3-Oxo-6 β-hydroxyolean-12-en-28-oic Acid, a New Triterpenoid from Commercial Tolu Balsam

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Examination of commercial tolu balsam revealed the presence of several triterpene acids, of which there are three main constituents. One of these has previously not been encountered in Nature and was shown to be 3-oxo-6β-hydroxyolean-12-en-28-oic acid (*1*), while the other two were identified as sumaresinolic acid (*2*) and oleanolic acid (*3*). The influence of oxygenation at C(6) on the mass spectrometric fragmentation reactions and on the chemical shifts of the methyl resonances in the NMR spectra of these compounds is discussed.

T he bled resin from *Myroxylon balsamum* (L.) Harms (Leguminosae), a tree endemic to the northern part of South America, is commercially available under the name of tolu balsam. It is commonly used as an additive to tobacco and as an ingredient in confectionery products and cosmetics. On examination of the commercial material,[1] a fraction comprising triterpene acids was isolated and shown to contain three main representatives.[2] The present paper deals with the identification of two of these and the structure determination of the third, which has evidently not been encountered in Nature before.

IDENTIFICATION AND STRUCTURE DETERMINATION

The triterpene acids were isolated from a fraction containing weak acids, derived from the hexane-soluble part of an ethanolic solution of tolu balsam by chromatography on silica gel. In order to obtain them in a pure state they were converted, using diazomethane, to the corresponding methyl esters and subsequently subjected to chromatography and recrystallization.

The methyl ester (*1a*) of the most abundant triterpene acid gave an infrared spectrum consistent with the presence of a hydroxyl group (3560, 3540 cm⁻¹), a carbomethoxy group (1730 cm⁻¹), an oxo group (1690 cm⁻¹), and a trisubstituted double bond (815 cm⁻¹). The NMR spectrum confirmed the presence of the carbomethoxy group (δ 3.64, 3H, s) and the trisubstituted double
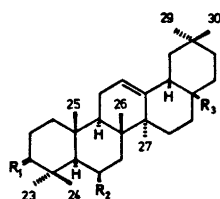
1	$R_1 = O$	$R_2 = OH$	$R_3 = COOH$
1a	$R_1 = O$	$R_2 = OH$	$R_3 = COOMe$
2	$R_1 = \beta\text{-}OH, H$	$R_2 = OH$	$R_3 = COOH$
2a	$R_1 = \beta\text{-}OH, H$	$R_2 = OH$	$R_3 = COOMe$
3	$R_1 = \beta\text{-}OH, H$	$R_2 = H$	$R_3 = COOH$
3a	$R_1 = \beta\text{-}OH, H$	$R_2 = H$	$R_3 = COOMe$
8	$R_1 = \beta\text{-}OH, H$	$R_2 = OAc$	$R_3 = COOMe$
9	$R_1 = \beta\text{-}OAc, H$	$R_2 = OAc$	$R_3 = COOMe$
10	$R_1 = \beta\text{-}OAc, H$	$R_2 = H$	$R_3 = COOMe$

Fig. 1.

bond (δ 5.36, 1H, m), and exhibits resonances due to seven tertiary methyl groups (δ 0.91 (3H), 0.94 (3H), 1.12 (6H), 1.17 (3H), 1.42 (3H), 1.50 (3H)). A broad signal at δ 4.49 could be ascribed to the proton on the same carbon as the hydroxyl group. The mass spectrum shows the following important peaks: m/e 484 (M), 466 (M $-$ H$_2$O), 451 (M $-$ H$_2$O $-$ CH$_3$), 425 (M $-$ COOCH$_3$), 424 (M $-$ HCOOCH$_3$), 407 (M $-$ H$_2$O $-$ COOCH$_3$), 302, 262 (fragment A), 249 (B), 247 (A $-$ CH$_3$), 203 (A $-$ COOCH$_3$), 189, 187 and 133 (A $-$ COOCH$_3$ $-$ C$_5$H$_{10}$).

