Molecular studies of fresh and aged triterpenoid varnishes
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6. Mass spectrometric analysis of triterpenoids in dammar and mastic under EI and APCI conditions

Abstract

The molecular information from mass spectra of a number of triterpenoids with different skeleton types obtained under EI(70 eV) and APCI conditions is compared. APCI mass spectra mainly provide molecular weight information. In addition, information about some frequently occurring functional groups in triterpenoids is obtained. The APCI cone voltage was found to influence the degree of fragmentation. In most cases, MS-MS under APCI conditions does not provide extra molecular information because fragment ions are formed which are similar for triterpenoids with different skeleton types.

6.1. Introduction

This thesis deals with the molecular identification of a large number of fresh and aged triterpenoid samples, as described in the previous chapters. Mass spectrometry is mainly used for this identification, therefore this chapter describes the mass spectrometric behaviour of the triterpenoids found in fresh resins and aged varnishes. The molecular information that is obtained from mass spectra will be discussed. The ionisation method used for the mass spectrometric analysis markedly determines the appearance of the mass spectrum. A number of methods to ionise a substance is available, of which electron ionisation (EI) is the most widely used. Fragmentation mechanisms of a large number of triterpenoid compounds under EI conditions are well-known and mass spectral libraries containing EI (70 eV) spectra are available [1-5]. The molecular identification of the samples described in the preceding chapters was therefore based on the

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corresponding EI spectra. Low (16 eV) and high (70 eV) voltage EI were used for direct temperature-resolved mass spectrometry (DTMS) and gas chromatography-mass spectrometry (GCMS) respectively. The molecular separation technique of high performance liquid chromatography interfaced to a mass spectrometer (HPLC-MS) was also used for the analysis of the triterpenoid samples. The triterpenoid analyte is dissolved in a HPLC eluent, such as a combination of acetonitrile and water, prior to introduction into the mass spectrometer, therefore it is relatively difficult to obtain a low pressure in the ion source. For this reason HPLC-EI-MS is hardly used, because EI can only be performed under low pressure (on the order of $10^{-4}$ Pa). The current trend in HPLC-MS is towards the use of ion sources which tolerate higher pressures, such as atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) [6]. Compared to the EI spectra (70 eV) obtained by GCMS, the spectra obtained under relatively high pressure are usually less reproducible and informative. This chapter compares the fragmentation behaviour of triterpenoids found in fresh resins and in aged varnishes under both EI and APCI conditions. APCI-MS spectra were obtained by performing HPLC-APCI-MS. The corresponding EI-MS (70 eV) spectra were obtained by collection of the HPLC fractions and subsequent analysis by GCMS after methylation. Identification of the HPLC fractions was based on the EI mass spectra, because fragmentation patterns are better understood under EI conditions. This chapter will first explain the fundamental aspects of the APCI ionisation technique and describe the effect of the instrumental parameters on the appearance of the mass spectrum under APCI conditions. Secondly, the fragmentation behaviour of a number of molecules with different triterpenoid skeleton types under EI conditions (70 eV) is reviewed and compared to their mass spectrometric behaviour under APCI-MS conditions. Finally, the information obtained by APCI-MS/MS is described and discussed.

