



SESQUITERPENE DILACTONES FROM *MIKANIA YPACARAYENSIS*

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Abstract—Aerial parts of *Mikania ypacarayensis* afforded, in addition to mikanolide and related known sesquiterpene dilactones, three new dilactones, a derived lactone hydroxy ester and a known germacranolide.

INTRODUCTION

Within the large genus *Mikania* of more than 400 species [1] functionalized derivatives of the sesquiterpene dilactone isabelin (**4a**) so far appear to be confined, with one exception (*Mikania urticaefolia* [2]) to members of the *Mikania scandens* complex as defined by Holmes [3], although not all members of the complex produce dilactones of this type [4–6]. Following our recent work on *Mikania dusenii* [7] we have now studied *Mikania ypacarayensis* Holmes and McDaniel, another member of the complex found in north-eastern Argentina and in southern Brazil and Paraguay.† Isolated from the aerial parts were mikanolide (**1a**) [9, 10], and 11 β H,13-dihydromikanolide (**1b**) [9], the two main lactone constituents, as well as deoxymikanolide (**2a**) [9], scandenolide (**2b**) and its 11 β H,13-dihydro derivative **2c** [9, 10], anhydroscandenolide (**3**) [2], 3 β -hydroxy- and 3 β -acetoxyisabelin (**4b,c**) [11] as well as the 3-methoxy analogue **4d**, miscandenin (**5a**) [9] and its 11 β ,13-dihydro derivative **5b**, the hydroxy ester lactone **6**, monolactone **7** previously found in *M. dusenii* [9] and the dilactones **8a** and **8b**. Compounds **4d**, **5b**, **6** and **8a,b** have not been described previously.

DISCUSSION

The structure of **4d**, which like methyl ester **6** is possibly an artefact, was easily deduced by comparing its ¹H NMR spectrum (Table 1) with the spectra of **4b** and **4c**, the OMe-3 β configuration being apparent from the coupling constants involving H-3 which is not only vicinally coupled to H-2a,b, but also allylically coupled to H-5 and homoallylically to H-6. The structure of **5b**

which was the major component of a binary mixture also containing **5a** was deduced in the same way, the value of $J_{7,11}$ (12 Hz) indicating α -orientation of the methyl group on C-11. This is also true for dihydroscandenolide (**2c**) (Table 1). Although this substance has been reported earlier from *Mikania scandens* [9] and from *M. micrantha* [12], high resolution NMR data for this assignment have not appeared previously.

The structure of ester **6** was clear from the mass spectrum which indicated incorporation of the elements of methanol into **5a** and from the ¹H NMR spectrum (Table 1) which when compared with that of **5a** exhibited the presence of a methoxyl singlet, a significant diamagnetic shift of the H-9b resonance and changes in the appearance of the H-13a,b signals whose allylic coupling to H-7 was reduced to less than 1 Hz.

