The σ contribution depends principally upon the polarity of the bonds around phosphorus, but also upon bond angles. This second parameter plays an increasingly important role as the electronegativity of A in PA_3 increases over that of phosphorus. For example Mark and Van Wazer[16] showed that the difference of chemical shift of 45 ppm between $P(OC_2H_5)_3$ and $P(OCH_2)_3CCH_3$ could be accounted for by a decrease in the latter of 1° between the O–P–O angles. The π contribution is simply proportional to the number of electrons in the d phosphorus orbitals.[15]

Considering the great similarity of the behavior of ligands as different from one another from the point of view of steric crowding as PF_3, $P(CH_3)_3$, $P(SCH_3)_3$, and $P(OCH_2)_3CC_2H_5$, we conclude that variation in bond angle is secondary in the cases described. On the other hand steric crowding could explain the shift toward higher fields observed in the case of the disubstituted derivatives $Ni(CO)_2(PA_3)_2$ with $PA_3 = P(C_2H_5)_3$ and $P(C_4H_9)_3$ studied by Meriwether and Leto.[4] The same concept could explain the particular shifts observed in the $Mo(CO)_{6-n}[P(C_2H_5)_3]_n$ series. A trend similar to that of trimethylphosphine derivatives is observed for the change from monosubstituted to *trans*-disubstituted cases, in which steric crowding phenomenon cannot occur. On the other hand, a chemical shift toward the higher fields is observed for the change from monosubstituted to *cis*-disubstituted and *fac*-trisubstituted compounds, when steric crowding can occur.

Finally we can assume that steric effect is a minor factor in the cases where the typical behavior defined above is observed and we can try to rationalize this behavior in terms of charge-transfer variations.

The chemical shift variation toward the lower fields is compatible either with a decrease of the σ ($P \rightarrow M$) charge transfer when the degree of substitution increases or with an increase of the π ($M \rightarrow P$) charge transfer, or else with a superposition of these two effects. Similarly, we can explain the difference between the values observed for the phosphorus atoms *trans* to a carbonyl group or to another phosphorus atom; indeed the carbonyl group is a better π-bonding ligand than phosphorus and this leads to a greater σ ($P \rightarrow M$) charge transfer or a smaller π ($M \rightarrow P$) charge transfer when the carbonyl group is in *trans* position relative to the phosphorus atom. On the ground of the theory of Letcher and Van Wazer it is obviously not possible to distinguish by what mechanism this charge transfer takes place since the two contributions σ and π occur in a similar way in the formulation of the chemical shift of ^{31}P.

In conclusion, we have considered that a study of structural signification of the chemical shift of the ^{31}P nucleus on an experimental ground, in the $M(CO)_{m-n}(PA_3)_n$ complexes, had necessarily to be performed in two stages. In the first stage it was a question of obtaining an overall description of the phenomenon by considering a sufficient number of derivatives and in the second stage of explaining the average behavior observed. The present work has been presented essentially as a contribution to the first stage.

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CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF WATERLOO, WATERLOO, ONTARIO, CANADA

Kinetics of Metalloporphyrin Formation in Glacial Acetic Acid

BY D. JAMES KINGHAM[1] AND DOREEN A. BRISBIN

Received December 23, 1969

Rate constants have been measured for the reaction $M + P \rightarrow MP$, where M is one of Fe(II), Co(II), Ni(II), or Cu(II) and P is the neutral hematoporphyrin IX molecule. The relative rates of reaction were found to be in the order Cu > Co > > Fe > > Ni, and the relative thermodynamic stabilities of the complexes are predicted to be in the order Ni > Cu ~ Co > Fe according to the ratio of intensities of the main bands in the visible spectrum. The activation parameters for metalloporphyrin formation are reported. The biological implications of the relative rates of formation and stabilities of the complexes are discussed.

Introduction

Hematoporphyrin IX is a macrocyclic "tetrapyrrole" structure with four methane, two propionic acid, and two ethanolic side chains as shown in Figure 1. A metal ion interacts with this tetradentate ligand by displacing the two central hydrogen atoms. The metal ion then occupies a position equidistant from the four nitrogen atoms[2] although in some cases it may lie to one side of the plane defined by them.[3-5]

Although little quantitative information[6] is available

[1] Department of Energy, Mines and Resources, Inland Waters Branch, Ottawa, Ontario, Canada.