These data, and in particular the mass spectrometric fragmentation pattern which is very similar to that of methyl sumaresinolate (*2a*), strongly indicates that the methyl ester (*1a*) is an olean-12-ene derivative, incorporating a hydroxyl and an oxo group in rings A/B. Moreover, the carbomethoxy group must be located in rings D/E, and the highly favoured loss of 59 mass units from the m/e 262 fragment implies that it is attached to C(17) rather than to C(20)[3,4] (*cf.* Scheme 1).
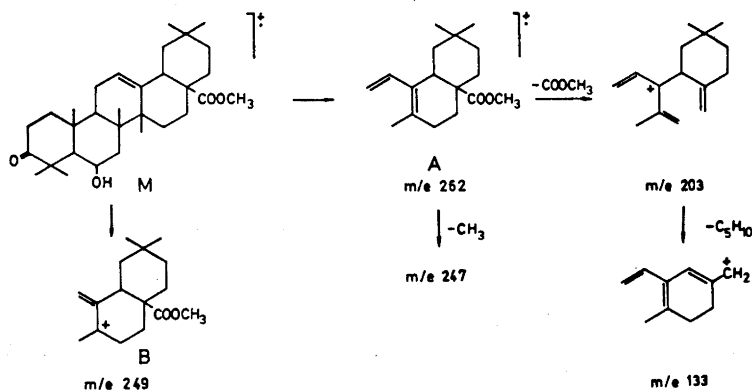
Scheme 1.

In view of these spectral results and the occurrence of sumaresinolic acid (*2*) in this material (*vide infra*), it seemed probable that the unknown acid (*1*) is structurally related to the former compound. Evidence for this was

obtained by converting the methyl ester (1a) and methyl sumaresinolate
(2a) to the corresponding triols (4), which proved to be identical. It followed
therefore that the methyl ester (1a) is either methyl 3β-hydroxy-6-oxo-olean-
12-en-28-oate (7) or the corresponding 3-oxo-6β-hydroxy derivative. Attempted
acetylation of the methyl ester (1a) with acetic anhydride in pyridine at 100°
for 2 h was unsuccessful, demonstrating that the hydroxyl group is sterically
hindered, and hence favouring the latter alternative. Conclusive evidence
was achieved by preparing methyl 3β-hydroxy-6-oxo-olean-12-en-28-oate (7).
This was carried out by acetylation of methyl sumaresinolate (2a) yielding
the 3β-acetate (5), which was oxidized with chromium trioxide in acetone,
and subsequently subjected to saponification. The 3β-hydroxy-6-oxo deriva-
tive (7) proved to be different from the unknown methyl ester (1a), and this
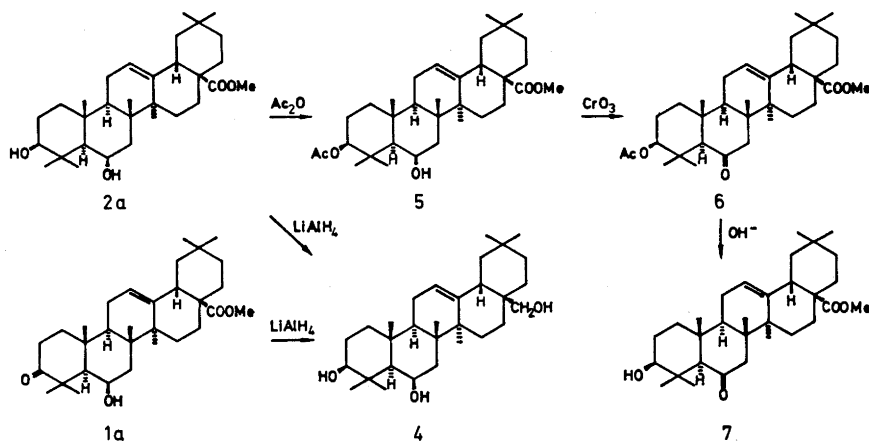established that the new acid is 3-oxo-6β-hydroxyolean-12-en-28-oic acid (1).

Fig. 2.

The second most abundant methyl ester was shown to be methyl suma-
resinolate (2a). The identification of this compound was accomplished by
converting it to methyl 3β-acetoxy-6-oxo-olean-12-en-28-oate (6), of which
reference material was available. The identity was further supported by
comparison of physical constants of several derivatives with corresponding
data from the literature.[5]

The least abundant methyl ester of the three main triterpene acids was
identified as methyl oleanolate (3a) by mixed melting point with authentic
material and by comparison of spectroscopic data.

