TABLE I

RX	Product	M.p., °C.	Yield, %[a]
Bromobenzene	3-Phenylpropanoic acid	48.5–49[b]	77
3-Bromotoluene	3-(3′-Tolyl)propanoic acid	43–44.5[c]	51
2-Bromotoluene	3-(2′-Tolyl)propanoic acid	102–104[d]	5
2-Bromonaphthalene	3-(2′-Naphthyl)-propanoic acid	134–135[e]	48
1-Bromonaphthalene	No reaction		
Bromomesitylene	No reaction		
2-Iodothiophene	3-(2′-Thenyl)-propanoic acid	45–46.5[f]	41
1-Bromobutane	Heptanoic acid	(B.p.) 222	32[g]
2-Chloro-2-methyl-propane	No reaction[h]		

[a] Based on propiolactone. [b] F. F. Blicke and D. G. Sheets, *J. Am. Chem. Soc.*, **70**, 3768 (1948). [c] V. Miller and H. Rohde, *Ber.*, **23**, 1899 (1890). [d] C. Grundman, *ibid.*, **81**, 513 (1948). [e] R. T. Arnold and R. Barnes, *J. Am. Chem. Soc.*, **65**, 2393 (1943). [f] G. Barger and A. P. T. Easson, *J. Chem. Soc.*, 2100 (1938). [g] Melting point of the anilide, 71°. R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," 4th Ed., John Wiley and Sons, Inc. New York, N. Y., 1956, p. 276. [h] Reaction carried out at −70°.

1,1-Di-*o*-anisyl-1,3-propanediol.—When *o*-anisyllithium was treated with propiolactone a 60% yield of 1,1-di-*o*-anisyl-1,3-propanediol, m.p. 105° (from ethanol), was obtained.

Anal. Calcd. for $C_{17}H_{20}O_4$: C, 70.8; H, 6.94. Found: C, 70.9; H, 6.91.

Benzyllithium with Propiolactone.—When one equivalent of benzyllithium[10] was cooled to 0° and treated with one equivalent of propiolactone, there was obtained, subsequent to hydrolysis and acidification, a 65% yield of 4-phenylbutanoic acid, identified by mixture melting point.[11]

Diphenylzinc with Propiolactone.—A solution of diphenylzinc[12] was cooled to 0° and treated dropwise with an equivalent of propiolactone. After hydrolysis and acidification there was obtained a 40% yield of 3-phenylpropanoic acid, m.p. 48°.

Allylmagnesium Bromide with Propiolactone.—To a solution of 0.1 mole of allylmagnesium bromide in 100 ml. of anhydrous ether, cooled to 0°, was added dropwise, with stirring, 7.2 g. (0.1 mole) of propiolactone in 50 ml. of anhydrous ether. After addition was complete the mixture was hydrolyzed with aqueous ammonium chloride. The aqueous layer was acidified with hydrochloric acid and extracted with three 50-ml. portions of ether. The combined ether extracts were dried over sodium sulfate and then subjected to distillation. The yield of 5-hexenoic acid, boiling at 202°, was 6.2 g. or 54%; $d^{25}{}_{25}$ 1.011; neut. equiv., 111.

Organocadmium Compounds.—General procedure: To 0.1 mole of the Grignard reagent in 100 ml. of anhydrous ether was added 19.1 g. (0.05 mole) of cadmium chloride. When color test I[13] was negative the ether solution was decanted into a second reaction flask. A 10-ml. aliquot was hydrolyzed with distilled water and titrated with 0.1 *N* hydrochloric acid to the methyl orange end point. The organocadmium solution was then cooled to 0° and one equivalent of propiolactone was added. After addition was completed the mixture was hydrolyzed with water, the aqueous layer acidified with concentrated hydrochloric acid, and the reaction product isolated by extraction with ether followed by distillation or crystallization from an appropriate solvent. Table I summarizes the results.

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(10) H. Gilman and H. A. McNinch, *J. Org. Chem.*, **26**, 3723 (1961).

(11) R. V. Christian, Jr., *J. Am. Chem. Soc.*, **74**, 1591 (1952).

(12) K. A. Kocheshkov, A. N. Nesmeyanov, and V. I. Potrosov, *Ber.*, **67**, 1138 (1934).

(13) H. Gilman and F. Schulze, *J. Am. Chem. Soc.*, **47**, 2002 (1925).

Spectral Studies of the "d⁶" Metalloporphyrins. Ligand and Solvent Field Effects on Ferrous and Cobaltic Mesoporphyrin IX[1]

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A new method for the preparation of ferrous mesoporphyrin IX has made possible the study of this complex under a variety of new conditions. The ferrous complexes, prepared in this manner, should serve as much better models for biological systems than those previously reported. The effect of solvents on the visible- and near-ultraviolet spectra of the cobalt mesoporphyrins has been investigated extensively. A profound solvent effect on the oxidation–reduction potentials of the cobalt porphyrins has been observed. These results have been interpreted with the aid of ligand field theory.

