# Acid–Base Behaviour of Mesoporphyrin IX Bis(methyl ester) and Copro-porphyrin I Tetrakis(methyl ester) in 95% (v/v) Dimethyl Sulphoxide–Water: Kinetic and Equilibrium Studies

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Individual equilibrium constants for ionisation of both protons from diprotonated mesoporphyrin IX bis(methyl ester) in 90 and 95% (v/v) $Me_2SO–H_2O$ containing hydrochloric acid have been obtained despite the fact that the first dissociation constant $(K_1)$ is smaller than the second $(K_2)$. The values in 95% (v/v) $Me_2SO–H_2O$ are $K_1$ 0.007 and $K_2$ 0.06 mol $l^{-1}$ which means that the monoprotonated porphyrin $(BH^+)$ is always present in deficit compared with the free base (B) and diprotonated species $(BH_2{}^{2+})$. Relaxation times for the equilibration between B and $BH_2{}^{2+}$ were measured using the temperature-jump method and forward and reverse rate coefficients for inter-conversion of $BH_2{}^{2+}$ and $BH^+$ have been obtained. The thermodynamically favourable protonation by hydronium ion of a pyrrole nitrogen atom in $BH^+$ occurs slowly and possible reasons for this are discussed.

PREVIOUSLY it was shown that the species present in solutions of mesotetraphenylporphyrin in 98, 90, and 80% (v/v) $Me_2SO–H_2O$ containing varying concentrations of hydrochloric acid were the free base and the diprotonated porphyrin, and the monoprotonated species was not detected.[1] This means that the first acid dissociation constant for the diprotonated porphyrin is much smaller than the second and only an overall equilibrium constant between the free base and diprotonated porphyrin could be measured. Although this is not the usual observation for a dibasic acid, diprotonated porphyrins often behave in this way.[2] Temperature-jump studies [1] of the equilibrium between mesotetraphenylporphyrin and the diprotonated form showed that the interconversion occurred slowly with relaxation times in the range 40—5 000 μs depending upon the acid concentration and this is exceptionally slow for proton transfers to and from nitrogen. The reaction involves the monoprotonated porphyrin as a low concentration intermediate and the kinetic analysis gave a value for the rate coefficient for ionisation of the diprotonated species to give the monoprotonated porphyrin. The value was extremely low. One way of explaining this is to assume that ionisation of the first proton from the diprotonated porphyrin is strongly thermodynamically unfavourable and in this case the rate coefficient in the forward direction would be low even if the reverse protonation occurred at the diffusion limit. This explanation is obviously compatible with the failure to observe the monoprotonated porphyrin. To find out whether this is the factor which determines the low rate of ionisation or whether some other explanation is required, it is necessary to study a porphyrin for which the mono-protonated species is detectable and then equilibrium constants for both ionisation steps can be measured. For mesoporphyrin IX bis(methyl ester) in mixtures of acetic acid or monobromopropionic acid with acetone, the free base and the mono- and di-protonated porphyrins have been observed spectrophotometrically and ionisation occurs in two well separated stages.[3] In this solvent the diprotonated form of mesoporphyrin IX bis(methyl ester) behaves as expected for a dibasic acid; the first dissociation constant is larger than the second. A similar observation has been made for coproporphyrin

I in aqueous solution and again both dissociations could be studied separately.[4] We have therefore made kinetic and equilibrium measurements with mesoporphyrin IX bis(methyl ester) (1) under the same conditions as used for mesotetraphenylporphyrin. We have made less detailed studies with coproporphyrin I tetrakis(methyl ester) (2). In the case of mesoporphyrin IX bis(methyl ester) we have been able to observe the monoprotonated porphyrin and this has permitted a much fuller analysis of the kinetic and equilibrium behaviour of this porphyrin than was possible for mesotetraphenylporphyrin.[1]



(1)



