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

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
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 an 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 stablisers (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.
Triterpenoid varnishes: the ageing process

**Fresh varnish:** ordered layers of triterpenoids

![Diagram of fresh varnish with TTP and Oxy TTP compounds]

**Stage 1 Oxidised varnish:** Oxygen uptake

Formation of excited and oxidised triterpenoids

![Diagram of oxidation process with light excitation and cross-links]

**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.
Chapter 1

References


