TABLE I

PROPERTIES OF NEW ISOPROPYLGERMANIUM DERIVATIVES

Compound[a]	B.p., °C.	M.p., °C.	d^{20}_4	n^{20}	Mol. wt. (camphor) Calcd.	Mol. wt. (camphor) Found	Halogen, % Calcd.	Halogen, % Found	Distilled at °C.	Distilled at Mm.
$(i\text{-}Pr_2GeO)_3$	321	44	...	....	524	540	(41.5	41.3% Ge)	138–139	1
$i\text{-}Pr_2GeF_2$	174	−24	1.222	1.414_6	196.8	202	19.3	19.2, 19.2	173.5	760[b]
$i\text{-}Pr_2GeCl_2$	203	−52	1.268	1.473_8	229.7	235	30.9	31.1, 30.9	203–204	756
$i\text{-}Pr_2GeBr_2$	234	−22	1.670	1.519	318.5	314	50.2	50.3, 50.5	60–61	1
$i\text{-}Pr_2GeI_2$	268	− 9	2.008	1.597	412.6	390	61.5	61.7, 61.4	84–86	1
$i\text{-}Pr_3GeOH$	216	−15	1.077	1.472	218.9	420[c]	(33.1	32.6% Ge)	65–66	1
$i\text{-}Pr_3GeF$	198	−65	1.069	1.440	220.9	225	8.6	8.3, 8.4	197–198	761
$i\text{-}Pr_3GeCl$	222	..	1.110	1.472	237.2	248	15.0	15.3, 15.4	221–222	761
$i\text{-}Pr_3GeBr$	234	−45	1.231	1.485_2	281.8	285	28.4	28.5, 28.4	234–235	751[d]
$i\text{-}Pr_3GeI$	254	4	1.446	1.524	328.8	320	38.5	38.8, 38.6	79–80	1
$i\text{-}Pr_3Ge(NCS)$	277	18	1.112	1.512	260.0	262	22.3	22.2, 22.3	84–86	1[e]
$(i\text{-}Pr_3Ge)_2O$	315 dec.	..	1.112	1.483_6	419.7	407	(34.4	34.6% Ge)	119–120	1

[a] All compounds are colorless. [b] B.p. of fluoride at 760 mm. is uncorrected. [c] Dimeric in camphor; Dumas mol. wt., 236. Later redistilled at 63–64° under 1 mm. [e] Later purified by fractional freezing.

ate in 30 ml. of benzene slowly evolved carbon dioxide. An hour of electromagnetic stirring and gentle reflux followed; after filtration, washing and distillation of solvent, 4.5 g. of crude organogermanium oxide resulted, a yield of 87%. Distilled at 1 mm. pressure, the end fraction was a clear liquid much more viscous than carbon tetrachloride and somewhat more so than triisopropylgermanium hydroxide. This oxide had little odor, formed a glass below 0° without a true m.p.; the oxide turned medium red at 315°.

Triisopropylgermanium Isothiocyanate.—Eighteen ml. of ethereal 3 M isothiocyanic acid and 3.5 g. of the hydroxide gave a product still containing soluble thiocyanuric acid; the sodium sulfate method was used.[6] Boiling at 84–86° under 1 mm. pressure, the product contained only 21.3% titratable NCS. Double purification of 1.8 g. of impure product by fractional freezing, with removal of the lower-melting portion, furnished 0.52 ml. of pure triisopropylgermanium isothiocyanate, m.p. 18 ± 1°, with the properties in Table I. An actual d^{30}_4 of 1.100 was the source of a calculated d^{20}_4 in the table. A rejected 1.20 ml. of material melted in the range 5–10°.

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[6] H. H. Anderson, THIS JOURNAL, **73**, 5439 (1951).

PHILADELPHIA 4, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

Electrostatic Effects in the Combination of Cobalt with Amino Acids[1,2]

BY CHARLES TANFORD AND WILLIAM S. SHORE

RECEIVED AUGUST 14, 1952

The association constants for complexing of cobalt with glycine, alanine, asparagine and arginine have been obtained from glass electrode measurements by Bjerrum's method. The difference between the results for arginine and alanine can be successfully treated by the Kirkwood–Westheimer theory on the basis of the electrostatic influence of the positively-charged guanidine group of arginine. The data for asparagine cannot be explained in this way, and indicate that the structure of the chelating group ($H_2N\text{-}CHR\text{-}COO^-$) in this molecule is abnormal. There is an appreciable difference between the association constants for glycine and those for alanine, for which no obvious explanation exists.

