  Bull. Chem. Soc. Jpn., 60, 1015—1019 (1987)

Concentration Quenching of Fluorescence from Bridged Synthetic Porphyrins

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(Received August 21, 1986)

Concentration quenching phenomena of singly and doubly bridged porphyrins and their zinc and magnesium complexes were studied. Hypothetical nonfluorescent dimer trap was suggested to be not a coplanarly stacked face-to-face dimer but a loosely interacting pair.

In the primary events of green plant photosynthesis, photons are absorbed by the antenna chlorophyll molecules and the resultant singlet excitation is transfered from molecule to molecule and ultimately trapped to the reaction center, where the efficient one-electron transfer from the special chlorophyll dimer to an appropriate acceptor takes place very rapidly. However, in vitro, chlorophylls and/or synthetic porphyrins exhibit the phenomenon of concentration quenching[1] of the excited singlet state whether in fluid solutions,[2,3] or in organized assemblies such as lipid monolayers,[4] multilayers,[5] lipid bilayer vesicles,[6] and detergent micelles,[7] or in a swollen polyethylene phase in the presence of other amphiphilic substances.[8] Whenever chlorophyll concentration is high enough for being comparable with that (ca. 10^{-1} M†) in the chloroplast, the singlet excited state is efficiently quenched, with complete annihilation of its photochemistry and usually that of the triplet also. Since singlet-singlet energy transfer between chlorophyll (or porphyrin) monomers (Eq. 1) has been well-established as a key step in photosynthesis and does not lead to the consumption of the excitation energy, it is reasonable to assume that dimers or aggregated form of chlorophyll or porphyrin is involved in the nonradiative decaying process of the concentration quenching. A possible molecular mechanism for the quenching process in homogeneous solutions has been suggested to involve capture of excitation energy by a nonfluorescent dimer trap[9] (Eq. 2—4).

$$^1P^* + P \xrightarrow{k_1} P + {}^1P^* \quad (1)$$

$$P + P \xrightarrow{K} P{-}P \quad (2)$$

$$^1P^* + P{-}P \xrightarrow{k_2} P + {}^1P{-}P^* \quad (3)$$

$$^1P{-}P^* \xrightarrow{k_d'} P{-}P \quad (4)$$

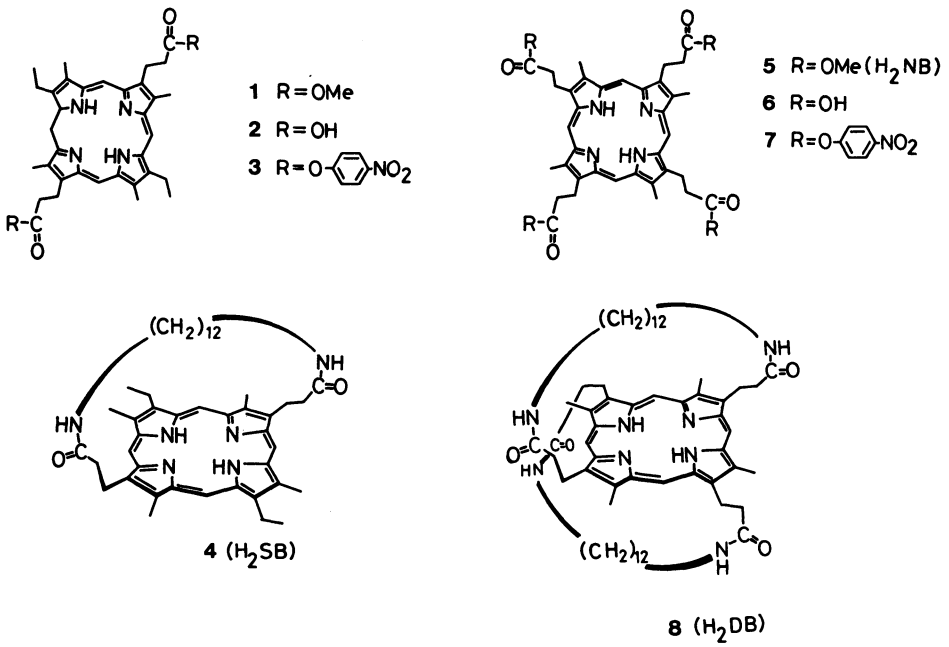
Although a large number of measurements on fluorescence lifetimes and fluorescence quantum yields have been reported in vivo and in vitro, relatively little is known about the steric effects around the porphyrin macrocycle upon the concentration quenching of fluorescence. Singlet energy transfer of chlorophyll and/or porphyrins may proceed via Förster-type inductive resonance-transfer mechanism (dipole–dipole interaction)[10] and thus may be independent of the steric hindrance around the macrocycles. However, one can expect *the steric effects on the formation of the hypothetical non fluorescent dimer* (Eq. 2). In order to obtain a better understanding of the quenching process and to make a model of an antenna chlorophyll function *in solution,* we have undertaken systematic studies on fluorescence properties of bridged porphyrins **H_2SB** and **H_2DB.**

