Electron Spin Resonance Spectra of Chloro(N-methyl-5,10,15,20-tetraphenylporphinato)copper(II) and Manganese(II) Complexes. Demethylation Mechanism Studies

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Received June 7, 1984

Abstract

ESR spectra of Cu(II) and Mn(II) complexes of N-methyl-5,10,15,20-tetraphenylporphyrin (NCH_3-TPPH) were studied. A large influence of the pyrrole nitrogen methylation on ESR parameters of Cu(NCH_3TPP)Cl and Mn(NCH_3TPP)Cl as compared to Cu(TPP) or Mn(TPP) was observed and discussed.

The five-coordinate forms of formula Mn(NCH_3-TPP)X, where X = Cl^- or solvent molecule, were defined by characteristic $g_\perp^{eff}$ values. The rhombic ESR Hamiltonian ($D \neq 0$, $E \neq 0$) was accounted for the observed ESR spectra of the five-coordinate forms contrary to the axial one ($D \neq 0$, $E = 0$) for Mn(TPP). Spontaneous demethylation of the Cu(NCH_3TPP)Cl complex and formation of Cu(TPP) was observed on the basis of ESR spectra. The decomposition of Mn(NCH_3TPP)Cl in the presence of di-n-butylamine (DBA) was analysed. The ESR experimental evidence was found for the presence of Mn(TPP)DBA and Mn(TPP)O_2 species as intermediates of this process.

Introduction

N-alkylporphyrins are formed as products of the interaction of cytochrome P-450 with a variety of substances [1]. This fact has drawn an increasing interest toward the chemistry of N-substituted porphyrins and corresponding metal complexes. Structural analysis undertaken via X-ray diffraction demonstrated clearly a pronounced deviation from planarity resulting from the nitrogen atom substitution [2]. The mono-N-methylporphyrin may serve as a model for the distorted porphyrin which incorporates the metal ion [3–6].

Coordination of some metal ions to N-methylporphyrins promotes the demethylation of the pyrrolic nitrogen and transfer of the CH_3^+ fragment to another nitrogen base present in solution [7–10]. This process should be essential in an understanding of alkyl fragment transfer within the molecule [11]. Some complexes of N-alkylporphyrins are strong alkylating agents which may show selective cytotoxicity in the case of leukaemia diseases [12].

We present here the results of electron-spin-resonance (ESR) studies of chloro(N-methyl-5,10,15,20-tetraphenylporphinato)copper(II) and manganese(II) complexes. (Abbreviations: Cu(NCH_3-TPP)Cl and Mn(NCH_3TPP)Cl). Particularly, we were interested in the influence of the N-methylation on ESR parameters of the complexes in comparison with the parent compounds, *i.e.* Cu(TPP) and Mn(TPP). The application of ESR allowed to elucidate some aspects of the demethylation process. The respective reaction intermediates were 'trapped' by ESR, whereas the widely-used electronic spectroscopy is not very sensitive in this respect as the visible region is almost identical for different metals and ligation states [3]. The ESR spectroscopy was also applied to elucidate the coordination of nitrogen bases to the N-alkylporphinato Cu(II) and Mn(II) complexes.

Experimental

Synthesis of N-methyl-5,10,15,20-tetraphenylporphyrin (NCH_3TPP)

The new procedure was worked out for the synthesis of NCH_3TPP. Dimethyl sulphate was used as a powerful methylating agent. In the typical procedure 250 mg $TPPH_2$ (FLUKA) was dissolved in 250 cm^3 of meta-(ortho)-dichlorobenzene (EGA-CHEMIE). The solution was refluxed and 1 cm^3 of $(CH_3)_2SO_4$ (INTERN. ENZYMES LTD.) was added. The reflux was continued for 1 h. The progress of the reaction was followed by visible spectra of the reaction mixture. The solution was cooled and neutralized with sodium carbonate. The solid residue was filtered off. The dichlorobenzene solution was chromatographed on acidic alumina (I grade, POCH). The first band was eluted by 10:1 v/v toluene–AcOEt and corresponded to unreacted

