Withanolides from *Jaborosa kurtzii*

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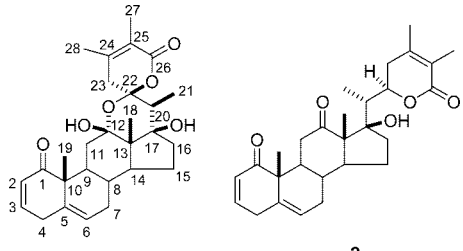
Received April 18, 2007

Two new withanolides were isolated and characterized from the aerial parts of *Jaborosa kurtzii*, namely, jaborosalactone 43 (**1**), with a spiranoid δ-lactone at C-22, and jaborosalactone 44 (**2**), a 12-oxowithanolide, which may function as a biosynthetic precursor to **1**. These new compounds were fully characterized by a combination of spectroscopic methods. Compound **1** showed selective phytotoxicity toward a dicotyledon species, *Lactuca sativa* (lettuce).

Withanolides are a group of oxygenated steroidal lactones isolated from several genera of the plant family Solanaceae. Their chemistry and occurrence have been reviewed.[1,2] Many of these compounds exhibit interesting biological activities, such as antifeedant,[3] insecticidal, and phytotoxic[4] properties. Several withanolides are inducers of the enzyme quinone reductase and, hence, show potential as cancer chemopreventive agents.[5]

Jaborosa Miers is a South American genus belonging to Solanaceae that comprises about 23 different species that grow mainly in Argentina.[6] As part of a project aimed at the search of withanolides with potential application as friendly environmental herbicides, we report herein on the isolation, characterization, and phytotoxic activity of two new withanolides from *Jaborosa kurtzii* Hunzinker et Barboza, named jaborosalactone 43 (**1**), related structurally to jaborosalactones 26–30,[7] and jaborosalactone 44 (**2**), a 12-oxowithanolide.

The aerial parts of *J. kurtzii* were air-dried and extracted with ethanol. After concentration and defatting, the residue was fractionated by a combination of chromatographic techniques, ultimately affording two new withanolides (**1** and **2**).



1

2

Jaborosalactone 43 (**1**) revealed a molecular formula of $C_{28}H_{36}O_6$ by high-resolution fast atom bombardment mass spectrometry (HRFABMS). The ^{1}H nuclear magnetic resonance (NMR) spectrum of **1** showed the characteristic signals of H-2, H-3, and H-6 for a 1-oxo-2,5-dienewithanolide at δ 5.86 dd (J = 10.0, 2.0 Hz), δ 6.79 ddd (J = 10.0, 5.0, 2.5 Hz), and δ 5.57 d (J = 5.9 Hz), respectively.[8] Corresponding olefinic signals at δ 127.7, 145.3, 135.9, and 124.1 and the signal at δ 203.7 assigned to a carbonyl group (C-1) were observed in the ^{13}C NMR spectrum. Signals because of the protons and carbons of rings C and D and the side chain were closely related to those of a group of spiranoid δ-lactone withanolides, jaborosalactones 26–30, which were isolated from *Jaborosa rotacea*.[7] The characteristic ^{1}H NMR signals for this type of compound are (i) two singlets at δ 1.90 and 1.93, corresponding to the two methyl groups of an α,β-unsaturated δ-lactone ring bearing methyl groups at positions C-24 and C-25, (ii) a doublet for the C-21 methyl group at δ 1.17 (J = 6.9 Hz), indicating the absence of a hydroxyl group at C-20, and (iii) the absence of any characteristic signal corresponding to the carbinyl hydrogen at C-22 in the 4–5 ppm region. The ^{13}C NMR spectrum showed a signal at δ 165.0 assigned to C-26, two olefinic signals at δ 147.2 and 120.1, corresponding to C-24 and C-25, respectively, and two signals for doubly oxygenated carbons at δ 100.1 and 103.5, which were assigned to C-12 and C-22, respectively. The chemical shifts of these signals were in good agreement with the presence of the hemiketal bridge between what must have originally been ketone functions at C-12 and C-22. This structure was confirmed by the cross-correlation peaks observed in the HMBC experiment. Key correlations were observed for H-11α (δ 1.73) and H-11β (δ 2.49) with C-12 (δ 100.1), for H$_3$-18 (δ 1.12) with C-12 (δ 100.1) and C-17 (δ 78.9), for H$_3$-21 (δ 1.17) with C-17 (δ 78.9), C-20 (δ 40.7), and C-22 (δ 103.5), and for H-23β (δ 2.19) with C-22 (δ 103.5), C-24 (δ 147.2), and C-25 (δ 120.1). Spectroscopic assignments were confirmed by distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY)-60, heteronuclear correlation (HETCOR), and heteronuclear multiple-bond correlation (HMBC) spectra. Thus, the structure of **1** was established as (20*S*,22*S*)-12α22-epoxy-12β,17β-dihydroxy-1-oxowitha-2,5,24-trien-26,22-olide.

