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

Cysteine Complexes with the Cobalt(III) Ion. I. The Mononucleate Structure of Cobalt(III) Bis-cysteinate

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Cobalt(III) bis-cysteinate has been prepared by the original method of Schubert and by a new method from hexamminecobalt(III) chloride. The results of analysis, titration and molecular weight determination indicate that the products obtained by both methods are identical and that the complex possesses a mononucleate structure of empirical formula, $HCoCy_2 \cdot 2H_2O$. No evidence was found for the binucleate structure suggested by earlier investigators.

Complexes of cobalt(III) formed in the reaction of cobalt(II) ion with alkaline solutions of cysteine in the presence of atmospheric oxygen were first studied by Harris,[3] Michaelis and his co-workers,[4-6] and Kendall and Holst.[7] The reaction is complicated, and many contradictory results exist in the earlier literature. Attempts to isolate the complexes met with little success until 1931–1933 when Schubert[8] described the preparation of several cysteine complexes of di- and trivalent cobalt. The isolation of these complexes in the pure state was found to be strongly dependent upon the concentration of reactants, the *p*H and the cobalt to cysteine molar ratio. Schubert described three interrelated cysteine complexes of cobalt(III), namely, a dark-brown complex of cobalt:cysteine ratio 1:2, and two isomeric complexes, green and red, in which the ratio was 1:3.

In the course of a study of the metal-catalyzed oxidation of cysteine by gaseous oxygen the cysteine complexes of cobalt(III) have been reinvestigated. Results significantly different from those reported by Schubert, and other workers, have been obtained. This paper is concerned with the formation and structure of cobalt(III) bis-cysteinate.

Historically, the cobalt(III) complexes of cysteine and thioglycolic acid are closely related, On mixing cobalt(II) ion with thioglycolic acid at *p*H 7–8, Michaelis and Schubert[9] found that, in entire absence of oxygen, a pale-green complex was formed in which the cobalt:thioglycolic acid ratio was 1:2. One-quarter of a mole of oxygen converted the green to an intensely dark-brown cobalt(III) complex, which was found to be dimeric by cryoscopic measurements carried out on the potassium salt. A binucleate structure, containing two hydroxo bridging groups, was therefore assigned to cobalt(III) bis-thioglycolate (Fig. 1). In the formation of cobalt(III) bis-cysteinate, from cobalt(II) ion and cysteine at *p*H 7–8, one-quarter of a mole of oxygen was absorbed, and the composition of the dark-brown complex was found to be $CoHCy_2 \cdot 5H_2O$, where "cy" represents $SCH_2CH(NH_2)COO$. Since the absorption spectra of the bis-thioglycolate and bis-cysteinate of cobalt(III) possessed a general similarity, Schubert tentatively formulated the bis-cysteinate as a binucleate structure, $[CoH_2Cy_2OH]_2 \cdot 8H_2O$, containing two hydroxo bridges, analogous to the bis-thioglycolate. Although quite insoluble in water, the complex dissolved in hydrochloric acid, and Schubert isolated a hydrochloride, $[CoH_2Cy_2OH]_2 \cdot 3HCl \cdot H_2O$. This observation, coupled with the spectral data, suggested that the amino group was not bound and that coördination to cobalt occurred by means of the sulfhydryl and carboxyl groups of cysteine.

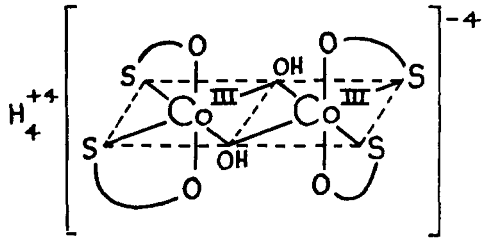

Fig. 1.—Structure of cobalt(III) bis-thioglycolate.

