sociation constants. Indeed, Azzari and Kraus[35] have shown recently that the Bjerrum equation fails completely in solutions of higher dielectric constant.

Acknowledgments.—The authors are indebted to Dr. C. A. Kraus of Brown University for suggesting this research topic and for valuable discussions and advice. Thanks are also due to Professor Robert H. Cole of Brown University for the use of his apparatus to measure dielectric constants of the solvent mixtures. Appreciation is expressed for solvents to The Dow Chemical Company for ethylene chloride, and the Calco Chemical Company for nitrobenzene.

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(35) M. Azzari and C. A. Kraus, *Proc. Natl. Acad. Sci.*, **42**, 590 (1956).

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Association of 4-Methylimidazole with the Ions of Cupric Copper and Zinc; with Some Observations on 2,4-Dimethylimidazole

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The 4-methyl and the 2,4-dimethyl derivatives of imidazole have been studied in their interactions with hydrogen, cupric and zinc ions, at different temperatures. The pH titrations, in the absence of Cu or Zn ions, gave pK' values which were extrapolated to zero ionic strength. Heats of reaction and standard entropies associated with these pK values were also determined. (ΔH° is in the range 8.5 ± 0.7 kcal./mole and ΔS° in the range -6.5 ± 0.8 cal. deg.$^{-1}$ mole^{-1}.) Similar titrations, in the presence of Cu(II) and Zn(II), showed that the number of molecules of both imidazole derivatives, bound to either of these ions, approached an upper limit of 4, at high imidazole concentrations. This number is the same as for unsubstituted imidazole. The intrinsic association constants log $\kappa_i(i = 1,2,3,4)$ at 25° were found for 4-methylimidazole to be 3.53, 3.31, 3.05 and 2.56, respectively, for combination with Cu(II), and 1.84, 2.35, 2.82 and 2.98 for combination with Zn(II). Approximate values for ΔH° in the combination of 4-methylimidazole with these ions have been estimated. Incomplete studies on the binding of 2,4-dimethylimidazole to copper indicated association constants of the same order of magnitude, but somewhat smaller, than those for 4-methylimidazole. A discussion is given of the stereochemistry of the copper and zinc complexes, and of some of the structural implications of the observed association constants.

The reactivity of the imidazole group of the histidyl residue in proteins has assumed great importance for the understanding of interactions between proteins and several metallic ions.[4-6] A previous report described the application of a method suggested by Scatchard to the interpretation of the equilibria between the parent imidazole and Cu(II) and Zn(II) ions.[7] A similar study has now been made of the corresponding equilibria of the 4-methyl derivative of imidazole, chosen because it is substituted in the same position as the imidazole ring in the histidyl residue, and thus represents a step toward the study of the metal-binding behavior of the histidyl residue in peptide chains. A fragmentary study has also been made of 2,4-dimethylimidazole, in order to determine whether the additional methyl group, attached to the carbon atom between the two nitrogens of the imidazole ring, would significantly alter the binding of the imidazole molecule to the metallic ions.

Materials and Methods

4-Methylimidazole was prepared as the crystalline salt, 4-methylimidazolium nitrate, by a modification of the method of Weidenhagen and Herrmann.[8] The free base was prepared, in the manner described by them, from acetol acetate, aqueous ammonia and formaldehyde in the presence of cupric acetate. The resulting copper complex of 4-methylimidazole was decomposed, and the copper precipitated as sulfide, by treatment with hydrogen sulfide at 50°. After removal of the precipitate, and cooling, dilute nitric acid was added cautiously to the solution, with constant stirring, until a pH between 2 and 3 was attained. (Further addition of nitric acid decreases both the yield and the purity of the product.) The solution was concentrated by evaporation to a small volume, *in vacuo* at 45–55°, filtered with charcoal and dried in a desiccator. The resulting crude product was recrystallized two or three times from acetone. The material was colorless and melted at 110.5–111.5°. Dedichen[9] reported a melting point of 110°. The purity of the material obtained by us was confirmed also by the constancy of the value of pK' obtained in the pH titrations (see below) for widely varying ratios of the concentration of free base to that of the conjugate imidazolium ion.

2,4-Dimethylimidazole also was prepared by the method of Weidenhagen and Herrmann[8]; the base released on removal of Cu(I) by sulfide was converted directly to the nitrate and the product was obtained by evaporation to dryness at low temperature. Recrystallization was from acetone or acetone–ether mixtures. Thrice recrystallized 2,4-dimethylimidazolium nitrate melted at 133–134°. The purity of the product was confirmed by the pH titrations discussed below.

The preparation of mixtures of methylimidazolium nitrates and Cu(II) or Zn(II) nitrates and their titration with NaOH were carried out as previously described.[7] The pH of each mixture was determined with a Beckman

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(3) Requests for reprints and inquiries concerning this paper should be addressed to Dr. J. T. Edsall, Biological Laboratories, Harvard University, Cambridge 38, Massachusetts.

(4) F. R. N. Gurd and D. S. Goodman, THIS JOURNAL, **74**, 670 (1952).

(5) C. Tanford, *ibid.*, **74**, 211 (1952).

(6) F. R. N. Gurd and P. E. Wilcox, *Advances in Protein Chem.*, **11**, 311 (1956).

(7) J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, THIS JOURNAL, **76**, 3054 (1954).

(8) R. Weidenhagen and R. Herrmann, *Ber.*, **68**, 1953 (1935). See also K. Hofmann, "Imidazole and its Derivatives" Part I, Interscience Publishers, Inc., New York, N. Y., 1953, Chapter II.

(9) G. Dedichen, *Ber.*, **39**, 1831 (1906).