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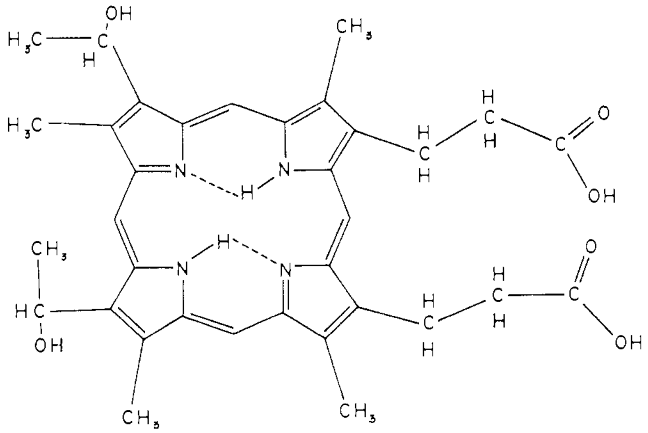

Figure 1.—Diagram of hematoporphyrin molecule.

on the rates of incorporation of different metal ions under comparable conditions, there is a great need for such information because of the biological importance of the complexes (the iron–hematoporphyrin complex is the "active" component of hemoglobin and vitamin B_{12} is a cobalt–porphyrin complex). The objective of this study was to determine the relative reaction rates under comparable conditions—a continuation of work completed on manganese.[7]

Because of the low solubility of hematoporphyrin IX in aqueous solutions kinetic studies by spectroscopic techniques could not be performed in aqueous solvents. While the solubility in water is enhanced by the addition of detergents, such detergents are thought to increase the nucleophilic nature of the porphyrin ring and thus enhance metalloporphyrin formation.[8] The rates of reaction of the α-, β-, γ-, and δ-substituted porphyrins of Fleischer, *et al.*,[9,10] and Dorough, *et al.*,[11] are also affected by the nature of the substituents. For these reasons, the relatively "pure" system of the metal acetates and neutral hematoporphyrin IX in glacial acetic acid was chosen for the present study.

Experimental Section

Materials.—Recrystallized hematoporphyrin free base (98% Spectro-Assay) was obtained from Mann Research Laboratories. The metal acetates other than iron were "Analar" reagents obtained from British Drug Houses Ltd., analyzed for metal content where necessary, and dissolved directly in glacial acetic acid. $Fe^{II}(OAc)_2$ solutions were prepared by dissolving finely divided, hydrogen-reduced iron powder directly in glacial acetic acid through which a stream of nitrogen gas was passed. Glacial acetic acid as analyzed using the Karl Fischer reagent contained less than 0.04% water.

Kinetic Techniques.—Solutions of metal acetates and hematoporphyrin were equilibrated in a thermostated bath and then mixed and transferred to the cell compartment of the spectrophotometer. Both the equilibration bath and cell compartment were maintained at the same constant temperature, ±0.01°, with accuracy estimated to be ±0.1°.

In the case of the Fe(II) experiments deoxygenated porphyrin solution was first placed in the absorption cell and nitrogen was bubbled through it. The $Fe^{II}(OAc)_2$ solution was then transferred to the cell by means of a thermostated pipet and the solutions were thoroughly mixed by the bubbling nitrogen. The nitrogen jet was then withdrawn to a position above the solution. The appearance of the metalloporphyrin was followed spectrographically using a Beckman DB-G recording spectrophotometer.

Results and Discussion

The spectra of neutral hematoporphyrin IX, cobalt hematoporphyrin IX, and iron hematoporphyrin IX are shown in Figure 2. The spectra of the Cu and Ni complexes are similar to that of the Co complex but the position and intensity of the α (longer wavelength) and β (shorter wavelength) bands in the visible region were shifted as indicated in Table I.

