Identification and Classification of some Porphyrins on the Basis of their X-ray Diffraction Patterns

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The close structural and chemical similarity of the various porphyrins makes differentiation between them by the usual chemical and physical methods difficult. Successful characterization, using infrared spectra, has been reported by Gray, Neuberger & Sneath (1950) and by Falk & Willis (1951). X-ray diffraction patterns, being dependent on composition, molecular configuration and crystal structure, suggested an alternative method of identifying these substances. The use of X-ray diffraction in a particular instance in this field was reported by Siedel & Winkler (1943) but no numerical data were quoted. It is surprising that, while the X-ray crystallography of the related phthalocyanin pigments has been extensively studied (Robertson, 1935, 1936; Robertson & Woodward, 1937, 1940), little attention has been paid to the porphyrins, though a preliminary report on tetramethylhaematoporphyrin has been given by O'Daniel & Damaschke (1942), on aetioporphyrin I by Christ & Harker (1942) and on tetrabenztriazaporphin by Barrett, Linstead, Tuey & Robertson (1939). One reason for this neglect may be the scarcity of the materials and the difficulty of obtaining pure specimens. Chromatographic methods, recently introduced (Nicholas & Rimington, 1949, 1951*a*; Nicholas, 1951; Chu, Green & Chu, 1951), have now simplified preparative procedures and provided sensitive criteria of purity. This paper describes an investigation of the X-ray diffraction patterns of powder samples of the methyl esters of such carefully purified porphyrins.

EXPERIMENTAL

Most of the samples crystallized as fine prisms or needles of microscopic size, and attempts at growing large crystals were unsuccessful. The Debye-Scherrer powder technique was therefore used throughout. The samples (about 10 μg.) were enclosed in thin-walled capillary tubes. The photographs were taken in a cylindrical camera 6 cm. diameter with filtered copper radiation. Diffraction patterns of some of the 'Waldenström' esters and of artificially prepared mixtures were recorded on a flat plate 6 cm. from the specimen. The diameters of the arcs were measured with a Hilger film measuring rule and the intensities estimated visually. Measurements of interplanar spacing were accurate to about 3%. The quality of the photographs was greatly influenced by the degree of crystallinity of the powder, diffuse bands being frequently encountered. When possible, definition was improved by repeated crystallization.

MATERIALS

Porphyrins are most readily prepared from natural sources, but these usually contain complex mixtures of different porphyrins. Even synthetic methods lead to mixtures which are difficult to separate. All specimens used for this work were rigorously purified by chromatography of their esters, recrystallization, etc. (Nicholas & Rimington, 1949, 1951*a*; Nicholas, 1951). Their origin was as follows.

Porphyrin esters of known structure

Protoporphyrin dimethyl ester IX. (*a*) Specimens prepared from haemin (Grinstein, 1947), m.p. 225°. We are grateful to Mr J. Scott and Dr J. Kench for providing some of these specimens. (*b*) An exceptionally well crystallized specimen, m.p. 227°, from faeces of a case of porphyria cutanea tarda (Macgregor, Nicholas & Rimington, 1952).

Mesoporphyrin dimethyl ester IX. Prepared from protoporphyrin, m.p. 212°.

Mesoporphyrin dimethyl ester I. Synthetic specimen from H. Fischer's collection for which we thank Prof. S. Goldschmidt; m.p. 190°.

Deuteroporphyrin dimethyl ester IX. Prepared from haemin (Fischer & Orth, 1937), m.p. 218°.

Coproporphyrin tetramethyl ester I. (*a*) Synthetic specimen from H. Fischer's collection, rechromatographed, m.p. 256°. (*b*) From calf meconium, m.p. 250–251°.

Coproporphyrin tetramethyl ester III. (*a*) From *Corynebacterium diphtheriae* (Gray & Holt, 1948); m.p. 160/178°. (*b*) Similarly prepared and kindly provided by Prof. J. Hale; m.p. 154/179°. (*c*) Isolated from pathological materials; m.p. 154/174°. (*d*) Isolated from pathological urine, m.p. 178/183°; crystallized spontaneously in the higher melting-point form.

