J. Pharm. Pharmacol. 2000, 52: 1361–1367
Received May 11, 2000
Accepted August 2, 2000

Solubilization of *meso*-Tetraphenylporphyrin Photosensitizers by Substitution with Fluorine and with 2,3-Dihydroxy-1-propyloxy Groups

SANDILE P. SONGCA AND B. MBATHA

Chemistry Department, University of Transkei, Private Bag X1, Unitra, Umtata 5117, Eastern Cape, South Africa

Abstract

The tetra(hydroxyphenyl)porphyrins (5,10,15,20-tetrakis(2-hydroxyphenyl)porphyrin (**3**), 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin (**4**), and 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (**5**) and the tetrahydroporphyrins (5,10,15,20-tetrakis(2-fluoro-3-hydroxyphenyl)porphyrin (**6**), 5,10,15,20-tetrakis(2,4-difluoro-3-hydroxyphenyl)porphyrin (**7**), and 5,10,15,20-tetrakis(3,5-difluoro-4-hydroxyphenyl)porphyrin (**8**)) have been reported as potential photosensitizers for photodynamic therapy. In particular, the tetrahydroporphyrin **7**, which has been in Phase III clinical trials for three years in the USA and Canada, has shown great promise for primary and recurrent head and neck cancer. It is expected to be on the market soon. A potentially limiting property of compounds **3**–**8** is their hydrophobicity, which renders them insoluble in aqueous media. We therefore set out to develop an approach for their solubilization in aqueous media.

The first attempt was to prepare analogues with fluorine substituents at the positions *ortho* to each of the four hydroxy groups on the *meso*-phenyl rings. This was expected to increase the acidity of the *ortho* hydroxy groups, and therefore the solubility of the compounds in hydroxylic media. This structural alteration resulted in a slight but significant decrease in the partition coefficients.

The second attempt was substitution of each of the hydroxy groups on the phenyl rings with 2,3-dihydroxy-1-propyloxy groups. This structural alteration resulted in a substantial decrease in the partition coefficients.

To harness the combined effect of these two structural variations porphyrins were prepared containing both 2,3-dihydroxy-1-propyloxy and *ortho* fluorine groups on the *meso*-phenyl rings. The partition coefficients decreased by more than two orders of magnitude. The most significant decrease was due to the substitution with 2,3-dihydroxy-1-propyloxy groups, rather than the substitution with fluorine, although fluorination alone contributed significantly. The effect of this method was demonstrated most clearly by comparison of the partition coefficients of *meta* and *para* isomers of these porphyrins.

Photodynamic therapy relies on two important properties of the photosensitizing drugs used in this method, notably porphyrins, chlorins and phthalocyanines; firstly their preferential tumour localization (Bonnett & Berenbaum 1989) and secondly their destruction of animal tissue in the presence of light and oxygen (Henderson & Dougherty 1992).

Correspondence: S. P. Songca, Chemistry Department, University of Transkei, Private Bag X1, Unitra, Umtata 5117, Eastern Cape, South Africa.

When porphyrins are used as photodynamic photosensitizers, one of the challenges is optimizing their amphiphilic properties in such a way that they are hydrophilic enough to be administered as aqueous solutions, so that they can quickly reach the tumours after administration, and be eliminated rapidly from the body after photodynamic therapy. At the same time they must be hydrophobic enough to be retained by, and preferentially localize in tumour tissue. As the porphyrin macrocycle is hydrophobic, the vast majority of approaches to

this challenge have relied on the introduction of varying degrees of hydrophilicity by introducing polar substituents to confer solubility in hydroxylic media, thus rendering the porphyrins amphiphilic (Kongshaug et al 1989).

One of the traditional ways of evaluating the amphiphilicity of medicinal drug compounds is to measure their partition coefficients (Leo 1986). In one approach to study this amphiphilic property for a number of porphyrins, the partition coefficients (**P**) between 2-octanol and phosphate-buffered saline (PBS, pH 7·3) have been measured. The partition coefficients of some of the most important photosensitizers varied over four-orders of magnitude in that study. The hydroxyphenyl porphyrins **3**, **4** and **5** (Figure 1), and the corresponding hydroxyphenyl chlorins were at the more hydrophobic extreme ($2–9\times10^{3}$), while protoporphyrin **1**, haematoporphyrin **2** (Figure 2) and haematoporphyrin derivative were at the more hydrophilic extreme ($2\times10^{-2}–6\times10^{1}$). No clear correlation was observed between the partition coefficients and biological activity in that study (Songca 1990).

