Synthesis and Characterisation by ^{1}H N.M.R. Spectroscopy of Diastereoisomeric Hydroxy- and Dihydroxy-alkylcobalamins

Ruth M. Dixon, Bernard T. Golding,* Oliver W. Howarth, and James L. Murphy

Department of Chemistry and Molecular Sciences, University of Warwick, Coventry, CV4 7AL, England

Stereoisomerically pure samples of (2*R*)- and (2*S*)-2-hydroxypropyl-, (2*R*)- and (2*S*)-2,3-dihydroxypropyl-, (3*S*)-3,4-dihydroxybutyl-, and (4*S*)-4,5-dihydroxypentyl-cobalamin have been synthesised by allowing cob(I)alamin to react with the appropriate optically pure alkylating agent; these alkylcobalamins and the mixtures of diastereoisomers obtained by allowing cob(I)alamin to react with the corresponding racemic alkylating agents have been characterised by high field ^{1}H n.m.r. spectroscopy.

We describe the first characterisation of both diastereoisomeric alkylcobalamins [*e.g.* (**2a**) and (**2b**)] from the reaction of a racemic alkylating agent with cob(I)alamin (**1**). Many authors have overlooked the consequences of such diastereoisomerism,

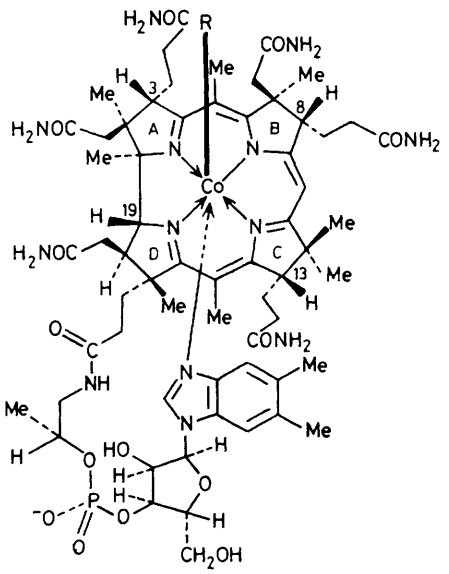
although diastereoisomeric alkylcobalamins have been proposed as intermediates in cob(I)alamin-catalysed reductions of unsaturated substrates leading to an excess of one enantiomer of product.[1] Alkylcobalamins that were probably mixtures of diastereoisomers, have been described as though they were single compounds. The rates of formation of diastereoisomers from cob(I)alamin and a racemic alkylating agent should differ, depending on the enantioselectivity of cob(I)alamin for the *R* and *S* alkylating agent. Furthermore, the rates of reactions of the resulting diastereoisomeric alkylcobalamins should be non-identical. These considerations are important in the context of the use of certain alkylcobalamins as model compounds for studying the mechanisms of adenosylcobalamin-dependent enzymic reactions.[2]

Alkylation of cob(I)alamin with 10 mol. equiv. of *rac*-3-chloropropane-1,2-diol gave 2,3-dihydroxypropylcobalamin (**2**) that was purified by phenol extraction and short column chromatography on silica gel.[3] The 400 MHz ^{1}H n.m.r. spectrum† of this material showed it to contain a *ca.* 2:3 ratio of (2*R*)-(**2a**) and (2*S*)-2,3-dihydroxypropylcobalamin (**2b**), respectively. The enantioselectivity of cob(I)alamin for enantiomers of 3-chloropropane-1,2-diol is therefore low. After recrystallising the mixture of (*R*)- and (*S*)-2,3-dihydroxypropylcobalamin from aq. acetone their ratio changed to 5:4 (*cf.* Figure 1a).