6.2. Effect of the instrumental parameters on the appearance of the APCI mass spectrum

APCI-MS/(MS) is often used as a very sensitive detection method, especially in the area of biomedical research where selected-ion monitoring (SIM) and multiple-reaction monitoring (MRM) are applied [7-10]. Moderately polar samples that are not too labile can be analysed by on-line HPLC using APCI [11]. As shown in Figure 1 [11], the HPLC solvent elutes from a capillary, surrounded by a coaxial flow of N$_2$ gas which nebulises the solution, into a heated region. The combination of nebuliser gas, HPLC eluent and heat form an aerosol, which begins to evaporate rapidly. Inside the source is a corona pin, which is held at high voltage
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(2.5-3.0 kV). The discharge that is produced by this high voltage ionises the solvent molecules eluting into the source. A region of reagent gas plasma is formed by a combination of collisions and charge transfer. Any sample molecule which elutes and passes through this plasma region of solvent ions can be ionised by transfer of a proton to form \((M+H)^+\) or \((M-H)^-\) ions. Therefore, the ratio of the proton affinity of the analyte and that of the eluent determines the response factor of the analyte. In addition, sample ions may fragment once they have left the ion source and have been extracted into the vacuum system. As the gas pressure is still relatively high at this point and the ions are being accelerated, collision induced dissociation (CID) can take place. Relatively little attention has been paid to the fundamental aspects of this type of fragmentation under APCI conditions. The voltage of the sample cone, as shown in Figure 1, can be regulated in order to adjust the degree of fragmentation. This CID “up front” is found to be very easy and reproducible and in some cases it has been used for the purpose of structural elucidation [11-14].

The effect of instrumental parameters, such as cone voltage, probe temperature, corona voltage and source temperature on the appearance of the mass spectra of triterpenoid compounds was investigated in the positive ion mode, by on-flow injection of oleanolic acid in acetonitrile/water (90/10). Only the cone voltage was observed to have an effect on the fragmentation patterns. As expected and illustrated in Figure 2(a), (b) and (c), increasing the cone voltage accelerates the ions, which may then gain internal energy by collision with surrounding gas molecules. The increased internal energy leads to more fragmentation. The specific triterpenoid fragments formed under APCI conditions in the positive ion mode will be described in detail below. The mass spectrometric behaviour of oleanolic acid under APCI conditions in the negative ion mode was also investigated (Figure 2(d)). In our experiments, the ion yield in the negative ion mode is approximately a

Figure 1  Schematic diagram of the experimental setup of an APCI interface.
factor 20 lower than that in the positive ion mode. This relatively low probability of producing a negatively charged ion under the high pressure conditions in APCI can be explained by the ready detachment of an electron when colliding with neutral molecules in the ion source. A high electron affinity of the analyte will increase the probability of formation. When a compound is formed in the negative ion mode, it can be concluded that its electron affinity is relatively high. Whereas functional groups are easily lost when the molecule was protonated in the positive ion mode (Figure 2(a), (b) and (c)), this phenomenon was not observed in the negative ion mode. Molecules are deprotonated without any further fragmentation. Especially in the cases of molecules with an acidic group or a hydroxyl group, spectra obtained in the positive and in the negative ion mode are complementary. Molecular mass information is obtained in the negative ion mode, whereas information on the presence of functional groups is gained by analysis in the positive ion mode.

6.3. Comparative EI-MS and APCI-MS studies of triterpenoid compounds

Table I presents the characteristic m/z values of the main triterpenoids found in fresh and aged dammar and mastic varnishes, as described in the preceding chapters, under APCI-MS and EI-MS (70 eV) conditions. Although some (stereo)isomeric compounds were separated by HPLC (8, 18 and 20), their exact identification could not be achieved by their mass spectra alone. The labels correspond to those used in the other chapters. Fragmentation of triterpenoids with different skeleton types under APCI-MS and EI-MS conditions is compared.

