

thiourea complexes, are assigned to C=O bending vibrations. These do not show the increase in frequency on complex formation that was observed for

the N—C=O bending vibration in dimethylformamide.^{13,19}

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Infrared and Proton Magnetic Resonance Spectra of Imidazole, α -Alanine, and L-Histidine Complexes in Deuterium Oxide Solution¹

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The infrared spectra of imidazole and imidazolium ion in D₂O have been assigned on the basis of isotopic substitution results. Spectral changes attendant upon coordination of imidazole and α -alanine with divalent metal ions in D₂O have been observed. Formation constants for monodentate coordination of alanine at lower pD values have been evaluated. The results for imidazole and alanine are employed in interpreting the infrared spectrum of L-histidine in D₂O as a function of pD. The structures of metal-histidine complexes as a function of solution pD have been inferred from infrared data, which indicate the presence of monodentate, bidentate, and tridentate 1:1 histidine complexes at various levels of solution acidity. At pD > 11 a histidine complex is formed involving coordination to the amino group and the deprotonated (anionic) imidazole moiety. The pD dependence of the infrared spectra provides information relating to the formation constants for complex formation; the magnitudes of certain frequency shifts provide information which relates to the enthalpy of formation of the coordinate bond. In particular, the shift in frequency of the antisymmetric carboxylate stretching mode upon coordination at the α -amino group is related to the strength of the metal-amino group interaction.

The interaction of metal ions with polypeptides and proteins is a subject of perennial interest. It is helpful in learning about such systems to understand first the interaction of metal ions with simple "model compounds" which are active components of the more complicated polypeptide chains. Histidine is one of the strongest metal coordinators among the amino acids and plays an important role in binding of metal ions by proteins. The imidazole group of histidine is of prime biological importance in that it is generally responsible for most of the buffering power of proteins in the physiological pH range and is a binding site for metal ions in insulin and serum albumin.^{3,4}

Many investigations of metal interaction with aqueous histidine have been reported in the literature. Most of this work has been concerned with the determination of stability constants.⁵⁻⁸ From a comparison of these stability constants with those of structurally related ligands, several authors have proposed possible structures for the aqueous histidine complexes.⁹⁻¹² In each instance, only indirect structural

evidence obtained from stability constant measurements has been cited. Furthermore, with the exception of recent work on Co(II)-histidine complexes by McDonald and Phillips,¹³ no investigation dealing with the structure of histidine complexes as a function of solution pH has been reported in the literature.

Infrared spectroscopy in aqueous medium furnishes interesting and useful information regarding the interaction of an amino acid or other similar ligand with metal ions. The ligand itself, in the absence of metal ion, may exhibit a spectrum which varies with the pH (or pD if the solvent is D₂O), *i.e.*, the spectra of acid and conjugate base forms of the ligand may differ significantly. Information regarding interaction of the ligand with metal ion can then be acquired by observation of the quantitative effect which the metal ion exerts on the pH (pD) dependence of the ligand spectrum. Infrared spectroscopy offers the singular advantage in this connection that the spectral changes seen are characteristic of protonated and deprotonated forms of specific functional groups, such as amino or carbonyl. Whereas in the more conventional titrimetric procedure for learning about formation constants, a single observable (the proton activity) is measured over the entire range of acidities, the infrared spectral method furnishes in the most fortunate cases separate data about the proton equilibria at each of the sites at which equilibrium with proton is established. The infrared method suffers from the disadvantage of requiring rather high concentrations of ligand and metal ion, however, and can therefore not be

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TABLE I
 INFRARED SPECTRA OF 1:1 METAL-IMIDAZOLE COMPLEXES IN D₂O^a (CM.⁻¹)

Zn(II) pD 6.3	Cu(II) pD 4.1	Ni(II) pD 5.9	Co(II) pD 7.2	Mn(II) pD 7.9	Cd(II) pD 7.8	Imidazole- <i>d</i> ₁ pD 10.9	Imidazolium- 1,3- <i>d</i> ₂ pD 1.4	Assignment
853 (sh)	852 (sh)	848 (sh)	848 (sh)	846 (sh)	...	840 (0.5)	869 (sh) ^b	In-plane ring mode
982 (4)	984 (3)	975 (4)	973 (4)	967 (4)	971 (sh)	950 (3)	...	In-plane ring mode
1083 (10)	1084 (10)	1082 (10)	1081 (10)	1078 (10)	1080 (10)	1071 (3)	1088 (3)	In-plane CH bend
1113 (1)	1113 (1)	1114 (1)	1113 (1)	1112 (1)	1112 (sh)	1110 (1)	1108 (sh)	In-plane CH bend
1330 (1)	1333 (1)	1327 (1)	1325 (1)	1323 (2)	...	1323 (2)	1368 (5)	Ring str.
1372 (4)	1374 (4)	1371 (5)	1370 (5)	1368 (5)	1369 (1)	1359 (3)	1399 (2)	Ring str.
1506 (1)	1507 (1)	1500 (1)	1498 (1)	1495 (2)	...	1486 (2)	1525 (sh)	Ring str.
1531 (2)	1533 (2)	1529 (2)	1527 (2)	1524 (2)	1526 (2)	1509 (1)	1550 (7)	Ring str.

^a Intensities estimated on a transmittance scale from 0 to 10; sh, shoulder. ^b Due in part to in-plane ND⁺ bend.

relied upon to yield formation constants with high precision. On the other hand, when a series of metal ions of the same charge is studied, the relative order of interaction with a given ligand can be determined with moderately good precision.

A second kind of information which the infrared method can yield derives from observation of the magnitudes of frequency shifts which accompany coordination to a metal ion, *e.g.*, shift of the carboxylate anti-symmetric frequency accompanying coordination of a metal ion at an α -amino group in an amino acid. Use of the infrared spectrum in following the pH (pD) dependence gives, in somewhat more detail than might otherwise be available, information about formation constants. Observation of frequency shifts, on the other hand, gives information which relates to the enthalpy of the coordination bond.

We report here the results of an infrared study, in D₂O as solvent, of the interaction of imidazole, α -alanine, and histidine with a number of divalent metal ions. The purpose in studying the first two ligands was to provide necessary background information for understanding the spectral features of the histidine systems. In addition to the infrared spectra, proton magnetic resonance (p.m.r.) data were obtained in certain of the systems, to provide corroborative data in support of the interpretations placed on the infrared results.

Experimental Section

Materials.—Imidazole was obtained from the Aldrich Chemical Co. and was purified by vacuum sublimation. DL- α -Alanine was obtained from General Biochemicals, Inc., and was purified by recrystallization from a boiling ethanol-water solution. L-Histidine (free base) was obtained from the Nutritional Biochemicals Corp. and used without further purification.

Metal ions were introduced in the form of anhydrous chloride or perchlorate salts.

Deuterium oxide (D₂O) of 99.8% isotopic purity was obtained from the Volk Chemical Co., Skokie, Ill.

Solution pD was adjusted using either NaOD (prepared by dissolving clean sodium metal in D₂O) or DCl, prepared by the method of Brown and Groot.¹⁴ Deuterioperchloric acid (DClO₄), prepared by an ion-exchange technique, was used for pD adjustment in solutions where the absence of chloride ion was desirable. Solution pD was measured on a Beckman Zeromatic pH meter equipped with a one-drop electrode assembly and calibrated with standard H₂O buffer solutions. In all cases, apparent

pD values were corrected for the deuterium isotope effect using the equation¹⁵ pD = meter reading + 0.40.

