[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WASHINGTON UNIVERSITY]

Pyridinate Complexes of Some Metallo-derivatives of Tetraphenylporphine and Tetraphenylchlorin[1]

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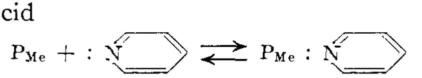
By the use of spectrophotometric techniques, it is shown that the zinc, cadmium, mercury and copper derivatives of tetraphenylporphine and the zinc derivative of tetraphenylchlorin form monopyridinate complexes. The magnesium derivatives of tetraphenylporphine and tetraphenylchlorin are best interpreted as forming dipyridinate complexes. The equilibrium constants, changes in heat content, and standard entropy changes for these reactions are determined. The results illustrate the primary dependence of metalloporphyrin complexing with base substances upon the nature of the central metal substituent.

The ability of iron metalloporphins[2] to form complexes with a variety of nitrogen bases is very well known.[3] Numerous other metallo-derivatives of the porphyrin ring systems (see Fig. 1) are also able to complex with basic substances, such as pyridine, but the resulting complexes are observed to vary widely in stability. The free base porphyrins, in which a metal is not present, do not complex with simple nitrogen bases. Complex formation between metalloporphyrins and base substances is thought to be an acid–base reaction in which the metalloporphyrin plays the role of a Lewis acid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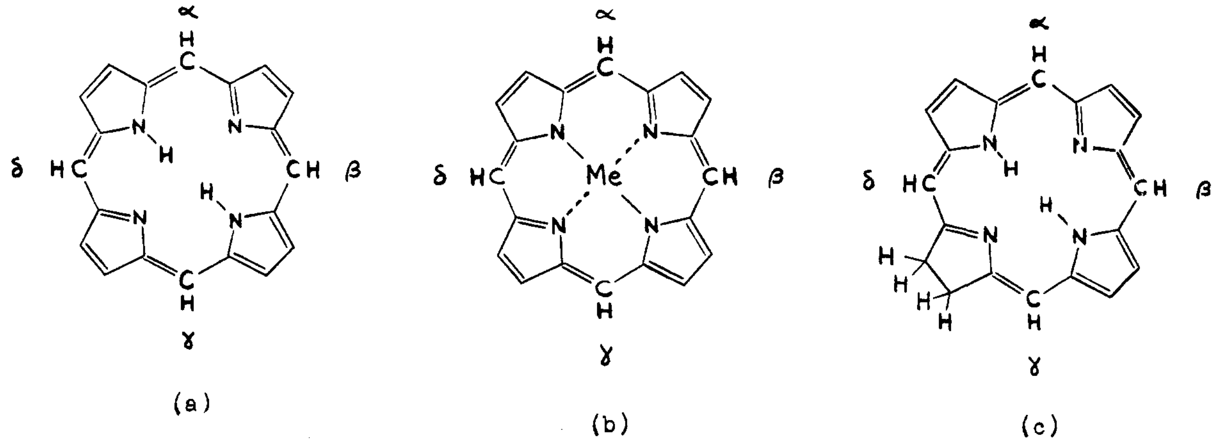

Fig. 1.—Porphyrin structures: (a) porphine free base; (b) a metalloporphine (divalent metal); (c) chlorin free base.

The orbital employed by the electron pair provided by the base is assumed to be a metal orbital in order to account both for the necessity of having a metal present, and for the variations in the extent of the reaction when the central metal is varied.

This type of complexing is of considerable biological importance, for most porphyrin systems play significant biochemical roles only as magnesium and iron metallo-derivatives, both of which readily enter into such reactions. Thus part of the bonding of the protein globin to heme is believed to be of this type, and the protein–chlorophyll extracts reported by Anson[4] and Smith[4] probably contain such bonding. Certainly the interaction of oxygen and ferroheme is a reaction of this type.

This article deals with a spectrophotometric study of the reaction with pyridine of the magnesium, zinc, cadmium, mercury and copper derivatives of $\alpha,\beta,\gamma,\delta$-tetraphenylporphine, and the magnesium and zinc derivatives of $\alpha,\beta,\gamma,\delta$-tetraphenylchlorin. The spectrographic data permit in most cases the calculation of the number of pyridine molecules which complex with a given metalloporphyrin, as well as the equilibrium constants, changes in heat content, and standard entropy changes associated with pyridinate formation. The use of corresponding porphines and chlorins makes possible a comparison of these two porphyrin systems with respect to their relative effect on complex formation.