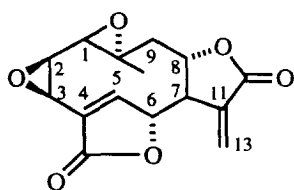
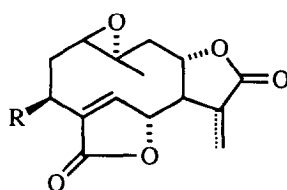
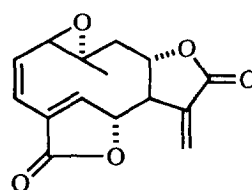
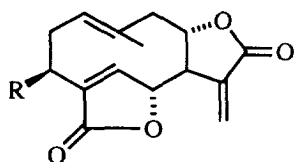
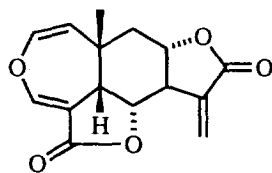
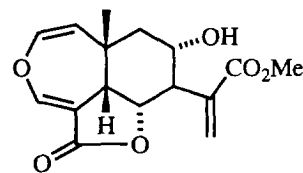
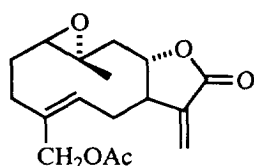
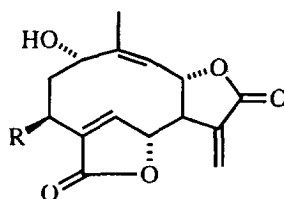
Finally, we deal with **8a** and **8b** which were closely related to mikaperiplocolide (**8c**) from *Mikania periplocifolia* [13], another member of the *M. scandens* complex. In the case of **8a** the empirical formula and comparison of the NMR spectrum (Table 1) with the NMR spectrum of **8c** [13] showed that there was no hydroxyl group on C-3; in the case of **8b** the C-3 hydroxyl group was acetylated. The biogenesis of **8a–8c** presumably involves action of a dioxygenase on a precursor of type **4** in a manner simulated chemically by the reaction of ¹O₂ with germacra-1(10), 4-dienolides.

EXPERIMENTAL

General. For sepn of mixts, HPLC with a differential refractometer was used. The columns employed were (A) a Beckman C18 (5 μ , 10 \times 250 mm) and (B) a Phenomenex Maxsil 10C8 (10 μ , 10 \times 500 mm). Retention times were measured from the solvent peak.

Plant material. Aerial parts of *Mikania ypacarayensis* Holmes and McDaniel were collected at the flowering stage in May 1989 near Ituzaingó, Corrientes province,

†The old binomial *M. trachypleura* B. L. Robins. for this species is illegitimate [8].

**1a****1b** 11 β H, 13-dihydro**2 a** R=H**b** R=OAc**c** R=OAc, 11 β H, 13-dihydro**3****4 a** R=H**b** R=OH**c** R=OAc**d** R=OMe**5 a****b** 11 β H, 13-dihydro**6****7****8 a** R=H**b** R=OAc**c** R=OH

Argentina. A voucher specimen (C. Catalán 92) is deposited in the Instituto Miguel Lillo, Tucumán.

Extraction and isolation. Flowers and leaves (245 g) were extracted with CHCl_3 at room temp. for 4 days to give 17 g of crude extract which was suspended in EtOH (150 ml) at 60°, diluted with H_2O (110 ml) and extracted successively with hexane (3×100 ml) and CHCl_3 (3×100 ml). Evapn of the hexane extract at red. pres. gave 4.5 g of residue which was not studied further. Evapn of the CHCl_3 extract gave 6.5 g of residue which was chromatographed over silica gel (300 g) using CHCl_3 with increasing amounts of EtOAc (15–45%), 148 frs being collected which were monitored by TLC.

Frs 22–33 (113 mg) were combined and processed by HPLC (using column B, MeOH– H_2O , 3:2, 2 ml min $^{-1}$). The peaks obtained were rechromatographed on column A (MeOH– H_2O , 4:3, 2 ml min $^{-1}$) to give 2.3 mg of **6** as a gum, R_f 7.5 min, and 5.3 mg of miscandenin (**5a**) as a crystalline solid, mp 240–245° (dec.), R_f 9 min, identified by comparison with authentic material.

Frs 36–43 (137 mg) were combined and chromatographed by HPLC using column B (MeOH– H_2O , 1:1, 2 ml min $^{-1}$). The peaks obtained were rechromatographed on column A (MeOH– H_2O , 1:1, 2 ml min $^{-1}$) to give 1 mg of a mixture of unidentified lactones, R_f 13.5 min, 1.2 mg of a mixture of **4b** and **7**, R_f 17.5 min, 1 mg of a mixture of **5a** and **5b**, R_f 13 min, 0.5 mg of **4c**, R_f 16 min, 1 mg of **4d**, R_f 18 min, and 1.5 mg of mikanolide, (**1a**), R_f 3.5 mg.

