A Novel Tricyclic Peroxide from the Photochemical Oxidation of Plastoquinone-1 [2,3-Dimethyl-5-(3-methylbut-2-enyl)-1,4-benzoquinone]

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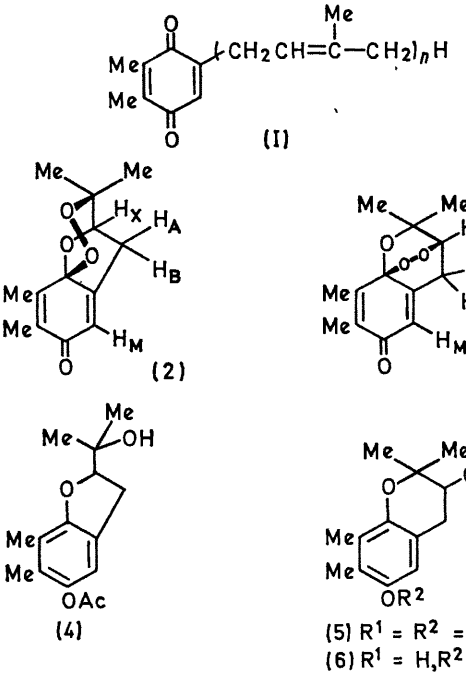
Summary Photochemical oxidation of plastoquinone-1, a model for the electron-transport quinones of plants, gives rise to the novel tricyclic peroxide, 4,5-dihydro-3,3,8,9-tetramethyl-4,9a-epoxy-9a*H*-1,2-benzodioxepin-7(3*H*)-one.

WE report the isolation and characterization of a novel tricyclic quinone peroxide from the photochemical oxidation of plastoquinone-1 [PQ-1; (**1**), $n = 1$]. This quinone serves as a model for the naturally occurring electron-transport quinone plastoquinone-9 [PQ-9; (**1**), $n = 9$], which is thought to function as an electron-carrier[1] in the subcellular photosynthetic organelles (chloroplasts) of plants. The *in vitro* photochemistry of electron-transport quinones such as PQ-9 is of great interest in view of the biological effects that have been attributed[2,3] to *in vivo* photochemical modification of these quinones.

Far-u.v. irradiation of chloroplasts results in loss of PQ-9 and disruption of several photosynthetic functions,[4] and u.v. irradiation of solid PQ-9 gives rise[5] to a dimer, also isolated from horse-chestnut leaves. We have subjected PQ-1,[6] to near-u.v. radiation (λ *ca.* 370 nm) under a variety of experimental conditions. Following aerobic irradiation of PQ-1 in benzene or isopropyl alcohol for 15 hr., the major photoproduct, not observed in a dark control reaction, was isolated (20—45%) by t.l.c. on silica gel. It was recrystallized from hexane–benzene as white crystals m.p. 153—155°; λ_{max} (EtOH) 234 nm (ϵ 10,250); ν_{max} (Nujol) 1685, 1640 cm^{-1}. The molecular formula, $C_{13}H_{16}O_4$, (by mass spectroscopy† and elemental analysis), and the positive reaction to a starch–iodide test, indicated that the photoproduct was a peroxide of PQ-1 engendered by photochemical O_2 addition: n.m.r. spectrum ($CDCl_3$) δ 1·12 and 1·70 (2s, each 3H, *gem*-dimethyls), 1·87 and 2·07 (2q, each 3H, $J_{homoallylic}$ 1·3, vinylic methyls), 2·77 (octet H_A, J_{AB} 18, J_{AX} 6, J_{AM} 2), 3·10 (ill-defined octet, H_B, J_{BA} 18, J_{BX} 1·2, J_{BM} 2), 4·30 (q, H_X, J_{XA} 6, J_{XB} 1·2), and 6·12 (t, H_M, $J_{MA} = J_{MB} = 2$c./sec). Structures (**2**) and (**3**) are consistent with these data.