(2)
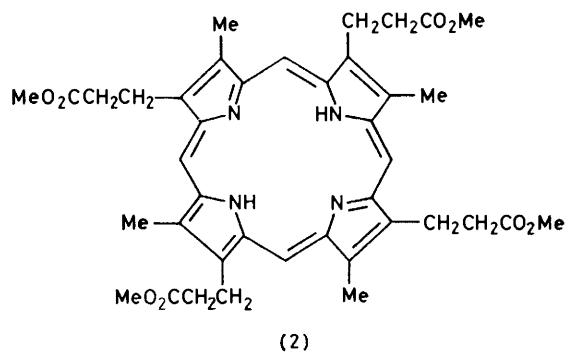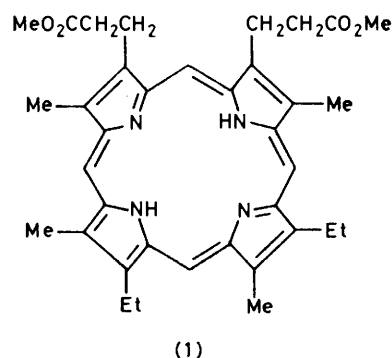
RESULTS

*Mesoporphyrin IX Bis(methyl ester).—Equilibrium studies.* The protonation of mesoporphyrin IX bis(methyl ester) was studied at 12.8 °C in 90% (v/v) $Me_2SO–H_2O$ at various hydrochloric acid concentrations. The ionic

strength was maintained constant at 0.08M by addition of potassium chloride. Studies were also made in 95% (v/v) $Me_2SO-H_2O$ containing hydrochloric acid and lithium chloride with a total ionic strength of 0.125M. Porphyrin concentrations were varied in the range $4 \times 10^{-6}$—$4 \times 10^{-5}$M. In 90% (v/v) $Me_2SO-H_2O$ containing 0.08M-HCl and in 95% (v/v) $Me_2SO-H_2O$ with 0.125M-HCl the visible spectra (350—650 nm) corresponded to the spectrum previously assigned to the diprotonated porphyrin.[3] In the absence of acid the spectrum of the free base was observed.[3,5] Spectra were recorded at intermediate acid concentrations and it was found that in both solvents quite good isosbestic points were observed at *ca.* 398 (Soret bands) and at *ca.* 530 and 600 nm. However at *ca.* 523 nm the optical density of a solution of mesoporphyrin IX bis-(methyl ester) in the presence of increasing concentrations of acid first increased and then subsequently decreased as the acid concentration was made higher. At this wavelength the spectra did not intersect at a single point. The variation in optical density with acid concentration in 95% (v/v) $Me_2SO-H_2O$ is shown in Figure 1 and similar results
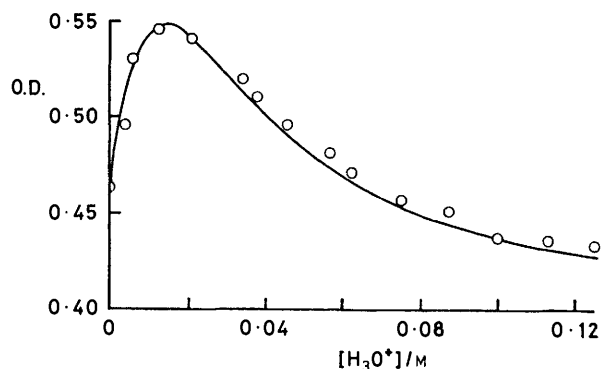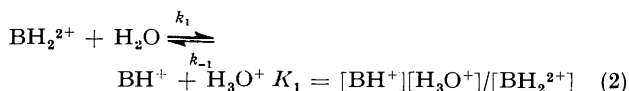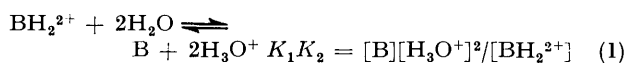


FIGURE 1 Optical density variation at 523 nm for a solution of mesoporphyrin IX bis(methyl ester) in 95% (v/v) $Me_2SO-H_2O$ in the presence of increasing concentrations of hydrochloric acid. The curve is a best fit plot of equation (4)

were obtained in 90% (v/v) $Me_2SO-H_2O$. Previous measurements[3] in mixtures of acetic or monobromo-propionic acid with acetone showed that the extinction coefficient of the monoprotonated porphyrin ($BH^+$) at 523 nm is higher than the extinction coefficients of the free base (B) and of the diprotonated species ($BH_2^{2+}$) at this wavelength. Hence the variation in optical density with acid concentration shown in Figure 1 is compatible with the presence of the monoprotonated porphyrin at intermediate acidities. The observation of quite good isosbestic points at other wavelengths is due to the fact that the spectrum of $BH^+$ at these wavelengths is similar to the spectrum of B or $BH_2^{2+}$ and the low concentration of $BH^+$ is then not detected. The results in Figure 1 were analysed to obtain values for the two dissociation constants of diprotonated mesoporphyrin IX bis(methyl ester) [$K_1$ and $K_2$ in equations (2) and (3) respectively]. The absorbance of a solution containing a mixture of B, $BH^+$, and $BH_2^{2+}$ is given by

