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A GUAIANOLIDE AND FOUR MELAMPOLIDES FROM *MELAMPODIUM LEUCANTHUM*

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Key Word Index—*Melampodium leucanthum*; Asteraceae; Heliantheae; sesquiterpene lactones; guaianolide dilactone; austinolide; melampolides; 4-epimelnerins A and B; 3α-hydroxyenhydrin; 9-desacetoxyleucanthinin.

Abstract—The aerial parts of *Melampodium leucanthum* from central Texas afforded a new guaianolide dilactone, which was named austinolide. Its structure was determined by spectral methods and the molecular structure was established by single crystal X-ray diffraction. In addition, two new 4βH-dihydrogermacranolides, 4-epimelnerins A and B, and two new melampolides, 3α-hydroxyenhydrin and 9-desacetoxyleucanthinin, were isolated. The aerial parts of a mountain population of *M. leucanthum* from Colorado afforded the known melampolides melampodin A acetate, leucanthin B and leucanthinin as well as 3α-hydroxyenhydrin. Peracid oxidation of leucanthinin provided the 4,5-monoepoxide derivative, the physical data of which were identical with 3α-hydroxyenhydrin.

INTRODUCTION

In our biochemical systematic study of the genus *Melampodium*, we have previously reported on the isolation and structure elucidation of melampolide-type sesquiterpene lactones and biogenitically related mono- and dilactones [1]. Here we describe the isolation of sesquiterpene lactones from the aerial parts of *M. leucanthum* obtained from a central Texas collection near Austin, Texas and a mountain population from Colorado. From the Texas collection, leucanthin A [2], melampodin A acetate [2], melampolidin [3] and leucanthinin (**7**) [3] as well as the diterpene lactones 17-acetoxyacanthoaustolide [4], 1,17-diacetoxy-5,6-dihydroxymelcantholide and 17-acetoxy-1,6,7-trihydroxymelcantholide [5] were isolated. In addition, we obtained a new guaianolide dilactone, austinolide (**1**), two new 4βH-dihydrogermacranolides, 4-epimelnerins A (**2**), B (**3**) as well as the C-4 epimer of 15-hydroxy-4α,15-dihydrorepandanolide-8-*O*-angelate (**4**) [6]. Furthermore, two new melampolides, 3α-hydroxyenhydrin (**5**) and 9-desacetoxyleucanthinin (**6**), were obtained. The Colorado collection contained melampodin A acetate [2], leucanthin B [2], leuncanthinin [3] and 3α-hydroxyenhydrin (**5**). The structures of the known and new compounds were determined by spectral methods as well as chemical correlation. The molecular structure of austinolide (**1**) was obtained by single crystal X-ray diffraction.

RESULTS AND DISCUSSION

Austinolide (**1**) $C_{22}H_{26}O_9$, a crystalline sesquiterpene dilactone, was isolated from fractions of intermediate polarity of the Texas *M. leuncanthum* terpenoid extract. The ^1H and ^{13}C NMR spectra showed signals typical of an α-methylene-γ-lactone (δ 6.33 *d*, 5.73 *d*, $J = 3.0$ Hz, and 134.05 *s*, 122.65 *t*). Irradiation of the H-13 signals located H-7 at δ 3.15 (*br d*, $J = 9.2$ Hz) and a broad signal at δ 5.91 ($W_{1/2} = 8$ Hz) was coupled to H-7 and was assigned to H-8. Based on chemical shift arguments, it had to be on a carbon bearing an ester side chain. The H-8 signal was further coupled to two doublets of doublets of doublets at δ 2.42 ($J = 15.2, 1.9, 1.9$ Hz) and 1.82 ($J = 15.3, 12.2, 3.4$ Hz) which are assigned to the two C-9 protons. The ester side chain was identified as a 3-acetoxy-2-hydroxy-2-methyl butyrate (**A**) by the diagnostic ^1H and ^{13}C NMR signals [3, 7] and mass spectral pattern with peaks at *m/z* 176 $[AH]^+$ and 258 $[M–AH]^+$ in support of the presence of this ester group. Irradiation of the broad multiplet at δ 5.69 ($W_{1/2} = 4$ Hz) affected a broadened doublet at δ 5.42 ($J \approx 7.0$ Hz) and a vinyl methyl signal at 2.00 (*q*, $J = 1.5$ Hz), allowing assignments of these signals as the olefinic H-3, the lactonic H-2 and the methyl group

