NOTES

A New Method for the Preparation of Hydroxoaquobis-(ethylenediamine)-cobalt(III) and Hydroxoamminebis-(ethylenediamine)-cobalt(III) Salts

By Fred Basolo and D. H. Steninger

The replacement of chloro (likewise bromo and iodo) ligands coördinated to cobalt(III) in the "Werner-type" complex compounds by hydroxide ion has in general been accomplished either by treatment of the complex with potassium hydroxide[1] or with a slurry of silver oxide.[2] Although both methods give satisfactory results it was found during a recent study[3] that hydroxoaquobis-(ethylenediamine)-cobalt(III) bromide, free of potassium chloride and potassium bromide, could be obtained in no greater than 65% yield from the reaction of *trans*-dichlorobis-(ethylenediamine)-cobalt(III) chloride and excess potassium hydroxide. The use of silver oxide instead of potassium hydroxide results in the formation of the desired product in almost the theoretical yield. However, this procedure is not convenient since it requires that the reaction mixture be ground in a mortar for approximately one hour followed by subsequent extraction of the product from the silver chloride and excess silver oxide.

It would appear that one of the strongly basic anion exchange resins[4] recently developed may be readily employed for the displacement of chloro ligands by hydroxide ion without the introduction of any impurities. The ionic chloride should be removed from solution and held by the resin as the hydrochloride. This then results in the formation of the complex hydroxide which is a strong base comparable to potassium hydroxide, and thus the hydroxide ion displaces the coördinated chloro groups which are in turn removed by the resin—the final product being the hydroxo hydroxide. This procedure was carried out successfully using Amberlite IR-A-400 resin for the synthesis of hydroxoaquobis-(ethylenediamine)-cobalt(III) and hydroxoamminebis - (ethylenediamine) - cobalt(III) salts in almost theoretical yields.

Experimental

Hydroxoaquobis-(ethylenediamine)-cobalt(III) Bromide.—A column of Amberlite IR-A-400 resin, in its original chloride form, 2 cm. in diameter and 60 cm. in length, is treated with 3 l. of 4% sodium hydroxide solution and then washed with distilled water to a *p*H of 8. A solution containing 5 g. of *trans*-dichlorobis-(ethylenediamine)-cobalt-(III) chloride in 100 cc. of water is then added to the column and followed by distilled water until the elutriant is only very faintly pink. The green color of the *trans*-dichloro compound readily changes to red as the salt progresses down the column. A total volume of 175 cc. of elutriant is collected in 30 min. and this solution has a *p*H of 12.5. Concentrated hydrobromic acid (approximately 4 cc.) is then added carefully adjusting the *p*H of the solution to 7. This solution is concentrated on a steam-bath to about 15 cc. and the concentrate is added slowly with vigorous stirring to 200 cc. of ice-cold absolute ethanol. The precipitate is collected on a filter, washed with alcohol and ether and dried at 75° to give 6 g. or 92% yield of the desired product.

Anal. Calcd. for $[\mathrm{Co\,en_2H_2O(OH)}]\mathrm{Br_2}$: Br, 42.74. Found: Br, 42.62.

Hydroxaquobis-(ethylenediamine)-cobalt(III) Sulfate.—This compound is prepared as described above except that the elutriant is neutralized with sulfuric instead of hydrobromic acid. A 90% yield of the desired sulfate was obtained.

Anal. Calcd. for $[\mathrm{Co\,en_2H_2O(OH)}]$: S, 10.34: Found: S, 10.53.

Hydroxoamminebis-(ethylenediamine)-cobalt(III) Bromide.—By this same procedure 5 g. of *cis*-chloroamminebis-(ethylenediamine)-cobalt(III) chloride was converted in 95% yield to the hydroxoamminebis-(ethylenediamine)-cobalt(III) bromide.

Anal. Calcd. for $[\mathrm{Co\,en_2NH_3OH}]\mathrm{Br_2}$: Br, 42.85. Found: Br, 42.78.

Hydroxoamminebis-(ethylenediamine)-cobalt(III) Sulfate.—Similarly a 94% yield of the corresponding sulfate was obtained by neutralizing the elutriant with sulfuric acid and precipitation of the desired product with absolute ethanol.

