Inorganica Chimica Acta, 55 (1981) 103–107

The Monomeric and Dimeric Copper(II) Complexes Containing Imidazole and Dipeptides

YASUO NAKAO

Chemical Laboratory, Faculty of Education, Okayama University, Okayama 700, Japan

WASUKE MORI, TAKESHI SAKURAI and AKITSUGU NAKAHARA

Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560, Japan

Received October 31, 1980

The mixed ligand copper(II) complexes with imidazole and glycylglycine or glycyl-β-alanine were prepared in neutral and basic solutions. The copper(II) complexes obtained in neutral solutions were mononuclear complexes, while those in basic solutions were binuclear complexes bridged by imidazolate. In the cases of the binuclear complexes, it was found that the two copper(II) ions are antiferromagnetically coupled with $-J$ values of 19–17 cm^{-1}. It has also been disclosed on the basis of visible and ESR spectral measurements that these dimeric structures are maintained in the concentration of 1.3×10^{-2} to 2×10^{-3} M/Cu.

Introduction

The imidazole group of histidine residue in proteins is one of the most important binding sites for copper in biological systems. Recently, the presence of copper(II)–imidazole binding was established by X-ray crystallographic studies of such proteins as pseudomonas *aeruginosa azurin* [1], *populus nigra var itlica plastocyanin* [2], and bovine erythrocyte superoxide dismutase (BESOD) [3]. One the other hand, considerable research has also been reported on imidazolate-bridged model copper(II) complexes in view of the active sites of BESOD [3]. Prior to these studies, several imidazolate (im)–copper(II) complexes such as $Cu_3(imH)_8(im)_2(ClO_4)_4$ [4], $Cu(imH)_2(im)Cl$ [5], and $Cu(im)_2$ [6], had been isolated as crystals. Imidazolate had also been considered to link two copper(II) in the complex 3 [7], though structure 3 appeared to be very unlikely as described previously [8].

We report here the new proposal of the structural formula for this complex, on the basis of our recent study on some other copper(II) complexes containing imidazole or N-methylimidazole and dipeptides. Further, magnetic susceptibilities over the temperature range of 4.2–295 K were determined for two imidazolate-bridged dimers containing glycylglycinate or glycyl-β-alaninate, and the results are compared with those of related dimeric copper(II) complexes [9–15].

Experimental

Materials

Glycyl-β-alanine

Glycyl-β-alanine was prepared by the procedure reported by Rao *et al.* [16].

Glycyl-γ-aminobutyric acid

Chloroacetyl-γ-aminobutyric acid was prepared by the same method as that for chloroacetyl-β-alanine, using γ-aminobutyric acid instead of β-alanine [16]. Mp. 73–74 °C. *Anal.* Found: C, 40.06; H, 5.66; N, 7.74%. Calcd for $C_6H_{10}O_3NCl$: C, 40.12; H, 5.62; N, 7.80%. Glycyl-γ-aminobutyric acid was obtained by a similar procedure to that for glycyl-β-alanine using chloroacetyl-γ-aminobutyric acid [16]. Recrystallization was carried out by using water–ethanol. Mp. 194–198 °C (dec.). *Anal.* Found: C, 44.68; H, 7.53; N, 17.48%. Calcd for $C_6H_{12}O_3N_2$: C, 44.98; H, 7.57; N, 17.49%.

Glycylsarcosine

Benzyloxycarbonylglycine was purchased from Protein Research Foundation, Osaka. Benzyl Sarcosinate *p*-Toluene-sulfonate was prepared from sarcosine, benzyl alcohol and *p*-toluenesulfonic acid monohydrate in the same way as described for benzyl β-alaninate *p*-toluenesulfonate [17]. This compound was used for the subsequent reaction without further purification. Benzyl N-Benzyloxycarbonylglycylsarcosinate was prepared in a similar method as that reported by Winitz *et al.* [18]. To a solution of 31.4 g of N-benzyloxycarbonylglycine, 47.9 g of benzyl sarcosinate *p*-toluenesulfonate and 13 cm^3 of triethylamine in 440 cm^3 of chloroform was added 31.0 g

of dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature overnight. After removal of precipitated N,N'-dicyclohexylurea, the filtrate was washed with water, 1 *M* HCl, saturated aqueous solution of $NaHCO_3$, and again with water. After the chloroform fraction had been dried over anhydrous magnesium sulfate, the filtrate was evaporated to dryness. The products obtained were recrystallized from ethyl acetate and petroleum benzine. Mp. 91.5–92.5 °C. *Anal.* Found: C, 64.58; H. 6.01; N, 7.57%. Calcd for $C_{20}H_{22}O_5N_2$: C, 64.84; H, 6.00; N, 7.56%. Glycylsarcosine was obtained by the catalytic reduction of benzyl N-benzyloxycarbonylglycylsarcosinate by using palladium black as a catalyst. Methanol was used as solvent with a small amount of acetic acid. This peptide was recrystallized from water–ethanol. Mp. 211–214 °C (dec.). *Anal.* Found: C, 41,07; H, 6.91; N, 19.41%. Calcd for $C_5H_{10}O_3N_2$: C, 41.08; H, 6.91; N, 19.17%.

Copper(II) complex containing glycylglycine and imidazole, [Cu(Gly·Gly)(imH)]·2H₂O, 1a

This was prepared by the method of Driver and Walker [7] from glycylglycine, imidazole and copper(II) hydroxide. *Anal.* Found: C, 28.36; H, 4.99; N, 18.88%. Calcd for $[Cu(C_7H_{10}O_3N_4)]\cdot 2H_2O$: C, 28.23; H, 4.75; N, 18.82%. The corresponding complex isolated by Driver and Walker had been reported as a monohydrate [7].

Copper(II) complex containing glycyl-β-alanine and imidazole, [Cu(Gly·β-Ala)(imH)]·2H₂O, 2a

This was prepared by the same method as described for $[Cu(Gly\cdot Gly)(imH)]\cdot 2H_2O$. Recrystallization from ethanol containing a small quantity of water gave violet-blue crystals. *Anal.* Found: C, 30.56; H, 5.43; N, 18.03%. Calcd for $[Cu(C_8H_{12}O_3N_4)]\cdot 2H_2O$: C, 30.81; H, 5.18; N, 17.97%.

Copper(II) complexes containing glycylglycine or glycyl-β-alanine and N-methylimidazole, [Cu(Gly·Gly)(N-Meim)]·2H₂O, 1b and [Cu(Gly·β-Ala)(N-Meim)]·1/2H₂O, 2b

These complexes were also prepared in the same way as that for $[Cu(Gly\cdot Gly)(imH)]\cdot 2H_2O$, and then recrystallized from aqueous ethanol. $[Cu(Gly\cdot Gly)(N\text{-}Meim)]\cdot 2H_2O$: *Anal.* Found: C, 30.86; H, 4.99; N, 17.99%. Calcd for $[Cu(C_8H_{12}O_3N_4)]\cdot 2H_2O$: C, 30.81; H, 5.18; N, 17.97%. $[Cu(Gly\cdot\beta\text{-}Ala)(N\text{-}Meim)]\cdot 1/2H_2O$: *Anal.* Found: C, 36.58; H, 4.92; N, 18.54%. Calcd for $[Cu(C_9H_{14}O_3N_4)]\cdot 1/2H_2O$: C, 36.29; H, 4.75; N, 18.82%.

Dimeric copper(II) complex containing glycylglycine and imidazole, Na[Cu(Gly·Gly)₂(im)]·6H₂O, 4a

This compound was prepared according to the method of Driver and Walker [7]. The same complex was also obtained by the following method. A mixture of glycylglycine (1.32 g, 0.01 mol), imidazole (0.69 g, 0.01 mol), and copper(II) acetate monohydrate (2.00 g, 0.01 mol) was dissolved in 20 cm^3 of water. The resulting solution was adjusted to pH 11–12 by using concentrated NaOH solution and stirred at room temperature for 1 h. After filtration, the filtrate was evaporated to dryness *in vacuo*. The product obtained was recrystallized from ethanol containing a small quantity of water. *Anal.* Found: C, 22.61; H, 4.59; N, 14.57; Cu, 22.1; Na, 3.8%. Calcd for $Na[Cu_2(C_{11}H_{15}O_6N_6)]\cdot 6H_2O$: C, 22.56; H, 4.66; N, 14.36; Cu, 21.7; Na, 3.9%.

