SUMMARY

1. β-Ketoadipic acid has been shown to arise by ring fission in the metabolism of phenol and benzoic acid by a vibrio species.

2. The keto acid is subsequently degraded by the vibrio to succinic acid.

The author wishes to express his thanks to Prof. F. C. Happold for suggesting the problem and for his interest and help during the work.

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Comparison of Haem *a*, the Dichroic Haem of Heart Muscle, and of Porphyrin *a* with Compounds of Known Structure*

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In his classical studies on the photochemical absorption spectrum of the respiratory ferment, Warburg (for review see Warburg, 1949) concluded that its prosthetic group is a haem with a formyl side chain, related to chlorocruorohaem ('Spirographis' haem) or to phaeohaem b_6 (iron complex of compound, formula III). The absorption spectra of the cytochromes *a*, a_1 and a_3 (the last named probably identical with cytochrome oxidase and the respiratory ferment) suggest that these are all derived from the same type of haem and possibly the same haem (cf. Lemberg & Legge, 1949). Attempts to isolate this haem from muscle have led to somewhat controversial results. It has been possible to isolate such a haem from heart muscle (Negelein, 1933; Roche & Bénévent, 1936), but there has been no complete agreement as to its properties. The porphyrin ('cryptoporphyrin') isolated by Negelein (1932*a*) from pigeon-breast muscle was later considered an artifact by this same author (1932*b*); this may well be so, but we have not been able to confirm Negelein's opinion that it arose through the action of light on protoporphyrin in acid solution during the isolation (cf. also Rawlinson & Hale, 1949). The divergences were probably due to the difficulties of isolation and to the lability of haem *a*, and not to the existence of a variety of haems *a* derived from different cytochromes, although such a possibility has not yet been definitely excluded.

At the time of Warburg's investigations only a small number of porphyrin derivatives with carbonyl groups in the side chains was known, but it has been greatly increased since, particularly through the work of Fischer. It appeared necessary to compare their properties and the properties of their haem compounds systematically with those of porphyrin *a* and haem *a*. For this it was necessary to reinvestigate the properties of the latter compounds. This study had just begun when, through the kindness of Dr W. A. Rawlinson, we were informed of the isolation of haem *a* and porphyrin *a* from ox-heart muscle and from *Corynebacterium diphtheriae* by an improved method (Rawlinson & Hale, 1949). We have, therefore, concentrated mainly on the comparison of these products with porphyrins and haems of known structure containing carbonyl groups in conjugation with the nucleus in one or two pyrrole rings. The three schools working on haem *a* (at the Department of Chemical Pathology, University

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College Hospital Medical School, London, at the Department of Biochemistry, University of Melbourne, and the Institute of Medical Research, Royal North Shore Hospital of Sydney), have collaborated for some time and presented three conjoint papers to the First International Congress of Biochemistry at Cambridge (see Rimington, Hale, Rawlinson, Lemberg & Falk, 1949). Close contact is maintained, but to minimize publication difficulties each group reports separately the work it has performed.

The properties of haemochromogen *a* support the previous assumption (Warburg, 1949) of an aldehyde (formyl) side chain. Stern and his co-workers (Stern & Wenderlein, 1934, 1935*a–c*, 1936; Stern & Molvig, 1936), have found correlations between the nature and orientation of carbonyl side chains on porphyrins, on the one hand, and the type of neutral porphyrin spectrum on the other. Porphyrins without such groups have, as a rule, an aetio-type spectrum in which the intensity of the bands increases stepwise from band I (that at longest wavelengths) to band IV (at shortest wavelengths). The order of intensities in this type of spectrum is conventionally stated as IV, III, II, I (Fig. 1*a*). The introduction of a single carbonyl group conjugated with the nucleus produces a rhodo-type spectrum (order of intensities: III, IV, II, I; cf. Fig. 1*b*). The conversion of the aldehyde or ketone into an oxime or hydrazone abolishes or greatly decreases the 'rhodofying effect' of the side chain. Introduction of a second carbonyl group on a pyrrole ring vicinal to the one substituted by the first, abolishes the effect of the first and leads to a spectrum of aetio type. Introduction of the second carbonyl group on a pyrrole ring opposite to the one substituted by the first, leading for example to carbonyl substitution on rings I and III, enhances the effect of the first with appearance of a spectrum similar to that of oxorhodoporphyrin (XXI), which we therefore call oxorhodo type (order of intensities III, II, IV, I; cf. Fig. 1*c*).

These correlations have been found to hold good in all instances examined, even where earlier data indicated exceptions, and together with other experimental data they allow certain conclusions about the structure of porphyrin *a* to be drawn. Such an approach to the problem is particularly appropriate in view of the great difficulty in obtaining haem *a* in a pure state in amounts sufficient for chemical studies. More evidence will, however, be required before the number of possible structures is limited to such a degree that an attempt at synthesis can be made with hope of success. One way in which we are seeking more evidence is through a systematic study of the infrared spectra.

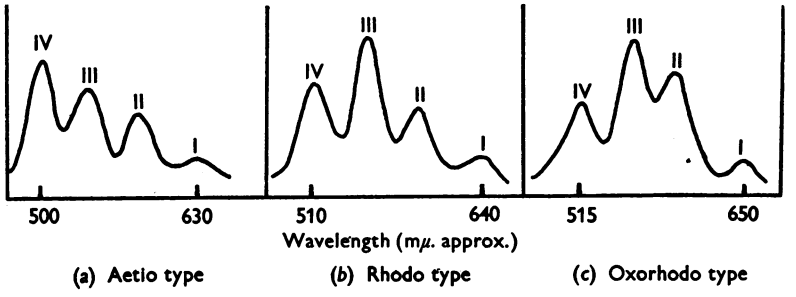


Fig. 1. Types of spectra of porphyrins in the visual region, in neutral solvents.

