Spiranoid Withanolides from *Jaborosa odonelliana*

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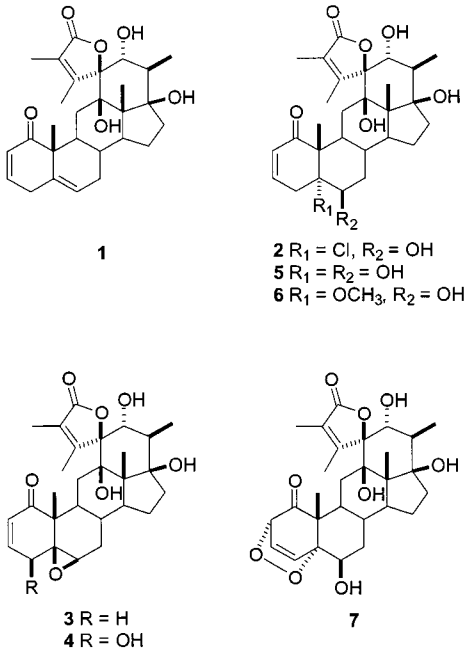
Six new spiranoid withanolides, (20*R*,22*R*,23*S*)-5α-chloro-6*β*,12*β*,17*β*,22-tetrahydroxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**2**), (20*R*,22*R*,23*S*)-5*β*,6*β*-epoxy-12*β*,17*β*,22-trihydroxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**3**), (20*R*,22*R*,23*S*)-5*β*,6*β*-epoxy-4*β*,12*β*,17*β*,22-tetrahydroxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**4**), (20*R*,22*R*,23*S*)-5α,6*β*,12*β*,17*β*,22-pentahydroxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**5**), (20*R*,22*R*,23*S*)-6*β*,12*β*,17*β*,22-tetrahydroxy-5α-methoxy-1-oxo-12,23-cycloergosta-2,24-dien-26,23-olide (**6**), and (20*R*,22*R*,23*S*)-6*β*,12*β*,17*β*,22-tetrahydroxy-2α,5α-epidioxy-1-oxo-12,23-cycloergosta-3,24-dien-26,23-olide (**7**), were isolated from the leaves of *Jaborosa odonelliana*. Compounds **2**−**7** were characterized by a combination of spectroscopic methods (1D and 2D NMR, MS) and molecular modeling.

The withanolides are a group of C-28 steroidal lactones isolated from several genera of Solanaceae. They exhibit a variety of biological activities, including antifeedant and cancer chemoprevention activities.[1−4] As part of a systematic investigation on the withanolides of Solanaceae growing in Argentina, we reported the isolation of the first withanolide with a spiranoid *γ*-lactone side chain, jaborosalactone P (**1**), from plants of *Jaborosa odonelliana*.[5] Later on, a related group of spiranoid withanolides were isolated from *J. runcinata* and *J. araucana*.[6] It has recently been shown that some of these spiranoid withanolides are potent inducers of the phase II enzyme quinone reductase; jaborosalactone P (**1**) was active both in vitro and in vivo, with a high chemoprevention index.[7] In search of new spiranoid withanolides, we studied the minor components of *J. odonelliana* collected in autumn and summer and isolated six new withanolides (**2**−**7**) structurally related to **1**.

In addition to the major component, jaborosalactone P (**1**), *J. odonelliana* plants collected in December (summer) gave withanolides **2**, **6**, and **7**, while plants collected in April (autumn) yielded **3**, **4**, **5**, and **6**. The ^{1}H and ^{13}C NMR spectra of these new compounds, jaborosolactones 10−15 (**2**−**7**) (Tables 1 and 2), showed patterns typical of the spiranoid lactone arrangement, from the resonances of carbons 23−28 and the low-field resonances of methyls 27 and 28, observed in most cases as quartets (J = 0.7−0.9 Hz) due to their mutual homoallylic coupling.[5,6] NMR spectral data for rings C, D and the side chain were coincident with those reported for **1**.[5] A 2-en-1-one arrangement was evident in compounds **2**−**6** from the signals between δ 5.79 and 6.90 in the ^{1}H NMR spectra. Highly diagnostic of the ring B substitution in **2** was the broad signal at δ 3.95, typical of H-6 in a 5α-chloro-6*β*-hydroxy arrangement previously found in several withanolides.[6,8] This functionality was corroborated by the signals at δ 81.6 and 73.8 in the ^{13}C NMR spectrum, assigned to C-5 and C-6, respectively. It is noteworthy that **2** was one of the major withanolides present in the plants collected in December but was not detected in the plants collected in April.



