of 3.6 to 3.9 amperes flowed through the circuit at a voltage of 3.8 to 4.0. The temperature of the anolyte was maintained at 40 to 45°. At the end of eight hours the oxidation was complete. The reaction mixture was worked up as in the case of the quinoline oxidation and copper nicotinate (9.6 g. or 60%) was obtained.

Summary

1. Quinoline has been oxidized electrolytically at a platinum anode in 75% sulfuric acid to quinolinic acid with a yield of 77%.

2. Nicotinic acid has been obtained by decarboxylation of quinolinic acid in cyclohexanol and also by the electrolytic oxidation of 3-picoline at a lead anode.

GUELPH, ONTARIO RECEIVED JUNE 17, 1946

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE JOHNS HOPKINS UNIVERSITY]

A Synthetic Ferroporphyrin Complex that is Passive to Oxygen[1]

BY ALSOPH H. CORWIN AND J. GORDON ERDMAN[2]

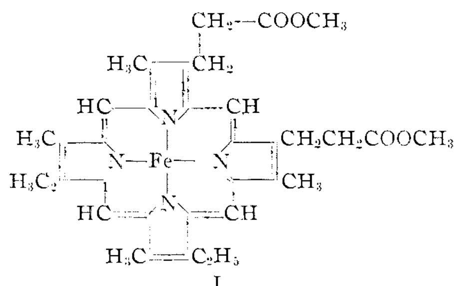
Hemoglobin possesses remarkable chemical properties other than its ability to combine reversibly with molecular oxygen. One of these is its passivity to oxygen, a property which has not been reported heretofore in ferroporphyrin complexes of known structure. Hemoglobin is a ferrous complex, potentially susceptible to oxidation, yet capable of combining with and transporting oxygen without being oxidized to the ferric state. An elucidation of the conditions under which passivity to oxygen may be expected should provide one of the specifications for the synthesis of compounds paralleling the chemical behavior of hemoglobin. The first step in a program with this objective would be the preparation of solutions of hemes, free from reducing agents, suitable for oxidation studies. This paper reports experimental conditions under which this objective may be attained. In the course of this work, conditions were discovered under which a ferroporphyrin complex coupled with pyridine is passive to atmospheric oxygen. The necessary reactions have been followed and the preparations analyzed spectroscopically.

Preparation of pure ferroporphyrins, or hemes, has proved difficult, due to their extreme sensitivity to oxidation. So far an absorption spectrum for one of these substances in a non-coördinating solvent has not been reported. Fischer, Treibs and Zeile[3] prepared crystalline hemes for the first time. They report powder spectra of three bands somewhat resembling the ferric porphyrin chlorides or hemins. Powder spectra are complicated by strong molecular interactions and by selective scattering of the particles.

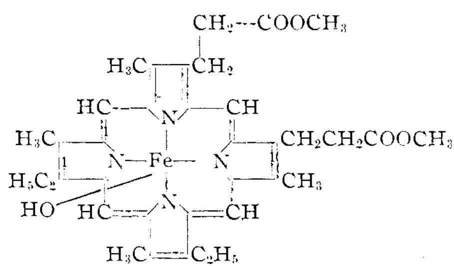
Spectra obtained by the addition of reducing agents such as hydrazine hydrate or sodium hydrosulfite to solutions of the ferric porphyrins in alkaline aqueous media or in nitrogen bases such as pyridine are complicated by the tendency of the iron to form complexes through its two remaining coördinate valencies. In the presence of large amounts of nitrogen bases the formation of the complex, or hemochromogen, is accompanied by a shift from para- to diamagnetism and the formation of a characteristic spectrum. In aqueous solutions the spectrum is spread out into a broad hump through partial complex formation with either water or the reducing agent which usually must be present in large excess.

Knowledge of the nature of the binding in the ferroporphyrins and their complexes is vital to our understanding of their action in living systems. The original purpose of the present work was to prepare a crystalline heme, to devise means for determining the spectrum in a non-coördinating solvent, and finally to examine its stability with respect to oxidation, both with and without a coördinating base. These objectives were attained by the use of special apparatus designed to exclude atmospheric oxygen during the manipulations.[4]

Mesoheme IX dimethyl ester, I, crystallized in red needles which possessed a peculiar golden lustre unlike any of the other iron compounds of



I



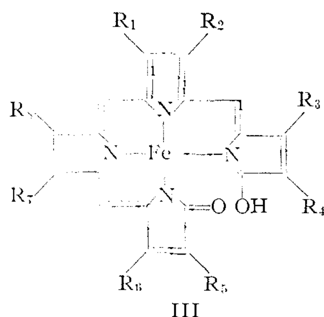
II

(1) Studies in the Pyrrole Series XVIII; Paper XVII, Erdman and Corwin, THIS JOURNAL, **68**, 1885 (1946).

