A plot of log $\{[Ag]_t/([Ag]_t - [Ag]_f\}$ against t should give a straight line with a slope of $2.3/(k[Ag]_f)$, whose intercept at $t = 0$ gives the initial concentration $[Ag]_0$ for the reaction step under consideration.

Case II.—The Ag(I) molarity is less than that of the particular Cr(III) species under consideration. Since the concentration of complexed chromic chloride species can now no longer be defined by the Ag(I) concentrations, a slightly different approach is used than in Case I, based on the following relations with $[Ag]_0$, $[Ag]_t$ and $([Ag]_0 - [Ag]_t)$ defined as in case I

$[CrCl_x]$ = initial concn. of the particular $[Cr(H_2O)_{6-n}Cl_n]^{(3-n)}$ species

$[CrCl_x] - [Ag]_0 + [Ag]_t$ = concn. of Cr species present at time, t

The equation for a second-order reaction for constant $[Ag]_0$ and $[CrCl_x]$ can then be written as

$$\log \frac{[CrCl_x]}{[Ag]_0} + \log \frac{[Ag]_t}{[Ag]_t + [CrCl_x] - [Ag]_0} = \frac{[Ag]_0 - [CrCl_x]}{2.3} kt \quad (7)$$

If the quantity $[CrCl_x] - [Ag]_0$ is written as U

$$\log \frac{[CrCl_x]}{[Ag]_0} + \frac{U}{2.3} kt = \log \frac{[Ag]_t + U}{[Ag]_t} \quad (8)$$

The plot of log $\{([Ag]_t + U)/[Ag]_t\}$ against time should give a straight line with a slope of $(-U/2.3)k$ and an intercept at $t = 0$ of log $([CrCl_x]/[Ag]_0)$.

The data of Tables I and II are expressed in the above terms. In cases where the silver concentration was in excess, the values of $[Ag]_t$ and of $([Ag]_t - [Ag]_f)$ were used; where the silver concentration was insufficient, values of $([Ag]_t + U)$ and of $([Ag]_t + U)/[Ag]_t$ were used. The data are presented in terms of either the first or the second of the two reaction steps; the very rapid rate of the first reaction precludes the detailed analysis possible for the second step.

Values of the rate constant k_2 were obtained graphically from the slopes of plots of either of two functions, log $\{[Ag]_t/([Ag]_t - [Ag]_f)\}$ or log $\{([Ag]_t + U)/[Ag]_t\}$ against time in accordance with the previous discussion. Although k_2 values could be obtained from the individual points, use of a graphical procedure served to smooth out variations in the data and gave more realistic values.

Values of k_1 were obtained by substituting the measured data into equation 7. The short time available for sampling did not permit more than one value for $[Ag]_t$ to be determined in each run, so that the graphical procedure could not be used. Sampling time was held to 30 sec. or less, this being the time required to drain completely the sampling pipet into a known sodium chloride solution which served to quench the reaction. All of the samples withdrawn had precipitated silver chloride present; the effect of this on the results obtained could not be ascertained, but is believed to be minor.

Values of $[Ag]_t$ were obtained by differential potentiometric titration, which method is capable of good accuracy; the results obtained were quite reproducible. To calculate k, it was also necessary to have values for either $[Ag]_f$ or $([CrCl_x] - [Ag]_0)$. Of these two, $[Ag]_f$ values are obviously available with greater accuracy for the second reaction. Since the latter approaches the final equilibrium value asymptotically, by waiting a sufficiently long time the difference between the final measured Ag(I) concentration and the true $[Ag]_f$ value can be made as small as desired. Values of log $\{[Ag_t/([Ag]_t - [Ag]_f)\}$, when plotted against time, gave a straight line only when the $[Ag]_f$ values used were essentially those of final equilibrium. If the experimental value of $[Ag]_f$ used was higher than the true value, the curve sloped upward with increasing time; if it was too low, the curve sloped downward. Consequently, an internal check is available for the deviation of $[Ag]_f$ from equilibrium. The latter is particularly useful in experiments involving very dilute silver solutions where the values of $[Ag]_f$ are difficult to measure accurately.

