MELFUSIN, A NEW GERMACROLIDE FROM *MELAMPODIUM DIFFUSUM*

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ABSTRACT.—Chemical investigations of *Melampodium diffusum* provided the known melampolide-type sesquiterpene lactone melampodinin (**5**) and the new germacrolide melfusin (**1a**). The structure of melfusin was established by nuclear magnetic resonance (^{13}C and ^{1}H nmr) mass spectrometry and chemical transformations. Melfusin represents the first germacrolide with a C-10 carbomethoxy group and a 2,3-double bond.

In our biochemical systematic study of the subtribe Melampodiinae (1–3), in which we analyzed the herbaceous parts of *Melampodium diffusum*, resulted in the isolation of melampodinin (**5**), a melampolide type sesquiterpene lactone, previously obtained from *M. americanum* (4) and *M. longipes* (5). In addition, a new sesquiterpene lactone, which we named melfusin, was isolated. Structural data for the new compound were obtained by physical methods, mainly nmr and ms, and by chemical modifications.

RESULTS AND DISCUSSION

Melfusin (**1a**), $C_{23}H_{26}O_{10}$, was a gum which exhibited spectral absorptions typical of an α-methylene-γ-lactone (ir at 1765 cm^{-1} and nmr doublets at 5.43 and 6.16 ppm). Further ir-absorptions at 3450, 1735, and 1715 cm^{-1} indicated the presence of hydroxyl(s) and ester function(s), respectively. The assignments of the basic skeletal arrangement of **1a** were mainly deduced from ^{1}H nmr spectral data obtained at 100 and 200 MHz, and ^{13}C nmr parameters combined with mass spectral fragmentation patterns. A three-proton singlet at 2.15 ppm together with a strong ms peak at *m/e* 43 indicated the presence of an acetate group in **1a**. Furthermore, a series of ms peaks at *m/e* 116 (AH), 99 (A'), and 71 (A'') in combination with diagnostic nmr absorptions (a one-proton quartet at 3.03 ppm, two three-proton methyl absorptions, a singlet at 1.52 ppm, and a doublet at 1.25 ppm) suggested the presence of an epoxy angelate moiety (6) in **1a**. A three-proton singlet at 3.71 ppm was assigned a carbomethoxy methyl which is typical for melampolides (3) and *cis,cis*-germacranolides (7) from *Melampodium* species. The acetylation product of melfusin contained one more acetate signal in the nmr spectrum and lacked a hydroxyl absorption in the ir spectrum, suggesting the presence of only one OH group in melfusin. The above data account for all but one oxygen in the molecule which must be an ether function, most likely representing an epoxide. Further assignments of the basic skeletal arrangement of melfusin were mainly deduced from detailed double resonance ^{1}H nmr and ^{13}C nmr experiments.

Irradiation of the multiplet at 3.41 ppm (H–7) changed the doublet of doublets at 5.69 ppm (H–8, $J_{7,8}=2.5$Hz) to a doublet, simplified the C–6 proton at 4.55 pp, ($J_{6,7}=11.5$Hz) to a doublet and collapsed the two H–13 doublets at 5.43 ppm ($J_{7,13a}=3.1$Hz) and 6.16 ppm ($J_{7,13b}=3.5$Hz) to singlets. Saturation of the H–8 signal at 5.69 ppm simplified the H–7 multiplet at 3.41 ppm and collapsed

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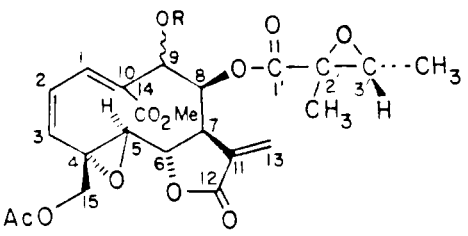
the doublet at 4.41 ppm (H-9, $J_{8,9}=3$Hz) to a broadened singlet. Irradiation of H-9 simplified H-8, as expected, and sharpened the most downfield broadened doublet at 5.97 ppm (H-1), which was part of an A,B-multiplet that was also affected when the broadened doublet at 5.51 ppm (H-3, $H_{2,3}=9.0$Hz) was irradiated. Saturation of the doublet of doublets at 4.55 ppm (H-6, $J_{5,6}=J_{6,7}=11.5$Hz) simplified, H-7 by loss of the large coupling, and the broad doublet at 3.23 ppm (H-5, $J_{5,6}=11.5$Hz) changed to a broad singlet. Irradiation of the broadened H-5 doublet sharpened the two-proton methylene absorption at 4.81