Uroporphyrin octamethyl ester I. From congenital porphyria urine (Rimington & Miles, 1951) rechromatographed upon $CaCO_3$, m.p. 293°.

Uroporphyrin octamethyl ester III. From turacin (Nicholas & Rimington, 1951*b*); m.p. 264°.

Porphyrin esters which are presumably mixtures or for which the structure has not been definitely established

Uroporphyrin 'Beresf.' Ester of a 'Waldenström' porphyrin (octacarboxylic only) from a case of acute porphyria; m.p. 260/261°. Yields coproporphyrins I and III on decarboxylation. Three different preparations from this patient were examined.

Uroporphyrin 'P.Wri.' Similar to above; m.p. 262°.

Uroporphyrin 'McLaugh.' Similar to above; m.p. 255/256°.

Uroporphyrin 'L.Walk.' Ester of a urinary porphyrin (octacarboxylic only) from a case of porphyria cutanea tarda; m.p. 260°. Yields coproporphyrins I and III on decarboxylation.

Uroporphyrin 'Ance.' Ester of a urinary porphyrin (octacarboxylic only) from a case of cutaneous porphyria (Dr J. Canivet); m.p. 272–278°.

Uroporphyrin 'Turp.' Similar to above; m.p. 272/279°.

Uroporphyrin 'Four.' Similar to above; m.p. 260/269°.

Uroporphyrin 'McCawl.' Ester of a urinary porphyrin (octacarboxylic only) from a case of porphyria cutanea tarda; m.p. 260°. Crystallizes in stout needles or fasciculi of fine, hair-like needles. Behaves abnormally on decarboxylation.

Uroporphyrin 'II.' Octamethyl ester of a porphyrin prepared synthetically by Dr S. F. McDonald of Cambridge; m.p. 256–258°. Constitution still uncertain but X-ray pattern strikingly similar to uroporphyrin 'McCawl.'

Uroporphyrin mixtures. The mixtures of uroporphyrins I and III were prepared by mixing $CHCl_3$ solutions of the two esters in the required proportions, evaporation to dryness, dissolving the residue in a little benzene and inducing crystallization by the slow addition of light petroleum. The mother liquor in each case contained only a trace of porphyrin ester. The more usual solvents, $CHCl_3$: methanol, were avoided as complete crystallization could not be obtained. It was, however, observed that crystallization from $CHCl_3$: methanol was poor until the composition 75% III isomer + 25% I isomer was reached; this mixture separated readily in very well formed crystals.

RESULTS AND DISCUSSION

Measurements for the interplanar spacings from different specimens of the same porphyrin were in satisfactory agreement in every case. Only one set of results is therefore reproduced under each heading in the tables. The patterns of some of the powder photographs are shown in Fig. 1 and the calculated interplanar spacings and relative intensities in Table 1.

A comparison of the series I and III isomers showed these to possess distinctly different crystal structures, consequent upon the differences in symmetry of arrangement of side chains between the series III and I types of molecules. The difference was marked for all the porphyrin isomers compared (Table 1).

Powder photographs of mechanical mixtures show all lines, characteristic of the various components, superimposed. In mixtures of coproporphyrin esters, the presence of about 15% of either isomer could be detected; this is the order of sensitivity given by other techniques (cf. Falk & Benson, 1953). If, however, two porphyrin isomers crystallize together from the same solvent, they either form crystalline solid solutions showing a distinct X-ray powder pattern or the presence of one isomer interferes with crystallization of the

