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Artificial ageing of varnish triterpenoids in solution

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Abstract

A new photochemical method is presented for the artificial ageing of the triterpenoid resins dammar and mastic, which are commonly used as painting varnishes. Varnish samples in solution are exposed to the radiation of a fluorescent tube device. The solvents dichloromethane (DCM) and acetone, and the photosensitisers Merocyanine 540 (MC540) and FotoFenton 2 (FF2) dissolved in acetonitrile (ACN) or DCM 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. This method provided information on the oxidation mechanisms of the model compounds hydroxydammarenone (**3**) and oleanolic acid (**8**). Furthermore, evidence was found that the formation of high molecular weight material is important for the yellowing behaviour of triterpenoid varnishes. This method of solvent ageing can be successfully used for the preparation of cross-linked fractions of aged dammar and mastic varnishes.

Keywords: Photochemistry; Triterpenoids

1. Introduction

Varnishes are often applied on paintings in order to increase the colour saturation and gloss, and to protect against ageing of the paint surface. The triterpenoid resins dammar (originating from the trees of the Dipterocarpaceae) and mastic (the bleed resin of *Pistacia lentiscus* L. (Anacardiaceae)) are frequently used as picture varnishes. However, dammar and mastic varnishes deteriorate in the course of time, leading to yellowed and brittle products. 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. Little is known about the molecular ageing processes of dammar and mastic resin on paintings, possibly due to a number of difficulties. First, 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]. The influence of these factors on the ageing process of triterpenoid varnishes is largely unknown. Secondly, 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. Earlier research on aged varnishes from paintings and artificially light aged varnishes indicated that oxidation, cross-linking and degradation processes take place during ageing [2–4]. Especially side chain oxidation of dammarane-type molecules and oxidation of C11, C17 and C28 of oleanane/ursane-type molecules have been 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 these aged varnishes. It is more straightforward to understand the oxidation processes, which are found to take place in paintings, by subjecting a single pure triterpenoid compound to ageing. Naturally, one would like to age the model compound as a film because a varnish ages on a painting as a film also. 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 was thought to complicate the investigation.

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 [5], which may initiate and generate ageing reactions in solutions of triterpenoids. This approach would allow for more control over the experiments as compared to those on films. The reagents move freely in the solution, not limited 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 varnish film. By ageing in solution, other disadvantages

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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 [6], which implies that an aged resin film is not uniform in chemical composition.

As radicals may be generated by light, we have investigated the artificial ageing of triterpenoids using photochemical methods. Photosensitisers were used, which are known to produce reactive species such as singlet oxygen, superoxide anion, OH radicals or alkyl radicals. The advantage of the application of photosensitisers 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 [5].

Photosensitisers are mainly used in 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 capacity of generating ageing processes in triterpenoids. In addition, two commercially available photosensitisers with different nodes of action were tested: Merocyanine 540 (MC540) and FotoFenton 2 (FF2) (2-hydroxyacetophenone oxime). These two specific photosensitisers were chosen because of their solubility in organic solvents. MC540 is a photosensitising dye of great therapeutic interest [7,8]. MC540 lacks specificity because it is reported to produce singlet oxygen in addition to other reactive oxygen species, including oxygen radicals [8–10]. FF2 resembles the hydroxyl radical formation of Fenton's reagent [5], but produces fewer side products. FF2 generates hydroxyl radicals by photochemical cleavage of the photosensitiser [11]. The 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.

Two pure triterpenoid compounds, hydroxydammarenone (**3**) and oleanolic acid (**8**), were solvent-aged. The compound **3** is abundant in fresh dammar and mastic resin, but **8** itself is not abundantly present in the fresh resins, However, its molecular structure is very similar to that of oleanonic acid, which is abundant in both the resins. Sample solutions were light exposed in a closed vial, to avoid 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 headspace with a large air to reagent ratio.

For the current experiments, the solutions were light-exposed in a fluorescent tube device. The chemical composition of the aged solutions was determined with gas chromatography mass spectrometry (GCMS), size exclusion chromatography (SEC) coupled with a diode array UV/VIS detector, and direct temperature-resolved mass spectrometry (DTMS). With this latter technique, heat is applied to the sample, which results in volatilisation and subsequent pyrolysis of non-volatile matter in the analyte. 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. Data can be presented as a full mass spectrum when all spectra are summarised, which provides a complete mass spectrometric overview of the sample [12].

2. Experimental

2.1. Sample preparation

Compound **3** (also called dipterocarpol) (Aldrich), **8** (Aldrich), dammar resin (A.J. van der Linde, Amsterdam, The Netherlands), mastic (H. Schmincke & Co., Erkrath, Germany), MC540 (Molecular Probes, Leiden, The Netherlands) and FF2 (Molecular Probes, Leiden, The Netherlands) were used for solvent ageing. Methanol (Merck, p.a.), ethanol (Merck, p.a.), dichloromethane (DCM) (Fluka, >99.8%, HPLC grade), acetone (Merck, p.a.), toluene (Aldrich, 99.5+%, A.C.S. reagent) and acetonitrile (ACN) (Sigma–Aldrich, 99.93%, HPLC grade) were used as solvents. Solutions of **3** and **8** 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). In the case of **3** and **8**, the molar ratio of triterpenoid to oxygen is approximately 1:50. MC540 was not completely soluble in DCM solutions. The aged mastic varnish was scraped off from the painting 'Painswick Beacon' by Philip Wilson Steer (Tate Gallery, N03884). The aged dammar varnish was removed from the painting '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)) by using a cotton swab wetted with ethanol. For analysis, this varnish was extracted from the swab with ethanol.

2.2. Ageing conditions

The glass vials containing triterpenoid solutions were irradiated in a device equipped with fluorescent tubes (TLd, 36 W, 96.5 (Philips), 13,000–13,500 lx), which emit UV/VIS radiation with a wavelength range from approximately

850 nm down to about 305 nm. The temperature was kept between 18 and 30°C with an average temperature of 25°C. The average temperature of solvent ageing of the sample, whose results are shown in Fig. 13, was about 37°C. After exposure, samples were stored in the freezer (−20°C).

2.3. DTMS

After solvent ageing, the solutions were diluted (10 times). Approximately 2 μl was applied to the DTMS probe by using a syringe (SGE, 5 μl). The aged dammar varnish solution was applied directly to the probe. In the case of fresh dammar and mastic resin and the aged mastic varnish, 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. All samples on the probe were dried in vacuo prior to introduction into 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 μm 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 s 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.

