Phytotoxic Withanolides from *Jaborosa rotacea*

Viviana E. Nicotra,[†] Natalia S. Ramacciotti,[†] Roberto R. Gil,[†,‡] Juan C. Oberti,[†] Gabriela E. Feresin,[§] Cecilia A. Guerrero,[§] Ricardo F. Baggio,[⊥] M. Teresa Garland,[∥] and Gerardo Burton*[,▽]

Departamento de Química Orgánica and IMBIV, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina, Instituto de Biotecnología, Facultad de Ingeniería, Universidad Nacional de San Juan, San Juan, Argentina, Departamento de Física, CNEA, Buenos Aires, Argentina, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Chile, and Departamento de Química Orgánica and UMYMFOR (CONICET-FCEN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EGA Buenos Aires, Argentina

Twelve new withanolides were isolated from the aerial part of *Jaborosa rotacea*: five had a spiranoid δ-lactone (**1**−**5**); one contained a 26,12-δ-lactone and a C-12−C-23 bond (**6**); five corresponded to trechonolide-type withanolides with configuration at C-23 opposite of those previously isolated (**7**, **8**, **10**−**12**); two of these have an additional oxido-bridge between C-21 and C-24; finally a withanolide with a hemiketal ring formed between a 21-hydroxyl and a 12-ketone (**13**) and the closely related jaborosalactone R were also isolated. New compounds were fully characterized by a combination of spectroscopic methods (1D and 2D NMR and MS). The structures of the spiranoid withanolide and of the epimer of trechonolide A were confirmed by X-ray diffraction studies. Compounds **4**, **5**, **6**, and **8** showed selective phytotoxicity toward monocotyledoneous and dicotyledoneous species.

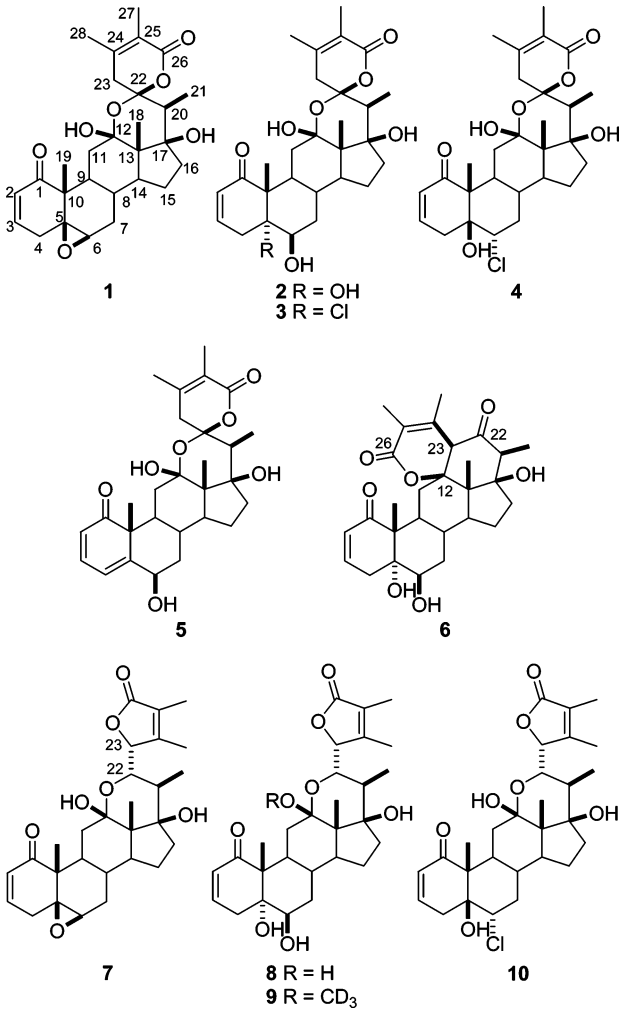
The withanolides are a group of naturally occurring C-28 steroids built on an ergostane skeleton functionalized at carbons 1, 22, and 26, commonly known as the withanolide skeleton. Their chemistry and occurrence has been the subject of several reviews.[1−3] Many withanolides exhibit a variety of biological activities, including antifeedant, insecticidal,[3] phytotoxic,[4] immunosuppressive,[5] and cancer chemoprevention properties.[6,7] While the presence of withanolides is almost a monopoly of Solanaceous plants, these compounds are not present in all members of the Solanaceae family. Thus far, members of 12 Solanaceous genera, all within the subfamily Solanoideae, have been shown to contain withanolides. *Jaborosa* Miers is a South American genus belonging to the Solanaceae that comprises about 23 different species, which grow mainly in Argentina. As part of our investigations of the withanolides of *Jaborosa* Miers species, we studied the withanolides from *Jaborosa rotacea* (Lillo) A. T. Hunziker et Barboza collected in Tucumán Province, Argentina.

Results and Discussion

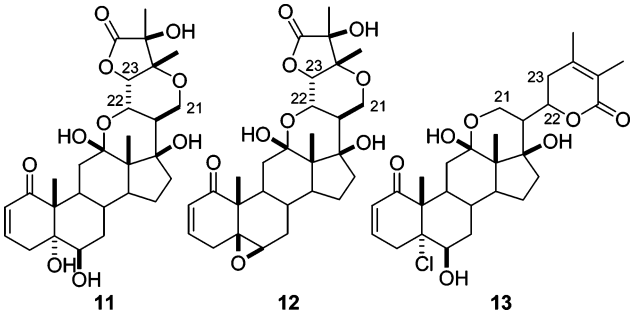
The aerial part of *Jaborosa rotacea* plants was air-dried and extracted with ethanol. After concentration and defattening, the residue was fractionated by a combination of chromatographic techniques, ultimately giving 12 new withanolides (**1**−**8**, **10**−**13**) and the known jaborosalactone R, previously isolated from *Jaborosa sativa*.[8]

Compound **1** revealed a molecular formula of $C_{28}H_{36}O_7$ by HRFABMS. The ^{1}H NMR spectrum of **1** exhibited only three signals at the low-field end, two olefinic protons at δ 6.02 (dd, J = 9.9 and 2.5 Hz) and 6.84 (ddd, J = 9.9, 6.2, and 2.2 Hz) typical of a 2-en-1-one system in ring A, and a doublet at δ 3.13 consistent with a 5β,6β-epoxy group, also supported by the small value of the coupling constant between H-6α and H-7β (J = 2.2 Hz).[9] The substitution pattern in ring B was further confirmed by the signals at δ 61.9 and 63.0 in the ^{13}C NMR spectrum (Table 1) that were assigned to C-5 and C-6, respectively. The chemical shift of the methyl group H_3-18 (δ 1.09) was indicative of the presence of a hydroxyl group with β configuration at C-17. The nonprotonated carbon signal at δ 78.9 assigned to C-17 confirmed this assumption.

Regarding the side chain, a singlet observed at δ 1.91 integrating for six hydrogens was assigned to the two methyls of an α,β-



* Corresponding author. Tel/Fax: (54-11) 4576-3385. E-mail: burton@qo.fcen.uba.ar.
† Universidad de Córdoba.
‡ Present address: Department of Chemistry, Carnegie Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213.
§ Universidad Nacional de San Juan.
⊥ Departamento de Física, CNEA.
∥ Universidad de Chile.
▽ Universidad de Buenos Aires.



11 **12** **13**

unsaturated δ-lactone bearing methyl groups at positions C-24 and C-25. The doublet for the C-21 methyl at δ 1.16 confirmed the absence of a hydroxyl group at C-20. The ^{1}H NMR spectrum did not show the characteristic signal corresponding to the carbinyl hydrogen at C-22 in the 4−5 ppm region. However, two nonprotonated carbon signals were observed at δ 100.0 and 103.5 in the ^{13}C NMR spectrum, corresponding to two acetal or ketal centers, assigned to C-12 and C-22, respectively. These data provided clear evidence for the hemiketal bridge between what must have originally been ketone functions at C-12 and C-22, resulting in a new six-membered ring with a β-oriented hydroxyl group at C-12 and a spiranoid center at C-22 upon formation of the δ-lactone. The resonances for H-23α and H-23β were observed as doublets exhibiting only the geminal mutual coupling, thus indicating that the methylene at this position did not have neighboring protons, in agreement with the proposed structure. The ^{13}C NMR spectrum showed two carbonyl groups at δ 202.6 and 164.5, assigned to C-1 and C-26, respectively. Also evident from the spectrum were the four olefinic carbon resonances at δ 129.4, 144.2, 146.7, and 120.4 corresponding to C-2, C-3, C-24, and C-25, respectively.

