[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

A New Complex of Cobalt and Cysteine and its Behavior with Hydrogen Peroxide

BY MAXWELL P. SCHUBERT

In connection with a colorimetric study[1] of the reaction between cobalt and cysteine at a *P*H between seven and eight, Michaelis discovered that the brown color of the solution made in the presence of air can be changed to a bright yellow by the addition of hydrogen peroxide, but did not publish his observation. In a more direct study of the chemistry of the complexes of cobalt and cysteine,[2] a complex was isolated, potassium cobalto biscysteinate, (I) $K_2[Co(SCH_2CHNH_2COO)_2]\cdot 2H_2O$, described as blue-green in color, which appeared to give the yellow peroxide reaction, but this yellow complex itself could not be isolated.

As the result of a more elaborate technique developed for working with compounds in the absence of air, it has been found that this potassium cobalto biscysteinate after recrystallization is really red-violet in color, and not only does not give the yellow peroxide reaction mentioned, but after the addition of hydrogen peroxide actually fades, finally giving a colorless solution. It has also been found possible with a higher proportion of cysteine and in a more strongly alkaline solution to separate a new complex, blue-violet in color, which can be formulated as (II) $K_4[Co(SCH_2CHNH_2COO)_3]\cdot 4H_2O$. In the air this potassium cobalto tris-cysteinate oxidizes to (III) $K_3[Co(SCH_2CHNH_2COO)_3]\cdot 3H_2O$, a very stable crystalline complex, which gives green aqueous solutions. This green complex, potassium cobalti tris-cysteinate, is the one which really gives the yellow color with hydrogen peroxide. The reaction occurs at room temperature and is very rapid, being complete in a few minutes in dilute aqueous solution. No other oxidant has been found which brings about this color change except sodium peroxide. The reaction can be used as a test for hydrogen peroxide, one hundredth of a millimole of the latter in ten cubic centimeters of solution giving a distinct yellow color with five cubic centimeters of a thousandth molar solution of the green complex.[3] The test has the disadvantage, of course, that it cannot be used in colored solutions.

The reason that the potassium cobalto biscysteinate made previously[2] appeared to give the yellow reaction with peroxide is that, due to the limitations of technique then available for working in oxygen-free atmosphere, the complex could not be recrystallized and so must have contained some II as an impurity.

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(1) L. Michaelis and S. Yamaguchi, *J. Biol. Chem.*, **83**, 367 (1929).

(2) M. Schubert, THIS JOURNAL, **53**, 3851 (1931).

(3) This reaction has also served as the basis for a colorimetric method for determining small amounts of potassium. A. Sobel and B. Kramer, *J. Biol. Chem.*, **100**, 561 (1933).

The yellow complex itself has also been crystallized and analysis of the potassium salt agrees with the formula (IV) $K_3[Co(SO_2CH_2CHNH_2COO)_3]\cdot 3H_2O$. The effect of the peroxide is thus to convert the cysteine in the green complex to a sulfinic acid or sulfone in the yellow.

To prove this a way was sought of breaking up the complex with sufficiently mild reagents so there might be a chance of recovering the suspected sulfinic acid from the decomposition products. By warming a solution of IV with ethylenediamine for ten minutes at 40°, a heavy crystalline precipitate separates whose analysis agrees with the formula (V) $[Co(NH_2CH_2CH_2NH_2)_3]\cdot[Co(SO_2CH_2CHNH_2COO)_3]\cdot 7H_2O$. This formulation was checked by making the compound independently from $Co(en)_3Br_3$ and $K_3[Co(SO_2CH_2CHNH_2COO)_3]$. The complex V is very insoluble, so that although only half of the sulfinic acid of the original complex had been displaced by ethylenediamine, the mildness of the conditions and the automatic removal of most of the cobalt from solution made it seem worth looking for the acid in the filtrate from V. The acid sought was actually isolated first as a very soluble barium salt, $Ba(SO_2CH_2CHNH_2COO)\cdot H_2O$, from which the free acid itself was obtained in a crystalline condition after removal of the barium. Both the barium salt and the free acid were titrated in acid solution with potassium permanganate and used up about 2.2 equivalents of oxygen per mole of sulfinate or enough oxidant to convert the sulfinate to a sulfonate. The new acid is α-amino-β-sulfinopropionic acid and on oxidation with permanganate gives cysteic acid. The use of the cobalt complexes here forms the basis for the synthesis of a sulfinic acid which would be difficult to make by the usual methods of organic chemistry but is probably very limited in scope.[4]

