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MELAMPOLIDES FROM *MELAMPODIUM* AND *SMALLANTHUS* SPECIES

V. CASTRO, J. JAKUPOVIC* and X. A. DOMINGUEZ†

Universidad de Costa Rica, Escuela de Quimica, San Jose, Costa Rica, *Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, F.R G; †ITESM, Sucursal de Correos "J", C P 64849 Monterrey, N. L., Mexico

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Key Word Index—*Melampodium cinereum* var *hirtellum*, *M cinereum*; *Smallanthus macvaughii*; Compositae, sesquiterpene lactones, melampolides; diterpenes, kaurane derivatives

Abstract—Investigation of three Mexican species belonging to the subtribe Melampodinae (Compositae, tribe Heliantheae) gave in addition to known compounds, six melampolides and three kaurane derivatives, one being a β-D-glucopyranoside. The structures were elucidated by high field NMR spectroscopy

INTRODUCTION

The subtribe Melampodinae contains several genera which can be characterized by the occurrence of melampolides [1]. *Melampodium*, *Acanthospermum*, *Ichthyothere* and *Smallanthus* in particular are rich in this type of sesquiterpene lactone. However, diterpenes are also frequent. As several *Polymnia* species have been transferred to *Smallanthus* [2] we have studied the constituents of one of them, *S. macvaughii* (J R Wells) H. Robinson, as well as *M. cinereum* DC var. *hirtellum* Stuessy and *M. cinereum* DC. The results are presented in this paper.

RESULTS AND DISCUSSION

The aerial parts of *S. macvaughii* afforded in addition to widespread compounds (see Experimental) the melampolides **5–7** [3] and **8** [3] as well as the kaurane derivatives **11–13**. The structure of **5** followed from its ^{1}H NMR spectrum (Table 1) which was similar to that of the

Table 1 ^{1}H NMR spectral data of compounds **1–6** (400 MHz, $CDCl_3$, δ-values)

H	1	2	3	4(60°)	5†	6‡
1	5 50 *br dd*	5.50 *br dd*		5 69 *br dd*	6 85 *dd*	6 84 *dd*
2	2.65 *m*	2 65 *m*			2.50 *m*	2 49 *dddd*
	2 33 *m*	2.30 *m*		*	2 40 *m*	2 25 *dddd*
3	2 88 *m*	2.88 *m*		*	2 97 *br d*	2 36 *ddd*
	2 38 *m*	2 36 *m*		1 93 *ddd*	2 37 *m*	2 03 *br ddd*
5	5 54 *br s*	5 54 *br s*		1 57 *ddd*	4 26 *d*	4 93 *br d*
6				4.86 *ddd*	4.74 *dd*	5 07 *dd*
7	3 11 *dddd*	3 12 *dddd*		3 11 *dddd*	3.07 *dddd*	2 62 *dddd*
8	5 56 *ddd*	5 58 *ddd*		5 38 *ddd*	5.71 *dd*	6.21 *dd*
9	2 68 *dd*	2 69 *dd*		2 72 *dd*	4.64 *br dd*	3.94 *dd*
	2 63 *dd*	2 63 *dd*		2 54 *dd*		
13	6.32 *d*	6 33 *d*		6 27 *d*	6.36 *d*	6.24 *d*
13′	5 65 *d*	5 66 *d*		5 64 *d*	5.79 *d*	5.63 *d*
14	4 12 *br s*	4 12 *br s*		4 16 *br s*	—	—
15	3 93 *br s*	4.00 *br s*		3 45 *br d*	5.21 *br s*	1.90 *br s*
					5.00 *br s*	
OR	2 49 *qq*	2 3 *m*	2.13 *m*	2.49 *qq*	6.10 *qq*	2.60 *qq*
	1 11 *d*	1 61 *m*	2.00 *m*	1 13 *d*	1.97 *dq*	1.18 *d*
	1 09 *d*	1 42 *m*	0 91 *d*	1 11 *d*	1 85 *dq*	1.15 *d*
	—	1 07 *d*	—	—	—	—
	—	0.87 *t*	—	—	—	—

*Obscured, †OMe 3.85 *s*, OH: 3 42 *d*, OOH 8 44 *br s*, ‡OMe· 3 80 *s*, OH 2.72 *d*