For characterising alkylcobalamins with chiral alkyl groups we anticipated that their diastereoisomers would show the greatest differences in their ^{1}H n.m.r. spectra for protons on the same side (*i.e.* β-face) of the corrin ring as the alkyl group. It is therefore important to identify the resonances from H-3, H-8, and H-13, because the chemical shifts of these protons may be very sensitive to subtle changes in the alkyl group. The 400 MHz ^{1}H n.m.r. spectrum† of MeCbl in D_2O shows 12 resonances in the region δ 3.0—4.8. For 9 of these we agree with the assignments of Hensens *et al.*,[4] which we supported by double irradiation experiments. We have assigned the remaining resonances with the aid of double irradiation and n.O.e. experiments to H-3 (δ 4.17, dd, *J* 2 and 9 Hz), H-8 (δ 3.40, dd, *J* 5 and 11 Hz), and H-13 (δ 3.01, dd, *J* 1 and 11 Hz). Recently, Ernst[5] and Battersby *et al.*[6] have located H-3, H-8, and H-13 in the ^{1}H n.m.r. spectrum of cobester, using different methods from those described here. They also found that δ for H-3 > δ (H-8) > δ (H-13). The values for the chemical shifts for H-3, H-8, and H-13 probably depend on the precise orientation of these protons with respect to the corrin chromophore (in the crystal structure of AdoCbl revealed by *X*-ray techniques these protons are quasi-equatorial on each 5-ring envelope[7]), which may be influenced by the sixth ligand attached to cobalt [R in (**2**)—(**5**)].

Alkylation of cob(I)alamin with (*R*)-glycerol 1-*O*-*p*-toluenesulphonate (prepared from 2,3-di-*O*-isopropylidene-*sn*-glycerol by tosylation and hydrolysis of the acetal[8]) gave (2*R*)-2,3-dihydroxypropylcobalamin (**2a**). The 400 MHz ^{1}H n.m.r. spectrum† (δ 2.7—4.7 region) of this substance is shown in Figure 1b. Assignments were made principally by n.O.e. difference experiments and comparison with the spectrum of MeCbl. Using these results the spectra (*cf.* Figure 1a) of the mixtures of (*R*)- and (*S*)-dihydroxypropylcobalamin (see above) can now be rationalised. The assignments to the (*S*)-isomer are supported by the synthesis of (2*S*)-2,3-dihydroxypropylcobalamin (**2b**) from (*S*)-glycerol 1-*O*-*p*-toluenesulphonate (prepared from L-ascorbic acid[9]) and examination of its ^{1}H n.m.r. spectrum. Note that H-13 and H-19 and all protons under the α-face of the corrin have almost identical chemical shifts in the two diastereoisomers. However, for (2*R*)-2,3-dihydroxypropylcobalamin H-3 is at δ 4.60, whereas it absorbs at δ 4.17 in the (*S*)-isomer. The proton H-8 absorbs at δ 3.40 in the (*R*)-isomer and at δ 3.29 in the (*S*)-isomer. These data suggest that the dihydroxypropyl group of (**2a**) and (**2b**) lies over the corrin ring in the vicinity of rings A and B. Changing chirality in the dihydroxypropyl group affects H-8 (ring B) and especially H-3 (ring A). Changes are also seen for the chemical shifts of some of the methyl groups of (**2a**) and (**2b**), but are always < 0.1 p.p.m.

The reaction of cob(I)alamin with 10 mol. equiv. of *rac*-epoxypropane gave a 3:1 mixture of (2*R*)- and (2*S*)-2-hydroxypropylcobalamin [the pure diastereoisomers (**3a**) and (**3b**) were prepared from (*R*)- and (*S*)-epoxypropane, respectively[10]]. Alkylation of cob(I)alamin with *rac*-2,2-dimethyl-4-(2′-hydroxyethyl)-1,3-dioxolan *p*-toluene-sulphonate[8] and hydrolysis of the acetal (**4c**) gave 3,4-dihydroxybutylcobalamin (**4**). This was a 1:1 mixture of (3*R*)- and