Infrared Spectra.—Spectra were obtained on a Beckman IR-7 spectrophotometer equipped with sodium chloride optics and an ordinate scale expansion unit. Eastman Kodak IRTRAN-2 plates as well as 0.025-mm. fixed thickness BaF₂ cells were employed. To attain balanced transmission, a variable transmittance shutter was placed in the reference beam of the spectrophotometer. Appropriate precautions were taken to ensure that the transmitted light intensity was adequate.

Proton Magnetic Resonance Spectra.—P.m.r. spectra were recorded at 60 Mc. with a Varian Associates Model A-60 spectrometer. For use as an internal standard, a 0.5–1.0% solution of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) prepared by the method of Tiers and Coon¹⁶ was employed. Chemical shifts were measured from the centers of multiplets and were taken to be negative in sign when appearing at a lower field than the internal standard.

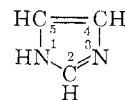
Results

Imidazole.—Infrared spectra of 1:1 metal-imidazole complexes in D₂O are presented in Table I along with corresponding spectra of imidazole and imidazolium ion.¹⁷ Band assignments were obtained by examining the infrared spectra of the deuterated derivatives of imidazole and imidazolium ion.¹⁸ The in-plane bending and ring stretching modes of imidazole undergo sizable shifts to higher frequencies upon formation of imidazolium ion. The spectra of the metal complexes are more characteristic of free imidazole than of imidazolium ion. These large frequency shifts indicate that upon protonation (and to a lesser extent, upon

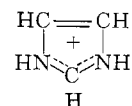
(15) K. Mikkelsen and S. O. Nielsen, *J. Phys. Chem.*, **64**, 632 (1960).

(16) G. V. D. Tiers and R. O. Coon, *J. Org. Chem.*, **26**, 2097 (1961).

(17) Imidazole is a five-membered heterocyclic ring containing two nitrogen atoms



By convention, the nitrogen atom in the 1 position is called the "pyrrole" nitrogen and that in the 3 position is called the "pyridine" nitrogen. Protonation of the pyridine nitrogen atom of imidazole results in formation of the imidazolium ion



In D₂O solution, proton bound to nitrogen in either form is rapidly exchanged.

(18) R. H. Carlson, Ph.D. thesis, University of Illinois, 1965. Imidazole-1,2,4,5-*d*₄ and imidazolium-*d*₅ ions were studied in addition to the N-deuterated species.

(14) H. C. Brown and C. Groot, *J. Am. Chem. Soc.*, **64**, 2223 (1942).

TABLE II

P.M.R. SPECTRA OF IMIDAZOLE AND ITS COMPLEXES IN D₂O^a

Compound	pD	2-CH	4,5-CH
Imidazole (0.2 M)	12.1	-7.76	-7.15
Imidazolium ion (0.2 M)	1.2	-8.75	-7.56
1:1 imidazole-ZnCl ₂ (0.2 M)	6.3	-8.15	-7.30
1:1 imidazole-CdCl ₂ (0.1 M)	8.1	-8.01	-7.29

^a Spectra measured in p.p.m. from internal DSS.

complex formation) the imidazole ring undergoes extensive π electron redistribution.

The proton resonance spectra of 1:1 Zn(II)- and Cd(II)-imidazole complexes are presented in Table II along with the corresponding spectra of imidazole and imidazolium ion. Each of these spectra shows a simple first-order AX₂ pattern in D₂O consisting of two peaks in the intensity ratio 1:2. The less intense peak is assigned to the no. 2 CH proton, while the other is assigned to the no. 4 and 5 CH protons, magnetically equivalent in solution because a rapid N-H proton exchange takes place with solvent. It is for this reason also that the NH proton of imidazole does not show a separate resonance in aqueous solution.

values <2 still show a sizable absorption band at 1412-1414 cm.⁻¹.

Loss of proton from the alanine cation in the pD range 0.5 to 4.8 is reflected in the disappearance of the band at 1735 cm.⁻¹, assigned to the C=O group, and appearance of the antisymmetric carboxylate mode at 1612 cm.⁻¹, characteristic of the alanine zwitterion. In the presence of metal ion, the pD dependence of this spectral change is shifted downward, indicating that an interaction occurs between metal ion and carboxyl group. A quantitative measure of the equilibria involved is most conveniently obtained by determination of the "half-reaction point," the pD value at which the concentrations of protonated and deprotonated forms involved are equal. The extinction coefficients of the 1735 and 1612 cm.⁻¹ bands were determined by measurements of optical densities at each extreme of the pD range. For 0.2 M ligand, the ratio $\epsilon_{1612}/\epsilon_{1735}$ is 3.27. The optical densities of each of the two bands, after correction for the extinction coefficient ratio, were then graphed vs. pD.²¹ The intersection of the two curves, which can be determined to within about ± 0.05 pD

TABLE III

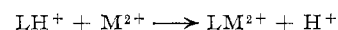
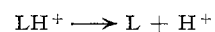
INFRARED SPECTRA OF DL- α -ALANINE IN D₂O^a (CM.⁻¹)

pD 0.5	pD 1.8	pD 4.8	pD 10.1	pD 10.8	Assignment
823 (3)	...	832 (sh)	830 (1)	829 (sh)	CCN str. + CC str.
911 (6) br	913 (1)	917 (1.5)	920 (1)	921 (sh)	CCN str. - CC str.
1018 (sh)	1022 (sh)	1015 (sh)	1021 (sh)	1018 (sh)	CH ₃ rock
1048 (4)	1049 (2.5)	1057 (1)	1054 (3)	1053 (3)	CH ₃ rock
1103 (2)	1103 (1.5)	1100 (2)	1108 (2)	1108 (1.5)	CCN out-of-phase str.
1269 (3)	1270 (4)	C-O str.
...	...	1292 (5)	1292 (5.5)	1290 (4)	CH bend
1327 (7)	1329 (5.5)	CH ₃ def.
...	1340 (sh)	1339 (10)	1336 (10)	1333 (9)	
...	1371 (6.5)	1369 (7)	
1379 (3)	1380 (4)	1377 (10)	1376 (6.5)	...	
1412 (3)	1413 (4)	1414 (9)	1415 (10)	1416 (10)	COO ⁻ sym. str.?
1468 (10)	1468 (10)	1466 (7.5)	1465 (6)	1464 (6)	CH ₃ def.
...	1575 (vs)	1573 (vs)	COO ⁻ antisym. str.
...	1615 (7)	1612 (vs)	1615 (vs)	...	C=O str.
1735 (vs)	1734 (vs)	1727 (1.5)	

^a Intensities estimated on a transmittance scale from 0 to 10; vs, very strong (more intense than a designation of 10); br, broad; sh, shoulder.

