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Osmium Tetroxide Oxidation of Protoporphyrin IX and Synthesis of Deuteroporphyrin IX 2,4-Diacrylic Acid

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Protoporphyrin IX has been oxidized with osmium tetroxide to 2,4-di(α,β-dihydroxy)ethyldeuteroporphyrin, which on reaction with sodium periodate formed 2,4-diformyldeuteroporphyrin. This dialdehyde on condensation with malonic acid gave deuteroporphyrin-2,4-diacrylic acid.

It is now commonly accepted that the biosynthetic precursor of protoporphyrin IX (Fig. 1) is coproporphyrinogen (isomer III),[2] but there are still some uncertainties about the oxidative decarboxylation of the two propionic acid chains and at what step the porphyrinogen ring is oxidized to a porphyrin. The oxidative decarboxylation could possibly proceed *via* a concerted mechanism, in which case deuteroporphyrin IX 2,4-diacrylic acid (hereafter referred to as diacrylic porphyrin) or its corresponding porphyrinogen would not be an intermediate in the biosynthesis of protoporphyrin IX. This diacrylic porphyrin was therefore synthesized as one approach to test this hypothesis.

The preparation of this diacrylic porphyrin has been reported by Fischer and Beer,[3] but no criteria of purity were given. The fact that their starting material, 2,4-diformyldeuteroporphyrin IX, had a rhodo type spectra shows it to be impure.[4] Lemberg and Parker[4] obtained a 5% yield of diformyldeuteroporphyrin IX by alumina chromatography of the reaction products between potassium permanganate and protoporphyrin IX dimethyl ester. Lemberg and Falk[5] claimed a 12% yield of the diformyl porphyrin by carefully controlling the reaction time of this oxidation. Several attempts were made to increase this yield by oxidizing protoporphyrin IX or hemin IX dimethyl ester with potassium permanganate, or with the neutral magnesium permanganate under a variety of conditions, but none of these experiments gave encouraging results.

However, when protoporphyrin IX dimethyl ester was oxidized with osmium tetroxide, a previously unknown diglycol, very easily purified through its low hydrogen chloride-number, was obtained in good yield. Fischer and Deilmann[6] obtained a very complex mixture of substances with this reaction from which they were able to isolate the diformyldeutero porphyrin in 6% yield. Under the experimental conditions here described, osmium tetroxide does not affect the porphyrin ring; however, if an excess of osmium tetraoxide is used substances absorbing at 640 and 490 mμ are obtained. The similarity of the spectrum to that of chlorins suggests that the osmium tetroxide has oxidized a peripheral double bond of a pyrrole nucleus giving a dihydroxy compound similar to those postulated by Fischer.[7] This interesting possibility will be studied further.

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[2] R. A. Neve, R. F. Labbe, and R. A. Aldrich, *J. Am. Chem. Soc.*, **78**, 692 (1956); S. Granick and D. Mauzerall, *Ann. N. Y. Acad. Sci.*, **75**, 115 (1958); L. Bogorad, *J. Biol. Chem.*, **233**, 516 (1958).

[3] H. Fischer and L. Beer, *Z. physiol. Chem., Hoppe-Seyler's*, **244**, 54 (1936).

[4] R. Lemberg and J. Parker, *Australian J. Exp. Biol. Med. Sci.*, **30**, 163 (1952).

[5] R. Lemberg and J. E. Falk, *Biochem. J.* **49**, 674 (1951).

[6] H. Fischer and K. Deilmann, *Z. physiol. Chem., Hoppe-Seyler's*, **280**, 186 (1944).

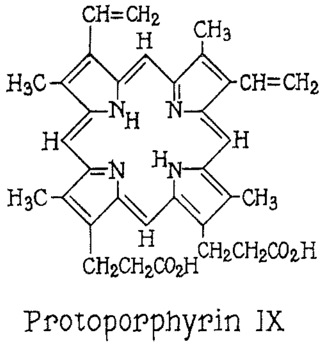
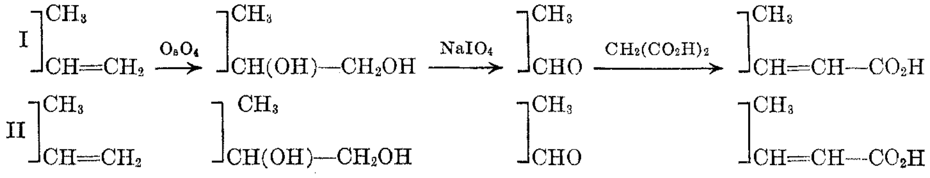


Fig. 1.

The diglycol, 2,4-di(α,β-dihydroxy)ethyldeuteroporphyrin IX dimethyl ester (presumably a mixture of the two pairs of enantiomorphs), was oxidized with sodium periodate to diformyldeuteroporphyrin IX dimethyl ester in high yield. The insolubility of the diformyl derivative relative to the glycols makes its purification a simple matter.