For cases where the silver was insufficient to react with all of the complexed chloride ion, it was necessary to know U. For the first reaction, U can be computed from the known initial concentrations of dichlorotetraaquochromium(III) and silver ions. The chromic species concentration was determined, immediately before adding the silver, by titration of the ionizable chloride with standard silver solution. In some cases the desired silver for $[Ag]_0$ was added to the titrated solution. In other cases, an amount of silver sufficient to precipitate the ionizable chloride and to furnish $[Ag]_0$ was added to a fresh sample of the analyzed chromium solution.

The value of U for the second reaction must be derived from that of U for the first reaction, and involves a small uncertainty due to two factors: the tendency for some of the dichlorotetraaquo complex to react with the silver when titrating the uncomplexed chloride, and the uncertainty in the "zero" time of the second reaction due to the mother–daughter relationship. It was found, when the quantity log $\{([Ag]_t + U)/[Ag]_t\}$ is plotted against time, that, as in the previous case, a straight line is obtained only when the value of U used had one particular value; otherwise, the line curved upwards or downwards with time depending on whether the value of U is too high or too low.

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ANN ARBOR, MICHIGAN

[CONTRIBUTION FROM DUQUESNE UNIVERSITY AND CHRISTIAN BROTHERS COLLEGE]

Some Metal Complexes of Glycine Peptides, Histidine and Related Substances[1]

BY NORMAN C. LI, BROTHER EDWARD DOODY AND JAMES M. WHITE

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An ion-exchange method was developed for obtaining the successive formation constants of the Co(II) complexes of glycylglycine, glycylglycylglycine and tetraglycine, and the results are in agreement with those obtained by the *p*H method. It is shown that the coördination sites of the glycine peptides toward Co(II) are probably the terminal amino group and the immediately adjacent peptide oxygen. The formation constants of Cu(II), Ni(II) and uranyl complexes of glycylglycine, glycine amide, histidine and histidine ester have been measured and compared. The predominant coördination sites of histidine toward Cu(II), Ni(II) and uranyl ions are probably the "pyridine" nitrogen of the imidazole group and the amino group. The rates of alkaline hydrolysis of histidine methyl ester have been determined in the presence and absence of metal ions and it was found that an increase in the stability of metal-ester complex is accompanied by an increase in the bimolecular rate constant.

Introduction

In glycine peptides the following functional groups must be considered as potential sites of coördination to metal ions: the terminal carboxyl group in its charged form, the terminal amino group, the peptide oxygen atom and the peptide nitrogen atom. The formulation of the metal

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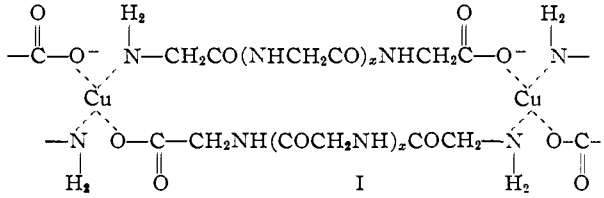
chelates of glycine peptides has been discussed in several publications.[2-5] Klotz, *et al.*,[2] suggested that coördination of glycine polypeptides with copper(II) ion takes place only with the terminal carboxyl and amino groups, giving rise to polymers of the type indicated by formula I



I

Manyak, *et al.*,[3] pictured the complexes of glycylglycine and glycylglycylglycine with Cu(II), Co(III) and Ni(II) ions as involving interactions at the amino nitrogen atom, the peptide nitrogen atom and the carboxyl group. Dobbie and Kermack[4] thought that Cu(II) ion coördinates to glycylglycine and glycylglycylglycine through the amino groups and peptide nitrogen atoms, with the displacement of protons from the latter nitrogen atoms. Rabin[5] proposed that the sites of coördination of the Co(II) and Mn(II) complexes of dipeptides are the amino group and the peptide oxygen atom, but that the Cu(II) complexes have several different structures, depending on the *p*H of the solution. There is thus considerable disagreement in the nature of the functional groups of glycine peptides associated with metal ions.

