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A Crystalline B_{12} Dimer from β-Cyano-Neocobyrate**

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Dedicated to Prof. Dr. Helmut Schwarz on the occasion of his 65th birthday

Nature's selection of the vitamin B_{12} coenzymes as ubiquitous organometallic cofactors is an intriguing and unsolved question.[1] Several biosynthetic pathways in aerobic and anaerobic microorganisms converge to provide vitamin B_{12} cofactors as uniquely structured natural cobalt complexes.[2–4] X-ray crystallography helped to elucidate not only the organometallic nature of coenzyme B_{12} (**1**), but first of all, the structure of the cyano-corrin vitamin B_{12} (**2**).[5,6] We report here the crystal structure of β-cyano-neocobyrate (**3**) and the discovery of a new structural motif for vitamin B_{12} derivatives. The dimeric nature of **3** provides insight in a remarkable structural feature of the natural corrinoids.

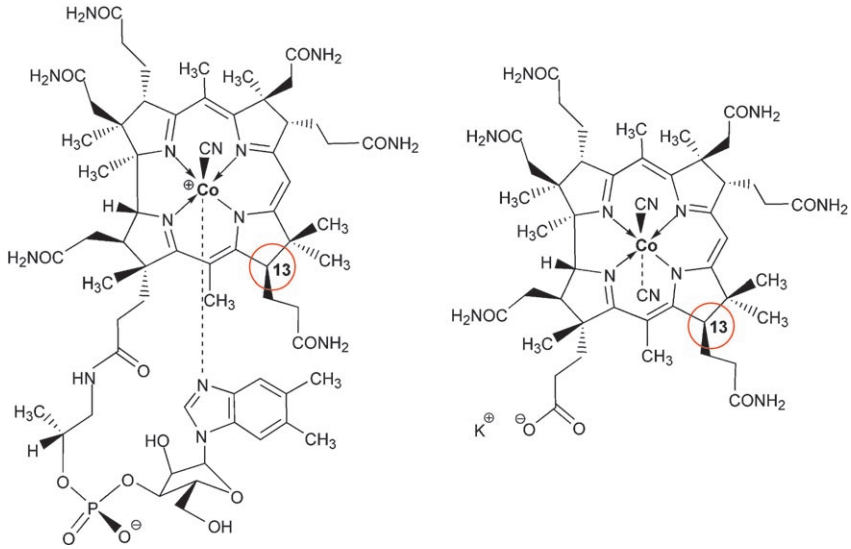

Figure 1. Structural formulae: left: neovitamin B_{12} (**4**), right: potassium dicyano-neocobyrate (**K-3a**); in vitamin B_{12} (**2**) and dicyano-cobyrate (**K-5a**) the configuration at C-13 is inverted (i.e., α and not β, see red circle).

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[**] B_{12} refers to a vitamin B_{12} derivative.

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Neovitamin B_{12} (**4**) was described in the early 1970s as isomerization product of vitamin B_{12} (**2**),[7] and it was identified by X-ray crystallography as the 13-epimer of **2** (see Figure 1).[8] Most of the "neocorrinoids" were only characterized by UV/Vis and CD spectra and by their chemical correlation with "normal" corrinoids. Acid-catalyzed epimerization inter-converts "neocorrinoids" and "normal" corrinoids selectively, and neocorrinoids typically predominate slightly in such equilibrations.[9,10] "Neocorrinoids" were also side-products in the total synthesis of cobyric acid (**5**),[9,11] but they appear not to be formed naturally by vitamin B_{12} biosynthesis.[2–4]

The dicyano-form **3a** of neocobyric acid (**3**) was obtained in 18% yield from vitamin B_{12} along with cobyric acid (20%).[12,13] Treatment of an aqueous solution of **3a** with acetic acid gave aquo-cyano-neocobyrate. Dimeric β-cyano-

neocobyrate (**3**–**3**) recrystallized in about 80% yield upon addition of acetone. The crystal structure of **3** was determined by using synchrotron radiation and diffraction data extending to a resolution of 0.95 Å. The asymmetric unit of the rhombohedral crystal contained four independent neocobyrate molecules, which were arranged as (two) very similarly structured (C_2-symmetric) dimers **3**–**3** (Figure 2). Dimerization was effected by coordination of the carboxylate function of the *f*-propionate of one corrin moiety to the Co centre (α-face) of the other.

