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A SPECTROSCOPIC STUDY OF HAEMATIN COMPOUNDS IN THE SORET REGION

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SUMMARY

1. The absorption spectra of various substituted haemin chlorides and alkaline haematins are given for the region 350–430 mμ.

2. Alkaline haematins (esters) in ether show a single Soret band. Haemin chlorides exhibit two absorption maxima in this region when the solvent is of low polarity such as in benzene or diethyl ether. In acetic acid only a single band is observed.

3. For haemin chlorides in ether the position of one band (S band) is dependent upon the electrophilic character of the substituents conjugated to the porphyrin ring. This band is major when the substituents are highly electrophilic. The other, shorter wavelength, band (S′ band) predominates where the substituents are weakly electrophilic or non-electrophilic and shows no correlation with the nature of the substituents.

INTRODUCTION

The spectrum of a porphyrin or its derivatives is usually characterised by a single absorption maximum of high extinction around 400 mμ in addition to the spectrum in the visible region. This is known as the Soret band. Recently we have obtained the prosthetic group of lactoperoxidase as an ether-soluble haemin chloride[1] and an examination of its spectrum in the Soret region has shown two distinct maxima which has prompted the present study.

As early as 1936 ADAMS[2] reported a spectrum of protohaemin showing a second maximum in the Soret region. More recently PAUL AND THEORELL[3] have indicated a shoulder on the Soret band of some "free ferriporphyrins" and the ferric complex of α, β, γ, δ-tetraphenylporphyrin in benzene has been reported[4] to have two bands in the Soret region. Apart from these observations this feature of porphyrin spectroscopy appears to have been little studied and where electrophilically substituted porphyrin derivatives are concerned no studies have been reported. This paper provides light absorption data between 350 mμ and 430 mμ for a series of variously substituted haemin chlorides and alkaline haematins and attempts some correlation between the structure of these haemins and their spectroscopic properties.

EXPERIMENTAL AND RESULTS

Absorption spectra

Measurements were made with a Hilger "Uvispek" Spectrophotometer, the wavelength scale being adjusted against the 546.1 mμ and 656.3 mμ lines of hydrogen. Most of this work has been carried out using ethereal solutions in which accurate extinction measurements are difficult to determine, hence no quantitative extinction data are given in this paper.

Solvents

Ether, not containing reducing agent, was used as supplied. Acetone, chloroform, benzene and acetic acid were of analytical reagent grade and were distilled prior to use. Pyridine was dried over potassium hydroxide and distilled.

Preparation of haemin chlorides

The following porphyrins, prepared by standard procedures, were converted into the corresponding haemin chloride dimethyl ester by the method of MORELL, BARRETT AND CLEZY[5]: 2,4-diformyldeuteroporphyrin dimethyl ester[6],4-formyldeuteroporphyrin-dimethyl ester[7], 2-formyl-4-vinyldeuteroporphyrin (chlorocruoroporphyrin) dimethyl ester[6], 2,4-diacetyldeuteroporphyrin dimethyl ester[8], 2 + 4-monoacetyldeuteroporphyrin dimethyl ester (obtained as a by-product of the 2,4-diacetyldeuteroporphyrin preparation and separated from it by alumina chromatography), 2,4-diethyldeuteroporphyrin (mesoporphyrin) dimethyl ester[9]. Protohaemin[10] and deuterohaemin[11] were converted to their dimethyl esters by allowing them to stand overnight in methanol containing 5 % (v/v) sulphuric acid. The esters were returned to ether and this solution was washed repeatedly with 5 % (w/v) aqueous hydrochloric acid.

The complete conversion of porphyrin into haematin is difficult both to achieve and to demonstrate. It is usual, therefore, to ensure the removal of any porphyrin remaining by extraction with aqueous HCl. However, in the case of porphyrin *a* ester its insolubility in 20 % HCl did not permit its separation from the haemin *a* ester. Also, since haemins, as distinct from prophyrins, cannot be esterified by diazomethane without alteration[12] the haemin *a* was prepared from porphyrin *a* free acid and then esterified by the methanol–sulphuric acid procedure.

