ploying synchrotron radiation and imaging plate detector technology, constituting by far the most accurate structure determination of a B_{12} ever reported, and yielding a molecular description with the precision and reliability of a very good small-molecule structure; (2) EXAFS measurements on crystals of the same batch used for the crystal structure analysis, as well as on solutions of $1^+ClO_4^-$, permitting a correlation between the solution spectra and the spectra from crystals of accurately known structure; and (3) the first detailed NMR investigations on a B_{12} derivative in H_2O, in particular of solutions of 1^+Cl^-, including 2D homo- and heteronuclear studies and assignment of signals due to the exchangeable amide protons of all nitrogens as well as measurements of amide proton exchange rates. These studies confirm the occupation of the axial coordination site at the Co(III) center by water, as well as the occurrence in solution of an intramolecular hydrogen bond to the axially coordinating water molecule, as observed in the crystal structure of $1^+ClO_4^-$. In addition, the recently reported[9] assignment for 1^+Ac^- was verified.

Results

Single-Crystal Structure Analysis of Aquocobalamin Perchlorate ($1^+ClO_4^-$). Crystals were grown from water after exchanging the chloride counterion of commercially available "aquocobalamin hydrochloride" by perchlorate.[17] We were not able to obtain suitable crystals from water–acetone. Cell dimensions were determined at ambient temperature[18] on a conventional four-circle diffractometer; intensity data (from the same crystal) were obtained with synchrotron radiation (λ = 0.65 Å) using a rotation camera with imaging plate detector. Since the cell dimensions refined against the imaging plate synchrotron data correlate strongly with the wavelength and the crystal–detector distance, they were regarded less reliable than the lattice constants obtained with the diffractometer.[19]

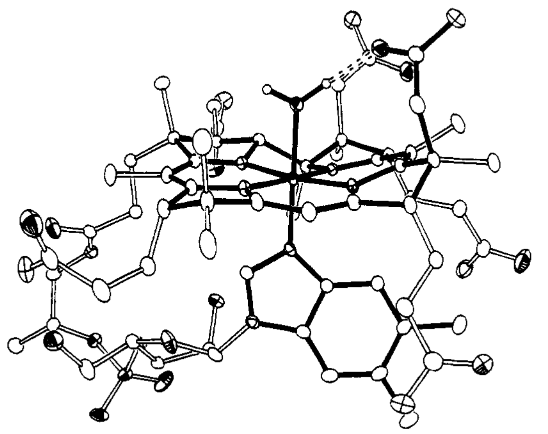

Figure 2. ORTEP-type representation[80b] of the 1^+ ion, as observed at room temperature in the crystal structure of $1^+ClO_4^-$. Note that thermal ellipsoids are drawn at the 10% probability level (as opposed to the usual 50%) in order to obtain a less clustered picture. Carbon atoms are represented as open ellipsoids, and principal axes have been drawn for nitrogen and principal axes plus shaded octants for oxygen, phosphorus and cobalt. The intramolecular hydrogen bond between the coordinated water molecule and the carbonyl oxygen of the c-acetamide is represented as a dashed bond. Hydrogen atoms were omitted with the exception of the two hydrogens on the coordinated water molecule, which have been observed in a difference electron density map and were included in the crystallographic refinement.

