Molecular Structure of Two Derivatives of the Germacranolide Sesquiterpene Lactone Melnerin

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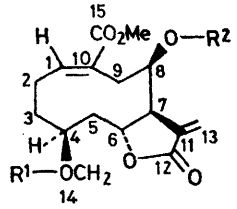
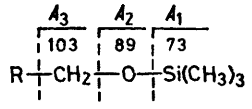
Two biomodified esters (1) and (2) of a new germacranolide sesquiterpene lactone, melnerin, have been isolated from the leaves of *Melapodium cinereum*. Their structures have been characterized by ^1H and ^{13}C n.m.r. spectroscopy, and gas chromatography–mass spectrometry of trimethylsilyl-derivatives, and single-crystal *X*-ray diffraction. Melnerin A, (1), $C_{20}H_{28}O_7$, with an isobutyrate side-chain, and melnerin B, (2), $C_{21}H_{30}O_7$ with a 2-methylbutyrate side-chain, co-crystallize in *ca.* 4 : 1 ratio, in the orthorhombic space group $P2_12_12_1$, with a = 9.322(2), b = 11.655(2), c = 19.032(4) Å, Z = 4. The crystal structure, which is disordered in the ester side-chain, was solved by direct phasing procedures and refined by weighted full-matrix least-squares to R 8.2% 1 711 reflections. The $\Delta^{1(10)}$-*cis*-germacrene ring has the same conformation as *cis*-cyclodecene. Substituents at C(4) (hydroxymethylene) and C(10) (methoxycarbonyl) are *syn* and α-oriented, while the ester group attached to C(8) is β-oriented. The α-methylene-γ-lactone, *trans*-fused at C(6) and C(7), is non-planar with right-handed chirality. The absolute configuration is assigned from biogenetic considerations and correlation with known structures.

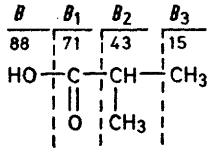
In our biochemical systematic studies of the genus *Melampodium* (Compositae, Heliantheae), combined



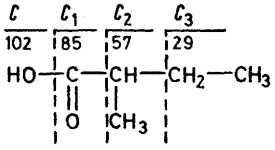
	R^1	R^2
(1)	H	B_1
(2)	H	C_1
(3)	$SiMe_3$	B_1
(4)	$SiMe_3$	C_1



(*A*)



(*B*)



(*C*)

with a search for antineoplastic plant constituents, we have carried out a detailed populational analysis for sesquiterpene lactones in the white-rayed complex of *Melampodium*. Our previous investigations have led to the isolation of melampolides [1,2] of the melampodin A type [1–6] as well as their biomodified dilactones of the melampodin B type.[6–8] From the leaves of *M. cinereum* var. *cinereum* two biomodified derivatives of a new sesquiterpene lactone, melnerin,‡ have been characterized by chemical, spectral, and single-crystal diffraction techniques. Melnerin A (1) and melnerin B (2) co-crystallize in *ca.* 4 : 1 ratio, a circumstance which was not recognized at the time *X*-ray analysis was undertaken, and which mitigates somewhat the precision of the crystal-structure results.

Chemical and Spectroscopic Characterization.—Standard column chromatographic procedures applied to several populations of *M. cinereum* provided a colourless, crystalline material with sharp melting point. Elemental analysis did not contradict the assumption of purity of the material which was named 'melnerin' and assigned formula (1) from analytical and spectral analysis.

The i.r. spectrum of the crystalline sample contained absorptions typical of α,β-unsaturated-γ-lactones (1 765 and 1 720 cm^{-1}), hydroxy-group(s) (3 500 cm^{-1}), and double bond(s) (1 660 cm^{-1}). Since the i.r. spectrum of an acetate derivative exhibited no OH absorption, and the ^1H n.m.r. spectrum showed one acetate signal, it was assumed that only one OH group must be present. Initial structural information on the skeletal arrangement (without side-chains) was deduced from correlation of 25.2 MHz ^{13}C and ^1H n.m.r. spectral data.

The ^{13}C n.m.r. data, obtained under proton noise decoupling (PND) and single-frequency off-centre decoupling (SFOCD) conditions,[9] indicated the presence of

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‡ Like (1) but with R^2 = H. It has not yet been isolated as such.

