Proton Nuclear Magnetic Resonance of Photoporphyrins; Assignments and Aggregation Behaviour

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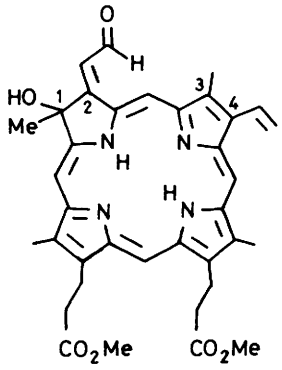
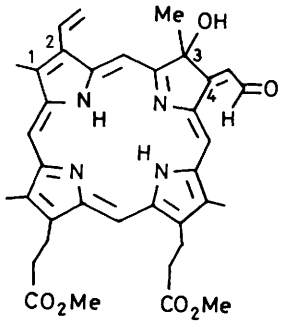
The ^{1}H n.m.r. signals of 2-devinyl-1,2-dihydro-1-hydroxy-2-(2-oxoethylidene)protoporphyrin IX and 4-devinyl-3,4-dihydro-3-hydroxy-4-(2-oxoethylidene)protoporphyrin IX (photoporphyrin A and B) have been assigned to specific pyrrolic substituents by observation of nuclear Overhauser enhancements. N.m.r. and visible spectra were concentration-dependent in some solvents (chloroform and methylene dichloride) but not in others (acetone and methanol) that could act as hydrogen-bond acceptors. The effects may be due to aggregation promoted by hydrogen bonding that is more extensive than the usual porphyrin π–π stacking.

Photoporphyrins A and B are derivatives of protoporphyrin IX prepared by limited aerobic photolysis. They provided a key intermediate in the synthesis of spirographis porphyrin,[1,2] and have been used as a model for natural chlorins.[3] Natural product haems d are similar saturated porphyrins bearing hydroxy groups.[4–6] We wanted to complete the assignment of the ^{1}H n.m.r. spectra of the photoporphyrins in order to understand the chemical shifts of the haems d. In the course of this study, concentration effects were observed that were greater than those associated with π–π stacking of porphyrins.[7,8] Stacking usually[9] causes chemical-shift differences of the order of 0.1—2 p.p.m. when the concentration changes from *ca.* 4 to 100mM. More pronounced changes occur in the chlorophyll series, where dimerization is promoted by hydrogen bonding.[10] Another such case may be the aggregation behaviour of haematoporphyrin derivatives (HPD).[11] It has been mentioned that porphyrins with hydroxy substituents demonstrate strong aggregation behaviour,[7] but details were not given or not readily accessible. Therefore, it seemed warranted to document this type of behaviour for the photoporphyrins.



photoporphyrin A



photoporphyrin B

Materials and Methods

Protoporphyrin IX dimethyl ester was photolysed[12] and the resulting photoporphyrins A and B were purified by h.p.l.c. (10 μm silica; column dimensions 4.6 × 250 mm; eluant $CHCl_3$ at 1 ml min^{-1}; detection at 400 or 670 nm). Isomer A was eluted at 700 s, isomer B at 530 s (0.3—0.6 μmol per injection). The isomers were stable when dry. Sample concentrations were determined by optical spectroscopy.[12] Chemical shifts (200 MHz; 20 ± 0.5 °C; ref. internal Me_4Si) were assigned by observation of nuclear Overhauser enhancements as described for other porphyrins.[5,6]

Results and Discussion

Table 1 lists observed chemical shifts. Assignments were made from the observation of nuclear Overhauser enhancements between nearest neighbour substituents. Table 2 lists results for a typical series of experiments on isomer B. Some resonances, such as that of the 3-methyl group on the saturated pyrrole ring, were assigned from their distinctive chemical shifts. Others were then assigned on the basis of the observation of an enhancement. For example, irradiation at the frequency of the 3-methyl signal produced an enhancement at δ 8.5, identifying this signal as due to the *meso* α-proton. Enhancements when the α-proton frequency was irradiated confirmed the assignment. Irradiation at the 4-ethylidene resonance frequency (δ 7.1) led to assignment of the β-proton frequency, irradiation at which then led to assignment of the 5-methyl signal, and so on. Not all enhancements expected on the basis of simple proximity were observed; their magnitude may have been below the detection limit. Additional series of irradiations were performed with acetone as solvent and with isomer A. The data obtained confirmed the original isomer assignments.[2]

Some tentative chemical-shift correlations were made possible by the assignments. The *meso*-proton at highest field was that next to the sp^3 pyrrole carbon atom, that at next highest field was near the aldehyde group, and the furthest downfield was furthest from the partially saturated pyrrole ring. The shifts were consistent with a decreased ring current near to the modified pyrrole ring. In common with some, but not all chlorins,[13] two NH proton signals were observed even at room temperature. This may be due to energy differences amongst tautomeric/resonance structures.