Confirmation of the structure of **1** and assignment of the configuration at C-12, C-17, C-20, and C-22 came from X-ray diffraction analysis. The diffraction data led to the structure depicted in Figure 1, where the orientation for the hydroxyl groups at positions 12 and 17 was established as β. The *S* configuration for C-20 and C-22 was also evident. The interatomic distances in the crystal structure indicate hydrogen bonds between the δ-lactone oxygen atom and the hydroxyl groups at C-12 and C-17.[10]

Table 1. ^{13}C NMR Data of Compounds **1**−**6** in $CDCl_3$[a]

C	1	2	3	4	5	6
1	202.6	204.0	200.9	199.7	204.9	203.8
2	129.4	128.6	128.4	127.6	126.8	128.3
3	144.2	141.5	141.6	142.0	139.9	141.4
4	32.80	35.7	37.2	31.5	118.3	35.8
5	61.9	77.3	80.4	77.3	157.5	77.2
6	63.0	73.9	74.2	65.9	73.4	73.8
7	30.3	32.6	32.4	37.7	39.0	32.4
8	29.2	29.4	29.5	34.6	29.9	30.5
9	41.9	38.4	39.2	42.4	46.2	39.0
10	48.0	51.8	52.5	54.9	53.1	51.5
11	37.2	37.0	36.9	37.0	35.4	32.2
12	100.0	100.2	100.3	99.5	100.1	89.6
13	48.0	48.1	48.1	48.2	47.7	50.3
14	47.4	47.3	47.2	47.5	46.2	48.5
15	21.9	22.0	21.9	21.8	22.1	22.7
16	34.1	34.2	34.2	34.2	34.1	34.7
17	78.9	79.1	79.1	78.8	78.5	88.4
18	12.2	12.5	12.8	12.4	12.3	11.7
19	14.9	15.5	16.2	8.8	19.6	15.3
20	40.7	40.7	40.7	40.8	40.6	51.0
21	10.0	10.1	10.1	10.0	10.1	7.2
22	103.5	103.9	103.9	103.6	103.6	207.7
23	39.3	39.2	39.3	39.3	39.2	54.8
24	146.7	147.9	148.0	146.3	146.8	123.2
25	120.4	120.1	120.1	120.6	120.4	146.7
26	164.5	165.3	165.3	164.1	164.7	165.4
27	12.3	12.1	12.2	12.3	12.5	12.3
28	20.5	20.5	20.6	20.5	20.5	21.2

[a] Chemical shifts (δ) downfield from TMS; 50.32 MHz.

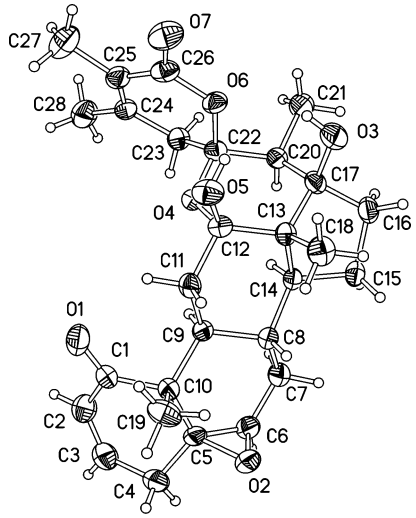


Figure 1. Displacement ellipsoid diagram (40% probability level) of jaborosalactone 26 (**1**) showing the numbering scheme used. Broken lines show the intramolecular O−H- - -O bonds.

The ^{1}H and ^{13}C NMR spectra of withanolides **2**−**5** were closely related to those of **1**, showing patterns typical of the spiranoid lactone arrangement at C-22, for resonances of carbons 20−28 and their protons. The almost identical ^{13}C NMR data (Table 1) for rings C and D and the side chain of compounds **1**−**5** indicated that structural differences were restricted to substituents in rings A and B. Furthermore, the presence of a 1-oxo-2-ene functionality in ring A was evident for the five compounds. The ^{1}H and ^{13}C NMR data of **2** were consistent with a 5α,6β-diol typical of many withanolides.[8] The small couplings in the H-6 resonance at δ 3.63 confirmed the axial orientation (β) of the 6-hydroxyl. The ^{13}C NMR spectrum showed the expected chemical shifts for signals of carbons C-5 and C-6 at δ 77.3 and 73.9, respectively. The ^{1}H and ^{13}C NMR data of **3** were consistent with a 5α-chloro-6β-hydroxy arrangement. Thus the broad singlet at δ 4.04 was assigned to equatorial H-6, and the unusually high chemical shift observed for H-4β at δ 3.53 (dt, J = 20.0 and 2.4 Hz) was indicative of a chlorine atom at C-5 with α-orientation.[9,11] The substitution pattern in ring B was further confirmed by the signals at δ 80.4 and 74.2 in the ^{13}C NMR spectrum that were assigned to C-5 and C-6, respectively. Highly diagnostic of the ring B substitution in **4** was the double doublet at δ 4.31 (J = 12.5 and 4.8 Hz) assigned to H-6 of a 5β-hydroxy-6α-chloro arrangement.[8] Analysis of the COSY and HSQC spectra allowed correlation of the H-6 signal with the hydrogens at position 7 (δ 2.24 and 1.47) and with C-6 at δ 65.9. The spin−spin coupling pattern of H-6 indicated its axial (β) orientation. Finally, the ^{1}H NMR spectrum of **5** presented signals at δ 6.04, 6.94, and 6.17 assigned to three olefinic protons at C-2, C-3, and C-4, respectively. In addition to these signals, the ^{1}H NMR spectrum displayed a carbinyl hydrogen signal at δ 4.57 (t, J = 2.7 Hz) assigned to H-6. The presence of the 1-oxo-2,4-diene-6β-hydroxy moiety was confirmed by the signals at δ 126.8, 139.9, 118.3, 157.5, and 73.4 in the ^{13}C NMR spectrum, assigned to C-2, C-3, C-4, C-5, and C-6, respectively.[12] The full and unambiguous proton and carbon NMR assignments for compounds **2**−**5** were confirmed using a combination of DEPT-135, COSY, and HETCOR or HSQC experiments. High-resolution mass measurements were in agreement with the proposed formulas. The occurrence of 5,6-chlorohydrins and diols together with the corresponding 5,6-epoxides is common among the withanolides, and their natural origin has been proven throughout the literature.[1−3]

Compound **6** revealed a molecular formula of $C_{28}H_{36}O_7$ by HRFABMS. Comparison of its ^{1}H and ^{13}C NMR spectra with those of compound **4** showed almost identical signals for all carbons and protons of rings A and B, indicating a 1-oxo-2-ene-5α,6β-diol

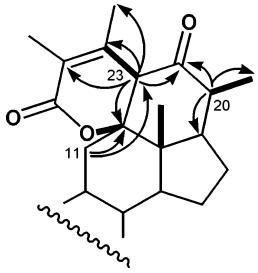


Figure 2. Relevant HMBC correlations (H→C) in the side chain of jaborosalactone 31 (**6**).

system. Five methyl signals were evident in the ^{1}H NMR spectrum of **6**, and the resonance of the H_3-19 methyl group at δ 1.29 was consistent with the substitution pattern in rings A and B. The high chemical shift of the H_3-18 methyl group (δ 1.26) suggested a hydroxyl group with β configuration at C-17, and the doublet for H_3-21 (δ 1.14) confirmed the absence of a hydroxyl group at C-20. The two methyl signals at δ 1.99 and 2.06 assigned to H_3-27 and H_3-28, respectively, are typical of an α,β-unsaturated lactone ring. Also evident in this spectrum was the methine singlet at δ 3.81 assigned to H-23, directly correlated to a carbon resonance at δ 54.8. In the ^{13}C NMR spectrum three carbonyl carbons were evident. The ketone carbonyls at δ 203.8 and 207.7 were assigned to C-1 of a 1-oxo-2-ene arrangement and to C-22, respectively. The lactone carbonyl at δ 165.4 was assigned to C-26 of an α,β-unsaturated δ-lactone. Besides the signals of C-5 and C-17 at δ 77.2 and 88.4, another oxygenated nonprotonated carbon was present at δ 89.6, attributed to C-12. These evidences led us to propose a bond between C-12 and C-23, resulting in a six-membered ring, an arrangement similar to that present in the spiranoid withanolides isolated from *J. odonelliana*,[13,14] *J. runcinata*, and *J.* araucana.[11] However, at variance with the spiranoid γ-lactone arrangement, **6** had a δ-lactone formed between the C-26 carboxyl and the C-12 hydroxyl. This structure was confirmed by the cross-correlation peaks observed in the COSY and HMBC experiments. The key correlations observed in the latter (Figure 2) were for H-23 (δ 3.81) with C-12 (δ 89.6), C-22 (δ 207.7), and C-24 (δ 123.2) and for H-11β (δ 1.64) with C-23 (δ 54.8). The configuration at C-23 was established from a NOESY experiment, in which the NOE correlations observed for H-23 with H-9, H-14, and H-20 indicated the 23*R* stereochemistry (see Supporting Information).

