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Proton and Carbon-13 NMR Studies on Coordination of ATP Nucleotide to Pd(II)glycyl-L-histidine Complex

HENRYK KOZŁOWSKI and EWA MATCZAK-JON
Institute of Chemistry, University of Wrocław, 14 Joliot-Curie, 50383 Wrocław, Poland
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The coordination sites and conformation in Pd(II)-Gly-His complex were examined by the ^1H and ^{13}C NMR methods.

The coordination equilibria in aqueous solutions containing the Pd(II) Gly-His and ATP in various molar ratios were investigated.

The essential influence of the purine ring on the chemical shifts of the H-2 imidazole proton of histidine residue has been found what allowed to conclude about the ternary complex conformation. The presence of the aromatic ring in the plane of the Pd(II) complex was found to affect the competitiveness in the complex coordination to the purine nitrogens N1 and N7.

The results of the magnetic resonance studies have been supported by absorption spectroscopy data.

Introduction

A number of works have been performed recently on the interaction of Pt(II) and Pd(II) ions with nucleosides, nucleotides and nucleic acids [1–25]. It was stated that in the isolated Pt(II) complexes with adenosine and its phosphates the main coordination site was the purine nitrogen N7 [3, 4]. In aqueous solutions, however, the interaction of the metal ion with both N1 and N7 nitrogens of purine base has been established with formation of monodentate and bidentate species [5–10]. The existing equilibria in solution depend strongly on the pH and the initial metal complex.

Guanosine and its derivatives both in solid [11, 12] and in solutions [5, 8, 9, 13–16] prefer the N7 as the coordination site of the metal ions. The chelation by the O6, N7 donors was also found to be likely, especially in solutions at higher pH regions [14–17]. The complex with the chelate coordination was also isolated from the solution [4]. The bonding of the metal ion with the O6, N7 donor pair was also found to exist in the Pt(II)–nucleic acid systems [18–20].

Complexes of Pd(II) with nucleosides and nucleotides are very close in their coordination behaviour to those of Pt(II) [21–25] and the results obtained for the former metal ion complexes can be often extended to the complexes of the latter metal ion.

In the present paper we report the results of the studies on the ATP coordination to the Pd(II)glycyl-L-histidine complex which contains the aromatic ring, imidazole, in the coordination sphere.

Experimental

Glycyl-L-histidine (Gly-His) and 5′-adenosine triphosphate (ATP), were used as obtained from Fluka A. G. K_2PdCl_4, 99% purity, was obtained by crystallization of KCl and $PdCl_2$ solution containing HCl.

The solutions were prepared in D_2O by mixing of the initial Pd(II)Gly-His 1:1 complex with ATP in molar ratios of 1:1 and 2:1, with a nucleotide concentration equal to 0.15 *M*.

The pH was established by DCl or KOD using a Mera-Elmat N-512 pH-meter. The NMR spectra were recorded on a JEOL PS-100 spectrometer operated at 100 MHz for ^1H NMR spectra and at 25 MHz, in the Fourier transform mode with proton noise decupling, for ^{13}C NMR spectra. The resonance signals in the ^{13}C NMR spectra of Gly-His and ATP were assigned according to the literature data [22, 26–29]. The chemical shifts of the resonance signals were denoted in relation to DSS or TMS standard for the ^1H NMR and ^{13}C NMR spectra, respectively. The ^1H NMR ABX spectra were analysed on the JEC-6 computer.

The absorption spectra of the 0.001 *M* Pd(II)Gly-His solutions were recorded on a UNICAM-SP-700 spectrophotometer.

