Withanolides with Phytotoxic Activity from *Jaborosa caulescens* var. *caulescens* and *J. caulescens* var. *bipinnatifida*[1]

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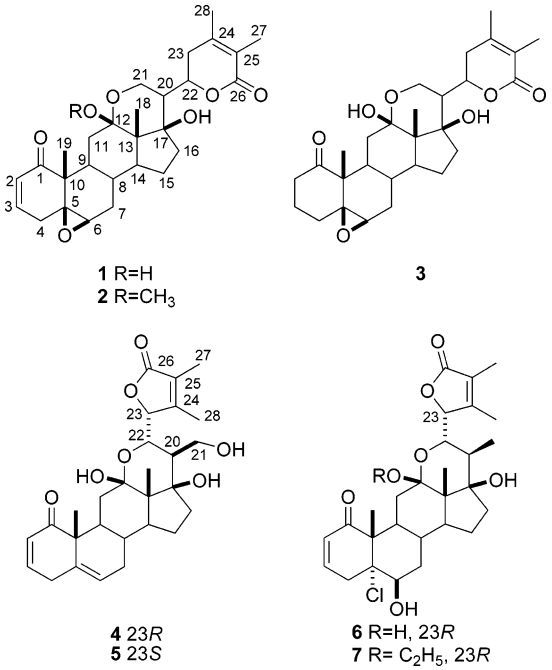
Seven new withanolides (**1**−**7**) were isolated from the aerial parts of *Jaborosa caulescens* var. *caulescens* and *Jaborosa caulescens* var. *bipinnatifida*. Three of the new compounds are related to jaborosalactones R, S, and T with a δ-lactone side chain and a hemiketal (or ketal) ring formed between a 21-hydroxyl and a 12-ketone (**1**−**3**). Compounds **4**−**7** are trechonolide-type withanolides with a γ-lactone side chain and a hemiketal (or ketal) ring formed between a 22-hydroxyl and a 12-ketone. Compounds **4** and **5** also contain a hydroxyl group at C-21. Compounds **1**, **2**, and **7** showed selective phytotoxicity toward monocotyledonous and dicotyledonous species.

The withanolides are a group of C-28 steroids built on an ergostane skeleton in which C-26 and C-22 or C-23 are appropriately oxidized in order to form a δ- or γ-lactone. They are known for the diversity encountered in the steroid nucleus and in the side chain, including formation of additional rings. Many of them exhibit a variety of biological activities such as inducers of the enzyme quinone reductase,[2] and insecticidal[3] and phytotoxic activity,[4−6] among others. *Jaborosa* Miers is a genus in the Solanaceae represented by 23 species that grow in South America. So far, 10 have been studied and reported to contain withanolides: *J. bergii*, *J. integrifolia*, *J. laciniata* (ex *Trechonaetes*), *J. lanigera*, *J. leucotricha*, *J. magellanica*, *J. odonelliana*, *J. sativa*, and *J. rotacea*.[3] Withanolides isolated from *Iochroma australe*,[4] *Jaborosa bergii*,[5] and *Jaborosa rotacea*[6] showed phytotoxic activity on crop and weed species, as well as selective effects on germination and radicle growth. Allelochemicals involved in weed−crop interference may serve as a source for natural herbicides or can be models for synthetic compounds. Continuing our investigations of the withanolides of *Jaborosa* species, we have studied the withanolides from two *Jaborosa caulescens* varieties, *J. caulescens* var. *caulescens* and *J. caulescens* var. *bipinattifida.* Both varieties contained withanolides with two different arrangements, thus far only found in *Jaborosa* species.



Results and Discussion

The dichoromethane extracts of the aerial parts of both varieties of *J. caulescens* were subjected to chromatographic purification to give seven new withanolides (**1**−**7**). *J. caulescens* var. *caulescens* gave the new compounds **1** and **2** along with four known withanolides with arrangements that have been shown to be significant chemical markers in the *Jaborosa* genus, trechonolide A,[7,8] jaborotetrol,[9,10] jaborosalatone R, and jaborosalactone S;[6,11] compounds **3**−**7** were isolated from *J. caulescens* var. *bipinnatifida*.

The ^{1}H and ^{13}C NMR spectra of withanolides **1**−**3** closely resemble those of jaborosalactones R, S, and T previously isolated from *J. sativa*[10] and jaborosalactone 37 isolated from *J. rotacea*.[6] Compounds **1**−**3** showed the typical ^{1}H and ^{13}C NMR profiles for the hemiketal (or ketal) ring formed between the 21-hydroxyl group and the 12-ketone and also showed a characteristic δ-lactone ring. This unique arrangement is characterized by the absence of the methyl-21 ^{1}H NMR doublet at high field and by the appearance of resonances in the ranges δ_H 3.57−3.99 (H_2-21), δ_C 99.3−102.8 (C-12), and δ_C 59.8−60.1 (C-21).

