A New 24-nor-Oleanane Triterpenoid from *Salvia carduacea*

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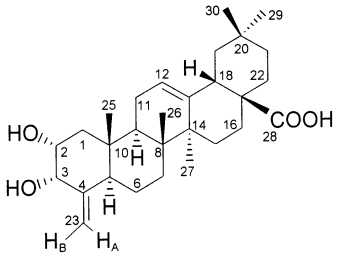
A new triterpenoid (**1**) has been isolated from the acetone extract of the leaves of *Salvia carduacea*, together with other compounds described previously. The structure of **1** [2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid] was elucidated by spectroscopic methods, particularly by extensive 1D and 2D NMR studies.

Salvia species (Labiatae) are pharmacologically active and used in folk medicine all around the world. Several plants of this genus have been associated with antibacterial, antitumor, and antioxidant activities and are used in the treatment of psoriasis, eczema, tuberculosis, and other diseases.[1] Continuing our studies on the terpenoid compounds from *Salvia* species,[2−4] we have now investigated the leaves of *Salvia carduacea* Benth., a plant that belongs to a group of annual *Salviae*, commonly known as chia species (also including *S. hispanica*, *S. tiliaefolia*, *S. columbariae*, etc.), native to southwestern North America (California, Texas, Mexico) and Central America. The use of chia, and particularly *S. hispanica* L., dates back to Mesoamerican history when the Aztec tribes appreciated its oil, roasted the edible seed, or soaked the seed in water to obtain a mucilaginous drink.[5] A literature survey showed that *S. carduacea* has not hitherto been studied chemically or biologically, except for its seed oil content.[6]

Column chromatography of the acetone extract of the leaves of *S. carduacea* (see Experimental Section) afforded β-sitosterol, large quantities of a mixture of ursolic and oleanolic acids,[7] another mixture of 2α-hydroxyursolic acid and 2α,3β-dihydroxyolean-12-en-28-oic acid (maslinic acid), which were characterized as their methyl ester derivatives,[7] and a new triterpenoid, whose structure **1** was elucidated as follows.



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Combustion analysis and low-resolution mass spectrometry established a molecular formula $C_{29}H_{44}O_4$ for **1**, and its IR spectrum showed carboxyl (3600−2650 br, 1690 cm^{-1}), hydroxyl (3430 cm^{-1}), and exocyclic methylene (3080, 1650, 898 cm^{-1}) absorptions. The ^1H NMR and COSY spectra of **1** revealed the presence of five C-Me singlets (δ 1.05, 0.82, 0.80, 0.70, and 0.63, 3H each signal), a trisubstituted olefinic double bond (δ 5.19, 1H, t, $J = 3.5$ Hz), an exocyclic methylene grouping (δ 4.94 and 4.60, 1H each, dd and t, respectively, $J_{gem} = 1.4$ Hz, $J_{allylic} = 1.2$ and 1.4 Hz, respectively), and a (C)−CH_2−CHOH−CHOH−(C) structural moiety (δ 1.22, 1H, dd, $J = 12.6$, 11.6 Hz; 1.68, 1H, dd, $J = 12.6$, 4.8 Hz; 3.68, 1H, ddd, $J = 11.6$, 4.8, 3.7 Hz; and 4.11, 1H, d, $J = 3.7$ Hz). The mass spectrum of **1** showed prominent ion fragments at *m*/*z* 248 (79%) and 203 (100%, base peak), characteristic of urs-12-ene or olean-12-ene derivatives[8] possessing a carboxyl group at the C-27, C-28, C-29, or C-30 position. These fragments are generated from the molecular ion (at *m*/*z* 456) by a retro-Diels−Alder fragmentation on the Δ^{12} double bond (ion at *m*/*z* 248) followed by loss of the carboxyl group (ion at *m*/*z* 203).[8]

All of the above data can be accommodated only on a 24-nor-olean-12-ene triterpenoid structure for compound **1**, with two secondary hydroxyl groups at the C-2 and C-3 positions, an exocyclic methylene involving the C-4 and C-23 carbons, and a carboxyl group placed at the C-27, C-28, C-29, or C-30 carbon.

