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The role of organic and inorganic indoor pollutants in museum environments on the degradation of dammar varnish

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Abstract

This paper investigates the effects of inorganic (NO₂ and O₃) and volatile organic acid (acetic acid) pollutants on the degradation of dammar varnish in museum environments.. Model paint varnish samples based on dammar resin were thus investigated, by Gas Chromatography – Mass Spectrometry (GC-MS), Dynamic Mechanical Analysis (DMA) and Atomic Force Microscopy (AFM). Dammar is a natural triterpenoid resin, commonly used as a paint varnish. Samples were subjected to accelerated ageing by different levels of pollutants (NO₂ and O₃ and acetic acid) over a range of relative humidity (RH) conditions and then analysed. The results revealed that as the dose of pollutant increased, so did the degree of oxidation and cross-linking of the resin. Most interestingly, it was shown for the first time that exposure to acetic acid vapour resulted in the production of an oxidised and cross-linked resin, comparable to the resin obtained under exposure to NO₂ and O₃. These conclusions were supported by the analyses of model varnishes exposed for about two years in selected museum environments, where levels of pollutants had been previously measured. Exposures were performed both within and outside selected microclimate frames for paintings. Results showed that varnishes placed within the microclimate frames were not always better preserved than those exposed outside the frames. For some sites, the results highlighted the protective effects of the frames from outdoor generated pollutants, such as NO₂ and O₃. For other sites, the results showed that the microclimate frames acted as traps for the volatile organic acids emitted by the wooden components of the mc-frames, which damage the varnish. The study permitted to understand how the

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37 degradation of organic materials can be affected by indoor pollutants, laying the basis for
38 an efficient preservation of our cultural heritage

39

40 **Keywords**

41 acetic acid; NO₂; O₃; GC-MS; AFM; DMA; museum environments; dammar resin.

42

43 **1. Introduction**

44 Paintings are among the most precious and visited works of art in museums, galleries and
45 historic houses. It is well known that light, pollution, and thermo-hygrometric variations
46 lead to the degradation of painting materials, threatening not only a painting's appearance,
47 but also its conservation, and that these phenomena also happen in protected museum
48 environments¹⁻⁸. A common approach used to protect paintings against these risks is to
49 enclose them in microclimate frames or showcases. These are specifically built frames or
50 showcases whose purpose is to protect the paintings. They have been used for many
51 years as an efficient means for "preventive conservation"⁹. They provide a fundamental
52 tool for the sustainable preservation of our cultural heritage, especially given the increased
53 demand for energy saving for conditioning enclosed environments. Although the protective
54 effect of microclimate frames against UV radiation, RH and thermal variations has been
55 studied¹⁰⁻¹³, disadvantages of airtight showcases in view of protection, corrosive self-
56 outgassing of volatile organic compounds (VOCs) and microbiological infection and growth
57 have been highlighted¹⁴. The protective effects against air pollutants, both those generated
58 within the frame, and those that infiltrate from the outside is in fact extremely important, as
59 most painting collections, especially in Europe, are hosted in historical buildings,
60 commonly located in city centres, with poor thermal isolation and high pollution levels.

61 This paper investigates the effects of pollutants in museum environments on the
62 degradation of organic materials in paintings. The research focused on the degradation of
63 dammar resin exposed to both organic and inorganic pollutants. Dammar resin has been,
64 and still is, commonly used as a varnish for paintings, and thus represents the first barrier
65 of a painting against the external environment¹⁵⁻¹⁷. Moreover the molecular composition of
66 dammar resin has been extensively studied, and it is known that it ages through
67 autoxidative pathways, in the same way as several other organic materials used in
68 paintings, such as drying oils and other terpenoid resins^{2, 18, 19} and references therein).
69 This study was undertaken within the European FP6 research project PROPAINT

70 (Improved Protection of Paintings During Exhibition, Storage and Transit, 2007-2009),
71 aimed at evaluating the protective effect of microclimate frames for paintings²⁰. In this
72 study the degradation in terms of oxidation, cross-linking, loss of solubility, embrittlement,
73 and surface changes of dammar was investigated under the effects of NO₂, O₃ and acetic
74 acid. The first two are pollutants present in urban atmospheres and can thus infiltrate in
75 museum environments. On the other hand volatile organic compounds (VOCs) are more
76 abundant in indoor environments²¹, and acetic acid is a VOC that is commonly present in
77 museums²²⁻²⁶. Although the degradation of natural triterpenoid varnishes in light and
78 darkness has been extensively studied^{1, 2, 18, 19, 27-40}, to date, there has only been one
79 study at the molecular level of the effects of environmental pollutants on the varnishes on
80 paintings⁴¹.

81 In our study, reference layers of dammar resin were prepared and subjected to
82 accelerated ageing at different levels of relative humidity and at different doses of the
83 investigated pollutants²⁰. To evaluate the protective effect of microclimate frames on
84 natural varnishes, reference layers were also exposed, both inside and outside of the
85 microclimate frames and showcases in museum environments, for on site natural ageing.
86 In this study Gas Chromatography Mass Spectrometry (GC-MS) was used to characterise
87 the soluble fraction of the resin in order to assess the cross-linking and oxidation reactions
88 taking place⁴². In addition, dynamic mechanical analysis (DMA) and atomic force
89 microscopy (AFM) were used for the physical characterisation of the resin.

90

91 **2. Experimental**

92 **2.1 Materials**

93 Dammar and Shellsol A used for the varnish preparation were supplied by Kremer
94 Pigmente, Germany. The raw resin was analysed and in the text is referred to as "raw".
95 The stainless steel foil (AISI 302, Fe/Cr18/Ni8) used as a support for the varnish replicas
96 was supplied by Goodfellow, England.

97

98 **2.2 Reagents**

99 N-hexane, dichloromethane, isooctane, methanol, acetone, tridecanoic acid and
100 hexadecane were provided by Sigma, Aldrich USA; N,O-bis(trimethylsilyl)-trifluoroacetamide
101 (BSTFA) with 1% of trimethylchlorosilane (Fluka, Switzerland), purity >97% (Sigma-
102 Aldrich, USA) was used without any further purification.

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103 The NO₂ source was from permeation tubes provided by Chalmers University of
104 Technology (Gothenburg, Sweden). Acetic acid purity 100% was obtained from Merck,
105 Millipore.

106

107 **2.3 Instrumentation**

108 The O₃ generator used for the accelerated ageing experiments was supplied by TE
109 Instruments (Model no. 165). The photometric O₃ analyzer (Model 400), which measured
110 the concentration of O₃ continuously during the accelerated ageing in the exposure
111 chambers was supplied by Advance Pollution Instrumentation Inc (API).

112

113 A 6890N GC System Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) was
114 coupled with a 5975 Mass Selective Detector (Agilent Technologies) single quadrupole
115 mass spectrometer and equipped with a PTV injector. The mass spectrometer was
116 operated in the EI positive mode (70 eV), transfer line: 280°C, investigated mass range:
117 *m/z* 50-850. The PTV injector was used in splitless mode and was kept at 320°C.
118 Chromatographic separation was performed with a chemically bound HP-5MS fused silica
119 capillary column (Agilent Technologies). The carrier gas was helium (99.995% purity),
120 used at a constant flow of 1.5 mL/min. The chromatographic oven was programmed as
121 follows: 80°C, isothermal for 2 min, 6°C/min up to 300°C, 300°C isothermal for 30 min.

122

123 Dynamic Mechanical Analysis (DMA) was used to measure the viscoelastic properties of
124 the varnish coatings on steel in terms of the glass transition temperature (*T_g*). The
125 rationale for measuring *T_g* is that changes in the chemical structure such as cross-linking
126 are detected by shifts in *T_g* to higher values. Measurements were taken in bending mode
127 (free length of 5 mm) from 30 to 200°C with a heating rate of 3°C/min and a frequency of 1
128 Hz, using a Rheometric Scientific Analyser (DMA) Mark 3

129

130 Atomic Force Microscopy (AFM) was performed using the Nanosurf® EasyScan 2 AFM in
131 dynamic mode to image the varnished strips. A cantilever (Nanosensors PPP-NCLR) with
132 a spring constant $k_{\text{tip}}=48$ N/m, resonance frequency $f_{\text{res}}=170$ kHz and a tip radius $r_{\text{tip}}<10$
133 nm was applied.

134

135 **2.4. Sample preparation**

136 *GC-MS analytical procedure.* Samples (200-450 µg) were removed using a scalpel from
137 the replicas. They were then extracted with 1000 µL dichloromethane and 500 µL
138 methanol at 25°C for 30 minutes in an ultrasonic bath. 5 µL of a tridecanoic acid solution
139 (derivatisation internal standard) were added to 50-500 µL of the extracted solution,
140 evaporated to dryness under a nitrogen flow and derivatised with 20 µL of N,O-
141 bistrimethylsilyl-trifluoroacetamide (BSTFA), 50 µL isooctane (solvent) at 60°C for 30 min.
142 2 µL of the derivatised solution were injected into the GC-MS. Sample blanks were run in
143 order to evaluate the effect of the sample matrix, systematically revealing the absence of
144 triterpenoids in the environmental contamination.

146 *DMA analysis of varnish replicas.* Samples (10 mms x 10 mms) were cut from the
147 varnished steel sheet (0.1 mm thick) and mounted in a single cantilever configuration.

149 *AFM analysis of varnish replicas.* Samples were cut from the varnished steel sheet (0.1
150 mm thick) and fixed onto a metallic holder using double-sided tape.

152 **2.5 Preparation of varnish replicas**

153 A series of test strips of stainless steel foil (0.1 mm thickness) were coated with a varnish
154 prepared by dissolving dammar in Shellsol A 40% w/w. The varnished steel sheets were
155 left lying flat for four days at room temperature in order to allow the varnishes to settle and
156 most of the solvents to evaporate. They were subsequently placed into a climate controlled
157 oven at 40°C and 50% RH for one week. Details on the preparation of the replicas are
158 reported elsewhere²⁰

159 The dry varnish replicas were wrapped in acid free paper and placed in air-tight sealed
160 aluminium envelopes, and stored in a fridge in anti-corrosion (Interceptor) bags before
161 analysis and artificial ageing. Two sets of varnish replicas not subjected to artificial ageing
162 were analysed as control samples in January 2008 and in June 2009, representing the
163 samples t0 January 2008 and t0 June 2009, respectively.

