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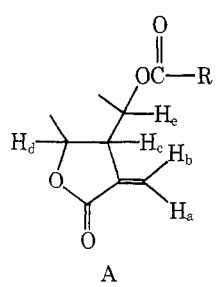
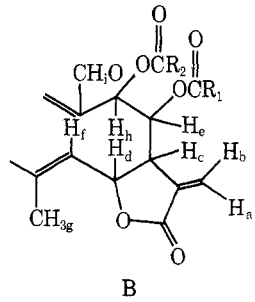
The isolation and structure determination of acanthospermal A (**1a**) from *Acanthospermum australe* (L.) Kuntze and acanthospermal B (**4a**) from *A. hispidum* DC. is reported. Both compounds belong to the melampolide subgroup of germacradienolides. **1a** is the first sesquiterpene lactone to possess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



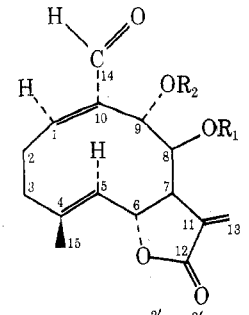
B

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.

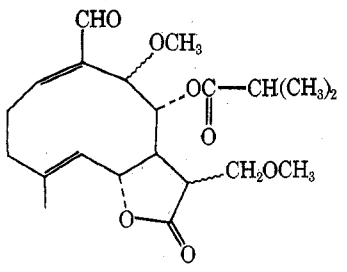
Irradiation at the frequency of H_d converted H_c into a broad singlet and also changed a broadened doublet at 4.39 ppm ($J = 10$ Hz, H_f) into a broadened singlet. The broadening was due to allylic coupling with a vinylic methyl (H_g) which appeared as a narrowly split doublet at 1.63 ppm. Irradiation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



1a, $R_1 = -C(=O)-CH(CH_3)_2$; $R_2 = -C(=O)-C(OH)(CH_3)_2$

b, $R_1 = -C(=O)-CH(CH_3)_2$; $R_2 = Ac$

c, R_1, R_2 reverse of **1a**



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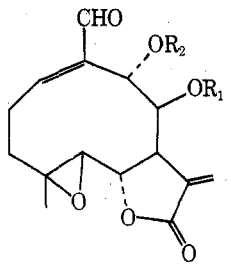
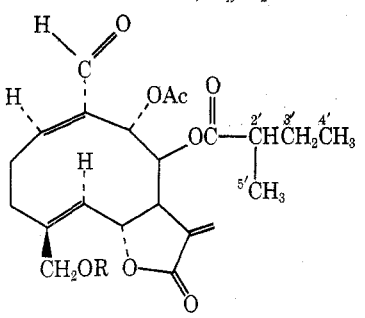
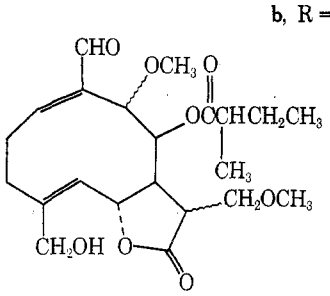


3, R_1, R_2 same as in **1a**



4a, R = H

b, R = Ac



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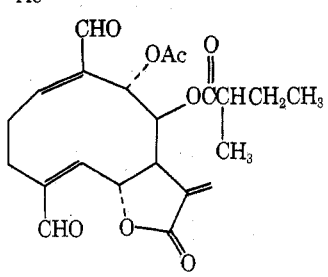

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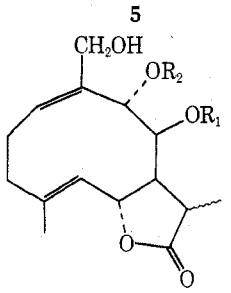
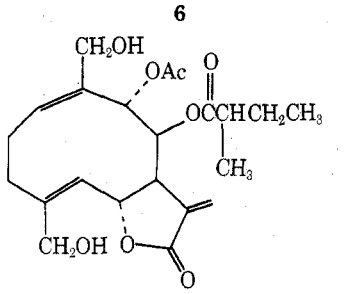




