Inorganica Chimica Acta, 55 (1981) 93–98
© Elsevier Sequoia S.A., Lausanne – Printed in Switzerland

Interaction of Vitamin B_1 (Thiamine hydrochloride) with Zn(II), Cd(II) and Hg(II) in Deuterated Dimethyl Sulfoxide

JAMES GARY and ADEGBOYE ADEYEMO*

Department of Chemistry, Tuskegee Institute, Ala. 36088, U.S.A.

Received July 28, 1980

The interactions of Vitamin B_1 with Zn(II), Cd(II), and Hg(II) have been studied in deuterated dimethyl sulfoxide at room temperature, employing ir, ^1H nmr and ^{13}C nmr techniques. These group 2B metal ions seem to bind directly to the Vitamin B_1 through N-3′ position of the pyrimidine ring (see Fig. 1). The interactions between Vitamin B_1 and these metal ions seem to be reasonably strong as reflected in the small upfield chemical shifts observed in ^1H nmr and downfield chemical shifts observed in ^{13}C nmr.

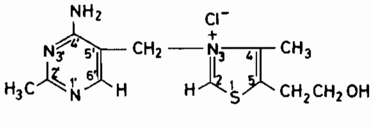


Fig. 1. Thiamine hydrochloride (Vitamin B_1).

Introduction

The biological importance of Vitamin B_1 (Thiamine hydrochloride) cannot be overemphasized. Vitamin B_1 is necessary in the diet of most vertebrates and some micro-organisms. Its deficiency causes beriberi in man and polyneuritis in birds. Vitamin B_1 occurs in cells largely as its active coenzyme form thiamine pyrophosphate, which serves as coenzyme for two classes of enzyme catalized reactions [1]. Aside from biological implications, the chemistry of interaction of Vitamin B_1 with these metal ions is very interesting, since Vitamin B_1 offers a variety of coordination sites.

The mechanism proposed for thiamine pyrophosphate action by Schellenberger [2] ushered in many investigations focused at specifying the role of metal ions. Before Schellenberger [2] made his famous proposal, it was generally assumed that the metal ion was involved in linking the coenzyme via its pyrophosphate residue to the enzyme [3], most especially phosphate or pyrophosphate containing coenzymes in which metal ion cofactor requirements could be shown.

Theophanides *et al.* [4] concluded that the role of the metal ion in enzymatic processes is to coordinate pyrimidine at N-1′ position as a result of their findings in the studies of interaction of thiamine and its phosphate esters with Pt(II) and Pd(II). Other investigators [5] have indicated that both the phosphate group and N-1′ position of the pyrimidine ring are involved in the interaction with certain metal ions. It thus appears from the results of the earlier investigators [4, 5] that any of the possible coordination sites of thiamine could be used for complex formation depending on the solvent, pH, cations and anions.

This study was designed to further understand metal ion interaction with thiamine. We hoped that the group 2B metal ions, especially mercury(II) would bind preferentially [6] through N-3′ position and/or the amino group of thiamine hydrochloride since mercury(II) has a high affinity for primary amines [10]. It was hoped that our findings would further facilitate a better understanding of the role of metal ions in the enzymatic processes.

Experimental

Materials

Thiamine hydrochloride and deuterated dimethyl sulfoxide were purchased from Aldrich Chemical Company. Cadmium chloride was obtained from Fisher Scientific Company, while mercuric chloride and zinc chloride were products of Baker Chemical Company.

Methods

^1H nmr spectra were recorded employing an EM-360 MHz nmr spectrometer. Tetramethylsilane (TMS) was used as an internal reference with DMSO-d_6 as solvent. Ir spectra were recorded on a Beckman 700 model Spectrometer. ^{13}C nmr spectra were recorded on Carbon-13 Fourier Transform-Twenty MHz NMR Spectrometer (CFT-20-MHz NMR Spectrometer) fully computerized and equipped with the optional alphanumeric printer.

