the vapor absorption spectrum. Light has given a vibrational analysis of transition (c) in the vapor in which he has concluded that excited state vibrations of 1110 and 662 cm.$^{-1}$ appear in the spectrum. The vibrational analysis of the crystal absorption spectrum is not in agreement with this, since vibrations of 457, 807, 1006 and 1091 were found. As is seen in Table V, a correspondence between many of the bands in transition (c) may be found by comparing vapor and crystal absorption spectra, so that the analysis which is proposed in this work is not inconsistent with the bands observed in the vapor spectrum.

Seshan[4] has studied transition (d) in the vapor and has found two sets of equally spaced bands, each having a 450 cm.$^{-1}$ spacing with a 230 cm.$^{-1}$ separation between corresponding members of the two sets. These bands are represented by the formula $\nu = 31780 + 450n - 230p$. The crystal absorption bands in this region are much more diffuse than the vapor absorption bands, and it is therefore difficult to state whether the 540 cm.$^{-1}$ interval deduced from the vibrational analysis of the crystal spectrum corresponds to the 450 cm.$^{-1}$ interval in the vapor spectrum.

Seshan has also found a series of very broad bands between 41000 and 45000 cm.$^{-1}$ with an average spacing of 450 cm.$^{-1}$. These bands correspond to the intense transition (ν_{max} 41000, ϵ_{max} 20900), which must therefore be π-π^* in nature.

A complete vibrational assignment for *p*-benzoquinone would be of considerable assistance in correlating vibrations in the ground and in the excited electronic states as has been done for biacetyl.[9] A more detailed interpretation of the vibrational structure of the absorption transitions must therefore await further work on this molecule.

Acknowledgments.—I am grateful to Dr. S. Nagakura and to Prof. J. R. Platt for helpful discussions concerning the assignment of the electronic transitions. The interest of Prof. D. S. McClure and Prof. A. B. F. Duncan is also acknowledged.

ROCHESTER 20, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY]

Metal Interaction with Sulfur-containing Amino Acids. II. Nickel and Copper(II) Complexes[1]

BY JAMES M. WHITE, RICHARD A. MANNING AND NORMAN C. LI

RECEIVED DECEMBER 30, 1955

The formation constants of the nickel(II) complexes of cysteine, glycine and cysteine esters have been measured and compared. The data indicate that the predominant binding sites in cysteine to nickel ion are the amino group and sulfhydryl ion. This interpretation is supported by the identical absorption spectra of the nickel(II) complexes of cysteine and cysteine ester. The rates of alkaline hydrolysis of glycine and cysteine esters have been determined in the absence and presence of metal ions and it was found that in every case an increase in the stability of metal–ester complex is accompanied by an increase in the bimolecular rate constant. The copper(II) complex of oxidized glutathione (GSSG) has been investigated polarographically in the region +0.01 to −0.01 v. (S.C.E.). GSSG forms only a 1:1 complex with copper ion: log k_1 = 14.6. This value is close to the log k_1k_2 value (15.1) of the copper complex of glycine and suggests that the binding sites in the GSSG complex are the two amino and α-carboxylate groups.

As part of a program of investigation of metal interaction with sulfur-containing amino acids,[2] studies were carried out to determine the sites of binding in cysteine to nickel ion. The rates of alkaline hydrolysis of glycine and cysteine esters in the absence and presence of various metal ions were investigated in order to determine the relationship between the stability of the metal–ester complexes and the bimolecular rate constants.

Li, Gawron and Bascuas[3] have suggested that in the zinc ion complex of oxidized glutathione (GSSG), the binding sites in GSSG are probably the two amino and α-carboxylate groups. Their postulated structure for the complex is based on a comparison of the value, log k_1 = 7.22, for this complex with the values, log k_1k_2 = 9.96 and log k_1k_2 = 8.10, for the zinc complexes of glycine and valine, respectively. Since there are such big differences in the values of these constants, we considered it worthwhile to study the GSSG complex with another metal ion; the copper(II) complex was investigated polarographically.