165 **2.6 Accelerated ageing**

166 The exposure chamber consists of three independent cylindrical chambers with a height of
167 about 22 cm and a diameter of approximately 21 cm. The three chambers are placed
168 inside a glass box and submerged in water at 22°C. For each individual chamber it is
169 possible to introduce gaseous pollutants, and regulate the relative humidity (RH). The

170 temperature is maintained by the water circulating between the chambers and the glass
171 box. The chambers are kept in a windowless room and very low levels of artificial light (i.e.
172 UV light almost zero, and visible light approximately 20 Lux). The pollutants investigated
173 were NO₂, O₃ and acetic acid at different doses, which were obtained by modifying the
174 flow rate and duration of the exposure. The flow rate varied from 0.5 to 2.0 cm/s and the
175 exposure time was 10, 20 or 30 days. An overview of the accelerated ageing experiments
176 is shown in Table 1.

177

178 **Table 1: Overview of the accelerated ageing exposures carried out with different**
179 **pollutants and values of Relative Humidity (RH) in the exposure chamber. C =**
180 **average concentration in the chamber; time = exposure duration.**

181

182 Accelerated ageing experiments with NO₂. The NO₂ was supplied through permeation
183 tubes as mentioned elsewhere²⁰. The average concentration of the gas in the chamber for
184 each experiment was estimated from the weight loss of the tubes, the constant air flow
185 rate through the chamber, and the exposure time.

186 Accelerated ageing experiments with O₃. The O₃ source was a UV – O₃ generator. The O₃
187 concentration in the chamber was continuously monitored by a photometric O₃ analyzer.

188 Accelerated ageing experiments with Acetic Acid. Two different types of accelerated
189 ageing experiments were carried out with acetic acid as the pollutant: one was performed
190 in the exposure chamber and the other in a closed glass vessel containing glacial acetic
191 acid.

192 Exposure chamber. The acetic acid source (for replicas AcA-20-21 and AcA-80-26) was a
193 vial filled with glacial acetic acid. The concentration in the chambers was estimated from
194 the weight loss of the vials, the flow rate and the exposure time.

195 Glass vessel. To cause severe damage to the varnish replicas, prolonged accelerated
196 ageing with glacial acetic acid was also performed. Two sets of varnish samples were
197 exposed to glacial acetic acid vapours in equilibrium with the pure liquid in a closed glass
198 vessel. The temperature was kept at 22°C and visible light exposure was approximately 20
199 Lux (UV light was zero). A small beaker filled with glacial acetic acid was placed at the
200 bottom of a glass vessel with a volume of about 10 L.

201 The varnish samples were placed in a vertical position in an open string rack on a
202 perforated porcelain plate, located approximately 10 cm from the bottom of the glass
203 vessel and about 8 cm above the surface of the glacial acetic acid. One set of samples
204 was removed after six weeks and placed in a climate chamber with a laminar pure air flow
205 of 0.1 cm³/s, RH = 50%.

206 The other set of samples was removed from the glass vessel after three months and
207 placed in the climate chamber with pure air for one week. The acetic acid concentration
208 was estimated at $4.4 \cdot 10^4$ $\mu\text{g/L}$. The resulting dose days for these two dammar replicas
209 were thus $0.8 \cdot 10^6$ ppm day for the 6-week exposure sample (glacial AcA 1.5) and $1.6 \cdot 10^6$
210 ppm day for the 3-month exposure sample (glacial AcA 3).

211

212 **2.7 Site exposures**

213 Ten sets of replicas were distributed to five end-user museums to test within selected
214 microclimate frames and rooms, and were exposed between November 2007 to April
215 2009. The environmental conditions (RH,T, pollutants and light) were measured in these
216 rooms during the exposure and are reported elsewhere⁴³. In each location, one set of
217 replicas was exposed inside the enclosures and another set outside, except in the National
218 Museum in Krakow where the replicas were exposed in two microclimate frames with
219 different air exchange rates.

220

221 **2.8 Quantitative measurements for GC-MS analysis**

222 Dammar triterpenoids are not commercially available. To perform quantitative
223 measurements by gas chromatography mass spectrometry it was thus not possible to refer
224 to calibration curves obtained by analysing the standard solutions of each component. As
225 in this study it was necessary to quantitatively estimate the relative amounts of each
226 triterpenoid and to determine the amount of free terpenoids extracted from the resin
227 samples, it was assumed that the calibration curves of all the compounds had the same
228 slope and intercept. Although this may be relatively true for similar compounds, it is not
229 rigorously correct for all triterpenoids. Nonetheless, by working always with similar
230 concentrations, the chromatographic areas were used to give estimates, in arbitrary units,
231 of the relative percentage content of each triterpenoid and to determine the total amount of
232 free terpenoids for each sample. This estimation, although not rigorous, proved very
233 efficient in depicting the ageing of dammar, by highlighting variations in the relative
234 amounts of some components with respect to others, as well as the loss of extractable
235 terpenoids.

236 To correctly determine the chromatographic area of the triterpenoid (*i*) in dammar resin
237 ($A_{(i)}$), thus avoiding problems in integration due to the partial overlapping of
238 chromatographic peaks, the area of a selected value of m/z for each molecule ($EIA_{(i)}$) was
239 measured and corrected for the relative abundance of the selected ion in the mass

240 spectrum of the pure component (RA): $A_{(i)} = EIA_{(i)} * 100 / RA$. The mass spectrum of the pure
241 component was obtained by separating the neutral and acidic components⁴⁴ and by
242 analysing the two fractions separately with a suitable chromatographic run, but the same
243 mass spectrometric conditions used in this study. The ions used and their relative
244 abundance in the mass spectra of the pure compounds for each dammar triterpenoid are
245 listed Table 2.

246

247 **Table 2 Ions used to evaluate the chromatographic area of each dammar**
248 **triterpenoid and their relative abundance in the mass spectra of the pure**
249 **compounds (alcoholic and acidic moieties were converted into the corresponding**
250 **TMS derivatives)**

251

252 The relative percentage content of a triterpenoid (*i*) (abbreviation: *Tr* %) in each resin
253 sample was thus evaluated by summing the area of each chromatographic peak detected
254 in the chromatogram (ΣA), and determining the percentage content of each
255 chromatographic area:

256

257 relative percentage content of terpenoid (i) = (*Tr*%) =
$$\frac{A_{(i)} \cdot 100}{\sum_i A_i}$$

258

259 Evaluating the total amount of free terpenoids (abbreviation (ΣTr) for each resin sample
260 involved correcting the chromatographic area for the sample weight, by taking each
261 analytical step into account.

262 The area of of the triterpenoid (*i*) ($A_{(i)}$) was corrected for the derivatisation internal
263 standard. The corrected area ($A_{(i)'}$) was then normalised for the sample weight, by taking
264 into account the sample weight (*W*), the volume of the extracted solution (*E*), the aliquot of
265 the derivatised (*EA*) extracted solution, the final volume of the derivatised solution (*D*) and
266 the injection volume (*I*).

267

268 normalised chromatographic area of triterpenoid (i) =
$$\frac{A_{(i)' } \cdot D \cdot E}{I \cdot EA \cdot W}$$

269

270 The sample weight was known with an accuracy of $\pm 1 \mu\text{g}$, and for all samples $2.0 \mu\text{L}$ were
271 taken with a $10.0 \mu\text{L}$ syringe (accuracy: $0.05 \mu\text{L}$). The data presented in this work are the
272 average values of three replicates. The RDS obtained for the *Tr* % were in most cases
273 below 10%, and in all cases the standard deviations obtained ranged between 0 and 1.

274 The total amount of free terpenoids, ΣTr is the sum of the normalised chromatographic
275 areas of each molecular component identified.

$$\text{total amount of free terpenoids} = \sum Tr = \sum_i \frac{A_{(i)} \cdot D \cdot E}{I \cdot EA \cdot W}$$

279 3. Results and discussion

280 3.1 Analysis of the artificially aged samples – effect of air pollutants on the 281 degradation of dammar

282 Dammaradienone, nor- β -amyrone, dammaradienol, nor- α -amyrone, oleanonic acid and
283 ursonic acid, dammarenolic acid, oleanonic and oleanolic aldehydes, ursonic and ursolic
284 aldehydes were identified in the GC-MS chromatogram (Figure 1 a) of the raw resin used
285 for the preparation of the replicas^{1, 2, 37}.

287 **Figure 1.** Chromatogram of a - raw dammar resin; b - dammar t0 January 2008

289 An analysis of the replicas that were not subjected to any artificial ageing, and that were
290 kept in the fridge and in darkness (t0 January 2008 - Figure 1 b - and t0 June 2009)
291 highlighted the fundamental role played by time in determining the molecular composition
292 of dammar varnish films. The Tr % of each compound identified in the chromatograms
293 and the $\sum Tr$ are reported in Table 3.

295 **Table 3** Tr % of dammar compounds determined in the raw, t0 January 2008, and t0
296 June 2009 samples, and corresponding $\sum Tr$

298 The $\sum Tr$ decreased considerably, indicating that oligomerisation and cross-linking
299 reactions were taking place. At the same time, free triterpenoids could have been
300 produced by an increased molecular weight, which the GC system might not be able to
301 detect. It has been proven that the oxidation of triterpenoids is characterised by the
302 incorporation of oxygen atoms, starting from one, up to more than six^{27, 28, 37}. When
303 oxygen atoms can be incorporated into more than one position of the same molecule,
304 isomers are generated, whose multiplicity explains why they fall under the detection limit of
305 the instrument. Moreover, when oxygen atoms are incorporated without eliminating
306 hydrogen atoms, hydroxyl moieties are generated. Every OH moiety is transformed after
307 silylation, into an -OSiMe₃ group. When a molecule incorporates one, two, or six oxygen
308 atoms in the form of alcoholic moieties, the corresponding molecular weights of the
309 derivatised molecules are increased by 89, 177, 529 Da, respectively. The resulting
310 compounds are soon too heavy and/or thermally unstable to be analysed by GC. Laser-

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311 assisted ionisation mass spectrometric based techniques have also shown that
312 compounds of a lower molecular weight are formed in an aged resin^{27, 28, 37, 40}, however,
313 no major peaks were identified in the chromatograms of the varnishes under investigation.
314 Most likely, a high number of compounds are formed with different molecular structures
315 and weights, leaving them below the detection limit of the instrument. All this helps to
316 explain why the chromatograms of aged replicas show some minor peaks, most of which
317 remain unidentified, although the main ones are those reported in Table 3.