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at C-4, respectively. Irradiation at δ 5.42 (H-2) affected a multiplet at δ 3.04 which was assigned to H-1. A doublet of doublets of doublets at δ 2.58 (J = 11.5, 11.5, 1.3 Hz) must be due to H-10, since irradiation of this signal affected H-1 and the two H-9 multiplets. The triplet at δ 4.55 (J = 9.1 Hz) was assigned to the lactonic H-6, since double resonance experiments affected H-7. Saturation of H-7 also affected an overlapping signal at δ 3.09, which was therefore assigned to H-5. In accord with structure **1**, the ^{13}C NMR DEPT experiments showed 22 carbon signals with four methyl, two methylene and nine methine carbon absorptions (see Table 2).

As in all sesquiterpene lactones from higher plants, an α-oriented H-7 in **1** was assumed [1]. Large coupling values (J = >9 Hz) for H-5, H-6 and H-7 suggested antiperiplanar orientations with an H-7α, H-6β, and H-5α. Therefore, the NOE between H-6 and H-10 suggested that H-10 is also β-oriented. Furthermore, the NOE between H-2 and H-5 as well as H-2 and H-1 were in accord with an α-orientation of these protons. The molecular structure and relative stereochemistry of **1** was further confirmed by single crystal X-ray diffraction, details of which will be presented at the end of this section.

4-Epimelnerins A(**2**) and B(**3**) and the C-4 epimer (**4**) of a melnerin-type compound [6] were isolated as a mixture which were separated by reverse phase HPLC. The three compounds showed very similar ^{1}H and ^{13}C NMR spectra for the medium ring portion and only differed in the absorptions due to the ester side chain.† The ^{1}H NMR spectrum of **2** obtained at 55° closely resembled that of melnerin A (**2a**) [9, 10], the molecular structure of which is known.

As in melnerin A, it showed two proton signals typical of the hydroxymethylene group at C-4 as a non-first-order AB pattern centred at δ 3.47. The H-4 multiplet, which appeared at δ 1.5 in melnerin A (**2a**), absorbed at δ 1.9 in **2**. The proton signals due to H-1, H-8 and H-6 gave similar chemical shifts but different coupling constants, suggesting different conformations of the flexible medium rings in **2** and **2a**. Furthermore, at ambient temperature the ^{1}H NMR spectrum of **2**–**4** showed a considerable broadening of signals. At lower temperatures additional signals appeared, indicating the presence of different conformations of these medium rings.

† It was pointed out by one of the reviewers of this paper that the ^{1}H NMR spectral data of compound **4** described in this paper was identical with the spectrum obtained in $CDCl_3$ of a lactone previously reported by Bohlmann *et al.* [8]. The previous ^{1}H NMR spectrum of this compound (lactone **25** in ref. [8] had been obtained in C_6D_6 at 75° and it was reported as a melnerin-type compound from *Smallanthus uvedalia*. Based on the above findings and the referee's comments, the stereogenic centre at C-4 in lactone **25** in ref. [8] should be revised to 15-hydroxy-4βH,15-dihydrorepandanolide-8-*O*-angelate (**4**) [6].

3α-Hydroxyenhydrin (**5**) was isolated from the Colorado collection as a mixture with leucanthinin (**7**). COSY and multipulse DEPT experiments aided the assignments of the spectral patterns of the lactone mixture. One set of signals corresponded to the values previously reported for leucanthinin [3] (Table 1). The other component differed from leucanthinin (**7**) by an upfield shift of the olefinic H-5 from δ 5.53 in **7** 3.17 in **5** and the signal for the C-4 methyl (H-15) was shifted from δ 1.97 to 1.71, respectively. These values strongly suggested that the C-4 double bond in **7** was replaced by an epoxide function in **5**. This was confirmed by peracid epoxidation of a mixture of **5** and **7** [2], the progress of the reaction being followed by ^{1}H NMR. The disappearance of the doublet at δ 5.53 (H-5) in favour of the doublet at δ 3.17 was complete after 28 hr, after which only signals for the new lactone **5** remained. Based on previous stereospecific transformations of melampolides to their 4-epoxides [2], the new compound must represent the 4-epoxide of leucanthinin (**5**). These assignments were supported by 2D ^{1}H NMR COSY and heteronuclear ^{1}H-^{13}C correlation experiments. The stereochemistry of the epoxide ring is in agreement with an attack from the outer face of the conformationally fixed C-4 double bond in **7** [2]. The relative stereochemistry at the carbon centres C-4 to C-9 of lactone **5** was based on comparison with the coupling constants for H-4 to H-9 of leucanthin **B**, which were in very good agreement. The configuration of the hydroxyl group at C-3 was assigned α based on coupling values for H-1 to H-3. This was further confirmed by a TAI-induced ^{1}H NMR shift experiment [11], which produced the expected downfield shift of $\Delta\delta$ 1.2 for H-3 along with a shift for H-2α from δ 3.10 to 3.38.

