Molecular studies of fresh and aged triterpenoid varnishes

The photograph on the cover is a detail of the panel painting “Madonna met Peer”, which is attributed to the Master of Frankfurt (Museum voor religieuze Kunst te Uden, the Netherlands). The photograph has been taken during the restoration of the painting by Kees Schreuder of the Stichting Restauratie Atelier Limburg (SRAL, Maastricht, the Netherlands).

Cover design in co-operation with Ellis Bartholomeus
and Henk Sodenkamp

The work described in this thesis was performed at the FOM-Institute for Atomic and Molecular Physics (Kruislaan 407, 1098 SJ Amsterdam, The Netherlands). This work is part of the research program of MOLART (Molecular Aspects of Ageing in Painted Art) and of FOM (Foundation for Fundamental Research on Matter), which is a subsidiary of the Dutch Organisation for Scientific Research (NWO). The Priority program MOLART is funded directly by NWO.

ISBN 90-801704-3-7

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. J.J.M. Franse,
ten overstaan van een door
het college voor promoties ingestelde commissie,
in het openbaar te verdedigen
in de Aula der Universiteit
op donderdag 25 maart 1999 te 13.00 uur
door

Gisela Annabel van der Doelen
geboren te Arnhem

Promotor: Prof. Dr. J.J. Boon

MOLART Reports

MOLART – Molecular aspects of Ageing of painted Art – is a 5-year co-operative project between art historians, restorers, analytical chemists and technical physicists funded by the Netherlands Organisation for Scientific Research (NWO). Technical support and advice is given by Shell-SRTCA (Amsterdam), AKZO-NOBEL (Arnhem), Instituut Collectie Nederland (ICN, Amsterdam) and the Dutch art museums. The project was launched on 1 February 1995 and will end early 2002. The object of MOLART is to contribute to the development of a scientific framework for the conservation of painted art on the molecular level. The focus of MOLART is the determination of the present chemical and physical condition of works of art produced in the period from the 15th to the 20th century. Studies of historical paint manufacturing and workshop practice must give insight into the nature of the painter's media and the painting technique used originally. Fundamental studies on varnishes, paints and colorants are undertaken to understand the molecular aspects of ageing since this is thought to be a main cause for the continued need to treat paintings.

This thesis is the first in a series of MOLART reports, that will summarise all research results obtained in the course of the project. Information on this series from MOLART can be obtained from the project coordinator Prof. Dr. J.J. Boon, FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands.

This thesis is based on the following publications:

Van der Doelen, G. A., and Boon, J. J., "Mass spectrometry of resinous compounds from paintings: characterisation of dammar and naturally aged dammar varnish by DTMS and HPLC/GCMS" in *Resins, Ancient and Modern. Pre-prints of the SSCR's 2*nd *Resins Conference*, Aberdeen, 13-14 September 1995, eds. Wright, M.M. and Townsend, J.H., Scottish Society for Conservation & Restoration, Aberdeen, 70-75 + errata.

Van der Doelen, G.A., Van den Berg, K.J., Boon, J.J., Shibayama, N., De la Rie and E.R., Genuit, W.J.L., "Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by HPLC-APCI-MS(/MS)", *Journal of Chromatography A* 809 (1998), 21-37.

Van der Doelen, G.A., Van den Berg, K.J. and Boon, J.J., "Comparative chromatographic and mass spectrometric studies of triterpenoid varnishes: fresh material and aged samples from paintings", *Studies in Conservation* 43 (4) (1998), 249-264.

Boon, J. J., and Van der Doelen, G. A., "Advances in the current understanding of aged dammar and mastic varnishes on the molecular level" in the Postprints of *Firnis, Material Aesthetik Geschichte, International Kolloquium*, Braunschweig, 15-17 June 1998, in press.

Carlyle, L., Binnie, N., Van der Doelen, G. A., Boon, J. J., McLean, B., and Ruggles, A., "Traditional painting varnishes project: preliminary report on natural and artificial aging and a note on the preparation of cross-sections" in the Postprints of *Firnis, Material Aesthetik Geschichte, International Kolloquium*, Braunschweig, 15-17 June 1998, in press.

Van der Doelen, G.A., Van den Berg, K.J. and Boon, J.J., "A comparison of weatherometer aged dammar varnishes and varnishes aged naturally on paintings", submitted to *Techne*.

Glossary

ACN	acetonitrile
APCI	atmospheric pressure chemical ionisation
CI	chemical ionisation
CID	collision induced dissociation
Da	dalton
DCM	dichloromethane
DTMS	direct temperature-resolved mass spectrometry
EI	electron impact, electron ionisation
eV	electronvolt
FF2	FotoFenton 2
GCMS	gas chromatography-mass spectrometry
HALS	hindered amine light stabiliser
HPLC	high performance liquid chromatography
IS	internal standard
$M^{+\cdot}$	molecular ion
$(M+H)^+$	protonated molecule
MALDI	matrix-assisted laser desorption/ionisation
MC540	Merocyanine 540
MS	mass spectrometry
MS-MS	tandem mass spectrometry
MW	molecular weight
m/z	mass/charge
NH_3/CI	ammonia chemical ionisation
rDA	retro-Diels-Alder
SEC	size exclusion chromatography
THF	tetrahydrofuran
TIC	total ion current
UV	ultraviolet (light)
VIS	visible (light)

Contents

1. Triterpenoid varnishes: the ageing process

Ageing of varnishes is one of the major problems encountered by painting conservators and museum curators, because it leads to yellowed products which can obscure colours, craze, become very brittle, and show a change in solubility. Because these physical changes are the consequence of molecular changes in the varnish, it is important to study these ageing processes on a molecular level. My research focuses on the molecular ageing process of the triterpenoid resins dammar and mastic, which are frequently used as picture varnishes. The molecular changes are investigated by comparative studies of fresh resins, aged varnishes from paintings and artificially aged varnishes. This chapter starts with a overview of the use of dammar and mastic resin as painting varnishes. A descriptive model is postulated for the molecular ageing of triterpenoid varnishes, which gives rise to the formulation of a number of research questions. This thesis addresses a number of these questions. The main research results will be described in this introduction, which gives it the character of a summary.

1.1. Use of dammar and mastic resin as painting varnishes

Natural resins are excretions or secretions of certain plants. Dammar and mastic are classed as triterpenoid resins, which implies that they consist of cyclic isoprenoid compounds with 30 carbon atoms. In addition, both resins contain a polymeric fraction. Dammar originates from trees of the Dipterocarpaceae and mastic is the resin from trees of the genus Pistacia of the Anacardiaceae family (more details in Chapter 2). These resins are often used as picture varnishes. They form a protective coat over a paint film and give a uniform surface to the work. In addition, they improve the optical qualities of the painted work of art by enhancing both the saturation of the colours and the overall gloss.

Mastic has been widely used as a picture varnish throughout the centuries, starting from the IX century. In the early centuries, the varnish was often prepared by dissolving the resin in linseed oil in combination with colophony and sandarac

[1]. Heat was often applied during this method of varnish preparation. Carlyle [2], who studied traditional British varnish recipes of the nineteenth century, reported that mastic was often used in this time period. It was primarily used as a spirit varnish, which is a solution of resin in a volatile solvent. The varnish was easily prepared by dissolution of the resin in turpentine or alcohol either with or without heat [2]. Owing to a reported tendency to become more yellow with age than dammar, mastic became less popular and was replaced, to an extent, by the somewhat paler dammar, which had been introduced into the market in 1827 [3]. Dammar is also applied as a spirit varnish, using mainly turpentine or, in the twentieth century, white spirit or turpentine [2, 4, 5]. Like mastic, dammar is a highly glossy varnish. In cases where the strong gloss of dammar varnish is not desirable, beeswax was often added [5]. In the nineteenth century the stickiness of dry dammar varnishes was considered a disadvantage. It was thought to be caused by the water content of dammar resin. In that time period recipes were developed, which solved this problem by heating the resin or the resin solution [5]. Today dammar varnishes are more often applied as painting varnishes than mastic varnishes [5].

The esthetic function of a triterpenoid varnish is lost due to changes during ageing. Natural varnishes change in colour, become more brittle and crack, which changes the appearance of the painted image. They may become completely opaque due to blanching. A recent comparative study of aged dammar and mastic varnishes, prepared according to traditional varnish recipes, pointed out that mastic varnish yellows especially strongly [6]. Due to these degradation processes, which lower the esthetic value of paintings, the removal of a degraded varnish, largely by dissolution in solvent, constitutes one of the major activities of painting conservators. This removal is complicated by solubility changes due to increased polarity of the triterpenoid compounds in the aged varnish film. Much research has been devoted to the development of synthetic resin varnishes, for example the low molecular weight ketone resins, which degrade much more slowly and are still easily removed after some time on the painting [7-9]. In North America these varnishes are used frequently, whereas in Europe they are not as widely popular as European painting conservators tend to be more conservative in adopting modern materials. Another trend in the use of triterpenoid varnishes is the addition of hindered amine light stabilisers (HALS), in order to stabilise triterpenoid varnishes. Fadeometer studies demonstrated that Tinuvin 292 stabilises triterpenoid varnishes, but only in an environment free of ultraviolet (UV) radiation. The varnish films remained completely soluble in cyclohexane and the original components of dammar resin were still present in slightly altered proportions after prolonged fadeometer ageing [10, 11].

There has long been a controversy surrounding the possible risks to the paint layer of varnish removal such as leaching and swelling [3, 12]. Therefore, the investigation of the effects of varnish removal is a very important subject. Knowledge of the varnish composition and the changes due to degradation will certainly support this area of research. Because changes in physical properties, such as yellowing and solubility change, are generally and fundamentally influenced by the chemical changes that occur with age, we have focussed on the study of ageing processes on a molecular level. This fundamental study is carried out to broaden and deepen the knowledge of the fate of triterpenoid resins when they are applied as varnishes on paintings. Since the major part of the fresh resins consists of triterpenoids, our research focuses mainly on the analysis of the triterpenoid fraction. When a solid molecular basis for the ageing processes in triterpenoid varnishes is obtained, further studies must make the link to the changes that occur in the physical characteristics of the varnishes. This link will not be made in the context of this PhD research.

1.2. Ageing of triterpenoid varnishes

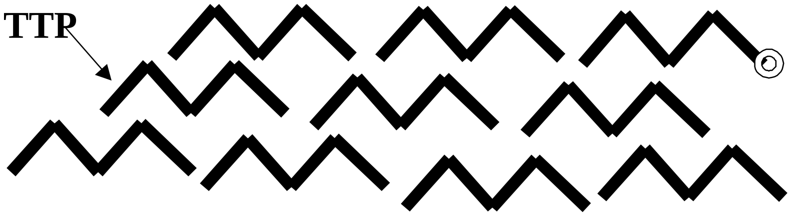
Surprisingly little is known about the molecular ageing processes of dammar and mastic resin on paintings, possibly due to a number of difficulties. First, fresh triterpenoid resins are already complex mixtures of a number of compounds. Ageing on paintings results in the formation of an even larger number of compounds, which are difficult to analyse. Secondly, the investigation of the ageing of varnish by sampling from actual paintings is complicated because of the many unknown factors, such as environmental effects and the varnish recipes used [2]. The influence of these factors on the ageing process of triterpenoid varnishes is largely unknown. This seems a serious problem so a complementary approach is often developed in which varnishes are subjected to various forms of artificial ageing, in order to control these factors. Degradation processes in artificially aged varnish films have been studied by De la Rie [13] and Zumbühl et al. [14, 15]. It is however not clear whether artificial ageing simulates the real degradation processes which occur on paintings. Therefore, this thesis focuses on fundamental molecular processes that occur during ageing of triterpenoid varnishes on paintings.

All organic compounds are susceptible to attack by molecular oxygen. This process is called autoxidation and is based on a free radical chain mechanism. Radical chain reactions are usually initiated by absorption of (UV)-light. The energy to initiate these reactions can also come from the thermal energy of the surroundings. In addition, air pollutants, such as ozone, are known to initiate autoxidation [16, 17]. In case of initiation by light energy, light has to be absorbed first. In a museum environment, a painting is not irradiated by light with a wavelength below 310 to 315 nm, because this radiation is absorbed completely by ordinary window glass [18]. Only certain compounds absorb UV-light above this wavelength, such as hydroperoxides. The RO-OH bond in hydroperoxides is relatively weak and easily broken, which results in radical formation. In addition, compounds with carbonyl groups are susceptible to light with a wavelength around 310 to 315 nm. Norris type reactions produce radicals through scission reactions. Once a radical is formed, it abstracts a hydrogen atom from an organic molecule to produce an alkyl radical. This highly reactive alkyl radical subsequently reacts rapidly with oxygen to form a peroxy radical, which in turn can abstract a hydrogen atom from an organic substrate. This radical chain ends when two radicals react with each other, which results in a cross-linked product [16, 17]. Both De la Rie [13] and Zumbühl et al. [14, 15] found evidence for the occurrence of oxidation and cross-linking processes during the artificial light ageing of a triterpenoid varnish.

Much research has been devoted to the ageing of synthetic resin films [19]. Although the chemical composition of synthetic resins is very different from that of the natural triterpenoid resins, similar ageing phenomena probably take place during ageing of these resin films. In addition to oxidation and cross-linking, photodegradation is a third phenomenon often encountered in the research area of synthetic resins, which leads to the degradation of the molecular structures [19]. Chain cleavage is often regarded in the polymer field as the main cause for polymer degradation, leading to embrittlement [19]. In addition, isomerisation may take place.

Theoretically, oxidation, cross-linking, degradation and isomerisation may occur during the ageing process of a triterpenoid varnish. Previously we introduced a simplified schematic model, which takes these different reactions into account [20] (Figure 1). This model attempts to visualise the molecular changes that triterpenoid varnishes undergo during ageing on paintings. The model is simplified, because the presence of the dammar and mastic polymer is not taken into account. The research described in this thesis focuses mainly on the triterpenoid fraction of dammar and mastic resin. The triterpenoids are schematically represented in Figure 1 by a **M** sign. It is assumed that there is a considerable degree of orientation of the triterpenoids forming flat sheet-line structures on the molecular level. This idea is extrapolated from orientational studies of sterols which have comparable carbon skeletal ring structures. The intermolecular forces are the result of the relatively weak Van der Waals interactions.

Fresh varnish: ordered layers of triterpenoids



Stage 1 Oxidised varnish: Oxygen uptake

Formation of excited and oxidised triterpenoids

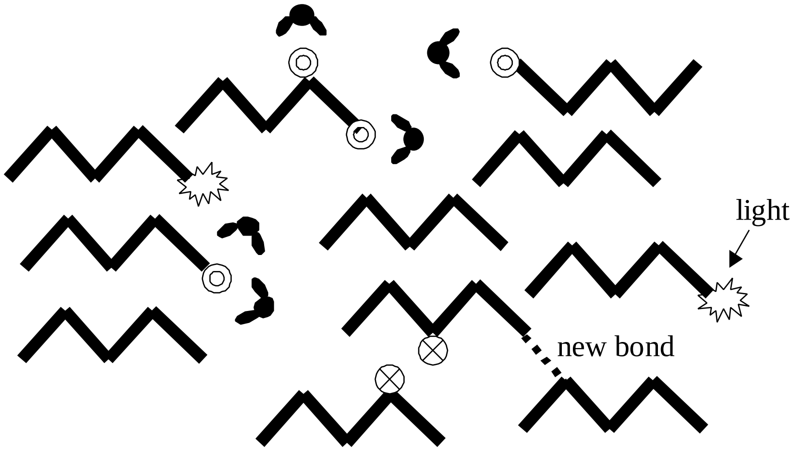
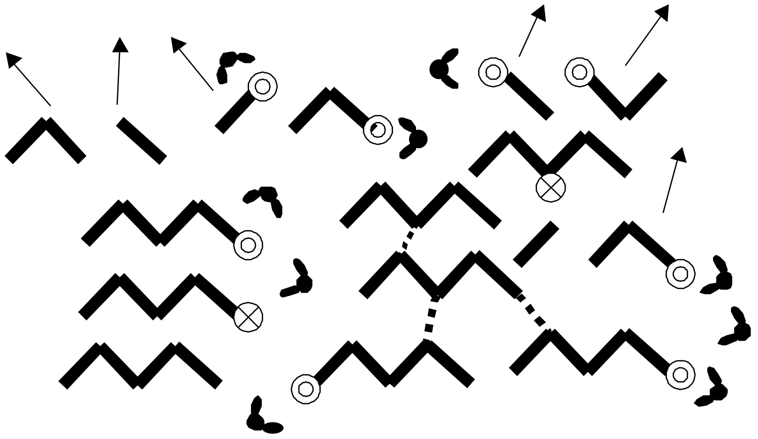


Figure 1 *Schematic model picturing different stages of ageing of triterpenoid varnishes. TTP indicates a tetra- or pentacyclic triterpenoid compound. Oxy TTP is a triterpenoid compound with oxygen containing functional groups (see second part of Figure 1 on the next page).*

Stage 1 Oxidised Varnish pictures the initial oxidation stages. The triterpenoid compounds undergo small modifications by light-induced excitation, free radical formation and oxygenation. The structural modifications disturb the sheet-like orientations of the triterpenoid compounds. Surface layers of the varnish contain oxidised triterpenoids, which attract water molecules. Hydrogen bonding interactions between polar groups and water molecules create weak “cross-links”. Excited molecules and free radicals not only react with oxygen but also form new covalent chemical bonds, thus forming cross-linked larger molecular weight complexes. Both types of cross-linking are likely to increase the stiffness of the varnish. It is likely that there is a difference in composition between the upper

Stage 2 Oxidised varnish: loss of C-skeletons
Emission of volatile oxidation products
Further cross-linking and O-insertion



Stage 3 Highly degraded varnish: H and O-loss
Polar and apolar regions in polymer fractions

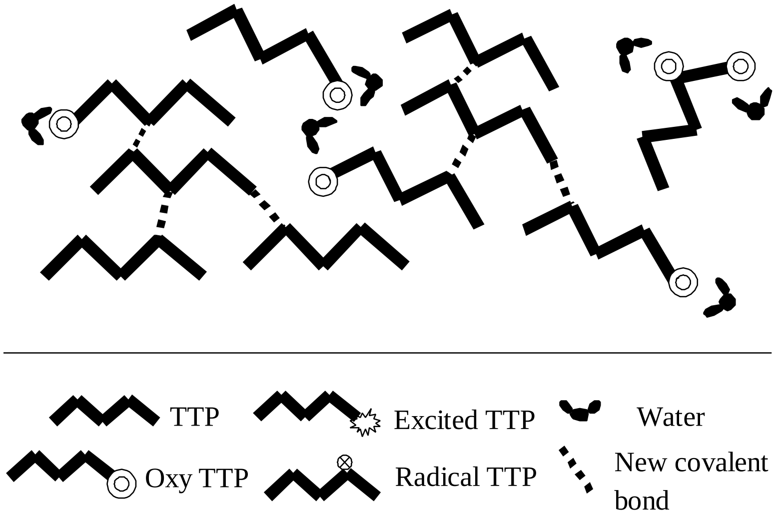


Figure 1 *(continued).*

layers in a varnish and deeper layers in the bulk. Most of the “damage” is probably near the air-varnish interface.

Stage 2 Highly Oxidised Varnish is characterised by further oxygen insertion, bond breaking and cross-linking processes. Triterpenoids in surface layers are further degraded forming smaller molecular species, which no more have the intact triterpenoid carbon ring skeleton. Very small photochemical degradation products may diffuse away and escape to the atmosphere (marked with arrows). The increasing number of oxygen containing functional groups on the triterpenoid compounds increases the number of absorbed water molecules. A change in mechanical properties, i.e. more brittle behaviour due to the increasing number of hydrogen bonding interactions is likely to result. Cross-linked higher molecular weight fractions now containing apolar and polar groups begin to play a larger role in the change in plasticity, which occurs in the transition from fresh to aged varnish. Chemically, this state is characterised by important changes in carbon speciation. Some carbon is lost from the varnish layer to the atmosphere; most of the carbon is now in new forms-smaller or more cross-linked-; a challenge for the analytical chemist. The relative amount of intact or slightly modified triterpenoids is strongly decreased compared to the initial varnish.

Stage 3 Highly degraded varnish describes a further stage of degradation. Elimination reactions in the higher molecular weight fractions could lead to formation of aromatic centers. Residual polar triterpenoids and polar parts of higher molecular weight fractions are linked by water molecules.

This molecular ageing model of triterpenoid varnishes on paintings gives rise to the formulation of a number of research questions, such as:

What type of oxidation products are formed?

To what degree does cross-linking take place?

To what degree does degradation take place?

Can we identify the compounds that are causing the aged triterpenoid varnish to be yellow?

These questions were addressed in the investigations, which were carried out in the context of this PhD research, and which will be described in this thesis. The main results regarding the research questions will be discussed below.

1.3. Thesis outline

In order to investigate how material has changed due to ageing both the chemical composition of the fresh starting material and the aged products must be investigated. The analysis of fresh dammar and mastic resin is described in Chapter 2.

After preparation of a dammar varnish, for which nowadays mainly white spirit is used, the varnish solution often gets cloudy. The reason for this cloudiness is unknown. The precipitate responsible for this cloudiness was investigated as described in the appendix of Chapter 2.

We chose to analyse varnishes from paintings, which have aged ‘naturally’. The study of these naturally aged varnishes unfortunately implies that a number of factors concerning the samples are unknown. Due to these unknown factors the interpretation of the analytical results of the aged varnishes is very complex, because it is possible that certain molecular structures are only formed under specific conditions. Therefore, a large number of aged varnishes was removed from paintings from several museum collections and private collections by using a swab and a suitable solvent. The varnish samples were analysed by several techniques in order to form a general idea about what components are present in aged varnishes. This investigation of aged varnishes from paintings is described in Chapter 3.

Artificial light ageing of painting materials is often used in conservation research. Unfortunately, it has never been investigated whether the various methods of artificial ageing available simulate the molecular ageing processes as found on paintings. Because the chemical composition of aged varnishes from paintings was identified in Chapter 3, different methods of artificial light ageing could be compared in view of the ageing products formed. Chapter 4 describes the investigation of the chemical composition of a number of artificially light aged triterpenoid varnishes.

Kinetic limitations due to the relatively low diffusion rates encountered in varnish films can be overcome by performing “ageing” experiments in solution. Chapter 5 presents a new method for the artificial ageing of triterpenoid samples. Varnish samples in solution are exposed to the light of a fluorescent tube device. Reactive species, such as reactive oxygen species or radicals, are formed in the solution, inducing molecular changes in the triterpenoid compounds. Because different processes go on during the ageing of the triterpenoid resins, exact oxidation mechanisms are presently difficult to disentangle. Therefore, pure triterpenoid standards were used to investigate the exact oxidation mechanisms.

Mass spectrometry is used as the main tool for the molecular identification of the triterpenoid samples. Therefore, the mass spectrometric behaviour of triterpenoids under different ionisation conditions is described in Chapter 6.

1.4. Main results: chemical changes in triterpenoid varnishes

1.4.1. Oxidation

Gas chromatography-mass spectrometry (GCMS), which allows the molecular separation and identification of complex samples, was used for the analysis of the aged varnishes. Because oxidation often results in the formation of more polar compounds with a higher molecular weight, high performance liquid chromatography-mass spectrometry (HPLC-MS) was used as well.

Chapter 2 demonstrates that the triterpenoid resins are complex samples. In the fresh state these resins already consist of a large number of different triterpenoid structures. The composition of the aged varnishes was found to be very different from that of the original fresh varnish (Chapter 3). It is still possible to discriminate between aged dammar and mastic varnish. GCMS and HPLC-MS demonstrated that oxidation takes place during ageing on the painting. Especially side chain oxidation of dammarane type molecules and oxidation of C11, C17 and C28 of oleanane/ursane type molecules was found to take place. A certain degree of variability in the composition of the aged triterpenoid varnishes from paintings was observed. The relative distributions of the oxidation products varied in the samples, which is probably caused by differences in the rate of progression of the degradation processes as varnish preparation methods and materials and subsequent display conditions for the paintings are never the same.

The oxidation products, which were formed after artificial light ageing of triterpenoid varnishes, were investigated (Chapter 4). Exposure by xenon-arc light and fluorescent tube light simulates the oxidation processes as found on paintings, only when irradiation by UV light is excluded. In the presence of UV light the oxidation processes that occur on paintings only take place to a limited degree and instead a number of new compounds are formed, which have not been found before on paintings. This chapter clearly demonstrates the importance of research on the molecular level.

Pure triterpenoid standards were subjected to a new method of inducing oxidation reactions in Chapter 5. The same oxidation products as found on paintings were formed, which enabled the confirmation of the exact oxidation mechanism of dammarane and oleanane/ursane type molecules.

1.4.2. Cross-linking

Quantitative studies by GCMS using an internal standard revealed that the total amount of triterpenoid compounds available for GCMS analysis diminishes greatly during ageing of triterpenoid varnishes on paintings. Evidence for the production of higher molecular weight material comes from direct temperature-resolved mass spectrometry (DTMS) analytical data of aged varnishes from paintings. DTMS gives information about the volatile and polymeric fraction of a sample. High molecular weight fractions were found to be formed during ageing of the triterpenoid varnishes on paintings (Chapter 3). Artificial light ageing and photochemical solvent ageing also result in the formation of a cross-linked fraction (Chapter 4 and 5). After a relatively long exposure period of the sample solution, a cross-linked fraction precipitates (Chapter 5). The method of solvent ageing can therefore be used for the easy preparation of a relatively large amount of the cross-linked fraction of aged dammar and mastic varnishes.

1.4.3. Degradation

Shortening of the side chain in dammarane type compounds was evident from GCMS studies of aged varnishes from paintings (Chapter 3). Photochemical ageing of pure triterpenoid standards in solution has confirmed this observation (Chapter 5). The degradation process is unlikely to stop here. We postulate that the photochemical oxidation process proceeds much further leading to bond breaking and disintegration of the triterpenoid carbon skeleton. HPLC-MS also showed a substantial unresolved envelope of compounds with relatively low masses, which indicates that degradation takes place during ageing on paintings (Chapter 3).

1.4.4. Yellowing

Aged varnishes from paintings were subjected to HPLC in combination with a multichannel array UV/VIS detector, which measures the light absorption of the purified triterpenoid substances from 190-900 nm. This work revealed that the oxidised triterpenoid compounds, identified with GCMS and HPLC-MS, do not absorb light in the blue light region, which implies that they are not important in the yellowing of aged varnishes. A fraction with relatively low molecular weight was found to absorb in the blue light region. Furthermore, analysis by size exclusion chromatography (SEC) coupled with the UV/VIS detector demonstrated that an unidentified, but possibly cross-linked or highly oxidised, fraction (900/1000 Da) exhibits a higher absorption in the blue region than the triterpenoid fraction (400 Da) (Chapter 3). The dammar and mastic resin solutions were found to become yellow after photochemical solvent ageing. SEC-UV/VIS analysis of a dammar sample that was solvent aged for a relatively long period of time demonstrated that the yellow colour of the solvent aged dammar sample was caused by the formation of relatively high molecular weight material (Chapter 5).

1.5. Implications for painting conservators

The descriptive model on the ageing of triterpenoid varnishes may give a better understanding of the molecular changes that occur during ageing on paintings. It may be used to rationalise the process of varnish removal. The most common procedure of varnish removal is by a solvent soaked swab. A key question is what happens to the higher molecular weight fractions under these conditions? Are they effectively removed or partially concentrated in layers that remain on the surface? As ageing proceeds, the remaining varnish was found to become more cross-linked. This may have a large influence on the solvent behaviour of the aged varnishes, because three-dimensional networks become rubbery and more difficult to dissolve in solvents. An experiment in which the varnish composition in a swab was compared to the composition of a scraped away chip of varnish, pointed out that high molecular weight materials demonstrate peculiar behaviour (Chapter 3). The cross-linked fraction was found to be less soluble in a common solvent used by painting conservators for varnish removal. This fraction was probably removed from the painting by means of mechanical action. This finding may have implications for the practice of partial cleaning. Partial cleaning advocates imply that varnish layers can be removed independently from each other leaving a last “protecting” layer behind. In our view this layer would be a mixture of highly aged and thus more difficult to dissolve fractions due to some degree of sorting out taking place in the swelling and dissolution process. The implication would be that microscopic layers of residues of varnish network polymers are concentrated on the surface. Our work on solvent aged varnishes indicates that these higher molecular weight fractions are important in the yellowing process of triterpenoid varnishes (Chapter 5). Clearly, more research is needed to elucidate the details of the varnish removal process on a microscopic level.

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2. Triterpenoid compounds in fresh dammar and mastic resin[1]

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

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[1] This chapter is based on the publications: Van der Doelen, G.A., van den Berg, K.J. and Boon, J.J., Comparative chromatographic and mass spectrometric studies of triterpenoid varnishes: fresh material and aged samples from paintings, Studies in Conservation 43 (4) (1998), 249-264, and Van der Doelen, G.A., Van den Berg, K.J., Boon, J.J., Shibayama, N., De la Rie and E.R., Genuit, W.J.L., Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by HPLC-APCI-MS(/MS), Journal of Chromatography A, 809 (1998), 21-37.

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

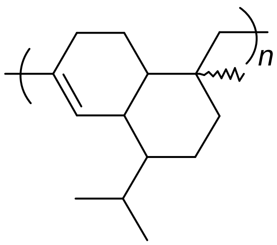


Figure 1 *Molecular structure of polycadinene.*

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

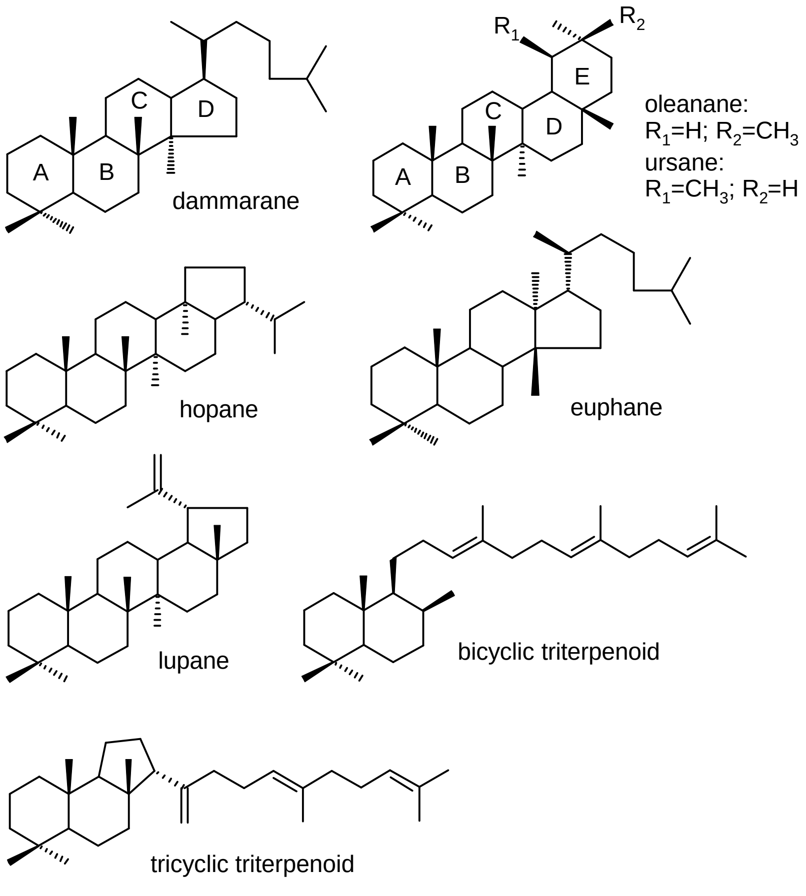
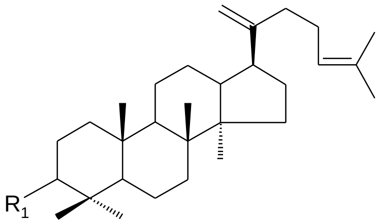
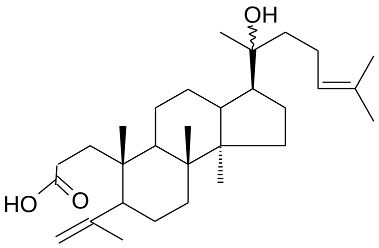


Figure 2 *Skeleton types of triterpenoids occurring in dammar and mastic resin.*

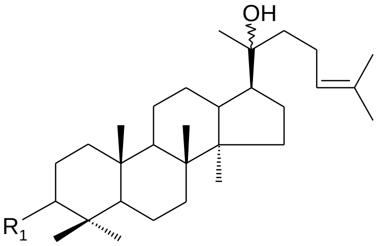
fresh dammar resin has been investigated extensively. Triterpenoids are usually classified according to their carbon skeleton type. Dammar consists largely of compounds of the tetracyclic dammarane skeleton series, but the pentacyclic oleanane, ursane and hopane derivatives are also present (Figure 2). 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].



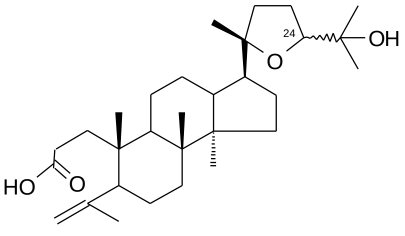
label	compound name	R_1	ref
3	dammaradienol	OH,H	[1, 8, 11, 12]
1	dammaradienone	O	[1, 11, 12]



label	Compound name	ref
5	dammarenolic acid	[1, 8, 11]

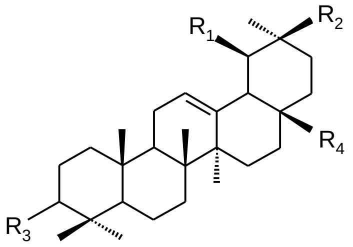


label	compound name	R_1	ref
8	hydroxydammarenone^1	O	[1, 8, 11]
22	dammarenediol^1	OH,H	[1, 8, 11, 12]

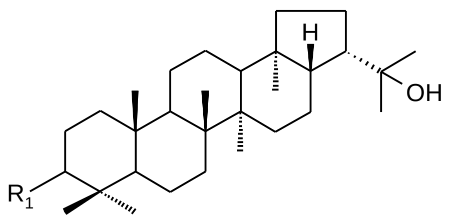


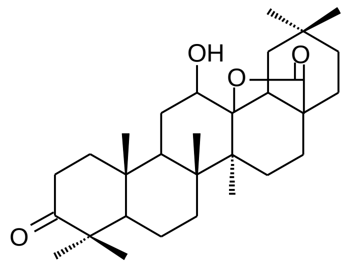
label	compound name	ref
4	shoreic acid^2 and eichlerianic acid^3	[8]

Figure 3 *Triterpenoids found to occur in dammar resin [1, 8, 10-17].*



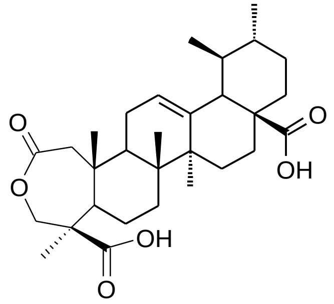
label	compound name	R_1	R_2	R_3	R_4	ref
14	nor-amyrone[4]	H/CH_3	H/CH_3	O	H	[1]
9	oleanonic aldehyde	H	CH_3	O	CHO	[1]
11	ursonic aldehyde	CH_3	H	O	CHO	[1]
43	oleanolic acid	H	CH_3	OH,H	COOH	[1]
-	ursolic acid	CH_3	H	OH,H	COOH	[1]
6	oleanonic acid	H	CH_3	O	COOH	[1]
10	ursonic acid	CH_3	H	O	COOH	[1, 8, 11]

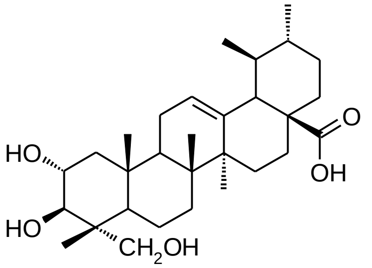




label	compound name	R_1	ref
12	hydroxyhopanone	O	[1, 8, 11]
-	3-acetoxy-22-hydroxy-hopanone	CH_3COO	[15]

label	compound name	ref
-	hydroxyoleanonic lactone	[8]





label	compound name	ref
-	23-hydroxy-2,3-secours-12-ene-2,3,28-trioic acid (2→23)-lactone	[16, 17]

label	compound name	ref
-	asiatic acid	[14]

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[1] Poehland et al., [8], found only hydroxydammarenone-I (*20r* configuration) and dammarenediol-II (20*s*), whereas Mills et al., [11], found both stereoisomers (*20r* and *20s*).

[2] This compound has the *24r* configuration.

[3] This compound has the *24s* 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.

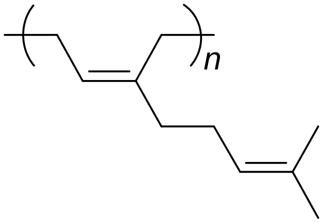
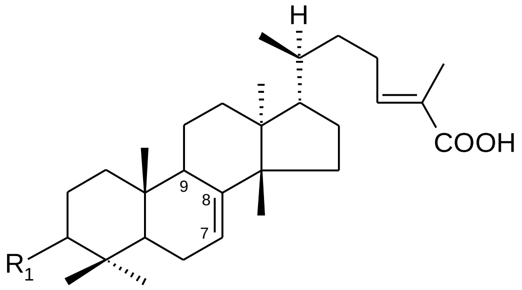
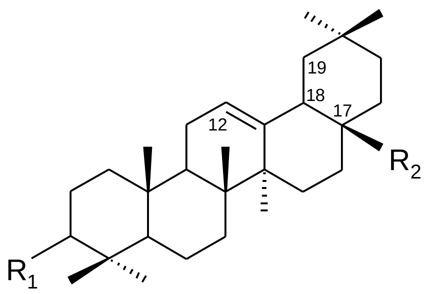


Figure 4 *Molecular structure of cis-1,4-poly-b-myrcene [36].*

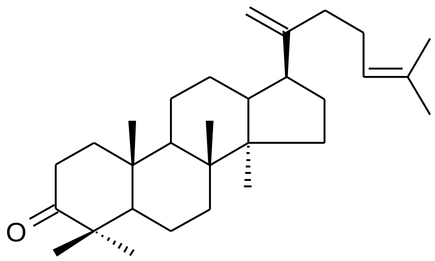
The polymer of mastic was identified as *cis*-1,4-poly-β-myrcene [36]. Its structure is shown in Figure 4. Mastic also contains a small fraction (approximately 2%) of essential oil, which was analysed by Papageorgiou et al. [35, 37]. A number of the triterpenoid constituents of gum mastic have been identified [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].

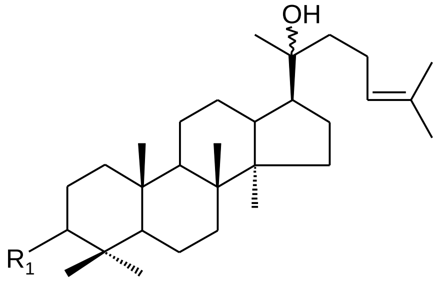




label	Compound name	R_1	ref
18/19	masticadienonic acid	O	[2, 38]
18/19	isomasticadienonic acid[1]	O	[2, 39]
-	masticadienolic acid	OH,H	[2]
-	3-epi-isomasticadienolic acid	OH,H	[2]
20/21	3-O-acetyl-2-epi-masticadienolic acid	CH_3COO	[2]
20/21	3-O-acetyl-2-epi-isomasticadienolic acid[1]	CH_3COO	[2]

label	compound name	R_1	R_2	ref
-	β-amyrin	OH,H	CH_3	[41]
15	β-amyrone	O	CH_3	[41]
-	nor-β-amyrin	OH,H	H	[41]
14	nor-β-amyrone	O	H	[41]
-	nor-olean-17-en-3-one[2]	O	H	[35]
-	28-hydroxy-β-amyrone	O	OH	[41]
9	oleanonic aldehyde	O	CHO	[41]
43	oleanolic acid	OH,H	COOH	[2]
6	oleanonic acid	O	COOH	[2, 39]
-	18αH-oleanonic acid	O	COOH	[2]
-	germanicol[3]	OH,H	CH_3	[41]
17	moronic acid[4]	O	COOH	[2]

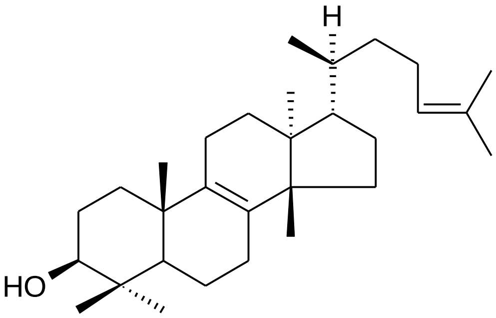


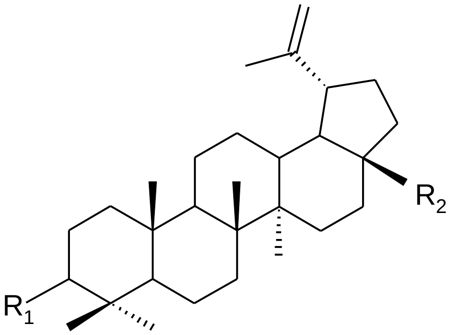


label	compound name	ref
1	dammaradienone	[41]

label	compound name	R_1	ref
8	hydroxydammarenone	O	[41]
-	3-acetoxy-hydroxydammarenone	CH_3COO	[41]

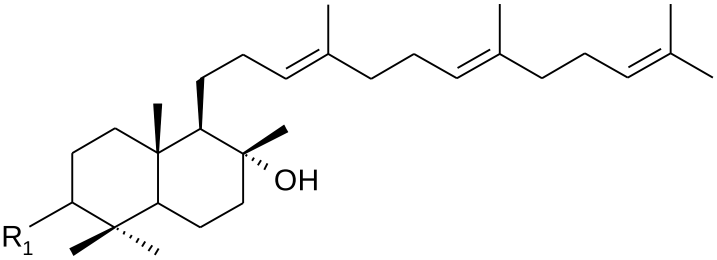
Figure 5 *Triterpenoids found to occur in mastic resin [2, 35, 38-41].*



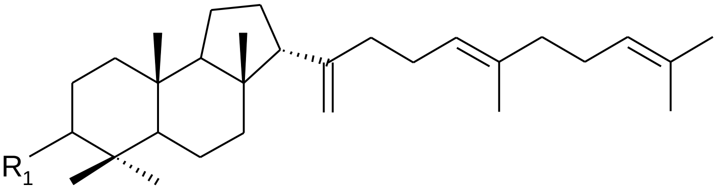


label	compound name	ref
-	tirucallol	[38, 41]

label	compound name	R_1	R_2	ref
-	nor-lupeone	O	H	[41]
-	lupeol	OH,H	CH_3	[41]



label	compound name	R_1	ref
16	(3L,8R)-3,8-dihydroxypoly poda-13E,17E,21-triene	OH,H	[40, 41]
13	(8R)-3-oxo-8-hydroxypoly poda-13E,17E,21-triene	O	[40, 41]



label	compound name	R_1	ref
-	3-hydroxy-malabarica- 14(26),17*E*,21-triene	OH,H	[41]
-	3-oxo-malabarica- 14(26),17*E*,21-triene	O	[41]

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[1] In case of the iso compounds, the double bond is located at C8.
[2] The double bond is located at C17.
[3] In case of germanicol, the double bond is located at C18.
[4] 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 (CH_2N_2) was used to methylate acid groups [52]. Nowadays, this method is often replaced by an alternative methylation method with trimethylsilyldiazomethane ($CHN_2Si(CH_3)_3$) [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.

a)

$$CHN_2Si(CH_3)_3 + CH_3OH \longrightarrow CH_3OSi(CH_3)_3 + CH_2N_2$$

$$CH_2N_2 + RCOOH \longrightarrow RCOOCH_3 + N_2$$

b)

$$RCOOH \xrightarrow{Si(CH_3)_3 \text{ reagent}} RCOOSi(CH_3)_3$$

$$ROH \xrightarrow{Si(CH_3)_3 \text{ reagent}} ROSi(CH_3)_3$$

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

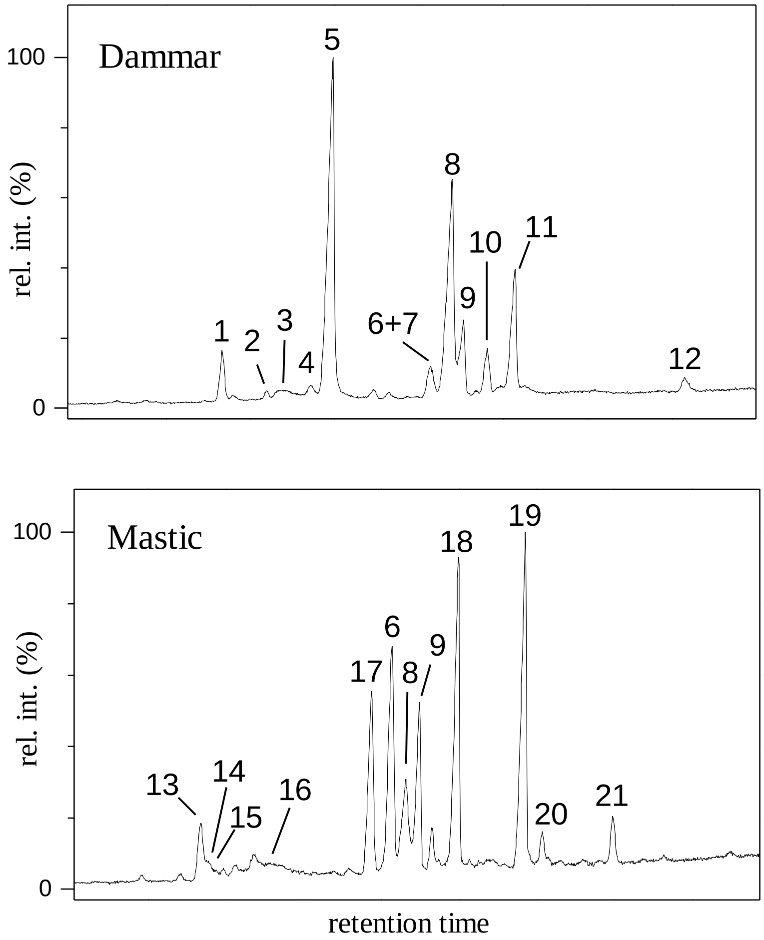

Figure 7 *Gas chromatograms of methylated fresh dammar and mastic resin (peak labels refer to table I).*

Table I *List of compounds identified in fresh triterpenoid resins by GCMS and HPLC-MS and their molecular weight (MW). Peak labels correspond to those used in Figure 3, 5, 7 and 8.*

Label	Compound name	MW
1	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424
2	Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)	410
3	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426
4	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)-dammaren-3-oic acid[1]	474
5	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[2])	458
6	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454
7	20,24-Epoxy-25-hydroxy-dammaran-3-one[1]	458
8	Hydroxydammarenone (20-hydroxy-24-dammaren-3-one[2])	442
9	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438
10	Ursonic acid (3-oxo-12-ursen-28-oic acid)	454
11	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438
12	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442
13	(8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene	442
14	Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)	410
15	β-Amyrone (3-oxo-olean-12-ene)	424
16	(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene	444
17	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454
18	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	454
19	Idem	454
20	3-O-Acetyl-3epi(iso)masticadienolic acid (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	498
21	Idem	498
22	Dammarenediol (20-dammar-24-ene-3β,20-diol[2])	444
23	Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)	440
24	Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)	440

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[1] The configuration at C-20 and C-24 was not determined.

[2] The configuration at C-20 was not determined.

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 α-amyrin, dammarenediol, oleanolic acid and ursolic acid for dammar, and like β-amyrin and tirucallol for mastic, were also found to occur to a small extent in fresh triterpenoid resins.

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

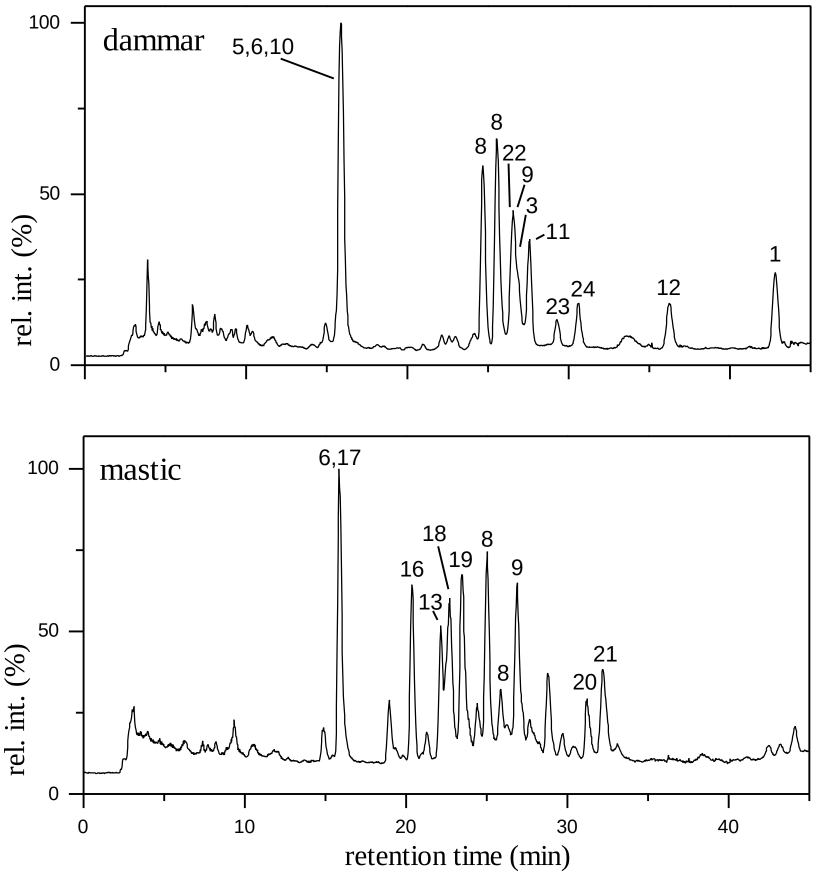

Figure 8 *Total ion current traces of reversed phase HPLC-APCI-MS, using the positive ion mode, of fresh dammar (a) and mastic (b) resin (peak labels refer to Table I).*

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 (NH_3/CI) DTMS. The hydroxyl group of hydroxydammarenone is easily eliminated under EI conditions, which complicates the molecular identification. This is not the case under NH_3/CI conditions as described in Chapter 6. The identification of the specific stereoisomers could not be achieved 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

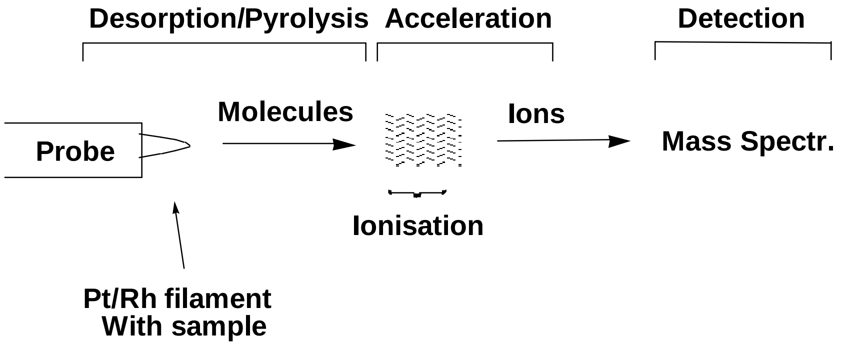

Figure 9 *Schematic diagram of the experimental setup of DTMS.*

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.

