Journal of Organometallic Chemistry, 437 (1992) 227–237
Elsevier Sequoia S.A., Lausanne
JOM 22879

Alkylcobalamins: formation by enantioselective alkylation of cob(I)alamin, ^{1}H NMR spectra, and conformational analysis of the alkyl group *

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(Received May 5, 1992)

Abstract

The ^{1}H NMR spectra of a series of alkylcobalamins, principally 2-oxy substituted, including adenosyl- and ribosylcobalamin, have been analysed with particular attention to the conformation of the alkyl moiety. The enantioselectivity of formation of some of these compounds from their chiral precursors has been determined (NMR analysis) and rationalized.

Introduction

Diol dehydrase catalyses the conversion of simple 1,2-diols into aldehydes [1] (see Scheme 1), a reaction that belongs to the group of enzyme-mediated molecular rearrangements requiring adenosylcobalamin (**1a**, AdoCbl, "B_{12} coenzyme") [2]. Mechanisms for these reactions *via* protein-bound free radicals have been proposed [3]. In support of some features of the proposed reaction pathway for diol dehydrase, it was shown [4] that photolysis and thermolysis, at both high and low pH, of 4,5-dihydroxy-2,2-dimethylpentyl(pyridine)cobaloxime (**2**) yields 4,4-dimethylpentanal (**3**). Homolysis of the cobalt–carbon bond of **2** gives the 4,5-dihydroxy-2,2-dimethylpentyl radical **4**, which undergoes a 1,5-H shift to afford the 1,2-dihydroxy-4,4-dimethylpentyl radical **5**. This is converted into the 1-formyl-3,3-dimethylbutyl radical **6** (by elimination of water at pH 3 or of hydroxide at pH 9) or the 1-(dihydroxymethyl)-3,3-dimethylbutyl radical **7** (by 1,2-dihydroxy shift) (refer to Scheme 2).

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e.g.

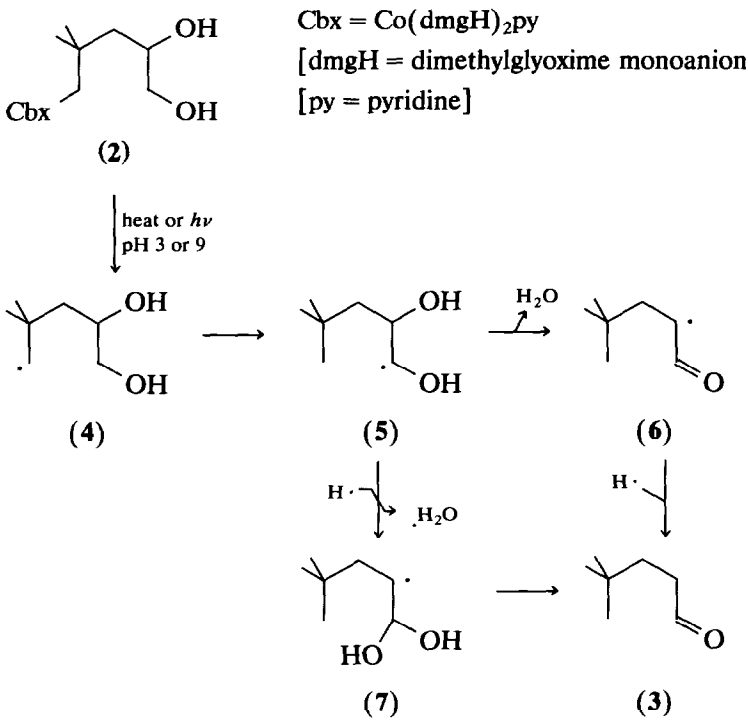
$$MeCHOHCH_2OH \xrightarrow[\text{dehydrase}]{\text{diol}} MeCH_2CH(OH)_2 \longrightarrow MeCH_2CHO + H_2O$$

Scheme 1. Diol dehydrase-mediated conversion of 1,2-diols into aldehydes.