Results and Discussion

Mesoporphyrin-II dimethyl ester (**1**) was prepared according to Chang's modified procedure.[11] Singly bridged porphyrin **4** (**H_2SB**) was synthesized in 45% yield by the reaction of 1,12-dodecamethylenediamine with mesoporphyrin-II bis(4-nitrophenyl) ester **3**,[12] which, in turn, was prepared by the treatment of mesoporphyrin-II dicarboxylic acid **2** with 4-nitrophenyl trifluoroacetate.[12] Coproporphyrin-I tetramethyl ester **5** (**H_2NB**) was hydrolyzed to the corresponding tetracarboxylic acid **6**, which was converted into tetrakis(4-nitrophenyl) ester **7.** The reaction of **7** with 1,12-dodecamethylenediamine in a dilute pyridine solution gave doubly bridged porphyrin **8** (**H_2DB**) in 8% yield. Dodecamethylene bridged structures of **H_2SB** and **H_2DB** were apparent from their 400 MHz ^{1}H NMR spectra; dodecamethylene protons in **H_2SB** appeared at δ −0.26 (4H, m), −0.12 (4H, m), 0.07 (4H, m), 0.14 (4H, m), 0.53 (4H, m), and 2.80 (4H, m) ppm; those in **H_2DB** appeared at δ −0.22 (8H, m), −0.11 (8H, m), 0.06 (8H, m), 0.18 (8H, m), 0.47 (8H, m), and 2.81 (8H, m) ppm. Almost the same chemical shifts of dodecamethylene protons in **H_2SB** and **H_2DB** indicate that a dodecamethylene bridge has a similar conformation in both porphyrins and thus may provide a similar degree of steric hindrance on one porphyrin side.

The optical absorption spectra and the fluorescence emission spectra of **H_2SB** and **H_2DB**, and their zinc and magnesium complexes were not significantly

† 1 M=1 mol dm^{-3}.



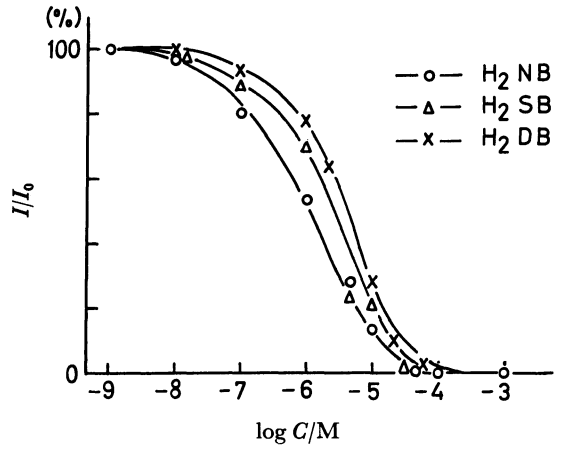


Fig. 1. Relative fluorescence yields of H_2NB, H_2SB, and H_2DB in CH_2Cl_2–CH_3OH (v/v=95/5) at 25 °C as a function of concentration.

altered by introduction of dodecamethylene bridge (Table 1). Relative fluorescence quantum yields were determined in CH_2Cl_2–MeOH (v/v=95/5) or CH_2Cl_2 at various concentration of porphyrins (Figs. 1—4). As shown in Fig. 1, there are small but distinct differences among **H_2NB**, **H_2SB**, and **H_2DB**. Critical concentrations of porphyrins and metalloporphyrins are defined as the concentration where an half of the intrinsic porphyrin fluorescence yields is lost by self-concentration quenching. The critical concentrations of **H_2NB**, **H_2SB**, and **H_2DB** are 1.1×10^{-6} M, 2.8×10^{-6} M, and 5.0×10^{-6} M, respectively (Table 2). Thus, the introduction of a dodecamethylene bridge roughly doubled the critical concentration of free base porphyrins in dichloromethane at room temperature.

