[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, OKLAHOMA STATE UNIVERSITY AND UNIVERSITY OF OREGON]

Complexes of Cobalt(III) with Cysteine and 2-Aminoethanethiol[1]

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The triscysteinato-N,S-cobaltate(III) anion, hydrogen triscysteinato-O,S-cobaltate(III) and hydrogen biscysteinato-N,S-cobaltate(III) are discussed with respect to acid-base properties, spectra and structures. The related complexes, hydrogen tris-(3-mercaptopropionato)-cobaltate(III) and tris-(2-aminoethanethio)-cobalt(III) also have been prepared and are compared to the cysteine complexes.

Mixtures of cysteine and cobalt(II) salts at appropriate *p*H values reduce gaseous oxygen readily, and reactions of this type are of great interest, because they are akin to those which supply to most living organisms the energy necessary for the maintenance of life. Although the reaction in question does not occur in living cells to a significant extent, it may be hoped that the study of it would reveal general principles, applicable to the interpretation of respiration processes. Since, furthermore, cysteine derivatives perform multifarious functions in the cell,[2] their possible interaction with oxygen is important in its own right. This paper presents a fuller description of the products of the reaction, on which the interpretation of mechanism must be based.

The reaction of oxygen with cysteine and cobalt(II) was first investigated by Michaelis and Barron[3] and, subsequently, by Michaelis and Yamaguchi,[4] Kendall and Holst[5] and Schubert.[6,7] Schubert succeeded in isolating complexes of cobalt(III) and cysteine from the reaction, and this accomplishment did much to clarify its nature. More recently, Neville[8,9] has provided interesting new data concerning these complexes. The present paper would amplify, and to some extent rectify, the last-mentioned reports.

Triscysteinato-N,S-cobaltate(III) (G-TRIS) and Tris-(2-aminoethanethio)-cobalt(III).—From cobalt (II) chloride, and excess cysteine, alkali, and oxygen, Schubert[7] isolated a green complex, $K_3(CoCys_3)\cdot 3H_2O$ (Cys = $[SCH_2CHNH_2COO]^=$). It is difficult to prepare this substance pure, because it is subject to rapid further oxidation by air.[7,9] In order to determine its absorption spectrum, its preparation was therefore undertaken from hexamminecobalt(III) chloride, cysteine and alkali in the absence of air, and the spectrum of the reaction mixture was determined directly. The best results were obtained when a large excess of cysteine was employed, in which case the spectra would remain unchanged for several hours and could be determined at leisure; as the proportion of cysteine was decreased, the spectrum altered more quickly, but substantially the same spectrum was obtained with only three moles of cysteine as with an excess of it if the absorption measurements were taken quickly after transfer to the spectrophotometer cell.

The absorption maxima were found to be at 274, 444 and 582 mμ and only a steadily rising absorption was found about 330 mμ. These results are quite different from those reported by Neville,[9] but there is an inconsistency in that report.[10] Inadequate protection of the complex from air may be the cause of the discrepancy, inasmuch as it has been observed in this work that exposure of the complex to air caused the development of absorption around 350 mμ.

An entirely analogous situation was found with 2-aminoethanethiol (henceforth called AET). Neville reported, for the *tris* complex of this substance, maxima at 580 and 340 mμ. In this work, the spectrum developed in excess AET was found to have maxima at 570 and 434 mμ, and no other maximum was observed to 300 mμ. It should be pointed out that substantially the same spectrum was developed in a variety of circumstances; in addition to the preparation from hexamminecobalt(III) chloride, the complex could be made in this case from cobalt(II), AET and a limited amount of oxygen, both in the presence of tris-(hydroxymethyl)-aminomethane as buffer and in its absence. It is felt, therefore, that the characteristics of the spectrum are well established, although the lability of the complex prevented obtaining accurate extinction data.[11]

Although these results demand a revision of the spectra previously reported, the spectra of the cysteine and AET complexes are still very similar, and there is no reason to change Neville's suggestion that N,S-coördination is involved in these complexes.

