117. *Thermodynamic Effects involved in the Metal-ion Chelation of Histidine, Histidine Methyl Ester, and 4(or 5)-Imidazolylacetic Acid.*

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Stepwise stability constants for the complexes of histidine, histidine methyl ester, and 4(or 5)-imidazolylacetic acid with Zn^{II}, Co^{II}, Ni^{II}, and Cu^{II}, determined potentiometrically, are reported at 0, 15, 25, and 40°. Calculated enthalpy and entropy factors are presented and discussed. Evidence indicates that histidine chelates involve the carboxyl oxygen atom, and exhibit considerable ring strain.

Amino-acid complex formation including histidine has been extensively studied.[1-6] Investigations incorporating temperature variation have been more sparse.[5,6] Complexes

[1] Albert, *Biochem. J.*, 1950, **47**, 531; 1952, **50**, 690.
[2] Hearon, Burk, and Schade, *J. Nat. Cancer Inst.*, 1949, **9**, 337; Maley and Mellor, *Nature*, 1950, **165**, 453.
[3] Li, Doody, and White, *J. Amer. Chem. Soc.*, 1957, **79**, 5859.
[4] Chakravorty and Cotton, *J. Phys. Chem.*, 1963, **67**, 2878.
[5] Chawla, Doctoral Diss., Kansas State Univ., 1962.
[6] Andrews and Romary, *J.*, 1964, 405.

of histidine methyl ester have been studied only briefly.[3,5,6] Complexes of 4(or 5)-imidazolylacetic acid have not been previously reported.

From data at several temperatures, stability constants can be resolved into enthalpy and entropy factors. As a first-order simplification, the enthalpy factor can be directly related to the strength of the metal-ion–ligand linkage, and the entropy factor to a statistical, or probability, effect.[7]

Experimental

Procedure.—The method and the potentiometric titration technique of Bjerrum [8] as modified by Albert [1] was used. Titrations at 0, 15, 25, and 40° were carried out in the same manner and with the same apparatus as previously described.[9]

Calculations were made with an IBM 1620 computer using Fortran programming.[10] Final values of stability constants were obtained from Bjerrum's approximations method, and also from the solution of simultaneous equations based on the formation function.[8,10] Both methods gave equivalent values.

The initial titration system ranged from 0·01 to 0·05M-ligand in known volume (about 80 ml.). The metal-ion concentrations were adjusted such that the molar ratio of ligand to metal-ion was maintained at 8 to 1. The ionic strength was 0·25 with potassium chloride. To avoid ionic-strength changes due to dilution during the titration, the titrant (0·5M CO_2-free potassium hydroxide) contained 0·25M-potassium chloride. All titrations were conducted in an atmosphere of purified and presaturated nitrogen.

Materials.—Histidine methyl ester dihydrochloride was prepared by heating a solution of histidine, methanol, and concentrated hydrochloric acid, under reflux, and was twice recrystallized from methanol–acetone. Histidine free base was purchased from Eastman Organic Chemicals, and was twice recrystallized from distilled water. Imidazolylacetic acid hydrochloride was as purchased from K and K Laboratories. Potentiometric titrations indicated a purity in excess of 99% for histidine and the ester, and 97·7% for imidazolylacetic acid. Purity of the ester was confirmed by base hydrolysis.

Solutions of the metal chlorides and all reagents were prepared from reagent grade material. Concentrations were accurately determined by analyses.

Discussion

Proton dissociation constants are presented in Table 1. Stability constants and thermodynamic values are reported in Table 2. Enthalpy values were obtained by least squares for $\log K_n$ against $1/T$. Entropy values were calculated by the Gibbs–Helmholtz equation. Several systems exhibited a rapid change in slope, near 25°, for the dependence of $\log K_n$ on temperature, as shown by nearly equal magnitudes of the stability constants at 25 and 40°. Reliable enthalpy factors cannot be calculated for these systems at 25°. For this reason all thermodynamic values are reported at 15°. It is estimated that the stability constants are correct to within $\pm 0{\cdot}05$ and the other values in Table 2 are correct to within $\pm 1{\cdot}0$ kcal./mole.

Chelate stabilities are greatest for histidine, least for imidazolylacetic acid, and reflect the relative basicities of the ligands.[11] The order of complex stabilities as regards the metal-ion is $Cu^{II} > Ni^{II} > Co^{II} > Zn^{II}$ in most cases, in agreement with Mellor and Maley's order [12] and Irving and Williams' order.[13]

The existence of 3 : 1 histidine methyl ester complexes is considered to indicate a terdentate nature for histidine and a bidentate nature for histidine methyl ester. Histidine complexes possess greater stability than corresponding ester complexes, yet histidine fails

[7] Parry in "The Chemistry of the Coordination Compounds," ed. Bailer, Reinhold, New York, 1956.

[8] Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941; *Chem. Rev.*, 1950, **46**, 381.

[9] Andrews, Lyons, and O'Brien, *J.*, 1962, 1776.

