mg, 0.69 mmol) in 60 ml of toluene was treated with 50 mg of *p*-toluenesulfonic acid. The flask was fitted with a Dean-Stark constant water separator and the mixture was heated at reflux for 10 min. After removal of the toluene in vacuo the residue was chromatographed on silica gel with benzene–ethyl acetate (8:1) and the band corresponding to **23** was isolated to obtain the product as a crystalline solid: 108 mg (98%); mp 67.5–68° (sublimation and then recrystallization from ether–pentane); ir ($CHCl_3$) 1740 (lactone), 1639 and 810 cm^{-1} (C=CH_2); NMR (60 MHz) δ 3.75 (t, J = <1 Hz, 2 H), 5.77 (dt, J = <1 Hz, 1 H), 6.40 (dt, J = <1 Hz, 1 H), 6.9–7.4 (m, 4 H); mass spectrum *m/e* (high resolution) calcd for $C_{10}H_8O_2$, 160.0524; found, 160.0527; *m/e* (rel intensity) 160 (M^+, 83), 131 (M − CHO, 100). A satisfactory elemental analysis for this compound could not be obtained despite repeated crystallizations form diethyl ether–pentane and sublimation (60°, 0.06 mm). The mass spectrum showed the presence of traces of higher molecular weight material in the purified compound which may have arisen via polymerization during the purification.

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Registry No.—**2,** 542-28-9; **3,** 27345-71-7; **4,** 24871-12-3; **8,** 56783-31-4; **10,** 42023-19-8; **11,** 56783-32-5; **12,** 55643-46-4; **12** MeI, 56783-33-6; **12** picrate, 56783-45-0; **13,** 3727-53-5; **14,** 56783-34-7; **15a,** 56783-35-8; **15a** MeI, 56783-36-9; **15a** picrate, 56783-46-1; **15b,** 56783-37-0; **15b** MeI, 56783-38-1; **15b** picrate, 56783-47-2; **16,** 16822-06-3; **17,** 56783-39-2; **18,** 56783-40-5; **19,** 56783-41-6; **21,** 56783-42-7; **22,** 56783-43-8; **23,** 56783-44-9; sodium α-formyl-δ-valerolactone, 53761-41-4; ethyl formate, 109-94-4; dimethylamine, 124-40-3; acetic–formic anhydride, 2258-42-6; 2,2,6,6-tetramethylpiperidine, 768-66-1; formic–pivalic anhydride, 10535-67-8.

References and Notes

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(2) Address correspondence to this author at the School of Pharmacy, University of Wisconsin, Madison, Wis. 53706.
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(25) Sodium formate (750 mg, 11 mmol), finely powdered and dried at 125° for 4 hr was mixed with pivaloyl chloride (1.20 g, 10 mmol) and 3 ml of anhydrous 1,2-dimethoxyethane in a tightly stoppered flask. The flask was heated at 47° for 45 min and then stirred overnight at room temperature. The mixture was filtered and used without further purification: NMR (60 MHz) δ 1.31 (s, 10 H), 9.07 (s, 1 H). Distillation of the anhydride at 18 mmHg, ambient temperature, resulted in decarbonylation and recovery of pivalic acid.

New Germacranolide Sesquiterpene Dilactones from the Genus *Melampodium* (Compositae)

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The isolation of four germacranolide sesquiterpene dilactones from the three white-rayed *Melampodium* species is reported. Melampodin B (**1a**) is found in all three species, and 4(5)-dihydromelampodin B (**4a**) only in *M. cinereum* DC. Cinerenin (**2a**) occurs in both, *M. cinereum* and *M. argophyllum* (A. Gray ex Robinson) Blake, and melampodin C (**3a**) is typical of the latter species. Artemetin is a common constituent of *M. cinereum* and *M. argophyllum*. The structures, configurations, and conformations of the new dilactones were determined by chemical transformations, correlations, and spectral methods.

In connection with our biochemical systematic study of the white-rayed complex of the genus *Melampodium* (Compositae, Heliantheae)[1] we have analyzed multiple populations of *M. cinereum* DC. and *M. argophyllum* (A. Gray ex Robinson) Blake for their sesquiterpene lactone content. In this communication we describe the isolation and structure elucidation of four closely related germacranolide type sesquiterpene dilactones, which we named melampodin B (**1a**), cinerenin (**2a**), melampodin C (**3a**), and 4(5)-dihydromelampodin B (**4a**). The flavonoid artemetin[2] is a common constituent in both *M. cinereum* and *M. argophyllum*.

Melampodin B and Derivatives. Melampodin B (**1a**), $C_{17}H_{18}O_7$, mp 226–228°, the major, most polar constituent, was present in most populations of *M. cinereum* and *M. argophyllum* and was also found in several west Texas populations of *M. leucanthum*.[3] The structure of melampodin B has been described in a previous communication[4] and was mainly deduced on the basis of correlations of 25.5-MHz ^{13}C and 300-MHz ^1H NMR spectra obtained in acetone-d_6 and pyridine-d_5. The ^{13}C NMR data were obtained under proton noise decoupled (PND) and single-frequency off-center decoupled (SFOCD) conditions.[5] The ^1H NMR spectral data of **1a**, which included extensive double resonance experiments, are tabulated in Table I.

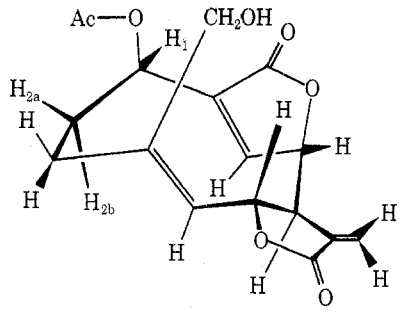
The stereochemical and conformational assignments in melampodin B require further comments. Two initial assumptions were made in the structural assignments of me-

