wave lengths with increase in *p*H from 3 to 7 and then slowly back.

3. The extinction of $\rangle C{=}N-$ absorption increases from *p*H 3 to 10.

4. There is no apparent breakdown after ultraviolet irradiation for as long as four hours.

ADRIAN, MICHIGAN
CINCINNATI, OHIO RECEIVED MARCH 31, 1941

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

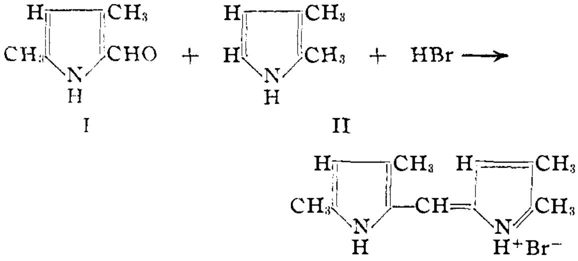
A Reinvestigation of the Configuration of Hemin[1]

BY ALSOPH H. CORWIN AND ROBERT H. KRIEBLE[2]

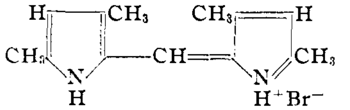
The preceding papers of this series[3] have developed a study of the anomalies which occur in the aldehyde synthesis of dipyrrylmethenes. They have shown that in an attempted synthesis of an unsymmetrical methene by this method, the possibility must be taken into account that either of two symmetrical methenes may result in addition to the normal product. An anomalous course of this type would be discovered readily by analytical methods in cases in which the three methenes differed in composition. More subtle methods would be required, however, in those cases in which the possible products were isomeric. In the proof of the structure of hemin two of the key porphyrins, meso-porphyrin and deutero-porphyrin, were prepared from intermediates which fall into this indeterminate class. Since deutero-porphyrin was used in the synthesis of hemin, its configuration has been reinvestigated with the possibility of this anomaly in mind.

The critical reaction in the deutero-porphyrin synthesis is the preparation of compound III, Chart I:

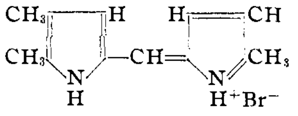
CHART I



I II III



IV



V

The products which might be formed in addition to compound III are represented by formulas IV and V.[3] All three of these substances have been prepared previously[4] but no attempt to compare them was made by the investigators who first prepared them. The methene salts themselves are not suitable for melting point comparison and a description of the properties of the free base of V was not available for comparison at the time that the synthesis of hemin was completed. The recorded melting points of the free bases of III and IV are 115° and 118–119°, respectively.

Compounds III, IV and V have now been prepared for the purpose of direct comparison of properties. Under mild condensing conditions in the presence of hydrogen chloride instead of hydrobromic acid and in an anhydrous medium, the yield of III (hydrochloride) was raised to 95%. The free base for identification was prepared in a yield of 83% making an over-all yield of over 78% from the pyrroles. This yield is structurally significant since neither of the symmetrical methenes can be formed from the unsymmetrical aldehyde synthesis in a yield greater than 50%.

Compounds IV and V were prepared by the reactions shown in Chart II.

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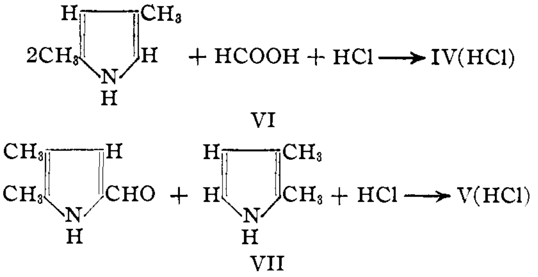
[1] Studies in the Pyrrole Series. V. This paper is from the doctoral dissertation of Robert H. Krieble, The Johns Hopkins University, 1939, and was presented at the Baltimore Meeting of the American Chemical Society, April, 1939.

[2] Mary Carroll Garvan Fellow; present address, Socony-Vacuum Oil Co., Paulsboro, N. J.