Results and Discussion

Metal Free Gly-His

In the whole pH region the glycine methylene proton spectrum is of A_2 type and that of α-CH and β-CH_2 protons of histidine is of ABX type. The H-2 and H-4 protons of the imidazole ring give single

TABLE I. ^{1}H NMR Parameters and Rotamer Populations for Gly-His Dipeptide and Its Complex with Pd(II) Ions. The populations obtained by Feeney's equations are given in parenthesis.

pH	ν_A	ν_B	ν_X	$\|J_{AB}\|$	J_{AX}	J_{BX}	ν_{CH_2} Gly	p_1	p_2	p_3
Gly-His										
1.6	3.38	3.26	4.83	15.6	5.7	7.9	3.82	0.48	0.28	0.24
								(0.53)	(0.25)	(0.23)
4.71	3.25	3.14	4.51	15.5	5.0	8.5	3.81	0.54	0.22	0.24
								(0.58)	(0.16)	(0.26)
8.25	3.11	2.98	4.43	14.9	4.4	8.5	3.52	0.54	0.16	0.30
								(0.55)	(0.10)	(0.35)
12.05	3.09	2.96	4.42	14.3	5.1	7.6	3.26	0.45	0.23	0.32
								(0.46)	(0.19)	(0.35)
Pd(II) Gly-His 1:1										
4.68	3.23	2.80	4.22	15.6	3.3	5.1	3.49	0.22	0.06	0.72
								(0.07)	(0.03)	(0.90)

resonance signals in the aromatic region of the spectra.

The rotational isomer populations around the C_α–C_β bond in histidine residue were determined by Pachler [30] and Feeney [31] approximations. The notations of rotamers are given in Fig. 1.

^{1}H NMR parameters of dipeptide aliphatic protons as well as the rotamer populations are listed in Table I.

The analysis of the ABX spectrum of the Gly-His reveals that the rotamer populations are almost constant in the whole pH range as was found for the other peptides which contain histidine residue on a C-terminal [32, 33]. The most stable rotamer of histidine residue is that one with two most bulky groups, *i.e.* carboxyl and imidazole ring, in *trans* position to each other (Table I, Fig. 1).

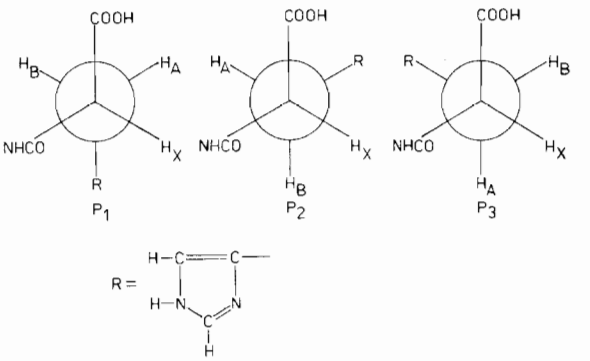

Figure 1. The rotamer notation of histidine residue in Gly-His.

Pd(II) Gly-His 1:1 Solutions

In the equimolar Pd(II): Gly-His solutions at pH over 2.5 the CH_2 group protons of glycine residue are shifted upfield (0.3 ppm) in comparison with the metal-free peptide in its zwitterionic form. The similar shift variations of glycine methylene protons in the presence of the metal ion have been observed also for the other Pd(II) Gly-X solutions as a result of Pd(II) ion coordination to amine and peptide linkage nitrogens [34–37].

The H-2 and H-4 protons of the imidazole ring give the signals shifted upfield 0.6 and 0.3 ppm, respectively, in comparison with the non-coordinated peptide. The Pd(II) coordination to peptide is complete above pH 4.

The changes in chemical shifts suggest three nitrogens, that of the imidazole ring (N1), that of the peptide linkage (N^-) and of the amine group to be the coordination sites of the Pd(II) ion. This suggestion was confirmed by the ^{13}C NMR spectrum of the 1:1 Pd(II): Gly-His solution at pH 5.38. The largest upfield variations of the chemical shifts in comparison with the metal-free peptide are observed for the carbons adjacent to those nitrogen donors, *i.e.* C_α gly, C-2 his and C=O of 8.13, 8.73 and 12.38 ppm, respectively.

The absorption spectra of the 1:1 Pd(II): Gly-His solutions with d–d transition energy equal to 33200 cm^{-1} and ϵ = 685 [M^{-1} cm^{-1}] at pH 4 confirmed the coordination of three nitrogens to the metal ion [37–40].