RCbl

R		
electron pair		(**1**)
1 2 3		
$CH_2CHOHCH_2OH$	mixture of diastereoisomers	(**2**)
$CH_2CHOHCH_2OH$	(2*R*)-isomer	(**2a**)
$CH_2CHOHCH_2OH$	(2*S*)-isomer	(**2b**)
1 2 3		
$CH_2CHOHMe$	mixture of diastereoisomers	(**3**)
$CH_2CHOHMe$	(2*R*)-isomer	(**3a**)
$CH_2CHOHMe$	(2*S*)-isomer	(**3b**)
1 2 3 4		
$CH_2CH_2CHOHCH_2OH$	mixture of diastereoisomers	(**4**)
$CH_2CH_2CHOHCH_2OH$	(3*S*)-isomer	(**4b**)
$CH_2CH_2CH(OCMe_2O)CH_2$	mixture of diastereoisomers or (*S*)-isomer	(**4c**)
1 2 3 4 5		
$CH_2CH_2CH_2CHOHCH_2OH$	mixture of diastereoisomers	(**5**)
$CH_2CH_2CH_2CHOHCH_2OH$	(4*S*)-isomer	(**5b**)
$CH_2CH_2CH_2CH(OCMe_2O)CH_2$	mixture of diastereoisomers or (*S*)-isomer	(**5c**)

† The spectra were recorded for solutions (*ca.* 0.02 mol dm^{-3}) in D_2O ($Me_3Si[CH_2]_3SO_3Na$ internal standard) or in CD_3OD (Me_4Si) using a Bruker WH-400 spectrometer. For compounds (**2b**) and (**5b**) differences between chemical shifts and coupling constants for their solutions in these solvents were ≤0.1 p.p.m. (δ values) and negligible (*J* values). N.O.e. experiments were successful with MeCbl in D_2O at 25 °C and cobalamin (**3b**) in CD_3OD at −10 °C.

