with the hydrogen chloride at a temperature above that at which methylcarbamyl chloride dissociates (93–94°).[5]

Summary

1. The vapor-phase reactions of primary and secondary monoamines with an excess of phosgene were investigated. A new method for the preparation of mono- and disubstituted carbamyl chlorides and of monoisocyanates from aliphatic, olefinic, alicyclic and aromatic amines is described.

2. Carbamyl chloride was prepared by the reaction between phosgene and ammonia at 500°. The carbamyl chloride was of high purity and was readily converted to cyanuric acid by heating.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ST. LOUIS UNIVERSITY]

Polarographic, Potentiometric and Conductometric Studies on the Aspartate and Alaninate Complexes of Copper

BY NORMAN C. LI AND EDWARD DOODY[1]

As a part of a general program on complex formation between different metallic and amino acid ions[2] this paper[3] presents the results on the aspartate and alaninate complex of copper. The methods used were polarographic, potentiometric and conductometric.

Several investigators have studied the complex ions formed between cupric and alaninate ions. Boorsook and Thimann[4] have deduced from spectrophotometric and electrometric data that the complex formed in alkaline solutions was CuA_3^-. Riley and co-workers[5,6] using a cupric ion concentration cell, have concluded that a very stable complex ion of the type CuA_3^- is formed; however, the data for alaninate solutions reported in the two papers do not agree. Using the dropping mercury electrode, Keefer[7] has reported the composition and stability of the alaninate complex of copper and has found the complex to be CuA_2, but it is desirable to extend the range of his studies to lower *p*H values by using buffer solutions.

Experimental

Reagent quality chemicals were used without further purification except for the alanine which was once recrystallized. A standard solution of cupric nitrate was prepared by dissolving about 0.025 mole of cupric nitrate in 250 ml. of water. To this solution excess potassium iodide was added and the liberated iodine was titrated with a standard solution of sodium thiosulfate to the starch endpoint. Stock solutions of the potassium aspartate and potassium alaninate were prepared from the amino acids and potassium hydroxide and kept in a refrigerator until used. The *p*H of all solutions for the polarographic studies was measured by means of a Leeds and Northrup *p*H meter, assembly number 7661. To minimize errors in calculating the aspartate concentration from the *p*H, the pK_2 and pK_3 for aspartic acid was determined at the same ionic strength using carbonate-free sodium hydroxide. A simple method for determining the pK_2 and pK_3 of aspartic acid and equation for calculating the concentration of the aspartate ion will be given later in the paper. For alanine Keefer's value[7] of 9.86 for pK_2 was used.

Polarographic measurements were made using a Fisher Elecdropode. An H-type cell was used, with a saturated calomel electrode, in a thermostat kept at 25.0°. Oxygen was removed from the solutions in the cell with a stream of hydrogen and during each run an inert atmosphere of hydrogen was maintained over the solution. Methyl red (0.025%) and brom cresol green (0.12%) were used as a maximum suppressor. In the case of the aspartate, all solutions were made up to 5×10^{-4} *M* cupric nitrate and sufficient potassium nitrate was added to keep the ionic strength constant at 1.0. In the case of alanine, the composition of the solutions was as follows: 0.04 *M* potassium alaninate, 5×10^{-4} *M* cupric nitrate, 0.06 *M* potassium dihydrogen phosphate plus sodium hydroxide. The reversibility of the electrode reaction was tested for each analysis by determining the slopes of plots of log $i/(i_d - i)$ against $E_{d.e.}$, or by determining the values of $(E_{3/4} - E_{1/4})$,[8] where $E_{3/4}$ and $E_{1/4}$ are the values of $E_{d.e.}$ at $i = (3/4)i_d$ and $i = (1/4)i_d$, respectively. Corrections were made for residual current. Half-wave potentials were reproducible to ±3 mv.

Potentiometric studies were made using a Cenco Titration–*p*H meter, with a glass electrode which had been cleaned thoroughly with dilute acid, allowed to stand in distilled water for a number of hours, and then calibrated immediately before use by means of a potassium acid phthalate buf-

(1) Brother Edward Doody of the Congregation of Christian Brothers.

(2) Li and Gormley, a paper presented at the Chicago meeting of the American Chemical Society, April 20, 1948.