9-Desacetoxyleucanthinin (**6**), $C_{21}H_{26}O_6$, was a gum with ^{1}H NMR signals very similar to those of leucanthinin [3]. The most evident differences between the ^{1}H NMR spectra of the two compounds were due to H-9. Compound **6** lacked the acetate methyl signal and the downfield doublet expected for a proton (H-9) attached to the carbon bearing the acetoxy group. Due to the lack of the deshielding acetate carbonyl at C-9, the H-8 doublet of doublet of doublets in **6** appeared slightly upfield at δ 6.36 (J = 9.8, 7.9, 1.5 Hz) when compared with the H-8 doublet of doublets at δ 6.65 in leucanthinin [3]. Furthermore, in **6** the signal at δ 6.36 (H-8) was coupled with two multiplets at δ 2.85 and 1.93, which based on COSY data could be assigned to the two methylene protons at C-9. These results were also supported by the ^{13}C NMR spectrum (Table 2), which showed two aliphatic methylene carbon signals in the DEPT spectrum, in accord with the structure of **6**.

The crystal structure of austinolide (**1**)

The crystal structure of **1** is illustrated in Fig. 1, in which the guaianolide skeleton is evident. The seven-membered ring is seen to be best described as the

Table 1. ^{1}H NMR data for austinolide (**1**), 4-epimelnerins A (**2**) and B (**3**), **4**, 3α-hydroxyenhydrin (**5**) and 9-desacetoxyleucanthinin (**6**)*

H	1	2	3	4	5	6
1	3.04 *m*†‡	6.93 *dd* (10.0, 6.9)	6.92 *dd* (9.4, 7.5)	6.93 *dd* (9.4, 7.5)	7.23 *dd* (10; 7.5)	6.90 *ddd* (9.5, 7.5, 2)
2a					3.11 *ddd* (14; 10; ~1)	2.71 *dd* (13; 8)
	5.42 *dbr* (7)	2.47 *m*	2.48 *m*	2.49 *m*		
2b					2.85 *ddd* (14; 8; 8)	2.17 *dd* (13; 10)
3a	5.69 *m* ($W_{1/2}$ = 4)	2.12 *m*	2.13 *m*	2.12 *m*		
					4.19 *br d* (6)	4.49 *brd* (6)
3b		1.31 *m*	1.32 *m*	1.34 *m*	—	—
4	—	1.93 *m*†	1.92 *m*†	1.90 *m*†	—	—
5a		1.93 *dd* (14.5; 5)†	1.92 *dd* (14.2, 5.3)†	1.93 *m*†		
	3.09†‡				3.18 *d* (9.5)	5.62 *brd* (11)
5b		1.48 *ddd* (15; 9.5; 4)	1.49 *m*	1.50 *ddd* (15; 9.5; 4)		
6	4.55 *t* (9.1)	4.77 *qbr* (~4)	4.74 *qbr* (~4.0)	4.76 *qbr* (~4)	4.39 *t* (9.5)	5.22 *dd* (11; 10)
7	3.15 *dbr* (9.2)	2.83 *mbr*	2.81 *brm*	2.8 *mbr*	3.0 *mbr*†	2.66 *dq* (10; ~2)
8	5.91 *m* ($W_{1/2}$ = 8)	5.52 *m* ($W_{1/2}$ = 18)	5.55 *ddd* (9.3, 5.7, 2.6)	5.59 *m* ($W_{1/2}$ = 18)	6.74 *dd* (10; 1)	6.36 *ddd* (9.8; 8; ~1.5)
9a	2.42 *ddd* (15; 1.9; 1.9)		2.90 *dd* (14; 5.6)			2.85 *ddd* (14; 8; 2.5)
		2.88 *dd* (14; 5.5)		2.80 *tbr* (~9)	5.82 *d* (10)	
9b	1.82 *ddd* (15; 12.2, 3.4)	2.74 *ddbr* (13.5; 9.5)	2.70 *dd br* (13.8, 10)			1.93 *dd* (14; 10.5)
10	2.58 *ddd* (12; 10.8; ~1.3)	—	—	—	—	—
13a	6.33 *d* (3)	6.26 *d* (2.1)	6.27 *d* (2.2)	6.28 *d* (2.1)	6.35 *d* (3.5)	6.24 *d* (3)
13b	5.73 *d* (3)	5.64 *d* (2)	5.64 *d* (1.8)	5.66 *d* (1.8)	5.86 *d* (3)	5.55 *d* (3)
15	2.00 *q* (~1.5)	3.46 ABq‡	3.47 *d* (6)	3.47 AB‡	1.72 *s*	1.88 *d* (1)
OAc	1.94 *s*	—	—	—	2.04 *s*	—
OMe	—	3.80 *s*	3.80 *s*	3.78 *s*	3.82 *s*	3.77 *s*
OCOR	5.12 *q* (6)	2.46 *hept* (7)	2.27 *sext.* (7)	6.04 *qq* (7; ~1.5)	3.01 *q* (5.5)†	3.04 *q* (5.5)
	1.22 *d* (6)	1.07 *d* (7)	1.05 *d* (7)	1.94 *dq* (7; ~1.5)	1.18 *d* (5.5)	1.25 *d* (5.5)
	1.32 *s*	1.10 *d* (7)	0.87 *t* (7.5)	1.79 *quint* (~1.5)	1.44 *s*	1.53 *s*
			1.61, 1.45 *m*			