$$BH_2^{2+} + 2H_2O \rightleftharpoons$$
$$B + 2H_3O^+ \quad K_1K_2 = [B][H_3O^+]^2/[BH_2^{2+}] \quad (1)$$

$$BH_2^{2+} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$$
$$BH^+ + H_3O^+ \quad K_1 = [BH^+][H_3O^+]/[BH_2^{2+}] \quad (2)$$

equation (4) where $\varepsilon_B$, $\varepsilon_{BH^+}$, and $\varepsilon_{BH_2^{2+}}$ are the extinction coefficients of B, $BH^+$, and $BH_2^{2+}$ respectively. The value

$$BH^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$$
$$B + H_3O^+ \quad K_2 = [B][H_3O^+]/[BH^+] \quad (3)$$
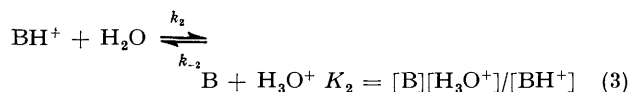
$$A = \frac{(\varepsilon_B K_2/[H_3O^+] + \varepsilon_{BH^+} + \varepsilon_{BH_2^{2+}}[H_3O^+]/K_1)}{(1 + K_2/[H_3O^+] + [H_3O^+]/K_1)} \times$$
$$\text{[Total porphyrin]} \quad (4)$$

of $\varepsilon_B$ ($3.0 \times 10^3$ l $mol^{-1}$ $cm^{-1}$) was determined in the absence of acid and $\varepsilon_{BH_2^{2+}}$ ($2.4 \times 10^3$ l $mol^{-1}$ $cm^{-1}$) was measured at high acid concentration. The values of $K_1$, $K_2$, and $\varepsilon_{BH^+}$ were treated as adjustable parameters in order to obtain a best fit of equation (4) to the experimental results. In Figure 1 which refers to measurements in 95% (v/v) $Me_2SO-H_2O$ the line is constructed with the values $K_1 = 0.007$ mol $l^{-1}$, $K_2 = 0.06$ mol $l^{-1}$, and $\varepsilon_{BH^+} = 7.8 \times 10^3$ l $mol^{-1}$ $cm^{-1}$. In 90% (v/v) $Me_2SO-H_2O$ the best fit to the experimental variation of optical density with acid concentration was obtained with $K_1 = 0.01$ mol $l^{-1}$, $K_2 = 0.03$ mol $l^{-1}$, and $\varepsilon_{BH^+} = 7.8 \times 10^3$ l $mol^{-1}$ $cm^{-1}$. In mixtures of acetic or monobromopropionic acid with acetone[3] the extinction coefficients at 523 nm are in the ratio $\varepsilon_B : \varepsilon_{BH^+} : \varepsilon_{BH_2^{2+}} = 4 : 7 : 3$ which is similar to the result obtained here. The best fit analysis shows that the first dissociation constant of diprotonated mesoporphyrin IX bis(methyl ester) ($K_1$) is smaller than the second dissociation constant ($K_2$) and hence the monoprotonated porphyrin is only present in low concentration. In 95% (v/v) $Me_2SO-H_2O$ the maximum concentration of $BH^+$ present is *ca.* 15% of the total porphyrin and in 90% (v/v) $Me_2SO-H_2O$, $BH^+$ reaches a maximum concentration of *ca.* 37% of the total porphyrin. In the previous experiments[1] with meso-tetraphenylporphyrin good isosbestic points were obtained on titration with acid. Hence, unless the spectrum of the monoprotonated form of mesotetraphenylporphyrin is almost identical with the spectrum of the free base or the diprotonated species at all wavelengths, this means that negligible concentrations of the monoprotonated porphyrin are present. For mesotetraphenylporphyrin, therefore, it was only possible to obtain an overall equilibrium constant between the free base and diprotonated porphyrin and this was calculated from optical density readings at $\lambda_{max}$ for these species. This procedure was also attempted for mesoporphyrin IX bis(methyl ester) by measuring the optical densities at 491 and 609 nm ($\lambda_{max}$ for B) and 405 and 544 nm ($\lambda_{max}$ for $BH_2^{2+}$) for solutions of the porphyrin in 90% (v/v) $Me_2SO-H_2O$ containing various concentrations of HCl. Concentrations of B and $BH_2^{2+}$ were calculated from the optical density readings by assuming that $BH^+$ was present in negligible concentrations. When the values of [B], [$BH_2^{2+}$], and [$H_3O^+$] were inserted into equation (1), the calculated value of $K_1K_2$ varied considerably (2—8 $\times$ $10^{-4}$ $mol^2$ $l^{-2}$) as expected if the monoprotonated porphyrin is present in significant concentrations.