---

*Author to whom correspondence should be addressed.

Preparation of the Solutions

One hundred and fifty milligrams of Thiamine hydrochloride were carefully transferred into a clean ^1H nmr tube before adding 3.0 ml DMSO-d_6. The tube with the contents was thoroughly shaken to insure complete dissolution after which TMS was added to serve as internal reference. Although the solution was not very clear, it was good enough for the experiment. After recording the ^1H nmr spectrum of the free thiamine hydrochloride, metal salt was added and almost immediately the solution became clear upon gentle shaking. The spectrum was recorded and it was observed that C-4′-NH_2 signal moved upfield. Metal salt was added continuously until the position and shape of C-4′-NH_2 signals became fixed when ligand:metal salt ratio = 1:1, at which point the C-4′-NH_2 signal became conspicuously broadened. The solutions used for the ^1H nmr spectra were also used for the ir spectra. The solutions for the ^{13}C nmr spectra were made employing similar methods.

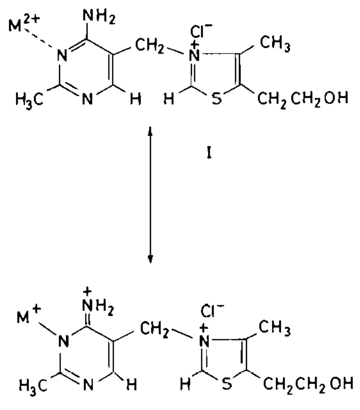
Results and Discussions

A number of important investigations [4–6] have been undertaken to identify the preferential binding sites of ligands with a variety of coordination sites. Identification of such preferential binding sites has been based on a significant broadening and chemical shift of signals of the protons in the immediate vicinity of the coordination site [4]. Chemical shifts [4] in the ^{13}C nmr signals in close proximity of the coordination site is also a criterion for the identification of such preferential binding site. Our conclusions have been drawn on these two important criteria.

^1H Nmr Spectra

The ^1H nmr chemical shifts for the free thiamine hydrochloride and its metal complexes are shown in Table I, while their spectra are shown in Fig. 2.

The ^1H nmr spectra of thiamine hydrochloride (Vitamin B_1) and those of its complexes were recorded in DMSO-d_6 so that a direct comparison of the chemical shifts of the free thiamine hydrochloride and its metal complexes could be made conveniently. Upon addition of metal salt, C-4′-NH_2 protons showed upfield chemical shifts which increased as more metal salt was added. This observation is interestingly unique since earlier studies [4, 18] performed on similar systems have shown downfield chemical shifts upon addition of metal salt. This phenomenon is a clear indication that metal may be binding at some sites other than N-1′ position of the pyrimidine ring. The large chemical shifts coupled with very conspicuous broadening of the C-4′-NH_2 protons is indicative of the fact that Zn(II), Cd(II), and Hg(II) may be binding through the N-3′ position and/or the amino group of the pyrimidine ring. Happe and Ward [9] observed upfield chemical shift for the signals of protons *ortho* and *para* to the coordination site. Binding through N-1′ position of the pyrimidine ring, in this work, is very unlikely since no chemical shift and broadening of C-6′-H signal was observed (see Fig. 2). Some earlier investigators in similar systems [7, 10] have claimed that mercury binds to the amino group of adenosine with loss of one protein in aqueous environment. Deuterated dimethyl sulfoxide (DMSO-d_6) has been used as solvent in this work and this probably explains why our observation is different from that of earlier investigators [7, 10]. Integration of our ^1H nmr spectra of the complexes show that the amino signal is still due to C-4′-NH_2 protons. In view of the fairly large chemical shift and the conspicuous broadening of the amino protons, the following resonance structures may be written (see structures I and II).