[10] Andrews, Hassler, and DeCou, *Comm. of A.C.M.*, 1963, **6**, 694.

[11] Calvin and Wilson, *J. Amer. Chem. Soc.*, 1945, **67**, 2003.

[12] Mellor and Maley, *Nature*, 1948, **161**, 436.

[13] Irving and Williams, *J.*, 1953, 3192.

TABLE 1.

Ionization constants for protonated ligands.*

	Temp.	pK for H_3li	pK for H_2li	pK for Hli
Histidine	0°	1·98	6·62	9·81
	15	1·79	6·24	9·46
	25	1·96	6·12	9·17
	40	1·85	5·88	8·86
Histidine methyl ester	0		5·84	8·02
	15		5·51	7·58
	25		5·39	7·34
	40		5·16	7·06
4(or 5)-Imidazolylacetic acid	0		3·13	7·86
	15		3·15	7·58
	25		3·24	7·40
	40		3·25	7·15

* Values for histidine and HME are in good agreement with previously reported values.[1,3,5,6]

TABLE 2.

Stepwise stability constants and thermodynamic values.

			log K_n				15°	
	M^{2+}	n *	0°	15°	25°	40°	$-\Delta H°$ (kcal./mole)	$T\Delta S°$ (kcal./mole)
Histidine	Zn	1	7·00	6·78	6·40	6·52	6·9	2·0
		2	5·96	5·55	5·02	5·07	11·4	−4·1
	Cu	1	12·8	11·2	10·7	10·5	27·4	−12·6
	Ni	1	9·28	8·79	8·50	8·30	11·4	0·2
		2	7·71	7·08	6·69	6·40	13·7	−4·4
	Co	1	7·30	7·10	6·77	6·56	8·0	1·4
		2	6·07	5·62	5·13	4·94	12·6	−5·2
Histidine methyl ester	Zn	1	5·29	4·82	4·40	4·36	9·2	−2·8
		2	4·71	3·93	3·78	3·69	9·2	−4·0
		3	1·90					
	Cu	1	9·84	9·12	8·32	8·14	18·3	−6·3
		2	7·10	6·54	5·82	5·72	16·0	−7·4
		3	2·42					
	Ni	1	7·32	6·65	6·02	5·95	16·0	−7·2
		2	5·88	5·14	4·30	4·34	16·0	−9·2
		3	3·59	2·76	2·50	2·11	17·2	−13·6
	Co	1	5·68	5·00	4·24	4·10	16·0	−9·4
		2	4·50	3·57	3·12	2·96	16·0	−11·3
		3	2·67	2·18				
4(or 5)-Imidazolyl-acetic acid	Zn	1	3·86	3·83	3·86	3·59	2·3	2·7
		2	3·43	3·32	3·24	3·33	0·0	4·4
		3	2·80	2·63	2·70		3·4	1·5
	Cu	1	7·02	7·34	7·00	6·72	3·4	6·3
		2	5·71	5·81	5·69	5·40	2·3	5·4
	Ni	1	4·65	4·83	4·70	4·34	2·3	4·1
		2	3·84	3·71	3·55	3·44	3·4	1·5
		3		2·28				
	Co	1	3·94	4·00	3·83	3·68	2·3	3·0
		2	3·04	3·03	2·98	2·63	3·4	0·6

* The symbol "n" refers to the ligand–metal-ion ratio of the complex.

to form 3 : 1 complexes. The 2 : 1 histidine complexes appear to fulfill the same co-ordination number of the metal-ion as do the 3 : 1 histidine methyl ester complexes.

Conflicting viewpoints have appeared in the literature as regards the sites of metal-ion binding on the histidine methyl ester molecule.[3,14] If the primary binding sites on the ester molecule are the carbonyl oxygen, and the side-chain nitrogen, a significantly different and smaller enthalpy contribution would be observed for ester complex formation than for histidine complex formation. The respective enthalpy contributions are observed to be similar. The lower enthalpy factors for the imidazolylacetic acid complexes, relative

[14] Kroll, *J. Amer. Chem. Soc.*, 1952, **74**, 2036; Bender and Turnquest, *ibid.*, 1957, **79**, 1889.

to those of the histidine and the ester complexes, show the effect of increased oxygen participation. For the complexes reported here, the primary binding sites for complex formation on the histidine methyl ester molecule appear to be the imidazole nitrogen, and the side-chain amino-nitrogen.

TABLE 3.

Thermodynamic effects for the replacement of histidine methyl ester with histidine, at 15°.

Reaction	M^{2+} *	$-\Delta H°$ (kcal./mole)	$T\Delta S°$ (kcal./mole)
$M(HME)A_{x-2} + Hd \longrightarrow HME + M(Hd)A_{x-3} + A$	Zn	−2·3	4·8
	Ni	−4·6	7·4
	Co	−8·0	10·8
	Cu	9·1	−6·3
$M(HME)_3 + 2Hd \longrightarrow M(Hd)_2 + 3HME$	Ni	−24·1	25·8

* M represents the metal-ion, Hd the histidine molecule, HME the histidine methyl ester molecule, and A a water molecule, or an inert salt anion. Charges on the species are omitted for convenience.

