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OLEANOLIC AND URSOLIC ACID DERIVATIVES FROM *POLYLEPIS AUSTRALIS*

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Key Word Index—*Polylepis australis*; Rosaceae; oleanolic acids; ursolic acids; triterpenes.

Abstract—Bark of *Polylepis australis* furnished a complex mixture of triterpene acids from which, after methylation, the methyl esters of five known and two new oleananes and five known ursanes were isolated. The esters of the two new oleananes were methyl 3β-hydroxyolea-9(11),12-dien-28-oate and methyl 2α,3β-hydroxy-11-oxo-olean-12-en-28-oate. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Polylepis (Rosaceae) is a characteristic high-Andean tree genus of approximately 15 species characterized by pinnately compound leaves with expanded sheathing petiole bases. There are no reports on its chemistry. As the bark of *Polylepis australis* Britt. is used by the indigenous population of northwestern Argentina to prepare an infusion for the treatment of diabetes we decided to investigate its constituents.

Extraction of the bark with chloroform gave a complex mixture of triterpene acids which were methylated prior to separation by HPLC. Present in largest amounts were methyl oleanolate and methyl ursolate. Present in smaller amounts, sometimes obtained in the form of binary mixtures, were methyl acetyloleanolate, methyl acetylursolate and the methyl esters **1a**, **1b**, **2** (methyl obtusilinate), **3**, **4a** (methyl maslinate) [1, 2], **4b** [3], **5** (methyl euscaphate) and **6** [2, 4]. The acids corresponding to esters **1b** and **3** are not known and while the acids corresponding to esters **1a** [5], **2** [6] and **5** [7, 8] have been reported, the properties of their methyl esters have not been recorded in the literature.

The structures assigned to the various methyl esters are based on ^{1}H NMR and mass spectrometry. Thus the ^{1}H NMR spectra of enones **1a**, **b** and **2** exhibited singlets of H-12 in the range δ 5.56–5.58 and a sharp singlet of H-9 near δ 2.3, while in the case of **4a**, **b**, **5** and **6** the signal of H-12 was a characteristic triplet ($J \sim 3.5$ Hz) near δ 5.3 with the signal of H-9 far upfield. In the case of enones **1a**, **b** the signal of H-18 was a *dd* (J = 14, 4 Hz) near δ 2.99 typical of 11-oxo-Δ^{12}-oleanones whereas in the case of **2** the H-18 signal was a *dd* (J = 11, 1 Hz) at δ 2.41 typical of Δ^{12}-ursanes. The ^{1}H NMR spectra of **1a**, **b**, **3** and **4a**,**b** exhibited seven methyl singlets of a methyl oleanolate while those of **2** and **6** contained the five methyl singlets and two methyl doublets of a methyl ursolate and that of ester **5** six methyl singlets and one methyl doublet. Esters **1a**, **2** and **3** exhibited the typical *dd* of axially orientated H-3 near δ 3.2; in compounds **1b**, **4a**, **b** and **6** the signal of H-3, now a doublet with J = 9 Hz, was shifted to slightly higher field and coupled to another axial proton (H-2$_{ax}$) near δ 3.6, the latter moving upfield in the case of methyl ether **4b** whereas in methyl euscaphate (**5**) equatorial H-3 at δ 3.41 was coupled to axial H-2 at δ 3.98. The ^{1}H NMR spectrum of ester **3** exhibited the typical mutually coupled (J = 6 Hz) vinylic signals of H-11 and H-12 at δ 5.57 and 5.54 as well as the *dd* of H-3$_{ax}$ at δ 3.21 and the *brdd* (J = 15, 3 Hz) of H-18 at δ 3.05, while in the MS a peak with m/z 299 corresponding to A [9–11] was prominent. Properties of two other oleananes of uncertain structure isolated in very small amounts are also listed in Section 2.

