cluster of such side-chain groups. Inasmuch as it contains only the imidazole group free to take part in the reactions studied here, Cbz-PHG-NH_2 represents a model for the behavior of an individual imidazole group in a protein. It represents a model in this sense for the reaction with NPA and for the formation of the first complex with Cu(II) or Zn(II) ion. In the formation of the higher complexes secondary interactions appear to have a part, and it is unlikely that the values of $\mathbf{k}_2$, $\mathbf{k}_3$ or $\mathbf{k}_4$ give a useful indication of the behavior of a cluster of imidazole groups in a protein.

Since identical sequences of three amino acid residues appear to be repeated only rarely in the peptide chain of a protein,[21] we may expect that each such residue will have different neighbors and so will be subject to different inductive effects transmitted through its peptide bonds. Strictly speaking, therefore, this peptide derivative represents a good model for the reactivity of the imidazole group only in the sequence -prolyl-histidyl-glycyl-. What range of properties to attribute to the individual side-chain groups of a given class in a protein, and how to correlate one measure of reactivity with another, are questions that must await the study of more model systems of this type.

Acknowledgments.—The technical assistance of Miss Reta Roth and Miss Shirley Light is gratefully acknowledged. We are indebted to Dr. Barbara W. Low for making available atomic models and for advice in the construction of molecular models.

NEW YORK, NEW YORK

[21] F. Šorm, B. Keil, V. Holeyšovský, V. Knesslová, V. Kosta, P. Mäsiar, B. Meloun, O. Mikeš, V. Tomášek and J. Vaněček, *Coll. Czechoslov. Chem. Commun.*, **22**, 1310 (1957).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE AND THE BUREAU OF MEDICAL RESEARCH, EQUITABLE LIFE ASSURANCE SOCIETY OF THE UNITED STATES]

Coördination Complexes and Catalytic Properties of Proteins and Related Substances. III. Effect of Zinc and Cupric Ions on the Reaction of *p*-Nitrophenyl Acetate with Glycylglycine[1,2]

BY WALTER L. KOLTUN AND FRANK R. N. GURD

RECEIVED JULY 18, 1958

Acetylglycylglycine is formed when *p*-nitrophenyl acetate (NPA) reacts with glycylglycinate (GG^-). When GG^- is present in excess the reaction follows first-order kinetics and provides a convenient means of determining the concentration of GG^- in systems containing Zn(II) or Cu(II) ions. The technique is compatible with measurements of complex formation by determinations of *p*H. The logarithms of the first and second association constants for GG^- with Zn(II) are 3.30 and 2.78, respectively, at 25° and ionic strength 0.16. The corresponding value for the formation of the complex denoted as $CuGG^-_2$ is 3.07.

Introduction

The potential advantages of studying the reactivity of polar side-chain and terminal groups in proteins by a combination of techniques applied concurrently were outlined in the first paper of this series.[3] It was shown by measurements on the Zn(II)-imidazole and Cu(II)-imidazole systems that kinetic and equilibrium measurements yielded identical results over a wide range of conditions. The rate of splitting of *p*-nitrophenyl acetate (NPA) by free basic imidazole was followed spectrophotometrically,[4,5] and the equilibrium concentration of free basic imidazole (Im) was determined from measurement of *p*H.[6] Detectable interference of one method of measurement with the other was avoided by using relatively small amounts of NPA.