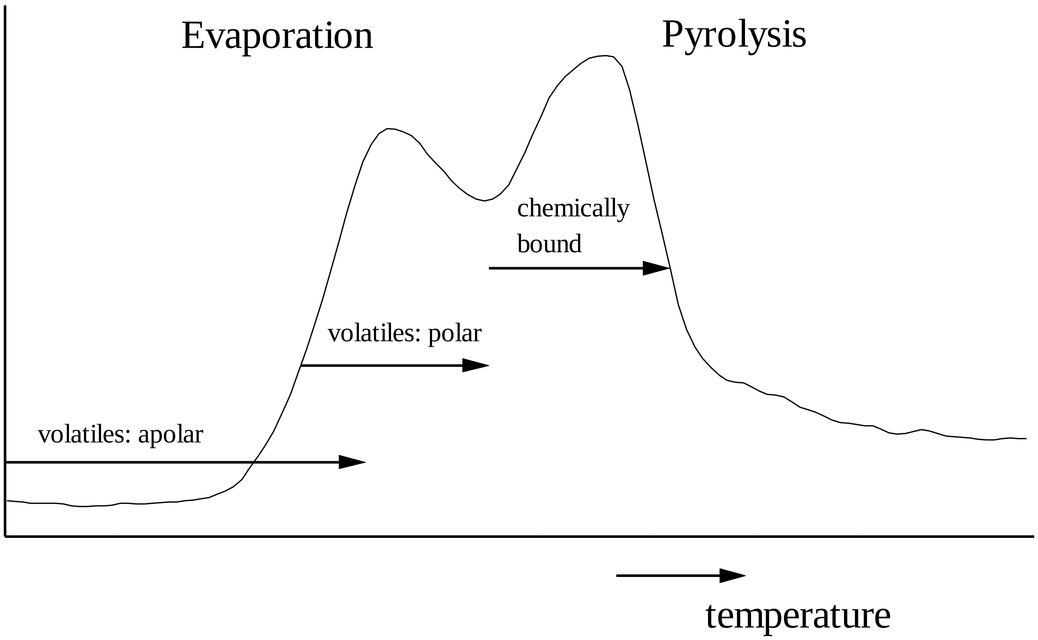

Figure 10 *Example of a DTMS total ion current.*

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 dammarenediol (**22**) are represented by a peak at m/z 426. The peak at m/z 440 represent a major fragment ion ($(M\text{-}H_2O)^{+\cdot}$) 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**).

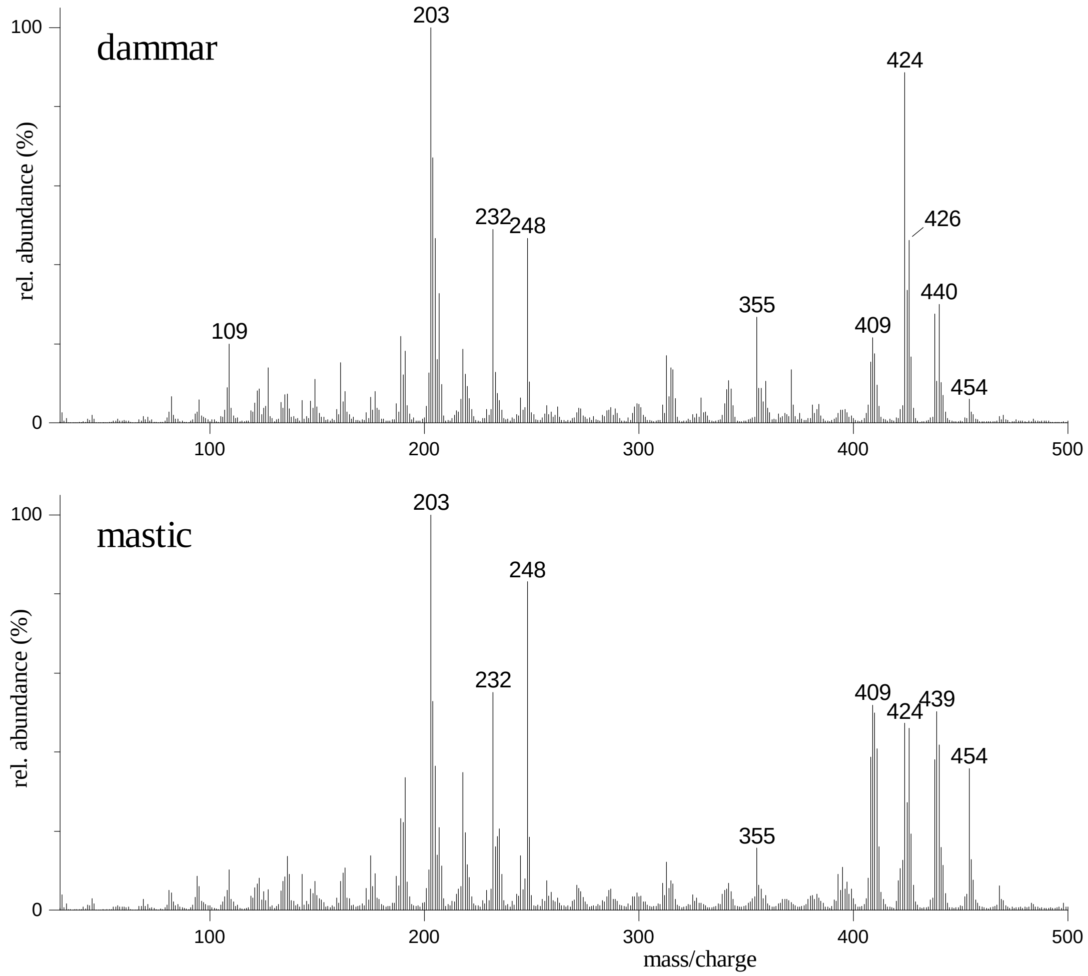

Figure 11 *EI(16 eV)-DTMS summation spectra of fresh dammar resin and mastic resin.*

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

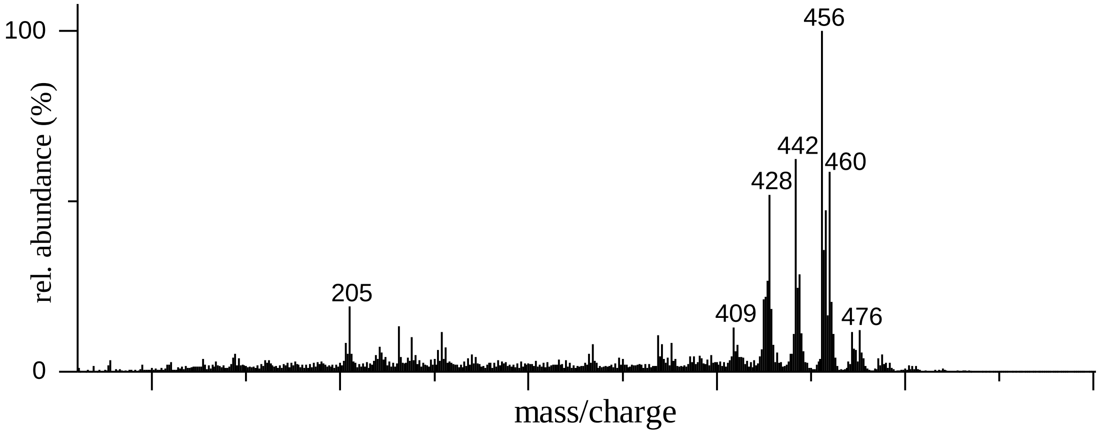

Figure 12 *NH_3/CI-DTMS summation spectrum of fresh dammar resin.*

probably represent $[M+NH_4-H_2O]^+$ 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 $[M+NH_4]^+$ ions of oleanonic/ursonic acid (**6**/**10**) and dammarenolic acid (**5**) respectively. The peak at m/z 458 represents the $[M+NH_4-H_2O]^+$ 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

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 C_{18} 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 an 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 (NH_3) 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.

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Appendix to chapter 2

Abstract

A dammar precipitate is usually formed when preparing a solution of dammar varnish. GCMS, DTMS and SEC analysis demonstrated that this precipitate consists of relatively polar, acidic material together with a fraction of dimerised or highly oxidised triterpenoid material.

1. Analysis of the precipitated fraction in fresh dammar varnish

Dammar varnishes are usually prepared by dissolving dammar in turpentine or white spirit. It is well known among painting conservators that dammar solutions can get cloudy and that precipitates can occur. The reason for this phenomenon is not exactly known. The water content of the resin or the presence of the dammar polymer (‘dammar wax’) is sometimes believed to be responsible [1]. There are a number of approaches in which painting conservators deal with these precipitates. Their measures range from a rejection of the whole varnish to filtering or dissolving the precipitates or shaking them up.

Wenders has studied these dammar precipitates [2]. She found that dammar films, which contain the precipitate, show more yellowing and cracking after artificial ageing compared to dammar films in which the precipitate was filtered out. It was also observed, that older dammar resins produced more precipitate. For this reason, it is recommended in conservation practice to use fresh dammar resin and to filter out the precipitate.

In collaboration with Wenders, various dammar precipitates were analysed and compared to fresh dammar resin in order to determine the chemical

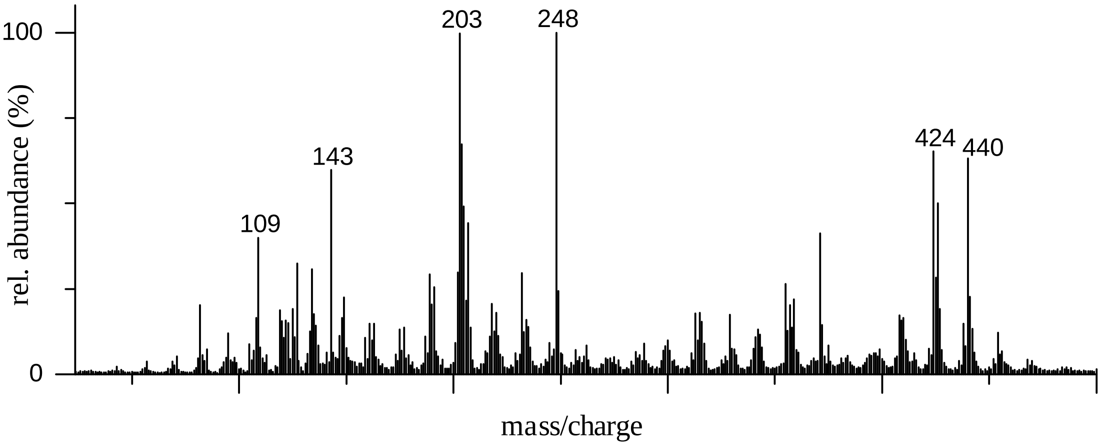

Figure 1 *EI(16 eV)-DTMS summation spectrum of the dammar precipitate formed after dissolution in white spirit.*

composition. The analytical results of the various dammar precipitates were found to be very similar. This thesis discusses the analytical results of one dammar precipitate. We refer to the thesis of Wenders for a discussion of the analytical results of precipitates from a variety of sources of dammar resin using a number of different solvents [2]. Both DTMS (16 eV) and GCMS show that there were only some small differences between the dammar resin and the dammar precipitate. As shown by DTMS (Figure 1), the precipitate contains a relatively large fraction of constituents with acidic groups, compared to the fresh resins (Chapter 2, Figure 11). The base peak at m/z 248 indicates the presence of a relatively large amount of ursonic/oleanonic acid. A relatively large amount of ocotillone type molecules is also present, as indicated by the peak at m/z 143.

Molecular weight information was obtained with Size Exclusion Chromatography (SEC). The particles of the SEC column packing have different size pores and pore networks, so that the analyte molecules are retained or excluded on the basis of their hydrodynamic molecular volume, which is a combination of their size and shape [3]. The precipitate was found to be soluble in tetrahydrofuran (THF). Figure 2 shows the SEC traces of fresh dammar resin (a) and of the dammar precipitate (b), using a UV detector at 240 nm and THF as the mobile phase. Fresh dammar resin mainly contains triterpenoid molecules with masses of approximately 400 to 500 dalton. The peak around 200 dalton is likely to be due to sesquiterpenoids. A broad hump at approximately 10,000 dalton points to the presence of the dammar polymer, polycadinene. In addition, a small peak is present at approximately 900 dalton, which may be caused by the presence of dimerised triterpenoids. However, the presence of dimerised triterpenoids could not be confirmed when studied by a number of mass spectrometric techniques (see page 71 of section 3.5.). It is possible that the peak at 900 Da represents strongly oxidised triterpenoids, which are relatively polar compounds with a lower molecular weight than 900 Da. These compounds will have more affinity for the polar eluent THF than the less polar triterpenoids, represented by a peak at 400/500 Da. This will result in a peak at a relatively shorter retention time, i.e. a peak at higher molecular weight. Compared to fresh resin, the precipitates contain a higher proportion of dimeric or highly oxidised and possibly trimeric triterpenoids, in addition to a triterpenoid fraction. Furthermore, the SEC results also indicate that the precipitate does not consist of polycadinene.

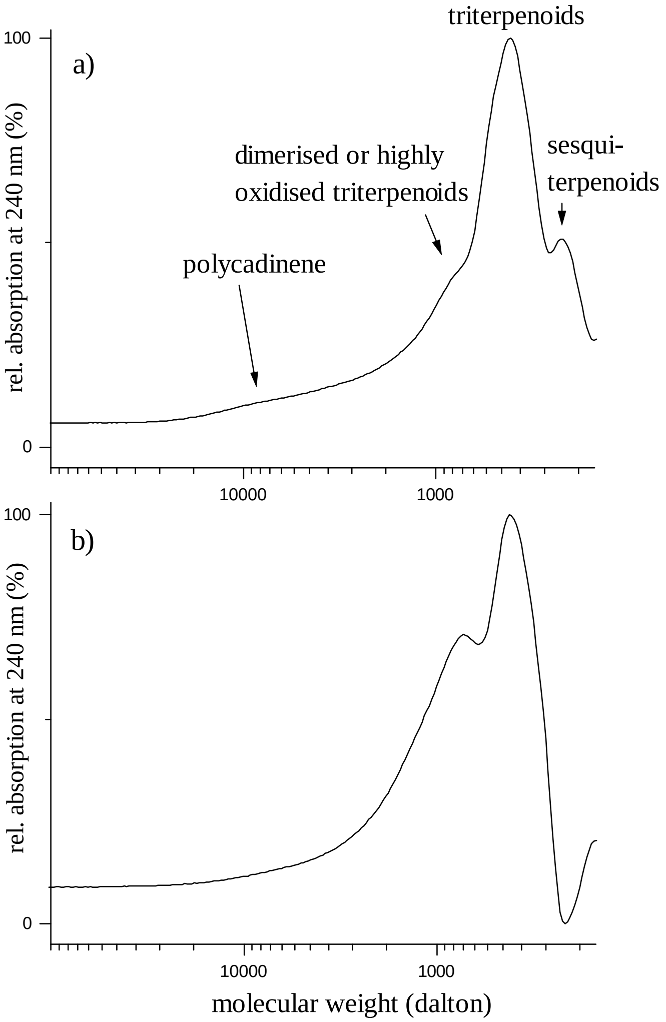

Figure 2 *SEC traces at 240 nm of fresh dammar resin (a) and of the dammar precipitate formed after dissolution in white spirit (b).*

2. Conclusions

According to the resin sampled it is likely that the precipitate that is often encountered by painting restorers when preparing a solution of dammar varnish consist of relatively polar, acidic material together with a fraction of dimeric or highly oxidised triterpenoid material. Contrary to what was believed, in our samples at least, the precipitate does not contain polycadinene.

3. Experimental

For the preparation of the dammar precipitate, one part dammar was put inside a filter in five parts of white spirit (Siedegrenzbenzin, Sb 140-200 ºC, Kremer Pigmente). After 24 hours a nearly clear, yellowish solution was formed. The filter was taken out, because there were no residues present. The following days, precipitates started to be formed, which were filtered after 8 weeks [2]. GCMS, DTMS and SEC experiments were carried out as described in Chapter 3.

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3. Aged triterpenoid varnishes from paintings[1]

Abstract

A large number of naturally aged yellowed varnishes taken from paintings from several museum collections were analysed by GCMS and HPLC-MS in order to identify oxidised triterpenoids, which are formed during the natural ageing processes. Discrimination between the different types of aged varnishes was achieved by Direct Temperature-resolved Mass Spectrometry (DTMS). Quantitative GCMS and HPLC-MS further showed that the triterpenoid fraction strongly decreases during ageing. It is likely that in addition to degradation, higher molecular weight fractions are being formed. DTMS analysis demonstrated that cross-linking takes place during ageing. Identification of the yellowing compounds of aged triterpenoid varnishes was attempted with HPLC-UV/VIS, showing that absorption between 390 and 430 nm was observed for compounds with a relatively low molecular weight. Analysis by SEC-UV/VIS demonstrated that an unidentified, but possibly cross-linked or highly oxidised, fraction (900/1000 Da) exhibits a higher absorption in the blue region than the triterpenoid fraction (400 Da). In a few samples strongly light-absorbing compounds were found, which are not stable in daylight. It is inferred that these compounds accumulate when varnish samples are stored in the dark, after removal from the painting.

3.1. Introduction

The chemical composition of triterpenoid resins is already complicated when it is freshly applied to the painting, as discussed in the previous chapter. The

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[1] This chapter is based on the publications: Van der Doelen, G.A., van den Berg, K.J. and Boon, J.J., Comparative chromatographic and mass spectrometric studies of triterpenoid varnishes: fresh material and aged samples from paintings, Studies in Conservation 43 (4) (1998), 249-264, and Van der Doelen, G.A., Van den Berg, K.J., Boon, J.J., Shibayama, N., De la Rie and E.R., Genuit, W.J.L., Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by HPLC-APCI-MS(/MS), Journal of Chromatography A, 809 (1998), 21-37.

ageing process on the painting increases the complexity of the varnish even more. Not much is known about the chemical composition of triterpenoid varnishes that have aged on paintings. However, it has been investigated whether it is possible to distinguish between dammar and mastic varnishes, once they have aged on a painting. Three methods of discriminating between aged dammar and mastic varnishes are described in literature. According to Mills et al. [1], it is possible to identify an aged mastic varnish by gas chromatography/mass spectrometry (GCMS) after methylation using the presence of moronic acid or tirucallol as marker compounds. Koller et al. [2] identified an aged mastic varnish by GCMS without prior derivatisation by the presence of noroleanenone. Both moronic acid, tirucallol and noroleanenone are absent in dammar resin. However, as was discussed in the previous chapter, we could not demonstrate noroleanenone in the different batches of fresh mastic resin from different suppliers. The third method of discriminating between aged dammar and mastic resin has been described by Halpine [3]. It is based on the presence of different trace amino acids in dammar and mastic resin, which can be successfully identified by amino acid analysis. The main difference between dammar and mastic is the presence or absence of hydroxyproline. Hydroxyproline is only present in mastic resin, not in dammar resin.

The degradation of dammar films, which were artificially aged, has been studied by De la Rie [4]. Surprisingly, it has not been investigated, whether artificial ageing simulates the same degradation processes that occur on paintings. The research approach of this thesis is to analyse varnishes from paintings, which have aged ‘naturally’ and compare the ageing processes to those occurring during artificial ageing. This chapter investigates aged varnishes from paintings and the next chapter will describe the analytical results of a number of artificially aged triterpenoid varnishes.

When investigating aged varnishes from paintings, many unknown factors are involved. Of all aged varnishes sampled here, it was not known what type of varnish was applied originally. The biological origin of especially dammar resin probably determines the chemical composition of the fresh resin and Wenders [5] reported that that older dammar resins contained more oxidised compounds. The kind of recipe used to prepare the varnish probably varied from studio to studio. Different solvents may have been used and in some cases the polymeric fraction may have been included in the varnish, whereas in other cases it could have been left out. Thus the chemical composition of varnish prior to application may have differed from painting to painting. Other unknown factors are the year of varnish application and thus the age of the varnish. The exact environmental conditions of the varnished paintings probably have a major effect on the ageing of the varnish layer, but can usually not be reconstructed. In most cases it was not known whether the varnish comprised a single layer or multiple layers. Another uncertainty is in the method of varnish sampling. It is not clear whether varnishes were completely removed by conventional methods using solvents. Certain parts of varnishes, like polymeric material, may be left behind on the painting, because of a lower solubility in the used solvent. However, mechanical action will aid in the removal of the varnish. The different solvents used for varnish removal probably have different abilities to dissolve the components of aged varnish films. Due to these unknown factors the interpretation of the analytical results of the aged varnishes is very complex, since it is possible that certain molecular structures are only formed under specific conditions. It is also possible that some of the unknown factors just mentioned only have an effect on the distribution of the ageing products and not on the kind of ageing products formed.

Therefore, a large number of aged varnishes were collected from more than 70 paintings of about 15 different museum collections and private collections from the Netherlands and the United Kingdom. They were analysed by several techniques in order to obtain a general idea about what components are present in aged varnishes. Because the varnishes were taken from paintings from a number of different environments, this broad collection of aged varnishes is considered to be representative of varnishes aged on paintings. The majority of the samples analysed were removed from paintings that were under restoration. In the opinion of the painting restorers involved, these degraded varnishes obscured the painted image underneath, and therefore can be considered to have aged considerably. The majority of the samples were removed using a swab and a suitable solvent. The choice for a specific solvent or specific solvent combination for varnish removal was made by the individual painting restorers involved. By using this sampling method we tried to stay as close as possible to the most common procedure of varnish removal. Scrapings of aged varnishes were also analysed in some cases.

This chapter deals with the analysis of this large number of aged varnishes taken from paintings. Direct temperature-resolved mass spectrometry (DTMS) was used as fingerprinting technique to make a survey of and to classify the different types of aged varnishes. Several chromatographic and mass spectrometric techniques, such as gas chromatography-mass spectrometry (GCMS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and DTMS, were used to determine the chemical composition of the aged triterpenoid varnishes. Subsequently, conclusions about the ageing processes that occur on paintings, such as oxidation and cross-linking, are drawn. High performance liquid chromatography (HPLC) and size exclusion chromatography (SEC), both coupled with a UV/VIS absorption detector, were used to obtain information about the spectroscopic characteristics. Finally, some implications concerning the removal of a varnish from a painting will be described.

3.2. Discrimination between several types of aged varnishes from paintings by DTMS

Direct Temperature-resolved Mass Spectrometry (DTMS) (Chapter 2) was applied to determine the nature of the 70 naturally aged varnishes. Figure 1 shows examples of the DTMS summation spectra of three different types of aged painting varnishes: an aged triterpenoid (a), diterpenoid (b) and synthetic cyclohexanone (c) varnish. The DTMS spectra of the different types of varnishes show several characteristic features. Aged triterpenoid varnishes (Figure 1(a)) are recognised by a high peak at m/z 143, which corresponds to the side chain of ocotillone-type molecules [6], and by the molecular ion region around m/z 410-460. At this point it is difficult to make a distinction between aged mastic and dammar varnishes, since their DTMS spectra are very similar. In order to identify the molecular fragments present in the DTMS spectrum, GCMS can be used to achieve molecular separation. Aged diterpenoid varnishes (Figure 1(b)) have a molecular ion region around m/z 300-330 [1] and show some characteristic peaks such as those at m/z 315, 253 and 299, indicative of the presence of 7-oxo-dehydroabietic acid (m/z 253, 299) and 7-oxo-15-OH-dehydroabietic acid (m/z 315) [7]. The DTMS spectra of synthetic varnishes (Figure 1(c)) are characterised by clusters of specific monomers. This specific varnish was identified as a synthetic cyclohexanone varnish, possibly Laropal K80 [8].

The DTMS data of the 70 aged varnish samples of unknown composition showed that 57 were of triterpenoid origin, 5 of diterpenoid origin, and 8 varnishes were identified as synthetic varnishes. Some of the aged varnish samples are not pure samples, since varnish layers can consist of different types of aged varnishes on top of each other. The presence of other compounds added during restoration is also possible. Those aged varnish samples which did not contain large amounts of ‘contaminating’ substances, such as waxes and fatty acids, were used for multivariate analysis, since these ‘contaminations’ hinder the separation of the varnishes by this technique. Multivariate analysis can be performed on a set of DTMS spectra, in order to deconvolute compositional differences between the individual spectra of the set. This method classifies the spectra and describes the similarity and dissimilarity of the samples. The output of this mathematical technique is a multivariate map, such as the one depicted in Figure 2. The geometric distances between the samples in the map point to differences in the distribution of mass peaks in the DTMS spectra, which in turn point to differences in chemical composition of the samples. The concept of this numerical treatment applied to sets of mass spectra has been outlined by Hoogerbrugge et al. [9], and Windig et al. [10]. In Figure 2, the relative distribution of the samples is plotted in a F1/F2 map (factor score map). The position in the F3 space is marked by an open

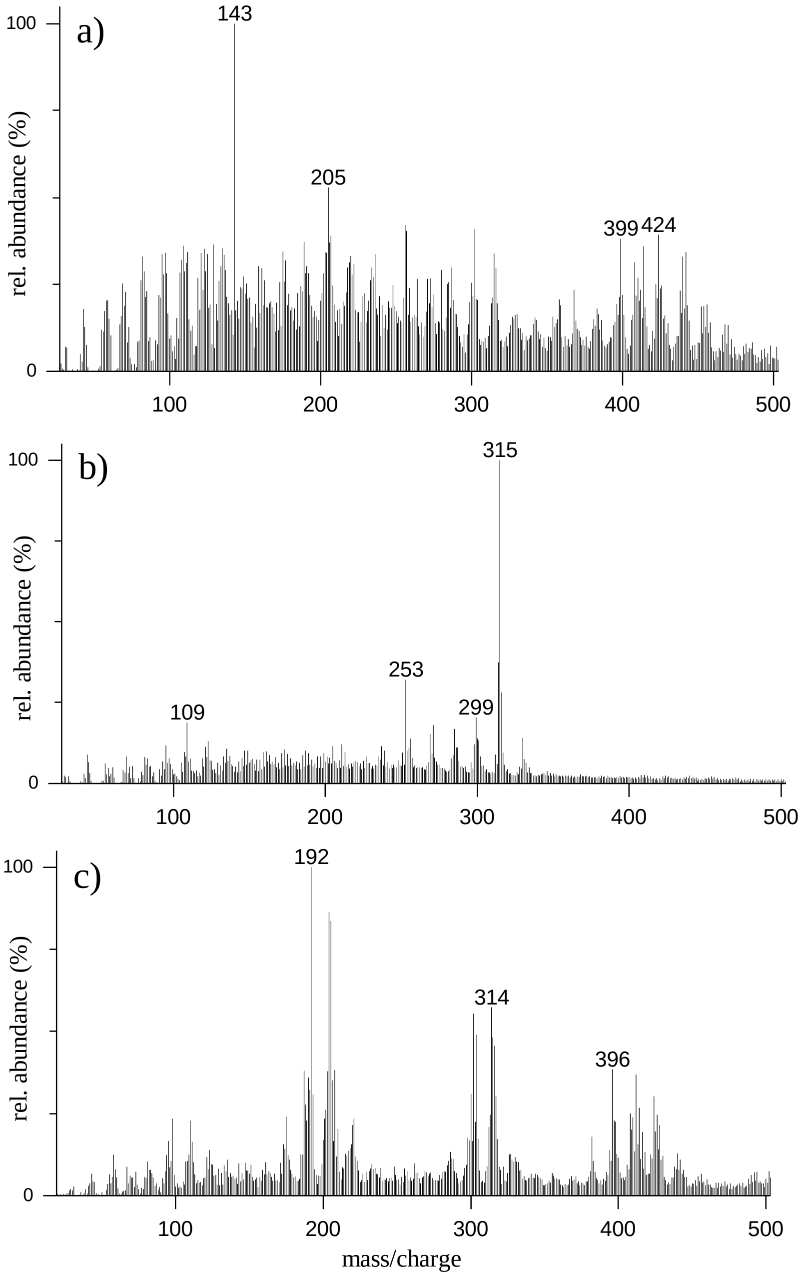

Figure 1 *Mass spectra of naturally aged triterpenoid (a), diterpenoid (b) and synthetic (cyclohexanone) (c) varnish, obtained by summation of all spectra of the DTMS dataset.*

symbol (positive score in F3) or a solid symbol (negative score in F3). The mass peaks responsible for the separate position of the synthetic varnishes, cyclohexanone (open up-triangle) and methylcyclohexanone (solid down-triangle) resin, are respectively m/z 192 and 204, and m/z 112, 206 and 220. Aged diterpenoid varnishes (open circles) are localised in the $F2^-$, $F3^+$ direction and are

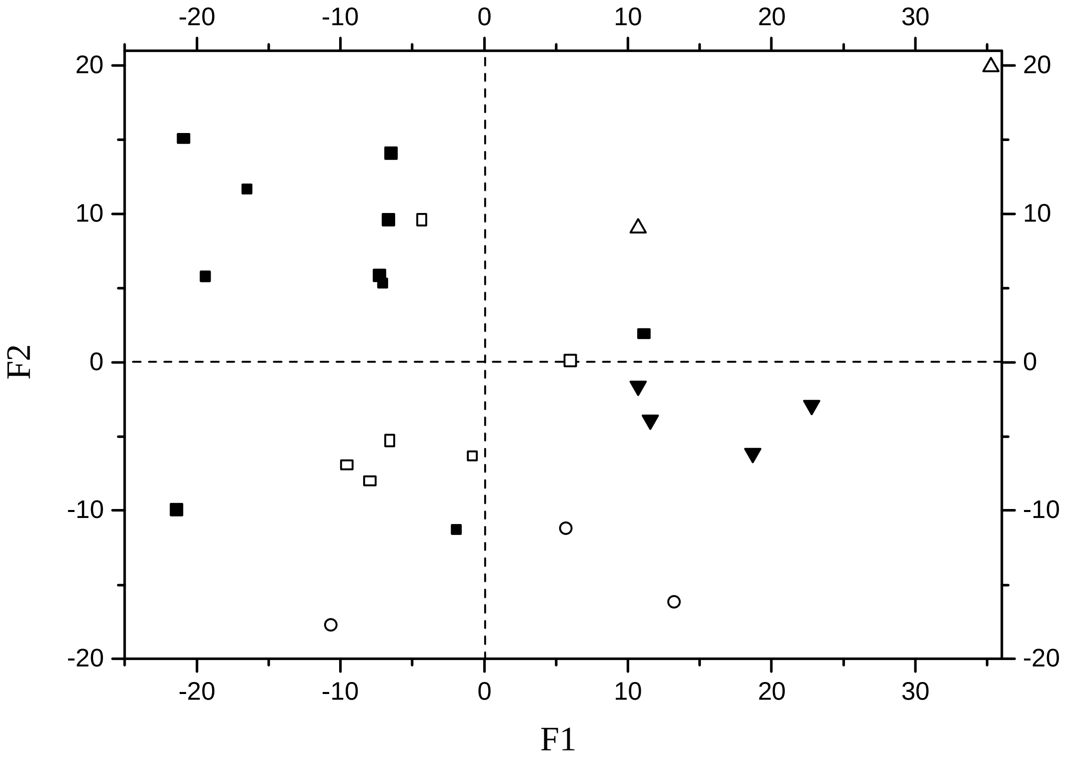

Figure 2 *Multivariate map resulting from principal component analysis of the DTMS fingerprints of aged cyclohexanone (open up-triangles), methylcyclohexanone (solid down-triangles), diterpenoid (open circles) and triterpenoid (open and solid squares) varnishes.*

characterised by mass peaks 315 and 253. Aged triterpenoid varnishes (open and solid squares) are predominantly present in the $F1^-$ direction. The difference between aged dammar and mastic varnishes is not sufficient to achieve a separate clustering in the score plot of Figure 2. The mass peaks responsible for discrimination of the triterpenoids in the direction $F1^-$ are plotted in Figure 3. Both aged dammar and mastic varnishes are characterised by a peak at m/z 143 and a molecular ion region between approximately m/z 400 and 470.

The aged triterpenoid varnishes were subjected to other analytical methods in order to determine their chemical composition. The ageing of diterpenoid varnishes has been studied by Van den Berg et al. [7]. A further study of the aged synthetic varnishes is outside of the scope of this thesis.

When the spectra of an aged dammar varnish is compared to the spectrum of fresh dammar resin (Chapter 2, Figure 11), it is evident that ageing alters the molecular composition of the triterpenoids significantly. One of the usual approaches in the identification of aged painting materials is to compare the aged material with “standards” prepared from fresh painting materials. The fact that the

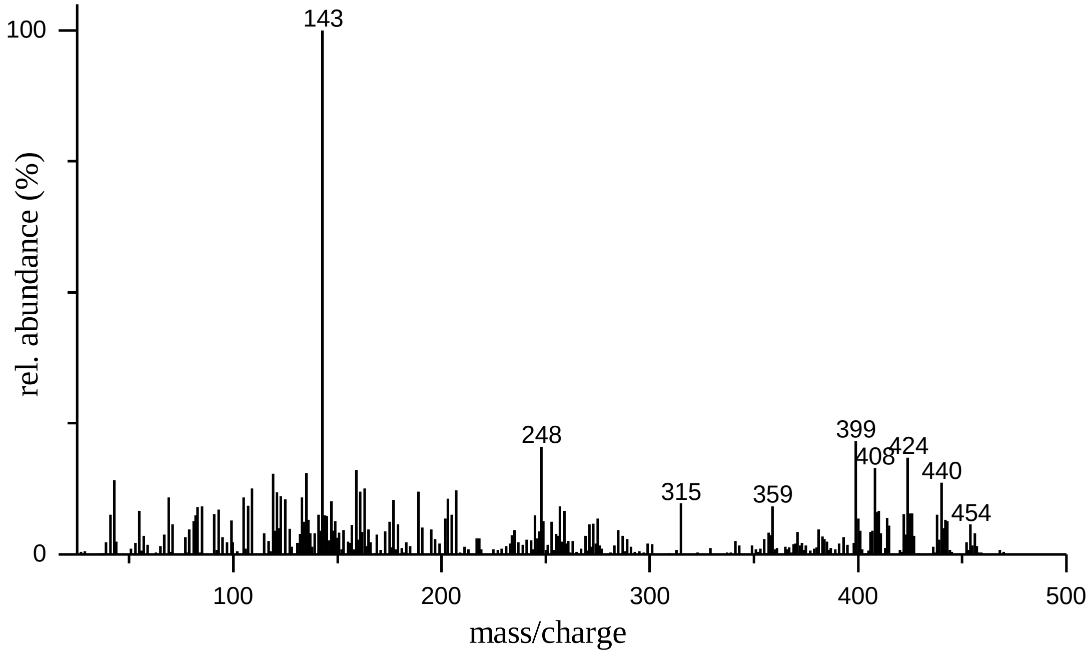

Figure 3 *Reconstructed mass spectrum of the negative first principal component (F1$^-$) which discriminates triterpenoid varnishes from all other varnishes analysed.*

DTMS spectra of the fresh resins do not resemble the DTMS spectra of the 57 aged triterpenoid varnishes from several museum collections, indicates that identification of aged painting materials on the basis of resemblance with fresh painting materials requires caution. It can be concluded that the differences between the several aged triterpenoid varnishes, due to factors such as the age or the preparation of the varnish, are small relative to the differences between fresh and aged resins.

3.3. *Oxidative changes in varnish composition*

3.3.1. *Analysis by GCMS*

The GCMS results of five methylated aged triterpenoid varnishes, which were representative of the whole triterpenoid sample set, are shown in Figure 4. The molecular identification of the peaks is given in Table I and is based on mass spectrometric literature [11-14]. Compound **25** is tentatively assigned by its mass

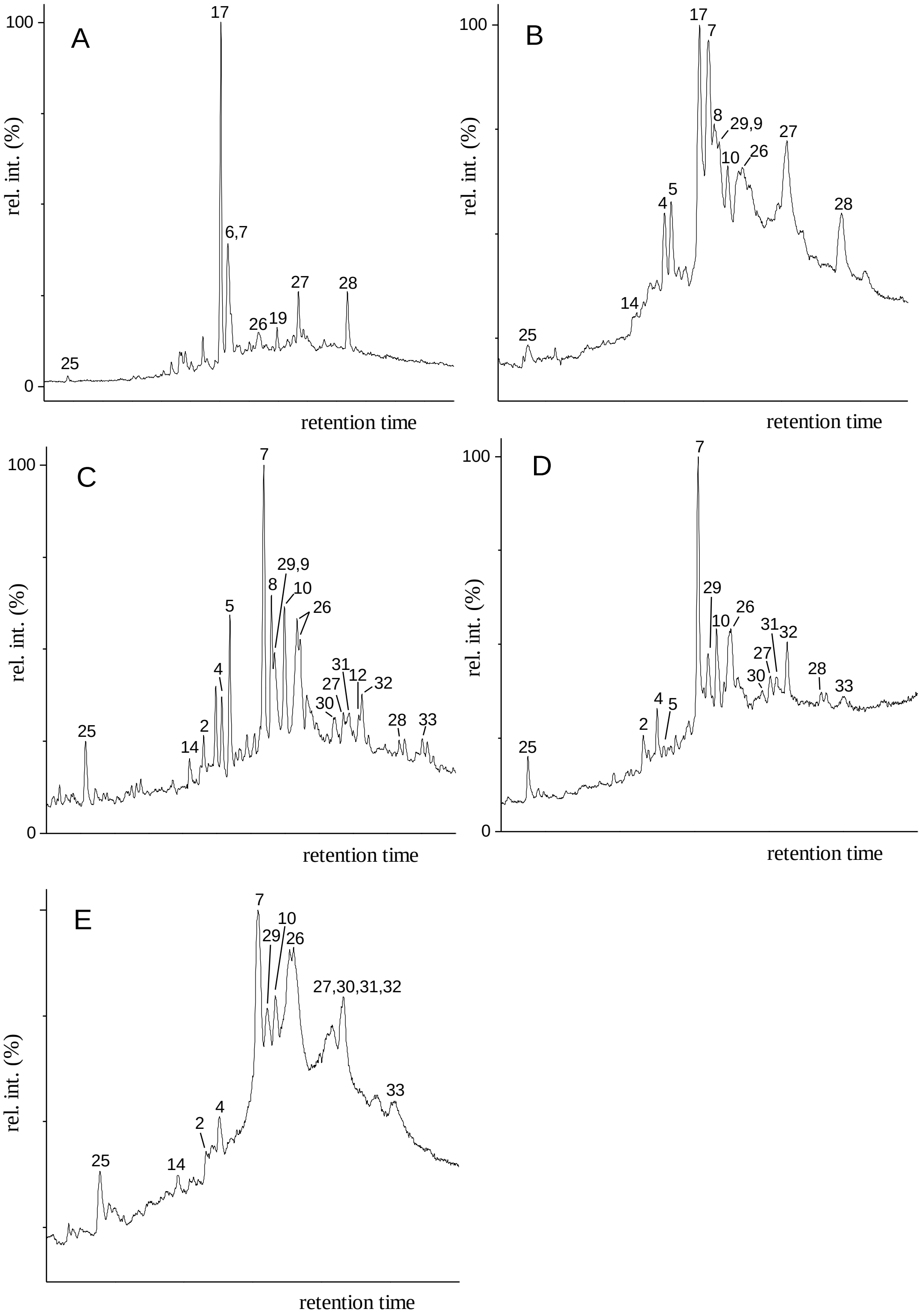

Figure 4 *Gas chromatograms of five methylated aged triterpenoid varnishes. Peak labels refer to the identified compounds of table I. Sample codes A to E refer to the codes that are used in the experimental section.*

spectrum and relatively short retention time. For easy comparison, the peak labels used in this table correspond to those of other figures of this thesis. The components of aged triterpenoid varnishes are not well resolved when analysed by

Table I *List of compounds, with their corresponding molecular weights (MW), identified in fresh and aged triterpenoid varnishes. Peak labels correspond to those used in other chapters.*

Label	Compound name	MW
1	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424
2	Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)	410
3	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426
4	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)dammaren-3-oic acid[1]	474
5	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[2])	458
6	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454
7	20,24-Epoxy-25-hydroxy-dammaran-3-one[1]	458
8	Hydroxydammarenone (20-hydroxy-24-dammaren-3-one[2])	442
9	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438
10	Ursonic acid (3-oxo-12-ursen-28-oic acid)	454
11	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438
12	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442
13	(8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene	442
14	Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)	410
15	β-Amyrone (3-oxo-olean-12-ene)	424
16	(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene	444
17	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454
18	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	454
19	Idem	454
20	3-O-Acetyl-3epi(iso)masticadienolic acid (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	498
21	Idem	498
22	Dammarenediol (20-dammar-24-ene-3β,20-diol[2])	444
23	Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)	440
24	Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)	440
25	Hexakisnor-dammaran-3,20-dione	358
26	3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone[2]	414
27	11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)	468
28	Oxidised oleanane type molecule	?
29	20,24-Epoxy-25-hydroxy-dammaran-3-ol[1]	460
30	17-Hydroxy-11-oxo-nor-β-amyrone (3,11-dioxo-17-hydroxy-28-nor-olean-12-ene)	440
31	17-Hydroxy-11-oxo-nor-α-amyrone (3,11-dioxo-17-hydroxy-28-nor-urs-12-ene)	440
32	11-Oxo-ursonic acid (3,11-dioxo-urs-12-en-28-oic acid)	468
33	Oxidised ursane type molecule	?

GCMS, indicating that the complexity of the resin has increased during ageing. Increase in polarity also gives rise to broader peaks in gas chromatography. Trimethylsilylation is often performed prior to GCMS analysis to shield the polar

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[1] The configuration at C-20 and C-24 was not determined.

[2] The configuration at C-20 was not determined.

carboxyl and hydroxyl groups, which results in narrower GC peaks [15]. This derivatisation technique was not used here, because the unknown silylated compounds of aged varnishes could not be identified by comparison with literature, which mainly consists of mass spectrometric investigations of methylated triterpenoids [11-14, 16]. Despite the poor separation by GC, the mass spectra of the majority of the components could be interpreted. The aged triterpenoid varnishes consisted of similar compounds. The distribution of these compounds differs slightly probably caused by the age of the varnish, the composition of the fresh material, remains of older varnishes and possibly other factors, which affect ageing. Some of the compounds are also present in the fresh resins, like **2**, **5**, **6**, **8**, **9**, **10**, **12**, **14**, **17** and **19** (Chapter 2) and are thus relatively stable. Compounds **4** and **7** were already present in fresh dammar resin, but are relatively more abundant in the aged varnishes. The triterpenoids **25**-**33** have not been reported before to occur in aged varnishes. The structures of the peaks labeled **28** and **33** in Figure 4 could not yet be determined unambiguously. Their mass spectra indicate strongly oxidised oleanane/ursane skeletons.

Figure 4 shows that varnishes A and B show similarities in their chemical composition. The main constituent of both varnishes is moronic acid, which was found to be a characteristic marker of mastic resin [1] (Chapter 2). Fresh mastic resin also contains some compounds with the dammarane skeleton. A small amount of oxidised triterpenoids with the dammarane skeleton (**4**, **5**, **7**, **8**, **25**, **26** and **29**) was found in the aged varnishes A and B. Furthermore, compounds with an oleanane skeleton (**6**, **27** and **28**) are present in this aged varnish. The oxidised species with the oleanane skeleton, **27** and **28**, are present in varnish A and B, whereas their corresponding isomers with the ursane skeleton, **32** and **33**, are not present. As discussed in the previous chapter, fresh mastic resin consists of molecules with the oleanane skeleton, whereas fresh dammar resin consists of both oleanane compounds and the isomeric ursane compounds. The presence or absence of compounds with the ursane skeleton can therefore be used for discriminating between aged dammar and mastic varnish. These observations were used to conclude that varnishes A and B are mainly composed of aged mastic resin. It is known that varnish A was applied in 1915 and is therefore more than 80 years old at the time of analysis. A small amount of ursonic acid is present in varnish B. This could either indicate the presence of some dammar resin or could be an indication that a certain degree of isomerisation occurs during ageing. Isomerisation would clearly obscure the distinction between aged dammar and mastic varnish. Tirucallol, which is present in fresh mastic resin and therefore was used as a marker for aged mastic varnishes [1], is not found in varnishes A and B. It is likely that this molecule oxidises during ageing, as will be explained below, and is only present in fresh mastic resin.

The other varnishes (C, D and E) exhibit an important similar feature in that the peak labeled **7** (ocotillone type molecules) is the highest of all total ion currents. As discussed in the previous chapter, molecules with the dammarane skeleton are present in both resins, but are more abundant in dammar resin. Ocotillone-type molecules, which have a dammarane skeleton, are therefore more abundant in aged dammar resin than in aged mastic resin. Varnish E contains a relatively large amount of the highly oxidised lactone with the dammarane skeleton (**26**) and large amounts of the oxidised oleanane and ursane skeleton type molecules (**27**, **30-32**). The presence of these compounds and the absence of moronic acid were used to conclude that varnishes C, D and E are probably aged dammar varnishes. There are other carbon skeletal types, which are specific for either dammar or mastic, but these are either hard to distinguish by their mass spectrum or only present in low amounts. Varnish E seems to be oxidised to a larger degree than the other aged dammar varnishes, because the oxidised compounds, such as **7**, **25-27**, **29-33**, are relatively abundant.

3.3.2. Analysis by HPLC-APCI-MS

As discussed in the previous section, GCMS after methylation does not resolve the constituents of aged varnishes very well. Since it is likely that more polar compounds are formed during the ageing process, GCMS is not the most appropriate method for the elucidation of all ageing products. HPLC-MS is suitable for the analysis of a much broader range of compounds. A number of aged varnishes from paintings were analysed by HPLC-APCI-MS. The reversed phase HPLC-APCI-MS total ion currents (TICs) of an aged dammar varnish and an aged mastic varnish (which also contains some components of another type of varnish made from diterpenoid resin) are shown in Figure 5(a) (dammar varnish D) and Figure 5(b) (mastic varnish B). The peak labels correspond to those used in Table I and in the other chapters. Since more polar compounds are expected to be formed by ageing, a more polar eluent is used to start the HPLC gradient in the analysis of the aged varnishes, compared to the eluents used to analyse the fresh resins (Chapter 2). The main constituents of the aged varnishes are well resolved by reversed phase HPLC, compared to the separation achieved by GCMS (Figure 4).

