Melampodinin, Leucanthinin, and Melampolidin, Three New Melampolides from *Melampodium* (Compositae)

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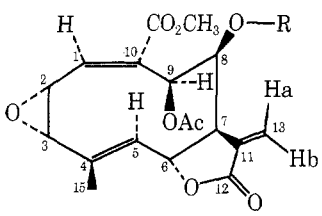
Received May 18, 1976

The isolation and structure elucidation of three melampolides from the genus *Melampodium* (Compositae, Heliantheae) is reported. Melampodinin (**2**) is typical of the yellow-rayed species, *M. americanum* L. Leucanthinin (**3a**) and melampolidin (**4**) are found in the white-rayed species, *M. leucanthum* Torr. and Gray. Compounds **2** and **4** represent the first sesquiterpene lactones with a 2-hydroxy-2-methyl 3-acetoxybutanoate side chain.

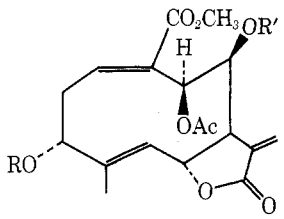
In our biochemical systematic study of the genus *Melampodium* (tribe Heliantheae, subtribe Melampodiinae),[2] we have previously reported the isolation and structural elucidation of several new sesquiterpene lactones[3,4] and dilactones.[5,6] In continuation of a detailed populational analysis of different species of *Melampodium* we now report on three new melampolides[7] from *M. leucanthum* Torr. and Gray and *M. americanum* L.

Results and Discussion

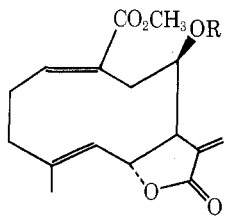
A. Melampodinin (2). Melampodinin (**2**), $C_{25}H_{30}O_{12}$, mp 208–210 °C, is the major constituent in a number of populations of the yellow-rayed species *M. americanum* from Mexico. The structure of **2** was mainly deduced on the basis of



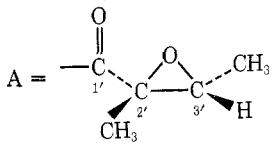
1, R = A
2, R = B

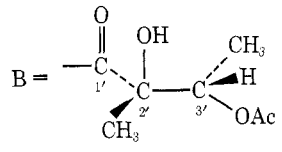


3a, R = H; R′ = A
b, R = Ac; R′ = A



4, R = B





correlations of physical parameters. The 100-MHz NMR spectrum of **2** exhibited two one-proton doublets at 5.79 (J = 3.0 Hz) and 6.19 ppm (J = 3.5 Hz) and a broad, featureless one-proton multiplet near 2.65 ppm that are characteristic of α,β-unsaturated γ-lactones, which was also indicated in the ir spectrum (1770 cm^{-1}). Melampodin A acetate (**1**)[3,4] and **2** exhibited gross NMR spectral similarities, except that the one-proton quartet at 3.05 ppm (J = 5.5 Hz), due to H-3′ in **1**, was replaced by a one-proton quartet at 5.18 ppm (J = 6.0 Hz) in **2**. In addition, **2** showed a sharp three-proton singlet at 1.89 ppm, typical for an acetate, and a one-proton singlet at 3.24 ppm, which must be due to a hydroxy hydrogen the presence of which was also evident from an OH absorption (3450 cm^{-1}) in the ir spectrum of **2**. The similarities of the NMR spectral parameters of the medium ring portions of **1** and **2** indicated that the structural differences between the two compounds must be situated in the side chain of the medium ring and most likely be due to a modification of the 2,3-epoxy-2-methylbutanoyl moiety (A) in **1** by the addition of acetic acid. This was corroborated by the appearance of mass spectral peaks at *m/e* 346 (M − $C_7H_{12}O_5$) and 159 (M − $C_7H_{11}O_4$) which indicated that seven carbon atoms have to be present in a single fragment in the side chain. Furthermore, the mass spectrum provided evidence for the presence of a side chain of type B in **2** by showing strong peaks at *m/e* 436 (M − 86, $C_4H_6O_2$) and 131 ($C_6H_{11}O_3$) which were attributed to the ions formed by fragmentations between C-2′ and C-3′ and C-1′ and C-2′ in B, respectively. The mass spectra of melampodin A acetate (**1**) and melampodinin (**2**) have very similar absorption patterns in the region due to the medium ring skeletal ions, which is further evidence for the structural similarity in the medium ring portions of **1** and **2**. Strong peaks at *m/e* 286 indicated sequential or simultaneous McLafferty rearrangements of the side chains from C-8 [epoxyangelic acid (116 mu) in **1** and 2-hydroxy-2-methyl 3-acetoxybutyric acid (176 mu) in **2**] and acetic acid (60 mu) from C-9 in **1** and **2**. Furthermore, in **1** and **2**, strong peaks appeared at *m/e* 227 which must be due to an additional loss of the carbomethoxy moiety from C-10 of the medium ring ions at 286. Peaks at *m/e* 198 indicated the loss of CO (28 mu), most likely from the lactonic portion of the ions due to *m/e* 226.

