**Identification of alkyl guaiacyl dehydroabietates as novel markers of wood
tar from Pinaceae in archaeological samples**

Lucile Bailly^{a*}, Pierre Adam^{a*}, Armelle Charrié^b, Jacques Connan^c

^a *Laboratoire de Biogéochimie Moléculaire, Institut de Chimie de Strasbourg UMR 7177, Université
de Strasbourg-CNRS, E.C.P.M., 25 rue Becquerel, 67200 Strasbourg, France*

^b *Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes LSMIS, Chimie de la
Matière Complexe UMR 7140, Université de Strasbourg-CNRS, 1 rue Blaise Pascal, 67008
Strasbourg, France*

^c *23, rue Antoine de Saint-Exupéry, 64000 Pau, France*

* Corresponding authors: Tel: +33 (0)3 68 85 28 04 (P. Adam); +33 (0)2 98 10 32 14 (L. Bailly).

E-mail addresses: padam@unistra.fr (P. Adam); bailly.lucile@orange.fr (L. Bailly).

ABSTRACT

Resinous materials from conifers from the Pinaceae family are frequently found in an archaeological context. These materials were used, notably, as waterproofing agent, as adhesive, or for the caulking of boats. Two main types of materials can be distinguished: resin, the exsudate of conifer tree, and wood tar obtained by dry distillation of wood. Molecular investigations of such materials aim, notably, at developing new molecular tools for the determination of their biological and geographical origin or of the manufacturing techniques, and for the discrimination between resin and wood tar. In this context, we report here the identification by synthesis of alkyl guaiacyl dehydroabietates, which occur in a series of recent and archaeological conifer wood tar samples from shipwrecks and Gallo-Roman tar preparation sites. They most likely result from the reaction of dehydroabietic acid and alkyl guaiacols, the latter being typical wood pyrolysis products formed during the preparation of wood tar. Alkyl guaiacyl dehydroabietates are therefore proposed to be specific molecular markers for conifer wood tar, allowing a clear molecular distinction between this material and resin to be made. They are also more resistant to the alteration undergone by archaeological samples than other compounds previously proposed as markers of conifer wood tar such as anhydrosugars or alkyl guaiacols.

Key words: Pinaceae, Wood Tar, Resin, Archaeology, Diterpenoids, Alkyl guaiacyl dehydroabietates.

1. Introduction

Resinous materials from conifers are relatively frequently found in an archaeological context. They have been extensively used since antiquity for various purposes, such as waterproofing jars and amphorae (Colombini et al., 2005a,b; Font et al., 2007; Romanus et al., 2009; Stacey et al. 2010; Jerković et al. 2011; Dimitrakoudi et al., 2011), caulking of boats or treatment of ropes (e.g. Evershed et al., 1985; Reunanen et al., 1989; Colombini et al., 2003; Connan and Nissenbaum, 2003; Connan and Charrié-Duhaut, 2010). They were also used as an adhesive (e.g. Regert and Rolando, 2002; Prokeš and Hložek, 2007; Hanuš and Ben-Yehoshua, 2013) and as ingredients in mummy balms (e.g. Łucejko et al., 2010; Łucejko et al., 2012). The various resinous materials from conifer comprise resin, which corresponds to the exudate of conifer trees, and wood tar which is prepared by dry distillation of conifer wood using various manufacturing techniques depending on the period and geographical regions (e.g. Aufan and Thierry, 1990; Egenberg, 1997; Beck et al., 2002; Loewen, 2005; Hjulström et al., 2006). Diagnostic molecular markers of Pinaceae-related archaeological material comprise mainly diterpenoids belonging to the abietane **1** (numbers refer to structures in the Appendix) and pimarane **2** series. Key issues in the study of such material in an archaeological context are the determination of their biological and geographical origin, mode of preparation and degree of alteration, as well as the discrimination between resin and wood tar. In this respect, some compounds have been proposed as molecular markers for wood tar. They comprise, in particular, anhydrosugars (e.g. levoglucosan **3**; Egenberg and Glastrup, 1999; Reunanen et al., 1996) and alkyl guaiacols **4-6** (Beck et al., 2002; Egenberg and Glastrup, 1999; Reunanen et al., 1996). These compounds are indeed pyrolysis products of the main constituents of wood: cellulose or hemicellulose (Oasmaa et al., 2003; Mohan et al., 2006; Demirbas, 2007) and lignin (Faix et al., 1990), respectively, and are therefore clearly diagnostic of organic materials originating from the thermal treatment of wood. The methyl ester of dehydroabietic

acid **7** has also been proposed as a specific marker of wood tar (Beck and Borromeo, 1990; Beck et al., 1997, 1999; Colombini et al., 2005a,b; Hjultsröm et al., 2006; Dimitrakoudi et al., 2011; Jerkovic et al., 2011; Izzo et al., 2013). It is postulated to originate from the esterification of dehydroabietic acid **7** with methanol formed during pyrolysis of wood (Hsu and Hixson, 1981; Hajaligol et al. 1982; Degroot et al., 1988, Hjulström et al., 2006).

In this context, we report here a series of compounds in Pinaceae wood tar samples which were identified as, respectively, methyl, ethyl, and propyl guaiacyl dehydroabietates (**8-10**; Fig. 1) from comparison with standards of the methyl and propyl homologues (**8**, **10**) prepared by synthesis. These compounds were identified in the frame of a study aimed at finding new molecular criteria for the characterization of coniferous materials found in an archaeological context (Bailly, 2015). The distributions of alkylguaiacol dehydroabietates in various archaeological samples including resinous materials found at sites dedicated to the preparation of conifer wood tar and on shipwrecks (coating of boat hulls, protection of ropes, waterproofing of amphorae or jars; Table 1) are discussed, as well as the use of these compounds as specific markers of Pinaceae wood tar.

2. Experimental

2.1. Samples

The archaeological/ancient resin and wood tar samples comprised several samples from shipwrecks and samples recovered from tar production sites. Additionally, one fresh resin from *Pinus pinaster* and a recent tar sample made from *Pinus sylvestris* were also investigated (Table 1).

2.2. Extraction and fractionation of extracts from resin and wood tar samples

2.2.1. Extraction procedure

No extraction procedure was necessary for resin and wood tar samples, which were generally completely soluble in CH_2Cl_2 , or in a mixture of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (6:4, v/v). In the case of samples comprising an insoluble part (e.g. ropes), the samples were either crushed in a mortar or cut into small pieces prior extraction via sonication ($\times 3$, 15 min) with, successively, a mixture of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (9:1, v/v), $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (6:4, v/v) and $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (1:1, v/v).

2.2.2. Fractionation and derivatization procedures

Two different fractionation and derivatization procedures were used. In the first procedure (2.2.2.1), the whole extracts were derivatized and a less polar fraction (F1d) separated by column chromatography. This fraction contains hydrocarbons but also functionalized compounds comprising ketones, acids (as methylester), alcohols (as acetates), up to compounds with a polarity of hexaesters. The GC-MS analysis of the F1d fraction gives thus an overview of the composition of the investigated substance (relative contribution of the various biomarker series: aromatic diterpenoids, resin acids, etc.). For a more detailed molecular investigation, the second procedure (2.2.2.2) involved first fractionating the underivatized extracts into several polarity sub-fractions using column and thin layer chromatography (TLC) prior GC-MS analysis.

