Artificial Photoaging of Triterpenes Studied by Graphite-Assisted Laser Desorption/Ionization Mass Spectrometry

by **Patrick Dietemann**[a]), **Michael J. Edelmann**[a]), **Christian Meisterhans**[b]), **Corina Pfeiffer**[a]), **Stefan Zumbühl**[c]), **Richard Knochenmuss**[a]), and **Renato Zenobi**[a])*

[a]) Laboratorium für Organische Chemie, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich
[b]) Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich
[c]) Berner Fachhochschule, Studerstrasse 56, CH-3004 Bern

Dedicated to Prof. *Albert Eschenmoser* on the occasion of his 75th birthday

Seven triterpenes were selected for study of their photoaging behavior by graphite-assisted laser-desorption/ionization mass spectrometry as the primary analytical tool. The triterpenes serve as simplified reference systems for complex triterpenoid resins, which are used as varnishes on paintings. Some of the triterpenes are main components of such natural resins; others were chosen for their particular functional groups or carbon skeleton to evaluate specific influences on the aging process. Progressive aging resulted in incorporation of oxygen and simultaneous loss of hydrogen, recognizable in the mass spectra by the appearance of signals with mass increments of 14 and 16 Da. Degradation of aging products also led to compounds with lower masses than the initial triterpenes. The general aging behavior of the triterpenes studied was found to be rather similar, although differences in reactivity, and, therefore, in the extent of oxidation were found, depending on the functional groups. Compounds with aliphatic ketone groups were much more reactive under the test conditions than others. A mixture of five triterpenes showed the highest reactivity. Yellowing, an important aspect of aging with regard to varnishes on paintings, was found to be related to a general breakdown of the initial triterpenes and was not caused by a single compound.

1. Introduction. – Naturally occuring triterpenoid resins such as dammar and mastic are widely used as varnishes on paintings [1]. Varnishes saturate the colors, give gloss to the paintings, and protect them from environmental influences. With time, the varnishes yellow, crack, become brittle, and are not able to fulfill their esthetic and protective functions, necessitating their removal and replacement. Because removal can damage the painting [2–4], it is desirable to understand the processes of aging, in the hope of devising methods to stop or slow the deterioration.

Natural resins are very complex mixtures of different compounds, mostly terpenoids [1][5][6]. With aging, the complexity increases even more. To understand the aging behavior of such complex mixtures, it is helpful to first study the aging behavior of individual components. In this work, several pure triterpenes were artificially aged to investigate the changes that occur during aging. For direct comparison with natural resins, some of the main components of dammar and mastic were chosen as reference triterpenes. Others were chosen for their particular functional groups to evaluate specific influences on the aging process.

The fundamental aging processes occurring in triterpenoid resins were extensively investigated by *de la Rie* [7][8]. Within the *MOLART* project (molecular aspects of aging in painted works of art) [9], *Van der Doelen et al.* have investigated the aging processes of triterpenoid resin varnishes on a molecular level [6] [10–12]. Earlier mass spectrometry work by *Zumbühl et al.* [13] [14] emphasized light-induced aging and the consequences for the mechanical behavior of dammar and mastic varnishes. These studies have led to a working model of the main processes that occur during aging of natural-resin films, but important aspects like yellowing and aging in darkness remain incompletely understood. Recent work compared the aging of dammar and mastic in light and darkness [15]. Oxidation was shown to proceed remarkably rapidly in darkness, which led to a revised model for yellowing of natural-resin varnishes.

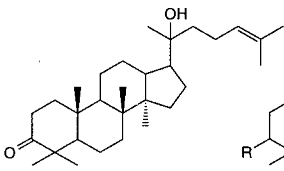
Aging of triterpenoid resins results in oxidation, polymerization, and decomposition of the initial compounds [6] [7] [10] [15]. Oxidation proceeds *via* autoxidative radical chain reactions, some of which are summarized here [14] [16] [17]:

$$\begin{array}{llllll} \text{Initiation:} & & \text{initiator} & \rightarrow & R^{\bullet} & (1) \\ \text{Propagation:} & R^{\bullet} + & O_2 & \rightarrow & ROO^{\bullet} & (2) \\ & ROO^{\bullet} + & RH & \rightarrow & ROOH + R^{\bullet} & (3) \\ \text{Branching:} & & ROOH & \rightarrow & RO^{\bullet} + {}^{\bullet}OH & (4) \\ \text{Termination:} & & \text{2 radicals} & \rightarrow & \text{non-radical products} & (5) \end{array}$$

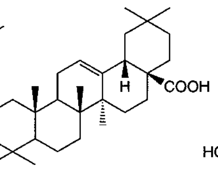
Once the radicals are formed (*Eqn. 1*), they rapidly react with O_2 (*Eqn. 2*). The resulting peroxyl radicals are relatively stable, thus *Eqn. 3* is rate-determining. Hydroperoxides can be homolytically cleaved by heat or light (*Eqn. 4*), and the chain is branched and propagated. This simple model describes the main radical oxidation pathways for organic compounds. There are, however, other reactions that proceed in parallel. Alkoxy radicals $RO^{\bullet}$, for example, can react to alcohols, ethers, or ketones. Addition to double bonds may compete with abstraction in *Eqn. 3* [16] [17]. Oxidation products like hydroperoxides also undergo non-radical 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 are nevertheless a consequence of these primary events [11] [18].