EXPERIMENTAL

Methods

Melting points were determined with a Gallenkamp electric hot-stage micro-melting point apparatus, and were corrected.

Absorption spectra were measured with a Hilger-Nutting visual spectrophotometer, a Beckman photoelectric spectrophotometer or a Beck-Hartridge reversion spectroscope, as indicated in the tables.

Dioxan was purified by the method of Eigenberger (1931). A solution of protoporphyrin ester in this dioxan did not change in spectroscopic properties after standing immersed in a boiling-water bath for 2 hr. in subdued light. This is a sensitive test; in the presence of traces of peroxides protoporphyrin is quickly oxidized, a strong absorption band appearing at 660 mμ. after a few minutes.

Hydrogen peroxide-ether. H_2O_2 (30%) was extracted with ether for several days in a liquid-liquid extractor, and the ethereal H_2O_2 solution dried over anhydrous Na_2SO_4. For titration 1 ml. was diluted to 100 ml. with distilled water; 10 ml. of this solution required 2·4 ml. 0·1 N-$KMnO_4$.

Hydrochloric acid concentrations. Because the familiar HCl number (Willstätter number), widely used in the purification of porphyrins, is stated in terms of % (w/v) HCl, this form is used instead of normality (1% HCl ≡ 0·274 N).

Haemins or their esters were prepared from the corresponding porphyrins or esters by the ferrous acetate-acetic acid method of Warburg & Negelein (1932*a*), except chlorocruorohaemin and haemin *a*, which were isolated from natural materials.

Oximes of porphyrins containing carbonyl groups were prepared as follows. The porphyrin ester was dissolved in pyridine, and to the solution, refluxing gently on a sand bath, an excess of a mixture of equivalent amounts of solid hydroxylamine hydrochloride and Na_2CO_3 was added. Refluxing was continued for 15 min., the solution cooled and the products taken into ether. After washing the ether solution free from pyridine with water, it was dried over anhydrous Na_2SO_4. When sufficient material was available the oximes were crystallized, as described below.

Substances used in this investigation

Monoacetyldeuteroporphyrin IX dimethyl ester. This was a small sample from the collection of the late Prof. H. Fischer, kindly made available by Prof. S. Goldschmidt. Its mode of preparation is not known to us, but its m.p. (243·5°) is close to that of the 4-acetyl isomer described by Fischer & Wecker (1942).

Oxime. A sample sufficient only for spectroscopy was made.

2:4-*Diacetyldeuteroporphyrin IX dimethyl ester* (*formula XI, Table* 2). Deuterohaemin, prepared from haemin by the resorcinol fusion method of Schumm (1928) was acetylated with acetic anhydride in the presence of $SnCl_4$ at 4° (Fischer & Zeile, 1929). From the crude diacetyldeuterohaemin, the porphyrin ester was prepared by the method of Grinstein (1947). After separation from by-products by ether-HCl fractionation, the ester was crystallized and twice recrystallized from $CHCl_3$-acetone; m.p. 236°.

Dioxime. About 5 mg. of the porphyrin ester was converted to the dioxime.

Monoformyldeuteroporphyrin IX dimethyl ester. Deuterohaemin, prepared as above, was converted by the method of Grinstein (1947) to deuteroporphyrin ester. This was saponified and purified by ether-HCl fractionation; after re-esterification and three crystallizations from $CHCl_3$-methanol its m.p. was 224°. From this deuteroporphyrin ester, deuterohaemin ester was prepared by the ferrous acetate method. The deuterohaemin ester was formylated by the dichloromethyl-ethyl-ether method of Fischer & Wecker (1942) (cf. also Fischer & Beer, 1936), and the crude formyldeuterohaemin converted to porphyrin by stirring for 10 min. in conc. H_2SO_4. The acid solution was thrown into crushed ice, and after further dilution the porphyrin was taken into ether. After washing the ether solution until neutral, the crude formyldeuteroporphyrin was extracted into 7% (w/v) HCl, precipitated by sodium acetate, collected at the pump and dried. Removal of iron from the crude formyldeuterohaemin by conc. H_2SO_4, by the ferrous acetate method (Warburg & Negelein, 1932*a*) and by the method of Grinstein (1947), gave products with similar absorption in the visible region. The crude monoformyldeuteroporphyrin was now esterfied with methanol saturated with dry HCl, and the ester crystallized from $CHCl_3$-methanol. From this material (m.p. 217°) extraction with hot methanol removed a small fraction of m.p. 221–224° and extraction of the remainder by $CHCl_3$, followed by two crystallizations from $CHCl_3$-methanol, led to a product of m.p. 231°. This presumably corresponds to the product of Fischer & Wecker (1942), of m.p. 232°, which they stated to be a mixture of the 2- and 4-formyl isomers. Neither of their two methods for the isolation of the 4-isomer (m.p. 260°) was successful in our hands. For our purposes the separation was not of great importance.

Oxime. From the porphyrin ester of m.p. 231° the oxime was prepared as previously described, and after recrystallization from $CHCl_3$-methanol had m.p. 222–223°. Fischer & Beer (1936) described an oxime of m.p. 226°; Fischer & Wecker (1942) described no simple derivatives of their porphyrin of m.p. 260°.