As the structure of cobalt(III) bis-cysteinate could not be regarded as having been firmly established, this paper describes work carried out to learn more about this complex. On the basis of analysis, titration, molecular weight determination and formation from hexamminecobalt(III) chloride, the structure of the bis-cysteinate is shown to be mononucleate. Determination of the nature of coördination in this complex will be described elsewhere.[10]

Experimental Materials and Methods

Materials.—Cysteine hydrochloride of C.P. grade was supplied by Eastman Organic Chemicals, Rochester, New York, and was used without further purification. Assay of fresh samples iodometrically, by ferricyanide, and by iron-catalyzed oxidation with gaseous oxygen, showed it to contain about 94% free sulfhydryl group. Throughout the investigation, standard solutions of cysteine were always stored under nitrogen and discarded after two days. Assays of four-day-old solutions showed that, even on storing under oxygen-free nitrogen, the percentage of free –SH group had fallen to 89. Cobalt(II) sulfate hexahydrate of C.P. grade was supplied by the J. T. Baker Chemical Company, Phillipsburg, New Jersey. Hexamminecobalt(III) chloride was prepared by the method of Inorganic Syntheses.[11] All other reagents employed were of C.P. grade.

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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] L. J. Harris, *Biochem. J.*, **16**, 739 (1922).

[4] L. Michaelis, *J. Biol. Chem.*, **84**, 777 (1929).

[5] L. Michaelis and E. S. G. Barron, *ibid.*, **83**, 191 (1929).

[6] L. Michaelis and S. Yamaguchi, *ibid.*, **83**, 367 (1929).

[7] E. C. Kendall and J. E. Holst, *ibid.*, **91**, 435 (1931).

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

[9] L. Michaelis and M. P. Schubert, *ibid.*, **52**, 4418 (1930).

[10] Paper II of this series, R. G. Neville and G. Gorin, *ibid.*, **78**, 4893 (1956).

[11] "Inorganic Syntheses," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 217.

Analyses for Cobalt.—Cobalt(II) sulfate was oxidized with hydrogen peroxide in dilute acid solution and precipitated as cobalt(III) hydroxide with sodium hydroxide.[12] The cobalt(III) hydroxide was dissolved in glacial acetic acid and sufficient 1-nitroso-2-naphthol (in 50% cetic aacid) added to precipitate the cobalt as $Co(C_{10}H_6O_2N)_3 \cdot 2H_2O$. Calcd. for $CoSO_4 \cdot 6H_2O$: Co, 22.40. Found: Co, 22.44.

For the determination of cobalt in the cysteine complexes of cobalt(III), a weighed amount of complex was decomposed to cobalt(II) ion by heating with a few ml. of concentrated nitric acid. Excess nitric acid was removed by heating with a small amount of concentrated sulfuric acid. On dilution with water, cobalt(II) ion was determined gravimetrically as above, or electrolytically.[13]

Assay of Cysteine.—Three different methods were used throughout to determine the amount of free sulfhydryl group in the cysteine hydrochloride.

1. **Iodometric Method.**—The iodometric determination as normally carried out tends to give high results, but Lavine[14] obtained accurate values with a solution of iodine in 1 *M* hydriodic acid (actually an equimolar mixture of 1 *M* HCl and 1 *M* KI). This method was employed as follows. Two 10-ml. aliquots of $KI-HCl-I_2$ mixture were taken, the solution being 0.025 *N* in iodine. To one aliquot 10 ml. of approximately 0.02 *M* cysteine solution was added. The other solution served as a control. Each solution was titrated with standard 0.025 *N* sodium thiosulfate, the difference between the two titration values being due to the iodine consumed by the cysteine. Starch was used as indicator. Found: -SH group, 95%.

2. **Ferricyanide Oxidation.**—Cysteine (0.04 *M*) was titrated with 0.04 *M* potassium ferricyanide. The end-point was located by making the solution alkaline with dilute ammonia,[7] then adding a few drops of 10% sodium nitroprusside. Ferricyanide was then added dropwise until the deep-purple nitroprusside-sulfhydryl complex disappeared. Found: -SH group, 94%.

According to Shinohara and co-workers,[15,16] and to Arnold,[17] the use of nitroprusside for sulfhydryl determination gives low values. In this work the results obtained agreed within 1% with those found by other methods.