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$TPPH_2$. NCH_3TPPH was eluted by $CHCl_3$ and crystallized from 1:1 v/v $CHCl_3$–ethanol. The yield of the procedure is 25–35%, depending on the concentration of dimethyl sulphate, and is much higher when compared with CH_3I methylating agent (di- and trimethyl substitution takes place in this case). The selectivity of the reaction is lower than for CH_3SO_3F methylating agent [6]. The methylated product, however, can be obtained using our method in a relatively short period of time. The product identity was confirmed by its characteristic ^1H NMR (NCH_3 shift = –4.10 ppm *vs.* TMS) and visible electronic spectra.

Synthesis of Copper(II) and Manganese(II) Complexes

The synthesis of $Mn(NCH_3TPP)Cl$ complex was carried out as described previously [3] with small modification: addition of an excess of $MnCl_2$ in ethanol to CH_2Cl_2 solution of NCH_3TPPH and subsequent addition of 2,4,6-trimethylpyridine. The product was chromatographed on silica gel (230–400 mesh MERCK) and once more recrystallized from 1:1 v/v chloroform–hexane. The synthesis of $Cu(NCH_3TPP)Cl$ was carried out as described by Stinson and Hambright [13] combining $CuCl_2$ and NCH_3TPPH in methanol. The powdered product was dissolved in $CHCl_3$ and chromatographed on silica gel. The decomposition product [Cu(TPP)] was eluted by $CHCl_3$. Methanol used as eluent quickly removed the $Cu(NCH_3TPP)Cl$ from the column. The methanolic solution was evaporated using a vacuum evaporator. Visible electronic spectra of $Mn(NCH_3TPP)Cl$ and $Cu(NCH_3TPP)Cl$ in different solvents were identical with those reported previously [3, 6].

Equipment

Visible electronic spectra were measured with a SPECORD UV–Vis spectrometer. ESR spectra were obtained on a JES-ME spectrometer using a nuclear magnetometer MJ 110R, frequency meter JES-SH-30X and ESR standards. A JEOL 100PS NMR spectrometer was used to check the ligand identity.

Sample Preparations

All solvents and ligands were reagent grade and were used as obtained: DMSO (FLUKA), $CHCl_3$ (POCH), CH_2Cl_2 (FLUKA), N-methylimidazole (MERCK), 1,2-dimethylimidazole (MERCK), di-n-butylamine (LOBA CHEMIE), pyridine and 2,4,6-trimethylpyridine (FLUKA), isoxazole (MERCK), acetonitrile (RIEDEL-DE HAËN). The complexes were weighed to get a concentration in the range 1–5 mmol dm^{-3}. In some cases the $Cu(NCH_3TPP)Cl$ complex was taken directly from the chromatographic column and frozen immediately to prevent

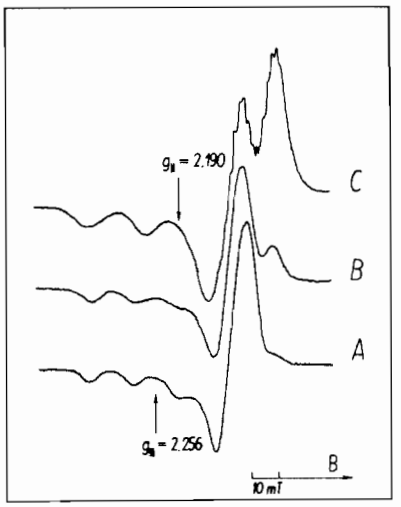

Fig. 1. ESR spectra of the reaction: $Cu(NCH_3TPP)Cl \xrightarrow[-Cl^-]{-CH_3^+} Cu(TPP)$ (Eqn. 1) at 130 K, ν = 9.250 GHz. Solvent: chloroform. A, 0.1 h; B, 3 h; C, 24 h after dissolution of $Cu(NCH_3TPP)Cl$ in chloroform, kept at room temperature. A, practically pure $Cu(NCH_3TPP)Cl$; C, practically pure Cu(TPP); B, a mixture of these complexes.