318 An evaluation of the degradation state of dammar resin needs to take into consideration
319 that several compounds may be related to each other, as a function of ageing. For
320 instance oleanolic aldehyde can be oxidised to oleanonic aldehyde, which can be oxidised
321 to oleanonic acid. In the same way, oleanolic aldehyde can be oxidised to oleanolic acid,
322 which can be oxidised to oleanonic acid (Figure 2 a). Dammaradienol, dammaradienone,
323 hydroxydammarone, and 20,24-epoxy-25-hydroxy-dammaren-3-one lay on a
324 hypothetical line of oxidation, where dammaradienol has the lower oxidation degree, and
325 20,24-epoxy-25-hydroxy-dammaren-3-one is the most highly oxidised. Dammarenolic acid,
326 which according to the literature is generated under UV light from the oxidation of
327 hydroxydammarone, can, under further oxidation, give rise to shoreic acid³⁴ (Figure 2 b).

328

329 **Figure 2. Some dammar compounds of different oxidation states. The arrow**
330 **indicates the passage from a less oxidised compound to a more oxidised**
331 **compound: this is not an actual reaction pathway, as it is only based on the**
332 **evaluation of the chemical structure of the reported compounds**
333

334 The *Tr* percentage data of the raw dammar resin compared to data of the t0 replicas
335 (Table 3) show that the relative amounts of dammarenolic acid, hydroxydammarone,
336 oleanonic and oleanolic aldehydes, ursonic and ursolic aldehydes, decrease with time,
337 while all other compounds, increase relatively with time, with the exception of
338 dammaradienone, which does not show any clear behaviour. This clearly indicates that
339 oxidation took place even though the replicas were kept in the dark and in the fridge in
340 closed envelopes. As a result, given the crucial role played by time in determining the
341 oxidation and cross-linking state of dammar resins films, only data of replicas artificially
342 aged within about one month were compared.

343 The *Tr* percentage of each dammar terpenoid identified in t0 January 2008 and the
344 artificially aged samples, together with $\sum Tr$ are reported in Table 4.

345

346 **Table 4. *Tr* % of each dammar terpenoid identified in the raw material, t0 January**
347 **2008 and the artificially aged samples, and $\sum Tr$**
348

349 The plot of the *Tr* percentage of dammaradienol, dammaradienone,
350 hydroxydammarenone, 20,24-epoxy-25-hydroxy-dammaren-3-ol and 20,24-epoxy-25-
351 hydroxy-dammaren-3-one versus the NO₂ dose, reveals that the relative amount of the
352 most oxidised compounds clearly increases compared to the least oxidised compounds,
353 when the NO₂ dose is increased (Figure 3).

354
355 **Figure 3. *Tr* % of dammaradienol, dammaradienone, hydroxydammarenone, 20,24-**
356 **epoxy-25-hydroxy-dammaren-3-ol and 20,24-epoxy-25-hydroxy-dammaren-3-one as**
357 **a function of the NO₂ dose at 50% (left) and 80% (right) RH in dammar replicas.**
358

359 The relative amounts of oleanolic and oleanonic acids, and ursolic and ursonic acids show
360 a similar behaviour under NO₂ ageing (Figure 4). This suggests that these compounds,
361 which show the same carbon skeleton structure (i.e. oleanane and ursane triterpenoids),
362 are degraded by NO₂ according to similar reaction patterns, although no meaningful trend
363 was observed. A similar behaviour was also observed in the dammarenic and shoreic
364 acids (Figure 4). The curing of the resin led to a significant decrease in the relative amount
365 of dammarenic acid and an increase in shoreic acid. The two compounds then behaved
366 very similarly under NO₂ ageing. Again, no significant trend was highlighted as an effect of
367 the increasing dose of NO₂. However, it is possible to say that the main degradation
368 reaction taking place under NO₂ exposure does not appear to be the oxidation of
369 dammarenic acid to shoreic acid.

370
371 **Figure 4. *Tr* % of oleanolic and oleanonic acids, ursolic and ursonic acids,**
372 **dammarenic and shoreic acids, as a function of the NO₂ dose at 50% and 80% RH**
373 **in dammar replicas**
374

375 The oxidising effect of NO₂ was confirmed by Dynamic Mechanical Analysis (DMA)
376 analyses. Measurements of the glass transition temperature (*T_g*) of the varnished films
377 were taken from the onset of the peak referred to as tan delta. The tan delta peak is the
378 ratio of inelastic (*E''*) to elastic components (*E'*) of the complex modulus and provides a
379 measure of the viscoelasticity of the coating. Previous work on *T_g* measurements for
380 dammar resin (DSC) has been reported by differential scanning calorimetry⁴⁵. The
381 advantage of DMA is that it is more sensitive than DSC and requires much less sample. In
382 addition, the measurements can be taken without removing the varnish from the steel
383 substrate.

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384 A comparison of the results obtained from the control sample (t0 January 2008) with the
385 aged samples showed that there was a clear shift in T_g from about 70°C to higher values
386 in the region of 80-90°C with increasing dose levels of NO₂. The shift in T_g to higher
387 values is caused by an increase in cross-linked and oxidized components, which leads to
388 a tighter structure. The RH values used during ageing also affected the T_g values; the T_g
389 of the sample exposed at 50% RH (NO₂ 426 ppm day) was in the region of 90°C and
390 higher than that for 80% RH (NO₂ 599 ppm day) where the T_g was closer to 80°C.

391 When comparing O₃, NO₂ and acetic acid, GC-MS data show that the pollutant that causes
392 the formation of a relatively higher amount of 20,24-epoxy-25-hydroxy-dammaren-3-one
393 is acetic acid, both with a high and low RH (Figure 5). Acetic acid is also responsible for
394 the highest amount of shoreic acid produced in the samples exposed at 20% RH (Figure
395 5).

396

397 **Figure 5. Comparison of the oxidizing effect of acetic acid, NO₂ and O₃ at 20% and**
398 **80% RH with GC-MS**

399

400 A comparison of the DMA curves of the varnish samples exposed to the different
401 pollutants is reported in Figure 6

402

403 **Figure 6. Comparison of the DMA curves of the varnish samples exposed to acetic**
404 **acid, NO₂ and O₃ at 20% and 80% RH**

405

406 DMA revealed that exposure to ozone particularly with a low RH value (20% RH and dose
407 of 117 ppm days) shifted the T_g to higher values in the region of 110°C. When comparing
408 the results of DMA and GC-MS, it is important to highlight that GC-MS is only able to look
409 at the soluble components of the varnishes, and the analysis is performed in bulk, whereas
410 DMA measures the sample in situ, and is more sensitive to surface effects. This could
411 explain why the effect of ozone at 20% RH is more pronounced. For NO₂ and acetic acid
412 exposure, the surface properties were influenced by RH. The tan delta curves are complex
413 in shape, which indicates that the varnishes were not truly polymeric. When the peak width
414 increases, the material becomes less homogeneous and contains areas with higher and
415 lower degrees of cross-linking. This is what may have been happening in the case of the
416 80% RH exposed samples compared to the 20% RH samples. In the case of the 20% RH
417 exposed samples, the shape of the curves is consistent with an increasing polymeric
418 component compared to the control sample.

419 The aggressive effect of O₃ is also highlighted in Figure 7 where the AFM image of the
420 sample exposed to O₃ 133 ppm day at 80% RH is shown in comparison to the control
421 sample t0 January 2008: ozone is responsible for the formation of a more degraded
422 surface with increased roughness compared to the control sample. This aspect is
423 extremely important, because surface deformations involve an increase contact with the
424 external environment, and, thus, increased vulnerability and further degradation by the
425 oxidizing agents.

426

427 **Figure 7. AFM images (topography left and deflection right) of the surface of**
428 **dammar varnish layer t0 January 2008 (above) and exposed to O₃ 133 ppm day at**
429 **80% RH (below)**

430

431 The higher effect of acetic acid with lower values of RH highlighted by GC-MS (Figure 5)
432 was also confirmed by DMA. In fact where RH was higher (80%), the measured *T_g* was in
433 the region of 80°C, while samples aged at lower RH (20%), showed a *T_g* value in the
434 region of 90°C.

435 A dose of about 25 ppm day of acetic acid has degrading effects on the natural resins that
436 are comparable to those of about 130 ppm day of O₃, and 599 ppm day of NO₂. This is
437 also highlighted by DMA analysis: the samples exposed to acetic acid (26 ppm day at 80%
438 RH) showed a *T_g* value in the region of 80°C, similar to values observed with higher doses
439 of NO₂ (599 ppm day 80% RH). Ozone and nitrogen dioxide are known oxidising agents ⁴⁶.

440 It is well known that organic acids are found in damaged layers of monuments and
441 buildings ⁴⁷, and that acetic and formic acids in museum environments corrode lead,
442 copper and some other metals and calcareous minerals ^{24, 26, 48-51}. However, very little is
443 known about the possible long-term degradation impact of these gases on organic
444 materials used in works of art. Studies so far have only been made on cellulose-based
445 materials ^{52, 53}.

446 One possible interpretation of the observed behaviour may be based on a study of the
447 catalytic effect of acetic acid on the autoxidation of methyl linoleate ⁵⁴. Methyl linoleate
448 autoxidation at 50°C in darkness was clearly favoured when acetic acid was present, the
449 higher the concentration, the higher the effect. According to the authors, acetic acid was
450 not acting as an oxidizing agent, but as a pro-oxidising agent, by catalyzing the
451 decomposition of the hydroperoxides formed during the autoxidation of methyl lineoleate.
452 The reaction mechanism proposed involved the formation of a hydrogen bond between the
453 carboxyl group in acetic acid with a hydroperoxide, thus lowering the dissociation energy

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454 of the oxygen-oxygen bond of the hydroperoxide. As triterpenoid resins age through
455 autoxidative pathways ⁴⁰, it can be hypothesized that acetic acid catalyzes the
456 decomposition of hydroperoxides formed as an effect of ageing, thus accelerating the resin
457 oxidation. Miyashita's paper ⁵⁴ also shows that the pro-oxidative effect of acetic acid
458 clearly decreases when water is added, as water probably promotes the acid dissociation.
459 This aspect is in agreement with the higher oxidizing efficacy that we observed for the
460 sample exposed at RH 20% compared to the sample exposed at RH 80% discussed
461 above.

462 As far as cross linking is concerned, although the artificially aged samples show a lower
463 content in free triterpenoids ($\sum Tr$) than the t0 January 2008, it is not possible to confirm
464 that there is a direct relationship between cross linking, the NO₂ dose and/or RH. By
465 comparing replicas artificially aged with O₃, NO₂ and acetic acid, GC-MS reveals that NO₂
466 is the most efficient in causing a decrease in the amount of free terpenoids detected
467 (Table 4).