I

II

Ir Spectra

Free thiamine hydrochloride absorbs very strongly between 3550 and 3150 cm^{-1}, which could be con-

TABLE I. ^1H NMR Chemical Shifts of Thiamine Hydrochloride and Its Zn(II), Cd(II), and Hg(II) Complexes in ppm (δ).

Compound	C-2-H	C-4′NH_2	C-6′-H	C-5′-CH_2	OCH_2	C-5-CH_2	C-4-CH_3	C-2′-CH_3
Thiamine·HCl	10.08	9.33	8.47	5.70	3.10	3.10	2.60	2.57
Zn(Th) Complex[a]	9.90	9.05	8.47	5.70	3.67	3.10	2.60	2.63
Cd(Th) Complex	9.90	9.10	8.47	5.70	3.67	3.10	2.60	2.60
Hg(Th) Complex	9.85	9.00	8.33	5.70	3.67	3.10	2.57	2.59

[a] Th is used for Thiamine for convenience.

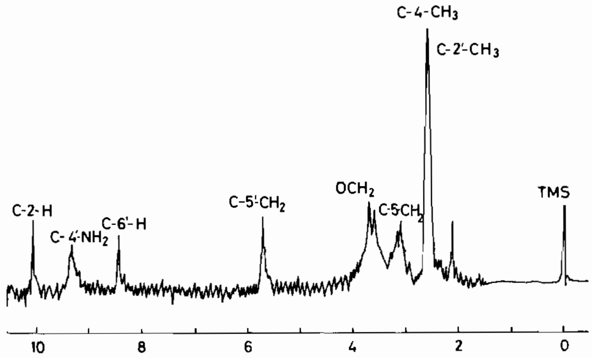


Fig. 2(a). ^{1}H nmr spectrum of the resulting solution of thiamine hydrochloride in DMSO-d_6.

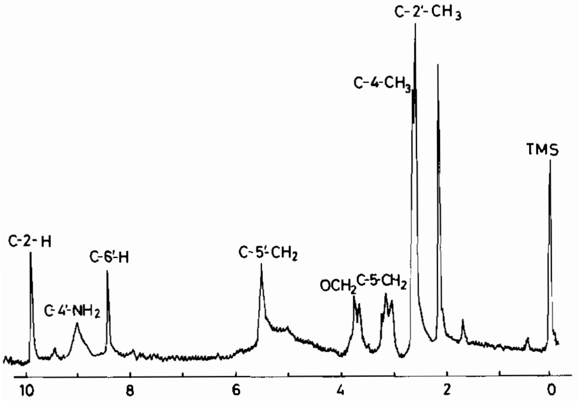


Fig. 2(b). ^{1}H nmr of the resulting solution of thiamine hydrochloride with zinc chloride.

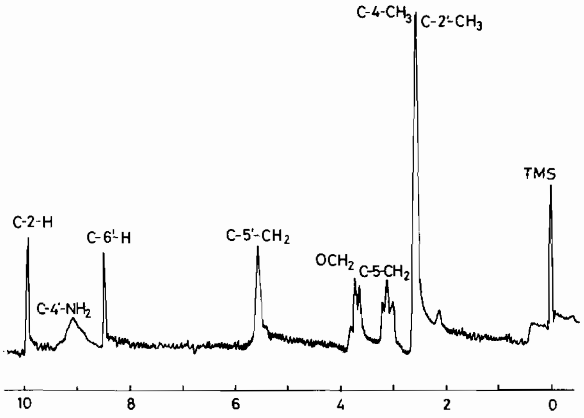


Fig. 2(c). ^{1}H nmr of the resulting solution of thiamine hydrochloride with cadmium chloride.