Histidine, like glycine peptides, contains four different functional groups which are potential sites of coördination to metal ions. These are: the charged carboxyl group, the uncharged amino group, the "pyrrole" nitrogen and the "pyridine" nitrogen atoms of the imidazole part of the molecule. Edsall, *et al.*,[6] from spectral evidence, have shown that the amino and imidazole groups are the chelating sites of Cu(II)–histidine complex, whereas Cohn and Townsend[7,8] suggested that Mn(II) ion tends to combine with the carboxyl and imidazole groups of histidine; both groups of investigators however made no distinction between the two different nitrogen atoms in imidazole. It is evident that more study on the nature of metal complexes of histidine is desirable.

This paper describes the use of ion-exchange, *p*H, conductometric and infrared methods for the study of metal complexes of glycine peptides, histidine and related substances. The complexing agents were chosen so as to provide further information concerning the sites of coördination of glycine peptides and of histidine to several metal ions.

Experimental

Materials.—Carrier-free Co^{60} was obtained as the chloride salt in a very dilute neutral solution. For the cation exchanger, Dowex-50, 8% cross-linked, 20–50 mesh was used. The resin was put through several alternate Na^+–H^+ cycles with 5% solutions of NaCl and HCl. After saturation with excess NaCl, the resin was equilibrated with excess 0.15 *M* NaCl solution. The resin was filtered through a büchner funnel, rapidly washed free of adhering salt solution with distilled water and air-dried. All experiments were conducted with the air-dried resin. Anhydrous UO_2Cl_2 and UCl_4 were gifts of Dr. H. R. Hoekstra. Stock solutions of $Cu(NO_3)_2$ and $Ni(NO_3)_2$ were standardized by conventional means; uranyl nitrate concentration was determined by precipitating with NH_4OH, igniting and weighing as U_3O_8. The glycine peptides, histidine and histidine methyl ester, all products of Mann Research Laboratories, were dried *in vacuo* over P_2O_5 before use. All other chemicals were of C.P. grade.

Procedure.—The ion-exchange experiments were carried out in a manner similar to that described by Schubert,[9] except that no buffer was used. The stock solutions of complexing agents were generally 0.1 *M* in glycine peptide, 0.02 *M* in NaOH, so that they serve as buffers as well. The flasks were agitated at 25°. After a three-hour shaking period, a time which was found sufficient for equilibration, a 4-ml. aliquot was removed from each flask and counted directly as liquid sample in a scintillation counter.

Measurements of *p*H were made with a Beckman Model G *p*H meter with external electrodes. Only freshly prepared solutions were used, and nitrogen gas was bubbled through the solution. Infrared spectra were taken with a Perkin–Elmer infrared spectrophotometer Model 21, using a sodium chloride prism.

The rates of alkaline hydrolysis of histidine methyl ester in the presence and absence of metal ions were measured by a conductometric method previously described.[10]

Results

Ion-exchange results at 25° on Co(II) complexes of glycine peptides, using Co-60, in tracer amount, are summarized in Table I. K^0_d and K_d are the distribution coefficients of the divalent metal cations,

TABLE I

ION-EXCHANGE EXPERIMENTS WITH Co(II) COMPLEXES OF

(a) Glycylglycine, GG

$(GG^-) = (A^-) \times 10^3$	$1/Kd^a$	$\frac{(K^0_d/K_d) - 1}{(A^-)} \times 10^{-3}$
0	2.0	
2.0	7.6	1.4
4.0	16.2	1.77
6.0	27.8	2.15
8.0	42.3	2.52
10.0	61.0	2.95

(b) Glycylglycylglycine (GGG)

$(GGG^-) = (A^-) \times 10^3$		
0	2.2	
0.92	4.67	1.17
1.84	8.59	1.53
3.68	18.3	1.94
5.52	33.4	2.51

(c) Tetraglycine (GGGG)

$(GGGG^-) = (A^-) \times 10^3$		
0	1.5	
1.03	3.54	1.34
2.06	7.00	1.80
3.44	12.3	2.12
4.82	19.25	2.49
5.51	24.0	2.76

[a] $1/K_d = \frac{\%\text{ Co-60 in aqueous phase}}{\%\text{ Co-60 in resin phase}} \times \frac{\text{mg. resin.}}{\text{ml. soln.}}$

(2) I. M. Klotz, I. L. Faller and J. M. Urquhart, *J. Phys. Colloid Chem.*, **54**, 18 (1950).

