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Unusual Triterpenoid Fatty Acyl Ester Components of Archaeological Birch Bark Tars

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Abstract: At least three series of previously unreported components of ancient birch bark tars have been identified by electron and chemical ionisation mass spectrometry as comprising betulin (lup-20(29)-en-3β,28-diol), lupa-2,20(29)-dien-28-ol and lupeol esterified to fatty acids. Since such components are not present in fresh bark or pyrolysates thereof it appears that they result from the intentional mixing of birch bark tar and animal fat in antiquity. © 1998 Elsevier Science Ltd. All rights reserved.

An increasing number of reports exist of the use in antiquity of anthropogenically altered natural materials, manufactured by the pyrolysis of plant materials.[1] These include tars and pitches produced from various coniferous tree genera, including pines and spruces,[2,3] while for angiosperms, the main product reported derives from birch bark.[4,5,6] Birch bark tar contains a high abundance of betulin, with lupenone and lupeol as minor constituents.[5,7] Such tars (or pitches) appear to have been used for a wide range of purposes, including hafting,[8] waterproofing[3] and repairing.[5] Very little is known about their exact method of manufacture, although chemical analysis and laboratory experiments indicate their production most likely involved the destructive distillation of wood or bark at temperatures in excess of 300°C. This serves to separate the volatile and pyrolysable components, leaving a brown tar residue, e.g. birch bark tar.[9] Recent work has examined the compositional changes tars undergo upon heating at various temperatures and characterised the chemical derivatives formed as a result of defunctionalisation and structural rearrangement.[10a]

Examination of samples of hard, black resinous materials recovered from excavations of Roman levels at Catterick (N. Yorkshire, UK; contents of a small, enamelled vessel) and West Cotton (Northants, UK; adhesive used to repair an ancient ceramic jar) by solvent extraction and high temperature gas chromatography (HT-GC)[11a] revealed a complex range of high and low molecular weight components. Preliminary HT-GC/MS[11b] identified free fatty acids, triterpenoids and two series of high molecular weight components eluting between 300 and 350 °C (Fig. 1), although electron ionisation (EI) mass spectra provided only partial structures for these latter components. Since the components of each series occurred with the same relative abundance as the $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ free fatty acids present in the samples it appeared that they may comprise triterpenes ester-linked to fatty acids. While the EI mass spectra contained fragment ions consistent with the loss of fatty acid moieties, it was not possible to identify either the triterpene or the position of esterification.

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The HT-GC retention times suggested that only one fatty acid was attached to each triterpene. In order to study the high molecular weight esters more closely the total lipid extract was fractionated by 'flash' adsorption column chromatography into three major fractions, namely acids (A), esters (B) and esters and alcohols (C).

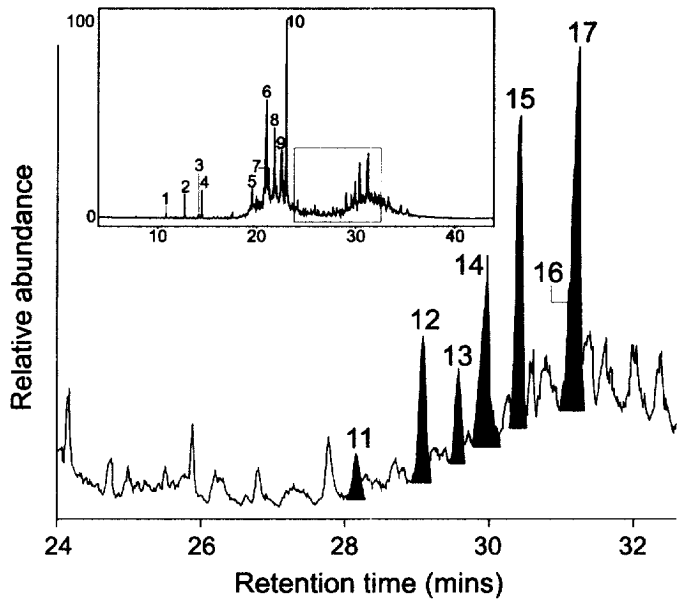

Figure 1. Partial HT-GC profile of the trimethylsilylated[21] total lipid extract of a Romano-British birch bark tar with the expanded section showing the distribution of high molecular weight esters. Key: **1 - 4** $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ fatty acids; **5** $C_{30}H_{48}$ triterpene of unknown structure possibly lupene, derived from dehydration of lupeol;[14] **6** lupa-2,20(29)-dien-28-ol;[15] **7** lupenone; **8** lupeol; **9** betulone; **10** betulin; **11, 12, 14** and **13, 15, 16** and **17** refer to two series of high molecular weight esters, identified by MS as triterpenols ester-linked to the fatty acids seen at short retention time.

Treatment of fractions B and C with methanolic sodium hydroxide followed by GC/MS analysis of trimethylsilyl ethers of the resulting free compounds identified lupa-2,20(29)-dien-28-ol as the major triterpene in fraction B and betulin (lup-20(29)-en-3β,28-diol) as the major component in fraction C. $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ fatty acids were also present in the alkali-treated extracts in the same distributions as seen in the total lipid extract, thus confirming the identity of the unknown compounds as triterpenol fatty acyl esters. The molecular weights were confirmed by GC/MS as *m/z* 634, 662, 688 and 690 for the lupa-2,20(29)-dien-28-ol esters and *m/z* 724, 752, 778 and 780 for the betulin fatty acyl esters (TMS ether derivatives).[11c] Other triterpenol fatty acyl esters, including those of lupeol (lup-20(29)-en-3β-ol), were also identified in fraction C albeit in lesser abundance. The identification of esters comprising both lupeol and lupa-2,20(29)-dien-28-ol confirms that esterification can occur at both the 3- and 28-positions. This also indicates that betulin could exist as bis-esters which would appear in fraction B together with the lupeol and lupa-2,20(29)-dien-28-ol, thus explaining the liberation of betulin from fraction B on alkali treatment. The betulin bis-esters are not seen in the HT-GC

chromatograms on account of their being retained beyond the upper temperature limit of the GC column. The structures for the lupa-2,20(29)-dien-28-ol and betulin-$C_{18:0}$ *n*-alkanoic acid esters are shown in Figure 2 together with the fragmentations giving rise to the most prominent ions. Further confirmation of the identities of the triterpenol fatty acyl esters was achieved by chemical ionisation/MS (CI/MS) which served to enhance the abundance of the higher mass pseudo-molecular and fragment ions.[13]

