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Proton Magnetic Resonance Study of some Cobalt(III) Amino Acid Complexes

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Proton magnetic resonance spectra were measured for some cobalt(III) amino acid complexes in D_2O and in ammonia solution [1]. In carboxylato coordinated $[Co(NH_3)_5OOCCH(R)NH_3]^{3+}$ compounds in D_2O, the chemical shifts of amino acid hydrogens are close to those of the free amino acid in the dipolar form, whereas upon deprotonation at NH_3^+ they are rather close to those of the anionic form of the free amino acid. The side-chain conformation of amino acid ligands has been deduced, where possible, from the three bond spin–spin coupling constants between the α- and β-protons. Chelation caused a side-chain conformational change, which seems to be brought about by the steric repulsion between the carbonyl oxygen and the α-substituent (R) of amino acid. For phenylalanine complexes, the effect of coordination upon side-chain rotamer distribution is greater than the effect of solution pH for the free amino acid. In pentaammine(L-histidine)cobalt(III) ion, the long-range coupling between the imidazole C_4 hydrogen and only one of the β-hydrogen has been observed, suggesting a restricted rotation about the C_β–C_γ bond.

Introduction

Though much work [2] has been done in an effort to clarify the factors which govern the side-chain conformation of amino acids in solution, it appears that little is known of them. Our initial aim in this work was to determine the conformation of amino acids coordinated to metal ions and thereby to gain a better understanding of the structures of amino acids in solution. Thus we chose at first some cobalt(III) complexes as samples, which are substitution-inert and have a well-defined stereochemistry around the metal ion.

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Experimental

Materials

Amino acids were purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo) and Katayama Chemical Industries Co., Ltd (Osaka). Deuterium oxide (99.8 atom% D minimum) and 20% ND_3 in D_2O (99 atom% D minimum) were obtained from Stohler Isotope Chemicals (Cal., U.S.A.) and E. Merck (Darmstadt, F.R.G.), respectively.

Synthesis of complexes

Most of the pentaammine(amino acid)cobalt(III) compounds were made after Shimura and coworkers [3] by using $[Co(NH_3)_5(H_2O)](ClO_4)_3$ [4] as the starting material. The reaction mixture was filtered after dilution with water and passed through a chromatographic column packed with a cation exchange resin SP-Sephadex C-25 (Na^+ form). Elution was made by 0.3 *M* sodium perchlorate. The red band corresponding to +3 charge was collected and concentrated to a small volume at room temperature. The precipitate was filtered and recrystallized from aqueous methanol to give analytically pure complexes. The identity of the compounds was confirmed by visible and circular dichroism spectra and chemical analysis. Since difficulties were encountered for the compounds containing serine, aspartic acid, and histidine, we prepared these compounds from $[Co(NH_3)_5(DMSO)](ClO_4)_3$ [5]*.

$[Co(NH_3)_5(L\text{-}serH)](ClO_4)_3 \cdot H_2O$

To a suspension of 5.2 g (0.01 *M*) $[Co(NH_3)_5(DMSO)](ClO_4)_3$ in 10 ml of DMSO was added 1.3 g (0.014 *M*) L-serine in 50 ml water, and a few drops of dilute $HClO_4$ was added to adjust the solution pH to

*Abbreviations: DMSO = dimethylsulfoxide, L-alaH = L-alanine, L-valH = L-valine, L-threoH = L-threonine, L-hisH = L-histidine, L-serH = L-serine, L-aspH = L-aspartic acid, L-asnH = L-asparagine, L-pheH = L-phenylalanine, en = ethylenediamine, and aaH = amino acid.

TABLE I. Analytical Data.

Compound	N	C	H
$[Co(NH_3)_5(L\text{-}alaH)](ClO_4)_3 \cdot H_2O$	15.04(15.29)[a]	6.43(6.55)	4.29(4.37)
$[Co(NH_3)_5(L\text{-}val)](ClO_4)_2 \cdot 2H_2O$	17.26(16.97)	12.22(12.13)	5.62(5.90)
$[Co(NH_3)_4(L\text{-}val)]Cl_2 \cdot 0.5H_2O$	21.86(21.74)	18.88(18.64)	7.14(6.88)
$[Co(NH_3)_4(L\text{-}threo)]Cl_2$	21.91(22.16)	15.05(15.20)	6.56(6.38)
$[Co(NH_3)_5(L\text{-}pheH)](ClO_4)_3 \cdot H_2O$	13.53(13.43)	17.04(17.28)	4.55(4.48)
$[Co(NH_3)_5(L\text{-}serH)](ClO_4)_3 \cdot H_2O$	15.06(14.86)	6.53(6.37)	4.35(4.28)
$[Co(NH_3)_4(L\text{-}ser)]Cl_2 \cdot H_2O$	21.69(21.88)	11.35(11.26)	6.32(6.30)
$[Co(NH_3)_5(L\text{-}hisH)](ClO_4)_3 \cdot HClO_4 \cdot NaClO_4 \cdot H_2O$	13.18(13.36)	8.71(8.59)	3.22(3.25)
$[Co(NH_3)_5(L\text{-}aspH)](ClO_4)_3 \cdot NaClO_4 \cdot H_2O$	11.79(11.74)	6.62(6.71)	3.30(3.38)
$[Co(NH_3)_5(L\text{-}asnH)](ClO_4)_3 \cdot H_2O$	16.41(16.55)	7.86(8.11)	4.01(4.25)
$[Co(NH_3)_5(L\text{-}asp)Co(NH_3)_5]Cl_5 \cdot LiCl \cdot 2H_2O$	22.64(22.78)	7.29(7.10)	6.21(5.92)