(2) Present address, Mellon Institute, University of Pittsburgh, Pittsburgh, Pennsylvania.

(3) Fischer, Treibs and Zeile, *Z. physiol. Chem.*, **195**, 1 (1931).

(4) *Cf.* Hieber, Sonnekalb and Becker, *Ber.*, **63**, 978 (1930).



the porphyrin. Dilute solutions in dioxane were deep purple-red. On exposure to air they almost instantly changed to the reddish brown color of ferric porphyrin hydroxide, or hematin, II.

Mesoheme in dioxane is readily cleaved by hydrogen chloride yielding the porphyrin dihydrochloride. Mesohematin is converted to mesohemin chloride. These reactions were utilized for determining the purity of the heme solutions.

The solution used for the calculation of the absorption curve contained about 90% heme and 10% hematin. The curve for the hematin was determined under identical conditions. Subtraction of the per cent. extinction coefficients yielded the curve for the pure mesoheme IX dimethyl ester (Fig. 1, Curve A) with peaks at 585, 540 mμ, and the great peak at 410 mμ. Because of the instability of the compound, the errors in the molar extinction coefficients are probably large (10%). The position of the great ultraviolet peak was determined but its extinction coefficient is not reported since at such dilutions the ratio of

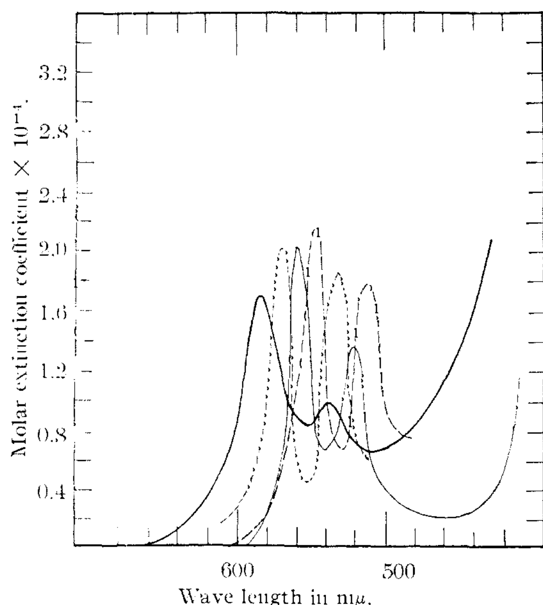


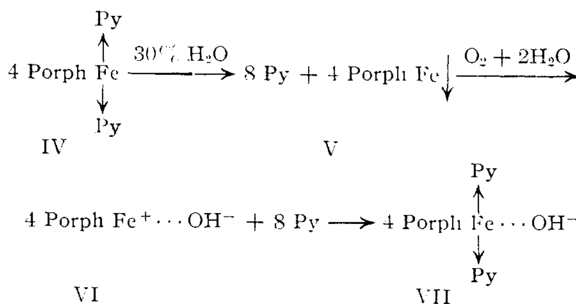
Fig. 1.—Absorption curves of divalent metallic complexes of mesoporphyrin IX dimethyl ester: ——— ferrous, ——— cupric, — — — nickelous, - - - - - zinc.

ferrous to ferric could not be determined. The curve is sufficiently good to show that the mesoheme spectrum contains only two bands in the visible. In this respect it resembles those of the other divalent metal complexes as determined by Stern and Dezelic.[5] Curves of the cupric, nickelous and zinc complexes are shown in Fig. 1 for purposes of comparison.

The complexes of the hemes and organic nitrogen bases, the hemochromogens, prepared by reduction of the ferric porphyrins react rapidly with air. The reaction is not simply an oxidation of the iron, for varying amounts of the hemin are destroyed. Warburg and Negelein[6] and Lemberg and co-workers[7] isolated from oxidation mixtures of protoheme a green substance believed to be ferric biliverdin hydroxide, or verdohematin, III.

We find that the addition of oxygen to solutions of mesoheme IX dimethyl ester prepared with various reducing agents shows a variety of results. In the case of reduction with sulfur dioxide a hitherto unreported compound is formed, possessing a characteristic three banded spectrum. For such reactions Lemberg proposes a mechanism involving the addition of oxygen to the iron. Excess reducing agent converts the oxygen to a peroxide complex. This strikes in to oxidize a bridge carbon. Such a mechanism does not suffice, however, to explain the variety of results obtained by varying the reducing agent.