Table I
^{1}H NMR Spectral Parameters[a] of Melampodin B and Analogs

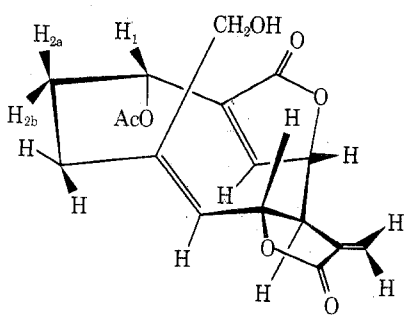
Compd	H-1	H-2[e]	H-3[e]	H-5	H-6	H-7	H-8	H-9	H-13a	H-13b	H-15	Miscellaneous
1a[d,f]	5.96 br dd (6.5; 6.5)	(a) 1.92 m (b) 2.45 m	2.2 m	5.99 br d (10.0)	4.99 dd (10.0; 10.0)	3.56 br ddd (10.0; 3.5; 3.0)	5.95 br	7.63 br	5.93 d (3.0)	6.47 d (3.5)	(a) 4.34 br d (15.0) (b) 4.40 br d (15.0)	2.02 (Ac) 6.60 (OH)
1b[d]	~5.9[g]	*g*	*g*	5.69 br (9.5)	4.93 dd (9.5; 9.5)	3.52 m	~5.9[g]	7.58 br	5.89 d (3.0)	6.41 d (3.5)	4.69 br[e]	2.02 (Ac)
1c[d]	5.83 br dd (7.0; 7.0)	*g*	*g*	6.60 br d (9.5)	5.12 dd (9.5; 9.5)	3.82 m	6.02 br	7.71 br	5.98 d (3.0)	6.47 d (3.5)	9.49 br	1.92 (Ac)
2a[d,f]	4.42 m	(a) 1.84 m (b) 2.34 m	(a) 2.08 m (b) 2.20 m	5.95 br d (9.0)	4.94 dd (10.0; 10.0)	3.52 br ddd (9.0; 3.5; 3.0)	5.97 br	7.58 br	5.96 d (3.0)	6.51 d (3.5)	(a) 4.3 br d (15.0) (b) 4.39 br d (15.0)	1.11 tr (5.5; CH_3'), 3.42 q[h] (5.5; CH_2'), 7.16 (OH)
2b[b]	4.33 m	*g*	*g*	5.47 br d (10.0)	4.60 dd (10.0; 10.0)	3.16 m	5.59 br	7.24 br	5.82 d (3.0)	6.48 d (3.5)	4.60 br[e]	1.21 tr (7.0; CH_3'), 3.53 q[h] (5.5; CH_2')
2c[c]	4.23 m	*g*	*g*	6.58 dd (10.0; 1.5)	4.72 dd (10.0; 10.0)	3.78 m	5.97 br	7.62 br	6.05 d (3.0)	6.38 d (3.5)	9.46 d (1.5)	1.07 tr (7.0; CH_3'), 3.44 q[h] (7.0; CH_2')
3a[c]	5.55 br dd (15.5; 6.5)	*g*	*g*	5.60 br d (10.0)	4.61 dd (10.0; 10.0)	3.49 m	5.86 br	7.54 dd (~1.0; ~1.0)	5.92 d (3.0)	6.27 d (3.2)	4.14 br[e]	1.13 d (7.0; CH_3')[i] 1.15 d (7.0; CH_3'')[i] 2.58 h (7.0; C-H′)
3b[b]	5.66 br dd (15.5; 6.5)	*g*	*g*	5.49 br d (10.0)	4.65 dd (10.0; 10.0)	3.16 m	5.57 br	7.14 br	5.80 d (3.0)	6.66 d (3.5)	4.61 br[e]	1.18 d (7.0; CH_3')[i] 1.19 d (7.0; CH_3'')[i] 2.11 (Ac) 2.53 h (7.0, CH′)
3c[b]	5.62[g]	*g*	*g*	6.41 d (10.0)	4.82 dd (10.0; 9.5)	3.38 m	5.62[g]	7.12 br	5.90 d (3.0)	6.52 d (3.5)	9.48 d (1.0)	1.14 d (7.0; CH_3')[i] 1.15 d (7.0); CH_3'')[i] 2.5 h (7.0; CH′)
4a[c]	5.30 m	*g*	*g*	*g*	3.50 m	3.37 m	5.92 br	8.03 d (1.5)	6.00 d (3.0)	6.31 d (3.5)	3.70 m[e]	2.02 (Ac)
4b[b]	5.37 ddd (10.0;5.0;1.5)	*g*	*g*	*g*	3.76 m	3.13 m	5.61 br	7.51 d (1.5)	5.82 d (3.0)	6.44 d (3.5)	3.87 d[e] (7.0)	2.06 (Ac)
5[c]	5.61 m	*g*	*g*	5.50 br d (10.0)	4.91 m	2.95 m	5.60 br	7.33			4.17 d (6.0)	1.40 d (8.0; C-11 CH_3) 2.08 (Ac) 4.91 m (H-11)
6[c]	5.39 ddd (5.5;5.5;1.0)	*g*	*g*	*g*	3.97 m	3.35 m	5.58 br	7.84 dd (1.0; 1.0)			3.37 d (6.0)	1.33 d (8.0; C-11 CH_3) 2.03 (Ac), 2.93 m (H-11)
7[c]	4.83 br dd (5.5; 11.5)	*g*	*g*	5.68 br d (9.5)	4.47 dd (9.5)	3.09 ddd (11.0; 9.5; 3.0)	5.73 dd (3.0; 1.5)	7.89 dd (1.5; ~1.0)			(a) 4.10 br d (10.0) (b) 4.27 br d (10.0)	3.5 d tr (11.0; 4.0; H-11) 3.93 d (4.0; 2 H-13[e])

[a] Spectra were run at 100 MHz except where indicated. Me_4Si was used as internal standard and values are recorded in parts per million relative to Me_4Si. Singlets are unmarked, multiplets are designated as follows: d, doublet; t, triplet; q, quartet; h, heptet; m, multiplet whose center is given; br, broad. Figures in parentheses are coupling constants or line separations in hertz. [b] $CDCl_3$. [c] Acetone-d_6. [d] Pyridine-d_5. [e] Intensity two protons. [f] Run at 300 MHz. [g] Obscured by superimposed signals. [h] Appearing as a doublet of a quartet owing to the nonequivalence of the C-2′ methylene hydrogens of the ethoxy group. [i] Pairs of diastereotopic methyls of the isobutyric acid moiety.

Chart I
Possible Configurations of Melampodin B



A

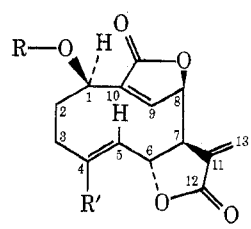


B

lampodin B. From biogenetic considerations and the cooccurrence of melampodin A,[3,6] a compound with known absolute configuration,[7,8] H-7 was assumed to have an α configuration and the C-4(5) double bond to adopt a trans configuration. From the inspection of models with a 4,5-cis double bond, a torsional angle of about 45° and a coupling constant between H-7 and H-8 greater than 5 Hz would have been predicted. In contrast, a skeletal arrangement with a 4,5-trans double bond dictates a torsional angle of 80° between H-7 and H-8, a value that correlates well with the observed coupling constant ($J_{7,8}$ = 2.5 Hz).

