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Chemical Evidence for Archaeological Frankincense: Boswellic Acids and their Derivatives in Solvent Soluble and Insoluble Fractions of Resin-Like Materials

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Abstract: α-Boswellic acid (3α-hydroxyolean-12-en-24-oic acid) and β-boswellic acid (3α-hydroxyurs-12-en-24-oic acid) and their acetates were identified in the solvent soluble fraction of samples of amorphous resin-like materials recovered from excavations at Qasr Ibrîm (Egyptian Nubia). The complementary 24-noroleana-3,12-diene and 24-norursa-3,12-diene were identified in pyrolysates of the residues where they were produced from the α- and β-boswellic acid and their acetates.
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The occasional finds of resinous materials, or their derivatives, at archaeological sites can provide valuable insights into the range of natural products exploited by ancient man for use as adhesives, sealants and in ritual activities.[1] The relatively durable nature of such materials means that they often survive over many millennia in a comparatively unaltered chemical state, making them amongst the most studied of organic remains of archaeological interest.[1,2] The characteristic biochemical constituents, particularly di- and triterpenoid components, allow their botanical origin to be defined with a high degree of certainty, often to genus level. Examples of such materials that have been identified chemically from archaeological excavations include pine resin and its derivatives, *Pistacia* resin, amber, copals, and by-products of heating of birch bark.[2]

Frankincense, also known as olibanum, is an aromatic gum-resin obtained from trees of the genus *Boswellia* (family Burseraceae), and is the best known of the ancient plant resins. It is a chemically complex material[3] characterised by a series of unusual triterpenoid acids, which include acetate derivatives of α-boswellic acid (3α-acetoxyolean-12-en-24-oic acid; **4**) and β-boswellic acid (3α-acetoxyurs-12-en-24-oic acid; **5**).[4] Although the importance of frankincense in antiquity is firmly established from documentary records,[5] as far as we are aware, no reports exist of the unequivocal chemical characterisation of frankincense from an archaeological site.[1,4a] Problems in recognising resinous materials, stem from their amorphous appearance, although failure to characterise ancient frankincense is surprising. An opportunity to undertake a search for ancient frankincense arose when a number of small pieces of resin-like material were recovered during dry sieving of the fill of the cellar of a house at Qasr Ibrîm (c. 400-700 AD).[6]

The compositions of selected pieces of the ancient material were investigated by conventional GC/MS[2] (solvent soluble components) and pyrolysis-GC/MS[7] (insoluble residue). Figure 1b shows the partial GC profile obtained from the solvent soluble fraction. GC/MS confirmed the identities of the major components to be β-boswellic acid (**3**) and the corresponding acetate (**5**),[9] the latter being the most dominant compound, together with, α-boswellic acid (**2**) and the corresponding acetate (**4**).[9] The distribution of compounds was immediately recognisable as deriving from frankincense, as evidenced by the GC profile of a sample of modern reference resin shown in Figure 1a.

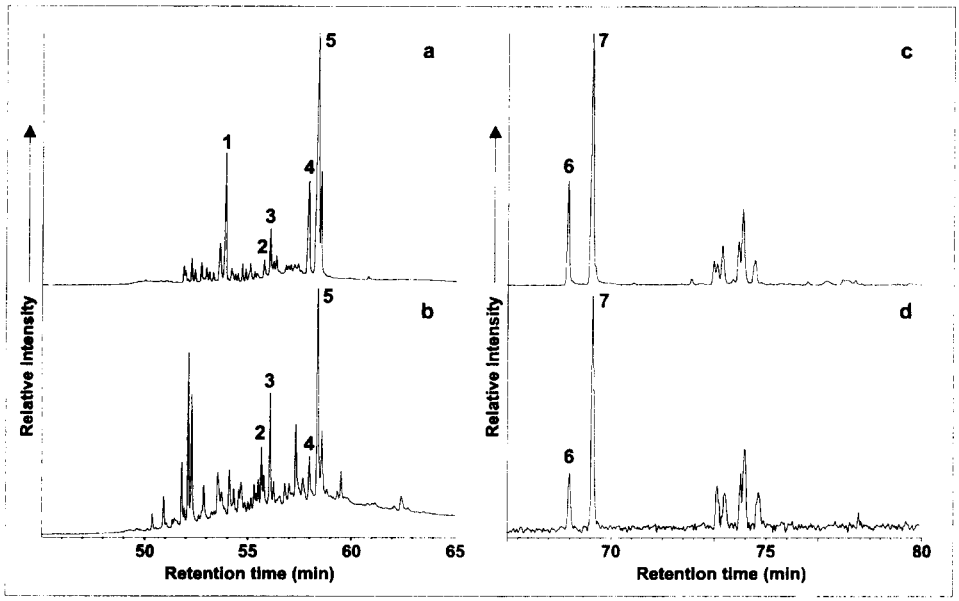
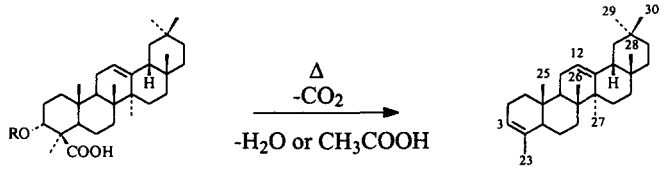


Figure 1. Partial GC profiles of the derivatised[8] total lipid extracts of (a) modern frankincense and (b) an archaeological resin sample from Qasr Ibrîm,[6] and partial mass chromatograms (*m/z* 218) of the pyrolysates of extracted residues of (c) frankincense and (d) the archaeological resin. Key:[9,10] **1** = β-amyrin, **2** = α-boswellic acid, **3** = β-boswellic acid, **4** = *O*-acetyl-α-boswellic acid, **5** = *O*-acetyl-β-boswellic acid, **6** = 24-noroleana-3,12-diene, **7** = 24-norursa-3,12-diene. The compounds eluting between 73 and 75 min (c, d) are triterpenoids including β-amyrin.