The analysis of the ABX spectrum of the peptide coordinated to Pd(II) indicates the essential change in conformation of the histidine residue upon the coordination of the metal ion (Table I). A p_3 rotational isomer (Fig. 1) with pseudoaxial position of the carboxyl group becomes the dominating rotamer in coordinated histidine residue. Its population increased about three times up to 0.72 (0.90) in comparison with that in metal-free peptide (Table I).

From the analysis of the molecular model it follows that the dominating form in solution in the pH region 4–9.5 (see also ref. 41) is the complex with tridentately coordinated peptide and the imidazole

TABLE II. ^{1}H NMR Results for Pd(II) Gly-His:ATP 1:1 Solutions.

Species	Chemical Shift in ppm from DSS		Molar Fractions at pH			
	H-2	H-8	6.18	7.18	7.85	9.47
ATP	8.20	8.49	0.31	0.51	0.83	0.86
Pd-N1	8.67	8.56	0.31	0.30	0.17	0.14
Pd-N7	8.30	8.84	0.08	0.07	–	–
Bidentate	9.01	9.24	0.30	0.12	–	–

TABLE III. Chemical Shifts (in ppm from DSS) for H-2 and H-4 Protons in Pd(II) Gly-His:ATP Solutions.[a]

Protons	Gly-His	Pd(II) Gly-His	Pd(II) Gly-His: ATP 1:1		Pd(II) Gly-His:ATP 2:1	
			Pd-N1	Bidentate Species	Pd-N1	Bidentate Species
H-2	8.47	7.84	7.21	6.89	7.27	6.89
				6.81		6.84
H-4	7.20	6.92	7.02	6.68	7.07	6.73
						6.69

[a]H-2 and H-4 proton signals of the Pd-N7 complex are overlapped with those of bidentate species (at 6.81 and 6.69 ppm) and are not specified in the table.

ring fixed in the complex plane. The fourth coordination site in complex molecule is occupied by the Cl^-, H_2O or OH^- and it can eventually be involved in the coordination of the nucleotide [23].

The A_2 type spectrum of the glycine residue signal could result from the almost complete planarity of the chelate ring formed by that residue [35, 40, 42, 43]. The fast exchange between the various chelate ring conformers, however, cannot be exluded.

Pd(II) Gly-His:ATP 1:1 Solutions

In the aromatic region of the ^{1}H NMR spectrum of the ATP solutions two singlet signals corresponding to the H-2 and H-8 protons of the purine ring are observed. The proton NMR spectra of the 1:1 Pd Gly-His:ATP solutions in the aromatic region are much more rich and one may observe several resonance lines originated from purine ring as well as from imidazole ring protons.

The set of the H-2 and H-8 purine proton signals may be used to elucidate the involvement of ATP in the Pd Gly-His coordination [22, 24]. The analysis of the chemical shifts, the changes of the signal intensities with pH variations and of the selective deuteration of the H-8 proton [22, 24] have allowed us to assign the obtained pairs of lines to the Pd-N1 and Pd-N7 monodentate species and to the Pd-N1 + Pd-N7 bidentate (Table II) (The notation Pd-N1 corresponds to the ATP coordination via N1 to the Pd Gly-His complex *etc.*).

From the analysis of the ^{1}H NMR spectra at various pH values it follows that the coordination of ATP to the Pd Gly-His complex is less efficient than that to the analogous Pd Gly-Asp complex, *i.e.* the amount of unbound ATP in the studied case at a given pH and molar ratio, is considerably higher than that found for systems containing the Pd Gly-Asp complex [24].

Determination of the influence of the imidazole ring on the chemical shifts of H-2 and H-8 ATP protons and that of the purine ring on the chemical shifts of H-2 and H-4 protons of imidazole has allowed us to draw some conclusions about the conformation of the Pd Gly-His-ATP complexes existing in solution. The effect of the diamagnetic currents of the purine ring on the H-2 and H-4 proton chemical shifts of histidine residue is considerable (Table III). The imidazole proton signals at 7.21 H-2 and 7.02 H-4 ppm corresponding to Pd-N1 complex are shifted by 0.63 ppm upfield and 0.10 ppm downfield, respectively, in comparison with those of the Pd Gly-His complex unbound to ATP.