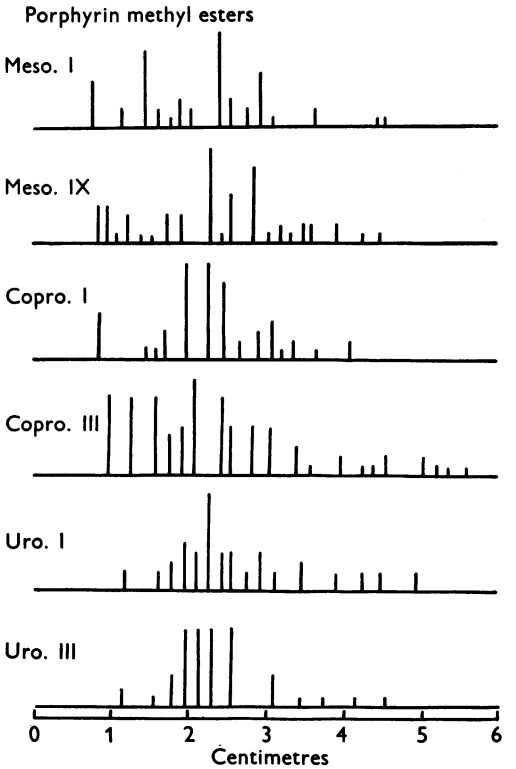


Fig. 1. Schematic representation of the positions and relative intensities of diffracted X-rays on the powder photographs of various porphyrin esters.

major constituent. In the case of uroporphyrin III, the presence of even 5% or less of the series I isomer results in a diffuse photograph showing one or two lines only. This method would seem, therefore, to provide an extremely sensitive criterion of purity.

Typical 'Waldenström' esters produced a characteristic powder pattern, with small variations in the spacing of the individual lines; Table 2 summarizes the results obtained from the various samples examined. Waldenström (1934, 1935) and Waldenström, Fink & Hoerburger (1935) and Mertens (1936, 1937) first isolated from acute porphyria urines a uroporphyrin different from uroporphyrin I; this was thought to be uroporphyrin III, since

Table 1. *X-ray diffraction patterns of porphyrin esters*

(Interplanar spacings in Ångström units.)

Coproporphyrin I	Coproporphyrin III	Uroporphyrin I	Uroporphyrin III	Protoporphyrin IX	Mesoporphyrin I	Mesoporphyrin IX	Deuteroporphyrin IX
2·32 W	1·71 W	1·93 W	2·08 W	1·88 W	2·09 W	2·12 W	1·68 W
2·60 W	1·77 W	2·12 W	2·28 W	1·98 W	2·13 W	2·22 W	1·74 M
2·77 W	1·83 W	2·23 W	2·51 W	2·12 W	2·59 W	2·41 W	1·85 W
2·91 W	1·88 W	2·42 W	2·72 W	2·43 W	3·02 W	2·62 W	1·96 W
3·02 M	2·07 W	2·73 M	3·02 M	2·63 W	3·19 S	2·70 W	2·06 M
3·20 M	2·15 W	3·02 M	3·63 S	3·04 M	3·38 W	2·82 W	2·18 W
3·51 W	2·21 W	3·21 M	4·02 S	3·45 S	3·65 M	2·94 W	2·24 W
3·74 S	2·36 W	3·41 W	4·35 S	3·66 S	3·85 S	3·09 W	2·37 W
4·10 S	2·62 W	3·69 M	4·67 S	3·99 M	4·58 W	3·29 S	2·51 W
4·67 S	2·76 M	3·87 M	5·18 M	4·17 M	4·87 M	3·65 S	2·62 M
5·41 M	3·06 S	4·13 S	5·91 W	4·82 M	5·22 W	3·83 W	2·75 W
5·83 W	3·29 S	4·46 M	8·12 W	5·61 M	5·75 W	4·10 S	3·23 S
6·42 W	3·66 S	4·80 M		6·07 M	6·37 S	4·90 M	3·47 S
	3·82 S	5·25 M		7·31 W	8·04 W	5·44 M	3·87 S
	4·44 S	5·79 W		8·19 M		6·15 W	4·04 M
	4·82 S	8·04 W		9·61 W		6·76 W	4·35 S
	5·25 M					7·76 M	4·58 W
	5·87 S					8·59 W	5·01 M
	7·31 S					9·36 M	5·47 M
	9·51 S						6·51 S
							8·59 S

Intensity scale: S = strong, M = medium, W = weak.

Table 2. *X-ray diffraction patterns of Waldenström type esters (McLaugh., Beresf. and P. Wri.) and urinary uroporphyrin esters from cases of cutaneous porphyria (L. Walk., Ance., Turp. and Four.)*

(Interplanar spacings in Ångström units.)

