COPPER COMPLEXES OF SUBSTITUTED HISTIDINES

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Approximate acid dissociation constants of 2-methyl-histidine and of 3-methyl-histidine have been measured and compared with those of histidine. The Cu(II) complexes of all three amino acids have been studied potentiometrically. The results obtained, though not of high absolute accuracy, are interpreted in terms of the structures of the complexes.

It has been shown by Leberman and Rabin [1] that complexes of histidine with Cu(II) differ from those with Zn(II), Co(II) and Ni(II). When the molar ratio of histidine to Cu(II) was unity an additional equivalent of alkali was required to titrate all the acid species present in aqueous solution, over and above that required when the ligand to Cu(II) ratio was two, or when Cu(II) was completely absent. Those authors were able to interpret their results in terms of the following equilibria,

$$Cu^{2+} + L^{\pm} \overset{K_1'}{\rightleftharpoons} CuL^{2+} \tag{1}$$

$$CuL^{2+} \overset{K_c}{\rightleftharpoons} CuL^{+} + H^{+} \tag{2}$$

$$Cu^{2+} + L^{-} \overset{K_1}{\rightleftharpoons} CuL^{+} \tag{3}$$

$$CuL^{+} \overset{K_c'}{\rightleftharpoons} CuL + H^{+}. \tag{4}$$

It was suggested that the complex species CuL^{2+} and CuL^{+} had the structures indicated in fig. 1, where for histidine R and R′ are hydrogen atoms. The dissociation corresponding to K_c' was believed by those authors to take place from a

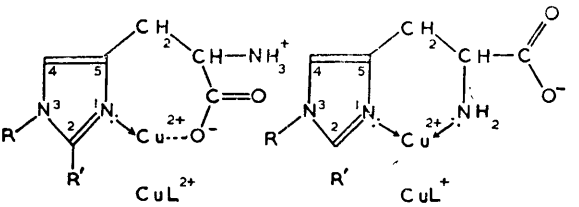

FIG. 1.

water molecule co-ordinated to the Cu(II) atom of the complex CuL^{+}. They attempted to exclude an alternative possibility, that this dissociation might arise from the uncoordinated imino group of the imidazole ring, by studying the corresponding Cu(II) complexes of 3-methyl-histidine. However, their sample of this compound was impure so that their result was inconclusive.

The same authors showed that complexes of Cu(II) with histamine also exhibit this additional acid dissociation. More recently,[2] they have studied

complexes of Cu(II) with 3-methyl-histamine and have found that such complexes are similar to those with histamine. It is therefore unlikely that an acid dissociation takes place from the imino group of the imidazole ring of the Cu(II)-histamine complexes.

The existence of the species CuL has been questioned by Perrin,[3] who suggests a third possible explanation of the results obtained when the Cu(II) to histidine ratio is unity. He was able to interpret his experimental data on the assumption that the complex CuL^+ is unstable in neutral or alkaline solution and dismutates into a cupric ion and the stable species CuL_2,

$$2CuL^+ \rightleftharpoons Cu^{2+} + CuL_2. \qquad (4')$$

On this basis he was able to calculate the three stability constants of the 1 : 2 Cu(II)-histidine complexes.

Pure synthetic 3-methyl-histidine and 2-methyl-histidine have been prepared and the dissociation constants of these compounds and of their 1 : 1 Cu(II)-ligand complexes have been measured by potentiometric titration, using the glass electrode. In order to simplify the nomenclature, the substituents in the imidazole ring are numbered in such a way that the alanine side chain is attached to the ring at the 5-position.

EXPERIMENTAL

The preparations of 2-methyl- and of 3-methyl-histidine have been outlined elsewhere.[4] Both compounds were obtained as the dihydrochlorides, samples of which gave the following analyses:

2-methyl-histidine dihydrochloride: C 34·6 %, H 5·5 %, N 16·8 %,

3-methyl-histidine dihydrochloride: C 35·3 %, H 5·6 %, N 17·6 %.

The calculated values for both compounds, based on the formula $C_7H_{13}N_3O_2Cl_2$, were C 34·9 %, H 5·3 %, N 17·4 %. The 2-methyl-histidine dihydrochloride was recrystallized and both compounds were dried before use.

Cupric chloride was of A.R. quality and the sodium hydroxide was freshly prepared from B.D.H. concentrated volumetric ampoules, by dilution with boiled-out distilled water. DL-Histidine monohydrochloride monohydrate was obtained from British Drug Houses Ltd.

TECHNIQUE

The aqueous solution of the amino acid was titrated against the standard alkali at room temperature, 18°C±1°C, using a Muirhead pH meter, type D-303-C. The latter was standardized using buffer solutions (pH 4·0 and 9·0) prepared from tablets supplied by the makers. Nitrogen, freed of oxygen, was bubbled through the solution during the titration and the initial concentration of the amino acid was always 0·01 M.

RESULTS

Since the precautions taken in these experiments were not as rigid as those taken by Leberman and Rabin,[1] it seemed advisable to repeat their experiments with histidine. Comparison of their results with those given here for histidine then provides a reasonable estimate of the probable absolute error in the values of the dissociation constants obtained for the substituted histidines and their Cu(II) complexes. The values of the acid dissociation constants of histidine, 2-methyl-histidine, and 3-methyl-histidine are given in table 1, together with values for histidine taken from the literature.[1] Comparison of the constants for histidine shows that the values of pK_{a3} for the substituted histidines are probably 0·2 unit too high.

