Molecular studies of fresh and aged triterpenoid varnishes
van der Doelen, G.A.

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2. Triterpenoid compounds in fresh dammar and mastic resin¹

Abstract

The chemical composition of fresh dammar and mastic was investigated by gas chromatography-mass spectrometry (GCMS) and high performance liquid chromatography-mass spectrometry (HPLC-MS) and compared to literature. Fifteen compounds with dammarane, oleanane, ursane and hopane skeletons were identified by GCMS and HPLC-MS in fresh dammar resin, whereas ten compounds with the euphane, oleanane and dammarane skeleton as well as two bicyclic triterpenoids were identified in fresh mastic resin. Direct temperature-resolved mass spectrometry (DTMS) was used as a fingerprinting technique and DTMS peaks of fresh dammar and mastic resin were identified.

2.1. Introduction

Terpenoids, which are widely distributed in nature in both the plant and animal kingdom, are made up of units of the 5-carbon compound isoprene. Triterpenoids are 30-carbon substances that often contain ring systems and a number of functional groups. The triterpenoid resins dammar and mastic mainly consist of triterpenoids together with a proportion of polymeric material.

A good knowledge of the composition of fresh dammar and mastic resin is necessary for ageing studies of these resins as addressed in the following chapters. To trace the molecular changes that are induced by ageing the chemical

composition of the fresh starting material has to be well known. Gas chromatography-mass spectrometry (GCMS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and direct temperature-resolved mass spectrometry (DTMS) will be used for the investigation of the aged materials. Therefore, the fresh resins were also studied with these techniques. GCMS analysis of dammar resin [1] and of the acidic components of mastic resin [2] has been published. HPLC has not been used before for the analysis of dammar and mastic resin. HPLC-MS enables the analysis of a broader compound range than GCMS and was therefore explored for its ability to separate the constituents of triterpenoid samples. Identification of the triterpenoid constituents separated by HPLC was performed by collection of the HPLC fractions, subsequent analysis by GCMS and identification by their EI (70 eV) mass spectra (Chapter 6).

The triterpenoid fraction of the resins has been investigated extensively as described in the literature review below. Subsequently, the analysis by GCMS, HPLC-MS and DTMS of fresh dammar and mastic resin will be presented.

2.2. Literature review of dammar resin

The triterpenoid resin dammar originates from the trees of the family Dipterocarpaceae, which grow in the Malay States and in the East Indies. “Damar” is the Malay word for resin or a torch made from resin [3]. A few hundred species produce dammar resin, of which probably the resin of only a few species is exported to the west. The resin may exude naturally to some extent onto the surface of the bark, but it is generally collected by wounding the tree by means of small incisions [4-6]. The resin production varies from tree to tree, ranging from a few kilograms to twenty to thirty kilograms per year [4]. The resin particles are sorted out by their particle size, pellucidity and degree of contamination [6, 7]. The most pure and clear batches of dammar are sold as “dammar mata kucing” (“cat’s eye”). The dammar resins are generally described by the name of the port from which they are exported, like Batavia, Padang and Singapore [7]. The identity of the botanical source is usually lost as the dammar passes through the various stages of marketing. Their main use is still in the manufacture of paper or wood varnishes and lacquers, and some paints, although consumption has inevitably declined over the years with the widespread use of synthetic materials [3]. Nowadays they are used especially as a varnish for the fine arts. The exported dammar appears to be of fairly consistent chemical composition, despite of its imprecise botanical origin (discussed further in section 2.4.). The dammar resin, which is used in the West, probably originates from the following genera of the family Dipterocarpaceae: Hopea, Shorea, Balanocarpus, and Vateria. The genus Canarium of the family
Triterpenoid compounds in fresh dammar and mastic resin

Burseraceae also yields a commercial product considered to be a dammar resin [3, 5, 8].