In the studies of the absorption spectra of metalloporphyrins especial significance has been attached to the relative intensities of the absorption bands. Since the intensities of the bands as well as their positions vary greatly with solvent, a detailed study of solvent–spectral relationships has been needed. Spectral studies of the ferrous complexes with simple molecules (dimethylglyoxime, phenanthroline, etc.), ferrous porphyrin complexes with proteins, and ferrous complexes of the porphyrins in the presence of nitrogenous bases (pyridine, ammonia) have been reported.[4] However, there exists very little data on the spectra of simple ferrous porphyrins owing to their marked instability in air and the difficulties in their preparation. The simple porphyrin complexes that have been prepared have usually been associated with powerful ligands capable of producing profound effects of their own in addition to the effect of the porphyrin ring. Common reducing agents employed in the preparation of ferrous porphyrin complexes (*i.e.*, hydrazine and dithionite) can serve as ligands, making it difficult to separate the spectral effects produced by these ligands.

Ferrous ion, with six d electrons, can exist either in a low spin configuration with no unpaired electrons or in a high spin configuration with four unpaired electrons; ferric ion can have one or five unpaired electrons. According to ligand field theory, ferric ion should exist in the high spin configuration except in the presence of

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(1) Porphyrin Studies XXVII. Paper XXVI, A. H. Corwin, *Trans. N. Y. Acad. Sci.*, **25**, 569 (1963); this work was supported in part by NIH grant A-2877(C3).

(2) Public Health Fellow, 1962–1963.

(3) Du Pont Fellow, 1962–1963.

(4) (a) R. J. P. Williams, *Chem. Rev.*, **56**, 299 (1956); (b) A. S. Brill and R. J. P. Williams, *Biochem. J.*, **78**, 246 (1961); (c) J. E. Falk, R. Lemberg, and R. K. Morton, Ed., "Haematin Enzymes," Pergamon Press, Inc., New York, N. Y., 1961.

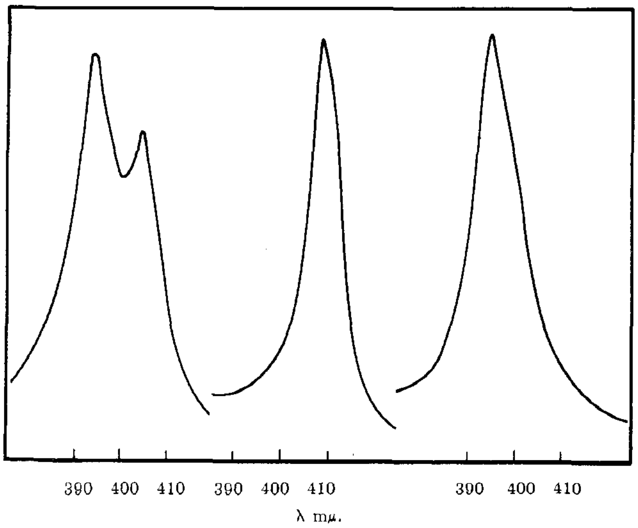

Fig. 1.—Left to right: mixture of cobaltous and cobaltic mesoporphyrin IX in 1-propanol; cobaltic mesoporphyrin IX in formamide; and cobaltous mesoporphyrin IX in 60% tetrahydrofuran–40% acetic acid (wave lengths in mμ).

very strong ligands.[5] For the ferrous case, however, relatively low field strengths are sufficient to produce the low spin configuration. The simple ferrous porphyrin complexes with amines, carbon monoxide, cyanide, azide ion, and pyridine have all been found to be low spin complexes.[6] Hemoglobin and some ferroporphyrin proteins have been shown to be high spin complexes or at least mixtures of the high and low spin forms. Their ligand field effects, therefore, exist close to the borderline of field strengths necessary to cause spin pairing. Identification of the low spin ferrous porphyrin is possible due to its strong, characteristic "hemochromogen type" spectrum (see Fig. 3). This is the method used in this paper. The spectrum of ferrous protoporphyrin IX, high spin, has been reported,[7] but the experimental details of the preparation remain unpublished. Corwin and Erdman[8] have published a spectrum of ferrous mesoporphyrin IX in dioxane but the band positions are at different wave lengths from those given in this paper.

Taylor[9] prepared cobaltous mesoporphyrin IX and found that it could be oxidized readily to cobaltic mesoporphyrin. He reported the visible spectrum of cobaltic and cobaltous mesoporphyrin in dilute base. Very little work has been published regarding cobaltic mesoporphyrin since Taylor's work even though it is isoelectronic with ferrous mesoporphyrin. According to ligand field theory, cobaltic ion should exist in the low spin configuration except in the presence of very weak fields. The only known paramagnetic cobaltic complexes are the cobaltic hexafluoride ion and cobaltic fluoride trihydrate.[10] We chose cobaltic mesoporphyrin as a model to compare with the more labile low spin ferrous porphyrins.

Cobaltic mesoporphyrin IX hydroxide was examined in a number of solvents and the positions of the visible and Soret bands, as well as the order of intensities of the α and β peaks, were determined (Table I). Surprisingly, wide variances were observed in both the location and order of the peaks. Cobaltous mesoporphyrin IX was obtained by reduction with iron in a sealed tube. The visible spectrum (Table II) agreed quite well with that of Taylor. However, we prefer to consider the visible spectrum as being composed of two bands rather than one as did Taylor. The Soret band of the cobaltous was displaced about 20 mμ toward shorter wave lengths relative to the cobaltic.