The HREIMS of **1** did not show a molecular ion, but revealed a M − H_2O fragment corresponding to $C_{28}H_{34}O_6$. The ^{1}H NMR spectrum showed the characteristic chemical shifts and multiplicities for the 1-oxo-2-ene system in ring A, where signals for H-2 and H-3 were clearly distinguished at δ 6.03 and 6.85, respectively. The correlations observed in the COSY experiment between the pairs H-2/H-4β, H-3/H-4α, and H-3/H-4β led to the assignment of H-4α and H-4β at δ 2.17 and 2.93, respectively. The doublet at δ 3.14 was consistent with a 5β,6β-epoxy group, also supported by the small value of the coupling constant between H-6β and H-7β (J = 2.2 Hz).[4] 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 molecular formula of compound **2** was determined by HREIMS as $C_{29}H_{38}O_7$. The ^{1}H NMR and ^{13}C NMR data were closely related to those of **1**, the major differences being a sharp three-proton singlet at δ_H 3.29 and a methyl carbon at δ_C 47.8 assigned to a methoxy group, the downfield shift of the C-12

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Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**−**7** in $CDCl_3$[a]

C	1	2	3	4	5	6	7
1	202.6	202.6	212.8	203.7	203.3	200.7	200.7
2	129.6	129.6	35.1	127.8	127.8	128.5	128.4
3	144.2	144.1	18.0	145.5	145.3	141.3	141.3
4	32.7	31.2	30.2	33.3	33.2	37.1	37.1
5	61.9	62.0	64.3	135.7	135.4	79.7[b]	80.1[b]
6	63.0	63.0	60.4	124.3	124.3	74.4	74.2
7	31.1	31.2	31.7	29.7	29.6	32.8	32.8
8	28.3	28.5	27.6	32.5	32.5	29.7	29.7
9	42.5	42.3	41.1	40.4	40.2	39.1	37.0
10	51.0	51.2	51.9[b]	49.9	49.7	52.2	52.3
11	37.6	31.2	36.5	36.5	36.6	36.3	30.5
12	100.5	102.8	100.4	99.3	99.0	99.3	102.1
13	48.0	48.2	51.3[b]	47.2	47.4	47.5	47.7
14	50.3	50.3	50.6	46.4	46.1	45.9	46.0
15	24.2	24.1	24.2	22.8	22.7	22.9	22.7
16	35.2	35.1	35.5	34.4	34.5	33.8	33.1
17	78.8	78.9	78.8	82.8	83.1	80.6[b]	80.4[b]
18	12.1	12.1	12.0	11.3	11.2	12.2	12.2
19	14.7	14.6	13.1	18.9	18.8	16.2	16.1
20	47.2	47.1	47.1	43.0	41.0	36.8	37.1
21	60.1	60.0	60.3	59.8	59.7	9.9	10.1
22	76.5	76.5	76.3	67.9	64.5	71.5	71.9
23	35.2	35.1	35.1	85.7	82.4	85.6	85.2
24	149.3	149.2	149.2	160.2	157.6	158.6	158.3
25	121.9	121.9	122.0	124.3	124.3	124.3	124.5
26	165.1	165.2	165.1	174.8	175.3	175.0	174.8
27	12.3	12.3	12.4	8.4	8.2	8.4	8.3
28	20.4	20.3	20.4	13.9	11.9	13.7	13.7
other		47.8[c]					55.2[d]
							15.5[d]

[a] Chemical shifts (δ) downfield from TMS, 50.32 MHz. [b]Assignments may be interchanged. [c]C-12 OCH_3. [d]C-12 OCH_2CH_3.

resonance from δ 100.5 to 102.8, and the upfield shift of the C-11 resonance from δ 37.6 to δ 31.2 (Table 1). These data were consistent with formation of a methyl ketal. The ease with which hemiketals like **1** react with methanol is well documented in the literature and supports the assumption that the methoxy derivative **2** was formed during the extraction procedure.[7,12]

The HRFABMS of compound **3** showed a molecular ion corresponding to the formula $C_{28}H_{38}O_7$. The ^1H NMR spectrum did not show olefinic protons, indicating a 2,3-dihydrowithanolide structure. The 5β,6β-epoxide was inferred from the broad signal at δ 3.15 corresponding to H-6, indicating a 1-oxo-5β,6β-epoxywithanolide. The ^{13}C NMR and DEPT spectroscopic data for **3** were in agreement with the proposed structure (Table 1).[13]

The ^1H NMR and ^{13}C NMR spectra of compounds **4**−**7** showed typical patterns of trechonolide-type withanolides with a γ-lactone side chain and a hemiketal (or ketal) ring formed between the 22-hydroxyl group and a 12-ketone. The ^1H NMR spectra showed the characteristic signals for H-23 as a broad singlet between 4.91 and 5.12, and the ^{13}C NMR spectra showed the signals corresponding to C-12 and C-23 in the δ 99.0−102.1 and 82.4−85.7 regions, respectively (Table 1).