6.3.1. Dammaranes

The mass spectra of hydroxydammarenone (8), a molecule with the dammarane skeleton, under EI (70 eV) and APCI conditions are shown in Figure 3. Complete elimination of the hydroxyl group at C20 as H₂O is observed under EI conditions (Figure 4). The side chain of the dammarane skeleton is cleaved at C22 (m/z 355) and ring C cleavage with concerted hydrogen transfer of the dammarane skeleton produces the fragment ion which is represented by a peak at m/z 205 [5]. Under APCI-MS conditions (Figure 3(b) and 4), the protonated molecule eliminates the hydroxyl group at C20 very easily. The presence of m/z 407 suggests that another molecule of water is lost possibly via the keto substituent. This is in accordance with the findings of Harrison [15], who states that ketones
show some elimination of water under chemical ionisation conditions using ammonia. The presence of acetonitrile adducts (represented by peaks at m/z 484 and m/z 466) assists in the molecular mass determination. Cleavage of ring C results in both fragment ion peaks at m/z 219 and m/z 205. Figure 3(c) shows the
Table I  List of compounds occurring in fresh triterpenoid resins and aged varnishes. The molecular weight, the characteristic m/z values of the compounds under APCI-MS conditions and the characteristic m/z values under EI (70 eV) conditions (corresponding methylated compounds) are listed. Labels are used consistently throughout this thesis. All (fragment) ion peaks that were found under APCI conditions, with an intensity higher than 10% of the base peak, are listed. In some cases, fragment ion peaks with an intensity lower than 10% of the base peak which are useful for identification purposes are also shown.

<table>
<thead>
<tr>
<th>label</th>
<th>Compound name</th>
<th>MW</th>
<th>M/z values of characteristic (fragment) ions of methylated compounds under EI (70 eV) (rel. int. %)</th>
<th>M/z values of characteristic (fragment) ions of compounds under APCI (cone 20 V) (rel. int. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Hydroxydammarenone (I or II) (20-hydroxy-24-dammaren-3-one)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>442</td>
<td>424(80), 355(39), 205(42), 109(100)</td>
<td>443(6), 425(100), 407(9), 219(21), 205(13)</td>
</tr>
<tr>
<td>5</td>
<td>Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>458</td>
<td>454(50), 385(48), 109(100)</td>
<td>441(100), 205(23), 191(44)</td>
</tr>
<tr>
<td>22</td>
<td>Dammarenediol (20-dammar-24-ene-3β,20-diol)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>444</td>
<td>426(35), 207(29), 189(12), 109(100)</td>
<td>427(30), 409(100), 219(20), 191(34)</td>
</tr>
<tr>
<td>3</td>
<td>Dammarradienol (3β-hydroxy-dammar-20,24-diene)</td>
<td>426</td>
<td>426(40), 207(84), 189(43), 109(100)</td>
<td>427(32), 409(100), 219(22), 191(42)</td>
</tr>
<tr>
<td>1</td>
<td>Dammarradienone (3-oxo-dammar-20(21),24-diene)</td>
<td>424</td>
<td>424(86), 205(65), 109(100)</td>
<td>425(100), 407(34), 245(24), 189(19)</td>
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<tr>
<td>26</td>
<td>3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone&lt;sup&gt;1&lt;/sup&gt;</td>
<td>414</td>
<td>414(100), 205(64), 99(78), 95(55)</td>
<td>415(100), 397(31), 379(4)</td>
</tr>
<tr>
<td>4</td>
<td>20,24-Epoxy-25-hydroxy-3,4-seco-4(28)-dammaren-3-oic acid&lt;sup&gt;2&lt;/sup&gt;</td>
<td>474</td>
<td>429(11), 143(100)</td>
<td>457, 439(100)</td>
</tr>
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<td>9</td>
<td>Oleanonic aldehyde (3-oxo-olean-12-en-28-al)</td>
<td>438</td>
<td>438(19), 232(48), 203(100)</td>
<td>439(100), 421(27), 411(9), 393(3)</td>
</tr>
<tr>
<td>11</td>
<td>Ursonic aldehyde (3-oxo-urs-12-en-28-al)</td>
<td>438</td>
<td>438(16), 232(21), 203(100)</td>
<td>439(100), 421(24), 411(17), 393(3)</td>
</tr>
<tr>
<td>23</td>
<td>Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)</td>
<td>440</td>
<td>440(9), 232(77), 203(100)</td>
<td>441(29), 423(100), 395(25), 205(14), 191(29)</td>
</tr>
<tr>
<td>24</td>
<td>Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)</td>
<td>440</td>
<td>440(5), 232(21), 203(100)</td>
<td>441(41), 423(100), 395(26), 205(16), 191(34)</td>
</tr>
<tr>
<td>6</td>
<td>Oleanonic acid (3-oxo-olean-12-en-28-oic acid)</td>
<td>454</td>
<td>468(25), 262(58), 203(100)</td>
<td>455, 437, 409(100)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>Ursonic acid (3-oxo-urs-12-en-28-oic acid)</td>
<td>454</td>
<td>468(18), 262(100), 203(77), 133(43)</td>
<td>455(100), 437, 409&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>17</td>
<td>Moronic acid (3-oxo-olean-18-en-28-oic acid)</td>
<td>454</td>
<td>468(48), 249(50), 189(100)</td>
<td>455(100), 437, 409&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>27</td>
<td>11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)</td>
<td>468</td>
<td>482(100), 317(49), 276(80), 257(49), 217(65)</td>
<td>469(100), 451(4), 423(29)</td>
</tr>
</tbody>
</table>
ammonia chemical ionisation (NH$_3$/CI) mass spectrum of hydroxydammarane
obtained by NH$_3$/CI-DTMS. The hydroxyl group is much more stable under these
ionisation conditions as indicated by the base peak at m/z 460 which represents
[M+NH$_4]^+$ ions. The peaks at m/z 442 and m/z 425 represent [M+NH$_4$-H$_2$O]$^+$ ions
and [M+H-H$_2$O]$^+$ ions, which indicates that the hydroxyl group is still a relatively
good leaving group. Additional elimination of water due to presence of the keto
group, as reported by Harrison [15], is not observed in this spectrum.