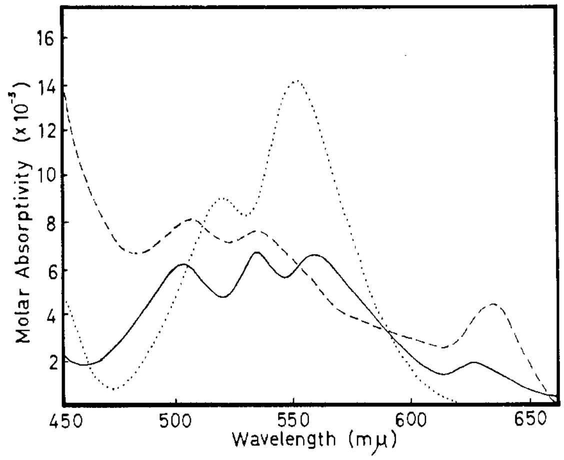

Figure 2.—Spectra of neutral hematoporphyrin (solid line), iron hematoporphyrin (dashed line), and cobalt hematoporphyrin (dotted line) in glacial acetic acid.

TABLE I

CHARACTERISTICS OF METALLOPORPHYRIN SPECTRA IN GLACIAL ACETIC ACID[a]

	Metalloporphyrin	Band			
		α	β	Other	α/β
λ_{max}, mμ	Mn	572	546	462	
ϵ $(\times 10^{-3})$		6.0	10.2	42.5	0.59
λ_{max}, mμ	Fe	530	501	628	
ϵ $(\times 10^{-3})$		6.68	6.85	3.44	0.98
λ_{max}, mμ	Co	550	520		
ϵ $(\times 10^{-3})$		14	9.0		1.56
λ_{max}, mμ	Ni	552	517		
ϵ $(\times 10^{-3})$		16.4	7.32		2.24
λ_{max}, mμ	Cu	562	526		
ϵ $(\times 10^{-3})$		15.7	9.6		1.64

[a] Molar absorptivities calculated on the basis of porphyrin monomer swamped with metal ion.

The reaction studied is essentially that described by Dempsey, *et al.*,[8] and Lowe and Phillips[12] which may be written: $M^{2+} + PH_2 \rightarrow MP + 2H^+$. With metal ion in large excess, plots of $-\log (A_\infty - A_t)$ *vs.* time gave good straight lines for at least 2 half-lives. A_∞ is the optical density of the solution at one of the metalloporphyrin maxima when $t \rightarrow \infty$ and A_t is the optical density at time t. $(A_\infty - A_t)$ may be shown to be directly proportional to the amount of unreacted por-

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[10] E. B. Fleischer and L. E. Webb, *ibid.*, **2**, 94 (1963).

[11] G. D. Dorough, J. R. Miller, and Frank M. Huennekens, *J. Amer. Chem. Soc.*, **73**, 4315 (1951).

[12] M. B. Lowe and J. N. Phillips, *Nature (London)*, **190**, 262 (1961).

phyrin at time *t*. The values of the pseudo-first-order rate constants were obtained from the slopes of the lines as indicated by Brisbin and Balahura[7] and they varied linearly with the concentration of metal ion for all metals but nickel. Figure 3 shows this linear relationship for Fe(II) and hematoporphyrin. The slopes of the lines are proportional to the metal ion concentrations and the multiplication of these slopes by 2.303/[Fe^{2+}] gives the second-order rate constant. The overall rate expression was thus found to be d[MP]/d*t* = $k[M^{2+}][PH_2]$ except for nickel for which it appears to be d[MP]/d*t* = $k[Ni^{2+}]^{1/2}[PH_2]$. The rate constants obtained are listed in Table II.

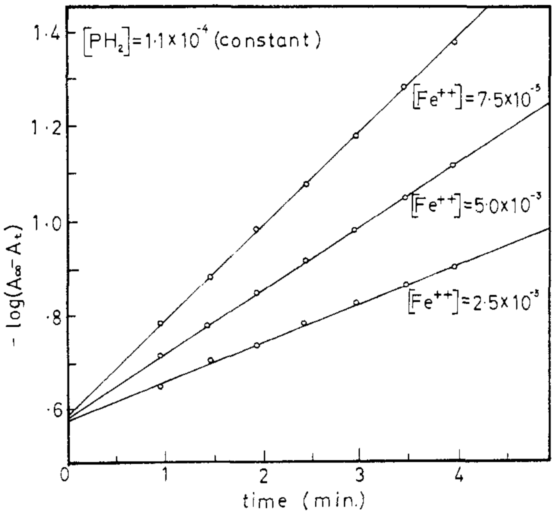

Figure 3.—Relationship between the slope *m* of the $-\log(A_\infty - A_t)$ *vs.* time plots and metal ion concentration. Data from Fe(II) at 45°.