The polymer of dammar was identified as polycadinene [9]. Its structure is shown in Figure 1. Unlike the triterpenoid fraction, this polymer is not soluble in alcohol. In the past, the alcohol insoluble part of dammar, was called β-resene, whereas the alcohol soluble part was called α-resene. In addition, dammar also contains a small sesquiterpenoid (C_{15}) fraction [1]. The triterpenoid fraction of

![Figure 1](image1.png)

**Figure 1** Molecular structure of polycadinene.

![Figure 2](image2.png)

**Figure 2** Skeleton types of triterpenoids occurring in dammar and mastic resin.
fresh dammar resin has been investigated extensively. Triterpenoids are usually classified according to their carbon skeleton type. Dammar consists largely of compounds of the tetracyclic dammarane skeleton series, but the pentacyclic oleanane, ursane and hopane derivatives are also present (Figure 2). The triterpenoid components of dammar as reported in literature are depicted in Figure 3 [1, 8, 10-17]. The labels correspond to those used in other figures of this thesis. Some contradictory observations exist in the literature concerning the occurrence of different stereoisomers in dammar resin. Poehland et al. [8] identified the triterpenoids dammarenediol-II and hydroxydammarenone-I, whereas Mills et al. [12] confirmed the presence of both stereoisomers dammarenediol-I and II, and hydroxydammarenone-I and II. Zumbühl et al. [18] used matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) to identify highly oxidised compounds in dammar resin. MALDI-MS only gives molecular weight information. It was concluded that up to six oxygens had been added to oleanonic and ursonic acid already in the fresh resin. However, it is disputable whether MALDI-MS offers sufficient resolution for the confident identification of these compounds. Dammars from other species of the dipterocarpaceae family have also been investigated [19-30]. Dammarane triterpenes are also found in resins from families other than the Dipterocarpaceae [31-33].

![Diagram of Triterpenoids in Dammar Resin](image)

Figure 3 Triterpenoids found to occur in dammar resin [1, 8, 10-17].
### Triterpenoid compounds in fresh dammar and mastic resin

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Label</th>
<th>Compound Name</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
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<td>nor-amyrone&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>H/CH&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>H</td>
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<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>O</td>
<td>CHO</td>
<td>[1]</td>
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<tr>
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<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>O</td>
<td>CHO</td>
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</tr>
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<td>H</td>
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<td>COOH</td>
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<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>OH, H</td>
<td>COOH</td>
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<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>O</td>
<td>COOH</td>
<td>[1]</td>
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<tr>
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<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>O</td>
<td>COOH</td>
<td>[1, 8, 11]</td>
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![Chemical structures](image)

<table>
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<th>R&lt;sub&gt;4&lt;/sub&gt;</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>hydroxyhopanone</td>
<td>O</td>
<td>[1, 8, 11]</td>
</tr>
<tr>
<td>-</td>
<td>3-acetoxy-22-hydroxy-hopanone</td>
<td>CH₃COO</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>hydroxyoleanonic lactone</td>
<td></td>
<td>[8]</td>
</tr>
</tbody>
</table>

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Label</th>
<th>Compound Name</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>23-hydroxy-2,3-secours-12-ene-2,3,28-trioic acid (2→23)-lactone</td>
<td>[16, 17]</td>
</tr>
<tr>
<td></td>
<td>asiatic acid</td>
<td>[14]</td>
</tr>
</tbody>
</table>

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1. Poehland et al., [8], found only hydroxydammarenone-I (20<sup>r</sup> configuration) and dammarendiol-II (20<sup>s</sup>), whereas Mills et al., [11], found both stereoisomers (20<sup>r</sup> and 20<sup>s</sup>).
2. This compound has the 24<sup>r</sup> configuration.
3. This compound has the 24<sup>s</sup> configuration.
4. It was not determined whether the compound had a nor-oleanane or nor-ursane skeleton.

**Figure 3 (continued).**
2.3. Literature review of mastic resin

Mastic is the resin of the mastic tree (*Pistacia lentiscus* L. from the Anacardiaceae family). Its natural distribution areas encloses the coastal regions of the Mediterranean. Its sub-species *Pistacia lentiscus* L. *var Chia* from the Greek island Chios is the major source of mastic resin [2]. Another species, *Pistacia atlantica* Desf., is regarded by some as a source of turpentine, which was traded under a variety of names such as Chios, Chio or Chian turpentine, or Cyprus Balsam [2, 34]. According to Koller et al. [35], this claim is based on a misunderstanding in the botanical literature. This species, which is cultivated in North Africa, does not deliver turpentine, but a dirty yellow resin somewhat similar to mastic resin. The source plant of the pistachio turpentine is the turpentine or terebinth tree *Pistacia terebinthus* L. Other Pistachio resins are the so-called Indian Bombay mastic from the species *Pistacia khinjuk* Stocks and *Pistacia cabulica* Stocks [35].

