Peroxyferolide: a Cytotoxic Germacranolide Hydroperoxide from *Liriodendron tulipifera*

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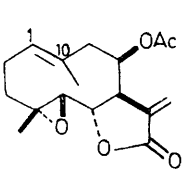
Summary An allyl hydroperoxy sesquiterpene lactone, peroxyferolide (**2**), from the leaves of *L. tulipifera* was characterized by spectral and chemical methods and prepared from lipiferolide (**1**) by photosensitized oxygenation.

THE crude ethanolic extract residue of the leaves of *L. tulipifera* L. (Magnoliaceae) possesses cytotoxic activity against Eagles' KB cells and antifeeding properties for the gypsy moth larvae (*Porthetria dispar* L.).† Two sesquiterpene lactones, lipiferolide (**1**) and epitulipinolide diepoxide [the 1,10-epoxide of lipiferolide (**1**)] were reported from this source.[1] Peroxyferolide‡ (**2**), $C_{17}H_{22}O_7$, m.p. 190 °C (decomp.), $[\alpha]_D^{20}$ + 20° (MeOH), isolated by chromatography on silicic acid, exhibited in the mass spectrum under chemical ionization conditions (isobutane) a peak at *m/e* 339 (36%) for (*M* + 1) and base peak at *m/e* 261 (*M* − H_2O − AcOH). The 90 MHz ^1H n.m.r. spectrum in [$^2H_6$]-acetone showed peaks at δ (rel. to Me_4Si) 4·37 (dd, *J* 4·4 and 9·9 Hz, H-1), 5·33 (1H, s br, H-14), 5·45 (1H, d br, *J* 2·4 Hz, H-14), and 10·6 (s br, hydroperoxide) along with characteristic peaks for other protons. The i.r. and u.v. spectra supported the acetate and αβ′-unsaturated γ-lactone components and extensive double irradiation n.m.r. experiments established the arrangement of the groups from C-5—C-8. In the ^{13}C n.m.r. spectrum 7 oxygenated carbon atoms were observed; two carbonyls, four singly protonated, and one unprotonated. The hydroperoxide carbon was located at δ (rel. to Me_4Si) 90·9 p.p.m. Thus, the seventh oxygen could not be bonded to a carbon atom, and led to consideration of a

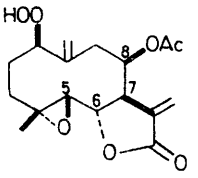
† The KB cell culture assay was obtained through the courtesy of the National Cancer Institute, N.I.H., following their protocol, and the insect antifeeding test was performed on third instar larvae according to unpublished results. Peroxyferolide showed an ED_{50} of 0·29 μg ml^{-1} and inhibited insect feeding only slightly (*ca.* 10%).

‡ Satisfactory elemental analyses and spectral data were obtained for all new compounds.

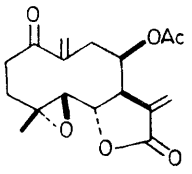
hydroperoxide, which was in accord with the elimination product obtained under acylation conditions.[2]



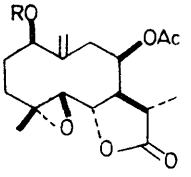
(1)



(2)



(3)



(4) R = H
(5) R = Ac

Peroxyferolide (**2**), ν_{OH} ($CHCl_3$) 3370 and 3510 cm^{-1}, with acetic anhydride and pyridine or with *N*-acetylimidazole yielded only anhydroperoxyferolide (**3**), $C_{17}H_{20}O_6$, m.p 157—158 °C, ν_{CO} ($CHCl_3$) 1670 cm^{-1} and λ_{max} (MeOH) 323 (log ϵ 1·48) and 212 (4·24) nm. Reduction of (**2**) with $NaBH_4$ gave the dihydrodeoxy-derivative (**4**), $C_{17}H_{24}O_6$, m.p. 165—166 °C, ν_{OH} (KBr) 3450 cm^{-1}, δ ($CDCl_3$, 60 MHz) 1·28 (d, *J* 6·2 Hz, H-13), 1·87 (s, OH exchangeable with D_2O), 2·13 (Ac), and 4·21 (dd, *J* 4·5 and 10 Hz, H-1 sharpened with D_2O), that gave the acetate (**5**), $C_{19}H_{26}O_7$, m.p. 121—122 °C, ν_{CO} ($CHCl_3$) 1735 cm^{-1} (double intensity), δ ($CDCl_3$) 2·01 (Ac) and 2·14 (Ac). These derivatives support the presence of an allylic hydroperoxide group. Preparation of (**2**) in good yield by photo-oxygenation[3] of (**1**) in ethanol by visible light and with Methylene Blue as sensitizer established the stereochemistry at C-1, since the conformation of (**1**) in solution is known[1] as is the stereochemistry of singlet oxygen addition. It follows that the remaining asymmetric centres of (**2**) were established by this conversion.

To our knowledge (**2**) is only the second hydroperoxide obtained from a natural source; the first, peroxy-Y base, a modified purine base from phenylalanine t-RNA, was isolated from animal and plant sources.[4] Since (**2**) has its origin in leaf material, it is probably produced by chlorophyll mediated singlet oxygen addition to (**1**), the major sesquiterpene lactone in this source, in a manner similar to the formation of ascaridole, the anthelmintic endoperoxide of Chenopodium oil.[5] Sesquiterpene lactones with exocyclic methylene allylic alcohols as artemorin[6] or ridentin[7] as well as the related $\alpha\beta$-unsaturated ketones may have arisen from the corresponding allylic hydroperoxides generated by sensitized photo-oxygenation.

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