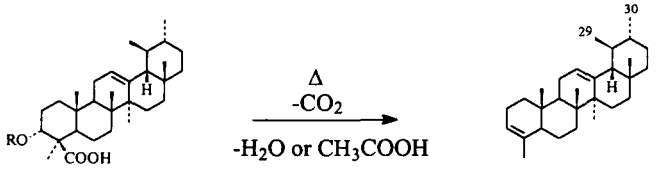
Further investigations of the archaeological resins focused on the solvent insoluble residue which was investigated using Curie-point (610°C) pyrolysis-GC/MS.[7] The pyrolysate of the insoluble residue of the ancient resin (Fig. 1d) is dominated by two products 24-noroleana-3,12-diene (**6**) and 24-norursa-3,12-diene (**7**). Identification of these two components was based on comparisons of mass spectra and GC retention times (co-injections) with those of synthetic compounds.[10] These products were also the dominant compounds released upon pyrolysis of the reference frankincense (Fig. 1c). The specific oleanane and ursane skeletons of these pyrolysis products indicate a precursor-product relationship (Scheme 1) with the characteristic frankincense

triterpenoid acids β-boswellic acid (**3**), α-boswellic acid (**2**) and their acetates (**4**, **5**). Formation of the dienes occurs via concomitant loss of carbon dioxide and either water or acetic acid from the triterpenoid acids which is favoured by the anti-periplanar conformation of the carboxyl and hydroxyl or acetate groups leading to formation of the Δ^3 double bond (Scheme 2). Heating of boswellic acids and their acetates under vacuum has been found previously to yield C_{29} diunsaturated hydrocarbons. However, until now these have only been partially characterised.[4a,b]



R=H; α-boswellic acid (**2**)
R=CH_3CO; *O*-acetyl-α-boswellic acid (**4**)

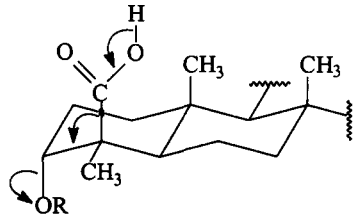
24-noroleana-3,12-diene (**6**)



R=H; β-boswellic acid (**3**)
R=CH_3CO; *O*-acetyl-β-boswellic acid (**5**)

24-norursa-3,12-diene (**7**)

Scheme 1



Scheme 2

These findings provide the first chemical characterisation of frankincense from an archaeological site. Chemical investigations of several other resin-like fragments recovered from the same deposit showed them to

comprise diterpenoid compounds characteristic of pine resin,[1,2] which provided circumstantial evidence that frankincense was possibly mixed with other ingredients by ancient incense-makers.[5a]

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8. Samples were derivatised using BSTFA with 1% (v/v) trimethylchlorosilane, 1h, 70°C.
9. **1** $M^{+\cdot}$ 498(2), 408(1), 393(3), 210(100), 203(35), 189(20); **2** $M^{+\cdot}$ 600(2), 585(3), 382(3), 292(50), 218(100), 203(30), 189(10); **3** $M^{+\cdot}$ 600(2), 585(3), 382(5), 292(85), 218(100), 203(10), 189(8); **4** $M^{+\cdot}$ 570(2), 352(4), 292(30), 218(100), 203(35), 189(7); **5** $M^{+\cdot}$ 570(2), 352(5), 292(40), 218(100), 203(12), 189(10).
10. The standards were prepared from 24-norolean-12-en-3α-ol and 24-norurs-12-en-3α-ol (Peakman, T. M.; ten Haven, H. L.; Rullkötter, J.; Curiale, J. A. *Tetrahedron* **1991**, *47*, 3779-3786 and 8941). The preparation of 24-noroleana-3,12-diene (**6**) is typical: 24-norolean-12-en-3α-ol (13 mg) and tosyl chloride (26 mg) were dissolved in dry pyridine (2 mL) and the reaction mixture left in the dark (5 days). After work-up the crude 24-norolean-12-en-3α-yl tosylate was taken up in dry THF and stirred (1 day) with potassium t-butoxide (190 mg) to afford a mixture (5 mg) of 24-noroleana-2,12-diene and 24-noroleana-3,12-diene (*ca.* 5:1), which were separated by reverse-phase HPLC. ^{1}H NMR 400 MHz ($CDCl_3$): 24-noroleana-3,12-diene (**6**) 0.848 (3H, s, $28H_3$), 0.854 (3H, s, $25H_3$), 0.871 (6H, s, $29H_3$ and $30H_3$), 1.056 (3H, s, $26H_3$), 1.154 (3H, s, $27H_3$), 1.640 (3H, d, J=1.2 Hz, $23H_3$), 5.225 (1H, t, J=3.4 Hz, 12H), 5.28 (1H, m, 3H); 24-norursa-3,12-diene (**7**) 0.805 (3H, d, J=6.6 Hz, $30H_3$), 0.817 (3H, s, $28H_3$), 0.868 (3H, s, $25H_3$), 0.914 (3H, s, $29H_3$), 1.085 (3H, s, $27H_3$), 1.099 (3H, s, $26H_3$), 1.640 (3H, broad s, $23H_3$), 5.165 (1H, dd, J=4.5, 1.5 Hz, 12H), 5.30 (1H, m, 3H). MS: 24-noroleana-3,12-diene (**6**) $M^{+\cdot}$ 394(22), 379(11), 218(100), 203(42), 189(11); 24-norursa-3,12-diene (**7**) $M^{+\cdot}$ 394(30), 379(11), 218(100), 203(19), 189(14).

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