TABLE I

COBALTIC MESOPORPHYRIN IX HYDROXIDE

Solvent[a]	λ, mμ α	λ, mμ β	Order	λ, mμ Soret
Pyridine	562	530	$\beta > \alpha$	419
4-Picoline	560	527	$\beta > \alpha$	418
Formamide	567	532	$\beta > \alpha$	417
Formamide + 1% imidazole	567	533	$\beta > \alpha$	416
Dimethylformamide + 1% imidazole	562	530	$\beta > \alpha$	417
0.1 *N* Sodium hydroxide	564	530	$\beta > \alpha$	412
Aniline	563	530	$\beta > \alpha$	419
Acetonitrile	567	533	$\beta = \alpha$	416
Nitromethane	562	529	$\beta = \alpha$	412
Acetic acid	562	529	$\alpha > \beta$	410
Water + 1% imidazole	562	532	$\alpha > \beta$	413
60% Tetrahydrofuran + 40% acetic acid	559	527	$\beta > \alpha$	410

[a] Solvents listed in decreasing $\beta:\alpha$ ratio.

TABLE II

COBALTOUS MESOPORPHYRIN IX

Solvent	λ, mμ α	λ, mμ β	Order	λ, mμ Soret
Dioxane	549	518	$\alpha \gg \beta$	396
Ethyl acetate	549	515	$\alpha \gg \beta$	389
Thiophene	556	522	$\alpha \gg \beta$	394
40% Acetic acid–60% tetrahydrofuran + iron + tetramethylammonium chloride in sealed tube[a]	550	520	$\alpha \gg \beta$	393

[a] For this solvent system the cobaltic form was observed in vessels open to the air; the cobaltous form was prepared by reduction in a sealed tube.

Upon examination of the cobaltic mesoporphyrin IX hydroxide in a variety of solvents we observed the presence of two Soret peaks in a number of instances (Table III). However, in some solvents (*e.g.*, dioxane, thiophene, and ethyl acetate, see Table II) only one Soret peak, that of cobaltous mesoporphyrin, was observed even though the solute was analytically pure cobaltic mesoporphyrin hydroxide. The ready transition of cobaltous to cobaltic has already been noted and we have observed the easy reversal of this reaction in certain solvents. Since the oxidation was readily reversible at room temperature, even in the presence of air, the effects of temperature changes and solvent mixtures were investigated.

The Soret region (Fig. 1) was used to estimate cobaltous (*ca.* 390 mμ) and cobaltic (*ca.* 410 mμ) porphyrins in mixtures. When N,N-dimethylformamide or ethanol solutions of the mixture were heated, the intensity of the 390-mμ peak increased at the expense of the 410-mμ peak. As the solution cooled to room temperature

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(5) L. E. Orgel, "An Introduction to Transition-Metal Chemistry Ligand Field Theory," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 50.

(6) R. J. P. Williams, "Haematin Enzymes," J. E. Falk, R. Lemberg, and R. K. Morton, Ed., Pergamon Press, Inc., New York, N. Y., 1961, p. 41.

(7) R. Lemberg and J. W. Legge, "Hematin Compounds and Bile Pigments," Interscience Publishers, Inc., New York, N. Y., 1949, p. 166.

(8) A. H. Corwin and J. G. Erdman, *J. Am. Chem. Soc.*, **68**, 2473 (1946).

(9) J. F. Taylor, *J. Biol. Chem.*, **135**, 569 (1940).

(10) C. J. Ballhausen, "Introduction to Ligand Field Theory," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 259.

TABLE III

COBALT MESOPORPHYRIN IX. MIXTURES OF Co(II) AND Co(III)[a]

	λ, mμ			
Solvent[b]	α	β	Soret₁	Soret₂
Methanol	558	525	408	390 sh
Benzyl alcohol	557	525	413	397
Chloroform[c]	560	527	414	393
Dimethyl sulfoxide	545	520	412	392
2-Picoline	546 broad		417	395
Ethanol	553	520 sh	410	389
n-Propyl alcohol	552	520 sh	411	390
n-Butyl alcohol	552	518 sh	412	391
t-Butyl alcohol	551	518 sh	410 sh	387
Dimethylformamide	550	520 sh	412	392
Tetrahydrofuran	551	525	412	395
Acetone	552	520 sh	414	389
Ethyl formate	550	518	411	389
Cyclohexyl acetate	553	518	412	391
1,2-Dichloroethane	553	520 sh	412	393

[a] The observation of two Soret bands implies that a mixture of cobaltous and cobaltic mesoporphyrin IX is present in these solvents. [b] Solvents in which mixtures of cobaltous and cobaltic mesoporphyrin were present. The solvents are listed approximately in decreasing cobaltic–cobaltous ratio. [c] Commercial chloroform containing methanol preservative.

in air the peaks slowly returned to near their original ratio. Those solvents which produce a high β to α ratio for cobaltic mesoporphyrin hydroxide (pyridine, 4-picoline, formamide, etc.) also have a powerful effect when added to other solutions causing the equilibrium to shift completely to the cobaltic form. Thus, the spectrum of cobaltic mesoporphyrin in 0.5% formamide in tetrahydrofuran is identical to that in pure formamide. A similar result is observed when one drop of pyridine is added to an ethanol solution of the cobalt mesoporphyrin mixture. In water, where only the cobaltic complex is present, the addition of a small quantity of pyridine causes complete conversion to the pyridine type spectrum (Fig. 2). It was apparent that the cobaltic mesoporphyrin is extremely sensitive to the presence of strong ligands and that they greatly alter its oxidation–reduction potential. Our results show a striking parallel to those of Caughey, *et al.*,[11] for the nickel(II) porphyrins.