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[1] Presented in part at the 129th, 131st and 132nd National Meetings, American Chemical Society, April, 1956, September, 1956, and September, 1957.

[2] E. S. G. Barron, *Adv. Enzymology*, **11**, 201 (1951).

[3] L. Michaelis and E. S. G. Barron, *J. Biol. Chem.*, **83**, 191 (1929).

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

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

[6] M. P. Schubert, THIS JOURNAL, **53**, 3851 (1931).

[7] M. P. Schubert, *ibid.*, **55**, 3336 (1933).

[8] R. G. Neville and G. Gorin, *ibid.*, **78**, 4891 (1956).

[9] R. G. Neville and G. Gorin, *ibid.*, **78**, 4893 (1956). Owing to a misunderstanding, the manuscripts of this paper and the preceding one, which were published under the name of one of the present authors, were not examined by him prior to publication. Questions and comments concerning them should therefore be addressed solely to R.G.N.

[10] Ref. 9, p. 4895, Table I, gives the wave lengths of the absorption maxima for G-TRIS as 280, 330 and 570 mμ, but it is stated elsewhere (p. 4894, lines 37–41) that, in solutions prepared from hexamminecobalt(III) chloride and cysteine at *p*H 10.5, "ratios from about 1:3 to 1:17 [cobalt to cysteine] were progressively greener in color, possessing absorption maxima at 440 and 580 mμ, characteristic of the green triscysteinate." It will be noted that the values mentioned in the quotation are close to those found in this work.

[11] E. Felder, E. Paoli and U. Tiepolo, *Il Farmaco, Ed. Sci.*, **10**, 863 (1955), reported the preparation of a solid tris AET-cobalt complex, but without experimental detail. A product presumably identical with theirs was obtained in this work when the reaction between cobalt(II), three moles of AET and oxygen was carried out in more concentrated solution (approximately 0.01 *M* in cobalt); this green product precipitated and did not dissolve in water sufficiently to impart to it even the slightest green color: apparently it is not identical with the complex described above.

Octahedral complexes with three ligands having dissimilar chelating groups can exist in two stereoisomeric forms[12]; in the case at hand, in one of them the three N groups (and also the three S groups) are at the corners of a triangular face (*fac*-NNN), and, in the other, two of the three similar groups are on opposite apices (*mer*-NNN). Each of these isomers is asymmetric and can exist in two enantiomorphic forms. Ordinarily, the two enantiomorphs would be produced in equal amounts, but not necessarily in this case, because the ligand is itself asymmetric. From the present evidence it is impossible to tell whether the complex obtained in this work is a single isomer, and, if so, which isomer; it would be instructive to study the optical activity of these complexes, but this is very difficult to do in such strongly colored substances.[13]

Hydrogen Triscysteinato-O,S-cobaltate(III) (R-TRIS).—From cysteine, cobalt(II), and oxygen at *p*H 5.5 Schubert[6] isolated a red complex, $Co(HCys)_3 \cdot 3H_2O$, and the preparation has been repeated by Neville,[9] who also reported the absorption spectrum. The preparation has again been effected in this work, and the spectrum checked; the position of the absorption maxima are in good agreement with those reported by Neville, although the intensities of absorption are considerably higher. Since the formula of the complex suggests that it would be formed from one gram-ion of cobalt and three moles of cysteine, Schubert's directions for the preparation are puzzling, since they prescribe taking these reagents and potassium hydroxide in a molar ratio of 1:1.92:4.32. Nevertheless, the procedure is valid, while, on the contrary, attempts to prepare the complex from more nearly stoichiometric proportions of reagents sometimes resulted in a product contaminated with brown material, presumably bis-complexes.