One of the disadvantages of using cobaloximes as models for cobalamins is that even under mild acidic conditions cob(II)aloxime is kinetically labile, rapidly dissociating to free dimethylglyoxime and aquated Co^{II} [5]. This complicates the interpretation of results for model systems like the one described. Therefore, we began the study of dihydroxyalkylcobalamins because the corrinoid of cob(II)-alamin is kinetically inert.

Ultraviolet-visible spectroscopy has often been used for the characterization of alkylcobalamins [6] and has the advantage of relatively high sensitivity and applicability to dilute solutions. The technique distinguishes between alkylcobalamins and cobalamins with a non-alkyl sixth ligand. However, the spectra of alkylcobalamins are all very similar and little information can be gained that is relevant to the structure and conformation of the σ-alkyl group. Likewise, infrared spectroscopy does not readily distinguish different alkylcobalamins because of the overwhelming contributions of vibrations from the corrin and nucleotide [7].

. High field ^1H NMR spectroscopy enables the structure and purity of alkylcobalamins to be determined and their conformation in solution to be assessed.



Scheme 2. Degradation of cobaloxime 2 to give 4,4-dimethylpentanal through radical intermediates.

Furthermore, diastereoisomeric alkylcobalamins can be distinguished (for a preliminary communication on these results see [8]). This paper describes in full our studies of the synthesis and characterization by ^{1}H NMR spectroscopy of a series of hydroxy- and dihydroxyalkyl-cobalamins, and some acetal derivatives of the diols, including an improved model compound for AdoCbl, methyl 5-deoxy-β-D-ribofuranos-5-ylcobalamin (RibCbl, **1b**). For other studies of the ^{1}H NMR spectra of alkylcobalamins, see [9–12].

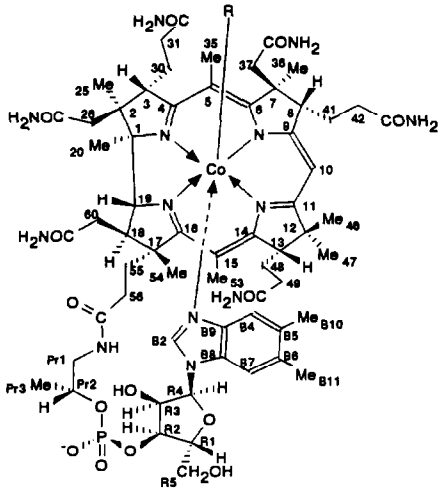
It was expected that cob(I)alamin, a chiral species, would show enantioselectivity in its reactions with racemic alkylating agents, giving an excess of one diastereoisomeric product. Early investigators [13] have overlooked this possibility, although Fischli [14] has shown how the chirality of cobalamin can be exploited to achieve preferential reduction of one prochiral face of a double bond. Bonhôte and Scheffold [15] recently obtained an excess of (*R*)-cyclopent-2-enol from the reaction of cob(I)alamin with epoxycyclopentane *via* a 2-hydroxycyclopentylcobalamin. Finke and his co-workers have obtained both diastereoisomeric α-alkylcobalamins from reaction of *rac*-chloroethylenecarbonate with cob(I)alamin [16]. The β-face of cobalamin possesses "sentinel groups" [17] (methyls at C-12 and C-17, acetamido groups at C-2, C-7 and C-18), which define a chiral cavity into which at least part of the substrate for alkylation of cob(I)alamin must enter. The relative rates of reactions with enantiomeric substrates will be governed by the substrate–cobalamin interactions for the two diastereoisomeric transition states. We describe results for several alkylating agents, including a series of epoxides, and an interpretation of the diastereoisomeric ratios observed. Some of the results have been given in a preliminary communication [8].