Cu(TPP) formation. The saturated solutions of $Mn(NCH_3TPP)Cl$ in acetonitrile–dibutylamine mixture (DBA concentration: 0.5 or 1 mol dm^{-3}) were prepared and degassed directly in ESR quartz tubes by freezing–thawing techniques. The samples were sealed to prevent any oxygen uptake and were heated (to 100 °C) for several hours. Every three hours the ESR spectra were taken. After completion of the reaction the sample was frozen in liquid nitrogen and opened to allow the oxygen addition. The separate sample of Mn(TPP) in DBA/acetonitrile mixture was prepared by reduction of Mn(TPP)Cl with sodium dithionite, as described previously [14].

Results and Discussion

$Cu(NCH_3TPP)X$ Complexes

The ESR spectra of $Cu(NCH_3TPP)X$ complexes are illustrated in Fig. 1. The spectra were obtained for frozen solutions using different solvents or mixtures of solvents to trace the solvation influence on ESR parameters. The $Cu(NCH_3TPP)Cl$ complex is unstable due to the spontaneous demethylation [8]. The freshly chromatographed samples (see Experimental) allow, however, the collection of the ESR spectra of the solution containing only the species of interest, *i.e.* $Cu(NCH_3TPP)Cl$. The demethylation was followed by ESR spectroscopy (Fig. 1); it may be described as

TABLE I. ESR Parameters for Cu(NCH_3TPP)Cl in Several Solvents (Cu(NCH_3TPP)X species) at 130 K.

Solvent	$g_\parallel$	$g_\perp$	$\lvert A_\parallel \rvert$ [10^{-4} cm^{-1}]
Chloroform	2.256	2.080	172
Pyridine	2.247	2.072	173
Methanol	2.240	2.060	174
1:4 v/v DBA–methanol	2.243	2.060	174
N-methylimidazole	2.252	2.084	158

TABLE II. ESR Parameters for Cu(TPP) in Several Solvents at 130 K.

Solvent	$g_\parallel$	$g_\perp$	$\lvert A_\parallel \rvert$ [10^{-4} cm^{-1}]
Chloroform	2.190	2.055	212
Pyridine	2.201	2.060	210
1:4 v/v DBA–methanol	2.200	2.058	211
In H_2TPP single crystal at 77 K[a]	2.190	2.045	211

[a]See Ref. 15.

$$Cu(NCH_3TPP)X \xrightarrow[-X]{-CH_3} Cu(TPP) \quad (1)$$

The following solvents were used: chloroform, pyridine, 4:1 v/v methanol–di-n-butylamine mixture. At room temperature the distinct signal of Cu(TPP) appears after 1 h. The spectrum of the same sample after 24 h demonstrates only Cu(TPP) signal. The ESR parameters of Cu(NCH_3TPP)X complexes are collected in Table I.

The ESR parameters are similar for chloroform, pyridine or methanol/DBA mixture solutions. The Cu(NCH_3TPP)(N-methylimidazole) complex presents, however, a different set of data characterized by a distinct decrease of $|A_\parallel|$ parameter. This effect is related to formation of a relatively strong Cu–N(3) of 1-methylimidazole bond. In this particular case the spontaneous demethylation is stopped; the respective ESR spectrum remains unchanged during a week. This fact strongly emphasizes the importance of the X ligand dissociation in a formation of an active intermediate [8, 10] in a sequence of reactions leading to the demethylation.