[a] ( ) = calcd.

2 to 3. The reaction was complete after heating the mixture for 2 to 3 hr at 80 to 90 °C, which brought about complete dissolution. After cooling to room temperature and filtering, the solution was diluted and loaded on a SP-Sephadex C-25 cation-exchange column (3 × 50 cm, Na^+ form). The complex was eluted with 0.3 *M* $NaClO_4$ containing a few drops of $HClO_4$ (pH = 4). Concentration of the eluent from the red band carrying +3 charge was effected by reloading the solution and eluting with a concentrated solution of $NaClO_4$ acidified with $HClO_4$. A few days refrigeration afforded large plate-like crystals.

$[Co(NH_3)_5(L\text{-}hisH)](ClO_4)_3 \cdot HClO_4 \cdot NaClO_4 \cdot H_2O$

To 5.2 g of $[Co(NH_3)_5(DMSO)](ClO_4)_3$ suspended in 10 ml DMSO was added 1.8 g (0.014 *M*) L-histidine in 50 ml water and the solution pH was adjusted by 1:1 $HClO_4$ to about 4. The reaction mixture was treated in the same way as above. The compound was recrystallized from a minimum quantity of water acidified with $HClO_4$ to a pH of 1 to 2. The imidazole ring in this compound is protonated.

$[Co(NH_3)_5(L\text{-}aspH)](ClO_4)_3 \cdot NaClO_4 \cdot H_2O$

The preparative procedure of this new compound was the same as that for the serine compound except that a small amount of sodium acetate was added to the reaction mixture and that heating at 80 to 90 °C was continued for 4.5 hr. Chemical analysis showed that the crystal contained one molecule of $NaClO_4$ and one water molecule of crystallization. The absorption maxima were 500 nm (ϵ = 67.3) and 348 nm (ϵ = 55.5) and the circular dichroism spectrum consisted of a single peak at 509 nm with $\Delta\epsilon$ = −0.1377.

$[Co(NH_3)_5(L\text{-}asp)Co(NH_3)_5]Cl_5 \cdot LiCl \cdot 2H_2O$

On top of the chromatographic column used for the above compound was left a red band carrying +5 charge. Elution of this band with concentrated LiCl solution yielded, after evaporation at room temperature, the desired complex. The absorption maxima were centered at 500 nm (ϵ = 142.4 based on this formula unit) and 350 nm (ϵ = 127.4). The circular dichroism spectrum was quite unlike other $[Co(NH_3)_5(L\text{-}aaH)]^{3+}$ types of complexes, which shows only one negative peak in the visible region, and contained three peaks at 540 nm ($\Delta\epsilon$ = +0.069) 466 nm ($\Delta\epsilon$ = −0.1432), and 347 nm ($\Delta\epsilon$ = −0.020).

Analytical data are summarized in Table I.

Measurements

Circular dichroism spectra were obtained on a JASCO J-40CS spectropolarimeter and NMR spectra were run either on a Varian T-60 spectrometer (36.4 °C) at 60 MHz or on a Hitachi R-22 spectrometer (30 °C) operating at 90 MHz. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate(DSS) was used as an internal standard. In the presence of DSS, all the phenylalanine complexes suffered from severe decrease in solubility. The complex concentration was about 0.18 to 0.20 Mol/l solvent. At these concentrations, the pH of the complex solution was measured at 2.60 to 2.96 for $[Co(NH_3)_5(L\text{-}hisH)]^{3+}$, 5.20 to 5.30 for $[Co(NH_3)_5(L\text{-}pheH)]^{3+}$, 3.25 to 3.74 for $[Co(NH_3)_5(L\text{-}alaH)]^{3+}$, 5.10 to 5.31 for $[Co(NH_3)_5(L\text{-}serH)]^{3+}$, 2.25 to 2.55 for $[Co(NH_3)_5(L\text{-}asnH)]^{3+}$, and 2.25 to 2.40 for $[Co(NH_3)_5(L\text{-}aspH)]^{3+}$ ion. The pH values cited above are direct pH-meter readings on a Hitachi-Horiba Model F-7 pH meter. Spectral analysis was made by a LAOCN 3 program using a HITAC 8700 electronic computer system at the Hiroshima University Computer Center.