2.4. GCMS

Methanolic extracts (5 mg/ml) were prepared from fresh dammar and mastic to eliminate the polymeric fraction from the analysis. An aliquot of 25 μl of these methanolic extracts was evaporated to dryness. In the case of the aged varnishes from paintings, approximately 0.5 mg was collected. In the case of the solvent-aged samples, an aliquot of 50 μl was evaporated to dryness. For methylation, according to the method of Hashimoto et al. [13], 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 min. After evaporation to dryness, the samples were dissolved in 25 μl DCM (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 min at 50°C, subsequently to 250°C at a rate of 8°C/min and from 250 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 s 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.

2.5. 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 tetrahydrofuran (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.

3. Results and discussion

3.1. Effect of solvents

In order to test whether certain solvents are capable of inducing changes in a reagent during light exposure, **3** was dissolved in six different solvents and light-exposed for 2 weeks. The solvents methanol, toluene, ACN, ethanol, DCM and acetone were tested. Fig. 1 shows the DTMS summation spectra of (a) pure **3** and those of (b) **3** after 2 weeks of exposure in ethanol, (c) acetone and (d) DCM. Compound **3** is represented by peaks at *m*/*z* 109, 205, 355 and 424 [14]. When **3** was dissolved in methanol, ethanol, toluene and ACN, no molecular changes were induced during the 2 weeks of light exposure, as shown for ethanol in Fig. 1(b). Rao et al. [15] demonstrated that the dammarane-type molecule, cabraleone, is A-ring oxidised, which results in a carboxylic group at C2 and an isopropyl group at C5, when a solution of this triterpenoid compound in methanol is irradiated by UV light. This specific A-ring oxidation is not observed in our own experiments, in which a methanol or an ethanol solution of **3** was light-exposed for 2 weeks. This implies that, in our experimental setup, this specific A-ring oxidation pathway is not accessible, pointing to very low UV light levels. Exposure in acetone and DCM resulted in major chemical changes as seen in Fig. 1(c) and (d). Fig. 1(c) shows that the characteristic mass peaks of **3** have almost disappeared and a number of new peaks are present at *m*/*z* 143, 399 and 443. These peaks are characteristic of an ocotillone-type molecule [14]. This oxidised triterpenoid is usually found as the main triterpenoid constituent of aged dammar varnishes from paintings [3]. A similar kind of side chain oxidation could also be achieved by Bielmann [16] after treatment with *p*-nitroperbenzoic acid in ether. Fig. 1(d) shows that some **3** is still present in addition to a certain amount of ocotillone-type molecules after exposure in DCM. Compared to the irradiated **3** in acetone, exposure in DCM results in a lower degree of oxidation.

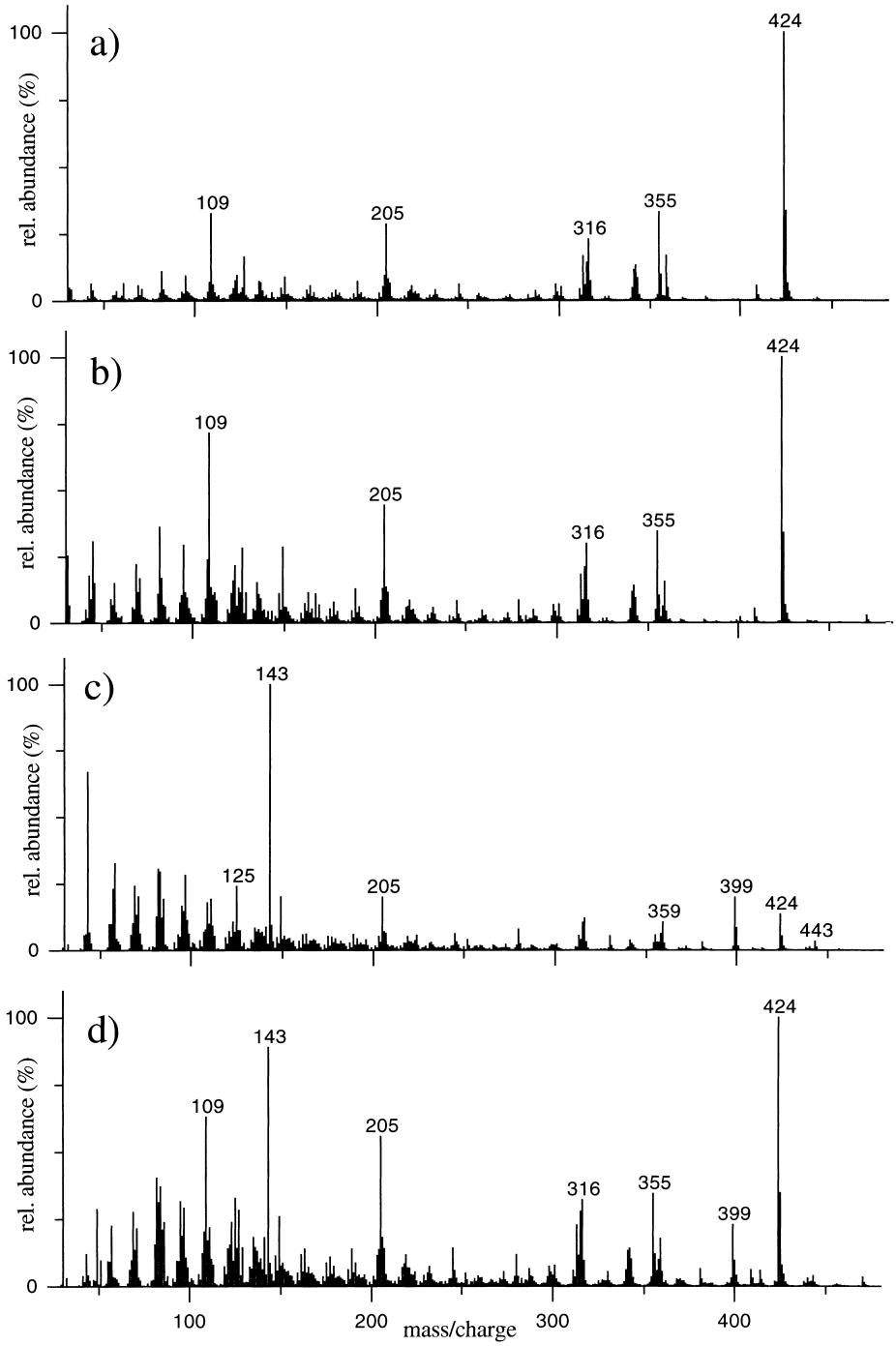

Fig. 1. DTMS summation spectra of (a) pure hydroxydammarenone, and hydroxydammarenone after 2 weeks of solvent ageing in (b) ethanol, (c) acetone, or (d) DCM.