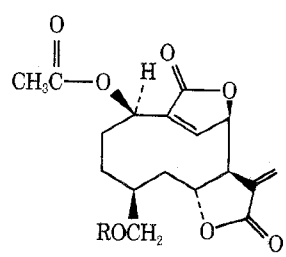
The stereochemical assignments at C-1 in **1a** are mainly based on the torsional angles between H-1 and the C-2a and C-2b protons, with the torsional angle between the H-1 and H-9 providing supporting evidence for the orientation of the side chain at C-1. The observed J values ($J_{1,2a}$ = $J_{1,2b}$ = 5.5 Hz) can be explained if the C-1 hydrogen bisects the two C-2 hydrogens with torsional angles between H-1 and H-2a and H-2b being approximately 45°. Stereomodels indicated that there exist two possible configurations each with two different conformations around the C_3–C_2–C_1 carbon centers. Both could be in agreement with the above J values (compare A and B in Chart I). In order to distinguish between the two possible configurations in melampodin B, the torsional angle dependence of the allylic coupling between the C-1 and C-9 protons was used. Maximum allylic coupling (>3.0 Hz) is observed when the two protons that are allylically coupled are perpendicular to one another.[9] The small coupling constant ($J_{1,9}$ = 1.0 Hz) found in melampodin B indicated that the torsional angle between H-1 and H-9 should be substantially smaller than 90°. From inspection of stereomodels of melampodin B a torsional angle of about 45° was derived which seems to be in good agreement with the observed coupling constants. Conversely, if the medium ring had contained an α-oriented C-1 substituent in a conformation as shown in B, the torsional angle between H-1 and H-9 would have been near 90°, thus a $J_{1,9}$ value of about 3 Hz should have been observed for melampodin B. Additional evidence for a syn orientation of the acetoxy group at C-1 and the hydroxyl group at C-15 as shown in Chart I, A, was derived from comparison of ir spectral data of melampodin B (**1a**) and cinerenin (**2a**). The OH absorption in the ir spectrum of **1a** appears as a sharp peak at 3450 cm^{-1}, indicating strong intramolecular hydrogen bonding between the OH group at C-15 and the C-1′ carbonyl function attached to C-1. This interaction does not occur in cinerenin, which has, as will be shown later, a β-oriented ethoxy group. On the basis of the above arguments, we tentatively assigned a β configuration of the acetoxy group at C-1 in **1a**, and a conformation as shown in A in Chart I.

Correlation of the spectral data of **1a** with its derivatives provided further support for the correctness of the previous structural assignments. Acetylation of **1a** caused a significant downfield shift of the broadened doublets at 4.34 and 4.40 ppm due to the two diastereotopic C-15 protons. Oxidation of **1a** with Sarett's reagent[10] resulted in a loss of the above absorptions and the appearance of an aldehyde proton signal at 9.49 ppm. The H-5 signal at 5.99 ppm in **1a** was shifted downfield to 6.60 ppm in **1c**, a position typical of a β hydrogen at an α,β-unsaturated aldehyde, thus indicating the presence of a primary, allylic alcohol group in **1a**.

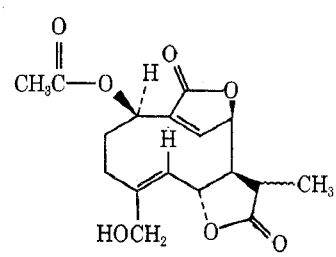


1a, R = Ac; R′ = CH_2OH **2a**, R = C_2H_5; R′ = CH_2OH
b, R = Ac; R′ = CH_2OAc **b**, R = C_2H_5; R′ = CH_2OAc
c, R = Ac; R′ = CHO **c**, R = C_2H_5; R′ = CHO

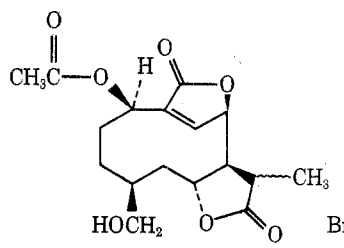
3a, R = $(CH_3)_2CHCO$; R′ = CH_2OH
b, R = $(CH_3)_2CHCO$; R′ = CH_2OAc
c, R = $(CH_3)_2CHCO$; R′ = CHO



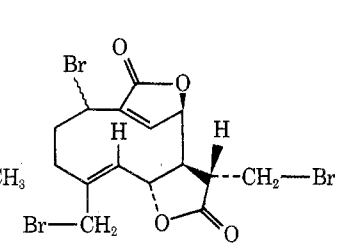
4a, R = H
b, R = Ac



5



6



7

The strong uv maximum at 215 nm and the observed allylic coupling between H-5 and H-15 in **1c** also corroborated the above assignments. The mass spectrum of melampodin B lacks a parent peak but shows intense peaks at m/e 274, 256 (base peak), and 228. The acetate **1b** gives a parent peak at m/e 376 and a peak at m/e 333 ($M^+ - 43$), which indicates the loss of an acylium ion (CH_3CO^+) from the parent ion. The fragment corresponding to m/e 274 ($M^+ - 102$) could be formed by a sequential or simultaneous loss

of CH_3COOH (60 mu) involving a McLafferty rearrangement of the C-1 acetoxy group and the elimination of ketene (42 mu) from C-15 in **1b**. Further loss of H_2O gives rise to the base peak at *m/e* 256 and the intense peak at *m/e* 228 in **1a** and **1b** must be due to the loss of CO (28 mu) from the fragment *m/e* 256.

Melampodin B was transformed into the tribromide **7** using a saturated solution of HBr in glacial acetic acid, involving substitution reactions at the allylic carbon centers C-1 and C-15 and an acid-catalyzed Michael addition at C-13. The observed coupling constants ($J_{1,2a}$ = 11.5, $J_{1,2b}$ = 5.5 Hz) for the H-1 doublet of doublets centered at 4.83 ppm indicates that one of the C-2 protons (H-2a) is anti periplanar to the C-1 proton while the torsional angle between H-2b and H-1 should be approximately 60°. Stereomodels indicated that these requirements can be met with H-1 adopting either an α or β orientation depending upon the conformation around the carbon atoms 1, 2, and 3 in **7**. Therefore, the configuration at C-1 in **7** could not be determined from the above coupling data. The multiplet at 3.50 ppm ($J_{7,11}$ = 11.0, $J_{11,13a} = J_{11,13b}$ = 4.0 Hz) in **7** was shown by double irradiation to be due to H-11. The large coupling constant ($J_{7,11}$ = 11.0 Hz) suggested that the protons have an anti periplanar orientation; therefore, since on biogenetic grounds H-7 was assumed to be α oriented, H-11 must have a β orientation. Spin decoupling experiments involving the signals of H-1, H-5, H-6, H-7, H-8, H-9, H-11, H-13, and H-15 verified the structural assignments of **7**. The similarities of the coupling constants of H-5, H-6, H-7, H-8, and H-9 in **1a** and **7** appear to be an expression of their stereochemical and conformational similarity.