For the bidentate species two signals corresponding to H-2 protons of histidine at 6.89 and 6.81 ppm are observed. They are derived from two complex molecules coordinated to ATP via N1 and N7 nitrogens. Analysis of the molecular model suggests that, most likely, the more upfield shifted signal corresponds to the coordination of the Pd Gly-His complex to the N7 site. The H-4 proton in bidentate species

TABLE IV. ^{13}C Chemical Shifts of Gly-His Carbons Upon Pd(II) Gly-His:ATP Complex Formation.

Carbons	Gly-His pH = 5.03	Pd(II) Gly-His 1:1 pH = 5.38	Pd(II) Gly-His: ATP 1:1 pH = 5.30		Pd(II) Gly-His:ATP 2:1 pH = 5.36	
			Pd-N1	Bidentate Species	Pd-N1	Bidentate Species
C_β-his	27.86	32.23	31.87	31.87	31.75	31.75
C-gly	41.21	49.34	49.22	49.22	49.22	49.22
C_α-his	55.04	55.41	55.53	55.53	55.53	55.53
C-4	117.54	123.48	122.75	124.09	122.75	121.17
C-5	130.15	132.70	133.43	131.93	132.82	130.64
C-2	134.04	142.77	140.35	136.83	139.94	136.10
C=O	167.16	179.54	179.05	179.05	179.05	179.05
COO^-	176.87	181.72	182.70	182.70	182.94	180.39

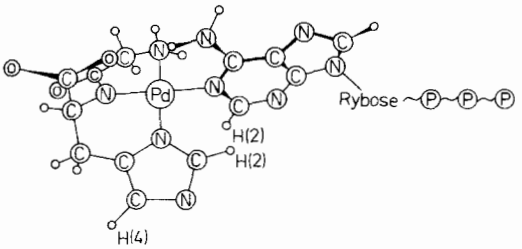

Figure 2. Pd(II) Gly-His-N1 ternary complex.

gives the signal with the same chemical shift value for both molecules of the coordinated Pd Gly-His complex.

Thus from the analysis of the obtained data it may be suggested that in the Pd-N1 monomer the purine ring is located over the H-2 imidazole proton which leads to its considerable upfield shift. Practically, no influence of the imidazole ring on the H-2 and H-8 ATP protons is observed. It is possible only if the purine and imidazole rings, or purine ring and Pd dipeptide complex plane, are nearly perpendicular to each other (Fig. 2). Analogous location of the nucleoside base ring in relation to metal complex plane was already found for some Cu(II) and Pd(II) complexes with nucleosides examined by the X-ray method [44, 45]. At such conformation of the ternary complex there are conditions for the formation of hydrogen bonding between the NH_2 group of purine base and the carboxyl group of peptide molecule.

In the bidentate species the chemical shifts of the H-2 histidine protons proceed also upfield. The slight difference between the chemical shifts of H-2 protons of both Pd(II) complexes coordinated to ATP in the bidentate form (via N1 and N7) indicates the similar, near perpendicular, location of both imidazole rings, *i.e.* complex planes in relation to the purine base plane.