The ^{13}C NMR, HSQC, and HMBC spectra of **1** confirmed the above deductions and established that the carboxyl group of this triterpenoid was at the C-28 position. The chemical shifts of the C-8, C-10−C-22, and C-26−C-30 carbons of **1** (see Experimental Section) were identical to those reported[9,10] for oleanolic acid, whereas the remaining carbons, except for C-7,[11] resonated at almost identical fields to those of przewanoic acid B (2α,3α-dihydroxy-12,-27-cyclo-24-nor-taraxera-4(23),14-dien-28-oic acid), a triterpenoid previously isolated[12,13] from *Salvia przewalskii*. Moreover, the HMBC spectrum of **1** showed connectivities between the C-23 carbon atom (δ 111.01 t) and the H-3 (δ 4.11 d) and H-5α (δ 2.05 br dd) protons and between the C-3 carbon (δ 75.34 d) and the H-2 proton (δ 3.68 ddd) and the H_2-1 (δ 1.68 dd and 1.22 dd) and H_2-23 (δ 4.94 dd and 4.60 t) methylene protons, whereas the C-25 carbon (δ 13.64) was correlated with both H_2-1 methylene protons and the H-5α and H-9α (δ 1.66 dd) methine protons, thus confirming the partial structure of ring A in this new nor-triterpenoid (**1**).

The 2α,3α-configurations of the two secondary hydroxyl groups of **1** were in agreement with the similarity of the chemical shift of its C-1−C-6, C-9, C-23, and C-25 carbons (δ 42.34 t, 68.68 d, 75.34 d, 149.99 s, 44.26 d, 20.00 t, 44.54 d, 111.01 t, and 13.64 q, respectively) and those reported for przewanoic acid B (δ 42.3 t, 69.2 d, 75.5 d, 150.2 s, 45.1 d, 20.4 t, 44.9 d, 111.3 t, and 14.5 q, respectively).[12,13] Moreover, the observed coupling constant values for the

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H-1α, H-1β, H-2β, and H-3β protons of **1** ($J_{1\alpha,1\beta}$ = 12.6 Hz, $J_{1\alpha,2\beta}$ = 11.6 Hz, $J_{1\beta,2\beta}$ = 4.8 Hz, and $J_{2\beta,3\beta}$ = 3.7 Hz) are compatible only with a spatial arrangement in which the H-1α and H-2β protons are axial substituents and the H-1β and H-3β protons are in an equatorial configuration.[14] This conclusion was strongly supported by NOE experiments, because irradiation at δ 3.68 (H-2β proton of **1**) caused NOE enhancements of the signals of the H-1α (+0.4%), H-1β (+2.2%), H-3β (+3.7%), and Me-25 (δ 0.63, +4.0%) protons, whereas on irradiating at δ 4.11 (H-3β) only the signals of the H-2β, H_B-23 (δ 4.94), and Me-25 protons were affected (NOE enhancements +3.4%, +3.4%, and +0.2%, respectively). The NOE observed between the H-2β and H_B-23 protons (see above) allowed the assignment of both C-23 methylene hydrogens, being the H_B-23 proton (δ 4.94), the pro-*Z* hydrogen.

Oleanane triterpenoids without the C-24 carbon, like **1**, are rare, and only a few compounds of this type have previously been isolated from natural sources, such as polygalasaponin XXVII, found in *Polygala japonica* (Polygalaceae),[11] vaccarosides F and H, extracted from *Vaccaria segetalis* (Caryophyllaceae),[15] and 23,28-dinor-18α-oleanane, isolated from an Egyptian petroleum sample.[16] To the best of our knowledge, compound **1** is the first example of a 24-nor-4(23),12-oleanadiene derivative isolated from a Labiatae species.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. The IR spectrum was obtained on a Perkin-Elmer Spectrum One spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$−$(CD_3)_2CO$ (9:1) solution on a Varian INOVA 400 apparatus at 400 and 100 MHz, respectively, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25) for protons and to the solvent signals (δ_{CDCl_3} 77.00) for carbons. ^{13}C NMR assignments were determined by gHSQC and gHMBC spectra. The MS was recorded in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analysis was made with a Carlo Erba EA 1108 apparatus. Merck Si gel no. 7734 (70−230 mesh) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis.

Plant Material. *Salvia carduacea* Benth. was cultivated at Torreblanca Experimental Station (Campo de Cartagena, Murcia, Spain). Original seeds of the species were obtained from the Department of Botany and Plant Sciences of the University of California, Riverside. Plant materials were collected in May 2000, and voucher specimens have been deposited in the Herbarium (CIDAHERB) of the Centro de Investigación y Desarrollo Agroalimentario, Murcia, Spain.