The condensation of the diformyl porphyrin with malonic acid was effected by the method of Fischer and Beer[3] with only minor changes. It is interesting that an attempt to condense the diformyl porphyrin with diethyl malonate (using piperidine–piperidinium acetate as catalyst) failed completely.



$$\begin{array}{lclclcl}
\text{I}\left]\begin{array}{l}CH_3\\ CH{=}CH_2\end{array}\right. & \xrightarrow{OsO_4} & \left]\begin{array}{l}CH_3\\ CH(OH)\text{—}CH_2OH\end{array}\right. & \xrightarrow{NaIO_4} & \left]\begin{array}{l}CH_3\\ CHO\end{array}\right. & \xrightarrow{CH_2(CO_2H)_2} & \left]\begin{array}{l}CH_3\\ CH{=}CH\text{—}CO_2H\end{array}\right. \\
\text{II}\left]\begin{array}{l}CH_3\\ CH{=}CH_2\end{array}\right. & & \left]\begin{array}{l}CH_3\\ CH(OH)\text{—}CH_2OH\end{array}\right. & & \left]\begin{array}{l}CH_3\\ CHO\end{array}\right. & & \left]\begin{array}{l}CH_3\\ CH{=}CH\text{—}CO_2H\end{array}\right.
\end{array}$$

Models of the diacrylic acid porphyrin showed that only the *trans* acrylic acid group could be made coplanar with the porphyrin ring. In confirmation of this, the infrared spectra showed a *trans* peak[8] at 973 cm.$^{-1}$ which was absent in the closely related coproporphyrin III. Bands were also present in the 1300 cm.$^{-1}$ region, but no new absorption was found in the *cis* regions. The double bonded carbon stretching band was at 1630 cm.$^{-1}$ This steric interference may also explain the lack of reaction of diethyl malonate with the diformylporphyrin. As proof of structure, the diacrylic acid porphyrin was reduced with sodium amalgam giving coproporphyrin III (after oxidation of the porphyrinogen with iodine) in 70% yield. The coproporphyrin was shown to be identical with the naturally occurring isomer III by infrared spectra, mixed melting points, and paper chromatography.[9]

EXPERIMENTAL

2,4-Di(α,β-dihydroxy)ethyldeuteroporphyrin (IX) *dimethyl ester.* Protoporphyrin dimethyl ester (550 mg.) was dissolved in dry dioxane (200 ml.) and osmium tetraoxide (500 mg.) in ether (25 ml.) followed by pyridine (0.35 ml.) was added. This solution was kept in the dark, under nitrogen, for 24 hr. After that time, the ether was removed *in vacuo,* a solution of sodium sulfite (1.1 g.) in water (20 ml.) was added, and the mixture heated on a boiling water bath for 40 min. After partial cooling, the solution was filtered and the black residue was dissolved in warm dioxane. The dioxane solution obtained from four similar preparations was concentrated *in vacuo,* taking care not to allow the mixture to dry. This mixture was extracted with water and the water insoluble material was dissolved in 500 ml. 0.5*N* hydrochloric acid and filtered. The acid solution was diluted with 1 l. of water and was extracted several times with ether and ethyl acetate (1/1, v/v). The organic solvent was back extracted with 0.17*N* hydrochloric acid (500 ml.). The combined acid solutions were neutralized with sodium acetate and the precipitate was centrifuged and washed with water.

The yield was 1.1 g. (45%) of dry 2,4-di(α,β-dihydroxy)-ethyldeuteroporphyrin dimethyl ester. In preliminary experiments with smaller amounts of protoporphyrin the yield was higher (74%).

A crystalline sample was obtained by dissolving a small part of the former product in a warm mixture of methanol and benzene (1/1, v/v) and filtering. Two thirds of the volume of petroleum ether (b.p. 30°–60°) was added and the solution left in the cold. The crystals, dried *in vacuo* melted sharply at 238–240°. The absorption spectrum (Table I) obtained in 1*N* hydrochloric acid agrees quantitatively with that of hematoporphyrin.

Anal. Calcd. for $C_{36}H_{42}N_4O_8$: C, 65.64; H, 6.43. Found: C, 65.10; H, 6.53. Nitrogen determinations by both Dumas and Kjeldahl methods gave low values. Porphyrins often give low values by the former method, but the latter method (with Van Slyke modification) is usually more successful.

