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Antimicrobial Constituents of *Udotea flabellum*

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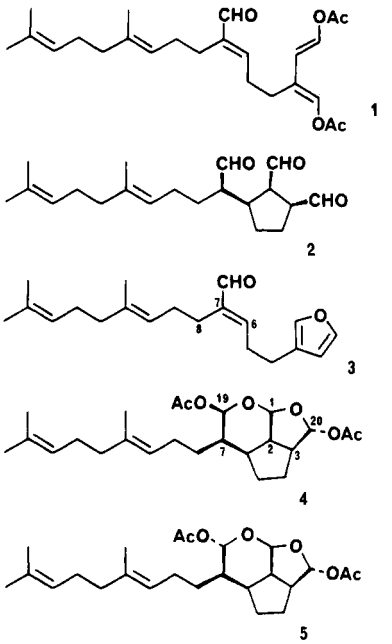
The calcareous green alga *Udotea flabellum* contains udoteafuran (3) and a mixture of cyclic acetals formed by the addition of water or ethanol to udoteatrial (2). The structures of the cyclic acetals 4–6, obtained by acetylation of crude antimicrobial fractions, were determined by analysis of spectral data, particularly the ^1H NMR data that included decoupling difference spectra, nuclear Overhauser enhancement difference spectra, and spin-lattice relaxation rates.

The calcareous green alga *Udotea flabellum* (Ellis and Solander) Howe is commonly encountered in shallow water in Florida[1] and Belize. The crude ethanolic extracts of *U. flabellum* showed moderate antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. Although all crude extracts tested have demonstrated antimicrobial activity, the compounds observed by thin-layer chromatography of fresh extracts of *U. flabellum* were not identical with those found in older ethanolic extracts. Working with fresh extracts of *U. flabellum*, Paul and Fenical[2] have isolated udoteal (1; see Chart I) as the major nonpolar metabolite. From material that had been preserved in ethanol for several months, we have isolated a complex mixture of compounds, best described as hydrates of udoteatrial (2), that is responsible for the antimicrobial activity of the crude extract of preserved material.

Chromatography on Florisil of the chloroform-soluble material from an ethanol extract of *U. flabellum* gave 12 fractions, four of which appeared to contain unusual secondary metabolites. Nonpolar fractions contained udoteafuran (3) but did not contain any detectable quantity of udoteal (1). More polar fractions contained a compound derived by addition of ethanol to the trialdehyde 2 while the most polar, antimicrobial fractions contained the hydrates of the trialdehyde 2. Since the hydrates seemed to undergo facile interconversion, we decided to approach the problem of structural elucidation by "freezing" the hydrates as the corresponding acetates. Acetylation of the fractions containing the hydrates with acetic anhydride in pyridine gave two major diacetates 4 and 5[3] while similar treatment of the ethanolate fraction gave one major acetate 6 (see Chart II).

Chart I



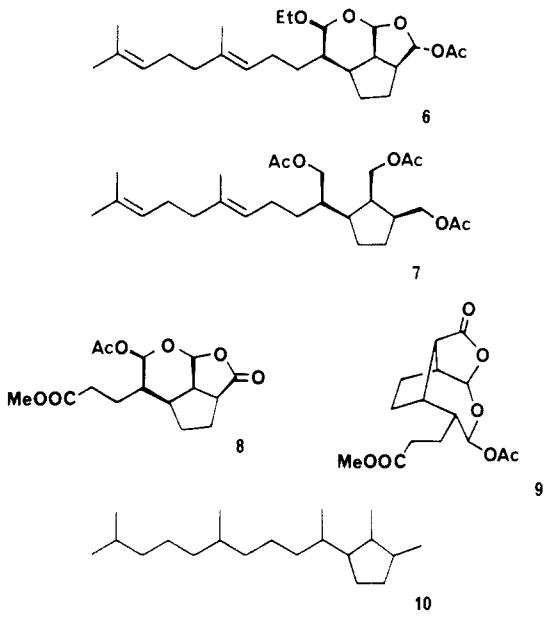
Udoteafuran (3) had the molecular formula $C_{20}H_{28}O_2$. The infrared (1680 cm^{-1}) and ultraviolet (224 nm) spectra both suggested the presence of an α,β-unsaturated aldehyde group. The ^{13}C NMR spectrum contained signals at δ 192.1 (d), 153.3 (d), and 143.7 (s) for the α,β-unsaturated aldehyde and at δ 143.0 (d), 138.9 (d), 123.5 (s), and 110.6 (d) for a β-substituted furan.[4] Comparison of the remaining signals at δ 136.0 (s), 131.2 (s), 124.2 (d), 123.2 (d), 39.6 (t), 29.3 (t), 26.8 (t), 26.6 (t), 25.5 (q), 24.2 (t), 23.8 (t), 17.5 (q), and 15.9 (q) with those of nerolidol[5] strongly

