Phytochemistry, Vol. 28, No. 2, pp. 527–530, 1989.
Printed in Great Britain.

0031–9422/89 \$3.00 + 0.00
© 1989 Pergamon Press plc.

GERMACRANOLIDES FROM *MIKANIA* SPECIES

V. CASTRO,* J. JAKUPOVIC and F. BOHLMANN

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, F.R.G.; * University of Costa Rica, Department of Chemistry, San Jose, Costa Rica

(*Received in revised form* 17 *July* 1987)

Key Word Index—*Mikania holwayana*; *M. cordifolia*; Compositae; sesquiterpene lactones; germacranolides; melampolides.

Abstract—The investigation of the aerial parts of *Mikania holwayana* afforded in addition to known compounds six new germacranolides while a reinvestigation of *M. cordifolia* gave several known melampolides and two new ones. The biogenetic relationships are discussed briefly.

INTRODUCTION

From the large genus *Mikania* (Compositae, tribe Eupatorieae) with *ca* 300 species, so far *ca* 20 species have been investigated chemically [1, 2]. The overall picture is not very uniform as most species contain sesquiterpene lactones but several only provide diterpenes. In continuation of our investigations of representatives of the tribe Eupatorieae we now have studied *Mikania holwayana* B. L. Robins. and reinvestigated *M. cordifolia* (L.F) Willd. The results are discussed in this paper.

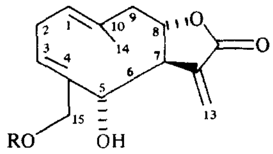
RESULTS AND DISCUSSION

Mikania holwayana is distributed in Central America. A sample was collected in Costa Rica (near La Palma, San Ramon). The extract of the aerial parts gave a complex mixture of 12,8α-germacranolides (**1a**–**1c**, **2a**, **2b** and **6**). The ^{1}H NMR spectrum of the mixture of **1a** and **1b**, which could not be separated and of **1c** (Table 1) indicated that these lactones differed only in the nature of the ester residue. Spin decoupling showed that 8,12-lactones with two further oxygen functions were present. The pair of broadened doublets at δ 4.73 and 4.64 indicated ester residues at C-15 and the chemical shifts of H-3 and H-5 required that a hydroxy group was at C-5. The stereochemistry was determined by NOE difference spectroscopy. Clear effects were observed between H-14, H-5 (9%), H-8 (8%) and H-2β (6%), between H-8, H-6β (6%), H-9β (5%) and H-14 (5%), between H-5, H-2β (4%), H-14 (5%) and H-6β (6%), between H-15 and H-3 (4%) as well as between H-1, H-7 (10%), H-2α (3%), H-3 (2%) and H-9α (10%). Inspection of Dreiding models showed that these data required the proposed configuration in a conformation with both C-14 and C-15 above the plane.

