Phytochemistry, Vol 24, No 8, pp 1747–1753, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

SESQUITERPENE AND DITERPENE LACTONES FROM *MELAMPODIUM LEUCANTHUM* AND THE MOLECULAR STRUCTURE OF 4(5)-DIHYDROMELAMPODIN B

LEOVIGILDO QUIJANO,* FRANK R. FRONCZEK and NIKOLAUS H. FISCHER†

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U S A.

(*Revised received* 12 *November* 1984)

Key Word Index—*Melampodium leucanthum*; Asteraceae; diterpene-γ-lactone; melcantholide derivatives; sesquiterpene lactones; melampolides; leucantholides.

Abstract—The aerial parts of *Melampodium leucanthum* afforded a novel diterpene-γ-lactone, 1,6,7-trihydroxy-17-acetoxymelcantholide, and two new sesquiterpene dilactones, 4(5)-dihydrocinerenin and 4(5)-dihydromelampodin C. The known melampolides, leucanthin A, melampodin A acetate, melampolidin and leucanthinin were also isolated. The structure determinations involved spectral and chemical methods. The molecular structure of 4(5)-dihydromelampodin B, determined by X-ray diffraction, is described.

INTRODUCTION

In an earlier paper we had reported the presence of an unidentified oil in central Texas populations of *Melampodium leucanthum* [1]. Similarities in the ^{1}H NMR patterns of this oil with spectra of diterpene lactones obtained from *M. diffusum* [2] and *M. longipilum* [3] suggested the need for a chemical reinvestigation of central Texas populations of *M. leucanthum*, in particular, the oily constituent(s). Non-polar chromatographic fractions of the crude terpenoid extract provided a mixture of the known melampolides leucanthin A and melampodin A acetate [1] followed by fractions containing melampolidin and leucanthinin [4]. From polar fractions, 4(5)-dihydromelampodin B (**4a**) [5] was obtained by spontaneous crystallization. The structure determination of **4a** had been based on ^{1}H NMR correlations with melampodin B [5], the molecular structure of which was subsequently established by single crystal X-ray diffraction [6]. Since the X-ray data of melampodin B required revision of the chiral centre C-1 and the configuration of the 4(5)-double bond from *trans* to *cis*, it became desirable to determine the X-ray structure of 4(5)-dihydromelampodin B to unambiguously establish its configurations, in particular at C-4. Its molecular structure will be described later in this paper.

Extended prep. TLC purification of the mother liquor of **4a** as well as of preceding fractions provided an oily diterpene the structure of which was determined by spectroscopic methods and chemical transformations, as will be discussed below.

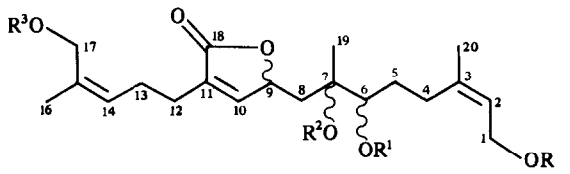
RESULTS AND DISCUSSION

1,6,7-Trihydroxy-17-acetoxymelcantholide‡ (**1a**), $C_{22}H_{34}O_7$, was isolated as a gum from the polar chromatographic fractions of *M. leucanthum*. The IR spectrum indicated a hydroxyl function(s) (3500 cm^{-1}) and possibly a γ-lactone moiety (1750 cm^{-1}). The presence of an acetate group in **1a** was verified by a three-proton ^{1}H NMR singlet at δ2.07 together with a base peak at *m/z* 43 (MeCO) in the mass spectrum. Acetylation of **1a** provided a mixture of acetate derivatives **1b–1d** which confirmed the presence of three hydroxyl groups in **1a**. *In situ* acylation with trichloroacetyl isocyanate (TAI) also gave three carbamate derivatives (**1e–1g**) which supported the above findings. The ^{1}H NMR spectrum of **1a** (Table 1) exhibited a broad methyl singlet at δ1.74 and a narrow three-proton doublet at δ1.75 (J = 1 Hz), suggesting the presence of two vinyl methyl groups. Another three-proton singlet at δ1.33 indicated a tertiary methyl group on an oxygen-bearing carbon. A doublet of doublets at δ3.42 (J = 11.0, 2.0 Hz) was assigned to a proton on a carbon bearing a secondary hydroxyl group. Two further doublets of doublets centred at δ4.18 and 4.03, which represented the AB part of an ABX pattern, were assigned to the methylene protons of an allylic primary hydroxyl group, since the signals shifted downfield upon acetylation. Two doublets of doublets appearing as broad triplets at δ5.58 and 5.37 indicated the presence of two vinyl protons. All the above ^{1}H NMR spectral features closely resembled those of 17-acetoxyacanthoaustralide (**5a**) which we had recently isolated from *M. longipilum* [3]. However, compounds **1a** and **5a** differed by the presence of one additional oxygen in **1a**. Furthermore, in lactone **5a** the β-hydrogen of the α,β-unsaturated lactone appeared as a broadened triplet at δ6.33 (H-10), whereas in **1a** the analogue signal absorbed as a broad singlet at δ7.14 (H-10). The differences in the splitting patterns of H-10 in **5a** and **1a** suggested that **1a** possesses an additional oxygen function at C-9. This was supported by

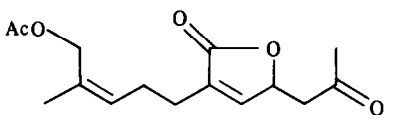
*Permanent address: Instituto de Quimica, UNAM, Mexico, D.F., Mexico.

