Anal. Silver salt: Ag found 61.55, calcd. 61.4%. Fractionation of the residue from the above gave V, b. p. 182–185° at 8 mm., mol. wt. in camphor 249, calcd. 242. *Anal.* Silver salt: Ag found 47.8; calcd. 47.37%.

1,3-Dithiane VI.—An excess of methylene chloride was added to 27 g. of trimethylene mercaptan in 500 cc. of absolute alcohol containing an equivalent amount of sodium ethylate. The reaction was completed at room temperature and the mixture poured into water and the solid high polymer filtered off. Extraction of the filtrate with ether gave a mixture of VI and low polymer from which VI sublimed readily in the molecular still at 80–90°, m. p. 53.3°, b. p. 207–208° (micro method), mol. wt. in camphor 126. An additional amount of VI was obtained from the high polymer by heating in a current of dry hydrogen chloride. The sulfone was obtained by oxidation of VI in 50 parts of acetic acid with perhydrol.

2-Methyl-1,4-dithiane VII was similarly obtained from propylene bromide and the sodium salt of ethylene mercaptan, mol. wt. in borneol 140, S found 48.05, calcd. 47.76%.

Trimethylene sulfide was prepared from 100 g. of trimethylene bromide with an excess of sodium sulfide (from sodium ethylate and hydrogen sulfide) in 800 cc. of absolute alcohol. Fractionation of the crude sulfide gave a 33% yield, m. p. −64 ± 1°, b. p. 93.6°, d 0/4 1.0371, d 25/41 0.0163 and n_D^{20} 1.5072. These properties agree fairly well with those reported by Trokhimovski.[13] From the residue 2% of its dimer IX was obtained.

Di-trimethylene-1,5-disulfide IX besides being found in the above was prepared from trimethylene mercaptan and bromide. It was separated from low polymer and its dimer by sublimation in the molecular still, keeping the bath at 90°, m. p. −15°, b. p. 245–246° (micro method) n_D^{20} 1.5747, d 0/4 1.1579, d 25/0 1.1476, mol. wt. in naphthalene 142. *Anal.* S found 43.33 and 43.02; calcd. 43.25%. The sulfone was obtained by oxidation with 30% hydrogen peroxide in acetic acid. The dimer of this X was sublimed from the still residue by raising the temperature to 170°, m. p. 46°, mol. wt. in camphor 285.

XI was obtained in 0.25 g. (0.6%) yield from 52 g. of tetramethylene bromide and 27 g. of trimethylene mercaptan. It was purified by sublimation below 100°, needles 2 cm. long, m. p. 57.5–58°, mol. wt. in naphthalene 154; S found 39.88, calcd. 39.51%. By raising the temperature of the still to 175° the dimer XII was obtained m. p. 61–62°, mol. wt. in camphor 336; S found 39.18, calcd. 39.51%. A mixture of XI and XII melted around 44°.

The dimeric ring XIII was obtained in two ways, from dimercapto-ethyl ether with ethylene bromide and from dichloroethyl ether with ethylene mercaptan, mol. wt. in borneol 341, in camphor 316; S found 38.56, calcd. 39.02%. No reactive groups could be detected.

The dimeric ring XIV from the sulfide mercaptan III and ethylene bromide showed mol. wt. 354 in borneol and 368 in camphor; S found 52.91, calcd. 53.33%.

The 22-membered dimeric ring XV was obtained from hexamethylene bromide and trimethylene mercaptan, m. p. 62°, mol. wt. found 386, minimum sublimation temperature 170°; S found 33.31, calcd. 33.68%.

(13) Trokhimovski, *J. Russ. Phys.-Chem. Soc.*, **48**, 880 (1916).

Summary

Sulfide di-mercaptans have been identified as by-products in the preparation of simple dimercaptans.

Rings, some monomeric and some dimeric, have been obtained by the reaction of a polymethylene halide with a polymethylene mercaptan and along with these high molecular weight polymers.

Exchange of radicals between an alkyl sulfide and an alkyl halide has been effected. This reaction has been used to break down polymers.