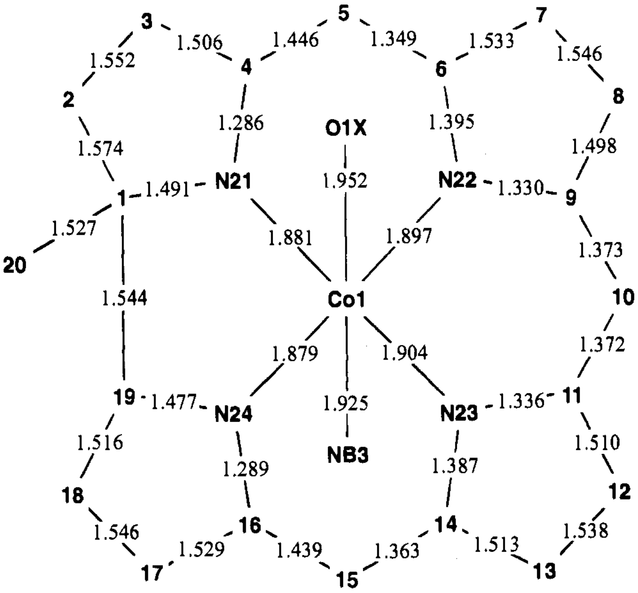

Figure 3. Bond lengths as observed in the crystal structure of $1^+ClO_4^-$. The ESD's for C–C and C–N bonds are 0.003 Å and for Co–N and Co–O bonds 0.002 Å.

The crystal structure was refined against a total of 22 867 reflections (Friedel equivalents not merged) with a maximum resolution of 0.8 Å. Refinement of 1165 parameters (subject to 649 restraints) converged at R-values of 0.045 for the 20 942 significant reflections and 0.050 for all 22 867 reflections. At the close of refinement, the average standard deviation for a carbon–carbon bond was 0.003 Å. Atomic coordinates are given in Table S2 (supplementary material). An ORTEP-type drawing of the aquocobalamin ion as observed in the crystal structure of $1^+ClO_4^-$ is shown in Figure 2. Selected bond lengths for the corrin ring and the cobalt coordination are given in Figure 3. The bonding distances of the conjugated system show the earlier noted[20a] symmetry about the Co–C10 line;

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(15) This concept has originally obtained early experimental support through work by Schrauzer[16a] and Pratt,[16f] who referred to the "mechanochemical" nature of steric factors governing Co–C bond cleavage. It has subsequently been advanced under a variety of names—butterfly-bending,[16a] upward conformational distortion,[16d] corrin butterfly conformational distortion,[16c] and conformational trigger mechanism[16e]—by several of the leading scientists in the B_{12} field, including Halpern,[41a] Marzilli,[16b] Glusker,[20] Golding,[16e] and Finke,[16c]; see, however, the critical statements by Brown et al.[16g] and Kräutler et al.[4j]

(16) (a) Schrauzer, G. N.; Grate, J. H.; Hashimoto, M.; Maihub, A. In *Vitamin B_{12}, Proceedings of the Third European Conference*; Zagalak, B., Friedrich, W., Eds.; W de Gruyter: Berlin, 1979; p 511. (b) Marzilli, L. G. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker, Inc.: New York, 1993; p 227. (c) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629. (d) Halpern, J. *Science* **1985**, *227*, 869. (e) Golding, B. T.; Rao, D. N. R. In *Enzyme Mechanisms*; Page, M. I.; Williams, A., Eds.; Royal Society of Chemistry: London, 1987; p 404. (f) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274. (g) Brown, K. L.; Brooks, H. B. *Inorg. Chem.* **1991**, *30*, 3420.

(17) Crystallization of "aquocobalamin hydrochloride" from water/acetone leads to the formation of chlorocobalamin crystals. Its crystal structure has (accidentially) been determined in the course of the present investigation.[19b] The result of this structure analysis will be reported elsewhere.

(18) We also tried to cool the crystals grown from water to cryotemperature (≈100 K). Although the crystals showed similar cell dimensions at low temperature, the cooling resulted in a deterioration of the diffraction pattern (increase of the mosaic spread), which compelled us to carry on at room temperature, where they exhibit favorable mosaicity and excellent stability in the X-ray beam, permitting the collection of several diffraction data sets from the same crystal.[19]

(19) (a) Following the data collection on the synchrotron, we also collected data on a rotating anode source with an imaging plate detector and on a sealed tube source with a conventional diffractometer, using the same crystal.[19b] By all standards, the synchrotron data were found to be superior to the other two data sets. A detailed comparison between the results of the three data collections will be given elsewhere. (b) Färber, G. Ph.D. Thesis, University of Graz, 1993.

(20) (a) Glusker, J. P. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, p 23. (b) Rossi, M.; Glusker, J. P. In *Molecular Structure and Energetics*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers: Weinheim, FRG, 1988; Vol. X, p 1.