Model G pH meter, first at 2.5° in a cold room and again at laboratory temperature. The instrument and solutions were allowed to reach temperature equilibrium for several hours before readings were taken. Measurements were repeated at five-minute intervals and care was taken to maintain the precision of the measurements at ±0.01 pH unit. The buffer described by Bates and Acree[10] containing $0.02M$ KH_2PO_4, $0.03M$ Na_2HPO_4 and $0.02M$ NaCl was used for calibration. The pH values assigned to this buffer at various temperatures were obtained by interpolation in Table II of the publication of Bates and Acree.[10]

Results

Dissociation of Hydrogen Ions.—Table I shows the pK' values for the dissociation of 4-methylimidazole and of 2,4-dimethylimidazole. The values for the former were obtained at 2.5 and 29°, and for the latter at 4 and 22°. Interpolated or extrapolated pK' values are given at 25°. Solutions were made up to $0.02M$ in total imidazole derivative and the ionic strength adjusted with sodium nitrate. The titration of 4-methylimidazole was carried out, at each ionic strength and temperature, for seven different ratios of imidazolium ion to free base, ranging from 6 to 0.17. The calculated pK' values for the seven solutions agreed within ±0.01, except occasionally at the extreme ends of the curve, where the deviation was sometimes twice as great. The titrations of 2,4-dimethylimidazole are somewhat less accurate, with a probable error of perhaps ±0.025 in the reported pK' values.

The results are given for several different ionic strengths ($\Gamma/2$) and are extrapolated to zero ionic strength according to a simplified version of the Debye–Hückel equation previously employed by Kirby and Neuberger.[11]

$$pK = pK' - 0.5\sqrt{\Gamma/2}/(1 + \sqrt{\Gamma/2})$$

The results of the present study are compared in Table II with their measurements, in which the hydrogen electrode was used. The results agree within experimental error.[12]

Table I

Apparent Dissociation Constants, pK', at Various Temperatures and Ionic Strengths for the Conjugate Acids of Two Imidazole Bases

Ionic strength	Temp., °C. 2.5	29	25
	4-Methylimidazolium ion		
0.16	8.20	7.60	7.69
.076	8.17	7.56	7.65
.034	8.15	7.55	7.64
.00	8.06	7.46	7.55
	2,4-Dimethylimidazolium ion		
	4	22	25
0.16	9.05	8.59	8.52
0.00	8.91	8.45	8.38

The values of $\Delta H°$ in Table II range from 7.7 to 9.2 kcal./mole. These values are somewhat larger than those which usually have been assumed as typical for imidazole groups.[13] The standard entropy of ionization is in all cases negative and of the order of −6 cal. deg.$^{-1}$mole^{-1}. In attempting to interpret these values, we note that the process involved is the transfer of a proton from an imidazolium ion to a water molecule to form an H_3O^+ ion. Both the imidazolium ion and the H_3O^+ ion tend to attract and orient water molecules, with an accompanying entropy decrease, around a positively charged center, but the orientation around the positively charged imidazolium nitrogen will be obstructed to some extent by the adjoining atoms of the ring; whereas no such obstruction exists around the H_3O^+ ion. Hence a moderate negative value of $\Delta S°$ is to be expected for a proton transfer of this type.[14]

Table II

Thermodynamic Values for the Hydrogen Ion Equilibria of Various Imidazoles at 25°

Conjugate acid of	pK at $\Gamma/2 = 0$	$\Delta F°$, kcal./mole	$\Delta H°$, kcal./mole	$\Delta S°$, cal. deg.$^{-1}$ mole^{-1}
Imidazole	6.95[a]; 6.97[b]	+ 9.5	+7.7[f]	−6.0
1-Methylimidazole	7.06[c]	+ 9.6		
2-Methylimidazole	7.86[a]	+10.7		
4-Methylimidazole	7.52[a]; 7.54[d]	+10.3	+8.6	−5.7
2,4-Dimethyl-imidazole	8.36[a]; 8.38[d]	+11.4	+9.2	−7.4
2,4,5-Trimethyl-imidazole	8.92[e]	+12.2		

[a] A. H. M. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1938). [b] J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, This Journal, **76**, 3054 (1954). [c] N. C. Li, J. M. White and E. Doody, *ibid.*, **76**, 6219 (1954). [d] Present investigation. [e] R. W. Cowgill and W. M. Clark, *J. Biol. Chem.*, 198, 33 (1952), give a pK' for this substance of 8.86 in 23.3% ethanol at 30° and ionic strength 0.2. Following their calculation, we have taken the value in water, at 25° and zero ionic strength, as 0.06 greater than this. [f] In reference 6, the value of $\Delta H°$ for imidazole was given incorrectly as 6.0 kcal./mole. The value listed here is based on the data given in that reference, and the calculation has been corrected. Values for $\Delta H°$ and $\Delta S°$ are based on temperature coefficients of pK values determined in the present investigation, or in reference *b* above.

Association of Cu(II) and Zn(II) with 4-Methylimidazole.—The results of the titration of mixtures of 4-methylimidazole with $Cu(NO_3)_2$ and $Zn(NO_3)_2$ are summarized in Tables III and IV. Throughout these measurements the ionic strength was maintained at 0.15–0.16. The first three columns in Table III, and the subhead and first column of Table IV, define the compositions of the mixtures; the next three columns show the results at 2.5°; and the last three show the results at 29 and 27°, respectively. The concentration of the imidazole derivative in the free base form is expressed as its negative logarithm, pA, and the average number of 4-methylimidazole molecules bound per metal ion is denoted by $\bar{\nu}$. The values of pA and $\bar{\nu}$ were obtained from the data by the method previously employed with imidazole.[7]

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[10] R. G. Bates and S. F. Acree, *J. Research Natl. Bur. Standards*, **30**, 129 (1943).

