Inorganica Chimica Acta, 25 (1977) 21–31

Interaction of Thiamine and its Phosphate Esters with Pt(II) and Pd(II)

N. HADJILIADIS, J. MARKOPOULOS, G. PNEUMATIKAKIS, D. KATAKIS
University of Athens, Inorganic Chemistry Laboratory, Navarinou 13A, Athens, Greece
T. THEOPHANIDES
University of Montreal, Department of Chemistry, Montreal, Québec H3C 3V1, Canada

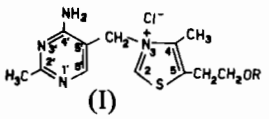
The reactions of Thiamine·HCl (ThH), Thiamine monophosphate·HCl (TMP) and Thiamine pyrophosphate (TPP) have been studied in aqueous solutions (pH ~3–4) at room temperature. Two types of complexes have been characterized by elemental analyses, conductivity measurements, ir, ^1H nmr and ^{13}C nmr spectra:

ionic 1:2 salts in which thiamine is present as a cation, and 1:1 complexes in which the organic ligand is directly coordinated to the metal ions.

The formulae of the ionic salts that were prepared are $[MX_4]^{2-}[Th]_2^+$ and $MThX_3$. The salts can easily be converted into 1:1 products by stirring in water at room temperature, while the TPP analogs require excess of ligand. The 1:1 complexes appear to have a zwitterionic structure and the metal is bound through N_1' of the pyrimidine ring of thiamine. These solid and stable complexes of thiamine are the first examples reported having a direct metal-to-nitrogen bond.

Introduction

Thiamine (Th) known also as Vitamin B_1 is an important dietary constituent for many animals. Its deficiency in man produces the disease beri-beri [1]. Its pyrophosphate ester (TPP) (Formula I) is the coenzyme cocarboxylase which catalyzes the decarboxylation of α keto-acids.



(I)

where, R=H (Thiamine), PO_3^{2-} (TMP) and $P_2O_7^{3-}$ (TPP)

This catalysis is enhanced by the presence of bivalent metal ions such as Mg(II), Mn(II), Co(II), *etc.* [2, 3].

In the mechanisms for the thiamine pyrophosphate action proposed [4] it is not as yet clear what role the metal ions play [5]. Shellenberger [6] proposed that the N_1' nitrogen of the pyrimidine ring might be involved in metal–nitrogen bonding.

Gallo *et al.* [2] and White and Drago [3] studied the interaction in solution between TPP and Ni(II) or Co(II) by ^1H nmr and ^{31}P nmr techniques and concluded that the N_1' nitrogen was indirectly bound to the metal *i.e.* through a water molecule. All solid metal complexes of thiamine reported so far in the literature are to our knowledge ionic complex salts [5, 7–9]. Marzotto *et al.* [10] stated that Cu(II) binds to pyrimidine moiety of thiamine in solution. However the crystal structure of the isolated adduct was shown to be an ionic salt [8]. The same was true for the U(VI)–thiamine complex [7]. As far as we know no solid complexes of TPP or TMP have so far been isolated either.

The present study was undertaken in order to gain some insight in the thiamine–metal interactions, which could help to understand the role of the metal ions in general in the enzymatic processes. Furthermore, it was felt that the new platinum complexes prepared might also be interesting as anti-tumour agents.

Results and Discussion

The reactions of thiamine and its analogs with Pt(II) and Pd(II) were carried out in aqueous solutions at pH ~3–4. This pH value was chosen because thiamine is unstable in neutral or alkaline solutions [1] and because at lower pH values the metal ion would compete unfavorably with protons for the N_1' site of the pyrimidine ring [11] and also precipitation of ionic species would be facilitated. The pK_a value [12] for the deprotonation of N_1' is ~5.

The general reaction may be represented as follows:

$$MX_4^{2-} + 2Th^+ \rightarrow [MX_4]^{2-}[Th]_2^+ \xrightarrow{-ThX} MThX_3 \quad (1)$$

where M = Pt(II), Pd(II) and X = Cl, Br.

The intermediates of formulae $[MX_4][Th]_2$ were only isolated when X = Br but they were con-

TABLE I. Analytical and Conductivity Data of the Complexes.

Compound	C%		H%		N%		Metal %		Halogen %		M.P.[b]	Molar conductance $ohm^{-1} cm^2 mol^{-1}$ (10^{-3} M at 20 °C)
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found		
$Pt(Th)Cl_3$	25.41	25.59	3.00	3.15	9.88	9.39	34.43	34.60	18.79	18.96	274 d	16.99
$Pt(Th)Br_3$	20.57	21.24	2.43	2.95	8.00	7.42	27.87	27.85	–	–	–	–
$[PtBr_4]^{2-}[Th]_2^+$	–	–	–	–	–	–	18.97	19.96	–	–	–	–
$Pd(Th)Cl_3$	30.13	29.95	3.56	3.84	11.72	11.59	22.26	22.19	22.28	21.55	237 d	25.11
$Pd(Th)Br_3$	23.57	24.11	2.78	3.21	9.17	8.70	17.41	17.45	–	–	–	–
$[PdBr_4]^{2-}[Th]_2^+$	30.06	29.28	3.76	4.05	11.69	10.91	11.12	11.56	–	–	–	–
$Pt(TMP)Cl_3$	22.27	22.86	2.78	3.36	8.66	8.83	30.16	–	16.47	–	230 d	31.59
$Pd(TMP)Cl_3$	25.81	25.40	3.23	3.52	10.04	10.16	19.07	–	19.09	–	228 d	26.73
$Pt(TPP)Cl_3$	19.82	20.07	2.61	2.52	7.71	7.36	26.84	–	14.66	–	–	–
$Pd(TPP)Cl_3$	22.57	22.90	2.99	3.19	8.78	8.94	16.69	–	16.68	–	–	–
$[PtCl_4]^{2-}[TPP]_2^+$	24.26	23.97	3.20	3.39	9.43	8.75	16.43	–	11.96	12.00	217 d	–
$[PdCl_4]^{2-}[TPP]_2^+$[a]	26.22	24.64	3.46	3.33	10.19	8.63	9.69	–	12.93	15.50	–	–

[a]This compound could not be isolated pure as the Pt(II) analog and may be a mixture with $Pd(TPP)Cl_3$. [b]d = Decomposition.

verted to the final product by stirring at room temperature for one day. In the case of TPP the final product was obtained by using excess of ligand.