Extinction coefficients for the cobaltic mesoporphyrin IX hydroxide are reported for a number of solvents (Table IV). Our values in base agree in most cases with those of Taylor. The comparison of the values obtained in N,N-dimethylformamide, where a mixture of cobaltous and cobaltic is present, with those for pure cobaltic in formamide is noteworthy.

TABLE IV

EXTINCTION COEFFICIENTS FOR COBALTIC MESOPORPHYRIN IX HYDROXIDE

Solvent	λ, mμ α	ε × 10⁻⁴	λ, mμ β	ε × 10⁻⁴	λ, mμ γ	ε × 10⁻⁵
Dimethylformamide[a]	551	1.45	523	1.06	(1) 412	0.64
					(2) 393	1.08
Pyridine	562	1.28	530	1.35	418	1.62
Formamide	567	1.07	532	1.21	416	1.47
0.1 *N* Sodium hydroxide	564	1.02	530	1.08	411	1.13
Water	561	1.07	529	1.05	412	1.18
Water + 1% imidazole	562	1.57	532	1.44	...	..
Dimethylformamide + 1% imidazole	562	0.92	530	1.09	417	1.03
Formamide + 1% imidazole	567	1.05	533	1.18	417	1.57

[a] Equilibrium mixture of Co(II) and Co(III) mesoporphyrin IX.

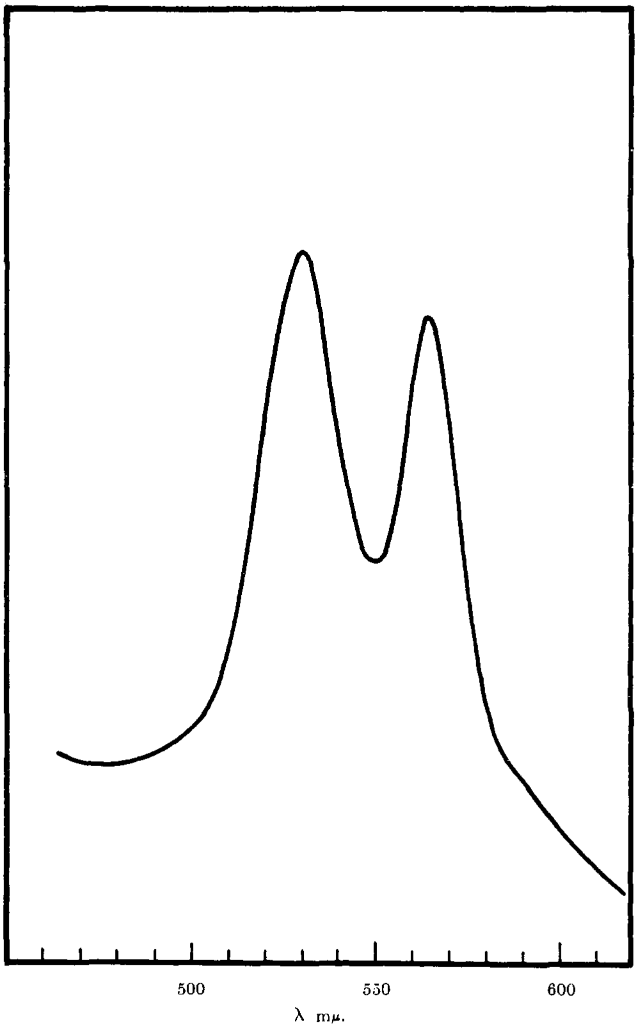

Fig. 2.—Cobaltic mesoporphyrin IX hydroxide in pyridine.

The spectra of mesoporphyrin IX ferric complexes in several solvents are reported (Table V). It is noteworthy that the ferric mesoporphyrin IX hydroxide in N-methylformamide (dielectric constant 180)[12] is apparently in the high spin configuration, according to the classification of Scheler, Schoffa, and Jung.[13] It is probable that in a solvent of such high dieletric constant the strength of the ligands surrounding the iron has been reduced, thus allowing the metal to adopt the high spin configuration.

Two types of Soret peak were observed for ferric mesoporphyrin IX chloride dimethyl ester (Table V). In the visible region, all of the ferric mesoporphyrin IX dimethyl ester solutions showed similar four-banded spectra, once again indicative of the high spin configuration. In some cases (alcohols), it was difficult to determine exact positions of bands II and III since these were only inflection points. Extinction coefficients for major bands of ferric mesoporphyrin IX chloride dimethyl ester were determined in several solvents (Table VI).

