Oleanane Triterpenoids from *Cedrela montana* (Meliaceae)

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Two new oleanane-type triterpenes, characterized as 3-oxo-11α,12α-epoxy-oleanan-28,13β-olide and 3-oxo-olean-11-en-28,13β-olide , were isolated from the fruits and seeds of *Cedrela montana* (Meliaceae). In addition, the known compounds oleanonic acid , a mixture of β-sitosterol and stigmasterol, and the limonoid photogedunin were also isolated. The structures of the new compounds were established by spectroscopic methods, including 2D NMR.

Introduction

The family Meliaceae, comprising about 50 genera and 1400 species, forms a large botanical family of mostly pantropical distribution. The trees are valued for their quality wood and resistance to the attack of several pest of insects (Banerji and Nigam, 1984). *Cedrela montana* Turczaninov (Meliaceae) belongs to the Meliaceae plant family highly prized in traditional medicine for their antitumor activity (Champagne *et al.*, 1992). No previous studies on the phytochemistry and biological activity of this species have been reported. We report here on the isolation and structural characterization of two new olean-28,13β-olides and the known β-sitosterol, stigmasterol, oleanonic acid and the limonoid photogedunin. Although oleanane triterpenoids are widely distributed, olean-28,13β-olides do not commonly occur in nature, to our knowledge only sixteen have been isolated from nature (Ahmad and Atha-ur-Rahman 1994) and three have been synthesized. (Barton and Holness, 1952; Kitagawa *et al.*, 1968, Majumder and Bagchi, 1983). Although the oleanonic acid triterpenoid has been isolated recently from *Cedrela odorata* (Campos *et al.*, 1991), this is the first report of oleanan-28,13β-olides from *Cedrela* species.

Results and Discussion

Fruits and seeds of *C. montana* were extracted with petroleum ether and dichloromethane followed in each case by CC over silica-gel (see Experimental). This procedure gave two new triterpene lactones named 3-oxo-11α,12α-epoxy-olean-28,13β-olide (**1**) and 3-oxo-olean-11-en,28,13β-olide (**2**). In addition the known β-sitosterol, stigmasterol, oleanonic acid (**3**) (Campos *et al.*, 1991) and photogedunin (**4**) (Céspedes *et al.*, 1999) were isolated. Identification of the known compounds was based on the comparison of their spectroscopic (^{1}H, ^{13}C and EIMS) and physical (m.p.) data reported in the literature.

Compound **1** was obtained as colorless crystals, m.p. 281 °C. Its molecular formula $C_{30}H_{44}O_4$ was deduced from the EIMS spectrum (M^+, *m/z* 468) and by quantification of the number of methyl, methylene, methine and quaternary carbon atoms revealed in the ^{13}C NMR and DEPT spectra (Table I).The IR spectrum of **1** displayed bands at 1770 and 873 cm^{-1} characteristic of an epoxy-γ-lactone moiety (Hui and Li, 1976; Kitagawa *et al.*, 1972 ; Majumder and Bagchi, 1983). A further band at 1708 cm^{-1} suggested the presence of a six-membered ring ketone, which was also supported by a resonance at δ 216.2 (s) for a carbonyl carbon in the ^{13}C NMR spectrum of **1** (Seo *et al.*, 1975). The ^{1}H NMR spectrum of **1** showed signals for

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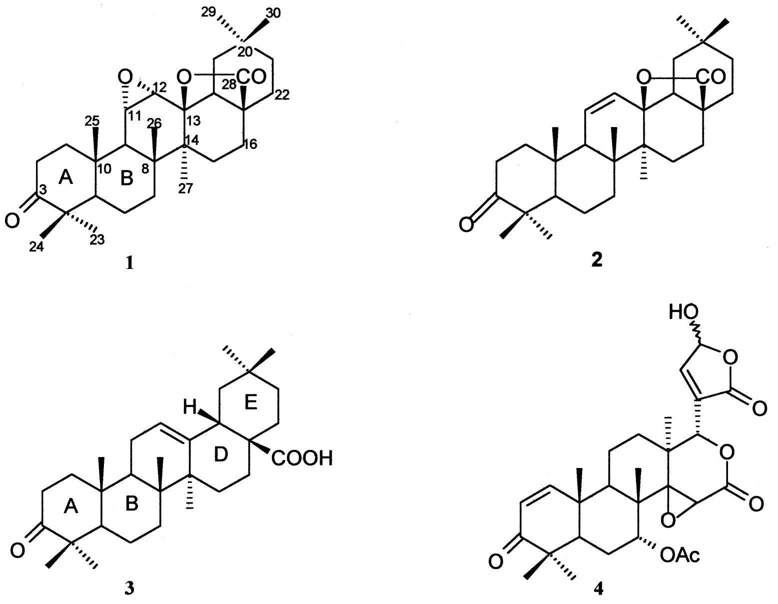