* Run at 400 MHz in $CDCl_3$ with TMS as int. standard. Figures in parentheses are coupling constants or line separations in Hz; compound **6** was run at 200 Hz.
† Overlapping signals.
‡ Non first order pattern.

Table 2. ^{13}C NMR spectral data of austinolide (**1**), 4-epi-melnerins A (**2**), B (**3**), **4**, 3α-hydroxyenhydrin (**5**) and 9-desacetoxyleucanthinin (**6**)*

C	1	2	3	4	5	6
1	47.2	146.4	146.4	146.2	147.7	139.7
2	77.7	29.3	29.4	29.4	29.9	31.5
3	125.4	27.4	27.5	27.3	69.8	73.7
4	146.9	48.2†	—	—‡	61.4	140.4
5	51.9	30.0	30.0	30.1	57.5	123.3
6	68.5	74.9	74.8	75.1	75.3	75.3
7	47.5	42.0	42.0	42.0	45.6	49.3
8	84.6	79.1	79.1	79.3	74.2	68.1
9	34.3	40.2	40.3	40.3	70.6	30.5
10	37.5	136.2	—‡	136.3	133.1	135.3
11	134.1	127.0	—‡	127.2	130.6	131.9
12	174.1	169.5	—‡	169.4	170.5	170.1
13	122.6	124.0	124.0	123.9	123.0	120.4
14	168.2	167.5	—‡	167.5	165.0	167.1
15	15.4	67.7	67.7	67.7	16.0	16.1
1′	175.8	175.9	—‡	166.4	168.4	168.7
2′	76.4	34.0	41.1	127.2	59.3	59.3
3′	72.9	18.9	26.4	139.6	59.8	59.9
4′	13.4	18.6	11.5	15.7	13.6	13.7
5′	22.9	—	16.5	20.3	19.1	19.2
CO_2Me	—	52.2	52.2	52.1	52.5	52.1
OCOMe	21.0					
OCOMe	169.6					

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT).

† Strongly broadened signal.

‡ Signals not observed due to high dilution of sample.

half-chair with C-5 on the local twofold axis. The cyclopentene ring is in the envelope conformation with C-1 at the flap position, while the lactone at C-6–C-7 is nearest the envelope with C-7 at the flap. The other lactone ring is in a twist-chair conformation with O-3 on the local twofold axis. A search of the Cambridge Structural Database (CSD) [12] for guaianolides containing a lactone at C-2–C-1–C-10 yielded only one structure, that of eufoliatorin [13], which has a C-1=C-10 double bond. Coordinates for that structure are not deposited in the CSD. The structure of anomalide hemihydrate, which has two mixed guaianolides with isomeric sidechains, has been reported [14]. It has a fused tetrahydrofuran unit at C-2-C-1-C-10, lacking the C=O at C-14. The OH group of austinolide is involved in an intermolecular hydrogen bond with acetyl carbonyl atom O-9 of the ester moiety at $x-1, y, z$. The O···O distance of the hydrogen bond is 2.894(2) Å, and the angle about H is 169(3)°. Thus, molecules of austinolide form hydrogen bonded chains in the **a** direction.