Qualitatively, it is easily demonstrated in numerous cases that complex formation of the type under consideration is a reversible reaction.[5] For example, if successively small amounts of pyridine are added to a 10^{-5} *M* benzene solution of zinc tetraphenylporphine, there occurs with each addition a change in the absorption spectrum of the zinc porphine until the pyridine concentration reaches about 0.05 *M*. Increasing the pyridine concentration above 0.05 *M* results in no appreciable change in the absorption spectrum, but decreasing the pyridine concentration by extraction

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[1] Fifth in a series on "Fundamental Properties of Porphyrin Systems." Previous articles: THIS JOURNAL, **72**, 3939, 4045 (1950); **73**, 4315 (1951); **74**, 3974 (1952).

[2] For the system of nomenclature employed, see *ibid.*, **73**, 4315 (1951), footnote 2.

[3] For a review of much of the work which has been done in this field, see Lemberg and Legge, "Hematin Compounds and Bile Pigments," Chapt. V, Interscience Publishers, Inc., New York, N. Y., 1949.

[4] M. L. Anson, *Science*, **93**, 186 (1941); E. L. Smith, *ibid.*, **88**, 170 (1938).

[5] R. L. Liss, M.S. Thesis, Washington University, 1949.

of the benzene solution with water results in a reversal of the previous changes, so that if sufficient extractions are performed, the absorption spectrum of the extracted benzene solution is identical to the original benzene solution. The appearance of the pure benzene solution is quite different from the 0.05 *M* pyridine solution; the former is pink in color, the latter a bluish-red (see Fig. 2 for spectra).

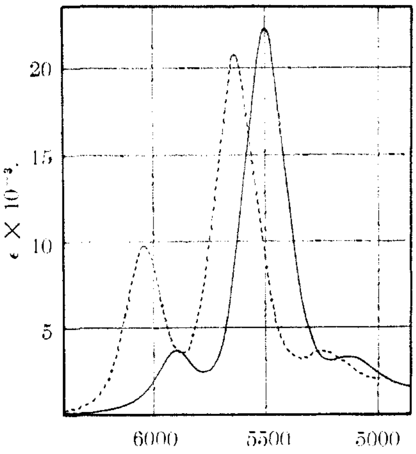

Fig. 2.—Absorption spectra at 29.9°: solid line, zinc tetraphenylporphine in benzene; dashed line, zinc tetraphenylporphine monopyridinate (1.24 *M* pyridine in benzene).

The changes in absorption spectra accompanying complex formation may be utilized to study the reaction quantitatively. Mathematical relations[6] useful in determining the equilibrium constant from the optical density of an equilibrium mixture at a chosen wave length are

$$K = \frac{(P_{\mathrm{Me}}:B)}{(P_{\mathrm{Me}})} \times \frac{1}{(B)} = \frac{R}{(B)} \tag{2}$$

$$F_c = X/A \tag{3}$$

$$R = \frac{F_c}{1 - F_c} = \frac{X}{A - X} \tag{4}$$

$$\frac{1}{X} = \frac{1}{KA(B)} + \frac{1}{A} \tag{5}$$

where K is the equilibrium constant for reaction (1), (B) is the equilibrium pyridine concentration, R is the ratio of complexed to uncomplexed metalloporphyrin, and F_c is the fraction of metalloporphyrin complexed. X and A are defined as $D_m - D_u$ and $D_c - D_u$, respectively, where D_m is the optical density of an equilibrium mixture at some given total metalloporphyrin concentration, and D_u and D_c are the optical densities which that total metalloporphyrin concentration would exhibit if complexing were zero or complete, respectively.

