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A Mass Spectrometry and Electron Paramagnetic Resonance Study of Photochemical and Thermal Aging of Triterpenoid Varnishes

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Photochemical and thermal aging of triterpenoid dammar and mastic resins used as varnishes on paintings were studied using graphite-assisted laser desorption/ionization mass spectrometry. This extends an earlier study on similar materials (Zumbühl et al., *Anal. Chem.* 1998, *70*, 707−715) that focused on photoaging. Progressive aging results in development of groups of signals spaced by 14 and 16 Da, indicating incorporation of oxygen as well as simultaneous loss of hydrogen. Oligomers up to tetramers are formed, while cleavage reactions lead to increased signal intensities in the mass ranges between the oligomers and below the monomers. No major differences were found between the mass spectra of samples aged in light or darkness, except that deterioration was faster in light. Electron paramagnetic resonance spectroscopy revealed similar and significant amounts of radicals in films of dammar stored either in light or in darkness. It is concluded that oxidative radical reactions also take place in darkness and that differences in light and dark aging pathways are minor, although rates may differ. These findings lead to a unified explanation for yellowing of natural resin varnishes, one of the major degenerative changes in the appearance of paintings. It is also shown that the commercially available, nominally fresh resins are already in an advanced stage of oxidation and degradation. Energy-rich substances are formed upon irradiation with sunlight and are believed to restart the autoxidative chain reactions, regardless of storage conditions. As a result, varnishes are oxidized quite quickly (months) even when kept in darkness.

Naturally occurring triterpenoid resins are widely used as varnishes on paintings.[1] Varnishes saturate the colors, add gloss and protect the paintings. With time, they yellow, crack, become brittle, and have to be removed and replaced. Because removal can damage the painting,[2−4] it is desirable to understand the processes occurring during aging, in hope of devising methods to stop or slow the deterioration.

Important fundamental studies of aging processes in triterpenoid resins have been reported by de la Rie[5,6] and van der Doelen et al.[7−11] These authors have described the main processes occurring in aging of natural resin films, but many aspects, such as yellowing and aging in darkness, remain incompletely understood.

Aging in light and darkness of two frequently used natural triterpenoid resins, dammar and mastic, are compared here. Previous work using similar methods[12,13] emphasized light-induced aging and the consequences for the mechanical behavior of these varnishes. The commercial resins were found to be partially oxidized. Light-induced polymerization was found to have occurred with subsequent decomposition of the polymers. These results are confirmed here and compared to samples aged in darkness. In addition, due to improved mass resolution, more precise conclusions about the aging processes can now be drawn.

The main analytical tool is graphite-assisted laser desorption/ionization time-of-flight mass spectrometry (graphite-assisted LDI-MS). This has been found to be uniquely suitable for direct analysis of triterpenoid varnishes, as well as their oxidation, polymerization, and decomposition products.[12−14] It requires no special sample preparation or derivatization and yields intact molecular (cationized) ions. It, therefore, provides a detailed and

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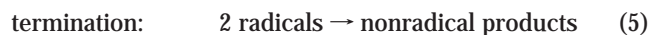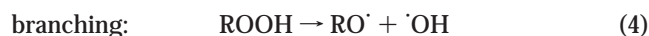† Laboratorium für Physikalische Chemie, ETH Zentrum.
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informative picture of the composition of a fresh or aged varnish sample.

Dammar and mastic consist mainly of triterpenoids, with lesser amounts of hydrocarbon polymers and sesquiterpenoids.[1,11,15] Autoxidation proceeds via radical chain reactions, as summarized here:[13,16,17]

initiation: $initiator \rightarrow R^{\cdot}$ (1)

propagation: $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$ (2)

      $ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$ (3)

branching: $ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH$ (4)

termination: 2 radicals $\rightarrow$ nonradical products   (5)
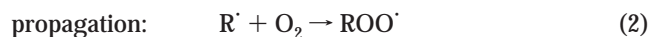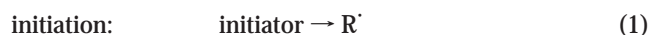
The peroxyl radicals are relatively stable, thus step 3 is rate-determining. Hydroperoxides are homolytically cleaved by heat or light (4), and the chain is branched and propagated. This simple model is believed to describe the main radical oxidation pathways for these compounds. Initiation via UV excitation of keto groups followed by alpha cleavage (Norrish reaction) has been proposed as a major initiation step.[6,12] In addition to the reactions shown, others proceed in parallel. Alkoxy radicals $RO^{\cdot}$ can react to form alcohols, ethers, or ketones. Addition to double bonds may compete with abstraction in step (3).[16,17] Products such as hydroperoxides also undergo nonradical reactions. As a result, many of the known aging products of dammar and mastic varnishes are not expected to form directly by reactions 1−5, but nevertheless, result from these primary events.[8,9]

Dark autoxidation is a known phenomenon in general (e.g., drying of oils or synthetic polymers). Absence of light does not alter the propagation pathways (reaction steps 2 and 3) but does decrease the initiation rate (reaction step 1).[17] Radical oxidation in darkness was believed to be of minor importance in varnishes because of rapid termination and insufficient initiation rates,[5] a view denoted below as the "conventional view". Although intuitively attractive, evidence for this was minimal. One indication was the solubility of photoaged varnishes in polar solvents, versus that of thermally aged varnishes in less polar solvents.[6] The trend was believed to correlate with degree of autoxidation,[5,18] but it can also be explained by an enhanced development of strongly polar compounds, such as acids, that is due to unrepresentative artificial aging methods, as by a lack of oxidation in darkness.

Another indication that the conventional view is questionable is the fact that oxidation also proceeds rapidly when UV light is excluded.[10,18] UV light was believed to be the major source of radicals in light aging (by Norrish reaction). Products attributed to this reaction were detected in samples artificially aged by UV light but were not abundant in naturally aged varnishes from paintings.[10] The authors of this study concluded that artificial photoaging simulates natural aging only when UV is excluded.[10]

Unfortunately, xenon-arc light sources equipped with a borosilicate and soda lime filters, as used by van der Doelen et al., still emit a large amount of UV radiation. Less UV-rich light sources, such as the fluorescence tubes used in this study or in that of Carlyle and van der Doelen et al.,[19] seem to simulate daylight through window glass much more reliably and do not support such extensive differences in realistic dark, light, or UV initiation mechanisms.

Another photoinduced initiation process is the absorption of long-wavelength light by impurities that transfer the energy to other compounds (e.g., peroxides, ketones), which then initiate the radical reactions of autoxidation.[17,20] Air pollutants such as NO_x have been suggested as another source of initiation.[21,22] Although the importance of this source is not yet completely clear, it is also a possible source of radicals in darkness.