A fully grown tree of the *Pistacia lentiscus* L. *var Chia* species produces 1 kg of resin yearly. After the resin is harvested, it is washed with large quantities of water and “green soap” in order to remove impurities. According to Koller et al. [35], this soap treatment can be disadvantageous with regard to the quality of the resin. The processing treatment causes the clear resin teardrops to turn yellow and cloudy.

\[
\text{cis-1,4-poly-}\beta\text{-myrcene} \quad \text{[36]}
\]

![Molecular structure of cis-1,4-poly-β-myrcene](image)

The polymer of mastic was identified as *cis*-1,4-poly-*β*-myrcene [36]. Its structure is shown in Figure 4. Mastic also contains a small fraction (approximately 2%) of essential oil, which was analysed by Papageorgiou et al. [35, 37]. A number of the triterpenoid constituents of gum mastic have been identified [2, 35, 38-41]. In the latter four of these investigations it is not mentioned whether the resin was obtained from *Pistacia lentiscus* L. or from its subspecies *Pistacia lentiscus* L. *var. Chia*. The triterpenoids present in mastic resin are of the tetracyclic euphane- and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type (Figure 2) [42]. In addition, bicyclic and tricyclic triterpenoids are also found to occur in mastic resin [40, 41]. The identified triterpenoids are depicted in Figure 5. Similar to dammar resin, Zumbühl et al. [18] report that the constituents of fresh
mastic resin are highly oxidised. Up to six oxygens were presumed to have been added to each of the presumed initial components of mastic resin. Much research has concentrated on the elucidation of the chemical composition of the galls of *Pistacia* L. [43-50].

**Figure 5** *Triterpenoids found to occur in mastic resin [2, 35, 38-41].*
Chapter 2

In case of the iso compounds, the double bond is located at C8.

The double bond is located at C17.

In case of germanicol, the double bond is located at C18.

In case of moronic acid, the double bond is located at C18.

Figure 5 (continued).
2.4. GCMS analysis

Gas chromatography-mass spectrometry (GCMS) is an established technique for the analysis of complex mixtures, holding a prime position in analytical chemistry because of its combination of sensitivity, wide range of applicability and versatility [51]. GCMS requires the sample under investigation to be volatile enough to pass the GC column in the gaseous phase, which excludes the analysis of high molecular weight material. Compounds must also be stable with respect to thermal decomposition and rearrangement in the gaseous phase. In the case of triterpenoids containing hydroxyl groups, dehydration may occur in the gas chromatograph, causing the components to be separated as their dehydration products. A number of derivatisation methods are developed to increase the volatility and gas-phase stability of specific, usually polar, compounds. In the past, treatment with the gas diazomethane (CH₂N₂) was used to methylate acid groups [52]. Nowadays, this method is often replaced by an alternative methylation method with trimethylsilyldiazomethane (CHN₂Si(CH₃)₃) [53]. The reagent reacts with methanol, which is added to the sample solution, forming trimethylsilylmethanol and diazomethane (Figure 6(a)). Diazomethane reacts with the sample in the usual way. Another derivatisation method is trimethylsilylation, which in addition to forming esters with the acid groups produces trimethylsilyl ethers with any hydroxyl group (Figure 6(b)). A serious disadvantage of this method is the susceptibility of the resulting derivatives to hydrolysis by traces of water [51]. In case of triterpenoid compounds, the disadvantage of this derivatisation method lies in the fact that these resulting mass spectra can not be compared with the major part of those reported in the literature. Methylation is more often used for these substances.

![Figure 6](image)

**Figure 6** GCMS derivatisation procedures: methylation of carboxyl groups by trimethylsilyldiazomethane (a) and trimethylsilylation of carboxyl and hydroxyl groups by a silylating agent (b).
Because the polymeric fraction of the fresh resins is too large to be analysed by GC, methanolic extracts of dammar and mastic were analysed by GCMS, in which only the triterpenoid fraction dissolves. Fresh dammar and mastic resin from different companies were analysed (dammar from Van der Linde, and Kremer, mastic from Schmincke, Kremer and Roberson). The analysis of different lumps of the resins showed that the chemical composition differs from one lump to another, varying mainly in the relative distributions of the components. In the case of dammar resin, this was also observed by de la Rie [1] and Wenders [54]. This is not surprising, since it is likely that, especially in the case of dammar, the lumps are not originating from one type of tree [4]. Wenders also found that the older dammar resins were more yellow and contained more oxidised compounds [54]. Especially old dammar solutions contained a large relative amount of oxidised triterpenoids. The triterpenoid compounds of the fresh resins are well separated by GC (Figure 7). The mass spectra were interpreted and compared with spectra