†To whom correspondence should be addressed.

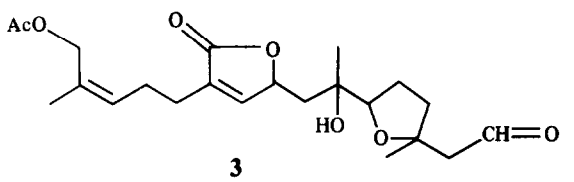
‡The name melcantholide has been reserved for the unsubstituted diterpene γ-lactone.



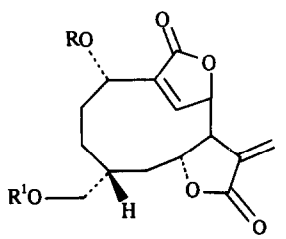
	R	R^1	R^2	R^3
1a	H	H	H	Ac
1b	Ac	H	H	Ac
1c	Ac	Ac	H	Ac
1d	Ac	Ac	Ac	Ac
1e	TAC*	H	H	Ac
1f	TAC	TAC	H	Ac
1g	TAC	TAC	TAC	Ac
1h	H	H	H	H



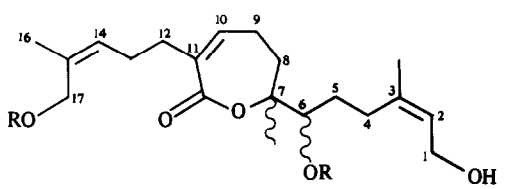
2



3

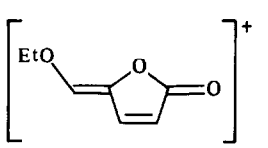


	R	R^1
4a	Ac	H
4b	Ac	Ac
4c	Ac	TAC
4d	Et	H
4e	Et	Ac
4f	i-Bu†	H



5a R = Ac

5b R = H



E

*TAC = —CO—NH—CO—CCl_3, † i-Bu = isobutyrate

the presence of a broadened one-proton doublet of a doublet at δ5.25 that was coupled to H-10 at 7.14. The chemical shift of H-9 was in agreement with a lactonic proton, suggesting an α,β-unsaturated γ-lactone moiety in **1a**. Further support for this structural arrangement was provided by the IR absorption at 1750 cm^{-1} and the deshielded proton absorption for H-10 at δ7.14. Hydrogens at the β-position in endocyclic α,β-unsaturated γ-lactone typically absorb below δ7.0 [5] which is also exemplified in the absorptions for H-9 in compound **4a** and analogues **4b–4f**.

The presence of an acetoxy group at C-17 in **1a** was derived from ^{1}H NMR spectral comparison with the known lactone **5a**. This was further supported by inspection of the acetate derivatives **1b–1d** which showed H-17 signals near δ4.57 as in **1a**. As expected, acetylation caused downfield shifts for the two protons at H-1 from δ4.03/4.18 in **1a** to δ4.48/4.56 in **1d** as well as H-6 in **1a** (δ3.42) to 4.84 in **1d**. This suggested the presence of hydroxyls at C-1 and C-6 in **1a**. *In situ* acylation of **1a** with TAI gave the carbamate derivatives **1e–1g** with different rates of formation as determined by ^{1}H NMR examination (Table 1). Since in TAI-derivative **1g** the absorption for the Me-7 group was distinctly shifted downfield (δ1.72) from the comparable Me-7 (δ1.33) in **1a**, a tertiary hydroxyl group had to be present at C-7. This was further supported by a periodate cleavage of the C-6, C-7 glycol moiety which gave a ketolactone, the ^{1}H NMR data

Table 1 ^{1}H NMR spectral data for the diterpene-γ-lactones **1a–1g** and derivatives **2** and **3** (200 MHz, $CDCl_3$, TMS as int. standard)*