The proton resonances of **2** were assigned with the aid of double-resonance experiments. Irradiation at the center of the broad multiplet at 2.65 ppm (H-7) caused collapse of the doublets at 5.79 (H-13a) and 6.19 ppm (H-13b) plus collapse of the narrower spacing ($J_{7,8}$ = 1.2 Hz) of the doublet of doublets at 6.65 ppm (H-8), and a substantial narrowing in the structure of the upfield portion (H-6) of the H-5,6 pattern centered near 5.25 ppm. Irradiation of the narrow doublet at 2.19 ppm (J = 1.0 Hz, C-4 methyl group) sharpened the low-field portion of the H-5,6 multiplet. Saturation of the H-9 doublet at 5.37 ppm ($J_{8,9}$ = 9.0 Hz) removed the wide spacing (J = 9.0 Hz) from the H-8 signal at 6.65 ppm. Irradiation at the signal at 7.00 ppm (H-1) narrowed the broadened doublet (J = 1.0 Hz) at 3.73 ppm (H-3) to two sharp lines and collapsed the narrower spacing of the H-2 doublet of doublets at 3.62 ppm. Irradiation at the center of the C-3′ methyl doublet at 1.24 ppm (J = 6.0 Hz) caused collapse of the quartet at 5.18 ppm (H-3′). The striking downfield shift in the resonance position of the H-3′ quartet (3.05 ppm in 1[3] and 5.18 ppm in **2**) and the presence of an additional acetate group in **2** suggested an attachment of the acetoxy group to C-3′ in **2**

Table I. ^{1}H NMR Parameters[a] of Melampodinin (2), Leucanthinin (3a), Its Acetate 3b, and Melampolidin (4)

