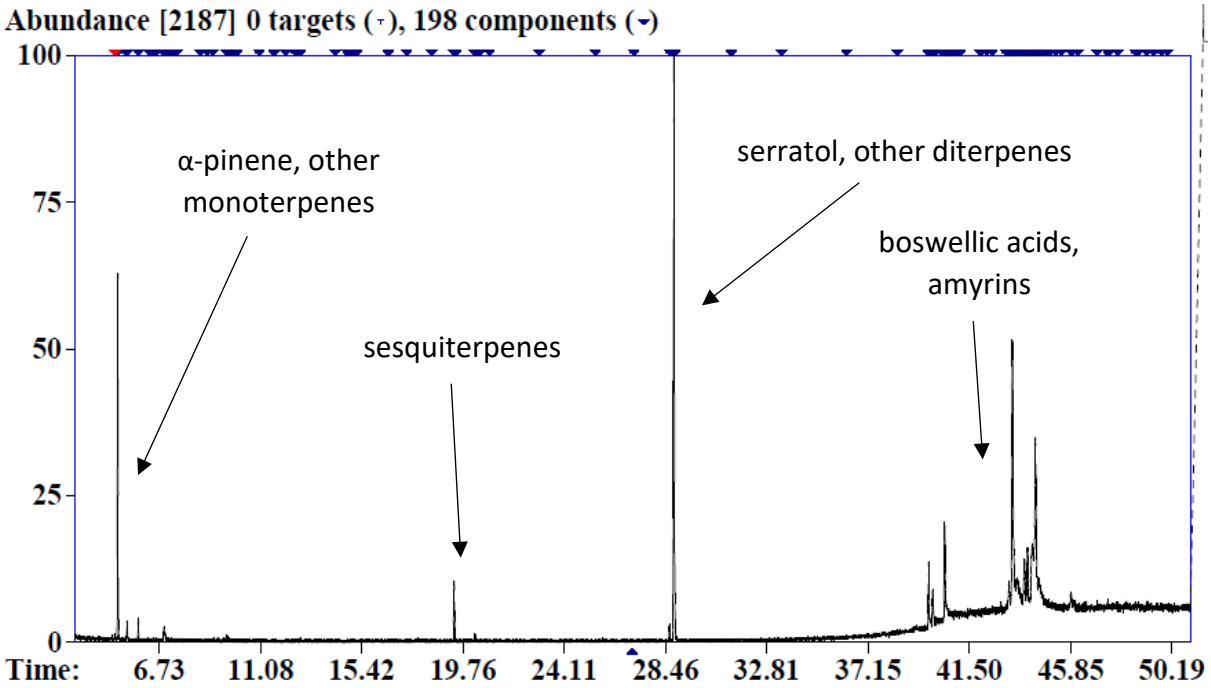



Figure 2.40. Chromatogram of an extract isolated via the chloroform maceration extraction of the Erigavo resin following the method in **Section 6.06**.

The major components of these extracts as determined by GC/MS analysis were α-pinene **25** (trace-13.4%), serratol **48** (31.5-46.3%), 24-norursa-3,12-diene **127** (5.1-10.7%), 24-norursa-3,12-dien-11-one **128** (16.4-20.9%) and α-amyrin acetate **129** (11.1-17.2%). The triterpene fraction was found to be rather inconsistent as well, with largely varying concentrations detected for amyrins and boswellic acids (figure 2.41). The methanol and chloroform extracts are quite unreliable for this fraction, with extract 3 showing only trace triterpenes other than the boswellic acids whereas 1 and 2 both show significant amyrin fractions. However, the diethyl ether and hexane extracts gave quite repeatable results in this case and produced oils of fairly concordant mass recoveries of oils compared to the resin mass.

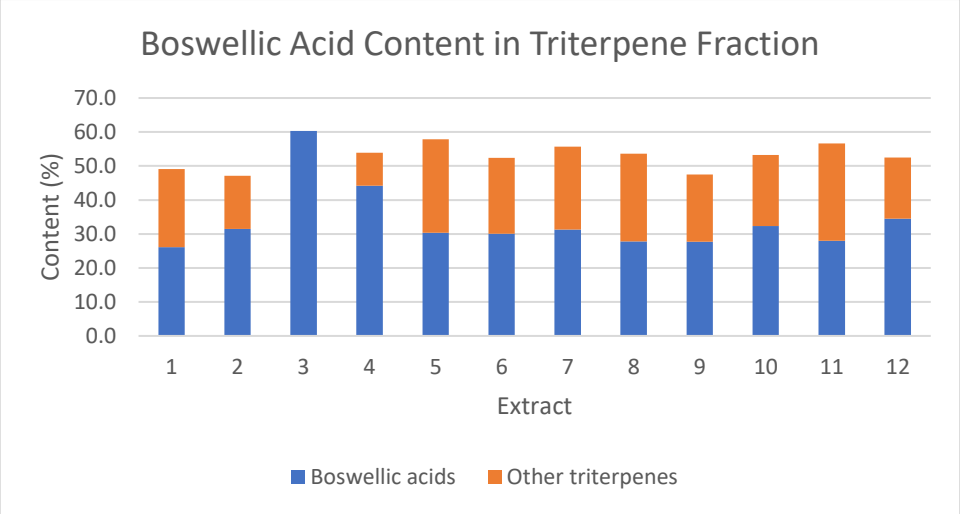



Figure 2.41. Boswellic acid content summary for extracts using methanol (1-3), chloroform (4-6), diethyl ether (7-9) and hexane (10-12). Content determined by GC/MS analysis.

### 2.2.4 Somaliland Resin

An interesting and possibly valuable trait of the Erigavo resin over the Gudmo Biyo Cas resin is the higher levels of serratol **48** detected on the chromatogram. Based on the weight of the extract compared to the resin mass, the Gudmo Biyo Cas resin has serratol levels of 6.0-10.9% whereas the Erigavo extracts had 14.9-31.5%. This is a significant difference, although it should be noted that these results do not mean that up to 31.5% of the Erigavo resin is made up of **48** because GC/MS gives poor estimates on heavy compounds due to their inability to enter the gas phase. Serratol however, is relatively volatile when compared to the heavier compounds in the oil, such as the boswellic acids and amyrins, so differences found in these compounds intensities on the chromatogram is useful.

To give an estimate on the quantity of **48** produced in this resin, the isolation experiments ran on the Gudmo Biyo Cas resin were repeated following the same method (**Section 6.12**). After extraction by maceration, removal of the acid fraction and purification by column chromatography, a yield of 6.6% of **48** when compared to the initial resin mass was collected. This is over 1.5 times higher than the levels in the Gudmo Biyo Cas resin, which is interesting as these 2 resins seem to be of the same species and grew in similar areas.

All three of the Somaliland resins have their own traits, with the North Coast sample being quite clearly from *Boswellia occulta* due to it producing the methoxydecane chemotype of essential oil. This fraction was also recovered in similar mass recoveries to the literature

data.[34] Further evidence is the large quantities of incensole extracted from this which was reported to make up a large part of the alcohol soluble fraction of this resin.[41] The other two resins have many similarities, both producing volatile fractions of the α-pinene chemotype. Furthermore, they both produce serratol in reasonable concentrations, which has been found to have anti-trypanosomal effects.[92] However, the Gudmo Biyo Cas resin produces a larger volatile fraction (7.3-7.9%) compared to the Erigavo resin (5.6-6.9%) whereas the Erigavo resin produces more serratol (6.6% compared to 4.4% for the Gudmo Biyo Cas). Apart from the higher levels of serratol, the Erigavo resin seemed to be worse than the Gudmo Biyo Cas resin, due to its unreliability in the production of consistent results for the alcohol soluble fraction and its lower essential oil fraction. It should be noted though that both resins gave fairly consistent oils in their mass recovery compared to the resin and their composition when subjected to hydrodistillation.

## 2.3 Sudanese Frankincense Composition

Four resins from Sudan were studied which were claimed to be *Boswellia papyrifera*. The resins each represented a different grade of the resin, with the highest grade being large pieces (above 6 mm diameter) which were generally pale in colour. The second grade were mostly smaller pieces (4-6 mm in diameter) but similar in colour and the third grade was smaller again (2-4 mm in diameter). The fourth grade had darker pieces of any size and contained some bark (figure 2.42). There is a fifth grade which was not studied in this work, but is made up of powder and granules less than 2 mm diameter mixed with bark.[93]

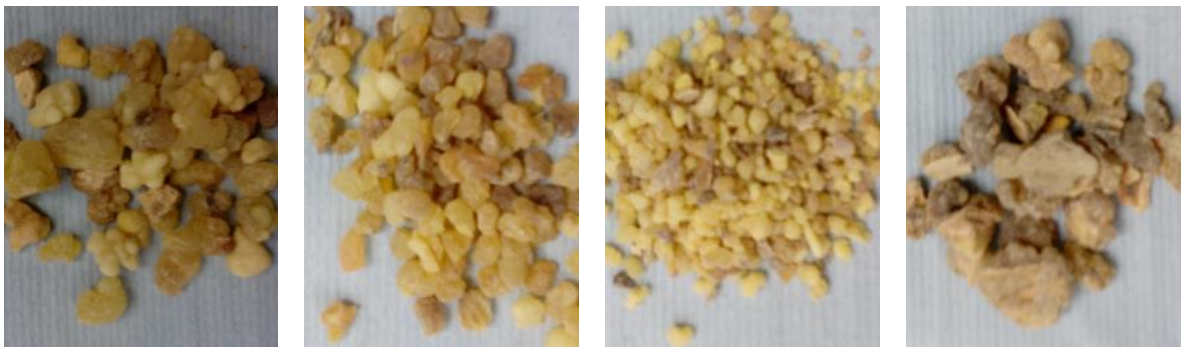


Figure 2.42. Sudanese Frankincense grades 1 (far left), 2 (middle left), 3 (middle right) and 4 (far right).

These resins were quite easy to mill due to their brittleness, which could be due to their low essential oil content. Each resin was hydrodistilled following the method in **Section 6.05**, at which point the samples had been exhausted. The resultant oils all were in relatively low mass

recoveries, with 1.7-2.0% for grade 1, 1.2-1.6% for grade 2, 1.9-2.0% for grade 3 and 1.2-1.4% for grade 4 when compared to the resin mass distilled. Furthermore, they all shared the octyl acetate chemotype and therefore are considered as Frankincense from *Boswellia papyrifera*. This species almost exclusively produces the octyl acetate chemotype in its essential oil, as discussed earlier and usually only produces small amounts of volatile oil. It is also known to have a strong diterpene fraction containing compounds such as incensole, incensole acetate and verticilla-4(20),7,11-triene.

When extracted by maceration experiments, quite large oils masses were isolated compared to the resin masses. Using solvents such as methanol, chloroform, diethyl ether and hexane as the extraction solvent gave oil yields of 48-71%, 44-69%, 31-74% and 55-60% for grades 1, 2, 3 and 4 respectively. The chemotype observed was similar for each, consisting mainly of incensole, incensole acetate and boswellic acids, mainly being *keto*-boswellic acids. However, unlike the other resins studied throughout this work, the *Boswellia papyrifera* extracts had no detectable amyrin content through GC/MS. This is an interesting observation and may have some significance to the resin's texture. For example, the Sudanese resins were quite brittle so were easy to mill a result. Therefore, it is possible that the stickiness of other resins, such as *Boswellia sacra, carterii* and *occulta* could be largely down to the presence of these amyrins.

### 2.3.1 Grade 1 Sudanese Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05**. As mentioned, the Grade 1 Sudanese Frankincense contained high levels of octyl acetate **50** (81-90%) in its essential oil (table 2.15 and figure 2.43). Furthermore, octanol **54** is also a major compound in this oil (5-6%), which will be produced through a similar biosynthetic route to octyl acetate. However, there are still terpenes such as those found in both the Omani and Somaliland resins. For example, limonene **33** (1.3-4.9%) and α-pinene **25** (0.7-1.1%) are very common across Frankincense resins and this sample is no exception, although in lower concentrations than most.

Table 2.15. Chemical profile of Grade 1 *Boswellia papyrifera* frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[56] |
|---|---|---|---|---|---|
| **25** | 932 | α-Pinene | High | 0.7-1.1 | 2.6 |
| **33** | 1030 | Limonene | High | 1.3-4.9 | 6.5 |
| **61** | 1053 | 1,8-Cineole | High | 0.3-0.6 | 2.2 |
| **54** | **1065** | **Octanol** | **High** | **5.3-6.2** | **8.0** |
| **-** | 1137 | Unknown Monoterpene | - | 0.4-0.9 | - |
| **50** | **1205** | **Octyl acetate** | **High** | **80.8-89.7** | **56.0** |
| **56** | 1950 | (*3E*)-Cembrene A | High | trace-0.5 | - |
| **58** | 2011 | Verticilla-4(20),7,11-triene | Medium | trace-1.7 | - |
| **49** | 2150 | Incensole | High | trace-0.7 | - |
| **53** | 2160 | Incensole acetate | High | trace-1.1 | - |
| **Mass Yield (%)[c]** | | | | **1.7-2.0** | **0.8** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled.

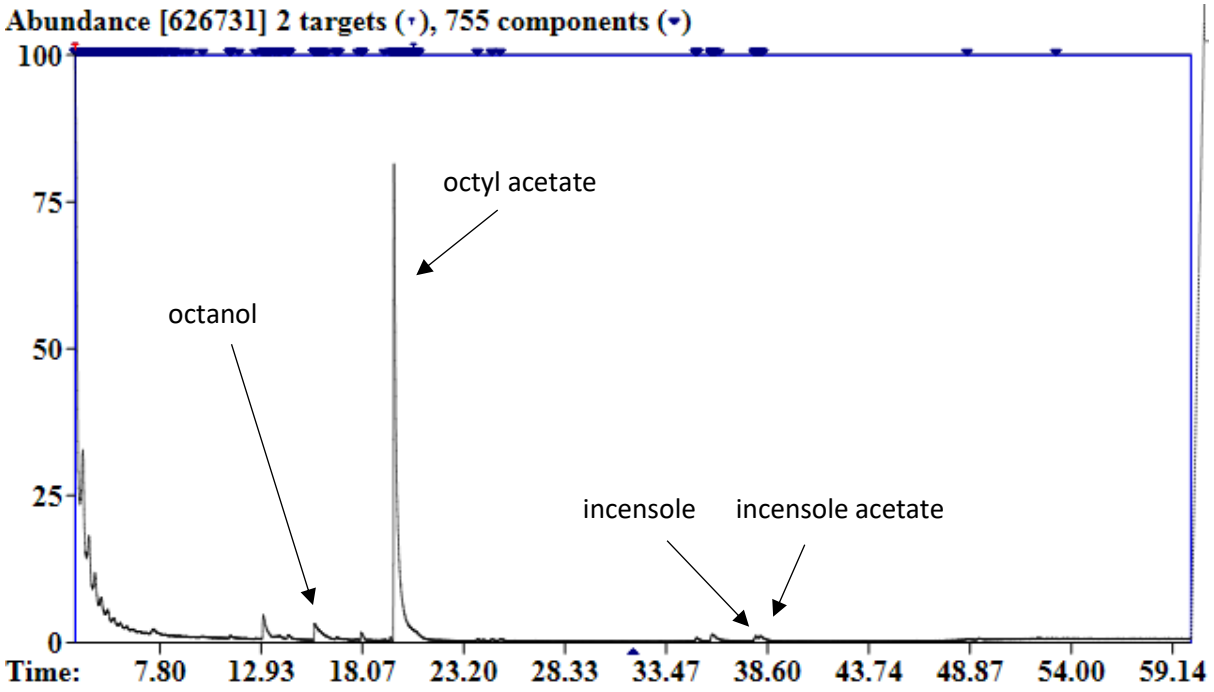



Figure 2.43. Chromatogram of the essential oil isolated via hydrodistillation of the grade 1 Sudanese resin following the method in **Section 6.05**.

With only around a 2% mass yield compared to the resin mass distilled, further work was required to uncover more information on the composition through solvent extractions.

Various solvents were used to extract the resin's oil via maceration, followed by filtration of the undissolved residue and evaporation of the filtrate to give thick, yellow oils following the method in **Section 6.06**. The use of different solvents resulted in different mass recoveries with 59-63%, 66-71%, 67-69% and 48-52% for methanol, chloroform, diethyl ether and hexane respectively (table 2.16).

Table 2.16. Chemical profiles of four separate maceration extraction experiments of the Grade 1 Sudanese resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **50** | 1203 | Octyl acetate | High | trace | trace | trace-1.9 | trace |
| **56** | 1950 | (*3E*)-Cembrene A | High | 1.5-1.7 | 1.1-1.6 | 1.2-1.9 | 0.9-1.1 |
| **-** | 1995 | Unknown Diterpene | - | 0.9-1.0 | 0.7-0.8 | 0.5-0.8 | 0.6 |
| **58** | 2010 | **Verticilla-4(20),7,11-triene** | **Medium** | **6.6-7.3** | **5.4-5.9** | **5.6-7.0** | **4.3-5.0** |
| **-** | 2039 | Unknown Diterpene | - | 0.9-1.1 | 0.5-0.8 | trace-0.8 | 0.5-0.6 |
| **-** | 2132 | Unknown Diterpene | - | 1.4-1.5 | 0.9-1.3 | 1.3-1.5 | 1.0-1.2 |
| **49** | **2144** | **Incensole** | **High** | **22.8-22.9** | **17.2-21.0** | **19.8-24.1** | **17.0-19.5** |
| **53** | **2160** | **Incensole acetate** | **High** | **20.7-22.1** | **17.9-19.9** | **18.6-22.4** | **16.3-17.5** |
| **154** | 2268 | Incensole acetate epoxide 1 | Low | trace | trace-0.4 | trace-0.4 | trace-0.3 |
| **155** | 2320 | Incensole acetate epoxide 2 | Low | trace | trace-0.2 | trace-0.4 | trace |
| **125** | **2999** | **24-Norursa-3,9(11),12-triene** | **High** | **3.7-3.9** | **4.4-5.6** | **2.5-4.6** | **5.9-6.4** |
| **126** | **3015** | **24-Noroleana-3,12-diene** | **High** | **4.4-4.6** | **4.1-5.5** | **2.7-4.6** | **5.9-6.7** |
| **127** | **3062** | **24-Norursa-3,12-diene** | **High** | **10.3-10.9** | **10.1-14.5** | **6.9-12.3** | **13.0-15.1** |
| **128** | **3309** | **24-Norursa-3,12-dien-11-one** | **High** | **23.8-24.5** | **28.2-29.6** | **27.8-29.5** | **29.3-30.3** |
| **Mass Yield (%)[c]** | | | | **59.0-63.0** | **65.7-71.0** | **66.7-68.7** | **47.5-51.5** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass extracted.

The general composition of these oils remained the same for each extract of this grade. An interesting difference for this sample compared to the other species discussed is the lack of amyrins detected, with the triterpene fraction being only made up of boswellic acids

according to the chromatograms (figure 2.44). This shows a strong diterpene fraction between around 25-30 minutes and a rich triterpene fraction between 39-44 minutes.

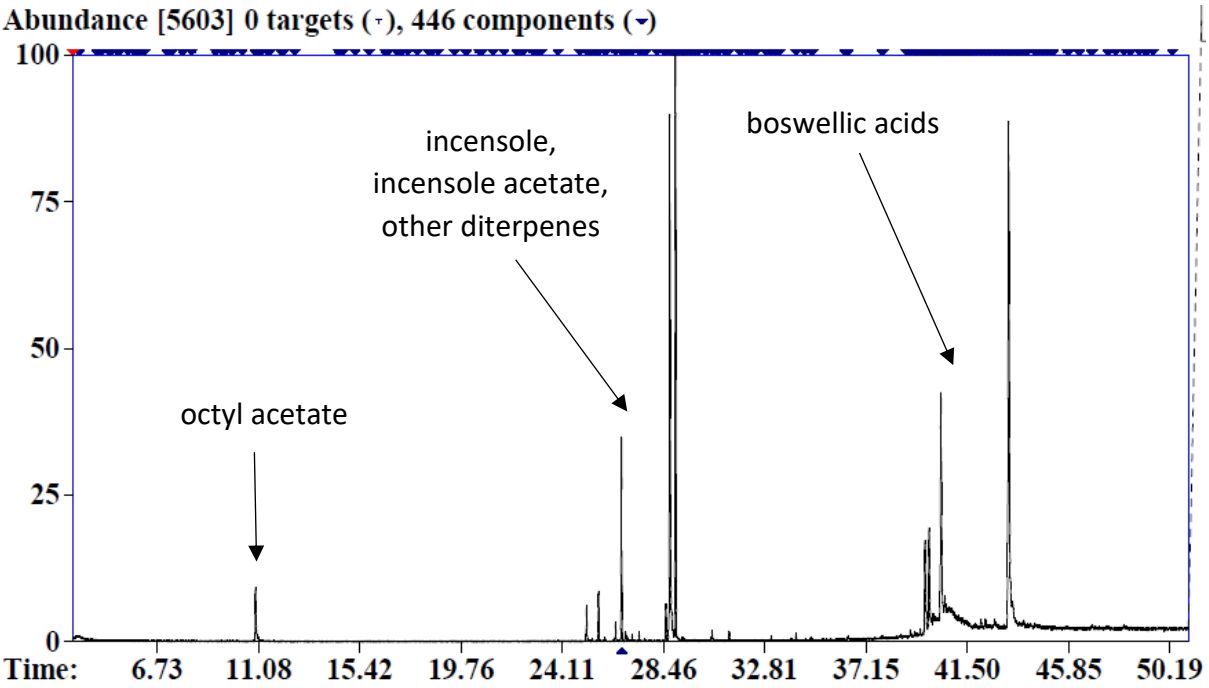



Figure 2.44. Chromatogram of an extract isolated via the chloroform maceration extraction of grade 1 Sudanese resin following the method in **Section 6.06**.

The major compounds found in these extracts were incensole **49** (17.0-24.1%), incensole acetate **53** (16.3-22.4%), 24-norursa-3,12-diene **127** (6.9-15.1%) and 24-norursa-3,12-dien-11-one **128** (23.8-30.3%). There was also a reasonable presence of verticilla-4(20),7,11-triene **58** detected (4.3-7.3%). Even when using different solvents of varying polarities, this fraction kept a fairly narrow range of concentrations of these compounds. When compared to the mass of the oil extracted to give an estimate on the size of the diterpene fraction, the chloroform and diethyl ether extracts showed the highest content, with 33.2-41.9% and 29.4-36.0% respectively. This is due to the higher extract mass, as the concentrations of the diterpenes remained similar throughout the extracts.

Incensole acetate's fragmentation pattern shows a parent ion peak at m/z = 348 (figure 2.45). According to the literature, cleavage of $C_3H_7$ gives rise to the peak at m/z = 305 and subsequent cleavage of $CH_3COOH$ allows for the loss of m/z = 60 giving the peak at m/z = 245. The peak at m/z = 288 is for the loss of $CH_3COOH$ directly from the parent ion.[91]

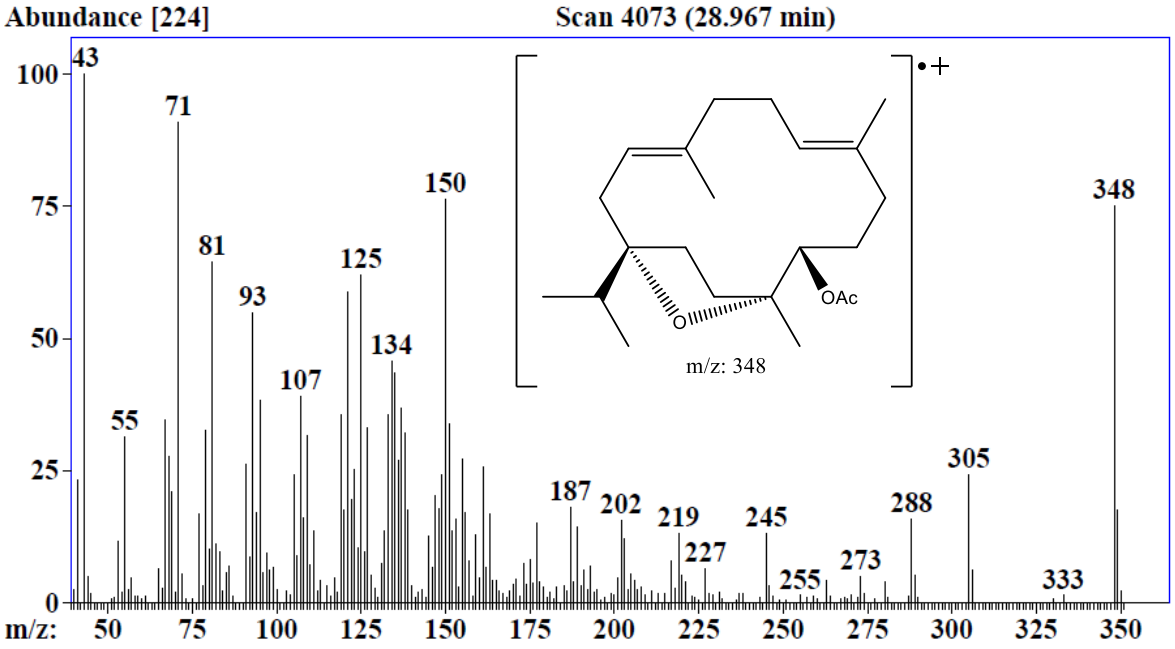



Figure 2.45. Fragmentation pattern of incensole acetate **53**, AI = 2160 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with chloroform following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[21]

Verticilla-4(20),7,11-triene was found as a major compound in these extracts, which was only previously detected in the North Coast, Somaliland resin in this study and was in small concentrations. The fragmentation pattern is quite similar to a lot of the cembrene type diterpenes that have nothing other than the core $C_{20}H_{32}$ structure. Its parent ion peak is detected at m/z = 272 and a very large peak at m/z = 257 shows the facile loss of a methyl which suggests it to be a tertiary methyl. A retro-Diels-Alder reaction stabilises this by opening up the 6-membered ring to give an acyclic diterpene (figure 2.46).[2,91]

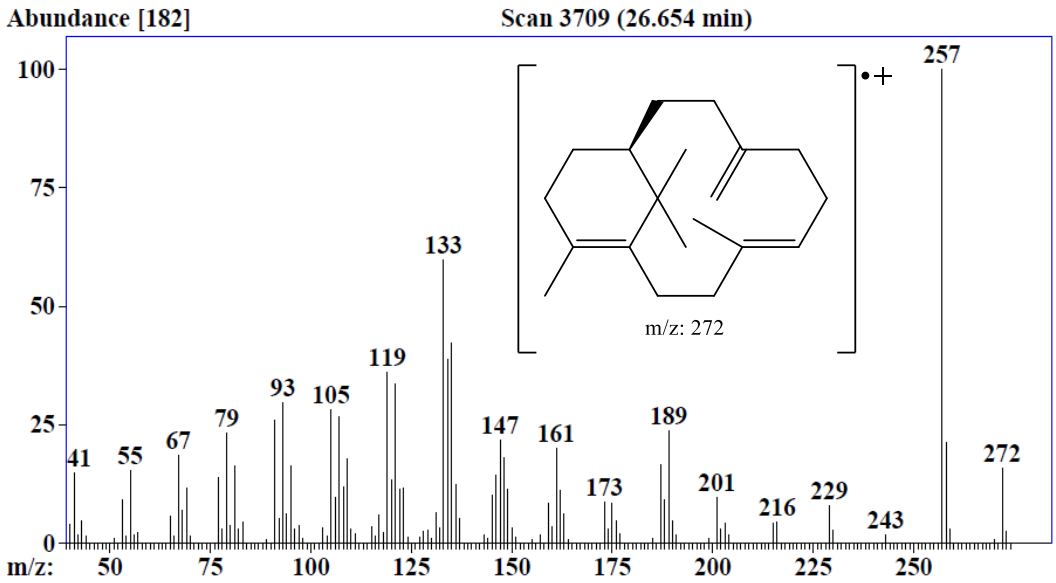



Figure 2.46. Fragmentation pattern of verticilla-4(20),7,11-triene **58**, AI = 2010 from the GC/MS analysis of an extract of grade 1 Sudanese resin, isolated through maceration with chloroform following the procedure in **Section 6.06**. Assignment confidence = medium. Literature comparison.[2]

As mentioned earlier, this grade of resin produced no detectable amyrins in the triterpene fraction and only found boswellic acid residue compounds. The β-keto-boswellic acids made up the largest part of this fraction (23.8-30.3%) followed by 11-unsubstituted β-boswellic acids (6.9-15.1%). The relative size of the triterpene fraction depended on the solvent used in the extraction. The hexane extracts were dominated by diterpenes whereas the methanol extracts had triterpenes as the majority of the composition. The diethyl ether and chloroform extracts were closer to a 50/50 split (table 2.17).

Table 2.17. Summary of the GC/MS determined content of extracts of grade 1 Sudanese Frankincense using different solvent. 1) Hexane; 2) Diethyl ether; 3) Chloroform; 4) Methanol.

| | **Content (%)** | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| **Diterpenes** | 57.0 | 48.9 | 53.0 | 43.6 |
| **Triterpenes** | 43.0 | 50.7 | 46.4 | 56.4 |

## 2.3.2 Grade 2 Sudanese Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** The Grade 2 Frankincense from Sudan gave a very similarly composed essential oil to Grade 1 for this species (table 2.18 and figure 2.47). With the oil being almost entirely

made up of octyl acetate and octanol (92-94%), only a small concentration was made up by the more common Frankincense oil compounds, such as α-pinene and limonene. A slightly lower mass of oil was yielded in this case however, with the mass making up a little less than 2% of the resin distilled. Like in the first grade's essential oil, incensole and incensole acetate were also both detected in low amounts (0-0.2%).

Table 2.18. Chemical profile of Grade 2 *Boswellia papyrifera* frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[56] |
|---|---|---|---|---|---|
| **25** | 932 | α-Pinene | High | 0.3-1.1 | 2.6 |
| **33** | 1032 | Limonene | High | 1.6-3.2 | 6.5 |
| **61** | 1051 | 1,8-Cineole | High | 0.6-1.1 | 2.2 |
| **54** | **1070** | **Octanol** | **High** | **7.1-9.6** | **8.0** |
| - | 1105 | Unknown Monoterpene | Medium | 0.4-0.6 | - |
| - | 1148 | Unknown Monoterpene | - | 0.6-0.7 | - |
| **50** | **1203** | **Octyl acetate** | **High** | **82.2-87.6** | **56.0** |
| - | 1418 | Unknown Monoterpene | - | 0.3-0.5 | - |
| **Mass Yield (%)[c]** | | | | **1.2-1.6** | **0.8** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled.

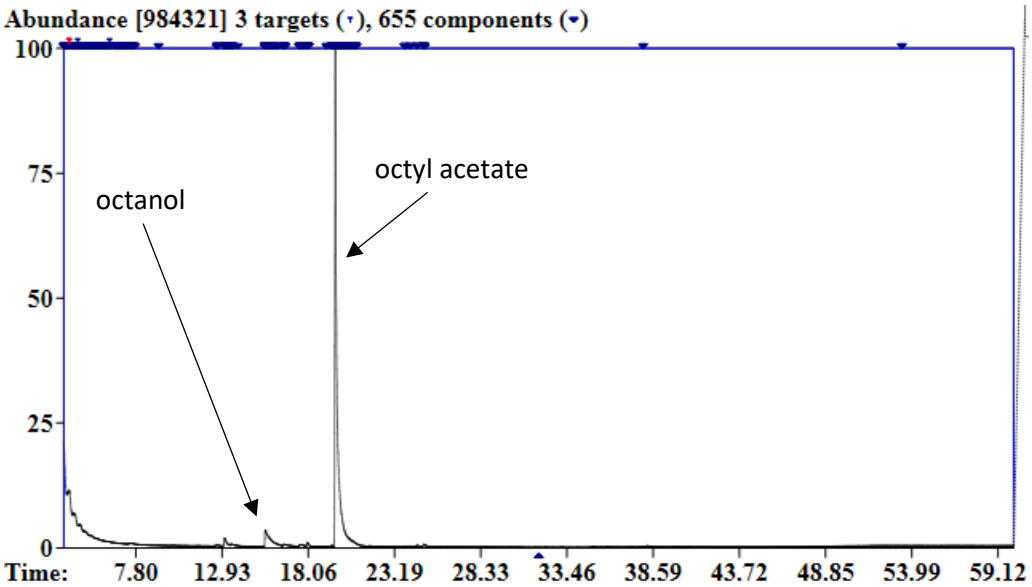



Figure 2.47. Chromatogram of the essential oil isolated via hydrodistillation of grade 2 Sudanese resin following the method in **Section 6.05**.

To compare this grade to the highest grade, solvent extractions were carried out to look at the resin fraction following the method in **Section 6.06**. These were again done by maceration, filtration and removal of the solvent as gave thick, gel-like yellow oils. The solvents used were methanol, chloroform, diethyl ether and hexane which gave mass recoveries of 63-65%, 60-68%, 64-69% and 44-47% respectively. These recoveries are similar to the highest grade, but the composition had some key differences (figure 2.48 & table 2.19).

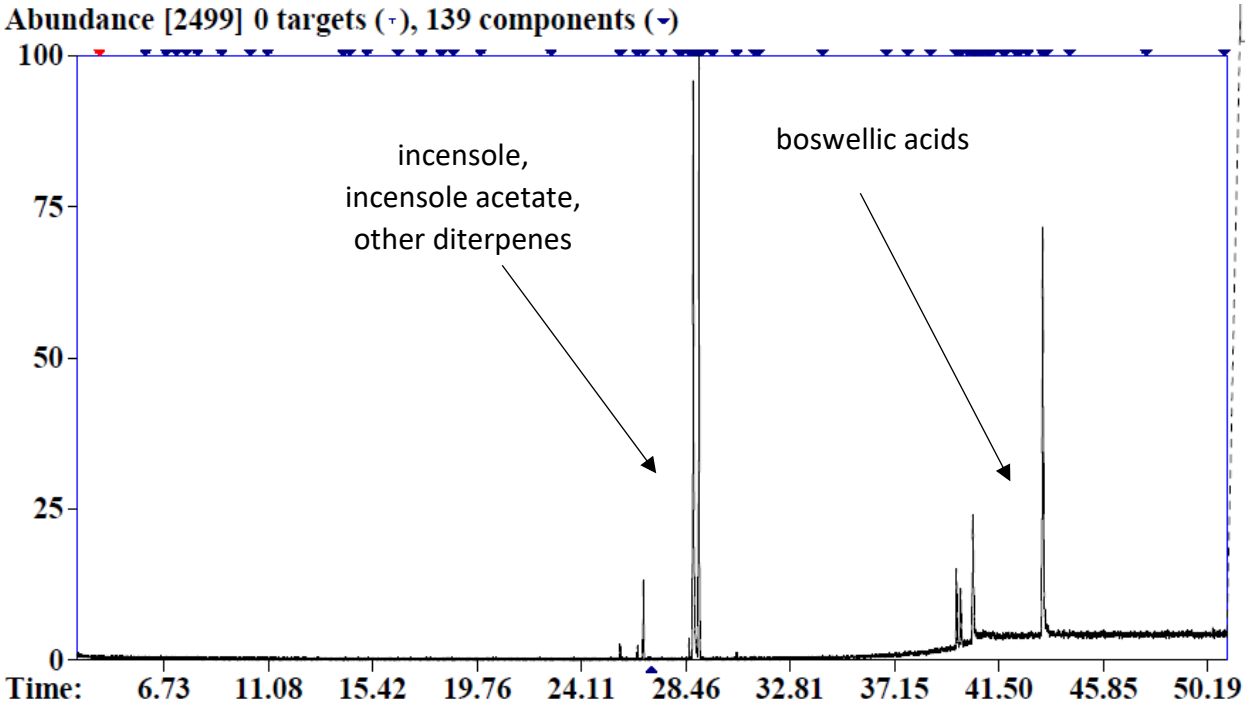



Figure 2.48. Chromatogram of an extract isolated via the chloroform maceration extraction of grade 2 Sudanese resin following the method in **Section 6.06**.

Table 2.19. Chemical profiles of four separate maceration extraction experiments of the Grade 2 Sudanese resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **54** | 1065 | Octanol | High | trace-0.1 | trace | trace | trace-0.6 |
| **50** | 1204 | Octyl acetate | High | trace-1.3 | trace | trace | trace-0.5 |
| **56** | 1950 | (*3E*)-Cembrene A | High | 0.8 | 0.4-0.7 | trace-0.6 | 0.5 |
| **-** | 1994 | Unknown Diterpene | - | 1.0-1.1 | 0.6-0.7 | 0.5-0.8 | 0.4 |
| **58** | 2010 | Verticilla-4(20),7,11-triene | Medium | 2.7-3.4 | 2.3-2.9 | 2.4-3.0 | 1.8-1.9 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **-** | 2132 | Unknown diterpene | - | 2.0-2.1 | 1.2-1.5 | 0.7-1.4 | 1.2-1.4 |
| **49** | **2145** | **Incensole** | **High** | **33.3-35.7** | **29.2-31.6** | **24.5-27.9** | **22.3-23.9** |
| **53** | **2160** | **Incensole acetate** | **High** | **22.6-24.5** | **22.7-23.4** | **22.1-25.5** | **14.9-16.6** |
| **154** | 2267 | Incensole acetate epoxide 1 | Low | 0.6 | trace-0.6 | trace-0.8 | 0.6-0.7 |
| **155** | 2319 | Incensole acetate epoxide 2 | Low | 0.2 | trace-0.3 | trace-0.3 | 0.3-0.4 |
| **124** | 2944 | 24-Noroleana-3,9(11),12-triene | High | trace-0.2 | trace | trace | 0.3-0.5 |
| **125** | 2999 | 24-Norursa-3,9(11),12-triene | High | 2.3-2.8 | 3.1-4.4 | 4.1-4.4 | 4.9-6.2 |
| **126** | 3015 | 24-Noroleana-3,12-diene | High | 2.3-3.0 | 2.3-3.7 | 2.6-4.1 | 5.7-6.1 |
| **127** | **3062** | **24-Norursa-3,12-diene** | **High** | **4.9-6.4** | **6.4-8.1** | **9.3-10.4** | **11.7-12.5** |
| **128** | **3310** | **24-Norursa-3,12-dien-11-one** | **High** | **21.2-22.2** | **26.6-27.2** | **25.9-27.3** | **31.0-33.3** |
| **Mass Yield (%)[c]** | | | | **63.0-65.3** | **59.6-68.0** | **64.0-69.0** | **44.0-47.5** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass extracted.

The chromatogram shows that the level of diterpenes found in this grade were quite high, especially for incensole **49** (22.3-35.7%) and incensole acetate **53** (14.9-25.5%). There was an observed decrease in the content of verticilla-4(20),7,11-triene **58** (1.8-3.4%) for this grade but overall, the diterpene fraction was significant for chloroform, diethyl ether and hexane extracts (53.7-68.7%). The methanol extracts were lower in diterpenes but still quite significant (42.6-45.5%). Although the levels of β-keto-boswellic acids remained similar, detected through the presence of 24-norursa-3,12-dien-11-one, the remaining boswellic acid fingerprint compounds showed a decrease when compared to the highest grade for the hexane and diethyl ether extracts (figures 2.49 and 2.50).

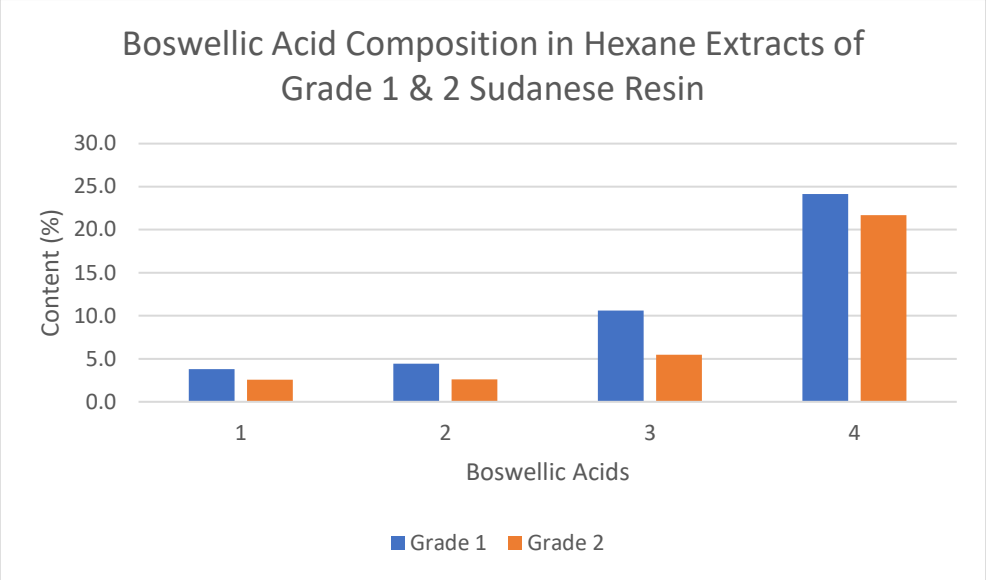



Figure 2.49. Boswellic acid comparison between the hexane extracts of the top two grades of Sudanese Frankincense. 1) 24-norursa-3,9(11),12-triene; 2) 24-noroleana-3,12-diene; 3) 24-norursa-3,12-diene; 4) 24-norursa-3,12-dien-11-one. Content estimated by GC/MS analysis.

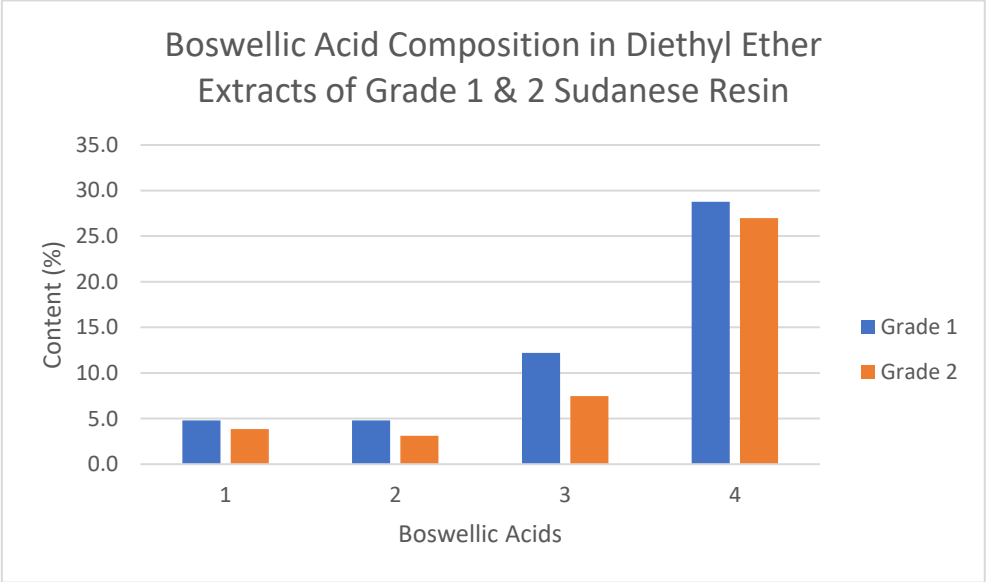



Figure 2.50. Boswellic acid comparison between the diethyl ether extracts of the top two grades of Sudanese Frankincense. 1) 24-norursa-3,9(11),12-triene; 2) 24-noroleana-3,12-diene; 3) 24-norursa-3,12-diene; 4) 24-norursa-3,12-dien-11-one. Content estimated by GC/MS analysis.

### 2.3.3 Grade 3 Sudanese Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** The grade 3 resin was the easiest to mill due to it being very brittle and in small pieces already. When distilled, the resultant oil was isolated in mass recoveries of around

2.0%. GC/MS analysis of these oils showed octyl acetate as the primary constituent (table 2.20 and figure 2.51). Once again, octyl acetate and octanol dominated the volatile oil's composition, but limonene was detected in lower concentrations. However, the diterpene verticilla-7(20),4,11-triene **58** was found as a major product.

Table 2.20. Chemical profile of Grade 3 *Boswellia papyrifera* frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[56] |
|---|---|---|---|---|---|
| **33** | 1033 | Limonene | High | trace-1.2 | 6.5 |
| **54** | **1070** | **Octanol** | **High** | **4.3-6.8** | **8.0** |
| **50** | **1200** | **Octyl acetate** | **High** | **84.4-86.5** | **56.0** |
| **-** | 1975 | Unknown diterpene | - | 0.7-0.8 | - |
| **58** | **2047** | **Verticilla-7(20),4,11-triene** | **Medium** | **3.9-4.6** | **-** |
| **49** | 2150 | Incensole | High | 0.3-0.6 | - |
| **53** | 2160 | Incensole acetate | High | 1.4-2.1 | - |
| **Mass Yield (%)[c]** | | | | **1.9-2.0** | **0.8** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled.

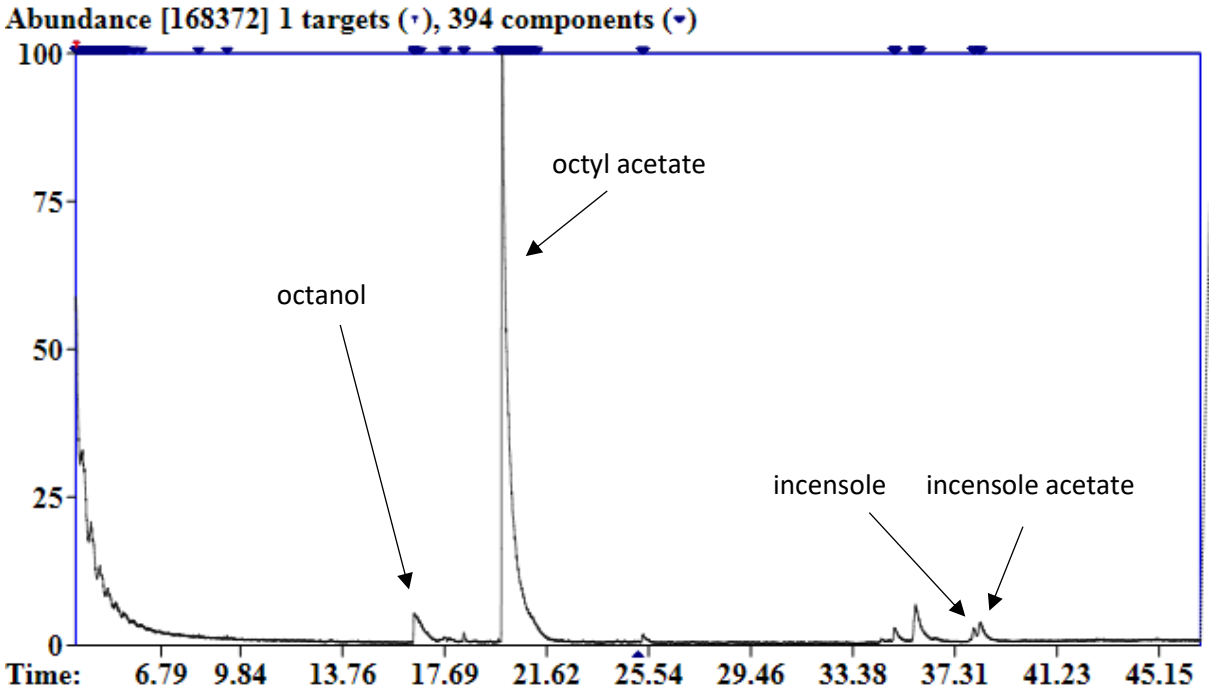



Figure 2.51. Chromatogram of the essential oil isolated via hydrodistillation of grade 3 Sudanese resin following the method in **Section 6.05**.

The majority of the chemical content in this oil was made up of octyl acetate, ranging from 84.4-86.5%. The content of octyl acetate and octanol combined made up over 90% of each of the grade 3 *Boswellia papyrifera* hydrodistilled samples, which is higher than was found in any other resin reported in this work. This oil also had incensole (0.3-0.6%) and incensole acetate (1.4-2.1%) in small concentrations, which can likely be extracted in much larger quantities using maceration or supercritical $CO_2$.

Maceration extractions in different solvents were carried out to study the non-volatile fraction of the resin following the method in **Section 6.06**. Using methanol, chloroform, diethyl ether and hexane, oils with mass recoveries of 57-66%, 68-74%, 64-68% and 31-35% respectively were isolated. The chromatogram shows a strong triterpene fraction, with a relatively weaker diterpene fraction, which explains why the hexane extracts are smaller for this resin (figure 2.52).