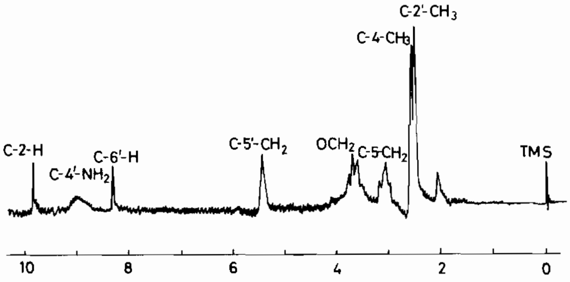


Fig. 2(d). ^{1}H nmr of the resulting solution of thiamine hydrochloride with mercuric chloride.

veniently assigned to OH, NH_2 and CH aromatic and alifatic stretching motions [4]. A medium absorption band appears between 2550 and 2130 cm^{-1} for the free thiamine hydrochloride. Other strong absorption bands occur at 1640, 1030, and between 820 and 750 cm^{-1}. The comparison of the ir spectrum (see Fig. 3) of free thiamine hydrochloride to the ir spectra of its metal complexes shows that the major functional groups, OH/OD and NH_2/ND_2 are intact. This observation further strengthens our claim that the metal ion does not bind to thiamine hydrochloride through OH or NH_2, leaving N-1' and N-3' positions as likely binding sites. Thiamine hydrochloride and its Zn(II)), Cd(II) and Hg(II) complexes show absorption bands at identical frequencies. This observation does not imply that there is no complexation between thiamine hydrochloride and the various metal ions. It only supports the fact that the metal ion is bonded to the ligand through nitrogen. This is consistent with the findings of Theophanides *et al.* [4]. The ND_2 bending band appears at about 1200 cm^{-1} in the ligand. Addition of metal salt did not effect any noticeable change. Again, this observation is in good agreement with the findings of the earlier investigators [4].

The band which appears at about 1035 cm^{-1} in free thiamine hydrochloride also appears in its Zn(II), Cd(II) and Hg(II) complexes. This observation further supports the non involvement of OH group in complex formation with metal ions. Earlier investigators [4, 19, 20] made similar conclusions. The band at 1640 cm^{-1} may be ascribed to the protonated or methylated pyrimidine ring stretching motion of thiamine hydrochloride [4].

^{13}C nmr chemical shifts are listed in Table II and the spectra in Fig. 4. The assignments for free thiamine hydrochloride have been published [14, 15] and are also listed in Table II for comparison. The spectra of free thiamine hydrochloride and its Zn(II), Cd(II), and Hg(II) complexes were recorded in DMSO-d_6. Theoretically, addition of metal salts to the solution of thiamine hydrochloride in DMSO-d_6 should result in downfield chemical shifts of carbons in the immediate vicinity of the coordination sites [6]. If our assumption that metal ions bind through N-3' position of the pyrimidine ring were correct, one would expect downfield chemical shifts for C-2', C-2'-CH_3 and C-4' carbons which are adjacent to N-3' position. In all these complexes, C-4', C-2' and C-2'-CH_3 carbons are the only carbons which show sig-

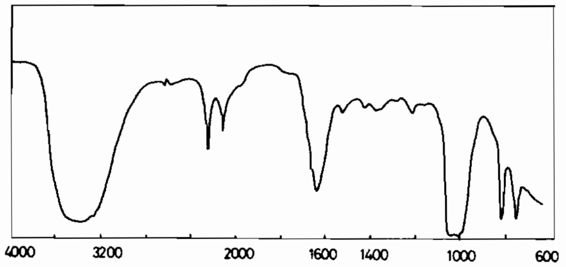


Fig. 3(a). Infrared spectrum of thiamine hydrochloride in DMSO-d$_6$ (cm^{-1}).

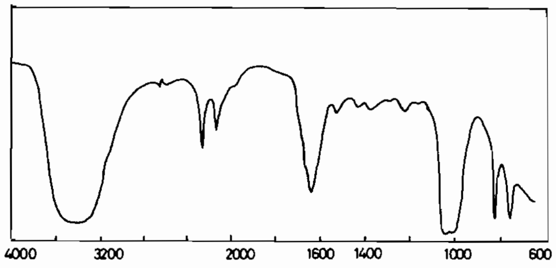


Fig. 3(c). Infrared spectrum of the resulting solution of thiamine hydrochloride with cadmium chloride in DMSO-d$_6$.

