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Isolation and Characterization of Peroxyferolide, a Hydroperoxy Sesquiterpene Lactone from *Liriodendron tulipifera*

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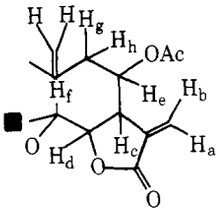
A naturally occurring germacranolide hydroperoxide, peroxyferolide, was assigned structure 1 from physical data, especially double-resonance ^1H NMR, and from chemical evidence. The allylic hydroperoxide function was supported by polarographic analysis, the preparation of anhydroderivative 3 under acetylation conditions, methylperoxyferolide (5) with methyl iodide and silver oxide, deoxyperoxyferolide (10) by mild reduction, and the presence of a characteristic absorption in the ^{13}C NMR. Formation of 1 from lipiferolide (2) by light-generated singlet oxygen confirmed the stereochemical assignments and established the configuration at the hydroperoxy-bearing carbon.

In screening ethanolic extracts of plants in a feeding test[2] for the larvae of the gypsy moth, *Lymantria dispar* L., it was found that the residue from the leaves of the tulip poplar, *Liriodendron tulipifera* L., showed antifeeding properties. On fractionating the crude extract a moderately active constituent,[3] peroxyferolide (1), was obtained and characterized to be the first naturally occurring germacranolide hydroperoxide[4] on the evidence reported herein. Previous work on this source had given lipiferolide (2) and epitulipinolide diepoxide (the 1,10-epoxide of 2) as the major sesquiterpene components.[5]

Peroxyferolide (1) was isolated by repeated column chromatography and crystallization from ethanol–chloroform. Elemental and chemical ionization mass spectral analyses established the molecular formula as $C_{17}H_{22}O_7$, and the infrared spectrum suggested hydroxyl, α,β'-unsaturated γ-lactone, ester, and olefinic functions. The ^1H NMR spectrum (Table I) showed a pair of one-proton doublets at 6.13 and 5.53 ppm split by 3.5 and 3.1 Hz, respectively, which are characteristic of α-methylene γ-lactones, and confirmed by preparation of a crystalline pyrazoline derivative that was too unstable for proper characterization. A three-proton singlet at 2.03 ppm supported an acetate as the ester function. The remainder of the molecule was assumed to be sesquiterpenoid.

Double-irradiation experiments clarified the arrangement of the substituents about the α,β'-unsaturated γ-lactone as shown in A, in which ■ designates a quaternary carbon. Irradiation of the doublet for H_a at 6.13 ppm caused the multiplet at 3.93 ppm to be simplified to a pair of triplets with J = 9.6, 3.1, and 3.1 Hz, and irradiation at 5.53 ppm (H_b) showed a similar collapse with coupling now 9.6, 3.5, and 3.1 Hz, thus locating H_c at 3.93 ppm. Saturation of this signal not only converted the H_a and H_b doublets to singlets but also changed



A

the one-proton triplet at 4.23 ppm to a doublet (J = 9.6 Hz) and the saw-tooth multiplet of eight-peaks at 5.95 ppm to a pair of doublets (J = 11.4 and 6.4 Hz). The lactonic proton H_d was assigned at 4.23 ppm, and H_e on the acetate-bearing carbon at 5.95 ppm in keeping with the chemical shifts observed for similar protons in other sesquiterpene lactones. Irradiation at 4.23 ppm collapsed the multiplet at 3.93 ppm (H_c) to a broadened quartet ($J \approx 3$ Hz) and the doublet at 2.98 ppm for H_f to a singlet. The pattern and chemical shift of H_f suggested it was adjacent to a quaternary carbon and most probably on a carbon with an epoxide oxygen (vide infra). Similar decoupling of H_e (5.95 ppm) caused the expected collapse of the H_c pattern at 3.93 ppm to a pair of triplets and of a one-proton (H_g) multiplet centered at 2.74 ppm; the A doublet (J = 17.2 Hz) of an AB quartet, further split into five peaks ($J \approx 2$ Hz) to a doublet split into four peaks. In addition, a change between 2.0 and 2.4 ppm was observed, but the region consists of overlapping peaks and was not clearly analyzable. Irradiation at ~2.2 ppm affected the large coupling for the pattern at 2.74 ppm and thus the hidden pattern corresponds to the second methylene proton H_h. Furthermore, the H_e multiplet at 5.95 ppm was changed to a pair of doublets and the one-proton broadened doublet at 5.33 ppm to a sharp doublet, as would be expected on elimination of allylic coupling. Finally, on irradiation of H_g (2.74 ppm), not only was