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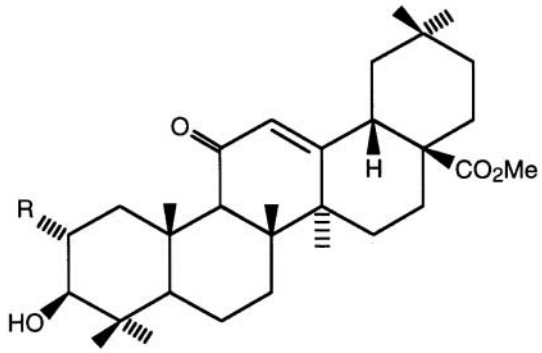

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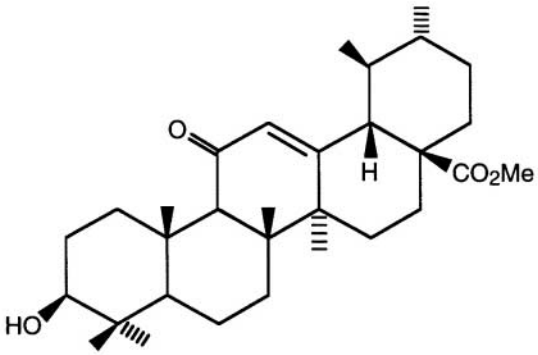
b R = OH
c R = OMe



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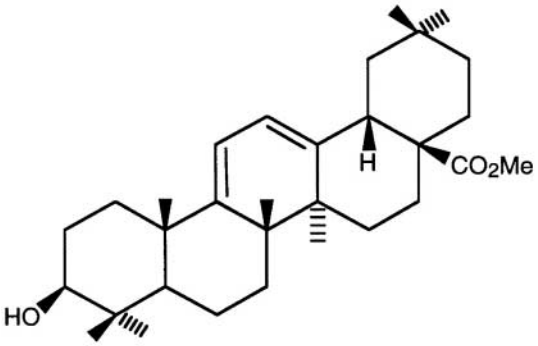

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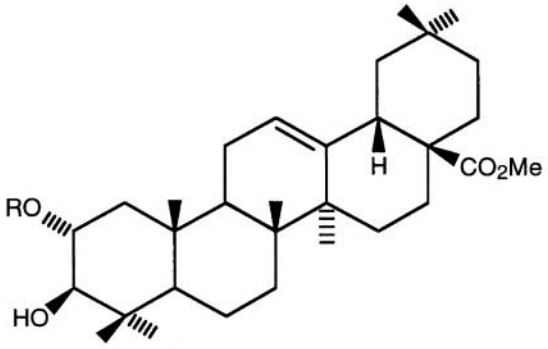

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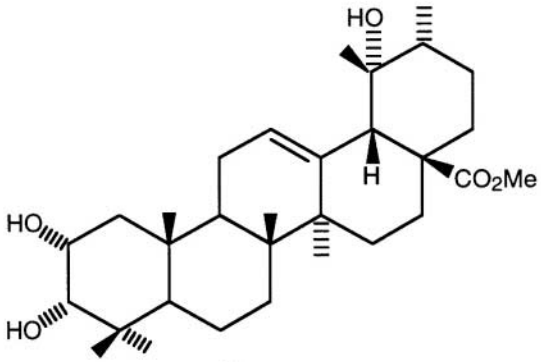
b R = Me



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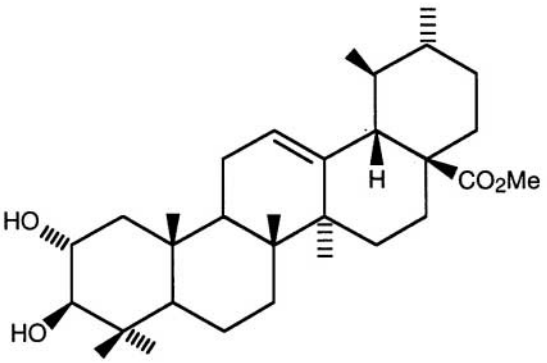