In C^2HCl_3 or $C^2H_2Cl_2$ solutions (Table 1), the n.m.r. spectra of both isomers were concentration-dependent to an extent not usually seen for porphyrins. The spectrum of octaethylporphyrin over the same concentration range shows shift changes of less than 0.005 p.p.m. and protoporphyrin IX dimethyl ester shows shift changes (not specific to any substituent) of less than 0.03 p.p.m. (although the spectrum of protoporphyrin IX shows more pronounced concentration effects at higher concentrations, in excess of 5mM). In the photoporphyrins, the signal of the *meso* δ-proton (in isomer A) or α-proton (in isomer B) was broadened up to 30 Hz in some spectra at room temperature (*cf.* 2 Hz in acetone). Precise chemical shifts showed considerable variation from sample to sample in halogenated solvents. The shifts were sensitive to concentration, moisture content (determined by the height of the residual water resonance), temperature, and trace

Table 1. Chemical shifts of photoporphyrins A and B[a]

Assignment	In $(CD_3)_2CO$	In $CDCl_3$[b]	Lit.[c]	Dilution shift[d]
Isomer A				
meso α-H	10.03	8.54	8.08	0.39
β-H	10.33	9.66	9.46	0.04
γ-H	10.32	9.72	9.54	0.12
δ-H	9.64	8.18	7.18	0.73
1-Me				
3-Me	2.35	1.68	1.31	0.12
5-Me	3.70	3.01	2.55	0.33
8-Me	3.54	3.34	3.22	0.05
CO_2Me	3.61	3.40	3.32	0.06
	3.61	3.69	3.71	0.01
	3.59	3.67	3.71	0.01
=*CH*CHO[e]	7.85	6.54	5.62	0.69
=CHC*H*O	11.24	10.29	9.94	0.27
C*H*=CH_2[f]	8.39	8.03	7.81	0.09
CH=C*H*$_2$	6.49	6.27	6.13	0.04
	6.30	6.16	6.06	0.02
C*H*$_2$$CH_2CO_2Me$[g]	4.46	4.30	4.17	0.02
	4.32	4.18	4.17	0.01
CH_2C*H*$_2$$CO_2Me$	3.32	3.19	3.19	0.01
	3.21	3.15	3.19	0.01
N*H*	−2.48	−3.38		0.28
	−2.62	−3.56		0.43
Isomer B				
meso α-H	9.70	8.52	7.39	1.02
β-H	9.98	9.02	8.20	0.67
γ-H	10.36	9.92	9.64	0.47
δ-H	10.26	9.79	9.52	0.36
1-Me	3.75	3.60	3.51	0.06
3-Me	2.32	1.69	1.05	0.64
5-Me	3.64	3.42	3.31	0.11
8-Me	3.55	3.42	3.25	0.08
CO_2Me	3.60	3.66	3.67	0.02
	3.59	3.65	3.65	0.02
=*CH*CHO[e]	7.86	7.11	6.31	0.57
=CHC*H*O	11.24	10.37	9.82	0.59
C*H*=CH_2[f]	8.29	7.81	7.46	0.15
CH=C*H*$_2$	6.46	6.21	6.00	0.09
	6.26	6.11	5.97	0.10
C*H*$_2$$CH_2CO_2Me$[g]	4.45	4.32	4.12	0.03
	4.32	4.23	4.12	0.06
CH_2C*H*$_2$$CO_2Me$	3.30	3.23	3.16	0.04
	3.21	3.17	3.16	0.01
N*H*	−2.48	−3.22		0.27
	−2.62	−3.45		0.31

[a] In p.p.m. from Me_4Si. [b] 1mM; 20 °C. [c] From ref. 2, in C^2HCl_3. [d] Chemical shift at 0.25 mM minus the shift at 1 mM. Positive values refer to resonances moved upfield with increasing concentration. [e] Spin-coupled doublets with J 7.8 Hz. [f] ABX subspectra with H_A *trans* to H_X and H_A downfield of H_B; J_{AX} 17, J_{BX} 12, J_{AB} 1 Hz. [g] Spin-coupled triplets with J 7 Hz.