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

Registry no.	Compd	H-1	H-5	H-6	H-7	H-8	H-13	H-14	H-15	Miscellaneous
61228-73-7	1[b]	4.37 dd (9.9, 4.4)	2.98 d (9.4)	4.23 t (9.6, 9.4)	3.93 m (9.6, 3.5) 3.1, 3.1)	5.95 dq (11.4, 6.4, 3.1)	6.13 d (3.5) 5.53 d (3.1)	5.45 d (2.4) 5.33 br d (1.7)	1.53 s	10.64 br s[c], 2.84 m (H-9*a*), 2.65 m (H-9*e*), 2.03 s (Ac)
41059-80-7	2[b]	5.28 br d (11)	2.86 d (8.5)	4.24 dd (8.5, 7.8)	3.35 m (7.8, 3.4, 3.1, 0.9)	5.49 m	5.96 d (3.4) 5.54 d (3.1)	1.74 br s (~1)	1.29 s	1.99 s (Ac)
61228-74-8	3[d]		2.63 d (9.2)	4.18 t (9.0)	~3.2 m	5.53 dq (7.8, 3.7, 1.5)	6.33 d (3.5) 5.65 d (3.1)	6.2 s ($w_{1/2}$ = 1.2) 5.77 br s ($w_{1/2}$ = 2.2)	1.52 s	2.07 s (Ac)
63511-98-8	5[d]	4.47 dd (9.2, 4.0)	2.90 d (8.9)	4.21 t (8.9)	~3.7 m	5.97 dq (10.6, 5.4, 2.5)	6.30 d (3.3) 5.57 d (2.9)	5.48 br d (2.2) 5.17 br d (~1.5)	1.54 s	3.80 s (MeO), 2.06 s (Ac)
61228-75-9	6[d]	4.20 dd (9.4, 4.6)	2.79 d (9.2)	4.10 t (9.3)	~3.0 m	5.63 dq (10.8, 5.3, 2.2)	1.28 d (6.2)	5.41 d (2.6) 4.93 br d (1.8)	1.54 s	2.13 s (Ac) 1.87 br s (OH)[c]
63511-99-9	7[e]	4.0–4.6 m	2.82 d (9.0)	4.15 t (9.2)	~2.8	4.0–4.6 m	1.24 d (6.2)	5.30 d (2.5) 4.92 br q (~1)	1.52 s	
61228-76-0	8[d]	5.39 dd (9.0, 4.8)	2.83 d (9.2)	4.10 t (9.2)		5.58 dq (10.8, 5.0, 2.0)	1.28 d (6.2)	5.49 d (2.4) 5.02 br s	1.55 s	2.14 (Ac) 2.01 (Ac)
63512-00-5	9[d]	4.17 dd (6.2, 4.8)	2.65 d (9.0)	4.10 t (9.3)		5.50 m (8.8, 7.0, 2.8)	1.29 d (6.3)	absent	1.63 s	2.13 (Ac)
63512-01-6	10[b]	~4.2 m	2.97 d (9.1)	4.23 t (9.4)	3.90 m (9.4, 3.5, 3.2, 3.0)	5.92 dq (11.4, 6.4 3.0)	6.13 d (3.5) 5.52 d (3.2)	5.35 br d (2.4) 5.10 br d (2)	1.54 s	2.8 m (H-9*a*) 2.03 s (Ac)
63512-02-7	11[f]	5.42 dd[g] (9.5, 5.1)	2.93 d (9.5)	4.21 t (9.5)	3.67 m (9.5, 3.5, 3.2, ~3)	5.86 dq (11.4, 5.1, 2.5)	6.29 d (3.5) 5.54 d (3.2)	5.51 br d (2.5) 5.09 br d (~1)	1.56 s	2.06 and 2.03 (2 Ac)

[a] Spectra were determined in stated solvent at 60 or 90 MHz with Me_4Si as internal standard. Chemical shifts (δ) are in parts per million, coupling constants (J) in Hz are given in parentheses, and multiplicities are designated by the following symbols: s, singlet; d, doublet; m, multiplet with center given; q, quartet; t, triplet; and br, broadened signal. [b] In acetone-d_6 at 90 MHz. [c] D_2O exchangeable. [d] In $CDCl_3$ at 60 MHz. [e] In acetone-d_6 at 60 MHz. [f] In $CDCl_3$ at 90 MHz. [g] Partially obscured by other peaks.