It finally was ascertained that the formation of bis-complexes would occur if the initial *p*H was excessively high; by mixing the reagents in proper sequence, as described in the Experimental section, the initial *p*H could be kept below 5, and the preparation of R-TRIS could be carried out from stoichiometric proportions of the reagents. To ensure the proper *p*H more reliably, the preparation also was carried out in sodium acetate–acetic acid buffer at *p*H 4.8–5.0. The stoichiometry of the reaction can be represented by equation 1

$$
\begin{aligned}
&4Co^{++} + 12H_3CysCl + O_2 + 20CH_3COO^- \longrightarrow \\
&\quad 4Co(HCys)_3 \downarrow + 2H_2O + 20CH_3COOH + 12Cl^- \qquad (1)
\end{aligned}
$$

The preparation also was executed in formate buffer of *p*H 4.2, but the reaction was slower; and in chloroacetate buffer of *p*H 2.6 the reaction was so slow that very little complex was formed. It had been wished to determine how strongly acid a medium would be required to prevent the reaction, but this limit could not be established, because of the increasing slowness with which the reaction occurred. On the other hand, it was found possible to dissolve R-TRIS in concentrated sulfuric acid and to recover it unchanged by dilution; still it is not clear whether the complex is thermodynamically stable in sulfuric acid or merely inert[14] toward its action. In either case, quite strong coördination is implied, and it is clear the R-TRIS is therefore very different from G-TRIS.

Schubert[6] was the first to propose that, in R-TRIS, the mercaptide and carboxylate groups are coördinated. Evidence in favor of this was adduced by Neville's preparation of a complex analogous to R-TRIS from N-formylcysteine.[9] In this work, additional evidence for Schubert's suggestion was obtained by preparing a complex from 3-mercaptopropionic acid, in exactly the same conditions as employed for cysteine. The two complexes are very similar in all respects; the spectra are nearly identical.

Schubert[6] isolated and analyzed a tripotassium salt of R-TRIS, and a trisodium salt has been obtained in this work; it is clear that the complex contains three replaceable protons. If the carboxylate and mercaptide groups are coördinated and the amino groups are free, it is they that would bear the protons in question. The solubility of the complex has been tested in buffers of increasing *p*H, and the complex begins to be appreciably soluble at *p*H values of 9 to 10; this is a reasonable value for the ionization of uncoördinated ammonium groups, which, in cysteine itself, have a pK_a of about 8.5.[15]

With respect to the structure of R-TRIS, the same considerations apply as in the case of G-TRIS, except that different groups are involved in the coördination. In this case, a mixture of isomers might be involved, but the inert character of the complex precludes the possibility that the isomers, once formed, would be in equilibrium with one another.

Now it is of interest to consider the question of how two different modes of coördination, N,S– and O,S–, become established in the different conditions in which G-TRIS and R-TRIS are formed. At the *p*H in which G-TRIS is formed, there is abundant qualitative evidence that complexes also can form between cobalt(II) and cysteine,[3,6] and Neville[16] has shown that in the bis-complex of cobalt(II) N,S-coördination obtains. This is also to be expected from general considerations, which indicate that the amino group is usually strongly bound to cobalt and that formation of a five-membered ring, which results from N,S-coördination, is favored.[17] It may accordingly be expected that, in the presence of excess cysteine, a triscysteinato-N,S-cobalt(II) complex would be formed

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[12] R. F. Trimble, *J. Chem. Ed.*, **31**, 176 (1954).

[13] Ref. 9 gives a diagram of the *mer*-NNN form as the formula for the cysteine complex, but other possibilities of isomerism were not considered. The structure as given in that reference is erroneously shown as being associated with three protons; the complex is decomposed by acid, as Neville himself has demonstrated, and can exist only in alkaline solution.

[14] H. Taube, *Chem. Revs.*, **50**, 69 (1951).

[15] The mode of ionization of cysteine is somewhat complex, and reference should be made to some discussion of the subject, *e.g.*, J. T. Edsall and J. Wyman, "Physical Biochemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1958, p. 458–504. Coördination of a group will greatly decrease the value of its basic ionization constant, $pK_b = pK_W - pK_a$, when, as in these cases, the strength of coördination is great; on the other hand, the acid–base properties of a group not directly coördinated will not be much affected.

[16] R. G. Neville, This Journal, **79**, 518 (1957).