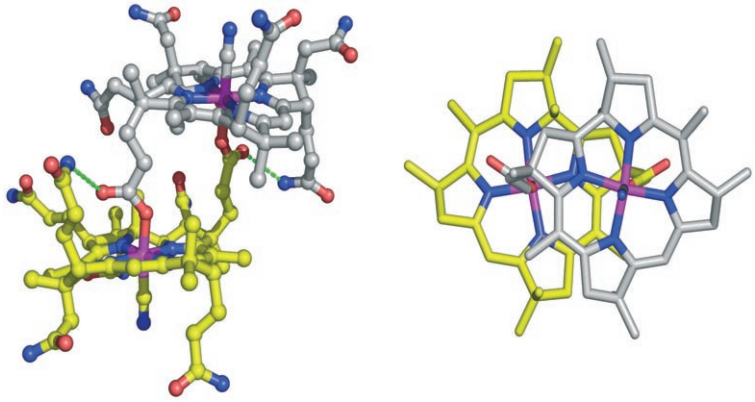

Figure 2. Model of the structure of the β-cyano-neocobyrate dimer (**3**–**3**). Left: ball-and-stick representation. Carbon atoms are shown in white and yellow, nitrogen in blue, oxygen in red and cobalt in magenta. Major components only are shown of the disordered side chains. The H bond between the carboxylate of the *f*-side chain and the *d*-propionamide is indicated by green dashes. Right: Stick representation of the dimer **3**–**3** viewed along an axis perpendicular to the dimer generating two-fold axis (acet- and propionamide side chains have been omitted for clarity).

This is the first structure of a corrinoid dimer in which the α-faces of the corrin moieties interact. In other corrinoid dimers, tetramethylene-bis(cobalamin)[14] and iodo-Co^{II}-cobester (see ref. [6]), the corrinoids form dimers via cobalt-coordinated ligands at the less encumbered β-faces. In the crystal structure of **3**, the distance between the almost parallel corrin rings is approximately 7 Å. The regions with greatest vertical overlap are ring D and the C15 *meso*-bridge (Figure 2). In a projection perpendicular to the corrin planes, the two Co atoms are displaced by roughly 4 Å. Close intra-dimer contacts exist between C1A···C1A′ (3.8 Å), O183···C1A′ (3.5 Å) and C12A···C12A′ (3.7 Å). The only polar contact between the corrin moieties in a dimer is an H-bond between N84 and O175' of the *d*- and *f*-side chains, respectively (for atom numbering see Supporting Information).

The geometry of the inner coordination sphere of the cobalt centre in **3** is comparable to that of β-aquo-α-cyano-cobyric acid (**5**):[6,15] The structure of the corrin ring in **3** is remarkably similar to that of the corrin moiety of neovitamin B_{12} (**4**) (Figure 3) and only the pucker of rings D differs significantly in **4** and **3**. Thus the fold angle of 22.5(5)° in **3** is only slightly smaller than the one in **4** (23.7°).[8,16] In contrast, the related comparison between α-cyano-β-aquo-cobyric acid (**5**) and vitamin B_{12} (**2**) revealed an increase of the fold angle from 4.3° (in **5**)[6,15] to 18.0° (in **2**), which was suggested to reflect steric strain excerted by the coordinated dimethylbenzimidazole (DMB) base in **2**.[17] The fold angle is characteristically larger in **3** than in **5** and in other "normal" corrinoids (average fold angle 7.5(5)°).[5,6]

The conformational switch in ring C of **3** breaks the pseudo-C_2 symmetry of the corrin ligand, observed in "normal" cobyrinic acid derivatives.[15] Concomitant with "inverting" the conformation of ring C, the corrin ligand of **3** locally adopts a "wave-shaped" (W)-conformation[1] and exhibits an increased folding angle. Indeed, similar conformational coupling between the macrocycle and (one of) the five-membered hydropyrrolic rings has been analyzed in crystal structures of the hydroporphyrinoid nickel-complex F430 and its C-12/C-13 epimer.[1,18]