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the following skeletal units: four $-CH_2-$, three each of $-C(:O)O-$ and $>CH-$, two each of $>C:$, $>CH-O-$, and Me, and one each of $:CH-$, $H_2C:$, $-CH_2-O-$, and $MeO-$. These data provided evidence for the presence of 20 carbon and 28 hydrogen atoms, in agreement with the empirical formula $C_{20}H_{28}O_7$ indicated from previous analytical results. Skeletal arrangement (1), exclusive of stereochemistry, was formulated with the aid of ^1H double-resonance experiments at 100 MHz (Table 1).

TABLE 1

^1H and ^{13}C n.m.r. spectral data

(*a*) ' Melnerin '

^1H N.m.r. (deuterioacetone): **6.87**dd (**5.0, 12.0**, H-1), **6.14**d (**2.0**, H-13b), **5.73**d (**1.5**, H-13a), **5.51**m (H-8), **5.23**m (H-6), **3.76** ($-CO_2CH_3$), **3.36**m (H-14), **2.44**m (H-1'),[a] **1.07**d (**7.0**, C-2'–Me),[a] **1.05**d (**7.0**, C-2'–Me),[a] **1.02**d (**7.0**, C-2'–CH_3),[b] **0.82**t (**7.5**, C-3'–CH_3)[b]

^{13}C N.m.r. (deuteriochloroform): **178.9** (C-1'), **169.7** (C-12), **167.2** (C-15), **145.2**d (C-1), **136.1** (C-11), **127.6** (C-10), **124.5**t (C-13), **76.5**d (C-6), **73.8**d (C-8), **68.1**t (C-14), **52.2**q ($-CO_2CH_3$), **43.2**d (C-7), **38.7**t (C-9), **35.8**d (C-4), **34.1**d (C-2'), **30.2**t, **29.1**t, **27.3**t (C-3, C-5, C-2), **18.9**q, **18.7**q (C-2'–CH_3)

(*b*) ' Melnerin ' acetate

^1H N.m.r. (deuteriochloroform): **6.88**dd (**5.0, 12.0**, H-1), **6.32**d (**2.0**, H-13b), **5.72**d (**1.5**, H-13a), **5.50**m (H-8), **5.10**m (H-6), **3.89**m (H-14), **3.83** ($-CO_2CH_3$), **3.0**m (H-7), **2.50**m (H-1'), **2.06** (Ac)

[a] Signals due to melnerin A; [b] Signals due to melnerin B. Singlets are unmarked, multiplets are designated as follows: d = doublet, t = triplet, q = quartet, m = multiplet whose centre is given. Values in parentheses are coupling constants or line separations (Hz) followed by the assignments.

However, these data were also consistent with a 7,8-lactone with side-chain OR^2 attached at C(6). At this point it was decided that the configurational and conformational ambiguities could best be resolved by *X*-ray analysis, especially since minor ^1H n.m.r. signals near 0.8 and 1.0 p.p.m. could not be assigned.

Concurrent with the crystallographic analysis, a new method for the detection of mixtures of closely related sesquiterpene lactones was developed, the gas chromatography–mass spectrometry (g.c.–m.s.) of trimethylsilyl derivatives. The g.c. trace of these derivatives of ' melnerin ' indicated that the sample was actually a *ca.* 4 : 1 mixture of two compounds differing by 14 mass units. The derivative of the major constituent, which we named melnerin A, exhibited a mass spectral pattern (Table 2) with a strong parent peak at *m/e* **452**, in full agreement with structure (1). The derivative of the minor constituent, melnerin B, showed a parent peak at *m/e* **466** due to the presence of an additional CH_2 unit. The derivatives of melnerin A and B gave strong peaks at *m/e* **381** which indicated loss of acylium ions Me_2CHCO^+ (**71**) and $C_4H_9CO^+$ (**85**) from the respective parent ions, which in turn suggested that the two compounds must have different ester side-chains. This was corroborated by the appearance of strong peaks at *m/e* **85** (base peak) and **57**, which were assigned to the respective fragments C_1 and C_2 in melnerin B. Furthermore, the peak at *m/e* **364** indicated loss of $C_4H_9CO_2H$ (*C*, **102**) from the parent ion by a McLafferty rearrangement. The previously unassigned ^1H n.m.r. doublet at 1.02 p.p.m. (C-2' Me) and triplet at 0.82 p.p.m. (C-3' Me) also supported the presence of a 2-methylbutanoate moiety (C_1) in melnerin B (2).