[11] A. H. M. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1938). Concerning the extrapolation of pK' values to zero ionic strength, see also A. Neuberger, *Proc. Roy. Soc.* (*London*), **A158**, 68 (1937).

[12] Much of the difference of about 0.02 unit may be due in any case to differences in methods of standardization. See also R. G. Bates, "Electrometric pH Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954, Chap. 4.

[13] E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, Chapters 4 and 5.

[14] Compare the discussion by E. J. King and G. W. King, This Journal, **78**, 1089 (1956), especially pp. 1095-1098.

TABLE III

TITRATION OF MIXTURES OF 4-METHYLIMIDAZOLIUM NITRATE (4-MeIm$^+$NO$_3^-$) AND CUPRIC NITRATE

Total molar concn. of 4-MeIm$^+$-NO$_3^-$	Cu-(NO$_3$)$_2$	NaOH	Results at 2.5° $pK' = 8.20$ pH	pA	$\bar{\nu}$	Result at 29° $pK' = 7.60$ pH	pA	$\bar{\nu}$
0.1354	0.0113	0.002377	3.93	5.15	0.21	3.82	4.66	0.21
.1354	.0113	.004754	4.28	4.80	0.42	4.16	4.32	0.42
.1354	.0113	.007131	4.50	4.59	0.63	4.39	4.10	0.62
.1354	.0113	.009508	4.72	4.38	0.84	4.58	3.92	0.83
.1354	.0113	.01664	5.25	3.88	1.46	5.09	3.44	1.44
.1354	.0113	.02377	5.70	3.45	2.08	5.48	3.07	2.03
.1354	.0113	.03090	6.16	3.02	2.65	5.90	2.68	2.55
.1354	.0113	.04041	6.76	2.46	3.27	6.38	2.24	3.08
.1354	.0113	.04754	7.13	2.13	3.55	6.65	2.01	3.34
.1354	.0113	.05705	7.48	1.83	3.73	6.95	1.76	3.50
.0200[a]	.0214	.007132	5.15	4.94	0.32	5.00	4.49	0.33
.0300[a]	.0136	.007132	5.18	4.66	0.52	5.02	4.22	0.52
.0600[a]	.0186	.02377	5.62	4.02	1.27	5.44	3.60	1.27
.0600[a]	.0186	.03090	5.99	3.75	1.65	5.80	3.34	1.64
.0600[a]	.0135	.03090	6.39	3.35	2.25	6.15	2.99	2.21
.0600[a]	.0186	.04754	6.96	3.14	2.52	6.72	2.79	2.41

[a] NaNO$_3$ added to bring total ionic strength to 0.15–0.16. The symbol pA, here and elsewhere in this paper, denotes the negative logarithm of the concentration of free basic imidazole.

TABLE IV

TITRATION OF MIXTURES OF 4-METHYLIMIDAZOLIUM NITRATE (4-MeIm$^+$NO$_3^-$) AND ZINC NITRATE

Total molar concentration of 4-MeIm$^+$NO$_3^-$ was 0.1354 and that of Zn(NO$_3$)$_2$ was 0.0111 in all solutions reported here.

Total NaOH added (mole/l.)	Results at 2.5° $pK' = 8.20$ pH	pA	$\bar{\nu}$	Results at 27° $pK' = 7.64$ pH	pA	$\bar{\nu}$
0.001418	5.43	3.64	0.11	5.01	3.50	0.10
.002836	5.70	3.38	.22	5.28	3.24	.20
.005138	5.90	3.19	.40	5.50	3.03	.38
.01028	6.13	2.93	.83	5.74	2.80	.78
.01541	6.26	2.86	1.27	5.88	2.68	1.20
.02055	6.38	2.76	1.69	6.00	2.58	1.61
.02569	6.48	2.68	2.13	6.10	2.50	2.03
.03084	6.60	2.58	2.54	6.20	2.42	2.44
.03597	6.74	2.46	2.93	6.33	2.31	2.80
.04614	7.08	2.17	3.55	6.62	2.07	3.39
.06166	7.61	1.72	3.85	7.07	1.72	3.77

From the data in Tables III and IV plots of $\bar{\nu}$ against pA (Bjerrum's "formation function") may be constructed. The curves so formed are nearly symmetrical about $\bar{\nu} = 2$, and it appears reasonable to assume that $\bar{\nu}$ approaches a limiting value of 4 at high concentrations of free 4-methylimidazole. The justification for this assumption lies partly in the approximate symmetry about $\bar{\nu} = 2$, referred to above, and partly in the extensive evidence that coördination complexes of Cu(II) and Zn(II) with nitrogenous bases generally approach a $\bar{\nu}$ value of 4. Similar conclusions concerning the Cu(II)–imidazole system[7] have been confirmed by the polarographic technique.[15]

The data in Tables III and IV have been analyzed by the method of Scatchard[7] from plots of log Q *versus* $\bar{\nu}$ where

$$Q = \bar{\nu}/(4 - \bar{\nu})\,(\mathrm{A}) \qquad (1)$$

These plots are shown in Figs. 1 and 2, derived from the data of Tables III and IV, respectively. The open circles represent points observed at 29 and 27°, respectively, and the closed circles at 2.5°. The

