Haematoporphyrin Derivative

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Summary Components of 'haematoporphyrin derivative,' a photosensitiser employed in clinical studies, have been separated as the free acids by reverse-phase high pressure liquid chromatography, and identified, the major components being *O,O'*-diacetylhaematoporphyrin and *O*-acetylhaematoporphyrin

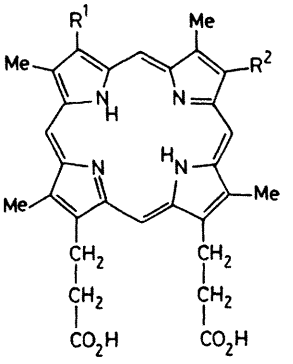
'Haematoporphyrin derivative' is the name given to a porphyrin preparation[1] which has been reported to be absorbed preferentially by tumour tissue, it appears to have potential in the detection (by fluorescence observations)[2] and the destruction (by virtue of its photodynamic effect)[3] of such tissue It is prepared by the action of sulphuric acid–acetic acid on haematoporphyrin dihydrochloride, and is a complex mixture, somewhat variable in composition We have sought to establish the chemical structures and biological activities of its individual components Because of the second objective we have worked with the porphyrin carboxylic acids throughout

Analysis of 'haematoporphyrin derivative' by h p l c using a reverse-phase column has led to the detection of protoporphyrin, haematoporphyrin, and the mono- and di-acetoxy derivatives of haematoporphyrin [4] This procedure has now been carried out on a preparative scale, and the products identified by comparison with authentic compounds 'Haematoporphyrin derivative' (294 mg) was fractionated on a column (200 g) of Merck Lichroprep RP-18 (25—40 μm) using aqueous methanol containing 4% acetic acid as eluant The components obtained, and the structures assigned to them, are shown in the Table

TABLE. Preparative separation of the components of 'haematoporphyrin derivative' (reverse-phase h.p.l.c., in order of elution).

Component	Wt. isolated/mg
Minor polar components	23[a]
Haematoporphyrin (**1**)	12[b]
O-Acetylhaematoporphyrin isomers (**2** and **3**)	42
3(8)-(1-Hydroxyethyl)-8(3)-vinyldeuteroporphyrin isomers (**4** and **5**)	13
O,*O*′-Diacetylhaematoporphyrin (**6**)	66
Minor components	17
3(8)-(1-Acetoxyethyl)-8(3)-vinyldeuteroporphyrin isomers (**7** and **8**)	20

[a] Contaminated with sodium chloride. [b] Estimated spectroscopically.



(**1**) $R^1 = R^2 = CH(OH)Me$
(**2**) $R^1 = CH(OH)Me$; $R^2 = CH(OAc)Me$
(**3**) $R^1 = CH(OAc)Me$; $R^2 = CH(OH)Me$
(**4**) $R^1 = CH(OH)Me$; $R^2 = CH{=}CH_2$
(**5**) $R^1 = CH{=}CH_2$; $R^2 = CH(OH)Me$
(**6**) $R^1 = R^2 = CH(OAc)Me$
(**7**) $R^1 = CH(OAc)Me$; $R^2 = CH{=}CH_2$
(**8**) $R^1 = CH{=}CH_2$; $R^2 = CH(OAc)Me$
(**9**) $R^1 = R^2 = CH{=}CH_2$

The major components are the *O*-monoacetyl (**2**, **3**) and *O*,*O*′-diacetyl (**6**) derivatives of haematoporphyrin. Products of elimination are present in modest amounts but only a trace of protoporphyrin was present. The components were identified by comparison with authentic samples of the free acids which were specially prepared and characterised for this work.† In addition, comparisons with the corresponding dimethyl esters were made. This allowed identification of the individual isomers (**4** and **5**) and (**7** and **8**).[5]

Some minor components remain unidentified, but all the fractions are available in a form (*i.e.* as the free acids) suitable for testing. We have developed an *in vivo* assay of biological activity in which the depth of necrosis in subcutaneously transplanted mouse tumours is measured after intravenous injection of porphyrin and exposure of the tumour to white light. The results of these biological assays will be reported elsewhere.

It may be noted that hydrolysis of the acetates occurs readily in alkaline solution. Thus, treatment of 'haematoporphyrin derivative' with 0·1M NaOH at room temperature for 1 h gives a mixture containing (**1**) (48%), (**4**) (30%), (**5**) (16%), and (**9**) (5%). This is an important observation since such treatment has, on occasion, been used to solubilise 'haematoporphyrin derivative' in water before injection.[6] Evidently, if reproducible results are to be obtained, the conditions for the treatment with base must be strictly defined.

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† Satisfactory elemental analyses were obtained for (**2**)/(**3**) (mixed isomers); (**5**) (isomers separated by preparative h.p.l.c.); (**6**); and (**8**). N.m.r. spectra (CD_3SOCD_3) for (**2**)—(**8**) accorded with the structures assigned.

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[4] R. Bonnett, A. A. Charalambides, K. Jones, I. A. Magnus, and R. J. Ridge, *Biochem. J.*, 1978, **173**, 693. The analytical separation is represented graphically in Figure 4 of this paper.
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