(1) Taylor, W. R. "Marine Algae of Florida"; Carnegie Institution: Washington DC, 1928; p 90.
(2) Paul, V. J.; Fenical, W. H., manuscript in preparation.
(3) A minor diacetate having an aldehyde and one ether ring was occasionally isolated.

(4) Cf.: Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1098.

0022-3263/81/1946-2435$01.25/0

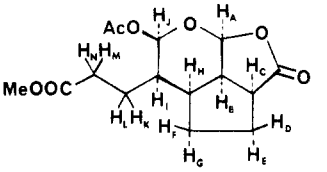
Chart II



suggested the presence of a terminal (*E*)-geranyl unit in udoteafuran (**3**). The ^{1}H NMR spectrum lent support to these assignments with three methyl signals at δ 1.55 (s, 3 H), 1.59 (s, 3 H), and 1.66 (s, 3 H), two olefinic proton signals at δ 5.07 (br t, 1 H, J = 7 Hz) and 5.10 (br t, 1 H, J = 7 Hz), furan proton signals at 6.28 (br s, 1 H), 7.24 (br s, 1 H), and 7.36 (br s, 1 H), and an aldehydic proton signal at δ 9.35 (s, 1 H). The β-proton on the α,β-unsaturated aldehyde gave rise to a quintet at δ 6.45 (1 H, J = 3.5 Hz) coupled to a four-proton signal at δ 2.62 (d, 4 H, J = 3.5 Hz) due to the protons allylic to the olefinic bond and the protons of the methylene adjacent to the furan group that exhibited virtual coupling to the olefinic proton.[6a] The *E* stereochemistry about the 6,7-olefinic bond was defined from the aldehyde proton chemical shift at δ 9.35 (lit.[7] (for *E*) δ 9.3–9.4 and (for *Z*) 10.0–10.1) and from ^{13}C NMR data. The predicted value of the chemical shift of C-8 should be δ 30–34 for the *Z* isomer and δ 22–26 for the *E* isomer;[8] there were no signals in the δ 30–34 region of the spectrum.

The major diacetate **4** ($[\alpha]^{22}_D$ −23° (*c* 2.1, $CHCl_3$)) had the molecular formula $C_{24}H_{36}O_6$.[9] The infrared spectrum indicated the presence of the acetate groups (1747 cm^{-1}), but no other carbonyl groups were indicated. The ^{13}C NMR spectrum provided confirmation for the presence of two acetate groups (δ 169.5, 169.1, 20.7, 20.6) while the signals at δ 135.4 (s), 130.8 (s), 123.9 (d), 123.2 (d), 39.3 (t), 26.3 (t), 25.2 (q), 25.1 (t), 17.2 (q), and 15.6 (q) could again be assigned to a terminal (*E*)-geranyl group. The presence of three acetal carbon signals at δ 101.8 (d), 100.5 (d), and 89.5 (d) and no other olefinic signals suggested that the diacetate **4** contained the partial structure AcO–CH–O–CH–O–CH–OAc set in a tricyclic ring system (i.e., two ether rings and one carbocyclic ring). The ^{1}H NMR spectrum contained signals at δ 1.60 (s, 3 H), 1.62 (s, 3 H), 1.69 (s, 3 H), 2.04 (s, 3 H), and 2.08 (s, 3 H) due to three vinyl and two acetate methyl groups, at δ 5.13 (br t, 2 H, J = 7 Hz) due to two olefinic protons, and at δ 5.72 (d, 1 H, J = 5 Hz), 5.93 (s, 1 H), and 6.20 (d, 1 H, J = 1.5 Hz) due to three acetal protons.