With GC/MS or HPLC/MS, individual compounds were identified in fresh or aged resins, from which detailed molecular level models for aging processes were developed.[10,11] It is very important, however, to note that these conclusions were drawn from only 10−20 identified compounds out of a mixture consisting of hundreds or thousands of components. Among the reasons for this are the sample derivatization or other necessary preparation steps that lead to bias or selectivity in the overall method. For example, it is striking how different GC/MS or HPLC/MS traces of the same varnish appear.[8,9] This detailed but very selective view of the aging process must be kept in mind when considering the conclusions drawn by these authors.

Graphite-assisted LDI-MS does not involve separation, derivatization, or other selective preparation steps and provides a good overview of the resins as a whole. Instead of observing <20 species after long preparation, hundreds or thousands are observed in an aged resin with LDI-MS in minutes. This overview capability allows certain aspects of aging like oxidation to be globally studied in a manner that is otherwise not possible.

Individual compounds that contribute to signals at each m/z were not identified in this study. However, in spectra of unaged resins, signals are observed at all of the m/z values expected from the literature. In addition, reference studies of individual triterpenes show that fragmentation is minimal and each substance yields only one signal in the mass spectrum.[14]

Considerable chemical information regarding aging is directly available from the mass increments observed, without full compound identification. For example, the main hydroxydammarenone ($M + Na^+$ at m/z 465) aging products identified by van der Doelen et al. should appear at m/z 483 (M + 18) and m/z 481 (M + 16), depending on the reaction mechanism. In our artificially and naturally aged dammar samples, the ion signal at m/z 483 is much weaker than that at m/z 481, which suggests that one mechanism, and not the other, is prevalent.

Interpretation of mass spectra in terms of possible aging pathways is also greatly assisted by comparison to aging results

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obtained with pure triterpenes,[14] and with GC- and HPLC/MS data from other authors.[6-8] Thus, LDI-MS and other methods such as GC/MS or HPLC/MS are complementary. In addition, this study reports the first measurement of radical concentrations in aged and fresh varnishes, and this is found to be strongly correlated with the changes observed in the mass spectra.

EXPERIMENTAL SECTION

The dipterocarpol reference triterpene was obtained from Fluka (Buchs, Switzerland; purity, > 97%) and was aged without further purification. Batavia dammar was obtained from Farbmühle Kremer (Aichstetten, Germany); the Chios mastic, from A. Grogg Chemie (Bern, Switzerland). The fresh mastic resin was harvested near Pyrgi on the island of Chios, Greece. The branch of the tree was wrapped in aluminum foil to protect the resin from sunshine. Naturally aged (25 years) dammar and mastic varnishes were provided by the Swiss Institute for Art Research (SIK) in Zürich.

For natural aging, dammar was dissolved in turpentine (1:3 wt %) and brushed on glass microscope slides. Film thickness was 15–25 μm. The photoaged samples were exposed to sunlight in a window facing south. The dark-aged samples were kept in a closed box in a drawer; thus, air circulation can be neglected.

The preparation of the samples for artificial aging is described in ref 12. Artificial aging of dipterocarpol and dammar or mastic films was carried out under daylight-simulating lamps (Power Twist True Lite, Duro Test 20TH12 TXC). The samples were aged for 300 h behind window glass. Subsequent thermal aging of the resin films was carried out in an oven for 200 h at 40, 60, or 80 °C.

The graphite-assisted laser desorption/ionization experiments were performed on a home-built 2-m linear time-of-flight mass spectrometer. Resolution was improved by delayed extraction[23] to ~600 at 500 Da. Ions were extracted using a 21 kV acceleration voltage and an empirically determined delay time of 180 ns. Desorption was performed using the 337-nm output from a nitrogen laser (VSL-337ND-T, Laser Science Inc., Franklin, MA).

Particle-assisted laser desorption/ionization was first demonstrated by Tanaka et al. using cobalt particles.[24] Graphite was introduced as a substrate by Sunner et al.[25] Other materials also work,[26,27] but graphite without addition of a liquid matrix was found to work best for resins.[12] The samples were prepared as follows: A suspension of 2-μm graphite particles (Aldrich, Buchs, Switzerland) in methanol was allowed to dry on the sample tip. A THF solution of the resin was pipetted onto the graphite and also allowed to dry. The sample quantity was varied empirically for the best signal and resolution. If too much sample was applied, the graphite appeared glossy, and no signal was obtained at all. The analytes were detected as alkali metal adducts. To avoid spectral confusion resulting from the occurrence of both Na^+ and K^+ adducts, Na^+ adduct formation was enhanced by the addition of a small amount of NaCl to the graphite/methanol slurry.

Contamination of the spectrometer with diffusion pump oil led to signals at m/z 413, 469, 483, and 507 (marked with crosses in figures), which interfered with the signals of the triterpenes. These signals appeared immediately after sample insertion and grew with time.

It should be noted that the use of graphite as a matrix can lead to contamination of the MALDI source and disruption in the operation of the turbomolecular pumps if it is placed below the ion source. The authors have found that the use of low laser powers and the application of thin graphite films minimizes the amount of sputtering in the ion source; however, care should be taken in the use of this methodology.

The cw-EPR spectra were recorded on a spectrometer from Bruker (ESP 300 E; microwave frequency, 9.4 GHz). The magnetic field was determined by a NMR Gaussmeter (ER 035 M, Bruker; Fällanden, Switzerland). All spectra were obtained at room temperature. The concentration of radicals in the pulverized resin samples were determined by comparing the integral of the absorption line to the linear regression of a series of four standard samples having known spin concentrations. The standard was the fourth line in the spectrum of $VO(acac)_2$ (Acros Organics; Basel, Switzerland; purity, 99%) dissolved in water-free toluene.

To avoid systematic errors, the usual recommendations for EPR signal area measurements[28,29] were taken into account (identical filling height, sample position in the resonator, measurement parameters). Due to physical differences between the calibration standard (toluene solution) and the dammar samples (powder), the remaining spin concentration uncertainty is estimated to be ±20%.

RESULTS AND DISCUSSION

Fragmentation in the spectrometer was found to be insignificant for all of the triterpenoid reference substances;[14] thus, each signal in the mass spectrum is believed to correspond to genuine components of the resin. Carboxylic acid protons can sometimes be exchanged for sodium, which leads to small additional signals at $(M + 2 Na - H)^+$. Although reproducibility is sometimes a problem in MALDI-MS, the results that were obtained by graphite-assisted LDI-MS were highly reproducible in terms of relative signal intensities.

