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TRITERPENOID ACIDS FROM *CUNILA LYTHRIFOLIA**

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Key Word Index-Cunila *lythrifolia*; Labiatae; ursene-type triterpenoid; 2β,3β,19α-trihydroxy-urs-12-en-28-oic acid; 2-*epi*-tormentic acid.

Abstract-Oleanolic, ursolic, 2α-hydroxyursolic and maslinic acids and the new 2-*epi*-tormentic acid (2β,3β,19α-trihydroxy-urs-12-en-2%oic acid) were isolated from the aerial parts of *Cunila lythrifolia.*

INTRODUCTION

Cunila lythrifolia Bentham, known in Mexico as poleo de campo, is widely used in traditional medicine for the relief of gastro-intestinal upsets [1]. Previous chemical work on the genus deals mainly with the terpenoid content of the essential oils [2–5]. In continuation of our studies on the constituents of Mexican plants [6–8], we report herein the isolation and structure elucidation of a new pentacyclic triterpenoid (1), 2-*epi*-tormentic acid, from the aerial parts of C. *lythrifolia.*

RESULTS AND DISCUSSION

Chromatographic separation of the chloroform extract of C. *lythrifolia* afforded β-sitosterol, oleanolic, ursolic, 2α-hydroxyursolic (5) and maslinic acids, clovandiol, a flavonoid identified as acacetin, and a new natural product (1), which was isolated as its methyl ester derivative (2) and characterized as 2-epi-tormentic acid.

Compound 2, analysed for $C_{31}H_{50}O_5$ (m/z 502 $[M^+]$). Its IR spectrum (see Experimental) showed hydroxyl group absorptions as well as bands corresponding to an olefinic and a carbonyl group. It formed an amorphous diacetate (3) and reacted with periodate confirming the vicinal diol nature of the compound. The mass spectral analysis exhibited important ions associated with the amyrins [9]. The peak at *m/z* 278 corresponded to the typical retro-Diels-Alder cleavage of the Δ^{12}-pentacyclic skeleton with a carbomethoxy group on C-17 and a hydroxyl substituent on ring D or E [10, 11]. This was confirmed by the peaks at m/z 260 $[278–H_2O]^+$, 219 $[278 - CO_2Me]^+$, 218 $[278 - HCO_2Me]^+$ and 201 $[278 - H_2O - CO_2Me]^+$. Other important ions were at *m/z* 179 and 146, corresponding to $[C_{11}H_{15}O_2]^+$ and $[C_{11}H_{14}]^+$, respectively, and which are characteristic of the presence of a tertiary hydroxyl function on C-19 in the urs-12-ene skeleton [10]. This fragmentation pattern also indicated that the two secondary hydroxyl groups were located in the A/B ring portion. The ^1H NMR spectrum of 2 showed signals for six tertiary methyl groups, two carbinol methine protons, assignable to H-3 (6 3.2) and H-2 (δ4.07) on biogenetic grounds [12], and one vinylic proton on C-12 (6 5.34). The location and a-disposition of the tertiary hydroxyl group at C-19 were established by the observed paramagnetic-induced shifts of the Me-29 (6 1.25) and Me-27 (6 1.22) signals, which were of the order reported for geminal (A6 = 0.36) and vicinal (A6 = 0.14) deshieldings in similar structural environments [8]. Splitting in the C-3 proton signal indicated that the interaction with H-2 is as a result of an axial-equatorial coupling (J_{ae} = 5 Hz). Furthermore, the coupling constants of H-2 are consistent with the hydroxyl proton being in a diequatorial (J_{ee} = 4 Hz) and axial-equatorial (J_{ae} = 4 Hz) relation with the two vicinal hydrogens at C-1 [12]. From a comparison of the ^1H NMR data of 2 with those of a large number of triterpenes [12–14], it is evident that the deshielding of the Me-24 ($\Delta\delta$ = 0.25) and Me-25 (AS = 0.35) groups was due to a hydroxyl substituent in a 1,3-diaxial relationship to them. Such chemical shifts for the methyl resonances and the observed multiplicities for the carbinol protons are possible only if both secondary hydroxyl groups are placed with a β-orientation. Therefore, the structure of the natural product was formulated as 2β,3β,19α-trihydroxy-urs-12-en-28-oic acid (1).