468 Accelerated ageing in the presence of glacial acetic acid which constitutes a high dose
469 confirms that this organic acid seems to have a strong oxidising power. When vapours of
470 glacial acetic acid were used for six weeks and three months, a clear decrease in the
471 relative amounts of dammaradienone, dammaradienol and hydroxydammaradienone was
472 observed, assisted by an increase in the amounts of 20,24-epoxy-25-hydroxy-dammaren-
473 3-one and 20,24-epoxy-25-hydroxy-dammaren-3-ol (Figure 8). The higher the dose, the
474 higher the observed oxidation, to an extent that after three months' of exposure, 20,24-
475 epoxy-25-hydroxy-dammaren-3-ol decreased compared to the six-week exposure, and
476 was most probably oxidised to 20,24-epoxy-25-hydroxy-dammaren-3-one. The same was
477 true for dammarenolic acid and shoreic acid, the former decreases under exposure to
478 glacial acetic acid vapours, and was oxidised most probably to shoreic acid, as indicated
479 by the increase in its relative amount (Figure 8). The higher the dose, the higher the effect

480

481 **Figure 8** *Tr* % of dammaradienol, dammaradienone, hydroxydammarenone, 20,24-
482 epoxy-25-hydroxy-dammaren-3-ol and 20,24-epoxy-25-hydroxy-dammaren-3-one,
483 dammarenolic and shoreic acid as a function of the dose of glacial acetic acid in
484 dammar replicas.

485

486 This is confirmed by DMA analyses (Figure 9): dammar varnish exposed to vapours from
487 glacial acetic acid for 1.5 and 3 months respectively, showed the largest shift in the tan
488 delta peak to values of 130°C and 150°C respectively, against 80°C obtained for the AcA
489 26 dose day at 80% RH. Moreover the shape of the DMA curves reveals that in the glacial

490 acetic acid exposed samples show a more typical polymeric behavior, indicating that a
491 polymeric network is present. This is in agreement with GC-MS data, as the samples show
492 an extremely low value of $\sum Tr$ compared to the control sample t0 June 2009.

493

494 **Figure 9. Comparison of the DMA curves of the varnish samples exposed to**
495 **vapours of glacial acetic acid for 1.5 and 3 months.**

496

497 3.2. Natural ageing at sites

498 The Tr percentage of dammar terpenoids, as well as the corresponding $\sum Tr$ of the
499 samples exposed inside and outside the microclimate frames and showcases are reported
500 in Table 5.

501

502 **Table 5. Tr % of the dammar terpenoids, as well as the corresponding $\sum Tr$ of**
503 **samples exposed at site**

504

505 On the basis of an evaluation of the molecular profile, as well as a comparison between
506 the $\sum Tr$, it is possible to evaluate whether the samples exposed inside are better
507 preserved (less cross-linked and/or less oxidised) than those exposed outside the
508 microclimate frames or showcases. For example, the dammar replicas exposed in the
509 Uffizi showed a relatively higher content of 20,24-epoxy-25-hydroxy-dammaren-3-one
510 inside the showcase than outside, whereas it was the other way round for
511 hydroxydammarenone. As 20,24-epoxy-25-hydroxy-dammaren-3-one has a higher
512 degree of oxidation than hydroxydammarenone, the varnish exposed inside the showcase
513 seems more oxidised than outside. The results of the oxidation and loss of soluble
514 triterpenoids for all the resins are summarised in Table 6. The concentrations of NO_2 , O_3 ,
515 acetic and formic acid for each frame are also reported. The data expressed as $\mu g/m^3$ are
516 an estimation for the period of exposure, based on the values calculated over one month.
517 Generally speaking, inorganic oxidising compounds were detected in low concentrations
518 inside the mc-frames, while higher concentrations were measured in the rooms⁵⁵, in fact
519 NO_2 concentrations were higher than the recommended values for museums ($10\mu g/m^3$)²³.
520 In contrast, higher levels of VOCs such as acetic and formic acids, toluene, p- and m-
521 xylenes, α -pinene, limonene and 3-carene were detected inside the mc-frames than in the
522 rooms. The environmental measurements are discussed in detail elsewhere⁴³.

523

524 **Table 6. Oxidation and loss of soluble triterpenoids for dammar samples exposed**
525 **on site and corresponding environmental parameters**⁴³

526
527 The evaluation of these data is extremely complex, and a comparison between different
528 locations is not possible, as there are several variables involved (light, pollutant levels,
529 temperature, RH, etc). With the exception of Cracow, in all the other locations, the light
530 and temperature conditions inside and outside the frames were the same or extremely
531 similar ⁴³. As a result, differences observed between the varnishes exposed inside and
532 outside the mc-frames would seem to be due to the pollutants present: the environment
533 inside the frames is characterised by higher amounts of organic acids than outside, and
534 lower amounts of inorganic pollutants ⁴³. Clearly the data show that the varnishes exposed
535 inside the frames are not always better preserved than those exposed outside, in terms of
536 oxidation, cross linking. The data obtained from the artificially aged sample strongly
537 suggests that the reason for this must lie in the higher concentrations of volatile organic
538 acids present in the frames.

539

540 **4. Conclusions**

541 This paper shows the effect at the molecular level of some inorganic and organic museum
542 pollutants on the degradation of dammar varnish. In particular the study shows for the first
543 time that acetic acid, which is a common pollutant in indoor environments, has damaging
544 effects on dammar. This result opens the way to an important question: are organic
545 materials in general subject to degradation under exposure to acetic acid? And which is
546 the effect of other organic indoor pollutants, such as formic acid? Although further work is
547 necessary to assess all this, on the basis of the results obtained in this study, it is already
548 possible to make some important suggestions to ensure the protection of painted objects
549 in museum environments, by improving the performance of microclimate frames as an
550 efficient means of preventive conservation. The impact of organic acids (acetic acid) on
551 sensitive materials inside microclimate frames could be significantly reduced by installing
552 barrier films over the emitting materials ⁵⁶ and emission-absorbing materials inside
553 microclimate frames ²⁰. In addition, the air exchange rate (AER) is a valuable parameter
554 for evaluating the performance of the microclimate frames ¹⁴, also regarding the possible
555 presence of VOCs. Frames with low AERs are advantageous in that they provide buffering
556 against external fluctuations in relative humidity and temperature. This could help to
557 reduce the impact of acetic acid (found to be the most damaging with a lower RH) as well
558 as limiting the ingress of oxidising pollutants, such as NO₂ and O₃. However, low AER
559 values may lead to problems: (1) where solvent molecules are still trapped within the
560 varnish, as this would lead to plasticizing effects on the varnish; and (2) where there are

561 high emissions of acetic acid, as this would also affect the varnishes. However, in
562 microclimate frames with high air exchange rates located in uncontrolled environments,
563 the ingress of oxidising agents and the loss of climate buffering (RH and T) could cause
564 damage to varnishes, and thus should be assessed in each individual case.

565

566 Acknowledgments

567 Dr. Mikkel Scharff and Dr. Tom Egelund of the Danish School of Conservation, Royal
568 Danish Academy of fine arts, Denmark are acknowledged for the preparation of the
569 samples and the useful discussion of the data. Thank also to Dr. S. Hudziak from
570 Electronic and Electrical Engineering Dept UCL for access and assistance with AFM, and
571 Dr. D. Morgan, Windsor Scientific UK, for advice in processing images. The research was
572 performed within the framework of the European FP6 research project PROPAINT
573 (Improved Protection of Paintings During Exhibition, Storage and Transit, 2007-2009)

574

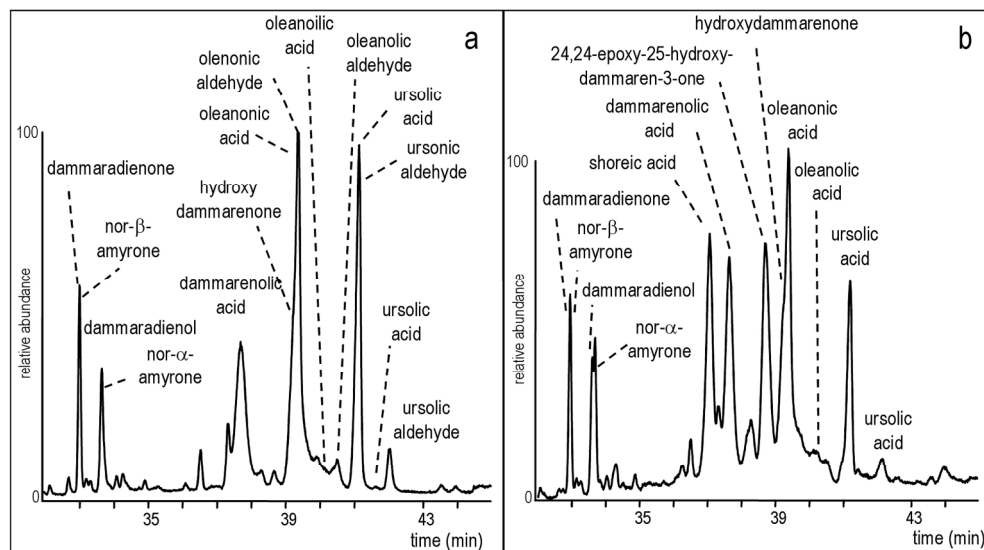
575 References

- 576 1. J. Mills and R. White, *Organic Chemistry of Museum Objects, Second Edition*
577 (*Conservation and Museology*, 2nd edn., Butterworth-Heinemann, 2000).
- 578 2. A. Andreotti, I. Bonaduce, M. P. Colombini, F. Modugno and E. Ribechini, *New Trends*
579 *Anal., Environ. Cult. Heritage Chem. FIELD Full Journal Title: New Trends in Analytical,*
580 *Environmental and Cultural Heritage Chemistry*, 2008, 389-423.
- 581 3. M. Bacci, C. Cucci, A.-L. Dupont, B. Lavédrine, M. Picollo and S. Porcinai,
582 *Environmental Science & Technology*, 2003, **37**, 5687-5694.
- 583 4. M. Odlyha, N. S. Cohen, G. M. Foster and R. H. West, *Thermochimica Acta*, 2000, **365** 53-
584 63.
- 585 5. O. F. van den Brink, G. B. Eijkel and J. J. Boon, *Thermochimica Acta*, 2000, **365**, 1-23.
- 586 6. M. Bacci, M. Picollo, S. Porcinai and B. Radicati, *Thermochimica Acta*, 2000, **365**, 25-34.
- 587 7. N. S. Cohen, M. Odlyha, R. Campana and G. M. Foster, *Thermochimica Acta*, 2000, **365**,
588 45-52.
- 589 8. M. Odlyha, N. S. Cohen and G. M. Foster, *Thermochimica Acta*, 2000, **365**, 35-44.
- 590 9. J. Shiner, in *Museum Microclimates*, eds. T. Padfield and K. Borchersen, The National
591 Museum of Denmark, Copenhagen Editon edn., 2007.
- 592 10. R. Boddi, P. Camera, C. Danti and F. Scieurpi, *Testing of a museum showcase equipped with*
593 *an active and passive system for control of internal hygrometric conditions, 6th*
594 *international conference on non-destructive testings and microanalyses for the diagnostics*
595 *and conservation of the cultural heritage*, Rome, 1999.
- 596 11. J. G. Holmberg and W. Kippes, *Conservation science 2002: papers from the conference held*
597 *in Edinburgh, Scotland 22-24 May 2002 Edinburgh, Scotland*, 2002.
- 598 12. F. Toledo, M. Sehn, M. Sousa, S. Brazolin and S. Hackney, *Museum microclimates*
599 *Conference 2007, Copenhagen*, 2007.
- 600 13. D. Thickett, R. Soek-Joo and S. Lambarth, *Museum Microclimates, Copenhagen*, 2007.
- 601 14. D. Camuffo, G. Sturaro and A. Valentino, *Thermochimica Acta*, 2000, **365**, 65-77.
- 602 15. E. R. De la Rie, *Anal. Chem. FIELD Full Journal Title: Analytical Chemistry*, 1989, **61**,
603 1228A-1233A, 1237A-1240A.