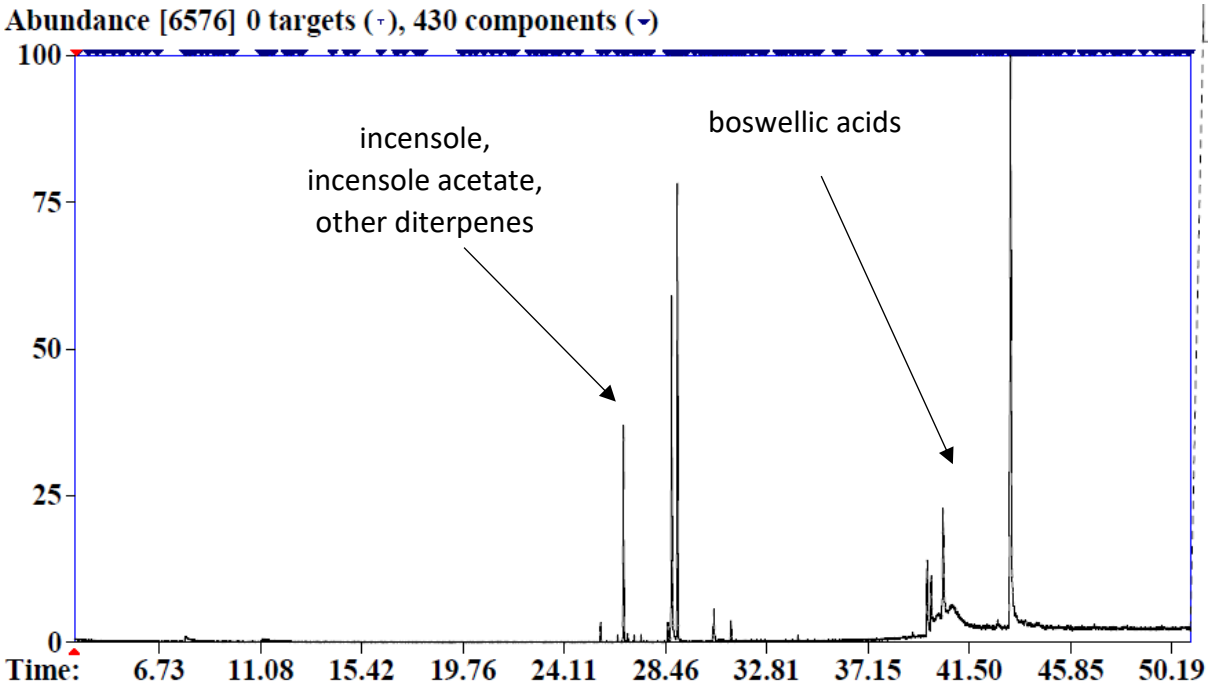



Figure 2.52. Chromatogram of an extract isolated via the chloroform maceration extraction of grade 3 Sudanese resin following the method in **Section 6.06**.

The levels for verticilla-4(20),7,11-triene **58** are quite high for these extracts (4.2-11.4%). Due to the relatively high amounts of this other diterpene, in addition to the fact this resin produces an overall, quite low diterpene fraction, the levels of incensole **49** (8.4-20.2%) and incensole acetate **53** (10.2-24.0%) are rather low compared to the other grades in this species.

However, the low mass recovery of the hexane extracts disguise this, with high concentrations of diterpenes in the resultant oil (table 2.21). Therefore, to give a better representation of the content extracted by each solvent, the concentrations need to be taken relative to the extract mass, to give an estimate for the content of each fraction (figure 2.53).

Table 2.21. Chemical profiles of four separate maceration extraction experiments of the Grade 3 Sudanese resin, each using a different solvent. 1) Methanol extract; 2) Chloroform extract; 3) Diethyl ether extract; 4) Hexane extract. Each experiment was performed in triplicate. Content estimated by GC/MS following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 |
| **54** | 1069 | Octanol | High | 0.1-0.2 | 0.2-1.4 | trace-1.5 | trace |
| **50** | 1209 | Octyl acetate | High | trace-0.2 | trace | trace | trace |
| **56** | 1948 | (*3E*)-Cembrene A | High | 1.0-1.1 | 0.6-0.7 | 0.7-0.8 | 0.4 |
| **-** | 1993 | Unknown diterpene | - | 0.5-0.6 | 0.3 | 0.3 | 0.1-0.2 |
| **58** | **2008** | **Verticilla-4(20),7,11-triene** | **Medium** | **10.2-11.4** | **6.8-7.4** | **7.8-9.0** | **4.2-4.7** |
| **-** | 2021 | Unknown diterpene | - | 0.7 | 0.4 | 0.4-0.5 | 0.2-0.3 |
| **-** | 2131 | Unknown diterpene | - | 1.2-1.3 | 0.8-0.9 | 0.8 | 0.5-0.6 |
| **49** | **2142** | **Incensole** | **High** | **17.7-20.2** | **12.8-14.6** | **14.3-15.1** | **8.4-9.6** |
| **53** | **2159** | **Incensole acetate** | **High** | **19.7-24.0** | **14.9-17.6** | **17.3-18.1** | **10.2-11.1** |
| **154** | 2265 | Incensole acetate epoxide 1 | Low | 0.9-1.1 | 1.0-1.8 | 1.5-1.7 | 1.0-1.4 |
| **155** | 2317 | Incensole acetate epoxide 2 | Low | 0.4-0.5 | 0.7-0.8 | 0.7-0.9 | 0.5 |
| **124** | 2942 | 24-Noroleana-3,9(11),12-triene | High | trace-0.2 | trace-0.3 | trace-0.2 | 0.6-0.7 |
| **125** | 2997 | 24-Norursa-3,9(11),12-triene | High | 3.1-4.1 | 4.2-5.0 | 3.7-4.4 | 7.3-8.7 |
| **126** | 3013 | 24-Noroleana-3,12-diene | High | 3.2-4.0 | 3.5-4.6 | 3.4-3.5 | 7.2-7.7 |
| **127** | **3060** | **24-Norursa-3,12-diene** | **High** | **7.7-7.8** | **8.5-10.8** | **6.6-10.5** | **15.1-16.3** |
| **128** | **3307** | **24-Norursa-3,12-dien-11-one** | **High** | **24.2-28.7** | **36.2-39.5** | **36.5-38.5** | **40.0-41.7** |
| **Mass Yield (%)[c]** | | | | **57.4-66.0** | **68.0-73.7** | **64.0-67.7** | **30.7-35.4** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass extracted.

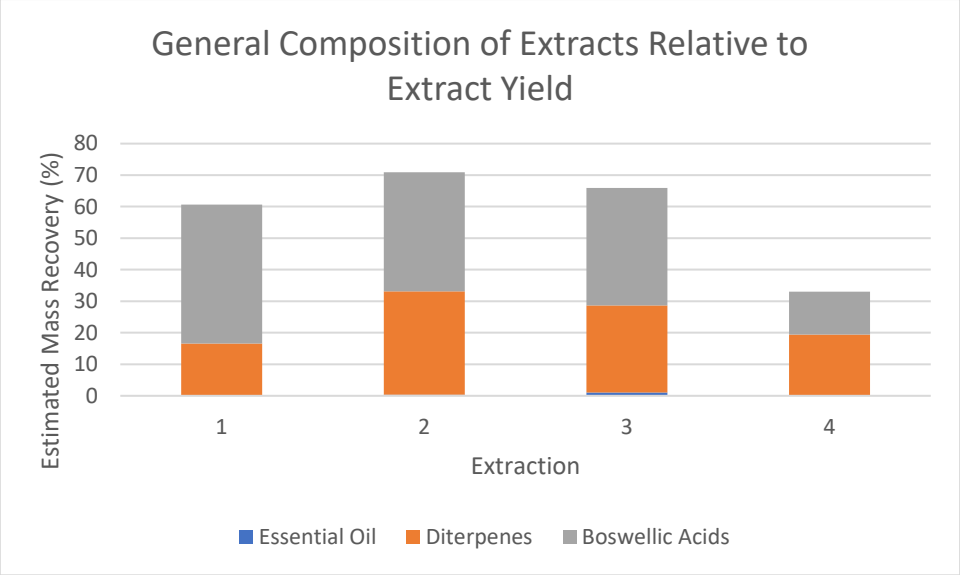



Figure 2.53. General oil composition from four separate maceration extractions using the grade 3 Sudanese Frankincense. Estimated mass recovery calculated based on mass recovery of oil and concentration of the different fractions. 1) Methanol extract; 2) chloroform extract; 3) diethyl ether extract; 4) hexane extract. Taken as averages over several experiments.

As shown in table 2.21 and figure 2.53, this grade of resin also contained high levels of triterpenes in the extracted oil. Again, the most abundant triterpenes are from the *keto*-boswellic acids, represented by the large detection of 24-norursa-3,12-dien-11-one in each extract (26.1-40.8%).

## 2.3.4 Grade 4 Sudanese Resin

Three hydrodistillation experiments were carried out for this resin following the method in **Section 6.05.** The grade 4 resin pieces from Sudan were larger than those of the grade 3 resin, however, its darker colour and the presence of more bark grades it as lower. When distilled, the oil yielded was a little over 1% of the resin's mass. Once again, the oil was dominated by octyl acetate and octanol (table 2.22 and figure 2.54). However, there was a significant increase in the levels diterpenes detected in this grade's oil, namely incensole acetate **53**.

Table 2.22. Chemical profile of Grade 4 *Boswellia papyrifera* frankincense essential oil, isolated through hydrodistillation. Carried out in triplicate. Some low content compounds omitted. Contents estimated by GC/MS following method (a) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | Literature Comparison (%)[56] |
|---|---|---|---|---|---|
| **33** | 1033 | Limonene | High | 0.3-3.8 | 6.5 |
| **46** | 1044 | (*E*)-β-Ocimene | High | 1.8-4.1 | 2.0 |
| **54** | **1070** | **Octanol** | **High** | **6.3-8.4** | **8.0** |
| **-** | 1122 | Unknown Monoterpene | Medium | trace-0.9 | - |
| **50** | **1205** | **Octyl acetate** | **High** | **54.3-74.1** | **56.0** |
| **55** | 1917 | Cembrene | High | trace-0.7 | - |
| **56** | 1950 | (*3E*)-Cembrene A | High | 1.1-3.4 | - |
| **58** | **2046** | **Verticilla-7(20),4,11-triene** | **Medium** | **2.4-3.4** | **-** |
| **49** | **2150** | **Incensole** | **High** | **2.1-6.0** | **-** |
| **53** | **2160** | **Incensole acetate** | **High** | **3.6-10.4** | **-** |
| **125** | 3005 | 24-Norursa-3,9(11),12-triene | High | 0.4-0.7 | - |
| **127** | 3070 | 24-Norursa-3,12-diene | High | 0.6-1.3 | - |
| **128** | 3320 | 24-Norursa-3,12-dien-11-one | High | 1.2-1.4 | - |
| **Mass Yield (%)[c]** | | | | **1.2-1.4** | **0.8** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass distilled.

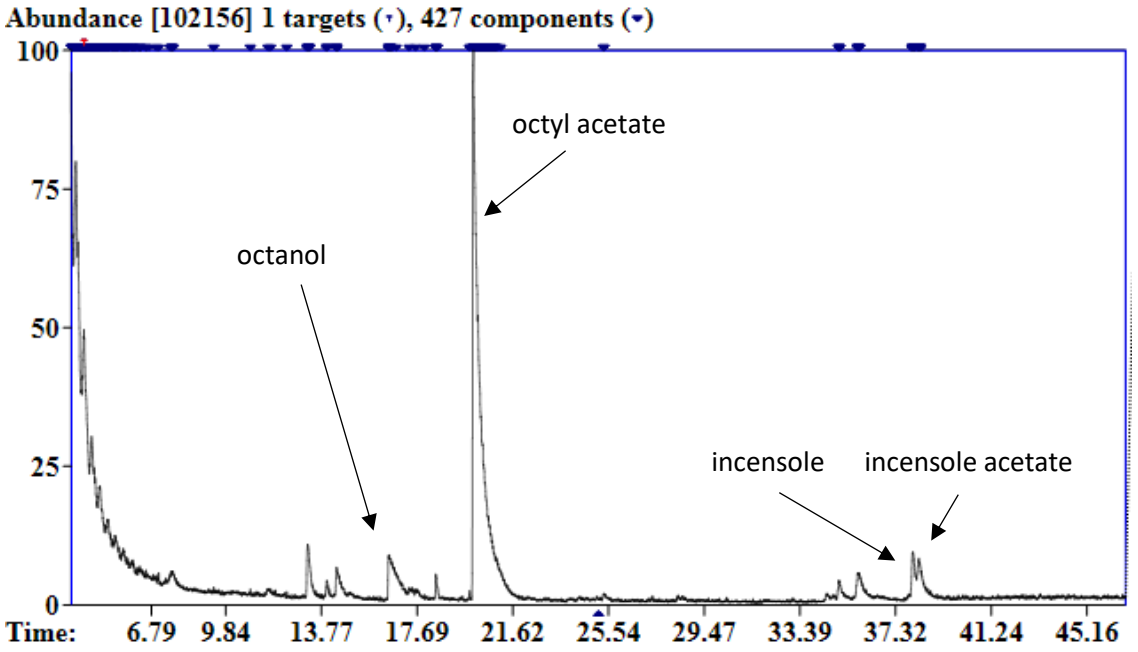



Figure 2.54. Chromatogram of the essential oil isolated via hydrodistillation of grade 4 Sudanese resin following the method in **Section 6.05**.

These oils showed high octyl acetate content (54.3-74.1%), as did the other *Boswellia papyrifera* samples. In addition, high levels of incensole (2.1-6.0%), incensole acetate (3.6-10.4%) and verticilla-7(20),4,11-triene (2.4-3.4%) were observed which shows that this grade produces a large diterpene fraction.

This grade of resin was also extracted using chloroform several times, giving a range of mass recoveries when compared to the resin mass (55-60%) following the method in **Section 6.06**. This recovery of oil is lower than the other grades studied for this resin. The chromatogram shows a very strong diterpene fraction which is promising for the isolation of compounds such as incensole and incensole acetate (figure 2.55).

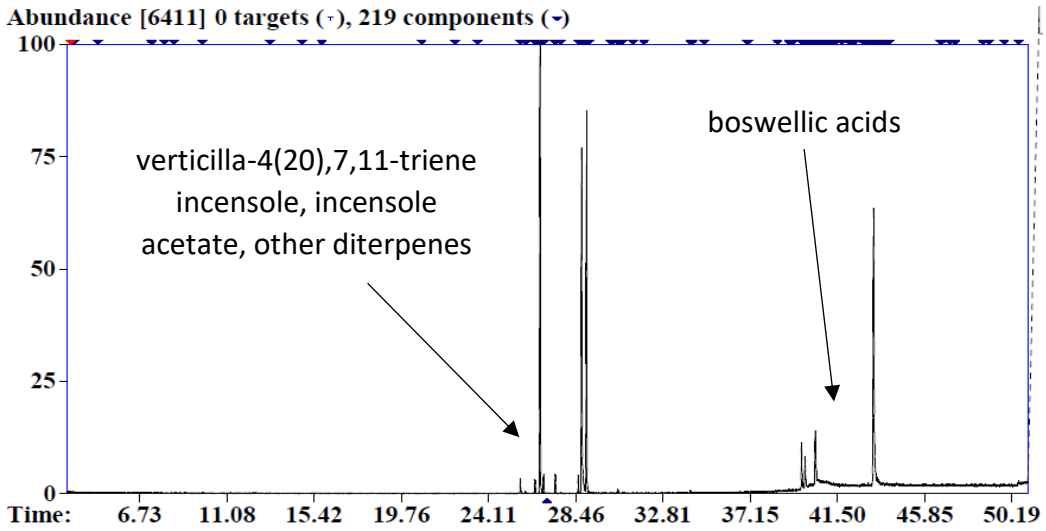



Figure 2.55. Chromatogram of the grade 4 Sudanese resin's solvent extract.

There is, however, the impurity of vertcilla-4(20),7,11-triene (18.2-22.1%), which was detected here in the largest quantities found in these resins. The diterpene fraction made up around 67% of the oil on average, with the remaining composition being from the boswellic acids, with trace essential oil content (table 2.23).

Table 2.23. Chemical profile of the chloroform maceration extract of the Grade 4 Sudanese resin. Carried out in triplicate. Content estimated by GC/MS following method (b) in **Section 6.02**. Some low content compounds have been omitted.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) |
|---|---|---|---|---|
| **56** | 1950 | (*3E*)-Cembrene A | High | 0.7-0.8 |
| **-** | 1994 | Unknown Diterpene | - | 0.7-0.8 |
| **58** | **2010** | **Verticilla-4(20),7,11-triene** | **Medium** | **18.2-22.1** |

| | | | | |
|---|---|---|---|---|
| **-** | 2022 | Unknown diterpene | - | 1.3-1.5 |
| **-** | 2058 | Unknown diterpene | - | 0.9-1.1 |
| **-** | 2132 | Unknown diterpene | - | 1.0-1.6 |
| **49** | **2144** | **Incensole** | **High** | **19.1-22.8** |
| **53** | **2160** | **Incensole acetate** | **High** | **17.3-19.4** |
| **154** | 2267 | Incensole acetate epoxide 1 | Low | 0.2-0.6 |
| **155** | 2319 | Incensole acetate epoxide 2 | Low | trace-0.2 |
| **124** | 2944 | 24-Noroleana-3,9(11),12-triene | High | 0.1 |
| **125** | 2999 | 24-Norursa-3,9(11),12-triene | High | 2.0-3.2 |
| **126** | 3015 | 24-Noroleana-3,12-diene | High | 2.2-2.5 |
| **127** | 3062 | 24-Norursa-3,12-diene | High | 3.7-5.8 |
| **128** | **3309** | **24-Norursa-3,12-dien-11-one** | **High** | **22.9-23.9** |
| **Mass Yield (%)[c]** | | | | **54.9-60.0** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass extracted.

This is a quite low boswellic acid content compared to the other grades of this resin, which shows how large the diterpene fraction is (figure 2.56). However, it should be noted that the relative amount of the extracts from this grade was also the lowest, meaning that a larger concentration of diterpenes in the extract was less significant per gram of resin than the other resins.

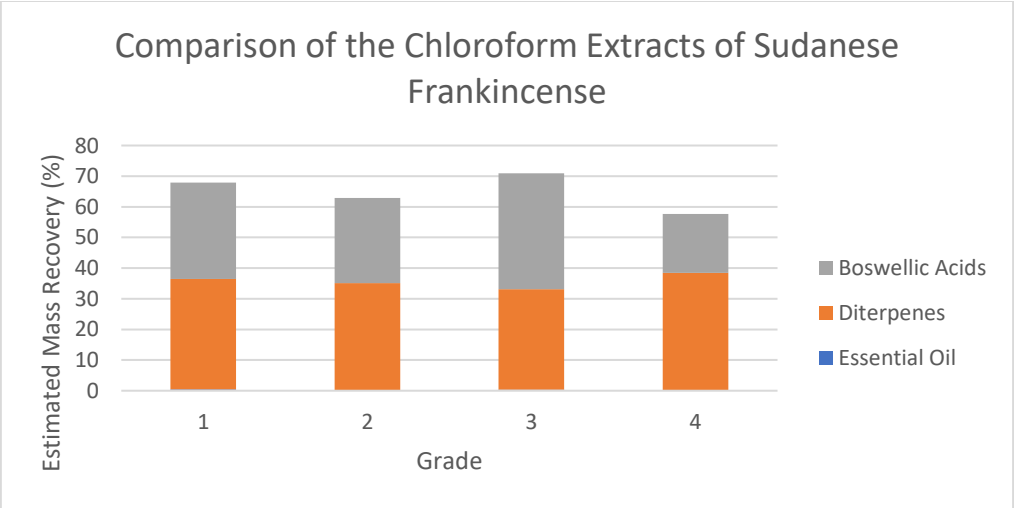



Figure 2.56. General composition of the chloroform extracts of grades 1-4 Sudanese Frankincense. Estimated mass recovery based on concentration of species compared to the mass recovery of the oil.

## 2.3.5 Sudanese Resin

This species of Frankincense is quite unique with regards to its chemical composition. Its volatile fraction is composed mainly of octyl acetate, a completely different type of compound to most species. Something in the biosynthesis clearly prioritises the formation of non-isoprene units for the bulk of the volatile oil. However, the resin is largely made up of cembrene type diterpenes and ursane and oleanane type triterpenes meaning that the majority of the products in the resin are created through the standard isoprene rule type additions. The common major compounds found in the oils isolated by distillation or solvent extraction are summarised (figure 2.57).

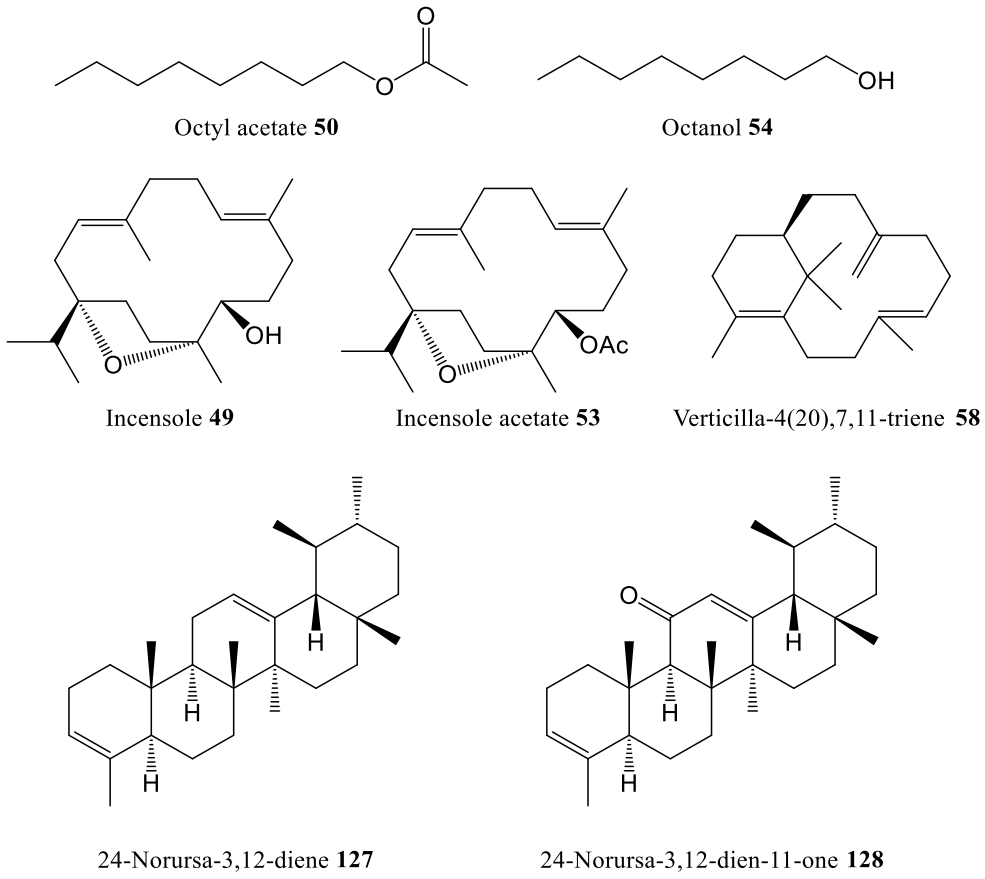



Figure 2.57. Common major products found in either the distillate of solvent extract of Sudanese frankincense.

Overall, the chloroform extracts seemed to have the highest concentration of diterpenes, except in the case of the diethyl ether extracts of the grade 2 resin (table 2.24). Despite having the largest diterpene fraction overall, the grade 4 resin detected relatively low concentrations of incensole and incensole acetate. This is due to the fact it had very high levels of verticilla-4(20),7,11-triene, which inflated the diterpene fraction. Grade 2 seems to produce the largest

amount of these key diterpenes according to GC/MS analysis. To give a better indication of the content of these compounds, isolation of high purity oils was necessary, which is discussed in **Chapter 4**. Interestingly, there were no amyrins detected in the Sudanese resins, which was found in all the other resins studied in this work. This lack of amyrins could potentially be used as a biomarker for the solvent extracts of these resins.

Table 2.24. Highest levels of incensole and incensole acetate found in Sudanese resin grades 1-4. 1) Grade 1 – chloroform extracts; 2) grade 2 – diethyl ether extracts; 3) grade 3 – chloroform extracts; 4) grade 4 – chloroform extracts. Content estimated by GC/MS analysis.

| **Content (%)** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| **Incensole** | 13.3-17.1 | 18.7-20.5 | 10.1-10.7 | 11.5-12.5 |
| **Incensole Acetate** | 12.4-15.9 | 14.6-15.7 | 12.2-12.9 | 10.1-11.6 |

# Chapter 3. Supercritical $CO_2$ Extractions

Various sets of extractions were carried out using supercritical $CO_2$ which allowed for the isolation of fractionated oils. This was done by adjusting the parameters in the extraction chamber were adjusted throughout the extraction and samples were typically collected every 30 minutes. The extraction vessels, which in the following experiments were either 100 mL or 2 L in size, were filled with finely ground mixtures of frankincense and a solid support, such as sand or basic alumina. The $CO_2$ flow rates were up to 10 g/min for the 100 mL extractor and up to 40 g/mL for the 2 L extractor, so the method had to be adapted for experiments that were scaled up to the 2 L extractor. All trials began at the 100 mL extractor scale, as to not waste resources while the experiments were fine tuned. Due to the complex nature of the compositions of the resins in this study, many trials were carried out, as discussed in **Sections 3.1-3.3**.

The Gudmo Biyo Cas resin, which was identified as *Boswellia carterii* was found to be high in essential oil from the hydrodistillation experiments earlier. Since hydrodistillation uses high temperature which can harm the compounds of interest, a milder system was tested. $CO_2$ extraction can be carried out at lower temperatures than standard distillation techniques, so a system was designed to extract this fraction using mild temperature and pressures. Care was required as increasing the pressure also increases the solvent power of the $CO_2$, which results in the undesired heavier oils being extracted alongside the essential oils.

Since the isolation of incensole and incensole acetate was an aim of this project, the four grades of *Boswellia papyrifera* and the *Boswellia occulta* resin were investigated. Due to the fractionation possibilities of a $CO_2$ extraction system, the essential oils were first extracted using mild pressures (c.a. 70-90 bar) before the pressure was increased up to c.a. 300 bar to strip the majority of the diterpene content. This also coelutes some of the triterpene matter, so frequent analysis of the resultant oils was required using GC/MS to give an extraction profile. The remainder of the triterpenes were also extracted by adding IMS as a co-solvent.

## 3.1 $CO_2$ Extractions on Gudmo Biyo Cas Resin

Due to its high essential oil content, the Gudmo Biyo Cas resin was taken forward for $CO_2$ trials. The resin has to be packed with a solid support in order to give adequate contact time

with $CO_2$ resulting in efficient extraction. Sand is often used as it is cheap, inert and aids dense packing. Trials were carried out using different ratios of sand to frankincense resin to see how it affected the yield and composition of the resultant extract. In these trials, a relatively low pressure was used in the extraction vessel in order to extract mainly essential oil content. Using too high a pressure results in too strong a solvent power, which will isolate more of the heavy oils. To compare the extraction efficiency to purity of the essential oil, pressures between 90 bar and 110 bar were used in this section using variations on method (a) described in **Section 6.08**. The results of extract mass using different packing ratios at 90 bar pressure are summarised below (table 3.1).

Table 3.1. $CO_2$ extractions of Gudmo Biyo Cas resin to isolate the essential oil fraction using different ratios of solid support. Conditions – 90 bar pressure, 40°C, 10g/min $CO_2$ flow rate, 30 minutes, 100mL extractor. Mass recovery is the oil mass relative to the resin mass.

| Resin/Sand | Mixture Packed (g) | Resin Packed (g) | Mass Recovery (%) |
|---|---|---|---|
| **1/3** | 105.9 | 26.3 | 4.5 |
| **1/2** | 96.5 | 32.2 | 7.1 |
| **1/1** | 82.2 | 41.1 | 4.5 |
| **2/1** | 75.2 | 50.1 | 1.3 |

The table clearly shows using a resin to sand ratio of 1 to 2 is the most efficient in terms of mass recovery. The issue with using low sand to resin ratios is poor contact time with the resin. This means the resin is not able to be fully extracted despite the $CO_2$ having significant solvent power as it passes through the matrix too quickly. At too high a ratio of sand to resin, a lower mass recovery is observed again. As the table shows, there a quite a large consequence of adding a solid support with regards to the amount of resin that can be added to the extractor. The best results came from processing 32.2 g of resin, as it was mixed with twice that mass in sand. One experiment used 50.1 g of resin, which is quite a lot more than the best experiment, but it is important to consider how much material is being processed per run, as on the larger scale, this could cause an increase in process time as potentially more extractions need to be carried out.

Next, trials were ran to find the optimal pressure and temperature for extracting the essential oil. Small amounts of lower volatility compounds also get extracted in the supercritical $CO_2$ extraction of the resin. This is because the $CO_2$ is able to partially extract some of these

triterpenes, even though it is relatively non-polar as a solvent. Furthermore, since the extractor has resin all the way from where the $CO_2$ enters to the end of the column, small amounts at the far end of the column, where the $CO_2$ has passed through will get extracted before some of the essential oil has as parts of the column have different lengths to travel through the column. This could be avoided more by using a blank of a solid support at the exit side of the column to hold on to more of the slower moving triterpenes. The problem with this though is it dramatically reduces the amount of resin that can be packed into the column, so it seemed best to exhaust the essential oil and distil it afterwards to give a purer essential oil than the $CO_2$ extract.

Extractions were carried out with a standard flow rate of 10 g/min of $CO_2$ for 30 minutes each in a 100 mL extractor. Six replica extractions were carried out at 90 bar and 40°C, four at 100 bar and 40°C, three at 100 bar and 50°C and four at 110 bar and 40°C. The extracts were sampled from the collector after the 30 minutes, weighed and then 5 mg were taken in heptane for GC/MS analysis (table 3.2).

Table 3.2. Chemical profile of several sets of separate $CO_2$ extraction trials on Gudmo Biyo Cas resin. Contents estimated by GC/MS following method (b) in **Section 6.02**. Conditions are as stated in table 2.26.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 90 bar, 40°C | 100 bar, 40°C | 100 bar, 50°C | 110 bar, 40°C |
| **148** | 923 | Tricyclene | High | 0.5-0.6 | 0.6-0.7 | 0.6-1.0 | 0.6-0.8 |
| **25** | **936** | **α-Pinene** | **High** | **33.6-42.3** | **38.0-41.1** | **40.9-46.9** | **37.7-41.5** |
| **26** | 953 | Camphene | High | 0.7-0.9 | 0.6-0.7 | 0.7-0.8 | 0.6-0.8 |
| **27** | 975 | Sabinene | High | 3.1-5.3 | 2.4-2.7 | 2.6-3.0 | 2.2-2.4 |
| **28** | **981** | **β-Pinene** | **High** | **5.5-8.6** | **4.6-5.2** | **5.2-6.2** | **6.0-8.4** |
| **29** | 990 | Myrcene | High | 3.6-4.5 | 4.2-4.6 | 4.9-5.6 | 4.0-5.9 |
| **30** | 1010 | α-Phellandrene | High | 0.6-0.7 | 0.6-0.7 | 0.7-0.8 | 0.4-0.6 |
| **32** | 1027 | *p*-Cymene | High | 0.9-1.0 | 0.8-0.9 | 1.0-1.1 | 0.6-1.6 |
| **33** | 1032 | Limonene | High | 3.1-6.4 | 3.7-7.0 | 3.6-4.6 | 1.9-2.6 |
| **149** | 1036 | (*Z*)-β-Ocimene | High | 1.1-1.2 | 1.0-1.1 | 1.3-1.5 | 1.0-1.4 |
| **151** | 1114 | *p*-1,3,8-Menthatriene | Medium | 1.7-2.0 | 1.2-1.5 | 1.4-1.7 | 1.2-1.6 |
| **-** | 1149 | Unknown Monoterpene | - | 1.6-1.9 | 1.1-1.5 | 1.7-2.1 | 1.0-1.5 |
| **134** | 1200 | Myrtenol | Medium | 0.8-1.0 | 0.6-0.7 | 0.7-0.9 | 0.5-0.7 |
| **37** | 1210 | Verbenone | High | 4.1-4.9 | 3.7-4.3 | 5.7-6.3 | 1.9-3.1 |
| **141** | 1287 | Bornyl acetate | High | 1.2-1.6 | 0.8-1.0 | 1.5-1.7 | 0.8-1.0 |
| **40** | 1425 | Caryophyllene | High | 0.7-0.8 | 0.7-0.8 | 1.1-1.3 | 1.1-1.5 |
| **150** | 1445 | 6,9-Guaiadiene | Medium | 0.6-1.2 | 1.1-1.4 | 1.6-2.0 | 0.7-1.1 |
| **41** | 1461 | α-Humulene | Medium | trace-0.5 | 0.4-0.7 | 0.6-0.7 | 0.5-0.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **156** | 1518 | γ-Cadinene | Medium | 0.9-2.0 | 0.8-0.9 | 1.2 | trace-1.1 |
| **43** | 1520 | δ-Cadinene | High | trace-1.2 | 1.1-1.2 | 1.4-1.6 | 1.3-2.3 |
| **152** | 1647 | epi-α-Cadinol | Medium | 1.5-1.8 | 1.3-1.7 | 1.4-1.7 | 1.6-1.9 |
| **-** | 2020 | Unknown Diterpene | - | 0.6-0.8 | 0.4-0.5 | trace-0.3 | 0.9-1.2 |
| **-** | 2143 | Unknown Diterpene | - | 0.5-0.7 | 0.6-0.7 | trace-0.3 | 0.7-0.9 |
| **48** | **2155** | **Serratol** | **High** | **9.0-11.9** | **12.3-14.1** | **3.9-8.2** | **13.1-14.4** |
| **-** | 3358 | Unknown Triterpene | - | trace-0.8 | 0.5-0.9 | trace-0.5 | 0.6-0.8 |
| **-** | 3367 | Unknown Triterpene | - | trace-0.9 | 0.7-1.1 | trace-0.7 | 0.6-1.3 |
| **-** | 3376 | Unknown Triterpene | - | trace-0.9 | 0.8-1.4 | trace-1.3 | 0.6-1.6 |
| **-** | 3391 | Unknown Triterpene | - | 1.4-3.0 | 2.0-3.4 | trace-1.9 | 1.7-3.7 |
| **Mass Yield (%)[c]** | | | | **5.1-7.0** | **7.7-9.1** | **3.1-4.1** | **8.4-9.1** |

a) Arithmetic index calculated using equation 1.3.
b) Assignment based on combined data mentioned in **Section 6.02**.
c) Based on the oil mass isolated relative to the resin mass extracted.

The highest yields of oil came from 100 bar at 40°C and 110 bar at 40°C. These both gave very similar chemical profiles with the 2 major compounds, α-pinene and serratol, having very similar concentrations (37.7-41.5 and 12.3-14.4 respectively). Increasing the temperature at 100 bar saw a decrease in the mass recovery of oil, which is due to the density of the $CO_2$ being decreased as an effect of the temperature change. At higher pressures, where $CO_2$ density is less affected by temperature, an increase in temperature would improve the solvent power.

These conditions were used as a guide when scaling up to the 2 L extractor, which allowed for the extraction of much more oil per run. Furthermore, the shape of the extractor and the flow rate of $CO_2$ more accurately represents the extraction on the pilot plant. Since the maximum flow rate available for the 2 L extractor was 40 g/min, the scaled up trials had a lower relative flow rate when compared to the size of the extractor than the 100 mL extraction trails, which operated at 10 g/min. If the method was directly scaled from the 100 mL method, to achieve the same result, a flow rate of 200 g/min would be required, which was not available on the instrument used. Therefore, an increase in pressure was required to compensate for the lower relative $CO_2$ flow rate, otherwise the extraction would take five times the amount of time to complete when compared to the 100 mL trials. This meant that to save time and $CO_2$, which is a necessary consideration when developing such a method from an economical point of view, trials were run at 140 bar and 40°C in the 2 L extractor following variations on method

(b) in **Section 6.08**, which gave concordant results when compared with the results from 100 and 110 bar at 40°C on the 100 mL extractor (table 3.3).

Table 3.3. Summary of 2 L $CO_2$ extraction. Extractions ran at 140 bar pressure, 40°C and 40 g/min $CO_2$ flow rate. Mass recovery is the oil mass relative to the resin mass. Essential oil content is determined by hydrodistillation of the extract.

| $CO_2$ Extraction | Time (minutes) | Mass Recovery (%) | Essential Oil Content (%) |
|---|---|---|---|
| **1** | 120 | 9.0 | 75.0 |
| **2** | 120 | 8.1 | 72.5 |
| **3** | 120 | 11.7 | 72.5 |
| **4** | 60 | 9.0 | 80.0 |
| **5** | 60 | 8.6 | 75.0 |
| **6** | 60 | 7.7 | 77.5 |
| **7** | 60 | 5.3 | 82.5 |

One of the challenges of scaling these extractions up is it can be difficult to keep the same tightness when it comes to packing the resin. Some of the extractions took twice as long to get to the high extraction yields due to this and oil yields ranged from 5.3-11.7%. If the extractor is not packed tightly enough, the $CO_2$ can pass through the system too easily resulting in less contact time with the resin, meaning less oil extracted. However, once packed tightly, high recoveries of oil can be collected as experiments 4 and 5 show in the above table. The essential oil content was approximately determined by the hydrodistillation of a small, known volume of the $CO_2$ extract. The oil yielded through distillation was measured by its volume against the volume of the total oil distilled and a percentage mass recovery was calculated. This method leaves behind non-volatiles so anything that was not part of the essential oil fraction would be left as residue in the distillation flask. Over the seven $CO_2$ extractions, the essential oil content was fairly consistent, ranging from 72.5-82.5%. The lower oil yields often gave higher essential oil content which is understandable because of the argument above regarding contact time with the resin. Assuming this is the case, 6 and 7 had the least $CO_2$ contact time as it passed through the extractor, meaning that only the lowest polarity oil could be extracted. This explains why the α-pinene levels are higher in 6 and 7 (45.8% and 48.8%) respectively and the levels of serratol and triterpenes are much lower. Serratol was found in large quantities in 1-5 ranging from 13.0%-26.3% and only was

found in small amounts in 6-7 at 2.7-3.5%. There was also only trace triterpene content found in 6-7 whereas 1-5 found it in a range of 4.5%-9.6%.

When compared to the hydrodistilled samples of the Gudmo resin, the $CO_2$ extracts are similar (table 3.4). The main difference in the $CO_2$ extracts is the essential oil content being less, as small amounts of higher molecular weight compounds have been extracted as well. The oil yielded came in similar amounts also with the hydrodistilled being between 7.3-8.3% and the $CO_2$ extracts ranging from 7.7-9.1% on the 100 bar, 40$^oC$ trial on the 100 mL extractor. However, there were some interesting differences between the hydrodistilled and $CO_2$ extracted oils. Many of the major components were the same, such as α-pinene, sabinene, β-pinene and limonene, but on the other hand the $CO_2$ extract had some surprising differences. The verbenone content in the $CO_2$ extracted oil was 3.8-5.3% and whereas the hydrodistilled oil was only 0.1-1.0%. In addition, myrcene was only detected in trace amounts in the hydrodistilled oil but was detected at a content of 2.4-5.3% in the $CO_2$ extracted oil. This could mean that in the hydrodistillation experiments, since the system involves boiled water, compounds such as myrcene and verbenone are thermally degraded, whereas the mild conditions of the $CO_2$ extraction allow for them to be isolated unharmed. Other hydrodistilled oils which have myrcene and verbenone content therefore may not show a full representation of the volatile fraction, due to these sorts of compounds potentially being degraded before they can be isolated. Another difference was the presence of a diterpene fraction in the $CO_2$ fraction, which was expected due to the stronger extraction potential of supercritical $CO_2$, with serratol contents between 2.7-26.3% in the oil extracted. It is therefore difficult to exhaust the resin of one pure fraction without partially extracting the next with $CO_2$, whereas distillation will not isolate non-volatile compounds except in very low amounts depending on the temperature of the experiment. Reducing the pressure for the $CO_2$ extraction could aid a higher purity essential oil but would extend the extraction time and use more $CO_2$.

Table 3.4. Chemical profiles of seven separate $CO_2$ extractions carried out using a 2 L extractor. Each extraction was run at 140 bar pressure, 40°C and 40 g/min $CO_2$ flow rate. Mass recovery is the oil mass relative to the resin mass. 1-3 carried out for 120 minutes and 4-7 carried out for 60 minutes. Content estimated by GC/MS following method (b) in **Section 6.02**.

| Compound Number | AI[a] | Compound | Assignment Confidence[b] | Content (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| **148** | 922 | Tricyclene | High | 0.6 | 0.6 | 0.5 | 0.8 | 0.9 | 1 | 1 |
| **25** | **934** | **α-Pinene** | **High** | **29.9** | **32.1** | **23.8** | **30.6** | **31.1** | **45.8** | **48.8** |
| **26** | 951 | Camphene | High | 0.6 | 0.6 | 0.4 | 0.5 | 0.7 | 0.8 | 0.9 |
| **27** | 973 | Sabinene | High | 2.6 | 3.9 | 2.5 | 2.9 | 3.6 | 4.2 | 4.3 |
| **28** | 980 | β-Pinene | High | 5.2 | 3.3 | 3.7 | 3.5 | 4.6 | 5.9 | 5.9 |
| **29** | 988 | Myrcene | High | 2.7 | 3.7 | 2.4 | 2.5 | 2.5 | 4.3 | 5.3 |
| **30** | 1008 | α-Phellandrene | High | 0.6 | 0.8 | 0.6 | 0.7 | 0.8 | 1.1 | 1.5 |
| **32** | 1026 | *p*-Cymene | High | 0.9 | 0.8 | 0.5 | 0.8 | 0.9 | 1.2 | 1 |
| **33** | 1030 | Limonene | High | 2.3 | 3.4 | 2.9 | 3.8 | 3.8 | 4.7 | 4 |
| **149** | 1034 | (*Z*)-β-Ocimene | High | 0.7 | 1.2 | 1.3 | 1.1 | 1.3 | 2 | 2.1 |
| **151** | 1112 | *p*-1,3,8-Menthatriene | Medium | 1.3 | 0.5 | 0.4 | 0.7 | 0.9 | 0 | 0 |
| **133** | 1144 | *trans*-Pinocarveol | Medium | 0.9 | 0.3 | 0.3 | 0.5 | 0.6 | 0.5 | 0.2 |
| **-** | 1148 | Unknown Monoterpene | - | 2.2 | 1.1 | 0.8 | 1.4 | 1.5 | 0.9 | 0.5 |
| **134** | 1198 | Myrtenol | Medium | 1 | 0.4 | 0.4 | 0.6 | 0.7 | 0.6 | 0.3 |
| **37** | 1209 | Verbenone | High | 4.5 | 4.2 | 3.8 | 3.9 | 4.1 | 5.3 | 5.1 |
| **141** | 1285 | Bornyl acetate | High | 1.1 | 0.5 | 0.5 | 0.6 | 0.8 | 0.9 | 0.7 |
| **38** | 1378 | α-Copaene | High | 0.5 | 0.4 | 0.5 | 0.5 | 0.6 | 0.5 | 0.5 |
| **65** | 1386 | β-Bourbonene | High | 0.7 | 0.6 | 0.9 | 0.8 | 1 | 1.1 | 1 |
| **39** | 1390 | β-Elemene | High | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.5 | 0.5 |
| **40** | 1423 | Caryophyllene | High | 1 | 1.1 | 1.5 | 1.1 | 1.2 | 1.4 | 1.6 |
| **150** | 1443 | 6,9-Guaiadiene | Medium | 1 | 1.3 | 1.2 | 0.9 | 0.8 | 1.4 | 1.4 |
| **41** | 1459 | α-Humulene | Medium | 0.6 | 0.6 | 0.8 | 0.6 | 0.7 | 0.8 | 0.9 |
| **156** | 1515 | γ-Cadinene | Medium | 0.8 | 0.6 | 0.6 | 0.6 | 0.6 | 0.7 | 0.7 |
| **43** | 1518 | δ-Cadinene | High | 1.4 | 1.2 | 1.5 | 1.2 | 1.2 | 1.4 | 1.3 |
| **147** | 1598 | Guaiol | Medium | 0 | 2 | 5.5 | 2.2 | 1.2 | 1.6 | 1.3 |
| **152** | 1644 | epi-α-Cadinol | Medium | 1.6 | 1.1 | 1.3 | 1.2 | 1.1 | 1.2 | 0.9 |
| **56** | 1957 | (*3E*)-Cembrene A | High | 0.7 | 0.8 | 1.2 | 1.1 | 1 | 0.7 | 0.5 |
| **-** | 2017 | Unknown Diterpene | - | 0.6 | 0.4 | 0.6 | 0.5 | 0.4 | 0.2 | 0.2 |
| **-** | 2140 | Unknown Diterpene | - | 1.3 | 1.4 | 2.5 | 2.1 | 1.6 | 0 | 0 |
| **48** | **2153** | **Serratol** | **High** | **13** | **21.4** | **26.3** | **24.1** | **18.9** | **3.5** | **2.7** |
| **-** | 3354 | Unknown Triterpene | - | 1.1 | 0.6 | 0.5 | 0.5 | 0.8 | 0 | 0 |
| **-** | 3363 | Unknown Triterpene | - | 1.7 | 0.9 | 0.8 | 0.8 | 0.9 | 0 | 0 |
| **-** | 3373 | Unknown Triterpene | - | 1.7 | 0.7 | 0.7 | 0.7 | 1 | 0 | 0 |
| **-** | 3386 | Unknown Triterpene | - | 3.1 | 2.6 | 2 | 2.1 | 2.8 | 0 | 0 |
| **Mass Yield (%)[c]** | | | | **9.0** | **8.1** | **11.7** | **9.0** | **8.6** | **7.7** | **5.3** |

a) Arithmetic index calculated using equation 1.3.

b) Assignment based on combined data mentioned in **Section 6.02**.

c) Based on the oil mass isolated relative to the resin mass distilled.

## 3.2 $CO_2$ Extractions on North Coast, Somaliland Resin

The resin from the North Coast of Somaliland, believed to be *Boswellia occulta* resin, had high incensole content without too much other compounds in the diterpene region. This means that isolation of incensole in high concentrations may be possible. However, an issue when extracting these resins with $CO_2$ is the thick oils that partially extract can cause blockages, so often a co-solvent was introduced earlier than planned. This set of extractions were variations on method (c) in **Section 6.08.** The higher purity incensole extracts (>60% determined by GC/MS) were combined for each experiment and the results are summarised below (table 3.5).

Table 3.5. Summary of $CO_2$ extractions with all high incensole content fractions combined. Mass recovery is the oil mass relative to the resin mass. Content estimated by GC/MS analysis following method (b) in **Section 6.02**.

| $CO_2$ Extraction | Mass Recovery (%) | Incensole Content (%) | Solid Support:Resin |
|---|---|---|---|
| **8** | 16 | 77 | Sand 3:1 |
| **9** | 21 | 71 | Sand 5:1 |
| **10** | 14 | 76 | Alumina 5:1 |
| **11** | 21 | 75 | Alumina 3:1 |
| **12** | 22 | 74 | Alumina 5:1 |
| **13** | 23 | 72 | Alumina 5:1 |
| **14** | 17 | 71 | Alumina 4:1 |

The first $CO_2$ extraction of this material (entry 8) was carried out with a 3:1 mix of sand to resin. The volatile fraction was exhausted over 7 hours at 70 bar pressure and 45$^{o}$C with a $CO_2$ flow rate of 10 g/min. This gave an oil that had a concentration of 63% incensole, and a mass recovery of oil of 8%. The pressure was increased to 300 bar for 2.5 hours, which gave another incensole rich fraction (76%) with a mass recovery of 13%. When IMS was introduced as a co-solvent at 1 mL/min and the $CO_2$ flow rate adjusted to 9 g/min, the fractions collected were dominated by the triterpene acids, but still contained some incensole. This means that the extraction could be optimised for isolating incensole efficiently, as the idea would be to extract as much of the incensole as possible before isolating the acid rich fraction. Furthermore, during the second phase, at 300 bar, there were significant signs of blocking in the pipes due to the thickness of the oil, so the co-solvent was introduced to aid solubility. Two incensole rich fractions were isolated in this extraction with a combined mass recovery of 21%, so this type of extraction was taken forward for optimisation (figure 3.01).