Numerous studies have been made comparing the affinity of related substances for hydrogen ion. It has been found that the majority of the results can be satisfactorily explained by the Kirkwood–Westheimer theory[3] involving electrostatic interaction between charged or dipolar substituents and the reacting proton. In the present paper an attempt is made to extend this treatment to the affinity of four amino-acids, all containing the chelating group $H_2N\text{-}CHR\text{-}COO^-$, for cobaltous ion.

Experimental

The amino acids used in this work were C.P. or the equivalent. Glycine and alanine were Dow Chemical Co. products, asparagine monohydrate and arginine (free base) were obtained from Pfanstiehl Chemical Co. The amino-acids were carefully dried and stock solutions of them in their zwitterion form ($^+H_3N\text{-}CHR\text{-}COO^-$) were prepared. Arginine was converted into this form by the addition of one equivalent of nitric acid.

Acid dissociation constants of the amino groups were obtained from complete titration curves at constant ionic strength of 0.15. CO_2-free potassium hydroxide[4] was used. These curves served also as criteria of purity for the amino-acids, all of which were judged to be at least 99% pure.

Metal complexing was studied by the method of Bjerrum,[5] involving pH measurements on solutions containing known concentrations of the zwitterion form of amino-acid, of added base, and of $Co(NO_3)_2$, with sufficient KNO_3 to bring the ionic strength to 0.15. The removal of ions from solution by complexing was not taken into account in computing ionic strength, since the error introduced in this way was shown by computation to be very small. A Beckman model G pH meter with external electrodes was used to make the measurements. All measurements were made at 25.15 ± 0.02°, under a stream of purified nitrogen, to avoid oxidation of the cobalt and contamination by CO_2.

Values of $\bar{n}$, the average number of amino-acid molecules associated with each cobalt ion, were computed from the pH readings in the customary manner,[5] using the acid dissociation constants previously determined. The results

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[1] Presented at the 122nd National Meeting of the American Chemical Society, Atlantic City, N. J., September 14–19, 1952.

[2] This investigation was supported by a research grant from the National Institute of Health, Public Health Service.

[3] J. G. Kirkwood and F. H. Westheimer, *J. Chem. Phys.*, **6**, 506 (1938); F. H. Westheimer and J. G. Kirkwood, *ibid.*, **6**, 513 (1938).

[4] I. M. Kolthoff, *Z. anal. Chem.*, **61**, 48 (1922).

