SYNTHESIS OF ADENOSYLCOBALOXIME, AN ORGANOCOBALT DERIVATIVE OF 5'-DEOXYADENOSINE

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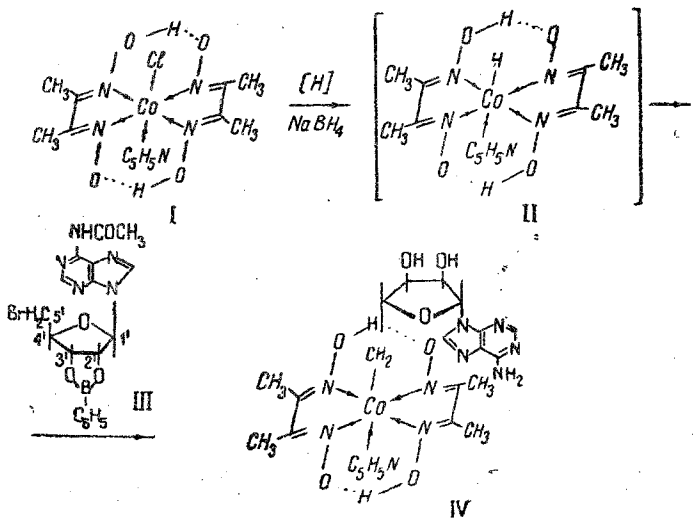
Among the natural metal-containing coenzymes (such as metalloporphyrins, cytochromes, etc.), there is one group of compounds that is distinguished by their structure from the chelate complexes that are characteristic for the transition metals. These compounds, which have recently been obtained from cell-free extracts of Clostridium tetanomorphum [1], have been called cobamide coenzymes and have proved to be similar in structure and chemical properties to the complexes cobalt corrins and vitamin B_{12}. X-ray analysis [2] has shown that a cobamide coenzyme contains a residue of the nucleoside adenosine in place of the cyanide group of cyanocobalamin, the $C_{5'}$ position of the adenosine being linked by a covalent bond to a reduced cobalt atom.

The cobamide coenzyme was almost the first natural organometallic compound isolated that possessed biocatalytic properties. The methylcobalamin synthesized later, as has been found, plays the part of a methyl group transferring agent in transmethylation reactions [3].

The present paper describes the synthesis and properties of adenosylcobaloxime (IV), a new organocobalt derivative of 5'-deoxyadenosine, containing a system of bonds analogous to that of the cobamide coenzyme. Model compounds with a similar system of bonds may evidently be adenosyl group transfer agents in various reactions of nucleophilic substitution taking place at the $C_{5'}$ position of an adenoside.

To synthesize the adenosylcobaloxime (IV) with a covalent C–Co bond, we used chloropyridine-bis-(dimethylglyoxime)cobalt (I) and N^6-acetyl-5'-bromo-5'-deoxyadenosine 2',3'-phenylboronate (III). In the complex (I), which was first obtained by Tschugaeff [Chugaev] [4], the cobalt atom is present, just as in the molecole of vitamin B_{12}, in the ligand field of five nitrogen atoms. The reduction of the complex (I) gives a hydride compound–cobaloxime$_s$ (II) which is analogous to vitamin B_{12s} [5, 6].

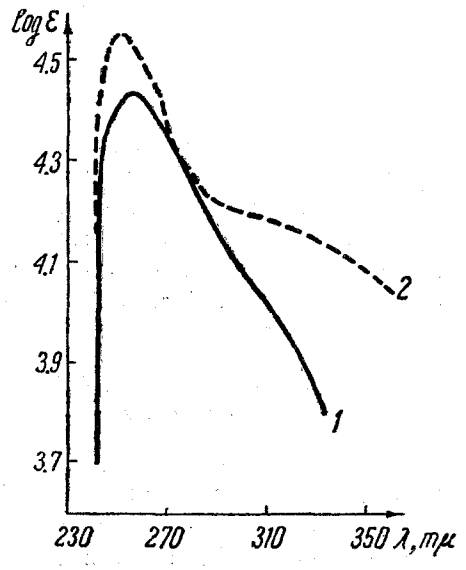


Cobaloxime$_s$ (II) was brought into reaction with the derivative of 5'-bromo-5'-deoxyadenosine which we obtained by the reaction of 5'-O-tosyladenosine 2',3'-phenylboronate with lithium bromide in acetic anhydride. The formation of nucleosides having a halogen or other similar group in position 5' is associated with some difficulties, in particular, the intramolecular cyclization of these compounds [7]. The tendency to intramolecular alkylation can be reduced by acetylating the N^6 amino group [8] and carrying out the reaction in acetic anhydride.

Compound (IV) was readily soluble in chloroform and other organic solvents and sparingly soluble in water, and was chromatographically individual. The UV spectrum of adenosylcobaloxime (IV) (in chloroform) showed an intense absorption maximum at λ_{max} 256 mμ (log ε 4.43) (figure).

Benzylcobaloxime (V) was obtained similarly by the reaction of cobaloxime$_s$ (II) with benzyl chloride, and its properties were similar to those of adenosylcobaloxime (IV). The UV spectrum of compound (V) (in chloroform) also shows an intense absorption maximum at λ_{max} 253 mμ (log ε 4.55) (see figure).