BALTIMORE, MD. RECEIVED JULY 30, 1934

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

Studies in the Chlorophyll Series. XIII. Nuclear Isomerism of the Porphyrins[1]

BY EMMA M. DIETZ AND TYRRELL H. WERNER

In paper IX of this series Conant and Bailey[2] reported the conversion of a simply constituted chlorin, chlorin f, to the corresponding porphyrin, isorhodoporphyrin, by the removal of two hydrogen atoms with potassium ferricyanide. The measurements of low temperature absorption spectra by Conant and Kamerling[3] similarly pointed to the view that porphyrins contain conjugated unsaturated systems which are partially hydrogenated in the chlorins. Conant therefore reiterated his former hypothesis that the fundamental nucleus of chlorophyll a, of the related phaeophorbides and of the chlorins is that of a reduced porphyrin ring, more specifically a reduced isorhodoporphyrin ring. This would lead to the assumption that the drastic alkali degradation of chlorophyll which produces rhodoporphyrin, but never isorhodoporphyrin, causes an isomerization as well as a dehydrogenation of the original chlorophyll nucleus.

This isomerism of rhodo- and isorhodoporphyrins is discussed at some length in paper IX. Verdoporphyrin is a second but much less stable

(1) This problem was suggested by President James B. Conant, to whom we are indebted for advice during its investigation.

(2) THIS JOURNAL, **55**, 798 (1933).

(3) *Ibid.*, **53**, 3522 (1931).

isomer of rhodoporphyrin, and pyrochloro- and phylloporphyrins are cited as other examples of unexplained porphyrin isomerism. Oddly enough, such isomers have never been reported by Fischer in his many synthetic preparations of porphyrins, even of rhodoporphyrin. They are found only among the degradation products of chlorophyll.

Rhodoporphyrin and isorhodoporphyrin are both stable substances with the same empirical formula, but differing in absorption spectrum and acid number. The isomeric verdoporphyrin, as described by Treibs,[4] changes spontaneously to rhodoporphyrin in indifferent solvents and in the solid state. This would seem to discredit his hypothesis that it contains two hydrogen atoms less than rhodoporphyrin. Two preparations from phaeophytin according to the careful directions of Treibs (except for the use of a Pyrex instead of a silver flask) gave in each case a product identical with isorhodoporphyrin, in P_H number and in every other property, and failed to give verdoporphyrin.

In paper IX Conant pointed out the possibility of two prototropic porphyrin isomers according to whether the two hydrogen atoms attached to nitrogens were on opposite or adjacent pyrrole rings. He suggested that the same completely coördinated complex metal salt should result from either isomer unless one made the unlikely assumption of electromers. The zinc complexes however were found to be different substances and each regenerated only the original porphyrin. Similarly, Treibs reported that verdophyllin regenerated verdoporphyrin on acid treatment.

Since iron is more certain to form coördinate compounds than zinc, particularly in the hemochromogen state, we prepared the pyridine and cyanohemochromogens of both rhodo- and isorhodoporphyrins. These again proved to be different compounds and regenerated only the original porphyrins, even after long heating or illumination with white or ultraviolet light. Similarly, no interconversion could be accomplished through the phyllins of both porphyrins, which complexes also had distinctly different spectra. Thus, there is no indication of a common metal complex of these porphyrins nor of any interconversion through such a derivative.

We have now made the interesting observation that it is possible to transform isorhodoporphyrin into rhodoporphyrin under conditions which indicate that the transformation is probably a prototropic isomerization. This isomerization was accomplished in 50% sulfuric acid at room temperature. In eighteen to forty-eight hours isorhodoporphyrin gave a 50% yield of rhodoporphyrin, which was identified by acid number and spectrum of the ester and of the iron and zinc complexes. The use of a nitrogen or oxygen atmosphere did not change the yield. The recovered material was actually isorhodoporphyrin, and not some closely similar isomer, since in a second reaction under the same conditions, it gave a 30% yield of rhodoporphyrin. Some transformation also takes place after several days' standing in cold concentrated hydrochloric acid, and more rapidly in glacial acetic acid with dry hydrogen bromide.