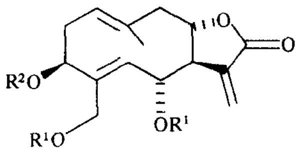
The ^{1}H NMR spectrum of **2a** and **2b** (Table 1), which could not be separated, showed highly broadened signals even at 110° in deuteriobenzene. The typical signals of the ester residue indicated that again a mixture of an isovalerate and a 2-methyl-butyrate was present. From the molecular formula ($C_{20}H_{28}O_6$) a lactone with two oxygen functions was very likely. Spin decoupling led again to 8,12-lactones and broadened signals at δ 4.41, 4.22, 4.10 and 4.08 indicated in addition to the acyloxy groups two further hydroxy groups. However, no clear assignment of all signals was possible and therefore we tried to get derivatives. Reaction of **2a**/**b** with acetone in the presence of sulphuric acid afforded the acetonides **4a** and **4b**. The ^{1}H NMR spectrum (Table 1) of this inseparable mixture now showed well resolved signals which could be assigned by spin decoupling. The couplings observed already led to the proposed configuration which was further ensured by NOE difference spectroscopy. Clear effects were obtained between H-7, H-5 (4%) and H-14 (6%), between H-8, H-1 (6%) and H-6 (15%), between H-6, H-15 (6%) and H-8 (5%) as well as between one of the acetonide methyls and H-3 (12%). Thus the structure and the stereochemistry of **4a**/**b** were settled. However, from the chemical shifts of H-15 in the spectrum of **2a**/**b** an acyloxy rather than free hydroxy group at C-15 was more likely. This assumption was confirmed by the downfield shift of H-3 and H-6 in the spectrum of the acetates of **2a**/**b** (**3a**/**b**) where at −40° the signals of the main conformer could be assigned by spin decoupling. Accordingly, the acetonide formation was accompanied by transesterification. In addition to **4a**/**b** the reaction of **2a**/**b** in acetone with sulphuric acid further afforded the esters **5a**/**b** as followed from the ^{1}H NMR spectrum (Table 1). Spin decoupling allowed the assignment of all signals and the observed NOE's led to the proposed stereochemistry especially by effects between H-8, H-6 (5%), H-1 (8%) and H-5 (12%) as well as between H-15 and H-3 (15%). Obviously, **5a**/**b** are formed by transesterification followed by allylic rearrangement. The ^{1}H NMR data are very close to those of the corresponding 3-desacyloxy derivative from an *Austroliabum* species [3].

The ^{1}H NMR spectrum of **6** (Table 1) indicated the presence of an 8,12-lactone with an isovaleryloxy group at C-15. Spin decoupling further showed that the signal δ 2.65 *dd* was due to H-5. Accordingly, a 4,5-epoxide was very likely. Many signals resemble those of a corresponding epoxy lactone from a *Schistostephium* species [4] and those of the isomeric epoxides obtained by epoxidation of desacetyllaurenobiolide [4].

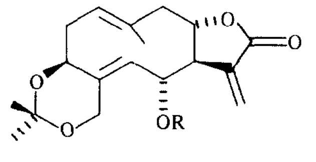
M. cordifolia Willd. has been studied previously [1, 5]. We now have investigated a sample which was collected in Costa Rica. The polar fractions afforded the melampol-



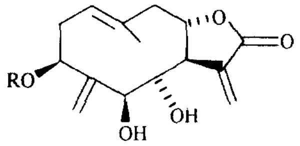
	1a	**1b**	**1c**
R	*i*Val	MeBu	*i*Bu



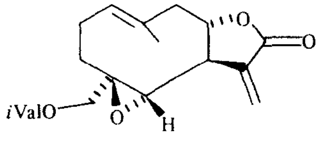
	2a	**2b**	**3a**	**3b**
R¹	*i*Val	MeBu	*i*Val	MeBu
R²	H	H	Ac	Ac



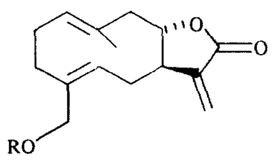
	4a	**4b**
R	*i*Val	MeBu



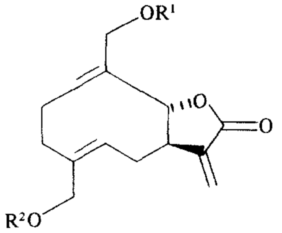
	5a	**5b**
R	*i*Val	MeBu

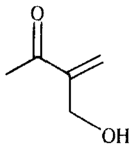
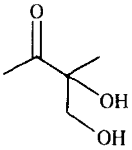
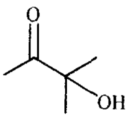
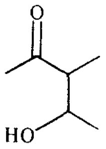
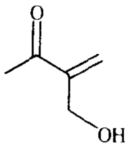


6



7



	8a	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**
R¹	H	H	H	H	H	H	H	Ac
R²	H	*i*Bu	Meacr					

ides **8a**–**8e** [6] and **8f**–**8h**. The ^{1}H NMR spectra of the latter three lactones (Table 2) were close to those of **8a**–**8e** where the stereochemistry was determined by the observed NOE's. If the chemical shifts of H-14 in the spectra of **8d** and **8h** were compared it was obvious that the latter was the corresponding 14-*O*-acetate.