The analytical results, melting points and conductivity measurement data are given in Table I.

The complexes were generally insoluble in organic solvents. In DMSO, however, they decomposed as follows:

$$M(Th)X_3 \xrightarrow{DMSO} [M(Th)(DMSO)X_2]X \qquad (2)$$

The solvent is replacing an X in the first coordination sphere. The 1:1 electrolyte values observed are consistent with this reaction.

The structures of the compounds prepared are discussed on the basis of ir, ^1H nmr and ^{13}C nmr spectra.

Ir Spectra

The ir tentative assignments are given in Tables II, III and the spectra in Fig. 1.

Thiamine HCl shows strong bands at 3450, 3435, 3270 and 3075 cm^{-1} due to OH, NH_2 and CH aromatic and aliphatic stretching motions. Hydrogen bonding and molecular stacking cause shifts of these bands to lower frequencies [13]. In the deuterated thiamine, a strong absorption is observed around 2400 cm^{-1} (Table II).

In the complexes $Pt(Th)Cl_3$ and $Pd(Th)Cl_3$ the strong bands at 3445, 3315, 3440 and 3320 cm^{-1} are due to OH stretchings and are moved to 2550 and 2500 in the deuterated compounds, respectively (NH/ND = 1.37–1.32). The higher bands are possibly due to H_2O of hydration, while the lowers to the OH terminal group of thiamine. The NH_2 stretchings for both complexes appear at 3100 and 3120 cm^{-1} shifting to 2300, 2270 cm^{-1} and 2300 cm^{-1}, respectively (NH/ND = 1.34–1.36). The bands at about 3000 cm^{-1} are not removed upon deuteration and are therefore assigned to C–H aromatic or aliphatic stretchings (See Table II).

These findings are consistent with the assumption that the metal ion is not bonded to the OH and NH_2 groups of thiamine. Additional support for this assumption is also obtained by looking at the NH_2 bending motion [14]. Both thiamine and its complexes with Pt(II) and Pd(II) show two bands in the region of 1600 cm^{-1}. At 1658 and 1608 for the ligand, at 1662 and 1615 for the Pt(II) complex and at 1660 and 1615 cm^{-1} for the Pd(II) analog. Both bands are removed upon deuteration to give only one band at 1648 for the ligand 1628 for Pt(II) and 1625 cm^{-1} for Pd(II). This band is attributed to the protonated or metalated pyrimidine ring stretching motion of thiamine, while the two bands of the undeuterated complexes are due to the coupling of the ring stretching with the NH_2 bending motion [14, 15]. The ND_2 bending band appears at 1200 cm^{-1} in the ligand and it is not affected by complex forma-

