

pared from cobalt(II) ion and cysteine in the presence of oxygen, as originally described by Schubert or by the metathetical reaction of cysteinate ion with the hexamminecobalt(III) ion. These results exclude the possibility, in the case of cysteine, of a desmotropic relationship between the cobalt(III)-sulfhydryl and cobalt(II)-disulfide structures suggested by Michaelis⁹ for the dark-brown thioglycolic acid complex with cobalt.

The difference between a hydroxo complex, such as that suggested by Schubert, and a hydrate lies in the number of replaceable hydrogen atoms. The empirical formulas for the bis-cysteinate may be written as $\text{HCoCy}_2 \cdot \text{H}_2\text{O}$, or $\text{H}_2\text{CoCy}_2\text{OH}$, according to whether the complex contains one or two hydrogen atoms which can be titrated in water, and hence are more strongly acidic than water. Titration of the acid form of the complex with base shows only one point of inflection, corresponding to only one replaceable hydrogen, and a neutralization equivalent of 326. For $\text{HCoCy}_2 \cdot 2\text{H}_2\text{O}$, the figure is 334. This evidence, coupled with the formation of only two ions on dissociation of the

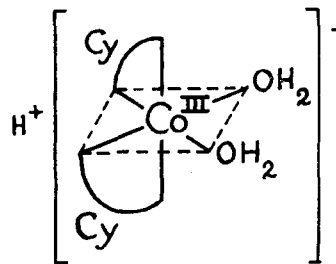


Fig. 2.—Structure of cobalt(III) bis-cysteinate.

sodium salt, corresponds with a mononucleate structure (Fig. 2) and eliminates the possibility of a binucleate structure possessing two hydroxo bridges. The complex may be represented in the conventional manner in which the central cobalt atom is surrounded octahedrally by two bidentate cysteine ligands, "cy," and two water molecules.

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

Cysteine Complexes with the Cobalt(III) Ion. II. Spectrophotometric Study of the Nature of Coördination in the Complexes of Cysteine with the Cobalt(III) Ion

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The three cysteine complexes of cobalt(III) first isolated by Schubert have been reinvestigated, their interrelationship demonstrated and their structures proved. Green cobalt(III) tris-cysteinate was prepared from hexamminecobalt(III) chloride, as in the case of the bis-cysteinate. This complex is stable only above about $p\text{H}$ 11; lowering the $p\text{H}$ to about 8 causes decomposition to the bis-cysteinate and cysteine. The bis- and green tris-cysteinate were shown, spectrophotometrically, to be quantitatively interconvertible by raising or lowering the $p\text{H}$ value. The nature of coördination in these complexes was deduced from a comparative study of the absorption spectra of the cobalt(III) chelates with thioglycolic acid, 2-mercaptoethylamine, aminoacetic acid and N-formylcysteine. Coördination in the bis- and green tris-cysteinate occurs through the sulfhydryl and amino groups of cysteine. In the isomeric red tris-cysteinate coördination to cobalt occurs *via* the sulfhydryl and carboxyl groups. Sodium cysteinate at $p\text{H}$ 12 converts the red tris-cysteinate irreversibly to the green isomer.

Although three complexes of cysteine with the cobalt(III) ion were isolated in the solid state by Schubert³ as long ago as 1931, no study of the nature of coördination in these compounds has been carried out. On the basis of a general similarity in the absorption spectra of the brown bis-thioglycolate and bis-cysteinate of cobalt(III), Schubert concluded that the sulfhydryl and carboxyl groups of cysteine were coördinated to the cobalt atom. The dissimilarity in absorption spectra of the red tris-cysteinate and tris-alaninate complexes of cobalt(III) appeared to support the same conclusion in the case of the red complex. No study was made with the green tris-cysteinate of cobalt(III). Martell and Calvin⁴ later suggested that coördination in these complexes probably oc-

curs *via* the amino and sulfhydryl groups of cysteine, since both nitrogen and sulfur are strong electron donors showing marked tendency to combine with cobalt. The carboxylate group of cysteine was thought not to be coördinated with cobalt for steric reasons.