Compound **7** revealed a molecular formula of $C_{28}H_{36}O_7$ by HRFABMS. The ^{1}H and ^{13}C NMR data of compound **7** were very similar to those of the known trechonolide A for rings A−D and the γ-lactone side chain.[15,16] The main difference observed was the downfield shift of the C-23 resonance from δ 82.4 for trechonolide A to δ 86.0 for **7** (Table 2). Smaller shifts were evident for the C-22 and C-24 resonances (from δ 68.7 and 157.1 to δ 70.9 and 158.2, respectively) as well as the chemical shifts for H-20 and H_3-21 (δ 2.29 and 1.00 to δ 2.05 and 0.75). The similarity of the NMR data suggested the same skeleton and substitution pattern for trechonolide A and **7** but a different configuration in the side chain (C-20, C-22, and/or C-23). The large coupling between H-22 and H-20 (J = 11.3 Hz) indicated an *anti* arrangement for these hydrogens, as found in all trechonolide-type withanolides, indicating the same configuration at C-20 and C-22 in **7** and trechonolide A. Therefore we considered that **7** should have an opposite configuration at the C-23 position. Circular dichroism data and the sign of the Cotton effect supported this assumption; thus trechonolide A showed a negative Cotton effect at 218 nm ($\Delta\epsilon$: −1.4), while compound **7** showed a positive Cotton effect at 218 nm ($\Delta\epsilon$: +1.6). Surprisingly, single-crystal X-ray analysis of compound **7** revealed an *R* configuration for C-23 (Figure 3),[10] the same as that reported for trechonolide A by Lavie et al.[15] and Fajardo et al.[16] Careful inspection of the published X-ray data for trechonolide A[15,16] showed that the C-23 configuration had been incorrectly assigned

Table 2. ^{13}C NMR Data for Compounds **7** and **9**−**13** in $CDCl_3$[a]

C	7	9[b]	10	11	12	13
1	202.6	204.5	200.0	205.0	202.9	200.8
2	129.5	128.3	127.3	128.1	129.7	128.6
3	144.2	141.8	142.8	142.5	144.7	141.3
4	32.7	35.4	31.7	35.4	32.6	37.1
5	61.9	77.2	77.4	77.3	61.5	79.9
6	63.1	73.8	65.9	74.2	63.0	74.5
7	30.2	33.1[c]	37.8	32.8	30.0	33.5
8	29.4	29.5	34.7	29.6	29.4	28.7
9	41.9	38.1	42.2	38.4	41.7	39.9
10	47.1	51.7	54.9	51.8	48.0	52.5
11	36.6	29.8	36.3	36.7	36.4	37.3
12	99.3	102.4	98.7	100.2	99.7	100.5
13	47.9	47.8	47.7	48.4	48.0	51.0
14	46.1	46.0	45.9	45.2	45.6	49.8
15	22.7	22.7	22.6	22.9	22.7	24.2
16	33.9	32.5[c]	33.9	33.6	33.5	35.4
17	80.6	80.5	80.3	78.1	77.9	79.0
18	9.9	12.0	12.0	11.5	11.3	12.6
19	14.8	15.4	8.8	15.6	14.4	16.3
20	35.7	36.3	35.7	37.4	37.3	47.6
21	11.9	10.0	9.9	61.9	61.8	59.7
22	70.9	71.4	70.8	64.7	64.5	76.6
23	86.0	85.9	85.5	80.4	80.4	35.2
24	158.2	158.7	157.5	81.2	81.3	149.4
25	124.1	124.1	124.4	77.6	77.5	121.9
26	174.8	175.0	174.7	177.7	178.1	165.2
27	8.4	8.3	8.4	11.5	11.5	12.4
28	13.4	13.4	13.3	15.7	15.5	20.5

[a] Chemical shifts (δ) downfield from TMS; 50.32 MHz. [b] Cl_3CD−CD_3OD (95:5); the spectrum corresponds to the 12-O-CD_3 derivative of **8**. [c] Assignments may be interchanged.

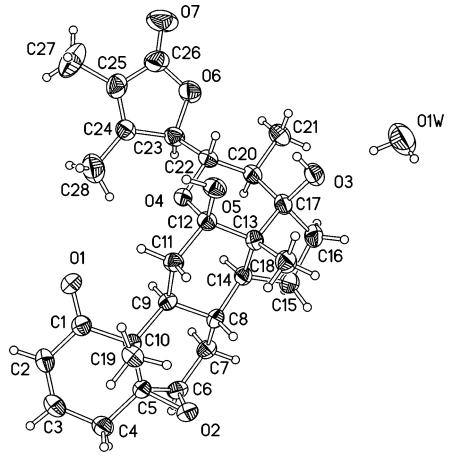


Figure 3. Displacement ellipsoid diagram (40% probability level) of jaborosalactone 32 (**7**).

in both cases and that it effectively was *S*. In view of the above, the structures of all previously known trechonolides that have been assigned the 23*R* configuration upon comparison with trechonolide A should now be revised. Both the carbon chemical shift of C-23 and the sign of the Cotton effect at 218 nm may be used as a direct indicator of the configuration at this position.

The FABMS of compound **8** showed a quasimolecular ion [M + H] at *m*/*z* 503, corresponding to a formula of $C_{28}H_{38}O_8$. However the NMR spectra obtained in deuterochloroform−methanol-d_4 (95:5) corresponded to the 12-*O*-trideuteromethyl derivative **9** being closely related to those of 12-*O*-methyljaborosotetrol previously isolated from *Jaborosa leucotricha*.[17] The ease with which hemiketals such as **8** react with methanol (in this case deuteromethanol) is well documented in the literature and reinforces the assumption of their artifactual nature.[15,17] The most remarkable difference between the spectra of **9** and 12-*O*-methyljaborosotetrol was the downfield shift of the C-23 resonance from δ 82.2 in the latter to δ 85.9, suggesting as in the previous case, an inverted configuration at C-23

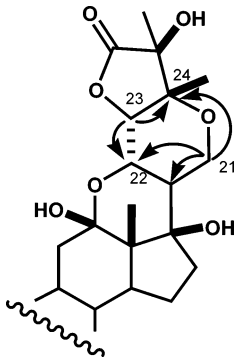


Figure 4. Relevant HMBC correlations (H→C) in the side chain of jaborosalactone 35 (**11**).

(i.e., 23*R* for **8**). This was confirmed by CD measurements that showed a positive Cotton effect for **8**. Thus the structure of **8** was established as the C-23 epimer of jaborosotetrol.

The ^{1}H and ^{13}C NMR data of compound **10** were closely related to those of jaborochlorotriol, previously isolated from *J. magellanica*;[18] the same differences observed between **7** and trechonolide A were evident in this case. Compound **10** showed different chemical shifts for H_3-21, C-20, C-24, and especially C-23 (from δ 82.14 for jaborochlorotriol to 85.5 for **10**) (Table 2). Circular dichroism measurements also showed a positive Cotton effect for this compound. These results in conjunction with the NMR data confirmed the structure of **10** as the C-23 epimer of jaborochlorotriol. The NMR assignments for **7**, the trideuteromethyl derivative **9**, and **10** were confirmed by DEPT, COSY, and HETCOR experiments.