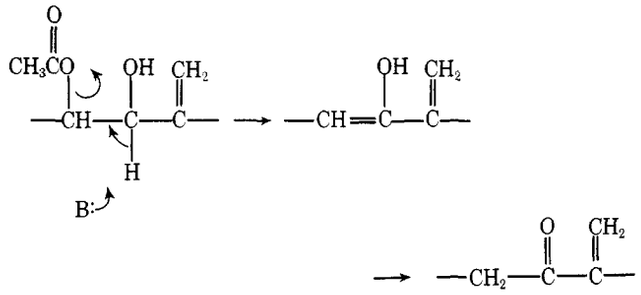
the H_e pattern affected, but the 2.0–2.4-ppm region containing H_h was changed, as was the conversion of the one-proton split singlets at 5.45 and 5.33 ppm to sharp singlets. The alterations are consistent with the presence of an olefinic methylene adjacent to the aliphatic protons, H_g and H_h.

The partial structure of peroxyferolide (1) derived from NMR studies was in agreement with the arrangement of substituents observed for lipiferolide (2) from C-5 through C-8. The presence of a three-proton singlet at 1.53, although 0.24-ppm downfield from a similar peak of lipiferolide, was taken as an epoxide methyl with the difference resulting from the nature of the olefinic group. Thus, placement of an oxirane ring between C-4 and C-5, and assuming a normal isoprenoid skeleton, requires a methyl at C-4. This extends the similarity in structure to lipiferolide (2) to C-4 with the one-proton doublet at 2.98 ppm assigned H-5. The molecular formula of peroxyferolide (1) requires seven double-bond equivalents of which six are met by substituents of partial structure from C-4 through C-10. The remaining unsaturation equivalent must be from a ring, either carbocyclic or ether forming, since two oxygens still need to be accommodated, neither of which are associated with IR absorption in the carbonyl region.

The ^{1}H NMR analysis also provided stereochemical information. The large coupling constant of 9.6 Hz between H-6 and H-7 supports a trans-fused lactone, and, since all well-characterized sesquiterpene lactones have β side chains at C-7, the absolute stereochemistry at C-6 and C-7 is as drawn in 1. Similarly, with $J_{7,8}$ = 3.1 Hz, a pseudo-equatorial H_8 is required that is further supported by the more downfield location of its pattern.[6]

Since hydroxyl absorption was observed for peroxyferolide (1) in the IR and ^{1}H NMR spectra, formation of an acetate with acetic anhydride and pyridine, and with acetylimidazole, was attempted. No acetylated product was formed, but, instead, an unstable derivative, anhydroperoxyferolide (3), was produced whose composition, $C_{17}H_{20}O_6$, corresponded to the loss of the elements of water. The ^{1}H NMR spectrum showed that the product retained the epoxide, unsaturated lactone,

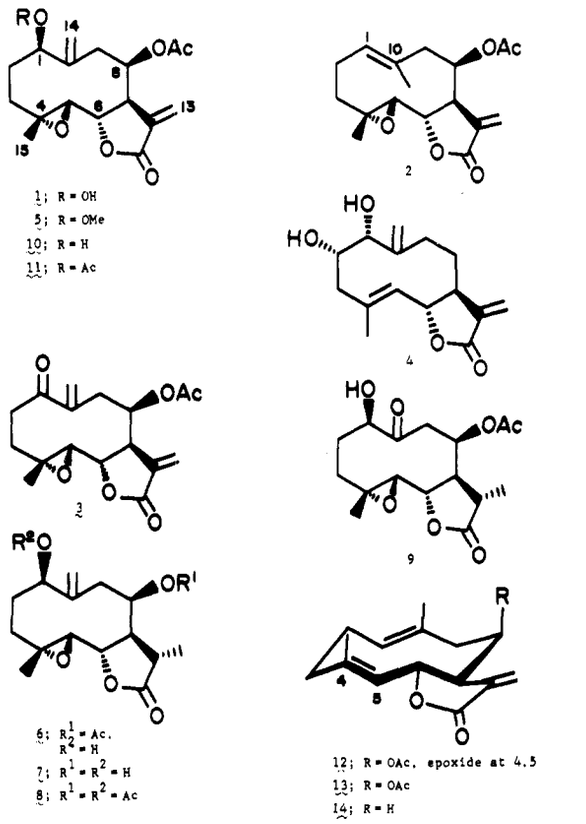
and original acetate, as the peak patterns associated with protons of these groups remained unaffected. There was, however, loss of the one-proton double doublet at 4.37 ppm in the transformation, and now the H-14 peaks became sharpened singlets. The UV and IR spectra, with intense ab-



sorption in the latter at 1670 cm^{-1}, suggested an α,β-unsaturated ketone, possibly arising from a 1-vinyl-1,2-glycol unit by elimination of acetate under basic conditions to form a pinacol rearranged product. The same reasoning was invoked for the conversion of verlotorin (4) to anhydroverlotorin.[7] Anhydroperoxyferolide was thus formulated as 3 with a ten-membered germacrane ring.

