Spiranoid Withanolides from *Jaborosa odonelliana* and *Jaborosa runcinata*

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Two new spiranoid withanolides, (20*R*,22*R*,23*R*)-12β,17β,22-trihydroxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**3**) and (23*R*)-5α,6β,12β,21-tetrahydroxy-1,22-dioxo-12,23-cycloergosta-2,17,24-trien-26,23-olide (**4**) were isolated from plants of *Jaborosa odonelliana* and *Jaborosa runcinata*, respectively. Compounds **3** and **4** were characterized by a combination of spectroscopic methods (1D and 2D NMR, MS) and molecular modelling.

Key words: Jaborosa, Withanolides, Jaborosalactone

Introduction

The withanolides are a group of natural C_{28}-steroidal lactones built on an intact or arranged ergostane framework that occurs mainly in plants of certain genera of *Solanaceae*. The first member of this group of compounds, withafarin A, was isolated from the well-known Indian medicinal plant, *Withania somnifera* [1] and its structure was fully elcidated by Lavie and coworkers in 1965 [2]. The withanolides exhibit a variety of biological activities as antifeedant, immunosuppressive and cancer chemoprevention activity [3].

Jaborosa Miers is a South American genus belonging to the *Solanaceae* family that comprises about 23 species, 11 of which are almost exclusively distributed in Argentina [4]. Previous studies on populations of *J. odonelliana*, *J. runcinata* and *J. araucana*, gave a group of withanolides with a spiranoid lactone ring in the side chain. The compounds isolated from *J. odonelliana* presented a 17,22-diol functionality and a 23*S* stereochemistry at the spiranoid center (*e.g.* Jaborosalactone P, **1**) [5], while those isolated from *J. runcinata* and *J. araucana* showed a 17(20)-ene-22-keto system and a 23*R* stereochemistry (*e.g.* Jaborosalactone 2, **2**) [6], in agreement with that found in **1**.

Studies on cancer chemopreventive activity of withanolides as inducers of phase II detoxification enzymes indicated that the spiranoid withanolides jaborosalactone P and jaborosalactone 1 were promising agents in terms of inducing potency and low toxicity [7].

Results and Discussion

Jaborosalactone 24 (**3**) was isolated as a minor component from the aerial part of *J. odonelliana*. The HREIMS showed a molecular ion corresponding to the formula $C_{28}H_{36}O_6$, whereas the EIMS showed peaks at *m/z* 299 (42) and 168 (31) corresponding to the cleavage between C-20-C-17 and C-23-C-12, distinctive for this type of structure [8]. ^{1}H and ^{13}C NMR chemical shift values in jaborosalactone 24 (**3**) were closely related to those reported for jaborosalactone P (**1**) [5]. In the low-field end of the ^{1}H NMR spectrum signals at $\delta = 5.81$, 6.73 and 5.56 were assigned to H-2, H-3 and H-6, respectively, of a 1-oxo-2,5-dienewithanolide. The typical pattern of the spiranoid arrangement was inferred from the resonances of carbons 23 – 28 (Table 1) and the low-field ^{1}H resonances of methyls 27 and 28, observed as quartets ($J = 1.0$ Hz) due to their mutual homoallylic coupling. Despite these similarities, detailed comparison of the NMR spectral data of **1** and **3** showed small but clear differences in the ^{13}C chemical shifts of the α,β-unsaturated-γ-lactone ring, and a downfield shift of H-22. This indicated that the side chain of jaborosalactone 24 (**3**) had an arrangement different from that found in **1**, possibly due to an inverted stereochemistry of the spiranoid center at C-23. The configuration at C-23 was confirmed to be *R* by comparison of the NOESY spectra of **1** and **3**. Thus, the correlation observed for the pair H-28/H-22 in the NOESY spectrum

Table 1. ^{13}C NMR spectral data of compounds **3** (125.77 MHz) and **4** (50.32 MHz) in $CDCl_3$.

C	3	4	C	3	4
1	203.8	203.3	2	127.6	128.8
3	145.0	141.9	4	33.3	34.3
5	135.4	79.7	6	124.9	73.8
7	34.5	31.9	8	32.9	29.5
9	39.9	38.3	10	50.0	51.6
11	30.0	35.4	12	78.9	75.2
13	50.8	48.9	14	43.6	46.4
15	23.3	23.3	16	32.7	25.3
17	83.5	165.6	18	10.7	14.8
19	17.9	15.1	20	40.7	127.3
21	11.8	58.2	22	69.0	193.5
23	94.5	91.0	24	163.1	161.9
25	126.7	128.1	26	171.6	173.4
27	8.6	18.9	28	15.2	12.1

C multiplicities were determined from DEPT data.