Dimeric copper(II) complex containing glycyl-β-alanine and imidazole, Na[Cu₂(Gly·β-Ala)₂(im)]·7H₂O, 4b

The complex 4b was prepared according to the same direction as described for the complex 4a, by using an equimolar amount of copper(II) hydroxide, glycyl-β-alanine and imidazole and NaOH aqueous solution [7]. Recrystallization was carried out by using ethanol containing a small quantity of water to give violet-blue crystals. *Anal.* Found: C, 24.72; H, 5.08; N, 13.38; Cu, 20.1%. Calcd for $Na[Cu_2(C_{13}H_{19}O_6N_6)]\cdot 7H_2O$: C, 24.72, H, 5.28; N, 13.31; Cu, 20.1%.

Attempts to synthesize the dimeric copper(II) complexes containing imidazole and glycyl-γ-aminobutyric acid or glycylsarcosine were unsuccessful.

Measurements

Melting points were determined on a micro melting point apparatus and are uncorrected. The visible and UV absorption spectra were recorded with a Hitachi 200-10 double beam spectrophotometer. The spectral data were obtained at room temperature in aqueous solution. The pH-adjustment was carried out by adding hydrochloric acid. Magnetic susceptibility at room temperature was determined by using a Gouy magnetic apparatus. The magnetic susceptibility measurements over the range of liquid helium to room temperature were carried out as described in our recent paper [19]. The ESR spectra were measured at 77 K on a JEOL-FX-1 X-band ESR spectrometer modulated at 100 kHz. The *g* values were determined by taking Li·TCNQ ($g = 2.0025$) as a standard, and the magnetic fields were calculated by the splitting of Mn(II) in MnO ($\Delta H_{3-4} = 86.9$ G). Aqueous ethanol (1:1 by volume) was used as a solvent. The concentration of the solutions was *ca.* 2×10^{-3} *M*/Cu.

Results and Discussion

Copper(II) Complexes Formed in Neutral Solution

The mixed ligand copper(II) complex 1a with glycylglycine and imidazole had been obtained by

TABLE I. Physical Properties of the Complexes.

Complex	Absorp. max.			μ_{eff} (K) (B.M.)	ESR parameters	
	λ_{max} (nm)	log ϵ	pH		$g_{\parallel}$	$-A_{\parallel}$(mK)
$Na[Cu_2(Gly\cdot Gly)_2(im)]\cdot 6H_2O$	618	1.94	10.2[a]	1.80(295)	–	–
$Na[Cu_2(Gly\cdot\beta\text{-}Ala)_2(im)]\cdot 7H_2O$	611	1.92	10.7[b]	1.79(287)	–	–
$[Cu(Gly\cdot Gly)(imH)]\cdot 2H_2O$	614	1.95	7.8	1.84(297)	2.22	16.9
$[Cu(Gly\cdot\beta\text{-}Ala)(imH)]\cdot 2H_2O$	611	1.91	7.8	1.85(297)	2.22	18.4
$[Cu(Gly\cdot Gly)(N\text{-}Meim)]\cdot H_2O$	614	1.95	7.8	1.83(297)	2.22	16.9
$[Cu(Gly\cdot\beta\text{-}Ala)(N\text{-}Meim)]\cdot 1/2H_2O$	609	1.89	7.4	1.95(298)	2.23	18.4
$[Cu(Gly\cdot Gly)(H_2O)]$	643	1.92	6.7[c]	–	2.24	15.7
$[Cu(Gly\cdot\beta\text{-}Ala)(H_2O)]$	633	1.87	6.6[c]	–	2.25	19.2

[a]

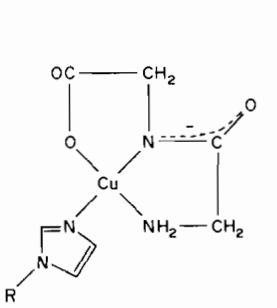
pH	λ_{max}(nm)
9.2	620
7.4	622
6.8	625
5.4	635

[b]

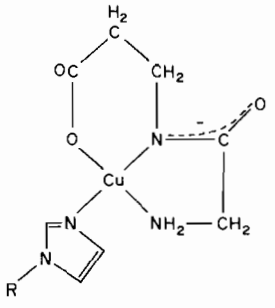
pH	λ_{max}(nm)
9.7	614
7.2	618
6.5	625
5.7	635

[c] O. Yamauchi, Y. Hirano, Y. Nakao and A. Nakahara, *Canad. J. Chem., 47,* 3441 (1969).