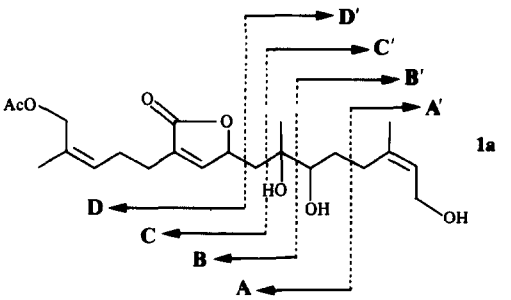
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**2**	**3**
H-1	4.03 *dd* (13, 7.5)	4.56 *dd* (13, 7)	4.48 *dd* (13, 7)	4.48 *dd* (13, 7)	4.84 *br d* (7)	4.64 *dd* (13, 7)	4.65 *dd* (13, 7)	—	9.80 *d* (3)
H-1′	4.18 *dd* (13, 8)	4.70 *dd* (13, 8)	4.56 *dd* (13, 8)	4.56 *dd* (13, 8)		4.83 *dd* (13, 7)	4.83 *dd* (13, 7)	—	
H-2	5.58 *br dd* (7, 8)	5.38 *br dd* (7, 8)	5.37 *br dd* (7, 8)	5.36 *br dd* (7, 8)	5.48 *br dd* (7, 8)	5.48 *br dd* (7, 8)	5.48 *br dd* (7, 8)	—	2.55, 2.65 *dd* (16, 3)
H-6	3.42 *dd* (11, 2)	3.40 *dd* (11, 2)	4.84 *dd* (10, 2.5)	4.84 *dd* (10, 2.5)	3.44 *br d* (10)	4.87 *br d* (10)	5.58 *br d* (10)	—	3.88 *dd* (8, 5)
H-8	1.75 *dd* (16, 5)	obs	1.75 *dd* (15, 5)	obs	~1.84 obs	obs	obs	2.64 *dd* (17, 7)	~1.84 obs
H-8′	1.92 *dd* (16, 9)	obs	1.89 *dd* (15, 8)	1.88 *dd* (16, 8)		obs	obs	3.04 *dd* (17, 7)	
H-9	5.25 *br dd* (9, 5)	5.23 *br t* (6)	5.19 *br t* (6.5)	5.19 *br t* (6)	5.24 *br t* (6)	5.22 *br t* (6)	5.24 *br t* (6)	5.29 *t* (7)	5.19 *br td* (5, 1)
H-10	7.14 *br s*	7.11 *br s*	7.14 *br d* (~1)	7.14 *br s*	7.10 *br s*	7.14 *br s*	7.11 *br s*	7.15 *br s*	7.13 *br d* (~1)
H-12	2.33 *br s*	2.35 *br s*	2.34 *br s*	2.34 *br s*	2.35 *br s*	2.35 *br s*	2.32 *br s*	2.34 *br s*	2.35 *br s*
H-13									
H-14	5.37 *br t* (6.5)	5.38 *br t* (6)	5.37 *br t* (6)	5.36 *br t* (6)	5.36 *br t* (6)	5.36 *br t* (6)	5.35 *br t* (6)	5.35 *br t* (6)	5.36 *br t* (6)
H-17	4.57 *br s*	4.58 *br s*	4.57 *br s*	4.57 *br s*	4.58 *br s*	4.58 *br s*	4.57 *br s*	4.57 *br s*	4.57 *br s*
H-16	1.75 *d* (~1)	1.75 *br s*	1.75 *d* (~1)	1.75 *br s*	1.76 *br s*	1.76 *br s*	1.75 *br s*	1.75 *d* (~1)	1.75 *d* (~1)
H-19	1.33 *s*	1.25 *s*	1.30 *s*	1.30 *s*	1.25 *s*	1.36 *s*	1.72 *s*	2.22 *s*	1.34 *s*
H-20	1.74 *br s*	1.75 *br s*	1.74 *d* (~1)	1.75 *br s*	1.81 *br s*	1.82 *s*	1.82 *br s*	—	1.28 *s*
OAc	2.07 *s*	2.06, 2.08 *s*	2.05, 2.07, 2.13 *s*	2.04, 2.07, 2.13, 2.17 *s*	2.08 *s*	2.07 *s*	2.08 *s*	2.07 *s*	2.07 *s*

*Figures in parentheses are coupling constants or line separations (*J*) in Hz.

(Table 1) and the mass spectrum of which were in full accord with structure **2**. Oxidation of the allylic alcohol at C-1 in **1a** with activated manganese dioxide did not provide the expected aldehyde but gave the furano-aldehyde **3**, the formation of which can be formulated as resulting from initial oxidation of the OH-1 group to the aldehyde which, subsequently, undergoes a conjugate addition reaction involving the OH-6 group to form the tetrahydrofuran derivative **3**.

All the chemical and ^{1}H NMR spectral data of the novel 1,6,7-trihydroxy-17-acetoxymelcantholide are in good agreement with the skeletal arrangement given in structure **1a**. The major mass spectral fragmentation pattern of **1a** is outlined in Fig. 1 and supports the above assignments. The stereochemistries at the Δ^2- and Δ^{14}-double bonds were assigned a Z-configuration based on ^{1}H NMR arguments which were obtained from ^{1}H NMR spectral comparisons with model compounds that were discussed in detail in our two previous papers [2, 3]. The configurations of the stereochemical centres C-6, C-7 and C-9 in **1a** could not be derived from the above spectral considerations and remain open.