Finally, the interpretation of the ^{13}C NMR spectrum of 3 supported the structural assignments because of its remarkable similarity with those of appropriate models, in particular with acetyl methyl pomolate (4) [15]. This comparison showed the predictable shifts attributable to β, γ and δ effects caused by the presence of an axial acetoxyl group at C-2. The ^{13}C NMR data are consistent with the two chair conformation of the cis-fused D and E rings for derivative 3 [16,17].

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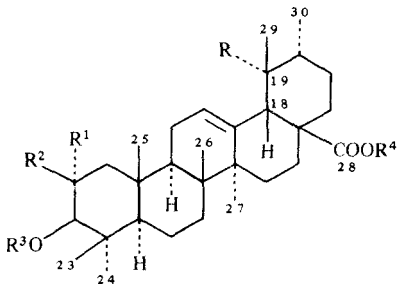

	R^1	R^2	R^3	R^4	R^5
1	H	OH	H	H	OH
2	H	OH	H	Me	OH
3	H	OAc	Ac	Me	OH
4	H	H	Ac	Me	OH
5	OH	H	H	H	H
6	OH	H	H	Me	H

EXPERIMENTAL

Plant material. Cunila lythrifolia was collected in Feb 1985, Km 30 Mexico City-Cuernavaca High-road (Morelos, Mexico). Reference specimens are deposited in the National Herbarium, Instituto de Biologia, UNAM, voucher No. 8812M.

Extraction and preliminary fractionation. Dried and finely powdered aerial parts of the plant (6.3 kg) were exhaustively extracted with $CHCl_3$ at room temp. for a week. After filtration, the extract was evapd to dryness yielding a residue (216 g). The crude extract was carefully chromatographed on a silica gel (2.5 kg deactivated with 10% H_2O) column, using an *n*-hexane–EtOAc gradient elution system. Fractions of 1 1 were collected.

Isolation procedure. The low polarity fractions 2679, eluted with n-hexane-EtOAc (9: 1), left a residue which crystallized when triturated with *n*-hexane–EtOAc (1: 1) to give *β*-sitosterol.

The medium polarity fractions 162-242 (12.4 g), eluted with *n*-hexane–EtOAc (4: 1), were rechromatographed over silica gel (300 g deactivated with 10% H_2O). From fractions 20–34, eluted with n-hexane-EtOAc (7:3) 112 mg of a powder pptd. mp 279–281°;100 mg of this residue were dissolved in MeOH and treated with an excess of CH_2N_2 in Et_2O at room temp. The mixture was left overnight and then evapd to yield upon trituration with Et_2O 97.5 mg of methyl ursolate. Both ursolic acid and its methyl ester derivative were identical in all respects with standard samples. Subsequent fractions 35–70, eluted with the same solvent system, were alkylated with CH_2N_2–Et_2O affording 150 mg of a mixture of methyl oleanolate and methyl ursolate. Part of this solid residual material (80 g) was taken and acetylated with Ac_2O–pyridine (1: 1) at room temp. for 24 hr. After work-up of the reaction mixture, the crude product was further chromatographed on a column in $CHCl_3$–Me_2CO (3: 1) affording the acetates of oleanolic acid (15.8 mg) and ursolic acid (64.3 mg) as their methyl esters derivatives. These were identified by comparison with authentic samples. Finally the fraction eluted with n-hexane-EtOAc (3:2) crystallized spontaneously to yield 25 mg of acacetin, mp 168-169" [18], which was identified by standard procedures.