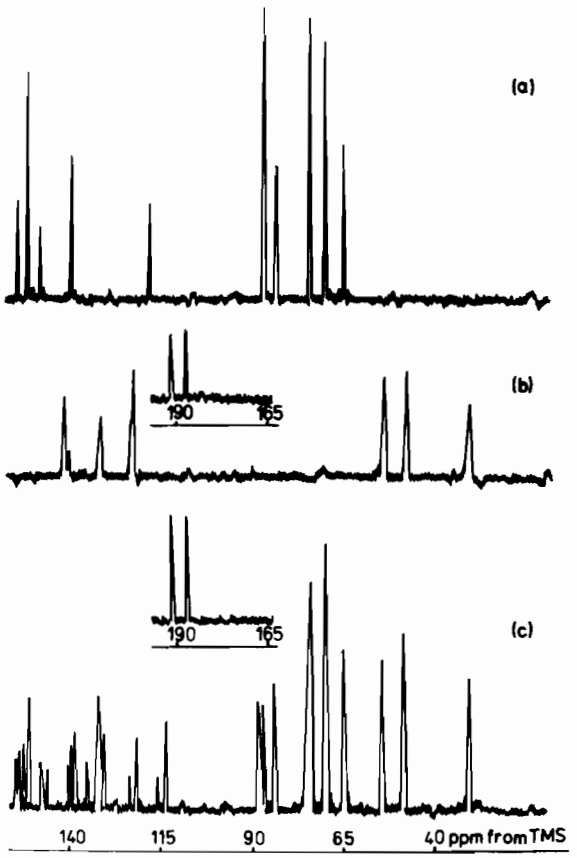

Figure 3. ^{13}C NMR spectra for ATP (a), Pd(II) Gly-His (b) and Pd(II) Gly-His:ATP 1:1 (c) solutions.

The ^{13}C NMR spectra of the Pd Gly-His:ATP 1:1 solution, Pd Gly-His complex and unbound ATP at pH about 5 are given in Fig. 3.

The double resonance signals obtained for the purine base carbons as well as for histidine C-2, C-4 and C-5 and ribose C-1 carbons (Tables IV, V) indicate the presence in 1:1 Pd Gly-His:ATP solution

TABLE V. ^{13}C chemical Shifts of 5'ATP Carbons upon Pd(II) Gly-His:ATP Complex Formation.

Carbons	5'-ATP pH = 5.40	Pd Gly-His:ATP 1:1 pH = 5.30		Pd Gly-His:ATP 2:1 pH = 5.36	
		Pd-N1	Bidentate Species	Pd-N1	Bidentate Species
C-5'	66.09	65.97	65.97	65.97	65.97
C-2'	71.06	70.82	70.82	70.94	70.09
C-3'	75.19	74.94	74.94	74.94	75.79
C-4'	84.65	84.41	84.41	84.41	84.41
C-1'	87.68	87.81	88.41	88.29	90.60
C-5	118.87	115.11	116.69	115.11	115.84
C-8	140.47	141.80	143.02	141.32	142.66
C-4	149.33	149.00	147.70	148.56	147.87
C-2	152.60	153.09	154.18	153.94	155.48
C-6	155.39	156.36	157.16	–	–

TABLE VI. ^{1}H NMR Results for Pd(II) Gly-His:ATP 2:1 Solutions.

Species	Chemical Shift in ppm from DSS		Molar Fractions at pH			
	H-2	H-8	5.49	6.48	7.89	12.81
ATP	8.27	8.46	–	–	0.36	1.0
Pd-N1	8.73	8.61	0.59	0.40	0.33	–
Pd-N7	8.39	8.89	0.05	0.22	0.09	–
Bidentate	9.11	9.40	0.23	0.22	0.01	–
Bidentate^{x}	9.01	9.31	0.14	0.16	–	–

of at least two major species. This is in agreement with ^{1}H NMR data presented above. At pH about 5 in 1:1 Pd Gly-His:ATP solution the two major species have been found to be the Pd-N1 monodentate and the Pd-N1 + Pd-N7 bidentate complexes (Table II). The ^{13}C chemical shift variations observed for ATP molecule upon coordination of palladium complex (Table V) support the conclusions obtained from ^{1}H NMR spectra and the values of these shift changes are similar to those reported by Lim and Martin for the analogous species [22]. The carbon spectrum presents no signals of the Pd-N7 monodentate because of its small concentration.

Comparison of the chemical shifts of the imidazole C-2, C-4 and C-5 carbons of the Pd Gly-His complex with those of ternary complex with ATP revealed that the coordination of nucleotide causes considerable upfield shift of C-2 carbon (2.42 and 5.94 ppm in monodentate and in bidentate species respectively).

Those chemical shift changes confirm the almost perpendicular position of the purine and imidazole rings both in monodentate and in bidentate species.

