[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

STUDIES IN THE CHLOROPHYLL SERIES. II. REDUCTION AND CATALYTIC HYDROGENATION

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The relationship between the magnesium-free chlorophyll derivatives and the porphyrins is a matter of considerable interest. While the structure of the porphyrins has been established by H. Fischer,[1] the constitution of the first decomposition products of chlorophyll (*e. g.*, the phaeophorbides, chlorin *e* and rhodin *g*) is still unknown. At the time this work was undertaken the only method of transforming the chlorophyll compounds into porphyrins was by means of drastic alkaline decomposition as employed by Willstätter. Within the last year several new reactions have been discovered which lead to the production of porphyrins from the chlorophyll series. The formation of a porphyrin in the pyrolysis of chlorin *e* was discussed in the first paper of this series.[2] In the course of the quantitative studies reported in the present paper, we have found that the complete hydrogenation of the chlorophyll compounds yields leuco compounds which on reoxidation with air form a mixture containing porphyrins. Fischer and Baumler[3] previously had discovered that a porphyrin is obtained by reducing phaeophorbide *a* with hydrogen iodide or sodium amalgam and reoxidizing with air. These transformations show the close relationship between the chlorophyll compounds and the porphyrins and seem to exclude the possibility suggested by Willstätter that the change from the one series to the other involved the synthesis of the carbon skeleton of the fourth pyrrole nucleus.

The characteristic structure of the porphyrins as given by Fischer contains a closed completely conjugated system of double linkages which includes linkages within and without the modified pyrrole nuclei.[4] Probably

[1] See particularly H. Fischer and A. Treibs, *Ann.*, **466**, 188 (1928), and H. Fischer and Schormüller, *ibid.*, **473**, 212 (1929).

[2] Conant and Hyde, *Science*, **70**, 1806 (1929); THIS JOURNAL, **51**, 3668 (1929).

[3] Fischer and Baumler, *Sitzb. d. Bayr., Akademie d. Wissenschaften*, March, 1929, p. 77; this paper had not come to our attention when our note in *Science* (Ref. 2) was published; see also *Ann.*, **474**, 65 (1929).

[4] Professor Linus Pauling has suggested to us that the characteristic spectrum of a porphyrin, which consists of a number of relatively very narrow bands, is due to the cyclic system which encloses and protects the chromophoric groups from thermal disturbances; the porphyrins might thus be considered analogous to the rare earths. At his further suggestion we have examined the spectra of a number of porphyrins and chlorophyll derivatives at liquid-air temperatures and observed a narrowing of bands and in the case of the two porphyrins examined a resolution of bands into lines. The further study of this phenomenon which is now in progress may possibly be of some service in establishing the structural relationships between the chlorophyll series and the porphyrins.

because of the closed cyclic conjugated system the porphyrins are not as reactive as the majority of highly colored organic compounds. For example, the combination of gaseous hydrogen and colloidal palladium on asbestos suspended in aqueous alkaline solution will reduce to the corresponding leuco compounds all the common dyestuffs which we have examined. Sodium hydrosulfite will usually bring about the same change. The porphyrins, however, occupy an almost unique place among colored organic substances by virtue of the fact that they are not reduced in alkaline aqueous solution by either hydrosulfite or hydrogen with palladized asbestos. On the other hand, the chlorophyll derivatives (the phaeophorbides, chlorin *e*, rhodin *g*) are reduced, as is also the bile pigment bilirubin.

Experiments with Hydrosulfite.—In all the experiments with sodium hydrosulfite, 0.1 *N* potassium hydroxide was used and the concentration of the organic material was approximately 0.5×10^{-3} mole per liter. This concentration gave in most cases quite a highly colored solution. The solution of sodium hydrosulfite was approximately 0.01 molar in 0.1 *N* potassium hydroxide and was freshly prepared before each series of experiments were made, being kept under a layer of xylene. Even under these conditions the reducing power was lost very rapidly. The concentration was determined approximately before use by titrating with ammoniacal copper sulfate solution. The first experiments were carried out by placing two cc. (containing 0.001 millimole) of the alkaline solution of the organic material in one arm of an H-tube and placing the desired amount of hydrosulfite solution in the other. The tube was then repeatedly evacuated (five or six times), and filled with oxygen-free nitrogen. This precaution was taken since it was expected that any reduction product would be sensitive to air. The contents of the two sides were mixed by tilting the tube and the tube was examined at noted intervals to observe the color. It was soon found that no apparent change takes place at room temperature but that the color change is rapid when the tube is heated in the steam-bath. The later experiments were carried out in a silica test-tube, which avoided possible complications due to contamination by metals from the glass. In a blank test in which the same quantity of hydrosulfite was heated alone under otherwise identical conditions, it was found that the reducing power of the hydrosulfite was completely lost in twenty to thirty minutes, as shown by titration with ammoniacal copper sulfate solution.

