TABLE I

	Specific rotations, degree				
	I	IIA[a]	II	III	IV
[Co trien Cl]$_2^+$	+120	0 to +5	−111	−100	−85
[Co en$_2$Cl$_2$]$^+$	+111	+ 57	− 21	...	−60
[Co en$_2$NH$_3$Cl]$^{++}$	+ 98.5	+103	−5∼0	...	...
[Co pn$_2$Cl$_2$]$^+$	+131	+ 53	− 10	...	...
K-*d*-SbO tart	+143	+143	0	...	...

[a] The rotations in column IIA include that due to the ionic SbO tart$^-$.

Anal. Calcd. for [Co en$_2$ Cl$_2$]Cl·H$_2$O, C, 15.81; H, 5.93; N, 18.41; Co, 19.41. Found: C, 15.87; H, 5.68; N, 18.37; Co, 18.99.

The rotation of a 0.05% aqueous solution was −0.035° at the D-line of sodium. Different samples of 0.05% solutions of L-[Co en$_2$Cl$_2$]Cl resolved by the standard *d*-bromocamphorsulfonate method gave rotations +0.11 to +0.134 for different samples.

A similar approach is under way using other stable metal complexes of the same type containing metal ions other than cobalt(III).

Our best thanks are due to the University of Illinois and to the National Science Foundation for providing funds to one of us (Das Sarma) and also to the kind help and coöperation of Mr. Josef Nemeth, Mrs. E. L. Fett and Mrs. L. Chang for the microanalysis for carbon, hydrogen and nitrogen.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MALAYA]

Compounds of Chromium(III) with Alanine

BY R. W. GREEN AND K. P. ANG

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The Van Slyke manometric method for determination of amino groups has been used to demonstrate the presence of N-coördinated alanine in solutions of alanine and chromium(III) salts. This has been combined with spectroscopic and isopiestic studies to show that solutions near *p*H 4.5 contain complexes in which each chromium atom is united to three alanine residues, of which one is in the form of a chelate ring and the other two are coördinated by their carboxyl groups only. It is possible to prepare solutions at *p*H 7–8 in which two alanine residues are chelated to each chromium atom, but higher degrees of chelation produce very sparingly soluble derivatives.

The first compound of chromium and alanine was prepared by Tchougaeff and Serbin,[1] who boiled together solutions of chloropentamminochromium(III) chloride and excess alanine until most of the ammonia had been driven off. The resulting deep red, faintly ammoniacal solution deposited rose colored needles whose composition corresponded to the formula Cr[CH$_3$·CH(NH$_2$)·COO]$_3$. Hugounenq and Morel[2] boiled concentrated solutions of amino acids with freshly precipitated chromium(III) hydroxide and obtained two products containing, respectively, two and three molecules of amino acid per chromium atom. In the same year, Ley and Ficken,[3] by adding three moles of sodium hydroxide to a hot solution of one mole chromium(III) chloride and three moles alanine, prepared two very sparingly soluble crystalline solids, one red and one violet, to which they gave the formulas, Cr(C$_3$H$_6$O$_2$N)$_3$ and Cr(C$_3$H$_6$O$_2$N)$_2$(OH)·H$_2$O. The same two solids, which, on account of their insolubility and inertness, are generally regarded as chelate compounds, were again prepared by Volshtein.[4] In each case, the amount of alkali present or added during the preparation was equivalent to the total chlorine of the chromium(III) chloride, so that the solutions from which the solids separated were approximately neutral in reaction.

More acid solutions have been studied by Shuttleworth,[5] who interpreted conductimetric evidence as indicating that the amino groups of glycine and alanine were not coördinated by chromium in dilute solutions, even on boiling. On the other hand, Serfass, Wilson and Theis[6] showed by spectrophotometric measurements that glycine reacted with chromium(III) ions much more readily than did acetate. Indeed, they found it to be more readily coördinated than any other substance they studied, except oxalic acid, which is well known to cause chelation. This paper describes the use of analytical, isopiestic and spectrophotometric methods to investigate the nature of chromium(III)–alanine solutions.

Experimental Methods

Chromium was determined in solution by oxidizing with sodium peroxide and comparing the optical density at 373 mμ with that of standard chromate solutions. Chromium(III) chloride and sulfate solutions were analyzed in this way for chromium, gravimetrically for chloride and sulfate, and also by titration of the hot solution with alkali in the presence of phenolphthalein.

Basic chromium(III) chloride solutions free from other electrolytes were made by adding to boiling chromium(III) sulfate solution the calculated quantities of barium chloride and barium hydroxide.

Absorption spectra were measured with a Beckman model DU spectrophotometer. Because solutions usually contained more than one chromium complex of unknown molecular formula, results are given as atomic extinction coefficients

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

where c is the concentration in g. atom Cr per liter. All the chromium solutions examined had two absorption maxima (Fig. 6), one near 400 mμ and one near 540 mμ. We shall use ϵ_1 and λ_1 to denote the atomic extinction coefficient and wave length of the first maximum, and ϵ_2 and λ_2 for the second maximum.

A Beckman *p*H meter was used for all *p*H determinations.

(1) L. Tchougaeff and E. Serbin, *Compt. rend.*, **151**, 1361 (1910).