1. Artificial Aging of Hydroxydammarenone. Understanding the aging behavior of reference compounds by themselves is very useful for interpreting aging experiments on natural mixtures of triterpenoids. Artificial photoaging of various pure triterpenes resulted in the formation of a multitude of aging products out of each single triterpene.[14] This is shown in Figure 1, using hydroxydammarenone, which is a major component of both dammar and mastic, as an example (also known as dipterocarpol, structure I).[8,9] Aging resulted in oxidation, polymerization, and decomposition. The remarkably wide variety of products indicates multiple radical attack points and a progressive reaction of initial products. A large number of oxygen atoms can, therefore, be incorporated. Incorporation of oxygen leads to signals having mass differences of 16 Da. The simultaneous loss of hydrogen (e.g., by allylic oxidation or oxidation from alcohols to acids) leads to increments of 14 Da. Different combinations of 14 and 16 Da

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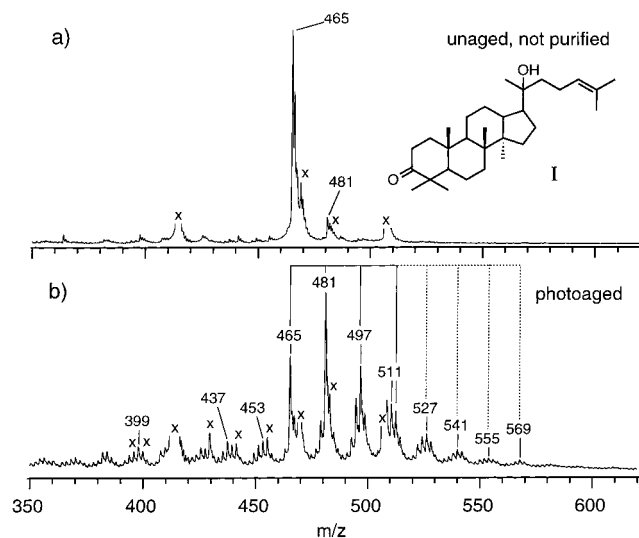
Figure 1. Laser desorption/ionization mass spectra of (a) unaged and (b) 300-h-photoaged hydroxydammarenone (structure I). The (M + Na)$^+$ signal is at m/z 465; a small amount of M + 16 appears at m/z 481. The typical oxidation pattern of a triterpene can be seen: groups of signals with mass increments of 14 (dashed lines) and 16 Da (solid lines), which indicate an incorporation of oxygen, a loss of hydrogen, and decomposition products due to cleavage reactions (m/z <460). Signals marked with crosses are contaminants in the spectrometer (m/z 413, 469, 483, 507). Signals marked with crosses at m/z <460 may have small contributions from contaminants.
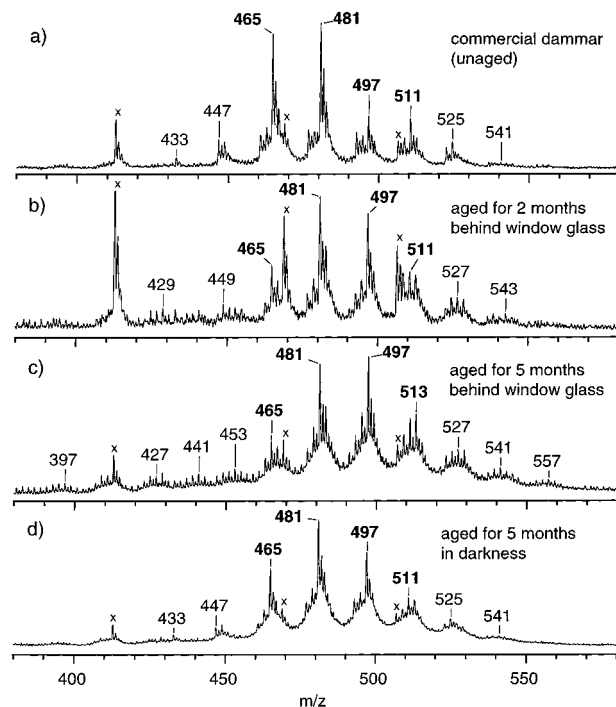

Figure 2. Laser desorption/ionization mass spectra of films of commercial and naturally aged dammar: (a) unaged dammar, (b) aged for 2 months, and (c) 5 months with daylight behind window glass, and (d) 5 months aged in darkness. Progressive oxidation can be observed even in darkness, most obviously at m/z 465, 481, 497, 513, and 527 (see text for more details). Signals marked with crosses are contaminants in the spectrometer (m/z 413, 469, 483, 507).

increments give signal groups that are broader and less distinct with increasing mass (cf., Figure 1). In aged hydroxydammarenone (signal at m/z 465), these groups of signals appear with maxima at m/z 481, 497, 513, 527, 541, 555, and 569.

Van der Doelen et al. found different aging products for varnishes artificially photoaged with or without UV light. Ketones are efficiently oxidized to acids via Norrish cleavage with UV light, but without UV, the side chain of dammaranes is oxidized instead.[10] In the case of hydroxydammarenone, the main aging products identified by van der Doelen et al. should appear in the MS at m/z 483 with and at m/z 481 without UV light. We found very little signal at m/z 483 under all of the aging conditions used in this study, and the same was true for naturally aged dammar (see chapter 2). It was concluded that natural aging can be well simulated with an appropriate choice of illumination, and oxidation does take place even in the absence of extensive Norrish initiation.

Low-molecular-weight products are produced by skeletal cleavages. For hydroxydammarenone, this leads to characteristic signal groups at m/z <465 but not to any dominant product. Dimerization or more extensive polymerization is also known to occur during artificial aging of hydroxydammarenone.[12] However, only minor amounts of tri- and tetramers were observed here.

2. Dammar. a. Unaged Dammar. Figure 2a shows the triterpenoid mass range of a graphite-assisted LDI mass spectrum of a typical dammar. A list of the known triterpenoid components of dammar is given in Table 1. The signals in the mass spectrum can be readily assigned to known components. In previous work,[12] the signal groups at higher masses (m/z 493, 509, 525) were interpreted as oxidation products of major dammar components known from GC/MS studies.[6] Thanks to improved mass resolution, it is now clear that most of the signals of these groups

Table 1. List of Compounds Identified in Commercial Dammar Resin by de la Rie, van der Doelen and Others (Refs. 6-8 and References Cited Therein) with Corresponding m/z Values

MW, Da	formula	m/z^a, (M + Na)$^+$	components^b
410	$C_{29}H_{46}O$	433	noramyrone
424	$C_{30}H_{48}O$	447	dammaradienone
426	$C_{30}H_{50}O$	449	dammaradienol
438	$C_{30}H_{46}O_2$	461	*oleanonic aldehyde, ursonic aldehyde*
440	$C_{30}H_{48}O_2$	463	oleanolic aldehyde, ursolic aldehyde
442	$C_{30}H_{50}O_2$	**465**	*hydroxydammarenone*, hydroxyhopanone
444	$C_{30}H_{52}O_2$	467	dammarenediol
454	$C_{30}H_{46}O_3$	477	*oleanonic acid, ursonic acid*
456	$C_{30}H_{48}O_3$	479	oleanolic acid, ursolic acid
458	$C_{30}H_{50}O_3$	**481**	*dammarenolic acid*, 20,24-epoxy-25-hydroxydammaran-3-one
470	$C_{30}H_{46}O_4$	493	hydroxyoleanonic lactone
474	$C_{30}H_{50}O_4$	**497**	shoreic acid, eichlerianic acid
486	$C_{32}H_{54}O_3$	509	3-acetoxy-22-hydroxyhopanone
488	$C_{30}H_{48}O_5$	**511**	asiatic acid
500	$C_{30}H_{44}O_6$	523	23-hydroxy-2,3-secours-12-ene-2,3,28-trioic acid (2→23)-lactone

^{a} m/z values that correspond to prominent signals in graphite-assisted LDI-MS are in bold print. ^{b} Compounds printed in italics were found to be main components by GC/MS or HPLC/APCI-MS.

correspond to known unoxidized minor components (cf., Table 1).