The ^{1}H NMR spectrum of subsequent fractions indicated mixtures of **1a**, **4a** and a new sesquiterpene dilactone, the ^{1}H NMR spectral data of which suggested the presence of the 4(5)-dihydro derivative of cinerenin [5]. Since attempted TLC separations were without success, the mixture was acetylated and the acetates separated by prep. TLC. The ^{1}H NMR data of 4(5)-dihydromelampodin acetate (**4b**) are summarized in Table 2. The structure of 4(5)-dihydrocinerinin acetate (**4e**) was deduced mainly from the ^{1}H NMR and mass spectral data by comparison with the spectral parameters of **4b** and cinerinin [5]. The mass spectrum of **4e** showed a molecular ion at *m/z* 364. Further peaks at 304 $[M-HOAc]^+$, 258 $[M-HOAc-EtOH]^+$ and 140 $[E]^+$ and diagnostic ^{1}H NMR signals indicated the presence of an ethyl and an acetyl moiety. The ^{1}H NMR spectral assignments, which were confirmed by decoupling experiments, also suggested that the ethoxy group in **4e** be attached to C-1, as indicated by a doublet of doublets at δ4.21 (J = 11, 5 Hz), the chemical shift indicating an ether proton at C-1 in contrast to a C-1 ester proton at δ5.38 in **4b**. Since the chemical shifts and couplings of the rest of the medium ring proton absorptions of this new lactone were nearly identical with those of **4b**, we suggest structure **4e**.



Fragment	*m/z* (rel int.)	Fragment	*m/z* (rel int.)
$[A]^+$	325 (-)	$[B'-2H_2O]^+$	93 (65)
$[A-AcOH]^+$	265 (2)	$[C'-2H_2O]^+$	137 (28)
$[B]^+$	281 (2)	$[D'-3H_2O]^+$	133 (52)
$[B-AcOH]^+$	221 (57)	$[D-CO_2]^+$	179 (18)
$[C]^+$	237 (1)	$[A'-H_2O]^+$	67 (33)
$[C-AcOH]^+$	177 (27)	$[-CH_2A'-H_2O]^+$	81 (46)
$[DH]^+$	224 (11)	$[B'-H_2O]^+$	111 (55)
$[DH-AcOH]^+$	164 (77)		

Fig. 1. Mass spectral fragmentation of **1a**

From a later collection of the same population of *M. leucanthum*, an additional sesquiterpene lactone was isolated besides the compounds described above. Major portions of the ^{1}H NMR spectrum of this new lactone

Table 2. ^{1}H NMR spectral data for the leucantholides **4a–4c**, **4e** and **4f** (200 MHz, $CDCl_3$, TMS as int. standard)*

	4a	**4c**	**4b**	**4e**	**4f**
H-1	5 41 *dd* (11, 5)	5 40 *dd* (11, 5)	5.38 *dd* (11, 5)	4.21 *dd* (11, 5)	5.37 *dd* (11, 5)
H-2a	2.10 *m*	—	—	~2.02 *m*	2.02 *m*
H-2b	1.68 *m*	—	—	—	~1.65 *m*
H-3a	1.8–1 9 *m*	2.19 *m*	—	—	—
H-3b	1 20 *tt* (13, 4.5)	1.25 *m*	1.20 *m*	1.12 *m*	—
H-5a	2.03 *m*	—	—	~1.95 *m*	—
H-5b	1 43 *ddd* (17, 11.5, ~2)	1.55 *dd* (17, 11)	1.43 *ddd* (16, 11.5, ~1.5)	1.42 *dd* (16, 11)	1.43 *dd* (16, 11)
H-6	3 92 *ddd* (10, 7, ~2)	3.80 *dd* (10, 7)	3.78 *dd* (9.5, 7)	3.77 *dd* (9, 8)	3.81 *ddd* (10, 6, ~1.5)
H-7	3 10 *dq* (10, ~3)	3.13 *dq* (10, ~3)	3.10 *dq* (9.5, 3)	3.09 *dq* (9 5, 3)	3.10 *dq* (10, 3)
H-8	5.60 *br s*	5.62 *br s*	5.60 *br s*	5 62 *br s*	5.59 *br s*
H-9	7.49 *d* (~1.5)	7.40 *d* (~1 5)	7.50 *d* (~1.5)	7.52 *d* (~1.5)	7.46 *d* (~1 5)
H-13a	6 47 *d* (3)	6 49 *d* (3)	6.46 *d* (3)	6.47 *d* (3)	6.44 *d* (3)
H-13b	5.82 *d* (3)	5 85 *d* (3)	5.82 *d* (3)	5.82 *d* (3)	5.80 *d* (3)
H-15(2H)	3 47 *br t* (5)†	4 10 *d* (6)†	3.86 *d* (7)†	3.85 *dd* (7, 3)†	3.45 *m*†
OAc	2.09 *s*	2 07 *s*	2.07, 2.06 *s*	2.06 *s*	—
R	—	—	—	3.46 *m*†	2 55 *hept* (7)
				1 28 *t* (7)	1.17, 1.16 *d* (7)

*Figures in parentheses are coupling constants or line separations (J) in Hz.
†Centre of non-first order AB pattern.

were nearly identical with the spectral data obtained for 4(5)-dihydromelampodin B (**4a**). The mass spectrum showed a molecular ion at *m/z* 364. Further peaks at 276 $[M-(Me)_2CHCOOH]^+$ and 71 $[(Me)_2CHCO]^+$ and diagnostic ^1H NMR signals, namely a heptet at δ2.55 ($J = 7$ Hz) and two methyl doublets at δ1.17 and 1.16 ($J = 7$ Hz), indicated the presence of an isobutyrate moiety at C-1. This suggests structure **4f** for the new lactone which represents the 4(5)-dihydro derivative of melampodin C [5].