TABLE II

RATES OF METALLOPORPHYRIN FORMATION, k_1 (M^{-1} SEC^{-1})

Temp, °C	Fe	Co	Ni[a]	Cu
16.8				6.63
20.0		4.57		
20.4				8.63
24.3				10.92
25.0		6.92		
28.1				15.52
30.0	0.367	10.37		
35.0	0.533	15.35		
39.5		20.75		
40.0	0.767			
45.0	1.05			
50.0	1.54		0.016	
55.0			0.022	
60.0			0.031	

[a] Units for Ni are $M^{-1/2}$ sec^{-1}.

The reaction could not be followed with porphyrin concentration in large excess over metal ion concentration because of the high extinction coefficients of porphyrin solutions throughout the visible spectrum. Low concentrations of both reactants could not be used because of the very slow rate of formation of the metalloporphyrin complex under such conditions.

It should be noted that the rate constants using this technique are independent of whether the ligand is dimerized or not. If the ligand proves to be a dimer in glacial acetic acid as has been suggested for hemin in aqueous solutions,[13,14] for example, then the quantity $(A_\infty - A_t)$ is still directly proportional to the amount of complex yet to be formed at time *t*. Where possible, job plots and mole ratio plots were done to determine the stoichiometry of the complex and indicated a metal to porphyrin ratio of 1:2. It must be remembered, however, that the concentration conditions for stoichiometric studies were in this case much different from those conditions under which rate constants could be measured. Because of the slow rate of nickel hematoporphyrin formation it was not feasible to determine the stoichiometry of the nickel–porphyrin complex in glacial acetic acid. Such determinations, should they become possible in the future, may help to determine the reason for the half-order dependence of the rate constant with nickel.

With Fe, Co, and Cu, pseudo-first-order rate constants varied linearly with metal concentration indicating that the reaction was first order with respect to metal ion. With Ni, however, such plots were nonlinear and did not pass through the origin. The pseudo-first-order rate constants varied linearly with $[Ni^{2+}]^{1/2}$ instead, indicating a half-order dependence on nickel. This relationship is indicated in Figure 4, in which the rate "constant" obtained from $2.303m/[Ni^{2+}]$ is not constant at all, whereas $2.303m/[Ni^{2+}]^{1/2}$ is. Since the reactions were analyzed by "pseudo-first-order" techniques with metal ion in large excess, a half-order dependence on nickel ion concentration could be the result of ion pairing in glacial acetic acid. Such ion pairing is not uncommon in this solvent. The rate of reaction would then be proportional to the square root

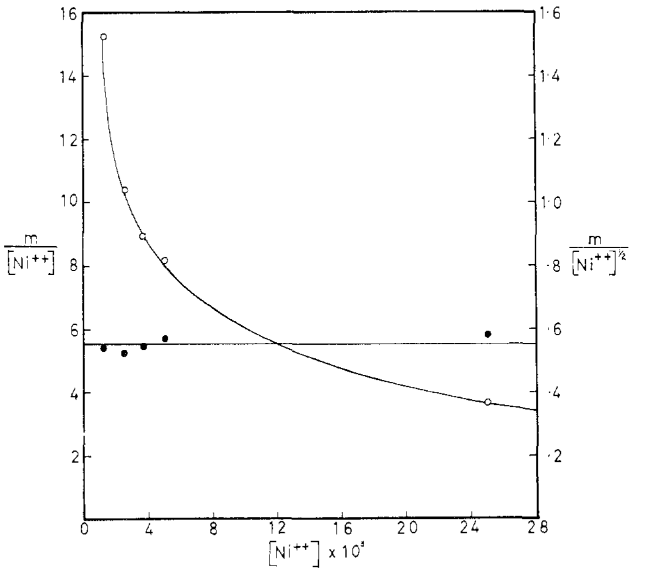

Figure 4.—Order of reaction in nickel ion. Whereas $m/[Ni^{2+}]^{1/2}$ is constant (solid circles), $m/[Ni^{2+}]$ is not (open circles).