![Dammar Chromatogram](image1)

![Mastic Chromatogram](image2)

**Figure 7** Gas chromatograms of methylated fresh dammar and mastic resin (peak labels refer to table I).
available in the literature [1, 2, 8, 41, 55-57]. Table I lists the compounds identified and their molecular weight (MW). The peak labels that are used in this table correspond to the peak labels used in Figure 3 and 5. The mass spectrometric behaviour of these triterpenoids under EI conditions is described in Chapter 6. Other compounds, which are not present in the gas chromatogram of Figure 7, like \(\alpha\)-amyrin, dammarenediol, oleanolic acid and ursolic acid for dammar, and like \(\beta\)-amyrin and tirucallol for mastic, were also found to occur to a small extent in fresh triterpenoid resins.

<table>
<thead>
<tr>
<th>Label</th>
<th>Compound name</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dammaradienone (3-oxo-dammar-20(21),24-diene)</td>
<td>424</td>
</tr>
<tr>
<td>2</td>
<td>Nor-(\alpha)-amyrone (3-oxo-28-nor-urs-12-ene)</td>
<td>410</td>
</tr>
<tr>
<td>3</td>
<td>Dammarradienol (3(\beta)-hydroxy-dammar-20,24-diene)</td>
<td>426</td>
</tr>
<tr>
<td>4</td>
<td>20,24-Epoxy-25-hydroxy-3,4-seco-4(28)-dammaren-3-oic acid(^1)</td>
<td>474</td>
</tr>
<tr>
<td>5</td>
<td>Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid(^2))</td>
<td>458</td>
</tr>
<tr>
<td>6</td>
<td>Oleanonic acid (3-oxo-olean-12-en-28-oic acid)</td>
<td>454</td>
</tr>
<tr>
<td>7</td>
<td>20,24-Epoxy-25-hydroxy-dammaran-3-one(^1)</td>
<td>458</td>
</tr>
<tr>
<td>8</td>
<td>Hydroxydammarenone (20-hydroxy-24-dammaren-3-one(^1))</td>
<td>442</td>
</tr>
<tr>
<td>9</td>
<td>Oleanonic aldehyde (3-oxo-olean-12-en-28-al)</td>
<td>438</td>
</tr>
<tr>
<td>10</td>
<td>Ursenic acid (3-oxo-12-ursen-28-oic acid)</td>
<td>454</td>
</tr>
<tr>
<td>11</td>
<td>Ursonic aldehyde (3-oxo-urs-12-en-28-al)</td>
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<tr>
<td>12</td>
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<td>13</td>
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<td>442</td>
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<td>14</td>
<td>Nor-(\beta)-amyrone (3-oxo-28-nor-olean-12-ene)</td>
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<tr>
<td>15</td>
<td>(\beta)-Amyrone (3-oxo-olean-12-ene)</td>
<td>424</td>
</tr>
<tr>
<td>16</td>
<td>(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene</td>
<td>444</td>
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<tr>
<td>17</td>
<td>Moronic acid (3-oxo-olean-18-en-28-oic acid)</td>
<td>454</td>
</tr>
<tr>
<td>18</td>
<td>(Iso)masticadienonic acid (3-oxo-13(\alpha),14(\beta),17(\beta)H,20(\alpha)H-lanosta-8,24-dien-26-oic acid or 3-oxo-13(\alpha),14(\beta),17(\beta)H,20(\alpha)H-lanosta-7,24-dien-26-oic acid)</td>
<td>454</td>
</tr>
<tr>
<td>19</td>
<td>Idem</td>
<td>454</td>
</tr>
<tr>
<td>20</td>
<td>3-O-Acetyl-3epi(iso)masticadienolic acid (3(\alpha)-acetoxy-13(\alpha),14(\beta),17(\beta)H,20(\alpha)H-lanosta-8,24-dien-26-oic acid or 3(\alpha)-Acetoxy-13(\alpha),14(\beta),17(\beta)H,20(\alpha)H-lanosta-7,24-dien-26-oic acid)</td>
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<tr>
<td>21</td>
<td>Idem</td>
<td>498</td>
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<tr>
<td>22</td>
<td>Dammarenediol (20-dammar-24-ene-3(\beta),20-diol(^2))</td>
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<tr>
<td>23</td>
<td>Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)</td>
<td>440</td>
</tr>
<tr>
<td>24</td>
<td>Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)</td>
<td>440</td>
</tr>
</tbody>
</table>