TABLE II. Chemical Shifts.

a) Pentaammine Complexes

	L-alaH		L-valH		L-threoH	L-pheH		L-serH	
	D_2O	ND_4OD	D_2O	ND_4OD	D_2O	D_2O	ND_4OD	D_2O	ND_4OD
α-H	3.93*q*	3.32*q*	3.69*d*	3.04*d*	3.77*d*	4.22	3.575	3.98	3.367
β-H	1.43*d*	1.16*d*	2.20	1.88	4.37*dq*	3.31	2.810	3.98	3.695
						3.14	2.861		3.637
other	3.90[a]		0.95[f]	0.86[f]	1.30[f]	3.82[a]		3.88[a]	
			1.00	0.78					
			3.93[a]		3.96[a]	7.45[c]	7.28[c]		
			2.87[b]		2.96[b]				

	L-hisH		L-asnH		L-aspH	
	D_2O	ND_4OD	D_2O	ND_4OD	D_2O	ND_4OD
α-H	4.17	3.55*t*	4.13	3.55*dd*	4.17*t*	3.58*t*
β-H	3.21	2.84*d*	2.90	2.52*d*	3.02*d*	2.45*d*
	3.45		2.97			
other	4.00[a]		3.92[a]		3.83[a]	
	2.97[b]		2.97[b]		2.83[b]	
	7.42[d]	6.90[d]*s*				
	8.66[e]	7.60[e]*d*				

b) Tetraammine and bimetallic compounds

	L-phe		L-ser		L-val		$[Co(en)_2(L\text{-phe})]^{2+}$ in D_2O	
	D_2O	ND_4OD	D_2O	ND_4OD	D_2O	ND_4OD	$(+)_{CD}$-form	$(-)_{CD}$-form
α-H	4.00	[h]	3.89	3.93	3.67[d]	3.57[d]	3.95	3.96
β-H	3.18	3.20[i]	3.95	3.93	2.27	2.25	3.31	3.31
	3.29						3.20	3.20
other	7.493[c]	7.33[c]			0.942[f]*d*	0.93[f]*d*	7.40[c]	7.40[c]
					1.175[f]*d*	1.14[f]*d*	2.60[g]	2.60[g]
							2.82[g]	2.82[g]

	$[Co(NH_3)_5(L\text{-asp})Co(NH_3)_5]^{5+}$	
	D_2O	ND_4OD
α-H	4.10*t*	3.54
β-H	2.91*d*	2.60
		2.41

d = doublet, *s* = singlet, *t* = triplet, *q* = quartet, *dq* = double quartet, *dd* = double doublet. a) *cis*-NH_3, b) *trans*-NH_3, c) C_6H_5, d) C(4)-H, e) C(2)-H, f) CH_3, g) CH_2CH_2, h) overlapped with solvent signal, i) impossible to analyse, because the chemical shift of α-hydrogen is unknown.

Results and Discussion

Chemical Shifts

It is well known [2] that in free amino acids the chemical shifts of α- and β-hydrogens move to higher magnetic fields as the pH is raised. This corresponds to the process of deprotonation of carboxylic and ammonium groups and the NMR titration experiments enabled one to determine the pK_a values of amino acids. The chemical shift values obtained in this work are given in Table II. As for free amino acids, the α- and β-proton resonances of $[Co(NH_3)_5(aaH)]^{3+}$ in D_2O appeared downfield of the corresponding resonances in ammonia solution, which may be due to deprotonation of NH_3^+ group of coordinated amino acids. The carboxylic group is

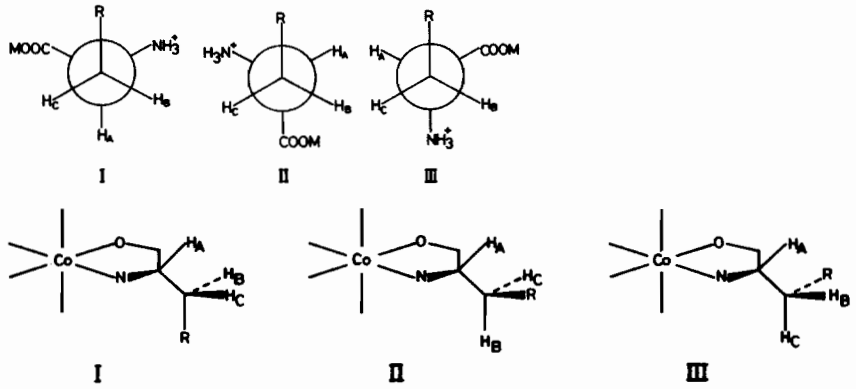

Fig. 1. Three rotational isomers of L-amino acids in (a) $[Co(NH_3)_5(L\text{-}aaH)]^{3+}$ and (b) $[Co(NH_3)_4(L\text{-}aa)]^{2+}$ or $[Co(en)_2(L\text{-}aa)]^{2+}$ ions. In (a) M stands for the $Co(NH_3)_5$ group.