2:4-*Diformyldeuteroporphyrin IX dimethyl ester* (*formula X*). This was prepared from pure protoporphyrin dimethyl ester (m.p. 230°) by OsO_4-H_2O_2 oxidation, by a modification of the method of Fischer & Deilmann (1944). Protoporphyrin ester (100 mg.) was dissolved in 40 ml. dioxan by heating on a steam bath. To this, 2 ml. of a solution of 1 g. OsO_4 in 100 ml. dry ether and 11 ml. H_2O_2-ether (cf. p. 675) were now added, and the mixture refluxed vigorously for 5 min., the protoporphyrin band at 630 mμ. now being no longer visible by the hand spectroscope. Longer heating decreases the yield. In a series of experiments the yields after 5, 15 and 90 min. refluxing were 12, 7·2 and 2·7%, respectively. When, instead of refluxing, the reaction mixture was allowed to stand for 30 min. at room temperature (19°) a yield of 8·7% was obtained. Unreacted protoporphyrin makes working up much more difficult, and the reaction must be stopped when the hand spectroscope shows that the protoporphyrin has just disappeared. This was done by pouring into 2 vol. water, from which the products were extracted with $CHCl_3$. The $CHCl_3$ solution was washed ten times with water, dried over anhydrous Na_2SO_4, and passed through an alumina column (British Drug Houses Ltd. 'For chromatographic adsorption analysis', or Savory & Moore 'nach Brockmann'). The diformyldeuteroporphyrin ester passed through the column faster than the various by-products, which mostly remained as a strongly adsorbed, dark-coloured band at the top of the column; on development with $CHCl_3$ most of the diformyl compound ran through before a small green-yellow band which followed it. The latter contains a porphyrin with a rhodo-type spectrum, and in addition a substance with a strong band at 660 mμ. The $CHCl_3$ solution of the diformyl compound was chromatographed again in the same way through fresh alumina, and, examined by the hand spectroscope, then appeared to have a pure diformyldeuteroporphyrin spectrum. The $CHCl_3$ solution was then concentrated *in vacuo* to about 2 ml. and, on standing for a short time, the ester crystallized. On recrystallization from $CHCl_3$-acetone, 12 mg. of material of m.p. 280° (decomp.) were obtained. The absorption spectrum in $CHCl_3$ was identical with that of Fischer & Deilmann's (1944) product as far as could be determined from their small published curve (cf. Table 1).

Dioxime. Prepared by the method of Fischer & Deilmann (1944). On crystallization from $CHCl_3$ its m.p. was 231–232°, unchanged by recrystallization.

Copper complex. This was prepared by the reaction of a solution of the porphyrin ester in glacial acetic acid with an acetic acid solution of copper acetate. The copper complex crystallized from the reaction mixture and was recrystallized from $CHCl_3$-methanol; m.p. 290°. Absorption spectrum in the reversion spectroscope: in $CHCl_3$, I, 597 mμ.; II, 553 mμ. Order of intensities: I, II. In pyridine-ether: I, 594·5 mμ.; II, 554·6 mμ. Order of intensities: I, II.

'*Oxorhodoporphyrin*' (*2-acetyl-4-ethyl-1:3:5:8-tetramethylporphin-6-carboxylic acid-7-propionic acid*) *dimethyl ester* (*formula XXI*). This was prepared by the method of Fischer & Krauss (1936). The porphyrin obtained after long and tedious ether-HCl fractionations had a spectrum similar to that found by Stern & Wenderlein (1935*a*). It was esterified

by methanol HCl, and the ester, crystallized from pyridine, had m.p. 280°. The yield was minute.

Oxime. This was prepared, in amounts sufficient for spectroscopic study only, by the method described above.

Porphyrin obtained by the action of daylight on protoporphyrin in HCl *solution.* Pure protoporphyrin (100 mg.) was dissolved in 200 ml. 10% (w/v) HCl, the solution transferred to Roux flasks so that the liquid layers were about 1 cm. deep, and the flasks allowed to stand on a shelf exposed to sunlight for 13 days. The solution was filtered and the porphyrins in the filtrate taken into ether by dilution with water. The ether was extracted exhaustively with 2% (w/v) HCl to remove unchanged protoporphyrin. The colour of the remaining ether solution was green with a pink tinge. Fractionation of this solution with increasing concentrations of HCl showed that the ether contained only one porphyrin, which was extracted quantitatively by 10% (w/v) HCl as a bright-green solution. The absorption curves in HCl and in ether gave no indication that more than one substance was present.

Spectrophotometric data for the porphyrin in ether are shown in Table 1, and for the pyridine haemochromogen prepared from it in Table 3. The porphyrin in 10% (w/v) HCl had the following bands (Hilger-Nutting visual spectrophotometer): I, 610; II, 565 mμ. Ratio I:II, 0·515.

Chlorocruoro ('Spirographis') porphyrin dimethyl ester (IX). A sample of chlorocruorohaemin prepared by Dr J. H. Hale from *Sabella pavonina* and *Branchiomma vesiculosum* was converted to the porphyrin by the ferrous acetate method of Warburg & Negelein (1932*a*). An ether solution of the product was extracted with 1·5% (w/v) HCl to remove any traces of protoporphyrin. The chlorocruoroporphyrin was esterfied with HCl-saturated methanol and crystallized from $CHCl_3$-ether; m.p. 270°.

Oxime. Prepared as described above. Unfortunately we were unable to obtain sufficient of this material for an analysis. No melting point for the analytically pure oxime ester has been found in the literature.

Chlorocruorohaem was extracted from the marine worm *Sabella starte indica* (formerly *Spirographis australiense*) collected on rock platforms on the coast about Sydney. Worms (120 g. of fresh worms) were minced and extracted with water. To the 500 ml. of aqueous extract so obtained, 2 l. of ice-cold acetone containing 10 ml. of 2N-HCl were added. The mixture stood at 0° for 2 hr., and then the precipitate of protein and other insoluble material was filtered off. The acetone was removed from the clear brown filtrate under vacuum, and the crude chlorocruorohaemin which separated from the remaining aqueous HCl was recovered at the centrifuge, dried *in vacuo* over $CaCl_2$ and crystallized from glacial acetic acid. Yield, 0·5 mg. No attempt was made to remove traces of protohaemin, which, although no doubt extracted from the body tissues of the worms by this procedure, could not be detected spectroscopically.