(1) This investigation was supported by research grants from the National Science Foundation and the American Philosophical Society.

(2) N. C. Li and R. A. Manning, THIS JOURNAL, **77**, 5225 (1955).

(3) N. C. Li, O. Gawron and G. Bascuas, *ibid.*, **76**, 225 (1954).

Experimental

Materials.—Cysteine methyl ester hydrochloride was prepared and purified in the manner described previously.[2] Oxidized glutathione, a Schwarz product, was dried *in vacuo* at 56° to constant weight. All other chemicals were C.P. reagent grade products. Stock solutions of copper(II) nitrate were analyzed by addition of excess KI and titration of the liberated iodine, stock solutions of nickel nitrate by precipitation with dimethylglyoxime.

Aqueous solutions were prepared from oxygen-free water and all measurements involving sulfur-containing compounds were carried out under an atmosphere of nitrogen. Only freshly prepared stock solutions of these were used.

Apparatus and Procedure.—Polarograms were obtained with a Sargent Recording Polarograph, Model XXI. Two potential measurements were made with an external potentiometer, after stopping the instrument, at points on the wave before and after half-wave potential. The half-wave potential was corrected for iR drop, using the minimum cell resistance value measured with a 60 cycle a.c. conductivity bridge. The characteristics of the capillary were: m = 2.33 mg. sec.$^{-1}$, t = 3.74 sec. (open circuit), at a height of the mercury column of 50 cm.

Measurements of *p*H were made in the apparatus and in the manner previously described.[2] Spectrophotometric

measurements were made with a Beckman quartz spectrophotometer, Model DU.

The rates of alkaline hydrolysis of glycine and cysteine esters in the absence and presence of various metal ions at 25.0° were measured by the conductivity method described by Daniels, *et al.*[4]

Results

Table I gives a summary of the formation constants of the nickel complexes obtained at an ionic strength of 0.15 and 25°. These were determined by the *p*H method of Bjerrum.[5] For the glycine ester complexes there are indications that higher order complexes exist. However, only values of log k_1 and log k_2 are recorded, because of hydrolysis of the ester at higher *p*H values.

TABLE I

FORMATION CONSTANTS OF NICKEL(II) COMPLEXES, 25°

	log k_1	log k_2
Cysteine	10.48	9.31
Cysteine methyl ester	8.95	8.45
Glycine	5.97	4.95
Glycine ethyl ester	2.49	2.09

Experiments using calcium nitrate were also carried out. When solutions containing cysteine or cysteine methyl ester were titrated with an alkali in the presence and absence of calcium ion, the curves obtained were identical. These results show that the interaction between calcium ion and sulfur-containing amino acids is weak.

Stricks and Kolthoff[6] report that oxidized glutathione (GSSG) is reduced at the dropping mercury electrode and that the polarographic waves exhibit different degrees of irreversibility depending on the *p*H of the solution. We have obtained many polarograms for mixtures of $Cu(NO_3)_2$ (10^{-4} to 10^{-5} *M*) and GSSG and found that under the experimental conditions listed in Table II, the copper(II) ion is reduced reversibly at the dropping mercury electrode without the interference of the GSSG reduction wave. In Table II the following symbols are used

T_{GSSG} = total concn. of GSSG in soln.

A = total concn. of free chelating agent in soln. The chelating agent is A^{-4}, the anion of GSSG and the concn. is calcd. by means of eq. 21 in ref. 3.

A plot of $E_{1/2}$ *vs.* $-\log A$ yields a straight line with slope 0.030. This means that GSSG forms only a 1:1 complex with copper(II) ion. The values of log k, where $k = (CuA^{-2})/(Cu^{+2})(A^{-4})$, are calculated by means of the equation[2]

$$(E_{1/2})_c - (E_{1/2})_s = -0.0296 \log k - 0.0296 \log A$$

and are listed in Table II.