A second pair of isomers can also be interconverted by acid. Thus, pyrochlorin-*e*-porphyrin in 50% sulfuric acid was changed in about 50% yield to its isomer, phylloporphyrin. Chlorin *f* and rhodoporphyrin were unaltered under the same conditions. Treibs has reported that verdoporphyrin changes readily to rhodoporphyrin in concentrated sulfuric acid.

If isorhodoporphyrin and rhodoporphyrin are indeed isomeric, as the evidence of the transformation with acid strongly indicates, it is probable that their metallic derivatives are also isomers. If one avoids the unlikely hypothesis of electromers, this would mean that the isomeric metallic derivatives either contained the metallic atom bound to only two nitrogen atoms, or else the cause of the isomerism is not to be found in the different positions of the hydrogens on the nitrogen atoms of the two porphyrins. If the isomerism of the porphyrins and the metallic derivatives becomes firmly established, it is evident that a very interesting problem awaits further investigation and that it may have wide bearing on the nature of complex metal derivatives.

Fischer, in a series of papers, has supported his contention that the fundamental nucleus of chlorophyll is a rearranged porphyrin ring by means of quantitative studies in catalytic hydrogenation.[5] He found that under certain conditions many chlorins and porphyrins absorbed 3 moles of hydrogen. He concluded, therefore, that they were isomeric in their nuclear structure.

(4) Treibs, *Ann.*, **506**, 10 (1933).

(5) Fischer and Helberger, *ibid.*, **480**, 260 (1930); Fischer and Lakatos, *ibid.*, **506**, 123 (1933).

On the other hand, a long line of evidence presented from this Laboratory has established that the chlorins are dihydroisoporphyrins, and following the argument just outlined, are therefore also dihydro derivatives of true porphyrins.

We have repeated and extended the investigation of catalytic hydrogenation of chlorophyll derivatives using different catalysts and conditions, and bringing into the study chlorin *f*, isorhodoporphyrin, rhodoporphyrin and pyrochloroporphyrin esters as well as certain other substances reported by Fischer.

The catalytic hydrogenation, using the Adams platinum oxide catalyst, was carried out on micro samples (2.5 mg.) in the ordinary Warburg apparatus adapted for the purpose. The results on other chlorophyll derivatives used as test substances checked fairly well our data of Paper II and were slightly higher than those of Fischer and Lakatos. Chlorin *e* ester took up 4 moles of hydrogen in going to the colorless leuco form, and chlorin *f* ester, 4.5. Isorhodoporphyrin ester absorbed 4.3 moles of hydrogen, going in 60% yield to rhodoporphyrin, while rhodo-, pyrro- and phylloporphyrin esters absorbed only 3.5 to 3.8 moles. In our experience the number of moles of hydrogen absorbed by a given compound varied by as much as 25% with the amount of Adams catalyst used.

The use of palladium black as catalyst in glacial acetic acid seemed to give more reliable results, and the data on test substances checked those of Fischer and Lakatos very well. Doubling the amount of catalyst used for a given sample of chlorin *e* ester had no appreciable effect on the result and the values are more nearly whole numbers than they were in the case of platinum oxide. Chlorin *e*, chlorin *f*, phyllo- and rhodoporphyrin esters absorbed 3.1 to 3.3 moles of hydrogen while isorhodoporphyrin and pyrochloroporphyrin esters absorbed 4.2 moles in going to the leuco form.

In any estimation of the value of the quantitative results of catalytic hydrogenation it must be borne in mind that the yields of regenerated porphyrin (after reoxidation of the leuco compound) are never more than 60%. It is extremely probable that in addition to the formation of a leuco compound, this compound itself is further reduced with cleavage of the molecule. Clar and Haurowitz report such complications in the catalytic hydrogenation of mesoporphyrin[6] and Fischer and Neumann for aetioporphyrin.[7] If this is the case, the trustworthiness of the results would depend on the further reduction of the leuco compound proceeding very much slower than the first process. We are confronted with the fact that in two instances porphyrins which appear to be isomeric (*e. g.*, rhodo- and isorhodoporphyrins) differ in the amount of hydrogen consumed. The difference varies from 0.8 to 1.2 moles of hydrogen. (It might be mentioned here that it was not possible to detect an extra double bond in isorhodoporphyrin with halogen acids nor to dehydrogenate rhodoporphyrin with mild reagents.) We may conclude that the compounds are not really isomeric but differ in their hydrogen content, or that the catalytic hydrogenation method is unreliable and the results are to be discarded. If the first alternative is accepted, then the acid transformations mentioned in the first part of this paper must be reductions. It seems to us very unlikely, indeed, that 50% sulfuric acid or concentrated hydrochloric acid should act as reducing agents. We are inclined to discard, therefore, the hydrogenation results.