Table 2. Nuclear Overhauser experiments on isomer B[a]

Expt.	Irradiation at δ	N.O.e. at δ	Assignment
1	1.7 (3-Me)	(a) 7.1	4-CHCHO
		(b) 10.4	4-CHCHO
		(c) 8.5	α-H
2	8.5 (α-H)	(a) 1.7	3-Methyl [corollary to expt. 1(c)]
		(b) 7.8	2-CH=CH_2
		(c) 6.2	2-CH=CH_2
3	7.1 (4-CH)	(a) 9.0	β-H
		(b) 10.4	4-CHO
4	9.0 (β-H)	(a) 7.1	4-ethylidene [corollary to expt. 3(a)]
		(b) 3.4	5-Me
5	3.4 (5- and 8-Me)	(a) 9.0	β-H [corollary to expt. 4(b)]
		(b) 9.8	δ-H
6	9.9 (γ-H)	(a) 4.2	6- or 7-CH_2 methylene
		(b) 4.3	6- or 7-CH_2 methylene
		(c) 3.2	6- or 7-CH_2 methylene
7	4.3 (6- and 7-CH_2)	(a) 9.9	γ-H [corollary to expt. 6(a,b)]
		(b) 3.4	5- + 8-Me
		(c) 3.2	6- + 7-CH_2
8	9.8 (δ-H)	(a) 3.4	8-Me [corollary to expt. 5(b)]
		(b) 3.6	1-Me
9	3.6 (1-Me)	(a) 9.8	δ-H *meso* [corollary to expt. 8(b)]
		(b) 7.8	2-CH

[a] At 20 °C; 1 mM; in C^2HCl_3. Each experiment produced several observed positive enhancements at the listed chemical-shift values [labelled (a), (b), *etc.* for cross-reference]. Certain results are marked as 'corollary to' another result because they confirmed the proximity of the protons. Because of overlap, it was not possible to assign individually the *6,7* methylene or the *5,8*-methyl protons in this case. The same problem occurred for the methyl ester protons in all cases.

impurities. In contrast, the shifts for solutions in acetone or 3:1 methanol–chloroform (chloroform for solubility) were concentration- and temperature-independent, and reproducible from sample to sample (less than 0.02 p.p.m. change for different batches or concentration differences from 0.25 to 1mM).

These observations are attributed to self-aggregation in the polar, but non-hydrogen-bonding solvents C^2HCl_3 and $C^2H_2Cl_2$. Resonances shift downfield upon dilution. At fixed porphyrin concentrations, shifts also depend on residual water content. Increasing temperature (20 to 50 °C) causes downfield shifts. In solvents capable of hydrogen bonding (acetone or chloroform–methanol) these effects are not present. The resonances most affected are those associated with the modified pyrrole, especially the neighbouring *meso*-protons, the aldehyde group, and the oxoethylidene protons. The chemical shifts originally reported[2] are noticeably upfield of the present observations, reflecting the more concentrated solutions (0.05M in chloroform) then used. Intramolecular hydrogen bonding had been suggested[2] to account for the shifts seen at a single experimental concentration. The observed concentration dependence implies that intermolecular effects are of major importance. In concentrated solutions in chloroform, the visible spectra show a shoulder at 650 nm on the expected 670 nm band, not present in the usual visible spectrum. It disappears upon dilution in chloroform or upon transferring the sample to acetone. It was not possible to reproduce this optical feature with all samples, although the frequency of appearance and the concentration and acetone sensitivity ruled out an instrumental or impurity artefact. Its appearance may relate to the precise moisture content of the solvent or the accumulation of non-absorbing impurities that prevent aggregation.

The observation of upfield shifts at high concentrations suggests that aggregation at least partially involves stacking of the aromatic planes. In this conformation, the protons will be affected by the shielding cone of the stacked macrocyclic ring current. The possibility of hydrogen bonding arises because of the introduction of a hydroxy and a carbonyl group that may

not be freely rotating. It is possible to speculate on structures for an aggregate, including questions of the role of the chiral centre, but there are insufficient data for detailed suggestions.

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