Results of spectrophotometric studies on the nickel(II) complexes of cysteine and cysteine methyl ester are presented in Fig. 1.

For each of the experiments on the rates of alkaline hydrolysis of the amino acid esters, the initial concentrations of amino acid ester anion, sodium hydroxide and divalent cation (when present) were always in the ratio of 1:1:1/3. Results of some typical experiments are shown in Fig. 2. From the lines 1a, 2a, 3a, it is seen that the alkaline hydrolysis is a second-order reaction. The specific rate constants are calculated from the slopes, s, of the lines and the initial concentrations, a, of the amino acid esters, according to the equation,[4] $s = (l/k)a$. Table III, column 5, summarizes the specific rate constants obtained at 25.0°. The formation constants of the ester complexes together with the values of the corresponding amino acid complexes are included for comparison.

TABLE II

POLAROGRAPHIC RESULTS OF COPPER-OXIDIZED GLUTATHIONE MIXTURES, 25°

Each solution contains 5×10^{-5} *M* $Cu(NO_3)_2$; (KOH) equal to 1/3 T_{GSSG}; sufficient KNO_3 to keep ionic strength of 0.15.

T_{GSSG}, *M*	$-E_{1/2}$(S.C.E.)	$E_{3/4}-E_{1/4}$	*p*H	$-\log A$	log k
0.0060	0.0114	0.030	3.17	13.73	14.63
.0033	.0074	.033	3.24	13.85	14.61
.0030	.0072	.034	3.21	13.95	14.70
.0023	.0038	.033	3.26	13.97	14.61
.0010	− .0004	.030	3.36	14.13	14.62
.0000	− .0146				

Ave. 14.64

TABLE III

FORMATION CONSTANTS *vs.* RATE CONSTANTS, 25°

Ester	M^{++}	log k_1k_2 (amino acid)	log k_1 (ester)	k_r, l. mole^{-1} sec.$^{-1}$
Glycine ethyl	None			0.73
ester	Ni^{++}	10.92	2.49	.92
			2.45 (methyl ester)	
	Cu^{++}	15.10	3.83 (methyl ester)	1.32
Cysteine methyl	None			0.31
ester	Ni^{++}	19.79	8.95	2.28
	Ca^{++}	[a]	[a]	0.39

[a] Very slight complex formation.

Discussion

The log k_1k_2 value of nickel complex of glycine is 6.34 units larger than that of glycine ethyl ester. This indicates that the carboxylate ion contributes appreciably to the stability of the glycine complex. Klotz, *et al.*,[7] compared the absorption spectra of copper ion complexes with glycine and with glycine ethyl ester and concluded that practically no cupric ion is bound to the ester complex. Actually the formation constants of the nickel and copper(II) complexes of glycine esters (see Tables I and III) are of the order of the values for the corresponding metal ion complexes of ammonia,[5] so that we may infer that glycine ester coördinates to the metal ion through the amino group only. We would expect therefore that, while the copper(II) glycine complex is of the MA_2 type, more than two glycine ester molecules can coördinate to each cupric ion. This has been observed, although the exact number of ester molecules in the complex cannot be ascertained on account of ester hydrolysis at higher *p*H.

(4) F. Daniels, J. H. Mathews, J. W. Williams, P. Bender, G. W. Murphy and R. A. Alberty, "Experimental Physical Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1949, p. 140.

(5) J. Bjerrum, "Metal-Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(6) W. Stricks and I. M. Kolthoff, THIS JOURNAL, **74**, 4646 (1952).