*Kinetics of proton transfer.* The protonation of meso-porphyrin IX bis(methyl ester) [equations (2) and (3)] was studied kinetically at 12.8 °C using the temperature-jump method. Measurements were made at different concentrations of hydrochloric acid in 90% (v/v) $Me_2SO-H_2O$ containing sufficient potassium chloride to maintain a constant ionic strength of 0.08 mol $l^{-1}$. The reaction was also studied in 95% (v/v) $Me_2SO-H_2O$ at an ionic strength of 0.125M kept constant by addition of LiCl. Porphyrin

concentrations were $5 \times 10^{-6}$ or $1 \times 10^{-5}$M. Chemical relaxation to the new equilibrium position after a rapid temperature jump of 1.2° was observed spectrophotometrically at *ca.* 427 nm where the absorbance is due mainly to the diprotonated species. Chemical relaxation times were found to be independent of the total concentration of porphyrin. The variations in reciprocal relaxation time with acid concentration in 90 and 95% (v/v) $Me_2SO-H_2O$ are shown in Figure 2. In our previous studies with mesotetraphenylporphyrin [1] and other meso-tetra-arylporphyrins [6] it was found that the reciprocal relaxation time varied linearly with the square of the acid concentration. For mesoporphyrin IX bis(methyl ester) this is not observed. Although the relaxations observed for mesoporphyrin IX bis(methyl ester) are slightly more rapid than the relaxations measured for other porphyrins, the curvature in Figure 2 is not due to the failure of the temperature-jump instrument to measure these rapid reactions. Accurately linear plots of reciprocal relaxation time against
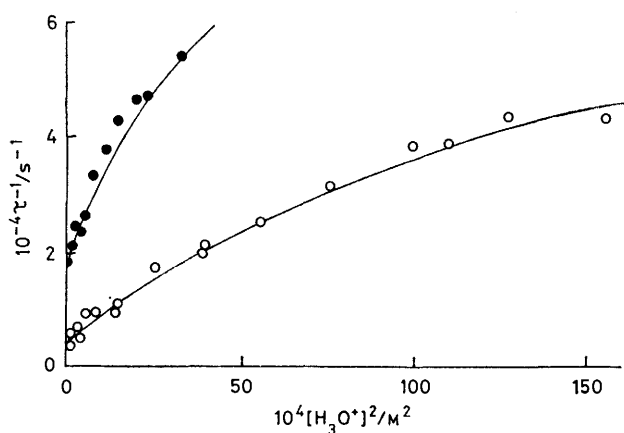
FIGURE 2  Reciprocal relaxation times for solutions of mesoporphyrin IX bis(methyl ester) in 90 (●) and 95% (○) $Me_2SO-H_2O$ containing hydrochloric acid. The curves are best fit plots of equation (5)

the square of the acid concentration were observed for mesotetraphenylporphyrin [1] and for mesotetrakis-($p$-sulphonatophenyl)porphyrin [7] up to reciprocal relaxation times of 2.5 and 3.0 × $10^4$ s$^{-1}$, respectively, which were the most rapid reactions followed because at higher acid concentrations the amplitudes of the relaxations for these porphyrins became very small. For mesoporphyrin IX bis(methyl ester) in 95% (v/v) $Me_2SO-H_2O$ there is strong curvature of the plot in Figure 2 at reciprocal relaxation times below 3.0 × $10^4$ s$^{-1}$. In order to check that the solution is heated by the temperature jump in a much shorter time than the time taken for chemical relaxation of the acid–base equilibrium of mesoporphyrin IX bis(methyl ester) to occur, the kinetics of ionisation of 4-chloro-2,6-dinitrophenol were studied. For this reaction, the chemical relaxation is extremely rapid and the equilibrium responds immediately to the change in temperature. The experiments are described in detail in a later section and it is concluded that the temperature rise following a discharge is 90% complete in 10 μs. The heating trace can be approximated by an exponential with a time constant of *ca.* 3 × $10^5$ s$^{-1}$ which is very much more rapid than the fastest reaction we have measured for mesoporphyrin IX bis(methyl ester). It is possible that the relaxations in 90% (v/v) $Me_2SO-H_2O$

are slightly coupled with the heating time, and the data in 95% (v/v) $Me_2SO-H_2O$ are therefore more reliable.