The *f*-propionic acid substituent of corrinoids typically displays less conformational disorder than the other propionic acid side chains.[19] In the structure of **3**–**3** the (C-171/C-172)-bond and the carboxylate function of the *f*-substituent are nearly eclipsed (see Figure 2). This conformation apparently is

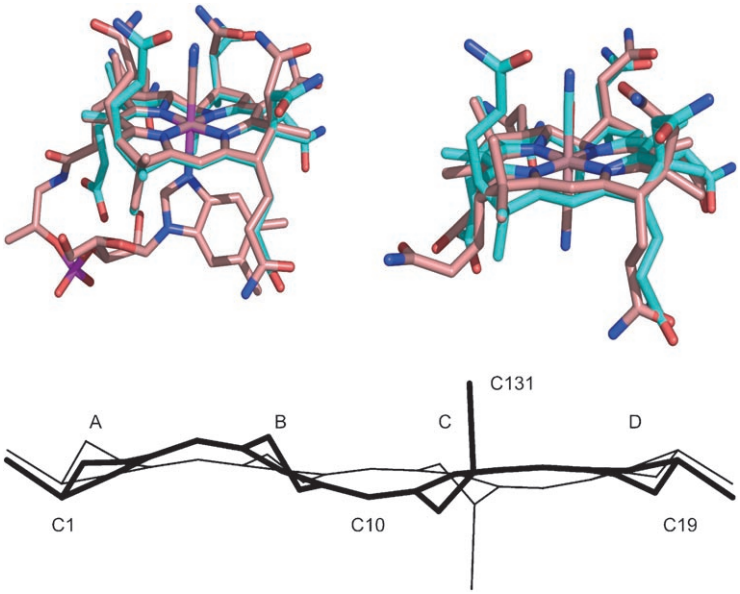

Figure 3. Top: Superposition of crystal structures (on the four corrin nitrogen atoms). Left: of β-cyano-neocobyric acid (**3**, cyan) and neovitamin B_{12} (**4**, pink). Right: of **3** (cyan) and α-cyano-β-aquo-cobyric acid (**5**, pink). In the structures of **3**, only the major components of the disordered side chains *b*, *d*, and *e* are shown for clarity. Bottom: Cylinder projection of β-cyano-neocobyric acid (thick lines) and α-cyano-β-aquo-cobyric acid (thin lines) based on a mean plane through the four corrin nitrogen atoms (for atom numbering, see Supporting Information).

required by the proper cobalt coordination of the *f*-carboxylate. NMR-analysis of β-cyano-neocobyric acid in aqueous solution (where water is the α-ligand) did not reveal a tendency to form dimers.

The formation, in the crystal, of an α-bridged dimer provides a new structural motif for B_{12} derivatives. While this motif is present in the neocobyrate **3**, the structure of **3** also indicates that its particular type of dimerization would be unlikely for the "normal" cobyric acid. A crude model of such an *f*-carboxylate bridged dimer (obtained by superimposing the structure of cobyric acid[15] onto each component of the neocobyric acid dimer) indicates dimerization to be obstructed by clashes of the *e*-propionamide side chains of each subunit, see Figure 4).

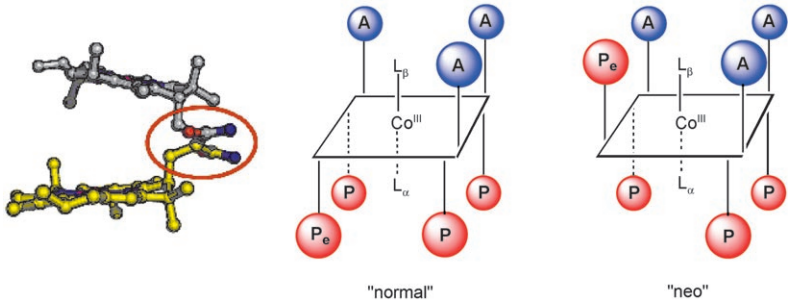

Figure 4. Left: Model of a cobyric acid dimer generated by superimposing the structure of cobyric acid on each component of the neocobyric acid dimer. All side chains except for the *e*-propionamide group were omitted. Carbon atoms are shown in white and yellow. Right: Symbolic stereo-models of "normal" and "neo"-corrinoids (A = acetamide group, P = propionamide (propionate) group, P_e = *e*-propionamide group).