McLaugh.	Beresf.	P. Wri.	L. Walk.	Ance.	Turp.	Four.
3·19 W	—	—	3·16 W	—	—	—
3·63 M	3·63 M	—	3·63 M	—	—	—
—	—	—	—	3·87 W	3·89 W	—
4·21 S	4·13 S	4·17 S	4·11 S	4·10 S	4·15 S	4·17 S
—	—	—	—	4·40 W	4·44 W	—
4·67 S	4·67 S	4·67 S	4·67 S	4·77 W	4·77 W	—
5·28 M	5·22 M	—	5·22 M	5·18 W	—	—
8·35 W	—	8·76 W	8·51 W	—	—	—

Intensity scale: S = strong, M = medium, W = weak.

Table 3. *X-ray diffraction patterns of crystalline solid solutions containing varying proportions of esters of uroporphyrins I and III*

(Interplanar spacings in Ångström units.)

Percentage of isomer I								
100	95	85	80	75–30	25	10	5	0
2·73 M	—	2·82 W	2·75 W	—	—	—	—	—
3·02 M	3·07 W	3·03 W	2·99 W	—	—	—	—	3·02 M
3·21 M	—	—	—	—	3·15 M	—	—	—
3·69 M	—	—	3·69 W	—	3·61 M	—	—	3·63 S
3·87 M	3·90 W	3·85 W	—	—	—	—	—	—
—	—	—	—	—	—	—	—	4·02 S
4·13 S	4·15 S	4·19 S	4·09 S	4·15–4·19 S	4·10 S	4·08 S	4·06 S	—
—	—	—	—	—	—	—	—	4·35 S
4·46 M	4·46 W	—	—	—	—	—	—	—
—	—	—	—	—	—	4·60 M	—	—
—	—	—	—	—	—	—	—	4·67 S
4·80 M	4·80 W	4·72 W	4·82 W	—	4·70 S	—	—	—
5·25 M	—	—	—	—	5·21 W	—	—	5·18 M

Intensity scale: S = strong, M = medium, W = weak.

coproporphyrin III was identified in the products of decarboxylation. Doubt has been cast, however, upon the homogeneity of the 'Waldenström' porphyrin by Grinstein, Schwartz & Watson (1945) and Watson, Schwartz & Hawkinson (1945). These workers considered the 'Waldenström' porphyrin to be uroporphyrin I accompanied by a heptacarboxylic porphyrin in smaller quantity. Methyl esters prepared from 'Waldenström' urinary porphyrins crystallize very readily and have fairly sharp melting points, usually lying within the range 250–260°, not far removed from the melting point (264°) of authentic uroporphyrin III (Nicholas & Rimington, 1951*b*).

All the 'Waldenström' esters used in the present work yielded both coproporphyrins I and III on decarboxylation. A series of mixed crystals, containing variable proportions of uroporphyrins I and III was therefore examined (Table 3). Decreasing the proportion of uroporphyrin I in the mixture resulted in progressively poorer crystallization; between 75 and 30 % only one powder line appeared, intermediate between the spacings of the strongest planes of the pure isomers. At 25 % uroporphyrin I content, there was a sudden improvement in crystallization and a well-defined diffraction pattern was produced. On decreasing the uroporphyrin I content further, crystallization was again poor and no sharp pattern was obtained until no series I isomer was present. It is of interest that the melting points of this series lie on a curve which exhibits a pronounced minimum, suggestive of a eutectic mixture, at a content of 75 % uroporphyrin I (Nicholas & Rimington, 1953).

The powder pattern of the 'Waldenström' esters resembled the pattern of the solid solutions at around 25 % uroporphyrin I composition. While deductions based on such few powder lines must necessarily have limited validity, the close agreement between the interplanar spacings, as well as relative intensities, lends support to the view that these 'Waldenström' esters are solid solutions of the two isomers containing around 25 % of uroporphyrin I and possibly accompanied by very markedly smaller quantities of other porphyrins (see Pl. 2 and Tables 2 and 3).