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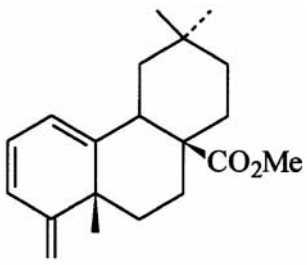
EXPERIMENTAL

General experimental procedures

^{1}H NMR spectra were recorded on Varian 500 MHz or 300 MHz instruments. Mass spectra were recorded on a MAT 90 instrument. Known compounds were identified by MS and ^{1}H NMR ($CDCl_3$). For separation of mixtures HPLC with a differential refractometer was used. The columns employed were (A) Altex Ultrasphere ODS (5 μ,



A

10×250 mm) and (B) Beckman C-18 (5 μ, 10×250 mm). Retention times were measured from the solvent peak.

Plant material

Bark of *Polylepis australis* Britt. was collected in May 1983 near La Quebradita, Tafí del Valle, Tucumán Province, Argentina. A voucher specimen (LIL 590509) is deposited in the herbarium of the Instituto Miguel Lillo, Tucumán.

Extraction and isolation

Ground bark (265 g) was extracted with CH_2Cl_2 (2×6 l) at room temperature for 6 days to give 4.43 g of crude extract (1.67%). The extract was divided into two portions. Portion I (0.64 g) was chromatographed over silica gel (30 g) using $CHCl_3$–Me_2CO 9:1, 40 frs being collected. Frs 2–4 were combined (80 mg). A portion (15 mg) was methylated with CH_2N_2 and processed by HPLC (column B, MeOH, 2 ml min^{-1}) to give 4.9 mg of the acetate of methyl oleanolate, R_t 20 min, and 1.1 mg of the acetate of methyl ursolate, R_t 21.7 min. Frs 7–36 were combined (0.42 g), methylated with CH_2N_2 and processed by HPLC (column A, MeOH, 1 ml min^{-1}) to give 172 mg of methyl oleanolate, R_t 28.5 min, and 121 mg of methyl ursolate, R_t 33 min.

Portion II (1.7 g) was washed 2× with hexane–ether (1:1). The insoluble material was dissolved in Et_2O and methylated with CH_2N_2 to give 0.87 g of methyl esters which were chromatographed (silica gel, 42 g) with hexane–EtOAc 7:3, 40 frs being collected which were monitored by TLC. Frs 12–20 were combined (539 mg). A portion (305 mg) was processed by HPLC (column B, MeOH, 2 ml min^{-1}) to give 7 peaks with R_ts of 6, 7.2, 8.5, 9.7, 19.6, 21.2 and 23.7 min. Rechromatography of peak 1 (col B, CH_3CN, 2.5 ml min^{-1}) gave 0.9 mg (R_t 4.5 min) of a tetrahydroxyoleanane $C_{30}H_{46}O_4$ with a primary hydroxyl group, probably on C-28, crude mp 230–236, MS PCI *m/z* (rel. int.): 471 (61.1, $M^+ + H$), 453 (91.8), 425 (40.5), 391 (100), 279 (30.6), 241 (44.5); ^{1}H NMR ($CDCl_3$): δ 3.65 (complex, 2H), 3.23 (*dd*, $J = 11$, 4.5 Hz, H-3_{ax}), 3.05 (complex, 2H), Me singlets at δ 1.08, 1.05, 1.01, 0.98, 0.98, 0.90 and 0.78. Rechromatography of peak 2 in the same manner gave **1a** contaminated by a small amount of **2** (8.3 mg, R_t 7 min), 1.5 mg (R_t 10 min) of a 1:1 mixture of **1a** and **2**, 2.1 mg (R_t 11 min) of a triterpene methyl ester $C_{32}H_{50}O_5$ as a gum (*vide infra*) and 2.8 mg of **3** (R_t 18 min). Rechromatography of peak 3 (col. B, CH_3CN–EtOAc 41:9, 1 ml min^{-1}) gave a 3:2 mixture of **1b** and **2** (R_t 9.5 min, 1.4 mg), **2** (2.3 mg, R_t 10.2 min and 1.1 mg, R_t 12 min) and a mixture (2.3 mg, R_t 16.5 min). Rechromatography of peak 4 (col. B, CH_3CN–EtOAc 41:9, 1.5 ml min^{-1}) gave 1.3 mg of methyl maslinate (**4a**, R_t 16.2 min). Peak 5 gave 6.2 mg of **4b**, Peak 6 gave 26 mg of methyl oleanolate and peak 7 gave 23 mg of methyl ursolate. The triterpene methyl ester from peak 2 was originally thought to be **1c** but this was contraindicated by detailed analysis of the ^{1}H NMR spectrum, MS PCI (*iso*-butane) *m/z* (rel. int.) 515 $[M + H]^+$ (65.2) ($C_{32}H_{50}O_5$), 497 $[M + H–H_2O]^+$ (91.6), 483 $[M + H–CH_3OH]^+$ (41.5), 467 (41.1), 249 (100), 235 (40.3), 189 (146). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.62 (*s*, H-11), 3.62 (*s*, 3H, OMe of methyl ester), 3.39 (*s*, 3H, OMe of methyl ether), 3.32 *dd* ($J = 13$, 4.5 Hz) coupled by 4.5 Hz to 3.26 *ddd* ($J = 13$,9, 4.5 Hz) and by 13 Hz to 0.74 *t* ($J = 13$ Hz); 3.26 *ddd* ($J = 4.5$, 9.5, 13) coupled by 4.5 Hz to 3.32 *dd*, by 9.5 Hz to 3.03 *d* and by 13 Hz to 0.71 *t*), 3.03 *d* ($J = 9$ Hz), 2.99 *brdd* ($J = 14$, 4 Hz, H-18 of oleanane), 2.34 (*s*, H-9 of 11-en-12-one), 2.03 *td* ($J = 14$, 14, 4 Hz), 1.34, 1.14, 1.04, 0.92, 0.91, 0.89, 0.83 (seven *s* and 3H), 0.71 *t* ($J = 13$ Hz).