Aging of the pure triterpenes 20-hydroxydammar-24-en-3-one (dipterocarpol; **1**) and 3-hydroxyolean-12-en-28-oic acid (**2**) was investigated by *van der Doelen* [10]. Solutions of the triterpenes spiked with photosensitizers were photoaged, and studied by gas chromatography/mass spectrometry (GC/MS) and direct temperature-resolved mass spectrometry (DTMS). Only a small number of aging products were found to be abundant. The products identified were similar or identical to those found in naturally aged varnishes removed from paintings.

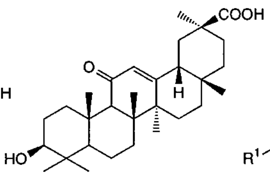
The goal of the present work was to expand the base of reference compounds, and to evaluate the influence of different functional groups or carbon skeletons on the aging behavior of pure triterpenes. The triterpenes investigated, in addition to **1** and **2**, were 3-oxoolean-12-en-28-oic acid (**3**), (3*β*,18*β*,20*β*)-3-hydroxy-11-oxoolean-12-en-29-oic acid (= 18*β*-glycyrrhetinic acid; **4**), 3*β*-urs-12-ene-3,28-diol (= uvaol; **5**), 3*β*-3-hydroxyurs-12-en-28-oic acid (= ursolic acid; **6**), and 3-oxours-12-en-28-oic acid (= ursonic acid; **7**). Compounds **1**–**3** are known components of dammar and mastic resins; **6** and **7** are components of dammar. The samples were analyzed primarily by graphite-assisted



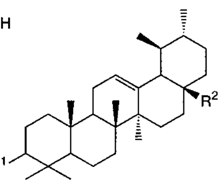
1



2 R = OH, H
3 R = O



4



5 R^1 = OH, H; R^2 = CH_2OH
6 R^1 = OH, H; R^2 = COOH
7 R^1 = O; R^2 = COOH

laser-desorption/ionization time-of-flight mass spectrometry (graphite-assisted LDI-MS). This method has been found to be uniquely suitable for direct analysis of complex mixtures such as varnishes [13][14], providing excellent spectra of starting materials, as well as oxidation, polymerization, and degradation products. It requires no special sample preparation or derivatization, and yields intact molecular ions (sodium adducts). It therefore provides a detailed and informative picture of the composition of fresh and aged triterpene or varnish samples. In contrast to *van der Doelen*'s work [10], triterpenes were aged as solids and without addition of photosensitizers.

2. Results and Discussion. – Graphite-assisted laser-desorption/ionization (graphite-assisted LDI-MS) results in intact desorption of the triterpenoids. Fragmentation in the spectrometer was found to be insignificant for all triterpene reference substances; thus, each signal in the mass spectrum of natural or aged samples is believed to correspond to one (or several) intact component(s). Carboxylic-acid protons can sometimes be exchanged for Na, leading to additional signals $[M + 2\,\mathrm{Na} - \mathrm{H}]^+$. While reproducibility of spectra sometimes is a problem in MALDI-MS in general, results obtained by graphite-assisted LDI-MS are always highly reproducible.

2.1. *General Progress of Aging.* Aging of the triterpenes results in a remarkably wide variety of products, indicating multiple radical-attack points and progressive reaction of initial products. H-Abstraction by attacking peroxyl radicals will preferentially produce the most stable radicals in tertiary, allylic, or oxygenated positions, first leading to a relatively small number of abundant products. With progressive oxidation, a triterpenoid will be oxidized in many different locations, since abstraction is not completely specific. A large number of O-atoms can, therefore, be incorporated, and a large variety of aging products is expected. Such a complex MS pattern was found for all artificially aged triterpenes measured by graphite-assisted LDI-MS, differing mainly in extent of oxidation. As the signal intensity of the initial triterpene decreases, groups of signals with mass increments of 16 or 14 Da develop (*Figs. 1–3*). Different numbers of O-atoms are incorporated, leading to signals with a mass difference of 16 Da. Simultaneous loss of H (*e.g.*, by allylic oxidation or oxidation from alcohols to acids) leads to mass increases of 14 Da. Different combinations of 14 and 16 Da increments lead to signal groups becoming broader and less distinct with increasing mass.

Aged dammar and mastic resin show the same grouped pattern [15], but, due to the complex composition of fresh resins, interpretation of the spectra is not clear. Since aging of a single triterpene already leads to a surprisingly large distribution of products, the complex composition of aged varnishes is not due to the formation of a few aging