α -Alanine.—Infrared spectra of α -alanine in D₂O as a function of solution pD are presented in Table III. Band assignments were obtained by comparing the aqueous spectra with the solid state spectra of DL- α -alanine assigned by Tsuboi and co-workers.¹⁹ For the most part, the aqueous spectra agree very well with the assignments given for the solid state spectra. The only assignment open to controversy is that of the symmetric carboxylate stretching mode, assigned by Tsuboi and co-workers to the band at 1411 cm.⁻¹. The KBr spectrum of alanine hydrochloride²⁰ also exhibits a band at 1410-1411 cm.⁻¹, which, however, cannot be due to carboxylate absorption. Furthermore, as the data in Table III show, the spectra of aqueous α -alanine at pD

unit, represents the half-reaction point. Assuming that simple equilibria of the form



are involved, the half-reaction point provides a reliable procedure for evaluating formation constants, subject to the uncertainties regarding the activity coefficients of the species involved in the equilibria at the rather high ionic strengths employed. It is a reliable procedure, in any case, for ascertaining the dissociation constants of the free ligand, as evidenced by the agreement attained with literature values based on more precise titrimetric procedures²¹ (*vide infra*), and for determining the relative order of interaction of metal ion with the

(19) M. Tsuboi, T. Takenishi, and A. Nakamura, *Spectrochim. Acta*, **17**, 634 (1961).

(20) A. Leifer and E. R. Lippincott, *J. Am. Chem. Soc.*, **79**, 5098 (1957).

(21) See F. L. Khalil and T. L. Brown, *ibid.*, **86**, 5113 (1964), for an exemplary figure.

TABLE IV

HALF-REACTION POINT (pD) AND MONODENTATE FORMATION CONSTANTS FOR 0.2 M α -ALANINE SOLUTIONS

Added electrolyte	Half-reaction point		Log K_f (COO ⁻)		
0.6 M NaClO ₄	2.75	2.85 ^a	...		
0.2 M Mn(ClO ₄) ₂	2.64		0.0		
0.2 M Co(ClO ₄) ₂	2.63		0.1		
0.2 M Zn(ClO ₄) ₂	2.53	2.57 ^a	0.5	0.3 ^b	
0.2 M Ni(ClO ₄) ₂	2.53		0.5		
0.2 M Cu(ClO ₄) ₂	2.39		0.9		
0.2 M Cd(ClO ₄) ₂	2.19	2.00 ^a	1.3	1.5 ^b	

^a Obtained from p.m.r. data. ^b Calculated from p.m.r. data.

TABLE V

INFRARED SPECTRA OF 1:1 METAL- α -ALANINE COMPLEXES IN D₂O^a (CM.⁻¹)

Cu(II) pD 7.4	Ni(II) pD 6.8	Co(II) pD 7.9	Zn(II) pD 9.2	Cd(II) pD 7.7	Assignment
897 (sh)					CCN str. - CC str.
933 (0.5)	932 (0.5)	932 (0.5)			
1051 (2)	1050 (2)	1049 (2)	1052 (2)	1049 (2)	CH ₃ rock
1111 (sh)	1115 (0.5)	1112 (0.5)	1113 (sh)	1111 (0.5)	CCN out-of-phase str.
1291 (sh)	1293 (sh)	1291 (sh)	1291 (sh)	1292 (sh)	CH bend
1330 (1)	1331 (2)	1331 (2)	1331 (2)	1333 (2)	CH ₃ def.
1371 (3)	1376 (2)	1374 (2)	1374 (2)	1376 (2)	
1401 (4)	1418 (4)	1416 (4)	1417 (4)	1416 (4)	COO ⁻ sym. str.?
1463 (3)	1465 (3)	1464 (4)	1465 (4)	1465 (4)	CH ₃ def.
1608 (10)	1594 (10)	1594 (10)	1592 (10)	1586 (10)	COO ⁻ anti-sym. str.

^a Intensities estimated on a transmittance scale from 0 to 10; uncertainty in frequencies ± 1 cm.⁻¹.

made by comparison with the spectra of the free ligand at various pD values. The antisymmetric carboxylate stretching mode is of particular interest and is discussed later.

The p.m.r. spectra of α -alanine and its 1:1 Zn(II) and Cd(II) complexes in D₂O are of the simple AX₃ variety. The coupling constant, $J_{\text{CH}_3-\text{CH}}$, is on the order of 7 c.p.s. and decreases slightly in going from acidic to basic medium. In a graph of the CH chemical shift vs. solution pD for solutions of 0.2 M α -alanine a break is observed in each curve in the pD range from 2 to 3. These breaks can be interpreted as resulting from proton ionization of the carboxyl (COOH) group.^{13,22,23} Ionization of the carboxyl proton results in an upfield shift of the CH resonance due to inductive and magnetic anisotropy effects.²⁴ The pD value at the midpoint of each break corresponds to the half-reaction point for cation dissociation into the free zwitterion or metal-zwitterion complex. The half-reaction points determined in this manner are listed in Table IV. The p.m.r. data are in good quantitative agreement with the infrared spectral data, within the precisions attainable.

Histidine.—Spectra of histidine in D₂O as a function of solution pD are presented in Table VI. Band assignments were obtained by comparing the spectra of histidine with the corresponding spectra of imidazole, 4-methylimidazole, and α -alanine. The assignment of

TABLE VI

INFRARED SPECTRA OF L-HISTIDINE IN D₂O^a (CM.⁻¹)

pD 0	pD 4.8	pD 8.1	pD 12.2	pD 14	Assignment
...	...	847 (sh)	837 (sh)	842 (sh)	CCN str. + CC str. (Al) + in-plane ring mode (Im)
874 (4) vbr	862 (1)	
...	...	943 (1)	945 (2)	952 (4)	In-plane ring mode (Im)
...	...	1005 (2)	1012 (4)	1010 (5)	Ring breathe (Im)
1022 (10)	1019 (1)	1020 (sh)	CH ₂ rock (Al)
1088 (sh)	...	1097 (2)	1097 (sh)	1082 (sh)	In-plane CH bend (Im)
1108 (2)	1108 (3)	1109 (1)	1103 (3)	1104 (5)	CCN out-of-phase str. (Al)
1274 (sh)	C—O str. (Al)
1321 (2)	1324 (3)	1317 (1)	1314 (sh)	...	CH bend (Al) + ring str. (Im)
...	1333 (sh)	1334 (3)	1327 (5)	1334 (5)	CH ₂ wag (Al)
1357 (sh)	1354 (2)	1351 (1)	
1411 (5)	1406 (10)	1408 (10)	1413 (10)	1418 (10)	CO ₂ ⁻ sym. str. (Al)?
1444 (5)	1443 (3)	1441 (3)	1442 (3)	1443 (sh)	CH ₂ bend (Al)
1603 (4)	...	1482 (3)	1483 (3)	1456 (6)	Ring str. (Im)
...	1625 (vs)	1619 (vs)	1579 (vs)	1579 (vs)	CO ₂ ⁻ antisym. str. (Al)
1737 (vs)	C=O str. (Al)

^a Intensities estimated on a transmittance scale from 0 to 10: vs, very strong band; sh, shoulder; br, broad band; Im denotes absorption characteristic of the imidazole moiety; Al denotes absorption characteristic of the α -alanine moiety.

ligand. Table IV lists the half-reaction points for a variety of α -alanine solutions.

It should be noted that the apparent increase in acidity of the carboxyl proton in the presence of metal ion implies that there is a monodentate interaction of the amino acid with metal ion, since in the range of pD values about 2.75 the amino group is completely protonated. Bidentate coordination occurs at pD values above approximately 5. The spectra of a number of 1:1 α -alanine-metal ion complexes in the pD range in which there is bidentate coordination are listed in Table V. The assignments of absorptions were

the symmetric carboxylate stretching vibration to the band at approximately 1410 cm.⁻¹ is doubtful, since a strong absorption still appears at this frequency in acidic solutions of histidine where the carboxylate group remains protonated.