The ^{1}H and ^{13}C NMR spectra of **11** revealed a 1-oxo-2-ene-5α,6β-dihydroxy substitution pattern from the characteristic signals corresponding to C-1, C-2, C-3, C-4, C-5, and C-6 and the corresponding protons. The chemical shifts and multiplicity observed for H-23 (δ 4.69 d), H-22 (δ 4.44 dd), C-12 (δ 100.2), C-22 (δ 64.7), and C-23 (δ 80.4) were indicative of a trechonolide-type skeleton; however some differences were evident. Only four methyl resonances were observed. The appearance of signals at δ 3.83 and 3.69 suggested an oxygenated function at C-21, consistent with the double triplet observed for H-20. The COSY spectrum showed the expected correlations between hydrogens at position 21 and H-20. Furthermore, the spin−spin coupling observed between H-20 and each of the hydrogens at C-21 (J = 11.6 and 3.3 Hz) suggested that carbons 20 and 21 were part of a ring. The chemical shifts of H_3-27 and H_3-28 were consistent with oxygenated substituents at C-24 and C-25; thus structure **11**, containing an oxygen bridge between C-21 and C-24, was proposed. The ^{13}C NMR and DEPT spectral data of **11** were in agreement with this structure; two carbonyl carbons were observed at δ 205.0 and 177.7 assigned to C-1 of the 1-oxo-2-ene system in ring A and C-26 of the five-membered saturated lactone in the side chain. The methylene resonance at δ 61.9 was assigned to C-21, and the oxygenated quaternary carbons at δ 81.2 and 77.6 were assigned to C-24 and C-25, respectively. A strong correlation in the HMBC spectrum between the ^{1}H double doublet at δ 3.83 (H-21α) and the carbon at δ 81.2 (C-24) confirmed the additional ring (Figure 4). The configuration of **11** at positions 23, 24, and 25, was assigned on the basis of spectroscopic evidence and biosynthetic considerations. The small coupling observed between H-22 and H-23 (J = 2.7 Hz) allowed us to discard those structures where H-22 and H-23 have an *anti* arrangement. Thus, assuming a 22*S* configuration as in most known withanolides,[19] the configuration at C-23 must be *S*, consistent with that found in compounds **7**−**9**. The strong NOE correlations of H-28 with H-21β, H-22, and H-23 and the absence of NOE between H-21β and H-23 are consistent with a 24*R* stereochemistry. Finally assuming that the compound originates in the addition of a 21-hydroxyl to a 24,25-epoxide, the configuration at C-25 may be assumed as *S*. Thus, the configuration of the side chain was assigned as (22*S**,23*S**,24*R**,25*S**).

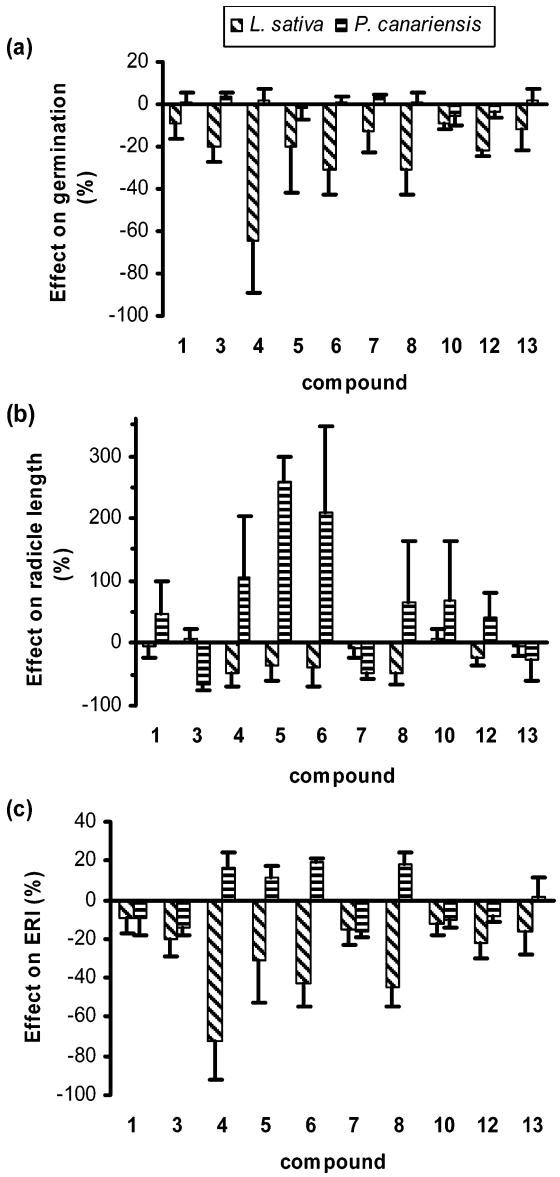


Figure 5. Effect of withanolides **1**, **3**−**8**, **10**, **12**, and **13** on (a) germination, (b) radicle length, and (c) emergence rate index (ERI) of *Lactuca sativa* (dicotyledon) and *Phalaris canariensis* (monocotyledon). The data are presented as percentage differences from the control (zero value); positive values represent stimulation of the studied variable and negative values represent inhibition. Withanolide concentration was 50 μg/mL in all cases.

Compound **12** revealed a molecular formula of $C_{28}H_{36}O_9$ by HRFABMS. The ^{1}H and ^{13}C NMR spectral data of **12** were very similar to those of **11**. A 5β,6β-epoxide was evident in the ^{1}H NMR spectrum from the doublet at δ 3.15 (J = 1.8 Hz), corresponding to H-6, in agreement with the signals at δ 61.5 and 63.0 in the ^{13}C NMR spectrum. The assignments for **12** were confirmed by DEPT, COSY, HETCOR, and NOESY spectra.

Compound **13** had ^{1}H and ^{13}C NMR data closely resembling those of jaborosalactones R, S, and T previously isolated from *Jaborosa sativa*,[8] differing only in the substitution pattern of ring B. On the other hand, the resonances from rings A and B were almost identical to those in compound **2**, especially the unusually high chemical shift observed for H-4β (δ 3.53 dt) and the broad singlet at δ 4.06 assigned to H-6α, consistent with a 5α-chloro-6β-hydroxy arrangement in ring B. The ^{13}C NMR and DEPT spectra of **13** were in agreement with the proposed structure.

Some withanolides have shown selective phytotoxic effects on monocotyledons and dicotyledons.[4,9] Thus jaborosalactone 18, isolated from *Jaborosa bergii*, inhibited radicle growth of several dicotyledons and stimulated it in monocotyledons (at 2×10^{-3} M) but had no effect on germination.[9] To evaluate the withanolides of *J. rotacea* as potential phytotoxic agents, their effects on germination, radicle growth, and emergence rate were assayed in *Lactuca sativa* (dicotyledon) and *Phalaris canariensis* (monocotyledon). The assays were performed according to the procedures optimized by Macias et al.[20] and the methodology of Ma et al.[21] The results are reported as percentage differences of germination (Figure 5a), radicle growth (Figure 5b), and emergence rate index[22] (Figure 5c) from controls. Compounds **4**, **5**, **6**, and **8** had opposite effects on the dicotyledon *L. sativa* and the monocotyledon *P. canariensis*. Compound **4** had the strongest inhibitory effect, selectively inhibiting germination, radicle growth, and emergence rate index of *L. sativa*, while its effect on *P. canariensis* did not differ significantly from controls. Compounds **5** and **6** had the highest stimulatory effect on radicle growth of *P. canariensis*. Compound **3** was the only withanolide that significantly inhibited radicle growth of *P. canariensis*.

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 in a Shimadzu-260 spectrophotometer. Circular dichroism spectra were measured on a Jasco J-810 spectropolarimeter. 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 at 200.13 (^{1}H) and 50.32 (^{13}C) MHz or a Bruker AM-500 at 500.13 (^{1}H) and 125.77 (^{13}C) MHz. Multiplicity determinations (DEPT) and 2D spectra (COSY, HETCOR, HMBC, HSQC, and NOESY) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from TMS internal standard. FABMS and HRFABMS were measured on a NBA-sodium matrix in a VG-ZAB mass spectrometer. Single-crystal X-ray measurements were performed on a Bruker SMART CCD diffractometer, with graphite-monochromated Mo Kα radiation. The structures were solved by direct methods with SHELXS97[23] and refined by full matrix least squares in F^2 using SHELXL97.[24] C-H hydrogen atoms were idealized at their expected positions (C−H: 0.93 Å) and allowed to ride; O-H hydrogens were found in the late difference Fourier synthesis and refined with isotropic displacement factors 1.2 times those of the oxygen to which they were attached. Molecular plots were drawn with XP, in the SHELXLTL-PC package.[25]

Chromatographic separations were performed by vacuum liquid chromatography, column chromatography (CC) on Si gel 60 (0.063−0.200 mm), radial chromatography with a radial Chromatotron Model 7924 T on Si gel 60 PF254 Merck (1 mm thick), preparative TLC on Si gel 60 F254 (0.2 mm thick) or Si gel reversed-phase C18 Merck, and preparative HPLC on a semipreparative HPLC (Spectra Physics) with a reversed-phase YMC ODS-A column (C-18), 22 × 250 mm (5 μm, 120 Å particle size), using a Shodex RI 71 detector.

Plant Material. The aerial parts of *Jaborosa rotacea* plants were collected in Tafí del Valle, Department Tafí Km. 90/91, Tucumán, Argentina, in March 1998. A voucher specimen is deposited at Museo Botánico, Universidad Nacional de Córdoba, under No. Barboza-144.