TABLE 2

Mass spectral data for trimethylsilyl derivatives of melnerin A and B; *m/e* value followed by intensity and assignment in parentheses

(*a*) Trimethylsilyl melnerin A **452** (**20.7**, M^+), **436** (**17.4**, $M - CH_3$), **420** (**34.7**, $M - CH_3OH$), **381** (**27.7**, $M - B_1$), **366** (**17.7**, $M - B_1 - CH_3$), **364** (**3.8**, $M - B$), **349** (**39.6**, $M - A_3$), **348** (**18.1**, $M - A_3H$), **316** (**22.0**, $M - A_3H - CH_3OH$), **274** (**7.0**, $M - B - A_2H$), **242** (**14.2**, $M - B - A_2H - CH_3OH$), **214** (**18.4**, $M - B - A_2H - CH_3OH - CO$), **103** (**7.9**, A_3), **73** (**8.3**, A_1), **71** (**100**, B_1), **43** (**37.5**, B_2)

(*b*) Trimethylsilyl melnerin B **466** (**13.1**, M^+), **451** (**12.2**, $M - CH_3$), **434** (**30.4**, $M - CH_3OH$), **381** (**26.8**, $M - C_1$), **364** (**3.1**, $M - C$), **363** (**6.8**, $M - A_3$), **349** (**33.3**, $M - C - CH_3$), **274** (**5.5**, $M - C - A_2H$), **242** (**10.6**, $M - C - A_2H - CH_3OH$), **214** (**14.4**, $M - C - A_2H - CH_3OH - CO$), **103** (**5.5**, A_3), **85** (**100**, C_1), **57** (**69.5**, C_2)

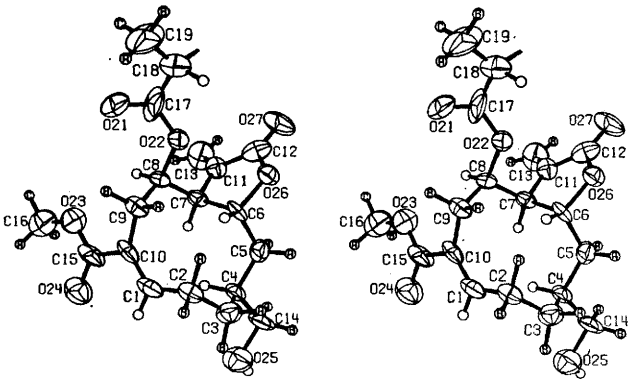

FIGURE 1 The β-face of the molecule

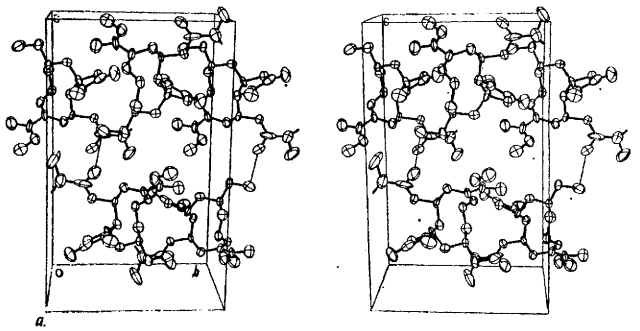

FIGURE 2 The packing of the molecules in the unit cell. A possible intermolecular hydrogen bond [O(21) · · · O(25) 2.80 Å] is shown, though the hydrogen atom was not observed

X-Ray Diffraction Results.—The structure was solved by direct methods and refined by full-matrix least-squares to *R* 8.2% by use of 1 711 reflections. Final atomic parameters are listed in Table 3, molecular dimensions in Table 4, and torsion angles in Table 5. Figure 1 shows the β-face of the skeleton, while Figure 2 shows the unit-cell packing. The skeleton is that of a $\Delta^{1(10)}$-*cis*-germacranolide with an α-methylene-γ-lactone *trans*-fused at C(6) and C(7). The hydroxymethylene group attached to C(4) and methoxycarbonyl-group attached to C(10) are *syn* and α-oriented while the carboxylate group attached to C(8) is β-oriented. The

TABLE 3

Fractional atomic co-ordinates (× 10^4)