TABLE III. Coupling Constants.

a) Pentaammine Complexes

		L-alaH		L-valH		L-threoH	L-pheH		L-serH
		D_2O	ND_4OD	D_2O	ND_4OD	D_2O	D_2O	ND_4OD	ND_4OD
$J_{\alpha\beta}$	*ca.*	7.0	6.6	4.55	4.8	3.45	5.81	3.911	5.225
$J_{\beta\gamma}$	*ca.*	7.0	6.6	7.0	6.1	6.75	8.44	9.925	4.144
$J_{\beta\beta'}$							−14.41	−13.799	−11.278

	L-hisH		L-asnH		L-aspH	
	D_2O	ND_4OD	D_2O	ND_4OD	D_2O	ND_4OD
$J_{\alpha\beta}$	5.75	6.20	5.77	6.16	5.5	6.5
$J_{\beta\gamma}$	8.59		5.30		5.5	6.5
$J_{\beta\beta'}$	−15.91		−11.15			

b) Tetraammine and bimetallic compounds

	L-val		$[Co(NH_3)_5(L\text{-}asp)Co(NH_3)_5]^{5+}$	
	D_2O	ND_4OD	D_2O	ND_4OD
$J_{\alpha\beta}$	3.60	3.80	6.0	4.612
$J_{\beta\gamma}$	7.0	6.9	6.0	8.113
$J_{\beta\beta'}$				−15.305

bonded to the $Co(NH_3)_5$ moiety so that protonation and deprotonation equilibrium of carboxylic group is irrelevant. An upfield shift in ND_4OD solution was found even for the bimetallic $[Co(NH_3)_5(L\text{-}asp)Co(NH_3)_5]^{5+}$ ion, in which both COO^- groups are coordinated to the $Co(NH_3)_5$ group. The phenyl signal of $[Co(NH_3)_5(L\text{-}pheH)]^{3+}$ behaved similarly. For $[Co(NH_3)_5(L\text{-}hisH)]^{3+}$, the imidazole C_2 and C_4* hydrogens suffered an upfield shift in ammonia, presumably due to imidazolium → imidazole. We note that for complexes of the type $[Co(NH_3)_5(aaH)]^{3+}$ in D_2O the shift values of the amino acid resonances are close to those of the corresponding resonances of free amino acid [2] in the neutral form. On the other hand, these complexes in ammonia solutions have chemical shifts which are rather close to those of the free amino acids in the anionic form. These observations appear rather surprising in view of the fact that in free amino acids the functional groups are in the form COO^- and NH_3^+ in D_2O and COO^- and NH_2 in ammonia while in

*The imidazole ring is numbered according to the nomenclature by IUPAC and IUPAC-IUB commission; *Biochemistry, 14*, 449 (1975).

TABLE IV. Results of Spectral Analysis and Fractional Populations. Error means the root-mean-square error estimated by LAOCN 3 and ν_0 is the measuring frequency.

	$[Co(NH_3)_5(L\text{-pheH})]^{3+}$			$[Co(NH_3)_4(L\text{-phe})]^{2+}$		$[Co(en)_2(L\text{-phe})]^{2+}$ in D_2O	
	ND_4OD	D_2O		D_2O		$(+)_{CD}$-form	$(-)_{CD}$-form
ν_1	0	0	0	0	0	0	0
ν_2	42.726	54.745	82.044	42.622	62.578	57.564	58.644
ν_3	43.624	64.921	98.167	49.455	73.961	67.501	67.392
J_{12}	3.911	6.01	5.81	4.32	3.97	4.44	4.26
J_{13}	9.925	8.30	8.44	8.75	8.19	8.48	8.38
J_{23}	−13.799	−14.62	−14.41	−15.20	−14.68	−14.80	−14.78
error	0.163	0.091	0.074	0.225	0.131	0.183	0.165
ν_0 (MHz)	60	60	90	60	90	90	90
p_I	21	17	18	28	36	29	32
p_{II}	67	52	53	56	51	54	53
p_{III}	12	31	29	16	13	17	15

	$[Co(NH_3)_5(L\text{-serH})]^{3+}$	$[Co(NH_3)_5(L\text{-hisH})]^{3+}$		$[Co(NH_3)_5(L\text{-asnH})]^{3+}$		$[Co(NH_3)_5(L\text{-asp})Co(NH_3)_5]^{5+}$
	ND_4OD	D_2O		D_2O		ND_4OD
ν_1	0	0	0	0	0	0
ν_2	−24.253	59.744	86.865	71.659	104.066	56.634
ν_3	−29.532	43.866	65.063	76.369	112.106	67.707
J_{12}	4.144	9.25	8.59	6.13	5.77	4.612
J_{13}	5.225	6.86	5.75	5.39	5.30	8.113
J_{23}	−11.278	−16.36	−15.91	−10.48	−11.15	−15.305
error	0.085	0.619	0.095	0.379	0.505	0.064
ν_0 (MHz)	90	60	90	60	90	60
p_I	62	0	16	42	46	31
p_{II}	14	39	29	32	25	50
p_{III}	24	61	55	26	29	19

