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Triterpenoids from *Salvia wagneriana*

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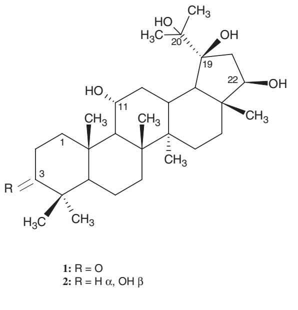
From the exudate of *Salvia wagneriana* Polak, beside the known ursolic acid, two pentaoxygenated triterpenoids were isolated. Their structures were determined as 3-oxo-11α,19β,20,22β-tetrahydroxy-lupane (**1**) and 3β,11α,19β,20,22β-pentahydroxy-lupane (**2**) using a combination of one- and two-dimensional NMR techniques.

1. Introduction

Various plants of the genus *Salvia* are used in folk medicine (Penso 1983) and some species are listed in modern Pharmacopoeias (Zepernick et al. 1984). Interesting compounds of this genus are flavonoids, essential oils, diterpenes and triterpenes, many of which possess anti-insect (antifeedant), anti-bacterial, anti-fungal, hallucinogenic and antioxidant activities (Tomás-Barberán and Wollenweber 1990; Rodríguez-Hahn et al. 1994; Cuvelier et al. 1996). During the course of our search on the constituents of various *Salvia* species (Romussi et al. 2001) we have investigated *Salvia wagneriana* Polak., an American species classified in the subgenus Calosphace, section Cardinales (Epling 1939). A literature survey revealed that no phytochemical studies had been performed on this species. In this communication we report on the isolation and identification of two new pentaoxygenated triterpenoids.

2. Investigations, results and discussion

Repeated column chromatography on Sephadex LH-20 and silica gel of the exudate of aerial parts of *S. wagneriana* yielded, beside ursolic acid, compounds **1** and **2**.



1: R = O
2: R = H α, OH β

The ^{13}C NMR spectrum of **1** (Table 1) exhibited 30 carbon resonances including eight methyls, eight methylenes, six methines, one oxo group (δ_C 218.8), two oxygenated tertiary carbons (δ_C 86.2 and 70.5) and five quaternary carbons. IR absorption bands at 3510, 3420 (br) and 1700 cm^{-1} confirmed the presence of hydroxyl and carbonyl groups. These results suggested a probable polyoxygenated triterpene. The ^1H- and ^{13}C NMR spectral data showed furthermore the presence of two methines [δ_C 71.2, 86.5; δ_H 3.90 (1 H, ddd, J = 11.0, 10.2, 4.8 Hz), 3.65 (1 H, dd, J = 10.2, 5.4 Hz)] attached to an oxygen function. From the ^1H–^1H–COSY, HSQC, HMBC spectral data the partial structures I: $(CH_3)C{-}CH_2{-}CH_2{-}CO{-}C(CH_3)_2{-}CH{-}$ [δ_C 16.8, 38.2, 41.9, 34.1, 218.8, 47.6, 27.4, 20.7, 55.2; δ_H 1.04 (3 H, s), 2.66 and 1.69 (1 H, ddd, J = 14.0, 7.8, 6.0 and 1 H, ddd, J = 14.0, 7.8, 7.6), 2.46 and 2.38 (1 H, m and 1 H, ddd, J = 14.0, 7.8, 7.8), 1.08 (3 H, s), 1.05 (3 H, s), 1.51 (1 H, m)]; II: $>CH{-}C(CH_3){-}CH_2{-}CH_2{-}$ [δ_C 54.8, 40.5, 16.2, 35.1 and 19.6; δ_H 1.53 (1 H, d, J = 10.2 Hz), 1.00 (3 H, s), 1.54 and 1.26 (both 1 H, m), 1.52 and 1.44 (both 1 H, m); III: $CH(OH){-}CH_2{-}CH{-}C(CH_3)<$ [δ_C 71.2, 39.2, 41.3, 49.7, 16.1; δ_H 3.90 (1 H, ddd, J = 11.0, 10.2, 4.8 Hz), 2.07 and 1.38 (1 H, m and 1 H, ddd, J = 12.0, 11.0, 10.2 Hz), 1.78 (1 H, ddd, J = 10.2, 10.2, 3.0 Hz); 0.90 (3 H,s)]; IV: $>CH{-}C(OH)(isopropyl){-}CH_2{-}CH(OH){-}C(CH_3){-}CH_2{-}CH_2{-}$ [δ_C 49.2, 86.2, 70.5, 23.7 27.5, 34.7, 86.5, 34.6, 27.1, 25.9, 30.9; δ_H 1.91 (1 H, m), 1.10 (3 H,s), 1.17 (3 H,s), 1.85 and 1.68 (both 1 H, m), 3.65 (1 H, dd, J = 10.2, 5.4), 1.13 (3 H, s), 1.35 (2 H, m), 1.39 and 1.08 (both 1 H, m)] were established. From the HMBC correlations (Table 1) these partial structures could be put in a lupane structure with an oxo group at the C-3 and four hydroxyl groups at C-11, C-19, C-20 and C-22. The proton signal at δ_H 3.90 (H-11) showed two axial/axial and one axial/equatorial coupling constants: thus this proton is β axial and the hydroxyl group at C-11 is α equatorial; the proton signal at δ_H 3.65 (H-22) was correlated in the Roesy spectrum with the methyl proton signals at δ_H 1.10 and 1.17 (29 and 30 CH_3); thus the H-22 must be α as the isopropyl group and the hydroxyl group β. From these facts the structure of **1** was determined as 3-oxo-11α,19β,20,22β-tetrahydroxy-lupane.

