Withanolides from *Jaborosa laciniata*

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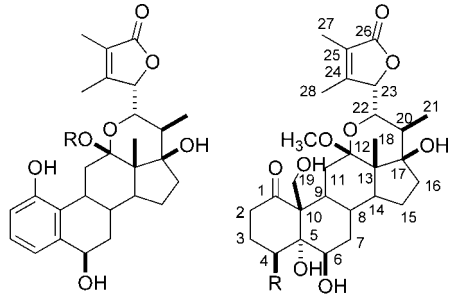
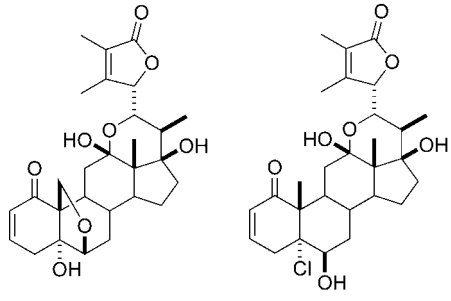
Six new trechonolide type withanolides (compounds **1**–**6**), together with trechonolide A, jaborotetrol, and 12-*O*-methyl jaborosotetrol, were isolated from the aerial parts of *Jaborosa laciniata.* The structures were elucidated on the basis of spectroscopic methods (1D and 2D NMR, MS).

The withanolides are a group of natural C-28 steroids that occur mainly in plants of certain genera of the Solanaceae. They exhibit a variety of biological effects such as antifeedant, immunosuppressive, and cancer chemoprevention activities.[1] *Jaborosa* Miers is a South American genus belonging to the Solanaceae family. The genus comprises 23 species, 11 of which are found almost exclusively in Argentina.[2] Previous studies on a population of *J. laciniata* (Miers) Hunz. (1987) (ex *Trechonaetes laciniata*) growing in Argentina gave trechonolide A and its 12-*O*-methoxy derivative trechonolide B, the first withanolides found with a γ-lactone side-chain and a hemiketal or ketal bridge formed by a 22-hydroxyl and a C-12 ketone.[3] Since then, many withanolides with this particular arrangement have been isolated from *Jaborosa* species.[1] Continuing our studies of withanolides from species of the genus *Jaborosa*, we reinvestigated *J. laciniata* (collected in the same region) and now report on the isolation of six new withanolides of the trechonolide type (**1**–**6**), together with the known trechonolide A,[3,4] jaborotetrol,[5] and 12-*O*-methyl jaborosotetrol.[6]





The ^{1}H and ^{13}C NMR spectra of compounds **1**–**6** all showed patterns typical of the trechonolide-type withanolides for rings C, D, and the side-chain.[3,4] The HRFABMS of compound **1** showed a protonated molecular ion at 471.2390 corresponding to the formula $C_{27}H_{35}O_7$. A phenolic A-ring was evident in compound **1** from the three ^{1}H NMR resonances between δ 6.60 and 6.99. The coupling pattern of the aromatic hydrogens (two double–doublets and a triplet) corresponded to three vicinal hydrogens. H-6 was observed as a broad singlet at δ 4.70 ($W_{1/2}$ = 2.8 Hz) indicating an equatorial orientation with small unresolved couplings to 7α-H and 7β-H. This type of functionality, with loss of the C-10 methyl, occurs in jaborosalactone Q[7] and jaborosalactone 7[6] isolated from *J. leucotricha*. The EIMS of **1** showed a small molecular ion at *m/z* 470 (0.5 %) and fragment ions at *m/z* 284 (2%) and 266 (6 %) originating from the cleavage of C-17–C-20 and C-22–O bonds followed by the loss of one or two molecules of water, respectively. The latter fragmentation was present in **1**–**6** and is characteristic of withanolides of the trechonolide type. The main differences observed between the NMR data of compounds **1** and **2** were the resonances for a methoxy group (a sharp three-proton singlet at δ 3.21 and a methyl carbon at δ 48.0) in the latter and the downfield shift of the C-12 resonance to δ 102.6. This indicated that compound **2** was the 12-*O*-methyl derivative of **1**, which was confirmed by the molecular ion observed in the HREIMS corresponding to $C_{28}H_{36}O_7$.