$[Co(NH_3)_5(aaH)]^{3+}$ they are $COOCo(NH_3)_5$ and NH_3^+ in D_2O and $COOCo(NH_3)_5$ and NH_2 in ammonia solution. We are, therefore, led to the conclusion that the influences of COO^- and $COOCo(NH_3)_5$ groups on the chemical shift of α- and β-protons are rather similar to each other.

The NMR spectra of pentaammine compounds when measured immediately after dissolution in D_2O exhibited, in addition to absorptions due to amino acid ligand, two broad resonances at about 3.8 to 4.0 ppm and at 2.8 to 3.0 ppm. These broad signals were assigned to *cis* and *trans* (to coordinated oxygen atom) ammine groups, based on their intensity ratio (4:1) and on the magnetic anisotropy effect [6] of the central cobalt(III) ion. The *trans* ammine hydrogens exchanged with deuterium and vanished much more rapidly than *cis* ones. This result is in accordance with the general trend that for $[Co(NH_3)_5X]$ ions the rate of the *trans* hydrogens is greater than that of the *cis* ones if the ligand X is a weak-field ligand [7].

The Δ- and Λ-forms of the $[Co(en)_2(L\text{-phe})]^{2+}$ ion are diastereoisomeric to each other and as such we may expect in principle a shift difference between them. Experimentally, however, no such difference could be found within our experimental uncertainties.

Conformation of Amino Acids

We can deduce the side-chain conformation of amino acids from analysis of the spectra due to α- and β-protons, if they give rise to an ABX type spectrum [2]. The observed three bond H_α–H_β coupling constants are related to the vicinal coupling constants in three minimum energy staggered rotational isomers of Fig. 1 weighted according to their fractional populations. Most workers have made the assumption that the *gauche* and *trans* vicinal coupl-

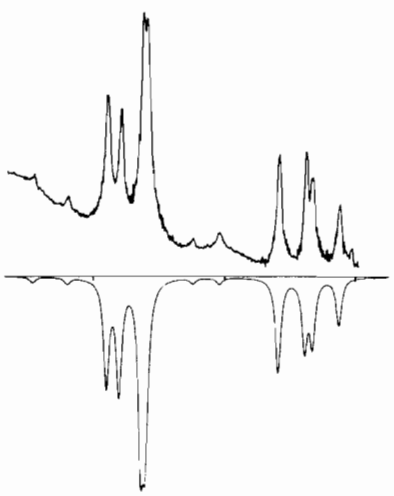

Fig. 2. The observed (upper) and calculated (lower) spectra of $[Co(NH_3)_5(L\text{-serH})]^{3+}$ in ammonia solution at 90 MHz. One division of abscissa is 20 Hz.

ing constants are the same for the three rotamers [8]. Thus, we obtain

$$J_{AB} = p_I J_g + p_{II} J_g + p_{III} J_t$$

$$J_{AC} = p_I J_g + p_{II} J_t + p_{III} J_g$$

$$p_I + p_{II} + p_{III} = 1$$

where most widely used values are J_g = 2.56 Hz and J_t = 13.60 Hz. Even with these equations, we do not know an *a priori* reason to infer which proton B or C is resonating at a higher magnetic field. This ambiguity has been resolved either by stereospecific deuteration at the β carbon atom [9] or by the combined use of $^3J_{HH}$ and the carboxyl carbon to β-hydrogen coupling constant $^3J_{^{13}C-C-C-H}$ [10]. Coupling constants obtained in this work are shown collectively in Table III. Table IV summarizes the results of spectral analysis for several compounds which gave rise to ABX type spectra. In the following we shall discuss the stereochemistry of these complexes in solution, which is inferred from the analysis.

L-Serine complexes

The $[Co(NH_3)_5(L\text{-serH})]^{3+}$ ion in D_2O and the $[Co(NH_3)_4(L\text{-ser})]^{2+}$ in ND_4OD exhibited only a single line at δ = 3.98 and 3.93, respectively. The latter compound in D_2O showed a quartet at δ = 3.89 due to α-proton and a singlet at 3.95 ppm due to β-protons. Figure 2 shows the observed and the calculated spectra of $[Co(NH_3)_5(L\text{-serH})]^{3+}$ in ND_4OD at 90 MHz. From the analysis of the ABX spectrum of Fig. 2, we obtained vicinal coupling constants of 5.225 and 4.144 Hz. The latter value corresponds to