J [Hz]: Compounds **1–3** 1,2 = 1,2′ = 7; 6,7 = 7,8 = 7,13 = 7,13′ ≈ 2.5; 8,9 = 10, 8,9′ = 7, 9,9′ = 14, compound **4**: 1,2 = 6; 1,2′ = 10, 4,5 = 2, 4,5′ = 9 5, 4,15 = 6; 5,5′ = 15; 5,6 = 5′,6 = 5, 6,7 = 7,8 = 3, 7,13 = 7,13′ = 2, 8,9 = 10; 8,9′ = 5; 9,9′ = 13 5; compound **5** 1,2 = 4 5, 1,2′ = 12, 3,3′ = 13, 5,6 = 8,9 = 10, 6,7 = 1; 7,8 = 2 5, 7,13 = 7,13′ = 2; 9,OH = 11; compound **6**. 1,2 = 8, 1,2′ = 10, 2,2′ = 13; 2,3 = 5.5; 2,3′ = 2′,3 = 2, 2′,3′ = 3,3′ = 12, 5,6 = 6,7 = 9,OH = 10; 7,8 = 2; 7,13 = 7,13′ = 3, 8,9 = 8 5

corresponding 5-hydroxy-9-*O*-acetate [4] the couplings being nearly identical. The presence of a hydroperoxide followed from the broadened singlet at δ8.44 The structure of **6** also followed from its ^{1}H NMR data (Table 1) which were similar to those of **7** [3] only the signals of the angelate moiety being replaced by those of an isobutyrate. As expected small shift differences were visible.

The structure of **11** also followed from its ^{1}H NMR spectrum (see Experimental) which was in part similar to that of 3β-acetoxy-16-hydroxy-*ent*-kaurane which has been isolated from an *Ichthyothere* species [5] However, the presence of a β-D-glucopyranoside clearly followed from typical ^{1}H NMR signals, especially from those of the tetraacetate **11**Ac obtained by acetylation While the chemical shift of H-17 was identical in the spectrum of the glucoside and the 3-*O*-acetate small differences of the H-19 signals indicated that a 3-*O*-glucoside was present. This was established by a NOE between H-3 and H-1′ (6%) while NOE's between H-18 and H-3 (3%) as well as between H-19 and H-3 (4%) excluded the 1-position for the oxygen function The axial orientation clearly followed from the observed small couplings

The ^{1}H NMR spectral data of **12** (see Experimental) were in part nearly identical with those of *ent*-kauran-16β-ol. However, the mass spectrum indicated the presence of additional hydroxy groups. A clear shift difference of H-17 in the spectra of **12** and *ent*-kauranol indicated the presence of a 9β-hydroxy group (Δ 0.13). The presence of a 3β-hydroxy group followed from the narrowly split triplet at δ3 41 which was as expected somewhat more down field as in the glucoside **11**Ac.

In the ^{1}H NMR spectrum of **13** (see Experimental) the H-3 signal was missing In agreement with the molecular formula the presence of the corresponding ketone was very likely. This was supported by a new downfield shifted multiplet at δ2 48 (H-2) as well as by the downfield shifts of the signals of H-18 and H-19. As again H-17 showed a singlet at δ1.23 a 9β-hydroxy group was present.

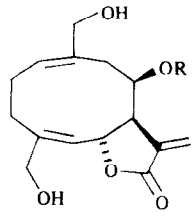
The extract of the aerial parts of *M. cinereum* var *hirtellum* gave in addition to phytol and sitosterol the melampolides **1**–**4** The ^{1}H NMR spectra (Table 1) indicated that the lactones **1**–**3** only differed in the nature of the ester group at C-8, **1** being an isobutyrate, **2** an isovalerate and **3**, which could not be separated from **2**, a methylbutyrate. The remaining signals were similar to those of the 15-*O*-acetate of **3**, which has been isolated from an *Acanthospermum* species where the 4*E*,1(10)*E*-configuration was established [6]. The upfield shift of the H-15 signals clearly showed that no ester group was present at this centre.

The signals in the ^{1}H NMR spectrum of lactone **4** (Table 1) could be assigned only at elevated temperature Spin decoupling indicated that this lactone had the same substitution pattern as **1** However, the H-6 signal now was a three-fold doublet which was coupled with a pair of three-fold doublets at 1 98 and 1 57. Accordingly, a 4,5-dihydro derivative of **1** was present. This also explains the flexibility of the compound. The configuration at C-4 could not be determined

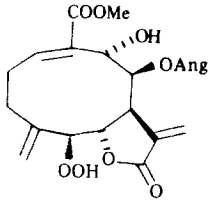
The extract of *M cinereum* gave in addition to phytol and sitosterol melampodin C (**10**) already isolated from this species [7, 8], **9** [9] and again the 4*E*,1(10)*E*-germacranolide **1** The results of this investigation clearly justify the placement of this species in *Smallanthus* which contain typical melampolides not present in *Polymnia* species [10]

EXPERIMENTAL

The aerial air-dried parts (vouchers deposited in the Herbarium of the Instituto Tecnologico at Monterrey, collected in



