SYNTHETIC STUDIES IN THE FIELD OF THE PORPHYRINS. SYNTHESIS OF A MESOPORPHYRIN AND OF A DIACETYLDEUTEROPORHYRIN

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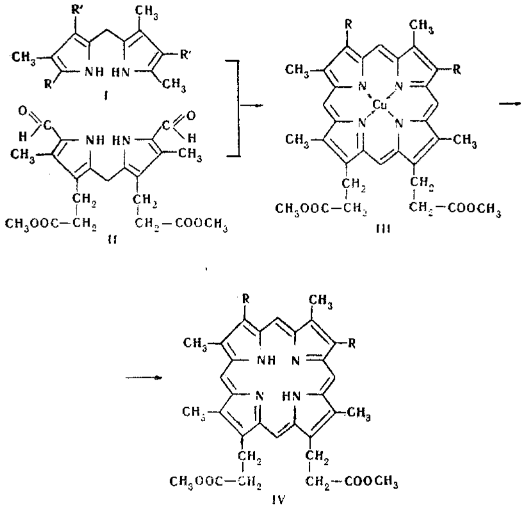
A method is proposed for the synthesis of porphyrins based on the cyclization of a tetrapyrrole system with methyl and formyl groups at the ends of the molecule. The synthesis of mesoporphyrin IX and diacetyldeuteroporphyrin IX has been effected.

In preceding communications we have described the synthesis of porphyrin systems [1, 2] from linear tetrapyrrole compounds. The synthesis of diacetyldeuteroporphyrin IX was effected via a bilene with a methyl group on the α-carbon atom at one end of the molecule while the α-carbon atom at the other end of the chain had no substituent.

The necessity for developing this method in the synthesis of diacetyldeuteroporphyrin IX was due to the considerable difficulties connected with the preparation of diacetyl-substituted dipyrrylmethane with two free α, α' positions. In spite of the well-known papers on the synthesis of porphyrins starting from α, α'-formyl-substituted and α, α'-free dipyrrylmethanes [3, 4], the synthesis of porphyrin derivatives of the type of III presents considerable difficulties. In the first place, the difficulties are due to the necessity for obtaining an α, α'-free unsymmetrical dipyrrylmethane. In the general case, in the synthesis of porphyrins of type III it is considerably simpler to prepare an unsymmetrical dipyrrylmethane in which a methyl group is left in one α position.

Another advantage of the method selected, as compared with the synthesis of porphyrins from α, α'-free and α, α'-diformyl-substituted dipyrrylmethanes, is the fact that in the preparation of porphyrins with a large number of different substituents synthesis from two unsymmetrical dipyrrylmethanes solely via the bilene gives only one required compound while the process starting from two dipyrrylmethanes can form at least a mixture of two isomers.

Continuing our investigations on the synthesis of the porphyrins via linear tetrapyrrole compounds, we have tested the possibility of obtaining porphyrins of type IX via a bilene having a methyl and a formyl group in the α positions of the pyrrole nuclei at the ends of the molecule. In the present work, using this method we have effected the synthesis of mesoporphyrin IX and diacetyldeuteroporphyrin IX. This route, analogous to that which we have used previously in the synthesis of diacetyldeuteroporphyrin IX (IV, R = $COCH_3$) should, as we have assumed, give a more stable bilene—stabilized by a formyl grouping—which could increase the yield of desired porphyrin. While retaining the main advantages of the method, this procedure has also permitted some simplification of the production of the bilene.