Infrared spectra are in complete agreement with the ionization constants (pK_a values of histidine in D₂O are 2.05, 6.53, 9.79, and 13.93 for D⁺ ionization from the carboxyl, imidazolium, amino, and imidazole groups,

(22) F. Taddei and L. Pratt, *J. Chem. Soc.*, 1553 (1964).

(23) R. J. Kula, D. T. Sawyer, S. I. Chan, and C. M. Finley, *J. Am. Chem. Soc.*, **85**, 2930 (1963).

(24) H. Spiesscke and W. G. Schneider, *J. Chem. Phys.*, **35**, 722 (1961).

TABLE VII
 INFRARED SPECTRA OF 1:1 HISTIDINE COMPLEXES IN D₂O^a (CM.⁻¹)

Cu(II) pD 5.0	Zn(II) pD 5.0	Cd(II) pD 5.0	Ni(II) pD 5.6	Co(II) pD 5.6	Mn(II) pD 7.5	Fe(II) pD 6.6	Assignment
835 (sh)	829 (sh)	...	831 (sh)	827 (sh)	823 (sh)	825 (sh)	CCN str. + CC str. (Al) + in-plane ring mode (Im)
860 (sh)	853 (sh)	856 (1)	859 (sh)	849 (sh)	843 (sh)	846 (sh)	
...	958 (4)	955 (4)	951 (2)	953 (2)	In-plane ring mode (Im)
979 (3)	977 (4)	965 (2)	970 (sh)	969 (sh)	963 (2)	964 (sh)	
1022 (sh)	...	1022 (1)	1033 (6)	1029 (8)	1022 (5)	1025 (6)	Ring breathe (Im) + CH ₂ rocking (Al)
1053 (4)	1051 (6)	1037 (2)	1051 (1)	1051 (1)	1048 (1) br	1051 (1)	
1086 (2)	1082 (1) br	1077 (sh)	1081 (6)	1080 (6)	1080 (3)	1080 (4)	In-plane CH bend (Im)
...	1100 (3)	1100 (3)	1100 (2)	1100 (2)	
1115 (7)	1116 (9)	1112 (4)	...	1111 (1)	1110 (2)	1111 (1)	CCN out-of-plane str. (Al)
1314 (2)	1318 (6)	1329 (4) br	1320 (10)	1321 (9)	1323 (8)	1322 (8)	
1331 (sh)	1335 (6)	...	1325 (sh)	CH bend (Al) + ring str. (Im)
1356 (sh)	1354 (sh)	1354 (1)	1355 (2)	1354 (2)	1352 (2)	1353 (2)	
1406 (10)	1411 (10)	1410 (10)	1415 (7)	1413 (9)	1412 (10)	1411 (10)	CH ₂ wag (Al)
1444 (9)	1443 (6)	1443 (3)	1445 (8)	1445 (10)	1443 (6)	1444 (8)	CO ₂ ⁻ sym. str. (Al)?
1500 (4)	1501 (5)	1492 (2)	1498 (3)	1496 (4)	1492 (4)	1493 (2)	CH ₂ bend (Al)
1598 (sh)	1600 (sh)	...	1599 (vs)	1597 (vs)	1592 (vs)	1595 (vs)	Ring str. (Im)
1616 (vs)	1621 (vs)	1622 (vs)	1614 (sh)	1615 (sh)	CO ₂ ⁻ antisym. str. (Al)

^a Intensities estimated on a transmittance scale from 0 to 10; vs, very strong; sh, shoulder; Im denotes absorption characteristic of imidazole moiety. Al denotes absorption characteristic of α -alanine moiety.

respectively^{25,26}) of histidine with regard to the ionization sequence of the ligand. At pD \sim 0, the presence of the strong band at 1737 cm.⁻¹, characteristic of the C=O stretch of a nonionized carboxyl group,²⁷ indicates the presence of the doubly charged histidine cation. At pD 4.8, the strong band at 1625 cm.⁻¹, characteristic of the antisymmetric stretching vibration of an ionized carboxylate group adjacent to a protonated amino group,²⁸ is associated with the presence of the singly charged histidine cation. At pD 8.1, the appearance of the bands at 1482 and 943 cm.⁻¹, assigned to the stretching and in-plane bending vibrations of the deprotonated (neutral) imidazole ring, respectively, indicates the presence of the histidine zwitterion. At pD 12.2, the strong band at 1579 cm.⁻¹, characteristic of the antisymmetric stretching vibration of an ionized carboxylate group adjacent to a neutral amino group,²⁸ supports the presence of the singly charged histidine anion. At pD \sim 14, the shifts in frequency of the ring stretching and in-plane ring bending vibrations of the imidazole moiety reflect proton ionization from the imidazole ring in forming a doubly charged histidine anion. It is thus apparent that unambiguous information regarding proton ionization from individual sites in amino acid systems can be obtained from infrared spectra.

Representative infrared spectra of some 1:1 metal-histidine complexes in D₂O are presented in Table VII. These spectra show essentially the same pD dependence as the spectrum of the free histidine ligand. As previously indicated, α -alanine forms a weak metal complex at low pD values (*i.e.*, 2.2–3.0) through mono-

dentate coordination at the carboxylate group. This same type of complex forms with histidine also. The pD range over which the monodentate histidine complex predominates in solution is a function of the driving force for coordination of metal ions to the other two coordination sites of histidine (*i.e.*, the imidazole ring and the amino group). From the observed proton ionization sequence of histidine it would seem reasonable to expect the imidazole ring to coordinate at a higher pD value than the carboxylate group, but at a lower value than the amino group. As the pD of a metal-histidine solution is increased above 3, bands corresponding to skeletal vibrations of the *coordinated* imidazole ring appear in the infrared spectra. The intensity of the band assigned to the ring-stretching vibration (1490–1501 cm.⁻¹) increases with pD until all of the imidazole moiety is coordinated. At pD values beyond this point the band intensity remains fairly constant.

The bands assigned to the breathing and in-plane bending modes of the coordinated imidazole ring appear with moderately high intensity in the 1035–1055 and 965–980 cm.⁻¹ regions, respectively. At somewhat higher pD values these bands are replaced by bands approximately 10–15 cm.⁻¹ lower in frequency. Also, in the same pD range, the band assigned to the CCN antisymmetric stretching vibration (1110–1115 cm.⁻¹) of the alanine moiety decreases in intensity and is replaced by a corresponding band at approximately 1100 cm.⁻¹. Furthermore, the band at 1615–1625 cm.⁻¹, assigned to the antisymmetric carboxylate stretch of the alanine moiety, is replaced by a band at 1590–1600 cm.⁻¹. These spectral changes at higher pD values (from 4 to 7), showing precisely the same pD dependence, result from coordination of the amino group of histidine. Spectral bands characteristic of amino coordination in the Cu(II)-, Zn(II)-, and Cd(II)-histidine complexes were not observed; precip-

(25) N. C. Li, P. Tang, and R. Mathur, *J. Phys. Chem.*, **65**, 1074 (1961).