[17] A. E. Martell and M. Calvin, "Chemistry of the Chelate Compounds," Prentice–Hall, New York, N. Y., 1955.

and that G-TRIS results from interaction of this complex with oxygen.

As the *p*H of the solution is lowered, protons compete with cobalt(II) for the amino group of cysteine more and more strongly and tend to disrupt the coördination; although, unfortunately, quantitative measurements of stability constants for cobalt(II)-cysteine complexes are not available, those found for nickel(II) have *pK* values of about 10,[18] and, with such binding constants, the competition of protons becomes critical about *p*H 5. Now, at this *p*H, the tendency for protons to add to the carboxylate group is still small, since the pK_b for that group is about 12,[15] and it can reasonably be supposed that, around *p*H 5 and below, some O,S-coördinated cobalt(II) complexes are present. Oxidation of these forms R-TRIS.

The concentration of O,S-coördinated complexes need not be, and probably is not, high; it should be noted in this connection that the formation of R-TRIS is rather slow. Furthermore, it has been found that the rate of formation of R-TRIS, as measured by the yield after rather long times of reaction, drops rather sharply with concentration; this is consistent with a mechanism which requires a triscysteinato-cobalt(II) complex as the rate-determining intermediate since, at low levels of complex formation, the concentration of this intermediate would be proportional to the third power of the initial cysteine concentration.

Hydrogen Biscysteinatocobaltate(III) (BIS).—A brown complex of empirical formula $CoH_3 \cdot 2Cys \cdot 4.5H_2O$ was first isolated by Schubert[6] from a 1:2 mixture of cobalt(II) and cysteine, which had been treated with oxygen at *p*H 8–9 and then made acid. The water is apparently bound very loosely; Neville, in repeating Schubert's work, obtained the dihydrate, and in this work both a dihydrate and a monohydrate have been obtained. Both Schubert and Neville have considered, and there seems to be no reason for doubting, that four positions in the coördination sphere of cobalt are occupied by two cysteine residues, and that two water molecules would occupy the other two positions.

Schubert proposed that this complex has a binucleate structure, with two μ-hydroxo bridges, and that the coördination involves the mercaptide and carboxylate groups; however, this assignment was a tentative one, based mainly on analogy with the complex of cobalt(III) with thioglycolic acid, that has a fairly similar spectrum.[19]

Neville[9] has shown that cryoscopic measurements on the monosodium salt of BIS accord well with a monomeric formula and that the spectrum of the complex, while indeed similar to that of the complex with thioglycolic acid, is even more closely similar to that of the complex of 2-aminoethanethiol. Neville's spectral data have been confirmed in this work. Accordingly, a mononucleate formula with N,S-coördination is indicated for BIS.

This formulation receives further support from a consideration of certain acid–base properties of BIS, namely, (1) the titration curve of BIS in excess sodium hydroxide, shown in Fig. 1, and the observations[6] that (2) BIS is soluble in dilute hydrochloric acid and that (3) an *unstable* trishydrochloride salt can be isolated from *concentrated* hydrochloric acid.

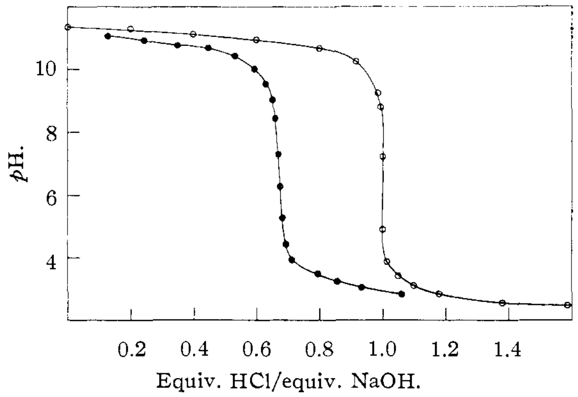

Fig. 1.—Titration of HBIS: ○, NaOH titrated with HCl; ●, NaOH + HBIS titrated with HCl.