The synthesis of mosoporphyrin IX was effected by the condensation of 5-carboxy-3, 4'-diethyl-4, 3', 5'-trimethyldipyrrylmethane (I, R = COOH, R' = C_2H_5) with 5, 5'-diformyl-3, 3'-bis(β-methoxycarbonylethyl)-4, 4'-dimethyldipyrrylmethane (II) in acetic acid in the presence of 56% hydriodic acid. The first compound was obtained by the debenzylation of 5-benzoxycarbonyl-3, 4'-diethyl-4, 3', 5'-trimethyldipyrrylmethane (I, R = $COOCH_2C_6H_5$, R' = C_2H_5) [5]. The second dipyrrylmethane was synthesized by a procedure developed previously [6]. The spectrum of the bilene formed had one strong absorption band at 493 nm. The ring-closure of the bilene system was effected with the aid of copper(II) ions. The yield of the copper complex of the dimethyl ester of mesoporphyrin IX (III, R = = C_2H_5) was 24.6%. The condensation of the dipyrrylmethanes I (R = COOH, R' = C_2H_5) and II in a mixture of methanol and chloroform in the presence of hydrobromic acid with subsequent cyclization of the bilene also led to the formation of the copper complex of the porphyrin III (R = C_2H_5). In this case, however, the yield did not exceed 3%.

In both cases, in addition to the copper complex of the dimethyl ester of mesoporphyrin IX, the formation of the copper complex of an etioporphyrin took place.

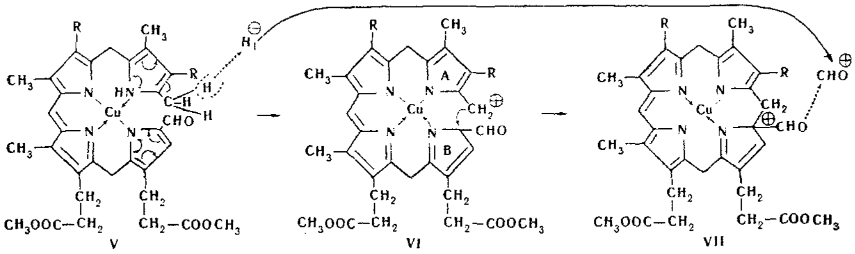
An etioporphyrin was also obtained by the decarboxylation of 5-carboxy-3, 4'-diethyl-4, 3', 5'-trimethyldipyrrylmethane (I, R = COOH, R' = C_2H_5). An attempt to introduce the formyl group into this dipyrrylmethane by means of phosphorus oxychloride and dimethylformamide in dichloroethane also led to the formation of etioporphyrin. In this case, 3-ethyl-2, 4-dimethylpyrrole-5-aldehyde was also obtained.

Subsequent treatment of the copper complex of mesoporphyrin IX with concentrated sulfuric acid gave the possibility of obtaining mesoporphyrin IX (IV, R = $= C_2H_5$). The porphyrin synthesized was identical with a sample obtained from blood hemin [7].

The production of the bilene in the synthesis of diacetyldeuteroporphyrin IX is complicated to a considerable extent by the presence of the deactiving acetyl group in the dipyrrylmethane I (R = H, R' = $COCH_3$). With the displacement of the electron cloud towards the oxygen atom of the acetyl group, a positive charge is induced on the α-carbon atom of the pyrrole nucleus which, in its turn, hinders the approach of the carbon atom of the formyl group of the dipyrrylmethane II. An increase in the concentration of protons in the solution facilitates the occurrence of the electrophilic reaction.

The possibility of performing this reaction successfully was confirmed by the condensation of 3-acetyl-2, 4-dimethylpyrrole with 3-acetyl-2, 4-dimethylpyrrole-5-aldehyde. In this case, the hydrobromide of 4, 4'-diacetyl-3, 5, 3', 5'-tetramethyldipyrrylmethane was isolated.

Under similar conditions, starting from the dipyrrylmethanes I (R = H, R' = $COCH_3$) and II we obtained the copper complex of diacetyldeuteroporphyrin IX. It was found that with a decrease in the concentration of hydrobromic acid the yield of porphyrin fell. It was shown by subsequent experiments that the replacement of hydrobromic acid by hydriodic acid promotes the bilene condensation and raises the yield of porphyrin. On treatment with sulfuric acid, the copper complex of the porphyrin can easily be converted into diacetyldeuteroporphyrin IX (IV, R = $COCH_3$).