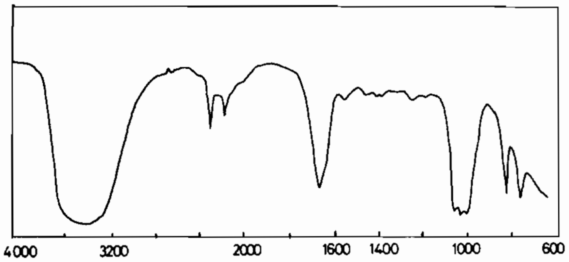


Fig. 3(b). Infrared spectrum of the resulting solution of thiamine hydrochloride with zinc chloride in DMSO-d$_6$.

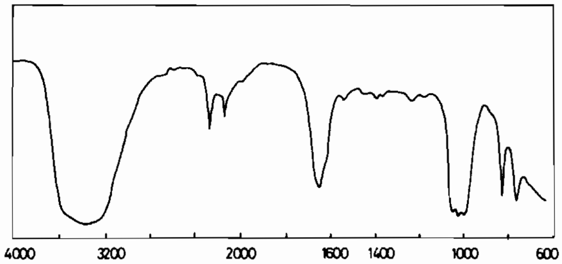


Fig. 3(d). Infrared spectrum of the resulting solution of thiamine hydrochloride with mercuric chloride in DMSO-d$_6$.

TABLE II. ^{13}C nmr Chemical Shifts of Thiamine·HCl and its Zn(II), Cd(II) and Hg(III) Complexes in ppm (δ).

Compound	4-CH$_3$	2'-CH$_3$	5-α-CH$_2$	5'-CH$_2$	5-β-CH$_2$		
Thiamine·HCl	11.63	21.13	29.33	49.88	59.56		
Hg(Th) Complex	11.69	2.20	29.30	49.81	58.55		
Cd(Th) Complex	11.68	21.40	29.32	49.82	59.51		
Zn(Th) Complex	11.67	21.42	29.32	49.83	59.53		
	C-5'	C-5	C-4	C-6'	C-2	C-4'	C-2'
Thiamine·HCl	105.28	135.31	142.13	146.65	155.23	161.23	163.13
Hg(Th) Complex	105.16	135.34	142.08	146.48	155.08	161.88	163.17
Cd(Th) Complex	104.91	135.33	141.99	146.54	154.90	161.89	163.19
Zn(Th) Complex	104.98	135.32	141.98	146.59	155.10	162.10	163.20

nificant downfield chemical shifts. Theophanides *et al.* [4] observed much larger downfield chemical shifts for carbons adjacent to the coordination site. It is, therefore, not unreasonable at this juncture to assume that the coordination site in our work may be different from that claimed by others [4] in view of the much smaller downfield chemical shifts observed. Thus ^{13}C nmr results have provided additional support for our assumption that metal ions bind through N-3' position, since only the carbons adjacent to the assumed coordination site are shifted downfield.

Summary

Theophanides *et al.* [4] observed a larger downfield chemical shift (0.5–0.65 ppm) for the proton adjacent to their ssumed coordination site (N-1'). In our work we observed upfield chemical shifts for C-4'-NH$_2$ (0.33 ppm), C-2'-CH$_3$ (0.06 ppm) and C-6'-H (0.16 ppm) protons which are *ortho* and *para*, respectively, to the assumed coordination site (N-3'). Binding through N-1' position is unlikely since a much larger chemical shift (0.5–0.65 ppm) would have been expected [4] if the nitrogen atom N-1' *ortho* to C-6'-H was involved in bonding with the metals. In the Pt(II)–pyrimidine complexes, for example, the downfield shifts of the *meta* and *para* protons are roughly only half of that of the *ortho* protons [17]. The upfield chemical shift and the conspicuous broadening observed for C-4'-NH$_2$ protons is a clear indication that metal ions may be binding through another site. Theophanides *et al.* [4] also observed large downfield chemical shifts for C-2'-

