JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Seco-eremophiladiolides and Eremophilane Glucosides from *Pittocaulon velatum*

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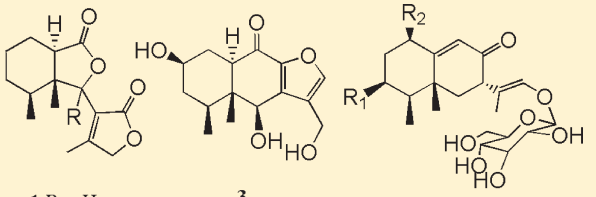
Supporting Information

ABSTRACT: Two seco-eremophiladiolides, velatumolide (**1**) and epi-velatumolide (**2**), the trihydroxyfuranoeremophilane velatumin (**3**), and three eremophilane glucosides (**4**−**6**) were isolated from *Pittocaulon velatum*, together with several known compounds. The structures of these compounds were elucidated by spectroscopic analysis and chemical reactions. The anti-inflammatory activity of the isolated compounds was investigated using the TPA-induced ear edema model.



1 R = Hα
2 R = Hβ

3

4 $R_1 = R_2 = H$
5 $R_1 = H$, $R_2 = OH$
6 $R_1 = OH$, $R_2 = H$

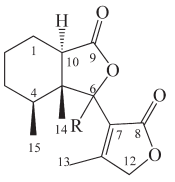
The genus *Pittocaulon* (Asteraceae, Senecioneae, Tussilaginineae) groups five species endemic to Mexico with one of them, *P. velatum*, also present in Guatemala.[1] Previous studies of the genus have shown that its secondary metabolites are closely related to those of the genus *Senecio*, from which it was segregated.[2] Thus, pyrrolizidine alkaloids have been reported in all five species of *Pittocaulon*,[3] and sesquiterpenes mainly of eremophilane skeleton have been found in *P. praecox*[4−6] and *P. bombycophole*.[7] The aim of this paper was to study the non-alkaloidal metabolites of *P. velatum* to further understand the chemistry of the genus, but also to search for anti-inflammatory agents since *Pittocaulon* species are used in Mexican folk medicine to treat rheumatism.[8] We report herein the isolation of six new compounds from *Pittocaulon velatum* (Greenm.) H. Rob. and Bretell var. *velatum*: velatumolide (**1**), epi-velatumolide (**2**), velatumin (**3**), and the eremophilane glucosides **4**−**6**. Four known furanoeremophilanes (**7**−**10**) and four flavonoids were also isolated. Structures of the isolated compounds were determined by spectroscopic methods. The anti-inflammatory activity of compounds **1**−**10** was evaluated using the 2-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema model of acute inflammation.

RESULTS AND DISCUSSION

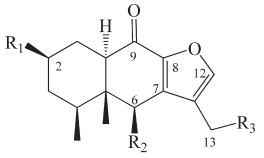
Velatumolide (**1**) had the molecular formula $C_{15}H_{20}O_4$ by HRFABMS. The IR spectrum presented evidence of γ-lactone rings (1777 and 1759 cm^{-1}), and the ^1H NMR data (Table 1) contained signals indicating three methyl groups (δ 2.22 s, 0.84 s, and 0.76 d), suggesting an eremophilanolide skeleton. In the ^1H NMR spectrum, the singlet at δ 4.98 was assigned to H-6 by its correlations, observed in the HMBC experiment, with C-5, C-10, and C-14, and the AB system at δ 4.76 (d, J = 17.6 Hz) and 4.69 (d, J = 17.6 Hz) was attributed to H_2-12 by its cross-peaks with C-7, C-11, and C-13. The HMBC spectrum also evidenced interactions of H-6, H_2-12, and H_3-13 with C-8 (δ 173.2) and of H-10 (δ 2.29, dd, J = 11.6, 3.2 Hz) with C-9 (δ 175.6), indicating the presence of the 8,12 and 9,6 γ-lactone rings, respectively. These functions could be the result of rupture of the C-8/C-9 carbon bond of the eremophilane skeleton, followed by their oxidation and cyclization. The relative configuration of **1** was determined on the basis of the NOESY interactions between H-6, H-4, and H-10 that indicated their α-orientation, since, on biogenetic grounds, the methyl groups 14 and 15 are β-oriented.[9] Therefore, the 8,12 lactone moiety is also β-oriented. The structure **1** of velatumolide was confirmed by X-ray spectroscopy (Figure 1).

