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TRITERPENOIDS AND FLAVONOIDS FROM *HYPTIS ALBIDA*[1]

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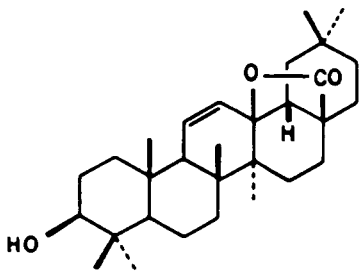
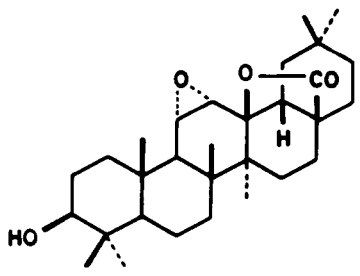
ABSTRACT—Investigation of the Me_2CO extract of the medicinal plant *Hyptis albida* led to the isolation of three triterpene lactones: 3β-hydroxyolean-28,13β-olide, 3β-hydroxy-11α,12α-epoxyolean-28,13β-olide, and the new 3β-hydroxyolean-11-en-28,13β-olide [**1**]. From the aerial parts four additional triterpenes (betulinic, oleanolic, ursolic, and acetyl oleanolic acids), along with five flavonoids (apigenin 7,4′-dimethyl ether, kaempferol 3,7,4′-trimethyl ether, ermanin, nevadensin A, and gardenin B) have been also isolated.

Hyptis albida H.B.K. is a tropical species found in Mexico. It grows extensively in the Balsas River basin where the plant is known as "salvia blanca" and is widely used in folk medicine for treating gastro-intestinal upsets and as a condiment in regional cuisine (1,2). The leaves are also reputed to act as a potent insect repellent (3). In the course of our chemical studies on Mexican Lamiaceae species (4,5), investigation of the Me_2CO extract of the aerial parts of this medicinal species resulted in the isolation and characterization of three triterpene lactones: the new 3β-hydroxyolean-11-en-28,13β-olide [**1**], and the known 3β-hydroxyolean-28,13β-olide [**2**] and 3β-hydroxy-11α,12α-epoxyolean-28,13β-olide [**3**]. In addition, four triterpenoids, including betulinic, ursolic, oleanolic [**4**], and acetyl oleanic [**5**] acids, along with five flavonoids: 5-hydroxy-7,4′-dimethoxyflavone, 5-hydroxy-3,7,4′-trimethoxyflavone, 5,7-dihydroxy-3,4′-dimethoxyflavone (ermanin), 5,7-dihydroxy-6,8,4′-trimethoxyflavone (nevadensin A), and 5-hydroxy-6,7,8,4′-tetramethoxyflavone (gardenin B) were also isolated from this species.

The new compound **1** had a molecular formula $C_{30}H_{46}O_3$ ($[M]^+$ at *m/z* 454) and its ir showed a γ-lactone (1754 cm^{-1}) with olefinic (1635 cm^{-1}) and hydroxyl (3611 cm^{-1}) groups. The ^1H-nmr spectrum exhibited signals for seven tertiary methyls, a secondary hydroxyl group (δ 3.20, 1H, dd, $J = 10, 6$ Hz) whose chemical shift and splitting pattern were typical of a 3β-equatorial OH, and two olefinic resonances centered at δ 5.37 (1H, dd, $J = 10$, 3 Hz) and 6.04 (1H, dd, $J = 10$, 1.5 Hz), which conformed to the typical AMX



1
2 11,12-dihydro



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[1]Part 2 in the series Chemical Studies on Mexican *Hyptis* Species. For Part I see Pereda-Miranda and Gascón-Figueroa (4).

system characteristic of a pentacyclic Δ^{11}-triterpen-28,13β-olide skeleton (6). The mass spectrum of **1** showed diagnostically important peaks at *m/z* 410 $[M-CO_2]^+$, 202, 207, and 189, which are common to some naturally occurring amyrins (4,7). The ^{13}C-nmr signals at δ 135.85 (C-11), 127.03 (C-12), and 89.82 (C-13) further confirmed the Δ^{11}-28,13β-olide system (6).

Additional evidence in support of the structural assignment for **1** has been secured from the following chemical correlations with the known compounds **2** and **3**. Hydrogenation of **1** with Raney Ni in EtOH was used to obtain the desired saturated oleanolic lactone **2**. These catalytic conditions also produced the hydrogenolysis of the Δ^{11}-28,13β-olide system affording oleanolic acid [**4**]. Epoxidation of compound **1** to yield derivative **3** was successful after treatment with H_2O_2/HOAc. Based on these results, we also attempted the preparation of compound **1** starting from oleanolic acid [**4**]. Treatment of **4** with Br_2/MeOH gave quantitatively the 12α-bromolactone **6** (4), which was easily dehydrohalogenated with 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (8) to afford **1**, whose physical and spectroscopic properties were identical with those observed for the natural product. These spectroscopic and chemical results led us to propose the structure of **1** as 3β-hydroxyolean-11-en-28,13β-olide.