The mass spectrometric information provided by the APCI-MS spectra is often insufficient to identify unknown molecules (Chapter 6). APCI is a relatively mild ionisation technique, which mainly provides molecular weight information. In order to determine the chemical structure of the compounds separated by HPLC, HPLC fractions can be collected and subsequently analysed with GCMS. This experimental approach was not possible in the case of the aged varnishes, because

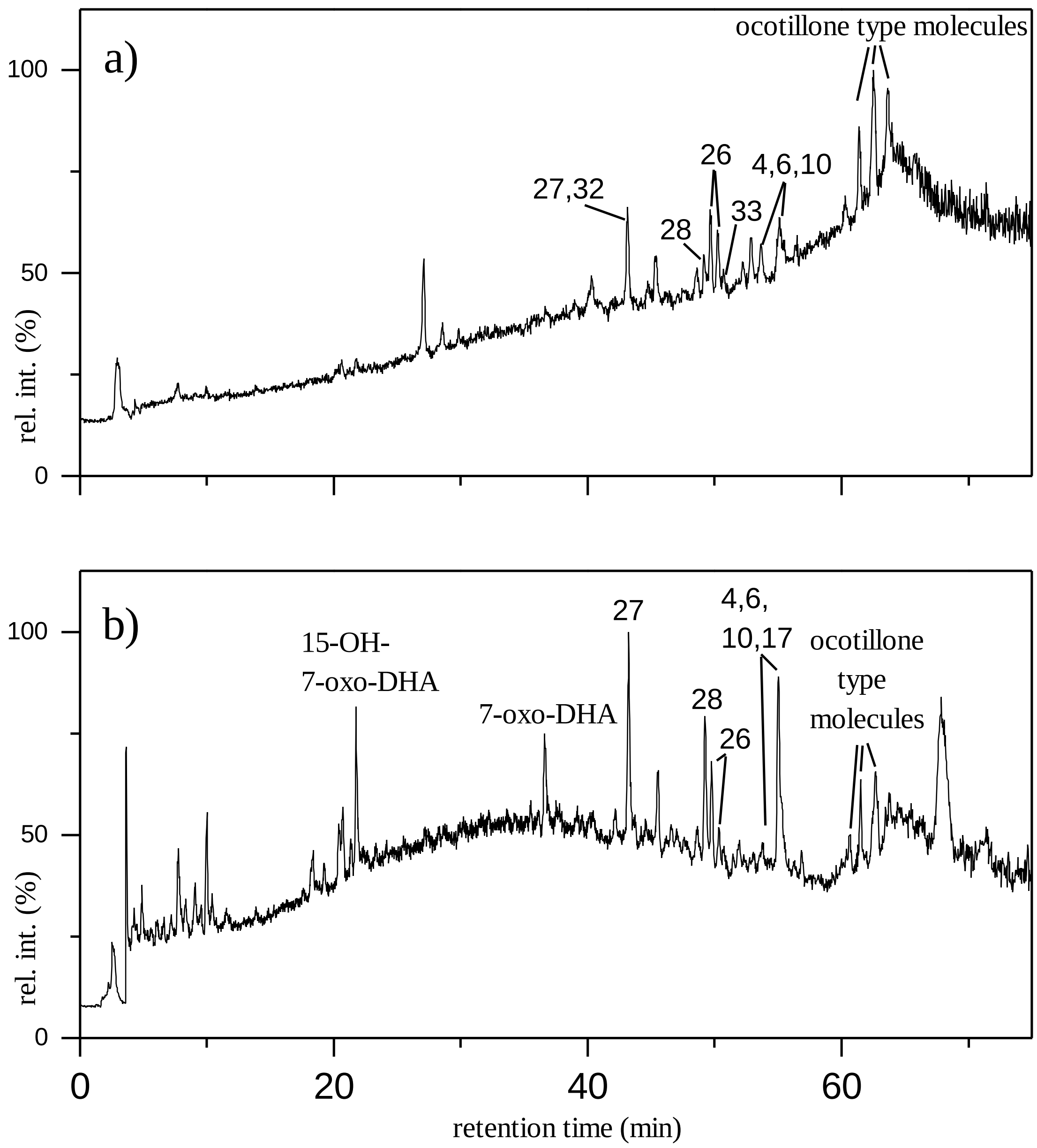

Figure 5 *Total ion current traces of reversed phase HPLC-APCI-MS, using the positive ion mode, of aged dammar varnish D (a) and aged mastic(/diterpenoid) varnish B (b) (peak labels refer to Table I).*

the amount of material that could be analysed by HPLC-MS was very low, as will be discussed below. With GCMS a number of constituents of these aged triterpenoid varnishes were already identified. Most of the constituents separated by HPLC could be tentatively identified, as listed in table I, with this GCMS information, the HPLC retention times, and the information obtained from the APCI-MS spectra, like the molecular mass information and the occurrence of some

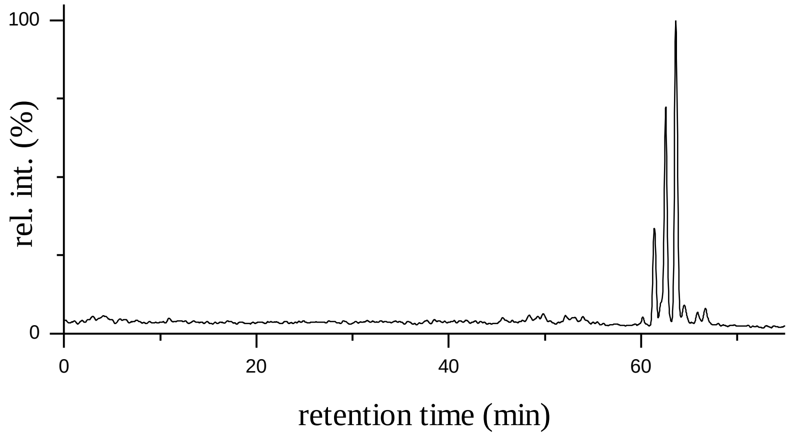

Figure 6 *Mass chromatogram of m/z 143, corresponding to compounds containing a hydroxyisopropylmethyltetrahydrofuran side chain (HPLC-APCI-MS analysis of the aged dammar varnish D).*

useful fragment ions. GCMS analysis indicated that ocotillone type molecules are the most abundant compounds of aged dammar varnishes. Since ocotillone type molecules contain two epimeric centers (C20 and C24), it is likely that a number of stereoisomers are present. In contrast to the GC separation, these stereoisomers were well separated by HPLC. Molecules of the ocotillone type could be traced by HPLC-APCI-MS by generating a mass chromatogram of their characteristic fragment ion of m/z 143, which corresponds to the oxidised side chain in ocotillone (Figure 6). Other oxidised stereoisomers with the dammarane skeleton (**4** and **26**) were also well resolved, whereas some isomeric compounds with the ursane/oleanane skeleton, such as **27**/**32** and **6**/**10**/**17**, were not separated by reversed phase HPLC. Since these compounds are well separated by GC, the HPLC fractions could be identified by off-line HPLC-GCMS. The aged mastic varnish of Figure 5(b) also contains two compounds which are indicative of the presence of an aged diterpenoid varnish (conifer resin), 15-hydroxy-7-oxodehydroabietic acid (15-OH-7-oxo-DHA) and 7-oxodehydroabietic acid (7-oxo-DHA) [7, 17]. It is probable that residues of older varnish layers were present on the painting from which this sample was taken. The components with a relatively short retention time, particularly present in Figure 5(b), could not be identified by their APCI-MS spectrum. It is possible that these compounds are smaller degradation products. Other unidentified peaks with longer retention times in Figure 5 were probably too large to be analysed by GC. These compounds could not be identified solely on the basis of their APCI-MS spectra. It is clear that the base line of the chromatogram is not straight. This base line was found to contain a large number of unresolved peaks over the total elution range, which could not be identified. It can be concluded from the HPLC-APCI-MS analysis of a number of aged varnishes from paintings that diversification during ageing leads to the formation of a large number of different species. The first twenty minutes of the chromatogram consists mainly of compounds with a relatively low molecular weight (150-300 Da), whereas the rest of the chromatogram mainly consists of compounds with molecular weights in the range of the molecular weight of triterpenoids. It is therefore likely that degradation of triterpenoids takes place during ageing.

As discussed before, moronic acid, which is only present in mastic resin and can be used as a marker to discriminate between aged dammar and mastic varnishes [1], is easily separated from the other components by GCMS. Under the HPLC conditions that were used here, moronic acid (**17**) co-elutes with two of its isomers, oleanonic (**6**) and ursonic acid (**10**), which show similar mass spectra under APCI conditions as moronic acid (see Chapter 6). The inability to separate these isomers excludes discrimination between dammar and mastic varnish by HPLC-APCI-MS on the basis of the occurrence of moronic acid. However, other molecular markers may be used for this discrimination. Since fresh dammar resin contains both compounds with the oleanane and ursane skeleton, whereas fresh mastic contains only the oleanane skeleton [1], this fact can also be used to make the distinction between the two types of varnishes. Two specific oxidised isomeric compounds, with either an oleanane or ursane skeleton, which could not be identified yet by their EI spectra, are often found in aged varnishes. Under APCI conditions these molecules are recognised by the protonated molecule, represented by a peak at m/z 469, and the characteristic fragment ion of m/z 233 (**28** and **33**) (Chapter 6). The two isomers with the oleanane and ursane skeleton are well resolved by HPLC-APCI-MS using acetonitrile and water as eluents and can be traced by generating mass chromatograms. Figure 7 shows that an aged dammar varnish (a) contains both isomers, whereas an aged mastic varnish (b) contains only the compound with the oleanane skeleton. HPLC-APCI-MS can thus be used for the discrimination between aged dammar and mastic varnish. There are other notable differences between the TICs of aged dammar and mastic varnish (Figure 5), such as the presence of peaks at lower retention times, around 27 and 68 minutes, which could not be identified. However, these differences are probably due to other factors, for example the number of years the varnishes have aged on the paintings, the environmental conditions, or the possible additions by painting restorers.

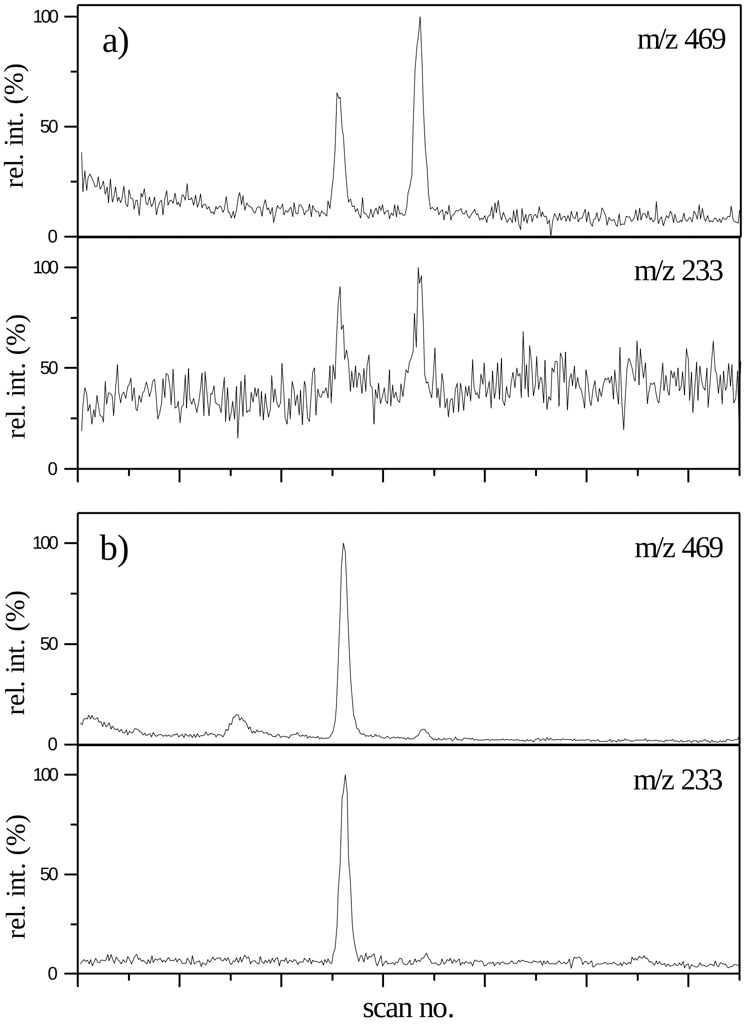

Figure 7 *Selected ion current profiles at m/z 469 and m/z 233 during the HPLC-APCI-MS analysis of aged dammar varnish (a) and aged mastic varnish (b).*

3.3.3. Molecular changes during ageing on a painting

The chemical reactions that have taken place in the ageing process can be rationalised on the basis of the molecules identified in fresh triterpenoid resins (Chapter 2) and aged triterpenoid varnishes (this chapter). Oxidation of the side chain appears to be a very important theme in the ageing of dammarane skeleton type molecules, leading to a side chain of the ocotillone-type (**5**, **7**, **29**) and to a γ-lactone in the side chain (**26**), as is shown in Figure 8 [18]. A compound with a short retention time (**25**) was identified tentatively by its mass spectrum. As is

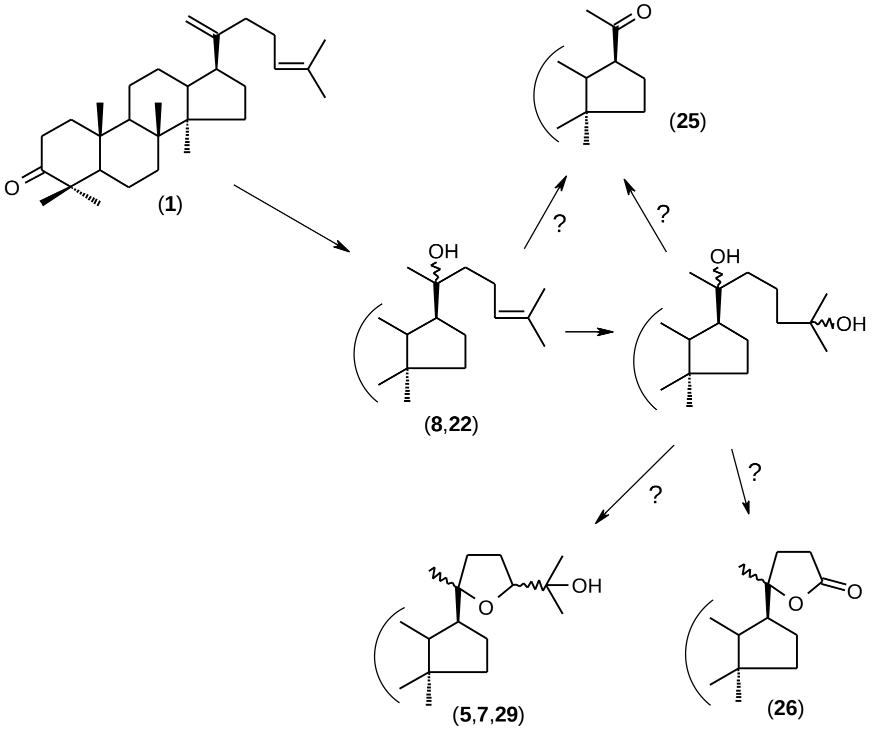

Figure 8 *Different stages of side chain oxidation occurring in dammarane type molecules.*

illustrated in Figure 8, this compound has a relatively short side chain compared to the other dammarane type molecules. According to Mills et al. [19] a compound with this same side chain, 3β-acetoxyhexakisnordammaran-20-one, is formed after either dehydration followed by ozonolysis, or direct oxidation of dammaranediol II monoacetate. The presence of this compound **25** indicates that, in addition to oxidation reactions, cleavage reactions take place during ageing, leading to compounds with a lower molecular weight. In principle, many more compounds can be formed with an appreciable vapor pressure and mobility, which will not be retained in the varnish film. These compounds are easily lost during the GCMS derivatisation procedure, due to the evaporation step. HPLC-MS lacks this derivatisation step, which implies that these compounds should be detected. As discussed above, HPLC-MS indeed shows a large envelope of unresolved peaks over the total elution range. A similar unoxidised side chain as the dammarane compounds is present in tirucallol. Therefore, the side chain of tirucallol is also likely to be oxidised during ageing, which explains why this compound was not found as such in aged mastic varnishes.

In the oleanane and ursane skeleton types, oxidation of C28 occurs readily, leading to an alcohol, an aldehyde (**9**) and finally a carboxylic acid group (**6**, **27**), as shown in Figure 9 for an oleanane type molecule. Subsequent decarboxylation

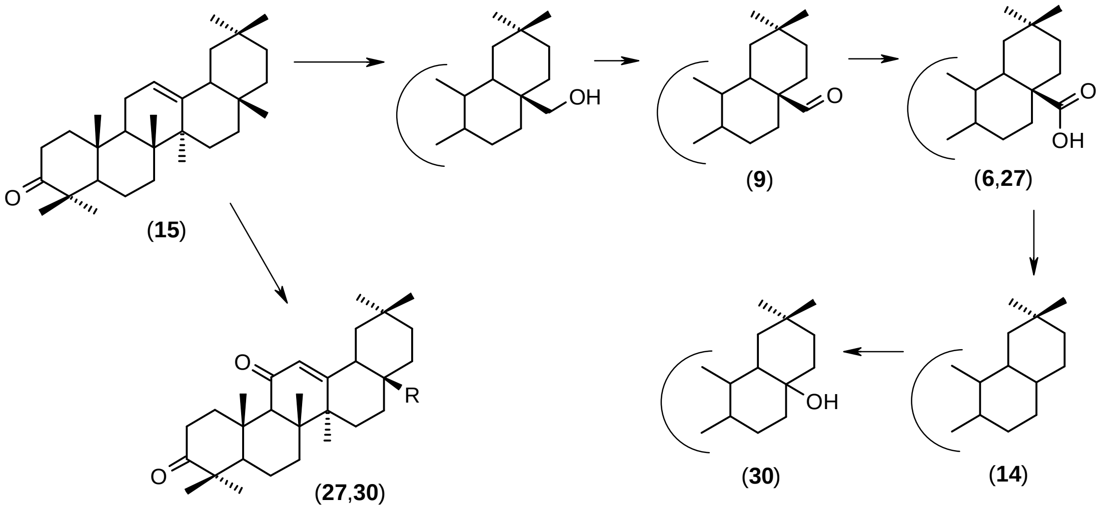

Figure 9 *Oxidation of an oleanane type molecule.*

leads to the loss of this carboxylic acid group (**14**) [20]. Oxidation of the C17 can then lead to a hydroxyl group at this site, as found in triterpenoids **30** and **31**. Furthermore, C11 can be oxidised to a conjugated keto group, as found in **27**, **30**, **31** and **32**.

The previous experiments by GCMS and HPLC-MS have demonstrated that both oxidation and chain cleavage processes take place during ageing on a painting. Chain cleavage is often regarded in the polymer field as the main cause for polymer degradation, leading to embrittlement. The formation of oxidised species during ageing will increase the polarity of the varnish, requiring a more polar solvent to remove an aged varnish. The formation of species with increased polarities is also likely to have an effect on the mechanical behaviour of a varnish, since the interaction between polar molecules is stronger. This intermolecular attraction in the varnish is increased by hydrogen bonding, which can be disrupted in the presence of moisture, leading to environmental stress cracking [21]. This last phenomenon is an important example of degradation by physical rather than chemical change. In contrast, Zumbühl et al. [22, 23] state that water acts as a plasticiser for oxidised triterpenoid varnishes. However, this conclusion was based on the investigation of artificially light aged varnishes, which may have other mechanical properties than varnishes aged ‘naturally’ on paintings. The chemical composition of artificially light aged varnishes will be discussed in the next chapter.

3.3.4. Structural interpretation of the DTMS data

Compared to a quick fingerprinting technique such as DTMS, GCMS after methylation is a rather time consuming method. In a DTMS experiment, all volatile triterpenoids are ionised and fragmented in the ion source at the same time, whereas during GCMS the different volatile triterpenoids are separated by a GC column before entering the ion source. The molecular identification of the different components of aged dammar and mastic varnish by GCMS makes it possible to identify mass fragments of the DTMS spectra of aged triterpenoid varnishes and explore whether it is possible to discriminate between aged dammar and mastic varnish by DTMS. Figure 10(a) and (b) show the DTMS spectra of the volatile parts of an aged dammar and mastic varnish (varnishes E and A, figure 4). The spectra of the volatile parts of the samples are depicted specifically in order to make comparisons with the GCMS data. It is important to realise that the acidic groups in the molecules are methylated prior to the GCMS experiments in order to render the acidic compounds more volatile, whereas in DTMS the sample is not derivatised. Naturally, samples can be derivatised prior to DTMS analysis, but this is not necessary. As has been pointed out before, dammar contains a relatively large amount of molecules with the dammarane skeleton. A large amount of ocotillone-type molecules, such as **7**, is characterised by peaks at m/z 143, which corresponds to the hydroxyisopropylmethyltetrahydrofuran side chain, and at m/z 399. The oxidised dammarane species with the lactonised side chain (**25**) is recognised by a peak at m/z 414. Thus, the intensity of peaks at m/z 143, 399 and 414 is relatively low in the DTMS spectra of aged mastic varnishes. As stated above, aged mastic varnish is recognised easily with GCMS by the presence of moronic acid (**17**). Unfortunately, oleanonic (**6**) and ursonic acid (**10**), which are also found in dammar resin, have the same molecular mass as moronic acid (454 Da). Methylation of the acid group is unlikely to have an effect on the ring C cleavage resulting in the characteristic peak at m/z 249 (methylated compound) or the peak at m/z 235 (underivatised compound). The peak at m/z 235 is only slightly present in the DTMS spectrum. Therefore, identification of moronic acid is not easy to achieve by DTMS, unless MS-MS is applied. The peaks in the molecular ion region of the aged dammar and mastic varnish are very similar. Their distribution probably depends on the age of the varnish and its environmental conditions during ageing. The same applies to the region between m/z 150 and m/z 250. A number of peaks, such as m/z 189, 205 and 248, are present in the spectra of both dammar and mastic varnish. Only the peak at m/z 163 may serve as a marker for aged mastic varnish. This peak probably represents a characteristic fragment ion of 28-norolean-17-en-3-one [11], which is formed by pyrolytic degradation of oleanonic acid [24] (Chapter 2). It is inferred that pyrolytic

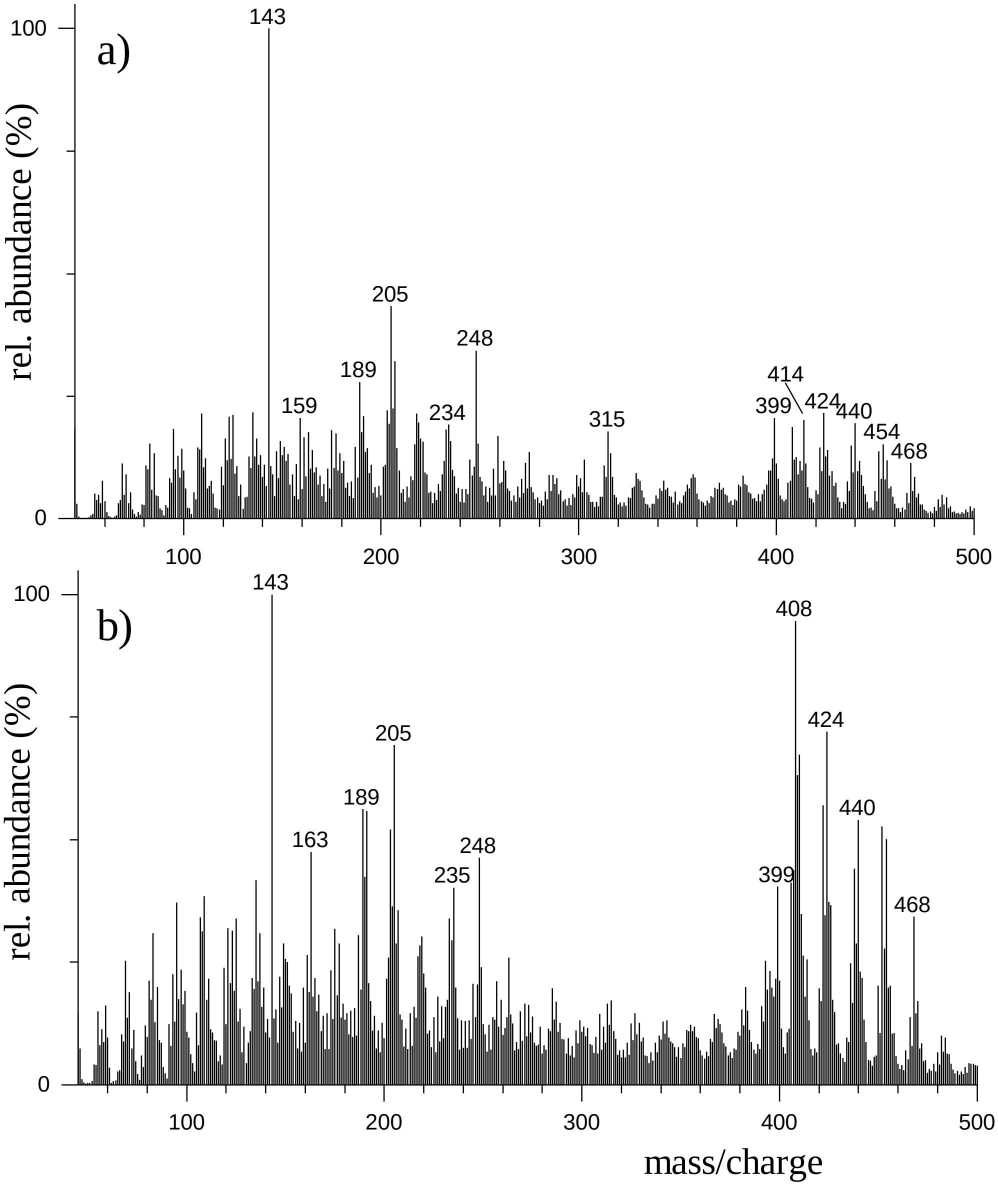

Figure 10 *DTMS spectra of the volatile part of an aged dammar varnish (varnish E) (a) and of an aged mastic varnish (varnish A) (b).*

degradation of moronic acid, which is highly abundant in aged mastic varnish, also gives rise to the formation of this compound represented by m/z 163. It can be concluded that DTMS gives some indications whether the aged varnish is a dammar or a mastic varnish. In the case of mixtures of these varnishes, interpretation of the DTMS spectrum becomes very complicated. Therefore, in order to get a conclusive answer on whether an aged varnish is of a mastic or dammar origin GCMS has to be performed.

3.4. Cross-linking

In the course of the GCMS analysis of a large number of aged triterpenoid varnishes, it was observed that the relative abundance of the compounds, expressed as TIC, is relatively low compared to that of fresh resins. To determine whether the amount of triterpenoids in varnishes decreases during ageing, the triterpenoid fractions of varnish B and D (Figure 4) were quantified by GCMS using an internal standard and were compared to those of fresh dammar and mastic resin. Figure 11 shows the gas chromatograms of these four samples. The methanol soluble constituents of the fresh resins and of varnish B and a solution of varnish D in isopropanol were used for analysis, since this latter solvent was used to remove the varnish from the painting. Figure 11 clearly demonstrates that the amount of triterpenoids has decreased dramatically as a result of ageing. It has to be realised that exact quantification of triterpenoids in the aged varnishes is difficult to achieve for a number of reasons. First, there is uncertainty about the percentage of triterpenoid material versus polymeric material in the fresh varnish. Besides the

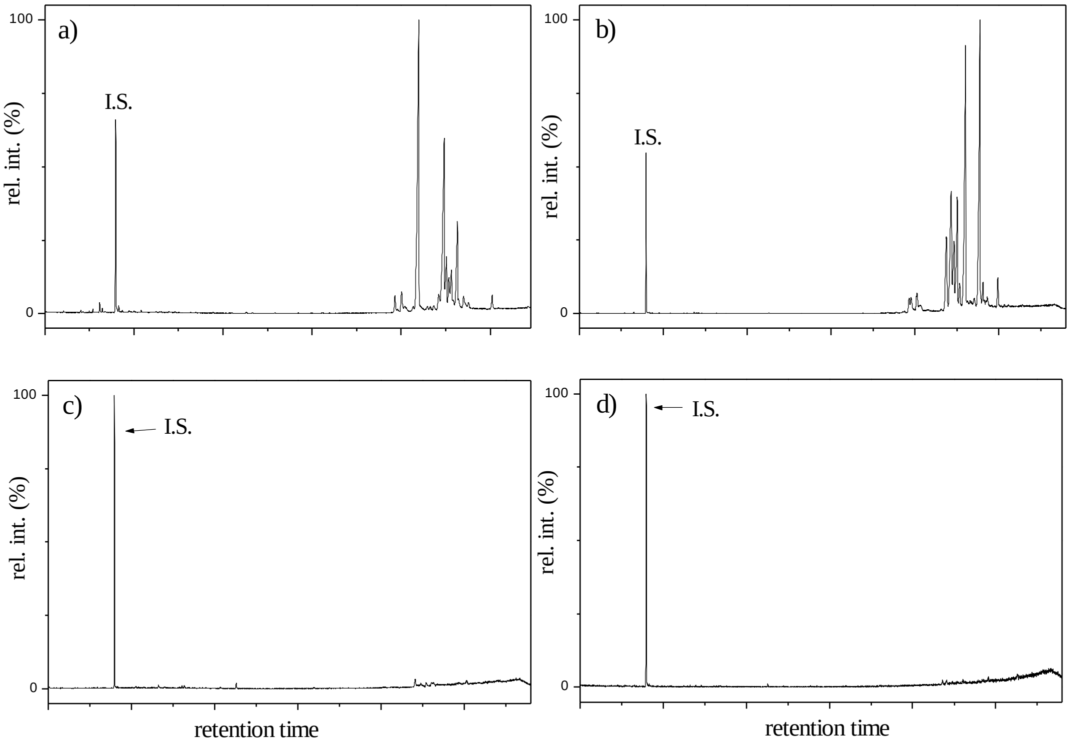

Figure 11 *Quantification of the triterpenoid fraction present in fresh dammar (a), mastic (b), aged dammar varnish D (c) and aged mastic varnish B (d) by GCMS using hexadecane as the internal standard (I.S.).*

biological variation, different varnish recipes will also have an effect. In some cases much effort is made to dissolve the complete resin, whereas in other cases the relatively insoluble part of the resin is discarded [5]. Secondly, the ageing process on a painting results in a number of different processes. Oxidation alters the molecular weight of the constituents of the resin. Degradation may lead to small volatile compounds and cross-linking also alters the molecular weight of the constituents. Thirdly, sampling of the aged varnish may have an effect on the percentage of triterpenoids in the total varnish sampled. When a degraded varnish is being removed from the painting, it is likely that some varnish residues are left behind on the painting, whereas other materials may be leached from the painting, for example, lipid material. Traces of lipid material were often present in the DTMS spectra of the aged varnishes. However, it is not clear whether this lipid material was intentionally added to the varnish or leached from the paint layer. Finally, the occurrence of different types of varnishes on top of each other and the addition of other materials during restoration are obviously complicating the exact quantification of the triterpenoids that are present in aged varnishes.

Despite the difficulties just described in achieving an exact quantification, Figure 11 clearly shows that the amount of triterpenoids is effected to a large extent by the ageing process. This may be partly explained by the phenomenon of chain cleavage, which occurs during ageing and gives rise to compounds with a lower molecular weight. GCMS analysis did not demonstrate the presence of large quantities of small compounds, but these may be lost during the derivatisation step. As discussed above, HPLC-MS of aged varnishes indicated that low molecular weight compounds are present. In addition to degradation processes, cross-linking may play a role during ageing. Loss of the triterpenoid fraction and formation of oligomers were previously demonstrated by SEC analysis of dammar films that were artificially aged in a weatherometer [4]. Loss of the triterpenoid fraction is also seen with HPLC-MS analysis of aged varnishes. When comparing the signal-to-noise ratio of the HPLC-MS results of the aged varnishes (Figure 5) to that of similar amounts of fresh resins (Chapter 2), it is clear that only a small part of the aged varnishes can be made visible by this technique. It is likely that the 'humps' in the baseline are either caused by the presence of numerous oxidised species and degradation products or the presence of a cross-linked fraction. It is also very well possible that compounds with a high molecular weight do not elute from the HPLC column or show a relatively long retention time. In addition, they may be difficult to ionise with APCI-MS and therefore difficult to detect.

Information on the occurrence of a cross-linked fraction as a result of ageing was obtained by DTMS. Figure 12 depicts the total ion currents of fresh mastic resin and the aged mastic varnish A. The shift towards higher scans (around scan no. 40), and thus higher temperature, after ageing on the painting indicates the formation of a more polar fraction, which is probably the result of the oxidation processes. In addition, this figure clearly demonstrates that the presence of a high molecular weight fraction (around scan no. 60) likely to have formed as a result of ageing. The same phenomenon was found in case of aged dammar varnishes. The MS data of the high molecular weight material (not shown) is very different from the spectrum of the dammar polycadinene polymer [25] and that of the mastic poly-β-myrcene polymer [26]. Fragment ions represented by peaks at m/z 91, 105 and 119 from branched benzenes are especially present in the high molecular weight fraction of aged mastic varnish, which suggests that aromatic rings may be present. At this point more research is necessary to identify these polymeric fractions. Chapter 5 will describe a method to prepare large quantities of this polymeric material needed for further investigations.

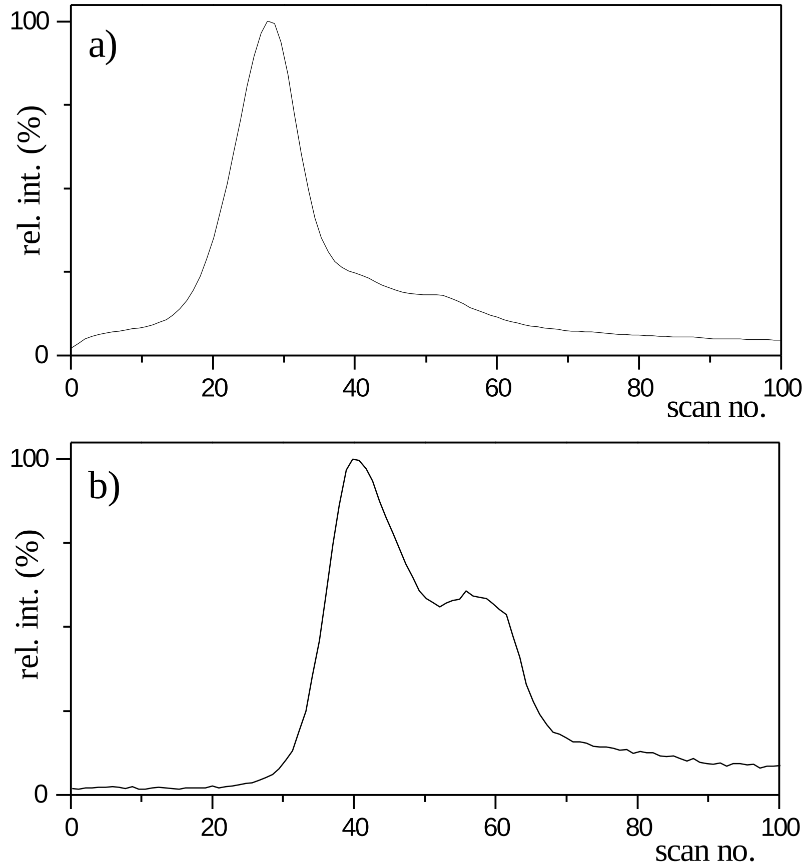

Figure 12 *DTMS total ion currents of fresh mastic resin (a) and aged mastic varnish A (b).*

3.5. Light absorbing characteristics of aged triterpenoid varnishes

Yellowing is one of the problems related to the ageing of triterpenoid varnishes. It would be interesting to identify the specific compounds responsible for the yellow appearance of an aged triterpenoid varnish. The molecular separation technique HPLC coupled with a diode array UV/VIS detector, was used for this purpose. In general, a compound will appear yellow to the eye, if an absorption band is present in the short wavelength part of the visible spectrum (400-430 nm) [27]. However, the solvent, in which the compound is dissolved when analysed by HPLC, can bring about a shift in the absorption exhibited by the compound, either to a longer or to a shorter wavelength [28]. Therefore, one has to keep in mind that the light absorption exhibited by constituents of varnishes on paintings can differ from the absorption seen by analysis of the constituents in dissolved state by HPLC-UV/VIS. In order to elucidate the spectroscopic changes of varnishes that occur during ageing, both fresh dammar and mastic resin were analysed and compared to aged triterpenoid varnishes. Methanolic extracts of fresh dammar and mastic resin were analysed by HPLC-UV/VIS. As discussed in the previous chapter, identification of the different compounds of fresh dammar and mastic resin, separated by reversed phase HPLC, was already achieved by collection of the HPLC fractions and subsequent analysis by GCMS. Figure 13 shows the absorption at 200 nm and the identification of the peaks is given in Table I. No absorption in the blue light region from 390 to 430 nm could be seen. The triterpenoid constituents of dammar and mastic show UV/VIS absorption spectra with absorbance maxima around 190-200 nm, due to isolated ethylenic bonds, and in most cases an additional absorption around 295 nm, due to ketone or aldehyde carbonyl groups [29]. GCMS already indicated that two compounds of mastic resin, **13** and **16**, are only present in very low abundance (Chapter 2, figure 7). Due to the presence of three isolated double bonds in the bicyclic diol (**16**) and its corresponding ketone (**13**), the absorption at 200 nm is much higher compared to that of the other triterpenoids of mastic resin.

Painting restorers often investigate the presence of natural resin varnishes by testing the fluorescence of the varnish layer. In our experiments, a scanning fluorescence detector was also used as a HPLC detector. The HPLC column was removed, so that the constituents of the samples were not separated prior to detection. For both the fresh resins and the aged varnishes, it was found that the excitation spectra were sensitive to a change in emission wavelength and that the emission spectra were sensitive to a change in excitation wavelength, which indicates the presence of several fluorescing chromophores. This latter phenomenon was also found by De la Rie to occur in artificially aged dammar

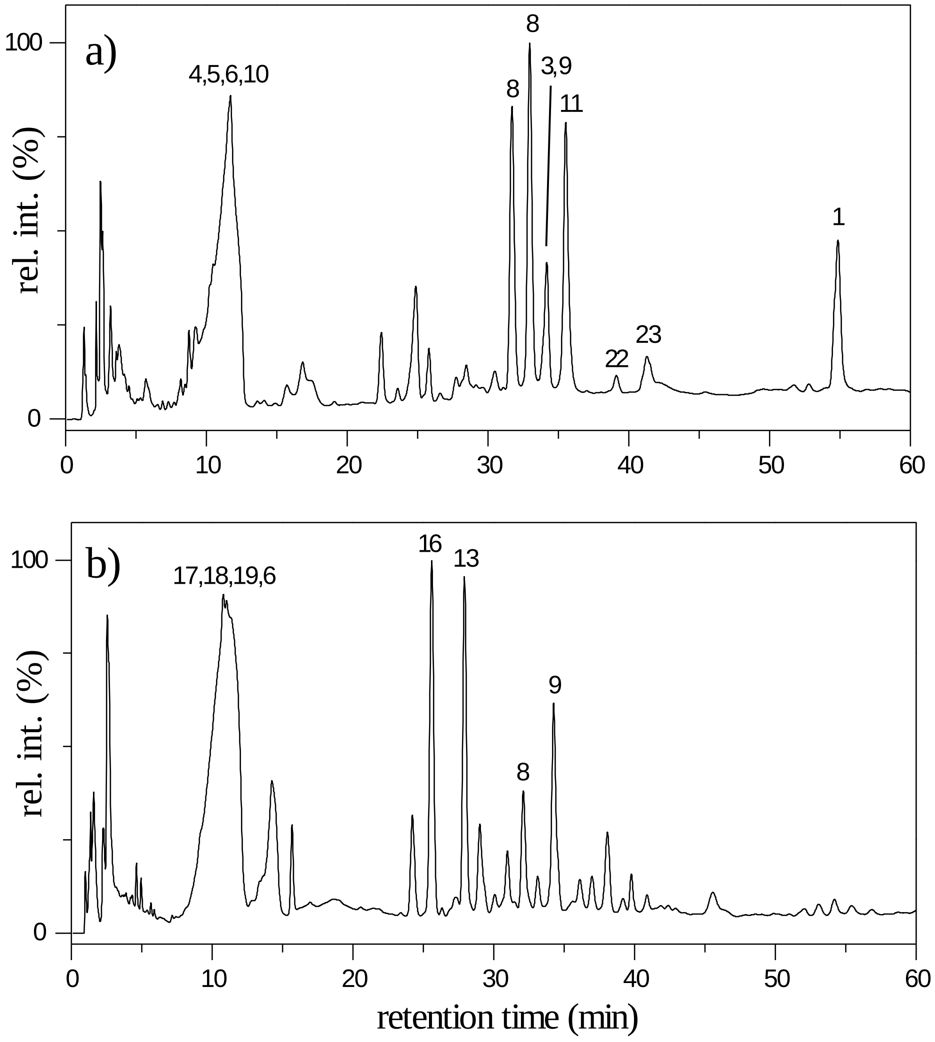

Figure 13 *HPLC-UV traces (200 nm) of methanolic extracts (5 mg/ml) of fresh dammar (a) and mastic resin (b) (peak labels refer to table I).*

varnishes [30]. The fluorescent behaviour of the individual constituents of triterpenoid varnishes was not investigated further, because this would entail an enormous amount of work with the experimental equipment available. For every compound represented by an HPLC peak, both the excitation and the emission wavelength would have to be scanned. For this purpose, a diode array fluorescent detector should be used, which simultaneously measures a large number of emission wavelengths for one specific excitation wavelength. In addition, stop-flow experiments should be carried out to scan the excitation wavelength.

Figure 14 shows the separation of the constituents of the aged dammar varnish E. The absorption at 200 nm is shown, together with an insert depicting the absorption in the range from 390 to 430 nm. The other aged varnishes were

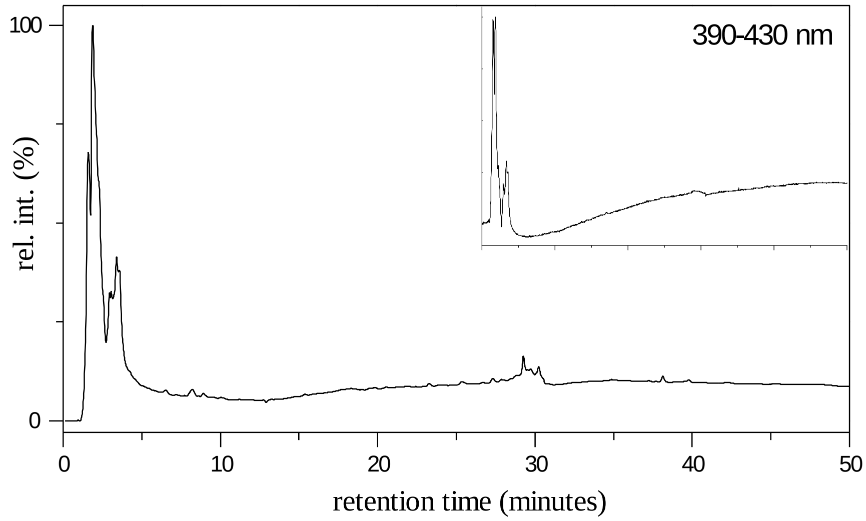

Figure 14 *HPLC-UV trace (200 nm) of a solution (5 mg/ml in ethanol/isooctane (75/25)) of aged varnish E, together with an insert showing the corresponding absorption in the range from 390 to 430 nm.*

analysed by the same technique, which resulted in similar chromatograms. Subsequent analysis of the HPLC fractions by GCMS showed that the oxidised triterpenoid compounds identified by GCMS eluted between 19 and 45 minutes. These compounds do not absorb light between 390 and 430 nm, so it can be concluded that they do not contribute to the yellow colour of an aged varnish. Figure 14 shows that mainly components with a very short retention time exhibit light absorbance both at 200 nm and in the range from 390 to 430 nm. The UV/VIS spectrum of these compounds has a maximum around 200 nm, but has a tail extending to much longer wavelengths exceeding 400 nm. Therefore, these components may induce a yellow colour. They also exhibit a fluorescence (excitation wavelength 337 nm and emission wavelength 411 nm). Because these compounds contribute both to the overall fluorescence and the yellow colour of an aged triterpenoid varnish, they may be important compounds from the restorer’s point of view. Molecules that exhibit a short retention time are either relatively small and/or relatively polar. It was not possible to identify these light-absorbing components by the GCMS method used here. Either the compounds were too polar to be analysed by the experimental GC conditions used or the compounds were too low in abundance and therefore below the GCMS detection threshold. It is known that trace amounts of highly light absorbing compounds can bring about a pronounced colour. It is possible that these components are small degradation products of triterpenoids. Previous HPLC-MS experiments already indicated that relatively small molecules elute at short retention times (Figure 5).

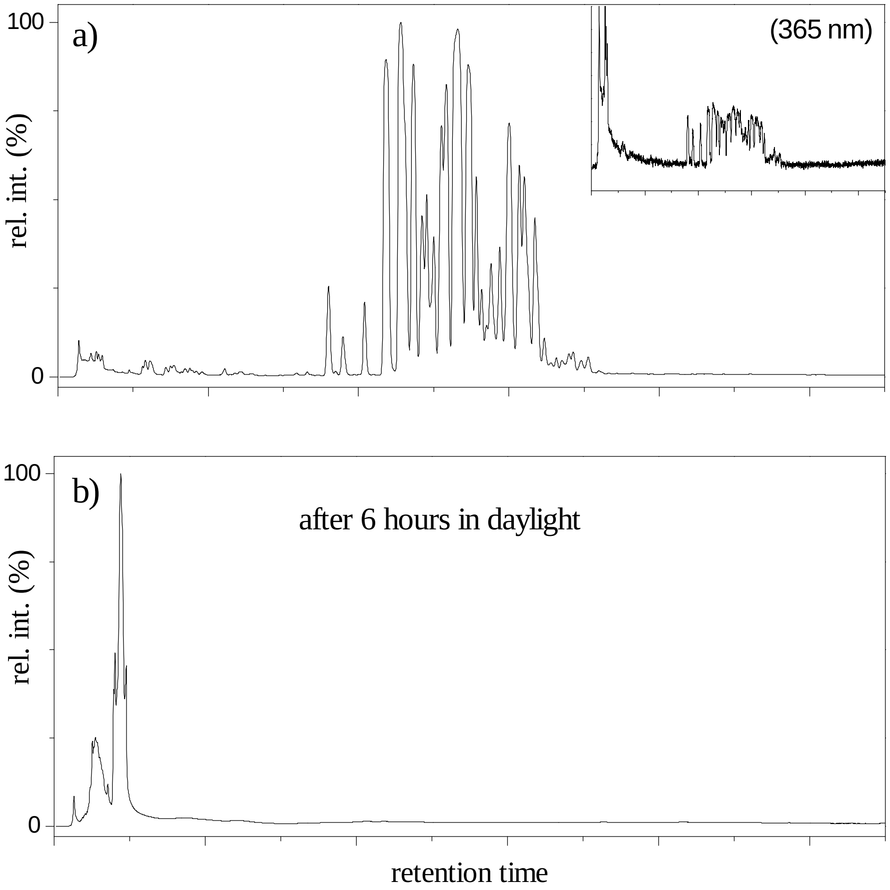

Figure 15 *HPLC-UV (trace at 200 nm and an insert of the trace at 365 nm) of an aged triterpenoid varnish, which was stored in the dark prior to analysis (a), and HPLC-UV trace (200 nm) of the same varnish sample after 6 hours of day light exposure (b).*

HPLC analysis of all aged triterpenoid varnishes produced chromatograms similar to the one that is shown in figure 14, except for three varnishes (Figure 15(a)). The absorbance at 200 nm is shown, for the maximum absorbance of all major peaks is around this wavelength. The UV trace of these three varnishes showed strongly light absorbing components eluting between 19 and 26 minutes. It was hypothesised that these compounds were accumulate in the dark, since these varnishes were the only ones that were (unintentionally) stored in the dark, for about one year at least, prior to analysis. Unfortunately, the changes that can take place in samples, after they have been taken from paintings, are often not considered during research. To check the light-stability of the strongly light-absorbing components a layer of one of these 'dark stored' varnishes (Figure 15(a)) was applied to a polyester film (Melinex®) and subjected to daylight through window glass. Figure 15(b) shows the results of the HPLC analysis of the sample after 6 hours of exposure. The results clearly show that the strongly light absorbing compounds are not stable in daylight through window glass. Therefore, it is possible that these light absorbing compounds can also be formed under light conditions, but they will react away quickly under the influence of light. It was very difficult to analyse these components by other molecular identification techniques, such as HPLC-MS, because the amount of material seemed to be very low. It is likely that their absorption coefficients are very high, which may be related to their instability. The strongly UV-light absorbing compounds of Figure 15(a) exhibit similar absorption spectra. Besides a maximum at 195-205 nm, a shoulder at 210-215 nm and a smaller maximum at 260-270 nm, they show a very small maximum around 365 nm. The HPLC-UV trace at 365 nm is also shown in Figure 15(a). The main absorbance at 195-205 nm indicates that the compounds probably contain isolated double bonds [29]. As was mentioned before, the polarity of the solvent of the analyte can produce a shift in the exact position of the absorption maxima. Therefore, it is not clear whether the absorbance at 365 nm exhibited by these components gives rise to a yellow colour.

Accelerated ageing experiments performed by De la Rie shows that yellowing occurs during heat ageing, especially when this is preceded by light ageing. This yellowing is easily reversed by exposure to (visible) light. It is possible that this yellowing caused by heat ageing is similar to the products formed by dark ageing found here. According to De la Rie it is likely that bleaching of the yellow films of dammar is the result of direct dissociation of double bonds by light [4].

As mentioned above, a yellow appearance is caused by light absorption around 400-430 nm. Size Exclusion Chromatography (SEC) was used to determine the molecular weights of the compounds of aged triterpenoid varnishes that absorb in this wavelength range. Figure 16 shows the SEC traces of a scraping of the aged mastic varnish B at 240 nm (solid line) and at 400 nm (dashed line). The trace at 240 nm shows that mainly two peaks are present, which are not well resolved. The peak at 400/500 Da represents the triterpenoid fraction. The peak with a maximum around 900/1000 Da can be attributed either to cross-linked dimerised triterpenoids or to highly oxidised triterpenoids, as already discussed in the appendix of Chapter 2. However, the presence of dimerised triterpenoids could not be confirmed by a number of mass spectrometric techniques, such as field desorption (FD), ammonia chemical ionisation (NH_3/CI) and matrix-assisted laser desorption/ionisation (MALDI). The SEC trace at 400 nm clearly shows that the SEC fraction around 900/1000 Da absorbs more light at 400 nm than the triterpenoid fraction. This may indicate that higher molecular weight fractions are relatively more responsible for the yellow appearance of an aged varnish.

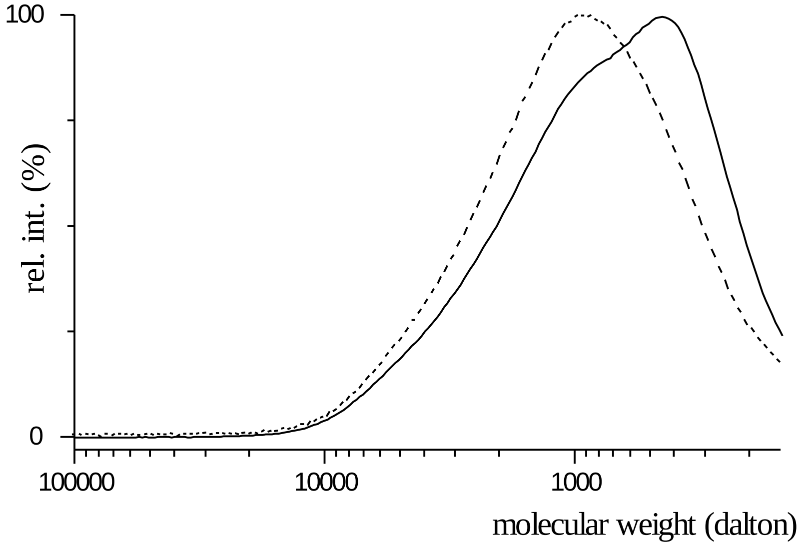

Figure 16 *SEC traces of the aged mastic varnish (labeled B) at 240 nm (solid line) and at 400 nm (dashed line).*

3.6. *Aspects of cleaning*

All techniques used here for the analysis of the aged varnishes showed that, in addition to oxidation and chain cleavage reactions, cross-linking reactions may occur during ageing of triterpenoid molecules on a painting. This observation has implications for the varnish removal practice, since it is obvious that it is relatively difficult to take higher molecular weight material into solution. Mechanical action may be necessary to remove certain parts of the varnish that are not easy to dissolve and it is feasible that a selectivity in the cleaning process can take place, leaving behind some high molecular weight residues. This also implies that there are unfortunate limitations to analysis of varnish samples that have been removed from paintings by means of a swab with solvent. It is likely that certain parts of the aged varnish are less soluble in the traditional solvents, as a result of possible oxidation and cross-linking reactions. Mechanical action will certainly aid in the removal of such parts, but it is not clear whether the complete varnish layer is removed or whether some residues are left behind. For analysis, the swabs were soaked in the same solvent, as was used for varnish removal. It is likely that those parts of the aged varnish, which were not soluble in the solvent, but were removed by means of mechanical action, cannot be retrieved for analysis, but remain in the swab. Removing the varnish by scraping will likely remove more of the insoluble fraction from the painting. Figure 17 shows the DTMS total ion currents of an aged varnish, which was removed both by the swab method (a) and by scraping (b). It is clear that certain parts of the aged varnish, probably those of relatively high molecular weight, are not soluble in the solvent used, ethanol, and can therefore not be analysed by using the swab method. Furthermore, Figure 17 demonstrates that mechanical action may be essential in the removal of aged triterpenoid varnishes from paintings. Therefore the analysis of scrapings gives a more complete view of the chemical composition of aged varnishes from paintings. The fact that one part of an aged varnish is still soluble in a frequently used solvent, whereas another part is not, implies that varnish removal can be quite a selective process. In this respect, the practice of thinning down a varnish layer may therefore selectively remove the soluble parts of an aged varnish, whereas the less soluble and probably higher molecular weight fractions may be concentrated on the painting. Clearly, more research is needed to elucidate the details of the varnish removal process on a microscopic level.