The high-resolution electron impact mass spectrometry (HREIMS) of **2** showed a molecular ion $[M]^+$ at m/z 452.2583, corresponding to an elemental formula of $C_{28}H_{36}O_5$. In the ^{1}H NMR spectrum, the resonances for the protons from rings A and B were almost identical to those in compound **1**, especially the three olefinic signals at δ 5.90, 6.80, and 5.62, corresponding to H-2, H-3, and H-6, respectively. The signals observed in the ^{13}C NMR spectrum at δ 202.9, 127.8, 145.3, 135.8, and 124.1 were assigned to C-1, C-2, C-3, C-5, and C-6, respectively. On the other hand, the resonances observed for rings C and D and the side chain were similar to those of 12-ketowithanolides previously isolated from *Jaborosa magellanica*.[9] The assignment of chemical shifts to this partial structure was based on data for a relevant number of related compounds previously reported in the literature.[9–11] The ^{1}H NMR spectrum showed the signals corresponding to five methyl groups at δ 1.20 s, 1.27 s, 0.93 d (6.9 Hz), 1.87 s, and 1.93 s, assigned to H$_3$-18, H$_3$-19, H$_3$-21, H$_3$-27, and H$_3$-28, respectively. The signal corresponding to the carbinyl proton at δ 4.56 ddd (12.4, 6.6, and 3.6 Hz) was assigned to H-22. The ^{13}C NMR and DEPT spectroscopic data for **2** were in agreement with the structure proposed. In addition to the signals assigned for rings A and B, the ^{13}C NMR spectrum showed two carbonyl carbons plus two additional olefinic carbons. The carbonyl signal at δ 165.8 was assigned to C-26 of an α,β-unsaturated δ-lactone, and the second carbonyl signal at δ 214.2 was assigned to the keto group at C-12. The signals observed at δ 78.4, 149.5, and 121.7 corresponded to C-22, C-24, and C-25, respectively. The spectroscopic assignments

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10.1021/np0701780 CCC: $37.00
Published on Web 08/22/2007

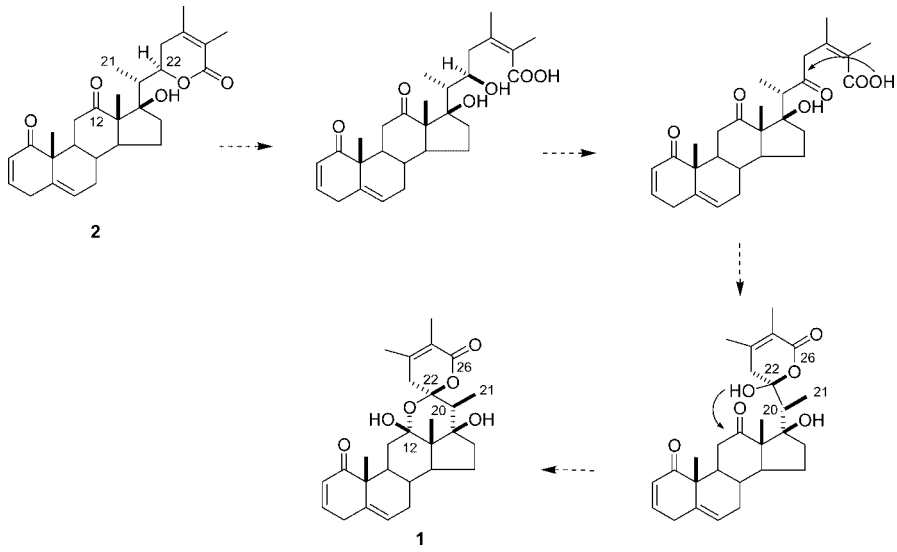

Figure 1. Possible biogenetic relationship between compounds **1** and **2**.