However, other studies on the effect of amphiphilic properties have reported some correlation between the partition coefficient and biological activity of photodynamic therapy photosensitizing compounds. A linear dependence of porphyrin uptake into V79 Chinese hamster cells on log(**P**) was observed for a number of monomeric porphyrins (Oenbrink et al 1988). In another study a parabolic relationship was observed between tumour localization and log(**P**) for a range of pure monomeric porphyrins, with a maximal tumour localization for most of the tissues studied at partition coefficient values of approximately one (Woodburn et al 1992). The monomeric porphyrins used in the studies by Oenbrink et al (1988) and Woodburn et al (1992) included protoporphyrin **1**, haematoporphyrin **2** and various derivatives of these compounds, e.g. methoxy and dialkylamido.

Several methods have been reported for the solubilization of the hydrophobic photosensitizing compounds used in photodynamic therapy, including substitution with hydrophilic substituents such as sulphonic acid groups (SO_3^-), trialkyl ammonium groups (NR_3^+), carboxylic acid groups (CO_2H), and recently glycosyl groups ($C_6H_6(OH)_4O$) (Momenteau et al 1994; Oulmi et al 1995). *Meso*-tetra(4-hydrocarboxyphenyl)porphyrin (Kongshaug et al 1989) and pthalocynanine tetrasulphonic acids (De Smidt et al 1993), for example, have been prepared. These compounds are extremely hydrophilic, dissolving in water, but insoluble in 2-octanol. In these two cases the hydrophilicity conferred to the compounds exceeds by far the hydrophobicity of the macrocyclic porphyrin or phthalocyanine nucleus. These compounds tend to exhibit poor photodynamic activity in both in-vivo and in-vitro bioassays (Kessel et al 1987; De Smidt et al 1993).

To overcome the difficulty of extreme hydrophilicity conferred to the hydrophobic porphyrin nucleus by four hydrophilic substituents on four *meso*-phenyl rings, porphyrins have been synthesized with degrees of substitution with hydrophilic substituents on the *meso*-phenyl rings, varying from 1 to 4, at the various different *meso*-positions of the porphyrin. Thus De Smidt et al (1993) prepared the mono-sulphonated, the 5,10-disulphonated and the tetra-sulphonated tetraphenylporphyrin. Similarly the mono-glycosylated, the two di-glycosylated (5,10- and 5,15-), the tri-glycosylated, and the tetra-glycosylated tetraphenylporphyrin were prepared by Momenteau et al (1994). In addition, to further enhance the hydrophilic/hydrophobic balance, which renders porphyrins amphiphilic,

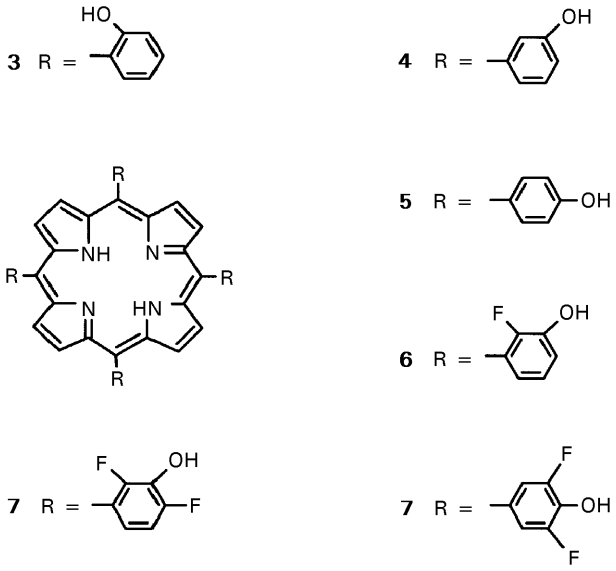

Figure 1. Structures of the tetrahydroxyphenylporphyrins and fluorinated tetrahydroxyphenylporphyrins **3**–**8**.