Our orienting experiments concerning oxidation of the ferrous iron to the ferric state by oxygen show that pure pyridine hemochromogen, IV, prepared by dissolving crystalline mesoheme in dry, purified pyridine, did not react with dry oxygen. Even the addition of just sufficient water to form the hydroxide ions required stoichiometrically did not cause rapid oxidation. Such solutions, after several weeks exposure to air, still retained the hemochromogen spectrum. Addition of approximately 30% water produced a salmon pink precipitate of the heme, V. This substance is probably a water complex. This quickly oxidized to hematin, VI, which dissolved to a brown-red solution of pyridine parahematin VII. The sequence of events in the oxidation in the presence of water is given in the following equation, which



$$\underset{\text{IV}}{4\ \text{Porph Fe}(\text{Py})_2} \xrightarrow{30\%\ H_2O} 8\ \text{Py} + \underset{\text{V}}{4\ \text{Porph Fe}\downarrow} \xrightarrow{O_2 + 2H_2O}$$

$$\underset{\text{VI}}{4\ \text{Porph Fe}^{+}\cdots OH^{-}} + 8\ \text{Py} \longrightarrow \underset{\text{VII}}{4\ \text{Porph Fe}(\text{Py})_2\cdots OH^{-}}$$

(5) Stern and Dezelic, *Z. physik. Chem.*, **A180**, 131 (1937).

(6) Warburg and Negelein, *Ber.*, **63**, 1816 (1930).

(7) Lemberg and co-workers, *Biochem. J.*, **27**, 1322 (1936); **32**, 140 (1938).

also clarifies the observed passivity in the absence of water.

This experiment shows that it is the water complex which is oxidized, not the pyridine complex.

The behavior of the heme in pyridine is in sharp contrast to that in dioxane. Anhydrous dioxane, like pyridine, should not provide a low energy electron acceptor capable of permitting the oxidation to proceed. However, the stability of the dioxane complex is sufficiently lower than that of the pyridine complex that our best efforts to exclude water were not sufficiently good to prevent the oxidation of the heme in dioxane solution.

The experiments cited above point to one of the conditions which may be of importance in considering the passivity of the blood and muscle hemoglobin to air oxidation. We have shown that a heme complex with a strong complex former, pyridine, is not oxidized in the presence of small amounts of water. The biological systems contain strong complex formers. They differ, however, in that the oxidation is apparently independent of the amount of water. A protein could reduce the amount of water available to the iron atom if it were a large concave surface capable of partially engulfing the heme molecule with suitable coördinating groups spatially arranged so as to be immediately available geometrically for attachment to the two free coördinating valencies of the iron.

The authors wish to acknowledge a grant from the Rockefeller Foundation which made this research possible.

Experimental

Protohemin IX Chloride.—The method used was a modification of that reported by Hans Fischer[8] as based on the original procedure of Nencki and Zaleski.[9] By using a very concentrated solution of hemoglobin the yield, based on glacial acetic acid, was increased by 60%, thus reducing the expense as well as the volume of liquid handled.

The hemoglobin concentrate was prepared from oxalated blood. This was chilled and the erythrocytes centrifuged down. The supernatant serum and the layer of leucocytes were removed with a pointed suction tube. The cells were then mixed with an equal volume of hypertonic sodium chloride solution (1.5%) and recentrifuged. The supernatant liquid was again pulled off and the cells laked with a little toluene. Two hundred seventy cubic centimeters of the concentrate were used per liter of glacial acetic acid: yield, 2.2 g.

The crude material was recrystallized from pyridine-chloroform acetic acid: yields, first recryst. 80%; subsequent recryst. 90%.

Magnified 125 ×, the material appeared as massive plates, the broad faces being slightly elongated parallelograms with oblique extinction.

Mesoporphyrin IX Dihydrochloride.—The present method of preparation from hemin involves the one-step reduction of the vinyl groups and removal of iron through the action of formic acid and colloidal palladium. The method is based on that reported by Taylor.[10] We are reporting the entire method for it involves several modifications which, in our experience, lead to higher and more consistent yields.

The palladium oxide catalyst was prepared according to the directions of Starr and Hixon.[11] The sticky mud-like material was dried only enough to permit its removal from the Hirsch filter funnel. The moist catalyst, containing about 30% water, was stored in a glass weighing bottle, care being taken that it did not dry out completely. Dried samples of catalyst were less active to varying degrees. Use of such samples required longer reaction times, the extent of which had to be determined spectroscopically in each case. The reaction mixtures, thus obtained, always contained smaller amounts of porphyrin, which, due to contamination with a brown dye, required greater labor in purification.