The dependence of reciprocal relaxation time on acid concentration for mesoporphyrin IX bis(methyl ester) can be explained in two ways. If it is assumed that the equilibrium in equation (2) is established slowly compared with the reaction in equation (3), the dependence of reciprocal relaxation time on acid concentration is given by equation (5). Alternatively if $BH^+$ is treated as a low

$$1/\tau = k_1[H_2O] + k_{-1}[H_3O^+]^2/(K_2 + [H_3O^+]) \quad (5)$$

concentration intermediate and the steady state approximation is applied to equations (2) and (3), equation (6) is obtained.

$$1/\tau = (k_1 k_2 [H_2O]^2 + k_{-1}k_{-2}[H_3O^+]^2)/(k_{-1}[H_3O^+] + k_2[H_2O]) \quad (6)$$

Equations (5) and (6) are of very similar form. Equation (5) can be rearranged to give (7) which is almost identical to equation (6) if, as in our case, the second term is small compared with the other terms in the numerator. It will be shown that both expressions (5) and (6) satisfactorily

$$1/\tau = (k_1 K_2 [H_2O] + k_1[H_2O][H_3O^+] + k_{-1}[H_3O^+]^2)/(K_2 + [H_3O^+]) \quad (7)$$

explain the dependence of reciprocal relaxation time on acid concentration. The curves in Figure 2 were constructed using equation (5) with best fit values for $k_1[H_2O]$, $k_{-1}$, $K_1 = k_1[H_2O]/k_{-1}$, and $K_2$. These are shown in the Table

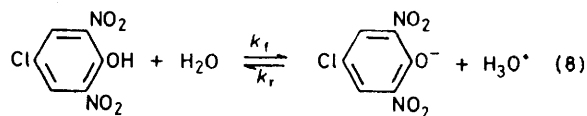Kinetic parameters for protonation of mesoporphyrin IX bis(methyl ester) [a]

| | $10^{-4}k_1$ [$H_2O$]/ s$^{-1}$ | $10^{-6}k_{-1}$/ l mol$^{-1}$ s$^{-1}$ | $K_1$/ mol l$^{-1}$ | $K_2$/ mol l$^{-1}$ |
|---|---|---|---|---|
| 90% (v/v) $Me_2SO-H_2O$ | 1.8 | 1.0 | 0.018 (0.01) [b] | 0.035 (0.03) [b] |
| 95% (v/v) $Me_2SO-H_2O$ | 0.45 | 0.5 | 0.009 (0.007) [b] | 0.058 (0.06) [b] |

[a] Rate coefficients and equilibrium constants were obtained from a best fit of equation (5) to the kinetic results. [b] The values in parentheses were obtained by spectrophotometric acid titration.

together with the values of $K_1$ and $K_2$ obtained from the spectrophotometric acid titration. The agreement between the two sets of results for $K_1$ and $K_2$ is within experimental uncertainty. Equation (6) can also be used to obtain a fit to the experimental variation of relaxation time with acid concentration and the following results are obtained: in 90% (v/v) $Me_2SO-H_2O$, $k_1[H_2O] = 1.8 \times 10^4$ s$^{-1}$, $k_{-2} = 1.0 \times 10^6$ l mol$^{-1}$ s$^{-1}$, and $K_1 K_2 = 6.0 \times 10^{-4}$ mol$^2$ l$^{-2}$; and in 95% (v/v) $Me_2SO-H_2O$, $k_1[H_2O] = 4.5 \times 10^3$ s$^{-1}$, $k_{-2} = 5.0 \times 10^5$ l mol$^{-1}$ s$^{-1}$, and $K_1 K_2 = 5.0 \times 10^{-4}$ mol$^2$ l$^{-2}$. From the individual values of $K_1$ and $K_2$ determined spectrophotometrically, values for $k_{-1}$ and $k_2$ can then be calculated. This means that values for all the rate coefficients in equations (2) and (3) are obtained by fitting equation (6) to the kinetic results whereas use of equation (5) gives results for $k_1$ and $k_{-1}$ only. The same values are obtained for $k_1$ and $k_{-1}$ whichever equation is used and these results are therefore considered to be reliable and are given in the Table. However, since there is no reason to choose equation (6) in preference to equation (5) the results obtained for $k_2$ and $k_{-2}$ by application of equation (6) may not be reliable and these are not discussed further.