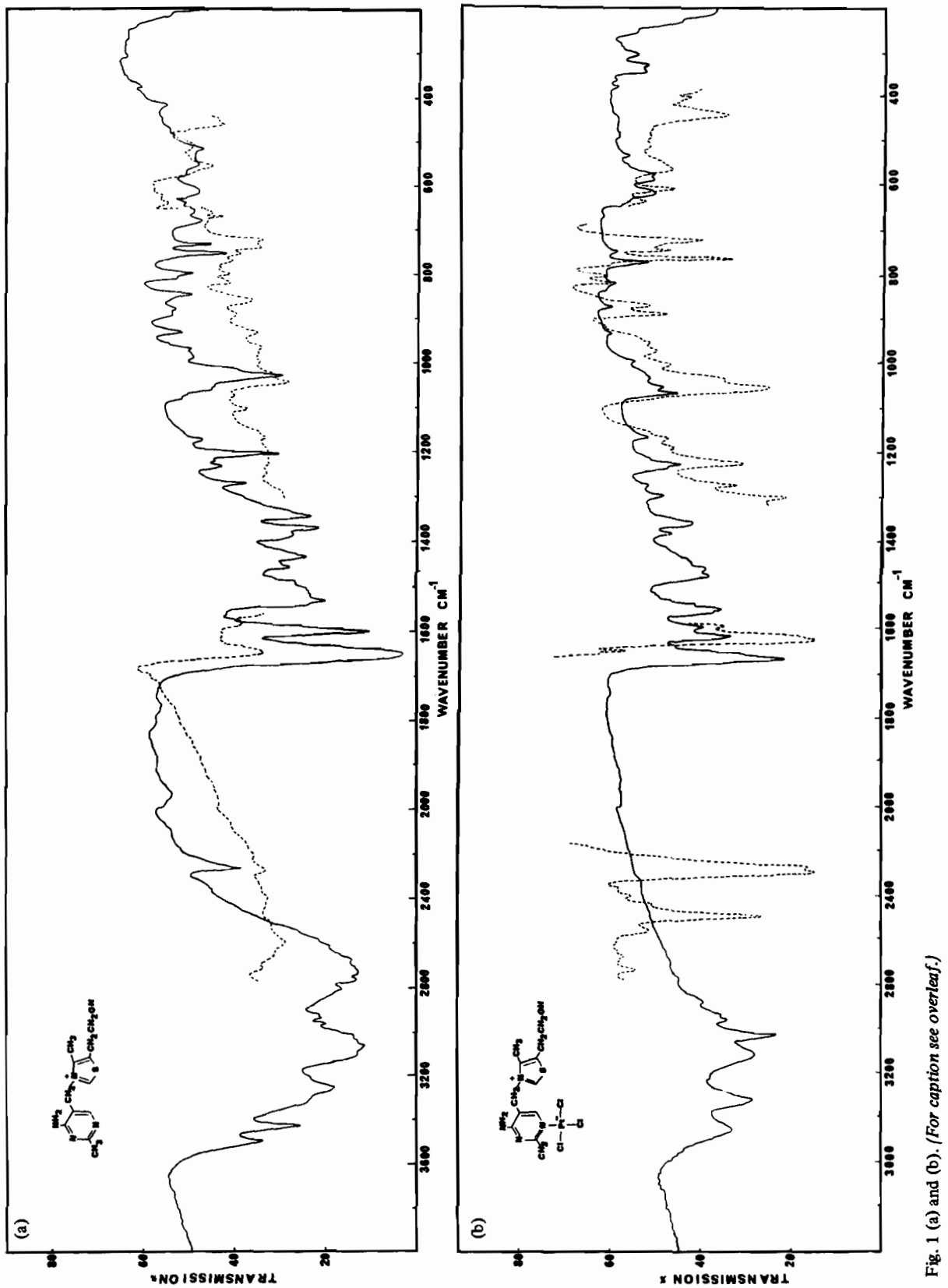


Fig. 1 (a) and (b). *(For caption see overleaf.)*

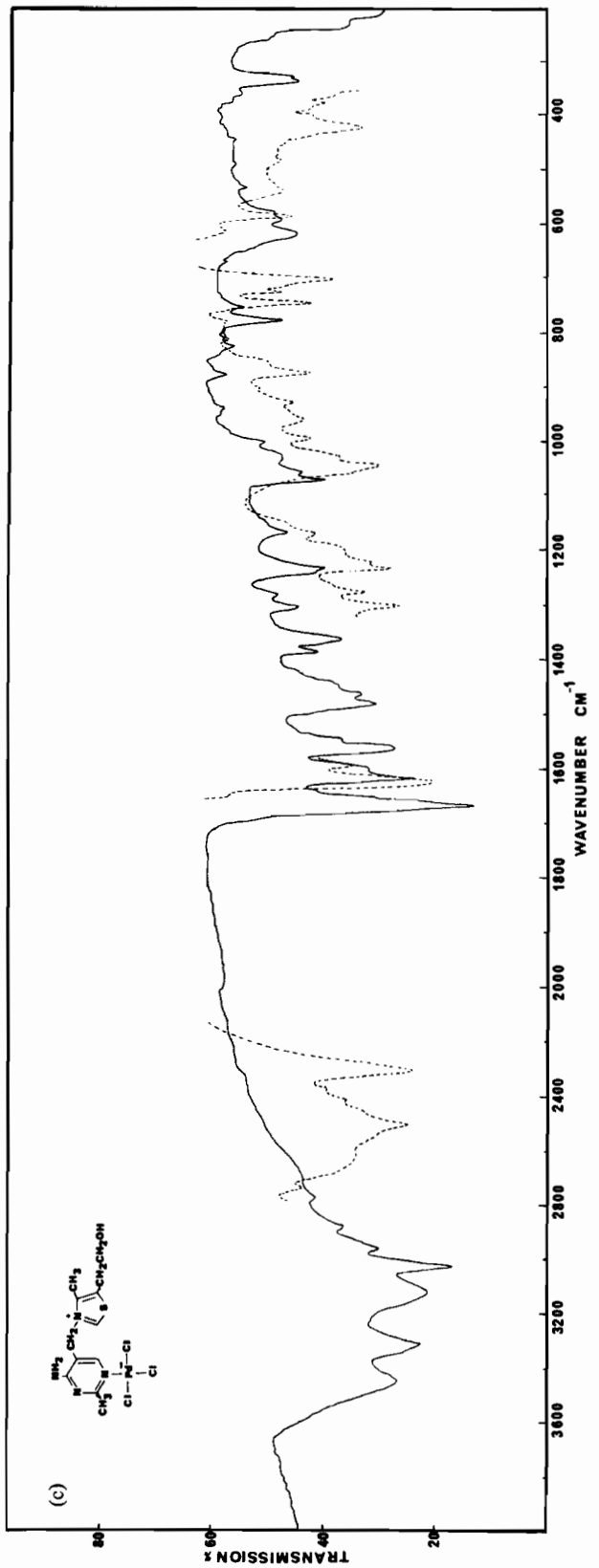


Figure 1. IR spectra of (a) Thiamine chloride hydrochloride, (b) $Pt(Th)Cl_3$ and (c) $Pd(Th)Cl_3$ in the region 4000–200 cm^{-1}. The solid lines correspond to the undeuterated, while the broken lines to the deuterated species.

TABLE II. Ir Tentative Assignments of Thiamine, Its Complexes and Deuterated Analogs.[a]

Assignments	Th•HCl	ThDCl deuterated	$Pt(Th)Cl_3$	$Pt(Th)Cl_3$ deuterated	$Pd(Th)Cl_3$	$Pd(Th)Cl_3$ deuterated
OH, NH_2, CH	3450 s	–	3445 s	–	3440 s	–
aromatic or	3435 s	–	3315 s	–	3320 s	–
aliphatic stretchings	3270 s	–	3100 s	–	3120 s	–
	3075 s	–	3020 vs	3020 s	3025 vs	3020 m
	–	–	2950 m	2950 m	2955 s	2972 m
	–	–	2870 m	–	2880 m	–
ND_2 or OD	–	2600 s	–	2550 w	–	2620 ssh
stretchings	–	2400 s	–	2490 s	–	2500 s
	–	2280 s	–	2300 s	–	2300 s
	–	–	–	2270 s	–	–
NH_2 bending +	1658 vs	–	1662 vs	–	1660 vs	–
ring stretching	–	1648 s	–	1628 s	–	1625 s
coupling	1608 s	–	1615 s	–	1615 s	–
skeletal C=C, C=N	1538 s	1575 m	1590 sh	1590 w	1590 sh	1592 w
vibrations	1505 sh	1560 m	1552 s	1550 s	1555 s	1550 s
	–	1200 s	–	1230 ms	–	1200 ms
C–OH stretching	1035 vs	–	1065 s	1055 s	1058 s	1048 s
	–	–	343 m	–	339 m	–
M–X stretchings	–	–	330 m	–	330 m	–

[a]vs = very strong, s = strong, m = medium, br = broad, w = weak, sh = shoulder.

tion. Protonation of a nitrogen atom of the pyrimidine ring causes a larger shift to higher frequencies of the ring stretching vibration than does metalation [16, 17]. This could indicate an interaction of a nitrogen pyrimidine ring with the metal on going from thiamine hydrochloride to the complexes. Other tentative assignments of the ring stretchings are given in Tables II, III.