(2) L. Hugounenq and A. Morel, *ibid.*, **154**, 119 (1912).

(3) H. Ley and K. Ficken, *Ber.*, **45**, 377 (1912).

(4) L. M. Volshtein, *Compt. rend. acad. sci. (U.S.S.R.)*, **54**, 321 (1946).

(5) S. G. Shuttleworth, *J. Intern. Soc. Leather Trades' Chem.*, **32**, 116 (1948).

(6) E. J. Serfass, C. D. Wilson and E. R. Theis, *J. Amer. Leather Chem. Assoc.*, **44**, 647 (1949).

Isopiestic measurements were made by the method of Robinson and Sinclair[7] at 25°, using silver dishes. The most dilute solutions were equilibrated for 7 days.

Alanine can be determined in solution by the manometric method of Van Slyke[8] for the estimation of free amino groups. Because of the sluggishness of most reactions involving chromium(III), the Van Slyke method offers a possible means of detecting combination between chromium and the amino groups of alanine. The following procedure was adopted. A sample (4 ml.) of solution containing approximately 2 mg. of alanine was introduced into the reaction chamber, followed by 1 ml. of saturated sodium acetate solution and 1 ml. glacial acetic acid. When the mixture had been degassed, 2 ml. of saturated sodium nitrite was introduced and the reaction was allowed to proceed for 4 minutes. The evolved gas was washed and measured in the usual way. From time to time a blank experiment was run with 4 ml. of water in place of the alanine solution, and the very small result was applied to the former as a correction.

A series of control experiments with known amounts of alanine gave a mean recovery of 100.2% with a standard deviation of 0.5% unaffected by the presence of chromium(III) chloride or sulfate. The method was then tested on the red or purple chromium(III)–alanine solutions, prepared by such methods as that of Ley and Ficken, which might be expected to contain coordinated alanine. Low Van Slyke nitrogen values were obtained, but, if the solution was heated for 1 hour with dilute sulfuric acid, it became green and gave a normal value for the amino nitrogen. Both these figures were reproducible with the same precision as before. Their difference was not due to differences in acidity during the Van Slyke determination, since at this stage both solutions were buffered to approximately the same *p*H by the sodium acetate. Moreover, it was possible by ion-exchange methods to separate from the chromium–alanine solution a fraction which gave no Van Slyke nitrogen before decomposition with acid, but a measurable value afterwards. This demonstrates that no decomposition of the chromium–nitrogen complex occurs during the short time of the Van Slyke reaction. Application of the above method to the same solution before and after treatment with hot dilute sulfuric acid can thus be taken to give the free and total amino nitrogen. The difference must represent the amount of alanine which is stably coördinated to chromium through its amino group. We shall use $\bar{n}$ to denote the average number of amino groups bound per chromium atom.

Sparingly soluble solid derivatives of chromium and alanine were brought into solution by heating with dilute sulfuric acid until the first red solution turned green. The solution was analyzed then for chromium and total alanine. Elementary analyses of the solids were also made by Dr. Zimmermann.[9]

Results

Neutral Solutions.—Solutions made by slowly adding 3 moles sodium hydroxide to boiling solutions of 1 mole chromium(III) chloride and r moles alanine all had *p*H values between 7 and 8 but varied in color from purple ($r = 2$) to bright red ($r = 10$). In a simple electrophoresis experiment all the chromium remained stationary and was apparently present in uncharged complexes. Van Slyke analyses gave values of $\bar{n}$ initially all lying between 1 and 2, but slowly decreasing with age. Solutions with $r > 3$ deposited a very sparingly soluble, red or violet crystalline solid. This precipitation occurred rapidly from the hot solution, but continued steadily even from a cold solution in a stoppered flask. In the presence of a sufficiently large excess of alanine, solutions, initially of molar concentration, slowly deposited solid until the chromium concentration had fallen as low as 0.01 g. atom Cr per liter.

(7) R. A. Robinson and D. A. Sinclair, THIS JOURNAL, **56**, 1830 (1934).

(8) D. D. Van Slyke, *J. Biol. Chem.*, **12**, 275 (1912); **83**, 425 (1929).

(9) Dr. W. Zimmermann, University of Melbourne, Australia.

If r was kept near 3 or greater than 10, solids corresponding to Ley and Ficken's two derivatives[3] could be obtained pure, as shown in Table I.

TABLE I

COMPOSITION OF CHROMIUM(III)–ALANINE COMPOUNDS

Calculated on weight after drying at 100°.

	$Cr(C_3H_6O_2N)_2(OH)\cdot H_2O$ Calcd.	Found	$Cr(C_3H_6O_2N)_3$ Calcd.	Found
Loss at 100°	7.35	7.12	0	0
Chromium	21.21	21.21	16.45	16.54
Amino N[a]	11.43	11.44	13.29	13.32
Carbon[b]	29.37	28.37	34.18	34.41
Hydrogen[b]	5.72	5.66	5.74	5.67
Nitrogen[b]	11.43	11.29	13.29	13.74

[a] Van Slyke. [b] Reference 9.