The ^{1}H NMR spectrum of compound **3** was closely related to that of 12-*O*-methyl jaborosotetrol,[6] with the main differences being the absence of a singlet for H-19 at the highfield end of the spectrum and the presence of an AB quartet at δ 3.52–3.91, indicative of an OH group at C-19. Final confirmation of the structure of **3** was provided by the ^{13}C NMR and DEPT data. Only four methyl groups were evident, corresponding to C-18 (δ 12.3), C-21 (δ 10.4), C-27 (δ 8.2) and C-28 (δ 12.1); the methylene signal at δ 63.3 was assigned to C-19.

The ^{1}H NMR of compound **4** did not show olefinic protons, indicating a 2,3-dihydrowithanolide, whereas the triplet at δ 3.76 (J = 1.9 Hz) was consistent with a 6β-hydroxy group.[6] Absence of a singlet for H-19 and the presence of an AB quartet at δ 4.50–3.71 as in compound **3** confirmed the hydroxy group at C-19. The ^{13}C NMR spectrum of **4** showed a methine at δ 72.7 and a nonprotonated carbon at δ 71.4, which were assigned to C-6 and C-5, respectively, as in **3**. An additional secondary hydroxyl at C-4 was inferred from the presence of the methine signal at δ 68.0; the H-4 broad triplet (δ 4.33, J = 1.8 Hz) typical of an equatorial hydrogen indicated a β-orientation for this hydroxyl.[8] A similar 4β,5α,6β-trihydroxy arrangement is also present in jaborosalactone X isolated from *J. leucotricha*.[9] As in the case of *J. leucotricha*, the simultaneous presence of 19-hydroxywithanolides and A-ring aromatic 19-norwithanolides is indicative of an oxidative degradation pathway for the loss of C-19.

The HRFABMS of **5** did not show a molecular ion, but revealed a MH-H_2O fragment corresponding to $C_{28}H_{35}O_7$. A 2-en-1-one

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arrangement was evident from the ^{1}H NMR signals at δ 5.86 and 6.95. Highly diagnostic of the ring B substitution was the doublet at δ 4.08 (J = 5.2 Hz) and the AB quartet at δ 4.01–3.91, assigned to H-6 and H-19, respectively, of a 6,19-epoxy functionality.[10] The signals for the oxygenated carbons observed at δ 77.1, 81.4, and 66.8 assigned to C-5, C-6, and C-19, respectively, confirmed the proposed structure. The 6,19-ether is an unusual functionality for a natural product, it may arise from the dehydration of a 6β,19-diol (as in **3**) or by nucleophilic attack of a 19-hydroxyl at position 6 of a 5α,6α-epoxide. Although we cannot determine at this time if compound **5** is a natural product or if it was formed during the isolation procedure, its natural origin is supported by the chemical stability of the 6β,19-diol functionality in compounds **3** and **4** (that did not cyclize spontaneously), and the absence of 5α,6α-epoxywithanolides in this plant.

The HRFABMS of **6** showed a MH ion corresponding to $C_{28}H_{38}ClO_7$. The ^{1}H NMR spectrum of compound **6** exhibited signals at δ 6.63 and 5.86 in the low-field region, typical of a 2-en-1-one system without substitution at C-4 and consistent with a 5α-chloro-6β-hydroxy arrangement.[11] Thus the broad singlet at δ 4.00 was assigned to equatorial H-6 and the signals at δ 78.6 and 74.5 in the ^{13}C NMR were assigned to C-5 and C-6, respectively. Comparison of the ^{13}C NMR spectrum with that of the 23*R* epimer Jaborosalactone 42, isolated from *J. caulescens* var. *bipinnatifida* showed differences only for carbons 22, 23, 24, and 28.[12] The upfield shift of C-23 from δ 85.6 in the 23*R* isomer to 82.2 in **6** confirmed the 23*S* configuration.[12,13]

NMR assignments for compounds **1**–**6** were confirmed using a combination of DEPT-135, COSY,and HETCOR experiments. Lavie et al.[3] and Fajardo et al.[4] had reported a 23*R* configuration for trechonolide A; however, after isolation of the true 23*R* epimer and careful inspection of the published X-ray data for trechonolide A, Nicotra et al. showed that its C-23 configuration had been incorrectly assigned and that it was *S*.[13] It is well established that the chemical shift of C-23 may be used as a direct indicator of the stereochemistry at this position, with the C-23 resonance shifted downfield in 23*R* trechonolide-type withanolides.[12,13] In the case of compounds **1**–**6**, the chemical shift of C-23 (ca. 82.4 ppm) was in agreement with a 23*S* configuration (Table 1).

