Biochem. J. (1989) 261, 277–280 (Printed in Great Britain)

Hydroporphyrins of the *meso*-tetra(hydroxyphenyl)porphyrin series as tumour photosensitizers

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Four new hydroporphyrins [the *o*, *m* and *p* isomers of 5,10,15,20-tetra(hydroxyphenyl)chlorin and 5,10,15,20-tetra(*m*-hydroxyphenyl)bacteriochlorin] related to the tetra(hydroxyphenyl)porphyrins have been prepared. They show the expected strong absorption bands in the red region of the visible spectrum and are found to be very effective tumour photosensitizers.

INTRODUCTION

There is currently worldwide activity in the development of a phototherapy for cancer (Dougherty, 1987*a*). An essential aspect of this effort is the design, synthesis and biological assay *in vivo* of new photosensitizers.

New photosensitizers are needed because of the inadequacies of haematoporphyrin derivative (HpD), which has been most commonly used in both biochemical and clinical studies in this area. HpD was first described by Lipson & Baldes (1960). It is prepared by the action of H_2SO_4 in acetic acid on haematoporphyrin at room temperature. This gives a purple solid (HpD Stage I), which is a complex mixture, but consists largely of haematoporphyrin diacetate (Bonnett *et al.*, 1981). For injection, this material is treated with alkali to give HpD Stage II. The treatment causes hydrolysis and elimination to give monomeric porphyrins such as haematoporphyrin, hydroxyethyl(vinyl)deuteroporphyrin and protoporphyrin; at the same time, condensation–polymerization reactions occur to give a high-molecular-mass fraction (fraction D) in which the bulk of the activity resides. We postulated that the composition of the material after treatment with base includes a mixture of dimers and oligomers involving ester, ether or carbon–carbon linkages (Berenbaum *et al.*, 1982). Although there has been some support for various of these proposals (Dougherty *et al.*, 1984; Kessel, 1986; Scourides *et al.*, 1987; Keir *et al.*, 1987), the limited information on the components of HpD Stage II indicates that it is a complex and variable mixture from which it has not yet proved possible to isolate a single highly active compound (Dougherty, 1987*b*).

The need therefore arises for 'second-generation' photosensitizers which embody the following characteristics: (i) lack of toxicity in the dark; (ii) uniform stable composition, and preferably a single substance; (iii) selective photosensitization of tumour tissue, a feature which seems to be related to amphiphilic properties; (iv) high triplet yield, with a triplet energy greater than 94 $kJ \cdot mol^{-1}$, the excitation energy for Δ_g singlet oxygen; (v) absorption in the red.

The last criterion arises because, due to scattering and absorption effects, the transmission of visible light through tissue is very low at 400 nm, but reaches a maximum in the 700–800 nm region (Wan *et al.*, 1981). Absorption in the red has been increased by (i) modifying the substitution pattern (Bonnett *et al.*, 1987) or (ii) by reducing the porphyrin to the chlorin or bacteriochlorin chromophore (Selman *et al.*, 1987; Kessel & Smith, 1989) or (iii) by more deep-seated changes in the structure, for example, to give phthalocyanine or naphthalocyanine systems (Ben-Hur & Rosenthal, 1985; Chan *et al.*, 1987).

Recently a series of porphyrins, namely the *o*, *m* and *p* isomers of 5,10,15,20-tetra(hydroxyphenyl)porphyrin (displayed structures **1**, **2** and **3** respectively) has been reported which fall into the first modification category (Berenbaum *et al.*, 1986). These substances showed promising activity and tissue selectivity in photonecrosis. Thus the *m*-isomer (**2**) was found to be 25–30 times as potent as HpD in sensitizing tumours in the '*in vivo*' bioassay.

On the basis of this substitution pattern, we now report the effect of a modification of the second category to give chlorin (structures **4**, **5** and **6**) and bacteriochlorin (structure **7**) analogues, a structural change which is accompanied by a dramatic increase in photonecrotic activity.