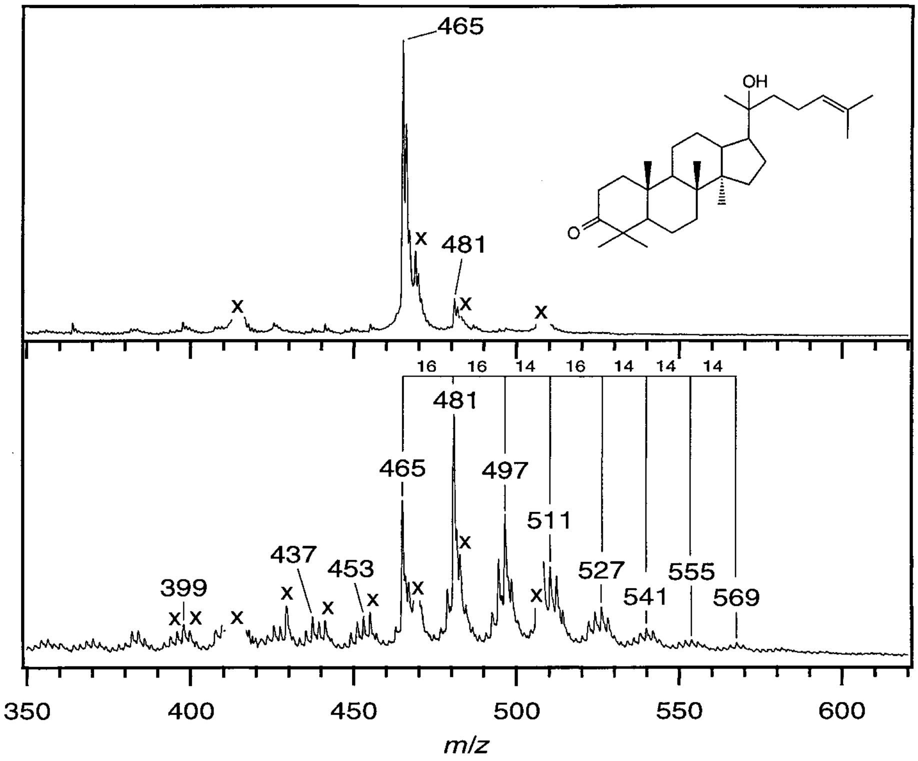


Fig. 1. *Graphite-assisted laser-desorption/ionization mass spectra of fresh* (upper) *and aged* (lower) *dipterocarpol* (**1**). The Na adduct $[M+\mathrm{Na}]^+$ of **1** appears at m/z 465. A small amount of $[M+16]^+$ at m/z 481 is visible in the unaged sample. Photoaging for 300 h results in oxidation and degradation of the initial compound. See text for more details. Signals marked with crosses are contaminants in the spectrometer (m/z 413, 469, 483, and 507). Signals with crosses at m/z <460 may have small contributions from contaminants.

products from a large number of initial compounds (as could be expected by the results of *van der Doelen* and *Boon* [6][10]), but rather the complexity of the aging products of each single component.

As mentioned above and in [15], the primary radical-oxidation reactions are followed by various secondary reactions. The most important among them are condensation reactions leading to polymers, rearrangement reactions [19], and cleavage reactions, *e.g.*, oxidative skeletal cleavage [11][12] or *retro-Diels-Alder* reactions [20], leading to products with lower molecular weights than the starting material. Although not all of these reactions have been shown to occur in aging of terpenes, their occurrence is very likely, and low-mass products can clearly be seen in the mass spectra, for example in that of aged dipterocarpol (*Fig. 1*). Polymerization of the triterpenes also occurred during aging, but was not studied in detail.

2.2. *Aging of Triterpenes with Aliphatic Ketone Groups.* The oxidation pattern is generally the same in all triterpenes, differing only in extent. However, especially strong oxidation occurred in all saturated ketones (**1**, **3**, and **7**). This was expected, because the light used for aging was filtered with a cutoff of *ca.* 315 nm (simulated

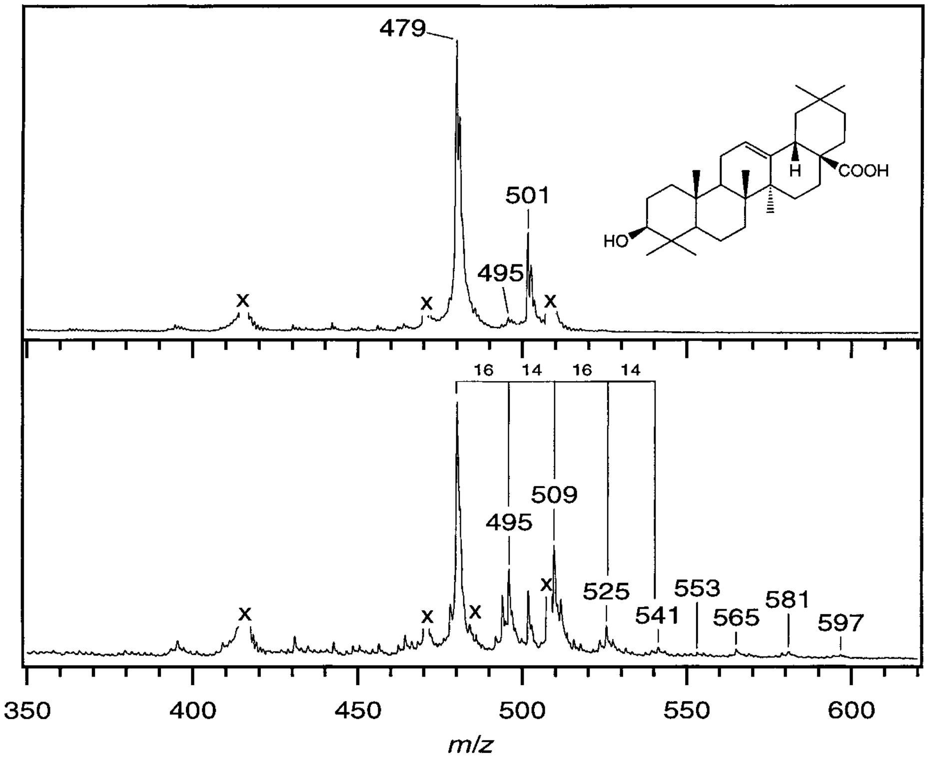


Fig. 2. *Graphite-assisted laser-desorption/ionization mass spectra of fresh* (upper) *and aged* (lower) *oleanolic acid* (**2**). The Na adduct $[M+\mathrm{Na}]^+$ of **2** appears at *m/z* 479. A small amount of $[M+16]^+$ at *m/z* 495 is visible in the unaged sample, together with the Na adduct of the carboxylic acid at *m/z* 501 ($[M+2\ \mathrm{Na}-\mathrm{H}]^+$). Photoaging results in basically the same oxidation pattern as for **1**, but the oxidation is not as advanced since autoxidation cannot be initiated by **2** itself. See text for more details. Signals marked with crosses are contaminants in the spectrometer.