In the study of the metal-catalyzed oxidation of cysteine with oxygen it was of great interest to establish the interrelationship and nature of coördination in the three complexes of cobalt(III). This paper presents the results of a detailed spectrophotometric study of cobalt(III) with cysteine, thioglycolic acid, aminoacetic acid, 2-mercaptoethylamine and N-formylcysteine. By comparing the ultraviolet absorption spectra of complexes in which the nature of coördination is unambiguous, the structures of the cysteine complexes of cobalt(III) were established. In addition, it has been shown that the brown bis- and the green tris-cysteinate are quantitatively, and reversibly, interconvertible. Evidence is also presented to show that the green and red tris-cysteinate of cobalt(III) are structural isomers and not stereoisomers.

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(2) Papers I, II and III, of this series are based on a portion of the thesis submitted by Roy G. Neville in 1954 to the University of Oregon in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) M. P. Schubert, *THIS JOURNAL*, **53**, 3851 (1931); **55**, 3336 (1933).

(4) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, New York, N. Y., 1952, p. 382.

Experimental Materials and Methods

Materials.—The chemicals listed were supplied by Eastman Organic Chemicals, Rochester, New York. All were of C.P. grade, and no further attempts at purification were made: cysteine hydrochloride, thioglycolic acid, aminoacetic acid, alanine, cystine, glutathione, tyrosine, 1-hexanethiol. S-Methylcysteine was prepared by methylation of cysteine with dimethyl sulfate in alkaline solution, according to the method of du Vigneaud.⁵ The recrystallized compound turned brown at 235–240°, and melted with decomposition at 248°. N-Formylcysteine was prepared by the method of Fruton and Clarke,⁶ by treating cystine in 90% formic acid with acetic anhydride and reducing the di-N-formylcysteine with zinc in dilute formic acid. Decomposition of the copper(I) salt with hydrogen sulfide yielded N-formylcysteine as colorless crystals, m.p. 87–89° dec. A small sample of 2-mercaptoethylamine, prepared from ethyleneimine,⁷ was kindly donated by Oregon State College, Corvallis, Oregon.

All compounds containing sulfhydryl groups were assayed for free -SH by the iodometric method of Lavine,⁸ and solutions used in quantitative work corrected accordingly.

Apparatus and Procedure.—Absorption spectra were determined using a Beckman spectrophotometer model DU, equipped with hydrogen and tungsten lamps. Stopped quartz cells, of thickness 10 mm., were used throughout. All solutions for spectrophotometric determination were made up directly by weight, with subsequent dilution if necessary.

General Qualitative Survey.—In cysteine three possibilities exist for the octahedral coordination of groups about the central cobalt atom: namely, coordination of -NH₂ and -COOH, -NH₂ and -SH, or -SH and -COOH. To establish which of these possibilities holds for the cysteine complexes of cobalt(III), a series of experiments was carried out using compounds containing either one or two of the above functional groups. At a given pH value, the ease with which each of these compounds formed a cobalt(III) complex with either cobalt(II) or cobalt(III) ion was determined.

One-ml. aliquots of 0.01 M cobalt(II) sulfate were treated at 20° with excess of an amino acid or thiol compound. Each solution was made progressively more alkaline by addition of 7.5 M potassium hydroxide, no attempt being made to exclude atmospheric oxygen. A similar series of experiments was carried out using 0.01 M hexamminecobalt(III) chloride. In alkaline solution, compounds possessing a free -SH group immediately formed a highly colored Co(III) complex. Compounds possessing -NH₂ or -COOH, or blocked sulfhydryl (e.g., -SCH₃), gave no reaction. By boiling solutions of methionine or aminoacetic acid for several hours low yields of Co(III) complex were produced. Since complexes form immediately on mixing either Co(II) or Co(III) ion with cysteine, it appears that coordination occurs through the -SH group.

Interconversion of Cobalt(III) Bis- and Green Tris-cysteinate.—A solution of the green tris-cysteinate, on exposure to air, rapidly turns brown by formation of bis-cysteinate. To establish whether this reaction is reversible, 1 g. of doubly recrystallized bis-cysteinate was mixed with a tenfold excess of cysteine hydrochloride dissolved in a small amount of water. The mixture was titrated to pH 10 with 6 M potassium hydroxide, using a Beckman pH meter. The bis-cysteinate was completely soluble at this pH value. A brisk stream of oxygen-free nitrogen was passed through the solution to expel dissolved oxygen. The temperature was gradually raised to 60°, then the whole allowed to cool, with the nitrogen still passing. The spectrum of this deep-green solution was identical with that of the green tris-cysteinate prepared by the method of Schubert.³ Addition of a few drops of 30% hydrogen peroxide caused the solution to change to the intense yellow characteristic of the sulfone.