The ^1H NMR spectrum of compound **4** presented signals at δ 5.87, 6.79, and 5.58 assigned to three olefinic hydrogens at C-2, C-3, and C-6, respectively. The carbon resonances observed at δ 128.8, 145.5, 135.7, and 124.3 assigned to C-2, C-3, C-5, and C-6, respectively, were in agreement with the substitution pattern for the A/B rings (Table 1).[10,14] The ^1H NMR spectrum showed three-proton singlets assigned to two tertiary methyls and two vinylic methyl groups. The missing C-21 methyl signal and the pair of double doublets at δ 3.99 (J = 12.4 and 1.8 Hz) and 3.73 (J = 12.4 and 2.6 Hz) indicated that C-21 was oxidized to a hydroxymethyl group.[15] This was consistent with the methylene carbon resonance at δ 59.8 in the ^{13}C NMR and DEPT spectra.[16] The large coupling constant between H-22 and H-20 (J = 11.3 Hz) indicated an *anti* relationship between these two hydrogens, as found in all trechonolide-type withanolides, indicating the same configuration at C-20 and C-22 (20*R*,22*S*). The high chemical shift of C-23 (δ 85.7) and the positive Cotton effect at 217 nm ($\Delta\epsilon$ +0.015) observed in the CD spectrum are in agreement with a 23*R* configuration according to what was previously established by NMR, circular dichroism, and X-ray crystallography on jaborosalactone 32 (epimer at C-23 of trechonolide A) isolated from *J. rotacea*.[6]

The ^1H and ^{13}C NMR data of compound **5** were almost identical to those of **4** for rings A−D and the γ-lactone side chain. The main difference observed was the downfield shift of the C-23 resonance from δ 85.7 in **4** to δ 82.4 in **5** (Table 1). Smaller shifts were evident for the C-22 and C-24 carbon resonances (from δ 67.9 and 160.2 to δ 64.5 and 157.6, respectively) as well as for H-20 from δ 2.02 to δ 2.25 and H_2-21 (from δ 3.99 and 3.73 to δ 4.15 and 3.96).[16] The similarity of the NMR data suggested the same skeleton and substitution pattern for **4** and **5** but opposite stereochemistry at C-23. The negative Cotton effect at 217 nm ($\Delta\epsilon$ −0.015) confirmed the 23*S* configuration.[6]

Compound **6**, $C_{28}H_{37}O_7Cl$, failed to show the molecular ion in the FAB mass spectrum, but a peak at *m*/*z* 503 corresponding to the [MH − H_2O] ion was observed. The ^1H and ^{13}C NMR spectroscopic data of **6** were consistent with a 5α-chloro-6β-hydroxy arrangement. Thus, the broad singlet at δ 4.05 was assigned to the equatorial H-6, and the unusually high chemical shift observed for H-4β at δ 3.52 (dt, J = 20.1 and 2.6 Hz) was indicative of a chlorine atom at C-5 with α-orientation.[5,10] The substitution pattern of ring B was further confirmed by the signals at δ 79.7 and 74.4 in the ^{13}C NMR spectrum that were assigned to C-5 and C-6, respectively (Table 1). The stereochemistry at positions C-20, C-22, and C-23 was established on the basis of NMR and CD data. Circular dichroism measurements again showed a positive Cotton effect at 219 nm. These results in conjunction with the NMR data confirmed the structure of **6** and the stereochemistry at C-23 as *R*.

Compound **7**, $C_{30}H_{41}O_7Cl$, showed a peak at *m*/*z* 503 corresponding to [MH − EtOH] in the FAB mass spectrum. A 12-*O*-ethoxy derivative of **6** was evident from the similarity of the corresponding NMR spectra and the presence of resonances corresponding to the ethoxy group at δ_H 1.14 (q, J = 6.6 Hz) and 3.48 (t, J = 6.6 Hz) and δ_C 15.5 and 55.2. The downfield shift of the C-12 signal from δ 99.3 to δ 102.1 and the upfield shift of C-11 from δ 36.3 to δ 30.5 (Table 1) confirmed the formation of the ethyl ketal. Hemiketals like **6** also react easily with alcohols, supporting the assumption that ethyl ketal **7** originated upon reaction of **6** with ethanol during the extraction procedure.