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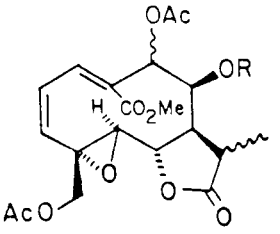
1b R = Ac



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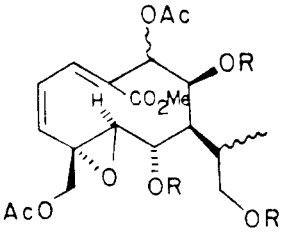
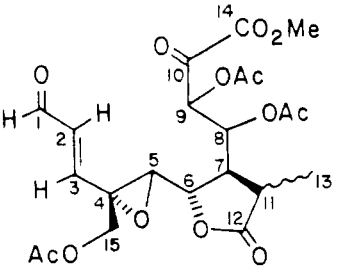


3a R = H
3b R = Ac



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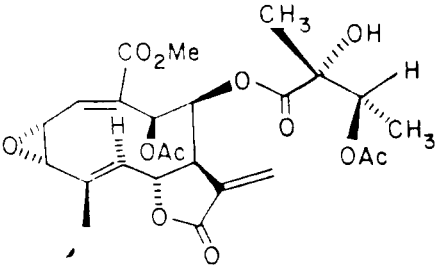

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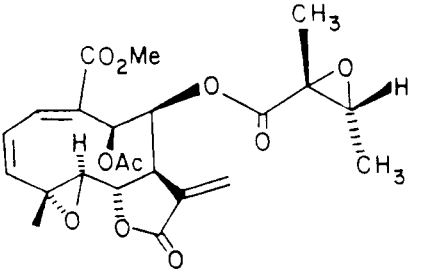

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ppm (H-15) and the doublet at 5.51 ppm (H-3), indicating a coupling not only between H-5 and the C-15 methylene protons but also to H-3. The above decoupling experiments strongly support a germacranolide skeleton for melfusin which, with the exception of C-7, must bear oxygen-substituted or olefinic carbons within the medium ring. Based on chemical shift arguments for the medium ring protons, double bonds must be present at the 1(10) and 2,3-positions. This was supported by a strong $n\rightarrow\pi^*$ uv absorption at 270 nm typical for an unsaturated ester. The unexpected reverse appearance of H-2 and H-3 in the ^{1}H nmr spectrum will be explained later. An epoxide function at the 4,5 position

TABLE 1. ^{1}H Nmr parameters of melfusin (1a) and derivatives.[a]

