Second Generation Tumour Photosensitisers: the Synthesis of Octa-alkyl Chlorins and Bacteriochlorins with Graded Amphiphilic Character

Raymond Bonnett,[a]* Alexander N. Nizhnik,[a] and Morris C. Berenbaum[b]

[a] *Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS, U.K.*
[b] *Department of Experimental Pathology, St. Mary's Hospital Medical School, London W2 1PG, U.K.*

Two series of polyhydroxy derivatives of hydroporphyrins are synthesised which have the hydroxy substituents at β-positions, or in a side chain: these amphiphilic systems are tested *in vivo* as tumour photosensitisers, and some are found to be very effective.

The photosensitiser used almost universally in clinical research in tumour phototherapy is haematoporphyrin derivative (HpD) or a commercial variant of it such as Photofrin II.[1] HpD is prepared by treating haematoporphyrin with 5% sulphuric acid in acetic acid, followed by base treatment of the resulting solid, and is a complex mixture, the active components of which are thought to be oligomers. A single highly active substance has not been isolated, and this, combined with the rather low selectivity for tumour tissue shown by HpD, has resulted in a search for second-generation sensitisers which are more effective and which are single substances.[1]

Previous studies led us to conclude that porphyrins with amphiphilic properties (*e.g.* as conferred by polar substituents such as hydroxy) are advantageous in this regard. Absorption in the red region is also an advantage since tissue penetration by red light is greater than by blue light.[2] Thus we have recently shown that tetra(*m*-hydroxyphenyl)porphyrin is about 25 times more effective than is HpD in tumour photonecrosis (and also shows better selectivity),[3] and that biological effectiveness is further enhanced on going to the corresponding chlorin and bacteriochlorin systems.[4] Here we outline approaches, illustrated for the octaethyl system, to the synthesis of hydroporphyrins which allow the number of hydroxy substituents to be varied.

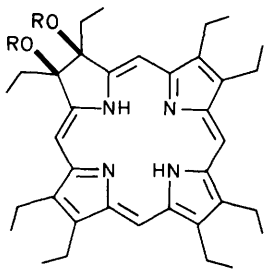
Treatment of octaethylporphyrin with osmium tetraoxide (1.6 mol) gave the 2,3-dihydroxychlorin[5] [(**1**), λ_{max} ($CHCl_3$) 643 nm, ε 41800] with a minor amount of the 7,8,17,18-tetrahydroxybacteriochlorin [(**2**), λ_{max} ($CHCl_3$) 715 nm, ε 53000]. The relative geometry of the opposite rings in the bacteriochlorin remains to be determined. The stereochemistry of (**1**) was demonstrated by the *X*-ray structure[6] of the corresponding cyclic osmate which was isolated as a highly crystalline bispyridine complex (**3**).

Pinacol rearrangement of the dihydroxychlorin (**1**) with fuming sulphuric acid[7] gave 75% of the β-oxochlorin (**4**),[8] borohydride reduction of which gave (87%) the hydroxy chlorin [(**5**), λ_{max} ($CHCl_3$) 642 nm, ε 43700].[9]

The hydroxychlorin (**5**) is a key intermediate in which the chromophoric system is protected against dehydrogenation by the geminal β-substitution at C-3; a similar comment applies to the dihydroxybacteriochlorins derived analogously from (**2**). Osmylation of the β-oxochlorin (**4**) gave the 7-oxo-17,18-dihydroxybacteriochlorin [(**6**), λ_{max} ($CHCl_3$) 693 nm, ε 50900]. Borohydride reduction of (**6**) gave the corresponding trihydroxybacteriochlorin (**7**); this was not obtained as a single substance, and is the only one of the hydroxy compounds described here which is not fully characterised.

The hydroxychlorin (**5**) was kept with 50% HBr–HOAc for 1h at 20 °C, and the solvent was removed *in vacuo* to give the green bromo compound (**8**). This was reacted at once with ethanol to give the corresponding ethyl ether [(**9**), 86%]. Similarly (**8**) reacted with ethylene glycol to give [(**10**), 56%], with glycerol to give [(**11**), 83%], and with D-glucose to give [(**12**), 64%, diastereoisomers], the last two reactions being carried out in anhydrous dimethylformamide.

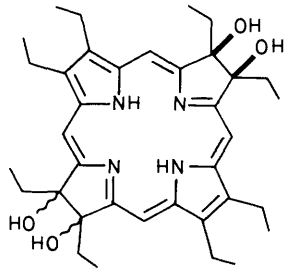
In this way a range of polyhydroxy hydroporphyrin derivatives is made available, specifically with one, two, three, or four hydroxy groups at β-positions, and with one, two, and four hydroxy groups in a side chain, and clearly others could be made in an analogous fashion. It was envisaged that,