Regarding the *O*-methyl derivatives **2**–**4**, Lavie et al. considered that the 12-*O*-methyl derivative of trechonolide A isolated from *J. laciniata* was an artifact originating from extraction with boiling methanol.[3] Although our extraction procedure did not involve heating with methanol at any stage, it has been shown that even small amounts of methanol that may be added to aid dissolution in less polar solvents can result in methyl ketal formation.[13] Thus, the possibility that the methoxy derivatives **2**–**4** and 12-*O*-methyl jaborosotetrol are (at least in part) artifacts of the isolation procedure cannot be discarded.

Experimental Section

General Experimental Procedures. Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 343 polarimeter. IR and UV spectra were measured on a Nicolet Magna 550 FT IR and a Hewlett-Packard 8451A spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 500 at 500.13 and 125.77 MHz in $CDCl_3$. Multiplicity determinations (DEPT) and 2D spectra (COSY, HETCOR) were obtained using standard Bruker software. Chemical shifts are given in ppm downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 mass spectrometer at 70 eV by direct inlet; HREIMS (70 eV) were measured on a VG-7070 mass spectrometer; FABMS and HRFABMS were measured on a VG-ZAB. HPLC separations were carried out on a YMC-Pack ODS-AQ column (250 × 10 mm i.d.) and a 7:3 mixture of MeOH:H_2O as eluant, with UV detection at 245 nm. Vacuum liquid chromatography (VLC) and column flash chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040–0.063 mm, respectively. TLC analyses were performed on silica gel 60 F254 (0.2 mm thick).

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

C	1	2	3	4	5	6
1	155.2 s	154.9 s	200.8 s	208.0 s	199.8 s	200.5 s
2	115.8 d	115.7 d	129.4 d	38.6 t	127.2 d	128.5 d
3	126.9 d	127.0 d	142.9 d	43.9 t	146.3 d	141.2 d
4	122.4 d	122.7 d	31.0 d	68.0 d	32.2 t	37.1 t
5	140.1 s	140.4 s	76.7 s	71.4 s	77.1 s	78.6 s
6	68.1 d	68.1 d	72.2 d	72.7 d	81.4 d	74.5 d
7	33.2 t	33.2 t	30.0 t	33.6 t	32.0 t	32.7 t
8	32.4 d	32.5 d	37.2 d	34.5 d	31.6 d	29.7 d
9	40.7 d	40.7 d	37.2 d	28.8 d	40.9 d	39.0 d
10	126.3 s	127.0 s	57.7 s	52.3 s	50.0 s	52.1 s
11	38.3 t	29.6 t	35.4 t	31.3 t	37.3 t	36.3 t
12	99.3 s	102.6 s	102.2 s	101.6 s	97.8 s	99.2 s
13	48.8 s	48.9 s	48.1 s	47.9 s	48.4 s	47.7 s
14	44.8 d	45.0 d	46.6 d	45.8 d	44.9 d	45.5 d
15	22.6 t	22.4 t	22.5 t	22.4 t	22.3 t	22.9 t
16	34.1 t	33.7 t	33.6 t	29.1 t	34.2 t	34.1 t
17	80.7 s	80.1 s	79.8 s	79.6 s	79.7 s	80.6 s
18	12.2 q	12.4 q	12.3 q	12.1 q	12.0 q	12.2 q
19			63.3 t	61.5 t	66.8 t	16.1 q
20	35.8 d	35.6 d	35.2 d	35.1 d	35.3 d	35.6 d
21	9.8 q	10.3 q	10.4 q	10.5 q	9.9 q	9.9 q
22	68.5 d	68.9 d	68.9 d	69.0 d	68.6 d	68.8 d
23	82.5 d	82.6 d	82.7 d	82.6 d	82.2 d	82.5 d
24	158.0 s	156.7 s	156.6 s	155.8 s	157.3 s	156.8 s
25	123.5 s	124.4 s	125.0 s	125.6 s	123.4 s	124.1 s
26	175.6 s	175.1 s	176.1 s	175.9 s	174.9 s	174.8 s
27	8.0 q	8.3 q	8.2 q	8.4 q	8.2 q	8.3 q
28	11.8 q	12.2 q	12.1 q	12.0 q	11.7 q	12.0 q
OCH_3		48.0 q	47.9 q	47.9 q		

[a] Chemical shifts (δ) downfield from TMS, 125.77 MHz.

Plant Material. Aerial parts of *J. laciniata* were collected in "Las Cuevas", Mendoza province, Argentina, in February 2002. A voucher specimen is deposited at the Museo Botánico Córdoba, Universidad Nacional de Córdoba, under CORD 306.