Acetone is known to act as a photosensitiser due to its keto group. Indig et al. [17] 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 [5]. Another ketone, benzophenone, is often used for the sensitised oxidation of lipids [18,19]. Acetone absorbs UV light with a wavelength up to 350 nm [20]. 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 [6]. 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 [20]. As mentioned above, the sample solution is not irradiated with light of this wave-

length. 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 $n \rightarrow \pi^*$ transition [21]. Compound **3** 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 postulated that DCM is a good substrate for this hydrogen abstraction, which gives rise to radical formation. A large number of radicals can be formed via this mechanism, which may explain the oxidation of **3** in DCM during irradiation. This hypothesis needs further investigation.

3.2. Effect of the addition of photosensitisers

It was investigated whether two specific photosensitisers, MC540 and FF2, could induce changes in a solution of **3** when irradiated by light. Compound **3** and a photosensitiser were dissolved in ACN because ACN was found to be a 'non-active' solvent during irradiation. Fig. 2 shows the DTMS summation spectra of **3** (a) in ACN, (b) in ACN with FF2, and (c) in ACN with MC540 after an exposure period of 2 weeks. Both photosensitisers are observed to induce changes in **3**. Fig. 2(b) shows that a certain amount of **3** is still present, deduced from the peak at *m*/*z* 424. In addition, peaks at *m*/*z* 143 and 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 [3]. This compound could also be produced by Mills et al. by oxidation of **3** with chromic acid [22]. 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

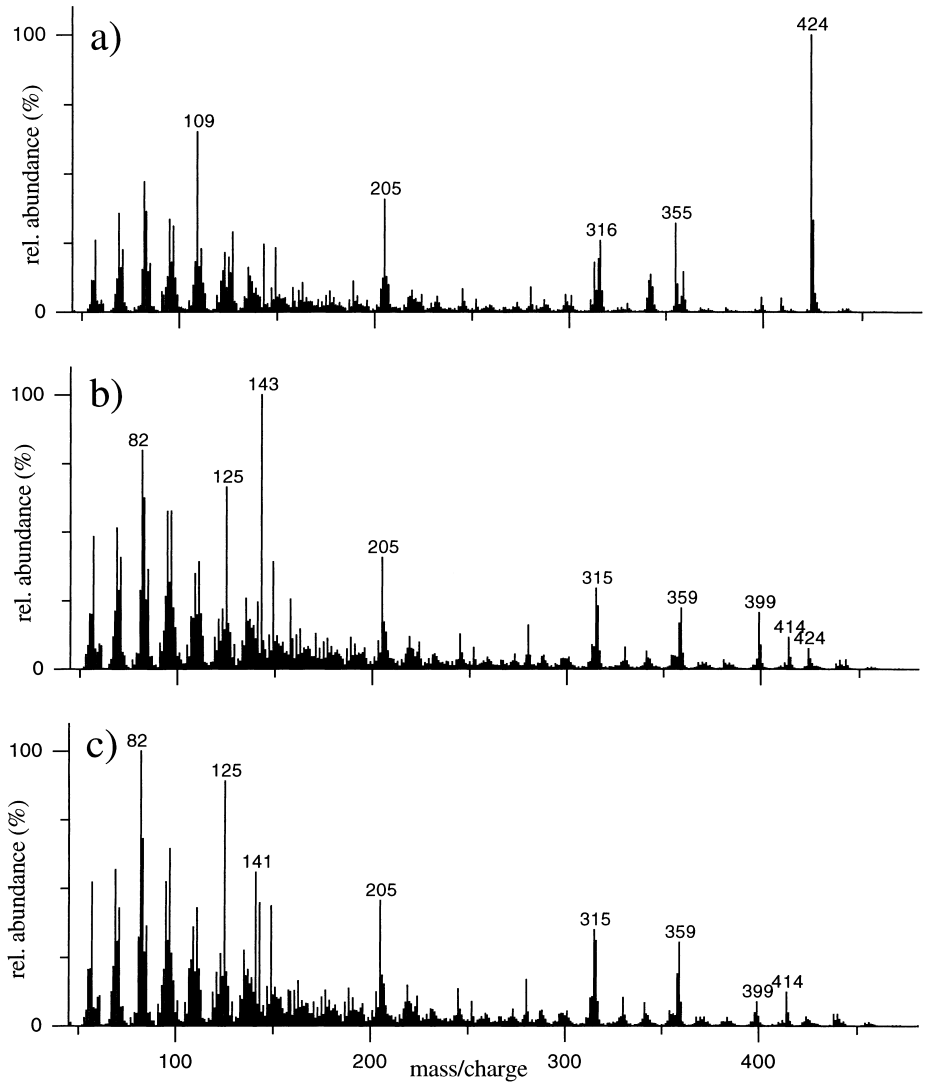

Fig. 2. DTMS summation spectra of hydroxydammarenone in ACN after 2 weeks of solvent ageing (a) without a photosensitiser, (b) with FF2, or (c) with MC540.

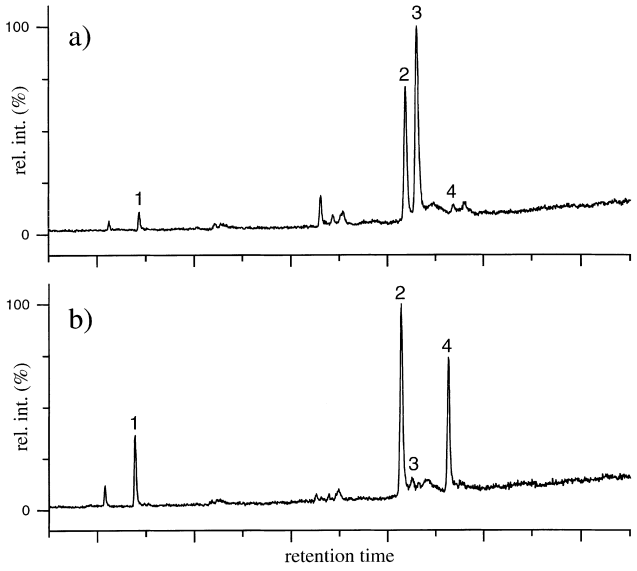

Fig. 3. Gas chromatograms of hydroxydammarenone and FF2 in ACN after (a) 1 week, or (b) 2 weeks of solvent ageing.

ocotillone-type side chain. The molecular ion region of the DTMS spectrum depicted in Fig. 2(c) mainly shows peaks that represent the lactonised molecule and ocotillone-type molecules. In addition, a peak at *m*/*z* 424 representing **3** is not present at all in Fig. 2(c), which indicates that MC540 induced relatively more changes than FF2. The DTMS spectra of Fig. 2(b) and (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 **3**, it was irradiated in ACN with FF2 for 1 and 2 weeks. GCMS (Fig. 3 and Table 1) demonstrates that, after 1 week of irradiation, **3** is partly oxidised to an ocotillone-type molecule (**2**). Small amounts of other compounds are present of which only a molecule with a shortened side chain (**1**) and the molecule with the lactonised side chain (**4**) could be identified. After 2 weeks of irradiation, almost all of **3** has been oxidised to mainly the same three compounds as mentioned above. The relative distribution of these compounds has changed. The molecule with the lactonised side chain (**4**) seems to have been formed mainly during the second week of irradiation. Probably, it is formed by oxidation of the ocotillone-type molecule (**2**) as shown in Fig. 4. The compound with the shortened side chain (**1**) is probably formed directly from **3**. All of these oxidised triterpenoids have been observed in aged varnishes from paintings [3].