The non involvement in bonding of the OH group with metals is further indicated by the non removal of the νC–OH band of the ligand in the complexes [7, 9]. This band appears at 1035 cm^{-1} in the ligand and at 1065 cm^{-1} in the complexes.

The absorptions of the phosphate groups [18] appear in the region 900–1150 cm^{-1}. Khalil and Brown [18] in ATP–metal complexes concluded from ir spectra that complexation occurred at the terminal phosphate group of ATP, because the band at 965 cm^{-1} was shifted to 995 cm^{-1} in the presence of metals or in alkaline solutions. In TMP new broad bands appear at 945 and 1130 cm^{-1}. In TPP and its complexes there are two very broad absorptions in this region with some distinguished maxima. No conclusion can be made of the possible M–O–P bonding here, due to the broadness of the bands in this region.

The M–Cl stretchings appear as medium bands at 343, 330 and 339 and 330 cm^{-1} for the complexes $Pt(Th)Cl_3$ and $Pd(Th)Cl_3$, respectively. In Pt(TMP)-Cl_3 they are found at 332 and 327 cm^{-1}, while in $Pt(TPP)Cl_3$ at 330 and 310 cm^{-1}. The complex $[PtCl_4]^{2-}[TPP]_2^+$ shows one strong band at 325 cm^{-1} with shoulders at 320 and 307 cm^{-1}. All the chloro bands are absent in the bromo analogs which should absorb at about 200 cm^{-1}, the lower limit of our instrument.

Nmr Spectra

The ^1H nmr chemical shifts are given in Table IV and the spectra in Fig. 2. In Table IV the assignments for thiamine taken from literature are given.

Although the nmr spectrum of thiamine HCl was recorded in D_2O and that of the complexes in DMSO-d_6, a comparison of the chemical shifts may still be made. The only proton which shows a large downfield shift upon complexation is the nitrogen atom adjacent to N_1' of the pyrimidine ring (C_6'–H). The ~0.5–0.65 ppm downfield shift of this proton is strong indication for a N_1'–metal covalent bond. This chemical shift is comparable to that observed in Pt(II)–nucleoside complexes by Kong and Theophanides [20]. A much smaller (0.15–0.20 ppm) downfield shift would have been expected if the nitrogen atom N_3' para to C_6'–H was involved in bonding with the metals. In the Pt(II)–pyridine complexes for example the downfield shifts of the meta and para protons are roughly only half of that of the ortho-protons [21]. Unfortunately, a ^{195}Pt–H coupling could not be observed for the complexes in DMSO-d_6. However, the C_6'–H signal is broad at the base (see Fig. 2). The thiazole C_2–H proton is not shifted significantly in the complexes, thus excluding an S–metal interaction. The C_2'–CH_3 protons are only slightly shifted and they are collapsed with the C_4–

TABLE III. Ir Tentative Assignments of the TMP, TPP, Their Platinum Complexes and Deuterated Analogs.[a]

Assignments	TMP	TMP deuterated	TPP	TPP deuterated	$Pt(TMP)Cl_3$	$Pt(TPP)Cl_3$	$Pt(TPP)Cl_3$ deuterated	$[PtCl_4]^{2-}[TPP]^{+}_2$
OH, NH_2 and	3230 sbr	3200 sbr	3178 sbr	–	3310 vs	3560 vs br	–	3200 vs br
CH aromatic or	3065 sbr	–	2965 sbr	–	3100 s	3320 vs	–	3050 vs
aliphatic stretchings	2820 sbr	–	2900 sbr	–	3010 m	3120 vs	–	–
	–	–	–	–	–	3020 vs	–	–
ND_2 or OD	–	2380 s	–	–	–	–	2600–2200	–
stretchings	–	2225 s	–	2000 vs br	–	–	vs br	–
	–	2120 s	–	–	–	–	–	–
	–	1950 s	–	–	–	–	–	–
NH_2 bending or ring	1662 s	–	1655 s	–	1665 s	1662 vs	–	1690 s
stretching coupling	– s	1610 s	–	1610 s	–	–	1633 s	1650 s
Skeletal C=C, C=N	1640 s	–	1623 s	–	1615 s	1615 s	–	1625 s
stretchings	1602 s	1570 s	1592 s	–	–	–	–	1600 w sh
	1510 vs	–	1558 m	–	–	–	1583 s	–
ND_2 stretching	–	1185 s	–	1200 s br	1542 s	1552 s	1560 w	1542 s br
P–O, P–O–P or	1072 s	–	1160 sbr	–	–	–	1200 s br	–
C–O–P stretchings	1055 m	–	–	–	1130 sbr	1100 s br	–	1095 s br
or bendings	1000 sbr	–	970 s	–	–	1050 s br	–	1060 s br
	950 s	–	957 s	–	–	–	–	1010 s br
	935 s	–	915 s	–	945 sbr	950 s br	–	975 s br
	–	–	882 s	–	–	910 s br	–	930 s br
	–	–	866 s	–	–	–	–	910 s br
	–	–	–	–	–	–	–	330 vs
	–	–	–	–	332 s	330 m		320 vs
M–X stretchings	–	–	–	–	327 ssh	308 m	–	307 s

[a]vs = very strong, s = strong, m = medium, br = broad, w = weak, sh = shoulder.

TABLE IV. ^{1}H Nmr Chemical Shifts of Thiamine•HCl and its Pt(II) and Pd(II) Complexes in ppm (δ).