Signal	2	3a	3b	4[e]	4
H-1	7.00 dd (1; 2.3)	7.03 dd (8.5; 8.5)	6.93 dd (8.0; 9.0)	6.63 m	6.85 brt (6.0)
H-2	3.62 dd (2.3; 3.6)	~2.7 m[f]	~2.85 m[f]	2.5–2.8[f]	(a) 2.0–2.4 m[f] (b) 2.8 m[f]
H-3	3.73 dd (1.0; 3.6)	4.55 m	5.31 m[f]	[f]	[f]
H-5	5.07–5.38 m	5.52 brd (10.0)	5.30 brd (10.0)	4.81 dd (1.0; 10.0)	5.03–5.12 m
H-6		5.18 dd (9.0, 10.0)	5.17 dd (10.0; 10.0)	5.09 dd (10.0; 10.0)	
H-7	2.65 m	2.85 m	~2.80 m[f]	2.48 m	2.6 m
H-8	6.65 dd (1.2; 9.0)	6.67 dd (1.5; 8.0)	6.68 dd (1.5; 8.2)	6.44 m	6.33 ddd (1.5; 7.5; 9.0)
H-9	5.37 d (9.0)	5.31 d (8.0)	5.32 d (8.2)	(a)[f] (b) 2.66 m	(a)[f] (b) 2.77 ddd (1.5; 7.5; 14.0)
H-13a	5.79 d (3.0)	5.73 d (3.0)	5.74 d (3.0)	5.49 d (3.0)	5.59 d (3.0)
H-13b	6.19 d (3.5)	6.23 d (3.5)	6.26 d (3.5)	6.14 d (3.5)	6.17 d (3.5)
H-3′	5.18 q (6.0)	3.00 q (5.5)	3.02 q (5.5)	5.15 q (6.5)	5.08 q (6.5)[f]
H-15	2.19 d (1.0)[c]	1.95 br[c]	2.03 d (1.0)[c]	1.62 d (1.2)[c]	1.93 d (1.0)[f]
C-2′-Me	1.25	1.45	1.46	1.24	1.35
C-2′-Me	1.24 d (6.0)	1.18 d (5.5)	1.19 d (5.5)	1.18 d (6.5)	1.23 d (6.5)
COO Me	3.82	3.77	3.82	3.51	3.77
Acetates	1.89; 1.99	1.98	2.00; 2.10	1.75	1.94
C-2′-OH	3.24				3.36

[a] Spectra were run in $CDCl_3$ at 100 MHz and Me_4Si was used as internal standard. 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; m, multiplet whose center is given; br, broad. Figures in parentheses are coupling constants or line separations in hertz. [b] Intensity two protons. [c] Intensity three protons. [d] Intensity four protons. [e] Run in C_6D_6. [f] Obscured by other signals.

which is in full agreement with the above mass spectral assignments. Since **2** does not form an acetate with Ac_2O in pyridine the hydroxy group must be tertiary, and be located at C-2′ which is in accord with the above spectral data. Therefore, by NMR and mass spectral analogy with melampodin A acetate (**1**), all the atoms of melampodinin have been accounted for, and the tentative structure **2** is suggested for melampodinin.

On the basis of the extreme similarity of the NMR parameters of **1** and **2** it appears that melampodinin exhibits the same configurational and conformational relationships around the medium ring as melampodin A acetate (**1**),[3,4] except the side chain at C-8. The stereochemistry at C-2′ and C-3′ in **2** could not be determined by spectroscopic methods. The side chain at C-8 in **2** could be possibly formed in a nucleophilic attack of an acetate at the C-3′ epoxide function of the epoxyangelic acid moiety (A) in **1** under opening of the epoxide ring to give the 2-hydroxy-2-methyl-3-acetoxy butyrate (B). Attack of a nucleophile at C-3′ is in agreement with our findings derived from neutron diffraction studies of melampodin A[8] that the epoxide group on the epoxyangelate side chain (A) in melampodin A is not symmetric. In melampodin A and therefore also in its acetate (**1**) the C-3′–oxygen bond is longer than the C-2′–oxygen bond, which should favor breaking of the C-3′–oxygen bond. A nucleophilic attack at the epoxyangelate side chain (A) should therefore occur with inversion of configuration at C-3′ to give directly the C-8 side group (B) as shown in **2**. Since the chiral centers in the epoxyangelate moiety in melampodin A[7] and therefore in its acetate (**1**) had been found to be 2′(*R*),3′(*R*) and nucleophilic attack at an epoxide group (here C-3′) generally occurs with inversion of configuration, the chirality at C-2′ and C-3′ in **2** should be *R* and *S*, respectively.

B. Leucanthinin (3a). Collections of *M. leucanthum* from Motley County, Texas, contained a crystalline compound which we named leucanthinin (**3a**), $C_{23}H_{28}O_{10}$ (high-resolution mass spectrum), mp 163–164 °C. The ir spectrum of **3a** contained absorptions typical of an α,β-unsaturated γ-lactone (1760 cm^{-1}) and a hydroxyl group (3480 cm^{-1}). Since the ir spectrum of the acetate (**3b**), $C_{25}H_{30}O_{11}$, lacked an OH absorption, **3a** must contain only one OH group.