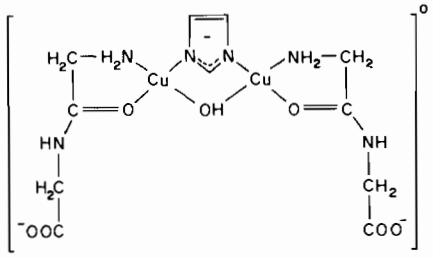
Driver and Walker [7]. The similar type of complexes 1b, 2a, and 2b were newly prepared by reactions between equimolar amounts of each component. The magnetic moments at room temperature and absorption maxima for the *d–d* transition bands of these complexes are given in Table I, together with those of glycylglycinato- and glycyl-β-alaninato-copper(II) complexes ($[Cu(Gly\cdot Gly)(H_2O)]$ and $[Cu(Gly\cdot\beta\text{-}Ala)(H_2O)]$). These complexes show normal magnetic moments as monomeric copper(II) compounds. The λ_{max} value for $[Cu(Gly\cdot Gly)(imH)]$ is in good agreement with that of Driver and Walker, *viz.* λ_{max} 610 nm and ϵ_{max} 89 [7]. As shown in Table I, the λ_{max} values of monomeric mixed ligand complexes 1a–2b are found in the range 609–614 nm, whereas those of $[Cu(Gly\cdot Gly)(H_2O)]$ and $[Cu(Gly\cdot\beta\text{-}Ala)(H_2O)]$ at 633–643 nm. This can be reasonably explained as due to the difference in donor set around copper(II) ion between $[Cu(Gly\cdot Gly)(H_2O)]$ or $[Cu(Gly\cdot\beta\text{-}Ala)(H_2O)]$ and the imidazole or N-methylimidazole containing complexes 1a–2b, since it is well known that the absorption maximum shifts to the shorter wavelengths as oxygen donors are replaced by nitrogen in square planar copper(II) complexes [20]. The ESR spectra for the complexes 1a–2b are also characteristic of monomeric copper(II) complexes. The ESR parameters $g_{\parallel}$ and $-A_{\parallel}$ values of these complexes are tabulated in Table I.



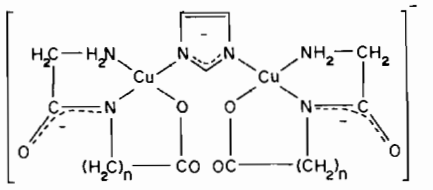
1a ; R = H
1b ; R = CH_3



2a ; R = H
2b ; R = CH_3



3



4a ; n = 1
4b ; n = 2

Dimeric Copper(II) Complexes Formed in Basic Solution

The dimeric copper(II) complex 4a containing glycylglycine and imidazole had already been isolated by Driver and Walker [7]. The same compound was also obtained by treating copper(II) acetate monohydrate with a mixture of glycylglycine and imidazole in a basic solution. For this compound the structural formula 3 which contained a bridged hydroxyl

ion as well as an imidazolate ion was previously proposed. However, it seems to be improbable from several reasons as pointed out in a previous letter [8]. Elemental analysis for Na, Cu, C, H, and N, shows that the complex must be $Na[Cu_2(C_{11}H_{15}N_6O_6)] \cdot 6H_2O$. The magnetic moment at room temperature was 1.80 B.M., which is close to the values for copper(II) complexes without interaction. In order to obtain detailed information on the structure of this compound, the magnetic susceptibility was measured over the temperature range 4.2–295 K (Fig. 1). The

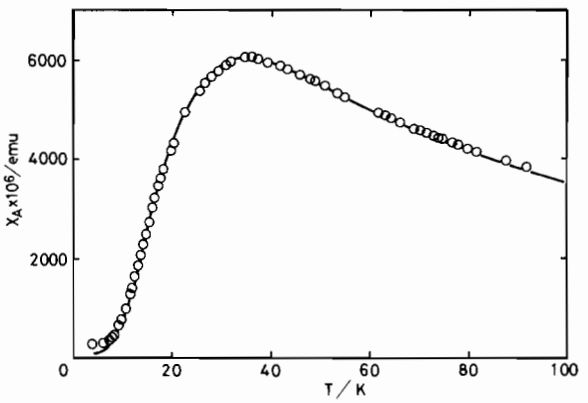


Fig. 1. The temperature-dependence of magnetic susceptibility of copper(II) complex 4a. The solid line shows theoretical susceptibility calculated by equation (1) with g = 2.08, $-2J/k$ = 55 K, $N\alpha = 60 \times 10^{-6}$ cgs emu.