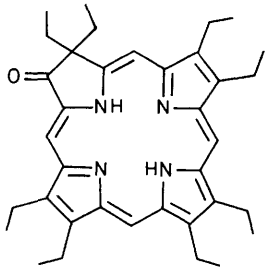
(**1**) R = H

(**3**) 2R = $>OsO_2 \cdot 2Py$

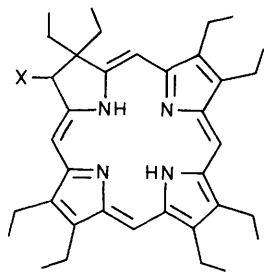
Py = pyridine



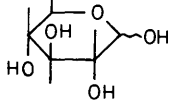
(**2**)

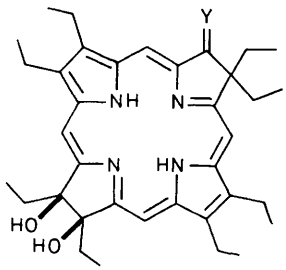


(**4**)



(**5**) X = OH
(**8**) X = Br
(**9**) X = OEt
(**10**) X = OCH_2CH_2OH
(**11**) X = $OCH_2CH(OH)CH_2OH$
(**12**) X = —O—CH_2





(**6**) Y = O
(**7**) Y = H, OH

Table 1. Tumour photonecrosis with hydroxyhydroporphyrins.[a]

Structure	Functionality	Dose/ μmol kg/$^{-1}$	λ[b] /nm	Depth of photonecrosis[c] [mm ± SE(*n*)]
(1)	(ββ-Dihydroxy)	12.5	645	5.69 ± 0.92 (9)
		1.56	645	0.86 ± 0.33 (9)
(7)	(βββ-Trihydroxy)	25	713	4.0 ± 0.29 (6)
		6.25	713	1.75 ± 0.52 (6)
(2)	(ββββ-Tetrahydroxy)	25	712.5	>7.29 ± 0.42 (6)
		3.12	712.5	2.72 ± 0.82 (6)
(6)	(ββ-Dihydroxy-β-oxo)	12.5	696	2.63 ± 0.60 (6)
		3.12	696	0.95 ± 0.59 (5)
(11)	(Side-chain dihydroxy)	12.5	646	4.36 ± 0.48 (7)
(12)	(Side-chain tetrahydroxy)	12.5	647	6.43 ± 0.53 (7)
	Photofrin II	100	625	3.03 ± 0.45 (8)
		50	625	1.94 ± 0.30 (12)

[a] The bioassay is described in ref. 3. The photosensitiser was introduced intraperitoneally in dimethyl sulphoxide. [b] Wavelength of irradiation, light dose 10 J cm^{-2}. [c] This column gives the index of activity of the photosensitiser. It records the depth (in mm) of destruction of a tumour of selected standard size after administration of the drug at the stated dose and irradiation with a light under standardised conditions. The index is quoted with a standard error (SE) which refers to the number of tumours in brackets.[3]

provided some of the compounds showed good photonecrotic activity in the *in vivo* tumour assay, it might be possible to vary their effectiveness by varying the number and type of such polar groupings to meet the requirements of different physiological situations.

Although the β-oxochlorin (**4**) is inactive, and the mono-β-hydroxy compound (**5**) gave erratic results (possibly associated with solubility problems), several of the compounds described here show marked activity (Table 1) in the *in vivo* tumour assay.[3] Results for Photofrin II are included for comparison. The results are encouraging, and will be reported in full elsewhere when tissue selectivities have been determined.

We are grateful to the S.E.R.C. and the Cancer Research Campaign for support. Dr. Nizhnik is on leave from the Lomonosov Institute of Fine Chemical Technology, Moscow, U.S.S.R., with the support of the British Council, whom we thank.

Received, 23rd August 1989; Com. 9/03616D

References

1 For review see: 'Photosensitising Compounds: Their Chemistry, Biology, and Clinical Use,' eds. G. Bock and S. Harnett, Ciba Foundation Symposium, 1989, **146**, in press.
2 S. Wan, J. A. Parrish, R. R. Anderson, and M. Madden, *Photochem. Photobiol.*, 1981, **34**, 679.
3 M. C. Berenbaum, S. L. Akande, R. Bonnett, H. Kaur, S. Ioannou, R. D. White, and U-J. Winfield, *Br. J. Cancer*, 1986, **54**, 717.
4 R. Bonnett, R. D. White, U-J. Winfield, and M. C. Berenbaum, *Biochem. J.*, 1989, **261**, 277.
5 K. R. Adams, Ph.D. Thesis, London, 1969.
6 R. Bonnett, M. B. Hursthouse, M. Motevalli, and A. N. Nizhnik, manuscript in preparation.
7 C. K. Chang and C. Sotiriou, *J. Org. Chem.*, 1987, **52**, 926. In our hands the use of perchloric acid in the pinacol rearrangement step (C. K. Chang and C. Sotiriou, *J. Org. Chem.*, 1985, **50**, 4989) leads to the formation of by-products.
8 R. Bonnett, M. J. Dimsdale, and G. F. Stephenson, *J. Chem. Soc. (C)*, 1969, 564.
9 A. M. Stolzenberg, P. A. Glazer, and B. M. Foxman, *Inorg. Chem.*, 1986, **25**, 983. These authors report that the β-oxochlorin could not be reduced with sodium borohydride.