2.2.2.1. Fractionation of the derivatized extracts

An aliquot of each crude extract obtained by filtration over celite was successively acetylated ($\text{Ac}_2\text{O}/\text{pyridine}$ 1:1 v/v, 60 °C, 2 h) and, after removal of the solvent and excess reagent under reduced pressure, methylated using a solution of diazomethane in Et_2O . The derivatized extracts were fractionated via liquid chromatography on silica gel, eluting successively with a

mixture of CH₂Cl₂/EtOAc (1:1 v/v) and CH₂Cl₂/CH₃OH (6:4 v/v), leading, respectively, to a first fraction (F1d) analysed using GC-MS, and a second more polar fraction not further investigated.

2.2.2.2. Fractionation of the underivatized extracts : Preparation of sub-fractions

The underivatized extracts were fractionated using column chromatography on silica gel into three fractions (F1u, F2u, F3u) eluted successively with a mixture of CH₂Cl₂/cyclohexane (1:9, v/v), CH₂Cl₂/EtOAc (1:1, v/v) and CH₂Cl₂/CH₃OH (6:4, v/v). F1u was further fractionated by way of TLC (SiO₂; CH₂Cl₂/cyclohexane; 1:9, v/v) into saturated (F1u.1, R_f > 0.80) and aromatic hydrocarbons (F1u.2, 0.80 > R_f > 0.10). F2u was fractionated using TLC (SiO₂; CH₂Cl₂) into “esters” (F2u.1, R_f > 0.70), “ketones” (F2u.2, 0.70 > R_f > 0.25), “alcohols” (F2u.3, 0.25 > R_f > 0.15) and “acids” (F2u.4, 0.15 > R_f > 0). F2u.3 was acetylated (Ac₂O/pyridine 1:1 v/v, 60 °C, 2 h) and F2u.4 methylated using diazomethane in Et₂O prior GC-MS. F3u was successively acetylated and methylated (see above) prior to purification on a silica gel column (elution with CH₂Cl₂/EtOAc; 1:1 v/v) and GC-MS.

2.3. Analytical methods

2.3.1. Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was carried out with a Thermo Scientific Trace Ultra gas chromatograph equipped with a TriPlus autosampler and a PTV “simili on-column” injector coupled to a ThermoScientific TSQ Quantum mass spectrometer. The source was set at 220 °C. The mass spectrometer was operating in the electron impact (EI) mode at 70 eV. GC separations were performed with a HP5-MS column (30 m × 0.25 mm; 0.1 µm film thickness) using He as

carrier gas (constant 1.1 mL/min). The temperature program was: 70 °C (1 min), to 100 °C (10 °C/ min), then to 300 °C (4 °C/min), isothermal at 300 °C.

2.3.2. Gas chromatography-high resolution MS (GC-HRMS)

GC-HRMS analysis was carried out with an Agilent Technologies 7890A gas chromatograph coupled to a JEOL Accu TOF GCV JMS-T100GCV mass spectrometer operating in the EI mode (70 eV) and equipped with a DB5-MS column (30 m × 0.25 mm; 0.25 µm film thickness) using He as carrier gas and the following temperature program: 40 °C-100 °C (10 °C/min), then to 300 °C (4 °C/min), isothermal at 300 °C.

2.3.3. Nuclear Magnetic Resonance (NMR)

NMR spectra were recorded using a Bruker Avance I 500 MHz spectrometer (500 MHz for ¹H; 125 MHz for ¹³C). Chemical shifts are reported in ppm relative to tetramethylsilane with the solvent used as internal standard (CHDCl₂: δ¹H 5.32 ppm; CD₂Cl₂: δ¹³C 53.8 ppm) and the coupling constants are expressed in Hz.

2.4. Synthesis of methyl and propyl guaiacyl dehydroabietates **8** and **10**

Methyl guaiacyl dehydroabietate **8** (resp. propyl guaiacyl dehydroabietate **10**) was prepared by reaction of dehydroabietic acid **7** (60 mg, 0.200 mmol; resp. 68 mg, 0.226 mmol) with methyl guaiacol **4** (176 mg, 1.27 mmol,) (resp. propyl guaiacol **6**; 198 mg, 1.19 mmol) at 100 °C using H₂SO₄ (50 µl) as catalyst. After 4 h, the reaction mixture was diluted with CH₂Cl₂ and washed with water (10 ml, ×3). The extract was evaporated under reduced pressure and filtered over a silica gel column (CH₂Cl₂/cyclohexane, 8/2, v/v), yielding impure methyl guaiacyl dehydroabietate **8** (resp. propyl guaiacyl dehydroabietate **10**) which was further purified by way of TLC on silica gel (cyclohexane/EtOAc, 95/5, v/v), furnishing 1.7 mg of

165 pure **8** (4.1 μmol , 2%) (resp. 2.9 mg of **10**; 6.5 μmol , 2.9%).

166

167 **Compound 8.** GC-MS (EI, 70 eV, Fig. 4a) m/z (rel. intensity) 420 (M^+ , 5%), 255 (12), 239
168 (35), 185 (19), 173 (90), 138 (100). ^1H -NMR (500 MHz; CD_2Cl_2): 1.20 (6H, d, $J = 7.0$ Hz, H-
16, H-17), 1.24 (3H, s), 1.35 (3H, s), 1.70 - 1.94 (6H, m), 2.04 (1H, ddd, $J = 4.5$; 12.5, 12.5
170 Hz), 2.34 (3H, s, H-27), 2.35 (1H, m), 2.39 (1H, dd, $J = 2.5$; 12.5 Hz, H-5), 2.81 (1H, sep, $J =$
171 7.0 Hz, H-15), 2.92 (2H, m, H-7), 3.77 (3H, s, H-28), 6.74 (1H, dd, $J = 1.5$; 8.0 Hz, H-25),
172 6.80 (2H, H-23, H-26), 6.90 (1H, d, $J = 1.5$ Hz, H-14), 6.98 (1H, dd, $J = 1.5$; 8 Hz, H-12),
173 7.18 (1H, d, $J = 8.0$ Hz, H-11). ^{13}C -NMR (125MHz; CD_2Cl_2): 16.9, 19.0, 21.5, 22.0, 24.2,
174 24.2, 25.3, 30.5, 33.9, 36.9, 37.4, 38.5, 45.4, 48.3, 56.1, 113.7, 121.3, 122.6, 124.2, 124.5,
175 127.2, 135.3, 137.0, 138.4, 146.2, 147.5, 151.3, 177.1.