Austinolide is only the second guaianolide isolated from the genus *Melampodium*, taxa of which typically contain melampolides and *cis,cis*-germacranolides and their biogenetic derivatives [1]. The guaianolide mikanocryptin was previously reported from *M. divaricatum* [15].

EXPERIMENTAL

Plant material. Melampodium leucanthum Torr. and Gray was collected on 20 August, 1972, 7 miles West of Austin on Ranch Road 2244 (N.H. Fischer no. 24; voucher deposited at LSU, U.S.A.). Crude syrup (10 g) was chromatographed over 200 g of silica gel and eluted with $CHCl_3$ and mixt. of $CHCl_3$–Me_2CO (5, 10, 20, 40, 60 and 80% Me_2CO). Two 250 ml frs of each mixt. were taken and all frs were monitored by TLC. Frs 1 and 2 eluted with $CHCl_3$ and fr. 3 with $CHCl_3$–Me_2CO (19:1), providing mixt. of leucanthin A, mel-

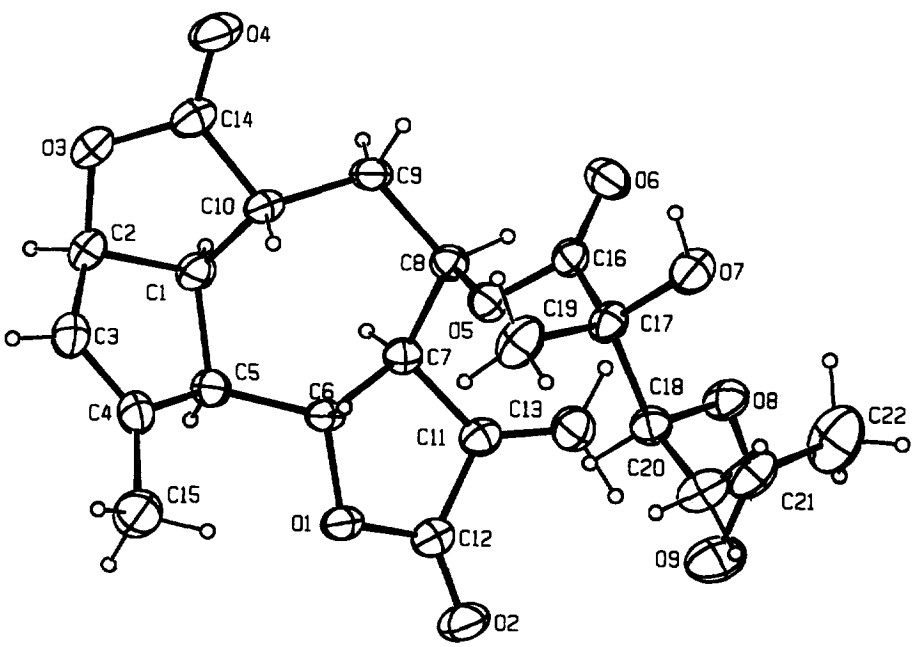
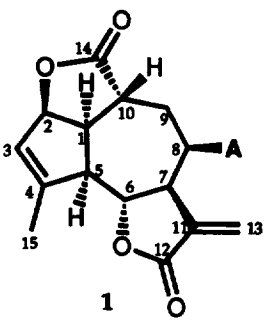
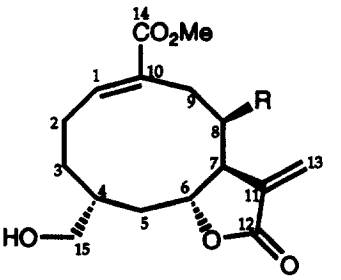


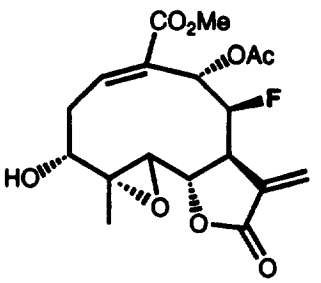
Fig. 1. The molecular structure of austinolide (**1**).



