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THE ACTION OF DIAZOMETHANE ON HAEMIN CHLORIDES

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SUMMARY

Haemin chlorides have been shown to react with diazomethane in ethereal solution to give a spectroscopically identifiable species with an absorption maximum near 600 mμ. This compound is decomposed by acid to give the porphyrin corresponding to the original haemin chloride.

Alkaline haematins or porphyrin–copper complexes are not affected by diazomethane.

INTRODUCTION

The most common and convenient method of preparing the methyl esters of porphyrins involves the use of diazomethane in ethereal solution. No side reactions have been reported during such methylation procedures which take place practically instantaneously, although it is known that long contact of diazomethane with formyl porphyrins in methanol produces a compound whose properties are consistent with those of a substituted ethylene oxide[1]. Attempts to employ diazomethane for the methylation of carboxylic acid groups in haemin chlorides has surprisingly led to major spectral alterations. Fortunately, however, haemins can be methylated by the more time-consuming method using anhydrous methanol containing hydrogen chloride or sulphuric acid.

Ethereal solution of haemins, when allowed to come into contact with diazomethane, immediately lose their characteristic red-brown colour and become greenish-yellow. This colour change is brought about by the disappearance of haemin absorption bands and their replacement by an absorption maximum in the vicinity of 600 mμ. This paper discusses, in some detail, the interaction of diazomethane with haemin chlorides and in particular describes the characterisation and properties of the product formed from the action of diazomethane on deuterohaemin chloride.

METHODS AND MATERIALS

Spectroscopy: Visible spectroscopic measurements were made with a Perkin-Elmer "350" Spectrophotometer, the wavelength scale being standardized against reduced cytochrome *c* (550 mμ). Infrared spectra were recorded as Nujol mulls using a Perkin-Elmer "Infracord" Spectrophotometer.

Melting points: Melting points are corrected and were determined using a Griffin and Tatlock hot-stage microscope.

Paper chromatography. The method of CHU, GREEN AND CHU[2] with slight modifications was used. Kerosene – chloroform (4.0:2.6) and kerosene – propanol (6:1) were used as solvent systems.

Solvents: All solvents were distilled prior to use with the exception of ether which did not contain a reducing agent and was used as supplied.

Hydrochloric acid: All percent concentrations refer to w/v.

Ethereal diazomethane: This was prepared by the gradual addition of 20 g of *N*-nitrosomethylurea[3] to 200 ml of 20 % aqueous potassium hydroxide layered under 500 ml of ether. When all the urea had reacted with the alkali the ethereal solution was separated from the aqueous phase and used as such.

Preparation of porphyrins and haemins: Deuterohaemin, protohaemin and monoformylhaemin chloride were prepared as their dimethyl esters by methods previously described[4].

Deuteroporphyrin dimethyl ester was obtained by the diazomethane methylation of the porphyrin free acid prepared by the removal of iron from deuterohaemin using the method of MORELL AND STEWART[5].

EXPERIMENTAL AND RESULTS

Action of diazomethane on haemin chlorides

About 0.3–0.4 mg of haemin chloride methyl ester was dissolved in 2 drops of chloroform and the solution diluted to 3 ml with ether. This was treated in a cuvette with 1 ml of ethereal diazomethane (see METHODS AND MATERIALS) and the reaction followed spectrophotometrically taking readings against a blank prepared by the addition of 1 ml of ethereal diazomethane to 3 ml of ether.

Repeated scanning of the visible spectrum showed that the reaction was substantially complete after 15 min. The haemin absorption maxima had disappeared and a new absorption maximum was apparent in the vicinity of 600 mμ, the actual position being dependent on the nature of electrophilic groups in conjugation with the tetrapyrrolic ring system. Monoformyl-, proto- and deuterohaemins after reaction with diazomethane developed maxima at 612 mμ, 595 mμ and 588 mμ respectively.

The use of ether, previously washed with ferrous sulphate to remove peroxides, or the careful drying of the ethereal diazomethane solution over potassium hydroxide pellets did not alter these results.

The hydroxy haematins (alkaline haematins) and the copper complexes of porphyrins were unaffected by ethereal diazomethane for periods up to 1 h.

As deuterohaemin appeared less likely to undergo side reactions with diazomethane and was available in large quantities, this haemin was selected for the further examination of this reaction.