	1a	1b	2a	2b	3a	3b[e]	4
H-1	5.97 brd (5)	6.01 dd (5.5, 1)	5.92[c]	5.1-6.1	5.65-5.8 m	5.89 m	9.60 d (7)
H-2	6.01 dd (9, 5)	6.07 dd (9, 5.5)	5.99[c]	5.1-6.1	5.98 dd (8, 5)	6.03 dd (9, 5)	6.40 dd (16, 7)
H-3	5.51 brd (9)	5.57 brd (9)	5.64 brd (9)	5.51 brd	5.65-5.8 m	5.44 brd (9)	7.59 d (16)
H-5	3.23 brd (11.5)	3.24 brd (11.5)	3.01 brd (11)	3.06 brd (11)	2.66 brd (10)	2.92 brd (11)	3.82 d (11)
H-6	4.55 dd (11.5, 11.5)	4.57 dd (11.5, 11.5)	4.62 dd (11, 11)	4.48 dd (11, 11)	4.20 dd (11, 11)	5.65 dd (11, 11)	4.50
H-7	3.41 qd (11.5, 2.5)	3.17 qd (11.5, 2.5)	ob	1.95-2.05 m	1.84 m	2.2 m	ob
H-8	5.69 dd (2.5, 3)	5.73 dd (2.5, 3)	4.12	5.18 dd (2.5)	3.97 dd (2.5)	5.30[e]	5.17
H-9	4.41 d (3)	5.45 d (3)	5.42 d (3)	5.45 d (3)	5.37 d (3)	5.14[e]	5.50
H-11	—	—	2.67 m	2.20 m	2.00 m	2.00 m	ob
H-13	a) 5.43 d (3.1) b) 6.16 d (3.5)	a) 5.53 d (3) b) 6.19 d (3.5)	1.20[d] d (7)	1.23[d] d (6.5)	1.11[d] d (7)	0.84[d] d (7)	1.25[d] d (6)
H-15[b]	4.81 brs	4.83 brs	4.78 br	4.78 brs	4.80 (15)	4.69 br (15.5)	5.05 (17)
H-3'	3.03 q (5.5)	3.03 q (5.5)	—	—	—	—	—
C-3'-CH_3	1.25 d (5.5)	1.25 d (5.5)	—	—	—	—	—
C-2'-CH_3	1.52	1.54	—	—	—	—	—
COOMe	3.71	3.63	3.60	3.59	3.58	3.55	3.76
OAc	2.15	2.09, 2.16	2.04, 2.13	2.05, 2.11, 2.13	2.00, 2.13	2.13, 2.10, 2.07, 2.03[f]	2.15, 2.09, 2.01

[a]Spectra were determined in $CDCl_3$ at 100 MHz with Me_4Si as internal standard. Chemical shifts (δ) in ppm, coupling constants (J) or line separations in Hz are given in parentheses; multiplicities are designated by the following symbols: singlets are unlabeled, d=doublet, m=multiplet, q=quartet, t=triplet, br=broadened signal, and ob=obscured signal.

[b]Center of a two-proton AB-signal of first or second order.

[c]Part of a non-first order AB system.

[d]Three-proton signal.

[e]H-12 appears as a doublet (J=6.5Hz) at 3.99 ppm.

[f]Six-proton signal.

would not only be in agreement with the chemical shift for H-5 (3.23 ppm) but would also account for the not yet assigned oxygen in melfusin. Detailed ^{13}C nmr studies involving proton noise decoupling, single-frequency off-center decoupling and selective decoupling experiments (8) allowed assignments of all 23 carbons in melfusin. The ^{13}C nmr parameters, which corroborated the ^{1}H nmr assignments, are summarized in table 2.

The general skeletal arrangement and oxidation pattern of melfusin was similar to the previously isolated melampolide leucanthin A (**6**) (9). Major differences between the ^1H nmr spectra of the two compounds are outlined below. The C–9 proton absorption in **1a** appeared as a broadened doublet at 4.41 ppm indicating the presence of a hydroxyl group at C–9. Indeed, acetylation of **1a** caused a downfield shift of H–9 to 5.45 ppm in **1b**. Furthermore, melfusin contained no C–4-methyl absorption but, instead, exhibited a two-proton methylene signal at 4.81 ppm suggesting the presence of an acyloxy function (acetate or epoxy-

TABLE 2. ^{13}C Nmr parameters for melfusin (**1a**).[a]

