

14 β ,17 β -DIHYDROXYWITHANOLIDES FROM *JABOROSA BERGII*

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(Received in revised form 25 March 1988)

Key Word Index—*Jaborosa bergii*; Solanaceae; 14 β ,17 β -dihydroxywithanolides; jaborosalactol M; jaborosalactone M; jaborosalactol N.

Abstract—From leaves of *Jaborosa bergii* five new withanolides were isolated and studied by spectroscopic (^1H and ^{13}C NMR, MS) and chemical methods. The new products were characterized as the epoxylactol and epoxylactone derivatives of 27-deoxy-2,3-dihydrojaborosalactone A having hydroxyl groups at C-14 β and C-17 β (jaborosalactol M and jaborosalactone M), the corresponding 2,3-dehydro derivatives, and the related 2,3,4,5-didehydro-6 β -hydroxy-epoxylactol (jaborosalactol N). The 14 β ,17 β -dihydroxywithanolides have not been previously reported.

INTRODUCTION

Jaborosa Miers. is a South American genus that comprises ca 12 different species growing mainly in Argentina [1]. From *Jaborosa bergii* Hieron, we have isolated five new withanolides, all of which had in common the presence of hydroxyl groups in positions C-14 and C-17 with the β -configurations. This would be the first time that naturally occurring 14 β ,17 β -dihydroxywithanolides have been reported.

RESULTS AND DISCUSSION

The ethyl ether extract of *J. bergii* was subjected to column chromatography [2] rendering, besides low polarity components and pigments, four fractions containing withanolides. Flash chromatography of fraction 2 gave the main components **1a** and **2a** which could not be separated either by TLC or reversed phase HPLC. The ^1H NMR spectrum of the mixture indicated that it consisted of a 1-oxo-5 β ,6 β -epoxywithanolide (**1a**) and its 2,3-dehydro derivative (**2a**) in a 2:1 ratio (Table 1). The ^{13}C NMR spectrum of the mixture confirmed this assumption and allowed the assignments of the individual resonances to compounds **1a** and **2a** (Table 2). Hydrogenation of the mixture afforded compound **1a** as the only product; its ^1H NMR spectrum (Table 1) presented a doublet at δ 4.99 (J = 11 Hz) which was assigned to H-26 of an epoxy lactol side chain. The coupling disappeared upon exchange with deuterium oxide, together with a doublet at δ 3.56 (J = 11 Hz) which corresponded to the lactol hydroxy group. The methyl groups at C-24 and C-25 resonated at δ 1.40 and 1.41, being consistent with the epoxylactol structure and the α -stereochemistry for this epoxide ring at the lactol side chain and for the C-26 hydroxyl group [3]. H-22 was observed as a double triplet at δ 4.04, and H-21 as a doublet at 0.97 indicating the absence of a hydroxyl group at C-20. The methyl-19 singlet (at δ 1.21) and the H-6 doublet at 3.22 (J = 3 Hz) corresponded to a 1-oxo-5 β ,6 β -epoxywithanolide. The methyl-18 singlet at δ 1.10 indicated the presence of hydroxyl groups at positions C-17 and/or C-14 [4].

The ^{13}C NMR spectrum of **1a** (Table 2) was in agreement with the above findings; the α -configuration for the C-26 hydroxyl and the 24,25-epoxide of **1a** (and **2a**) was further supported by the close similarity of the ^{13}C chemical shifts of C-22 through C-28 with those of Nic-2 [5]. The presence of tertiary hydroxyl groups at C-14 and C-17 was evidenced by the signals at δ 87.5 and 88.5, respectively. The high chemical shift values of these carbons suggested that probably both hydroxyl groups were oriented towards the same face of the steroid skeleton [5, 6]. Comparison of the chemical shift values for the D-ring carbons as well as for C-18, C-20 and C-21 with those of related withanolides having 14 α ,17 β -[4], 14 α ,17 α - and 14 β ,17 α -[6] configurations was not successful, suggesting the possibility of a 14 β ,17 β -orientation for the tertiary hydroxyls.