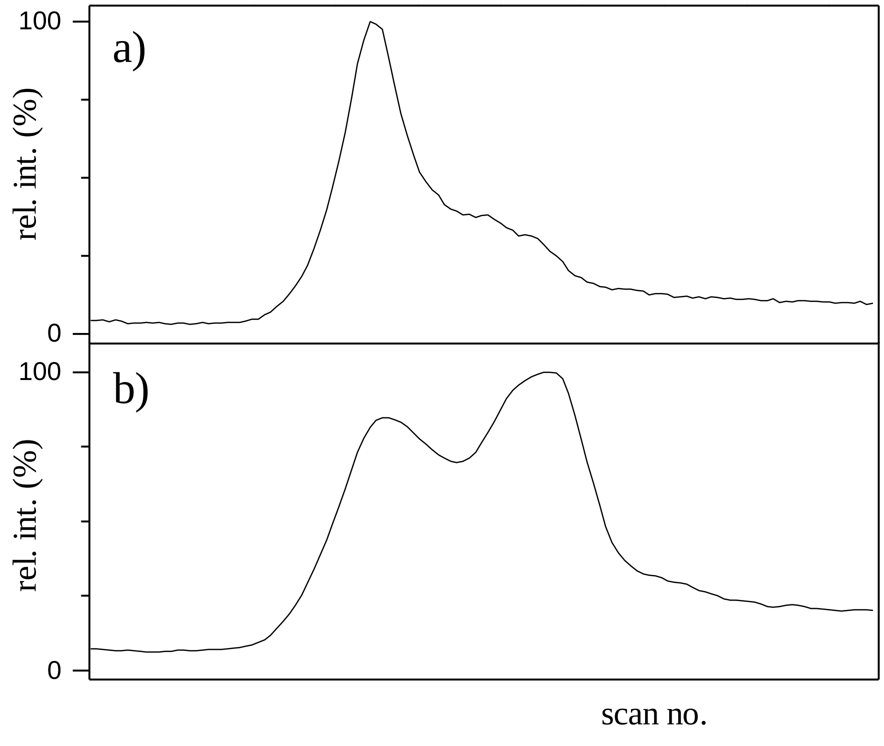

Figure 17 *DTMS total ion currents of an aged varnish that was removed by the swab method (a) and by scraping (b).*

3.7. Conclusions

Direct Temperature-resolved Mass Spectrometry (DTMS) was demonstrated to be a very useful method to discriminate between ‘naturally’ aged diterpenoid, triterpenoid and synthetic varnishes. Peaks occurring in DTMS spectra that represent fragment ions of compounds with a dammarane skeleton were identified. Since these latter compounds are relatively more abundant in dammar than in mastic, the corresponding peaks can be used to (tentatively) identify the aged dammar or mastic varnish. GCMS and HPLC-MS analysis demonstrated that ageing leads to complex mixtures. The aged varnishes were found to contain similar oxidised triterpenoid compounds in varying relative distributions. The positions in the molecules where oxygen is incorporated and molecules are cleaved during natural ageing could be identified. GCMS, HPLC-MS and DTMS all suggest that, in addition to oxidation and chain cleavage reactions, cross-linking reactions are likely to occur during ageing on a painting. All these processes give rise to the formation of specific fractions in aged varnishes, which may have different solubility characteristics. Especially the cross-linked fraction was found to be less soluble in a common solvent used by painting restorers for varnish removal.

HPLC-UV/VIS shows that the oxidised triterpenoids do not contribute to the yellow colour of an aged varnish. Absorption between 390 and 430 nm is only exhibited by compounds that elute at short retention times. These components are probably relatively small molecules, which could not be identified as yet. SEC analysis shows that an unidentified, but possibly cross-linked or highly oxidised, fraction (900/1000 Da) exhibits a higher absorption in the blue region than the triterpenoid fraction (400 Da). Identification of compounds responsible for the yellow colour of a substance can be very difficult, since it is known that colour can be induced by the presence of only trace amounts of highly light absorbing compounds. Three samples of aged varnishes were found which showed strongly light absorbing species, which are not stable in daylight. It is inferred that these compounds accumulate in the dark, after the sample has been taken from the painting.

3.8. Experimental

3.8.1. Samples

Methanolic extracts were prepared of Dammar (A.J. van der Linde, Amsterdam) and Mastic (H. Schmincke & Co., Erkrath). Aged varnishes from paintings were kindly provided by the following persons: Mr. R. Hoppenbrouwers (SRAL, Maastricht, NL), Mr. P. Dik (private restorer, Laren, NL), Dr. J. Townsend (Tate Gallery, London, UK), Ms. E. Hendriks and Ms. E. Reissner (Frans Halsmuseum, Haarlem, NL), Ms. C. Perez and Mr. R. Boitelle (Van Gogh Museum, Amsterdam, NL), Ms. A.M. Roorda Boersma-Pappenheim (private restorer, Rotterdam, NL), Ms. M. Zeldenrust and Mr. L. Sozzani (Rijksmuseum, Amsterdam, NL), Ms. L. Struick van der Loeff (Kröller-Müller Museum, Otterlo, NL) and Ms. L. Abraham (Restauratoren Kollektief, Amsterdam, NL). The aged varnishes were removed from paintings using a cotton swab wetted with a suitable solvent (solvents that are commonly used for varnish removal, such as propan-2-ol (isopropanol), ethanol, acetone and combinations of propan-2-ol and 2,2,4-trimethylpentane (isooctane)). Before use the cotton swabs were extracted with both hexane and acetone. The varnish, labeled A, was scraped off from the painting. One varnish, which is labeled B, was removed by applying a mixture of acetone and mineral spirits (70/30) to the varnish layer. After pressing down a sheet of polyester film on the surface, the varnish was removed mechanically (‘peeled off’). Varnish B was scraped off from the polyester film and dissolved in methanol for analysis.

For analysis the other varnishes were dissolved in the same solvents as were used for removal from the paintings using an ultrasonic bath. These solutions were directly subjected to DTMS and HPLC-MS experiments. GCMS experiments were performed after methylation. Varnishes A to E were studied in more detail. They were taken from the following paintings:

A. “Painswick Beacon” by Philip Wilson Steer (Tate Gallery, N03884). It was documented that a varnish was applied in 1915 (personal communication, Dr. J. Townsend, Tate Gallery, London).

B. “Supper at Emmaus” by Jan Steen (Rijksmuseum, inv. no. SK-A-1932).

C. “De zeeslag bij Nieuwpoort” by Willem Van der Velde de Oude (Nederlands Scheepvaartmuseum Amsterdam, inv. no. 1990.0949, in permanent loan (het Vaderlandsch Fonds ter Aanmoediging van ‘s Lands Zeedienst).

D. “Stadsgezicht” by Metzelaar (private ownership).

E. “De Heliaden bewenen de dode Phaëthon”, by Nicolaes de Helt alias Stocade (Frans Halsmuseum, inv. no. 81-369b).

For the DTMS experiment of which the results are shown in Figure 17, a varnish scraping of the painting “Deel van de triomfstoet met gevangenen, meegevoerde oorlogsbuit” by Pieter de Grebber (Oranjezaal, Huis ten Bosch) was used. An additional varnish sample was removed from this painting using a swab wetted with ethanol.

3.8.2. DTMS

About 50-100 μg of the aged varnish scrapings and fresh mastic resin were homogenised in approximately 100-200 μl of ethanol. An aliquot (about 2 μl) of the resulting suspensions was applied to the DTMS probe by using a syringe (SGE, 5 μl). The solutions containing aged varnishes were applied directly to the probe. DTMS analysis (16 eV) was performed as described in Chapter 2.

3.8.3. Multivariate analysis

Principal component analysis (PCA) of the DTMS spectra was performed on a SUN/SPARC workstation using a modified ARTHUR package (1978 version, Infometrix, Seattle) [31]. The concept of this numerical treatment applied to sets of mass spectra has been outlined by Hoogerbrugge et al. [9], and Windig et al. [10].

3.8.4. GCMS

For methylation the samples were first evaporated to dryness (approximately 0.5 mg). Aliquots of 250 μl of methanol, 25 μl of benzene and 10 μl of TMSdiazomethane were added [32]. This mixture was left at room temperature for 30 minutes. After evaporation to dryness, the sample was dissolved in 20 μl of dichloromethane (1 μl injection). For the quantification experiments, 0.12 mg of both fresh and aged varnishes was methylated and dissolved in 1 ml dichloromethane containing hexadecane (4 μg/ml) as an internal standard. On-column 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 (Fisons Instruments, series 8565 HRGC MEGA 2) coupled directly to the ion source of a JEOL DX-303 double focusing mass spectrometer (E/B). Some samples, of which the gas chromatograms are shown in Figure 11, 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. 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 with a rate of 8 °C/min and from 250 °C to 350 °C with 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) and accelerated to 10 kV. The mass spectra were interpreted and compared with spectra available in the literature.

3.8.5. HPLC

HPLC-UV/VIS

The aged varnish solutions with concentrations in the order of a few micrograms per milliliter 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 C_{18} column (Merck: LiChrospher 100 RP-18, 5 µm, 250 x 4 mm I.D.), which was kept at 35 °C, a diode-array UV/VIS detector (Waters Model 996) (190-800 nm wavelength range) and a scanning fluorescence detector (Waters Model 474). 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 an second isocratic period of 10 minutes using a flow rate of 0.8 ml/min. For the HPLC-UV/VIS analysis of aged triterpenoid varnishes, eluent A was a mixture of 50% water and 50% 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 14 minutes, going to eluent C in 1 minute, followed by a second isocratic period of 5 minutes using a flow rate of 0.8 ml/min.

HPLC-APCI-MS(-MS)

For the HPLC-MS analysis of the aged varnishes, eluent A was a mixture of 80% water and 20% acetonitrile, eluent B was acetonitrile. Separation was achieved with a linear gradient from A to B in 60 min, followed by an isocratic period of 15 minutes, using a flow rate of 0.8 ml/min. The HPLC fractions of the aged varnishes were collected and subsequently analysed after methylation by GCMS in order to identify the HPLC separated compounds. HPLC-APCI-MS analysis was performed as described in Chapter 2.

3.8.6. SEC

The SEC equipment consisted of a solvent delivery system (LDC/Milton Roy Model CM4000), a 20 µl valve loop injector (Applied Biosystems Model 480) and a diode-array UV/VIS detector (Waters Model 996). Samples were dissolved in stabilised THF and chromatographed with stabilised THF as eluent at 1 ml/min on a 30 cm PLgel 5 µm MIXED-D column (7.5 mm I.D.) (Polymer Laboratories). For the calibration of the system molecular weight standards of polystyrene (Polymer Laboratories) were used.

Acknowledgements

We are grateful to the Stichting Restauratie Atelier Limburg, Frans Halsmuseum, Van Goghmuseum, Rijksmuseum, Kröller-Müller Museum, Tate Gallery (London) and the Nederlands Scheepvaartmuseum Amsterdam, Mr. R. Hoppenbrouwers (Stichting Restauratie Atelier Limburg), Mr. P. Dik (private restorer, Laren), Ms. C. Perez and Mr. R. Boitelle (Van Gogh Museum), Ms. E. Hendriks and Ms. E. Reissner (Frans Halsmuseum), Ms. A.M. Roorda Boersma-Pappenheim (private restorer, Rotterdam), Ms. M. Zeldenrust and Mr. L. Sozzani (Rijksmuseum), Ms. L. Struick van der Loeff (Kröller-Müller Museum), Dr. J.H. Townsend (Tate Gallery, London) and Ms. L. Abraham (Restauratoren Kollektief) for providing the aged varnishes.

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4. Artificially light aged varnishes[1]

Abstract

The chemical composition of a number of artificially light aged triterpenoid varnishes was investigated by direct temperature-resolved mass spectrometry (DTMS) and gas chromatography-mass spectrometry (GCMS). The chemical composition of varnish samples that had been irradiated in a xenon-arc lighting device by UV-free light is similar to that of aged varnishes from paintings. Samples, which had been xenon-arc light aged by light with a UV component, predominantly consist of compounds, which have not been found in aged triterpenoid varnishes from paintings. These compounds were identified by their electron impact fragmentation patterns, which indicated the presence of oxidised A-rings. Therefore, exposure to xenon-arc light simulates the oxidation and cross-linking processes as found on paintings, only when irradiation by UV light is excluded. The amount of UV light is also important for the fluorescent tube light ageing of triterpenoid varnishes. Under low UV conditions the ageing processes as found on paintings are simulated, whereas under higher UV conditions the A-ring oxidation take place as well.

4.1. Introduction

As discussed in the previous chapter, more than 50 degraded dammar and mastic varnishes from paintings from about 15 different museum collections and private collections in the Netherlands and the United Kingdom were analysed in order to determine their chemical composition. These aged varnishes were found to

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[1] This chapter is partly based on the publications: Van der Doelen, G.A., Van den Berg, K.J. and Boon, J.J., "A comparison of weatherometer aged dammar varnishes and varnishes aged naturally on paintings", submitted to Techne, and Carlyle, L., Binnie, N., Van der Doelen, G. A., Boon, J. J., McLean, B., and Ruggles, A., "Traditional painting varnishes project: preliminary report on natural and artificial aging and a note on the preparation of cross-sections" in the Postprints of *Firnis, Material Aesthetik Geschichte, International Kolloquium*, Braunschweig, 15-17 June 1998, in press.

contain similar oxidation products in varying relative distributions. The investigation of the ageing of varnish by sampling from actual paintings is complicated because of the many unknown factors, such as environmental effects and the varnish recipes used [1], which may be important. This seems a serious problem so a complementary approach is often developed in which varnishes are subjected to various forms of artificial ageing, in order to control these factors.

Numerous pessimistic statements concerning the use of artificial ageing can be cited [2], for example the paper by Brown [3], who states that “the majority of users do not believe that they properly simulate or predict service”. To the best of my knowledge, it has never been investigated whether the various methods of artificial ageing available today simulate the molecular ageing processes as found on paintings[2]. This chapter therefore investigates the chemical composition of artificially light aged varnishes and compares it to our earlier data on aged varnishes from paintings.

Most commercially available ageing devices, such as weatherometers and fadeometers use xenon-arc light sources. According to Feller [2] xenon arc radiation, filtered to resemble sunlight or daylight through window glass, has become the most widely accepted source in tests of general photochemical stability. Examples of the use of this ageing technique in conservation science are the investigation of the long term stability of synthetic resins [4], the influence of light stabilisers [5, 6] and the investigation of the effects of solvents on artificially aged egg tempera films [7]. A commercially available artificial ageing technique has the advantage that the ageing conditions, such as relative humidity and temperature, are accurately regulated and known. It could be argued that they create ageing conditions that are too severe for the ageing of relatively vulnerable organic painting materials. Therefore, there is a trend in conservation science to use a ‘milder’ form of artificial ageing by using fluorescent tubes and lower illuminances [8-10, 26]. Ordinary fluorescent tubes have the added advantage that they emit very little infrared and thus the test samples do not experience temperatures much above 25-28ºC [2]. Artificial ageing setups, which use fluorescent tubes, are usually self-constructed in the different laboratories. Consequently, the experimental ageing conditions may vary among laboratories, which unfortunately may complicate the interlaboratory comparison of the analytical results.

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[2] We choose to use the term “artificial ageing” instead of the term “accelerated ageing”. “Accelerated ageing” implies that processes are only accelerated. In many ageing studies it has not been investigated whether the processes that are induced by the ageing device are similar to the processes that one attempts to simulate. We believe that in those cases where it is demonstrated that the same processes are simulated, it is valid to use the term “accelerated ageing”. In other cases the term “artificial ageing” is more appropriate.

This chapter evaluates four different artificial ageing conditions by comparing the chemical composition of artificially aged dammar and mastic varnishes to that of ‘naturally’ aged varnishes (as discussed in the previous chapter). The question whether a varnish undergoes the same oxidation and cross-linking reactions when exposed to these different methods of ageing is investigated. Dammar and mastic varnishes were subjected to the different types of artificial ageing conditions that are frequently used in conservation science using a commercially available xenon-arc lighting device with or without an additional UV filter, and two fluorescent tube lighting devices developed at the Canadian Conservation Institute (CCI). Direct temperature-resolved mass spectrometry (DTMS) and on column capillary gas chromatography-mass spectrometry (GCMS) were used to identify the constituents of the aged varnish samples.

4.2. Xenon-arc light ageing of varnish resins

4.2.1. Introduction

Dammar varnish has been xenon-arc light aged in the past in order to investigate the changes in chemical composition and in physical properties such as solubility, yellowness, gloss and surface roughness [11, 12]. In these investigations, daylight through window glass was simulated using a combination of filters. Another investigation on the ageing of dammar and mastic varnishes uses a filter which simulates daylight [13, 14]. The xenon-arc light ageing studies by De la Rie et al. pointed out that dammar and mastic varnishes can be stabilised by the addition of hindered amine light stabilisers (HALS) [5, 6]. This stabilisation is only successful in an ageing environment free of ultraviolet (UV) light. This was achieved with an additional UV-absorbing filter (Acrylite OP-2) (plexiglass) added to the filters used for simulating daylight through window glass.

For our investigation, triterpenoid varnishes, which had been artificially light aged in a xenon-arc lighting device, were kindly provided by Prof. Dr. De la Rie of the National Gallery of Art (Washington DC, USA). Two experimental light ageing conditions were tested. The first setup simulated daylight through window glass, which has been commonly used for the study of dammar varnish and uses a combination of a borosilicate inner and a sodalime outer filter, which transmits light down to about 315 nm [2]. The second setup utilised the Acrylite OP-2 filter (plexiglass), in addition to the two filters just mentioned, which results in the filtering of all light below approximately 380 nm [5].

4.2.2. Ageing of dammar and mastic

Figure 1 shows the total ion currents (TICs) and their corresponding total summation spectra of the direct temperature-resolved mass spectrometry (DTMS) analysis of two dammar varnishes, which were aged for 370 hours without (Figure (a)) or with (Figure (b)) the additional plexiglass UV-filter. For comparison, the DTMS results of fresh dammar resin are shown in Figure 1(c). The internal standard perylene, which is represented by a molecular ion peak at m/z 252, was added to the samples. The single ion currents of the ions with a mass of 252 and 253 dalton are subtracted from the TICs of Figure 1. Two peaks are present in the TICs. As described in the previous chapters, the first peak represents volatile (triterpenoid) material. At higher scan numbers and thus higher temperature the cross-linked fraction pyrolyses, which is represented by the second peak. When comparing the TICs of the artificially aged varnishes (Figure 1(a) and (b)) and of the fresh dammar varnish (Figure 1(c)), it is observed that, especially in the case of the varnish aged without the additional UV-filter, the cross-linked fraction has increased relative to the triterpenoid fraction during the artificial ageing process. When the spectra of Figure 1 are compared, it is obvious that the peak ratio internal standard/analyte has increased after artificial ageing. Compared to the fresh resins, less material is analysed. Cross-linking may result in the formation of a three-dimensional covalently bonded macromolecular system, which is difficult to desorb with DTMS, because more than one chemical bond has to be broken [15]. Furthermore, the pyrolysis yield of high molecular weight material is often much less than a hundred percent, because reactive pyrolysis fragments may recombine to form thermally stable material, which does not desorb from the DTMS probe. Zumbühl et al. and De la Rie, who used matrix–assisted laser desorption/ionisation (MALDI-MS) [13, 14] and size exclusion chromatography (SEC) [11] respectively, found that higher molecular weight material with a mass up to 1500 dalton [13] is formed after xenon-arc light ageing.

When comparing the total summation spectra of the dammar varnishes, aged without or with the additional filter (Figure 1(a) and (b)), a difference in the ion distribution is observed. The spectrum of the varnish that was aged without the additional UV filter (Figure 1(a)) is different from that of a varnish aged on a painting (Chapter 3, Figure 1(a)). The peak at m/z 143 is relatively small and the abundant peaks at m/z 396, m/z 410 and m/z 426 in the molecular ion region of Figure 1(a) (this chapter) are not abundant in the spectra of aged varnishes from paintings (Figure 1(a) of Chapter 3). The spectrum of the varnish that was aged with the additional UV filter (Figure 1(b)) clearly resembles the spectrum of an aged varnish from a painting, such as a base peak at m/z 143 and a similar distribution of ions in the molecular ion region. These DTMS data indicate that

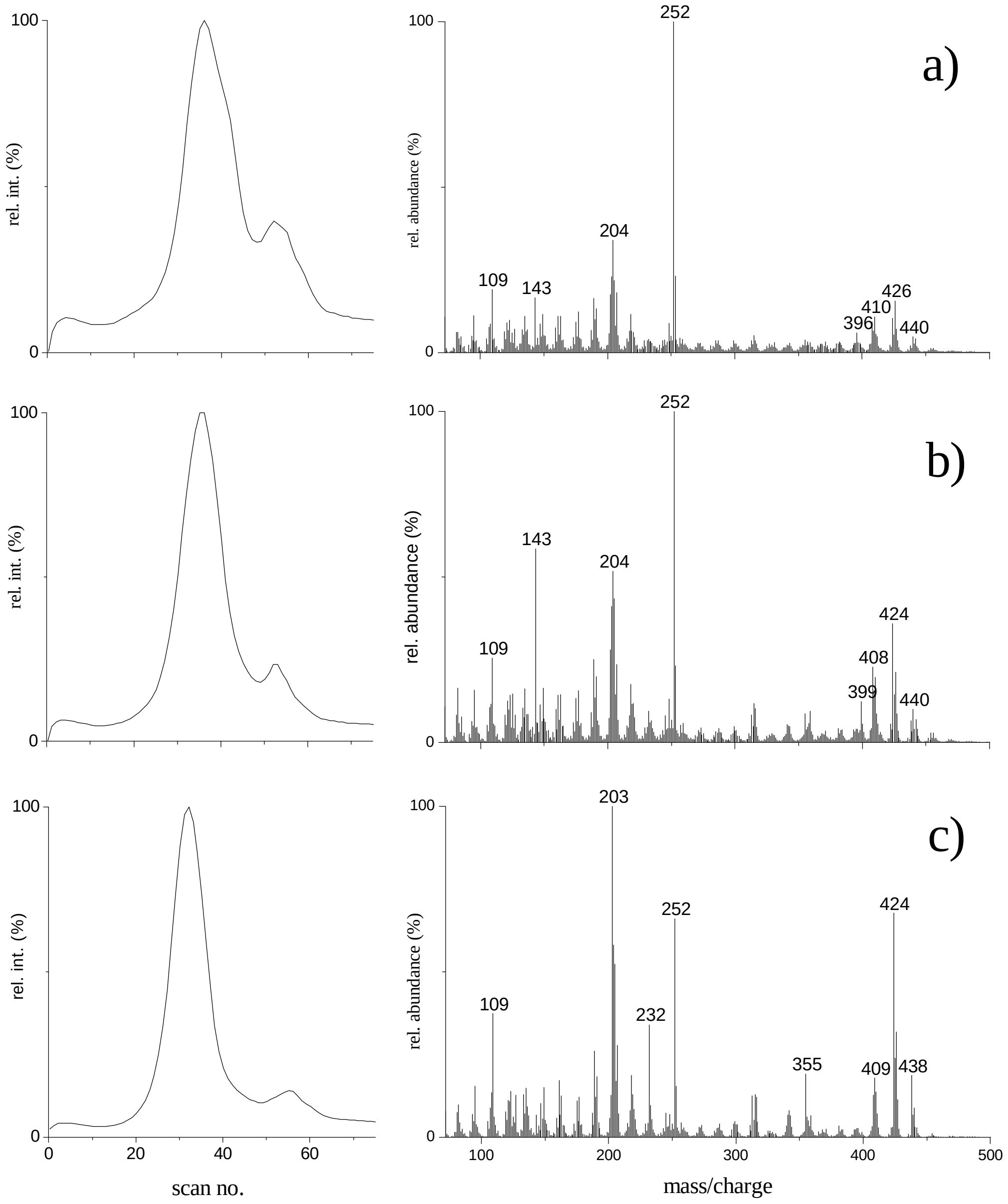

Figure 1 *The DTMS TICs and total average mass spectra (scan no. 20-60) of dammar varnishes, which were xenon-arc light aged for 370 hrs without (a) or with an additional UV filter (b). In addition, the DTMS results of fresh dammar resin (c) are depicted. The internal standard perylene was added to all samples, which is recognised by the molecular ion peak at m/z 252. The single ion currents of the ions with a mass of 252 and 253 dalton are subtracted from the TICs.*

there are differences in the chemical composition of the two xenon-arc light aged varnishes. GCMS analysis was applied for the molecular identification of the constituents of these varnishes.

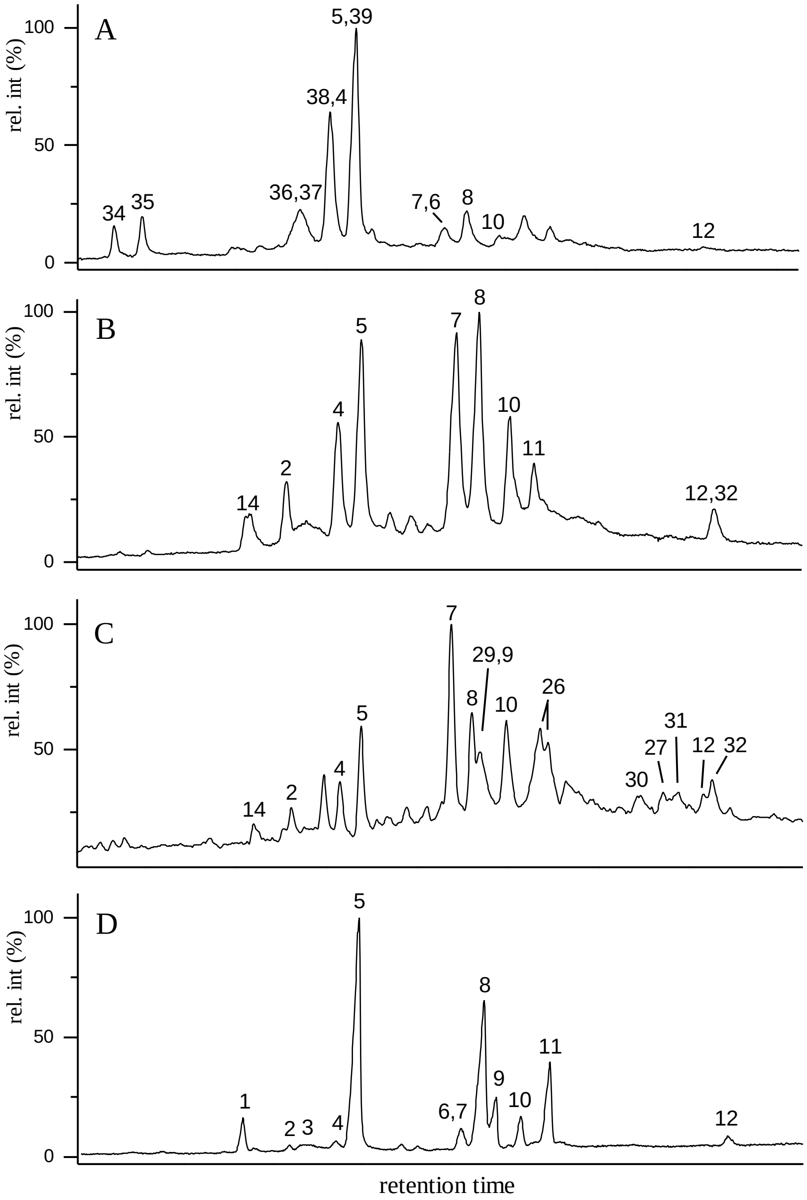

Figure 2 *Gas chromatograms of dammar varnishes xenon-arc light aged for 370 hrs without (a) or with an additional UV filter (b). In addition, the gas chromatograms of a 'naturally' aged dammar varnish (c) (which was labeled C in Chapter 3) and of fresh dammar resin (d) are depicted. Peak labels refer to those of Table I and II.*

GCMS was carried out in order to separate and identify the oxidation products, which are formed after ageing in the xenon-arc lighting device. Samples were methylated prior to GCMS analysis in order to render the acidic triterpenoids more volatile. Trimethylsilylation was not used for derivatisation, because the

Table I *List of compounds, with their corresponding molecular weights (MW), identified in triterpenoid varnish samples. Peak labels correspond to those used in other figures of this thesis.*

Label	Compound name	MW
1	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424
2	Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)	410
3	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426
4	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)dammaren-3-oic acid[1]	474
5	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[2])	458
6	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454
7	20,24-Epoxy-25-hydroxy-dammaran-3-one[1]	458
8	Hydroxydammarenone (20-hydroxy-24-dammaren-3-one[2])	442
9	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438
10	Ursonic acid (3-oxo-12-ursen-28-oic acid)	454
11	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438
12	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442
13	(8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene	442
14	Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)	410
15	β-Amyrone (3-oxo-olean-12-ene)	424
16	(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene	444
17	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454
18	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	454
19	Idem	454
20	3-O-Acetyl-3epi(iso)masticadienolic acid (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	498
21	Idem	498
22	Dammarenediol (20-dammar-24-ene-3β,20-diol[2])	444
23	Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)	440
24	Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)	440
25	Hexakisnor-dammaran-3,20-dione	358
26	3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone[2]	414
27	11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)	468
28	Oxidised oleanane type molecule	?
29	20,24-Epoxy-25-hydroxy-dammaran-3-ol[1]	460
30	17-Hydroxy-11-oxo-nor-β-amyrone (3,11-dioxo-17-hydroxy-28-nor-olean-12-ene)	440
31	17-Hydroxy-11-oxo-nor-α-amyrone (3,11-dioxo-17-hydroxy-28-nor-urs-12-ene)	440
32	11-Oxo-ursonic acid (3,11-dioxo-urs-12-en-28-oic acid)	468
33	Oxidised ursane type molecule	?
34	3,4-seco-28-nor-olean-12-en-3-oic acid	428
35	3,4-seco-28-nor-urs-12-en-3-oic acid	428
36	3,4-seco-olean-12-en-3,28-dioic acid	472
37	20,24-Epoxy-25-hydroxy-3,4-seco-dammaran-3-oic acid[1]	476
38	Dihydro-dammarenolic acid (20-hydroxy-3,4-seco-24-dammaren-3-oic acid[2])	460
39	3,4-seco-urs-12-en-3,28-dioic acid	472
40	3,4-seco-3-carboxy-25,26,27-trisnor-dammarano-24,20-lactone[2]	432

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[1] The configuration at C-20 and C-24 was not determined.
[2] The configuration at C-20 was not determined.

unknown silylated compounds of artificially light aged varnishes could not be identified by comparison with literature, which mainly consists of mass

Table II *List of triterpenoid compounds found in fresh dammar resin, aged dammar varnishes from paintings and dammar varnishes aged with four different artificial light ageing methods. Labels correspond to those used in Table I.*

Label of compounds listed in Table I	Fresh dammar resin	Dammar varnishes aged on paintings (Chapter 3)	Dammar varnish: xenon-arc light aged with UV	Dammar varnish: xenon-arc light aged without UV	Dammar varnish: fluorescent tube light aged (Biltz)[1]	Dammar varnish: fluorescent tube light aged (Down)[2]
1	●					
2	●	●		●	●	
3	●					
4	●	●	●	●	●	●
5	●	●	●	●		
6	●		●			
7	●	●	●	●	●	●
8	●	●	●	●		
9	●	●				
10	●	●	●	●	●	●
11	●			●		
12	●	●	●	●	●	●
13						
14		●		●		
26		●			●	●
27		●			●	●
28						
29		●			●	
30		●			●	
31		●			●	
32		●		●	●	●
33						
34			●			●
35			●			●
36			●			●
37			●			●
38			●			
39			●			●
40						●

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[1] This dammar varnish was prepared by dissolution of dammar resin in turpentine.

[2] This dammar varnish was prepared by dissolution of dammar resin in 50% xylene/50% mineral spirits (18% aromatic) with the addition of Tinuvin 292 (3%).

spectrometric investigations of methylated triterpenoids [16-20]. Figure 2 depicts the chromatograms of the dammar varnishes, which were aged for 370 hours without (a) or with (b) the additional plexiglass filter. For comparison, the chromatograms of an aged dammar varnish from a painting (varnish C as described in Chapter 3) and a fresh dammar resin are shown in Figure 2(c) and (d) respectively. Table I lists the compounds identified on the basis of their electron impact (EI) fragmentation patterns [16-21], and their molecular weight (MW). Table II summarises the occurrence of the triterpenoid compounds identified in fresh dammar resin (Chapter 2), in aged dammar varnishes from paintings (Chapter 3) and in the dammar varnishes aged with the different artificial light ageing methods.

A major difference between the chemical composition of the varnish that was xenon light aged with some UV present (Figure 2(a)) and the varnish aged on a painting (Figure 2(c)), is the degree of oxidation of the side chain of the dammarane type compounds. Aged varnishes from paintings mainly contain dammarane compounds with the hydroxyisopropylmethyltetrahydrofuran side chain or the lactonised side chain (see Figure 3(a)) (**4**, **7**, **26** and **2**). In case of the varnish, which was aged in the xenon-arc lighting device with some UV light present, the non-oxidised form of this dammarane side chain (**5**, **8** and **38**) is much more abundant than the hydroxyisopropylmethyltetrahydrofuran side chain (**4**, **7** and **37**). The compound with the lactonised side chain (**26**) is not present at all in Figure 2(a). During 'natural' ageing, oleanane type molecules are oxidised at C11 and at C28 (**27**, **30**, **31** and **32**) as discussed in the previous chapter[3]. These oxidation products were not present in the varnish, which was aged in the xenon-arc lighting device with some UV light present.

Another major difference is the presence of a number of abundant compounds in the xenon-arc light aged material (Figure 2(a)) (**34**, **35**, **36**, **37**, **38** and **39**), which have not been found to occur in aged varnishes from paintings. The identification of these compounds is based on their GC retention times and mass spectra. The molecular structures and mass spectra of a number of these newly found compounds are depicted in Figure 4. Strikingly, all of these compounds have an oxidised A-ring with a carboxylic acid group at C2. The mass spectra of compounds **34**, **35**, **36** and **39** show peaks which are representative of oleanane/ursane type molecules with either a hydrogen (m/z 204 for **34** and **35**) or a carboxylic acid group (m/z 262 and m/z 203 for **36** and **39**) at C17 (see Chapter 6). Compounds with these characteristic fragment ions are also present in the fresh resin (**2**, **6**, **10** and **14**). Compounds **34**, **35**, **36** and **39** all have molecular ion peaks, which are 32 mass units higher than the compounds found in the fresh resin. Furthermore, the presence of the peaks at m/z 355 (**34** and **35**) and at m/z 413 (**36** and **39**) indicates $(M\text{-}87)^{+\cdot}$ ions. As will be discussed in Chapter 6, a methylated carboxylic group at C2 gives rise to this fragment under electron impact conditions [21]. The mass spectra of compounds **37** and **38** were very similar to those of **4** and

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[3] We define these oxidation processes of oleanane/ursane type and dammarane type molecules as the typical processes occurring during varnish ageing on paintings.

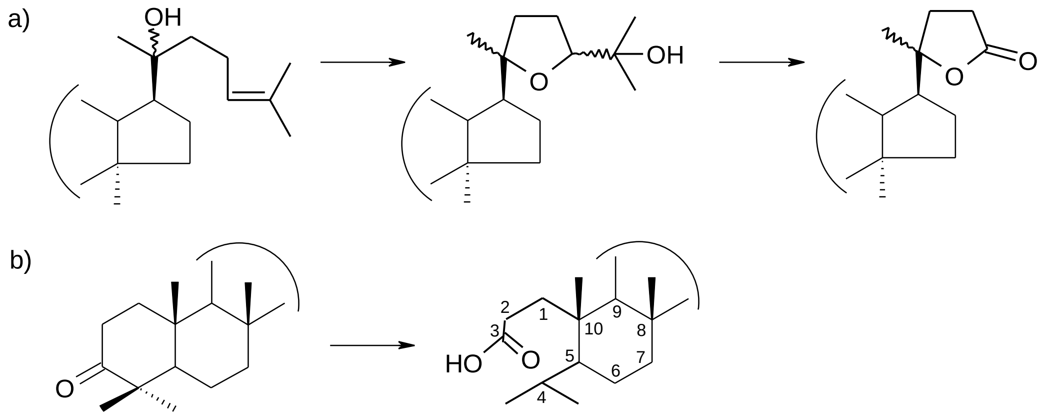

Figure 3 *Oxidation processes of triterpenoids. Side chain oxidation of dammarane compounds, as found to occur during ageing on paintings (a). Oxidation of the A-ring, as found to occur in triterpenoid samples during irradiation with UV light in aqueous acetic acid or in methanol and during artifical light ageing in the presence of UV light (b).*

5, which are found in fresh dammar resin (Figure 2(d)). The main difference is that a number of peaks present in the spectra of **37** (m/z 413 and m/z 431) and **38** (m/z 387 and m/z 456) are 2 mass units higher than those of **4** (m/z 411 and m/z 429) and **5** (m/z 385 and m/z 354). The difference between the dammarane type compounds **37**/**38** and **4**/**5** is the presence of an isopropyl group (**37** and **38**) instead of an isopropenyl group (**4** and **5**) at C5. Compounds **4**, **5**, **37** and **38** all contain an oxidised A-ring. The dammar varnish, which was aged without the additional UV filter (Figure 2(a)) also contains compounds **4** and **5.** These compounds however are already present in fresh dammar resin (Figure 2(d)). This latter type of A-ring opening, which results in an oxidised A-ring with an isopropenyl group at C5, probably takes place during biosynthesis or after excretion by exposure to outdoor conditions. The UV exposed dammar varnish contains only small amounts of triterpenoids that do not have an oxidised A-ring (**6**, **7**, **8**, **10** and **12**). In contrast, the majority of the triterpenoid constituents of the ‘naturally’ aged dammar varnish (Figure 2(c)) contains a keto group on C3 (**2**, **7**, **8**, **9**, **10**, **12**, **14**, **26**, **27**, **30**, **31** and **32**).

Arigoni et al. [22] and Rao et al. [23] investigated the photochemical cleavage of a number of triterpenoid compounds, such as α- and β-amyrone, 3-oxo-25,26,27-trinordammarano-24,20-lactone and cabraleone. It was found that irradiation with UV light in either aqueous acetic acid [22] or methanol [23] resulted in the cleavage of the ketone group at C3 into a carboxylic acid, as illustrated in Figure 3(b). This type of photochemical reaction also takes place during xenon-arc light ageing in the presence of UV light. Carbonyl groups are known to undergo scission reactions, such as the Norris type I reaction, under the influence of UV light [24]. For example, compound **38** is abundantly present in Figure 2(a). It is inferred that this compound is formed by oxidation of one of the most abundant compounds of fresh dammar, hydroxydammarenone (**8**). This same observation holds for compounds with an oleanane or ursane skeleton. All oleanane and ursane type triterpenoids found in the dammar varnish, which was aged in the xenon-arc lighting device with some UV light present, contain an oxidised A-ring (**34**, **35**, **36** and **39**). Other experiments (not shown here) demonstrate that this A-ring oxidation has already taken place even after 100 hours of weatherometer ageing. After 2000 hours of weatherometer ageing, these A-ring oxidised compounds are still the main constituents of the weatherometer aged material.

Because UV light seems to be generating this specific type of A-ring oxidation, it is interesting to investigate the chemical composition of the varnish that was aged by xenon-arc light with the additional UV filter. Figure 2(b) shows that the oxidised triterpenoid constituents of the UV filtered xenon-arc light aged dammar (**4**, **7** and **32)** resemble those found in ‘naturally’ aged varnishes. In addition, a number of unoxidised triterpenoids are present, which are also constituents of fresh dammar resin (**2**, **5**, **8**, **10**, **11** and **12)**. This implies that the specific type of A-ring oxidation, which gives rise to an oxidised A-ring with an isopropyl group, only occurs during xenon-arc light ageing when UV light is present. Feller [2] provides many examples, which illustrate that the type of compounds formed during ageing depends on the wavelengths of the light source used. The increased temperature in the xenon-arc lighting device may also have an effect on the reaction kinetics. In order to determine the exact role of UV light and temperature on the formation of different oxidation products, additional experiments have to be carried out.

We have investigated whether the above findings, as described for dammar resin also apply to the xenon-arc light ageing of mastic varnish. Figure 5 shows the gas chromatogram of a mastic varnish, which was aged for 600 hours with an additional plexiglass filter (a), and that of an aged mastic varnish from a painting (varnish A as described in Chapter 3) (b). Peak labels refer to those of Table I. The constituents of the xenon-arc light aged mastic varnish clearly resemble those formed during ageing on a painting. It can be concluded that xenon-arc light ageing with the exclusion of UV light by means of a plexiglass filter simulates the molecular ageing processes as found on paintings.

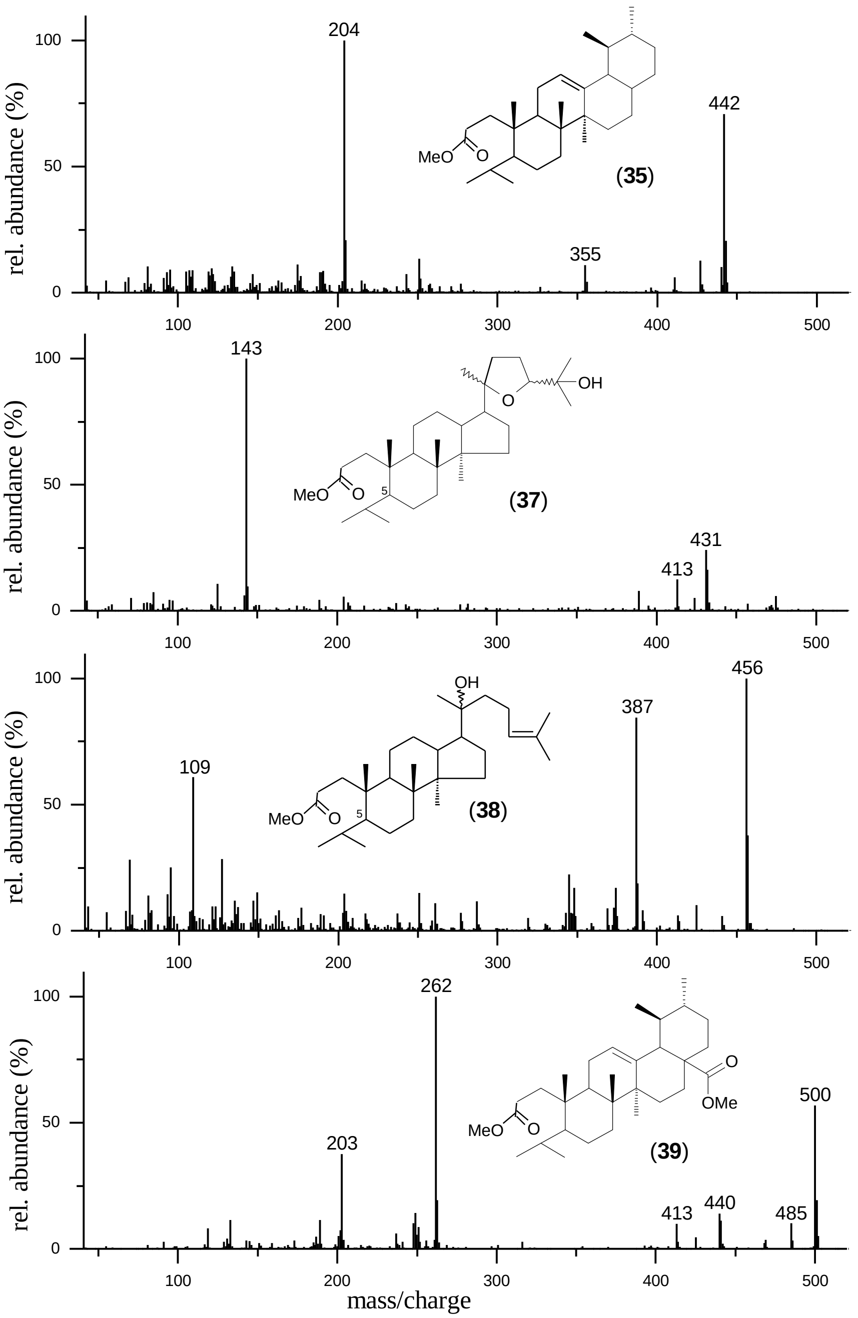

Figure 4 *Molecular structures and mass spectra (70 eV) of a number of triterpenoid compounds, which were identified in a varnish sample aged in a xenon-arc lighting device with some UV light present.*

The A-ring oxidation, which results in a carboxylic acid group at C2 and an isopropyl group at C5, was not found in the more than 50 aged triterpenoid varnishes from paintings. The conditions under which these varnishes from paintings were aged are not exactly known. For some years, some of the aged

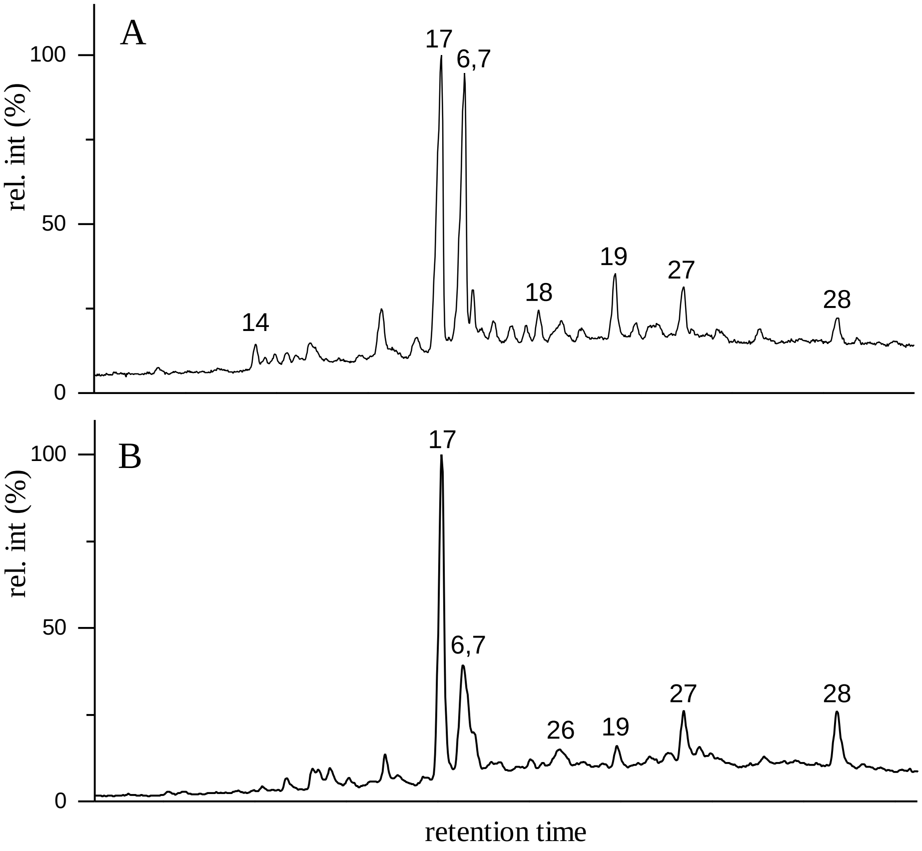

Figure 5 *Gas chromatograms of two mastic varnishes. A represents the TIC of a mastic varnish aged for 600 hours in a xenon-arc lighting device with an additional plexiglass filter. B represents the TIC of a 'naturally' aged mastic varnish from a painting. Peak labels refer to those of Table I.*

varnishes may have been protected from UV light by filters. However, many museums and probably a number of private collectors do not use these UV filters. Some paintings may have been lit by daylight through window glass, whereas others have been lit by other sources of light. This collection of more than 50 aged triterpenoid varnishes from paintings from about 15 museum and private collections in the Netherlands and the United Kingdom can therefore be regarded as representative of a large number of display conditions. Even in the case of a severely degraded varnish that was taken from a painting, which was hung next to a window and consequently was lit directly by the sun for several hours per day without any UV filtering, products of this specific A-ring oxidation were not found.

4.2.3. *Interpretations of earlier research*

Earlier investigations on the degradation process of dammar varnish applied xenon-arc light ageing simulating daylight through window glass [11, 13, 14]. Analysis by infrared spectroscopy (IR) and determination of acid numbers pointed out that a large amount of acid groups were formed during xenon-arc light ageing [11, 13, 14]. Nuclear magnetic resonance (NMR) measurements also indicated that ageing leads to complete disappearance of the ketones [13, 14]. The investigations did not use additional UV filtering. These analytical results agree with our findings that all triterpenoid keto groups at C3 are oxidised to acid groups (Figure 3(b)) after ageing in a xenon-arc lighting device in the presence of UV light. Therefore, compared to the ageing processes found on paintings, much more carboxylic acid groups are formed during this type of xenon-arc light ageing, due to extensive A-ring opening reactions. The formation of a large number of acid groups is likely to have implications for the solubility behaviour and the mechanical properties of a film of dammar varnish. Carboxylic acids are polar groups, which will cause a change in solubility towards more polar solvents. Furthermore, the presence of these polar groups will cause the resin to be more sensitive towards moisture. Acid groups will also give rise to stronger intermolecular forces in the film, which can lead to cracking. Physical changes, such as solubility change, increased sensitivity towards moisture, and cracking are also known to occur during “natural” ageing of triterpenoid varnishes. We believe that these physical changes are more pronounced when all A-rings are oxidised. Furthermore, the extensive A-ring opening may also result in relatively more flexible structures compared to the rigid multiring units, which are still found in aged varnishes from paintings [13]. It is therefore almost certain that dammar and mastic varnishes, which are aged in the xenon-arc lighting device in the presence of UV light, result in varnish films that are not comparable to aged varnish films from paintings.