Compound	C_2–H	C_6'–H	NH_2	C_5'–CH_2	O–CH_2	C_5–CH_2	C_4–CH_3	C_2'–CH_3
Thiamine•HCl	9.62	8.00	–	5.53	3.79	3.10	2.56	2.50
Pt(Th)Cl_3	9.73	8.65	8.40	5.55	3.76	3.12	2.56*	2.56*
Pt(Th)Br_3	9.70	8.50	8.07	5.55	3.72	3.18	2.60*	2.60*
Pd(Th)Cl_3	9.74	8.52	8.19	5.65	3.70	3.20	"	"
Pd(Th)Br_3	9.75	8.62	8.07	5.60	3.70	3.20	"	"

*Masked by the DMSO-d_6 band at 2.6 ppm.

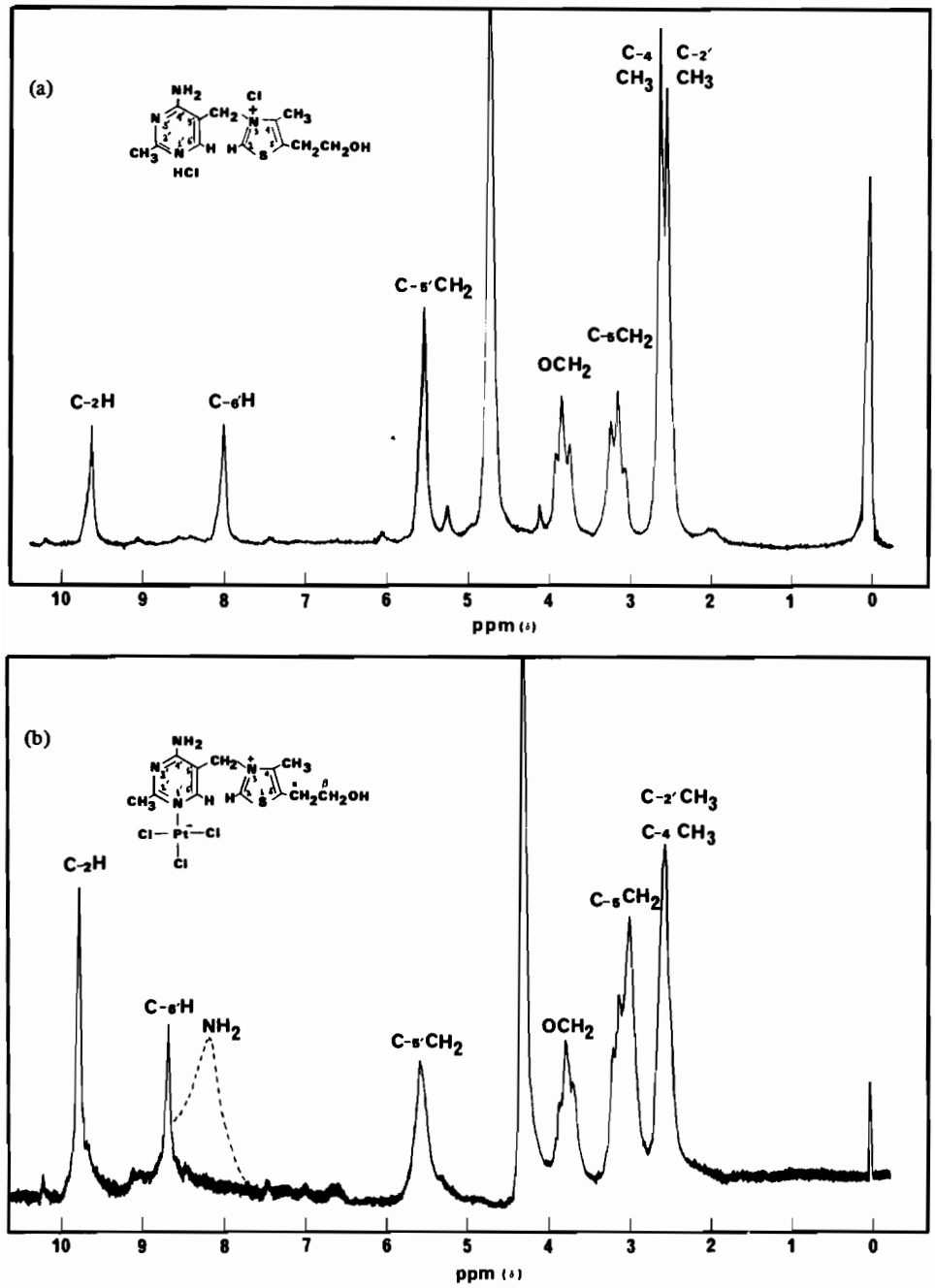


Fig. 2 (a) and (b). *(For caption see overleaf.)*

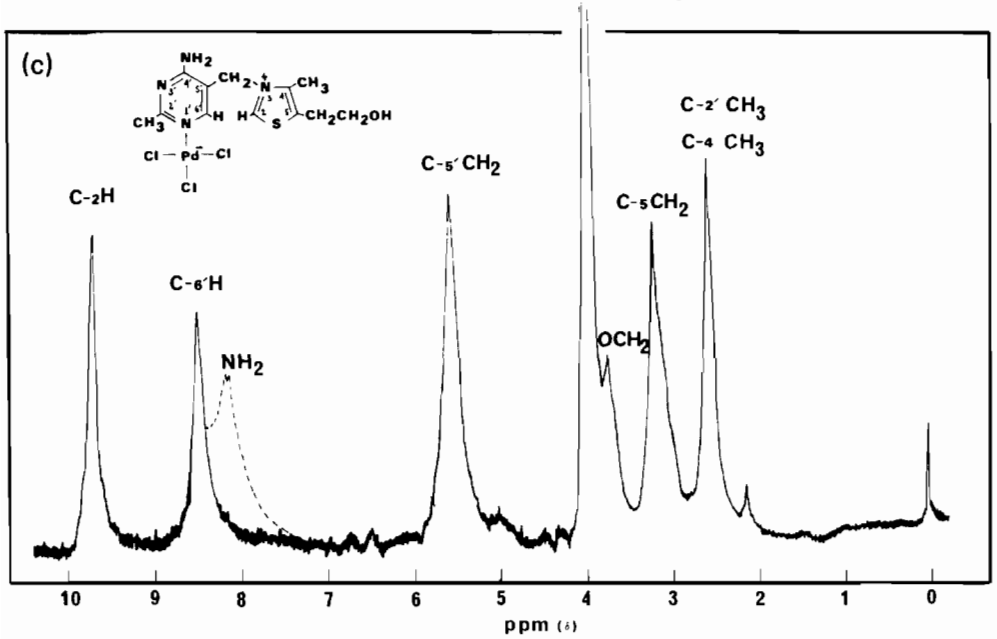


Figure 2. ^{1}H nmr spectra of (a) thiamine hydrochloride in D_2O, (b) $PtThCl_3$ in DMSO-d_6 + 2 drops D_2O and (c) $PdThCl_3$ in DMSO-d_6 + 2 drops D_2O. The broken line in the (b) and (c) spectra represents the NH_2 group shown in DMSO-d_6 without addition of D_2O.

CH_3 protons and the non deuterated protons of the solvent (DMSO-d_6) at ~2.6 ppm.

In the ^{1}H nmr studies of White and Drago [3] and Gallo *et al.* [2] the observed shifts of the C_6–H proton upon addition of the metal were much smaller than expected for the fully coordinated species. Gallo *et al.* [2] concluded that the metal is coordinated to N_1' by the intervention of a water molecule. The situation is not similar here.

^{13}C Nmr

The ^{13}C nmr chemical shifts are given in Table V and the spectra in Fig. 3. The assignments for the ligand have been recently published [22, 23] and are also given in Table V for comparison purposes.

The spectra were recorded in D_2O for the ligand and DMSO-d_6 for the complexes. Complexation causes downfield shifts of carbons adjacent to the coordination sites [24]. The more downfield shifted peaks are those of the $C_{2'}$–CH_3 carbon (4.9–5.6 ppm) and of the C-6′ carbon (12.6–13.5 ppm). The peak of the ligand in D_2O at 169.5 ppm is due to both C-2′ and C-4′. In the complex $Pt(Th)Cl_3$ in DMSO-d_6 two peaks appear at 170.6 and 164.2 and in the complex $Pd(Th)Cl_3$ at 170.3 and 164.2 ppm which are assigned to C-2′ and C-4′ respectively.