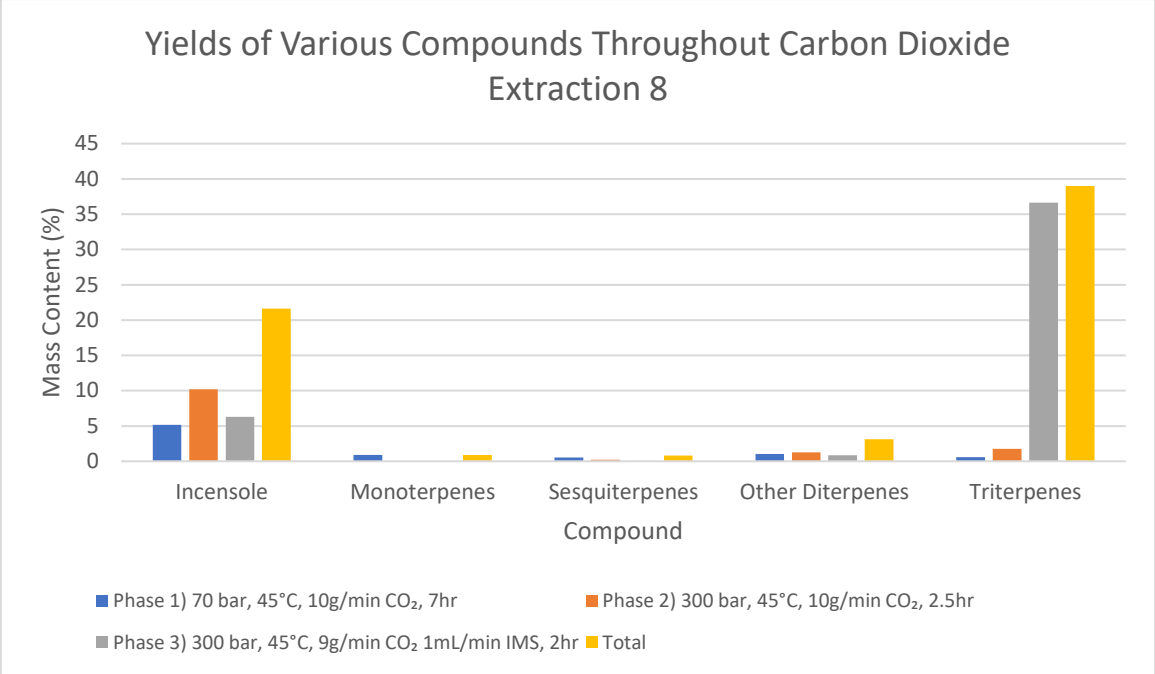



Figure 3.01. Summary of a $CO_2$ extraction which isolated two incensole rich fractions. Extraction consists of three successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

In extraction 9, a higher ratio of sand to resin (5:1) was used to see if this would help with reducing blockages and increasing incensole purity. This extraction used the same temperature, flow rate and pressure, but added a 1% flow of heptane. This was to aid solubility without changing the relative polarity of the $CO_2$ too much. Over 3 hours at 70 bar, an incensole rich fraction (62%) was collected with a mass recovery of 11%. Following this, the pressure was increased to 300 bar and the heptane flow rate was increased to 2%. This gave another incensole rich oil (65%) with a mass recovery of 23%. A final phase using a gradient co-solvent was carried out with flow rates of 5%, 10% and then 20% of heptane compared to $CO_2$ flow rate over 90 minutes. This gave an oil with 41% incensole content and a mass recovery of oil compared to the resin mass of 10%. Overall, a mass recovery of 44% was achieved, which means some non-volatiles have been left behind. However, it is likely that very little incensole content was left meaning what was left behind would be triterpene acids mainly, which are not desired in these experiments (figure 3.02).

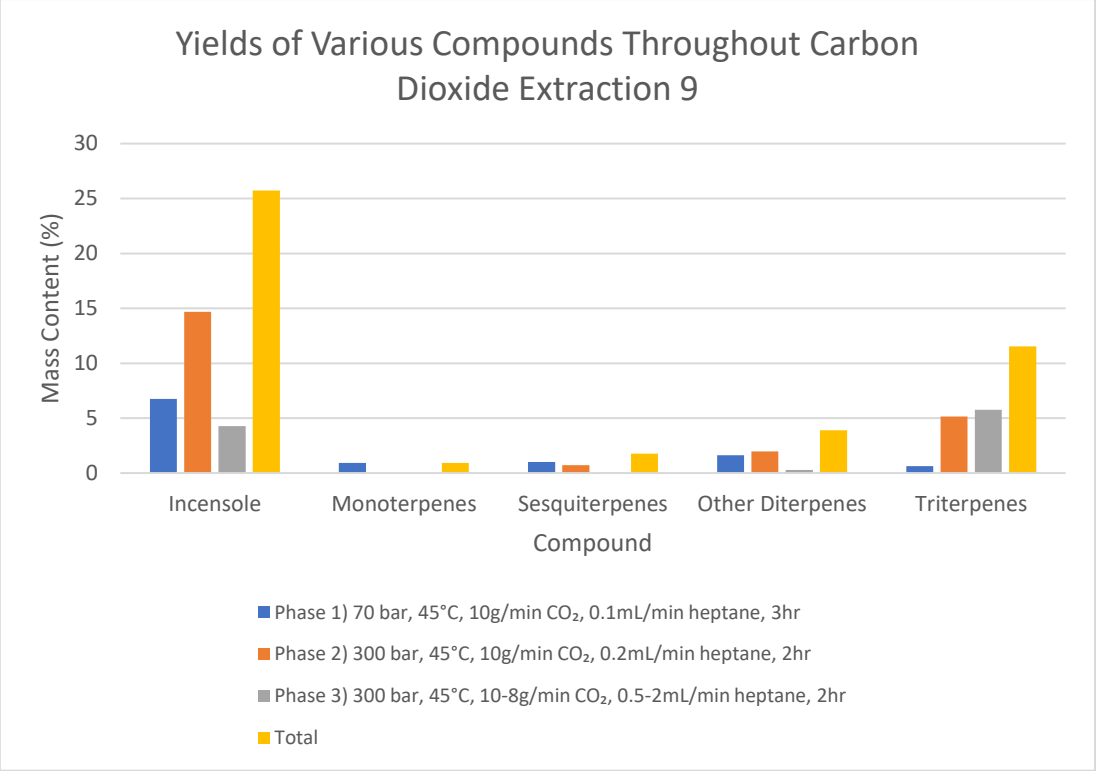



Figure 3.02. Summary of a $CO_2$ extraction which isolated 2 incensole rich fractions. Extraction was performed with 3 successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

Extractions 10-14 used basic alumina as the solid support instead of sand. Theoretically, the basicity of the alumina should have helped retain some of the acid constituents, allowing for the neutral components to be extracted in higher purity. Using alumina did however have a consequence on the packing as alumina is less dense than sand, meaning that less resin could be extracted than when packed with sand as weight ratios were used for mixing.

Extraction 10 used basic alumina and had similar conditions to the previous extractions, which gave several fractions with high incensole content (figure 3.03). The $CO_2$ flow rate remained the same, as did the temperature and pressure at 10 g/min $CO_2$, 45$^{o}$C and 70 bar pressure. This was carried out for 4.5 hours to give an incensole fraction with a concentration of 42% and a mass recovery of 4%. This is notably less than the first phase of extraction when using sand as the packing agent. This can be explained by the relatively low concentration of incensole which may be due to hydrogen bonding between incensole and the basic alumina, as the fraction is mainly made up of more volatile compounds. After this, a 1% flow of heptane compared to the $CO_2$ flow rate was introduced to aid solubility in the pipes to avoid blockages. 1 hour later, the pressure was increased to 300 bar and over 3 hours, this gave an oil very high

in incensole (76%) with the mass recovery for this fraction being 14%. Before fully exhausting the resin, the heptane flow rate was increased to 10% for 1 hour to attempt to extract the remainder of the incensole while avoid much of the heavier oils. This fraction had a reasonable amount of oil (3% mass recovery) that was high in incensole (69%) according to GC/MS analysis.

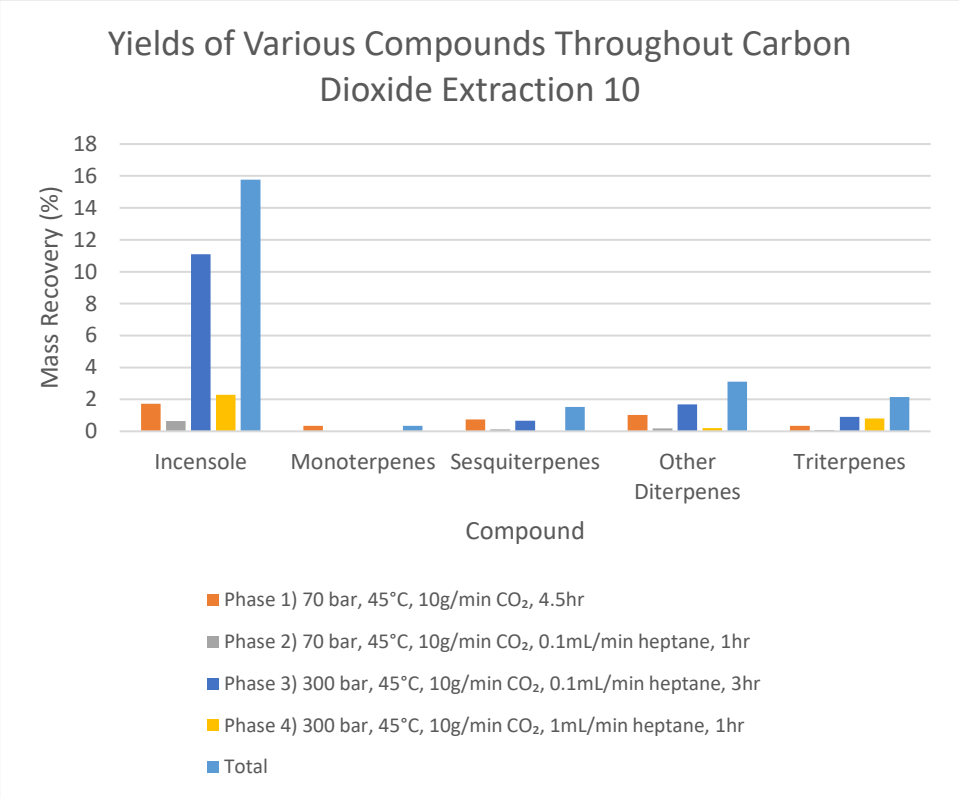



Figure 3.03. Summary of a $CO_2$ extraction which isolated two incensole rich fractions and one essential oil fraction with reasonable incensole levels. Extraction was carried out over four successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

Extraction 11 used a mixture of basic alumina and resin in a 3:1 ratio to allow for more resin to be extracted in one run. Using less basic alumina compared to the previous extraction meant the interactions between the components and the alumina was weaker. This resulted in two incensole rich fractions. The volatile fraction, which was extracted over 6 hours, gave a mass recovery of 8%, with an incensole concentration of 63%. After this phase was exhausted, a 1% flow of heptane was added to the system and 1 hour later, the pressure was increased to 300 bar. Over 2.5 hours, this yielded a 15% mass recovery that was 75% incensole. After this, there was very little incensole left to extract, which was illustrated when

the co-solvent ratio was increased and only very minor concentrations of incensole were then detected (figure 3.04).

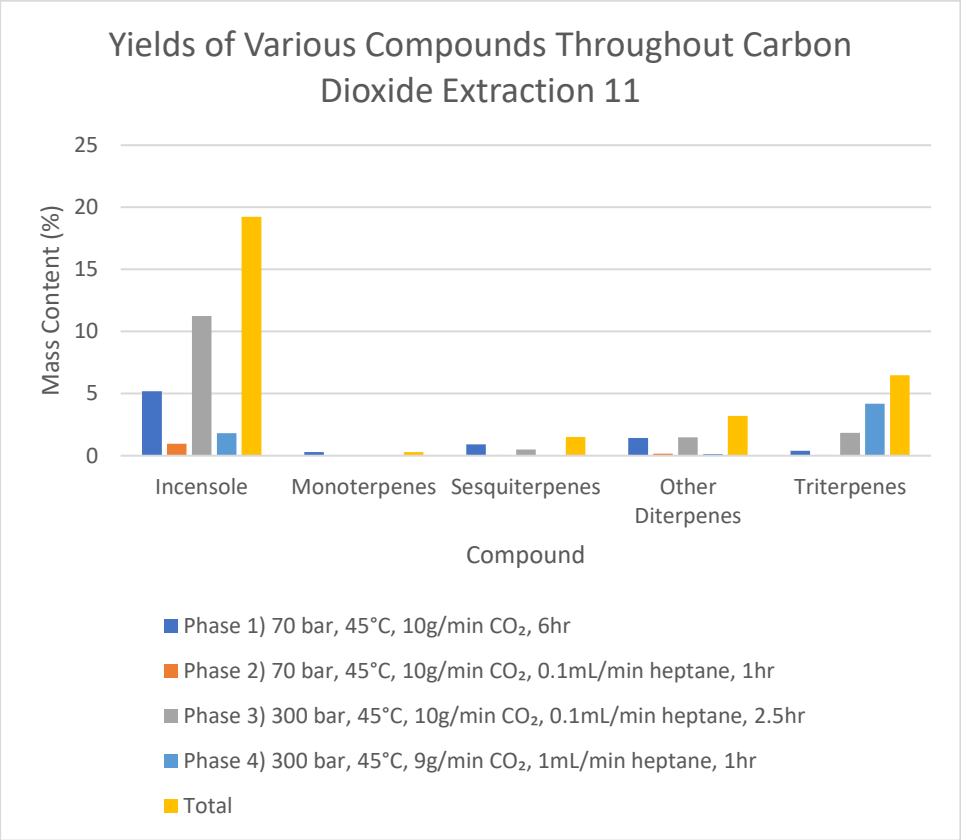



Figure 3.04. Summary of a $CO_2$ extraction which isolated two incensole rich fractions. Extraction consisted of four successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

The next extraction, number 12, applied the same pressure and $CO_2$ flow rate as done previously, but the extraction was performed at room temperature. Over 5 hours, this only yielded a 3% mass recovery with incensole being in 42% concentration. However, this meant the essential oil had been exhausted, so when a 1% flow of heptane was added, the pressure was increased to 300 bar and the temperature was risen to 50°C, 3 hours of flow yielded a fraction containing 80% incensole and a mass recovery of 16%. This was followed by 1 hour of a 10% flow of heptane, then 2 hours of 10% IMS to exhaust the resin. The 10% heptane phase gave a 59% incensole oil content with 5% mass recovery. This method worked well as the second phase gave a very high purity extract after solvent evaporation (figure 3.05).

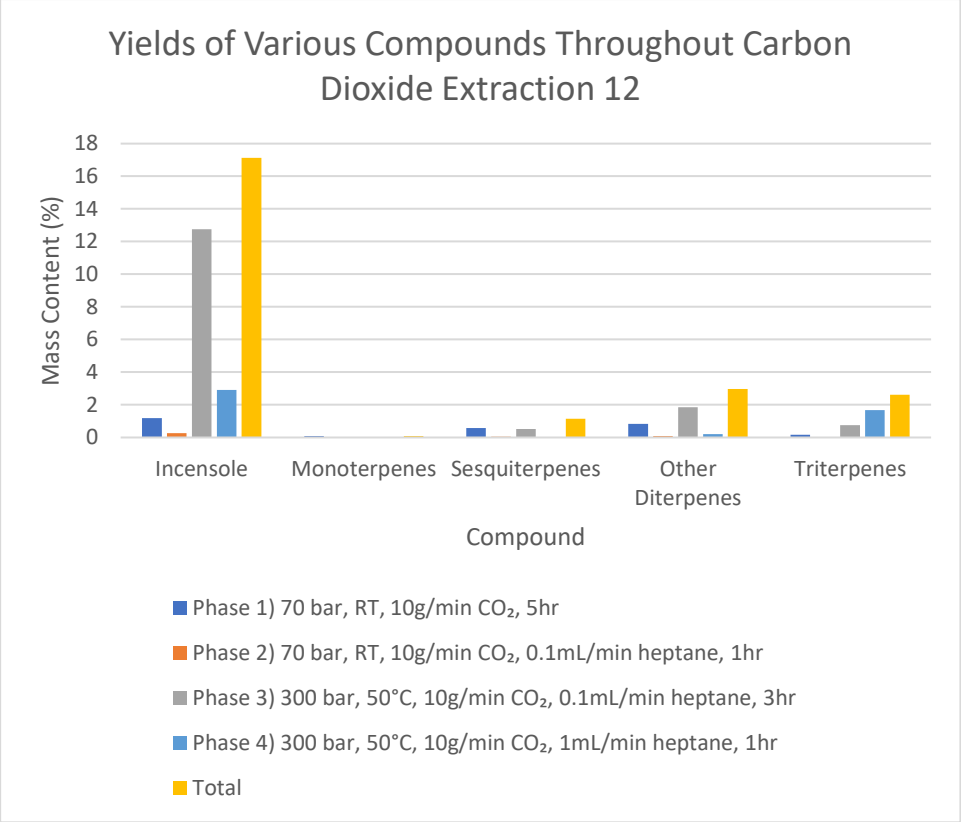



Figure 3.05. Summary of a $CO_2$ extraction which isolated one very rich incensole fraction. Extraction consisted of five successive phases, the last of which was to exhaust the resin and was almost completely made up of triterpenes so was omitted from the chart. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

Following this, extraction 13 used a higher pressure, which was set to 90 bar for the first phase. This was accompanied by a 10 g/min flow rate of $CO_2$ at 40°C (figure 3.06). Over 4 hours, this yielded a low incensole content fraction (31%) with a mass recovery of 5%. When the pressure was increased to 300 bar and the temperature to 50°C, a larger incensole content fraction was isolated. A 1% flow of IMS was used for the second half of this 5-hour segment, which resulted in an oil with a mass recovery of 22%, of which, the incensole content was 70%. This almost fully exhausted the incensole content of the resin as in the final phase, where a 10% IMS flow rate was applied, only a very small incensole content (2%) was detected in the boswellic acid dominated fraction. This was a positive result as this experiment produced 1 major fraction of incensole, which could be theoretically taken forward for further purification. Furthermore, it may be possible to isolate this incensole rich fraction faster, by adding the 1% flow of IMS at the same time as increasing the pressure to 300 bar.

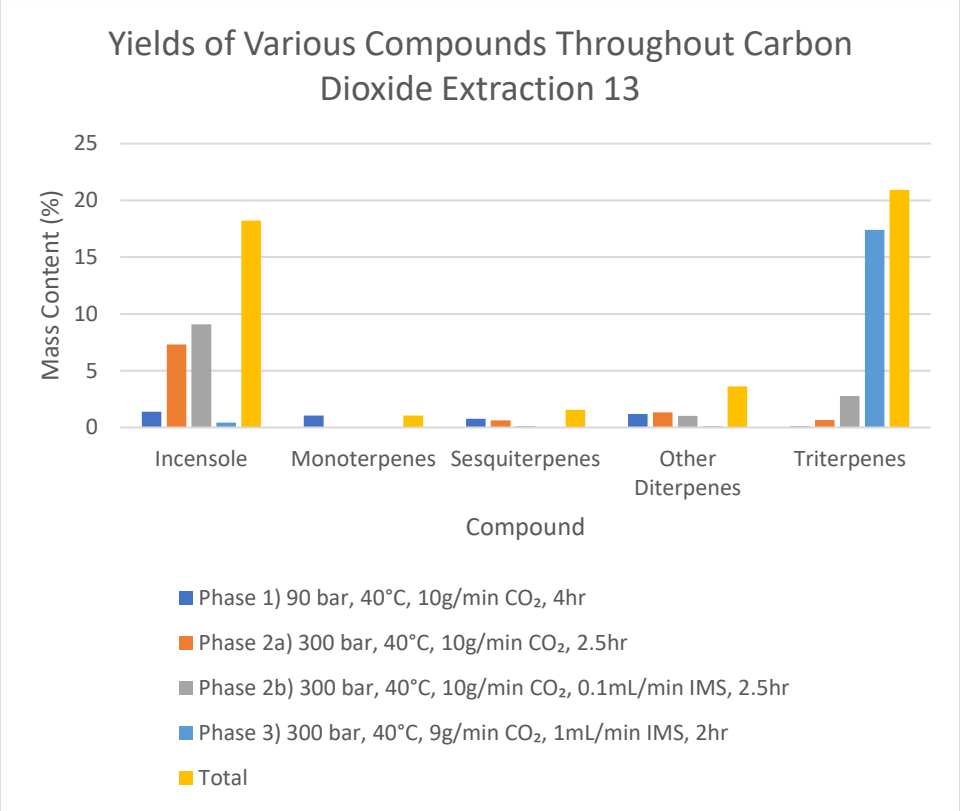



Figure 3.06. $CO_2$ extraction which gave one major incensole fraction by combining phases 2a and 2b. Extraction consisted for three main successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

Due to the encouraging results found in extraction 13, it was repeated, using a larger amount of resin to alumina (alumina 4:1 resin) to see if more resin could be processed without adversely affecting the extraction to make it more cost effective. The experiment remained quite similar with regards to the different phases of the extraction. The first phase, using 90 bar pressure, 10 g/min $CO_2$ and 40°C over 4.5 hours yielded a strong incensole fraction (67%) with a mass recovery of 10%. Therefore, the ratio of basic alumina clearly has an effect, as in the 5:1 ratio experiment ran prior, the incensole was mostly retained until the 300 bar pressure phase. Next, the pressure was increased to 300 bar and 50°C for 5.5 hours, of which, the final 1.5 hours had an IMS 1% flow rate to aid solubility. This fraction gave an 8% mass recovery of 62% incensole concentration. After this, once again the 10% IMS phase gave only low incensole content (2%) in a large boswellic acid fraction. Although, the incensole was not retained as well as in the 5:1 packing of basic alumina to resin, this experiment still produced two good incensole fractions that could be combined and purified. The mixture was well

exhausted of the incensole content while mostly avoiding extraction of the heavy triterpenes (figure 3.07).

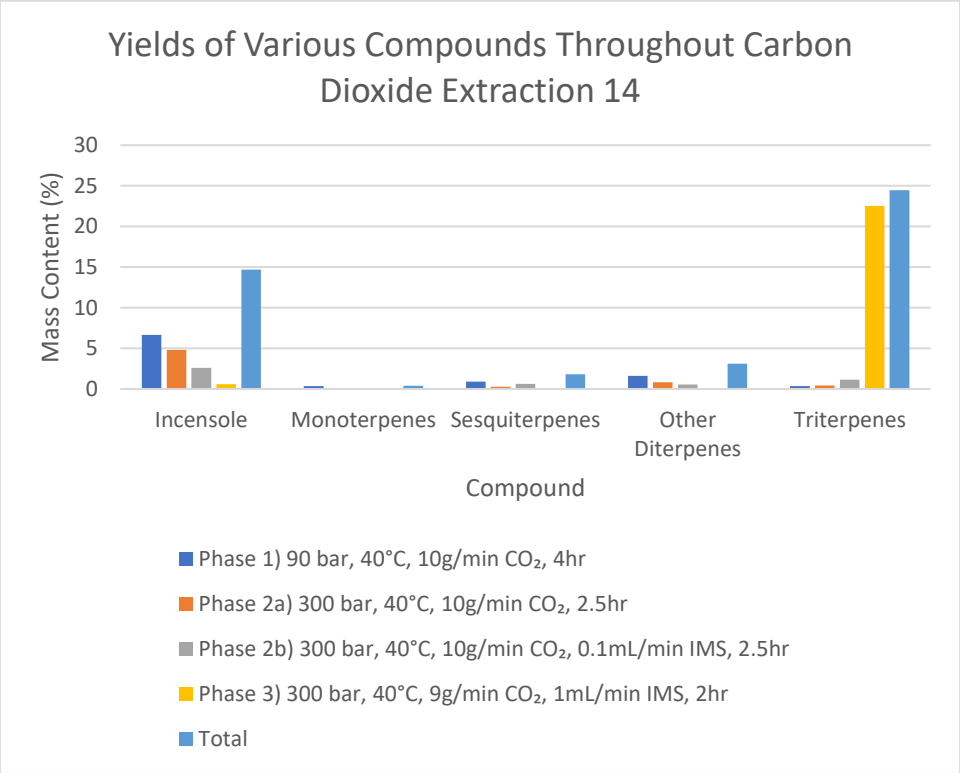



Figure 3.07. $CO_2$ extraction using 4:1 ratio of basic alumina to resin which produced incensole rich fractions to be combined. Extraction consisted of three main successive phases. Mass content refers to the GC/MS estimated mass of the compounds in the oil relative to the resin mass.

These extractions all had a similar outcome, where the first phase would be able to remove the essential oil before increasing the pressure to remove the bulk of the incensole fraction. Using basic alumina was useful for not disturbing the boswellic acid fraction as much until the 10% co-solvent phase. It should be noted that when using sand, only the last 1-2 fractions of second extraction phase, before the 10% co-solvent introduction, contained large concentrations of boswellic acids. Furthermore, the major compound in these samples was still incensole. This means that sand could be used as a cost-effective solid support, but the extract would require additional care in the following steps to purify.

Using the above experiments as a guide, a faster extraction method was tried using the same size $CO_2$ extractor (100 mL). The North Coast, Somaliland resin was milled finely and mixed with basic alumina in a 1:4 ratio. The essential oil fraction was firstly extracted for 30 minutes at 90 bar pressure, using a 10 g/min $CO_2$ flow rate at 40°C. This fraction contained little

incensole and was mainly made up of methoxydecane (50%). The pressure was then increased to 300 bar and the temperature to 50°C for 90 minutes. This gave a rich incensole fraction of 64% content and a 10% mass recovery. Then a 10% flow rate of IMS was introduced for 1 hour and this exhausted the vast majority of the incensole giving a mass recovery of 23% with an incensole content of 43%. This fraction contained significant amounts of boswellic acids (47%) but was added to the mixture as there were further purification steps carried out and the incensole content was high. The extraction was continued to extract the remaining boswellic acid content and to analyse to check that all the incensole had been removed. Overall, the fractions that were combined from this step were taken between 60-210 minutes and gave a mass recovery of 34% with a 50% content of incensole. (figure 3.08)

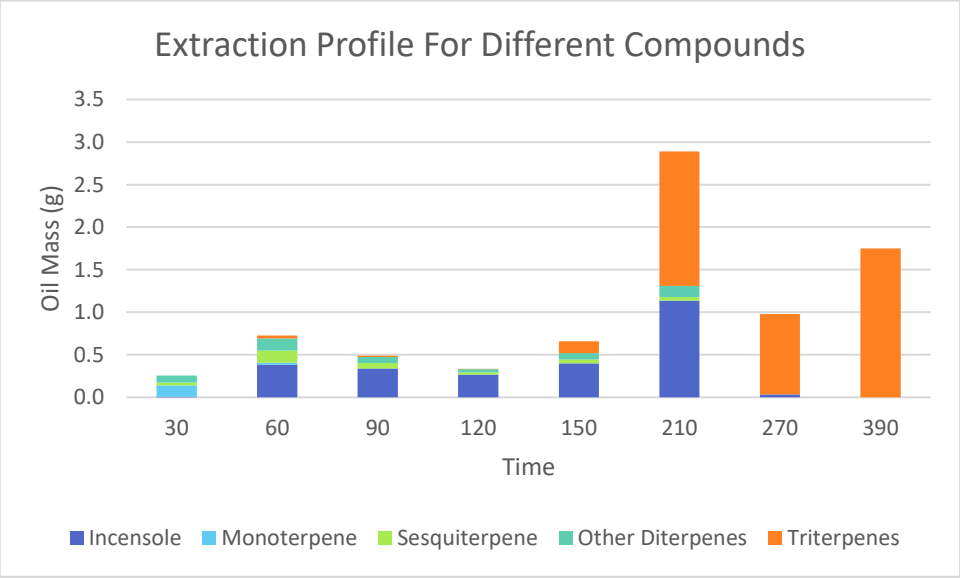



Figure 3.08. Extraction profile of incensole extraction using $CO_2$. Masses were estimated by GC/MS calculations compared to oil mass. Conditions: 0-30 min = 90 bar, 40°C, 10g/min $CO_2$; 30-120 min = 300 bar, 50°C, 10 g/min $CO_2$; 120-210 min = 300 bar, 50°C, 9 g/min $CO_2$, 1 mL/min IMS; 210-390 min = 300 bar, 50°C, 8 g/min $CO_2$, 2 mL/min IMS.

The combined fractions were evaporated onto basic alumina and loaded into the extractor. At 300 bar pressure, 50°C and a 10 g/min flow of $CO_2$, the mixture was extracted for 2.5 hours before introducing a 1% flow of IMS into the system to aid solubility. After another 3 hours, the matrix was exhausted by adding a 10% flow of IMS followed by a 20% flow for 1 hour each (figure 3.09). To remove the volatile impurities, the next step would be hydrodistillation, meaning that boswellic acids would remain in the residue with the incensole. Therefore, fractions with any significant boswellic acid content were not taken. This left 9 fractions which

were collected between 30-270 minutes, which gave a mass recovery of 20% when compared to the original resin used in the previous extraction, with incensole content being 80% as determined by GC/MS.

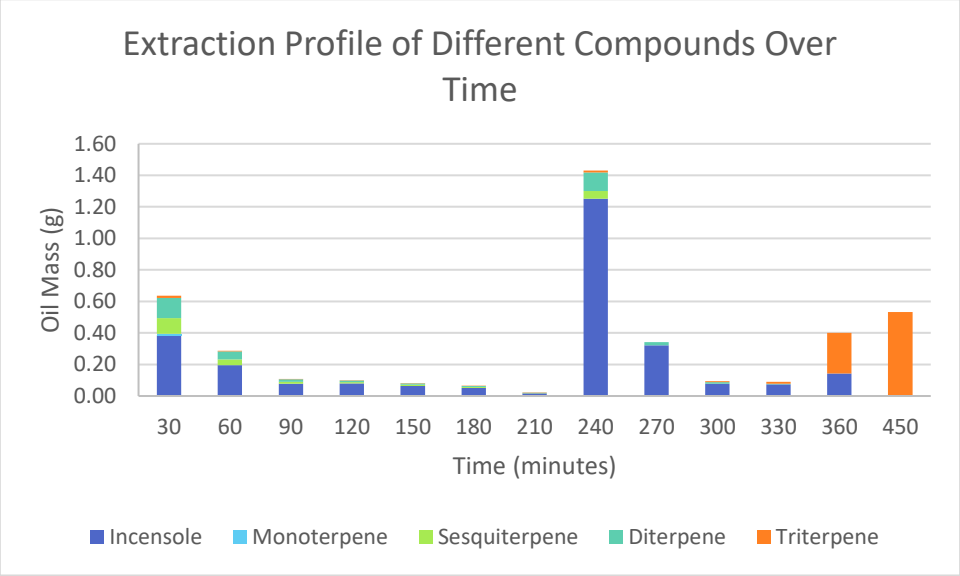



Figure 3.09. Extraction profile of incensole extraction using $CO_2$. Masses were estimated by GC/MS calculations compared to oil mass. Conditions: 0-150 min = 300 bar, 50°C, 10 g/min $CO_2$; 150-330 min = 300 bar, 50°C, 10 g/min $CO_2$, 0.1mL/min IMS; 330-360 min = 300 bar, 50°C, 9 g/min $CO_2$, 1 mL/min IMS; 360-450 min = 300 bar, 50°C, 8 g/min $CO_2$, 2 mL/min IMS.

Next, the combined fractions were hydrodistilled for 3 hours to remove to volatile components. After cooling, the oil was extracted with dichloromethane 3 times and then evaporated to give the residue fraction. This fraction had a mass recovery of 18% compared to the original resin mass extracted and the concentration of incensole was 95%, by GC/MS, with only some minor diterpene impurities remaining. Overall, a good yield of incensole was obtained and the method could be developed to use a thin film evaporator with multiple fractionation capability, meaning that isolating fractions containing the heavy oils would also be practical.

A further three other extractions were performed where the whole extraction would be intended to be faster. This resulted in a higher boswellic acid content in the same fractions that were incensole rich, but as mentioned, the use of a thin film evaporator could potentially isolate high purity fractions. The extractions are summarised below (table 3.6).

Table 3.6. Summary of the incensole rich fractions from three separate $CO_2$ extractions. It should be noted that the time stated is from the start until the end of the high incensole content fractions and not for the entire extraction. Mass recovery is the mass of oil relative to the resin mass. Content estimated by GC/MS analysis following method (b) in **Section 6.02**.

| No. | Time (minutes) | Mass recovery (%) | Incensole content (%) | Notes |
|---|---|---|---|---|
| **15** | 120 | 29 | 70 | 2:1 sand/resin, 0-1% IMS flow main phase |
| **16** | 60 | 31 | 65 | 2:1 sand/resin, 5% IMS flow main phase |
| **17** | 120 | 28 | 55 | 1:1 sand/resin, 5% IMS flow main phase |

Extraction 15 used a 2:1 ratio of sand to resin and applied a 300 bar pressure, a flow rate of 10 g/min $CO_2$ and was ran at 50°C. After 1 hour, a 1% flow of IMS was introduced. After 210 minutes total, a 20% flow of IMS was used to exhaust the acid fraction. All fractions of the main phase were collected to make up the tabulated values for mass recovery and incensole concentration (figure 3.10). The time taken for this extraction was still quite slow, but the incensole fraction does not need to be high purity at this stage. Therefore, an increase in the co-solvent flow rate was applied for the following extractions.

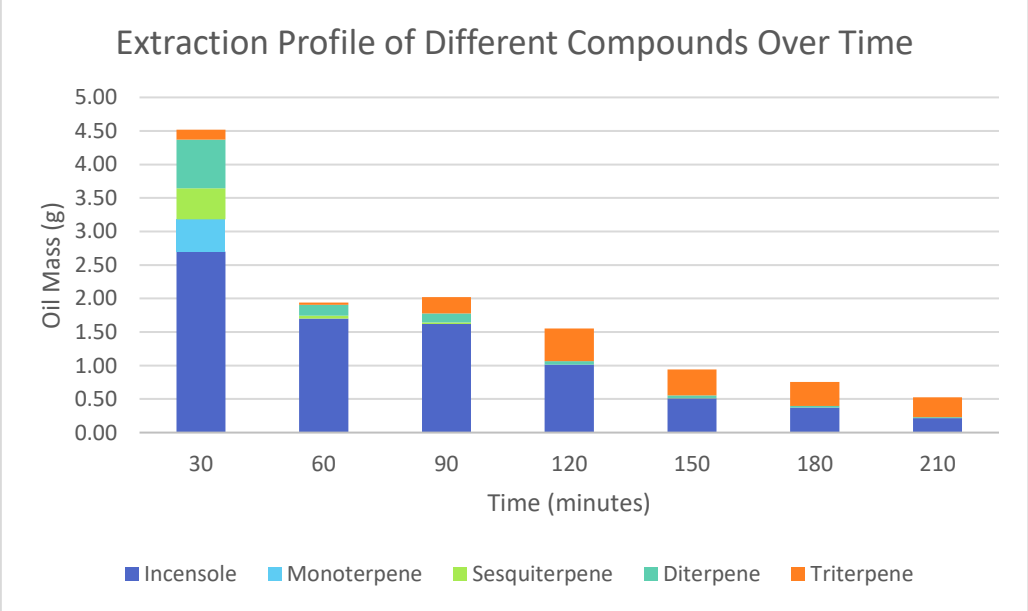



Figure 3.10. Results from $CO_2$ extraction 15 for isolating incensole. Masses were estimated by GC/MS calculations compared to oil mass.

Extraction 16 also used a packing ratio of 2:1 of sand to resin. The pressure and temperature remained the same as in extraction 15, at 300 bar and 50°C respectively. However, the $CO_2$ flow rate was reduced to 9.5 g/min to allow for a 0.5 mL/min flow of IMS, which meant a 5% flow rate of the co-solvent. This was ran for 90 minutes before exhausting the resin for 2 hours with a 20% flow of co-solvent. The fractions in the main phase were collected every 30 minutes and those that were combined were the first 60 minutes of the extraction. This mix had an incensole content of 65% with a mass recovery of 31%. The final fraction of this phase still had incensole, however it was dominated by triterpenes (71%). This type of extraction is very efficient, giving significant incensole fraction after 1 hour (figure 3.11).

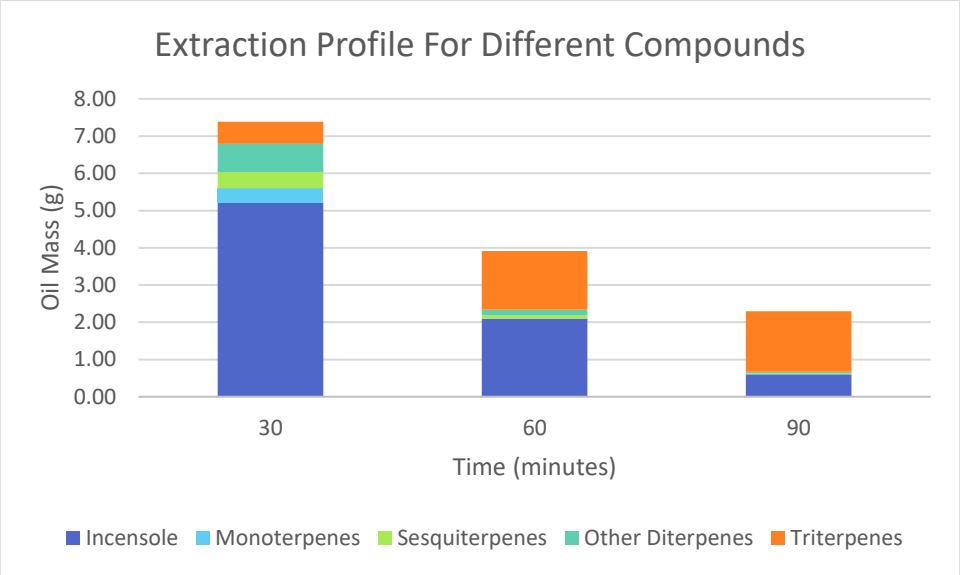



Figure 3.11. Summary of the composition of the extracts at various stages of extraction 16. Masses were estimated by GC/MS calculations compared to oil mass.

Extraction 17 used the same conditions as extraction 16, set at 300 bar, 50°C, 9.5 g/min $CO_2$ and 0.5 mL/min IMS. However, the packing ratio was changed to 1:1 resin to sand to test the practicality of extracting more resin per run. This resulted in a less efficient extraction, most likely due to the contact time of the solvent with the resin being reduced (figure 3.12). These conditions were applied for 120 minutes before exhausting the resin with a 10% co-solvent flow for 30 minutes followed by a 20% flow for 120 minutes. Upon analysis, after 60 minutes there was a mass recovery of 19% and incensole levels of 59%. This is significantly lower than the previous experiment at the same time stamp. Furthermore, after 90 minutes, the mass recovery was still lower, at 28%, with the incensole content being reduced to 55% and triterpene levels of 25%. Therefore, it seems that a 1:1 ratio of the solid support to resin

allows for poor contact time of the $CO_2$ to the resin and poorer selectivity, as the resin is partially extracted over all fractions when packed this way.

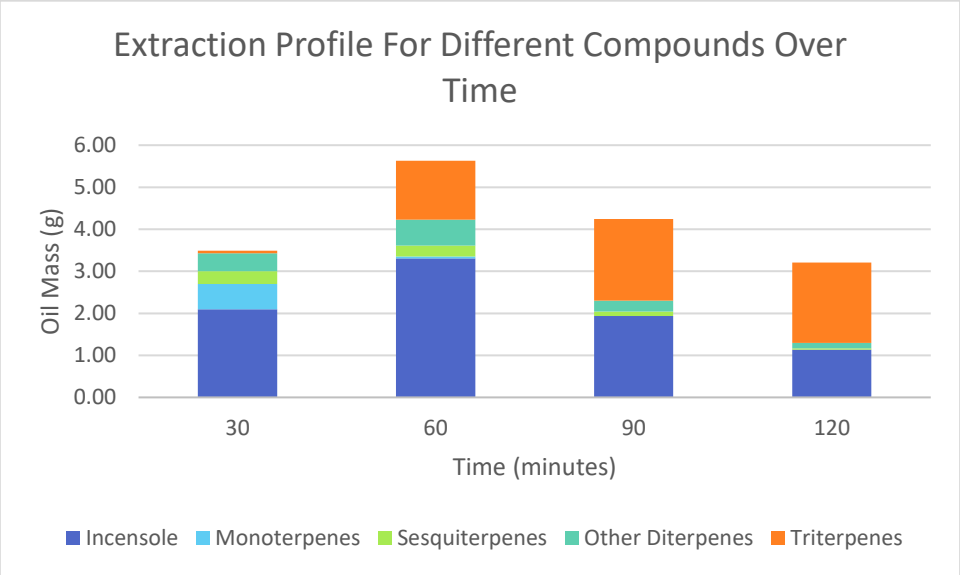



Figure 3.12. Summary of the main phase of the $CO_2$ extraction 17. Masses were estimated by GC/MS calculations compared to oil mass.

Due to the positive results from $CO_2$ extraction 16, three more extractions were carried out to test its repeatability. Each of these were ran at the same pressure, temperature and flow rates of 300 bar, 50°C and 9.5 g/min $CO_2$ with a 0.5 mL/min IMS flow. After 1 hour the extractions were stopped, the oil was isolated and analysed by GC/MS (table 3.7).

Table 3.7. Results for three separate, identical $CO_2$ extractions of the *Boswellia occulta* resin. Mass recovery is the oil mass relative to the resin mass. Content estimated by GC/MS analysis following method (b) in **Section 6.02**.

| No. | Mass recovery (%) | Incensole content (%) |
|---|---|---|
| **18** | 19 | 47 |
| **19** | 27 | 57 |
| **20** | 27 | 43 |

Although these results show good incensole content with reasonable mass recoveries, the inconsistency in the resin is clearly highlighted. In 16, the mass recovery and incensole content was higher (31% and 65% respectively) than all 3 of the repeatability trials.

Overall, the $CO_2$ trials on this resin worked well, as every experiment was able to isolate significant fractions of incensole. The essential oil fraction of this resin is relatively small (c.a.

2% of the resin mass) and can be simply distilled due to this fraction having a generally much lower boiling point to incensole. The main heavy oil impurities arise from the boswellic acids, namely the keto-boswellic acids, which are more difficult to remove completely due to the large concentration of them. These have a much higher boiling point than incensole, so would likely be the residue fraction of a distillation that involves several collectors. Using $CO_2$, the boswellic acid fraction can be mainly undisturbed, as shown in previous experiments where they were often only isolated in higher contents after the introduction of a significant co-solvent (10-20% IMS). This is because of the low relative polarity of $CO_2$ as a solvent. The heavy oil fraction has many polar compounds, which are not very soluble in $CO_2$ until the polarity is dramatically increased by adding IMS which is a polar solvent.

## 3.3 $CO_2$ Extractions on Sudanese Resins

$CO_2$ extractions were also carried out on each of the four grades of *Boswellia papyrifera* resin. Two types of extraction were performed using a 100 mL extractor, both packed with resin and sand at a 2 to 1 ratio. The first set of extractions, which followed method (d) in **Section 6.08**, passed 10 g/min of $CO_2$ through the extractor at 300 bar pressure and 50$^{o}$C for 4 hours to remove a large portion of the diterpene fraction, without extracting too much of the triterpene compounds. Samples were collected every 30 minutes during this 'supercritical phase'. After this, a 10% flow of IMS was used for 2 hours as a co-solvent to remove the remainder of the extractable material, leaving just the polysaccharides behind. This allowed for fractionation of the diterpene fraction away from the triterpene fraction. Low pressures were not used as there was very little in the essential oil fraction of these resins, meaning that removing it would be wasteful on $CO_2$, time and would result in some losses of incensole and incensole acetate to the essential oil fraction.

All the resins gave similar extraction profiles during the supercritical phase, with regards to the mass of oil extracted. After 240 minutes, the mass recoveries ranged from 17.1-24.7% compared to the resin mass (figure 3.13).

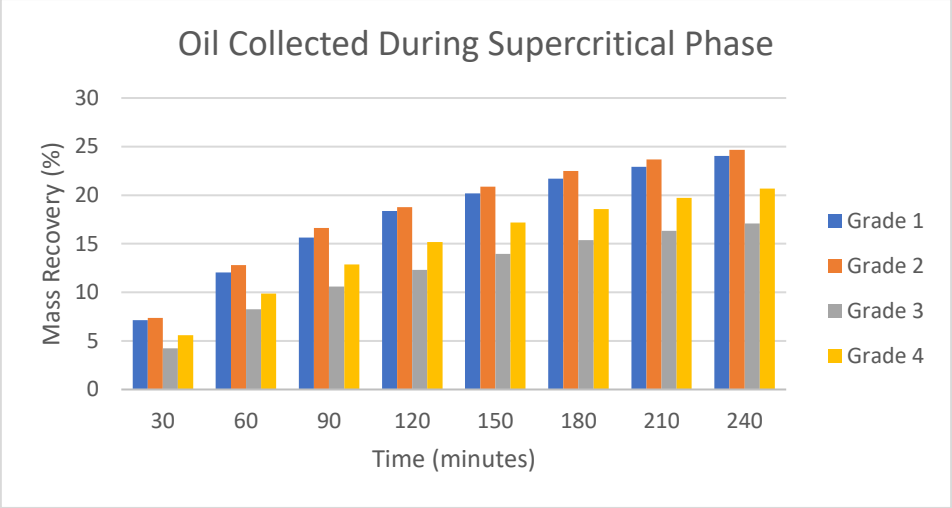



Figure 3.13. Comparison of the mass recovery of oil for each grade of *Boswellia papyrifera* resin throughout the extraction. Mass recovery is the oil mass relative to the resin mass.

Samples were taken every 30 minutes and submitted for GC/MS analysis to study the composition (table 3.8). Throughout the entire supercritical phase, all resins had incensole and incensole acetate as the dominating compounds in the extracts with their combined concentrations ranging between 55.5-79.1%. The main impurities were the boswellic acids, which grew over the course of each extraction and other diterpenes, such as verticilla-4(20),7,11-triene. With the desired compounds making up the bulk of the oil extracted, the mixture could be distilled to higher purity. However, the Sudanese resins have other diterpenes present in reasonable concentrations, which would be difficult to separate on the larger scale.

Table 3.8. Summary of the samples taken at each time stamp during $CO_2$ extraction of *Boswellia papyrifera* resins. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. In = incensole, InAc = incensole acetate.

| **Time (minutes)** | **Content (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Grade 1** | | **Grade 2** | | **Grade 3** | | **Grade 4** | |
| | **In** | **InAc** | **In** | **InAc** | **In** | **InAc** | **In** | **InAc** |
| **30** | 19.8 | 45.7 | 24.1 | 48.1 | 12.0 | 43.5 | 23.5 | 47.3 |
| **60** | 32.3 | 43.0 | 36.9 | 42.2 | 20.5 | 41.8 | 35.5 | 40.2 |
| **90** | 40.5 | 35.4 | 43.2 | 29.6 | 29.6 | 32.0 | 45.2 | 31.0 |
| **120** | 46.2 | 30.3 | 46.8 | 25.0 | 35.4 | 28.9 | 49.7 | 27.4 |
| **150** | 47.5 | 27.0 | 51.0 | 19.0 | 38.8 | 25.9 | 42.1 | 20.9 |
| **180** | 47.6 | 24.7 | 52.5 | 16.7 | 41.7 | 22.0 | 46.9 | 19.7 |
| **210** | 47.3 | 23.4 | 61.8 | 15.4 | 39.9 | 18.0 | 51.3 | 19.7 |
| **240** | 46.9 | 22.1 | 62.1 | 13.5 | 40.7 | 14.9 | 52.9 | 17.3 |

The second set of extractions, which followed method (e) in **Section 6.08** passed $CO_2$ through the extractor at 9.5 g/min, with a pressure of 300 bar and temperature of 50°C. The packing ratio was again 2 to 1 of sand to resin, finely milled. The major difference in this extraction as opposed to the previous is that a co-solvent was used immediately from the start of this second set. A 5% flow of IMS was used as the co-solvent which allowed for less fractionation, but more of a full picture of the contents of the resin that are extractable by $CO_2$ more quickly. Each extraction was carried out for 2 hours and the mixture was collected every 30 minutes. This required evaporation under vacuum to remove the IMS to give a mass of the extract. Once a weight had been determined and a sample had been submitted for GC/MS, the following sample could be added to its predecessor to give a cumulative composition and mass of the oil as the extraction went on (figures 3.14 and table 3.9).

