Synthesis and Properties of a Bis[(porphyrin-2-yl)methyl] Ether, a Model for the Oligomers in Haematoporphyrin Derivative (HpD)

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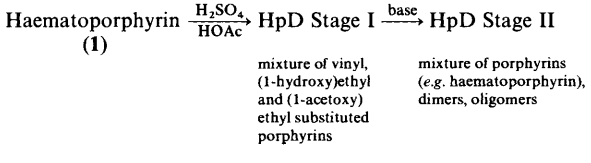
A model bis[(porphyrin-2-yl)methyl] ether (**3**) has been prepared and its spectroscopic and chemical properties have been examined in relation to those of the tumour photosensitiser, haematoporphyrin derivative.

Haematoporphyrin derivative (HpD) has the remarkable property of localising with some degree of selectivity in tumour tissue, and there is considerable current interest in its application both to the diagnosis and to the phototherapy of cancer.[1] HpD is prepared in two stages. Haematoporphyrin (**1**), generally as its dihydrochloride, is treated with 5% sulphuric acid in acetic acid[2] to give a purple amorphous solid (Stage I) which is a mixture of acetylation and elimination products [haematoporphyrin diacetate (**2**) is the main component].[3] This solid is then treated with base, and brought back to neutrality before injection (HpD Stage II). During this process hydrolysis and



elimination reactions of the pseudo-benzylic acetate functions occur to give porphyrin monomers [haematoporphyrin, hydroxyethyl(vinyl) deuteroporphyrin and protoporphyrin] but at the same time a higher molecular weight fraction is

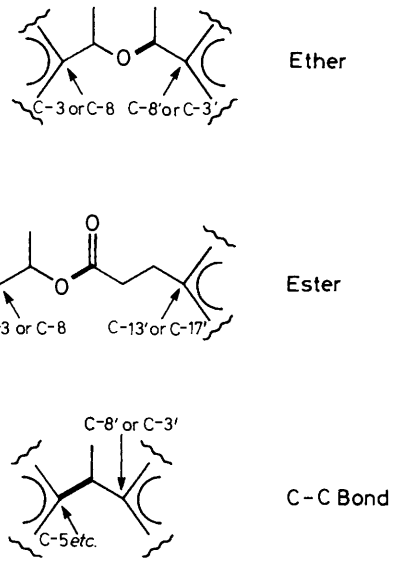
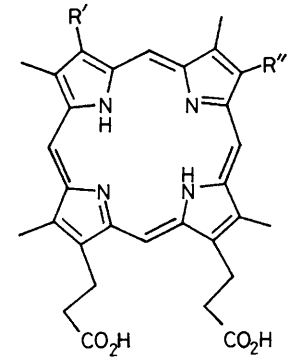


Figure 1. Postulated internuclear linkages in haematoporphyrin derivative (Stage II).

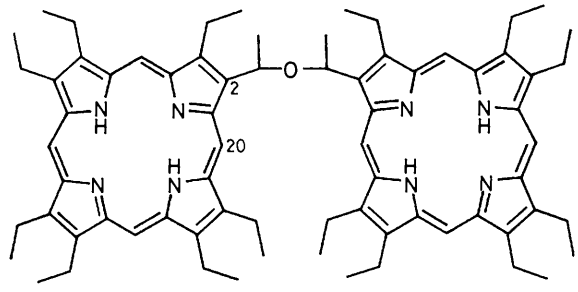
generated. We proposed that this material was dimeric or oligomeric involving ether, ester or *meso*-carbon-benzylic carbon bonds between porphyrin rings (Figure 1).[4,5] The first two of these suggestions have attracted support. The ether structure has been favoured by some workers,[6,7] while others have favoured the ester structure or a mixed ether-ester.[8] Some evidence exists for the initial formation of an ester with subsequent conversion into an ether.[9] While it seems to us that the ether structure is the most likely, it is being discussed in the absence of information about the chemistry of the basic system, which is a rather unusual one. Earlier syntheses have been directed towards multi-functional systems which possess biological activity.[10] We have now prepared the model bis-[(porphyrin-2-yl)methyl] ether (**3**): this is not expected to be effective in the *in vivo* photonecrosis assay but allows the chemistry of the system to be defined without interference from other functional groups.

Treatment of the dihydroxychlorin (**4**)[11] with hydrogen chloride in aqueous dioxane[12] gave in 62% yield the benzylic alcohol (**5**), which on reaction with 50% HBr/HOAc[13] in CH_2Cl_2 gave the corresponding bromide (**6**). (This bromide could alternatively be made from heptaethyl-2-vinylporphyrin[14] using the same reagent.) The bromide is a sensitive compound: it was not purified but, after removal of reagent and solvent under reduced pressure was treated in dichloromethane under nitrogen with the alcohol (**5**) in the presence of pyridine (25 °C, 2 h) to give 31% of the bis[(porphyrin-2-yl)methyl] ether (**3**) as reddish brown crystals, mp 268–271 °C, from chloroform–methanol after column chromatography.