daylight through window glass), and ketone absorption bands extend to *ca.* 330 nm. Photoexcitation of ketones is followed by α-cleavage (*Norrish* Type I), radicals are formed, and autoxidative radical chain reactions are initiated. This reaction was considered to be the most important source of radicals in degradation of varnishes [7] [13]. However, *van der Doelen et al.* showed evidence that naturally aged varnishes from paintings contain only small amounts of *Norrish* reaction products [12]. Since radicals are also present in varnishes stored in darkness, and autoxidation takes place without any light [15], it was recently concluded that ketone cleavage is not the only source for radicals, and oxidation proceeds in a UV-free environment or even in darkness. Nevertheless, the strong oxidation of all ketones studied here strongly suggests that *Norrish* reactions are important in degradation of varnishes of paintings when they are exposed to indoor daylight. Presumably, the much lower light intensity in natural aging merely leads to small concentrations of *Norrish* products, which react further to form a large variety of different compounds with progressive oxidation, *i.e.*, detection of a single *Norrish* product with GC/MS is difficult. Moreover, ketones

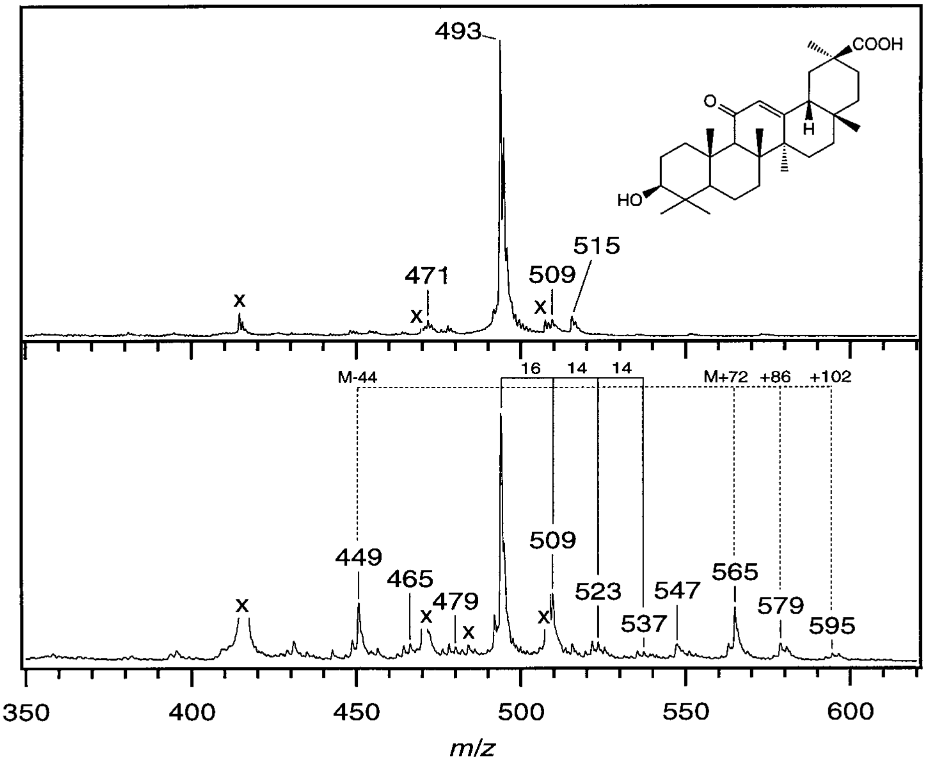


Fig. 3. *Graphite-assisted laser-desorption/ionization mass spectra of fresh* (upper) *and aged* (lower) *18β-glycyrrhetinic acid* (**4**). The Na adduct $[M+Na]^+$ appears at m/z 493. A small amount of $[M+16]^+$ at m/z 509 is visible in the unaged sample, together with the Na adduct of the carboxylic acid at m/z 515 ($[M+2\,Na-H]^+$). Photoaging leads to a slightly different oxidation pattern as for the other triterpenes (signals connected by dashed lines). See text for more details. Signals marked with crosses are contaminants in the spectrometer.

formed by autoxidation and not contained in the original resins also can undergo *Norrish* cleavage. Closer inspection of the gas chromatograms in [12] in fact reveals numerous unresolved and unidentified signals that could be *Norrish* products.

2.3. *Aging of Triterpenes without Ketone Groups.* Compounds **2**, **5**, and **6** show weak oxidation after photoaging (*cf. Fig. 2*) although they do not absorb at wavelengths >315 nm. Since the triterpenes were not purified, they may contain traces of absorbing compounds (*e.g.*, ketones) that act as sensitizers, either by direct formation of radicals or by energy transfer to other compounds that then decompose [16]. Once oxidation is started, new ketones probably develop and propagate the oxidation as described above. This model explains why the triterpenes lacking a ketone group are also oxidized with time, but to a lesser extent compared to the ketones. It would also explain why the general oxidation pattern is the same.