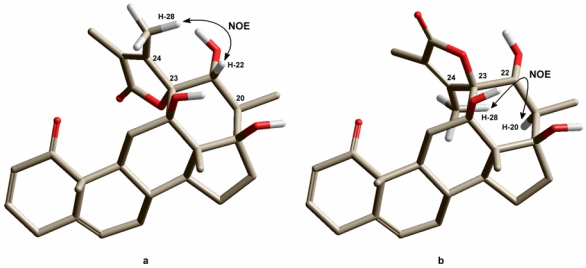
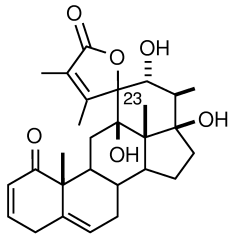


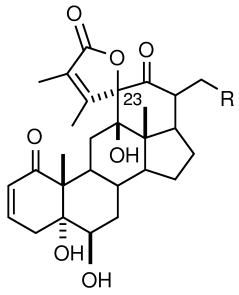
Fig. 1. AM1 calculated structures of a) jaborosalactone 24 (**3**) and b) jaborosalactone P (**1**), indicating relevant NOEs observed.

of **3** was only possible in the 23*R* stereoisomer, while a correlation for the pair H-28/H-20 was observed in the NOESY spectrum of **1** in agreement with the 23*S* stereochemistry assigned to this compound (Fig. 1).

Jaborosalactone 25 (**4**), was isolated as a minor component from the aerial part of *J. runcinata*. The ^1H and ^{13}C NMR spectral data of **4** were very similar to those of jaborosalactone 2 (**2**) [6], however the absence of a singlet for H-21 in the high-field end of the ^1H NMR spectrum and the appearance of an AB quartet at 4.21 – 4.33 ppm indicated the presence of an isolated C-21 hydroxymethylene group. This functionality has been previously found in jaborosalactones 4 and 5 isolated from *J. runcinata* [6]. The ^{13}C NMR spectrum (Table 1) showed only four methyl groups that were coincident with C-18, C-19, C-27 and C-28 in **2**. The methylene signal at 58.2 ppm confirmed the presence of a hydroxyl group at C-21. The molecular ion was absent in the EIMS, but a peak at *m/z* 480 (1%) corresponding to the ion $[M\text{-}H_2O]^+$ was observed. A significant fragment was at *m/z* 355 (4%), corresponding to the cleavage between C-23 and C-22 and the



1 23*S*
3 23*R*



2 R=H
4 R=OH

subsequent loss of the γ-lactone ring and CO. The FABMS (glycerol) of jaborosalactone 25 (**4**) showed a $[M+1]^+$ ion at *m/z* 499 consistent with the formula $C_{28}H_{34}O_8$.

Experimental Section

General experimental procedures

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively, or a Bruker AM-500 at 500.13 and 125.77 MHz, respectively. Multiplicity determinations (DEPT-135) and 2D spectra (COSY-45 and NOESY) were obtained using standard Bruker software. Chemical shifts are given in (δ) downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 mass spectrometer at 70 eV by direct inlet; FABMS and HREIMS (70 eV) were measured on a VG ZAB-BEQQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 550 FT IR and a Hewlett-Packard 8451A spectrophotometer, respectively. AM1 calculations were performed with the MOPAC module in Chem3D 8.0 (Cambridge Soft). Melting points were taken on a Fisher-Johns apparatus and are uncorrected. HPLC separations were carried out on a YMC-Pack ODS-AQ column (250 × 10 mm ID) and a mixture of MeOH – H_2O (70:30) as eluant, with UV detection at 245 nm. Vacuum liquid chromatography (VLC) and column flash chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040 – 0.063 mm, respectively. TLC analysis was performed on silica gel 60 F254 (0.2 mm thick).

Plant material

Whole *J. runcinata* plants were collected in March 1995 in El Jagüel, departamento Paraná, Entre Ríos Province, Argentina. Aerial parts of *J. odonelliana* were collected in April and December 1996 in Salta Province, Argentina. Voucher specimens of both species are deposited at the Museo Botánico, Universidad Nacional de Córdoba under No. CORD 248 (*J. runcinata*) and CORD No. 25540 (*J. odonelliana*).