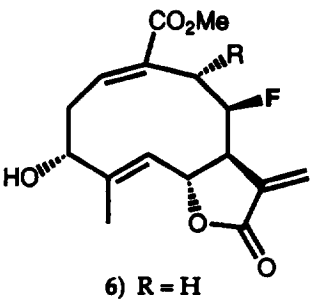
1



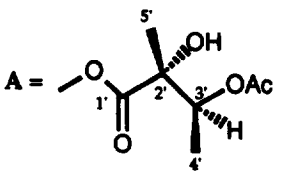
2) R = B
2a) 4-epimer of 2
3) R = D
4) R = E

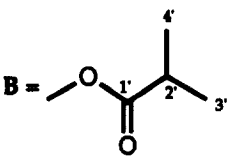


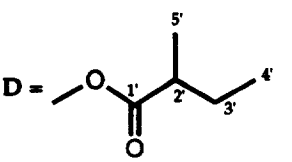
5

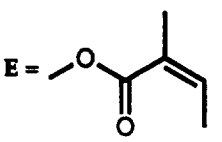


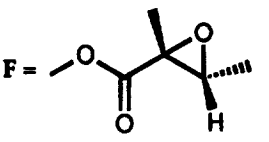
6) R = H
7) R = OAc











ampodin A acetate and melampolidin as shown by ^1H NMR analysis [2, 3]. Fr. 5 (1.15 g), which eluted with $CHCl_3$–Me_2CO (9:1), was chromatographed over 50 g of silica gel using mixtures of $CHCl_3$–EtOAc of increasing polarity, all frs being monitored by TLC. Similar frs were combined and further purified by prep. TLC. Fr. 5-14′-15′ contained melampolidin [3] and prep. TLC of frs 5-16′-19′ and 5-20′-23′ (Et_2O, 2 ×) afforded 3α-hydroxyenhydrin (**5**). From frs 5-32′-34′ and 5-35′-38′ leucanthinin (**7**) [3] and the new melampolide 9-desacetoxyleucanthinin (**5**) were isolated. Prep. TLC of frs 5-32′-34, 5-35′-38′, 5-39′-47′ and 5-48′-56′ (Et_2O, 3 ×) gave a mixt. of **2**, **3**, **4**. Semiprep. C_{18} reverse phase HPLC using MeOH–H_2O (2:3) as mobile phase permitted sepn of **2**, **3** and **4**. From the same frs the dilactone **1** as well as 1,17-diacetoxy-5,6-dihydroxymelcantholide were isolated [5]. Chromatographic fr. 8 was eluted with $CHCl_3$–Me_2CO (4:1) and frs 9 and 10, eluted with $CHCl_3$–Me_2CO (3:2), contained a mixt. of the known diterpene lactones 17-acetoxyacanthoaustralide [4], 17-acetoxy-isoacanthoaustralide [4] and 17-acetoxy-melacantholide [5] (^1H NMR) and 17-acetoxy-1, 6,7-trihydroxymelcantholide [5], as shown by ^1H NMR analysis.

Another collection of *M. leuncanthum* was obtained on 22 May, 1985 in Pueblo County, Colorado; Pueblo Reservoir, Boggs Flat, public use area on the south side of the reservoir at an elevation of about 1450 m. Vouchers are deposited at the Herbarium of Colorado State University (Voucher numbers: CSU 53104 and CSU 53239). Dried ground leaves (340 g) were extracted with CH_2Cl_2 and worked up as described before [2], providing 15 g of crude terpenoid extract.

Part of this extract (7 g) was fractionated by CC on silica gel, eluting with CH_2Cl_2 and mixt. of CH_2Cl_2 with increasing amounts of Me_2CO. After further purification, frs 13–18 provided 500 mg of a crystalline mixt. ($R_f = 0.80$, CH_2Cl_2–Me_2CO; 4:1). Spectral data (^1H and ^{13}C NMR and EIMS) established their identity as melampodin A acetate and leucanthin B [2]. Frs 20–21 gave, after further purification, 150 mg of a lactone mixt. ($R_f = 65$, CH_2Cl_2–Me_2CO; 4:1). ^1H NMR spectral data indicated a 1:1 mixt. of leucanthinin (**7**) and 3α-hydroxyenhydrin (**5**).