Effects of the dodecamethylene bridge were much more pronounced in zinc porphyrins, **ZnNB, ZnSB,** and **ZnDB** (Fig. 2). Fluorescence concentration

Table 1. Absorption and Emission spectra of Nonbridged and Bridged Porphyrins[a)]

Porphyrin	Absorption/nm		Emission nm	
	Soret band ($\varepsilon\times10^{-5}$)	Q-bands ($\varepsilon\times10^{-3}$)		
H_2NB	398 (1.48)	496 (10.8), 533 (8.8), 570 (5.6), 620 (5.0)	620	686
H_2SB	398 (1.47)	497 (9.7), 534 (7.5), 567 (5.7), 623 (4.7)	622	687
H_2DB	402 (1.48)	500 (11.0), 531 (7.2), 568 (4.3), 622 (3.8)	622	688
ZnNB	406 (4.04)	533 (21), 570 (32)	578	624
ZnSB	404 (3.95)	533 (20), 570 (30)	578	627
ZnDB	404 (4.09)	533 (19), 569 (26)	578	630
MgNB	411 (4.16)	546 (18), 582 (17)	587	627
MgSB	409 (4.17)	545 (19), 582 (23)	587	627
MgDB	408 (4.26)	545 (20), 582 (27)	588	628

a) Measured in CH_2Cl_2 at 10^{-5} M at 25 °C.

quenching of **ZnNB** occurred at more dilute conditions compared with that of **H_2NB** and the critical concentration of **ZnNB** was only 5.2×10^{-8} M. Zinc-complexes of porphyrins have been shown to be five-coordinate in a number of studies,[13] and we expect the same to be true for **ZnNB** and **ZnSB.** Therefore, the concentration quenching of zinc porphyrins may be in part due to the formation of a dimer (or larger aggregate) bridged by coordination of one ester or amide group to other central zinc. The presence of the dodecamethylene bridge over the porphyrin macrocycle must result in retardation of such coordination, thus suppressing the concentration quenching. Accordingly, doubly bridged zinc porphyrin **ZnDB** showed the nearly same critical concentration (2.0×10^{-6} M) as that of the corresponding free base, **H_2DB.**

While effects of the dodecamethylene bridge upon the concentration quenching of Mg-porphyrins were much less than those of Zn-porphyrins in a CH_2Cl_2-MeOH (v/v=95/5) solution (Fig. 3), those became more prominent in an aprotic nonpolar solution (CH_2Cl_2) (Fig. 4). This may be ascribed to a much stronger coordination of methanol to the central magnesium, which leads to monomerization of Mg-porphyrin.

Fluorescence lifetimes of these nonbridged and bridged porphyrins and metalloporphyrins were measured by the time-correlated single-photon-counting technique. For all of the solution studied, the fluorescent decay is a single exponential within experimental error (Table 3). In accordance with the results of the fluorescence quantum yields, fluores-

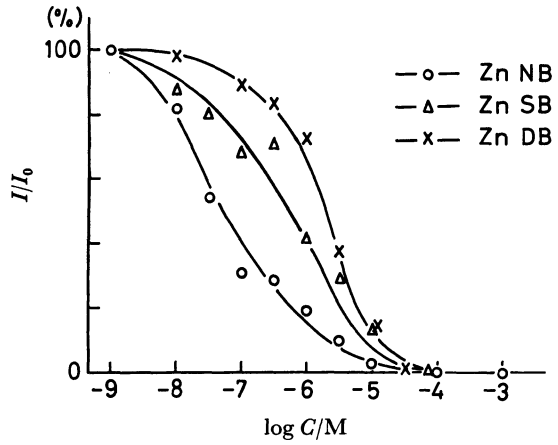


Fig. 2. Relative fluorescence yields of ZnNB, ZnSB, and ZnDB in CH_2Cl_2-CH_3OH (v/v=95/5) at 25 °C as a function of concentration.