Since the chemical shifts and couplings of the medium ring proton absorptions of the two new dilactones **4d** (identified as **4e**) and **4f** were nearly identical with the spectral parameters of **4b**, their configurational and conformational structures must be the same as 4(5)-dihydromelampodin B (**4b**), the molecular structure of which was established in the course of this study and will be discussed below.

Crystal structure analysis of 4(5)-*dihydromelampodin B* (**4a**).

The structure of 4(5)-dihydromelampodin B is illustrated in Fig. 2. It is nearly identical to that of the previously described melampodin B [6] and will, thus, not be discussed in detail here. The only notable conformational change brought about by hydrogenation is an increase in the torsion angle about C-4–C-5 from 1° to 65° and concomitant changes in torsion angles about the bonds flanking C-4–C-5. The remainder of the molecule is, essentially, unaffected with bond distances (estimated standard deviations 0.004–0.006 Å) and angles (estimated standard deviations 0.2–0.4°) showing excellent agreement. The lactone ring at C-8–C-10 in 4(5)-dihydromelampodin B is slightly less planar, with the sum of five endocyclic torsion angle magnitudes 29°, vs. 12° for melampodin B. The intermolecular hydrogen bond, which has an O . . . O distance of 2.851(4)Å in melampodin B, is shortened to 2.804(4)Å in crystals of the dihydro compound, with H. . . . O distance 1.85 Å and O–H . . . O angle 175°.

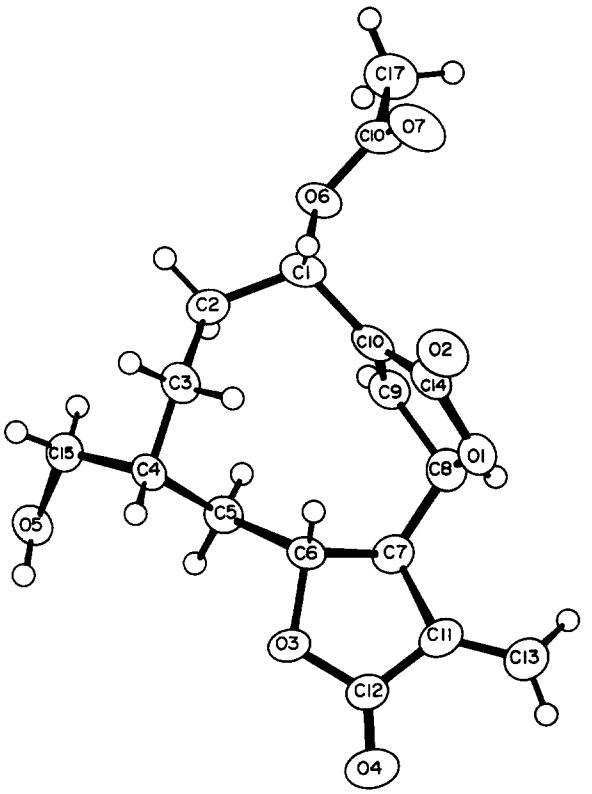


Fig. 2 The β-face of 4(5)-dihydromelampodin B.

EXPERIMENTAL

Melampodium leucanthum Torr. and Gray was collected on 18 June 1973 on the west side of Mount Bonnell in Austin, Texas, U.S.A. (Sanderson 438 E, voucher deposited at the University of Texas, U.S.A.). The crude syrup (9.0 g), preadsorbed on 30 g silica gel (35–70 mesh), was chromatographed on a column packed with 250 g silica gel (70–230 mesh) using $CHCl_3$ and mixtures of $CHCl_3$–Me_2CO (5, 10, 20, 40, 80%) as eluant; 100 ml fractions were taken and monitored by TLC. Fraction 4 crystallized from $CHCl_3$–Et_2O to give 490 mg of a mixture of leucanthin A and melampodin A acetate (3:2) [1]. Fraction 6 provided 120 mg melampolidin [4] and fraction 13 gave leucanthinin [4]. Fractions 37–39 (218 mg) were rechromatographed over 10 g silica gel. After TLC purification (Et_2O, ×4) of the polar fractions, 55 mg of the diterpene lactone **1a** was obtained. Crystallization of fraction 42 with $CHCl_3$ gave 20 mg 4(5)-dihydromelampodin B (**4a**). TLC purification of the mother liquor of fraction 42 (Et_2O, ×7) provided 45 mg **1a**. Fraction 43, after acetylation followed by prep. TLC (CH_2Cl_2–Me_2CO, 19:1, ×3) gave the acetates **1b**–**1d**. Further TLC purification of the triacetate **1c** (Et_2O–petrol, 4:1, ×3) allowed the isolation of 4(5)-dihydromelampodin B acetate (**4b**) and the new sesquiterpene lactone 4(5)-dihydrocinerenin (**4d**) which was identified as its acetate (**4e**).