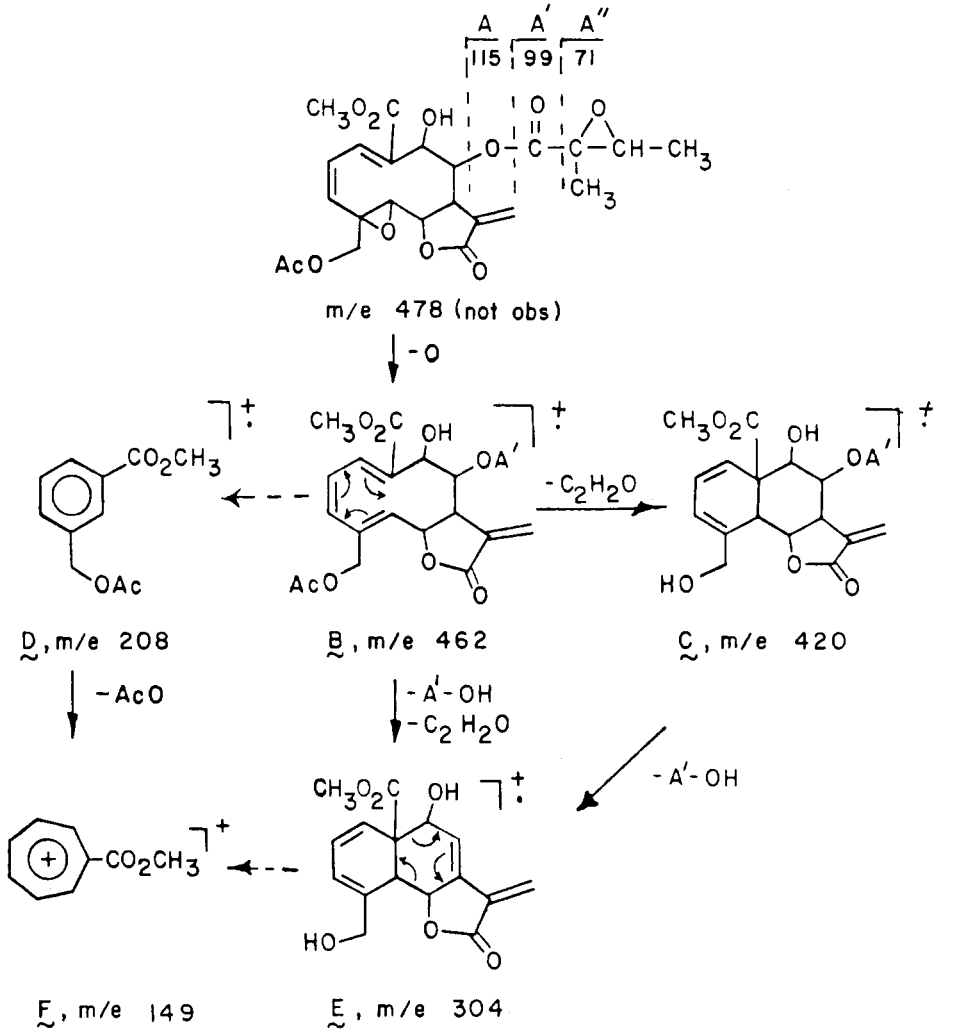
Carbon	δ, multiplicity	Carbon	δ, multiplicity
1	125.78d	13	119.66tr
2	119.34d	14	170.60s
3	124.36d	15	66.08tr
4	53.75s	CO_2CH_3	52.48q
5	44.49d	1^3	168.98s
6	76.35d	2^3	59.38s
7	38.54d	3^3	60.17d
8	72.30d	2^3–CH_3	18.84q
9	70.70d	3^3–CH_3	13.40q
10	135.31s	CH_3	
11	133.74s	(acetate)	20.88q
12	170.96s	CO (acetate)	168.83s

[a]Spectra were obtained in $CDCl_3$ at ambient temperature at 50.32 MHz. Chemical shifts (δ) are in ppm relative to TMSi as internal standard as determined by proton noise decoupling. Peak multiplicity was obtained by off-resonance decoupling (3.5 ppm above TMSi). Multiplicities are designated by the following symbols: s=singlet, d=doublet, tr=triplet, q=quartet.