These two solids were insoluble in organic solvents but very slightly soluble in water with slow decomposition. For example, stirring tris-(alanine)-chromium(III) with cold water for 15 minutes gave a solution containing 0.001 g. atom Cr per liter. When first prepared the solution had $\bar{n} = 2.76$, but this value decreased steadily and after 12 days had reached 0.60. On boiling, the solution deposited a basic precipitate resembling chromium(III) hydroxide. The basic bis-(alanine) solid derivative was even less soluble, although quite concentrated solutions (1 *M*) with $\bar{n} = 2.0$ were easily prepared by Ley and Ficken's method. Clearly, then, there are at least two chromium(III)–alanine species with $\bar{n} = 2$, and it seems likely that the insoluble one is a polymeric olated form, while the soluble species is a simpler molecule.

Although the above two insoluble derivatives could be obtained pure by careful control of the experimental conditions, it was more usual to find a product of intermediate composition which we were unable to separate into simpler components. For example, successive fractions of solid filtered from one solution during a period of two months increased regularly from $\bar{n} = 2.09$ to $\bar{n} = 2.70$, while $\bar{n}$(solution) decreased from 2.00 to 1.64. The products appeared homogeneous under the microscope and in sedimentation tests in mixed organic liquids. In view of the known tendency of chromium(III) to form large aggregates by olation, it cannot be said with certainty that these sparingly soluble substances are simple mixtures. They may form a continuous series of macromolecules in which the chromium atoms are linked together by hydroxyl or amino acid bridges in varying proportions. It is at least certain that they separate from solution, not by a process of fractional crystallization, as was implied by earlier workers,[3] but as a result of slow reactions between soluble complexes and free alanine.

The solutions themselves were evidently complicated mixtures. Those with $\bar{n}$ near 1.0 gave on dialysis a red dialysate ($\bar{n} > 1.5$) and a greenish-purple residual solution ($\bar{n} < 0.6$) which on evaporation yielded a glassy green solid with a molecular weight too high to be measured by the isopiestic method. Solutions with $\bar{n}$ nearer 2.0 were completely dialyzable, but ion-exchange experiments showed them to contain more than one chromium species. We were unable to isolate or identify any definite species in these solutions, but it seems prob-

able that they ranged from fairly simple complexes with one or two chelated alanine residues per chromium atom to highly basic olated colloidal aggregates with a very low proportion of coördinated alanine.

Solutions at Lower *p*H.—A common method of studying complex formation, which has been used with success in connection with the coördination of amino acids by divalent metals,[10] namely, the potentiometric titration of the metal ion with alkali in the presence of the complexing agent, is excluded here by the slowness of the reactions involved. The following modification was used: A solution of normal chromium(III) chloride (1 *M*) was boiled under reflux for 24 hours with 3 moles of alanine. It was then divided into 20 portions to which sodium hydroxide was added in amounts of from 0 to 3 moles per mole $CrCl_3$. The solutions were all adjusted to the same volume and heated in stoppered tubes on the water-bath for 24 hours. By this time the contents of the tubes had come near to equilibrium. They were cooled, and immediately the *p*H and absorption spectrum were measured, and some were analyzed for coördinated amino groups. It was found that a plot of $\bar{n}$ against ϵ_2 gave a smooth, almost linear curve which could be used for interpolating $\bar{n}$ for the other solutions. The results are shown in Figs. 1–4.

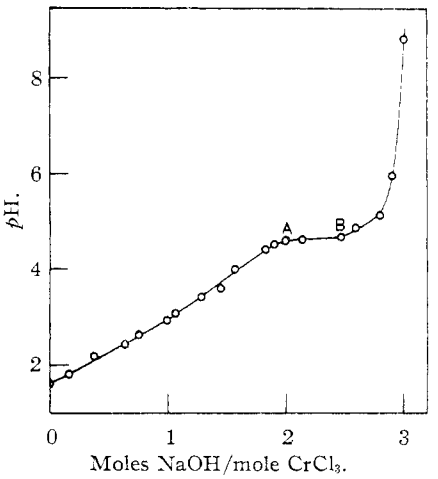


Fig. 1.—Changes in *p*H caused by adding sodium hydroxide to a boiling solution of 1 mole of $CrCl_3$ and 3 moles of alanine.

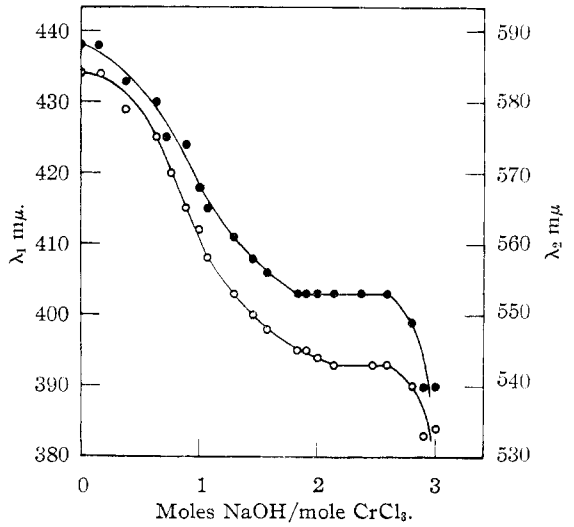


Fig. 2.—Changes in wave length of the two absorption maxima caused by adding sodium hydroxide to a boiling solution of 1 mole of $CrCl_3$ and 3 of moles alanine: ●, λ_1; ○, λ_2.