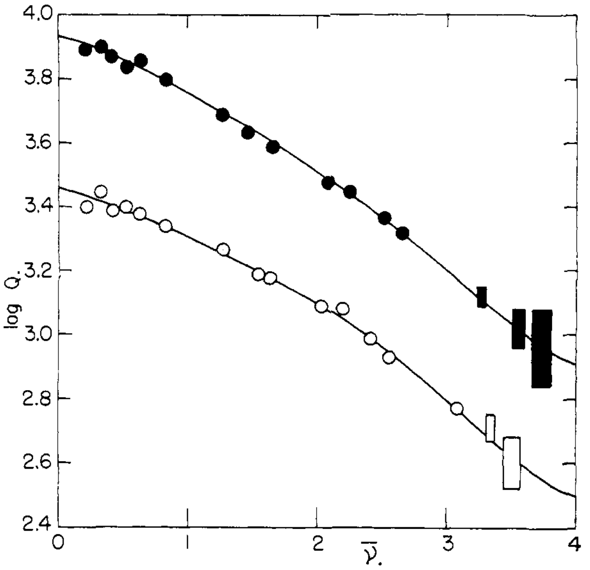

Fig. 1.—Values of the Scatchard function log Q for the binding of Cu(II) to 4-methylimidazole as a function of the mean number ($\bar{\nu}$) of imidazole molecules bound per Cu ion: upper curve data at 2.5°; lower curve data at 29°. Increased probable error of the data at high $\bar{\nu}$ values is indicated by the increased height of the rectangles on the right-hand side of the curves. Circles and rectangles are experimental values; curves are drawn on the basis of the constants listed in Table V.

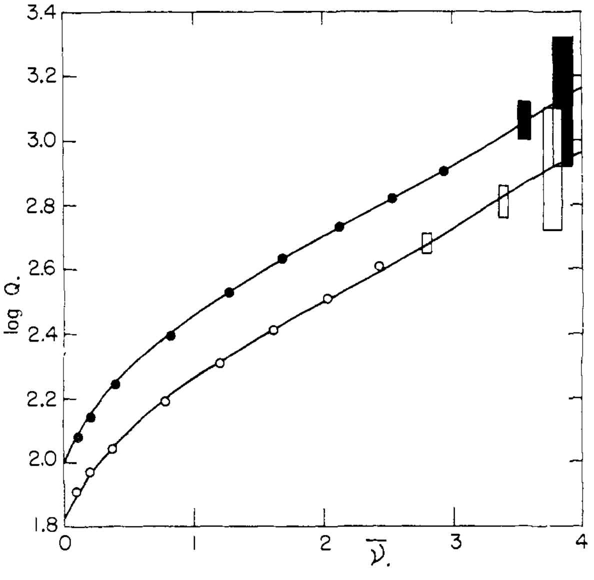

Fig. 2.—Log Q for the binding of Zn(II) to 4-methylimidazole. The data should be compared with those for Cu(II) in Fig. 1: upper curve data at 2.5°; lower curve data at 27°.

circles are drawn to represent the probable accuracy with which log Q is measured. Errors in the pH measurements affect most directly the value of (A) in equation 1. The computed value of (A) depends both on the measurement of the pH in the experimental mixture and on the value of pK' determined separately. Taking the precision of both values as ±0.01 unit, the uncertainty in the value

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[15] N. C. Li, J. M. White and E. Doody, THIS JOURNAL, **76**, 6219 (1954).

of log Q may be expected to be ±0.02 unit throughout most of the range of conditions studied. The circles in the figures rather overestimate the uncertainties in the values of $\bar{\nu}$. As $\bar{\nu}$ approaches 4, errors in (A) begin to affect the value of $\bar{\nu}$ much more sharply, and in turn the computation of Q becomes abruptly subject to large errors. The rectangles in Figs. 1 and 2 show the maximum limits of the parameters when pK' and the pH measurement are both ascribed errors of ±0.01 unit.

If we accept the assumption that the maximum value of $\bar{\nu}$ is 4, the relation of $\bar{\nu}$ to (A) may be defined[7] in terms of four successive association constants, k_1, k_2, k_3 and k_4

$$\bar{\nu} = \frac{k_1(\mathrm{A}) + 2k_1k_2(\mathrm{A})^2 + 3k_1k_2k_3(\mathrm{A})^3 + 4k_1k_2k_3k_4(\mathrm{A})^4}{1 + k_1(\mathrm{A}) + k_1k_2(\mathrm{A})^2 + k_1k_2k_3(\mathrm{A})^3 + k_1k_2k_3k_4(\mathrm{A})^4} \quad (2)$$

Instead of the k values, it is often illuminating to express the results in terms of the "intrinsic association constants," κ_1, κ_2, κ_3 and κ_4, which are defined by the relations

$$\kappa_1 = k_1/4;\ \kappa_2 = 2k_2/3;\ \kappa_3 = 3k_3/2;\ \kappa_4 = 4k_4 \quad (3)$$

The factors $^1/_4$, $^2/_3$, $^3/_2$ and 4 in (3) are statistical factors. For a system in which all the ligand sites are equivalent and also react independently with the ligand, all the four κ values would be identical. In the systems studied here, this is far from being the case, and the differences in the successive κ values give a measure of the character of the interactions in the binding of the ligand molecules. As previously shown,[7] the value of log κ_1 is equal to the limiting value of log Q when $\bar{\nu}$ approaches zero, and the value of log κ_4 is equal to the limiting value of log Q when $\bar{\nu}$ approaches the maximum value of 4. The values of log κ_2 and log κ_3 may also be computed from such data as those shown in Figs. 1 and 2, as previously described. The k and κ values are constants for a given ionic strength and temperature; the values in Tables V and VI below refer specifically to ionic strength 0.16.