These equations are most conveniently applied as follows: A series of benzene solutions are prepared in which the pyridine concentration is varied from zero to any necessary value, but the total metalloporphyrin concentration in each solution is kept the same. The optical densities of the solutions are determined at any wave lengths where the extinction coefficients of the two species differ appreciably (the equations become indeterminate when $\epsilon_c = \epsilon_u$). For a series of such solutions, a set of values for X is readily determined by subtracting the optical density of the solution containing zero pyridine concentration from the optical densities of the other solutions. If the K of the reaction is fairly large, the value of A will also be known, for the optical density will become a constant in the solutions containing the higher pyridine concentrations, and this constant value will be D_c, which is required for the evaluation of A. With both A and X known, R may be calculated from equation (4), and the equilibrium constant from equation (2). (The value of (B) in equation (2) will be given by $B_0 - F_cC_t$, where B_0 is the original pyridine concentration of the mixture, and C_t is defined in footnote 6. Thus C_t must be known if B_0 and F_cC_t are comparable in value.)

If the K of the reaction is relatively small, the value of D_c would not be known since even in pure pyridine the metalloporphyrin would not be completely complexed. The general expression (5) may then be employed. A plot of $1/X$ against $1/B$ will give a straight line from which the equilibrium constant may be calculated by evaluating the slope and intercept. In this case the value of C_t need not be known.

It should be emphasized that the above equations would fail if the uncomplexed metalloporphyrin were dimerized, if there were any association between the uncomplexed and complexed metalloporphyrins, or if the reaction led to a complex other than the monopyridinate. If the equations fail for the latter reason, the number of base molecules involved in the complex may be determined by methods which depend upon the nature of the complexing. For example, if dipyridinate formation should occur in which the constant for the association of the first pyridine is much larger than for the second, the problem may be treated as two monopyridinate problems using any of the equations (2) through (5). There are many other possible cases, some of which are complicated by the presence of more than two porphyrin species in the solution. There will be no occasion to treat such cases here.

Experimental

Apparatus.—All optical density readings were taken on a Beckman spectrophotometer model DU equipped with a specially constructed cell compartment which was thermostated to within ±0.1° by circulating water from a Precision Scientific Constant Temperature Bath No. 66600. The equilibrium mixtures were prepared in reaction cells made from 1 cm. square mandrel Pyrex tubing and equipped with ground glass caps. The cells were calibrated with reference to a standard cell so that corrections for transparency at the wave lengths employed and for cell thickness could be applied. (The corrections were of the order of 1 to 2% of the observed optical densities.)

Materials.—The metalloporphyrins in benzene solution were prepared by methods given previously.[2] The pyridine used was Mallinckrodt A.R., redistilled from barium oxide. The benzene employed was redistilled Mallinckrodt A.R.

Procedure.—Stock solutions of pyridine in benzene covering a wide range of pyridine concentrations were prepared. Two ml. each of a stock metalloporphyrin solution and an

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(6) These relations may be derived by the reader from Beer's law, $D = \epsilon C$, and the condition that $C_c + C_u = C_t$, where D is the optical density (log I/I_0) of a solution of 1-cm. thickness, ϵ is the molar extinction coefficient, C is concentration in moles/liter, and the subscripts c, u and t refer to complexed, uncomplexed, and total metalloporphyrin, respectively. Similar equations for a more general case are derived by W. M. Clark, *et al.*, *J. Biol. Chem.*, **135**, 561 (1940).

appropriate pyridine solution were pipetted into a Pyrex reaction cell. Several reaction mixtures were made up simultaneously, each having the same total metalloporphyrin content, but a different pyridine concentration.

Three cells, containing, respectively, pure benzene, the metalloporphyrin in pure benzene, and the metalloporphyrin in an appropriate pyridine concentration, were placed in the cell compartment of the Beckman and allowed to come to temperature equilibrium. Optical density readings were taken and recorded at previously selected wave lengths where the difference in extinction coefficients of the complexed and uncomplexed metalloporphyrins was large. The cell containing the pyridine was replaced by another of different concentration, and after temperature equilibrium was attained, readings were recorded for the new mixture at the chosen wave lengths. This latter procedure was repeated for all the remaining equilibrium mixtures. Since it was found that the wave length dial setting in relation to the spectral segment selected by the monochromator changed slightly as heat evolved by the tungsten light warmed up the back plate of the Beckman instrument, it was necessary to set the wave length each time to repeat the original optical density reading of the pure benzene solution, rather than merely to reset the wave lengths indicated on the dial.[7] The pure benzene solution of the metalloporphyrin was thus always left in the Beckman as a reference solution.