Two grams of recrystallized hemin, finely powdered in an agate mortar, was suspended in 165 cc. of 90% C. P. formic acid in a 500-ml. boiling flask. Damp catalyst containing 460 mg. of palladium oxide was added, a glass cold-finger suspended in the neck, and the flask immediately plunged into an electrically heated glycerol-bath to a depth such that the liquid levels within and without the flask were the same. Several boiling sticks were added to prevent bumping. The formic acid began to reflux within two or three minutes. In about fifteen minutes the brown suspension changed to a deep purple-red solution. The mixture was refluxed for one and one-half hours though spectroscopic assay of aliquots taken during several runs indicated that the reaction was practically complete after half an hour. Marked decomposition became evident after two to three hours.

The cooled solution was filtered through a large sintered glass funnel to remove the catalyst and then poured slowly with efficient stirring into 600 ml. of 30% ammonium acetate solution. After a half hour, the precipitate was centrifuged down, washed with distilled water, and dissolved in 140 ml. of 2% aqueous ammonia. Twenty-four cubic centimeters of 30% disodium tartrate was added with thorough stirring, and the mixture allowed to stand for a half hour. The bright red precipitate was centrifuged out, and the supernatant liquid carefully pulled off. The latter varied from brown to the pale amber-red of disodium mesoporphyrin. The precipitate was redissolved in 140 ml. of 2% ammonia and treated as above. In most cases the mother liquor contained only disodium mesoporphyrin. If it was still brown the process was repeated a third time.

The wet residue was heated on the steam-bath to 100° and 150 cc. of boiling 25% hydrochloric acid added with vigorous stirring. Fine purple needles of the mesoporphyrin dihydrochloride began to separate almost immediately. After cooling overnight in the icebox the solid was filtered by suction and washed with a little cold 2½% hydrochloric acid. The entire funnel and contents were placed in a vacuum desiccator over potassium hydroxide until thoroughly dry. The mesoporphyrin dihydrochloride was then lifted out as a thin brittle purple microcrystalline cake. Concentration of the mother liquor yielded an additional small amount of the dihydrochloride; average yield 1.78 g. or 90.5%.

Since the reaction time in the above preparation was reduced, it was felt desirable to prove that the product was not protoporphyrin IX by comparing it with a sample of the latter prepared by a method not involving catalytic reduction. A small sample of protoporphyrin IX was therefore prepared by dehydration of hematoporphyrin which was in turn obtained by the reaction of acetic acid-hydrogen bromide on hemin. Comparisons were made of melting points and spectra of the dimethyl ester, its copper complex and of the reformed pyridine hemochromogens and mixed melting points were taken. These comparisons prove conclusively that the reduction in the above preparation yielded mesoporphyrin.

Mesoporphyrin IX Dimethyl Ester.—Two grams of mesoporphyrin dihydrochloride was added to 2 liters of absolute methanol containing 10 g. of dry hydrogen chloride. The mixture was refluxed for one hour, filtered while hot through a large sintered glass funnel, and then poured slowly and with stirring into 3 liters of 2½% sodium bi-

(8) Hans Fischer, "Organic Syntheses," **21**, 53 (1941).

(9) Nencki and Zaleski, *Z. physiol. Chem.*, **30**, 390 (1900).

(10) Taylor, *J. Biol. Chem.*, **135**, 570 (1940).

(11) Starr and Hixon, "Organic Syntheses," **16**, 77 (1936).

carbonate solution containing 1 kg. of finely crushed ice and 4 g. of celite. A filter was prepared by pouring 1 g. of celite, suspended in water, through an 8-cm. Büchner funnel. The layer of celite thus deposited prevented some of the porphyrin which floats on the surface from clogging the filter paper. The celite suspension of the porphyrin was washed thoroughly with distilled water and immediately dried in a vacuum desiccator.

The dry celite cake was broken into a fine powder and packed into a chromatograph tube in the usual manner. Chloroform was forced through the column under several pounds pressure. In this way a very concentrated solution, free from celite, was obtained. The filtrate was concentrated to about 20 ml. and hot methanol added to incipient precipitation. On cooling, the porphyrin separated in fine glistening purple leaves. It was filtered by suction, washed with 26 ml. of 1:9 chloroform-methanol, and dried in a vacuum desiccator; yield 1.78 g. or 97.5%, m. p. 213.5-216° (cor.).