All efforts at gaining chemical support for the presence of a 1,2-glycol system were unsuccessful. These included acetonide (via acetone or 2,2-dimethoxypropane) and phenylborate ester preparation. However, treatment of peroxyferolide (1) with methyl iodide and silver oxide produced a monomethoxy derivative still containing seven oxygens, but no longer showing hydroxyl absorption in the IR spectrum. It was recovered unchanged after an acetylation attempt. Clearly the seventh oxygen was not hydroxyl. The methoxy derivative was formulated from evidence to follow as the methyl peroxide 5.

On reduction of peroxyferolide (1) with sodium borohydride the major product contained one less oxygen and two additional hydrogens. The ^1H NMR spectrum showed no peaks for the H-13 protons, but instead a three-proton doublet at 1.32 ppm and an upfield shift for H-7, indicating the expected



reduction of the lactonic α-methylene. The remainder of the spectrum, except for the H-7 absorption as stated, stayed essentially unchanged; thus, the loss of oxygen was from a position little affecting the proton spectrum. When sodium borodeuteride was used, only two deuteriums were incorporated, at positions 11 and 13, confirming that additional carbons were not reduced and that the hydroxyl group was not formed from an unsaturated function. The evidence available refuted the presence of a glycol in peroxyferolide (1) and required consideration of a hydroperoxide. The borohydride product was formulated as 6 (stereochemical assignment at C-1 open) with the C-13 methyl placed α on the basis of similar reductions on related compounds.[6] A minor by-product of the borohydride reduction was assigned structure 7 on spectral evidence and by conversion to an acetate derivative 8 identical to the acetate prepared from 6. With dihydrodeoxyperoxyferolide (6) it was possible to provide chemical proof for the exocyclic double bond to C-14, as ozonolysis of 6 gave formaldehyde (identified as the dimedone derivative) and the α-hydroxy ketone 9.

Support for a hydroperoxide group in peroxyferolide (1) was obtained from the ^{13}C NMR spectrum (Table II), since three peaks were found in the methylene region at 34.1, 32.1, and 26.6 ppm, each appearing as triplets in off-resonance and undecoupled spectra. Only seven oxygenated carbons could be assigned, six of which corresponded to the partial structure from C-4 to C-8 previously established by the ^1H NMR experiments. The seventh oxygen-bearing carbon, uniquely located at 90.9 ppm, had to contain the two remaining oxygens. Chemical tests on peroxyferolide (1) for a hydroperoxide were positive; e.g., a deep blood-red color rapidly formed with ferrous thiocyanate,[8] and iodine was readily liberated from ethanolic potassium iodide. Furthermore, the characteristic loss of 16 mass units for hydroperoxides was observed in the mass spectrum,[9] and polarographic analysis in nonaqueous medium for peroxides and hydroperoxides[10] gave a half-wave potential of −0.66 V, a value within the range of −0.61 to −0.96 V reported for hydroperoxides. On mild reduction of peroxyferolide (1) with acidic potassium iodide, deoxyperoxyferolide (10) was formed, which was easily acetylated to 11.

Table II. ^{13}C NMR Spectra of Peroxyferolide and Related Compounds[a]

Carbon atom	1[b]	2[b]	10[e]	11[e]
1	90.9 d	129.1 d	78.2 d	78.0 d
2	34.1 t[c]	44.0 t[c]	33.3 t[c]	33.5 t[c]
3	32.1 t[c]	36.4 t[c]	30.3 t[c]	31.2 t[c]
4	60.7 s	61.8 s	60.4 s	60.0 s
5	64.4 d	66.8 d	63.8 d	63.9 d
6	76.4 d	76.4 d	75.6 d	75.4 d
7	46.7 d	49.4 d	45.8 d	45.8 d
8	67.0 d	74.5 d	66.2 d	66.4 d
9	26.6 t[c]	24.5 t[c]	29.8 t[c]	27.8 t[c]
10	142.9 s	132.0 s	146.2 s	141.3 s
11	136.1 s	137.9 s	134.5 s	134.3 s
12	169.3 s	169.1 s	168.7 s	168.3 s
13	120.4 t	121.9 t	120.8 t[d]	119.4 t[d]
14	120.1 t	19.7 q	117.0 t[d]	120.9 t[d]
15	18.5 q	17.2 q	18.3 q	18.3 q
CH_3CO	20.5 q	20.6 q	20.6 q	21.0 q, 20.6 q
CH_3CO	170.2 s	169.9 s	169.9 s	169.8 s, 169.2 s

[a] Assignments of multiplets were made by single frequency off-resonance spin decoupling. Peak assignments were based on comparison with related compounds in our possession and by single-frequency irradiation of known proton resonances. [b] In Pyr-d_5. [c,d] Not designated, may be interchanged. [e] In $CDCl_3$.