The specimen 'L. Walk.' was obtained from a case of porphyria cutanea tarda and had m.p. 260°. It appeared to be quite similar to the 'Waldenström' porphyrins examined and gave an identical powder pattern. The urinary uroporphyrins from the three French cases of cutaneous porphyria were kindly placed at our disposal by Dr J. Canivet of the Saint Louis Hospital, Paris. They were selected for comparison because other methods (Nicholas & Rimington, 1953; Falk & Benson, 1953) indicated that in each the series I isomer of uroporphyrin predominated (less markedly in 'Four'). None had experienced any porphyria symptom other than photosensitization and formation of bullae. The ester melting points were 'Ance.' 272/278°, 'Turp.' 272/279° and 'Four.' 260°; thus the first two resembled the urinary uroporphyrin of congenital porphyria, while the specimen from 'Four.' had a melting point similar to that of a typical 'Waldenström' porphyrin ester. The specimens from 'Ance.' and 'Turp.' gave X-ray powder patterns indicative of a mixture of 80–95 % uroporphyrin I with 5–20 % of uroporphyrin III, whilst with 'Four.' only a single line at 4·17 Å appeared, as is found in mixtures of the two isomers extending from 80 to 25 % uroporphyrin I (Pl. 2 and Tables 2 and 3). Other evidence (Nicholas & Rimington, 1953) supported the conclusion that the proportion of uroporphyrin I in this material is around 75–79 %.

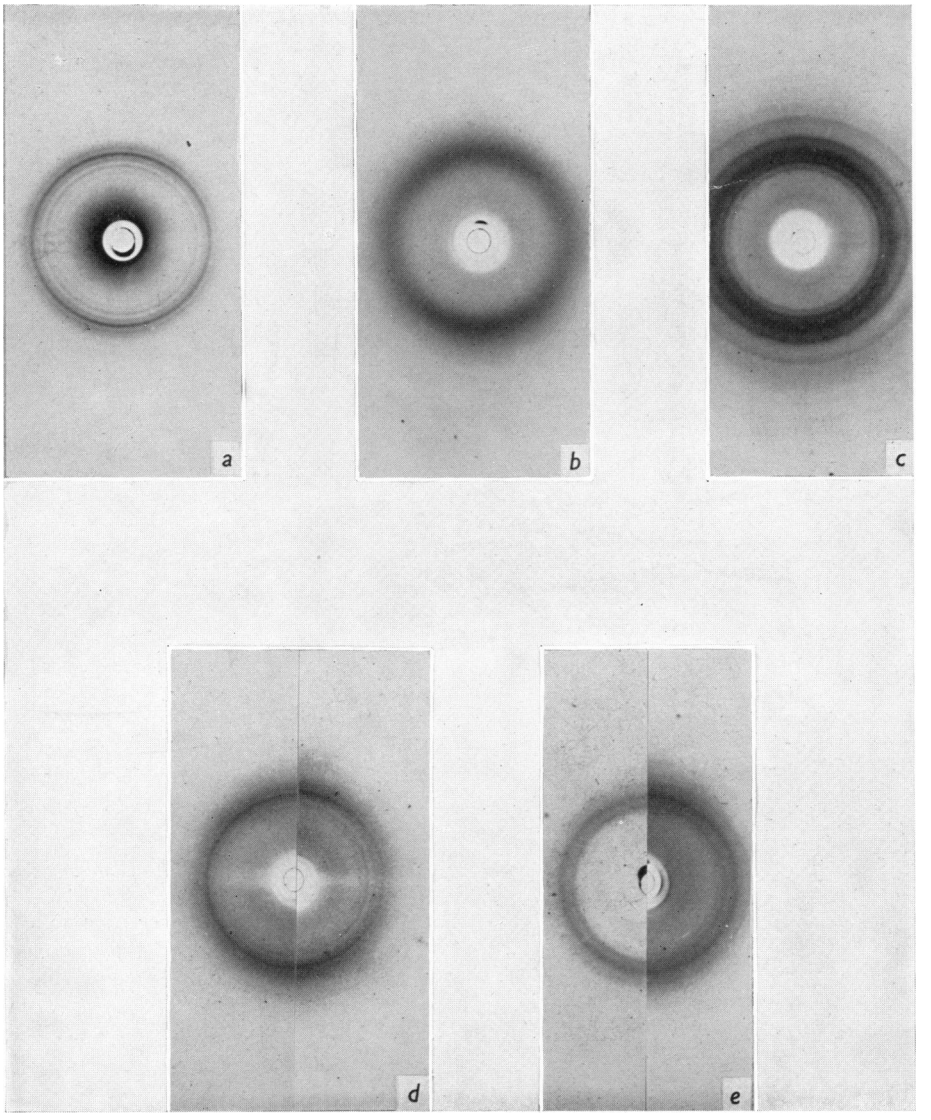
The specimen of urinary porphyrin 'McCawl.' is of special interest. This was a well crystallized methyl ester, m.p. 260°, which by elementary, methoxyl and chromatographic analyses was shown to consist of only octacarboxylic porphyrin of the uro type. The appearance of the crystals was unusual for uroporphyrin and, after melting, the mass recrystallized on the slide and remelted at the same temperature as before. The behaviour of this uroporphyrin on decarboxylation was also unusual and preliminary results have been inconclusive. The powder pattern was different from that of all the other octacarboxylic porphyrins examined except a synthetic specimen made by MacDonald (1952) (Table 4). Careful comparison showed identity with this ester. The structure of MacDonald's porphyrin is uncertain, but the synthetic route used might be expected to lead to uroporphyrin II as the main product. The infrared spectra of the synthetic and the natural porphyrins also proved to be identical (MacDonald, private communication). The elucida-