The final bit of evidence that was sought to prove that the yellow complex really contains the sulfino acid was to make the complex from its component parts, that is, from a cobalt salt and the sulfino acid; but no reaction was apparent with any simple cobaltous salt even in alkaline solution or with trivalent cobalt in the form of the hydroxide. Finally it was found that if hexammine cobalti chloride be warmed with the sulfino acid there is obtained after a time masses of hair-like yellow crystals whose analysis agrees with the formula (VI) $[Co(NH_3)_6][Co(SO_2CH_2CHNH_2COO)_3]\cdot 4H_2O$, a compound analogous to V. This complex was also made from hexammine cobalti chloride and the yellow potassium salt IV by double decomposition, thus showing that the α-amino-β-sulfinopropionic acid is capable under proper conditions of forming the same complex nucleus as is obtained from the green cobalti tris-cysteinate ion by the action of hydrogen peroxide.

The probable structure of IV brings up some points worthy of considera-

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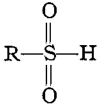
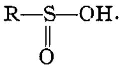
[4] There has recently appeared some work showing that by oxidation of cysteine with iodine under proper conditions this sulfinic acid seems to be formed but it could not be separated in pure form, D. Simonsen, *J. Biol. Chem.*, **101**, 35 (1933).

tion. The reactions of sulfinic acids indicate that they can be regarded as existing in either one of two forms possibly in tautomeric equilibrium: A,  $R{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O}{\|}}{S}}{-}H$ B,  $R{-}\underset{\underset{\displaystyle O}{\|}}{S}{-}OH$. The fact that the yellow complex IV can be formed so quickly, easily and smoothly from the green complex III, a very stable complex in which the sulfur is directly attached to the cobalt, makes it appear more likely that in IV the sulfino acid exists as the isomer A. If it existed in the form B the reaction with peroxide would involve the disruption of the bond between sulfur and metal in the complex III with the insertion of an oxygen atom. Furthermore, if it existed in the form B in the complex it would be difficult to understand why the oxidation should stop at the sulfinic acid stage and not go on to the sulfonic acid stage which would be a much more stable oxidation level of the sulfur atom. In the form A, however, this is easier to understand since the sulfur has no free electron pairs and oxidation to the sulfonic acid would involve a breaking of the metal to sulfur link. Finally, if the form B existed in the complex it might be expected that cysteic acid would give similar complexes since its structure is so similar but under the conditions under which the sulfino acid gave rise to VI cysteic acid gave no apparent reaction at all. The evidence thus seems to favor the existence of the sulfino acid in the complex in the form A.

Krishna and Singh[5] have described a method for the quantitative estimation of sulfinates based on precipitation of the ferric salts in acid solution. The new sulfinic acid which has just been described gives no insoluble ferric salt either in acid or neutral solution, but a deep orange-red color appears, probably due to the formation of a complex. This may be the reason that ferric iron does not precipitate the new sulfinic acid.

In a previous paper,[2] there was described a complex identical in composition with the green complex III but giving cherry-red solutions. This red complex gives no reaction with hydrogen peroxide. The nature of the isomerism involved here is not clear but certainly seems not to be stereoisomerism.

Experimental Part

For the preparation of the cobalto complexes an air-tight metal tank, 40 × 40 × 45 cm. was constructed with a plate glass top and front. In the sides were two holes into which were fastened a pair of heavy rubber gloves having a heavy rim of rubber at their open ends to serve as a gasket. The back of the tank was made removable, being bolted in place against a rubber gasket. At one side of the tank on a shelf was set a small toy motor fitted with an electric fan to circulate the gas content so air could be more quickly displaced. A gas inlet and an outlet were fitted with heavy-walled suction tubing. Finally there was another outlet connected to a suction pump which could be attached to a suction flask fitted with a stopcock permitting the application of

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[5] S. Krishna and H. Singh, THIS JOURNAL, **50**, 792 (1928).

suction as desired. In this tank is placed all that is needed for the preparations, the back of the tank is bolted in place and a stream of nitrogen and hydrogen, freed of oxygen by passage over heated copper, is passed through the tank. Usually these gases were run overnight to displace all oxygen. The phosphorus cloud test for oxygen described by von Wartenberg[6] and for which a positive test is claimed down to 0.002% oxygen was used to determine when oxygen had been displaced from the tank. The gases were passed through the tank continuously during operations, which in no case were commenced until the gas issuing from the tank and passed over phosphorus showed no trace of cloud.