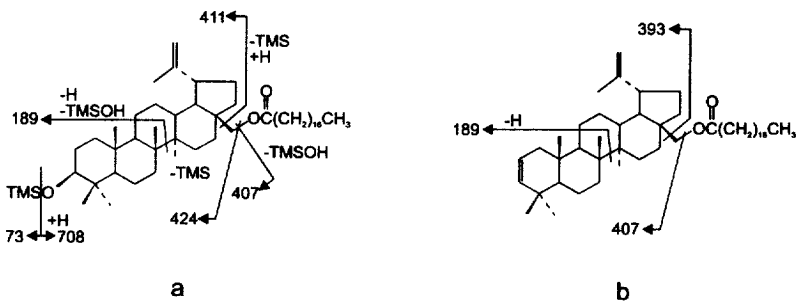

Figure 2. Structures and EI/MS fragmentations for (a) betulin stearate and (b) lupa-2,20(29)-dien-28-yl stearate.

Although betulin is readily recognised as the major component of birch bark tar,[6,7] lupa-2,20(29)-dien-28-ol has not been so widely reported.[10a,14] Formation of the latter from betulin occurs by removal of the hydroxyl functionality at C-3 via a 1,2-elimination[18] as demonstrated during the synthetic production of birch bark tar in a glass tube at *ca.* 600°C.[10a] The presence of an appreciable abundance of triterpenoid fatty acyl esters in these Romano-British birch bark tars is noteworthy since they have not been reported in tars from earlier periods[10,14b] perhaps suggesting a later technological development. The distribution of free fatty acids in the extract resembled that commonly recognised in animal fats with palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids being present in approximately equal abundance, together with smaller amounts of $C_{14:0}$ and $C_{18:1}$ acids. The fatty acids in fraction A were prepared as their methyl esters and analysed by GC-combustion-isotope ratio MS (GC-C-IRMS)[16] to obtain $\delta^{13}C$ values for the individual $C_{16:0}$ and $C_{18:0}$ *n*-alkanoic acids. Comparison with values previously obtained for reference fats from animals raised on diets isotopically representative of the archaeological period[16] showed the most likely sources of the fats from the Catterick and West Cotton vessels to be ruminant ($C_{16:0}$, -27.9; $C_{18:0}$, -32.3) and non-ruminant ($C_{16:0}$, -25.8; $C_{18:0}$, -26.7) animals, respectively. Notwithstanding the different origins of the fats in the two samples, their presence in similarly high abundance indicates their addition to the birch bark tar was intentional, presumably to provide a material with more desirable properties. Formation of the triterpene fatty acyl esters would occur *via* an acid catalysed condensation during the heating of animal fat and birch bark (or the tar derived therefrom) at high temperatures to produce the modified resin/adhesive. The presence of small amounts of water would have been sufficient to catalyse the reaction. The lupa-2,20(29)-dien-28-ol fatty acyl esters would more readily form *via* pyrolytic 1,2-deacylation of the betulin bis-esters than through acylation of the preformed lupa-2,20(29)-dien-28-ol.[17]

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11. (a) GC analyses were performed on an HP 5890 gas chromatograph, coupled to an Opus V PC using HP Chemstation software. Samples were introduced by on-column injection into a 15 m x 0.32 mm i.d. fused silica capillary (DB1 stationary phase; immobilised dimethyl polysiloxane, 0.1 μm film thickness; J & W Scientific). The temperature programme was: 50 °C (2 min) - 350 °C at 10 °C min^{-1} (10 min). H_2 was used as carrier gas. FID was used to monitor the column effluent. (b) GC-EI/MS analyses were performed using a Finnigan 4500 quadrupole mass spectrometer directly coupled to a Carlo Erba 5160 Mega series gas chromatograph with on-column injection. Operating conditions were: ion source, 170°C; emission current, 400 μA and electron energy, 70 eV, GC-MS interface temperature, 350°C. Spectra were recorded over the range *m/z* 50-850 every 1.5 s. The GC operating conditions were the same as those described above except that helium was used as carrier gas. (c) Samples were derivatised using *N,O*-bis(trimethylsilyl)trifluoroacetamide, 1h, 70°C.
12. **A** 708(0.4), 690(3), 496(3), 442(0.5), 424(18),407(23), 393(9), 367(6), 271(8), 229(16), 217(29), 203(64), 189(100), 177(42), 135(54), 121(51), 95(72), 81(62), 69(69), 55(46); **B** M^+ 690(4), 424(1), 406(27), 393(21), 363(4), 324(2), 270(4), 257(5), 229(13), 216(21), 204(22), 203(46), 189(100), 187(45), 161(20), 159(20), 147(27), 135(36), 121(45), 107(49), 95(62), 81(48), 69(49), 57(54), 55(46).
13. GC-CI/MS conditions were as follows: NH_4 CI (BOC, micrographic grade); source pressure, 0.4 mTorr; source temperature, 150 °C; electron energy, 40 eV; emmission current, 350 μA; *m/z* range 100-1000.
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