The temperatures chosen for the measurements were in the neighborhood of 20, 30 and 40°.

ΔH and $\Delta S°$ Determinations.—ΔH was calculated in the usual way from the variation of the equilibrium constant with temperature. $\Delta S°$ was calculated from this ΔH and the $\Delta F°$ determined from the equilibrium constant.

Absorption Curves of the Complexed and Uncomplexed Metalloporphyrins.—The absorption curves were determined with the aid of a Beckman spectrophotometer by taking optical density readings every 50 Å. by even fifties, and plotting the results in terms of extinction coefficient against wave length. The curves are shown in Figs. 2 through 8.

TABLE I

Temperature 29.9°, total zinc tetraphenylporphin concentration (C_t) 2.91 × 10^{-5} mole/liter

Initial pyridine concn. B_0, moles/liter × 10^3	Observed optical density D_m	X $(D_m - D_u)$	F_c (X/A)	Equilibrium pyridine concn. (B) $(B_0 - F_cC_t)$ × 10^3	Equilibrium constant $\frac{F_c}{1-F_c} \times \frac{1}{B}$
		At 5510 Å.			
0	0.647 (D_u)	0.000	0.0		
1240	.278 (D_c)	.369(A)	1.0		
1.24	.349	.298	0.808	1.22	3450
0.620	.396	.251	.681	0.600	3560
.248	.479	.168	.456	.235	3560
.124	.536	.111	.301	.115	3740
		At 5640 Å.			
0	0.252 (D_u)	0.000	0.0		
1240	.610 (D_c)	.358(A)	1.0		
1.24	.551	.299	0.834	1.22	4120
0.620	.504	.252	.704	0.599	3970
.248	.423	.171	.478	.234	3910
.124	.368	.116	.315	.115	4000
		At 6050 Å.			
0	0.036 (D_u)	0.000	0.0		
1240	.282 (D_c)	.246(A)	1.0		
1.24	.234	.198	0.806	1.22	3410
0.620	.206	.170	.691	0.600	3730
.248	.152	.116	.472	.234	3820
.124	.114	.078	.317	.115	4030
				Average K	3780±200

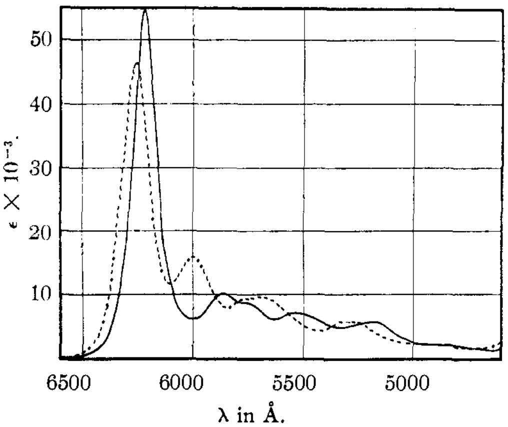

Fig. 3.—Absorption spectra at 29.9°: solid line, zinc tetraphenylchlorin in benzene; dashed line, zinc tetraphenylchlorin monopyridinate (1.24 M pyridine in benzene).

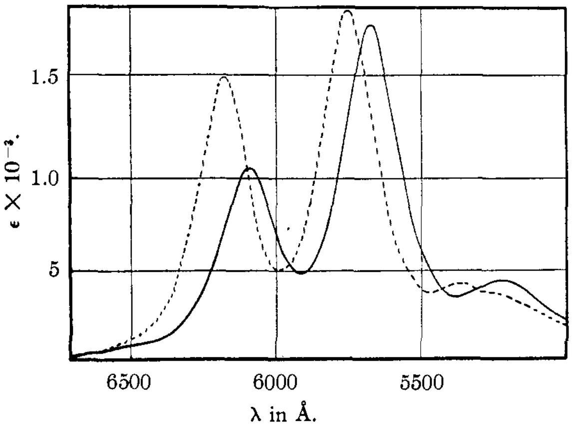

Fig. 4.—Absorption spectra at 20.3°: solid line, cadmium tetraphenylporphine in benzene; dashed line, cadmium tetraphenylporphine monopyridinate (1.24 M pyridine in benzene).