It has been seen that the amino group of cysteine has a pK_b of about 5.5, and, if it was *not* coördinated, a fairly stable hydrochloride salt should be formed at *p*H values no lower than, say, 3.5; the fact that the hydrochloride salt is formed only in *concentrated* acid indicates that the basic properties of the amino group have been repressed by coördination, and strong coördination at that.

In excess base, BIS is converted to the monovalent anion, and back-titration with acid shows that a proton is taken up by this anion at *p*H 4-3; this is in accord with what might be expected for the uptake of a proton by an uncoördinated carboxylate group. It should be noted, by the way, that BIS is therefore an acid of considerable strength, albeit sparingly soluble.

BIS contains a carboxylate group, which, together with the two mercaptide groups, balance the charge of the cobalt ion. This makes possible the easy assumption of another proton, and the solubility of the complex in dilute hydrochloric acid is thus explained. It is, again, entirely reasonable that this process should take place at *p*H 1-0.

One more feature of interest should be noticed in the titration curve: there is no inflection in the region *p*H 8-10. This indicates that the aquo groups which presumably occupy the free coördination positions around the cobalt ion are not acidic, as they are, for instance, in the tetrammine and bisethylenediamine complexes.[20,21]

Five positional isomers are possible for a biscysteinato-N,S-complex.[12] Neville[9] has suggested that the complex has a *cis*-diaquo formula, but, again, other possibilities for isomerism were not considered. A *cis*-diaquo structure is supported by the possibility of converting BIS into G-TRIS,[9] but the transformation occurs so slowly that a rearrangement should not be excluded. A definite structural assignment cannot be made at this time.

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[18] J. M. White, R. A. Manning and N. C. Li, THIS JOURNAL, **78**, 2367 (1956).

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

[20] J. Bjerrum and S. E. Rasmussen, *Acta Chem. Scand.*, **6**, 1265 (1952).

[21] S. E. Rasmussen and J. Bjerrum, *ibid.*, **9**, 735 (1955).

Experimental

Materials.—All reagents were of analytical reagent grade, except as otherwise specified. L-Cysteine hydrochloride hydrate was obtained from the California Foundation for Biochemical Research ("cfp" grade) or from Schwarz Laboratories (C.P. grade); determination of mercapto content by the methods of Lavine[22] or of Waddill and Gorin[23] indicated values above 96% of theoretical. 3-Mercaptopropionic acid was obtained from the Aldrich Chemical Company and purified by vacuum distillation, only the middle third of the fraction boiling at constant temperature being retained for use. Hexamminecobalt(III) chloride was prepared according to "Inorganic Syntheses."[24] Nitrogen was of commercial grade and purified by passage through alkaline pyrogallol or acid vanadous solutions. Deaerated water was prepared by boiling distilled water, passing a stream of nitrogen through it, allowing it to cool and storing it out of contact with the air.

Preparation of BIS.—The preparation was carried out both from cobalt(II) chloride according to Schubert[7] and from hexamminecobalt(III) chloride according to Neville,[9] except that acidification of the reaction mixture was controlled carefully with a *p*H meter and stopped at *p*H 2; an excess of hydrochloric acid must be avoided, since BIS is soluble in it. By this means, the complex could be obtained in good yield without adding alcohol to promote its separation. The preparations were dried in a desiccator over concentrated sulfuric acid at room temperature for 1–2 days.

Titration of BIS in Excess Sodium Hydroxide.—BIS, 61 mg. was dissolved in 4 ml. of 0.1 *M* sodium hydroxide and titrated with 0.1 *M* hydrochloric acid. The *p*H was measured with a Beckman *p*H meter, which was checked before and after the titration with standard buffers. The results are shown in Fig. 1.