Epi-velatumolide (**2**) had the same molecular formula ($C_{15}H_{20}O_4$) and IR spectrum as velatumolide (**1**). The ^1H and ^{13}C NMR data of both compounds were very similar (Table 1). In the ^1H NMR spectrum of **2**, the signal of H_2-12 appeared as a singlet (δ 4.73), while in **1** it was observed as an AB system, and those of H-10 and H_3-14 were shifted to low field ($\Delta\delta$ 0.75 and 0.24 ppm, respectively) compared to the same signals in compound **1**. In the ^{13}C NMR, the signals of C-4, C-5, C-6, and C-10

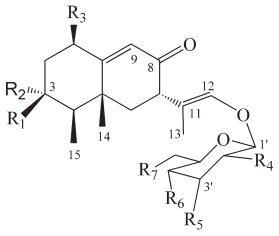
Received: March 2, 2011
Published: June 10, 2011

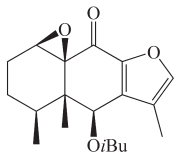


1 R = Hα
2 R = Hβ



3 $R_1 = R_2 = R_3 = OH$
8 $R_1 = H, R_2 = O$*i*Bu, $R_3 = H$
9 $R_1 = H, R_2 = O$*i*Val, $R_3 = H$





10

4 $R_1 = R_2 = R_3 = H, R_4 = R_5 = R_6 = R_7 = OH$
4a $R_1 = R_2 = R_3 = H, R_4 = R_5 = R_6 = R_7 = OAc$
5 $R_1 = R_2 = H, R_3 = R_4 = R_5 = R_6 = R_7 = OH$
5a $R_1 = R_2 = H, R_3 = R_4 = R_5 = R_6 = R_7 = OAc$
6 $R_2 = R_3 = H, R_1 = R_4 = R_5 = R_6 = R_7 = OH$
6a $R_2 = R_3 = H, R_1 = R_4 = R_5 = R_6 = R_7 = OAc$
7 $R_1 = R_3 = H, R_2 = R_4 = R_5 = R_6 = R_7 = OH$

appeared shifted upfield ($\Delta\delta$ −2.1, −3.9, −2.0, and −4.6 ppm, respectively), while that of C-14 was displaced to low field ($\Delta\delta$ 5.9 ppm) in relation to those of **1**. Since the interactions observed in the COSY and HMBC experiments were the same for both compounds, it was evident that they should have different configurations. Thus, in the NOESY spectrum of compound **2** the cross-peaks between H-4 and H-10 and between H_3-14 and H-6 showed that H-10 was on the α-side of the molecule while H-6 was on the β-side. Therefore, compound **2** was the 6-epimer of compound **1**. This conclusion was confirmed by X-ray spectroscopy (Figure 2).

The IR spectrum of velatumin (**3**) showed strong bands at 3348 and 1662 cm^{-1} characteristic of OH and conjugated carbonyl groups. The presence of 15 carbon atoms was evident in the ^{13}C NMR spectrum (Table 1), in agreement with the molecular formula $C_{15}H_{20}O_5$ (HRFABMS). The ^1H NMR spectrum (Table 1) revealed the presence of an aromatic proton (δ 7.48 s) and of a secondary (δ 1.19, d, J = 6.8 Hz) and a tertiary (δ 0.80 s) methyl group, suggesting a furanoeremophilane skeleton. The signals of the hydrogen geminal to oxygenated functions H-2 (δ 3.61, dddd, J = 11.6, 11.6, 3.6, 3.6 Hz), H-6 (δ 4.93 s), and H_2-13 (δ 4.64, d, J = 13.6 Hz; δ 4.59, d, J = 13.6 Hz) were

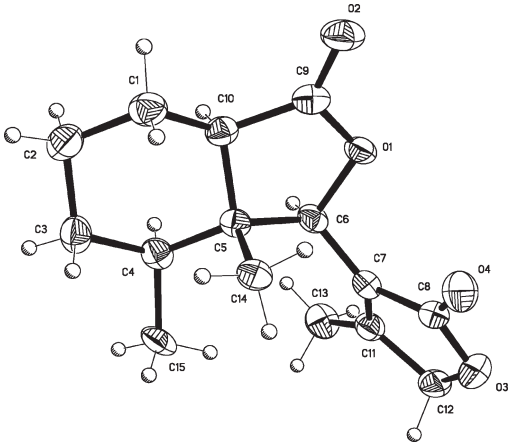


Figure 1. ORTEP projection of velatumolide (**1**).

Table 1. NMR Spectroscopic Data of Compounds 1−3[a]

	1[b]		**2**[b]		**3**[c]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	1.95 brd (11.2)	18.9	1.95 brd (12.4)	18.9	2.30 ddd (13.2, 3.6, 3.2)	30.9
1b	1.42 qd (12.8, 3.2)		1.38 dd (12.4, 3.6)		1.29 ddd (13.2, 12.8, 11.6)	
2a	1.80 m	25.2	1.81 brd (12.4)	25.1	3.61 dddd (11.6, 11.6, 3.6, 3.6)	70.2
2b	1.31 tt (12.3, 4.0)		1.26 m			
3a	1.56 brd (13.2)	29.9	1.52 m	29.9	1.66 brd 12.0	41.9
3b	1.21 brq (13.2)		1.23 da (12.4)		1.26 ddd (12.8, 12.0, 11.6)	
4	1.88 m	38.5	1.50 m	36.4	1.89 dqd (12.8, 6.8, 3.2)	42.6
5		50.4		46.5		51.2
6a	4.98 s	84.4	5.05 s	82.4	4.93 s	75.3
6b						
7		121.6		122.8		140.4
8		173.2		172.2		147.4
9		175.6		176.8		188.5
10	2.29 dd (11.2, 3.2)	52.4	3.04 dd (12.0, 3.2)	47.8	2.48 dd (12.4, 3.2)	54.5
11		163.3		163.1		128.7
12a	4.76 d (17.6)	73.2	4.73 s	72.8	7.48 s	146.8
12b	4.69 d (17.6)		4.73 s			
13a	2.22 s	14.1	2.15 s	13.2	4.64 d (13.6)	56.0
13b					4.59 d (13.6)	
14	0.84 s	9.3	1.08 s	15.1	0.80 s	19.1
15	0.76 d (6.8)	16.9	0.80 d (6.4)	16.7	1.19 d (6.8)	6.8