Extraction and Isolation. The air-dried powdered aerial parts of *J. rotacea* (1000 g) were extracted exhaustively with EtOH, and the resulting extract was concentrated at reduced pressure. The residue (244.67 g) was defatted by partition in hexane−MeOH−H_2O (10:9:1), the MeOH−H_2O phase was washed with hexane (3 × 400 mL), and the MeOH was evaporated at reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 400 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue (18.2 g) was initially fractionated by vacuum liquid chromatography. Elution with hexane−EtOAc mixtures of increasing polarity (100:0 to 0:100) afforded a fraction containing withanolides, and elution with EtOAc−MeOH (100:0 to 50:50) afforded two fractions containing withanolides. The fractions collected were I (2.86 g, hexane−EtOAc, 1:3), II (1.62 g, EtOAc−MeOH, 97.5:2.5), and III (2.31 g, EtOAc−MeOH, 92.5:7.5). Fraction I was subjected to CC with hexane−EtOAc, 2:1, yielding three fractions containing withanolides; compound **1** (30 mg) precipitated from the most polar fraction. Further fractionation of the remaining fractions by CC eluting with CH_2Cl_2−MeOH mixtures of increasing polarity gave compounds **3** (5 mg), **7** (50 mg), **10** (8.5 mg), and **12** (10 mg). Fraction II was separated by CC with hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to give a mixture that was further fractionated by preparative reversed-phase HPLC (water−MeOH, 1:1). This led to the isolation of (in order of elution) **11** (3 mg), **8** (10 mg), **2** (16 mg), and **13** (5 mg). The mixtures from fraction II, showing similar TLC behavior, were pooled and processed by radial chromatography with CH_2Cl_2−MeOH mixtures of increasing polarity and preparative reversed-phase TLC (water−acetonitrile, 1:1), yielding compound **6** (5 mg) and jaborosalactone R (4 mg). Column chromatography of fraction III with CH_2Cl_2−EtOAc and EtOAc−MeOH mixtures of increasing polarity gave compound **5** (12 mg) and **4** (15 mg). All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy.

Seed Germination Bioassays. Seeds of *Lactuca sativa* (lettuce) and *Phalaris canariensis* (birdseed) were obtained from Instituto Nacional de Tecnología Agropecuaria (INTA, San Juan, Argentina); they were surface sterilized with 1.5% (v/v) bleach for 1 min and washed with sterile deionized water. Bioassays were conducted according to the methodology of Ma.[21] Briefly, germination was carried out in sterile flasks over a disk of sterile filter paper. Compounds were added to each germination flask (10 μg/flask) in $CHCl_3$ solution, and the solvent was evaporated; seeds were added followed by sterile deionized water (200 μL) and the flasks sealed with plastic wrap to prevent moisture loss. Four replicates—20 seeds each—were prepared for each treatment, and germination was conducted at 25 °C in the dark for 4 days. Germination was considered positive when the radicle had protruded from the seed coat at least 1 mm. After 4 days, seeds with roots were frozen at −10 °C for 24 h to stop growth and facilitate handling and root elongation measurement. Inhibitory and stimulatory effects were calculated according to Ma et al.[21] Emergence rate index (ERI) was determined according to Schmueli and Goldberg.[22] Data are reported as difference from controls, and statistical significance between groups was determined using ANOVA ($p < 0.05$).

Jaborosalactone 26 ((20*S*,22*S*)-5*β*,6*β*:12α,22-diepoxy-12*β*,17*β*-dihydroxy-1-oxo-witha-2,24-dien-26,22-olide) (1): colorless crystals (hexane−EtOAc), mp 218−220 °C; $[\alpha]^{21}_D$ +3.3 (*c* 0.0036, $CHCl_3$); UV (MeOH) λ_{max} 219 nm; IR (dry film) ν_{max} 3500, 1703, 1673, 1382 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.84 (1H, ddd, J = 9.9, 6.2, 2.2, H-3), 6.02 (1H, dd, J = 9.9, 2.5, H-2), 3.13 (1H, d, J = 2.2, H-6), 2.97 (1H, dt, J = 18.6, 2.5, H-4*β*), 2.81 (1H, d, J = 17.9, H-23α), 2.24 (1H, dd, J = 14.2, 4.4, H-11α), 2.12 (1H, d, J = 17.9, H-23*β*), 2.08 (1H, br d, J = 14.2, H-7*β*), 1.93 (1H, m, H-20), 1.91 (6H, s, H-27, H-28), 1.89 (1H, dd, J = 18.6, 6.4, H-4α), 1.85 (1H, m, H-16α), 1.68 (1H, m, H-11*β*), 1.67 (1H, m, H-14), 1.64 (2H, m, H-8, H-16*β*), 1.59 (2H, m, H-15), 1.27 (1H, m, H-9), 1.22 (1H, m, H-7α), 1.22 (3H, s, H-19), 1.16 (3H, d, J = 6.9, H-21), 1.09 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; FABMS *m*/*z* 507 $[MNa]^+$ (76), 467 (MH − H_2O, 30), 341 (15), 313 (12), 166 (3), 158 (2), 150 (8), 145 (10), 144 (8), 132 (2), 128 (4), 123 (24); HRFABMS *m*/*z* 507.2364 (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

Crystallographic Data and Data Collection Parameters.[10] Colorless prismatic crystals recrystallized from hexane−EtOAc. $C_{28}H_{36}O_7$, M = 484.56, orthorhombic, space group $P2_12_12_12_1$ (#19); cell constants a = 6.4596(5) Å, b = 12.2100(10) Å, c = 30.375(3) Å; V = 2395.7-(3) Å^3, D_c(Z=2) = 1.341 g cm^{-3}; crystal dimensions 0.26 × 0.18 × 0.14 mm, reflections measured 17 738, reflections unique 5431, reflections observed ($I > 2\sigma(I)$) 2591; R = 0.046 and R_w^2 = 0.087.

Jaborosalactone 27 ((20*S*,22*S*)-12α,22-epoxy-5α,6*β*,12*β*,17*β*-tetrahydroxy-1-oxo-witha-2,24-dien-26,22-olide) (2): colorless crystals (hexane−EtOAc), mp 194 °C (dec); $[\alpha]^{21}_D$ +11.8 (*c* 0.0029, $CHCl_3$); UV (MeOH) λ_{max} 225 nm; IR (dry film) ν_{max} 3449, 1735, 1720, 1380 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.60 (1H, ddd, J = 9.9, 5.1, 2.6 Hz, H-3), 5.85 (1H, dd, J = 9.9, 2.2 Hz, H-2), 3.63 (1H, br s, H-6), 3.32 (1H, dt, J = 19.5, 2.6 Hz, H-4*β*), 2.82 (1H, br d, J = 18.3 Hz, H-23α), 2.53 (1H, dd, J = 14.3, 3.6 Hz, H-11α), 2.26 (1H, d, J = 18.3 Hz, H-23*β*), 2.06 (1H, dd, J = 19.5, 5.1 Hz, H-4α), 2.00 (1H, m, H-20), 1.94 (1H, m, H-9), 1.93 (3H, s, H-27), 1.90 (3H, s, H-28), 1.86 (1H, m, H-14), 1.85 (1H, m, H-8), 1.60 (2H, m, H-7), 1.59 (1H, m, H-15α), 1.52 (1H, m, H-11*β*), 1.50 (1H, m, H-15*β*), 1.28 (3H, s, H-19),

1.15 (3H, d, J = 6.9 Hz, H-21), 1.11 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; FABMS m/z 525 $[MNa]^+$ (29), 485 (MH − H_2O, 9), 439 (8), 341 (11), 166 (8), 150 (7), 145 (6), 128 (7), 123 (11); HRFABMS m/z 525.2483 (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

Jaborosalactone 28 ((20*S*,22*S*)-5α-chloro-12α,22-epoxy-6*β*,12*β*,-17*β*-trihydroxy-1-oxo-witha-2,24-dien-26,22-olide) (3): colorless crystals (hexane−EtOAc), mp 185−187 °C; $[\alpha]^{21}_D$ −0.6 (c 0.0045, $CHCl_3$); UV (MeOH) λ_{max} 224 nm; IR (dry film) ν_{max} 3394, 1698, 1459, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 6.66 (1H, ddd, J = 10.1,5.1, 2.0 Hz, H-3), 5.89 (1H, dd, J = 10.1, 2.6 Hz, H-2), 4.04 (1H, br s, H-6), 3.53 (1H, dt, J = 20.0, 2.4 Hz, H-4*β*), 2.84 (1H, d, J = 18.1 Hz, H-23α), 2.64 (1H, dd, J = 13.9, 4.2 Hz, H-11α), 2.52 (1H, dd, J = 20.0, 5.1 Hz, H-4α), 2.29 (1H, d, J = 18.1 Hz, H-23*β*), 2.07 (1H, m, H-9), 2.06 (1H, q, J = 6.7 Hz, H-20), 1.96 (3H, s, H-28), 1.92 (1H, m, H-7*β*), 1.91 (3H, s, H-27), 1.91 (2H, m, H-8, H-14), 1.84 (1H, m, H-16α), 1.68 (1H, m, H-16*β*), 1.66 (1H, br d, J = 12.8 Hz, H-7α), 1.59 (1H, m, H-15α), 1.52 (1H, t, J = 13.9 Hz, H-11*β*), 1.51 (1H, m, H-15*β*), 1.35 (3H, s, H-19), 1.17 (3H, d, J = 6.7 Hz, H-21), 1.14 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; FABMS m/z 543 $[MNa]^+$ (85), 503 (MH − H_2O, 14), 341 (15), 340 (11), 166 (17), 150 (7), 145 (5), 128 (5), 123 (15); HRFABMS m/z 543.2141 (calcd for $C_{28}H_{37}O_7NaCl$, 543.2125).