Spectral Measurements. General.—Spectra were scanned with a Beckman Model DK-1 spectrophotometer, and the location of, and absorption at, the maxima checked with a Beckman Model DU spectrophotometer. Silica cells with a 10-mm. light path and a ground glass stopper were used. When "anaerobic conditions" were employed, the glass stopper was removed and a rubber cap fitted over the mouth of the cell; through the cap were inserted two hypodermic needles, which provided entrance and exit to a stream of purified nitrogen. The sample was introduced by means of a hypodermic syringe under a stream of nitrogen; the flow then was interrupted, and the needles were stoppered.

Preparation of G-TRIS.—Hexamminecobalt(III) chloride, 0.01 *M*, cysteine hydrochloride, 0.05 *M*, and sodium hydroxide, 0.15 *M* were mixed in deaerated water and heated under nitrogen at 50°. The intensity of absorption did not increase after 2 hr., indicating complete reaction; spectral maxima: 582 mμ, extinction 269; 444, 568; 279, 16.4×10^3. The proportion of cysteine could be reduced to 0.03 *M* without substantial alteration in the spectrum, if measurements were taken quickly after transferring to the spectrophotometer cell.

Preparation of Tris-(2-Aminoethanethio)-cobalt(III). (1) From Hexamminecobalt(III) Chloride.—Hexamminecobalt (III) chloride, 0.0268 g. (0.100 mmole), 0.1135 g. (1.00 mmole) of AET hydrochloride and 1.21 g. (10.0 mmoles) of tris-(hydroxymethyl)-aminomethane were dissolved in 100 ml. of deaerated water and heated under nitrogen at 50°. At 2 hr. intervals, an aliquot sample was withdrawn (while a stream of nitrogen was passed over the surface of the solution) and transferred to the anaerobic cell; extinction values then were measured in the region 350–625 mμ within 15 minutes of the time of transfer. It was found that the reaction was complete after 24 hr.; position of maxima, 570 and 434 mμ; extinction values, 620 and 1.38×10^3, respectively.

The tris complex also was prepared in this way from 0.0375 g. (0.033 mmole) of AET hydrochloride and 0.0268 g. (0.0100 mmole) of hexamminecobalt(III) chloride, *i.e.*, with a 3.3:1 ratio of AET to cobalt; position of maxima, 570 and 434 mμ; extinction values, 568 and 1.24×10^3, respectively.

(2) From Cobalt(II) Chloride in Buffered Solution.—Cobalt(II) chloride hexahydrate, 0.0119 g. (0.050 mmole), 0.0568 g. (0.50 mmole) of AET hydrochloride and 1.21 g. (10.0 mmoles) of tris-(hydroxymethyl)-aminomethane were dissolved in 100 ml. of deionized but not deaerated water. The color developed in a few minutes; position of maxima, 570 and 434 mμ; extinction values, 640 and 1.46×10^3, respectively.

The complex also was prepared in the above manner, with the exception that only a 4-fold excess AET hydrochloride was used. The spectrum was quite fleeting and was determined within 15 minutes of the time of mixing; position of maxima, 570 and 434 mμ; extinction values, 580 and 1.3×10^3, respectively.

(3) From Cobalt(II) Chloride in Unbuffered Solution.—Cobalt(II) chloride hexahydrate, 0.0119 g. (0.050 mmole) and 0.1135 g. (1.00 mmole) of AET hydrochloride were weighed into a 100-ml. volumetric flask and dissolved in deionized but not deaerated water; 1.6 ml. of 1 *M* sodium hydroxide was added and the solution then was diluted to the mark. The complex formed rapidly; position of maxima, 570 and 434 mμ; extinction values, 612 and 1.36×10^3, respectively. The above solution was diluted to a cobalt concentration of 5×10^{-5} molar, and the absorption was determined in an effort to locate and measure maxima below 300 mμ, but the absorption was still too great to permit the delineation of any maximum in this region.

Preparation of R-TRIS.—In the preparation of this complex, the yield is very critically dependent on the concentration of the reagents, and it is accordingly necessary to control the volume of the reaction mixture very carefully.