TABLE V

CALCULATED INTRINSIC ASSOCIATION CONSTANTS FOR $\mathrm{Cu^{II}}$ AND $\mathrm{Zn^{II}}$ WITH 4-METHYLIMIDAZOLE

	$\mathrm{Cu^{II}}$(2.5°)	$\mathrm{Cu^{II}}$(29°)	$\mathrm{Zn^{II}}$(2.5°)	$\mathrm{Zn^{II}}$(27°)
Log κ_1	3.93	3.46	2.01	1.83
Log κ_2	3.69	3.24	2.53	2.33
Log κ_3	3.36	2.99	3.01	2.80
Log κ_4	2.90	2.50	3.15	2.95

The curves drawn in Figs. 1 and 2 were computed from values of the intrinsic association constants listed in Table V. To obtain these, approximate values of κ_1 and κ_4 were first obtained by extrapolation and values of κ_2 and κ_3 were estimated roughly from the values of log Q corresponding to $\bar{\nu}$ = 1.5 and $\bar{\nu}$ = 2.5, respectively. The values reported in Table V were obtained by successive approximations, to give the best fit between the observed and calculated curves.

In general, a plot of log Q *versus* $\bar{\nu}$ yields most directly the values of κ_1 and κ_4. In the present study, based on pH measurements, κ_1 may be determined with considerable accuracy but, as Figs. 1 and 2 show, the extrapolation to obtain κ_4 is more uncertain. The uncertainty in κ_4 means that methods of general applicability for estimating temporary values of κ_2 and κ_3 from limiting slopes[7,16] cannot be exploited conveniently. It was found that the value of the product $\kappa_2\kappa_3$ was fixed by the experimental data with considerably less uncertainty than the value of either κ_2 or κ_3 alone. The same point has been emphasized by the very careful studies of Roughton, Otis and Lyster[17] on the combination of sheep hemoglobin with oxygen—a problem mathematically identical with that considered here, if we substitute hemoglobin for Cu(II) or Zn(II), and the concentration of free oxygen in solution, or its partial pressure, for (A).

We estimate the probable range of error of the successive log κ values given in Table V to be ±0.04, ±0.10, ±0.10 and ±0.20, respectively. The error in the product log ($\kappa_2\kappa_3$), however, is estimated at ±0.05–0.06. If anything, we believe that this estimate overstates the probable errors. Standard enthalpy changes, $\Delta H°$, for the first three steps of the complex formation with Cu(II) are computed to be −6.7, −6.4 and −5.3 kcal./mole and with Zn(II) are −2.8, −3.1 and −3.1 kcal./mole. The values for the first step are subject to an uncertainty of about ±1 kcal./mole, and for the second and third steps they may be as great as ±3 kcal./mole. No significance can be attached to the apparent trend in the values for the successive steps. The calculation of $\Delta H°$ values for the fourth step scarcely appears justified, in view of the probable errors in the values of κ_4. For imidazole[7] the corresponding value for the first stage of the interaction with Cu is approximately −4.9 kcal./mole, while the value for imidazole and Zn is also negative but decidedly smaller.[18] We note that the combination of a Cu or a Zn ion with an imidazole nitrogen—or rather the displacement of a water molecule and its substitution by an imidazole molecule at one of the coördination positions around the ion—is thus an exothermic process. The heat liberated in the reaction, however, is significantly less than that produced by the combination of a proton with a basic nitrogen of imidazole or one of its methylated derivatives (see Table II). In view of the uncertainty of the data it appears unprofitable to calculate standard entropy changes ($\Delta S°$) for the interactions of 4-methylimidazole with Cu and Zn ions. It may be estimated with some confidence, however, that for the first step of the complex formation with Cu(II) $\Delta S°$ is probably negative.

Association of Cu(II) with 2,4-Dimethylimidazole.—Because of the high pK value of the 2,4-dimethyl derivative (Table II) and the tendency of uncomplexed Cu(II) to hydrolyze at pH values above about 6,[6] only the first step of the association could be determined with assurance. The measurements were made at ionic strength 0.16 and at 26 or 20.5°. Three values of $\bar{\nu}$ between 0.23 and 0.44 were obtained at pH values ranging between 5.37 and 5.72, and the values of log k_1 were

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(16) For a general discussion of these and other methods see J. Z. Hearon and J. B. Gilbert, This Journal, **77**, 2594 (1955).

(17) F. J. W. Roughton, A. B. Otis, and R. L. J. Lyster, *Proc. Roy. Soc.* (*London*), **B144**, 29 (1955). Also personal communications from F. J. W. Roughton to J. T. Edsall.

(18) The value for the copper–imidazole interaction was incorrectly given in our previous paper[7] as −2.2 kcal./mole.

computed to be 3.89, 3.91 and 3.95. Since some contribution from the second step of association was to be expected, judging from analogy with the 4-methylimidazole system (see Fig. 3, below), we estimate that the value of log k_1 at about 25° is 3.8 ± 0.1. This is equivalent to log κ_1 = 3.2 ± 0.1.[7] Very rough estimates from the titration data gave values for log κ_2, log κ_3 and log κ_4 which were of the order of 3.0, 2.6 and 2.4, respectively. These values, however, should not be taken as indicating anything more than the order of magnitude of the constants involved. Values of $\bar{\nu}$ as high as 3.6 were estimated for the solutions containing the highest concentrations of 2,4-dimethylimidazole relative to Cu, but the *p*H in these solutions was as high as 7.8–7.9. Although by working at low total concentrations of cupric ion—of the order of 0.006 *M*—it was possible to attain such high *p*H values without precipitation of cupric oxide, accurate evaluation of the data would require careful correction for the binding of some of the added hydroxyl ion by copper (see the detailed discussion in reference 7). We have therefore refrained from presenting the detailed data for the 2,4-dimethylimidazole system. The available evidence indicates that the association constants involved are all of the same order of magnitude but somewhat lower than those found for imidazole and 4-methylimidazole.[19]