Jaborosalactone 29 ((20*S*,22*S*)-6α-chloro-12α,22-epoxy-5*β*,12*β*,-17*β*-trihydroxy-1-oxo-witha-2,24-dien-26,22-olide) (4): white amorphous powder; $[\alpha]_D$ +4.2 (c 0.002, $CHCl_3$); UV (MeOH) λ_{max} 223 nm; IR (dry film) ν_{max} 3383, 1796, 1675, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 6.73 (1H, ddd, J = 10.0, 5.5, 2.0 Hz, H-3), 6.05 (1H, dd, J = 10.0, 2.0 Hz, H-2), 4.31 (1H, dd, J = 12.5, 4.8 Hz, H-6), 2.83 (1H, dt, J = 20.4, 2.5 Hz, H-4*β*), 2.82 (1H, m, H-23α), 2.60 (1H, dd, J = 20.4, 5.5 Hz, H-4α), 2.31 (1H, br d, J = 17.3 Hz, H-23*β*), 2.24 (1H, dt, J = 13.4, 4.6 Hz, H-7*β*), 1.93 (1H, q, J = 6.8 Hz, H-7*β*), 1.88 (3H, d, J = 0.9 Hz, H-28), 1.86 (3H, s, H-27), 1.84 (1H, m, H-16α), 1.84 (1H, m, H-16*β*), 1.72 (1H, m, H-14), 1.69 (1H, m, H-8), 1.60 (1H, m, H-11α), 1.55 (1H, m, H-15α), 1.55 (1H, m, H-15*β*), 1.47 (1H, m, H-7α), 1.40 (1H, m, H-9), 1.20 (3H, s, H-19), 1.16 (3H, d, J = 6.8 Hz, H-21), 1.15 (1H, m, H-11*β*), 1.08 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; FABMS m/z 543 $[MNa]^+$ (4, too weak for accurate mass determination), 341 (73), 313 (50), 125 (27), 111 (29).

Jaborosalactone 30 ((20*S*,22*S*)-12α,22-epoxy-6*β*,12*β*,17*β*-trihydroxy-1-oxo-witha-2,4,24-trien-26,22-olide) (5): white amorphous powder; $[\alpha]_D$ −32.0 (c 0.0016, UV (MeOH) λ_{max} 223 nm; IR (dry film) ν_{max} 3270, 1701, 1690, 1572, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 6.94 (1H, dd, J = 9.7, 6.0 Hz, H-3), 6.17 (1H, d, J = 6.0 Hz, H-4), 6.04 (1H, d, J = 9.7 Hz, H-2), 4.57 (1H, t, J = 2.7 Hz, H-6), 2.78 (1H, d, J = 18.1 Hz, H-23α), 2.17 (1H, dd, J = 11.6, 3.0 Hz, H-8), 2.12 (1H, dd, J = 14.3, 4.3 Hz, H-11α), 2.05 (1H, d, J = 18.1 Hz, H-23*β*), 2.02 (1H, dt, J = 12.0, 3.0 Hz, H-7*β*), 1.92 (1H, m, H-20), 1.89 (3H, s, H-27), 1.87 (3H, s, H-28), 1.83 (1H, m, H-16α), 1.81 (1H, m, H-11*β*), 1.71 (1H, t, J = 10.1 Hz, H-16*β*), 1.66 (1H, d, J = 10.9 Hz, H-14), 1.57 (2H, m, H-15), 1.45 (3H, s, H-19), 1.23 (1H, m, H-9), 1.19 (3H, s, H-18), 1.15 (3H, d, J = 6.7 Hz, H-21), 1.11 (1H, td, J = 12.0, 3.6 Hz, H-7α); ^{13}C NMR (50.32 MHz), see Table 1; FABMS m/z 507 $[MNa]^+$ (10), 342 (12), 341 (31), 166 (10), 150 (12), 144 (4), 136 (80), 132 (2), 128 (3), 123 (11); $CHCl_3$; HRFABMS m/z 507.2341 (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

Jaborosalactone 31 ((20*S*,23*R*)-12*β*,26-epoxy-5α,6*β*,17*β*-trihydroxy-26-oxo-12α,23-cycloergosta-2,24-dien-1,22-dione) (6): white amorphous powder; $[\alpha]_D$ −25.9 (c 0.0005, $CHCl_3$); UV (MeOH) λ_{max} 220 nm; IR (dry film) ν_{max} 3430, 1715, 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 6.62 (1H, ddd, J = 10.2, 5.0, 2.3 Hz, H-3), 5.87 (1H, dd, J = 10.2, 2.3 Hz, H-2), 3.81 (1H, br s, H-23), 3.66 (1H, t, J = 2.7 Hz, H-6), 3.34 (1H, dt, J = 19.8, 2.7 Hz, H-4*β*), 2.94 (1H, q, J = 6.5 Hz, H-4*β*), 2.75 (1H, dd, J = 13.2, 3.0 Hz, H-11α), 2.26 (1H, td, J = 10.7, 7.5 Hz, H-14), 2.07 (1H, ddd, J = 19.8, 5.0, 0.9 Hz, H-4α), 2.06 (3H, d, J = 1.0 Hz, H-28), 2.04 (1H, m, H-16α), 2.03 (1H, m, H-8), 1.99 (3H, s, H-27), 1.96 (1H, td, J = 11.2, 3.0 Hz, H-9), 1.81 (2H, m, H-15α, H-16*β*), 1.76 (1H, dt, J = 13.9, 2.5 Hz, H-7*β*), 1.66 (1H, m, H-7α), 1.66 (1H, m, H-15*β*), 1.64 (1H, t, J = 12.7 Hz, H-11*β*), 1.29 (3H, s, H-19), 1.26 (3H, s, H-18), 1.14 (3H, d, J = 6.5 Hz, H-21); ^{13}C NMR (50.32 MHz), see Table 1; FABMS m/z 507 $[MNa]^+$ (19), 485 $[MH]^+$ (24), 383 (25), 341 (21), 313 (20), 166 (13), 158 (8), 150 (19), 145 (11), 144 (7), 132 (9), 128 (11), 123 (35); HRFABMS m/z 507.2346 (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

Jaborosalactone 32 ((22*S*,23*R*)-5*β*,6*β*:12α,22-diepoxy-12*β*,17*β*-dihydroxy-1-oxo-witha-2,24-dien-26,23-olide) (7): colorless crystals (hexane−EtOAc), mp 180−182 °C; $[\alpha]^{21}_D$ +25.3 (c 0.099, $CHCl_3$); CD (0.002, MeOH) $\Delta\epsilon$ +1.6 (218 nm); UV (MeOH) λ_{max} 217 nm; IR (dry film) ν_{max} 3490, 1736, 1672, 1383, 1101, 1012, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.87 (1H, ddd, J = 9.9, 6.2, 2.2 Hz, H-3), 6.02 (1H, dd, J = 9.9, 2.6 Hz, H-2), 4.88 (1H, br s, H-23), 4.18 (1H, dd, J = 11.3, 2.6 Hz, H-22), 3.15 (1H, d, J = 2.2 Hz, H-6), 2.96 (1H, dt, J = 18.6, 2.6 Hz, H-4*β*), 2.24 (1H, dd, J = 13.5, 4.4 Hz, H-11α), 2.07 (1H, m, H-7*β*), 2.05 (3H, s, H-28), 2.05 (1H, m, H-20), 1.92 (1H, m, H-14), 1.90 (1H, dd, J = 18.6, 6.2 Hz, H-4α), 1.79 (3H, s, H-27), 1.78 (1H, t, J = 13.5 Hz, H-11*β*), 1.77 (1H, m, H-15α), 1.62 (1H, m, H-8), 1.60 (2H, m, H-16), 1.54 (1H, m, H-15*β*), 1.43 (1H, m, H-9), 1.35 (1H, br t, J = 11.6 Hz, H-7α), 1.22 (3H, s, H-19), 1.01 (3H, s, H-18), 0.75 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR (50.32 MHz), see Table 2; FABMS m/z 507 $[MNa]^+$ (20), 485 $[MH]^+$ (15), 467 (MH − H_2O, 100), 341 (15), 166 (7), 158 (3), 150 (5), 145 (11), 144 (5), 128 (9), 123 (14); HRFABMS m/z 507.2378 (calcd for $C_{28}H_{36}O_7Na$, 507.2359).

