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PORPHYRINS. 33.* PROTON MAGNETIC RESONANCE SPECTRA OF HEMATOPORPHYRIN DERIVATIVES. PROPERTIES OF A DIMETHOXY DERIVATIVE (DIMEGIN) IN AQUEOUS SOLUTION

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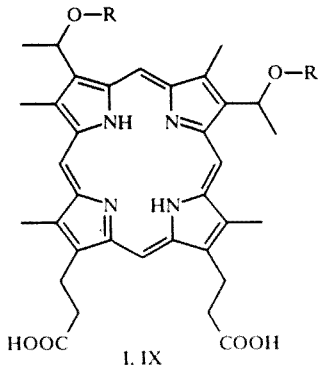
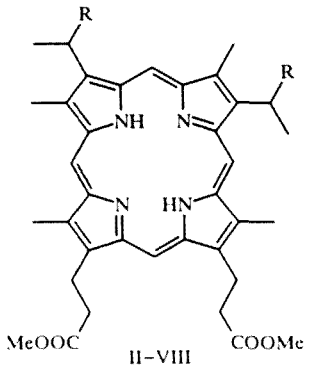
The PMR spectra of derivatives of hematoporphyrin IX have been investigated in organic and aqueous solution. It has been shown that the presence of the two chiral centers at positions 2 and 4 of the macrocycle is displayed in the specific splitting of the signals of the meso protons. The structures of dimeric associates of 2,4-di(α-methoxyethyl)-deuteroporphyrin IX (dimegin) have been studied in aqueous solution over a wide pH range and have the structure of a "skewed sandwich" according to PMR data.

Extension of the previously developed method [2] of replacing the OH groups in hematoporphyrin IX (I) has led to the synthesis of compounds (II)-(VIII) in which the substituents at positions 2 and 4 have other N-, S-, and O-containing groups in place of hydroxyl groups. Among the compounds synthesized, some possessed a marked radio-protective action [3] and the water-soluble salt of one of them, viz. 2,4-di(α-methoxyethyl)deuteroporoporphyrin IX (IX), called dimegin by us, has been successfully applied for the photodestruction of vessels of the eye in ophthalmology [4].



I, IX



II–VIII

I R = H; II R = OMe; III R=OAc; IV R = NMe_2; V R = $N(Me)CH_2Ph$; VI R = NEt_2; VII R = Im (1-imidazolyl); VIII R = $SC(S)NBu_2$; IX R = Me

The aim of the present work was to study the PMR spectra of the compounds obtained and particularly their behavior in aqueous solution, since the photophysical characteristics and display of physiological activity of porphyrins is linked with their structure in biological media. The PMR spectra of the synthesized derivatives of hematoporphyrin IX, as the dimethyl ethers, are given in Tables 1 and 2.

*For Communication 32, see [1].

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TABLE 1. Parameters of the PMR Spectra of Hematoporphyrin IX Dimethyl Ether Dimethyl Ester (II)

Chemical shifts, δ, ppm					
meso H	Ring CH_3, OCH_3 and $COOCH_3$	CH_2CH_2CO	$CH(CH_3)$	$CH(CH_3)$	NH
a)					
10,55 (1H)	3,71 (3H)	4,42 (4H)	6,05 q	2,26 d	-3,70
10,51 (1H)	3,67 (6H)	3,30 (4H)	(2H)	(6H)	
10,13 (1H)	3,66 (3H)		J = 6,6 Hz	2,25 d	
10,08 (1H)	3,65 (3H)			(6H)	
	3,61 (3H)				
b)					
	3,71 and 3,669				
	3,665 and 3,66				
	3,65 and 3,618				
	3,614				
c)					
11,45	3,875 and 3,865	4,72 m	6,43 m	2,336 d	
11,408	3,728 and 3,714	3,33 t		2,33 d	
11,407	3,65 and 3,642	3,31 t		2,24 d	
11,36	3,638 and 3,623			2,22 d	
11,194	3,613 and 3,60				
11,19	3,58 and 3,542				
11,089	3,538				
11,059					
d)					
11,19	3,91 and 3,74	4,51 t	6,14	2,17	
11,17	3,73 and 3,71	3,17 t	6,12	2,12	
11,13	3,69 and 3,685	3,14 t		2,06	
11,11	3,673 and 3,665			2,05	
10,95	3,669				
10,94					
10,73					
10,72					

Conditions of plotting spectra:

a) in $CDCl_3$, 2 mg porphyrin in 0.4 ml solution;

b) in $CDCl_3$, 0.4 mg porphyrin in 0.4 ml solution, methyl proton signal region;

c) in CF_3COOD;

d) in $CDCl_3$ + 1% CF_3COOD.

