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FOUR NEW *CIS,CIS*-GERMACRANOLIDES FROM CYTOTOXIC FRACTIONS OF *MELAMPODIUM CINEREUM*

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Key Word Index—*Melampodium cinereum*; *cis,cis*-germacranolides; antineoplastic agents; cytotoxicity.

Abstract—The aerial parts of *Melampodium cinereum* afforded an antineoplastic crude extract from which, besides the known dilactones cinerenin and melampodin B, four new *cis*-1(10)-*cis*-4,5-germacranolides, melcanthins D–G, were isolated from cytotoxic fractions.

INTRODUCTION

A biochemical systematic study [1, 2] combined with our search for antineoplastic constituents within members of the family Compositae resulted in crude terpenoid extracts from *Melampodium cinereum* DC. (NSC No. B-900555) which exhibited no cytotoxicity but significant antineoplastic activity *in vivo* against experimental lymphocytic leukemia P-388 (PS) cells (T/C = 186 at 37.5 mg/kg; ED_{50} = 23 μg/ml)*. The major constituents, melampodin B (**1**) and cinerenin (**2**), which had previously been obtained from the crude syrup by spontaneous crystallization, exhibited no PS-activity. Systematic chromatographic fractionation over Si gel led to cytotoxic fractions which were analysed for their chemical constituents. This paper reports the isolation and characterization by spectral methods of the four new sesquiterpene lactones from the cytotoxic fractions of *M. cinereum*.

RESULTS AND DISCUSSION

The structures of the new compounds were deduced by comparison of the physical parameters, mainly NMR and MS, of the new natural products with those of the co-occurring known *cis,cis*-germacranolide melcanthin A (**3**) which had previously been isolated from *Melampodium leucanthum* [3].

Melcanthin D (**4**), $C_{22}H_{26}O_9$, displayed in the 200 MHz ^{1}H NMR spectrum signals which were nearly identical with those for the medium ring portion of melcanthin A (**3**) the parameters of which are also listed in Table 1 for better comparison. In addition, NMR signals at δ 1.80 (vinyl methyl) and 5.51 and 5.94 (two vinyl protons) together with diagnostic MS peaks at *m/e* 69 ($\mathbf{B}_1$) and 41 ($\mathbf{B}_2$) suggested the presence of a methacrylate ester in **4** instead of the angelate moiety in **3**. Information about the attachment of the acetoxy function, either at C-8 or C-9 in **4**, of the medium ring was obtained from MS data of melcanthin D, which gave a significant peak at *m/e* 170 tentatively assigned to the MS fragment given in Scheme 1 [3]. This suggested that in melcanthin D the acetate moiety was attached to C-9 as in melcanthin A and the methacrylate was at C-8.

Melcanthin E (**5**), $C_{22}H_{28}O_9$, exhibited ^{1}H NMR parameters nearly superimposable with those of the medium ring absorptions of melcanthins A (**3**) and D (**4**) (Table 1). Differences appeared for the ester side chain signals with NMR and MS data indicating the presence of an isobutyrate moiety. A six-proton doublet at δ 1.0 (J = 6.0 Hz) together with a heptet at 2.45 (J = 6.0 Hz) and diagnostic MS peaks at *m/e* 71 ($\mathbf{A}_1$) and 43 ($\mathbf{A}_2$), respectively, were in agreement with the presence of an isobutyrate moiety in **5**. The same MS arguments for the attachment of the acetate group to C-9 used above for melcanthin D can also be applied to melcanthin E (**5**).

Melcanthin F (**6**) and G (**7**) could not be separated by reverse phase HPLC and were therefore analysed as a mixture. Although the NMR spectral parameters of this mixture indicated a double pattern for most signals due to the medium ring protons, the absorptions caused by the ester side chain were relatively sharp suggesting that compounds **6** and **7** differed by attachment of the ester functions, that is, acetate at C-9 and 2-methylbutanoate ($\mathbf{D}_1$) at C-8 in **6** and vice versa in **7**. Examples for similar positional isomers have previously been described for other sesquiterpene lactone mixtures [4]. Additional evidence for the attachment of the 2-methylbutanoate to C-9 in **7** was provided by a MS peak at *m/e* 212 assigned to the fragment shown in Scheme 1.

Lack of material did not permit further chemical and spectral investigations as well as biological tests of the newly isolated compounds.