176 **Compound 10.** GC-MS (EI, 70 eV, Fig. 4b) m/z (rel. intensity) 448 (M^+ , 3%), 255 (8), 239
177 (18), 185 (14), 173 (73), 166 (100), 137 (12). ^1H -NMR (500 MHz; CD_2Cl_2): 0.95 (3H, t, $J =$
178 7.5 Hz, H-29), 1.20 (6H, d, $J = 7.0$ Hz, H-16, H-17), 1.25 (3H, s), 1.35 (3H, s), 1.64 (2H, td, J
179 = 7.5; 7.5 Hz, H-28), 1.70 – 1.94 (6H, m), 2.35 (1H, m), 2.03 (1H, ddd, $J = 3.5$; 13.0, 13.0
180 Hz), 2.38 (1H, dd, $J = 2.0$, 13.0 Hz, H-5), 2.57 (2H, t, $J = 7.5$ Hz, H-27), 2.81 (1H, sep, $J =$
181 7.0 Hz, H-15), 2.93 (2H, m, H-7), 3.78 (3H, s, H-30), 6.74 (1H, dd, $J = 2.0$, 8.0 Hz, H-25),
182 6.80 (1H, d, $J = 2.0$ Hz, H-23), 6.82 (1H, d, $J = 8.0$ Hz, H-26), 6.89 (1H, d, $J = 1.5$ Hz, H-14),
183 6.98 (1H, dd, $J = 1.5$; 8.0 Hz, H-12), 7.18 (1H, d, $J = 8.0$ Hz, H-11). ^{13}C -NMR (125 MHz;
184 CD_2Cl_2): 13.6, 16.5, 18.6, 21.6, 23.8, 23.8, 24.7, 24.9, 30.1, 33.5, 36.5, 36.9, 37.9, 38.1, 45.0,
185 47.8, 55.6, 112.6, 120.3, 122.2, 123.8, 124.2, 126.9, 134.8, 138.0, 141.5, 145.8, 147.0, 150.9,
186 176.7.

187

188 **3. Results and discussion**

3.1. Identification of alkyl guaiacyl dehydroabietates 8-10 and related compounds

Three representative examples of gas chromatograms of F1d fractions isolated from tar samples altered to different levels and under different conditions (#2924, #2864, #2757; Table 1) are shown in Fig. 2a-c. Sample #2924 was found as a solid material in the recovery pipe of a traditional tar kiln dated to the XIXth century (Gensel, 2005, unpublished results) and was unambiguously identified as wood tar of *Pinus halepensis* (Connan and Tengberg, 2006, unpublished results; Bailly, 2015). As an interesting feature, the recovery pipe was a barrel shotgun from the Napoleon army. Taking into account the context of the sampling in the heart of the barrel shotgun, the tar was well protected from alteration processes such as oxidation or leaching. It could thus be considered as an unaltered wood tar sample. Sample #2864 was found in a Gallo-Roman site at Vielle-Saint-Girons (Aquitaine, France; Table 1; Jacques, 2011). This sample corresponded to a piece of resinous material collected at a site where conifer wood tar was prepared by heating conifer wood (most likely from *Pinus pinaster*) in jars (so-called “Dolia”) with an external fire, as attested to from the occurrence of a characteristic fire-place and tar-stained fragments of broken jars mixed with pieces of solidified tar. This sample was exposed to atmospheric conditions since its preparation and had undergone oxidative degradation. Sample #2757 was collected from an anchor cable from the *Mary Rose* shipwreck (Evershed et al., 1985; Robinson et al., 1987) and was postulated to correspond to conifer wood tar. It corresponded to a highly dispersed material exposed to severe alteration under the conditions prevailing in an anoxic marine environment (see below).

The distributions of the constituents of the F1d fractions (Fig. 2a-c) were dominated by diterpenic acids and related diterpenic aromatic hydrocarbons generally found in conifer

wood tar (e.g. Evershed et al., 1985; Beck and Borromeo, 1990; Egenberg et al., 2002; Connan and Nissenbaum, 2003; Hjulström et al., 2006; Connan and Charrié-Duhaut, 2010) comprising, notably, abietic acid **11**, pimarane derivatives such as isopimaric acid **12**, dehydroabietic acid **7**, retene **13**, methylretene **14**, tetrahydroretene **15**, 18- and 19-norabietatrienes **16** and **17**, as well as some of their typical oxidation products such as didehydroabietic acid **18**, 7-oxodehydroabietic acid **19** and its related alcohol **20** (e.g. Egenberg et al., 2003). The latter were proportionally more abundant in sample #2864, which was exposed to oxidative alteration conditions for a long period of time. In the case of sample #2757, the predominant diterpenic acid corresponds to tetrahydroabietic acid **21** (Fig. 2 c). This compound has been observed in conifer wood tar collected from ropes found on shipwrecks (e.g. Reunanen et al., 1990). It is likely not a genuine product present in the resinous material used for the treatment of the anchor cable but rather a diagenetic transformation product of abietic acid formed under the anoxic/suboxic conditions prevailing in the marine sediments where the shipwreck was buried (Reunanen et al., 1990; Tavendale et al., 1997). The distribution was also characterized by a high abundance of 19-norabietatriene **17**. Such a predominance was never encountered in freshly prepared conifer wood tar and can be explained by microbially-mediated diagenetic transformation of abietic acid (e.g. Simoneit et al., 1986; Martin et al., 1999).

Detailed investigation of the ester fractions (F2u.1) from the underivatized extracts from the same samples using GC-MS revealed, besides early eluting diterpenoids and some unidentified compounds, the occurrence of two series of late-eluting compounds (Fig. 3). The two series could also be detected in a recent tar sample (#2745, Stockholm tar) as well as in archaeological tar samples identified as such, either on the basis of their provenance (i.e. samples from wood tar preparation sites: sample #2924, Sainte Anne du Castellet; samples

237 #2843, 2864, 2886, 2887, Petre du Bin; Table 1) or on molecular criteria (i.e. occurrence of
238 alkyl guaiacols and anhydrosugars; see Section 3.3 and Table 2).

239 *Monoterpenyl esters of resin acids*

240 The mass spectra of compounds from the first eluting series (filled and empty triangles
241 in Fig. 3) are characterized by an intense fragment at m/z 137 and M^+ at m/z 436 or m/z 438
242 (spectra not shown). These compounds have been detected by Czechowski et al. (1996) and
243 Yamamoto et al. (2006) and were proposed, based on interpretation of the mass spectra, to
244 correspond to monoterpenyl esters of dehydroabietic acid **22** ($[M^+]$ at m/z 436) and of
245 diunsaturated tricyclic diterpenic acids from the abietic and pimaric series **23**, **24** ($[M^+]$ at m/z
246 438). The monoterpenyl moieties were proposed to correspond mainly to borneol **25**,
247 isoborneol **26**, or fenchol **27**, based on the wide occurrence of these monoterpenoid alcohols
248 in conifers (Czechowski et al., 1996; Yamamoto et al., 2006). None has, however, been
249 unambiguously identified yet. These hypotheses were supported by our own investigations
250 using GC-HRMS. Indeed, the molecular formulae for the members of this series are,
251 respectively, $C_{30}H_{44}O_2$ ($[M^+]$ at m/z 436) and $C_{30}H_{46}O_2$ ($[M^+]$ at m/z 438). In addition, the
252 formula obtained for the fragment at m/z 137 ($C_{10}H_{17}$) is compatible with that of a
253 monoterpenyl moiety with two degrees of unsaturation (rings or double bonds). The
254 monoterpenyl diterpenic esters were detected in all the recent and archaeological samples
255 investigated i.e. resin and wood tar samples.

