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Short Communication

Liquid Ammonia as a New Solvent for Cobalamins. Preparation of Anhydrous Ammin- and Cyanocobalamin, and Synthesis of Alkynylcobalamins

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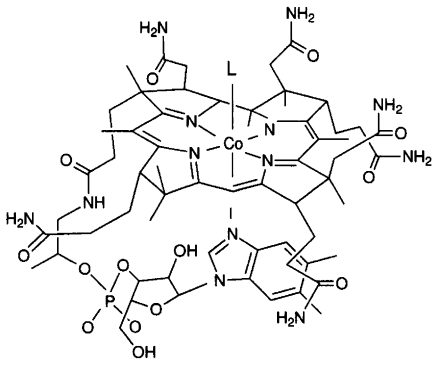
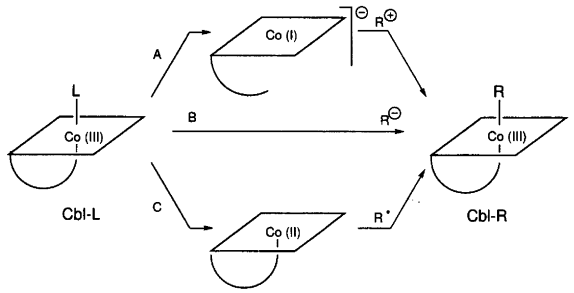
The inorganic chemistry of cobalamins **1** (Cbl-L) deals mainly with the variation of their axial ligands. Especially for the synthesis of organylcobalamins Cbl-R (R = alkyl, alkenyl, alkynyl) i.e. for derivatives with C-bonded axial ligands, the three possibilities in Scheme 1 exist.[1,2]

Until now, the only representative of alkynylcobalamins, ethynylcobalamin (**2**) (Cbl-CCH) has been prepared according to path A (Scheme 1) using bromoethyne as a source of the ligand.[3] As a by-product bromoethenylcobalamin (Cbl-CHCHBr) is formed, but the ethynyl compound can be isolated in pure form.[4]

We considered a direct synthesis of alkynylcobalamins according to path B (Scheme 1) to be more convenient. Reactions with cobalamins are however normally performed in aqueous solutions in which alkynyl anions



1 (Cbl-L)



Scheme 1.

cannot be handled. Liquid ammonia is on the other hand an excellent solvent for these anions.[5–7] Since liquid ammonia is a solvent similar to water, it can as well be expected to be suitable for cobalamins, since the corrin moiety of **1** contains seven carboxylic amide groups.

Cobalamins are indeed easily dissolved in liquid ammonia. In addition, by repeatingly condensing and evaporating the ammonia, crystal water can be removed from the compounds or reduced considerably. For instance, when solutions of cyanocobalamin are evaporated four times in a row a product of the composition Cbl-CN · ½NH_3 (**4**) is obtained.

In aquacobalamin ($[Cbl\text{-}OH_2]^+Cl^- \cdot 17H_2O$, **5**)[8] not only the solvate water but also the axial aqua ligand is substituted by ammonia resulting in ammincobalamin ($[Cbl\text{-}NH_3]^+Cl^- \cdot ½NH_3 \cdot H_2O$, **6**). The hydrated form of ammincobalamin is known and the electronic spectrum of **6** in water was similar to the spectrum described in the literature.[9,10] The now obtained practically anhydrous form of **6** proved to be well suited for the synthesis of alkynylcobalamins. The axial ligands of both ammincobalamin in the ammono system and of aquacobalamin

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in the aquo system (ammonia and water, respectively) are good leaving groups. They differ, however, in their acidity, since unlike water, ammonia does not protonate alkynyl anions. Thus the known[3,4] ethynyl-**2** and the hitherto unknown phenylethynylcobalamin **3** were prepared from ammincobalamin and the respective alkynyl anions in liquid ammonia by a simple displacement reaction.

Experimental

Apparatus. For all operations in liquid ammonia as a solvent specially constructed glassware, already described in detail, was used.[11] The reactions and all handling of the substances were carried out under minimal exposure to light.

Starting material. Aquacobalamin hydrochloride, cyanocobalamin, acetylene and phenylacetylene were commercially available products. The sodium alkynyls were obtained by deprotonation of the respective alkynes using sodium amide.[5–7] Solvents used were all analytical grade and distilled prior use. For the column chromatography Amberlite XAD-2 (20–50 mesh) from Serva was used. Paper strips for the paper chromatography were from Schleicher–Schüll as well as the paper no. 2040 for the paper electrophoresis. The paper chromatography was performed in water-saturated 2-butanol using Cbl-CN with the R_f value 1.0 as a reference,[3] and the paper electrophoresis in 0.5 M acetic acid with Cbl-CN and factor B (cyanoaquacobinamide) with R_f values of 0.0 and 1.0, respectively, as references.