angelate) in **1a**. The attachment of an acetoxy group at C–15 was supported by a strong ms peak at *m/e* 208, which had been assigned to ion *D* (scheme 1). Chemical evidence for the presence of the acetoxy group at C–15 was obtained from the ^1H nmr spectrum of the 11,13-dihydromelfusin derivative **2a**. Compound **2a** lacked the epoxyangelate group and exhibited a H–8 absorption at 4.12 ppm due to a proton attached to a hydroxyl-containing C–8. Removal of the epoxyangelate moiety from C–8 upon $NaBH_4$ reduction of **1b** can be used as strong evidence for the attachment of the acetoxy group to C–15 instead of C–8. Furthermore, this experiment established that melfusin must represent a 6,12-γ-lactonized germacranolide. The unprecedented reductive removal of the epoxyangelate at C–8 most likely involves initial hydride attack at the epoxyangelate carbonyl followed by cleavage of the newly formed hemiacetal to give compound **2a**, a reaction similar to the reductive opening of the lactone function leading to the other major product **3a**.

The major mass spectral fragmentations of melfusin are summarized in scheme 1. The parent peak is not observed for **1a** and the acetate **1b** but is present in the spectra of the 11,13-dihydroderivatives **2a** and **2b** as well as the ozonolysis product **4**. A strong driving force for the loss of oxygen appears to be the formation of the decalin skeleta *C* and *E* from which the stable aromatic ions *D* and *F* may be formed by the retro Diels-Alder processes.

The above spectral assignments established the basic skeletal arrangement of melfusin exclusive of configurational and conformational considerations. These



SCHEME 1. Major mass spectral fragmentations of melfusin.

will now be discussed below. A significant difference existed between the ^{1}H-nmr absorptions for H–1 at 5.97 ppm in melfusin and the H–1 signals of known melampolides of types **5** and **6**, which generally exhibit a diagnostic H–1 absorption near 7 ppm. The appearance of H–1 in melfusin near 6 ppm indicated trans-attachment of H–1 and the $COOCH_3$ group at C–10 suggesting the presence of a 1(10)-*trans* double bond in **1a**. The coupling constant between H–2 and H–3 ($J_{2,3}$=9.0Hz) suggested a 2,3-cis-double bond in melfusin. However, the ^{1}H nmr chemical shifts for H–2 (6.01 ppm), and H–3 (5.51 ppm) are contrary to absorptions of doubly unsaturated esters in which, due to resonance deshielding by the carbomethoxy group, normally the γ-H (H–3 in **1a**) absorbs downfield from the β-hydrogen (H–2 in **1a**). Stereomodels of melfusin revealed, however, that the plane of the 2,3-double bond in **1a** is nearly perpendicular to the 1(10)-double bond thus interrupting conjugation of the 2,3-double bond with the

α,β-unsaturated ester moiety. Therefore, the 2,3-double bond protons shifts appeared within the range of isolated olefinic absorptions. Further evidence for the presence of a 2,3-double bond was provided by ozonolysis of compound **2b**, which resulted in compound **4**. Detailed decoupling experiments of **4**, which are summarized in table 1, fully supported its structure and verified the presence of a 2,3-double bond in **2b** and, therefore, in **1a**.