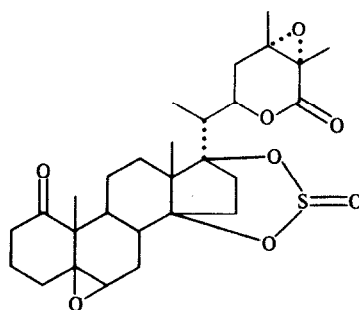
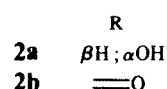
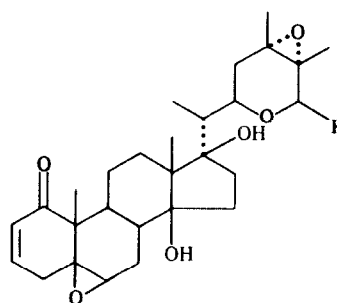
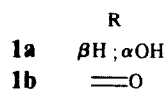
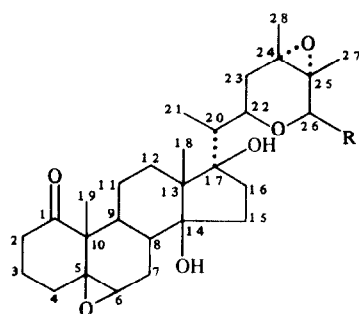
The pyridine induced shifts observed in the ^1H NMR spectrum were significant for H-18 and H-21 (Table 1); the downfield shift of H-18 (0.24 ppm) indicated that at least one of the tertiary hydroxyls (either C-14 or C-17) should have the β -orientation [4, 7].

Final proof for the 14 β ,17 β -configuration was obtained as follows: lactol **1a** was oxidized with Jones reagent to the corresponding epoxylactone **1b** [8]. This had ^1H and ^{13}C NMR spectra (Tables 1 and 2) closely related to those of **1a**. The ^1H NMR spectrum showed the disappearance of the signal of H-26, and the H-22 signal was shifted downfield to δ 4.92. In the ^{13}C NMR spectrum, C-26 appeared at δ 170.0, while C-22 was shifted to 74.8. Treatment of lactone **1b** with thionyl chloride in pyridine at -12° [9] afforded the cyclic sulphite **3**. Considering that the pyridine induced shifts had established that either the C-14 or the C-17 hydroxyl should have a β -orientation, formation of the cyclic ester indicated that both groups were β -oriented. Further analysis of the ^1H NMR spectrum of **3** indicated that H-18 was shifted downfield 0.28 ppm with respect to lactone **1b**. This may be explained considering that the methyl hydrogens are positioned in the deshielding zone of the sulphur-oxygen double bond which points away from C-18. No deshielding of H-18 was observed in the cyclic sulphite of a 14 α ,17 α -dihydroxy-steroid [9]. The spectral data for

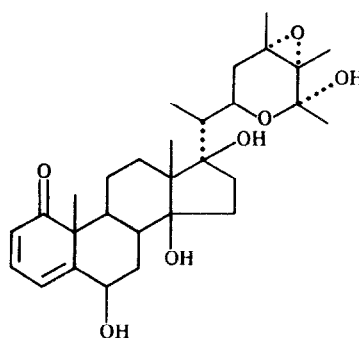
Table 1. ^1H NMR spectral data for relevant protons of compounds 1–4

H	1a	1b	2a	2b	3	4
2			6.10 <i>dd</i> $J=10; 4\text{ Hz}$	6.10 <i>dd</i> $J=10; 4\text{ Hz}$		6.01 <i>dd</i> $J=9.5; 1\text{ Hz}$
3			6.85 <i>m</i>	6.85 <i>m</i>		6.92 <i>dd</i> $J=9.5; 6\text{ Hz}$
4						6.14 <i>dd</i> $J=6; 1\text{ Hz}$
6	3.22 <i>d</i> $J=3\text{ Hz}$	3.23 <i>d</i> $J=3\text{ Hz}$	3.22 <i>d</i> $J=3\text{ Hz}$	3.23 <i>d</i> $J=3\text{ Hz}$	3.21 <i>d</i> $J=3\text{ Hz}$	4.62 <i>br s</i>
18	1.10 <i>s</i> [1.34]	1.12 <i>s</i> [1.33]	1.11 <i>s</i> [1.34]	1.14 <i>s</i>	1.40 <i>s</i>	1.18 <i>s</i>
19	1.21 <i>s</i>	1.23 <i>s</i>	1.26 <i>s</i>	1.27 <i>s</i>	1.23 <i>s</i>	1.49 <i>s</i>
21	0.97 <i>d</i> $J=7\text{ Hz}$ [1.39]	1.01 <i>d</i> $J=7\text{ Hz}$ [1.19]	0.97 <i>d</i> $J=7\text{ Hz}$ [1.39]	1.01 <i>d</i> $J=7\text{ Hz}$	1.05 <i>d</i> $J=7\text{ Hz}$	0.96 <i>d</i> $J=7\text{ Hz}$
22	4.04 <i>dt</i> $J=12; 4\text{ Hz}$ [4.84]	4.92 <i>dt</i> $J=12; 4\text{ Hz}$ [5.22]	4.04 <i>dt</i> $J=12; 4\text{ Hz}$	4.92 <i>dt</i> $J=12; 4\text{ Hz}$ [5.22]	4.98 <i>m</i>	4.07 <i>m</i>
26	4.99 <i>d</i> $J=11\text{ Hz}$ [5.48]		4.99 <i>d</i> $J=11\text{ Hz}$ [5.52]			5.00 <i>d</i> $J=11\text{ Hz}$
27	(1.41) <i>s</i>	1.57 <i>s</i>	(1.41) <i>s</i>	1.57 <i>s</i>	1.55 <i>s</i>	(1.42) <i>s</i>
28	(1.40) <i>s</i>	1.48 <i>s</i>	(1.40) <i>s</i>	1.48 <i>s</i>	1.49 <i>s</i>	(1.40) <i>s</i>
26-OH	3.56 <i>d</i> $J=11\text{ Hz}$		3.56 <i>d</i> $J=11\text{ Hz}$			3.56 <i>d</i> $J=11\text{ Hz}$