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- 604 16. J. K. Delaney, E. R. de la Rie, M. Elias, L.-P. Sung and K. M. Morales, *Stud. Conserv. FIELD Full Journal Title:Studies in Conservation*, 2008, **53**, 170-186.
- 605
- 606 17. R. S. Berns and E. R. De La Rie, *Stud. Conserv. FIELD Full Journal Title:Studies in Conservation*, 2003, **48**, 251-262.
- 607
- 608 18. P. Dietemann and C. Herm, *Org. Mass Spectrom. Art Archaeol. FIELD Full Journal Title:Organic Mass Spectrometry in Art and Archaeology*, 2009, 131-163.
- 609
- 610 19. D. Scalarone and O. Chiantore, *Org. Mass Spectrom. Art Archaeol. FIELD Full Journal Title:Organic Mass Spectrometry in Art and Archaeology*, 2009, 327-361.
- 611
- 612 20. E. Dahlin, T. Grøntoft, S. López-Aparicio, M. Odlyha, M. Scharff, T. Larsen, G. Andrade, A. T. García, A. Ortega, P. Mottner, M. Obarzanowski, R. Kozłowski, J. Czop, M. P. Colombini, I. Bonaduce, D. Thickett, S. Hackney, J. Wadum, A. H. Christensen, M. Ryhl-Svendsen and S. Jakiela, *PROPAINT - Improved Protection of Paintings during Exhibition, Storage and Transit. Final Activity Report*, 2010.
- 613
- 614
- 615
- 616
- 617 21. P. Bruno, M. Caselli, G. de Gennaro, S. Iacobellis and M. Tutino, *Indoor Air*, 2008, **18**, 250-256.
- 618
- 619 22. R. E. Hodgkins, C. M. Grzywacz and R. L. Garrell, *e-PS*, 2011, **8**, 74-80.
- 620 23. G. Thomson, *The Museum Environment*, Butterworth - Heinemann, Oxford 1986.
- 621 24. M. Ryhl-Svendsen, *Journal of Cultural Heritage*
- 622 2008, **9**, 285-293.
- 623 25. M. Ryhl-Svendsen and J. Glastrup, *Atmospheric Environment*, 2002, **36**, 3909-3916.
- 624 26. P. Brimblecombe and C. M. Grossi, *Chemistry Central Journal*, 2012, **6**, 21.
- 625 27. P. Dietemann, 2003.
- 626 28. P. Dietemann, M. J. Edelmann, C. Meisterhans, C. Pfeiffer, S. Zumbuhl, R. Knochenmuss and R. Zenobi, *Helv. Chim. Acta FIELD Full Journal Title:Helvetica Chimica Acta*, 2000, **83**, 1766-1777.
- 627
- 628
- 629 29. P. Dietemann, M. Kaelin, C. Sudano, R. Knochenmuss and R. Zenobi, *Chimia FIELD Full Journal Title:Chimia*, 2001, **55**, 972-980.
- 630
- 631 30. P. Dietemann, M. Kaelin, S. Zumbuehl, R. Knochenmuss, S. Wuelfert and R. Zenobi, *Anal. Chem. FIELD Full Journal Title:Analytical Chemistry*, 2001, **73**, 2087-2096.
- 632
- 633 31. P. Dietemann, S. Zumbuhl, R. Knochenmuss and R. Zenobi, *Adv. Mass Spectrom. FIELD Full Journal Title:Advances in Mass Spectrometry*, 2001, **15**, 909-910.
- 634
- 635 32. D. Scalarone, M. C. Duursma, J. J. Boon and O. Chiantore, *J. Mass Spectrom. FIELD Full Journal Title:Journal of Mass Spectrometry*, 2005, **40**, 1527-1535.
- 636
- 637 33. D. Scalarone, J. van der Horst, J. J. Boon and O. Chiantore, *J. Mass Spectrom. FIELD Full Journal Title:Journal of Mass Spectrometry*, 2003, **38**, 607-617.
- 638
- 639 34. C. Theodorakopoulos, J. J. Boon and V. Zafirooulos, *Int. J. Mass Spectrom. FIELD Full Journal Title:International Journal of Mass Spectrometry*, 2009, **284**, 98-107.
- 640
- 641 35. C. Theodorakopoulos and V. Zafirooulos, *Appl. Surf. Sci. FIELD Full Journal Title:Applied Surface Science*, 2009, **255**, 8520-8526.
- 642
- 643 36. C. Theodorakopoulos, V. Zafirooulos, J. J. Boon and S. C. Boyatzis, *Appl. Spectrosc. FIELD Full Journal Title:Applied Spectroscopy*, 2007, **61**, 1045-1051.
- 644
- 645 37. G. A. van der Doelen, University of Amsterdam, 1999.
- 646 38. G. A. van der Doelen and J. J. Boon, *J. Photochem. Photobiol., A FIELD Full Journal Title:Journal of Photochemistry and Photobiology, A: Chemistry*, 2000, **134**, 45-57.
- 647
- 648 39. G. A. van der Doelen, K. J. van den Berg, J. J. Boon, N. Shibayama, E. Rene de la Rie and W. J. L. Genuit, *J. Chromatogr., A FIELD Full Journal Title:Journal of Chromatography, A*, 1998, **809**, 21-37.
- 649
- 650
- 651 40. P. Dietemann, C. Higgitt, M. Kälin, M. J. Edelmann, R. Knochenmuss and R. Zenobi, *Journal of Cultural Heritage*, 2009, **10** 30-40.
- 652
- 653 41. R. H. West, M. Odlyha, K. Pratt, A. Roberts and S. Hutton, *Surface and Interface Analysis*, 2004, **36**, 862 - 865.
- 654

- 655 42. M. P. Colombini, A. Andreotti, I. Bonaduce, F. Modugno and E. Ribechini, *Accounts of*
656 *Chemical Research*, 2010, **395** 715-727.
- 657 43. S. López-Aparicio, T. Grøntoft, M. Odlyha, E. M. Dahlin, P. Mottner, D. Thickett, M. Ryhl-
658 Svendsen, N. Schmidbauer and M. Scharff, *e-PS*, 2010, **7**, 59-70.
- 659 44. A. Andreotti, I. Bonaduce, M. P. Colombini, G. Gautier, F. Modugno and E. Ribechini,
660 *Analytical Chemistry*, 2006, **78**, 4490-4500.
- 661 45. M. Schilling, *Studies in Conservation*, 1989, **34**, 110-116.
- 662 46. D. Kleya, M. Kleinmannb, H. Sander manc and S. Krupad, *Environmental Pollution*, 1999,
663 **100**, 19-42.
- 664 47. C. Sabbioni, N. Ghedinia and A. Bonazza, *Atmospheric Environment*
665 2003, **37**, 1261-1269.
- 666 48. J. Tétreault, *Airborne Pollutants in Museums, Galleries, and Archives: Risk Assessment,*
667 *Control Strategies and Preservation Management*, Canadian Conservation Institute, Ottawa,
668 2003.
- 669 49. K. Linnow, L. Halsberghe and M. Steiger, *Journal of Cultural Heritage*, 2007, **8**, 44-52.
- 670 50. L. T. Gibson, B. G. Cooksey, D. Littlejohn and N. H. Tennent, *Analytica Chimica Acta*,
671 1997, **337**, 253-264.
- 672 51. J. Tetreault, E. Cano, M. van Bommel, D. Scott, M. Dennis, M. G. Barthes-Labrousse, L.
673 Minel and L. Robbiola, *Studies in Conservation*, 2003, **48**, 237-250.
- 674 52. A.-L. Dupont and J. Tétreault, *Studies in Conservation*, 2000, **45**, 201-210.
- 675 53. M. Strlič, I. Kralj Cigić, A. Možir, G. de Bruin, J. Kolar and M. Cassar, *Polymer*
676 *Degradation and Stability*, 2011, **96**, 608-615.
- 677 54. K. Miyashita and T. Takagi, *Agricultural and Biological Chemistry*, 1987, **51**, 1179-1181.
- 678 55. T. Grøntoft, M. Odlyha, P. Mottner, E. Dahlin, S. Lopez-Aparicio, S. Jakiela, M. Scharff, G.
679 Andrade, M. Obarzanowski, M. Ryhl-Svendsen, D. Thickett, S. Hackney and J. Wadum,
680 *Journal of Cultural Heritage*, 2010, **11**, 411-419.
- 681 56. E. Uhdea and T. Salthammera, *Atmospheric Environment*, 2007, **41**, 3111-3128.
- 682
- 683
- 684



Chromatogram of a - raw dammar resin; b - dammar t0 January 2008
173x96mm (300 x 300 DPI)