2.4. *Aging of 18β-Glycyrrhetinic Acid* (**4**). The structure of **4** contains an unsaturated ketone that absorbs beyond 350 nm, thus even stronger oxidation is expected than for the ketones. Nevertheless, this compound was only weakly oxidized when irradiated (*Fig. 3*), comparable to compounds without ketone groups (*e.g.*, oleanolic acid (**2**), *Fig. 2*). It seems that irradiated 18β-glycyrrhetinic acid (**4**) does not undergo the same reactions. This is also indicated by an aging pattern differing from those of the other triterpenes: relatively intense signals appear at *m*/*z* 449 ($M - 44$) and *m*/*z* 565, 579, and 595 ($M + 72$, $M + 86$, and $M + 102$, resp.). The signal at *m*/*z* 449 ($M - 44$) could be explained by loss of CO_2, but this is in contrast to the aging behavior of all other triterpenes with carboxylic-acid groups, which showed no decarboxylation. It seems therefore probable that the $C{=}C{-}C{=}O$ group is involved in the processes leading to the special aging pattern of **4**, although the signals cannot yet be assigned unambiguously.

2.5. *Aging of Oleanonic Acid* (**3**) *and Ursonic Acid* (**7**). Artificial aging of oleanonic acid (**3**) led to very informative results. This was the only triterpene studied that showed pronounced yellowing. Orange and brown spots of different sizes appeared, and white crystals were found in the center of the brown spots. Mass spectra of samples from the different colored regions are depicted in *Fig. 4*. The white crystals are pure **3**, while the yellow regions consist of both oxidized and unoxidized **3**. The acid is totally broken down in the brown spots. Thus, the discoloration is related to a general breakdown of **3**. This is also suggested by the UV/VIS spectrum of the aged **3**, which is structureless above 320 nm, having only a steadily decreasing tail up to wavelengths >400 nm. The broadness and lack of structure is probably due to the presence of a variety of compounds. Similar behavior was observed for aged dammar resins [7] and is a result of unspecific oxidation pathways [5] [7] [15] [21].

All triterpenes were aged without further purification since mass spectra revealed only small amounts of impurities, and there was no indication that they lead to a different aging behavior than in resins. It must be kept in mind that resins are complex mixtures of many compounds that can influence each other, *e.g.*, by sensitization (see *Sect. 2.3*). Aged oleanonic acid (**3**), however, showed a very strong and inhomogenous discoloration and oxidation. Since **3** was synthesized by oxidation of oleanolic acid (**2**) with H_2CrO_4, and was first aged without special purification, the inhomogeneities were presumably initiated by traces of catalytic or sensitizing residues from the synthesis that may not be representative of natural resins. The potential catalysts fall into two categories: oxidized organic by-products, or traces of Cr compounds. To evaluate these possibilities, comparative aging experiments were carried out. Oleanonic acid (**3**) was purified by crystallization or by HPLC. To have a second system for comparison with **3**, ursonic acid (**7**) was synthesized from ursolic acid (**6**; these substances have very similar structures). This synthesis was carried out analogously to that of **3**, under the same conditions and with the same reagents. Thus, similar by-products should be formed, and aging results should be directly comparable. Ursonic acid **7** was also purified by crystallization.

Semiquantitative determination of Cr concentrations in samples of **3** and **7** was performed by inductively-coupled-plasma optical-emission spectroscopy (ICP-OES). At a concentration of 12 ppm, Cr was found in the unpurified oleanonic acid (**3**). In the crystallized acid, the concentration was 2 ppm. Unpurified ursonic acid (**7**) had a Cr concentration *ca.* 9 times higher (105 ppm) than unpurified **3**.

Crystallized oleanonic acid (**3**) was less oxidized by aging than unpurified **3**, although oxidation was still strong. HPLC-purified **3** showed only weak oxidation. No yellowing occurred in either sample. The crystallized ursonic acid (**7**) also showed