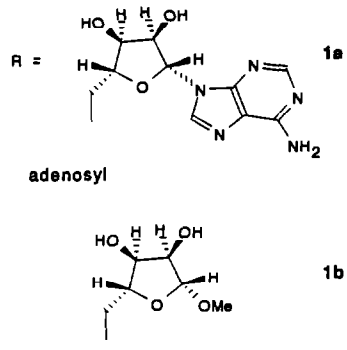
^{1}H NMR spectra of alkylcobalamins and their assignment

Examination of the ^{1}H chemical shifts of the alkylcobalamins **1** and **8–19** (see Fig. 1) showed that most of the resonances from the corrin and nucleotide change only slightly on alteration of the alkyl ligand. However, the methine protons, H-3, H-8, H-13 and H-19 show relatively greater shifts from one cobalamin to another (see Table 1).

The signals for methylcobalamin were assigned as follows: the ribose and propanolamine protons were assigned by a decoupling sequence similar to that used by Hensens *et al.* [9] for adenosylcobalamin, starting with the doublet at δ 6.28 (R1–H) and the doublet at δ 1.22 (Pr1–H). This left four unassigned signals in the region between δ 4.6 and 2.8, corresponding to the four methine protons H3, H-8, H-13 and H-19. These were assigned by nOe difference spectroscopy, irradiating methyl groups previously assigned by Hensens *et al.* [9] and observing the effects on the methine protons. Incidentally, the sequence of nOe experiments allowed us to confirm most of the assignments in [9]. Similar nOe experiments were also carried out on (*S*)-2,3-dihydroxypropyl- (**12b**) and (*S*)-3,4-dihydroxybutyl-cobalamin (**13b**). The resonances for the other cobalamins were assigned by direct comparison with these spectra.

The resonances for alkyl ligands are shown in Table 2. These were assigned by decoupling and difference spectroscopy. In general, protons on the first and second carbon atoms from the cobalt atom (α and β positions, respectively) are shifted to high field, the shielding effect of the corrin system being an important





methyl	**8**
ethyl	**9**
propyl	**10**
(*R*)-2-hydroxypropyl	**11a**
(*S*)-2-hydroxypropyl	**11b**
(*R*)-2,3-dihydroxypropyl	**12a**
(*S*)-2,3-dihydroxypropyl	**12b**
(*R*)-3,4-dihydroxybutyl	**13a**
(*S*)-3,4-dihydroxybutyl	**13b**
(*R*)-4,5-dihydroxypentyl	**14a**
(*S*)-4,5-dihydroxypentyl	**14b**
(*R*)-5,6-dihydroxyhexyl	**15a**
(*S*)-5,6-dihydroxyhexyl	**15b**
(*R*)-2-hydroxy-3,3-dimethylbutyl	**16a**
(*S*)-2-hydroxy-3,3-dimethylbutyl	**16b**
(*R*)-2-cyclohexyl-2-hydroxyethyl	**17a**
(*S*)-2-cyclohexyl-2-hydroxyethyl	**17b**
neopentyl	**18**
octyl	**19**

Fig. 1. Structures of alkylcobalamins.

influence. The protons β to the cobalt atom are shifted to higher field than those in the α position, because they lie over the unsaturated portion of the corrin.

Conformational analysis of alkylcobalamins

The solution structure of alkylcobalamins is of interest, because the first step in adenosylcobalamin-dependent enzymic reactions, after binding of the substrate to the enzyme, is generally agreed [18] to be the homolysis of the cobalt–carbon bond of the coenzyme, activated by a conformational change in the enzyme. It was therefore important to determine whether the coenzyme is unusual in having a

Table 1

Selected methine resonances for alkylcobalamins (RCbl): δ (J, Hz)

RCbl	C3-H	C8-H	C13-H	C19-H
1a	4.10	3.31 (11.2, 4.9)	2.90 (9, 1)	4.23
1b	4.08	3.39	3.13	4.08
8	4.17 (9.1, 2.3)	3.40 (10.9, 5.0)	3.02 (11.2, 1)	3.94 (9.7)
12a	4.05 (9.1)	3.40 (11.1, 4.8)	3.22 (9.4)	4.60 (10.6)
12b	4.04 (9.1)	3.29 (11.1, 5.0)	3.22 (10)	4.17 (10.4)
13a [a]	4.23 (8.7)	3.62 (10, 5.5)	3.24 (8.9, 3.3)	4.17 (11)
13b [a]	4.22 (8.7)	3.61 (9.9, 5.4)	3.25 (8.9, 3.3)	4.17 (11.3)
17a	4.03 (9)	3.44 (11, 5)	3.19 (10)	4.69 (11)
17b	4.03 (9)	3.25	3.2	4.17 (11)
9–11, 14 [a], **15,16,18,19**	4.03–4.09 (9)	3.20–3.43 (11)	3.19–3.22 (9–11)	4.14–4.58 (10–11)