Extraction and isolation

Leaves and stems (1.15 g) of *J. odonelliana* were extracted as previously described [8]. The residue obtained after evaporation of the combined extracts was initially fractionated by vacuum liquid chromatography using hexane-EtOAc mixtures of increasing polarity (100:0-0:100) as eluant.

The fraction eluted with hexane-EtOAc (40:60) was further fractionated by flash chromatography and purified by HPLC yielding jaborosalactone P (**1**) (176 mg) and jaborosalactone 24 (**3**) (3.4 mg).

The dried and pulverized aerial parts of *J. runcinata* (935 mg) were extracted and fractionated as previously described [6]. The fraction eluted with hexane-EtOAc (20:80) was further purified by flash chromatography using mixtures of CH_2Cl_2 – MeOH (100:5-100:10) yielding jaborosalactone 25 (**4**) (4.5 mg).

*Jaborosalactone 24 (**3**)*

Amorphous solid. – UV/vis (MeOH): $\lambda_{max}(\log\varepsilon) =$ 223 nm (3.07). – IR (dry film): $\tilde{\nu} =$ 3458, 2966, 1747, 1676, 1390, 1270, 1006, 728 cm^{-1}; ^1H NMR (500.13 MHz, $CDCl_3$, assignments based on ^1H-^1H-COSY): $\delta = 1.06$ (s, 3H, 18-H), 1.16 (d, $J = 6.4$ Hz, 1H, 21-H), 1.16 (s, 3H, 19-H), 1.48 (m, 1H, 11β–H), 1.80 (m, 1H, 7α-H), 1.99 (q, $J = 1.0$ Hz, 3H, 27-H), 2.02 (m, 1H, 7β–H), 2.09 (m, 1H, H-20), 2.15 (q, $J = 1.0$ Hz, 3H, 28-H), 2.46 (dd, $J = 10.4$, 2.6 Hz, 1H, 11α–H), 2.83 (dd, $J = 21.0$, 5.0 Hz, 1H, 4α–H), 3.24 (dt, $J = 21.0$, 2.5 Hz, 1H, 4β-H), 4.03 (d, $J = 11.1$ Hz, 1H, 22-H), 5.56 (dd, $J = 4.1$, 2.0 Hz, 1H, 6-H), 5.81 (dd, $J = 10.0$, 2.5 Hz, 1H, 2-H), 6.73 (ddd, $J = 10.0$, 5.0, 2.5 Hz, 1H, 3-H). – ^{13}C NMR (125.77 MHz) see Table 1. – MS (EI, 70 eV): *m/z* (%) = 468 (4) [M^+], 450 (2), 432 (2), 299 (42), 283 (10), 265 (4), 168 (31), 107 (18), 97 (21), 43 (100); HREIMS *m/z*= 468.2512 [M^+] ($C_{28}H_{36}O_6$, requires 468.2516).

*Jaborosalactone 25 (**4**)*

White solid. – Mp 253 – 255 °C. – UV/vis (MeOH): $\lambda_{max}(\log\varepsilon) =$ 226 nm (3.25). – IR (dry film): $\tilde{\nu} =$ 3450, 1742, 1673, 1381, 1254, 1018 cm^{-1}. – ^1H NMR (200.13 MHz, $CDCl_3$, assignments based on ^1H-^1H-COSY): $\delta = 1.14$ (s, 3H, 18-H), 1.26 (s, 3H, 19-H), 2.03 (q, $J =$ 1.0 Hz, 3H, 27-H), 2.10 (dd, $J = 19.2$, 5.0 Hz, 1H, H-4α), 2.25 (q, $J = 1.0$ Hz, 3H, 28-H), 2.64 (m, 1H, 16-H), 3.25 (dt, $J = 19.2$, 2.2 Hz, 1H, 4β-H), 3.66 (t, $J = 2.6$ Hz, 1H, 6-H), 4.21 (d, $J = 12.2$ Hz, 1H, 21b-H), 4.33 (d, $J = 12.3$ Hz, 1H, 21a-H), 5.82 (dd, $J = 10.1$, 2.2 Hz, 1H, 2-H), 6.60 (ddd, $J = 10.1$, 5.0, 2.2 Hz, 1H, 3-H). – ^{13}C NMR (50.32 MHz) see Table 1. – MS (EI, 70 eV): *m/z* (%) = 480 (1) [M^+ - H_2O] (1), 355 (4), 107 (10), 97 (20), 43 (100); FABMS (glycerol) *m/z* 499 $[M+H]^+$.

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