\(^1\) The configuration at C-20 and C-24 was not determined.
\(^2\) The configuration at C-20 was not determined.
A specific triterpenoid that was found by Koller et al. [35] to be a very stable and specific marker for mastic resin, 28-norolean-17-en-3-one, was not observed in any of the mastic resin analysed in our laboratory. Pastorova [58] demonstrated that this specific compound is formed on pyrolysis of oleanonic acid, which is a major constituent of mastic resin. 28-Norolean-17-en-3-one is formed by decarboxylation of oleanonic acid and subsequent migration of the double bond by a 1,3-H shift. Koller used the split GC injection mode at a temperature of 260ºC. It is possible that pyrolytic degradation of oleanonic acid took place in the injector.

GCMS also demonstrated that fresh dammar contains a small sesquiterpenoid fraction. The analysis of this fraction falls outside the scope of this thesis.

2.5. **HPLC-APCI-MS analysis**

Compounds that can not be rendered volatile for GC analysis, such as very polar, ionic or large compounds, can often be analysed by High-Performance Liquid Chromatography (HPLC). This technique is suitable for the analysis of a much broader range of compounds. In contrast to GC, the temperature is kept around room temperature, which enables the analysis of thermolabile compounds. In addition, no chemical derivatisation is required. Another advantage of HPLC over GC is the possibility of using UV/VIS detection. A UV/VIS detector gives information about the UV/VIS absorbing characteristics of the compounds eluting from the HPLC column. This detector can be useful for the investigation of the yellowing characteristics of varnishes. A major disadvantage of HPLC over GC is the lower resolving power of HPLC. As will be described in Chapter 6, atmospheric pressure chemical ionisation (APCI) can be used for interfacing HPLC with mass spectrometry.

Mixtures of triterpenoids have been successfully separated by reversed phase HPLC, using mainly acetonitrile/water or methanol/water combinations as eluents [59-61]. The methanolic extracts of fresh dammar and mastic resin were analysed by reversed phase HPLC-APCI-MS. A combination of acetonitrile and water as the eluent system was found to resolve the main constituents of fresh dammar and mastic resin. Figure 8 depicts the reversed phase HPLC-APCI-MS total ion currents (TIC) of fresh dammar and mastic resin. Mass spectrometric detection was performed in the positive ion mode, because detection in the negative ion mode is far less sensitive as described in Chapter 6. As was the case in
Triterpenoid compounds in fresh dammar and mastic resin

GCMS experiments, the relative distributions of the compounds vary when different lumps of resins are analysed. As discussed in Chapter 6, mass spectra obtained under APCI conditions do not give sufficient information in order to identify unknown compounds. Therefore, HPLC fractions were collected and subsequently analysed by GCMS (off line HPLC-GCMS), in order to identify the constituents of the resins by their EI (70 eV) spectra [1, 2, 8, 41, 55-57]. Table I lists the compounds identified and their molecular weight. The peak labels that are used here correspond to those presented in Figure 3 and 5. The mass spectrometric behaviour of these triterpenoids under APCI conditions is described in Chapter 6. The two stereoisomers of hydroxydammarenone (8) were identified by ammonia chemical ionisation (NH₃/CI) DTMS. The hydroxyl group of hydroxydammarenone is easily eliminated under EI conditions, which complicates the molecular identification. This is not the case under NH₃/CI conditions as described in Chapter 6. The identification of the specific stereoisomers could not be achieved.