(3) A. R. Manyak, C. B. Murphy and A. E. Martell, *Arch. Biochem.*, **59**, 373 (1955).

(4) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246, 257 (1955).

(5) B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).

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

(7) M. Cohn and J. Townsend, *Nature*, **173**, 1090 (1954).

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

(9) J. Schubert, *J. Phys. Chem.*, **56**, 113 (1952).

(10) J. M. White, R. A. Manning and N. C. Li, THIS JOURNAL, **78**, 2367 (1956).

Co(II), between the resin and solution phases in the absence and presence of the complexing agent, respectively. The ionic strength of each solution was kept constant at 0.15 by adding NaCl.

If we assume that no complex is adsorbed by the resin and consider the possible complexes in solution of Co(II) with a ligand, A^-, we may define the distribution coefficients by the expressions

$$K_d = \frac{(\text{Co(resin)})}{(\text{Co}^{++}) + (\text{CoA}^{+}) + (\text{CoA}_2) + \ldots} \quad (1)$$

$$K_d^0 = \frac{(\text{Co(resin)})}{(\text{Co}^{++})} \quad (2)$$

By introducing the term $k_i = (\text{MA}_i)/(\text{MA}_{i-1})\cdot(\text{A}^-)$, equation (3) is derived readily

$$y = \frac{(K_d^0/K_d) - 1}{(\text{A}^-)} = k_1 + k_1k_2\,(\text{A}^-) + \ldots \quad (3)$$

From equation 3 it is seen that $y = k_1$ and independent of (A^-), if only 1:1 complex exists. On the other hand, if a plot of y *vs.* (A^-) gives a straight line, with a finite slope, it may be assumed that 1:2 complex also exists. The values of k_1 and k_1k_2 may then be obtained from the intercept and slope of the straight line, respectively. The data of Table I, when plotted in this manner, yield straight lines, from which the values of log k_1 and log k_1k_2 have been calculated. The Co(II) complexes of the three glycine peptides also have been studied by the Bjerrum pH method[11] at an ionic strength of 0.15 and the results, together with the ion-exchange results, are listed in Table II.

TABLE II

FORMATION CONSTANTS OF Co(II) COMPLEXES OF GLYCINE PEPTIDES, μ = 0.15, 25°

Peptide	log k_1			log k_1k_2	
	pK	Ion-exchange	pH	Ion-exchange	pH
Glycylglycine	8.17	3.00	3.08	5.28	5.30
Glycylglycylglycine	8.09	2.95	3.14	5.46	5.44
Tetraglycine	7.94	3.02	3.00	5.49	5.50

All the values of log k_1 and log k_1k_2, determined by the ion-exchange method in this investigation, are in good agreement with those determined by the pH method. For these complexes, therefore, two conclusions may be made: (1) the complex itself is not taken up by the resin to any noticeable extent, and (2) the nature and stability of complexes of radioactive metal cations are identical with complexes of non-radioactive metal cations. The assumption that no cobalt complex is adsorbed by the resin, therefore, is justified and equation 1 is valid.

Formation constants of cobalt(II)–glycylglycinate complexes have been extensively determined by other investigators, using the pH titration method (Table III). Our value of log k_1k_2, determined by the pH method, is in exact agreement with that of ref. *c*, and differs by 0.58, 0.49 and 0.28, respectively, from those of ref. *a*, *b* and *d*, in Table III. The difference probably is due to the difference in ionic strengths. The formation constants of cobalt(II)–glycylglycylglycinate complexes also have been determined by Evans and Monk (ref. *a*): log k_1k_2 = 5.60, in fair agreement with our results.