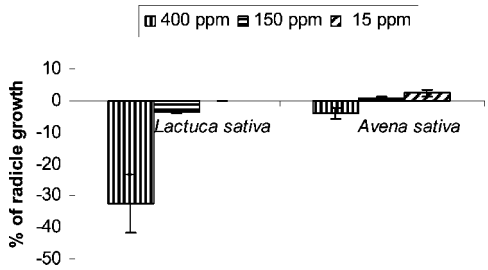

Figure 2. Effect of withanolide **1** at different concentrations on the radicle length of *L. sativa* (dicotyledon) and *A. sativa* (monocotyledon). The data are presented as percentage differences from the control (zero value); positive values represent stimulation, and negative values represent inhibition.

of **2** were confirmed by the COSY-60 and HETCOR spectra. Accordingly, the structure of compound **2** was identified as (20*R*,22*R*)-17β-hydroxy-1,12-dioxowitha-2,5,24-trien-26,22-olide.

Biogenetically, compound **1** may arise from compound **2**, through a doubly oxygenated precursor at the C-22 position to give the δ-lactone and a hemiketal ring upon cyclization with a 12-ketone (Figure 1). The occurrence of closely related mechanisms was proposed in the biosynthesis of other C-12 substituted withanolides found in the genus *Jaborosa*, namely, the trechonolide-type withanolides,[12] 21-hydroxylated withanolides that form an additional ring through a cyclic hemiketal between OH-21 and a 12-ketone,[13] and the C-13 spiranoid withanolides.[8] The 12-oxowithanolides occur rarely in nature, and only three examples are known in the genus *Jaborosa*: jaboromagellone, jaboromagellonine, and projaborol, which were isolated from *Jaborosa magellanica* together with trechonolide-type withanolides.[9]

Several withanolides isolated from different species of *Jaborosa* have shown selective phytotoxic activity toward dicotyledon species. To evaluate this bioactivity, the major component of *J. kurtzii*, compound **1**, was tested on seed germination and radicle length on both a dicotyledon (*Lactuca sativa*) and a monocotyledon (*Avena sativa*) as standard target species in the range concentration of 15–400 ppm. Compound **1** did not produce a significant effect on the germination of the assayed species. However, significant inhibition of radicle growth was observed at 400 ppm on *L. sativa* (32 %), while on *A. sativa*, the corresponding inhibition was quite small (4%) (Figure 2). A similar selective inhibition effect on dicotyledon species was reported for jaborosalactol 18 isolated from *Jaborosa bergii*[4] and for jaborosalactone 29 isolated from *Jaborosa rotacea*.[7]

Experimental Section

General Experimental Procedures. Melting points were measured on a mercury thermometer apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1010 polarimeter. UV spectra were obtained using a Shimadzu-260 spectrophotometer. IR spectra were obtained in a Nicolet 5-SXC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer operating at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C and on a Bruker AVANCE II AV-400 operating at 400.13 MHz for ^{1}H. Multiplicity determinations (DEPT) and 2D spectra (COSY, HETCOR, and HMBC) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from tetramethylsilane (TMS) as the internal standard. FABMS and HRFABMS were measured on an NBA-sodium matrix in a VG-ZAB mass spectrometer. EIMS and HREIMS were determined at 70 eV on a VG Auto Speccon mass spectrometer. Chromatographic separations were performed by vacuum-liquid chromatography, column chromatography on silica gel 60 (0.063–0.200 mm), radial chromatography with a radial Chromatotron Model 7924 T on silica gel 60 PF_{254} Merck (1 mm thick), and preparative thin-layer chromatography (TLC) on silica gel 60 F_{254} (0.2 mm thick) plates.

Plant Material. The aerial parts of *J. kurtzii* were collected in Departamento Lujan, km 1086, Mendoza, Argentina, in February 2002. A voucher specimen is deposited at Museo Botánico Córdoba, Universidad Nacional de Córdoba, under Oberti CORD 862. This plant material was identified by Gloria Barboza.