(1) In Acetate Buffer.—Cysteine hydrochloride hydrate, 3.53 g. (0.0200 mole), was dissolved in 6.00 ml. of 1.00 *M* cobalt(II) chloride, and 13.6 g. (0.100 mole) of sodium acetate trihydrate was added. The initial *p*H was 5.1. The reaction mixture was placed at the bottom of a 100-ml. Berzelius beaker and stirred with a magnetic stirring bar for 12 hr.; in order to minimize evaporation, the reaction vessel was placed under a larger inverted beaker together with a crystallizing dish containing pure water. When the reaction was stopped, the mixture was a thick reddish-brown paste; the *p*H was 4.8. Twenty milliliters of 5.75 *M* hydrochloric acid was added, the mixture filtered and the residue washed with 50 ml. of water. Dry weight of residue (determined on an aliquot portion after drying at 100° for 3 hr.) was 2.58 g. (95%). The residue was dissolved in 1.1 l. of 0.05 *M* potassium hydroxide and gave a deep burgundy-red solution; this was filtered and acidified with concentrated hydrochloric acid to *p*H 2. The precipitate formed had a unique appearance: it was stringy, gelatinous, exceedingly voluminous and very difficult to filter; a judicious combination of centrifugation and filtration must be employed to effect filtration and washing in a reasonable time. After washing and pressing on the filter, the product was dried over sulfuric acid *in vacuo*. The dry product has a green metallic sheen. Calcd. for $Co(HCys)_3\cdot 2H_2O$: Co, 12.92; C, 23.74; H, 9.23. Found: Co, 12.9; C, 23.4; H, 9.26.

(2) According to Schubert.—The proportion of reagents and procedure were as described[6]; the initial *p*H was 4.2. Air was passed through the reaction mixture for 1 hr., and the mixture then allowed to stand 24 hr.; at the end of this time, the *p*H had not changed. The red precipitate was filtered and the residue treated as described in (1).

(3) From Stoichiometric Proportions.—Cobalt chloride, 6.00 ml. of 1.00 *M* solution, was mixed with 1.10 ml. of 7.70 *M* sodium hydroxide; cysteine hydrochloride, 3.00 g. (0.0190 mole) was treated with 3.00 ml. of 7.70 *M* sodium hydroxide and added to the cobalt solution. The subsequent procedure was as described in (1).

Spectrum of R-TRIS.—All the preparations given above gave substantially the same spectrum; maxima, 510 mμ, ϵ 24,700; 417, 8,400; 270, 9,800; 225, 8,300.

Sodium Salt of R-TRIS.—R-TRIS, 1 g. was stirred for 1 hr. with 1 l. of 0.1 *N* sodium hydroxide, the solution was filtered, the salt precipitated by adding alcohol and dried in air. The dry salt looked green, gave red solutions. Calcd. for $Na_3(CoCys_3)\cdot 6H_2O$: Na, 11.63. Found: Na, 11.6.

Preparation and Spectrum of Hydrogen Tris-(3-mercaptopropionato)-cobaltate(III).—The preparation was carried out as for R-TRIS in acetate buffer. Calcd. for $Co(SCH_2CH_2COOH)_3$: Co, 15.87; C, 28.88; H, 4.04. Found: Co, 15.7; C, 29.05; H, 4.24. Spectral maxima: 510 mμ, ϵ 22,600; 415, 6,400; 270, 8,200.

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

[23] H. G. Waddill and G. Gorin, *Anal. Chem.*, **30**, 1069 (1958).

[24] J. Bjerrum and J. P. Reynolds, *Inorg. Syntheses*, **2**, 217 (1946).

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[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

The Formation of a Dinuclear Cr(III) Species by Oxidation of Chromous Solutions[1]

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The dissolved chromic species formed when chromous perchlorate is oxidized in aqueous solution by molecular oxygen is shown by visible spectrum and behavior toward a cation-exchange resin to be the same as the hydrolytic polymer which forms first when chromic solutions are refluxed. Analysis of the solution for chromium, perchlorate and free acid shows the species to have a charge of +2 per Cr atom. Ion-exchange experiments indicate that +4 is the most likely charge for the entire species, which is consistent with its being dinuclear Cr(III) species with the two Cr atoms bound by either an O or two OH bridges. This same product can be formed by oxidation of Cr^{++} with Tl^{+3} and in lower yield by ClO_3^-, HClO, $Cr_2O_7^{=}$ and H_2O_2. It is absent when the oxidation is performed with Fe^{+3}, Cu^{++}, Cl_2 or Br_2. It is believed that the dimer results from reaction of the intermediate Cr(IV) with Cr^{++}.