Extraction and Isolation. Dried and powdered leaves of *S. carduacea* (1.1 kg) were extracted with Me_2CO (2 × 6 L) at room temperature for 1 week. After filtration and concentration of the extract in vacuo at low temperature (40 °C) 51 g of residue remained. This residue was subjected to dry column chromatography on Si gel with a solvent gradient from 100% petroleum ether (bp 50−70 °C) to 100% EtOAc. The fractions eluted with petroleum ether−EtOAc (9:1) were decolorized by filtration through a pad of a mixture (1:1) of activated charcoal and Celite, eluting with EtOAc. Evaporation of the solvent and crystallization from MeOH yielded β-sitosterol (350 mg).

From the fractions eluted with petroleum ether−EtOAc (3:1 to1:1) large amounts of a mixture of ursolic and oleanolic acids[7] (4 g, in a 4:1 ratio, respectively) were isolated, whereas the fractions eluted with EtOAc−petroleum ether (4:1) yielded, after methylation with diazomethane and column chromatography [Si gel, petroleum ether−EtOAc (2:1) as eluent], 48 mg of a 3:1 mixture of methyl maslinate[7] (2α,3β-dihydroxyolean-12-en-28-oic acid methyl ester) and methyl 2α,3β-dihydroxy-urs-12-en-28-oate,[7] respectively.

A part (650 mg) of the residue obtained from the fractions eluted with EtOAc−petroleum ether (3:2) arising from the initial chromatography was subjected to column chromatography [Si gel Merck 0.040−0.063 mm, petroleum ether−EtOAc (3:2) as eluent], yielding pure **1** (34 mg).

β-Sitosterol was identified by its physical (mp, $[\alpha]_D$) and spectroscopic (^{1}H NMR) data, and the mixtures of ursolic and oleanolic acids and that of methyl maslinate and 2α-hydroxy-ursolate were characterized by a careful study of their ^{1}H NMR spectra.[7]