Further information which led to the structure of leucanthinin was deduced from correlations of 25.2-MHz ^{13}C NMR and ^{1}H NMR spectral data and mass spectral fragmentation patterns. The ^{13}C NMR data (see Experimental Section) obtained under proton noise decoupling and single-frequency off-center decoupled conditions[9] and the ^{13}C chemical shift considerations indicated that leucanthinin contains 23 carbon atoms and possesses the following skeletal systems: five of >CHO–, four of each of $-CH_3$ and $-C(=O)O$, three of >C=, two of –CH= and one each of $-CH_2-$, >CH, >CO–, $-OCH_3$, and $H_2C=$.

The ^{1}H NMR parameters of leucanthinin (**3a**) were similar in many respects to those of melampodin A and its acetate (**1**).[3] The major differences between the ^{1}H NMR spectra of **1** and **3a** were observed for the proton signals assigned to H-2 and H-3 (compare Table I and table in ref 3). Instead of the absorptions centered at 3.65 (H-2) and 3.75 ppm (H-3) in **1**, in **3a** multiplets at 2.7 (2 protons) and 4.55 ppm (1 proton) were observed. In **3a** the appearance of the signals at 7.03 ppm (H-1) as a broadened doublet of doublets indicated the presence of two protons at C-2. Indeed, double irradiation at the

multiplet at 2.7 ppm collapsed the H-1 signal at 7.03 ppm to a broadened singlet and also caused the multiplet at 4.55 ppm (H-3) to collapse. From chemical shift considerations, H-3 in **3a** must be attached to a carbon atom bearing an oxygen function, possibly an OH group. A downfield shift of the H-3 absorption from 4.55 ppm in **3a** to 5.31 ppm in acetate **3b** corroborated the above assignments. Since double irradiation experiments involving the signals of H-5, H-6, H-7, H-8, H-9, and the two H-13 of **3a** produced results as expected,[3,4] leucanthinin must be represented by formula **3a** exclusive of stereochemistry.

The high-resolution mass spectra of leucanthinin (**3a**) and the acetate **3b** exhibited peaks which verified the above NMR spectral arguments. Leucanthinin gave a weak parent peak at *m/e* 464.1687 and a peak at *m/e* 404.1488 (M − $C_2H_4O_2$) indicative of the loss of CH_3COOH from the parent ion. The peaks at *m/e* 348.1193 (M − $C_5H_8O_3$) and 116.0495 ($C_5H_8O_3$) are in accord with the loss of epoxyangelic acid from the medium ring skeleton of **3a**. The fragment corresponding to *m/e* 288.1012 ($C_{16}H_{16}O_5$) could be formed by sequential or simultaneous McLafferty rearrangements of the epoxyangelic acid ($C_5H_8O_3$) from C-8 and CH_3COOH from C-9. The peak at *m/e* 229.0858 ($C_{14}H_{13}O_3$) was assigned the radical ion derived from the ion *m/e* 288 by a loss of the carbomethoxy group (59 mu) from C-10 of the medium ring. Intense peaks at *m/e* 99.0463 ($C_5H_7O_2$) and 81.0360 (C_5H_5O) and the base peak at *m/e* 71.0519 (C_4H_7O) were in agreement with the acylium ion derived from the epoxyangelic acid moiety at C-8 in **3a** and the fragments derived from the latter acylium ion by the loss of H_2O (18 mu) and CO (28 mu), respectively. Besides the above assignments a number of other diagnostic mass spectral peaks were in accord with the skeletal arrangement derived for leucanthinin.