[a] These spectra were obtained in CD_3OD.

particularly strained Co–C bond. In the crystal, adenosylcobalamin has a Co–C_α–C_β bond angle of 121–125° [19], whereas (*R*)- (**12a**) and (*S*)-2,3-dihydroxypropylcobalamin (**12b**) have angles of 120° and 114°, respectively [20]. The Co–C bond lengths are not significantly different. The NMR coupling constants for the 2,3-dihydroxypropyl ligands suggest that the torsion angles are similar in the crystal and in solution for both **12a** and **12b**, although a rotation of *ca.* 10° is required in the *S*-isomer to fit the observed coupling constants (see Fig. 2). This can be explained if the hydrogen bond between the β-hydroxyl group and one of the acetamido side chains, found in the crystal [20], is absent in solution. The larger angle for Co–C_α–C_β in the coenzyme can be explained by the greater steric bulk of the adenosyl ligand, interacting with groups projecting from the β-face of the corrin. These "sentinel groups" determine where the β-ligand must lie.

To explore the effect of the adenine ring of AdoCbl on the Co–C_α–C_β angle (in the AdoCbl crystal, the adenine lies over the β-methyl at C-12 [17,19]) methyl 5-deoxy-β-D-ribofuranos-5-ylcobalamin (RibCbl, **1b**) was synthesized (from methyl 5-deoxy-5-iodo-β-D-ribofuranoside, along with the corresponding cobaloxime, methyl 5-deoxy-β-D-ribofuranosyl-5-ylcobaloxime, RibCbx, **1c**). In these compounds, the relatively bulky adenine is replaced by a methoxy group. Although we have been unable to obtain crystals of RibCbl suitable for X-ray diffraction, the crystal structure of RibCbx showed Co–C–C to be 123° and Co–C to be 201.5 pm [21]. This suggests that steric factors involving the ribosyl group are primarily responsible for the unusual Co–C–C angle in AdoCbl. The ^{1}H NMR signals for the ribosyl protons are shown in Table 2 for AdoCbl, RibCbl and RibCbx.

Using CHEM-X, a probability contour map was generated by plotting the most probable torsion angle around the C_α–C_β bond against that of the Co–C_α bond, and led to the Newman projection shown (Fig. 3). This is in accord with the observed coupling constants and a similar ribose conformation was observed for both AdoCbl and RibCbx in the crystal [17,19,21]. The least hindered channel of the corrin appears to be towards the C–D ring junction, bounded by two sentinel methyl groups, and the crystal structures show that this channel is occupied by the β-ligand in the cobalamins, AdoCbl, (*R*)- and (*S*)-2,3-dihydroxypropylcobalamin [19,20]. It is likely that this preference persists in solution, since rotation of a bulky