Figure 8  Total ion current traces of reversed phase HPLC-APCI-MS, using the positive ion mode, of fresh dammar (a) and mastic (b) resin (peak labels refer to Table I).
by these methods. Two compounds, which have not been found before in dammar resin, i.e. oleanolic and ursolic aldehyde (23 and 24), were identified by their EI spectrum. The relative amount of these compounds varied from one resin lump to another and these compounds were also detected by GCMS. It is evident that some of the triterpenoids which contain an acid group (compounds 5, 6, 10, and 17) in fresh dammar as well as in mastic resin are not well resolved by this acetonitrile/water eluent system. The addition of an acidic compound to the eluent may improve the separation. Decreasing the pH will suppress the ionisation of acids and allows the acids to be separated by the reversed-phase method [62]. The other triterpenoids with hydroxyl, keto and/or aldehyde groups are well separated. Interestingly, the two stereoisomers hydroxydammarenone I and II (8), which are abundantly present in dammar resin, are well separated by reversed phase HPLC. This separation could not be achieved by GCMS. The highly oxidised compounds, mentioned by Zumbühl et al. [18], in fresh dammar and mastic resin were not detected by GCMS nor by HPLC-MS analysis. It is likely that these compounds are not volatile enough to elute from the GC column used here. However, these types of polar compounds should have been detected by HPLC-MS. It is possible that these specific compounds are present only in very low amounts in fresh dammar and mastic resin. The relatively high response by MALDI-MS found by Zumbühl et al., may be caused by the high amount of oxygen that is inserted in the molecule. Acidic compounds easily form sodium adducts under MALDI-MS conditions and therefore give a relatively high response. It is also possible that the solvent tetrahydofuran (THF), which was used for the MALDI-MS experiments, has induced some molecular changes in the resin. THF is known to create radicals when irradiated by light, which may react with the analyte. Sample solutions in THF are therefore not very stable.

2.6. DTMS analysis

Complex samples, such as aged varnishes from paintings, are often difficult to analyse with ‘standard’ methods, such as GCMS and HPLC-MS, because they are complex mixtures of complex molecular structures, which are difficult to dissolve or are totally insoluble in solvents. Direct-temperature resolved mass spectrometry (DTMS) is a technique that is suitable for these complex materials [63]. Figure 9 depicts the different stages of a DTMS experiment. With this technique, heat is applied to the sample, which results in volatilisation and subsequent pyrolysis of the analyte. Heating of the probe, which is equipped with a Pt/Rh (9/1) filament (100 micron diameter), takes place within the ion source, which allows immediate ionisation of the volatilised molecules [63]. Different
Triterpenoid compounds in fresh dammar and mastic resin

Types of ionisation techniques can be applied, such as electron impact (EI) and chemical ionisation (CI). Mainly low voltage EI (16 eV) DTMS is performed for the analysis of the triterpenoids in order to minimise fragmentation and obtain molecular information. A JEOL SX-102 double focusing mass spectrometer (B/E) is used for the DTMS experiments. Since the ion source is held at vacuum ($10^{-4}$ Pa), oxidation (burning) of the sample inside the source is impossible. A separation is achieved between the low molecular weight fraction (volatiles) and the polymeric fraction of the sample by gradually raising the temperature of the probe. Low molecular weight compounds such as di- and triterpenoids, fatty acids and waxes are volatilised at low temperature, whereas polymeric material such as the oil network polymer, polysaccharides and proteins are released at higher temperature by pyrolytic degradation. A number of metals, such as sodium, potassium, lead, mercury, cadmium, zinc are detected and in the case of certain pyrolysis wires iron is detected as well [64, 65]. Inorganic pigments are first reduced and the corresponding metal ions subsequently desorb from the probe. Compounds are released from the probe at a certain temperature and every second a mass spectrum is recorded. The resulting total ion current (TIC) gives information about the volatile and polymeric part of the sample (Figure 10). Data can be presented as a full mass spectrum when all spectra are summarised, which provides a complete mass spectrometric overview of the sample. Advantages of DTMS are the sensitivity (picomole range), the broad compound class acceptance, the absence of chemical workup and the short analysis time (in the order of minutes). Pyrolysis Mass Spectrometry (PYMS) is a related technique in which the total sample is pyrolysed. Samples suited for PYMS analysis predominantly contain polymeric material. The temperature resolution obtained by DTMS, resulting in the separation of the volatile and the polymeric part of the sample, is not achieved by PYMS.