EXPERIMENTAL

Melampodium cinereum DC. var. *cinereum* (T. F. Stuessy and N. H. Fischer No. 2015; voucher deposited at OS; USA: Texas: Duval County on route 359, 8.6 miles northeast

*Cytotoxicity against KB (human carcinoma of the nasopharynx) cell culture and *in vivo* inhibitory activity against lymphocytic leukemia P-388 (PS) in rats were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institute of Health.

Table 1. ^{1}H NMR spectral data* of melcanthin A (**3**), D (**4**), E (**5**) and the mixture of F and G (**6** and **7**)

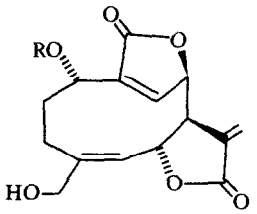
Assignment	3	4	5	6,7
H-1	7.15 *br. t* (8)	7.15 *br. t* (8)	7.14 *br. t* (8)	7.1 *br. t* (8)
H-2, H-3	2.25–2.80 *m*	2.20–2.80 *m*	2.20–2.80 *m*	1.75–1.95 *m*
H-5	5.64 *br. d* (9.0)	5.62 *br. d* (8.0)	5.61 *br. d* (8.0)	5.05, 5.07 *d* (8)
H-6	5.42 *br.*	5.40 *br.*	5.42 *br.*	5.13, 5.15 *br.*
H-7	3.15 *m*	3.13 *m*	3.07 *m*	2.5 *m*
H-8	5.88 *dd* (2.0, 4.0)	5.80 *dd* (2.0, 4.0)	5.77 *dd* (2.0, 4.0)	5.75, 5.77 *dd* (2, 4)
H-9	6.04 *d* (4.0)	6.01 *d* (4.0)	6.01 *d* (4.0)	5.91 *d* (4)
H-13a	5.81 *d* (3.0)	5.78 *d* (3.0)	5.73 *d* (3.0)	5.77, 5.79 *d* (3)
H-13b	6.36 *d* (3.0)	6.34 *d* (3.0)	6.34 *d* (3.0)	6.05, 6.08 *d* (3)
H-15	4.11 *d* (1.0)	4.10 *d* (1.0)	4.10 *d* (1.0)	3.38
COOMe	3.65	3.61	3.69	3.16
Acetate	2.08	2.06	2.08	1.43
C-2′-Me	1.74 *m*	1.80 *m*	1.00 *d* (6.0)	0.7 *d* (6)
C-3′-Me	1.92 *dq* (1.5, 7.0)	—	—	0.5 *t* (7)
H-2′	—	—	2.45 *hept* (6.0)	1.7 *m*
H-3′	6.06 *qq* (1.5, 7.0)	—	—	—
H-3′a	—	5.51 *dq* (1.5)	—	—
H-3′b	—	5.94 *dq* (1.5)	—	—

* Spectra of compounds **3**, **4** and **5** were run in $CDCl_3$ and the mixture of **6** and **7** in benzene-d_6 at 200 MHz. TMS was used as internal standard and values are recorded in ppm relative to TMS. Multiplets are given by the usual symbols. Figures in parentheses are coupling constants or line separations in Hertz.

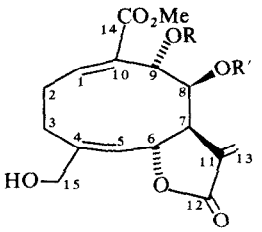
of Hebbronville, 19 July, 1973). Dried stems and leaves (1475 g) were extracted with cold $CHCl_3$ as described before [5] providing a crude extract (~ 50 g) from which upon standing for several days a mixture of melampodin B (**1**) and cinerenin (**2**) precipitated [1,2]. After filtration of the precipitated dilactones **1** and **2**, the residual syrup (5 g) was chromatographed over 250 g Si gel using *n*-propyl acetate as an eluant collecting 25 ml fractions. Fractions 28–37 (800 mg) were cytotoxic and therefore combined for further prep. layer chromatography on Si gel with Et_2O–hexane (9:1) as eluting solvent. Two overlapping bands were obtained which were shown by NMR analysis to consist still of lactone mixtures necessitating further separations by reverse phase HPLC. Separations were carried out on a semipreparative μ-Bondapak C-18 reverse phase column (7.8 mm i.d. × 30 cm, 10 μm particle size). Eluant was MeOH–H_2O (1:1) at a flow rate of 1.2 ml/min. and a pressure of 1700 psi. A UV-detector was used. The effluent fractions contained the compounds in the following order of elution: melcanthins D (**4**), E (**5**), A (**3**) followed by a mixture of melcanthins F and G (**6** and **7**). The fractions were concd *in vacuo* for the removal of MeOH and the residual H_2O was extracted with CH_2Cl_2. Drying of the CH_2Cl_2 layer over Na_2SO_4 and evapn of the solvent yielded the pure compounds **3** (10 mg), **4** (8 mg), **5** (8 mg) and the mixture of **6** and **7** (5 mg).