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 (**5** and **6**) [3]. These compounds are probably formed by oxidation of triterpenoid compounds with a keto group at C3. However, it is unknown 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 **3**, used for the solvent ageing experiments, contains a keto group at C3 and is, therefore, a good precursor for A-ring oxidation. GCMS demonstrates that this A-ring oxidation does not take place during solvent ageing (Fig. 3). In addition, the majority of oxidised triterpenoids found in aged varnishes from paintings contain a keto group at C3 [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, is 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 'non-active' solvent ACN, it was investigated whether an additional photosensitiser affects the rate and the type of oxidation reactions that occur during light exposure in the 'active' solvent DCM. Fig. 5 shows the gas chromatograms of **3** (a) in DCM, (b) in DCM with FF2, and (c) in DCM with MC540 after an exposure period of 2 weeks. Table 1 lists the compounds identified. Fig. 5(a) shows that, after irradiation in DCM, **3** is still the main compound, in addition to a small amount of an ocotillone-type molecule (**2**), which indicates a small degree of oxidation. The presence of FF2 and MC540 clearly has an effect on the rate of the oxidation reactions. Fig. 5(b) shows that **3** has been oxidised to an ocotillone-type molecule (**2**), a lactonised molecule (**4**) and a molecule with a shortened side chain (**1**) in the presence of FF2. After exposure in the presence of MC540 (Fig. 5(c)), only the lactonised molecule (**4**) and the molecule with a shortened side chain (**1**) are present. As described above, the molecule with the lactonised side chain (**4**) is probably formed via oxidation of the ocotillone-type molecule (**2**). 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 **3** result in the same oxidation processes, as depicted in Fig. 4. 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 solution in DCM or acetone with or without an additional photosensitiser did not result in oxidation of the triterpenoids.

Fresh dammar and mastic resin also contain molecules with other carbon skeleton types, such as the isomeric oleanane/ursane skeletons. Compound **8** was used as the second model compound. Fig. 6 shows the gas chromatograms of **8** after exposure in DCM with FF2 for (a) 1 week and (b) 2 weeks. Table 1 lists the compounds identified. After 1 week of solvent ageing, **8** is still the main compound with some traces of oxidised compounds. Small amounts of compounds with additional keto groups at C3 (**7**) and C11 (**9**) are formed (Fig. 7). Another compound was formed after 1 week of ageing (**10**), which became the main compound after 2 weeks of ageing. This compound is formed when the double bond at C12 of **8** becomes oxidised and a keto group is formed at this position (Fig. 7). This compound has only

Table 1
List of compounds, with their corresponding molecular weights (MW), identified in triterpenoid samples

Label	Compound name	MW
1	Hexakisnor-dammaran-3,20-dione	358
2	20,24-Epoxy-25-hydroxy-dammaran-3-one[a]	458
3	Hydroxydammarenone (20-hydroxy-24-dammaren-3-one[b])	442
4	3-Oxo-25,26,27-trisnor-dammarano-24,20-lactone[b]	414
5	20,24-Epoxy-25-hydroxy-3,4-seco-4(28)dammaren-3-oic acid[a]	474
6	Dammarenolic acid (20-hydroxy-3,4-seco-4(28),24-dammaradien-3-oic acid[b])	458
7	Oleanonic acid (3-oxo-olean-12-en-28-oic acid)	454
8	Oleanolic acid (3-hydroxy-olean-12-en-28-oic acid)	456
9	11-Oxo-oleanolic acid (3-hydroxy-11-oxo-olean-12-en-28-oic acid)	470
10	3-Hydroxy-12-oxo-olean-28-oic acid	472
11	Nor-α-amyrone (3-oxo-28-nor-urs-12-ene)	410
12	Nor-β-amyrone (3-oxo-28-nor-olean-12-ene)	410
13	17-Hydroxy-11-oxo-nor-β-amyrone (3,11-dioxo-17-hydroxy-28-nor-olean-12-ene)	440
14	17-Hydroxy-11-oxo-nor-α-amyrone (3,11-dioxo-17-hydroxy-28-nor-urs-12-ene)	440
15	Dammaradienone (3-oxo-dammara-20(21),24-diene)	424
16	Dammaradienol (3β-hydroxy-dammara-20,24-diene)	426
17	Oleanonic aldehyde (3-oxo-olean-12-en-28-al)	438
18	Ursonic acid (3-oxo-12-ursen-28-oic acid)	454
19	Ursonic aldehyde (3-oxo-urs-12-en-28-al)	438
20	Hydroxyhopanone (21β,22-hydroxy-3-hopanone)	442
21	3,4-Seco-2-carboxy-25,26,27-trisnor-4(28)-dammareno-24,20-lactone[b]	430
22	20,24-Epoxy-25-hydroxy-dammaran-3-ol[a]	460
23	11-Oxo-oleanonic acid (3,11-dioxo-olean-12-en-28-oic acid)	468
24	11-Oxo-ursonic acid (3,11-dioxo-urs-12-en-28-oic acid)	468
25	Oxidised oleanane-type molecule	
26	(8R)-3-Oxo-8-hydroxy-polypoda-12E,17E,21-triene	442
27	Moronic acid (3-oxo-olean-18-en-28-oic acid)	454
28	(Iso)masticadienonic acid (3-oxo-13α,14β,17βH,20αH-1anosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-1anosta-7,24-dien-26-oic acid)	454
29	Idem	454
30	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
31	Idem	498
32	3,12-Dioxo-olean-28-oic acid	470

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

been found as traces in aged varnishes from paintings [23]. Therefore, 2 weeks of ageing of **8** 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 a norursane skeleton (**11** and **12**). These compounds are oxidised during ageing on the painting to compounds **13** and **14** [3]. The