Discussion

The results of the present study with 4-methylimidazole show the same striking contrast in behavior between Cu(II) and Zn(II) as was found in the study with unsubstituted imidazole.[7] In both cases the imidazole compound shows increasing affinity for Zn(II) as the degree of complex formation increases; in other words there are "positive interactions" between the coördinated imidazole groups. With Cu(II) the opposite behavior is seen and the interactions are negative. This contrasting behavior appears to be the rule with all the simple imidazoles that have been tested, including the 1-methyl derivative,[15] and is indeed characteristic of the behavior of other systems in which nitrogen is the donor atom of the ligand molecule.[6]

A consequence of the contrasting behavior with Cu(II) and with Zn(II) is illustrated in Figs. 3 and 4 which show the per cent. of each metal ion in each complex as a function of the concentration of the free base 4-methylimidazole. The curves were computed from the *k* values for 29 and 27° corresponding to the κ values given in Table V. The conversion from κ values to *k* values was made according to equation 3. Noteworthy is the much greater overlapping of the regions of existence of the Zn(II) complexes. Indeed the Zn(II) system moves from 50% in the form of the uncomplexed ion to 50% in the form of the quaternary complex when the concentration of the uncombined base is increased about fivefold. By contrast the corresponding change in the Cu(II) system requires an increase of approximately 200-fold.

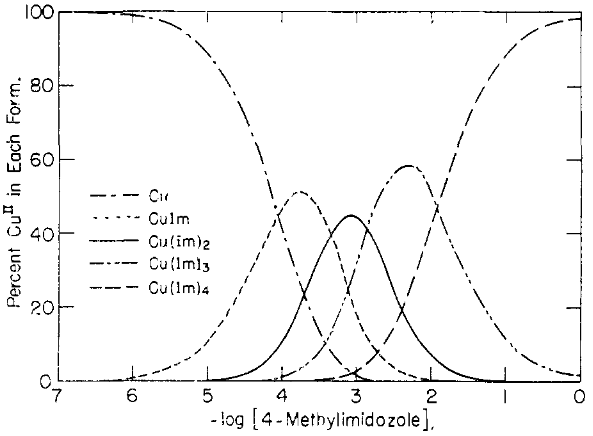

Fig. 3.—Relative concentrations of Cu(II) ion and of its various complexes with 4-methylimidazole, plotted as a function of the negative logarithm of the concentration of free basic 4-methylimidazole.

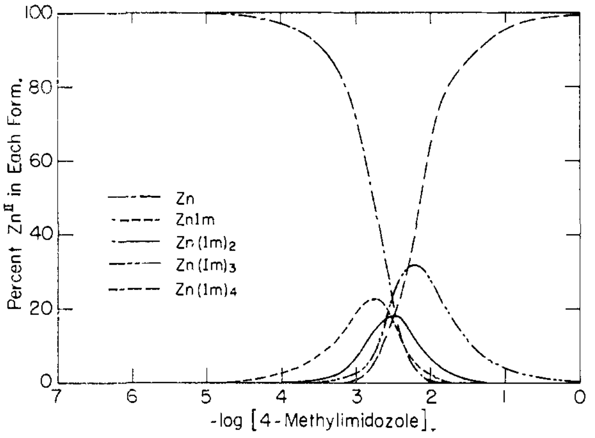

Fig. 4.—The relative concentrations of Zn(II) and its complexes with 4-methylimidazole, plotted in the same manner as the data of Fig. 3.

The successive log *k* values for the association of Cu(II) with four different imidazole derivatives and of Zn(II) with two of them are brought together in Table VI. For the sake of comparison the values are extrapolated or interpolated to correspond with 25° and in each case the ionic strength is 0.15–0.16.

TABLE VI

SUCCESSIVE ASSOCIATION CONSTANTS FOR Cu(II) AND Zn(II) WITH SOME IMIDAZOLES[a]

Log *k* values for ionic strength 0.15–0.16 and 25°

	Cu(II) complexes				Zn(II) complexes	
	Imidazole[b]	1-Methylimidazole[c]	4-Methylimidazole	2,4-Dimethylimidazole	Imidazole[b]	4-Methylimidazole
Log k_1	4.33	4.22	4.13	3.8	2.57	2.44
Log k_2	3.54	3.54	3.49		2.36	2.53
Log k_3	2.82	2.89	2.87		2.22	2.64
Log k_4	2.03	2.21	1.96		2.01	2.38

[a] Note that the values given in this table are log *k* values as defined by equation 2. They are related by equation 3 to the log κ values given in Table V. [b] J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, THIS JOURNAL, **76**, 3054 (1954). [c] N. C. Li, J. M. White and E. Doody, *ibid.*, **76**, 6219 (1954).

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(19) Further details concerning the work on 2,4-dimethylimidazole are given in the Honors Thesis of Raymond F. Chen, submitted to the Board of Tutors in Biochemical Sciences, Harvard University, 1955.