(3) Presented at the 116th meeting of the American Chemical Society, Atlantic City, Sept., 1949.

(4) Boorsook and Thimann, *J. Biol. Chem.*, **98**, 671 (1932).

(5) Riley and Gallafent, *J. Chem. Soc.*, 2029 (1931).

(6) Ferrel, Ridgion and Riley, *ibid.*, 1440 (1934).

(7) Keefer, THIS JOURNAL, **68**, 2329 (1946).

(8) Kolthoff and Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

fer at pH 4.00. It was noted that the pH continued to change for about four minutes after each addition of solution, but the results of titrations were reproducible. Conductometric measurements were made using a Fisher Conductivity Bridge, Model RC 1 B.

Discussion

Kolthoff and Lingane[8] have shown that the reduction to a metallic state (amalgam) of a cupric complex may be represented by

$$CuA_p^{(2-pb)+} + 2e + Hg = Cu(Hg) + pA^{-b} \quad (1)$$

where Cu(Hg) represents the amalgam formed on the dropping mercury electrode, and A^{-b} is the complex forming ion. If the above reaction is rapid and reversible at the dropping mercury electrode, then at 25° the following relationship holds

$$\Delta E_{1/2}/\Delta \log (A^{-b}) = -0.0296\, p \quad (2)$$

where $E_{1/2}$ is the half-wave potential and p is the number of amino acid ions attached to each cupric ion. Thus from the slope of the curve obtained by plotting $E_{1/2}$ *vs.* the activity of the complex forming amino acid ion, the value of p may be determined and the formula of the complex obtained. The dissociation constant

$$K_c = (Cu^{++})(A^{-b})^p/(CuA_p^{(2-pb)+}) \quad (3)$$

is calculated by means of the relationship

$$(E_{1/2})_c - (E_{1/2})_s = 0.0296 \log K_c(f_c k_s)/(f_s k_c) - p\, 0.0296 \log C_A f_A \quad (4)$$

in which the subscripts c and s refer to the complex and simple metal ions, respectively, f is the activity coefficient and k is proportional to the square root of the diffusion coefficient of the ion.

The results of the polarographic analyses of solutions containing cupric nitrate (5×10^{-4} M) and varying concentrations of aspartate ion are given in Table I. Only single waves are observed. The electrode reactions for the complexes indicate that two electrons are involved, as shown by the values of the slopes of the plots of $\log i/(i_d - i)$ *vs.* E or by the values of $(E_{3/4} - E_{1/4})$. Thus in these cases the cuprous complexes are not stable, in contrast to the cupric complexes of ammonia, thiocyanate, pyridine and chloride,[8] which are reduced first to the cuprous state before being further reduced to the amalgam.

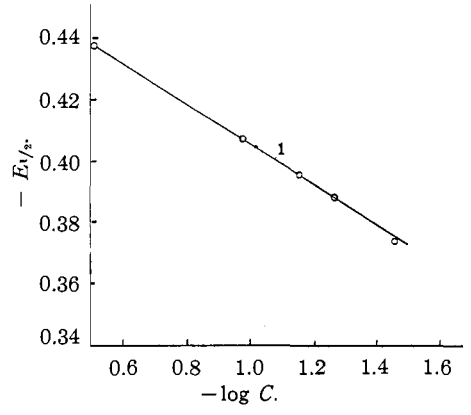

Fig. 1.—Variation of half-wave potential with concentration of aspartate.

TABLE I

HALF WAVE POTENTIAL OF THE COPPER ASPARTATE COMPLEX AS A FUNCTION OF THE ASPARTATE ION CONCENTRATION

Total aspartic acid, molar	pH μ = 1.0	$E_{3/4} - E_{1/4}$	$-E_{1/2}$ *vs.* S. C. E. pK_2 = 3.70,	$-\log$ As^a pK_3 = 9.61
0.33	10.90	0.034	0.438	0.504
.12	10.50	.031	.408	.977
.08	10.60	.033	.397	1.145
.06	10.69	.031	.388	1.265
.04[a]	10.50	.031	.375	1.461
.00	...	...	−0.014	...

[a] 0.44 M potassium nitrate as S. E.

