

MINOR CONSTITUENTS FROM *ORTHOSPHENIA MEXICANA*

ANTONIO G. GONZÁLEZ,\* L. SAN ANDRÉS, A.G. RAVELO, J.G. LUIS, I.A. JIMÉNEZ,  
Centro de Productos Naturales Orgánicos "Antonio González", Instituto Universitario de Química Orgánica,  
Carretera La Esperanza 2, La Laguna, 38206 Tenerife, Canary Islands, Spain  
and X.A. DOMÍNGUEZ

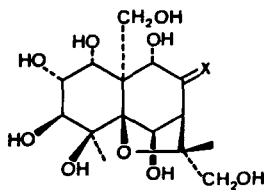
Instituto Tecnológico y de Estudios Superiores de Monterrey, Mexico

ABSTRACT.—This paper reports the isolation and structural elucidation of two minor products from *Orthosphenia mexicana*: orthosphenin [4], a new cassinic acid alkaloid sesquiterpene ester with an evoninol nucleus, and a new norisopimaradiene diterpene 8 of a type found only for the second time in the Celastraceae, where abietatriene diterpenes are the norm.

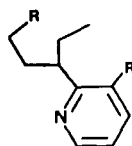
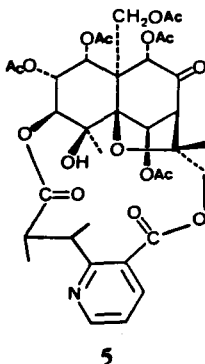
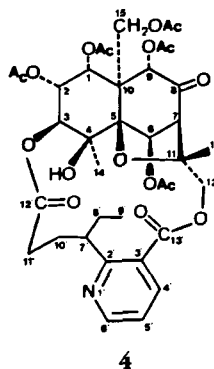
Polyester sesquiterpene alkaloids are frequently found in the Celastraceae and are viewed as chemotaxonomical markers (1). Structures incorporating nicotinic (2), evoninic (3), wilfordic (4), hydroxy-wilfordic (4), cathaic (5), and cassinic (6) acid moieties characterize such compounds as alkaloids. The macrocyclic al-

kaloids have three basic sesquiterpene cores: evoninol [1], euonyminol [2] and iso-euonyminol [3].

This paper reports the isolation and structural elucidation of two minor products from *Orthosphenia mexicana* Standley (Celastraceae) (7): a new cassinic acid alkaloid sesquiterpene ester



- 1 X=O
- 2 X=α-OH; β-H
- 3 X=α-H; β-OH



- 6 R=COOH
- 7 R=CH<sub>2</sub>OAc

with an evoninol nucleus, orthosphenin [4], and a new norisopimaradiene diterpene **8** of a type found only for the second time in the Celastraceae (8), where abietatriene diterpenes are the general rule (1). An earlier study of this plant afforded new triterpenes (9–12), sesquiterpenes (13), and sesquiterpene alkaloids (2), some of which have shown interesting cytostatic (14) or antibiotic (15) activity in preliminary assays.

Repeated chromatography of the hexane extract of the roots of *O. mexicana* on Si gel gave compound **4** as an off-white amorphous solid, formula  $C_{36}H_{45}O_{17}$ . This compound had signals for an OH group in its ir spectrum and could not be acetylated under normal conditions, thereby indicating the presence of a tertiary alcohol group. The  $^1H$ -nmr spectra of **4** and evonin [5] (16) have four acetate methyl signals with the same chemical

shifts, two singlets for protons allylic to a ketone at  $\delta$  5.54 and 3.01, assigned to H-9 and H-7, and very similar signals in general (see Table 1). These data show that the sesquiterpene nucleus of **4** has the basic structure of evoninol [1]. This was confirmed when  $LiAlH_4$  reduction of **4** in THF under reflux followed by acetylation with  $Ac_2O$  in pyridine gave a mixture of three products which were separated by preparative tlc and identified as euonyminol octa-acetate, iso-euonyminol octa-acetate (17, 18) and the diacetate of diol **7**, a reduction product of cassinic acid [6] earlier obtained from *Cassine metabelica* Loes. (Celastraceae) (6). The  $^1H$  nmr spectrum of compound **4** had a methyl triplet at  $\delta$  0.68 ( $J=7$  Hz), COSY experiments showed the presence of a  $>CH-CH_2-CH_3$  grouping, and  $^{13}C$ -nmr data confirmed the proposed structure. We suggest the

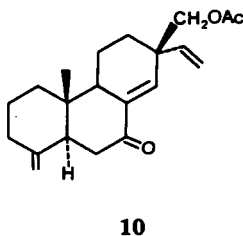
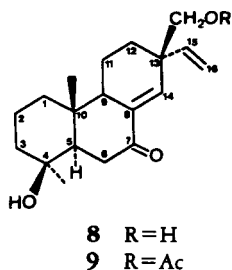
TABLE 1.  $^1H$ -<sup>a</sup> and  $^{13}C$ -nmr<sup>b</sup> Data for Orthosphenine [4] ( $CDCl_3$ ).

