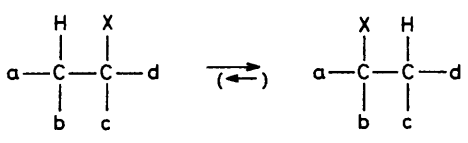
The Crystal Structures of (*R*)- and (*S*)-2,3-Dihydroxypropylcobalamin; Comparison with the Structure of Adenosylcobalamin

Nathaniel W. Alcock,[a] Ruth M. Dixon,[b] and Bernard T. Golding*[b]

[a] *Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K.*
[b] *Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU, U.K.*

The crystal structures of (*R*)- and (*S*)-2,3-dihydroxypropylcobalamin show Co–C_α–C_β angles of 119.6 ± 1.7 and 113.6 ± 2.1° and Co–C bond lengths of 2.002(23) and 2.079(30) Å, respectively; comparison with the structure of the B_{12} coenzyme adenosylcobalamin suggests that the coenzyme's Co–CH_2–C angle of 125° is abnormal.

Adenosylcobalamin (AdoCbl, a coenzyme form of vitamin B_{12}) assists the enzymic catalysis of a number of molecular rearrangements of the type shown in equation (1),[1] where X = OH, NH_2, or a carbon derived group (*e.g.* $CH\overset{+}{N}H_3CO_2^-$) and a, b, c, d, are substituents (*e.g.* a = OH, b = c = d = H, when X = OH or NH_2). Most investigators accept that the first step



(1)

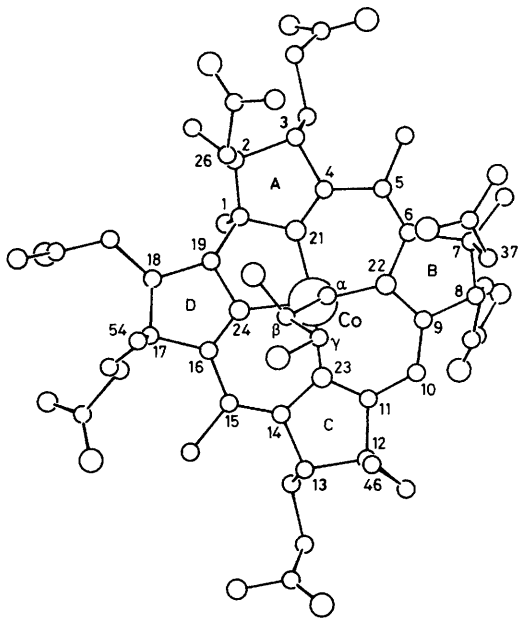


Figure 1. View of part of molecule (**1a**) (lacking the nucleotide loop), showing the atomic numbering of corrin atoms and the disposition of the 2,3-dihydroxypropyl group.

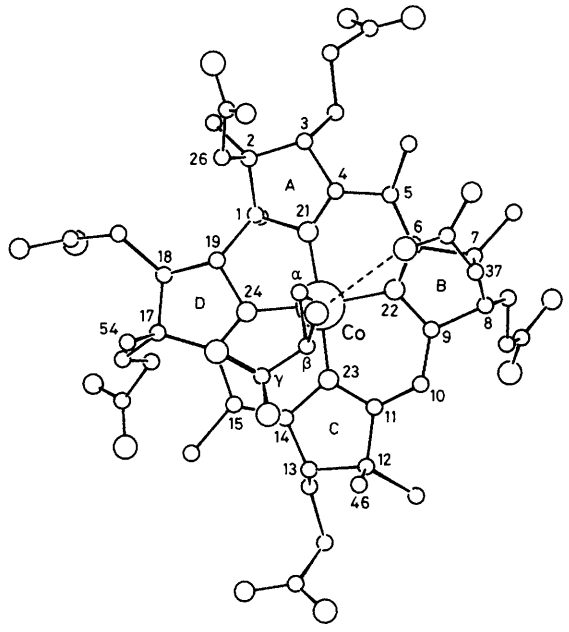


Figure 3. View of part of molecule (**1b**) (lacking the nucleotide loop) showing the atomic numbering of corrin atoms, the disposition of the 2,3-dihydroxypropyl group, and the hydrogen bond from C_β-OH to the ring B acetamido oxygen; the oxygen at C_γ is disordered.