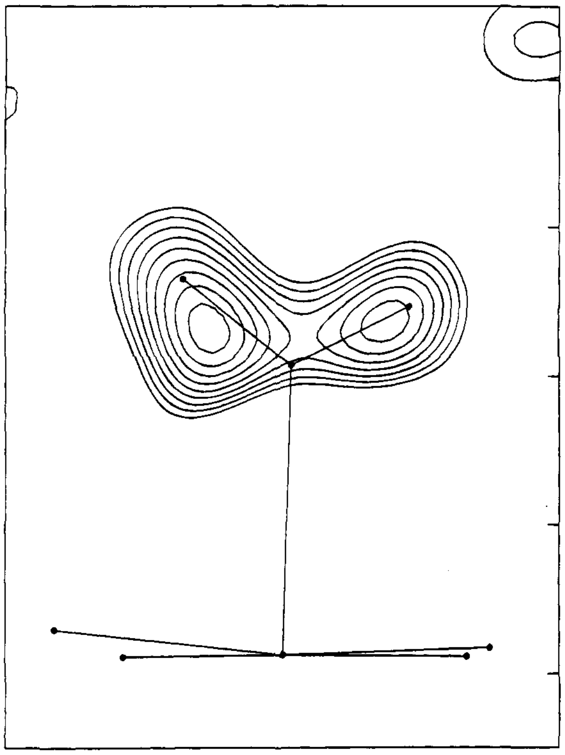

Figure 4. "Omit" map for the hydrogen atoms of the cobalt-coordinated water molecule in the crystal structure of $1^{+}ClO_4^{-}$. The figure shows the result of a ΔF Fourier synthesis with the final coordinates, excluding the two protons. The hydrogen atom positions indicated in the figure were obtained by refining the H-coordinates subject to an O—H distance restraint of 0.95 Å; lowest contour 0.1 eÅ^{-3}, increment 0.02 eÅ^{-3}, rms deviation of electron density from the mean value 0.06 eÅ^{-3}.

deviations from this symmetry are small but statistically significant (χ^2 = 28 for six degrees of freedom). These quantities agree remarkably well with theoretical values proposed more than 30 years ago[21] on the basis of very crude bond orders. A thermal motion analysis[22] for the corrin ring and its directly attached substituents yields bond length corrections of 0.001−0.002 Å.

The most noteworthy features of the crystal structure of $1^{+}ClO_4^{-}$ are (1) the short axial Co—N(DMB) distance of 1.925 (0.002) Å, which is the shortest axial Co—N distance observed so far in a cobalamin; (2) the large upward folding angle[23] of 18.71(0.07)°; (3) the base tilt angle[25] of 5.46(9)°; (4) an intramolecular hydrogen bond between the cobalt-coordinated water molecule and the carbonyl oxygen atom of the c-acetamide (O39···H1(O1x), 1.78 Å; O39···O1x, 2.662 Å). A very similar intramolecular hydrogen bond has been observed in the only other crystal structure of a B_{12} molecule with a water axially coordinating the metal center, i.e., for aquocyanocobyric acid;[26] and (5) direct observability in a difference Fourier synthesis of the electron density originating from the two protons of the cobalt-coordinated water molecule (Figure 4).