When we now turn to a consideration of the relation between the chlorins and the *iso* and *true* porphyrins, we again have two alternatives depending on whether or not we regard the hydrogenation results as reliable. These results show no difference between the hydrogen absorption of chlorins and true porphyrins (less than 0.4 mole) and indicate that isoporphyrins are dehydrogenation products of true porphyrins. If we accept these results, then chlorophyll and the chlorins are still regarded as dihydroisoporphyrins in basic structure, as has so often been maintained by this Laboratory, but they are also isomeric with true porphyrins as Fischer postulates. It might be pointed out that it is still possible that the chromophoric group in the chlorins is a reduced porphyrin ring with the extra hydrogen atoms compensated by a loss of hydrogen in some other part of the molecule.

We prefer the other alternative in which the hydrogenation results are discarded and the isomerization of the *true* and *iso* porphyrins is credited. We therefore continue to assume that the fundamental nucleus of chlorophyll and the chlorins is that of a dihydroisoporphyrin, but would add that it is also that of a dihydroporphyrin, these being of an equal state of hydrogenation.

(6) Clar and Haurowitz, *Ber.*, **66**, 332 (1933).

(7) Fischer and Neumann, *Ann.*, **494**, 243 (1932).

We are indebted to Mrs. G. Ware Wellwood for the microanalyses reported in this paper.

Experimental

Zinc Salt of Isorhodoporphyrin Dimethyl Ester.—Isorhodoporphyrin dimethyl ester was prepared from phaeopurpurin 7 methyl ester as described by Fischer, Süs and Klebs.[8] To 100 mg. of ester in 10 cc. of chloroform, a hot solution of 30 mg. of zinc acetate in 10 cc. of methyl alcohol was added and the solution evaporated to a small volume. Crystallization of the zinc salt was aided by the addition of methyl alcohol. The beautiful red needles were recrystallized from chloroform–methyl alcohol.

The spectrum in ether (1 mg. in 30 cc.; 5-cm. tube): I, 610.8–586.5; II, 565–536; III, shadow at 519.0. E. A. 444. Order: I, II, III.

Anal. Calcd. for $C_{34}H_{36}O_4N_4Zn$: C, 64.80; H, 5.76; Zn, 10.38. Found: C, 65.20, 64.93; H, 5.51, 5.80; Zn, 10.39, 10.38.

Zinc Salt of Rhodoporphyrin Dimethyl Ester.—This was prepared exactly as was the zinc salt of isorhodoporphyrin ester. The rhodoporphyrin ester was obtained from phaeophytin as described by Treibs and Wiedemann.[9]

The spectrum in ether: I, 604–582; II, 562.5–533; III, shadow at 514.7. E. A. 439.0. Order: I, II, III.

Anal. Calcd. for $C_{34}H_{36}O_4N_4Zn$: C, 64.80; H, 5.76; Zn, 10.38. Found: C, 64.69, 64.57; H, 5.66, 5.50; Zn, 10.36, 10.42.

Iron Salt of Isorhodoporphyrin Dimethyl Ester.—To 100 mg. of ester in 10 cc. of chloroform, a few crystals of sodium chloride and 10 cc. of a hot solution of freshly prepared ferrous acetate in acetic acid were added, and the solution was heated on a steam-bath for fifteen minutes. The chloroform was distilled off, the iron salt separating on cooling. It was recrystallized three times from chloroform–methyl alcohol.

The spectrum of the hemochromogen in pyridine–hydrazine solution: I, 586–559; II, 538–522. Order of intensity, I, II.