[3] THIS JOURNAL, **58**, 1081 (1936); **58**, 1086 (1936); **59**, 1973 (1937); **62**, 418 (1940).

[4] III, Fischer and Kirstahler, *Ann.*, **466**, 183 (1928); IV, Piloty, Krannich and Will, *Ber.*, **47**, 2544 (1914); V, Fischer and Nüssler, *Ann.*, **491**, 170 (1931).

Chart II



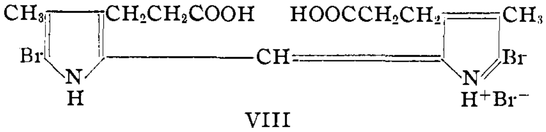
Since neither of these reactions can lead to an unsymmetrical methene, the structures are inferred from the analyses. The melting points found for the three materials together with the mixed melting points are shown in Table I.

Table I

III		IV		V		III
83°		116–8°		116°		83°
	53°		86°		72°	

It will be noted that the melting point of the base of the hemin intermediate, compound III, is 83° instead of 115° as reported by Fischer and Kirstahler.[4] The identity of this material is established by its high yield from the starting pyrroles and by the method of logical exclusion, *i. e.*, it is the third of three isomers (as established by analysis) all of which are different (as established by melting points and mixed melting points) and two of which have already been assigned two of the three possible formulations. Thus it seems necessary to revise the physical properties of 3,5,4′,5′-tetramethyl dipyrrylmethene which have appeared in the literature.[5]

The melting point of 115° reported by Fischer and Kirstahler[4] for the hemin intermediate, compound III, is much closer to those of compounds IV and V than it is to that found for authentic compound III. Because of this discrepancy in melting points, the conclusions drawn as to the structure of deutero-porphyrin and therefore of hemin cannot be accepted as conclusive until it is learned whether the hemin synthesis was performed with one of the symmetrical methenes IV or V instead of with compound III as formulated.[6] The deutero-porphyrin from methene V was prepared by Fischer and Nüssler[4] and its dimethyl ester was found to melt at 285°. Since the natural product is recorded as melting at 220°,[7] it seems safe to eliminate compound V as a possible intermediate. Accordingly deutero-porphyrins were prepared from methenes III and IV by condensation with compound VIII:



VIII

Our preparations of natural deutero-porphyrin dimethyl ester, after chromatographic purification, melted at 224–224.5°. The deutero-porphyrin from methene IV, deutero-porphyrin XIII by Fischer's system of designation,[8] gave a dimethyl ester melting at 243–243.5° and showed a depression to 220° with the natural product. The dimethyl ester of the deutero-porphyrin from compound III, deutero-porphyrin IX,[8] melted at 223.5–224° after several purifications and showed no depression with the natural ester. These syntheses confirm the configuration assigned to hemin by Fischer's laboratory. They indicate that Fischer and Kirstahler[4] used methene III when they synthesized deutero-porphyrin even though they did not correctly characterize its free base. Any attempt to explain this discrepancy would be speculative, especially since their published directions for the preparation are not detailed. It should be noted, however, that an accidental excess of 2,4-dimethyl-5-formylpyrrole could have led to formation of some of compound IV.

The authors wish to acknowledge the assistance of Mr. Kurt Fisher in the preparation of some of the compounds used in this study. One of us (R. H. K.) also wishes to express his thanks for a grant-in-aid from the Hynson, Westcott and Dunning Research Fund.

Experimental Part

2,4-Dimethylpyrrole (Pyrrole VI).—The methods in the literature[9] for this preparation give unreliable yields. The following procedure gives a markedly increased yield and is reliable.

Ninety-five grams of 2,4-dimethyl-3,5-dicarbethoxypyrrole is mixed with 145 g. of 85% potassium hydroxide and ground in a mortar. The mixture is placed in a steel

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(5) Fischer and Endermann, *Ann.*, **545**, 148 (1940), have been so generous as to indicate their concurrence in this conclusion, preliminary notice of which appeared in the lithoprinted abstracts of the proceedings of the Baltimore Meeting of the American Chemical Society.