With the allylic hydroperoxide function of peroxyferolide (1) characterized, formation of the anhydro-derivative **3** under acylating conditions is understood to arise by elimination of acetate from the transient acetyl peroxyferolide, since pyridine alone is inert. Similar facile eliminations have been reported for hydroperoxides α to a ketonic[11] or aromatic group.[12] The stability of methylperoxyferolide (**5**), in comparison, rests with the poor leaving property of methoxide vs. acetate.

Preparation of peroxyferolide (1) was accomplished in high yield by photooxygenation of lipiferolide (**2**)[5] by visible light and Methylene Blue as sensitizer. This confirmed the stereochemical assignments for carbons 4 through 8, and requires that the hydroperoxy group be placed β at C-1, since the solution conformation of lipiferolide as in **12** has been related chemically to epitulipinolide (**13**),[5] which in turn has been related to costunolide (**14**) by circular dichroism. Costunolide from nuclear Overhauser effect analysis was shown to have the olefins crossed and the methyl groups up.[13] The stereochemistry of singlet-oxygen addition has been established to proceed via a cis cyclic mechanism, with the oxygen approaching perpendicular to the olefinic plane. Thus, for peroxyferolide the oxygen introduction would be to the β position.

Prior to the isolation of peroxyferolide, two hydroperoxides were characterized from nature, 3α,22α-dihydroxy-7α-hydroperoxystigmast-5-ene from horse-chestnut (*Aesculus hippocastanum*),[15] and peroxy-Y base, an elaborated tRNA purine from several plant and animal sources.[9b] A third, verlotorin, from *Artemisia verlotorum*, was reported as **4**, but recent work requires it be revised to an allylic hydroperoxide.[16] Peroxyferolide (1) is therefore the first recognized sesquiterpene hydroperoxide from nature. The genesis of these products is unknown, but an artifactual origin seems unlikely as plant material from different parts of the country varied in their content of peroxyferolide (some were totally devoid), yet processing was the same. Lipiferolide (**2**) was found in all samples. Chlorophyll-mediated oxygenation appears probable, since peroxyferolide (1) was obtained from leaves in which lipiferolide (**2**) is the most abundant germacranolide, and chlorophyll is known to be an effective sensitizer.[14] A spinach-leaf preparation has already been reported to convert α-terpinene to ascaridole, an endoperoxide.[17]

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The UV spectra were determined in MeOH on a Cary Model 15 instrument, and IR spectra were obtained on a Beckman 4230 or Perkin Elmer 257 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian A-60A or Bruker HX-90E instrument; the latter equipped for Fourier transform analysis was also used for ^{13}C NMR determinations. Mass spectra were measured on AEI MS-902 and MS-9, Finnigan 1015, or DuPont 21-491 spectrometers. CD spectra were taken in MeOH on a Durrum-Jasco ORD/UV-5 spectropolarimeter with Sproul Scientific SS-20 modification, and specific rotations on a Perkin-Elmer 241 photoelectric polarimeter. Elemental analyses were by the Scandinavian Microanalytical Laboratory, Herley, Denmark. Silica gel G (E. Merck) was used for TLC with H_2SO_4–Et_2O (1:4) as a spray reagent followed by heating, or by 0.3% aqueous $KMnO_4$.

Isolation of Peroxyferolide (1). The dried powdered leaves of *Liriodendron tulipifera* L. were percolated with EtOH. The residue remaining after removal of the solvent at reduced pressure was partitioned and the 10% aqueous MeOH fraction was chromatographed on silicic acid as already described.[5] The column fraction (285 mg) preceding lipiferolide (**2**) was rechromatographed on a column of 17 g of silica gel G (E. Merck) prepared from TLC grade adsorbent by powdering the dried cake as prepared for plate pouring and sieved through a 50 mesh screen. Elution with 8% EtOH in $CHCl_3$ and collection of 5-mL fractions gave in fractions 16–23 one-spot material, R_f 0.17 on TLC with the same system as used in the column. The residue (101 mg) was crystallized from EtOH–$CHCl_3$ to give colorless needles (45 mg, 0.02% from dried leaves) of peroxyferolide (1): mp 190 °C softens and then decomposes gradually on further heating up to 300 °C without melting; $[\alpha]^{22}_D$ +30° (*c* 0.30, MeOH); CD curve $[\theta]_{257}$ −2000 and $[\theta]_{214}$ −31 300; UV end absorption (ϵ_{215} 9000); IR ($CHCl_3$) bands at 3515 and 3370 (OH), 1770 (lactone C=O), 1740 (acetate C=O), 1665 and 1640 (C=C), and 1210–1250 (C–O) cm^{-1}; chemical ionization mass spectrum (isobutane) *m/e* 339 (32%, MH$^+$, $C_{17}H_{22}O_7$ requires 338), 323 (9, MH − O), 321 (24, MH − H_2O), 305 (6, MH − H_2O_2), 279 (43, MH − AcOH), 263 (72, MH − O − AcOH), 261 (100, MH − H_2O − AcOH), and 245 (72, MH − H_2O_2 − AcOH).