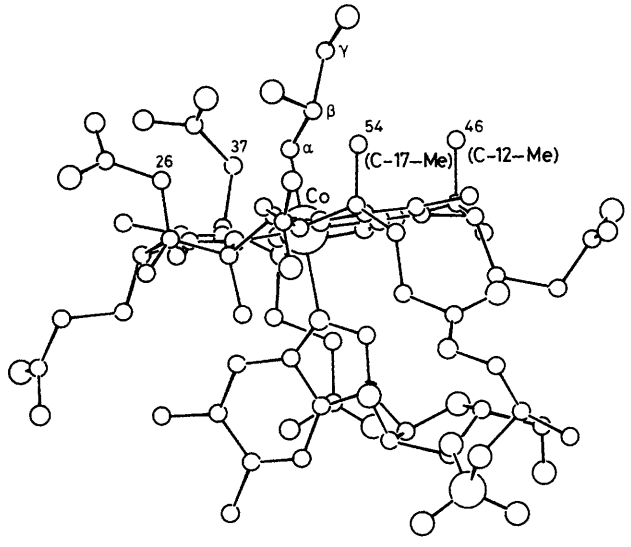


Figure 2. View of molecule (**1a**) showing the atomic numbering of sentinel groups and the disposition of the 2,3-dihydroxypropyl group.

in the catalytic cycle is homolysis of the Co–C σ-bond of AdoCbl to give cob(II)alamin and the adenosyl radical, which abstracts a hydrogen atom from a substrate molecule.[1] It has been suggested that the Co–CH_2–C (Co–C_α–C_β) bond angle of 125 ± 3° in AdoCbl, determined by crystal structure analysis,[2,3] shows that the coenzyme is strained by intramolecular steric interactions.[2,4] It was proposed that this strain aids homolytic cleavage in the enzymic reactions.[4] In a recently proposed alternative theory of the mechanism of AdoCbl-dependent enzymic reactions, the 125° angle was taken to imply unusual bonding in alkylcobalamins which was described as carbenoid for C_α.[5] Studies of alkylcobaloximes have shown that the Co–C_α–C_β angle can vary widely [*e.g.* 114.2° in isopropyl(pyridine)cobaloxime, 130.3° in neopentyl(pyridine)cobaloxime] and the variations have been ascribed to steric effects (repulsion between σ-alkyl group and dimethylglyoximate ligands).[6] It is therefore important to establish whether the Co–C_α–C_β angle in AdoCbl is typical or atypical for alkylcobalamins. To attempt to answer this question, we have determined the single crystal structures† of the diastereoisomeric 2,3-dihydroxypropylcobalamins[7] [*R*-isomer (**1a**): see Figures 1 and 2; *S*-isomer (**1b**): see Figures 3 and 4].

The structures of the cobalamin portion of these molecules are very similar to the cobalamin of AdoCbl (*cf.* ref. 3).

† *Crystal data* for (**1a**) (*R*-form, from diglyme–water): $C_{65}H_{95}CoN_{13}O_{16}P \cdot ca.15H_2O$, M = 1404.42 (excluding water), orthorhombic, space group $P2_12_12_1$ with a = 24.779(7), b = 21.008(7), c = 16.049(5) Å, U = 8354(5) Å^3, Z = 4; Mo-K_α radiation (λ = 0.7169 Å), μ(Mo-K_α) = 2.83 cm^{-1}, R = 0.085 for 2266 reflections [$I/\sigma(I) > 3.0$] collected by a Syntex $P2_1$ four-circle diffractometer, and corrected for Lorentz and polarisation effects.

Crystal data for (**1b**) (*S*-form, from acetone–water): orthorhombic, space group $P2_12_12_1$ with a = 24.43(3), b = 21.13(3), c = 16.08(2) Å, U = 8299(18) Å^3, Z = 4; Mo-K_α radiation (λ = 0.71069 Å), μ(Mo-K_α) 2.83 cm^{-1}. R = 0.151 for 2299 reflections [$I/\sigma(I) > 3.0$] collected as for *R*-isomer. In contrast to the *R*-form which was stable and diffracted more strongly, the *S*-isomer diffracted weakly and decayed during data collection. For both isomers hydrogen atoms were placed at calculated positions without refinement. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

Note added in proof: Further refinement of the crystallographic data for adenosylcobalamin has given values of 124.0 ± 0.5° for the Co–C_α–C_β angle and 1.985(9) Å for the Co–C bond length (H. J. Savage, unpublished work).