Atom	X	Y	Z
C(1)	9 401(11)	4 241(7)	8 406(5)
C(2)	10 419(10)	4 951(6)	8 018(5)
C(3)	10 301(10)	4 747(7)	7 222(5)
C(4)	8 788(10)	4 757(6)	6 913(4)
C(5)	8 154(10)	5 926(6)	6 793(4)
C(6)	7 747(10)	6 632(6)	7 444(5)
C(7)	6 526(9)	6 124(6)	7 885(4)
C(8)	6 678(9)	6 264(6)	8 680(4)
C(9)	7 967(10)	5 720(7)	9 031(4)
C(10)	8 323(11)	4 554(6)	8 826(5)
C(11)	5 266(11)	6 801(7)	7 612(5)
C(12)	5 753(9)	7 872(7)	7 245(4)
C(13)	3 892(11)	6 579(9)	7 684(6)
C(14)	8 797(11)	4 210(7)	6 188(5)
C(15)	7 393(13)	3 690(7)	9 153(5)
C(16)	5 345(15)	3 268(10)	9 827(5)
C(17)	6 356(14)	8 064(11)	9 374(5)
C(18)	6 654(14)	9 318(7)	9 433(6)
C(19)	7 053(18)	9 711(9)	10 132(6)
C(20)	5 645(20)	9 910(8)	9 000(8)
O(21)	5 748(10)	7 439(7)	9 830(3)
O(22)	6 789(7)	7 553(4)	8 790(3)
O(23)	6 350(8)	4 061(5)	9 551(4)
O(24)	7 583(8)	2 611(5)	9 075(3)
O(25)	9 177(8)	3 023(5)	6 274(3)
O(26)	7 225(6)	7 781(4)	7 189(2)
O(27)	5 130(7)	8 722(5)	7 020(3)

TABLE 4

Bond lengths (Å) and bond angles (°)

(*a*) Distances

C(1)–C(2)	1.46(1)	C(10)–C(15)	1.47(1)
C(2)–C(3)	1.54(1)	C(11)–C(12)	1.50(1)
C(3)–C(4)	1.53(1)	C(11)–C(13)	1.31(1)
C(4)–C(5)	1.50(1)	C(12)–O(26)	1.38(1)
C(4)–C(14)	1.52(1)	C(12)–O(27)	1.23(1)
C(5)–C(6)	1.53(1)	C(14)–O(25)	1.44(1)
C(6)–C(7)	1.53(1)	C(15)–O(23)	1.31(1)
C(6)–O(26)	1.50(1)	C(15)–O(24)	1.28(1)
C(7)–C(8)	1.53(1)	C(16)–O(23)	1.42(1)
C(7)–C(11)	1.51(1)	C(17)–O(21)	1.27(1)
C(8)–C(9)	1.51(1)	C(17)–O(22)	1.32(1)
C(8)–O(22)	1.52(1)	C(17)–C(18)	1.49(1)
C(9)–C(10)	1.45(1)	C(18)–C(19)	1.46(1)
C(10)–C(1)	1.33(1)	C(18)–C(20)	1.43(2)

(*b*) Angles

C(10)–C(1)–C(2)	129.7(8)	C(7)–C(11)–C(12)	111.1(8)
C(1)–C(2)–C(3)	111.4(7)	C(7)–C(11)–C(13)	128.3(8)
C(2)–C(3)–C(4)	116.3(7)	C(12)–C(11)–C(13)	120.5(9)
C(3)–C(4)–C(5)	115.4(7)	C(11)–C(12)–O(26)	106.8(7)
C(3)–C(4)–C(14)	109.9(7)	C(11)–C(12)–O(27)	133.8(8)
C(5)–C(4)–C(14)	104.1(6)	O(26)–C(12)–O(27)	120.4(7)
C(4)–C(5)–C(6)	117.3(6)		
C(5)–C(6)–C(7)	114.3(6)	C(4)–C(14)–O(25)	107.6(7)
C(5)–C(6)–O(26)	107.3(6)	C(10)–C(15)–O(23)	117.3(7)
C(7)–C(6)–O(26)	106.3(6)	C(10)–C(15)–O(24)	123.0(9)
C(6)–C(7)–C(8)	115.6(7)	O(23)–C(15)–O(24)	119.7(9)
C(6)–C(7)–C(11)	100.8(7)	C(18)–C(17)–O(21)	126.4(10)
C(8)–C(7)–C(11)	110.9(7)	C(18)–C(17)–O(22)	116.6(9)
C(7)–C(8)–C(9)	117.7(7)	O(21)–C(17)–O(22)	117.0(10)
C(7)–C(8)–O(22)	104.3(5)	C(17)–C(18)–C(19)	115.1(9)
C(9)–C(8)–O(22)	107.5(6)	C(17)–C(18)–C(20)	107.9(10)
C(8)–C(9)–C(10)	117.1(7)	C(19)–C(18)–C(20)	122.9(10)
C(1)–C(10)–C(9)	126.0(8)	C(8)–O(22)–C(17)	122.6(7)
C(1)–C(10)–C(15)	120.8(7)	C(15)–O(23)–C(16)	119.4(8)
C(9)–C(10)–C(15)	113.2(8)	C(6)–O(26)–C(12)	111.4(6)