(26) R. B. Martin, *Science*, **139**, 1198 (1963).

(27) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., Methuen and Co., Ltd., London, John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 234–242.

(28) K. Nakamoto, Y. Morimoto, and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 309 (1963).

itation precluded further measurements at high pD values.

To obtain more quantitative information regarding histidine complexation as a function of solution pD, optical density measurements were carried out on the imidazole ring stretching and the alanine CCN out-of-phase stretching bands of the complexes. These two absorptions represent attendant spectral changes accompanying imidazole and amino coordination, respectively. For each of the complexes listed in Table VII, the optical density ($\log I_0/I$) of the imidazole ring stretching vibration, obtained by the base-line technique, was graphed as a function of solution pD. The pD at which the optical density equaled one-half of its maximum value was taken as the half-reaction point for imidazole coordination. The half-reaction point for amino coordination in the histidine complexes was obtained by plotting the optical densities of the CCN out-of-phase stretching vibration at 1110–1115 cm^{-1} (characteristic of the protonated amino group) and the corresponding CCN vibration at 1100 cm^{-1} (characteristic of the coordinated amino group) against solution pD. The ratio of the extinction coefficient of the protonated form of the CCN stretching mode to that of the coordinated form was found to be approximately 1.4; therefore, the optical density of the former band was divided by 1.4 before plotting. Table VIII lists the half-reaction points for imidazole and amino coordination in a series of metal-histidine solutions.

TABLE VIII

HALF-REACTION POINTS (pD) FOR 1 M HISTIDINE SOLUTIONS

Added electrolyte (1 M)	Half-reaction point ^a	
	Imidazole coordination	Amino coordination
CuCl ₂	3.28	...
ZnCl ₂	3.88	...
NiCl ₂	4.02	4.14
CoCl ₂	4.25	4.77
CdCl ₂	4.88	...
FeCl ₂	5.16	5.98
MnCl ₂	5.61	7.19

^a Uncertainty in half-reaction points is ± 0.10 pD unit.

Proton Magnetic Resonance Spectra.—Proton magnetic resonance (p.m.r.) spectral data for L-histidine²⁹ and its 1:1 Zn(II) and Cd(II) complexes in D₂O are presented in Table IX. The spectrum of histidine in acidic solution strongly resembles the simple AX₂YZ type. Only a small splitting of the center peak of the triplet indicated that the spectrum should not be classified as strictly a first-order type. The protons of the imidazole moiety show first-order splitting with the H-5 proton splitting the H-2 proton into a doublet and *vice versa*. The spectrum of L-histidine in alkaline solution is of the more complicated ABCXY pattern, as are the spectra of the Zn(II) and Cd(II) complexes,

(29) The imidazole moiety of histidine is numbered in the following manner

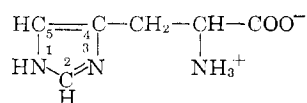


TABLE IX

P.M.R. SPECTRA OF L-HISTIDINE AND ITS COMPLEXES IN D₂O (1 M)^a

Compound	pD	Imidazole moiety		Alanine moiety	
		H-2	H-5	CH	CH ₂
Histidine	1.7	-8.84	-7.59	-4.47	-3.56
Histidine	5.5	-8.65	-7.45	-4.12	-3.39
Histidine	8.6	-7.79	-7.06	-3.99	-3.17
Histidine	12.4	-7.65	-6.95	-3.54	-2.92
1:1 Cd-histidine	5.9	-8.06	-7.20	-4.05	-3.27
1:1 Zn-histidine	5.0	-8.20	-7.25	-4.10	-3.34
1:1 Zn-histidine	11.8	-7.26	-6.90	-3.64	-3.00

^a Spectra measured in p.p.m. from internal DSS.

where now only the protons of the imidazole moiety show simple first-order splitting.

The p.m.r. data are in consonance with the ionization sequence of histidine supported by the infrared measurements. Proton ionization results in an upfield shift of the C-H resonances due to inductive and magnetic anisotropic effects.²⁴ With histidine, the proton group closest to the ionizing site shows the largest upfield shift for a given ionization. The data in Table IX reveal that the CH proton of the alanine moiety shows the largest upfield shift in the pD range 1.7 to 5.5 and 8.6 to 12.4 where proton ionization from the carboxylate and amino groups occur, respectively. The protons of the imidazole moiety show the largest upfield shift in the pD range 5.5 to 8.6 where proton ionization from the imidazole ring occurs.

Discussion

Imidazole.—The infrared spectra indicate that upon protonation imidazole undergoes an extensive electron redistribution resulting from inductive electron withdrawal operating at the pyridine nitrogen. Protonation of imidazole should also induce a shift in the CH proton resonances to lower field, as observed (Table II). Ring frequency shifts, therefore, may serve as a spectral probe with which to measure the relative bond strengths of metal-imidazole interaction; the stronger the metal-imidazole bond, the more closely should the ring frequencies approach those observed for imidazolium ion. As the data in Table I show, the in-plane ring bending and ring stretching frequencies of imidazole complexes increase in the following order³⁰: Imid < Mn(II) < Cd(II) < Co(II) < Ni(II) < Zn(II) < Cu(II) < D⁺. This is interpreted to mean that the strength of the metal-imidazole bond increases in the above order in going from Mn(II) to Cu(II). This order of metal-imidazole bond strength is in good agreement with the Irving-Williams order of stability of transition metal complexes of nitrogen-containing ligands^{31,32}: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II). The order of chemical shift to lower field in the p.m.r. spectra of imidazole and its Zn(II) and Cd(II) complexes is the same as the infrared frequency order and confirms the fact that Zn(II) interacts more strongly with

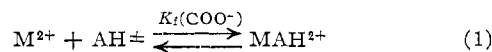
(30) The frequencies recorded in Table I were obtained from an average of four scans and are relatively accurate to within ± 0.5 cm^{-1} .

(31) H. Irving and R. J. P. Williams, *Nature*, **162**, 746 (1948).

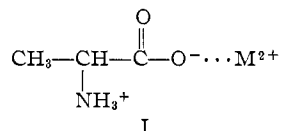
(32) H. Irving and R. J. P. Williams, *J. Chem. Soc.*, 3192 (1953).

imidazole than does Cd(II). Quantitative application of the results with imidazole will be made in subsequent discussion of histidine complexes.

α -Alanine.—A quantitative measure of the relative strength of metal interaction with the carboxylate group of α -alanine in D₂O can be obtained by evaluating the formation constants of the reaction



where AH[±] represents the alanine zwitterion and MAH²⁺ represents the *monodentate* complex (I).