(10) A. Albert, *Biochem. J.*, **47**, 531 (1950); **50**, 690 (1952).

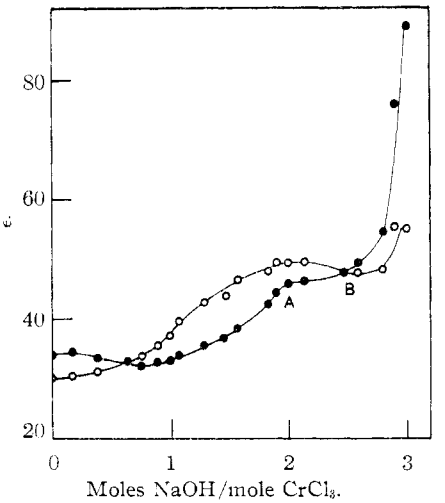


Fig. 3.—Changes in atomic extinction coefficient caused by adding sodium hydroxide to a boiling solution of 1 mole $CrCl_3$ and 3 moles alanine: ●, ϵ_1; ○, ϵ_2.

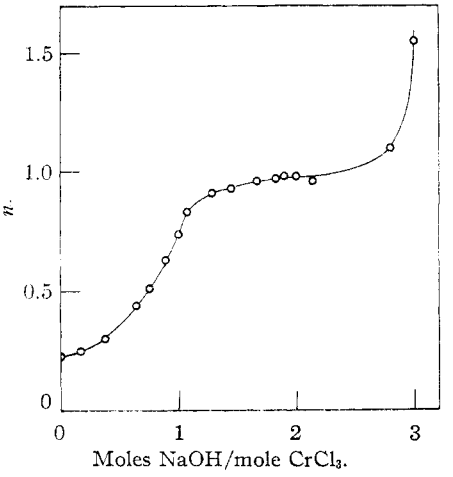


Fig. 4.—Changes in degree of chelation, $\bar{n}$, caused by adding sodium hydroxide to a boiling solution of 1 mole of $CrCl_3$ and 3 moles of alanine.

Between A and B (Fig. 1), all the chromium moved toward the cathode. Since the reaction was carried out at 100° and the measurements were made at 25°, we cannot use the data for calculating stability constants, but we can make deductions from the qualitative features of the graphs.

The most striking is the change of direction which appears in all the curves between A and B (2.0 to 2.5 moles of alkali). The *p*H curve is almost horizontal in this region. Below A, $\bar{n}$ increased from 0.23 in the most acid solution to 1.0 at *p*H 4.6, and no insoluble derivatives were formed. Solutions be-

tween A and B deposited as much as 40% of their total chromium as pure $Cr(C_3H_6O_2N)_3$, but beyond B, as the *p*H increased again, the solid began to be contaminated with the hydroxo derivative, $Cr(C_3H_6O_2N)_2(OH)\cdot H_2O$; and, when 2.90 moles of alkali had been added, $\bar{n}$(solid) had fallen to 2.43. At this point, 90% of the total chromium had been converted to insoluble chelate and had been precipitated in quantities only slightly less than 1 g. atom Cr for every equivalent of alkali in excess of 2.

It is clear that a good yield of tris-(alanine)-chromium(III) is best obtained by adding about 2.4 moles of sodium hydroxide to a hot concentrated solution of 1 mole of $CrCl_3$ and 3 moles of alanine. At higher *p*H values, hydroxyl ions begin to be coördinated by the chromium, and the higher derivative is only obtained pure by using a wastefully large excess of alanine.

Since the solution at A (Fig. 1) evidently possessed unusual properties, a portion was diluted and titrated as rapidly as possible in the cold with 0.1 *N* NaOH. The *p*H titration curve is compared in Fig. 5 with that for pure alanine and with the last section of the curve of Fig. 1, the high temperature titration. In this cold titration no solid derivative was precipitated. On the contrary, the titrated solution on standing suffered a rapid decrease in $\bar{n}$ and soon deposited chromium(III) hydroxide.

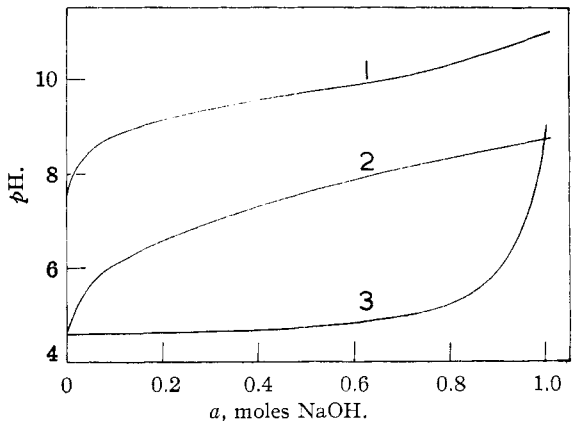


Fig. 5.—*p*H titration: 1, one mole alanine titrated with *a* moles NaOH; 2, solution A (Fig. 1) titrated in the cold with *a* moles NaOH per Cr atom; 3, solution A (Fig. 1) titrated at 100° with *a* moles NaOH per Cr atom.