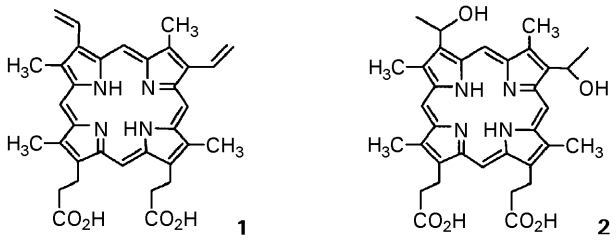

Figure 2. Structures of protoporphyrin (**1**) and haematoporphyrin (**2**).

porphyrins containing extended hydrophilic and hydrophobic character at opposite ends of the porphyrin nucleus have been synthesized. Thus porphyrins containing glycosylated *meso*-phenyl rings at positions 5 and 15, and meso-phenyl rings, or alkyl chains at positions 10 and 20 have been synthesized (Momenteau et al 1994; Oulmi et al 1995). In contrast to the porphyrins that are symmetrically tetra-substituted with extremely hydrophilic substituents, these compounds tend to exhibit mediocre partition coefficients between 2-octanol and PBS (roughly from 1 to 50), and moderate to excellent photodynamic activity in-vivo and in-vitro (Kessel et al 1987; De Smidt et al 1993).

In this paper we describe the results of a method which we have developed for moderate solubilization of the hydrophobic porphyrins **3**, **4**, and **5**, in aqueous media. We started by studying the effect of fluorine substituents at the positions *ortho* to each of the four hydroxy groups. This was followed by a study on the effect of substitution of each of the hydroxy groups with 2,3-dihydroxy-1-propyloxy groups. Thus, porphyrins have been prepared that retain the symmetrical tetra-substitution pattern of **3**, **4**, and **5**, but incorporate the various moderately solubilizing substituents (Songca et al 1997; Songca & Mbatha 2000), resulting in significantly reduced partition coefficients. However, because of the moderate hydrophilicity conferred by the *ortho* fluorine and the 2,3-dihydroxy-1-propyloxy substituents, the partition coefficients of these compounds are not so low as to render these compounds water soluble and insoluble in organic media. On the contrary, it was found that the partition coefficients were still insufficiently low ($\pm 5\times10^{-1}$), and these compounds were still relatively hydrophobic.

Materials and Methods

Materials

2-Octanol, analytical grade, was used for the partitioning of the various porphyrin solutes. In all cases the porphyrin solutes had to be sonicated in the 2-octanol using a Deacon FS00B ultrasonic bath, to dissolve as much of the porphyrin as possible. The equilibration of the two layers by agitation was carried out on a Stuart Scientific shaker at 500 rev min^{-1}. The PBS solution was prepared by dissolving the tablets (Merck) in de-ionized water, followed by boiling the solution for 5 min and cooling down to room temperature overnight. PBS solutions obtained in this way were kept at room temperature and used within seven days. Analytical grade methanol and HCl were used to prepare the 1·2 M methanolic HCl solution. This solution was stored at room temperature and used within seven days. Electronic spectra were measured on a Perkin Elmer Lambda II UV and visible spectrophotometer, using a matched pair of 100-mm path length quartz window cuvettes. Microsoft Excel 97 was used to record the data and to perform the calculations.

Synthesis of the porphyrin compounds

The porphyrins 5,10,15,20-tetrakis[2-(2,3-dihydroxy-1-propyloxy)phenyl]porphyrin (**9**), 5,10,15,-20-tetrakis[3-(2,3-dihydroxy-1-propyloxy)phenyl]-porphyrin (**10**), 5,10,15,20-tetrakis[4-(2,3-dihydroxy-1-propyloxy)phenyl]porphyrin (**11**), 5,10,-15,20-tetrakis[2-fluoro-3-(2,3-dihydroxy-1-propyloxy)phenyl]porphyrin (**12**), 5,10,15,20-tetrakis[3,5-difluoro-3-(2,3-dihydroxy-1-propyloxy) phenyl]porphyrin (**13**), and 5,10,15,20-tetrakis [2,4-difluoro-3-(2,3-dihydroxy-1-propyloxy)phenyl] porphyrin (**14**) (see Figure 3), were prepared from the corresponding tetra(hydroxyphenyl)porphyrins, as described by Mbatha (1999) and Songca & Mbatha (2000).