**Figure 9** Schematic diagram of the experimental setup of DTMS.
Figure 11 shows the low voltage EI (16 eV) DTMS summation spectra of fresh dammar and mastic resin. As was the case in GCMS and HLPC-MS experiments, the relative distributions of the mass peaks vary when different lumps of resins are analysed. The molecular identification of the different components of fresh dammar and mastic resin by GCMS enables the allocation of mass fragments of the DTMS spectra of these compounds. It has to be kept in mind however that the triterpenoid samples were methylated prior to GCMS experiments to render acidic triterpenoids more volatile, whereas the samples are underivatised when analysed by DTMS. Naturally, samples can be derivatised prior to DTMS analysis, but this is not necessary. The peak at m/z 109 is caused by the side chain cleavage of compounds with the dammarane skeleton, such as 1, 3, 5, 8, and 22, which are highly abundant in dammar resin. Peaks at m/z 203, 232, 248 and 409 represent fragment ions from compounds with the oleanane or ursane skeleton with either an aldehyde or an acid group at C28 (6, 9, 10, and 11). Oleanonic, ursonic and moronic acid (6, 10, and 17) give a molecular ion peak at m/z 454, whereas oleanonic and ursonic aldehyde (9 and 11) give a molecular ion peak at m/z 438. Peaks at m/z 355 and 424 are mainly caused by the presence of hydroxy-dammarenone (8), which is highly abundant in dammar resin. Dammaradienol (3) and dammarenediols (22) are represented by a peak at m/z 426. The peak at m/z 440 represent a major fragment ion ((M-H\textsubscript{2}O\textsuperscript{+}) of dammarenolic acid (5). The peak at m/z 439 is characteristic for mastic resin and represents fragment ions of isomasticadienonic and masticadienonic acid (18 and 19).

Figure 10 Example of a DTMS total ion current.
The molecular identification of compounds is facilitated by the complementary use of ammonia chemical ionisation (NH$_3$/CI) and EI ionisation. Whereas EI promotes fragmentation of a molecule yielding structural information, NH$_3$/CI mainly produces [M+H]$^+$ or [M+NH$_4$]$^+$ ions yielding molecular weight information. The proton affinities of the main functional groups of triterpenoids, such as ketones and carboxylic acids, are lower than that of ammonia. Thus, proton transfer from NH$_4^+$ is not to be expected although the ammonium adduct will still be formed [66]. Figure 12 shows the NH$_3$/CI-DTMS spectrum of fresh dammar resin. It is clear that relatively less fragmentation takes place compared to EI ionisation. The main peaks such as those at m/z 456, m/z 442 and m/z 460 represent [M+NH$_4$]$^+$ ions of oleanonic/ursonic aldehyde (9/11), dammaradienone (1) and hydroxydammarenone/hydroxyhopanone (8/12) respectively. The ion represented by a peak at m/z 428 could not be identified yet. A number of peaks
probably represent \([\text{M+NH}_4\cdot\text{H}_2\text{O}]^+\) ions, such as \(m/z\) 426, \(m/z\) 442 and \(m/z\) 444, indicative of dammaradienol/dammarenediol (3/22) hydroxydammarenone/hydroxyhopanone (8/12) and dammarenediol (22) respectively, because a hydroxyl group is a good leaving group (see Chapter 6). The peaks at \(m/z\) 472 and \(m/z\) 476 represent the \([\text{M+NH}_4]^+\) ions of oleanonic/ursonic acid (6/10) and dammarenolic acid (5) respectively. The peak at \(m/z\) 458 represents the \([\text{M+NH}_4\cdot\text{H}_2\text{O}]^+\) ion of dammarenolic acid (5). These peaks, which represent acidic triterpenoids, are not abundantly present in the NH\(_3\)/CI spectrum, despite their relatively high abundance in fresh dammar resin as demonstrated by the gas chromatogram of Figure 7. This can be explained by the fact that NH\(_3\)/CI is known to be a selective ionisation medium [66].

2.7. Conclusions

GCMS and HPLC-MS were found to be valuable tools for the analysis of the triterpenoid fraction of fresh dammar and mastic resin. In contrast to the GC results, triterpenoids with an acid group were not resolved, but the stereoisomers hydroxydammarenone I and II were well resolved by the HPLC system used. Two compounds were found to be present in dammar resin, which have not been reported before, oleanolic and ursolic aldehyde. Some compounds, which were mentioned in literature to be constituents of dammar and mastic resin, such as the highly oxidised ursane and oleanane type compounds and 28-norolean-17-en-3-one, could not be demonstrated by us in the fresh dammar and mastic resins that we analysed.
2.8. **Experimental**

2.8.1. **Materials**

Methanolic extracts (5 mg/ml) were prepared of Dammar (A.J. van der Linde, Amsterdam, The Netherlands) and Mastic (H. Schmincke & Co., Erkrath, Germany, “Chios Mastic of first choice, extra light”). Resins from other suppliers (Kremer Pigmente, Aichstetten, Germany and Roberson, London, England) were also analysed, but the analytical results thereof are only described in section 3.4. and not shown.