Sumaresinolic acid (2) is a rare triterpenoid and has previously, to our
knowledge, only been isolated from Sumatra benzoin gum.[6] Its structure
was determined by Ružicka et al.,[5,7] and the stereochemistry at C(3), C(5),
and C(6) was clarified by Djerassi et al.[8]

A CHARACTERISTIC FRAGMENTATION REACTION OF SUMARESINOLIC ACID DERIVATIVES

The mass spectra of all available sumaresinolic acid derivatives show a small but significant peak, the position of which is dependent on the substitution at C(17) ($-COOH$, $-COOCH_3$, $-CH_2OH$), but unaffected by variation of the substituents at C(3) and C(6) ($=O$, $-OH$, $-OAc$). Since it is absent in the spectra of α- and β-amyrin derivatives, lacking oxygenation at C(6), it is reasonable to assume that the formation of the corresponding ion is triggered by the oxygen function at C(6) and involves cleavage of ring B with charge retention by the larger fragment containing rings C, D, and E. Several mechanisms, which are based on this assumption, can be formulated for each of these C(6) substituents. It is, however, impossible to elucidate the details by labelling studies in this particular case, as the part of the molecule where the initial reaction steps occur is lost as a neutral fragment.

The 6-oxo derivatives may give rise to the "CDE" ion by the reaction shown in Scheme 2, which involves a McLafferty rearrangement with transfer of a hydrogen to the oxo function, followed by a retro-Diels-Alder cleavage of ring B. A similar reaction sequence has, on the basis of labelling studies, been postulated for the formation of the m/e 70 ion from menthone.[9] 6-Oxo steroids do not give rise to an ion of this type, thus supporting the formulated mechanism.[10]
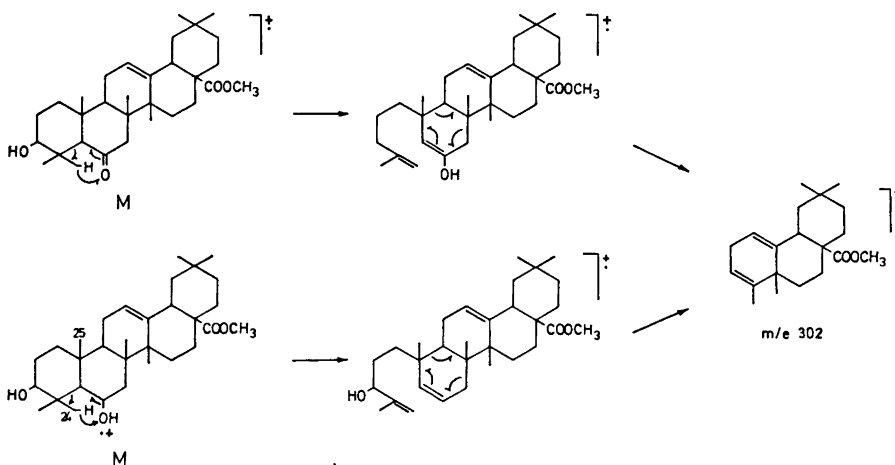
Scheme 2.

The formation of the "CDE" ion from the 6-hydroxy and 6-acetoxy derivatives is most probably associated with the elimination of water and acetic acid. When the hydrogen involved is derived from the 24 or 25 positions, the dehydration or the deacetylation may be followed by cleavages of the 7,8 and 9,10 bonds, leading to the "CDE" ion as visualised in Scheme 2.

INFLUENCE OF 6β-HYDROXYL AND 6β-ACETOXYL SUBSTITUENTS ON THE SHIFTS OF THE METHYL SIGNALS IN THE NMR SPECTRA

The effect of different substituents on the chemical shifts of the methyl groups in olean-12-ene derivatives has previously been studied by several workers.[11-14] As these investigations do not deal with 6β-hydroxyl and 6β-acetoxyl substituents, it was of interest to consider their effects (cf. Ref. 12) The NMR spectra of compounds (2a), (5), (8), and (9), together with data for methyl oleanolate (3a) and methyl oleanolate acetate (10), for which the chemical shifts of each methyl group are known, were studied for this purpose.