A comparison of the ESR parameters for Cu(NCH_3TPP)X and Cu(TPP) in the same solvents (see Tables I and II) demonstrates a large decrease of the $|A_\parallel|$ parameter and increase of the $g_\parallel$ parameter of the first complex. This effect reflects pronounced changes in the symmetry of the closest surrounding of Cu(II) in Cu(NCH_3TPP)X in relation to Cu(TPP). The latter has a planar geometry with Cu(II) in the porphyrin plane [16]. The X-ray structure of Cu(NCH_3TPP)X has not yet been determined, however it should resemble the structures of other complexes of general formula M(NCH_3TPP)X. In such cases the Cu(II) ion is five-coordinated. The geometry is based on a square pyramid considerably distorted in the basis due to methylation. The Cu(II) should be displaced above the plane defined by four pyrrole nitrogens. The direct mixing of 4s metal orbital to the ground state of Cu(II) caused by a symmetry lowering is responsible for the observed changes of $A_\parallel$ parameters. A similar effect has been observed for Schiff base Cu(II) complexes [17, 18].

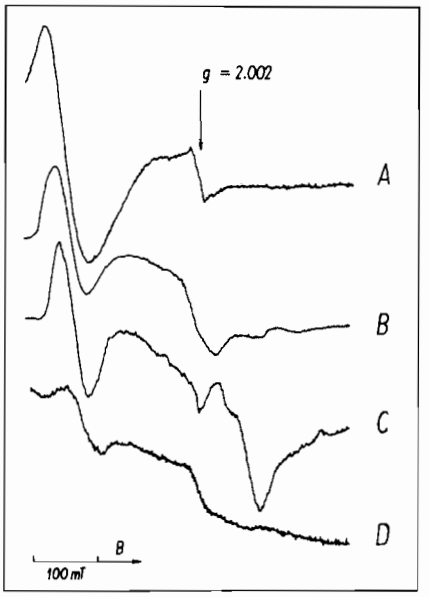

Fig. 2. ESR spectra of the powdered Mn(NCH_3TPP)Cl at 130 K, ν = 9.250 GHz. A, crystallized from 1:1 v/v acetonitrile–methanol mixture; B, after fast evaporation from chloroform solution; C, crystallized slowly from chloroform; D, sample C after heating at 400 K during 40 min interval.

Mn(NCH_3TPP)X Complexes

The observed ESR spectra (Figs. 2, 3, 4, Table III) are characteristic for the Mn(II) ion when the spin Hamiltonian

$$\hat{H}_{sp} = \beta B g \hat{S} + D(\hat{S}_z^2 - 35/12) + E(\hat{S}_x^2 - \hat{S}_y^2)$$

with $D \neq 0$ and $E \neq 0$ is postulated. In all cases the stronger signal in the $g_\perp^{eff}$ = 3.2–5.2 region and weaker one in the $g_\parallel^{eff}$ = 2 region are observed. In some cases the higher field transitions are also observed (see below). Hyperfine splitting from ^{55}Mn is

TABLE III. ESR Parameters for $Mn(NCH_3TPP)Cl$ in Several Solvents ($Mn(NCH_3TPP)X$ species) at 130 K.

Solvent[a]	$g_\perp^{eff}$	$A_\perp^{eff}$ [10^{-4} cm^{-1}]
N-methylimidazole	3.24	85
Di-n-butylamine (DBA)	3.25	84
1:1 v/v chloroform–DBA	3.80	84
1:1 v/v acetonitrile–DBA	4.05	
1 mol dm^{-3} DBA in acetonitrile	4.15	
1,2-dimethylimidazole	4.15	85
Pyridine	4.38	
DMSO	5.05	
DMF	5.10	
Quinoline	5.20; 4.02	
Isoxazole	5.14	
Sym-collidine	5.20	
Ortho-dichlorobenzene	5.20	
Chloroform[b]	4.9–5.2	

[a]In each case the electronic visible spectra have been checked. No noticeable solvent influence has been observed. [b]Depending on crystallization – see text.