The object of the present study is to apply the same combination of techniques to a model compound representing the N-terminal α-amino group of a peptide chain. In this case the simplest model is a peptide itself. We have chosen glycylglycine, GG, as a model because its metal complexes have been studied in some detail.[7-10]

We have again made use of the rate of splitting of NPA, in this case to measure the concentration of the unbound anion, (GG^-). Although this reaction leads to the irreversible formation of N-acetylglycylglycine, $AcGG^-$, such small quantities of GG^- are consumed in the reaction that a detectable change in the over-all equilibrium of GG with metal ion is avoided.[8] The kinetic and equilibrium measurements on the Zn(II)-GG system are mutually confirmatory in the same sense as the previous studies on the systems containing imidazoles.[3,11] The combined measurements on the Cu(II)-GG system make possible some unambiguous conclusions confirming part of the general picture originally suggested by Dobbie and Kermack.[8]

Materials and Methods

Glycylglycine was "chromatographically pure," supplied by Mann Research Laboratories, Inc., New York, N. Y. Other reagents were of analytical grade prepared and

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[1] This investigation was supported by research grant No. H-2739 from the National Heart Institute, U. S. Public Health Service.

[2] Presented in part at the 133rd National Meeting, American Chemical Society, San Francisco, California, April 13-18, 1958.

[3] W. L. Koltun, R. N. Dexter, R. E. Clark and F. R. N. Gurd, THIS JOURNAL, **80**, 4188 (1958).

[4] M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1652 (1957).

[5] T. C. Bruice and G. L. Schmir, *ibid.*, **79**, 1663 (1957).

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

[7] N. C. Li and E. Doody, *ibid.*, **76**, 221 (1954).

[8] H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246 (1955).

[9] S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1117 (1956).

[10] M. Fried, F. R. N. Gurd and W. L. Koltun, in preparation.

[11] W. L. Koltun, R. E. Clark, R. N. Dexter, P. Katsoyannis and F. R. N. Gurd, THIS JOURNAL, **81**, 294 (1959).

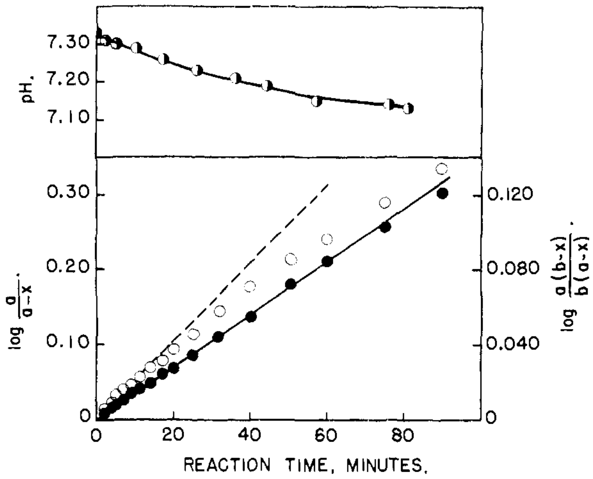

Fig. 1.—Reaction of NPA with GG^-: ◑, pH values; ○, first-order kinetics, left hand ordinate; ●, second-order kinetics, right hand ordinate.

standardized as described previously.[3] Acetylglycylglycine was a gift from Dr. Melvin Fried.

Kinetic and pH measurements were made as previously described.[3] The total volume of solution in each experiment after addition of NPA was usually 2.00 ml. These solutions were prepared with a calibrated Syringe Microburet, Model SB2, supplied by Micro-Metric Instrument Co., Cleveland, Ohio. Sufficient NaCl was added to bring the final ionic strength to 0.16. The 0.200 ml. of NPA solution was added from a micropipet which was rinsed out by drawing up the solution several times with the aid of a greased syringe connected to the micropipet by a short length of rubber tubing. Measurements in the Beckman Model DU Spectrophotometer were made with fused silica cells of internal dimensions 3 × 10 × 25 mm. supplied by Pyrocell Manufacturing Co., New York, N. Y. Glass covers normally were used. The pH measurements were made in a small jacketed vessel with Radiometer electrodes G222B and K130. The Spinco Model R Paper Electrophoresis apparatus was used. The ninhydrin reagent was composed of 0.2 g. of ninhydrin dissolved in a solution of 180 ml. of 1-butanol, 20 ml. of H_2O and 0.5 ml. of glacial acetic acid.