The spectra of the haemin chlorides in ether were determined between 350 mμ and 650 mμ and the maxima are given in Table I. Some representative curves in the Soret region are recorded in Fig. 1.

Alkaline haematins

Ethereal solutions of the alkaline haematin esters (hydroxyhaematin esters) were prepared by shaking the haemin esters in ether with two equal volumes of 1 N NaOH. The spectra of the alkaline haematins in ether were determined between 350 mμ and 630 mμ and the maxima are given in Table I.

Pyridine haemochromes

To characterise the haemins their pyridine haemochromes were prepared and their absorption maxima are reported in Table II, together with some literature values. For solubility reasons the pyridine haemochrome esters were prepared with equal

TABLE I

ABSORPTION MAXIMA OF HAEMIN CHLORIDES AND ALKALINE HAEMATINS

Haemin	*Haemin chlorides absorption maxima (mμ)*							*Alkaline haematins maxima (mμ)*		
	S'	*S*	*W*	*III*	*II*	*I*	*S/S'*	*S*	*II*	*I*
2,4-Diformyl deuterohaemin	360	425	517	550	590	645	1.51	425	587	630
Haemin *a**	370/80	416	505	550	600	660	1.26	410	573	621
2-Formyl-4-vinyl deuterohaemin (chlorocruorohaemin)	370/80	415	510	548	605/10	650	1.24	406	577	620
4-Formyldeuterohaemin	365/70	411	507	547	600	650	1.32	404	573	616
2,4-Diacetyldeuterohaemin	360/65	417	515	544	590	640	1.54	411	580	605/10
2 + 4-Monoacetyldeuterohaemin	365/75	406	507	540	585	633	1.29	401	574	619
2,4-Divinyldeuterohaemin (protohaemin)	381/2	407	512	539/40	585	638	0.97	399	570	595/600
2,4-Diethyldeuterohaemin (mesohaemin)	370	397	506/8	534	585	632/4	0.84	392	504	590
Deuterohaemin	370	399	505	530	570	626/8	0.96	391	560	585

* Substituents affecting spectrum are 4-vinyl (extended) and 8-formyl.

volumes of pyridine and 0.01 N aqueous sodium hydroxide with sodium dithionite as reducer. The pyridine concentration which is somewhat higher than normally used is possibly responsible for the slight discrepancies between values published here and those reported earlier. In the Soret region only single bands were found for all haemochromes.

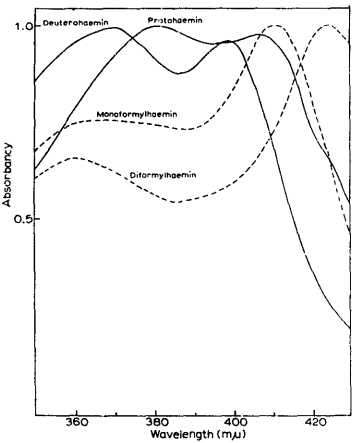

Fig. 1. Spectra in the Soret region of deuterohaemin, protohaemin, monoformylhaemin and diformylhaemin. Solvent ether. Absorbancies are arbitrarily adjusted so that maxima are equal at 1.0.

DISCUSSION

An examination of the spectroscopic properties in ether of a variety of haemin chlorides has revealed the presence of two absorption peaks in the Soret region—one about 360–380 mμ and the other between 400 mμ and 430 mμ. The position of the band at longer wavelength is dependent on the electrophilic character of substituents in conjugation with the tetrapyrrolic ring, while the position of the second maximum is independent of the electrophilic character of the substituents. This is in contrast to the finding of PAUL AND THEORELL[3] who report the position of both bands in the Soret region of some "free ferriporphyrins" to be dependent on the structure of the compound. These authors do not list the haemins examined or the conditions under which the measurements were made. Since the position of the Soret band in other porphyrin derivatives is dependent on the electrophilic character of substituents we regard the longer wavelength maxima of haemin chlorides as being the Soret band (S band), while we shall refer to the other maxima as the S' band.