Anal. Calcd for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55. Found: C, 60.26; H, 6.60.

Pyrazoline of Peroxyferolide (1). A 40-mg sample of 1 in 5 mL of $CHCl_3$ was treated overnight with 24 mL of Et_2O containing ~0.1 g of diazomethane at 5 °C. Removal of the solvent and crystallization of the residue several times from Me_2CO–Et_2O gave colorless cubes, mp 177–178 °C (d, with effervescence), which decomposed rapidly on handling and storage. The NMR spectrum (Pyr-d_5, 60 MHz) contained changes expected for a pyrazoline derivative, e.g., loss of the H-13 doublets, simplification of the H-7 pattern to a pair of doublets (3.85 ppm, J = 2, 10 Hz), and a large downfield shift of the H-6 triplet from 4.51 to 5.45 ppm (J = 10 Hz), which requires the diazene group to be placed α.[18]

Attempted Acetylation of Peroxyferolide (1). (A) By Ac_2O/Pyr. A 50-mg sample of 1 was dissolved in 20 mL of Ac_2O and 1 mL of Pyr at room temperature. The following day ice and 10 mL of 1% $NaHCO_3$ were added. The mixture was extracted with $CHCl_3$ and the extract washed with dilute HCl, $NaHCO_3$, and H_2O. The $CHCl_3$ residue was crystallized from $CHCl_3$–*i*-Pr_2O and Et_2O–EtOH to yield fine needles of anhydroperoxyferolide (**3**): mp 157–158 °C; R_f 0.58 on TLC with Me_2CO–$CHCl_3$ (1:3); $[\alpha]^{22}_D$ −24° (*c* 0.50, MeOH); UV λ_{max} 323 nm (ϵ 30) and 212 (17 000); IR ($CHCl_3$) no OH bands but peaks at 1770 (lactone), 1740 (acetate), 1685 and 1670 cm^{-1} (unsaturated C=O); MS (EI) *m/e* 320 (0.2%, M$^+$, $C_{17}H_{20}O_6$ requires 320), 278 (0.4, M − CH_2CO), 277 (0.9, M − Ac), 260 (0.9, M − AcOH), and 43 (100, Ac).

Anal. Calcd for $C_{17}H_{20}O_6$: C, 63.74; H, 6.29. Found: C, 63.74; H, 6.42.

(B) By Acetylimidazole. Peroxyferolide (**1**, 10 mg) and 5 mg of acetylimidazole in 2 mL of $CHCl_3$ were refluxed for 2 h and then diluted with 18 mL of $CHCl_3$ and washed with H_2O. Chromatography of the reaction residue on 5 g of silica gel with 5% EtOH in $CHCl_3$ removed the imidazole, and the 6 mg of effluent residue was crystallized from *i*-Pr_2O–EtOH to give **3** identical with the product obtained with Ac_2O/Pyr.

Methylation of Peroxyferolide (1) to 5. A 50-mg sample of 1 in $CHCl_3$ was stirred with 270 mg of MeI and 250 mg of Ag_2O for 16 h at room temperature. The mixture was filtered and the colorless filtrate on evaporation left a residue that was crystallized from EtOH–Et_2O to give **5** as colorless needles: mp 175–176 °C; R_f 0.61 on TLC with 8% EtOH in $CHCl_3$; $[\alpha]^{22}_D$ +30.2° (*c* 0.43, MeOH); IR no OH bands, 1778 (lactone), 1742 (acetate), 1667 and 1643 cm^{-1} (olefins); chemical ionization MS (NH_3) *m/e* 370 (100%, MNH_4^+, $C_{18}H_{24}O_7$ requires 352), 353 (2, MH), 340 (10, MNH_4 − CH_2O), 338 (7, MNH_4 − MeOH), and 310 (3, MNH_4 − AcOH), and with isobutane *m/e* 353 (15, MH$^+$), 305 (4, MH − MeOOH), 293 (67, MH − AcOH), 262 (64, MH − AcOH − MeO), and 245 (100, MH − AcOH − MeOOH), but electron impact MS gave no useful spectrum.