The curves of Figs. 1–5 now provide some evidence of the nature of the chromium complexes in solution. Since simple chromium(III) ammines are not usually formed directly in aqueous solution,[11] and since the carboxylate ion coördinates readily with chromium, we may safely assume that the bound amino groups represent chelated alanine. This chelation is found even at *p*H 1.6 and increases with *p*H and hence with zwitterion concentration in the free alanine. However, at *p*H 4.6, when the degree of chelation, $\bar{n}$, has reached exactly 1.0, addition of more alkali causes the hot solution to precipitate tris-(alanine)-chromium(III), with $\bar{n} = 3$. This abrupt change in $\bar{n}$ suggests that the Van Slyke figure is not giving a complete picture of the complexes in solution and that the Cr atom of the principal species at A may coördinate as many as three alanine molecules, even though only one is chelated. Confirmation comes from two sources, the method of continuous variations and a study of the osmotic properties of the solution.

Continuous Variations.—The method of continuous variations, introduced by Job[12] and extended by Vosburgh and Cooper,[13] consists in measuring the optical densities of a series of mixtures of solutions of A and B, both of concentration *M* moles per liter. In this case, A was $^2/_3$ basic chromium(III) chloride, B was alanine, and the mixtures were made by taking $10x$ ml. of B and $10(1 - x)$ ml. of A. Since even basic chromium(III) chloride solutions are as acid as *p*H 1.7, while the isoelectric point of alanine is 6.1, mixtures of the two will vary widely in *p*H. The cationic form of alanine cannot coördinate with a metal; and in the *p*H range 1.7–4.7 covered by our experiment, the proportion of zwitterion increases from 18% to over 99%. It therefore becomes necessary to inquire how this will affect the interpretation of the results.

Let the reaction be represented by

$$A + nB \rightleftarrows AB_n$$

and let the equilibrium concentrations of free metallic ion and total free alanine be c_1 and c_2, respectively. Since *p*H varies with x, the concentration of free alanine in the zwitterionic form can be written as $c_2 f(x)$. Finally, let Y be the difference between the observed optical density and the value calculated for no reaction. It can easily be shown that Vosburgh and Cooper's conclusions will be unaffected by *p*H changes if $c_1 c_2 f'(x)/M^2 f(x)$ is small in comparison with $(1 - x)$.

In the work described here, calculations from the observed *p*H values and from the known ionization constants of alanine[14] showed that, when Y was a maximum, $f'(x)/f(x)$ was approximately unity. The strong enhancement of the extinction coefficient of chromium in the presence of alanine, and the fact that the *p*H was higher than that found in even the most basic solutions of chromium(III) chloride both make it probable that c_1 was very small and justify our neglecting the correcting term. This is further supported by the osmotic measurements described below. We shall assume, therefore, that Vosburgh and Cooper's theory[13] can be applied to our results.

These authors have shown that, when there is a possibility of more than one coördination compound being formed in solution, the continuous variations calculations should be made at carefully selected wave lengths. As in the previous section, we found it necessary to carry out the reaction in hot solution. Figure 6 shows the results of a preliminary study in which atomic extinction coefficients were measured for $^2/_3$ basic chromium(III) chloride, alone and after having been heated for 48 hours with 1, 2 and 3 moles of alanine. The last three spectral curves all intersect at much the same

(11) J. C. Bailar and J. B. Work, THIS JOURNAL, **67**, 176 (1945).

(12) P. Job, *Compt. rend.*, **180**, 928 (1925).

(13) W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 437 (1941).

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

wave length, a circumstance which is unfavorable to Vosburgh and Cooper's method of selecting wave lengths, except when investigating the possibility of a tris-(alanine)-chromium(III) species in solution. In that case we choose wave lengths of 400 and 540 mμ for maximum difference between curves C and D.

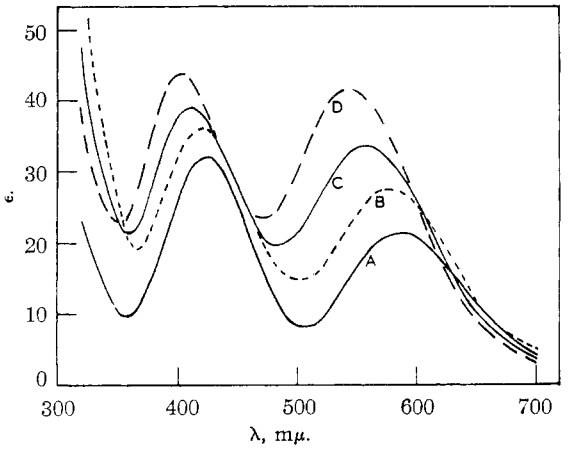


Fig. 6.—Absorption spectra: A, ²/₃ basic chromium(III) chloride, $Cr(OH)_2Cl$, after boiling; B, C, D, respectively, represent the same solution after boiling with 1, 2, 3 moles of alanine per atom Cr.