Phaeoporphyrin a_5 *dimethyl ester (formula II)* was a sample from the collection of Fischer. Amounts of 10 mg. each were converted into the oxime and into crystalline phaeohaemin a_5.

Phylloerythrin methyl ester (formula IV) was a pure sample prepared from sheep bile by Prof. C. Rimington.

Haemin a. This was isolated in minute quantities from ox hearts by the method of Rawlinson & Hale (1949). The haemochromogen had spectroscopic properties identical with those described by these authors.

RESULTS

Porphyrins

Spectrophotometric data concerning the porphyrins and their oximes are shown in Tables 1 and 2. Among the compounds of known structure, the spectra all conform with Stern's correlations. The spectra of phaeoporphyrin a_5 (II) and phylloerythrin (IV), which contain the isocyclic ring, and of their oximes, are discussed below. All the other oximes were aetio type except that of oxorhodoporphyrin (XXI), which retains the 'rhodofying' carbonyl group. Porphyrin *a* and its oxime have spectra of the same types as oxorhodoporphyrin and its oxime, and the possible significance of this is discussed below.

As shown in Table 1, spectra in chloroform, in comparison with other organic solvents, tend towards oxorhodo type, i.e. bands II and III are intensified in relation to band IV and the absorption maxima are moved to slightly longer wavelengths. This effect of chloroform has been observed consistently in many porphyrins besides those shown here.

Haemins

Haemochromogen spectra of the compounds studied are shown in Table 3. We have confirmed the finding of Fischer & Krauss (1936) that oxorhodohaemochromogen has only a single absorption band in the visible region, and have found that the haemochromogen of phylloerythrin haemin dimethyl ester has only a very faint second band in the visible region (Table 3). In the same table the spectra are shown of the haemochromogens obtained after reaction with hydroxylamine or with hydrazine.

Reaction with hydroxylamine

To a solution of the haemin in 0·5 ml. pyridine, 1·5 ml. of distilled water were added, followed by about 3 mg. of a mixture of equivalent amounts of solid hydroxylamine hydrochloride and sodium carbonate. Solution of the solids was effected by gentle rotation, and the solution allowed to stand for 30 min. at about 20°. A pinch of sodium dithionite was added for full reduction before measuring the haemochromogen absorption bands of the product.

Under these conditions the formyl compounds and haemochromogen *a* reacted with hydroxylamine with a considerable shift of absorption bands towards shorter wavelengths; the acetyl compounds and phaeohaemin a_5 and phylloerythrin haemin gave little or no shift (Table 3).

Reaction with hydrazine

This reaction was carried out under the same conditions as the hydroxylamine reaction, using hydrazine sulphate instead of hydroxylamine

Table 1. *Absorption spectra of porphyrins and porphyrin oximes*

(Δ Band I indicates the magnitude of the shift to shorter wavelengths of this band on oxime formation. *A*, aetio type; *R*, rhodo type; *O*, oxorhodo type. Negelein's 'cryptoporphyrin', Rawlinson & Hale's porphyrin *a* and our product from the action of light on protoporphyrin were the free porphyrins; the remainder were methyl esters.)