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

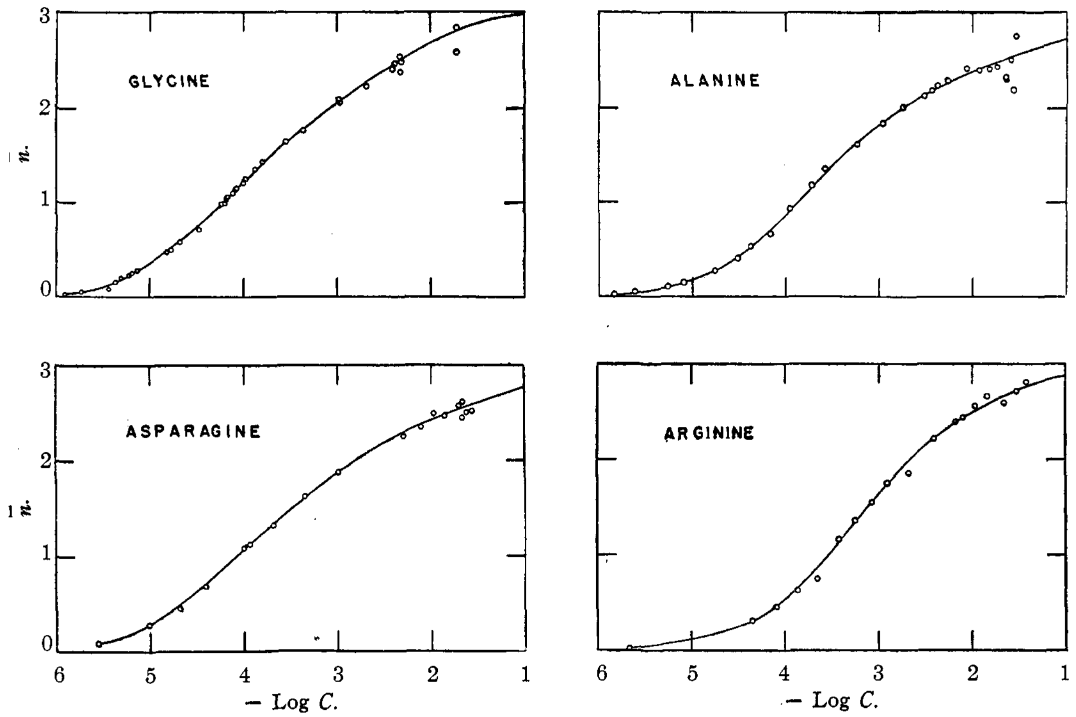

Fig. 1.—Formation curves for cobalt–amino-acid complexes at ionic strength 0.15 and 25°.

were recorded as formation curves (Fig. 1), *i.e.*, as plots of $\bar{n}$ *versus* the logarithm of the concentration of the basic form of the amino-acids ($H_2N-CHR-COO^-$).

Results and Discussion

The formation curves for the four amino acids are plotted in Fig. 1. It is seen that in each case $\bar{n}$ tends to a maximum of 3, the same value as found for cobalt with other chelating substances, such as ethylenediamine.

The formation constants were determined from these curves by the method of successive approximation used by Bjerrum.[5] His second method of obtaining temporary constants was used.[6] The constants so calculated, as well as the observed acid dissociation constants of the amino groups are listed in Table I. It is estimated that the values of pK_H are accurate to ±0.01 and those of log k_1 and log k_2 to ±0.03. (No values of log k_2 and log k_3 could be found to fit any formation curve, when a value of log k_1 was chosen which differed by 0.05 from the one given in Table I.) The values of log k_3 are less precise, since the experimental points at the upper end of the formation curves show considerable scatter.

TABLE I

OBSERVED ASSOCIATION CONSTANTS,[a] μ = 0.15

	pK_H'	log k_1'	log k_2'	log k_3'
Glycine	9.69	4.65	3.78	2.38
Alanine	9.78	4.27	3.45	1.75
Asparagine	8.84	4.55	3.58	1.83
Arginine	9.11	3.87	3.20	2.08

[a] These constants refer to the equilibria, $Co^{++} + A \rightleftarrows Co^{++}A$, $Co^{++}A + A \rightleftarrows Co^{++}A_2$, and $Co^{++}A_2 + A \rightleftarrows Co^{++}A_3$, where A represents the basic form of the amino-acid, $H_2N-CHR-COO^-$.

The constants in Table I have been corrected to zero ionic strength by means of activity coefficients estimated either from pertinent experimental data[7-12] or from calculations by means of the Debye–Hückel theory for ions[13] or the Kirkwood and Roberts–Kirkwood equations for zwitterions.[14] For several of the activity coefficients two or three independent estimates could be made, which were always found to agree within ±0.02 or better in log γ, except in the case of log $\gamma_{Co^{++}}$, which is uncertain by ±0.05. The activity coefficients of the various forms of asparagine were taken to be the same as for the corresponding forms of alanine.

The corrected constants are listed in Table II, with figures in parentheses representing, where available, previously-determined values of these constants. The agreement of these with our values is very satisfactory, except that Monk's values for the first association constants of Co^{++} with glycine and alanine are higher than those reported here. This is due in part to a difference of 0.04 in the standardization of the pH scale,[15] and in part to Monk's use of generalized activity coefficients,

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(6) Reference 5, p. 36.

(7) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1950, pp. 369, 423, 547–578.

(8) R. G. Bates, *Chem. Revs.*, **42**, 1 (1948).

(9) B. B. Owen, THIS JOURNAL, **56**, 24 (1934).

(10) A. C. Batchelder and C. L. A. Schmidt, *J. Phys. Chem.*, **44**, 888 (1940).

(11) P. K. Smith, A. C. Taylor and E. R. B. Smith, *J. Biol. Chem.*, **122**, 109 (1937).

(12) L. F. Nims and P. K. Smith, *ibid.*, **101**, 401 (1933).