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

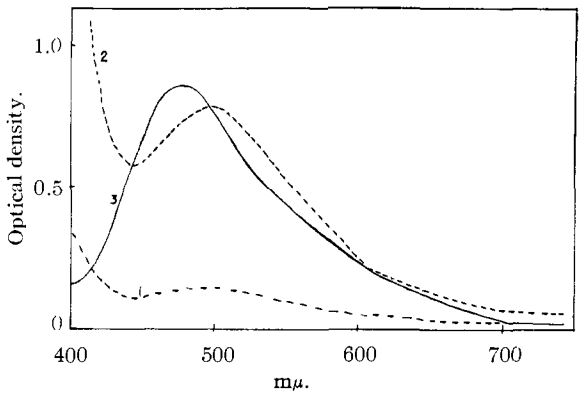


Fig. 1.—Absorption spectra of nickel chelates of cysteine methyl ester. In the following the first number indicates the molar ratio of cysteine methyl ester to total nickel; the second is the *p*H: curve (1). 0.2, 5.66; curve (2), 1.0, 5.79; curve (3), 2.0, 8.36. A 1:8 Ni^{++} cysteine methyl ester soln., *p*H 9.53, gives a curve identical with curve (3); similarly for a 1:2.3 Ni^{++}–cysteine soln., *p*H 11.27.

On the other hand, the log k_1k_2 of the nickel complex of cysteine is only 2.39 units larger than that of cysteine methyl ester. In the case of zinc complexes, Li and Manning[2] found that log k_1k_2 of cysteine complex is 2.46 units larger than that of cysteine methyl ester and concluded that the sites of binding in cysteine to zinc are the amino group and sulfhydryl ion. Our data on the formation constants of the nickel complexes of cysteine and cysteine methyl ester therefore indicate that the predominant binding sites in cysteine to nickel ion are also the amino group and sulfhydryl ion.

From Fig. 1 it is seen that curves (1) and (2) exhibit the same general contour and the same peak and minimum. Within experimental errors, the two curves differ only because of mass action. Since the spectra for the 1:8 nickel–cysteine methyl ester mixture coincide with curve 3 (1:2 mixture), it can be concluded that the highest order complex is 1:2, the same as for the cysteine complex. The 1:2.3 nickel–cysteine curve also follows curve 3 closely. This indicates that the sites of binding in cysteine complexes are the same as in cysteine ester complexes. The carboxylate ion in cysteine, therefore, cannot be appreciably involved in the binding with nickel ion.

The value of log k (14.64) for the copper(II) complex of oxidized glutathione is close to the value, log k_1k_2 = 15.10, for the copper complex of glycine. Since it is known that in the latter complex, the two amino and two α-carboxylate groups in the *two* glycinate ions coördinate to one cupric ion, it is reasonable to suggest that in the oxidized glutathione complex, the two amino and two α-carboxylate groups in *one* GSSG anion coördinate to one cupric ion. This is supported by the finding that copper forms only a 1:1 complex with oxidized glutathione.

Cystine is similar to oxidized glutathione in that it also contains a disulfide linkage, as well as two amino and two α-carboxylate groups in the same molecule. It would be of interest to determine whether the sites of binding in the cystine complex are the same as in the oxidized glutathione com-

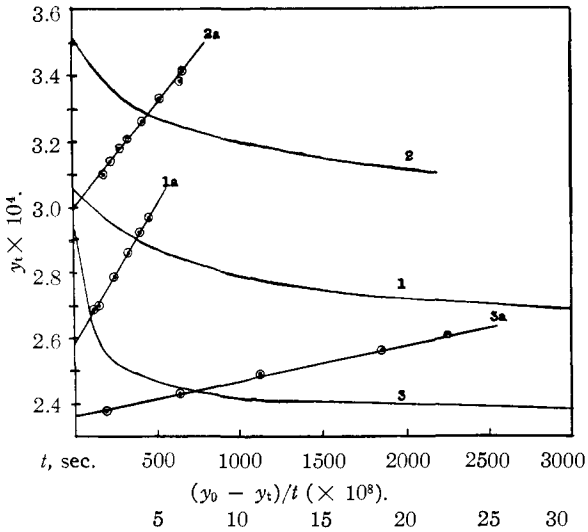
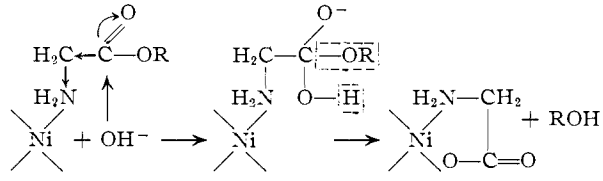


