484. *Metal Complexes of Histamine and Some Structural Analogues. Part I.*

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The chelating abilities of histamine, 2-2′-aminoethylpyridine, 4-2′-aminoethylthiazole, 3-2′-aminoethylpyrazole, 4-aminomethylimidazole, and 2-aminomethylpyridine with Cu(II), Ni(II), and Co(II) have been studied potentiometrically. The ligands generally form bis-complexes with Cu(II), and tris-complexes with Ni(II) and Co(II), when hydrolysis or oxidation does not interfere with complex-formation. 3-2′-Aminoethylpyrazole forms only 1 : 1 complexes with all three metals. Approximate formation constants are given.

An investigation of the chelating properties of histamine and a number of its structural analogues has been undertaken. The ligands are interesting in that they exhibit different degrees of physiological activity.[1] A number of generalisations may be made concerning histamine activity as a function of molecular structure [2] and these are enumerated since they provide the pattern on which the chelation studies are based: (1) Other aromatic rings may replace imidazole, but all the highly active compounds contain a tertiary nitrogen atom as part of the aromatic ring. (2) The number of carbon atoms between the primary amino-group and the aromatic ring is critical. When attached at the 4-position of

[1] Hofmann, "Imidazole and Derivatives," Part I, Interscience Publ. Inc., New York, 1953.
[2] Lee and Jones, *J. Pharmacol. Exp. Therap.*, 1949, **95**, 71.

imidazole the 2-aminoethyl side chain confers maximum activity; any variation in chain length diminishes the activity. (3) The side chain must be attached at the 4-position in the case of imidazole: 1- and 2-2′-aminoethylimidazole are biologically inactive. (4) The presence of a basic nitrogen atom in the side chain is usually necessary. *N*-Alkylation of this primary amino-group in general reduces the activity in proportion to the size and number of the attached groups. (5) Ring substitution also reduces histaminic potency to a degree depending on the position of the substituent.

Knowing how structural modifications of the histamine molecule affect its biological activity, we have investigated how the same modifications affect the nature and stability of complexes formed between these ligands and Cu(II), Ni(II), and Co(II) ions, since Lyons and Andrews [3,4] have indicated the possible rôle of metal ions as sites of binding of histamine to proteins. For histamine itself a number of metal complexes have been reported. Albert,[5] Mickel and Andrews,[6] Hares *et al.*,[7] and von Schalien [8] have given stability constants and other thermodynamic data.

The present paper deals with the effects on the chelating ability, first, of changing the aromatic portion, and secondly, of shortening the side chain. The ligands discussed are histamine, 2-2′-aminoethylpyridine, 4-2′-aminoethylthiazole, 3-2′-aminoethylpyrazole, 4-aminomethylimidazole, and 2-aminomethylpyridine.

Experimental

Materials.—Histamine was obtained from Eastman Kodak Co. and recrystallised from benzene. 2-2′-Aminoethylpyridine was prepared as described by Kirchner *et al.*[9] 4-2′-Aminoethylthiazole and 3-2′-aminoethylpyrazole were given by Dr. Reuben G. Jones, Eli Lilly Laboratories. 4-Aminomethylimidazole dihydrochloride was prepared by Turner's method,[10] and 2-aminomethylpyridine according to Craig and Hixon's instructions.[11]

Metals were used in the form of their perchlorates.

Determinations.—Dissociation constants for the ligands were obtained by potentiometric titration at 25·0° ± 0·1°; 5·00 ml. of standard (*ca.* 0·1N) perchloric acid (2·00 ml. when ligand dihydrochlorides were used) and 15·00 ml. of approx. 1×10^{-2}M-organic base, made up to 50·00 ml. with carbon dioxide-free water, were titrated with 0·1000N-carbonate-free sodium hydroxide. Stirring was by a stream of nitrogen which had been passed through Fieser's solution to remove oxygen and carbon dioxide and then pre-saturated with aqueous vapour at 25·0°.

Formation constants for the metal complexes were obtained from similar titrations with 5·00 ml. of $1{\cdot}000 \times 10^{-2}$M-metal ion present; the initial volume was again 50·00 ml., the ionic strength being 0·01—0·02.

It was found necessary to obtain the lower dissociation constants of the thiazole, pyrazole, and aminomethylpyridine ligands spectrophotometrically by the method of Andon *et al.*[12] This was carried out at 20° ± 1°, a Unicam S.P. 500 spectrophotometer being used with matched 1 cm. silica cells.