Fig. 1. Chemical Structures of 3-oxo-11α,12α-epoxy-olean-28,13β-olide (**1**), 3-oxo-olean-11-en,28,13β-olide (**2**), oleanonic acid (**3**) and photogedunin (**4**).

seven tertiary methyl groups at δ 0.93 , 1.00, 1.11 (6H), 1.19, 1.06, and 1.10 ppm and one, two proton signal at δ 3.07 (br, s) which agreed with those reported for oleanane triterpenoids possessing an 11α,12α epoxy group (Hui and Li, 1976; Kitagawa *et al.*, 1968; Majumder and Bagchi, 1983). Furthermore, the ^{13}C NMR spectrum exhibited resonances at δ 52.5 (d), 57.0 (d), 87.3 (s) and 179.2 (s) which were assigned to C-11, C-12, C-13, and C-28, respectively, and agreed closely with those reported for 11α,12 α-epoxy-olean-28,13β-olides (Ikuta and Morikawa, 1992; Iwamoto *et al.*, 1985). Moreover, the resonances at δ 52.5 (d) and 57.0 (d) attributed to the epoxide group, correlated with the signal at δ 3.07 (br, s) in the HETCOR spectrum. In addition, the ^{13}C NMR and DEPT spectra of **1** revealed the presence of seven methyls, five methines, nine methylenes and nine quaternary carbons which provided further evidence for a pentacyclic triterpene skeleton. On the basis of the above spectra evidence the structure of compound **1** was established as 3-oxo-11α,12α-epoxy-oleanan-28,13β-olide.

Compound **2,** was isolated as a gummy solid. Its molecular formula $C_{30}H_{44}O_3$ determined by EIMS (M^+, *m/z* 452) was confirmed by ^{13}C NMR and DEPT analysis. The IR spectrum of **2** displayed absorption bands at 1765, 1705 and 1640 cm^{-1} assigned to a γ-lactone, a six-membered ring ketone and a double bond groups respectively. The ^{1}H NMR spectrum of **2** (Table I) exhibited signals for seven tertiary methyl groups, characteristic for oleanan-28,13β-olides (Hui and Li, 1976; Ikuta and Morikawa, 1992).Further signals were observed at δ 6.04 (d, d, J = 10.6 and 1.3 Hz) and 5.46 (d, d, J = 10.2 and 3.1 Hz), indicating the presence of a –CH–CH=CH group in the structure of **2.** The chemical shifts and coupling constants agree closely with those reported for 3β-hydroxyolean-11-en-28,13β-olide isolated from *Hyptis albida* (Pereda-Miranda and Delgado, 1990). Furthermore, the ^{13}C NMR spectrum of **2** displayed resonances at δ 135.2 (d), 127.4 (d), 89.6 (s) and 180.0 for C-11, C-12, C-13 and C-28 respectively which confirmed the olean-11-en,28,13β-olide structure (Pereda-Miranda and Delgado, 1990). All the above mentioned spectral data established the structure of compound **2** as 3-oxo-olean-11-en,28,13β-olide.

Experimental

General

Melting points, uncorrected. ^{1}H-NMR: TMS as int. standard. CC: silica-gel 60, 70–230 mesh. TLC: precoated silica-gel 60 F_{254} (Merck, 1.0 mm). Spots were visualized by UV (254 nm) and 10% $CeSO_4–H_2SO_4$ reagent followed by heating.

Table I. ^{1}H and ^{13}C NMR spectral data for **1** and **2** ($CDCl_3$, TMS as int. standard)[a,b].

	Compound **1**		Compound **2**	
Position	δ_H	δ_C	δ_H	δ_C
1		38.7 t		39.0 t
2ax	2.67 ddd (16.1, 12.0, 6.9)	33.8 t	2.65 ddd (16.0, 11.1, 7.2)	33.8 t
2eq	2.44 ddd (16.1, 6.6, 3.3)		2.44 ddd (16.0, 6.7, 3.7)	
3		216.2 s		216.8 s
4		47.5 s		47.6 s
5		54.7 d		54.6 d
6		18.7 t		18.8 t
7		30.5 t		30.4 t
8		40.6 s		41.4 s[c]
9		50.1 d	2.00 br,s	52.5 d
10		36.2 s		36.1 s
11	3.07 br s	52.5 d	5.46 dd (10.2, 3.1)	135.2 d
12	3.07 br s	57.0 d	6.04 dd (10.2, 1.3)	127.4 d
13		87.3 s		89.6 s
14		41.2 s		41.5 s[c]
15		26.7 t[c]		25.4 t
16		21.2 t		21.2 t
17		43.8 s		44.0 s
18	2.34 dd (13.6, 3.6)	49.6 d		50.5 d
19	1.87 t (13.5)	37.8 t	1.81 t (13.5)	37.3 t
20		31.5 s		31.4 s
21		34.3 t		34.3 t
22		27.0 t[c]		27.1 t
23	1.10 s	25.9 q	1.08 s	26.0 q
24	1.06 s	21.1 q	1.06 s	20.8 q
25	1.19 s	16.4 q	1.05 s	17.3 q
26	1.11 s	18.7 q	1.10 s	18.1 q
27	1.11 s	19.8 q	1.11 s	18.6 q
28		179.2 s		180.0 s
29	1.00 s	33.2 q	0.98 s	33.3 q
30	0.93 s	23.6 q	0.89 s	23.5 q