Anal. Calcd for $C_{18}H_{24}O_7$: C, 61.35; H, 6.86. Found: C, 60.95; H, 6.89.

$NaBH_4$ Reduction of Peroxyferolide (1). A 350-mg sample of 1 in 45 mL of absolute EtOH was treated with 80 mg of $NaBH_4$ for 20 min. The mixture was neutralized with dilute HOAc and evaporated to dryness, and the residue was mixed with water and extracted with $CHCl_3$. The chloroform solubles were chromatographed on 14 g of silica gel with Me_2CO–$CHCl_3$ (1:3) and the effluent residue (200 mg) was crystallized to give **6** from Et_2O–$CHCl_3$, mp 165–166 °C, or from *i*-Pr_2O–MeOH, mp 132–133 °C: R_f 0.34 with 10% EtOH in $CHCl_3$; $[\alpha]^{22}_D$ −21.6° (*c* 0.51, MeOH); IR (KBr) 3450 (OH), 1765 (lactone), 1735 (acetate), and 1635 cm^{-1} (olefin); MS (CI, isobutane) *m/e* 325 (8%, MH, $C_{17}H_{24}O_6$ requires 324), 307 (3, MH − H_2O), 265 (100, MH − AcOH), and 247 (24, MH − H_2O − AcOH).

Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.81; H, 7.49.

Elution of the column with Me_2CO–$CHCl_3$ (2:3) gave 28 mg of a minor polar compound **7** that was crystallized from *i*-Pr_2O–EtOH: mp 101–102 °C; R_f 0.24 on TLC with Me_2CO–$CHCl_3$ (2:3); $[\alpha]^{25}_D$ −30.9° (*c* 0.55, MeOH); IR (KBr) 3200–3350 (OH), 1745 (lactone), and 1625 (olefin); MS (CI, isobutane) 283 (33%, MH, $C_{15}H_{22}O_5$ requires 282), 265 (100, MH − H_2O), and 247 (17, MH − $2H_2O$).

Anal. Calcd for $C_{15}H_{22}O_5 \cdot {}^2/_3H_2O$: C, 61.20; H, 7.99. Found: C, 60.97; H, 8.05.

Acetylation of 7 with Ac_2O/Pyr under the usual conditions produced diacetate 8.

$NaBD_4$ Reduction of Peroxyferolide (1). Compound 1 (90 mg) in 12 mL of EtOD was treated with 25 mg of $NaBD_4$ for 30 min. After the usual workup including chromatography, the product was recrystallized several times from Et_2O–$CHCl_3$, mp 165–166 °C. ^1H NMR ($CDCl_3$) showed loss of one proton between 2.3 and 2.8 ppm and the 3-proton doublet at 1.28 of 6 changed to a 2-proton broadened singlet at δ 1.26; MS (CI, isobutane) *m/e* 327 (21%, MH^+, $C_{17}H_{22}D_2O_6$ requires 326), 325 (0), 309 (5, MH − H_2O), 267 (100, MH − AcOH), and 249 (25, MH − H_2O − AcOH).

Acetylation of Dihydrodeoxyperoxyferolide (6). A 50-mg sample of 6 was dissolved in 3 mL each of Ac_2O and Pyr at room temperature. The next day ice was added and the mixture extracted with $CHCl_3$. The extract was washed with dilute H_2SO_4, $NaHCO_3$, H_2O. The oily $CHCl_3$ residue was crystallized from Et_2O–EtOH to give 35 mg of 8: mp 121–122 °C; $[\alpha]^{22}_D$ +35.7° (*c* 0.42, MeOH); IR ($CHCl_3$) 1775 (lactone), 1730 and 1735 (acetate), and 1640 (olefin); MS (EI) no M^+ peak at 366, *m/e* 306 (3%, M − AcOH) and 246 (2, M − 2AcOH).

Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.04; H, 7.22.

Ozonolysis of 6 to 9. A stream of 3% O_3 in O_2 was bubbled through 5 mL of AcOH containing 40 mg of 6 at ~10 °C for 5 min. The reaction mixture was diluted with 50 mL of H_2O and distilled. The distillate (15 mL) was passed into 15 mL of cold saturated aqueous dimedone. After storing overnight in the cold, the crystalline precipitate, as needles (24 mg, mp 189–170 °C), was collected and found to give an undepressed mixture melting point with the dimedone derivative of formaldehyde.

