

## GERMACRANOLIDES FROM *MIKANIA GRAZIELAE*\*

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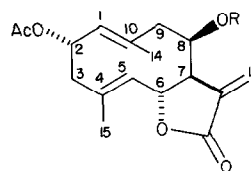
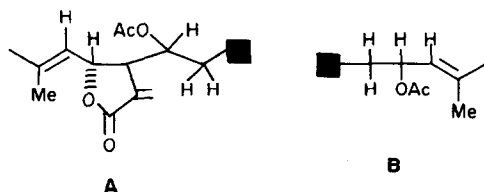
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**Key Word Index**—*Mikania grazielae*; Compositae; sesquiterpene lactones; germacranolides; 2 $\alpha$ -acetoxyeupatolide; 2 $\alpha$ -acetoxylaurenobiolide.

**Abstract**—*Mikania grazielae* afforded two new germacranolides which were identified as 2 $\alpha$ -acetoxyeupatolide and 2 $\alpha$ -acetoxylaurenobiolide.

In a continuation of our investigation of *Mikania* species[1], we have studied the constituents of the aerial parts of *M. grazielae* K. et R. In addition to  $\alpha$ -copaene, longifolene, alloaromadendrene, caryophyllene, germacrene D,  $\alpha$ -humulene, squalene, phytol, spathulenol, lup-12-en-3 $\beta$ -ol, stigmaterol, oleanolic acid, naringenin and the corresponding 4'-O-methyl ether, two sesquiterpene lactones were isolated, the germacranolides 1 and 3. The mass spectrum showed that the main compound 1 was a hydroxyacetate having the molecular formula C<sub>17</sub>H<sub>20</sub>O<sub>5</sub>. The IR spectrum also indicated a hydroxyl, an acetyl and a  $\gamma$ -lactone group. The <sup>1</sup>H NMR spectrum (Table 1) showed the presence of a methylene lactone by the typical downfield doublets of H-13. Acetylation afforded the diacetate 2. Its <sup>1</sup>H NMR spectrum (Table 1) showed that a broadened doublet in the spectrum of 1 was shifted downfield. This allowed the assignment of the relative positions of the oxygen functions by spin decoupling. Irradiation at  $\delta$ 2.92 showed that this signal was that of H-7. Subsequent further decouplings established sequence A, while starting with the three-fold doublet at  $\delta$ 5.67, sequence B was assigned.

The combination of these two fragments led to 2. The stereochemistry at C-6 and C-7 followed from the typical couplings of  $J_{5,6}$  and  $J_{6,7}$  (Table 1) while the small couplings of  $J_{7,8}$ ,  $J_{8,9\alpha}$  and  $J_{8,9\beta}$  showed that



- 1 R = H  
2 R = Ac

the oxygen function at C-8 was  $\beta$ -orientated. The large coupling  $J_{1,2}$  agreed with a 2 $\alpha$ -orientation of the acetoxy group if a conformation was assumed with both the C-4 and C-10 methyl groups above the plane. Furthermore, the <sup>1</sup>H NMR signal of H-2 showed the same splitting as that of similar germacranolides like dihydrotamulipin-A-acetate[2], where the conformation has been confirmed by NOE. Accordingly, 1 is 2 $\alpha$ -acetoxyeupatolide.

The minor lactone was a diacetate, as followed from the spectral data. The <sup>1</sup>H NMR spectrum (Table 1) was similar to that of laurenobiolide[3]. Spin decoupling allowed the assignment of all signals, while the stereochemistry at C-2 and C-5 through C-8 was deduced from the couplings observed. Again, a conformation was assumed with the methyl groups at C-4 and C-10 above the plane. The couplings of H-8 were 9 Hz and less than 1 Hz, indicating that the angle H-8, H-9 $\beta$  should be nearly 90° which was in agreement with a model. Again, the couplings of H-2 agreed best with an  $\alpha$ -orientation of the acetoxy group. Following the Geissman rule [4], the presence of an 8,12-*trans*-lactone was supported by the CD-curve of the pyrazoline 4 obtained after addition of diazomethane. As followed from the <sup>1</sup>H NMR spectrum (Table 1), 4 was the isomer formed by a  $\beta$ -attack of diazomethane (downfield shift of the H-6 signal). Accordingly, 3 was identified as 2 $\alpha$ -acetoxylaurenobiolide.

