# Studies on Cobaltammines

## VIII. The Infrared Spectrum of the Cobalthexammine(III) Ion in Aqueous Solutions of some Electrolytes

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In previous works in this series it has been shown that the cobalthexammine(III) ion can bind various ligands in its second sphere of coordination. The question then arises: what kind are the bonding forces in these outer-sphere complexes? There are — in addition to mere electrostatic attraction — two possibilities: hydrogen bonding *via* the ammonia groups and an electron transfer bonding. The latter effect can be detected [1] by ultraviolet spectroscopy, whereas infrared spectroscopy may be a useful tool for the detection of hydrogen bonding.

Hydrogen bonding should shift the N—H stretching vibration towards lower frequencies. In order to investigate this effect $Co(NH_3)_6Cl_3$ has been dissolved in heavy water or in heavy water containing $Na_2SO_4$ or KF. Heavy water must be used, as ordinary water absorbs light too strongly in the actual frequency region (3500—2800 $cm^{-1}$). The chloride must be used to achieve sufficently high concentrations of the complex. The experimental conditions were the same as before [2], but for the use of 34 $\mu$ platinum spacers and the fact that the instrument was run with "routine" settings. In order to suppress the isotope exchange, a small quantity of conc. perchloric acid was added to the heavy water (pH $\approx$ 2). In the case of the fluoride solutions pH was then by necessity higher (pH $\approx$ 7). One should therefore expect a rapid isotope exchange [3,4]. However, only a slow fading of the intensity of the N—H absorption was observed (also at 2950 $cm^{-1}$). One possible explanation of this phenomenon may be that the fluoride ions block the positions where hydroxide ions can coordinate and thus the reaction path proposed by Block and Gold [4] would be obstructed.

Some representative results are given in Fig. 1. The most striking feature is the position of the main peak at 3400 $cm^{-1}$. This is decidedly higher than any position

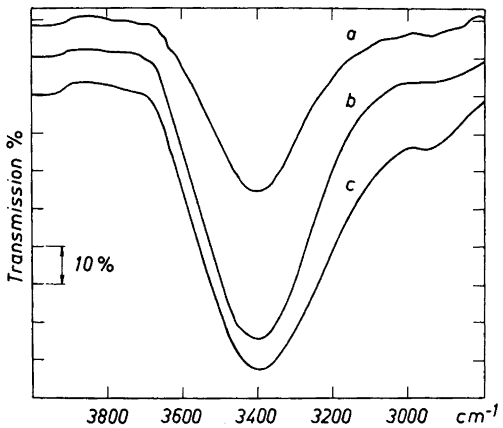


*Fig. 1.* Curve a: 0.06 m $Co(NH_3)_6Cl_3$ dissolved in 1.0 m $Na_2SO_4$. Curve b: 0.19 m $Co(NH_3)_6Cl_3$. Curve c: 0.19 m $Co(NH_3)_6Cl_3$ dissolved in 4.0 m KF. The curves are displaced with 10 % intervals along the ordinate scale to increase the readability.

of N—H stretching vibrations reported for solid cobalthexammine(III) salts (*e.g.* Ref. [5]). It rather corresponds to the position reported (Ref. [6], p. 259) for "free" $R—NH_3^+$ ions in solution. A small peak is apparent at $\sim$ 2950 $cm^{-1}$ for all solutions studied. It is most clearly resolved for the fluoride solutions for which a broadening of the 3400 $cm^{-1}$-peak is observed. This peak at $\sim$2950 $cm^{-1}$ probably corresponds to hydrogen-bonded species. However, as the extinction coefficient of this peak should be expected to be greater than that of the non-hydrogen-bonded one (Ref. [7], p. 101), we are forced to conclude that neither water nor the dissolved anions coordinate *via* hydrogen bonds to any greater extent. This conclusion is obviously in accordance with the one that can be reached from the position of the main peak.

This somewhat astonishing result is probably related to the nonlinearity of the Co—N—H bonds. If a strong hydrogen bond is to be formed, the N—H— —B bond should be linear (Ref. [7], p. 101 and Ref. [8]). Then the atom B will not be in a position as close as possible to the cobalt atom. If the attraction from the cobalt centre supersedes the attraction of the N—H proton, a bent N—H bond will result,

which may not change the N—H vibration frequency much.

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## A New γ-Glutamylpeptide, γ-L-Glutamyl-S-(prop-1-enyl)-L-cysteine, in the Seeds of Chives (*Allium schoenoprasum*)

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The precursor of the lachrymatory factor was isolated in this laboratory two years ago from onion bulbs (*Allium cepa*) and was characterized chemically as (+)-S-(prop-1-enyl)-L-cysteine sulphoxide [1]. In larger amounts this sulphoxide is present in onion bound with the γ-carboxyl group of L-glutamic acid [2] (γ-L-glutamyl-S-(prop-1-enyl)-L-cysteine sulphoxide). We have now isolated the corresponding peptide with a reduced S-atom from the seeds of chives. The isolation of the pure peptide met with difficulties because of its instability. The isolation was finally performed as follows.

1 kg of ground seeds were thoroughly extracted with 70 % ethanol, the amino acids and peptides were separated on an Amberlite IR-120 column, and 0.5 N acetic acid was added to the evaporated ammonia eluate. 200 mg of Johns-Manville filter gel was added to the turbid solution, and the mixture filtered. The fractionation of the solution was performed using a 4.9 × 59 cm column filled with Dowex 1 × 8 resin in acetate form. The eluant was at first 0.5 N acetic acid, after 170 fractions 1 N acetic acid, after 516 fractions 2 N acetic acid, and after 801 fractions 1 N HCl. 1025 fractions of 20.3 ml each were taken in all. Several unknown spots occurred on the paper chromatograms in the fractions separated.

Peptide 12 (the peptide in question) emerged from the column in fractions 594—633. The fractions were evaporated to dryness by lyophilization. The residue was dissolved in 70 % v/v isopropanol and was fractionated with this solvent on a 6.4 × 36.5 cm cellulose powder column. Peptide 12 emerged from the column in fractions 59—81, the volume of the fractions being 18.4 ml. The fractions were evaporated to dryness *in vacuo*, and the peptide was crystallized from an acetone-water mixture. The yield was 1.02 g of white needle-shaped crystals. Since the peptide is to some degree unstable, losses were probably considerable. Several faint spots which travelled slower than the peptide occurred on the paper chromatogram developed with butanol-acetic acid-water as solvent. When the peptide was separated by cutting off the corresponding zone and running a new chromatogram with the same solvent system, the same faint spots appeared again. The peptide accordingly decomposed to some extent during the preparation of the chromatogram.

*Structure elucidation of peptide 12.* The γ-glutamyl bond in the peptide was established by determining the free α-amino and carboxyl groups according to Linko [3]. 79.8 % of ammonia and 96.3 % of carbon dioxide were split off by the effect of ninhydrin. According to the method of Buchanan [4], 90 % of the theoretical amount of $NH_3$ was split off.

An enzyme preparation of calf kidney hydrolyzed the peptide in a phosphate buffer at pH 7.4. Glutamic acid and a sulphur-containing amino acid which travelled at much the same speed as S-propyl-cysteines were established by paper chro-