Certain comparisons of thermodynamic values between histidine and histidine methyl ester chelates are summarized in Table 3. The positive entropy effects strongly indicate a greater degree of chelation by histidine in its complexes, and appear to confirm a terdentate nature for histidine. Steric hindrance to complexing by the ester molecule also may be reflected in the entropy effects. If histidine is terdentate, it must be capable of forming a fused ring chelate involving both a five-membered ring (side-chain nitrogen and carboxyl oxygen) and a six-membered ring (side-chain nitrogen and imidazole nitrogen). The suggestion that the carboxyl group is involved in histidine complexes has been made before by Irving and Weber,[15] who reasoned from stability constants reported by Mickel and Andrews,[16] Albert,[1] Chakravorty and Cotton,[4] and Andrews and Romary.[6]

Schwarzenbach [17] has stated that chelates having fused rings with more than one member in common will be less stable, because of ring strain, than similar chelates having rings with only one member in common. Mild ring strain might result in a weaker overall metal-ion–ligand linkage with histidine than with its methyl ester. This could account for the unfavourable enthalpy changes shown in Table 3. The favourable entropy changes in Table 3 still would occur because three metal-ion co-ordination sites would be occupied by histidine, as opposed to only two by the ester.

The histidine–Cu^{II} complex is unique. The large enthalpy change is opposed by a large negative entropy change, but still results in the most stable of the 1 : 1 complexes. Addition of a second histidine molecule proceeds irregularly, and with difficulty, and is complete only at relatively high free ligand concentrations. A reliable stability constant value can be obtained only for the 1 : 1 complex. Albert [1] has noted comparable peculiarities for the histidine–Cu^{II} system. Jonassen *et al.*[18] reported almost identical behaviour for the titration of the diethylenetriamine–Cu^{II} system, and reasoned that the first ligand molecule attaches to copper(II) normally, but the second does not attach to copper(II) through all three of its donor sites. The same explanation appears valid for histidine–copper(II).

The difference between successive stability constants is greater for histidine methyl ester–copper(II) than for the other ester–metal-ion systems. Mickel and Andrews [16] have considered similar evidence for histamine–metal-ion systems to indicate steric resistance to the square planar configuration by histamine. When the donor sites of histidine are forced to fit a planar configuration, greater ring strain will occur than for other configurations, because two of the ligand donor sites will have to be *trans* to each other. Such

[15] Irving and Weber, *J.*, 1959, 2560.
[16] Mickel and Andrews, *J. Amer. Chem. Soc.*, 1955, **77**, 5291.
[17] Schwarzenbach in "Advances in Inorganic Chemistry and Radiochemistry," eds. Emeléus and Sharpe, Academic Press, New York, 1961, Vol. III; *Helv. Chim. Acta*, 1952, **35**, 2344.
[18] Jonassen, LeBlanc, and Rogan, *J. Amer. Chem. Soc.*, 1950, **72**, 4968.

an intensified ring strain might be the cause of the unfavourable entropy change observed for the replacement of ester by histidine in the 1 : 1 copper(II) complex. Schwarzenbach [17] has pointed out that the chelate entropy effect is greatest for chelate rings free of strain, and that strained cyclic structures can exhibit a negative chelate entropy effect.

The large favourable enthalpy factor for copper(II)–histidine would not be expected from previous considerations. A marked enthalpy contribution to the total chelate effect, unique to chelates having copper(II) as the central metal-ion, is known to exist [19] and has been confirmed calorimetrically.[7] The 1 : 1 histidine–copper(II) chelate might be another example.

Chakravorty and Cotton [4] have expressed the opinion that the carboxyl oxygen of histidine makes a relatively minor contribution towards binding to copper(II) and to nickel(II). Our findings indicate the opposite to be true.

Entropy contributions are small compared with the enthalpy factors, for the histidine and the histidine ester complexes. Entropy factors are greatest for the 1 : 1 complexes. This is not so in general for the enthalpy factors. Bjerrum [8] has reasoned that on a purely statistical basis successive stepwise stability constants should be expected to decrease in magnitude. Such a decrease would be manifested in the entropy factor rather than the enthalpy factor.

Entropy contributions become more predominant and consistently positive for the imidazolylacetic acid complexes. Imidazolylacetic acid apparently forms six-membered strain-free chelate rings and offers little steric hindrance to complexing. Uusitalo [20] has observed that for complexes of ligands possessing both nitrogen and oxygen donor atoms, entropy and enthalpy contributions are approximately equal, and that favourable entropy effects dominate and increase as complex stabilities decrease.

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[19] Calvin and Bailes, *J. Amer. Chem. Soc.*, 1946, **68**, 949; Spike and Parry, *ibid.*, 1953, **75**, 2726, 3770.
[20] Uusitalo, *Finska Kemistsamfundets Medd.*, 1958, **67**, 101 (*Chem. Abs.*, 1959, **53**, 13743).