The polar fractions 243-285 (7.3 g), eluted from the original column with *n*-hexane–EtOAc (7:3) were resolved by CC over silica gel (240g). The elution was accomplished with *n*-hexane–EtOAc (3:2). An additional crop of the binary mixture of oleanolic and ursolic acid (530 mg) was obtained from fractions 20–29. Fractions 46-79 afforded a crystalline powder which after recrystallization from EtOH–$CHCl_3$ (1: 1) yielded 53 mg of clovandiol, mp 150–152°. This was identified by standard sample comparison [19]. Fractions 296–344 (8.12 g), eluted with *n*-hexane–EtOAc (3:2) from the initial fractionation, were treated with CH_2N_2–Et_2O. The reaction mixture was chromatographed over silica gel (250 g), using $CHCl_3$–Me_2CO (3:2) as the eluent mixture. Fractions 28–41 crystallized spontaneously to afford 189.3 mg of 2-*epi*-tormentic acid methyl ester (2). Oil, $[\alpha]_D^{25}$ + 16.3, (EtOH; *c* 0.22); IR $\nu_{max}^{CHCl_3}$ cm-': 3621, 3029, 2933, 1718, 1458, 1381, 1231, 1199, 1149, 1048, 1030, 972, 929; ^{1}H NMR (80 MHz, $CDCl_3$): SO.71 (3H, *s*, Me-26), 0.95 (3H, *d*, *J* = 7 Hz, Me-30), 1.03 (6H, *s*, Me-23, -24), 1.22 (3H, *s*, M&27), 1.25 (3H, s, M-29), 1.27 (3H, *s*, Me-25), 2.60 (1H, *s*, $W_{1/2}$ = 6 Hz, H-18), 3.20 (1H, *d*, *J* = 5 Hz, H-3), 3.60 (3H, *s*, CO_2Me), 4.07 (1H, *ddd*, *J* = 5, 4, 4 Hz, H-2), 5.35 (1H, *m*, H-12); EIMS 75 eV, m/z (rel. int.): 502 $[M]^+$ (3.7), 484 (3.1), 469 (2.2), 466 (2), 442 (15.8), 424 (4.1), 409 (2.1), 370 (4.7), 352 (3.4), 278 (2.9), 262 (10), 260 (15), 250 (15.8), 223 (20.5), 219 (17.5), 218 (15.2), 207 (13.9), 205 (22.8), 201 (32.4), 179 (65.5), 146 (34.2). 69 (38.5), 55 (42.1). 43 (100). Finally, fractions 43-50 were combined to afford a solid residue. which after recrystallization from MeOH yielded 245 mg of a mixture of methyl 2*α*-hydroxyursolate (6) and methyl maslinatc [20].

Acetylation of 2. Compound 2 (145 mg) was dissolved in 5 ml Ac_2O and 1 ml pyridine. After 2 hr, the reaction mixture was worked-up as usual to yield 135 mg 3 (oil). $[\alpha]_D^{25}$ + 37.3 (EtOH; *c* 0.214); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3622, 3030, 2935, 1733, 1628, 1458, 1370, 1255, 1055, 1028,907; ^{1}H NMR (80 MHz, $CDCl_3$): 60.71 (3H, *s*, Me-26), 0.90 (3H, *s*, Me-23), 0.94 (3H, *d*. *J* = 7 Hz, Me-30). 1.05 (3H, *s*, Me-24), 1.18 (3H, *s*, Me-25), 1.21 (3H, s, Me-27), 1.25 (3H. s, Me-29), 2.02 (3H, *s*, MeCO–), 2.58 (1H, s, $W_{1/2}$ = 5 Hz, H-18), 3.60 (3H, *s*, –CO_2Me), 5.32 (1H, *m*, H-12); ^{13}C NMR (20.0 MHz, $CDCl_3$): 42.03 (C-l), 69.68 (C-2), 78.14 (C-3), 37.39 (C-4), 51.48 (C-5), 18.22 (C-6), 32.88 (C-7), 40.19 (C-8). 47.79 (C-9). 36.81 (C-10), 23.81 (C-11), 128.83 (C-12), 138.45 (C-13), 41.51 (C-14), 28.25 (C-15), 25.57 (C-16), 48.05 (C-17), 53.44 (C-18), 73.14 (C-19), 41.33 (C-20), 26.11 (C-21). 37.46 (C-22), 29.16 (C-23). 17.70 (C-24). 15.92 (C-25), 16.85 (C-26). 24.58 (C-27), 178.29 (C-28), 27.43 (C-29), 16.10 (C-30), 170.57 (–OCOMe), 170.12 (–OCOMe), 21.18 (–OCOMe), 20.77 (–OCOMe), 55.30 (–CO_2Me); EIMS 75 eV, m/z (rel. int.): 586 $[M]^+$ (3.1), 568 (2.4). 526 (15.4), 510 (2.3), 466 (4.0), 454 (5.2). 320 (4.8), 307 (3.6), 260 (10.1), 203 (4.2), 201 (20.5), 179 (44.8). 146 (26.7), 119 (17.5), 81 (20.2). 69 (15.4). 55 (20.0), 43 (100).

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