The signals of the ester residue of **8g** indicated a 3-hydroxy-2-methylbutyrate as followed from spin decoupling while the signals of the lactone moiety were close to those of **8b**. The lactones **8a**–**8f** recently were isolated from the same species [7]. We thank Prof. J. B. Harborne for providing us with the data prior to publication. The data of **8f** nicely agree with ours.

If the structures of the germacranolides isolated from the two *Mikania* species are inspected it seems to be very likely that all lactones are derived from the common precursor **7**. Epoxidation would lead to **6** and oxidation with allylic rearrangement to **1a**/**b**. Double allylic oxidation would give **2a**/**b**. Similarly, allylic oxidation of **7** accompanied with double bond isomerization would lead

Table 1. ^{1}H NMR spectral data of compounds **1a–1c**, **2a/b**, **3a/b**, **4a/b**, **5a/b** and **6** ($CDCl_3$, 400 MHz, δ-values)

H	**1a/b** (57°)	**1c**	**2a/b** (C_6D_6, 110°)	**3a/b** (40°)	**4a/b**	**5a/b**	**6** (57°)
1	5.29 *br d*	5.27 *br d*	4.82 *br m*	5.23 *br dd*	5.15 *br d*	5.40 *m*	5.46 *br dd*
2α	2.63 *br dd*	2.64 *br dd*	2.37 *m*	3.05 *m*	2.67 *ddd*	2.68 *ddd*	2.42 *m*
2β	3.02 *br ddd*	3.02 *br ddd*	2.06 *m*	2.10 *m*	2.49 *br d*	2.38 *br d*	2.25 *m*
3	6.17 *br dd*	6.17 *br dd*	4.22 *br s*	5.44 *br dd*	4.68 *br d*	5.75 *br d*	2.42 *m* 1.22 *m*
5	4.38 *br dd*	4.36 *br d*	5.00 *br m*	5.04 *br d*	4.48 *dddd*	4.61 *br d*	2.65 *dd*
6α	2.09 *ddd*	2.07 *ddd*	—	—	—	—	2.15 *m*
6β	1.76 *ddd*	1.78 *ddd*	4.10 *br dd*	5.35 *br dd*	5.00 *dd*	3.91 *br ddd*	1.72 *m*
7	2.73 *m*	2.71 *m*	2.51 *br m*	2.91 *m*	2.94 *m*	3.04 *m*	2.92 *m*
8	4.24 *ddd*	4.26 *ddd*	3.57 *br m*	4.26 *br ddd*	3.96 *ddd*	4.37 *ddd*	4.41 *ddd*
9α	2.01 *dd*	2.03 *dd*	1.77 *br m*	1.91 *br dd*	1.92 *dd*	1.82 *dd*	2.10 *dd*
9β	2.72 *br d*	2.72 *br d*	2.79 *br d*	2.97 *br d*	3.03 *br dd*	2.92 *br dd*	2.88 *br dd*
13	6.34 *d*	6.35 *d*	6.42 *br s*	6.42 *d*	6.38 *d*	6.51 *br s*	6.36 *d*
13′	5.67 *d*	5.67 *d*	6.10 *br s*	5.97 *d*	5.90 *d*	6.11 *br s*	5.61 *d*
14	1.79 *br s*	1.80 *br s*	1.29 *br s*	1.72 *br s*	1.74 *br s*	1.70 *br s*	1.76 *br s*
15	4.73 *br d*	4.73 *br d*	4.41 *br m*	4.79 *br d*	4.60 *dd*	6.20 *br s*	4.54 *d*
15′	4.64 *br d*	4.60 *br d*	4.05 *br m*	4.71 *br d*	4.10 *ddd*	5.30 *br s*	3.94 *d*
OR	2.19 *d* 2.37 *tq*	2.55 *qq*	2.06 *m* 2.28 *tq*	2.22 *d* 2.37 *m*	2.22 *dd* 2.37 *m*	2.29 *d* 2.47 *tq*	2.29 *d*
	2.09 *m* 1.70 *m*	1.19 *d*	0.91 *d* 1.68 *m*	2.10 *m* 1.70 *m*	2.17 *dd* 1.70 *m*	2.17 *m* 1.77 *m*	2.15 *m*
	0.99 *d* 1.50 *m*		1.40 *m*	0.97 *d* 1.50 *m*	2.10 *m* 1.48 *m*	1.01 *d* 1.53 *m*	1.00 *d*
	1.18 *d*		1.09 *d*	1.18 *d*	0.96 *d* 1.15 *d*	2.28 *d* 1.24 *d*	
	0.93 *t*		0.88 *t*	0.92 *t*	1.41 *s* 0.91 *t*	(OH) 0.96 *t*	
					1.38 *s*		
					Acetonid		