2.8.2. **GCMS**

An aliquot of 16 µl of the methanolic extracts was evaporated to dryness and subsequently methylated according to the method of Hashimoto et al. [53]. Aliquots of 250 µl of methanol, 25 µl of benzene and 10 µl of TMSdiazomethane were added. This mixture was left at room temperature for 30 minutes. After evaporation to dryness, the sample was dissolved in 1 ml of dichloromethane. GCMS data were obtained with a fused silica BPX5 column (SGE) (25 m × 0.32 mm i.d., 0.25 µm film thickness) in a gas chromatograph (Carlo Erba, series 8565 HRGC MEGA 2). Samples were introduced on the GC column, in quantities of 50 µl, by a Fisons/Carlo Erba Cold On-Column Large Volume Injection System in combination with a Fisons/Carlo Erba AS800 autosampler. The column was coupled directly to the ion source of a JEOL DX-303 double focusing mass spectrometer (E/B). Helium was used as the carrier gas with a linear velocity of approximately 26 cm/s. The temperature was programmed for 2 minutes at 50 °C, subsequently to 250 °C at a rate of 8 °C/min and from 250 °C to 350 °C at a rate of 3 °C/min. A JEOL MP-7000 data system was used for data acquisition and processing. The mass spectrometer was scanned from m/z 40-700 with a 1 second cycle time. Ions were generated by electron impact (70 eV), extracted at 3 kV and postaccelerated to 10 kV. The mass spectrometric information was interpreted and compared with spectra available in the literature.

2.8.3. **HPLC-MS**
Chapter 2

**HPLC**

The methanolic extracts were analysed by HPLC-MS. The HPLC equipment consisted of a solvent-delivery system (HP1090, Hewlett-Packard) and a Rheodyne 7125 injection valve equipped with a 20 µl loop, connected to a C18 column (Merck: LiChrospher 100 RP-18, 5 µm, 250 x 4 mm I.D.), which was kept at 35 °C. For the analysis of the fresh resins, eluent A consisted of a mixture of 20% water and 80% acetonitrile, eluent B was a mixture of 2% water and 98% acetonitrile and eluent C was acetonitrile. Separation was achieved with a linear gradient from A to B in 30 min, followed by an isocratic period of 9 min, going to eluent C in 1 minute, followed by a second isocratic period of 10 minutes using a flow rate of 0.8 ml/min. The HPLC fractions were collected and subsequently analysed after methylation by GCMS in order to identify the HPLC separated compounds.

**APCI-MS(-MS)**

The outlet from the HPLC system was connected directly to the APCI interface of a VG Quattro II mass spectrometer (Micromass/Fisons Instruments). For system control and data processing, MassLynx software (Micromass/Fisons VG) was used. The source and APCI probe temperatures were maintained at 150 °C and 350°C respectively, and the corona discharge was kept at 3.5 kV. The cone voltage was set at 20 V.

### 2.8.4. **DTMS**

About 50-100 µg of fresh dammar and mastic resin were homogenised in approximately 100-200 µl ethanol. An aliquot (about 2 µl) of the resulting suspensions of the resins were applied to the DTMS probe with a syringe (SGE, 5 µl) and dried in vacuo prior to introduction in the ion source. DTMS analysis was performed in a JEOL SX-102 double focusing mass spectrometer (B/E) using a direct insertion probe equipped with a Pt/Rh (9/1) filament (100 micron diameter). Ions were generated by electron impact (16 eV) or chemical ionisation (NH₃) in an ionisation chamber kept at 180 °C and were accelerated to 8 kV. The mass spectrometer was scanned from m/z 20-1000 (EI) or from m/z 60-1000 (CI) with a 1 second cycle time. The probe filament was temperature programmed at a rate of 0.5 A/min to an end temperature of about 800 °C (1 A). Data were acquired using the JEOL MP-7000 data system.
References


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