Crystallographic Data and Data Collection Parameters.[10] Colorless prismatic crystals recrystallized from hexane−EtOAc. $C_{28}H_{36}O_7 \cdot H_2O$, M = 502.58, orthorhombic, space group $P2_12_12_12_1$ (#19); cell constants a = 7.4703(6) Å, b = 15.0140(11) Å, c = 21.9821(17) Å; V = 2465.5(3) Å^3, $D_c(Z=2)$ = 1.354 g cm^{-3}; crystal dimensions 0.34 × 0.14 × 0.12 mm, reflections measured 14 823 reflections unique 5558, reflections observed ($I > 2\sigma(I)$) 2839; R = 0.042 and R_w^2 = 0.077.

Jaborosalactone 33 ((22*S*,23*R*)-12α,22-epoxy-5α,6*β*,12*β*,17*β*-tetrahydroxy-1-oxo-witha-2,24-dien-26,23-olide) (8): colorless crystals (hexane−EtOAc), mp 191−192 °C; $[\alpha]^{21}_D$ +26.2 (c 0.0144, $CHCl_3$); UV (MeOH) λ_{max} 224 nm; IR (dry film) ν_{max} 3425, 1736, 1685, 1380, 1107, 1018, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$−CD_3OD, 95:5, 200.13 MHz, corresponds to the 12-O-CD_3 derivative **9**) δ 6.61 (1H, ddd, J = 10.2, 5.1, 2.2 Hz, H-3), 5.86 (1H, dd, J = 10.2, 2.6 Hz, H-2), 4.95 (1H, br s, H-23), 3.77 (1H, dd, J = 11.3, 3.3 Hz, H-22), 3.71 (1H, br s, H-6), 3.31 (1H, dt, J = 19.7, 2.6 Hz, H-4*β*), 2.76 (1H, dd, J = 13.2, 4.0 Hz, H-11α), 2.13 (3H, s, H-28), 2.13 (1H, m, H-14), 2.11 (1H, m, H-4α), 2.11 (1H, m, H-20), 2.06 (1H, br t, J = 10.2 Hz, H-9), 1.83 (3H, s, H-27), 1.82 (1H, m, H-8), 1.73 (1H, m, H-7*β*), 1.73 (1H, m, H-16α), 1.60 (1H, m, H-15α), 1.56 (2H, m, H-7α, 16*β*), 1.51 (1H, m, H-15*β*), 1.35 (1H, t, J = 12.8 Hz, H-11*β*), 1.31 (3H, s, H-19), 1.00 (3H, s, H-18), 0.82 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR (50.32 MHz, corresponds to the 12-O-CD_3 derivative **9**), see Table 2; FABMS m/z 503 $[MH]^+$ (48), 341 (61), 166 (10), 123 (19); HRFABMS m/z 503.2227 (calcd for $C_{28}H_{36}O_7Na$, 503.2644).

Jaborosalactone 34 ((22*S*,23*R*)-6α-chloro-12α,22-epoxy-5*β*,12*β*,-17*β*-trihydroxy-1-oxo-witha-2,24-dien-26,23-olide) (10): white amorphous powder; $[\alpha]_D$ +7.15 (c 0.0112, $CHCl_3$); UV (MeOH) λ_{max} 219 nm; IR (dry film) ν_{max} 3406, 1738, 1670, 1383, 1044, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.76 (1H, ddd, J = 10.0, 5.1, 2.5 Hz, H-3), 6.01 (1H, dd, J = 10.0, 1.8 Hz, H-2), 4.86 (1H, br s, H-23), 4.31 (1H, dd, J = 12.4, 5.1 Hz, H-6), 4.18 (1H, dd, J = 11.3, 2.2 Hz, H-22), 2.87 (1H, dt, J = 20.5, 2.5 Hz, H-4*β*), 2.61 (1H, dd, J = 20.5, 5.1 Hz, H-4α), 2.23 (1H, dt, J = 13.2, 4.2 Hz, H-7*β*), 2.02 (1H, m, H-20), 2.01 (1H, s, H-28), 2.01 (1H, m, H-14), 1.78 (3H, s, H-27), 1.74 (1H, m, H-16α), 1.68 (1H, m, H-8), 1.62 (1H, m, H-11α), 1.62 (1H, m, H-11*β*), 1.60 (3H, m, H-9, H-15), 1.55 (1H, m, H-16*β*), 1.49 (1H, m, H-7α), 1.20 (3H, s, H-19), 1.00 (3H, s, H-18), 0.79 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR (50.32 MHz), see Table 2; FABMS m/z 193 (30), 179 (24), 165 (24), 163 (25), 136 (61), 123 (40), 121 (44), 111 (51).

Jaborosalactone 35 ((22*S,23*S**,24*R**,25*S**)-12α,22:21,24-diepoxy-5α,6*β*,12*β*,17*β*,25-pentahydroxy-1-oxo-with-2-en-26,23-olide) (11):** white amorphous powder; UV (MeOH) λ_{max} 221 nm; IR (dry film) ν_{max} 3420, 1772, 1670, 1382, 1051 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 6.64 (1H, ddd, J = 10.1, 5.0, 2.0 Hz, H-3), 5.88 (1H, dd, J = 10.1, 2.7 Hz, H-2), 4.69 (1H, d, J = 2.7 Hz, H-23), 4.44 (1H, dd, J = 11.6, 2.7 Hz, H-22), 3.83 (1H, dd, J = 12.1, 3.8 Hz, H-21α), 3.71 (1H, br s, H-6), 3.69 (1H, t, J = 12.1 Hz, H-21*β*), 3.27 (1H, dt, J = 19.7, 2.3 Hz, H-4*β*), 2.65 (1H, dd, J = 13.4, 4.3 Hz, H-11α), 2.28 (1H, td, J = 11.6, 3.3 Hz, H-20), 2.18 (1H, td, J = 12.8, 4.3 Hz, H-9), 2.16 (1H, m, H-14), 2.14 (1H, m, H-4α), 1.89 (1H, m, H-16α), 1.85 (1H, m, H-8), 1.75 (1H, td, J = 14.3, 2.2 Hz, H-7*β*), 1.71 (1H, t, J = 13.4 Hz, H-11*β*), 1.69 (1H, m, H-15α), 1.62 (1H, m, H-16*β*), 1.56 (1H, m, H-7α), 1.48 (1H, m, H-15*β*), 1.35 (3H, s, H-27), 1.33 (3H, s, H-28), 1.32 (3H, s, H-19), 1.05 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 2; FABMS m/z 231 (42), 215 (25), 155 (26), 136 (41), 117 (100).

Jaborosalactone 36 ((22*S,23*S**,24*R**,25*S**)-5α,6*β*:12α,22:21,24-triepoxy-12*β*,17*β*,25-trihydroxy-1-oxo-with-2-en-26,23-olide) (12):** colorless crystals (hexane−EtOAc), mp 210−212 °C; $[\alpha]^{21}_D$ −13.10 (*c* 0.048, $CHCl_3$); UV (MeOH) λ_{max} 221 nm; IR (dry film) ν_{max} 3480, 1774, 1664, 1381, 1097, 1053, 768 cm^{-1}; ^1H NMR ($CDCl_3$, 200.13 MHz) δ 6.88 (1H, ddd, J = 12.9, 6.6, 2.2 Hz, H-3), 6.05 (1H, dd, J = 12.9, 2.6 Hz, H-2), 4.59 (1H, d, J = 2.9 Hz, H-23), 4.40 (1H, dd, J = 12.1, 2.9 Hz, H-22), 3.80 (1H, dd, J = 12.1, 4.0 Hz, H-21α), 3.65 (1H, t, J = 12.1 Hz, H-21*β*), 3.15 (1H, d, J = 1.8 Hz, H-6), 2.95 (1H, dt, J = 18.3, 2.2 Hz, H-4*β*), 2.30 (1H, dd, J = 13.5, 4.4 Hz, H-11α), 2.17 (1H, m, H-20), 2.06 (1H, m, H-7*β*), 1.94 (1H, m, H-14), 1.87 (1H, dd, J = 18.3, 6.6 Hz, H-4α), 1.83 (1H, m, H-11*β*), 1.64 (1H, m, H-15α), 1.62 (2H, m, H-16), 1.58 (1H, m, H-8), 1.44 (1H, m, H-7α), 1.43 (1H, m, H-9), 1.40 (1H, m, H-15*β*), 1.32 (3H, s, H-27), 1.31 (3H, s, H-28), 1.22 (3H, s, H-19), 1.00 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 2; FABMS *m*/*z* 539 $[MNa]^+$ (13), 499 (MH − H_2O, 16), 343 (11), 166 (8), 158 (3), 150 (7), 145 (6), 136 (80), 128 (6), 123 (23); HRFABMS *m*/*z* 539.2255 (calcd for $C_{28}H_{36}O_9Na$, 539.2257).