(6) This critical point of the argument was, unfortunately, dismissed by Fischer and Endermann, ref. 5, p. 151, with the statement, "The question as to whether this was a printing error or an error in writing could no longer be determined; but this error has nothing to do with the sequence of the substituents."

(7) Fischer and Hummel, *Z. physiol. Chem.*, **181**, 127 (1929).

(8) Fischer and Stangler, *Ann.*, **459**, 63 (1927).

(9) See Fischer and Orth, "Die Chemie des Pyrrols," Vol. 1, Akademische Verlagsgesellschaft, Leipzig, 1934, p. 42.

bomb of 300-cc. capacity and stirred to a paste with 70 cc. of water. The bomb is sealed and placed in an oil-bath at 160° for four to five hours. When cooled, one clear, light brown liquid phase is found with a crystalline layer of potassium carbonate beneath. The liquid is decanted and the residue is extracted three times with ether. The combined liquids are steam distilled and the distillate is extracted several times with ether. The extract is dried over potassium carbonate, the ether is removed and the pyrrole is distilled *under vacuum*, b. p. 72° (25 mm.). During the distillation and upon removing the vacuum, the pyrrole is protected from the air with illuminating gas. It must be stored in an inert atmosphere; yield, 35 g. or 95%.

2,3-Dimethyl-4-carbethoxy-5-carboxylpyrrole. (a) Preparation of Aminobutanone Hydrochloride.—The standard method[10] was modified to permit the isolation of a tin complex of aminobutanone which contained only a fraction of the tin used in the reduction.

Three and seven-tenths liters of 35% hydrochloric acid in a five-liter balloon flask fitted with mechanical stirring is cooled to 15° in a wash-tub of ice–salt mixture; 544 g. of mossy tin and 5 g. of stannous chloride are added; 460 g. of isonitrosomethyl ethyl ketone[11] is added fast enough to maintain the solution at 15°, after which the solution is cooled to 0° and allowed to stand several hours during which time a tin complex of aminobutanone crystallizes out. This is filtered off (dry weight 760 g.) and dissolved in 4 liters of water in a 12-liter balloon flask. The *p*H of the solution, originally about 0.8, is adjusted to 1.8 by the addition of sodium hydroxide solution with violent stirring. Hydrogen sulfide is then blown in as rapidly as possible, precipitating the tin as stannic sulfide. The addition is continued until a test sample gives no further precipitate on the addition of a few drops of sodium hydroxide and saturation with hydrogen sulfide. The solution is then filtered through a bed of paper pulp. The tedious isolation of the crystalline product is omitted and the filtered solution used directly in the pyrrole condensation.

(b) The Ring Closure.—The directions given below embody a six-fold increase in yield from isonitroso-methyl ethyl ketone over that recorded[12] and, in addition, permit a reasonably pure product to be filtered directly from the reaction mixture.

A 12-liter balloon flask is fitted with a mechanical stirrer, two dropping funnels and a wash-tub as a water-bath; 884 g. of sodium oxalacetic ester and 4 liters of water are placed in the flask and the temperature is raised to 85°. The *p*H is lowered to about 6 by the addition of dilute hydrochloric acid. The solution of aminobutanone hydrochloride is added over a period of two hours through one dropping funnel, while a 1% solution of sodium hydroxide is added through the other at a rate just sufficient to maintain the *p*H at 6. The pyrrole begins to precipitate almost immediately. After the addition is complete, the flask is allowed to remain one hour at the same temperature and then the reaction mixture is filtered while still hot. The product is washed with 200 cc. of water and dried forty-eight hours in a vacuum desiccator. The yield is 360 g. which is 35% of the theoretical based on isonitrosomethyl ethyl ketone or 65% based on aminobutanone. The product melts with decomposition at 203°. After several recrystallizations this may be raised to 210°.