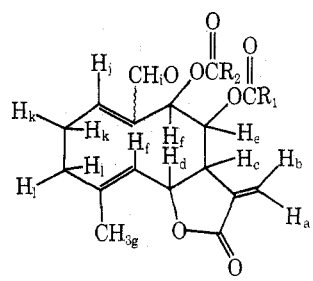
7, R_1, R_2 same as in **1a**

8

also converted a doublet of doublets at 5.10 ppm (H_h, J_1 = 9, J_2 = 2 Hz) into a doublet (J = 2 Hz). The smaller coupling of H_h could be traced to the aldehyde proton H_i. The chemical shift of H_h suggested that it might be either olefinic, with allylic coupling to the aldehyde proton (in which case H_h would have to be β to the aldehyde and less deshielded than usual) or under an ester oxygen with W coupling to the aldehyde proton as in frutescin.[2] The ambiguity was decided in favor of the second alternative by a single-frequency off-resonance decoupling experiment in the ^{13}C NMR spectrum (Table II); irradiation at the frequency of H_h collapsed a doublet at 67.8 ppm, clearly associated with carbon attached to oxygen by a single bond, and not one of the doublets (at 159.2 and 126.8 ppm) identifiable with –CH=. In a similar vein, irradiation at the frequency of H_e collapsed a doublet at 72.0 ppm, thus showing that in spite of its unusually low shift H_e was attached to a carbon atom carrying two carbons and one oxygen. Consequently partial structure A could be expanded to B.

The NMR spectrum further exhibited a one-proton doublet of doublets (H_j) at 5.79 ppm (J_1 = 10, J_2 = 9 Hz), presumably the proton β to the aldehyde function.[3] The identity of this signal was confirmed by single-frequency off-resonance decoupling in the ^{13}C NMR spectrum which resulted in collapse of the doublet at 159.2 ppm to a singlet. Irradiation at the frequency of H_j also simplified two well-separated multiplets at 2.54 and 1.78 ppm which were obviously associated with geminally coupled protons (H_k). Decoupling experiments further showed that the methylene group of H_k was adjacent to another methylene group whose protons (H_l) appeared as multiplets at 1.80 and 1.41 ppm.

Consideration of these results permitted extension of B to C. In accordance with this formula, epoxidation of acanthospermal A gave a monoepoxide (**3**), in whose NMR spectrum (Table I) the split vinylic methyl signal was replaced by a sharp methyl signal at 1.70 ppm and the H_d and H_f frequencies had shifted upfield to 4.27 and 2.60 ppm, respectively.



C

The nature of R_1 and R_2 was deduced as follows. The NMR spectrum exhibited two methyl doublets at 0.86 and 0.87 ppm, each coupled to a one-proton multiplet at 2.11 ppm, thus pointing to the possibility of an isobutyryl side chain. This was confirmed by the loss of 88 mass units and the appearance of peaks corresponding to 87, 71, and 43 mass units in the mass spectrum. The second ester side chain had to correspond to $C_4H_7O_3$ to fit the molecular formula, the extra oxygen atom deriving from a hydroxyl group (ir frequency at 3510 cm^{-1}) which appeared to be tertiary (carbon singlet at 72.2 ppm) and could not be accommodated in the ten-membered ring. Since the NMR spectrum displayed two additional methyl singlets at 1.22 and 1.28 ppm, presumably methyls on carbon carrying single-bonded oxygen, it was concluded that the second side chain was α-hydroxyisobutyrate. In accord with this conclusion, the high-resolution mass spectrum also showed an important peak corresponding to loss of α-hydroxyisobutyric acid; moreover, the base peak corresponded to the combined loss of isobutyric and α-hydroxyisobutyric acid.

The following experiments permitted placement of these two ester side chains. Attempted acetylation of acanthospermal A with pyridine–acetic anhydride furnished a substance **1b**, $C_{21}H_{26}O_7$, by replacement of the α-hydroxyisobutyryl side chain with an acetyl function (NMR spectrum, see Table I). Hydrolysis of **1a** with sodium methoxide in methanol gave a single product $C_{21}H_{30}O_7$ whose NMR spectrum (Table I) fitted in well with structure **2** (exclusive of stereochemistry). Apart from methanol addition to the