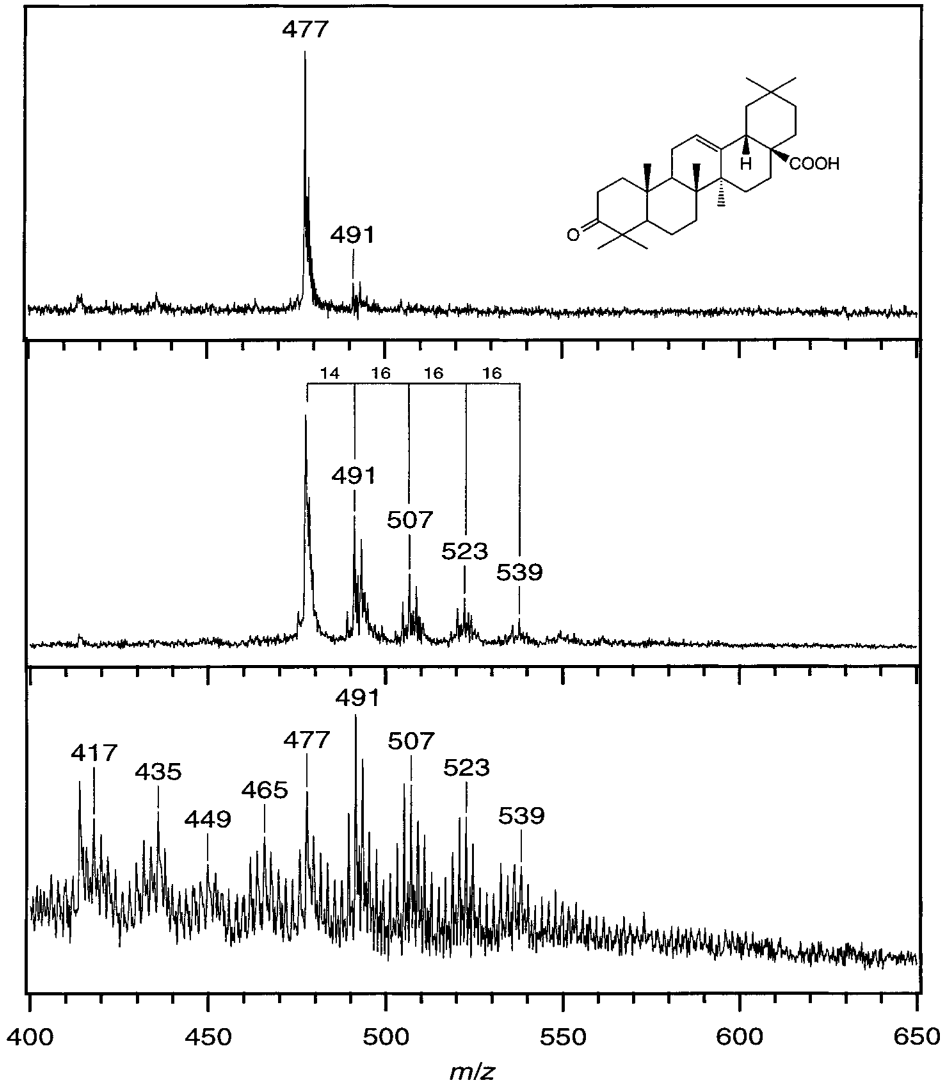


Fig. 4. *Graphite-assisted laser-desorption/ionization mass spectra of aged oleanonic acid* (**3**). The Na adduct of **3** appears at *m/z* 477. Strong yellowing occurred during aging, and brown spots with white crystals in their centers developed. Samples were taken from white crystals (*upper*), yellow region (*middle*), and brown spots (*lower*). Degradation proceeds in two steps: first oxygen is incorporated (*middle*), and then cleavage reactions of oxidized intermediates lead to compounds with masses smaller than that of oleanonic acid (*lower*). The discoloration is related to a general breakdown of the initial oleanonic acid.

weaker oxidation than the unpurified, but the degree of oxidation in **7** was generally much lower compared to that in **3**. No yellowing occurred during aging of **7**. These results imply that traces of Cr are not the only important factors for oxidation, not only because Cr concentrations were very low, but also because oxidation of **7** was weaker than that of **3** despite a much higher Cr concentration. Since purity does matter, other minor by-products probably play a sensitizing role. It is, therefore, surprising that differences in reactivity of **3** and **7** are that large, although similar oxidation by-products from the synthesis are expected. The role of impurities is an aspect in aging that needs to be further investigated.

2.6. *Aging of a Mixture of Five Triterpenes.* To simulate a simplified 'resin', equimolar amounts of **1**–**5** were mixed and artificially aged. Compounds **1**–**3** are components of dammar and mastic. Results are depicted in *Fig. 5*. The different triterpenes show different signal intensities in the mass spectrum of the unaged mixture, although they all were present in the same concentrations. This is not surprising since sensitivity is related to the strength of Na adducts, which is generally greater for more polar compounds. This can clearly be seen in *Fig. 5:* the combined signal of dipterocarpol (**1**) and uvaol (**5**) at *m*/*z* 465 has the same intensity as that of oleanonic acid (**3**), although it is caused by two substances. The signals of **2** and **3** have similar intensities, thus there is no big difference between an OH and a C=O group with respect to mass-spectral sensitivity. 18*β*-Glycyrrhetinic acid (**4**) reveals the most intense signal. It is not clear whether this is a polarity effect of the additional O-atom, or is specific to the