The starting compounds (5,10,15,20-tetrakis(2-hydroxyphenyl)porphyrin (**3**), 5,10,15,20-tetrakis (3-hydroxyphenyl)porphyrin (**4**), and 5,10,15,-20-tetrakis(4-hydroxyphenyl)porphyrin (**5**), the required starting materials for the synthesis of the porphyrins **9**–**11**) were prepared as described by Bonnett et al (1987) and the fluorinated porphyrins (5,10,15,20-tetrakis(2-fluoro-3-hydroxyphenyl) porphyrin (**6**), 5,10,15,20-tetrakis(2,4-difluoro-3-hydroxyphenyl)porphyrin (**7**), and 5,10,15,20-tetrakis(3,5-difluoro-4-hydroxyphenyl)porphyrin (**8**) (see Figure 1), the required starting materials

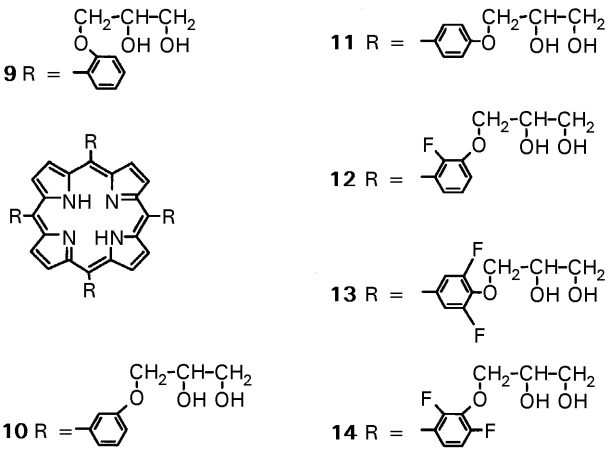

Figure 3. Structures of*meso*-tetrakis(2,3-dihydroxy-1-propyloxyphenyl)porphyrins and fluorinated derivatives **9**–**14**.

for the synthesis of the porphyrins **12**–**14**) were prepared as described by Bonnett et al (1987) and by Songca et al (1997).

Measurement of partition coefficients

A typical experiment is described. 5,10,15,20-Tetrakis(2-hydroxyphenyl)porphyrin (100 mg) was dissolved in 2-octanol (25 mL) in a 100-mL conical flask. The solution was filtered into a 100-mL separating funnel, on a sintered glass filter funnel, to remove any undissolved porphyrin. PBS (20 mL) was added. The separating funnel was agitated for 10 min on a shaker to thoroughly mix the two layers and then the two layers were allowed to separate (2–5 min). Samples of the aqueous (15 mL) and organic layers (500 μL) were taken. The aqueous layer was diluted to 50 mL with a 1·2 M solution of HCl in methanol. The organic layer was treated with PBS (15 mL) and then diluted to 50 mL with a 1·2 M solution of HCl in methanol. Electronic spectra were measured (350–750 nm), using 100-mm path length cuvettes. The absorbance of the Soret (445–455 nm), as well as the red band (640–660 nm), was recorded. The partition coefficients were calculated using the absorbance of the Soret band. The absorbance of the red band was used to double-check the calculations of the partition coefficients. These measurements were carried out in triplicate and the mean values are reported.

Results and Discussion

The partition coefficients of compounds **3**–**14** were measured between 2-octanol and aqueous PBS using a suitable modification of the traditional "shake flask" method (Kessel 1977; Leo 1986). Table 1 lists the partition coefficients of the porphyrins measured using this method. Comparison of the partition coefficients of **4** and **5** with those of **6** and **8** showed that the expected effect of *ortho* fluorine substituents, i.e. to decrease the hydrophobicity, was only slightly realised (Bonnett & Songca 1995). However, comparison of the partition coefficients of **3**, **4** and **5** with those of **9**, **10** and **11** showed that the expected decrease in the hydrophobicities of the porphyrins due to substitution with 2,3-dihydroxy-1-propyloxy groups was substantial.