(11) (a) J. Bjerrum, "Metal-Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941. (b) A. E. Martell and M. Calvin, "Chemistry of Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952.

TABLE III

LITERATURE VALUES FOR FORMATION CONSTANTS OF Co(II)–GLYCYLGLYCINATE COMPLEXES

Ref.	Temp., °C.	Ionic strength	log k_1	log k_1k_2
a	25	0	3.49	5.88
b	25	0.015[e]	3.23	5.79
c	26	0.075–0.20[e]	3.04	5.30
d	25	1.0	2.73	5.02

[a] C. B. Monk, *Trans. Faraday Soc.*, **47**, 297 (1951); W. P. Evans and C. B. Monk, *ibid.*, **51**, 1244 (1955). [b] S. P. Datta and B. R. Rabin, *ibid.*, **52**, 1117 (1956); *Biochim. Biophys. Acta*, 19, 572 (1956). [c] J. B. Gilbert, M. C. Otey and J. Z. Hearon, THIS JOURNAL, **77**, 2599 (1955). [d] C. Tanford, D. C. Kirk, Jr., and M. K. Chantooni, Jr., *ibid.*, **76**, 5325 (1954). [e] Ionic strength of initial solution only.

The formation constants of Cu(II), Ni(II) and uranyl complexes of glycine amide, glycylglycine, histidine and histidine methyl ester have been determined by pH method and the results are summarized in Table IV.

TABLE IV

FORMATION CONSTANTS OF SOME METAL COMPLEXES OF GLYCYLGLYCINE, HISTIDINE AND RELATED SUBSTANCES

μ = 0.15–0.25, 25°

	$pK_{NH_3^+}$	log k_1			log k_2	
		Cu^{++}	Ni^{++}	UO_2^{++}	Cu^{++}	Ni^{++}
Glycine amide	8.06	5.51	4.18	5.15	4.21	3.09
Glycylglycine	8.17	6.04[a]	4.49[a]			3.42
Histidine	6.17[b]	10.60	8.79	7.71	8.00	7.05
	9.20					
Histidine methyl ester	5.38[b] 7.33	9.10	6.73	5.76	6.70	5.11

[a] Taken from ref. 11b. [b] pK_{ImH^+}.

The rates of alkaline hydrolysis of histidine methyl ester in the presence and absence of metal ions were determined. For each of the rate experiments, the initial concentration of ester, sodium hydroxide and divalent cation (when present) were always in the mole ratio 1:1:1/3. Table V summarizes the specific rates obtained at 25° together with the formation constants of metal chelates with histidine ester.

TABLE V

KINETICS OF THE ALKALINE HYDROLYSIS OF HISTIDINE METHYL ESTER

Initial concn. of histidine ester, M	M^{++}	k_r, l. mole^{-1} sec.$^{-1}$	log k_1 (ester)
0.00478	None	0.62	
.00382	Ni	1.57	6.73
.00382	Cu	2.84	9.10

In order to determine whether the amide nitrogen or carbonyl oxygen in glycine peptides is involved in coördination with metal ions, we have taken the infrared spectra of various organic compounds, which contain an amide group, (a) in ethanol solutions, and (b) in ethanol solutions containing a metal salt. The reasoning is as follows: if the carbonyl oxygen coördinates to the metal cation, then the "C=O" bond will be weaker and hence its characteristic frequency will be lower in

(b) than in (a). On the other hand, if the amide nitrogen coördinates to the cation, then the above lowering will not be expected to take place.

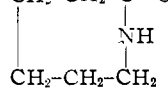
The carbonyl stretching frequencies may be considered to occur in the region 1800–1500 cm.$^{-1}$. These frequencies however may be associated with other types of motion, and it is fortunate that ethanol does not absorb appreciably in the above frequency region. Table VI lists the infrared results obtained.