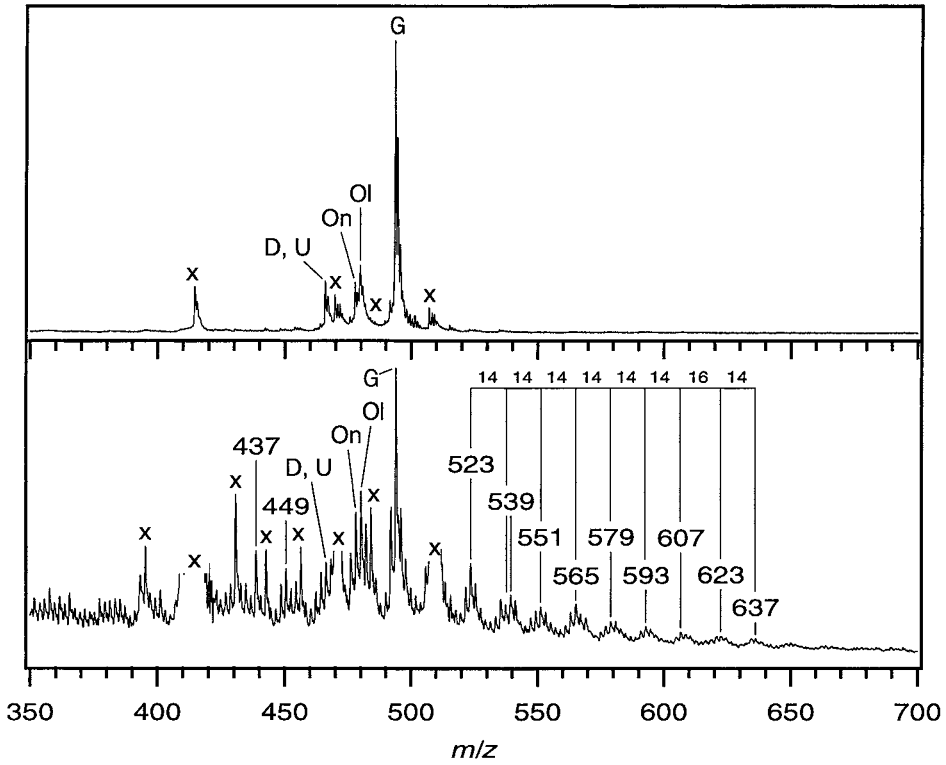


Fig. 5. *Graphite-assisted laser-desorption/ionization mass spectra of fresh* (upper) *and aged* (lower) *equimolar mixture of dipterocarpol* (**1**: D, *m*/*z* 465), *uvaol* (**5**: U, *m*/*z* 465), *oleanonic acid* (**3**: On, *m*/*z* 477), *oleanolic acid* (**2**: Ol, *m*/*z* 479), and *18β-glycyrrhetinic acid* (**4**: G, *m*/*z* 493). Different signal intensities indicate different efficiencies of Na-adduct formation. Photoaging leads to the same oxidation pattern as observed for the single triterpenes. See text for more details. Signals marked with crosses are contaminants in the spectrometer.

unsaturated keto group. The different location of the acid group in the structure could also lead to different Na-adduct-formation efficiencies due to different steric factors.

Artificial aging of the mixture of the triterpenes led to very strong oxidation and yellowing. The initial triterpenes are almost totally broken down and a large variety of compounds is formed. The signal groups with 14 and 16 Da mass increments observed in aged pure triterpenes are nevertheless preserved in the mixture. Substances with masses of up to more than 700 Da are formed. The same reactive species as in **3** probably also induce the strong oxidation and yellowing in the mixture, since **3** is contained in the mixture. Nevertheless, it is evident that the mixture is more reactive than the single components alone. This is presumably because the mixture contains more different functional groups and more combinations of groups in proximity that can react with each other. Natural resins like dammar or mastic are much more complex mixtures, and their reactivity is therefore very high. As varnishes on paintings, they oxidize and decompose very fast (within months), almost independently of storage conditions [15].

3. Conclusions. – Artificial aging of individual triterpenes with simulated window-filtered daylight results in strong oxidation of compounds with saturated ketone groups while compounds without such a group are only weakly oxidized. It is, therefore, concluded that ketones are the main initiators of autoxidation (*via Norrish* cleavage) under these conditions. Surprisingly, the one unsaturated ketone studied did not react in the same way and was only weakly oxidized.

The general pattern of oxidation is almost the same in all the triterpenes studied: different numbers of O-atoms are incorporated while H is eliminated. This results in a large number of compounds with mass increments of 14 or 16 Da. Seven O-atoms or more can be incorporated. Cleavage reactions also lead to a broad distribution of compounds with smaller masses than the initial compounds.

Oleanonic acid (**3**) was found to be very reactive, but this is highly dependent on purity. Energy-rich by-products from the synthesis could be responsible for this effect. Traces of Cr left from the synthesis do not act as catalysts. Ursonic acid (**7**) generally showed significantly weaker reactivity than **3**, although their structures are very similar. It is not obvious why this difference in reactivity is so large.

With regard to varnishes on paintings, a major aspect of aging is yellowing. Unpurified oleanonic acid (**3**) and its mixture with the other triterpenes strongly yellowed during aging. This yellowing is related to a general breakdown of the initial compounds and is not caused by a single compound formed in a well-defined reaction.

Comparison of the aging behavior of single triterpenes, a mixture of five triterpenes, and the complex natural triterpenoid resins [15] reveals the same basic processes: oxidation and degradation. With increasing compositional complexity, the reactivity of the reference substances increases, thus even greater reactivity is then expected in natural resins.

In pure triterpenes, all aging products must evolve from a single compound. Since photoaging of even a single triterpene results in a remarkably complex pattern of many different products, it is obvious that the complex composition of aged varnishes is caused not only by the complexity of the fresh resin, but even more by the complexity of the aging-product formation of each individual component.

The authors wish to thank *K. Hametner* for performing the Cr-content determination. We are grateful to Prof. *D. Arigoni* and Dr. *C. Herm* for stimulating and fruitful discussions. Financial support of the *Swiss National Science Foundation* (project No. 20-52422.98) is gratefully acknowledged.

Experimental Part

Materials. The triterpene reference substances dipterocarpol (**1**; >97%) and 18β-glycyrrhetinic acid (**4**, >97%) were obtained from *Fluka*, oleanolic acid (**2**; >97%) and uvaol (**5**; *ca.* 95%) were obtained from *Sigma*, and ursolic acid (**6**; >90%) was obtained from *Aldrich*. They were aged without further purification.

Synthesis of Oleanonic Acid (**3**) *and Ursonic Acid* (**7**). A 0.54M *Jones* reagent soln. was prepared [22]: CrO_3 (54 g, 0.1 mol) was dissolved in conc. H_2SO_4 (46.5 ml), keeping the temp. at 0°, and this soln. was diluted to 1000 ml with H_2O.

The acid (0.199 g, 0.44 mmol), was dissolved in acetone (4 ml). Dropwise addition of *Jones* reagent (0.25 ml) caused the color of the soln. to change from white to green-blue, indicating the formation of Cr^{III}. Further addition of acetone (4 ml) and dropwise addition of *Jones* reagent (0.34 ml) resulted in a green-blue fine precipitate in a yellowish soln., indicating unreacted Cr^{VI}. After several min, the soln. clarified. After 40 min, the reaction was quenched by addition of H_2O (5 ml) and aq. $Na_2S_2O_3$ soln. (1M, 5 ml), and extracted with Et_2O (3 × 20 ml). The org. phase was washed with HCl (1M, 2 × 20 ml) and dried ($MgSO_4$). Removal of the solvent at reduced pressure gave 0.230 g of product. For further investigations, the product was purified by either crystallization from Et_2O or HPLC (*Vydac* reverse-phase *C_{18}* column at r.t., with MeCN/H_2O 5 : 1, at a flow rate of 0.8 ml/min, UV detection at 240 nm).