Frs 44–49 (754 mg) were recrystallized from heptane–EtOAc (1:4) to give 317 mg of mikanolide, mp 216–220°, identified by comparison with authentic material. Additional mikanolide could be recovered from the mother liquor.

Frs 50–59 (254 mg) were recrystallized from heptane–EtOAc (1:4). A portion of this material was purified by HPLC using column A (MeOH– H_2O , 2 ml min $^{-1}$) to give 15 mg of a mixture of **1a** and **1b**, R_f 7.5 min. Frs 60–73 (187 mg) were combined. TLC (heptane–EtOAc, 1:1) afforded 37 mg of a lactone mix-

Table 1. ^1H NMR spectra of **2c**, **4d**, **5b**, **6** and **8a,b** (500 MHz, CDCl_3)

H	2c *	4d	5b †	6 *	8a	8b
1	3.02 <i>dd</i> (11.5, 2.5)	5.13 <i>dquint</i> (12.5, 1)	4.78 <i>d</i> (8.5)	4.82 <i>br dd</i> (8, 8, 1)	4.40 <i>br d</i> (9.5)	4.67 <i>br d</i> (9.5)
2a	2.25 <i>ddd</i> (15, 2.5, 2.5)	2.65 <i>ddd</i> (13.5, 12.5, 4.5)	6.16 <i>d</i> (8.5)	6.12 <i>br d</i> (8)	2.62 <i>m</i>	2.71 <i>ddd</i> (15, 9.5, 4)
2b	1.77 <i>ddd</i> (15, 11.5, 4)	2.50 <i>ddd</i> (13.5, 2, 1.5)	—	—	2.62 <i>m</i>	2.02 <i>br dd</i> (15, 3)
3	5.73 <i>dddd</i> (4, 2.5, 1.5, 1.5)	4.54 <i>dddd</i> (4.5, 2, 1.5, 1.5)	7.51 <i>br d</i> (3)	7.47 <i>br d</i> (3)	1.81 <i>br ddd</i> (14.5, 4, 4)	5.78 <i>dddd</i> (3, 2, 1.5, 1.5)
5	7.96 <i>t</i> (1.5)	7.06 <i>t</i> (1.5)	3.47 <i>br dd</i> (7.3)	3.42 <i>br ddd</i> (7.5, 3, 1.5)	7.09 <i>t</i> (1.5)	7.15 <i>t</i> (2)
6	5.42 <i>q</i> (1.5)	5.31 <i>q</i> (1.5)	4.65 <i>dd</i> (10.5, 7)	4.94 <i>br dd</i> (10, 7.5)	5.34 <i>brq</i> (1.5)	5.42 <i>q</i> (1.5)
7	2.69 <i>br dd</i> (12.5, 10.5, 1.5)	3.27 <i>dddd</i> (8.5, 3.5, 3.5, 1.5)	1.78 <i>ddd</i> (12, 11, 11)	2.18 <i>br dd</i> (10, 7.5)	3.23 <i>dddd</i> (10, 3, 3, 1)	3.27 <i>dddd</i> (10, 3, 3, 1)
8	4.62 <i>ddd</i> (10, 10, 4.5)	4.46 <i>ddd</i> (8.5, 8, 4)	4.01 <i>ddd</i> (12, 12, 3.5)	4.14 <i>ddd</i> (11, 10, 4)	4.90 <i>t</i> (10.5)	4.89 <i>t</i> (10.5)
9a	2.07 <i>dd</i> (14.5, 10)	3.10 <i>ddd</i> (14, 8, 1.5)	2.19 <i>br dd</i> (12.5, 3.5)	1.94 <i>ddd</i> (13, 4, 1.5)	5.18 <i>br d</i> (10)	5.22 <i>br d</i> (10.5)
9b	2.01 <i>dd</i> (14.5, 4.5)	1.92 <i>dd</i> (14, 4)	1.81 <i>t</i> (12)	1.59 <i>dd</i> (13, 11.5)	—	—
11	2.95 <i>dq</i> (12.5, 7)	—	2.55 <i>dq</i> (12, 7)	—	—	—
13	1.37 <i>d</i> ‡ (7)	6.49 <i>d</i> (3.5) 5.89 <i>d</i> (3.5)	1.37 <i>d</i> ‡ (7)	6.44 <i>br d</i> (1) 5.83 <i>br s</i>	6.36 <i>dd</i> (3.5, 0.5) 5.67 <i>dd</i> (3.5, 0.5)	6.39 <i>br d</i> (3) 5.69 <i>d</i> (3)
14‡	1.16 <i>s</i>	1.61 <i>br s</i>	1.31 <i>s</i>	1.30 <i>s</i>	1.83 <i>br s</i>	1.83 <i>d</i> (1.5)
Ac‡	2.15 <i>s</i>			3.70 <i>s</i> (OMe)		2.20 <i>s</i>