The mother liquors were evaporated to dryness, yielding 68 mg. of crude but crystalline material.

Recrystallization.—The above material was dissolved in 20 ml. of boiling chloroform and 50 ml. of boiling methanol slowly added, care being taken that none of it ran down the walls. A clear but highly supersaturated solution was thus obtained. The glass stopper was immediately inserted and the flask plunged into a 4-liter beaker of water heated to 63°. After about a half hour clusters of sparkling purple crystals began to appear on the walls. After cooling overnight the temperature was slowly depressed by addition of ice and finally salt. The crystals were filtered off, washed with 1:9 methanol-chloroform and vacuum dried; yield 1.64 g. or 92.2%; m. p. 213.5-215.5° (cor.).

Evaporation of the mother liquors yielded 42 mg. of crude but crystalline material.

Magnified 125×, the pure product appeared as very elongated plates showing oblique extinction under crossed nicols. The crystals possess a striking purplish green lustre.

Chromatographic adsorption on activated alumina did not alter the melting point or appearance.

Mesoheme IX Dimethyl Ester.—Experimentation led to the following procedure as the simplest and most satisfactory by which the pure substance could be obtained.

The nitrogen used as an inert atmosphere was purified by passage through a modified form of the train described by Corwin and Ellingson.[12] The modifications were the following. The lead acetate solution was replaced by pumice chips impregnated with lead acetate. The sulfuric acid bottle was replaced by a tower containing calcium chloride followed by magnesium perchlorate. The sodium ketyl of benzophenone was prepared in xylene in place of the more volatile toluene. The mineral oil and paraffin mixture was replaced by a tower packed with paraffin chips. The use of solids instead of liquids in three of the operations decreased the back pressure which sometimes was troublesome in the older train. The nitrogen was supplied to the train through a constant pressure valve set at two pounds.

Deoxygenation of solvents each time they were used proved tedious. Storage vessels were prepared by sealing fine capillary inlets into the bottoms of a set of boiling flasks. A positive pressure of nitrogen was applied to these tubes at all times during storage. Whenever the stopper was lifted, nitrogen bubbled up through the liquid, thus protecting it from air. Due to the internal pressure of nitrogen, a leak around the stoppers did not cause danger of contamination. Water, acetic acid, and methanol were each refluxed for one hour in a stream of nitrogen and then distilled directly into the storage vessels. Reflux and distillation were carried out in a one-piece glass still. Transfers from the storage vessels were accomplished by means of a medical syringe, the steel needle of which was replaced by a long, thin, glass capillary tube. The barrel was rinsed with several small portions of the liquid before an actual transfer was made.

The reaction was carried out in the apparatus shown in Fig. 2. The air was removed by progressively sweeping from the left, being sure that each inlet and exit line had been swept clean of air. In the last stages, the apparatus was cautiously flamed to remove any oxygen adsorbed on the inner walls. It was finally cooled by sweeping with nitrogen.

A slow stream of nitrogen was passed in through A. The glass stopper of chamber II was lifted and 115 mg. of mesoporphyrin dimethyl ester and 100 mg. of sodium acetate introduced through a small powder funnel. Two milliliters of acetic acid was added by means of the syringe and the stopper replaced. The stopper of inlet E, chamber I, was removed and 1.5 cc. of acetic acid, 2 ml. of distilled water, and several lengths of iron wire added (Mallinckrodt "reagent"): C, 0.014%; Mn, 0.025; P, 0.001; Si, 0.001; S, 0.026; Fe, 99.9%. The mixture was then refluxed gently until an estimated 50 mg. of iron had dissolved. Loss of liquid was avoided by cooling the upper portion of the chamber with compressed air. The flow of nitrogen was then increased and the solution concentrated to about 0.5 ml. Three milliliters of oxygen-free acetic acid was added and the mixture heated to boiling. Part of the ferrous acetate separated as a suspension of fine white crystals. The porphyrin-acetic acid mixture was warmed by means of an oil-bath until the porphyrin dissolved completely. The hot solution of ferrous acetate was then forced over in small portions (nitrogen entering at D and escaping at B). The solution was stirred after each addition by reversing the flow of nitrogen (A to C). After complete addition of the ferrous acetate, the solution was gently refluxed for several minutes. The color turned from dull red to brilliant almost purple-red. Meanwhile 3 ml. of water was placed in compartment I, heated to boiling and swept with nitrogen to remove any contaminating traces of oxygen. This was forced over in small portions into the heme solution, the addition requiring about one hour. Fine red needles began to separate. The mixture was cooled slowly and allowed to stand for one hour. The crystals were blown into suspension with nitrogen and filtered on the sintered glass disk in chamber III, the mother liquors being removed at B. Wash water was prepared in chamber I as above. In small portions it was forced into chamber II, refluxed for better washing, and finally passed through the filter. About 18 ml. of water was used in all. The crystals were finally washed with two 1.5-ml. portions of methanol prepared in the same manner as the water. The product was dried on the filter disk by forcing a stream of nitrogen through it for several hours. Drying was speeded by wrapping chamber III in a towel and warming with an infrared lamp.