Anal. Calcd. for $[\mathrm{Co\,en_2NH_3OH}]\mathrm{SO_4}$: S, 10.37. Found: S, 10.11.

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[1] Werner, *Ber.*, **40**, 282 (1907).

[2] Werner, *Ann.*, **386**, 186 (1912); Lamb and Yngve, This Journal, **43**, 2352 (1921).

[3] Basolo, *ibid.*, **72**, 4393 (1950).

[4] Davies and Nancollas, *Nature*, **165**, 237 (1950).

Critical Solution Temperatures for Sulfur and Butyl Phthalate

By Maxey Brooke

In an attempt to extend the rather limited data on solubility of sulfur in organic solvents, several experiments were made.

The chemicals were Eastman Kodak Company *n*-butyl phthalate, b. p. 203–206° (20 mm.). No attempt at purification was made. The sulfur was a very pure specimen of natural brimstone having the analysis: S, 99.983; carbonaceous matter, 0.015; ash, 0.002. The determinations were made in a modified aniline point apparatus (ASTM designation D 611-47T) consisting of a 20 × 175 mm. test-tube for a mixing chamber, loosely stoppered. Holes were bored in the stopper to admit a thermometer meeting ASTM specification 1C-49 and a stirring device. This assembly was heated in a glycerol-bath and illuminated from behind with a fluorescent light. Dibutyl phthalate and sulfur were weighed into the test-tube. The thermometer and stirrer were immersed in the mixture. The test-tube was placed in the cold glycerol-bath which was then heated at a rate not exceeding 5° per minute. The sulfur–dibutyl phthalate mixture was constantly stirred manually until it was clear and homogeneous. The heat source was removed from the bath and the mixture was allowed to cool. A distinct cloud-point marked the separation of the two materials. Temperature measurements were taken at this point. On reheating and recooling it was possible to duplicate the temperature of separation within a half a degree. Each determination was made in triplicate and the averaged results appear in Table I.

TABLE I

CRITICAL SOLUTION TEMPERATURES FOR SULFUR AND BUTYL PHTHALATE

Mole % dibutyl phthalate	Mole % sulfur (S_8)	Critical soln. temp., °C.
97.54	2.46	61.1
94.98	5.02	91.1
91.45	8.55	127.8
87.26	12.74	161.1
87.14	12.86	164.4
84.02	14.98	197.8

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Preparation of Oxythiamine Diphosphate and Oxythiamine Monophosphate[1]

BY LEOPOLD R. CERECEDO AND ALBERT J. EUSEBI

In a previous paper,[2] oxythiamine was shown to be a potent thiamine antagonist. These findings have been confirmed and extended by us and by other workers.[3,4,5,6] In contrast, experiments carried out in this Laboratory[7,8] with chloro- and bromoxythiamine, in which the hydroxyl group of the thiazole moiety of oxythiamine was replaced by halogen, showed these substances to be not toxic to mice, even when administered at high levels. This observation suggested that the hydroxyl group was necessary for the anti-thiamine effect of oxythiamine. Furthermore, since it is well known that thiamine functions *in vivo* as the diphosphate, the idea suggested itself that oxythiamine exerted its anti-thiamine effect in the form of the diphosphate. In this paper, we wish to report the preparation of the diphosphate and the monophosphate of oxythiamine.

Phosphate Salt of Oxythiamine Diphosphate.—This substance was prepared according to a modification of the method which Karrer and Viscontini[9] used for the preparation of thiamine diphosphate.