Results and Discussion

Reaction of GG^- with NPA.—The consumption of GG^- in the reaction was demonstrated by the ninhydrin test after paper electrophoresis of a reaction mixture containing initially 0.005 M NPA, 0.005 M total GG and borate buffer of pH 8.44 and ionic strength 0.135. After the reaction mixture had stood for 40 hr., 20 μl. was placed on a strip of Schleicher and Schüll No. 2043A paper in the Spinco Model R paper electrophoresis apparatus and run for 150 minutes at 2.5 milliamperes. A buffer containing 0.02 M sodium acetate and 0.002 M acetic acid (pH 5.63) was used. After spraying the dried paper with ninhydrin no color could be detected on the strip. A control strip bearing half as much GG as had been present initially in the reaction mixture showed on spraying a distinct yellow band near the point of application.

In order to identify the reaction product positively, 132 mg. of recrystallized GG was dissolved in 1 ml. of 1 M NaOH and added with strong stirring to 200 mg. of NPA dissolved in 3 ml. of 95% ethanol at about 40°. After 30 minutes the deep yellow mixture was acidified with 3 drops of 12 M HCl. The solution was evaporated to dryness under reduced pressure and acetylglycylglycine (AcGG) crystallized twice from 95% ethanol at 2°. The m.p. was 184-185°. The m.p. of an authentic sample was the same, as was the mixed m.p. (reported[12] 186–187°).

The results of a kinetic study of the reaction between GG^- and NPA are shown in Fig. 1. Here the initial concentration of GG^- was 0.00138 M, of NPA 0.00100 M and of ethanol 0.95%. The initial concentration of $GG^\pm$ was 0.00862 M and the pH 7.33. Part of the reaction mixture was observed in the pH vessel, part in the spectrophotometer using fused quartz inserts to reduce the light path to 1 mm. The upper part of Fig. 1 shows the course of the pH changes. The lower part shows the results of the spectrophotometric measurements interpreted according to first-order kinetics (open circles, left-hand ordinate) and second order kinetics (filled circles, right-hand ordinate). Each reading of the optical density at 400 mμ was corrected to the value corresponding to the initial pH, 7.33. For this purpose the value of pK' for p-nitrophenol of 7.12 was used.[3] That part of the optical density due to hydrolysis catalyzed by hydroxyl ions was then estimated from appropriate values of k_w and subtracted to give values corresponding to the reaction under study.[3] The resulting values were applied by standard methods to the expressions

$$2.303 \log \frac{a}{a-x} = k_1 t \qquad (1)$$

and

$$\frac{2.303}{(b-a)} \log \frac{a(b-x)}{b(a-x)} = k_2 t \qquad (2)$$

for first- and second-order processes, respectively. Here a represents the initial concentration of NPA, b that of GG^- and $(a - x)$ and $(b - x)$ the corresponding values at any time t. The first- and second-order rate constants are represented by k_1 and k_2, respectively.

Figure 1 shows that the first-order plot develops curvature at an early stage. By contrast, the lower curve in Fig. 1 shows that the second-order kinetics are followed satisfactorily for approximately one hour. The value of k_2 from equation 2 is 8.55 l. mole^{-1} min.$^{-1}$.

Implicit in the foregoing treatment is the assumption that $AcGG^-$ does not catalyze the splitting of NPA. This point was demonstrated directly by following the reaction in a pH 6.93 phosphate buffer of ionic strength 0.04 containing 0.005 M sodium acetylglycylglycinate.

Standardization of Kinetic Measurements of (GG^-).—In applying kinetic measurements to the determination of (GG^-) in the Zn(II)-GG and Cu(II)-GG systems, we have chosen to maintain the initial concentration of NPA much less than that of the GG^-. Under such conditions the reaction is pseudo first-order throughout much of its course and the initial slope of a first-order plot may be determined with certainty. This procedure has been followed throughout the rest of this study, so that the treatment of the kinetic data is formally identical to that used in the first two papers of this

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(12) M. Bergmann, V. du Vigneaud and L. Zervas, *Ber.*, **62**, 1912 (1929).