The ratio metal : ligand for metal complexes of histamine and of 3-2′-aminoethylpyrazole in solution was checked spectrophotometrically by the method of continuous variations.[13]

Formation data for the systems were obtained by Bjerrum's method,[14] the calculations being based upon those of Maley and Mellor [15] adapted for our own systems. Formation constants were calculated by either of two methods, depending on the complexity of the system. For

[3] Lyons and Andrews, *J. Colloid Sci.*, 1955, **10**, 370.
[4] Andrews and Lyons, *Science*, 1957, **126**, 561.
[5] Albert, *Biochem. J.*, 1952, **50**, 690.
[6] Mickel and Andrews, *J. Amer. Chem. Soc.*, 1955, **77**, 323, 5291.
[7] Hares, Fernelius, and Douglas, *J. Amer. Chem. Soc.*, 1956, **78**, 1816.
[8] von Schalien, *Suomen Kem.*, 1958, **31**, *B*, 372; 1959, **32**, *B*, 148.
[9] Kirchner, McCormick, Cavallito, and Miller, *J. Org. Chem.*, 1949, **14**, 388.
[10] Turner, *J. Amer. Chem. Soc.*, 1949, **71**, 2801.
[11] Craig and Hixon, *J. Amer. Chem. Soc.*, 1931, **53**, 4368.
[12] Andon, Cox, and Herington, *Trans. Faraday Soc.*, 1954, **50**, 1918.
[13] Vosburgh and Cooper, *J. Amer. Chem. Soc.*, 1941, **63**, 437.
[14] Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.
[15] Maley and Mellor, *Austral. J. Sci. Res.*, 1949, **2**, *A*, 92, 579.

the Cu(II) complexes [with the exception of Cu(II)–3-2′-aminoethylpyrazole], Irving and Rossotti's " correction term " method [16] was used. For the nickel(II) and cobalt(II) systems, which showed greater complexity, the constants were evaluated by the method of successive approximations.[14]

Results

Dissociation Constants.—All the ligands show two dissociation constants when their acid solutions are titrated with sodium hydroxide to pH 11. The calculated dissociation constants are given in Table 1.

TABLE 1. *Dissociation constants of the ligands in aqueous solution at* 25·0°, $\mu = 0{\cdot}015$.

Ligand	pK_1'	pK_2'	Ligand	pK_1'	pK_2'
Histamine	5·94	9·80	3-2′-Aminoethylpyrazole	2·02 *	9·61
2-2′-Aminoethylpyridine	3·80	9·52	4-Aminomethylimidazole	4·71	9·37
4-2′-Aminoethylthiazole	1·68 *	9·53	2-Aminomethylpyridine	1·85 *	8·62

* Determined spectrophotometrically.

For 2-2′-aminoethylpyridine and 4-2′-aminoethylthiazole complete stability data were obtained only with copper(II); hydrolysis and the precipitation of basic material made it impossible to obtain the upper portions of the formation curves with nickel(II) and cobalt(II). The failure of the copper(II) titration curves to rejoin those of the ligands at high pH indicated a tendency to hydrolysis even with this metal.

Slight hydrolysis was sometimes noted for other systems; for this and other reasons the formation curves obtained for copper(II) and nickel(II) were not symmetrical. Formation constants are therefore given only to $\pm 0{\cdot}1$ log unit.

With cobalt titrations it was obvious that incomplete removal of oxygen led to oxidation, particularly in alkaline solution, so that approximate values are given for log K_1 only.

The 3-2′-aminoethylpyrazole system differed from the others in the shapes of the titration and formation curves. All the complexes showed a strong tendency to be hydrolysed, since more protons were liberated on titration than could come from the total amount of mineral acid present. This prompted a re-investigation of the metal–ligand ratio spectrophotometrically. The results indicated that, at all pH's studied and all ratios of metal to ligand used, 3-2′-aminoethylpyrazole forms no complexes other than with a 1 : 1 ratio. They must contain hydroxyl groups and are being further examined.

TABLE 2. *Formation constants in aqueous solution at* 25·0° *and* $\mu = 0{\cdot}01$—$0{\cdot}02$.

	Cu			Ni			Co
	log K_1	log K_2	log β_2	log K_1	log K_2	log β_2	log K_1
Histamine	$9{\cdot}5_5$	6·5	16·0	6·8	5·1 [a]	11·9	5·2
2-2′-Aminoethylpyridine	7·3	5·4	12·7	5·2	3·3	8·5	~3·8
4-2′-Aminoethylthiazole	7·4	5·3	12·7	5·6	4·0	9·6	~4·1
3-2′-Aminoethylpyrazole	~7·5	—	—	~5·7	—	—	—
4-Aminomethylimidazole	$9{\cdot}0_5$	7·8	16·8	6·0	5·0 [b]	11·0	~4·8
2-Aminomethylpyridine	~9·3	7·9	~17·2	7·3	6·3 [c]	13·6	~5·8

log K_3 = (*a*) 3·1, (*b*) 3·3, (*c*) 5·5.
log β_3 = (*a*) 15·0, (*b*) 14·3, (*c*) 19·4.