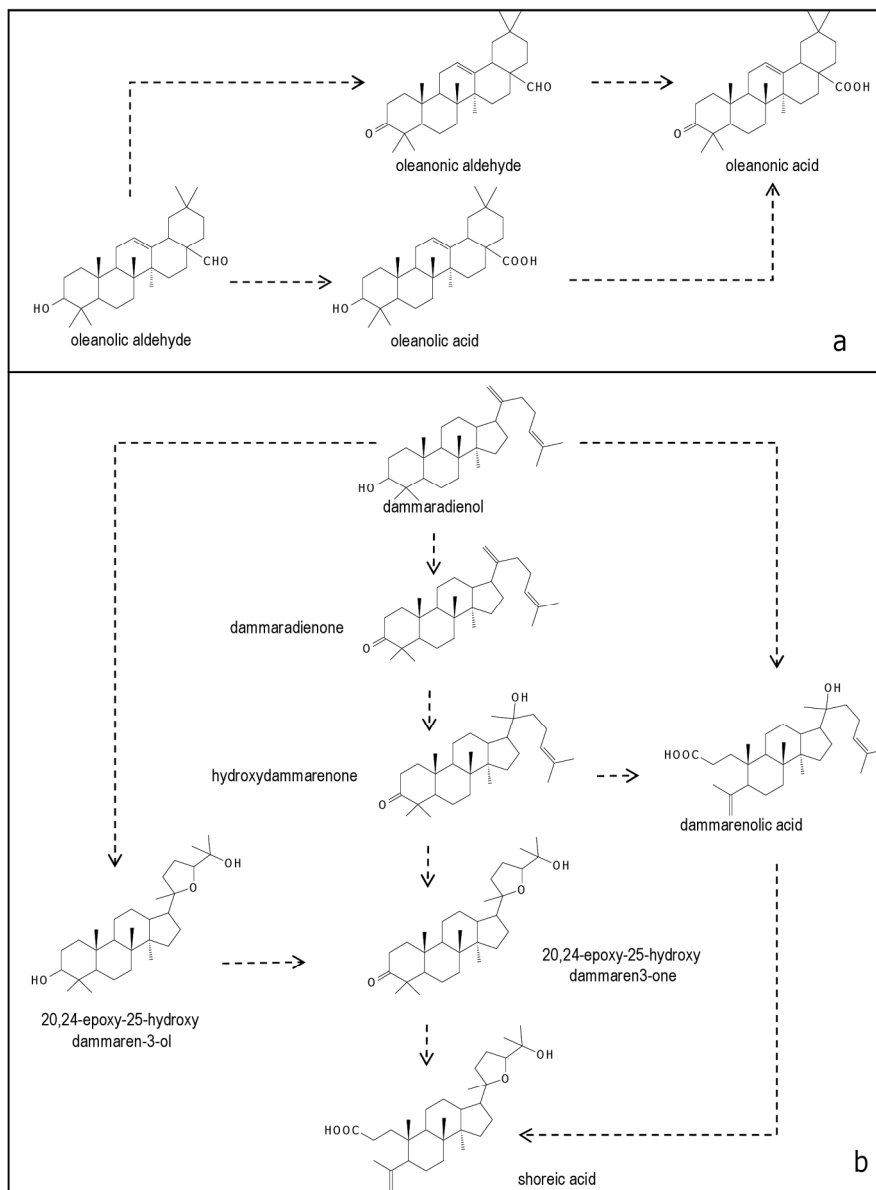


Figure 2. Some dammar compounds of different oxidation states. The arrow indicates the passage from a less oxidised compound to a more oxidised compound: this is not an actual reaction pathway, as it is only based on the evaluation of the chemical structure of the reported compounds
173x232mm (300 x 300 DPI)

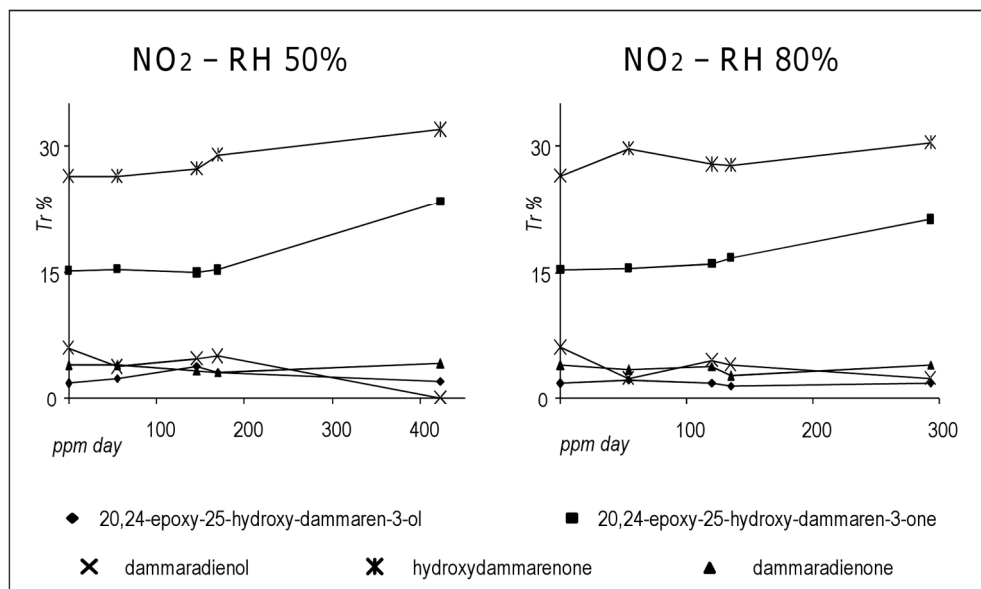


Figure 3. Tr % of dammaradienol, dammaradienone, hydroxydammarenone, 20,24-epoxy-25-hydroxy-dammaren-3-ol and 20,24-epoxy-25-hydroxy-dammaren-3-one as a function of the NO₂ dose at 50% (left) and 80% (right) RH in dammar replicas
173x104mm (300 x 300 DPI)

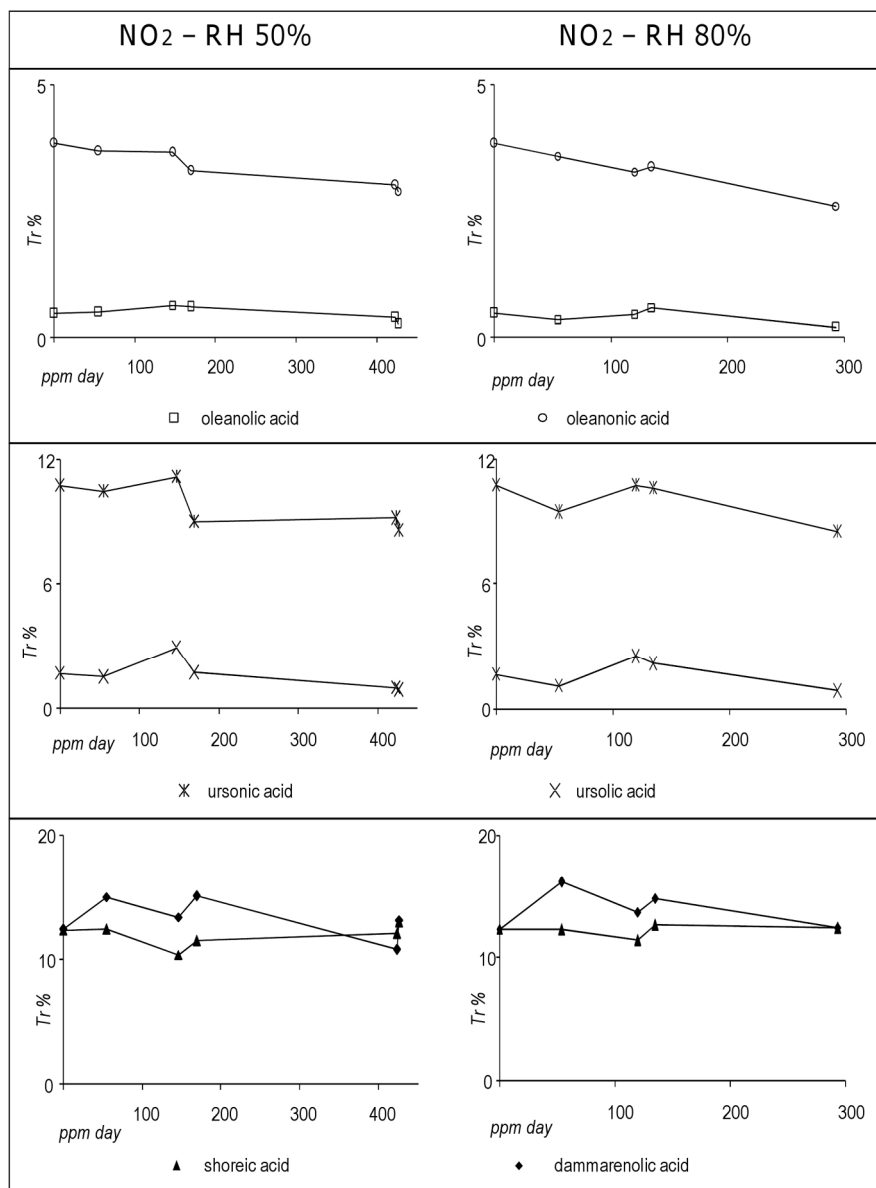


Figure 4. Tr % of oleanolic and oleanonic acids, ursolic and ursonic acids, dammarenolic and shoreic acids, as a function of the NO₂ dose at 50% and 80% RH in dammar
173x232mm (300 x 300 DPI)

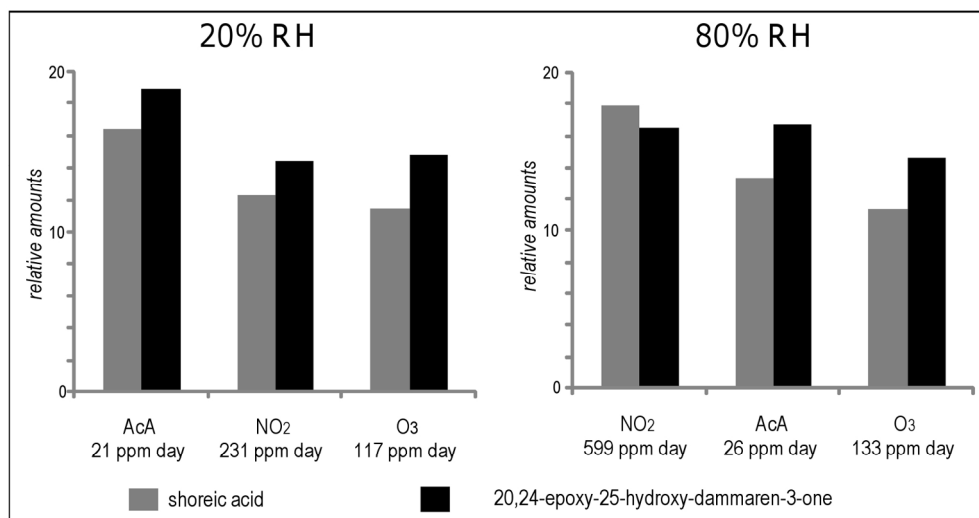


Figure 5. Comparison of the oxidizing effect of acetic acid, NO₂ and O₃ at 20% and 80% RH with GC-MS 173x93mm (300 x 300 DPI)