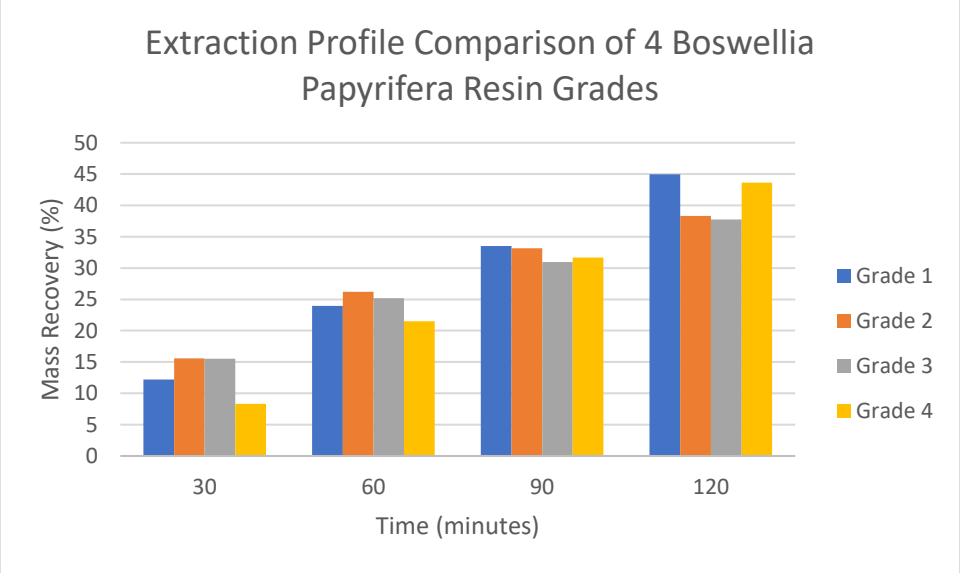



Figure 3.14. Extraction profile for the four grades of Sudanese resin under $CO_2$ extraction using a co-solvent. Mass recovery is the oil mass relative to the resin mass.

Table 3.9. Summary of the samples taken at each time stamp during $CO_2$ extraction using a co-solvent of IMS of *Boswellia papyrifera* resins. Content estimated by GC/MS analysis following method (b) in **Section 6.02**. In = incensole, InAc = incensole acetate.

| **Time (minutes)** | **Content (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grade 1 | | Grade 2 | | Grade 3 | | Grade 4 | |
| | In | InAc | In | InAc | In | InAc | In | InAc |
| **30** | 22.8 | 28.2 | 28.4 | 31.1 | 19.1 | 27.8 | 25.6 | 40.6 |
| **60** | 21.2 | 20.2 | 31.6 | 24.1 | 22.1 | 25.5 | 25.6 | 23.0 |
| **90** | 19.7 | 17.2 | 30.0 | 21.4 | 21.2 | 19.8 | 23.0 | 17.8 |
| **120** | 16.3 | 12.9 | 26.1 | 18.0 | 18.2 | 17.3 | 19.0 | 13.9 |

Each of the resins extracted similar amounts of oil respective to the amount of resin packed into the extractor (37.7-45.0%). However, looking at the GC/MS data profile, grade 2 seems to contain the largest amount of incensole and incensole acetate with the triterpene concentrations varying quite broadly over the 4 resin's extracts 36.9-58.9%. As expected, the triterpene content began quite low and grew as the extraction went on and the more easily extractable diterpenes started out in high concentration and plateaued throughout the extraction.

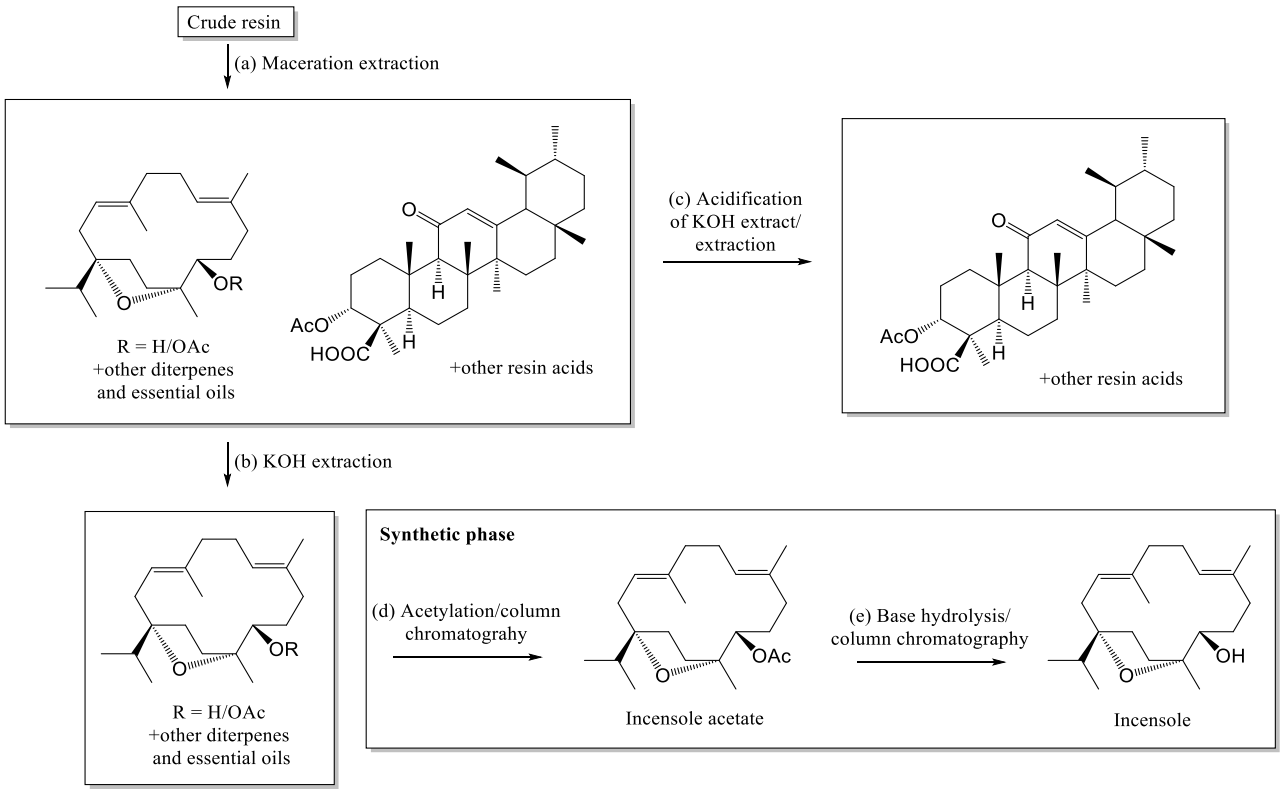
There are advantages and disadvantages to each method. The first method allowed for much purer extracts, with high purity diterpene fractions throughout the whole supercritical phase. The second extraction extracted a much larger portion of the triterpenes early on but was much faster at exhausting the diterpene fraction as a result. However, this means that further purification would be required. To do this, the difference in volatility could be exploited to distil the diterpenes away from the heavy triterpene acids. In addition, even though the use of a co-solvent improves the extraction efficiency, it adds another impurity to the mixture, as the IMS needs to be removed in order to get a pure extract, which adds the additional step of evaporation of the solvent. Although the extraction was long for the first set and only yielded a part of the diterpene fraction, the pressure could potentially be increased to increase the solvent power of the $CO_2$. This would likely see improved mass recoveries, however the triterpene fraction would once again be more pronounced in the extract. This would give a balance between the two extractions though, giving an extract free from solvent residue with a large amount of incensole and incensole acetate yielded at a fast rate.

# Chapter 4. Isolation of Incensole Acetate from *Boswellia occulta* and *papyrifera* resins

Incensole and its acetate have some interesting biological activity as discussed in **Section 1.6** and therefore isolating the acetate in high quantity was one of the aims of this project. As found through GC/MS analysis of the solvent extracts of the available resins, all four of the *Boswellia papyrifera* samples had reasonable diterpene fractions, including incensole and incensole acetate. In addition, the North Coast material from Somaliland which is likely *Boswellia occulta* also contained high amounts of incensole, so trials were performed to isolate these fractions. In these experiments, the method in **Section 6.09** was used.

To isolate the incensole and incensole acetate, each resin that contained either compound was ground to a fine powder and separately extracted with chloroform and the combined extracts were washed with a KOH (2% aq.) solution to generate a neutral organic fraction and an acid fraction. For the *Boswellia papyrifera* resins, the neutral fractions contained incensole, incensole acetate and a variety of other diterpenes. *Boswellia occulta's* neutral fraction had a composition mainly consisting of incensole and various amyrins. The acid fraction was acidified using concentrated HCl and subsequently re-extracted with chloroform and after isolation the crude acid oil, was weighed to test the size of this fraction compared to the overall resin mass. This fraction made up a large content of the resin (up to 46% mass recovery compared to the resin mass), and GC/MS analysis showed they contained very little incensole and incensole acetate (<1%) and was mainly comprised of boswellic acids. Since the acetate was the main target, the neutral fractions in each case were acetylated using pyridine, acetic anhydride and a catalytic amount of 4-dimethylaminopyridine at reflux for 1 hour. This gave a crude mixture of incensole acetate, some triterpenes and diterpenes. Since incensole was the only diterpene in this oil that could be acetylated under these conditions, it was now simpler to purify by column chromatography as it eluted easily through the silica gel column in comparison to the unacylated components. In comparison, incensole elutes more slowly, with several of the other diterpenes and triterpenes co-eluting, meaning that it is more difficult to isolate in high purity by chromatography, which results in a loss in yield and a lower purity. If incensole is required in high purity the isolated incensole acetate can be easily

converted back to incensole by hydrolysis in a refluxing KOH (5% aq.) and isopropanol mixture (1:1 ratio) for 4 hours followed by column chromatography (Scheme 4.1).




Scheme 4.1. Isolation of incensole acetate and then incensole from *Boswellia papyrifera* and *Boswellia occulta*. Experiment conditions: a) 3 x $CHCl_3$, b) 2% KOH (2% aq.), c) conc. HCl/2 x $CHCl_3$ d) $Ac_2O$, Pyridine, DMAP, $CH_2Cl_2$, reflux, 1 hr, e) KOH (5% aq.)/IPA (1:1 mixture), reflux, 6 hr.

Performing the acetylation step on the neutral fractions of *Boswellia papyrifera and Boswellia occulta* resins resulted in the isolation of crude incensole acetate with mass recoveries between 26-30% compared to the resin mass. After column chromatography, using 2-4% $Et_2O$ in petroleum spirits (40-60) as an eluent, pure incensole acetate fractions were isolated with mass recoveries of 9-15% compared to the resin mass, with grade 4 *Boswellia papyrifera* resulting in the highest content of incensole acetate. This was analysed by $^1H$ (figure 4.01), $^{13}C$ (figure 4.02) and HSQC (figure 4.03) NMR spectroscopy. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.92 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz), 1.13 (s, 3H), 1.53 (dddd, 1H, *J* = 14.2, 10.5, 5.1, 1.6 Hz), 1.57 (s, 3H), 1.61 (s, 3H), 1.62 (m, 1H), 1.64 (m, 1H), 1.66 (m, 1H), 1.78 (m, 1H), 1.86 (m, 1H), 1.89 (m, 1H), 1.93 (sep, 1H, *J* = 6.8 Hz), 2.05 (dd, 1H, *J* = 13.5, 5.0 Hz), 2.07 (s, 3H), 2.17 (m, 2H), 2.18-2.19 (m, 2H), 2.21-2.22 (m, 1H), 4.89 (d, 1H, *J* = 10.4 Hz), 5.18 (m, 1H), 5.19 (m,

1H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta_C$ 16.0, 17.7, 18.0, 18.0, 21.3, 22.0, 24.8, 27.7, 30.3, 32.0, 33.4, 34.9, 35.6, 38.5, 76.6, 83.1, 89.2, 121.1, 125.4, 133.2, 135.2, 171.2.

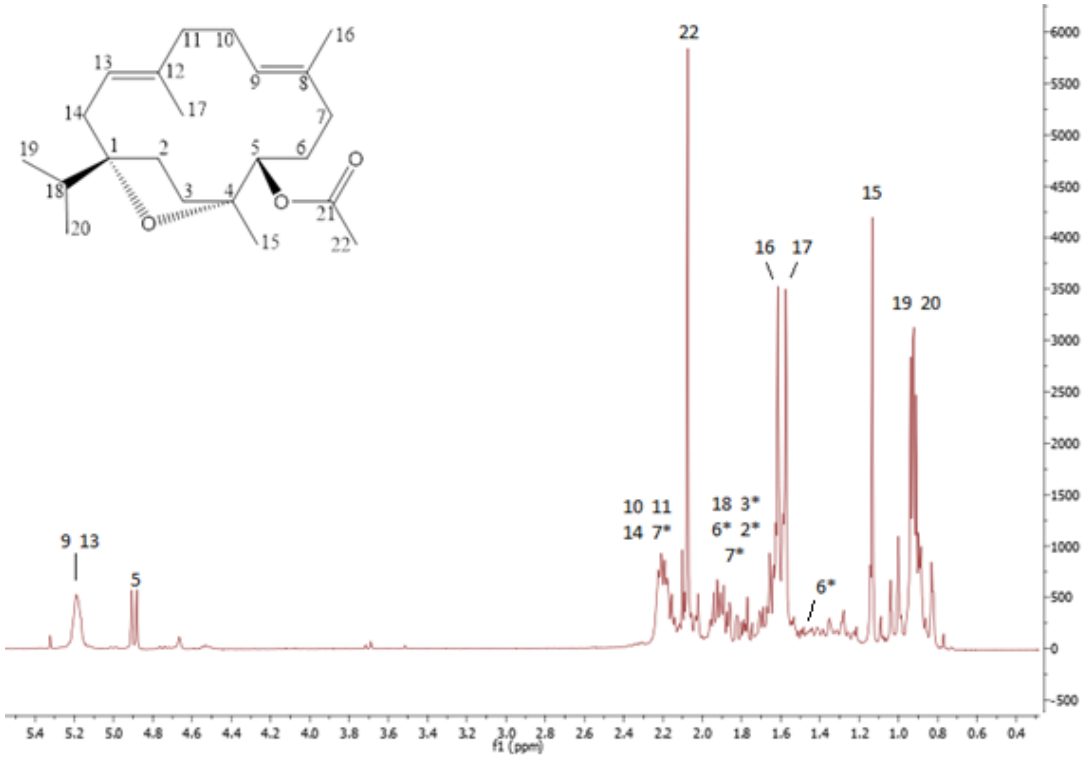



Figure 4.01. $^1H$ NMR (400 MHz, $CDCl_3$) of incensole acetate which corresponds to the literature. Peak numbers correspond to their position on the compound. *Protons from $CH_2$ groups where each CH produces a separate signal.

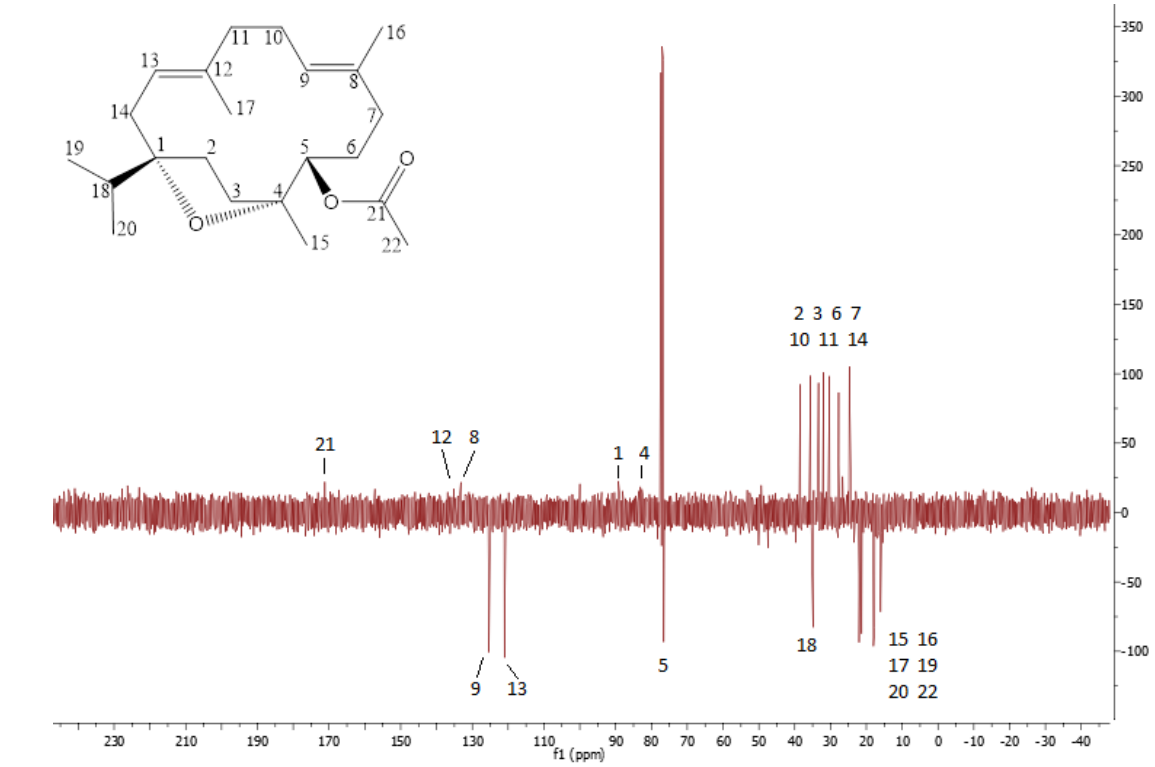



Figure 4.02. $^{13}C$ NMR (100 MHz, $CDCl_3$) of incensole acetate which corresponds to the literature. Peak numbers correspond to their position on the compound.

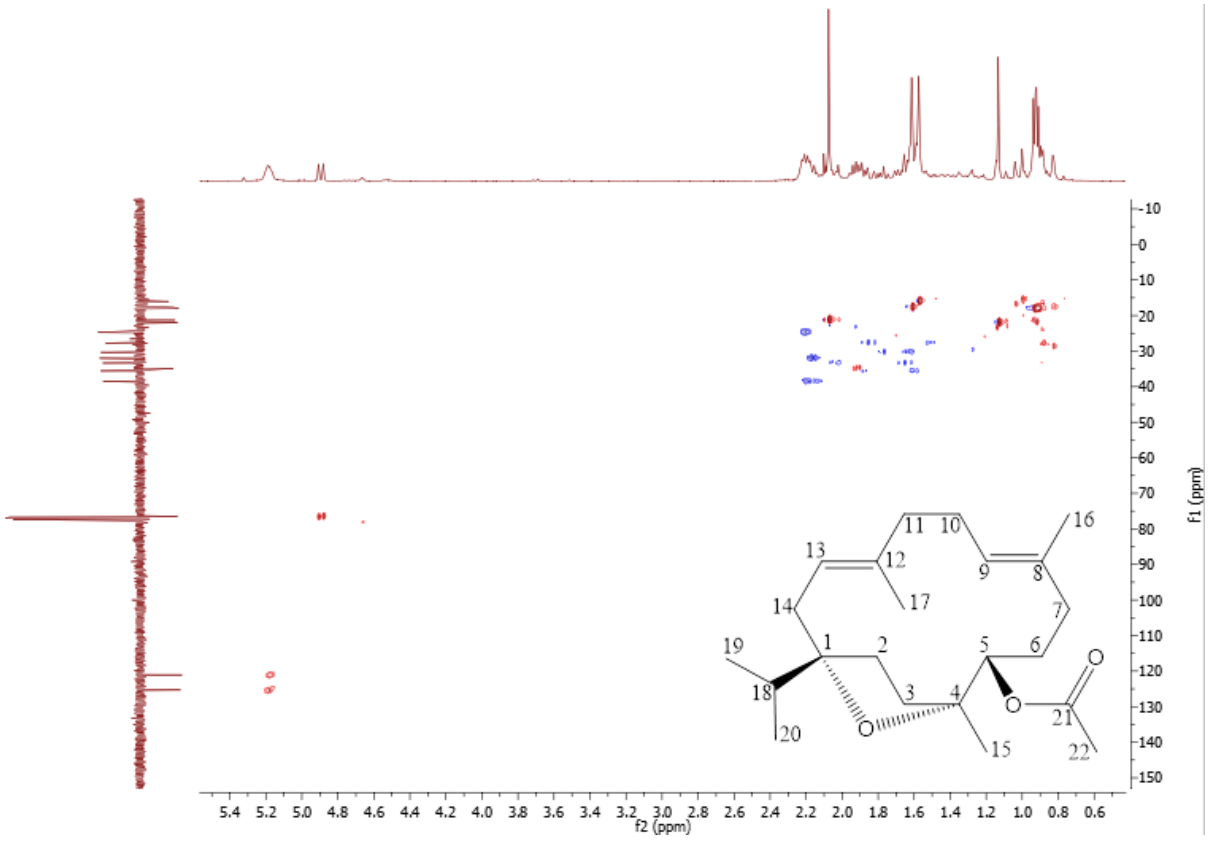



Figure 4.03. HSQC NMR ($CDCl_3$) of incensole acetate confirming the $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) peak overlaps.

Using a combination of the literature NMR and GC/MS data that showed this to be the peak with an m/z of 348 was considered proof of the isolated oil being incensole acetate. The $^{13}C$ spectrum determined there were twenty-two carbon atoms in this molecule, including twelve carbons that were substituted with an even number of protons and ten carbons that were substituted with an odd number of protons, as detected by the phase separation ability of DEPTQ. The quaternary carbons were simple to assign, as they displayed low intensity peaks and due to the nuclear spin, point upwards on a DEPTQ $^{13}C$ NMR spectrum. Furthermore, the lack of a response on the HSQC supports this, as there are no protons on these carbons, so there were no $^{1}H$ NMR peaks to correlate to the $^{13}C$ spectrum for carbons 1, 4, 8, 12 and 21. The $^{1}H$ NMR spectrum shows some simple peaks for diagnosis as well as some more difficult multiplets. The proton on carbon 5 gives a sharp doublet at around 4.9 ppm and the alkene CH atoms form two overlapping triplets at around 5.2 ppm. The main difference between incensole acetate and incensole's $^{1}H$ NMR is the carbon 5 proton's peak, which is at around 3.3 ppm for incensole due to being substituted here by an OH group instead of an OAc. Integration of four sharp singlets at 1.13 ppm, 1.57 ppm, 1.61 ppm and 2.07 ppm have relative intensities to show they are $CH_3$ groups. Furthermore, a doublet of doublets at around 0.9 ppm is evident of the two $CH_3$ groups on the isopropyl functionality. However, several of the $CH_2$ groups that are part of the cembrene ring show two responses, likely due to the interactions these protons have with different parts of the compound due to the fact that the ring system locks them in place, one above and one below. One such proton is the H6β proton, which is similar to the splitting observed in incensole, as discussed earlier in **Section 2.2.1**. This proton is shifted higher than the same proton in incensole (1.53 and 1.34 ppm for incensole acetate and incensole respectively) due to the presence of the carbonyl in the acetate and as a result, is slightly obscured by the methyl singlet at 1.57 ppm (figure 4.04).[70]

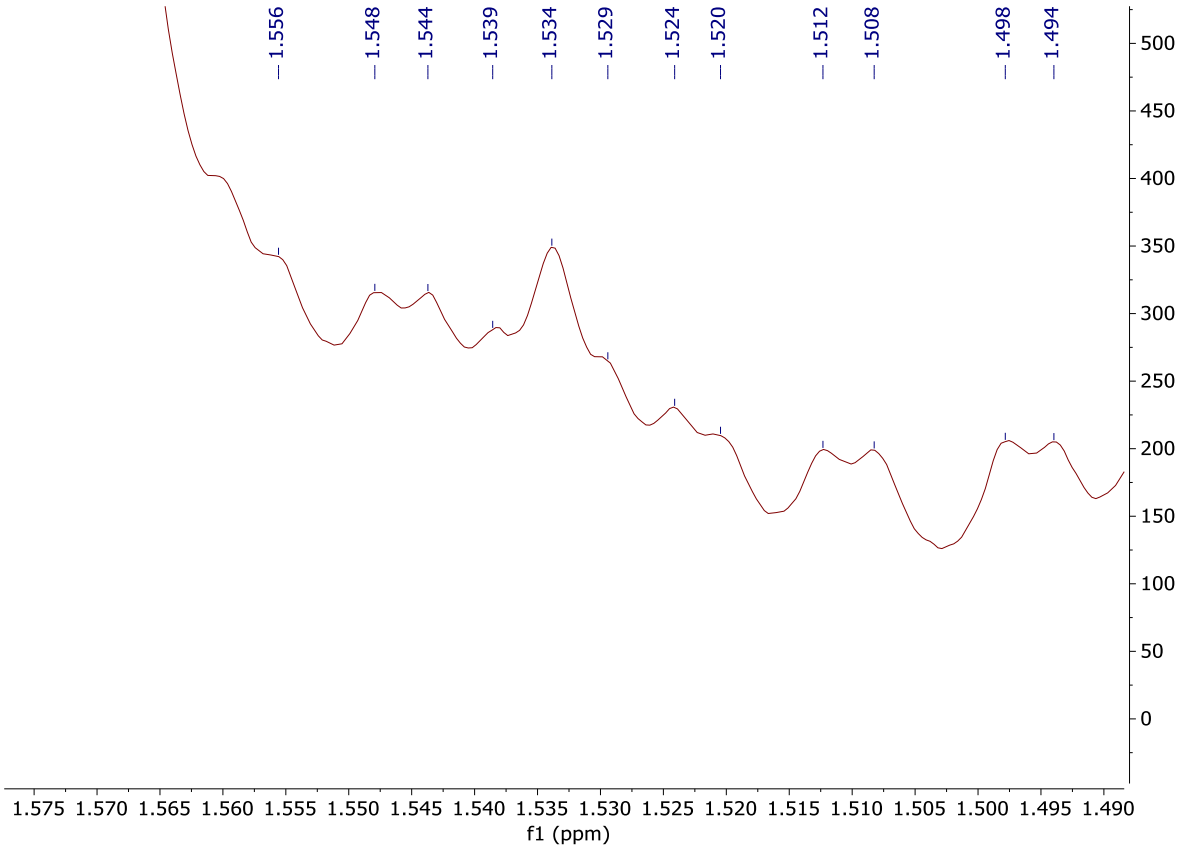
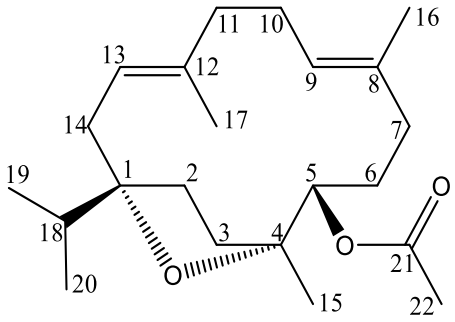



Figure 4.04. Characteristic dddd splitting for the H6β proton on incensole acetate found by $^{1}H$ NMR (400 MHz, $CDCl_3$).

As was the case for incensole, the H6α signal is overlapped by other signals in the same region so was not fully characterised (1.86 ppm). The full assignment of peaks to atoms is summarised (table 4.1).

Table 4.1. Structural assignment of incensole acetate via $^{1}H$, $^{13}C$ and HSQC NMR spectroscopy compared to a literature source. N.s – Not specified.




| Carbon No. | $^{13}C$ NMR (ppm) | | $CH_n$ | $^{1}H$ NMR (ppm) | | Multiplicity/J-Coupling (Hz) | |
|---|---|---|---|---|---|---|---|
| | Literature[57] | Observed | | Literature[57] | Observed | Literature[57] | Observed |
| **1** | 89.23 | 89.22 | C | - | - | - | - |
| **2** | 30.33 | 30.31 | $CH_2$ | 1.78<br>1.61 | 1.78<br>1.64 | N.s<br>N.s | m<br>m |
| **3** | 35.59 | 35.59 | $CH_2$ | 1.89<br>1.59 | 1.89<br>1.62 | N.s<br>N.s | m<br>m |
| **4** | 83.07 | 83.06 | C | - | - | - | - |
| **5** | 76.57 | 76.56 | CH | 4.87 | 4.89 | dd, *J* = 10, 0.8 | d, *J* = 10.4 |
| **6** | 27.73 | 27.74 | $CH_2$ | 1.85<br>1.51 | 1.86<br>1.53 | m<br>dddd, *J* = 14.0, 10.0, 5.6, 1.6 | m<br>dddd, *J* = 14.2, 10.5, 5.1, 1.6 |
| **7** | 33.37 | 33.33 | $CH_2$ | 2.02<br>1.63 | 2.05<br>1.66 | dd, *J* = 14.0, 5.6<br>m | dd, *J* = 13.5, 5.0<br>m |
| **8** | 133.20 | 133.21 | C | - | - | - | - |
| **9** | 125.35 | 125.34 | CH | 5.18 | 5.19 | m | m |
| **10** | 24.76 | 24.76 | $CH_2$ | 2.19 | 2.21-2.22 | m | m |
| **11** | 38.49 | 38.49 | $CH_2$ | 2.15 | 2.18-2.19 | m | m |
| **12** | 135.21 | 135.09 | C | - | - | - | - |
| **13** | 121.05 | 121.04 | CH | 5.16 | 5.18 | m | m |
| **14** | 32.01 | 32.00 | $CH_2$ | 2.17 | 2.17 | m | m |
| **15** | 22.02 | 22.04 | $CH_3$ | 1.11 | 1.13 | s | s |
| **16** | 17.66 | 17.68 | $CH_3$ | 1.59 | 1.61 | m | s |
| **17** | 16.00 | 16.02 | $CH_3$ | 1.55 | 1.57 | m | s |
| **18** | 34.92 | 34.90 | CH | 1.90 | 1.93 | sep, *J* = 6.8 | sep, *J* = 6.8 |
| **19** | 18.00 | 18.02 | $CH_3$ | 0.89 | 0.92 | d, *J* = 6.8 | d, *J* = 6.8 |
| **20** | 18.04 | 18.06 | $CH_3$ | 0.91 | 0.93 | d, *J* = 6.8 | d, *J* = 6.8 |
| **21** | 171.17 | 171.16 | C | - | - | - | - |
| **22** | 21.29 | 21.32 | $CH_3$ | 2.05 | 2.07 | s | s |

Hydrolysis of incensole acetate was be performed to isolate incensole free from the impurities in the crude neutral fraction. Incensole acetate was dissolved in isopropanol (IPA) before adding an equal amount of KOH (5% aq.) solution. Quite a large amount of IPA is required to dissolve incensole acetate (c.a. 50 mL per 1 g), but a water miscible solvent is required for this

step to facilitate the hydrolysis. The mixture was heated to reflux for 4 hours resulting in full consumption of the starting material into the desired alcohol.

After isolating crude incensole oil, it was purified using column chromatography, with a mobile phase of 10-30% $Et_2O$ in petroleum spirits (40-60). Performing this hydrolysis on each of the incensole acetate oils isolated prior to this resulted in mass recoveries of 6-12% of incensole compared to the initial resin mass. The yields of these reactions based on the starting material of incensole acetate being converted and subsequently purified to isolate incensole ranged from 77-92%.

The data for each step of this experiment from extraction until isolation of purified incensole acetate has been summarised (table 4.2). The fourth grade of *Boswellia papyrifera* gave the highest mass of incensole and the acetate back, meaning that the diterpene fraction must contribute little to the overall grade. The pale colour and larger size of the higher-grade material could be influenced more by the acid fraction, due to its large size in proportion to the overall resin mass. The GC/MS analysis of the acid fractions of the four grades of *Boswellia papyrifera* showed very similar acid fractions, but there was a range in the size of the acid fractions relative to the resin mass (27-46%). Grades 1-3 had similarly sized acid fractions, between 43-46% and grade 4 was noticeably lower, at 35%. Grades 1-3 of *Boswellia papyrifera* share a similar pale colour, so the similarity in the acid fraction content could be what leads to this. The *Boswellia occulta* sample had the lowest result for acid fraction size, at 27% compared to the resin mass. All the grades of *Boswellia papyrifera* and the *Boswellia occulta* resin had acid fractions dominated by β-11-keto-boswellic acids (46.1-50.5%), followed by β-boswellic acids (20.9-22.9%), α-boswellic acids (11.8-12.9%), 11-methoxy/hydroxyl and 9,11-dehydro-β-boswellic acids (10.4-12.4%) and all had small concentrations of 11-methoxy/hydroxyl and 9,11-dehydro-α-boswellic acids (0.5-1.0%). Therefore, the fraction which is not detected by GC/MS may have a lot to do with the size of the resin may be due to small concentrations of pigments which can have a large effect.

Table 4.2. Summary of the isolation of incensole and its acetate from *Boswellia papyrifera* and *Boswellia occulta* frankincense. See scheme 4.1 for experimental steps taken. Mass recoveries based on the initial resin mass.

| Step | | *Boswellia papyrifera* | | | | *Boswellia occulta* |
|---|---|---|---|---|---|---|
| | | Grade 1 | Grade 2 | Grade 3 | Grade 4 | |
| **a** | Resin (g) | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| **b** | Neutral fraction (g) | 2.9 | 2.6 | 2.9 | 3.0 | 2.5 |
| **c** | Acid fraction (g) | 4.3 | 4.6 | 4.4 | 3.5 | 2.7 |
| **d** | Incensole acetate (g) | 1.4 | 1.2 | 0.9 | 1.5 | 1.4 |
| | Mass recovery (%) | **13.9** | **11.8** | **9.1** | **15.1** | **14.0** |
| **e** | Incensole (g) | 0.9 | 0.9 | 0.6 | 1.2 | 1.1 |
| | Mass recovery (%) | **9.4** | **8.6** | **6.3** | **12.2** | **11.0** |

Isolating these compounds on the larger scale is not as simple unfortunately, due to the high cost of running purification through standard column chromatography experiments. The main cost comes from the large volume of solvent required to carefully isolate a pure compound. Furthermore, there is a difficulty in the size of the column if there is too large an amount of an oil to purify. Silica is required usually in around 10-20 times the mass of the crude oil, which requires very large columns when compared to the amount of product to be purified. If significantly less silica is used relative to the crude oil, the separation will be very poor, as the resultant mixture eluted will contain both high and low running compounds. Therefore, a simple and scalable method was required.

## 4.1 Scalable Incensole Acetate Isolation Trials from *Boswellia occulta*

The main idea would be to extract all the incensole/incensole acetate from the resin using maceration or $CO_2$ and convert any incensole to the acetate. Purification could then be achieved by successive distillations and performing column chromatography using only a small amount of stationary phase to give a high incensole acetate fraction. Several columns could be carried out to counter the effect of overloading and the stationary phase could be recycled after removing the slow running compounds using a polar solvent such as methanol.

Due to its simpler diterpene fraction, the *Boswellia occulta* resin was chosen over the Sudanese samples to investigate to begin with. The general method followed for this section was the protocols described in **Section 6.10**. Extractions were carried out by maceration, followed by filtration to give a crude extract . Extracts of this resin were acetylated using acetic

anhydride, pyridine and DMAP under reflux. This typically gave oils with incensole acetate concentrations of around 45% by HPLC. However, different mass recoveries were observed depending on the extraction solvent. Extractions with petroleum spirits (40-60) gave 40-50% mass recoveries of crude essential oil compared to the resin mass whereas chloroform and methanol were around 56% and 64% respectively. This means that based on these approximate values, around 18-28% incensole acetate content was detected by HPLC in these extracts when compared to the resin mass. Although this was useful information going forward to the purification stages, it should be noted that HPLC gives only an estimate on the content within the samples taken, and these estimations do not take into account undetected compounds. The impurities at this stage were mainly comprised of heavier compounds, which are more polar, such as amyrins and boswellic acids. This means a purer product could be achieved using simple techniques such as column chromatography or distillation. In a standard column chromatography experiment using normal phase silica gel, the desired diterpene, incensole acetate elutes faster than the triterpene impurities, but slower than the volatile constituents, such as methoxydecane. As for distillation, heavier compounds tend to boil at higher temperatures, meaning incensole acetate and methoxydecane could be isolated as separate distillates and the boswellic acids and amyrins could be left behind as a residue.

Several stationary phases were tried for use in flash column chromatography trials based on method (c) in **Section 6.10**. Filtering over silica, basic alumina and acidic alumina all yielded purer oils with less colour that were less viscous, indicating less contamination from heavier compounds. Around 5 g of crude incensole acetate, prepared as previously described was mixed with 5 g of the solid support in petroleum spirits (40-60) and filtered over a further 20 g of the solid support, which was eluted with 30 mL of 10% $Et_2O$ in petroleum spirits (40-60). The resultant mixture was evaporated to give a higher purity incensole acetate product. Acidic alumina and basic alumina gave mass recoveries of 20% and 15-21% respectively, relative to the initial resin mass, with high incensole acetate content (62-67% and 62-71% by HPLC). Using silica, however, gave only a very small mass of oil of around 1% compared to the initial resin mass. After further elution with another 50 mL of 10% $Et_2O$ in petroleum spirits (40-60), similar results to the alumina supports were found (70% incensole acetate by HPLC) but with a lower mass yield of around 7% compared to the resin mass. After further elution purity was decreased. Since silica was found to have a higher loading capacity for these oils, trials were

continued based on this solid support as it is the cheapest of the three solid supports to buy. Furthermore, because of silica gel's higher loading capacity as mentioned, less would have to be handled, which would be more practical on the larger scale. Next, a suitable mobile phase and quantity of silica relative to the amount of crude incensole acetate would need to be determined to optimise the purity and the resultant oil yield of the isolation of incensole acetate.

Various experiments were tried on crude incensole acetate mixtures. Typically, 5 g of a stock of crude incensole acetate oil (approximately 45% by HPLC) was dissolved in petroleum spirits (40-60) and thoroughly mixed with silica before filtering over a bed of silica. The pre-mixing with silica was to allow for better contact time with the solid support and therefore, theoretically result in a purer product. The oil was eluted with varying mobile phases, all using diethyl ether and petroleum spirits (40-60) in varying relative polarity. Just like in a regular column chromatography experiment, higher polarity mobile phase would result in more of the polar compounds eluted, which are mostly derived from boswellic acids. Therefore, a lower volume of diethyl ether was used and a larger volume of petroleum spirits (40-60) to avoid stripping too many impurities back in to the incensole acetate filtrate. Once the solvent was removed, the oils' estimated mass recoveries were calculated by comparing the oil mass to the mass of the resin (table 4.3).

Table 4.3. Summary of incensole acetate fractions by filtering crude mixture through silica gel columns. Mass recovery is the oil mass relative to the resin mass. Content estimated by HPLC analysis.

| **Trial Number** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| **Silica mixed with oil (g)** | 5 | 5 | 2.5 | 5 | 5 | **5** | 5 |
| **Silica in column (g)** | 5 | 7.5 | 7.5 | 10 | 10 | **5** | 5 |
| **$Et_2O$ in petroleum spirits (40-60) (%)** | 5 | 10 | 10 | 10 | 20 | **10** | 20 |
| **Mobile phase volume (mL)** | 50 | 40 | 40 | 90 | 60 | **30** | 30 |
| **Mass recovery (%)** | 24 | 19 | 19 | 15 | 14 | **28** | 28 |
| **Incensole acetate content (%)** | 70 | 71 | 66 | 72 | 75 | **70** | 57 |

All of the experiments resulted in an increase in the incensole acetate purity, as expected (57-75%). However, the balance between mass recovery and purity would be the determining factor for going forward. Trials 1, 6 and 7 all gave high mass recoveries, but 7 had a large drop in incensole acetate content as a result of its good mass yield. Trials 2-5 all had less oil yielded and did not see a large increase in purity when compared to 1 and 6. Experiments 4 and 5

were carried out using 30 mL of mobile phase successively, due to the larger amount of silica being used, which gave a profile for the experiment. Between each 30 mL of mobile phase used, the resultant filtrate was concentrated under vacuo to isolate the oil. These oils were weighed and their incensole acetate content analysed by HPLC, before eluting the column with another 30 mL solvent into the same flask for analysis (table 4.4).

Table 4.4. Cumulative incensole acetate fractions obtained by successive elution through a larger bed of silica.

| **Mobile phase (mL)** | **4** | | **5** | |
|---|---|---|---|---|
| | MR[a] (%) | InAc[b] (%) | MR[a] (%) | InAc[b] (%) |
| **30** | 1 | 3 | 1 | 3 |
| **60** | 10 | 70 | 14 | 75 |
| **90** | 15 | 72 | 22 | 60 |

a) Mass recovery is the oil mass relative to the resin mass

b) Incensole acetate content estimated by HPLC analysis following the method in **Section 6.03**

After the initial 30 mL of solvent, both 4 and 5 had very low mass recoveries, as the fraction obtained was mainly essential oil, which passes through silica very quickly, even in non-polar solvents. In both cases, reasonable fractions were isolated after 60 mL of solvent, with 5 having a higher mass recovery, due to using a more polar mobile phase of 20% diethyl ether in petroleum spirits (40-60) as opposed to 10% diethyl ether in petroleum spirits (40-60). However, a further elution in trial 5 resulted in a significant drop in purity, as more of the slower running compounds had also eluted. After 3 elutions in trial 4, no more were carried out as further elution would require too large a volume of solvent for the process to be convenient and cheap and reasonable incensole acetate content had already been isolated.

Trial 6 seemed to give the best results out of the trials ran with incensole content at 70% with a mass recovery of 28% compared to the resin. Although 2, 4 and 5 gave higher detectable incensole acetate on HPLC (71-75%), the difference was only small, and the overall mass of the isolated oil was lower than in 6. Trials 1 and 6 gave very similar results, with mass recoveries compared to the resin of 24% and 28% respectively, with both having an incensole acetate content of 70% as determined by HPLC. These two experiments both used the same masses of silica for the purification but differed in the mobile phase. Trial 1 used 50 mL of 5% $Et_2O$ in petroleum spirits (40-60) and trial 6 used 30 mL of 10% $Et_2O$ in petroleum spirits (40-60), meaning that trial 6 would be the more practical method moving forward, due to having

to use less solvent. This result was convenient as it used a low amount of silica and solvent, which on the larger scale will result in a more economical procedure.

Since experiment 6 gave the best results, it was used as a template for scaling the process up from the 10 g scale to the 100 g scale of resin extracted as described in steps (a) to (c) of **Section 6.11**. Thus 100 g of *Boswellia occulta* resin was milled before extracting successively three times by maceration followed by filtration, first using 200 mL of petroleum spirits (40-60) followed by two more extractions with 100 mL of petroleum spirits (40-60). After evaporation of the combined filtrates, mass recoveries were obtained between 40-44% compared to the resin mass. The next step was to acetylate the crude oil, which was performed using acetic anhydride, pyridine and DMAP. Even at this scale, the reaction was still complete after 1 hour, which is the length the reactions were allowed on the smaller scale. This shows that this step is scalable, along with having similar mass recoveries of oil compared to the resin at this point, being around 40-45%. Following this, as done in trial 6, the crude incensole acetate oil was dissolved in the minimum volume of petroleum spirits (40-60) and mixed with silica. In this case, 50 g of silica was used, as it directly scales up by a factor of 10 compared to the experiment in trial 6. This mixture of solution and silica was filtered over a column of 50 g more silica and eluted with 300 mL of 10% $Et_2O$ in petroleum spirits (40-60) and the combined filtrates were concentrated in vacuo to give pale yellow oils which contained reasonable incensole acetate content. The mass recoveries of these oils compared to the resin mass were between 15-24% and had incensole acetate concentrations between 63-70%. The difference in mass recovery highlights the inconsistency in the resin, but a likely reason is just that more bark was in the lower mass recovery extraction, as the resin contains much larger amounts of incensole. It is also likely that the resin itself produces varying concentrations of different compounds due to environmental factors and harvesting methods. Understanding what factors result in an increase in the production of these valuable diterpenes would be useful in obtaining a more ideal resin, but that is out of the scope of this work due to the number of variabilities and the difficulty in monitoring them in a controlled environment.

Although this method worked well to get a good mass recovery of the incensole acetate fraction, when scaled up it became more difficult to perform well. This is because of the pre-mixing with the stationary phase, as when adding this mixture to the column, quite a large

amount of petroleum spirits (40-60) would need to be used to wash all the mixture across to the column and there was a higher chance of the column's stationary phase being disturbed. Therefore, additional trials at the 10 g scale were carried out without mixing with stationary phase before the column was performed following step (d) **Section 6.10** instead of step (c) while keeping the other steps the same. This allowed for less solvent to be used and made it simpler to perform on a larger scale, as the stationary phase would remain undisturbed. This also allowed for the column to be re-used, as the slow running fraction could be collected in MeOH and the column's relative polarity could be reset with $Et_2O$ followed by petroleum spirits (40-60). Approximately a 1:1 ratio of MeOH volume to silica volume was required to sufficiently flush the column, meaning not much solvent was required and the silica could be used again continuously. This flush step was also quite time efficient, as it could be carried out while evaporating the eluted incensole acetate fraction to isolate the oil.

Four samples of incensole acetate oils were prepared from four separate 10 g resin samples, extracted by $CHCl_3$, $Et_2O$, MeOH and IMS by maceration following various steps in **Section 6.10**. The $Et_2O$ extract had the acid fraction removed by KOH extraction before evaporating the organic to retrieve a neutral fraction containing incensole. Each of the four resultant oils were acetylated by using pyridine, acetic anhydride and DMAP to give a crude incensole acetate product. Each crude oil was dissolved in the minimum amount of petroleum spirits (40-60) at room temperature before carefully adding to the column packed with 5 g of silica. The mixtures were eluted with 30 mL of 10% $Et_2O$ in petroleum spirits (40-60) and the resultant mixtures were evaporated. After this first step of purification, the oils had similar incensole acetate concentrations of 54-62% as determined by HPLC. After the column was flushed and reset with MeOH followed by $Et_2O$ and petroleum spirits (40-60), the oils were applied to the column again. After a total of three successive isolations through the column by the same method, a set of high purity incensole acetate fractions were obtained (table 4.5). Each washing of the column with MeOH was isolated and analysed by HPLC to assess the incensole acetate content left behind in the column after the elution with the 10% $Et_2O$ in petroleum spirits (40-60). Fortunately, very little of the desired diterpene was detected in the polar fraction (around 1% in each) meaning very little incensole acetate was lost during successive purifications while quite large fractions were removed. These combined MeOH

flush fractions had mass recoveries of up to 22% compared to the resin mass, which is a large amount of impurities to remove from these complex oils.

Table 4.5. Comparison of four separate experiments to isolate incensole acetate fractions following the protocol in **Section 6.10**. Initial extraction solvent represents the solvent used to extract the resin, which was the only variable in the method. The mass recovery is the oil mass relative to the resin mass. Content estimated by HPLC analysis following the method in **Section 6.03**.

| Initial extraction solvent | Mass recovery (%) | Incensole acetate content (%) |
|---|---|---|
| **Dichloromethane** | 30 | 67 |
| **Diethyl ether** | 14 | 73 |
| **Methanol** | 20 | 64 |
| **Methylated Spirits** | 14 | 74 |

Each experiment gave a similar detectable concentration of incensole acetate, with a narrow range of 64-74%. However, the dichloromethane extract appears to give the best results in this test, with a significantly larger amount of oil yielded. As expected, each successive purification by column chromatography found an increased purity of incensole acetate, as detected by HPLC at around 6.8 minutes at 210 nm, but quite a lot of oil mass was lost via this. It is likely that not all the matter in the oil is detected by HPLC, even when detecting over several wavelengths using a diode array detector (figures 4.05-4.07) which would explain the large losses in oil mass for only small increases in purity. Furthermore, the HPLC data supported this, as the MeOH flush fractions which would elute any remaining incensole acetate only contained small amounts which was usually less than 1% content, except for the extraction using IMS, which detected a 4% content in the oil. Most notably, the IMS extract lost nearly half of its oil mass between the first and third purification step (table 4.6).