The full and unambiguous proton and carbon NMR assignments for compounds **1**−**7** were made using a combination of DEPT-135, COSY, and HETCOR or HSQC experiments. These results support that *J. caulescens* var. *caulescens* and *J. caulescens* var. *bipinnatifida* are closely related chemically, as the same type of withanolides have been isolated from both varieties. The main differences observed are the presence of trechonolide-type withanolides with a C-21 hydroxymethyl group in the *bipinnatifida* variety and the different stereochemistry at the C-23 position. Thus, *J. caulescens* var. *caulescens* produced only 23*S* withanolides (trechonolide A and jaborosotetrol), while *J. caulescens* var. *bipinnatifida* had 23*R* withanolides (**4**, **6**, and **7**) as major components. Although compound **1** is the probable biosynthetic precursor of **3**, only the latter could be isolated from *J. caulescens* var. *bipinnatifida*; this could indicate a very active reducing system that would characterize this variety.

To evaluate the major compounds isolated from both *J. caulescens* varieties as potential phytotoxic agents, we assayed the effect of **1**, **2**, **7**, trechonolide A, and jaborotetrol on germination and radicle length of *Lactuca sativa* (dicotyledon) and *Avena sativa* (monocotyledon). The tested compounds had no effect on the germination of either *Latuca sativa* or *Avena sativa* seeds, with

Table 2. Effect of Withanolides **1**, **2**, and **7**, Trechonolide A, and Jaborotetrol on Radicle Length of *Lactuca sativa* (Dicotyledon) and *Avena sativa* (Monocotyledon)

	effect on radicle length (%)[a]					
	Lactuca sativa			*Avena sativa*		
treatment	15 ppm	150 ppm	400 ppm	15 ppm	150 ppm	400 ppm
1	−1.6 ± 0.5	−46.2 ± 10.3	−58.6 ± 28.7	−13.9 ± 4.6	2.4 ± 0.7	−36.5 ± 11.5
2	−8.7 ± 2.0	−22.7 ± 4.9	−74.7 ± 30.2	15.3 ± 5.5	1.5 ± 0.5	−24.5 ± 10.0
7	2.7 ± 0.7	−25.7 ± 6.9	−65.1 ± 24.0	3.5 ± 1.2	−11.2 ± 4.0	−32.2 ± 13.7
trechonolide A	3.7 ± 0.9	−8.4 ± 1.8	2.5 ± 0.6	2.5 ± 0.8	−11.7 ± 2.9	4.0 ± 1.3
jaborotetrol	−3.9 ± 1.1	−5.5 ± 1.4	−4.0 ± 1.1	−10.2 ± 3.6	2.7 ± 0.8	−5.9 ± 2.2

[a] The data are presented as percentage differences from the control (zero value); negative values represent inhibition, and positive values represent stimulation. No effect was observed on germination.

treated seeds and controls showing over 80% germination. On the other hand, compounds **1**, **2**, and **7** caused strong inhibition of radicle elongation on the dicotyledon *Latuca sativa* at 150 and 400 ppm, and a dose-dependent effect was observed; the effect of trechonolide A and jaborotetrol was small (Table 2). As observed with other withanolides,[6] the effect on monocotyledons was much smaller; thus significant but low inhibitory effects on radicle elongation of *Avena sativa* were observed for **1**, **2**, and **7** only at 400 ppm. At 150 ppm these compounds were inactive, while **2** was marginally stimulatory at 15 ppm.

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 (1D and 2D) 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 MHz (^{1}H). ^{13}C multiplicity determinations (DEPT) and 2D spectra (COSY, HETCOR, and HSQC) were obtained using standard Bruker software. Chemical shifts are given in ppm (δ) downfield from TMS as internal standard. FABMS and HRFABMS were measured on a NBA-sodium matrix in a VG Auto Speccon (EBE) mass spectrometer. Electron-impact mass spectra were determinated 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 Merck silica gel 60 PF_{254} (1 mm thick), and preparative TLC on Merck silica gel 60 F_{254} (0.2 mm thick) or Merck silica gel reversed-phase C_{18}.

Plant Material. *J. caulescens* var. *caulescens* was collected in Departamento Las Heras, Mendoza, Argentina, in February 2000. A voucher specimen is deposited at Museo Botánico Córdoba, Universidad Nacional de Córdoba, under No. Barboza, Oberti, Filippa CORD 211. *J. caulescens* var. *bipinnatifida* was collected in Refugio del Peñón, Departamento General Sarmiento, La Rioja, Argentina, in February 1998 and February 2001. A voucher specimen is deposited at Museo Botánico Córdoba, Universidad Nacional de Córdoba, under No. Barboza, Oberti, Romanutti CORD 237.