Chemical shifts are in ppm measured in deuteriochloroform. Values in brackets are for deuteropyridine solutions. Values in parentheses may be interchanged.



3



4

Table 2. ^{13}C NMR chemical shifts of compounds 1, 2 and 4

C	1a	1b	2a	4
1	212.4	212.2	203.3	191.7
2	(30.7)	30.8	128.8	126.3
3	30.5	30.8	144.5	140.0
4	35.9	36.0	32.9	118.3
5	65.3	65.3	61.5	148.1
6	61.7	61.6	64.0	73.4
7	26.0	25.9	25.6	29.7
8	34.7	40.0	35.2	34.9
9	38.6	38.7	40.3	44.8
10	51.8	51.7	48.2	53.2
11	18.9	19.0	18.9	19.0
12	21.6	21.4	21.4	21.1
13	50.6	50.3	50.6	51.0
14	87.5	87.4	87.4	87.5
15	(31.6)	(30.0)	(31.6)	(30.2)
16	36.3	37.1	36.0	35.7
17	88.5	88.4	88.6	88.8
18	14.4	14.6	14.4	14.4
19	14.1	14.3	14.1	20.2
20	41.7	41.5	41.8	41.5
21	9.8	9.0	9.8	9.9
22	64.0	74.8	64.0	64.4
23	(31.9)	(31.6)	(31.9)	(32.5)
24	(63.7)	61.2	(63.9)	(63.6)
25	(63.5)	63.0	(63.2)	(63.4)
26	91.4	170.0	91.5	91.6
27	16.5	13.6	16.5	16.6
28	18.9	17.8	18.8	18.9

All assignments are based in APT spectra and comparison with related compounds. Values in parentheses may be interchanged.

compound **2a** were obtained from the spectra of the mixture of **1a** and **2a**.

From the less polar fraction containing withanolides (fraction 1), flash chromatography and preparative reverse phase-HPLC afforded a mixture of two withanolides that could not be separated further. ^1H NMR analysis of the mixture indicated that it consisted of lactone **1b** and its 2,3-dehydro derivative **2b** (Table 2). Hydrogenation of the mixture gave pure lactone **1b**, identical (^1H and ^{13}C NMR) to the lactone obtained by Jones oxidation of lactol **1a** (see above).

Finally, fraction 3 was further fractionated by flash chromatography using ethyl acetate-hexane affording compound **4**. The ^1H NMR spectrum of **4** (Table 1) presented signals between δ 6.01 and 6.92 and at 4.62 which corresponded to a 1-oxo-2,4-diene-6 β -hydroxy-withanolide [10]. Signals for H-18 and the side chain hydrogens were closely related to those of **1a** indicating the presence of an epoxylactol side chain and hydroxyl groups at positions C-14 β and C-17 β . The ^{13}C NMR spectrum closely resembled the spectra of **1a** and **2a** for the carbons of ring D and the side chain. The four resonances at δ 126.3, 140.0, 118.3 and 148.1 were assigned to the olefinic carbons C-2, C-3, C-4 and C-5, respectively; C-1 appeared at δ 191.7 and C-6 at 73.4. It is noteworthy that the only other withanolide reported with the A/B-ring substitution pattern of **4**, was jaborosalactone B, isolated from *Jaborosa integrifolia* [10].