TABLE I

REACTION OF NPA WITH GLYCYLGLYCINATE IN THE PRESENCE OF Zn(II)

Composition: Initial total molar concn. of ZnCl$_2$	GG$^{\pm}$	NaOH	A. Kinetic results: $k_1 \times 10^2$, min.$^{-1}$	$-$log (GG$^-$)	$\bar{\nu}$	B. Equilibrium results: pH	$-$log (GG$^-$)	$\bar{\nu}$
0.0324	0.0324	0.0081	0.100	3.94	0.247	5.82	3.90	0.246
.0324	.0324	.0121	.179	3.69	.367	6.15	3.67	.367
.0162	.0324	.0081	.252	3.54	.482	6.26	3.50	.480
.0162	.0324	.0121	.476	3.27	.714	6.59	3.24	.715
.0162	.0324	.0162	.742	3.07	.947	6.89	3.04	.944
.0162	.0324	.0178	.809	3.04	1.04	6.98	2.96	1.04
.0162	.0324	.0211	1.17	2.87	1.22	7.26	2.81	1.21
.0162	.0648	.0263	2.68	2.51	1.44	7.04	2.51	1.43
.0162	.0648	.0316	4.56	2.28	1.63	7.28	2.38	1.69
.0162	.0648	.0369	5.60	2.19	1.88	7.40	2.10	1.97
.0162	.0648	.0422	9.64	1.96	1.92	7.82	1.97	1.94
.0081	.0648	.0263	8.87	1.94	2.00	7.51	2.03	2.08
.0081	.0648	.0369	17.4	1.70	2.11	7.98	1.70	2.12
.0081	.0648	.0422	23.1	1.58	1.95	8.19	1.58	1.86

series.[3,11] Values of k_2 were computed from the expression

$$k_2 = k_1/(\mathrm{GG}^-)$$

where $k_1 = k_{\mathrm{obsd}} - k_w$. Here k_{obsd} is the observed value of the first-order rate constant. Values of k_1 were determined using an initial concentration of NPA of 1.0×10^{-4} M and concentrations of GG$^-$ between 0.00154 and 0.00969 M. The mean value of k_2 from 11 determinations of k_1 was 8.77 l. mole^{-1} with an average deviation of 0.44 l. mole^{-1} min.$^{-1}$. In all cases the initial slope was maintained for at least 50% of the reaction. Even in the far less favorable case illustrated in Fig. 1 the initial slope of the first-order plot (broken line) yielded a value of k_2 of 8.69 l. mole^{-1} min.$^{-1}$. These values are in satisfactory agreement with the value of 8.55 l. mole^{-1} min.$^{-1}$ computed directly from the second-order kinetic treatment.

Measurement of Free Glycylglycinate in the Presence of Zn(II) Ions.—Solutions were prepared by mixing standard solutions of GG$^{\pm}$, ZnCl$_2$ and NaOH with sufficient NaCl to give a final ionic strength, of 0.16. The general procedure was identical to that used previously,[3] except that solutions were made up to a total volume of 2.00 ml. The pH values of the experimental solutions after completion of the kinetic measurements were practically identical to those of control solutions from which NPA had been omitted. The average deviation of pairs of pH readings was slightly over 0.01 unit. The logarithm of the association constant for hydrogen ion with the α-amino group of GG$^-$ under these conditions was found by titration to be 8.14 ± 0.01.

Table I lists the composition of the solutions employed, kinetic results (A) and equilibrium results (B). Values of $-$log (GG$^-$) computed[3] from the separate measurements are listed in appropriate columns and may be seen to be in reasonably good agreement. Clearly the two methods of measurement are mutually compatible for the Zn(II)-system, as they have been shown to be for the Zn(II)-imidazole and Cu(II)-imidazole systems.[3,11] The results show no evidence that the Zn(II)-GG complexes themselves have any tendency to react with NPA.