Dammars that were obtained from different sources showed the same patterns, differing only in relative concentrations of the compounds. This moderate degree of heterogeneity was also found by other researchers with other methods.[6,7,15] The signals

at m/z 465, 481, 497, and 511 showed the largest variations, which is not surprising, because they are oxidation products. A quantitative comparison of relative concentrations should not be made using relative signal intensities in graphite-assisted LDI-MS, GC/MS or HPLC/MS, because detection efficiencies differ for different compounds. As a result, the characteristic resin pattern is different with each method[6,8,9] (cf., Table 1).

b. Aging of Dammar in Sunlight. Parts b and c of Figure 2 show the progressive oxidation of dammar films that were aged in sunlight behind window glass. The oxidation pattern of dammar is clearly very similar to that of hydroxydammarenone, taking into account the larger number of starting compounds. The most abundant signals are the same as hydroxydammarenone and its corresponding oxidation products at m/z 465, 481, 497, 513, 527, and 541 (see Figure 1).

Progressive oxidation leads to a shift of the base peak from m/z 465 to m/z 481 (M + 16, 2 months) and 497 (M + 32, 5 months). The ratios of more highly oxidized components to starting materials (e.g., m/z 513/511, m/z 527/525) increase with time, and compounds with $m/z > 530$ develop. Decomposition leads to a relatively uniform distribution of products having masses lower than the original triterpenes (Figure 2b,c).

c. Aging of Dammar in Darkness. Dammar films prepared simultaneously from the same raw materials as the photoaged samples were stored in darkness for 5 months. A representative mass spectrum is shown in Figure 2d. Significant oxidation has taken place in this relatively short time, even though the only light exposure was during the one week drying period, in normal room conditions with no direct sunlight. After 2 months, no significant change was found, thus oxidation occurred in darkness and not while drying. Air pollutants, such as nitrous oxide, ozone, or sulfur oxides, are known to influence the deterioration of art objects;[21,22] however, the storage conditions used in this study certainly did not expose the samples to atypically contaminated air. In fact, the conditions may even be considered too mild, with less exposure to circulated pollutants than in most museum environments. The spectrum in Figure 2d is remarkably similar to that of dammar aged in daylight through window glass for 2 months; the relative intensities of the signals indicating the extent of oxidation (m/z 465, 481, 497, 511/513, 525/527) are almost the same. The similarity is so strong that the question arises whether the main oxidation mechanisms in darkness could be the same as with exposure to light, only slower.

d. Artificial Aging of Dammar. The nature of light and dark aging pathways were also studied using artificially aged films of dammar. These were samples of the same series studied by Zumbühl et al.,[12,13] but stored in darkness for 3 years afterward. These naturally aged samples were then further photoaged for 300 h. Some of the photoaged samples were subsequently aged at 80 °C (and at 40 or 60 °C, results not shown) for 200 h in an oven (see Figure 3). This thermal treatment was intended to enhance dark reactions that would otherwise take place over a long period of time. 80 °C is a rather high temperature for artificial aging of dammar, but qualitative changes in aging behavior are only expected above 100 °C.[5] Qualitatively, the aging characteristics obtained at 80 °C and discussed in this paper were the same as those that were observed at 40 and 60 °C to a lesser extent.
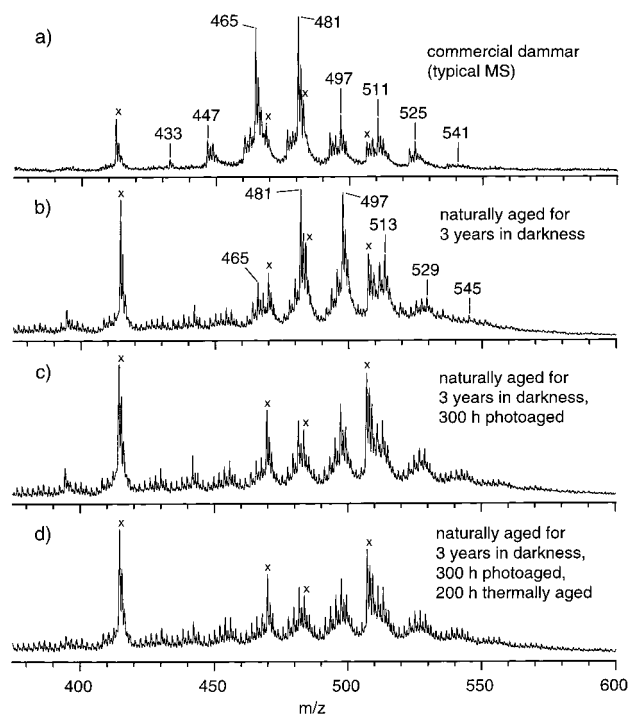

Figure 3. Laser desorption/ionization mass spectra of commercial and artificially aged dammar. No general mass shift due to incorporation of oxygen is visible in the spectra, except signals at m/z 481 and 497. See text for more details. Signals marked with crosses are contaminants in the spectrometer.

A mass spectrum of commercial dammar is presented in Figure 3a. Natural aging in darkness for 3 years results in strong oxidation and decomposition (see Figure 3b). Signals of oxidation products (e.g., m/z 481, 497, 513, and $m/z > 525$) are strong, and those of unoxidized triterpenes are low (e.g., m/z 447, 465). Additionally, decomposition products are visible at all $m/z < 460$, which is similar to aging in daylight.

Further photoaging of these naturally pre-aged varnishes does not lead to a general and major shift to higher masses, as observed between panels a and b of Figure 3; however, changes are observed for m/z 481 and 497 which decrease in intensity. New substances fill in the regions between the main signal groups which, nevertheless, remain well separated.

Additional thermal aging of the photoaged samples at 80 °C for 200 h also does not change the appearance of the mass spectra. In the conventional view, photooxidation products would be expected to decompose in darkness, leading to a general shift toward lower masses. This is not observed, even though the spectrum does fill in slightly; therefore, it is probable that both mass-increasing and mass-decreasing reactions are operative in darkness. This supports the hypothesis that the main reactions are similar to those in photoaging, that is, radical oxidation.