The nature of the solvent plays an important role in the shape of the curve in the Soret region. Fig. 2 shows the spectra of protohaemin chloride in five solvents. In benzene and ether two well developed bands are clearly shown, while in chloroform

and acetone the S band has degenerated to an inflexion. In acetic acid only one band is seen. This latter solvent is the one that has been commonly employed to record haemin spectra which perhaps explains why this double absorption maximum in the Soret region has not been reported upon more frequently. Fig. 2 shows the effect of solvent on the spectrum of monoformylhaemin chloride. Since ether showed the development of these two absorption maxima to the best advantage it was chosen as the solvent to study a range of variously substituted haemin chlorides.

TABLE II

SPECTRAL DATA OF PYRIDINE HAEMOCHROMES

Corresponding haematin dimethyl ester	*Pyridine haemochromes* Absorption (mμ)*					
	Found				*Literature*	
	Soret band	*β-band*	*α-band*	*β-band*	*α-band*	*References*
2,4-Diacetyldeutero-haematin	430	540	573	539.7	575.2	13
2,4-Diformyldeutero-haematin	450	548–9	584	549.7	584.3	13
2,4-Diethyldeutero-haematin (meso haematin)	412–3	518	547	518	547	14
Deuterohaematin**	407	515	545	513.3	544.9	15
2,4-Divinyldeutero-haematin (protohaematin)	420	525	556	525	558	14
2-Formyl-4-vinyldeutero-heamatin (chlorocruorohaematin)	434	532–4	585.5	545.1	583.1	13
Monoacetyldeutero-haematin***	422–3	526	570	530	571	13
4-Formyldeuterohaematin	428	530	578–9	548	578	13
Haematin a§	427	None	585	None	587	13
Oxorhodoporphyrin 2-acetyl-6-(des-propionic acid) carboxylic deutero-porphyrin				None	582	13

* The pyridine haemochromes were prepared in pyridine–0.01 N NaOH + $Na_2S_2O_4$ (1:1, v/v).
** 1,3,5,8-Tetramethyl-6,7-di-proprionic acid porphin–Fe^{3+} complex.
*** A mixture of the 2- and 4-isomers.
§ Substituents on the 2-, 4- and 8-positions are hydroxyalkyl, extended vinyl and formyl respectively. Other positions are substituted as for deuterohaematin.

Some correlations in Table I deserve attention. We have already mentioned the relationship of the position of the S band with the electrophilic character of substituents conjugated with the tetrapyrrolic ring—the more electrophilic the substituent the further the S band lies to longer wavelengths. It is interesting to note the conflicting position of diacetyldeuterohaemin, in what might be termed the "electrophilic series". From the visible maxima (α-band of pyridine haemochrome, Table II;

band III of haemin chloride, Table I; alkaline haematin, band I, Table I) the electrophilic character of diacetyldeuterohaemin is judged to be less than of chlorocruorohaemin and monoformylhaemin, but if the Soret region of the spectrum is considered the order is changed and diacetyldeuterohaemin lies above chlorocruorohaemin and monoformylhaemin (pyridine haemochrome, Table II; alkaline haematin and S band of haemin chloride, Table I). The reason for this inconsistency is not clear.

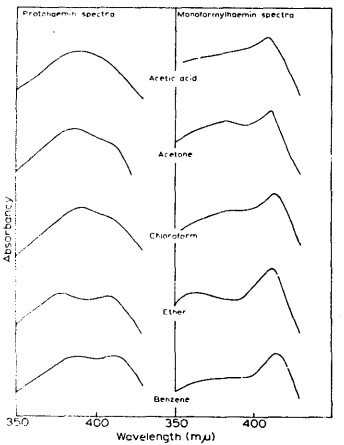

Fig. 2. The effect of solvents on the spectra of protohaemin and monoformylhaemin.