Table 2

Selected alkyl resonances for alkylcobalamins, RCbl

RCbl	R	Solvent	δ (ppm)		J (Hz)	
1a	Adenosyl	D_2O	C1	5.56	C1–C2	2.9
			C2	4.54	C2–C3	5.1
			C3	3.74	C3–C4	6.4
			C4	2.54	C4–$C5_\alpha$	0
			$C_{5\alpha}$	1.55	C4–$C5_\beta$	8.8
			$C5_\beta$	0.37	$C5_\alpha$–$C5_\beta$	9.6
			A2	8.19		
			A8	8.00		
1b	Ribosyl	D_2O	C1	4.41	C1–C2	2.83
			C2	4.20		
			C3	3.68		
			C4	2.53		
			$C5_\alpha$	1.45		
			$C5_\beta$	0.77		
			OMe	3.19		
1c	Ribosyl	$CDCl_3$	C1	4.63	C1–C2	1.05
	RibCbx (cobaloxime)		C2	3.99	C2–C3	5.6
			C3	3.84	C3–C4	4.3
			C4	3.38	C4–$C5_\alpha$	3.1
			$C5_\alpha$	2.21	C4–$C5_\beta$	10.6
			$C5_\beta$	1.11	$C5_\alpha$–$C5_\beta$	8.9
			OMe	3.29		
8	Methyl	D_2O		−0.12		
12a	(*R*)-2,3-Dihydroxypropyl	D_2O	$C1_\alpha$	0.48	$C1_\alpha$–$C1_\beta$	9.1
			$C1_\beta$	1.13	$C1_\alpha$–C2	2
			C2	1.66	$C1_\beta$–C2	7.3
			$C3_\alpha$	2.72	C2–$C3_\alpha$	7.3
			$C3_\beta$	2.81	C2–$C3_\beta$	4.2
					$C3_\alpha$–$C3_\beta$	11.2
12b	(*S*)-2,3-Dihydroxypropyl	D_2O	$C1_\alpha$	0.48	$C1_\alpha$–$C1_\beta$	8.8
			$C1_\beta$	1.54	$C1_\alpha$–C2	3.7
			C2	1.62	$C1_\beta$–C2	5.6
			$C3_\alpha$	2.72	C2–$C3_\alpha$	4.0
			$C3_\beta$	2.81	C2–$C3_\beta$	6.9
					$C3_\alpha$–$C3_\beta$	11.2
13a	(*R*)-3,4-Dihydroxybutyl	CD_3OD	$C1_\alpha$	0.47	$C1_\alpha$–$C1_\beta$	12
			$C1_\beta$	1.58		
			$C2_\alpha$	−0.15		
			$C2_\beta$	0.34		
			C3	3.05		
			$C4_\alpha$	3.2		
			$C4_\beta$	3.2		
13b	(*S*)-3,4-Dihydroxybutyl	CD_3OD	$C1_\alpha$	0.86	$C1_\alpha$–$C1_\beta$	6.9
			$C1_\beta$	1.23	$C1_\alpha$–$C2_\alpha$	5.0
			$C2_\alpha$	−0.15	$C1_\alpha$–$C2_\beta$	12.5
			$C2_\beta$	0.34	$C1_\beta$–$C2_\alpha$	13
			C3	3.08	$C1_\beta$–$C2_\beta$	5.0
			C4	3.15	$C2_\alpha$–$C2_\beta$	13
					$C2_\alpha$–C3	4.7
					$C2_\beta$–C3	7.4
					C3–$C4_\alpha$	5
					C3–$C4_\beta$	5
					$C4_\alpha$–$C4_\beta$	12.5

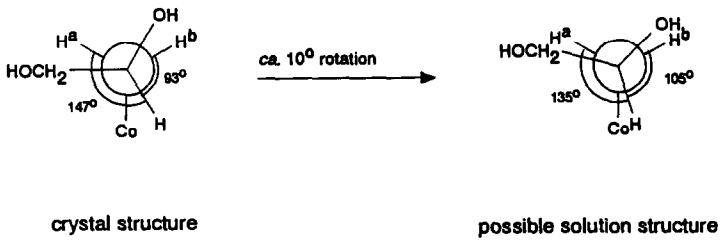


Fig. 2. Newman projection of (*S*)-2,3-dihydroxypropylcobalamin, **12b**.

ligand would be very unfavourable. Indeed, measurement of relaxation times by Hogenkamp *et al.* [22] showed that the carbon bonded to cobalt in adenosylcobalamin has a much shorter T_1 than that of methylcobalamin, while ethylcobalamin has an intermediate value. This suggests that rotation becomes progressively more difficult as the alkyl ligand becomes larger. The nOe spectra for AdoCbl showed only four adenosyl/corrin nOes [12], all four being related to interactions of the ribosyl protons, A11–H with C17–Me, A11–H and A14–H with C12–Me, and A15–Ha with C19–H. From these observations, it can be deduced that the ribose ring of AdoCbl is sited in the C–D channel, as it is in the crystal.