2,3-Dimethylpyrrole (Pyrrole II).—The hydrolysis and decarboxylation of 2,3-dimethyl-4-carbethoxy-5-carboxylpyrrole were accomplished by the method used for the preparation of 2,4-dimethylpyrrole. 2,3-Dimethylpyrrole is considerably more stable to air and light than the 2,4 isomer. The boiling point is about the same, yield, 80%.

2,4-Dimethyl-5-formylpyrrole (Pyrrole I).—The method was essentially that given in the literature.[13] Substitution of anhydrous ether for chloroform as a solvent for the reaction was found to be desirable and the hydrolysis gave a cleaner product when performed with sodium bicarbonate than when sodium hydroxide is used; yield, 75% after recrystallization, m. p. 91°.

2,3-Dimethyl-5-formylpyrrole (Pyrrole VII).—This was prepared in the same manner as the 2,4 isomer; yield, 50%; pink flakes, m. p. 127.5–128°.

3,5,4′,5′-Tetramethyldipyrrylmethene (Compound III). (a) The Hydrochloride.—Nine hundred and fifty milligrams of 2,3-dimethylpyrrole and 1.23 g. of 2,4-dimethyl-5-formylpyrrole are dissolved in 10 cc. of absolute alcohol and hydrogen chloride is blown through in short bursts until further addition causes no further precipitation of red powder. After standing for several hours, the precipitate is filtered and washed with a few cc. of cold alcohol and then with ether. The yield is 2.25 g. or 95%. The substance decomposes at 222°.

(b) Alternative Synthesis.—The preparation was repeated with identical quantities of 2,4-dimethylpyrrole and 2,3-dimethyl-5-formylpyrrole with the same results.

Under the microscope, the finely divided, brick-red powder is seen to be made up of fine red needles, bearded with still finer ones.

(c) The Free Base.—Five hundred milligrams of the methene salt is dissolved in a minimum amount of water (about 50 cc.) at room temperature. Two drops of concentrated ammonia solution are added to the resulting orange solution, causing a heavy yellow turbidity. On rubbing the walls of the flask with a spatula, a yellow semi-crystalline solid falls out in the course of about fifteen minutes. The free base is filtered, dried in vacuum and recrystallized by dissolving in a few cc. of acetone at 40° and cooling to −80°. The purity of the hydrochloride is best measured by the melting point of the crude free base. A m. p. of 78–81° is usual. The pure free base melts at 82–83°. Yield of the crude product is 350 mg. or 83%. If the melting point of the crude is much below 78° it may be purified by recrystallization from glacial acetic acid but only at the cost of a great loss of product. The bases of the products prepared by methods (a) and (b) have identical melting points and give no depression when mixed.

When crystallized from acetone or from methyl alcohol, the methene forms large, orange, pleochroic rhombs which do not appear to have an extinction between crossed nicols. The material looks beautifully pure under the microscope.

Anal. Calcd. for $C_{13}H_{16}N_2$: C, 77.95; H, 8.05. Found: C, 77.80; H, 8.15.

The instability of the free base is to be emphasized.

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[10] Künne, *Ber.*, **28**, 2036 (1895).

[11] "Organic Syntheses," Vol. X, p. 22 (biacetyl monoxime).

[12] Fischer and Kutscher, *Ann.*, **481**, 199 (1930).

[13] Fischer and Zerweck, *Ber.*, **55**, 1949 (1922).

Under water it may be kept for several days but the dry material darkens in a few hours' exposure to air. This base is markedly less stable than its symmetrical isomers.

