Melampodin, A New Germacranolide from *Melampodium Leucanthum* Torr. and Gray var. *Leucanthum* (*Compositae*)

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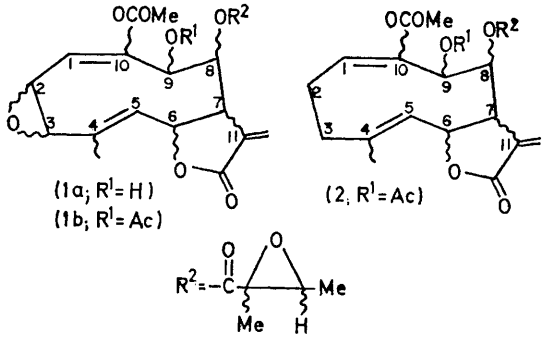
Summary The structure of melampodin, a new sesquiterpene lactone isolated from *Melampodium leucanthum* Torr. and Gray. var. *leucanthum*, has been elucidated on the basis of physical properties.

SYSTEMATIC study of the three white-rayed species of the genus *Melampodium* (*Compositae, Heliantheae*)[1] from Texas and neighbouring areas has led to the isolation of a number of new, highly oxygenated, sesquiterpene lactones. Chloroform extraction of *Melampodium leucanthum* Torr. and Gray var. *leucanthum*† yielded a new substance, which we named melampodin. Melampodin (**1a**), $C_{21}H_{24}O_9$,‡ m.p. 210—211°, $[\alpha]_D^{24}$ + 155° (*c* 0·7; MeOH), showed the following spectral data: strong u.v. end absorption; ν_{max} (KBr) 3400

† The plants were collected in El Paso County, Texas. The vouchers are deposited in the University of Texas at Austin Herbarium (A.S. Tomb 231). We thank Dr. T. F. Stuessy (now at Ohio State University) for collecting and authenticating the collections and for valuable discussions and correspondence.

‡ Satisfactory elemental analyses and molecular weights (mass spectral data) were obtained.

(OH), 1760 (γ-lactone), 1720 (αβ-unsat. ester), and 1670 and 1630 cm^{-1} (double bonds). Treatment of (**1a**) with acetic anhydride–pyridine gave melampodin acetate (**1b**), $C_{23}H_{26}O_{10}$,‡ m.p. 175—177°; ν_{max} (KBr) 1780, 1765, 1747, and 1245 cm^{-1}. The absence of an OH absorption from the i.r. spectrum of the monoacetate (**1b**) shows that (**1a**) contains only one hydroxy-group.

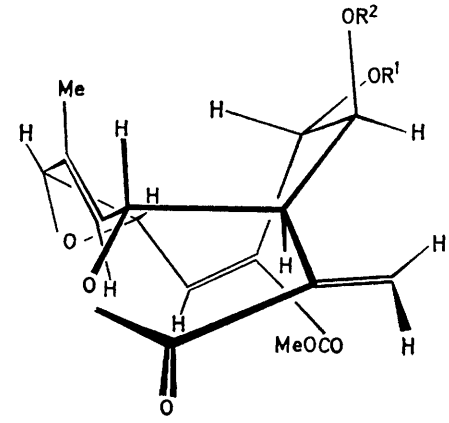


The 100 MHz n.m.r. spectra of (**1a**) and (**1b**) exhibit a number of signals (doublets near δ 6·2 and 5·6 p.p.m., and a broad, featureless, 1H multiplet near 2·7 p.p.m.) that are characteristic of αβ-unsaturated sesquiterpene γ-lactones. The base peak of the mass spectrum of (**1a**) (*m/e* 245, $C_{14}H_{13}O_5^+$) is consistent with such a formulation, and the observation of major fragments of *m/e* 59 ($C_2H_3O_2^+$) and 361 ($M^{+\cdot} - C_2H_3O_2\cdot$) suggests that the skeletal carbon atom lost in the base peak is present as a carboxy-group that has undergone methylation; this is corroborated by the appearance of a 3H singlet at δ 3·85 in the n.m.r. spectrum of (**1a**).

Mass-spectral fragments at *m/e* 115 ($C_5H_8O_2^+$) and 305 ($M^{+\cdot} - C_5H_8O_2\cdot$) indicate that the remaining five carbon atoms of (**1a**) are present in a single fragment. The n.m.r. spectra of (**1a**) and (**1b**) exhibit a 3H singlet at δ 1·5, a 3H doublet (*J* 5·4 Hz) at 1·2, and a sharp, 1H quartet (*J* 5·4 Hz) at δ 3·05; as similar patterns, including the compressed methine–methyl coupling have been reported[2] recently for the 2,3-epoxy-2-methylbutanoyl moiety (fragment R²) of uvedalin (**2**) and a number of its derivatives, the same fragment (or an optical isomer) must represent the remaining C_5 unit. The base peak (*m/e* 245) thus arises from the simultaneous or sequential loss of $\cdot CO_2Me$ (59 m.u.) and $C_4H_9CO_2H$ (116 m.u.) from the molecular ion (*m/e* 420).