The ^{13}C results provide additional support for the assumption that the metal ions are coordinated through N_1', since only the carbon atoms in the neighbourhood of this nitrogen are affected. The other carbon signals remain practically unaffected. Unfortunately, the complexes of TMP and TPP were not soluble enough to record their ^{13}C nmr spectra.

TABLE V. ^{13}C Nmr Chemical Shifts of Thiamine•ClH and its Pt(II) and Pd(II) Complexes in ppm (δ).

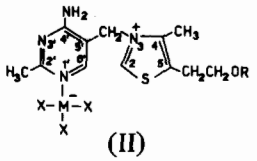
Compound	4–CH_3	2′–CH_3	5–α–CH_2	Bridged CH_2	5–β–CH_2
Thiamine•HCl	12.2	22.1	30.7	50.9	62.5
$Pt(Th)Cl_3$	11.5	26.0	29.4	50.9	60.6
$Pd(Th)Cl_3$	10.8	26.7	28.7	50.9	59.9

	C–5′	C–5	C–4	C–6′	C–2	C–4′	C–2′
Thiamine•HCl	109.1	140.0	147.0	149.4	159.0	169.5	169.5
$Pt(Th)Cl_3$	108.3	138.4	144.8	162.0	158.8	164.2	170.6
$Pd(Th)Cl_3$	106.3	137.5	144.3	162.9	160.9	164.2	170.3

Conclusion

The chemical analyses of the complexes fit the general formula MLX_3 where M = Pt(II), Pd(II); L = Thiamine, TMP or TPP and X = Cl, Br. The organic ligand presumably replaces one chloride or bromide of the original square planar Pt(II) or Pd(II) complex.

Ir spectra do not indicate bonding of the metal through the OH and NH_2 groups of the ligands. They rather indicate interaction with the pyrimidine moiety and more specifically coordination through N_1'. Stronger support for metal coordination through N_1' is provided by the ^1H nmr and ^{13}C nmr spectra. This finding is in agreement with previous results [2, 3, 6] and suggests the following zwitterionic structures for the compounds isolated:



(II)