Comparison of the trend of the values of log k_1 for the 1:1 Cu(II) complexes in Table VI with the trend of the pK values listed in Table II shows that the order of *decreasing* affinity for Cu(II) is the same as that of *increasing* affinity for hydrogen ion. The decreasing trend of the values for Cu(II) is considerably gentler than the increasing trend for hydrogen ions. The contrasting picture is considerably accented if we broaden the comparison to include ammonia which behaves much like the imidazole derivatives with respect to Cu(II) but falls beyond trimethylimidazole with respect to hydrogen ion.[6]

The absence of a strong trend in affinity for Cu(II) paralleling the basicity of the imidazole derivative might be taken as an indication not that no such underlying tendency exists, but that it is obscured by the effects of steric hindrance operating in the opposite sense. However, if steric hindrance were such a powerful factor in the formation of the 1:1 complexes we should expect that far larger effects would be evident in the formation of the higher Cu(II) complexes containing two, three or four ligand molecules crowded around the central metal atom. No such trend is evident in Table VI.

Cowgill and Clark[20] have studied the effect of substitution on the tendency of imidazoles to coördinate with Fe(III) in ferrimesoporphyrin. The coördination appeared to be strongly inhibited in those derivatives such as 2-methylimidazole and 4,5-dimethylimidazole in which both nitrogen atoms are adjacent to a carbon atom that carries a substituent. On the other hand, derivatives in which one nitrogen atom is not so hindered were shown to combine readily with the porphyrin. Presumably 4-methylimidazole is in this category, although it was not studied by Cowgill and Clark; it would coördinate at the nitrogen atom in position 1 (in the alternative numbering system these positions would be 5 and 3, respectively), and so on. Possibly there is some tendency for similar preferences in the coördination of imidazole derivatives with free metal ions. The analogy should not be carried far, however, because Cowgill and Clark also showed that among those imidazole derivatives that were effective in forming complexes there was a general tendency for the strength of coördination to increase with increasing pK', an effect opposite to that found in the present study.

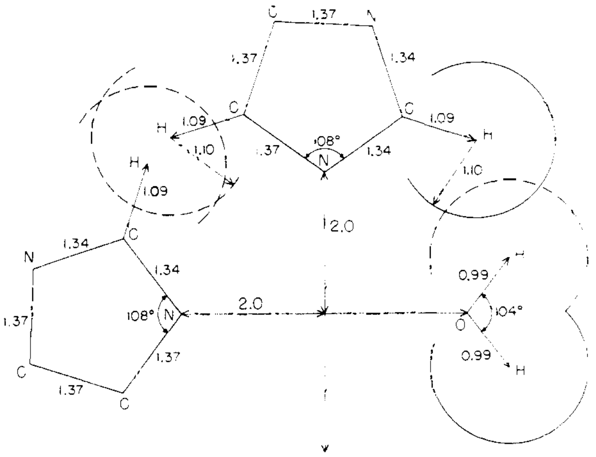

Fig. 5.—Diagram of the steric relations of two imidazole rings and a water molecule, coördinated around a central Cu ion. The Cu–N bonds and the Cu–O bond are assumed to be 2.0 Å. long. In the drawing all these bonds are assumed coplanar. The dotted arcs of circles on the upper left indicate the overlapping van der Waals radii of the hydrogen atoms on two adjoining imidazole rings. A slight overlapping is also shown to exist between the van der Waals radii of the hydrogen atoms of the attached water molecule on the right and the neighboring imidazole ring (see text).

Some further comments on the stereochemistry of these complexes may be offered. In general, tetra-coördinated zinc complexes are tetrahedral, while those of copper are generally planar[6,21] but may sometimes correspond to a more or less distorted tetrahedron. The strongly positive interactions which are apparent in the binding of many organic bases to the Zn(II) ion are present to about the same extent in imidazole and 4-methylimidazole; the extra methyl group apparently produces no added hindrance to the binding of the successive imidazole molecules to the zinc ion. This appears not unreasonable if the methyl groups in the complex are oriented outward, away from the nitrogen which is attached to the copper, which must then be the nitrogen in position 1. On the other hand if the Cu(II) complexes are square and planar, some difficult steric problems arise, in the placing of three or four imidazole rings about a central cupric ion. We take the Cu–N distance as approximately 2.0 Å., which appears reasonable in the light of available data.[22] In constructing a model for the imidazole ring, the detailed X-ray diffraction study by Donohue, Lavine and Rollett[23] of histidine hydrochloride monohydrate gives by far the most definite information available. The imidazole ring in this crystal is in the positively charged (imidazolium) form. The Cu(II) imidazole complexes should be fairly closely similar in electron distribution because of the positive charge on the copper (basic imidazole may well be considerably different in structure, since the removal of the positive charge should have important repercussions on the electron distribution in the ring). We may adopt a simplified model suggested by Donohue, *et al.*, as being compatible with their data within the limits of experimental error. It is illustrated in the two imidazole groups around the Cu atom in Fig. 5. All the internal bond angles are taken as being very close to 108°, and all C and N atoms shown are coplanar within very narrow limits (within 0.017 Å. in the case of histidine hydrochloride monohydrate). An attached carbon in the 4-position, as in histidine and 4-methylimidazole, is also coplanar with the atoms of the ring. Allowing for the usual C–H bond distances and van der Waals radii for the hydrogen atoms, it proves to be impossible to place four imidazole rings, even without substituent

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[20] R. W. Cowgill and W. M. Clark, *J. Biol. Chem.*, **198**, 33 (1952).

[21] See, for instance, A. F. Wells, "Structural Inorganic Chemistry," 2nd Ed., Oxford, 1950; also J. C. Bailar, Jr. (editor) "The Chemistry of the Coördination Compounds," Reinhold Publ. Corp., New York, N. Y., 1956.

[22] See, for instance, the discussion by J. Bjerrum, C. J. Ballhausen and C. I. Jørgensen, *Acta Chem. Scand.*, **8**, 1275 (1954).