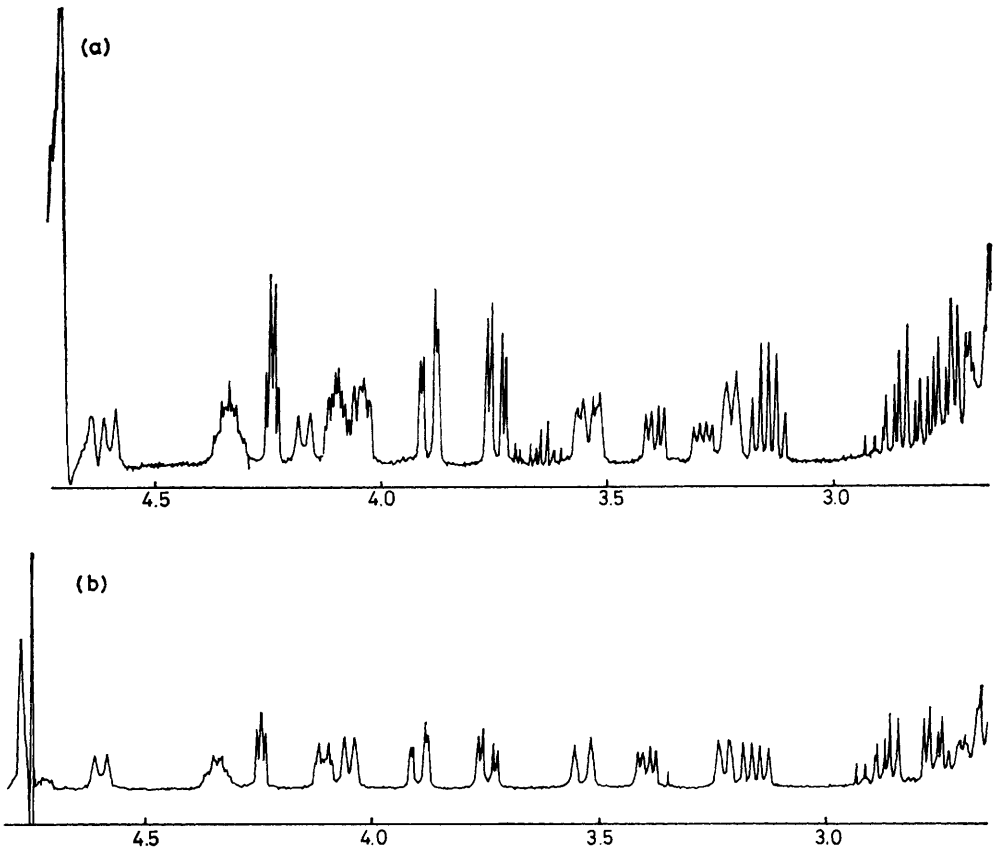


Figure 1. 400 MHz ^{1}H n.m.r. spectra (δ 2.7—4.7) of (a) *rac*-2,3-dihydroxypropylcobalamin and (b) (2*R*)-2,3-dihydroxypropylcobalamin. The peaks between *ca.* δ 3.6 and 3.7 in spectrum (a) are due to 3-chloropropane-1,2-diol. The triplet at δ 2.91 in each spectrum is due to $Me_3Si[CH_2]_3SO_3Na$.

(3*S*)-diastereoisomers according to its ^{1}H n.m.r. spectrum† and comparison with (3*S*)-3,4-dihydroxybutylcobalamin (**4b**) [prepared from (*S*)-2,2-dimethyl-4-(2′-hydroxyethyl)-1,3-dioxolan[8,11]]. With 4,5-dihydroxypentylcobalamin (**5**), it was not possible to distinguish the (4*R*)- and (4*S*)-diastereoisomers by examining the resonances from H-3, H-8, H-13, and H-19 of the corrin, because C-4 is too far removed from the corrin. However, these diastereoisomers can still be readily distinguished [(**5**) is a 1 : 1 mixture of diastereoisomers] because the diastereotopic protons at C-2 of the dihydroxypentyl group have quite different chemical shifts in each diastereoisomer [(*R*)-isomer: δ −0.24 and +0.29; (*S*)-isomer: δ −0.37 and +0.38 p.p.m.]. Cobalamin (**5**) was prepared from *rac*-2,2-dimethyl-4-(3′-hydroxypropyl)-1,3-dioxolan toluene-*p*-sulphonate[8] *via* acetal (**5c**). The (*S*)-diastereoisomer (**5b**) was obtained from (*S*)-glutamic acid *via* (*S*)-pentane-1,2,5-triol.[8,12]

We thank the S.E.R.C. for financial support and access to the regional n.m.r. service. We are grateful to Dr. H. A. O. Hill for a copy of ref. 4 prior to publication.

Received, 8th November 1982; Com. 1263

References

1 A. Fischli and J. J. Daly, *Helv. Chim. Acta*, 1980, **63**, 1628.
2 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.
3 D. Dolphin, *Methods Enzymol*, 1971, Vol. XVIII, Part C, p. 34.
4 O. D. Hensons, H. A. O. Hill, C. E. McClelland, and R. J. P. Williams, in 'B_{12},' vol. 1, ed. D. Dolphin, Wiley–Interscience, New York, 1982, ch. 13.
5 L. Ernst, *Liebigs Ann. Chem.*, 1981, 376.
6 A. R. Battersby, C. Edington, C. J. R. Fookes, and J. M. Hook, *J. Chem. Soc., Perkin Trans.* 1, 1982, 2265.
7 J. P. Glusker, in 'B_{12},' vol. 1, ed. D. Dolphin, Wiley–Interscience, New York, 1982, ch. 3.
8 B. T. Golding, T. J. Kemp, C. S. Sell, P. J. Sellars, and W. P. Watson, *J. Chem. Soc., Perkin Trans.* 2, 1978, 839.
9 M. E. Jung and T. J. Shaw, *J. Am. Chem. Soc.*, 1981, **102**, 6304.
10 B. T. Golding, D. R. Hall, and S. Sakrikar, *J. Chem. Soc., Perkin Trans.* 1, 1973, 1214; see also B. T. Golding, P. J. Sellars, and A. K. Wong, *J. Chem. Soc., Chem. Commun.*, 1977, 570.
11 D. A. Howes, M. H. Brookes, D. Coates, B. T. Golding, and A. T. Hudson, *J. Chem. Res.*, 1983 (*S*)9; (*M*)0217.
12 U. Ravid, R. M. Silverstein, and L. R. Smith, *Tetrahedron*, 1978, **34**, 1449.