It is known that hematoporphyrin IX may have four isomers differing in the stereoisomerism of the hydroxyethyl substituents at positions 2 and 4 (RR, SS, RS, SR). In the case of the dimethyl ether dimethyl ester of the hematoporphyrin (II) in dilute and in acid solution (Table 1) and also of the amino derivatives (IV)-(VII) (Table 2) this isomerism is displayed in the meso proton region of the PMR spectra by the presence of two groups of signals, differing in some cases by up to 1 ppm, and containing 4 signals and 2 signals of double intensity respectively. [On plotting the spectrum of porphyrin (II) on instruments of 360 and 400 MHz 8 signals were clearly displayed in the meso proton region characterizing the presence of two isomers]. The same spectrum, but with smaller differences of chemical shifts, was observed for hematoporphyrin in acidified aqueous solution. A similar spectroscopic display of optically active isomers has been observed for certain chlorophyll derivatives also having asymmetric carbon atoms [5]. A broadening of the signals of the meso protons occurs in acid solution.

The spectrum of dimegin in acid has the characteristic spectrum of meso protons mentioned above (6 lines) and two doublets for protons of the methyl group of the $CH(CH_3)(OCH_3)$ substituent. Furthermore, one of the doublets, in its turn, was split into two (Fig. 1 and Table 3). The signals of the methyl and hydroxymethyl protons are disposed in a very narrow group and their intensities also correspond to the presence of two isomers.

The behavior of the PMR signals of dimegin on gradually increasing the solution pH is very interesting. Initially one of the signals of the meso protons with double intensity is broadened and is shifted towards high field, then the other, and the signals of single intensity are shifted further. Simultaneously the high field signals of the methyl protons are broadened and shifted towards high field (Fig. 2). To all appearances, the increase of pH thaws the motion of the bulky substituents in the dimegin molecule, and this process begins at a lower pH for one of the isomers than for the other (a difference of 0.1-0.2 ppm overall). The dynamic character of this process was confirmed by temperature measurements as heating the sample led to narrowing of the lines.

TABLE 2. Parameters of the PMR Spectra of Derivatives of the Dimethyl Ester of Hematoporphyrin IX in $CDCl_3$ Solution

Compound, R	Chemical shifts, δ, ppm					
	meso H	CH_3	CH_2CH_2CO	$CH(CH_3)$	R	NH
III $OCOCH_3$	10,46 (1H) 10,40 (1H) 10,10 (1H) 10,05 (1H)	3,79 (3H), 3,75 (3H), 3,67 (6H), 3,64 (3H), 3,61 (3H)	4,41 (2H) 4,38 (2H) 3,29 (2H) 3,28 (2H)	7,54 (2H) 2,33 (6H)	2,30 (6H)	- 3,75
IV $N(CH_3)_2$	10,80 (1H) 10,79 (1H) 10,10 (1H) 10,05 (1H)	3,73 (6H), 3,66 (6H), 3,65 (3H), 3,64 (3H)	4,42 (4H) 3,29 (2H) 3,28 (2H)	4,82 br. 2,15 br.	2,66 (6H)	- 3,72
V $NMe(CH_2Ph)$	11,095 (1H) 11,06 (3H) 10,09 (2H) 10,05 (2H)	3,80 (6H), 3,75 (6H) 3,70 (6H), 3,67 (12H) 3,65 (6H)	4,45 (4H) 4,43 (4H) 3,30 (8H)	5,04 (4H) 2,22 (12H)	2,58 (CH_3) 3,63 (CH_2) 7,21 (Ph) m 7,48 (Ph) m	- 3,67
VI $N(CH_2CH_3)_2$	11,15 (1H) 11,14 (1H) 11,12 (1H) 11,11 (1H) 10,07 (2H) 10,02 (2H)	3,69 (6H), 6,7 (12H) 3,65 (6H), 3,64 (12H)	4,42 (8H) 3,30 (4H) 3,28 (4H)	5,28 (4H) 2,10 (6H) 2,08 (6H)	3,10 q 1,21 t	- 3,72
VI dication	11,11 (2H) m 11,02 (1H) 10,54 (1H)	3,71 (6H) m, 3,63 (6H) 3,533 (3H), 3,526 (3H)	4,40 (4H) 3,17 (4H)	5,65 (2H) m 2,63 (6H)	3,95 q , 1,55 t	
VII 1-Imidazolyl	10,14 (2H) 10,09 (2H) 9,70 (1H) 9,66 (1H) 9,64 (1H) 9,62 (1H)	3,65 (12H) 3,64 (6H) 3,59 (3H) 3,58 (3H) 3,48 (6H) 3,42 (3H) 3,40 (3H)	4,40 (4H) 3,29 (4H) 4,38 (4H) 3,26 (4H)	6,99 (4H) m 2,65 (6H) 2,62 (6H)	8,13 (4H) m 7,30 (8H) m	- 3,70
VIII $SC(S)NBu_2$	10,51 (1H) 10,47 (1H) 10,09 (1H) 10,05 (1H)	3,84 (3H) 3,81 (3H) 3,67 (6H) 3,65 (3H) 3,63 (3H)	4,41 (4H) 3,28 (4H)	7,24 (2H) m 2,53 (6H)	4,04 (3H) t 3,71 (3H) t 1,71 (4H) m 1,35 (4H) m 0,85 (6H) t	- 3,68
H in (D_2O + DCl)	11,21 (1H) 11,20 (1H) 11,19 (1H) 11,18 (1H) 10,93 (2H) 10,90 (2H)	3,75 (12H) 3,70 (6H)	4,56 (4H) 3,32 (4H)	6,71 (2H) 2,05 (6H)		