(d) **Attempt to Duplicate Fischer and Kirstahler's[4] Preparation.**—Two hundred and fifteen milligrams of 2,3-dimethylpyrrole and 280 mg. of 2,4-dimethyl-5-formylpyrrole are dissolved in 0.4 cc. of absolute alcohol at the boiling point. The flask is removed from the flame while 1.0 cc. of 48% hydrobromic acid is added, then brought to a boil again and let stand at room temperature for one hour. A brick-red powder precipitates which is washed with 1 cc. of alcohol and then a little dilute hydrobromic acid. The melting point of its free base prepared as described under (c) is 45–46°. The filtrate, on dilution with water, gives more red precipitate, the free base of which softens at 63° and gradually becomes more liquid over the next 15°. This free base is dissolved in 1 cc. of boiling alcohol, diluted with hot water to turbidity, heated until clear and allowed to stand at room temperature for several hours. A few orange needles appear. These are filtered off and dried, m. p. 101–105°. Mixed m. p. with compound IV, base, melting at 113° is 108°. Mixed m. p. with compound V, base, melting at 109° is 93°. The crystal form differs from compounds III and V but resembles compound IV. All attempts to repeat this experiment failed. It is believed that the appearance of the high melting product was caused by the accidental use of an excess of 2,4-dimethyl-5-formylpyrrole which is known to condense with itself under these conditions to form compound IV.

3,5,3',5'-Tetramethyldipyrrylmethene (Compound IV). (a) **The Hydrochloride.**—One hundred and fifty cubic centimeters of absolute ether and 10 cc. of 90% formic acid are placed in a 500-cc. three-neck flask fitted with mechanical stirring, a dropping funnel and two tubes for the introduction of hydrogen chloride and carbon dioxide; 14 g. of 2,4-dimethylpyrrole in 20 cc. of absolute ether is placed in the dropping funnel and a few cc. are run into the solution. Hydrogen chloride is then blown through in small bursts giving the solution a yellow turbidity due, presumably, to the formation of the insoluble pyrrole hydrochloride. The addition of hydrogen chloride is continued until further addition causes only a slight increase in turbidity. A few drops more of the pyrrole solution are then added. After stirring a few minutes a thin film of red oil is formed on the walls of the flask, leaving the solution clear. After standing several hours the oil sets to a somewhat sticky crystalline mass. More 2,4-dimethylpyrrole and hydrogen chloride are then added in the above manner, allowing the reaction to stand half an hour after each addition to crystallize the resulting product. This requires three or four hours. Too much hydrogen chloride causes the methene salt to dissolve, too much pyrrole added at one time results in occlusion and decreased yields. After the reaction is complete, the walls of the flask are encrusted with large red needles of the methene. The ether is decanted and the flask is allowed to stand overnight in a vacuum desiccator over concentrated sodium hydroxide solution to remove excess acid which tremendously increases the solubility of the methene. The product is broken up and recrystallized from a minimum amount of boiling absolute alcohol; yield, 10–11 g. or about 50%, after recrystallization; decomposition point, 226°.

(b) **Dimorphism of the Hydrochloride.**—One hundred milligrams of the red needles of compound IV hydrochloride was dissolved in boiling alcohol and the flask placed in a Dewar flask filled with water at 79°. Twelve hours later, beautiful large blue rhombohedral (almost cubic) crystals had come down. While blue by reflected light, these are yellow by transmitted light. These crystals give a free base identical with that from the red crystals, m. p. 116°, mixed m. p., no depression.

It was found that the red and blue crystals could be interconverted; 1.5 g. of the red crystals was dissolved in a minimum amount of hot absolute alcohol and allowed to cool for a few hours in a Dewar flask. This time red crystals were obtained. The process of warming to boiling and slowly cooling was repeated many times. Sometimes red crystals were obtained, sometimes blue. Occasionally a mixture was observed. This rapidly went over entirely to the blue form. Either form could be obtained at will by seeding the hot, saturated solution with a crystal of the desired form before cooling. The blue form crystallized much less rapidly than the red and was much less soluble in alcohol. The solutions of each form were identical in color and their spectra showed the same absorption edge at the same concentration.

The fact that the blue form, while less soluble in alcohol, also crystallized less rapidly, made it possible to interconvert the two forms. A boiling alcohol solution saturated with respect to the red form was supersaturated with respect to the blue. On long standing at the boiling point a precipitate of blue crystals appeared. On the other hand, a boiling alcohol solution saturated with respect to the blue form could be concentrated by evaporation past the saturation point of the red form, whereupon red crystals appeared due to their much higher rate of crystallization.