Pd(II) Gly-His:ATP 2:1 Solutions

Analysis of the ^{1}H NMR spectra of the Pd Gly-His:ATP 2:1 solutions (Table VI) revealed the presence of bidentate and Pd-N1 monodentate species, as well as the small amount of the Pd-N7 species. Moreover, the presence of additional pair of signals was observed at 9.11 (H-2) and 9.40 (H-8) ppm for purine protons which suggests the presence of some other bidentate form of the ternary complex. The difference of chemical shifts of H-2 and H-8 protons of both dimers, however, is very small and could be most probably related to the stacking of the aromatic rings [46].

Analysis of the ^{1}H NMR spectra in various pH ranges indicates that in the system examined even in excess of the Pd Gly-His complex the formation of a bidentate species is quite difficult, contrary to the systems examined earlier, where under the analogous solution conditions the bidentate form was the dominating species [22, 24]. That is likely to be combined with more difficult coordination of the Pd Gly-His complex to ATP via nitrogen N7.

In the studied Pd Gly-His:ATP 1:1 and 2:1 solutions the monodentate Pd-N7 exists as a minor species only, though the coordination of ATP to Pd Gly-His via N7 can be stabilized in the bidentate form (Tables II, VI).

The variations of chemical shifts of H-2 and H-4 histidine residue protons upon the ATP coordination are analogous to those found in the 1:1 Pd Gly-His:

ATP system (Table III, see discussion in previous section).

The conclusions obtained from the ^{1}H NMR spectra are fully confirmed by the ^{13}C NMR spectra (Table IV, V). In ^{13}C NMR spectra, however, separate signals corresponding to two bidentate species have not been observed (Table V), as was the case for the ^{1}H NMR spectra (Table VI).

Conclusions

The results obtained have shown that the introduction of the more bulky groups to the initial Pd(II) dipeptide complex may change their coordination ability towards the nucleic acid constituents quite considerably. The aromatic ring of histidine residue could be also the cause of some additional stacking interactions with the nucleotide molecules and it will also influence the coordination conditions in the systems studied.

This could be of a critical importance in the antitumor action of Pt(II) complexes where the competitive N1 or N7 coordination sites are thought to be interacting with the drug molecule.

The use of the aromatic ring in the initial complex allows us also to conclude that the purine ring plane is almost perpendicular to the complex plane.