Where, R = H, PO_3^{2-}, $P_2O_7^{3-}$
M = Pt(II), Pd(II) and X = Cl, Br

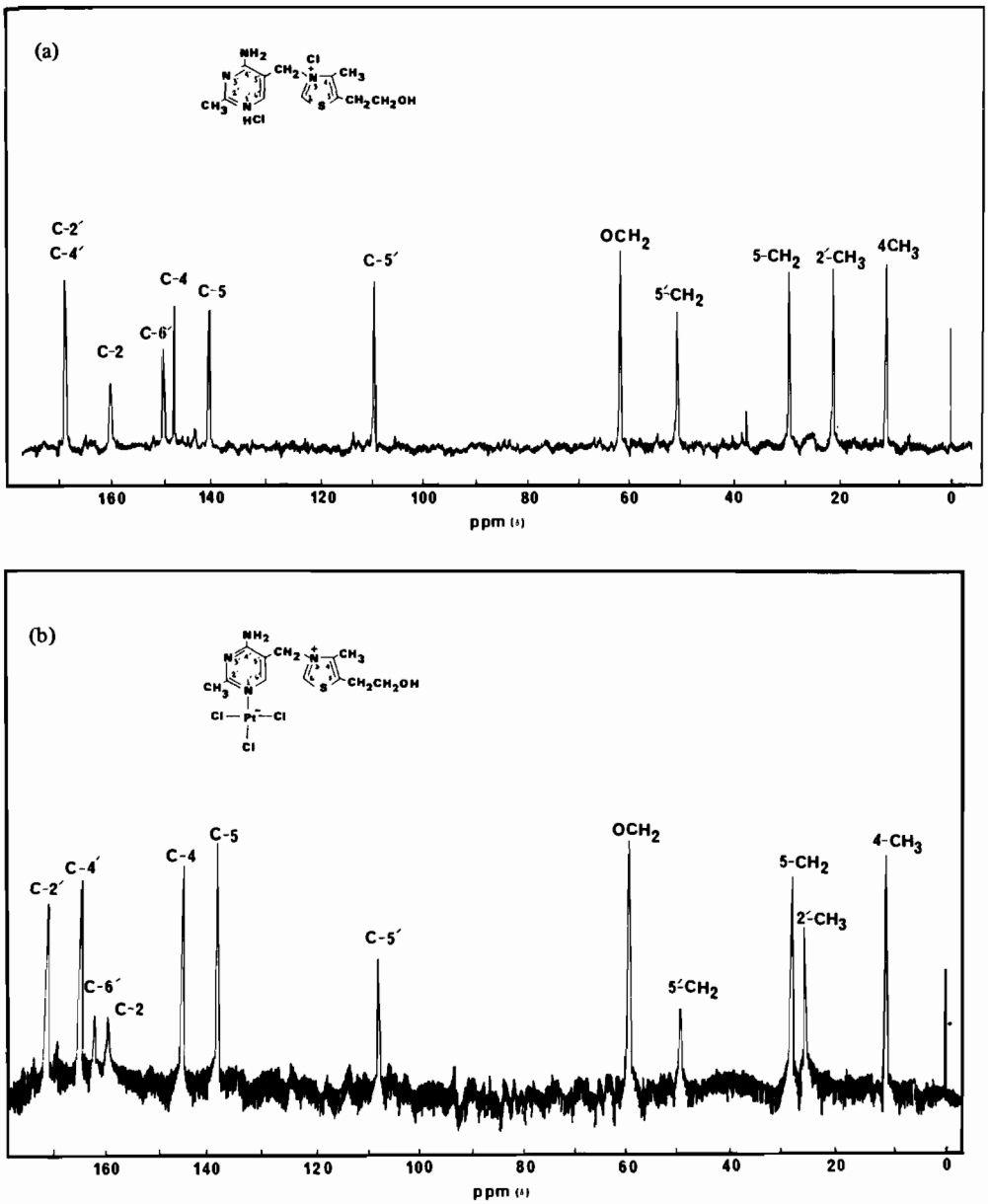


Fig. 3 (a) and (b). *(For caption see overleaf.)*

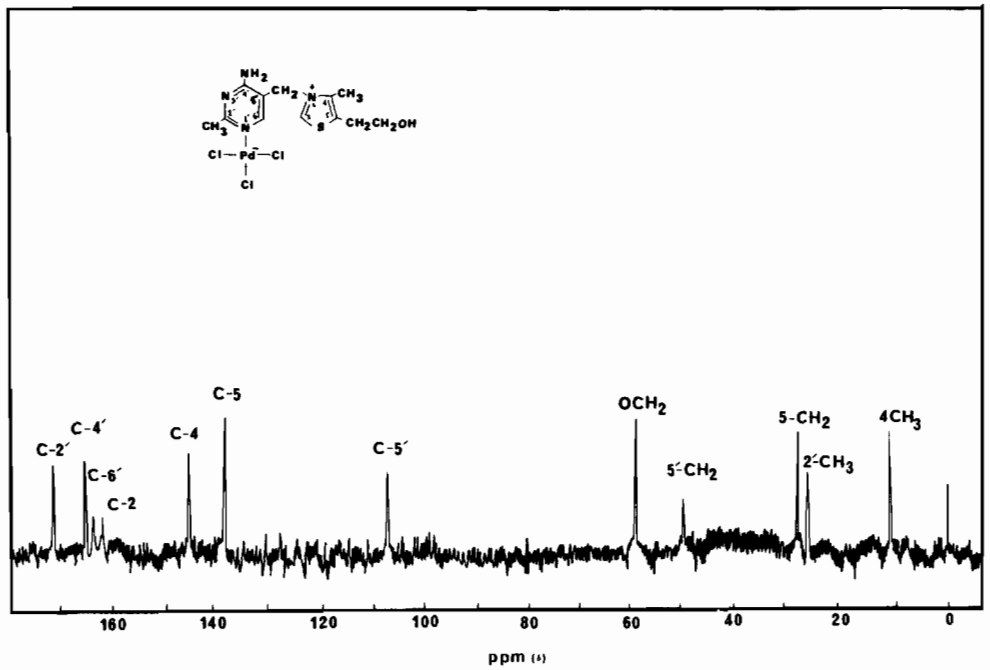


Figure 3. ^{13}C nmr spectra of (a) thiamine hydrochloride in D_2O, (b) $PtThCl_3$ in DMSO-d_6 and (c) $Pd(Th)Cl_3$ in DMSO-d_6.

Our results do not show S–metal interaction. This is not unexpected since even in thiazole–metal complexes nitrogen is the usual binding site [25], and the thiophene S is known [26] to be unreactive towards Pt(II).

To our knowledge the complexes reported here are the first solid adducts of thiamine having a direct metal to ligand bond and could substantiate suggestions of other workers [2, 3, 6] that the role of the metal ion in enzymatic processes is to coordinate pyrimidine at the $N_{1'}$ position.

Experimental

Materials

Thiamine hydrochloride, thiamine monophosphate hydrochloride and thiamine pyrophosphate were purchased from Sigma Chemical Co. Potassium chloroplatinate (K_2PtCl_4) was a Johnson Matthey and Mallory Ltd. product, while palladous chloride ($PdCl_2$) was from Merck Chemical Co.