Melcanthin D (**4**), $C_{22}H_{26}O_9$, gum; UV, λ_{max}^{MeOH} nm: 220 ($\varepsilon = 0.96 \times 10^4$); CD ($c$ 3.24×10^{-4}, MeOH), $[\theta]_{214} - 2.2 \times 10^4$, $[\theta]_{237} + 7.4 \times 10^3$; IR, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1755 (lactone), 1725 (ester) and 1640 (double bonds); MS 70 eV *m/e* (rel. int.): 434 (–, M^+), 392 (0.9, M – C_2H_2O), 374 (0.2, M – CH_3COOH), 288 (3.6, M – $C_2H_4O_2$–$C_4H_6O_2$), 256 (10.1), 228 (13.5), 211 (7.9), 199 (10.4), 183 (5.9), 170 (1.8), 161 (8.2), 128 (13.3), 109 (5.7), 91 (7.0), 77 (7.1), 69 (100), 43 (9.5), 41 (15.2). [Calc. for $C_{20}H_{24}O_8$: 392.1471 Found: (MS, M – C_2H_2O) 392.1452].

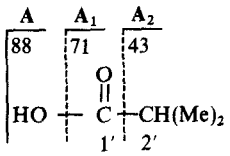
Melcanthin E (**5**), $C_{22}H_{28}O_9$: gum; UV, λ_{max}^{MeOH} nm: 218 ($\varepsilon = 1.75 \times 10^4$); CD ($c$ 6.0×10^{-5}, MeOH), $[\theta]_{210} - 2.1 \times 10^4$; $[\theta]_{235} + 7.6 \times 10^3$; IR, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH) and 1755 (lactone); MS 70 eV *m/e* (rel. int.): 436 (–, M^+), 376 (0.7, M – CH_3COOH), 288 (8.6, M – $C_2H_4O_2$–$C_4H_8O_2$), 260 (10.9), 256 (19.6), 228 (23.7), 211 (12.5), 170 (0.9), 161 (13.7), 149 (8.7), 128 (25.9), 91 (16), 83 (23.6), 77 (15), 71 (100), 43 (75).

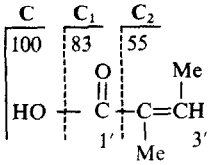


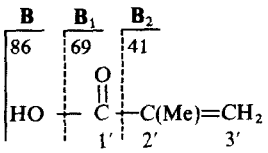
1 R = Ac
2 R = Et

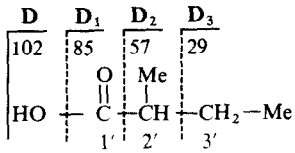


	3	4	5	6	7
R	Ac	Ac	Ac	Ac	D′
R′	C′	B′	A′	D′	Ac









	R	*m/e* (int.)
3	Ac	170 (0.3)
4	Ac	170 (0.9)
5	Ac	170 (1.8)
6	Ac	170 (2.1)
7	D_1	212 (5.3)

Scheme 1

[Calc. for $C_{20}H_{24}O_7$: 376.1522 Found: (MS, M $-$ $C_2H_4O_2$) 376.1490.]

Melcanthin F (**6**) *and* G (**7**), $C_{23}H_{30}O_9$, gum; MS 70 eV *m/e* (rel. int.): 450 (0, M^+), 390 (0.3, M $-$ CH_3COOH), 348 (0.8), 288 (6.4), 270 (6.7), 256 (20.5), 228 (27.9), 212 (5.3), 200 (14.2), 170 (2.1), 161 (16.5), 128 (27.5), 105 (11.7), 91 (11.3), 85 (100), 77 (12.4), 57 (84), 43 (27). [Calc. for $C_{18}H_{20}O_7$: 348.1209 Found: (MS, M $-$ $C_5H_{10}O_2$) 348.1216.]

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