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WITHANOLIDES FROM *JABOROSA LEUCOTRICHA*

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Key Word Index—*Jaborosa leucotricha*; Solanaceae; withanolides;19-norwithanolides; jaborosalactones.

Abstract—From leaves of *Jaborosa leucotricha* three new withanolides (20*S*,22*R*)-1,6β,17α,27-tetrahydroxy-19-norwitha-1,3,5(10),24-tetraenolide, (20*R*,22*R*)-5α,6β,14β,17β-tetrahydroxywitha-2,24-dienolide and (22*R*, 23*S*)-5α,6β,17β-trihydroxy-12β-methoxy-12,22-epoxiergosta-2,24-dien-26,23-olide have been isolated and fully characterized by spectroscopic methods.

INTRODUCTION

The withanolides are a group of C_{28}-steroids found exclusively within the botanical family Solanaceae, although a few related compounds have been found in other families [1]. The aerial parts of *Jaborosa leucotricha* (Speg.) contain, among others, several 19-hydroxywithanolides and a ring A aromatic withanolide [2, 3]. Continuing our investigation on the constituents of this plant, we have isolated two new withanolides named jaborosalactone 7 (**1**) and jaborosalactone 8 (**2**), the known jaborotetrol (**3**) previously isolated from *Jaborosa magellanica* [4] and its 12-*O*-methyl derivative (**4**).

RESULTS AND DISCUSSION

The FAB-mass spectrum (*m*-nitrobenzylalcohol, KCl) of jaborosalactone 7 (**1**), $C_{27}H_{36}O_6$, showed a $[M+K]^+$ ion at *m/z* 495 (18%). The ^1H NMR and ^{13}C NMR chemical shifts of compound **1** (Tables 1 and 2) were closely related to those of jaborosalactone Q (**5**) [2]. A phenolic A-ring was evident from the ^1H NMR resonances between δ 6.63 and 7.09, while H-6 was observed as a broad singlet at δ 4.76. Regarding the side chain, the presence of a singlet at δ 2.05 (CH_3-28) and a broad singlet at δ 4.38 (CH_2-27) were consistent with a 27-hydroxylated α,β-unsaturated lactone ring. The main difference with the NMR data of compound **5** was the downfield shift of H-22 (from δ 4.45 in **5** to δ 4.68 in **1**). This observation, in combination with the non-protonated carbon signal appearing at δ 85.1 in the ^{13}C NMR spectrum, indicated the presence of a hydroxyl group at the 17α-position. Selective irradiation at δ 2.35 (H-20) collapsed the 3.5 Hz coupling in the H-22 signal, turning it into a triplet (8.8 Hz), while irradiation of H-22 (δ 4.68) turned the signal at δ 2.56 (H-23) into a broad singlet, thus confirming the equivalence between H-23α and H-23β. DEPT spectral data and ^1H-^1H correlations (COSY-45) were consistent with the proposed structure.

The ^1H NMR spectrum of jaborosalactone 8 (**2**) (Table 1) exhibited, in the low field region, signals at δ 5.86 and 6.62 typical of a 2-en-1-one system. The broad singlet at δ 3.62 was assigned to the 6α-H of a 5α,6β-diol. At the highfield end of the spectrum, two methyl signals were observed at δ 1.86 and 1.94 indicating an α,β-unsaturated δ-lactone bearing methyl groups at positions C-24 and C-25. The CH_3-18 singlet appearing at δ 1.12 was consistent with the presence of hydroxyl groups at positions 17β and/or 14β. Final confirmation of structure **2** was provided by the ^{13}C NMR and DEPT spectra (Table 2). The resonances for non protonated carbons at δ 88.0 and 88.1 were assigned to C-14 and C-17, respectively, these high chemical shift values are characteristic of a 14β,17β-diol. The only other withanolides reported with this substitution pattern in ring D have been isolated from *Jaborosa bergii* [5]. The FAB-mass spectrum (*m*-nitrobenzylalcohol, KCl) of jaborosalactone 8 showed an $[M+K]^+$ ion at *m/z* 527 (24%), consistent with the formula $C_{28}H_{40}O_7$.