[23] J. Donohue, L. R. Lavine and J. S. Rollett, *Acta Cryst.*, **9**, 655 (1956).

methyl groups, around the central Cu(II) ion, if it is assumed that all the rings are coplanar, and lie in the plane defined by the C–N bonds. The nature of the steric hindrance involved is shown in Fig. 5, in which the C–H distance is taken as 1.09 Å., and the van der Waals radius of the hydrogen atoms as 1.10 Å. The marked overlapping shown between the van der Waals radii of the adjacent imidazole rings in Fig. 5 obviously cannot be obviated by any reasonable change in the assumed dimensions. Presumably some or all of the imidazole rings must be rotated out of the plane of the figure, around the Cu–N bonds, so as to make mutual accommodation easier. Probably there is also some distortion of the orientation of the Cu–N bonds, so that they do not all lie in a single plane.[24]

The insertion of a methyl group in the 4-position on the ring gives rise to new stereochemical problems and also to new possibilities of isomerism, as illustrated in Fig. 6. To minimize steric hindrance it seems probable that the binding will occur chiefly with the 1-nitrogen, as for the imidazole ring shown on the right hand side of Fig. 6. This places the methyl group as far away as possible from the central copper ion. In the binding of 2,4-dimethylimidazole, however, it is inevitable that one of the methyl groups must be on a nitrogen atom immediately adjacent to the copper ion. Nevertheless the rather incomplete studies reported here on 2,4-dimethylimidazole indicate only a slight decrease in its tendency to coördinate with copper as compared with imidazole or 4-methylimidazole. These findings reinforce the conclusion that the Cu–N bonds cannot be coplanar in the tri- and tetra-coördinated complexes. X-ray diffraction and spectroscopic studies on these complexes may serve to clarify these problems, to which we can give no definite answer here.[25]

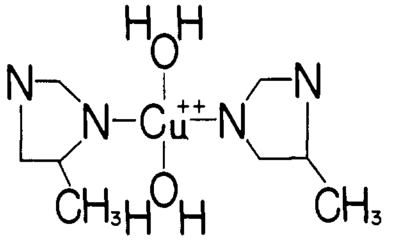

Fig. 6.—Combination of 4-methylimidazole with Cu(II), showing the two possible isomeric arrangements; the one shown on the right is presumably preferred. The other two coördination positions are shown as being occupied by water molecules.

Acknowledgment.—We are indebted to Mr. Charles Lipson for much work on the preparation and purification of the imidazole derivatives studied in this paper, and for some of the preliminary measurements upon them.

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[24] Bjerrum, *et al.*,[22] conclude that the $Cu(NH_3)_4^{++}$ complex, and other analogous complexes, are distorted octahedral structures, with attached water molecules above and below the plane defined by the 4 Cu–N bonds. If this is true for the imidazole complexes, the stereochemical problem is not very much changed from that outlined in the text here. The two attached water molecules, however, may make the attachment of 4 imidazole molecules involve an even tighter fit than we have suggested.

[25] Compare the structure of the $CuCl_4^=$ ion in cesium chlorocuprate, reported by L. F. Helmholz and R. F. Kruh, THIS JOURNAL, **74**, 1176 (1952).

CAMBRIDGE AND BOSTON, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTHERN CALIFORNIA]

Amine Chemistry of the Pentaborane B_5H_9

BY ANTON B. BURG

RECEIVED NOVEMBER 13, 1956

The structure of B_5H_9 suggests certain modes of reaction which its amine chemistry appears to confirm. It adds trimethylamine at −78° to form $B_5H_9 \cdot 2Me_3N$, which on heating *in vacuo* is partly dissociated into the original components and partly converted to Me_3NBH_3 and $(BH)_x$. On heating with excess Me_3N, the adduct yields $2Me_3NBH_3$ and BH polymers incorporating Me_3N, which lends thermoplastic character. Either Me_2NH or aminoboron hydrides such as Me_2NBH_2 or $(Me_2N)_2BH$ remove BH_3 from B_5H_9 on heating, and much of the new $(Me_2N)_3B_3H_4$ (m.p. 95°; b.p. est. 220°) is formed. Small yields of the new liquid $(Me_2N)_2B_4H_6$, of similar volatility, also are obtained. Both compounds, which seem to represent a large new class, are presumed to have cross-ring structures wherein all B and N atoms are four-coördinate. The $(Me_2N)_2B_3H_4$ is especially unreactive and thermally stable but can be converted by heat to still more inert new materials.

The structure of the pentaborane B_5H_9[1,2] is such that the removal of two BH_3 groups from the molecule—as by the use of a tertiary amine to form $2R_3NBH_3$—could occur by breaking only bonds of order less than one, and without shifting any hydrogen atoms from one boron atom to another. The remainder of the molecule then would be a B_3H_3 unit which could not persist as a simple B–B–B chain hydride, but would convert immediately to a high-polymer form. These ideas correlate with some experimental results obtained by the author in the year 1937, during a period of collaboration with Professor H. I. Schlesinger at the University of Chicago. It was found that the adduct $B_5H_9 \cdot 2(CH_3)_3N$ would form slowly at −79° and upon warming *in vacuo* it would decompose in two ways: dissociation into the original components and the formation of $(CH_3)_3NBH_3$ and a yellow residue. The two ways of decomposition, occurring simultaneously, suggested the possibility that the adduct had two forms with different types and strengths of bonding. On heating with more trimethylamine, the yellow solid became almost colorless, with slight production of $(CH_3)_3NBH_3$, and approached the composition $(BH)_x$, containing only a very

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[1] K. Hedberg, M. E. Jones and V. Schomaker, THIS JOURNAL, **73**, 3538 (1951).

[2] W. J. Dulmage and W. N. Lipscomb, *ibid.*, **73**, 3539 (1951).