The 4,5-epoxide function in **1a** was assigned a trans-configuration on the following grounds. It had been outlined before that melfusin contained a 6,12-lactone group with $J_{7,13}$ couplings > 3 Hz which, by the application of Samek's rule (10), suggest a trans-lactone in **1a**. The large couplings between H–5, H–6 and H–7 ($J_{5,6}=J_{6,7}=11.5$Hz) supported the above assignment and the large J-values also indicated that the three protons had to adopt a near antiperiplanar orientation. Based on the biogenetic theory (11) that all sesquiterpene lactones from higher plants have an H–7 α-configuration, H–6 must be β-oriented, that is, a trans-lactone must be present in **1a**. However, application of Geissman's rule (12) would predict a *cis*-6,12-lactone based on the strong positive CD-band at 270 nm. The absorption at 270 nm could possibly have resulted from two overlapping bands, one negative band caused by the α-methylene-γ-lactone chromophore and a more intense positive absorption due to the α,β-unsaturated methyl ester. The presence of a *cis*-4,5-epoxide which is biosynthetically derived from a *cis*-4,5-double bond (heliangolide skeleton) was excluded on the basis of stereomodel inspections and ^{1}H nmr couplings. In a *cis*-4,5-epoxide the dihedral angles between H–6 and H–7 would be near 90° dictating a small $J_{6,7}$-value which is contrary to the experimental findings. Furthermore, in a heliangolide-type medium ring the $J_{7,13}$-values would be < 3 Hz (10) but were found to be > 3 Hz. The above data suggest that melfusin contains a medium ring skeleton which is biogenetically derived from a 1(10)-*trans*-4,5-*trans*-cyclodecadiene (germacrolide) ring system. The anti-periplanar arrangement of H–5 and H–6β ($J_{5,6}=11.5$Hz) in **1a** necessitated that H–5 must be α; therefore, the C–15 attached to the *trans*-4,5-epoxide moiety must be placed above the plane of the medium ring. The conformation around the 1(10)-double bond could not be derived from the nmr spectral data.

The configuration of H–8 in melfusin was determined from the couplings between H–7α and H–8 ($J_{7,8}=2.5$Hz). Model considerations allowed assignments based on correlations between the observed J-values and the angles between H–7α and H–8, which were near 130° when H–8 was assumed α and $\sim$ 150° for H–8β. Although dihedral angles derived from stereomodels and ^{1}H nmr J-values have to be treated with great caution, particularly in highly stained medium rings like germacrolides, application of the Karplus correlation was in good agreement with a dihedral angle $\sim$ 130° suggesting that H–8 be α. This was independent of the conformation of the 1(10)-double bond. Similar considerations for the determination of the configuration at C–9 of **1a** were not conclusive. Assuming an α-oriented carbomethoxy group (C–14) attached to C–10 and H–8α, inspection of stereomodels indicated that the dihedral angles between H–8α and both H–9α and H–9β were $\sim$ 60°, not allowing a configurational assignment for C–9. Assuming a crown-conformation for melfusin with C–14 and C–15 being placed above the plane of the medium ring, an arrangement typical for 6,12-lactonic germacrolides (11), gave dihedral angles near 90° for H8α,H9α and $\sim$ 40° for H8α,H9β. The observed value for $J_{8,9}$ (3Hz) was intermediate between the values expected for $J_{8\alpha,9\alpha}$ and $J_{8\alpha,9\beta}$, a problem which had recently been encountered in a germacrolide with similar nmr parameters (13).

Melfusin is the first germacrolide with C–14 being a carbomethoxy function. Future finding will have to show whether this type of compound represents a biogenetic link between germacrolides and melampolides which co-occur in the subtribe Melampodiinae (11).

EXPERIMENTAL[3]

PLANT MATERIAL.—*Melampodium diffusum* Cass. (Asteraceae-Heliantheae) was collected on September 1, 1976, in Mexico: Guerrero, road to Ayatla, *ca.* 1 mile south of Tierra Colorado (Hartman-Funk No. 4211). A voucher is deposited at the Herbarium of the Ohio State University at Columbus, Ohio, U.S.A.

ISOLATION OF SESQUITERPENE LACTONES **1a** and **5**.—Dried leaves (280 g) were extracted with chloroform and worked up as described before (9). The resulting 3.37 g of crude syrup was chromatographed on silica gel (70 g) and eluted with chloroform and mixtures of chloroform with increasing amounts of acetone (2.5, 5.0, 10.0, 20.0, 40.0, 80.0%); 200 ml fractions were collected and monitored by tlc. Fractions 4–5 eluted with chloroform containing 2.5% acetone provided 350 mg of melampodinin (**5**) (4, 5).