Based on the assumption that the effect of each substituent is additive, and that hydroxyl and acetoxyl groups do not cause shifts of remote methyl groups (C(27), C(29), and C(30)), the assignments shown in Tables 1 and 2 are proposed.

Table 1. Methyl frequencies (δ) in methyl sumaresinolate derivatives.[a]

Com-pound	Substituent	Methyl group						
		23	24	25	26	27	29	30
2a	3β-OH; 6β-OH	1.07	1.17	1.29	1.03	1.11	0.91	0.93
5	3β-OAc; 6β-OH	0.95	1.25	1.33	1.03	1.11	0.92	0.95
8	3β-OH; 6β-OAc	1.05	0.96	1.28	0.98	1.12	0.93	0.93
9	3β-OAc; 6β-OAc	0.93	1.02	1.30	0.93	1.11	0.93	0.93

[a] 60 MHz spectra, CDCl$_3$ solutions.

Table 2. Effect of 6β-hydroxyl and 6β-acetoxyl substituents on the chemical shifts of methyl groups.[a,b]

Substituent	Methyl group					
	23	24	25	26	27	29 − 30
6β-OH	0.08	0.38	0.39	0.29	− 0.04	0
6β-OAc	0.06	0.16	0.37	0.22	− 0.03	0

[a] 60 MHz spectra, CDCl$_3$ solutions.
[b] The down-field shifts chosen as positive.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 instrument, and NMR spectra on a Varian A-60A spectrometer at 60 MHz. Rotations were measured on a Perkin-Elmer 141 Polarimeter. Low resolution mass spectra were obtained on an LKB 9000 instrument at 70 eV with an ion-source temperature of 290°. Mass numbers are only given for mass spectral peaks which are regarded to be important. The high-resolution measurements were performed on an Atlas SM 1 instrument through the courtesy of Dr. Ryhage,

Masspektrometrilaboratoriet, Karolinska Institutet, Stockholm. Liquid chromatography was performed on silica gel Merck 0.05 – 0.20 mm, activity I, and thin-layer chromatography on silica gel Merck GF. Microanalyses by Dr. Kirsten, Centrala Analyslaboratoriet, University of Uppsala.

Isolation. The hexane-soluble part (32 %) of commercial tolu balsam, dissolved in ethanol (81.9 g per 100 ml solution),[1] was first treated with saturated aqueous sodium hydrogen carbonate to remove the strong acids (12 %), and then with aqueous sodium hydroxide (10 %) to give a fraction containing weak acids (2 %). This fraction was chromatographed on silica gel, and three crude acids (*1, 2,* and *3*) were eluted with a mixture of hexane and acetone (1:0 – 0:1). These were treated with diazomethane in ether and subsequently chromatographed on silica gel (hexane/acetone) to give the corresponding methyl esters *1a* (0.4 %), *2a* (0.3 %), and *3a* (0.2 %).

The least polar of these, on recrystallization from acetonitrile, gave methyl oleanolate (*3a*), m.p. 199 – 201°, mixed m.p. 198 – 201°; $[\alpha]_D(CHCl_3)$ +72° (*c* 0.98); infrared, NMR, and mass spectra identical with those of authentic material.

The second most polar ester, methyl 3-oxo-6β-hydroxyolean-12-en-28-oate, (*1a*), on recrystallization from a mixture of hexane and isopropylether, had m.p. 190 – 191°; $[\alpha]_D(CHCl_3)$ +33° (*c* 0.98); (Found: C 76.95; H 10.00. $C_{31}H_{48}O_4$ requires C 76.82; H 9.98). M = 484.3517; $C_{31}H_{48}O_4$ requires 484.3552; ν_{max} (KBr): 3560, 3540, 2950, 1730, 1690, 1462, 1430, 1265, 1230, 1195, 1162, 1118, 815 cm⁻¹; δ (CDCl₃): 0.91 (3H,s), 0.94 (3H,s), 1.12 (6H,s), 1.17 (3H,s), 1.42 (3H,s), 1.50 (3H,s), 2.92 (1H,q), 3.64 (3H,s), 4.49 (1H, broad), 5.36 (1H,m); *m/e* (%): 484 (10), 466 (6), 451 (1), 425 (5), 424 (6), 407 (4), 302 (2), 262 (53), 249 (10), 247 (5), 203 (100), 189 (25), 187 (10), 133 (14).