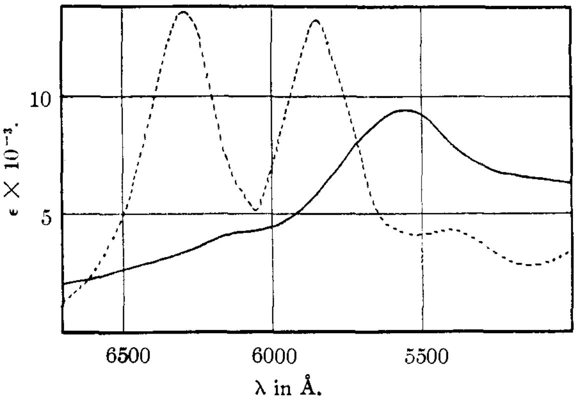

Fig. 5.—Absorption spectra at 30°: solid line, mercury tetraphenylporphine in benzene; dashed line, mercury tetraphenylporphine monopyridinate (6.2 M pyridine in benzene).

Discussion

Optical density measurements on a set of equilibrium mixtures of pyridine and zinc tetraphenylporphine at 29.9° are given in Table I. The ap-

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[7] This difficulty, which was most serious in the red region of the spectrum, is corrected by the use of one of the water-cooled lamp housings with which the Beckman Spectrophotometers are now equipped.

TABLE II

PYRIDINATE COMPLEXES OF VARIOUS METALLO-DERIVATIVES OF TETRAPHENYLPORPHINE AND TETRAPHENYLCHLORIN

Porphyrin	Metal	Pyridines in complex	Equilibrium constants[a] 20.3°	29.9°	39.8°	ΔH, cal./mole	ΔS^0 (29.9°)
Tetraphenylporphine	Mg	2	....	2000 (est.)	....	....	....
			0.656 ± 0.039^b	0.577 ± 0.03^b	0.496 ± 0.025^b	−2600	− 9.8
	Zn	1	6220 ± 610	3720 ± 270	2320 ± 130	−9200	−14
	Cd	1	3890 ± 290	2690 ± 270	1520 ± 110	−8700	−13
	Hg	1	16.5 ± 1.2	12.1 ± 0.7	9.90 ± 0.5	−4900	−11
	Cu(II)	1	....	0.05 ± 0.02	....	....	....
Tetraphenylchlorin	Mg	2	....	4000 (est.)	....	....	....
			0.942 ± 0.091^b	0.833 ± 0.047^b	0.706 ± 0.039^b	−2600	− 9.0
	Zn	1	8030 ± 250	5270 ± 300	3060 ± 110	−9000	−13

[a] For the reaction P_{Metal} + pyridine $\rightleftarrows$ P_{Metal} : pyridine unless otherwise noted. The ± limits placed on the values of the constants represent the average deviations for 8 determinations in the case of zinc tetraphenylchlorin and copper tetraphenylporphine, and 12 determinations in all other cases. [b] For the reaction P_{Mg} : pyridine + pyridine $\rightleftarrows$ P_{Mg} (pyridine)$_2$.

plication of equations for monopyridinate formation (eq. 2, 3 and 4) to these data, also given in Table I, shows that the reaction involved is

$$P_{\text{zinc}} + \text{pyridine} \rightleftarrows P_{\text{zinc}} : \text{pyridine}.$$

To illustrate the sensitivity of the data to the type of equilibrium assumed, if we calculate an equilibrium constant on the basis of the reaction

$$P_{\text{zinc}} + 2\ \text{pyridine} \rightleftarrows P_{\text{zinc}}\ (\text{pyridine})_2$$

the values from the data at 6050 Å. are 2.9 × 10^6, 6.7 × 10^6, 19 × 10^6 and 40 × 10^6 for the four equilibrium mixtures. Since equations 2, 3 and 4 would fail if the uncomplexed zinc tetraphenylporphine were dimerized, or if there were any association between the uncomplexed and complexed metalloporphines, we may exclude these reactions from consideration.