4.3. *Fluorescent tube light ageing of varnish resins*

4.3.1. *Introduction*

A large number of historical sources have been analysed by Carlyle [1] in order to study traditional recipes for artists’ oil painting materials. These sources included recipes for binding media and varnishes. Carlyle and coworkers subsequently started an investigation of the effect of different varnish preparation methods on the appearance of the varnish [25]. For this purpose, a large number of varnishes, made according to different traditional recipes, were artificially aged at the Canadian Conservation Institute (CCI) in two self-constructed fluorescent tube lighting devices. These devices were developed in order to subject materials to relatively mild light ageing regimes compared to that of xenon-arc light ageing. Consequently, the exposure times were in the order of a few years, which is relatively long compared to those of xenon-arc light ageing, which are usually in the order of a number of weeks. Two different devices were used for artificial light ageing. Both were equipped with banks of fluorescent light tubes kept on for twenty-four hours a day, but the light levels and UV content differed considerably. The device that was developed by Biltz et al. [26] produces relatively high light levels (2300 lux) and negligible UV (15 μW/l). The device that was developed by Down [9] contains substantially lower light levels (750 lux) and a higher level of UV (190 μW/l). The majority of the samples were aged for about two and a half years in the Biltz apparatus. Tinuvin 292 was added to some varnishes in order to investigate its stabilizing ability. These latter varnishes were light aged for about one year in the Down apparatus. Half of the varnishes were covered with aluminum foil in order to obtain a set of light-protected, i.e. dark aged, control samples.

4.3.2. Ageing of dammar and mastic varnishes

Figure 6 shows the DTMS total ion currents (TICs) and total summation spectra of a dammar (a) and mastic varnish (b) that were aged in the Biltz apparatus. Comparable to aged varnishes from paintings (Chapter 3), a cross-linked fraction (around scan 50) is formed during this type of fluorescent tube ageing. Both DTMS spectra resemble those of aged dammar and mastic varnish from paintings (Chapter 3). The aged dammar varnish shows the specific marker peaks for the oxidised dammarane triterpenoids, such as m/z 143, m/z 399 and m/z 414. The molecular ion region of the DTMS spectrum of the artificially aged mastic varnish is similar to that of the ‘naturally’ aged mastic varnish. In addition, the possible marker for mastic varnish, the peak at m/z 163, is present. In order to investigate the oxidation products formed during ageing in the Biltz apparatus, GCMS was carried out on an artificially aged dammar and mastic varnish as shown in Figure 7(a) and (b). Labels correspond to those used in Table I and II. When comparing these chromatograms to those of aged varnishes from paintings as depicted in Figure 2(c) and 5(b), it is clear that the artificial light ageing in the Biltz apparatus has induced similar oxidation reactions as those occurring on

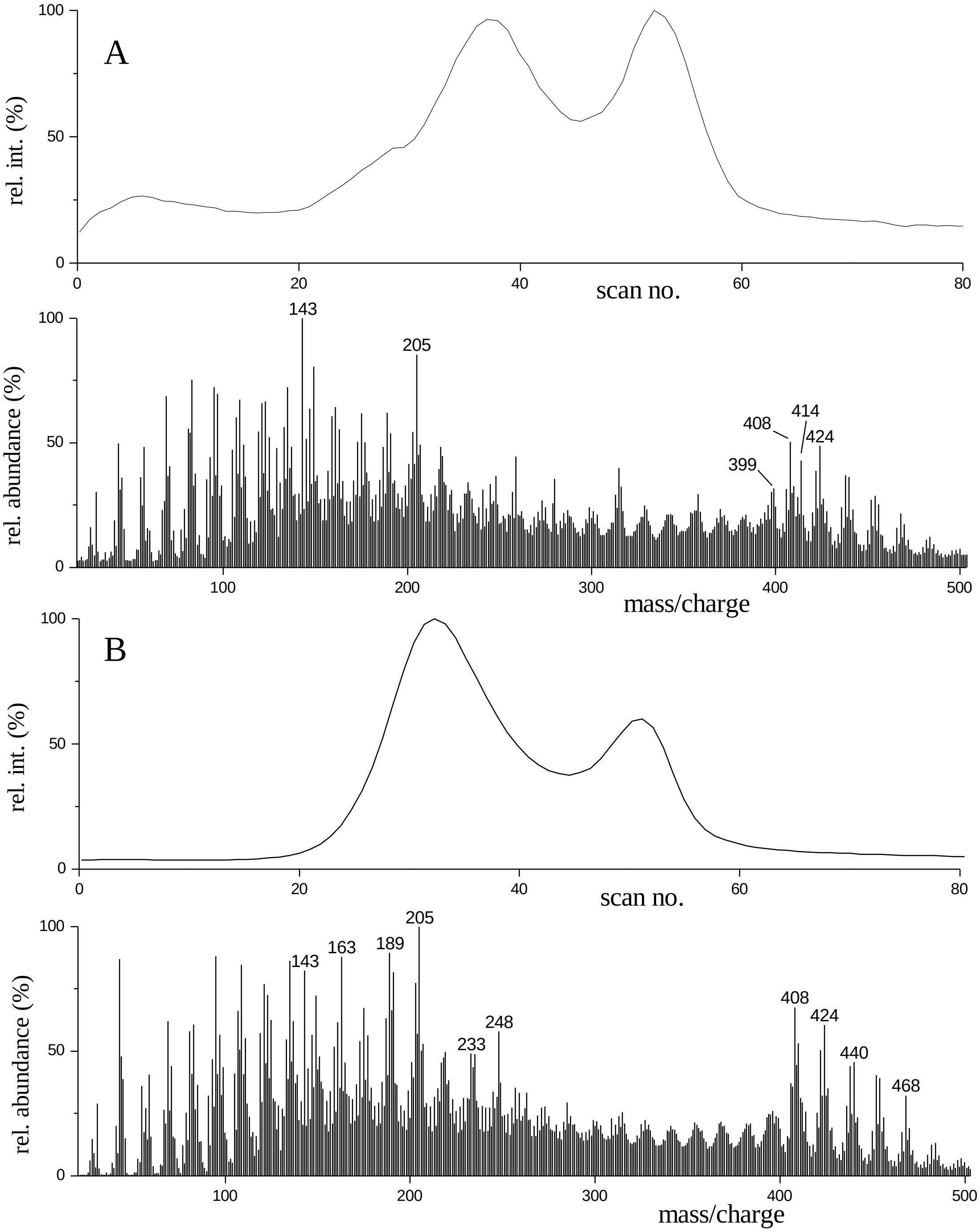

Figure 6 *The DTMS TICs and total summation mass spectra (scan no. 20-60) of a dammar (a) and mastic varnish (b), which were light aged in the Biltz fluorescent tube lighting device.*

paintings. The specific type of A-ring oxidation, which was found to occur during xenon-arc light ageing with some UV light present, does not take place in the Biltz apparatus.

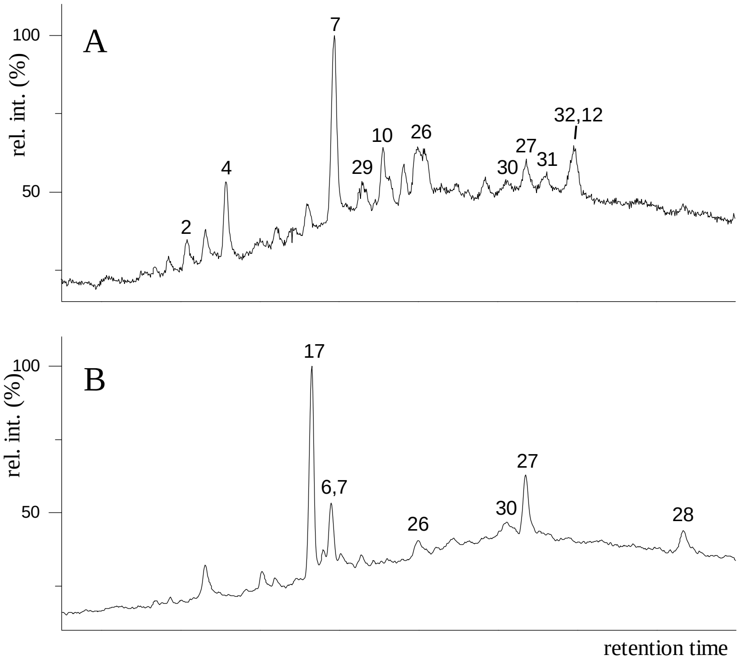

Figure 7 *Gas chromatograms of dammar (a) and mastic varnish (b) aged in the Biltz apparatus Peak labels refer to those of Table I and II.*

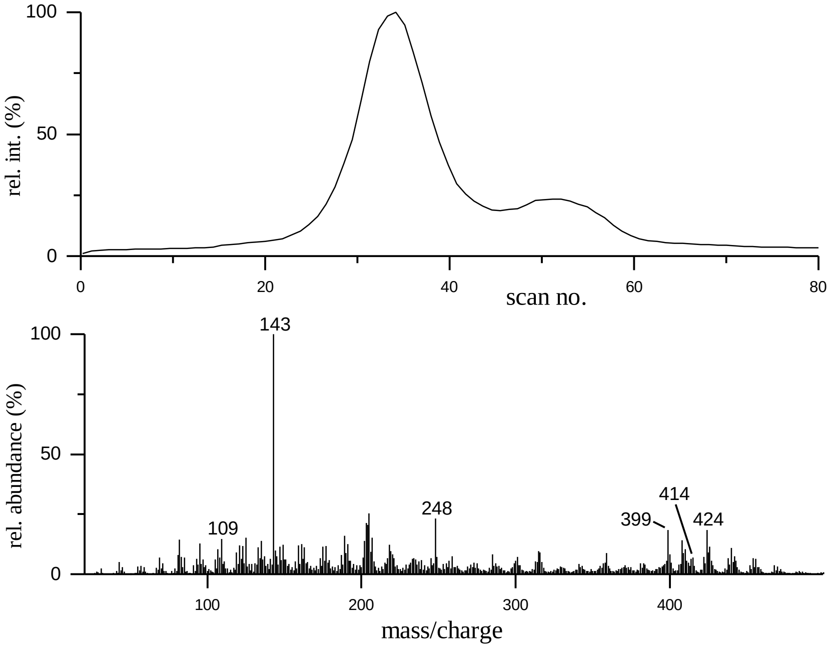

Figure 8 *The DTMS TIC and total average mass spectrum (scan no. 20-60) of a light-protected control sample of dammar (Biltz apparatus).*

The control samples, covered from the fluorescent tube light of the Biltz apparatus, can be considered to be artificially aged in the dark. Figure 8 shows the

DTMS TIC and total summation spectrum of the light-protected control sample of dammar. When this TIC is compared to that of the corresponding light aged dammar varnish (Figure 6(a)), it is clear that the light aged varnish contains a higher relative amount of cross-linked material. The light-protected control sample (Figure 8) contains a relatively large amount of ocotillone type molecules and a relatively small amount of the lactonised molecules compared to the corresponding light aged dammar varnish (Figure 6(a)). During ageing, hydroxydammarenone is first oxidised to an ocotillone type molecule (with its characteristic mass spectrometric markers m/z 143 and 399) and subsequently to a molecule with a lactonised side chain (corresponding to the marker m/z 414) (figure 3(a)). Thus, oxidation has also taken place in the light-protected controls, although the light exposed material is clearly oxidised to a larger degree. We conclude that ageing processes do take place in the dark as well, but to a minor extent than when placed in the light. It can be argued that light induced reactive species in the atmosphere in the lighting device are the cause of the oxidation of the light-protected control samples.

4.3.3. Effect of the light stabiliser (Tinuvin 292) in fluorescent tube light aged dammar varnish

Carlyle and coworkers had also prepared varnishes, which contain the light stabiliser Tinuvin 292, to investigate its effect on the appearance of the varnish [25]. The varnishes had been artificially light aged in the Down lighting apparatus, which emits a higher proportion of UV light than the Biltz lighting apparatus. This provided us with the opportunity to investigate the stabilizing capability of Tinuvin 292 in a dammar varnish. DTMS and GCMS were used for analysis. Figure 9 shows the DTMS TIC and the total summation spectrum of the Tinuvin 292 containing dammar varnish. The TIC shows a large peak around scan 53, which indicates that Tinuvin 292 does not inhibit the cross-linking processes. The DTMS spectrum is very complex, showing a large number of peaks. Some mass spectrometric markers, which are found in aged varnishes from paintings, such as the peaks at m/z 399 and 414, are present. GCMS was performed in order to identify the triterpenoid fraction (Figure 10). The labels used in Figure 10 correspond to those used in Table I and II. This sample consists of a large number of oxidised compounds. Oxidised triterpenoids such as those found in aged varnishes from paintings (**7**, **26**, **27** and **32**) are present. In addition, the A-ring oxidised triterpenoids (**35**, **36**, **37** and **39)** were identified. The previous section demonstrated that predominantly A-ring oxidised compounds are formed during

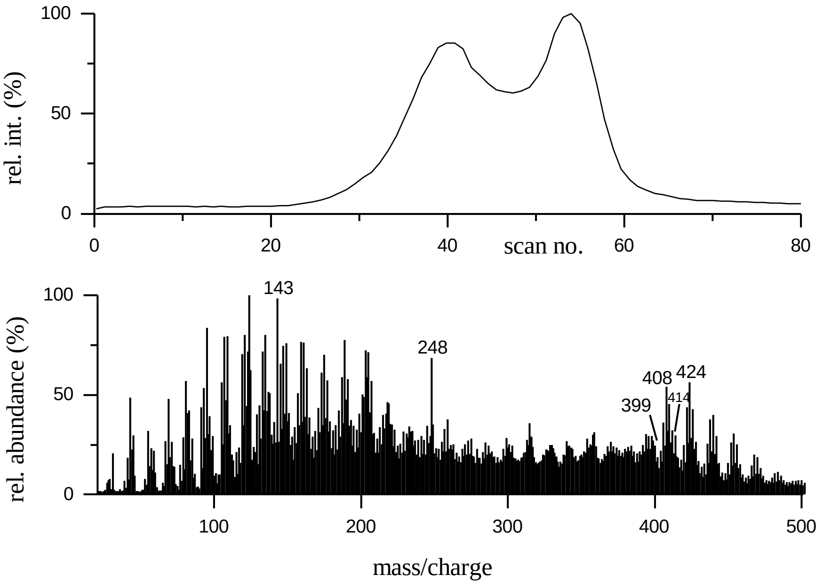

Figure 9 *The DTMS TIC and total summation spectrum (scan no. 20-60) of a Tinuvin 292 containing dammar varnish, which was light aged in the Down apparatus.*

xenon-arc light ageing with UV light present, instead of the oxidation products found in aged varnishes from paintings. During ageing in the Down apparatus (Figure 10), both the specific type of A-ring oxidation and the oxidation processes occurring on paintings are generated (see footnote in section 4.2.2.). For example, compound **40**, which has both an oxidised A-ring and a lactonised side chain, is a product of both types of oxidation reactions. This specific type of A-ring oxidation did not occur during light ageing in the Biltz apparatus although this machine has a substantially higher light level. The Down apparatus emits a higher proportion of UV light. Therefore it can be concluded that the presence of this UV light is the cause of the A-ring oxidation. Additional experiments have to be carried out to determine the exact effects of UV light of specific wavelengths on the oxidation of triterpenoid varnishes. As with xenon-arc light ageing in the presence of UV light [5], Tinuvin 292 is not capable of inhibiting the oxidation processes generated by light exposure in the Down apparatus (Figure 10).

Figure 11 shows the DTMS TIC and total summation spectrum of the corresponding light-protected control sample of the Tinuvin 292 containing dammar varnish. When the spectrum is compared to that of fresh dammar resin (Figure 1(c)), it is clear that oxidation and cross-linking reactions are completely blocked in the dark when Tinuvin 292 is present. As discussed before, without the addition of Tinuvin 292, oxidation reactions proceed in the dark but to a lower extent than when placed in the light (figure 8).

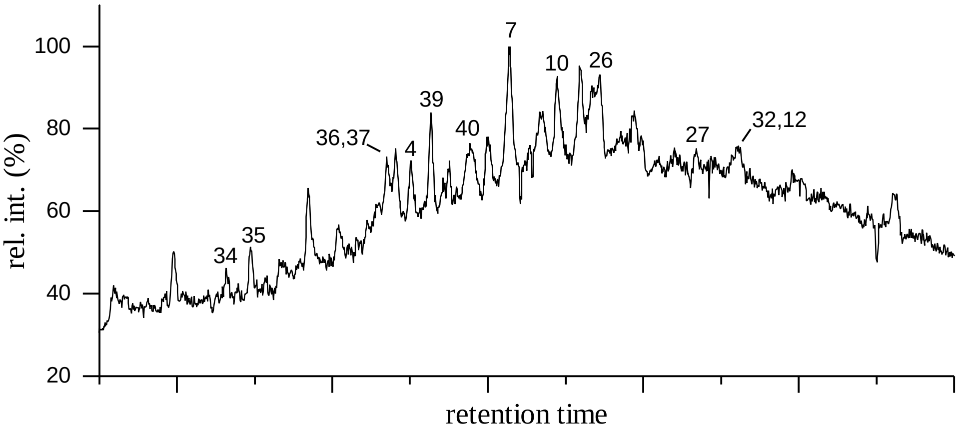

Figure 10 *Gas chromatogram of the Tinuvin 292 containing dammar varnish that was aged in the Down apparatus. Peak labels refer to those of Table I and II.*

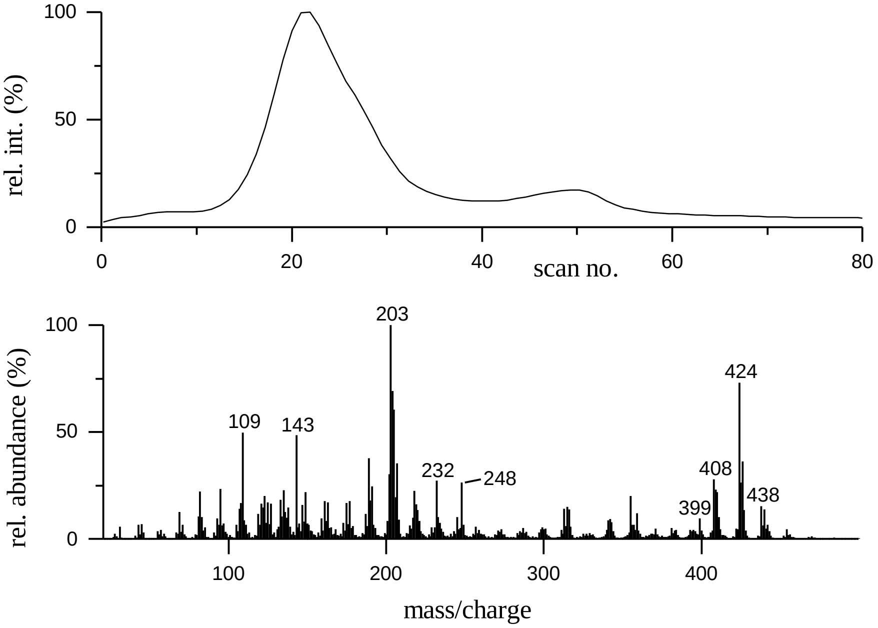

Figure 11 *The DTMS TIC and total summation spectrum (scan no. 10-60) of the light-protected control sample of the Tinuvin 292 containing dammar varnish.*

4.3.4. Effects of different methods of preparation of the varnish

A large number of varnishes were prepared at the CCI according to several recipes. A selection of these varnishes, which were aged in the Biltz apparatus [26] were analysed by DTMS [25]. DTMS indicated that ageing in the bottle for a year prior to varnish application did not seem to have any effect on the chemical composition of these varnishes after artificial light ageing. The chemical composition of a set of varnishes where the resin remained the same, but the solvents were varied (turpentine versus the modern solvents, mineral spirits and xylene), was almost identical, which indicates that the different solvents do not have a substantial effect on the type of oxidation processes that occur during ageing.

4.4. Conclusions

Four different light ageing conditions were applied for the artificial ageing of dammar and mastic varnish, in order to determine whether similar oxidation and cross-linking products are formed compared to those found in aged varnishes from paintings. The absence of UV light was found to be essential for the simulation of the molecular changes that were found to occur during ‘natural’ ageing. Analysis by DTMS and GCMS showed that two experimental ageing setups, a xenon-arc lighting device equipped with an additional UV filter (plexiglass) and the fluorescent tube lighting device (negligible UV) developed by Biltz et al. (CCI), can generate molecular ageing processes which simulate those of ‘natural’ ageing. The other two artificial light ageing setups, a xenon-arc lighting device without the additional UV filter and the fluorescent tube lighting device developed by Down (CCI), emit light with some UV light present. Irradiation by light with a UV component gives rise to the formation of A-ring oxidised compounds. This specific type of A-ring oxidation was not found to occur during ageing on paintings. Table II summarises which compounds are formed during these different methods of ageing. Both the A-ring oxidation and the oxidation reactions that are commonly found to take place on paintings occur during light ageing in the fluorescent tube lighting device developed by Down. Tinuvin 292 is not able to inhibit these oxidation processes generated by light exposure in the Down apparatus. Xenon-arc light ageing without the additional UV filter predominantly gives rise to A-ring oxidation. Extensive A-ring oxidation of triterpenoid varnishes will probably have an impact on the solubility and mechanical behaviour of the artificially aged varnishes. It is therefore advisable to exclude UV light for the artificial light ageing of triterpenoid varnishes. The exact effect of UV light of specific wavelengths on the oxidation processes in triterpenoid varnishes needs further research.

4.5. Experimental

4.5.1. Xenon-arc light ageing

The dammar and mastic resins used for xenon-arc light ageing were purchased from Kremer. The resins were dissolved in toluene (40 w/w%). Glass plates were used as substrates for the varnish films. An Atlas Ci65A xenon arc Weather-ometer with a borosilicate inner and soda lime outer filter glass was used. In some cases the UV component was removed from the light hitting the films by mounting Acrylite OP2 UV absorption filters (plexiglass) in front of the samples. The xenon arc output was controlled at 420 nm and was maintained at an irradiance of 0.90 W/m^2. Other settings were: black panel temperature 50-55°C, dry bulb temperature 30-35°C, relative humidity 44%. The dammar varnishes were aged for 370 hours both with or without the additional UV filter. The mastic varnish was aged for 600 hours with the additional UV filter. After ageing, samples were scraped from the glass plates.

4.5.2. Fluorescent tube light ageing

Dammar and mastic resin were purchased from O.J. Innes Corporation (New York, "Singapore No.1") and Cornelissen (London) respectively. Different recipes were used to prepare the varnishes. The dammar varnish, of which the results are depicted in Figures 6, 8 and 9, were prepared by dissolution in 50% xylene/50% mineral spirits (18% aromatic) (31 wt/vol% resin concentration). The Tinuvin 292 containing dammar samples (3% by weight of the resin) were prepared similarly. The dammar and mastic varnishes, of which the results are depicted in Figure 6(b) and 7, are prepared by dissolution in turpentine (31 wt/vol% resin concentration). These latter samples were aged in the bottle for one year. Glazed white ceramic tiles were used as substrates for the varnish films. Elemental analysis showed that there is no titanium white present in the tiles.

Both the Biltz apparatus and the Down apparatus were equipped with banks of fluorescent lights (40 W, Duro-Test #1157 Vita-lite). The experimental ageing conditions are described in the publications by Biltz et al. [26] and Down et al. [9]. The Tinuvin containing samples were aged for 7920 hours in the Down apparatus (750 Lux, 190 μW/l). The other samples were aged for 20184 hours in the Biltz apparatus (2300 lux, 15 μW/l). Following light exposure, all samples were stored in the dark for a few years (ambient temperature, normal atmosphere).

4.5.3. DTMS

A 12.8 ng/µl solution of the internal standard perylene (Aldrich, 99+%) in tetrahydrofuran was prepared. The xenon-arc light aged dammar varnishes and fresh dammar resin were subsequently dissolved in the internal standard solution (0.5 µg/µl). An aliquot of 2 µl was applied to the DTMS probe by using a syringe (SGE, 5 µl). About 50-100 µg of the fluorescent tube light aged varnishes was homogenised in approximately 100-200 µl of ethanol. An aliquot (about 2 µl) of the resulting suspensions was applied to the DTMS probe by using a syringe (SGE, 5 µl). DTMS analysis (16 eV) was performed as described in Chapter 2.

4.5.4. GCMS

The GCMS analysis of fresh resins and the aged varnishes is described in Chapter 2 and 3 respectively. For methylation, according to the method of Hashimoto et al. [27], aliquots of 250 µl of methanol, 25 µl of benzene and 10 µl of TMSdiazomethane were added to approximately 0.2 mg of the sample. This mixture was left at room temperature for 30 minutes. After evaporation to dryness, the sample was dissolved in 20 µl of dichloromethane (1 µl injection). On-column 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) 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 320 °C at a rate of 3 °C/min, at which temperature it remained for another 10 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), and accelerated to 10 kV. The mass spectra were interpreted and compared with spectra available in the literature.

Acknowledgements

Shell Research and Technology Centre (Amsterdam, The Netherlands), and the National Gallery of Art (Washington DC, USA) are acknowledged for providing the opportunity to use their HPLC-MS equipment.

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5. Artificial ageing of varnish triterpenoids in solution

Abstract

A new method is presented for the artificial ageing of triterpenoid samples. Varnish samples in solution are exposed to the radiation of a fluorescent tube device. The solvents dichloromethane and acetone, and the photosensitisers Merocyanine 540 and FotoFenton 2 dissolved in acetonitrile or dichloromethane were found to generate reactive species, which induce similar oxidation and cross-linking processes in the triterpenoid samples as found in varnish films on paintings. Information was obtained on the oxidation mechanisms of the model compounds hydroxydammarenone and oleanolic acid. Hydroxydammarenone is first oxidised to an ocotillone type molecule, which is subsequently oxidised to a lactonised molecule. In addition, a compound with a shortened side chain is formed, probably from the ocotillone type molecule. Oxidation of the A-ring was not observed. The solvent ageing of oleanolic acid first results in the formation of small amounts of compounds with additional keto groups at C3 and C11. In addition, the double bond at C12 of oleanolic acid becomes oxidised and a keto group is formed at this position. The compound formed by this latter process is the main compound after prolonged solvent ageing. Decarboxylation of the carboxylic acid at C17 of oleanolic acid was not observed. This method of solvent ageing can be successfully used for the preparation of cross-linked fractions of aged dammar and mastic varnishes, in addition to the investigation of the oxidation mechanism of triterpenoids.

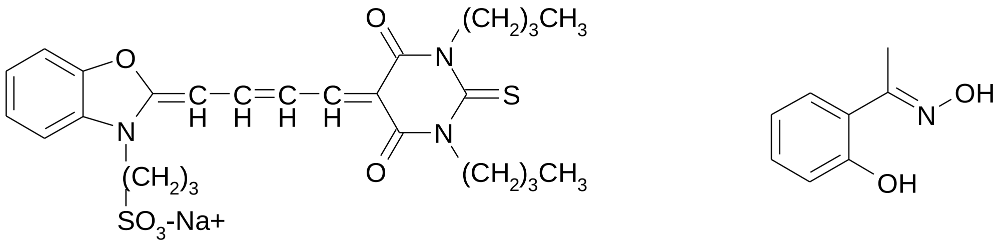
5.1. Introduction

Dammar and mastic are complex mixtures of a large number of triterpenoids with different carbon skeletons. As discussed in Chapter 2, both fresh dammar and mastic resin consist of more than 10 triterpenoid compounds. Films of varnishes subjected to either ‘natural’ or artificial ageing, see Chapter 3 and 4, result in even more complex mixtures due to oxidation and cross-linking processes. Especially side chain oxidation of dammarane type molecules and oxidation of C11, C17 and C28 of oleanane/ursane type molecules was found to take place. However, it is difficult to assess the exact oxidation mechanisms of the triterpenoid constituents by investigation of the chemical composition of ‘naturally’ or artificially aged varnishes. It is more straightforward to model the oxidation processes, which are found to take place in paintings, by subjecting a single pure triterpenoid compound to these ‘ageing’ processes. Naturally, one would like to age the model compound as a film, because a varnish ages on a painting as a film as well. However, unlike a fresh resin solution, the solution of one pure compound in a solvent does not produce a homogeneous solid film. The addition of compounds with good film forming properties, such as an acrylate, may improve this, but this addition may complicate the investigation of the ageing processes. Therefore, a new approach was developed to investigate the ageing of triterpenoids in a dissolved state.

Specific types of reactive species can be generated in solution, which initiate and generate ageing reactions in triterpenoid solutions. Therefore, this approach allows for more control over the modelling experiments. The reagents can move freely in the solution, unlimited by low diffusion rates encountered in solid films. Therefore, the rate of the ageing reactions in the solution is no doubt higher than that in a solid film. By ageing in solution, other disadvantages related to the ageing of films are avoided. For example, the comparison of films is complicated when their surfaces are not constant. Subsequently the availability of oxygen and light energy is not constant. Another disadvantage is the observation that photochemical damage is largely a surface phenomenon [1], which implies that an aged resin film is not uniform in chemical composition.

Ageing processes are thought to occur via radical reactions. Photochemical methods, which generate a large number of radicals or other reactive species in the reagent solution, were investigated for their capability to ‘artificially age’ triterpenoids. Photosensitisers can be used for this purpose, which are compounds that create these reactive species in the presence of light. The photosensitiser absorbs at the irradiating wavelength. It may subsequently transfer its energy to either a substrate or to molecular oxygen. Furthermore, it may exchange an electron or hydrogen atom with the substrate or exchange an electron with oxygen. Depending on the type of photosensitiser used, singlet oxygen, superoxide anion, OH radicals and alkyl radicals can be produced [2]. The advantage of these photochemical methods is that specific radicals may be produced in high yield. Induction is strictly dependent on the dose of radiation and stops immediately once irradiation is ended [2]. Two types of photosensitisation reactions exist: radical photosensitised oxidation (type I) and the oxidation by singlet oxygen (type II). Polyunsaturated fatty acids and cholesterol give rise to characteristic peroxidation products depending on the type of photosensitisation reaction [3, 4]. This dependence on type of photosensitisation reaction may also apply to the peroxidation of triterpenoids.

Photosensitisers are mainly used in the biomedical research in order to study peroxidation processes. Systems such as membranes or whole cells contain numerous substances that may act as a photosensitiser. A number of photosensitisers are ketones. A solvent such as acetone can therefore act as a photosensitiser. We investigated a number of solvents for their capability of generating ageing processes in triterpenoids. In addition, two commercially available photosensitisers with different actions were tested: Merocyanine 540 (MC540) and FotoFenton 2 (FF2) (2-hydroxyacetophenone oxime) (figure 1). These two specific photosensitisers were chosen, because of their solubility in organic solvents. MC540 is a photosensitizing dye of great therapeutic interest [5, 6]. MC540 lacks specificity, because it is reported to produce singlet oxygen in addition to other reactive oxygen species, including oxygen radicals, thus giving rise to both type I and type II reactions [6-8]. FF2 resembles the hydroxyl radical formation of Fenton's reagent [2], but produces fewer side products. FF2 generates hydroxyl radicals by photochemical cleavage of the photosensitiser [9]. This method of solvent ageing was used to gain insight into the oxidation and cross-linking mechanisms of triterpenoids. By using relatively large amounts of these photosensitisers and applying relatively long exposure times, we investigated whether this photochemical approach could simulate the natural ageing process of triterpenoid samples as seen on paintings.



MC 540 FF2

Figure 1 *Molecular structure of Merocyanine 540 (MC540) and FotoFenton 2 (FF2).*

Two pure triterpenoid compounds, hydroxydammarenone and oleanolic acid, were solvent aged. Hydroxydammarenone is abundant in fresh dammar and mastic resin. Oleanolic acid itself is not abundantly present in the fresh resins, but its molecular structure is very similar to that of oleanonic acid. Sample solutions were light exposed in a closed system, a jar containing a lid. The lid was necessary, because of the rapid evaporation of the organic solvents suitable for dissolving triterpenoids. Therefore, the amount of oxygen decreases during the reaction period, which complicates the investigation of the reaction kinetics. In order to reduce this problem, we used small volumes of reaction products in relatively large vials, thereby creating a large oxygen to reagent ratio. For future experiments, an ageing device should be constructed, which allows the irradiation of solutions under constant oxygen pressure.

For the current experiments, the solutions were light exposed in a fluorescent tube device (13,000-13,500 lux), which was constructed by R. Hoppenbrouwers of the Stichting Restauratie Atelier Limburg (SRAL). UV/VIS radiation is emitted with a wavelength range from approximately 850 nm down to about 305 nm. The chemical composition of the aged solutions was determined with direct temperature-resolved mass spectrometry (DTMS), gas chromatography mass spectrometry (GCMS) and size exclusion chromatography (SEC) coupled with a diode array UV/VIS detector. This chapter first describes the effect of a number of different solvents on the light ageing of hydroxydammarenone. Second, the effect of the two photosensitisers on the solvent ageing of hydroxydammarenone and oleanolic acid is investigated. Third, it is investigated whether the solvent ageing of fresh dammar and mastic simulates the ageing processes as found on paintings. Finally, some observations concerning cross-linking processes are described.

5.2. *Effect of solvents*

In order to test whether certain solvents are capable of inducing changes in a reagent during light exposure, hydroxydammarenone was dissolved in six different solvents and light exposed for two weeks. The solvents methanol, toluene, acetonitrile (ACN), ethanol, dichloromethane (DCM) and acetone were tested. Figure 2 shows the DTMS summation spectra of pure hydroxydammarenone (a) and of hydroxydammarenone after two weeks of exposure in ethanol (b), acetone (c) and DCM (d). As will be described in Chapter 6, hydroxydammarenone is represented by peaks at m/z 109, m/z 205, m/z 355 and m/z 424. When hydroxydammarenone was dissolved in methanol, ethanol, toluene and ACN, no molecular changes were induced during the two weeks of light

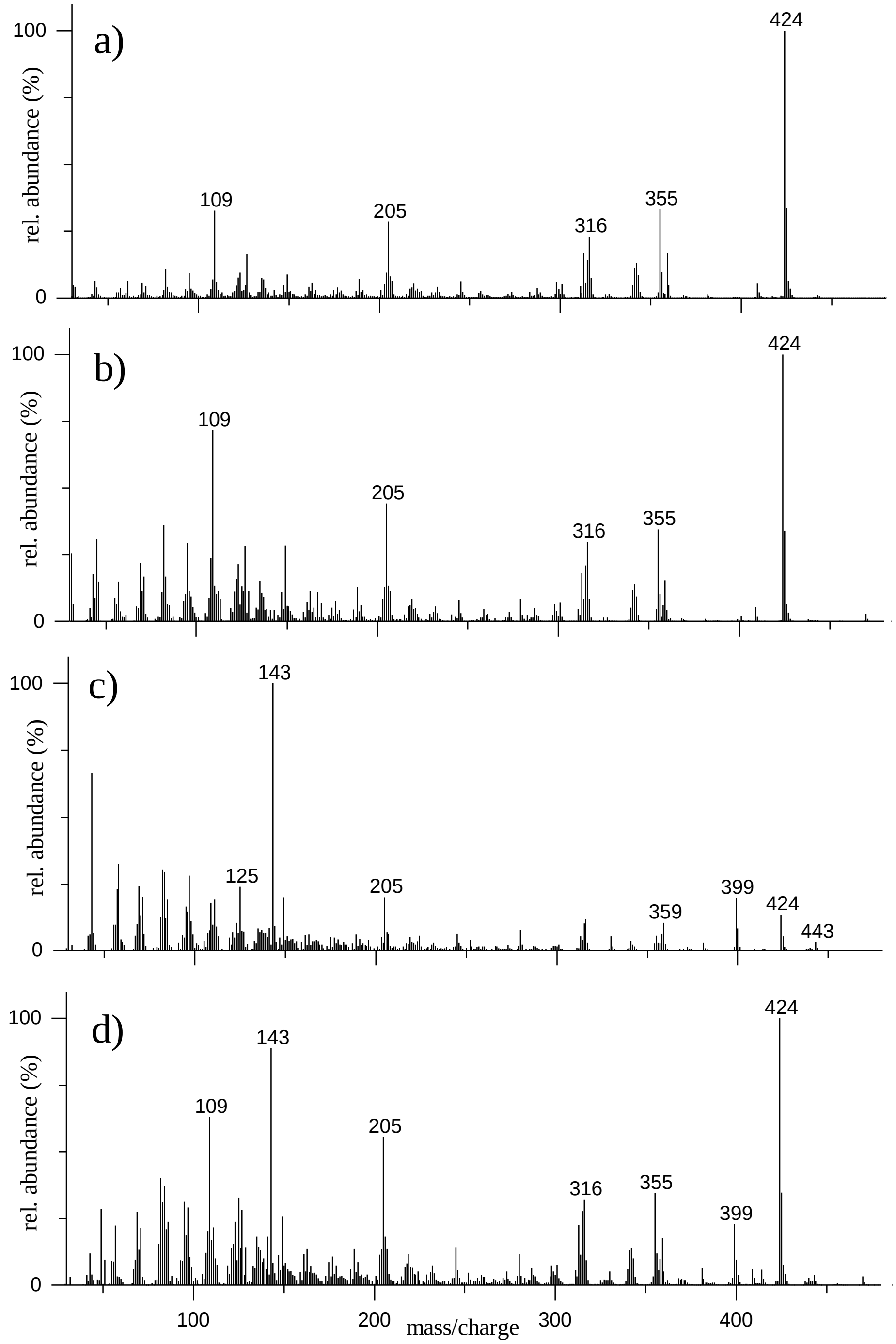


Figure 2 *DTMS summation spectra of pure hydroxydammarenone (a) and hydroxydammarenone after two weeks of solvent ageing in ethanol (b), DCM (c), or acetone (d).*

exposure, as shown for ethanol in Figure 2(b). Rao et al. [10] demonstrated that the dammarane type molecule, cabraleone, is A-ring oxidised when a solution of this triterpenoid compound in methanol is irradiated by UV light. This A-ring oxidation is not observed in our own experiments, in which a methanol or ethanol solution of hydroxydammarenone was light exposed for two weeks. This implies that in our experimental setup the A-ring oxidation pathway is not accessible, probably due to very low UV light levels. Exposure in acetone and DCM resulted in major chemical changes as seen in Figure 2(c) and (d). Figure 2(c) shows that the characteristic mass peaks of hydroxydammarenone have almost disappeared and a number of new peaks at m/z 143, m/z 399 and m/z 443 are present. As described in Chapter 6, these peaks are characteristic of an ocotillone type molecule. This oxidised triterpenoid is usually found as the main triterpenoid constituent of aged dammar varnishes from paintings, as described in Chapter 3. A similar kind of side chain oxidation could also be achieved by Bielmann [11] after treatment with p-nitroperbenzoic acid in ether. Figure 2(d) shows that some hydroxydammarenone is still present in addition to a certain amount of ocotillone type molecules after exposure in DCM. Compared to the irradiated hydroxydammarenone in acetone, exposure in DCM results in a lower degree of oxidation.

How can we rationalise the oxidising abilities of acetone and dichloromethane? Acetone is known to act as a photosensitiser due to its keto group. Indig et al. [12] demonstrated that acetone can be optically excited by a mercury lamp, forming triplet acetone. Carbonyl compounds after excitation can remove a hydrogen atom from a substrate giving rise to an alkyl radical [2]. Another ketone, benzophenone, is often used for the sensitised oxidation of lipids [13, 14]. Acetone absorbs UV light with a wavelength up to 350 nm [15]. The fluorescent tube device used for light exposure emits radiation with a wavelength down to about 305 nm. The borosilicate glass sample vials absorb UV radiation with wavelengths below 315 nm, but transmit UV radiation with longer wavelengths [1]. Therefore, acetone directly absorbs the UV radiation, acts as a photosensitiser and probably oxidises the triterpenoid sample.

The action of DCM is not entirely clear. DCM absorbs UV light below 240 nm [15]. As mentioned above, the sample solution is not irradiated with light of this wavelength. However, triterpenoids usually contain keto groups and in some cases aldehyde groups. These functional groups absorb UV radiation at relatively high wavelengths (270-300 nm), which results in an $n \rightarrow p^*$ transition [16]. Hydroxydammarenone was found to have an absorption band between 260 and 330 nm. The triterpenoid itself can therefore absorb UV light during light exposure and subsequently abstract a hydrogen atom from a substrate. It is inferred that DCM is a good substrate for this hydrogen abstraction, which gives rise to radical formation. The resulting $CHCl_2^\bullet$ radical may subsequently expel a chlorine radical forming a carbene (:CHCl). A large number of radicals can be formed via this mechanism, which may be responsible for the oxidation of hydroxydammarenone in DCM during irradiation. This hypothesis needs further investigation.

5.3. Effect of the addition of photosensitisers

Photosensitisers added to the reagent solution can generate specific types of radicals or reactive oxygen species when irradiated by light. This could give more control on the reaction process, because certain photosensitisers only generate one specific type of reactive oxygen species or radical species [2]. It was investigated whether two specific photosensitisers, Merocyanine 540 (MC540) and FotoFenton 2 (FF2), could induce changes in hydroxydammarenone when irradiated by light.

Hydroxydammarenone and a photosensitiser were dissolved in ACN, because ACN was found to be a 'non-active' solvent during irradiation. Figure 3 shows the DTMS summation spectra of hydroxydammarenone in ACN (a), in ACN with FF2 (b), and in ACN with MC540 (c) after an exposure period of two weeks. Both photosensitisers are observed to induce changes in hydroxydammarenone. Figure 3(b) shows that a certain amount of hydroxydammarenone is still present, deduced from the peak at m/z 424. In addition, peaks at m/z 143 and m/z 399 point to the formation of an ocotillone type molecule. A peak at m/z 414 is known to represent the oxidised triterpenoid with a lactonised side chain, as described in Chapter 3. This compound could also be produced by Mills et al. by oxidation of hydroxydammarenone with chromic acid [17]. A number of peaks are present, which could not be assigned yet, such as m/z 82, 125, 315 and 359. GCMS analysis showed that these peaks represent trace compounds, which could not be identified by their 70 eV mass spectra. It is possible that the DTMS peak at m/z 125 represents a compound with a dehydrated ocotillone type side chain. The molecular ion region of the DTMS spectrum depicted in Figure 3(c) mainly shows peaks that represent the lactonised molecule and ocotillone type molecules. In addition, a peak at m/z 424 representing hydroxydammarenone is not present at all in Figure 3(c), which indicates that MC540 induced relatively more changes than FF2. The DTMS spectra of Figure 3(b) and 3(c) show similar peaks, which indicates that MC540 and FF2 probably induce similar oxidation reactions, independent of the type of reactive species generated.

In order to get information about the oxidation mechanism of hydroxydammarenone, it was irradiated in ACN with FF2 for one week and two weeks. GCMS (figure 4 and Table I) demonstrates that after one week of irradiation hydroxydammarenone (**8**) is partly oxidised to an ocotillone type molecule (**7**). Small amounts of other compounds are present of which only a molecule with a shortened side chain (**25**) and the molecule with the lactonised side chain (**26**)

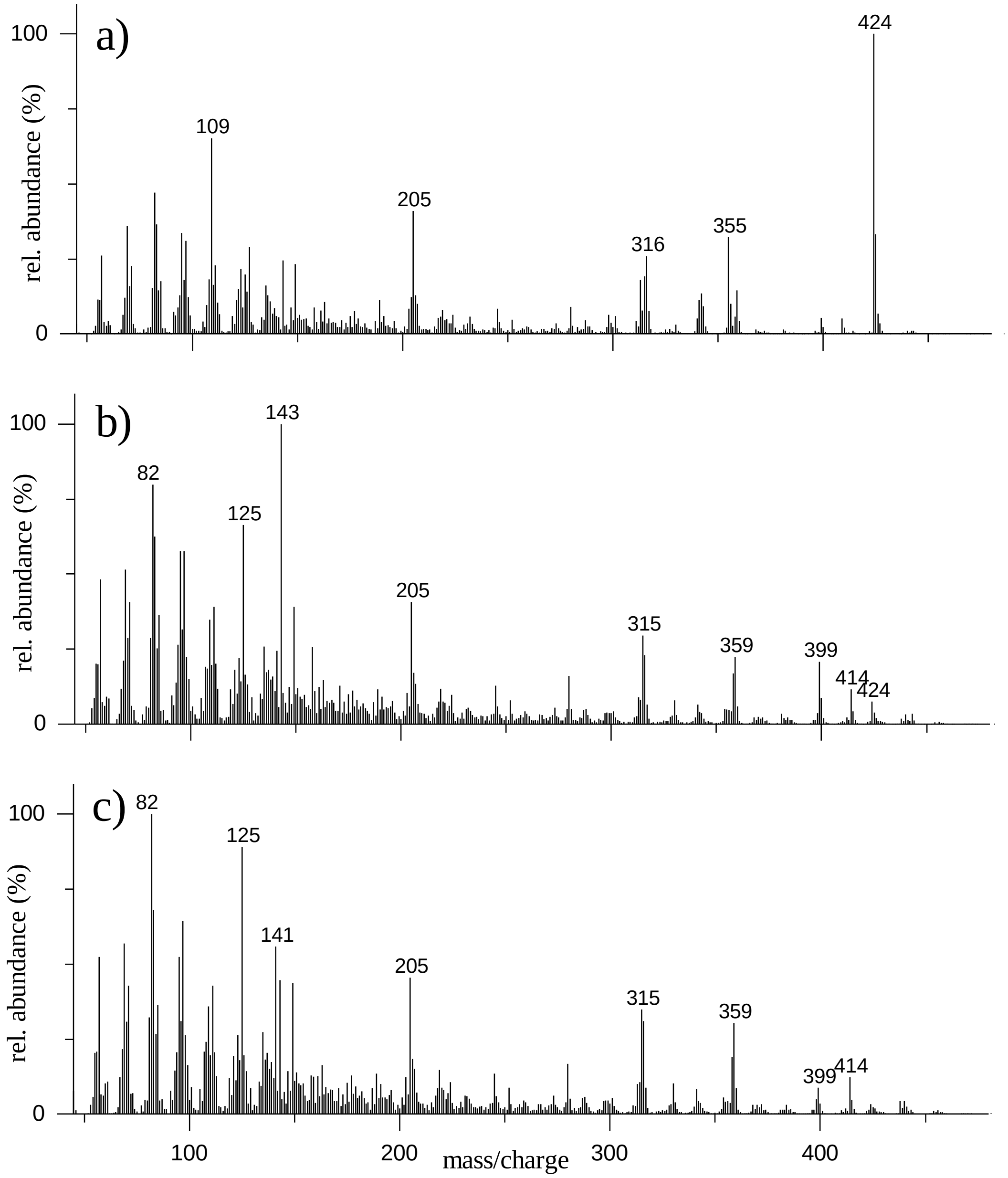


Figure 3 *DTMS summation spectra of hydroxydammarenone in ACN after two weeks of solvent ageing without a photosensitiser (a), with FF2 (b), or with MC540 (c).*

could be identified. After two weeks of irradiation, almost all of the hydroxydammarenone has been oxidised to mainly the same three compounds as just mentioned. The relative distribution of these compounds has changed. The molecule with the lactonised side chain (**26**) seems to have been formed mainly during the second week of irradiation. Probably it is formed by oxidation of the ocotillone type molecule (**7**) shown in Figure 5. The compound with the shortened side chain (**25**) is probably formed directly from hydroxydammarenone (**8**).

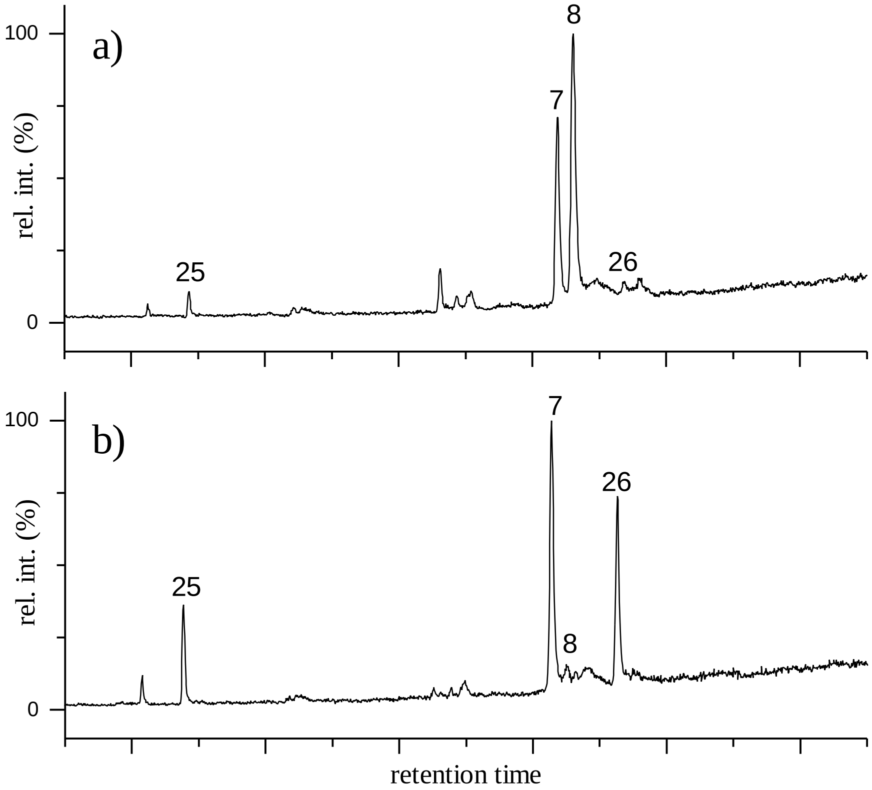


Figure 4 *Gas chromatograms of hydroxydammarenone and FF2 in ACN after one week (a), or two weeks (b) of solvent ageing.*

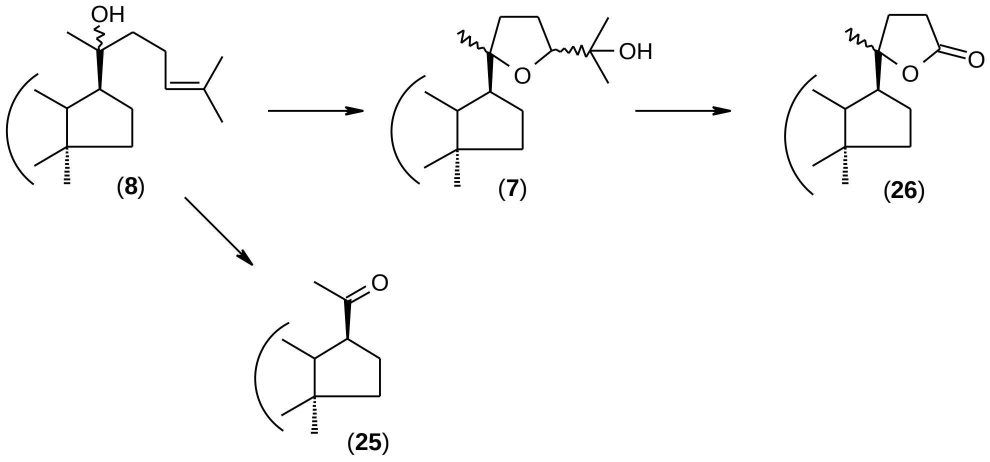


Figure 5 *Proposed oxidation mechanism of hydroxydammarenone.*

Table I *List of compounds, with their corresponding molecular weights (MW), identified in triterpenoid samples. Peak labels correspond to those used in other figures of this thesis.*

Label	Compound name	MW
1	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424
2	Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)	410
3	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426
4	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)dammaren-3-oic acid[1]	474
5	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[2])	458
6	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454
7	20,24-Epoxy-25-hydroxy-dammaran-3-one[1]	458
8	Hydroxydammarenone (20-hydroxy-24-dammaren-3-one[2])	442
9	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438
10	Ursonic acid (3-oxo-12-ursen-28-oic acid)	454
11	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438
12	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442
13	(8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene	442
14	Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)	410
15	β-Amyrone (3-oxo-olean-12-ene)	424
16	(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene	444
17	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454
18	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	454
19	Idem	454
20	3-O-Acetyl-3epi(iso)masticadienolic acid (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	498
21	Idem	498
22	Dammarenediol (20-dammar-24-ene-3β,20-diol[2])	444
23	Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)	440
24	Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)	440
25	Hexakisnor-dammaran-3,20-dione	358
26	3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone[2]	414
27	11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)	468
28	Oxidised oleanane type molecule	?
29	20,24-Epoxy-25-hydroxy-dammaran-3-ol[1]	460
30	17-Hydroxy-11-oxo-nor-β-amyrone (3,11-dioxo-17-hydroxy-28-nor-olean-12-ene)	440
31	17-Hydroxy-11-oxo-nor-α-amyrone (3,11-dioxo-17-hydroxy-28-nor-urs-12-ene)	440
32	11-Oxo-ursonic acid (3,11-dioxo-urs-12-en-28-oic acid)	468
33	Oxidised ursane type molecule	?
34	3,4-seco-28-nor-olean-12-en-3-oic acid	428
35	3,4-seco-28-nor-urs-12-en-3-oic acid	428
36	3,4-seco-olean-12-en-3,28-dioic acid	472
37	20,24-Epoxy-25-hydroxy-3,4-seco-dammaran-3-oic acid[1]	476
38	Dihydro-dammarenolic acid (20-hydroxy-3,4-seco-24-dammaren-3-oic acid[2])	460
39	3,4-seco-urs-12-en-3,28-dioic acid	472
40	3,4-seco-2-carboxy-25,26,27-trisnor-dammarano-24,20-lactone[2]	432
41	3,4-seco-2-carboxy-25,26,27-trisnor-4(28)-dammareno-24,20-lactone[2]	430
42	3,12-dioxo-olean-28-oic acid	470
43	Oleanolic acid (3-hydroxy-olean-12-en-28-oic acid)	456
44	11-Oxo-oleanolic acid (3-hydroxy-11-oxo-olean-12-en-28-oic acid)	470
45	3-hydroxy-12-oxo-olean-28-oic acid	472

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[1] The configuration at C-20 and C-24 was not determined.