*Coproporphyrin I Tetrakis(methyl ester).*—A much less detailed study was made for this porphyrin but the results which were obtained were quite similar to those observed for mesoporphyrin IX bis(methyl ester). On titration with acid in 90 or 95% (v/v) $Me_2SO-H_2O$ the spectrum of solutions of coproporphyrin I tetrakis(methyl ester) changed from the spectrum of the free base to the spectrum of the diprotonated species. Isosbestic points were observed at *ca.* 536 and 606 nm but on the edge of the Soret region at *ca.* 450 nm the spectra did not cross at a single isosbestic point. This probably means that a low concentration of the monoprotonated species is present. Chemical relaxations for solutions of coproporphyrin 1 tetrakis(methyl ester) ($7.5 \times 10^{-6}$M) in 95% (v/v) $Me_2SO-H_2O$ containing hydrochloric acid (0.005—0.125M) were observed at 430 nm and the relaxation times were in the same range as those observed for mesoporphyrin IX bis(methyl ester). The reciprocal relaxation times did not increase linearly with the square of the acid concentration but could be fitted using equation (5) with the following values: $k_1[H_2O] = 4.5 \times 10^3 s^{-1}$, $k_{-1} = 3.3 \times 10^5 l\,mol^{-1}\,s^{-1}$, $K_1 = 0.014$ mol $l^{-1}$, and $K_2 = 0.094$ mol $l^{-1}$.

*Proton Transfer for 4-Chloro-2,6-dinitrophenol.*—Very few proton transfers have been studied in $Me_2SO-H_2O$ mixtures [8,9] and it was therefore necessary to study a simple reaction involving protonation at nitrogen or oxygen for comparison with the results for mesoporphyrin IX bis(methyl ester). We attempted to find a reaction which behaves as a normal proton transfer in aqueous solution [10] and which could be studied in $Me_2SO-H_2O$ mixtures. A suitable reaction involving an amine could not be found; however the ionisation of 4-chloro-2,6-dinitrophenol [equation (8)] could be studied. Equilibrium constants for reaction (8) were determined spectrophotometrically in 98, 95, and 90% (v/v) $Me_2SO-H_2O$ at 12.8 °C and an ionic strength of 0.125M maintained by addition of lithium chloride. The total concentration of 4-chloro-2,6-dinitrophenol was $1.0 \times 10^{-4}$M and observations of the degree of dissociation at hydronium ion concentrations in the range 0.000 5—0.01M were made at 476 nm where the anion absorbs strongly. The equilibrium constant for reaction (8) had values of $2.6 \pm 0.1 \times 10^{-3}$, $2.7 \pm 0.1 \times 10^{-3}$, and $3.2 \pm 0.1 \times 10^{-3}$ mol $l^{-1}$ in 98, 95, and 90% (v/v) $Me_2SO-H_2O$, respectively.

$$Cl \underset{NO_2}{\overset{NO_2}{\diagdown}} OH + H_2O \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Cl \underset{NO_2}{\overset{NO_2}{\diagdown}} O^- + H_3O^+ \quad (8)$$

The kinetics of the dissociation of 4-chloro-2,6-dinitrophenol were studied under the same conditions using the temperature-jump method. Following a temperature jump of 2.1° from an initial temperature of 10.7 °C the change in optical density at 476 nm was observed for solutions of 4-chloro-2,6-dinitrophenol in 98 and 95% (v/v) $Me_2SO-H_2O$. In 90% (v/v) $Me_2SO-H_2O$ the change in optical density was small and no detailed measurements were made. In 98 and 95% (v/v) $Me_2SO-H_2O$ the change in optical density occurred simultaneously with the temperature jump. The usual method of identifying an observed change in optical density after a temperature jump with the perturbation of a particular chemical equilibrium is to show that the relaxation time varies with the concentration of reactants in the way expected for that equilibrium. In the present

case this is not possible because the relaxation occurs within the heating time and relaxation times cannot be determined. Hence in order to show that the observed rapid change in optical density after a temperature jump corresponded to a displacement of equilibrium (8), the variation of the total change in optical density (relaxation amplitude) with the reactant concentration was investigated. For equilibrium (8) under our conditions the amplitude of the relaxation is given by equation (9) in which [ROH] is the total concentration of dissociated and undissociated forms of 4-chloro-2,6-dinitrophenol, $\Delta H$ and $K$ are the enthalpy and equilibrium constant for the reaction, $T$ is the reaction temperature, and $\Delta T$ is the size of the temperature jump. Equation (9) predicts that the relaxation amplitude