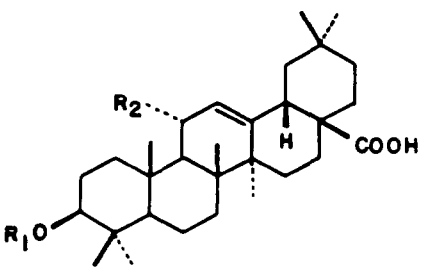
The hydrogenolysis process observed for compound **1** would represent the reverse transformation for the likely biosynthesis of this type of Δ^{11}-28,13β-olide moiety. For this reason some authors have assumed this system could be derived secondarily from an 11-hydroxy-Δ^{12}-17β-COOH precursor **7** during the isolation procedures (7). However, from a biogenetic point of view, the occurrence of **2** and **3** in this species, as well as the presence of this type of triterpene lactone in other related members of *Hyptis* (4), provides supporting evidence for the natural occurrence of **1**. It is important to note that the flavonoid content of *H. albida* is characterized by compounds with methoxylation of C-3, C-6, and C-8 positions of the apigenin and kaempferol nucleus. The 6,8-dimethoxylated flavonoids have a restricted distribution within the Lamiaceae, being found in genera such as *Sideritis*, *Mentha*, and *Thymus* (9,10). Therefore, these flavonoids could be of outstanding taxonomic interest.

Finally, all the compounds isolated from *H. albida* were inactive in the brine shrimp lethality bioassay ($LC_{50}>700$ ppm) (11).

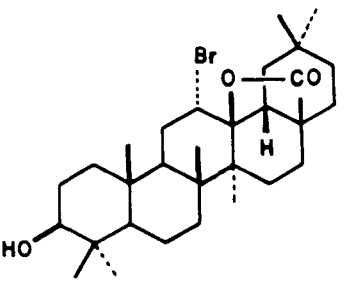
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For details on methods, see Pereda-Miranda and Gascón-Figueroa (4). Brine shrimp lethalities were determined as previously described (11).

PLANT MATERIAL.—The aerial parts of *H. albida* were collected in November 1987, in Michoacan, Mexico. Voucher specimens are deposited in the National Herbarium, Instituto de Biologia, UNAM, voucher No. 8528 M.



4 $R_1=R_2=H$
5 $R_1=Ac$, $R_2=H$
7 $R_1=H$, $R_2=OH$



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EXTRACTION.—Dried and finely powdered aerial parts of the plant (6 kg) were exhaustively extracted with Me_2CO at room temperature. After filtration, the solvent was evaporated, yielding a gum (327 g).

ISOLATION PROCEDURE.—The crude extract was chromatographed on a Si gel (2 kg) column, using a hexane-EtOAc gradient elution system. Fractions of 500 ml were collected. The low polarity fractions 22–30, eluted with *n*-hexane–EtOAc (4:1), contained a solid residue, which upon crystallization from EtOAc yielded 1.8 g (0.09% dry wt) of β-sitosterol. Fractions 43–50, eluted with *n*-hexane–EtOAc (7:3), crystallized to yield 230.4 mg (0.0115% dry wt) of kaempferol 3,7,4′-trimethyl ether, mp 146–147°, which was identified by standard procedures. Fractions 51–55 (198.4 mg) were resolved by cc, using $CHCl_3$-Me_2CO (9:1) as the eluent mixture, to give 45 mg (0.0007% dry wt) of oleanolic acid acetate [**5**], which was identical to an authentic sample.

The medium polarity fractions 56–76 (20.4 g), eluted with *n*-hexane–EtOAc (7:3), were rechromatographed over Si gel (800 g). Elution with *n*-hexane–CH_2Cl_2 (4:1) left a residue which crystallized to give 314.2 mg (0.0157% dry wt) of apigenin 7,4′-dimethyl ether, mp 174°, which was identical in all respects with an authentic sample. Subsequent fractions, eluted with *n*-hexane–CH_2Cl_2 (7:3), were combined to afford 36.5 mp (0.00182% dry wt) of betulinic acid. The *n*-hexane–CH_2Cl_2 (3:2) elution afforded 648.7 mg of a mixture of oleanolic and ursolic acids. This material was separated using the methodology previously described (4) to yield 64.8 mg (0.001% dry wt) of oleanolic acid [**4**] and 544.7 mg (0.009% dry wt) of ursolic acid. Both triterpenoids, as well as their methyl ester derivatives, were identical to standard samples.