Table I. ^{1}H NMR Data for γ-Lactone **8**



H	shift, δ	multiplicity; J, Hz (coupled signal)[a]	R_1, s^{-1} (±10%)[9]
A	5.94	d; 5 (B)	0.55
B	2.98	ddd; 10 (H), 8 (C), 5 (A)	0.7
C	3.24	t; 8 (B), 8 (E), <1* (D)	0.9
D	2.32	dd; 13 (E), 6 (F), <1* (C), <1* (G)	1.7
E	1.80	m; 13 (D), 13 (F), 9 (C), 8 (G)	1.7
F	1.41	m; 13 (E), 13 (G), 11 (H), 6 (D)	1.7
G	2.16	m; 13 (F), 8 (E), 7 (H), <1* (D)	1.7
H	2.22	m; 11 (F), 10 (B), 9 (I), 7 (G)	1.3
I	~1.75	not resolved	1.4
J	6.15	d; 1 (I)	0.7
K	~1.75	not resolved	1.4
L	~1.75	not resolved	1.4
M	2.38	m, not first order	1.0
N	2.40	m, not first order	1.0
OMe	3.84	s	0.7
OAc	2.12	s (NOE to F)	0.8

[a] An asterisk indicates that irradiation of signal causes a sharpening of the signal indicated.

Reduction of the diacetate **4** with lithium aluminum hydride in refluxing ether followed by acetylation of the product gave a triacetate **7** as the major product. Analysis of the ^{1}H NMR spectrum (C_6D_6) of the triacetate **7** revealed the presence of three AcO–CH_2–CH groups; irradiation at δ 1.54 decoupled signals at δ 4.10 (dd, 1 H, J = 12, 6 Hz) and 4.26 (dd, 1 H, J = 12, 6 Hz) to give an AB quartet, irradiation at δ 2.05 decoupled signals at δ 4.02 (dd, 1 H, J = 13, 7 Hz) and 4.17 (dd, 1 H, J = 13, 5 Hz), and irradiation at δ 2.20 decoupled signals at δ 3.90 (dd, 1 H, J = 13, 7 Hz) and 4.18 (dd, 1 H, J = 13, 9 Hz).

Ozonolysis of the diacetate **4** followed by oxidation of the product with Jones' reagent and methylation of the resultant acids with ethereal diazomethane solution gave a γ-lactone **8** as the major product. The γ-lactone **8** (mp 77.5 °C) had the molecular formula $C_{15}H_{20}O_7$. The infrared spectrum contained a γ-lactone band at 1775 cm^{-1} and a much stronger ester band at 1740 cm^{-1}. The γ-lactone **8** had been formed by oxidation of one of the acetoxyacetal groups to a γ-lactone during the oxidation reaction. We were able to determine the structure and stereochemistry of the γ-lactone **8** from a detailed analysis of the 360-MHz ^{1}H NMR spectrum (Table I).

Spin-decoupling studies were performed to locate the chemical shift, multiplicity, and coupling constants for each signal except those due to protons I, L, and K which could not be resolved.[10] In addition, the coupling between the

(5) Wenkert, E.; Buckwalter, B. L.; Burfitt, I. R.; Gasic, M. J.; Gottlieb, H. E.; Hagaman, E. W.; Schell, F. M.; Wovkulich, P. M. In "Topics in Caron-13 NMR Spectroscopy"; Levy, G. C., Ed.; Wiley: New York, 1976; p 92.