Position	H	C	Position	H	C
1 . . . . .	5.69 d (3)	69.6–75.1	15 . . . . .	4.52–4.82 d <sub>AB</sub> (13.1)	60.44
2 . . . . .	5.15 t (3)		2' . . . . .	—	131.0
3 . . . . .	5.02 d (3)		3' . . . . .	8.42 dd (1.7, 8.3)	124.8
4 . . . . .	—		4' . . . . .	7.27 dd (4, 8.3)	138.3
5 . . . . .	—	95.5	5' . . . . .	8.83 dd (1.7, 4)	121.0
6 . . . . .	6.72 s	69.6–75.1	6' . . . . .	4.36 m	154.1
7 . . . . .	3.01 s	62.43	7' . . . . .	4.36 m	42.44
8 . . . . .	—	188.9	8' . . . . .	1.80 <sup>c</sup>	29.0
9 . . . . .	5.54 s	69.6–75.1	9' . . . . .	0.68 t (7)	12.07
10 . . . . .	—	52.6	10' . . . . .	—	29.9
11 . . . . .	—	86.6	11' . . . . .	—	31.9
12 . . . . .	1.61 d (1)	19.3–24.0	12' . . . . .	—	166.7–172.2
13 . . . . .	3.64 d (12)	70.1	13' . . . . .	—	
14 . . . . .	1.24 s	19.3–24.0	MeOOC } 2.08, 2.14 2.22	1.89, 2.04 2.08, 2.14 2.22	

<sup>a</sup> $^1H$ -nmr data were obtained in COSY experiments ( $CDCl_3$ , 200 MHz).

<sup>b</sup> $^{13}C$ -nmr data are based on DEPT experiments and correlations.

<sup>c</sup>Overlapping signal.



name orthosphenine for this new sesquiterpene alkaloid, the second cassinic acid derivative to be isolated.

Compound **8** has formula  $C_{19}H_{28}O_3$  and signals in the ir and uv spectra for an  $\alpha, \beta$ -unsaturated keto grouping with the  $\beta$  proton appearing in the  $^1H$ -nmr spectrum at  $\delta$  6.74 as a broad singlet. When **8** was acetylated in the normal way, the monoacetate **9** was obtained. The continued presence of OH signals in its ir indicate the presence of a tertiary group. Compounds **8** and **9** both have signals for two AB doublets centered at  $\delta$  3.51 and 3.32 and 3.96 and 3.87, respectively; signals for an ABX vinylic system appear in the spectra of both compounds and indicate a norisopimaradiene diterpene of the same type as those previously isolated (**8**). Compound **8** was related with the already-known product **10**, the acetate **9** being dehydrated with thionyl chloride in pyridine at  $0^\circ$  to give three products, one of which, in tlc, had an  $R_f$  value identical with that of **10**. From these data the tertiary OH group could be positioned on C-4, but its stereochemistry is still unknown, despite a study of its methyl shifts in different solvents that proved inconclusive. However, as the product was easily dehydrated and gives three products, the OH is probably axial.

## EXPERIMENTAL

**PLANT MATERIAL.**—The plant was collected in northeastern Mexico, and a voucher specimen (No. 7723) was deposited with the Department of Biology, Instituto Tecnológico, Monterrey, Mexico.

The hexane extract (5 g) of the roots of *O. mexicana* yielded the known substances netzahualcoyondiol, netzahualcoyone, and pristimerin and also two new products, orthosphenine [**4**] (19 mg) and the norditerpene **8** (3 mg).

**ORTHOSPHENINE** [**4**].—Compound **4** was obtained as a pale yellow amorphous solid:  $m/z$   $[M]^+$  775.2717 (calcd for  $C_{37}H_{45}O_{17}N$ , 775.2748); uv  $\lambda$  max (EtOH) nm 221, 270; ir  $\nu$  max  $cm^{-1}$  3670, 3430, 3010, 2950, 2920, 1740, 1595, 1560, 1365, 1215, 1145, 1085, 1070, 1040, 1005, 865;  $^1H$  and  $^{13}C$  nmr see Table 1; eims  $m/z$  (rel. int.)  $[M]^+$  775 (3), 716 (6), 542 (4), 276 (10), 248 (5), 236 (10), 220 (100), 192 (75), 178 (26), 147 (32), 121 (69), 106 (56), 93 (13), 79 (4).

Compound **4** (19 mg), dissolved in dry THF, was added to a suspension of  $LiAlH_4$  in dry THF at room temperature while being stirred under an argon atmosphere, and the mixture was then kept at the same temperature for 24 h. EtOAc was added carefully, and the excess of hydride was eliminated in an ice bath. Evaporation at reduced pressure followed by acetylation of the crude product with  $Ac_2O$  in pyridine at  $70^\circ$  for 20 h, followed by filtration and elimination of the reagents by repeated evaporation at reduced pressure with  $C_6H_6$  and EtOH, left a substance which was chromatographed to yield iso-euonyminol octa-acetate, euonyminol octa-acetate, and a diacetate **7** derived from cassinic acid by reduction.