Frs 21–38 (212 mg) of the original chromatogram of portion II were combined. HPLC of the mixture (col. b, MeOH–H_2O 9:1, 1.5 ml min^{-1}) gave methyl euscaphate (**5**, 3.6 mg, R_t 19.5 min), **4b** (49 mg, R_t 40.5 min) and **6** (10.6 mg, R_t 45 min, crude mp 122–125°).

Methyl 3*β-hydroxy*-11-*oxo-olean*-12-*en*-28-*oate* (**1a**)

Gum; MS PCI (*iso*-butane) *m/z* (rel. int.) 485 $[C_{31}H_{48}O_4 + H]^+$ (17.6), 467 (13.0), 249 (6.7), 235 (100). ^{1}H NMR ($CDCl_3$), 300 MHz) δ 5.61 (*s*, H-12), 3.61 (*s*, 3H, OMe of ester), 3.19 (*dd*, J = 10,5 Hz, H-3_{ax}), 2.98 (*brdd*, 13.5, 3.5 Hz, H-18), 2.81 (*dt*, 13, 3 Hz), 2.29 (*s*, H-9), 1.34, 1.08, 0.97, 0.92, 0.91, 0.89, 0.79 (all *s* and 3p).

Methyl 2*a,3b-dihydroxy*-11-*oxo-olean*-12-*en*-28-*oate* (**1b**)

Obtained only in admixture with **2** as a gum. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.66 (*s*, H-12), 3.63 (*s*, 3H, OMe of ester), 3.61 (*m*, H-2_{ax} partially obscured by -OMe signal of **2**), 3.02 *d* ($J = 11$ Hz, H-3_{ax}), 2.96 (*m* (H-18) H-, 1.36, 1.22, 1.08, 1.05, 0.95, 0.93, 0.91 (all *s* and 3p).