Extraction and Isolation. The air-dried powdered aerial parts of *J. caulescens var. caulescens* (800 g) were extracted exhaustively with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue (28 g) was dissolved in 1000 mL of EtOH, and a 4% aqueous solution of lead acetate (1000 mL) was added.[17] After 2 h at room temperature, the insoluble material was filtered and the filtrate concentrated in vacuo to remove the alcohol. Partition of the remaining aqueous solution with CH_2Cl_2 (4 × 500 mL) followed by drying, filtration, and evaporation of the CH_2Cl_2 extract gave 3.6 g of crude extract, which was chromatographed on Kieselgel 60-G. Elution with hexane−EtOAc mixtures of increasing polarity (100:0−0:100) and EtOAc−MeOH (100:0−95:05) gave five fractions containing withanolides. Further column chromatography purifications of this fraction led to the isolation of trechonolide A (300 mg), **2** (3.6 mg), **1** (100 mg), jaborotetrol (57 mg), and a mixture (23 mg), which was processed by preparative TLC (CH_2Cl_2−MeOH, 95:05), yielding jaborosalactone S (2.5 mg) and jaborosalactone R (3.4 mg).

The air-dried, powdered aerial parts of *J. caulescens* var. *bipinnatifida* (300 g) were extracted exhaustively with EtOH, and the extract was concentrated at reduced pressure. The residue was defatted by partition in hexane−MeOH−H_2O (10:9:1), the MeOH−H_2O phase was washed with hexane (3 × 150 mL), and the MeOH was evaporated at reduced pressure. The residue was diluted with H_2O and extracted with CH_2Cl_2 (3 × 150 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The residue from the plant collected in 1998 (7.6 g) was initially fractionated by vacuum-liquid chromatography over silica gel. Elution with hexane−EtOAc mixtures of increasing polarity (100:0 to 0:100) and elution with EtOAc−MeOH (100:0 to 50:50) afforded two fractions containing withanolides. Fraction I (600 mg) was fractionated by column chromatography on silica gel using hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to give a mixture that was further fractionated by preparative reversed-phase HPLC (CH_3CN−H_2O, 2:1) giving compound **4** (21 mg). Fraction II (1 g) was chromatographed over Kieselgel 60-G twice; the first column chromatography was eluted with hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity and the second column chromatography was eluted with CH_2Cl_2−EtOAc and EtOAc−MeOH mixtures of increasing polarity to give **5** (10 mg). The residue from the plant collected in 2001 (4 g) was fractionated by vacuum-liquid chromatography over silica gel. Elution with hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity afforded two fractions containing withanolides. The mixture from fraction I (472 mg) was separated by column chromatography with CH_2Cl_2−MeOH mixtures of increasing polarity to give a mixture that was further fractionated by preparative TLC (CH_2Cl_2−MeOH, 95:05). This led to the isolation of **6** (22 mg) and **7** (30 mg). The mixture from fraction II (322 mg) was processed by radial chromatography with CH_2Cl_2−MeOH mixtures of increasing polarity and preparative TLC (CH_2Cl_2−MeOH, 95:05), giving compound **3** (8.4 mg).

Seed Germination Bioassays. Seeds of *Lactuca sativa* and *Avena sativa* were obtained from Laboratorio de Semillas (Facultad de Ciencias Agropecuarias, UNC, Argentina). Bioassays were carried out as described previously.[5,6] Germination and root length values of treated and control experiments were analyzed by Student's *t*-test ($p < 0.05$).

Jaborosalactone 38 [(20*R*,22*R*)-5β,6β:12α,21-diepoxy-12β,17β-dihydroxy-1-oxowitha-2,24-dien-26,22-olide] (1): colorless crystals (hexane−EtOAc), mp 237−239 °C; $[\alpha]^{21}_D$ +56.5 (*c* 0.034, MeOH); UV (MeOH) λ_{max} 240 nm (log ϵ 4.10); IR (dry film) ν_{max} 3419, 2930, 1696, 1668, 1125 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.85 (1H, ddd, J = 9.9, 6.5, 2.2 Hz, H-3), 6.02 (1H, dd, J = 9.6, 2.6 Hz, H-2), 4.33 (1H, ddd, J = 12.4, 8.0, 3.3 Hz, H-22), 3.89 (1H, dd, J = 11.2, 5.5 Hz, H-21β), 3.57 (1H, t, J = 11.2 Hz, H-21α), 3.14 (1H, d, J = 2.2 Hz, H-6), 2.93 (1H, dt, J = 18.6, 2.9 Hz, H-4β), 2.92 (1H, m, H-20), 2.59 (1H, t, J = 14.6 Hz, H-23β), 2.25 (1H, m, H-11α), 2.19 (1H, d, J = 14.6 Hz, H-23α), 2.17 (2H, m, H-4α, H-7β), 1.93 (3H, s, H-28), 1.92 (1H, m, H-11β), 1.87 (3H, s, H-27), 1.74 (2H, m, H-16), 1.71 (1H, m, H-8), 1.55 (2H, m, H-15), 1.35 (1H, m, H-9), 1.31 (1H, m, H-7α), 1.29 (1H, m, H-14), 1.24 (3H, s, H-19), 1.04 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 466 (M − H_2O, 9), 448 (1), 415 (0.5), 368 (0.5), 313 (2), 299 (2), 279 (2), 269 (2), 253 (2), 236 (3), 125 (28), 107 (18), 97 (18), 91 (17), 69 (20), 55 (62), 43 (100); HREIMS *m*/*z* 466.2351 [M − H_2O]$^+$ (calcd for $C_{28}H_{34}O_6$, 466.2355).