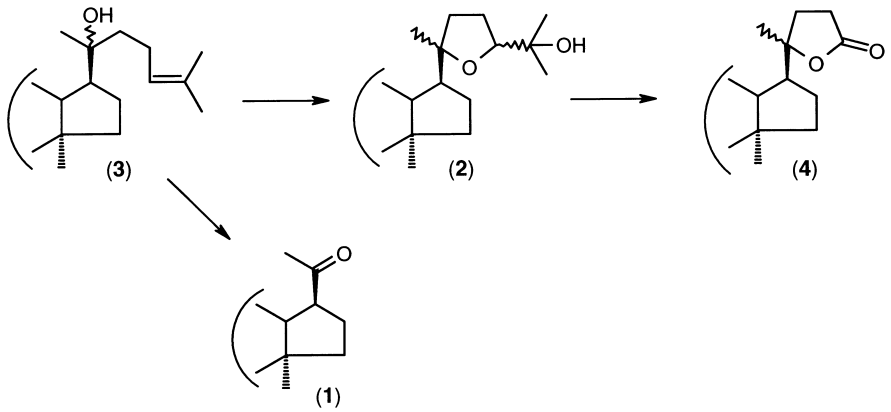

Fig. 4. Proposed oxidation mechanism of hydroxydammarenone.

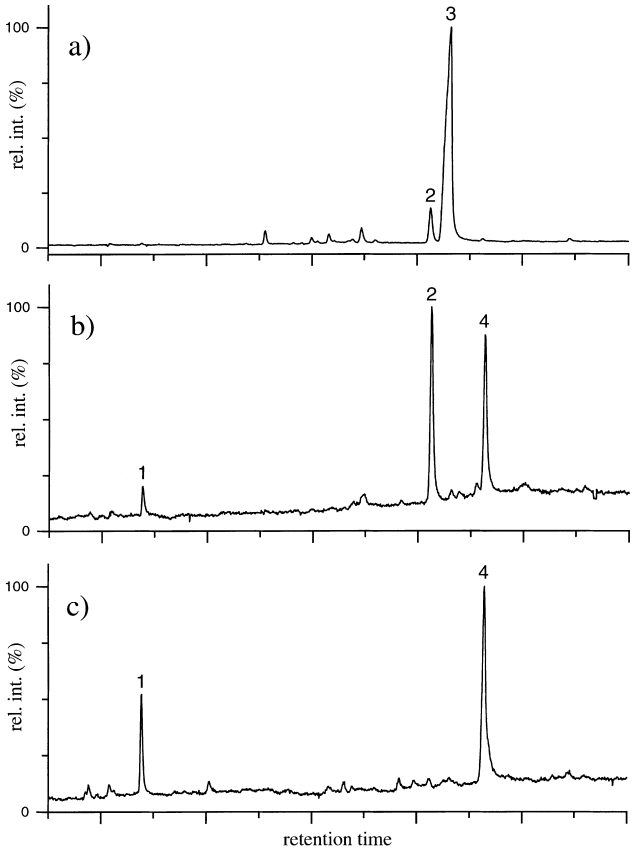

Fig. 5. Gas chromatograms of hydroxydammarenone in DCM after 2 weeks of solvent ageing (a) without a photosensitiser, (b) with FF2, or (c) with MC540.

noroleanane/norursane-type molecules are reported to be formed by decarboxylation of compounds with a carboxylic acid at C17 [24]. However, it is unknown whether this decarboxylation process takes place during biosynthesis by the action of enzymes, after excretion, or afterwards as a result of ageing on a painting. The model compound **8**, 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

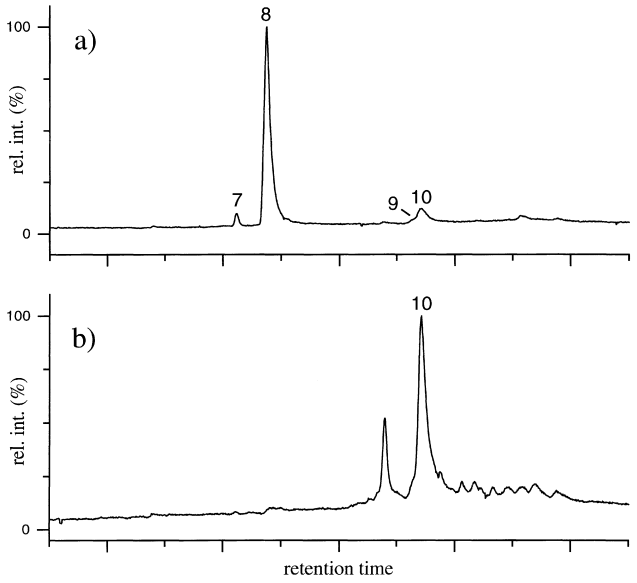

Fig. 6. Gas chromatograms of oleanolic acid and FF2 in DCM after (a) 1 week, or (b) 2 weeks of solvent ageing.

demonstrates that the 28-nor-derivative of the oleanane compound was not formed during solvent ageing (Fig. 6), which implies that the carboxylic acid group at C17 is not eliminated. In addition, the majority of the oxidised oleanane/ursane-type triterpenoids found in aged varnishes from paintings contain a carboxylic acid group at C17 [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.

3.3. Solvent ageing of dammar and mastic resin

The application of solvent ageing to **3** and **8** by combinations of solvents and photosensitisers as described above

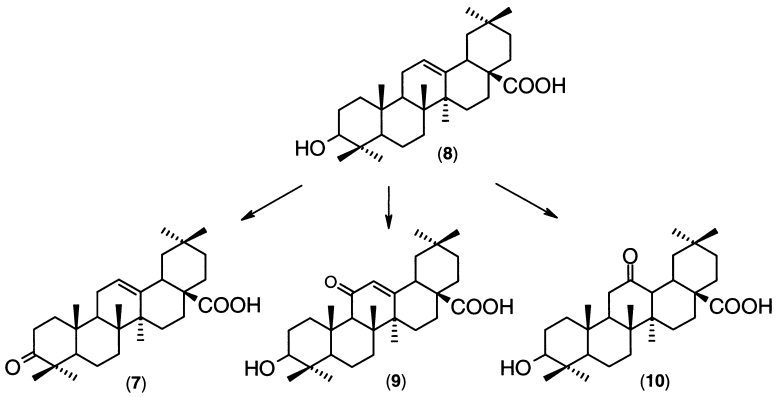

Fig. 7. Proposed oxidation mechanism of oleanolic acid.

