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PEROXYCOSTUNOLIDE AND PEROXYPARTHENOLIDE: TWO CYTOTOXIC GERMACRANOLIDE HYDROPEROXIDES FROM MAGNOLIA GRANDIFLORA. STRUCTURAL REVISION OF VERLOTORIN AND ARTEMORIN.[1]

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As part of a screening program of Mississippi flora for biologically active compounds, the ethanolic extract of the leaves of Magnolia grandiflora L. (Magnoliaceae) yielded two novel cytotoxic germacranolide hydroperoxides, peroxycostunolide (1) and peroxyparthenolide (2).[2]

Peroxycostunolide (1) was isolated from the costunolide (3) column fraction by repeated chromatography, then crystallized from diethyl ether--abs. ethanol as colorless prisms; mp 141°(softens but decomp. temp. indeterminate) and $[\alpha]_D^{25}$ +171° (c, 0.20 acetone). The chemical ionization mass spectrum (i-butane) showed peaks at m/e 265 (12%, MH^+, $C_{15}H_{20}O_4$ requires 264), 249 (78, MH-O), 247 (60, MH-H_2O) and 231 (100, MH-H_2O_2), while the IR spectrum (KBr) contained peaks at 3440 and 3350 (OH), 3090 (unconj. C=CH_2), 1745 (C=O) and 1660 cm^{-1} (C=C). The NMR spectrum (acetone-d_6) possessed absorption at δ 6.03 (d, J 3.2Hz, H-13c), 5.52 (d, 3.2, H-13t), 5.39 (br d, 8.9, H-5), 5.18 and 5.03 (split s, 1H each, H-14), 4.44 (t, 9.7, H-6), 4.04 (dd, 3.5 and 9, H-1), 1.70 (d, 1.3, Me-15), and 10.51 ppm (br s, OOH, D_2O exchanged). The spectral evidence was consistent with the presence of an α,β'-unsat. γ-lactone, an olefinic methyl and an allylic hydroperoxide[3] with an exocyclic methylene. Relevant stereochemical assignments were in accord with the coupling constants. The broad-band decoupled CMR spectrum (pyridine-d_5) contained fifteen peaks of which only three were assignable to oxygenated carbons and located at 170.4 (C-12), 80.9 (C-6) and 91.5 ppm (C-1), the latter uniquely characteristic of a hydroperoxide-bearing allylic carbon.[4] Further support for a hydroperoxide group was obtained from an intense ferrous thiocyanate test[5] and by ready liberation of I_2 from ethanolic KI solution. The functional groups, molecular formula and biogenetic consideration indicated a germacranolide of structure 1 as likely for peroxycostunolide. Conversion of 1 to ketone 4, mp 123-124° from ethanol--diethyl ether, $[\alpha]_D$ +143.7° (c, 0.16 chloroform) by acetic anhydride and pyridine was chemical evidence for an allylic hydroperoxide.[3]

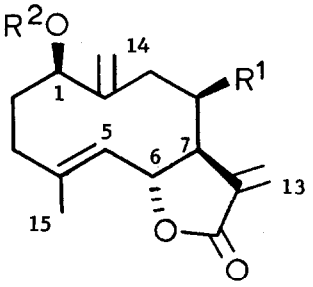
Reduction of peroxycostunolide (1) with triphenylphosphine formed the hydroxy compound 5: mp. 120-121°, $[\alpha]_D$ +89° (c, 0.10 chloroform), with chemical ionization (i-butane) mass spectral peaks at m/e 249 (23%, MH^+, $C_{15}H_{20}O_3$ requires 248) and 232 (100, MH-OH); and NMR (chloroform-d) peaks at δ 6.16 (d, J 3.5Hz, H-13c), 5.43 (d, 3.2, H-13t), 5.24 (br d, 10, H-5), 5.19 and 4.85 (s, 1H each, H-14), 4.39 (t, 9.9, H-6), 3.97 (br t, ~6, H-1) and 1.71 (d, 1.3, Me-15). The CMR spectrum (chloroform-d) showed the C-1 peak at 78.2 ppm, and the IR spectrum (chloroform) contained absorption at 3600 (OH), 1760 (C=O), 1670 and 1640 cm^{-1} (2 C=C). The changes in physical properties from 1 to 5 are in accord with a conversion of a hydroperoxy to a hydroxy compound.

Photooxygenation[6] of costunolide (3) in ethanol with visible light and methylene blue as sensitizer produced peroxycostunolide (1) in excellent yield, thereby confirming the absolute structure of 1 except for C-1 stereochemistry. Since the solution conformation of costunolide[7] has been determined to be as represented in 3, and the oxygen addition in the ene reaction occurs cis and perpendicular to the olefinic plane,[6] the hydroperoxy group in 1 must be placed β, with configuration at C-1 as R. It follows that the hydroxyl of 5 is also β.