Action of diazomethane on deuterohaemin chloride dimethyl ester

Small scale reaction: 100 mg of deuterohaemin chloride dimethyl ester was dissolved in 25 ml chloroform and treated with an equal volume of ethereal diazomethane. An immediate change in the absorption spectrum of the solution was noted. The spectrum of this diazodeuterohaematin, which was formed by allowing deuterohaemin to remain in contact with diazomethane for 1 h is given in Fig. 1. This compound appears quite stable in ethereal solution and has been kept for several weeks at room temperature without apparent change. It gives a pyridine haemochrome in 20% aqueous pyridine with sodium dithionite as reducing agent, and this haemochrome is spectroscopically identical with pyridine deuterohaemochrome. The diazodeuterohaematin is readily soluble in 25% HCl whereupon the two-banded diazohaematin spectrum is converted into a three-banded spectrum (maxima at 595 mμ, 566 mμ and 550 mμ, Fig. 1). With time it was observed that the 566-mμ maximum, originally the most intense band, slowly faded while the maxima at 595 mμ and 550 mμ became stronger. This change was greatly accelerated upon dilution of the acidic solution. Adjustment of the pH of the solution with sodium acetate enabled a porphyrin to be extracted into ether. Spectroscopically and paper chromatographically this porphyrin was identical with deuteroporphyrin. The aqueous phase left after extraction of the porphyrin contained yellow by-products devoid of sharp spectral bands. This fraction was not investigated.

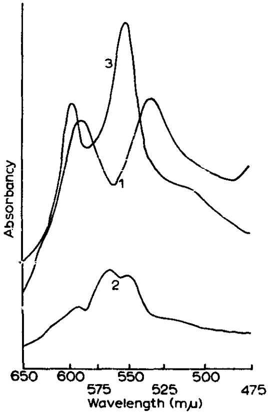


Fig. 1. Spectra: 1, diazodeuterohaematin in ether; 2, diazodeuterohaematin shortly after extraction into 25% HCl showing the development of acid porphyrin bands; 3, the acid porphyrin bands produced after 3 h in dilute HCl. The pigment concentrations are not equivalent.

Large scale reaction: 1.3 g of deuterohaemin chloride dimethyl ester was dissolved in 1 l of chloroform which was then diluted by the addition of 3 l of ether. To this mixture was added 500 ml of ethereal diazomethane and the whole allowed to stand for 15 min, when the hand spectroscope showed at this stage only one major band (approx. 585 mμ). The ethereal solution was then extracted repeatedly with 25% HCl until the extracts were no longer coloured. The ethereal phase which contained unaltered haemin was washed once with water, evaporated to half its volume and again treated with 500 ml of ethereal diazomethane as above. Recovered haemin from this treatment, after washing and reduction of solution volume, was again subjected to fresh ethereal diazomethane as above. No significant amounts of haemin were recovered after this treatment.

All 25 % HCl extracts, which were dark violet in colour and showed the characteristic three-banded spectrum referred to above, were combined and diluted with 2 volumes of water. After 3 h the 566 mμ maximum had disappeared leaving the 595 mμ and 550 mμ maxima which are typical of an acid porphyrin spectrum. After careful pH adjustment, first with sodium hydroxide and finally with sodium acetate, an ethereal solution of a porphyrin was obtained. This ethereal solution was washed repeatedly with water to free it of acetic acid. The porphyrin was then purified by acid extraction (2 × 2 %; 1 × 5 % HCl) which left a small amount of haemin in the ether. This was rejected. The porphyrin was returned to ether with aqueous sodium acetate and the ethereal solution again freed of acetic acid by repeated water washings. After a brief treatment with ethereal diazomethane to ensure that the porphyrin was fully esterified the ethereal porphyrin solution was washed with aqueous sodium carbonate and water and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a residue which was obtained crystalline from chloroform–methanol. Repeated recrystallization from the same solvent mixture gave 0.5 g of fine needles, m.p. 220–222° undepressed by admixture with deuteroporphyrin dimethyl ester, m.p. 222–224°. The visible and infrared spectra of this porphyrin were identical with those of deuteroporphyrin dimethyl ester and furthermore the two porphyrins had the same R_F values in two solvent systems (see METHODS AND MATERIALS). (Found: C, 70.7; H, 6.2; N, 10.45; O, 11.8 %. $C_{32}H_{34}O_4N_4$ requires C, 71.35; H, 6.4; N, 10.4; O, 11.9 %.)

Preparation of copper complex

50 mg of porphyrin dissolved in 50 ml of acetic acid was heated on a steam bath for 10 min in the presence of excess cupric acetate. After cooling this solution, the copper complex was transferred to ether and the ethereal solution washed with water and 5 % HCl to remove acetic acid and porphyrin respectively. The bright red copper complex was purified by recrystallisation from chloroform–methanol, m.p. 229–232° undepressed upon admixture with the copper complex of deuteroporphyrin dimethyl ester, m.p. 233–235°. The visible and infrared spectra of these two complexes were identical. (Found: C, 63.8; H, 5.25; N, 9.3 %. $C_{32}H_{32}O_4N_4Cu$ requires C, 64.0; H, 5.4; N, 9.3 %.)

DISCUSSION

Haemin chlorides when subjected to diazomethane in ethereal solution are almost instantaneously transformed into a species which can be identified spectroscopically by an absorption maximum near 600 mμ. Since porphyrins or their copper complexes are not affected by this reagent it can be assumed that the diazomethane attacks the iron of the haemin chloride. Furthermore since a pyridine haemochrome is produced from diazohaematins by the action of aqueous pyridine in the presence of dithionite it would seem that the iron is still present in such a compound. This leads to the conclusion that diazomethane displaces the chloride from the iron. The spectroscopic properties of the diazohaematin are also in accord with this conclusion.