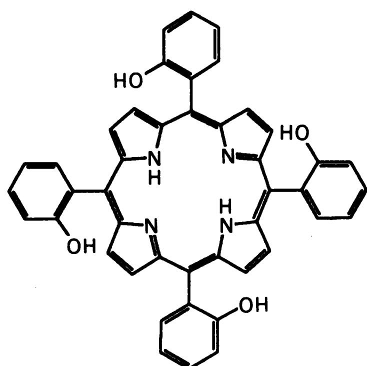
MATERIALS AND METHODS

Chlorins

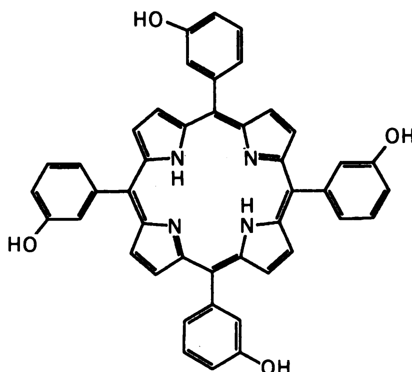
The porphyrin (**1**, **2** or **3**; Bonnett *et al.*, 1987) was reduced with di-imide using the method of Whitlock *et al.* (1969), without protection of the phenolic functions.

Thus the *p* isomer (**3**; 163 mg), *p*-toluenesulphonylhydrazide (90 mg), anhydrous K_2CO_3 (300 mg) and anhydrous pyridine (11.25 ml) were stirred with N_2 flushing for 20 min. The mixture was heated (100–105 °C) for 6.5 h under N_2; further quantities of the hydrazide (97 mg in 0.3 ml of anhydrous pyridine on each occasion) were added after 2 and 4 h. The reaction mixture was treated with ethyl acetate (75 ml) and distilled water (37.5 ml) and digested on the steam bath for 1 h. The organic layer was separated and washed in turn with HCl (2 M, 75 ml), distilled water (75 ml) and saturated $NaHCO_3$ solution (75 ml). *o*-Chloranil (total 166 mg) was added in portions

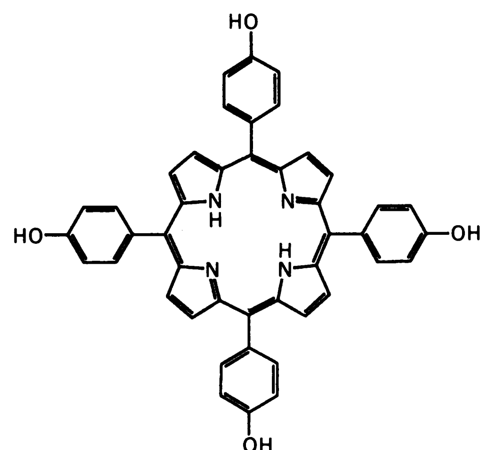
Abbreviations used: abbreviations for the porphyrins are provided below the structural formulae and full chemical names are displayed in the text; other abbreviation: HpD, haematoporphyrin derivative.



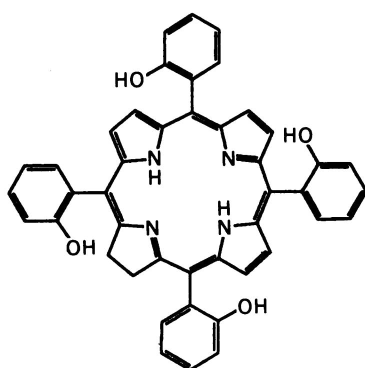
(1) 5,10,15,20-Tetra(*o*-hydroxyphenyl)porphyrin (*o*-THPP)



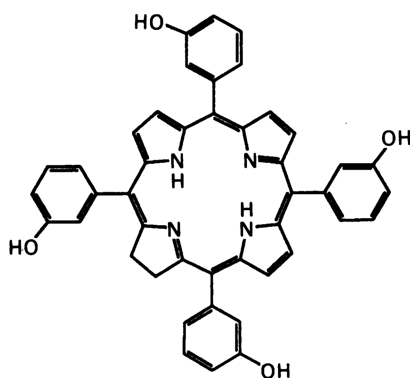
(2) 5,10,15,20-Tetra(*m*-hydroxyphenyl)porphyrin (*m*-THPP)