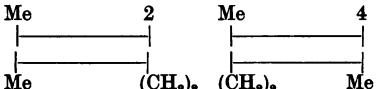
Compound	Formula	Solvent	Absorption maxima (mμ.) and ratios of intensities (in parentheses) I	II	III	IV	Spectral type	Δ Band I (mμ.)
Monoformyldeuteroporphyrin	(VIII)	Dioxan*	640 (0·223)	578·5 (0·850)	551 (1·430)	512 (1·0)	*R*	—
		Chloroform*	641 (0·175)	580 (1·037)	555 (1·790)	515 (1·0)	*R–O*	—
Monoformyldeuteroporphyrin oxime		Dioxan*	630 (0·225)	575 (0·558)	540 (0·948)	504 (1·0)	*A*	10
		Dioxan†	628 (0·225)	573 (0·507)	539 (0·944)	503 (1·0)	*A*	12
		Chloroform*	630 (0·213)	574 (0·598)	543 (1·010)	506 (1·0)	*R–A*	11
2:4:Diformyldeuteroporphyrin	(X)	Dioxan*	650 (0·184)	593 (0·458)	559 (0·585)	523 (1·0)	*A*	—
		Chloroform‡	651 (0·228)	595 (0·490)	562 (0·600)	525 (1·0)	*A*	—
		?§	648 (0·267)	594 (0·466)	561 (0·580)	525 (1·0)	*A*	—
2:4-Diformyldeuteroporphyrin dioxime		Dioxan*	639 (0·309)	583 (0·485)	546 (0·785)	512 (1·0)	*A*	11
		Chloroform‡	640 (0·285)	582 (0·485)	550 (0·820)	514 (1·0)	*A*	11
Monoacetyldeuteroporphyrin		Chloroform‡	634 (0·193)	578 (0·735)	548 (1·145)	511 (1·0)	*R*	—
Monoacetyldeuteroporphyrin oxime		Chloroform‡	622 (0·237)	570 (0·500)	536 (0·732)	502 (1·0)	*A*	12
2:4-Diacetyldeuteroporphyrin	(XI)	Dioxan*	639 (0·246)	588 (0·425)	552 (0·525)	516 (1·0)	*A*	—
		Chloroform‡	640 (0·258)	588 (0·460)	552 (0·545)	518 (1·0)	*A*	—
2:4-Diacetyldeuteroporphyrin dioxime		Dioxan*	625 (0·220)	576 (0·409)	537 (0·588)	503 (1·0)	*A*	14
		Chloroform‡	626 (0·253)	573 (0·484)	538 (0·632)	506 (1·0)	*A*	14
Oxorhodoporphyrin	(XXI)	Dioxan‖	637 (0·270)	583 (1·280)	557·5 (2·395)	515 (1·0)	*O*	—
Oxorhodoporphyrin oxime		Dioxan†	631 (0·089)	575 (0·892)	548 (1·747)	509 (1·0)	*R*	6
Protoporphyrin irradiation product		Ether‡	640 (0·161)	583 (0·515)	548 (0·807)	511 (1·0)	*A*	—
'Cryptoporphyrin'		?¶	648–639	591–579·5	559–549	520–505	*R*	—
Phylloerythrin	(IV)	Dioxan*	637·5 (0·224)	583·5 (0·984)	559 (1·469)	519 (1·0)	*O–R*	—
		Dioxan‖	634 (0·218)	581·5 (1·000)	557·5 (1·517)	517·5 (1·0)	*O–R*	—
Phylloerythrin oxime		Dioxan*	628·5 (0·145)	574 (0·675)	549 (0·993)	512 (1·0)	*R–A*	9
Phaeoporphyrin a_5	(II)	Dioxan‖	634 (0·202)	583 (1·343)	562 (1·710)	521 (1·0)	*O*	—
		Chloroform‡	639 (0·310)	588 (1·550)	568 (1·640)	525 (1·0)	*O*	—
Phaeoporphyrin a_5 oxime		Dioxan†	625 (0·150)	573 (0·833)	550 (1·117)	512 (1·0)	*R*	9
		Chloroform‡	627 (0·170)	576 (1·100)	556 (1·370)	517 (1·0)	*O*	12
Porphyrin *a*		Dioxan*	648 (0·400)	582 (1·040)	557 (1·640)	515 (1·0)	*O*	—
		Pyridine**	647 (0·301)	582 (1·097)	559 (1·512)	516 (1·0)	*O*	—
Porphyrin *a* oxime		Pyridine**	643 (0·229)	578 (0·724)	551 (1·233)	512 (1·0)	*R*	4
Porphyrin *a* ester		Chloroform‡	645 (0·275)	585 (1·310)	562 (1·780)	518·5 (1·0)	*O*	—

* Determined by the Beckman photoelectric spectrophotometer.
† From Stern & Wenderlein (1936).
‡ Determined by Hilger-Nutting visual spectrophotometer.
§ Calculated from small curve published by Fischer & Deilmann (1944).
‖ From Stern & Wenderlein (1935*a*).
¶ From Negelein (1932*a*).
** Calculated from Rawlinson & Hale (1949).

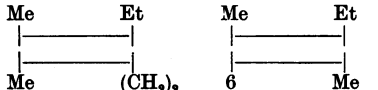
Table 2. *The effect of certain substituents on the wavelength of maximal absorption, and on the type of spectrum in the visual region, of porphyrin methyl esters and their oximes in dioxan solution*

(*A*, aetio type; *R*, rhodo type; *O*, oxorhodo type.)

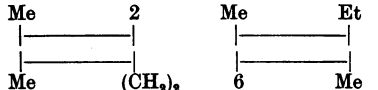
Formula



	Substituents at positions		Band I max. (mμ.)	Spectral type (cf. Fig. 1)
	2	4		
V	—H	—H	618*†	*A*
VI	—C_2H_5	—C_2H_5	620*†	*A*
VII	—CH:CH_2	—CH:CH_2	630*†	*A*
VIII	—H	—CHO	640*	*R*
	—H	—C:N.OH	630* 628†	*A*
IX	—CHO	—CH:CH_2	641* 639†	*R*
	—C:N.OH	—CH:CH_2	635·5*	*A*
X	—CHO	—CHO	650*	*A*
	—C:N.OH	—C:N.OH	639*	*A*
XI	—$CO.CH_3$	—$CO.CH_3$	639*	*A*
	—$C(:N.OH).CH_3$	—$C(:N.OH).CH_3$	625*	*A*

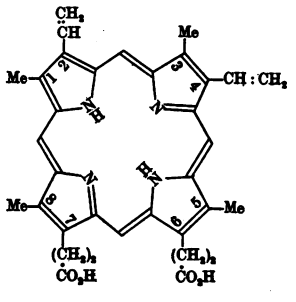


	Substituents at position 6		
XII	—H	620†	*A*
XIII	—CH:CH_2	624†	*A*
XIV	—CHO	635·5†	*R*
XV	—$CO.CH_3$	632†	*R*
XVI	—$CO.C_6H_5$	627†	*R*
XVII	—$COOCH_3$	632†	*R*
XVIII	—CH:CH.$COOCH_3$	624·5†	*R*

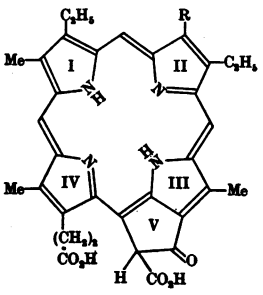


	Substituents at positions			
	2	6		
XIX	—C_2H_5	—$COOCH_3$	632†	*R*
XX	—CH:CH_2	—$COOCH_3$	638·5†	*R–O*
XXI	—$CO.CH_3$	—$COOCH_3$	637†	*O*
	—$C(:N.OH)CH_3$	—$COOCH_3$	631†	*R*
XXII	—$COCH_3$	—$COCH_3$	637†	*O*
XXIII	—CHO	—$COOCH_3$	Unknown	Unknown

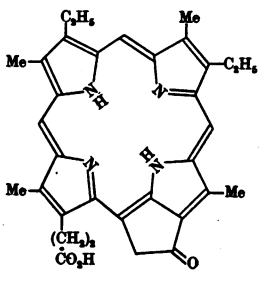
* Measured for this study (cf. Table 1). † From the data of Stern *et al.* (1934–6).