1 **2** **3** **4**
R *i*Bu *i*Val MeBu *i*Bu
4α,5H



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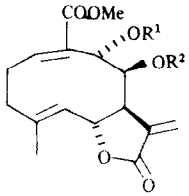
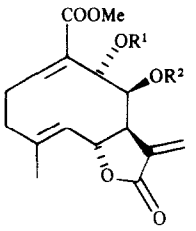


6 **7** **8**
R^1 H H Ac
R^2 *i*Bu Ang Epang
4α,5β epo



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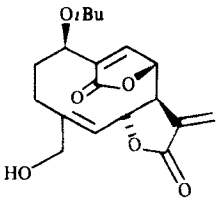

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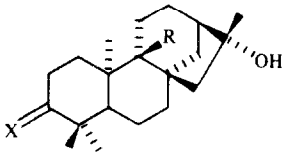

11 **11**Ac **12** **13**
X β-(β-OGlc),H β-[β-OGlc(OAc)$_4$],H β-OH,H O
R H H OH OH

N Mexico) were extracted with MeOH–isopropylether–petrol (1 1 1) and the extracts obtained were worked-up and sepd as reported previously [11]

The extract of *S macvaughii* (350 g, voucher 8327, collected in Laguma de Sanchez, N L., Act 1987) was sepd by CC into 7 fractions (petrol, Et_2O–petrol mixts and Et_2O–MeOH, 9·1). TLC of fr 1 gave 2 mg curcumene, fr 2 gave 5 mg caryophyllen-epoxide and 3 mg γ-curcumene endoperoxide TLC of fr 3 afforded 1 mg *ent*-kaurenic acid and 1 mg of its Δ^9-derivative as well as 2 mg 1β-hydroxygermacra-4 (15),5*E*,10 (14)-triene. Fr 4 gave 1 mg 4-hydroxycarvone and fr 5 by TLC (C_6H_6–CH_2Cl_2–Et_2O, 9 9 2, ×3) 1 mg **8**, 2 mg **7**, 4 mg **6** (R_f 0.45) and 0 5 mg **5** (R_f 0 3) Fr **6** gave by TLC (C_6H_6–$CHCl_2$–Et_2O, 2 2 1, ×3) 1 mg **13** (R_f 0 4) and 1 mg **12** (R_f 0 3) The most polar fraction gave 300 mg **11**

The extract of 400 g aerial parts of *M. cinereum* var. *hirtellum* (voucher 8221, collected in Mamulique, N L., Oct 1987) gave by CC and TLC 20 mg phytol, 30 mg sitosterol and a polar fraction which gave by HPLC (RP 8, MeOH–H_2O, 3 2, flow rate 3 ml/min) 1 mg **4** (R_t 6 4 min), 1 mg **1** (R_t 7 0 min) and 1 mg of a mixt of **2** and **3** (*ca* 1 1)

The extract of 650 g aerial parts of *M cinereum* (voucher 8118, collected in Escobedo, N L, July 1986) gave by CC and TLC 10 mg phytol, 15 mg sitosterol and a polar fraction which gave by HPLC (as described above) 1 mg **10** (R_t 5 2 min), 1 mg **9** (R_t 5 8 min) and 1 mg **1** (R_t 7 0 min).

Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material and/or with the data in the lit.

14,15-*Dihydroxy*-8β-*isobutyryloxygermacra*-1(10)E,4E-11(13)-*trien*-12,6α-*olide* (**1**) Colourless gum, MS *m/z* (rel. int)· 350.173 $[M]^+$ (0 7) (calc for $C_{19}H_{26}O_6$ 350.173), 332 $[M-H_2O]^+$ (10), 244 $[332-RCO_2H]^+$ (42), 71 $[RCO]^+$ (100).

14,15-*Dihydroxy*-8β-*isovaleryl and* [2-*methylbutyryl*]-*oxygermacra*-1(10)E,4E,11(13)-*trien*-12,6α-*olide* (**2** and **3**) Colourless gum, MS *m/z* (rel int) 346 178 $[M-H_2O]^+$ (2) (calc. for $C_{20}H_{26}O_5$· 346 178), 244 $[346-RCO_2H]^+$ (4), 226 $[244-H_2O]^+$ (4), 85 $[RCO]^+$ (34), 57 $[85-CO]^+$ (100)

14,15-*Dihydroxy*-8β-*isobutyryloxygermacra*-1(10) E,11(13)-*dien*-12,6α-*olide* (**4**) Colourless gum, MS *m/z* (rel int) 264 136 $[M-RCO_2H]^+$ (2 5) (calc for $C_{15}H_{20}O_4$ 264 136), 246 $[264-H_2O]^+$ (4 5), 71 $[RCO]^+$ (100)