In applying the continuous variations method to the chromium(III)–alanine system, $10x$ ml. M alanine and $10(1 - x)$ ml. ²/₃ basic M chromium(III) chloride, $Cr(OH)_2Cl$, were placed in a series of stoppered tubes and heated for 48 hours on the water-bath. The contents were then cooled, tested for pH, diluted, and the optical density and total chromium concentration were measured. In Figs. 7 and 8 the difference, Y, between observed optical density and the value for no reaction is plotted against x for a number of wave lengths. The maximum occurs at different values of x as the wave length is varied, indicating the presence of more than one chromium–alanine complex.[13] Gould and Vosburgh[15] have pointed out that, although it is theoretically incorrect to use Y calculated in the above manner when more than one colored complex is present, nevertheless in practice reliable results are still obtained. Assuming that to hold true here, we conclude from Figs. 7 and 8 that the solutions examined contain at least two, and probably three, species, in which three, two or one molecule of alanine, respectively, are coördinated to each chromium atom. Their relative proportions in a solution such as A (Fig. 1) can be estimated by isopiestic measurements.

Isopiestic Measurements.—A solution similar to that represented by A in Fig. 1 was prepared by slowly adding to a boiling solution of 1 mole of normal chromium(III) sulfate and 6 moles of alanine, first 1 mole of barium chloride and then two moles of barium hydroxide. The pH of the centrifuged solution was 4.6, and Van Slyke measurements gave $\bar{n} = 1$, as before. A conductimetric titration with silver nitrate gave an end-point corresponding to one chloride ion per chromium atom.

(15) R. K. Gould and W. C. Vosburgh, THIS JOURNAL, **64**, 1630 (1942).

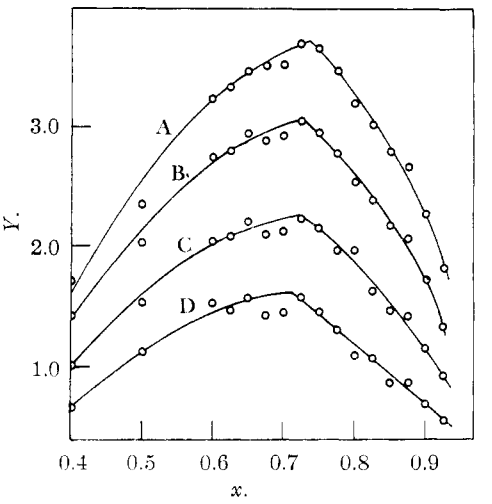


Fig. 7.—Continuous variations—plot of Y against x: A, 400 mμ; B, 410 mμ; C, 420 mμ; D, 430 mμ.

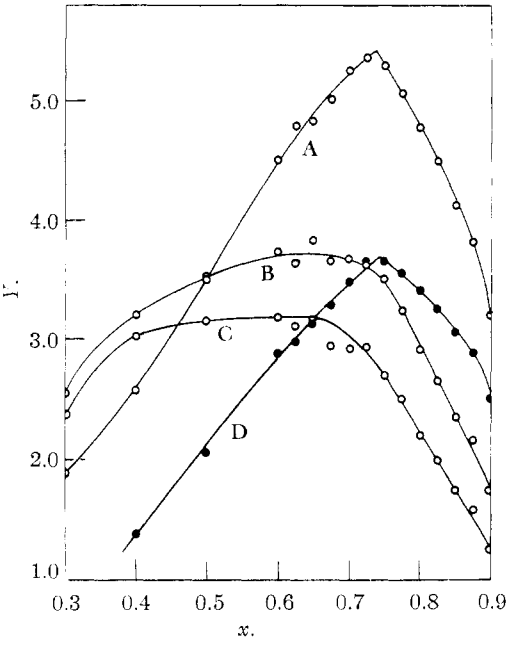


Fig. 8.—Continuous variations—plot of Y against x: A, 530 mμ; B, 570 mμ; C, 580 mμ; D, 500 mμ.

If we assume the absence of polynuclear complexes, the solution should therefore consist of free alanine, chloride ions and $Cr(Alanine)_z^+$, where we should expect z to lie between 2 and 3. The solution was equilibrated by the isopiestic method[7] against solutions of sodium chloride ranging from 0.1 to 2.0 molal. If m_1 is the molality of sodium chloride solution in equilibrium with a solution of m_2 g. atom Cr per 1000 g. water, then

$$2\phi_1 m_1 = 2\phi_2 m_2 + \phi_3 m_2(3 - z)$$

where ϕ_1, ϕ_2, ϕ_3 are the osmotic coefficients of sodium chloride, the complex chloride and alanine, respectively. At pH 4.6 the alanine is almost entirely in the zwitterion form, so that ϕ_3 is almost exactly unity[16] and the equation reduces to

$$z = 3 + 2\phi_2 - 2\phi_1 m_1/m_2$$

(16) R. A. Robinson, *J. Biol. Chem.*, **199**, 71 (1952).