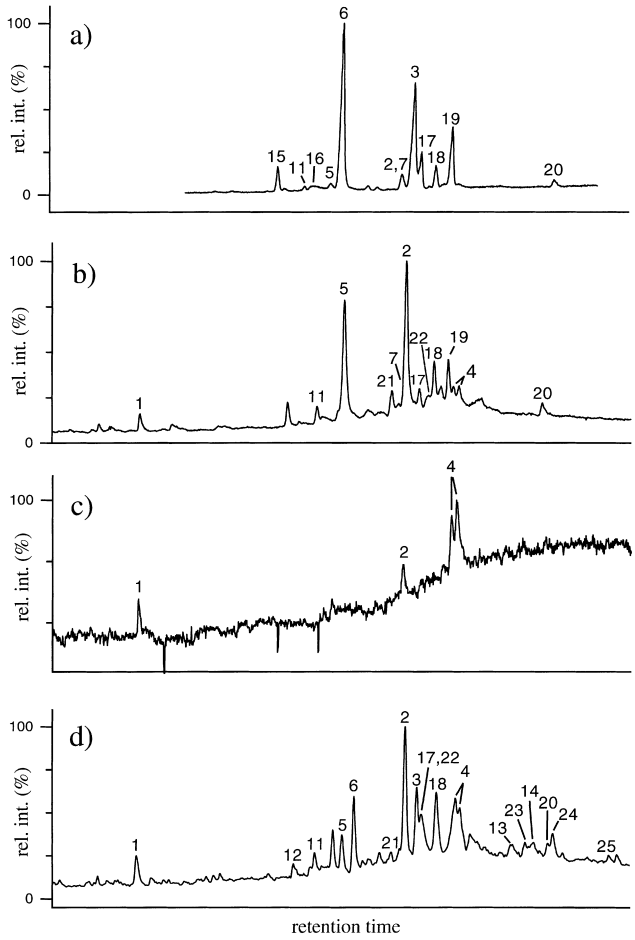

Fig. 8. Gas chromatograms of (a) fresh dammar, (b) dammar with FF2 in DCM after 1 week or (c) 2 weeks of solvent ageing and (d) the gas chromatogram of a dammar varnish aged on a painting.

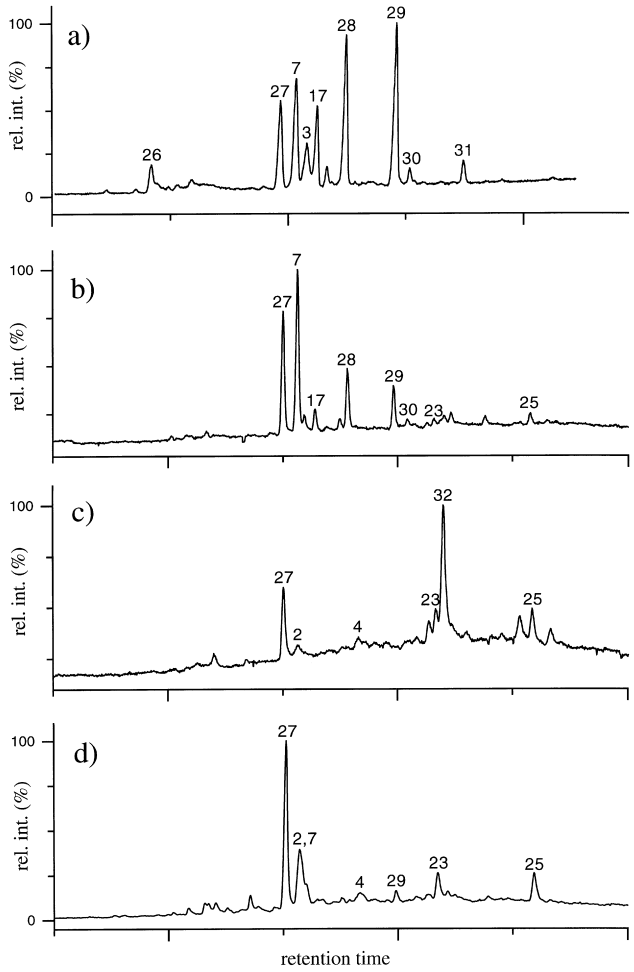

Fig. 9. Gas chromatograms of (a) fresh mastic, (b) mastic with FF2 in DCM after 1 week or (c) 2 weeks of solvent ageing and (d) the gas chromatogram of a dammar varnish aged on a painting.

resulted in similar dammarane and oleanane-type oxidation products as were found in aged varnishes from paintings [3]. 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 the new solvent ageing method. Of all the six solvents tested, only DCM was able to dissolve the resins completely due to polymeric fractions present in both resins. Therefore, dammar and mastic were irradiated in DCM with FF2 for 1 and 2 weeks. Fig. 8 (dammar) and Fig. 9 (mastic) depict the gas chromatograms of (a) the fresh resins, after solvent ageing for (b) 1 week and (c) 2 weeks. For comparison, the gas chromatograms of a typical aged dammar and mastic varnish from a painting are shown (Figs. 8(d) and 9(d)) [3]. Table 1 lists the compounds identified. The dammar and mastic solutions had turned yellow already after 1 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 (Fig. 8(a)) and those of the 1-week solvent-aged dammar (Fig. 8(b)). Mainly, side chain oxidation of the dammarane-type molecules has taken place, resulting in the oxidation of compounds **3**, **6** and **15** to compounds **1**, **2**, **4**, **5**, **21** and **22**. Compound **21** has been found only in trace amounts in aged dammar varnishes from paintings [23]. Part of the oleanane/ursane-type compounds with the aldehyde side chain (**17** and **19**) is oxidised to compounds with an acid group (**7** and **18**) as deduced from the peak ratios of these compounds. These modifications were also found to take place during ageing of varnishes on paintings (Fig. 8(d)) [3]. As seen from the signal to noise ratio, the dammar sample that was solvent-aged for 2 weeks (Fig. 8(c)) contains only traces of triterpenoid compounds, as will be described below. Only some highly oxidised dammarane-type molecules (**1**, **2** and **4**) are detected by GCMS because dammarane-type compounds are the major constituents of fresh dammar resin (Fig. 8(a)).

The dammar solution that was exposed for 1 week resembles the painting varnish most, when the gas chromatograms of the solvent-aged and a naturally aged varnish are compared. The dammar solution that was exposed for

2 weeks (Fig. 8(c)) contains a relatively higher amount of the dammarane-type compound with the lactonised side chain (**4**) than of the dammarane-type compound with the ocotillone side chain (**2**). Of the more than 10 aged dammar varnishes from paintings analysed by GCMS earlier [23], the lactonised compound was less abundant than the ocotillone-type compound. Consequently, 2 weeks of solvent ageing results in a light aged 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 **13**, **14**, **23**, **24** and **25**, are usually present in small amounts in aged varnishes from paintings (Fig. 8(d)). These compounds are not observed in gas chromatograms of the solvent-aged samples (Fig. 8(b) and (c)). It is possible that these oxidised compounds are formed some time after 1 week, but in amounts below the detection limit, and therefore, are not present in Fig. 8(c). Only dammarane-type compounds are present in Fig. 8(c) because oleanane/ursane-type molecules are much less abundant than dammarane-type molecules in dammar resin (Fig. 8(a)). 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. It could be that the activation energy of the oxidation reactions of the oleanane/ursane compounds is relatively high.