1,6,7-*Trihydroxy*-17-*acetoxymelcantholide* (**1a**). Gum, $C_{22}H_{34}O_7$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1750, 1420; EIMS (probe) *m/z* (rel int.): 368 $[M-CH_2CO]^+$ (0.4), 350 $[M-HOAc]^+$ (1), 332 $[M-HOAc-H_2O]^+$ (1), 314 $[M-HOAc-2H_2O]^+$ (1), 296 $[M-HOAc-3H_2O]^+$ (0.4), 281 $[B]^+$ (2), 265 $[A-HOAc]^+$ (2), 251 $[296-CO_2H]^+$ (5), 224 $[DH]^+$ (11), 221 $[B-HOAc]^+$ (57), 203 $[B-HOAc-H_2O]^+$ (24), 179 $[D-CO_2]^+$ (18), 177 $[C-HOAc]^+$ or $[B-HOAc-CO_2]^+$ (27), 164 $[DH-HOAc]^+$ (77), 163 $[D-HOAc]^+$ (25), 159 $[B-HOAc-H_2O-CO_2]^+$ (18), 137 (28), 135 (49), 133 (52), 121 (34), 111 (55), 105 (30), 93 (65), 91 (43), 81 (46), 79 (36), 69 (31), 68 (32), 67 (33), 55 (33), 43 (100).

6,7-*Dihydroxy*-1,17-*diacetoxymelcantholide* (**1b**). Gum, $C_{24}H_{36}O_8$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730 (br), 1600, 1230; EIMS (probe) *m/z* (rel. int.): 332 $[M-2HOAc]^+$ (0.6), 281 $[B]^+$ (2), 224 $[DH]^+$ (3), 221 $[B-HOAc]^+$ (17), 203 $[B-HOAc-H_2O]^+$ (6), 177 $[C-HOAc]^+$ or $[B-HOAc-CO_2]^+$ (7), 164 $[DH-HOAc]^+$ (9), 163 $[D-HOAc]^+$ (6), 161 (9), 159 (5), 137 (7), 135 (8), 133 (8), 121 (7), 111 (17), 109 (14), 105 (7), 93 (12), 91 (8), 81 (17), 79 (11), 69 (10), 68 (8), 67 (11), 55 (15), 43 (100).

7-*Hydroxy*-1,6,17-*triacetoxymelcantholide* (**1c**). Gum, $C_{26}H_{38}O_9$, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3500, 1750, 1730, 1600, 1230; EIMS (probe) *m/z* (rel. int.): 392 $[M-HOAc-CH_2CO]^+$ (0.5), 374 $[M-2HOAc]^+$ (0.5), 314 $[M-3HOAc]$ 296 $[M-3HOAc-H_2O]^+$ (0.5), 281 $[B]^+$ (1), 251 $[M-3HOAc-H_2O-CO_2H]^+$ (1), 221 $[B-HOAc]^+$ (9), 203 $[B-HOAc-H_2O]^+$ (3), 177 $[B-HOAc-CO_2]^+$ (4), 164 $[DH-HOAc]^+$, 163 $[D-HOAc]^+$ (4), 161 (5), 159 (3), 137 (11), 135 (7), 133 (8), 121 (6), 119 (6), 111 (5), 109 (10), 105 (6), 93 (12), 91 (9), 81 (15), 79 (13), 69 (7), 68 (8), 67 (11), 55 (10), 43 (100)

1,6,7,17-*Tetra-acetoxymelcantholide* (**1d**). Gum, $C_{28}H_{40}O_{10}$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1730, 1600, 1230; EIMS (probe) *m/z* (int. rel.): 392 $[M-HOAc-2CH_2CO]^+$ (1), 374 $[M-2HOAc-CH_2CO]^+$ (1), 332 $[M-2HOAc-2CH_2CO]^+$ (0.5), 314 $[M-3HOAc-CH_2CO]^+$ (2), 279 $[M-3HOAc-OAc]^+$ (1), 296 $[M-4HOAc]^+$ (0.6), 281 $[B]^+$ (1), 251 $[M-4HOAc-CO_2H]^+$ (2), 221 $[B-HOAc]^+$ (14), 203 $[B-HOAc-H_2O]^+$ (5), 177 $[B-HOAc-CO_2]^+$ (7), 164 $[DH-HOAc]^+$, (7), 163 $[D-HOAc]^+$ (6), 161 (8), 159 (6), 137 (12), 135 (8), 133 (9), 121 (9), 119 (8), 111 (7.4), 109 (15), 107 (7), 105 (6), 95 (11), 93 (13), 91 (9), 81 (7), 79 (11), 69 (8), 68 (7), 67 (8), 55 (13), 43 (100).