Relaxation amplitude =
$$\frac{K[ROH]\Delta H\Delta T}{RT^2} \frac{[H_3O^+]}{(1 + K[H_3O^+])^2} \quad (9)$$

will go through a maximum as the hydronium ion concentration is increased. The experimental variation in 98% (v/v) $Me_2SO-H_2O$ is shown by the points in Figure 3. The
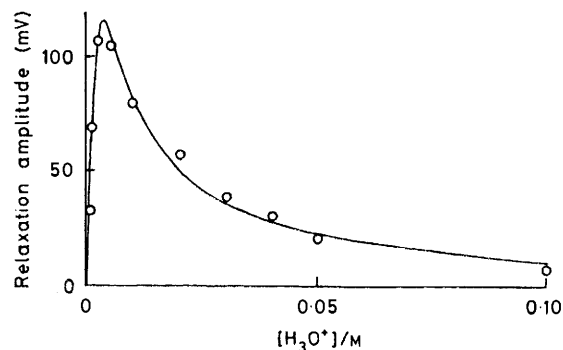


FIGURE 3 Variation of the amplitude of relaxation for the dissociation of 4-chloro-2,6-dinitrophenol in 98% (v/v) $Me_2SO-H_2O$. The curve is a plot of equation (10). An amplitude of 10 mV is equivalent to an optical density change of 0.001

curve is a plot of equation (10) with $K = 2.6 \times 10^{-3}$ l mol$^{-1}$ which is the same value of $K$ as was determined by spectro-

Relaxation amplitude =
$$1.8 \times 10^5[H_3O^+]/(1 + K[H_3O^+])^2 \quad (10)$$

photometric equilibrium measurements. Since the experimental results are well reproduced by equation (10) it is established that the observed changes in optical density after a temperature jump correspond to displacement of equilibrium (8).

Chemical relaxation of equilibrium (8) occurs at least as rapidly as the temperature jump and from this conclusion it is possible to estimate lower limits for the rate coefficients in equation (8). The trace of optical density against time following a perturbation of equilibrium (8) corresponds to a heating curve which can be roughly approximated by an exponential with a time constant of *ca.* $3 \times 10^5 s^{-1}$. Similar time constants are obtained over a range of acid concentrations, as expected if the chemical relaxation occurs as rapidly as the temperature rise. Hence chemical relaxation of equilibrium (8) occurs with a reciprocal relaxation time $\tau^{-1} > 3 \times 10^5 s^{-1}$ at acid concentrations of *ca.* $1 \times 10^{-3}$ mol $l^{-1}$. The reciprocal relaxation time under our conditions is given by equation (11), in which $K$ is the equilibrium

constant of the reaction. Using this equation, the result $k_r > ca.$ 1 × 10$^8$ 1 mol$^{-1}$ s$^{-1}$ is obtained for protonation of 4-chloro-2,6-dinitrophenolate ion by hydronium ion in 98 and 95% (v/v) Me$_2$SO–H$_2$O.

$$1/\tau = k_f[H_2O] + k_r[H_3O^+] = k_r(K + [H_3O^+]) \quad (11)$$

## DISCUSSION

The equilibrium results obtained for mesoporphyrin IX bis(methyl ester) show that this porphyrin behaves differently from mesotetraphenylporphyrin. For meso-tetraphenylporphyrin the monoprotonated species was not detectable at any acidity which means that the first dissociation constant of the diprotonated porphyrin is very much smaller than the second. For mesoporphyrin IX bis(methyl ester) the monoprotonated species can be detected and although the first dissociation constant is smaller than the second the difference is not as large as for mesotetraphenylporphyrin. For both porphyrins the monoprotonated species will always be present in low concentration and this is unusual behaviour for a dibasic acid but commonly observed for porphyrins.[2] The equilibrium behaviour of mesoporphyrin IX bis(methyl ester) depends upon the solvent and in mixtures of acetone with acetic or monobromopropionic acid the monoprotonated form can be made the predominant species by choosing a mixture with the appropriate composition.[3] The values of the dissociation constants given in the Table show that the maximum concentration of the monoprotonated species present will be higher in 90 than in 95% (v/v) Me$_2$SO–H$_2$O.