Figure 5 shows the EI and APCI-MS spectra of an octillone type
stereoisomer. The configuration at C20 and C24 of the octillone type molecule
could not be determined as yet. Under EI conditions, cleavage of ring C with
concerted hydrogen transfer occurs, which results in a fragment ion peak at m/z
205 (Figure 6). The side chain is cleaved at C17 and C24, which produces the
fragment ion peak at m/z 399 and the base peak at m/z 143. In addition to water
elimination, the fragment ion peak at m/z 143 is also present in the APCI-MS
spectrum and is therefore indicative of the presence of this hydroxyisopropyl-
methyltetrahydrofuran side chain.

The APCI-MS spectra of other molecules with the dammarane skeleton
(22, 3, 1, 26 and 4) showed similar results. Cleavage of ring C, with the exception
of compounds 26 and 4, and loss of hydroxyl, aldehyde and keto groups are mainly
observed. 3,4-\textit{A}-seco-triterpenoids, such as dammarenolic acid (5), contain a

Figure 3  Mass spectra of hydroxydammarenone obtained by GC-(EI)MS (70 eV)
(a), HPLC-APCI-MS (b), and NH$_3$/CI-DTMS (c) ("*0.25" indicates that the actual
peak intensity at m/z 425 is a factor of four higher).
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Figure 4  Proposed principal mass spectral fragments of hydroxydammarenone under EI and APCI conditions.

Figure 5  Mass spectra of an ocotillone type molecule obtained by EI (70 eV) (a), and HPLC-APCI-MS (b).

carboxylic acid group at C2 of the A ring. These compounds form characteristic fragment ions under EI conditions (Figure 7) [16]. These fragments ions are not found under APCI conditions. Triterpenoids 5 and 1 show fragment ion peaks at m/z 191, m/z 245 and m/z 189, which could not be assigned to particular fragmentation mechanisms as yet.
6.3.2. Oleananes and ursanes

The mass spectra of oleanonic aldehyde (9) obtained by EI (70 eV) and HPLC-APCI-MS are shown in Figure 8. Under EI conditions, the aldehyde substituent is eliminated to a certain extent (Figure 9). A typical retro-Diels-Alder (rDA) rearrangement takes place, producing peaks at m/z 232 and m/z 203 [1, 2].
Figure 8 Mass spectra of oleanonic aldehyde obtained by EI (70 eV) (a), and HPLC-APCI-MS (b) (“*0.25” indicates that the actual peak intensities at m/z 480 and m/z 439 are four times higher).