Computation of Successive Association Constants for the Zn(II)-Glycylglycinate System.—In Fig. 2 are plotted values of $\bar{\nu}$ against $-$log GG$^-$ taken from Table I. Values derived from kinetic measurements are shown in open circles and those from equilibrium measurements in closed circles. The curve is computed for $\mathbf{k}_1 = 2.00 \times 10^3$ and $\mathbf{k}_2 = 6.00 \times 10^2$ (log $\mathbf{k}_1$ = 3.30; log $\mathbf{k}_2$ = 2.78). The computed curve gives a satisfactory fit at least up to $\bar{\nu} = 1.8$. This indicates that if a third step occurs, $\mathbf{k}_3$ must be distinctly smaller than $\mathbf{k}_1$ and $\mathbf{k}_2$. Similar conclusions may be drawn from the conductometric studies of Li and Doody.[8] Flood and Lorås found that the Zn(II)-glycine system behaved similarly, and listed values corresponding to log $\mathbf{k}_1$, log $\mathbf{k}_2$ and log $\mathbf{k}_3$ of 4.80, 4.14 and 2.54, respectively.[13] These authors consider the third constant least accurate, but from their published observations it seems unlikely that it is seriously in error. The present results do not rule out a parallel value for log $\mathbf{k}_3$ in the

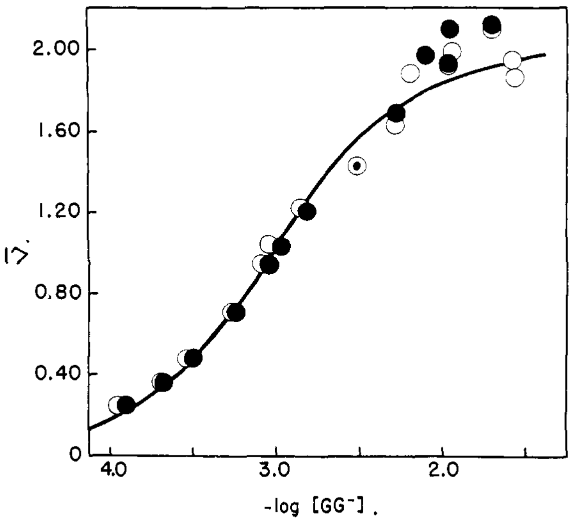

Fig. 2.—Plot of $\bar{\nu}$ *vs.* $-$ log (GG$^-$) for Zn(II)-glycylglycinate. Values derived from kinetic measurements, ○; from equilibrium measurements, ●.

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[13] H. Flood and V. Lorås, *Tidsskr. Kjemi Bergvesen Met.*, **5**, 83 (1945).

GG system of, say, 1.0–1.3. Such low constants are not accessible to accurate measurement by the present combination of techniques.

Although the values of $\mathbf{k}_1$ and $\mathbf{k}_2$ for the Zn(II)–GG system are distinctly smaller than those for the Zn(II)–glycine system, it seems safe to conclude that both systems contain chelate complexes. Otherwise the characteristic sequence of four steps with comparable values of the equilibrium constants[14] are to be expected. In both cases, moreover, the association constants are greater than the corresponding constants for ammonia[15] or imidazole.[3]

Cu(II)-Complexes of Glycylglycine.—Dobbie and Kermack[8] discovered that the first step in complex formation between Cu(II) ions and GG^-

$$Cu^{++} + GG^- \overset{\mathbf{k}_{I,+}}{\rightleftharpoons} CuGG^+ \tag{4}$$

is followed by the displacement of a hydrogen ion from the peptide bond, an interpretation since substantiated by the use of structural analogs.[9,16,17]

$$CuGG^+ \overset{\mathbf{k}_{I,0}}{\rightleftharpoons} CuGG + H^+ \tag{5}$$

Detailed information about the formation of the second complex, CuGG, may be found elsewhere.[8–10,18] Under the conditions of the present study log $\mathbf{k}_{I,+}$ is found to be 4.96 and $p\mathbf{k}_{I,0}$ is 3.90.[10] The magnitudes of these constants mean that the equilibrium expressed by equation 6 is

$$CuGG + GG^- \overset{\mathbf{k}_{II,-}}{\rightleftharpoons} CuGG_2^- \tag{6}$$

accessible to measurement without complications due to the steps described in equations 4 and 5.