Methods

Ir spectra were recorded in a Beckman 2050 model spectrophotometer. The positions of the bands are given within ±2 cm^{-1}. ^1H nmr spectra were recorded on a Varian T60 high resolution spectrometer. TMS was used as an internal reference when DMSO-d_6 was used as solvent, while in the D_2O spectra TMS was an external reference. ^{13}C nmr spectra were obtained on a Bruker WH-90 spectrometer operating in Fourier transform mode with proton noise decoupling at frequency of 22.62 Hz. Chemical shifts were measured relative to internal DMSO-d_6 and converted to the TMS scale using $\delta_c^{DMSO\text{-}d_6} = +39.6$ ppm. All ^{13}C chemical shifts are expressed in ppm downfield from TMS. Chemical shifts are estimated to be accurate to ±0.1 ppm. Conductivity measurements were performed using an E365B conductoscope, Metrohm Ltd., Herisau, Switzerland. The melting points were determined on a Fisher John's melting point apparatus and are uncorrected.

Microanalyses

These were performed in the Laboratories of the National Research Foundation of Greece in Athens by Dr. Mantzos.

Preparation of the Complexes

General procedure

(1.2×10^{-3} *M*) of K_2PtCl_4 in 10 ml of H_2O were mixed with 10 ml of (4.8×10^{-3} *M*) of the ligand (metal: ligand ratio = 1:4), after fixing the pH of both solutions at ~3.5 using 0.1 N KOH or HCl solutions. The resulting solution was left at room temperature for one day, during which time the color of the solution became first yellow from red depositing finally yellow crystalls. These were filtered, washed with small quantities of water, acetone and ether and dried first at room temperature in the

presence of $CaCl_2$ under vacuum, and finally at 110 °C in P_2O_5 under vacuum. The yield was ~80%.

The palladium analogs were obtained by dissolving $PdCl_2$ in 0.5 N HCl or 0.5 N HBr by heating and then adjusting the pH in the desired value by adding 0.5 N KOH.

The bromine analogs $[MX_4]^{2-}[Th]_2^+$ of both Pt(II) and Pd(II) were immediatedly precipitated upon mixture of the metal ion solutions with the ligands. Further stirring at room temperature produced the 1:1 products.

In the case of TPP when the metal:ligand ratio was 1:2 no precipitate was separated after several days at room temperature. The resulting yellow solution was evaporated to dryness and washed with a mixture of $CH_3OH:H_2O$=1:1 at 0 °C to remove KCl. The compound thus isolated was the $[PtCl_4]^{2-}[TPP]_2^+$ complex salt. Preparation of $Pt(TPP)Cl_3$ required again excess of ligand. The analog $[PdCl_4]^{2-}[TPP]_2^+$ was too unstable to be isolated and purified and it was easily converted into $Pd(TPP)Cl_3$.

Deuteration of the Ligands and the Complexes

The ND_2, OD deuterated ligands were prepared by exposing them to an atmosphere saturated with D_2O in a desiccator under vacuum. The ND_2, OD deuterated complexes were either prepared as above or prepared in D_2O (pD = 3.5) solutions from the beginning.

Acknowledgements

The microanalyses were performed by Dr. Mantzos of the National Research Foundation of Greece.

References

1 F. A. Robinson "The Vitamin Co-Factors of Enzyme Systems", Pergamon Press, Oxford, London, *etc.* (1966).
2 A. A. Gallo, I. L. Hansen, H. Z. Sable and T. J. Swift, *J. Biol. Chem., 247,* 5913 (1972).
3 W. D. White and R. S. Drago, *Inorg. Chem., 10,* 2727 (1971).
4 L. O. Krampitz, *Annu. Rev. Biochem., 38,* 213 (1969).
5 P. T. Talbert, J. A. Weaver and P. Hambright, *J. Inorg. Nucl. Chem., 32,* 2147 (1970).
6 A. Schellenberger, *Angew. Chem., 6,* 1024 (1967).
7 A. Marzotto, G. Bandoli, D. A. Clemente, F. Benetollo and L. Galzigna, *J. Inorg. Nucl. Chem., 35,* 2769 (1973).
8 M. R. Caira, G. V. Fazakerley, P. W. Linder and L. R. Nassinibeni, *Acta Cryst., B30,* 1660 (1974).
9 G. V. Fazakerley and J. C. Russell, *J. Inorg. Nucl. Chem., 37,* 2377 (1975).
10 A. Marzotto, M. Nicolini, A. Signor and L. Galzigna, *Atti. Acad. Peloritana Pericolanti, Classe Sci. Fis. Mat. Nat., 50,* 79 (1970).
11 J. Kraut and H. J. Reed, *Acta Cryst., 15,* 747 (1962).
12 J. Suchy, J. J. Mieyal, G. Bantle and H. Z. Sable, *J. Biol. Chem., 247,* 5905 (1972).
13 C. H. Willits, J. C. Decius, K. L. Dille and B. E. Christensen, *J. Am. Chem. Soc., 77,* 2569 (1955).
14 N. Hadjiliadis and T. Theophanides, *Can. Spectr., 16,* 135 (1971).
15 M. Tsuboi, Y. Kyogoku and T. Shimanouchi, *Bioch. Bioph. Acta, 55,* 1 (1962).
16 R. Fogglizo and A. Novak, *Spectr. Acta, 26A,* 2281 (1970).
17 A. Lautié and A. Novak, *J. Chim. Phys., 10,* 1492 (1971).
18 F. L. Khalil and T. L. Brown, *J. Am. Chem. Soc., 86,* 5113 (1964).
19 K. Kotera, *Chem. Pharm. Bull., 1,* 440 (1965).
20 (a) P. C. Kong and T. Theophanides, *Inorg. Chem., 13,* 1167 (1974).
(b) *Ibid., 13,* 1981 (1974).
21 J. Chatt and A. D. Westland, *J. Chem. Soc. A,* 88 (1968).
22 A. A. Gallo and H. Z. Sable, *J. Biol. Chem., 249,* 1382 (1974).
23 R. E. Echals and G. C. Levy, *J. Org. Chem., 39,* 1321 (1974).
24 T. Yasui and T. Ama, *Bull. Chem. Soc. Japan, 48,* 3171 (1975).
25 J. A. Weaver, P. Hambright, P. T. Talbert, E. Kang and A. N. Thorpe, *Inorg. Chem., 9,* 268 (1970).
26 Yu. N. Kukushkin and Ts. Todorova, *Russ. J. Inorg. Chem., 14,* 1486 (1969).