The ratio of the intensities of S/S′ shows some interesting correlations with the structure of the haemin. When a powerful electrophilic group such as formyl or acetyl group is present in the haemin chloride the ratio of S/S′ is greater than unity, while the ratio is less than 1 in protohaemin, deuterohaemin and mesohaemin. It is also interesting to note that when two electrophilic groups are present as in 2,4-diformyl-deuterohaemin or 2,4-diacetyldeuterohaemin the ratio S/S′ is about 1.5 while in the monoformylated or monoacetylated haemins the ratio is lower, 1.2–1.3. Although this correlation should be tested with a wider range of compounds, it may be of diagnostic value in providing some information about the structure of a haemin from its spectrum in the Soret region.

The alkaline haematin spectra show but a single band in the Soret region, the position of which correlates with the electrophilic character of the haemin substituents conjugated with the ring system. The extinction of this band is lower than the S band of the corresponding haemin chloride which is possibly due to association. The position of the alkaline haematin maximum lies 6–8 mμ to shorter wavelength than the maximum of the S band of the haemin chloride. This hypsochromic shift might be expected in terms of the relative electronegativities of chlorine and oxygen.

The reason for the two bands in the Soret region of the haemin chloride spectra is

not apparent. However, it may be relevant to point out that in both alkaline haematins and haemin chlorides there appears to be a relationship between the absorption maxima in the visible region of the spectrum with the bands near 400 mμ. Alkaline haematins have one band in the Soret region and two maxima in the visible, the position of all three being dependent on the structure of the parent porphyrin. The haemin chlorides have two absorption maxima in the Soret region and four maxima in the visible region of the spectrum. As already pointed out above, the position of the S band is dependent on the nature of the substituent groups while the position of the S' band is not. In the visible region the band III position is structure-dependent while band IV is not. It is difficult to be certain about the correlations of position of bands II and I with structure as these bands are not always very sharp and band II is asymmetric.

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REFERENCES

1 D. B. MORELL AND P. S. CLEZY, *Biochim. Biophys. Acta*, 71 (1963) 157.
2 S. A. ADAMS, *Biochem. J.*, 30 (1936) 2016.
3 S. K. PAUL AND H. THEORELL, *Acta Chem. Scand.*, 8 (1954) 1087.
4 G. D. DOROUGH, J. R. MILLER AND F. M. HUENNEKENS, *J. Am. Chem. Soc.*, 73 (1951) 4315.
5 D. B. MORELL, J. BARRETT AND P. S. CLEZY, *Biochem J.*, 78 (1961) 793.
6 R. LEMBERG AND J. PARKER, *Australian J. Expti. Biol. Med. Sci.*, 30 (1952) 153.
7 J. FISCHER AND C. WECKER, *Z. Physiol. Chem.*, 272 (1942) 1.
8 H. FISCHER AND K. ZEILE, *Ann. Chem.*, 468 (1929) 99.
9 K. G. STERN, *J. Biol. Chem.*, 112 (1936) 661.
10 H. FISCHER, *Organic Synthesis*, 21 (1941) 53.
11 H. FISCHER AND H. ORTH, *Die Chemie des Pyrrols*, Vol. 2, Part 1, Leipzig, 1937, p. 414.
12 P. S. CLEZY AND D. B. MORELL, *Biochim. Biophys. Acta*, 71 (1963) 150.
13 R. LEMBERG AND J. E. FALK, *Biochem. J.*, 49 (1951) 674.
14 R. LEMBERG AND J. LEGGE, *Haematin Compounds and Bile Pigments*, Interscience Publishers, Inc., New York, 1949, p. 177.
15 H. FISCHER AND H. ORTH, *Die Chemie des Pyrrols*, Vol. 2, Part 1, Leipzig, 1937, p. 415.