Anal. Calcd. for $C_{34}H_{36}O_4N_4FeCl$: C, 62.24; H, 5.53. Found: C, 60.81, 60.91; H, 5.36, 5.47.

Iron Salt of Rhodoporphyrin Dimethyl Ester.—This was prepared exactly as was the iron salt of isorhodoporphyrin ester.

The spectrum of the hemochromogen in pyridine–hydrazine solution: I, 575–556; II, 535–520. Order of intensity, I, II.

Anal. Calcd. for $C_{34}H_{36}O_4N_4FeCl$: C, 62.24; H, 5.53. Found: C, 61.03, 61.37; H, 5.80, 5.36.

Transformation of Isorhodo- to Rhodoporphyrin

In 50% Sulfuric Acid.—A solution of 150 mg. of isorhodoporphyrin in 25 cc. of cold 50% sulfuric acid was saturated with nitrogen and allowed to stand forty-eight hours at room temperature. The product was transferred to ether and fractionated. Rhodoporphyrin was extracted with 4% hydrochloric acid. The residue had the spectrum of unchanged isorhodoporphyrin. This residue was taken to dryness and redissolved in 50% sulfuric acid. After forty-eight hours the further conversion to rhodoporphyrin was about 30%, showing that the residue in the first reaction was really isorhodoporphyrin and not a closely similar isomer formed in the reaction.

Exactly the same results were obtained in an atmosphere of oxygen or air as in nitrogen. The yield of rhodoporphyrin was about 60% and the product was methylated with diazomethane and recrystallized three times from chloroform–methyl alcohol. The acid number and spectrum as well as the spectra of the iron and zinc complexes checked those of an authentic sample of rhodoporphyrin ester.

Anal. Calcd. for $C_{34}H_{38}O_4N_4$: C, 72.08; H, 6.71. Found: C, 70.5; H, 6.89.

The compound showed a tendency to hold water but the analyses continued low in carbon even after long drying and weighing in a dry atmosphere. Chlorin *f* was unchanged in 50% sulfuric acid whether in a nitrogen or oxygen atmosphere, as was also rhodoporphyrin itself under the same conditions.

In Other Acid Media.—The conversion took place also at room temperature in concentrated sulfuric acid, in 70% sulfuric acid (both with some decomposition), in concentrated aqueous hydrochloric acid and in methyl alcohol, chloroform or acetic acid, saturated with dry hydrogen chloride or hydrogen bromide. In several cases another unidentified porphyrin of unsharp acid number accompanied the rhodoporphyrin, but this was not isolated. The hemins of both porphyrins were similarly treated but did not react as long as they remained unhydrolyzed by the medium. If the extra double bond in isorhodoporphyrin indicated by the hydrogenation results were especially reactive it might be expected to add halogen acids. Therefore, the products obtained by reaction in methyl alcohol plus dry hydrogen chloride or bromide were transferred to ether with ice water and dilute sodium acetate and the solutions concentrated *in vacuo*. Analysis showed the complete absence of halogen.

Effect of Mild Oxidizing Agents.—Rhodoporphyrin and isorhodoporphyrin esters, their zinc salts and their pyridine and cyanohemochromogens were illuminated with white and ultraviolet light in the following solvents: pyridine, chloroform, acetone, methyl alcoholic potassium hydroxide, sodium amylate, arochlor plus sulfur, arochlor plus selenium, nitrobenzene and oleum plus sulfur. In all instances in which any changes took place they were evident only as a fading or darkening of the solutions, indicating extensive decomposition. In no case was any interconversion of one isomer to the other detected. Heating the same compounds at 100° in methyl alcoholic potassium hydroxide, sodium amylate, diphenyl, arochlor plus sulfur or selenium, and nitrobenzene also failed to produce any conversion, though often destruction of material resulted.

Transformation of Pyrochloro- to Phylloporphyrin.—This was carried out in 50% sulfuric acid on material obtained from the action of hydriodic acid in glacial acetic acid on pyrochlorin e[10] and carefully fractionated to remove phylloporphyrin. After forty-eight hours at room tem-

(8) Fischer, Süs and Klebs, *Ann.*, **490**, 87 (1931).