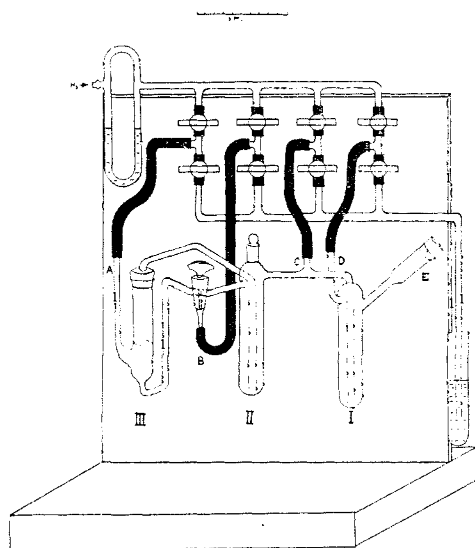


Fig. 2.

(12) Corwin and Ellingson, THIS JOURNAL, 64, 2101 (1942).

While the product was drying a transfer container was prepared from a large battery jar. A tube was led to the bottom from a tank of liquid carbon dioxide. The end of the tube was covered with a folded towel to prevent convection currents from being set up by the flow of gas. The top of the jar was covered with several thicknesses of toweling and the air replaced by carbon dioxide.

Storage ampoules were prepared from 5-mm. Pyrex tubing, the bulbs being about 8-mm. external diameter, and the stems about 5-cm. long. These were carefully cleansed and weighed to 0.1 mg. Each was baked out in nitrogen and placed in the jar.

Compartment III was quickly disconnected from the reaction apparatus and placed in the jar of carbon dioxide. The flow of gas was increased and a small amount of the crystalline material introduced into each ampoule by means of a small spatula and filling tube. Each ampoule was then attached to a line from a high vacuum system and the pump turned on. The ampoules were removed from the jar, evacuated to about 5×10^{-2} mm., and sealed off. As soon as the sealing was complete the bulbs were plunged into cold water to prevent heat from diffusing back and damaging the sample. The weights of the samples were obtained by reweighing the bulb and stem; yield 60 mg. or 47.8%.

Suspended in boiled out mineral oil the crystals appeared under the microscope as fine needles with clean surfaces and oblique extinction. They seem to grow in sheaves and were occasionally branched. By transmitted light they were bright red, by reflected light golden.

Determination of the Spectrum.—The measurement was made with the instruments described by Erdman and Corwin,[1] the only change being that the cell thickness was reduced to 1.38 mm. so as to permit the use of more concentrated solutions.

A small volumetric flask was constructed with a fine capillary inlet at the bottom for nitrogen. An ampoule of the heme was introduced and the flask swept with nitrogen for several hours. Oxygen-free dioxane was distilled in against the flow of nitrogen. The ampoule was broken with a carborundum pointed rod. The solution was mixed by the flow of nitrogen. Meanwhile a cuvette equipped with a nitrogen inlet was swept and flamed. Transfers of the solution were made with a fine siphon tube backed by nitrogen pressure. Nitrogen was passed through the upper part of the cuvette throughout the measurement. The peak at 595 was measured first. After every third reading the intensity of this peak was rechecked. When a decline in absorption appeared, the solution was discarded. Three refills were necessary for the range of 220 mμ covered in the study.

Maxima

I		II		III	
λ	$\epsilon \times 10^{-4}$	λ	$\epsilon \times 10^{-4}$	λ	$\epsilon \times 10^{-4}$
595	1.68	550	0.953	410	5.0

Minima

I		II	
λ	$\epsilon \times 10^{-4}$	λ	$\epsilon \times 10^{-4}$
0.565	0.812	510	0.648

Action of Oxygen on Pyridine Mesohemochromogens Prepared by Several Different Methods.—Anhydrous oxygen-free pyridine was prepared by treating the C. P. product with potassium hydroxide pellets for several months and then distilling under nitrogen, as with the solvents in the preceding section. A solution of mesohemin chloride was prepared containing 0.3 mg./ml. Spectrum: 640–623, 552–545, 525–510 mμ.