256 *Alkyl guaiacyl dehydroabietates 8-10*

257 Three compounds (**8-10**) constituted the second eluting series from the ester fractions
258 (Fig. 3). They could be detected, although in variable relative amounts, in all the recent and
259 archaeological tar samples, but were absent from the resin samples (Table 2). Their relative
260 proportions also varied, some samples being characterized by the clear predominance of

compound **8**, whereas compounds **8-10** were present in almost equal proportions in most samples (Table 2). The mass spectra of **8-10** are characterized by M^+ at m/z $420 + n \times 14$, with $n = 0-2$ (Fig. 4a-c), the occurrence of an important common fragment at m/z 239 and of an even fragment at m/z $138 + n \times 14$ ($n = 0-2$), respectively. Based on their molecular formulae ($C_{28}H_{36}O_3$, $C_{29}H_{38}O_3$, $C_{30}H_{40}O_3$, respectively) obtained from GC-HRMS, on the presence of an important fragment at m/z 239 (this fragment being also predominant in the spectra of esters related to dehydroabietic acid **7**) and on the co-occurrence of methoxyphenol derivatives **4** ($[M^+]$ at m/z 138), **5** ($[M^+]$ at m/z 152, m/z 137;) and **6** ($[M^+]$ at m/z 166, m/z 137), we initially postulated that the compounds could correspond to alkyl guaiacyl dehydroabietates **8-10**. This hypothesis was confirmed by comparison of the mass spectra and chromatographic behaviour (coinjection on a HP5-MS GC column) of synthesized reference compounds **8** and **10** with those of the alkyl guaiacyl dehydroabietates occurring in the wood tar samples.

7-oxo-derivatives of the alkyl guaiacyl dehydroabietates 28-30

Besides, another series of three compounds **28-30**, paralleling the identified alkyl guaiacyl dehydroabietates **8-10** was also detected in the ketone fractions from samples showing clear signs of oxidative alteration (e.g. #2864). Their mass spectra showed features very similar to those of alkyl guaiacyl dehydroabietates **8-10** (predominant fragments at m/z $138 + n \times 14$) but with fragments ascribed to the diterpenic moiety in the spectra of alkyl guaiacyl dehydroabietates (e.g. m/z 173, 239, 255), as well as the molecular ions shifted upwards by 14 Da (e.g. Fig. 4d). We therefore propose that these compounds could correspond to the 7-oxo-derivatives (**28-30**) of the alkyl guaiacyl dehydroabietates **8-10**.

3.2. Alkyl guaiacyl dehydroabietates **8-10** and related compounds **28-30** as specific markers of conifer wood tar

Alkyl guaiacyl dehydroabietates **8-10** are formed by esterification of dehydroabietic acid **7** with alkyl guaiacols **4-6** (Fig. 5), the latter being typical compounds formed by thermal degradation of lignin upon pyrolysis of wood (Faix et al., 1990). Alkyl guaiacols are generally found in fresh, unaltered softwood tar (Beck et al., 2002) and their presence in archaeological tar samples has sometimes been reported (Egenberg and Glastrup, 1999; Reunanen et al., 1996). It follows that alkyl guaiacyl dehydroabietates **8-10** can be considered as specific markers for wood tar from Pinaceae. They have the advantage over alkyl guaiacols **4-6** as being less easily lost during ageing due to their lower volatility and lower solubility in water. All the samples in Table 2 containing **8-10**, therefore, necessarily comprise a wood tar component. It appeared also that the relative proportions of the methyl-, ethyl- and propyl homologues (**8-10**) are not identical in all the samples investigated (Table 2). In some cases, only the methylated homologue **8** could be detected (e.g. coating of ropes from the “Jeanne Elisabeth”, Table 2). This might be due to differences in the manufacturing techniques. It is indeed likely that the temperature, notably, influences the relative proportions of the various homologues since higher temperatures would possibly favor the pyrolytic cleavage of the C2 or C3 alkyl chain of the higher homologues.

Similarly to alkyl guaiacyl dehydroabietates **8-10**, the 7-oxo derivatives **28-30**, formed by autoxidation during ageing of the archaeological material, share the same source specificity and can be considered as diagnostic markers of altered oxidized tar. In samples exposed to severely oxidizing conditions, autoxidation might further affect the alkyl side-chain of the alkyl guaiacyl moiety, leading to compounds with a ketone functionality (e.g. **31**, **32**) or a carboxylic acid group (**33**) at the benzylic position (Charrié-Duhaut et al., 2000).

Such compounds which would also be specific markers of wood tar could, however, not be detected in the investigated samples. In aquatic environments, one might envisage that the ester functionality of alkyl guaiacyl dehydroabietates and related compounds is ultimately hydrolyzed.

3.3. Reliability of the novel markers with respect to other wood tar markers

Other molecular markers have been proposed to allow discrimination between resin and tar samples. They comprised, in particular, anhydrosugars (e.g. levoglucosan **3**), which are pyrolysis products of cellulose and hemicellulose (Oasmaa et al., 2003; Mohan et al., 2006; Demirbas, 2007). These compounds can indeed be found in tar but not in resin (Egenberg and Glastrup, 1999; Reunanen et al., 1996). However, due to their solubility in water and their biodegradability, they can easily disappear in the case of archaeological wood tar samples depending on environmental conditions, notably in the case of samples found in an aquatic context. In this respect, we examined a series of coniferous materials used for caulking of boat hulls or treatment of ropes from shipwrecks which had been exposed to aquatic environments (Table 1). These materials were identified as wood tar or as a mixture of wood tar and other materials (e.g. #2767 and #2768; Bailly, 2015; Table 2). Such mixtures correspond to the so-called “stuff” (Charrié-Duhaut et al., 2009). Most of the tar samples used for the treatment of ropes did not contain anhydrosugars (Table 2) probably because the coniferous material was highly dispersed on the ropes and was therefore exposed to severe leaching and bacterial degradation. Anhydrosugars could, however, be detected in some of the substances used for caulking the hull of boats and anhydrosugars occurred only in trace amounts in the material used for the waterproofing of amphorae from the Mazotos shipwreck (Demesticha, 2011; Table 2). Since both types of materials were exposed to similar environmental conditions (aquatic medium), differences in the anhydrosugar content might

also be explained by way of the extent of the degradation undergone by the samples and by the methods used for their preparation. Indeed, the temperature to which wood tar is submitted during preparation depends on the mode of preparation and might influence the initial anhydrosugar content. Since the temperature values reached using the double-pot method are higher than those reached in tar kilns (Beck et al., 2002), one might expect a lower initial content of anhydrosugars in tar prepared via the double-pot method due to the fact that sugars are thermally labile. In this respect, tar from the Mazotos shipwreck dating back to the 4th century BC and most likely prepared in jars (double-pot or related methods; Aufan and Thierry, 1990; Kurzweil and Todtenhaupt, 1992; Beck et al., 2002) possibly did have an initial anhydrosugar content lower than that of tar found in shipwrecks from the XVIIIth century (e.g. “L’Aimable Grenot” and “La Dauphine”) which was most likely prepared in tar kilns (Aufan and Thierry, 1990; Egenberg, 1997; Hjulström et al., 2006). This possibly explains to some extent why anhydrosugars were detected only in trace amount in tar from the Mazotos shipwreck. The tar samples from Vielle Saint-Girons (except #2843 and #2887, Table 2) did not contain anhydrosugars, although they were not found in an aquatic context. However, these samples were exposed to rain and atmospheric conditions for several centuries. In addition, they were prepared by heating conifer wood in jars, as was probably also the case with the Mazotos shipwreck, using a process which might possibly result in lower initial anhydrosugar content (see above).

