An Improved Synthesis of Porphyrin C

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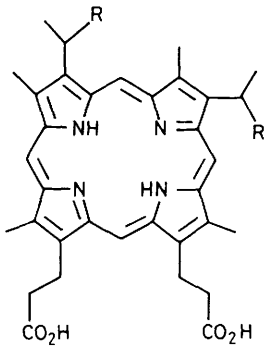
A synthesis of porphyrin c from haematoporphyrin in high yield and purity is described.

Porphyrin c (**3**) has been shown to localize in and to sensitize tumours to photoirradiation both *in vivo* and *in vitro*.[1—3] Although a number of procedures exist in the literature[4—9] for the preparation of porphyrin c, all result in low yields of relatively impure products that require purification. We originally prepared porphyrin c from haemin by the method of Neilands and Tuppy,[4] a two-step procedure involving formation of the dibromide adduct of protoporphyrin, 2,4-di-(1-bromoethyl)deuteroporphyrin (**2**), and treatment of this with L-cysteine at 160 °C to obtain crude porphyrin c. We have developed both analytical and preparative h.p.l.c. procedures for the purification of porphyrin c and for the separation of the free acid form of the diastereoisomers of porphyrin c.[10] However, we now report an improved synthesis of (**3**) from haematoporphyrin (**1**), that yields porphyrin c of high purity. The following procedure also utilizes the dibromide adduct (**2**) as an intermediate, and is a simple one-step procedure under mild conditions.



(1) R = OH
(2) R = Br
(3) R = $SCH_2CH(CO_2H)NH_2$

Typically, haematoporphyrin dihydrochloride (1 g) (from Roussel UCLAF, Sydney, Australia) and L-cysteine hydrochloride (2 g) (BDH, Melbourne, Australia) in a 100 ml flask were dried *in vacuo*. Hydrogen bromide in glacial acetic acid (45% w/v; 10 ml) was added and the mixture stirred rapidly at 21 °C for 40 min. The solvent was removed under high vacuum and the flask containing the residue submerged in a boiling water bath for 45 min while still connected to high vacuum (0.01 mmHg). The residue was cooled and dissolved in oxygen-free, ice-cold sodium hydroxide (1 M), and the porphyrin was precipitated by the addition of ice-cold oxygen-free hydrochloric acid (5 M) to pH 3.5. The porphyrin was isolated by centrifugation and was washed three times with oxygen-free, ice-cold water. The solid was dried *in vacuo* to give typically *ca.* 95% of the theoretical yield. During the preparation and handling of porphyrin c it is essential to minimize exposure of the porphyrin solutions to light and oxygen, in order to prevent oxidation. The porphyrin c was found by h.p.l.c.[2] to be *ca.* 95% pure, and its identity was confirmed by h.p.l.c. comparison with authentic samples, elemental analysis, ^1H and ^{13}C n.m.r.,[2] and fast atom bombardment mass spectroscopy.[10] This synthetic procedure takes advantage of the good nucleophilic properties of the cysteinyl thiol group and the volatility of the protonating reagent in order to give an essentially quantitative conversion of haematoporphyrin into porphyrin c. We found that, as in the preparation of protoporphyrin from haematoporphyrin,[11] impurities present in commercial haematoporphyrin samples are also converted into porphyrin c, resulting in high product purity. Samples of porphyrin c prepared by the above procedure, and by that of Neilands,[4] have been found to contain at least 4 diastereoisomers.[10] This method of synthesis has been used to incorporate ^{35}S into porphyrin c using ^{35}S-labelled L-cysteine.

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