Table I
^{1}H NMR Spectra of Acanthospermals and Derivatives[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-14	H-15	Misc
1a	6.80 dd (10, 9)	2.84 m (14, 10, 10, 2) 2.5 m[c] (14, 9, 2)	2.50 dd[c] (14, 2) 2.09 ddd (14, 10, 2)	5.00 d br (10)	5.07 t (10)	2.63 m[c]	6.73 dd (9, 1.5)	5.15 dd (9, 2)	6.25 d (3.5) 5.73 d (3.5)	9.45 d (2)	2.00 br	1.08 d (7)[d] (H-3′); 1.27[b], 1.29[b] (H-3′); 2.50 m[c] (H-2′)
1a[e]	5.79 dd (10, 9)	2.54 m (14, 10, 10, 2) 1.78 m (14, 9, 2)	1.80 dd (14, 2) 1.41 ddd (14, 10, 2)	4.39 d br (10)	4.97 t (10)	2.30 m	6.99 dd (9, 1.5)	5.10 dd (9, 2)	6.22 d (3.5) 5.66 d (3.5)	9.00 d (2)	1.63 br	0.86 d (7), [b] 0.87 d (7)[b] (H-3′); 1.22, [b] 1.28[b] (H-3″); 2.11 m (H-2′)
1b	6.77 dd (10, 9)	2.81 m (14, 10, 10, 2) 2.46 m[c]	2.59 m[c] 2.07 ddd (14, 10, 2)	4.91 d br (10)	5.05 t (10)	2.59 m[c]	6.70 dd (9, 1.5)	5.25 dd (9, 2)	6.25 d (3.5) 5.73 d (3.5)	9.47 d (2)	2.00 br	1.09 d (7)[d] (H-3′) 1.95[b] (Ac); 2.46 m[c] (H-2′)
2	6.78 dt (10, 9)	2.66 m[c] 2.30 m[b]	2.39 m[c] 2.05 ddd (14, 10, 2)	4.90[c]	4.90[c]	2.30[c]	6.12 dd (9, 1.5)	3.56 dd (9, 2)	3.70[f]	9.43 d (2)	1.90 br	1.20 d (7)[d] (H-3′) 2.39 m[b] (H-2′) 3.35[b] (OMe)
3	6.97 dd (10, 9)	3.17 2.70 m	2.43 m 1.24 m	2.60 d br (10)	4.27 t	2.80 m	6.78 dd (9, 1.5)	5.64 dd (9, 2)	6.35 d (3.5) 5.87 d (3.5)	9.52 d (2)	1.70 br	1.06 d (7), [b] 1.08 (7)[b] (H-3′); 1.32, [b] 1.34[b] (H-3″); 2.50 m (H-2′) 1.60 (–OH)
4a	6.81 dd (10, 9)	2.89 m[c] 2.65 m[c]	2.89 m[c] 2.00 ddd (14, 10, 2)	5.03 br (10)	5.27 t (10)	2.65 m[c]	6.69 dd (9, 1.5)	5.32 dd (9, 2)	6.26 d (3.5) 5.78 d (3.5)	9.45 d (2)	4.50[f]	0.87 t (7)[b] (H-4′); 1.09 d (7)[b] (H-5′) 1.60 m, 1.43 m (H-3′) 1.98[b] (Ac); 2.35 m (H-2′)
4a[e]	5.73[c]	2.63 m (14, 10, 10, 2) 1.80 m (14, 9, 2)	2.48 dd (14, 10) 1.41 ddd (14, 10, 2)	4.44 d br (10)	5.21 t (10)	2.32 m	7.00 dd (9, 1.5)	5.30 dd (9, 2)	6.23 d (3.5) 5.75 d (3.5)	9.05 d (2)	4.21[f]	0.68 t (7)[b] (H-4′); 0.90 d (7)[b] (H-5′); 1.00 m, 1.19 m (H-3′) 1.50[b] (Ac), 2.07 m (H-2′)
4b	6.78 dd (10, 9)	2.92 m (14, 10, 10, 2) 2.5–2.7[c]	2.5–2.7[c] 2.12	2.86 d br (10)	5.08 t (10)	2.5–2.7[c]	6.68 dd (9, 1.5)	5.28 dd (9, 2)	6.28 d (3.5) 5.82 d (3.5)	9.47 d (2)	5.00[f]	0.85 t (7)[b] (H-4′), 1.08 d (7)[b] (H-5′), 1.45 m, 1.60 m (H-3′), 1.95[b], 2.12[b] (Ac), 2.36 m (H-2′)

5	6.80 dd (10, 9)	2.82 m	2.66 m	4.98 d br (10)	5.05 t (10)	2.55 m	6.11 d br (9)	3.79 d br	3.70[f]	9.50 d (2)	4.40[f]	0.94 t (7)[b] (H-4'); 1.17 d (7)[b] (H-5'); 1.52 m, 1.70 m (H-3'); 2.47 m (H-2'); 3.10, [b] 3.37[b] (OMe)
		2.32 m	2.00 m									
6	6.78 dd (10, 9)	2.76 m[c]	2.76 m[c]	6.05 d br (10)	5.00 t (10)	3.20 m	6.71 dd (9, 1.5)	5.00 dd (9, 2)	6.36 d (3.5)	9.48 d (2)	10.22 br	0.83 t (7)[b] (H-4'); 1.05 d (7)[b] (H-5'); 1.45 m, 1.61 m[c] (H-3'); 1.90 (Ac); 2.33 m (H-2')
		1.61 m[c]	1.82 dd (14, 10, 2)						5.89 d (3.5)			
7	5.75 dd (10, 9)	2.42 m (14, 10, 10, 2)	2.27 m[c]	4.90 d br (10)	5.00 t (10)	2.27 m[c]	5.78 dd (9, 1.5)	5.34 d (9)	1.25 d[b] (7)	4.20[f]	1.94 br	1.17 t (7)[b] (H-3'); 1.32 d (H-3''); 2.17 m (H-2''); 2.58 m (H-11)
		1.94 m[c]	1.94 m[c]									
8	5.75 dd (10, 9)	2.1–2.8 m[c]	2.1–2.8 m[c]	5.00 d br (10)	5.20 t (10)	2.1–2.8 m[c]	5.70 dd (9, 1.5)	5.42 d (9)	1.25 d[b] (7)	4.23[f]	4.44[f]	0.90 t (7)[b] (H-4'); 1.15 d (7)[b] (H-5')
		2.8 m[c]	1.85 ddd (14, 10, 2)									1.44 m, 1.64 m (H-3'); 2.1–2.8 m (H-2' and H-11)