To establish that these complexes were quantitatively interconvertible, their spectra were examined in the region 320–620 m μ . To 10 ml. of 0.05 M cysteine hydrochloride 0.2 M sodium hydroxide was added to pH 8. The mixture was added to 5 ml. of 0.01 M hexamminecobalt(III) chloride, and the whole diluted to 50 ml. On warming to 50° the

bis-cysteinate formed rapidly. The spectrum of this solution was determined at a Co concn. of 5×10^{-4} M.

The same experiment was repeated at pH 11. The spectrum was that of the green tris-cysteinate. On lowering the pH to 8 (by addition of a trace of concd. sulfuric acid to maintain constant volume), the brown bis-cysteinate was produced, the spectrum being identical with that of the bis-cysteinate originally prepared at pH 8. The bis- and green tris-cysteinate are thus quantitatively interconvertible.

Conditions of Formation of Green Cobalt(III) Tris-cysteinate.—To investigate the conditions of formation of these complexes, in particular the green tris-cysteinate, an apparatus was constructed in which the complexes were prepared in absence of oxygen. The apparatus consisted essentially of a Thunberg tube connected with a system of six gas-washing bottles.

Solutions for investigation were prepared as follows. Aliquots of 0.5 ml. of 0.005 M hexamminecobalt(III) chloride were placed in the side-arms of a number of Thunberg tubes. Appropriate amounts of cysteine, previously titrated with sodium hydroxide to pH 10.5, were placed in each of the tubes and the total volume made up to 5.0 ml. to give solutions 8.33×10^{-4} M in Co and of pH about 10. In this way, Co:cysteine ratios varying from 1:1 to 1:17 were prepared. Dissolved oxygen in the solutions was removed by means of a water-pump connected to the tube *via* a three-way stopcock. Nitrogen from a cylinder was purified from traces of oxygen by passage through a wash-bottle containing alkaline pyrogallol, then through five tubes containing Fieser's solution.⁹ This nitrogen was admitted to the solutions, which were then mixed and allowed to stand at 20° for two hours. The spectrum (between 380 and 620 m μ) of each solution was then determined by placing the Thunberg tubes directly into the Beckman spectrophotometer, no correction being made for the roundness of the tubes. In this region, the spectra were identical with those obtained using optically-flat quartz cells. Solutions of ratio 1:1 to 1:3 were brown, but ratios from about 1:3 to 1:17 were progressively greener in color, possessing absorption maxima at 440 and 580 m μ , characteristic of the green tris-cysteinate. At ratios greater than 1:5 the spectrum was that of the pure tris-cysteinate. No change in these spectra was observed even on standing for 168 hours. In entire absence of oxygen, therefore, the green tris-cysteinate is stable.

Cobalt(III) Tris-cysteinate (Green Isomer).—Specimens of this complex, prepared from cobalt(II) ion and cysteine by the method of Schubert,³ were found always to be contaminated with bis-cysteinate and were thus unsuitable for spectral work. Solutions for spectrophotometric use were prepared directly from hexamminecobalt(III) chloride.

Cobalt(III) Tris-cysteinate (Red Isomer).—This complex was prepared using a modification of the Schubert method. Potassium hydroxide (7.5 M, 10 ml.) in 15 ml. of water was added to 10 g. of cysteine hydrochloride dissolved in 40 ml. of water. This mixture was added to 33 ml. of 1 M cobalt(II) sulfate containing 9 ml. of 7.5 M potassium hydroxide, the whole was quickly stirred then filtered. Air was passed through the filtrate for one hour. After 24 hours, the precipitate was filtered, washed with water followed by ethanol, then dried in the air; yield 90%. Owing to its insolubility in dilute acids and water, this complex was not recrystallized. The complex was soluble in dilute sodium hydroxide, but heating this solution resulted in partial decomposition to the bis-cysteinate. Cobalt determination by electrolytic deposition corresponded to the anhydrous tris-cysteinate.

Anal. Calcd. for H₃[Co(SCH₂(NH₂)CHCOO)₃]: Co, 14.10. Found: Co, 14.15, 14.08 (2 detns.).

Substitution of hexamminecobalt(III) chloride for cobalt(II) sulfate in the above preparation gave bis-cysteinate only.