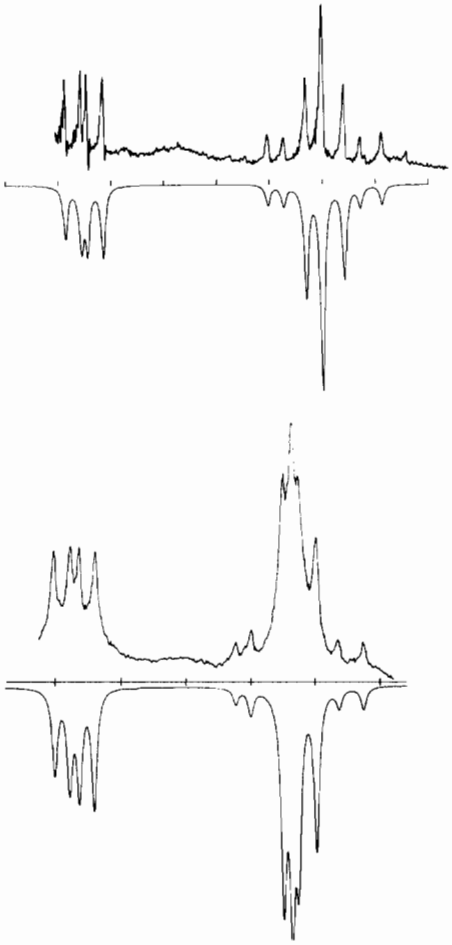

Fig. 3. The 90 MHz spectra of (a) $[Co(NH_3)_5(L\text{-pheH})]^{3+}$ in D_2O and (b) $[Co(NH_3)_4(L\text{-phe})]^{2+}$ in D_2O. One division of abscissa is 20 Hz.

the β-proton resonating at a higher magnetic field. The α-proton resonances are upfield of the β-proton resonances and this shift pattern is the same as for free serine in a pH region >4 [11]. At lower pH values, the shift pattern is reversed in free serine. Further, free serine J_{AC} value is always larger at any pH [11, 9] than J_{AB}. The larger value of the observed vicinal coupling constants is close to the free serine J_{AC} at a neutral or lower pH region. These facts point to the assignment of the proton having a larger $J_{\alpha\beta}$ value to the C proton of Fig. 1. This assignment leads to the fractional population as given in Table IV. The dominant isomer is the one in which three functional groups are close to each other. The stability of this isomer has been noted for free serine and interpreted as a result of hydrogen bonds between these functional groups [11].

L-Phenylalanine compounds

All the phenylalanine compounds investigated here yielded an ABX type spectrum due to CH_2CH

moiety. Typical examples of the spectra are illustrated in Fig. 3, along with the simulated spectra. The larger values of the two observed $J_{\alpha\beta}$ are rather constant over these complexes and close to the corresponding free phenylalanine value. This is in line with a similar constancy of the vicinal couplings found for this amino acid [12]. This suggests that the dominant rotational isomer in these complexes may be the same as that of free amino acid. Thus we assigned the proton which is situated at a higher field and showing a larger vicinal coupling constant to the C proton of Fig. 1. The fractional populations are summarized in Table IV. It is noted in Table IV that p_I and p_{III} vary from compound to compound but p_{II} is rather invariant. In free amino acid, they change little with solution pH and assume almost constant values. About 50% of free phenylalanine exists as a structure in which the bulky phenyl and carboxyl groups are *trans* to each other. Steric hindrance between these groups may be proposed as a possible cause in stabilizing the structure II. The same type of repulsion between $COOCo(NH_3)_5$ and phenyl groups seems to be an important factor in determining the rotamer distribution of coordinated phenylalanine.

It is also noted in Table IV that chelation of phenylalanine makes the rotamer I more abundant than II. This may be rationalized by invoking the repulsion between the phenyl group and the carboxyl C=O group. In $[Co(NH_3)_5(L\text{-pheH})]^{3+}$, free rotation of $C_6H_5CH_2CH(NH_3^+)$ group around the C_o–C_α bond is possible, which is, of course, also the case with free phenylalanine. Upon chelation, however, it is not feasible. The five-membered chelate ring formed is known [13] to be almost planar and the carbonyl group is so oriented that the steric repulsion between the carbonyl oxygen and the phenyl group is greater in rotamer III than in rotamer I, which will tend to make the former rotamer least stable. For $[Co(NH_3)_5(L\text{-pheH})]^{3+}$ ion, in which phenylalanine is coordinated to cobalt(III) only through the carboxyl group, this type of repulsion is irrelevant, hence $p_{III} > p_I$. The situation will be seen from Fig. 1. The result for $[Co(NH_3)_5(L\text{-pheH})]^{3+}$ in ND_4OD is most unusual and at present we have no explanation for it.