The upfield shifts on C12–Me and H-13 in the coenzyme compared to other cobalamins is due to paramagnetic shielding by the adenine system which lies over ring C, again indicating the similarity of the solution and crystal structures. By contrast, H-13 of RibCbl appears at a similar chemical shift to that of other alkylcobalamins (*cf.* Table 1). However, C12b–Me of RibCbl experiences deshielding, analogous to deshielding of C12b–Me observed in alkylcobalamins with 2- and 3-hydroxy substitution. The crystal structure of (*S*)-2,3-dihydroxypropylcobalamin showed the C3-hydroxyl elevated over ring C [20], suggesting that the oxygen is close enough to cause this effect. The C3–OH of RibCbl is structurally related to C3–OH in (*S*)-2,3-dihydroxypropylcobalamin.

There is a downfield shift of the resonance of H-19 in the (*R*)-2-hydroxyalkylcobalamins compared to the (*S*)-isomers (> 0.35 ppm). The crystal structure [20] of (*R*)-2,3-dihydroxypropylcobalamin shows a close contact between the 2-hydroxyl group and H-19, which causes Van der Waal's deshielding of H-19 (*cf.* [23]). This effect is even more pronounced with RibCbl, H-19 being observed more downfield than H-19 in AdoCbl. It is possible that the reduced bulk of the methylribosyl ligand allows it to fit more snugly into the C–D channel, thus allowing greater contact with H-19.

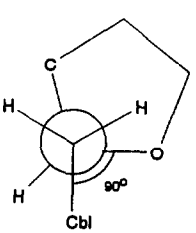


Fig. 3. CHEM-X generated Newman projection for AdoCbl, **1a**.

Table 3

Enantioselectivity of cob(I)alamin towards racemic alkylating agents

Alkylating agent	Yield (%)	$(R:S)$ [a]	R_{CN} [b]
Methyloxirane	52	3:1	1.2
(Hydroxymethyl)oxirane	–	1.8:1	0.8
Cyclohexyloxirane	–	1.3:1 [c]	1.4
tert-Butyloxirane	88	4.3:1 [c]	1.5
3-Chloropropane-1,2-diol	58	0.6:1 [d]	0.8
4-(2-*O*-Tosylethyl)-2,2-dimethyl-1,3-dioxolan	75	1.0:1	0.9
4-(3-*O*-Tosylpropyl)-2,2-dimethyl-1,3-dioxolan	–	1.0:1	1.6
4-(4-*O*-Tosylbutyl)-2,2-dimethyl-1,3-dioxolan	–	1.0:1	1.6

[a] Diastereomeric ratio. [b] $R_{CN} = R_f$ (RCbl)/R_f (CNCbl). [c] Ratio may be $(S:R)$ rather than $(R:S)$. [d] 1.1:1 after recrystallization.

When the chiral centre on the alkyl ligand was further from the cobalt atom than the second carbon atom, the resonances of the corrin protons of the diastereoisomers could not be distinguished. The diastereoisomers could, however, still be distinguished because the chemical shifts of protons on the alkyl ligand were distinct (Table 2).