Extraction and Isolation. The dried and pulverized aerial parts of *J. laciniata* (730 g) were triturated and macerated successively with ether (1 mL/g of plant, 3 days) and ethanol (1 mL/g of plant, 3 days) at room temperature. The residue obtained after evaporation of the combined extracts (86 g) was initially fractionated by vacuum liquid chromatography using EtOAc:hexane mixtures of increasing polarity (0:1–1:0) as eluant; the fraction eluted with 4:1 EtOAc:hexane contained the withanolides. Flash chromatography of this fraction with EtOAc:hexane mixtures of increasing polarity gave 5 fractions (eluting with 4:1 and 7:3 EtOAc:hexane) that were purified by HPLC, leading to the isolation of **3** (3.0 mg), **4** (18.3 mg), **5** (4.9 mg), and **6** (11.8 mg). Further elution with 9:1 EtOAc:hexane gave trechonolide A (3.5 mg), **1** (330 mg), **2** (196 mg), jaborotetrol (48 mg), and 12-*O*-methyl-jaborosotetrol (44 mg). Known withanolides were identified by comparison of their ^{1}H and ^{13}C NMR spectra with those described in the literature.[3–6]

Jaborosalactone 45 ((22*S*,23*S*)-12α,22-epoxy-1,6β,12β,17β-tetrahydroxywitha-1,3,5(10),24-tetraen-26,23-olide) (1): white solid (EtOAc); mp 202–203 °C; $[\alpha]^{20}_D$ –33.5 (*c* 0.16 MeOH); UV(MeOH) λ_{max} (log ε) 215 (3.90), 287 (3.05) nm; IR (dry film) ν_{max} 3388, 2938, 1740, 1526, 1270, 1027, 742 cm^{-1}; ^{1}H NMR δ 6.99 (1H, t, J = 7.7 Hz, H-3), 6.85 (1H, dd, J = 7.7, 1.5 Hz, H-4), 6.60 (1H, dd, J = 7.7, 1.5 Hz, H-2), 4.89 (1H, brs, H-23), 4.70 (1H, brs, $W_{1/2}$ = 2.8 Hz, H-6), 4.12 (1H, dd, J = 11.0, 2.0 Hz, H-22), 3.08 (1H, dd, J = 13.2, 3.6 Hz, H-11α), 2.46 (1H, m, H-9), 2.35 (1H, m, H-20), 2.20 (1H, m, H-14), 1.99 (3H, s, H-28), 1.99 (1H, m, H-8), 1.89 (1H, m, H-16a), 1.84 (1H, dt, J = 13.4, 2.9 Hz, H-7β), 1.79 (3H, s, H-27), 1.63 (1H, m, H-15a), 1.61 (1H, m, H-16b), 1.56 (1H, t, J = 13.2 Hz, H-11β), 1.51 (1H, m, H-15b), 1.35 (1H, dt, J = 13.4, 2.9 Hz, H-7α), 1.06 (3H, s, H-18), 1.02 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR see Table 1; EIMS *m/z* 470 $[M]^+$ (0.5), 452 (2), 434 (22), 284 (2), 283 (12), 266 (6), 265 (23), 168 (7), 55 (100); HRFABMS *m/z* 471.2390 $[M + H]^+$ (calcd for $C_{27}H_{35}O_7$, 471.2383).

12-*O*-Methyl-jaborosalactone 45 ((22*S*,23*S*)-12α,22-epoxy-1,6β,17β-tetrahydroxy-12β-methoxywitha-1,3,5(10),24-tetraen-26,23-olide) (2): white solid (EtOAc); mp 195–196 °C; $[\alpha]^{20}_D$ –56.5 (*c* 0.17 MeOH); UV(MeOH) λ_{max} 218 (3.8), 287 (3.00) nm; IR (dry film) ν_{max} 3415,

