TABLE II

THIOSULFATE FORMATION FROM SULFIDE AND "DISULFIDE"

The test system of pH 7.2 contained 15 μmoles Na_2S or 7.5 μmoles Na_2S_2, 400 μmoles phosphate and catalyst as indicated in a final volume of 3 ml. Incubation was carried out with shaking in Warburg vessels for 15 min at 37° and thiosulfate then determined colorimetrically[6]. The values obtained were corrected for corresponding "blanks" (0.10–0.25 μmole $S_2O_3^{2-}$ in case of sulfide and 1.1–1.5 μmoles $S_2O_3^{2-}$ in case of "disulfide").

Catalyst	*Substrate*	$S_2O_3^{2-}$ *formed* μ*moles*
Hemin (32.5 μg)	Sulfide	1.37
Hemoglobin (10 mg)	Sulfide	1.85
Liver extract*	Sulfide	1.50
Hemin (32.5 μg)	"Disulfide"	0.10
Hemoglobin (10 mg)	"Disulfide"	0.13
Liver extract*	"Disulfide"	1.43

* Prepared according to BAXTER *et al.*[2]. Each vessel contained 0.34 g liver (fresh weight).

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Crystalline synthetic porphyrin *c*

Synthetic porphyrin *c* was prepared more than twenty years ago by THEORELL[1] who heated protoporphyrin with L-cysteine in mineral acid solution. ZEILE AND MEYER[2], on the other hand, heated protoporphyrin with HBr and subsequently fused the crude adduct with L-cysteine. Both procedures gave non-crystalline products with the correct analytical data and the expected properties for the structure given in Fig. 1.

In this laboratory, during the course of preparation of porphyrin *c* to be used for metal-ion complexing studies, two modifications in the method of ZEILE AND MEYER[2] were introduced which led ultimately to a crystalline product. Thus in the present instance (a) the porphyrin–HBr adduct was fused with L-cysteine in an oil bath under controlled conditions instead of over an open flame, and (b) the crude

product obtained after fusion was subjected to partition chromatography with butanol–acetic acid–0.02 *N* HCl on a Celite column.

1 g crystalline protohemin was sealed in a glass tube with 20 ml 40 % HBr in acetic acid. After heating for 1.5 days at 37° the tube was opened and the contents poured out into a crystallizing dish. The solvent and excess HBr were removed under slightly reduced pressure in the presence of NaOH in a desiccator. Several additions of 10-ml volumes of glacial acetic acid were introduced over a period of 2–3 days in order to remove the last traces of HBr.

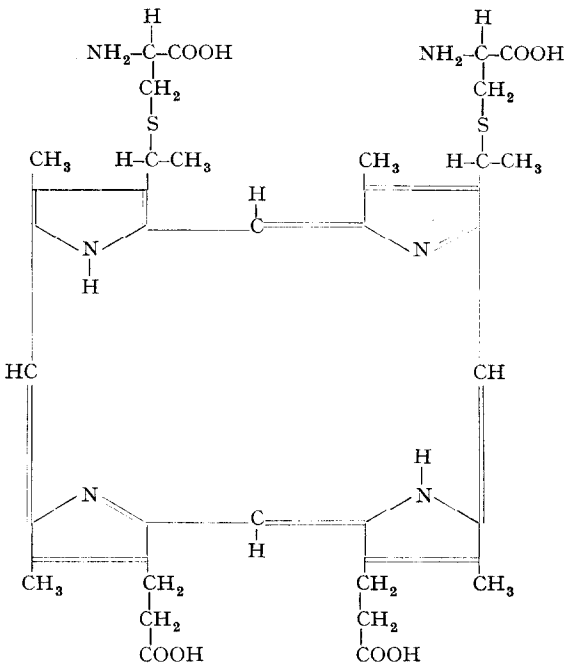

Fig. 1. Structure of porphyrin *c*.

The tacky material was thoroughly stirred with 3 g L-cysteine·HCl and the mixture stored under full vacuum overnight. A dry, crumbly product was obtained.

An oil bath was heated to 160° and the sample, contained in a beaker suspended by a wire handle, was introduced. The mixture, which melted almost instantly, was stirred for 3 min and then allowed to cool to room temperature. The crude product was dissolved in water and dil. NaOH then added to give a pH of 4. The precipitated porphyrin was collected by centrifugation and dried in a desiccator.

A 3.2 × 20 cm partition column of purified Celite with butanol–acetic acid–0.02 *N* HCl (4 : 1 : 5) as solvent was prepared as described by TUPPY AND PALÉUS[3]. A 200-mg sample of the crude product was dissolved in the minimum quantity of external solvent and applied to the surface of the column. Three principal bands were found. The fast and intermediate-rate bands were of about equal intensity; the slowest flowing band was a minor constituent. The intermediate-rate band only was collected and the porphyrin extracted from the organic layer with three 12-ml portions of 1 *N* HCl. The combined extracts were adjusted to pH 4 with 6 *N* NaOH

and set aside in the dark for crystallization. After standing overnight the rosettes were filtered, washed with a small amount of cold water and dried in a desiccator. The yield was about 100 mg, *i.e.* about 50 % of the total material applied to the column. Additional 200-mg batches of crude porphyrin were purified by passage through the same column.

The rosettes did not melt below 300°. The isolated compound was highly fluorescent and gave a positive ninhydrin reaction. The solubility, titration and spectral data were, in general, in good agreement with those reported by THEORELL[4] for the natural product. The titration curve closely resembled that shown by THEORELL[4] (Fig. 2 p. 244), *i.e.* the mid-points of the first and second buffer zones were at pH 5.6 and 9.2, respectively. The neutral equivalent calculated at pH 7.2 was 806, a figure in excellent agreement with the theoretical value of 804. The shape of the light-absorption curve in 1 *N* HCl was the same as that reported by THEORELL[4] (Fig. 1, p. 243) and the extinction coefficient of $1.65 \cdot 10^4$ at 553 mμ was confirmed. The Soret maximum in 1 *N* HCl was at 406 mμ and gave an extinction coefficient of $3.12 \cdot 10^5$ in contrast to the value $2.48 \cdot 10^5$ reported for natural porphyrin *c*. A single, elongated spot was observed on paper chromatograms developed with a lutidine–water (1 : 1) solvent. The R_F ratio for porphyrin *c*/protohemin was 1.0.

A number of different samples dissolved at varying concentrations in very dilute HCl did not transmit sufficient light to permit an exact determination of the specific rotation. The substance can be assumed to be optically active since natural cysteine was employed and racemization would not have been anticipated during the synthesis. Neither the method of preparation nor the available characterization data will allow an entirely unequivocal choice between an α or a β attachment of the sulfur atom to the porphyrin side chain. However, it has been found that the C–S lyase[5] of *Albizzia lophanta* splits synthetic porphyrin *c* either at a negligible rate or not at all. Although this enzyme cleaves a number of S-alkyl cysteines, the branched-chain derivatives appear to be acted upon much less readily than the corresponding straight-chain S-alkyl compounds[6]. The failure of the C–S lyase to attack our preparation would therefore favor the structure shown, *i.e.*, the α-isomer.

Analyses

Found: N, 10.71, 10.62; S, 7.94. Calc. for $C_{40}H_{48}N_6S_2O_8$: N, 10.44; S, 7.97.

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