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[11] W. S. Caughey, R. M. Deal, B. D. McLees, and J. O. Alben, *J. Am. Chem. Soc.*, **84**, 1735 (1962).

[12] G. R. Leader and J. F. Gormley, *ibid.*, **73**, 5731 (1951).

[13] W. Scheler, G. Schoffa, and F. Jung, *Biochem. Z.*, **329**, 232 (1957).

TABLE V

FERRIC MESOPORPHYRIN IX CHLORIDE DIMETHYL ESTER

Solvent	Dielectric constant	λ, mμ Absorption maxima					
N-Methylformamide	180[a]	612	568	530	485		392 s[e]
Formamide–acetic acid		619	570	530	493		392 s
Dimethyl sulfoxide	45[b]	620	570	530	495		392 s
Methanol	34[c]	615		530	492		389 s
Dimethylformamide–acetic acid		625	570	530	500		394 s
Methyl ethyl ketone	18.5[d]	632	580	532	503	sh[f]	375 broad
1-Butanol–acetic acid		625		530	500		395 s
Benzene-acetic acid		634	580	534	505	400	378
Acetic acid	7.1[c]	630	580	528	500	385 very broad	
Tetrahydrofuran–acetic acid		632	580	532	504	403	375
Tetrahydrofuran		635	582	534	505	396	371
Dioxane	2.3[c]	635	580	533	505	395	372
1,2-Dichloroethane		638	580	534	505	sh	376 broad
Xylene	2.3[d]	637	582	534	506		

[a] G. R. Leader and J. F. Gormley, *J. Am. Chem. Soc.*, **73**, 5731 (1951). [b] "The Merck Index," 7th Ed., P. G. Stecher, Ed., Merck and Company, Rahway, N. J., 1960, p. 373. [c] L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1961, p. 1158. [d] "Handbook of Chemistry and Physics," 39th Ed., C. D. Hodgman, Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1957, pp. 2331–2340. [e] Sharp. [f] Shoulder.

TABLE VI

EXTINCTION COEFFICIENTS FOR FERRIC MESOPORPHYRIN IX CHLORIDE DIMETHYL ESTER

Solvent	"630" Extinction coefficients × 10^{-4}	"530"	"500"	Soret × 10^{-5}	
60% Benzene–40% acetic acid	0.47	0.93	0.88	0.60	0.77
60% 1-Butanol–40% acetic acid	.34	.67	.79	1.41	
60% Dimethylformamide–40% acetic acid	.37	.66	.79	1.52	
60% Formamide–40% acetic acid	.36	.53	.79	..	
60% Tetrahydrofuran–40% acetic acid	.45	.88	.86	0.41	0.53

Since reduction with mercury has been used successfully to prepare pyridine hemochromogens (ferrous mesoporphyrin IX dipyridine complex) we attempted to prepare other ferrous complexes by this method. The hemochromogen was prepared successfully in a xylene solution containing only about 3% pyridine. The hemochromogen could not be obtained in similar experiments with N-methylformamide and tetrahydrofuran as the major solvent. It was impossible to obtain an observable concentration of ferrous porphyrin with mercury and bromide or chloride as the reductant, in the absence of nitrogenous bases. It has been shown that mercury and chloride reduce a sufficient amount of the ferric porphyrin to the ferrous state to allow equilibration with the porphyrin acid salt.[14]

A facile reduction was obtained with iron in the presence of acetic acid as a reducing agent. Varying concentrations of tetrahydrofuran and acetic acid together with iron powder, tetramethylammonium chloride, and mesohemin chloride were used to determine the optimum conditions for the preparation of the ferrous complex. In solutions containing 80 or 100% tetrahydrofuran, no reduction could be observed even after three days. In solutions containing 80 or 100% acetic acid, reduction was observed; however, a destructive side reaction also occurred. The side reaction was not observed to occur in solutions containing 60% or less acetic acid. This competing reaction produces a material which has absorption peaks at 730 and 483 mμ. Experiments with 60% dioxane–40% acetic acid showed that it was unnecessary to add chloride. Because the spectra of the ferrous compounds are very different from the ferric, the reduction could be easily observed. When the seal was broken, the ferrous complex rapidly reverted to the ferric state, and the ferrous chloride slowly oxidizd to ferric chloride. In sealed tubes, the ferrous complex was stable for an indefinite period.

Reduction was effected in a variety of solvents (Table VII). Generally, in solutions of low dielectric constant (benzene and tetrahydrofuran, see Fig. 3), the ferrous porphyrin spectrum consisted of two well defined peaks, the α and β, somewhat reminiscent of the hemochromogen spectrum but with significant differences. The α to β ratio was much less than that of the pyridine hemochromogen; the peaks were displaced towards higher wave lengths and generally broader peaks were observed. In solutions of higher dielectric constant (N-methylformamide, N,N-dimethylformamide) the contrast to the pyridine hemochromogen spectrum was marked. Here (Fig. 4) the α and β peaks were less defined, almost merging into a single broad peak in the same region. The α to β ratio was almost unity. The spectrum resembled that of hemoglobin. In most cases the Soret was a single sharp peak near 400 mμ.