Figure 8(b) shows that the protonated molecule obtained under APCI-MS conditions is relatively stable. In addition to adduct formation with acetonitrile (represented by a peak at m/z 480), some elimination of water occurs from (M+H)+ to generate the species represented by a peak at m/z 421, which is due to the presence of the keto or the aldehyde substituent. Elimination of 28 Da from (M+H)+ (to generate a species represented by a peak at m/z 411) can be best explained by the loss of CO from the aldehyde substituent. This loss is also observed in HPLC-APCI-MS analysis of phenolic compounds which bear an aldehyde substituent [10]. According to Madhusudanan [17], the rDA rearrangement takes place under chemical ionisation (CI) conditions and this leads to ions corresponding to both the diene part and the dienophile part of the molecule (Figure 9). This is unlike the EI spectra where rDA reaction gives only the diene...
ion. The APCI mass spectra are somewhat more complex. In Figure 8(b) there are a number of peaks present around m/z 200. Other molecules with an oleanane or the isomeric ursane skeleton, such as oleanolic acid (figure 2(c)), show similar fragment ion peaks in this mass range which are not seen when these molecules are analysed under EI conditions. The presence of fragment ion peaks in this mass range can be best explained by hydrogen migration through the molecule resulting in the “formation” of a double bond at another location in the molecule than at C12. When a molecule is protonated under APCI conditions, this proton can be positioned on a double bond since this feature has a relatively high proton affinity [15]. It is likely that protonation of the double bond under APCI conditions facilitates the hydrogen migration. According to Budzikiewicz et al., this type of migration in the molecular ion also occurs occasionally under EI conditions [1]. The migration of hydrogen resulting in new locations of double bonds has also been found to occur in acyl lipids containing unsaturated fatty acids during the collision induced dissociation (CID) processes in low energy MS-MS experiments [18]. When the HPLC-APCI-MS experiments were repeated, it was observed that

Figure 9  Proposed principal mass spectral fragments of oleanonic aldehyde under EI, CI and APCI conditions.
the relative distribution of the smaller fragment ion peaks did not reproduce very well in contrast to the molecular ion region. The same fragment ion peaks were observed, but their intensity ratios differed. The ion life time prior to mass analysis is relatively long in HPLC-APCI-MS experiments. In addition, the gas involved is relatively dense, which results in multiple collisions [12]. It is likely that these factors give rise to the formation of ions with varying internal energies, which results in a poor reproducibility of fragment ion peaks under APCI conditions. Furthermore, the fragment pattern is also influenced by the conditions of the chromatographic separation. When an HPLC gradient is used, the atmospheric conditions change continuously, which is very likely to have an effect on the fragmentation behaviour of the analytes. The condition of the HPLC column changes due to its age and usage history; this may have an effect on the retention times of analytes and consequently on the corresponding APCI conditions.

Other compounds with the oleanane or ursane skeleton (11, 23, 24, 6, 10, 17, 27 and 32, Table I) showed a similar behaviour under APCI-MS conditions as just discussed for oleanonic aldehyde. In addition to hydroxy, aldehyde and keto groups, the carboxylic acid substituents (in the 17 position) were eliminated as formic acid (6, 10, 17, 27 and 32) to some degree. The loss of formic acid is energetically more favourable than the loss of water and carbon monoxide together [19]. However, carboxylic acid groups on other positions, present on triterpenoids with other skeleton types (5, 4, 18 and 20), were not split off under APCI conditions. Furthermore, fragment ions represented by peaks around m/z 200, which were probably induced by double bond migration, were observed. However, these fragment ion peaks did not reproduce well and were of low intensity. When a hydroxyl group was present at C3 (23 and 24), water was eliminated and fragment ions represented by peaks at m/z 205 and m/z 219 were found, which were probably formed in a rDA reaction. Compounds with a keto group on C11, such as 27 and 32, show characteristic fragment ions under EI conditions, as illustrated for 11-oxo-oleanonic acid (27) in Figure 10 [1, 4]. These fragment ion peaks were not present in the APCI spectra.