^{13}C-NMR Data of **3** ($CDCl_3$, 75 MHz): 14.91 (*s*); 16.88 (*s*); 19.46 (*d*); 21.34 (*s*); 22.82 (*d*); 23.40 (*s*); 23.45 (*d*); 25.73 (*s*); 26.36 (*s*); 27.59 (*d*); 30.57 (*q*); 32.07 (*d*); 32.31 (*d*); 32.94 (*s*); 33.71 (*d*); 34.03 (*d*); 36.71 (*q*); 39.01 (*d*); 39.39 (*q*); 40.69 (*t*); 41.65 (*q*); 45.73 (*d*); 46.49 (*q*); 46.80 (*t*); 47.33 (*q*); 55.23 (*t*); 122.32 (*t*); 143.54 (*q*); 183.61 (*q*); 216 (*q*).

Aging Procedure. All triterpenes were dissolved in freshly distilled THF, spread on glass microscope slides and allowed to dry. Artificial aging 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.

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

ICP-OES Measurements were performed on a *IRIS* instrument (*Thermo Jarell Ash*, Germany).

Sample Preparation for MS Measurements. A suspension of 2-µm graphite particles (*Aldrich*) in MeOH was allowed to dry on the sample tip. A THF soln. of the triterpene was pipetted onto the graphite and also allowed to dry. The sample quantity was varied empirically for best signal and resolution. 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 addition of a small amount of NaCl to the graphite/MeOH slurry.

Contamination of the spectrometer with diffusion pump oil led to signals at *m/z* 413, 469, 483, and 507 that interfered with the signals of the triterpenes. These signals appeared immediately after sample insertion and increased with time.

REFERENCES

[1] R. de la Rie, *Anal. Chem.* **1989**, *61*, 1228A.
[2] N. Stolow, in 'On Picture Varnishes and their Solvents', R. L. Feller, N. Stolow, E. H. Jones, National Gallery of Art, Washington, 1985, pp. 47–116.
[3] D. Erhardt, J.-S. Tsang, 'Cleaning, Retouching and Coatings' (IIC Congress), Brussels, 1990, pp. 93–97.
[4] K. Sutherland, *Studies in Conservation* **2000**, *45*, 54.
[5] J. S. Mills, R. White, 'The Organic Chemistry of Museum Objects', 2nd. edn., Butterworth-Heinemann, Oxford, 1994.
[6] J. Boon, G. van der Doelen, 'Postprints of Firnis, Material Aesthetik Geschichte', International Kolloquium, Braunschweig, 1998, pp. 92–104.

[7] R. de la Rie, *Studies in Conservation* **1988**, *33*, 53 – 70.
[8] R. de la Rie, Ph. D. thesis, University of Amsterdam, 1988.
[9] J. J. Boon, 'Project on Molecular Aspects of Ageing in Painted Works of Art, Progress Report 1995 – 1999', 1999, www.amolf.nl/research/biomacromolecular mass spectrometry/molart/molart.html.
[10] G. A. van der Doelen, Ph. D. thesis, University of Amsterdam, 1999.
[11] G. A. van der Doelen, K. J. van den Berg, J. J. Boon, *Studies in Conservation* **1998**, *43*, 249 – 264.
[12] G. A. van der Doelen, K. J. van den Berg, J. J. Boon, accepted in *Techne.*
[13] S. Zumbühl, R. Knochenmuss, S. Wülfert, F. Dubois, M. J. Dale, R. Zenobi, *Anal. Chem*. **1998**, *70*, 707.
[14] S. Zumbühl, R. D. Knochenmuss, S. Wülfert, *Z. für Kunsttechnologien und Konservierung* **1998**, *12*, 205.
[15] P. Dietemann, M. Kälin, S. Zumbühl, R. Knochenmuss, S. Wülfert, R. Zenobi, submitted to *Anal. Chem.*
[16] G. Scott, 'Atmospheric Oxidation and Antioxidants', Elsevier Science Publishers B.V., Amsterdam, 1993.
[17] R. L. Feller, 'Accelerated Aging, Photochemical and Thermal Aspects', The Getty Conservation Institute, Los Angeles, 1994.
[18] G. A. van der Doelen, K. J. van den Berg, J. J. Boon, N. Shibayama, R. E. de la Rie, W. J. L. Genuit, *J. of Chromatogr. A* **1998**, *809*, 21.
[19] I. Agata, E. J. Corey, A. G. Hortmann, J. Klein, S. Proskow, J. J. Urspung, *J. Org. Chem*. **1965**, *30*, 1698.
[20] A. Melera, D. Arigoni, A. Eschenmoser, O. Jeger, L. Ruzicka, *Helv. Chim. Acta* **1956**, *39*, 441.
[21] O. S. Privett, M. L. Blank, J. B. Covell, W. O. Lundberg, *J. Am. Oil Chem. Soc*. **1961**, *38*, 22.
[22] G. Cainelli, G. Cardillo, 'Chromium Oxidations in Organic Chemistry', Springer Verlag, New York, 1984.
[23] M. L. Vestal, P. Juhasz, S. A. Martin, *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1044.

Received May 3, 2000