Fig. 2.—Alkaline hydrolysis of cysteine methyl ester (ester concn., 0.00382 *M*): Curves 1, 2, 3 are plots of y_t *vs.* *t* for hydrolysis of cysteine methyl ester in the presence of: no divalent cation, Ca^{++}, Ni^{++}, respectively. Lines 1a, 2a, 3a are the corresponding plots of y_t *vs.* $(y_0 - y_t)/t$. y_t and y_0 are the conductances at times *t* and 0, respectively.

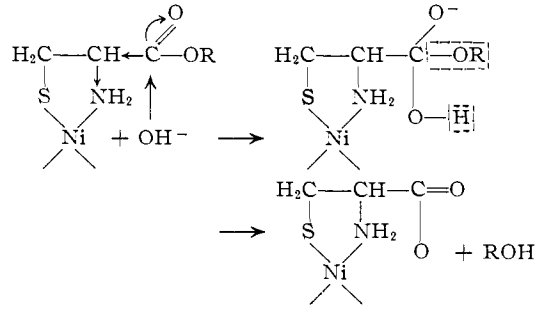
plex. Unfortunately no quantitative formation constant studies can be carried out for the metal–cystine complexes, because of precipitation. We have however carried out extensive potentiometric and conductometric titrations of cystinate with zinc and with copper(II) ions in the manner described by Li and Doody,[8] and in each case one break only occurs in the curve at the ratio cystinate: M^{++} = 1:1. These data indicate that cystine, like oxidized glutathione, also forms a 1:1 complex only with metal ion.

From Table III it is seen that an increase in log k_1 (ester) is accompanied by an increase in the specific rate constant, k_r. Part of the hydrolysis of the amino acid esters in the presence of nickel ion probably proceed as

Glycine ester



Cysteine ester



The coördination with nickel of the amino group in

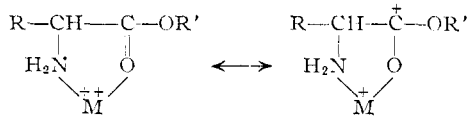
(8) N. C. Li and E. Doody, THIS JOURNAL, **76**, 225 (1954).

glycine ester results in an electron pull away from the carbon of the carbonyl group, thus facilitating attack by OH^-. The cupric complex of glycine ester is more stable than the nickel complex, resulting in a higher specific rate constant, as is seen in Table III. The values of log k_1 of the glycine ester complexes are about the same as those of the corresponding ammonia complexes, and for this reason we have not drawn the glycine ester complex as a chelate.

On the other hand, we have shown that the nickel complex of cysteine ester is probably a chelate, with the amino group and sulfhydryl ion as the predominant binding sites. On comparing the thermodynamic and kinetic data on the glycine and cysteine esters, it may seem strange at first that the specific rate constants for the alkaline hydrolysis of cysteine ester, in the presence of nickel ion, is not larger than 2.28 l.-mole^{-1} sec.$^{-1}$. If one remembers however that the net charges for the NiA_2 complexes are +2 and 0 for glycine ester and cysteine ester, respectively, then it is reasonable to assume that the net charge on the complex would facilitate the alkaline hydrolysis of glycine ester, but not cysteine ester.

The slower hydrolysis of cysteine ester, relative to glycine ester, both esters in the absence of divalent cation, is likewise explained. The negative charge of the cysteine ester anion inhibits the attack by OH^-, whereas no such inhibiting factor exists for the uncharged glycine ester. Calcium ion does not form complex with cysteine ester, and the data show that calcium does not catalyze the hydrolysis of the ester.