Formation of $CuGG_2^-$.—A series of solutions of the compositions listed in Table II were studied.

TABLE II

REACTION OF NPA WITH GLYCYLGLYCINATE IN THE PRESENCE OF Cu(II)

Composition: Initial total molar concn. of CuCl$_2$	GG$^\pm$	NaOH	Equilibrium results: pH	$-\log$ (GG$^-$)	Kinetic results: $k_1 \times 10^2$	$-\log$ (GG$^-$)	log $\mathbf{k}_{II,-}$
0.0100	0.0162	0.0249	7.92	3.11	0.597	3.17	3.06
.0100	.0202	.0228	6.69	3.58	.220	3.60	3.21
.0100	.0202	.0228	6.78	3.49	.265	3.52	3.15
.0100	.0202	.0228	6.78	3.49	.279	3.50	3.04
.0100	.0202	.0249	7.24	3.18	.598	3.17	3.01
.00975	.0197	.0243	7.24	3.19	.600	3.17	2.98
.0100	.0202	.0249	7.23	3.19	.531	3.22	3.16
.0100	.0202	.0260	7.55	3.03	.804	3.04	3.16
.00975	.0197	.0263	7.87	2.77	1.22	2.86	3.06
.0100	.0202	.0270	7.94	2.71	1.44	2.79	2.96
.0100	.0202	.0291	8.54	2.56	2.20	2.60	2.91
.0100	.0202	.0291	8.60	2.50	2.14	2.61	2.96
.0100	.0248	.0270	7.35	2.90	1.05	2.92	3.14
.0100	.0248	.0291	7.75	2.63	1.84	2.68	3.16
.0100	.0248	.0312	8.03	2.55	2.73	2.51	3.04
.0100	.0248	.0333	8.53	2.21	3.80	2.36	3.19
						Av. log $\mathbf{k}_{II,-}$ =	3.07

The conditions were chosen to maintain the pH between 6.6 and 8.6 to avoid the presence of significant quantities of $CuGG^+$ (equation 5) and of basic complexes such as $CuGGOH^-$ (equation 7; $p\mathbf{k}_{I,-} > 9$).[8,10] Assuming that the concentrations

$$CuGG \overset{\mathbf{k}_{I,-}}{\rightleftharpoons} CuGGOH^- + H^+ \tag{7}$$

of these two species may be neglected, (GG^-) may be computed from the equilibrium pH measurements using equations 8-11 combined to give equation 12

$$(\text{Total Cu}) = (CuGG) + (CuGG_2^-) \tag{8}$$

$$(\text{Total GG}) = (GG^-) + (GG^\pm) + (CuGG) + 2(CuGG_2^-) \tag{9}$$

$$(\text{Total NaOH}) = (GG^-) + 2(CuGG) + 3(CuGG_2^-) \tag{10}$$

$$pH = \log \mathbf{k}'_{GG^\pm} + \log (GG^-)/(GG^\pm) \tag{11}$$

$$(GG^-) = \frac{(\text{Total Cu}) + (\text{Total GG}) - (\text{Total NaOH})}{\mathbf{k}'_{GG^\pm}(H^+)} \tag{12}$$