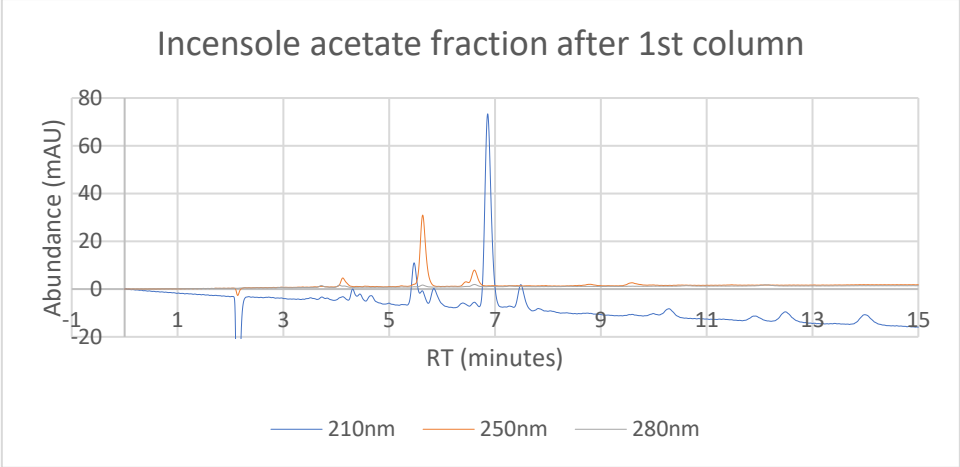



Figure 4.05. Chromatogram of crude incensole acetate (RT ~6.8 minutes) at 210, 250 and 280 nm after first column chromatography experiment from the IMS extract.

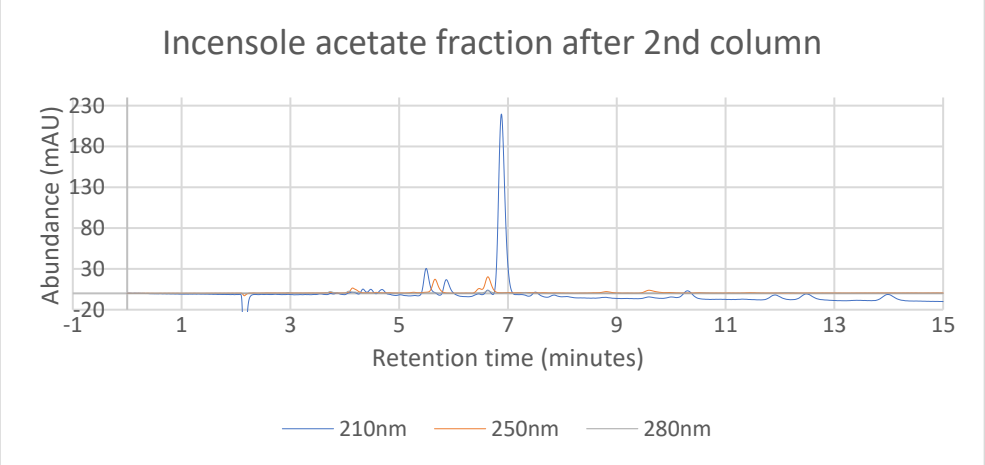



Figure 4.06. Chromatogram of crude incensole acetate (RT ~6.8 minutes) at 210, 250 and 280 nm after second column chromatography experiment from the IMS extract.

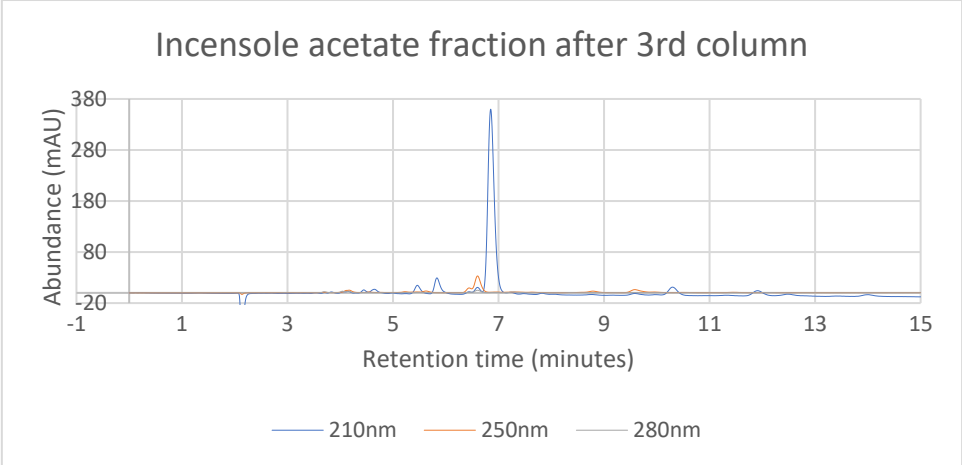



Figure 4.07. Chromatogram of crude incensole acetate (RT ~6.8 minutes) at 210, 250 and 280 nm after third column chromatography experiment from the IMS extract.

Table 4.6. Successive purifications using flash column chromatography of the IMS extracted oil following the method in **Section 6.10**. Mass recovery is the oil mass relative to the resin mass. Content estimated by HPLC analysis.

| | **Successive purification steps** | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **Mass recovery (%)** | 26 | 20 | 14 |
| **Incensole acetate (%)** | 59 | 65 | 74 |

Further purification was achieved by distillation and column chromatography using basic alumina as the stationary phase. Various crude incensole acetate samples were freshly prepared for vacuum distillation using a Kugelrohr, obtained by MeOH (i), $CHCl_3$ (ii) and petroleum spirits (40-60) (iii) extraction and subsequently purified by column chromatography using basic alumina as the stationary phase. Each extraction was separate from each other, and started with the maceration of the milled resin and followed steps (a), (b), (d), (e) and (f) in **Section 6.10**. After evaporating the solvent under vacuo, the mass recoveries of oil compared to the resin was 56%, 52% and 39% for the MeOH, $CHCl_3$ and petroleum spirits (40-60) extracts respectively, with each oil having a similar content of incensole detected. Each oil had a similar incensole content, but based on the differing mass recoveries, showed some variance with the MeOH extract having the highest content (figure 4.08).

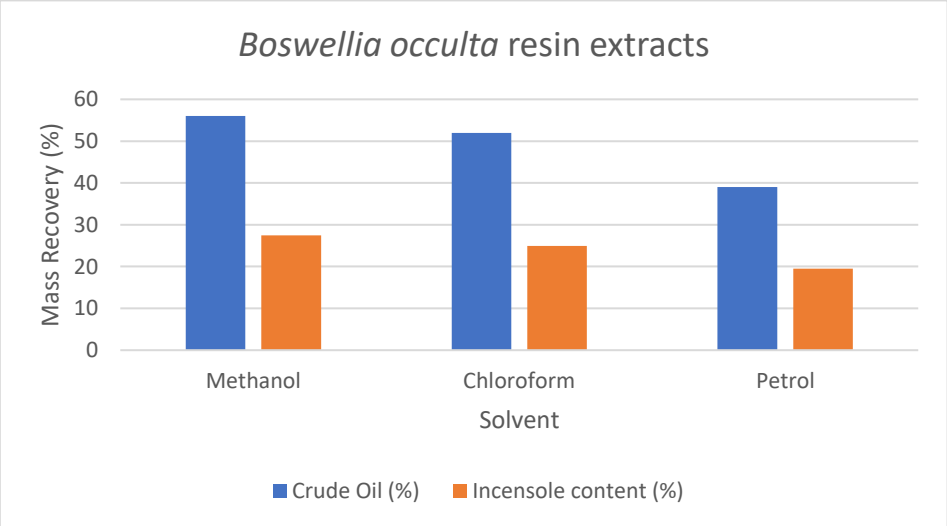



Figure 4.08. Comparison of the different extracts of *Boswellia occulta* resin. The Mass recovery is based on the resin mass. The crude oil represents the whole oil extracted and the incensole content illustrates what portion of the crude oil is incensole as detected by HPLC.

Each extract was acetylated using acetic anhydride, pyridine and DMAP which gave very similar incensole acetate contents in the oils (45-47% as detected by HPLC). However, there were quite different mass recoveries recorded compared to the resin mass (41-64%), meaning these HPLC readings would need to be adjusted to give a true representation of the incensole acetate content with respect to the resin mass. The mass recoveries at this stage were 64%, 56% and 41% for the MeOH, $CHCl_3$ and petroleum spirits (40-60) extracts respectively, so applying this to the incensole acetate content gives estimates of incensole acetate content similar to the incensole content shown for the previous step. However, the MeOH and $CHCl_3$ extracted oils increased in mass a noteworthy amount whereas the petroleum spirits (40-60) extracted oil remained quite similar. (figure 4.09). This likely means that the MeOH and $CHCl_3$ extracts contain more compounds that can be acetylated under the conditions applied and therefore had a larger set of compounds increasing in mass due to OH groups being replaced with OAc groups.

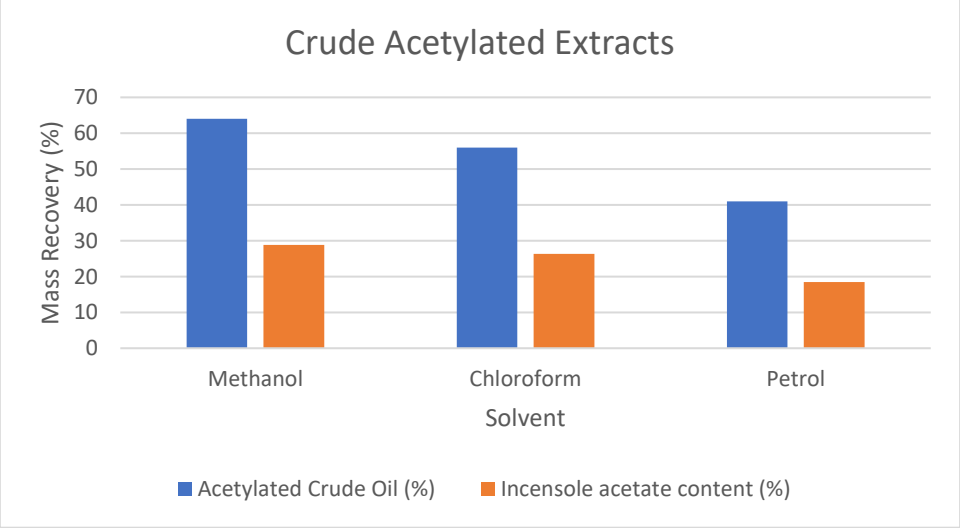



Figure 4.09. Comparison of the different crude acetylated oils from the extracts of *Boswellia occulta* resin. The Mass recovery is based on the resin mass. The acetylated crude oil represents the whole oil isolated after the reaction and the incensole acetate content illustrates what portion of this oil is incensole acetate as detected by HPLC.

These oils were each subjected to three successive column chromatography flushes with 10% $Et_2O$ in petroleum spirits (40-60) as described in step (d) of **Section 6.10**. This resulted in the isolation of oils with mass recoveries of 38%, 31% and 20% for the MeOH, $CHCl_3$ and petroleum spirits (40-60) trials with regards to the resin mass. Again, the HPLC data is shown as representative compared to the resin mass in, but this time the incensole acetate content

is a much larger part of the oil as performing column chromatography removed a significant amount of heavy compounds that elute more slowly than the desired diterpene (figure 4.10).

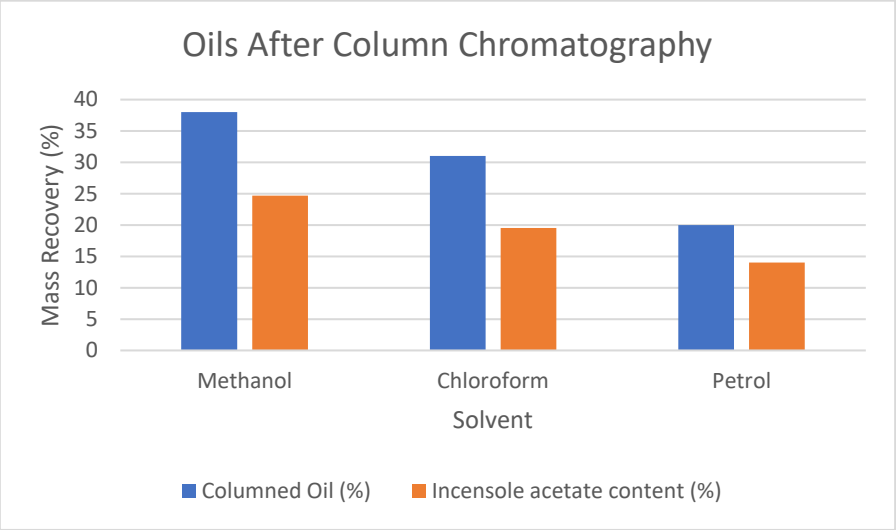



Figure 4.10. Comparison of the different acetylated oils after column chromatography using the extracts of *Boswellia occulta* resin. The Mass recovery is based on the resin mass. The columned oil represents the whole oil isolated at this stage and the incensole acetate content illustrates what portion of this oil is incensole acetate as detected by HPLC.

Finally, the oils were each subjected to vacuum distillation using a Kugelrohr at 125°C for 25 minutes, following the protocol in step (e) in **Section 6.10** to remove the volatile constituents. The residue fraction was collected and gave mass recoveries of 32%, 26% and 16% for the MeOH, $CHCl_3$ and petroleum spirits (40-60) trials with regards to the resin mass. The MeOH and $CHCl_3$ trials both gave oils with 67% incensole acetate content, but the petroleum spirits (40-60) experiment had the highest content at 76% at the cost of having a lower mass recovery (figure 4.11)

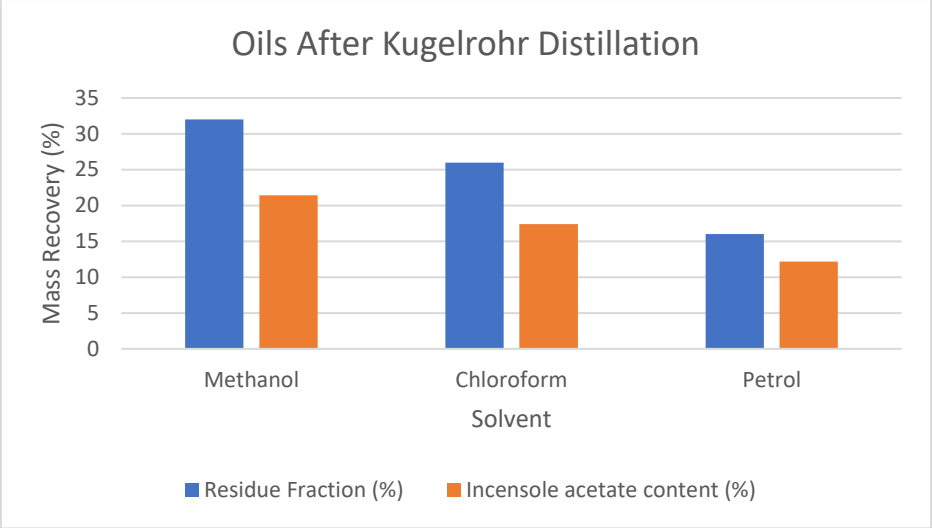



Figure 4.11. Comparison of the different acetylated oils after column chromatography and Kugelrohr distillation using the extracts of *Boswellia occulta* resin. The mass recovery is based on the resin mass. The residue fraction represents the whole oil isolated at this stage and the incensole acetate content illustrates what portion of this oil is incensole acetate as detected by HPLC.

All distillations gave a higher purity fraction of incensole acetate. However, removal of some diterpene impurities and similar running heavy oils proved difficult. GC/MS analysis indicated that a significant concentration of the impurities came from boswellic acids, therefore another column chromatography experiment was attempted on each of the three oils. Each of the three oils were separately dissolved in petroleum spirits (40-60), mixed thoroughly with 5 g of basic alumina (based on half the initial resin mass) and added separately to columns packed with 20 g basic alumina. Elution with 60 mL of 10% $Et_2O$ in petroleum spirits (40-60) and evaporation of solvent led to the isolation of three oils (table 4.7).

Table 4.7. Incensole acetate fractions after further purification via column chromatography using basic alumina as the stationary phase. Three separate experiments were performed with extraction solvent being the only variable following the protocol in **Section 6.10**. Mass recovery is the oil mass relative to the resin mass. Content estimated by HPLC analysis.

| Trial | Mass recovery (%) | Incensole acetate content (%) |
|---|---|---|
| **Methanol** | 20 | 83 |
| **Chloroform** | 16 | 82 |
| **Petroleum spirits (40-60)** | 10 | 80 |

This column chromatography step emulates the base extraction performed with 2% KOH solution and showed a significant rise in incensole acetate content for the methanol and

chloroform prepared oils (figures 4.12 and 4.13 respectively). The petroleum spirits (40-60) prepared oil (figure 4.14) saw a small increase in purity, with a lower decrease in the mass of the resultant oil which could be due to the oil containing a lower abundance of boswellic acid impurities.

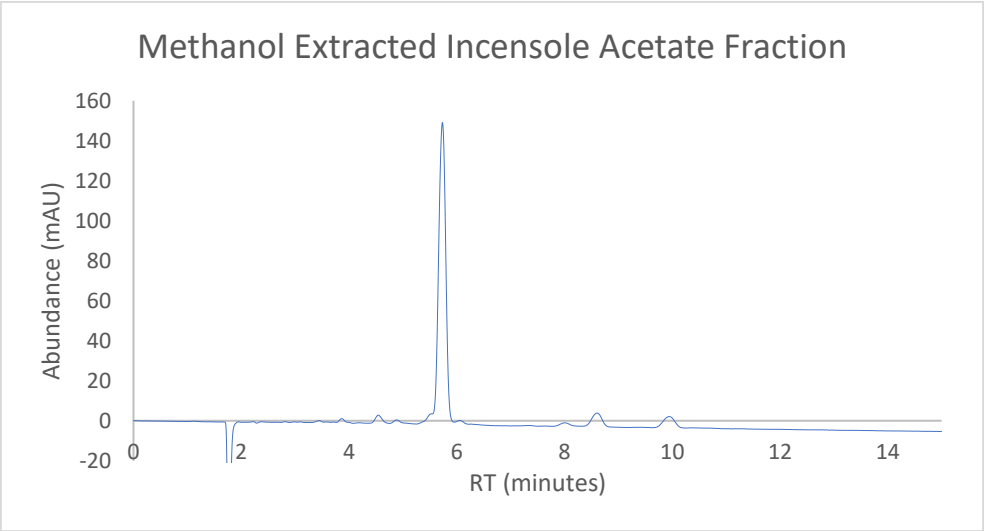



Figure 4.12. Chromatogram of incensole acetate (RT = 5.8 minutes) isolated from the methanol extract of *Boswellia occulta*. Detected at 210 nm, 83%. Solvent front at RT = 1.8 minutes.

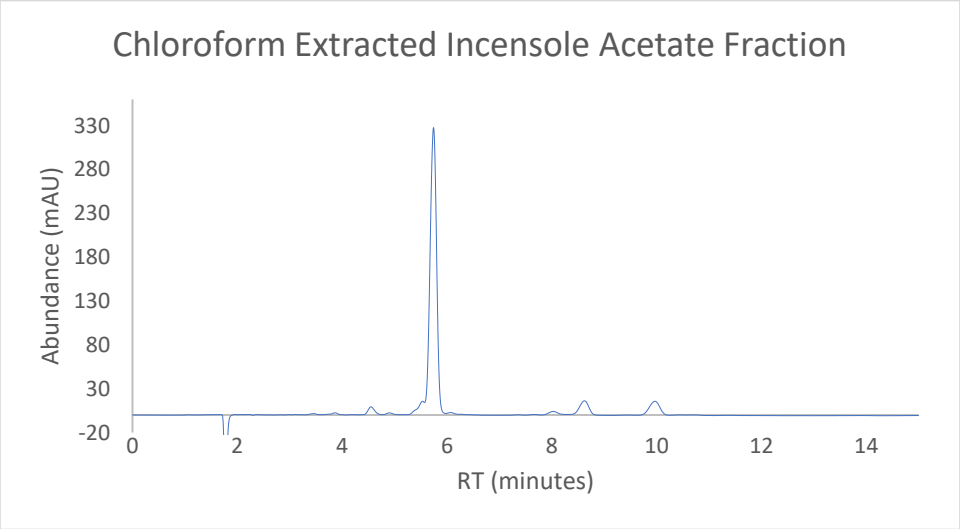



Figure 4.13. Chromatogram of incensole acetate (RT = 5.8 minutes) isolated from the chloroform extract of *Boswellia occulta*. Detected at 210 nm, 82%. Solvent front at RT = 1.8 minutes.

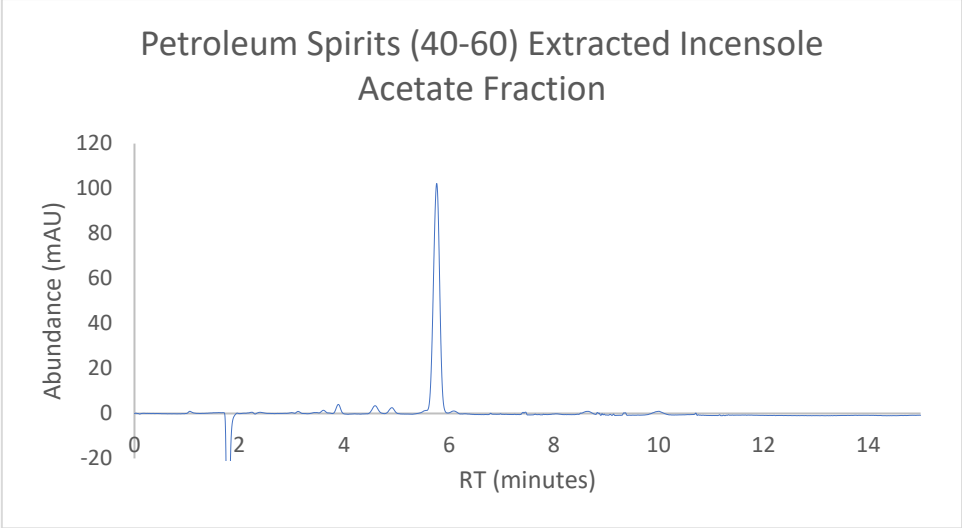



Figure 4.14. Chromatogram of incensole acetate (RT = 5.8 minutes) isolated from the petroleum spirits (40-60) extract of *Boswellia occulta*. Detected at 210 nm, 80%. Solvent front at RT = 1.8 minutes.

This suggests that the chloroform and methanol prepared extracts contained more of the hard to remove, lower running compounds such as the boswellic acids and amyrins. With a purity of 80-83% detected by HPLC at 210 nm, this version of the method was by far the best. Adding the basic alumina filtration seemed to help retain some of the faster running acids on silica and gave much cleaner chromatograms for the oils.

Due to the fairly significant mass removed by filtration over basic alumina, another trial was performed, where the acid fraction was removed by extraction with KOH solution. Firstly, the milled resin was extracted three times successively by maceration in $Et_2O$ following step (a) in **Section 6.10**. The resultant solution of oil in $Et_2O$ was extracted by a 2% solution of KOH using a separating funnel following step (b) in **Section 6.09**. The organic layer was collected, dried and evaporated to yield an oil of 21% mass recovery compared to the resin mass, which will be referred to as the neutral fraction. The aqueous layer was collected by acidifying using concentrated HCl and extracting three times with $CHCl_3$. After drying and evaporating, the acid fraction was subjected to HPLC analysis and showed less than 1% incensole acetate content, so the neutral fraction was taken to the next step. The neutral fraction was acetylated following step (b) in **Section 6.10,** using acetic anhydride, pyridine and DMAP and the resultant oil had a mass recovery of 23% compared to the resin mass, with an incensole acetate content of 56%. This oil was purified over three successive column chromatography experiments as described in step (d) of **Section 6.10** and the resultant oil had a mass recovery

of 14% compared to the resin mass. This oil was in high incensole acetate purity and was then distilled under vacuum using a Kugelrohr, as described in step (e) of **Section 6.10** at 125°C for 25 minutes to remove the volatile constituents. The residue fraction was collected and had a mass recovery of 12% compared to the resin mass. This oil had an incensole acetate content of 76% which was lower than the oils isolated in the trials prior to this which were columned over a basic alumina bed in addition to the three successive silica columns (figure 4.15). However, extraction by KOH is cheaper than using basic alumina to remove the acidic constituents, so that is something to consider if this process would be scaled up.

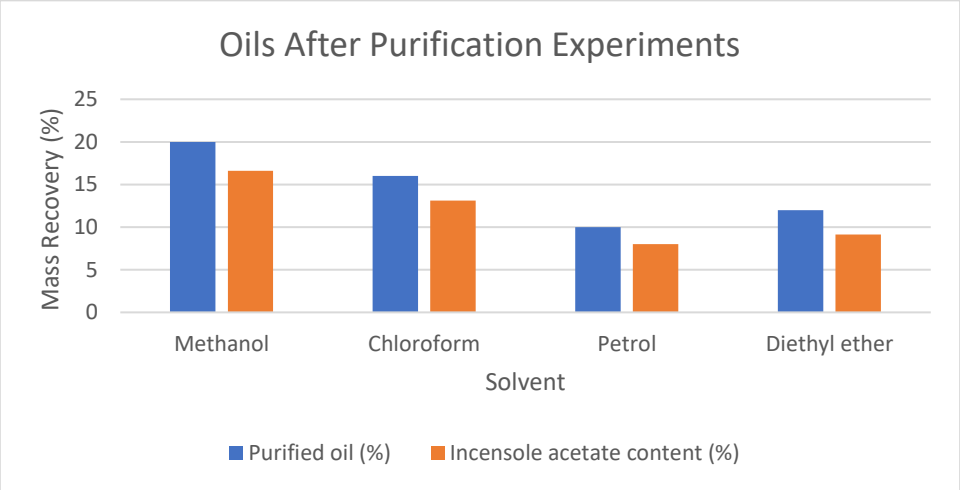



Figure 4.15. Oils isolated through extractions using the solvents stated, followed by a series of steps to acetylate the oil and subsequently isolate high purity oils using simple scalable methods as described in this section. Mass recovery refers to the oil mass compared to the resin. Incensole acetate content was determined by HPLC.

The distillate fractions of the Kugelrohr experiments were low in mass compared to the large residue fractions and were combined for GC/MS analysis to test their incensole acetate content for each experiment (table 4.8).

Table 4.8. Volatile fractions distilled in four separate experiments which followed the same method for I, II and III and an adapted method as described in this section. Each oil was initially extracted using a different solvent; I) MeOH; II) $CHCl_3$, III) Petroleum spirits (40-60); IV) $Et_2O$. Mass recovery is the oil mass relative to the resin mass. Content estimated by GC/MS analysis following method (b) in **Section 6.02**.

| | Mass recovery (%) | Incensole acetate content (%) | Methoxydecane content (%) |
|---|---|---|---|
| **I** | 2 | 10 | 55 |
| **II** | 2 | 22 | 32 |
| **III** | 2 | 26 | 44 |
| **IV** | 1 | 15 | 52 |

The low mass recovery of all the distillates from these Kugelrohr experiments indicates only a small part of the oil was removed during the distillation. This resin is known to only contain a small volatile fraction, so this makes sense. Furthermore, GC/MS analysis showed the incensole acetate content in this fraction was quite low (10-26%) and was mainly dominated by methoxydecane (32-55%), the biomarker compound for *Boswellia occulta's* essential oil.

## 4.2 Scalable Incensole Acetate Isolation from *Boswellia papyrifera*

The incensole acetate content in the Sudanese resins was compared using the same, simple methods. As found in **Section 4**, the Sudanese resins give a range of around 9-15% incensole/incensole acetate content when purified by standard column chromatography, depending on the grade.

To give an insight on how much incensole and incensole acetate there is in each resin compared to each other, 50 mg of each resin was taken and extracted in 10 mL chloroform thoroughly. The extract was then analysed by GC/MS. This gave an idea on what content can be found compared to the resin mass, as resins with more incensole and incensole acetate should give larger absorbances, even if their relative concentration is lower (figure 4.16).

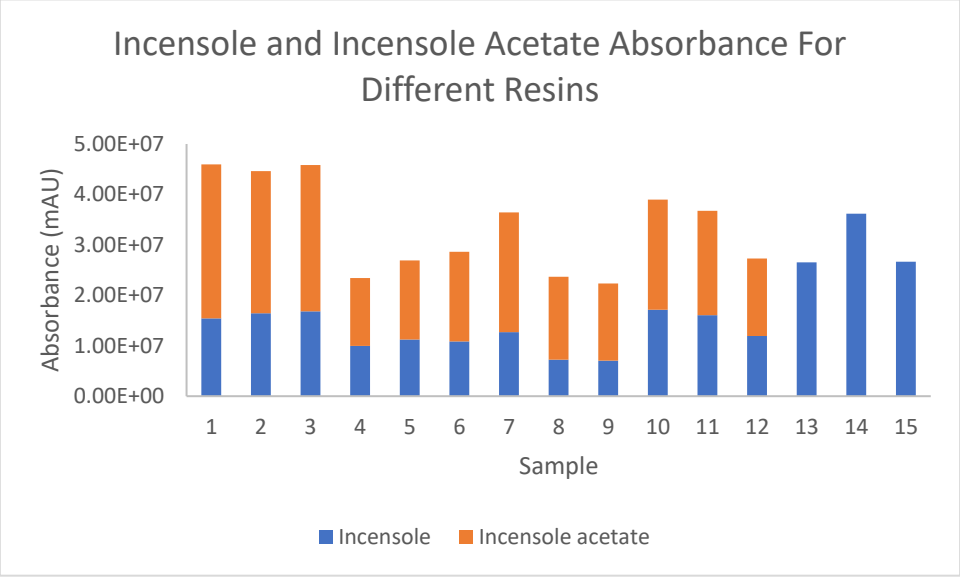



Figure 4.16. Significant diterpene content in Sudan frankincense grade 1 (i-iii), grade 2 (iv-vi), grade 3 (vii-ix) and grade 4 (x-xii) and *Boswellia occulta* chloroform extracts as determined by GC/MS following method (b) in **Section 6.02**.

Overall, it seems that the cumulative content of incensole and incensole acetate is largest in grade 1 of the Sudanese frankincense. Grade 4 of the Sudanese material also has a large

concentration of these oils. The *Boswellia occulta* resin has the most incensole content, but does not produce incensole acetate in detectable levels. However, its simpler chromatogram made it the choice for the main purification trials in this section. Investigations to assess how the quality of incensole acetate fractions in grades 1-4 of *Boswellia papyrifera* resin compared with *Boswellia occulta* resin following steps (a), (b), (d) and (e) in **Section 6.10** were carried out.

Thus, 10 g of each grade of *Boswellia papyrifera* resin was finely milled and then separately extracted exhaustively with $CHCl_3$. After evaporation, the oils were each acetylated using acetic anhydride, pyridine and DMAP to convert all the incensole to incensole acetate. This resulted in oils with mass recoveries between 60-69% compared to the resin mass. This was followed by three successive flushes through a column packed with 5 g of silica using 10% $Et_2O$ in petroleum spirits (40-60) (30 mL) as an eluent, which after evaporation yielded oils containing reasonable concentrations of the key diterpene. After each successive flush through the column, the isolated oil was analysed by HPLC to test how much the purification step had improved the purity of oil with regards to incensole acetate content (figures 4.17-4.19).

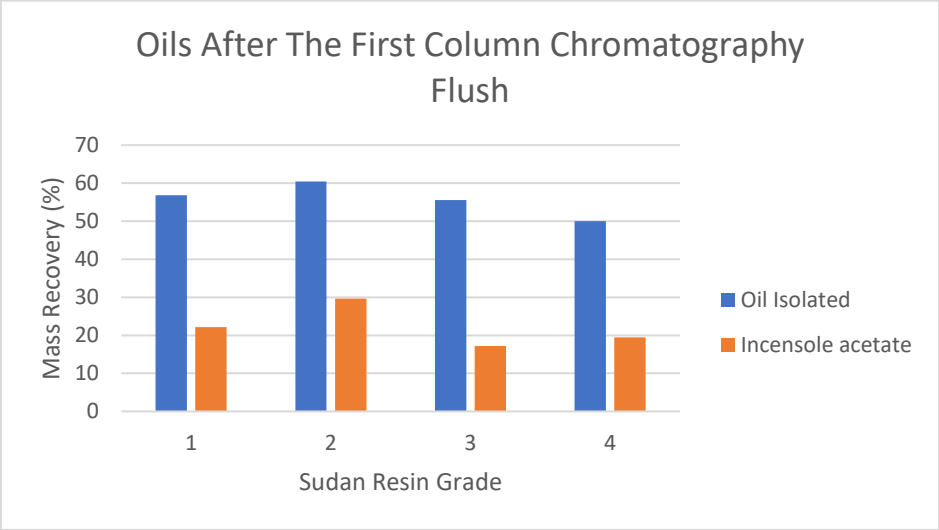



Figure 4.17. Incensole acetate content compared to the oil isolated after the first column chromatography experiment as determined by HPLC. Mass recovery is based on the resin mass.

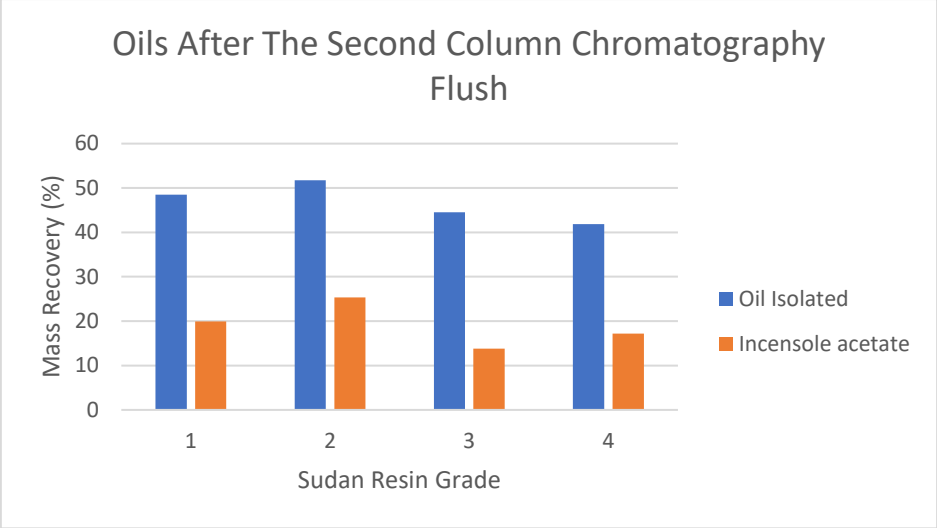



Figure 4.18. Incensole acetate content compared to the oil isolated after the second column chromatography experiment as determined by HPLC. Mass recovery is based on the resin mass.

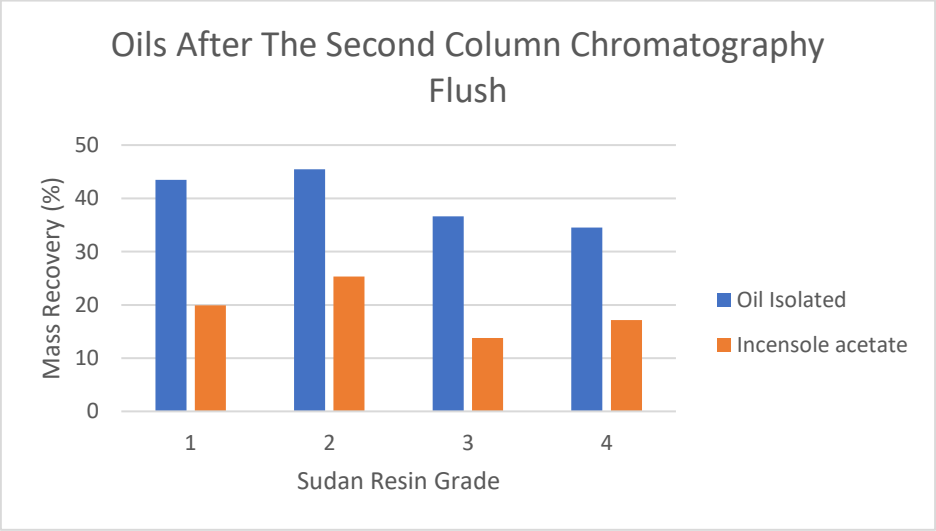



Figure 4.19. Incensole acetate content compared to the oil isolated after the third column chromatography experiment as determined by HPLC. Mass recovery is based on the resin mass.

However, as the graphs show, the incensole acetate content as determined by HPLC did not change a lot throughout the three successive flushes whereas the mass of the oil isolated was significantly reduced after each flush. This indicates that the Sudanese resins may contain reasonable amounts of compounds that are not detected through the HPLC experiments that were carried out in this work.

To further purify the oils, they were separately distilled at 150$^{o}$C for 25 minutes at around 4 mBar using a Kugelrohr to give a residue fraction high in incensole acetate. Each step of this experiment was repeated for 10 g of the *Boswellia occulta* resin to compare to (table 4.9).

Table 4.9. Summary of the incensole acetate fractions of grades 1-4 of the *Boswellia papyrifera* resins and the *Boswellia occulta* resin. Mass recovery is the oil mass relative to the resin mass.

| | **Mass recovery (%)** | **Incensole acetate content (%)** |
|---|---|---|
| **Grade 1** | 35 | 44 |
| **Grade 2** | 38 | 58 |
| **Grade 3** | 30 | 40 |
| **Grade 4** | 29 | 59 |
| ***B. occulta*** | 23 | 76 |

These results show that this isolation method for incensole acetate is much more suited to the *Boswellia occulta* resin. Most of the impurities in the Somaliland based incensole acetate are small impurities that add up to reduce the purity of the oil, but the Sudanese resins have some difficult to remove diterpenes and some seem to have retained large concentrations of triterpenes (figures 4.20-4.24).

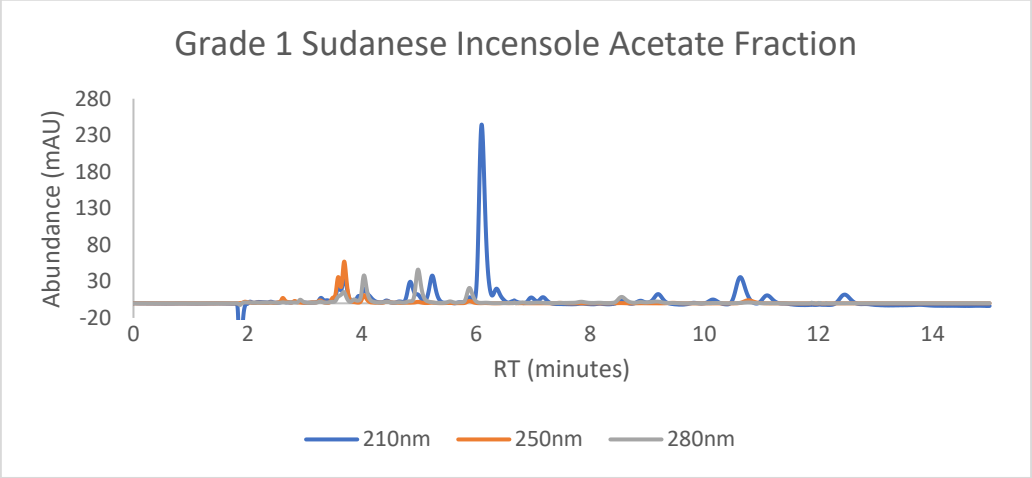



Figure 4.20. RP-HPLC-DAD analysis of the Grade 1 Sudanese's incensole acetate fraction isolated.

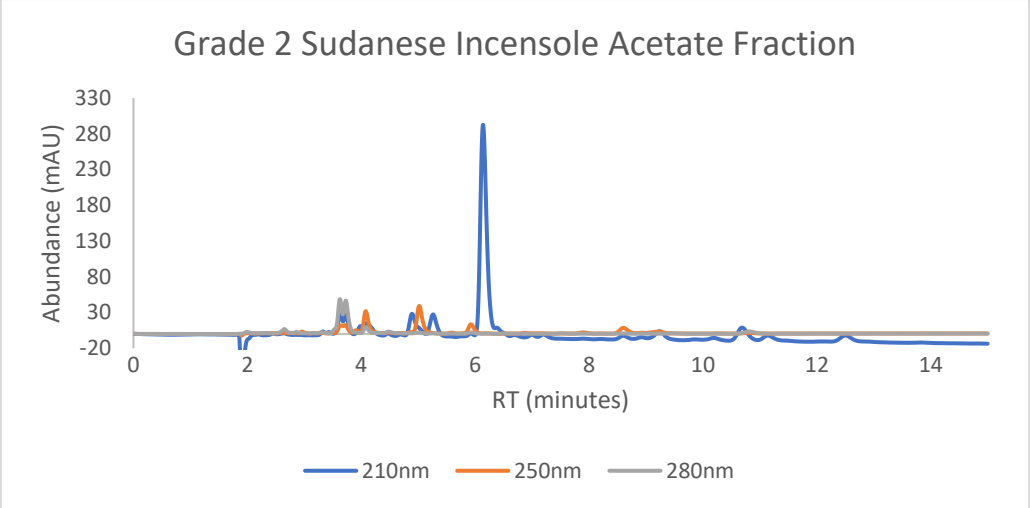



Figure 4.21. RP-HPLC-DAD analysis of the Grade 2 Sudanese's incensole acetate fraction isolated.

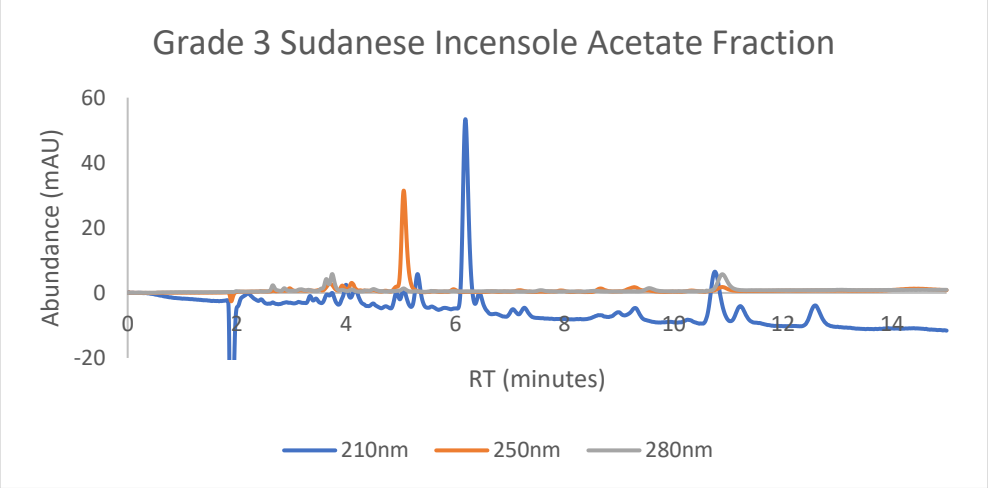



Figure 4.22. RP-HPLC-DAD analysis of the Grade 3 Sudanese's incensole acetate fraction isolated.

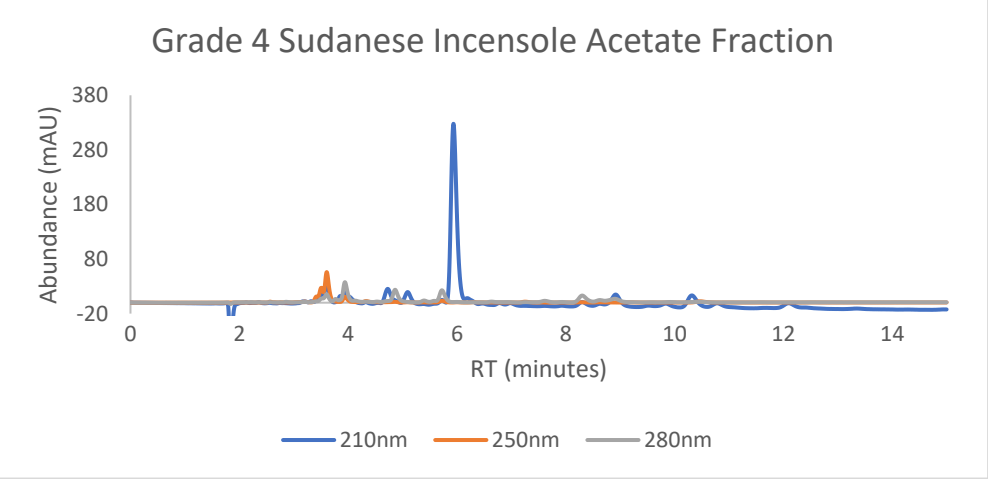



Figure 4.23. RP-HPLC-DAD analysis of the Grade 4 Sudanese's incensole acetate fraction isolated.

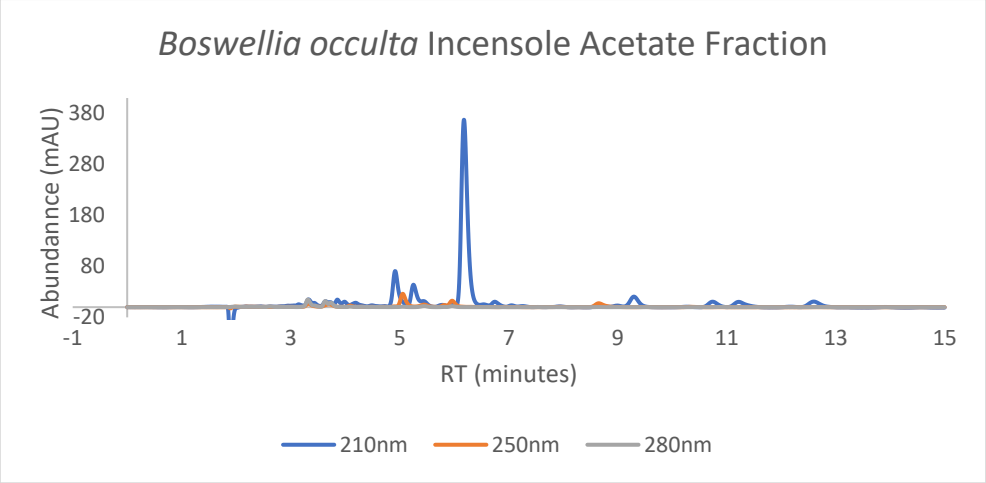



Figure 4.24. RP-HPLC-DAD analysis of *Boswellia occulta's* incensole acetate fraction isolated.

It can be clearly seen that at 210 nm, 250 nm, and 280 nm, the Sudanese based incensole acetate fractions have retained far more impurities than the *Boswellia occulta* sample prepared by the same method.

The highest incensole acetate content was found in the second and fourth grade for the Sudanese resin by HPLC. This is contrary to what was discovered in the GC profiles of the Sudan resins in **Section 2.3**. Grade 1 was also supposed to be high in these diterpenes but showed quite low content and relatively low purity. Therefore, further work would be required to make Sudanese resins viable for this type of purification method.

This resin is fairly complex chemically, as many of the frankincense species are. Therefore, using only simple methods to get incensole acetate content of up to 83% by HPLC was considered a success. Each step did very little to leave any of this diterpene behind, while increasing its overall purity and in the cases of column chromatography, used material which could be used multiple times to improve the cost implications of these methods. The polar fractions after column chromatography and the distillates from the Kugelrohr experiments all had very little incensole acetate in. To increase the purity more on the large scale, thin film distillation would be required. This would allow for more surface interactions, which would theoretically distil at a narrower range for each compound. If the temperature was increased to where the incensole acetate would leave the residue fraction, it would allow for only a small amount of the heavy oils to be collected with it. Furthermore, more volatile compounds could be collected separately from the incensole acetate fraction resulting in purer fractions.

Isolating an incensole acetate fraction this way and successively distilling would, in theory increase the purity even more when compared to the Kugelrohr method.

# Chapter 5. Conclusion

There were two main aims for this study. The first was to give a detailed description of compositions of various frankincense species through different methods of extraction to identify compounds of interest. The second was to develop methods to efficiently isolate these as either high purity compounds or fractions containing high content in the desired compounds. The main methods available were distillation, extraction by supercritical $CO_2$, maceration and Soxhlet, organic reactions, column chromatography and liquid-liquid extraction.