Similarity between light and dark reactions is also found in the polymeric portion of the mass spectrum, as shown in Figure 4. For semiquantitative comparison of the degree of polymerization, the spectra were normalized to the integrated signal in the mass range m/z 474−560 (monomeric triterpenes). Both artificial light and dark aging further increased the proportion of polymers somewhat, but did not change the detectable distribution.
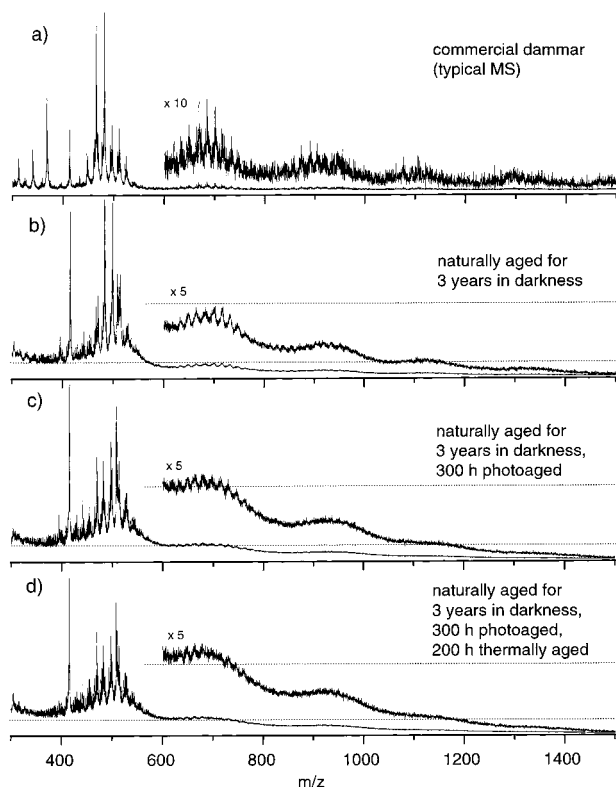
Figure 4. Oligomeric mass range of the laser desorption/ionization mass spectra of Figure 3. The dashed lines are referenced to the integrated intensities of the triterpene region and facilitate comparison of the relative amounts of oligomers. Natural aging for 3 years leads to a larger difference, as compared to bulk dammar, than does subsequent artificial aging.

Dammar contains di-, tri-, and tetramers of triterpenoids, but the regions between the oligomers are well-filled-in, which indicates a substantial cleavage of smaller units off the polymers. Quasi-specific cleavage reactions lead to the increased formation of substances with masses about 1.5, 2.5, and 3.5 times the masses of the triterpenoids. As for the monomeric triterpenoids, varying amounts of incorporated oxygen are indicated by signal groups having mean mass differences of ~14 Da, which also broaden and become less distinct with progressive aging. Signal groups spaced by 14 Da are typical for samples containing highly oxidized components [see, for example, aged hydroxydammarenone at m/z >520 (Figure 1)].

Half-integer oligomers could be formed by the oxidative rearrangement of substances with oleanane or ursane skeletons, which results in the formation of taraxarenes (Figure 5, structures II→III→V). This reaction was observed to occur in solutions of amyrin in sunlight.[30] In Figure 5, the reaction is adapted to oleanonic acid (structure II). Substances with taraxarene skeletons can easily undergo retro-Diels−Alder reaction (Figure 5, as demonstrated for α amyrin derivative by Melera et al.[31]). The addition of the resulting fragments to another triterpenoid would lead to compounds in the appropriate mass range. Taraxarenes have not yet been detected in aged varnishes, but their formation

seems likely, because intermediate III is expected to be easily formed by autoxidation. Moreover, it is an intermediate in the formation of 11-oxo-oleanonic acid (structure IV), which is a known component of aged varnishes.[8,9]

e. Determination of Radical Concentrations in Dammar. All of the results presented to this point indicate a strong similarity of light and dark aging processes, as noted also by Feller.[17] Because aging with light proceeds largely via radical mechanisms, dark-aged samples should also contain radicals in substantial quantities. Significant amounts of radicals were, indeed, found in all of the tested dammar samples (see Table 2 and the representative EPR spectrum of Figure 6).

Dammar that was recently exposed to light contained the most radicals, as was expected from mechanisms such as the homolytic cleavage of peroxides or ketones (Norrish). Commercial bulk dammar and films stored in darkness contained about one-third as many radicals. A steady-state concentration apparently develops, which is dependent on only the recent aging history. The most oxidized, photoaged sample, D, did not show a significantly higher concentration of radicals than the less oxidized samples, A and C. These concentrations are quite high as compared to those observed in autoxidative degradation of synthetic polymers.[32]

Especially, the radical concentrations in dark-aged samples are remarkably high. Contrary to the conventional view, storage in darkness does not lead to extensive termination reactions and radical quenching. Autoxidation, therefore, will continue in darkness. Assuming first-order kinetics, the rate of autoxidation in these samples will be one-third of that of the light-exposed sample, as is qualitatively consistent with the mass spectra of Figure 2. Unfortunately, the peak positions (g values between 2.0053 and 2.0067) were between the typical values for peroxyl (~2.015) and carbon-centered radicals (2.0025−2.0030).[33] The active species is, therefore, not immediately clear.

3. Mastic. a. Fresh vs Commercial Mastic. The mass spectrum of very fresh, colorless mastic is depicted in Figure 7a. Like dammar, mastic consists of triterpenoids and some polymeric hydrocarbons.[7,15] Some components of mastic are the same as in dammar. A list of the known triterpenoid mastic components is given in Table 3. This fresh mastic consists mainly of components with masses from 438 to 444 Da (m/z 461−467) and 454 Da (m/z 477). Components with smaller masses have only low signal intensities. The signal at m/z 499 is probably caused by the disodium adducts of the carboxylic acids of m/z 477[14] (see above).

A spectrum of commercially available mastic is depicted in Figure 7b. This mastic is made up of many more compounds than fresh mastic, most of them oxidized and appearing at higher masses than the nominal primary components. In addition, in the oligomer mass range, the distribution is much broader and less distinct in commercial mastic. In fresh mastic, the dimers are more pronounced (Figure 8). This spectrum (Figures 7b and 8b) is representative of commercially available mastic. It is obviously in an advanced stage of oxidation (compare, also, the artificially aged samples below), although nominally and for conservation purposes, it is considered "fresh". One reason may be that the resins are irradiated with daylight and the autoxidation is started when it is still on the tree. This is also true for dammar, but it is

(30) Agata, I.; Corey, E. J.; Hortmann, A. G.; Klein, J.; Proskow, S.; Ursprung, J. J. *J. Org. Chem.* **1965**, *30*, 1698.
(31) Melera, A.; Arigoni, D.; Eschenmoser, A.; Jeger, O.; Ruzicka, L. *Helv. Chim. Acta* **1956**, *39*, 441.