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR spectra were measured at 100.1 and 25.2 MHz respectively. Chemical shifts are given in ppm downfield from TMS (internal standard). Mass spectra were determined by direct inlet in a Varian-Mat CH7-A computerized spectrometer. HPLC separations were performed in a Micromeritics liquid chromatograph with a refractive index detector, using an Altex Ultrasphere C-18 5 μm (250 \times 10 mm) column and MeOH-H₂O 7:3 at 3 ml/min as eluent.

Plant material and isolation procedure. Whole *Jaborosa bergii* plants were collected in Liborio Luna, San Luis province, Argentina. A voucher specimen has been deposited in the herbarium of IMBIV, Universidad Nacional de Córdoba under No 24806 (A. T. Hunziker, 16-XII-1984). Dried and pulverized leaves (3.9 kg) were extracted with Et₂O at room temp. The residue (67 g) obtained after evapn of the solvent was chromatographed on silica gel. Initial elution with hexane-C₆H₆ mixtures of increasing polarity followed by CHCl₃ afforded low polarity components and pigments. Elution was continued with CHCl₃-MeOH mixtures (99:1 to 19:1) rendering four fractions containing withanolides.

[17R,20R,22R,24S,25S,26R]-5,6 β :22,26:24,25-Triepoxy-14 β ,17,26-trihydroxy-5 β -ergostan-1-one (*jaborosalactol M*) (**1a**). Part of fraction 2 (250 mg) was purified by flash chromatography (CH₂Cl₂-MeOH 28:1) rendering a mixture of **1a** and **2a** (15 mg) that was hydrogenated in EtOH using 10% Pd/C (1.5 mg) at room temp. and atm. pres. for 1 hr. The catalyst was filtered off and the solvent evapd. The residue was pure **1a** (15 mg) as white crystals from EtOAc, mp 234-236°. MS m/z (rel. int.): 472 [M - H₂O]⁺ (1), 454 [M - 2 H₂O]⁺ (4), 436 [M - 3 H₂O]⁺ (5), 228 (9), 227 (31), 143 (65), 138 (14), 137 (68), 127 (30), 125 (62), 109 (100).

[17R,20R,22R,24S,25S]-5 β ,6 β :24,25-Diepoxy-14 β ,17-dihydroxy-1-oxo-withanolide (*jaborosalactone M*) (**1b**). Flash chromatography of fraction 1 (1.4 g) as above, and prep. HPLC of the less polar fraction (38 mg) rendered a mixture of **1b** and **2b** (5 mg) which was dissolved in EtOH (2 ml) and hydrogenated as above to yield pure **1b** (5 mg) identical (TLC, ^1H and ^{13}C NMR) to that obtained upon oxidation of **1a** (see below).

[17R,20R,22R,24S,25S,26S]-22,26:24,25-Diepoxy-6 β ,14 β ,17,26-tetrahydroxyergosta-2,4-dien-1-one (*jaborosalactol N*) (**4**). Flash chromatography of a portion of fraction 3 (40 mg) using EtOAc-hexane (9:1) afforded pure **4** (8 mg) as white crystals from EtOAc, mp 183-185° (dec). MS m/z (rel. int.): 470 [M - H₂O]⁺ (1), 452 [M - 2 H₂O]⁺ (3), 434 [M - 3 H₂O]⁺ (3), 143 (6), 127 (5), 125 (63), 109 (68), 43 (100).

Preparation of 1b from 1a. Compound **1a** (10 mg) was dissolved in Me₂CO (1 ml) and treated with 8N CrO₃-H₂SO₄ (0.5 ml) at 0° for 4 min. Dilution with H₂O and extractive work-up afforded pure **1b** (9 mg).

Preparation of 3 from 1b. Compound **1b** (5 mg) was dissolved in dry pyridine (0.8 ml) and treated with a soln of freshly distilled SOCl₂ (0.1 ml) in dry pyridine (0.5 ml) at -12° for 2 min. Dilution with H₂O and extractive work-up afforded compound **3** (3 mg) homogeneous by TLC, as an amorphous powder of mp 119-123° (dec). MS m/z (rel. int.): 470 [M - SO₂]⁺ (5), 452 [M - H₂SO₃]⁺ (23), 292 (100), 264 (37), 64 (89). (Analysis. Found, S: 6.3%; C₂₈H₃₈O₈S, requires S: 6.0%).

Acknowledgements—We thank Prof. A. T. Hunziker (Universidad Nacional de Córdoba) for classification of the plant. We are also indebted to CONICET (Argentina) and The Organization of the American States for partial financial support.

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