Cinerenin and Derivatives. Cinerenin (**2a**), $C_{17}H_{20}O_6$, mp 161–163°, is a common constituent in *M. cinereum* and *M. argophyllum*. The ir spectrum of **2a** contained absorptions typical of α,β-unsaturated γ-lactones (1775, 1750 cm^{-1}) while signals at 3450 and 1665 cm^{-1} indicate a hydroxyl group and double bonds, respectively. The ir spectrum of the acetate **2b** exhibited no OH absorption indicating the presence of only one OH group in cinerenin. The OH group had to be primary since oxidation of **2a** with Sarett's reagent gave an aldehyde (**2c**). The NMR spectral features of **2c** were similar to those of the aldehyde **1c** derived from melampodin B (see Table I). Treatment of cinerenin with HBr in glacial acetic acid gave the tribromide **7** which had previously been obtained from melampodin B. This conversion provided strong evidence that **2a** must have a skeletal arrangement similar to melampodin B and the structural difference between the two compounds should be restricted to the side chain at C-1.

Further information which led to the final structure of cinerenin was deduced from correlations of 25.2-MHz ^{13}C NMR, ^1H NMR spectral data, and mass spectral fragmentation patterns. The ^{13}C NMR data obtained under PND and SFOCD conditions and the ^{13}C chemical shift considerations indicated that cinerenin contains 17 carbon atoms and possesses the following skeletal systems: three each of >C= and >CHO, two each of –C(=O)O, –CH=, C–CH_2C, and C–CH_2O, and one each of H_2C=, >CHC, and –CH_3. Extreme similarities of most ^{13}C NMR parameters of **1a** and **2a** strengthened the chemical evidence that melampodin B and cinerenin must have a structurally similar medium ring skeleton. Major differences were apparent for the signals due to C-1 and the possible two-carbon unit attached to C-1. From chemical shift considerations and the residual splitting patterns in the SFOCD spectra of **2a** (triplet at about 65 ppm and quartet at 15.7 ppm) the presence of an ethoxyl moiety in **2a** was suggested. Comparison of the chemical shifts and the splitting patterns of the 300-MHz spectra of melampodin B and cinerenin provided further strong evidence for the structure as shown in **2a**. Double irradiation experiments on cinerenin in acetone-d_6 at 100 MHz led to the structural assignments as summarized for **2a** in Table I. The major differences between the ^1H NMR spectra of **1a** and **2a** were observed for the proton signals due to the medium-ring side chain at C-1. In melampodin B, an acetate methyl signal was observed; instead a three-proton triplet at 1.11 ppm (C-2′) and a two-proton quartet centered at 3.42 ppm (C-1′) are present in cinerenin. In **2a** and its derivatives the quartet due to the two C-1′ methylene hydrogens showed a double pattern, appearing as a narrow-spaced doublet of a quartet, thus indicating the diastereotopic relationship of the two C-1′ methylene hydrogens in cinerenin and analogs. The mass spectral data of **2a** corroborated the above structural assignments. Cinerenin showed major mass spectral peaks at *m/e* 274, 256, and 228, typical of the melampodin B skeleton, and a parent peak at *m/e* 320. The peak at *m/e* 274 could result from a loss of ethanol (46 mu) from the parent ion *m/e* 320 by a McLafferty rearrangement, whereas the peaks at *m/e* 256 and 228 would be due to the subsequent loss of H_2O (18 mu) and CO (28 mu) from the ion *m/e* 274.

Unlike melampodin B, which carries an acetoxy group at C-1, cinerenin contains a C-1 ethoxy substituent. Cinerenin could represent an artifact of melampodin B, possibly by the introduction of the C-1 ethoxy group in the isolation procedure which involves lead acetate in ethanol–water. However, when **1a** was treated under conditions as applied in the isolation process, it was recovered quantitatively and no cinerenin was detected.

The stereochemistry at C-1 in **1a** was shown to have a β configuration of the acetoxy group which might be different in **2a**. In the ir spectrum of **1a**, the OH absorption appears as a sharp peak at 3450 cm^{-1} while in cinerenin the OH band is broadened. It could be argued that the alcohol group at C-15 undergoes intramolecular hydrogen bonding involving the C-1′ carbonyl function in **1a** while in cinerenin, owing to the lack of a C-1′ carbonyl group, the OH absorption is broadened, possibly owing to a stronger intermolecular contribution to the hydrogen bonding of the hydroxyl group at C-15. Alternatively, intermolecular hydrogen bonding in **2a** could be due to the α orientation of the ethoxy group at C-1. The remoteness of the involved atoms would not allow hydrogen bonding between the C-15 hydroxyl group and the C-1 oxygen. However, since the ^1H NMR coupling constants of H-1, H-5, H-6, H-7, H-8, and H-9 in the two compounds indicated close similarity for the corresponding proton interactions, the stereochemistries in the medium-ring skeleton including C-1 in cinerenin can be considered identical with that of melampodin B. This is evident from the 300-MHz ^1H NMR spectral patterns of the signals due to H-1, H-2a, H-2b, and the two H-3. The small allylic coupling between the H-1 and H-9 signals in cinerenin ($J_{1,9} \sim 1.0$ Hz) indicated that the torsional angle between the two protons is less than 90°, as in melampodin B. From the inspection of stereomodels it was learned that these conditions can be best met when the substituent at C-1 in **2a** is β oriented; thus, the ethoxy group at C-1 in cinerenin seems to have a β configuration. Since both melampodin B and cinerenin appear to have the same configuration at C-1, it is suggestive that in **2a** the ethoxy group is biosynthesized by a reductive process of the acetoxy carbonyl carbon in **1a**. Processes of this kind are rare in terpenoids and, to the best of our knowledge, cinerenin represents the first sesquiterpene lactone containing an ether-linked side chain.