Phaeophorbide *a* and chlorin *e* were reduced by sodium hydrosulfite at 80° in about ten minutes, yielding a pink solution which with air became green, while longer heating with excess hydrosulfite produced a similar green color. The green solutions were spectroscopically similar to but not identical with the initial solutions. Phaeophorbide *b* and rhodin *g* did not give a pink phase; there appeared to be a slight color change, but there was no

clear indication of a further change on exposure to air. Isochlorophyllin (*a* plus *b*) showed no change. It is interesting that the introduction of magnesium prevents the action of hydrosulfite. Bilirubin changed from a light yellow-orange to a reddish-orange, which with air reverted to the yellow-orange. A rather broad absorption band in the green appeared on reduction; this disappeared on exposure to air, the spectrum again being like that of the initial material.

Some idea of the minimum number of moles of hydrosulfite required to produce the changes was obtained from a series of experiments using varying proportions of hydrosulfite. For example, with phaeophorbide *a* five moles per mole produced a definite brown color, whereas with three moles no noticeable change of the initial olive-green could be observed. The change with five moles was not so definite as with ten moles, and it would, therefore, appear that somewhere between these limits lies the minimum amount of hydrosulfite necessary to reduce this substance under these conditions. Approximately seven moles per mole of substance seemed to be required with the phaeophorbides, chlorin *e*, rhodin *g* and bilirubin. We cannot conclude from this, however, that these numbers correspond to a stoichiometric relationship in the reduction process; a large excess of hydrosulfite may be required in order to obtain a sufficiently high rate of reaction or in order to compensate for the partial destruction of the hydrosulfite which occurs at this temperature.

It was evident from our experiments that both the typical porphyrins from the blood pigments (protoporphyrin and mesoporphyrin) as well as two porphyrins from chlorophyll (cyanoporphyrin and erythroporphyrin) were not affected by hydrosulfite at 80°, whereas the magnesium-free chlorophyll derivatives were attacked although they were not reduced to colorless compounds.

Reduction with Hydrogen.—The action of hydrogen in the presence of palladium on asbestos was studied in a special apparatus which enabled us to measure with a fair degree of accuracy the number of moles of hydrogen consumed even with such small samples as 3–6 mg. This apparatus, which was developed in this Laboratory, will be the subject of a separate paper, since it promises to be of value in connection with any problem involving absorption of hydrogen or oxygen by very small quantities of material. It was composed entirely of glass and the pressure differences were read on a differential scale as in the Warburg apparatus for measuring oxygen consumption.[5] The palladium catalyst was prepared by adding 10 cc. of a 1% solution of palladous chloride to a small amount of asbestos suspended in water and reducing with the required amount of hydrazine hydrochloride and alkali. The catalyst was filtered off, thoroughly washed and finally suspended in a total volume of 100 cc. of water. At the beginning of an experiment, 1-cc. portions of the suspension were added to the alkali contained in each of the two reaction bottles of the apparatus (one of which served as the blank control) which were connected by the differential manometer. The sample was contained in a small glass tube so arranged

[5] Warburg, *Biochem. Z.*, **152,** 51–63 (1924).

that it could be broken *after* the equilibration of catalyst and solvent with hydrogen was complete. The apparatus was standardized by reducing well-known substances of high purity in aqueous alkali with hydrogen and palladium on asbestos. In a typical run 0.0148 millimole of quinone gave a final reading on the differential manometer of 4.6 cm. (the liquid in the manometer was oil of cloves), corresponding to 0.0026 millimole of hydrogen (at 23°, 760 mm.) per cm. The other results with the same compound gave 0.0025 and 0.0024. Indigo tetrasulfonate and anthraquinone disulfonate gave similar results. Dimethylacrylic acid in glacial acetic acid with a platinum oxide catalyst gave values of 0.0026 and 0.0027 millimole per cm.