Although the activity coefficients of the ions are not known, the ionic strength of the solutions was kept constant at 1.0, hence the activity coefficient of the aspartate ion would remain nearly constant and, as a first approximation, may be assumed to be unity. In most cases the diffusion coefficients of the simple and complex ions are nearly equal so that the ratio of k_s/k_c may also be assumed to be unity. Hence equation 4 may be simplified to

$$(E_{1/2})_c - (E_{1/2})_s = 0.0296 \log K_c - p\, 0.0296 \log C_A \quad (5)$$

from which the concentration dissociation constant K_c of the complex may be calculated.

In Table I the concentration of the aspartate ion was calculated from the concentration of free aspartic acid, pH of solution and pK_3 of aspartic acid by means of the relationship

$$pK_3 = p\text{H} + \log[C/(R^{--}) - 1] \quad (6)$$

where K_3 is the third dissociation constant of aspartic acid, C is the concentration of free aspartic acid and was obtained by subtracting the concentration of aspartic acid combined with cupric ion (assumed $CuAs_2$) from the total aspartic acid present. The difference between pK_2 and pH values is about 7, so that we need not consider pK_2.

The pK_2 and pK_3 of aspartic acid given in Table I was taken to be equal to the pH of solutions made up of: (a) 10 ml. of 0.04987 M aspartic acid + 2.12 ml. of 0.1175 N KOH and (b) 10 ml. of 0.04987 M aspartic acid + 6.37 ml. of 0.1175 N KOH, respectively, at total ionic strength of 1.0, potassium nitrate being used as the indifferent electrolyte to keep the ionic strength constant. Experience gained in this Laboratory has shown that the results of pK_2 and pK_3 obtained by this simple method are as accurate as other methods using complicated and cumbersome equations.

The number of groups, p, coördinated to each cupric ion is obtained from the slope of the plot of equation 2, shown in Fig. 1. The slope is 0.065, indicating that two groups are coördinated. The average dissociation constant for the aspartate complex was calculated by means of equation 5 to

be 6.3×10^{-16}. The free energy of formation of the complex, $\Delta F = RT \ln K$, is -20.8 kcal.

In the case of alanine we followed a procedure somewhat similar to that described by Laitinen and co-workers[9] for the glycinate complex. Equation 6 may be modified to give

$$(E_{1/2})_c - (E_{1/2})_a = 0.0296 \log K_c + p\,0.0296\,(pK_2 - p\mathrm{H}) - p\,0.0296 \log C_{Al} \quad (7)$$

It is evident that the half-wave potential of the complex ion is a linear function of pH and that a buffer must be used in order to prevent the depletion of hydrogen ion concentration at the dropping electrode surface. The results are given in Table II and the average dissociation constant is 1.5×10^{-15}, which is to be compared with the value 0.98×10^{-15} obtained by Keefer for the cupric alaninate. Free energy of formation of the

TABLE II

INDIFFERENT ELECTROLYTE 0.06 M KH_2PO_4 PLUS NaOH; 5×10^{-4} M $Cu(NO_3)_2$; 0.04 M POTASSIUM ALANINATE PUT INTO SOLUTIONS

pH	$-E_{1/2}$ vs. S. C. E.	$E_{3/4} - E_{1/4}$	$K_c \times 10^{15}$
6.92	0.193	0.034	1.7
7.30	.216	.033	1.7
7.60	.236	.033	1.5
8.41	.285	.039	1.2
8.92	.314	.033	1.3

complex is calculated to be -20.3 kcal. The plot of $E_{1/2}$ *vs.* pH is shown in Fig. 2, and the slope $\Delta E/\Delta p$H is 0.059. From equation 7 the theoretical slope for $p = 2$ is 0.0592. The agreement is excellent. This finding verifies Keefer's work[7] on alaninate.