The formation constants, $K_f(\text{COO}^-)$, of the monodentate α -alanine complexes can be calculated in the following manner: From (1), the expression for $K_f(\text{COO}^-)$ can be written as

$$K_f(\text{COO}^-) = \frac{[\text{MAH}^{2+}]}{[\text{AH}^{\pm}][\text{M}^{2+}]} \quad (2)$$

The constant, K_1 , for proton dissociation from the carboxyl group of α -alanine is expressed as

$$K_1 = \frac{[\text{AH}^{\pm}][\text{H}^+]}{[\text{AH}_2^+]} \quad (3)$$

where AH₂⁺ represents the alanine cation. Solving for [AH[±]] and substituting into eq. 2 gives

$$K_f(\text{COO}^-) = \frac{[\text{MAH}^{2+}][\text{H}^+]}{[\text{M}^{2+}]K_1[\text{AH}_2^+]} \quad (4)$$

At the half-reaction point, [AH₂⁺] = 0.10 M, [M²⁺] = X, [MAH²⁺] = 0.2 - X. K_1 , corrected for the deuterium isotope effect, is 1.95×10^{-3} .²⁶ Substituting these values into eq. 4 gives

$$K_f(\text{COO}^-) = \frac{[0.2 - X][\text{D}^+]}{X[1.95 \times 10^{-3}][0.1]} \quad (5)$$

The formation constants of the monodentate metal-alanine complexes were calculated from eq. 5 using a simple iterative technique²¹ to obtain the correct values of X. The quantity [D⁺] in eq. 5 is the deuterium ion concentration at the half-reaction point obtained from Table IV. Values of $K_f(\text{COO}^-)$ are listed in Table IV; correction for ionic strength effects, using an extended form of the Debye-Hückel equation,³³ yields log K_f values which are 0.5 larger than those listed in Table IV. The correction is probably not very reliable quantitatively, but the fact that it is in the direction of a larger value for K_f indicates that the apparent increase in acidity of the carboxyl proton is in fact due to complex formation. The constant K_1 for proton dissociation from the carboxyl group of α -alanine was also

(33) J. N. Butler, "Ionic Equilibrium A Mathematical Approach," Addison-Wesley, Inc., Reading, Mass., 1964, p. 432. The calculation of $-\log \gamma$ involved the following assumptions about the monodentate complexes: (a) The complex behaves as two 1:1 electrolytes. (b) The resultant activity coefficient calculated for the complex is an average of the individual activity coefficients calculated for the carboxylate and amino termini of the complex. The calculation of corrected K_f values seems reasonable, but in any case, the relative values of K_f in the series of metal ions studied are essentially unaffected by the assumptions made.

TABLE X

ANTISYMMETRIC CARBOXYLATE STRETCHING MODE FREQUENCY SHIFTS vs. PSEUDO-FORMATION CONSTANTS FOR COORDINATION AT THE AMINO GROUPS OF ALANINE AND HISTIDINE

Metal ion	1:1 alanine complexes		1:1 histidine complexes	
	Log $K_f'(\text{NH}_2)$	$\Delta\nu$, cm. ⁻¹	Log $K_f'(\text{NH}_2)$	$\Delta\nu$, cm. ⁻¹
Cd(II)	1.5	12
Mn(II)	3.1	...	2.6	12
Zn(II)	4.1	18
Co(II)	4.2	20	5.0	18
Ni(II)	4.9	20	5.7	20
Cu(II)	7.3	34

evaluated from the spectral data. Values of $pK_1 = 2.75$ and 2.85 were obtained from infrared and p.m.r. data, respectively. Correction for the deuterium isotope effect²⁶ yields values of 2.38 and 2.48 in H₂O, in very good agreement with the value of 2.34 reported in the literature.³⁴

The antisymmetric carboxylate stretching frequency of α -alanine increases from 1573 cm.⁻¹ for the anion at pD 10.8 to 1612 cm.⁻¹ for the zwitterion at pD 4.8. This increase in carboxylate frequency can be ascribed to the inductive effect of the positively charged amino group of the zwitterion.²⁸ It follows that the metal ion forming the strongest bond with the amino group of α -alanine should exhibit the highest antisymmetric carboxylate stretching frequency in its complexes. As previously mentioned, carboxylate coordination does not produce a shift in frequency of the antisymmetric carboxylate stretching vibration; hence, in spectra of the aqueous metal-alanine chelates the frequency of this band must be governed by the relative strength of metal-amino group interaction. Table V shows that the antisymmetric carboxylate stretching frequency decreases in the following order: Cu(II) > Ni(II) \approx Co(II) > Zn(II) > Cd(II); this is evidence that the relative enthalpies of the amine-metal interactions decrease in the above order. If the formation constant $K_f(\text{COO}^-)$ for monodentate coordination at the carboxylate group is subtracted from the value for bidentate 1:1 complex formation,³⁵⁻³⁷ the result, which we label $K_f'(\text{NH}_2)$, should reflect the relative driving force for complex formation at the amino group. Log $K_f'(\text{NH}_2)$ is tabulated in Table X along with the frequency shift $\Delta\nu = (\nu_{\text{complex}} - \nu_{\text{anion}})$ for the antisymmetric carboxylate stretching frequency of the alanine chelates. The values of 1:1 formation constants of the alanine complexes derived from different studies are widely different, but the smooth trend in the data is evident. It would appear that the antisymmetric carboxylate stretching frequency of the complex may prove a useful measure of the relative enthalpies of coordinate bond formation at the amino group, since the entropy change for formation of a bidentate complex from the monodentate can be expected to remain constant or vary monotonically with ΔH in a

(34) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York and London, 1961.

(35) H. Irving and L. D. Pettit, *J. Chem. Soc.*, 1547 (1963).

(36) D. J. Perkins, *Biochem. J.*, **57**, 702 (1954).

(37) L. E. Maley and D. P. Mellor, *Nature*, **165**, 453 (1950).