L-Histidine complex

The $[Co(NH_3)_5(L\text{-hisH})]^{3+}$ ion gave rise to a typical ABX spectrum, as shown in Fig. 4, when dissolved in D_2O while an AX_2 type spectrum is obtained in ND_4OD. For D_2O solution, we note that the chemical shift values of the α-, β-, C_2-, and C_4-protons and the vicinal coupling constants are similar to the corresponding values of the acidic form of free histidine [14]. Therefore it seems reasonable to assign the proton with larger vicinal coupling constant to the B proton of Fig. 1. Namely, we assume that the rotamer distribution of histidine in this compound is similar to that of free histidine in the acidic form. This assumption is most probable in view of the fact that (i) the solution pH used for NMR measurement was about 2.60 to 2.96 and (ii) at this pD the imidazole $N_1(\pi)$ nitrogen is protonated [15], for which pK_a is reported at 6.0.

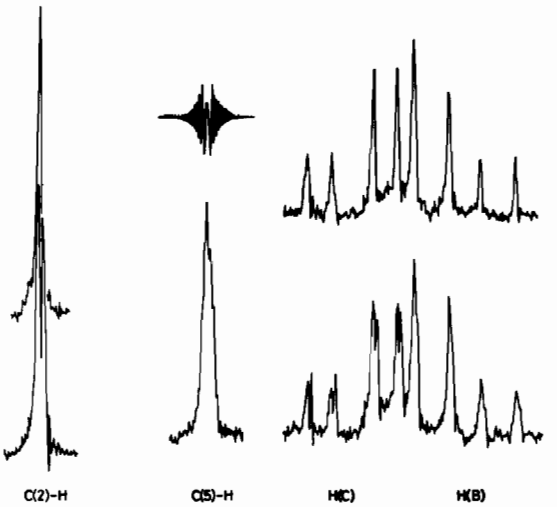

Fig. 4. Decoupling experiment for $[Co(NH_3)_5(L\text{-hisH})]^{3+}$ in D_2O. Irradiation at C(5)-H simplifies both C(2)-H and H(C) resonances.

It is seen in Fig. 4 that the four low-field lines of the AB part of the spectrum are each split into doublets, all the doublet spacings being equal to 0.9 Hz. The imidazole ring protons appear as a broad singlet at δ = 7.42, and a sharp doublet at δ = 8.66 with a splitting of 1.3 Hz. It is well established that the histidine C(2) proton resonates at a field 0.7 to 1.1 ppm lower than the C(5) proton and they are spin–spin coupled with a coupling constant of 1.3 Hz [14]. We can therefore safely assign the doublet resonating at a lower magnetic field to the C(2) proton and a broad singlet at higher field to the C(5) proton. The broad nature of the C(5) resonance is correlated to the splitting of the resonance of one of the β-protons. A decoupling experiment was performed to confirm which proton, the C(2) or the C(5) proton, is coupled to the methylene proton. Irradiation of the C(5) singlet reduced the four low-field doublets of the AB part of the spectrum to four singlets and at the same time the C(2) doublet collapsed to a singlet, see Fig. 4. Thus it is evident that the C(5) proton and one of the β protons are coupled with J = 0.9 Hz and that the C(2) and C(5) protons are coupled with J = 1.3 Hz. The C(5) proton is coupled to only one of the methylene protons. This can be taken as a clear indication that the imidazole ring assumes some preferred conformation with respect to the methylene group. Otherwise, the C(5) proton should couple equally to both protons of the methylene group.

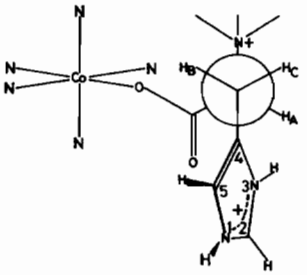

Fig. 5. The structure of $[Co(NH_3)_5(L\text{-hisH})]^{3+}$ ion in D_2O proposed by NMR spectrum.

The most probable overall structure of the complex ion may be inferred as follows. Firstly, of the three rotational isomers generated by rotation about the C_α–C_β bond, the most abundant isomer is II of Fig. 1 as mentioned above. This implies that we assigned the four low-field doublets of the AB part to the C proton. Secondly, the allylic coupling in the fragment CH_2–C(4)=C(5)–H can be used to determine the stereochemistry about the imidazole ring with respect to the methylene group. The variation of the allylic coupling constant with stereochemistry has been studied both experimentally and theoretically [16]. The result in brief is: the coupling between H(C) and C(5)-H would be maximal when the C_β–H(C) bond is parallel to the *pi* electron of the C=C bond while it would be minimal when they are mutually perpendicular. The imidazole ring plane in Fig. 5 is therefore aligned toward the C_β–H(B) bond so that the allylic coupling between H(C) and C(5)-H becomes larger than that between H(B) and C(5)-H. The most probable structure of the $[Co(NH_3)_5(L\text{-hisH})]^{3+}$ ion will be the one depicted in Fig. 5.