A 5*β*,6*β*-epoxide was evident in the ^{1}H NMR spectrum of **3**, from the doublet at δ 3.13 (J = 2.5 Hz) corresponding to H-6, in agreement with the signals at δ 62.0 and 63.1 in the ^{13}C NMR spectrum. The double-doublet at δ 6.90 and the two doublets at δ 6.08 and 3.76 in the ^{1}H NMR spectrum of **4** indicated a substituent at C-4. The signals at δ 69.3, 62.7, and 63.7 in the ^{13}C NMR spectrum were assigned to C-4, C-5, and C-6, respectively, of a 4*β*-hydroxy-5*β*,6*β*-epoxywithanolide. The main difference between the ^{1}H NMR spectra of **3** and **5** was the downfield shift of the H-6 resonance from δ 3.13 in **3** to δ 3.51 in **5**. The presence of ^{13}C signals at δ 77.7 and 74.4, assigned to C-5 and C-6, respectively, were consistent with a 5α,6*β*-dihydroxy functionality in compound **5**. The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **5**; however, the presence of a methoxy group at C-6 was inferred from the sharp three-proton singlet observed at δ 3.04 in the ^{1}H NMR spectrum and a signal at δ 49.9 in the ^{13}C NMR spectra. The NMR spectral assignments for **2**−**6** were confirmed by DEPT and

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Table 1. ^{1}H NMR Spectral Data for Relevant Protons of Compounds **2**−**7** in Cl_3CD[a]

H	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[b]	**7**[b]
2	5.84 dd (10.0; 2.3)	5.93 dd (10.0; 2.5)	6.08 d (10.0)	5.82 dd (10.2; 2.4)	5.79 dd (10.2; 2.7)	4.40 dd (6.38; 1.37)
3	6.61 ddd (10.0; 5.0; 2.3)	6.80 ddd (10.0; 6.0; 2.5)	6.90 dd (10.0; 5.7)	6.55 ddd (10.2; 5.1; 2.4)	6.50 ddd (10.2; 5.2; 2.7)	6.64 dd (8.40; 6.38)
4α	2.49 dd (20.1; 5.0)	1.99 dd (19.0; 6.0)	3.76 d (5.7)	2.1 dd (19.7; 5.1)	2.38 dd (20.0; 5.2)	
4*β*	3.50 dt (20.1; 2.3)	2.95 dt (19.0; 2.5)		3.30 dt (19.7; 2.4)	3.00 dt (20.0; 2.7)	6.99 dd (8.40; 1.37)
6	3.95 br s	3.13 d (2.5)	3.25 br s	3.51 br s	3.91 t (2.5)	3.98 t (2.73)
7α	1.73 m	1.45 m	1.84 m	1.59 m	1.59 m	1.50 m
7*β*	2.15 m	2.07 m	2.23 m	2.23 m	1.80 m	1.73 m
18	1.14 s	1.11 s	1.10 s	1.14 s	1.17 s	1.17 s
19	1.34s	1.19 s	1.36 s	1.26 s	1.26 s	1.22 s
20	2.15 m	2.09 m	1.96 m	2.10 m	2.16 m	2.09 m
21	1.17 d (6.6)	1.17 d (7.0)	1.21 d (7.0)	1.17 d (6.6)	1.21 d (6.4)	1.17 d (6.2)
22	4.19 d (12.3)	4.22 d (12.4)	4.20 d (12.4)	4.19 d (12.4)	4.26 d (12.3)	4.18 d (12.3)
27	1.82 s	1.85 q (0.7)	1.82 q (0.7)	1.82 s	1.83 s	1.78 q (0.9)
28	2.30 s	2.22 q (0.7)	2.21 q (0.7)	2.30 s	2.31 s	2.35 q (0.9)
5-OCH_3					3.04 s	

[a]Chemicals shifts (*δ*) downfield from TMS, *J* couplings (in parentheses) in Hz. [b]500.13 MHz. [c]200.13 MHz.