TABLE VII

FERROUS MESOPORPHYRIN DIMETHYL ESTER

(40% Acetic acid in all cases except pyridine and pyridine–xylene)

Solvent	λ, mμ α	λ, mμ β	Order	λ, mμ Soret
60% N-Methylformamide	558	527	$\alpha > \beta$	402
60% Formamide	554	521	$\alpha > \beta$	
60% Dimethyl sulfoxide	550	522	$\alpha > \beta$	410 404 split
60% Dimethylformamide	556	528	$\alpha > \beta$	
60% Methanol	557	523	$\alpha > \beta$	399
60% Acetic anhydride	558	525	$\alpha > \beta$	400 broad
60% 1-Butanol	558	525	$\alpha > \beta$	401
60% Methyl ethyl ketone	558	525	$\alpha > \beta$	398
Pyridine	547	518	$\alpha >>> \beta$	406
60% Tetrahydrofuran	556	523	$\alpha >> \beta$	399
60% Dioxane	558	528	$\alpha = \beta$	406
60% Benzene	559	525	$\alpha > \beta$	401
2% Pyridine in xylene	548	518	$\alpha >>> \beta$	

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(14) A. H. Corwin and R. Singh, *J. Org. Chem.* **28**, 2476 (1963).

We report extinction coefficients (Table VIII) for the α and β peaks of ferrous mesoporphyrin IX dimethyl ester in several solvents. While the precision of these measurements is good, it is realized that the values are probably low because some of the porphyrin was adsorbed on the finely divided metal surface. The degree of adsorption varies with solvent. We estimate, however, that this effect did not cause an error in the extinction coefficients of more than 10%.

TABLE VIII

EXTINCTION COEFFICIENTS FOR FERROUS MESOPORPHYRIN IX DIMETHYL ESTER

Solvent	λ, mμ α	$\epsilon \times 10^{-4}$	λ, mμ β	$\epsilon \times 10^{-4}$
60% Benzene–40% acetic acid	559	0.91	525	0.80
60% 1-Butanol–40% acetic acid	558	.70	525	.64
60% Dimethylformamide-40% acetic acid	556	.77	528	.73
60% Tetrahydrofuran–40% acetic acid	556	1.27	523	1.04

Studies at higher temperatures (30–80°) indicate that the reduction is reversed on heating. Limited work has been undertaken in this area because heating increased the evolution of hydrogen. Even at room temperature, considerable hydrogen evolution was observed with 60% methanol–40% acetic acid. This method obviously is limited to nonaqueous systems for this reason.

Magnetic measurements on the ferrous complexes should prove extremely interesting. While our method of preparation lends itself admirably to spectral studies, it cannot be used without modification for magnetic studies by the Gouy method. The presence of excess iron and ferrous chloride in unknown quantities would vitiate such measurements. Paramagnetic resonance studies are being used on this system. Here, the technique may enable us to separate the signals of the different paramagnetic species.

Discussion

We have noted the presence of two Soret peaks for cobalt mesoporphyrin in a number of solvents. This is interpreted to indicate the presence of a mixture of cobaltic and cobaltous porphyrins in these solvents. We also have noted the ready formation of cobaltous mesoporphyrin from cobaltic in some solvents but not in others. Either the explanation of Pauling[15] or ligand field theory can be used to rationalize our results. The presence of strong ligands (pyridine, water, imidazole, etc.) at the fifth and sixth positions of the octahedral complex destabilizes the cobaltous relative to the cobaltic. Weaker ligands (tetrahydrofuran, ketones, dimethylformamide, 2-picoline, etc.) do not interact with the metal strongly enough to prevent the acceptance of a seventh d electron in the d_{z^2}-orbital. Especially striking is the contrast between 2-picoline which leads to cobaltous and pyridine which leads to cobaltic mesoporphyrin IX. Even with Co(III) in the absence of Co(II) we observe that solvent changes reverse the α–β order. Therefore, it is no longer possible to regard the α–β order of a given metalloporphyrin as a fixed property as others[16] have.