On the basis of the similarity of the ^1H NMR parameters of **1** and **3a**, it appears that leucanthinin exhibits the same configurational and conformational relationships as melampodin A acetate (**1**) except at C-3. The hydroxy group at C-3 in **3a** was assigned an α configuration on the basis of the torsional angles between H-3 and the two C-2 hydrogens. The observed J values ($J_{2a,3} = J_{2b,3} \simeq 4.0$ Hz) can only be explained if the C-3 hydrogen bisects the two hydrogens at C-2 with about 60° torsional angles. Stereomodels show that the only possible configuration which is in agreement with the experimental data must have a β proton at C-3. Therefore, the configurational structure **3a** is suggested for leucanthinin.

C. Melampolidin (4). Collections of young shoots of *M. leucanthum* from Presidio County, Texas, gave, after repeated chromatography, a gummy material that was pure by TLC. The ir spectrum of this noncrystalline compound which we named melampolidin, $C_{23}H_{30}O_9$ (high-resolution mass spectrum), indicated an OH group (3450 cm^{-1}) which resisted acetylation with pyridine–acetic anhydride suggesting the presence of tertiary hydroxyl group in **4**. The 100-MHz ^1H NMR spectrum of **4** in $CDCl_3$ showed doublets at 5.59 (J = 3.0 Hz) and 6.17 ppm (J = 3.5 Hz) as well as a broad multiplet at 2.6 ppm, that are characteristic of α,β-unsaturated γ-lactones. Further ^1H NMR spectral assignments were made by double irradiation experiments on **4** in C_6D_6. Strong irradiation at the center of the multiplet at 2.48 ppm (H-7) caused the doublets at 5.49 (H-13a) and 6.14 ppm (H-13b) to collapse and the multiplet at 6.44 ppm (H-8) to simplify by the loss of the small coupling to give a doublet of doublets (J = 7.0 and 9.5 Hz). In addition, the doublet of a doublet at 5.09 ppm (H-6) simplified to a doublet. In return, when H-6 was irradiated, the C-7 proton signal at 2.48 ppm and the doublet of doublets at 4.81 ppm (H-5) lost their wide spacing (J = 10.0 Hz). When the C-5 proton signal at 4.81 ppm was saturated, H-6 became a doublet (J = 10.0 Hz) and the C-4 methyl doublet at 1.62 ppm (J = 1.2 Hz) collapsed to a singlet. Irradiation of the C-3′ methyl doublet at 1.18 ppm (J = 6.5 Hz) caused collapse of the quartet at 5.15 ppm (H-3′). The positioning of the latter two signals together with the occurrence of a methyl singlet at 1.24 ppm strongly suggested the presence of a 2-methyl-2-hydroxy-3-acetoxybutyrate moiety (B) in **4**. This was corroborated by the appearance of strong mass spectral fragments at *m/e* 274.1213 (M − $C_7H_{12}O_5$) and 159.0663 ($C_7H_{11}O_4$) which indicated that seven carbon atoms are present in a single fragment. The base peak at *m/e* 131.0728 ($C_6H_{11}O_3$) must be due to fragmentation between C-1′ and C-2′ of the side chain B of compound **4** and peaks at *m/e* 117.0553 ($C_5H_9O_3$) and 99.0435 ($C_5H_7O_2$) indicated the loss of ketene (42 mu) and acetic acid (60 mu), respectively, from the side chain fragment $C_7H_{11}O_4$ (159 mu). Other MS peaks verified the presence of side chain B in **4**.

