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GERMACRANOLIDES OF *MIKANIA CORDATA*

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Key Word Index—*Mikania cordata*; Compositae; germacranolide; sesquiterpenoid; 11*β*-hydroxy-13-chloromikanolide; diepoxygermacranolide.

Abstract—Germacranolides from *Mikania cordata* grown in the Philippines, were investigated and four new compounds, including a chlorinated germacranolide, and mono- and di-epoxygermacranolides having one or two lactone rings, were isolated and their structures characterized.

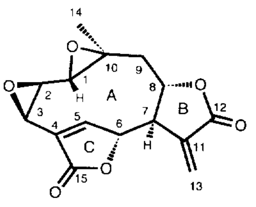
INTRODUCTION

Mikania cordata (Burm. f.) B. L. Robinson (*M. scandens* (L.) Will.) is commonly called river vine in the Philippines. The plant has been used for medicinal purpose, such as sore eyes, coughs, infections of stomach and intestines, itching and for snake and scorpion bites [1]. One of the constituents, scandenolide, was reported to exhibit an antiinflammatory property [2]. Many studies on the constituents have revealed the presence of germacranolide-type sesquiterpenoids, mikanolide, dihydromikanolide [3, 4], scandenolide, dihydroscandenolide, miscandenin [4] and deoxymikanolide [4, 5], along with other constituents including sterols, triterpenoids, and flavonoids. This paper deals with the isolation of four new germacranolides (**5**–**8**) besides known ones (**1**–**4**), from the leaves of *M. cordata* collected in the Philippines.

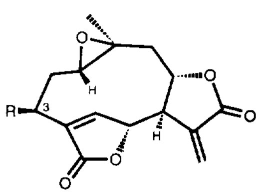
RESULTS AND DISCUSSION

The isolation of the eight germacranolides (**1**–**8**) was carried out principally according to the procedure described in ref. [4]. Four compounds were identified as mikanolide (**1**), dihydromikanolide (**2**), deoxymikanolide (**3**) and scandenolide (**4**) by comparison of the spectral data with those in the literature.

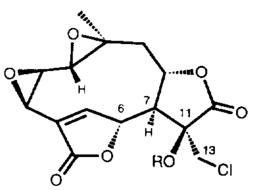
Compound **5** afforded a $[M + NH_3]^+$ peak at m/z 359 (100%) as well as a $[M + 2 + NH_3]^+$ peak at m/z 361 (33%). Based on the positive flame reaction with copper, **5** seemed to be a halogenated compound. The elemental analysis also suggested a molecular formula containing a chlorine atom, $C_{15}H_{15}O_7Cl$. Signals in the ^1H NMR spectrum from a germacrane ring observed with almost the same chemical shifts as in **1** and **2** and two sets of five-membered lactone rings were suggested by the ^{13}C NMR spectrum. Therefore, **5** was considered to retain the same germacrane framework as in **1** and **2**. As for the characteristic feature in the NMR spectrum of **5**, two doublet signals assignable to a methylene group (δ4.18, *d*, $J = 11$ Hz; 4.30 *d*, $J = 11$ Hz) and a broad signal at δ10.16 from a hydroxyl proton were observed. In the ^{13}C NMR spectrum, corresponding methylene and carbinyl carbon signals were observed at δ44.4 (*t*) and 77.0 (*s*), respectively, and chlorine and hydroxyl groups were considered to be located at either C-11 or C-13. Employing the usual acetylation procedure, **5** afforded an acetate (**5a**), in which the corresponding methylene protons were detected with almost the same chemical shifts at δ4.25 and 4.34. The hydroxyl group was thus assigned to C-11, and consequently, the chlorine atom to C-13. Orientation of the



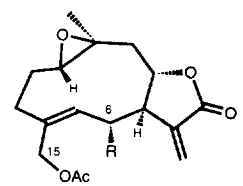
1
2 (11, 13-dihydro)



3 : R=H
4 : R=OAc
6 : R=OH



5 : R=H
5a : R=Ac



7 : R=H
8 : R=OH

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11-hydroxyl group was determined to be β, based on evidence that H-6 showed an upfield shift in **5a** (-0.7 ppm) owing to the introduction of the acetyl group. Cross-peaks between H-13a/H-7 and H-13b/H-6 in **5a** were also consistent with the β-orientation of the hydroxyl group.