I Protoporphyrin IX.



II R = —CH_3. Phaeoporphyrin a_5.
III R = —CHO. Phaeoporphyrin b_6.



IV Phylloerythrin.

Table 3. *Absorption spectra of haemochromogens, before and after reaction with hydrazine and with hydroxylamine*

(All measurements were made by the Beck-Hartridge reversion spectroscope. Δ Band I indicates the magnitude of the shift to shorter wavelengths of this band after reaction.)

		Haemochromogen		Haemochromogen after hydroxylamine reaction			Haemochromogen after hydrazine reaction		
Haemin of	Formula	Band I (mμ.)	Band II (mμ.)	I	II	Δ Band I (mμ.)	I	II	Δ Band I (mμ.)
Monoformyldeuteroporphyrin	—	578	548	560	530	18	560	530	18
2:4-Diformyldeuteroporphyrin	X	584·3	549·7	563	533	21	558	527	24
Monoacetyldeuteroporphyrin	—	571	530	569·5	530	1·5	571	530	Nil
2:4-Diacetyldeuteroporphyrin	XI	575·2	539·7	573	530·1	2·2	575·2	539·7	Nil
Chlorocruoroporphyrin	IX	583·1	545·1	561·4	529·5	22	553	523	30
Oxorhodoporphyrin	XXI	582	—	582	—	Nil	582	—	Nil
Haemin *a*	—	587	—	570	533	17	569	530	18
Haemin from protoporphyrin irradiation product	—	576	530	—	—	—	—	—	—
'Cryptohaemin'	—	582	531	—	—	—	—	—	—
Phaeohaemin a_5	—	587	(555·5)	587	(555)	Nil	587	(555)	Nil
Phylloerythrin haemin	—	584	(538)	584	(538)	Nil	584	(538)	Nil

hydrochloride. In these experiments the formyl compounds and haemochromogen *a* again reacted with a shift of absorption bands to shorter wavelengths, but the acetyl compounds, and phaeohaemin *a* and phylloerythrin did not (Table 3).

Warburg, Negelein & Haas (1930) had noticed a similar reaction with chlorocruorohaemin; they reported that the (pyridine hydrazine) haemochromogen was unstable, the 585 mμ. band wandering to 560 mμ. 'under conditions we do not yet understand'.

A similar shift in the absorption bands of haemochromogen *a* caused by the action of cysteine (Rawlinson & Hale, 1949) had already indicated the presence of a formyl group in this material. Cysteine causes a similar shift in the absorption bands of phaeohaemin a_5 in weakly alkaline solution at 75° (cf. also Fischer & Mittermair, 1941). In 25% pyridine solution, however, cysteine does not shift the absorption bands of phaeohaemochromogen a_5 at room temperature.

The spectroscopic changes in our hydroxylamine and hydrazine reactions are thus almost certainly not due to the reaction of a keto group, but confirm the presence of a formyl group (cf. Rawlinson & Hale, 1949).

Porphyrin from action of daylight on protoporphyrin in hydrochloric acid solution

Negelein (1932*a*) isolated from pigeon-breast muscle a porphyrin which he called 'cryptoporphyrin' (cf. Table 1). A little later (1932*b*) he reported, giving no details of either experimental method or products, that by the action of light on protoporphyrin in hydrochloric acid solution a porphyrin identical with 'cryptoporphyrin' was obtained. He concluded that 'cryptoporphyrin' was an artifact arising from protoporphyrin during the isolation from the muscle. We therefore made an attempt to repeat Negelein's irradiation experiment (see Experimental section), choosing daylight in the absence of more specific information. Our product was neither in band position nor in spectroscopic type identical with Negelein's (1932*a*) 'cryptoporphyrin' (Table 1), nor were the corresponding haemochromogens identical (Table 3). Though we may have obtained a different product, in our irradiation experiment, from Negelein's (1932*b*) product, the band positions of our 'irradiated protoporphyrin' lie close enough to those of Negelein's (1932*a*) 'cryptoporphyrin' to make it conceivable that by direct vision spectroscopy the substances might be thought to be identical.

Chlorocruoroporphyrin ('Spirographis' porphyrin)

The structure (IX, Table 2) for this porphyrin rests on very strong evidence (Fischer & Seemann, 1936; Fischer & Wecker, 1942; Fischer & Deilmann, 1944). The porphyrin itself has been the subject of some careful spectrophotometric studies (Fischer & Seemann, 1936; Stern & Molvig, 1936), but the oxime and other derivatives have been studied solely by direct vision spectroscopy, from which only unreliable estimates of relative intensities can be made. Reports based on such observations have not been consistent with the correlations of Stern and coworkers. Thus Fischer & Seemann (1936) reported no movement of band I (in pyridine) and a rhodo-type spectrum after oxime formation. Warburg & Negelein (1932*a*, *b*), however, did observe a shift of the absorption bands to shorter wavelengths on oxime formation.

By spectrophotometric measurements (Table 4) we have found in the oxime an aetio-type spectrum and a marked shift to shorter wavelengths, in accordance with Stern's correlations.