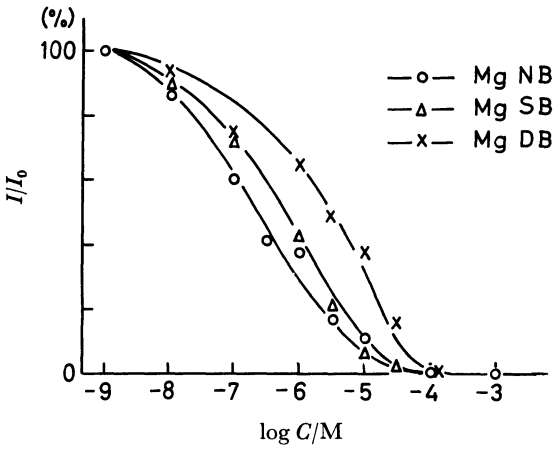


Fig. 3. Relative fluorescence yields of MgNB, MgSB, and MgDB in CH_2Cl_2-CH_3OH (v/v=95/5) at 25 °C as a function of concentration.

Table 2. Critical Concentration[a] of Porphyrin Fluorescence Quenching

Central metal	NB	SB	DB
H_2	1.1×10^{-6}	2.8×10^{-6}	5.0×10^{-6}
Zn	5.2×10^{-8}	5.6×10^{-7}	2.0×10^{-6}
Mg	2.5×10^{-7}	6.3×10^{-7}	3.2×10^{-6}
Mg[b]	7.9×10^{-8}	1.0×10^{-6}	7.1×10^{-6}

a) Defined in the text, M in CH_2Cl_2-MeOH (v/v, 95/5) at 25 °C. b) In CH_2Cl_2 at 25 °C.

Table 3. Fluorescence Lifetimes[a] (ns) of Bridged Porphyrin

Central metal	NB	SB	DB
H_2	11.5	12.4	14.0
Zn	1.69	1.71	2.04
Mg	6.3	6.7	7.2

a) 10^{-5} M in CH_2Cl_2, aerated conditions. Excitation at soret band in Table 1, measured at 620 nm (free base), at 578 nm (Zn complexes), and at 587 nm (Mg complexes), and measured on a HORIBA Model NAES 1100 nanosecond lifetime fluorometer at 25 °C.

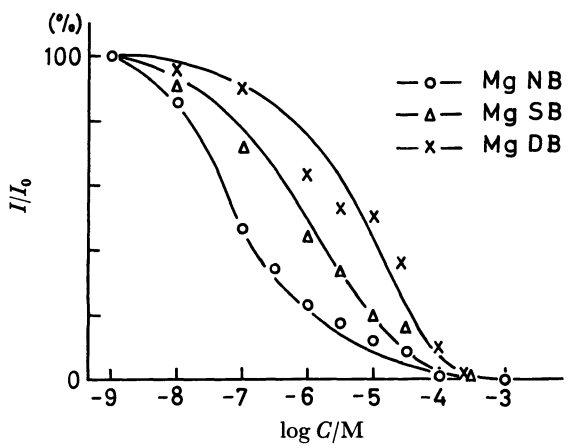


Fig. 4. Relative fluorescence yields of MgNB, MgSB, and MgDB in CH_2Cl_2 at 25 °C as a function of concentration.

Table 4. Relative Fluorescence Quantum Yields[a] in Micellar System

Porphyrin	SDS[b]	CTAB[c]	Brij-58
H_2NB	1.0	1.0	1.0
H_2SB	1.5	1.1	1.07
H_2DB	4.5	1.3	1.12

a) Relative fluorescence quantum yields of **H_2SB** and **H_2DB** to that of **H_2NB** measured at 25 °C. b) Porphyrin concentration was 1×10^{-6} M, and detergent concentration was 6×10^{-2} M. b) Sodium dodecyl sulfate. c) Hexadecyltrimethylammonium bromide.

cence lifetime increased in the order of **H_2NB<H_2SB<H_2DB, ZnNB<ZnSB<ZnDB,** and **MgNB<MgSB<MgDB.** At 10^{-5} M, the introduction of a dodecamethylene bridge increased the singlet lifetimes of **H_2NB** and **H_2SB** by ca. 1.5 ns.