Austinolide (**1**). $C_{22}H_{26}O_9$, colourless crystals; mp 238–239°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3670, 3610, 1760, 1740, 1715; EIMS (probe) *m/z* (rel. int.): 416 $[M-H_2O]^+$ (9), 390 $[M-CO_2]^+$ (1), 356 [416 − AcOH]$^+$ (1), 348 [390 − ketene]$^+$ (8), 330 [390 − AcOH]$^+$ (2), 259 [M − A]$^+$ (12), 258 [M–AH]$^+$ (13), 240 [258 − H_2O]$^+$ (11), 214 [390 − AH]$^+$ (14), 212 $[C_{14}H_{12}O_2]^+$ (29), 185 (11), 169 (11), 167 (14), 159 (13), 142 (11), 131 (100), 91 (33), 89 (48), 80 (23), 71 (29), 43 (53).

4-*Epimelnerin A* (**2**). $C_{20}H_{28}O_7$, gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3450, 1755, 1730, 1715, 1640, 1600; EIMS (probe) *m/z* (rel. int.): 348 [M − MeOH] (3), 320 [M–$Me_2C{=}C{=}O$]$^+$ (2.0), 319 (9), 292 [M − BH]$^+$ (0.6), 278 (2), 260 (7), 242 [M − BH − MeOH − H_2O]$^+$ (7), 215 (7), 214 (10), 213 (3.2), 212 $[C_{14}H_{12}O_2]^+$ (1), 149 (21), 71 (100), 43 [Ac]$^+$ (36).

4-*Epimelnerin B* (**3**). $C_{21}H_{30}O_7$, gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1760, 1715, 1640; EIMS (probe) *m/z* (rel. int.): 362 [M − MeOH]$^+$ (2), 334 [M − HCO_2Me]$^+$ (1), 333 (5), 292 [M − DH]$^+$ (0.7), 279 (5), 278 (2), 261 [M − D − MeOH]$^+$ (3), 260 [M − DH − MeOH]$^+$ (6), 243 (2), 242 [M − DH − MeOH − H_2O]$^+$ (7), 215 (5), 214 [M − DH − HCO_2Me − H_2O]$^+$ (8), 213 $[C_{14}H_{13}O_2]^+$ (2), 212 $[C_{14}H_{12}O_2]^+$ (1), 149 (60), 85 (100), 57 (92).

15-*Hydroxy*-4β*H*,15-*dihydrorepandanolide*-O-*angelate* (**4**). $C_{21}H_{28}O_7$, gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1760, 1715, 1600; EIMS (probe) *m/z* (rel. int.): 360 [M − MeOH]$^+$ (0.2), 293 [M − E]$^+$ (2.0), 261 (3), 243 (1.3), 242 [M − EH − MeOH − H_2O]$^+$ (0.6), 233 [M − EH − CO_2Me]$^+$ (1.4), 215 [M–EH–CO_2Me–H_2O]$^+$ (5), 149 (10), 83 (100), 55 (21).

3α-*Hydroxyenhydrin* (**5**). $C_{23}H_{28}O_{11}$, mp 155–160° (dec); IR ν_{max}^{KBr} cm^{-1}: 3536, 3484, 1775, 1744, 1721, 1692, 1264, 1142, 1005 cm^{-1}; EIMS *m/z* (rel. int.): 480 [M]$^+$ (0.4) [M − AcOH]$^+$ (41), 364 [M − FH]$^+$ (100), 305 [M − FH − CO_2Me]$^+$ (36), 304 [M − FH − AcOH]$^+$ (14), 245 (15), 71 (13), 43 [Ac]$^+$ (75).

9-*Desacetoxyleucanthinin* (**6**). $C_{21}H_{26}O_6$, colourless gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1760, 1730, 1710; EIMS (probe) *m/z* (rel. int.): 291 [M − F]$^+$ (2), 290 [M − FH]$^+$ (5), 272 [M − FH − H_2O]$^+$ (4), 244 [M − FH − H_2O]$^+$ (11), 231 [M − FH − CO_2Me]$^+$ (12), 230 (10), 214 (11), 115 (19), 83 (85), 71 (68), 55 (34).

Epoxidation of leucanthinin (**7**). A soln of 100 mg of 1:1 mixt. of **5** and **7** in 2 ml of $CDCl_3$ and 100 mg of *m*-CPBA (85%) was reacted at ambient temp, the reaction being monitored by ^1H NMR. After completion of the reaction (28 hr), usual work up [2] and purification gave 60 mg of lactone **5**.