[a] Run in $CDCl_3$ at 270 MHz on a Bruker HX-270 instrument with Me_4Si as internal standard, unless otherwise specified. Values are in parts per million: d, doublet; t, triplet; br, broadened singlet; m, multiplet. Unmarked signals are singlets. [b] Intensity three protons. [c] Signal partially obscured or superimposed. [d] Intensity six protons. [e] Run in C_6D_6. [f] Intensity two protons, center of AB system.

Table II
^{13}C NMR Spectra of Acanthospermal A and B[a]

1a	Assignment[b]	4a	Assignment[b]
193.5 d	C-14	193.9 d	C-14
176.2	C-1'	176.0	C-1'
175.4	C-1''	170.3	Ac
169.0	C-12	168.4	C-12
159.2 d	C-1[c]	158.4 d	C-1
140.8	C-10	141.5	C-10
138.3	C-4	141.0	C-4
134.2	C-11	134.1	C-11
126.8 d	C-5	128.5 d	C-5
121.8 t	C-13	122.0 t	C-13
74.9 d	C-6[c]	73.6 d	C-6
72.2	C-2''	70.3 d	C-8
72.0 d	C-8	68.1 d	C-9
67.8 d	C-9[c]	60.4 t	C-15
51.0 d	C-7	51.2 d	C-7
36.9 t	C-3[c]	41.4 d	C-2'
34.1 d	C-2'	32.4 t	C-3
26.8 t	C-2	27.6 t	C-3'
26.8 q	C-3'[c]	26.6 t	C-2
26.8 q	C-3'[c]	20.7 q	Ac
19.0 q	C-3''	16.8 q	C-5'
18.8 q	C-3''	11.5 q	C-4'
16.8 q	C-15[c]		

[a] Run in $CDCl_3$ on Bruker HX-270 instrument. Unmarked signals are singlets. [b] Assignments based on predicted shifts and comparisons with data in the literature and in our files. [c] Assignment established by single frequency off-resonance decoupling.

methylene group of the conjugated lactone, the α-hydroxyisobutyryl group had been replaced by a methoxyl. The signal of H_h now appeared at 3.56 ppm, whereas the chemical shift of H_e (6.12 ppm) indicated that the corresponding carbon atom retained the remaining ester side chain, i.e., the isobutyrate unit. Therefore, in acanthospermal A the isobutyrate side chain must be at C-8 and the easily displaced α-hydroxyisobutyrate chain at C-9.

Before delineating the stereochemistry of acanthospermal A, we shall discuss acanthospermal B (**4a**), $C_{22}H_{28}O_8$ (high-resolution mass spectrum), $[\alpha]_{Hg}^{25}$ −33°, whose spectral properties were very similar to those of **1a** and indicated the presence of an α,β-unsaturated lactone, an α,β-unsaturated aldehyde, two ester side chains, and a hydroxyl group. Comparison of the chemical shifts of the various protons[4] showed the essential identity of the basic germacradiene system, but in acanthospermal B the vinylic methyl of **1a** was replaced by a hydroxymethylene group (AB quartet at 4.50 shifted downfield to 5.00 ppm on acetylation to **4b**).

The two ester groups were also different. One was an acetate (singlet at 1.98 ppm); the second—a five-carbon unit to be accommodated in the molecular formula—was an α-methyl butyrate as evidenced by the presence of a methyl doublet (1.09 ppm) coupled to a one-proton multiplet at 2.35 ppm. This was also coupled to two one-proton multiplets at 1.60 and 1.43 ppm, each of which was coupled in turn to a methyl triplet at 0.87 ppm. In accordance with these deductions the high-resolution mass spectrum exhibited diagnostically important peaks at *m/e* 318.1121 (M − $C_5H_{10}O_2$), 300.0994 (M − $C_5H_{10}O_2$ − H_2O), 276.1004 (M − $C_5H_{10}O_2$ − C_2H_2O), 258.0886 (M − $C_5H_{10}O_2$ − $C_2H_2O_2$), and 240.0787 (M − $C_5H_{10}O_2$ − $C_2H_4O_2$ − H_2O) and the base peak at *m/e* 85 (C_5H_9O).