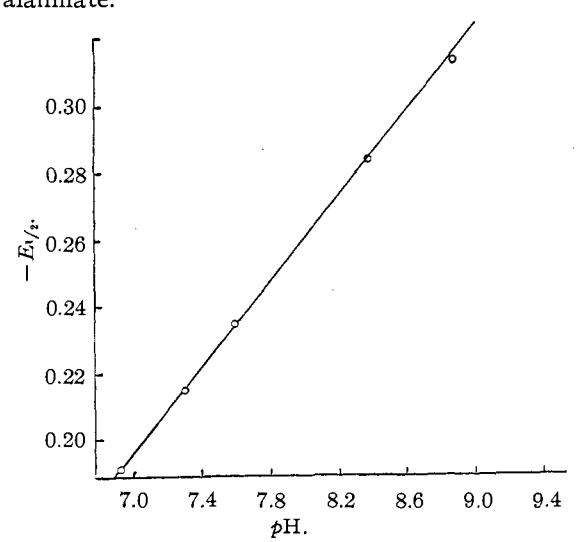

Fig. 2.—Change of half-wave potential with pH of alaninate solutions.

The potentiometric titrations of aspartate and alaninate with copper are shown in Fig. 3. Figure 4 shows the potentiometric pH titrations of copper with aspartate and alaninate. In each case the curves show that the ratio of copper to the amino acid ion is about 1:2, confirming the polarographic results. There is, however, a difference in this ratio resulting from the direction of titration, the average ratios being 1:2.19 in Fig. 3 and 1:2.0 in Fig. 4.

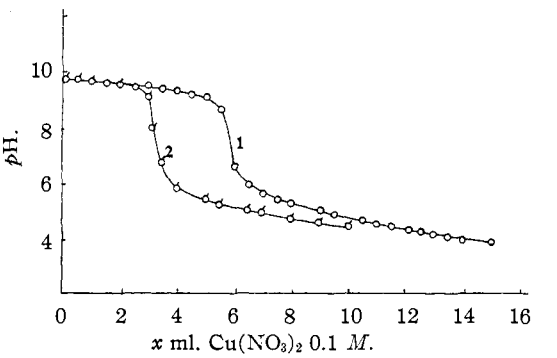

Fig. 3.—Potentiometric titration: curve 1, 2.5 ml. 0.5 M potassium aspartate, diluted to 50 ml. + x ml. Cu^{++}, 0.1 M; curve 2, 2.5 ml. 0.3 M potassium alaninate, diluted to 50 ml. + x ml. Cu^{++}, 0.1 M.

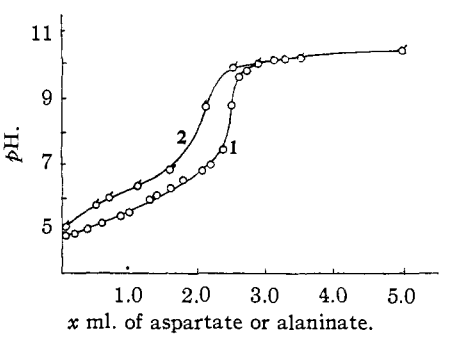

Fig. 4.—Potentiometric titration: curve 1, 6.25 ml. 0.1 M Cu^{++}, diluted to 50 ml. + x ml. 0.5 M potassium aspartate; curve 2, 1.0 ml. 0.1 M Cu^{++}, diluted to 50 ml. + x ml. 0.1 M potassium alaninate.

Groups of curves which are typical of the conductometric titrations of the amino acid ions with copper are shown in Fig. 5. The ordinates are the reciprocal of resistance, corrected for dilution. Figure 6 shows the conductometric titration of copper with the amino acid ions.

As seen from Figs. 5 and 6, there are two breaks in each curve. Table III gives the ratios of amino acid ions to copper, the first and second numbers

TABLE III

CONDUCTOMETRIC STUDIES OF RATIOS OF AMINO ACID IONS TO COPPER SHOWING DIFFERENCES RESULTING FROM DIRECTION OF TITRATION

	Ratio	
Cu^{++} added to aspartate	1.85: 0.98	Fig. 5, curve 1
Aspartate added to Cu^{++}	0.95: 2.1	Fig. 6, curve 1
Cu^{++} added to alaninate	2.0 : 0.96	Fig. 5, curve 2
Alaninate added to Cu^{++}	1.05: 2.1	Fig. 6, curve 2

(9) Laitinen, Onstott, Bailar and Swann, THIS JOURNAL, 71, 1550 (1949).