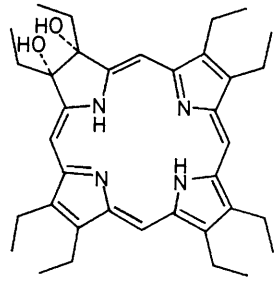
The mass spectrum (FAB) showed a strong molecular ion at 1 083.732 ($C_{72}H_{90}N_8O$ + H requires 1 083.732). The electronic spectrum in chloroform was similar in so far as λ_{max} values were concerned to that of the alcohol (**5**): the molecular extinctions of (**3**) were greater than those of (**5**), but the increase was less than two-fold [(**3**) λ_{max} 400 nm, ε 232 000; (**5**) λ_{max} 400



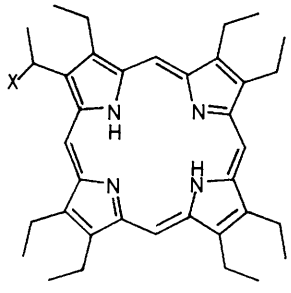
(**1**) R′ = R″ = -CH(OH)Me
(**2**) R′ = R″ = -CH(OAc)Me



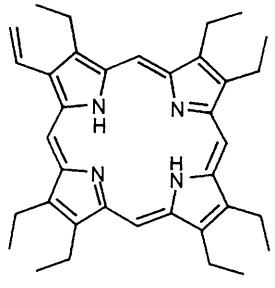
(**3**)



(**4**)



(**5**) X = OH
(**6**) X = Br
(**8**) X = OMe
(**9**) X = OAc



(**7**)

Scheme.

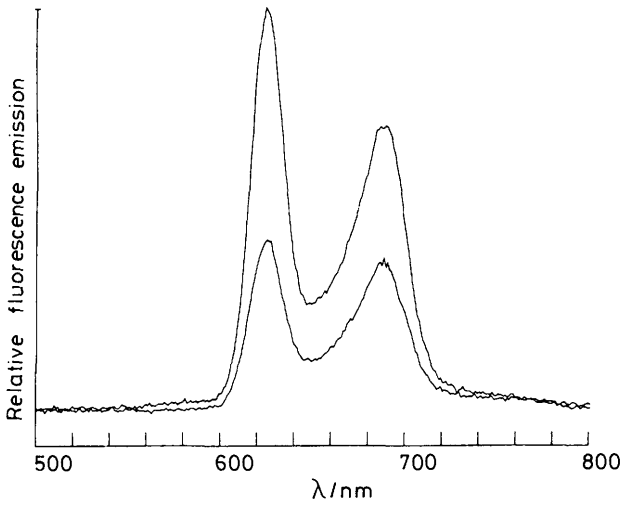


Figure 2. Relative fluorescence emission from the alcohol (**5**) (upper line) and the dimer (**3**) (lower line) at 2.5×10^{-5}M in ethanol-free chloroform: excitation wavelength 400 nm.

nm, ε 148 000]. At concentrations of 2.5×10^{-5}M the fluorescence emission for the dimer was less than that for the alcohol (Figure 2) but this difference disappeared at lower concentrations (*ca.* 10^{-6}M). This leads us to the view that aggregation, with attendant concentration quenching effects, persists to greater dilutions for the dimer (**3**) than for the alcohol (**5**).

The NMR spectra of the alcohol (**5**) and the dimer (**3**) showed considerable differences. In acidic media ($CDCl_3$–CF_3CO_2D) the C-20 proton of (**5**) was shifted downfield (δ 11.29) of the other *meso* signals (two singlets, 1 × 2 H, 1 × 1 H, in the δ 10.6 region): for the dimer (**3**) this signal (now due to C-20 and C-20′) was a sharp singlet which had been further shifted downfield to δ 11.86, although the other *meso*-protons (now resolved into three sharp singlets: 10.675, 10.64, 10.60) remained in the δ 10.6 region. On the basis of the inspection of models, we attribute the extra deshielding observed for the C-20 and C-20′ protons of (**3**) to the effect of the ring current of the *other* ring. In principle, (**3**) should consist of a mixture of diastereoisomers. We have not yet detected evidence for such isomers; attempted HPLC separations have given single sharp peaks.

The chemical reactions of the ether (**3**) may be summarised as follows. It is readily handled without decomposition, and does not suffer rapid autoxidation in solution in the dark. On pyrolysis it eliminates water to give the vinyl derivative (**7**) cleanly. It is unchanged by sodium borohydride or by lithium aluminium hydride (tetrahydrofuran, reflux). Resistance to the latter reagent as a distinction[15,16] between ethers and esters in this series (see Scheme) is thus validated. However the ether (**3**) is readily hydrogenolysed to give octaethylporphyrin and the 1-hydroxyethyl derivative (**5**) on catalytic hydrogenation over 10% palladium on charcoal. The ether is unaffected by base (*e.g.* NaOH in aqueous THF–Me_2CO) and by 6M HCl in aqueous methanol (1:1) at room temperature. On refluxing in 1M HCl–MeOH cleavage occurs to give the methoxy derivative (**8**) as the major product. At room temperature acetic acid [containing 30% CH_2Cl_2] does not affect the ether, but 50% HBr–HOAc causes rapid cleavage: quenching with methanol results in clean conversion into the methoxy derivative (**8**), while work-up with triethylamine, or with aqueous sodium acetate, gives the 1-acetoxyethyl derivative (**9**).