Hydrolysis of acanthospermal B with sodium methoxide–

methanol afforded a substance $C_{22}H_{32}O_8$ (**5**) as the result of methanol addition to the lactone and replacement of the acetate by methoxyl. Just as in the case of **1a**, displacement of methoxyl was accompanied by an upfield shift of the H-9 signal from 5.15 to 3.79 ppm, whereas the shift of H-8, from 6.69 to 6.11 ppm, was considerably less and not compatible with conversion of an ester to an ether function. Consequently acanthospermal B had formula **4a** exclusive of stereochemistry.

We now turn to the stereochemistry of **1a** and **4**, which because of the similarity of chemical shifts and coupling constants had to be the same. The chemical shift of the aldehyde proton (H-14) which appeared near 9.45 ppm indicated clearly that the 1(10) double bond was cis rather than trans, a trans aldehyde proton being found at 10 ppm or higher.[5,6] To determine the geometry of the 4,5 double bond, acanthospermal B was oxidized (MnO_2) to the dialdehyde **6**, whose NMR spectrum (Table I) exhibited the new aldehydic proton at 10.22 ppm indicating that the 4,5 bond was trans. Studies of possible NOE's between the C-15 aldehyde proton of **6** (or the C-15 methyl group of **1a** or the $-CH_2OH$ of **4a**) and H-5 produced the negative results expected for a trans double bond. Hence the acanthospermals belong to the melampolide[7] subgroup of germacranolides.[8]

If the usual assumption be made that the C-7 side chain is equatorial and β as in all sesquiterpene lactones of authenticated stereochemistry, the large values of $J_{5,6}$ and $J_{6,7}$ (see Table I) require that H-6 be trans to H-7 and β, and that H-5 be trans to H-6 and α, i.e., that the lactone ring be trans fused. This conclusion is reinforced by the magnitude of $J_{7,13a}$ and $J_{7,13b}$ (>3 Hz) which according to Samek's rule[12] (apparently applicable to melampolides[2,7,9-11]) indicates the presence of a trans lactone ring. Such a lactone might be expected to exhibit a negative Cotton effect if the absolute configuration is as depicted in the formulas.[13] However, the α,β-unsaturated aldehyde chromophore seems to exert a dominant effect on the CD curves which display a negative maximum at 224 nm (θ −5400 for **1a** and −40200 for **4a**), the much weaker Cotton effect of the unsaturated lactone function usually found near 250 nm having been swamped.

The stereochemistry at C-8 and C-9 was deduced by comparison of the observed coupling constants with those deduced from dihedral angles in Dreiding models. The small value of $J_{7,8}$ (1.5 Hz) can be accounted for only by α orientation of H-8, whereas the large coupling constant between H-8 and H-9 (9 Hz) shows that H-9 is trans to H-8 and β. This stereochemistry places H-9 and H-14 into a W relationship if the aldehyde carbonyl is oriented such that there is maximum overlap between the π orbitals of the 1(10) carbon–oxygen double bonds, an arrangement which accounts for the long-range coupling between H-9 and H-14.

In this orientation of the aldehyde carbonyl group, H-8 lies in the plane of the carbonyl, relatively close to the carbonyl oxygen, and should be strongly deshielded as actually observed. That this was the correct explanation for the paramagnetic shift of H-8 could be verified experimentally. $NaBH_4$ reduction of **1a** gave the tetrahydro derivative **7** whose NMR spectrum exhibited the H-8 signal at a normal frequency of 5.78 ppm and H-1 at 5.75 ppm, as expected. Similarly, $NaBH_4$ reduction of **4a** gave **8** which had H-8 at 5.70 and H-1 at 5.75 ppm.

Thus not only the oxidation pattern, but also the stereochemistry of the acanthospermals is identical with that of five other melampolides whose stereochemistry has been established by X-ray analysis,[7,10] either directly or by chemical correlation.[9,11] Possible implications of this finding will be discussed elsewhere.