The polar fractions 77–110 (60 g), eluted with *n*-hexane–EtOAc (3:2) from the original column, were rechromatographed on Si gel (1.2 kg). The elution was accomplished with *n*-hexane–EtOAc (4:1), and eluates of 250 mg were collected. This procedure afforded two subfractions (i and ii), which were subsequently resolved by cc. Rechromatography of subfraction i (14 g) was performed with *n*-hexane–EtOAc (4:1), and fractions of 50 ml were collected. Eluates 9–13 were washed with Et_2O to give 384.4 mg (0.0192% dry wt) of gardenin B, mp 180–181°, which was closely followed in the chromatographic elution by 12.5 mg (0.0002% dry wt) of the oleanolic lactone **2**, mp 278°. Subfraction ii (25.32 g) was resolved by Si gel cc (550 g), starting elution with $CHCl_3$ and then with increasing amounts of Me_2CO. Fractions 9–12 eluted with $CHCl_3$ afforded 72 mg (0.0036% dry wt) of nevadensin A, mp 195–196°, identified by direct comparison with an authentic sample. Fractions 14–16, eluted with $CHCl_3$-Me_2CO (9:1), crystallized spontaneously to yield 239.5 mg (0.0119% dry wt) of ermanin, mp 232–234°. Fractions 19–23 afforded 66 mg (0.0033% dry wt) of compound **1**. Finally, subsequent fractions 25–34 yielded upon trituration with Et_2O 150 mg (0.0075% dry wt) of the lactone **3**, mp 270–271°, which was identified by comparison with an authentic sample.

Compound **1**.—Colorless crystals: mp 260°, $[\alpha]_D$ −6.66° ($CHCl_3$, $c=0.15$); ir ν max ($CHCl_3$) cm^{-1} 3611, 2935, 1754, 1635, 1469, 1388, 1029, 868; ^1H nmr (80 MHz, $CDCl_3$) δ 0.80 (3H, s, Me-24), 0.90 (3H, s, Me-30), 0.93 (3H, s, Me-29), 1.00 (6H, s, Me-23, -25), 1.09 (6H, s, Me-26, -27), 3.23 (1H, dd, $J=10$, 6 Hz, H-3), 5.37 (1H, dd, $J=10$, 3 Hz, H-11), 6.04 (1H, dd, $J=10$, 1.5 Hz, H-12); ^{13}C nmr (20.0 MHz, $CDCl_3$) δ 39.00 (C-1), 27.26 (C-2), 78.89 (C-3), 38.39 (C-4), 54.95 (C-5), 17.75 (C-6), 34.49 (C-7), 41.54 (C-8), 53.34 (C-9), 36.47 (C-10), 135.85 (C-11), 127.03 (C-12), 89.82 (C-13), 41.74 (C-14), 27.08 (C-15), 21.41 (C-16), 44.10 (C-17), 50.66 (C-18), 37.36 (C-19), 31.46 (C-20), 33.30 (C-21), 31.30 (C-22), 27.81 (C-23), 14.95 (C-24), 15.77 (C-25), 19.00 (C-26), 18.31 (C-27), 178.35 (C-28), 25.48 (C-29), 23.59 (C-30); eims *m/z* (rel. int.) $[M]^+$ 454 (87.8), 439 (5), 436 (7), 426 (7), 421 (10), $[M-CO_2]^+$ 410 (71), 395 (7), 392 (6), 310 (10), 300 (10), 290 (7), 285 (9), 255 (18), 241 (12), 239 (8), 234 (9), 229 (10), 227 (10), 225 (7), 221 (4), 219 (6), 215 (15), 207 (8), 203 (17), 202 (14), 201 (20), 189 (37), 43 (100). Found C 79.19, H 10.08, $C_{30}H_{46}O_3$ requires C 79.24, H 10.11%.

CATALYTIC HYDROGENATION OF **1**.—A solution of **1** (10 mg) in EtOH (4 ml) was hydrogenated over Raney Ni (14 mg) at room temperature for 1 h. The product obtained after the usual workup was separated by tlc developing with *n*-hexane–EtOAc (4:1) to afford the oleanolic lactone **2** (6.2 mg) and oleanolic acid **4** (1.8 mg). Both compounds, as well as their acetylated derivatives, were identical to the natural products and identified by direct comparison with authentic samples (4).

Epoxidation of **1**.—Compound **1** (50 mg) was dissolved in CH_2Cl_2 (10 ml) and a mixture of 30% H_2O_2 (2.5 ml) and glacial HOAc (2.5 ml) was added uniformly at room temperature. The mixture was stirred for 2 days. Usual workup of the reaction gave **3** (38.7 mg), which was identical in all respects with an authentic sample.

Bromination of **4**.—A solution of **4** (1 g) in MeOH (50 ml) was treated with Br_2 (1.25 g) in MeOH (50 ml). After 30 min the solution was cooled in an ice bath to give colorless needles

(987.9 mg) of a product which melted at 225–226° and showed identical ^{1}H-nmr and mass spectra to those previously described for bromo lactone **6** (4,12).

Dehydrobromination of **6**.—Compound **6** (500 mg) was added to a solution of DBU (350 mg) and 5 ml of *o*-xylene which was allowed to remain at 165° for 12 h. The Et_2O extract of the reaction mixture was acidified with 5% HCl, washed with 5% aqueous Na_2CO_3, dried over anhydrous sulfate, and evaporated in vacuo. Crystallization of the residue from $CHCl_3$-MeOH (10:1) yielded 305 mg of a white crystalline material which was identical by ir, nmr, and mmp with the natural product **1**.

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