Periodate oxidation of 2,4-di(α,β-dihydroxy)ethyldeuteroporphyrin (IX) *dimethyl ester.* To this diglycol (900 mg.), dissolved in 500 ml. of warm dioxane, a solution of sodium periodate (1.15 g.) in water (200 ml.) was added and the mixture left in the dark for 22 hr. Water was added (200 ml.) and the precipitate centrifuged, washed with methanol, and dried *in vacuo;* yield: 680 mg. The absorption spectrum shows the presence of about 20% of monoformylporphyrin. This crude diformyl derivative was extracted with a mixture of 200 ml. of chloroform and 300 ml. of dioxane (solution A) and the insoluble material was then dissolved in 300 ml. of warm chloroform (solution B).

(a) *2-(or 4) Formyl-4-(or 2)-(α,β-dihydroxy)ethyldeuteroporphyrin* IX *dimethyl ester.* Aqueous methanol (45% methanol V/V) was gradually added to solution A so that a mixture of mono and diformyl derivatives (preponderantly diformyl) was precipitated, while in solution there remained only a mixture of the two possible monoformyl derivatives. After diluting the solution with water, the porphyrins were extracted with chloroform; this solution was washed with water and dried with sodium sulfate. The solubility of this porphyrin, the position of its absorption bands (Table II), and the rhodo type spectrum agree with its formulation as the monoglycolmonoformyl derivative

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(7) H. Fischer and H. Orth, *Die Chemie des Pyrrols,* Vol. II, part 1, Leipzig, 1937, pp. 269–274.

(8) L. J. Bellamy, *The Infrared Spectra of Complex Molecules,* 2nd ed., J. Wiley & Sons, Inc., New York, 1958, p. 45 ff.

(9) J. E. Falk, E. I. B. Dresel, A. Benson, and B. C. Knight, *Biochem. J.,* **63,** 87 (1956).

TABLE I

SPECTRA OF 2,4-DI(α,β-DIHYDROXY)ETHYLDEUTEROPHORPHYRIN IX DIMETHYL ESTER

		Maxima					Minima			
		I	II	III	IV	V				
$CHCl_3$ + 20% CH_3OH	λ in mμ	621	568	535	500	400	603	551	519	457
	$\epsilon_M \times 10^{-4}$	0.362	0.65	0.86	1.385	19.1				
	ratio	0.261	0.476	0.621	1.00	13.7	0.05	0.10	0.19	0.10
1*N* HCl	λ in mμ	592	(567)	550		402.5	582		450	
	$\epsilon_M \times 10^{-4}$	0.52	(0.55)	1.56		38.0				
	ratio	0.33	(0.35)	1.00		24.35	0.20		0.028	

TABLE II

SPECTRA OF 2-(OR 4)FORMYL-4-(OR 2)(α,β-DIHYDROXY)ETHYLDEUTEROPORPHYRIN IX DIMETHYL ESTER

Solvent	Maxima					Minima			
	I	II	III	IV	V				
$CHCl_3$									
λ in mμ	643	580	558	519	415	622	572	538	472
Absorbancy ratio	0.20	1.00	1.63	1.00	15.5	0.10	0.81	0.63	0.26

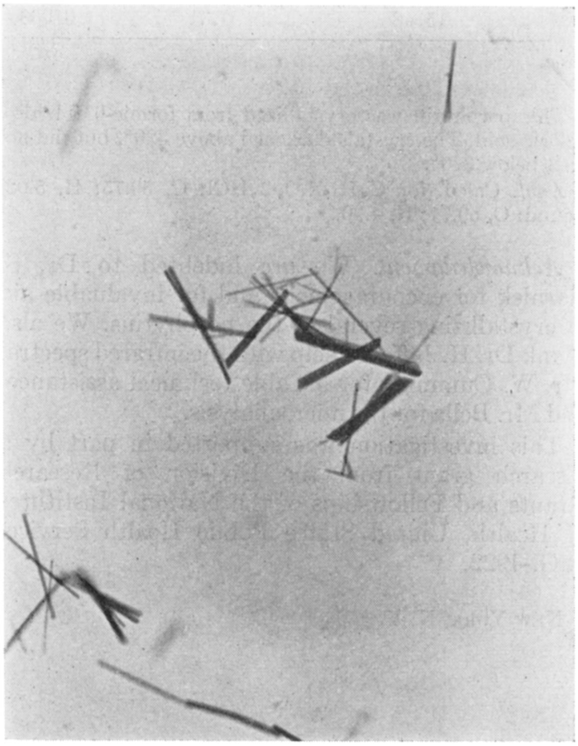

Fig. 2. 2,4-Diformyldeuteroporphyrin IX. Dimethyl ester (×800)

Fig. 3. Deuteroporphyrin IX. 2,4-Diacrylic acid dihydrochloride (×800)