X-Ray data of austinolide (**1**). A colourless needle of dimensions 0.04 × 0.07 × 0.35 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with CuK_α radiation (λ = 1.54184 Å), and a graphite monochromator. Crystal data are: $C_{22}H_{26}O_9$, M_r = 434.4, orthorhombic space group $P2_12_12_1$, a = 7.6607(8), b = 11.4311(9), c = 23.998(2) Å, V = 2101.6(6) Å^3, Z = 4, d_c = 1.373 g cm^{-3}, T = 24°. Intensity data were measured by ω–2θ scans of variable rate, designed to yield $\lambda = 25\sigma(I)$ for all significant reflections. Two octants of data were collected within the limits $2 < \theta < 75°$. Data reduction included corrections for background, Lorentz, polarization, and absorption effects. Absorption corrections (μ = 8.6 cm^{-1}) were based on ψ scans, with minimum relative transmission coefficient 80.4%. Of 4222 unique data, 3605 had $\lambda > 3\sigma(I)$ and were used in the refinement.

The structure was solved by direct methods using RANTAN [16] and refined by full-matrix least squares, treating nonhydrogen atoms anisotropically, using the Enraf–Nonius MolEN programs [17].

Table 3. Positional parameters and their estimated s.d.s. for austinolide (**1**)

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
O1	0.4941(2)	0.9572(1)	0.60131(6)	2.79(3)
O2	0.7321(2)	0.8451(1)	0.60942(7)	3.68(3)
O3	−0.1529(2)	1.1992(1)	0.67381(7)	3.61(3)
O4	−0.3136(2)	1.0521(2)	0.70614(7)	3.90(3)
O5	0.1254(2)	0.7662(1)	0.64047(5)	2.38(2)
O6	−0.0278(2)	0.6254(1)	0.68344(6)	3.45(3)
O7	−0.0513(2)	0.4985(1)	0.58802(7)	3.59(3)
O8	0.2989(2)	0.5046(1)	0.61899(6)	2.92(3)
O9	0.5756(2)	0.5366(2)	0.59238(8)	4.68(4)
C1	0.1297(3)	1.1104(2)	0.67831(8)	2.49(4)
C2	0.0309(3)	1.2194(2)	0.65920(9)	3.20(4)
C3	0.0675(3)	1.2248(2)	0.5984(1)	3.70(5)
C4	0.2049(3)	1.1592(2)	0.5848(1)	3.37(4)
C5	0.2774(3)	1.0960(2)	0.63528(8)	2.56(4)
C6	0.3218(2)	0.9674(2)	0.62637(8)	2.21(3)
C7	0.3284(2)	0.8946(2)	0.68079(8)	2.18(3)
C8	0.1601(3)	0.8282(2)	0.69222(8)	2.24(3)
C9	0.0094(3)	0.9073(2)	0.71048(8)	2.49(4)
C10	−0.0132(3)	1.0168(2)	0.67475(8)	2.35(3)
C11	0.4879(3)	0.8199(2)	0.67265(8)	2.48(3)
C12	0.5885(3)	0.8703(2)	0.62593(9)	2.70(4)
C13	0.5409(3)	0.7270(2)	0.7001(1)	3.41(4)
C14	−0.1765(3)	1.0858(2)	0.68761(9)	2.98(4)
C15	0.2882(4)	1.1494(3)	0.5288(1)	5.47(6)
C16	0.0396(3)	0.6633(2)	0.64242(8)	2.37(3)
C17	0.0453(3)	0.6030(2)	0.58508(9)	2.54(4)
C18	0.2357(3)	0.5700(2)	0.57112(9)	1.56(4)
C19	−0.0262(3)	0.6839(2)	0.53955(9)	3.74(5)
C20	0.2546(3)	0.4970(2)	0.51885(9)	3.48(5)
C21	0.4726(3)	0.4923(2)	0.6238(1)	3.48(4)
C22	0.5166(4)	0.4160(3)	0.6723(1)	5.73(6)
H7O	−0.161(4)	0.508(2)	0.594(1)	5.4(7)

Hydrogen atoms were located using difference maps and refined isotropically, except for those of the methyl group C15, which were placed in calculated positions. Convergence was achieved with $R = 0.0366$, $R_w = 0.0444$, and GOF = 1.791. The absolute configuration was determined by refinement of the mirror-image structure under identical circumstances, yielding $R = 0.0368$, $R_w = 0.0447$, and GOF = 1.801. The crystal structure is illustrated in Fig. 1, and its coordinates are tabulated in Table 3.

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