(6) (a) Jackman, L. M.; Sternhell, S. "Applications of NMR Spectroscopy in Organic Chemistry"; Pergamon Press: Oxford, 1969; p 147. (b) *Ibid.*, Chapter 4-2.

(7) Faulkner, D. J. *Synthesis* **1971**, 175.

(8) These values assume that the substitution of an aldehyde for a methyl group at C-19 causes a −6- to −10-ppm shift at C-8 and that for C-8 $\Delta\delta_Z - \Delta\delta_E$ = 8 ppm. Examples from: Wehrli, F. W.; Nishida, T. *Fortschr. Chem. Org. Naturst.* **1979**, *36*, 1.

(9) The molecular formulas of acetates **4–6** were deduced from mass measurement of the highest visible ion (M − AcOH or M − EtOH) coupled with the ^{13}C NMR data.

(10) The decoupling difference spectrum obtained on irradiation at δ 2.39 consisted of a sharp singlet at δ 1.75, suggesting that protons I, K, and L had identical chemical shifts.

methylene groups in the side chain (protons K–N) was not first order. The acetal proton signal at δ 5.94 was coupled to a methine proton signal at δ 2.98 that was in turn coupled to a signal at δ 3.24 due to the methine proton adjacent to the lactone carbonyl. At first sight, each of the protons at δ 2.98 and 3.24 appeared to be coupled to one additional proton, making it difficult to differentiate between structures **8** and **9** for the γ-lactone. We were able to differentiate between methylene and methine protons on the five-membered ring by measuring spin-lattice relaxation rates.[11] The R_1 values for the methine protons are smaller than those for the methylene protons on the cyclopentane ring, indicating that the signal at δ 2.22 (coupled to the δ 2.98 proton) was due to a methine proton. Further support for structure **8** was provided by the observation of a small but significant coupling between protons C and D. Irradiation of the acetate methyl signal gave a positive nuclear Overhauser enhancement (NOEDS)[11] of the signal at δ 1.41 due to proton F, indicating that the acetate group must be positioned above the cyclopentane ring. A small coupling constant (~1 Hz) indicated that the dihedral angle between protons I and J must be in the range 80–100°. Examination of a molecular model of the γ-lactone **8** revealed an excellent agreement between the measured coupling constants and those predicted from dihedral angle measurements.[6b]

Having determined the structure of the γ-lactone **8** we were able to apply that information to the structural elucidation of the diacetate **4**. We could assume the same stereochemistry about the cyclopentane, tetrahydrofuran, and tetrahydropyran rings but needed to define the stereochemistry at the acetoxy–acetal centers. The ^{1}H NMR signals for the acetal protons were differentiated by using decoupling difference measurements.[11] The signal at δ 6.22 (d, 1 H, J = 1.5 Hz) was coupled to a methine proton signal at δ 1.84 (qd, 1 H, J = 7, 7, 7, 1.5 Hz), and the signal at δ 5.72 (d, 1 H, J = 5 Hz) was coupled to a methine proton signal at δ 2.71 (td, 1 H, J = 7, 7, 5 Hz) which was in turn coupled to a methine proton signal at δ 2.76 (t, 1 H, J = 7 Hz); irradiation of the two-proton complex at δ 2.7–2.8 caused both acetal proton signals at δ 5.72 and 5.93 to become sharp singlets. Thus the signals at δ 6.22, 5.93, and 5.72 were assigned to protons at C-19, C-20, and C-1, respectively. The small coupling constants associated with C-19 and C-20 proton signals require 75–100° dihedral angles to vicinal protons at C-7 and C-3, respectively, resulting in the stereochemistry shown for the diacetate **4**.