Table 4. *Comparison of X-ray diffraction patterns of esters of a synthetic uroporphyrin preparation (MacDonald, 1952) and of a urinary uroporphyrin from a case (McCawl.) of porphyria cutanea tarda*

(Interplanar spacings in Ångström units.)

Synthetic	McCawl.
2·05 W	2·21 W
2·21 W	2·57 W
2·54 W	2·90 M
2·91 W	3·08 M
3·07 W	3·29 M
3·27 W	3·47 W
3·45 W	3·59 W
3·60 W	3·82 S
3·82 M	4·15 S
4·13 S	4·46 S
4·51 S	5·31 W
	9·03 W

Intensity scale: S = strong, M = medium, W = weak.



Powder photographs of uroporphyrin methyl esters. (*a*) Pure uroporphyrin I; (*b*) uroporphyrin III containing 5% uroporphyrin I; (*c*) pure uroporphyrin III; (*d*) composite photograph; left side: crystalline solid solution containing 90% uroporphyrin I and 10% uroporphyrin III; right side, urinary uroporphyrin ester from a case of cutaneous porphyria; (*e*) composite photograph; left side, crystalline solid solution containing 25% uroporphyrin I and 75% uroporphyrin III; right side, 'Waldenström' type porphyrin from acute porphyria urine.

tion of the structure of this material awaits the outcome of further work which is in progress.

The present work has demonstrated that powder patterns can be used to characterize porphyrins of established structure and to detect the presence of unusual porphyrins by departure from the standard patterns. Certain points of similarity between the powder patterns of various porphyrin esters are evident from Fig. 1. Full interpretation of the X-ray diffraction patterns of these substances will, however, have to wait upon the completion of studies on single crystals.

SUMMARY

1. The Debye-Scherrer powder patterns of pure porphyrin esters of known structure and configuration have been recorded.

2. A qualitative analysis of mixtures of porphyrin isomers has been made. It is shown that the powder pattern affords a very sensitive criterion of purity.

3. Powder patterns of methyl esters of porphyrins from various natural sources have been compared with recorded reference patterns.

4. The nature of the 'Waldenström' porphyrin present in urines from cases of acute porphyria is discussed in the light of the present findings.

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Studies on the 'Waldenström Porphyrin' of Acute Porphyria Urines

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Since the naturally occurring uroporphyrins have not yet been synthesized, conclusions as to their structure are based upon their chemical reactions, particularly their partial decarboxylation to the corresponding coproporphyrins. Identification of the coproporphyrin isomer indicates to which series the parent uroporphyrin belonged.

The first uroporphyrin (m.p. of octamethyl ester 290°), isolated by Fischer (1915) from the urine of a patient with congenital porphyria, yielded coproporphyrin I and was thus designated uroporphyrin I, and from supporting evidence (summarized by Nicholas & Rimington, 1951*a*) was assigned the structure porphin-1:3:5:7-tetraacetic acid-2:4:6:8-