Extraction and Isolation. The air-dried powdered aerial parts of *J. kurtzii* (210 g) were exhaustively extracted with EtOH, and the solvent was evaporated at reduced pressure. The residue (19.8 g) was defatted by partition in hexane/MeOH/H_2O (10:3:1), with the resultant MeOH/H_2O phase washed with hexane (3 × 100 mL) and MeOH evaporated at reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue (5.95 g) was fractionated initially by vacuum-liquid chromatography. Elution with hexane/EtOAc mixtures of increasing polarity (from 80:20 to 0:100) afforded four fractions containing withanolides: fractions III, IV, V, and VI (hexane/EtOAc, from 3:2 to 3:7). From fraction V, compound **1** (400 mg) precipitated. Fractions III–VI (2.58 g) were pooled and chromatographed on silica gel 60 G. Elution with CH_2Cl_2/EtOAc mixtures of increasing polarity (from 90:10 to 0:100) and EtOAc/MeOH (95:05) afforded a mixture (256 mg) that was further processed by radial chromatography with CH_2Cl_2/MeOH mixtures of increasing polarity (from 100:0 to 80:20), yielding compound **2** (8 mg).

Jaborosalactone 43 [(20*S*,22*S*)-12α,22-Epoxy-12β,17β-dihydroxy-1-oxowitha-2,5,24-trien-26,22-olide] (1): colorless crystals (hexane/EtOAc), mp 167 °C (dec). $[\alpha]_D^{21}$ +12.9 (*c* 0.044, MeOH). UV (MeOH) λ_{max} (log ε): 227 (4.01) nm. IR (dry film) ν_{max}: 3455 (s), 2960 (m), 1694 (s), 1676 (s), 1384 (m), 983 (m), 765 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ: 6.79 (1H, ddd, J = 10.0, 5.0, 2.5 Hz, H-3), 5.86 (1H,

dd, J = 10.0, 2.0 Hz, H-2), 5.57 (1H, d, J = 5.9 Hz, H-6), 3.29 (1H, m, H-4β), 2.84 (1H, dd, J = 21.0, 4.2 Hz, H-23α), 2.83 (1H, m, H-4α), 2.83 (1H, m, H-20), 2.48 (1H, dd, J = 11.7, 2.5 Hz, H-11β), 2.30 (1H, m, H-16α), 2.19 (1H, d, J = 17.9 Hz, H-23β), 2.00 (1H, m, H-7β), 1.94 (3H, s, H-28), 1.92 (3H, s, H-27), 1.82 (1H, m, H-14), 1.80 (1H, m, H-9), 1.80 (1H, m, H-16β), 1.73 (1H, m, H-11α), 1.54 (2H, m, H-15), 1.52 (1H, m, H-7α), 1.51 (1H, m, H-8), 1.21 (3H, s, H-19), 1.18 (3H, d, J = 6.9 Hz, H-21), 1.12 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 50.32 MHz) δ: 203.7 (C, C-1), 165.0 (C, C-26), 147.2 (C, C-24), 145.3 (CH, C-3), 135.9 (C, C-5), 127.7 (CH, C-2), 124.1 (CH, C-6), 120.1 (C, C-25), 103.5 (C, C-22), 100.1 (C, C-12), 78.9 (C, C-17), 50.0 (C, C-10), 47.8 (CH, C-14), 47.5 (C, C-13), 40.7 (CH, C-20), 40.4 (CH, C-9), 39.3 (CH_2, C-23), 37.2 (CH_2, C-11), 34.1 (CH_2, C-16), 33.3 (CH_2, C-4), 32.3 (CH, C-8), 29.8 (CH_2, C-7), 21.9 (CH_2, C-15), 20.4 (CH_3, C-28), 18.7 (CH_3, C-19), 12.1 (CH_3, C-18), 11.9 (CH_3, C-27), 10.0 (CH_3, C-21). FABMS m/z: 491 $[M + Na]^+$ (15), 342 (14), 341 (100), 313 (34), 291 (17), 176 (35), 155 (23), 154 (54), 152 (27), 137 (64), 136 (58), 125 (26), 123 (24), 111 (23). HRFABMS m/z: $[M + Na]^+$ 491.2399 (calcd for $C_{28}H_{36}O_6Na$, 491.24095).