The minor diacetate **5** also had the molecular formula $C_{24}H_{36}O_6$. The infrared spectrum appeared almost identical, except in the fingerprint region, with that of diacetate **4**. Reduction of the diacetate **5** with lithium aluminum hydride followed by acetylation of the product gave the triacetate **7**, identical in all respects with the sample obtained from diacetate **4**, indicating the presence of the same carbon skeleton and the same absolute stereochemistry at carbons 2, 3, 6, and 7. The ^{1}H NMR spectrum of diacetate **5** was similar to that of the diacetate **4** with the exception of the signals for the acetal protons. Using spin-decoupling experiments, we could assign the signals at δ 5.67 (d, 1 H, J = 5 Hz), 5.93 (d, 1 H, J = 5 Hz), and 5.98 (s, 1 H) to the protons at C-1, C-19, and C-20, respectively. While the chemical shifts and coupling constants of the C-1 and C-20 proton signals were almost identical in diacetates **4** and **5**, the difference in chemical shift and coupling constant of the C-19 proton signals suggested a change of configuration at that center. Comparison of the ^{13}C NMR spectra of diacetates **4** and **5** also suggested a change in the stereochemistry of C-19 as indicated by a change from δ 89.5 in diacetate **4** (axial OAc) to δ 92.9 in diacetate **5** (equatorial OAc).[12] We have therefore assigned structure **5** to the minor diacetate.

The acetate **6** had the molecular formula $C_{24}H_{38}O_5$. The infrared spectrum contained a band at 1735 cm^{-1} due to the acetate group. The ^{1}H NMR spectrum contained signals at δ 1.20 (t, 3 H), 3.53 (dq, 1 H, J = 10, 7, 7 Hz), and 3.86 (dq, 1 H, J = 10, 7, 7, 7 Hz) due to the ethoxy group, at δ 1.60 (s, 6 H), 1.68 (s, 3 H), and 2.05 (s, 3 H) assigned to three vinyl methyl groups and one acetoxy methyl group, at δ 5.09 (br t, 1 H, J = 7 Hz) and 5.12 (br t, 1 H, J = 7 Hz) due to the vinyl protons, and at δ 4.94 (d, 1 H, J = 2 Hz), 5.68 (d, 1 H, J = 5 Hz), and 5.86 (s, 1 H) due to the acetal protons. Irradiation of the two methine protons at δ 2.66 (dt, 1 H, J = 7, 7, 5 Hz) and 2.74 (br t, 1 H, J = 7, 7 Hz) caused the signals at δ 5.68 and 5.86 to become sharp singlets, indicating that the substitution pattern about the tetrahydrofuran ring was the same as in diacetates **4** and **5**. The signal at δ 4.94 must therefore be due to the C-19 acetal proton in a compound analogous to diacetate **4** with ethoxy replacing acetoxy at C-19.

The ^{1}H NMR spectra of the crude chromatographic fractions (prior to acetylation to obtain acetates **4–6**) contained many broad signals in the δ 4.5–6.0 region and a variable number of small signals in the aldehyde region. We concluded that "udoteatrial" existed as an equilibrating mixture of hydrates and ethanolates. We could separate the ethanolates from the hydrates but could not obtain pure compounds. Attempts to dehydrate the hydrate mixture were also unsuccessful.

Comparison of these results with those of Paul and Fenical[2] led to the conclusion that udoteal (**1**) was unstable to storage in aqueous ethanol. Since the acetates **4–6** were all optically active, udoteatrial (**2**) must be a natural product and not a degradation product of udoteal (**1**). The cyclization of a linear diterpene precursor to form the cyclopentane ring of udoteatrial (**2**) is reminiscent of the biosynthesis of the iridoid monoterpene skeleton. Udoteatrial (**2**) and its derivatives are the first representatives of a novel diterpene skeleton **10** that we have named "udoteane". The formal name for udoteatrial (**2**) is (2*R**,3*S**,6*R**,7*R**)-(10*E*)-10,14-udoteadiene-1,19,20-trial.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 10-cm microcell. ^{1}H NMR spectra were recorded on a Varian HR-220 NMR spectrometer (for **3** only) or on a 360-MHz spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet FT data system by Dr. John M. Wright for the UCSD NMR Facility. ^{13}C spectra were recorded on a Varian CFT-20 NMR spectrometer; all chemical shifts are reported with respect to Me_4Si (δ 0). Low-resolution mass spectra were recorded on a Hewlett-Packard 5930A mass spectrometer. High-resolution mass spectra were supplied by the Chemistry Department at UCLA. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were either spectral grade or distilled from glass prior to use.