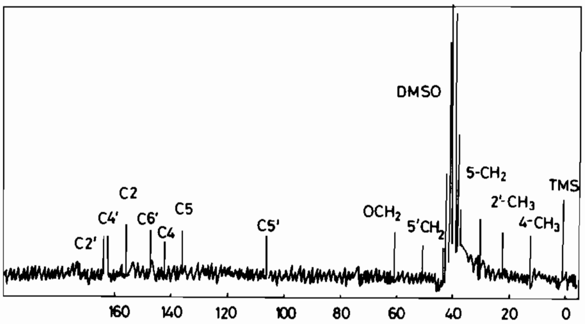


Fig. 4(a). ^{13}C nmr spectrum of thiamine hydrochloride in DMSO-d_6.

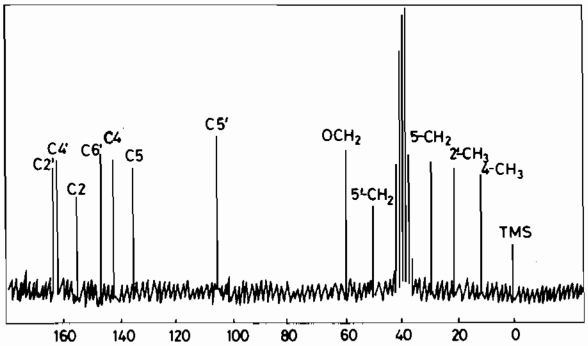


Fig. 4(b). ^{13}C nmr spectrum of the resulting solution of thiamine hydrochloride with zinc chloride.

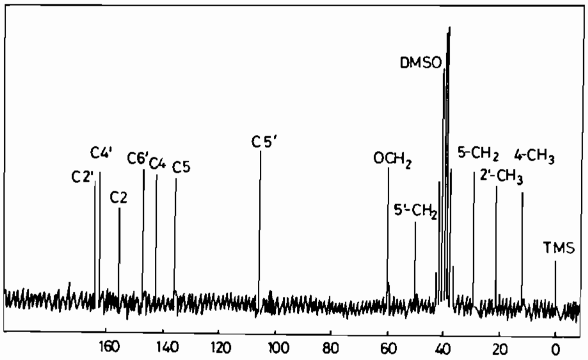


Fig. 4(c). ^{13}C nmr spectrum of the resulting solution of thiamine hydrochloride with cadmium chloride.

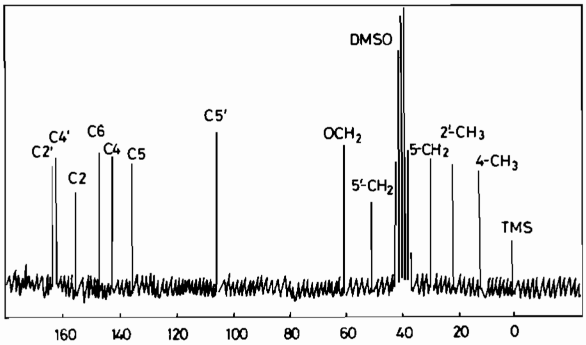


Fig. 4(d). ^{13}C nmr of the resulting solution of thiamine hydrochloride with mercuric chloride.