Table 2. ^{13}C NMR Spectral Data of Compounds **2**−**7** in Cl_3CD[a]

C	**2**[b]	**3**[c]	**4**[b]	**5**[b]	**6**[b]	**7**[b]
1	200.3	203.0	201.1	203.8	202.3	204.9
2	128.3	128.8	131.2	129.0	129.2	78.4
3	141.1	145.0	142.5	141.3	138.5	126.3
4	36.9	33.3	69.3	36.1	27.3	141.2
5	81.6	62.0	62.7	77.7	81.4	81.2
6	73.8	63.1	63.7	74.4	68.3	66.3
7	34.2	29.7	34.2	31.5	34.3	34.1
8	29.5	29.1	29.7	29.3	29.0	28.7
9	38.5	41.7	44.2	37.6	37.2	38.5
10	50.5	50.3	47.6	50.0	50.0	47.8
11	31.2	30.5	30.4	29.7	31.5	30.9
12	82.6	82.0	82.6	82.4	82.4	81.5
13	53.3	53.4	50.1	52.0	53.0	50.6
14	44.1	44.1	41.4	44.1	44.8	44.7
15	23.2	23.1	23.1	23.4	23.4	23.0
16	32.6	32.4	31.3	33.0	33.2	33.6
17	83.0	82.8	82.6	82.9	82.9	82.7
18	13.0	12.8	12.7	13.0	12.9	13.0
19	15.4	14.7	17.1	14.6	14.7	18.4
20	40.8	40.5	40.5	40.6	40.7	40.6
21	11.2	11.2	11.2	11.2	11.3	11.2
22	71.1	71.8	71.5	71.9	71.7	71.2
23	97.0	97.0	97.1	97.6	97.3	97.4
24	158.1	157.0	157.8	158.4	157.5	159.2
25	130.8	130.5	130.5	131.0	130.9	129.4
26	173.5	173.2	173.5	171.0	173.0	172.9
27	9.4	9.6	9.4	9.6	9.4	8.9
28	18.6	18.7	18.7	18.3	18.5	18.6
OCH_3					49.9	

[a] Chemical shifts (*δ*) downfield from TMS. [b] 125.77 MHz. [c]50.32 MHz.

COSY 45 spectra. High-resolution mass measurements were in agreement with the proposed formulas.

The NMR spectra of **7** differed from the above in the substitution pattern of ring A. When compared with **1** and the jaborosalactones described above (**2**−**6**), a downfield shift was observed for the signals corresponding to the two olefinic protons (which appeared at *δ* 6.64 and 6.99) as well as a smaller coupling constant between them (8.4 Hz). These signals and the presence of a carbonyl carbon at *δ* 204.9 and two methines at *δ* 126.3 and 141.2 in the ^{13}C NMR spectrum indicated a carbonyl group and a nonconjugated double bond in ring A. Also evident in this spectrum were a nonprotonated carbon at *δ* 81.2 and a methine at *δ* 78.4, in correspondence with a signal at *δ* 4.40 in the ^{1}H NMR. These data were consistent with the presence of a peroxy bridge between C-2 and C-5. The signals at *δ* 4.40 and 6.64 were assigned to H-2 and H-3, respectively, from the cross-peak observed in the COSY 45 spectrum. The presence of a 6*β*-hydroxy group was inferred from the triplet at *δ* 3.98 in the ^{1}H NMR spectrum and the signal at *δ* 66.3 in the ^{13}C NMR. Similar endoperoxy systems have been found in physalins K and G, isolated from *Physalis alkekengi*,[9] which differ in the configuration of the peroxide bridge (α and *β*, respectively). The main difference observed in the ^{13}C NMR of these diastereomers was the chemical shift of CH_3-19; in the α-stereoisomer this carbon appeared at *δ* 18.4, being coincident with that found for **7**. AM1 calculations (Hyperchem 5.1) predicted the distance between H-4 and H-19 as 2.64 Å for the α-endoperoxide (Figure 1, Supporting Information). Thus the strong correlation observed in the NOESY spectrum of **7** for the pair H-4 (*δ* 6.99)/H-19 (*δ* 1.22) was indicative of the 2α,5α-configuration. Mass measurements were in accordance with the structural assignments; the HREIMS showed a molecular ion corresponding to the formula $C_{28}H_{36}O_9$, whereas the EIMS showed peaks at *m*/*z* 348 (1) and 168 (14) corresponding to the cleavage between C-20−C-17 and C-23−C-12. The latter fragment was present in the mass spectra of all the spiranoid withanolides isolated from *J. odonelliana* and appears to be distinctive for this type of structure.