On treating the tris-cysteinate with excess hot sodium cysteinate at pH 12 the green isomer formed rapidly. Lowering the pH to 6 gave the bis-cysteinate.

Cobalt(III) Tris-N-formylcysteinate.—This complex was prepared by a procedure similar to that described above, using 0.22 g. of N-formylcysteine, in 1 ml. of water. After adjusting to pH 6 with 7.5 M potassium hydroxide, cobalt(II) sulfate (0.77 ml., 1.0 M) was added.

(5) V. du Vigneaud, H. S. Loring and H. A. Craft, *J. Biol. Chem.*, **105**, 481 (1934).

(6) J. S. Fruton and H. T. Clarke, *ibid.*, **106**, 667 (1934).

(7) E. J. Mills and M. T. Bogert, *THIS JOURNAL*, **62**, 1173 (1940).

(8) T. F. Lavine, *J. Biol. Chem.*, **109**, 141 (1935).

(9) L. F. Fieser, "Experiments in Organic Chemistry," Heath and Co., Boston, Mass., 1941, p. 395.

Anal. Calcd. for $H_3[Co(SCH_2(NH_2)CHCOO)_3]$: Co, 11.71. Found: Co, 11.73, 11.74 (2 detns.).

Cobalt(III) Tris-aminoacetate.—Two isomeric forms of this complex exist, a violet dihydrate and a rose-red monohydrate.¹⁰ The violet isomer was prepared by the method of Ley and Winkler,¹¹ from cobalt(III) hydroxide and aminoacetic acid, and was obtained as deep-violet crystals of composition $[Co(OOCCH_2NH_2)_3] \cdot 2H_2O$. From the filtrate the rose-red monohydrate was obtained in small yield. For spectrophotometric determinations, the violet complex was recrystallized twice from boiling water then dried at 80°.

The red isomer was prepared by a new method from hexamminecobalt(III) chloride. Aminoacetic acid (2.0 g., 0.0263 mole) and hexamminecobalt(III) chloride (2.34 g., 0.0087 mole) were dissolved in 150 ml. of water. Potassium hydroxide (6.6 M) was added to pH 10, the solution boiled under reflux for three hours, then evaporated to the point of crystallization. The deep rose-red crystals of tris-aminoacetate monohydrate, after recrystallizing twice from water, were dried at 80° and used without further purification. The spectrum of this complex was identical with that of the monohydrate prepared by the method of Ley and Winkler.

Cobalt(III) Bis-thioglycolate.—The potassium salt of this complex was prepared using a modification of the method of Michaelis and Schubert.¹² For spectrophotometric use, solutions were prepared by direct weighing.

Complexes of Cobalt(III) with 2-Mercaptoethylamine.—Owing to the difficulty of isolation of pure specimens of cobalt(III) complexes with 2-mercaptoethylamine, solutions for spectrophotometric use were prepared from cobalt(II) ion as described below.

Spectrophotometric Determinations.—Bis-complexes of cobalt(III) with cysteine, 2-mercaptoethylamine and thioglycolic acid were prepared from 1:2 molar ratios of 0.01 M cobalt(II) sulfate and 0.01 M solutions of chelating agent, dilute potassium hydroxide being added to raise the pH to 8. After air oxidation for five minutes, spectra were determined at a Co concn. of 5×10^{-4} M.

The green tris-complexes of cysteine and 2-mercaptoethylamine were prepared by mixing 1 ml. of 0.01 M hexamminecobalt(III) chloride with from 3 to 10 ml. of chelating agent, dilute potassium hydroxide being added to pH 11.5. The solution was then diluted almost to 100 ml. with water, heated to 60° to effect formation of the complex, cooled, and the solution made up to 100 ml. to give a Co concn. of 1×10^{-4} M. For convenience, spectral measurements were

made at a Co concn. of 1×10^{-2} M in the case of the violet and red tris-aminoacetates. Solutions of the red tris-cysteinate and tris-N-formylcysteinate 5×10^{-3} M in Co, at pH 10, were prepared by direct weighing.

All spectra were measured in the range 210–700 m μ . Absorption maxima and extinction coefficients are listed in Table I.