[2] The configuration at C-20 was not determined.

Fresh dammar resin and aged dammar varnishes both contain compounds with an oxidised A-ring, which contains a carboxylic acid group at C2 and an isopropenyl group at C5 (**4** and **5**) (Chapter 2 and 3). These compounds are probably formed by oxidation of triterpenoid compounds with a keto group at C3. However, it is not known whether this type of A-ring oxidation takes place during biosynthesis by enzymatic action, after excretion, or afterwards as a result of ageing on a painting. The model compound hydroxydammarenone, used for the solvent ageing experiments, contains a keto group on C3 and is therefore a good precursor for the A-ring oxidation. GCMS demonstrates that this A-ring oxidation does not take place during solvent ageing (Figure 4). In addition, the majority of the oxidised triterpenoids found in aged varnishes from paintings contains a keto group at C3 (Chapter 3), which implies that this A-ring oxidation is not likely to take place during ageing on paintings. The oxidised A-ring is probably formed during biosynthesis of the resin in the tree and therefore tree dependent, or formed during exposure to the relatively harsh outdoor conditions after exudation of the resin from the tree.

After establishment of the effect of photosensitisers in the ‘nonactive’ solvent ACN, it was investigated whether an additional photosensitiser effects the rate and the type of oxidation reactions that occur during light exposure in the ‘active’ solvent DCM. Figure 6 shows the gas chromatograms of hydroxydammarenone in DCM (a), in DCM with FF2 (b), and in DCM with MC540 (c) after an exposure period of two weeks. Table I lists the compounds identified. Figure 6(a) shows that hydroxydammarenone (**8**) is still the main compound, in addition to a small amount of an ocotillone type molecule (**7**), after irradiation in DCM, which indicates a small degree of oxidation. The presence of FF2 and MC540 clearly has an effect on the rate of the oxidation reactions. Figure 6(b) shows that hydroxydammarenone has been oxidised to an ocotillone type molecule (**7**), a lactonised molecule (**26**) and a molecule with a shortened side chain (**25**) in the presence of FF2. After exposure in the presence of MC540 (Figure 6(c), only the lactonised molecule (**26**) and the molecule with a shortened side chain (**25**) are present. As described above, the molecule with the lactonised side chain (**26**) is probably formed via oxidation of the ocotillone type molecule (**7**). Therefore, it can be concluded that MC540 has accelerated the oxidation reactions to a higher degree than FF2. It can also be concluded that the different combinations of solvents and photosensitisers used for the oxidation of hydroxydammarenone result in the same oxidation processes, as depicted in Figure 5. Another important observation was the fact that light is necessary for induction of the molecular changes. Storage in the dark at room temperature of a triterpenoid

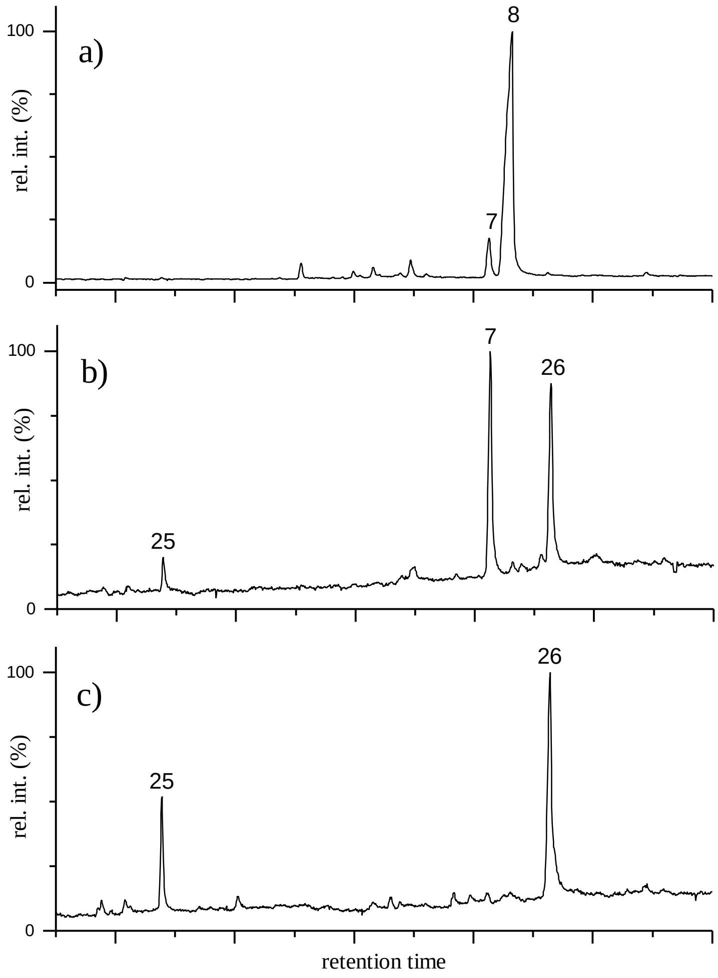

Figure 6 *Gas chromatograms of hydroxydammarenone in DCM after two weeks of solvent ageing without a photosensitiser (a), with FF2 (b), or with MC540 (c).*

solution in DCM or acetone with or without an additional photosensitiser did not result in triterpenoid oxidation.

Fresh dammar and mastic resin also contain molecules with other carbon skeleton types, such as the isomeric oleanane/ursane skeletons. Oleanolic acid was used as the second model compound. Figure 7 shows the gas chromatograms of oleanolic acid after exposure in DCM with FF2 for one week (a) and two weeks (b). Table I lists the compounds identified. After one week of solvent ageing, oleanolic acid (**43**) is still the main compound with some traces of oxidised compounds. Small amounts of compounds with additional keto groups at C3 (**6**) and C11 (**44**) are formed (figure 8). In addition, a compound is formed which is represented by peaks at m/z 235, 263 and 470 (compound is not labeled). This unidentified compound is probably related to the unidentified oleanane type

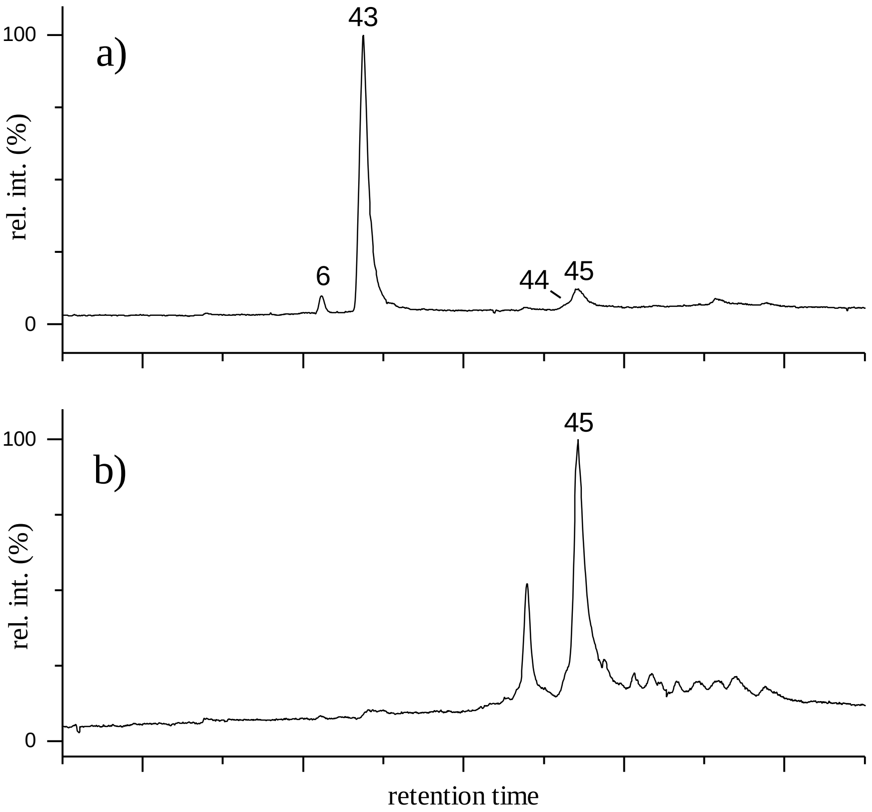

Figure 7 Gas chromatograms of oleanolic acid and FF2 in DCM after one week (a), or two weeks (b) of solvent ageing.

molecule (**28**), which is often found in aged varnishes from paintings and represented by peaks at m/z 233, 263 and 468. The difference between these two unidentified compounds is probably the presence of the keto or hydroxyl group on C3. Another compound was formed after one week of ageing (**45**), which became the main compound after two weeks of ageing. The double bond at C12 of oleanolic acid (**43**) becomes oxidised and a keto group is formed at this position (figure 8). This compound has only been found in traces in aged varnishes from paintings. Therefore, two weeks of ageing of oleanolic acid in DCM with FF2 results in the formation of compounds that are oxidised to a higher degree than those found in aged varnishes from paintings.

Fresh dammar resin and aged dammar varnishes both contain compounds with a noroleanane or norursane skeleton (**2** and **14**) (Chapter 2 and 3). These compounds are oxidised during ageing on the painting to compounds **30** and **31** (Chapter 3). The noroleanane/norursane type molecules are reported to be formed by decarboxylation of compounds with a carboxylic acid at C17 [18]. However, it is not known whether this decarboxylation process takes place during biosynthesis

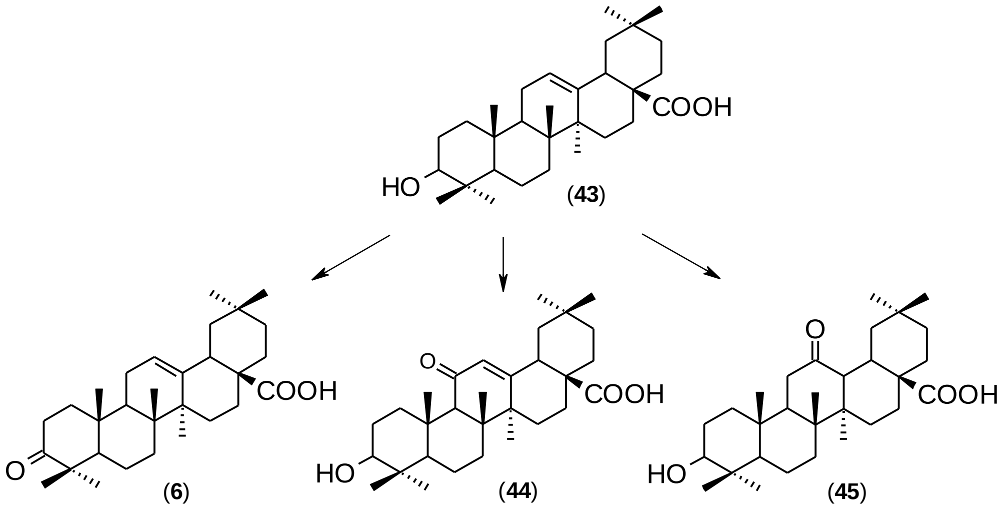

Figure 8 *Proposed oxidation mechanism of oleanolic acid.*

by the action of enzymes, after excretion, or afterwards as a result of ageing on a painting. The model compound oleanolic acid, used for the solvent ageing experiments, contains a carboxylic acid group on C17 and is therefore a good precursor compound to study whether decarboxylation can be induced. GCMS demonstrates that the 28-nor-derivative of the oleanane compound was not formed during solvent ageing (Figure 7), which implies that the carboxylic acid group at C17 is strongly bound in oleanolic acid. In addition, the majority of the oxidised oleanane/ursane type triterpenoids found in aged varnishes form paintings contains a carboxylic acid group at C17 (Chapter 3), which implies that decarboxylation is not likely to take place during ageing on paintings. The noroleanane/norursane type molecules are probably formed during biosynthesis of the resin in the tree and are therefore tree dependent, or formed during exposure to the relatively harsh outdoor conditions after exudation of the resin from the tree.

5.4. Solvent ageing of dammar and mastic resin

The application of solvent ageing to hydroxydammarenone and oleanolic acid by combinations of solvents and photosensitisers was described above. Similar dammarane and oleanane type oxidation products were formed as were found in aged varnishes from paintings. The conclusive test, for investigating whether this solvent ageing method simulates the ageing processes as found on paintings, is to subject dammar and mastic resin to this ageing method. Of all six solvents tested, only DCM was able to dissolve the complete resins due to the polymeric fraction present in both resins. Therefore, dammar and mastic were irradiated in DCM with FF2 for one and two weeks. Figure 9 (dammar) and 10

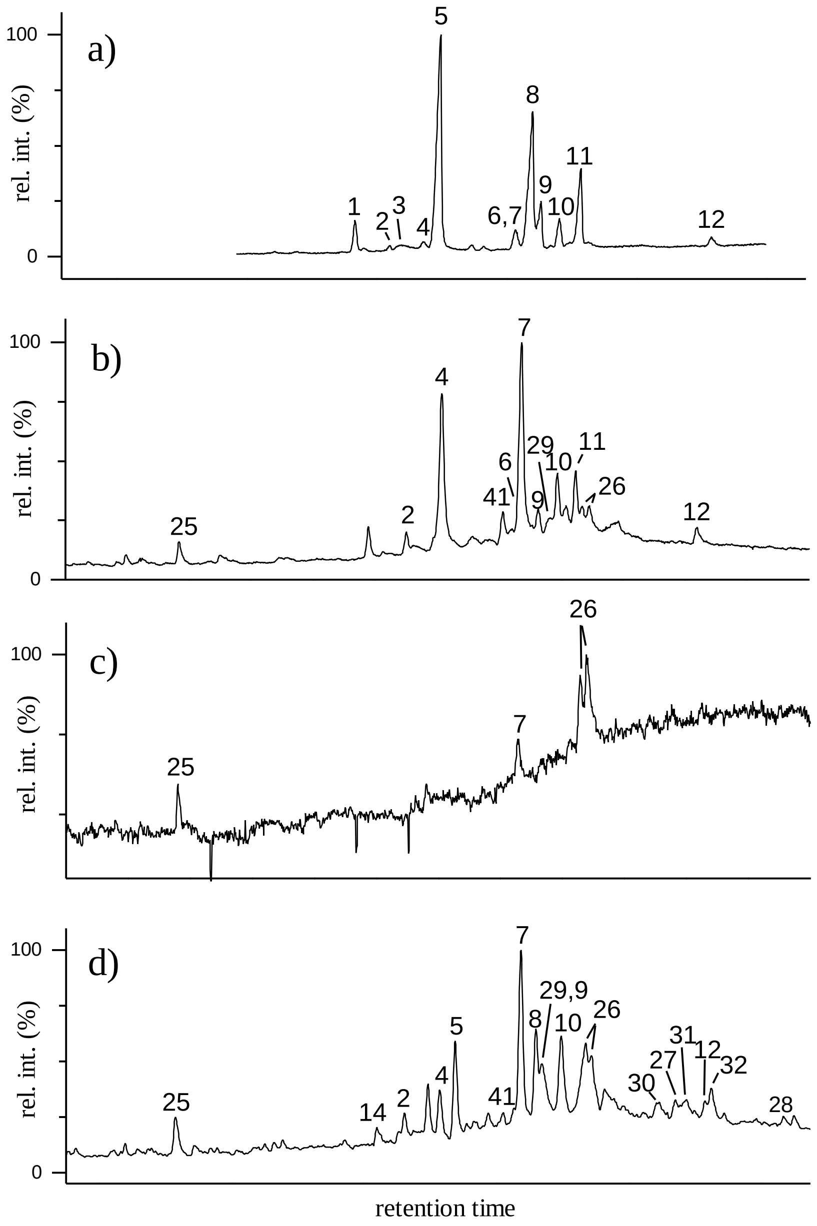

Figure 9 *Gas chromatograms of fresh dammar (a), dammar with FF2 in DCM after one week (b) or two weeks (c) of solvent ageing, and the gas chromatogram of a dammar varnish aged on a painting (d).*

(mastic) depict the gas chromatograms of the fresh resins (a), after solvent ageing for one week (b) and two weeks (c). For comparison the gas chromatograms of an aged dammar and mastic varnish from a painting are shown (Figure 9(d) and 10(d)). Table I lists the compounds identified. The dammar and mastic solutions had turned yellow after only one week of solvent ageing. This colour aspect will be dealt with below. A number of changes are apparent when comparing the chromatograms of fresh dammar (Figure 9(a)) and of the one week solvent aged dammar (Figure 9(b)). Mainly side chain oxidation of the dammarane type molecules has taken place, resulting in the oxidation of compounds **1**, **5** and **8** to compounds **4**, **7**, **25**, **26**, **29** and **41**. Compound **41** has been found only in trace amounts in aged dammar varnishes from paintings. Part of the oleanane/ursane type compounds with the aldehyde side chain (**9** and **11**) is oxidised to compounds with an acid group (**6** and **10**) as deduced from the peak ratios of these compounds. These modifications were also found to take place during ageing of varnishes on paintings (Figure 9(d)), as described in Chapter 3. As seen from the signal to noise ratio, the dammar sample that was solvent aged for two weeks (Figure 9(c)) contains only traces of triterpenoid compounds, as will be described below. Only some highly oxidised dammarane type molecules (**7**, **25** and **26**) are detected by GCMS, because dammarane type compounds are the major constituents of fresh dammar resin (Figure 9(a)).

The dammar solution that was exposed for 1 week resembles the painting varnish most, when the gas chromatograms of the solvent aged and naturally aged varnish are compared. The dammar solution that was exposed for two weeks (Figure 9(c)) contains a relatively higher amount of the dammarane type compound with the lactonised side chain (**26**) than of the dammarane type compound with the ocotillone side chain (**7**). More than 10 aged dammar varnishes from paintings have been analysed by GCMS (Chapter 3). In all cases, the lactonised compound was less abundant than the ocotillone type compound. Consequently, two weeks of solvent ageing results in a dammar sample that is relatively more oxidised than the aged dammar varnishes that are usually found on paintings. A number of oxidised oleanane/ursane type molecules, such as **27**, **28**, **30**, **31** and **32**, are usually present in small amounts in aged varnishes from paintings (Figure 9(d)). These compounds are not observed in the gas chromatograms of the solvent aged samples (Figure 9(b) and 9(c)). It is possible that these oxidised compounds are formed some time after one week, but formed in amounts below the detection limit and therefore not present in Figure 9(c). Only dammarane type compounds are present in Figure 9(c), because oleanane/ursane type molecules are much less abundant than dammarane type molecules in dammar resin (Chapter 2). Another explanation, for the absence of the oxidised oleanane/ursane type compounds in the solvent aged samples, is the possibility that the oxidation of dammarane type molecules is relatively more accelerated than the oleanane/ursane type molecules during solvent ageing. Possibly the activation energy of the oxidation reactions of the oleanane/ursane compounds is relatively high.

After one week of solvent ageing of mastic resin (Figure 10(b)), the relative amount of a large number of compounds has decreased (**8**, **9**, **13**, **18**, **19**, **20** and **21**). Strikingly, all of these compounds, except **9**, have a tetracyclic euphane or

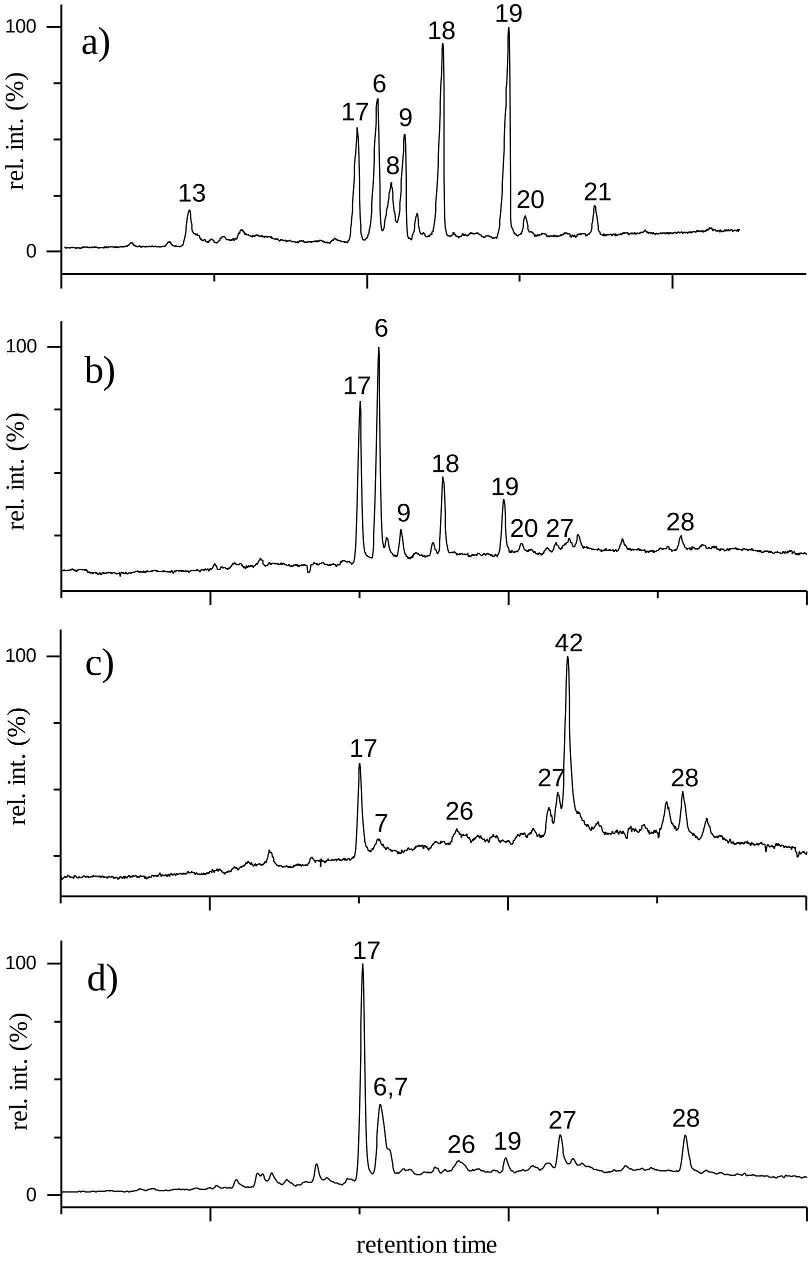

Figure 10 *Gas chromatograms of fresh mastic (a), mastic with FF2 in DCM after one week (b) or two weeks (c) of solvent ageing, and the gas chromatogram of a dammar varnish aged on a painting (d).*

dammarane structure (or a bicyclic structure (**13**)). Two compounds, **6** and **17**, were found to be relatively stable after one week of ageing and traces of oxidised oleanane type molecules are formed (**27** and **28**). Oxidised compounds with a tetracyclic structure are not formed. The fate of these tetracyclic structures is not known. Processes such as degradation, cross-linking or isomerisation may give rise to compounds with other cyclic structures. After two weeks of solvent ageing

(Figure 10(c)) the only compound left of fresh mastic resin is moronic acid (**17**), which is confirmed to be a very stable compound and a useful marker for aged mastic varnish [19]. Oxidised compounds with the dammarane (**7** and **26**) or oleanane skeleton (**27** and **28**) are present. A main constituent becomes the oxidised oleanane type molecule (**42**), which is found only in trace amounts in aged varnishes from paintings (Figure 10(d), not labeled). One week of solvent ageing of mastic resembles the aged mastic varnish from a painting best, as is the case with the solvent ageing of dammar resin. Two weeks of solvent ageing results in a chemical composition of the resin that is relatively more oxidised than the aged varnishes that are usually found on paintings.

5.5. Cross-linked fractions in solvent aged resins

Earlier it was noted that the signal to noise ratio of the gas chromatograms of dammar resin in particular decreased during solvent ageing. This phenomenon has also been observed in aged varnishes from paintings (Chapter 3) and is probably caused by cross-linking processes. DTMS was used to obtain additional information on the occurrence of these cross-linking processes. Figure 11 and 12

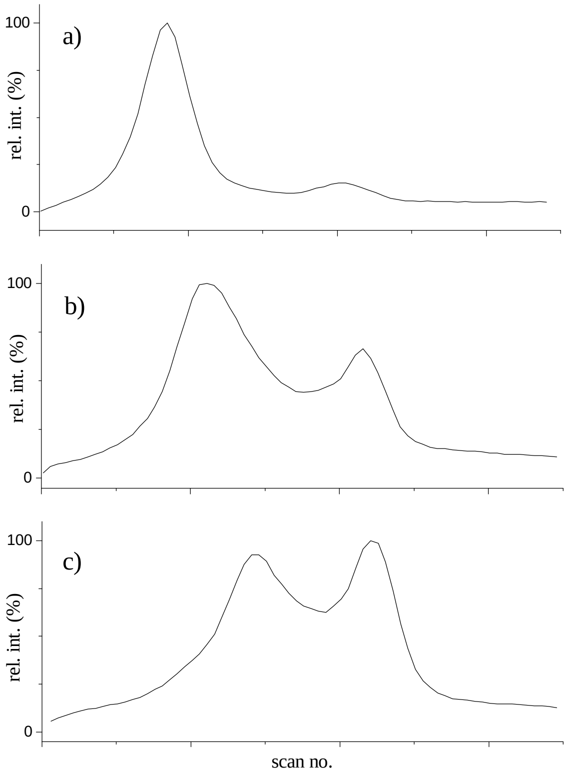

Figure 11 *DTMS total ion currents of fresh dammar resin (a) and of dammar after one week (b) or two weeks (c) of irradiation in DCM with FF2.*

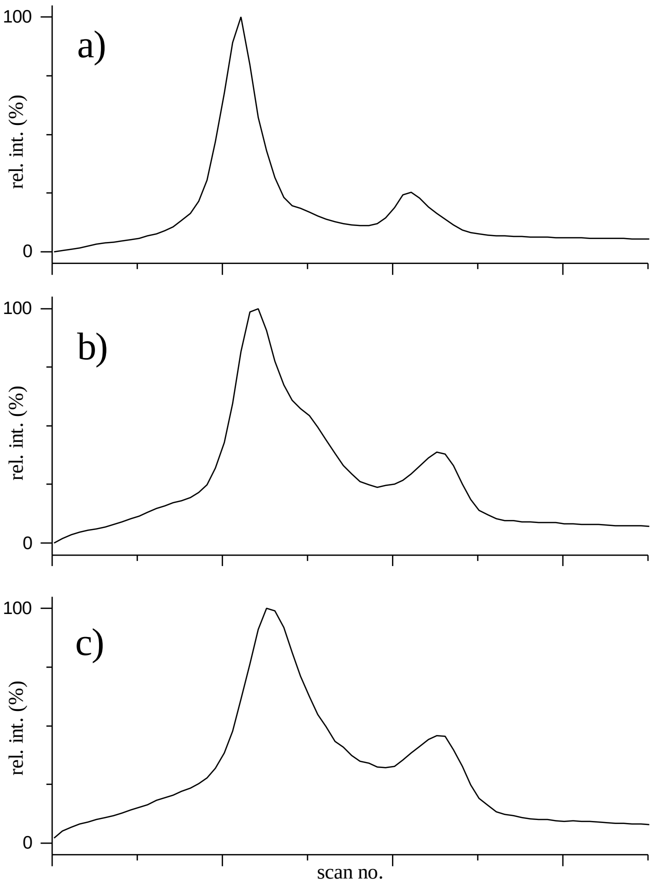

Figure 12 *DTMS total ion currents of fresh mastic resin (a) and of mastic after one week (b) or two weeks (c) of irradiation in DCM with FF2.*

show the DTMS TICs of the dammar (Figure 11) and mastic (Figure 12) samples that had been solvent aged in DCM with FF2 for one and two weeks. Two main peaks are present in the TICs. As described in the previous chapters, the first peak represents volatile (triterpenoid) material. At higher scan numbers and thus higher temperature the cross-linked fraction pyrolyses, which is represented by the second peak. Particularly in the case of dammar, the TICs clearly show that the cross-linked fraction increases during solvent ageing. The same process also occurs in mastic resin, but to a lower extent. The DTMS TICs of the pure compounds, hydroxydammarenone and oleanolic acid, contain a peak at relatively high temperature as well (not shown), which indicates that these pure compounds also cross-link during solvent exposure.

The dammar and mastic resin solutions were found to become yellow after solvent exposure in DCM with as well as without an additional photosensitiser. A yellow appearance of the sample is caused by light absorption around 400-430 nm. Size Exclusion Chromatography (SEC) in combination with a UV/VIS diode array detector was used to investigate the spectroscopic characteristics of some solvent aged samples. Figure 13(a) shows the SEC traces of the dammar sample that had been solvent aged in DCM with FF2 for one week at 240 nm (solid line) and at 400 nm (dashed line). For comparison, the SEC trace at 240 nm of fresh dammar resin (dotted line) is shown. The trace at 240 nm of the solvent aged dammar shows that two peaks are present, which are not well resolved. The peak at 400 dalton represents the triterpenoid fraction. The (shoulder) peak at 900/1000 dalton can be attributed either to cross-linked dimerised triterpenoids or to highly oxidised triterpenoids, as already discussed in Chapter 3. The SEC trace at 400 nm clearly shows that the SEC fraction at 900/1000 dalton absorbs more light at 400 nm than the triterpenoid fraction. These results are similar to the results obtained with scrapings of aged varnishes from paintings (Chapter 3).

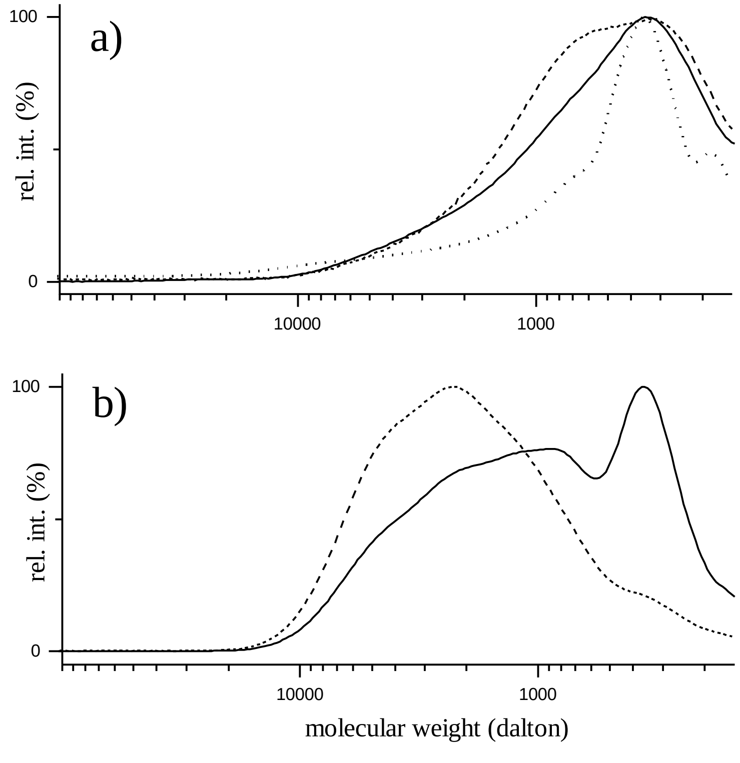

Figure 13 *SEC traces at 240 nm (solid line) and at 400 nm (dashed line) of dammar after one week of solvent ageing in DCM with FF2 (a). In addition, the SEC trace at 240 nm (dotted line) of fresh dammar resin is shown in Figure (a). Figure (b) shows the SEC traces at 240 nm (solid line) and at 400 nm (dashed line) of dammar after three and a half weeks of solvent ageing in DCM (b).*

One dammar sample was exposed in DCM for a relatively long period of time (three and a half weeks). After exposure the dammar solution had turned dark yellow to brown. Figure 13(b) shows the SEC traces of this dammar sample at 240 nm (solid line) and at 400 nm (dashed line). It is clear that a high degree of cross-linking has taken place during this relatively long period of light exposure in DCM. Higher molecular weight material up to 10 KDa has been formed. The SEC trace at 400 nm clearly demonstrates that the higher molecular weight fractions absorb light in the blue light region. A much higher degree of cross-linking has taken place after three and a half weeks of solvent ageing as compared to ageing on paintings (Chapter 3). This dammar sample, which was aged for a relatively long period of time, is not comparable to that of a varnish aged on a painting. In general, the chance of cross-linking is relatively high in the solvent ageing method. Molecules have much more freedom of movement in solution. Secondly, the solvent ageing method leads to the generation of a relatively large amount of radicals or other reactive species in the resin solution, which increases the probability of radical recombination (quenching) resulting in cross-linking. Thirdly, the concentration of oxygen decreases in the vials during light exposure, which may cause a shift to occur from oxidation to cross-linking reactions.

It was observed that a yellowish precipitate was deposited on the glass jar after some period of solvent ageing of dammar in DCM with or without a photosensitiser. This precipitate was found to be soluble in tetrahydrofuran (THF). The solvent ageing conditions were exactly the same as used above for the dammar ageing in DCM with FF2, except for a higher temperature of the fluorescent tube device during exposure. The temperature was about 10-15 degrees higher than in the experiments described above, which resulted in an increased reaction rate. The type of oxidation reactions was found to be the same. In general, the temperature was found to be a very important factor for the rate of the ageing reactions. Under these experimental conditions the precipitate was observed to be formed already after one week of solvent ageing. DTMS analysis of the nine days exposed dammar in DCM solution (a) and the precipitate formed (b) shows that the precipitate consists of cross-linked material, because it appears later in the temperature profile (figure 14). The total summation spectrum of the precipitate (Figure 14(c)) shows a large number of peaks, which is indicative of complex material. Figure 14(d) depicts the DTMS spectrum of the cross-linked fraction of an aged dammar varnish from a painting. The spectra of the solvent aged (Figure 14(c)) and “naturally” aged cross-linked fraction (Figure 14(d)) are very similar. An important difference between the two spectra of Figure 14(c) and (d) is the presence of the peaks at m/z 36 and m/z 38 in the spectrum of the solvent aged material. These peaks point to the elimination of hydrogen chloride, which

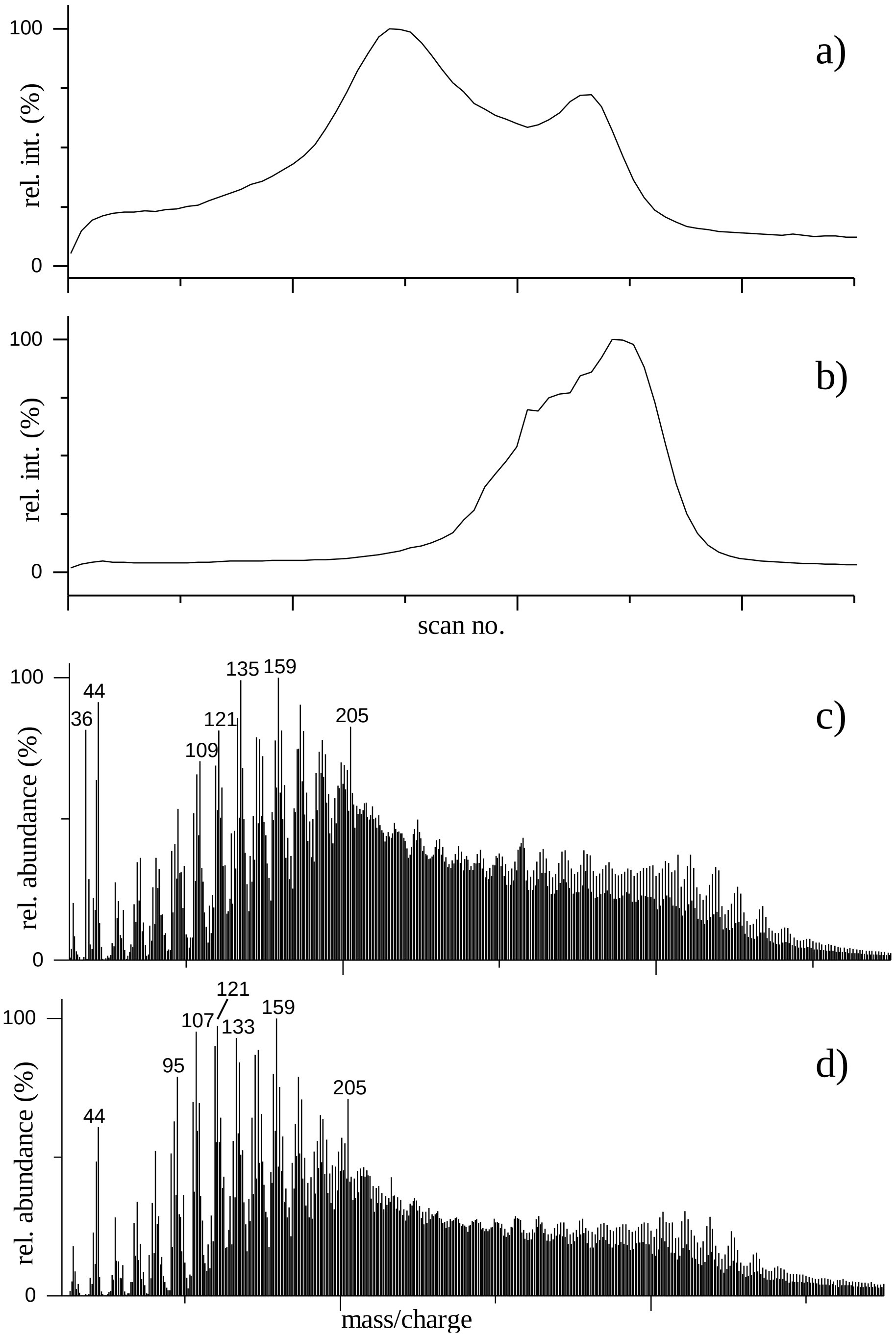

Figure 14 *DTMS total ion currents of dammar and FF2 in DCM after nine days of irradiation (DCM soluble fraction) (a) and of the precipitate formed after solvent ageing (DCM insoluble, THF soluble fraction) (b). Figure (c) and (d) show the summation spectra of the precipitate (c) and of the cross-linked fraction of an aged dammar varnish from a painting (d) respectively.*

indicates that some incorporation of chlorine occurs during solvent ageing. This observation is consistent with the hypothesis that DCM itself is involved in the formation of radicals.

The chemical structure of the higher molecular weight fraction will be investigated further with Nuclear Magnetic Resonance Spectroscopy (NMR). The method of solvent ageing is very well suited for the preparation of a large batch of this cross-linked material needed for NMR.

5.6. Conclusions

Dissolution in the solvents DCM and acetone and subsequent light exposure induces changes in triterpenoid substances, like oxidation and cross-linking, which are similar to the molecular changes seen during “natural” ageing of triterpenoid varnishes on paintings. Other solvents, such as methanol, ethanol, acetonitrile and toluene, were found not to induce oxidation reactions during light exposure of triterpenoid samples. The photosensitisers MC540 and FF2 were also found to be useful for the simulation of natural oxidation processes either in ‘active’ or ‘non-active’ solvents. Light is the driving force of these ageing processes. Information was obtained on the oxidation mechanisms of the model compounds hydroxydammarenone and oleanolic acid. Hydroxydammarenone is first oxidised to an ocotillone type molecule, which is subsequently oxidised to a lactonised molecule. In addition, a compound with a shortened side chain is formed, probably from the ocotillone type molecule. Only side chain oxidation of this dammarane type molecule occurs during solvent ageing. Oxidation of the A-ring was not observed. The solvent ageing of oleanolic acid first results in the formation of small amounts of compounds with additional keto groups at C3 and C11. In addition, the double bond at C12 of oleanolic acid becomes oxidised and a keto group is formed at this position. The compound formed by this latter process is the main compound after prolonged solvent ageing. Decarboxylation of the carboxylic acid at C17 of oleanolic acid was not observed. SEC in combination with UV/VIS diode array detection demonstrated that the yellow colour of a dammar sample, which was light exposed in DCM for a relatively long period of time, was caused by the formation of relatively high molecular weight material. This chapter leaves a number of questions unresolved, such as the role of specific reactive species generated by the ‘active’ solvents and the photosensitisers and the effect of different wavelengths of the radiation.

Nevertheless, the photochemical technique of solvent ageing can be regarded as a useful technique for a number of reasons. Information can be gained on the type of oxidation products that are formed during ageing. It can be determined which compounds can be used as stable markers for aged triterpenoid varnishes. Furthermore, it can be used as a preparation technique for the cross-linked fraction of aged dammar and mastic varnishes. Finally, this technique is very simple from an experimental point of view, very rapid, without film forming problems and controllable, because there is no reaction without light. It should be investigated whether it can also be successfully used for the artificial ageing of other painting materials. A disadvantage of the current investigations is that kinetic measurements were not possible due to the decreasing oxygen concentration. A device that controls the oxygen concentration is presently being developed in our laboratory.

5.7. Experimental

5.7.1. Sample preparation

Hydroxydammarenone (also called dipterocarpol) (Aldrich), oleanolic acid (Aldrich), dammar resin (A.J. van der Linde, Amsterdam, The Netherlands), mastic (H. Schmincke & Co., Erkrath, Germany), Merocyanine 540 (Molecular Probes, Leiden, the Netherlands) and FotoFenton 2 (Molecular Probes, Leiden, the Netherlands) were used for solvent ageing. Methanol (Merck, p.a.), ethanol (Merck, p.a.), dichloromethane (Fluka, >99.8%, HPLC grade), acetone (Merck, p.a.), toluene (Aldrich, 99.5+%, A.C.S. reagent) and acetonitrile (Sigma-Aldrich, 99.93%, HPLC grade) were used as solvents. Hydroxydammarenone and oleanolic acid solutions were made in the concentration of 1 mg/ml. Photosensitisers were added in a concentration of 0.2 mg/ml. Dammar and mastic solutions were made in a concentration of 2 mg/ml. Photosensitisers were added in a concentration of 0.4 mg/ml. Solutions of 300 μl were irradiated in borosilicate glass vials (Chromacol, type 1, class A, 4 ml content). MC540 was not completely soluble in DCM solutions. The aged varnishes of which the gas chromatograms are depicted in Figures 9(d) and 10(d) are described in Chapter 3 (varnish A and C).

5.7.2. Ageing conditions

The glass vials containing triterpenoid solutions were irradiated in a fluorescent tube device, which was constructed by R. Hoppenbrouwers of the Stichting Restauratie Atelier Limburg (SRAL). The device was equipped with fluorescent tubes (TLd, 36 Watt, 96.5 (Philips), 13,000-13,500 lux). The temperature was kept between 18°C and 30°C with an average temperature of 25°C. The average temperature of the solvent ageing of the sample, of which the results are shown in Figure 14, was about 37°C. After exposure, samples were stored in the freezer.

5.7.3. DTMS

After solvent ageing, the solutions were diluted (10 times). Approximately 2 µl was applied directly to the DTMS probe. In the case of fresh dammar and mastic resin, about 50-100 µg was homogenised in approximately 100-200 µl ethanol. An aliquot (about 2 µl) of the resulting suspensions was applied to the DTMS probe by using a syringe (SGE, 5 µl. All samples on the probe were 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) in an ionisation chamber kept at 180 °C and were accelerated to 8 kV. The mass spectrometer was scanned from m/z 20-1000 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 800 °C. Data were acquired using a JEOL MP-7000 data system.

5.7.4. GCMS

The GCMS analysis of fresh dammar and mastic resin is described in Chapter 2. An aliquot of 50 µl of the solvent aged samples was evaporated to dryness. For methylation, according to the method of Hashimoto et al. [20], aliquots of 250 µl of methanol, 25 µl of benzene and 10 µl of TMSdiazomethane were added to the samples. The mixtures were left at room temperature for 30 minutes. After evaporation to dryness, the samples were dissolved in 25 µl dichloromethane (1 µl injection). GCMS experiments were performed as described in Chapter 4.

5.7.5. SEC

SEC experiments were carried out as described in Chapter 3.

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6. Mass spectrometric analysis of triterpenoids in dammar and mastic under EI and APCI conditions[1]

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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[1] This chapter is based on the publication: Van der Doelen, G.A., Van den Berg, K.J., Boon, J.J., Shibayama, N., De la Rie and E.R., Genuit, W.J.L., Analysis of fresh triterpenoid resins and aged triterpenoid varnishes by HPLC-APCI-MS(/MS), Journal of Chromatography A, 809 (1998), 21-37.