Since both fluorine substitution and substitution with 2,3-dihydroxy-1-propyloxy groups resulted in decreases in hydrophobicity, albeit to different degrees, we decided to synthesize derivatives of **3**, **4** and **5** substituted with fluorine as well as 2,3-dihydroxy-1-propyloxy groups to harness the combined effect of these structural alterations. Comparison of the partition coefficients of **3**, **4** and **5** with those of **12**, **13** and **14** shows that the combined effect of substitution with fluorine as well as 2,3-dihydroxy-1-propyloxy was to decrease the hydrophobicity dramatically. However, the decrease in hydrophobicity due to the combined effect of fluorine and 2,3-dihydroxy-1-propyloxy substitution was still relatively insignificant when compared with the effect of substitution with substituents such as sulphonic acid, trialkyl ammonium, carboxylic acid and glycosyl groups, and these compounds were still relatively hydrophobic. They dissolved in 2-octanol, ethanol and methanol, but not in water or PBS at pH 7.

Table 1. Partition coefficients of the porphyrins between 2-octanol and PBS at a pH value of 7·3.

Porphyrin *meso* substituent	Porphyrin number	P
2-Hydroxyphenyl	**3**	$3{\cdot}79\times10^{4}$
3-Hydroxyphenyl	**4**	$5{\cdot}39\times10^{4}$
4-Hydroxyphenyl	**5**	$5{\cdot}94\times10^{4}$
2-Fluoro-3-hydroxyphenyl	**6**	$3{\cdot}99\times10^{4}$
2,4-Difluoro-3-hydroxyphenyl	**7**	$2{\cdot}61\times10^{4}$
3,5-Difluoro-4-hydroxyphenyl	**8**	$3{\cdot}34\times10^{4}$
2-(2,3-Dihydroxy-1-propyloxy) hydroxyphenyl	**9**	$4{\cdot}67\times10^{4}$
3-(2,3-Dihydroxy-1-propyloxy) hydroxyphenyl	**10**	$6{\cdot}77\times10^{4}$
4-(2,3-Dihydroxy-1-propyloxy) hydroxyphenyl	**11**	$8{\cdot}67\times10^{4}$
2-Fluoro-3-[2,3-dihydroxy-1-propyloxy]phenyl	**12**	$4{\cdot}50\times10^{2}$
3,5-Difluoro-4-[2,3-dihydroxy-1-propyloxy]phenyl	**13**	$4{\cdot}10\times10^{2}$
2,4-Difluoro-3-[2,3-dihydroxy-1-propyloxy]phenyl	**14**	$3{\cdot}20\times10^{2}$

Comparison of analogous porphyrins

The combined effect of both these structural alterations is perhaps most appropriately illustrated by a comparison of the partition coefficients of the tetrahydroxyphenylporphyrins, the fluorinated tetrahydroxyphenylporphyrins, and fluorinated tetra(2,3-dihydroxy-1-propyloxy)phenylporphyrins in which the hydroxy groups are at the same position. The series of porphyrins used in such a comparison are shown in Figure 4. A graphical illustration of this comparison is shown in Figure 5.

The "shake flask" method

We developed a modification of the "shake flask" method for the measurement of the partition coefficients of the porphyrins **3**–**14**. The method is particularly suitable for the determination of the