(9) Treibs and Wiedemann, *ibid.*, **471**, 193 (1929).

(10) Conant, Hyde, Moyer and Dietz, THIS JOURNAL, **53**, 365 (1931).

perature in nitrogen the yield of phylloporphyrin was about 50%. The acid number, and acid and ether spectra checked those of authentic phylloporphyrin, and the spectrum of the residue was that of unchanged starting material.

Catalytic Hydrogenation.—The standard Warburg apparatus was modified slightly so that glacial acetic acid could be used as manometer liquid, and so that the manometer could be conveniently emptied while evacuating and filling with hydrogen. Ordinarily, the manometer level is adjusted by exerting pressure by means of a clamp on a rubber tube connected at the bottom of the manometer and containing excess manometer liquid. Since glacial acetic acid evaporates very rapidly through rubber, it was found advisable to replace the clamp and rubber tube by a stopcock, connected to a small glass leveling tube by rubber tubing. In this manner the leveling device could be closed off during evacuation. The system was evacuated through one arm of the manometer and filled with hydrogen through the other, the process being repeated five times. The substance (2.5 mg.) was introduced into the side arm in a glass capsule at the beginning of the experiment and the Adams platinum oxide catalyst (0.25 to 6 mg., preferably 0.5 mg.) and 5 cc. of glacial acetic acid were placed in the Warburg vessel. The system was completely equilibrated with hydrogen before the substance was added. A duplicate manometer and vessel containing acetic acid only, served to record changes in barometric pressure. Four determinations with dimethylacrylic acid as a standard substance gave very satisfactory results. The hydrogenation of this substance with Adams catalyst required thirty minutes with 6 mg. of catalyst and 0.97 mole of hydrogen was absorbed. Varying the amount of catalyst merely caused a nearly proportional change in rate. A similar variation in the amounts of catalyst used with chlorophyll compounds caused considerable change in the leuco point as is evident from the accompanying table. It was always noted that a disproportionate amount of hydrogen was absorbed during the disappearance of the last small fraction of color. With 0.5 mg. of catalyst, the hydrogenation required from one to three hours.

Yields were determined in several cases by diluting the filtered acetic acid solution, reoxidizing by standing in air overnight, to 10 cc. with pyridine, and then comparing with a weighed sample similarly dissolved, in a comparison spectroscope. The yield of rhodoporphyrin from isorhodoporphyrin was about 57%; that of rhodoporphyrin from chlorin *f*, about 50%; the reoxidation of reduced solutions of rhodo and pyrroporphyrins gave between 60 and 65% recovery of the original porphyrins. It might be mentioned here that the product of reoxidation of leucopyrochloroporphyrin is phylloporphyrin.

Using palladium black prepared by the Willstätter method[11] as catalyst, it was found that doubling the amount used had no significant effect on the number of moles of hydrogen absorbed. The hydrogenation required about six hours for 2.5 mg. of substance and 5 mg. of catalyst.

Substance, ester	Pt catalyst (0.5–6 mg.)	Pd catalyst
Phylloporphyrin monomethyl	4.0	3.3
Pyrochloroporphyrin monomethyl		4.1
Pyrroporphyrin monomethyl	4.0, 3.6	
Rhodoporphyrin dimethyl	4.1, 3.7, 3.8	3.1
Isorhodoporphyrin dimethyl	5.4, 5.1, 4.8	4.0
Chlorin *f* dimethyl	4.6, 4.4, 4.5	3.2, 3.3
Chlorin *e* trimethyl	3.7, 4.0, 4.2	3.2, 3.2

Summary

1. The transformation of isorhodo- to rhodoporphyrin and pyrochloro- to phylloporphyrin in acid media is reported as evidence for a prototropic isomerization in both instances.

2. The metal complexes of these porphyrins (the pyridine and cyanohemochromogens) are also isomeric and regenerate only the original porphyrins.

3. Catalytic hydrogenation data indicate a difference of two hydrogen atoms between *iso* and *true* porphyrins. The significance of such data and their bearing on the state of oxidation of the nucleus, are discussed.

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(11) Willstätter and Waldschmidt-Leitz, *Ber.*, **54**, 123, 137 (1921).