Compound **6** afforded a $[M + 1]^+$ peak at *m/z* 293.104, suggesting the molecular formula to be $C_{15}H_{16}O_6$, 42 mu less than **4**. In the ^1H NMR spectrum, each signal showed similar coupling pattern to **4**, although the upfield shift was observed in the signal assignable to the carbinyl methine proton (H-3; -0.50 ppm). In the ^{13}C NMR spectrum, C-3 was shifted upfield (-1.8 ppm), and C-2 and C-4 were downfield ($+4.3$ ppm and $+5.3$ ppm) in comparison with those of **4**. The upfield and downfield shifts in the ^{13}C NMR spectrum were considered to be a result of the absence of the acetyl group at the C-3 hydroxyl, and **6** was characterized as deacetylscandenolide.

The molecular formula of **7** was determined to be $C_{17}H_{22}O_5$ based on the CI-mass spectrum ($[M + 1]^+$ at *m/z* 307.155). The presence of one acetoxyl group was suggested by the signals at δ_H 2.05 and δ_C 20.6, 170.6. No C-6 carbinyl proton signal, observed at δ5.67–6.18 in **5** and **6**, was present in the ^1H NMR spectrum of **7**. Connection of H-7 (δ3.10) to H-5 (δ5.62) via C-6 methylene protons at δ2.16 and 2.81 was indicated in the ^1H-^1H COSY spectrum, suggesting C-6 to be a methylene group. The acetoxyl group was located at the primary hydroxyl at C-15 based on the presence of geminal coupling signals of hydroxylmethylene protons in the lower field at δ4.70 and 4.91 (*d*, $J = 12$ Hz each).

Compound **8** afforded a $[M + Na]^+$ peak at *m/z* 345.133, suggesting the molecular formula, $C_{17}H_{22}O_6$ with one more oxygen than in **7**. The NMR data for ring B and the 1,10-epoxy group were similar to those of **7**. In the ^1H-^1H COSY spectrum, however, H-7 (δ3.28) was linked to H-5 (δ5.75) via a hydroxymethine proton at δ4.71, indicating that the hydroxyl group was located at C-6. Orientation of the hydroxyl group was assigned to be α, based on the coupling constants of H-6 ($J_{6,7} = 10$ Hz and $J_{6,5} = 9$ Hz).

Table 1. ^{13}C NMR spectral data of compounds **1**, **4**–**7** and **8** (δ ppm, in pyridine-d_5, 100 MHz)

C	**1**	**4**	**5**	**6**	**7**	**8**
1	58.3 *d*	58.0 *d*	58.7 *d*	59.0 *d*	61.5 *d*	61.6 *d*
2	56.1 *d*	28.7 *t*	55.4 *d*	33.0 *t*	25.1 *t*	24.5 *t*
3	51.0 *d*	66.4 *d*	51.3 *d*	84.6 *d*	30.8 *t*	29.9 *t*
4	130.8 *s*	132.4 *s*	130.6 *s*	137.7 *s*	135.6 *s*	135.1 *s*
5	148.8 *d*	146.7 *d*	149.4 *d*	147.3 *d*	129.5 *d*	135.0 *d*
6	83.8 *d*	82.1 *d*	77.2 *d*	82.7 *d*	32.4 *t*	70.6 *d*
7	50.5 *d*	49.6 *d*	52.2 *d*	50.5 *d*	43.9 *d*	50.3 *d*
8	77.2 *d*	77.7 *d*	78.4 *d*	78.6 *d*	79.5 *d*	77.9 *d*
9	43.5 *t*	42.6 *t*	43.0 *t*	43.8 *t*	45.0 *t*	44.8 *t*
10	57.4 *s*	56.6 *s*	57.6 *s*	57.0 *s*	56.9 *s*	57.0 *s*
11	138.4 *s*	135.9 *s*	77.0 *s*	138.0 *s*	139.8 *s*	137.7 *s*
12	167.9 *s*	167.8 *s*	173.5 *s*	168.1 *s*	169.2 *s*	169.6 *s*
13	122.6 *t*	122.6 *t*	44.6 *t*	122.3 *t*	122.6 *t*	125.4 *t*
14	21.4 *q*	19.7 *q*	21.3 *q*	20.5 *q*	21.5 *q*	21.3 *q*
15	170.9 *s*	169.3 *s*	171.4 *s*	171.1 *s*	61.4 *t*	62.7 *t*
OAc	169.5 *s*				170.6 *s*	170.6 *s*
	19.2 *q*				20.6 *q*	20.5 *q*

Four new germacranolides were isolated as minor components from the plant materials collected at Mt. Makiling, along with four previously known compounds from the same species. The presence of a chlorine atom in **5** may be a result of the environmental conditions in the area of the collection.