[a] 400 MHz for protons and 100 MHz for carbons. Chemical shifts are in ppm; J values in Hz are in parentheses. Assignments are based on COSY, HSQC, and HMBC experiments. [b] $CHCl_3$. [c] MeOH-d_4.

dx.doi.org/10.1021/np200188w |*J. Nat. Prod.* 2011, 74, 1584–1589

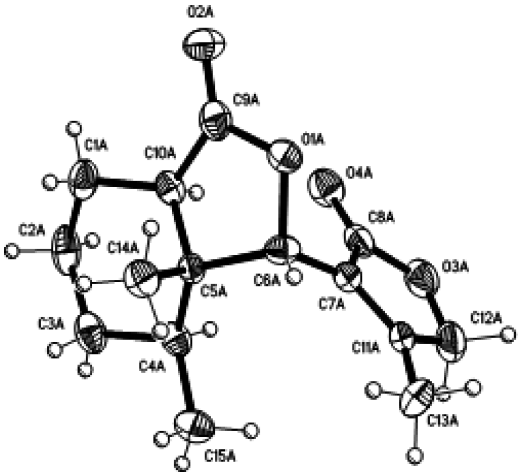


Figure 2. ORTEP projection of epi-velatumolide (**2**).

assigned on the basis of their interactions observed in the COSY and HMBC experiments. The 9-keto function was deduced by the correlation between H-10 (δ 2.48, dd, J = 12.4, 3.2 Hz) and C-9 (δ 188.5) observed in the HMBC spectrum. Finally, and considering the β-orientation of the 14 and 15 methyl groups,[9] the NOESY experiment indicated the β-orientation of the OH groups, since NOE effects of H-2 and H-6 with H-10 and H-4 reveled that they were α-oriented.

Compound **4** had the molecular formula $C_{21}H_{32}O_7$ (HRFABMS) and UV absorptions at 216 and 240 nm. The IR spectrum exhibited bands indicating OH (3380 cm^{-1}) and conjugated carbonyl groups (1658 cm^{-1}). The ^{13}C NMR spectrum displayed 21 carbon signals, including those of a sugar moiety: five CH (δ 103.9, 78.4, 77.9, 74.6, and 71.4) and one CH_2 (δ 62.7). The remaining 15 carbons were assigned, based on ^1H and 2D NMR data, to an eremophilane-type sesquiterpene with a carbonyl group (δ 202.4) and two trisubstituted double bonds (δ 124.6 CH, 173.9 and 114.9 C, 142.6 CH). The HMBC experiment placed the carbonyl group at C-8 and the double bonds at C-9 and C-11, respectively. The location of the sugar was determined by the cross-peak between the anomeric proton (δ 4.48, d, J = 8.0 Hz) and C-12 (δ 142.6), observed in the same experiment. The sugar was identified as β-D-glucose ($[\alpha]^{25}_D$ +44.5) by the axial−axial coupling constants of H-1′, H-2′, H-3′, and H-4′ observed in the acetyl derivative **4a**. The correlation of H-7 with H-12 observed in the NOESY experiment suggested the *E* configuration of the C-11 double bond, and that between H-7 and H_3-14 the β-orientation of H-7. Since the Cotton effects observed in the CD experiment were comparable to those reported for 4-ene-3-ketosteroids,[10] the absolute configuration of compound **4** should be 4*S*, 5*R*, 7*S*.

Compound **5** had the molecular formula $C_{21}H_{32}O_8$, one oxygen atom more than **4**, and exhibited ^1H and ^{13}C NMR spectra (Table 2) very similar to those of **4**. The presence of an OH at C-1 in **5** was supported by the chemical shifts of H-1 (δ 4.49) and C-1 (δ 73.5) and by the cross-peaks between H-1, C-5, and C-9 observed in the HMBC experiment. The NOESY spectrum, unfortunately, was not useful to establish the configuration at C-1 in compounds **5** and **5a**. Nevertheless, the β-axial orientation of the OH was suggested by the small α-equatorial coupling constant of H-1 (J = 2.8 Hz) and was supported by the CD curve of **5**, which exhibited the same pattern as 6β-hydroxy-4-ene-3-ketosteroids.[10] Therefore, the absolute configuration of **5** should be 1*R*, 4*S*, 5*R*, 7*S*.