The behaviour of mesoporphyrin IX bis(methyl ester) has permitted a more detailed analysis than was possible for mesotetraphenylporphyrin. For both porphyrins proton transfer occurs slowly. This means that the slow proton-transfer behaviour observed for mesotetra-phenylporphyrin cannot be attributed to the presence of the aromatic substituents in the porphyrin ring and appears to be a general feature of the behaviour of porphyrins under our conditions. The rate coefficients for ionisation of the diprotonated forms of meso-porphyrin IX bis(methyl ester) and coproporphyrin I tetrakis(methyl ester) in 95% (v/v) Me$_2$SO–H$_2$O are ca. 20-fold larger than the value found for mesotetraphenyl-porphyrin.[1] This is compatible with the observation that proton exchange for coproporphyrin esters in chloroform in the presence of trifluoroacetic acid is rapid on the n.m.r. time scale whereas slow exchange is observed for mesotetraphenylporphyrin.[11]

One explanation for the slow ionisation of diproton-ated mesotetraphenylporphyrin to the monoprotonated porphyrin is that the ionisation is very strongly thermo-dynamically unfavourable and the forward reaction would then occur slowly even if the reverse protonation of the monoprotonated porphyrin was diffusion controlled ($k$ ca. 1 × 10$^{10}$ 1 mol$^{-1}$ s$^{-1}$). However we can now eliminate this as a possible explanation since for meso-porphyrin IX bis(methyl ester) we have determined that the rate coefficient for the reverse thermodynamically favourable protonation of BH$^+$ is well below the diffusion

limit with $k_{-1}$ 5.0 × 10$^5$ 1 mol$^{-1}$ s$^{-1}$ in 95% (v/v) Me$_2$SO–H$_2$O. It is necessary to explain why this protonation occurs more slowly than expected [10] for thermodynamic-ally favourable protonation at nitrogen. One possibility may be that proton transfers in Me$_2$SO–H$_2$O mixtures do not reach the diffusion limit even when in aqueous solution the same reactions are diffusion controlled. In 70% (v/v) Me$_2$SO–H$_2$O it has been shown [8] that proton-ation of an amine by hydronium ion is diffusion con-trolled with a rate coefficient 2.9 × 10$^{10}$ 1 mol$^{-1}$ s$^{-1}$. However, in 98.5—100% (v/v) Me$_2$SO–H$_2$O containing trifluoromethanesulphonic acid, proton exchange of benzylamines is slow and can be observed by n.m.r.[9] Our experiments with 4-chloro-2,6-dinitrophenol show that in 98 and 95% (v/v) Me$_2$SO–H$_2$O, protonation at oxygen occurs with a rate coefficient which is close to the diffusion limit and the same is also probably true for protonation of normal amines [10] under our conditions. It therefore seems likely that the slow proton-transfer behaviour of porphyrins relates to some particular property of the porphyrin ring. We have previously mentioned two possibilities : attack by hydronium ion may be hindered by an intramolecular hydrogen bond [1] in the monoprotonated porphyrin or alternatively the high activation energy could result from changes in resonance stabilisation [1] or buckling [12] of the porphyrin ring which occur on protonation.

## EXPERIMENTAL

Porphyrins were commercial samples. The solvent mixtures were made up by volume from Me$_2$SO (AnalaR) and doubly distilled water. Potassium and lithium chlorides were dried before use.

Spectra were run on a Perkin-Elmer 402 or Unicam SP 8000 spectrophotometer. Measurements at fixed wave-length were made with a Unicam SP 500 instrument.

The temperature-jump measurements have been described previously.[1] In the present experiments it was necessary to determine the heating time of solutions after a tem-perature jump more accurately than previously and this was carried out using solutions of 4-chloro-2,6-dinitrophenol in 95% (v/v) Me$_2$SO–H$_2$O containing hydrochloric acid (1 × 10$^{-3}$M) and lithium chloride (0.124M). With a 20 kV discharge from a 0.01 μF capacitor, 90% of the temperature rise (2.1°) was completed within 10 μs.

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