Potassium cobalto biscysteinate can now be prepared, in the tank just described, free of the complex II so that the only reaction that it gives with hydrogen peroxide is a gradual fading; 6.4 g. of cysteine hydrochloride is dissolved in 20 cc. of 1 *M* cobalt chloride, and 20 cc. of 6.4 *M* potassium hydroxide is added. Seventy-five cc. of alcohol is stirred in and after standing an hour the precipitate is filtered off on a Buchner funnel. This product is dissolved in 30 cc. of water, the solution filtered and the salt reprecipitated with 40 cc. of alcohol. The precipitate appears almost red. After two hours the precipitate is again filtered off with suction, and washed with 50% alcohol and finally with alcohol. The tank is opened and the product quickly placed in a desiccator over sulfuric acid at 1 mm. pressure, 2 g. being obtained after drying.

Anal. Calcd. for (I): Co, 14.35; S, 15.57; N, 6.81; K, 18.96. Found: Co, 14.11; S, 15.75; N, 6.86; K, 20.50.

The **potassium cobalto tris-cysteinate** is prepared similarly, starting, however, with 10 g. of cysteine hydrochloride, 20 cc. of 1 *M* cobalt chloride and 40 cc. of 6.4 *M* potassium hydroxide. On addition of 100 cc. of alcohol a violet precipitate gradually appears which is filtered off with suction after an hour. The precipitate is dissolved in 30 cc. of water, filtered and reprecipitated with 40 cc. of alcohol. The violet product is filtered off after two hours, washed with 50% alcohol, then with alcohol and transferred from the tank to a desiccator which is evacuated to 1 mm. After drying 1 to 2 g. is obtained.

Anal. Calcd. for (II): Co, 9.26; S, 14.90; N, 6.52; K, 24.22. Found, for separate preparations: Co, 9.28, 9.16; S, 15.56, 15.54; N, 6.02, 6.83; K, 24.26, 17.15.

The barium cobalto tris-cysteinate is prepared from the potassium salt just described and barium chloride. The salt is quite insoluble and is washed with water and alcohol, the tank opened and the preparation dried as usual.

Anal. Calcd. for $Ba_2[Co(SCH_2CHNH_2COO)_3]\cdot 4H_2O$: Co, 7.73; S, 12.57; N, 5.50; Ba, 36.05. Found, for separate preparations: Co, 6.89, 7.59; S, 12.26, 12.69; N, 5.43, 5.58; Ba, 32.99, 37.35.

The remaining complexes, being cobalti complexes, are all prepared without the use of the tank.

To prepare potassium cobalti tris-cysteinate 25 g. of cysteine hydrochloride is dissolved in 52 cc. of 1 *M* cobalt chloride and 100 cc. of 6.6 *M* potassium hydroxide is added, the mixture being cooled and air bubbled through for about five hours and then 200 cc. of alcohol is added. After standing a few hours the mixture is filtered, the precipitate sucked as dry as possible and then dissolved in 150 cc. of water. The solution is filtered and 150 cc. of alcohol is slowly stirred in. After standing a few hours masses of green crystals separate having a bar-like shape sometimes with queer kinks. These are filtered and washed with 50% alcohol. After drying at 1 mm. over sulfuric acid 25 to 27 g. is obtained.

Anal. Calcd. for (III): Co, 10.05; S, 16.35; N, 7.16; K, 19.93. Found: Co, 9.86; S, 15.87; N, 6.76; K, 20.52.

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[6] Von Wartenberg, *Z. Elektrochemie*, **36**, 295 (1930).

The corresponding barium salt is easily crystallized by adding barium chloride to a solution of the potassium salt. It forms long, thin, transparent green needles.

Anal. Calcd. for $Ba_3[Co(SCH_2CHNH_2COO)_3]_2 \cdot 7H_2O$: Co, 8.62; S, 14.03; N, 6.14; Ba, 30.01; C, 15.78; H, 3.24. Found: Co, 8.42; S, 14.76; N, 6.29; Ba, 31.29; C, 15.56; H, 3.26.

The yellow complex, potassium cobalti tris-α-amino-β-sulfinopropionate, is prepared from potassium cobalti tris-cysteinate. Ten grams of the latter salt is dissolved in 100 cc. of water. To this solution there is added dropwise, keeping the mixture well cooled in running water, 13 cc. of 30% hydrogen peroxide (superoxol) diluted with an equal volume of water. The green color changes to yellow. After thirty minutes the solution is heated and when the temperature reaches 50 to 60°, 35 cc. of 3 *M* hydrogen chloride is added slowly, stirring vigorously, until the gelatinous precipitate which forms at first crystallizes to short, fine, yellow needles. After cooling these are filtered off, suspended in 25 cc. of water and just enough potassium hydroxide added (5–7 cc. of 6.6 *M* potassium hydroxide) to cause complete solution. This is filtered, 100 cc. of alcohol is slowly stirred in and the mixture set on ice for a few hours. Long, thin and narrow, rectangular, yellow bars separate. These are filtered off and washed with alcohol. The yield varies between wide limits for unascertained reasons, the average being about 5 g. after drying *in vacuo*.