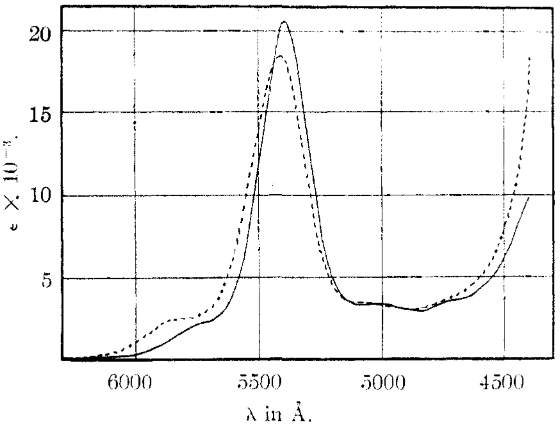

Fig. 6.—Absorption spectra at 29.9°: solid line, copper tetraphenylporphine in benzene; dashed line, copper tetraphenylporphine in 6.4 *M* pyridine solution in benzene. (The porphine is complexed as a monopyridinate to the extent of about 25%.)

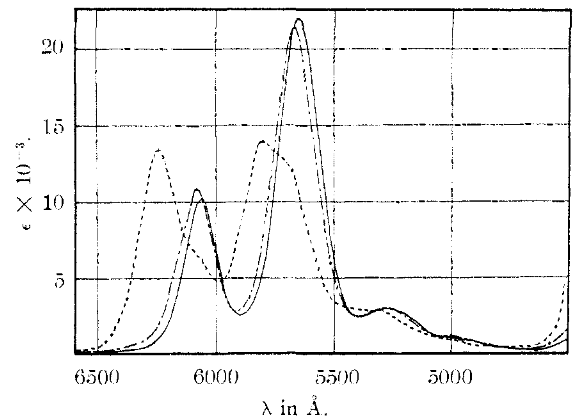

Fig. 7.—Absorption spectra at 29.9°: solid line, magnesium tetraphenylporphine in benzene; dashed-dotted line, magnesium tetraphenylporphine monopyridinate (0.0124 to 0.031 *M* pyridine in benzene); dashed line, magnesium tetraphenylporphine in 6.2 *M* pyridine solution in benzene. (The porphine is complexed as a dipyridinate to to the extent of about 78%.)

The cadmium and mercury derivatives of tetraphenylporphine, and the zinc derivative of tetraphenylchlorin gave results which also were consistent only with the assumption of monopyridinate formation. The calculated constants are given in Table II. The spectral curves for these uncomplexed and complexed metalloporphyrins are shown in Figs. 3 through 5.

Optical density data taken on mixtures of pyridine and magnesium tetraphenylporphin follow a different pattern from the cases discussed above. For example, if the optical density at 6075 Å. is followed, it is found that the optical density for a 2 × 10^{-5} *M* magnesium tetraphenylporphine solution increases as the pyridine concentration is increased from 0.0 to 0.0124 *M*. In the range 0.0124 to 0.031 *M* pyridine, the optical density remains essentially constant. Finally, as the pyridine concentration is raised to higher values, the optical density decreases to a value lower than that observed in pure benzene (see Fig. 7).

Such behavior is entirely inconsistent with monopyridinate formation (the optical density should change in only one direction under such conditions), but is consistent with the assumption that monopyridinate formation with a fairly high association constant is followed by dipyridinate formation with a low second association constant. Assuming that the optical density values observed in the range 0.0124 to 0.031 *M* pyridine concentration are those of magnesium tetraphenylporphine monopyridinate, the second association constant was cal-

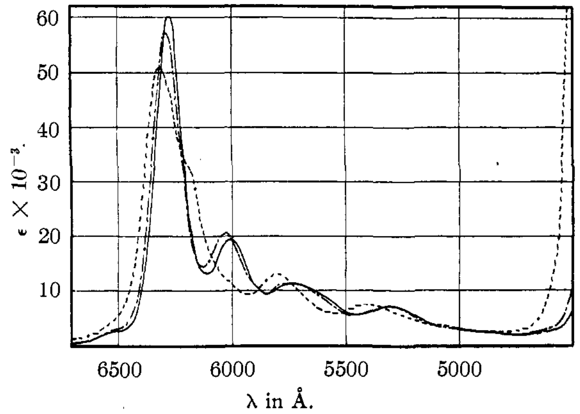

Fig. 8.—Absorption spectra: solid line, magnesium tetraphenylchlorin in benzene; dashed-dotted line, magnesium tetraphenylchlorin monopyridinate (0.0124 M pyridine in benzene); dashed line, magnesium tetraphenylchlorin in 6.2 M pyridine solution in benzene. (The chlorin is complexed as a dipyridinate to the extent of about 84%.)