Experimental

Bis[1-(3,7,8,12,13,17,18-*heptaethylporphyrin*-2-*yl*)*ethyl*]-*ether* (**3**).—3,7,8,12,13,17,18-Heptaethyl-2-(1-hydroxyethyl)-porphyrin (**5**) (30 mg, 0.05 mmol) was treated in darkness for 12 h with freshly prepared 50% hydrogen bromide in glacial acetic acid (6 ml) and dry dichloromethane (6 ml). The solvent was removed under high vacuum to give the intermediate bromide (**6**) which was not isolated but dissolved *immediately* in dichloromethane (5 ml) and added to solid compound (**5**) (30 mg, 0.05 mmol). The solution was then stirred at room temperature in darkness for 2 h, after which time pyridine (1 ml) was added and stirring was continued for a further 15 min. The solvent was removed under high vacuum and the residue was flash chromatographed on silica, eluting with chloroform. Fractions containing the ether were evaporated and crystallised from chloroform–methanol to give reddish brown microprisms (18 mg, 31%), m.p. 268–271 °C (Found $[M + H]^+$ 1 083.7316. $C_{72}H_{91}N_8O$ requires 1 083.7316); $\lambda_{max}(CHCl_3)$ 400 (ε 232 000), 499 (20 000), 535 (16 200), 566 (11 200), and 618 nm (5 800); δ_H(250 MHz, $CDCl_3$) 10.78 (s, 2 H, *meso*-H), 10.10 (s, 2 H, *meso*-H), 9.89 (s, 2 H, *meso*-H), 9.87 (s, 2 H, *meso*-H), 6.74 (q, *J* 6 Hz, 2 H, $OCHCH_3$), 4.28–3.92 (m, 16 H, CH_2CH_3), 3.84–3.36 (m, 8 H, CH_2CH_3), 2.85 (br m, 4 H, CH_2CH_3), 2.58 (d, 3 H, *J* 6 Hz, $OCHCH_3$), 2.05–1.74 (m, 21 H, CH_2CH_3), 1.70 (m, 15 H, CH_2CH_3), 0.88 (m, 6 H, CH_2CH_3), and −3.80 (br s, 4 H, NH).

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References

1 For review of current activity see: 'Photosensitising Compounds: Their Chemistry, Biology and Clinical Use,' eds. G. Bock and S. Harnett, Ciba Foundation Symposium, 1989, **146**, in the press.
2 R. L. Lipson and E. J. Baldes, *Arch. Dermatol.*, 1960, **82**, 508.
3 R. Bonnett, R. J. Ridge, P. A. Scourides, and M. C. Berenbaum, *J. Chem. Soc., Chem. Commun.*, 1980, 1198.
4 R. Bonnett and M. C. Berenbaum, Porphyrin Photosensitisation Workshop, Washington, 1981 published as *Adv. Exptl. Med. Biol.*, 1983, **160**, 241.
5 M. C. Berenbaum, R. Bonnett, and P. A. Scourides, *Br. J. Cancer*, 1982, **45**, 571.
6 T. J. Dougherty, W. R. Potter, and K. R. Weishaupt, *Adv. Exp. Med. Biol.*, 1984, **170**, 301.
7 P. A. Scourides, R. M. Bohmer, A. H. Kaye, and G. Morstyn, *Cancer Res.*, 1987, **47**, 3439.
8 D. Kessel, *Photochem. Photobiol.*, 1986, **42**, 193.
9 C. J. Byrne, L. V. Marshallsay, and A. D. Ward, *Photochem Photobiol.*, 1987, **46**, 575.
10 R. K. Pandey and T. J. Dougherty, *Photochem. Photobiol*, 1988, **47**, 769.
11 K. R. Adams, Ph.D. Thesis, London, 1969.
12 C. K. Chang and C. Sotiriou, *J. Org. Chem.*, 1987, **52**, 926.
13 G. D. Mikhailov, V. A. Zubtsov, T. I. Samsonova, A. N. Nizhnik, and A. F. Mironov, *Otkrytiya Izobret.*, 1988, **3**, 82 [*Chem. Abstr.*, 1988, **108**, 134380].
14 R. Bonnett, P. Cornell, and A. F. McDonagh, *J. Chem. Soc., Perkin Trans. 1*, 1976, 794.
15 D. Kessel, P. Thompson, B. Musselman, and C. K. Chang, *Cancer Res.* 1987, **47**, 4642.
16 T. J. Dougherty, *Photochem. Photobiol.*, 1987, 569.

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