Finally, we examined the relative fluorescence quantum yields of porphyrins in micellar systems such as sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide (CTAB), and Brij-58. It is interesting to note that the fluorescence quantum yield of **H_2DB** was retained 4.5 times as much as that of **H_2NB** in a SDS micelle. This result may suggest the steric hindrance around the porphyrin macrocycles becomes increasingly important in the more ordered and highly viscous environment.

In all the cases examined here, the steric hindrance imposed by the dodecamethylene bridge diminished the concentration quenching phenomena of porphyrin and metalloporphyrin to some extent. However, the concentration quenching was still observed even in the doubly bridged system under much more dilute conditions than those of antenna chlorophyll in chloroplast. Since a stacked face-to-face dimer cannot be formed from **H_2DB** and its metal complexes, it seems unnecessary to form such a compact dimer for the self-concentration quenching. In other words, the hypothetical nonfluorescent dimer may have a very loose structure.

Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were recorded on a JASCO model 402-G spectrometer. 400 MHz ^{1}H NMR spectra were recorded on a JEOL GX-400, and chemical shifts are reported in parts per million on the δ scale from internal tetramethylsilane. Elemental analyses are performed at the Microanalytical Laboratory of Kyoto University. Preparative separations were usually performed by flash column chromatography on silica gel (Merk, Kieselgel 60 H, Art. 7736). Fluorescence spectra and fluorescence quantum yields were measured under aerobic conditions on a Shimadzu model RF 502 fluorometer. Fluorescence lifetimes were measured by the time-correlating single-photon-counting technique on a HORIBA NAES 1100 model.

1,12-Dodecamethylenediamine, 4-nitrophenyl trifluoroacetate (Aldrich), SDS, CTAB, and Brij-58 were commercially available and were used without further purification. Mesoporphyrin-II dimethyl ester (**H_2NB**),[11] mesoporphyrin-II dicarboxylic acid (**1**),[12] mesoporphyrin-II bis-(4-nitrophenyl) ester (**2**),[12] and coproporphyrin-I tetramethyl ester (**5**)[13] were prepared according to the reported procedures. Coproporphyrin-I tetracarboxylic acid (**6**) was prepared by acid-catalyzed hydrolysis of **5**.[12]

Preparation of Singly-Bridged Porphyrin H_2SB. To 400 mL of dry pyridine (maintained at 65—70 °C) were added six equimolar portions of mesoporphyrin-II bis(4-nitrophenyl) ester (**2**) (646 mg, 0.8 mmol) and 1,12-dodecamethylenediamine (169 mg, 0.8 mmol) under argon atmosphere at 2 h intervals. After completion of the last reagent addition, the reaction mixture was left overnight at 65 °C. The pyridine was removed on the rotary evaporator. The residue was dissolved in CH_2Cl_2–MeOH (v/v=5/1) and filtered through alumina short column. Filtrate was concentrated and then separated on flash column chromatography using CH_2Cl_2–MeOH (v/v=20/1) as the eluant. First porphyrin fraction was singly bridged porphyrin , **5 (H_2SB)**, yield 262 mg, 45%; mp> 300 °C. IR (KBr) 2860 and 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=10.11 (s, 2H, meso H), 10.07 (s, 2H, meso H), 4.80 (br., 2H, -CONH-), 4.66 (dd, 2H, -C$\underline{H}_2$CH$_2$CO-), 4.11 (dd, 2H, -C$\underline{H}_2$CH$_2$CO-), 4.10 (q, 4H, -C$\underline{H}_2$CH$_3$), 3.25 (dd, 2H, -CH$_2$C$\underline{H}_2$CO-), 3.07 (dd, 2H, -C$\underline{H}_2$CH$_2$CO-), 1.87 (t, 6H, -CH$_2$C$\underline{H}_3$), and -3.78 (br, 2H, N$\underline{H}$). Chemical shifts due to the dodecamethylene bridge were discussed in the text.