(c) **The Free Base.**—The free base of compound IV was prepared by the process described for compound III. The yield is nearly quantitative; m. p. of the crude product, 114–118°. After recrystallization from acetone as described above, light yellow pleochroic needles were obtained which did not appear to extinguish when rotated between crossed nicols; m. p. 116–118°.

4,5,4',5'-Tetramethyldipyrrylmethene (Compound V). (a) **The Hydrochloride.**—One and fifteen hundredths grams of 2,3-dimethyl-5-formylpyrrole and 0.88 g. of 2,3-dimethylpyrrole are dissolved in 5 cc. of alcohol; 1 cc. of 35% hydrochloric acid is added slowly under cooling. The solution turns deep red, followed almost immediately by a precipitation of reddish-purple crystals. After standing at room temperature for an hour the mixture is filtered and the product washed with a little alcohol and then ether, yield, 1.7 g. or 80%. Under the microscope the crystals are seen to be small octahedra which are red by transmitted light and blue by reflected; decomposition point, 212°.

An attempt to prepare this material by condensation of 2,3-dimethylpyrrole with 90% formic acid in absolute alcohol gave ammonium chloride as the only recognizable product.

(b) **The Free Base.**—This was prepared by the same process used for compounds III and IV; yield, nearly quantitative; melting point of the crude product, 115°. After recrystallization from acetone as described above, light orange rectangular plates were obtained, m. p. 116°.

The Mixed Melting Points.—The hydrochlorides were not suitable for purposes of identification. Mixed melting points were taken with the free bases: compound III, m. p. 83°, mixed with compound IV, 116–118°, depressed to 53°; compound IV, 116–118°, mixed with compound V, 116°, depressed to 86°; compound V, 116°, mixed with compound III, 83°, depressed to 72°.

"Natural" Deuteroporphyrin Dimethyl Ester.—One gram of deuterohemin[14] is powdered in an agate mortar and dissolved in 500 cc. of boiling glacial acetic acid containing 5 cc. of 35% hydrochloric acid; 100 mg. of powdered iron is added in small batches over ten minutes. The brown solution turns deep violet. A sample should show no absorption at 545 mμ in pyridine-hydrazine hydrate solution. The mixture is poured into 1500 cc. of water and the porphyrin is precipitated by the addition of sodium hydroxide; 5 g. of "Celite Filter Aid" is added and the solution is filtered. After sucking as dry as possible on the Büchner funnel, it is placed in a vacuum desiccator overnight. The porphyrin is extracted from the "Celite" with 500 cc. of methanol containing 5 g. of dry hydrogen chloride. The solution is refluxed for one hour; 50 cc. of benzene is added and the solution washed by shaking with one liter of 2% sodium carbonate solution, then one liter of water. After drying the benzene solution with anhydrous sodium sulfate it is passed over a chromatograph with "Alorco" less than 80-mesh activated alumina as an adsorbent. The chromatograph is developed with benzene containing 1% methanol. The porphyrin ester moves down the column as a sharp, well-defined band, leaving some colored impurities at the top of the column. The band is cut from the column and the porphyrin ester eluted with 25% methanol in benzene. The eluate is filtered first through hardened paper then through sintered glass under pressure. The porphyrin ester is crystallized by boiling off the benzene on the hot-plate with constant addition of absolute methanol until a precipitate begins to appear on the walls. A little more benzene is added, the solution is brought again to a boil and allowed to cool to room temperature. The deutero-porphyrin dimethyl ester begins to crystallize almost immediately in long, red, glittering needles. The product is filtered and dried for one hour in a vacuum at 100°; m. p. 224.5°; yield 570 mg.

Anal. Calcd. for $C_{32}H_{34}O_4N_4$: C, 71.35; H, 6.36. Found: C, 71.35; H, 6.47.