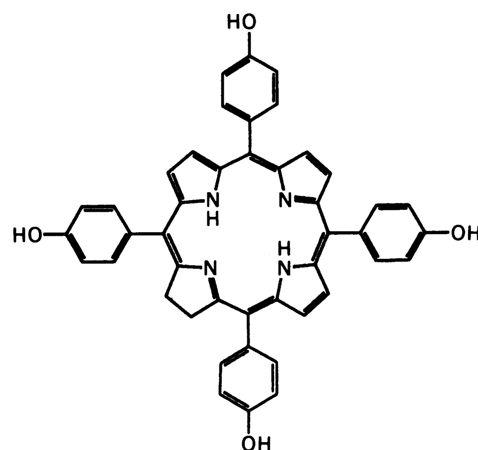
(3) 5,10,15,20-Tetra(*p*-hydroxyphenyl)porphyrin (*p*-THPP)



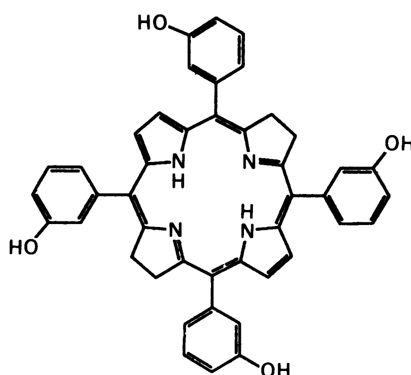
(4) 5,10,15,20-Tetra(*o*-hydroxyphenyl)chlorin (*o*-THPC)



(5) 5,10,15,20-Tetra(*m*-hydroxyphenyl)chlorin (*m*-THPC)



(6) 5,10,15,20-Tetra(*p*-hydroxyphenyl)chlorin (*p*-THPC)



(7) 5,10,15,20-Tetra(*m*-hydroxyphenyl)bacteriochlorin (*m*-THPBC)

to the stirred organic solution at room temperature until the absorption peak at ~ 735 nm had just disappeared. The solution was washed with aqueous $NaHSO_3$ (5%; 2 × 75 ml), distilled water (75 ml), NaOH (0.01 M, 100 ml), and saturated $NaHCO_3$ (80 ml), and dried (over anhydrous Na_2SO_4). The filtered solution was evaporated to dryness, and the residue was crystallized from methanol/water to give 77 mg (middle cut, 47%) of *5,10,15,20-tetra(p-hydroxyphenyl)chlorin* (**6**) as a purple solid. (Found, by fast-atom-bombardment mass spectrometry: $M+H^+$, 681.253; $C_{44}H_{32}N_4O_4+H$ requires 681.250). Light-absorption maxima in methanol [ϵ (molar absorption coefficient)]: 285 (17000), 295 (16900), 418 (143000), 520 (10000), 550 (9000), 597 (5100) and 651 nm (18600 litre·mol^{-1}·cm^{-1}). N.m.r.: δ (p.p.m.) ([^{2}H]chloroform/[$^2H_6$]dimethyl sulphoxide) 9.22

Table 1. Tumour photonecrosis with chlorins and a bacteriochlorin of the *meso*-tetra(hydroxyphenyl) series

Photosensitizer	Dose (μmol/kg body wt.)	Wavelength* (nm)	Depth of tumour necrosis (mm) (mean ± S.E.M.)
o-THPC (**4**)	6.25	652	4.16 ± 0.27 (14†)
	3.125	652	3.69 ± 0.74 (9)
	1.56	652	0.31 ± 0.31 (8)
	0.78	652	0 (8)
m-THPC (**5**)	0.75	652	5.41 ± 0.39 (19)
	0.375	652	3.79 ± 0.28 (6)
	0.2	652	0.13 ± 0.05 (12)
	0.1	652	0.042 ± 0.024 (12)
p-THPC (**6**)	6.25	653	3.50 ± 0.54 (10)
	3.125	653	2.13 ± 0.50 (10)
m-THPBC (**7**)	6.25	741	Died within 2 h of irradiation, tumour visibly blackening
	3.125	741	
	1.562	741	
	0.39	741	5.22 ± 1.21 (8)
	0.19	741	0.11 ± 0.05 (7)

* The total energy administered was 10 J·cm^{-2} throughout.
† Number of tumours in parentheses.