MELFUSIN (**1a**).—Fraction 6 eluted with chloroform-acetone (9:1) provided 150 mg of a gummy material which, after repeated purification by preparative layer chromatography (plc) on 1 mm silica gel plates (ether), gave 50 mg of pure gummy **1a**: uv, λ max (MeOH) 270 nm (ε, 3200), 205 nm (ε, 9800), cd (*c*, 2.0 x 10^{-4}, MeOH), $[\theta]_{215}-57{,}400$, $[\theta]_{270}+56{,}200$; ir (film), ν max 3450, 1765, 1745, 1735, 1715 cm^{-1}; ms (ei) *m/e* 462 (5.5, M–16), 420 (100.0, M–16–C_2H_2O), 360 (2.5), 304 (6.7, 420–116), 286 (6.8, 304–18), 227 (26.0, 286–59), 209 (13.5, 227–18), 149 (93.5, $C_9H_9O_2$), 119 (21.2, C_5H_7O), 115 (4.2, $C_5H_7O_3$), 100 (27.8), 99 (8.7, $C_5H_7O_2$), 91 (40.0, C_7H_7), 83 (26.8), 71 (35.2, C_4H_7O), 55 (14.8), 43 (52.3, C_2H_3O).

Anal. Calcd. for $C_{23}H_{26}O_{10}$: MW, 462.1518. Found: MW (MS) 462.1543.

ACETATE (**1b**).—A solution of 50 mg of **1a** in 1 ml of acetic anhydride and 0.5 ml of pyridine was allowed to react at room temperature overnight. After the usual workup, the residual acetate (55 mg) was purified by plc (ether); uv, λ max (MeOH) 270 nm (ε, 4,900), 205 nm (15,900) cd (*c*, 8.5 x 10^{-5}, MeOH), $[\theta]_{220}-68{,}500$, $[\theta]_{270}+127{,}200$; ir (film) ν max 1780–1715 (carbonyls) cm^{-1}; ms (ei) *m/e* 504 (6.7, M–O), 426 (100, M–O–$C_2H_4O_2$), 389 (2.3, M–O–$C_5H_7O_3$), 346 (2.7, M–O–C_2H_2O–$C_5H_5O_3$), 304 (2.4), 286 (6.4), 255 (6.4), 227 (42), 209 (21.5), 166 (3.6), 149 (46.6), 119 (13.6), 99 (2.9), 91 (11.7), 71 (9.6).

REDUCTION OF **1B** WITH SODIUM BOROHYDRIDE.—To a solution of 50 mg of the acetate **1b** in 4 ml of methanol was added 65 mg of $NaBH_4$ at room temperature; the mixture was allowed to react for 15 min. The mixture was then diluted with water, acidified with 5% aqueous HCl and extracted with ethyl acetate. The washed and dried extracts were evaporated, and the residual mixture was separated by plc on silica gel (ether) to give two major products. Compound (**2a**) was a gum: uv, λ max (MeOH), 270 nm (ε, 3,100), 200 nm (ε, 2,400), cd, (*c*, 1.14 x 10^{-4} MeOH), $[\theta]_{230}-33{,}500$, $[\theta]_{270}+59{,}500$; ir, ν max (film) 3500, 1770, 1635 cm^{-1}; ms (ei) *m/e* 424 (0.9, M^+), 408 (14.8, M–O), 366 (100, M–O–C_2H_2O), 348 (5.5, M–O–$C_2H_4O_2$), 324 (11.8, M–O–$2C_2H_2O$), 306 (7.2, M–O–C_2H_2O–$C_2H_4O_2$), 288 (4.8, M–O–$2C_2H_4O_2$), 256 (3.9), 229 (32), 228 (8.3), 211 (4.4), 166 (5.7), 165 (6.2), 149 (57), 119 (18), 91 (17), 43 (28).