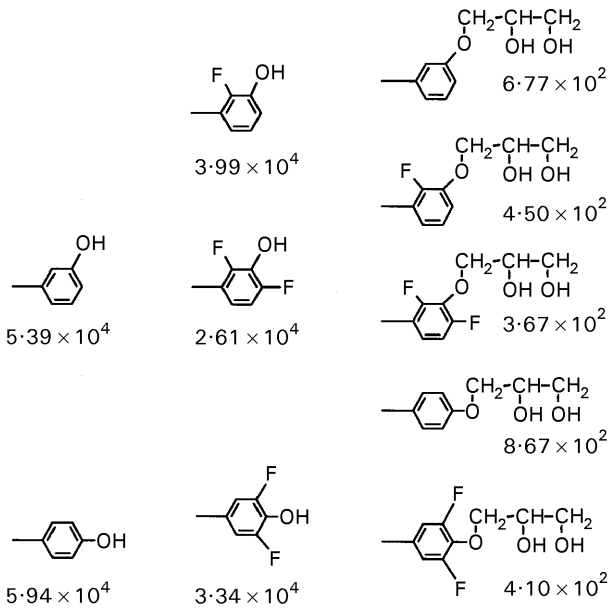

Figure 4. The *meso*-substituents of structures of the series of porphyrins in which the hydroxy groups are in the same positions. The partition coefficient of each porphyrin is given below each structure.

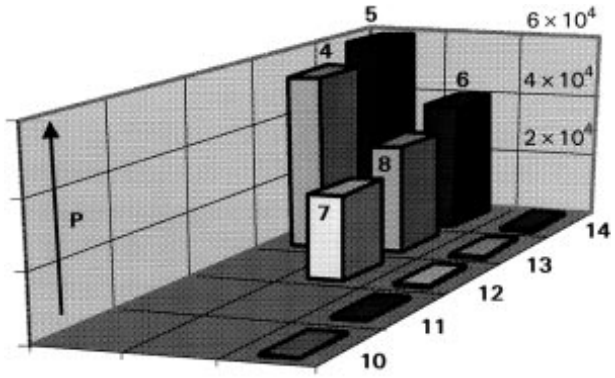

Figure 5. The combined effect of the two structural variations. P = partition coefficient.

partition coefficients of the more lipophilic porphyrin compounds, with partition coefficients of roughly up to 70 000 by UV and visible spectrophotometry, between 2-octanol and aqueous buffer systems. The method involves equilibration of a high concentration of the porphyrin initially dissolved in the organic solvent, between 2-octanol and the buffer system, followed by allowing a period for complete separation of the layers. The layers are then separated and analysed under identical conditions. To achieve this it proved effective to select the best conditions for analysis of the aqueous samples and then match these conditions with samples of the organic layer.

Due to very low concentrations of most of the porphyrins studied in the aqueous layers at pH 7·3, analysis of the aqueous layer required large samples (15 mL). These samples were then dissolved in a 1·2 M solution of HCl in methanol to give clear yellowish-green solutions of the dications of the porphyrins and the characteristic electronic absorption spectra shown in Figure 6b. To bring the organic phase samples to conditions identical to those under which the aqueous phase samples were measured, small, accurately measured amounts (0·5–2·0 mL) of the organic phase were taken and diluted with appropriate quantities of the methanolic HCl solution and the aqueous buffer system, to give the same characteristic electronic spectra shown in Figure 6a. The small amounts taken from the organic layers and the subsequent dilutions were calculated to give final solvent system compositions exactly matching those of the diluted aqueous layer samples and to give final porphyrin concentrations that are fairly close to those of the aqueous layer samples. All electronic spectrophotometric measurements were carried out using 100-mm path length cells to optimize sensitivity. The absorbance of the Soret bands (445–455 nm) were measured and used in subsequent calculations of the partition coefficients.