Hydrazine Hydrate.—One drop of 92% solution added to 3 ml. of the hemin solution. Color is orange-red. Spectrum: 556–543, 527–504 mμ. Pure dry oxygen rapidly destroys the hemochromogen spectrum, the solution becoming pale yellow after about fifteen minutes.

Hydrogen Sulfide. A few milliliters of hydrogen sulfide were passed into 3 ml. of stock hemin solution. The spectrum of the hemochromogen formed rapidly. The excess hydrogen sulfide was swept out with nitrogen and the solution treated with oxygen. The color and spectrum changed rapidly. After ten seconds the solution was greenish black. Spectrum: I 656–640, II 555–548, III 530–525 mμ; order of intensities I, II, III. After three minutes the solution was deep green. Spectrum: I 655–641, II 553–548, III 532–526 mμ; order of intensities I, III, II. After twenty minutes the solution was brownish green. Spectrum: I 656–641, II 553–548, III 532–526 mμ; order of intensities, I, III, II (shadow). Hydrogen sulfide added to the final solution did not alter the spectrum.

Sulfur Dioxide.—A few milliliters of sulfur dioxide were passed into 3 ml. of the stock hemin solution. The solution became warm due to the formation of the sulfur dioxide-pyridine complex. The hemochromogen spectrum formed immediately. Pure oxygen was passed through the mixture. After five minutes: spectrum: I 584–575, II 558–548, III 540–510; order of intensities II, III (smeared), I (shadow). After thirty minutes: spectrum: I 589–573, II 555–543; order of intensities I, II. The reaction goes well only in purified pyridine. A little moisture seems to be necessary.

Sodium Hydrosulfite.—One milliliter of the stock hemin solution was diluted with 2 ml. of water. One milligram of sodium hydrosulfite causes immediate formation of the hemochromogen spectrum. Pure oxygen was passed through the mixture for one hour. The color slowly changed to amber-brown; spectrum I 563–548, II 530–506 mμ (probably hematin).

Ascorbic Acid.—One milliliter of the stock hemin solution was diluted with 2 ml. of oxygen-free water. Several milligrams of ascorbic acid was added. The spectrum of the hemochromogen appeared within a few seconds. Oxygen was passed through the mixture for twenty minutes. The solution gradually became a deep clear green; spectrum I 660–630, II 533–522 mμ; order of intensities I, II.

From Mesoheme Dimethyl Ester.—One of the ampoules of heme was broken under pyridine. The solution was bright orange-red and showed an intense hemochromogen spectrum.

Three cubic centimeters of the solution was treated with oxygen for a half hour without change in either type or intensity of the spectrum. A drop of water was added and the solution again treated with oxygen. The hemochromogen spectrum persisted. The solution was allowed to stand to the air for several weeks. At the end of that time the larger portion was converted to the ferric state but a hemochromogen spectrum was still visible.

Three milliliters of the stock solution was treated with 3 ml. of oxygen-free water. A bright pink precipitate formed. When exposed to air it rapidly redissolved, giving the brownish red spectrum of the ferric complex.

Summary

1. The preparation of mesoheme IX dimethyl ester is described. The preparation requires the use of special apparatus to exclude atmospheric oxygen.

2. The absorption spectrum of this substance is recorded. It is shown to resemble that of other divalent metallic complexes.

3. The contrasting behavior of mesoheme and mesohematin in the presence of hydrogen chloride may be used as an assay of purity.

4. Grounds are presented for predicting that hemes coördinated with a base in the absence of molecules capable of accepting electrons and the absence of peroxide should be passive to oxygen.

5. It is shown experimentally that such passivity occurs with pure pyridine hemochromogen in the absence of water or in the presence of the

amount of water required stoichiometrically. An excess of water permits rapid oxidation.

6. The possible relationship between this phenomenon and the observed passivity of hemoglobin is discussed.

BALTIMORE 18, MD. RECEIVED APRIL 22, 1946

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK AND CO., INC.]