magnetic parameters can be estimated as g = 2.08, $N\alpha = 60 \times 10^{-6}$ cgs emu and $-J = 19$ cm^{-1} from the best fit of the χ_A values to the Bleaney–Bowers equation [21],

$$\chi_A = \frac{N\beta^2 g^2}{3kT} \times \frac{1}{1 + 1/3\exp(-2J/kT)} + N\alpha \qquad (1)$$

where J denotes the exchange integral between copper(II) ions in binuclear copper(II) complexes. This shows that there is an antiferromagnetic exchange interaction between the two copper(II) ions in the complex. Other imidazolate-bridged copper(II) complexes exhibit $-J$ values of 20–90 cm^{-1} [9–15]. On the other hand, it was disclosed that there is no hydroxide ion-bridge as in 3, since the μ-hydroxo-bridged copper(II) complexes generally display quite strong antiferromagnetic interactions [12, 22–24]. Therefore, it is concluded that the present complex has an imidazolate-bridged dimeric structure, 4a, in which glycylglycinate is coordinated around each copper(II) through the terminal amino nitrogen, deprotonated amide nitrogen and the carboxylate oxygen. This mode of coordination may be well accepted in the light of the knowledge established through the studies of oligopeptide–copper(II) complexes [25]. The same type of binuclear copper(II) complex 4b with glycyl-β-alanine has also been isolated. This compound exhibits a similar magnetic property ($-J$ = 17 cm^{-1}) to the complex 4a as illustrated in Fig. 2. The $-J$ values for these dimeric copper(II) complexes are close to that reported for the 4Cu(II) derivatives of BESOD ($-J$ = 26 cm^{-1}), in which copper(II) was substituted for zinc(II) [26].

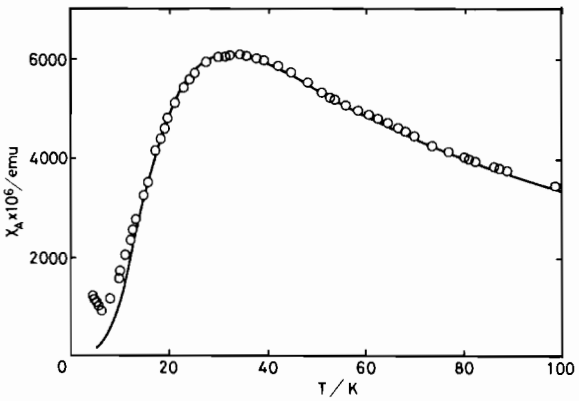


Fig. 2. The temperature-dependence of magnetic susceptibility of copper(II) complex 4b. The solid line shows theoretical susceptibility calculated by equation (1) with g = 2.00, $-2J/k$ = 50 K, $N\alpha = 60 \times 10^{-6}$ cgs emu.

The attempts to isolate the complexes corresponding to 4a containing glycyl-γ-aminobutyrate or glycylsarcosinate instead of glycylglycine were unsuccessful. The results also support the validity of structural formula 4-a and -b for dimeric complexes of glycylglycinate and glycyl-β-alaninate, since it is assumed that either glycyl-γ-aminobutyrate or- sarcosinate would not behave as a stable tridentate ligand.

The absorption maxima for the d–d transition band of these dimeric complexes are listed in Table I. These values are in reasonable agreement with those for the monomeric copper(II) complexes mentioned above. Evidently this is attributed to the same coordination environment around copper(II) both in monomeric and dimeric complexes. Furthermore, these absorption spectra of the binuclear complexes obey Beer's law even in the concentration of 1.3×10^{-2} to 2×10^{-3} M/Cu, suggesting no dissociation of the dimeric structures occurs in the same concentration. However, these d–d bands are shifted bathochromatically with the decrease of pH as is clear from Table I, indicating that the imidazolate bridge is broken in the low pH. The ESR spectra at 77 K of the complex 4a in 50% aqueous ethanol are illustrated in Fig. 3. At pH 9.7, the spectrum exhibits a broader peak in the ΔM_S = 1 region, whereas [Cu(Gly·Gly)(imH)] gives spectrum characteristic of monomeric copper(II) complex. It is considered that the prominent broadening given in Fig. 3(a) stems from the antiferromagnetic spin–spin coupling through the imidazolate-bridge. The lack of the half-

Fig. 3. ESR spectrum of complex 4a at 77 K, (a): pH 9.7, (b): pH 5.4.

field absorption in the $\Delta M_s = 2$ region and non-appearance of seven-hyperfineline coupling structure manifest that the interaction of the two copper(II) ions is rather strong as indicated in $-J$ values (19 cm^{-1}). The ESR spectra of $[Cu_2(Gly\cdot\beta\text{-}Ala)_2(im)]^-$, 4b, exhibited similar behavior. The monomeric complex 1a gave the broadened spectrum similar to Fig. 3(a) at an elevated pH, whereas the spectrum of [Cu(Gly·Gly)(N-Meim)], 1b, did not change with elevating pH. These ESR spectral behaviors clearly indicates that the dimeric structures bridged by imidazolate are retained in basic solution for both the glycylglycinate and glycyl-β-alaninate systems.