J[Hz]: Compounds **1a–1c**: 1,2β = 11; 2α,2β = 6α,6β = 9α,9β = 14; 2α,3 = 9; 2β,3 = 4.5; 5,6α = 6β,7 = 8,9α = 12; 5,6β = 3; 6α,7 = 2; 7,8 = 8,9β = 4; 7,13 = 3; 7,13′ = 2.5; 15,15′ = 12.5; compounds **2a**, **2b**, **3a**, **3b**: 1,2α = 1,2β = 9; 2α,3 = 2β,3 = 8; 5,6 = 6,7 = 10; 7,8 = 8,9β = 4; 7,13 = 2.8; 7,13′ = 2.5; 8,9α = 11; 9α,9β = 14; 15,15′ = 13; compounds **4a** and **4b**: 1,2α = 12; 2α,2β = 15; 2α,3 = 5.5; 3,5 = 5,15 = 5,15′ = 1; 3,15′ = 2; 5,6 = 6,7 = 10; 7,8 = 8,9β = 4.5; 7,13 = 2.8; 7,13′ = 2; 8,9α = 11; 9α,9β = 13; 15,15′ = 14; compounds **5a/b**: 1,2α = 10.5; 2α,2β = 14; 2α,3 = 5; 5,6 = 2; 6,7 = 10; 6,OH = 3; 7,8 = 2; 8,9α = 11.5; 8,9β = 5; 9α,9β = 13; compound **6**: 1,2α = 1,2β = 8; 5,6α = 11; 5,6β = 4; 7,8 = 8,9β = 4; 7,13 = 3; 7,13′ = 2.5; 8,9α = 11; 9α,9β = 12; OCOR: *i*Bu: 2,3 = 2,4 = 7; *i*Val: 2,3 = 2,5 = 7.

Table 2. ^{1}H NMR spectral data of compounds **8g** and **8h** ($CDCl_3$, 400 MHz, δ-values)

H	**8g**	**8h**
1	5.62 *br dd*	5.61 *br dd*
5	5.56 *br dd*	5.54 *br dd*
6	2.80 *m*	2.82 *m*
6′	2.35 *m*	2.30 *m*
7	2.83 *m*	2.82 *m*
8	3.86 *ddd*	3.89 *ddd*
9	2.74 *dd*	2.72 *dd*
9′	2.49 *dd*	2.51 *dd*
13	6.24 *d*	6.24 *d*
13′	5.55 *d*	5.56 *d*
14	4.15 *br s*	4.59 *br d* 4.53 *br d*
15	4.65 *br d*	4.73 *br d*
15′	4.47 *br d*	4.54 *br d*
OR	3.88 *dq*	6.23 *dt*
	2.47 *dq*	5.82 *dt*
	1.23 *d*	4.33 *br s*
	1.18 *d*	2.08 *s* (OAc)

J[Hz]: 1,2 = 1,2′ = 8; 5,6 = 5; 5,6′ = 11; 7,8 = 9; 7,13 = 3.5; 7,13′ = 3; 8,9 = 8,9′ = 3.5; 9,9′ = 15; 15,15′ = 12; (compound **8h**: 14,14′ = 12.5; compound **8g**: OR: 2,3 = 2,5 = 3,4 = 7; compound **8h**: OR: 3,3′ 3,4 = 3′,4 = 1.5).

to **8a–8h** The altered configuration of the 1(10)-double bond seems to be typical for germacranolides oxygenated at C-15.