A further increase in solution pH leads to a dynamic equilibrium of the whole series of isomeric forms of the molecules linked to one another in associates. These isomers become indistinguishable in the PMR spectra. Even at concentrations of $5 \cdot 10^{-4}$ M at pH 6.6-11 the proportion of associates in dimegin solutions is very significant. Four lines of the meso protons are located at 7.3-10.5 ppm, two of them are very broad (20 Hz) (Table 3, Fig. 3). The residues of propionic acid are clearly nonequivalent, the signals of the CH groups joined to the porphyrin ring are broad, and both groups are markedly shifted in the direction of high field. The position of the proton signals of the OCH_3 groups of the substituents at positions 2 and 8 may be determined only from the integrated intensity and the tendency to change the values of their chemical shifts on making acid solutions alkaline. However, the methyl group and $CH(CH_3)$ group signals have the usual chemical shifts and are fairly narrow.

It may be proposed that the dimegin associates have the structure of a skewed sandwich in which under the shielding influence of the π current the partner molecules contact only a portion of the porphyrin molecule including the propionic acid residues and two meso protons.

The problem of the mutual orientation of the porphyrin rings in dimers and porphyrin metal complexes assumes particular importance when considering the mechanism and the rate of the electron transfer reaction in biological systems. Calculations [6] on a one-electron model, in which the electron being transferred is in a spheroidal potential pit of specific depth, have shown that the thermal matrix of electron transfer is very sensitive to the mutual orientation of the porphyrin rings. On the other hand, the experimental data available show that the geometry of the skewed stack is a fundamental property of porphyrin–porphyrin interactions, being found in solution, in the solid, in dimers, and in more complex associates [7].

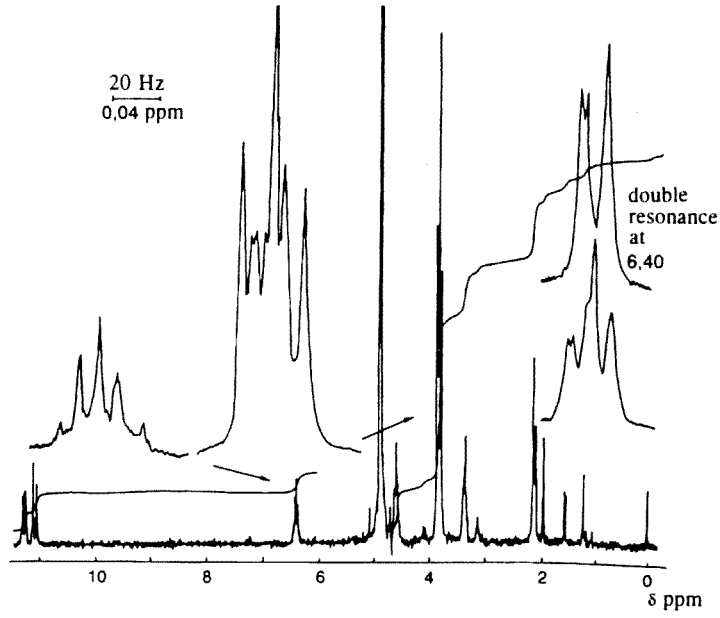

Fig. 1. PMR spectrum of dimegin in solution in DCl in D_2O.