3. **Iron-catalyzed Oxidation by Oxygen.**—Cysteine is completely oxidized to cystine by gaseous oxygen in the presence of catalytic amounts of iron salts in alkaline solution.[18] This reaction was used as an assay method.

Twenty ml. of 0.04 *M* cysteine in phosphate buffer (*p*H 7.0) was mixed with 2 ml. of 0.01 *M* ferrous ammonium sulfate in the oxygen-absorption apparatus described elsewhere.[19] The rate of oxygen uptake was very slow, and after 24 hr. only about one-half of the cysteine had been oxidized. Another 2 ml. of ferrous ammonium sulfate was added and the whole allowed to stand, with stirring, for a further 24 hours. Oxygen uptake was then complete since no more absorption occurred during a further 24-hr. period, even on adding 2 ml. of the ferrous solution.

The results of the three methods of sulfhydryl determination in cysteine may be summarized: Iodometric method, 95%; Ferricyanide method, 94%; Iron-catalyzed oxidation method, 94%.

Cobalt(III) Bis-cysteinate.—This complex was prepared from cobalt(II) ion by a method similar to that employed by Schubert[8] and by a new method from hexamminecobalt(III) chloride.

1. **Formation from Cobalt(II) Ion.**—Cysteine hydrochloride (5 g.) was dissolved in 20 ml. of water, and 7.5 ml. of 7.5 *M* potassium hydroxide was added. This solution was then poured into 16 ml. of 1 *M* cobalt(II) sulfate containing 4.5 ml. of 7.5 *M* potassium hydroxide. The mixture was stirred, filtered and air passed through the filtrate for 1 hr. Concentrated hydrochloric acid was then added dropwise until the dark-brown complex crystallized. Fifty ml. of 95% ethanol was added and the whole set on ice. The crystals were filtered, washed with a little cold water followed by ethanol, then dried in the air. The complex was recrystallized by dissolving in potassium hydroxide and reprecipitating with hydrochloric acid, followed by ethanol, as above.

Analysis by the electrolytic deposition method gave Co, 17.61% (3 detns.). Calcd. for $CoHCy_2 \cdot 2H_2O$: Co, 17.63.

2. **Formation from Hexamminecobalt(III) Chloride.**—Hexamminecobalt(III) chloride (10.0 g., 0.0373 mole) was dissolved in the minimum amount of hot water (100 ml.). To this solution were added 12.55 g. of cysteine hydrochloride (0.0746 mole, 94% -SH) in 35 ml. of water and with stirring 8.37 g. of potassium hydroxide (0.1492 mole) in 15 ml. of water. The mixture was heated at 60–70° until all evolution of ammonia had ceased (about 2 hr.), then concd. hydrochloric acid was added dropwise until the solution reached *p*H 1.0. The micro-crystalline precipitate of bis-cysteinate was filtered, washed several times with 50-ml. aliquots of cold water, then with 95% ethanol and dried in the air; yield 11.0 g. (88%). The complex was recrystallized by dissolving in potassium hydroxide and precipitating with concd. hydrochloric acid, as described above.

Analysis by the electrolytic deposition method gave Co, 17.64. Calcd. for $CoHCy_2 \cdot 2H_2O$: Co, 17.63.

Determination of Ammonia Liberated in Preparation from Hexamminecobalt(III) Chloride.—To establish that the complex prepared by both methods was identical, it was necessary to demonstrate that in the preparation of the bis-cysteinate from hexamminecobalt(III) ion and cysteine hydrochloride under basic conditions all the ammonia coördinated to cobalt was quantitatively replaced by water. The following equation was assumed to hold in the formation of this complex

$$[Co(NH_3)_6]Cl_3 + 6KOH + 2[HSCH_2CH(\overset{+}{N}H_3)COOH]Cl^{-} = K[Co(SCH_2CH(NH_2)COO)_2(H_2O)_2] + 5KCl + 6NH_3 + 4H_2O$$

According to this equation, six moles of ammonia is liberated per mole of hexamminecobalt(III) chloride. Two series of experiments were carried out. In one series, six moles of potassium hydroxide was used; and in the other, seven moles of potassium hydroxide per mole of hexamminecobalt(III) chloride. One-g. samples of cobalt(III) complex were used in every case. The mixture was boiled and the evolved ammonia swept out with a stream of nitrogen and absorbed in 200 ml. of standard (0.2500 *N*) hydrochloric acid. The residual acid was back-titrated with standard (0.1000 *N*) sodium carbonate. Found: 5.97 moles of ammonia evolved per gram atom of Co (3 detns.).