Five grams of 85% phosphoric acid was heated in a small beaker to 300–310°, or until crystals appeared on its walls. The acid was allowed to cool and then placed in an oil-bath previously heated to 135–140°. After temperature equilibration, 4 g. of oxythiamine was added all at once and kept at this temperature for 20 minutes with constant stirring. The fused mass was allowed to cool somewhat and then washed twice with 50 ml. of acetone before it solidified. The solid was dissolved in 50 ml. of water containing 4 drops of concentrated phosphoric acid, and precipitated with 150 ml. of acetone. After standing overnight, the dark brown oil was dissolved in 15 ml. of water plus 4 drops of concentrated phosphoric acid, and decolorized with Norit A, filtered, washed with water, and brought to a volume of 75 ml. The solution was treated with 150 ml. of acetone to give an initial dense cloudiness. It was then placed in the refrigerator for 6 hours, after which the turbid supernatant was decanted and discarded. The yellowish oil which remained was taken up in 15 ml. of phosphoric acid and decolorized a second time with a small amount of Norit A, filtered, washed and brought to a volume of 25 ml. The material was then precipitated with 125 ml. of acetone and kept in the refrigerator overnight. After removing the supernatant, the colorless oil was dissolved in 20 ml. of phosphoric acid, and precipitated with 100 ml. of acetone. A further fractionation was then carried out by dissolving the oil in 25 ml. of phosphoric acid and precipitating with 50 ml. of acetone. After removal of the supernatant, the glassy appearing material was taken up in 10 ml. of phosphoric acid and precipitated with an equal mixture of absolute alcohol and acetone. The precipitate was treated with 50 ml. of absolute alcohol and placed in the refrigerator. After two days, with occasional scratching, the material became crystalline and could be filtered. It was washed with absolute alcohol, acetone and ether, and then dried *in vacuo* over sulfuric acid for 2 days; yield 1.7 g. (27.5%). The compound started to brown at 190° and decomposed at 245–255°.

Anal. Calcd. for $C_{12}H_{20}O_{12}N_3P_3S$: C, 27.53; H, 3.82; N, 8.03; free P, 5.93; hydrolyzable P, 11.87; total P, 17.80. Found: C, 27.83; H, 4.06; N, 7.77; free P, 5.65; hydrolyzable P, 11.62; total P, 17.70.

Oxythiamine Monophosphate.—Thiamine diphosphate, on treatment with nitrous acid gases in an aqueous medium, loses one phosphoric acid group quite rapidly. The second phosphate group is more difficult to remove. The deamination and dephosphorylation of thiamine diphosphate was carried out according to the procedure used in the preparation of oxythiamine from thiamine.[2]

Four grams of cocarboxylase phosphate salt, prepared according to the method of Karrer and Viscontini,[9] was dissolved in 50 ml. of water, and nitrous acid gases passed into the solution for four hours. The mixture was allowed to stand overnight, and the process repeated on the second and on the third day. The excess acid was removed by passing the solution through a column of the resin Amberlite IR-4B[10] which was kept cold. The effluent was acidified with *N* HCl and concentrated to an oil *in vacuo* at room temperature. The oil was taken up in alcohol, precipitated with an excess of ether, and left in the refrigerator. As no precipitate formed, the alcoholic solution was treated with an excess of ether. After standing for five hours in the refrigerator, the slightly turbid supernatant was decanted, the precipitate dissolved in 10 ml. of 0.1 *N* HCl, the solution decolorized with Norit A, the Norit washed, and the volume brought to 20 ml. with the washings. The solution was treated with 175 ml. of acetone and kept in the refrigerator overnight. The resultant precipitate was taken up in absolute alcohol, and hydrogen chloride gas passed in at ice-bath temperature to saturation. The solution was then treated with ether to give a dense cloudiness. After standing overnight, the precipitate was treated with 20 ml. of absolute alcohol to aid crystallization; yield 1.1 g. The compound was very hygroscopic and melted at 185–196°.

Anal. Calcd. for $C_{12}H_{18}O_5N_3PSCl_2$: C, 34.45; H, 4.31; free P, 0; hydrolyzable P, 0; total P, 7.41. Found: C, 35.05; H, 4.49; free P, 0, hydrolyzable P, 0; total P, 7.18.

The analysis indicated that the product probably contained a very small amount of free oxythiamine. However, it was sufficiently pure for use with certain enzyme systems and for experiments *in vivo*. The results of these studies and of those with oxythiamine diphosphate will be reported in a separate paper.

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(10) Supplied by Rohm and Haas Company, Philadelphia, Pa. This technique for the removal of excess acid has been used with success by Mr. G. Bonvicino of the Organic Chemistry Department in the preparation of oxythiamine from thiamine.