Feeding deterrant activity of the major components **1** and **2** was studied against the stored grain pest *Tribolium castaneum*. The bioassays were performed as previously described incorporating the products into the larval diet in concentrations of 500 ppm, and the number of larvae, pupae, and adults were recorded every 10 days.[4] Significative development delay was assessed by no superposition of the fiducial limits between DT_{50} (necessary time to reach the adult stage for 50% of the exposed larvae) of treated larvae and controls. Only jaborosalactone P (**1**) produced a significative delay in the development of neonatae larvae, the DT_{50} being 55.59, 82.57, and 53.00 days for the control, **1**, and **2**, respectively.

Compounds **2** and **6** were less active than jaborosalactone P (**1**) as to their potential to induce quinone reductase in cultured murine hepatoma cells (Hepa 1c1c7). These results are described in a separate publication.[7]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz or a Bruker AM-500 at 500.13 and 125.77 MHz. Multiplicity determinations (DEPT) 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 Hyperchem 5.1 (Hyperchem Inc.). 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 i.d.) 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. Aerial parts of *Jaborosa odonelliana* were collected in April and December (1996) in Salta, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad Nacional de Córdoba [CORD No. 25540].

Extraction and Isolation. Fresh leaves and stems (600 g in April and 1700 g in December) were triturated and macerated succesively with ether (1 mL/g of plant, 3 days) and ethanol (1 mL/g of plant, 3 days) at room temperature. The residues obtained after evaporation of the combined extracts (20.0 g from plants collected in April and 59.7 g from plants collected in December) were initially fractionated by vacuum liquid chromatography using hexane−EtOAc mixtures of increasing polarity (100:0−0:100) as eluant. Fractions eluted with 30:70, 20:80, and 10:90 hexane−EtOAc mixtures contained mixtures of withanolides (as determined by ^{1}H NMR). Each of these fractions was further fractionated by flash chromatography, and the resulting withanolides were further purified by preparative TLC and HPLC. This led to the isolation of the known withanolide **1**[5] and six new withanolides. Plants collected in December gave (in order of elution) **1** (176 mg), **7** (6 mg), **2** (89 mg), and **6** (11.5 mg), while plants collected in April yielded (in order of elution) **1** (132 mg), **4** (18 mg), **5** (12 mg), **6** (4 mg), and **3** (2.2 mg).

Jaborosalactone 10 (2): white crystals (EtOAc−hexane), mp 264−265 °C; $[\alpha]^{25}_D$ −7.37° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (3.17) nm; IR (dry film) ν_{max} 3458, 2939, 1738, 1687, 1383, 1261, 1098 cm^{-1}; ^{1}H NMR (500.13 MHz), see Table 1; ^{13}C NMR (125.77 MHz), see Table 2; EIMS *m/z* 484 [M − 2 H_2O]$^+$ (0.4), 448 (2), 430 (4), 412 (3), 351 (1), 168 (20), 152 (33), 107 (12), 97 (8), 43 (100); FABMS (glycerol) *m/z* 521 [M + H]$^+$ (77); HREIMS *m/z* 484.2020 (calcd for $C_{28}H_{33}O_5Cl$, 484.2017).