Table 4. *Absorption spectra of chlorocruoroporphyrin ('Spirographis' porphyrin) ester and its oxime*

Compound	Solvent	Absorption maxima (mμ.) and ratios of intensities (in parentheses) I	II	III	IV	Δ Band I (mμ.)
Porphyrin ester	Chloroform	644 (0·209)	584 (0·917)	558·5 (1·400)	518·5 (1·0)	—
	Dioxan	641 (0·213)	583 (0·728)	554 (1·210)	515 (1·0)	—
	Dioxan*	639 (0·223)	581 (0·770)	553 (1·204)	514 (1·0)	—
Porphyrin ester oxime	Chloroform	634 (0·270)	579 (0·558)	546 (0·928)	511 (1·0)	10
	Dioxan	635·5 (0·277)	580 (0·537)	543 (0·874)	508 (1·0)	5·5
	Ether	636 (0·294)	580 (0·469)	541 (0·870)	505 (1·0)	—

* From Stern & Molvig (1936); the remainder of the measurements were made by the Beckman spectrophotometer.

DISCUSSION

All side chains having double bonds in conjugation with the porphyrin nucleus shift the absorption spectrum towards longer wavelengths, the shift being particularly noticeable in band I. As shown in Table 2, aldehyde and acrylic acid groups have a stronger effect in this direction than keto groups, which in turn are stronger than vinyl groups.

Further, a $\rangle C{=}O$ group in an aldehyde, a ketone or a carboxylic acid side chain, causes enhancement of the absorption of band III relative to that of band IV with the appearance of a rhodo-type spectrum (cf. Fig. 1*b*). The acrylic acid side chain also causes a rhodo-type spectrum to appear; unfortunately only one reliable example for this effect of the acrylic acid group is available (XVIII). Such substituents which, singly, cause the appearance of a rhodo-type spectrum have been called (Lemberg, Falk, Rimington, Hale & Rawlinson, 1949) 'rhodofying' groups. The vinyl group, and aldoxime and ketoxime groups are not rhodofying. Thus (XIII) has an aetio-type spectrum and while (VIII), (IX) and (XIV–XVIII) have rhodo-type spectra, the oximes of (VIII) and (IX) have spectra of aetio type. In addition, the large shift of absorption bands to longer wavelengths due to carbonyl groups is partly reversed on oxime formation, the oxime group in its effect both on wavelength of absorption and on type of spectrum thus resembling a vinyl group.

Two rhodofying groups on vicinal pyrrole rings, e.g. (III), (X) and (XI), abolish each other's rhodofying effect, and the aetio-type spectrum is found. The introduction of the second carbonyl group does not abolish, but slightly enhances, the shift to longer wavelengths caused by the first. This shift is reversed on dioxime formation, the spectrum however remaining of aetio type (dioximes of (III), (X) and (XI)). Two rhodofying groups on diagonally opposed pyrrole rings, e.g. rings I and III, however, enhance each other's rhodofying effect, and the oxorhodo-type spectrum results, as in (XXI) and (XXII). The former (XXI) was called by Fischer 'oxorhodoporphyrin'; its oxime has, as would be expected, not oxorhodo-type but rhodo-type spectrum. Unfortunately, this is the only example of this particular change on oxime formation in a compound of known structure.

A vinyl group diagonally opposed to a rhodofying group does not intensify the effect of the latter sufficiently to cause oxorhodo-type spectrum. Thus in (XX) the spectrum is rhodo type, not oxorhodo type, though band II is distinctly stronger than in the related ethyl compound (XIX).

These correlations are quite consistent among the simple porphyrins (i.e. excluding compounds containing the isocyclic ring which is characteristic of the chlorophyll derivatives). The oxime of chlorocruoroporphyrin (IX) was not studied by Stern, and according to non-quantitative published reports, had a rhodo-type spectrum, thus providing an apparent anomaly. It has, in fact, an aetio-type spectrum (Table 4).

Phaeoporphyrin a_5 (II) and phylloerythrin (IV), which contain isocyclic ring keto groups, require separate discussion. Their spectra are shown in Table 1. The cyclic, β-keto acid structure in phaeoporphyrin a_5 is very strongly rhodofying, the compound having a very marked oxorhodo-type spectrum. Phylloerythrin has lost the carboxyl group, and its spectrum is intermediate between rhodo type and oxorhodo type (band II ≏ band III); its ring keto group, while less strongly rhodofying than the carboxylated keto ring, exerts a much stronger effect in this direction than do keto groups in open chains attached to the pyrrole ring (cf. (XV), which has a true rhodo-type spectrum).

The oxime of phaeoporphyrin a_5 has a true rhodo-type spectrum, and that of phylloerythrin an aetio-rhodo type (band III ≏ IV).

It is thus apparent that the ring carboxyl group, which as written is not in conjugation with the nucleus, enhances the rhodofying effect of the ring keto group. Further, when the keto group is masked by oxime formation, the carboxyl group itself exerts a rhodofying effect. To explain this it is suggested that phaeoporphyrin a_5 and its oxime exist largely in the enol form.

Nothing is known about the equilibrium between these keto and enol forms, but the fact that on enolization the isocyclic ring becomes fully conjugated may favour the enol form.

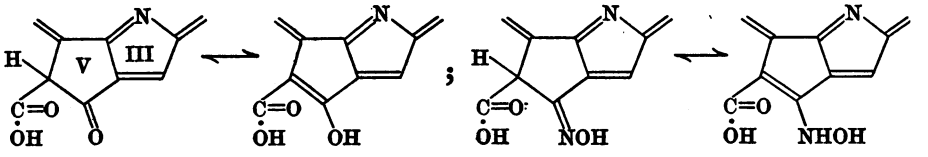


Fig. 2. Partial formulae of phaeoporphyrin a_5 and its oxime.