Anal. Calcd. for (IV): Co, 8.64; S, 14.05; N, 6.14; K, 17.12. Found: Co, 8.67; S, 14.57; N, 6.05; K, 18.25.

The corresponding free acid is very insoluble in water and can be made by addition of hydrogen chloride, to a hot solution of the potassium salt. It separates as tiny light yellow needles.

Anal. Calcd. for $H_3[Co(SO_2CH_2CHNH_2COO)_3]$: Co, 11.45; S, 18.63; N, 8.15; C, 20.97; H, 3.52. Found: Co, 11.56; S, 19.04; N, 7.96; C, 21.29; H, 3.81.

A barium salt was also prepared from the potassium salt. It is difficultly soluble in cold water and separates from water in rosets of needles and rectangular yellow plates.

Anal. Calcd. for $Ba_3[Co(SO_2CH_2CHNH_2COO)_3]_2 \cdot 6H_2O$: Co, 7.64; S, 12.44; Ba, 26.63. Found: Co, 7.49; S, 12.55; Ba, 27.49.

If 5 g. of the potassium salt of this complex be dissolved in 25 cc. of water, 4 cc. of ethylenediamine be added and the mixture warmed in a beaker of water at 60°, there is formed after about five to ten minutes a dense crystalline mass of long, flat, yellow, rectangular plates of the double complex V.

Anal. Calcd.: Co, 13.45; S, 10.94; N, 14.36. Found: Co, 13.38; S, 10.90; N, 14.07.

The same insoluble compound can be made by double decomposition between solutions of $[Co(en)_3]Br_3$ and $K_3[Co(SO_2CH_2CHNH_2COO)_3]$. Found: Co, 13.00; S, 10.96.

If, instead of separating the insoluble yellow crystals, the heating with ethylenediamine be continued for an hour, the crystals slowly go into solution again and from the resulting clear solution $[Co(en)_3]Br_3$ could be precipitated by addition of hydrogen bromide. But the sulfinic acid which had been displaced from coördination with the cobalt by the ethylenediamine could not be isolated. Probably the prolonged heating with ethylenediamine caused decomposition of the compound wanted.

The following procedure gives an easy and rapid method for the separation of the barium salt of the sulfinic acid: 12.5 g. of $K_3[Co(SO_2CH_2CHNH_2COO)_3]$ is dissolved in 60 cc. of water, 8 cc. of ethylenediamine added and the mixture warmed in a beaker of water at 45° for ten minutes. It is quickly cooled, the double complex described above filtered off, and to the filtrate 40 cc. of 1 *M* barium chloride is added. This causes a separation of a gummy precipitate. This need not be filtered off separately but 50 cc. of alcohol is added slowly with efficient stirring. This causes a further separation of a non-crystalline unidentified yellow compound. The whole mass is now filtered and to the filtrate there is added 150 cc. of alcohol. This causes a separation of the barium sulfinate in an almost pure condition. It may be dissolved in water and reprecipitated with alcohol for further purification. This salt is very soluble in water, has a tendency to get gummy in the process of solution and usually has a little yellow color. This, however, is not due to the presence of any cobalt complexes. After drying 6 g. of the product is obtained.

Anal. Calcd. for $Ba(SO_2CH_2CHNH_2COO)\cdot H_2O$: Ba, 44.85; S, 10.45; N, 4.57; C, 11.76; H, 2.31. Found for independent preparations: Ba, 43.81, 44.82; S, 10.28, 10.36; N, 4.67, 4.63; C, 10.65, 11.17; H, 2.23, 2.42.

The free sulfinic acid itself is made from the dried barium salt by weighing this out, dissolving in water, adding the calculated quantity of sulfuric acid, centrifuging off the barium sulfate, evaporating the solution to small volume and precipitating the acid with alcohol. It separates as rectangular plates and the product after filtration is perfectly white.

Anal. Calcd. for $HO_2SCH_2CHNH_2COOH\cdot H_2O$: S, 18.70; N, 8.18; C, 21.04; H, 5.31. Found: S, 18.84; N, 8.20; C, 21.42; H, 5.37.