The n.m.r. spectra of (**1b**) and (**2**) exhibit gross similarities of the remaining signals, except that the 4H multiplet near 2·5 p.p.m. in (**2**) is replaced by a less complex, two-proton pattern near 3·7 p.p.m. As all the carbon atoms of (**1b**) have been accounted for, and no bands corresponding to ketonic groups are observed in the i.r. spectrum of (**1b**), the substitution of an oxygen atom in (**1b**) for two hydrogen atoms in (**2**) must signify the presence of an epoxide function at C-2 and C-3; and the structure (**1b**) may be drawn, tentatively, by analogy to (**2**). The geometry of the two double bonds§ and the 2,3-epoxide fusion was determined by *X*-ray crystallography.[3]

The proton resonances of (**1a**) and (**1b**) were assigned completely with the aid of double-resonance and the paramagnetic shift reagent, tris-(2,2,6,6-tetramethyl-3,5-heptanedionato)europium[4] [Eu(dpm)$_3$]. In the n.m.r. spectrum of (**1a**), overlaps obscure the structure of several resonances. Sequential additions of small amounts of a solution of Eu(dpm)$_3$ in deuteriochloroform caused (a) the separation of a doublet of doublets (δ 6·21, *J* 8·5, 1·5 Hz), assigned to H-8, from the H-13$_{trans}$ doublet (δ 6·19, $J_{7,13trans}$ 3·0 Hz); (b) emergence of the AB portion of a more extensively coupled system near 5·2 p.p.m.; the low-field portion of this pattern separated as a very broad doublet ($J_{5,6}$ 10 Hz, broadened by coupling to the C-4 methyl group), which was assigned as H-5, and the high-field portion appeared as a sharp doublet of doublets ($J_{5,6}$ 10 Hz, $J_{6,7}$ *ca.* 10 Hz), assigned to H-6; (c) emergence of a wide, poorly resolved pattern, assigned to H-9, from the strong resonance at δ 3·85 due to the CO_2Me group; (d) simplification of the doublet of doublets at δ 6·92 (H-1, *J* 2·0, 1·2 Hz) to a sharp doublet (*J* 2·0 Hz), presumably by specific, paramagnetic decoupling¶ of H-1 from H-3, and (e) partial separation of the narrow multiplet at 3·6 p.p.m. into an apparent doublet (*J ca.* 3 Hz) and doublet of doublets (*J ca.* 3 and *ca.* 2 Hz), interpreted as arising from H-3 and H-2, respectively. The n.m.r. spectral parameters in the Table were determined by inspection, and verified by double-irradiation.



(1c)

Acetylation of (**1a**) causes a striking downfield shift in the resonance position of the H-9 pattern, which is also simplified by the loss of the O-H coupling; the hydroxy-group is thus located on C-9, as in (**2**). Additionally, in the spectrum of (**1b**) the H-3 resonance experiences sufficient displacement to low field of the H-2 signal that these two are viewed as separate multiplets, and the H-8 pattern, which

§ The *cis*-double bond ($\Delta^{1(10)}$) in melampodin must already have been formed in the living plant since the isolation and work-up conditions involved gentle procedures: cold chloroform extraction and treatment of the crude extract with 5% Pb(OAc)$_4$ in 50% aqueous ethanol, followed by crystallization. The question remains whether the *cis*-double bond is formed at an early biosynthetic stage or is generated later in a photochemical *trans-cis*-isomerization due to the high irradiation of the plants by the sun.

¶ A similar, nonspecific effect has been observed in the n.m.r. spectra of dimethylformamide and of trimethyl phosphite in the presence of bis-(2,4-pentanedionato)nickel.[5]

TABLE

N.m.r. spectral parameters of melampodin (**1a**), *melampodin acetate* (**1b**), *and uvedalin* (**2**)

	(1a)[a,b]		(1b)[a,b]		(2)	
Signal	δ (p.p.m.)	*J*/Hz	δ (p.p.m.)	*J*/Hz	δ (p.p.m.)	*J*/Hz
H-1	6·92 (dd)	1.2; 2·0	7·04 (dd)	*ca.* 1; 2·5	6·92 (m)	
H-2	3·56—3·69 (c)		3·65 (dd)	2·5; 3·8	2·1—2·8 (m)	
H-3			3·75 (dd)	*ca.* 1; 3·8		
H-5	5·10—5·39 (c)	9·8	5·10—5·42 (c)		4·96 (br-d)	
H-6		9·8, *ca.* 10			5·10 (dd)	
H-7	2·58 (m)		2·73 (m)		2·8 (m)	
H-8	6·21 (dd)	1·5; 8·5	6·70 (dd)	1·2; 9·0	6·55 (dd)	1·4; 8·4
H-9	3·98 (dd)	8·5; 11·0	5·41 (d)	9·0	5·33 (d)	8·4
H-13$_{cis}$	5·60 (d)	3·0	5·75 (d)	3·0	5·61 (d)	3·0
H-13$_{trans}$	6·19 (d)	3·0	6·25 (d)	3·5	6·12 (d)	3·5
H-3	3·05 (q)	5·5	3·05 (q)	5·5	2·99 (q)	5·4
4-Me	2·10 (br)		2·18 (d)	1·0	1·96 (br)	
2′-Me	1·54		1·48		1·44	
3′-Me	1·27 (d)	5·5	1·20 (d)	5·4	1·14	
9-OH or Ac	2·95 (d)	11·0	2·00		1·96	
CO_2Me	3·85		3·85		3·72	