Compound **6** showed the same molecular formula ($C_{21}H_{32}O_8$) and very similar NMR spectra to those of compound **5**. The only difference between these two compounds was the position of the OH group on the A ring. Thus in compound **6**, H-3 resonated at δ 3.85 and C-3 appeared at δ 74.6 in the NMR spectra. The interaction between H_3-15 (δ 1.08, d, J = 7.2 Hz) and C-3 (δ 74.6), observed in the HMBC spectrum, indicated the presence of an OH at C-3. H-3 was α-equatorial, as deduced from the small coupling constants observed in the ^1H NMR spectrum of the acetyl derivative **6a** (H-3, δ 5.07, q, J = 2.8 Hz). The H-3β axial coupling constants (J = 11.0, 11.0, 4.5 Hz) have been reported for the 3-epimer of **6** (compound **7**, isolated from *P. praecox*).[6] The CD spectrum of compound **6** showed the same profile as the one reported for **7**,[6] which was comparable to those of 4-ene-3-ketosteroids.[10] This could mean that the structural alteration, due to the ε-OH group, remote from the chromophore, did not influence the Cotton effect.[11] Therefore the absolute configuration of compound **6** should be 3*S*, 4*R*, 5*R*, 7*S*.

Structures of the known compounds **7**,[6] **8**,[12] **9**,[4] **10**,[13] naringenine,[14,15] aromadendrin,[16] kaempferol,[17] and taxifolin[18] were determined by comparison of their physical and spectroscopic features with those reported in the literature.

Compounds **1**−**10** were tested using the TPA-induced model of acute inflammation.[19] None of these compounds showed any relevant activity; therefore, the anti-inflammatory action of *P. velatum* is probably due to its flavonoids, whose activity has been described.[20,21]

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 MHz or a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane as internal standard. EIMS were determined on a Bruker Daltonics Analysis 3.2 mass spectrometer. FABMS were obtained on a JEOL JMS-SX102A mass spectrometer operated with an acceleration voltage of 10 kV, and samples were desorbed from a nitrobenzyl alcohol matrix using 6 kV xenon atoms. HRFABMS were performed at 10 000 resolution using electric field scans and polyethylene glycol ions (Fluka 200 and 300) as reference material. Circular dichroism was obtained on a Jasco J-720 spectropolarimeter. X-ray crystallographic analyses were carried out on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods using the program SHELXS. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included at calculated positions and were not refined. Column chromatography was carried out under vacuum (VCC) on silica gel G 60 (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was performed on silica gel 230−400 (Macherey-Nagel, Germany). Analytical TLC was carried out on Si gel 60 GF_{254} or RP-18W/UV_{254} (Macherey-Nagel, Germany), and preparative TLC on Si gel GF_{254}, layer thickness 2.0 mm, or RP-18W/UV_{254}, layer thickness 1.0 mm.

Plant Material. *Pittocaulon velatum* (stems and roots) was collected in Taxco, Guerrero, México, in April 2007. A voucher specimen (MEXU 1205023) has been deposited at the Herbario del Instituto de Biología, Universidad Nacional Autónoma de México.

Table 2. NMR Spectroscopic Data of Compounds 4−6[a]

	4[b]		5[c]		6[c]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.41 tdd (15.0, 6.0, 1.5)	34.0	4.49 t (2.8)	73.5	2.85 dddd (14.0, 14.0, 4.8, 1.2)	28.6
1b	2.28 brd (15.0)				2.13 dt (14.0, 3.2)	
2a	1.88 m	27.5	1.96 dq (13.6, 3.2)	33.9	2.00 brd (14.0)	34.9
2b	1.48 m		1.68 tt (13.6, 3.2)		1.70 brt (14.0)	
3a	1.54 m	31.4	1.86 td (13.6, 3.2)	26.0	3.85 m	74.6
3b	1.54 m		1.38 m			
4	1.47 m	45.2	1.46 m	44.8	1.49 qd (7.2, 2.8)	47.9
5		40.9		40.4		40.9
6a	1.94 dd (14.0, 5.5)	43.2	1.92 dd (12.0, 7.2)	44.6	1.90 dd (13.2, 5.6)	43.8
6b	1.90 dd (14.0, 13.5)		1.91 dd (12.0, 11.6)		1.85 t (13.2)	
7	3.06 dd (13.5, 5.5)	48.4	3.16 dd (11.6, 7.2)	48.8	3.07 dd (13.2, 5.6)	47.9
8		202.4		203.3		202.5
9	5.70 d (1.5)	124.6	5.79 brs	127.1	5.74 d (1.2)	124.4
10		173.9		170.7		174.7
11		114.9		114.8		114.9
12	6.29 brq (1.0)	142.6	6.31 q (1.2)	142.7	6.26 q (1.2)	142.7
13	1.54 d (1.0)	10.9	1.53 d (1.2)	10.8	1.53 d (1.2)	10.9
14	1.20 s	16.3	1.35 s	18.4	1.39 s	19.4
15	0.92 d (6.5)	15.4	0.94 d (6.8)	15.5	1.08 d (7.2)	12.4
1′	4.48 d (8.0)	103.9	4.49 d (7.6)	103.9	4.48 d (7.6)	103.9
2′	3.38−3.28 m	78.4[d]	3.38−3.28 m	78.3[d]	3.35−3.28 m	78.3[d]
3′	3.38−3.28 m	77.9[d]	3.38−3.28 m	77.9[d]	3.35−3.28 m	77.9[d]
4′	3.38−3.28 m	74.6[d]	3.38−3.28 m	74.6[d]	3.35−3.28 m	71.6[d]
5′	3.38−3.28 m	71.4[d]	3.38−3.28 m	71.4[d]	3.35−3.28 m	71.4[d]
6′a	3.85 dd (11.5, 1.0)	62.7	3.85 dd (12.0, 1.6)	62.7	3.85 dd (12.0, 2.0)	62.7
6′b	3.65 dd (11.5, 5.5)		3.65 dd (12.0, 5.2)		3.65 dd (12.0, 5.2).	