ACETATE (**2b**).—Acetylation of **2a** provided the acetate **2b** as a gum: uv, λ max (MeOH), 270 nm (ε, 1,650), 200 nm (ε, 1,150); cd (*c*, 4.5 x 10^{-5}, MeOH), $[\theta]_{225}-22{,}800$, $[\theta]_{268}+46{,}600$; ir, ν max (film) broad, intense band at 1740 cm^{-1}; ms (ei) *m/e* 466 (0.2, M^+), 450 (5, M–O), 408 (100, M–O–C_2H_2O), 348 (16.6, M–O–C_2H_2O–$C_2H_4O_2$), 316 (10.3), 306 (6.4), 289 (27), 288 (8.6), 256 (7.1), 229 (61), 228 (18), 211 (16.7), 166 (5.4), 165 (10.4), 150 (9.7), 149 (88), 119 (20), 91 (12.5), 43 (37).

THE SECOND REDUCTION PRODUCT, **3a**.—Compound **3a** showed the following physical data: uv, λ max (MeOH), 270 nm (ε, 2,500), 200 nm (ε, 2,400), cd (*c*, 6.5 x 10^{-5}, MeOH), $[\theta]_{227}-23{,}700$, $[\theta]_{270}+66{,}500$; ir, ν max (film) 3450, 1735, 1715 cm^{-1}; ms (ei) *m/e* 428 (0.1, M^+), 412 (0.3, M–O), 394 (1.4, M–O–H_2O), 352 (2.2, M–O–$C_2H_4O_2$), 334 (3.8, M–O–H_2O–$C_2H_4O_2$), 315 (0.8), 292 (1.1),

[3]Melting points were performed in capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. 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 Hewlett-Packard GC–MS–DS 5985 and the high-resolution mass spectra were run on a Varian MAT 711 instrument (70 eV ionizing voltage). The samples were introduced via the direct inlet tube. Nmr spectra were taken on a Bruker WH 200 and a Varian HA-100 instruments. The voucher specimens are on deposit in the Ohio State University Herbarium at Columbus, OH.

275 (3.2), 274 (2.3), 257 (3.3), 233 (6.0), 215 (12.5), 209 (14.6), 166 (4), 165 (4.2), 150 (23), 149 (100), 119 (17.6), 91 (26), 43 (53.6).

ACETATE (**3b**).—Acetylation of **3a** by the procedure outlined above gave the gummy acetate **3b**: uv, λ max (MeOH), 270 nm (ε, 2,600), 200 nm (ε, 2,400); cd (*c*, 8.0 x 10^{-5}, MeOH), $[\theta]_{225}$−27,700, $[\theta]_{265}$+69,250; ir, ν max (film) broad, intense band centered at 1740 cm^{-1}; ms (ei) *m/e* 538 (0.2, M–O), 496 (0.3, M–O–C_2H_2O), 436 (5.4, M–O–C_2H_2O–$C_2H_4O_2$), 418 (4.7, M–O–$2C_2H_4O_2$), 376 (6.3, M–O–$2C_2H_4O_2$–C_2H_2O), 334 (1.5, M–O–$2C_2H_4O_2$–$2C_2H_2O$), 316 (23), 300 (12), 299 (62.6), 284 (12), 274 (6.2), 257 (64), 256 (45), 240 (25), 239 (28), 209 (4.7), 215 (31), 198 (37.5), 197 (100), 166 (4.3), 165 (8.1), 149 (48), 119 (19), 91 (11.5), 43 (38).

OZONOLYSIS PRODUCT (**4**).—A solution of 8 mg of **2b** in CS_2–CH_2Cl_2 was ozonized at −50° and worked up to provide 7 mg of compound **4**: uv, λ max (MeOH), 225 nm (ε, 1,250); cd (*c*, 6.0 x 10^{-5}, MeOH), $[\theta]_{235}$−4,000, $[\theta]_{285}$+2,200; ir, ν max (film) 1780, 1750, 1735, 1685 cm^{-1}; ms (ei) *m/e* 498 (0.3, M^+), 482 (1, M–O), 440 (27.5, M–O–C_2H_2O), 409 (16), 381 (13.2), 367 (61), 353 (11.5), 339 (12.2), 321 (16), 279 (32.6), 267 (11), 263 (53), 261 (32), 231 (21.6), 169 (13), 43 (100).

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