![Figure 10](image.png)

**Figure 10** Principal mass spectral fragments of 11-oxo-oleanonic acid under EI conditions.
6.3.3. **Euphanes**

The main fragment ion peaks of compounds with the euphane skeleton, present in mastic resin, such as 18 and 20, are formed by elimination of a methyl group, methanol or an acetoxy group. Other minor fragmentation pathways are described by Papageorgiou et al. [20]. Under APCI conditions, the acetoxy group elimination also takes place (20), but the other eliminations are not observed. Instead of the elimination of a methyl group and methanol, water is lost in the case of 18. Other fragment ion peaks were found under APCI conditions, at m/z 127, 125, 247 and 191, which could not be identified as yet. Under EI conditions, a fragment ion peak at m/z 127 is found to be indicative of methyl masticadienonate [20]. This characteristic fragment ion was also formed under APCI conditions.

6.3.4. **Hopanes**

Triterpenoids with the hopane skeleton type give characteristic fragment ions under EI conditions, as illustrated for hydroxyhopanone (12) in Figure 11 [2]. These fragment ions were not formed under APCI conditions. In addition to some functional group eliminations, hydroxyhopanone (12) showed a fragment ion peak at m/z 179, which could not be identified as yet.

![Figure 11](image)

**Figure 11** Principal mass spectral fragments of hydroxyhopanone under EI conditions.

6.4. **Fragmentation behaviour under APCI-MS-MS conditions**

Low energy HPLC-APCI-MS-MS spectra were obtained with a triple quadrupole mass spectrometer, using argon as the collision gas. Figure 12(a) depicts the APCI-MS-MS spectrum of an ion of 425 Da, which is formed by
protonation of hydroxydammarenone (8) followed by the loss of a water molecule. Figures 12(b) and 12(c) show the APCI-MS-MS spectra of ions of 459 Da and of 439 Da, which correspond to a protonated ocottillone type molecule and protonated oleanonic aldehyde (9) respectively. All MS-MS spectra show peaks representing ring cleavage fragment ions which indicate that molecules of triterpenoid origin

Figure 12 Mass spectra of hydroxydammarenone (a), an ocottillone type molecule (b), and oleanonic aldehyde (c) obtained by HPLC-APCI-MS-MS.
were analysed [21]. The ocothillone type molecule also produces the characteristic fragment ion of m/z 143 under MS-MS conditions. In addition to the elimination of functional groups, mainly non-specific fragment ion peaks below m/z 240 are produced, as seen in Figure 12. When the pressure of the collision gas (argon) is relatively low, fragmentation of the selected protonated molecule is minimal leading to fewer ring cleavage fragment ions. When the pressure was increased, fragmentation again only produced small fragment ion peaks but now with a higher intensity. Unfortunately, these fragment ion peaks observed were similar for triterpenoids with dammarane, oleanane and ursane skeletons, which are often isomeric compounds. The fragment ion peaks of the APCI-MS-MS spectra are therefore of little diagnostic value for the molecular identification of these structures. In most cases, as in Figure 12(a) and 12(c), the MS-MS spectra resembled neither the APCI-MS spectra nor the EI spectra. The fragment ions represented by peaks in the MS-MS spectra (produced in a collision cell) and even those in the APCI-MS spectra (high pressure in the source) are generated by collision induced dissociation processes. Ions that are represented by peaks in the APCI-MS spectra have reached the detector and can be considered as relatively “cool” and stable, because unstable “hotter” ions are likely to be destroyed by the multiple collisions in the dense gas of the ion source. In the APCI-MS spectra of triterpenoids mostly peaks representing protonated molecules ((M+H)^+) were observed. In the process of MS-MS analysis of these ions, the internal energy is increased in a short time. The unimolecular dissociation of these excited (M+H)^+ ions apparently preferentially leads to ring cleavage ions because peaks representing C-ring rearrangement ions have a very low relative intensity in the spectra.