Table VI

Absorption Maxima in Cm.$^{-1}$ in the Region 1800-1625 Cm.$^{-1}$ in Ethanol Solution

	Cm.$^{-1}$	Mode
A, ε-Caprolactam[e]	1655	*a*
A + $ZnCl_2$	1630	*a*
A + UO_2Cl_2	1630	*a*
A + UCl_4	1625	*a*
B, Urea	1675	*b*
	1628	*c*
B + $ZnCl_2$	1668	*b*
	1640;1605	*c*
B + UO_2Cl_2	1641	*b*
	1585	*c*
B + UCl_4	1650	*b*
	1577	*c*
C, Acetamide	1685	*b*
	1634	*c*
C + $ZnCl_2$	1670	*b*
	1615;1588	*c*
C + UO_2Cl_2	1679	*b*
	1605	*c*
C + UCl_4	1678	*b*
	1585	*c*
D, Acetanilide	1685	*b*
	1610	*d*
	1560	*c*
D + $ZnCl_2$	1681	*b*
	1610	*d*
	1560	*c*
D + UO_2Cl_2	1682	*b*
	1611	*d*
	1560	*c*
D + UCl_4	1680	*b*
	1610	*d*
E, Benzamide	1680	*b*
	1620	*d*
	1589	*c*
E + $ZnCl_2$	1680	*b*
	1620	*d*
	1585	*c*
E + UO_2Cl_2	1680	*b*
	1620	*d*
	1585	*c*
E + UCl_4	1665	*b*
	1610	*c*

[a] ν (C=O). [b] Amide I band: ν(C=O) and δ(N-H). [c] Amide II band: δ(N-H) and ν(C=O). [d] ν(C=C). [e] ε-Caprolactam (or 2-oxohexamethylenimine):



Discussion

As shown in Table VI, the "carbonyl" frequencies, whether pure or impure, in compounds A, B and C are definitely lowered when metallic salts are present. This indicates that there is interaction between these compounds with the zinc, uranyl and U(IV) ions, and that in these compounds the carbonyl oxygen is involved in coördination with these metal ions. Our conclusion on the site of coördination of the urea–zinc chloride complex in ethanol solution is in agreement with that reached by Penland, *et al.*,[12] who investigated the infrared spectra of the same complex by KBr disk technique. Penland, *et al.*, moreover, showed that metal-to-nitrogen bond does not exist in the zinc–urea complex.

Our infrared data indicate that acetanilide and benzamide do not complex appreciably with Zn(II) and uranyl ions. The presence of the phenyl group, which may be considered to be electron-attracting, may increase the double-bond character of the carbonyl bond and therefore lessen the tendency of the carbonyl oxygen to coördinate with the metal ions. It will be noted that the 1680 cm.$^{-1}$ peak of benzamide is shifted to 1665 cm.$^{-1}$ in the presence of UCl_4, whereas $ZnCl_2$ and UO_2Cl_2 do not affect the 1680 cm.$^{-1}$ band. This may be because of the high charge of the U(IV) ion, which would therefore have a greater tendency to form complexes.

In view of the infrared data on organic compounds containing the amide group, both in the presence and absence of metal cations, we may infer that in glycine amide and in glycylglycine, the preferable site of binding in the amide group toward zinc, uranyl and U(IV) ions is the carbonyl oxygen. We did not take infrared spectra of the Cu(II) complexes; however, we are preparing solid Cu(II) and Zn(II) complexes of glycylglycinate, and we plan to study the proton nuclear magnetic resonance absorption characteristics of these compounds.