On this basis then it is not surprising that copper complexes and alkaline haematins are not affected by diazomethane. Copper, co-ordinated with tetrapyrrolic molecules does not react with other ligands, while under the neutral conditions of this reaction it is to be expected that the chloride of a haemin chloride would be displaced much more readily than the hydroxyl group of an alkaline haematin.

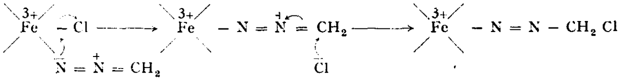
However, the diazohaematin is remarkable in that aqueous acid removes iron from the molecule to yield the porphyrin corresponding to the original haematin. The overall reaction then, brings about the removal of iron from the haemin chloride. Normally the removal of iron from a Fe^{3+}-porphyrin complex is extremely difficult and can only be achieved in rather special cases and when very strong acids are used, *e.g.* monoformyldeuterohaemin is converted to its porphyrin by 36 N sulphuric acid[6]. The more usual procedure in such a transformation is to reduce the iron to the ferrous state which readily gives up its metal under acid conditions. Ferrous acetate in acetic acid at 80° (see ref. 7) or the more convenient and milder ferrous sulphate procedure of MORELL AND STEWART[5] are the reducing agents generally employed.

In the present case, since the iron atom can be removed from the diazohaematin by aqueous acid it appears that at some stage of this reaction the oxidation state of the iron is changed from Fe^{3+} to Fe^{2+}. Most of the findings presented here can be rationalised in terms of the following mechanism.

The structure of diazomethane is usually represented as a hybrid of the forms:

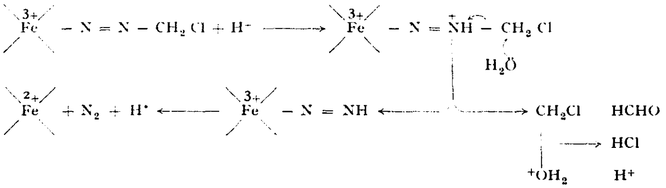
$$\overset{-}{C}H_2 - \overset{+}{N} \equiv N \longleftrightarrow CH_2 = \overset{+}{N} = \overset{-}{N}$$

and the diazohaematin might be formed in the following way:

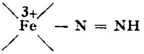


This species would then be responsible for the absorption maximum near 600 mμ characteristic of diazohaematins.

In aqueous acids the diazohaematin might be imagined to decompose by the following scheme:



and the ferrous complex so obtained converted under the acid conditions to the free porphyrin.

The free radical decomposition of  is analogous to the proposed mechanism of phenylation of aromatic hydrocarbons by phenylhydrazine in the presence of silver oxide[8].

$$\mathrm{Ph} - \mathrm{NH} - \mathrm{NH_2} \xrightarrow{\mathrm{Ag_2O}} [\mathrm{Ph} - \mathrm{N} = \mathrm{NH} \longrightarrow \mathrm{Ph}^{\cdot} + \mathrm{N_2} + \mathrm{H}^{\cdot}$$

When diazodeuterohaematin is dissolved in 25% hydrochloric acid a three-banded spectrum is obtained. Two of these maxima are almost certainly due to small amounts of acid porphyrin. It is not possible to say with certainty whether the third maximum, at 566 mμ is due to diazodeuterohaematin itself (the change from 588 mμ being attributed to solvent difference) or whether it is due to another intermediate along the decomposition pathway.

A consideration of general interest is that in this reaction a haematin is converted to a porphyrin in an aqueous medium. MORELL AND STEWART[5] have reported earlier that removal of iron from haematins in acetic acid is seriously retarded as the water concentration rises. If the mechanism proposed here for the decomposition of diazohaematin is essentially correct it might be supposed that the presence of water affects not the actual removal of iron but its reduction by ferrous sulphate.

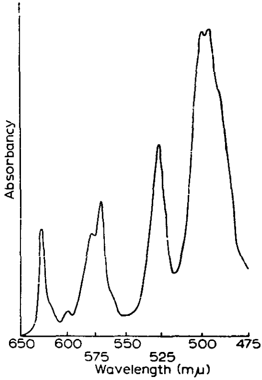


Fig. 2. The spectrum of deuteroporphyrin dimethyl ester in ether demonstrating band IV as a doublet.

One last point deserves comment. The spectrum of deuteroporphyrin presented in this paper (Fig. 2) shows very clearly that band IV is a doublet. This does not appear to have been reported before. Haematoporphyrin and mesoporphyrin also show this spectroscopic feature but protoporphyrin, monoformyldeuteroporphyrin and diacetyldeuteroporphyrin, all of which are electrophilically substituted, exhibit only a single band.

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