Experimental Section

Experimental details have been specified previously.[14]

Extraction of *Acanthospermum australe*. Above-ground parts of *A. australe* (L.) Kuntze, wt 6.3 kg, collected by Mr. R. Lazor on July 16, 1969 along the Dog Lake Fire Tower Road near Tallahassee, Fla. (Lazor no. 3742), was extracted with $CHCl_3$ and worked up in the usual manner.[15] The crude gum, wt 15 g, was chromatographed over 500 g of silicic acid (Mallinckrodt 100 mesh), 50-ml fractions being collected. The $CHCl_3$–MeOH (3%) eluates (fractions 10–15) gave a gummy residue which was fairly homogeneous and was purified by repeated preparative TLC over silica gel (Merck PF 254–356) using $CHCl_3$–MeOH (6%) to give pure acanthospermal A (**1a**, 1.1 g) as a colorless gum which could not be induced to crystallize: $[\alpha]_{Hg}^{25}$ −54° (*c* 0.328, $CHCl_3$); CD curve $[\theta]_{300}$ 0, $[\theta]_{250}$ −8590, $[\theta]_{235}$ −31500, $[\theta]_{224}$ −54400, $[\theta]_{215}$ −401100, $[\theta]_{205}$ 0 (last reading); ir bands at 3510 (–OH), 1770, 1620 (conjugated lactone), 1740, 1730 (esters), 1690 (conjugated aldehyde), 1460, 1065, 990, and 880 cm^{-1}; uv strong end absorption rising from 250 nm onwards (ϵ_{230} 8700, MeOH). For unknown reasons, the carbon analysis was consistently low, but the high-resolution mass spectrum afforded the correct composition.

Anal. Calcd for $C_{23}H_{30}O_8$: C, 63.58; H, 6.96; O, 29.46; mol wt, 434.1940. Found: C, 61.58; H, 6.64; O, 29.01; mol wt, 434.1975 (MS).

Extraction of *Acanthospermum hispidum*. Above-ground parts of *A. hispidum* DC., wt 5 kg, collected by Mr. R. F. Doren on August 9, 1972 in Gadsden County, Fla. (Doren no. 1500), was extracted with $CHCl_3$ and worked up in the usual manner. The crude gum, wt 10 g, was dissolved in $CHCl_3$ and chromatographed over 400 g of silicic acid, 50-ml fractions being collected. The $CHCl_3$–MeOH (2%) eluates gave a gummy residue, wt 1 g, which appeared to be reasonably homogeneous and was purified by preparative TLC (silica gel, $CHCl_3$–MeOH, 6%) to give 0.6 g of acanthospermal B (**4a**) as a colorless gum: $[\alpha]_{Hg}^{25}$ −33° (*c* 0.092, $CHCl_3$); CD curve $[\theta]_{300}$ 0, $[\theta]_{250}$ −8940, $[\theta]_{235}$ −24600, $[\theta]_{224}$ −40200, $[\theta]_{215}$ −29100, $[\theta]_{208}$ 0 (last reading); ir bands at 3480 (–OH), 1750, 1630 (conjugated lactone), 1740, 1730 (esters), 1685 (conjugated aldehyde), 1450, 1370, 1130, 990, and 910 cm^{-1}; uv strong end absorption (ϵ_{230} 14000).

Anal. Calcd for $C_{22}H_{28}O_8$: C, 62.59; H, 6.65; O, 29.85; mol wt, 420.1783. Found: C, 62.85; H, 6.71; O, 30.44; mol wt, 420.1766 (MS).

Preparation of 1b and 4b. Acetylation of 0.1 g of **1a** in 1 ml of pyridine and 1 ml of acetic anhydride followed by the usual workup gave a gum (**1b**, 0.06 g) which was purified by preparative TLC (silica gel, $CHCl_3$–MeOH, 4%) and had ir bands at 1770, 1740, 1690, 1620, 1220, 1150, and 990 cm^{-1}. The low-resolution mass spectrum had significant peaks at *m/e* 390 (M^+), 348 (M − C_2H_2O), 330 (M − $C_2H_4O_2$), 319 (M − C_2H_2O − CHO), 302 (M − $C_4H_8O_2$), 260 (M − C_2H_2O − $C_4H_8O_2$), 242 (base peak, M − $C_4H_8O_2$ − $C_2H_4O_2$), 231 (M − $C_4H_8O_2$ − C_2H_2O − CHO), 213 (M − $C_4H_8O_2$ − $C_2H_4O_2$ − CHO), and 71 (C_4H_7O).

Anal. Calcd for $C_{21}H_{26}O_7$: 64.60; H, 6.71; O, 28.68. Found: C, 63.82; H, 6.75; O, 28.27.

Acetylation of 0.05 g of **4a** in the same manner and purification of the crude product by preparative TLC ($CHCl_3$–MeOH, 4%) gave **4b** as a gum. It had ir bands at 1770, 1740, 1690, 1620, 1360, 1230, and 990 cm^{-1}.

Anal. Calcd for $C_{24}H_{30}O_9$: C, 62.33; H, 6.54; O, 31.13. Found: C, 61.62; H, 6.42; C, 30.50.