(s, 4 × OH), 8.62, 8.42, 8.23 (d, s, d, *J* 4.5 Hz, pyrrole β-H), 7.90, 7.65 (d, d, *J* 8 Hz, 4-benzenoid H), 7.16 (two overlapping d, *J* 8 Hz, benzenoid H), 4.17 (s, 2 × CH_2), and −1.53 (bs, 2 × NH).

Similarly, the *m isomer* (**5**) was obtained from methanol/water as a purple solid (37%). (Found: $M+H^+$, 681.251). Light-absorption maxima in methanol (ϵ): 284 (16900), 306 (15600), 415 (146000), 516 (11000), 543 (7300), 591 (4400) and 650 nm (22400 litre·mol^{-1}·cm^{-1}). N.m.r.: δ (p.p.m.) ([^{2}H]chloroform/[$^2H_6$]dimethyl sulphoxide) 9.16 (s, 4 × OH), 8.66, 8.46, 8.28 (d, s, d, *J* 4.5 Hz, pyrrole β-H), 7.1–7.7 (m, benzenoid H), 4.21 (s, 2 × CH_2) and 1.64 (bs, 2 × NH).

The *o-chlorin* (**4**) was obtained as a purple crystalline solid (64%) from methanol/water. Like the starting porphyrin (**1**), it was a mixture of atropisomers (Gottwald & Ullmann, 1969), which were not individually isolated. R_F (1% methanol in chloroform; Merck silica gel) 0.23, 0.29, 0.38, and 0.43. (Found: $M+H^+$, 681). Light-absorption maxima in methanol (ϵ): 415 (90700), 515 (8400), 542 (5600), 597 (3800) and 651 nm (16000 litre·mol^{-1}·cm^{-1}). N.m.r.: δ (p.p.m.) ([$^2H_6$]dimethyl sulphoxide) 9.49 (s, 4 × OH), 8.50, 8.25, 8.15 (d, s, d, *J* 5 Hz, pyrrole β-H), 7.02–7.97 (m, benzenoid H), 4.12 (s, 2 × CH_2), and −1.57 (s, 2 × NH).

To obtain *bacteriochlorin* (**7**), *m*-THPP (109 mg) was reduced with *p*-toluenesulphonylhydrazide (483 mg) in anhydrous pyridine (7.5 ml) as described above, except that further additions of the hydrazide (60 mg in 2 ml of anhydrous pyridine) were made every 1.5 h, and refluxing was continued for a total of 12 h. Digestion was effected with ethyl acetate (100 ml) and distilled water (50 ml), and the separated organic layer was washed in turn with HCl (2 M, 50 ml), H_3PO_4 (56%, 4 × 50 ml), distilled water (50 ml) and saturated $NaHCO_3$ (50 ml) and dried (over anhydrous Na_2SO_4). The resulting solid was crystallized from methanol/water to give *5,10,15,20-tetra-(m-hydroxyphenyl)bacteriochlorin* as a green solid (29 mg, 26%). (Found: M^+ 682.260; $C_{44}H_{34}N_4O_4$ requires 682.258). Light-absorption maxima in methanol (ϵ): 352 (92000), 361 (114000), 372 (129000), 516 (50000) and 735 nm (91000 litre·mol^{-1}·cm^{-1}). N.m.r.: δ (p.p.m.) ([$^2H_6$]dimethyl sulphoxide) 9.69 (s, 4 × OH), 7.95 (d, *J* 2 Hz, pyrrole β-H), 7.47, 7.21, 7.18, 7.04 m, benzenoid H), 3.95 (s, 8 × CH_2), and −1.54 (bs, 2 × NH). The n.m.r. spectrum revealed minor contamination by the corresponding chlorin.