[a] In $CDCl_3$. [b] Chemical shifts are in p.p.m. (δ scale) relative to Me_4Si as an internal standard. Singlets are unmarked, multiplets are described as follows: d = doublet, t = triplet, c = overlapping parts of ABX systems, br = broad, q = quartet, m = multiplet. [c] Data from ref. 2.

is adjacent to the new substituent, is deshielded away from overlap with the H-13$_{trans}$ doublet. Accordingly, the double-irradiation experiments were performed on (**1b**).

In the spectrum of (**1b**), irradiation at the centre (δ 2·73) of the broad multiplet assigned to H-7 caused collapse of the doublets at δ 5·75 (H-13$_{cis}$) and 6·25 (H-13$_{trans}$), plus collapse of the narrower spacing ($J_{7,8}$ 1·5 Hz) of the doublet of doublets at δ 6·70 (H-8), and a substantial narrowing in the structure of the upfield portion (H-6) of the H-5,6 pattern centred near 5·3 p.p.m. Irradiation of the narrow (*J* 1·0 Hz) doublet at δ 2·18 (4-Me) sharpened the low-field portion of the H-5,6 multiplet; saturation of the doublet ($J_{8,9}$ 9·0 Hz) at δ 5·41 (H-9) removed the corresponding, wide spacing from the H-8 pattern. Irradiation at δ 7·04 (H-1) narrowed the broadened doublet (*J ca.* 1 Hz) at 3·75 (H-3) to two, sharp lines, and collapsed the narrower ($J_{1,2}$ *ca.* 2·5 Hz) spacing in the doublet of doublets at δ 3·65 (H-2), to form a symmetrical, AB pattern (H-2,3; $J_{2,3}$ 3·8 Hz) centred at 3·7 p.p.m. Irradiation of other signals produced results as expected.

From biogenetic considerations, it is assumed that H-7 is below the plane of the rings in (**1a**). The small value of $J_{7,8}$ suggests a dihedral angle of *ca.* 90° relating these two protons, placing H-8 down in the planar projection, although probably twisted outward in the actual conformation. The large (*ca* 10 Hz) value of $J_{6,7}$ indicates approximately antiparallel disposition of H-5 to H-6, consistent with *trans*-fusion of the lactone ring (H-6 up), whereas the larger $J_{8,9}$ value (> 8 Hz) signals a dihedral angle near 180° between H-8 and H-9; the relatively large, downfield shift of the H-8 signal upon acetylation of the hydroxy-group also suggests close proximity of O-9 to H-8.

Considerable strain is evident in (**1a**) and (**1b**). From the substantial coupling (*ca.* 10 Hz) of H-5 to H-6 one must infer a conformation for (**1a**) having the C(4)–C(5) bond twisted substantially out of the plane of the ring system, which brings H-5 and H-6 into the indicated, antiparallel relationship.

Exposure to Eu(dpm)$_3$ caused major displacements of the signals of H-1,2,3,5,9, and the C-4 methyl protons, and somewhat smaller effects upon the resonances of H-7 and H-8. The relatively strong shifting of the C-4 methyl proton resonances locates the europium atom and, thus, the hydroxy-group (to which the Lewis acid co-ordinates most strongly[4b]) on the same side of the medium ring as the C-4 methyl group. This concurs with the coupling data above in specifying (**1c**) as the favoured conformation of (**1a**) [and certainly (**1b**) as well] in solution. Relative shifts suggest that the epoxide oxygen atoms behave as fairly typical ethers[4b] in their ability to co-ordinate competitively with Eu(dpm)$_3$.

Single-crystal *X*-ray diffraction methods[3] have verified these configurational assignments, and established that the conformation in the solid state is essentially the same. The stereochemistry of the epoxymethylbutyryl moiety (R[1]) could not be determined by spectroscopic methods. On the basis of the extreme similarity of the n.m.r. parameters of (**1b**) and (**2**) (Table), it appears that uvedalin and related compounds[2,6] exhibit the same configurational and conformational relationships as melampodin.

Acknowledgment is made to the University Council on Research, Louisiana State University (N.H.F.) for support of this work, and to W. E. Wegner for assistance in determining some of the n.m.r. spectral data. We thank Professor D. Rogers and Dr. S. Neidle for helpful discussions and suggestions.

(*Received, September 29th,* 1971; *Com.* 1699.)

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