2931, 1733, 1277, 1469, 1013, 742 cm^{-1}; ^1H NMR δ 7.03 (1H, t, J = 7.7 Hz, H-3), 6.88 (1H, dd, J = 7.7, 0.7 Hz, H-4), 6.63 (1H, dd, J = 7.7, 0.7 Hz, H-2), 4.92 (1H, brs, H-23), 4.71 (1H, brs, H-6), 3.86 (1H, dd, J = 11.0, 1.5 Hz, H-22), 3.40 (1H, dd, J = 13.2, 3.5 Hz, H-11α), 3.21 (3H, s, OCH_3), 2.41 (1H, m, H-9), 2.35 (1H, m, H-20), 2.21 (1H, m, H-14), 2.05 (3H, s, H-28), 2.01 (1H, m, H-8), 1.85 (1H, m, H-16a), 1.84 (1H, m, H-7β), 1.83 (3H, s, H-27), 1.63 (1H, m, H-15a), 1.61 (1H, m, H-16b), 1.51 (1H, m, H-15b), 1.37 (1H, dt, m, H-7α), 1.36 (1H, m, H-11β), 1.05 (3H, s, H-18), 1.02 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR see Table 1; EIMS m/z 484 $[M]^+$ (1), 466 (2), 452 (1), 434 (23), 284 (3), 283 (12), 265 (22), 168 (6), 125 (19), 107 (30), 55 (100); HREIMS m/z 484.2453 (calcd for $C_{28}H_{36}O_7$, 484.2461).

Jaborosalactone 46 ((22*S*,23*S*)-12α,22-epoxy-5α,6*β*,17*β*,19-tetrahydroxy-12*β*-methoxy-1-oxowitha-2,24-dien-26,23-olide) (3): amorphous solid; $[\alpha]^{20}_D$ −25.4 (*c* 0.16 MeOH); UV(MeOH) λ_{max} (log ε) 220 (3.80) nm; IR (dry film) ν_{max} 3440, 1720, 1670, 1375, 1096 cm^{-1}; ^1H NMR δ 6.65 (1H, ddd, J = 10.1, 5.0, 2.2 Hz, H-3), 5.82 (1H, dd, J = 10.1, 2.2 Hz, H-2), 4.91 (1H, brs, H-23), 3.91 (1H, d, J = 11.6 Hz, H-19a), 3.74 (1H, dd, J = 11.2, 1.5 Hz, H-22), 3.60 (1H, brs, H-6), 3.52 (1H, d, J = 11.6 Hz, H-19b), 3.17 (1H, dt, J = 19.6, 2.2 Hz, H-4β), 3.17 (3H, s, OCH_3), 3.20 (1H, m, H-11α), 2.32 (1H, m, H-20), 2.30 (1H, m, H-9), 2.21 (1H, dd, J = 19.6, 5.0 Hz, H-4α), 2.04 (3H, s, H-28), 1.90 (3H, s, H-27), 1.60 (1H, m, H-11β), 1.68 (1H, m, H-7β), 1.54 (1-H, dt, m, H-7α), 1.03 (3H, s, H-18), 1.02 (3H, d, J = 6.7 Hz, H-21); ^{13}C NMR see Table 1; EIMS m/z 496 (M - $2H_2O$, 0.5), 482 (2), 465 (1), 284 (2), 266 (1), 55 (100); HRFABMS m/z 497.2532 $[M - 2H_2O + H]^+$ (calcd for $C_{29}H_{37}O_7$, 497.2539).

Jaborosalactone 47 ((22*S*,23*S*)-12α,22-epoxy-4*β*,5α,6*β*,17*β*,19-pentahydroxy-12*β*-methoxy-1-oxowith-24-en-26,23-olide) (4): white solid (EtOAc); mp 220–222 °C; $[\alpha]^{20}_D$ –32.3 (*c* 0.17 MeOH); UV(MeOH) λ_{max} (log ε) 226 (4.37) nm; IR (dry film) ν_{max} 3450, 1740, 1700, 1260, 1014 cm^{-1}; ^1H NMR δ 4.90 (1H, brs, H-23), 4.50 (1H, d, J = 9.4 Hz, H-19a), 4.33 (1H, br t, J = 1.8 Hz, H-4α),[8] 3.76 (1H, t, J = 1.9 Hz, H-6), 3.72 (1H, dd, J = 11.2, 1.10 Hz H-22), 3.71 (1H, d, J = 9.4 Hz, H-19b), 3.10 (3H, s, 12-OCH_3), 2.74 (2H, m, H-2), 2.74 (1H, dt, J = 14.3, 3.0 Hz, H-11α), 2.62 (1H, m, H-3a), 2.50 (1H, m, H-3b), 2.33 (1H, m, H-20), 2.06 (1H, dd, H-14), 2.03 (3H, s, H-28), 1.96 (3H, s, H-27), 1.91 (1H, m, H-8), 1.81 (1H, m, H-16a), 1.74 (1H, m, H-9), 1.67 (1H, m, H-15a), 1.67 (1H, m, H-11β), 1.66 (1H, m, H-7α), 1.62 (1H, m, H-16b), 1.58 (1H, m, H-7β), 1.48 (1H, m, H-15b), 1.02 (3H, d, J = 6.6 Hz, H-21), 0.95 (3H, s, H-18); ^{13}C NMR see Table 1; EIMS m/z 532 (M – H_2O, 0.5), 514 (2), 363 (2), 349 (2), 345 (1), 168 (2), 43 (100); HRFABMS m/z 533.2756 $[M + H - H_2O]^+$ (calcd for $C_{29}H_{41}O_9$, 533.2751).