Instrumental. The NMR spectra were recorded in D_2O at 298 K on Bruker AM 360 and Bruker AC 250 spectrometers using standard Bruker software for water suppression and TMS as external standard for the ^1H- and $^{13}C\{^1H\}$-spectra. For the IR spectra in a KBr pellet, Perkin Elmer 325 and 577 spectrometers were used, while for the electronic spectra Perkin Elmer 554 and a Varian Gary 17 spectrophotometers were used.

Synthesis

Anhydrous cyanocobalamin. Cyanocobalamin hydrate (250 mg) was dissolved in liquid ammonia (250 ml). The solvent was evaporated and the procedure repeated three times. Prior elementary analysis the product was dried under a high vacuum. Cbl-CN·$\frac{1}{2}NH_3$, $C_{63}H_{89.5}CoN_{14.5}O_{14}P$ (**4**): Found: C55.23; H6.94; N15.01. Calcd.: C55.52; H6.62; N15.18.

Ammincobalamin. By the above described procedure **5** gave $[Cbl\text{-}NH_3]^+Cl^- \cdot \frac{1}{2}NH_3H_2O$, $C_{62}H_{94.5}ClCoN_{14.5}O_{15}P$ (**6**) (1408.4). Found: C52.80; H7.12; N14.42 Calcd.: C52.87; H6.76; N14.48.

Ethynylcobalamin. To the red solution of ammincobalamin (200 mg, 0.14 mmol) in 100 ml of liquid ammonia was added 1.0 mmol of sodium acetylide. This resulted in a colour change of the solution to yellow–brown. The mixture was refluxed for 30 min then filtered and the solvent evaporated. The yellow–brown residue was dissolved in water (20 ml) affording a red solution which was added to a column filled with Amberlite XAD-2. It was washed with water to free the product from salts and eluted with methanol.[12,13] The methanol was removed, and the red product was characterized by elementary analysis and spectroscopic methods. It was also tested with paper chromatography and paper electrophoresis, classical methods in corrin chemistry. Cbl-CCH·12 H_2O, $C_{64}H_{113}CoN_{13}O_{26}P$ (**2**). Found: C48.39; H7.30; N11.46. Calcd.: C48.46; H7.18; N11.48. The compound is very light-sensitive in aqueous solution. Electronic spectrum (λ_{max}/nm): 270, 279, 289 (sh), 340, 362, 524 ans 556 (sh) (sh = shoulder).

Phenylethynylcobalamin. The same procedure as above was followed, giving a light-red solution after the addition of the sodium phenyl acetylide. The product was eluted from the XAD-2 column with methanol, disolved in water and extracted several times with ether. It was finally recrystallized from water–acetone. Cbl-CCC_6H_5·9 H_2O, $C_{70}H_{111}CoN_{13}O_{23}P$ (**3**). Found: C52.71; H5.92; Co3.67; N11.22; P1.93. Calcd.: C52.79; H7.03 Co3.70; N 11.43; P1.94. Electronic spectrum (λ_{max}/nm): 268 (sh), 328, 345 (sh) 382, 502, 530 (sh).

The IR spectra of **2** and **3** were similar to the IR spectra of cobalamins, with the addition of signals which are related to the b-axial ligand.[14] The stretching vibration of the triple bond appeared at 1985 and 2020 cm^{-1}, respectively, and **3** showed in addition characteristic signals for a monosubstiuted aromatic ring at 750 and 690 cm^{-1}. For reasons of comparison Cbl-CN was measured, giving a signal for the triple bond at 2030 cm^{-1}. The electronic spectrum of **2** in water was comparable to the data described in the literature.[15] The paper electrophoresis of **2** and **3** in 0.5 M acetic acid confirmed the negatively charged alkyl ligand, with R_f values of 0.0, and the paper chromatography showed R_f values of 1.4–1.6, which are in good agreement with published data of alkyl cobalamins.[13]

The phenyl group of **3** was additionally identified in the ^1H and ^{13}C NMR spectra.[16,17] In the aromatic region of the^1H NMR spectrum, in addition to the signals of the corrin moiety there was a multiplet at 7.1–7.2 ppm of the intensity of five protons and in the ^{13}C NMR spectrum there were signals at 131.7, 129.4 and 127.8 ppm.

Liquid ammonia is an excellent solvent for cobalamines; its application gives an opportunity to isolate pure corrins, which are difficult or even impossible to synthesize by conventional methods.

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