Alkyl guaiacols (**4-6**), which are also considered as specific markers for wood tar might be rapidly lost upon ageing due to their volatility and partial water solubility. Like anhydrosugars, they were not systematically detected in the archaeological tar samples containing alkyl guaiacyl dehydroabietates (Table 2).

The occurrence of native methyl ester of dehydroabietic acid **7** in a coniferous material has also been proposed to be characteristic of conifer wood tar (Beck and Borromeo, 1990; Beck et al., 1997, 1999; Colombini et al., 2005a,b; Hjulström et al., 2006; Dimitrakoudi et al., 2011; Jerkovic et al., 2011; Izzo et al., 2013). Indeed, dehydroabietic acid **7** is often the predominant diterpenic compound found in archaeological conifer resin and tar samples. Its related methylated derivative, occurring in small amounts in tar, is postulated to be formed by esterification of dehydroabietic acid **7** with MeOH originating from the pyrolysis of wood (Hsu and Hixson, 1981; Hajaligol et al. 1982; Degroot et al., 1988, Hjulström et al., 2006). The presence of this compound in a sample should, however, be interpreted with care since it might also be formed, to some extent by esterification of dehydroabietic acid **7** during sample handling when MeOH is used as extraction solvent or as eluent for chromatography. In addition, this compound may occur in small amounts in native resins (Beck and Borromeo, 1990; Hjulström et al., 2006).

Aromatic hydrocarbons related to diterpenes may also be considered for distinguishing between tar and resin since these compounds can be formed by thermal processes during the preparation of wood tar and are not of biosynthetic origin. However, besides thermal treatment, they can also be formed by natural diagenetic alteration affecting resinic acids in sediments and/or under aquatic conditions (e.g. Simoneit, 1977; Wakeham et al., 1980; Simoneit et al., 1986, Hynning et al., 1993; Tavendale et al., 1997) so, they cannot be considered as unambiguous tar-diagnostic markers since they might be encountered in both resins and tars found on shipwrecks (e.g. Reunanen et al., 1990). Nevertheless, it should be mentioned that some diterpenic aromatic hydrocarbons, comprising in particular, methylated derivatives of retene (**14**), are postulated to be formed by thermal processes during the preparation of wood tar (Connan and Nissenbaum, 2003) and can, thus, be considered as molecular indicators of thermal processes and not as early diagenetic transformation products

of abietic acid. In this respect, such compounds have, to our knowledge, never been found in recent sediments.

4. Conclusions

Alkyl guaiacyl dehydroabietates (**8-10**) were identified from comparison with synthetic standards in a recent wood tar from Pinaceae as well as in a series of archaeological materials derived from Pinaceae from different periods. The materials were collected from tar preparation sites and from shipwrecks, where they were used for the coating of hulls, the protection of ropes and the waterproofing of jars and amphorae.

Alkyl guaiacyl dehydroabietates (**8-10**) result from the esterification between dehydroabietic acid **7** and alkyl guaiacols (**4-6**), the latter being typical wood pyrolysis products, and are therefore proposed to represent specific molecular markers for tar as opposed to resin. In this respect, these compounds could not be detected in any (recent or ancient) conifer resin investigated. They exclusively occurred in samples made from conifer wood, whatever the mode of tar preparation involved (e.g. in jars or in tar kilns), and which had undergone different alteration processes to various degrees. They also appear to be more resistant to alteration than other compounds previously proposed as markers of conifer wood tar, such as anhydrosugars or alkyl guaiacols. The latter can indeed easily be lost by way of biodegradation (anhydrosugars), leaching (anhydrosugars, alkyl guaiacols) or evaporation (alkyl guaiacols). Contrary to most of the aromatic diterpenoids which are sometimes used to identify wood tar, alkyl guaiacyl dehydroabietates cannot be formed via diagenetic or thermal alteration of resin. In addition, contrary to methyl dehydroabietate, no process related to sample handling can account for their presence in coniferous materials.

403

404 **Acknowledgements**

405 E. Motsch and P. Wehrung (Université de Strasbourg) are thanked for GC-MS analysis and L.
406 Allouche and B. Vincent (Université de Strasbourg) for NMR measurements. P. Schaeffer
407 (CNRS/Université de Strasbourg) is thanked for helpful comments on the manuscript, and
408 two anonymous reviewers for constructive suggestions. We thank E. Veyrat and D. Sanders,
409 as well as the ADRAMAR (Association pour le Développement de la Recherche en
410 Archéologie MARitime) and the DRASSM (Département des Recherches Archéologiques
411 Subaquatiques et Sous-Marines) for the samples from “L’Aimable Grenot” and “La
412 Dauphine”, the Mary Rose Trust for the samples from the “Mary Rose”, S. Demesticha for
413 the samples from the Mazotos shipwreck, A. Kurzweil and the Museumsdorf Düppel in
414 Berlin and the archaeological museum of Biskupin for reference wood tar samples, P.
415 Jacques, for resins and wood tars from the Gallo-Roman archaeological site in Vielle-Saint-
416 Giron, P. Grandjean for the samples from the “Jeanne-Elisabeth” shipwreck, A. Gensel for
417 the tar sample from Sainte Anne du Castellet (Var, SE of France). L.B. thanks the French
418 Ministère de l’Enseignement Supérieur et de la Recherche for a doctoral fellowship.

419

420 **References**

421 Aufan, R., Thierry, F., 1990. Histoire des Produits Résineux Landais: Goudrons, Poix et
422 Brays Gras depuis l’Antiquité, en Buch, Born et Marensin. Société Historique et
423 Archéologique d’Arcachon et du Pays de Buch, Arcachon.

424 Bailly, L., 2015. Caractérisation Moléculaire et Isotopique de Goudrons et Résines
 425 Archéologiques Dérivés de Conifères en Contexte Maritime. PhD Thesis, Université de
 426 Strasbourg, France.

427 Beck, C.W., Borromeo, C., 1990. Ancient pine pitch : Technological perspectives from a
 428 Hellenistic shipwreck. *Masca Research Papers in Science and Archaeology* 7, 51-58.

429 Beck, C.W., Stout, E.C., Jänne, P., 1997. The pyrotechnology of pine tar and pitch inferred
 430 from quantitative analyses by gas chromatography-mass spectrometry and ^{13}C nuclear
 431 magnetic resonance spectrometry. In: Brzeziński, W., Piotrowski, W. (Eds.), *Proceedings of*
 432 *the first International Symposium on Wood Tar and Pitch*, Biskupin, Poland, July 1st-4th
 433 1993. State Archaeological Museum, Warsaw, pp. 181-182.