The molecular formula of 12-*O*-methyl jaborosotetrol (**4**) was determined by HR-EIMS as $C_{29}H_{40}O_8$. The EI mass spectrum of this compound showed a molecular ion at *m/z* 516 (57%), a peak at *m/z* 486

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Table 1. ^{1}H NMR spectral data for relevant protons of compounds **1**, **2** and **4** (in $CDCl_3$, δ from TMS)

H	1	2	4
2	6.63 *dd* (7.7; 1.5)	5.86 *dd* (9.8; 2.5)	5.82 *dd* (10.3; 2.7)
3	7.09 *t* (7.7)	6.62 *ddd* (9.8; 5.0; 2.5)	6.60 *ddd* (10.3; 3.0; 2.7)
4α	6.95 *dd* (7.7; 1.5)	2.06 *dd* (20.1; 2.8)	2.60 *dd* (15.0; 3.0)
4β	—	3.26 *dt* (20.1; 2.5)	3.20 *dt* (15.0; 2.7)
6α	4.76 *br s* ($W_{1/2}$ = 3.0)	3.62 *br s* ($W_{1/2}$ = 2.9)	3.67 *br s* ($W_{1/2}$ = 3.3)
18	0.92 *s*	1.12 *s*	0.98 *s*
19	—	1.30 *s*	1.26 *s*
20	2.35 *m*	—	2.30 *m*
21	1.06 *d* (7.0)	1.10 *d* (7.3)	1.00 *d* (6.6)
22	4.68 *td* (8.2; 2.0)	4.76 *ddd* (10.0; 6.9; 2.9)	3.72 *dd* (12.0; 1.5)
23	2.56 *d* (8.2)	—	4.88 *br s* ($W_{1/2}$ = 3.0)
27	4.38 *brs*	1.86 *s*	1.86 *s*
28	2.05 *s*	1.94 *s*	2.02 *s*
OMe	—	—	3.16 *s*

Coupling constants (in parentheses) in Hz.
Assignments are based on COSY-45 spectra and coupling constants.

Table 2. ^{13}C NMR spectral data of compounds **1**, **2** and **4** (in $CDCl_3$, δ from TMS)

C	1	2	4
1	154.7	205.3	203.7
2	115.6	128.2	128.2
3	127.1	142.2	141.8
4	123.1	35.5	32.8
5	140.6	77.3	77.3
6	68.1	74.0	74.0
7	34.3	29.7	29.8
8	29.9	35.0	29.4
9	33.0	36.8	38.3
10	126.7	51.9	51.4
11	25.5	22.2	35.1
12	(33.3)	27.4	102.0
13	44.1	50.7	48.0
14	48.9	88.0	45.6
15	23.2	31.9	22.7
16	36.8	36.9	33.8
17	85.1	88.1	80.0
18	15.5	14.9	10.6
19	—	15.5	15.7
20	42.8	41.6	35.1
21	9.4	9.3	12.2
22	79.3	77.5	69.1
23	(33.0)	32.7	82.7
24	154.2	151.4	156.2
25	125.2	121.1	125.1
26	167.1	167.9	175.6
27	57.5	12.2	8.4
28	20.0	20.4	12.1
OMe	—	—	47.5

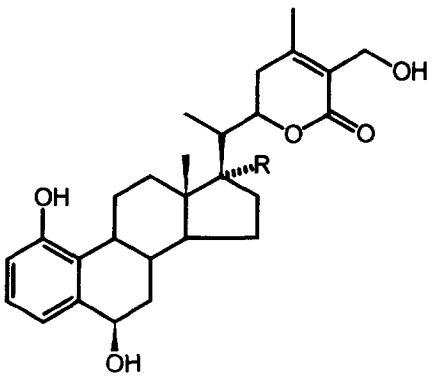
Values in parentheses may be interchanged. DEPT spectra were in agreement with the assignments given.

(29%) arising from the loss of methanol and a peak at *m/z* 373 (46%) corresponding to the cleavage between C-23 and C-22 followed by the loss of water. The ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) were closely related to those of jaborotetrol (**3**) [4], the main differences observed were the appearance of resonances for a methoxy group (a sharp three-proton singlet at δ 3.16 and a methyl carbon at δ 47.5) and the downfield shift of the C-12 resonance to δ 102.0 (as compared to **3**). The 12-*O*-methyl derivative of trechonolide A (a closely related (22 → 12)-hemiketalic withanolide) isolated by Lavie *et al.* [6] from *Jaborosa laciniata* was considered by the authors to be an artifact originating from extraction with boiling methanol, although our extraction procedure does not involve heating with methanol at any stage and is carried out at room temperature, the high reactivity of these hemiketals strongly suggests that the methoxy derivative **4** may be formed during the extraction procedure.

The isolation of jaborosalactone 7 (**1**) further supports the degradation route for withanolides previously proposed for this plant, which implied oxidative elimination of a C-19 methyl substituent and formation of an aromatic A ring [3]. Thus, compound **1** would derive from the corresponding 17α-hydroxywithanolide jaborosalactone L (**6**) a major component of *J. leucotricha* [7].