Melampodin C. Melampodin C (**3a**), $C_{18}H_{22}O_7$, mp

199–201°C, cooccurred with melampodin B in *M. argophyllum*, a rare species in the mountains of northern Mexico. ^{1}H NMR spectral parameters and the mass spectral patterns of the new compound exhibited gross similarities with those of melampodin B. The mass spectra of melampodin C and its acetate (**3b**) showed major peaks at *m/e* 274 ($M^+ - 88$), 256, and 228 and parent peaks at *m/e* 362 and 404, respectively, suggesting that **3a** possesses a melampodin B type ring skeleton with the grouping $C_4H_7O_2$ (88 mu) attached to the medium ring. This was further substantiated by the conversion of **3a** into the tribromide **7** and by double irradiation experiments involving H-1, H-5, H-6, H-7, H-8, H-9, and the two H-13 signals in **3a**. In addition, **3a** exhibited two three-proton doublets at 1.13 and 1.15 ppm, respectively. The heptet at 2.5 ppm was coupled to the above two methyl doublets, indicating the presence of an isopropyl group in the side chain. The empirical formula, $C_{19}H_{22}O_7$, together with the chemical, mass spectral, and ^{1}H NMR data, only allows the presence of an isobutyrate group at C-1 in **3a**. On the basis of the extreme similarity of the NMR parameters of **1a** and **3a** and their derivatives, it appears that melampodin C exhibits the same configurational and conformational relationships as melampodin B.

4(5)-Dihydromelampodin B. This new compound (**4a**), $C_{17}H_{20}O_7$, mp 204–205°, was isolated from several populations of *M. cinereum*. It showed ir absorptions similar to those of melampodin B, indicating a hydroxyl group (3400 cm^{-1}), a γ-lactone (1785 cm^{-1}), an α,β-unsaturated ester (1750 cm^{-1}) and double bonds (1665 cm^{-1}). Treatment of **4a** with acetic anhydride in pyridine gave a monoacetate (**4b**), $C_{19}H_{22}O_8$, mp 195–196°. The absence of an OH absorption from the ir spectrum of **4b** indicated the presence of only one OH group in **4a**. The mass spectra of **4a** and **4b** gave parent peaks at *m/e* 336 and 378, respectively. The major peaks at *m/e* 276, 258, and 230 in **4a** and **4b** differed from those of melampodin B (**1a**) and **1b** (*m/e* 274, 256, and 228) by two mass units, strongly suggesting that **4a** represented a dihydro derivative of melampodin B. Further evidence concerning the structure of **4a** was provided by correlations of ^{1}H NMR spectra of melampodin B (**1a**), the acetate (**1b**), and the compounds **4a** and **4b** which involved detailed double-resonance experiments. In **4a**, doublets at 6.00 and 6.31 ppm and a multiplet at 3.37 ppm signified that it represents an α,β-unsaturated γ-lactone. Irradiation of the multiplet at 3.37 (H-7) collapsed the doublets at 6.00 and 6.31 (H-13a and H-13b), simplified the multiplet at 3.50 (H-6), and sharpened the broadened singlet at 5.92 ppm (H-8). Irradiation of the H-8 signal affected the multiplet at 3.37 (H-7) and collapsed the downfield doublet at 8.03 ppm (H-9) to a broadened singlet. At this point, the gross similarities between the ^{1}H NMR data of the new compound and melampodin B (**1a**) were apparent. The major ^{1}H NMR spectral differences between **1a** and **4a** were observed in the C-6 and C-15 proton signals. In melampodin B, the C-6 lactonic proton appeared as a sharp triplet at 4.99 while the H-6 signal in **4a** represented a complex multiplet at 3.50 ppm. This implied that more than one proton is attached to C-5 in **4a**. Further evidence that **4a** represents a dihydro derivative of melampodin B was provided by a two-proton signal at 3.70 ppm suggesting the presence of methylene protons (two C-15 protons) which are coupled to a proton at C-4. Double irradiation of **4b** at the center of the signals at 3.87 ppm (C-15 protons) affected the envelope at about 2.00 ppm, while irradiation at 2.00 ppm (H-4) caused the doublet at 3.87 ppm to collapse, indicating that **4a** and **4b** contain a proton at C-4. The mass spectral data and the above ^{1}H NMR spectral assignments suggest that the structural differences between melampodin B and **4a** lies in the absence of a 4(5) double bond in **4a**.

The stereochemistry of the new chiral center at C-4 in **4a** could not be obtained from the above spectral data. If melampodin B represents the biological precursor for the dihydro compound **4a**, then, from a fixed conformation of **1a** with a β-oriented C-15 moiety, the biological reducing reagent should attack from the outer face of the 4(5) double bond in **1a** and directly lead to a product with an α-oriented H-4, as is shown for **4a**.

Experimental Section[11]

Isolation of Melampodin B (1a) and Cinerenin (2a). A collection of *M. cinereum* DC. var. *cinereum* was made on July 19, 1973 (T. F. Stuessy and N. H. Fischer, No. 2015) 8.6 miles northeast of Hebbronville, Duval County, Texas, on route 359.

Dried leaves (1475 g) were extracted with cold chloroform and worked up as described before.[3] The crude syrup was allowed to stand at room temperature for several days, resulting in a partial crystallization of the syrup. Filtration and washing of the residue with ether gave a yellow, crystalline solid. Repeated trituration of this material with hot ethyl acetate (EtOAc) left 2.7 g of crude melampodin B (**1a**): mp 226–228° dec.; strong uv end absorption; CD (c 6.2×10^5, MeOH), $[\theta]_{216}$ -8.3×10^3, $[\theta]_{238}$ $+5.4 \times 10^3$, $[\theta]_{277}$ -5.4×10^2; ir ν_{max} (Nujol) 3420 (OH), 1780 (γ-lactone), 1730 (ester), 1655 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 274 (M − 60), 256 (M − 18 − 60, base peak), 245, 228, 227, 210, 165, 162, 91, and 43.

Anal. Calcd for $C_{17}H_{18}O_7$: C, 61.07; H, 5.43; mol wt, 334. Found: C, 61.28, H, 5.63.

The combined ethyl acetate extracts provided a final yield of 4.0 g of crude cinerenin (**2a**). Recrystallization from EtOAc gave colorless crystals: mp 161–163°; uv λ (MeOH) 205 nm (ϵ 2.7×10^{-4}) (end absorption); CD (c 6.25×10^{-5}, MeOH), $[\theta]_{218}$ -4.1×10^4, $[\theta]_{240}$ $+4.7 \times 10^4$; ir ν_{max} (Nujol) 3400 (broad, OH), 1770 (γ-lactone), 1750 (α,β-unsaturated ester), 1660 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 320 (M^+), 274 (M − 46), 256 (M − 18 − 46), 228, 227, 199, 179, 165, 147, 112 (base peak), 91, and 43; ^{13}C NMR (acetone-d_6)[12] 173.2 (C-14),[13a] 169.6 (C-12),[13a] 153.9 d (C-9), 148.1 (C-10), 136.6 (C-4), 133.0 (C-11), 122.3 d (C-5), 122.3 t (C-13), 79.1 d (C-8), 73.4 d (C-6),[13b] 73.2 d (C-1),[13b] 65.3 t (C-1′),[13c] 65.1 t (C-15),[13c] 49.5 d (C-7), 28.3 t (C-3), 23.2 t (C-2) and 15.7 q (C-2′).