To achieve a detailed description of the compositions of all the resins available, the essential oils were firstly examined. The essential oils could all broadly be characterised as 3 chemotypes: α-pinene, octyl acetate or methoxydecane. All three *Boswellia sacra* resins and both *Boswellia carterii* resins were of the α-pinene variety. This is fairly typical of these two species, however there were some differences found in these fractions. The *Boswellia sacra* essential oils were a very high purity of α-pinene (up to 80.1%), however the *Boswellia carterii* essential oils made up a larger content relative to the overall mass of the resin (up to 8.3%). The resin responsible for the highest essential oil content was the resin from Gudmo Biyo Cas, which was one of the *Boswellia carterii* resins. The Gudmo Biyo Cas resin therefore became of considerable interest for developing methods for the isolation of this fraction. Extraction of the essential of this resin by alternative techniques to protect the thermally unstable constituents of the oil were quite thoroughly explored using supercritical $CO_2$. Under mild conditions, high mass yields compared to the resin were isolated with high essential oil contents, which could simply be distilled under a vacuum to give pure fractions of volatile oils. The method was scaled up from the 100 mL extractor scale to the 2 L extractor scale, which after adjusting for the different flow rates available on the 2 L $CO_2$ rig, can be directly scaled up to the pilot plant scale. This method for essential oil extraction provides a good template for exhausting resins of their volatile constituents while mostly not disturbing the heavier oils, to be fractionated separately if desired.

The remaining five resins produced essential oils belonging to the other two chemotypes determined during this work: octyl acetate for four grades of *Boswellia papyrifera* and methoxydecane for the *Boswellia occulta* resin. Although these fractions were not of

particular interest and also were produced in low content compared to the resin mass (up to 2.7%), this was the first work known at the time of writing that compared the compositions of different grades of *Boswellia papyrifera*. Therefore, the results regarding these resins and their compositions can be used as a template for the four grades studied. However, the main reason for the interest in these resins, including the *Boswellia occulta* sample was due to the presence of incensole in each of their essential oils and the presence of incensole acetate in all except for the *Boswellia occulta* sample. Due to these being diterpenes, which are much less volatile than monoterpenes and sesquiterpenes, the low content of them is not definitive of the whole resin and extractions of the bulk content of the resin showed this, with high diterpene content isolated from each of these resins. This made these resins of considerable interest, as one of the main focuses of this project was to isolate key compounds within these resins, with incensole acetate being the most desirable due to the interesting properties of this compound, discussed in **Section 1.6**. A number of methods were therefore developed in this work, each with their own advantages, involving a simple scalable method for the isolation of this compound in high yields and purity at low cost.

Each of the *Boswellia papyrifera* and the *Boswellia occulta* samples were extracted by maceration, followed by filtration to assess their resinous fractions. GC/MS analysis determined high diterpene content in all of them. Incensole can be easily converted to its acetate in high yields, so after the crude extracts had been acetylated and subsequently purified by column chromatography using the method in **Section 6.09**, incensole acetate yields of 9-15% were isolated compared to the resin mass. Since column chromatography can require large quantities of solvent and silica gel, which makes it expensive and can be impractical to scale up, other methods to isolate high purity incensole acetate oils were desired. Several methods of extraction using supercritical $CO_2$ were developed for these diterpene fractions. Extracting at 300 bar pressure gave fractions high in incensole acetate content. Using a co-solvent to aid solubility (c.a. 5-10% of the flow rate) isolated fractions of slightly lower purity, but the extraction times were as low as an hour to exhaust the resin of its diterpene fraction and could be distilled simply to remove the other fractions of the oil. In contrast, a more controlled extraction using only a small amount of co-solvent (c.a. 1% of the flow rate) yielded very high incensole/incensole acetate content fractions in high oil masses. For example, a mass recovery compared to the resin of 22% was isolated for an oil with 80%

incensole content as determined by GC/MS from the *Boswellia occulta* resin. The *Boswellia papyrifera* diterpene fraction was quite diverse, so proved difficult to get as high purity as the *Boswellia occulta* resin, which the main impurities were amyrins and boswellic acids, which should be simple to fractionate using a thin film evaporator.

A method that utilised simpler techniques that could be scaled up for the isolation of incensole acetate was also of interest for this work. Successful trials followed variations on the general procedure in **Section 6.10** which consisted firstly of maceration of the milled resin in a suitable solvent followed by filtration. Evaporation of the solvent isolated oils of around 40-60% mass recovery compared to the resin mass depending on the extraction solvent, with up to 40% incensole acetate content by HPLC. Since incensole acetate was both simpler and faster to purify than incensole by column chromatography, the crude mixture was acetylated simply with pyridine, acetic anhydride and DMAP at reflux for one hour. After isolation of the resultant mixture of incensole acetate and other resinous compounds, column chromatography was used to remove the slower eluting impurities (5 g silica and 30 mL of 10% $Et_2O$ in petroleum spirits (40-60) per 10 g of resin mass extracted). The column could be simply regenerated afterwards for successive purifications by firstly exhaustively extracting the column by flushing methanol through (2 mL methanol per 1 g of silica gel). The relative polarity was reset by flushing $Et_2O$ followed by petroleum spirits (40-60) through (1 mL solvent per 1 g silica gel). It was found that the *Boswellia occulta* resin gave highest purities of incensole acetate through this method, as the *Boswellia papyrifera* resin contained a rich diterpene fraction with other cembrene type compounds which were difficult to separate. As a result, optimizations were based around the *Boswellia occulta* resin and after distillation under a vacuum to remove the volatiles and column chromatography over basic alumina (5 g basic alumina mixed with crude oil and 20 g basic alumina in the column, 30 mL of 10% $Et_2O$ in petroleum spirits (40-60) per 10 g resin mass extracted), high content incensole acetate fractions (83% HPLC) in high mass recoveries compared to the resin mass (up to 20%) were isolated. This was considered a success as all impurity fractions contained very little incensole acetate content and further purification using a thin film evaporator could potentially isolate even higher purity fractions. Variations on these methods were used on the 100 g scale and results were concordant with the 10 g scale, affording mass recoveries of 15-17% based on the resin mass with high incensole content (63-70% determined by HPLC). The 100 g scale

methods have not been tested further than the maceration followed by acetylation and subsequent column chromatography steps, so going further and subjecting to vacuum distillation and basic extraction with either basic alumina or 2% KOH solution would likely result in even higher purities of incensole acetate.

## 5.1 Final Remarks

Detailed compositional investigations were carried out on *Boswellia sacra,* and *carterii* frankincense resins as well as the first comparison of the highest four grades of *Boswellia papyrifera.* Furthermore, a relatively newly discovered species, *Boswellia occulta,* was investigated at length for its high value diterpene fraction. Incensole acetate was made semi-synthetically and subsequently isolated in high purity using simple, cost-effective techniques that can be directly scaled up over several steps. As a result, the methods developed hitherto can be used as a template for future investigations such as purification of the incensole acetate fractions using a thin film evaporator.

# Chapter 6. Experimental Methods

The methods undertaken throughout this work are detailed as written in the following segments. These experimental methods are described as general procedures and details on the exact parameters are discussed throughout **chapters 2-4**. Unless otherwise noted, reactions were stirred and monitored by TLC. TLC plates were visualized using iodine, phosphomolybdic acid or under UV light. All anhydrous reactions were conducted under a static argon atmosphere using oven dried glassware that had previously been cooled under a constant stream of nitrogen. Reagents, dry solvents and starting materials were purchased from commercial suppliers and used without further purification. Flash column chromatography was performed on Davisil® silica gel (35-70 microns) with the eluent specified in each case, TLC was conducted on precoated E. Merck silica gel 60 F254 glass plates.

The raw materials used were all frankincense resins, which consisted of two *Boswellia carterii*, one *Boswellia occulta*, four *Boswellia papyrifera* and three *Boswellia sacra* resins.

***Boswellia carterii*** – Samples named Gudmo biyo cas and Erigavo throughout this work.

The Gudmo biyo cas sample was harvested from the town Gudmo biyo cas, which is in the Sanaag region of Somaliland. Approximately 5 kg of the resin was initially supplied of various sizes and shades of gold to dark brown before another 28 kg of a similar size and colour mix was supplied later on in the project, after finding particular interest in both the essential oil and diterpene fractions. The resin was supplied by Asli Maydi Exports Ltd and contained various resin piece sizes of up to around 3 cm. There were some larger clumps up to around 5 cm and above, which seemed to be where the resin had merged together, likely from heat during storage and transport.

The Erigavo sample was harvested from Erigavo, the capital city of the Sanaag region of Somaliland. Approximately 5 kg of the resin was supplied by Asli Maydi Exports Ltd and was various sizes of up to around 3 cm diameter and different shades of gold to brown. There were some larger clumps up to around 5 cm and above, which seemed to be where the resin had merged together, likely from heat during storage and transport.

***Boswellia occulta*** – This sample was referred to as the North Coast resin or *Boswellia occulta* sample throughout this work.

The North Coast sample was harvested from the North Coast of Somaliland close to Xiis in the Sanaag region of Somaliland. Approximately 5 kg of the resin was supplied by Asli Maydi Exports Ltd and was various sizes of up to around 3 cm diameter and different shades of gold to brown. There were some larger clumps up to around 5 cm and above, which seemed to be where the resin had merged together, likely from heat during storage and transport.

It should be noted that the two *Boswellia carterii* samples and the *Boswellia occulta* sample were thought to all be *Boswellia carterii* when supplied. It was only through the differing compositions and importantly, the unique essential oil fraction of the North Coast resin found during this work, that it was assumed to be the more recently discovered species, *Boswellia occulta*. Furthermore, all three of these samples were harvested from within about 30 miles of each other, which gives further reason that the North Coast sample is of a different species.

***Boswellia papyrifera*** – Grades 1-4 of this resin was supplied by Juniper Global Ltd. Each sample was about 2.5 kg in mass and were from the Gol region of South Sudan. The grades were consisted with the literature definitions for this species.[93]

Grade 1-3 were mixtures of white and gold pieces. Grade 1, which is the highest grade, were pieces of resin that were over 6 mm or more in diameter. Grade 2 was a mixture of 4-6 mm diameter pieces of resin. Grade 3 was a mixture of 2-4 mm diameter pieces of resin. Grade 4 was a mixture of pieces of any size, but were various shades of brown and some darker gold pieces.

***Boswellia sacra*** – Three grades of this resin were supplied, each of which were about 1 kg in mass. These three samples were all kindly supplied by Dhofar University in Salalah.

The Hoojri grade sample was a mixture of pale yellow pieces up to about 1 cm in diameter. This is the highest grade of *Boswellia sacra* resin and was harvested from Samhan Mountain in the Dhofar region of Oman.

The Najdi grade sample was a mixture of different shades of brown and were up to around 1 cm in diameter. This is the second highest grade of *Boswellia sacra* and was harvested from Hafid Mountain in the Dhofar region of Oman.

The Sha'abi grade resin was a mixture of different shades of brown and black and were up to around 1 cm in diameter. This is the fourth highest grade of *Boswellia sacra* and was harvested from Maghseel, slightly inland from the coastal side, which is in the Dhofar region of Oman.

Often it was found that resin pieces had stuck together, likely from the heat from where they were harvested. This meant that there were larger pieces than mentioned here, but the resin sizes were based on the size of the 'tear' shapes the resin naturally forms as the resin drips from the plant.

## 6.01 Handling of Resin and Prepping for Experiments

All resin samples were stored in a cool dry place. The resins were freshly milled when possible before each experiment using a clean, dry pestle and mortar. To give the most even spread of the colour and size of the resin, a larger portion of evenly mixed pieces were taken and milled, before sampling for the experiment. Multiple extractions were often done in the same day to avoid wasting the resin while ensuring the experiment used fresh resin. Furthermore, in the cases of carrying out multiple extrations using the same parameters, a much larger portion was milled (up to 10 times the mass required for a single experiment) and stored in a cool, dry, dark place. Care was taken to prepare samples that consisted of evenly distributed selections of resin in an attempt to make the data reproducible. In cases where the resin was quite soft and difficult to effectively mill, the resin was cooled using a small amount of liquid nitrogen or dry ice until it was brittle and simple to mill.

## 6.02 GC/MS Analysis

There were two GC/MS machines and methods used during this work based on the availability of the machines. Analysis of the hydrodistilled oils was carried out using machine and method (a) and all other oils were analysed using machine and method (b):

a) This GC/MS system consisted of a Trace1300 GC/MS equipped with a Restek rxi-5ms column (30 m x 0.25 mm inner diameter), Thermo ISQ mass spectrometer and an RSH autosampler. Oven temperature programmed to rise from 60$^{o}$C to 300$^{o}$C at a rate of 6$^{o}$C/min; injector temperature at 250$^{o}$C; interface temperature, 280$^{o}$C; carrier gas, He; average velocity, 29 cm/s; flow rate, 10 ml/min; splitless.

b) This GC/MS system consisted of an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer (EI detector) equipped with a Zebron ZB-5ms column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness). Oven temperature programmed to rise from 60°C to 300°C at a rate of 6°C/min; injector temperature at 250°C; interface temperature, 280°C; carrier gas, He; average velocity, 37 cm/s; flow rate, 53.8 ml/min; split ratio 50:1.

The GC/MS data was all analysed using AMDIS software.[16] The sum of the area of all the peaks was calculated before calculating the ratio of an individual peak compared to the total area of all peaks to give the content for each compound. This is an estimate, as there are several factors that influence the accuracy of these results. The baseline can interfere with peak area, more significantly for smaller peaks. If there are a large number of low content peaks taken into consideration, this will skew the content profile, as lower content peaks will be considered larger than they may be in reality, whereas the major peaks will be slightly less with regards to their ratio with the entire oil. Furthermore, less volatile compounds reach the gas phase less easily, resulting in an inaccurate detection of their content and sometimes no detection at all. This was observed during the isolation of pure serratol in **Section 2.2.2** with lower serratol being isolated than the chemical profile given by GC/MS showed was in the oil. This technique is still useful for giving an overview of the major compounds and general chemical profile in the extract, but is much more accurate when analysing volatile mixtures.

Compounds were tentatively assigned based on their fragmentation patterns and their relative retention indices. The relative retention index was calculated using the equation 1.3 in **Section 1.3.2** to provide a universal retention time which was useful for the identification of volatiles. Along with literature data on a certain oil's general composition, this data was also compared to the NIST database and in some cases, pure compounds were confirmed by NMR spectroscopy.[21] Each fragmentation pattern included in this work has a reference, where possible, in the figure description which contains a fragmentation pattern for the compound in question, which is consistent with the data reported here.

## 6.03 HPLC Analysis

The HPLC system consisted of an SR-3000 solvent rack, a TCC-3000SD thermostatted column compartment, a DAD-3000 Diode Array Detector, an LPG-3400SD quaternary pump and a

WPS-3000SL analytical autosampler. Before each injection the column was equilibrated for 15 minutes with a flow of 1 mL/min of acetonitrile containing 0.1% acetic acid and the temperature of the column oven was set to 40$^{o}$C. Each sample was made up as a 1 mg/mL solution in acetonitrile and injected into an Acclaim Reverse-Phase C18 (150 mm x 4.6 mm) column at a rate of 1 mL/min. The mobile phase used was acetonitrile containing 0.1% acetic acid and the elution continued for 15 minutes, at which point no new material was detected, as was tested for over an hour under these conditions in some cases. The Diode Array Detector allowed for detection at several wavelengths during the same experiment, so chromatograms were ran at 210, 250 and 280 nm, although most was detected at 210 nm.

Analysis was carried on Chromeleon software, where peak areas were given and the content of a specific peak could be calculated based on its relative size compared to the total area of all peaks. HPLC was used primarily to assess the incensole acetate content in an oil. Therefore, to characterize which peak was incensole acetate, a sample purified via column chromatography was used as a reference standard. A reference standard of incensole was also made through purificaton of an extract containing incensole. This was used to know the retention time for incensole so HPLC could be used to determine whether the acetylation or hydrolysis reactions were complete. These reactions will be detailed later in this chapter. Impurities were no characterised, but with GC/MS information on these extracts, they could generally be assumed to be either diterpene impurities, amyrins or essential oil.

## 6.04 NMR Spectroscopy

A 500MHz Bruker NMR ultrashield magnet system was used for the NMR experiments with an internal deuterium lock at ambient temperature at 400/100 MHz with internal references of δH 7.26 and δC 77.016 ppm for $CDCl_3$. This was operated using a Bruker Advance 3 console with Topspin 3.2 software. Typically, 30-40 mg of sample was taken in 0.7 mL $CDCl_3$ and submitted for $^{1}H$, $^{13}C$ and HSQC NMR spectroscopy and structural assignment was carried out using MNova software.

## 6.05 Hydrodistillation

All hydrodistillations were carried out using standard Clevenger apparatus where excess condensed water was fed back to the round bottom flask in a reverse dean-stark setup until

completion. The distilled oil sits in a small solvent reservoir above the water due to its lower density and being immiscible with water. After cooling the oil was collected by carefully using the tap of the Clevenger to decant the water layer separately. The resultant volatile oil was weighed and submitted for GC/MS analysis following method (a) in **Section 6.02**. All hydrodistillation experiments were carried out using 15+/-0.3 g of resin and 150 mL of water. All distillations appeared to be complete after around 45 minutes by visual inspection, but were left for 90 minutes total distillation time in order to ensure completion of the experiment.

*Example procedure:*

A finely milled sample of Hoojri grade *Boswellia sacra* (15.1 g) in water (150 mL) was hydrodistilled for 90 minutes and the resultant distillate was collected as the essential oil fraction (1.1 g, 7.2% mass recovery compared to the resin mass) as a pale yellow non-viscous oil. GC/MS analysis of this oil showed α-pinene (76.6%), sabinene (3.9%), limonene (3.0%), trans verbenol (2.7%) and d-3-carene (2.3%) as the major constituents.

## 6.06 Extraction by Maceration

Different solvents were used in different experiments to allow for comparison of the oil mass isolated relative to the resin's mass and GC/MS determined the content of various compounds. All of these extractions were carried out at room temperature and were on the same scale. Different scales were trialled, but it made no noticeable difference in the product's relative mass and GC/MS determined content. The extraction process followed these steps:

i) The chosen solvent (40 mL) was added to the resin (1+/-0.03 g) in a 100 mL conical flask.

ii) The flask was swirled by hand for 30 seconds before the mixture was filtered into a round bottom flask (250 mL).

iii) Any residue on the filter paper was added back to the conical flask carefully with a metal spatula.

iv) More of the same solvent was added to the conical flask (40 mL), carefully pouring over the spatula to get any residue off and back into the flask.

v) Steps ii-iv was repeated.

vi) Step ii was repeated a final time. This exhausted the resin of all that would go into that particular solvent at room temperature. It should be noted that longer maceration time was trialled (up to 24 hours stirred with a stirrer bar) and no difference was found in oil mass or GC/MS determined content, so swirling for 30 seconds was chosen for the efficiency.

vii) The combined filtrates were then concentrated under vacuo on a rotovap at 35$^{o}$C and the resultant oil was weighed. To ensure no solvent residue was remaining, the oil was attached to a stronger vacuum (~4 mBar) which had a cold trap submersed in liquid nitrogen to allow a stronger vacuum. This was left for 30 minutes before weighing again and this process was continued until there was no more difference in weight between weighing.

*Example procedure*:

The finely milled grade 2 *Boswellia papyrifera* resin (1.0 g) was extracted by maceration in $CHCl_3$ (3 x 40 mL) followed by filtration. The combined filtrates were evaporated in vacuo and weighed (0.7 g, 70% mass recovery compared to resin mass) giving a yellow viscous oil. GC/MS analysis following method (b) in **Section 6.02** showed incensole (27.9%), 24-norursa-3,12-dien-11-one (25.9%), incensole acetate (22.1%) and 24-norursa-3,12-diene (9.7%) as the major compounds.

## 6.07 Extraction by Soxhlet

In a typical Soxhlet extractor, a sample of 10 g milled resin was placed in a porous extraction thimble. Connected below the Soxhlet extractor was a round bottom flask with 100 mL of the required solvent. With a reflux condenser added to the top of the apparatus, the mixture was heated to reflux and the warm, condensed solvent extracts the resin continuously while the thimble filters the resin out of the bulk of the solvent, which is syphoned back into the round bottom flask. After 1 hour, the resin had been exhausted and the mixture was left to cool before evaporating the solvent in vacuo to give just the extract oil. The oil containing flask was repeatedly weighed and re-attached to the vacuum for periods of 30 minutes until no change in the mass was recorded. This viscous, yellow oil was typically submitted for GC/MS following method (b) in **Section 6.02**.

## 6.08 Extraction by Supercritical $CO_2$

All $CO_2$ work carried out during this project were carried out at Suprex Ltd. Both of the $CO_2$ rigs were Thar systems, consisting of an ABPR 200 (pressure regulator), a P10 co-solvent pump and a P50 $CO_2$ pump and were identical apart from different sized extractors and separators. The 100 mL extractor was coupled to a 25 mL separator and the 2 L extractor was coupled to a 500 mL separator for collection of the extract. Temperature, $CO_2$ flow rate, co-solvent flow rate and pressure were controlled Process Suite Software. All extractions were performed using a 100 mL or 2 L extractor as stated and the resin was finely milled before thoroughly mixing with the solid support. Both $CO_2$ and co-solvent were introduced before the ABPR which connected directly to the extraction chamber as was shown in figure 1.07 in **Section 1.3.4.1**. Variables such as flow rate of $CO_2$ and IMS, pressure, temperature, solid support choice, packing ratio and extraction time were subject to change throughout method development and are detailed in each case throughout the discussion in **Chapter 3**. After each experiment, the system was depressurized, the extraction residue was removed and then stored. All oils were subjected to GC/MS analysis following method (b) in **Section 6.02**.

*a) Essential oil $CO_2$ extraction trials with 100 mL extraction chamber size example:*

This method was performed to extract the essential oil fraction from *Boswellia carterii* resin (Gudmo Biyo Cas). To a 100 mL extractor vessel, a 2:1 mixture of sand and finely milled resin was added (87.9 g total, 29.3 g resin). After sealing the extraction chamber and heating to 40$^oC$, $CO_2$ was allowed to enter the system until a pressure of 100 bar was reached, at which point the flow rate of $CO_2$ was set to 10g/min and the resultant extract was taken after 30 minutes by tapping off the collection chamber, which had a back pressure of 20 bar to aid the collection of the product as a pale yellow, non-viscous oil (2.7 g, 9% mass recovery compared to the resin mass).

*b) Essential oil $CO_2$ extraction trials with 2 L extraction chamber size example:*

This method was performed to extract the essential oil fraction from *Boswellia carterii* resin (Gudmo Biyo Cas). To a 2 L extractor vessel, a 2:1 mixture of sand and finely milled resin was added (1610.0 g total, 536.7 g resin). After sealing the extraction chamber and heating to 40$^oC$, $CO_2$ was allowed to enter the system until a pressure of 140 bar was reached, at which point the flow rate of $CO_2$ was set to 40 g/min and the resultant extract was taken after 60

minutes by tapping off the collection chamber, which had a back pressure of 20 bar to aid the collection of the product as a pale yellow, non-viscous oil (48.4 g, 9% mass recovery compared to the resin mass).

c) *Incensole $CO_2$ extraction trials example:*

This method was performed to extract the incensole rich diterpene fraction from the *Boswellia carterii* resin (north coast, Somaliland). To a 100 mL extractor vessel, a 2:1 mixture of sand and finely milled resin was added (109.7 g total, 36.6 g resin). After sealing the extraction chamber and heating to 50$^{o}$C, $CO_2$ was allowed to enter the system until a pressure of 300 bar was reached, at which point the flow rate of $CO_2$ was set to 9.5 g/min and a flow rate of IMS as a co-solvent was set to 0.5 mL/min. The resultant extracts were taken after 60 minutes by tapping off the collection chamber, which had a back pressure of 20 bar to aid collection. After evaporation of the IMS by vacuo, a yellow viscous oil was isolated (10.0 g, 27% mass recovery compared to the resin mass).

*d) Slower $CO_2$ extraction without a co-solvent in the main phase example*:

This method was performed to extract the rich diterpene fraction containing incensole and incensole acetate from the four grades of *Boswellia papyrifera* resin (Sudan). To a 100 mL extractor vessel, a 2:1 mixture of sand and finely milled grade 1 *Boswellia papyrifera* resin was added (99.2 g total, 33.1 g resin). After sealing the extraction chamber and heating to 50$^{o}$C, $CO_2$ was allowed to enter the system until a pressure of 300 bar was reached, at which point the flow rate of $CO_2$ was set to 10 g/min. The resultant extracts were collected, weighed and submitted for GC/MS analysis separately every 30 minutes by tapping off the collection chamber, which had a back pressure of 20 bar to aid collection, resulting in a semi-viscous, pale yellow oil (4 hour supercritical total yield: 8.0 g, 24% mass recovery compared to the resin mass). After 4 hours, the $CO_2$ flow rate was reduced to 8 g/min and a flow rate of 2 mL/min of IMS was introduced and the resultant extract was collected every 15 minutes for 1 hour into a separate single flask. After evaporation of the combined extracts by vacuo, a yellow, viscous oil was isolated (co-solvent fraction yield: 15.6 g, 47% mass recovery compared to the resin mass).

*e) Faster $CO_2$ extraction using a co-solvent in the main phase example*:

This method was performed to extract the rich diterpene fraction containing incensole and incensole acetate from the four grades of *Boswellia papyrifera* resin (Sudan). To a 100 mL extractor vessel, a 2:1 mixture of sand and finely milled grade 4 *Boswellia papyrifera* resin was added (107.3 g total, 35.8 g resin). After sealing the extraction chamber and heating to 50$^{o}$C, $CO_2$ was allowed to enter the system until a pressure of 300 bar was reached, at which point the flow rate of $CO_2$ was set to 9.5 g/min and a flow rate of IMS as a co-solvent was set to 0.5 mL/min. The resultant extracts were collected into a single flask every 30 minutes by tapping off the collection chamber, which had a back pressure of 20 bar to aid collection. After evaporation of the IMS by vacuo, a yellow, viscous oil was isolated (15.6 g, 43.6% mass recovery compared to the resin mass). It should be noted that each 30-minute sample was evaporated, weighed and submitted for GC/MS analysis before the next extract was added so the extracts were initially collected separately to allow for adequate evaporation time, before successively adding each extract.

## 6.09 Isolation of Pure Incensole Acetate and Incensole from *Boswellia papyrifera* and *Boswellia occulta* Resin

Isolation of incensole acetate and then conversion back to incensole was performed over several successive steps, to be detailed. Briefly, the experimental steps were: a) extraction by maceration with $CHCl_3$, b) removal of the acidic constituents by extraction with 2% KOH, c) acetylation of the neutral fraction using acetic anhydride, pyridine and DMAP, d) flash column chromatography of crude incensole acetate, e) base hydrolysis using 1:1 of 5% KOH solution and IPA, f) flash column chromatography of crude incensole. This experiment was carried out on *Boswellia occulta* and grades 1-4 of *Boswellia papyrifera*. A specific example using grade 3 *Boswellia papyrifera* is described below. Solvent volumes and molar equivalents were the same for each experiment and 10.0 g was used in each case. In all cases, to ensure there was no residual solvent in the isolated oils, the flask was continually attached to a strong, liquid nitrogen assisted vacuum (~4 mBar) for periods of 30 minutes before weighing and returning to the vacuum for a further 30 minutes until there was no change in oil mass.

*a) Extraction by maceration example:*

The maceration step carried out followed the same steps as **Section 6.06**, except on a different scale, which remained consistent for all experiments following this method. Less solvent was required per gram of resin was used than in **Section 6.06**, as the extraction solvent mixes and extracts the resin very easily. The finely milled grade 3 *Boswellia papyrifera* resin (10.0 g) was extracted at room temperature by maceration in $CHCl_3$ (3 x 20 mL) followed by filtration. The combined filtrates were evaporated in vacuo to give an oily solid and weighed (7.37 g, 74% mass recovery compared to resin mass).

*b) Removal of the acidic constituents by extraction with 2% KOH example:*

Next, 7.37 g of crude extract was dissolved in $Et_2O$ (100 ml) and extracted twice with an equal volume of aqueous KOH (2%, 2 x 100 mL). The $Et_2O$ layer was washed with 1M HCl (50 ml), dried ($MgSO_4$) and evaporated under vacuum to give the neutral fraction as a pale yellow, viscous oil (2.85 g, 29% mass recovery compared to resin mass). The combined aqueous extracts were acidified (pH 1) with HCl (conc.) and extracted with $CHCl_3$ (3 x 50 mL) and the combined extracts were dried ($MgSO_4$) and evaporated under vacuum to give the acid fraction as a yellow solid (4.44 g, 44% mass recovery compared to resin mass). GC/MS analysis following method (b) in **Section 6.02** was carried out on the acid fraction to check incensole and incensole acetate were not lost into this fraction (trace incensole, 0.1% incensole acetate, >99% boswellic acids).

*c) Acetylation of the neutral fraction using acetic anhydride, pyridine and DMAP example:*

All molar equivalents were based on if the crude neutral fraction was pure incensole. Acetic anhydride (27.9 mmol, 2.64 ml, 3.00 equiv.) was added dropwise top a stirred solution of crude incensole extract (2.85 g) dissolved in $CH_2Cl_2$ (5 mL) and pyridine (37.2 mmol, 3.00 ml, 4.00 equiv.) at room temperature under a nitrogen atmosphere. A catalytic amount DMAP (56.8 mg, 0.46 mmol, 0.05 equiv.) was added and the resultant mixture was heated to reflux. After one hour, the reaction was complete (TLC and HPLC showed no more incensole). The mixture was cooled, diluted with $Et_2O$ (50 mL) and transferred to a separating funnel then washed with aqueous $NaHCO_3$ (Sat. 2 x 50 mL) and 2 M HCl (aq. 2 x 25 mL). The organic layer

was collected, dried over $MgSO_4$ and concentrated in vacuo to give crude incensole acetate as a pale yellow, viscous oil (2.86 g, 29% mass recovery compared to resin mass).

*d) Flash column chromatography of crude incensole acetate example*:

Crude incensole acetate (2.86 g) was purified by flash column chromatography on Davisil® silica gel (35-70 microns), using $Et_2O$ in petroleum spirits (40-60) as an eluent (gradient 2%, 4%). TLC was conducted on precoated E. Merck silica gel 60 F254 glass plates using $Et_2O$/hexane (10%). Similar fractions that seemed pure (by visual inspection) were collected in a single flask and evaporated in vacuo to give pure incensole acetate **53** as a colourless, viscous oil ($R_f$ =0.21, 0.91 g, 9% mass recovery compared to resin mass). NMR data was collected on the sample based on the method in **Section 6.04**.

$^1$H NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.92 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz), 1.13 (s, 3H), 1.53 (dddd, 1H, *J* = 14.2, 10.5, 5.1, 1.6 Hz), 1.57 (s, 3H), 1.61 (s, 3H), 1.62 (m, 1H), 1.64 (m, 1H), 1.66 (m, 1H), 1.78 (m, 1H), 1.86 (m, 1H), 1.89 (m, 1H), 1.93 (sep, 1H, *J* = 6.8 Hz), 2.05 (dd, 1H, *J* = 13.5, 5.0 Hz), 2.07 (s, 3H), 2.17 (m, 2H), 2.18-2.19 (m, 2H), 2.21-2.22 (m, 1H), 4.89 (d, 1H, *J* = 10.4 Hz), 5.18 (m, 1H), 5.19 (m, 1H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta_C$ 16.0, 17.7, 18.0, 18.0, 21.3, 22.0, 24.8, 27.7, 30.3, 32.0, 33.4, 34.9, 35.6, 38.5, 76.6, 83.1, 89.2, 121.1, 125.4, 133.2, 135.2, 171.2.

*e) Base hydrolysis using 1:1 of 5% KOH solution and IPA example:*

A solution of KOH (aq. 5%, 50 mL) was added slowly over 5 minutes to a stirred solution of incensole acetate **53** (0.91 g, 2.61 mmol) dissolved in IPA (50 mL). The resultant mixture was heated to reflux for 4 hrs, at which point TLC indicated the completion of the reaction. The resultant mixture was evaporated in vacuo, then dissolved in EtOAc (50 mL). This was washed with 2M HCl (2 x 25 mL). After drying ($MgSO_4$), filtration and evaporation under vacuo gave crude incensole **49** as a pale yellow, viscous oil (0.8 g, 8.0% mass recovery compared to resin mass).

*f) Flash column chromatography of crude incensole example:*

Crude incensole (0.80 g) was purified by flash column chromatography on Davisil® silica gel (35-70 microns), using $Et_2O$ in petroleum spirits (40-60) as an eluent (gradient 10%, 20%, 30%). TLC was conducted on precoated E. Merck silica gel 60 F254 glass plates using

$Et_2O$/hexane (30%). Similar fractions that seemed pure (by visual inspection) were collected in a single flask and evaporated in vacuo to give pure incensole **49** as a colourless, viscous oil ($R_f$ = 0.25, 0.63 g, 6.3% mass recovery compared to resin mass). NMR data was collected on the sample based on the method in **Section 6.04**.

$^1$H NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.91 (s, 3H), 0.93 (s, 3H), 1.09 (s, 3H), 1.34 (dddd, 1H, *J* = 13.9, 10.3, 5.4, 1.9 Hz), 1.52 (s, 3H) 1.61 (ddd, 1H, *J* = 11.3, 7.8, 4.0 Hz), 1.64 (s, 3H), 1.77 (m, 1H), 1.86 (m, 1H), 1.90 (br s, 1H), 1.93 (m, 1H), 1.98 (s, 1H), 2.06 (m, 1H), 2.06 (m, 1H), 2.13 (br dd, 1H, *J* = 8.2, 4.1 Hz), 2.13-2.19 (m, 2H), 2.13-2.19 (m, 2H), 2.19 (m, 1H), 3.32 (d, 1H, *J* = 10.2 Hz), 5.09 (m, 1H, *J* = 6.5 Hz), 5.13 (m, 1H, *J* = 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 100MHz): $\delta_C$ 16.1, 18.0, 18.1, 18.2, 20.7, 24.8, 30.6, 30.7, 32.4, 33.7, 34.8, 36.4, 38.6, 75.6, 84.2, 88.6, 121.8, 125.1, 134.2, 134.2.

## 6.10 Isolation of High Incensole Acetate Content Oils – 10 g Scale

A general procedure for the isolation of high incensole acetate fractions is outlined from grades 1-4 of *Boswellia papyrifera* and *Boswellia occulta* resin. These experiments were performed over several steps and during the discussion, various purification steps were sometimes not carried out for comparisons to be made during the development of this method, as described throughout the discussion in **Section 4.1-4.2**. Briefly, the full set of steps were a) extraction by maceration, b) acetylation of crude oil using acetic anhydride, pyridine and DMAP, c) low quantity stationary phase column chromatography - method 1, d) low quantity stationary phase column chromatography - method 2, e) vacuum distillation using a Kugelrohr, f) extraction of acidic constituents using basic alumina. The examples used will be from one experiment that used all the steps except step (d) and a separate experiment that used step (d). In all cases, to ensure there was no residual solvent in the isolated oils, the flask was continually attached to a strong, liquid nitrogen assisted vacuum (~4 mBar) for periods of 30 minutes before weighing and returning to the vacuum for a further 30 minutes until there was no change in oil mass. The scale remained the same throughout these experiments with 10+/-0.2 g of resin being used for each.

*a) Extraction by maceration example:*

The maceration step carried out followed the same steps as **Section 6.06**, except on a different scale which remained consistent for all experiments following this method. Less

solvent was required per gram of resin was used than in **Section 6.06**, as the extraction solvent mixes and extracts the resin very easily. The finely milled *Boswellia occulta* resin (10.01 g) was extracted by maceration in $CHCl_3$ (3 x 20 mL) followed by filtration. The combined filtrates were evaporated in vacuo to give a yellow, viscous oil and weighed (5.75 g, 57% mass recovery compared to resin mass).

*b) Acetylation of crude oil using acetic anhydride, pyridine and DMAP example:*

All molar equivalents were based on if the crude neutral fraction was pure incensole. Acetic anhydride (31.7 mmol, 3.00 ml, 1.69 equiv.) was added dropwise top a stirred solution of crude incensole extract (5.75 g) dissolved in $CH_2Cl_2$ (5 mL) and pyridine (37.2 mmol, 3.00 ml, 1.98 equiv.) at room temperature under a nitrogen atmosphere. A catalytic amount DMAP (0.12 g, 0.98 mmol, 0.05 equiv.) was added and the resultant mixture was heated to reflux. After one hour, the reaction was complete (TLC and HPLC). The mixture was cooled, diluted with $Et_2O$ (50 mL) and transferred to a separating funnel then washed with aqueous $NaHCO_3$ (Sat. 2 x 50 mL) and 2M HCl (aq. 2 x 25 mL). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuo to give crude incensole acetate as a yellow, viscous oil (5.60 g, 56% mass recovery compared to resin mass).

*c) Low quantity stationary phase column chromatography - method 1 example:*

The ratio of stationary phase used in these trials remained the same for simplicity at half the mass of the resin extracted. In this example the stationary phase is silica, but as detailed throughout the discussion, varied between silica, basic alumina and acidic alumina. Crude incensole acetate (5.60 g) was dissolved in the minimum volume of petrol and mixed with silica gel (5 g) before adding to the silica gel column (5 g). The system was eluted with $Et_2O$ in petroleum spirit (40-60) as mobile phase (10%, 30 mL) as one fraction, which was evaporated in vacuo to give incensole acetate fraction as a pale yellow, semi-viscous oil (3.10 g, 31% mass recovery compared to the resin mass). HPLC analysis showed oil was 63% incensole acetate. The impurities were likely a combination of essential oil, minor diterpene impurities and amyrins.

*d) Low quantity stationary phase column chromatography - method 2 example:*

As noted at the start of this section, this step was applied to a different experiment, which used 9.96 g of resin and followed steps (a) and (b) to get crude incensole acetate (5.25 g, 53% mass recovery compared to the resin mass). Crude incensole acetate (5.25 g) was dissolved in the minimum volume of petrol and purified by column chromatography (5 g silica) using $Et_2O$ in petroleum spirits (40-60) as the mobile phase (10%, 30 mL). This was collected as a single fraction and evaporated in vacuo to yield incensole acetate fraction 1 as a pale yellow, viscous oil (4.36 g, 44% mass recovery compared to the resin mass). The silica gel column was then eluted with MeOH (10 mL) into a single fraction to be added to later in this step. The column's polarity was then reset using $Et_2O$ (10 mL) followed by petroleum spirits (40-60) (10 mL). Incensole acetate fraction 1 was then dissolved in the minimum amount of petrol and added again to the same silica gel column and eluted the same way as earlier in this step using $Et_2O$ in petroleum spirits (40-60) as the mobile phase (10%, 30 mL). This was again collected as a single fraction and evaporated in vacuo to give incensole fraction 2 as a pale yellow, semi-viscous oil (3.66 g, 37% mass recovery compared to the resin mass). The silica gel column was again eluted with MeOH (10 mL) into the MeOH fraction from before and the column's polarity was again reset with $Et_2O$ (10 mL) followed by petroleum spirits (40-60) (10 mL). Incensole acetate fraction 2 was purified the same was as incensole acetate 1 was using $Et_2O$ in petroleum spirits (40-60) as the mobile phase (10%, 30 mL) and after evaporation of the collected fraction gave incensole acetate fraction 3 as a pale yellow, semi-viscous oil (3.02 g, 30% mass recovery compared to the resin mass). The silica gel column was again eluted with MeOH (10 mL) into the combined MeOH fractions, which after evaporation gave a yellow, oily solid (2.06 g, 21% mass recovery compared to the resin mass). Each successive purified incensole acetate fraction and the MeOH fraction was submitted for HPLC following the method in **Section 6.03** and the MeOH fraction showed very low incensole acetate content (<1%) whereas the desired diterpene fraction contained 67% incensole acetate. The impurities were likely a combination of essential oil, minor diterpene impurities and amyrins.

*e) Vacuum distillation using a Kugelrohr example:*

There were two Kugelrohr distillation methods discussed throughout this work varying only by operating at different temperatures. The method detailed in this section operated at 125$^{o}$C

and the other method operated at 150°C with all other parameters remaining the same, except the oil distilled.

The crude incensole acetate fraction (3.10 g) was added to the residue flask of the Kugelrohr apparatus and four fractionation chambers were added end on end in the heating chamber. The Kugelrohr was attached to a vacuum system with a cold trap cooled by liquid nitrogen before heating. The fractionation glassware was turned slowly throughout the experiment and the system heated to 125°C for a period of 25 minutes, at which point the distillation was complete. The system was returned to atmospheric pressure and the flasks were allowed to cool before weighing and sampling for HPLC analysis (67% incensole acetate in the residue fraction) following the method in **Section 6.03**. The impurities were likely a combination of minor diterpene impurities and amyrins. This resulted in an incensole fraction as a pale yellow, semi-viscous oil in the residue flask (2.65 g, 26% compared to the resin mass) and 4 pale yellow, non-viscous volatile fractions (combined total of 0.22 g, 2% mass recovery compared to the resin mass, contained 22% incensole acetate by GC/MS analysis following method (b) in **Section 6.02**).

*f) Extraction of acidic constituents using basic alumina example:*

This step was performed similarly to step (c) and is essentially flash column chromatography using basic alumina as the stationary phase. An incensole acetate fraction (2.65 g) was dissolved in the minimum volume of petrol and mixed with basic alumina (5 g). This mixture was added to a column of basic alumina (20 g) and eluted with $Et_2O$ in petroleum spirits (40-60) as the mobile phase (10%, 60 mL). This was collected as a single fraction and evaporated in vacuo to give an incensole acetate rich fraction as a very pale yellow, semi-viscous oil (1.57 g, 16% mass recovery compared to the resin mass, 82% purity by HPLC following the method in **Section 6.03**). The impurities were likely a combination of minor diterpene impurities and amyrins.

## 6.11 Isolation of High Incensole Acetate Content Oils – 100 g scale

This scaled up experiment followed the parts of the procedure in **Section 6.10**, but on a 100 g scale of *Boswellia occulta* resin (north coast, Somaliland) and was performed several times. However, further method development was made after these experiments so there are some slight differences between this method and the method in **Section 6.10**. Briefly the steps were

a) extraction by maceration, b) acetylation of crude oil using acetic anhydride, pyridine and DMAP, c) low quantity silica gel column chromatography - method 1. The parameters for this experiment were directly scaled up from the same steps in **Section 6.10**. In all cases, to ensure there was no residual solvent in the isolated oils, the flask was continually attached to a strong, liquid nitrogen assisted vacuum (~4 mBar) for periods of 30 minutes before weighing and returning to the vacuum for a further 30 minutes until there was no change in oil mass.

*a) Extraction by maceration example:*

The maceration step carried out followed the same steps as **Section 6.06**, except on a different scale which remained consistent for all experiments following this method. Less solvent was required per gram of resin was used than in **Section 6.06**, as the extraction solvent mixes and extracts the resin efficiently. Finely milled *Boswellia occulta* resin (100 g: North Coast of Somaliland) was extracted by maceration successively with petroleum spirits (40-60) (1 x 200 mL and 2 x 100 mL) and filtered through a Buchner funnel. The combined filtrates were concentrated in vacuo to give a crude incensole extract as a yellow, viscous oil (40 g, 40% mass recovery compared to the resin mass).

*b) Acetylation of crude oil using acetic anhydride, pyridine and DMAP example:*

All molar equivalents were based on if the crude neutral fraction was pure incensole. Acetic anhydride (0.21 mol, 20.0 ml, 1.62 equiv.) was added dropwise over 10 minutes to a stirred solution of the crude incensole extract (40 g) dissolved in $CH_2Cl_2$ (40 mL) and pyridine (0.25 mol, 20.0 ml, 1.90 equiv.) at room temperature under a nitrogen atmosphere. A catalytic amount DMAP (0.80 g, 6.55 mmol, 0.05 equiv.) was added and the resultant mixture was heated to reflux. After one hour, the reaction was complete (TLC and HPLC). The mixture was cooled, diluted with $Et_2O$ (100 mL) and transferred to a separating funnel then washed with aqueous $NaHCO_3$ (Sat. 250 mL) and 2M HCl (150 mL). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuo to give crude incensole acetate as a yellow, viscous oil (40 g, 40% mass recovery compared to the resin mass).

*c) Low quantity silica gel column chromatography - method 1 example:*

Crude incensole acetate (40 g) was purified by flash column chromatography on Davisil® silica gel (35-70 microns). This oil was dissolved in a minimum volume of petroleum spirits (40-60)

and mixed with silica gel (50 g). This mixture was added to a silica gel column (50 g of silica gel) and eluted with $Et_2O$ in petroleum spirits (40-60) (10%, 300 mL). The elutant was collected as a single fraction and concentrated in vacuo to give a rich incensole acetate fraction as a pale yellow, semi-viscous oil (15 g, 15% mass recovery compared to the resin mass, 70% purity determined by HPLC following the method in **Section 6.03**). The impurities were likely a combination of essential oil, minor diterpene impurities and amyrins.

## 6.12 Isolation of Serratol

Serratol was isolated separately from both the Gudmo Biyo Cas and Erigavo resins (*Boswellia carterii)* to compare the mass recoveries of serratol compared to the resin mass. Experiments began with maceration in methanol followed by filtration (a) to give the crude extract after evaporation in vacuo. This was followed by removal of the acid constituents with 2% KOH solution (b), resulting in a neutral fraction, free of the boswellic acids. Column Chromatography (c) was then required to remove the remaining impurities, such as amyrins and essential oils to give a semi-viscous, clear and colourless oil. In all cases, to ensure there was no residual solvent in the isolated oils, the flask was continually attached to a strong, liquid nitrogen assisted vacuum (~4 mBar) for periods of 30 minutes before weighing and returning to the vacuum for a further 30 minutes until there was no change in oil mass.

*a) Extraction by maceration example:*

The maceration step carried out followed the same steps as **Section 6.06**, except on a different scale which remained consistent for all experiments following this method. Less solvent was required per gram of resin was used than in **Section 6.06**, as the extraction solvent mixes and extracts the resin very easily. The finely milled Gudmo Biyo Cas resin (5.12 g) was extracted by maceration in MeOH (3 x 20 mL) followed by filtration. The combined filtrates were evaporated in vacuo and weighed to give an orange, viscous oil (2.83 g, 55% mass recovery compared to resin mass).

*b) Removal of the acidic constituents by extraction with 2% KOH example:*

Next, 2.83 g of crude extract was dissolved in $Et_2O$ (100 ml) and extracted twice with an equal volume of aqueous KOH (2%, 2 x 100 mL). The $Et_2O$ layer was washed with 1M HCl (50 ml),

dried ($MgSO_4$) and evaporated under vacuum to give the neutral fraction as a yellow, viscous oil (1.50 g, 29% mass recovery compared to resin mass).

*c) Flash column chromatography of crude serratol example*:

The crude neutral fraction (1.50 g) was purified by flash column chromatography on Davisil® silica gel (35-70 microns), using $Et_2O$ in petroleum spirits (40-60) as an eluent (gradient 5%, 10%, 15%). TLC was conducted on precoated E. Merck silica gel 60 F254 glass plates using $Et_2O$/hexane (20%). Similar fractions that seemed pure (by visual inspection) were collected in a single flask and evaporated in vacuo to give pure serratol **48** as a colourless, viscous oil ($R_f$ = 0.27, 0.22 g, 4.3% mass recovery compared to resin mass). NMR data was collected on the sample based on the method in **Section 6.04**.

$^1$H NMR ($CDCl_3$, 400 MHz): $\delta_H$ 0.95 (t, 6H, *J* = 6.9 Hz), 1.57 (s, 3H), 1.59 (s, 3H), 1.61 (s, 3H), 1.65 (t, 2H, *J* = 7.6 Hz), 1.72 (m, 1H, *J* = 6.8 Hz), 1.79 (t, 1H, *J* = 7.2 Hz), 1.93 (t, 1H, 7.5 Hz), 1.97 (t, 1H, *J* = 7.0 Hz), 2.11 (s, 1H), 2.12 (m, 2H), 2.14 (br s, 1H), 2.14 (t, 1H, *J* = 7.8 Hz), 2.20 (br s, 1H), 2.22-2.23 (m, 2H), 2.34 (t, 1H, *J* = 7.1 Hz), 4.91 (br t, 1H, *J* = 6.1 Hz), 5.02 (br t, 1H, *J* = 6.8 Hz), 5.27 (br t, 1H, *J* = 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta_C$ 15.1, 15.2, 16.4, 16.6, 16.8, 23.8, 24.8, 33.5, 34.6, 34.7, 34.9, 39.5, 39.9, 76.9, 120.9, 123.2, 125.9, 133.3, 135.6, 136.7.