12-*O*-Methyljaborosalactone 38 [(20*R*,22*R*)-5β,6β:12α,21-diepoxy-12β-methoxy-17β-hydroxy-1-oxowitha-2,24-dien-26,22-olide] (2): white, amorphous powder; $[\alpha]^{21}_{D}$ +71.7 (*c* 0.019; MeOH); UV (MeOH) λ_{max} 221 nm (log ϵ 4.00); IR (dry film) ν_{max} 3429, 3058, 1705, 1686, 1383, 1204, 1128, 741 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.85 (1H, ddd, *J* = 10.0, 6.4, 2.4 Hz, H-3), 6.04 (1H, dd, *J* = 9.9, 2.6 Hz, H-2), 4.34 (1H, ddd, *J* = 12.4, 8.0, 3.3 Hz, H-22), 3.80 (1H, dd, *J* = 11.3, 5.5 Hz, H-21β), 3.59 (1H, t, *J* = 11.0 Hz, H-21α), 3.29 (3H, s, OCH_3-12), 3.14 (1H, d, *J* = 2.2 Hz, H-6), 2.96 (1H, dt, *J* = 18.3, 2.9 Hz, H-4β), 2.76 (1H, m, H-20), 2.60 (1H, m, H-23β), 2.52 (1H, dd, *J* = 13.5, 4.4 Hz, H-11α), 2.15 (1H, m, H-23α), 2.13 (1H, m, H-7β), 2.03 (1H, m, H-16β), 1.94 (3H, s, H-28), 1.89 (2H, m, H-4α, H-15β), 1.86 (3H, s, H-27), 1.75 (1H, m, H-16α), 1.74 (1H, m, H-8), 1.57 (1H, m, H-11β), 1.54 (1H, m, H-15α), 1.32 (1H, m, H-14),1.30 (2H, m, H-7α, H-9), 1.24 (3H, s, H-19), 0.98 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 466 (M − MeOH, 1), 448 (4), 430 (1), 415 (1), 329 (1), 312 (1), 297 (1), 279 (3), 253 (2), 237 (2), 223 (2), 125 (28), 107 (19), 97 (15), 91 (16), 69 (18), 55 (56), 43 (100); HREIMS *m*/*z* 498.2639 (calcd for $C_{29}H_{38}O_7$, 498.2618).

Jaborosalactone 39 [(20*R*,22*R*)-5β,6β:12α,21-diepoxy-12β,17β-dihydroxy-1-oxowith-24-en-26,22-olide] (3): colorless crystals (hexane−EtOAc), mp 156−158 °C; $[\alpha]^{21}_{270}$ +16.3 (*c* 0.00003, MeOH); UV (MeOH) λ_{max} 213 nm (log ϵ 4.03); IR (dry film) ν_{max} 3454, 1708, 1385, 1306, 1101, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 4.33 (1H, ddd, *J* = 11.7, 8.1, 3.4 Hz, H-22), 3.84 (1H, dd, *J* = 11.1, 5.3 Hz, H-21β), 3.59 (1H, t, *J* = 11.1 Hz, H-21α), 3.15 (1H, brs, H-6), 2.94 (1H, ddd, *J* = 13.3, 8.1, 5.3 Hz, H-20), 2.67 (1H, ddd, *J* = 14.5, 9.6, 6.9 Hz, H-2β), 2.61 (1H, brt, *J* = 14.2 Hz, H-23β), 2.35 (1H, ddd, *J* = 14.5, 8.7, 5.3 Hz, H-2α), 2.26 (1H, dt, *J* = 14.6, 3.2 Hz, H-7β), 2.16 (1H, dd, *J* = 14.2, 2.0 Hz, H-23α), 2.11 (1H, m, H-16α), 2.03 (1H, m, H-4β), 1.94 (3H, s, H-28), 1.91 (1H, m, H-3α), 1.87 (3H, s, H-27), 1.83 (1H, m, H-3β), 1.79 (1H, dd, *J* = 14.3, 7.3 Hz, H-16β), 1.68 (1H, t, *J* = 13.8 Hz, H-11β), 1.65 (1H, m, H-8), 1.63 (1H, m, H-15α), 1.48 (1H, dd, *J* = 13.8, 5.0 Hz, H-11α), 1.39 (1H, m, H-9), 1.35 (1H, m, H-7α), 1.31 (1H, m, H-14), 1.28 (1H, m, H-4α), 1.17 (3H, s, H-19), 0.99 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 468 (M − H_2O, 15), 450 (M − $2H_2O$, 7), 432 (12), 311 (19), 265 (15), 223 (7), 165 (10), 159 (17), 149 (36), 125 (37), 91 (33), 83 (40), 73 (96), 69 (65), 55 (100); HRFABMS *m*/*z* 486.2594 (calcd for $C_{28}H_{38}O_7$, 486.2618).