Anal. Calcd for $C_{17}H_{20}O_6$: C, 63.74; H, 6.29; mol wt, 320. Found: C, 63.54; H, 6.20.

Melampodin B Acetate (1b). A solution of **1a** (103 mg) was heated in 1 ml of pyridine until all of the crystals were dissolved and then 1 ml of Ac_2O was added. The solution was left overnight at room temperature and then evaporated under reduced pressure to give a white residue. Water (5 ml) and a drop of concentrated HCl were added and the slurry was extracted three times with 50-ml portions of $CHCl_3$; the combined $CHCl_3$ extracts were dried ($MgSO_4$), filtered, and evaporated. The residual white powder was recrystallized from EtOAc, providing 70 mg of **1b**: mp 202–204°; uv λ_{max} (MeOH) 206 nm (ϵ 3×10^4); CD (c 7.1×10^{-4}, MeOH) $[\theta]_{216}$ -2.9×10^4, $[\theta]_{233}$ $+4.5 \times 10^4$; ir ν_{max} (Nujol) 1790, 1770 (γ-lactones), 1735, 1230 (acetate), 1660 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 376 (M^+), 333 (M − 43), 274 (M − 42 − 60), 256 (M − 18 − 42 − 60), 228, 165, 162, 147, 91, and 43 (base peak).

Anal. Calcd for $C_{19}H_{20}O_8$: C, 60.64; H, 5.32; mol wt, 376. Found: C, 60.55; H, 5.41.

11(13)-Dihydromelampodin B (5). A solution of 100 mg of **1a** in 90 ml of MeOH was hydrogenated for 1 hr over 5 mg of 10% Pd/C. After filtration and evaporation, the residue was chromatographed over silica gel using EtOAc as an eluent. The fractions were analyzed by TLC and combined appropriately. The crude residue was recrystallized from EtOAc, providing 30 mg of 11(13)-dihydromelampodin B (**5**): mp 200–202°; ir ν_{max} (Nujol) 3420 (OH), 1775 (γ-lactone), 1735 and 1245 cm^{-1} (acetate).

Anal. Calcd for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99; mol wt, 336. Found: C, 60.90; H, 5.95; mol wt, 336 (MS).

Oxidation of 1a with Sarett's Reagent. Melampodin B (100 mg) in 50 ml of acetone under nitrogen was treated with an excess of Sarett's reagent[10] in acetone. After 4 hr the brown precipitate was filtered and the acetone evaporated. Water (5 ml) was added

and the slurry extracted twice with 75 ml of $CHCl_3$ and once with 50 ml of EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated. The impure brownish crystals were dissolved in acetone and chromatographed over silica gel–CH_2Cl_2. The column was eluted with CH_2Cl_2, CH_2Cl_2–EtOAc (1:1), and finally with pure EtOAc. The recovered crystals were recrystallized from acetone–2-propanol, providing 45 mg of colorless, crystalline **1c**: mp 241–245° dec; uv λ_{max} (MeOH) 215 nm (ϵ 3.2 × 10^4); ir ν_{max} (Nujol) 1775 (γ-lactone), 1740 (acetate), 1660 (double bonds), 1700, and 1240 cm^{-1}.

Anal. Calcd for $C_{17}H_{16}O_7$: C, 61.45; H, 4.82; mol wt, 332. Found: C, 61.26; H, 4.84; mol wt, 332 (MS).

Reaction of 1a with Lead(II) Acetate. Melampodin B (100 mg) was stirred overnight at room temperature in a mixture of 50 ml of 5% lead(II) acetate in H_2O and 50 ml of EtOH. The solution was filtered and the filtrate evaporated to approximately 30 ml and then extracted three times with 30 ml each of $CHCl_3$. The combined $CHCl_3$ extracts were dried and filtered and the solvent evaporated. A crude white residue was left, which after recrystallization from acetone gave unchanged **1a**, characterized by melting point, mixture melting point, ir, and NMR with authentic material.

Reaction of 1a with HBr. A solution of melampodin B (100 mg) in 25 ml of glacial acetic acid and 3.0 ml of a saturated solution of HBr in glacial acetic acid was refluxed for 20 hr. The solvent was evaporated and ethyl ether was added which resulted in the precipitation of a white solid. Recrystallization from 2-propanol yielded 85 mg of pure **7**: mp 215–217°; ir ν_{max} (Nujol) 1775 (γ-lactone), 1650 (double bonds), 1200, and 1000 cm^{-1}.

Anal. Calcd for $C_{15}H_{15}O_4Br_3$: C, 36.08; H, 3.01; Br, 48.10; mol wt, 499. Found: C, 36.1 ; H, 3.07; Br, 48.00; mol wt, 499 (MS).

Cinerenin acetate (2b) was obtained from 100 mg of **2a** as described above for **1b**. Recrystallization of the crude material from 2-propanol gave 60 mg of pure **2b**: mp 187–189°; ir ν_{max} (Nujol) 1780 and 1770 (γ-lactones), 1740, 1235, 1225 (acetate), and 1665 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 362 (M^+), 333 (M − 29), 316 (M − 46), 274 (M − 42 − 46, base peak), 256 (M − 18 − 42 − 46), 228, 178, 165, 162, 147, 112, and 43.

Anal. Calcd for $C_{19}H_{22}O_7$: C, 62.97; H, 6.12; mol wt, 362. Found: C, 62.89; H, 6.11.

Oxidation of 2a with Sarett's Reagent. To a solution of 105 mg of **2a** in 25 ml of acetone in a nitrogen atmosphere an excess of Sarett's reagent was added. After 4 hr, the brown precipitate was filtered and the acetone was removed by evaporation. Water (5 ml) was added and the slurry was extracted twice with 50 ml of $CHCl_3$ and once with 50 ml of EtOAc. The combined organic extracts were dried ($MgSO_4$) and evaporated. The crude brownish crystals were taken up in acetone and chromatographed over silica gel; the column was eluted with CH_2Cl_2, CH_2Cl_2–EtOAc (1:1), and finally with pure EtOAc, which gave colorless crystals. Recrystallization from 2-propanol–$CHCl_3$ gave 50 mg of pure **2c**: mp 218–221° dec; uv λ_{max} (MeOH) 216 nm (ϵ 2.7 × 10^4); ir ν_{max} (Nujol) 1770 (γ-lactones), 1700 (α,β-unsaturated aldehyde), 1655 (double bonds), and 1110 cm^{-1}.

Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70; O, 30.16; mol wt, 318. Found: C, 63.93; H, 5.59; O, 30.35; mol wt, 318 (MS).

Reaction of **2a** (105 mg) with HBr under conditions described above for **1a** gave 80 mg of **7**.

Isolation of Melampodin C (3a) from *M. argophyllum*. A collection of *M. argophyllum* was made on July 3, 1974 (T. F. Stuessy No. 3599) 30 miles southeast of the Coahuila-Nuevo León border on route 53 in the state of Nuevo León, Mexico. Dried leaves (1350 g) were extracted and worked up as described before.[3] The crude syrup (22 g), when allowed to stand at room temperature for 3 weeks, partially crystallized, providing 2.5 g of crude melampodin B. The remaining crude syrup (10 g) was chromatographed over 300 g of silica gel (Brinkmann 7734) collecting 20-ml fractions. The column was eluted using the following solvent mixtures: 1000 ml of CH_2Cl_2–EtOAc (9:1); 500 ml of CH_2Cl_2–EtOAc (4:1); 450 ml of CH_2Cl_2–EtOAc (2:1); 700 ml of CH_2Cl_2–EtOAc (1:1); 300 ml of EtOAc; 500 ml of 5% MeOH in EtOAc; and 500 ml of 15% MeOH in EtOAc. The following fractions were collected and combined according to TLC analysis. Fractions 21–40 contained 610 mg of artemetin (5-hydroxy-3,4′,5′,6,7-pentamethoxyflavone). Fractions 71–100 provided 1.23 g of melampodin C (**3a**). Recrystallization from EtOAc–Et_2O gave colorless crystals: mp 199–201°; uv λ (MeOH) 205 nm (ϵ 2.9 × 10^{-4}) (end absorption); CD (*c* 5.5 × 10^{-5}, MeOH) $[\theta]_{218}$ −5.2 × 10^4, $[\theta]_{238}$ +3.8 × 10^{-4}, $[\theta]_{280}$ −9.3 × 10^2; ir ν_{max} (Nujol) 3450 (OH), 1770 (γ-lactone), 1725 (ester), and 1655 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 362 (M^+), 344 (M − 18), 274 (M − 88), 256 (M − 18 − 88), 228, 165, 147, 91, 43 (base peak).

Anal. Calcd for $C_{19}H_{22}O_7$: C, 62.97; H, 6.12; O, 30.91; mol wt, 362. Found: C, 63.07; H, 6.00; O, 30.77.

Fractions 116–130 gave 890 mg of **2a** and 3.0 g of **1a** was obtained from fractions 140–160.

Melampodin C acetate (3b) was obtained from 170 mg of **3a** as described above for **1b**. Recrystallization of the crude product from 2-propanol provided 160 mg of **3b**: mp 151–153°; ir ν_{max} (Nujol) 1780, 1765 (γ-lactones), 1225 cm^{-1} (acetate); low-resolution mass spectrum *m/e* 404 (M^+), 361 (M − 43), 316 (M − 88), 274 (M − 42 − 88), 256, (M − 18 − 42 − 88), 228, 165, 147, 91, 71, 43 (base peak).

Anal. Calcd for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98; O, 31.65; mol wt, 404. Found: C, 62.53; H, 5.96; O, 31.57.

Oxidation of Melampodin C with Sarett's Reagent. Melampodin C (150 mg) was dissolved in 25 ml of acetone under nitrogen and an excess of Sarett's reagent, suspended in acetone, was added. After 4 hr, the brown precipitate was filtered and the acetone was removed by evaporation. The crude residue was chromatographed over 25 g of silica gel. The column was eluted with 300 ml of CH_2Cl_2–EtOAc (9:1), then with 200 ml of CH_2Cl_2–EtOAc (1:1). Evaporation of later fractions gave a white powder. Recrystallization from EtOAc provided 71 mg of the pure aldehyde **3c**: mp 207–209°; ir ν_{max} (Nujol) 1785 (γ-lactone), 1735 (ester), 1245, and 1145 cm^{-1}; low-resolution mass spectrum *m/e* 360 (M^+), 359 (M^+ − 1) 329, 315, 274, 256, 212, 91, 77, 71, 43, 29 (base peak).

Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59; O, 31.08; mol wt, 360. Found: C, 63.46; H, 5.64; O, 30.84.

Reaction of **3a** (100 mg) with HBr under conditions described above for **1a** gave 60 mg of compound **7**.

Isolation of 4(5)-Dihydromelampodin B (4a). A collection of *M. cinereum* var. *cinereum* was first made on Oct 4, 1971 (N H. Fischer No. 12) 12 miles south of George West, Texas, on Highway 59 and again on July 7, 1973 (N. H. Fischer No. 29). NMR spectra of the crude extracts indicated the presence of the same constituents in the above collections.

The dried leaves (200 g) were extracted and worked up as described before,[3] providing 18 g of crude terpenoid-containing syrup. The crude syrup (9 g) was chromatographed over 300 g of silica gel (Baker 3405) collecting 15-ml fractions and using the following solvent mixtures: fractions 1–40 (CH_2Cl_2–EtOAc, 9:1), 41–60 (CH_2Cl_2–EtOAc, 4:1), 61–100 (CH_2Cl_2–EtOAc, 3:1), 100–170 (CH_2Cl_2–EtOAc, 1:1), 171–190 (CH_2Cl_2–EtOAc, 1:4), 191–220 (pure EtOAc), 220–260 (5% MeOH in EtOAc), and 260–289 (MeOH–EtOAc, 1:1). The following fractions were combined according to TLC analysis. Fractions 40–57 gave 50 mg of artemetin, mp 159–160°, identical with an authentic sample by mixture melting point and spectral comparison (ir, NMR). Fractions 227–261 provided 300 mg of 4(5)-dihydromelampodin B (**4a**): mp 204–205°; uv λ_{max} (EtOH) 202 nm (ϵ 3.2 × 10^{-4}); CD (*c* 1 × 10^{-5}, MeOH) $[\theta]_{220}$ −106 × 10^3, $[\theta]_{265}$ 1.60 × 10^3; ir ν_{max} (Nujol) 3400 (OH), 1785 (γ-lactone), 1750 (ester), and 1665 cm^{-1} (double bonds); low-resolution mass spectrum *m/e* 336 (M^+), 276 (M − 60), 258 (M − 18 − 60), 230, 228, 201, 165, 162, 91, 43 (base peak).