Permanganate titrations were run on both the free acid and acidified solutions of the barium salt. The permanganate solution was 0.0190 *M*.

	Barium salt		Free acid	
Acid or barium salt, mg.	231.5	207.5	237.9	208.7
$KMnO_4$, cc.	17.51	15.58	32.68	28.65
Ox. equiv. per mole acid or Ba salt	2.19	2.36	2.23	2.23

The end-point is considered reached when a drop of permanganate causes a pink color to suffuse the entire solution for a few seconds.

The only successful method which could be found for inducing this sulfinic acid to reform the original complex nucleus from which it had been split out is as follows. Four to five grams of the barium salt was dissolved in 50 cc. of water and a solution of 4 g. of potassium sulfate in about 50 cc. of water was added. The barium sulfate was centrifuged off and the solution added to a solution of 3 g. of $[Co(NH_3)]Cl_3$ in 100 cc. of water. This mixture is warmed at 45° for two to three hours. Heavy masses of hair-like crystals separate. These are filtered off, washed with water, and dried *in vacuo*.

Anal. Calcd. for (VI): Co, 15.83; S, 12.88; N, 16.90; C, 14.49; H, 5.55. Found: Co, 16.40; S, 13.10; N, 16.13; C, 16.00; H, 5.66.

The same crystals were obtained when a hot solution of 2.5 g. of $K_3[Co(SO_2\cdot CH_2\cdot CHNH_2COO)_3]$ in 50 cc. of water and 3 g. of $[Co(NH_3)_6]Cl_3$ in 100 cc. of water are mixed and digested until the precipitate crystallizes.

Anal. Found: Co, 15.29; S, 12.67; N, 17.33; C, 15.90; H, 5.44.

If in the preceding synthesis of the complex from the sulfinic acid, cysteic acid be substituted, no reaction occurs at all.

All of the analytical work in connection with this paper was done by Mr. Bitterlich, to whom thanks are due.

Summary

Some new complexes of cobalt and cysteine are described. One of these undergoes a very unusual reaction with hydrogen peroxide, giving rise to a cobalt complex of the sulfinic acid corresponding to cysteine. This com-

plex has been isolated and the sulfinic acid, after being dislodged from the cobalt, crystallized as such. In colorless solutions this reaction can be used as a sensitive test for hydrogen peroxide.

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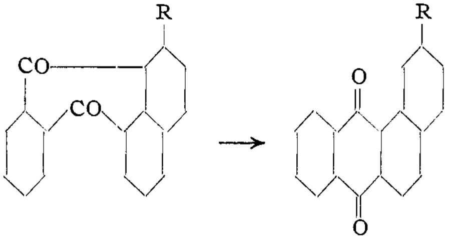
The Conversion of Phthaloylnaphthalenes and Naphthoyl-2-benzoic Acids into Benzanthraquinones[1]

BY LOUIS F. AND MARY FIESER

According to a recent patent[2] 1,8-phthaloyl-2-naphthol, I (R = OH), rearranges when heated with sulfuric acid to give an hydroxy-1,2-benzanthraquinone which probably has the structure of II.



I II

It seemed to us a matter of interest to study this reaction further, both for the purpose of establishing the course of the reaction and the structures of the quinones mentioned but not described in the patent, and with the idea of gaining further evidence of the structures of the benzanthraquinones previously obtained by us[3] by the action of aluminum chloride on methylated naphthoyl-2-benzoic acids.

If the rearrangement follows the course indicated, 2-methyl-1,8-phthaloylnaphthalene, I (R = CH_3), a compound which we recently obtained synthetically,[4] would be expected to yield 2′-methyl-1,2-benzanthraquinone. The product is indeed identical with a quinone which was first prepared by Cook,[5] and to which he assigned the structure indicated with the reservation that the evidence rendered this structure very probable, but not certain. Since there remained some element of doubt in the matter, we turned to the dimethyl derivatives. The first of these to be considered was the phthaloylnaphthalene ("pleiadenedione") derivative, VIII, which we had previously synthesized[4] from 2,6-dimethylnaphthalene,

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(1) Sixth paper.
(2) I. G. Farbenind., German Patent 555,081 (1932).
(3) Fieser and Peters, THIS JOURNAL, **54**, 3742 (1932).
(4) Fieser and Fieser, *ibid.*, **55**, 3010 (1933).
(5) Cook, *J. Chem. Soc.*, 2551 (1929).