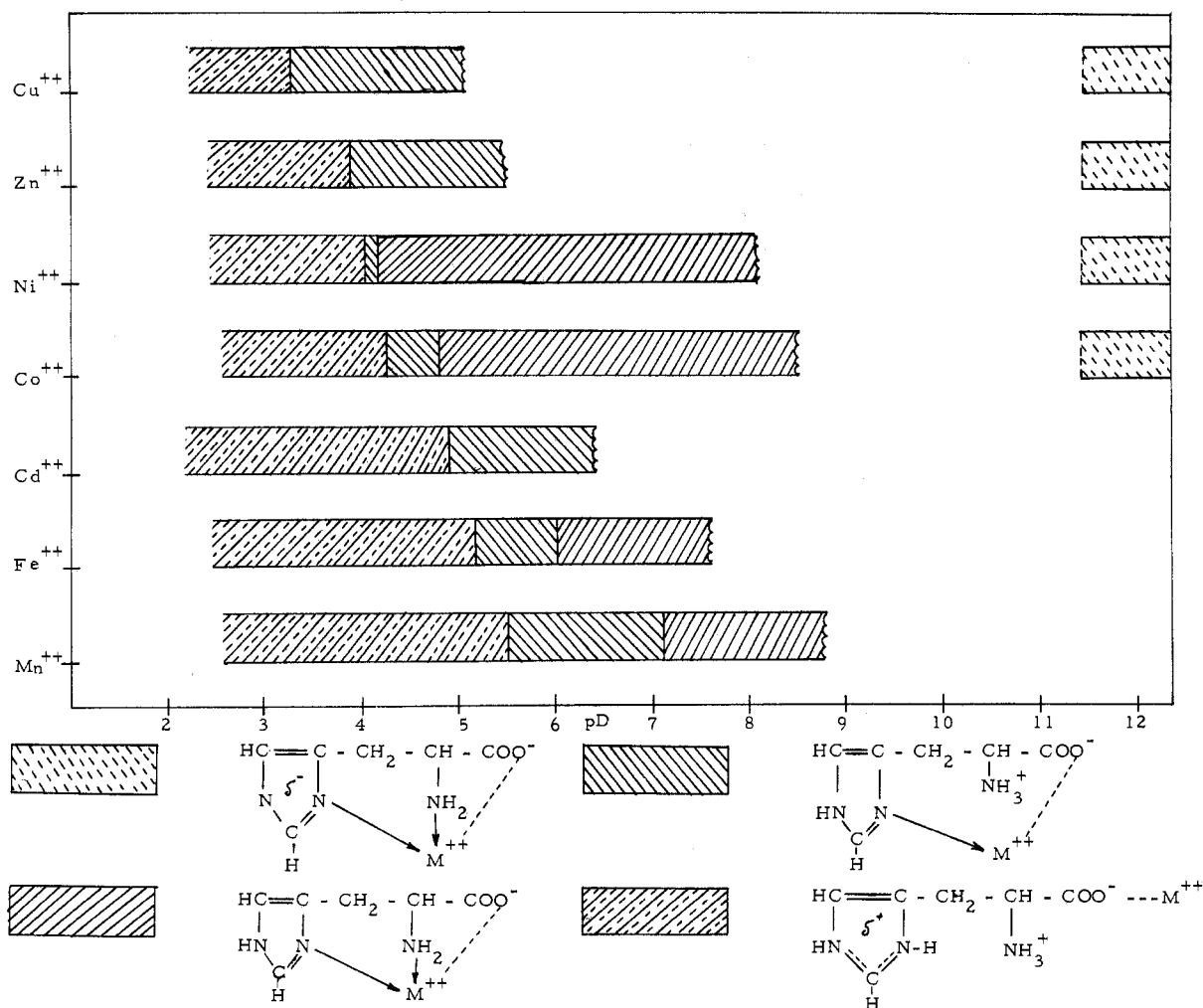


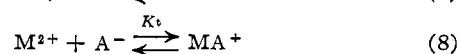
Figure 1.—Structures of 1:1 metal ion-histidine complexes in D₂O as a function of pD. Open regions in the mid-pD range are indicative of low solubility.

series of divalent metal ions. Such relationships are well-known in other types of systems.³⁸

Histidine.—The structure of the metal-histidine complexes as a function of pD can be established from the half-reaction points for imidazole and amino coordination listed in Table VIII. Figure 1 shows in a schematic fashion the structure of the histidine complexes as a function of pD; the various regions represent different structural forms of the complexes in solution. The infrared spectra of the Cu(II), Zn(II), and Cd(II) complexes presented in Table VII are representative of the bidentate metal-histidine chelates, while the spectra of the Ni(II), Co(II), Mn(II), and Fe(II) complexes are representative of the tridentate chelates. The infrared spectra suggest that at pD > 11 complex formation with the metal ions Cu(II), Zn(II), Ni(II), and Co(II) involves coordination to the amino group and to the ionized (anionic) imidazole ring.³⁹ Ionization of the "pyrrole" hydrogen (proton at the N-1 position) of the imidazole moiety in the presence of the above metal ions at pD > 11 is evidenced by a shift in frequency of the imidazole ring vibrations. The shifts are very similar to those

noted in the spectrum of *free* histidine at pD ~ 14. The amino group is apparently still coordinated at pD > 11; the antisymmetric carboxylate stretching vibration of the complexes lies well above the frequency of 1579 cm.⁻¹ observed in the spectrum of free histidine at pD 12.2 for a carboxylate group adjacent to a free amino group. The observed dependence of the structure of the Co(II)-histidine complex on pD is in good agreement with the conclusions drawn by McDonald and Phillips¹⁸ from contact shift measurements. These authors were able to detect a monodentate Co(II)-histidine complex at pH < 4, a tridentate (octahedral) complex in the pH range from 4 to 10, and a bidentate (tetrahedral) complex at pH 11 involving coordination to the amino groups and to the ionized (anionic) imidazole ring.

Formation constants can be calculated for the bidentate and tridentate metal-histidine complexes from the half-reaction points listed in Table VIII. "Successive" formation constants for the reactions



(38) M. D. Joesten and R. S. Drago, *J. Am. Chem. Soc.*, **84**, 3817 (1962).

(39) With solutions of the Cu(II), Zn(II), Co(II), and Ni(II) complexes, the precipitate of metal hydroxide and metal hydroxyhistidine ultimately formed upon addition of sufficient NaOD redissolves at pD values > 11.

TABLE XI
SUCCESSIVE FORMATION CONSTANTS OF
1:1 METAL-HISTIDINE COMPLEXES^a

Metal	Log K_b^b	Log K_t^c
Cu(II)	4.1	...
Zn(II)	3.1	...
Ni(II)	3.0	8.7 (8.7) ^c
Co(II)	2.4	7.4 (6.9) ^c
Cd(II)	2.9	...
Mn(II)	0.9	3.5

^a Temperature 32°; $\mu = 1-1.5$. ^b Internal reliability of $\pm 0.10 \log K$ unit. ^c Reference 5.

are listed in Table XI. In these equilibria, AH_2^+ , AH^\pm , and A^- represent the singly-charged cation, zwitterion, and anion of L-histidine, respectively, and MAH_2^{3+} , MAH^{2+} , and MA^+ represent the monodentate, bidentate, and tridentate histidine complexes, respectively. The formation constants ($\log K_m$) of the monodentate metal-histidine complexes are taken to be the same as those found for monodentate coordination with α -alanine (Table IV). From (7), the expression for K_b can be written

$$K_b = \frac{[MAH^{2+}]}{[M^{2+}][AH^\pm]} \quad (9)$$

The constant, K_2 , for proton dissociation from the singly charged histidine cation in D_2O is

$$K_2 = 2.96 \times 10^{-7} = \frac{[AH^\pm][H^+]}{[AH_2^+]} \quad (10)$$

Solving for $[AH^\pm]$ gives

$$[AH^\pm] = \frac{K_2[AH_2^+]}{[H^+]} \quad (11)$$

Substitution of eq. 11 into eq. 9 gives

$$K_b = \frac{[MAH^{2+}][H^+]}{[M^{2+}]K_2[AH_2^+]} \quad (12)$$

Now from (6)

$$[AH_2^+] = \frac{[MAH_2^{3+}]}{K_m[M^{2+}]} \quad (13)$$

Substitution of eq. 13 into eq. 12 gives

$$K_b = \frac{[MAH^{2+}][H^+]K_m}{K_2[MAH_2^{3+}]} \quad (14)$$

At the half-reaction point for bidentate coordination

$$K_b = \frac{[H^+]K_m}{K_2} \quad (15)$$

The formation constants ($\log K_b$) of the bidentate metal-histidine complexes were calculated from eq. 15 where $[H^+]$ is the proton (deuterium ion) concentration at the half-reaction point for imidazole coordination. The formation constants ($\log K_t$) of the tridentate metal-histidine complexes were calculated using the same procedure as above where K_m and K_2 in eq. (15) are replaced, respectively, by K_b and $K_3 = 1.62 \times 10^{-10}$ for proton dissociation from the histidine zwitterion. The value of $[H^+]$ is now the proton (deuterium ion) concentration at the half-reaction point for amino coordination.