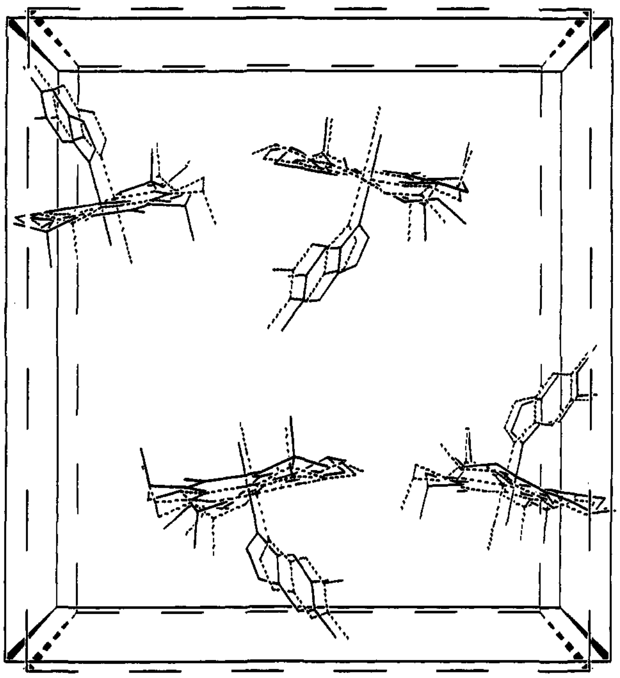

Figure 5. Superposition of the crystal structures of aquocobalamin perchlorate ($1^{+}ClO_4^{-}$) (full lines) with vitamin B_{12} (**2**) (dashed), projected down the crystallographic a-axis. Side chains and solvent molecules have been omitted for clarity.

$1^{+}ClO_4^{-}$ crystallizes in the orthorhombic space group $P2_12_12_1$ with four cobalamin units plus 100 solvent water molecules per unit cell. The B_{12} molecules are packed into the orthorhombic $P2_12_12_1$ unit cell in a way characteristic for the majority of other "complete corrinoids".[4,7] This preferred mode of packing can be exemplified by comparing the crystal structure of $1^{+}ClO_4^{-}$ with that of the vitamin **2**.[4a,7] Figure 5 shows a superposition of the unit-cell content of the crystal structures of $1^{+}ClO_4^{-}$ and **2**, projected down the crystallographic a-axis. Only the corrin rings and the two cobalt ligands are shown for each B_{12} molecule to obtain a less cluttered picture. The structural similarity in the positions of the B_{12} molecules in the unit cell also extends to part of the solvent domain, which shows the perchlorate counterion in the crystal structure of $1^{+}ClO_4^{-}$ at the same position as the (only) acetone molecule in the structure of **2**. Also some—but by no means all—of the solvent water molecules are observed in similar positions in the two structures: 8 of the 25 solvent water molecules of the aquocobalamin structure are observed within 1 Å of one of the 12 fully occupied solvent sites in the vitamin structure. As a consequence, the hydrogen-bonding network is quite different between the two crystal structures, which is remarkable in view of the very similar packing of the cobalamin molecules. A listing of all intra- and intermolecular hydrogen bonds observed in the crystal structure of $1^{+}ClO_4^{-}$ is given in Table S4 (supplementary material).

Figure 6 shows a superposition of the molecules of $1^{+}ClO_4^{-}$ (full line) with **2** (dashed), as observed in their respective crystal structures. The similarity in the overall molecular conformation is in line with the above packing resemblance, although there are differences in the conformations of some of the side chains; particularly noteworthy is the difference in the conformation of the c-acetamide side chain, which—relative to **2**—swings around by almost 180° to form an intramolecular hydrogen bond to the Co-coordinated water molecule. Differences exceeding 1 Å between corresponding atoms are also observed for the b- and g-side chains and the ribose hydroxy group OR8. The latter group is found to be

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(21) Hodgkin, D. C. *Proc. R. Soc. London, Ser. A* **1962**, *288*, 294.

(22) Dunitz, J. D.; Shoemaker, V.; Trueblood, K. N. *J. Phys. Chem.* **1988**, *92*, 856.

(23) The upward folding angle is defined[24] as the dihedral angle between the planes through atoms N21, C4, C5, C6, N22, C9, C10 and C10, C11, N23, C14, C15, C16, N24, respectively (see Figure 1).

(24) Lenhert, P. G. *Proc. R. Soc. London, Ser. A* **1968**, *303*, 45.

(25) The base tilt angle has been defined[7] as one-half the difference between the two axial Co—N—C angles, i.e., $(\phi_{Co1-NB3-B9} - \phi_{Co1-NB3-B2})/2$ (see Figure 1 for a definition of the atom numbering).

(26) Venkatesan, K.; Dale, D.; Hodgkin, D. C.; Nockolds, C. E.; Moore, F. H.; O'Connor, B. H. *Proc. R. Soc. London, Ser. A* **1971**, *323*, 455.