(32) Fischer, H. Personal communication.
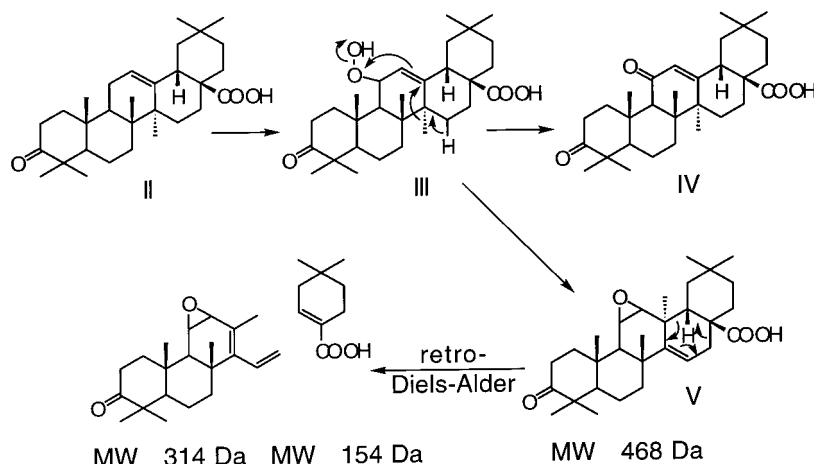(33) In *Landolt-Boernstein (New Series)*; Fischer, H., Ed.; Springer: New York; Vol. II/9a−II/9c, II/17g.

Figure 5. Reactions proposed to take place during the aging of dammar and mastic. Oxidative rearrangement III→V was demonstrated to occur in aging of solutions of β-amyrin with sunlight by Agata et al.[30] Structures of type V can very easily undergo a retro-Diels−Alder reaction (demonstrated for a derivative by Melera et al.[31]). Reactions of these types could occur in the aging of dammar and mastic components having an oleanane and ursane skeleton. The peroxide III is an intermediate formed by autoxidation of oleanonic acid (II) that can also be oxidized to IV. Structure IV was found in naturally aged varnishes from paintings.[8]

Table 2. Radical Concentrations in Samples of Commercial and Aged Dammar, Determined by cw-EPR

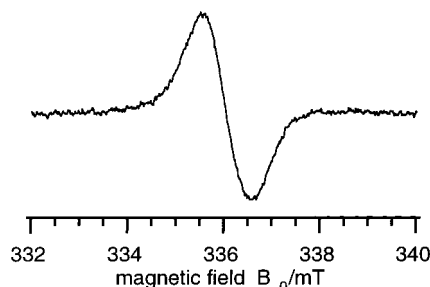|	aging conditions	radical concn[a] nmol/g resin	radical concn[a] ppm (assuming MW = 500 g/mol)
A	commercial dammar, stored in darkness	26	13
B	6 months naturally aged in daylight	72	36
C	6 months naturally aged in darkness	24	12
D	artificially photoaged for 600 h, stored in darkness for 1 year	25	12

[a] The estimated uncertainty is ± 20%.

Figure 6. EPR spectrum of a dammar sample (g value, 2.0053), aged as a film in sunlight behind window glass for six months. Experimental parameters: microwave frequency, 9.43040 GHz; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz; microwave power, 2 mW.

especially so for mastic, which is washed and usually dried in the sun after harvest.[34] After the washing and drying process it is already yellow, but truly fresh mastic is colorless.

b. Artificial Aging of Mastic. Three years of natural dark aging results in strong oxidation and decomposition, as in dammar (see Figures 7c, 3b). Signals of oxidation products (m/z >510) are strong, and those of unoxidized triterpenes are low (e.g. m/z 447, 461−467, 477). Especially prominent in mastic are decomposition products at m/z <460. If the mass spectrum of this 3-year-old varnish is compared to fresh mastic resin (Figure 7a), it is

obvious that these are two different materials from a chemical point of view.

As for dammar, further photoaging of these naturally pre-aged mastic varnishes does not have a large effect on the mass spectrum, nor does thermal aging at 80 °C of the photoaged samples for 200 h (see Figures 7 and 8). This is true for the triterpenoid and the polymeric regions. Once again, these findings are in contrast to the conventional view, and all of them point to strong similarities between dark and light reactions. Artificial aging clearly continues the trend from fresh to commercial mastic in all aspects of oxidation, polymerization, and degradation. When compared to aged dammar, the signal groups of the monomeric triterpenoids of aged mastic are broader and less distinct (Figures 3d, 7e). The polymeric part of mastic is surprisingly different from dammar, because hardly any tri- or tetramers are visible in the spectra (Figures 4 and 8).

4. Old Naturally Aged Varnishes. The mass spectra of 25-year-old dammar and mastic varnishes, naturally aged as films on a glass plate, are depicted in Figure 9. The characteristic processes observed above in short-term natural and artificial aging are also found here: oxidation, polymerization, and decomposition of the triterpenoids. The spectra look very similar to the artificially aged samples. The space between the initial signal groups is well-filled-in, as in artificial aging (insets in Figure 9). When compared to dammar, the signal groups of the monomeric triterpenoids are again broader and less distinct in mastic. The dominant signals at m/z 481 and 497 are slightly more prominent in dammar after

(34) Koller, J.; Baumer, U.; Grosser, D.; Schmid, E. In *Arbeitshefte des Bayerischen Landesamtes für Denkmalpflege*; Walch, K., Koller, J., Eds.; Karl M. Lipp Verlag: München, 1997; Vol. 81, pp 347−358.

Table 3. List of Compounds Identified in Mastic Resin by van der Doelen and Others (Refs. 7, 8 and References Cited Therein) with Corresponding *m/z* Values

MW, Da	formula	m/z^a $(M + Na)^+$	components[b]
410	$C_{29}H_{46}O$	433	nor-β-amyrone, norlupeone
412	$C_{29}H_{48}O$	435	nor-β-amyrin
424	$C_{30}H_{48}O$	447	dammaradienone, β-amyrone, 3-oxomalabarica-14(26),17E,21-triene
426	$C_{30}H_{50}O$	449	tirucallol, β-amyrin, germanicol, lupeol, 3-hydroxymalabarica-14(26),17E,21-triene
438	$C_{30}H_{46}O_2$	**461**	oleanonic aldehyde
440	$C_{30}H_{48}O_2$	**463**	28-hydroxy-β-amyrone
442	$C_{30}H_{50}O_2$	**465**	*hydroxydammarenone, (8R)-3-oxo-8-hydroxypolypoda-13E,17E,21-triene*
444	$C_{30}H_{52}O_2$	**467**	*(3L,8R)−3,8-dihydroxypolypoda-13E,17E,21-triene*
454	$C_{30}H_{46}O_3$	**477**	*oleanonic acid, moronic acid, masticadienonic acid, isomasticadienonic acid,* 18α*H*-oleanonic acid
456	$C_{30}H_{48}O_3$	479	oleanolic acid, 3-epimasticadienolic acid, 3-epiisomasticadienolic acid
486	$C_{32}H_{54}O_3$	509	3-acetoxy-hydroxydammarenone
498	$C_{32}H_{50}O_4$	521	3-O-acetyl-2-epi-masticadienolic acid, 3-O-acetyl-2-epiisomasticadienolic acid