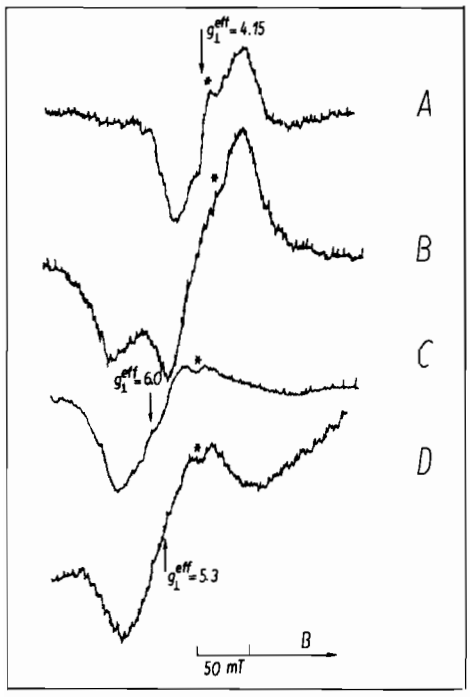

Fig. 3. ESR spectra of the reactions of $Mn(NCH_3TPP)Cl$ (Eqn. 2 – see text), at 130 K, ν = 9.250 GHz. A, saturated solution of $Mn(NCH_3TPP)Cl$ in solution of 0.5 mol dm^{-3} DBA in CH_3CN. B, sample A heated for 3 h at 373 K at anaerobic conditions; C, sample A heated for 24 h at 374 K at anaerobic conditions; D, sample C after oxygen addition ($Mn(TPP)O_2$ is formed). Note: asterisk* – signal from small admixture of Fe(III).

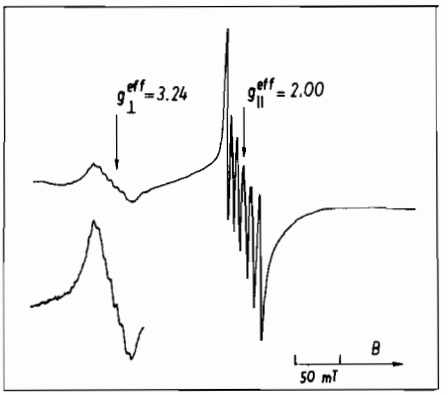

Fig. 4. ESR spectrum of $Mn(NCH_3TPP)Cl$ in N-methylimidazole at 130 K, ν = 9.250 GHz. Note: lines in parallel part of the spectrum (g = 2 region) result partially from the solvolysis of the complex. The gradual solvolysis was confirmed by UV–VIS spectra.

resolved into the $g_\perp^{eff}$ and $g_\parallel^{eff}$ bands but only for few systems (see Table III).

The $\hat{\tilde{D}}$ tensor is non-axial in the case of all complexes under investigation. The following conditions were imposed on spin Hamiltonian parameters in order to explain the observed patterns of ESR spectra: $D \gg h\nu$ and $E/D \approx 0.3$ [19, 20], particularly when a nitrogen base is coordinated to Mn(II). The $g_\perp^{eff}$ values depend strongly on the axial ligand coordination (see Table III). The methyl group of NCH_3-TPP ligand provides effective stereochemical blocking against coordination of a sixth ligand [2a] and the $Mn(NCH_3TPP)X$ complexes are five-coordinate in solution. The $g_\perp^{eff}$ values are determined by the E/D ratio [19–21]. These values are lowest for strong nitrogen bases as solvents; in this case the ligand X properties are more similar to those displayed by pyrrole nitrogens of NCH_3TPP ligand and the axial distortion of the ligand field decreases relatively to the non-axial field. In contrast to the $Mn(NCH_3TPP)X$ complexes, the Mn(TPP) complex has only strong axial distortion: $g_\perp^{eff} = 6$, $D \gg h\nu$, $E = 0$, and weak solvation effect [22, 23].

The powder samples of $Mn(NCH_3TPP)Cl$ were examined by ESR. The strong solvation effect was observed in this case (see Fig. 2). The samples crystallized from chloroform exhibit at X-band the transitions about 0.13T, 0.33T, 0.40T, 0.44T, 0.47T. The positions and intensities of these lines depend on the way of crystallization. High field lines are absent for the crystals obtained from other solvents; the heating of the samples presenting high field ESR lines leads to decay of these lines. The observed spectra (Fig. 2) are related to chloroform adduct formation; the $\hat{\tilde{D}}$ tensor is modified in the presence of $CHCl_3$ in crystals, which results in new ESR transitions [19–21].