According to Kroll,[9] the actual intermediate complex required for the hydrolysis of amino acid esters is



He studied the cobalt induced hydrolysis of methyl esters of glycine and cysteine at *p*H 7.9 (Co^{++}, 0.016 *M*; ester concn., 0.016 *M*) and reported the following specific rate constants: $k_{\text{glycine ester}}$ = 0.0262 sec.$^{-1}$ at 25°, $k_{\text{glycine ester}}$ = 0.0156 sec.$^{-1}$ at 25.4°, $k_{\text{cysteine ester}}$ = 0.0121 at 25°. The inhibition of ester hydrolysis for cysteine ester was attributed to the strong competition of sulfhydryl ion for the metal ion, so that the binding of the carbonyl oxygen to the metal ion is decreased. In these experiments Kroll used 0.14 *M* tris-(hydroxymethyl)-aminoethane as buffer (ester concn., 0.016 *M*) and did not take into account the interaction between the metal ion and the buffer constituent.

Benesch and Benesch[10] found that the reactivity of tris toward silver is of the same order as that of ammonia. We have found that the formation constants of copper(II) and nickel complexes with tris and with glycine esters are also of the same order of the values for the corresponding metal complexes of ammonia. In the light of these observations, Kroll's conclusion about the inhibition of cysteine ester hydrolysis is subject to reservations.

Acknowledgments.—The authors greatly appreciate support of the National Science Foundation and the American Philosophical Society which made this work possible.

(9) H. Kroll, THIS JOURNAL, **74**, 2036 (1952).

(10) R. E. Benesch and R. Benesch, *ibid.*, **77**, 2749 (1955).

PITTSBURGH, PA.

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, TEXAS SOUTHERN UNIVERSITY]

Interaction of Palladium(II) Nitrate and of Palladium(II) Sulfate Solutions with 1,2,3-Benzotriazole[1]

BY RAY F. WILSON, LOUBERTA E. WILSON AND LAWRENCE J. BAYE

RECEIVED JANUARY 16, 1956

A quantitative study of the reaction of palladium(II) sulfate and of palladium(II) nitrate solutions with 1,2,3-benzotriazole has been made. In these reactions, precipitation tests and elemental analyses showed that two palladium substitution compounds and two palladium substitution, coördination compounds were formed.

In a previous study, the preparation of two palladium coördination compounds that are formed in the interaction of palladium(II) chloride with 1,2,3-benzotriazole has been reported.[2] Palladium(II) chloride has been determined both gravimetrically and amperometrically using 1,2,3-benzotriazole as a precipitant.[3]

The purpose of the present study was to investigate the interaction of palladium(II) nitrate and of palladium(II) sulfate systems with 1,2,3-benzotriazole in an attempt to elucidate the chemistry of the reactions.

Experimental

Materials and Preparation of Solutions.—A dilute nitric acid solution of palladium(II) and a dilute sulfuric acid solution of palladium(II) were prepared from palladium metal powder, obtained from A. D. Mackay, Inc. These solutions were standardized using modifications of the Gilchrist-Wichers scheme.[4]

Standard 1,2,3-benzotriazole solution was prepared as described by Wilson and Wilson.[2] All other materials used were C.P. reagent grade chemicals.

Analytical Methods.—The determinations of carbon, hydrogen and nitrogen content in the compounds reported under "Procedure" were performed by Laboratory of Microchemistry, Tiedcke, N. J. Analyses of all other elements were carried out in our laboratory using conventional

(1) This work was supported by a grant from the National Science Foundation.

(2) R. F. Wilson and L. E. Wilson, THIS JOURNAL, **77**, 6204 (1955).

(3) R. F. Wilson and L. E. Wilson, *Anal. Chem.*, **28**, 93 (1956).

(4) W. F. Hillebrand, G. E. F. Lundell, H. A. Bright and J. I. Hoffman, "Applied Inorganic Analysis," John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 338–383.