One dimensional ^1H- and ^{13}C NMR spectra of **2** (Table 2) showed that this compound has a very similar structure to **1** and differs only in ring A. All these results were con-

Table 1: NMR spectral data for compound 1 (δ values, $CDCl_3$, ^{13}C-NMR at 150 MHz, ^{1}H NMR at 600 MHz)

C	^{13}C	DEPT	^{1}H (J in Hz)	HMBC correlations of the C
1	41.9	CH2	2.66 ddd (14.0, 7.8, 6.0), 1.69 ddd (14.0, 7.8, 7.6)	2.46, 2.38, 1.53, 1.51, 1.04
2	34.1	CH2	2.46 m, 2.38 ddd (14.0, 7.8, 7.8)	2,66, 1.69
3	218.8	C	–	2.66, 1.69, 2.46, 2.38, 1.05, 1.08
4	47.6	C	–	2.46, 1.52, 1.51, 1.44, 1.08, 1.05
5	55.2	CH	1.51 m	2.66, 1.69, 1.54, 1.53, 1.52, 1.44, 1.26, 1.08, 1.05,1.04
6	19.6	CH2	1.52 m, 1.44 m	–
7	35.1	CH2	1.54 m, 1.26 m	1.52, 1.44, 1.00
8	40.5	C	–	1.54, 1.26, 1.00, 0.90
9	54.8	CH	1.53 d (10.2)	3.90, 2.66, 2.07, 1.69, 1.38, 1.04, 1.00
10	38.2	C	–	2.66, 2.46, 2.38, 1.69, 1.53, 1.51, 1.04
11	71.2	CH	3.90 ddd (11.0, 10.2, 4.8),	1.53, 2.07, 1.38
12	39.2	CH_2	2.07 m 1.38 ddd (12.0, 11.0, 10.2)	3.90, 1.91, 1.78
13	41.3	CH	1.78 ddd (10.2, 10.2, 3.0)	1.39
14	49.7	C	–	2.07, 1.53, 1.38, 1.35, 1.00, 0.90
15	30.9	CH_2	1.39 m, 1.08 m	1.35, 0.90
16	25.9	CH_2	1.35 m	1.91
17	34.6	C	–	1.91, 1.85, 1.78, 1.68, 1.13
18	49.2	CH	1.91 m	1.13
19	86.2	C	–	1.91, 1.85, 1.78, 1.68, 1.17, 1.10
20	70.5	C	–	1.85, 1.17, 1.10
21	34.7	CH_2	1.85 m, 1.68 m	1.91
22	86.5	CH	3.65 dd (10.2, 5.4)	1.91, 1.85, 1.68, 1.35, 1.13
23	27.4	CH_3	1.08 s	1.05
24	20.7	CH_3	1.05 s	1.08
25	16.8	CH_3	1.04 s	2.66, 1.69, 1.53, 1.51
26	16.2	CH_3	1.00 s	1.54, 1.53, 1.26
27	16.1	CH_3	0.90 s	1.78, 1.39, 1.08
28	27.1	CH_3	1.13 s	3.65, 1.91
29	23.7	CH_3	1.10 s	1.17
30	27.5	CH_3	1.17 s	1.10