V VI VII

The considerable differences in the yields of the mesoporphyrin IX and diacetyldeuteroporphyrin IX must be considered on the basis of the reactivities of the bilene compounds formed as intermediates. It may be assumed that the mechanism of the ring-closure of the bilene consists of three main stages. In the first of them the anionic detachment of a hydride ion H^- and the formation of the carbocation VI takes place. It is known, further, that a tetrapyrrole system of the type of the bilene V can undergo ring closure without great stresses [8]. The formation of a complex of the bilene with bivalent copper, which forms an unstable tetrahedron readily changing into a planar structure, also favors the approach of the terminal atoms of the molecule [9]. In the second stage, the carbocation VI is stabilized by an electrophilic attack of the carbon atom in the α position of ring B. In the following moment, the intermediately-formed compound VII splits off a CHO^+ group and changes into the more stable macrocycle the oxidation of which leads to the corresponding porphyrin. The possibility of the splitting out of a formyl group from a pyrrole nucleus has been shown in the performance of the porphyrin condensation [10]. The carbocation CHO^+ adds the hydride ion H^- and gives a molecule of formaldehyde.

In the mechanism considered, the limiting reaction determining the rate of cyclization and the yield of porphyrin is the elimination of the hydride ion. This elimination, in its turn, depends on the size of the negative charge induced on the α-carbon atom of the pyrrole ring A. In the synthesis of mesoporphyrin IX the presence of an ethyl group in the neighboring β position favors the formation of a negative charge on the α-carbon atom. In the production of diacetyldeuteroporphyrin IX, however, when in compound V (R = $COCH_3$) the acetyl group draws the electron cloud from the pyrrole nucleus, the elimination of the hydride ion is opposed. These considerations are in good agreement with the results obtained in experiment.

To determine the concentration of the dimethyl ester of diacetyldeuteroporphyrin IX and its copper complex in solution and in the reaction mixture, a spectral method of analysis of these compounds has been developed. This enabled us to determine with sufficient accuracy what amounts of porphyrin are formed in the reaction and to shorten the time of the determination considerably. In the determination the dimethyl ester of diacetyldeuteroporphyrin IX we used the most intense band in its visible spectrum—the Soret line at 424 nm. The change in the density of the porphyrin solutions in chloroform with known concentrations makes it possible to calculate the coefficient K. This magnitude, equal to the ratio of the concentration of porphyrin to the density of the solution, was constant within the experimental error, amounting to 504. The performance of control experiments showed the satisfactory accuracy of the method, the error of which does not exceed 2–3%.

Similarly, for the copper complex at 420 nm we found a value of K of 416. In the determination of this compound in the reaction mixture, it is necessary first to isolate the required porphyrin by means of preparative paper chromatography. In this case the magnitude of K, as control experiments showed, must be multiplied by 1.2. The minimum amount of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin IX in the sample is 2–2.5 μg. The error of this method does not exceed 5%.

The investigations carried out permit the conclusion that for the synthesis of alkyl-substituted porphyrins of the type of mesoporphyrin **IX** it is desirable to use α, α'-diformyl-substituted and α-carboxy-α'-methyldipyrrylmethanes and for porphyrins having strong electron-accepting groups of the type of diacetyldeuteroporphyrin **IX** it is desirable to use α-formyl-α'-methyl- and α, α'-unsubstituted dipyrrylmethanes.

EXPERIMENTAL

Copper complex of the dimethyl ester of mesoporphyrin IX (III, R = C_2H_5). A) A solution of 100 mg of 5-carboxy-3,4'-diethyl-4,3',5'-trimethyldipyrrylmethane (I, R = COOH, R' = C_2H_5) in 50 ml of glacial acetic acid was treated with 140 mg of 5,5'-diformyl-3,3'-bis(β-methoxycarbonylethyl)-4,4'-dimethyldipyrrylmethane (II) in 15 ml of acetic acid and then with 0.8 ml of 56% hydriodic acid. The solution was left to stand in a current of nitrogen at 20° C for 1 hr and then, after the addition of 1.5 g of calcined sodium acetate and 1.5 g of copper acetate, it was heated to the boil for another hour. The acetic acid was distilled off in vacuum, and water, followed by saturated ammonia solution, was added to the residue. The colored substances were extracted with chloroform. The solution was evaporated and transferred to a column (d = 20 mm, h = 150 mm) of alumina of activity grade **IV**. The copper complex of the porphyrin was eluted with benzene and crystallized from a mixture of chloroform and methanol (10 : 1). Yield 56 mg (24.6%). Mp 216.5–217.5° C. According to the literature [11], mp 224° C. Found, %: C 65.72; H 6.46; N 8.49. Calculated for $C_{36}H_{40}H_4O_4Cu$, %: C 65.90; H 6.14; N 8.53.