The most polar methyl ester, on recrystallization from acetonitrile, gave methyl sumaresinolate (*2a*), m.p. 222 – 223.5° (reported:[5] 220 – 221°); $[\alpha]_D(CHCl_3)$ +53° (*c* 1.03) (reported:[5] +46.7°); ν_{max} (KBr): 3550, 3470, 2955, 1710, 1462, 1430, 1255, 1165, 1065, 1032, 1020, 818, 654 cm⁻¹; δ (CDCl₃): 0.91 (3H,s), 0.93 (3H,s), 1.03 (3H,s), 1.07 (3H,s), 1.11 (3H,s), 1.17 (3H,s), 1.29 (3H,s), 2.90 (1H,q), 3.14 (1H,t), 3.62 (3H,s), 4.54 (1H,broad), 5.30 (1H,m); *m/e* (%): 486 (5), 468 (4), 453 (2), 450 (2), 435 (2), 427 (4), 426 (5), 302 (3), 262 (52), 249 (4), 247 (5), 203 (100), 189 (29), 187 (13), 133 (19).

Olean-12-en-3β,6β,28-triol (4). Methyl sumaresinolate (*2a*, 15 mg) in ether (4 ml) and lithium tetrahydroaluminate (30 mg) were refluxed for 3 h. Excess reagent was destroyed with ethyl acetate, and the reaction mixture was diluted with aqueous sulphuric acid (20 %) and extracted with ether. Chromatography on silica gel yielded olean-12-en-3β,6β,28-triol (*4*, 10 mg), which after repeated recrystallization from nitromethane had m.p. 274.5 – 277° (reported:[8] 266 – 271°); ν_{max} (KBr): 3450, 2950, 2925, 2865, 1460, 1385, 1368, 1125, 1090, 1045, 1005, 818 cm⁻¹; *m/e* (%): 458 (4), 440 (2), 428 (2), 427 (3), 409 (2), 391 (1), 274 (2), 243 (2), 234 (20), 216 (10), 203 (100), 189 (8), 187 (6), 133 (8). Reduction of methyl 3-oxo-6β-hydroxyolean-12-en-28-oate (*1a*) with lithium tetrahydroaluminate gave the same triol (*4*), m.p. 274 – 277°; mixed m.p. 273 – 277° with the above material. The IR and mass spectra were identical.

Methyl 3β-acetoxy-6β-hydroxyolean-12-en-28-oate (5). Methyl sumaresinolate (*2a*, 120 mg) was treated with acetic anhydride (5 ml) and pyridine (10 ml) at 100° for 2 h. The solution was diluted with water and extracted with ether to give methyl 3β-acetoxy-6β-hydroxyolean-12-en-28-oate (*5*, 128 mg), m.p. 222 – 225° (reported:[5] 227°); δ(CDCl₃): 0.92 (3H,s), 0.95 (6H,s), 1.03 (3H,s), 1.11 (3H,s), 1.25 (3H,s), 1.33 (3H,s), 2.06 (3H,s), 2.92 (1H,q), 3.64 (3H,s), 4.33 (1H,t), 4.55 (1H,broad), 5.36 (1H,m); *m/e* (%): 528 (2), 469 (2), 468 (4), 453 (1), 450 (2), 435 (1), 409 (1), 408 (1), 407 (1), 391 (2), 302 (2), 262 (67), 249 (6), 247 (5), 203 (100), 189 (24), 187 (10), 133 (14).

Methyl 3β-acetoxy-6-oxo-olean-12-en-28-oate (6). Methyl 3β-acetoxy-6β-hydroxyolean-12-en-28-oate (*5*, 110 mg) in acetone (10 ml) was stirred with chromium trioxide (70 mg) in dilute sulphuric acid (10 %, 3.5 ml) for 1 h. Work up in the usual way and recrystallization from acetonitrile furnished methyl 3β-acetoxy-6-oxo-olean-12-en-28-oate (*6*), m.p. and mixed m.p. 284 – 287°; $[\alpha]_D(CHCl_3)$ +48° (*c* 0.96); δ (CDCl₃): 0.78 (3H,s), 0.93 (12H,s), 1.27 (3H,s), 1.30 (3H,s), 2.06 (3H,s), 2.22 (1H,s), 2.49 (1H,d J = 13), 2.90 (1H,q), 3.63 (3H,s), 4.44 (1H,t), 5.33 (1H,m); *m/e* (%): 526 (4), 467 (5), 466 (9), 451 (5), 407 (9) 302 (2), 262 (45), 249 (8), 247 (5), 203 (100), 189 (22), 187 (11), 133 (13).