Ligation and Demethylation of Mn(NCH_3TPP)Cl

The sensitivity of ESR parameters on the axial coordination allowed us to identify the species formed during Mn(NCH_3TPP)Cl reactions. A systematic titration of Mn(NCH_3TPP)Cl with di-n-butylamine (DBA) in chloroform solution was carried out. The equilibrium:

$$Mn(NCH_3TPP)Cl + DBA \rightleftarrows Mn(NCH_3TPP)DBA^+ + Cl^-$$

was observed and the corresponding spectral parameters were found (see Table III). In the quinoline solution of Mn(NCH_3TPP)Cl two species with $g_\perp^{eff}$ = 5.2 and $g_\perp^{eff}$ = 4.2 exist, probably due to small equilibrium constants for the quinoline ligand; in the case of collidine the steric hindrance connected with the methyl groups prevents any collidine coordination ($g_\perp^{eff}$ = 5.2).

In the presence of DBA the Mn(NCH_3TPP)Cl complex undergoes demethylation in acetonitrile solution (see Experimental) similarly to the other complexes of NCH_3TPP^- ligand [8, 10, 13]. The ESR spectra allow us to investigate the reaction stage (see Fig. 3). The following processes are proposed for the observed ESR results:

$$\underset{\mathbf{A}}{Mn(NCH_3TPP)Cl} + DBA \rightleftarrows \underset{\mathbf{B}}{Mn(NCH_3TPP)DBA^+}$$

$$\downarrow +DBA$$

$$\underset{\mathbf{C}}{Mn(TPP)DBA} + CH_3DBA^+ \xrightarrow{O_2} \underset{\mathbf{D}}{Mn(TPP)O_2} \xrightarrow[RT]{} \underset{\mathbf{E}}{Mn(TPP)^+} \quad (2)$$

Too low solubility of Mn(NCH_3TPP)Cl in acetonitrile precludes the collection of the ESR spectrum for **A**. The species **B** was identified in 1 mol dm^{-3} DBA in acetonitrile ($g_\perp^{eff}$ = 4.15). The demethylation reaction was carried out at 100 °C; the demethylation product **C** is defined as Mn(TPP)-DBA complex with $g_\perp^{eff}$ = 6 [22, 23]. This complex was obtained separately directly from $Mn(TPP)^+$ (complex of Mn(III)) by the reduction with sodium dithionite. In both cases identical ESR spectra were obtained. An addition of oxygen (**C** to **D** step) results in the appearance of a new signal ($g_\perp^{eff}$ = 5.3). Its position is consistent with formation of $Mn(TPP)O_2$ species [24, 25]. The oxidation of $Mn(TPP)O_2$ leads to Mn(III) complex $Mn(TPP)^+$ (form **E**) which is silent in ESR. The ESR results generally confirm the reaction mechanism proposed earlier by Lavallee [9]. ESR spectroscopy, however, clarifies the structure of the reaction intermediates, especially the direct product of demethylation (**C**) and the first step of oxidation (**D**).

Conclusion

The observations recorded here indicate an essential influence of the pyrrole nitrogen methylation on the ESR parameters. Due to the methylation the symmetry of Cu(NCH_3TPP)Cl and Mn(NCH_3TPP)Cl is lowered in comparison with Cu(TPP) and Mn(TPP), respectively. The rhombic distortion in the porphyrine plane introduces noticeable changes in the ESR spectra of the Mn(II) complexes; the spin Hamiltonian E parameter becomes significant and forms the $g_\perp^{eff}$ parameters. Hence, the parameters may be used for investigation of the coordination effect of X ligand in Mn(NCH_3TPP)X and identification of the species formed during reactions of the complex (*e.g.* demethylation reaction).

Acknowledgement

This work was supported by the Polish Academy of Sciences (Problem MR. I-9).