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References

1 D. J. Hodgson, "Progress in Inorganic Chemistry", ed. by S. J. Lippard, Vol. *23,* New York, Wiley (1977), p. 211.
2 L. G. Marzilli, "Progress in Inorganic Chemistry", ed. by S. J. Lippard, Vol. *23,* New York, Wiley (1977), p. 255.
3 A. Terzis, N. Hadjiliadis, R. Rivest, T. Theophanides, *Inorg. Chim. Acta, 12,* L-5 (1975).
4 J. Dehand, J. Jordanov, *Chem. Comm.,* 598 (1976).
5 S. Mansy, B. Rosenberg, A. J. Thomson, *J. Am. Chem. Soc., 95,* 633 (1972).
6 N. Hadjiliadis, P. Kourounakis, T. Theophanides, *Inorg. Chim. Acta, 7,* 226 (1973).
7 Pi Chang Kong, T. Theophanides, *Inorg. Chem., 13,* 1981 (1974).
8 Pi Chang Kong, T. Theophanides, *Bioinorg. Chem., 5,* 51 (1975).
9 W. M. Scovell, T. O'Connor, *J. Am. Chem. Soc., 99,* 120 (1977).
10 W. M. Scovell, R.Reaoch, *J. Clin. Hematol. Oncol., 7,* 427 (1977).
11 R. W. Gellert, R. Bau, *J. Am. Chem. Soc., 97,* 7379 (1975).
12 D. M. L. Goodgame, I. Jeeves, F. L. Phillips, A. C. Skapski, *Biochim. Biophys. Acta, 378,* 153 (1975).
13 Pi Chang, Kong, T. Theophanides, *Inorg. Chem., 13,* 1167 (1974).
14 N. Hadjiliadis, T. Theophanides, *Inorg. Chim. Acta, 16,* 77 (1976).
15 R. S. Tobias, G. Y. Chu, H. J. Peresie, *J. Am. Chem. Soc., 97,* 5305 (1975).
16 G. Y. Chu, R. S. Tobias, *J. Am. Chem. Soc., 98,* 2641 (1976).
17 G. Pneumatikakis, N. Hadjiliadis, T. Theophanides, *Inorg. Chim. Acta, 22,* L-1, (1977).
18 M. M. Millard, J. P. Macquet, T. Theophanides, *Biochim. Biophys. Acta, 402,* 166 (1975).
19 J. P. Macquet, T. Theophanides, *Bioinorg. Chem., 5,* 59 (1975).
20 J. P. Macquet, T. Theophanides, *Inorg. Chim. Acta, 18,* 189 (1976).
21 D. J. Nelson, P. L. Yeagle, T. L. Miller, R. B. Martin, *Bioinorg. Chem., 5,* 353 (1976).
22 M. C. Lim, R. B. Martin, *J. Inorg. Nucl. Chem., 38,* 1915 (1976).
23 R. Ettore, *Inorg. Chim. Acta, 25,* L-9 (1977).
24 H. Kozłowski, *Inorg. Chim. Acta, 24,* 215 (1977).
25 H. Kozłowski, S. Wołowiec, *Biochim. Biophys. Acta* (in press).
26 D. E. Dorman, J. D. Roberts, *Proc. Natl. Acad. Sci. U.S., 65,* 19 (1970).
27 W. Horsley, H. Sternlich, J. S. Cohen, *J. Am. Chem. Soc., 92,* 680 (1970).
28 W. Woelter, G. Sokolowski, U. Weber, U. Weser, *Europ. J. Biochem., 58,* 159 (1975).
29 M. Tanokura, M. Tasumi, T. Miyazawa, *Biopolym., 15,* 393 (1976).
30 K. G. Pachler, *Spectrochim. Acta, 19,* 2085 (1963).
31 J. Feeney, *J. Magn. Res., 21,* 473 (1976).
32 B. J. Dale, D. W. Jones, *J. Chem. Soc. Perkin II,* 1190 (1976).
33 T. Tran, K. Lintner, F. Toma, S. Fermandjian, *Biochim. Biophys. Acta, 492,* 245 (1977).
34 H. Kozłowski, B. Jeżowska-Trzebiatowska, *Chem. Phys. Lett., 42,* 246 (1976).
35 H. Kozłowski, M. Jeżowska, *Chem. Phys. Lett., 47,* 452 (1977).
36 H. Kozłowski, G. Formicka-Kozłowska, B. Jeżowska-Trzebiatowska, *Org. Magn. Res., 10,* 146 (1977).
37 L. Rassmussen, C. K. Jørgensen, *Acta Chem. Scand., 22,* 2313 (1968).
38 T. P. Pitner, E. W. Wilson, R. B. Martin, *Inorg. Chem., 11,* 738 (1972).
39 B. Jeżowska-Trzebiatowska, G. Formicka-Kozłowska, H. Kozłowski, *Bull. Acad. Polon. Sci., ser. sci. chim., 26,* 561 (1978).
40 H. Kozłowski, *Inorg. Chim. Acta, 31,* 135 (1978).
41 P. J. Morris, R. B. Martin, *J. Inorg. Nucl. Chem., 33, 2913 (1971).*
42 J. W. Chang, R. B. Martin, *J. Phys. Chem., 73,* 4277 (1969).
43 Y. Sugiura, T. Takeyma, H. Tanaka, *Chem. Lett.,* 491 (1976).
44 E. Sinn, Ch. M. Flynn, R. B. Martin, *Inorg. Chem., 16,* 2403 (1977).
45 D. Szalda, T. J. Kistenmacher, *Acta Cryst., B33,* 865 (1977).
46 K. G. Wagner, R. Lawaczeck, *J. Magn. Res., 8,* 164 (1972).