The sesquiterpene lactones isolated from *M. grazielae* show relationships to the members of *Mikania* in

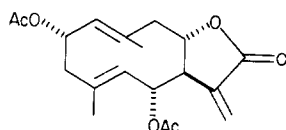
\*Part 418 in the series "Naturally Occurring Terpene Derivatives". For Part 417 see Bohlmann, F., Borthakur, N., Robinson, H. and King, R. M. (1982) *Phytochemistry* 21, (in press).

Table 1.  $^1\text{H}$  NMR spectral data of compounds 1–4 (400 MHz,  $\text{CDCl}_3$ , TMS as int. standard)

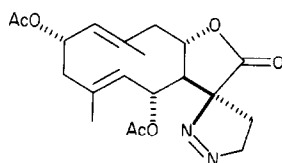
	1	2	3	4*
H-1	4.97 <i>brd</i>	5.01 <i>brd</i>	4.99 <i>brd</i>	4.99 <i>brd</i>
H-2	5.68 <i>ddd</i>	5.67 <i>ddd</i>	5.65 <i>ddd</i>	5.62 <i>ddd</i>
H-3 $\beta$	2.71 <i>dd</i>	2.75 <i>dd</i>	2.65 <i>dd</i>	2.61 <i>dd</i>
H-3 $\alpha$	2.14 <i>dd</i>	2.20 <i>dd</i>	2.13 <i>brdd</i>	2.02 <i>dd</i>
H-5	4.89 <i>brd</i>	4.99 <i>brd</i>	4.87 <i>d br</i>	4.68 <i>brd</i>
H-6	5.20 <i>dd</i>	5.08 <i>dd</i>	5.36 <i>dd</i>	5.57 <i>dd</i>
H-7	2.80 <i>ddd</i>	2.92 <i>dddd</i>	3.11 <i>dddd</i>	2.80 <i>dd</i>
H-8	4.62 <i>brd</i>	5.74 <i>ddd</i>	4.01 <i>dd</i>	5.00 <i>ddd</i>
H-9 $\alpha$	2.71 <i>dd</i>	2.84 <i>dd</i>	2.52 <i>brdd</i>	2.55 <i>dd</i>
H-9 $\beta$	2.27 <i>dd</i>	2.31 <i>dd</i>	2.72 <i>brd</i>	3.03 <i>brd</i>
H-13	6.35 <i>d</i>	6.32 <i>d</i>	6.40 <i>d</i>	2.31 <i>ddd</i>
H-13'	5.58 <i>d</i>	5.61 <i>d</i>	5.95 <i>d</i>	1.86 <i>m</i>
H-14	1.78 <i>d</i>	1.83 <i>d</i>	1.83 <i>brs</i>	1.82
H-15	1.75 <i>brs</i>	1.65 <i>brs</i>	1.74 <i>brs</i>	1.77 <i>brs</i>
OAc	2.05 <i>s</i>	2.07 <i>s</i>	2.09 <i>s</i>	2.07 <i>s</i>
			2.08 <i>s</i>	1.88 <i>s</i>

\*4.85 *ddd* and 4.67 *ddd* (H-16)

$J(\text{Hz})$ : Compounds 1 and 2: 1,2 = 2,3 $\alpha$  = 10; 2,3 $\beta$  = 5.5; 3 $\alpha$ ,3 $\beta$  = 11; 5,6 = 10; 6,7 = 8.5; 7,8 = 1; 7,13 = 3.5; 7,13' = 3; 8,9 $\alpha$  = 5.5; 8,9 $\beta$  = 2; 9 $\alpha$ ,9 $\beta$  = 14.5; compound 3: 1,2 = 2,3 $\alpha$  = 10; 2,3 $\beta$  = 6; 3 $\alpha$ ,3 $\beta$  = 11; 5,6 = 6,7 = 10; 7,8 = 6; 7,13 = 3; 7,13' = 2.5; 8,9 $\alpha$  = 9; 9 $\alpha$ ,9 $\beta$  = 13; compound 4: 8,9 $\alpha$  = 10; 8,9 $\beta$  = 1.5; 13,13' = 13.5; 13,16 = 4.5; 13,16' = 9.5; 13',16 = 10.5; 13',16' = 7.5.