Jaborosalactone 48 ((22*S*,23*S*)-6*β*,19:12α,22-diepoxy-5α,12*β*,17*β*-trihydroxy-1-oxowitha-2,24-dien-26,23-olide) (5): amorphous solid; $[\alpha]^{20}_D$ –42.8 (*c* 0.18 MeOH); UV(MeOH) λ_{max} 227 (3.8) nm; IR (dry film) ν_{max} 3447, 1720, 1685, 1119 cm^{-1}; ^1H NMR δ 6.95 (1H, ddd, J = 10.0, 5.0, 2.3 Hz, H-3), 5.86 (1H, dd, J = 10.0 and 2.3 Hz, H-2), 4.83 (1H, brs, H-23), 4.08 (1H, d, J = 5.2 Hz, H-6), 4.01 (1H, d, J = 8.9 Hz, H-19a), 3.91 (1H, d, J = 8.9 Hz, H-19b), 3.91 (1H, dd, J = 11.0, 1.8 Hz, H-22), 2.82 (1H, dt, J = 19.6, 2.3 Hz, H-4β), 2.65 (1H, m, H-11α), 2.61 (1H, dd, J = 19.6, 5.0 Hz, H-4α), 2.30 (1H, m, H-20), 1.89 (3H, s, H-28), 1.83 (1H, m, H-7β), 1.76 (3H, s, H-27), 1.66 (1H, m, H-11β), 1.33 (1H, dt, m, H-7α), 1.03 (3H, s, H-18), 1.00 (3H, d, J = 6.8 Hz, H-21); ^{13}C NMR see Table 1; EIMS m/z 482 (M - H_2O, 0.5), 446 (1), 331 (1), 314 (1), 266 (0.5)297 (1), (30), 135 (100); HRFABMS m/z 483.2391 $[MH - H_2O]^+$ (calcd for $C_{28}H_{35}O_7$, 483.2383).

Jaborosalactone 49 ((22*S*,23*S*)-5α-chloro-12α,22-epoxy-6*β*,12*β*,17*β*-trihydroxy-1-oxowitha-2,24-dien-26,23-olide) (6): amorphous solid; $[\alpha]^{20}_D$ –73.5 (*c* 0.17 MeOH); UV(MeOH) λ_{max} (log ε) 233 (3.82) nm; IR (dry film) ν_{max} 3450, 1745, 1670, 1254, 1008 cm^{-1}; ^1H NMR δ 6.63 (1H, ddd, J = 10.0, 5.0, 2.1 Hz, H-3), 5.86 (1H, dd, J = 10.0, 2.5 Hz, H-2), 4.87 (1H, brs, H-23), 4.09 (1H, dd, J = 11.0, 2.4 Hz, H-22), 4.00 (1H, brs, H-6), 3.48 (1H, dt, J = 20.0, 2.5 Hz, H-4β), 2.49 (1H, dd, J = 20.0, 5.0 Hz, H-4α), 2.45 (1H, m, H-11α), 2.33 (1H, m, H-20), 2.13 (1H, m, H-9), 2.12 (1H, m, H-14), 1.98 (1H, m, H-7β), 1.98 (3H, s, H-28), 1.87 (1H, s, H-16a), 1.84 (3H, s, H-27), 1.78 (1H, m, H-8), 1.65 (1H, m, H-15a), 1.58 (1H, m, H-15b), 1.55 (1H, m, H-7α), 1.46 (1H, m, H-16b), 1.31 (3H, s, H-19), 1.04 (3H, s, H-18), 1.00 (3H, d, J = 6.6 Hz, H-21); ^{13}C NMR see Table 1; EIMS m/z 484 (M – HCl, 1), 466 (4), 448 (7), 430 (4), 315 (14), 299 (14), 266 (3) 281 (10), 168 (3), 55 (100); HRFABMS m/z 521.2306 $[M + H]^+$ (calcd for $C_{28}H_{38}ClO_7$, 521.2313).

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