3,5,3′,5′-Tetramethyl-4,4′-dibromodipyrrylmethene Hydrobromide.[15]—Five hundred milligrams of 3,5,3′,5′-tetramethyldipyrrylmethene hydrochloride (compound IV) is dissolved in a boiling mixture of 50 cc. of carbon tetrachloride and 35 cc. of chloroform. To the boiling mixture is added 0.22 cc. of bromine in a little carbon tetrachloride. The solution is boiled until no more hydrogen bromide fumes come off or for about a minute. It is then placed in an ice-water-bath for about an hour. The bromomethene hydrobromide begins to precipitate immediately. It is filtered off, washed with ether and air-dried; yield 815 mg. or 83%. Additional product may be obtained by removing the residual chloroform from the mother liquors.

3,5,4′,5′-Tetramethyl-4,3′-dibromodipyrrylmethene.[16]—This is prepared by the preceding method, using 3,5,4′,5′-tetramethyldipyrrylmethene (compound III). The yield is the same. The product is a very finely divided red powder.

Intermediates in the Preparation of 3,3′-Dipropionic Acid-4,4′-Dimethyl-5,5′-dibromodipyrrylmethene (Compound VIII). (a) **2,4-Dimethyl-3-propionic Acid-5-carbethoxypyrrole.**[17]—One cubic centimeter of 10% palladium chloride solution is dissolved in 50 cc. of distilled water and 4 g. of Norit and 100 mg. of sodium acetate is added. The mixture is hydrogenated at room temperature and approximately atmospheric pressure for five to six hours. The palladinized Norit is filtered off and added to the reagents for reduction immediately.

Ten grams of 2,4-dimethyl-3-acrylic acid-5-carbethoxypyrrole is dissolved in 200 cc. of distilled water containing 1.8 g. of sodium hydroxide. The mixture is shaken on the hydrogenator at atmospheric pressure for fifteen to sixteen hours. The catalyst is then filtered off and washed with distilled water and the pyrrole precipitated from the filtrate by addition of 4% hydrochloric acid to the congo red endpoint. The product is filtered off, washed with distilled water and dried in a vacuum desiccator; yield, 7.8 g. of a product melting at 153°.

(b) **2-Bromomethyl-3-propionic Acid-4-methyl-5-carbethoxypyrrole.**[17]—The yields of this product are more reliable when a few per cent. of acetic anhydride is added to remove traces of water from the glacial acetic acid used as solvent for the bromination.

(c) **3,3′-Dipropionic Acid-4,4′-dimethyl-5,5′-dicarboxyldipyrrylmethane.**—The yield and purity of the dibromomethene in the last step of this series are increased if the methane dicarboxylic acid is purified until colorless. This may be achieved by solution in the minimum amount of boiling 70% acetone and crystallization. Peculiarly, this substance is nearly insoluble in water but is much more soluble in aqueous acetone than in dry acetone.

Deutero-porphyrin XIII.—One gram of 3,3′-dipropionic acid-4,4′-dimethyl-5,5′-dibromodipyrrylmethene hydrobromide[18] (compound VIII) and 840 mg. of 3,5,3′,5′-tetramethyl-4,4′-dibromodipyrrylmethene hydrobromide are ground up with 4.3 g. of benzoic acid. The mixture is divided among three Pyrex test-tubes which are immersed in an oil-bath at 180–182° for one hour, forming a black, sticky tar. The tar is rubbed with 5 cc. of boiling glacial acetic acid, added immediately after removing the tubes from the oil-bath. The extraction is repeated four or five times until all the black solid has been dissolved or suspended in acetic acid. The extracts are combined and diluted with 150 cc. of peroxide-free ether in a two liter separatory funnel; 500 cc. of water is added. After shaking, the ether layer, which contains the porphyrin, is removed. In the water layer a great deal more solid has now been precipitated. This is filtered off, crushed in a mortar and extracted several times with 10-cc. portions of boiling acetic acid until the extract is clear. This extract is diluted with another 150 cc. of ether and separated by the addition of the aqueous filtrate. The aqueous layer is again filtered, the residue extracted with acetic acid and the process repeated. The third ether extract should show only a faint porphyrin spectrum. The combined ether ex-

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(14) Fischer and Hummel, *Z. physiol. Chem.*, **181**, 127 (1929).