A strong mass spectral fragment at *m/e* 215.1059 ($C_{14}H_{15}O_2$) indicated the loss of $\cdot CO_2CH_3$ (59 mu) from the ion due to the medium ring skeleton (*m/e* 274.1213); the presence of a carbomethoxy moiety was corroborated by the appearance of a methyl singlet at 3.77 ppm ($-CO_2CH_3$) in the NMR spectrum. In addition, a broadened triplet at 6.85 ppm in the ^1H NMR spectrum ($CDCl_3$) of melampolidin suggested the presence of an α,β-unsaturated methyl ester, the absorption at 6.85 ppm being due to the β hydrogen (H-1) of the conjugated ester. In $CDCl_3$, irradiation of the broadened triplet at 6.85 ppm affected the envelope at 2.0–2.4 and the multiplet near 2.8 ppm indicating the presence of two protons at C-2 in **4**. When the center of the H-8 multiplet at 6.33 ppm ($J_{7,8}$ = 1.5, $J_{8,9b}$ = 7.5, $J_{8,9a}$ = 9.0 Hz) was saturated, the H-7 signal at 2.6 ppm sharpened and the one-proton multiplet (H-9) at 2.77 ppm ($J_{9,1}$ = 1.5, $J_{9,8}$ = 7.5, $J_{9a,9b}$ = 14.0 Hz) simplified to a broadened doublet (J = 14.0 Hz). The remaining wide spacing (J = 14.0 Hz) of the broadened H-9 doublet indicated a geminal coupling, suggesting the presence of two hydrogens at C-9, one of the two H-9 hydrogens very likely being a part of the envelope at 2.0–2.4 ppm.

The above NMR spectral assignments together with the high-resolution mass spectral fragmentation patterns, in particular the peaks at *m/e* 274 and 215, are in full agreement with a medium ring skeleton as shown in **4**. Furthermore, on the basis of biogenetic relationships and the similarity of the NMR parameters, melampolidin (**4**) should exhibit the same configurational and conformational relationships around the medium ring as the other melampolide sesquiterpene lactones (**1**, **2**, and **3a**). The stereochemical considerations related to the C-8 side chain B in **2** also apply to the side chain in **4**. Finally, it is noteworthy that melampolidin represents the first melampolide from *Melampodium* lacking a C-9 oxygen function.

Experimental Section[10]

Melampodin A acetate (1)[3,4] gave significant low-resolution mass spectral peaks (20 eV, 160 °C) at *m/e* (rel intensity) 462 (1.6), 420 (0.6), 402 (58), 347 (2.5), 306 (9.4), 287 (26.1), 286 (60.0), 272 (23.3), 271 (36.6), 258 (32.4), 227 (95.8), 226 (100.0), 211 (48.6), 200 (89.5), 190 (49.3), 176 (43.0), 171 (43.7), 159 (9.8), 95 (11.3), 71 (10.6), 43 (48.0).

Melampodinin (2). A collection of *Melampodium americanum* L. was made on Dec 6, 1974, 8.5 miles north of Palma Sola on route 180, Veracruz, Mexico (Stuessy and Roberts No. 3171). Dried leaves (1012 g) were extracted and worked up as previously described,[4] providing 2.5 g of crude, terpenoid-containing syrup which was chromatographed over 100 g of silica gel using CH_2Cl_2/EtOAc (9:1) as elutant; 15-ml fractions were taken and all fractions were monitored by TLC. Fractions 11–20 contained about 3 mg of artemetin which was identical with authentic material by melting point and mixture melting point.[6] Fractions 36–60 provided 75 mg of **2**: mp 203–205 °C; uv λ_{max} (MeOH) 213 nm (ϵ, 1.7×10^4); CD (c 3.8×10^{-5}, MeOH) $[\theta]_{213}$ -1.5×10^5, $[\theta]_{246}$ $+3.2 \times 10^4$; ir ν_{max} (Nujol) 3500 (OH), 1770 (γ-lactone), 1730 (ester), 1670 and 1650 cm^{-1} (double bonds); significant low-resolution mass spectral peaks (20 eV, 120 °C) at *m/e* (rel intensity) 522 (0.8), 504 (1.3), 478 (1.4), 462 (4.9), 436 (34.2), 346 (2.0),

304 (22.0), 286 (99.0), 272 (39.7), 271 (24.4), 258 (35.2), 227 (89.0), 226 (100.0), 211 (31.6), 200 (66.7), 190 (26.2), 176 (61.3), 171 (23.4), 159 (17.2), 131 (61.3), 95 (10.4), 71 (15.3), 43 (51.4).