Collection and Extraction of *Udotea flabellum*. Samples of *U. flabellum* were collected by hand (−1 m) at Lighthouse Reef, Belize, Carrie Bow Cay, Belize, and near Key West, FL. The blade portions of the plants were stored in ethanol at 0 °C for several months. The ethanol was decanted from the sample (no. 77-069) obtained at Lighthouse Reef, and the solids were Soxhlet extracted with ethanol. The combined ethanol extracts were evaporated

(11) Hall, L. D.; Saunders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.

(12) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 461.

to obtain an aqueous suspension which was extracted with chloroform. The chloroform extract was dried over sodium sulfate and the solvent evaporated to obtain a dark brown gum (22.3 g, 3.1% dry weight).

Chromatographic Separations. A portion (21 g) of the chloroform extract was preadsorbed on Florisil and applied to the top of a column (100 × 5 cm diameter) of Florisil. Fractions eluted with 5–10% ether in hexane contained a mixture of phytol and udoteafuran. The combined fractions (357 mg) were acetylated by using the procedure described below to obtain a mixture of phytol acetate and udoteafuran. The crude reaction product was chromatographed on silica gel plates (10% ether in hexane) to obtain udoteafuran **3** (73 mg, 0.01% dry weight). The first fraction (490 mg) eluted with ether contained an ethoxy acetal. A portion of the ethoxy acetal (20 mg) was acetylated as described below and the product purified by LC on μ-Porasil (3:2 hexane–ether) to yield the acetate **6** (17 mg, equivalent to 0.055% dry weight). The second fraction (980 mg) eluted with ether contained a mixture of the ethoxy acetal and hydrates of udoteatrial. The third fraction (1.21 g) eluted with ether contained the mixture of hydrates of udoteatrial. A portion (880 mg) of the hydrate fraction was dissolved in pyridine (2 mL) and acetic anhydride (1 mL) and the solution was allowed to stand at room temperature for 24 h. The solvents were removed under high, vacuum and the residue was partitioned between ether (3 × 25 mL) and water (25 mL). The combined ether extracts were dried over sodium sulfate, and the solvent was evaporated to obtain an oil that was chromatographed by LC on μ-Porasil to obtain the diacetate **4** (650 mg, equivalent to 0.11% dry weight) and the diacetate **5** (72 mg, equivalent to 0.012% dry weight).

Udoteafuran (3): oil; UV (MeOH) 224 nm (ε 17 400); IR ($CHCl_3$) 2720, 1680, 1635, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 3 H), 1.59 (s, 3 H), 1.66 (s, 3 H), 2.0 (m, 6 H), 2.27 (t, 2 H, J = 7 Hz), 2.57 (d, 4 H, J = 3.5 Hz), 5.07 (br t, 1 H, J = 7 Hz), 5.10 (br t, 1 H, J = 7 Hz), 6.28 (s, 1 H), 6.45 (m, 1 H, J = 3.5 Hz), 7.24 (br s, 1 H), 7.36 (br s, 1 H), 9.35 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 192.1 (d), 153.3 (d), 143.7 (s), 143.0 (d), 138.9 (d), 136.0 (s), 131.2 (s), 124.2 (d), 123.5 (s), 123.2 (d), 110.6 (d), 39.6 (t), 29.3 (t), 26.8 (t), 26.6 (t), 25.5 (q), 24.2 (t), 23.8 (t), 17.5 (q), 15.9 (q); mass spectrum, m/z 300, 219 (M − 81), obsd m/z 300.2103, $C_{20}H_{28}O_2$ requires m/z 300.2089.