(15) Fischer, *Sitzungsber. d. Bayer. Akad. d. Wiss.*, 415 (1915).

(16) Fischer and Kirstahler, *Ann.*, **466**, 182 (1928).

(17) Fischer and Andersag, *ibid.*, **450**, 216 (1926).

(18) Fischer and Andersag, *ibid.*, **458**, 135 (1927).

tracts are washed many times with 1% acetic acid. The ether solution is then extracted twice with 1.5% hydrochloric acid. The porphyrin remaining in the ether can be extracted with 10% hydrochloric acid. It was not investigated further. The deutero-porphyrin in the 1.5% acid extract is precipitated from the aqueous solution by the addition of sodium acetate and filtered off with the aid of "Celite." It is esterified as described under "natural" deutero-porphyrin ester. The benzene solution of the ester is evaporated to dryness under vacuum and the residue is dissolved in moist, peroxide-free ether containing a few drops of acetic acid. The ester is then extracted with 1.5% hydrochloric acid until no more color goes into the acid layer. The residual porphyrin in the ether can be extracted with 10% hydrochloric acid but was not investigated further. The deutero-porphyrin ester is extracted from the aqueous layer with benzene by the addition of sodium acetate to neutralize the acid. The benzene layer is washed with water and dried with sodium sulfate. It is then chromatographed and crystallized as described under "natural" deutero-porphyrin ester; yield, about 40 mg.; m. p. 243–243.5°; mixed m. p. with "natural" ester, m. p. 224.5°, depressed to 220°.

Anal. Calcd. for $C_{32}H_{34}O_4N_4$: C, 71.35; H, 6.36. Found: C, 71.27; H, 6.29.

Deutero-porphyrin IX.—Six hundred and ten milligrams of 3,3′-dipropionic acid-4,4′-dimethyl-5,5′-dibromodipyrrylmethene hydrobromide (compound VIII) and 510 mg. of 3,5,4′,5′-tetramethyl-4,3′-dibromodipyrrylmethene hydrobromide are ground with 2.62 g. of benzoic acid. The condensation, isolation, esterification and purification are carried out as described for deutero-porphyrin XIII dimethyl ester. The yield is 37 mg. of a product melting at 218°. All attempts to raise this melting point by further recrystallization, acid fractionation or chromatographing failed. It was suspected that the product still contained some brominated deutero-porphyrin which had not been debrominated in the melt. Accordingly 20 mg. of the product was dissolved in 7 cc. of benzene and placed in a Warburg flask under hydrogen with about 2 mg. of Busch catalyst in the side arm. The catalyst was reduced, then mixed with the substrate. The uptake of hydrogen was measured from this point. After 0.3 equivalent of hydrogen had been absorbed, the reduction was interrupted and the solution shaken with air to oxidize any leuco-porphyrin that might have been formed. It was then chromatographed and the porphyrin crystallized in the usual way; m. p. 223.5–224°; mixed m. p. with "natural" ester, m. p. 224.5°, no depression.

Summary

1. A scrutiny of the literature suggests that the synthesis of the unsymmetrical tetramethylmethene used in the synthesis of deutero-porphyrin and hemin may be another example of an anomalous aldehyde synthesis of dipyrrylmethenes. The anomaly is suggested by the closeness of the reported melting points of the symmetrical and unsymmetrical isomers.

2. The three tetramethyl-methenes which might result from the aldehyde synthesis have been prepared and their structures established.

3. The unsymmetrical aldehyde synthesis proceeds without anomaly in anhydrous alcohol.

4. Contrary to the original statement in the literature, the melting point of the unsymmetrical methene differs from those of the symmetrical isomers by more than 30°.

5. In spite of the melting point discrepancy, the deutero-porphyrin prepared from the unsymmetrical methene is identical with natural deutero-porphyrin, confirming its configuration as deutero-porphyrin IX which was assigned by Fischer's laboratory.

6. A new method for the proof of structure of dipyrryl methenes is presented.

7. Deutero-porphyrin XIII has been synthesized.

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