Almost the same stereochemistry has been deduced by Blomberg *et al.* [15c] from ^{15}N and ^{13}C NMR of ^{15}N labelled histidine, simply on the ground that they observed different values for the couplings $^3J(^{15}N_\pi$–$^1H_{\beta B})$ and $^3J(^{15}N_\pi$–$^1H_{\beta C})$.

They argued that at pH < 6.2 the carboxylate group is turned over the imidazole ring plane where a positive charge is located. The most stable isomer at this pH region is thus such that the positively charged ammonium and imidazolium groups are *trans* to each other. Kainosho and Ajisaka [9] have established by stereoselective deuteration that in the cationic form of free histidine the most abundant isomer is II of Fig. 1, which is in line with our deduction. As to the conformation around the C_β–C_γ bond, it can be expected that the banana-bond character of the C_4=C_5 double bond will make the C(5)–H bond eclipse with either H(B) or H(C). An attractive interaction between COO group and imidazole group may be the primary determining factor in rotamer stability and this will stabilize the conformation in which these groups are in close proximity [17].

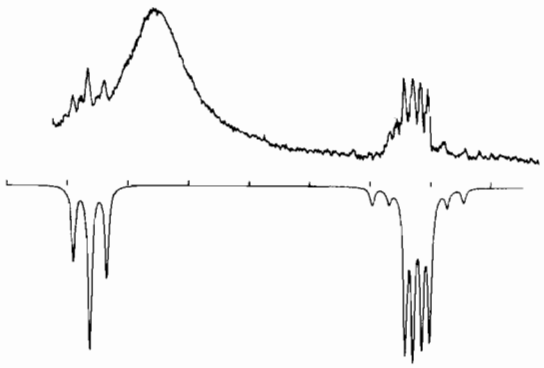

Fig. 6. The 90 MHz spectrum of $[Co(NH_3)_5(L\text{-asnH})]^{3+}$ in D_2O. One division of abscissa is 20 Hz.

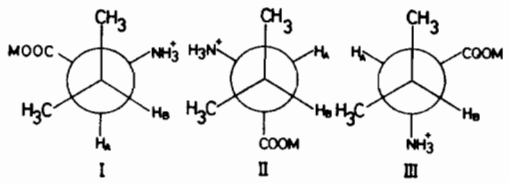

Fig. 7. Three staggered conformations of L-valine.

L-Asparagine complex

The pentaammine compound of this amino acid in ND_4OD gave rise to a double doublet (J = 6.16 Hz) at δ 3.55 and a doublet (J = 6.16 Hz) at δ 2.52. From their intensity ratio (1:2) these resonances can be assigned to α- and β-hydrogens. In D_2O this compound yielded a typical ABX spectrum, as given in Fig. 6, from which we obtained the spectral parameters as shown in Table IV. The vicinal coupling constants are close to those of free amino acid in the acidic form [9], so that we assigned the proton with a larger vicinal coupling constant and resonating at higher magnetic field to the C proton of Fig. 1. This assignment leads to the rotamer distribution given in Table IV, where the most stable isomer is the one in which the three functional groups are brought together in close proximity. The stability of this rotamer may result either from the hydrogen bond between the amido oxygen and the coordinated *cis* ammine group or from water-structure. The latter possibility is suggested by Kainosho and Ajisaka [9].

L-Valine complexes

The $[Co(NH_3)_4(L\text{-val})]^{2+}$ ion has $J_{\alpha\beta}$ values of 3.6 Hz (in D_2O) and 3.8 Hz (in ND_4OD). These values are considerably smaller than the free amino acid value (4.3 to 5.1 Hz). This points to the decrease of the rotamer population p_{III} of Fig. 7, in which H_α and H_β are mutually *trans*. The conformation of free valine in aqueous solution has been studied by Tran-

Dinh *et al.* [18] and by Feeney and coworkers [19]. Their conclusion is the same; the most abundant rotamer is the one where one of the methyl groups are in the $\langle COO^-, NH_3^+ \rangle$ zone, which is illustrated by the rotamers I and II of Fig. 7. The chelation of valine through COO and NH_2 groups drives the rotamer equilibrium off from III toward I and II, which can be interpreted as for the case of chelated L-phenylalanine. In rotamer III, the carbonyl group is fixed to protrude to one of the methyl groups and the non-bonding interaction between the carbonyl oxygen and the methyl group will tend to destabilize this rotational isomer. This type of interaction is absent in monodentate amino acid ligand and the $J_{\alpha\beta}$ and $J_{\beta\gamma}$ values of $[Co(NH_3)_5(L\text{-valH})]^{3+}$ are close to the free ligand values.

The α hydrogen of valinato ligand in $[Co(NH_3)_4(L\text{-val})]^{2+}$ is deuterated in ammonia solution. The rate constant for this process can be obtained by following the intensity decrease of the doublet due to this hydrogen. The pseudo-first-order rate constant at 36.4 °C was 2.0×10^{-4} sec^{-1}.

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