(13) Using exclusion radii as given by J. Kielland, THIS JOURNAL, **59**, 1675 (1937).

(14) J. G. Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934); R. M. Roberts and J. G. Kirkwood, THIS JOURNAL, **63**, 1373 (1941).

(15) C. B. Monk (*Trans. Faraday Soc.*, **47**, 285 (1951)) has assigned pH values of 3.97 and 9.14, respectively, to 0.05 *M* potassium acid phthalate and to 0.05 *M* borax, but has used pK_H values given in ref. 9 and 12, which were obtained on cells without liquid junction. Cells of this type lead to values of 4.005 and 9.19 for the pH values of the two buffer solutions (ref. 8).

rather than the experimentally derived ones used in this paper.

TABLE II

ASSOCIATION CONSTANTS CORRECTED TO $\mu = 0$

	pK_H	$\log k_1$	$\log k_2$	$\log k_3$
Glycine	9.78	5.08	4.02	2.39
	(9.778)[a]	(5.23)[b]	(4.02)[b]	
Alanine	9.86	4.70	3.69	1.76
	(9.867)[c]	(4.82)[b]	(3.66)[b]	
Asparagine	8.92	4.98	3.84	1.84
Arginine	8.99	3.84	3.24	2.08
	(9.012)[d]			

[a] Ref. 9. [b] C. B. Monk, *Trans. Faraday Soc.*, **47**, 297 (1951). [c] Ref. 11. [d] Ref. 10.

From the previous discussion, the pK_H values of Table II should be accurate to ±0.02, the $\log k_1$ and $\log k_2$ values to ±0.05, while the $\log k_3$ values are considerably less precise.

The difference in $\log k_1$ between arginine and alanine has been successfully treated by the Kirkwood–Westheimer theory.[3] It has been assumed that the guanidine group of arginine is so far removed from the chelating group at the other end of the molecule that the structure of this group is not altered. If the guanidine group were uncharged the value of $\log k_1$ would be the same for arginine as for alanine, and the observed difference is thus assumed to be due to electrostatic interaction between the charge on the guanidine group and the cobalt ion. Using the Kirkwood–Westheimer theory, then, the distance R between the charge on the guanidine group and the cobalt ion can be computed, proper allowance being made for the double charge on Co^{++}. This value of R can then be compared with values calculated from the structure of the molecule on the basis that it is fully extended (R_{max}) or that there is free rotation about single bonds (R_{free}).

In making these calculations we have followed closely the method of Westheimer and Shookhoff.[16] In computing R_{max} and R_{free}, the positive charge on the guanidine group has been located, on the average, on the carbon atom of that group. The cobalt ion has been placed 3 Å. from the α-carbon atom, at an angle of 150° with the α–β carbon–carbon bond. The C–N distance in the guanidine group has been given the value 1.33 Å.

The results of the calculations are given in Table III, together with similar calculations for the distance between the guanidine charge and the α-amino proton in the zwitterion form of arginine (^+H_3N–CHR–COO$^-$), based on our pK_H values, and the distance between the guanidine charge and the carboxyl proton in the acid form of arginine (^+H_3N–CHR–COOH), based on pK_H values for the carboxyl groups of arginine[10] and alanine.[11] The calculated R values in every case fall between R_{max} and R_{free}, as they should.

TABLE III

INTERCHARGE DISTANCES IN ARGININE (IN Å.)

Species[a]	R	R_{max}	R_{free}
^+G–$(CH_2)_3$–CH(NH_2)–COO$^-$Co^{+2}	7.7	9.1	6.3
^+G–$(CH_2)_3$–CH(COO$^-$)–NH$_3^+$	6.3	7.5	5.0
^+G–$(CH_2)_3$–CH(NH$_3^+$)–COOH	7.2	8.5	5.4

[a] The letter G represents the guanidinium group. The distance computed is that between the charge on this group and the ion (H$^+$ or Co^{+2}) at the extreme right of the species formula.

There are numerous errors inherent in these calculations. The values of R depend upon whether or not electrostriction is taken into account in calculating molar volumes.[17] Different values of R would also have been obtained if arginine had been compared to norleucine, say, instead of to alanine. Finally, R_{max} and R_{free} for the cobalt-arginine complex depend on the accuracy of the location of the cobalt ion. To estimate the effect of these errors, a number of alternative calculations have been made, from which it appears most unlikely that figures differing from those of Table III by more than 0.3–0.4 Å. could have been obtained.[18]

It can therefore be concluded that the effect of the charged guanidine group in the combination of Co^{++} with arginine is, like its effect in the dissociation of H$^+$, largely an electrostatic one.