434 Beck, C.W., Stout, E.C., Bingham, J., Lucas, J., Purohit, V., 1999. Central European pine tar
 435 technologies. *Ancient Biomolecules* 2, 281-293.

436 Beck, C.W., Stout, E.C., Lee, K., Todtenhaupt, D., Kurzweil, A., 2002. Ancient pine tar
 437 technology: correlations between manufacturing methods and composition. In: Jerem, E.,
 438 Biro, K.T. (Eds.), *Archaeometry 98: Proceedings of the 31st symposium*, Budapest, April 26 -
 439 May 3, 1998, *Archaeolingua, BAR - Central European Series* 1. Archaeopress, Oxford, pp.
 440 179-184.

441 Charrié-Duhaut, A., Lemoine, S., Adam, P., Connan, J., Albrecht, P., 2000. Abiotic oxidation
 442 of bitumen under natural conditions. *Organic Geochemistry* 31, 977-1003.

443 Charrié-Duhaut, A., Connan, J., Darnell, M., Spangenberg, J., Szymczyk, E., Bissada, A.,
 444 Albrecht, P., 2009. Molecular and isotopic characterization of organic samples from the
 445 wreck of the Saint-Etienne merchant ship (XVIIIth century): Identification of pitch, fat, hair
 446 and sulfur. *Organic Geochemistry* 40, 647-665.

447 Colombini, M.P., Giachi, G., Modugno, F., Pallecchi, P., Ribechini, E., 2003. The
 448 characterization of paints and waterproofing materials from the shipwrecks found at the
 449 archaeological site of the Etruscan and Roman harbor of Pisa (Italy). *Archaeometry* 45, 659-
 450 674.

451 Colombini, M.P., Giachi, G., Modugno, F., Ribechini, E., 2005a. Characterisation of organic
 452 residues in pottery vessels of the Roman age from Antinoe (Egypt). *Microchemical Journal*
 453 79, 83-90.

454 Colombini, M.P., Modugno, F., Ribechini, E., 2005b. Direct exposure electron ionization
 455 mass spectrometry and gas chromatography/mass spectrometry techniques to study organic
 456 coatings on archaeological amphorae. *Journal of Mass Spectrometry* 40, 675-687.

457 Connan, J., Maurin, B., Long, L., Sebire, H., 2002. Identification de poix et de résine de
 458 conifère dans des échantillons archéologiques du lac de Sanguinet: Exportation de poix en
 459 Atlantique à l'époque gallo-romaine. *Revue d'Archéométrie* 26, 177-196.

460 Connan, J., Nissenbaum, A., 2003. Conifer tar on the keel and hull planking of the Ma'agan
 461 Mikhael Ship (Israel, 5th century BC): identification and comparison with natural products
 462 and artefacts employed in boat construction. *Journal of Archaeological Science* 30, 709-719.

463 Connan, J., Charrié-Duhaut, A., 2010. Etude géochimique d'enduits de surface, de tissus de
 464 calfatage, de blocs de poix et de bitume des épaves gallo-romaines du Parc Saint-Georges à
 465 Lyon. *Archaeonautica* 16, 147-172.

466 Czechowski, F., Simoneit, B.R.T., Sachanbiński, M., Chojcan, J., Wołowiec, S., 1996.
 467 Physicochemical structural characterization of ambers from deposits in Poland. Applied
 468 Geochemistry 11, 811-834.

469 Degroot, W.F., Pan, W.P., Dalilur Rahman, M., Richards, G.N., 1988. First chemical events
 470 in pyrolysis of wood. Journal of Analytical and Applied Pyrolysis 13, 221-231.

471 Demesticha, S., 2011. The 4th-Century-BC Mazotos Shipwreck, Cyprus: A Preliminary
 472 Report. International Journal of Nautical Archaeology 40, 39-59.

473 Demirbas, A., 2007. The influence of temperature on the yields of compounds existing in bio-
 474 oils obtained from biomass samples via pyrolysis. Fuel Processing Technology 88, 591-597.

475 Dimitrakoudi, E.A., Mitkidou, S.A., Urem-Kotsou, D., Kotsakis, K., Stephanidou-
 476 Stephanatou, J., Stratis, J.A., 2011. Characterization by gas chromatography-mass
 477 spectrometry of diterpenoid resinous materials in Roman-age amphorae from Northern
 478 Greece. European Journal of Mass Spectrometry 17, 581-591.

479 Egenberg, I.M., 1997. Kiln-produced tar. In: Brzeziński, W., Piotrowski, W. (Eds.),
 480 Proceedings of the first International Symposium on Wood Tar and Pitch, Biskupin, Poland,
 481 July 1st-4th 1993. State Archaeological Museum, Warsaw, pp. 141-148.

482 Egenberg, I.M., Glastrup, J., 1999. Composition of kiln-produced tar. In: Bridgland, J.;
 483 Brown, J. - ICOM Committee for Conservation (Eds.), Preprints of triennial meeting (12th),
 484 Lyon, 29 August - 3 September 1999. James & James, London, pp. 862-867.

485 Egenberg, I.M., Isaksson S., Hennius, A., 2002. Organic geochemical evidence for pine tar
 486 production in Middle Eastern Sweden during the Roman Iron Age. *Journal of Archaeological*
 487 *Science* 33, 283-294.

488 Egenberg, I.M., Holtekjølén, A.K., Lundanes, E., 2003. Characterisation of naturally and
 489 artificially weathered pine tar coatings by visual assessment and gas chromatography-mass
 490 spectrometry. *Journal of Cultural Heritage* 4, 221-241.

491 Evershed, R.P., Jerman, K., Eglinton, G., 1985. Pine wood origin for pitch from the Mary
 492 Rose. *Nature* 314, 528-530.

493 Faix, O., Meier, D., Fortmann, I., 1990. Thermal degradation products of wood. *Holz als Roh-*
 494 *und Werkstoff* 48, 281-285.

495 Font, J., Salvadó, N., Butí, S., Enrich, J., 2007. Fourier transform infrared spectroscopy as a
 496 suitable technique in the study of the materials used in waterproofing of archaeological
 497 amphorae. *Analytica Chimica Acta* 598, 119-127.

498 Hajaligol, M.R., Howard, J.B., Longwell, J.P., Peters, W.A., 1982. Product compositions and
 499 kinetics for rapid pyrolysis of cellulose. *Industrial & Engineering Chemistry Process Design*
 500 *and Development* 21, 457-465.

501 Hanuš, L.O., Ben-Yehoshua, S., 2013. Analysis of resinous material present on an alabaster
 502 lid excavated from Nabataean Moa in the Arava valley. *Arabian Archaeology and Epigraphy*
 503 24, 232-236.

504 Hjulström, B, Isaksson, S., Hennius, A., 2006. Organic geochemical evidence for pine tar
 505 production in Middle Eastern Sweden during the Roman Iron Age. *Journal of Archaeological*
 506 *Science* 33, 283-294.