References

1 (a) F. DeMatteis, A. H. Jackson, A. H. Gibbs, K. R. N. Rao, A. Atton, S. Weerasinghe and C. Hollands, *FEBS Lett., 142,* 44 (1982);
(b) F. DeMatteis, C. Hollands, A. H. Gibbs, N. De Sa and M. Rizzardini, *FEBS Lett., 145,* 87 (1982);
(c) P. R. Ortiz de Montellano, H. S. Beilan and K. L. Kunze, *Proc. Natl. Acad. Sci. U.S.A., 78,* 1490 (1981);
(d) P. R. Ortiz de Montellano, K. L. Kunze and B. A. Mico, *Mol. Pharmacol., 18,* 602 (1980).
2 (a) O. P. Anderson and D. K. Lavallee, *Inorg. Chem., 16,* 1634 (1977);
(b) D. K. Lavallee, A. B. Kopelove and O. P. Anderson, *J. Am. Chem. Soc., 100,* 3025 (1978).
3 D. K. Lavallee, *Bioinorg. Chem., 6,* 219 (1976).
4 D. K. Lavallee and M. J. Bain-Ackerman, *Bioinorg. Chem., 9,* 311 (1978).
5 M. J. Bain-Ackerman and D. K. Lavallee, *Inorg. Chem., 18,* 3358 (1979).
6 D. K. Lavallee and A. Gebala, *Inorg. Chem., 13,* 2004 (1974).
7 B. Shears and P. Hambright, *Inorg. Nucl. Chem. Lett., 6,* 678 (1970).
8 D. K. Lavallee, *Inorg. Chem., 15,* 691 (1976).
9 D. K. Lavallee, *Inorg. Chem., 16,* 955 (1977).
10 D. Kuila and D. K. Lavallee, *Inorg. Chem., 22,* 1095 (1983).
11 H. Ogoshi, E. I. Watanabe, N. Koketzu and Z. I. Yoshida, *J. Chem. Soc., Chem. Commun.,* 943 (1974).
12 J. D. Doi, D. K. Lavallee, S. C. Srivastava, T. Prach, R. Richards and R. A. Fawwaz, *Int. J. Appl. Rad. Isotopes, 32,* 877 (1981).
13 C. Stinson and P. Hambright, *Inorg. Chem., 15,* 3181 (1976).

14 L. J. Boucher and H. K. Garber, *Inorg. Chem., 9,* 2644 (1970).
15 P. T. Manoharan and M. T. Rogers, in Teh Fu Yen (ed.), 'ESR of Metal Complexes', Plenum Press, New York, 1969, p. 143.
16 (a) E. B. Fleischer, *J. Am. Chem. Soc., 85,* 667 (1963); (b) E. B. Fleischer, C. K. Miller and L. E. Webb, *J. Am. Chem. Soc., 86,* 2342 (1964).
17 H. Yokoi, *Bull. Chem. Soc. Jpn., 47,* 3037 (1974).
18 J. Jezierska and B. Jeżowska-Trzebiatowska, *Bull. Acad. Pol. Sci., Chem.,* in press.
19 R. D. Dowsing and J. F. Gibson, *J. Chem. Phys., 50,* 294 (1969).
20 R. D. Dowsing, J. F. Gibson, D. M. L. Goodgame, M. Goodgame and P. J. Hayward, *J. Chem. Soc., A:,* 1242 (1969).
21 R. B. Birdy, G. Brun, D. M. L. Goodgame and M. Goodgame, *J. Chem. Soc., Dalton Trans.,* 149 (1979).
22 B. B. Wayland, L. W. Olson and Z. U. Siddiqui, *J. Am. Chem. Soc., 98,* 94 (1976).
23 Ch. A. Reed, J. K. Kouba, C. J. Grimes and S. K. Cheung, *Inorg. Chem., 17,* 2666 (1978).
24 B. M. Hoffman, Ch. J. Weschler and F. Basolo, *J. Am. Chem. Soc., 98,* 5473 (1976).
25 B. M. Hoffman, T. Szymanski, T. G. Brown and F. Basolo, *J. Am. Chem. Soc., 100,* 7253 (1978).