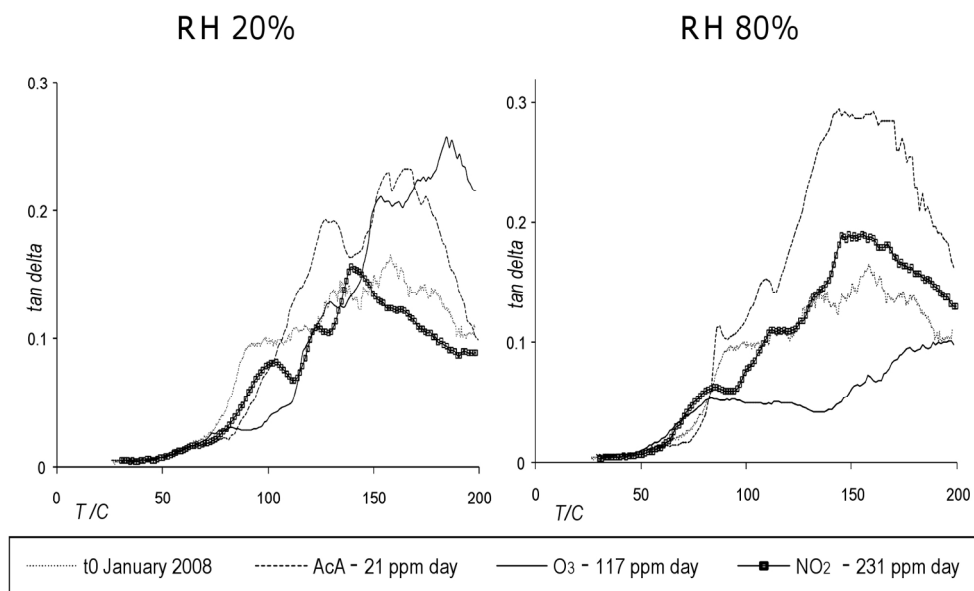


Figure 6. Comparison of the DMA curves of the varnish samples exposed to acetic acid, NO₂ and O₃ at 20% and 80% RH
173x107mm (300 x 300 DPI)

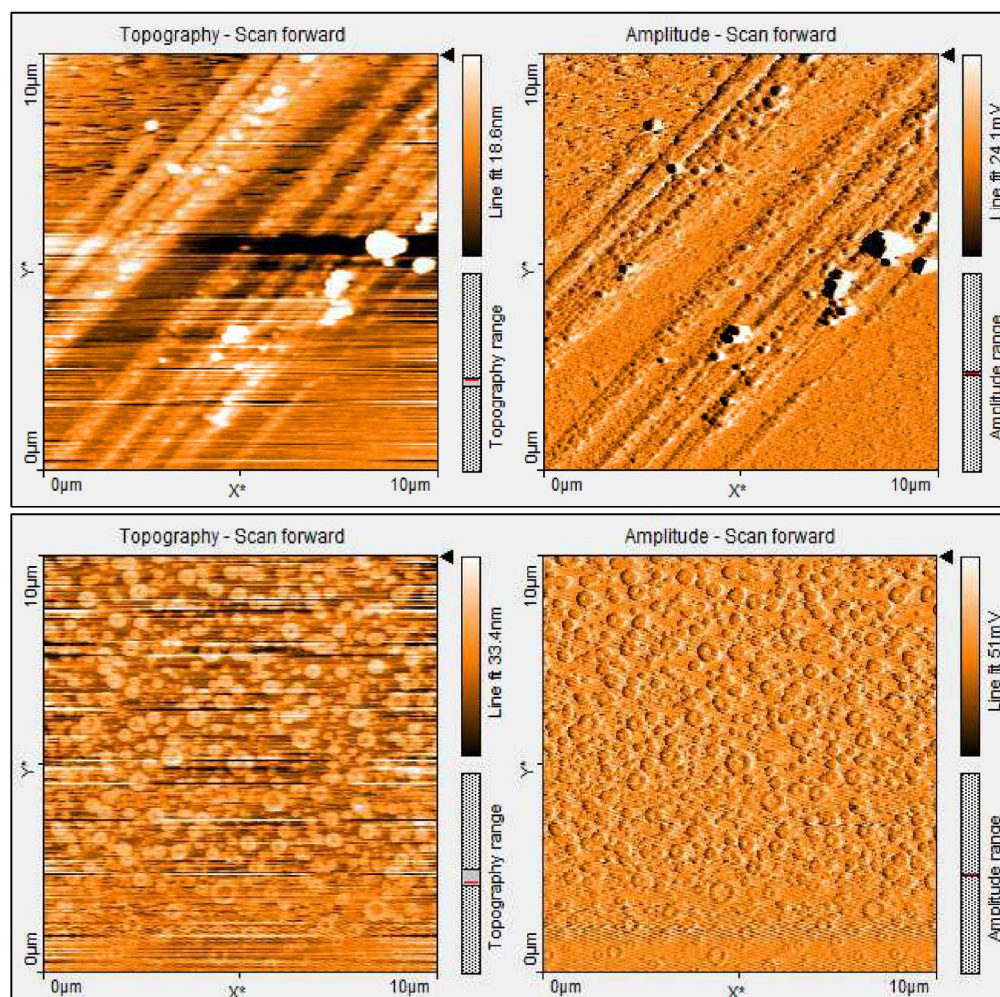


Figure 7. AFM images (topography left and deflection right) of the surface of dammar varnish layer to January 2008 (above) and exposed to O₃ 133 ppm day at 80% RH (below) 173x171mm (300 x 300 DPI)

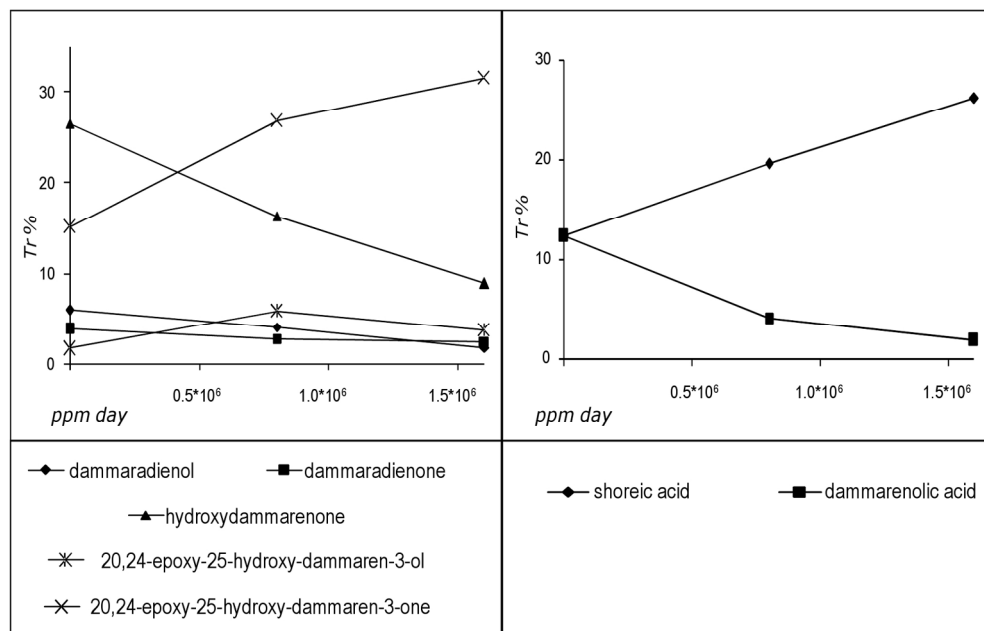


Figure 8. Tr % of dammaradienol, dammaradienone, hydroxydammarenone, 20,24-epoxy-25-hydroxy-dammaren-3-ol and 20,24-epoxy-25-hydroxy-dammaren-3-one, dammarenolic and shoreic acid as a function of the dose of glacial acetic acid in dammar replicas
173x111mm (300 x 300 DPI)

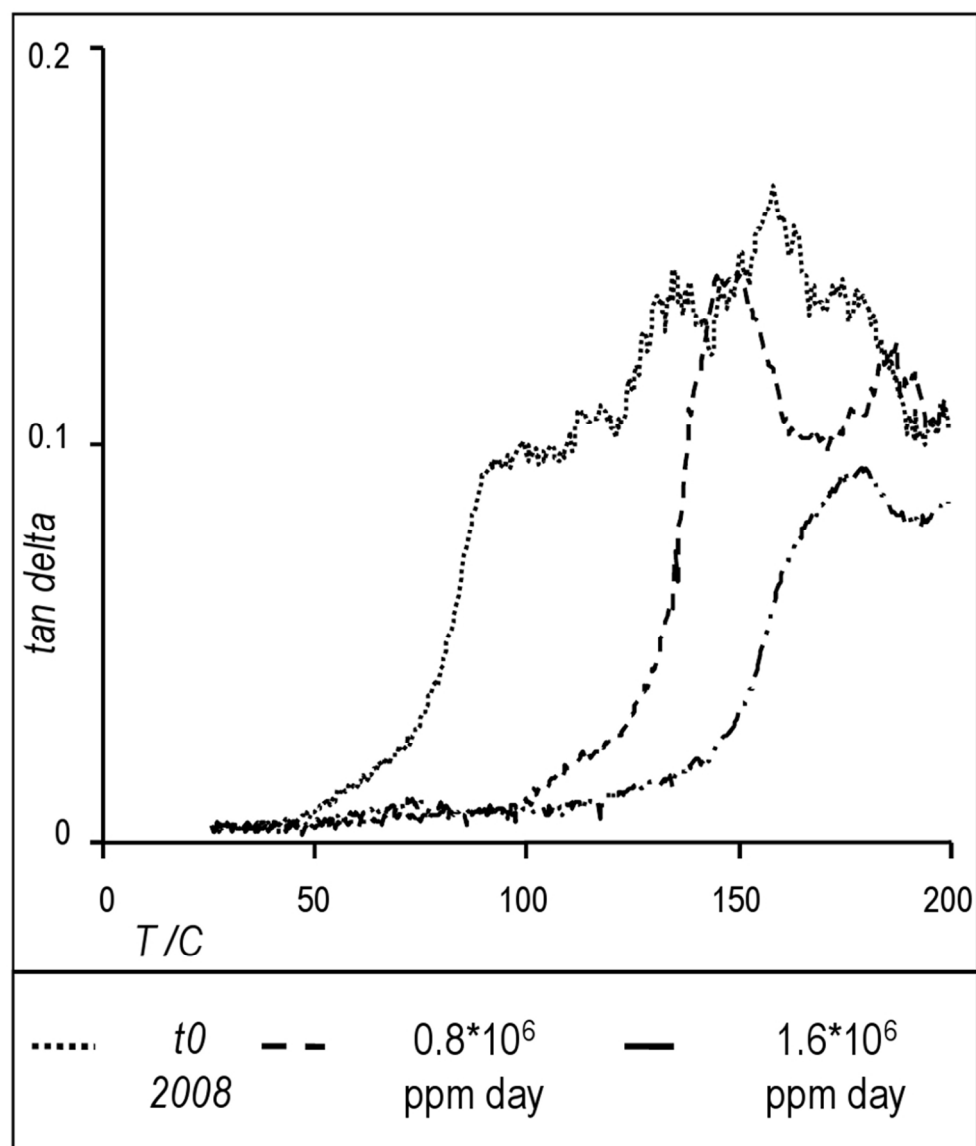


Figure 9. Comparison of the DMA curves of the varnish samples exposed to vapours of glacial acetic acid for 1.5 and 3 months
82x96mm (300 x 300 DPI)

Table 1: Overview of the accelerated ageing exposures carried out with different pollutants and values of Relative Humidity (RH) in the exposure chamber. C = average concentration in the chamber; time = exposure duration.