Discussion of Results

The interconversion of the bis- and green tris-cysteinate may be explained in the following manner. When hexamminecobalt(III) ion and cysteine are mixed in dilute solution (about 10^{-3} M) in the region of pH 10, there exists an equilibrium comprising green tris-cysteinate, bis-cysteinate and sodium cysteinate, which constitutes a buffer sys-

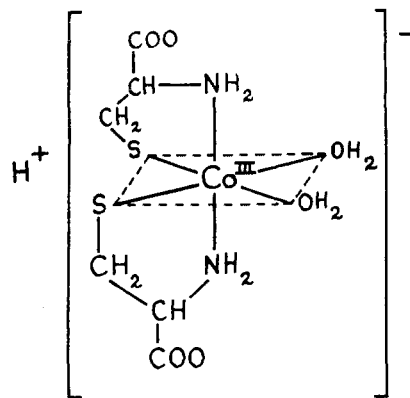


Fig. 1.—Bis-cysteinatodiaquocobaltate(III).

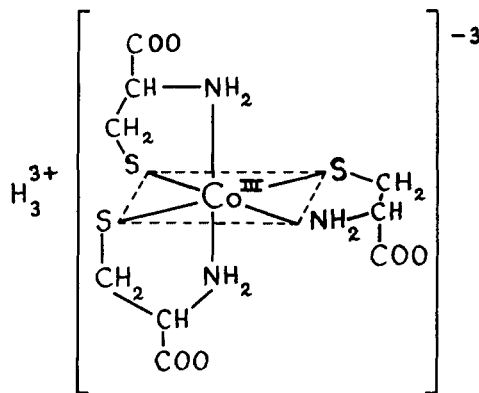


Fig. 2.—Tris-cysteinatocobaltate(III), green isomer.

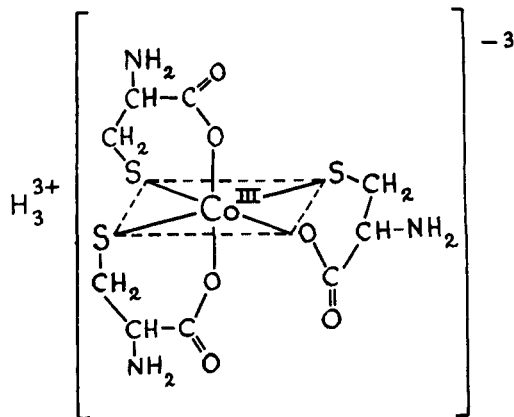


Fig. 3.—Tris-cysteinatocobaltate(III), red isomer.

TABLE I

SPECTRAL DATA FOR COBALT(III) COMPLEXES

Complex of Co(III)	Concn. (moles/l. $\times 10^{-4}$)	Absorption max., m μ	Extinction coefficient ($\times 10^3$)
Bis-cysteinate	2	280	7.50
	2	350	5.51
	2	440	2.25
Bis-(2-mercaptoethylamine)	5	280	6.8
	5	350	4.8
	5	440	1.8
Bis-thioglycolate	5	350	4.3
Tris-aminoacetate (violet)	50	380	0.134
	50	540	0.108
Tris-aminoacetate (red)	50	540	0.050
Tris-(2-mercaptoethylamine)	5	340	4.18
	5	580	0.34
Tris-cysteinate (green)	1	280	18.5
	1	330	3.68
	1	570	0.30
Tris-cysteinate (red)	0.5	270	4.9
	.5	410	3.4
	.5	505	8.8
Tris-(N-formylcysteinate)	0.5	270	3.2
	.5	420	2.9
	.5	490	5.2

(10) H. Krebs and R. Rasche, *Z. anorg. Chem.*, **276**, 236 (1954).

(11) H. Ley and H. Winkler, *Ber.*, **42**, 3894 (1909).

(12) L. Michaelis and M. P. Schubert, *THIS JOURNAL*, **52**, 4418 (1930).

tem. At 1:1-1:3 ratios, the equilibrium lies in favor of the bis-cysteinate but as the cobalt:cysteine ratio is increased above 1:3 progressively more of the green complex is formed. At ratios greater than about 1:5 the equilibrium is shifted totally in favor of the tris-cysteinate. Above pH 11, a 1:3 ratio gives pure tris-cysteinate showing that chelation of the third cysteine residue is strongly pH-dependent. From these observations it is clear that the third cysteine molecule is not so tightly bound to cobalt as are those in the bis-cysteinate, which is not decomposed in strong acid. Lowering the pH from 11 to 8, even in concentrated solutions, causes immediate dissociation of the tris-cysteinate to the bis-complex and cysteine. The corresponding cobalt(III) chelates with 2-mercaptoethylamine behave in the same manner. Comparison of the spectra of the bis- and green tris-complexes at pH 8 and 11 show that these compounds are quantitatively interconvertible. The marked instability toward oxygen of the green tris-complexes of cysteine and 2-mercaptoethylamine may be explained by the ease of dissociation of the third ligand, which is then oxidized to the corresponding disulfide and does not recombine with the cobalt.