The α-configuration of the *e*-side chain in "normal" corrinoids and the absence of an acetamide group at ring C have been suggested to be important, first of all, for the proper function of coenzyme B_{12}.[1,20] A crystal structure of neocoenzyme B_{12} (**6**) is not available and **6** still functions as cofactor in some B_{12}-dependent enzymes, for example, diol dehydratase.[21] However, the crystal structures of coenzyme B_{12} (**1**)[5,6] and of neovitamin B_{12} (**4**)[8] indicated the *e*-propionamide side chain in **6** and the organometallic 5′-deoxyadenosyl-functionality to compete for the same region of space.[20]

In **5** and other "normal" corrinoids all four propionamide (propionate) groups are oriented to the α-face of the corrin ligand, including the e-side chain at C-13. The latter is reoriented to the β-face in the neocorrinoids (see Figure 4).[22] As shown here, the altered substituent pattern of the "neo"-corrinoid **3** sets the stage for dimer formation. Natural B_{12} derivatives, in contrast, are "notoriously" monomeric,[5] and typically differ also in this respect from other natural porphyrinoids. These have often been observed in (ligand bridged) dimeric states (e.g. in Fe^{III}–porphyrins[23]) or as higher aggregates (e.g. with chlorophylls[24]).

Carboxylate-bridging, as observed in **3–3**, is the basis of aggregation of Fe^{III}-heme in β-haematin. Carboxylate bonded heme dimers were identified as the building blocks of β-haematin, providing a structural model for hemozoin, the "detoxified" disposal form of heme in *Plasmodium malaria*.[25,26] The crystal structure of **3–3** reminds of features of the proposed (dimer) structure of hemozoin. Recently, vitamin B_{12}-derivatives were indicated to act as remarkable inhibitors of hemozoin formation,[27] opening a possible alternative for the treatment of strains of *Pl. malaria*, which have become resistant to chloroquine and other relevant anti-malarials.[28] The structural motif of a carboxylate bridged B_{12} dimer **3–3** may also add a new facet to this subject.

Experimental Section

Preparation of β-cyano-neocobyric acid (3): The title compound was prepared in its dicyano-form (**3a**) from vitamin B_{12} (**2**, Hoffmann-La Roche)[12] and was identified by 500 MHz ^{1}H NMR, UV/Vis- and CD spectra (see Supporting Information): UV/Vis (Hitachi-U3000, in H_2O, $c = 5.25 \times 10^{-5}$ M, λ_{max} ($\log \varepsilon$): 279.0 (3.87), 310.5 (3.95), 368.5 (4.33), 581.5 nm (3.84 L mol^{-1} cm^{-1}); CD (JASCO-J715, in H_2O, $c = 5.25 \times 10^{-5}$ M, λ_{max} and λ_{min}($\Delta\varepsilon$): 223.0 (2.8), 237.0 (−2.3), 281.0 (13.1) 309.0 (18.6), 341 (−11.5), 377.0 (−5.8), 406.0 (9.3), 436.0 (12.1), 530.0 (−12.7), 575 nm (−12.2 L mol^{-1} cm^{-1}).

Recrystallization and crystal-structure determination of β-cyano-neocobyric acid (3): Cyano-neocobyric acid was obtained by treatment of **3a** with dilute aqueous acetic acid and precipitation with acetone. The dark red powder of **3** was dissolved in water and crystals of β-cyano-neocobyric acid (**3**) grew upon addition of acetone. Diffraction data were collected at the EMBL beam line BW7b at DESY in Hamburg (Germany). Details of the data collection and structure refinement are given in the Supporting Information. CCDC 641650 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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