The values in the first four columns of Table II, together with the separately determined value of 8.14 for log $\mathbf{k}'_{GG^\pm}$, yield the values of $-\log (GG^-)$ listed in column 5. The results of the kinetic measurements are listed as k_1 in column 6 and the corresponding values of $-\log(GG^-)$ in column 7. The agreement between the equilibrium and kinetic methods is shown in columns 5 and 7. The last column shows values of log $\mathbf{k}_{II,-}$ computed from the values in columns 1, 2 and 7 used in conjunction with equations 6, 8, 9 and 11. The average value of log $\mathbf{k}_{II,-}$ is 3.07. This value obtained at 25° and ionic strength 0.16 is in satisfactory agreement with the value of 3.26 reported by Dobbie and Kermack at 20° and at a lower and uncontrolled ionic strength.[8] The present method of determining $\mathbf{k}_{II,-}$ draws on both kinetic and pH measurements and avoids such heavy reliance on the pH measurements alone.

The foregoing computations of $\mathbf{k}_{II,-}$ from kinetic measurements involve calculations of (CuGG) and $(CuGG_2^-)$ without making use of the known quantities of NaOH added (column 3). The values of (GG^-), (CuGG) and $(CuGG_2^-)$ drawn from the above computations may be introduced into equation 10 to yield computed values of the concentration of total NaOH added. Such computed values were found to agree with the experimental quantities within less than 1% on the average, and in no case did the difference exceed 2.3%.

The self-consistency of the computation of $\mathbf{k}_{II,-}$ from kinetic measurements, the agreement between the kinetic and equilibrium measurements of (GG^-), and the agreement between the experimental and calculated quantities of total NaOH added all confirm the picture of successive equilibria described by equations 4-6.

Dobbie and Kermack also added support to their original interpretation of pH measurements by making supplementary measurements, in their case, with the copper electrode.[8] This served best to substantiate their interpretation in terms of the processes described in equations 4 and 5. The

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[14] F. R. N. Gurd and P. E. Wilcox, *Adv. in Protein Chem.*, **11**, 311 (1956).

[15] J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," Haase and Son, Copenhagen, 1941.

[16] M. Fried and F. R. N. Gurd, Abst. 132nd National Meeting, American Chemical Society, New York, N. Y., Sept. 8–13, 1957, p. 29C.

[17] The notation for the equilibrium constants designates the species formed by the subscripts; the roman numeral shows the number of molecules of ligand combined with the metal ion, and +, − or 0 represents the charge borne by the complex. For the ligand alone the association constant for the combination of a proton with the α-amino group is designated by $\mathbf{k}'_{GG^\pm}$.

[18] B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).

present technique appears to be better suited for interpreting unambiguously the subsequent step in complex formation.

The reaction of NPA with the basic form of the α-amino group of a peptide provides both a measure of the reactivity of the group and a means of modifying it by acetylation. The reaction should be able to supply valuable information about molecules containing reactive groups belonging to both the categories represented by the α-amino group and the imidazole group. With excess NPA the former class should be converted to the subsequently unreactive acetylated form. If such a flexible use of the NPA technique were not suitable for a particular investigation, presumably a purely catalytic technique could be used. Speck and Forist[19] have shown that cupric ion inhibits the glycinate-catalyzed dealdolization of diacetone alcohol.

Although CuGG does not react with NPA at a measurable rate, conversion to basic complexes of the type of $CuGGOH^-$ leads to rapid splitting of the NPA. In a subsequent paper this finding will be described in detail, together with other observations on complexes of peptides with Cu(II) ion.

Acknowledgments.—We wish to thank Dr. Melvin Fried for the gift of acetylglycylglycine and for helpful discussions. The technical assistance of Miss Reta Roth and Miss Shirley Light is gratefully acknowledged.

[19] J. C. Speck, Jr., and A. A. Forist, THIS JOURNAL, **79**, 4659 (1957).

NEW YORK, N. Y.