Compound **7** was isolated as an amorphous solid:  $m/z$   $[M - C_2H_4]^+$  265.1328 (calcd for  $C_{14}H_{19}O_4N$ , 265.1342); uv  $\lambda$  max (EtOH) nm 258; ir  $\nu$  max  $cm^{-1}$  2950, 2920, 2840, 1735, 1450, 1360, 1225, 1035, 960, 785;  $^1H$  nmr ( $CDCl_3$ , 200 MHz)  $\delta$  0.76 (3H, t,  $J = 7$  Hz), 1.93, 1.96 (2  $\times$  3H, s), 4.12 (4H, m), 7.10 (1H, m), 7.64 (1H, dd,  $J = 1.75$  Hz), 8.57 (1H, br s); eims  $m/z$  (rel. int.)  $[M]^+$  293 (4),  $[M - C_2H_4]^+$  265 (14),  $[M - Ac]^+$  250 (38),  $[M - OAc]^+$  234 (20), 220 (15), 206 (23), 193 (63), 190 (45), 178 (100).

**7-OXO-19-NORISOPIMARA-8(14),15-DIENE-4 $\beta$ ,17-DIOL** [**8**].—Compound **8** was isolated as

an amorphous off-white solid:  $[\alpha]_D -7.41^\circ$  (0.054,  $\text{CHCl}_3$ );  $m/z$   $[\text{M}]^+$  304.2045 (calcd for  $\text{C}_{19}\text{H}_{28}\text{O}_3$ , 304.2096); uv  $\lambda$  max (EtOH) nm 237; ir  $\nu$  max  $\text{cm}^{-1}$  3600–3140, 2920, 2840, 1715, 1665, 1595, 1450, 1405, 1375, 1265, 1225, 1065, 1035, 1005, 910, 885;  $^1\text{H}$  nmr ( $\text{CDCl}_3$ , 200 MHz)  $\delta$  1.01 (3H, s), 1.10 (3H, s), 2.54 (1H, m), 3.32, 3.51 (2H, d,  $J = 11$  Hz), 4.97, 5.02 (2H, dd<sub>AB</sub>,  $J_{\text{BX}} = 10$ ,  $J_{\text{AB}} = 8.08$ ,  $J_{\text{AX}} = 18$  Hz), 5.76, 5.85 (1H, dd,  $J_{\text{BX}} = 10$ ,  $J_{\text{AX}} = 18$  Hz), 6.74 (1H, br s); eims  $m/z$  (rel. int.)  $[\text{M}]^+$  304 (17), 273 (100), 255 (24), 185 (10), 149 (41), 147 (23), 145 (34), 133 (29), 123 (29), 121 (63), 105 (41), 93 (37), 91 (46), 57 (50), 55 (77).

Product **9** was isolated as an amorphous yellow solid: ir  $\nu$  max  $\text{cm}^{-1}$  3620–3140, 2920, 2840, 1730, 1675, 1605, 1460, 1410, 1375, 1235, 1035, 915;  $^1\text{H}$  nmr ( $\text{CDCl}_3$ , 200 MHz) 1.01 (3H, s), 1.10 (3H, s), 2.09 (3H, s), 2.53 (1H, m), 3.87, 3.96 (2H, d<sub>AB</sub>,  $J = 11$  Hz), 4.98, 5.03 (2H, dd<sub>AB</sub>,  $J_{\text{AX}} = 18$ ,  $J_{\text{BX}} = 10$ ,  $J_{\text{AB}} = 8$  Hz), 5.77, 5.86 (1H, dd,  $J_{\text{AX}} = 18$ ,  $J_{\text{BX}} = 10$  Hz), 6.77 (1H, br s);  $^1\text{H}$  nmr (pyridine- $d_5$ , 200 MHz) 1.03 (3H, s), 1.21 (3H, s), 2.02 (3H, s), 3.87, 3.96 (2H, d<sub>AB</sub>,  $J = 11$  Hz), 6.61 (1H, br s); eims  $m/z$  (rel. int.)  $[\text{M}]^+$  346 (12), 273 (23), 268 (10), 171 (10), 165 (17), 149 (43), 133 (24), 121 (54), 119 (30), 105 (48), 93 (34), 91 (48), 79 (41), 71 (44), 69 (52), 57 (81), 55 (100).

DEHYDRATION OF **9**.—Two drops of  $\text{SOCl}_2$  were added to compound **9** (1.8 mg) dissolved in dry pyridine and placed in an ice bath. After 30 min the reaction mixture was poured over ice and extracted with EtOAc. The organic layer was washed three times with a 5% HCl solution and three times more with a saturated solution of  $\text{NaHCO}_3$ . It was left to dry under  $\text{Na}_2\text{SO}_4$ . Tlc showed at least three significant spots, one of which agreed with **10**, as described elsewhere (8).

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