The fact that on enolization, (*a*) the carboxyl >C=O becomes conjugated with the nucleus, and (*b*) that ring V is brought into conjugation with the remainder of the porphyrin nucleus, may well explain the very strong oxorhodo-type spectrum of phaeoporphyrin a_5 and the rhodo-type spectrum of its oxime. Similarly, consideration of the additional resonance on enolization could explain not only the strong rhodo-type spectrum of phylloerythrin (band II ≏ III), but also the tendency towards rhodo type in its oxime (band III ≏ IV).

Possible structures for porphyrin a

As was found by Rawlinson & Hale (1949), porphyrin *a* has an oxorhodo-type spectrum, and its oxime is of rhodo type. From the knowledge of porphyrin compounds now available, this might be explained in three different ways:

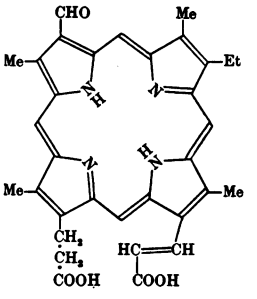
(1) The porphyrin has only one 'rhodofying' group. In this case the group should be a β-keto acid group in an isocyclic ring, as in phaeoporphyrin a_5 (II).

(2) There are two 'rhodofying' groups on opposite pyrroles, one of which should not be able to form an oxime (e.g. a carboxyl group as in (XXI) or an acrylic acid side chain as in (XVIII)).

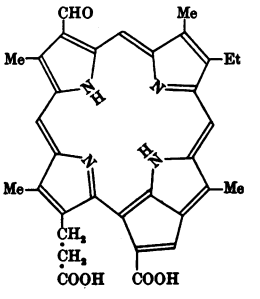
(3) There are two 'rhodofying' groups, one of which is a keto group in an isocyclic ring as in (1).

Such an assumption as (3) appears attractive, since compounds of this type are known in nature (bacteriochlorophyll), though in the tetrahydroporphin, not in the porphin series. Against assumption (1) is the fact that no compound of this type is known which has absorption bands as far shifted towards the red as has porphyrin *a*. Assumption (2) was supported not only on the ground of the similarity of the absorption spectra of porphyrin *a* and oxorhodoporphyrin (XXI), but also by the observation that the haemochromogen of oxorhodoporphyrin is so far the only haemochromogen of known structure which, like haemochromogen *a*, shows only a single absorption band. This single band is, moreover, in a position close to that of haemochromogen *a*. The iron complex of the 'formylrhodoporphyrin' (XXIII) might be expected to give a haemochromogen still more similar to that of haemin *a*.

However, evidence obtained by the London workers (cf. Rimington, Hale, Rawlinson, Lemberg & Falk, 1949) made such a hypothesis unlikely. They had found that oxime formation after reaction of porphyrin *a* with either diazoacetic ester or hydriodic acid caused the appearance of porphyrins of aetio type with shifts of band I of 17 and 19 mμ. respectively. A carboxylic acid group on a β-position on a pyrrole does not appear



XXIV



XXV

likely to be changed in these reactions, the evidence pointing rather to an ethylenic double bond. These reactions are also difficult to explain on the basis of assumption (3), since the keto group in the isocyclic ring does not react with hydriodic acid or diazoacetic ester. The only side chain with an ethylenic bond which is known to be rhodo-forming (as required by the rhodo-type spectrum of porphyrin *a* oxime) is the acrylic acid group, and the structure (XXIV), or possibly a similar compound containing a modified isocyclic nucleus (XXV), would be consistent with all evidence so far available. Other possibilities remain. The formula with one formyl group and one vinyl group in one ring and a vinyl group in the vicinal pyrrole ring, first suggested (without evidence) by Warburg (cf. Warburg, 1949) and independently by the London workers is also not excluded, since nothing is known about the spectroscopic type of porphyrins which contain vinyl and formyl on one ring. Although the vinyl group has usually no 'rhodofying' effect, it may possess it when on the same pyrrole ring as the aldehyde group in porphyrin *a*, or the aldoxime group in its oxime.

Finally, the behaviour of the haemochromogens to hydroxylamine and particularly to hydrazine (cf. Table 3) supplies reliable evidence that one of the 'rhodofying' groups is formyl and not an acetyl or an isocyclic ring keto group. The possibility that both a formyl and a keto group are present, on opposite pyrrole rings, is not excluded on the evidence from the haemochromogens alone, but such structures would not be consistent with the properties of porphyrin *a* and the products of its reactions with hydroxylamine, hydriodic acid and diazoacetic ester.

SUMMARY

1. The absorption spectra of haemin *a*, isolated from ox-heart muscle, and the related porphyrin *a* have been compared with those of a number of haemins and porphyrins of known structure.

2. The absorption spectra of the porphyrins of known structure and their oximes are completely in accordance with the correlations found by Stern and co-workers between structure and type of spectrum. Chlorocruoroporphyrin ('Spirographis' porphyrin) oxime, which, according to the literature, provided an exception, was shown in fact to be in accordance with these correlations. Though Negelein's 'cryptoporphyrin' from pigeon-breast muscle may well have been an artifact, we have not been able to confirm the particular evidence which led him to believe this.

3. The properties of the porphyrins and haemochromogens support previous evidence for the presence of an aldehyde (formyl) side chain in haemin *a*, and do not indicate the presence of a keto group in an isocyclic ring or in an open (e.g. acetyl) side chain.

4. Structures for porphyrin *a* which are consistent with all the evidence available, are those of 4-ethyl-2-formyl-1:3:5:8-tetramethylporphin-6-acrylic acid-7-propionic acid, or the same compound where the γ-methene-bridge carbon and the α-carbon of the acrylic acid are joined with loss of two H atoms to form a five-membered ring. More direct evidence upon the structure of the natural compound is, however, required.

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