Coproporphyrin-I Tetrakis(4-nitrophenyl) Ester (7). Coproporphyrin-I tetracarboxylic acid (**6**) (654 mg, 1 mmol) was dissolved in dry pyridine (300 mL) under nitrogen atmosphere. 4-Nitrophenyl trifluoroacetate (3.3 g, 14 mmol) was added to the above solution and the resulting solution was stirred at 50—55 °C for 3 h, and then at 10 °C for 12 h. Microcrystalline solids were precipitated, which were collected and washed with pyridine and hexane, and dried under vacuum to give **7**, (1.49 g, 88%): mp, 204—206 °C; IR (KBr) 1770 (ester C=O), 1500 and 1350 (NO_2) cm^{-1}. ^{1}H NMR δ=10.22 (s, 8H, meso H), 7.94 (d, 8H), 6.88 (d, 8H), 4.62 (t, 8H, C$\underline{H}_2$-CH$_2$CO-), 3.68 (t, 8H, CH$_2$C$\underline{H}_2$CO-), 3.62 (s, 12H, CH$_3$), 3.65 (br, 2H, NH).

Doubly Bridged Porphyrin, 8 (H_2DB). Doubly bridged porphyrin **8 (H_2DB)** was synthesized in the same fashion with **5** by the reaction of **7** (890 mg, 0.8 mmol) with 1,12-dodecamethylenediamine (320 mg, 1.6 mmol). Separation by flash column chromatography over silica gel using CH_2Cl_2–MeOH (v/v=5/1) gave **8**, (63 mg, 8%) as the first fraction. **8 (H_2DB)**; mp >300 °C; IR (KBr) 2860 and 1640 (amide C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$–CD_3OD, v/v=20/1) δ=10.7 (s, 4H, meso), 4.87 (br., 4H, -CONH-), 4.65 (dd, 4H, -C$\underline{H}_2$CH$_2$CO-), 4.15 (dd, 4H, -C$\underline{H}_2$CH$_2$CO-), 3.62 (s, 12H, -CH$_3$), 3.19 (dd, 4H, -CH$_2$C$\underline{H}_2$CO-), 3.00 (dd, 4H, -CH$_2$C$\underline{H}_2$CO-), and −3.75 (br, 2H, NH). Chemical shifts due to the dodecamethylene bridge was discussed in the text.

References

1) For review, see: K. Sauer, in "Bioenergetics of Photosynthesis", ed. by Govindjee, Academic Press, New

York (1975).

2) W. F. Watson and R. Livingston, *J. Chem. Phys.*, **18**, 802 (1950).

3) M. J. Yuen, L. L. Shipman, J. J. Katz, and J. C. Hindman, *Photochem. Photobiol.*, **32**, 281 (1980); J. A. Anton, P. A. Loach, and Govindjee, *ibid.*, **28**, 235 (1978).

4) W. D. Bellamy, A. G. Tweet, and G. L. Gaines, *J. Chem. Phys.*, **41**, 2068 (1964); T. Trosper, R. B. Park, and K. Sauer, *Photochem. Photobiol.*, **7**, 451 (1968).

5) A. R. Kelly and G. Porter, *Proc. Roy. Soc.*, **A 315**, 149 (1970); A. R. Kelly and L. K. Paterson, *ibid.*, **A 324**, 117 (1971).

6) G. S. Beddard, S. E. Carlin, and G. Porter, *Chem. Phys. Lett.*, **43**, 27 (1976); A. G. Lee, *Biochemistry*, **14**, 4397 (1975).

7) E. Lehoczki and K. Csatorday, *Biochem. Biophys. Acta*, **396**, 86 (1975).

8) G. R. Seely and V. Senthilarthpan, *J. Phys. Chem.*, **87**, 373 (1983), and references cited therein.

9) G. S. Beddard and G. Porter, *Nature*, **260**, 366 (1976).

10) N. J. Turro, "Modern Molecular Photochemistry," The Benjamin/Cummings Publishing Co. Inc. (1978), Chapt. 9, p. 296.

11) C. K. Chang, *J. Am. Chem. Soc.*, **99**, 2819 (1977).

12) J. P. Collman, F. C. Anson, C. E. Barnes, C. S. Bencosme, T. Geiger, E. R. Evitt, R. P. Kreth, K. Meier, and R. B. Pettman, *J. Am. Chem. Soc.*, **105**, 2694 (1983).

13) A. H. Jackson, G. W. Kenner, and J. Wass, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1475.

14) J. L. Hoard in K. M. Smith, ed. "Porphyrins and Metalloporphyrins," Elsevier, New York (1975), Chapt. 8, p. 345.