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# Chapter 8. Appendix

This section consists of the fragmentation patterns of the rest of the compounds that were mentioned in this thesis that were not discussed. When used alongside their arithmatic index, calculated using equation 1.3 in **Section 1.3.2**, the compounds could be tentatively assigned using literature references. The assignment confidence is in the figure description and each has the citation of the litereature reference fragementation pattern in the figure description. All the fragmentation patterns here were produced in this work and are from experiments that were discussed throughout the thesis, as mentioned in the figure descriptions.

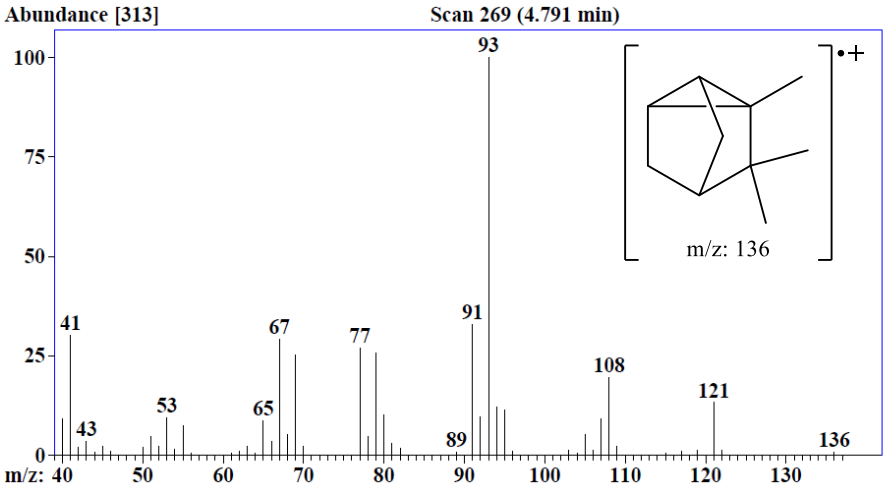


Figure A.01. Fragmentation pattern of tricyclene **148**, AI = 921 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

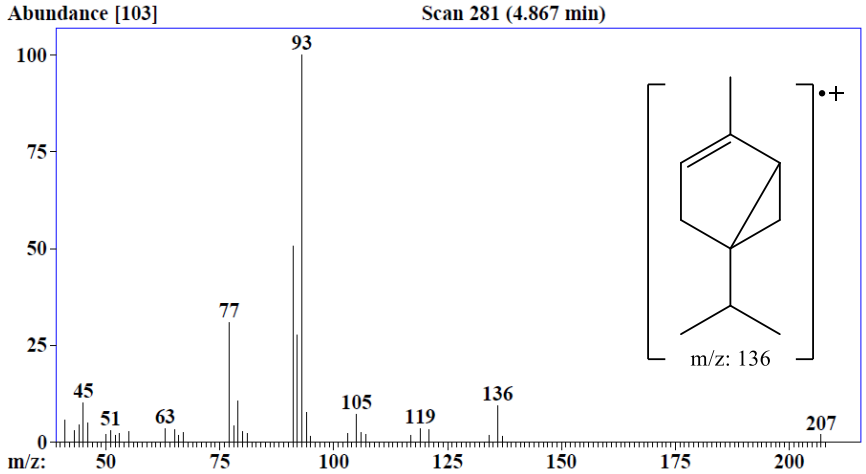


Figure A.02. Fragmentation pattern of thujene **24**, AI = 924 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

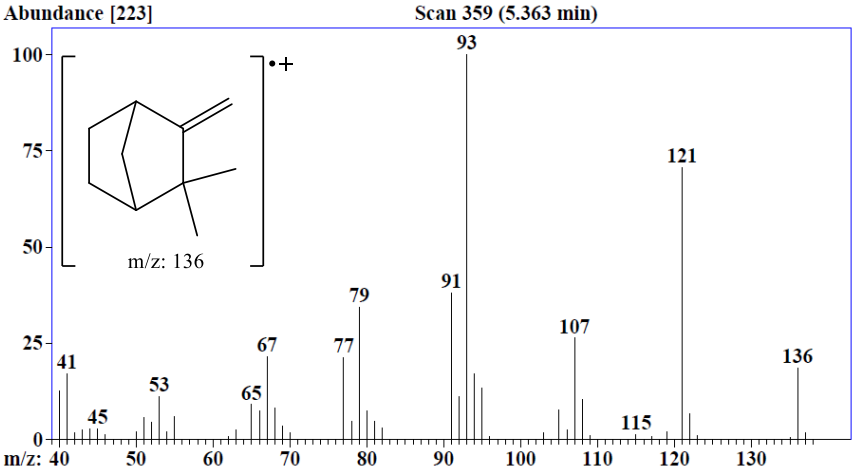


Figure A.03. Fragmentation pattern of camphene **26**, AI = 946 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

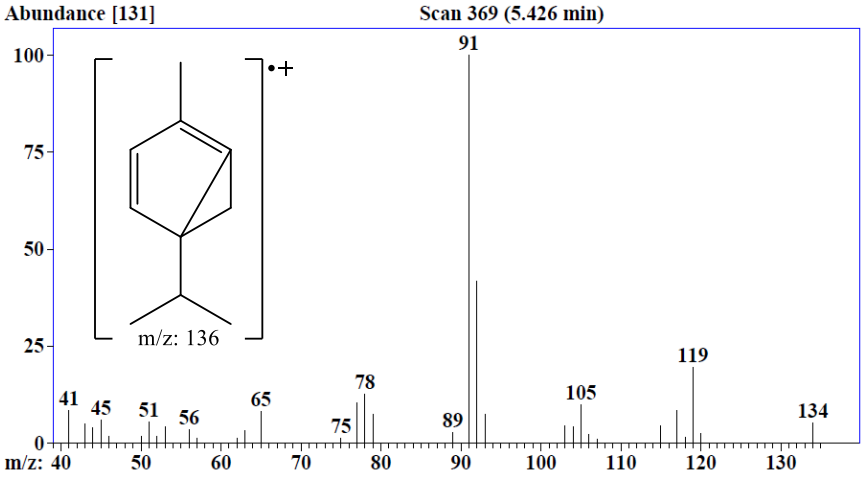


Figure A.04. Fragmentation pattern of thuja-2,4(10)-diene **153**, AI = 953 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

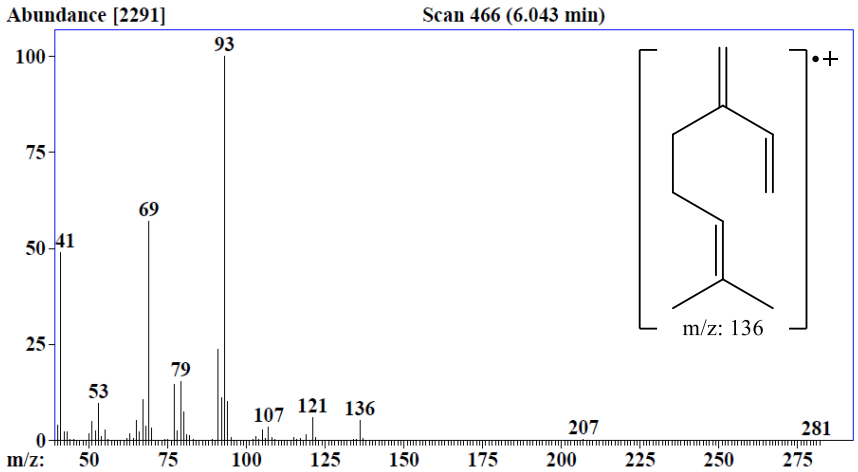


Figure A.05. Fragmentation pattern of myrcene **29**, AI = 988 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = high. Literature comparison.[21]

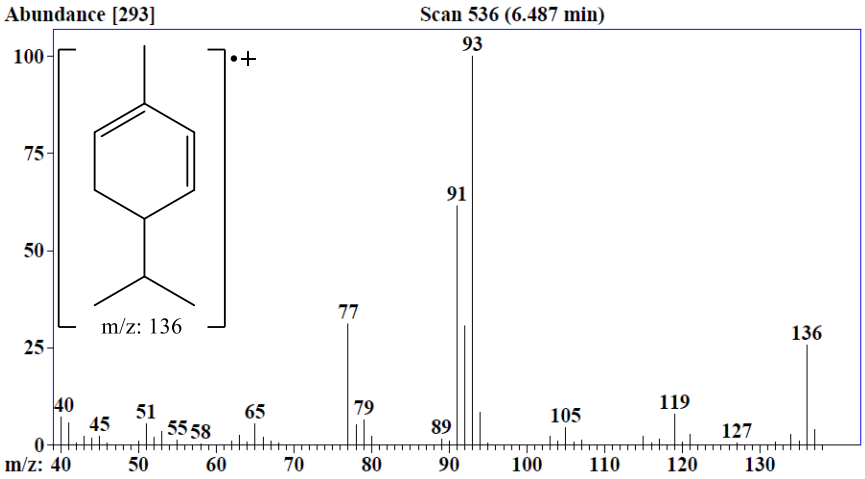


Figure A.06. Fragmentation pattern of α-phellandrene **30**, AI = 1002 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

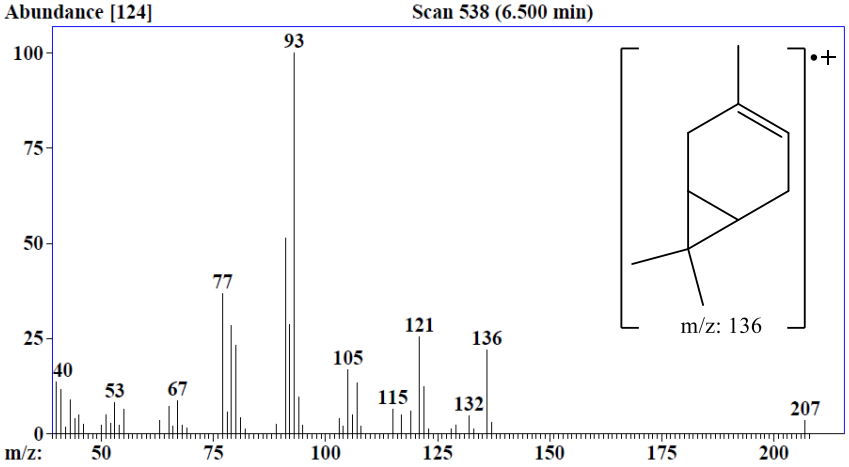


Figure A.07. Fragmentation pattern of δ-3-carene **31** at AI = 1010 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

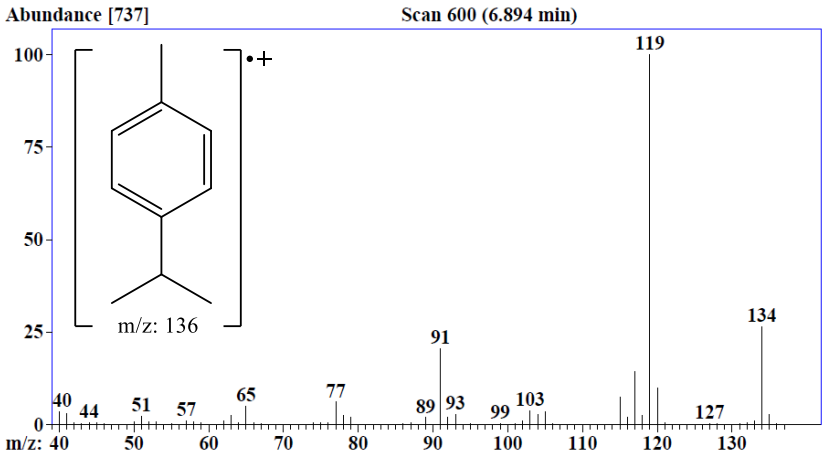


Figure A.08. Fragmentation pattern of *p*-cymene **32**, AI = 1020 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

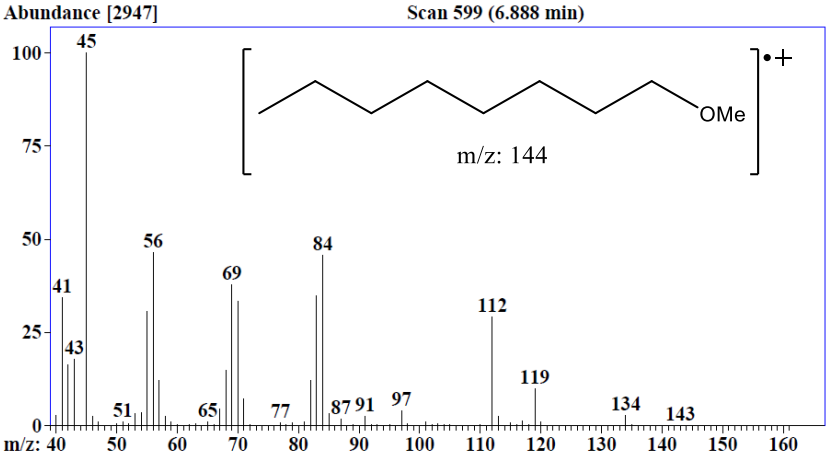


Figure A.09. Fragmentation pattern of methoxyoctane **69**, at AI = 1023 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Upon electron ionization, there is facile loss of $CH_3OH$ meaning there is minimal detection of the parent ion peak at m/z = 144. Assignment confidence = high. Literature comparison.[87]

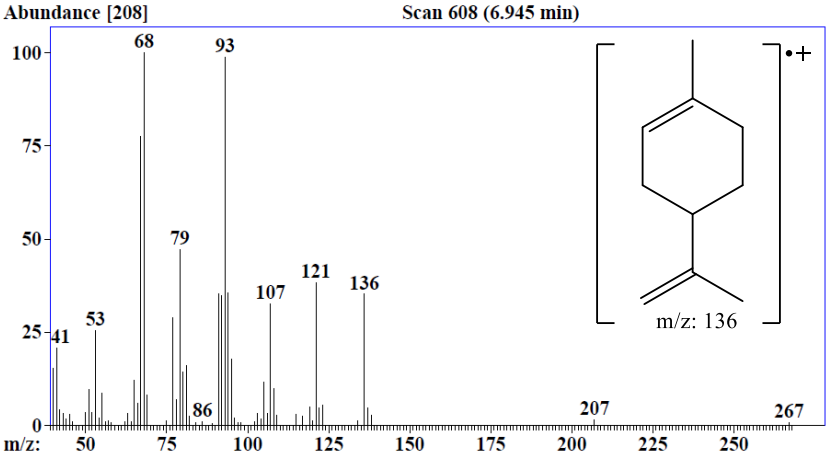


Figure A.10. Fragmentation pattern of limonene **33** at AI = 1030 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

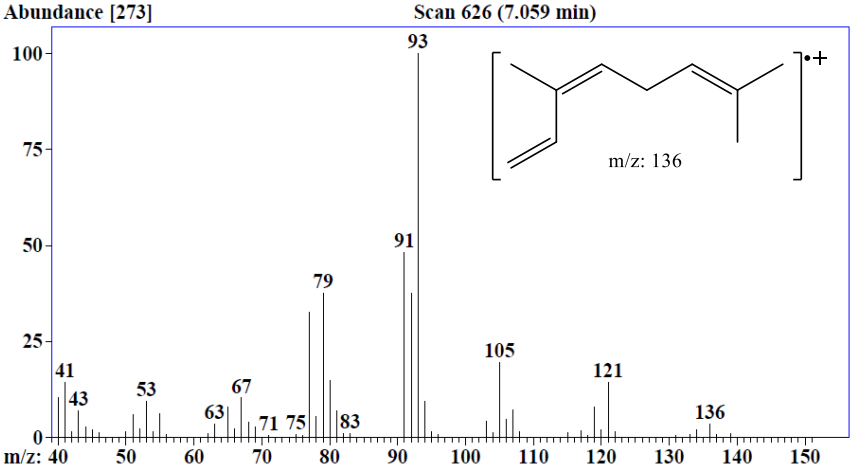


Figure A.11. Fragmentation pattern of (*Z*)-β-ocimene **149**, at AI = 1036 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = high. Literature comparison.[21]

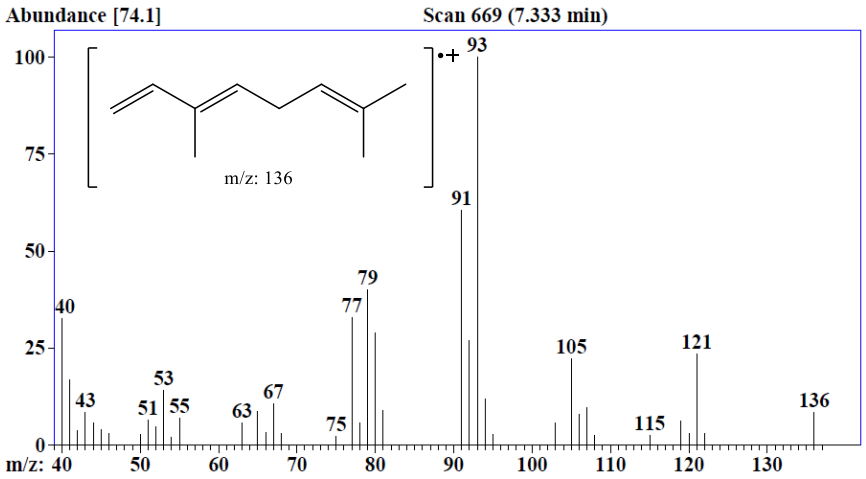


Figure A.12. Fragmentation pattern of (*E*)-β-ocimene **46**, AI = 1044 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

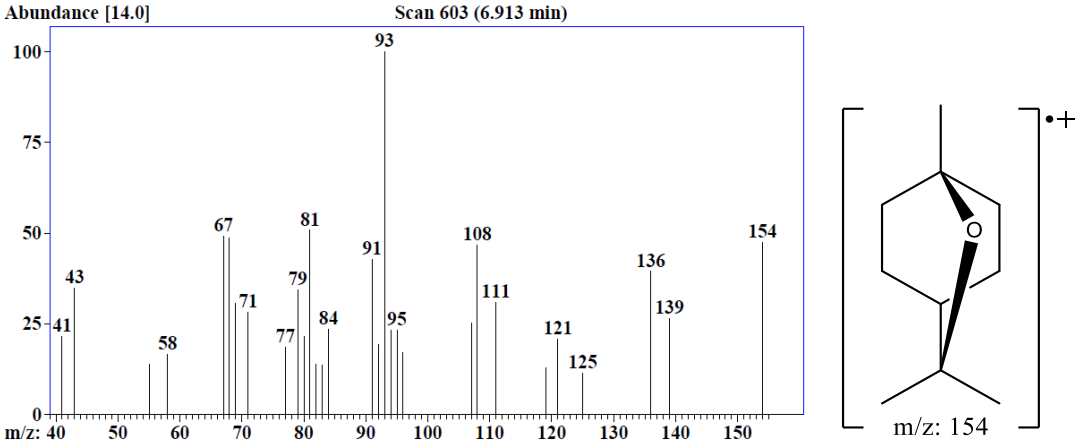


Figure A.13. Fragmentation pattern of 1,8-cineole **61**, AI = 1053 from the GC/MS analysis of an oil isolated through hydrodistillation of the grade 1 Sudanese resin following the procedure in **Section 6.05**. There is slight overlap with limonene. Assignment confidence = high. Literature comparison.[21]

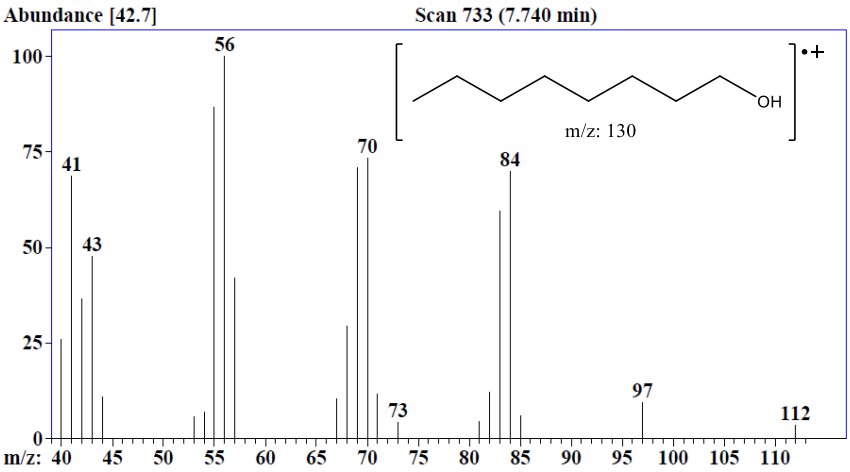


Figure A.14. Fragmentation pattern of octanol **54**, AI = 1070 from the GC/MS analysis of an oil isolated through hydrodistillation of the grade 1 Sudanese resin following the procedure in **Section 6.05**. There is loss of $H_2O$ upon electron ionization resulting in no detection for the parent ion at m/z = 130. Assignment confidence = high. Literature comparison.[21]

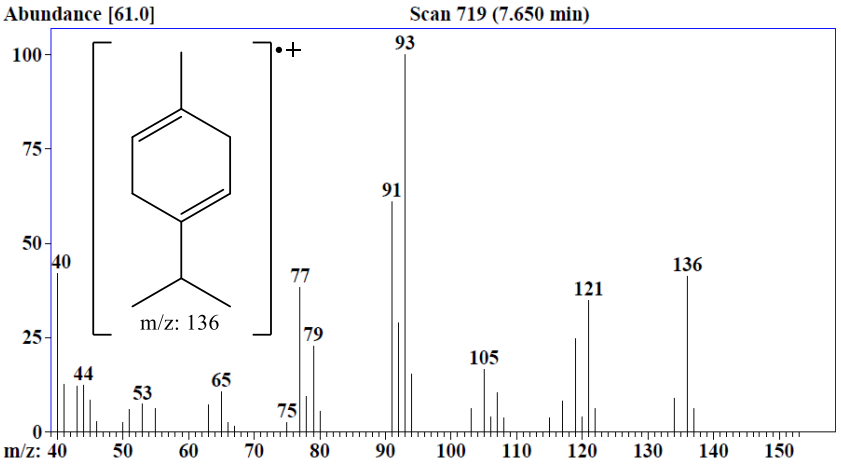


Figure A.15. Fragmentation pattern of γ-terpinene **62**, at AI = 1104 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

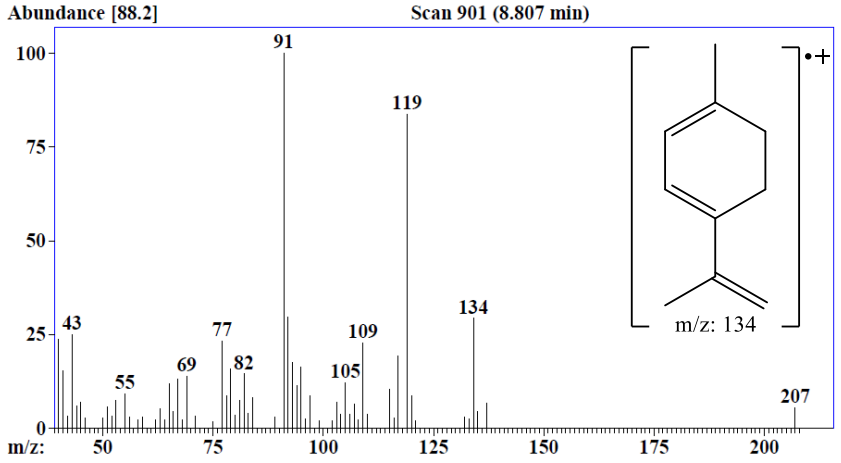


Figure A.16. Fragmentation pattern of *p*-1,3,8-menthatriene **151**, AI = 1112 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

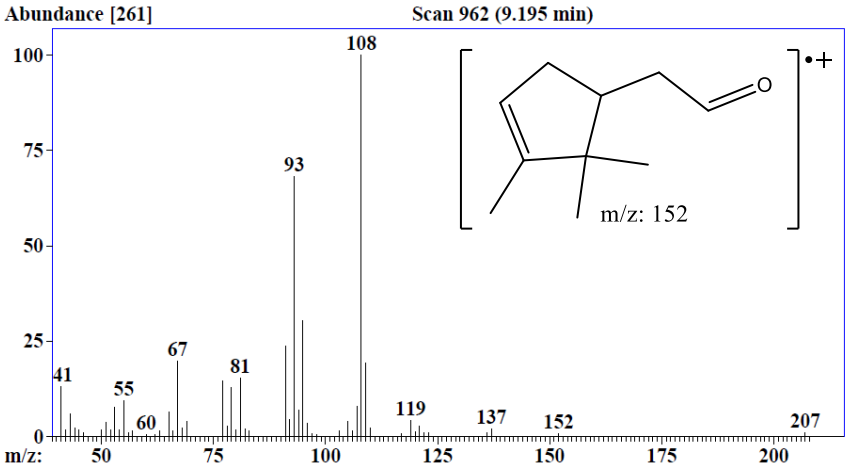


Figure A.17. Fragmentation pattern of α-campholenal **132** at AI = 1125 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

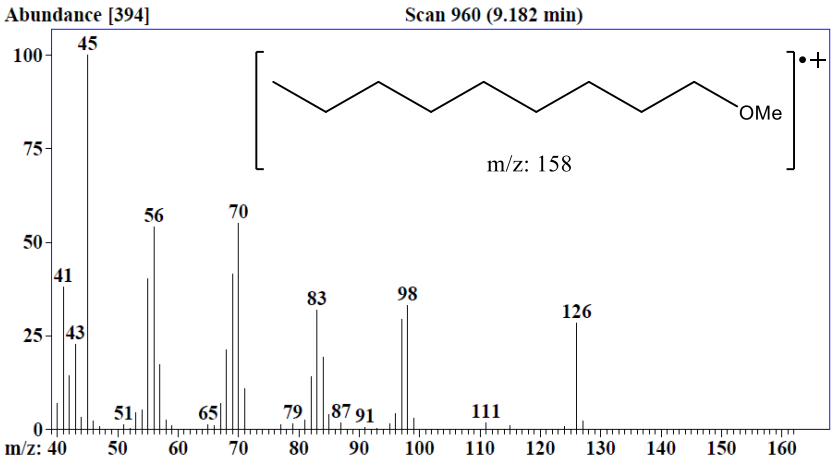


Figure A.18. Fragmentation pattern of methoxynonane **70**, at AI = 1130 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Upon electron ionization, there is facile loss of $CH_3OH$ meaning there is minimal detection of the parent ion peak at m/z = 158. Assignment confidence = high. Literature comparison.[87]

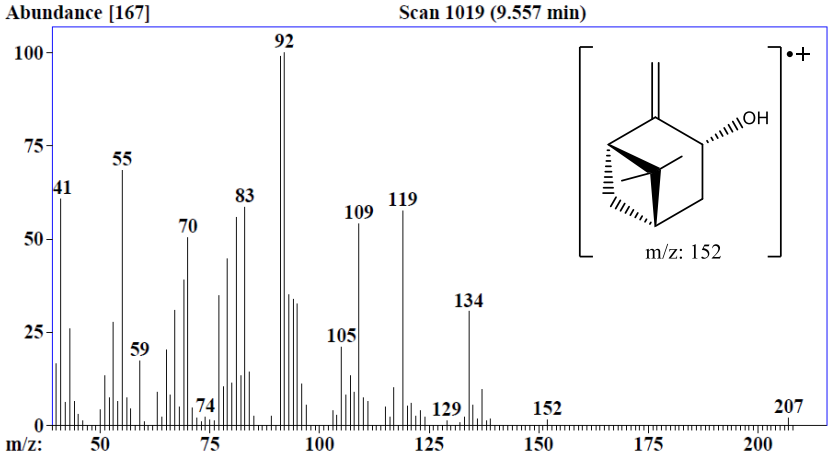


Figure A.19. Fragmentation pattern of *trans*-pinocarveol **133**, at AI = 1136 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

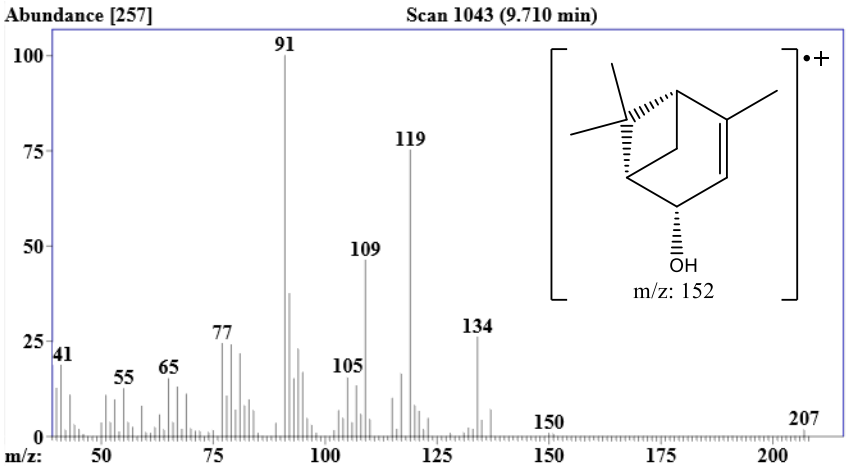


Figure A.20. Fragmentation pattern of *cis*-verbenol **35**, at AI = 1138 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

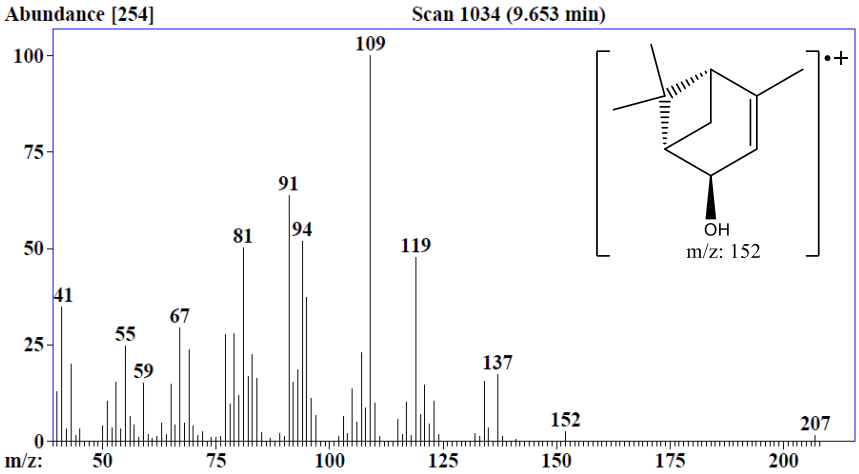


Figure A.21. Fragmentation pattern of *trans*-verbenol **130** at AI = 1141 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

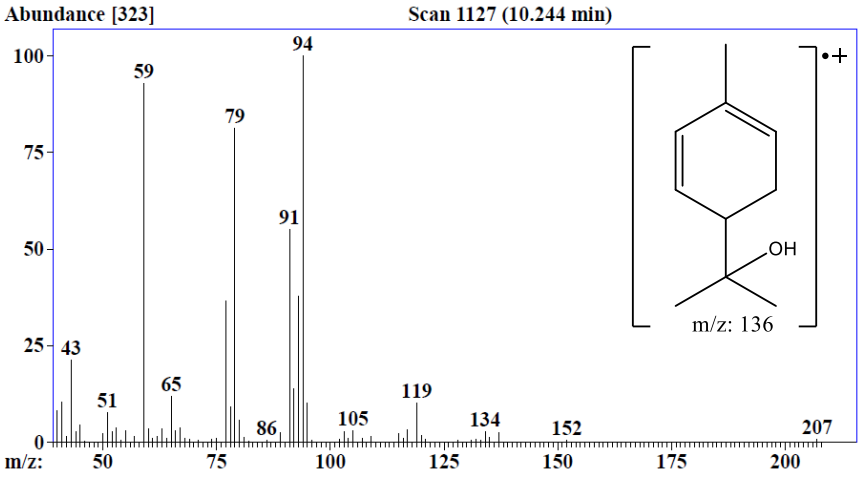


Figure A.22. Fragmentation pattern of *p*-mentha-1,5-dien-8-ol at AI = 1180 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

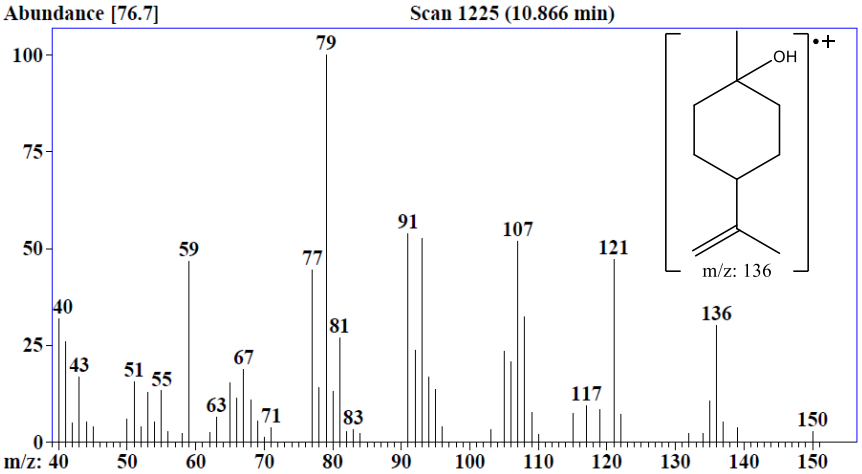


Figure A.23. Fragmentation pattern of α-terpineol **64**, AI = 1190 from the GC/MS analysis of an oil isolated through hydrodistillation of the Erigavo resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

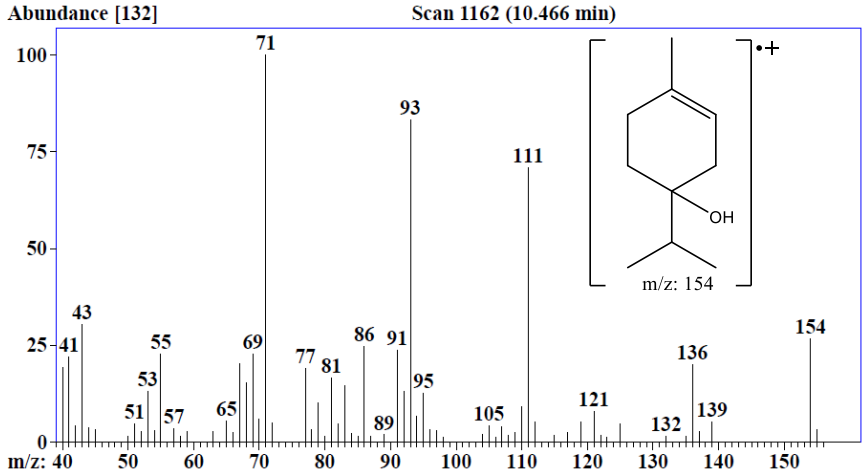


Figure A.24. Fragmentation pattern of 4-terpineol **63** at AI = 1201 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

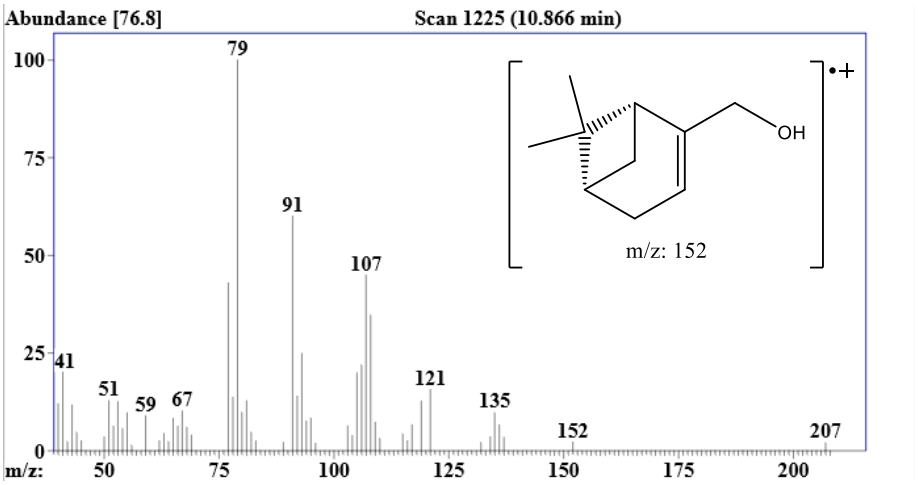


Figure A.25. Fragmentation pattern of myrtenol **134**, at AI = 1201 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

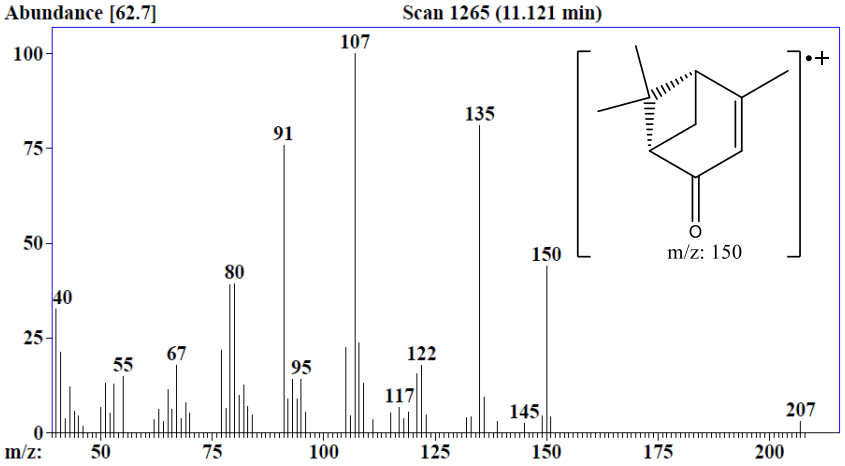


Figure A.26. Fragmentation pattern of verbenone **37**, at AI = 1205 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

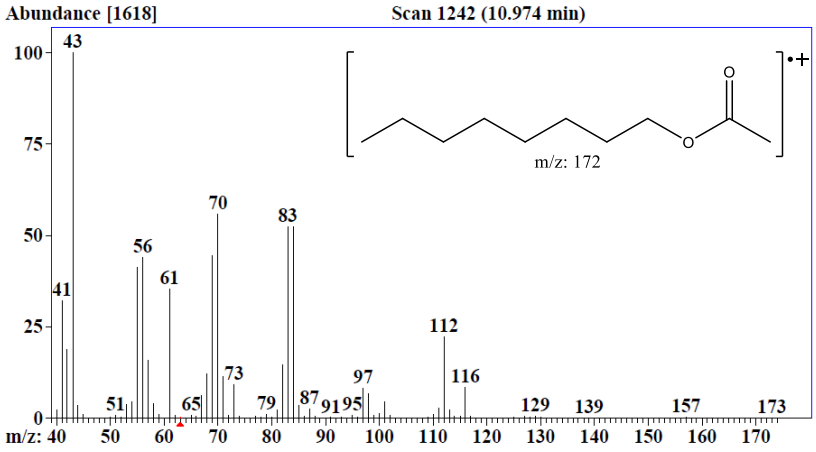


Figure A.27. Fragmentation pattern of octyl acetate **50**, AI = 1205 from the GC/MS analysis of an oil isolated through hydrodistillation of the grade 1 Sudanese resin following the procedure in **Section 6.05**. There is loss of $CH_3COOH$ upon electron ionization resulting in minimal detection for the parent ion at m/z = 172. Assignment confidence = high. Literature comparison.[21]

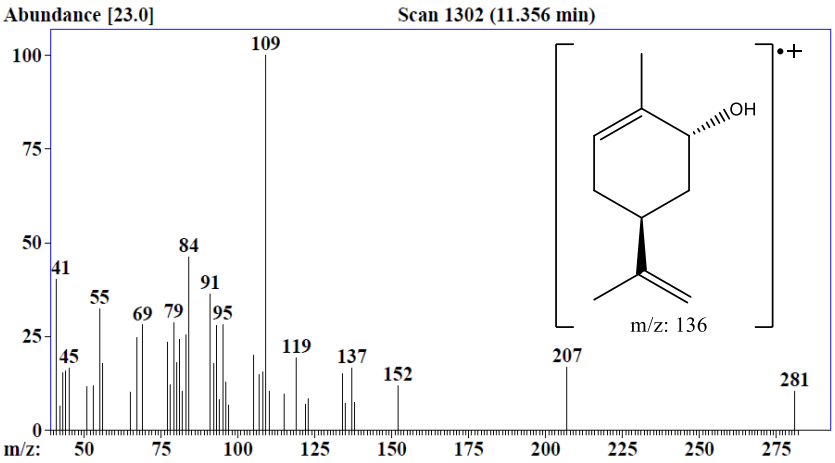


Figure A.28. Fragmentation pattern of *trans*-carveol **140** at AI = 1218 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

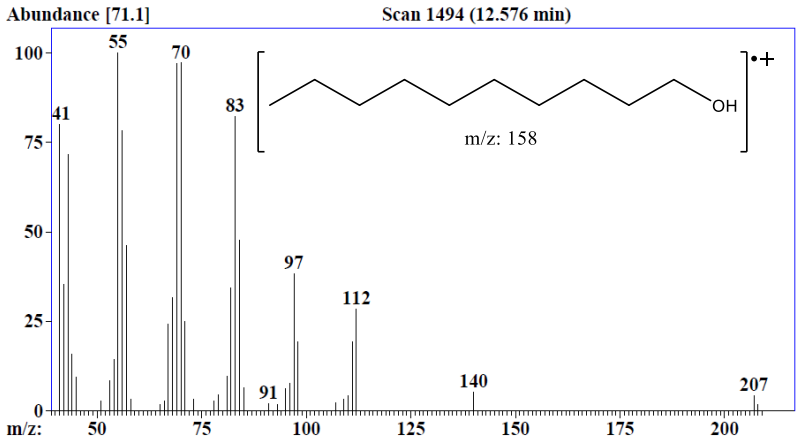


Figure A.29. Fragmentation pattern of decanol **72** at AI = 1270 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. There is facile loss of $H_2O$ upon electron ionization resulting in minimal detection at m/z = 158. Assignment confidence = high. Literature comparison.[21]

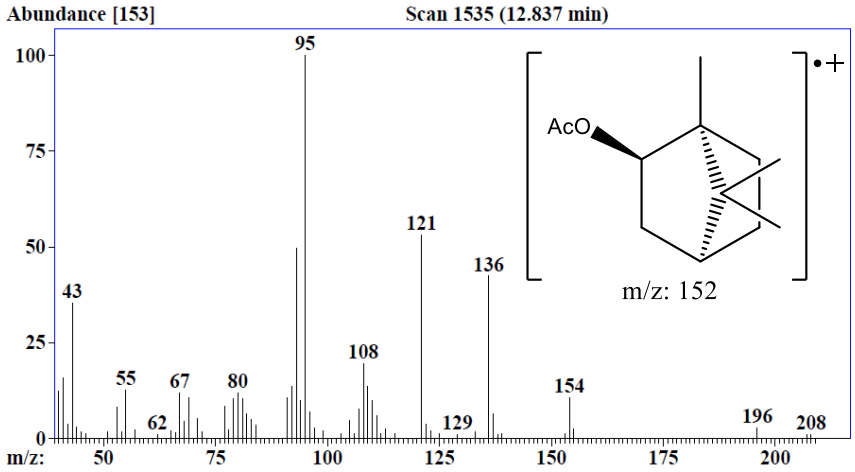


Figure A.30. Fragmentation pattern of bornyl acetate **141**, at AI = 1285 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

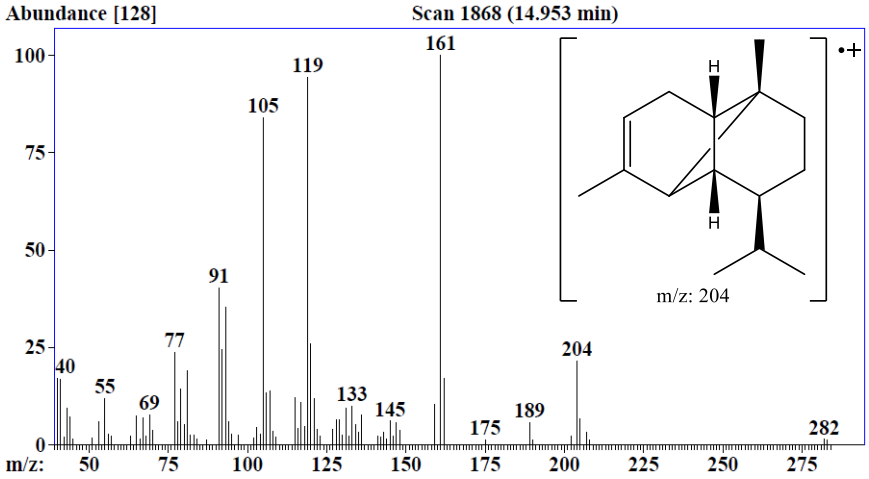


Figure A.31. Fragmentation pattern of α-copaene **38**, at AI = 1382 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

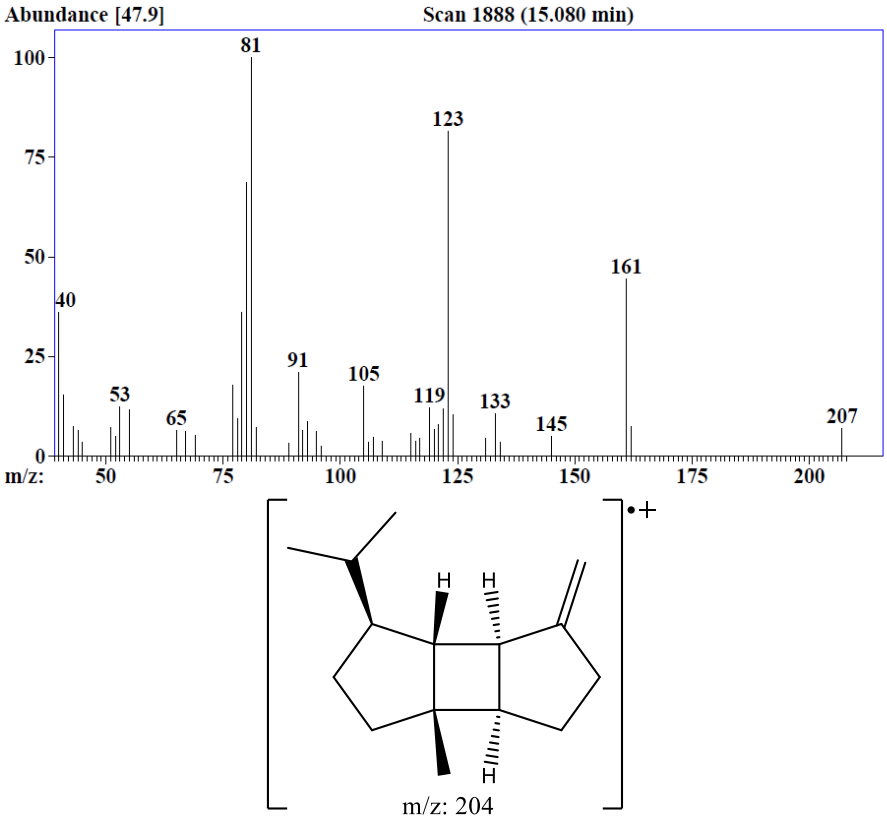


Figure A.32. Fragmentation pattern of β-bourbonene **65**, at AI = 1387 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade rebousin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

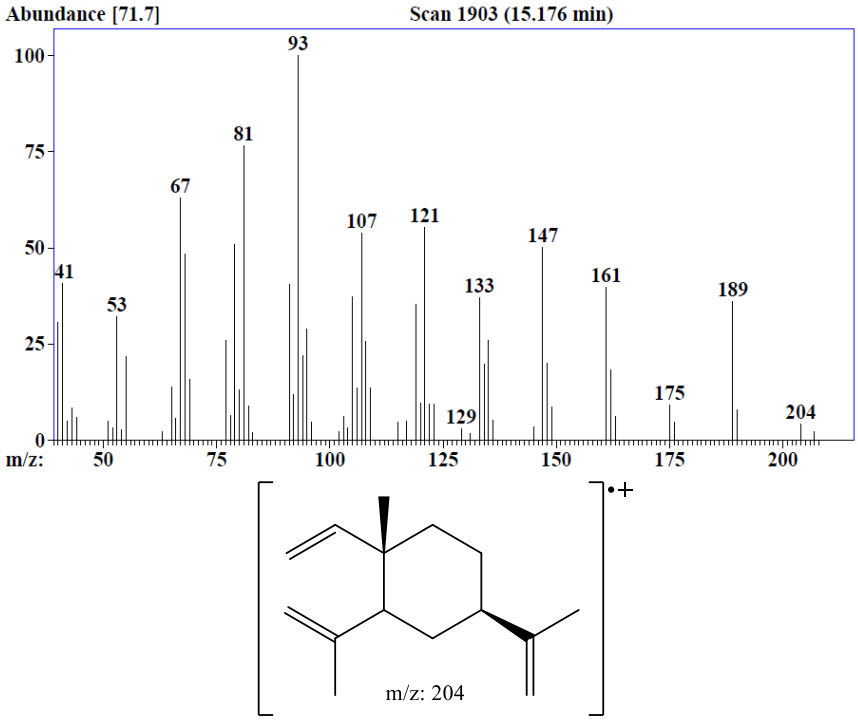


Figure A.33. Fragmentation pattern of β-elemene **39** at AI = 1390 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