3



4

the *Kanimia* group [1], where desacetyl-laurenobiolide was the main constituent. By contrast, higher oxygenated lactones were isolated from most of the other sesquiterpene lactone-containing species [1]. Further investigations may show whether a separation of the large genus may be indicated.

## EXPERIMENTAL

The air-dried aerial parts (100 g) collected in north-eastern Brazil (voucher RMK 8159, deposited in the U.S. National Herbarium, Washington) were extracted with  $\text{Et}_2\text{O}$ -petrol (1:2) and the resulting extract was separated first by CC (Si gel) and further by repeated TLC (Si gel). Compounds were identified by comparing the  $^1\text{H}$  NMR spectra with those of authentic material. The following compounds were isolated: 15 mg  $\alpha$ -copaene, 25 mg longifolene, 20 mg alloaromadendrene, 20 mg caryophyllene, 260 mg germacrene D, 20 mg  $\alpha$ -humulene, 40 mg squalene, 25 mg phytol, 10 mg spathulenol, 10 mg lup-12-en-3 $\beta$ -ol, 20 mg stigmaterol, 10 mg oleanolic acid, 120 mg naringenin-4'-*O*-methyl ether, 5 mg naringenin, 250 mg 1 ( $\text{Et}_2\text{O}$ -petrol, 3:1 several times) and 5 mg 3 (same solvent).

2 $\alpha$ -Acetoxyeupatolide (1). Colourless crystals, mp 146° ( $\text{Et}_2\text{O}$ -petrol), IR  $\nu_{\text{max}}^{\text{CHCl}_3}$ ,  $\text{cm}^{-1}$ : 3590 (OH), 1760, 1660 (methylene lactone), 1730, 1230 (OAc); MS  $m/z$  (rel. int.):

306 [ $\text{M}^+$ ] (1), 288 [ $\text{M} - \text{H}_2\text{O}^+$ ] (11), 246.126 [ $\text{M} - \text{HOAc}^+$ ] (20) ( $\text{C}_{15}\text{H}_{18}\text{O}_3$ ), 228 [ $288 - \text{HOAc}^+$ ] (100), 213 [ $228 - \text{Me}^+$ ] (44), 200 [ $228 - \text{CO}^+$ ] (15).

$$[\alpha]_{\text{D}}^{25} = \frac{589}{+59} \frac{578}{+62} \frac{546}{+72} \frac{436 \text{ nm}}{+141} (\text{CHCl}_3; c 0.76).$$

Compound 1 (10 mg) was heated for 1 hr with 0.1 ml  $\text{Ac}_2\text{O}$  at 70°. TLC ( $\text{Et}_2\text{O}$ -petrol, 3:1) afforded 10 mg 2, colourless gum; for  $^1\text{H}$  NMR see Table 1.

2 $\alpha$ -Acetoxy-laurenobiolide (3). Colourless gum, IR  $\nu_{\text{max}}^{\text{CHCl}_3}$ ,  $\text{cm}^{-1}$ : 1770, 1660 (methylene lactone), 1740, 1260 (OAc); MS  $m/z$  (rel. int.): 288 [ $\text{M} - \text{HOAc}^+$ ] (1.5), 228.115 [ $288 - \text{HOAc}^+$ ] (100), 213 [ $228 - \text{Me}^+$ ] (12), 200 [ $228 - \text{CO}^+$ ] (15).

$$[\alpha]_{\text{D}}^{25} = \frac{589}{+56} \frac{578}{+58} \frac{546}{+67} \frac{436 \text{ nm}}{+128} (\text{CHCl}_3; c 0.37)$$

To 5 mg 3 in 1 ml  $\text{Et}_2\text{O}$  excess  $\text{CH}_2\text{N}_2$  was added. After 5 min, evaporation afforded the pyrazoline 4, CD (MeCN)  $\Delta\epsilon_{322} - 6.9$ .

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