To establish the structure of adenosylcobaloxime (I) we used the reactions that have been applied to the investigation of the cobamide coenzyme, hydrolytic decomposition in the presence of potassium cyanide and in the presence of hydrochloric acid. In both cases, the adenine liberated was identified chromatographically on paper in various systems of solvents and from its UV absorption spectrum (SF-4 spectrophotometer). The chromatography was carried out on FN-1 paper with the solvent systems shown in the table.



UV spectra (in chloroform). 1) Adenosylcobaloxime; 2) benzylcobaloxime.

In the acid hydrolyzate, in addition, a compound having a carbohydrate nature was found chromatographically [R_f 0.43 in system 1; revealed with a 5% solution of silver nitrate in 25% aqueous ammonia (9:1)].

Experimental

Chloropyridine-bis-(dimethylglyoxime)cobalt (I). A mixture of 5.0 g of cobaltous chloride and 5.5 g of dimethylglyoxime was dissolved in 200 ml of boiling ethyl alcohol. The hot filtrate was mixed with 3.4 ml of pyridine. The crystals that deposited were separated off and washed with water, alcohol, and ether. The yield of the complex (II) was 6.07 g (71.6%), mp 222–224° C (from alcohol). UV spectrum (in chloroform): λ_{max} 260 mμ (log ε 4.34), R_f 0.87 (system 1).

Found, %: C 39.50; H 4.65. Calculated for $C_{13}H_{19}N_5O_4CoCl$, %: C 39.65; H. 4.86.

N^6-Acetyl-5'-bromo-5'-deoxyadenosine 2', 3'-phenylboronate (III). A solution of 1.4 g of 5'-O-tosyladenosine 2', 3'-phenylboronate was heated with 1.08 g of lithium bromide in a glycerol bath at 100° C for 1 hr. The cooled solution was mixed with 50 ml of chloroform and washed once with a solution of sodium sulfite and with water, and was then evaporated to dryness in vacuum. The residue was dissolved in a small amount of benzene and precipitated with n-hexane. The yield of product (IV) was 0.71 g (55.5%). UV spectrum (in methanol): λ_{max} 270 mμ (log ε 3.99). Qualitative tests for halogen and boron were positive.

Found, %: C 47.73; H 4.23. Calculated for $C_{18}H_{17}N_5O_4BBr$, %: C 47.20; H 3.74.

Adenosylcobaloxime (IV). With stirring in an atmosphere of nitrogen, 0.06 g of sodium borohydride in 10 ml of ethyl alcohol was added to a suspension of 0.20 g of the complex (I) in 20 ml of ethyl alcohol. After the formation of the blue-green hydride (II), a solution of 0.24 g of the bromide (III) in 5 ml of ethyl alcohol was added and the mixture was stirred for 30 min at 18–20° C and then for 10 min at 30° C and was evaporated to dryness in vacuum. The residue was dissolved in a small amount of chloroform and was precipitated with n-hexane. The yield of substance (IV) was 0.37 g (97.5%) R_f 0.89 (system 1). UV spectrum (in chloroform): λ_{max} 256 mμ (log ε 4.43).

Found, %: C 44.99; H 5.24. Calculated for $C_{23}H_{31}N_{10}O_7Co$, %: C 44.69; H 5.05.

Benzylcobaloxime (V). This substance was obtained in a similar manner to adenosylcobaloxime (IV) from 0.20 g of the complex (I) and 0.10 g of benzoyl chloride. The yield of (V) was 0.21 g (93%), R_f 0.83 (system 1).

Found, %: C 51.82; H 5.73. Calculated for $C_{20}H_{26}N_5O_4Co$, %: C 52.29; H 5.71.

Chromatographic Identification of the Products of the Hydrolysis of Adenosylcobaloxime (I)

System No.	Solvent	R_f		
		Hydrolyzate (KCN)	Adenine	Acid Hydrolyzate
1	n-butanol–acetic acid–water (4:1:5)	0.59	0.59	0.59
2	n-butanol–water (21:4)	0.45	0.45	0.45
3	n-butanol–ethyl alcohol–water (10:3:7)	0.61	0.61	0.61

Decomposition of adenosylcobaloxime (IV) with potassium cyanide. Adenosylcobaloxime (IV) was treated with a 0.1 N aqueous solution of potassium cyanide, left in the dark at 18–20° C for 24 hr, neutralized with a 1 N solution of hydrochloric acid to pH 5, evaporated in vacuum, and chromatographed on FN-1 paper in various solvent systems (see table) with adenine as a reference standard. Then the hydrolyzate was chromatographed on Whatman 3 mm paper in system 1. The spot corresponding to adenine was eluted with water. The absorption was measured in a SF-4 spectrophotometer (λ_{max} 260 mμ).

Decomposition of adenosylcobaloxime (IV) with hydrochloric acid. Adenosylcobaloxime (IV) was heated in a 1 N solution of hydrochloric acid at 100° C for 1 hr. The cooled solution was passed through a column (10 × 1 cm) of Dowex 50B × 2 resin (200–400 mesh in the H^+ form). It was eluted with water. The eluate was concentrated in vacuum and chromatographed on FN-1 paper in system 1. A sugar with R_f 0.43 was detected in the solution. Then it was eluted again with 0.1 N ammonia solution. All the fractions were chromatographed on paper in various systems of solvents (table). The spots corresponding to adenine were eluted with water. The absorption was measured in a SF-4 spectrophotometer (λ_{max} 260 mμ).

Summary

The synthesis and properties of adenosylcobaloxime, a new organocobalt derivative of 5'-deoxyadenosine, have been described.

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