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solns, on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively. Multiplicity determinations (DEPT) and 2D spectra (COSY) were obtained using standard Bruker software. Chemical shifts are given in ppm downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 at 70 eV by direct inlet, FABMS and HREIMS were measured on a VG ZAB-BEQQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 560 FT-IR and a Hewlett-Packard 8451A spectrophotometer, respectively. CC was performed on Kieselgel S (0.031–0.063 mm); TLC was performed on Si gel 60 F254 (0.2 mm thick).



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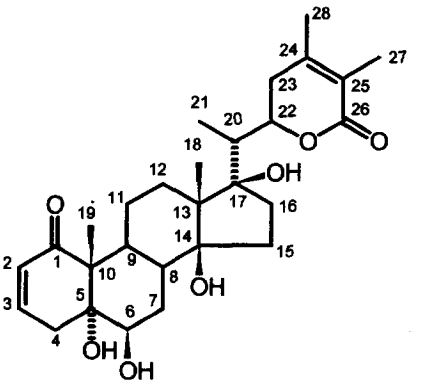

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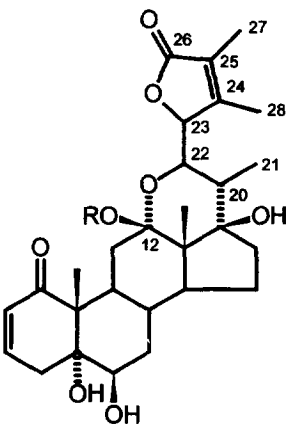

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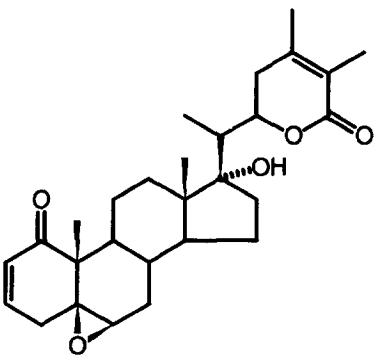
4 R=Me



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Plant material and isolation procedure. Whole *Jaborosa leucotricha* plants, were collected in Lujan, Carrizal, Mendoza Province, Argentina, during the autumn (May 1992). A voucher specimen is deposited at the herbarium of Universidad Nacional de San Luis, Argentina. Dried and pulverized aerial parts (1.2 kg) of *J. leucotricha* were extracted and fractionated as previously described [3]. Further fractionation of the most polar frs by prep. TLC yielded compounds **1–4**.

Jaborosalactone 7 (**1**). (7 mg); crystals from EtOAc–hexane, mp 185–186°; UV λ_{max}^{MeOH} nm: 222, 278. IR ν_{max}^{KBr} cm^{-1} 3406, 2910, 1690, 910. EIMS *m/z* (rel.int.): 438 $[M-H_2O]^+$ (9), 420 $[M-2H_2O]^+$ (9), 251 $[M-H_2O-$side chain$]^+$ (65). FABMS (*m*-nitrobenzylalcohol, KCl), *m/z* (rel. int.): 495 $[M+K]^+$ (18).

Jaborosalactone 8 (**2**). (4 mg); crystals from EtOAc–hexane, mp 181–182° (dec.); UV λ_{max}^{MeOH} nm: 224. IR ν_{max}^{KBr} cm^{-1} 3500, 2963, 1688, 1050. EIMS *m/z* (rel. int.): 470 $[M-H_2O]^+$ (2), 452 $[M-H_2O]^+$ (9), 434 $[M-3H_2O]^+$ (15), 345 $[M-H_2O-$side chain$]^+$ (9), 327 $[M-2H_2O-$side chain$]^+$ (41), 238 (100). FABMS (*m*-nitrobenzylalcohol, KCl), *m/z* (rel. int.): 527 $[M+K]^+$ (24). HREIMS *m/z*: 470.2661 $[M\text{-}H_2O]^+$ (calcd for $C_{28}H_{38}O_6$: 470.2668).

Jaborosotetrol (**3**). (9 mg); had ^1H and ^{13}C NMR spectra identical to those described previously [4].

12-O-*Methyljaborosotetrol* (**4**). (7 mg); crystals from EtOAc, mp 210–215°; UV λ_{max}^{MeOH} nm: 226. IR ν_{max}^{KBr} cm^{-1} 3543, 2950, 1750, 1019. EIMS *m/z* (rel.int.): 516 $[M]^+$ (57), 498 $[M-H_2O]^+$ (28), 486 $[M-CH_2O]^+$ (29), 485 $[M-MeO]^+$ (100), 480 $[M-2H_2O]^+$ (13), 444 $[M-4H_2O]^+$ (39), 373 $[M-H_2O-$side chain$]^+$ (46), 355 $[373\text{-}H_2O]^+$ (20). HREIMS *m/z* 516.2721 $[M]^+$ (calcd for $C_{29}H_{40}O_8$: 516.2723).

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