Methyl 3β-hydroxy-6-oxo-olean-12-en-28-oate (7). Methyl 3β-acetoxy-6-oxo-olean-12-en-28-oate (*6*, 30 mg) was added to a solution of aqueous potassium hydroxide (45 %, 1 ml) in ethanol (3 ml) and refluxed for 45 min under nitrogen. Work up, chromatography

on silica gel and recrystallization from acetonitrile gave methyl 3β-hydroxy-6-oxo-olean-12-en-28-oate (*7*, 22 mg), m.p. 208 – 210° (reported:[5] 205 – 206°), δ (CDCl$_3$): 0.78 (3H,s), 0.93 (9H,s), 1.05 (3H,s), 1.23 (3H,s), 1.26 (3H,s), 2.90 (1H,q), 3.15 (1H,t), 3.63 (3H,s), 5.37 (1H,m), m/e (%): 484 (11), 469 (1), 466 (1), 451 (1), 425 (5), 424 (5), 407 (3), 302 (3), 262 (50), 249 (9), 247 (5), 203 (100), 189 (21), 187 (9), 133 (14). Thin layer chromatography (SiO$_2$, hexane/acetone 1:0.1) proved this compound (*7*) to be different from methyl 3-oxo-6β-hydroxyolean-12-en-28-oate (*Ia*).

Methyl 3β,6β-diacetoxyolean-12-en-28-oate (9). Acetyl chloride (1.5 ml) was carefully added to a stirred solution of methyl sumaresinolate (*2a*, 100 mg) in *N*-dimethyl aniline (3 ml) kept at 0°. The reaction mixture was left at room temperature for 20 h. It was then cooled to 0° and diluted with ether, cold aqueous sulphuric acid (5 %) and water. Chromatography on silica gel gave methyl 3β,6β-diacetoxyolean-12-en-28-oate (*9*, 22 mg), m.p. 254 – 257° (reported:[5] 258°); δ (CDCl$_3$): 0.93 (12H,s), 1.02 (3H,s), 1.11 (3H,s), 1.30 (3H,s), 2.05 (3H,s), 2.06 (3H,s), 2.90 (1H,q), 3.54 (3H,s), 4.45 (1H,t), 5.38 (1H,m), 5.56 (1H,t); m/e (%): 570 (3), 510 (8), 495 (1), 451 (5), 450 (7), 435 (5), 407 (1), 391 (4), 302 (2), 262 (66), 249 (13), 247 (6), 203 (100), 189 (21), 187 (17), 133 (12).

Methyl 3β-hydroxy-6β-acetoxyolean-12-en-28-oate (8). Methyl 3β,6β-diacetoxyolean-12-en-28-oate (*9*, 20 mg) was added to a solution of aqueous potassium hydroxide (45 %, 1 ml) in ethanol (5 ml) and left at room temperature for 1 h under nitrogen. Work up in the usual way and recrystallization from acetonitrile gave methyl 3β-hydroxy-6β-acetoxyolean-12-en-28-oate (*8*, 19 mg), m.p. 132 – 135° (reported:[5] 134 – 135°); δ (CDCl$_3$): 0.93 (6H,s), 0.96 (3H,s), 0.98 (3H,s), 1.05 (3H,s), 1.12 (3H,s), 1.28 (3H,s), 2.04 (3H,s), 2.91 (1H,q), 3.17 (1H,t), 3.66 (3H,s), 5.39 (1H,m), 5.62 (1H,t), m/e (%): 528 (3), 468 (8), 453 (2), 450 (3), 435 (2), 409 (2), 391 (2), 302 (4), 262 (63), 249 (7), 247 (5), 203 (100), 189 (18), 187 (18), 133 (16).

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