crystal contains two slightly different molecules in a partially disordered lattice; melnerin A contains an isobutyrate side-chain [methyl attached to C(18)] and melnerin B contains a 2-methylbutyrate side-chain [ethyl attached to C(18)]. The configuration shown in Figure 1 as **4**(*S*), **6**(*R*), **7**(*S*), **8**(*R*) [and in melnerin B, **18**(*R*)], is assigned on biogenetic grounds as previously outlined for **4**(5)-dihydromelampodin B,[8] and is also consistent with that of melampodin A, for which the absolute configuration has been experimentally determined.[2] We therefore assume that Figure 1 represents the absolute configurations of melnerin A and B and by implication the (as yet unknown) diol melnerin.

The conformation of the cyclodecene ring can be analysed in terms of the endocyclic torsion angles.[10] Thus, the torsion angles (Table 6) of melampodin A [1,2] and enhydrin [11] display a single pattern representative

TABLE 5

Torsion angles (°)

C(10)–C(1)–C(2)–C(3)	−119	C(7)–C(6)–O(26)–C(12)	−18
C(1)–C(2)–C(3)–C(4)	48	C(6)–O(26)–C(12)–O(27)	−175
C(2)–C(3)–C(4)–C(5)	79	C(6)–O(26)–C(12)–C(11)	6
C(2)–C(3)–C(4)–C(14)	−163	O(26)–C(12)–C(11)–C(7)	9
C(3)–C(4)–C(5)–C(6)	−70	O(26)–C(12)–C(11)–C(13)	−173
C(14)–C(4)–C(5)–C(6)	170	O(27)–C(12)–C(11)–C(7)	−170
C(4)–C(5)–C(6)–C(7)	−65	O(27)–C(12)–C(11)–C(13)	8
C(4)–C(5)–C(6)–O(26)	177	C(12)–C(11)–C(7)–C(6)	−19
C(5)–C(6)–C(7)–C(8)	143	C(12)–C(11)–C(7)–C(8)	104
C(5)–C(6)–C(7)–C(11)	−98	C(13)–C(11)–C(7)–C(6)	164
O(26)–C(6)–C(7)–C(8)	−99	C(13)–C(11)–C(7)–C(8)	−73
O(26)–C(6)–C(7)–C(11)	21	C(7)–C(8)–O(22)–C(17)	152
C(6)–C(7)–C(8)–C(9)	−61	C(9)–C(8)–O(22)–C(17)	−83
C(6)–C(7)–C(8)–O(22)	58	C(8)–O(22)–C(17)–O(21)	−5
C(11)–C(7)–C(8)–C(9)	−175	C(8)–O(22)–C(17)–C(18)	175
C(11)–C(7)–C(8)–O(22)	−56	O(22)–C(17)–C(18)–C(19)	−142
C(7)–C(8)–C(9)–C(10)	−45	O(22)–C(17)–C(18)–C(20)	76
O(22)–C(8)–C(9)–C(10)	−162	O(21)–C(17)–C(18)–C(19)	37
C(8)–C(9)–C(10)–C(1)	105	O(21)–C(17)–C(18)–C(20)	−105
C(8)–C(9)–C(10)–C(15)	78	C(1)–C(10)–C(15)–O(23)	−179
C(9)–C(10)–C(1)–C(2)	−4	C(1)–C(10)–C(15)–O(24)	2
C(15)–C(10)–C(1)–C(2)	178	C(9)–C(10)–C(15)–O(23)	3
C(3)–C(4)–C(14)–O(25)	65	C(9)–C(10)–C(15)–O(24)	−176
C(5)–C(4)–C(14)–O(25)	−171	C(10)–C(15)–O(23)–C(16)	174
C(5)–C(6)–O(26)–C(12)	106	O(24)–C(15)–O(23)–C(16)	−7