Oxidation of **1a** *with periodic acid.* A satd soln of H_5IO_6 in Et_2O (2 ml) was added to a soln of **1a** (15 mg) in 1 ml Et_2O, the reaction being monitored by TLC. When the reaction was completed the mixture was diluted with Et_2O, washed with H_2O, dried and the solvent evaporated under red. pres. The residue was purified by TLC (CH_2Cl_2–Me_2CO, 9:1) giving 12 mg **2**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1720; EIMS (probe) *m/z* (rel. int.): 280 $[M]^+$ (0.4), 238 $[M-CH_2CO]^+$ (4), 220 $[M-HOAc]^+$ (31), 205 $[M-HOAc-Me]^+$ (13), 202 $[M-HOAc-H_2O]^+$ (28), 177 $[M-HOAc-MeCO]^+$ (27), 163 $[M-HOAc-CH_2COMe]^+$ (28), 162 (100), 160 (43), 159 (76), 149 (43), 145 (35), 133 (64), 131 (92), 117 (94), 105 (49), 91 (57), 43 (58).

Oxidation of **1a** *with* MnO_2. To a soln of **1a** (23 mg) in CH_2Cl_2, 100 mg activated MnO_2 was added and stirred for 20 hr. The MnO_2 was filtered; the solvent evaporated and the residue separated by TLC (CH_2Cl_2–Me_2CO, 9:1). The less polar compound was identified as **2**, obtained previously from the oxidation with H_5IO_6. The more polar compound was an aldehyde (**3**), gum, $C_{22}H_{32}O_7$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730 (br), 1600, 1230; EIMS (probe) *m/z* (rel. int.): 365 $[M-43]^+$ (0.3), 364 $[M-CO_2]^+$ (0.5), 306 $[M-C_2H_3O-CH_2CO]^+$ (2), 305 $[M-OAc-CO_2]^+$ (2), 304 $[M-HOAc-CO_2]^+$ (5), 289 $[M-HOAc-CO_2-Me]$ (1), 281 $[B]^+$ (2), 237 $[C]^+$ (1), 221 $[B-HOAc]^+$ (43), 203 $[B-HOAc-H_2O]^+$ (10), 185 $[D'']^+$ (2), 177 $[C-HOAc]^+$ (5), 171 $[C'']^+$ (3), 164 $[D-OAc]^+$ (10), 163 $[D-HOAc]^+$ (8), 161 (14), 159 (5), 135 (11), 133 (13), 127 $[B'']^+$ (42), 121 (7), 119 (6), 111 (4), 109 (28), 107 (6), 105 (7), 95 (7), 93 (7), 91 (12), 81 (61), 75 (15), 69 (11), 68 (10), 67 (11), 55 (21), 43 (100).

4(5)-*Dihydromelampodin B acetate* (**4b**). $C_{19}H_{22}O_8$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1735, 1600, 1230; EIMS (probe) *m/z* (rel int.): 378 $[M]^+$ (4), 336 $[M-CH_2CO]^+$ (1), 318 $[M-HOAc]^+$ (1), 276 $[M-HOAc-CH_2CO]^+$ (7), 258 $[M-2HOAc]^+$ (6), 240 (5), 230 $[M-2HOAc-CO]^+$ (10), 214 $[M-HOAc-CO_2]^+$ (3), 212 $[C_{14}H_{12}O_2]^+$ (5), 162 (6), 149 (7), 123 (6), 111 (9), 91 (8), 79 (7), 67 (7), 55 (8), 43 (100).

4(5)-*Dihydrocinerenin acetate* (**4e**). $C_{19}H_{24}O_7$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1735, 1600, 1240; EIMS (probe) *m/z* (rel. int.): 364 $[M]^+$ (0.6), 336 $[M-CO]^+$ (2), 322 $[M-CH_2CO]^+$ (4), 304 $[M-HOAc]^+$ (14), 286 $[M-HOAc-H_2O]^+$ (6), 276 $[M-HOAc-CO]^+$ (5), 258 $[M-HOAc-EtOH]^+$ (8), 240 $[M-HOAc-EtOH-H_2O]^+$ (7), 234 (9), 230 $[M-HOAc-EtOH-CO]^+$ (6), 215 (5), 214 (4), 206 (13), 179 (18), 178 (27), 140 $[E]^+$ (37), 112 $[E-CH_2{=}CH_2]^+$ (75), 111 $[E-Et]^+$ (21), 97 (18), 83 (17), 68 (18), 55 (23), 43 (100).

4(5)-*Dihydromelampodin C* (**4f**). Gum, $C_{19}H_{24}O_7$. Compound **4f** was isolated from the same population of *M. leucanthum*, collected on 7 July 1973 (Sanderson, 438F). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1760, 1735; EIMS (probe) *m/z* (rel. int.): 364 $[M]^+$ (3), 293 $[M-iso\text{-}Bu]^+$ (0.6), 276 $[M-iso\text{-}BuOH]^+$ (1), 258 $[M-iso\text{-}BuOH-H_2O]^+$ (2), 240 $[M-iso\text{-}BuOH-2H_2O]^+$ (2), 230 $[M-iso\text{-}BuOH-H_2O-CO]^+$ (7), 214 $[M-iso\text{-}BuOH-H_2O-CO_2]^+$ (2), 213 (4), 212 $[C_{14}H_{12}O_2]^+$ (3), 111 (17), 71 $[iso\text{-}Bu]^+$ (100), 43 $[Me\text{–}C{\equiv}O]^+$ (36).