[a] Assignments are based on COSY, DEPT, HSQC, and HMBC experiments. Measured in MeOH-d_4. Chemical shifts are in ppm; J values in Hz are in parentheses. [b] 500 MHz for protons and 125 MHz for carbons. [c] 400 MHz for protons and 100 MHz for carbons. [d] Exchangeable signals.

Extraction and Isolation. Dried and ground stems (1.5 kg) and roots (490 g) were extracted separately with MeOH at room temperature. The stem extract (300 g) was fractionated by VCC eluted with a hexane−EtOAc−MeOH gradient system to afford mixtures A−F from hexane and 19:1, 9:1, 4:1, 7:3, and 1:1 hexane−EtOAc fractions, respectively. Mixture G was collected with EtOAc and 19:1 EtOAc−MeOH and 9:1 EtOAc−MeOH mixtures. Compounds **8** (28.3 mg)[12] and **9** (45 mg)[4] were isolated from A (25 g) and B (31 g), respectively, after purification by VCC (hexane) followed by RPTLC (MeOH−H_2O, 3:2 × 4) on 100 mg portions of each mixture. Fraction C (35 g, VCC: hexane−EtOAc gradient system) gave C1 (hexane) and C2 (hexane−EtOAc, 9:1). Fraction C1 (1.28 g) yielded compounds **8** (10 mg) and **10** (18 mg)[13] by FCC (benzene−EtOAc, 49:1) followed by RPTLC (MeOH−H_2O, 3:2 × 4). Fraction C2 furnished a solid, which by crystallization produced 678 mg of velatumolide (**1**). Fraction D (9.2 g, VCC: hexane−acetone gradient system) afforded D1 (hexane−acetone, 9:1), D2 (hexane−acetone, 4:1), and D3 (hexane acetone, 7:3). Fraction D1 (800 mg, FCC: CH_2Cl_2−EtOAc, 49:1) produced 200 mg of **1** and a solid, which yielded by crystallization 45 mg of epi-velatumolide (**2**). Fraction D2 (1.5 g, FCC: CH_2Cl_2−EtOAc, 49:1) yielded **2** (50 mg). Fraction D3 (900 mg, FCC: hexane−EtOAc, 4:1) gave naringenine (6 mg).[14,15] Fraction E (27 g, VCC: hexane−EtOAc gradient system) afforded 370 mg of naringenine, 230 mg of aromadendrin,[16] and 23 mg of kaempferol.[17] Fraction F (14.3 g, VCC: hexane−EtOAc gradient system) produced aromadendrin (10 mg), kaempferol (400 mg), and a mixture (290 mg) that by TLC (hexane−acetone, 3:2 × 2) furnished aromadendrin (10 mg), taxifolin (10 mg),[18] and a solid, which yielded velatumin (**3**; 22.6 mg) by recrystallization. Fraction G (35 g, VCC: EtOAc−MeOH gradient system) yielded fractions G1 (EtOAc), G2 (EtOAc−MeOH, 49:1), and G3 (EtOAc−MeOH, 9:1). Fraction G1 (320 mg), by FCC (CH_2Cl_2−MeOH, 19:1) followed by RPTLC (H_2O−MeOH, 3:2 × 3), afforded compound **4** (10 mg). Fraction G2 (1.5 g) was fractionated by two successive FCC (CH_2Cl_2−MeOH, 9:1, and EtOAc−MeOH, 19:1) to obtain G2a−G2c. Fraction G2a (50 mg, RPTLC: H_2O−MeOH, 3:2 × 3) yielded compounds **5** (8 mg) and **6** (12 mg); G2b (38 mg, RPTLC: H_2O−MeOH, 4:1 × 4) gave compound **6** (15 mg); and G2c (49 mg, RPTLC: H_2O−MeOH, 4:1 × 4) produced compound **7** (9 mg).[6] Compounds **5** (5 mg) and **6** (35 mg) were isolated from G3. The methanolic extract of roots (58 g) was fractionated following the procedures described above and afforded **5** (10 mg), **6** (18 mg), **7** (10 mg), **8** (57 mg), **9** (42 mg), **10** (12 mg), naringenine (150 mg), aromadendrine (123 mg), and kaempherol (87 mg).

*Velatumolide (**1**):* colorless prisms (hexane−EtOAc); mp 170−172 °C; $[\alpha]^{25}_D$ +116.4 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 217 (3.96) nm; IR ($CHCl_3$) ν_{max} 1776, 1759 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 264 $[M]^+$ (2), 218 (6), 110 (100); HRFABMS *m/z* 265.1447 $[M + H]^+$ ($C_{15}H_{21}O_4$ requires 265.1440).