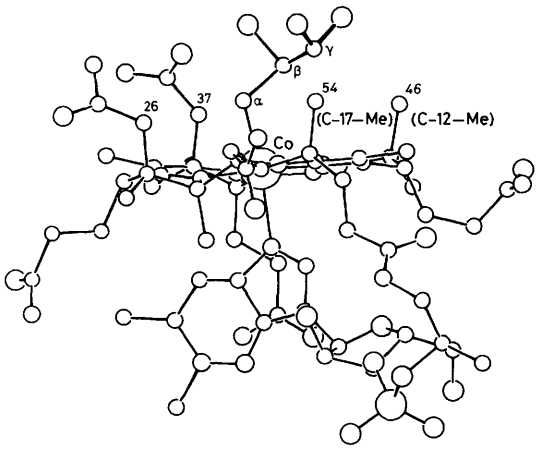


Figure 4. View of molecule (**1b**) showing the atomic numbering of sentinel groups and the disposition of the 2,3-dihydroxypropyl group.

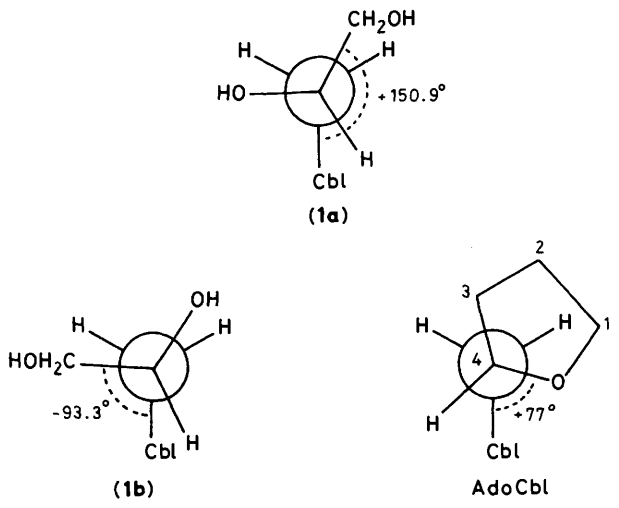


Figure 5. Newman projections showing (**1a**) (along the C_β–C_α bond), (**1b**) (along the C_β–C_α bond), and AdoCbl (along C-4–C-5 bond) *n.b.* the positions of hydrogen atoms are inferred.

However, the Co–C_α–C_β bond angles in (**1a**) and (**1b**) are 119.6 ± 1.7 and 113.6 ± 2.1°, respectively. Their Co–C bond lengths are for (**1a**) 2.002(23) and for (**1b**) 2.079(30) Å and do not differ significantly from that in AdoCbl [2.03(6) Å]. Both compounds adopt similar conformations for their Co–C_α–C_β–C_γ fragment, as shown by Newman projections in Figure 5, except that the OH at C_β and CH_2OH group are of course interchanged from one isomer to another. In each case the nearest atom to the corrin is the hydrogen at C_β, for obvious steric reasons. With AdoCbl, it is also the hydrogen at C_β (C-4 of ribose) that is the nearest substituent to the corrin, but the ribose ring oxygen rather than C-3 [corresponding to CH_2OH in (**1b**): *n.b.* AdoCbl and (**1b**) have corresponding chirality at C_β] is closer to the corrin. The ribose ring is approximately perpendicular to the corrin and nestles in a channel between rings C and D. Cobalamin (**1b**) uses this channel for its CH_2OH group, and the C_β–OH is positioned so that it can hydrogen bond to the carbonyl oxygen of the c-acetamido group. AdoCbl cannot form such a hydrogen bond and this may be an important reason for the difference in crystal structure between it and (**1b**). The preference of AdoCbl and (**1b**) for the channel between rings C and D may be because it is bounded by the smaller 'sentinel' groups (two methyls rather than two acetamido groups or one acetamido and one methyl). The arrangement adopted by AdoCbl allows the adenine to lie partly over ring C (N-7 of the adenine is almost directly above C-46, the β-methyl group at C-12), the plane of the adenine system being approximately parallel to the plane of the corrin. This may be a favourable arrangement because of hydrophobic bonding between portions of adenine and ring C in close contact (but not too close! see below). Furthermore, the pyrimidine ring of the adenine projects away from the periphery of the corrin. Another important difference beween AdoCbl and (**1b**) is that in the former cobalamin C_α–C_β lies nearly over the Co–N-24 bond (torsional angle φ = 16°), whereas for the latter compound C_α–C_β is over the Co–N-23 bond (φ = 10.7°). Cobalamin (**1a**) has its C_α–C_β above Co–N-24 like AdoCbl. The substituents at C_β are then accommodated by placing the H in the channel between rings C and D and the CH_2OH group above this channel, whilst the hydroxy group is very close to H-19. A similar hydrogen bond to that observed for (**1b**) is not formed, because this would require the CH_2OH group to point down at ring C.