Products formed when acidified chromous perchlorate solutions are oxidized have been investigated previously. Taube and Myers[2] found that the oxidation product depends on the nature of the oxidizing agent. Reagents such as Fe^{+3} or H_2O_2 produce hexaquo chromic ion, $Cr(H_2O)_6^{+3}$, but reagents such as $Co(NH_3)_5Cl^{++}$, I_2 or Br_2 produce the complex $Cr(H_2O)_5X^{++}$ (X being Cl, I, Br, respectively). They showed that the halogen atom in the final complex originated in the oxidizing agent, thereby indicating that the reaction proceeds with halogen atom transfer rather than by electron transfer alone. A third type of product of the oxidation of chromous perchlorate solutions was reported by Ardon and Stein.[3] It is obtained by oxidation of acidic chromous perchlorate solution with molecular oxygen. The blue-green solution so obtained was shown to contain chromium only in the +3 oxidation state which, however, was neither $Cr(H_2O)_6^{+3}$ nor one of its mononuclear substitution products. It was concluded that this product must be a hydrolytic polymer of Cr^{+3}.

Polynuclear hydrolysis products of Cr^{+3} are known to exist in aged or boiled solutions of chromic salts.[4] A characteristic property of these products is the slow rate of their formation and decomposition. In contrast to mononuclear hydrolysis products, such as $Cr(H_2O)_5OH^{++}$ which are in rapid equilibrium with the hexaquo chromic ion and are immediately converted to it by increasing acidity, the hydrolytic polymers react only very slowly with acid and persist for long periods in highly acid solution. It is generally agreed that these polynuclear compounds consist of a number of chromic ions, with water molecules of hydration, connected by "ol" bridges, $Cr\text{–}\overset{H}{O}\text{–}Cr\text{–}\overset{H}{O}\text{–}$, or "oxo" bridges, Cr–O–Cr–O–. Various structural formulas have been proposed for these products,[4] but none of them ever was proved. Neither crystallization, nor identification of individual compounds has been reported. The main difficulty in investigating these compounds is their extreme solubility and the fact that the solutions usually contain more than one component.

Laswick and Plane[5] succeeded in separating the boiled solutions of chromic perchlorate by means of ion-exchange chromatography into three main components: (1) unchanged hexaquo chromic ion $Cr(H_2O)_6^{+3}$ which constituted the main soluble component at all times and could be eluted from cation-exchange resin (Dowex 50) by 1 *M* $HClO_4$; (2) a polynuclear blue-green species which was formed quickly upon boiling the solution and attained a steady concentration after a few minutes of boiling, and could not be eluted with 1 *M* $HClO_4$, but was eluted with a solution of 0.02 *M* $La(ClO_4)_3$ at *p*H 2; (3) a second, green, polynuclear species which formed in the boiling solution at a much slower rate and was not eluted by 0.02 *M* $La(ClO_4)_3$ but could be eluted by 0.2 *M* $La(ClO_4)_3$ at *p*H 2. The visible absorption spectrum of each compound was measured and the various concentrations in the boiling solutions were determined.

A study of the formation of polynuclear Cr(III) by the reaction between oxygen and chromous ion is important for an understanding of the mechanism of this and possibly other oxidations by molecular oxygen. The purpose of the present work is to identify the reaction product, making use of the chromatographic method employed by Laswick and Plane,[5] to learn whether this product is identical with one of those obtained in boiled chromic

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(5) J. A. Laswick and R. A. Plane, Paper presented at 133rd National Meeting of the American Chemical Society, San Francisco. 1958.