507 Hsu, C.C., Hixson, A.N., 1981. C₁ to C₄ oxygenated compounds by promoted pyrolysis of
 508 cellulose. *Industrial & Engineering Chemistry Product Research and Development* 20,
 509 109-114.

510 Hynning, P.A., Remberger, M., Neilson, A.H., 1993. Identification and quantification of 18-
 511 nor- and 19- norditerpenes and their chlorinated analogues in samples of sediment and fish.
 512 *Journal of Chromatography A* 643, 439-452.

513 Izzo, F.C., Zendri, E., Bernardi, A., Balliana, E., Sgobbi, M., 2013. The study of pitch via gas
 514 chromatography–mass spectrometry and Fourier-transformed infrared spectroscopy: the case
 515 of the Roman amphoras from Monte Poro, Calabria (Italy). *Journal of Archaeological Science*
 516 40, 595-600.

517 Jacques, P., 2011. Production de poix à la période antique. Le site de Petre de Bin à Vielle
 518 Saint-Girons. *Mémoire en Marensin* 22, 123-134.

519 Jaouen, M., Poletto, A., 2012. Jeanne-Elisabeth, 1755, *Arkaeos*, [http://www.arkaeos.fr/les-](http://www.arkaeos.fr/les-operations/article/jeanne-elisabeth-1755)
 520 [operations/article/jeanne-elisabeth-1755](http://www.arkaeos.fr/les-operations/article/jeanne-elisabeth-1755).

521 Jerković, I., Marijanović, Z., Gugić, M., Roje, M., 2011. Chemical profile of the organic
 522 residue from ancient amphora found in the Adriatic sea determined by direct GC and GC-MS
 523 analysis. *Molecules* 16, 7936-7948.

524 Kurzweil, A., Todtenhaupt, D., 1992. Technologie der Holzteergewinnung. *Acta Praehistorica*
 525 et *Archaeologica* 23, 63-91.

526 L'Hour, M., Veyrat, E., Daeffler, M., Hulot, O., Peaudecerf, H., 2010. Les épaves corsaires de
 527 la Natière. Archéologie sous-marine à Saint-Malo, *La Natière*,
 528 [http://www.epaves.corsaires.culture.fr/#/fr/uc/accueil/t=Les%20%C3%A9paves%20corsaires](http://www.epaves.corsaires.culture.fr/#/fr/uc/accueil/t=Les%20%C3%A9paves%20corsaires%20de%20la%20Nati%C3%A8re.%20Arch%C3%A9ologie%20sous-marine%20%C3%A0%20Saint-Malo)
 529 [%20de%20la%20Nati%C3%A8re.%20Arch%C3%A9ologie%20sous-](http://www.epaves.corsaires.culture.fr/#/fr/uc/accueil/t=Les%20%C3%A9paves%20corsaires%20de%20la%20Nati%C3%A8re.%20Arch%C3%A9ologie%20sous-marine%20%C3%A0%20Saint-Malo)
 530 [marine%20%C3%A0%20Saint-Malo](http://www.epaves.corsaires.culture.fr/#/fr/uc/accueil/t=Les%20%C3%A9paves%20corsaires%20de%20la%20Nati%C3%A8re.%20Arch%C3%A9ologie%20sous-marine%20%C3%A0%20Saint-Malo).

531 Loewen, B., 2005. Resinous paying materials in the French Atlantic, AD 1500–1800. History,
 532 technology, substances. *International Journal of Nautical Archaeology* 34, 238-252.

533 Łucejko, J.J., Modugno, F., Ribechini, E., Colombini, M.P., 2010. Wood tar and wood
 534 resinous extracts as ingredients of Egyptian embalming materials: a GC/MS study. In:
 535 Proceedings of 4th International Conference of Cost Action IE0601, Interaction between
 536 Wood Science and Conservation of Cultural Heritage, Izmir, Turkey, 20-22 October 2010.

537 Łucejko, J.J., Lluveras-Tenorio, A., Modugno, F., Ribechini, E., Colombini, M.P., 2012. An
 538 analytical approach based on X-ray diffraction Fourier transform infrared spectroscopy and
 539 gas chromatography/mass spectrometry to characterize Egyptian embalming material.
 540 *Microchemical Journal* 103, 110-118.

541 Martin, V.J., Yu, Z., Mohn, W.W., 1999. Recent advances in understanding resin acid
 542 biodegradation: microbial diversity and metabolism. *Archives of Microbiology* 172, 131-138.

543 Mohan, D., Pittman, C.U., Steele, P.H., 2006. Pyrolysis of wood/biomass for bio-oil: A
 544 critical review. *Energy & Fuels* 20, 848-889.

545 Monaghan, J., 2010. Guernsey's Roman ship: small island, big problem. 6th International
 546 Conference on Small Island Cultures, Guernsey, 22-25 June 2010, Book of Abstracts, pp. 35-
 547 39.

548 Oasmaa, A., Kuoppala, E., Solantausta, Y., 2003. Fast pyrolysis of forestry residue. 2.
 549 Physicochemical composition of product liquid. *Energy & Fuels* 17, 433-443.

550 Prokeš, L., Hložek, M., 2007. Identification of some adhesives and wood pyrolysis products
 551 of archaeological origin by direct inlet mass spectrometry. *Chemia. Analityczna* 52, 701-714.

552 Regert, M., Rolando, C., 2002. Identification of archaeological adhesives using direct inlet
 553 electron ionization mass spectrometry. *Analytical Chemistry* 74, 965-975.

554 Reunanen, M., Ekman, R., Heinonen, M., 1989. Analysis of Finnish pine tar and tar from the
 555 wreck of frigate St. Nikolai. *Holzforschung* 43, 33-39.

556 Reunanen, M., Ekman, R., Heinonen, M., 1990. Long term alteration of pine tar in a marine
 557 environment. *Holzforschung* 44, 277-278.

558 Reunanen, M., Ekman, R., Hafizoglu, H., 1996. Composition of tars from softwoods and
 559 birch. *Holzforschung* 50, 118-120.

560 Robinson, N., Evershed, R.P., Higgs, W.J., Jerman, K., Eglinton, G., 1987. Proof of a pine
 561 wood origin for pitch from Tudor (Mary Rose) and Etruscan shipwrecks: application of
 562 analytical organic chemistry in archaeology. *Analyst* 112, 637-644.

563 Romanus, K., Baeten, J., Poblome, J., Accardo, S., Degryse, P., Jacobs, P., De Vos, D.,
 564 Waelkens, M., 2009. Wine and olive oil permeation in pitched and non-pitched ceramics:

565 relation with results from archaeological amphorae from Sagalassos, Turkey. *Journal of*
566 *Archaeological Science* 36, 900-909.

567 Simoneit, B.R.T., 1977. Diterpenoid compounds and other lipids in deep-sea sediments and
568 their geochemical significance. *Geochimica et Cosmochimica Acta* 41, 463-476.

569 Simoneit, B.R.T., Grimalt, J.O., Wang, T.G., Cox, R.E., Hatcher, P.G., Nissenbaum, A.,
570 1986. Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers
571 and coals. *Organic Geochemistry* 10, 877-889.