Careful examination of the crystal structures shows why there is an increase in the Co–C_α–C_β angles between (**1b**) on the one hand, and (**1a**) and AdoCbl on the other. The arrangement described for (**1b**) engenders no severe steric interactions and profits from an intramolecular hydrogen bond. Thus it has a 'normal' angle. For (**1a**) there is close contact between C_β-OH and H-19, causing an increase of the Co–C_α–C_β angle to 119.6°. With AdoCbl, steric interactions between the ribose and corrin are minimised by placing the ribose in the C–D channel. This causes the adenine to lie over ring C and too close contact (N-9 · · · · · · C-46 = 3.71 Å) is avoided by increasing the Co–C_α–C_β angle to 125°.

The proximity of H-19 and C_β-OH in cobalamin (**1a**) is reflected in its 400 MHz ^{1}H n.m.r. spectrum (10 mM in D_2O), which shows a downfield shift of H-19 (δ 4.60) compared to its position (δ 4.17) in the spectrum of cobalamin (**1b**). Previously,[7] we assigned these resonances to H-3, but they have been corrected on the basis of nuclear Overhauser effect difference experiments.[8] These observations suggest that the predominant conformation of cobalamin (**1a**) [and (**1b**)] in water is similar to that in the crystal, although the hydrogen bond of (**1b**) will probably not persist in solution.

Received, 3rd January 1985; Com. 019

References

1 Recent reviews: B. T. Golding, in 'B_{12},' vol. 1, ed. D. Dolphin, Wiley-Interscience, New York, 1982, ch. 15; J. M. Pratt, *ibid.*, ch. 10; J. Halpern, *ibid.*, ch. 14; B. Zagalak, *Naturwissenschaften*, 1982, **69**, 63; B. T. Golding, in 'Comprehensive Organic Chemistry,' vol. 5, eds. D. Barton and W. D. Ollis, Pergamon, Oxford, 1979, ch. 24.4; J. Halpern, *Science*, 1985, **227**, 869.
2 J. P. Glusker, in 'B_{12},' vol. 1, ed. D. Dolphin, Wiley-Interscience, New York, 1982, ch. 3.
3 P. G. Lenhert, *Proc. R. Soc. London, Ser. A*, 1968, **303**, 45.
4 J. M. Pratt, in 'B_{12},' vol. 1, ed. D. Dolphin, Wiley-Interscience, New York, 1982, ch. 10.
5 J. J. Rooney, *J. Chem. Res.*, 1984, 48.
6 N. Bresciani-Pahor, L. G. Marzilli, L. Randaccio, P. J. Toscano, and E. Zangrando, *J. Chem. Soc., Chem. Commun.*, 1984, 1508; see also M. F. Summers, L. G. Marzilli, N. Bresciani-Pahor, and L. Randaccio, *J. Am. Chem. Soc.*, 1984, **106**, 4478.
7 R. M. Dixon, B. T. Golding, O. W. Howarth, and J. L. Murphy, *J. Chem. Soc., Chem. Commun.*, 1983, 243.
8 R. M. Dixon and B. T. Golding, unpublished observations.