The value of $\log K_b = 4.1$ obtained for the bidentate Cu(II)-histidine complex is somewhat lower than the

value of 5.0 obtained by Datta and Rabin¹⁰ using titrimetric techniques. Formation constants for other *bidentate* metal-histidine complexes have not been reported. Values of $\log K_t$ presented here are in good agreement with values found in the literature for 1:1 metal-histidine complexes.^{5,7} Differences between the present results and conclusions based on titrimetric data are not unexpected, since we are required to employ rather high ionic strength and a solvent isotope effect of unknown magnitude²⁵ is also involved.

The value for K_b represents the apparent formation constant for coordination at the imidazole group of histidine. These values are somewhat larger than those found in the literature for coordination at imidazole itself,^{40,41} because of the chelate effect resulting from the prior monodentate coordination at carboxylate. The comparison is rather rough because of the rather large uncertainty in both K_t (COO^-) and K_b values.

By analogy with the procedure followed above for alanine, subtraction of $\log K_b$ from $\log K_t$ yields a $\log K_t'$ (NH_2) value which should serve as a measure of the relative driving force for coordination at the amino group. The data for the frequency shifts in the antisymmetric carboxylate stretching mode *vs.* the corresponding $\log K_t'$ (NH_2) values are tabulated in Table X. Where a comparison is possible, the frequency shifts in the alanine and histidine systems are about the same, but somewhat larger K_t' (NH_2) values obtain for the histidine complexes. The difference, which is not large, very probably arises from somewhat more favorable entropy effects in the histidine systems, since the histidine is already bidentate through coordination at the imidazole and carboxylate groups.

Proton Magnetic Resonance Spectra.—The p.m.r. spectral data presented in Table IX support a bidentate chelate structure for the 1:1 Zn(II)- and Cd(II)-histidine complexes at pD 5.0 and 5.9, respectively. The imidazole resonances of the complexes lie between those observed for free histidine at pD 5.5 and 8.6 where the imidazole ring is protonated and non-protonated, respectively. This is consistent with metal coordination to the imidazole moiety, for Zn(II) and Cd(II) interact less strongly with the ring than does a proton and, hence, produce a smaller downfield chemical shift. The imidazole resonances lie further downfield in the Zn(II) complex than in the Cd(II) complex, indicating that Zn(II) interacts to a greater extent with the imidazole moiety than does Cd(II).

Infrared data suggest that at pD >11 Zn(II) forms a 1:1 histidine complex involving primary coordination to the amino group and to the ionized (anionic) imidazole ring. P.m.r. data are in agreement with formation of this type of complex, for the imidazole resonances in the Zn(II) complex at pD 11.8 (Table IX) lie considerably upfield from those observed in the Zn(II) complex at pD 5.0, and in the spectra of free

(40) R. B. Martin and J. T. Edsall, *J. Am. Chem. Soc.*, **80**, 5033 (1958).

(41) B. L. Mickel and A. C. Andrews, *ibid.*, **77**, 5291 (1955).

histidine at pD 8.6 and 12.4, where the imidazole ring is nonprotonated. The upfield shift is indicative of an increase in electron density about the ring protons at pD >11, as a result of ionization of the "pyrrole" hydrogen. P.m.r. data support amino coordination in the Zn(II)-histidine complex at pD 11.8, for the alanine resonances lie between those observed for free histidine at pD 8.6 and 12.4, where the amino group is protonated and nonprotonated, respectively. Furthermore, the alanine resonances lie upfield of those observed in the Zn(II) complex at pD 5.0. These facts are consistent with replacement of the proton on the amino group by the less electronegative Zn(II) ion in going from pD 5.0 to 11.8.

In summary, the particular value of the infrared spectral data, and the ancillary p.m.r. data, is that the structures of the coordinated species at a given pD are deducible from correlation of the spectra with the spectra of free ligand as a function of pD. The evaluation of formation constants is not very precise, but the agreement of the results in this area with the expectations based on more precise methods is sufficiently good to lend support to the interpretations placed on the infrared data. In addition, observation of certain frequency shifts in favorable cases, such as the anti-symmetric carboxylate stretching mode, yields information which relates closely to the relative enthalpies of coordinate bond formation.

CONTRIBUTION FROM THE INSTITUTE FOR ATOMIC RESEARCH AND DEPARTMENT OF CHEMISTRY
IOWA STATE UNIVERSITY, AMES, IOWA

A Redetermination of the Crystal Structure of CsCuCl₃^{1a}

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The crystal structure of CsCuCl₃ has been redetermined by single crystal X-ray diffraction techniques. Data were taken by scintillation counter methods and the atomic parameters were refined by anisotropic least-squares methods. The final parameters are essentially those originally reported by Wells. The structure consists of distorted hexagonally close-packed layers of cesium and chlorine atoms with the copper atoms occupying octahedral holes. The Cu-Cu distance is a very short 3.062 Å, but direct copper-copper bonding is ruled out by overlap integral calculations. The close approach of the coppers is considered to be a result of the copper atoms sharing faces of their chlorine octahedra instead of edges as has been found in all other halogen-bridged copper compounds. This interpretation differs from that of Wells. Several other metal-halogen systems having chain structures similar to that found in CsCuCl₃ are considered and the overlap integrals for metal-metal interaction calculated. From the calculations it is apparent that d-d metal-metal bonding for the elements at the right of the transition metal series is unlikely. The implications of this are related to the much discussed Cu₂(C₂H₃O₂)₂·2H₂O.

Introduction

The structure determination of CsCuCl₃ was first attempted by Klug and Sears in 1946,^{2a} but they were unsuccessful in solving the structure. Wells^{2b} next investigated the problem and obtained a model which gave an agreement factor, $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, of 20% on the basis of 60 reflections.

Because of their interesting structural and magnetic features, several related copper compounds have recently been investigated in this laboratory, namely, LiCuCl₃·2H₂O,^{3,4} KCuCl₃, and NH₄CuCl₃.⁵ These structures all consisted of Cu₂Cl₆²⁻ dimers associated into chains by long unsymmetric Cu-Cl bridges and are quite different from the CuCl₃⁻ infinite chain found for CsCuCl₃ by Wells. Thus CsCuCl₃ should represent a

different and particularly simple system to study magnetically because of its essentially one-dimensional character. Preliminary to such a study we decided to carry out a more accurate determination of its structure, which is being reported here. A magnetic investigation of this compound is currently underway, by B. C. Gerstein of this laboratory.

Experimental Section

Dark red-brown crystals of CsCuCl₃ were obtained by evaporating to dryness a solution containing copper and cesium chlorides. A molar excess of copper chloride was used to prevent formation of Cs₂CuCl₄. The crystals appeared as hexagonal prisms often capped by hexagonal pyramids.

Weissenberg photographs exhibited 6/mmm Laue symmetry. The only systematic extinction observed was $l \neq 6n$ for 00 l reflections, indicating the space group P6₃22 and confirming Wells' observation. The lattice constants were determined by a least-squares extrapolation treatment of twelve reflections in the back-reflection region observed using a single crystal orienter. The orienter was previously aligned with an aluminum single crystal. The lattice constants found are: $a = b = 7.2157 \pm 0.0005$ and $c = 18.1777 \pm 0.0010$ Å.

Complete three-dimensional data to $\sin \theta/\lambda = 0.904$ were taken with zirconium-filtered molybdenum K α radiation using a General

(1) (a) Work was performed at the Ames Laboratory of the U. S. Atomic Energy Commission. Contribution No. 1780. (b) Deceased, Oct. 9, 1963.

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