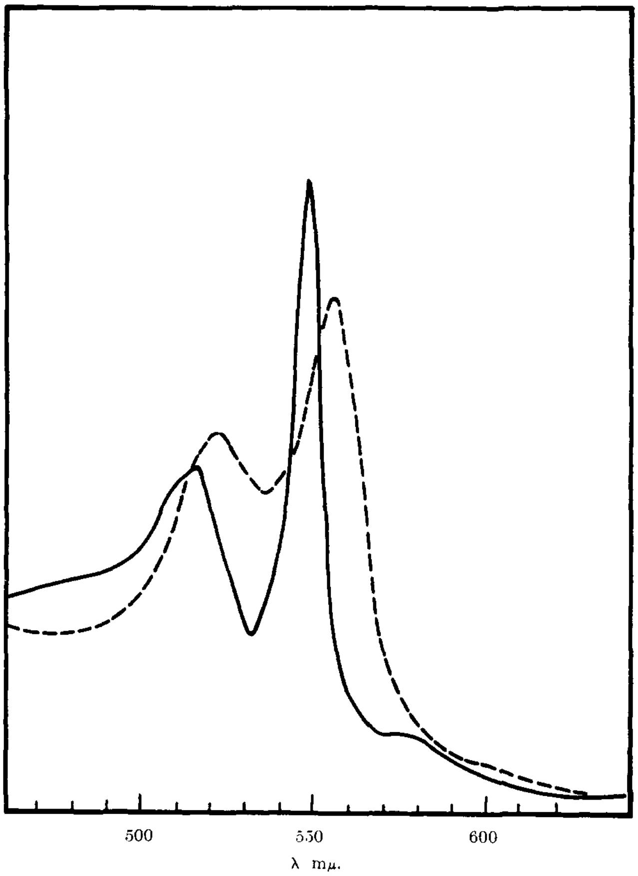

Fig. 3.—Spectra of pyridine "hemochromogen," solid line; and mesoheme IX in 60% tetrahydrofuran–40% acetic acid, broken line (wave lengths in mμ).

The spectra of the ferric porphyrins used in the preparation of all of the ferrous complexes have been recorded (Table V). All of these spectra are indicative of the high spin configuration. While the high spin ferric configuration is favored under solvent conditions that favor low spin ferrous configuration, the spectra of ferrous complexes observed in high dielectric constant media are high spin types. We interpret the gradual change of spectrum with increasing dielectric constant to be due to increasing amounts of the high spin ferrous complex. This phenomenon has been observed in other ferrous porphyrin complexes.[17] Fig. 4 shows the striking resemblence of the spectrum of our ferrous complex in N,N-dimethylformamide (also in N-methylformamide) to that of hemoglobin.[18] If we accept the spectrum of hemoglobin as a reference standard for a ferrous spectrum of high spin type, the solutions of ferrous mesoporphyrin in media of high dielectric constant show residual absorption at the β band which is attributed to the persistance of some low spin complex. Because of the high energy required to promote the metal in cobaltic mesoporphyrin IX hydroxide to the high spin state, this change does not occur. For this reason the behavior of the cobaltic complex constitutes a model for the behavior of the

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(15) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, pp. 148–150.

(16) See for example, J. E. Falk, *Chromatog. Rev.*, **4**, 142 (1962), and R. J. P. Williams, *Chem. Rev.*, **56**, 299 (1956).

(17) L. E. Orgel, "Haematin Enzymes," J. E. Falk, R. Lemberg, and R. K. Morton, Ed., Pergamon Press, Inc., New York, N. Y., 1961, p. 5.

(18) D. Drabkin in "Haemoglobin Symposium" in memory of Sir Joseph Barcroft, Interscience Publishers, Inc., New York, N. Y., 1949, p. 35.

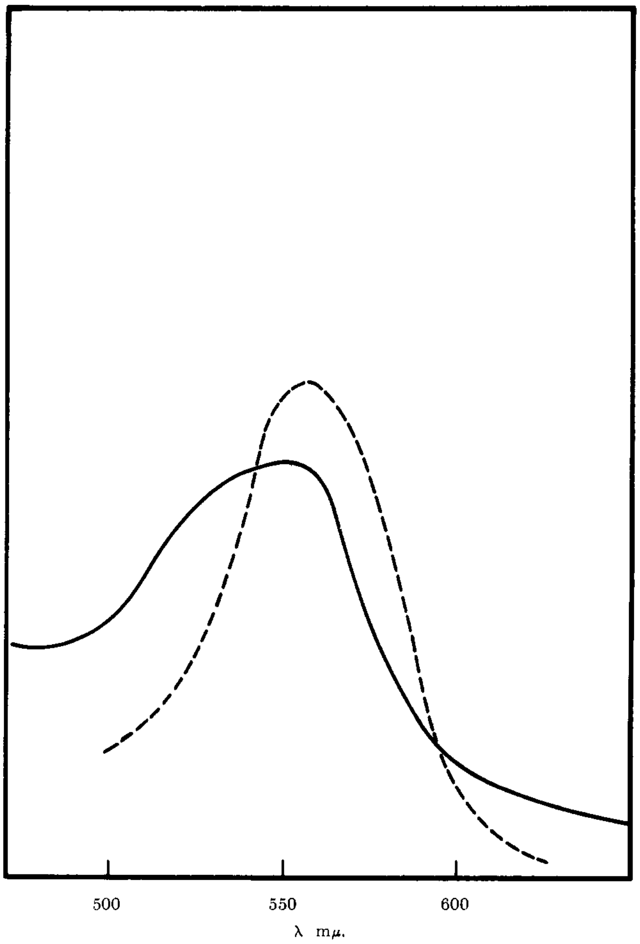

Fig. 4.—Spectra of mesoheme IX in 60% N,N-dimethylformamide–40% acetic acid, solid line; hemoglobin, broken line.

isoelectronic low spin ferrous complexes. Deviations of the behavior of the ferrous complex from that of the cobaltic complex which are actually observed are a measure of the degree of change of the ferrous complex from the low spin to high spin case.