TABLE 3. *Continuous variation results for histamine and* 3-2′-*aminoethylpyrazole complexes.*

Ligand	Metal	pH	$\lambda_{max.}$ (mμ)	Metal : ligand ratio of absorbing species
Histamine	Cu	5·0	680	1 : 1
		9·0	600	1 : 2
3-2′-Aminoethylpyrazole	Cu	4·5	690	1 : 1
		8·0	570	1 : 1
	Ni	11·0	470	1 : 1
	Co	10·0	*	1 : 1

* No peak in visible region; values taken at $\lambda = 400$ mμ.

Formation Constants.—The formation constants of the metal complexes are given in Table 2, and relevant spectrophotometric data in Table 3.

[16] Irving and Rossotti, *J.*, 1953, 3397.

Discussion

The aminoethyl-substituted pyridine, thiazole, and pyrazole are considerably less basic than histamine, owing to the lower basicities of their heterocyclic rings than of imidazole. The basicities (pK') of the free heterocyclic rings are [17] imidazole 7·03, pyridine 5·23, thiazole 2·53, and pyrazole 2·53. When these rings are attached to the 2-aminoethyl side chain their basicities are lowered somewhat, as may be seen from Table 1. It is assumed that in all the ligands pK_1' represents the dissociation from the heterocyclic ring and pK_2' that of the side chain. When the aminoethyl side chain is replaced by aminomethyl, in both cases, there is a decrease in basicity similar to that encountered in passing from trimethylene- to ethylene-diamines,[18] presumably owing to the smaller separation between the two nitrogen atoms. Both pK_1' and pK_2' become less but the effect is greater, by about one unit, with pK_1'.

The dissociation constants for histamine agree with other published values; those for the two pyridine-containing ligands are close to the recent values of Goldberg and Fernelius,[19] except that our figure for the pK_1' of 2-2′-aminoethylpyridine is much lower than the values quoted by them for 30° and 40°; they quote no values for 10° and 20°.

Formation Constants.—The constants for the histamine complexes are very similar to those obtained by Mickel and Andrews.[6] Those for aminoethyl- and aminomethyl-pyridine do not differ much from the values of Goldberg and Fernelius [19] when allowance is made for the difference in conditions.

For all the ligands the Irving–Williams [20] order of stabilities is followed. The order Cu > Ni > Co is maintained for all comparable values of log K and log β.

All the ligands with the aminoethyl side chain can form six-membered chelated rings similar to that of histamine, and it might be expected that the dissociation constants would govern the stability of the chelates. When the aminoethyl derivatives are compared, histamine, which is by far the most basic ligand, always forms the most stable complexes. With the other three ligands, however, there is no parallel between base strength and stability; only the pyridine and thiazole derivatives may be compared here since the pyrazole forms 1 : 1 complexes. Other factors such as bond angle and $d\pi$–$p\pi$ bonding differences must play a part.

With the aminomethyl derivatives the pyridine-containing complexes are more stable than those with an imidazole residue, in spite of the higher basicity of the latter. Once more, when heterocyclic rings of different types are compared, other considerations may outweigh those of basicity.

In both cases where corresponding aminomethyl and aminoethyl derivatives can be compared, log (K_1/K_2) is less for the five-membered (aminomethyl) than for the six-membered chelated ring (aminoethyl). This is also true for log (K_2/K_3) with the nickel complexes of the imidazole-containing ligands; the comparison cannot be made in the pyridine series since no value of log K_3 could be obtained for nickel–2-aminoethylpyridine. A similar tendency is found when values for histamine and 2-aminomethylbenzimidazole are compared; [21] it should be noted that with the last ligand no value for log K_3 was obtainable with nickel, although this is possible with the unsubstituted 2-aminomethylimidazole.

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[17] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.
[18] Irving, Williams, Ferrett, and Williams, *J.*, 1954, 3494.
[19] Goldberg and Fernelius, *J. Phys. Chem.*, 1959, **63**, 1246.
[20] Irving and Williams, *J.*, 1953, 3192.
[21] Irving and Weber, *J.*, 1959, 2560.