EXPERIMENTAL

The air-dried plant material (collected in Costa Rica, near La Palma, San Ramon, vouchers deposited in the National herbarium of Costa Rica) was extracted with a mixture of $MeOH–Et_2O$–petrol (1 : 1 : 1). The extracts obtained were treated with MeOH and the soluble parts were separated by CC (silica gel) and further by TLC (silica gel, PF 254) or HPLC (RP 8, *ca* 100 bar, flow rate *ca* 3 ml/min). The extract of *M. holwayana* (500 g, voucher 118843) gave by CC two polar fractions (1 and 2) (Et_2O–MeOH, 19:1 and Et_2O–MeOH, 7:3). TLC (Et_2O) of fraction 1 afforded two bands (1/1 and 1/2). HPLC ($MeOH–H_2O$, 3:2) of the more polar band (1/2) afforded 35 mg **1a/b** (R_t 8.5 min) and 12 mg **1c** (R_t 7.0 min). Repeated TLC of fraction 1/1 (Et_2O–petrol, 11:9) gave 0.5 mg **6** (R_f 0.17). Repeated TLC of fraction 2 (Et_2O) gave 30 mg **2a/b** (R_f 0.13).

The extract of *M. cordifolia* (1 kg, voucher 116211) was treated as above. CC gave two polar fractions (1: Et_2O–MeOH, 19:1 and 2: Et_2O–MeOH, 7:3). TLC of fraction 1 gave 5 mg **8c** and 7 mg **8b**. Flash chromatography of fraction 2 gave two fractions (2/1: Et_2O, 2/2: Et_2O–MeOH, 3:1). TLC of fraction 2/1 ($Et_2O–CH_2Cl_2$–MeOH, 79:20:1, three developments) gave 11 mg **8h** (R_f 0.65), 44 mg **8e** and 46 mg **8f** (R_f 0.48). HPLC of fraction 2/2 ($MeOH–H_2O$, 3:2) afforded 267 mg **8d** (R_t 2.6 min), 1 mg **8g** (R_t 2.3 min) and 5 mg **8a** (R_t 2.0 min). Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

5α-Hydroxy-15-isovaleryloxy and [2-methylbutyryloxy]-germacra-1 (10)E,3Z,11(13)-*trien*-12,8*α-olide* (**1a/b**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone), 1740 (CO_2R); MS *m/z* (rel. int.): 348.194 $[M]^+$ (2.5) (calc. for $C_{20}H_{28}O_5$: 348.194), 246 $[M-RCO_2H]^+$ (8), 228 $[246-H_2O]^+$ (7.5), 190 (95), 85 $[RCO]^+$ (41), 57 $[85-CO]^+$ (100).

5α-hydroxy-15-isobutyryloxygermacra-1(10)E,3Z,11(13)-*trien*-12,8*α-olide* (**1c**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone), 1740 (CO_2R); MS *m/z* (rel. int.): 334.178 $[M]^+$ (6) (calc. for $C_{19}H_{26}O_5$: 334.178), 246 $[M-RCO_2H]^+$ (17), 228 $[246-H_2O]^+$ (20), 190 (100), 71 $[RCO]^+$ (94); $[\alpha]_D^{24}$ −7 ($CHCl_3$; *c* 0.11).

3β,6α-Dihydroxy-15-isovaleryloxy and [2-methylbutyryloxy]-germacra-1(10)E,4E,11(13)-*trien*-12,8*α-olide* (**2a/b**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1760 (γ-lactone), 1725 (CO_2R); MS *m/z* (rel. int.): 364.189 $[M]^+$ (0.7) (calc. for $C_{20}H_{28}O_6$: 364.189), 262 $[M-RCO_2H]^+$ (8), 244 $[262-H_2O]^+$ (47), 216 $[244-CO]^+$ (100), 215 $[244-CHO]^+$ (83), 85 $[RCO]^+$ (49), 57 $[85-CO]^+$ (88). To 10 mg **2a/b** in 5 ml Me_2CO containing 1 drop of conc. H_2SO_4 10 mg dry $CuSO_4$ was added. After 2 hr TLC (Et_2O–petrol, 11:9) gave 2 mg **4a/b** (R_f 0.38) and 2.5 mg **5a/b** (R_f 0.32).