After 1 week of solvent ageing of mastic resin (Fig. 9(b)), the relative amount of a large number of compounds had decreased (**3**, **17**, **26**, **28**, **29**, **30** and **31**). Strikingly, all of these compounds, except **17**, have a tetracyclic euphane or dammarane structure (or a bicyclic structure (**26**)). Two compounds, **7** and **27**, were found to be relatively stable after 1 week of ageing and traces of oxidised oleanane-type molecules are formed (**23** and **25**). 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 2 weeks of solvent ageing (Fig. 9(c)), the only compound of fresh mastic resin left is moronic acid (**27**), which confirms that this compound is photochemically very stable, and consequently, a useful marker for aged mastic varnish [25]. Oxidised compounds with the dammarane (**2** and **4**) or oleanane skeleton (**23** and **25**) are present. A main constituent becomes an oxidised oleanane-type molecule (**32**), which is found only in trace amounts in aged varnishes from paintings (Fig. 9(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 aged varnishes from paintings analysed earlier [3,23].

3.4. 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 decrease during solvent ageing. This phenomenon has also been observed in aged varnishes from paintings and is postulated to be caused by cross-linking processes [3]. DTMS was used to obtain additional information on the occurrence of these cross-linking processes. Figs. 10 and 11 show the DTMS TICs of the dammar (Fig. 10) and mastic (Fig. 11) samples that had been solvent-aged in DCM with FF2 for 1 and 2 weeks. Two main peaks are present in the TICs. 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 lesser extent. The DTMS TICs of the pure compounds, **3** and **8**, 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. SEC in combination with a UV/VIS diode array detector was used to investigate

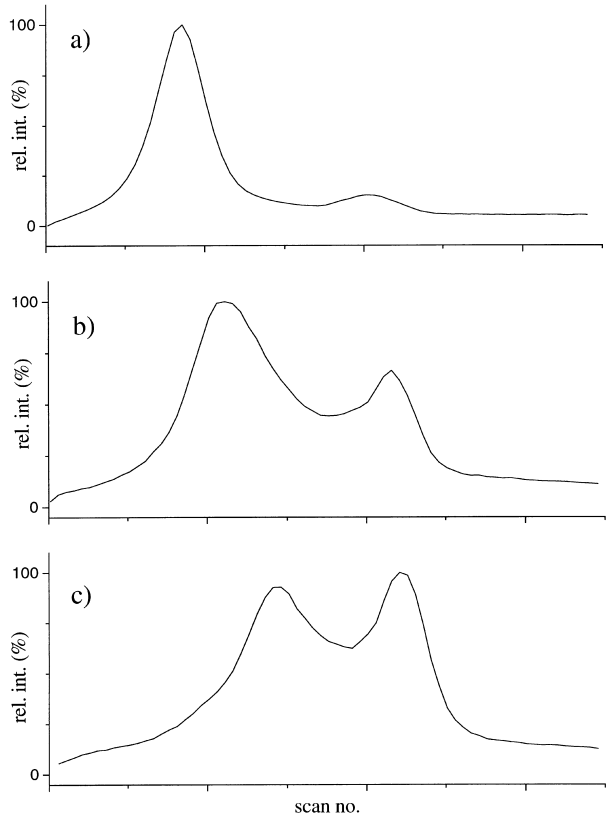

Fig. 10. DTMS total ion currents of (a) fresh dammar resin and of (b) dammar after 1 week or (c) 2 weeks of irradiation in DCM with FF2.

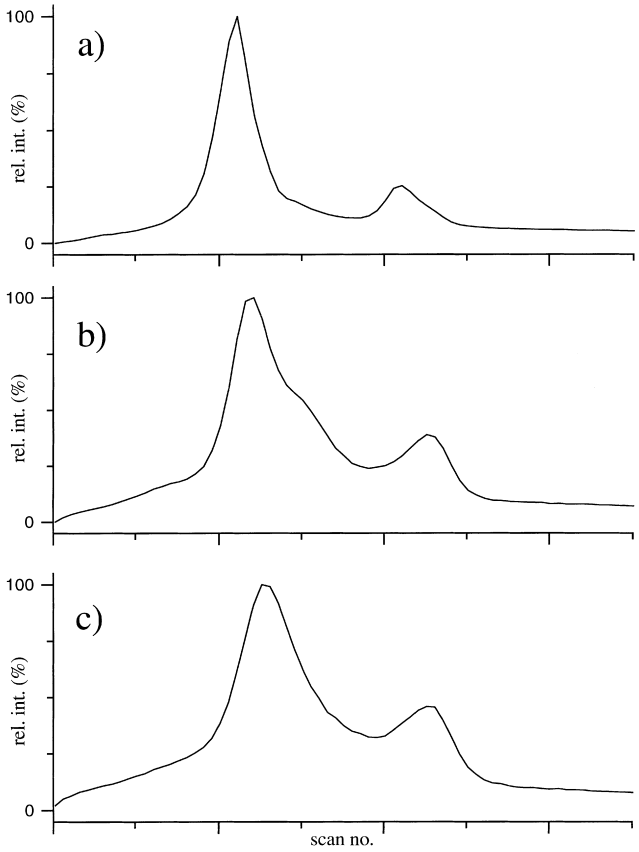

Fig. 11. DTMS total ion currents of (a) fresh mastic resin and of (b) mastic after 1 week or (c) 2 weeks of irradiation in DCM with FF2.