The results obtained on titrating 3-methyl-histidine dihydrochloride alone and with half, and one, molar equivalents of cupric chloride are shown in fig. 2. It

TABLE—1. ACID DISSOCIATION CONSTANTS OF HISTIDINE AND SUBSTITUTED HISTIDINES

		results of Leberman and Rabin			present work		
	temp. °C	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a1}	pK_{a2}	pK_{a3}
histidine	25	1·82	6·00	9·16	—	—	—
histidine	18	—	—	—	—	6·1	9·4
2-methyl-histidine	18	—	—	—	1·7	7·2	9·5
3-methyl-histidine	18	—	—	—	1·7	6·1	9·6

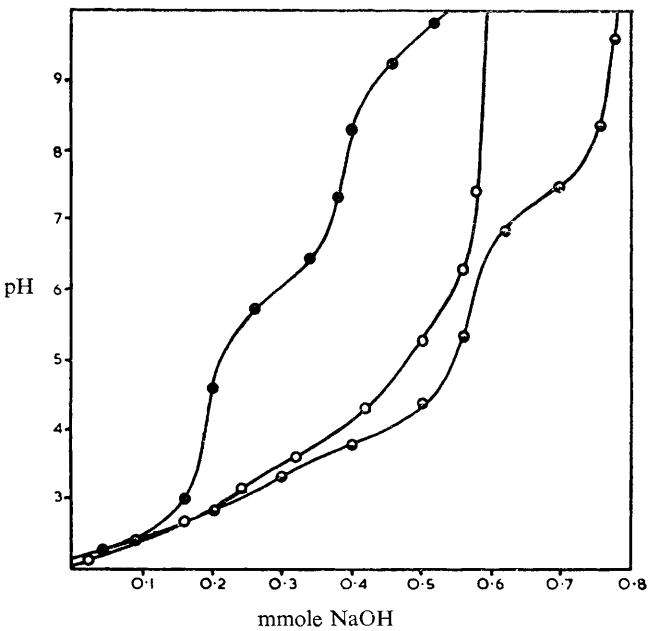

FIG. 2.—Titration curves of 3-methyl-histidine dihydrochloride (0·20 mmole in 20 ml of water) alone ●, in the presence of 0·10 mmole of $CuCl_2$ ○, and of 0·20 mmole of $CuCl_2$ ◒. Only a limited number of the experimental points are marked.

is clear that 3-methyl-histidine, like histidine itself, gives rise to an additional acid species when the ratio of Cu(II) to ligand is unity. Analogous curves were obtained for histidine and for 2-methyl-histidine. From the titration data values of the stability and acid dissociation constants of the various cupric complexes were calculated according to the methods described by Leberman and Rabin.[1] The results are collected in table 2.

TABLE 2.—STABILITY AND ACID DISSOCIATION CONSTANTS OF COPPER COMPLEXES OF HISTIDINE AND SUBSTITUTED HISTIDINES

		results of Leberman and Rabin				present work			
ligand	temp. °C	$\log_{10} K_1$	$\log_{10} K_1'$	pK_c	pK_c'	$\log_{10} K_1$	$\log_{10} K_1$	pK_c	pK_c
histidine	25	10·37	5·02	3·81	7·18	—	—	—	—
histidine	18	—	—	—	—	10·6	4·9	3·9	7·4
2-methyl-histidine	18	—	—	—	—	10·7	5·8	4·7	7·5
3-methyl-histidine	18	—	—	—	—	10·7	4·8	3·7	7·5

DISCUSSION

As will be seen from table 1, the pK_a values for histidine and 3-methyl-histidine are similar. However, the value of pK_{a2} for 2-methyl-histidine is appreciably higher than for the other two amino acids. The increased basicity of the imidazole ring when a methyl group is substituted in the 2-position is readily explicable in terms of the electron-donor properties of this group and its close proximity to the nitrogen atoms of the ring. On the other hand, methylation of one of these nitrogens has much less effect on the value of pK_{a2}, possibly because of the increased distance of the substituent from, and lack of conjugation with, the second nitrogen atom. Other factors, such as resonance considerations, may also be involved.

It is evident from table 2 that the stability constant of the complex CuL^{2+} is very much less than that of CuL^{+}. Since the imidazole nitrogen atom -1 is a co-ordinating site common to both species (fig. 1) the former complex must be more dependent than the latter on the electron-donor properties of this nitrogen atom. Substitution of a methyl group in the 2-position of the imidazole ring of histidine should, therefore, increase the value of $\log_{10} K_1'$, while the increased stability of CuL^{2+} relative to CuL^{+} should increase the value of pK_c. The experimental results are in agreement with these expectations.

The stability constants K_1 of the complex CuL^{+} are practically identical for all three amino acids. The additional acid dissociation, according to Leberman and Rabin,[1] involves removal of a hydrogen ion from a water molecule attached to the Cu(II) atom of this complex, while, according to Perrin,[3] the additional acidity arises from the breakdown of CuL^{+} and the formation of the more stable CuL_2 and a cupric ion. No attempt has been made here to calculate the constants of the 1 : 2 Cu(II)-ligand complexes by the method of Perrin,[3] which he has not given in detail. However, the effect of substituents in the imidazole ring on the stability of the complex CuL_2 would be expected to be even less than on that of CuL^{+}. It is, therefore, unlikely that the results obtained with the substituted histidines would enable a decision to be made between these alternative explanations.

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[1] Leberman and Rabin, *Trans. Faraday Soc.*, 1959, **55**, 1660.
[2] Leberman and Rabin, *Nature*, 1960, **185**, 768.
[3] Perrin, *Nature*, 1959, **184**, 1868.
[4] Mackay and Shepherd, *Brit. J. Pharmacol.*, 1960, **15**, 552.