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(13) D. G. Davis and R. F. Martin, *J. Amer. Chem. Soc.*, **88**, 1365 (1966).
(14) A. C. Maehly and A. Akeson, *Acta Chem. Scand.*, **12**, 1254 (1958).

of nickel acetate added if $Ni(OAc)_2$ is the predominant species and $NiOAc^+$ is the species that interacts with the porphyrin moiety so that $[NiOAc^+] = K^{1/2}[Ni(OAc)_2]^{1/2}$. K is the dissociation constant for the reaction $Ni(OAc)_2 \rightleftharpoons NiOAc^+ + OAc^-$. It is assumed here that $[NiOAc^+] \simeq [OAc^-]$ since the contribution of OAc^- from the self-ionization of glacial acetic acid, which is exceedingly small, is quite negligible. The unique behavior of nickel would thus simply reflect a significantly lower dissociation constant than for the other metal acetates. Such a conclusion would be in keeping with the higher crystal field stabilization energy of nickel complexes.

Activation energies and entropies for Fe(II), Co(II), Ni(II), and Cu(II) were found to be 15.2, 14.6, 14.3, and 12.8 kcal/mol and −12, −7, −20, and −12 cal deg^{-1} mol^{-1}, respectively.

There is a considerable variation (0.0082 $M^{-1/2}$ sec^{-1} to 37 M^{-1} sec^{-1} for Ni and Cu, respectively, extrapolated to 40°) in the rate constants. Crystal field stabilization energies have been used to explain the rates of reaction of coordination compounds both qualitatively by Orgel[15] and quantitatively by Basolo and Pearson.[16] In order to see if a correlation exists with these metal ions in glacial acetic acid, the spectra of concentrated solutions of the metal acetates were compared with those of the hexaaquometal complexes. In each case the spectra were quite similar, indicating a six-coordinate metal reactant. If the reaction then proceeds *via* an S_N2 mechanism as suggested by the substitution studies of Phillips,[2] then the metal ion changes from a six-coordinate reactant to a seven-coordinate transition state during the formation of the complex. The changes in crystal field stabilization energy related to such a change in metal ion coordination number are compared to the log of the forward rate constants in Figure 5 and suggest at least one reason for the rate sequence observed.

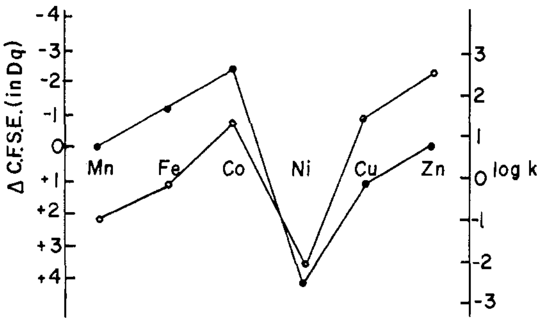

Figure 5.—Comparison of changes in crystal field stabilization energies (solid circles) with the log of the forward rate constant (open circles) for metalloporphyrin formation at a given temperature (extrapolated to 40°). The value for Zn is from unpublished results and that for Mn is from Brisbin and Balahura.[7]

It is interesting to note that the rate of metalloporphyrin formation is much faster for cobalt than iron (30 times as fast at 35°). The cobalt–hematoporphyrin complex is probably also the more stable of the two since the ratio of the intensities of the α to β bands is 1.56 for the cobalt complex and only 0.98 for that of iron. According to Falk,[6] who investigated metalloporphyrin stabilities by a series of displacement studies, these ratios are directly proportional to the stabilities of the complexes. Since cobalt reacts with hematoporphyrin more quickly than iron to form a more stable complex than iron under these conditions, then it might also do so under other comparable conditions. In view of the greater importance of iron–porphyrin complexes in biological systems, the effect of cobalt and other metal ions must be more fully considered and investigated. The biological implications of these results could be serious in areas where the diet is low in iron and possibly artificially high in cobalt.

Acknowledgments.—We are pleased to acknowledge the support of this work by the National Research Council of Canada.

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