Desacetyl-5-*desoxyrepandin* E-5β-*hydroperoxide* (**5**) Colourless gum, MS *m/z* (rel int) 322 113 $[M-OAng]^+$ (1 5) (calc for $C_{16}H_{19}O_7$ 323.113), 291 $[323-MeOH]^+$ (3), 273 $[291-H_2O]^+$ (2), 258 $[291-O_2H]^+$ (2 3), 83 $[RCO]^+$ (100)

Desacylpolymatin A-isobutyrate (**6**) Colourless gum, MS *m/z* (rel. int) 290 116 $[M-RCO_2H]^+$ (6) (calc for $C_{16}H_{18}O_5$ 290.116), 258 $[290-MeOH]^+$ (10), 71 $[RCO]^+$ (100)

3β,16α-*Dihydroxy*-ent-*kaurane*-3-*O*-β-D-*glucopyranoside* (**11**). Colourless crystals, mp 263–266°, MS *m/z* (rel int) 288 245 [M−glucose]$^+$ (11) (calc. for $C_{20}H_{32}O$ 288 245), 271 $[C_{20}H_{31}]^+$ (100), 255 (21), 137 (81), 69 (86); ^{1}H NMR (MeOD) 3 3 (*m*, H-3), 1.32 (*s*, H-17), 0 99 (*s*, H-18), 0 84 (*s*, H-19), 1 32 (*s*, H-20), 4.39 (*d*, H-1′, *J* = 8 Hz), 3 18–3 35 (*m*, H-2′–H-5′), 3 82 (*dd*, H-$6_{1'}$, *J* = 2, 12), 3.60 (*dd*, H-$6_{2'}$, *J* = 12, 5 5); ^{13}C NMR (MeOD, C-1–C-20) 35.3, 28.0, 87.5, 40 2, 50 5, 20 8, 43 0, 46 4, 58 0, 39 2, 19 0, 25.3, 49 7, 38.5, 58 8, 79 8, 24 5, 29 1, 22 7, 18.5, *O*Glc (1′–6′) δ106.4, 75 8, 78 3, 71 7, 77 6, 62 8. Acetylation (DMAP, pyridine, Ac_2O) gave the tetraacetate **11**Ac, colourless gum, MS *m/z* (rel. int) 618.342 $[M]^+$ (5) (calc for $C_{34}H_{50}O_{10}$. 618 340), 331 $[glcAc]^+$ (44), 271 $[331-HOAc]^+$ (100), 270 $[C_{20}H_{30}]^+$ (76), 255 $[270-Me]^+$ (44), 169 (95), 109 (95), ^{1}H NMR ($CDCl_3$) δ3 20 (*t*, H-3, *J* = 2 5 Hz), 1.36 (*s*, H-17), 0 81 (*s*, H-18), 0 81 (*s*, H-19), 1 01 (*s*, H-20), 4.51 (*d*, H-1′, *J* = 8), 5.01 (*dd*, H-2′, *J* = 8, 10), 5 18 (*dd*, H-3′, *J* = 9.5, 10), 5 04 (*dd*, H-4′, *J* = 9.5, 10), 3 66 (*ddd*, H-5′, *J* = 10, 6, 2 5), 4 09 (*dd*, H-$6_{1'}$, *J* = 12, 2 5), 4 23 (*dd*, H-$6_{2'}$, *J* = 12, 6), 2 07, 2.02, 2.00 (2 ×) (*s*, OAc)

3β,9β,16α-*Trihydroxy*-ent-*kaurane* (**12**). Colourless gum, MS *m/z* (rel. int.) 322 251 $[M]^+$ (1 3) (calc for $C_{20}H_{34}O_3$· 322.251), 304 $[M-H_2O]^+$ (81 2), 286 $[304-H_2O]^+$ (1 7), 263 $[M-C_3H_7O]^+$ (100), 245 $[263-H_2O]^+$ (10), ^{1}H NMR ($CDCl_3$). 3.41 (*t*, H-3, *J* = 2 5 Hz), 1 22 (*s*, H-17), 0.94 (*s*, H-18), 0.83 (*s*, H-19), 1 03 (*s*, H-20)

9β,16α-*Dihydroxy*-ent-*kauran*-3-*one* (**13**). Colourless gum, MS *m/z* (rel. int) 320.235 $[M]^+$ (1 3) (calc for $C_{20}H_{32}O_3$ 320 235), 302 $[M-H_2O]^+$ (4), 287 $[302-Me]^+$ (2), 261 $[M-C_3H_7O]^+$ (100), 259 $[287-CO]^+$ (8), 135 (31), ^{1}H NMR ($CDCl_3$): 2.48 (*m*, H-2), 1 23 (*s*, H-17), 1 08 (*s*, H-18), 1.07 (*s*, H-19), 1.02 (*s*, H-20).

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