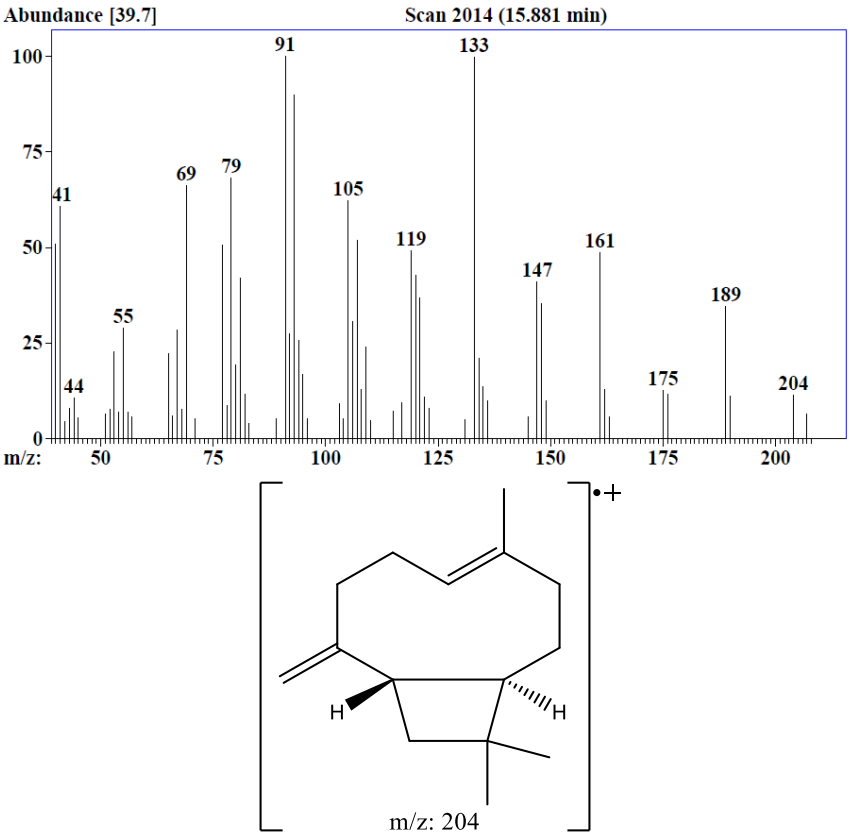


Figure A.34. Fragmentation pattern of caryophyllene **40**, at AI = 1425 from the GC/MS analysis of an oil isolated through hydrodistillation of the Najdi grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

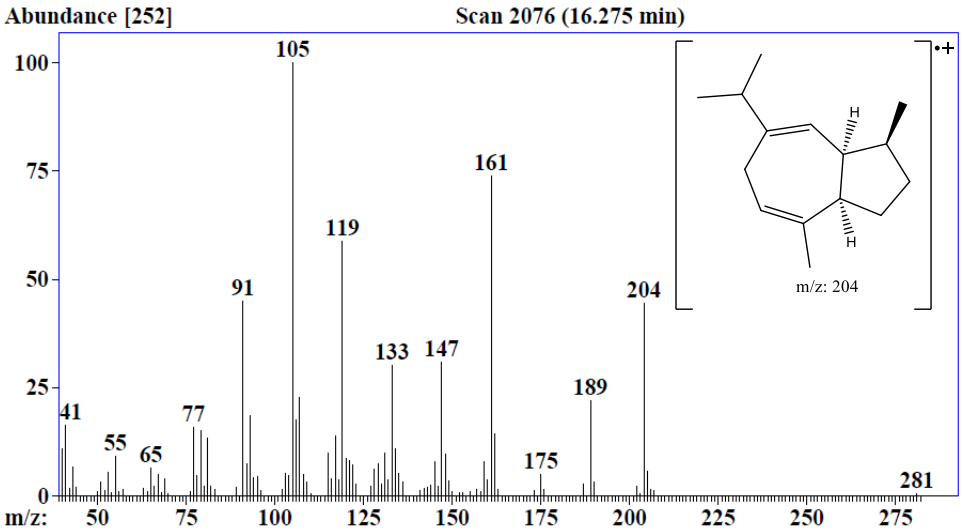


Figure A.35. Fragmentation pattern of 6,9-guaiadiene **150** at AI = 1442 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

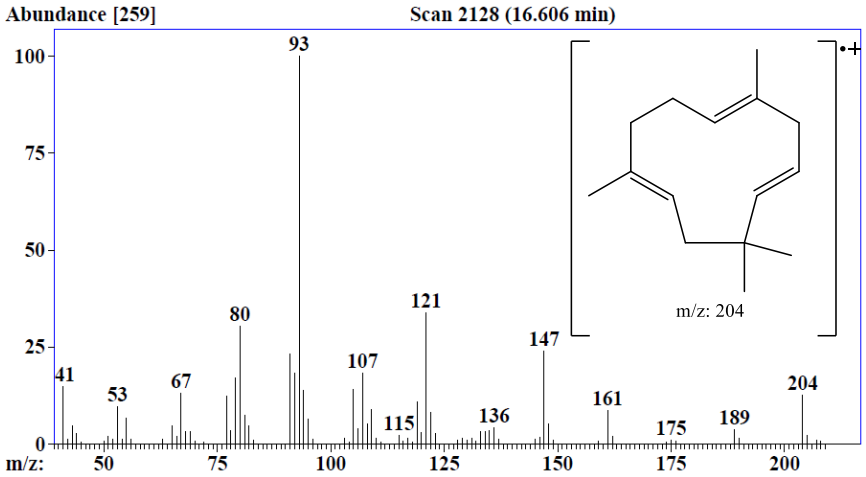


Figure A.36. Fragmentation pattern of α-humulene **41**, at AI = 1459 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

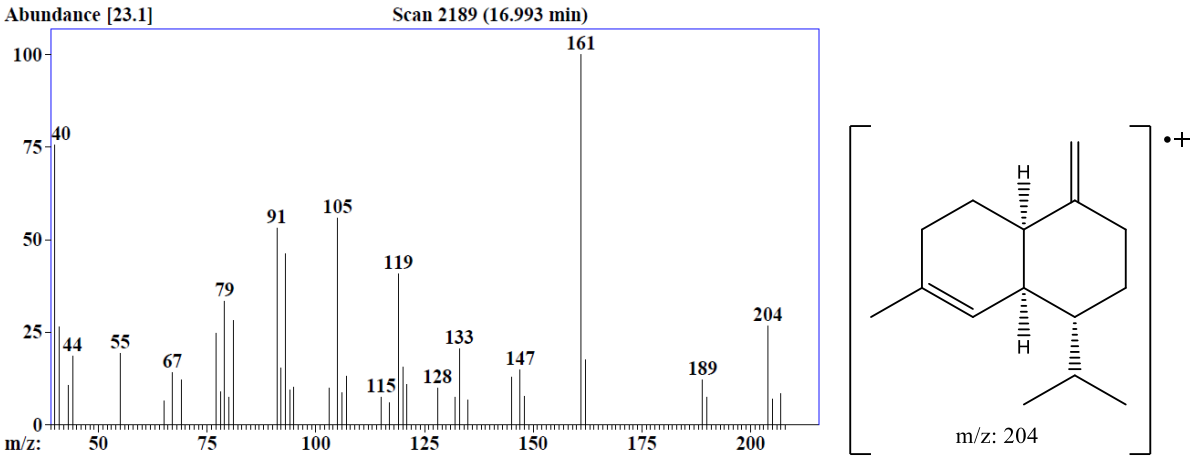


Figure A.37. Fragmentation pattern of γ-muurolene **143**, at AI = 1480 from the GC/MS analysis of an oil isolated through hydrodistillation of the Sha'abi grade resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

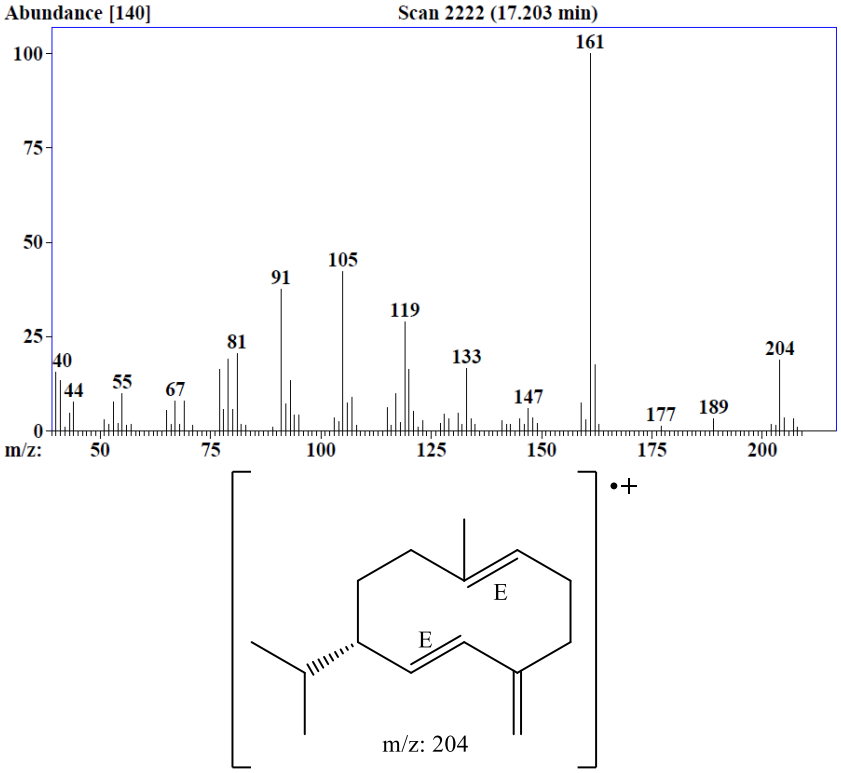


Figure A.38. Fragmentation pattern of germacrene D **145**, at AI = 1496 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

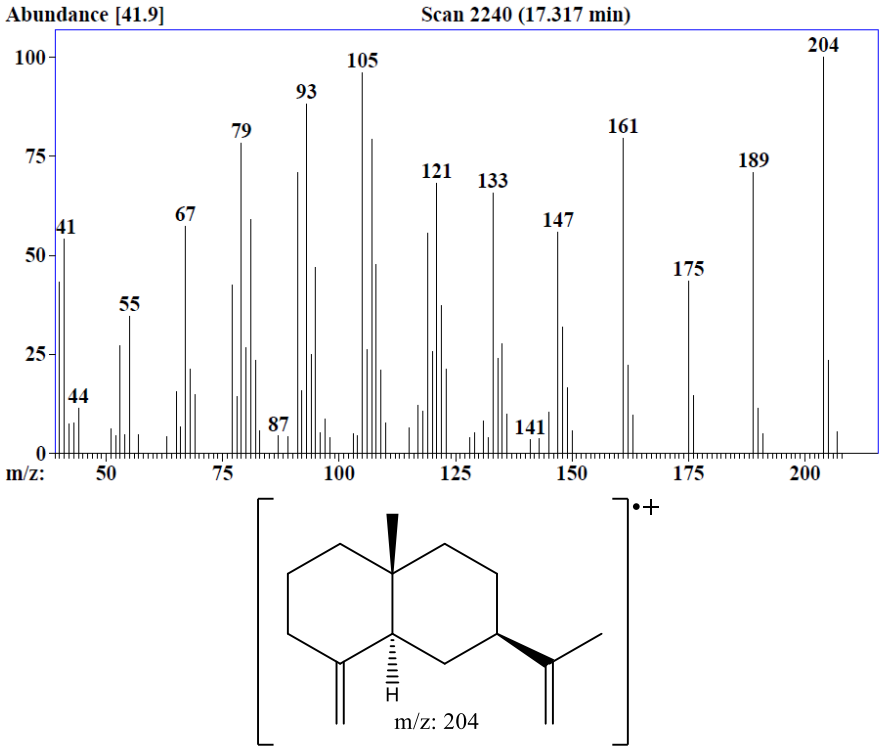


Figure A.39. Fragmentation pattern of β-selinene **42** at AI = 1499 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

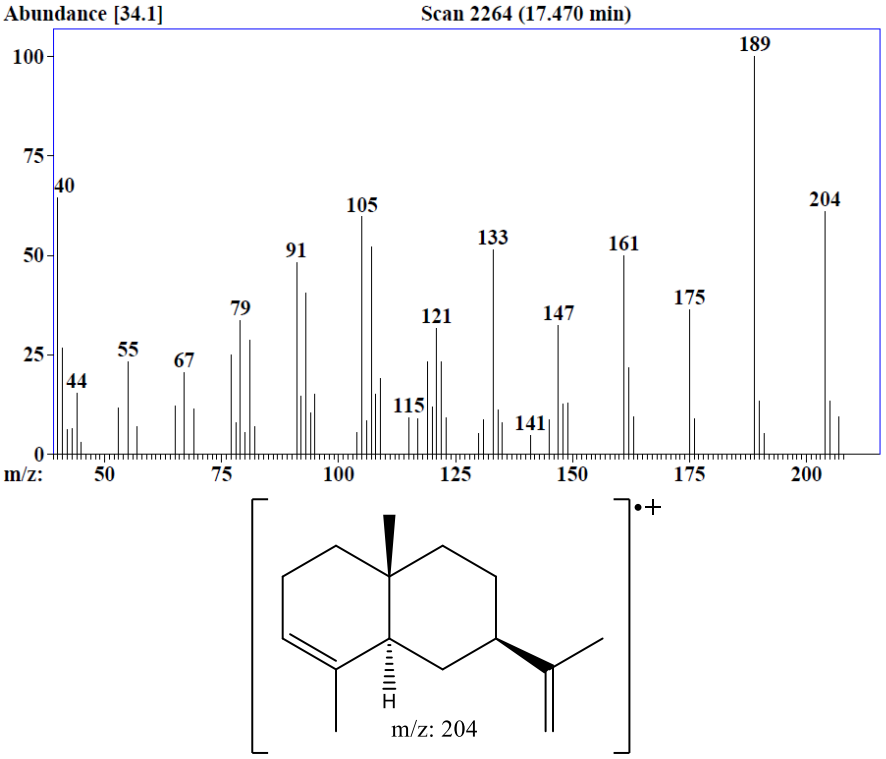


Figure A.40. Fragmentation pattern of α-selinene **135**, at AI = 1500 from the GC/MS analysis of an oil isolated through hydrodistillation of the Hoojri grade resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

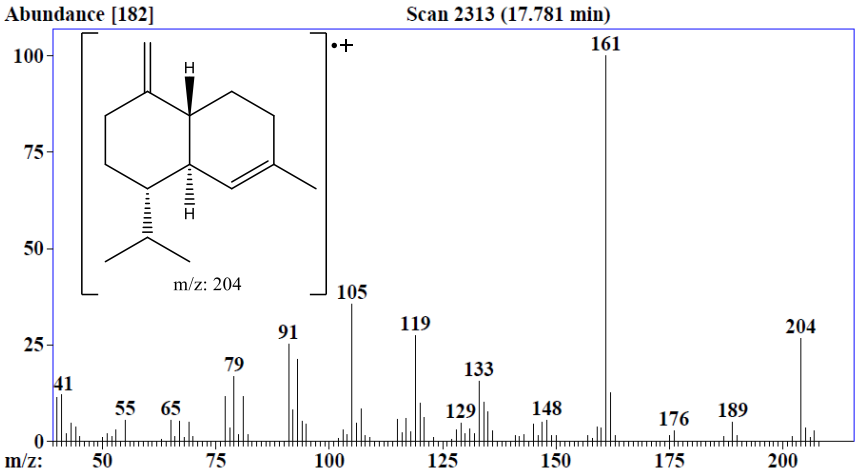


Figure A.41. Fragmentation pattern of γ-cadinene **160** at AI = 1515 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

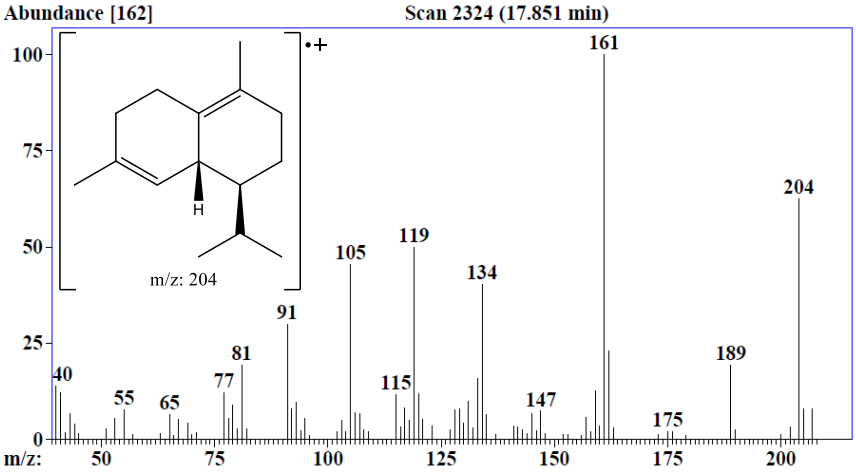


Figure A.42. Fragmentation pattern of δ-cadinene at **43** AI = 1518 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = high. Literature comparison.[21]

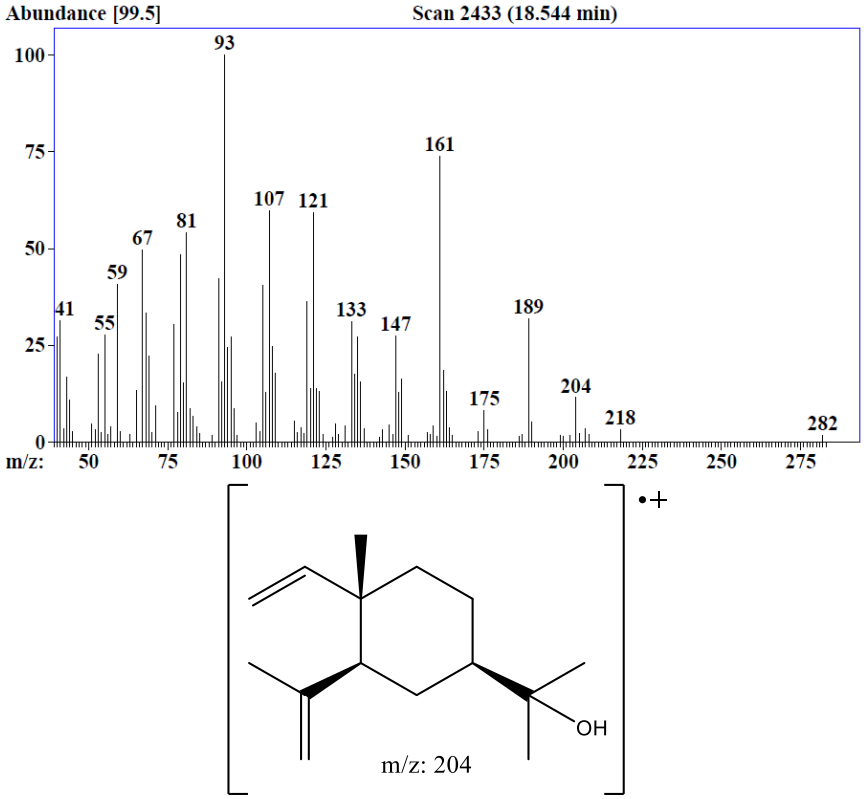


Figure A.43. Fragmentation pattern of elemol **146**, at AI = 1555 from the GC/MS analysis of an oil isolated through hydrodistillation of the *Boswellia occulta* resin following the procedure in **Section 6.05**. Assignment confidence = medium. Literature comparison.[21]

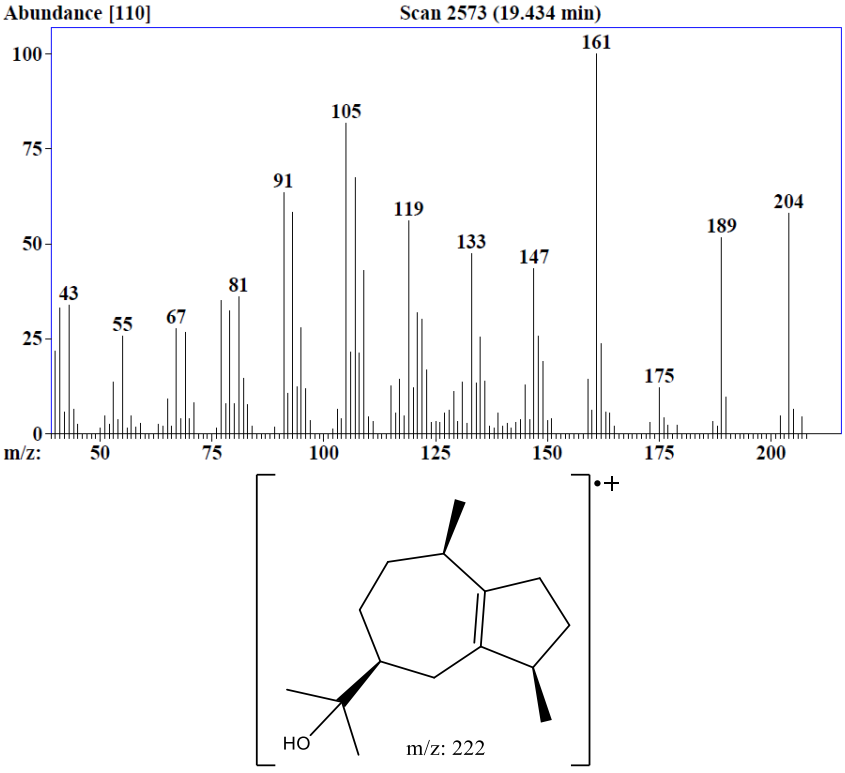


Figure A.44. Fragmentation pattern of guaiol **147** at AI = 1598 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

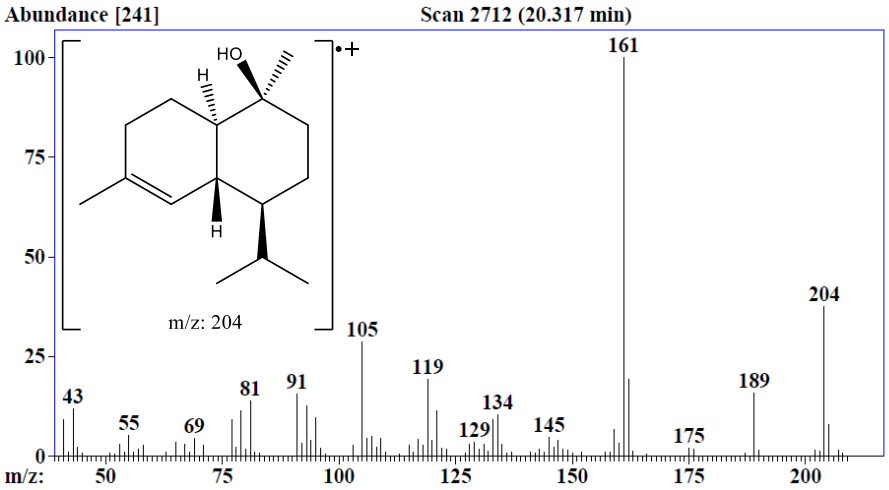


Figure A.45. Fragmentation pattern of *epi*-α-cadinol **152** at AI = 1644 from the GC/MS analysis of an oil isolated through supercritical $CO_2$ extraction of the Gudmo biyo cas resin following method (b) in **Section 6.08**. Assignment confidence = medium. Literature comparison.[21]

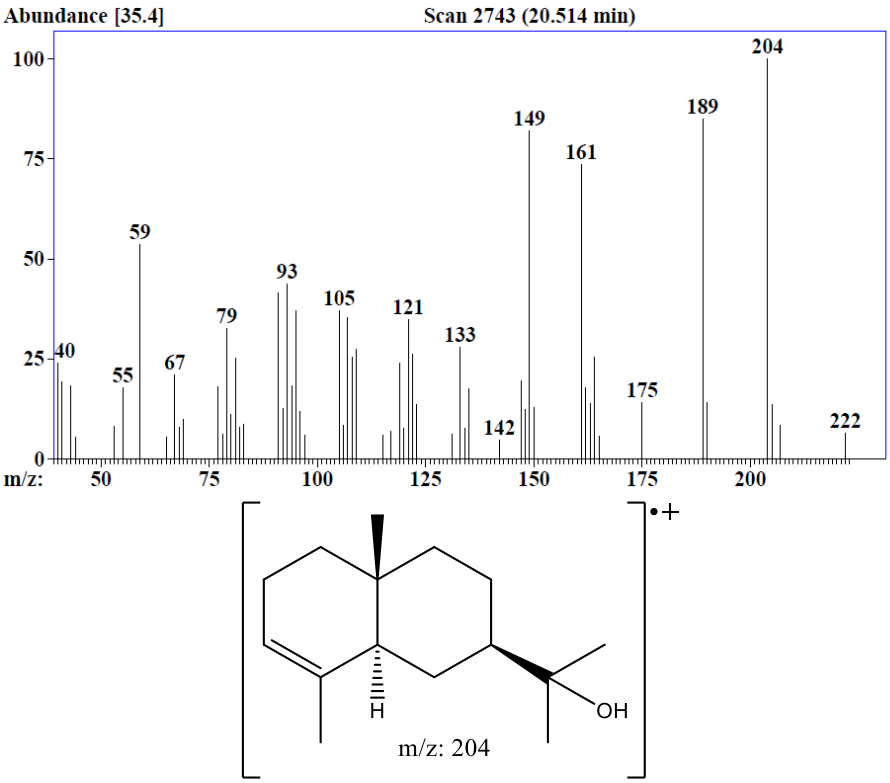


Figure A.46. Fragmentation pattern of α-eudesmol **144**, at AI = 1653 from the GC/MS analysis of an extract isolated through maceration of the *Boswellia occulta* resin with hexane following the procedure in **Section 6.06**. Assignment confidence = high. Literature comparison.[21]

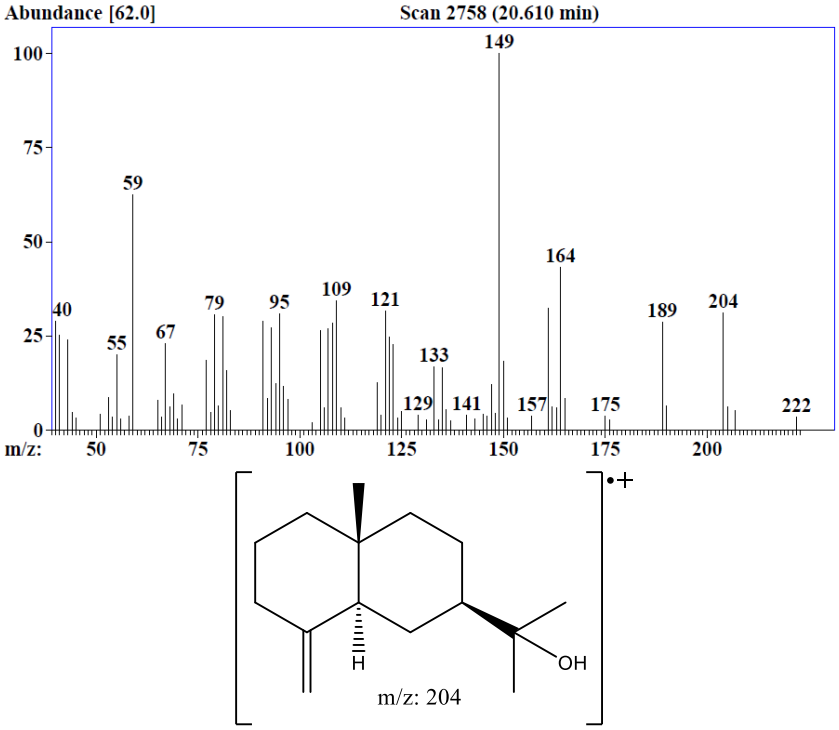


Figure A.47. Fragmentation pattern of β-eudesmol **142**, at AI = 1659 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Najdi grade resin following the method in **Section 6.07**. Assignment confidence = high. Literature comparison.[21]

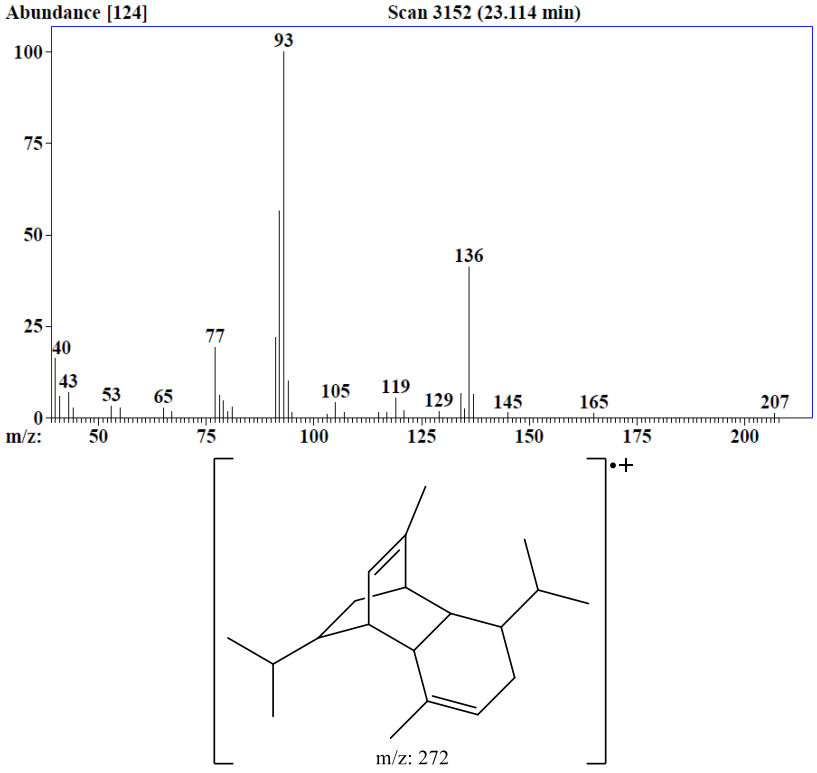


Figure A.48. Fragmentation pattern of an α-phellandrene dimer **131** at AI = 1815 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Sha'abi grade resin following the method in **Section 6.07**. Assignment confidence = low. Literature comparison.[15]

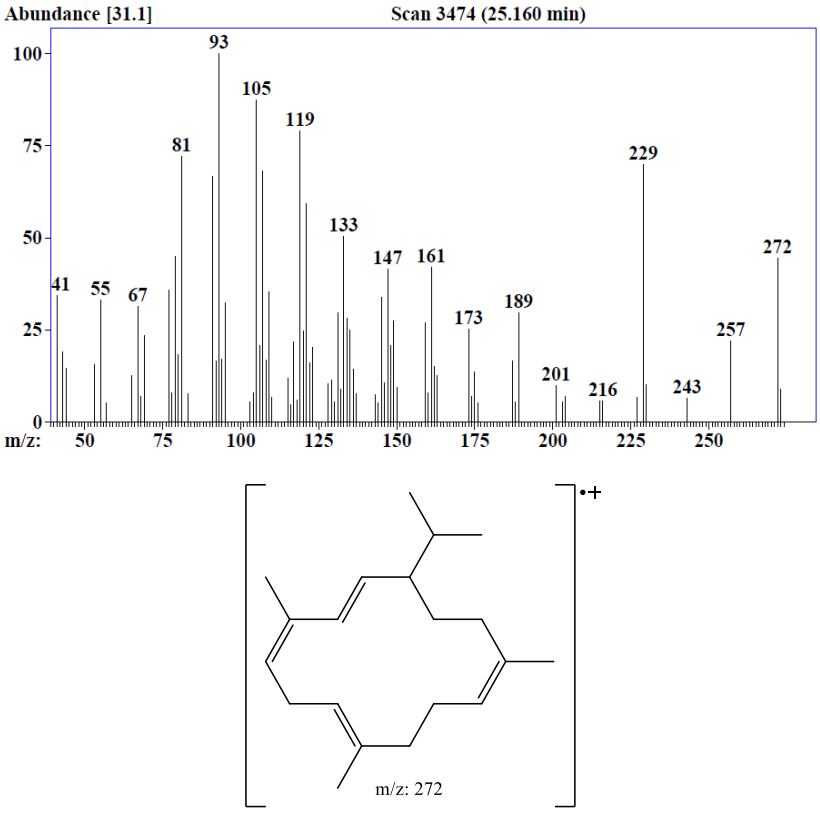


Figure A.49. Fragmentation pattern of cembrene **55**, AI = 1917 from the GC/MS analysis of an oil isolated through hydrodistillation of the grade 1 Sudanese resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

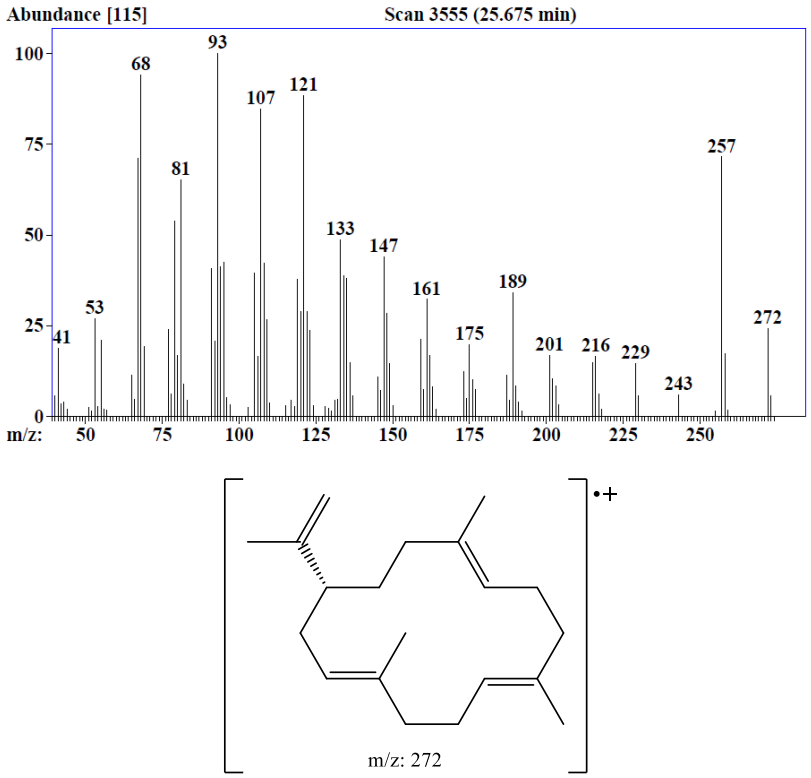


Figure A.50. Fragmentation pattern of (3*E*)-Cembrene A **56**, AI = 1950 from the GC/MS analysis of an oil isolated through hydrodistillation of the grade 1 Sudanese resin following the procedure in **Section 6.05**. Assignment confidence = high. Literature comparison.[21]

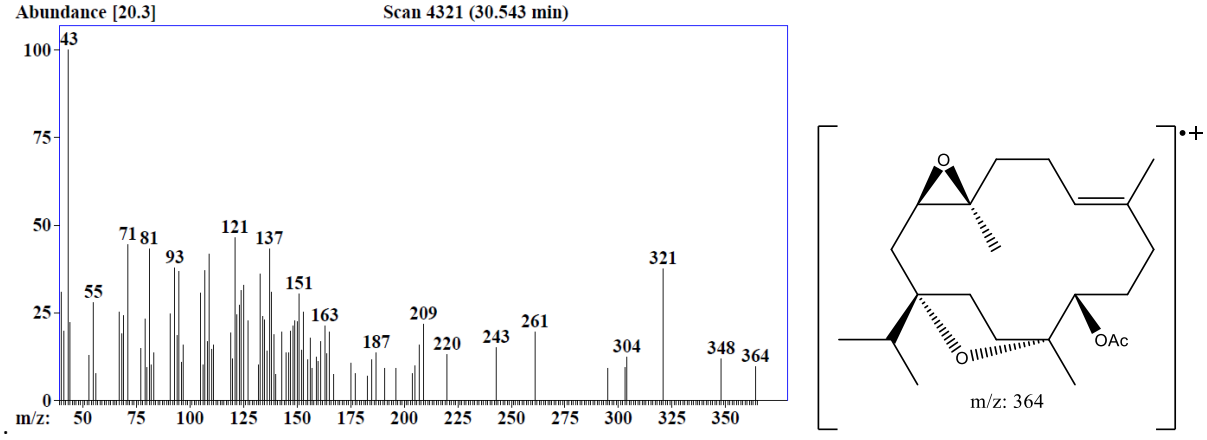


Figure A.51. Fragmentation pattern of incensole acetate epoxide 1 **154**, at AI = 2268 from the GC/MS analysis of an extract isolated through maceration with chloroform following the procedure in **Section 6.06**. No literature comparison so this is purely speculative. Assignment confidence = low. Although this compound has been found in *Boswellia papyrifera* in the literature.[94]

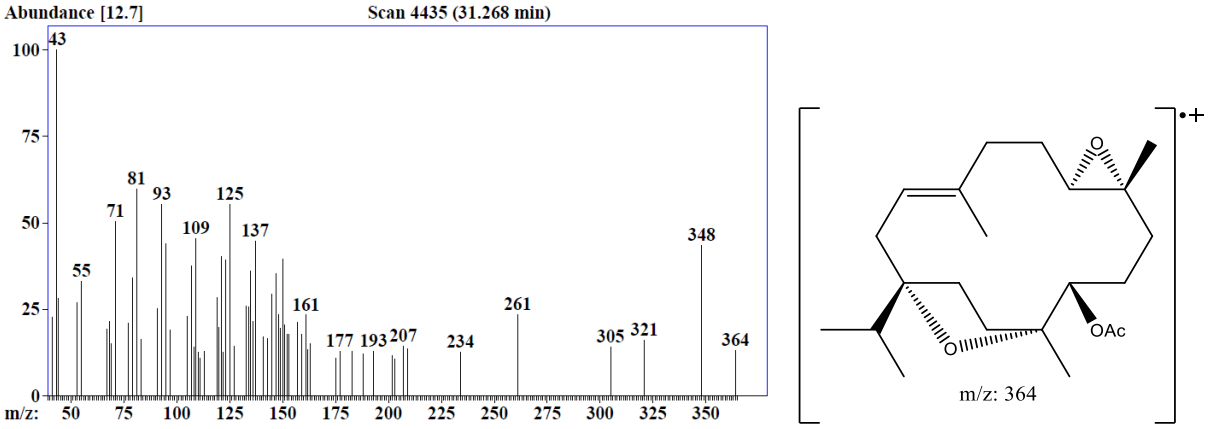


Figure A.52. Fragmentation pattern of incensole acetate epoxide 2 at AI = 2320 from the GC/MS analysis of an extract isolated through maceration with chloroform following the procedure in **Section 6.06**. No literature comparison so this is purely speculative. Assignment confidence = low. Although this compound has been found in *Boswellia papyrifera* in the literature.[94]

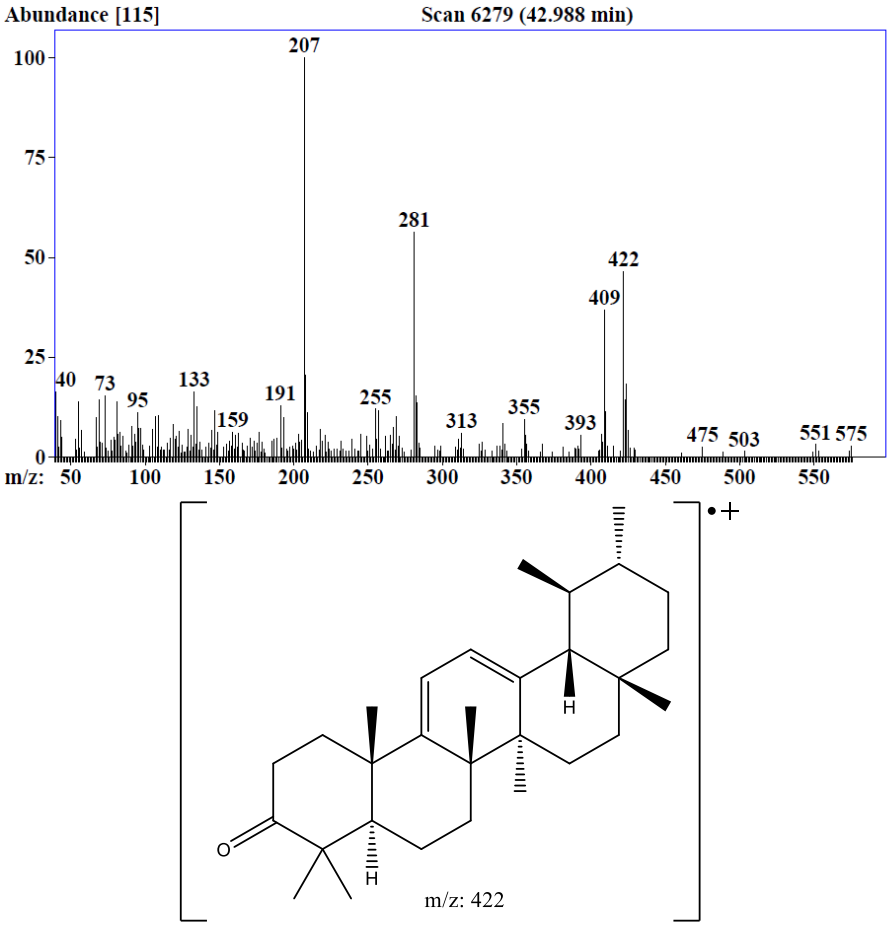


Figure A.53. Fragmentation pattern of Ursa-9(11),12-dien-3-one **136**, at AI = 3283 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. No literature comparison so this is purely speculative. Assignment confidence = low.

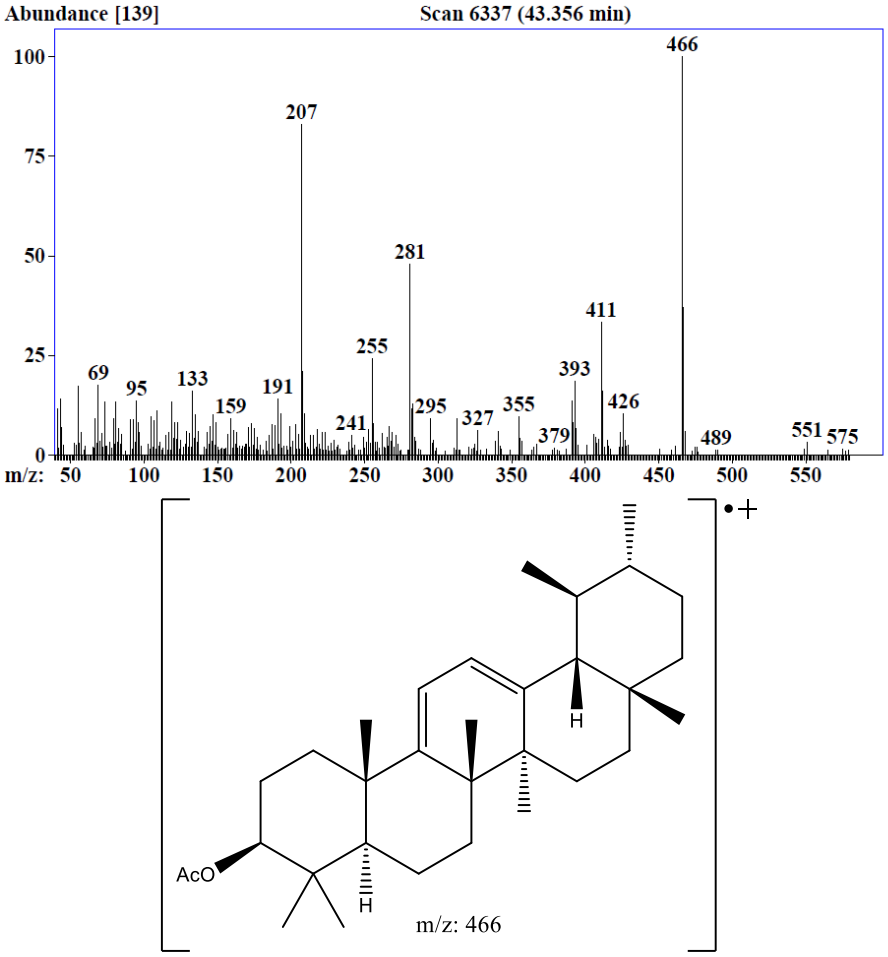


Figure A.54. Fragmentation pattern of Ursa-9(11),12-dien-3-yl acetate **137**, at AI = 3310 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. No literature comparison so this is purely speculative. Assignment confidence = low.

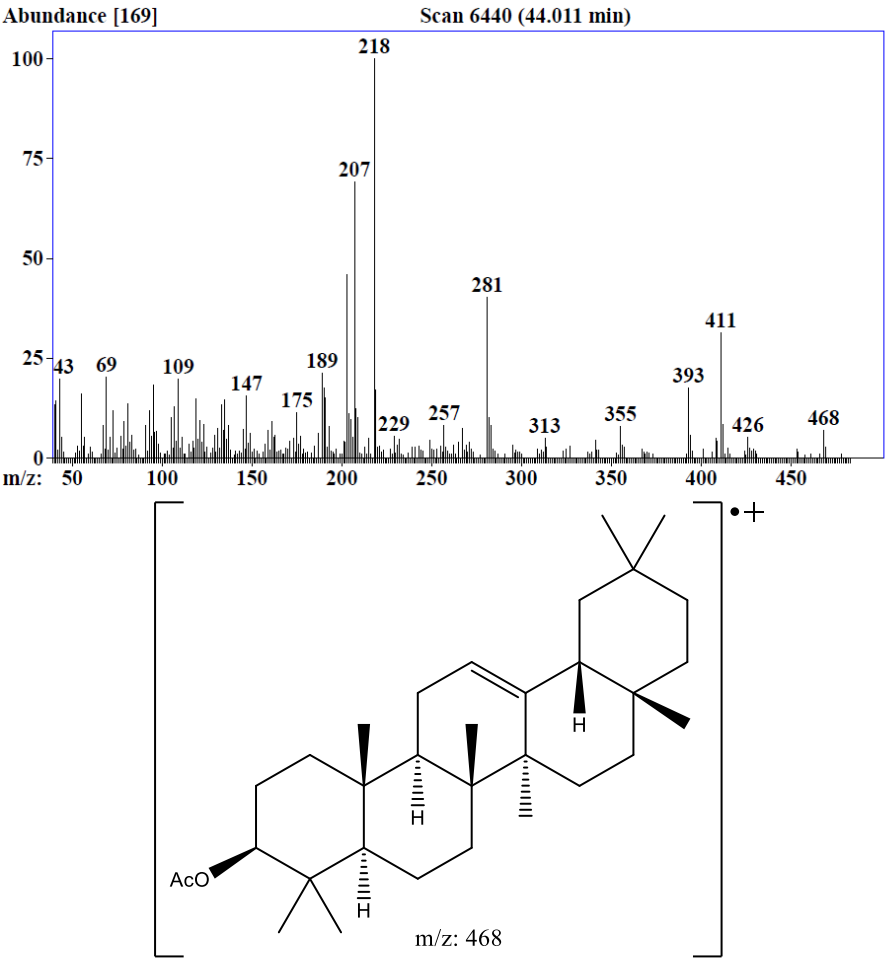


Figure A.55. Fragmentation pattern of β-amyrin acetate **138**, at AI = 3347 from the GC/MS analysis of an extract isolated via the acetone Soxhlet extraction of the Hoojri grade resin following the method in **Section 6.07**. Assignment confidence = medium. Literature comparison (in the supplementary information).[85]