The nonvolatile residue from the still was combined with material of a repeat ozonolysis and chromatographed on 5 g of silica gel with Me_2CO–$CHCl_3$ (1:4). The effluent material, TLC R_f 0.43 with Me_2CO–$CHCl_3$ (2:3), weighing 34 mg was crystallized from Et_2O–EtOH to give 22 mg of 9: mp 168–169 °C; $[\alpha]^{25}_D$ −45.8 (*c* 0.48, MeOH); UV λ_{max} 297 nm (ϵ 42) and end absorption ϵ_{210} 230; IR ($CHCl_3$) 3470 (OH), 1775 (lactone), 1740 (acetate), and 1710 (ketone); and positive tests with periodic acid reagent and 2,4-dinitrophenylhydrazine.

Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.87; H, 6.90.

Reduction of Peroxyferolide (1) to 10. A 200-mg sample of 1 in 25 mL of MeOH was stirred 1 h with 2 mL of 10% aqueous KI and then at 0 °C treated with 0.1 mL of AcOH for 1 h. The residue after evaporation of solvent was taken up in 25 mL of $CHCl_3$ and extracted successively with 5% $NaHCO_3$, 5% $Na_2S_2O_3$, and H_2O. The $CHCl_3$ residue was purified by preparative TLC on silica gel PF-254 with EtOH–$CHCl_3$ (1:19), R_f 0.74 after triple development. The band was eluted with MeOH–$CHCl_3$ (1:1) and the extract residue crystallized from *i*-Pr_2O–EtOH to give 148 mg of deoxyperoxyferolide (10): mp 169–171 °C; $[\alpha]^{22}_D$ +17° (*c* 0.30, MeOH); IR ($CHCl_3$) 3600 and 3500 (OH), 1770 (lactone), 1750 (acetate), 1670 (conjugated olefin), 1645 cm^{-1} (olefin); MS (EI) *m/e* 322 (33%, M^+, $C_{17}H_{22}O_6$ requires 322), 304 (14, M − H_2O), 280 (11, M − CH_2CO), 262 (28, M − AcOH), 244 (22, M − H_2O − AcOH), 127 (100) and 43 (74, Ac), and CI (isobutane) *m/e* 323 (15, MH^+), 305 (100, MH − H_2O) and 245 (5, MH − H_2O − AcOH).

Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.14; H, 6.81.

Acetylation of Deoxyperoxyferolide (10) to 11. A 100-mg sample of 10 was dissolved in 0.1 mL of Ac_2O and 0.1 mL of Pyr. After 6 h at room temperature, the reaction residue on reduced pressure evaporation was dissolved in 5 mL of $CHCl_3$ and extracted successively with 0.1 M HCl, 5% $NaHCO_3$, and H_2O. Recrystallization of the $CHCl_3$ residue from Et_2O–EtOH gave 88 mg of 11: mp 112–115 °C; $[\alpha]^{22}_D$ +52° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) 1780 (lactone) and 1740 cm^{-1} (double intensity, acetate); MS (EI) *m/e* 364 (2, M^+, $C_{19}H_{24}O_7$ requires 364), 322 (3, M − CH_2CO), 304 (30, M − AcOH), 262 (24, M − AcOH − CH_2CO), 244 (36, M − 2AcOH) and 43.

Anal. Calcd for $C_{19}H_{24}O_7$: C, 62.62; H, 6.64. Found: C, 62.24; H, 6.81.

Photooxygenation of Lipiferolide (2) to 1. 2 (102 mg, 0.33 mmol) and Methylene Blue (1.3 mg) were dissolved in 7 mL of absolute EtOH, placed in a U-shaped reaction tube fitted with a sintered glass frit at the bottom, and connected to an oxygen source in a closed system. Oxygen was circulated via the frit by a peristatic pump and measured manometrically. The reaction tube was placed into a 4-L silver-lined Dewar flask and 10 cm from a Sylvania DWY 650 W quartz-halogen lamp. Cooling water passed into the Dewar was maintained at 16 ± 1 °C. After 5 h, O_2 uptake ceased at 0.34 mmol and the crystalline precipitate (40 mg) of peroxyferolide (1) was collected. The filtrate was passed through a short silica gel (5 g) column to remove the dye, and the residue was combined with the crystals and recrystallized from EtOH–$CHCl_3$ to give 86 mg of 1. The product showed the same IR, UV, NMR, mass spectra, melting point characteristic, $[\alpha]_D$, and TLC mobility as peroxyferolide (1) from nature.

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