X-Ray data of 4(5)-*dihydromelampodin B.* A crystal 0.16 × 0.40 × 0.40 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with MoKα radiation (λ = 0.71073 Å) and a graphite monochromator. Crystal data are: $C_{17}H_{20}O_7$, M_r = 336.3, monoclinic space group $P2_1$, a = 9.512(3), b = 7.398(1), c = 12.341(3) Å, β = 106.24(3)°, Z = 2, d_c = 1.340 g/cm^3, μ(MoKα) = 0.98 cm^{-1}. Data were collected by $\omega-2\theta$ scans of variable speed, designed to yield $I = 50\sigma(I)$ for all significant reflections. One quadrant of data having $1° < \theta < 25°$ was measured at 23°, yielding 1625 unique reflections, of which 1244 had $I > 3\sigma(I)$, and were used in the refinement. Data reduction included corrections for background, Lorentz and polarization effects; absorption effects were negligible.

The similarity of the unit cell dimensions and diffraction patterns of melampodin B and 4(5)-dihydromelampodin B indicate near isomorphism and coordinates of 20 atoms of melampodin B were successfully used as an initial phasing model. Refinement was by full matrix least-squares with wts $w = \sigma^{-2}(F_0)$, treating non-hydrogen atoms anisotropically Hydrogen atoms were located by difference maps and included as

Table 3. Coordinates for no-hydrogen atoms. 4(5)-Dihydromelampodin B

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
O-1	0.7328 (2)	−0.1377*	0.1195 (2)	C-6	0.7880 (3)	0.0683 (6)	0.3330 (3)
O-2	0.6060 (3)	0.0473 (5)	−0.0150 (2)	C-7	0.8148 (3)	−0.1356 (6)	0.3244 (3)
O-3	0.9324 (2)	0.1429 (4)	0.3917 (2)	C-8	0.7116 (4)	−0.2220 (6)	0.2200 (3)
O-4	1.1663 (3)	0.0719 (5)	0.4209 (2)	C-9	0.5553 (3)	−0.1794 (6)	0 2128 (3)
O-5	0.5887 (2)	0.3489 (5)	0.5555 (2)	C-10	0.5023 (3)	−0.0630 (6)	0 1305 (3)
O-6	0.2388 (2)	−0.0734 (4)	0.0928 (2)	C-11	0.9760 (3)	−0 1432 (8)	0.3321 (3)
O-7	0 2165 (3)	−0.0978 (5)	−0 0919 (2)	C-12	1.0386 (3)	0.0297 (7)	0.3846 (3)
C-1	0.3637 (3)	0.0471 (6)	0.1077 (3)	C-13	1.0495 (4)	−0.2693 (8)	0.2966 (3)
C-2	0.3672 (3)	0.1662 (6)	0.2090 (3)	C-14	0.6113 (4)	−0.0377 (6)	0.0678 (2)
C-3	0.4971 (4)	0.3016 (6)	0.2373 (3)	C-15	0.5077 (3)	0.3518 (7)	0.4398 (3)
C-4	0.5969 (3)	0.2994 (6)	0.3596 (3)	C-16	0.1792 (4)	−0.1396 (7)	−0.0104 (3)
C-5	0.6776 (3)	0.1211 (6)	0.3954 (2)	C-17	0.0600 (4)	−0.2691 (8)	−0.0119 (4)

Estimated standard deviations in the least significant digits are shown in parentheses.
*The *y*-coordinate of O-1 was fixed to define the origin of the unit cell.

fixed contributions. Convergence was achieved with $R = 0.038$, $R_w = 0.050$, maximum residual density 0.18 e/Å^3 and extinction coefficient $1.2(3) \times 10^{-6}$. Coordinates are given in Table 3

Acknowledgements—We thank Dr. Sanderson for plant collections. This research was supported, in part, by USDA–SEA cooperative agreement No. 58-7B30-2-399.

REFERENCES

1. Fischer, N. H., Wiley, R. A., Lin, H. N., Karimian, K. and Politz, S. M. (1975) *Phytochemistry* **14**, 2241
2. Quijano, L. and Fischer, N. H. (1984) *Phytochemistry* **23**, 833.
3. Quijano, L. and Fischer, N. H. (1984) *Phytochemistry* **23**, 829.
4. Fischer, N. H., Wiley, R. A., Perry, D. L. and Haegele, K. D (1976) *J. Org Chem.* **41**, 3956.
5. Perry, D. L. and Fischer, N. H. (1975) *J. Org. Chem.* **40**, 3480.
6. Fronczek, F. R., Watkins, S. F., Fischer, N. H and Klimash, J. W. (1980) *J Chem. Soc. Perkin Trans.* 2, 1425.