The results obtained with the chlorophyll compounds and the porphyrins are summarized in Table I. As in the case of the experiments with sodium hydrosulfite it is clear that the porphyrins are different in their behavior from the magnesium-free chlorophyll derivatives and bilirubin. The small amounts of hydrogen recorded in the last column of Table I in the case of a few porphyrins are within the experimental error. Although the quantitative results were not entirely reproducible, it appears that the

TABLE I

SUMMARY OF RESULTS OBTAINED IN EXPERIMENTS ON THE ACTION OF HYDROGEN AND PALLADIUM CATALYST IN ALKALINE SOLUTION AT 23°

Substance	Millimole of substance	Millimole of H_2 absorbed	Approx. time in hours for reduction	Mole of H_2 per mole of substance
Phaeophorbide *a*	0.0068	0.0109	60	1.6
Phaeophorbide *a*	.0036	.0061	40	1.7
Phaeophorbide *b*	.0036	.0057	42	1.7
Phaeophorbide *b*	.0033	.0057	40	1.73
Chlorin *e*	.0052	.0096	36	1.8
Chlorin *e*	.0041	.0023	30	0.57
Chlorin *e*	.0028	.0018	42	0.6
Chlorin *e*	.0030	.0039	24	1.3
Chlorin *e*	.0078	.0100	49	1.3
Rhodin *g*	.0059	.0086	48	1.45
Rhodin *g*	.0018	.0028	50	1.6
Bilirubin	.0068	.016	35	2.3
Bilirubin	.0044	.0073	30	1.6
Cyanoporphyrin	.0089	.0032	15	0.36
Cyanoporphyrin	.0054	.00053	50	.10
Erythroporphyrin	.012	.00159	12	.13
Erythroporphyrin	.0065	.00035	48	.05
Protoporphyrin	.0054	.00	60	.00
Protoporphyrin	.014	.00	20	.00
Mesoporphyrin	.0057	.00	72	.00
Phylloporphyrin	.006	.00	20	.00

NOTE.—In calculating the millimoles of substance the following molecular weights were employed: phaeophorbide *a*, 610; phaeophorbide *b*, 624; chlorin *e*, 628; rhodin *g*, 611; bilirubin, 584.

phaeophorbides, chlorin *e* and rhodin *g* take up approximately two moles of hydrogen; the results with chlorin *e* were the least satisfactory. The reduction of the magnesium-free chlorophyll derivatives under these conditions does not lead to the formation of leuco compounds; the reduced solutions are still colored as in the case of the action of hydrosulfite. In this connection it should be pointed out that the reductions brought about by hydrogen in the presence of palladized asbestos are not catalytic hydrogenations but reduction processes at a level of intensity of the hydrogen electrode. Only special linkages are attacked by *reducing* agents as contrasted with *hydrogenating agents;* for example, neither sodium hydrosulfite[6] nor palladium on asbestos in alkaline solution with hydrogen will add hydrogen to such double bonds as those which occur in allyl alcohol and dimethylacrylic acid.

Catalytic Hydrogenation.—Although the porphyrins are not attacked by the two reducing agents just mentioned, they may be converted into leuco compounds by a number of procedures, as is well known. Fischer, for example,[7] catalytically reduced the methyl ester of uroporphyrin in acetic acid using a platinum catalyst. Kuhn[8] has also studied the catalytic reduction of the porphyrins in acetic acid solution and has shown that the leuco compounds formed are reoxidized to the same porphyrin on exposure to air. Using our same apparatus we have carried out a few experiments on the catalytic hydrogenation of the magnesium-free chlorophyll derivatives in acetic acid using the platinum oxide catalyst of Adams.[9]

The results are summarized in Table II. In every case the final solutions were colorless and on exposure to air became colored again. A distinction of considerable importance between the phaeophorbides, chlorin *e* and rhodin *g* on the one hand, and the porphyrins on the other hand, is the fact

TABLE II

EXPERIMENTS ON THE CATALYTIC HYDROGENATION IN GLACIAL ACETIC ACID OF CERTAIN CHLOROPHYLL DERIVATIVES AND PORPHYRINS

Substance	Millimole of substance	Millimole of H_2 absorbed	Time of reduction in hours	Moles of H_2 per mole of substance
Phaeophorbide *a*	0.0058	0.0214	3	3.7
Phaeophorbide *b*	.0057	.0223	6	3.9
Chlorin *e*	.0068	.02407	12	3.54
Chlorin *e*	.00479	.01832	19	3.82
Rhodin *g*	.0052	.0182	18	3.5
Cyanoporphyrin	.0061	.01805	6	2.96
Erythroporphyrin	.0067	.0247	4	3.7
Phylloporphyrin	.0083	.0292	3	3.5

[6] Conant and Cutter, *J. Phys. Chem.*, **28**, 1096 (1924).

[7] Fischer and Zerweck, *Z. physiol. Chem.*, **137**, 242 (1924).

[8] Kuhn and Seyffert, *Ber.*, **61**, 2509 (1928).

[9] Adams and Shriner, THIS JOURNAL, **45**, 2171 (1923).

that the chlorophyll derivatives did not revert to the original substances on shaking the reduced solutions with air, whereas the porphyrins were regenerated.