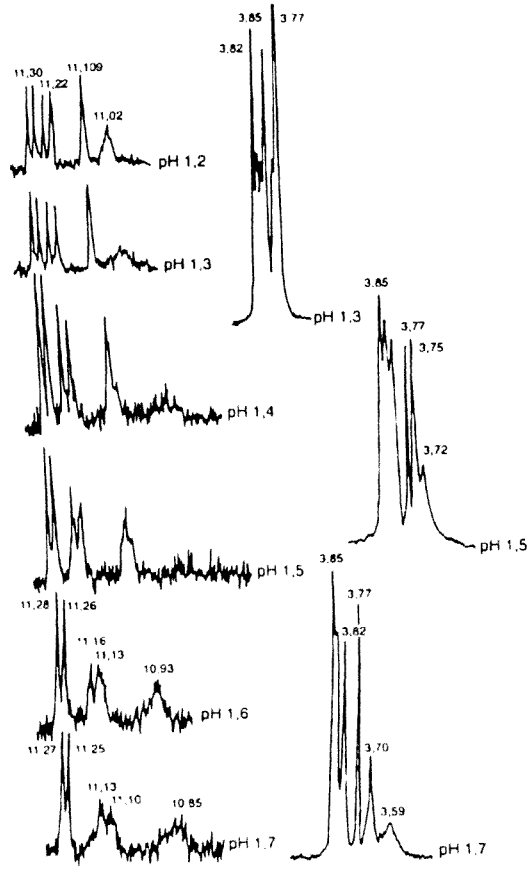

Fig. 2. Shape of the PMR signals of the meso protons and methyl protons of dimegin in hydrochloric acid solution at pH 1.2 to 1.7.

TABLE 3. Values of the PMR Spectra of Dimegin (IX) and Hematoporphyrin (I) in Various Solvents

Conditions	Chemical shifts, δ, ppm				
	meso H	ring CH_3	OCH_3	CH_2CH_2CO	$CH(CH_3)$
(IX) diacid in $CDCl_3$	10,04 (1H) 10,08 (1H) 10,51 (1H) 10,52 (1H)	3,58 (3H) 3,62 (6H) 3,64 (3H)	3,696 (3H) 3,705 (3H)	4,41 (4H) 3,34 (4H)	6,04 (2H) 2,25 (6H)
(IX) dication in $CDCl_3$	10,67 (1H) 10,70 (1H) 10,89 (2H) 11,12 (1H) 11,13 (1H) 11,18 (1H) 11,20 (1H)	3,653 (6H) 3,659 (3H) 3,665 (6H) 3,674 (6H) 3,688 (3H)	3,70 (3H) 3,72 (3H) 3,882 (3H) 3,886 (3H)	4,45 (8H) 3,24 (4H) 3,195 (4H)	6,07 (2H) 2,13 (6H) 6,045 (2H) 2,00 (6H)
(IX) diacid in DCl (pH < 1)	11,06 (2H) 11,12 (2H) 11,23 (1H) 11,26 (1H) 11,29 (1H) 11,315 (1H)	3,78 (6H) 3,81 (6H) 3,82 (9H) 3,83 (3H)	3,84 (3H) 3,85 (3H) 3,86 (6H)	4,60 (8H) 3,36 (8H)	6,40 (4H) 2,15 (6H) 2,12 (6H)
(IX) disodium salt in D_2O, pH 10.2	7,43 (1H) 9,11 (1H) 9,96 (1H) 10,53 (1H)	3,34 (3H) 3,57 (3H) 3,64 (3H) 3,66 (3H)	3,67 (3H) 3,87 (3H)	2,73 (4H) 2,44 (2H) 2,58 (6H)	6,42 (2H) 2,16 (2H)
(IX) disodium salt in D_2O, pH pH 10.2, 306 K	9,18 (1H) 9,55 (1H) 10,16 (1H) 10,37 (1H)	3,48 (3H) 3,54 (3H) 3,59 (3H) 3,62 (3H)	3,71 (3H) 3,73 (3H)	2,73 (2H) 2,87 (2H) 3,18 (2H) 3,32 (2H)	6,20 (2H) 2,34 (6H)
(I) in D_2O + DCl	11,21 (1H) 11,20 (1H) 11,19 (1H) 11,18 (1H) 10,93 (2H) 10,90 (2H)	3,75 (12H) 3,70 (6H)		4,56 (4H) 3,32 (4H)	6,71 (2H) 2,05 (6H)