Preparation of 2 and 5. A solution of 0.1 g of **1a** in 10 ml of anhydrous MeOH containing 0.08 g of CH_3ONa was stirred at room temperature in a nitrogen atmosphere, the reaction being monitored by TLC. After 1 hr, when the starting material had disappeared completely, the solution was acidified with dilute acetic acid, diluted with water, and extracted with ethyl acetate. The washed and dried extract was evaporated and the residue purified by preparative TLC ($CHCl_3$–MeOH, 6%). The gummy product (**2**, 0.03 g) had ir bands at 1770–1720 (broad), 1690, 1620, 1460, 1390, 1310, and 990 cm^{-1}. The mass spectrum exhibited significant peaks at *m/e* 394 (M^+), 365 (M − CHO), 306 (M − $C_4H_8O_2$), 277 (M − $C_4H_8O_2$ − CHO), 71 (C_4H_7O), and 43 (base peak).

Anal. Calcd for $C_{21}H_{30}O_7 \cdot \frac{1}{2}H_2O$: C, 62.53; H, 7.69; O, 29.75. Found: C, 62.88; H, 7.41; O, 29.71.

Treatment of 0.06 g of **4a** with MeOH–MeONa in a similar fashion and purification of the product by preparative TLC ($CHCl_3$–

MeOH, 5%) gave 22 mg of gummy **5**, ir bands at 3510, 1770, 1730, 1690, and 990 cm^{-1}. The mass spectrum exhibited significant peaks at *m/e* 424 (M^+), 395 (M − CHO), 322 (M − $C_5H_{10}O_2$), 304 (M − $C_5H_{10}O_2$ − H_2O), 293 (M − $C_5H_{10}O_2$ − CHO), 85 (base peak, C_5H_9O), and 57 (C_4H_9).

Anal. Calcd for $C_{22}H_{30}O_8$: mol wt, 424.2097. Found: mol wt, 424.2106 (MS).

Acanthospermal A Epoxide (3). A solution of 0.05 g of **1a** in 5 ml of $CHCl_3$ was stirred with 0.05 g of *m*-chloroperbenzoic acid at room temperature for 48 hr and extracted with $CHCl_3$. The extracted was washed with sodium metabisulfite and water, dried, and evaporated. Purification of the crude product by preparative TLC ($CHCl_3$–MeOH, 8%) yielded **3** as a gum, ir bands at 3500, 1770, 1730, 1690, 1620, and 990 cm^{-1}. The mass spectrum exhibited significant peaks at *m/e* 450 (M^+), 362 (M − $C_4H_8O_2$), 347 (M − $C_4H_7O_3$), 276 (M − C_4H_7O − $C_4H_7O_3$), 260 (M − $C_4H_7O_3$ − $C_4H_7O_2$), 71 (C_4H_7O), 59 and 43 (base peak).

Anal. Calcd for $C_{23}H_{30}O_9$: mol wt, 450.1890. Found: mol wt, 450.1894 (MS).

$NaBH_4$ Reductions of 1a and 4a. A solution of 0.05 g of **1a** and 0.05 g of $NaBH_4$ in 10 ml of MeOH was stirred at 0° for 4 hr, acidified with dilute acetic acid, evaporated at reduced pressure, diluted with water, and extracted with ethyl acetate. The washed and dried extract was evaporated and the residue was purified by preparative TLC ($CHCl_3$–MeOH, 8%) to give **7** as a gum, ir bands at 3540, 3500, 1770, 1740, 1460, 1370, and 990 cm^{-1}. The mass spectrum exhibited significant peaks at *m/e* 438 (M^+), 350 (M − $C_4H_8O_2$), 324 (M − $C_4H_8O_3$), 316 (M − $C_4H_8O_3$ − H_2O), 246 (M − $C_4H_8O_2$ − $C_4H_8O_3$), 228 (base peak, M − $C_4H_8O_2$ − $C_4H_8O_3$ − H_2O), 71, 59, and 43.

Anal. Calcd for $C_{23}H_{34}O_8$: mol wt, 438.2253. Found: mol wt, 438.2257 (MS).

Reduction of 0.1 g of **4a** with 0.1 g of $NaBH_4$ followed by workup in the same way gave, after preparative TLC ($CHCl_3$–MeOH, 8%), **8** as a gum, ir bands at 3540, 3490, 1770, 1760, 1730, 1460, 1230, and 990 cm^{-1}. The mass spectrum exhibited significant peaks at *m/e* 424 (M^+), 322 (M − $C_5H_{10}O_2$), 280 (M − $C_5H_{10}O_2$ − C_2H_2O), 262 (M − $C_5H_{10}O_2$ − $C_2H_4O_2$), 244 (M − $C_5H_{10}O_2$ − $C_2H_4O_2$ − H_2O), 85 (C_5H_9O), 57 (base peak), and 43.