Anal. Calcd for $C_{25}H_{30}O_{12}$: C, 57.47; H, 5.79; mol wt, 522. Found: C, 57.51; H, 5.80; mol wt (MS), 522.

Leucanthinin (3a). *Melampodium leucanthum* was collected in Motley County, Texas, 18 miles north of Dickens on Farm road 3203 on June 25, 1974 (Stuessy-Stuessy 3560). Dried leaves (570 g) were extracted with cold $CHCl_3$ and worked up as described before.[4] From the combined $CHCl_3$ extracts 2.8 g of crude material was obtained. The crude syrup (1.0 g) was chromatographed over 100 g of silica gel (Merck 0.05–0.2 mesh) using *n*-propyl acetate as eluent and taking 15-ml fractions. The progress of the chromatographic run was monitored by TLC. Fraction 15–25 contained a material which was homogeneous by TLC. The fractions were combined and evaporated in vacuo providing a crystalline material (40 mg). Recrystallization from $CHCl_3$–Et_2O gave pure **3a:** mp 163–164 °C; uv λ_{max} (MeOH) 226 nm (ϵ 5.6 × 10^3); CD (*c* 2.1 × 10^{-4}, MeOH) $[\theta]_{222}$ −62 × 10^3, $[\theta]_{243}$ +3 × 10^3; ir ν_{max} (neat) 3480, 1760, 1740, 1720, 1670, 1630 cm^{-1}; ^{13}C NMR ($CDCl_3$) 169.4, 167.9, 167.5, 164.8 (>C=O); 145.0 d (-CH=); 140.5, 133.7, 130.9 (=C<); 122.7 d (=CH–); 121.0 t ($=CH_2$); 74.6 d, 73.6 d, 70.9 d, 70.6 d, 59.6 d (HCO); 59.1 (>CO); 52.0 d (>CH); 50.6 q ($-OCH_3$); 32.0 t ($-CH_2-$); 20.8 q, 19.1 q, 15.8 q, 13.6 q ($-CH_3$). The mass spectrum showed significant peaks at *m/e* 464.1687 (M^+), 404.1488 (M − CH_3COOH), 348.1193 (M − $C_5H_8O_3$), 288.1012 (M − $C_5H_8O_3$ − CH_3COOH), 229.0858 (M − $C_5H_8O_3$ − CH_3COOH − $C_2H_3O_2$), 183.0814 ($C_{13}H_{11}O$), 131.0491 (C_9H_7O), 116.0495 ($C_5H_8O_3$), 99.0463 ($C_5H_7O_2$), 71.0519 (C_4H_7O, base peak).

Anal. Calcd for $C_{23}H_{28}O_{10}$: mol wt, 464.1682. Found: mol wt (MS), 464.1687.

The acetate **3b** [uv λ_{max} (MeOH) 211 nm (ϵ 7.2 × 10^3); ir ν_{max} (neat) 1770, 1738, 1720, 1680, 1235, 1140 and 990 cm^{-1}] showed no parent peak but exhibited significant mass spectral peaks at *m/e* 446.1647 (M − CH_3COOH), 288.1002 (M − $C_5H_8O_3$ − CH_3COOH − CH_2CO), 270.0889 (M − $C_5H_8O_3$ − $2CH_3COOH$), 229.0866 ($C_{14}H_{13}O_3$), 183.0825 ($C_{13}H_{11}O$), 131.0466 (C_9H_7O), 116.0493 ($C_5H_8O_3$), 99.0422 ($C_5H_7O_2$), 81.0343 (C_5H_5O), 71.0486 (C_4H_7O, base peak).