*In acetone- d_6 .†From mixture with **5a**.

‡Intensity three protons.

ture which was processed by HPLC using column A ($\text{MeOH-H}_2\text{O}$, 2 ml min^{-1}) to give 8 mg of crystalline **3**, R_f 7.5 min, 4 mg of crystalline scandenolide (**2b**), R_f 8.4 min, both identified by comparison with authentic material, and 2 mg of a complex mixture, R_f 11.4 min.

Frs 74–77 (71 mg) were combined and rechromatographed over silica gel (10 g) using heptane–EtOAc 1:1, 25-ml frs being collected. Frs 8–10 (15 mg) of the rechromatogram were scandenolide (**2b**). Frs 12–14 (9 mg) were deoxymikanolide (**2a**) identified by comparison with authentic material, frs 18–20 (5 mg) were crude **8a** and frs 22–25 were crude **8b**.

Several recrystallizations of frs 78–92 (366 mg) of the original chromatogram from heptane–EtOAc afforded 32 mg of pure **2a**. The residue from the mother liquors contained a mixture of **2a** and **2c**. Frs 93–109 (223 mg) of the original chromatogram on repeated recrystallization from heptane–EtOAc afforded 5 mg of pure **2c** as a crystalline solid, mp 282–285°, identified by mass and ^1H NMR spectra. More, slightly impure **2c** could be recovered from the mother liquors. Frs 110–148 (223 mg)

of the original chromatogram on repeated processing by HPLC afforded only complex lactone mixtures.

3 β -Methoxyisabelin (4d). Gum; PCI-MS m/z (rel. int.) 291 $[\text{M} + \text{H}]^+$ (100); ^1H NMR spectrum in Table 1.

11 β H,13-dihydromiscandenin (5b). Obtained only as the major component of a mixt. also containing **5a**; PCI-MS m/z (rel. int.) 277 $[\text{M} + \text{H}]^+$ of **5** (100), 275 (25, $[\text{M} + \text{H}]^+$ of **5a** (25); ^1H NMR spectrum in Table 1.

Methyl ester 6. Gum; PCI-MS m/z (rel. int.): 307 $[\text{M} + \text{H}]^+$ (100); IR ν^{film} cm^{-1} : 1754, 1657; ^1H NMR spectrum in Table 1.

3-Dehydroxymikaperiplocolide (8a). Mp 235–239°; PCI-MS m/z (rel. int.): 277 $[\text{M} + \text{H}]^+$ (100), 259 (88); IR ν^{KBr} cm^{-1} : 3450, 1750, 1650; ^1H NMR spectrum in Table 1.

3-Acetoxymikaperiplocolide (8b). Mp 237–239°; IR ν^{KBr} cm^{-1} : 3450, 1750, 1650. The sample was lost in an attempt to determine the MS.

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