Anal. Calcd for $C_{22}H_{32}O_8$: mol wt, 424.2097. Found: mol wt, 424.2102 (MS).

Oxidation of 4a to 6. A solution of 0.05 g of **4a** in 10 ml of spectral grade $CHCl_3$ was stirred at room temperature with 0.1 g of active MnO_2, the reaction being monitored by TLC. After 24 hr, when the reaction did not appear to proceed further, the mixture was filtered and the precipitate washed repeatedly with $CHCl_3$. The combined filtrate and washings were evaporated and the residue developed as a preparative TLC plate using $CHCl_3$–MeOH (6%) as solvent. The major band yielded 40 mg of starting material. A minor band yielded 6 mg of the dialdehyde **6** as a gum, ir bands at 1770, 1730, 1690, 1680, 1460, 1240, and 1000 cm^{-1}. The mass spectrum exhibited significant bands at *m/e* 360 (M − 2CHO), 258 (360 − $C_5H_{10}O_2$), 85 (C_5H_9O), and 57.

Anal. Calcd for $C_{22}H_{26}O_8$: mol wt, 418.1628. Found: mol wt, 418.1632 (MS).

Registry No.—**1a,** 56689-33-9; **1b,** 56679-16-4; **2,** 56679-17-5; **3,** 56679-18-6; **4a,** 56679-19-7; **4b,** 56679-20-0; **5,** 56679-21-1; **6,** 56679-22-2; **7,** 56679-23-3; **8,** 56679-24-4.

References and Notes

(1) This work was supported in part by Grant CA-13121 from the U.S. Public Health Service through the National Cancer Institute.
(2) W. Herz and S. V. Bhat, *Phytochemistry*, **11**, 1829 (1972).
(3) In $CDCl_3$ this signal appeared at 6.80 ppm. A similarly large diamagnetic shift on passing from $CDCl_3$ to C_6D_6 was observed earlier for H-2 of frutescin.[2]
(4) Since the results of spin-decoupling experiments on **4a** were similar to those performed on **1a**, they are not discussed in detail.
(5) For references see W. Herz and R. P. Sharma, *J. Org. Chem.*, **40**, 192 (1975).
(6) The proximity of the H-1 signal to H-8 (in $CDCl_3$) and to H-13b (in C_6D_6) interfered with attempts to verify the expected nuclear Overhauser effect between H-1 and H-14.
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Synthesis of Tabtoxinine-δ-lactam

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The synthesis is described of tabtoxinine-δ-lactam, an amino acid produced by various Pseudomonad species and also formed on hydrolysis of tabtoxin. The key intermediate in the synthesis is 1-anisyl-6-methoxycarbonyl-3-methylene-2-piperidone, which is easily obtained by application of the α-methylenelactam rearrangement to dimethyl 1-anisyl-2,5-piperidinedicarboxylate. Epoxidation gave a mixture of cis and trans oxides which were individually treated with ammonia. From the trans epoxide, the major isomer, the corresponding 3-aminomethyl-3-hydroxy compound was isolated. Removal of the anisyl protecting group gave the amino acid, *cis*-3-aminomethyl-6-carboxy-3-hydroxy-2-piperidone, identical with tabtoxinine-δ-lactam. This synthesis confirms the structure of, and establishes the aminomethyl and carboxy groups as cis in, the natural amino acid.

Tabtoxinine-δ-lactam (**1**) is an amino acid produced by various Pseudomonad species and is one of the compounds found in the hydrolysis of tabtoxin (**2**) or isotabtoxin (**3**).[1,2] The other hydrolysis products are tabtoxinine (**4**) and threonine (**5**).[1-3] Tabtoxin (**2**), the chlorosis-inducing exotoxin produced by *Pseudomonas tabaci, P. coronafaciens,* and other phytopathogenic *Pseudomonas,* is the component responsible for the toxicity of these bacteria to various plants (e.g., tobacco, soybean, oat, timothy). Tabtoxin (**2**) is relatively unstable, and at room temperature and pH 7 the biological activity of toxic solutions decreases with a half-life of about 1 day[3] as ready translactamization occurs to the more stable and nontoxic δ-lactam isomer, isotabtoxin (**3**).[1,3] Presented here is the total synthesis of (±)-tabtoxinine-δ-lactam (**1**) which further confirms the structure assigned to isotabtoxin (**3**) and to tabtoxin (**2**), and establishes the relative stereochemistry as shown in structures **1, 2, 3,** and **4.**