Qualitatively, the decreasing difference between alanine and arginine, when $\log k_2$ and $\log k_3$ are considered, is in complete agreement with this view.

It is evident that the same kind of treatment of the difference between asparagine and alanine would not be successful. If the principal effect of the amide group of asparagine were electrostatic, then $\log k_1$ and pK_H would be shifted in the same direction, whereas the observed effects are in opposite directions. This indicates that the presence of the amide group in asparagine changes the structure of the chelating group. Such a change in structure has, in fact, been recently suggested on the basis of the organic chemistry of asparagine.[19] The new structure suggested is a ring structure, which would tend to force the chelating oxygen and nitrogen onto the same side of the connecting C–C bond, and, as a result, might be expected to increase the affinity for metals, relative to that for hydrogen ion.

The alternative explanation, that the relatively high affinity of asparagine for cobalt indicates that it acts as a tridentate group (with the third bond from the cobalt to the amide oxygen) can be excluded at once, since this would lead to a maximum of 2 coördinated asparagine molecules, which is incompatible with the data of Fig. 1.

Finally, the not inconsiderable difference between glycine and alanine should be noted, as well as the fact that alanine has a lower affinity for

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(16) F. H. Westheimer and M. W. Shookhoff, THIS JOURNAL, **61**, 555 (1939).

(17) *Cf.* E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 157. The procedure adopted here has been to allow 3 cc. for electrostriction in each calculation. The major portion of the experimentally observed electrostriction (which may amount to as much as 20 cc.) can unquestionably be ascribed to the solvent rather than to the amino acid.

(18) If $\log k_1$ for arginine is compared with the value for glycine, instead of that for alanine, a difference of 0.7 Å. is found. However, it is quite unlikely that a long chain unbranched α-amino-acid would have properties differing from alanine by as much as glycine does. In any event, even an error of 0.7 Å. would not affect the validity of our conclusions.

(19) F. C. Steward and J. F. Thompson, *Nature*, **169**, 739 (1952).

cobalt than does glycine, but a higher affinity for hydrogen ion. No obvious explanation exists for these observations.

Acknowledgment.—The authors wish to express their appreciation to Dr. F. H. Westheimer for helpful discussion of the calculations involving the Kirkwood–Westheimer theory.

IOWA CITY, IOWA

[CONTRIBUTION FROM THE CHEMISTRY LABORATORIES OF NORTHWESTERN UNIVERSITY]

Mechanism of Substitution Reactions in Complex Ions. II. Stereochemical Changes Accompanying Some Reactions of Cobalt(III) Complexes[1,2]

BY FRED BASOLO, BOB D. STONE[3,4] AND RALPH G. PEARSON

RECEIVED APRIL 23, 1952

The reactions of several geometric isomers of cobalt(III) complexes with various reagents have been studied. A semi-quantitative estimate of the relative amounts of *cis* and *trans* isomers produced *in situ* was made by means of a spectrophotometric determination. A discussion is given showing various structural changes to be expected on the basis of four different reaction mechanisms. However, the results of this study show clearly that no one of these mechanisms is operative in all substitution reactions.

The mechanisms whereby ligands attached covalently to the central metallic ion in hexacovalent complexes are removed and replaced by other groups is of considerable interest. Although this problem has received considerable attention,[5] little can be said as to how these substitution reactions actually proceed. One plausible approach toward obtaining useful data in this connection is that utilized as early as 1912 by Werner.[6] He investigated the reactions of the geometric isomers of several complexes of cobalt(III) with various reagents and qualitatively estimated the ratios of the isomeric products isolated from these reactions. Unfortunately, Werner was unable to correlate his findings with any reaction mechanism(s) and concluded that there was no relationship between the position occupied by the incoming group and that of the outgoing group.

Since rearrangements of cobalt(III) complexes could occur during isolation, it can be argued that Werner's observations need not be a true measure of the immediate products of reaction. Because of this, it was decided that some of these reactions should be reinvestigated using milder reaction conditions and some technique which will allow an estimate of the ratios of geometric isomers as they are produced. Since geometric isomers of cobalt (III) complex ions usually have different absorption spectra,[7] a spectrophotometric technique was applied to get a semi-quantitative estimate of the relative amounts of *cis* and *trans* isomers obtained as products of reaction. In addition, several reactions were investigated which had not been included in Werner's study.