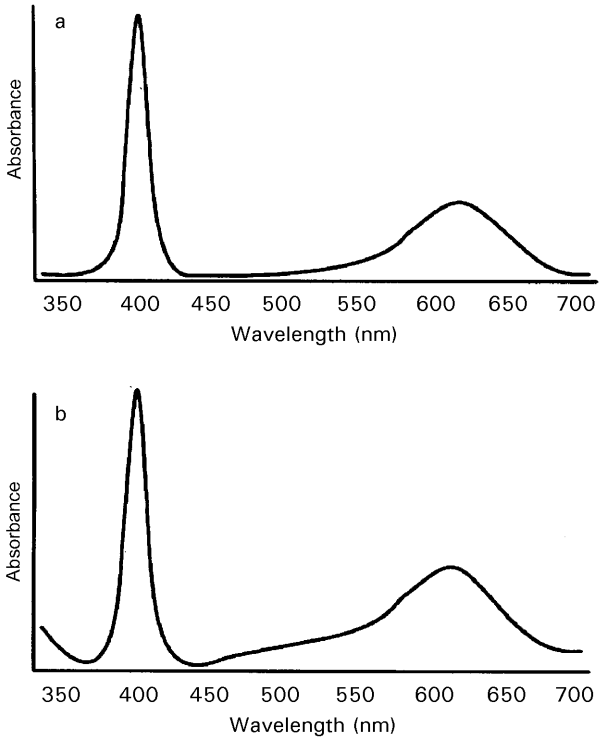

Figure 6. Characteristic electronic spectra of the dications of the porphyrins in a solution of phosphate buffered saline in 1·2 M methanolic HCl (PBS: $CH_3OH/HCl = 8:17$, v/v). a. Typical organic layer sample. b. Typical aqueous layer sample.

Calculation of the partition coefficients

The partition coefficients were calculated as

$$P = \frac{A(org.) \times d(org.)}{A(aq) \times d(aq)} \quad (1)$$

where A(org.) is the absorbance of the organic layer sample, A(aq) is the absorbance of the aqueous layer sample, d(org.) is the dilution factor for the organic layer sample, and d(aq) is the dilution factor for the aqueous layer sample.

The dilution factor is the ratio of the final volume of the sample to the volume of sample:

$$d(org.) = \frac{Vf(org.)}{V(org.)} \quad (2)$$

$$d(aq) = \frac{Vf(aq)}{V(aq)} \quad (3)$$

where Vf(org.) is the final volume of the sample from the organic layer, V(org.) is the volume of the sample of the organic layer, Vf(aq) is the final volume of the sample from the aqueous layer, V(aq) is the volume of the sample of the aqueous layer. Substitution of equation 1 with equations 2 and 3 gave equation 4, which is the equation used for the calculation of the partition coefficients:

$$P = \frac{A(org.) \times Vf(org.) \times V(aq)}{A(aq) \times V(org.) \times Vf(aq)} \quad (4)$$

In all the cases, however, the samples from the organic and aqueous layers were diluted to the same final volume, 50 mL. Thus Vf(org.) = Vf(aq), and equation 4 could be simplified to equation 5:

$$P = \frac{A(org.) \times V(aq)}{A(aq) \times V(org.)} \quad (5)$$

Partition coefficients

The partition coefficients of the *ortho*, *meta*- and *para*-tetrahydroxyphenyl porphyrins **3**–**5**, the fluorinated porphyrins **6**–**8**, and the tetra(2,3-dihydroxy-1-propyloxy)phenylporphyrins **9**–**14**, have been reproducibly measured between 2-octanol and PBS (pH = 7·3) (Table 1). A comparison of the results obtained from these measurements shows that the PBS system was sensitive to the structural features of the porphyrin solutes. The partition coefficients of the porphyrins decreased by a factor of approximately 130 as a result of the combined effect of the *ortho* fluorine substituents and conversion of the hydroxy groups of the tetrahydroxyphenyl porphyrins to 2,3-dihydroxy-1-propyloxy groups.

Conclusion

The presence of the fluorine substituents adjacent to hydroxy groups in the fluorinated tetrahydroxyphenylporphyrins **6**–**8** caused a slight increase in the hydrophilicity of these compounds, relative to the unfluorinated analogues **3**–**5**. However, the transformation of the hydroxy functional groups to 2,3-dihydroxy-1-propyloxy groups caused a substantial increase in the hydrophilicity. The combined effect of the presence of the fluorine substituents adjacent to the hydroxy groups and transformation of the hydroxy groups to 2,3-dihydroxy-1-propyloxy groups was to increase the hydrophilicity by more than two-orders of magnitude.

The substantial increase in hydrophilicity presents sufficient motivation for these compounds to be subjected to biological assays against cancer cell models in-vivo. Such biological studies would establish the extent to which the porphyrins **6**–**14** retain the biological activity that has been observed with the porphyrins **3**–**5** (Berenbaum et al 1986).

Acknowledgements

This work was supported by a generous grant from the National Research Foundation, by the University of Transkei, by the Luthuli Memorial Trust, and by the United Nations Education and Training Programme for Southern Africa. A generous grant from Sentrachem, and the support from the University of London and from Rhodes University are warmly acknowledged.