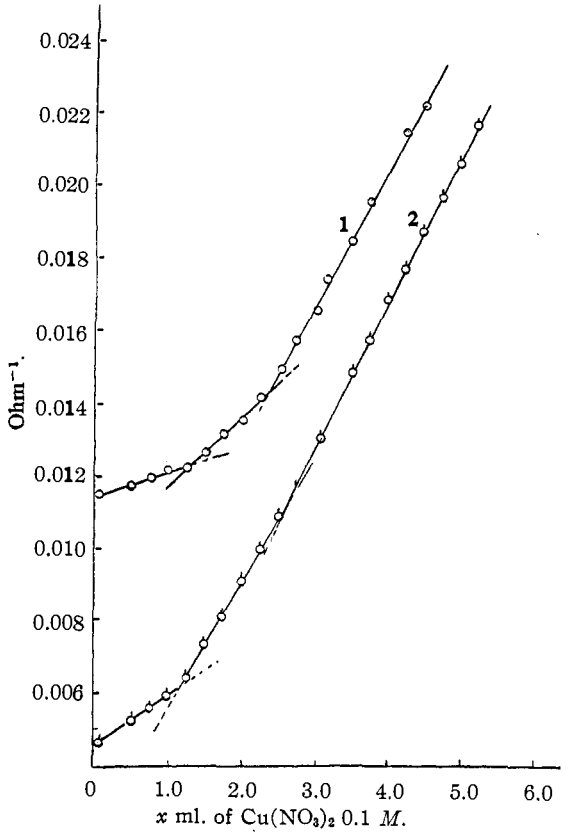

Fig. 5.—Conductometric titration: curve 1, 0.8 ml. 0.3 *M* potassium aspartate + 12 ml. water + *x* ml. Cu^{++}, 0.1 *M*; curve 2, 0.8 ml. of 0.3 *M* potassium alaninate + 12 ml. water + *x* ml. Cu^{++}, 0.1 *M*.

correspond to the first and second breaks, respectively, in the conductometric curves. The results obtained show that for the aspartate and alaninate complexes, one mole of the amino acid ions is coördinated to each cupric ion when the latter is in excess. When the amino acid ions are in excess two moles are coördinated to each cupric, and this result confirms the polarographic and potentiometric data.

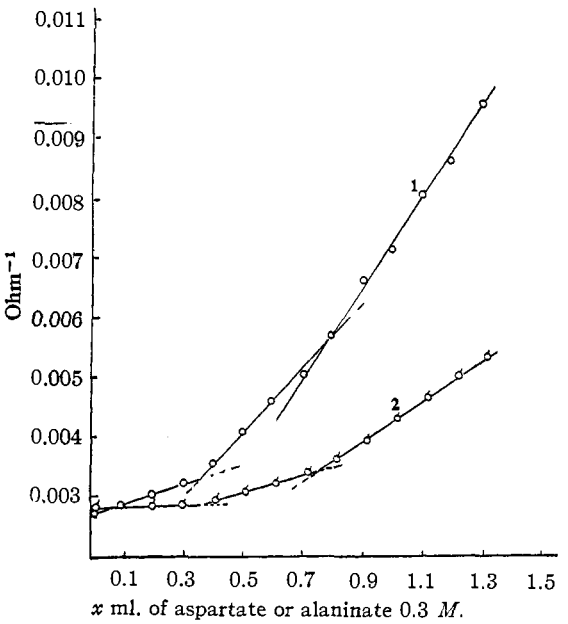

Fig. 6.—Conductometric titration: curve 1, 1.1 ml. 0.1 *M* $Cu(NO_3)_2$ + 23 ml. water + *x* ml. 0.3 *M* potassium aspartate; curve 2, 1.1 ml. 0.1 *M* $Cu(NO_3)_2$ + 23 ml. water + *x* ml. 0.3 *M* potassium alaninate.

Acknowledgment.—The authors wish to express their appreciation to Merck, Inc., for supplying the amino acids for our general research program on amino acid complexes.

Summary

The formulas, dissociation constants and free energies of formation of the aspartate and alaninate complexes of copper(II) have been obtained by polarographic methods. The composition of the aspartate and alaninate complexes have been confirmed by potentiometric and conductometric measurements. The conductometric results show further that the complex CuA, where A is the amino acid ion, is formed when cupric ion is in excess.

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