Jaborosalactone 44 [(20*R*,22*R*)-17β-Hydroxy-1,12-dioxowitha-2,5,24-trien-26,22-olide] (2): white amorphous powder. $[\alpha]_D^{21}$ +18.6 (c 0.043, MeOH). UV (MeOH) λ_{max} (log ε): 227 (4.00) nm. IR (dry film) ν_{max}: 3460 (s), 2930 (m), 1693 (s), 1681 (s), 1666 (m), 1386 (m), 1126 (m), 1003 (m), 738 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ: 6.80 (1H, ddd, J = 10.1, 5.1, 2.6 Hz, H-3), 5.90 (1H, ddd, J = 10.1, 2.9, 1.1 Hz, H-2), 5.62 (1H, d, J = 5.9 Hz, H-6), 4.56 (1H, ddd, J = 12.4, 6.6, 3.6 Hz, H-22), 3.32 (1H, m, H-4β), 3.20 (1H, dd, J = 17.5, 5.8 Hz, H-11α), 2.89 (1H, m, H-4α), 2.83 (1H, m, H-20), 2.56 (1H, dd, J = 17.5, 11.9 Hz, H-11β), 2.52 (1H, m, H-23α), 2.26 (1H, m, H-23β), 2.21 (1H, m, H-9), 2.14 (1H, m, H-7β), 2.02 (1H, m, H-16β), 1.95 (1H, m, H-14), 1.93 (3H, s, H-28), 1.87 (3H, s, H-27), 1.82 (1H, m, H-8), 1.73 (1H, m, H-7α), 1.63 (1H, m, H-16α), 1.61 (2H, m, H-15), 1.27 (3H, s, H-19), 1.20 (3H, s, H-18), 0.93 (3H, d, J = 6.9 Hz, H-21). ^{13}C NMR ($CDCl_3$, 50.32 MHz) δ: 214.2 (C, C-12), 202.9 (C, C-1), 165.8 (C, C-26), 149.5 (C, C-24), 145.3 (CH, C-3), 135.8 (C, C-5), 127.8 (CH, C-2), 124.1 (CH, C-6), 121.7 (C, C-25), 83.6 (C, C-17), 78.4 (CH, C-22), 59.0 (C, C-13), 50.7 (C, C-10), 48.5 (CH, C-14), 43.6 (CH, C-20), 42.3 (CH, C-9), 40.0 (CH_2, C-11), 34.1 (CH_2, C-23), 33.3 (CH_2, C-4), 33.1 (CH_2, C-16), 32.2 (CH, C-8), 30.4 (CH_2, C-7), 23.0 (CH_2, C-15), 20.4 (CH_3, C-28), 18.5 (CH_3, C-19), 18.0 (CH_3, C-18), 14.0 (CH_3, C-21), 12.3 (CH_3, C-27). EIMS m/z: 452 $[M]^+$ (13), 434 $[M - H_2O]^+$ (6), 419 (16) 299 (19), 281 (16), 243 (20), 152 (26), 137 (26), 125 (100), 121 (28), 109 (44), 97 (39), 91 (30), 64 (24). HREIMS m/z: $[M]^+$ 452.2583 (calcd for $C_{28}H_{36}O_5$ 452.2563).

Seed Germination Bioassays. Seeds of *L. sativa* (lettuce) and *A. sativa* (oats) were obtained from the Laboratorio de Semillas (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina). Bioassays were carried out as reported previously.[14] Germination and root length values of treated and control experiments were analyzed by the Student's t test ($p < 0.05$).

Acknowledgment. This work was supported by grants from CONICET (Argentina), SeCyT-UNC, and Agencia Córdoba Ciencia. N.S.R. thanks Agencia Córdoba Ciencia for a fellowship. We thank Prof. G. E. Barboza (IMBIV-CONICET) for the collection and identification of plant material and Prof. A. Gutiérrez Ravelo (Instituto de Bioorgánica Antonio González, Universidad de La Laguna, Tenerife, Spain) for the mass spectra.

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NP0701780