Jaborosalactone 40 [(22*S*,23*R*)-12α,22-epoxy-12β,17β,21-trihydroxy-1-oxowitha-2,5,24-trien-26,23-olide] (4): white, amorphous powder; $[\alpha]^{21}_{270}$ +58.0 (*c* 0.00002, MeOH); CD MeOH Δϵ (nm) +0.015 (217); UV (MeOH) λ_{max} 242 nm (log ϵ 4.20); IR (dry film) ν_{max} 3434, 1736, 1395, 1088 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.79 (1H, ddd, *J* = 9.9, 5.1, 2.6 Hz, H-3), 5.87 (1H, dd, *J* = 9.9, 2.2 Hz, H-2), 5.58 (1H, brd, *J* = 5.5 Hz, H-6), 4.99 (1H, brs, H-23), 4.55 (1H, dd, *J* = 11.3, 4.4 Hz, H-22), 3.99 (1H, dd, *J* = 12.4, 1.8 Hz, H-21a), 3.73 (1H, dd, *J* = 12.4, 2.6 Hz, H-21b), 3.29 (1H, brd, *J* = 21.2 Hz, H-4β), 2.85 (1H, dd, *J* = 21.2, 4.8 Hz, H-4α), 2.49 (1H, dd, *J* = 12.8, 3.7 Hz, H-11α), 2.21 (3H, s, H-28), 2.02 (2H, m, H-14, H-20), 2.01 (1H, m, H-16β), 1.99 (1H, m, H-7β), 1.97 (1H, m, H-9), 1.82 (3H, s, H-27), 1.77 (1H, m, H-11β), 1.63 (2H, m, H-7α, H-16α), 1.62 (2H, m, H-15), 1.49 (1H, m, H-8), 1.22 (3H, s, H-19), 1.04 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 466 (M − H_2O, 9), 451 (3), 448 (2), 355 (19), 297 (24), 281 (8), 253 (5), 173 (6), 159 (9), 150 (7), 145 (8), 135 (22), 121 (13), 107 (16), 97 (18), 91 (19), 69 (18), 55 (82), 43 (100).[16]

Jaborosalactone 41 [(22*S*,23*S*)-12α,22-epoxy-12β,17β,21-trihydroxy-1-oxowitha-2,5,24-trien-26,23-olide] (5): white, amorphous powder; $[\alpha]^{21}_{270}$ +24.0 (*c* 0.00002, MeOH); CD MeOH Δϵ (nm) −0.015 (217); UV (MeOH) λ_{max} 240 nm (log ϵ 4.20); IR (dry film) ν_{max} 3434, 1743, 1388, 1109 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.77 (1H, ddd, *J* = 9.9, 5.1, 2.6 Hz, H-3), 5.82 (1H, ddd, *J* = 9.9, 2.9, 1.1 Hz, H-2), 5.52 (1H, brd, *J* = 5.8 Hz, H-6), 5.12 (1H, brs, H-23), 4.71 (1H, dd, *J* = 11.3, 2.2 Hz, H-22), 4.15 (1H, dd, *J* = 12.4, 1.5 Hz, H-21a), 3.96 (1H, dd, *J* = 12.4, 3.3 Hz, H-21b), 3.24 (1H, brd, *J* = 21.2 Hz, H-4β), 2.83 (1H, dd, *J* = 21.2, 5.1 Hz, H-4α), 2.35 (1H, dd, *J* = 10.6, 2.2 Hz, H-11α), 2.25 (1H, brd, *J* = 11.3 Hz, H-20), 2.21 (3H, s, H-28), 2.05 (1H, m, H-16β), 1.90 (1H, m, H-7β), 1.92 (1H, m, H-14), 1.82 (3H, s, H-27), 1.76 (1H, m, H-9), 1.72 (1H, m, H-11β), 1.68 (1H, m, H-16α), 1.59 (1H, m, H-15β), 1.51 (1H, m, H-7α), 1.42 (1H, m, H-8), 1.37 (1H, m, H-15α), 1.22 (3H, s, H-19), 1.03 (3H, s, H-18); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 466 (M − H_2O, 18), 448 (10), 433 (10), 355 (5), 297 (53), 281 (13), 253 (7), 237 (9), 159 (14), 137 (25), 125 (12), 121 (21), 107 (22), 91 (32), 79 (26), 69 (21), 55 (93), 43 (100).[16]