Methyl obtusilinate (**2**)

Crude mp 150–158°. EI MS *m/z* (rel. int.) 484 (14.3, $C_{31}H_{48}O_4$), 317 (49.4), 276 (24.7), 257 (16.1), 248 (5.7), 235 (5.8), 217 (12.0), 193 (5.2), 192 (11.1), 189 (8.4), 175 (16.6), 174 (5.3). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.58 (*s*, H-12), 3.59 (*s*, 3H, OMe of ester), 3.20 (*dd*, $J = 11$, 5 Hz, H-3_{ax}), 2.78 (*ddd*, $J = 13.5$, 3.5, 3.5 Hz), 2.41 (*dd*, $J = 11.5$, 1 Hz, H-18), 2.28 (*s*, H-9), 1.29, 1.12, 0.98, 0.91, 0.79 (all *s* and 3H), 0.96 (*d*, 3H, $J = 6.5$ Hz), 0.86 (*d*, 3p, $J = 6$ Hz).

Methyl 3*β-hydroxyolean*-9(11),12-*dien*-28-*oate* (**3**)

Crude mp 172–180°. MS EI *m/z* (rel. int.) 468 (100, $C_{31}H_{48}O_3$), 450 (6.1), 409 (12.1, M–CO_2Me),

408 (13.4, M–CO_2Me–H), 393 (16.7), 299 (255), 239 (17.1), 189 (10.9). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.57 (*d*, J = 6 Hz, H-11), 5.54 (*d*, J = 6 Hz, H-12), 3.63 (*s*, 3H, -OMe), 3.21 (*dd*, J = 11.5, 4.5 Hz, H-3$_{ax}$), 3.05 (*brdd*, J = 15, 3 Hz, H-18), 1.16, 1.01, 1.01, 0.94, 0.94, 0.89, 0.79 (all *s* and 3H).

Methyl 2α-*methoxy*-3β-*hydroxyolean*-12-*en*-28-*oate* (**4b**)

This ester, prepared as a derivative of the corresponding acid from *Epilobium hirsutum* [3], has not been characterized adequately; mp without recrystallization 224–227°. MS PCI (isobutane) *m*/*z* (rel. int.): 501 ($[C_{32}H_{52}O_4+H]^+$) (21.3), 483 (100), 469 (77.3), 467 (46.0), 453 (44.3), 451 (73.0), 263 (36.6). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.27 (*t*, J = 3.5 Hz, H-12), 3.61 (*s*, 3H, -OMe of ester), 3.36 (*s*, 3H, -OMe of ether), 3.20 (*ddd*, J = 11, 9.5, 4.5 Hz, H-2$_{ax}$), 3.03 (*d*, J = 9.5 Hz, H-3$_{ax}$), 2.84 (*brdd*, J = 13.5, 4 Hz, H-18), 1.11, 1.03, 0.95, 0.91, 0.88, 0.81, 0.70 (all *s* and 3 H).

Methyl 2α, 3α, 19α-*trihydroxyursan*-12-*en*-28-*oate* (*methyl euscaphate*) (**5**)

Gum; MS EI *m*/*z* (rel. int.): 502 (4, $C_{31}H_{50}O_5$), 484 (4.1), 469 (5.4), 442 (20.3), 424 (4.3), 370 (6.0), 278 (6.8), 278 (6.8), 262 (10.5), 260 (14.5), 250 (11.7) 236 (10.2), 223 (17.3), 205 (28.8), 203 (14.3), 191 (7.9), 189 (9.3), 187 (20.6), 185 (10.5), 179 (100). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.35 (*t*, J = 3.5 Hz, H-12), 3.98 (*ddd*, J = 11.5, 3.5, 3.5 Hz, H-2$_{ax}$), 3.58 (*s*, 3H, -OMe), 3.41 (*brd*, J = 3 Hz, H-3$_{eq}$), 2.59 *brs* (H-18), 2.50 (*dt*, J = 13, 13, 5 Hz), 1.34 (*dd*, J = 12, 3.5 Hz, H-1β), 1.26, 1.20, 1.01, 0.95, 0.85, 0.67 (all *s* and 3H) 0.85 (*d*, and 3H J = 6.5 Hz, H-30).

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