TABLE 6

Endocyclic torsion angles (°) of some cyclodecene systems

	(*A*)	(*B*)	(*C*)	(*D*)
ω(10–1)	4	13	−4	−3
ω(1–2)	−56	−97	−116	−116
ω(2–3)	14	75	48	55
ω(3–4)	−54	−92	79	68
ω(4–5)	155	147	−70	−76
ω(5–6)	−101	−119	−65	−53
ω(6–7)	76	95	143	147
ω(7–8)	−71	−63	−61	−62
ω(8–9)	−44	−56	−45	−52
ω(9–10)	125	132	105	109

(*A*) Melampodin, inverted from (ref. 1*b*); (*B*) enhydrin (ref. 11); (*C*) melnerin A and B (present work); (D) *cis*-cyclodecene, renumbered and inverted from ref. 12.

of the highly strained $\Delta^{1(10)}$-*cis*-Δ^{4}-*trans*-germacradiene system [*trans*-epoxidation of the 4(5) double bond, as in enhydrin, does not alter the gross ring conformation]. Significantly, the torsion-angle spectrum of the melnerins shows the same pattern except at ω(**3**—**4**) and ω(**4**—**5**). Therefore, we suggest that the biogenetic precursor of melnerin (and its derivatives) is a $\Delta^{1(10)}$-*cis*-Δ^{4}-*trans*-germacradiene (melampolide) which undergoes hydrogenation at the 4(5) double bond. This relieves strain at

[10] J. D. Dunitz, *Perspectives in Structural Chem.*, 1968, **2**, 7.
[11] G. Kartha, K. T. Go, and B. S. Joshi, *J.C.S. Chem. Comm.*, 1972, 1327.
[12] O. Ermer, H. Eser, and J. D. Dunitz, *Helv. Chim. Acta*, 1971, **54**,2 469.

the *trans*-double-bond by allowing C(4) to flip over and the C(3)–(4) and C(4)–(5) single bonds to rotate, thus moving the attached C(14) from an initial β-orientation to the final α-orientation. Considerable energy is thus released from the highly strained melampolide system [1] since the resulting melnerins have the same conformation as *cis*-cyclodecene,[12] a mono-olefin with little inherent strain.

All bond lengths in the melnerin cyclodecene ring are as expected; mean of seven C(sp^3)–C(sp^3) 1.52(1), mean of two C(sp^2)–C(sp^3) 1.46(1), and the single C(sp^2)–C(sp^2) 1.33(1) Å. The endocyclic bond angles in the melnerins show a marked relation to those of *cis*-cyclodecene. The mean C(sp^3)–C(sp^2)–C(sp^2) angle of 128° is identical in both systems, and the mean of eight C(sp^3)–C(sp^3)–C(sp^3) angles, 115.5 in the melnerins and 116.5° in *cis*-cyclodecene, does not differ significantly. However, whereas the angles around the unsubstituted *cis*-cyclodecene ring show a monotonic increase to and decrease from a single maximum of 120°, the angles in the melnerins show a random but relatively flat distribution. Considering also the similarities in the endocyclic torsion angles, one can conclude that the total bending (Baeyer) and torsion (Pitzer) strains in these two cyclodecene systems are comparable though distributed somewhat differently.

The lactone ring is decidedly non-planar, as evidenced by the sum of the five endocyclic torsion angle moduli (73°). Although the sign of the Cotton effect arising from the $n\rightarrow\pi^*$ transition of the α-methylene-γ-lactone chromophore is unknown, the chromophore has right-handed chirality (positive torsion angle). As discussed by Cox and Sim,[13] this C:C–C:O torsion angle (ω_2, +8°) and the endocyclic C_α–C_β–C_γ–O torsion angle (ω_3, 21°) are correlated in sign, and fall in the upper right quadrant of their plot (Figure 3 of ref. 13).