References

Berenbaum, M. C., Akande, S. L., Bonnett, R., Ioanou, S., White, R. D., Winfield, U.-J. (1986) *meso*-Tetra(hydroxyphenyl)porphyrins, a new class of potent tumour photosensitisers with favourable selectivity. Br. J. Cancer 54: 717–725

Bonnett, R., Berenbaum, M. (1989) Tumour photochemotherapy. Spectrum 218: 8–10

Bonnett, R., Songca, S. P. (1995) New fluorinated photosensitisers based on the *meso*-tetra(hydroxyphenyl)porphyrins. In: Brault, D., Jori, G., Moan, J., Ehrenberg, B. (eds) Photodynamic Therapy of Cancer II. Proc. SPIE 2325: 150–154

Bonnett, R., Ioannou, S., White, R. D., Winfield, U.-J., Berenbaum, M. C. (1987) *meso*-Tetra(hydroxyphenyl) porphyrins as tumour photosensitisers: chemical and photochemical aspects. Photobiochem. Photobiophys. (Suppl.) 45–56

De Smidt, P. C., Versluis, A. J., van Berkel, T. J. C. (1993) Properties of incorporation, redistribution, and integrity of porphyrin-low-density-lipoprotein complexes. Biochemistry 32: 2916–2922

Henderson, B. W., Dougherty, T. J. (1992) How does photodynamic therapy work? Photochem. Photobiol. 55: 145–157

Kessel, D. (1977) Effects of photoactivated porphyrins at the cell surface of leukemia L1210 cells. Biochemistry 16: 3443–3448

Kessel, D., Thompson, P., Saatio, K., Nantwi, K. D. (1987) Tumour localisation and photosensitisation by sulphonated derivatives of tetraphenylporphyrin. Photochem. Photobiol. 45: 787–790

Kongshaug, M., Moan, J., Brown, S. B. (1989) The distribution of porphyrins with different tumour localising ability among human plasma proteins. Br. J. Cancer 59: 184–188

Leo, A. J. (1986) Some advantages of calculating octanol-water partition coefficients. J. Pharm. Sci. 76: 166–168

Mbatha, B. (1999) Solubilization of Porphyrins used in Photodynamic Therapy of Cancer. MSc Thesis. University of Transkei

Momenteau, M., Oulmi, D., Maillard, P., Croisy, A. (1994) In vitro photobiological activity of a new series of photosensitizers: the glycoconjugated porphyrins. In: Brault, D., Jori, G., Moan, J., Ehrenberg, B. (eds) Photodynamic Therapy of Cancer II, Proc. SPIE 2325: 13–23

Oenbrink, G., Jurgenlimke, P., Gabel, D. (1988) Accumulation of porphyrins in cells: influence of hydrophobicity aggregation and protein binding. Photochem. Photobiol. 48: 451–456

Oulmi, D., Maillard, P., Guerquin-Kern, J. L., Huel, C., Momenteau, M. (1995) Glycoconjugated porphyrins. 3. Synthesis of flat amphiphilic mixed meso-(glycosylated-aryl)arylporphyrins and mixed *meso*-(glycosylated-aryl) alkylporphyrins bearing some mono- and disaccharide groups. J. Org. Chem. 60: 1554–1564

Songca, S. P. (1990) A Study of some Porphyrin Photosensitisers Used in Cancer Photodynamic Therapy. MSc Thesis. University of London

Songca, S. P., Bonnett, R., Maes, C. (1997) A convenient route to new fluorinated photodynamic therapeutic photosensitisers based on *meso*-tetra(hydroxyphenyl)porphyrins. S. Afr. J. Chem. 50: 40–43

Songca, S. P., Mbatha, B. (2000) Synthesis of solubilised *meso*-tetrahydroxyphenylporphyrin photosensitisers. S. Afr. J. Chem. In press

Woodburn, K. W., Stylli, S., Hill, J. S., Kaye, A. H., Reiss, J. A., Phillips, D. R. (1992) Evaluation of tumour and tissue distribution of porphyrins for use in photodynamic therapy. Br. J. Cancer 65: 321–328