*Epi-velatumolide (**2**):* colorless prisms (hexane−EtOAc); mp 195−197 °C; $[\alpha]^{25}_D$ +80.8 (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.04) nm; IR ($CHCl_3$) ν_{max} 1777, 1759 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 264 $[M]^+$ (3), 218 (10), 110

dx.doi.org/10.1021/np200188w | *J. Nat. Prod.* 2011, 74, 1584–1589

(100); HRFABMS m/z 265.1445 $[M + H]^+$ ($C_{15}H_{21}O_4$ requires 265.1440).

*Velatumin (**3**):* colorless needles (EtOAc); mp 222−224 °C; yellow oil; $[\alpha]^{25}_D$ −27.3 (c 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 275 (4.27) nm; IR (KBr) ν_{max} 3348, 1662 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRFABMS m/z 281.1385 $[M + H]^+$ ($C_{15}H_{21}O_5$ requires 281.1389).

*Compound **4**:* white powder; mp 118−120 °C; $[\alpha]^{25}_D$ +13.0 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.55), 240 (3.85) nm; IR (KBr) ν_{max} 3380, 1658, 1617 cm^{-1}; CD (MeOH) $\Delta\varepsilon_{\lambda max}$ $+201.6_{204}$, $+154.5_{239}$, -28.1_{274} (c 8.5×10^{-5} M); ^1H NMR and ^{13}C NMR data, see Table 2; FABMS m/z 419 $[M + Na]^+$, 235, 41; HRFABMS m/z 419.2059 $[M + Na]^+$ ($C_{21}H_{32}O_7Na$ requires 419.2046).

*Compound **5**:* white powder; mp 128−130 °C; $[\alpha]^{25}_D$ +11.5 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.69), 236 (3.92) nm; IR (KBr) ν_{max} 3460, 1660, 1617 cm^{-1}; CD (MeOH) $\Delta\varepsilon_{\lambda max}$ $+294.0_{205}$, -51.5_{251} (c 9.5×10^{-5} M); ^1H NMR and ^{13}C NMR data, see Table 2; EIMS m/z 250 $[M - C_6H_{10}O_5]^+$ (55), 233 (20), 125 (100); HRFABMS m/z 413.2174 $[M + H]^+$ ($C_{21}H_{33}O_8$ requires 413.2175).

*Compound **6**:* white powder; mp 145−147 °C; $[\alpha]^{25}_D$ +37.0 (c 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.69), 241 (4.05) nm; IR (KBr) ν_{max} 3413, 1657, 1605 cm^{-1}; CD (MeOH) $\Delta\varepsilon_{\lambda max}$ $+331.2_{204}$, $+213.9_{240}$, -48.3_{276} (c 7.7×10^{-5} M); ^1H NMR and ^{13}C NMR data, see Table 2; EIMS m/z 250 $[M - C_6H_{10}O_5]^+$ (75), 125 (100); HRFABMS m/z 413.2185 $[M + H]^+$ ($C_{21}H_{33}O_8$ requires 413.2175).

Acetylation of Compounds 4−6. Compounds **4**, **5**, and **6** (10 mg) were acetylated in an excess of anhydrous pyridine−acetic anhydride to obtain compounds **4a** (11 mg), **5a** (10 mg), and **6a** (9 mg), respectively.

Hydrolysis of Compounds 4−6. Compounds **4a**, **5**, and **6** (10 mg) were hydrolyzed with 2 N HCl at 70 °C (2 h for **4a** and 40 min for **5** and **6**). The reaction mixtures were extracted with CH_2Cl_2, and the aqueous layers were evaporated and purified by FCC (EtOAc−MeOH, 3:2) to obtain D-glucose:[22] 2.0 mg from **4a**, $[\alpha]^{25}_D$ +44.5 (c 0.18, H_2O); 2.5 mg from **5**, $[\alpha]^{25}_D$ +47.3 (c 0.23, H_2O); and 2.7 mg from **6**, $[\alpha]^{25}_D$ +55.0 (c 0.27, H_2O).

*Compound **4a**:* ^1H NMR ($CDCl_3$, 500 MHz) δ 2.31 (1H, brtd, J = 14.0, 7.5 Hz, H-1a), 2.24 (1H, brd, J = 14.0 Hz, H-1b), 1.88 (1H, m, H-2a), 1.47 (1H, m, H-2b), 1.54 (2H, m, H_2-3), 1.44 (1H, m, H-4), 1.94 (1H, dd, J = 13.0, 5.0 Hz, H-6a), 1.82 (1H, dd, J = 14.5, 13.0 Hz, H-6b), 2.92 (1H, dd, J = 14.5, 5.0 Hz, H-7), 5.72 (1H, d, J = 1.0 Hz, H-9), 6.15 (1H, brd, J = 1.0 Hz, H-12), 1.57 (3H, d, J = 1.0 Hz, H_3-13), 1.15 (3H, s, H_3-14), 0.91 (3H, d, J = 6.0 Hz, H_3-15), 4.69 (1H, d, J = 8.0 Hz, H-1′), 5.12 (1H, dd, J = 9.5, 8.0 Hz, H-2′), 5.22 (1H, t, J = 9.5 Hz, H-3′), 5.13 (1H, t, J = 9.5 Hz, H-4′), 3.73 (1H, ddd, J = 9.5, 5.0, 2.5 Hz, H-5′), 4.27 (1H, dd, J = 12.5, 5.0 Hz, H-6′a), 4.13 (1H, dd, J = 12.5, 2.5 Hz, H-6′b), 2.09 s, 2.06 s, 2.03 s, 2.01 s (3H each, Ac); HRFABMS m/z 565.2646 $[M + H]^+$ ($C_{29}H_{41}O_{11}$ requires 565.2649).