Tumour necrosis

The PC6 plasma cell tumour, obtained initially from the Chester Beatty Research Institute, was grown by inoculating (0.3–0.6) × 10^6 cells subcutaneously in female BALB/c mice. It was used about 2 weeks later, when it was 12–13 mm in its longest diameter and 6–7 mm deep. Sensitizers were injected in dimethyl sulphoxide intraperitoneally (2.5 μl·g^{-1}) and, 24 h later, skin over the tumours was depilated, the mice anaesthetized and tumours exposed to light (10 J·cm^{-2}). Wavelengths for illumination were the longest-wavelength absorption peaks in the red in solutions of the sensitizer in fetal-calf serum, and were 652–653 nm for the compounds **4–6** and 741 nm for compound **7**. The light source was a copper vapour laser with an output of 10–12 W (Cu 10 laser: Oxford Lasers, Oxford, U.K.) pumping a D2 10K dye laser (Oxford Lasers). The dye was Rhodamine 640 (Applied Photophysics, The Royal Institution, London, U.K.) for illumination at 652–653 nm and LDS-722 (Exciton, Dayton, OH, U.S.A.) for illumination at 741 nm. Light intensity at the tumour surface was kept below 0.3 W·cm^{-2}, where thermal effects were undetectable.

At 24 h after illumination, 0.2 ml of 1% Evans Blue (Sigma) in saline was given intravenously and tumours were removed 1 h later and fixed in formol/saline. The fixed tumours were sliced at right angles to the surface, and the depth of necrosis measured with a dissecting microscope fitted with an eyepiece graticule, as described elsewhere (Berenbaum *et al.*, 1982).

RESULTS AND DISCUSSION

Di-imide reduction of the porphyrins (**1**, **2** and **3**) gives a mixture of the corresponding dihydro- and tetrahydroporphyrins. Dehydrogenation with *o*-chloranil cleanly removes the tetrahydro compound ($\lambda_{max.} \sim 735$ nm) to give a useful route to the chlorins (**4**, **5** and **6**) without the necessity for protection of the phenolic functions. Under more forcing conditions, di-imide reduction of the *m* isomer (**2**) gives mainly the corresponding bacteriochlorin (**7**).

As expected, these compounds are soluble in polar solvents, and they all have strong absorption in the red region of the visible spectrum. The chlorins have maxima at ~650 nm with molar absorption coefficients of about 20000 litre·mol^{-1}·cm^{-1}; in the bacteriochlorin (**7**) the long-wavelength band in methanol has shifted to 735 nm (741 in fetal-calf serum), with a much increased absorption coefficient (91000 litre·mol^{-1}·cm^{-1}).

The tumour photonecrosis results are summarized in Table 1. Although the advantages of absorption in the red region of the visible spectrum are well recognized, this is the first report of results for a series of reduced porphyrins based on a porphyrin substitution pattern of established photobiological activity.

All four sensitizers show considerable activity and, even with these limited results, appear to be considerably more potent as tumour photosensitizers than are the corresponding tetra(hydroxyphenyl)porphyrins. This is especially evident with the *p* and *m* isomers, where substantial tumour necrosis is produced with doses at which *p*- and *m*-tetra(hydroxy)phenylporphyrin are quite ineffective (Berenbaum *et al.*, 1986). Whether these compounds will prove to be useful in clinical tumour phototherapy will depend largely on whether their effects are relatively selective for tumours as compared with normal tissues.

We are grateful to Scotia Pharmaceuticals Ltd. and to the Cancer Research Campaign for support. The new compounds described here are the subject of British Patent Application no. 88/05849.

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Received 13 March 1989/12 April 1989; accepted 24 April 1989