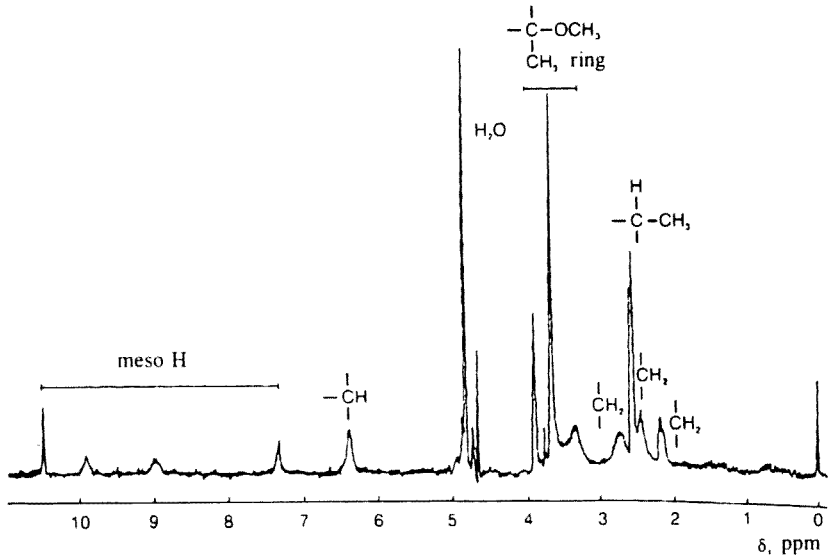

Fig. 3. PMR spectrum of dimegin in aqueous solution at pH 6.6; concn. = $1.25 \cdot 10^{-3}$ M.

We have calculated the ring currents for a rough assessment of the geometry of the dimer using measurements of the chemical shifts of the meso protons, of the protons of the propionic residues, and the protons of the ring methyl groups in dimegin monomer (in chloroform solution) and in the dimer (in aqueous alkaline solution at pH 10.2 and concentration $1.25 \cdot 10^{-3}$ M). The shifts under the influence of the π currents in dimer, z = 3.8 Å, x = 3.6 Å, y = 2.5 Å.

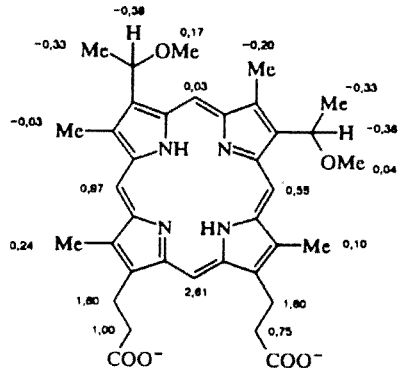

Fig. 4. Changes of chemical shifts ($\Delta\delta$) of the dimegin protons under the influence of π currents in the monomeric state (in chloroform) and in water at pH 10.2.

Finally, at concentrations of 10^{-3} M it is impossible to exclude the formation of more complex associates than dimers since the broad lines indicate the variation of intermolecular interactions in the different isomers of the associates.

On increasing the experimental temperature, a narrowing and a shift of signals towards low field is observed. At T = 306 K, the chemical shifts of the meso proton signals are 10.37, 10.16, 9.55, and 9.18 ppm. To all appearances, an increase in temperature of neutral and alkaline solutions of dimegin leads to an increase in the rate of conversion of the molecular forms from one to another, i.e., to an averaging of the chemical shifts of the proton signals, and on the other hand to the destruction of complex associates and dimers and thereby to a decrease in the mutual influence of the π currents.

EXPERIMENTAL

The PMR spectra were plotted using Bruker WM 250, WM 360, and AM 400 instruments.

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