EXPERIMENTAL

General. Mps: uncorr. ^1H NMR: 400 MHz and ^{13}C NMR: 100 MHz, pyridine-d_5, with TMS as int. standard. For TLC, silica gel and Sephadex LH-20 CC, the following solvent systems were used, 1: C_6H_6–Me_2CO (5:2), 2: $CHCl_3$–MeOH–H_2O (7:2:1, bottom layer), 3: $CHCl_3$–MeOH (15:1) for (LH-20), 4: EtOAc–hexane (3:1). For reversed phase CC (YMC–gel or Fuji-gel), the following solvent systems were used, 5: MeOH–H_2O (7:3), 6: MeCN–H_2O (3:2). Spray reagent: 10% H_2SO_4.

Plant material. The leaves of *Mikania cordata* (Burm. f.) B. L. Robinson were collected in Mt. Makiling, Los Baños, Laguna in January 1988 and were authenticated at the National Museum in Manila. Voucher specimens are deposited at the Herbaria of the National Museum, the Institute of Chemistry, University of the Philippines at Los Baños (voucher No. 57479) and the UST Research Center for the Natural Sciences (voucher No. 887).

Extraction and isolation. Ground air-dried leaves of *M. cordata* (21.5 kg) were extracted with $CHCl_3$ (215 l). The extract was concd *in vacuo* to yield a crude extract (2.1 kg). Four batches of 300 g each of crude extract were dissolved in 4 × 900 ml hot MeOH, then mixed 4 × 1.8 l 4% $Pb(OAc)_2$ soln. The mixture was shaken and allowed to stand for 2 days, then filtered over Celite. The entire filtrate was concd *in vacuo* to 1.2 l yellowish brown suspension. The concd extract was partitioned with $CHCl_3$ (3 × 1 l), followed by *n*-BuOH (3 × 800 ml). The $CHCl_3$ layer was dried with Na_2SO_4. Both extracts were separately concd *in vacuo* to give yellowish brown syrups ($CHCl_3$ extract, 56.6 g; BuOH extract, 25.1 g). The $CHCl_3$ and BuOH extracts were subjected to repeated CC to afford **1** (18 mg), **2** (18 mg), **3** (37 mg), **4** (67 mg), **5** (31 mg), **6** (20 mg), **7** (65 mg) and **8** (46 mg).

11β-*Hydroxy*-13-*chloromikanolide* (**5**). Mp 239–243° (dec.); $[\alpha]_D^{23}$ + 27.9° (MeOH; *c* 1.0). UV λ_{max}^{MeOH} nm (ε): 212 (12,500). CI-MS (NH_3) *m/z* (rel. int.): 359 $[M + NH_3, C_{15}H_{15}O_7Cl + NH_3]^+$ (100), 360 $[M + 1 + NH_3]^+$ (15), 361 $[M + 2 + NH_3]^+$ (33). Elem. anal. (Found: C, 52.71; H, 4.38. $C_{15}H_{15}O_7Cl$ requires: C, 52.71; H, 4.42%). When **5** was put on the tip of a copper wire and burned in the bunzen burner, a green flame was observed (Beilstein test).

11β-*Hydroxy*-13-*chloromikanolide acetate* (**5a**). Compound **5** (10 mg) was dissolved in pyridine (0.2 ml) and Ac_2O (1 ml) and stirred overnight at room temp. The

Table 2. ^{1}H NMR spectral data of compounds **1**, **4**–**8** and **9** (δ in pyridine-d_5, 400 MHz)