2α,3α-Dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (1): colorless needles ($CHCl_3$−*n*-hexane), mp 278−280 °C; $[\alpha]^{18}_D$ +101.5° (*c* 0.459, MeOH); IR (KBr) ν_{max} 3430 (OH), 3600−2650 br, 1690 (COOH), 3080, 1650, 898 (exocyclic methylene), 2940, 1460, 1385, 1050, 818, 755 cm^{-1}; ^{1}H NMR [$CDCl_3$−$(CD_3)_2CO$ (9:1)] δ 5.19 (1H, t, *J* = 3.5 Hz, H-12), 4.94 (1H, dd, J_{gem} = 1.4 Hz, $J_{23B,5\alpha}$ = 1.2 Hz, H_B-23, pro-*Z* hydrogen), 4.60 (1H, t, J_{gem} = $J_{23A,5\alpha}$ = 1.4 Hz, H_A-23, pro-*E* hydrogen), 4.11 (1H, d, $J_{3\beta,2\beta}$ = 3.7 Hz, H-3β), 3.68 (1H, ddd, $J_{2\beta,1\alpha}$ = 11.6 Hz, $J_{2\beta,1\beta}$ = 4.8 Hz, $J_{2\beta,3\beta}$ = 3.7 Hz, H-2β), 2.74 (1H, dd, $J_{18\beta,19\alpha}$ = 13.9 Hz, $J_{18\beta,19\beta}$ = 4.2 Hz, H-18β), 2.05 (1H, br dd, $J_{5\alpha,6\alpha}$ = 2.1 Hz, $J_{5\alpha,6\beta}$ = 11.9 Hz, H-5α), 1.68 (1H, dd, J_{gem} = 12.6 Hz, $J_{1\beta,2\beta}$ = 4.8 Hz, H-1β), 1.66 (1H, dd, $J_{9\alpha,11\alpha}$ = 2.3 Hz, $J_{9\alpha,11\beta}$ = 11.8 Hz, H-9α), 1.53 (1H, t, J_{gem} = $J_{19\alpha,18\beta}$ = 13.9 Hz, H-19α), 1.22 (1H, dd, J_{gem} = 12.6 Hz, $J_{1\alpha,2\beta}$ = 11.6 Hz, H-1α), 1.00 (1H, dt, J_{gem} = 13.6 Hz, $J_{15\alpha,16\alpha}$ = $J_{15\alpha,16\beta}$ = 2.8 Hz, H-15α), 1.05 (3H, s, Me-27), 0.82 (3H, s, Me-30), 0.80 (3H, s, Me-29), 0.70 (3H, s, Me-26), 0.63 (3H, s, Me-25), overlapped signals whose δ values were established from the HSQC spectrum at 1.89 (1H, m, H_B-11), 1.88 (1H, m, H_B-16), 1.85 (1H, m, H_A-11), 1.66 (1H, m, H_B-22), 1.63 (1H, m, H-15β), 1.52 (1H, m, H_A-16), 1.47 (1H, m, H_A-22), 1.42 (1H, m, H_B-7), 1.38 (1H, m, H-6α), 1.30 (1H, m, H-6β), 1.22 (2H, m, H_A-7 and H_B-21), 1.11 (1H, m, H_A-21), 1.05 (1H, m, H-19β); ^{13}C NMR [$CDCl_3$−$(CD_3)_2CO$ (9:1)] δ 181.68 (s, C-28), 149.99 (s, C-4), 143.81 (s, C-13), 122.20 (d, C-12), 111.01 (t, C-23), 75.34 (d, C-3), 68.68 (d, C-2), 46.14 (s, C-17), 45.56 (t, C-19), 44.54 (d, C-9), 44.26 (d, C-5), 42.34 (t, C-1), 41.72 (d, C-18), 40.90 (s, C-14), 39.13 (s, C-8), 37.60 (s, C-10), 33.56 (t, C-21), 32.84 (q, C-29), 32.17 (t, C-22), 30.73 (t, C-7), 30.44 (s, C-20), 27.28 (t, C-15), 25.76 (q, C-27), 23.92 (t, C-11),[10] 23.31 (q, C-30), 22.67 (t, C-16),[10] 20.00 (t, C-6), 16.89 (q, C-26), 13.64 (q, C-25); EIMS *m*/*z* 456 $[M]^+$ (1), 441 (0.5), 438 (0.7), 423 (2), 420 (1), 248 (79), 233 (9), 203 (100), 189 (18), 175 (13), 173 (13), 161 (13), 147 (14), 145 (15), 133 (44), 119 (36), 105 (42), 91 (39), 79 (28), 69 (31), 55 (38), 41 (34); *anal.* C 76.38%, H 9.83%, calcd for $C_{29}H_{44}O_4$, C 76.27%, H 9.71%.

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(10) It is of interest to indicate that the assignment of the C-11 and C-16 carbon atom resonances of **1** (δ 23.92 and 22.67 t, respectively) was supported by the HMBC spectrum, which showed connectivities between the carbon at δ 23.92 and the H-9α and H-12 protons and between the carbon at δ 22.67 and the H-18β and the C-15 and C-22 methylene protons. These unequivocal assignments seem to be in

contradiction with those reported[9] for oleanolic acid and its 3-epi, 3-oxo, or 2α-hydroxy derivatives (Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575), in which the C-16 carbon is always downfield shifted with respect to the C-11 carbon (δ 23.5−23.4 vs 23.2−23.1, respectively).

(11) The chemical shift of the C-7 carbon in **1** (δ 30.73) was very similar to those reported for the same carbon in other structurally related triterpenoids, like polygalasaponin XXVII [2β-hydroxy-24-nor-4(23),-12-oleanadiene-28-carboxy-3β-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, δ_{C-7} 31.9; see: Zhang, D.; Miyase, T.; Kuroyanagi, M.; Umehara, K.; Ueno, A. *Chem. Pharm. Bull.* **1996**, *44*, 173−179] and ilekudinol B [2α,3β-dihydroxy-24-nor-urs-4(23),12-dien-28-oic acid, δ_{C-7} 32.0; see: Nishimura, K.; Fukuda, T.; Miyase, T.; Noguchi, H,; Chen, X.-M. *J. Nat. Prod.* **1999**, *62*, 1061−1064].

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(13) The difference in the chemical shifts of the C-7 carbon for **1** (δ 30.73) and przewanoic acid B (δ 36.7) must be attributed to the absence in the latter of a methyl substituent on C-14, which precludes a shielding γ-effect on the C-7 carbon with respect to **1**.

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