Acknowledgements

We thank Miss Mieko Kawai for technical assistance. This work was supported in part by a Grants-in-Aid for Scientific Researches (A) (No. 443012) and (C) (No. 454198).

References

1 E. T. Adman, R. E. Stenkamp, L. C. Sieker and L. H. Jensen, *J. Mol. Biol., 123,* 35 (1978).
2 P. M. Colman, H. C. Freeman, J. M. Guss, M. Murata, V. A. Norris, J. A. M. Ramshaw and M. P. Ven-Katappa, *Nature, 272,* 319 (1978).
3 J. S. Richardson, K. A. Thomas, B. H. Rubin and D. C. Richardson, *Proc. Nat. Acad. Sci. USA, 72,* 1349 (1975).
4 G. Ivarsson, B. K. S. Lundberg and N. Ingari, *Acta Chem. Scand., 26,* 3005 (1972).
5 B. K. S. Lundberg, *Acta Chem. Scand., 26,* 3902 (1972).
6 J. A. Jarvis and A. F. Wells, *Acta Crystallogr., 13,* 1027 (1960).
7 R. Driver and W. R. Walker, *Austral. J. Chem., 21,* 671 (1968).
8 W. Mori, A. Nakahara and Y. Nakao, *Inorg. Chim. Acta, 37,* L507 (1979).
9 G. Kolks, C. R. Frihart, H. N. Rabinowitz and S. J. Lippard, *J. Am. Chem. Soc., 98,* 5720 (1976).
10 G. Kolks and S. J. Lippard, *J. Am. Chem. Soc., 99,* 5804 (1977).
11 Chi-L. O.' Young, J. C. Dewan, H. R. Libienthal and S. J. Lippard, *J. Am. Chem. Soc., 100,* 7291 (1978).
12 M. S. Haddad and D. N. Hendrickson, *Inorg. Chem., 17,* 2622 (1978).
13 M. S. Haddad, E. N. Duesler and D. N. Hendrickson, *Inorg. Chem., 18,* 141 (1979).
14 H. M. J. Hendricks and J. Reedijk, *Inorg. Chim. Acta, 37,* L509 (1979).
15 M. Suzuki, H. Kanatomi, H. Koyama and H. Murase, *Inorg. Chim. Acta, 44,* L41 (1980).
16 K. R. Rao, S. M. Birnbaum, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem., 198,* 507 (1952).
17 N. Izumiya and S. Makisumi, *Nippon Kagaku Zasshi, 78,* 1768 (1957).
18 M. Winitz, L. B. Frankenthal, N. Izumiya, S. M. Birnbaum, C. G. Baker and J. P. Greenstein, *J. Am. Chem. Soc., 78,* 2423 (1956).
19 Y. Nakao, W. Mori, N. Okuda and A. Nakahara, *Inorg. Chim. Acta, 35,* 1 (1979).
20 A. Nakahara, *Bull. Chem. Soc. Japan, 32,* 1195 (1959).
21 B. Bleany and K. D. Bowers, *Proc. Roy. Soc., A214,* 451 (1952).
22 H. Okawa and S. Kida, *Bull. Chem. Soc. Japan, 44,* 1172 (1971).
23 H. Okawa, S. Kida, Y. Muto and T. Tokii, *Bull. Chem. Soc. Japan, 45,* 2480 (1972).
24 H. Okawa, T. Tokii, Y. Nonaka, Y. Muto and S. Kida, *Bull. Chem. Soc. Japan, 46,* 1462 (1973).
25 A. Nakahara, O. Yamauchi and Y. Nakao, 'Bioorganic Chemistry', Vol. 4, E. E. van Tamelen (ed.), Academic Press (1978), p. 349, and references cited therein.
26 J. A. Fee and R. G. Briggs, *Biochim. Biophys. Acta, 400,* 438 (1975).