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

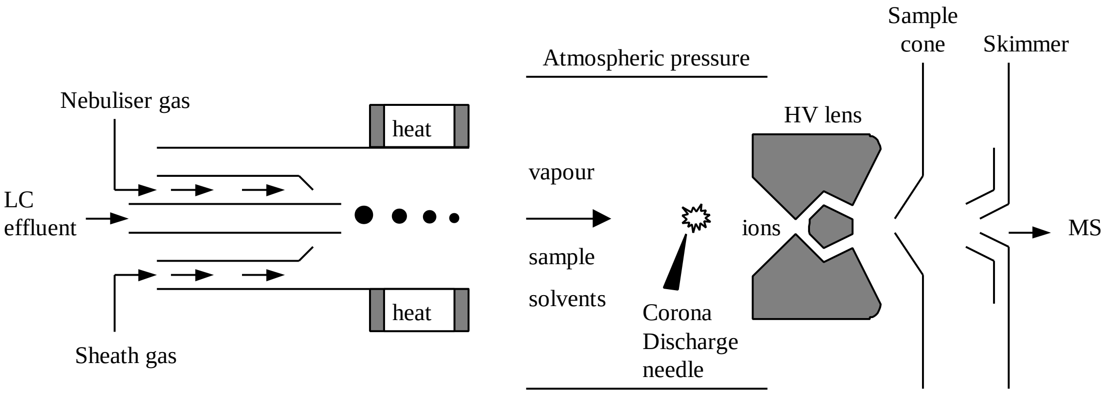

Figure 1 *Schematic diagram of the experimental setup of an APCI interface.*

(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 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 H_2O 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

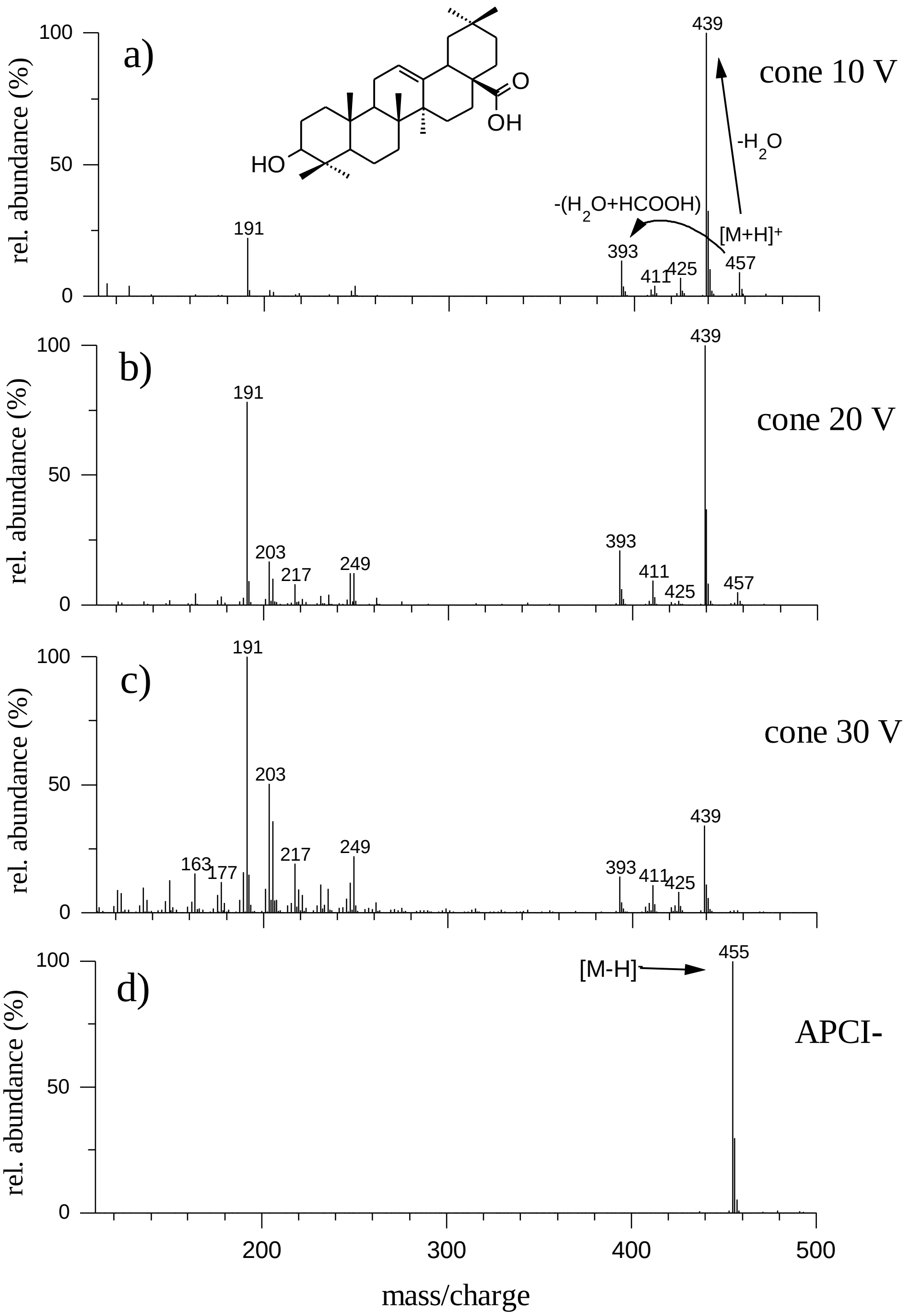

Figure 2 *Mass spectra of oleanolic acid obtained by HPLC-APCI-MS: in the positive ion mode with increasing cone voltage (a-c), in the negative ion mode (d).*

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.*

label	Compound name	MW	M/z values of characteristic (fragment) ions of methylated compounds under EI (70 eV) (rel. int. %)	M/z values of characteristic (fragment) ions of compounds under APCI (cone 20 V) (rel. int. %)
8	Hydroxydammarenone (I or II) (20-hydroxy-24-dammaren-3-one[1])	442	424(80), 355(39), 205(42), 109(100)	443(6), 425(100), 407(9), 219(21), 205(13)
5	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[1])	458	454(50), 385(48), 109(100)	441(100), 205(23), 191(44)
22	Dammarenediol (20-dammar-24-ene-3β,20-diol[1])	444	426(35), 207(29), 189(12), 109(100)	427(30), 409(100), 219(20), 191(34)
3	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426	426(40), 207(84), 189(43), 109(100)	427(32), 409(100), 219(22), 191(42)
1	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424	424(86), 205(65), 109(100)	425(100), 407(34), 245(24), 189(19)
26	3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone[1]	414	414(100), 205(64), 99(78), 95(55)	415(100), 397(31), 379(4)
4	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)-dammaren-3-oic acid[2]	474	429(11), 143(100)	457, 439(100)
9	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438	438(19), 232(48), 203(100)	439(100), 421(27), 411(9), 393(3)
11	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438	438(16), 232(21), 203(100)	439(100), 421(24), 411(17), 393(3)
23	Oleanolic aldehyde (3-hydroxy-olean-12-en-28-al)	440	440(9), 232(77), 203(100)	441(29), 423(100), 395(25), 205(14), 191(29)
24	Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)	440	440(5), 232(21), 203(100)	441(41), 423(100), 395(26), 205(16), 191(34)
6	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454	468(25), 262(58), 203(100)	455, 437, 409(100)[3]
10	Ursonic acid (3-oxo-urs-12-en-28-oic acid)	454	468(18), 262(100), 203(77), 133(43)	455(100), 437, 409[3]
17	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454	468(48), 249(50), 189(100)	455(100), 437, 409[3]
27	11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)	468	482(100), 317(49), 276(80), 257(49), 217(65)	469(100), 451(4), 423(29)

Table I (continued).

32	11-Oxo-ursonic acid (3,11-dioxo-urs-12-en-28-oic acid)	468	482(65), 317(100), 276(53), 257(46)	469(100), 451, 423[3]
18	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	454	468(30), 453(100), 421(21) or 468(26), 453(100), 421(18)	455(100), 437(45) or 455(98), 437(100), 127(20), 125(27)
20	3-O-Acetyl-3epi(iso)masticadienolic acid (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3α-acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid)	498	512(22), 497(26), 437(100) or 512(22), 497(34), 437(100)	439(100), 247(17), 191(56) or 439(100)
12	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442	442(11), 424(8), 409(11), 384(27), 207(28), 189(81), 149(100)	443(18), 425(100), 407(12), 179(10)
16	(3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene	444	426(7), 218(30), 190(47), 175(45), 137(59), 94(66), 81(97), 69(100)	427(58), 409(100)
13	(8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene	442	424(8), 218(28), 175(23), 137(52), 94(54), 81(100), 69(89)	425(100), 407(7), 219(10), 205(18), 191(13)

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[1] The configuration at C-20 was not determined.
[2] The configuration at C-20 and C-24 was not determined.
[3] The exact relative intensities could not be determined since this compound was not separated from other compounds with the HPLC conditions used here.

ammonia chemical ionisation (NH_3/CI) mass spectrum of hydroxydammarenone 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 $[M+NH_4-H_2O]^+$ ions and $[M+H-H_2O]^+$ 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 ocotillone type stereoisomer. The configuration at C20 and C24 of the ocotillone 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

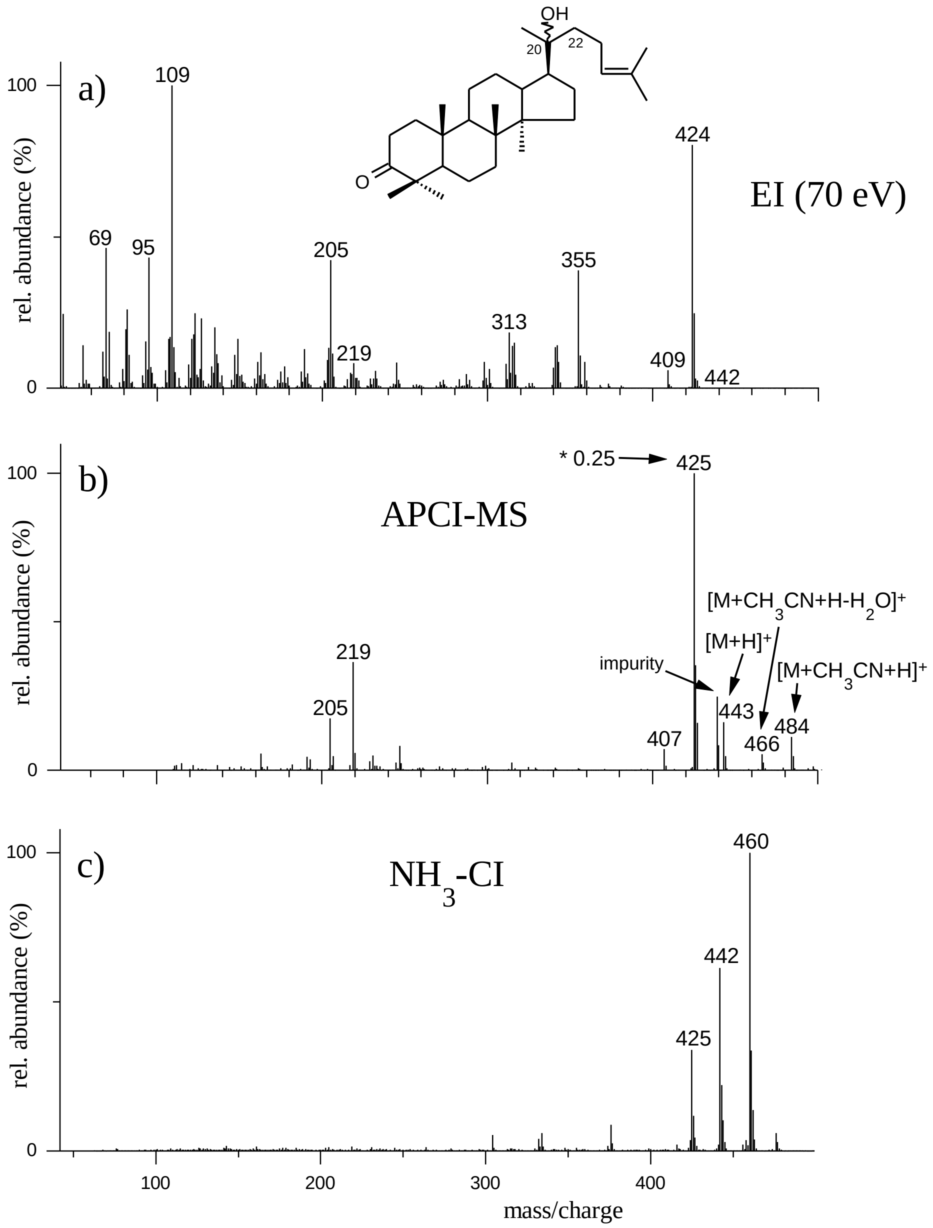

Figure 3 *Mass spectra of hydroxydammarenone obtained by GC-(EI)MS (70 eV) (a), HPLC-APCI-MS (b), and NH_3/CI-DTMS (c) ("*0.25" indicates that the actual peak intensity at m/z 425 is a factor of four higher).*

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-A-*seco*-triterpenoids, such as dammarenolic acid (**5**), contain a

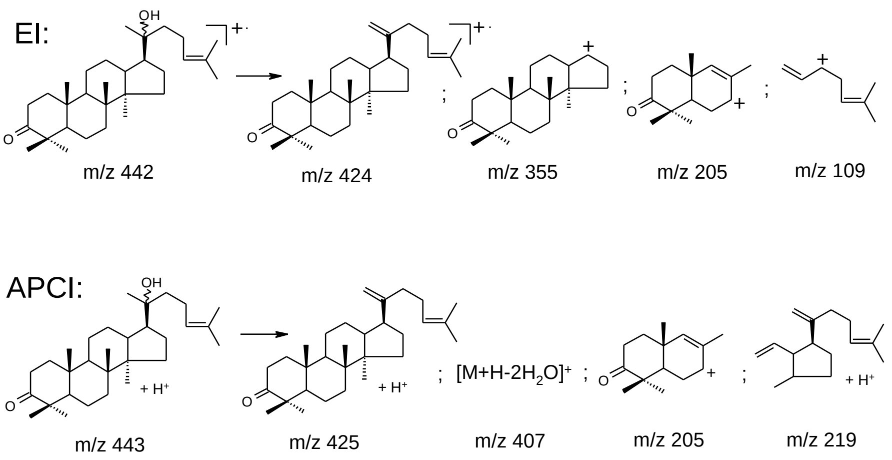

Figure 4 *Proposed principal mass spectral fragments of hydroxydammarenone under EI and APCI conditions.*

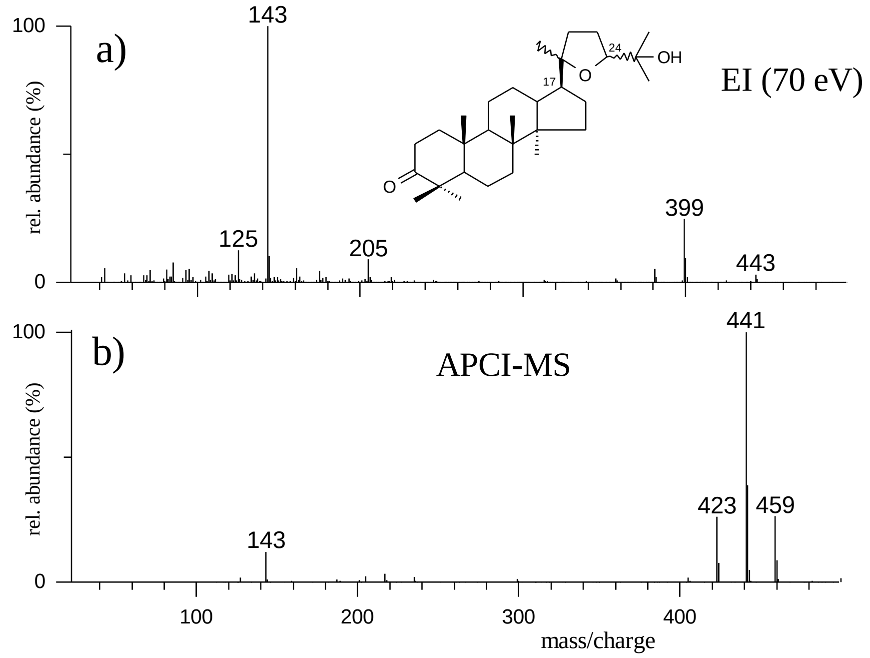

Figure 5 *Mass spectra of an ocotillone type molecule obtained by EI (70 eV) (a), and HPLC-APCI-MS (b).*

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.

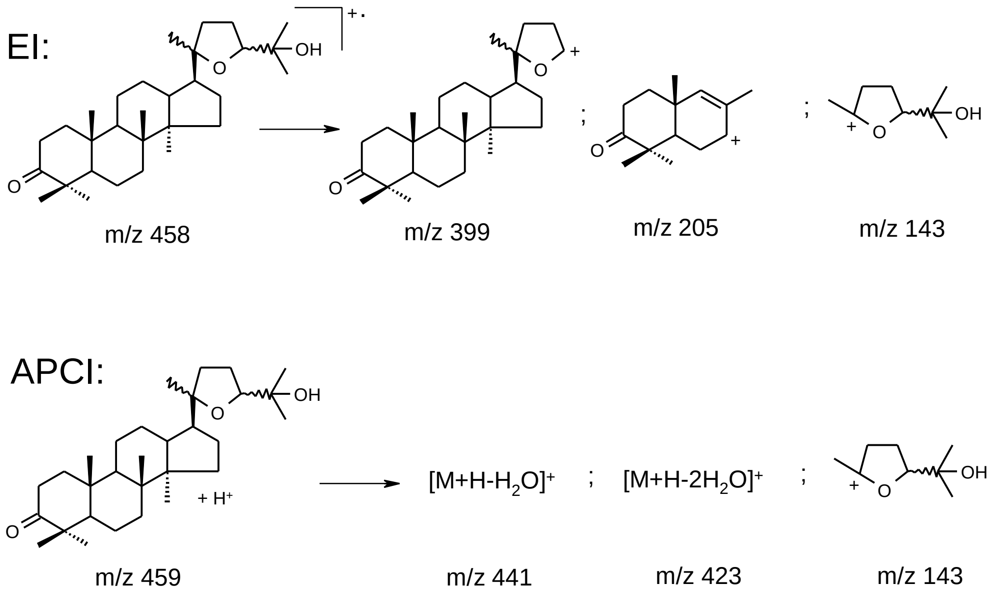

Figure 6 *Proposed principal mass spectral fragments of an ocotillone type molecule under EI and APCI conditions.*

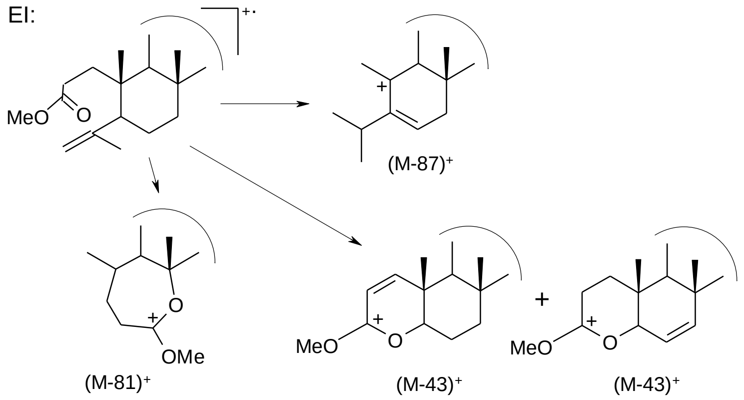

Figure 7 *Fragmentation mechanism of 3,4-A-seco-triterpenoids under EI conditions.*

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].

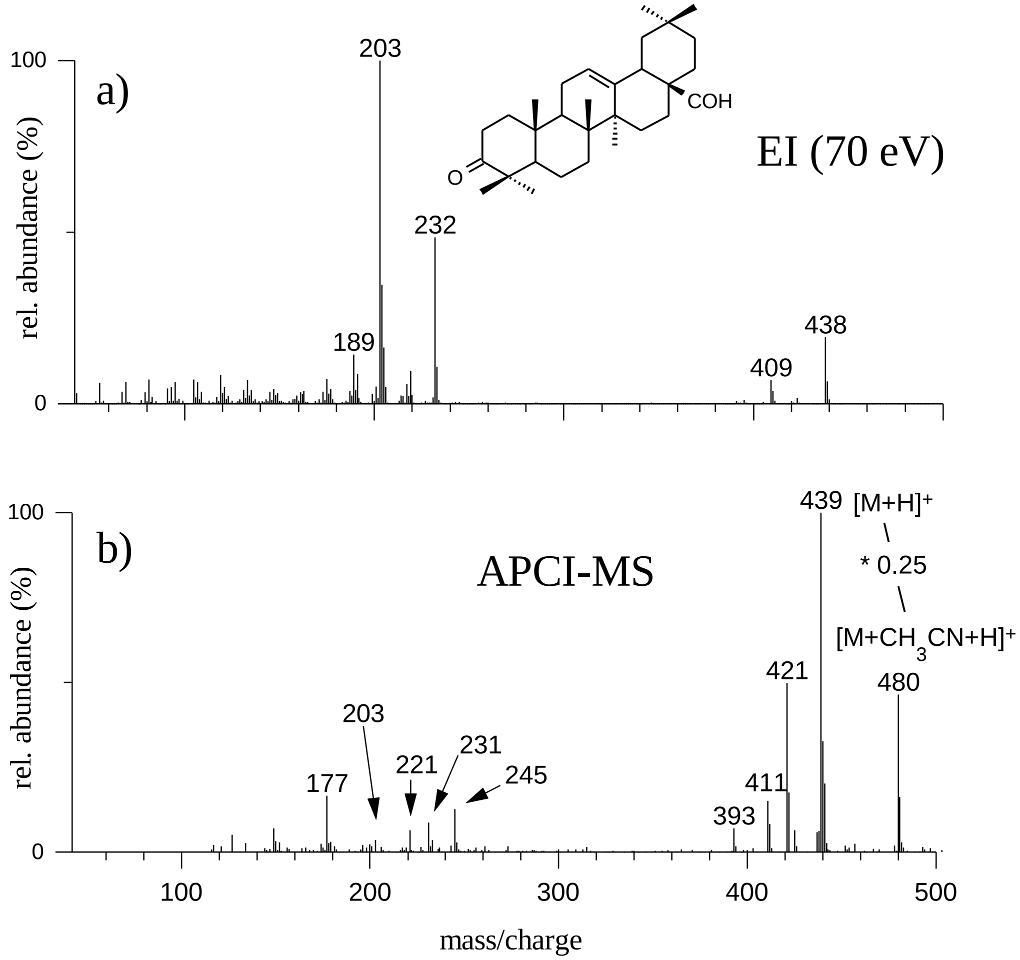

Figure 8 *Mass spectra of oleanonic aldehyde obtained by EI (70 eV) (a), and HPLC-APCI-MS (b) ("*0.25" indicates that the actual peak intensities at m/z 480 and m/z 439 are four times higher).*

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

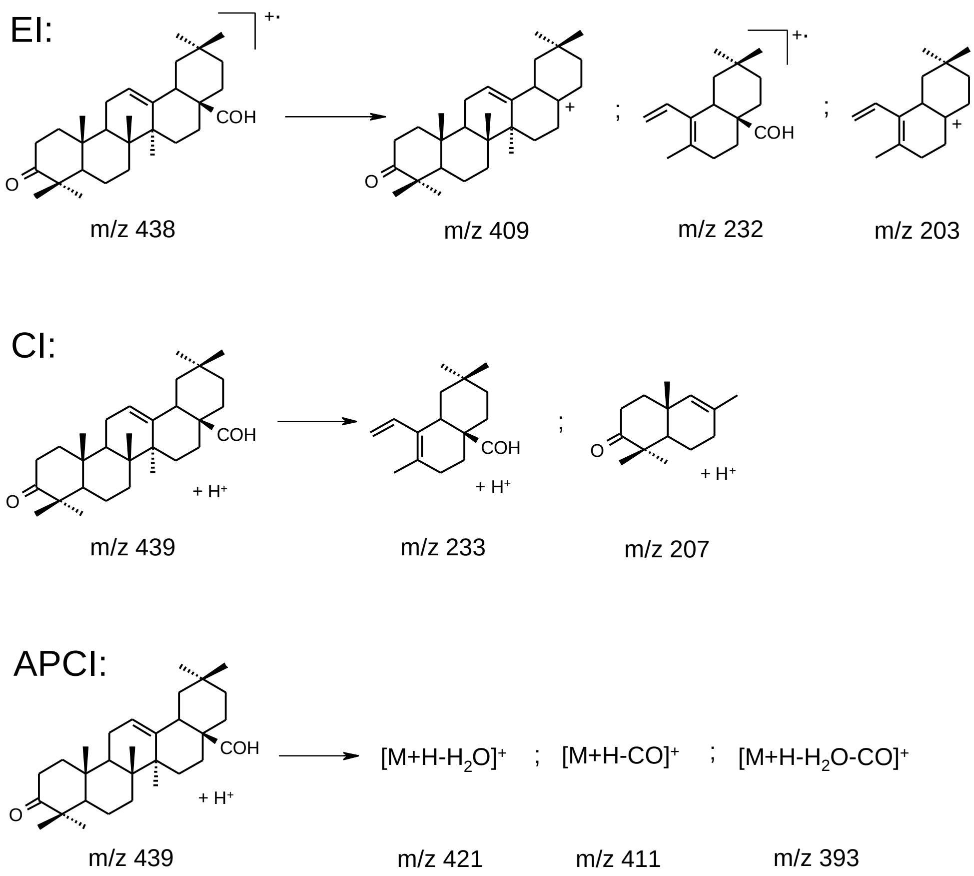

Figure 9 *Proposed principal mass spectral fragments of oleanonic aldehyde under EI, CI and APCI conditions.*

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 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.

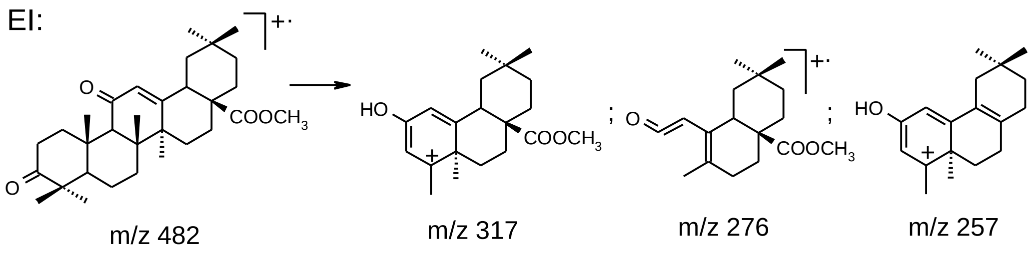

Figure 10 *Principal mass spectral fragments of 11-oxo-oleanonic acid under EI conditions.*

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.

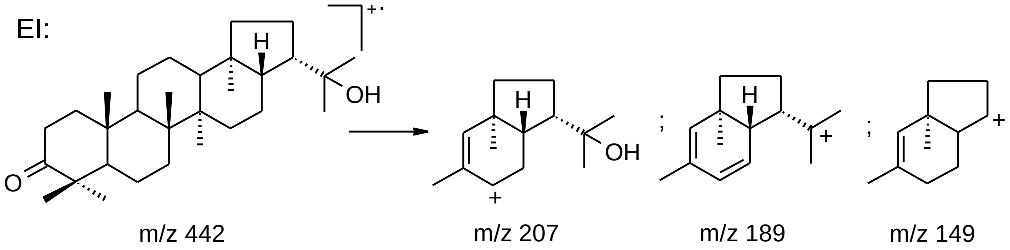

Figure 11 *Principal mass spectral fragments of hydroxyhopanone under EI conditions.*

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

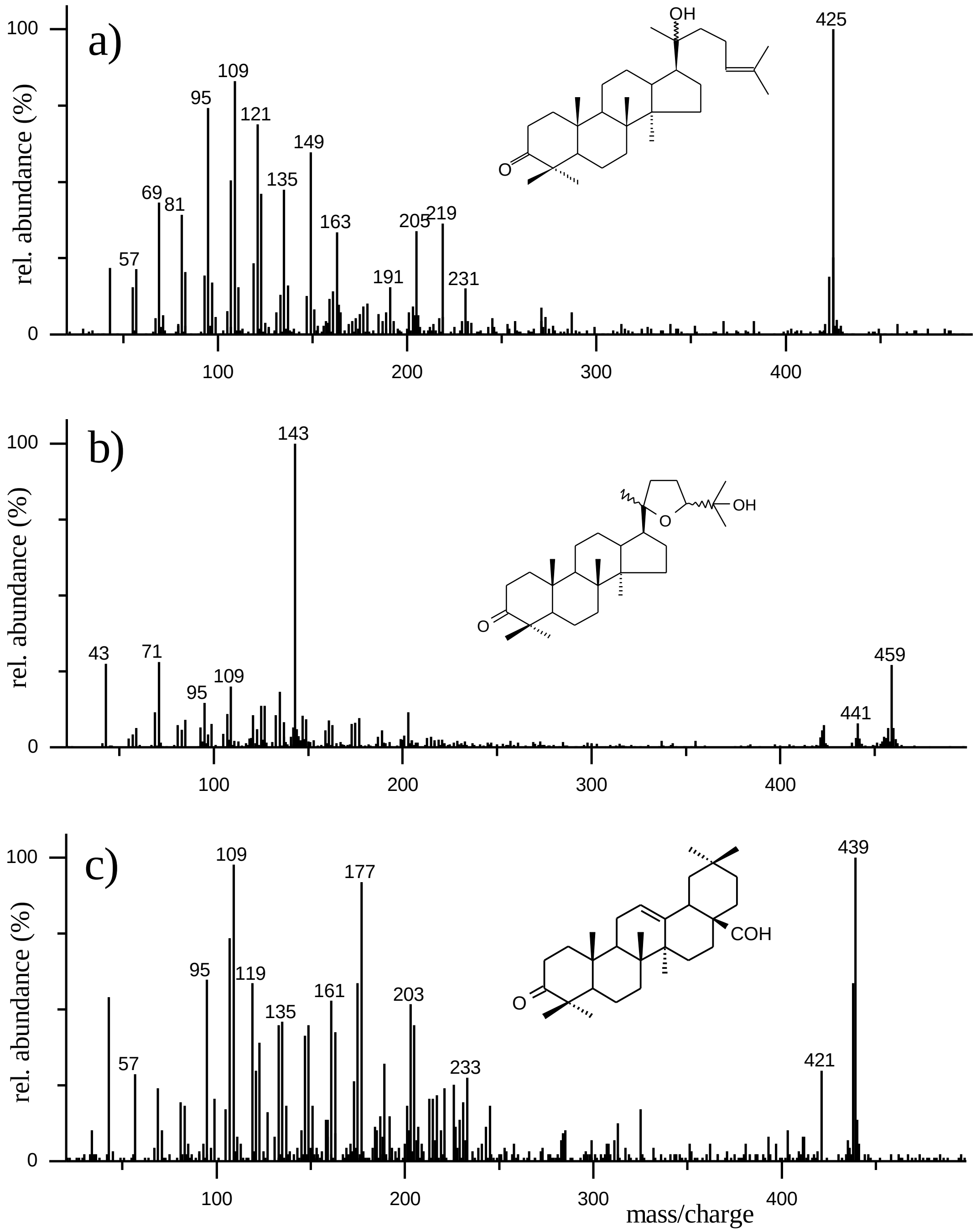

Figure 12 *Mass spectra of hydroxydammarenone (a), an ocotillone type molecule (b), and oleanonic aldehyde (c) obtained by HPLC-APCI-MS-MS.*

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 ocotillone 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 were analysed [21]. The ocotillone 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

12(c)) as well as ring C cleavage of dammarane type compounds (Figure 12(a)) are observed to occur to a smaller extent when the analytes are protonated.

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 $\pm 0.3 * 10^{-3}$ mbar and a collision energy of 40 V (in case of Figure 3 and 8) or 50 V (in case of Figure 5).

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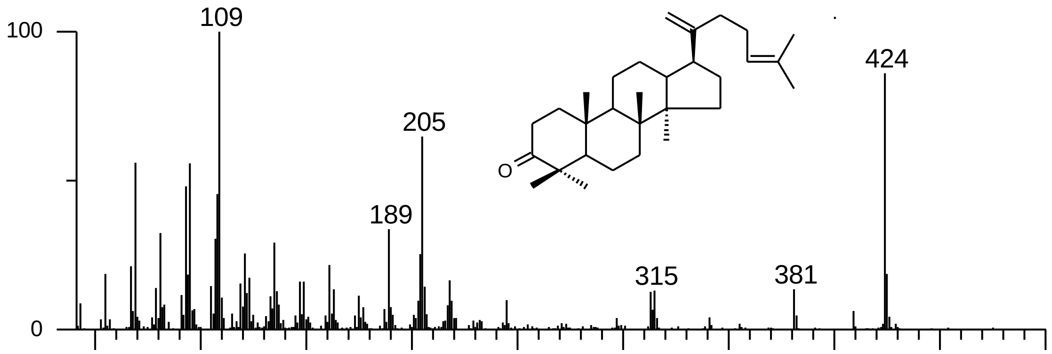
Atlas of mass spectra of triterpenoid compounds in varnishes

Mass spectra were obtained under electron ionisation conditions (70 eV). Peak labels refer to those used in the figures and tables of the preceding chapters. The mass spectra of some triterpenoid compounds (**15**, **23** and **40**) are not included in this atlas, due to low relative intensity or insufficient separation of these compounds necessary for obtaining a good quality of the spectra. Some mass spectra have a relatively low signal to noise ratio, due to the low relative intensities of the corresponding compounds (**30**, **31**, **32** and **33**).



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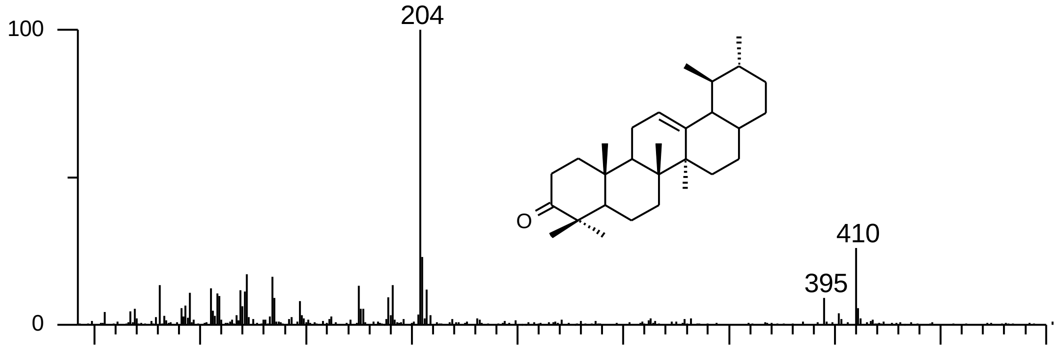
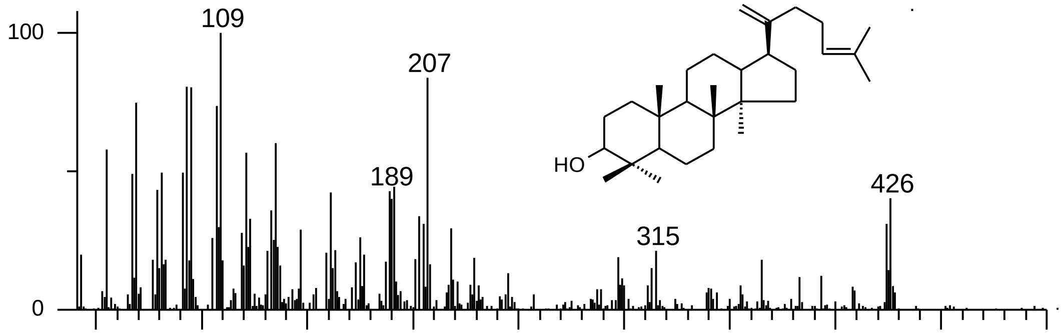
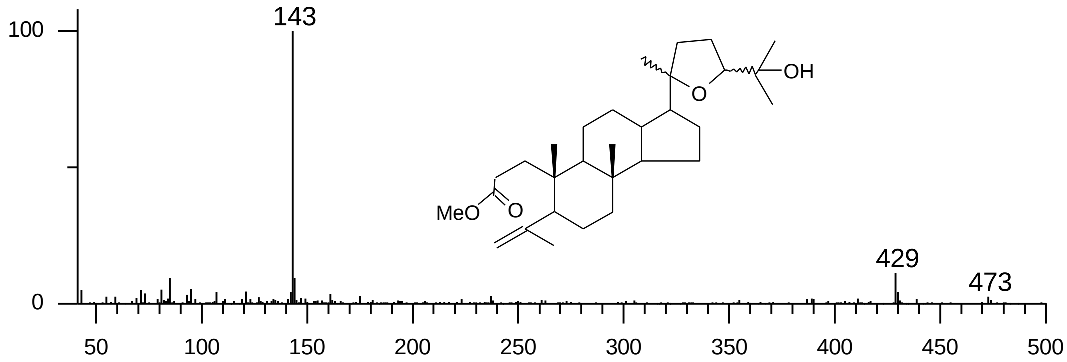


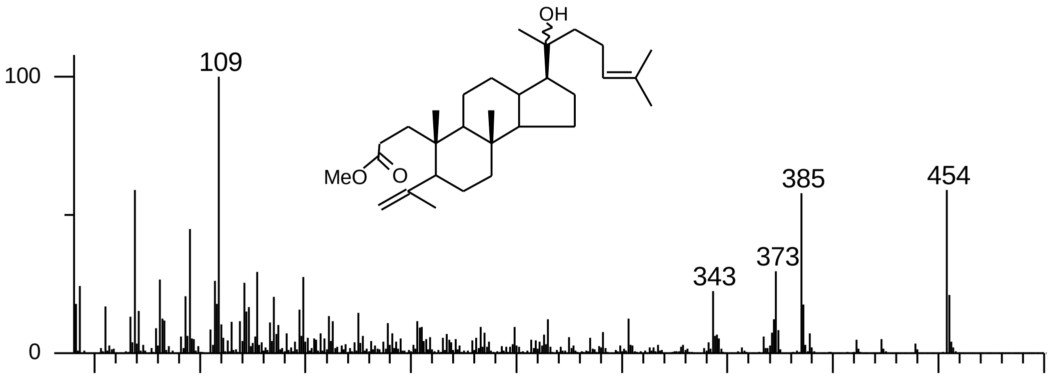
2. Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)



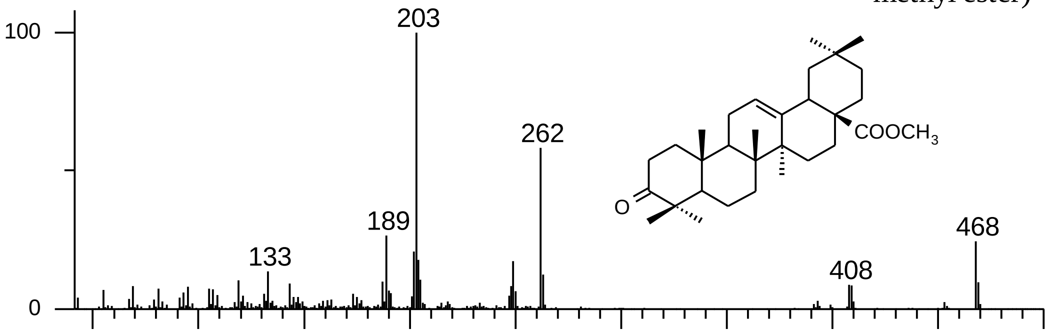
3. Dammaradienol (3β-hydroxy-dammara-20,24-diene)



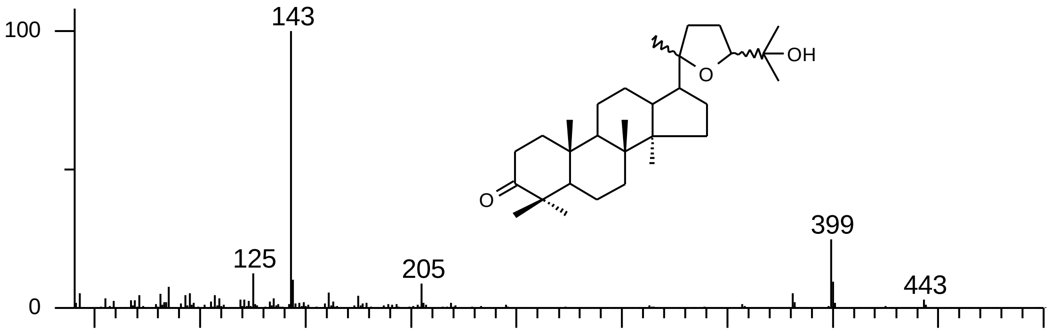
4. 20,24-Epoxy-25-hydroxy-3,4-seco-4(28)dammaren-3-oic acid methyl ester



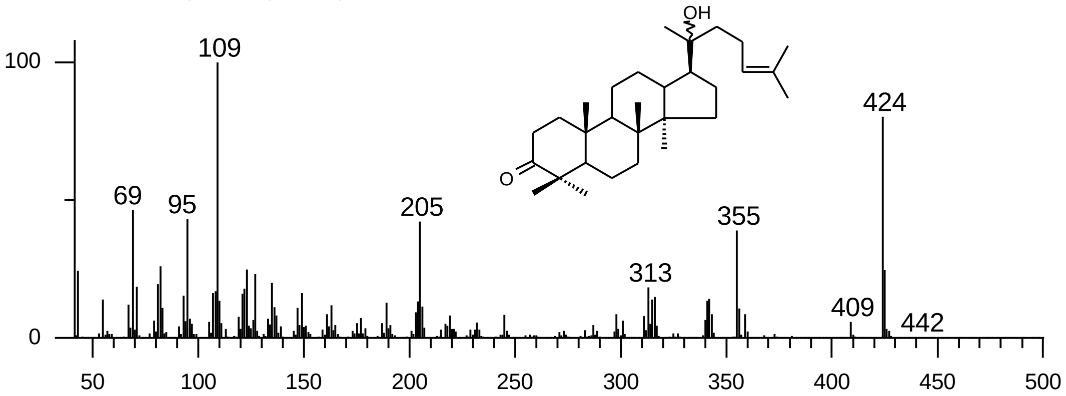
5. Dammarenolic acid methyl ester (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid methyl ester)



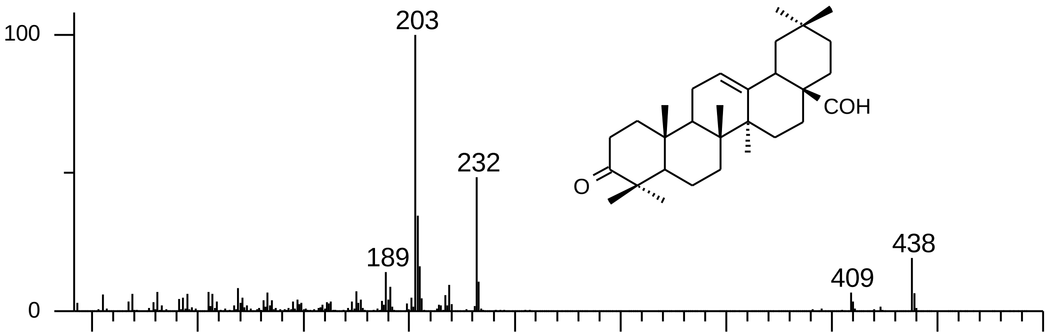
6. Oleanonic acid methyl ester (3-oxo-olean-12-en-28-oic acid methyl ester)



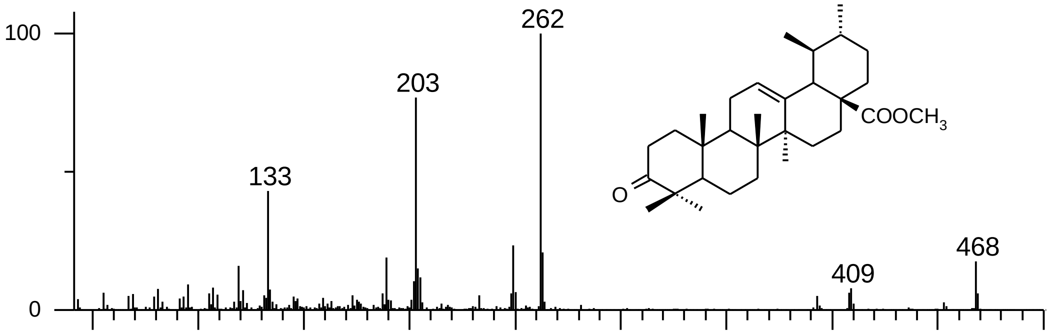
7. 20,24-Epoxy-25-hydroxy-dammaran-3-one



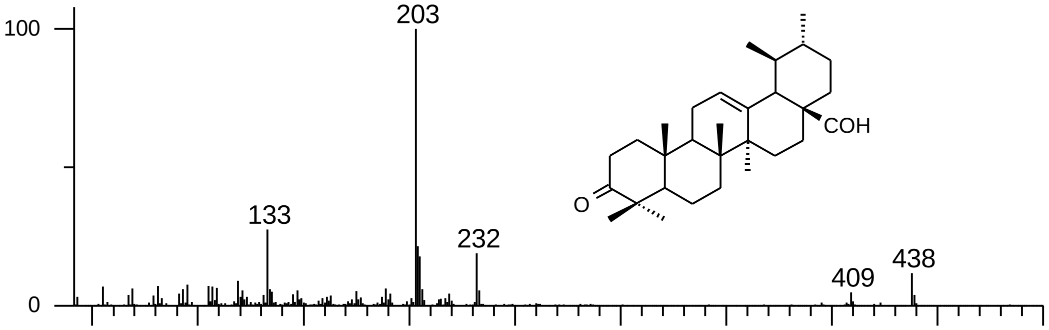
8. Hydroxydammarenone (20-hydroxy-24-dammaren-3-one)



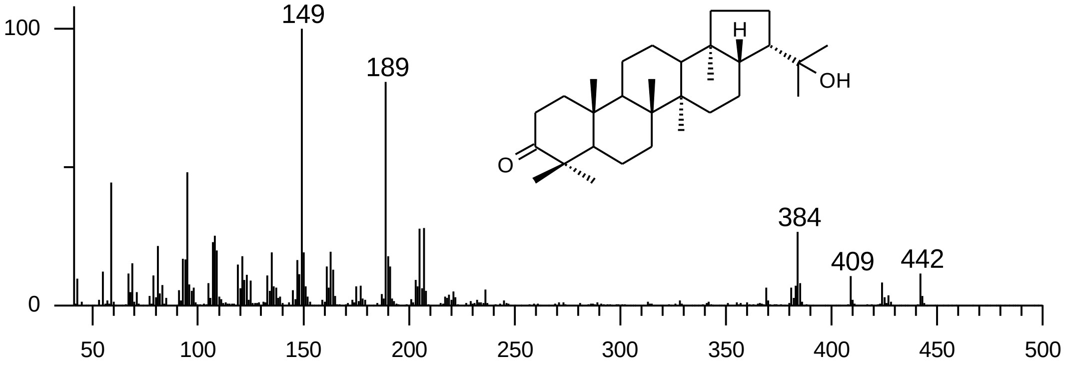
9. Oleanonic aldehyde (3-oxo-olean-12-en-28-al)



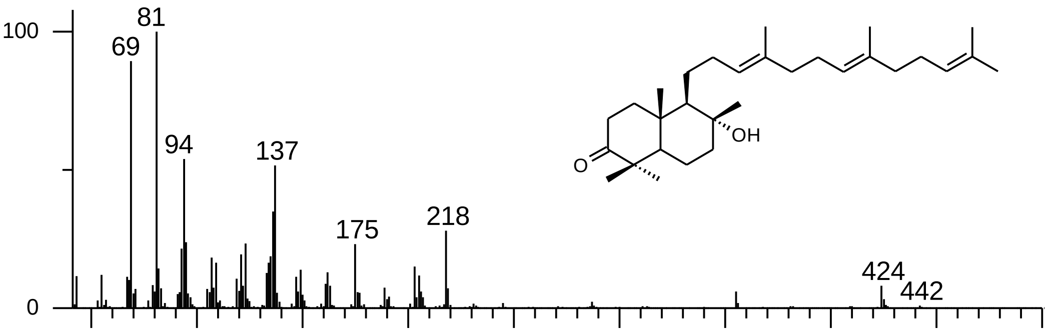
10. Ursonic acid methyl ester (3-oxo-12-ursen-28-oic acid methyl ester)



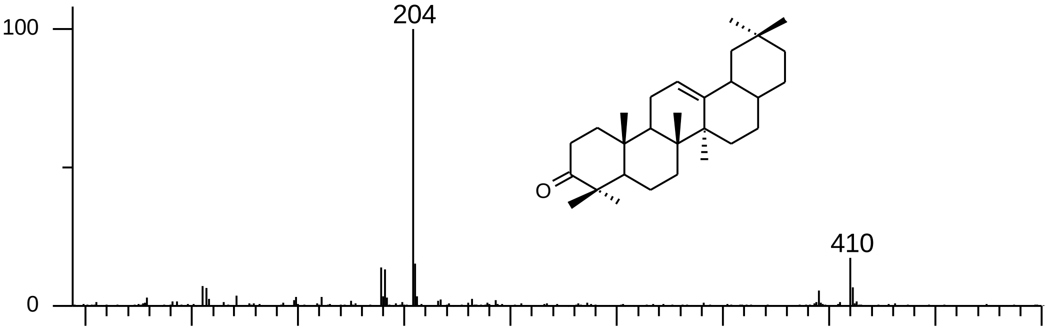
11. Ursonic aldehyde (3-oxo-urs-12-en-28-al)



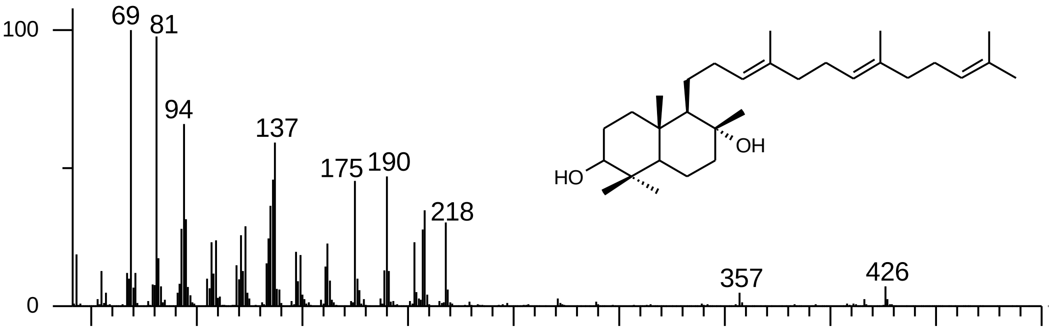
12. Hydroxyhopanone (21β,22-hydroxy-3-hopanone)



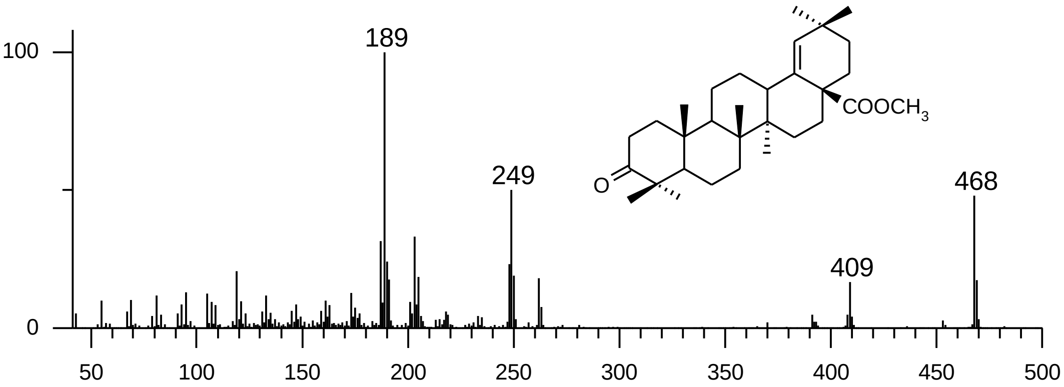
13. (8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene



14. Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)



16. (3L,8R)-3,8-Dihydroxy-polypoda-13E,17E,21-triene



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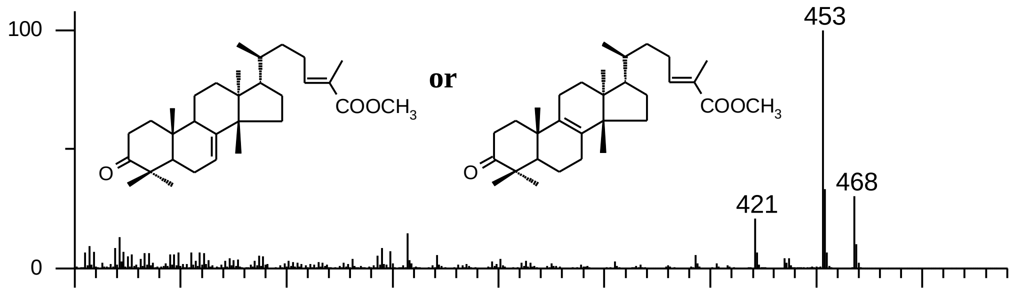
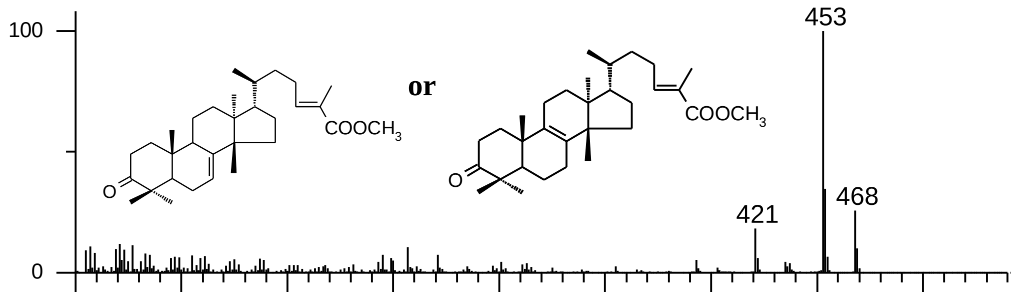
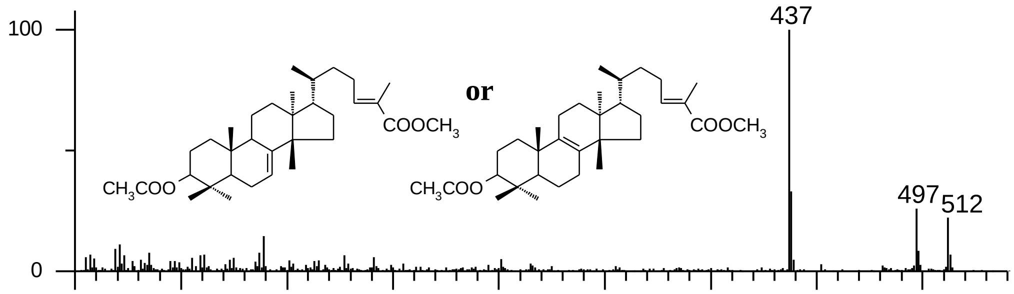