The osmotic coefficient of sodium chloride, ϕ_1, is well known,[17] and, if the chromium complexes are mononuclear, ϕ_2 for the chloride, $Cr(Alanine)_z^+ Cl^-$, will probably lie somewhere between the two extremes represented by lithium chloride and cesium chloride.[17] In Fig. 9, the plotted experimental points show the values of z obtained when ϕ_2 is put equal to ϕ(NaCl) at the same molality. The other two curves are calculated in the same way with $\phi_2 = \phi$(LiCl) and $\phi_2 = \phi$(CsCl). It is seen that z never falls below 2 and probably lies quite near 3 over a wide concentration range. The decrease in z with dilution must denote the change from $Cr(Alanine)_3^+$ to $Cr(Alanine)_2^+$ by aquation, probably accompanied by a decrease in $\bar{n}$, the degree of chelation. This is confirmed by measurements of the absorption spectrum. In the more concentrated solutions this remains constant for long periods, but in dilute solutions the two maxima gradually become less and move toward longer wave lengths, indicating loss of carboxyl or amino groups, or both, from the complex ion.

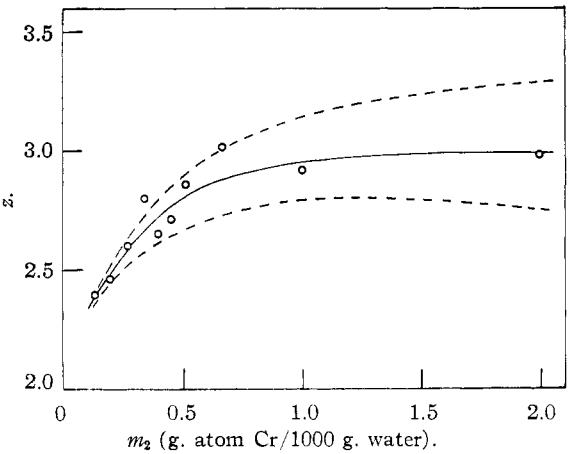
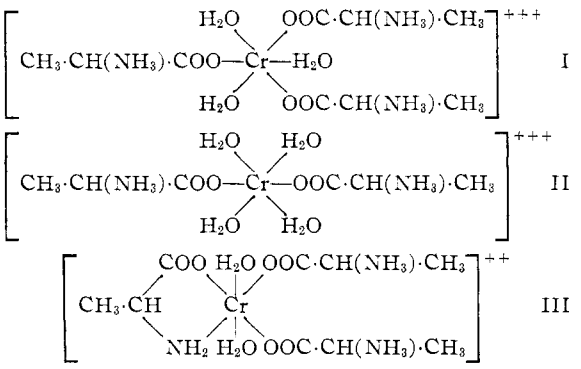


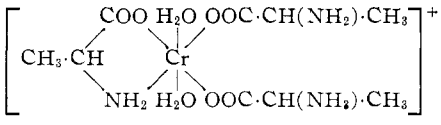
Fig. 9.—Values of z in $Cr(Alanine)_z^+Cl^-$ calculated from isopiestic measurements. The plotted points and the continuous curve have been derived by assuming the osmotic coefficient of the complex chloride to be equal to ϕ(NaCl). The upper broken curve is derived in the same way from ϕ(LiCl) and the lower from ϕ(CsCl).

Discussion

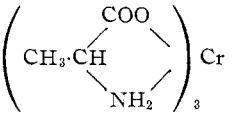
It appears from the evidence of the Van Slyke determinations that a certain amount of chelation occurs in boiled solutions of Cr(III) and alanine even at low pH values. The isopiestic and continuous variations methods, which do not distinguish between chelation and simple coördination, indicate the presence of two or three molecules of alanine per chromium atom. The observed results can be most simply explained by postulating the presence in acid solution of such species as I, II and III, their relative amounts depending on pH and concentration.



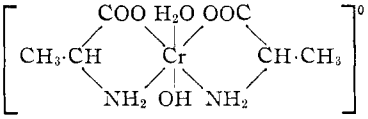
The proportion of III increases as alkali is added to the hot solution until, after the addition of two equivalents, every chromium atom carries one alanine chelate ring (A, Fig. 1). In fairly strong solutions the predominant species probably now has the formula IV, with one proton distributed between the



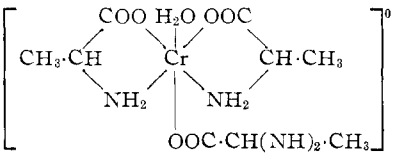
two free amino groups. Addition of alkali to the dilute solution in the cold simply titrates the remaining $-NH_3^+$ group, which is a slightly stronger acid than that in free alanine (Fig. 5). Chelation must occur very slowly, if at all, in the cold, and the uncharged form of IV gradually decomposes, ultimately to $Cr(OH)_3$. In concentrated solutions at 100°, on the other hand, chelation is the favored reaction and occurs as soon as the $-NH_3$ group begins to be titrated. This depresses the high temperature pH titration curve in the form characteristic of strong complex formation (Fig. 5). The precipitated solid is the pure tris-(alanine) chelate, V.



When the amount of alkali added to the hot solution is increased beyond B (Fig. 1), some of the complex bound alanine of IV may be displaced by hydroxyl, with simultaneous chelation of more amino groups, to produce some such species as VI.