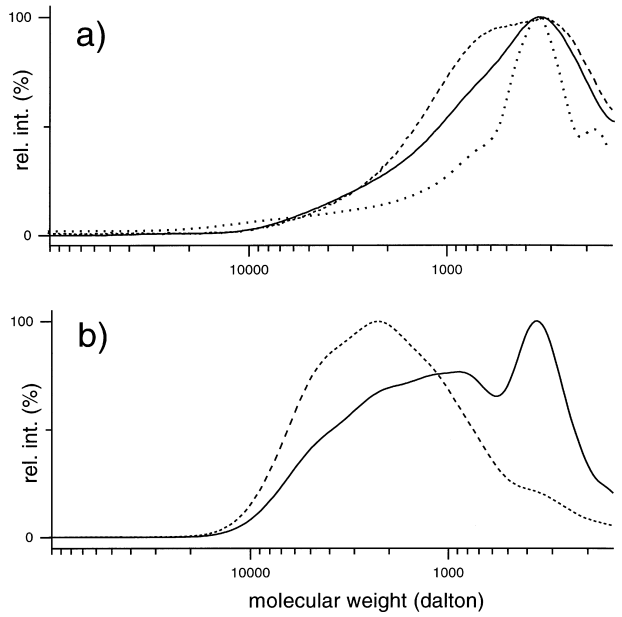

Fig. 12. (a) SEC traces at 240 nm (solid line) and at 400 nm (dashed line) of dammar after 1 week of solvent ageing in DCM with FF2. In addition, the SEC trace at 240 nm of fresh dammar resin (dotted line) is shown in (a). (b) SEC traces at 240 nm (solid line) and at 400 nm (dashed line) of dammar after 3.5 weeks of solvent ageing in DCM.

the spectroscopic characteristics of some solvent-aged samples. Fig. 12(a) shows the SEC traces of the dammar sample that had been solvent aged in DCM with FF2 for 1 week at 240 nm (solid line) and at 400 nm (dashed line). For comparison, the SEC trace of fresh dammar resin at 240 nm (dotted line) is shown. The trace of the solvent-aged dammar at 240 nm shows that two peaks are present, which are not well resolved. The peak at 400 Da represents the triterpenoid fraction. The (shoulder) peak at 900/1000 Da may be attributed to cross-linked dimerised triterpenoids. The SEC trace at 400 nm clearly shows that the SEC fraction at 900/1000 Da absorbs more light at 400 nm than the triterpenoid fraction. These results are similar to the results obtained with aged varnishes from paintings [23].

One dammar sample was exposed in DCM for 3.5 weeks. After exposure, the dammar solution had turned dark yellow/brown. Fig. 12(b) shows the SEC traces of this dammar sample at 240 nm (solid line) and 400 nm (dashed line). It is clear that a high degree of cross-linking has taken place during this longer period of light exposure in DCM. A 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 3.5 weeks of solvent ageing as compared to ageing on paintings [23]. This dammar sample, which was aged for a relatively long period of time, is not directly comparable to that of a varnish aged on a painting. In general, the chance of cross-linking may be higher in the solvent ageing method because 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 from oxidation to cross-linking reactions.

It was observed that a yellowish precipitate was deposited on the glass vial after some period of solvent ageing of dammar in DCM with or without a photosensitiser. This precipitate was found to be soluble in THF. The solvent ageing conditions were exactly the same as used above for dammar ageing in DCM with FF2, except for a higher temperature of the fluorescent tube device during exposure. The temperature was higher by about 10–15°C than in the experiments described above, which probably resulted in an increased reaction rate. The type of oxidation reactions was found to be the same. In general, 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 form already after 1 week of solvent ageing. DTMS analysis of (a) the dammar exposed in DCM solution for 9 days and (b) the precipitate formed shows that the precipitate

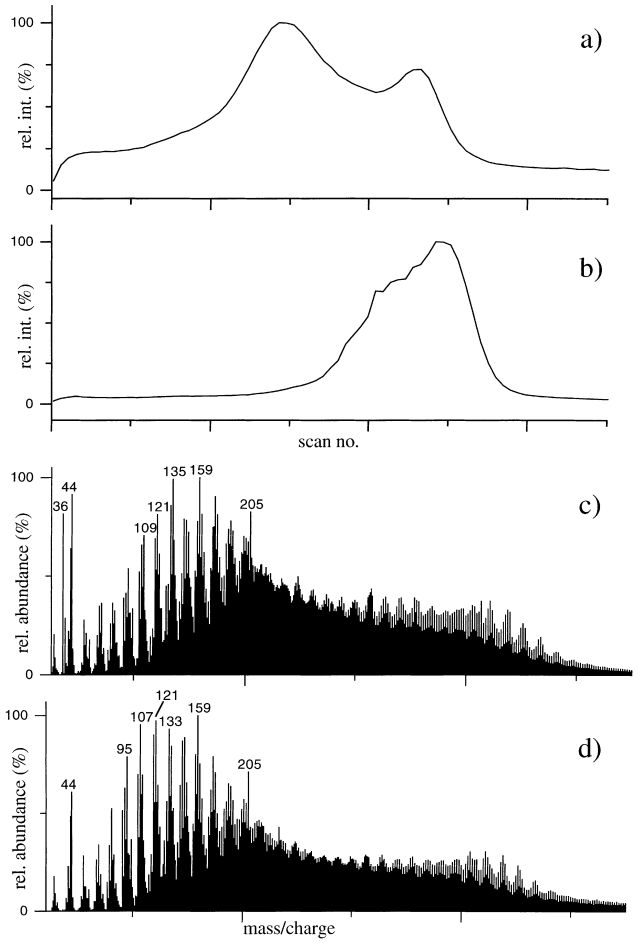

Fig. 13. (a) DTMS total ion currents of dammar and FF2 in DCM after 9 days of irradiation (DCM soluble fraction); and (b) those of the precipitate formed after solvent ageing (DCM insoluble, THF soluble fraction). (c) and (d) show the summation spectra of the precipitate and those of the cross-linked fraction of an aged dammar varnish from a painting, respectively.

consists of cross-linked material because it appears later in the DTMS TIC/temperature profile (Fig. 13). The total summation spectrum of the precipitate (Fig. 13(c)) shows a large number of peaks, which is indicative of complex material. Fig. 13(d) depicts the DTMS spectrum of the cross-linked fraction of an aged dammar varnish from a painting. The spectra of the solvent-aged (Fig. 13(c)) and the 'naturally' aged cross-linked fraction (Fig. 13(d)) are strikingly similar. An important difference between the two spectra of Fig. 13(c) and (d) is the presence of the peaks at *m*/*z* 36 and 38 in the spectrum of the solvent-aged material. These peaks point to the elimination of hydrogen chloride, which indicates that some incorporation of chlorine occurs during solvent ageing in DCM. 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.

4. Conclusions

Dissolution in the solvents DCM and acetone and subsequent light exposure induce 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, ACN 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 **3** and **8**. Compound **3** 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 occurs during solvent ageing of this dammarane-type molecule. Oxidation of the A-ring did not occur. Solvent ageing of **8** 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 **8** is 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 **8** 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 a relatively high molecular weight material.

A number of questions are left 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 low-cost 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 as to which compounds can be used as stable markers for aged triterpenoid varnishes. Furthermore, it can be used as a preparative 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. 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. Current research in our laboratory demonstrates that this ageing technique can be used successfully to investigate the ageing characteristics of several paint materials such as the stability of the blue coloring agent indigo and the oxidation and cross-linking processes of oil paint.

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