The very close similarity in the spectra of the

bis- and green tris-complexes of cysteine and 2-mercaptoethylamine, together with the quantitative interconversion data, lead to the formulation of the bis- and green tris-cysteinate as sulfhydryl- and amino-coordinated complexes (Figs. 1 and 2).¹³ In N-formylcysteine, the formyl group renders the nitrogen atom less basic than it is in cysteine resulting in sulfhydryl- and carboxyl-coordination. The similarity in spectra of the tris-complexes of cysteine and N-formylcysteine indicates that this mode of coordination occurs in the red triscysteinate (Fig. 3).

Treatment of the red tris-cysteinate with hot sodium cysteinate at pH 11-12 results in rapid formation of the green isomer. Lowering the pH to 6 gives the bis-cysteinate. The red tris-cysteinate is actually prepared at pH 6, from cobalt(II) ion and cysteine, but the above observation shows that, once formed, sulfhydryl- and amino-coordination is favored. The red and green tris-cysteinate are thus not interconvertible.

Acknowledgment.—We wish to express our thanks to the Research Corporation for financial assistance in this work.

(13) Paper I of this series discusses the structure of the bis-cysteinate.

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

The Constitution of the Pyridine Monocarboxylic Acids in their Isoelectric Forms

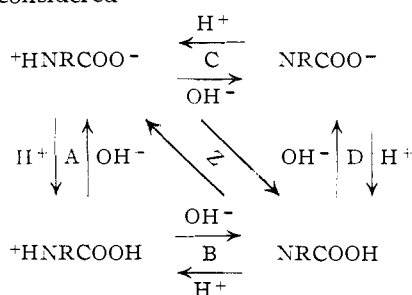
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Absorption spectra of the pyridine monocarboxylic acids, their methyl esters and N-methylbetaines demonstrate that in aqueous solutions of the acids near the isoelectric point the predominant species is the dipolar ion. From the dissociation constants of the methyl esters, it is found that less than 10% of the isoelectric form is present as uncharged molecules.

Introduction

In an aqueous solution of a pyridine monocarboxylic acid, NR₃COOH, the following equilibria must be considered



The cationic species behaves as a dibasic acid for which two thermodynamic dissociation constants K_1 and K_2 can be measured by the usual methods. These are related to the constants of the above equilibria by

$$K_1 = K_A + K_B \quad (1)$$

$$\frac{1}{K_2} = \frac{1}{K_C} + \frac{1}{K_D} \quad (2)$$

$$K_Z = \frac{K_A}{K_B} = \frac{K_D}{K_C} \quad (3)$$

Values of K_1 and K_2 have been determined for all three acids,^{1,2} and several workers¹⁻³ have shown that the changes in absorption spectra with pH of the aqueous solutions can be correlated with the two dissociation constants.

The equilibrium Z is independent of pH, and none of the individual constants K_A , K_B , K_C , K_D or K_Z can be evaluated from pH measurements on the acids alone. However, two conflicting views have recently been expressed as to the constitution of the predominant species in isoelectric solutions. Hughes, Jelinek and Ambrose³ state that nicotinic acid is not present as zwitterion in solution; Evans, Herington and Kynaston² support that opinion by referring to the dissociation of the carboxyl group in terms of K_2 . This implies a very small value for K_Z . On the other hand Jaffé⁴ has concluded from

(1) H. H. G. Jelinek and J. R. Urwin, *J. Phys. Chem.*, **58**, 548 (1954).

(2) R. F. Evans, E. F. G. Herington and W. Kynaston, *Trans. Faraday Soc.*, **49**, 1284 (1953).

(3) E. B. Hughes, H. H. G. Jelinek and B. A. Ambrose, *J. Phys. Colloid Chem.*, **53**, 414 (1949).

(4) H. H. Jaffé, *THIS JOURNAL*, **77**, 4445 (1955).