CH_3 (4.9–5.6 ppm) and C-6′ (12.6–13.5 ppm) carbons which are adjacent to their assumed coordination site. We observed downfield shifts for C-4′ (0.87 ppm), C-2′ (0.07 ppm) and 2′-CH_3 (0.29 ppm) carbons which are adjacent to the coordination site (N-3′). Thus the results from both the proton and carbon-13 NMR may imply that the preferential binding site in these systems may be different from that claimed by earlier investigators [4]. Theophanides *et al.* [4] observed a large downfield chemical shift for C-6′-H proton and on this basis concluded that N-N-1′ position of the pyrimidine ring was the preferred coordination site. Several authors [7, 10] have claimed that mercury also binds to the amino group. In this work, we have no evidence for such claim, probably due to the fact that our studies were carried out in DMSO-d_6, while theirs were done in an aqueous environment. We claim that there is only one binding site (N-3′) in these systems, suggesting only one type of complex (1:1). Wang and Li [13, 18] and other authors [6, 12] have made similar claims.

Our inability to observe larger downfield ^{13}C nmr chemical shifts for the carbons adjacent to the coordination site may be due to the fact that the reactions are very slow. This being the case, a much longer time may be required to effect any significant change in the carbon skeleton of these systems. A much larger chemical shift would have been expected if coordination were through N-1′ position of the pyrimidine ring [4].

Ir spectra did not indicate the involvement of OH and NH_2 groups of the ligand in bonding with the metal [4]. Interaction with the pyrimidine ring, more specifically, coordination through N-3′, seems to be evident. It thus appears from this work and the previous ones [4, 5], that any of the possible coordination sites of thiamine hydrochloride may be used in complex formation, depending on the solvent, pH, cations and anions involved.

Acknowledgements

The authors wish to thank Dr. F. Johnson of Auburn University for his assistance in obtaining ^{13}C nmr spectra. We would also like to express our gratitude to Dr. Courtney J. Smith and Mrs. Zettie J. McCrea for making this work possible.

References

1 A. Lehninger, 'Biochemistry', Worth Publishers, New York (1970).
2 A. Schellenberger, *Angew. Chem.*, *6*, 1024 (1967).
3 E. F. Roger, *Ann. N.Y. Acad. Sci.*, *98*, 412 (1962).

4 N. Hadjiliadis, J. Markopoulos, G. Pneumatikakis, D. Katakis and T. Theophanides, *Inorg. Chim. Acta, 25,* 21 (1977).

5 A. A. Gallo, I. L. Nansen, H. A. Sable and T. J. Swift, *J. Biol. Chem.,* 5913 (1972).

6 L. S. Kan and N. C. Li, *J. Am. Chem. Soc., 92,* 16 (1970).

7 B. P. Block and J. C. Bailar, *J. Am. Chem. Soc., 73,* 4722 (1951).

8 R. Gramer and R. Drago, *J. Am. Chem. Soc., 92,* 66 (1970).

9 J. Happe and R. Ward, *J. Chem. Phys., 39,* 1211 (1963).

10 G. Eichhorn and P. Clark, *J. Am. Chem. Soc., 85,* 4020 (1963).

11 A. C. Plaush and R. R. Sharp, *J. Am. Chem. Soc., 98,* 25 (1976).

12 R. B. Simpson, *J. Am. Chem. Soc., 86,* 2059 (1964).

13 S. M. Wang and N. C. Li, *J. Am. Chem. Soc., 88,* 4592 (1966).

14 A. A. Gallo and H. Z. Sable, *J. Biol. Chem., 249,* 1382 (1974).

15 R. E. Echols and G. C. Levy, *J. Org. Chem., 39,* 1821 (1974).

16 T. Yasu and T. Ama, *Bull. Chem. Soc. Japan, 48,* 1371 (1965).

17 J. Chatt and A. D. Westland, *J. Chem. Soc. A,* 88 (1968).

18 S. M. Wang and N. C. Li, *ibid.,* 5069 (1968).

19 A. Marzotto, G. Bandoli, D. A. Clemente, F. Benetollo, and L. Galzigna, *J. Inorg. Nucl. Chem., 35,* 2769 (1973).

20 G. V. Fazekenley and J. C. Russel, *J. Inorg. Nucl. Chem., 37,* 2377 (1975).