572 Stacey, R., Cartwright, C., Tanimoto, S., Villing, A., 2010. Coatings and contents:
573 investigations of residues on four fragmentary sixth-century BC vessels from Naukratis
574 (Egypt). *The British Museum Technical Research Bulletin* 4, 1-26.

575 Tavendale, M.H., McFarlane, P.N., Mackie, K.L., Wilkins, A.L., Langdon, A.G., 1997. The
576 fate of resin acids-2. The fate of resin acids and resin acid derived neutral compounds in
577 anaerobic sediments. *Chemosphere* 35, 2153-2166.

578 Wakeham, S.G., Schaffner, C., Giger, W., 1980. Polycyclic aromatic hydrocarbons in recent
579 lake sediments—II. Compounds derived from biogenic precursors during early diagenesis.
580 *Geochimica et Cosmochimica Acta* 44, 415-429.

581 Yamamoto, S., Otto, A., Krumbiegel, G., Simoneit, B.R.T., 2006. The natural product
582 biomarkers in succinite, glessite and stantienite ambers from Bitterfeld, Germany. *Review of*
583 *Palaeobotany and Palynology* 140, 27-49.

584

585

Figure captions

Fig. 1. Structures of alkyl guaiacyl dehydroabietates (**8-10**) present in recent and archaeological conifer wood tar samples.

Fig. 2. Gas chromatograms of the F1d fractions isolated from (a) a historical wood tar (#2924) collected from a recovery pipe of a tar kiln; (b) a Gallo-Roman conifer wood tar sample (#2864) collected from Vielle-Saint-Girons; (c) a resinous material sampled from an anchor cable from the “Mary Rose” shipwreck (#2757). Carboxylic acids were analysed as methyl esters.

Fig. 3. (a) Gas chromatograms of the ester fractions (F2u.1) isolated from (a) a historical wood tar (#2924) collected from the recovery pipe of a tar kiln; (b) a Gallo-Roman conifer wood tar sample (#2864) collected from Vielle Saint-Girons; (c) a resinous material sampled from an anchor cable from the “Mary Rose” shipwreck (#2757). Filled triangles correspond to monoterpenyl esters of dehydroabietic acid. Empty triangles correspond to monoterpenyl esters of pimaric and abietic acids and of related isomers.

Fig. 4. Mass spectra (EI, 70eV) (a-c) of alkyl guaiacyl dehydroabietates (**8-10**) identified in conifer wood tar and (d) of 7-oxo methyl guaiacyl dehydroabietate **28** detected in a Gallo-Roman conifer wood tar sample (#2864) collected from Vielle Saint-Girons.

Fig. 5. Mode of formation of alkyl guaiacyl dehydroabietates (**8-10**) and of related 7-oxo-derivatives (**28-30**) by processes involving esterification of dehydroabietic acid **7** with alkyl guaiacols, typical lignin pyrolysis products formed by thermal degradation of wood during the preparation of conifer wood tar.

Table 1

Recent and archaeological conifer resin and wood tar samples investigated.

609 **Table 2**

610 Occurrence of anhydrosugars (e.g. **3**), alkyl guaiacols (**4-6**), and alkyl guaiacyl
611 dehydroabietates (**8-10**) in the conifer resin and wood tar samples investigated (✓, alkyl
612 guaiacyl dehydroabietate homologues **8, 9, 10** present in ca. equal amount.

613

Table 1

Origin of sample	Date	Site	Sample type	N°.
Mazotos shipwreck ^a	IV th century BC	Mazotos (Cyprus Island)	Coating of amphorae	2832, 2834, 2836
Guernsey shipwreck ^b	280	St Peter Port (Guernsey, Great-Britain)	Sample from the load	1668
“Mary Rose” shipwreck ^c	1545	Isle “of Wight, Great-Britain	Coating of ropes	2856, 2857, 2858
“La Dauphine” shipwreck ^d	1704	Saint Malo, France	Coating of ropes	2749, 2750, 2751
			Samples from coating of hull	2754, 2755
“L’Aimable Grenot” shipwreck ^d	1748	Saint Malo, France	Samples from coating of hull	2768, 2767
“Jeanne Elisabeth” shipwreck ^e	1755	Villeneuve-lès-Maguelone, France	Coating of ropes	2838, 2839
			Sample from a cauldron	2826
Origin of sample	Age	Sample type		N°.
Vielle-Saint- Giron, France ^f	III-V th century AD	Samples from a Gallo-Roman tar preparation site		2843, 2864, 2886, 2887
Sainte-Anne du Castellet, France ^g	1820	Sample from a tar kiln (<i>Pinus halepensis</i>)		2924
Teste de Buch, Gironde, France	XVI th century AD	Resin		2841
Vielle-Saint- Giron, France	IV-V th century AD	Resin		2842
Fresh wood tar	Modern	Stockholm tar (<i>Pinus sylvestris</i>)		2745
Fresh resin	Modern	Resin from <i>Pinus pinaster</i> (Landes, France)		2769

^a Demesticha (2011) ; ^b Connan et al. (2002), Monaghan (2010); ^c Evershed et al. (1985), Robinson et al. (1987) ; ^d l’Hour et al. (2010); ^e Jaouen and Poletto (2012); ^f Jacques (2011); ^g Gensel (2005) unpublished results, Connan and Tengberg (2006) unpublished results, Bailly (2015).

Table 2

Type	Origin	Sample	Anhydro sugars	Methoxy phenols	Alkyl guaiacyl dehydroabietates 8-10
In containers on shipwrecks	St Peter Port-Guernsey	1668	-	-	✓
	Jeanne-Elisabeth	2826	Trace	✓	✓
Coating of amphorae	Mazotos shipwreck	2832	Trace	✓	✓
		2834	Trace	-	✓
		2836	✓	✓	✓
Coating of boat hull	La Dauphine	2754	✓	-	✓
		2755	✓	-	✓
	L'Aimable Grenot	2767	✓	-	✓
		2768	✓	-	✓
Coating of ropes	Mary Rose	2756	-	-	✓
		2757	-	-	✓
		2758	-	✓	✓
	La Dauphine	2749	-	✓	8, 9
		2750	-	-	8, 9
		2751	-	✓	8, 9
		2752	-	-	✓
	Jeanne-Elisabeth	2838	-	-	8
		2839	-	-	8
Wood tar from archaeological tar preparation site	Vielle-Saint-Girons, France (III-V th century BC)	2843	Trace	✓	✓
		2864	-	-	✓
		2886	-	-	✓
		2887	Trace	-	✓
Wood tar from historical tar preparation site	Sainte-Anne du Castellet, France (<i>Pinus halepensis</i>)	2924	✓	✓	✓
Fresh wood tar	Stockholm tar (<i>Pinus sylvestris</i>)	2745	✓	✓	8, 9
Fresh resin	Landes, France (<i>Pinus pinaster</i>)	2769	-	-	-
Ancient resins	Teste de Buch, Gironde,	2841	-	-	-
	Vielle-Saint-Girons	2842	-	-	-

Appendix

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

R: monoterpenyl

23

24

(+) OH

25

HO (-)

26

27

28

29

30

31

32

33

8: R= CH₃

9: R= C₂H₅

10: R = C₃H₇

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5