4a/b: Colourless gum; MS *m/z* (rel. int.): 404 $[M]^+$ (1), 346 $[M-Me_2CO]^+$ (6), 244 $[346-RCO_2H]^+$ (6), 85 $[RCO]^+$ (76), 57 $[85-CO]^+$ (100).

5a/b: Colourless gum; MS *m/z* (rel. int.): 346.178 $[M-H_2O]^+$ (2) (calc. for $C_{20}H_{26}O_5$: 346.178), 244 $[346-RCO_2H]^+$ (15), 85 $[RCO]^+$ (100).

10 mg **2a/b** in 0.5 ml Ac_2O were heated for 2 hr at 70°. Usual work-up and TLC (Et_2O–petrol, 11:9) gave 8 mg **3a/b**: colourless gum; MS *m/z* (rel. int.): 448.210 $[M]^+$ (1.5) (calc. for $C_{24}H_{32}O_8$: 448.210), 389 $[M-OAc]^+$ (0.7), 346 $[M-RCO_2H]^+$ (0.2), 244 $[346-HOAc, ketene]^+$ (2.5), 85 $[RCO]^+$ (100).

15-*Isovaleryloxy-4β,5α-epoxygermacra*-1(10)E,11(13)-*dien*-12,8*α-olide* (**6**). Colourless gum; MS *m/z* (rel. int.): 348.194 $[M]^+$ (0.5) (calc. for $C_{20}H_{28}O_5$: 348.194), 246 $[M-RCO_2H]^+$ (8), 228 $[246-H_2O]^+$ (8), 85 $[RCO]^+$ (73), 57 $[85-CO]^+$ (100).

14-*Hydroxy*-15-[*2-methyl-3-hydroxybutyryloxy*]-*germacra*-1(10)E,4Z-11(13)-*trien*-12,8*α-olide* (**8g**). Colourless gum; CIMS (isobutane): 247 $[M-RCO_2H+1]^+$ (1), 219 $[247-CO]^+$ (20), 73 $[MeCH(OH)CHMe]^+$ (100).

14-*Acetoxy*-15-[*2-hydroxymethylacryloyloxy*]-*germacra*-1(10)E,4Z-11(13)-trien-12,8*α-olide* (**8h**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone), 1700 ($C=CCO_2R$); MS *m/z* (rel. int.): 331 $[M-OAc]^+$ (2.5), 289 $[M-RCO_2]^+$ (4), 229 $[289-HOAc]^+$ (24), 228 $[M-RCO_2H, HOAc]^+$ (28), 85 $[RCO]^+$ (42), 57 $[85-CO]^+$ (100).

REFERENCES

1. Herz, W., Subramaniam, P. S., Murari, R., Dennis, N. and Blount, J. F. (1977) *J. Org. Chem.* **42**, 1720.
2. Castro, V., Jakupovic, J. and Bohlmann, F. (1986) *Phytochemistry* **25**, 1750.
3. Jakupovic, J., Baruah, R. N., Bohlmann, F., King, R. M. and Robinson, H. (1986) *Planta Med.* 204.
4. Bohlmann, F., Jakupovic, J., Ahmed, M. and Schuster, A. (1983) *Phytochemistry* **22**, 1623.
5. Bohlmann, F., Natu, A. A. and Mahanta, P. K. (1978) *Phytochemistry* **17**, 483.
6. Boeker, R., Bohlmann, F. and Schmeda-Hirschmann, G. (1987) *Plant Med.*, 39.
7. Gutierrez, A., Oberti, J. C., Sosa, U. E. and Herz, W. (1987) *Phytochemistry* **26**, 2315.