Anal. Calcd for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99; mol wt, 336. Found: C, 61.01; H, 6.31.

4(5)-Dihydromelampodin B acetate (4b) was obtained from 100 mg of **4a** as described for **1b**. Recrystallization of the crude product from EtOAc–Et_2O provided 95 mg of **4b**: mp 195–196°; ir ν_{max} (Nujol) 1777 (γ-lactone), 1245 (acetate), and 1070 cm^{-1}; low-resolution mass spectrum *m/e* 378 (M^+) 335 (M − 43), 276 (M − 42 − 60), 258 (M − 18 − 42 − 60), 230, 165, 162, 149, 111, 91, and 43 (base peak).

Anal. Calcd for $C_{19}H_{22}O_8$: C, 60.32; H, 5.82; O, 33.86; mol wt, 378. Found: C, 60.40; H, 5.80, O, 33.73.

4(5),11(13)-Tetrahydromelampodin B (6). A solution of 100 mg of 4(5)-dihydromelampodin B in 75 ml of MeOH and 5 mg of 10% Pd/C were placed in a 100-ml round-bottom flask. After removal of air in vacuo the stirred mixture was hydrogenated under rapid uptake of hydrogen for about 15 min. The reaction was terminated after 1 hr; filtration and evaporation of MeOH provided a syrup which was chromatographed by preparative layer chromatography using propyl acetate as developing solvent. The band at R_f 0.3 was extracted from the silica gel and the resulting crude material recrystallized from acetone, giving 35 mg of **6**: mp 220–222°; ir ν_{max} (Nujol) 3500 (OH), 1770, 1750 (γ-lactone), 1725, and 1240 cm^{-1} (acetate).

Anal. Calcd for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55; mol wt, 338. Found: C, 60.42; H, 6.27; mol wt, 338 (MS).

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Registry No.—**1a**, 51419-54-6; **1b**, 51212-98-7; **1c**, 56650-61-4; **2a**, 56650-62-5; **2b**, 56650-63-6; **2c**, 56650-64-7; **3a**, 56650-65-8; **3b**, 56650-66-9; **3c**, 56650-67-0; **4a**, 56650-68-1; **4b**, 56650-69-2; **5**, 56650-70-5; **6**, 56650-71-6; **7**, 56650-72-7; lead(II) acetate, 301-04-2; HBr, 10035-10-6.

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(12) The spectra were determined on a Varian XL-100-15 spectrometer operating Fourier transform mode with proton decoupling. Me_4Si was used as internal standard and the values are in parts per million relative to Me_4Si. The number of lines in the single-frequency off-center decoupled spectra are designated as follows: d, doublet; t, triplet; q, quartet. Unmarked signals are singlets.
(13) (a)–(c) Vice versa.

Acanthospermal A and Acanthospermal B, Two New Melampolides from *Acanthospermum* Species[1]

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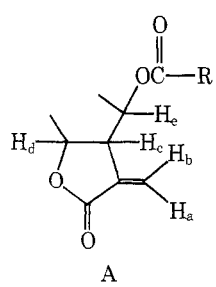
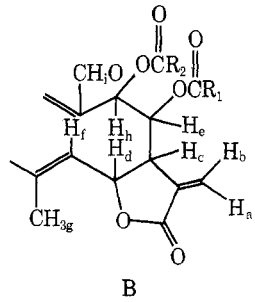
The isolation and structure determination of acanthospermal A (**1a**) from *Acanthospermum australe* (L.) Kuntze and acanthospermal B (**4a**) from *A. hispidum* DC. is reported. Both compounds belong to the melampolide subgroup of germacradienolides. **1a** is the first sesquiterpene lactone to possess an α-hydroxyisobutyric acid ester side chain.

In continuation of our search for sesquiterpene lactones with potential biological activity in Compositae we have examined two local *Acanthospermum* species (tribe Heliantheae, subtribe Melampodiinae). This resulted in the isolation of two closely related noncrystalline melampolides, acanthospermal A (**1a**) from *Acanthospermum australe* (L.) Kuntze and acanthospermal B (**4a**) from *A. hispidum*



A



B

DC. Structures and stereochemistry were established by chemical transformations and extensive use of ^1H and ^{13}C NMR spectrometry.

Acanthospermal A (**1a**), $C_{23}H_{30}O_8$ (high-resolution mass spectrum and elemental analysis), $[\alpha]^{25}_{Hg}$ −54°, was an α,β-unsaturated aldehyde (ir band at 1690 cm^{-1}, NMR signal at 9.45 ppm) and an α,β-unsaturated lactone of the type shown in A as evidenced by the usual criteria (strong uv end absorption due to superposition of the two chromophores, ir bands at 1780 and 1620 cm^{-1}, narrowly split NMR doublets of H_a and H_b at 6.25 and 5.73 ppm). Attempts to locate H_c by spin decoupling were complicated by overlapping of signals in the $CDCl_3$ spectrum, but a solution of **1a** in benzene-d_6 afforded excellent separation of signals (see Table I) and permitted determination of the entire carbon framework.

The location of H_c as a multiplet at 2.30 ppm was established by double irradiation at the frequency of H_a and H_b. Irradiation at the frequency of H_c collapsed H_a and H_b into singlets and also converted a triplet at 4.97 ppm ($J_1 = J_2 =$ 10 Hz) into a doublet and a narrowly split doublet of doublets at 6.99 ppm ($J_1 = 9$, $J_2 = 1.5$ Hz) into a clean doublet ($J = 9$ Hz). Thus H_d and H_e were at 4.97 and 6.99 ppm, respectively, or the reverse. The chemical shift of the lower field proton suggested that it was under an ester rather than under the lactone oxygen, especially since the ir spectrum indicated the presence of additional carbonyl functions near 1740 cm^{-1} associated with esters. Hence the signal at 4.97 ppm was provisionally assigned to H_d and the signal at 6.99 ppm to H_e. The reason for the unusual paramagnetic shift of H_e will be discussed subsequently.

Irradiation at the frequency of H_d converted H_c into a broad singlet and also changed a broadened doublet at 4.39 ppm ($J = 10$ Hz, H_f) into a broadened singlet. The broadening was due to allylic coupling with a vinylic methyl (H_g) which appeared as a narrowly split doublet at 1.63 ppm. Irradiation at the frequency of H_e slightly sharpened H_c and