culated with the aid of equation (5). A plot of the data at 6250 Å. is shown in Fig. 9. The straight line plot which results shows that in the range of pyridine concentration above 0.031 M a pyridine molecule adds to the species present in 0.031 M pyridine. It is not possible to determine exactly what happens in the range of pyridine concentration 0.0 to 0.0124 M, because the two spectral curves from which the calculations must be made are so similar that the precision is rather poor. The data, however, fit best the assumption of monopyridinate formation. The association constant may be estimated by determining the pyridine concentration required to give an optical density half way between the optical density values at 0.0 and 0.0124 M pyridine concentration. At this point, R is equal to 1.0, and the association constant is equal to the reciprocal of the pyridine concentration. The first association constant given in Table II was estimated in this way. It is to be noted that the second association constant is so low that even in pure pyridine the magnesium tetraphenylporphine is not completely dicomplexed. The 6.2 M pyridine spectral curve in Fig. 7 clearly shows the presence of two species. The magnesium derivative of tetraphenylchlorin is analogous to the porphine derivative in its behavior toward pyridine (see Table II and Fig. 8).

The collected data of Table II illustrate the marked dependence of pyridinate formation upon the nature of the metal substituted in the center of the metalloporphine. The change from the porphine to the chlorin structure has a comparatively small effect, resulting in slightly higher association constants in the limited number of cases studied. The standard entropy changes in all cases are negative, and fairly comparable in value, as would be expected in a series of association processes as similar as these. The magnitude of the entropy effect is such that about 9 kcal. of heat must be liberated per mole in the process before the metalloporphyrin may be classed as a moderately good complexing agent toward pyridine.

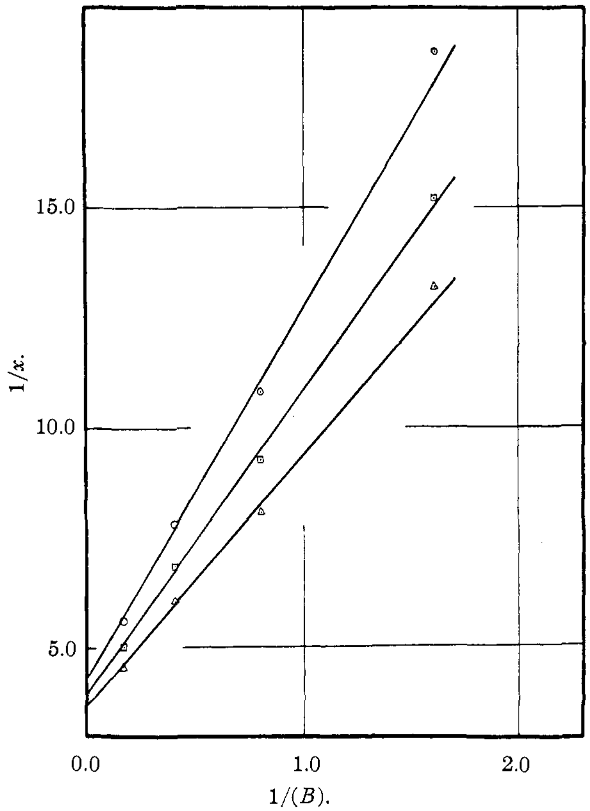

Fig. 9.—Determination of the second association constant for magnesium tetraphenylporphine dipyridinate: ⊙, 39.8°; ⊡, 29.9°; ◬, 20.3°.

The strongest complexing agent of this type that we have so far observed is cobalt(III) tetraphenylporphine chloride. The monopyridinate[8] of this compound is not appreciably dissociated at a concentration of 4 × 10^{-5} M, which corresponds to an association constant of greater than 10^7. Other metallotetraphenylporphines we have investigated briefly are the copper(II) derivative, which forms a monopyridinate with an association constant at 30° of about 0.05 (see Fig. 6), the nickel(II) derivative, which forms a monopyridinate with an association constant of similar magnitude, and the silver(II) derivative, which is a much weaker Lewis acid toward pyridine than the copper derivative.

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[8] William Parr, work in progress in this Laboratory.