EXPERIMENTAL

Chemical and Spectral Data.—A collection of *M. cinereum* var. *cinereum* (Stuessy–Fischer No. 2013) was made on July 19, 1973 in Duval County, Texas, 1.4 miles S.W. of San Diego on route 44.* Dried leaves (345 g) were extracted and worked up as described previously.[4] The crude terpenoid-containing syrup was chromatographed over silica gel using CH_2Cl_2 with increasing amounts of EtOAc as eluents. Crystalline samples were obtained from the appropriate fractions in the following elution sequence: artemetin [8] (25 mg), cinerenin [8] (20 mg), ' melnerin ' (405 mg), 4(5)-dihydromelampodin B[8] (100 mg), and melampodin B[8] (30 mg). Recrystallization of ' melnerin ' from isopropyl alcohol gave colourless crystals, m.p. 194—195 °C; i.r. absorptions (Nujol) 3 500, 1 765, 1 720, and 1 660 cm^{-1}, n.m.r. spectra in Table 1 (Found C, 63.05; H, 7.5. Calc. for ' melnerin ', $C_{20}H_{28}O_7$: C, 63.2; H, 7.4. Calc. for 80% $C_{20}H_{28}O_7$/20% $C_{21}H_{30}O_7$: C, 63.35; H, 7.5%).

Acetylation of ' melnerin ' with Ac_2O in pyridine provided the acetate as a gum, i.r. absorptions (Nujol) *ca.* 1 700 and 1 600 cm^{-1}, ^1H n.m.r. spectrum in Table 1.

* Voucher specimens are on deposit in the Louisiana State University Herbarium at Baton Rouge, Louisiana.

[13] P. J. Cox and G. A. Sim, *J.C.S. Perkin II*, 1977, 255.

' Melnerin ' was silylated with an excess of *N,O*-[bis(trimethylsilyl)]trifluoroacetamide at 60 °C for *ca.* 3 h.[14] This derivative (2—5 μl) was chromatographed on a 6 ft × 4 mm (i.d.) silanized glass column packed with 1% SE 30 Gas Chrom Q (100—120 mesh), and the mass spectra of the g.c. fractions were obtained on an interfaced LKB-9000 mass spectrometer at 20 eV. The g.c.-trace showed two major peaks in *ca.* 4 : 1 ratio. The major component (3) had the lower g.c. retention time and exhibited the mass spectrum shown in Table 2. The minor component (4), with higher g.c. retention time, showed the mass spectrum in Table 2.

Apparatus.—M.p.s were measured in capillaries on a Thomas–Hoover. I.r. spectra were obtained on Perkin-Elmer 621 and u.v. spectra on Cary 14 spectrophotometers. Low-resolution mass spectra were obtained on an LKB 9000 mass spectrometer at 20 eV. ^1H and ^{13}C n.m.r. spectral data were recorded on Varian HA 100 and XL 100 FT spectrometers. The ^1H signal assignments were obtained from the mononuclear decouple resonance experiments. The ^{13}C resonance designations were derived from the proton noise decoupled (PND) and the single-frequency off-centre decoupled (SFOCD) mode spectral data. A small amount of Me_4Si was employed as internal standard for both ^1H and ^{13}C n.m.r. data.

X-*Ray Crystal Structure of Melnerin A and B*

Crystal Data.—$C_{20}H_{28}O_7$/$C_{21}H_{30}O_7$, M = 380/394. Orthorhombic, a = 9.322(2), b = 11.655(2), c = 19.032(4), U = 2 068 Å^3, D_c = 1.22—1.27 g cm^{-3} for Z = 4. Mo-K_α radiation λ = 0.710 73 Å; μ(Mo-K_α) = 0.99—1.02 cm^{-1}, estimated transmission range 0.93—0.97. Space group $P2_12_12_1$.

Crystals were obtained by very slow cooling of a hot, saturated solution of ' melnerin ' in isopropyl alcohol. A colourless, transparent crystal *ca.* 0.34 × 0.36 × 0.57 mm^3 was mounted on a glass fibre with [110] nearly parallel to the ϕ axis of an Enraf–Nonius CAD 4 diffractometer with pulse-height analyser. All measurements were carried out with graphite monochromated Mo-K_α radiation. Accurate centring of fifteen reflections with $2\theta > 20°$ led to a least-squares determination of lattice parameters. The intensity at each reciprocal lattice point in the *hkl* octant for which $6° < 2\theta < 50°$ ($0.074 < \sin\theta/\lambda < 0.595$) was measured by the ω—2θ scan technique. The 2θ scan range was computed as $(2.0 + 2.1\tan\theta)°$, and this range was then extended 25% on each end to produce background counts. The aperture setting was computed as $(5.2 + 2.1\tan\theta)$ mm. In each of these formulae, θ is the calculated peak centre. From a rapid prescan (5° min^{-1}) of each reflection the integrated intensity $I = I_t + I_b$ was calculated where I_t is the total number of counts over the scan range and I_b is the estimated background count over the scan range. For $I < 75$, the reflection was judged unobserved. For $2\,000 < I < 75$, a new scan speed was calculated to yield $I \simeq 2\,000$, but the maximum allowable scan time was 300 s. Reflections with $I > 2\,000$ were accepted without additional scanning. Throughout data collection, two reflections were measured periodically to monitor electronic and crystal instabilities, neither of which was detected. In addition, a third reflection was monitored for angular displacement. No movement greater than 0.1° in any angle was detected.