Experimental

Spectral Measurements.—All measurements were made at room temperature with a Beckman model DU spectrophotometer in silica cells having a 1-cm. light path. Molecular extinction coefficients were calculated from the familiar equation

$$\epsilon = 1/cd \log_{10} (I_0/I)$$

Compounds.—The compounds used were prepared by the procedures described in the literature and purified either by recrystallization or reprecipitation by the addition of ethanol-ether to a concentrated aqueous solution of the salt.[8] Although the compounds showed the correct analysis, there was no assurance that any of them consisted of a single isomeric form. However, many of these isomers are separated by differences in solubility, and considerable care was taken to discard intermediate fractions, retaining only the least and most soluble fractions.

Procedure.—Solutions of the *cis* and *trans* isomers were prepared using as solvent a stock solution containing the desired reagent. After reaction took place, the absorption spectra of the reaction mixtures between 300 and 600 mμ were determined by comparison with blanks of the same stock solution. For example, to study the reaction of chloroamminebis-(ethylenediamine)-cobalt(III) ions with sodium hydroxide

$$[\mathrm{Coen_2NH_3Cl}]^{+}{}_{2} + \mathrm{OH^-} \longrightarrow [\mathrm{Coen_2NH_3OH}]^{+}{}_{2} + \mathrm{Cl^-}$$

the chloroammine complexes were dissolved in a 0.5 *M* solution of sodium hydroxide and the spectra of the reaction products were determined by comparison with the sodium hydroxide solution. In addition, the absorption spectra of the *cis*- and *trans*-hydroxoamminebis-(ethylenediamine)-cobalt(III) ions which were used as standards, were determined by the same procedure in the same stock solution. The standards used were actually the *cis*- and *trans*-aquamminebis-(ethylenediamine)-cobalt (III) ions which in alkali react to yield hydroxo complexes

$$[\mathrm{Coen_2NH_3H_2O}]^{+3} + \mathrm{OH^-} \longrightarrow [\mathrm{Coen_2NH_3OH}]^{+2} + \mathrm{H_2O}$$

and it is assumed that this proton transfer does not alter the configuration of these complexes.

The absorption spectra shown in Fig. 1 may serve to illustrate the method employed to estimate the relative amounts of *cis* and *trans* isomers present in a given reaction mixture.[9] For example, the maximum difference in the spectra of *cis*- and *trans*-hydroxoamminebis-(ethylenediamine)-cobalt(III) ions is at 370 mμ. At this wave length the molecular extinction coefficient of the *cis* standard is 77 as compared to 53 for the *trans* complex. Since the extinction coefficient of the reaction mixture of the *cis*-chloroammine complex is also 77, it is concluded that the product is 100% *cis*-$[\mathrm{Coen_2NH_3OH}]^{+2}$. The extinction coefficient of the reaction mixture of the *trans*-chloroammine

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[1] Paper I of this series: F. Basolo, J. G. Bergmann and R. G. Pearson, *J. Phys. Chem.*, **56**, 22 (1952).

[2] Presented at the New York Meeting of the American Chemical Society, September, 1951.

[3] Atomic Energy Commission Pre-doctoral Fellow, 1950–1952.

[4] Taken in part from a thesis submitted by Bob D. Stone in partial fulfillment of the requirements for the Ph.D. degree, 1952.

[5] For a review see, J. C. Bailar, Jr., *Chem. Revs.*, **19**, 67 (1936).

[6] A. Werner, *Ann.*, **386**, 1 (1912).

[7] Y. Shibata, *J. Coll. Sci. Imp. Univ. Tokyo*, 37 (1915); F. Basolo, THIS JOURNAL, **72**, 4393 (1950); Y. Shimura, *ibid.*, **73**, 5079 (1951).

[8] Gmelin, "Handbuch der anorganischen Chemie," No. 58B (Kobaltammine) Berlin, 1930.

[9] The detailed absorption spectra which were used to determine the relative amounts of *cis* and *trans* isomers reported in Table I are available in the doctorate thesis of Bob D. Stone.