Examination of molecular models of (**2**) and (**3**) indicated, on the basis of the dihedral angles between H_A, H_B, and H_X, that J_{AX} should be much larger[7] than J_{BX} for (**2**) whereas J_{AX} and J_{BX} should be almost equal for (**3**). These considerations supported structure (**2**), and this assignment was confirmed when the product of $NaBH_4$ reduction of the quinone peroxide, after acetylation with pyridine–Ac_2O (room temp., overnight or 30 min., 100°), was identified as the dihydrobenzofuran (**4**) rather than the chromanyl acetate (**5**). Cyclic secondary alcohols with the same *gem*-dimethyl substitution pattern as (**6**) are acetylated[8] under these conditions whereas tertiary alcohols are not.[9] The acetylated material was isolated as a colourless oil, λ_{max} (EtOH), 283 (ϵ 3000), 289 nm (3100); ν_{max} (film), 3450, (OH stretch), 1760 cm^{-1} (acetate C=O): *m/e* 264 (M^+) *m/e* 59 ($C_3H_7O^+$): n.m.r. ($CDCl_3$) δ 1·20 and 1·33 (2s, each 3H, *gem*-dimethyls), 2·01 and 2·14 (2s each 3H, ring methyls), 2·29 (s, 3H, $COCH_3$), 3·12 (br d, 2H, J 9c./sec, $-CH_2-$), 4·57 (t, 1H, J 9 c./sec, methine), and 6·66 (s, 1H, aromatic H). The signal due to the tertiary hydroxy-proton is observed in $(CD_3)_2SO$ as a sharp singlet, disappearing with D_2O, at δ 4·52.



These data are in complete accord with structure (**4**). There is no splitting of the signal due to the hydroxy-proton as would be expected for a secondary alcohol[10] such as (**6**). The protons of the dihydrobenzofuran ring give rise to a splitting pattern and coupling constants comparable to those observed for the dihydrobenzofuran columbianetin[11] and the dihydrobenzofuran obtained by mild oxidative cyclization of colupulone.[12]

The formation of the peroxide (**2**) from plastoquinone-1 contrasts with the recently reported[13] formation of hydroperoxides by near-u.v. irradiation of menaquinones. These *in vitro* photo-oxidations and the observation of

† 70 ev Spectrum: parent peak at *m/e* 236 (27% of base peak). Base peak at 189. Major peak at 204 (62%), corresponding to loss of O_2 from parent peak.

photochemically induced lipid peroxidation in both mitochondria[14] and isolated chloroplasts,[15] suggest that photooxidations of electron-transport quinones may also occur *in vivo*.

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[1] M. Avron in "Current Topics in Bioenergetics," ed. D. R. Sanadi, Academic Press, New York, 1967, vol. 2, p. 1. An alternative view of the photosynthetic process is described by A. Arnon in "Biological Oxidations," ed. T. P. Singer, Interscience, New York, 1968, p. 123.
[2] H. Werbin and E. T. Strom, *J. Amer. Chem. Soc.*, 1968, **90**, 7296.
[3] A. F. Brodie in "Biochemistry of Quinones," ed. R. A. Morton, Academic Press, New York, 1965, p. 384; J. Jagger, *Photochem. and Photobiol.*, 1964, **3**, 451.
[4] K. E. Mantai and N. I. Bishop, *Biochim. Biophys. Acta*, 1967, **131**, 350; N. Shavit and M. Avron, *ibid.*, 1963, **66**, 187; A. Trebst and E. Pistorious, *Z. Naturforsch*, 1965, **20b**, 885.
[5] V. H. Eck and A. Trebst, *Z. Naturforsch*, 1963, **18b**, 446.
[6] P. M. Scott, *J. Biol. Chem.*, 1965, **240**, 1374.
[7] S. Sternhell, *Quart. Rev.*, 1969, **23**, 236, and references cited therein.
[8] R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1957, 1131.
[9] L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, 1967, p. 958.
[10] O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.*, 1964, **86**, 1256.
[11] Spectrum No. 310 in "High Resolution N.M.R. Spectra Catalogue," Varian Associates, Palo Alto, California, 1962.
[12] D. N. Cahill and P. V. R. Shannon, *J. Chem. Soc.* (*C*), 1969, 936.
[13] C. D. Snyder and H. Rapoport, *J. Amer. Chem. Soc.*, 1969, **91**, 731.
[14] A. Ottolenghi, F. Bernheim, and K. M. Wilbur, *Arch. Biochem. Biophys.*, 1955, **56**, 157.
[15] R. L. Heath and L. Packer, *Arch. Biochem. Biophys.*, 1968, **125**, 189.