The oxidation product of the hydrogenated chlorophyll compounds is a complex mixture. Porphyrins appear to be formed as judged by the spectra of the products obtained after fractionating with hydrochloric acid according to the Willstätter–Mieg procedure.[10] We have isolated small quantities of two crystalline porphyrins from the hydrogenation of chlorin *e* in acetic acid and subsequent oxidation with air. The analyses and spectra are given below.

Porphyrin from chlorin *e:* acid number, about 0.7. Spectrum in ether (0.1 g. in 5 liters), 100-mm. layer: (very faint) 638—630; 593- - -587.6—575.8; 557.2- - -550—540.5- - -534.4; 523.8- - -520—492.4- - -488.4; E. A., 445.4.

Anal. Calcd. for $C_{34}H_{38}O_5N_4$: C, 70.0; H, 6.5. Found: C, 70.4; H, 6.6.

Porphyrin from chlorin *e:* acid number about 5. (Insufficient amounts for analysis.) Spectrum in ether, 100-mm. layer: (faint) 646.9—644; 635.4—630.3; 591.8- - -579.9—575.3; 561.3- - -559.0—548.5- - -544.9; 525.5- - -522.8—507.2; E. A. 444.1.

The resistance of the porphyrins to mild reducing agents as well as the fact that the leuco compounds are reoxidized and reconverted to the original material indicates a particularly stable arrangement of linkages in the porphyrin molecule. The behavior of the phaeophorbides, chlorin *e* and rhodin *g* shows clearly that they contain a more reactive unsaturated system. They appear to contain groupings which, like those present in the dipyrrylmethenes, are easily attacked by mild reducing agents. If one accepts the conclusion that the carbon skeleton of the porphyrins is already present in the phaeophorbides, it seems probable that one of the four modified pyrrole nuclei does not have its unsaturation so located as to form part of the cyclic conjugated system characteristic of porphyrins. As a result, the incompletely conjugated systems are readily attacked by mild reducing agents but on reoxidation of these products porphyrins are not formed. A drastic hydrogenation followed by oxidation is necessary before the porphyrin structure is formed. The quantitative results (Table II) show that the degree of total unsaturation of the porphyrins and chlorophyll compounds is the same as measured by the formation of the leuco compounds in acetic acid. The difference thus appears to rest in the location of the various linkages. Two relatively reactive double bonds are present in the chlorophyll series and one or two more that are only attacked by catalytic hydrogenation. These latter are part of the chromophoric group present in the *colored reduction* products of the chlorophyll series formed by the action of the mild reducing agents.

[10] Willstätter and Mieg *Ann.*, **350**, 1 (1906).

In connection with the experiments with hydrosulfite, we have made another observation which shows the reactivity of the chlorophyll compounds as compared with the porphyrins. When the phaeophorbides or chlorin *e* or rhodin *g* in ether solution are shaken with an aqueous solution of sodium acid sulfite, a rapid reaction takes place in which the intensity of the color is much diminished and the shade changes to a reddish-brown, some of the material passing into the aqueous layer. The porphyrins do not show this behavior. We propose to investigate this reaction further.

Summary

1. The action of sodium hydrosulfite in alkaline aqueous solution at 80° on chlorophyll derivatives and porphyrins has been examined. The magnesium-free chlorophyll derivatives are reduced with a change of color; the porphyrins and the magnesium-containing chlorophyll compound isochlorophyllin are not affected. Similar results were obtained with hydrogen and palladium on asbestos suspended in an aqueous alkaline solution at 23°. The amount of hydrogen absorbed was measured in a special micro apparatus. The magnesium-free chlorophyll compounds took up approximately two moles.

2. Catalytic hydrogenation of the porphyrins and the chlorophyll compounds proceeds to leuco compounds in glacial acetic acid. The number of moles of hydrogen absorbed is 3 to 4. On reoxidation with air the porphyrins are regenerated; the chlorophyll compounds yield a mixture of products including some porphyrins.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF TEXAS]

AN INVESTIGATION OF THE BASES IN THE KEROSENE DISTILLATE OF CALIFORNIA PETROLEUM[1]

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Introduction

Very little is known regarding the nature of the nitrogen compounds in crude petroleum, and these are at times confused with products in *dis-*

[1] This paper contains results obtained at the Chemical Laboratory, University of Texas, in an investigation on "The Nitrogen Compounds in Petroleum," listed as Project 20 of American Petroleum Institute Research. Financial assistance in this work has been received from a research fund of the American Petroleum Institute donated by John D. Rockefeller. This fund is being administered by the Institute with the cooperation of the Central Petroleum Committee of the National Research Council.

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