(b) *2,4-Diformyldeuteroporphyrin* IX *dimethyl ester*. Solution B was treated gradually with 350 ml. of methanol and the precipitated porphyrin was centrifuged, washed with methanol, and dried; yield: about 300 mg. of pure diformyl derivative. The spectrum does not show any trace of the Soret band of the monoformyl, or glycol derivative. This porphyrin was easily crystallized from hot pyridine (Fig. 2). This porphyrin appeared to crystallize with pyridine, most of which was lost on drying at 60° *in vacuo* over phosphorus pentoxide. The dried crystals melted at 300° dec.; lit., m.p. 301–303°,[4] 280°,[4] and 303–305°,[6] all with decomposition. Melting points were observed with polarized light on a microscope hot stage and are corrected. The spectrum of this compound (Table III) (in chloroform) is in good agreement with that reported by Lemberg and Parker,[4] the bands being somewhat sharper.

The yield was increased by working up the mother liquors and reoxidizing with sodium periodate.

Anal. Calcd. for $C_{34}H_{34}N_4O_6$: C, 68.67; H, 5.76; $C_{34}H_{34}N_4O_6.C_5H_5N$: C, 69.31; H, 5.82. Found: C, 69.13; H, 6.16.

Deuteroporphyrin IX *2,4-Diacrylic Acid.* Diformyldeuteroporphyrin IX dimethyl ester (150 mg.) was dissolved in boiling pyridine (100 ml.) containing piperidine (0.03 ml.). The temperature was decreased to 95° and a solution of malonic acid (6 g.) in pyridine (60 ml.) containing piperidine (0.1 ml.) was added dropwise during 6 hr. After that time the temperature was raised to the boiling point and an additional amount of malonic acid (2 g. in 20 ml. of pyridine + 0.02 ml.

TABLE III

SPECTRUM OF 2,4-DIFORMYLDEUTEROPORPHYRIN IX DIMETHYL ESTER

Solvent	Maxima I	II	III	IV	V	Minima			
$CHCl_3$ in mμ	650.5	596	563	527	437	631	581	550	482
Absorbancy ratio	0.265	0.470	0.580	1.00	10.12	0.07	0.23	0.40	0.23

TABLE IV

SPECTRA OF DEUTEROPORPHYRIN IX 2,4-DIACRYLIC ACID

Solvent		Maxima I	II	III	IV	V	Minima			
Pyridine	in mμ	641	586	554	517	428	621	572	537	479
	$\epsilon_M \times 10^{-4}$	0.577	0.805	1.36	1.49	15.1	0.14	0.40	0.73	0.48
	ratio	0.387	0.540	0.913	1.00	10.13	0.094	0.27	0.49	0.32
0.1*M* KOH +	in mμ	635	581	552	515	402[a]	615	567	534	477
10% pyridine	$\epsilon_M \times 10^{-4}$	0.432	0.705	1.15	1.15	9.90	0.17	0.51	0.61	0.46
	ratio	0.375	0.613	1.00	1.00	8.60	0.15	0.44	0.53	0.40
6.0*M* HCl +	in mμ	611		566		423[a]		597		485–495
+10% pyridine	$\epsilon_M \times 10^{-4}$	0.708		1.66		25.7		0.52		0.11
	ratio	0.426		1.00		15.48		0.31		0.066

[a] These bands were measured in solvents not containing pyridine.

piperidine) was added dropwise during 1 hr. Upon cooling, 800 ml. of petroleum ether (b.p. 30–60°) were added and after standing in the cold, the solution was centrifuged. The precipitate was shaken twice with a mixture of chloroform and ether (2/3, v/v) and centrifuged. The insoluble material showed on paper chromatography[9] (using 2,6-lutidine (50 ml.) and 0.5*N* ammonia (35 ml.) as liquid phase) that it was mainly composed of a dicarboxylic acid porphyrin with only a trace of tricarboxylic acid porphyrin.

This residue was dissolved in 100 ml. of 0.5*N* potassium hydroxide and kept for 64 hr. in the dark at 3° to hydrolyze the two methyl ester groups. The alkaline solution was then acidified with acetic acid and the porphyrins collected by centrifugation, washed with water several times and dissolved in 100 ml. of 0.01*N* potassium hydroxide. This solution was kept frozen. Attempts to dry the free porphyrin led to considerable alteration. Paper chromatography showed that this product was composed of a tetracarboxylic acid porphyrin with a trace only of a pentacarboxylic porphyrin. The spectra of deuteroporphyrin diacrylic acid in different solvents follow (Table IV); the spectrum in pyridine disagrees with the spectrum reported by Fischer and Beer.[3]

This porphyrin was crystallized from formic-6*M* hydrochloric acid. The crystals darkened above 320°, but did not melt below 340°.

Anal. Calcd. for $C_{36}H_{34}N_4O_8 \cdot 2\ HCl$: C, 59.75; H, 5.02. Found: C, 59.71; H, 4.90.

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