Pollutant	exposure period (From/To)	RH (%)	concentration (ppm)	time (days)	dose ppm day	Sample name
NO ₂	27.12.2007 to 08.01.2008	50	5	10	55	NO2-50-55
NO ₂	27.12.2007 to 08.01.2008	50	15	10	146	NO2-50-146
NO ₂	27.12.2007 to 08.01.2008	50	17	10	170	NO2-50-169
NO ₂	29.01.2008 to 18.02.2008	50	21	20	423.	NO2-50-423
NO ₂	29.01.2008 to 18.02.2008	50	21	20	426	NO2-50-426
NO ₂	08.01.2008 to 18.01.2008	80	5	10	55	NO2-80-54
NO ₂	08.01.2008 to 18.01.2008	80	12	10	120	NO2-80-120
NO ₂	08.01.2008 to 18.01.2008	80	13	10	136	NO2-80-135
NO ₂	19.01.2008 to 10.03.2008	80	15	20	293	NO2-80-293
NO ₂	13.03.2008 to 11.04.2008	80	21	29	599	NO2-80-599
O ₃	13.03.2008 to 11.04.2008	80	4	29	115	O3-80-133
Acetic Acid	11.04.2008 to 28.04.2008	80	2	17	26	AcA-80-26
NO ₂	14.04.2008 to 05.05.2008	20	11	21	231	NO2-20-231
O ₃	14.04.2008 to 14.05.2008	20	4	27	117	O3-20-117
Acetic Acid	14.05.2008 to 02.06.2008	20	1	19	21	AcA-20-21

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Table 2 Ions used to evaluate the chromatographic area of each dammar triterpenoid and their relative abundance in the mass spectra of the pure compounds (alcoholic and acidic moieties were converted into the corresponding TMS derivatives)

Dammar triterpenoid	<i>m/z</i> used to evaluate the chromatographic area	relative abundance of the selected <i>m/z</i> in the mass spectrum of the pure compound %
dammaradienone	109	6.5
nor- β -amyrone	204	14.6
dammaradienol	189	4.8
nor- α -amyrone	204	24.2
shoreic acid	143	24.8
dammarenolic acid	443	1.4
20,24-epoxy-25-hydroxy-dammaren-3-one	143	27.9
hydroxydammarenone	109	6.5
oleanonic acid	408	11.0
oleanonic aldehyde	232	3.2
20,24-epoxy-25-hydroxy-dammaren-3-ol	143	30.6
oleanolic acid	203	13.5
oleanolic aldehyde	203	10.4
ursonic acid	409	3.4
ursonic aldehyde	232	1.5
ursolic acid	203	6.0
ursolic aldehyde	203	13.6

Table 3 *Tr* % of dammar compounds determined in the raw, t0 January 2008, and t0 June 2009 samples, and corresponding $\sum Tr$

compound	<i>Tr</i> %		
	raw	t0 January 2008	t0 June 2009
nor- β -amyrone	1	2	3
nor- α -amyrone	1	3	4
dammaradienone	6	4	6
dammaradienol	5	6	8
dammarenolic acid	15	12	0
hydroxydammarenone	25	26	17
20,24-epoxy-25-hydroxy-dammaren-3-ol	0	2	5
oleanolic acid	0	1	1
ursolic acid	0	2	4
shoreic acid	0	12	12
20,24-epoxy-25-hydroxy-dammaren-3-one	0	15	22
oleanonic acid	2	4	4
ursonic acid	9	11	13
oleanonic aldehyde	13	1	1
oleanolic aldehyde	2	0	0
ursonic aldehyde	18	0	0
ursolic aldehyde	3	0	0
$\sum Tr$	10548142	9411015	46677

Table 4. *Tr* % of each dammar terpenoid identified in the raw material, t0 January 2008 and the artificially aged samples, and $\sum Tr$

compound	t0 January 2008	NO2-50-55	NO2-50-146	NO2-50-169	NO2-50-423	NO2-50-426	NO2-80-54	NO2-80-120	NO2-80-135	NO2-80-293	AcA-20-21	NO2-20-231	O3-20-117	O3-80-133	NO2-80-599	AcA-80-265	glacial AcA 1.5	glacial AcA 3
dammaradienone	4	4	3	3	4	4	3	4	3	4	3	3	3	3	3	3	3	3
nor- β -amyrone	2	2	2	2	3	2	2	2	2	2	10	2	2	2	2	2	2	3
dammaradienol	6	4	5	5	0	0	2	5	4	2	2	4	6	6	4	4	4	2
nor- α -amyrone	3	3	2	2	2	2	2	2	2	2	2	3	2	2	2	2	3	4
shoreic acid	12	12	10	12	12	13	12	11	13	12	16	12	12	11	18	13	20	26
dammarenolic acid	12	15	13	15	11	13	16	14	15	13	12	17	17	17	18	16	4	2
20,24-epoxy-25-hydroxy-dammaren-3-one	15	15	15	15	24	20	15	16	17	21	19	15	15	15	17	17	27	32
hydroxydammarenone	27	27	27	29	30	32	30	28	28	30	20	25	23	24	26	26	16	9
oleanonic acid	4	4	4	3	3	3	4	3	3	3	4	4	4	4	3	4	3	3
oleanonic aldehyde	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
20-24-epoxy-25-hydroxy-dammaren-3-ol	2	2	4	3	2	2	2	2	1	2	3	1	2	1	0	2	6	4
oleanolic acid	1	1	1	1	0	0	0	1	1	0	1	1	1	1	0	0	0	1
oleanolic aldehyde	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ursonic acid	11	11	11	9	9	9	10	11	11	9	12	12	12	12	9	11	10	12
ursonic aldehyde	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
ursolic acid	2	2	3	2	1	1	1	3	2	1	2	2	2	2	0	1	2	1
ursolic aldehyde	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$\sum Tr$	941101 5	478477 9	431847 6	630825 9	587039 3	739478 0	739130 0	436233 2	612017 2	397787 2	369360 0	208875 3	771213 1	174341 9	151642 7	631669 9	9978 2	4737 9

Table 5. *Tr* % of the dammar terpenoids, as well as the corresponding Σ *Tr*of samples exposed at site

compound	Tate Britain (UK)		National Museum of Krakow (PL)		Museo de Bellas Artes de Valencia (ES)		Statens Museum for Kunst, Copenhagen (DK)		Uffizi Gallery (IT)	
	in	out	NF	LEO	in	out	in	out	in	out
dammaradienone	4	6	5	7	3	3	6	3	3	3
nor- β -amyron	3	3	3	3	2	2	3	2	2	2
dammaradienol	6	7	7	11	2	2	4	2	5	5
nor- α -amyron	3	4	3	4	2	3	3	3	3	3
shoreic acid	19	14	16	12	18	20	9	19	16	17
dammarenolic acid	6	4	4	4	6	6	2	5	4	5
20,24-epoxy-25-hydroxy-dammaren-3-one	27	26	22	24	24	26	27	27	29	26
hydroxydammarenone	4	3	11	6	14	15	20	18	11	13
oleanonic acid	5	5	4	2	4	4	5	4	4	4
oleanonic aldehyde	0	0	0	0	0	0	0	0	0	0
20,24-epoxy-25-hydroxy-dammaren-3-ol	5	7	5	6	11	5	3	3	8	6
oleanolic acid	1	1	1	0	0	0	0	0	1	2
oleanolic aldehyde	0	0	0	0	0	0	0	0	0	0
ursonic acid	13	15	14	15	13	14	14	12	11	12
ursonic aldehyde	0	0	0	0	0	0	0	0	0	0
ursolic acid	3	4	4	3	0	0	0	1	3	4
ursolic aldehyde	0	0	0	0	0	0	0	0	0	0
Total Amount of Free Triterpenoids	131737	193460	123329	153064	85548	154548	106057	138586	386746	123745

[View Online](#)**Table 6. Oxidation and loss of soluble triterpenoids for dammar samples exposed on site and corresponding environmental parameters (López-Aparicio et al., 2010)**

site	exposure location *	environmental parameters (López-Aparicio et al., 2010)						results	
		NO ₂ (µg/m ³)	O ₃ (µg/m ³)	Acetic acid (µg/m ³)	Formic acid (µg/m ³)	Light exposure	light levels (Dose 1 year Lux Hours)	Oxidation	loss of soluble terpenoids
Tate Britain (UK)	in	2	1	543	15	yes		-	-
	out	26	3	106	2	yes		+	+
National Museum of Krakow (PL)	NF**	0	1	502	10	no		-	+
	LEO***	2	3	317	0	yes		+	-
Museo de Bellas Artes de Valencia (ES)	in	0	3	435	156	no		+	+
	out	27	8	47	10	no		-	-
Statens Museum for Kunst, Copenhagen (DK)	in	4	1	1079	161	no	1303571	+	+
	out	12	12	43	18	no	2105769	-	-
Uffizi Gallery (IT)	in	2	0	81	51	yes	1955357	+	-
	out	14	2	33	7	yes	1955357	-	+

*in= inside the frame or showcase; out = outside the frame or showcase; LEO = Leonardo frame;

NF= new frame;

**AER= 14.9

***AER=0.39