^{a} *m/z*-values that correspond to prominent signals in graphite-assisted LDI-MS are in bold print. ^{b} Compounds printed in italics were found to be main components by GC/MS or HPLC/APCI-MS.
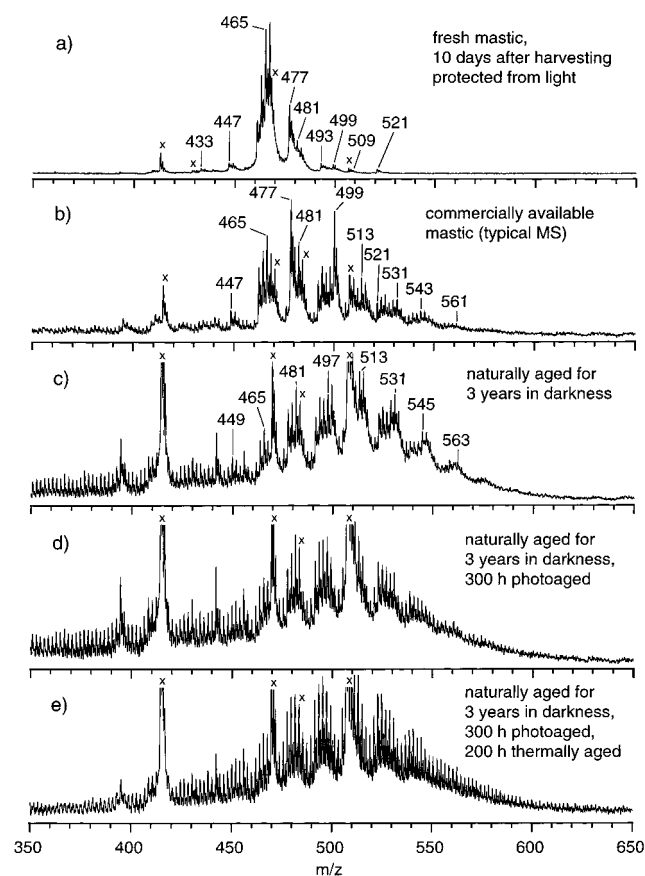

Figure 7. Laser desorption/ionization mass spectra of (a) fresh, colorless mastic direct from Chios, (b) commercially available mastic, and (c−e) artificially aged mastic. Commercially available mastic, nominally fresh, largely consists of oxidized compounds that are not contained in the initial resin. The artificially aged samples are from the same series. The same changes as in dammar occur during aging. See text for more details. Signals marked with crosses are contaminants in the spectrometer.

Figure 8. Oligomeric mass range of the laser desorption/ionization mass spectra of Figure 7. The dashed lines are referenced to the integrated intensities of the triterpene region and facilitate comparison of the relative amounts of oligomers. The largest differences are found between fresh and commercial mastic. Further aging results in comparatively slight increase in oligomers.

25 years of natural aging than after the artificial aging performed here. The molecular weight of the majority of the visible compounds is <1500 Da.

Polymerization is not more extensive than in the artificially aged samples, again indicating simultaneous polymerization and decomposition processe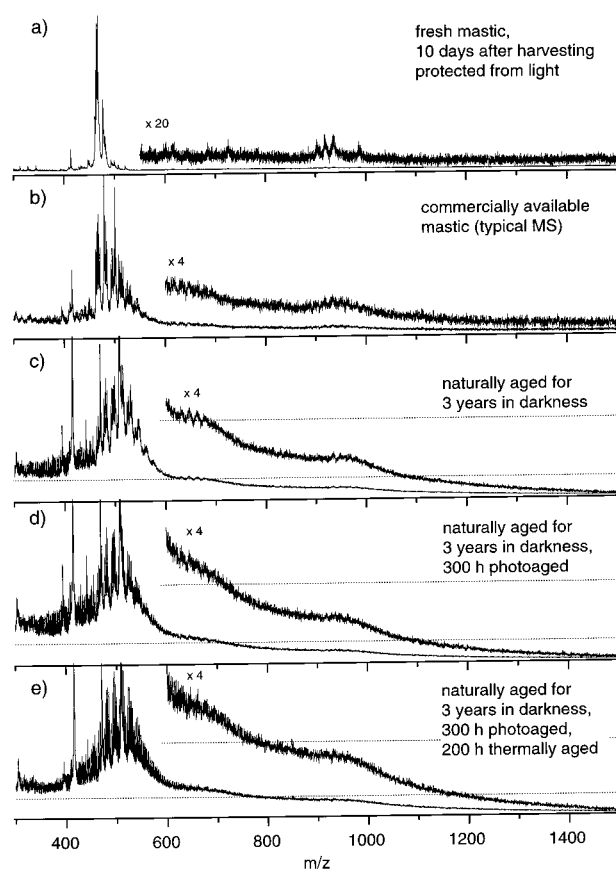s. The mass distribution of oligomers in mastic is comparable to the short-term naturally and artificially aged samples, but in dammar, an interesting phenomenon is observed: the number of signal groups in the oligomeric mass range is doubled. It is not yet clear which differences in the dammar resin and/or aging history of a varnish lead to one or the other pattern. In naturally aged varnishes from paintings, both patterns have been found, but because the age and aging history
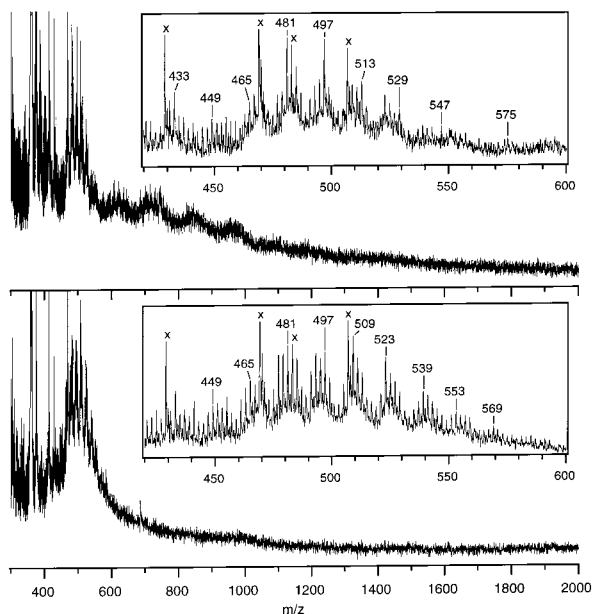
Figure 9. Laser desorption/ionization mass spectrum of 25-year-old naturally aged dammar (upper) and mastic (lower) varnishes. The insets show the mass ranges of the monomeric triterpenes (cf., Figures 3 and 7). The same changes as those observed for short-term natural and artificial aging can be seen: broad distribution of compounds with indistinct signal groups in the monomeric mass range and decreasing amounts of oligomers up to tetramers.

of these varnishes is not known, no conclusions can be drawn yet from the samples we have studied.