Diacetate 4: oil; $[\alpha]_D$ −23° (c 2.1, $CHCl_3$); IR (CCl_4) 1748, 1370, 1355, 1150, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (s, 3 H), 1.62 (s, 3 H), 1.69 (s, 3 H), 2.04 (s, 3 H), 2.09 (s, 3 H), 2.71 (td, 1 H, J = 7, 7, 5 Hz), 2.76 (t, 1 H, J = 7, 7 Hz), 5.13 (br t, 2 H, J = 7 Hz), 5.72 (d, 1 H, J = 5 Hz), 5.93 (s, 1 H), 6.20 (d, 1 H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 169.5 (s), 169.1 (s), 135.4 (s), 130.8 (s), 123.9 (d), 123.2 (d), 101.8 (d), 100.5 (d), 89.5 (d), 50.5 (d), 40.2 (d), 39.3 (t), 38.5 (d), 37.3 (d), 31.7 (t), 29.6 (t, 2 C), 26.3 (t), 25.2 (q), 25.1 (t), 20.7 (q), 20.6 (q), 17.2 (q), 15.6 (q); mass spectrum, m/z 360 (M − AcOH), 300 (M − 2 AcOH), obsd m/z 360.2310, $C_{22}H_{32}O_4$ requires m/z 360.2301.

Diacetate 5: oil; $[\alpha]_D$ +36° (c 1.4, $CHCl_3$); IR (CCl_4) 1748, 1370, 1360, 1150, 1100, 1005, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.59 (s, 6 H), 1.67 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.76 (m, 2 H), 5.08 (br t, 2 H, J = 7 Hz), 5.64 (d, 1 H, J = 5 Hz), 5.93 (d, 1 H, J = 5 Hz), 5.98 (s, 1 H); ^{13}C NMR (C_6D_6) δ 169.4 (s), 169.3 (s), 136.5 (s), 131.3 (s), 124.8 (d), 124.2 (d), 101.8 (d), 101.6 (d), 92.9 (d), 50.2 (d), 40.4 (d), 40.1 (t), 38.3 (d), 35.7 (d), 32.1 (t), 31.4 (t), 30.0 (t), 27.1 (t), 26.8 (q), 26.6 (t), 21.0 (q), 20.8 (q), 17.7 (q), 16.1 (q); mass spectrum, m/z 360 (M − AcOH), 300 (M − 2 AcOH), obsd m/z 360.2300, $C_{22}H_{32}O_4$ requires m/z 360.2301.

Acetate 6: oil; IR (CCl_4) 1740, 1375, 1360, 1235, 1145, 1080, 975 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (t, 3 H, J = 7 Hz), 1.60 (s, 6 H), 1.68 (s, 3 H), 2.05 (s, 3 H), 2.68 (m, 2 H), 3.53 (dq, 1 H, J = 10, 7, 7 Hz), 3.86 (dq, 1 H, J = 10, 7, 7 Hz), 4.94 (d, 1 H, J = 2 Hz), 5.09 (br t, 1 H, J = 7 Hz), 5.12 (br t, 1 H, J = 7 Hz), 5.68 (d, 1 H, J = 5 Hz), 5.86 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 170.1, 135.2, 131.3, 124.6, 124.5, 103.3, 101.6, 95.9, 63.9, 50.3, 40.0, 39.1, 39.0, 31.8, 30.3, 29.5, 29.2, 26.8, 25.9, 25.8, 21.5, 18.4, 16.8, 16.0; mass spectrum, m/z 360 (M − EtOH), 346 (M − AcOH).