*Compound **5a**:* ^1H NMR ($CDCl_3$, 500 MHz) δ 5.39 (1H, t, J = 2.5 Hz, H-1), 2.00 (1H, m, H-2a), 1.69 (1H, tt, J = 14.0, 3.5 Hz, H-2b), 1.76 (1H, dt, J = 14.0, 2.5 Hz, H-3a), 1.47 (1H, m, H-3b), 1.48 (1H, m, H-4), 1.95 (1H, dd, J = 13.0, 4.5 Hz, H-6a), 1.86 (1H, dd, J = 14.0, 13.0 Hz, H-6b), 3.01 (1H, dd, J = 14.0, 4.5 Hz, H-7), 5.94 (1H, s, H-9), 6.14 (1H, q, J = 1.0 Hz, H-12), 1.55 (3H, d, J = 1.0 Hz, H_3-13), 1.26 (3H, s, H_3-14), 0.95 (3H, d, J = 6.5 Hz, H_3-15), 4.69 (1H, d, J = 8.0 Hz, H-1′), 5.11 (1H, dd, J = 9.5, 8.0 Hz, H-2′), 5.22 (1H, t, J = 9.5 Hz, H-3′), 5.12 (1H, t, J = 9.5 Hz, H-4′), 3.73 (1H, ddd, J = 9.5, 4.5, 2.5 Hz, H-5′), 4.27 (1H, dd, J = 12.5, 4.5 Hz, H-6′a), 4.13 (1H, dd, J = 12.5, 2.5 Hz, H-6′b), 2.09 s, 2.06 s, 2.03 s, 2.02 s, 2.01 s (3H each, Ac); HRFABMS m/z 623.2700 $[M + H]^+$ ($C_{31}H_{42}O_{13}$ requires 623.2704).

*Compound **6a**:* ^1H NMR ($CDCl_3$, 400 MHz) δ 2.62 (1H, dddd, J = 14.0, 14.0, 5.2, 1.6 Hz, H-1a), 2.17 (1H, brd, J = 14.0 Hz, H-1b), 2.09−2.01 (1H, m, H-2a), 1.68 (1H, brd, J = 14.0 Hz, H-2b), 5.07 (1H, q, J = 2.8 Hz, H-3), 1.65 (1H, m, H-4), 1.95 (1H, dd, J = 13.0, 4.4 Hz, H-6a), 1.80 (1H, dd, J = 14.0, 13.0 Hz, H-6b), 2.94 (1H, dd, J = 14.0, 4.4 Hz, H-7), 5.79 (1H, d, J = 1.2 Hz, H-9), 6.15 (H, brd, J = 1.2 Hz, H-12), 1.56 (3H, d, J = 1.2 Hz, H_3-13), 1.34 (3H, s, H_3-14), 0.99 (3H, d, J = 6.4 Hz, H_3-15), 4.69 (1H, d, J = 7.6 Hz, H-1′), 5.12 (1H, dd, J = 9.2, 7.6 Hz, H-2′), 5.22 (1H, t, J = 9.2 Hz, H-3′), 5.13 (1H, t, J = 9.2 Hz, H-4′), 3.76 (1H, ddd, J = 9.2, 4.8, 2.4 Hz, H-5′), 4.27 (1H, dd, J = 12.4, 4.8 Hz, H-6′a), 4.13 (1H, dd, J = 12.4, 2.4 Hz, H-6′b), 2.12 s, 2.09 s, 2.06 s, 2.03 s, 2.02 s (3H each, Ac); HRFABMS m/z 623.2701 $[M + H]^+$ ($C_{31}H_{43}O_{13}$ requires 623.2704).

Crystal Data of 1 (ref 23): $C_{15}H_{20}O_4$, M_r 264.31, monoclinic, space group $P2_1$, a = 9.423(1) Å, α = 90°, b = 32.275(5) Å, β = 116.120(2)°, c = 7.288(1) Å; γ = 90°, V = 1356.5(4) Å^3, Z = 4, D_c = 1.294 Mg/m^3, F(000) = 568; crystal dimensions/shape/color 0.466 × 0.252 × 0.208 mm/prism/colorless. Reflections collected 21 466, independent reflections 3299. Number of parameters refined 349; final R indices $[I > 2\sigma(I)]$ R_1 = 0.0535, wR_2 = 0.1371; R indices (all data) R = 0.0662, wR_2 = 0.1465. Remarks: twinned crystal: 05438/04562.