5. Varnish Yellowing. Yellowing occurs predominantly in darkness; yellowed varnishes are bleached when exposed to light even for short time.[5,7] The processes leading to this yellowing and bleaching are not well-understood, and no chromophores have been clearly identified.[5,7,17] A straightforward explanation for varnish's yellowing is the formation of unsaturated ketones. Some indication for this was found by using infrared spectroscopy: absorption in the region of unsaturated carbonyl compounds is enhanced after the aging of varnishes, especially after heat aging;[5] however, it was not clear how these ketones should be formed in darkness, because oxidation was believed not to occur without light. In addition, it was shown that previously photoaged varnishes undergo strong yellowing when thermally aged in an oxygen-free atmosphere or even in vacuo.[5,35] Thus, yellowing is a process proceeding in two steps: oxidation to colorless precursors, which react further to yellow compounds in a non-oxidative second step. Aldol addition followed by the elimination of the resulting hydroxy group as water was postulated as the non-oxidative reaction, as supported by evidence for dehydration reactions being involved in the yellowing process.[5] As an example, quinoid structures could arise from the condensation of two diketone molecules.[36]

Radical oxidation reactions taking place in darkness, as found here, support the hypothesis of unsaturated ketones being a major cause of yellowing. Alternating autoxidation and condensation reactions lead directly to the expected products (Figure 10).
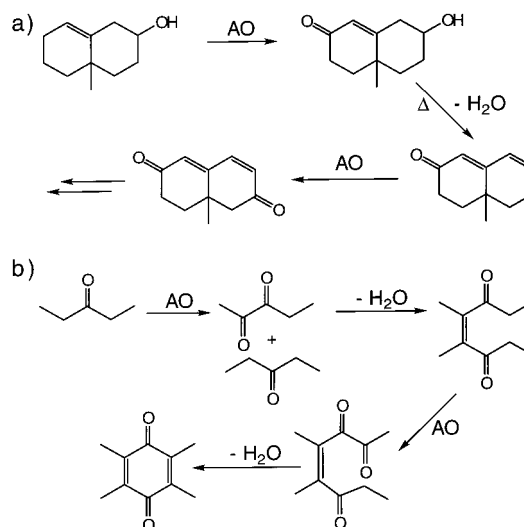
(35) Privett, O. S.; Blank, M. L.; Covell, J. B.; Lundberg, W. O. *J. Am. Oil Chem. Soc.* **1961**, *38*, 22−27.

(36) Formo, M. W. In *Bailey's Industrial Oil and Fat Products*; Swern, D., Ed.; John Wiley & Sons: New York, 1979; Vol. 1, pp 722−724.

Figure 10. Autoxidation (AO) occurring in darkness gives a straightforward explanation for yellowing. C=C bonds are converted to unsaturated ketones via allylic oxidation, and elimination of hydroxy groups enlarges the unsaturated system. Subsequent autoxidation could lead to conjugated diketones, which are yellow (a). Combination of condensation reactions with autoxidation can also lead to unsaturated diketones or quinones (b).

Autoxidative allylic oxidation and elimination of water, as shown by de la Rie,[5] could lead to diketones with several conjugated double bonds (Figure 10a) that absorb in the blue range, thus giving a yellow cast to the material. Elimination of water is favored adjacent to unsaturated systems, and hydroxy groups could also be introduced by autoxidation. Formation of quinones, as suggested by Formo,[36] seems reasonable, because the radical intermediates are stabilized by double bonds, and the 1,2-diketone is formed in the right position needed for cyclization (Figure 10b). This explanation of yellowing through condensation (dimerization of two ketones) is also consistent with the fact that the polymeric fraction of aged triterpenoid varnishes shows a stronger absorption at 400 nm than does the monomeric fraction.[5,11]

This theory of autoxidation as a contributor to yellowing is consistent with prior results. Oxidation is much stronger in light than it is in darkness, but new yellow compounds are simultaneously bleached; therefore, yellowing in light is not as evident as in darkness, even if the absolute rate of oxidation is higher. Yellowing in non-oxidative environments could be a result of condensation reactions and an enlargement of the preformed unsaturated ketone systems by water elimination without the formation of new ketones, as suggested by de la Rie.[5]

As mentioned in part 2d, aging of amyrin with sunlight resulted in the formation of a taraxarene product (structure V in Figure 5). The taraxarene skeleton was shown to be unstable relative to the amyrin skeleton.[30] Thus, the energy of the initial peroxide was to some extent stored in the structure of the product. This opens up an explanation for fast oxidation and degradation of triterpenoid varnishes on paintings: exposure of the resins to light induces formation of energy-rich substances (e.g., peroxides), which decompose and, presumably, initiate autoxidation, even without light. Because commercially available fresh resins are in an advanced stage of oxidation, especially mastic, they very probably contain many such compounds. Thus, oxidation is apparently initiated by daylight while the resin is still on the tree

or during harvesting and processing. After the varnish is applied to the painting, autoxidation continues quite quickly regardless of storage conditions, because induction has already taken place.

CONCLUSIONS

Films of dammar and mastic were naturally aged in light or darkness. These were compared to films artificially aged by light and heat, using graphite-assisted LDI-MS and EPR spectrometry. A comparison to 25-year-old naturally aged varnishes revealed essentially the same aging processes in both natural and artificial aging. The triterpenoids oxidize, polymerize, and decompose.

The mass spectra obtained from dammar and mastic samples aged in light or darkness were surprisingly similar. Oxidation takes place very easily and on a short time scale. Dammar is oxidized to a significant extent even after five months. Oxidation also proceeds in darkness without photoinitiation of the oxidative radical chain reaction, but at a lower rate. This is supported by quantitative EPR measurements that show that the radical concentration in commercial dammar and dammar films stored in darkness is one-third of the concentration of films recently exposed to light, which is rather high. The dark radical concentration was the same in all of the samples, regardless of the aging history before their dark storage.

Radical oxidation chain reactions proceeding in darkness support the hypothesis of unsaturated ketones being the cause of yellowing. Formation of yellow compounds can plausibly be explained by these reactions. Although oxidation occurs at a higher rate in light, yellowing is thought to be stronger in darkness, only because the colored compounds are photobleached.

The results shown here, as well as earlier studies, lead to the conclusion that radical initiation in dammar and mastic resins very probably occurs during harvesting and processing. As a result, autoxidation can no longer be stopped, and natural resin varnishes deteriorate relatively quickly on paintings, regardless of the care taken in storing or exhibiting them. A practical implication is that such resins should be harvested with as little exposure to light as possible, and processed in ways that do not generate radicals or radical precursors. They should then be far less susceptible to autoxidative aging. Studies to verify this hypothesis are currently under way in our laboratory.