H	**1**	**4**	**5**	**5a**	**6**	**7**	**8**
1	3.48 *s*	3.04 *dd* (12, 2)	3.47 *d* (1)	3.51 *br s*	3.53 *dd* (11, 12)	2.96 *dd* (10, 2)	2.96 *dd* (10, 2)
2a	3.51 *d* (3)	1.83 *ddd* (15, 12, 14)	3.51 *dd* (3, 1)	3.50 *d* (3)	2.03 *m*	1.56 *m*	1.66 *m*
2b		2.38 *ddd* (15, 3, 2)			2.53 *ddd* (14, 2.4, 2.0)	2.07 *m*	2.11 *m*
3a	4.25 *ddd* (3, 2, 1)	5.84 *dddd* (4, 3, 2, 1)	4.26 *dt* (3, 2)	4.21 *brd* (3)	5.34 *dtd* (4, 2.4, 1.5)	2.28 *m*	2.31 *m*
3b						2.40 *m*	2.45 *m*
5	7.82 *dd* (2, 1)	7.68 *dd* (2, 1)	7.88 *t* (2)	7.74 *br s*	8.17 *d* (2, 1)	5.62 *br t* (8)	5.75 *d* (9)
6a	5.53 *ddd* (3, 2, 1)	5.48 *td* (2, 1)	6.18 *q* (2)	5.48 *m*	5.67 *td* (2.0, 1.5)	2.16 *m*	4.71 *dd* (10, 9)
6b						2.81 *m*	
7	3.53 *dq* (8, 3)	3.49 *dtd* (8, 3, 2)	3.41 *dd* (9, 2)	3.37 *dd* (9, 2)	3.70 *dtd* (9, 3, 2)	3.10 *m*	3.28 *m*
8	5.08 *ddd* (11, 8, 5)	4.65 *ddd* (10, 8, 5)	5.26 *ddd* (11, 9, 4)	5.37 *m*	5.07 *ddd* (11, 9, 4)	4.67 *m*	4.56 *m*
9a	2.23 *dd* (14, 11)	2.18 *dd* (15, 10)	2.21 *dd* (14, 11)	2.28 *d* (10)	2.22 *dd* (14, 11)	2.10 *dd* (14, 11)	2.20 *d* (8)
9b	2.30 *dd* (14, 5)	2.21 *dd* (15, 5)	2.40 *dd* (14, 4)	2.28 *d* (10)	2.34 *dd* (14, 4)	2.23 *dd* (14, 5)	2.20 *d* (8)
13a	6.41 *br d* (3)	6.46 *dd* (3, 1)	4.18 *d* (11)	4.25 *d* (11)	6.48 *br d* (3)	6.36 *br d* (3)	6.54 *br d* (2)
13b	5.94 *br d* (3)	6.01 *dd* (3, 1)	4.30 *d* (11)	4.34 *d* (11)	5.97 *br d* (3)	5.66 *br d* (3)	6.52 *br d* (2)
14	1.23 *s*	1.24 *s*	1.25 *s*	1.19 *s*	1.42 *s*	1.35 *s*	1.46 *s*
15						4.70 *br d* (12) 4.91 *br d* (12)	4.88 *d* (12) 5.12 *d* (12)
OAc		2.20 *s*		2.17 *s*		2.05 *s*	1.98 *s*
Others			10.16 *br s* (11-OH)				4.80 *br s* (6-OH)

Coupling constants (*J* in Hz) given in parentheses.

mixt. was concd with toluene *in vacuo* to dryness, and purified by prep. TLC, eluting with $CHCl_3$–MeOH (20:1), to give **5**-acetate (**5a**, 5 mg).

3β-*Hydroxydeoxymikanolide* (**6**). Solid, $[\alpha]_D^{26}$ + 45.1° (pyridine; *c* 1.1). UV λ_{max}^{MeOH} nm (ε): 206 (11,00). CI-MS (NH_3) *m/z*: 293.104, $C_{15}H_{16}O_6$ + H requires 293.102; 235 (31), 163 (40), 112 (100), 97 (47), 84 (77).

Cordatolide (**7**). Solid, $[\alpha]_D^{26}$ + 109.9° (pyridine; *c* 1.1), UV λ_{max}^{MeOH} nm (ε): 205 (6800), CI-MS (NH_3) *m/z*: 307.155, $C_{16}H_{18}O_7$ + H requires 307.154; 247 (77), 229 (100), 201 (22), 126 (28).

6α-*Hydroxycordatolide* (**8**). Solid, $[\alpha]_D^{29}$ + 128.6° (pyridine; *c* 1.25). FAB-MS *m/z*: 345.133, $C_{17}H_{22}O_6$ + Na requires 345.132; 115 (36), 91 (33), 81 (30), 79 (45), 43 (100).

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