A total of 2 098 unique reflection intensities were measured, of which 1 080 were considered observed, having

[14] D. L. Perry, D. M. Desiderio, and N. H. Fischer, *Org. Mass. Spectrometry*, in the press.

$I > 3\sigma(I)$. The variance of each intensity was estimated as $\sigma(I)^2 = I_t + I_b$. Lorentz and polarization (Lp) corrections were applied to the integrated intensities to yield structure amplitudes $|F_o|$. The variance of each structure amplitude was estimated as $\sigma(|F_o|)^2 = \sigma(I)^2/4ILp$.

Structure Solution and Refinement.—Routine application of the multiple-solution phasing procedure MULTAN [15] generated 64 phase sets, and the E map based on the set with the highest combined figure-of-merit (2.88) showed 26 of the non-hydrogen atoms in a reasonable configuration. Residual electron density in the appropriate area of a ΔF map was interpreted as the missing carbon, C(20), of an isobutyrate function. Isotropic full-matrix weighted ($w = \sigma(|F_o|)^{-2}$ least-squares refinement with all 27 non-hydrogen atoms converged to R 13.5%.* A ΔF map indicated the positions of all hydrogen atoms except the hydroxy hydrogen. All refinement was carried out using the programs of ref. 16.

Conversion of all non-hydrogen atom thermal parameters to the anisotropic mode, and inclusion of the hydrogen parameters (isotropic) reduced R to 8.4%. However, refinement was halted when it was observed that a large number of the hydrogen atoms would not settle into physically meaningful positions with reasonable thermal values. Further, four of the non-hydrogen atoms in the isobutyrate group [C(18)—(20), O(21)] had unreasonable thermal parameters, to the extent that the ellipsoid for C(18) was non-positive definite.

At this point the gas chromatography–mass spectral data were obtained, from which it was concluded that the C(20) 'methyl' carbon was in fact disordered methyl–ethyl groups and that the remainder of the molecule, identical in the two derivatives, was also slightly disordered to varying degrees at each atom but worst at the three atoms C(18), C(19), and O(21). No discernable methyl group was obvious at any of the C(20) hydrogen positions on a ΔF map, and it is probable the ethyl groups are themselves disordered about C(20).

Refinement was allowed to continue, with hydrogen atoms fixed in idealized positions. In the final cycle of least squares, 1 711 reflections contributed, of which 631 had $|F_c| \geqq |F_o| > 0$ The final error-of-fit, with 245 variables, was 3.04, and the final R and R' values were 8.2 and 6.5% respectively. All parameters except those of the non-positive-definite thermal parameters of C(18) shifted $<0.5\sigma$. Observed and final calculated structure factors and thermal parameters are listed in Supplementary Publication No. SUP 22179 (14 pp., 1 microfiche).†

We thank the National Science Foundation, the National Cancer Institute, and the Robert A. Welch Foundation for financial support, Dr. Tod Stuessy of Ohio State University for collecting and identifying the plant material, and Dr. D. M. Desiderio for assistance in obtaining gas chromatography–mass spectral data.

[7/999 *Received*, 13*th June*, 1977]

† See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1977, Index issue.

* $R' = [\Sigma w \Delta^2/\Sigma w|F_o|^2]^{1/2}$ where $\Delta = |F_o| - |F_c|$; $\Sigma w\Delta^2$ is the function minimized in least squares. The error of fit is $[\Sigma w\Delta^2/(N_O - N_V)]^{1/2}$ where N_O is the no. of reflections and N_V the no. of parameters varied.

[15] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

[16] 'X-'Ray' Program System, eds. J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, Technical Report TR 192, Computer Science Center, University of Maryland, 1972.