The Partial Synthesis of Dehydrocorticosterone Acetate

BY LEWIS HASTINGS SARETT

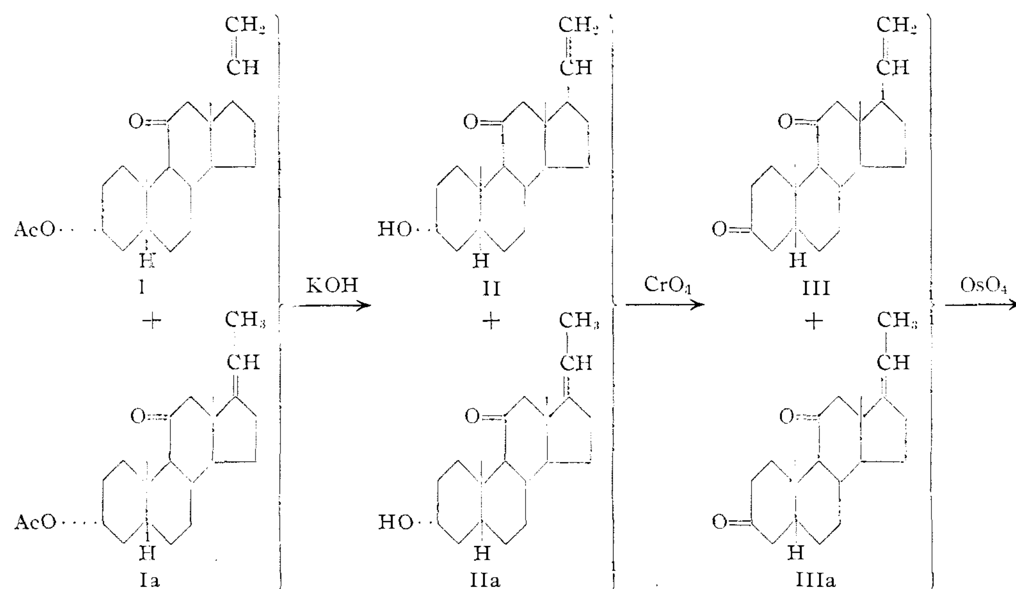
Dehydrocorticosterone, a member of the adrenal cortical hormone group, was first isolated from cortical extracts by Kendall and co-workers.[1] Its partial synthesis from desoxycholic acid has been accomplished by Lardon and Reichstein.[2] This synthesis employs the reaction of a 3-acetoxy-11-keto-*etio*-cholanic acid chloride with diazomethane as a means of introducing the required ketol side chain. This has also been accomplished by lead tetraacetate oxidation.[3]

The availability of $\Delta^{17,20}$- and $\Delta^{20,21}$-3(α)-acetoxy-11-ketopregnenes[4] has now made possible the utilization of another method for constructing the ketol side chain.

The crude crystalline mixture of $\Delta^{17,20}$- and $\Delta^{20,21}$-3(α)-acetoxy-11-ketopregnenes (I and Ia), obtained by the method previously described[4] was saponified to the corresponding mixture of 3(α)-hydroxy-11-ketopregnenes (II and IIa), which upon oxidation with chromic acid afforded the corresponding mixture of 3,11-diketopregnenes (III and IIIa). Hydroxylation with osmium tetroxide by the method of Criegee[5] gave a mixture of pregnanedioldiones from which the pair of isomeric 20,21-diols could immediately be separated. This was effected by subjecting the mixture to mild esterification with succinic anhydride followed by separation of the acidic from the neutral fraction.

The neutral fraction consisted essentially of a pregnanediol-17,20-dione-3,11,[6] (IV), which was best isolated as the readily crystalline 20-acetate, (V). The structure of this dioldione was shown to be that given by formula IV through chromic acid oxidation. Two products were obtained, *etio*-cholanetrione-3,11,17 (VII) and pregnanol-17-trione-3,11,20 (VI). VII could also be obtained by oxidation of *etio*-cholanol-3(α)-dione-11,17.[4]

The $\Delta^{4,5}$-unsaturated derivative of IV was also prepared. Bromination of V gave 4-bromopregnandiol-17,20-dione-3,11 acetate-20 (XXI) as the crystalline alcohol complex. Refluxing this product with pyridine gave pregnene-4-diol-17,20-dione-3,11 acetate-20 (XXII), saponification of



(1) Mason, Myers and Kendall, *J. Biol. Chem.*, **114**, 613 (1936).

(2) Lardon and Reichstein, *Helv. Chim. Acta*, **26**, 747 (1943).

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(4) Sarett, *J. Biol. Chem.*, **162**, 591 (1946).

(5) Criegee, *Ann.*, **522**, 75 (1936); Criegee, Marchand and Wannowius, *ibid.*, **550**, 99 (1942).

(6) The stereochemical configuration of this compound has not yet been determined.