Melampolidin (4). Collections of young shoots of *M. leucanthum* were made in Presidio County, Texas, 2.3 miles south of Marfa on Highway 67 on July 24, 1973 (Stuessy-Fischer No. 2044). Dried plant material (95 g) yielded 420 mg of crude syrup which was worked up and chromatographed as described above. Fraction 7–12 gave 95 mg of melampolidin (4) as a gum: uv λ_{max} (MeOH) 222 nm (ϵ 1.2 × 10^4); CD (*c* 8.4 × 10^{-5}, MeOH) $[\theta]_{218}$ −58 × 10^3, $[\theta]_{249}$ −3 × 10^3, $[\theta]_{265}$ −7 × 10^3; ir ν_{max} (neat) 3450, 1760, 1740, 1710, 1670, 1630 cm^{-1}; significant mass spectral peaks at *m/e* 432.1798 (M − H_2O), 274.1213 (M − $C_7H_{12}O_5$), 242.0935 ($C_{15}H_{14}O_3$), 215.1059 ($C_{14}H_{15}O_2$), 159.0663 ($C_7H_{11}O_4$), 131.0728 ($C_6H_{11}O_3$, base peak), 117.0553 ($C_5H_9O_3$), 99.0435 ($C_5H_7O_2$), 91.0550 (C_7H_7), 71.0513 (C_4H_7O). Since in the mass spectrum no parent peak was obtained for 4 the M − H_2O data were used for the determination of the empirical formula.

Anal. Calcd for $C_{23}H_{30}O_9$: mol wt, 432.1798. Found: mol wt (MS), 432.1784.

Acknowledgment is made to the National Science Foundation (Grant GB-42644) for support of this work. The authors thank Judy Abraham and Joseph Abraham for technical assistance, Professor N. S. Bhacca for NMR data, and Professor Tod Stuessy, The Ohio State University, for collecting and identifying the plant material.

Registry No.—**1,** 35878-52-5; **2,** 60295-53-6; **3a,** 60295-54-7; **3b,** 60295-55-8; **4,** 60295-56-9.

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(10) Melting points were performed in capillaries on a Thomas-Hoover and are uncorrected. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were taken on a Perkin-Elmer Model 621 spectrophotometer and ultraviolet spectra were obtained on a Cary Model 14 spectrophotometer. The CD spectra were determined on a Durram-Jasco J-20 spectrometer. Low-resolution mass spectra were obtained on a Hitachi Perkin-Elmer Model RMS-4 and the high-resolution mass spectra were run on a CEC 21-1108 instrument (70 eV ionizing voltage). The samples were introduced via the direct inlet tube. The ^{13}C NMR 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. The voucher specimens are on deposit in the Louisiana State University Herbarium at Baton Rouge, La.

Mass Spectrometry of Cytokinin Metabolites. Per(trimethylsilyl) and Permethyl Derivatives of Glucosides of Zeatin and 6-Benzylaminopurine

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Received March 15, 1976

The mass spectra of the Me_3Si and permethylated derivatives of a comprehensive series of synthetic isomeric glucosides of the cytokinins zeatin and 6-benzylaminopurine (6-BAP) have been recorded by combined gas chromatography–mass spectrometry. Comparison of these spectra with those obtained for a number of glucosyl metabolites of zeatin and 6-BAP allows unambiguous structural assignments to be made. Detailed analysis of the mass spectral fragmentation patterns indicate that, a priori, it should be possible to assign the sugar ring size (furanose vs. pyranose) in such compounds on the basis of characteristic fragment ion intensities. Mass spectra of the Me_3Si derivatives show more significant isomer differences than the corresponding permethylated compounds and their method of preparation appears less prone to multiple product formation. A thermal 1,3 migration of the sugar moiety from N_3 to N_9 was observed in the GC–MS of the derivatives of the 3-β-D-glucopyranoside of 6-BAP.

Phytohormones, and in particular the adenine derived cytokinins, evoke their biological responses at extremely low concentrations and occur free in plants in minute amounts. For example, the natural cytokinin zeatin (1) induces growth of carrot phloem tissue at concentrations of less than 0.1 μg/l. (5 × 10^{-10} M).[1] Zeatin-related compounds, e.g., N^6-(3-methyl-2-butenyl)adenosine, are ubiquitous, although minor, components of *t*-RNA hydrolysates from animals, plants, and

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