It is interesting to contrast the solvent effects on the oxidation–reduction potentials of the ferrous and cobaltic mesoporphyrin. In the case of cobaltic, any effect of the dielectric constant is obscured by the powerful ligand effect. In a large number of solvents, the oxidation–reduction potential of the cobalt mesoporphyrin system lies close to that of molecular oxygen in air. Most ferrous complexes have been prepared in the presence of powerful ligands which alter the oxidation–reduction potential of the iron in a direction favoring the ferrous form. Our experiments with calomel point to this. However, even in the presence of strong ligands the ferrous becomes more difficult to obtain as the dielectric constant of the medium increases. Our observations show that both the solvent field and the ligand field must be considered in determining the spectral properties of iron–porphyrin complexes.

Experimental

Cobaltous Mesoporpyrin IX.—The method is based on that reported by Taylor.[19] Cobaltous acetate tetrahydrate (0.5 g., 2×10^{-3} mole) was dissolved in glacial acetic acid (300 ml.) with heating. Mesoporphyrin IX[20] (0.350 g., 0.62×10^{-3} mole) was dissolved in hot glacial acetic acid (300 ml.) with stirring. Deoxygenation was effected by passing nitrogen through the hot solutions. The cobaltous acetate solution was added to the porphyrin solution in several portions (*ca.* 50 ml. each) with vigorous stirring. Heating and stirring were continued for 30 min. After cooling to room temperature the mixture was filtered with suction and the precipitate washed with acetic acid and then ether. The product was dried *in vacuo;* yield, 0.33 g., 84%. The spectrum of cobaltous mesoporphyrin obtained was identical to that reported by Taylor.

Cobaltic Mesoporphyrin IX Hydroxide.—Cobaltous mesoporphyrin IX (*ca.* 0.1 g.) was suspended in *N* sodium hydroxide solution (20 ml.) and stirred vigorously for 1.5 hr. to complete oxidation. A small amount of solid was then removed by filtration. The solution was adjusted to pH 5.5 with glacial acetic acid. The precipitate was collected by filtration, washed with water, and dried *in vacuo*. The yield was nearly quantitative.

Anal.[21] Calcd. for $C_{34}H_{37}O_5N_4Co$ (640.61): C, 63.74; H, 5.82; Co, 9.20. Found: C, 64.13; H, 5.60; Co, 9.29.

Ferrous Mesoporphyrin IX and Ferrous Mesoporphyrin IX Dimethyl Ester.—Mesohemin IX chloride and mesohemin IX chloride dimethyl ester were prepared by the method of Erdman and Corwin.[22] For this preparation of ferrous mesoporphyrin IX (or dimethyl ester) from the ferric derivative, the porphyrin complex (*ca.* 3 mg.) was dissolved in glacial acetic acid (50 ml.). A 1-ml. sample of this solution was transferred by hypodermic syringe to a 13 × 100 mm. Pyrex test tube in which the neck had been drawn to a 1-mm. bore. Analytical grade iron powder (1–2 mg.) and in some cases tetramethylammonium chloride (10–20 mg.) were added together with glacial acetic acid (1 ml.) and the selected solvent (3 ml.). The tubes were cooled and sealed. Then the tubes were positioned horizontally on a reciprocating shaker and shaken at the rate of 120 shakes per minute at room temperature. Reduction to the ferrous complex was determined spectrophotometrically, after centrifuging the tube, by observing the disappearance of the ferric band at 630 mμ and the appearance of the strong ferrous peak at 550–560 mμ. Complete reduction was generally achieved in 2–12 hr. depending upon the exact conditions employed. The salmon pink color of the ferrous complex was replaced immediately by the dull brown color of the ferric complex upon opening the tubes.

Determinations of spectra for the region 350–450 mμ were made on samples containing approximately 0.10 as much porphyrin, but the same quantities of other reagents.

Spectra.—All spectra were taken on a Beckman DK-2 recording spectrophotometer. The spectra of cobalt and ferric compounds were taken in matched quartz cells while those of ferrous complexes were taken in sealed Pyrex test tubes. The pyrex tubes were found to be transparent over the region 900–320 mμ. Solvent *vs.* solvent determinations agreed within 5% transmission for the Pyrex tubes and computed "path lengths" varied from 1.62–1.81 cm. Temperature studies were made using a Beckman cell compartment heating unit. The wave-length calibration of the instrument was checked with a mercury source.

Determination of Extinction Coefficients for Ferrous Mesoporphyrin IX.—The ferrous porphyrin was prepared in sealed tubes as described. An arbitrary index mark was made on the tube. The spectrum was recorded using a similar sealed tube containing solvent as the reference. The neck of the tube was cracked off, the tube cleaned, and then filled with a solution of cobaltic mesoporphyrin IX hydroxide of known concentration in pyridine. Comparison spectra were taken in the region 600–500 mμ with the index mark at the same position. Effective path lengths for each Pyrex tube were then calculated for wave lengths 562 and 530 mμ. Agreement was excellent (2%). These path lengths were then used to calculate the extinction coefficients for the ferrous complexes.

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[19] J. F. Taylor, *J. Biol. Chem.*, **135**, 569 (1940).

[20] Prepared by alkaline catalytic reduction, E. W. Baker, M. Ruccia, Mary Lachman, and A. H. Corwin, unpublished.

[21] Microanalyses were performed by Mr. Joseph Walter.

[22] J. G. Erdman and A. H. Corwin, *J. Am. Chem. Soc.*, **69**, 750 (1947).