Hazai et al. identified pentacyclic triterpenes by GC-(EI)MS-MS using a triple Q-MS (low energy collisions). In contrast to our observations, their collision-activated dissociation spectra were found to be similar to EI spectra [22]. It can be concluded that when these triterpenoid molecules are ionised by EI, both low energy and high energy collisions give rise to the same fragmentation behaviour. The major difference between these GC-MS-MS experiments and our HPLC-APCI-MS-MS experiments (using the positive ion mode) is the fact that in the latter case the molecules are ionised by protonation (CI). As stated by Harrison, the fragmentation mode of the even-electron protonated molecules is quite different from the fragmentation modes of the odd-electron molecular ions formed by EI ionisation [15]. Since the MS-MS spectra of Figure 12(a) and 12(c) do not resemble the corresponding EI spectra (Figure 3(a) and 8(a)) protonation of a triterpenoid molecule is likely to be an important factor determining the fragmentation behaviour under MS-MS conditions. Retro-Diels-Alder rearrangement in the 12-unsaturated oleanane or ursane type compounds (Figure
6.5. Conclusions

APCI-MS is found to be a mild ionisation method, in which predominantly protonated molecules are formed. Loss of water is observed to a large extent for molecules which contain a hydroxyl group and to a much smaller extent for molecules with a keto group. Triterpenoids with an aldehyde substituent show some loss of CO. The dammarane skeleton type molecules, which have a saturated ring system, predominantly show cleavage of ring C, similar to EI. The fragmentation of the 12-unsaturated oleanane/ursane skeleton type molecules is more complex, probably because of hydrogen migration. This latter phenomenon prevents the identification of the double bond position and it complicates the interpretation of the spectra. Under negative ionisation conditions, deprotonated molecules are formed without any other fragmentation, which gives molecular mass information. In conclusion, in addition to molecular mass information, APCI-MS spectra provide extra information about some frequently occurring functional groups in triterpenoids.

Additional MS-MS produces fragment ion peak patterns which are characteristic for molecules of the triterpenoid class. Unfortunately, identification of specific triterpenoids is not possible, since the MS-MS fragment ion peaks are similar for a number of different triterpenoids. HPLC-APCI-MS-MS of the aged triterpenoid varnishes provided information about the functional groups present, but this information is already available in the APCI-MS spectra.

6.6. Experimental

6.6.1. Materials

Oleanolic acid (Aldrich) was dissolved in methanol and analysed by HPLC-APCI-MS. Fresh triterpenoid resins and aged triterpenoid varnishes were prepared as described in the preceding chapters.
6.6.2. **EI/CI**

EI spectra were obtained from GCMS experiments of triterpenoid samples as described in Chapter 2 and 3. The CI spectrum of hydroxydammarenone was obtained by NH$_3$/CI-DTMS as described in Chapter 2.

6.6.3. **APCI**

The outlet from a HPLC system (as described in Chapter 2), using a mixture of acetonitrile and water as the eluent, was connected directly to the APCI interface of either a VG Quattro (Figure 3, 5, and 8) or a VG Quattro II (Figure 2) 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 (in the case of Figure 2(d), and 5(b)) or at 30 V (in the case of Figure 3 and 8). HPLC-MS-MS experiments were carried out using argon at a pressure of 1.7 * 10$^{-3}$ mbar ± 0.3 * 10$^{-3}$ mbar and a collision energy of 40 V (in case of Figure 3 and 8) or 50 V (in case of Figure 5).

**References**


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