Crystal Data of 2 (ref 23): $C_{15}H_{20}O_4$, M_r 264.31, monoclinic, space group $P2_1$, a = 6.488(1) Å, α = 90°, b = 32.300(5) Å, β = 116.314(3)°, c = 7.308(1) Å; γ = 90°, V = 1372.9(5) Å^3, Z = 4, D_c = 1.27 Mg/m^3, F(000) = 568; crystal dimensions/shape/color 0.396 × 0.268 × 0.266 mm/prism/colorless. Reflections collected 13 763, independent reflections 13 763. Number of parameters refined 350; final R indices $[I > 2\sigma(I)]$ R_1 = 0.0685, wR_2 = 0.1085; R indices (all data) R = 0.1009, wR_2 = 0.1299. Remarks: twinned crystal: 07231/03769.

■ ASSOCIATED CONTENT

Supporting Information. ^1H, ^{13}C, and 2D NMR spectra of velatumolide (**1**), epi-velatumolide (**2**), velatumin (**3**), and compounds **4**−**6**, as well as the ^1H NMR spectra of the acetyl derivatives **4a**−**6a** are available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We are indebted to R. Gaviño, I. Chávez, H. Ríos, Á. Peña, E. Huerta, R. Patiño, B. Quiroz, L. Velasco, J. Pérez, A. Toscano, C. Márquez, E. Ríos, and A. Nieto for technical assistance.

■ REFERENCES

(1) Olson, M. E. *J. Torrey Bot. Soc.* **2005**, *132*, 173–186.
(2) Robinson, H.; Brettell, R. D. *Phytologia* **1973**, *26*, 451–453.
(3) Marín-Loaiza, J. C.; Ernst, L.; Beuerle, T.; Theuring, C.; Céspedes, L. C.; Hartmann, T. *Phytochemistry* **2008**, *69*, 154–167.
(4) Bohlmann, F.; Zdero, C. *Chem. Ber.* **1976**, *109*, 819–825.
(5) Ortega, A.; Romero, M.; Díaz, E. *Rev. Latinoam. Quím.* **1975**, *6*, 136–142.
(6) Arciniegas, A.; Pérez-Castorena, A. L.; Gastélum, E.; Villaseñor, J. L.; Romo de Vivar, A. *Heterocycles* **2009**, *78*, 1253–1263.
(7) Maldonado, J, I.; Arciniegas, A.; Pérez-Castorena, A. L.; Villaseñor, J. L.; Romo de Vivar, A. *Heterocycles* **2008**, *75*, 3035–3042.
(8) Martínez, M. *Las Plantas Medicinales de México*, Ed.; Botas: México, 1959; pp 470−471.
(9) Richards, J.; Hendrickson, J. *Biosynthesis of Terpenes, Steroids and Acetogenins*; W. A. Benjamin Inc.: New York, 1964; Vol. 8, pp 225−237.
(10) Gawrońsky, J. K. *Tetrahedron* **1982**, *38*, 3–26.

(11) Lightner, D. A.; Gurst, J. *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*; John Wiley & Sons: New York, 2000; Chapter 11, pp 337−391.
(12) Villarroel, L.; Torres, R.; Gavin, J.; Reina, M.; De la Fuente, G. *J. Nat. Prod.* **1991**, *54*, 588–590.
(13) Wang, Y.; Yuan, C.-S.; Han, Y.-F.; Jia, H. Z.-J. *Pharmazie* **2003**, *58*, 349–352.
(14) Shen, C.-C.; Chang, Y.-S.; Ho, L.-K. *Phytochemistry* **1993**, *34*, 843–845.
(15) *Dictionary of Natural Products*; Chapman Hall, CRS Press, on line http//dnp.chemnetbase.com, 2008 (accessed on June 2008).
(16) Takahashi, H.; Li, S.; Harigaya, Y.; Onda, M. *Chem. Pharm. Bull.* **1988**, *36*, 1877–1881.
(17) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389–1397.
(18) Nonaka, G. I.; Goto, Y.; Kinjo, J. E.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 1105–1108.
(19) Arciniegas, A.; Pérez-Castorena, A. L.; Nieto-Camacho, A.; Villaseñor, J. L.; Romo de Vivar, A. *J. Mex. Chem. Soc.* **2009**, *78*, 229–232.
(20) Zhang, X.; Hung, T. M.; Phuong, P. T.; Ngoc, T. M.; Min, B.-S.; Song, K. S.; Seong., Y. H.; Bae, K. *Arch. Pharm. Res.* **2006**, *29*, 1102–1108.
(21) Ueda, H.; Yamazaki, C.; Yamazaki, M. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 119–125.
(22) Fu, X.; Li, X.-C.; Smillie, T. J.; Carvalho, P.; Mabusela, W.; Syce, J.; Johnson, Q.; Folk, W.; Avery, M. A.; Khan, A. *J. Nat. Prod.* **2008**, *71*, 1749–1753.
(23) Crystallographic data for the structures **1** (CCDC 827112) and **2** (CCDC 827113) have been deposited with the Cambridge Crystallographic Data Centre. 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).