Jaborosalactone 42 [(22*S*,23*R*)-5α-chloro-12α,22-epoxy-6β,12β,-17β-trihydroxy-1-oxowitha-2,24-dien-26,23-olide] (6): colorless crystals (hexane−EtOAc), mp 203−204 °C; $[\alpha]^{21}_{D}$ +17.2 (*c* 0.0099 $CHCl_3$); UV (MeOH) λ_{max} 220 nm (log ϵ 4.19); IR (dry film) ν_{max} 3420, 1732, 1685, 1417, 1201, 1107, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.66 (1H, ddd, *J* = 10.0, 5.1, 2.2 Hz, H-3), 5.91 (1H, dd, *J* = 10.0, 2.6 Hz, H-2), 4.92 (1H, brs, H-23), 4.14 (1H, dd, *J* = 11.3, 2.9 Hz, H-22), 4.05 (1H, brs, H-6), 3.52 (1H, dt, *J* = 20.1, 2.6 Hz, H-4β), 2.52 (1H, dd, *J* = 20.1, 5.1 Hz, H-4α), 2.57 (1H, dd, *J* = 13.2, 4.0 Hz, H-11α), 2.25 (1H, brt, *J* = 12.8 Hz, H-9), 2.16 (1H, m, H-20), 2.12 (1H, m, H-14), 2.10 (3H, s, H-28), 2.02 (1H, m, H-7β), 1.84 (1H, m, H-8), 1.81 (3H, s, H-27), 1.63 (1H, m, H-7α), 1.60 (1H, t, *J* = 13.2 Hz, H-11β), 1.36 (3H, s, H-19), 1.05 (3H, s, H-18), 0.87 (3H, d, *J* = 6.6 Hz, H-21); ^{13}C NMR (50.32 MHz), see Table 1; FABMS *m*/*z* 503 (MH − H_2O, 100), 485 (2), 567 (5), 351 (10), 288 (10), 154 (70), 138 (27), 136 (57), 125 (5), 121 (13), 111 (11); HRFABMS *m*/*z* 503.2204 $[MH - H_2O]^+$ (calcd for $C_{28}H_{36}O_6Cl$, 503.2200).

12-*O*-Ethyljaborosalactone 42 [(22*S*,23*R*)-5α-chloro-12α,22-epoxy-12β-ethoxy-6β,17β-trihydroxy-1-oxowitha-2,24-dien-26,23-olide] (7): colorless crystals (hexane−EtOAc), mp 200−202 °C; $[\alpha]^{21}_{D}$ +26.6 (*c* 0.0016 $CHCl_3$); UV (MeOH) λ_{max} 221 nm (log ϵ 4.15); IR (dry film) ν_{max} 3437, 1738, 1685, 1380, 1105, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200.13 MHz) δ 6.65 (1H, ddd, *J* = 10.2, 5.1, 2.2 Hz, H-3), 5.90 (1H, dd, *J* = 10.2, 2.6 Hz, H-2), 4.91 (1H, brs, H-23), 4.04 (1H, brs, H-6), 3.78 (1H, dd, *J* = 11.3, 2.9 Hz, H-22), 3.52 (1H, dt, *J* = 20.1, 2.6 Hz, H-4β), 3.48 (2H, q, *J* = 6.6 Hz, OCH_2CH_3-12), 2.81 (1H, dd, *J* = 13.2, 3.7 Hz, H-11α), 2.52 (1H, dd, *J* = 20.1, 5.1 Hz, H-4α), 2.13 (1H, m, H-9), 2.12 (1H, m, H-14), 2.12 (3H, s, H-28), 2.11 (1H, m, H-20), 1.97 (1H, m, H-7β), 1.87 (1H, m, H-8), 1.82 (1H, m, H-16β), 1.81 (3H, s, H-27), 1.62 (1H, m, H-7α), 1.61 (1H, m, H-16α), 1.60 (2H, m, H-15), 1.35 (3H, s, H-19), 1.32 (1H, m, H-11β), 1.14 (3H, t, *J* = 6.6 Hz, 12-OCH_2CH_3), 1.01 (3H, s, H-18), 0.90 (3H, d, *J* = 6.6 Hz, H-21); ^{13}C NMR (50.32 MHz), see Table 1; EIMS *m*/*z* 468 (7), 466 (14), 448 (47), 446 (65), 430 (22), 315 (48), 313 (86), 299 (44), 297 (41), 277 (37), 251 (29), 152 (100), 121 (34), 109 (29), 91 (27), 69 (22), 55 (30); HRFABMS *m*/*z* 503.2250 [MH − EtOH]$^+$ (calcd for $C_{28}H_{36}O_6Cl$, 503.2200).

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