Reduction and Acetylation of Diacetate 4. A solution of the diacetate **4** (22 mg, 0.053 mmol) in anhydrous ether (5 mL) was added to a stirred suspension of lithium aluminum hydride (150 mg) in anhydrous ether (5 mL), and the reaction mixture was stirred for 2 h at room temperature. After excess reagent had been destroyed by dropwise addition of ethyl acetate, the product was partitioned between 2 N hydrochloric acid (10 mL) and ethyl acetate (2 × 10 mL). The organic extract was washed with water until neutral and dried over sodium sulfate, and the solvent was evaporated to obtain the crude alcohol (16 mg). The alcohol was immediately dissolved in acetic anhydride (1 mL) and pyridine (2 mL), and the solution was stirred at room temperature overnight. The solvents were evaporated under vacuum, and an ethereal solution of the residue was passed through a short plug of silica gel to remove polar byproducts. The product was purified by LC on μ-Partisil with 1:1 ether–hexane as eluant to obtain the triacetate **7**: 12 mg (50% from **4**); colorless oil; $[\alpha]_D$ −4° (c 0.23, $CHCl_3$); IR (CCl_4) 1735, 1360, 1240, 1035 cm^{-1}; ^1H NMR (C_6D_6) 1.58 (s, 3 H), 1.64 (s, 3 H), 1.69 (s, 9 H), 1.71 (s, 3 H), 3.90 (dd, 1 H, J = 13, 7 Hz), 4.02 (dd, 1 H, J = 13, 7 Hz), 4.10 (dd, 1 H, J = 12, 6 Hz), 4.17 (dd, 1 H, J = 13, 5 Hz), 4.18 (dd, 1 H, J = 13, 9 Hz), 4.26 (dd, 1 H, J = 12, 4 Hz), 5.24 (br t, 2 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 170.8 (s), 170.7 (s), 170.6 (s), 135.2 (s), 131.0 (s), 124.1 (d), 123.8 (d), 65.5 (t), 65.2 (t), 60.8 (t), 44.7 (d), 42.1 (d), 40.5 (d), 39.5 (t), 37.8 (d), 29.8 (t), 26.9 (t), 26.5 (t), 25.4 (q), 24.7 (t), 24.3 (t), 20.7 (q, 3 C), 17.4 (q), 15.7 (q); mass spectrum, obsd m/z 450.2990, $C_{26}H_{42}O_6$ requires m/z 450.2981.

Reduction and Acetylation of Diacetate 5. The diacetate **5** (26 mg, 0.062 mmol) was reduced and acetylated by using the established procedure above to obtain the triacetate **7** (3 mg, 10% from **5**), identical in all respects ($[\alpha]_D$ −4° (c 0.22, $CHCl_3$)) with the material obtained from diacetate **4**.

Ozonolysis of Diacetate 4. A stream of ozone in oxygen was bubbled into a solution of the diacetate **4** (75 mg, 0.18 mmol) in ethyl acetate (20 mL) at −78 °C until a blue solution was obtained. After the saturated solution was allowed to stand at −78 °C for 5 min, excess ozone was removed in a stream of nitrogen, and the solvent was evaported. The resulting oil was dissolved in acetone (10 mL) and Jones reagent added until an orange solution resulted. Excess Jones reagent was reduced with 2-propanol and neutralized with sodium bicarbonate. The solids were removed by filtration, and the solvent was evaporated. The residue was dissolved in ether (5 mL) and excess diazomethane solution added. After 30 min at room temperature, the solvent was evaporated and the product purified by LC on μ-Porasil with 1:2:2 ether–dichloromethane–hexane as eluant to obtain the γ-lactone **8**: 19 mg (34% from **4**); mp 77.5 °C; $[\alpha]_D$ −28° (c 0.4, $CHCl_3$); IR (KBr) 1775, 1740, 1225, 1140, 965 cm^{-1}; ^1H NMR (see Table I); mass spectrum, m/z 281 (M − 31), 270 (M − 42), 253 (M − 59), 239, 224, 221.

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