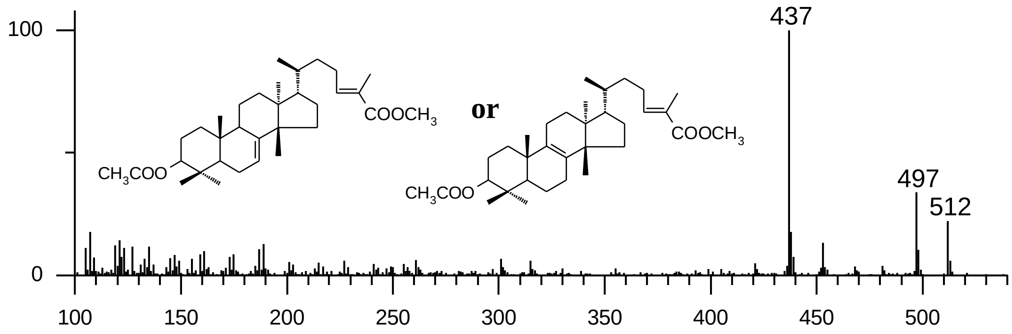
18. (Iso)masticadienonic acid methyl ester (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid methyl ester or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid methyl ester)



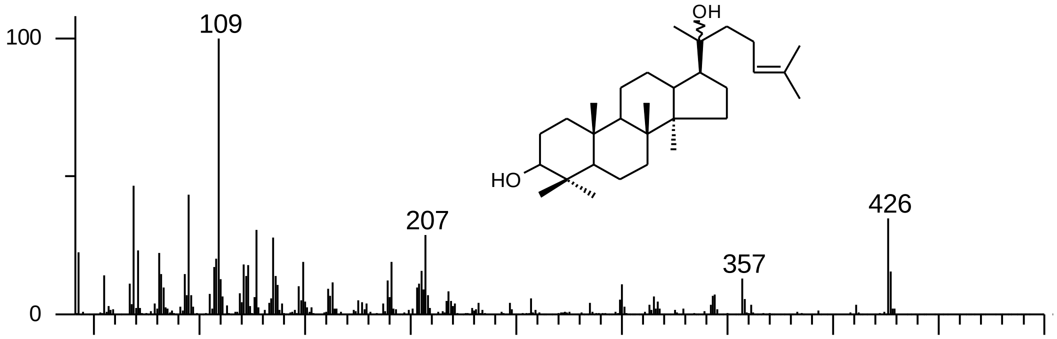
19. (Iso)masticadienonic acid methyl ester (3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid methyl ester or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid methyl ester)



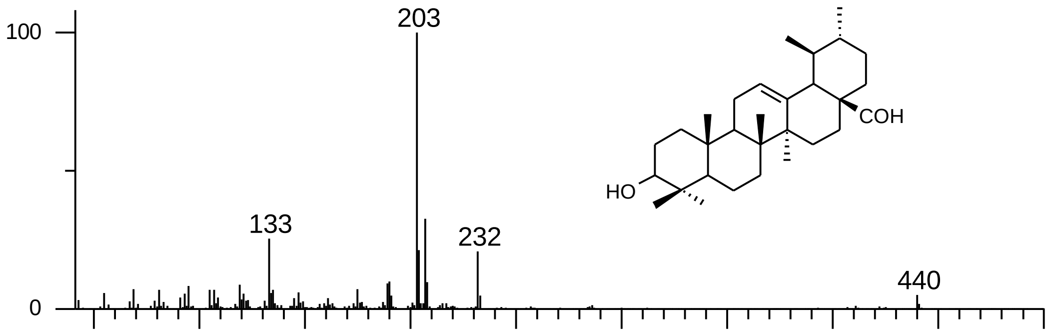
20. 3-O-Acetyl-3epi(iso)masticadienolic acid methyl ester (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid methyl ester or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid methyl ester)



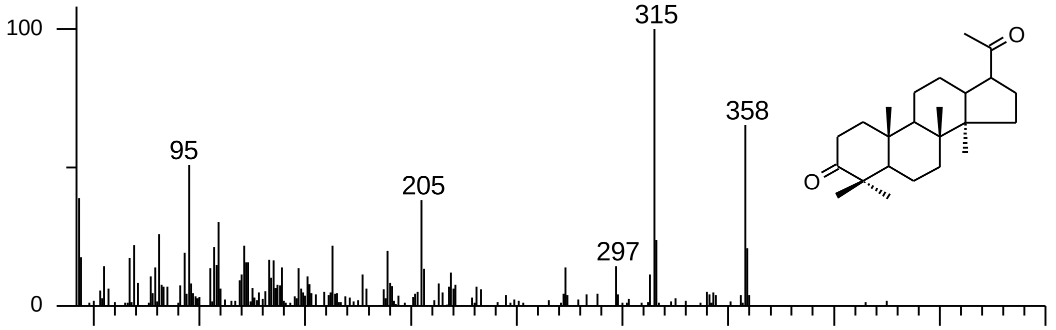
21. 3-O-Acetyl-3epi(iso)masticadienolic acid methyl ester (3α-acetoxy-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid methyl ester or 3α-Acetoxy-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic acid methyl ester)



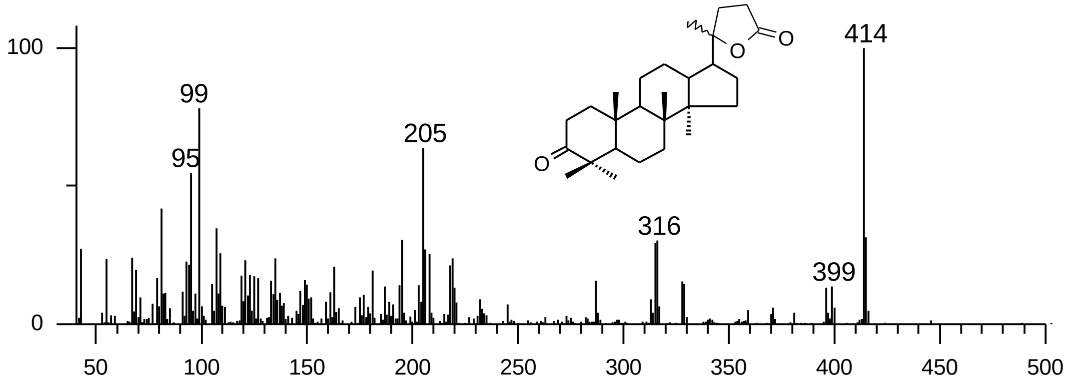
22. Dammarenediol (20-dammar-24-ene-3β,20-diol)



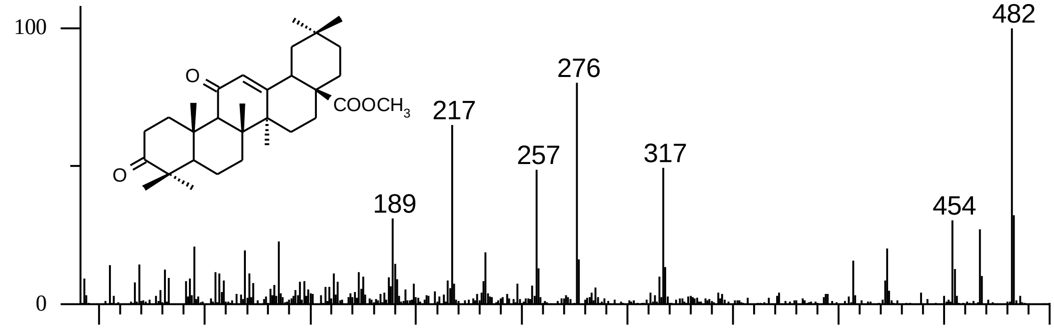
24. Ursolic aldehyde (3-hydroxy-urs-12-en-28-al)



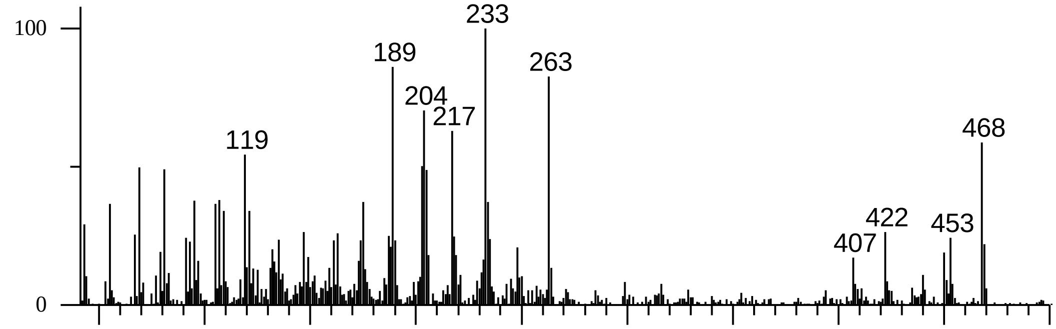
25. Hexakisnor-dammaran-3,20-dione



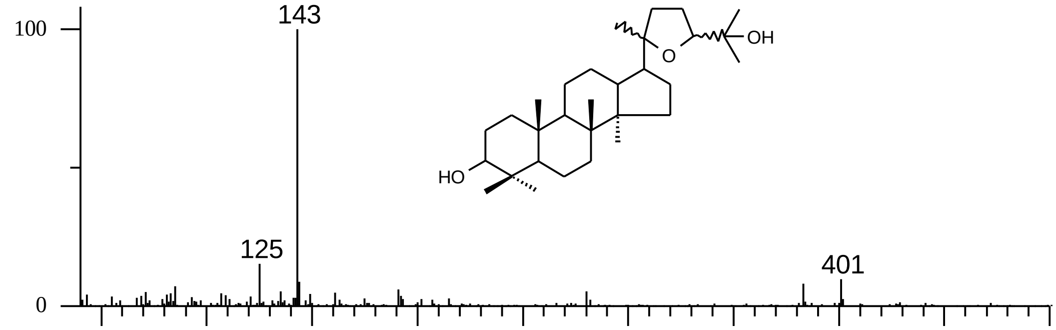
26. 3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone



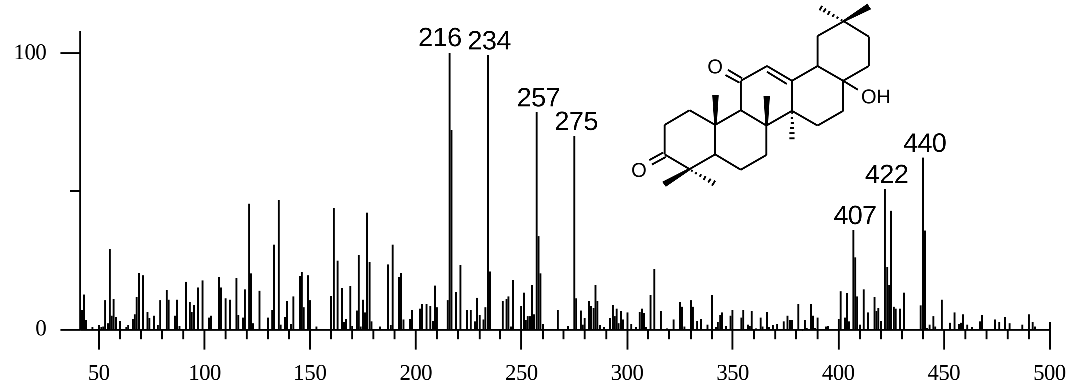
27. 11-Oxo-oleanonic acid methyl ester (3,11-dioxo-olean-12-en-28-oic acid methyl ester)



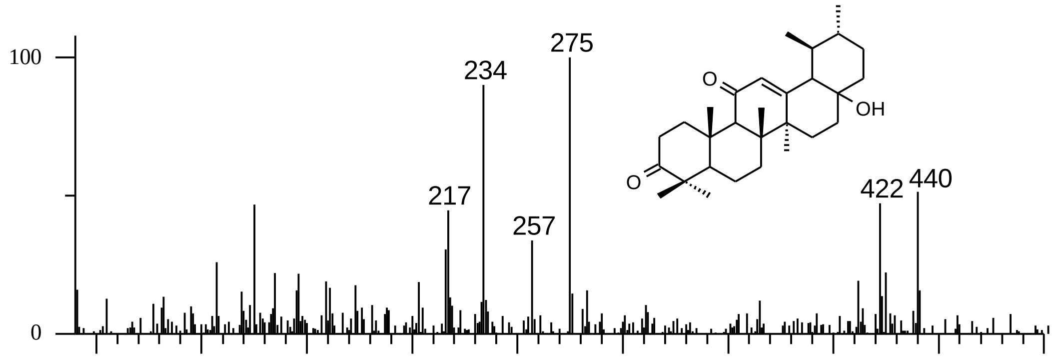
28. Oxidised oleanane type molecule



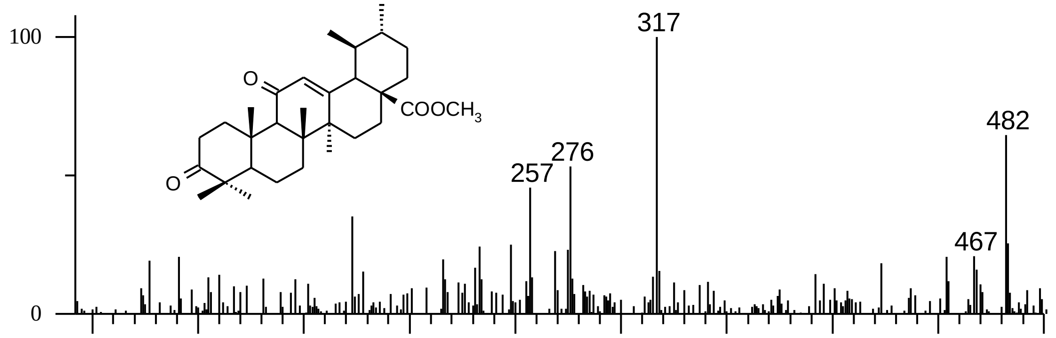
29. 20,24-Epoxy-25-hydroxy-dammaran-3-ol



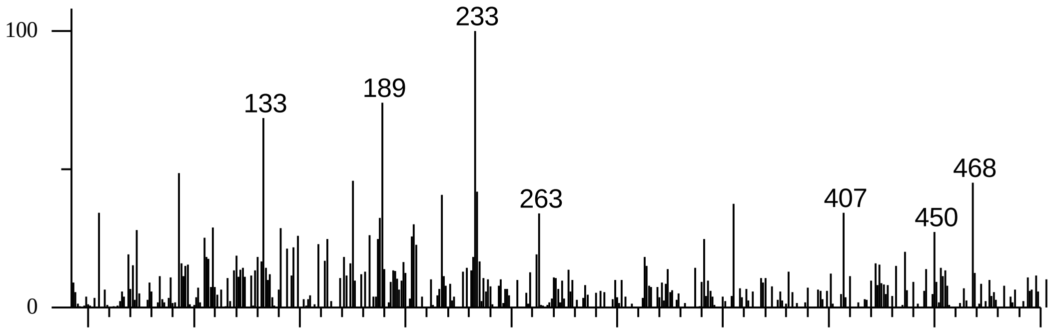
30. 17-Hydroxy-11-oxo-nor-β-amyrone (3,11-dioxo-17-hydroxy-28-nor-olean-12-ene)



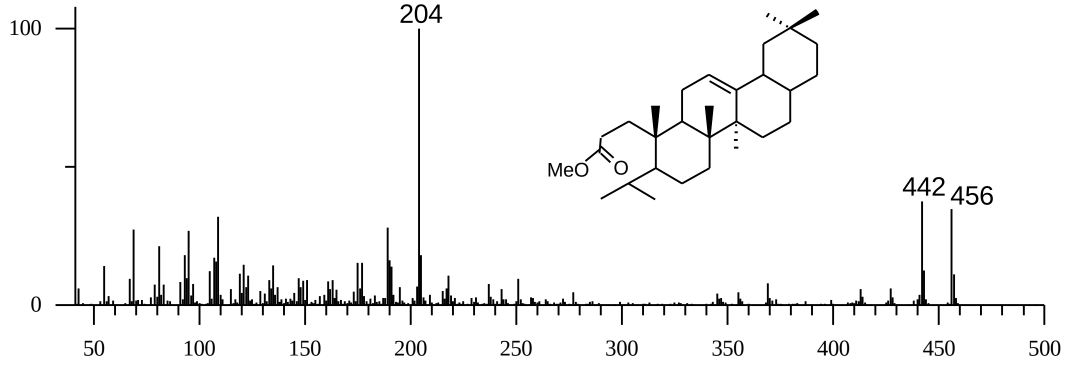
31. 17-Hydroxy-11-oxo-nor-α-amyrone (3,11-dioxo-17-hydroxy-28-nor-urs-12-ene)



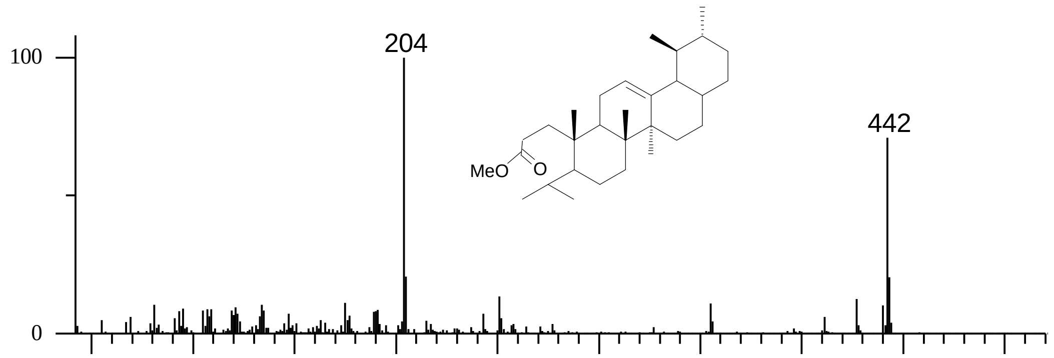
32. 11-Oxo-ursonic acid methyl ester (3,11-dioxo-urs-12-en-28-oic acid methyl ester)



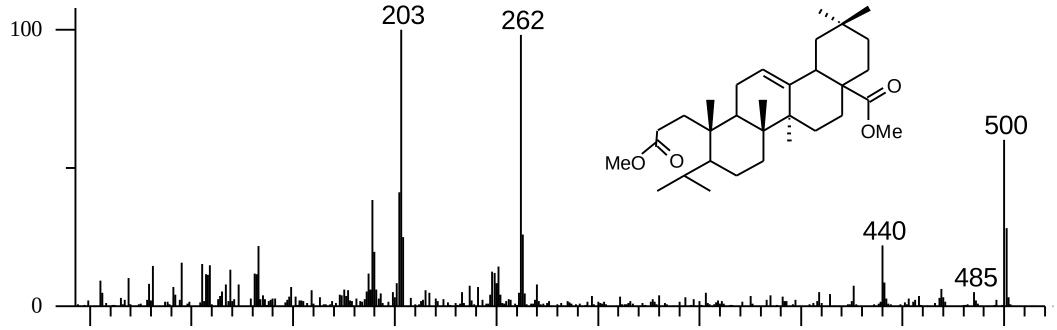
33. Oxidised ursane type molecule



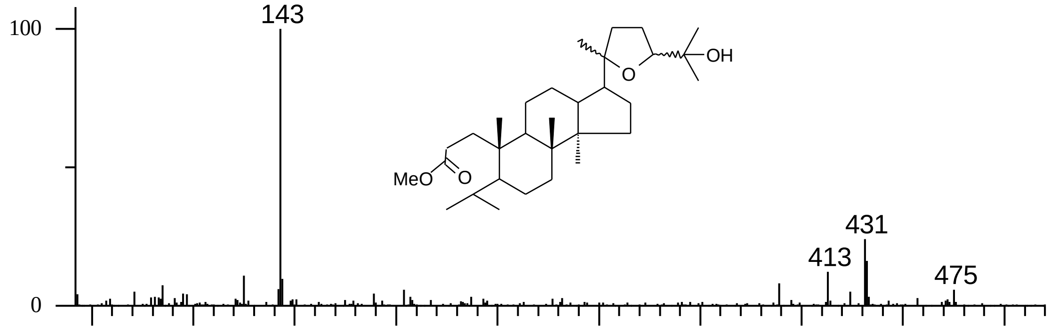
34. 3,4-Seco-28-nor-olean-12-en-3-oic acid methyl ester



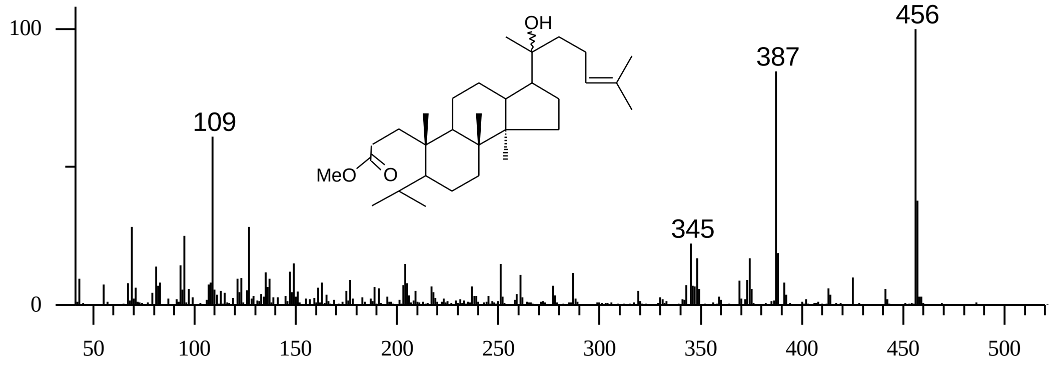
35. 3,4-Seco-28-nor-urs-12-en-3-oic acid methyl ester



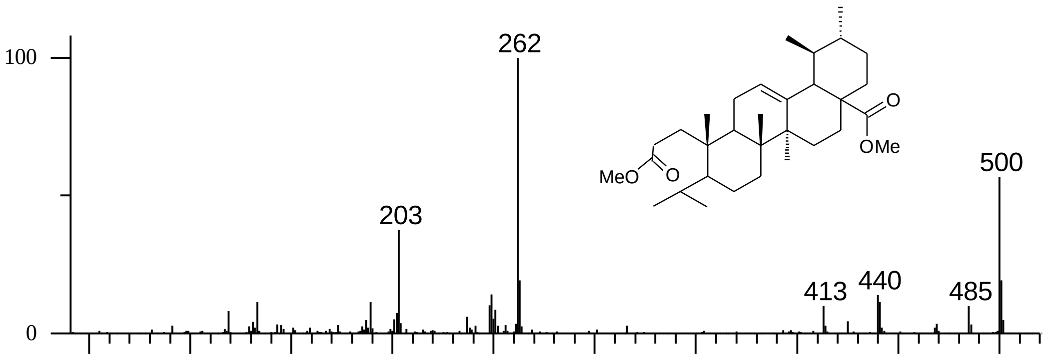
36. 3,4-Seco-olean-12-en-3,28-dioic acid dimethyl ester



37. 20,24-Epoxy-25-hydroxy-3,4-seco-dammaran-3-oic acid methyl ester

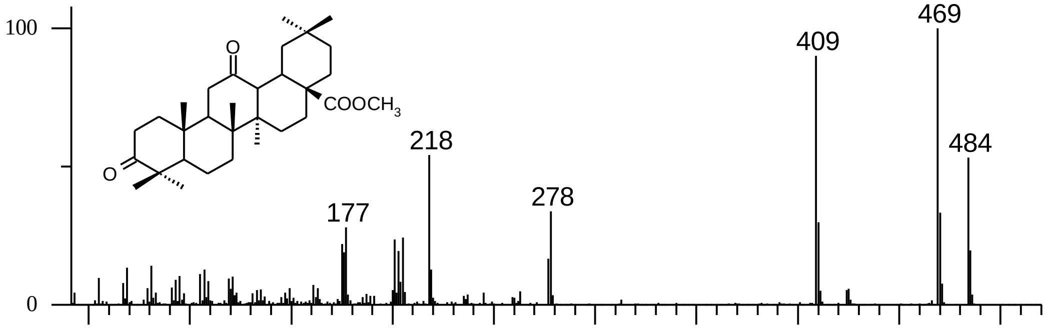


38. Dihydro-dammarenolic acid methyl ester (20-hydroxy-3,4-seco-24-dammaren-3-oic acid methyl ester)

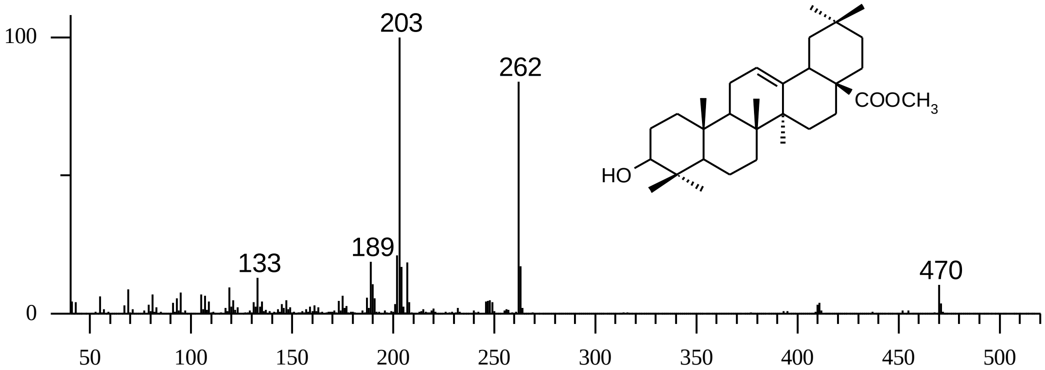


39. 3,4-Seco-urs-12-en-3,28-dioic acid dimethyl ester

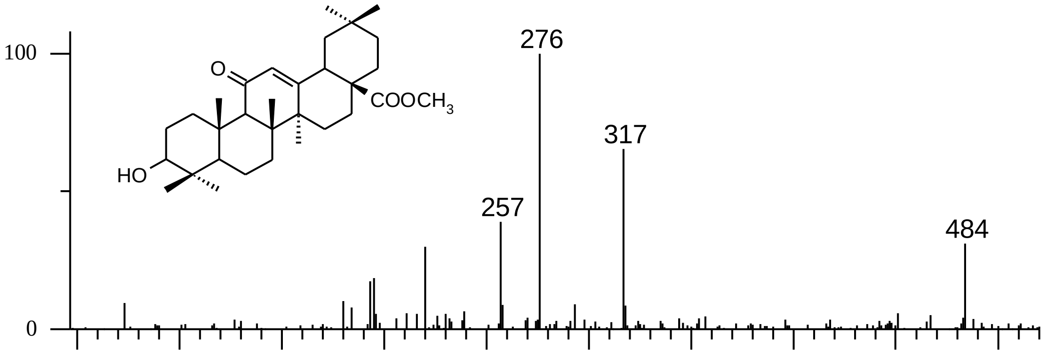
41. Methyl ester of 3,4-seco-2-carboxy-25,26,27-trisnor-4(28)-dammareno-24,20-lactone



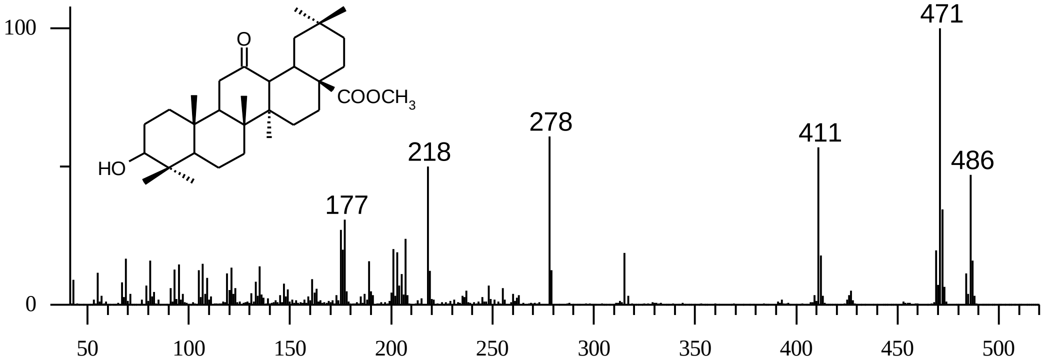
42. 3,12-Dioxo-olean-28-oic acid methyl ester



43. Oleanolic acid methyl ester (3-hydroxy-olean-12-en-28-oic acid methyl ester)



44. 11-Oxo-oleanolic acid methyl ester (3-hydroxy-11-oxo-olean-12-en-28-oic acid methyl ester)



45. 3-Hydroxy-12-oxo-olean-28-oic acid methyl ester

Summary

The natural triterpenoid resins, dammar and mastic, are often used as painting varnishes. Degradation of these natural varnishes results in yellow and brittle films, which obscure the painted images underneath. The main objective of this thesis is to investigate the molecular changes that occur in triterpenoid varnishes during ageing on paintings. For this purpose, the chemical composition of both fresh and aged varnishes from paintings was investigated. In addition, it was investigated whether triterpenoid varnishes, which were aged according to several artificial light ageing methods, have similar chemical compositions as varnishes aged on paintings. Chapter 1 gives a short introduction on the use of dammar and mastic as painting varnishes and introduces a hypothetical model for the molecular changes due to ageing of triterpenoid varnishes.

The investigation of fresh dammar and mastic resin is a necessary prestudy for the questions addressed in the chapters dealing with aged triterpenoid material. To trace the molecular changes that are induced by ageing the chemical composition of the fresh starting material has to be well known. The chemical composition of fresh dammar and mastic is investigated by gas chromatography-mass spectrometry (GCMS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and direct temperature-resolved mass spectrometry (DTMS) in Chapter 2. Fifteen compounds with dammarane, oleanane, ursane and hopane skeletons are identified in fresh dammar resin, whereas ten compounds with the euphane, oleanane and dammarane skeleton as well as two bicyclic triterpenoids are identified in fresh mastic resin.

In the Appendix of Chapter 2, the dammar precipitate, which is usually formed when preparing a solution of fresh dammar varnish, is characterised. GCMS, DTMS and size exclusion chromatographic (SEC) analysis demonstrate that this precipitate consists of relatively polar, acidic material together with a fraction of dimerised or highly oxidised triterpenoid material. In contrast to earlier beliefs, the precipitate does not consist of the dammar polymer polycadinene.

A large number of aged yellowed varnishes were collected from paintings from several museum collections and private collections (Chapter 3). DTMS was

demonstrated to be a useful technique for the discrimination between aged triterpenoid, diterpenoid and synthetic varnishes. A number of aged triterpenoid varnishes were analysed by GCMS and HPLC-MS in order to identify oxidised triterpenoids, which are formed during the natural ageing processes. These aged triterpenoid varnishes were found to contain similar oxidation products in varying relative distributions. These distributions most likely depend on a number of unknown factors, such as the age of the varnish, the environmental conditions in the museum and the restoration history of the painting. GCMS, HPLC-MS and DTMS all suggest that, in addition to oxidation and chain cleavage reactions, cross-linking reactions occur during ageing on a painting. The positions in the molecules where oxygen is incorporated and molecules are cleaved during natural ageing could be identified. It is likely the ageing process gives rise to the formation of specific fractions in aged varnishes, which may have different solubility characteristics. Especially the cross-linked fraction was found to be less soluble in a common solvent (ethanol) used by painting restorers for varnish removal. Yellowing is one of the problems induced by varnish degradation. Identification of the yellowing compounds of aged triterpenoid varnishes was attempted with HPLC in combination with UV/VIS diode array detection, showing that absorption between 390 and 430 nm is observed for compounds with a relatively low molecular weight. In addition, analysis by SEC-UV/VIS demonstrates that an unidentified, but possibly cross-linked or highly oxidised, fraction (900/1000 Da) exhibits a higher absorption in the blue region than the triterpenoid fraction (400 Da).

Despite the fact that artificial ageing is often applied in conservation science, it has never been investigated whether the various methods of artificial ageing available simulate the molecular ageing processes as found on paintings. In Chapter 4, GCMS and DTMS are used to investigate the molecular composition of a number of artificially light aged varnishes, which is subsequently compared to the chemical composition of aged varnishes from paintings. Exposure by xenon-arc light simulates the oxidation and cross-linking processes as found on paintings, only when irradiation by UV light is excluded. In the presence of UV light the oxidation processes that occur on paintings only take place to a limited degree and instead a number of new compounds are formed, which have not been demonstrated before, to the best of our knowledge, on paintings. These compounds were identified by their electron impact fragmentation patterns, which indicated the presence of oxidised A-rings. The amount of UV light is also important for the fluorescent tube light ageing of triterpenoid varnish films. Under low UV conditions the ageing processes as found on paintings are simulated, whereas under high UV conditions the A-ring oxidation takes place as well. It is therefore advisable to exclude UV light for the artificial light ageing of triterpenoid varnishes.

It is difficult to assess the exact oxidation mechanisms of the triterpenoid constituents by investigation of the chemical composition of ‘naturally’ or artificially aged varnishes, due to the complexity of these samples. It is more straightforward to model the oxidation processes, which are found to take place in picture varnishes, by subjecting a single pure triterpenoid compound to these ‘ageing’ processes. However, it is difficult to cast a film of a pure triterpenoid compound. Therefore, a new method is explored in Chapter 5, in which triterpenoid samples are “aged” in solution by exposing them to the radiation of a fluorescent tube light device. Reactive species, such as reactive oxygen species or radicals, are formed in the solution, inducing molecular changes in the triterpenoid compounds. The solvents dichloromethane (DCM) and acetone, and the photosensitizers Merocyanine 540 and FotoFenton 2 dissolved in acetonitrile or dichloromethane were found to generate reactive species by irradiation, which induce similar oxidation and cross-linking processes in the triterpenoid samples as found on paintings. Information was obtained on the oxidation mechanisms of the model compounds hydroxydammarenone and oleanolic acid. SEC in combination with UV/VIS diode array detection demonstrated that the yellow colour of a dammar sample, which was light exposed in DCM for a relatively long period of time, was caused by the formation of relatively high molecular weight material. This method of solvent ageing can be successfully used for the investigation of the oxidation mechanism of triterpenoids and the preparation of the cross-linked fraction of aged dammar and mastic varnishes.

Mass spectrometry is the main technique used in this thesis for the molecular identification of triterpenoids. Chapter 6 describes the mass spectrometric behaviour of the triterpenoids found in fresh resins and aged varnishes. The molecular information of mass spectra obtained under electron ionisation (EI) and atmospheric pressure chemical ionization (APCI) conditions is compared for a number of triterpenoids with different types of skeletons. EI promotes fragmentation of a molecule and yields structural information, whereas APCI-MS 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. An atlas is provided in this thesis, which consists of the EI spectra (70 eV) of 42 triterpenoid compounds identified in fresh and aged triterpenoid varnishes.

Samenvatting

De triterpenoïde boomharsen dammar en mastiek worden veelvuldig toegepast als schilderijvernissen. Ten gevolge van degradatieprocessen worden deze natuurlijke harsen na verloop van tijd geel en treedt er verbrossing op, waardoor het schilderij minder “leesbaar” wordt. Het bestuderen van de moleculaire veranderingen die optreden tijdens de veroudering van triterpenoïde vernissen op schilderijen vormt het belangrijkste onderwerp van dit proefschrift. Tevens is bestudeerd of de chemische samenstelling van verschillende kunstmatig verouderde vernissen vergelijkbaar is met de chemische samenstelling van “natuurlijk” verouderde vernissen afkomstig van schilderijen. In Hoofdstuk 1 wordt het gebruik van dammar en mastiek als schilderijvernissen kort uiteengezet en wordt tevens een hypothetisch model geïntroduceerd, welke de moleculaire veranderingen in triterpenoïde vernissen ten gevolge van veroudering beschrijft.

Om de moleculaire veranderingen ten gevolge van veroudering te traceren is het noodzakelijk te weten wat de chemische samenstelling is van het verse uitgangsprodukt, de verse dammar- en mastiekhars. De analytische technieken gas chromatografie-massaspectrometrie (GCMS), hoge druk vloeistof chromatografie-massaspectrometrie (HPLC-MS) en directe temperatuurs-opgeloste massaspectrometrie (DTMS) zijn gebruikt voor de analyse van de verse harsen (Hoofdstuk 2). Vijftien triterpenoïde verbindingen met dammaraan, oleanaan, ursaan of hopaan skeletten zijn geïdentificeerd in geval van dammarhars en tien triterpenoïde verbindingen met euphaan, oleanaan of dammaraan skeletten evenals twee bicyclische triterpenoïden in geval van mastiekhars.

Bij de bereiding van verse dammarvernis oplossingen wordt door restaurateuren vaak een hinderlijke neerslag waargenomen. De karakterisering van dit neerslag met behulp van GCMS, DTMS en een chromatografische techniek die moleculen op grootte scheidt (SEC) wordt in de Appendix van Hoofdstuk 2 beschreven. Het neerslag blijkt te bestaan uit een relatief polaire, zure triterpenoïd fractie en een gedimeriseerde of hoog geoxideerde triterpenoïd fractie. In tegenstelling tot vroegere opvattingen bestaat de neerslag niet uit het dammar polymeer polycadineen.

De analyse van een groot aantal verouderde, vergeelde vernissen afkomstig van schilderijen uit diverse museumcollecties en privé-collecties is beschreven in Hoofdstuk 3. DTMS bleek een zeer geschikte methode te zijn om verouderde triterpenoïde, diterpenoïde en synthetische schilderijvernissen te onderscheiden. Een groot aantal triterpenoïde vernissen is met GCMS en HPLC-MS geanalyseerd om geoxideerde triterpenoïden te kunnen identificeren. Op deze manier konden de plaatsen in de moleculen waar zuurstof-inbouw plaatsvindt en waar bindingen worden verbroken getraceerd worden. De verouderde triterpenoïde vernissen bleken dezelfde oxidatieprodukten te bevatten, maar in verschillende verhoudingen. Deze verhoudingen zijn waarschijnlijk afhankelijk van een aantal onbekende factoren, zoals de ouderdom van het vernis, de bewaarcondities en restauratiegeschiedenis van het schilderij. Het blijkt dat naast oxidatie- en degradatieprocessen ook ‘cross-linking’ reacties een rol spelen tijdens de veroudering. Waarschijnlijk leidt het verouderingsproces tot de vorming van bepaalde fracties in vernissen met verschillende oplosbaarheidseigenschappen. Vooral de ‘gecross-linkte’ fractie bleek minder goed oplosbaar te zijn in een voor vernisverwijdering gebruikelijk oplosmiddel (ethanol). Vergeling is een van de grootste problemen van de veroudering van schilderijvernissen. Met behulp van HPLC in combinatie met UV/VIS diode array detectie werd aangetoond dat verbindingen met een relatief laag moleculair gewicht een absorptie vertonen in het 390-430 nm gebied. Met SEC-UV/VIS is het mogelijk om verbindingen met hogere molecuulgewichten te analyseren. SEC-UV/VIS analyse toonde aan dat een tot nu toe ongeïdentificeerde, maar waarschijnlijk ‘gecross-linkte’ of hoog geoxideerde, fractie (900/1000 Da) relatief meer absorptie van blauw licht vertoont dan de triterpenoïd fractie (400 Da).

Ondanks het feit dat kunstmatige veroudering veelvuldig is toegepast voor de bestudering van de verouderingsverschijnselen van schilderijmaterialen, is het nooit onderzocht of de verschillende kunstmatige verouderingsmethoden dezelfde moleculaire processen genereren als die op schilderijen optreden. In Hoofdstuk 4 wordt de analyse van een aantal kunstmatig verouderde triterpenoïde vernissen met behulp van GCMS en DTMS beschreven. De chemische samenstelling van deze vernissen is vervolgens vergeleken met de chemische samenstelling van ‘natuurlijk’ verouderde vernissen van schilderijen. Het bleek dat de vaak gebruikte xenon booglamp-belichtingsapparatuur alleen bij gebruik van een UV filter dezelfde moleculaire oxidatie- en ‘cross-linking’ processen genereert als op schilderijen. Zonder UV filter worden voornamelijk bepaalde typen geoxideerde verbindingen gevormd, die nog niet eerder in verouderde schilderijvernissen gevonden zijn. Deze verbindingen werden geïdentificeerd op basis van de electron-ionisatie massaspectra en blijken allemaal een geoxideerde A-ring te bevatten. Voor kunstmatige veroudering met behulp van TL-buizen geldt hetzelfde. Bij gebruik van licht met een laag UV gehalte worden dezelfde processen gegenereerd als op schilderijen. Bij een hoog UV gehalte treedt tevens A-ring oxidatie van de triterpenoïden op. Het gebruik van een UV filter bij de kunstmatige veroudering van triterpenoïde vernissen lijkt daarom essentieel voor een correcte simulatie.

Het is niet eenvoudig de exacte oxidatiemechanismen van triterpenoïde verbindingen te ontrafelen door de chemische samenstelling van ‘natuurlijk’ of kunstmatig verouderde dammar- of mastiekvernissen te onderzoeken. Deze verouderde monsters bevatten namelijk een groot aantal componenten die tijdens de veroudering gevormd zijn. Het is veel eenvoudiger om oxidatieprocessen te modelleren aan de hand van de veroudering van één zuivere triterpenoïd verbinding. Het is echter lastig om van één zuivere triterpenoïd verbinding een dunne laag te maken. Vandaar dat in Hoofdstuk 5 een nieuwe techniek beschreven wordt, waarbij triterpenoïde monsters ‘verouderd’ worden in oplossing door ze bloot te stellen aan belichting van TL-buizen. Hierbij worden reactieve verbindingen, zoals radicalen of vormen van reactieve zuurstof, gegenereerd in de oplossing, welke vervolgens moleculaire veranderingen teweegbrengen in de triterpenoïde verbindingen. De oplosmiddelen dichloormethaan (DCM) en aceton, en de fotosensitizers Merocyanine 540 en FotoFenton 2 opgelost in acetonitril of dichloormethaan, bleken bij belichting dezelfde oxidatie- en ‘cross-linking’ processen in triterpenoïde verbindingen te induceren als in schilderijvernissen. Met deze techniek werd informatie verkregen over het oxidatiemechanisme van de triterpenoïde modelverbindingen, hydroxydammarenone en oleanolic acid. Met SEC-UV/VIS werd aangetoond dat de gele kleur van een dammar monster, wat relatief lang belicht was in DCM, veroorzaakt wordt door de aanwezigheid van een fractie met een relatief hoog moleculair gewicht. Deze methode van verouderen in oplossing bleek uitermate geschikt voor de bestudering van de oxidatiemechanismen van triterpenoïde verbindingen en voor de bereiding van een ‘gecross-linkte’ fractie van verouderde dammar- en mastiekvernissen.

Voor de moleculaire identificatie van triterpenoïde verbindingen is in dit proefschrift voornamelijk massaspectrometrie gebruikt. In Hoofdstuk 6 wordt daarom het fragmentatiegedrag beschreven van de triterpenoïde verbindingen die voorkomen in verse harsen en verouderde vernissen. De moleculaire informatie die verkregen kan worden uit de massaspectra, opgenomen onder electron-ionisation (EI) of atmosferische druk chemische ionisatie (APCI) condities, wordt besproken voor een aantal triterpenoïden met verschillende skelettypen. EI induceert fragmentatie van het molecuul en verschaft moleculaire structuurinformatie. De APCI-MS spectra geven voornamelijk moleculairgewichtsinformatie, naast informatie over de aanwezigheid van een aantal veel voorkomende functionele groepen. De mate van fragmentatie kan met behulp van het APCI “cone voltage” beïnvloed worden. In de meeste gevallen kon met additionele MS-MS experimenten onder APCI condities geen aanvullende informatie verkregen worden, omdat vergelijkbare MS-MS fragmenten voor triterpenoïde verbindingen met verschillende skelettypes gevormd worden. Het proefschrift eindigt met een atlas van de EI spectra (70 eV) van 42 triterpenoïde verbindingen geïdentificeerd in verse harsen en verouderde vernissen.

Dankwoord

Leuk zo’n dankwoord, eindelijk iets persoonlijks in dit, voor de meeste van u, vrij droge boekje. De afgelopen jaren zijn vooral leuk en leerzaam geweest. Om te beginnen bij de werkomgeving, het FOM Instituut AMOLF. Ik heb dit instituut altijd als heel prettig ervaren, waar dingen goed geregeld zijn, mensen vriendelijk met elkaar omgaan en er veel mogelijkheden worden geboden om onderzoek te doen, cursussen te volgen en conferenties te bezoeken. Ook bij mijn promoter Jaap Boon waren er onderzoeksmogelijkheden ten overvloede. Jaap, het heeft me altijd verbaasd dat je in een kwartiertje werkoverleg me weer voor maanden/jaren werk kon bezorgen. Vanwege jouw hoge mate van creativiteit, enthousiasme en het vermogen om over bepaalde grenzen heen te kunnen kijken was je betrokken bij veel uiteenlopende projecten. Ik waardeer het enorm dat ik ondanks deze werkdrukte op ieder moment van de dag kon binnenlopen en je altijd klaar stond om vragen te beantwoorden. Bedankt! Een andere steunpilaar in wetenschappelijk en persoonlijk opzicht was Klaas Jan van den Berg. Het was erg waardevol om altijd bij iemand je ei te kunnen leggen!

Ik kwam op het AMOLF met twee linkerhanden wat betreft technische aangelegenheden (“een baco...eh...wat is dat?”). Het AMOLF is daarin een harde leerschool gebleken (“Oh, doet de HPLC het niet, nou haal ‘m dan even uit elkaar”). Mijn natuurlijke aanleg om alle technische apparaten als black boxes te beschouwen, heb ik dan ook snel moeten onderdrukken en na 4 jaar moet ik toegeven dat ik er nu zelfs de lol van inzie als een technisch hoogstandje het begeeft. Met technische hoogstandjes ben ik aangekomen bij de vaklui van onze groep. Jos, Gert, Leo, Jerre, Marc en Annebeth, jullie zorgden voor de broodnodige randvoorwaarden van het doen van onderzoek. Zonder jullie was ik nu nog steeds bezig met het openschroeven van de HPLC-pomp. Bedankt! In het bijzonder wil ik Tina hartelijk bedanken voor het corrigeren van mijn teksten.

Het werken in het multidisciplinaire MOLART team heb ik altijd één van de aantrekkelijke kanten van dit promotieonderzoek gevonden. Hoewel het soms lastig was om elkaars taal te begrijpen, heb ik de multidisciplinaire samenwerking altijd als erg interessant en grensverleggend ervaren. Ik vond het erg leuk om een kijkje te krijgen in de wereld van restaurateuren en kunsthistorici en wil alle leden van het MOLART team (Prof. Dr. van Asperen de Boer, Prof. Dr. van de Wetering, Anne, Margriet, Mireille, René Hop, Ken, Karin, René B., Arie, Alan en Sibylle) bedanken voor de leerzame en vooral ook gezellige tijd. In het bijzonder wil ik René Hoppenbrouwers noemen met wie ik een ideale samenwerking heb gehad. René, dankzij jouw onverwoestbare humeur was het altijd een plezier om naar Maastricht te gaan. Tevens wil ik ook alle restaurateuren, die genoemd zijn aan het eind van hoofdstuk 3, nogmaals bedanken voor het beschikbaar stellen van het grote aantal verouderde vernissen.

Collaboration with colleagues from other research centers was one of the nicest aspects of my promotion period. I thank Prof. Dr. René de la Rie for his invitation to spend one month at the National Gallery of Art in Washington. I enjoyed this visit enormously! During my stay Nobuko Shibayama and I formed a close ‘LCMS’ team. Nobuko, it was a real pleasure to collaborate with you! Another institute that I was able to visit was the Canadian Conservation Institute in Ottawa. I was very lucky to meet and collaborate with Dr. Leslie Carlyle. Thank you Leslie, I shall never forget your kind hospitality during my stay. Ook in Nederland was er een nauw samenwerkingsverband. Mijn bijzondere dank gaat uit naar Dr. Wim Genuit (Shell Research and Technology Center Amsterdam) voor de plezierige samenwerking en de mogelijkheid om me allerlei massaspectrometrische technieken eigen te maken.

Het feit dat ik met veel plezier kan terugkijken op mijn promotieperiode is misschien nog wel het meest te danken aan de MacroMol groep. Werken in het kippenhok was niet altijd even makkelijk, maar wel altijd erg gezellig. Alle MacroMollers (Oscar, Jorrit, Georgiana, Sander, Nicolas, Marc, Gerard, Ivana, Jos, Gert, Inez, Parisz, Linda, Piet, Jerre, Leo, Annebeth, Klaas Jan, Ron, Vincent, Janine, Jaap, Liz, Muriel, Marieke, Erik en Jan) bedankt voor de goede sfeer!

Tot slot wil ik mijn ouders bedanken voor het feit dat ze er altijd voor me zijn! Het is heerlijk om te beseffen dat ‘the next generation’ zo’n liefdevolle opa en oma zal krijgen. Met ‘the next generation’ ben ik in meerdere opzichten aangekomen bij de belangrijkste persoon die ik wil bedanken. Maarten, mijn hart wil nu heel poëtisch gaan doen, maar daar is dit proefschrift misschien niet de meest geschikte plaats voor. Dank je wel!