This is probably still soluble but, by virtue of its hydroxyl group, can undergo olation polymerization, either with another molecule of VI, or with the uncharged form of IV. The insoluble macromolecular product would then have a value of $\bar{n}$ between 2 and 3, as we have observed. Neutral solutions prepared by Ley and Ficken's method contain only uncharged complexes. If the concentration of excess alanine is high, $\bar{n}$(solution) is initially 2.0 and the predominant species in solution may be VI or VII. According to their relative proportions,



(17) R. A. Robinson and R. H. Stokes, *Trans. Faraday Soc.*, **45**, 612 (1949).

the solution will deposit V, or an intermediate solid with $\bar{n}$ between 2 and 3, or the pure basic bis-(alanine) derivative. Formation of the insoluble olated form of VI must be a reaction of higher order than the simple ring closure necessary to produce V from VII. Hence, as the solution becomes more and more dilute with age, the speed of the olation reaction will decrease more rapidly than that of the chelation reaction. This explains the increase in $\bar{n}$(solid) for successive fractions collected from the same solution. The corresponding decrease in $\bar{n}$(solution) can be explained by postulating a parallel aquation reaction in which chromium–oxygen and chromium–nitrogen bonds are broken in the same way as in dilute solutions.

SINGAPORE

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF SAN DIEGO STATE COLLEGE]

Spectrophotometric Study of the Hydrolysis Constants of the Negative Ions of Some Aryl Imidazoles

BY HAROLD WALBA AND ROBERT W. ISENSEE

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A special spectrophotometric method for determining the hydrolysis constants of some very weak acids in water at 25.0° is given. Medium and salt effects are considered. A linear relationship between the pK_h and the square root of the ionic strength is observed for 2-phenylbenzimidazole. A pK_a of 14.2 is estimated for the acidity constant of imidazole. The effect of resonance on the acid strengths of the aryl imidazoles is discussed.

The amphiprotic nature of imidazole and its derivatives has long been known. Whereas considerable quantitative data on their base strengths may be found in the literature,[1] such data are lacking for the acid strengths. In this study we have obtained quantitative measures of the acid strengths in water of the following compounds: 2-phenylbenzimidazole, 2,4(or 2,5)-diphenylimidazole, benzimidazole, 2-phenylimidazole and 4(or 5)-phenylimidazole. The weakness of these compounds as acids as well as their low water solubility suggested a spectrophotometric study.

$$R^- + HOH = HR + OH^- \qquad (1)$$

The equilibrium studied was the hydrolysis reaction (1) where HR represents the acid and R^- its conjugate base. The classical equilibrium constant for this reaction is the hydrolysis constant, K_h, defined for the case in which water is the solvent by

$$K_h = c_{OH^-}(c_{HR}/c_{R^-}) \qquad (2)$$

where c is the concentration in moles/liter of the species indicated by the subscripts. The classical acidity constant, K_a, is related to K_h by

$$K_a = K_w/K_h \qquad (3)$$

where K_w is the appropriate ionization product of water and is defined by

$$K_w = c_{H^+}\, c_{OH^-} \qquad (4)$$

The hydrolysis equilibrium can be followed spectrophotometrically if the spectrum of the neutral molecule differs from that of the negative ion. The relationship between the spectral data and the concentrations of the neutral acid and its ion has been derived by Stenstrom and Goldsmith[2] and is given by the equation[3]

$$c_{HR}/c_{R^-} = (A^- - A)/(A - A^\circ) \qquad (5)$$

where A^- is the absorbancy of the negative ion, A is the absorbancy of the solution containing un-ionized acid and its negative ion in equilibrium, and A° is the absorbancy of the un-ionized acid. Substituting for c_{RH}/c_{R^-} in (2) one obtains

$$K_h = c_{OH^-}(A^- - A)/(A - A^\circ) \qquad (6)$$

To solve (6) for K_h a knowledge of the absorbancy of the negative ion is needed. In the case of acids having an acidity constant greater than 10^{-10}, the absorbancy of the negative ion may be obtained from solutions with relatively small concentrations of base (about 10^{-2} N). In such cases there is little uncertainty caused by the medium effect, that is, the shifting of the absorption curve with changes in salt concentration. For the aryl imidazoles that are the subject of this paper the medium effect could not be neglected. For instance, even the strongest acid of the series, 2-phenylbenzimidazole, requires a concentration of base greater than 1 N to be converted to the negative ion. At this relatively high concentration of base a medium effect was detectable. Hence the value obtained for the absorbancy could not be used without error in calculations involving lower concentrations of base, which are the ones required for the equilibrium measurements. In addition 2-phenylimidazole and 4(or 5)-phenylimidazole are such weak acids that even in 3.5 N base they are still far from being neutralized.

The equation that is derived below enables the absorbancy of the negative ion to be calculated directly from experimental values even for acids too weak to be neutralized in experimentally attainable concentrations of base. It also helps to eliminate uncertainties caused by optical medium effects.

The relationship between the classical and the thermodynamic hydrolysis constants is given by

$$K_h = K_h^\circ/F_h \qquad (7)$$

(1) K. Hofmann, "Imidazole and Its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1953, pp. 15, 251.

(2) W. Stenstrom and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).

(3) In the original derivation[2] the molecular extinction coefficient, ϵ, rather than the absorbancy was used. Absorbancy may be substituted for molecular extinction coefficient if all the absorbancies in a series of measurements are obtained at a constant stoichiometric concentration of acid, c, and constant cell length, d. Absorbancy = log (I_0/I) where log $(I_0/I) = \epsilon cd$.