Table 2: NMR spectral data for compound 2 (δ values, $CDCl_3$, ^{13}C-NMR at 150 MHz, ^{1}H-NMR at 600 MHz)

C	^{13}C	DEPT	^{1}H (*J* in Hz)	HMBC correlations of the C
1	40.9	CH_2	2.57 ddd (15.0, 3.6, 3.0), 1.15 m	3.20, 1.60, 1.20, 1.04
2	27.5	CH_2	1.60 m, 1.20 m	3.20, 2.57, 1.15
3	78.4	CH	3.20 dd (10.2, 5.1)	2.57, 1.60, 0.99, 0.80, 0.76
4	39.4	C	–	1.60, 1.20, 0.99, 0.80, 0.76
5	56.0	CH	0.76 nd (12.0)	2.57, 1.54, 1.22, 1.15, 1.04, 0.99, 0.80
6	18.1	CH_2	1.55 m, 1.45 m	1.54
7	36.0	CH_2	1.54 m, 1.22 m	1.55, 1.45,1.42, 0.98, 0.76
8	40.7	C	–	3.95, 1.55, 1.77, 1.54, 1.45, 1.22, 1.41, 1.08, 0.98, 0.91
9	55.7	CH	1.42 d (10.2)	3.95, 2.57, 2.10, 1.36,1.15, 1.04, 0.98, 0.76
10	38.9	C	–	2.57, 1.42, 1.04, 0.76
11	71.3	CH	3.95 ddd (11.0, 10.2, 4.8)	2.10, 1.36
12	39.6	CH_2	2.10 m, 1.36 ddd (12.0, 11.0, 10.2)	1.92
13	41.1	CH	1.77 ddd (10.2, 10.2, 3.0)	3.95, 2.10, 1.36, 1.92
14	49.8	C	–	2.10, 1.77, 1.41, 1.36, 1.08, 0.98, 0.91
15	31.0	CH_2	1.41 m, 1.08 m	1.77, 1.36, 0.91
16	25.9	CH_2	1.36 m	1.92
17	34.7	C	–	1.92, 1.86, 1.68, 1.14
18	49.5	CH	1.92 m	2.10, 1.86, 1.77, 1.36, 1.14
19	86.2	C	–	1.92, 1.77, 1.19, 1.11
20	70.4	C	–	1.86, 1.19, 1.11
21	34.8	CH_2	1.86 m, 1.68 m	1.92
22	86.5	CH	3.67 dd (10.0, 5.0)	1.92, 1.86, 1.68, 1.36, 1.14
23	28.2	CH_3	0.99 s	3.20, 0.80, 0.76
24	15.4	CH_3	0.80 s	3.20, 0.99, 0.76
25	16.6	CH_3	1.04 s	2.57, 1.15, 0.76
26	16.7	CH_3	0.98 s	1.54, 1.42
27	16.3	CH_3	0.91 s	1.77, 1.41, 1.08
28	27.0	CH_3	1.14 s	1.92
29	23.8	CH_3	1.11 s	1.19
30	27.6	CH_3	1.19 s	1.11