B) A solution of 60 mg of **I** (R = COOH, R' = C_2H_5) and 60 mg of **II** in 18 ml of ethanol and 18 ml of chloroform was treated with 8 drops of 41.5% hydrobromic acid. The reaction mixture was allowed to stand for 1 hr in an atmosphere of nitrogen at 10° C and then 100 mg each of sodium and copper acetates were added and the mixture was heated in the water bath in a current of nitrogen for 13 hr and then for another 6 hr with the bubbling of air. The residue after the elimination of the solvent was separated on a column of alumina. The copper complex of mesoporphyrin IX was eluted with petroleum ether–chloroform (1 : 1) and then with chloroform. Yield 3 mg (3%), Mp 210–211.5° C. Spectrum in chloroform: I 565 nm, **II** 526 nm (**I** > **II**).

Dimethyl ester of mesoporphyrin IX (IV, R = C_2H_5). The copper complex of the dimethyl ester of mesoporphyrin IX (35 mg) was dissolved in 0.5 ml of concentrated sulfuric acid and the solution was kept for 5 min. Then it was diluted with 5 ml of water, made alkaline with 10% of caustic soda, and extracted with chloroform. The chloroform solution was washed with water and evaporated. The residue was transferred to a column of alumina of activity grade V. The mesoporphyrin IX was eluted with chloroform and recrystallized from a mixture of chloroform and methanol (10 : 1). Yield 19 mg (60.2%). Mp 212–214° C. According to the literature [11], mp 216° C. The substance obtained and an analytical sample prepared from blood hemin had the same R_f values on paper chromatography in the kerosine–chloroform (1 : 8) system (R_f 0.78) and on alumina of activity grade **IV** in petroleum ether–diethyl ether (1 : 1) (R_f 0.72). Spectrum in chloroform: **I**) 623; **II**) 568; **III**) 535; **IV**) 500 nm (**I** < **II** < **III** < **IV**). Found, %: C 72.51; H 7.04; N 9.37. Calculated for $C_{36}H_{42}N_4O_4$, %: C 72.69; H 7.12; N 9.42.

Copper complex of the dimethyl ester of diacetyldeuteroporphyrin IX (IV, R = $COCH_3$). A) A suspension of 3.37 mg of 3,4'-diacetyl-4,3',5'-trimethyldipyrrylmethane (**I**, R = H, R' = $COCH_3$) and 4.96 mg of 5,5'-diformyl-3,3'-bis-(β-methoxycarbonylethyl)-4,4'-dimethyldipyrrylmethane (**II**) in 0.2 ml of methanol was treated with 0.3 ml of 56% hydriodic acid. The resulting solution gradually acquired a dark red color. To monitor the reaction, samples were taken from the reaction mixture every 30 min which, after dilution with water and extraction with chloroform, were deposited on a plate of silica. It was found that after 1.5 hr the initial dipyrrylmethane had disappeared from the solution. In ethanol the product had a maximum in the visible region at 510 nm. The reaction mixture was diluted with 10 ml of water, the hydriodic acid was neutralized with sodium acetate, and the bilene was extracted with chloroform. The chloroform extract was dried with sodium sulfate and the solvent was driven off in vacuum. The residue was dissolved in 10 ml of acetic acid and the solution was treated with 300 mg of sodium acetate and 200 mg of copper acetate. The reaction mixture was stirred for 5 hr and then, with the passage of air, was heated in the water bath for 6 hr. The solution was diluted with water and the colored substances were extracted with chloroform, the extract being dried with sodium sulfate and evaporated. It was found by preparative paper chromatography that the yield of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin **IX** was 273 μg (3.26%). The substance obtained had the same R_f value on paper chromatography in the chloroform–kerosine (1 : 1.15) system as an analytical sample of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin IX that we had synthesized previously [1,2]. Visible spectrum in chloroform: I) 586, **II**) 546 nm (**I** > **II**).