Titration of Cobalt(III) Bis-cysteinate.—The number of replaceable hydrogen atoms per atom of Co was determined by converting air-dried samples of bis-cysteinate (prepared by both methods) to the sodium derivative, by addition of two equivalents of standard base, then back-titrating with standard hydrochloric acid. The titration was conducted under nitrogen. In a typical experiment, 0.0334 g. of complex was equivalent to 1.02 atoms of hydrogen. For a mononucleate structure (see later) of mol. wt. 334.3, this corresponds to a H : Co ratio of 1 : 1.

Cryoscopic Determination of Molecular Weight.—The acid form of the bis-cysteinate was too insoluble for direct determination of molecular weight. Addition of one equivalent of sodium hydroxide gave the monosodium salt, $Na[Co(Cy)_2(H_2O)_2]$, which possessed markedly greater solubility. In solution, two ions are present per molecule of complex, Na^+ and $[Co(Cy)_2(H_2O)_2]^-$.

Cobalt(III) bis-cysteinate (0.3160 g.) was titrated under nitrogen to *p*H 7.0 with 0.104 *N* sodium hydroxide. The solution was diluted to 25.00 ml. with distilled water and the freezing point determined using a standard Beckmann apparatus. Assuming the complex to be mononucleate with a molecular weight of 334.3, the expected depression of freezing point for 0.3343 g. per 25 ml. would be 0.1484° (*i.e.*, 0.0742° per ion). In two determinations, using 0.3160 g. of complex, the depressions were 0.139 and 140°, corresponding to 1.98 and 2.00 ions, respectively.

Results and Discussion

The evidence presented shows that cobalt(III) bis-cysteinate, $H[Co(Cy)_2(H_2O)_2]$, may be pre-

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[12] A. I. Vogel, "A Text-book of Quantitative Inorganic Analysis," Longmans, Green and Co., London, 1947, p. 547.

[13] W. F. Hillebrand, "Applied Inorganic Analysis," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 419.

[14] T. F. Lavine, *J. Biol. Chem.*, **109**, 141 (1935).

[15] K. Shinohara and M. Kilpatrick, *ibid.*, **105**, 241 (1934).

[16] K. Shinohara, *ibid.*, **109**, 665 (1935).

[17] V. Arnold, *Z. physiol. Chem.*, **70**, 300 (1911).

[18] O. Warburg and S. Sakuma, *Arch. ges. Physiol.* (*Pfluger's*), **200**, 203 (1923).

[19] Paper III of this series.

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 Michaelis[9] 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 $HCoCy_2 \cdot H_2O$, or H_2CoCy_2OH, 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 $HCoCy_2 \cdot 2H_2O$, the figure is 334. This evidence, coupled with the formation of only two ions on dissociation of the 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.

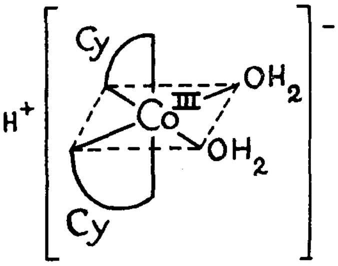

Fig. 2.—Structure of cobalt(III) bis-cysteinate.

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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*H 11; lowering the *p*H to about 8 causes decomposition to the bis-cysteinate and cysteine. The bis- and green tris-cysteinates were shown, spectrophotometrically, to be quantitatively interconvertible by raising or lowering the *p*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-cysteinates 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*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 Schubert[3] 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 Calvin[4] later suggested that coördination in these complexes probably occurs *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 coordination 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-cysteinates are quantitatively, and reversibly, interconvertible. Evidence is also presented to show that the green and red tris-cysteinates of cobalt(III) are structural isomers and not stereoisomers.

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(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.