[a] δ in ppm and *J* (in parentheses) in Hz.

[b] Assignments were based on ^{1}H–^{1}H COSY and HETCOR experiments and comparison with spectroscopy data reported in the literature (Ikuta and Morikawa, 1992; Pereda-Miranda and Delgado, 1990; Seo *et al.*, 1975).

[c] Assignments could be reversed. Chemical shifts were determined at 500 (^{1}H) and 75 (^{13}C) MHz. Carbon multiplicities were determined by DEPT experiments.

Plant material

Fruits and seeds from *Cedrela montana* were collected along the road to Puente de Piedra (Cundinamarca, Colombia) in December 1994. The plant was identified by M. Sc. Maria E. Morales. Voucher specimen has been deposited at the Herbario Nacional Colombiano. Universidad Nacional de Colombia. No. 289.532

Extraction and isolation

Dried fruits and seeds (2.65 kg) were extracted successively with petroleum ether (b.p. 40–60°) (10 l) and CH_2Cl_2 (7 l). After evaporation of solvents, green syrups A (35 g) and B (28 g) were obtained respectively.

The petroleum ether (b.p. 40–60°) extract A (33.5 g dry wt.) was chromatographed on a silica gel column (600 g) eluting with petroleum ether and mixtures of CH_2C1_2–MeOH (8:2 v/v). 120 fractions of 500 ml each were collected and pooled according to their TLC patterns to give several major fractions. Fractions 50–55, after crystallization from MeOH, yielded a mixture of β-sitosterol and stigmasterol (m.p. 134–136 °C). Fractions 75–91 (650 mg) were purified by preparative TLC and eluted with petroleum ether–CH_2Cl_2 (7:3 v/v) to give after crystallization from CH_2Cl_2–MeOH, oleanonic acid **(3)** (450 mg) as white crystals (m.p. 183 °C) (Campos *et al.,* 1991)

The CH_2Cl_2 extract B (27 g dry wt) was subjected to flash chromatography on silica gel (486 g) and eluted with petroleum ether, CH_2Cl_2 and MeOH. 171 fractions of 500 ml each were collected and pooled according to their TLC patterns to give several major fractions. Fractions 50–60 purified by preparative TLC afforded 300 mg of a mixture of β-sitosterol and stigmasterol. Fractions 79–83 were separated by preparative TLC using petroleum ether–CH_2Cl_2 (7:3 v/v) giving 100 mg of 3-oxo-11α,12α-epoxy-olean-28,13β-olide (**1**) and 4 mg of 3-oxo-olean-11-en-28,13β-olide (**2**). Fractions 98–108 (1.8 g) were rechromatographed on a silica gel column (40 g), eluted with petroleum ether and mixtures of CH_2Cl_2–MeOH giving 15 mg. of photogedunin (**4**) (Céspedes *et al*., 1998) as a white solid (m.p. 286 °C).

3-Oxo-11α,12α-epoxy-oleanan-28,13β-olide (**1**)

M.p. 281 °C (CH_2Cl_2–MeOH), IR_{KBr} (cm^{-1}): 2945, 2860, 1770, 1708, 1477, 1449, 1392, 1139, 873. EIMS (probe) 70 eV *m/z* (rel. int.): 468 $[M]^+$ (50), 453 (26), 450 (15), 440 (10), 424 (5), 422 (20), 407 (15), 368 (12), 263 (50), 248 (20), 233 (100), 204 (65), 189 (75).

3-Oxo-olean-11,12-en-28,13β-olide (**2**)

Gummy solid. IR_{KBr} (cm^{-1}): 2940, 2850, 1765, 1705, 1640, 1610, 1470, 1390, 1035, EIMS (probe) 70eV *m/z* (rel. int.): 452 $[M]^+$ (100), 408 (80), 255 (18), 201 (12), 203 (10), 193 (50), 189 (59), 187 (30).

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