[CONTRIBUTIONS FROM THE APPLIED SCIENCE LABORATORY AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CINCINNATI]

The Stability of Platinum–Styrene Complexes

BY JOHN R. JOY AND MILTON ORCHIN

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The reaction of styrene with ethylene platinous chloride was used to prepare a series of substituted styrene–platinum complexes of the general formula $(X—C_6H_4CH{=}CH_2PtCl_2)_2$, where X = H, 3-Cl, 3-$CH_3O$, 4-$CH_3O$, 3-$NO_2$, 4-$NO_2$ and 4-CH_3. The analogous 1-dodecene complex also was prepared. When dissolved in alcoholic hydrogen chloride the dimeric complex splits into an ionic one, *e.g.*, $(C_{12}H_{24}PtCl_2)_2 + 2HCl \rightarrow 2H(C_{12}H_{24}PtCl_3)$. The addition of styrene to this system results in the equilibrium: $X—C_6H_4CH{=}CH_2 + (C_{12}H_{24}PtCl_3)^- \rightleftarrows (X—C_6H_4CH{=}CH_2PtCl_3)^- + C_{12}H_{24}$. The equilibrium constant for this reaction (starting from either side) was evaluated by means of ultraviolet spectrophotometry. When this constant was plotted against the Hammett sigma values for the various substituents a U-shaped curve resulted. All substituents were stabilizing relative to styrene. This fact is consistent with the presence of a double bond between the platinum and the olefinic ligand, the sigma and pi bonds being affected approximately equally but in opposite directions by the substituent.

Introduction and Background

The Structure of Platinum–Olefin Complexes.—A study of platinum–olefin coördination complexes was undertaken in an effort to obtain quantitative data on the factors affecting the stability of the metal–olefin bond. The complexes under consideration are of two types, ionic complexes, such as Zeise's salt,[1] $K(C_2H_4PtCl_3)$, and non-ionic, dimeric complexes, such as ethylene platinous chloride,[2] $(C_2H_4PtCl_2)_2$.

The concept of the bonding between the metal and the olefin as developed by Dewar[3] to describe the silver–olefin complexes was extended by Chatt and Duncanson[4] to the platinum compounds. The platinum ion is divalent; its complexes are square planar and involve $5d6s6p^2$ hybrid orbitals of platinum. In both the ionic and non-ionic complexes the olefin is oriented perpendicularly to the plane of the complex. The bonding pi-electrons of the olefin overlap an unoccupied $5d6s6p^2$ hybrid orbital of platinum, forming a sigma-bond. The platinum in turn donates a pair of electrons in a 5d6p hybrid orbital into the empty anti-bonding pi-orbital of the olefin, forming a pi-bond. The double bond which results frequently has been called a dative double bond.

Baenziger, Dempsey and Holden[5,6] showed, by means of X-ray studies on the analogous palladium complexes, that the dimeric compounds have a bridged structure with two chloride ions linking the two central atoms. This is represented by the structure I, where Un represents any coördinating olefin.[7]

Evaluation of Relative Stabilities.—One of the most striking reactions of the platinum–ethylene complexes is the rapid displacement of ethylene by another less volatile olefin and the formation of a new complex

$$Un + (C_2H_4PtCl_3)^- \longrightarrow (UnPtCl_3)^- + C_2H \quad (1)$$

$$2Un + (C_2H_4PtCl_2)_2 \longrightarrow (UnPtCl_2)_2 + 2C_2H_4 \quad (2)$$

Anderson[2] used these reactions to prepare complexes of various olefins including that with styrene.

It appeared reasonable that were a non-volatile olefin reacting with the complex of another high-

[1] Zeise, *Pogg. Ann.*, **9**, 632 (1827).

[2] J. S. Anderson, *J. Chem. Soc.*, 1042 (1936).

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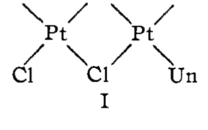
[7] The systematic name for this structure, when Un is ethylene, is 1,3-bis-(ethylene)-2,4-dichloro-μ-dichlorodiplatinum. The systematic name for the ionic form, $K(C_2H_4PtCl_3)$, is potassium ethylenetrichloroplatinate(II).



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