Jaborosalactone 37 ((20*R*,22*R*)-5α-chloro-12α,21-epoxy-6*β*,12*β*,-17*β*-trihydroxy-1-oxo-witha-2,24-dien-26,22-olide) (13): colorless crystals (hexane−EtOAc), mp 205 °C; $[\alpha]^{21}_D$ +62.85 (*c* 0.0047, $CHCl_3$); UV (MeOH) λ_{max} 227 nm; IR (dry film) ν_{max} 3420, 1685, 1655, 1381, 1304, 1045, 960, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 500.13 MHz) δ 6.66 (1H, ddd, J = 10.1, 5.0, 2.4 Hz, H-3), 5.92 (1H, dd, J = 10.1, 2.6 Hz, H-2), 4.36 (1H, ddd, J = 12.2, 8.4, 3.0 Hz, H-22), 4.06 (1H, br s, H-6), 3.98 (1H, dd, J = 11.4, 5.0 Hz, H-21*β*), 3.66 (1H, t, J = 11.0 Hz, H-21α), 3.53 (1H, dt, J = 19.9, 2.4 Hz, H-4*β*), 2.87 (1H, ddd, J = 11.0, 8.4, 5.0 Hz, H-20), 2.65 (1H, dd, J = 13.6, 4.2 Hz, H-11α), 2.59 (1H, br t, J = 14.4 Hz, H-23*β*), 2.53 (1H, dd, J = 19.9, 5.0 Hz, H-4α), 2.25 (1H, dd, J = 13.2, 2.4 Hz, H-23α), 2.23 (1H, m, H-9), 2.11 (1H, dd, J = 14.2, 9.5 Hz, H-16α), 2.02 (1H, td, J = 9.2, 2.0 Hz, H-7α), 2.00 (1H, td, J = 8.8, 2.0 Hz, H-8), 1.95 (3H, s, H-28), 1.88 (3H, s, H-27), 1.81 (1H, dd, J = 14.2, 7.1 Hz, H-16*β*), 1.69 (1H, br d, J = 10.6 Hz, H-7*β*), 1.68 (1H, t, J = 13.0 Hz, H-11*β*), 1.61 (1H, m, H-15α), 1.57 (1H, m, H-14), 1.55 (1H, m, H-15*β*), 1.39 (3H, s, H-19), 1.08 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 2; FABMS *m*/*z* 543 $[MNa]^+$ (8, too weak for accurate mass), 521 $[MH]^+$ (9, too weak for accurate mass), 505 (15), 503 (MH − H_2O, 25), 166 (10), 136 (86), 123 (27).

Acknowledgment. This work was supported by grants from CONICET (Argentina), Universidad de Buenos Aires, SeCyT-UNC, Agencia Córdoba Ciencia, and FONCYT. M.T.G. and R.F.B. acknowledge grants FONDECYT 1020802 and CONICYT-FONDAP 11980002 (Chile). G.E.F. thanks CICITCA-UNSJ (project 21F/466) for a grant. V.E.N. thanks SeCyT-UNC for a fellowship. We thank Prof. G. E. Barboza (IMBIV-CONICET) for identification of the plants, Prof. J. A. Palermo (University of Buenos Aires) for the HPLC separations, and M. Ruiz (INTA, San Juan) for technical assistance.

Supporting Information Available: Relevant NOE correlations in **6**; final fractional atomic coordinates of **1** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Anjaneyulu, A. S. R.; Rao, D. S.; Lequesne, P. W. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science, B.V.: Amsterdam, 1998; Vol. 20, pp 135−261.
(2) Ray, A. B.; Gupta, M. *Prog. Chem. Org. Nat. Prod.* **1994**, *63*, 1−106
(3) Veleiro, A. S.; Oberti, J. C.; Burton, G. In *Studies in Natural Products Chemistry, (Part L) Bioactive Natural Products*; Atta-ur-Rahman Ed.; Elsevier Science Publishers: Amsterdam, 2005; Vol. 32, pp 1019−1052.
(4) Vaccarini, C. E.; Bonetto, G. M.; *J. Chem. Ecol.* **2000**, *26*, 2187−2193
(5) Budhiraja, R. D.; Sudhir, S. *J. Sci. Ind. Res.* **1987**, *46*, 488−491.
(6) Kennelly E. J.; Gerhause C.; Song L. L.; Graham J. G.; Beecher C. W. W.; Pezzuto J. M.; Kinghorn A. D. *J. Agric. Food Chem.* **1997**, *45*, 3771−3777.
(7) Misico R. I.; Song L. L.; Veleiro A. S.; Cirigliano A. M.; Tettamanzi M. C.; Burton G.; Bonetto G. L.; Nicotra V. E.; Silva G. L.; Gil R. R.; Kinghorn A. D.; Pezzuto J. M. *J. Nat. Prod.* **2002**, *65*, 677−680.
(8) Bonetto, G. M.; Gil, R. R.; Oberti, J. C.; Veleiro, A. S.; Burton, G. *J. Nat. Prod.* **1995**, *58*, 705−711.
(9) Nicotra, V. E.; Gil, R. R.; Vaccarini, C.; Oberti, J. C.; Burton, G. *J. Nat. Prod.* **2003**, *66*, 1471−1475.
(10) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 255336 (compound **1**) and 255337 (compound **7**). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(11) Cirigliano, A. M.; Veleiro, A. S.; Bonetto, G. M.; Oberti, J. C.; Burton, G. *J. Nat. Prod.* **1996**, *59*, 717−721.
(12) Monteagudo, E.; Burton, G.; González, C.; Oberti, J. C.; Gros, E. G. *Phytochemistry* **1988**, *27*, 3925−3928.
(13) Monteagudo, E. S.; Oberti, J. C.; Gros E. G.; Burton, G. *Phytochemistry* **1990**, *29*, 933−935.
(14) Cirigliano, A. M.; Veleiro, A. S.; Oberti, J. C.; Burton, G. *J. Nat. Prod.* **2002**, *65*, 1049−1051.
(15) Lavie, D.; Besalle, R.; Pestchanker, M. J.; Gottlieb, H. E.; Frolow, F.; Giordano, O. S. *Phytochemistry* **1987**, *26*, 1791−1795.
(16) Fajardo, V.; Freyer, A.; Minard, R.; Shamma, M. *Tetrahedron* **1987**, *43*, 3875−3880.
(17) Misico, R. I.; Veleiro, A. S.; Burton, G.; Oberti, J. C. *J. Nat. Prod.* **1996**, *59*, 66−68.
(18) Fajardo, V.; Podesta, F.; Shamma, M.; Freyer, A. *J. Nat. Prod.* **1991,** *54*, 554−563.
(19) Burton, G.; Oberti, J. C. *Kurtziana* **2000**, *28*, 81−93.
(20) Macías, F.; Castellano, D.; Molinillo, J.; *J. Agric. Food Chem.* **2000**, *48*, 2512−2521
(21) Ma, Y. Q.; Cheng, J. M.; Inanaga, S.; Shui, J. F. *Agron. J.* **2004**, *96*, 1349−1356
(22) Shmueli, M.; Goldberg, D. *Hortscience* **1971**, *6*, 563−565
(23) Sheldrick, G. M. *SHELXS-97*, Program for Structure Resolution; University of Göttingen: Germany, 1997.
(24) Sheldrick, G. M. *SHELXL-97*, Program for Structure Refinement; University of Göttingen: Germany, 1997.
(25) Sheldrick, G. M. *SHELXTL*-PC version 5.0; Siemens Analytical X-ray Instruments, Inc.: Madison, WI, 1994.