firmed from the $^1H-^1H$–COSY, HSQC, and HMBC spectral data. The signals at δ_C 27.5 (δ_C 1.60 and 1.20), 78.4 (δ_H3.20), 39.4, 28.2 (δ_H 0.99), 15.4 (δ_H 0.80) and 56.0 (δ_H 0.76) were assigned to the 2-CH_2, 3-CH(OH), 4-C, 23-CH_3, 24-CH_3 and 5-CH respectively from HMBC spectral data. The 3-H proton signal at δ_H 3.20 showed one axial/axial and one axial/equatorial coupling constant and thus is α axial and the hydroxyl group at 3-C is β equatorial. Compound **2** was thus established as 3β,11α,19β,20,22β-pentahydroxy-lupane.

As far as we know neither isomeric oxo-tetrahydroxy- nor pentahydroxy-lupanes were yet described.

3. Experimental

3.1. General procedures

Sephadex LH-20 (Pharmacia) and silica gel 60 (Merk 230–400 mesh) were used for CC. The melting points are uncorrected and were measured on a melting point apparatus Tottoli-Apparat Büchi. ^{1}H and ^{13}C NMR spectra were performed with a BRUKER DRX 600 spectrometer using TMS as internal standard. IR spectra were obtained using a Perkin-Elmer 1310 spectrophotometer. The optical rotation was recorded on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp 589 nm and 1 dm microcell. Elemental C, H analyses were obtained on a Carlo Erba Model EA 1110 CHNS-O analyzer. All the results were in an acceptable range. Aluminium sheets silica gel 60 F_{254} (Merck, 0.2 mm thick) with $CHCl_3$–MeOH (12 : 0.4, v/v) as eluent were used for TLC and spots were detected by spraying 50% H_2SO_4, followed by heating.

3.2. Plant material

Aerial parts of *S. wagneriana* Polak were collected in spring 2000 from the collection of *Salvia* species established and available in the Hanbury Botanical Gardens of La Mortola, Ventimiglia (Italy) and a voucher specimen is deposited at the Herbarium of the Gardens.

3.3. Extraction, isolation and characterization of the compounds

Fresh aerial parts (2.85 kg) were immersed in CH_2Cl_2 for 20 s. After filtration the extraction solvent was removed under reduced pressure. The exudate (22 g) was chromatographed on Sephadex LH-20 columns, (43 × 4 cm, 2 g portions, analytical TLC control) using $CHCl_3$–MeOH (6 : 4) as eluent to give in order of elution fractions with mixture of ursolic acid and **1** (5 g) and then fractions with ursolic acid, **1** and **2** (8.4 g). These two fractions groups were chromatographed on silica gel columns (47 × 3.5 cm, 4.5 g portions, analytical TLC control) using mixtures of increasing polarity of *n*-hexane-$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH. Elution with $CHCl_3$ afforded first fractions with ursolic acid and then fractions with **1**. Elution with $CHCl_3$–MeOH (95 : 5) afforded fractions with **2**. Yields: 2.3 g of crude ursolic acid, which was recrystallized from EtOH (identified by direct TLC and IR comparison with authentic sample), 1.06 g and 0.16 g of crude **1** and **2** respectively, which were recrystallized from MeOH–H_2O.

Compound **1** (3-oxo-11α,19β,20,22β-tetrahydroxy-lupane): colourless crystals, m. p. 188–189 °C. $[\alpha]_D^{20}$ + 16 ($CHCl_3$, c 1.00). IR ν_{max}^{KBr} cm^{-1}: 3510, 3420 br (–OH), 1700 (>C=O), 1463, 1388, 1375, 1368. ^{1}H and ^{13}C NMR: Table 1.

Compound **2** (3β,11α,19β,20,22β-pentahydroxy-lupane): colourless crystals, m.p. 191–193 °C. $[\alpha]_D^{20}$ + 37 ($CHCl_3$, c 1.00). IR ν_{max}^{KBr} cm^{-1}: 3425 br (–OH), 1470, 1395, 1380, 1365. ^{1}H and ^{13}C NMR: Table 2.

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