B) The condensation of 16.9 mg of the dipyrrylmethane **I** (R = H, R' = $COCH_3$) with 25 mg of the α, α'-diformyl-substituted dipyrrylmethane **II** in 15 ml of methanol and 6.6 ml of 41.5% hydrobromic acid led to the formation of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin **IX**. Yield 810 μg (1.9%).

C) In a similar reaction with a small amount of hydrobromic acid (3 drops) the yield of IV (R = $COCH_3$) was 335 μg (0.8%).

Hydrobromide of 4,4'-diacetyl-3,5,3',5'-tetramethyldipyrrylmethene. With stirring and ice-water cooling, 1 ml of 41.5% hydrobromic acid was added to a suspension of 50 mg of 3-acetyl-2,4-dimethylpyrrole and 60 mg of 3-acetyl-2,4-dimethylpyrrole-5-aldehyde in 1 ml of methanol. The reaction mixture was left for 2 hr and the precipitate that had deposited was separated off. The substance was crystallized from a small amount of methanol. Yield 122 mg (92%). The compound did not melt below 330° C. Spectrum in ethanol: λ_{max} 476 nm. Found, %: C 56.10; H 5.82; N 7.60; Br 22.03. Calculated for $C_{17}H_{20}N_2O_2 \cdot HBr$, %: C 55.89; H 5.79; N 7.67; Br 21.87.

Etioporphyrin I. A) A well-stirred mixture of 0.45 g of 5-carboxy-3,4'-diethyl-4,3',5'-trimethyldipyrrylmethane (I, R = COOH, R' = C_2H_5), 1.35 g of sodium acetate, and 2.03 g of potassium acetate was heated at 150–160° C for 45 min, after which the evolution of carbon dioxide had ceased. The reaction mixture was diluted with 50 ml of water and the product was extracted with chloroform. The chloroform solution, after purification on columns of silica and alumina and crystallization yielded 4 mg of etioporphyrin. Spectrum in chloroform: **I**) 626; **II**) 572; **III**) 538; **IV**) 498 nm (**I** < **II** < **III** < **IV**). Found, %: C 80.10; H 8.16; N 11.74. Calculated for $C_{32}H_{34}N_4$, %: C 80.33; H 7.95; N 11.71.

B) The etioporphyrin was also isolated by the treatment of a solution of the dipyrrylmethane I (R = COOH, R' = C_2H_5) in dichloroethane with dimethylformamide and phosphorus oxychloride and subsequent heating. The spectral characteristics and the R_f values on a silica plate in the n-heptane–chloroform (5 : 1) system of the etioporphyrins obtained in experiments (a) and (b) were identical.

Method for the quantitative determination of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin IX. To determine the concentration of this substance in a reaction mixture, preparative chromatography on Whatman No. 3 paper has been developed. After the trial of several systems the best was found to be chloroform–n-propanol–n-decane (15 : 0.1 : 30). At the start of a chromatogram 120 mm wide a definite volume of the reaction mixture (usually 0.04–0.06 ml) is deposited by means of a micropipet. The amount of solution depends on the concentration of the copper porphyrin complex. In parallel with the solution under investigation, an analytical sample of the copper complex of the dimethyl ester of diacetyldeuteroporphyrin IX is deposited on the paper. The chromatogram is run in the system mentioned and is dried, and after the zones have been outlined in UV light they are cut out. The required zone is cut into small pieces and is extracted three times with hot chloroform. The solution is evaporated to a volume of 5 ml. The density of this solution at the Soret line is determined on an SF-4 spectrophotometer. The concentration of the porphyrin copper complex is determined from the value of K. Knowing this concentration and the total volume of the reaction mixture the amount of porphyrin is determined. To find the correction to the coefficient K, known amounts of an analytically pure sample are chromatomatographed in the same way. This correction was found to be 1.2.

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