Jaborosalactone 11 (3): amorphous solid; $[\alpha]^{25}_D$ −14.0° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (3.25) nm; IR (dry film) ν_{max} 3452, 2926, 1739, 1672, 1452, 1382, 1261, 1137, 1097 cm^{-1}; ^{1}H NMR (200.13 MHz), see Table 1; ^{13}C NMR (50.32 MHz), see Table 2; EIMS *m/z* 484 [M]$^+$ (0.6), 315 (2), 297 (2.5), 168 (10), 152 (17), 135 (6), 107 (10), 97 (9), 43 (100); HREIMS *m/z* 484.2470 (calcd for $C_{28}H_{36}O_7$, 484.2461).

Jaborosalactone 12 (4): amorphous solid; $[\alpha]^{25}_D$ −10.8° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.07) nm; IR (dry film) ν_{max} 3450, 2928, 1737, 1671, 1460, 1383, 1268, 1146, 1089 cm^{-1}; ^{1}H NMR (500.13 MHz), see Table 1; ^{13}C NMR (125.77 MHz), see Table 2; EIMS *m/z* 500 [M]$^+$ (0.4), 468 (0.4), 331 (4), 297 (3), 168 (16), 152 (23), 107 (10), 97 (11), 43 (100); FABMS (glycerol) *m/z* 501 [M + H]$^+$ (100); HREIMS *m/z* 500.2419 (calcd for$C_{28}H_{36}O_8$, 500.2410).

Jaborosalactone 13 (5): amorphous solid; $[\alpha]^{25}_D$ −10.1° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.25) nm; IR (dry film) ν_{max} 3432, 2924, 1734, 1671, 1465, 1380, 1260, 1080 cm^{-1}; ^{1}H NMR (500.13 MHz), see Table 1; ^{13}C NMR (50.32 MHz), see Table 2; EIMS *m/z* 502 [M]$^+$ (0.7), 484 (0.6), 334 (10), 168 (16), 152 (22), 107 (8), 97 (8), 43 (100); FABMS (glycerol) *m/z* 503 [M + 1]$^+$ (100); HREIMS *m/z* 502.2570 (calcd for $C_{28}H_{38}O_8$, 502.2566).

Jaborosalactone 14 (6): white crystals (EtOAc−hexane), mp 269−270 °C; $[\alpha]^{25}_D$ −6.5° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.36) nm; IR (dry film) ν_{max} 3444, 2927, 1724, 1669, 1439, 1377, 1261, 1083 cm^{-1}; ^{1}H NMR data (500.13 MHz), see Table 1; ^{13}C NMR data (125.77 MHz), see Table 2; EIMS *m/z* 480 [M − 2 H_2O]$^+$ (0.4), 347 (24), 331 (16), 152 (22), 107 (8), 97 (8), 43 (100); FABMS (glycerol) *m/z* 517 [M + H]$^+$ (100); HREIMS *m/z* 480.2520 (calcd for $C_{29}H_{36}O_6$, 480.2512).

Jaborosalactone 15 (7): amorphous solid; $[\alpha]^{25}_D$ −6.3° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.44) nm; IR (dry film) ν_{max} 3476, 2929, 1746, 1671, 1448, 1384, 1081 cm^{-1}; ^{1}H NMR data (500.13 MHz), see Table 1; ^{13}C NMR data (125.77 MHz), see Table 2; EIMS *m/z* 516 [M]$^+$ (0.4), 448 (0.4), 348 (1), 168 (14), 152 (27), 107 (10), 97 (9), 43 (100); HREIMS *m/z* 516.2351 (calcd for $C_{28}H_{36}O_9$, 516.2359).

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Supporting Information Available: AM1 calculated structure for compound **7** (rings A/B) indicating relevant NOEs observed. This material is available free of charge via the Internet at http://pubs.acs.org.

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