Peroxyparthenolide (2) was isolated by repeated chromatography of the parthenolide (6) column fraction and crystallized from acetone--diethyl ether: mp 190 (d), $[\alpha]_D^{25}$ +27° (c, 0.21 acetone), with chemical ionization (ammonia--methane) mass spectral peaks at m/e 298 (86%, MNH_4^+, $C_{15}H_{20}O_5$ requires 280) and 282 (100, MNH_4-O); and NMR (acetone-d_6) peaks at δ 6.05 (d, J 3.5, H-13c), 5.60 (d, 3.2, H-13t), 5.41 and 5.28 (s, 1H each, H-14), 4.33 (dd, 4.2 and 10.5, H-1), 3.92 (t, 9.2, H-6), 3.37 (m, H-7), 2.93 (d, 8.9, H-5), 1.43 (s, Me-15) and 10.51 ppm (br s, OOH, D_2O exchanged). The IR spectrum (KBr) has absorption at 3440 and 3350 (OH), 3090 (unconj C=CH_2), 1748 (C=O) and 1660 cm^{-1} (C=C). From the physical data structure 2 was suggested and preparation of peroxyparthenolide (2) by methylene blue mediated photooxygenation of parthenolide (6)[8] confirmed the assignment. This includes the stereochemistry, since parthenolide (6) and costunolide (3) as the 11,13-dihydro derivatives have been interrelated by conversion to a common diepoxide;[9] and by a recent X-ray analysis on parthenolide (6) itself.[10]

On examining the properties of hydroxy lactone 5 with those published for artemorin (7),[11] it appears that the substances are identical. Furthermore, verlotorin[11] with proposed structure 8 has properties very similar to those of peroxycostunolide (1). Comparison of the published spectral data, melting point characteristics, tlc mobility, color formation in strong acid and chemical properties, confirmed the identity of artemorin with compound 5, and verlotorin with peroxycostunolide (1).[12] Ketone 4 was found to be identical with anhydroverlotorin[13] by similar comparison. Henceforth, by deferring to established practice, we will refer to these compounds by their earlier names.

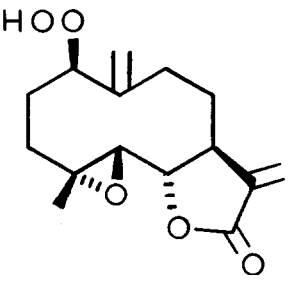
There are to date three naturally occurring sesquiterpene hydroperoxides; the other, peroxyferolide (9),[3] was also obtained from a Magnoliaceae. Verlotorin (1) was initially isolated from Artemisia verlotorum Lamotte a Composite, and suggests that the hydroperoxides, of yet uncertain origin, may have wide distribution.



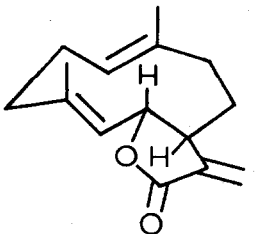
1; $R^1 = H$, $R^2 = OH$

5; $R^1 = R^2 = H$

9; $R^1 = OAc$, $R^2 = OH$



2



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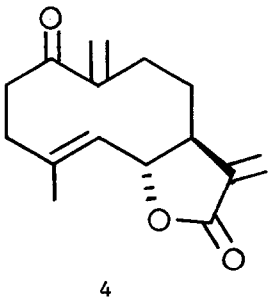
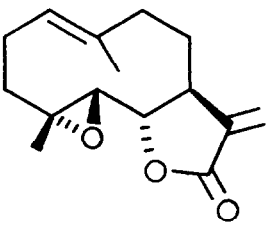
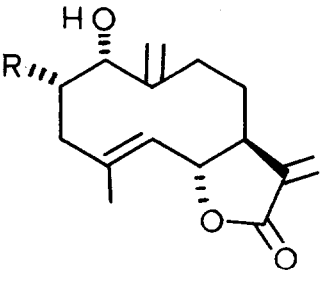


4



6



7; R = H

8; R = OH

REFERENCES AND FOOTNOTES

1. This study was supported in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi. All new compounds showed satisfactory elemental composition by mass spectrometry or elemental analysis. NMR spectra were taken at 90 MHz for protons and 22.63 MHz for carbon in stated solvents with TMS as internal standard.

2. Cytotoxic activity was determined against Eagle's KB cells through the courtesy of the National Cancer Institute, N.I.H., following their protocol. Peroxycostunolide (1) and peroxyparthenolide (2) showed activities of ED50 2.7 μg/ml and 2.8 μg/ml, respectively.

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13. T. A. Geissman and K. H. Lee, Phytochemistry 10, 419 (1971).