As seen from Table II, the Co(II) complexes of glycylglycine, glycylglycylglycine and tetraglycine are about equally stable. This means that the three glycine peptides probably have common coördination sites, and that the sites cannot be the terminal amino and charged carboxylate groups. Otherwise, the chelate rings which Co(II) forms with GG, GGG and GGGG would be 8-, 11- and 14-membered respectively, and the stability of the three peptide complexes would not be expected to be about equal. While it is possible that each of these complexes involves only a linkage to $-NH_2$ and not to carboxylate or any other group at all, this is not likely, since the value of log k_1k_2 for Co(II)–ammonia complex is only 3.6.[11]

The common coördination sites of the glycine peptides toward Co(II) are probably the terminal amino group and the immediately adjacent amide group. Since it has been shown from infrared measurements, both in ethanol solution and in KBr disc,[12] that urea forms oxygen-to-metal bond with Zn(II), and since the affinity of Zn(II) toward many ligands is close to that of Co(II),[11b] it is safe to conclude that the coördination site in the immediately adjacent amide group, mentioned above, is the peptide oxygen.

From Table IV it is seen that the Cu^{++} and Ni^{++}

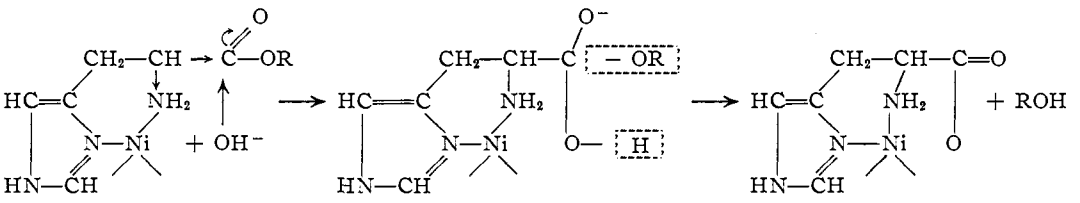
(12) R. B. Penland, S. Mizushima, C. Curran and J. V. Quagliano, This Journal, **79**, 1575 (1957).

complexes of glycine amide are only a little less stable than the corresponding metal complexes of glycylglycine, indicating that the coördinating sites of glycylglycine are probably the same as those of glycine amide. The carboxylate group in glycylglycinate therefore is probably not involved in the binding with these metal cations.

It is also seen in Table IV that the formation constants of the complexes of histidine methyl ester are about 1.5–2 log units less than the corresponding complexes of histidine. This difference in stability is about equal to the difference in stability between the cysteine and cysteine methyl ester complexes of Ni(II), and is much smaller than the difference, $\Delta \log k_1 = 3.5$, between the glycine and glycine ester complexes of Ni(II).[10] By comparing the difference in stability of cysteine and cysteine ester complexes with the corresponding difference between the glycine and glycine ester complexes, White, *et al.*,[10] came to the conclusion that the predominant binding sites in cysteine to Ni(II) ion are the amino group and sulfhydryl ion (carboxyl group not involved). Our present results on the difference in stability of histidine and histidine ester complexes therefore may be interpreted to mean that the predominant binding sites in histidine toward Cu(II), Ni(II) and uranyl ions are the imidazole and amino groups.

By comparing the formation constants of cadmium and copper(II) complexes of imidazole and 1-methylimidazole, Li, White and Doody[13] came to the conclusion that the binding site in imidazole is the "pyridine" nitrogen rather than the "pyrrole" nitrogen. From what has been said above regarding the binding sites in histidine, we may now point to the "pyridine" nitrogen of the imidazole group and the amino group as the coördination sites of histidine.

The data of Table V show that an increase in log k_1 of the metal complex of histidine methyl ester is accompanied by an increase in the specific rate constant, k_r. Part of the hydrolysis of the histidine ester in the presence of nickel ion probably proceeds as



The coördination with nickel of the ester results in an electron pull away from the carbon of the carbonyl group, thus facilitating attack by OH^-. It is of interest to point out that the rate constants for the alkaline hydrolysis of glycine ester and of histidine ester are about equal. However, while Ni^{++} catalyzes the rate of hydrolysis of glycine ester by 26%,[10] it increases the rate of hydrolysis of histidine ester by 250%. This large difference in the catalytic effects of Ni is probably because glycine ester is a monodentate ligand,[10] while the complex of histidine ester is probably a chelate. Cu(II) forms a more stable chelate with histidine ester than does Ni(II). The greater catalytic effect of Cu(II) on the hydrolysis of histidine ester then is as expected.

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