Enantioselectivity of cob(I)alamin towards racemic alkylating agents

Cob(I)alamin was treated with an excess of various monosubstituted epoxides, and other chiral alkylating agents, and the proportions of the two diastereoisomers formed (see Table 3) were estimated by integration of suitable NMR signals. The highest ratios were observed for t-butyloxirane and methyloxirane. If the chiral centre was further from the cobalt than two carbon atoms, no differentiation was seen. A relatively low enantioselectivity was shown towards cyclohexyloxirane. This was not due to an inhomogeneous reaction mixture, since increasing the proportion of methanol did not alter the diastereoisomeric ratio. The enantiomers of methyloxirane experience different environments as they approach the cobalt atom, presumably *via* the least hindered channel between rings C and D. The *R*-isomer is favoured because the methyl group points away from the more crowded ring D. In contrast, when cyclohexyloxirane approaches, the interactions between the cobalamin and the cyclohexyl ring are spread over both rings C and D, and the enantiomers are not so well differentiated. In all three cases, the preferred isomer was *R*. This was proved for methyloxirane because its pure enantiomers were available and were individually reacted with cob(I)alamin to give pure samples of **11a** and **11b**. Preference for the *R*-enantiomer was inferred in the other cases by observing a downfield shift of H-19 for the major products **16a** and **17a**.

Experimental

^{1}H NMR spectroscopy

The alkylcobalamin (*ca.* 7 mg, 5×10^{-3} mmol) was dissolved in D_2O, or [$^2H_4$]methanol (0.5 ml) to give a *ca.* 10 m*M* solution. All solutions were protected from light. The ^{1}H NMR spectra were obtained on a Bruker WH 400 spectrome-

ter, operating under ASPECT 2000 control, using a spectral width of 5376 kHz with 32 K data points. This gave a digital resolution of 0.33 Hz per point and an acquisition time of 3 s. Presaturation of the residual HOD peak for 2.5 s increased the total recycle time to 5.5 s. The spectra were processed with a Lorenzian to Gaussian transformation (time constant 0.5–1 s), or with exponential multiplication (0.2–0.8 Hz).

Decoupling experiments were done either directly, with continuous-wave irradiation of the chosen frequency, or in the difference mode. In this, 8–12 transients were recorded with decoupling of the chosen frequency, then the same number were recorded with the irradiation off-resonance, set as close as possible to the on-resonance frequency. The two sets of data were subtracted, and the cycle was repeated.

Nuclear Overhauser experiments were also run in the difference mode. Low power irradiation of the chosen frequency for 2.5 s, followed by acquisition of the FID was repeated 8–12 times. Then the same number of transients were subtracted with irradiation at an off-resonance frequency. The irradiation was then returned to the original frequency and the cycle was repeated, typically 100–200 times.

Alkylcobalamins

Except for neo-pentylcobalamin, which was prepared by the method of Schrauzer and Grate [24], these were prepared in the manner described below for methyl 5-deoxy-β-D-ribofuranos-5-ylcobalamin starting from either cyano- or hydroxocobalamin. For water-soluble alkylating agents, water was used as solvent. Otherwise, up to 20% ethanol was added to aid solubilization of the alkylating agent. Products were obtained as chromatographically pure crystalline solids that were characterized by ^{1}H NMR and, in some cases, FAB mass spectrometry. In experiments where the enantioselectivity of alkylation was measured, initial NMR measurements were made on the alkylcobalamin obtained from column chromatography (*i.e.* before recrystallization). The importance of this was shown with 2,3-dihydroxypropylcobalamin, where the ratio of *R/S* diastereoisomers was 0.6 : 1 before recrystallization, but changed to 1.1 : 1 after recrystallization from aqueous acetone. The majority of the alkylating agents were prepared by standard methods. Methyl 5-deoxy-5-iodo-β-D-ribofuranoside was prepared as follows.

Methyl 5-deoxy-5-iodo-2,3-O-isopropylidene-β-D-ribofuranoside [25,26]

Under nitrogen, to triphenylphosphite methiodide [27] (6.51 g, 14.4 mmol) in dry dimethylformamide (4 ml) was added a solution of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (2.38 g, 11.6 mmol, prepared from D-ribose [28]) in dry toluene (15 ml) and pyridine (0.1 ml). The resultant orange solution was stirred at room temperature under a nitrogen atmosphere for 1 h. The solution was washed with 0.1 *M* sodium hydroxide solution until colourless, then with water and brine. The organic layer was dried ($MgSO_4$) and the solvent was removed. The residue was purified by column chromatography on silica (100 g) using ether/light petroleum (1 : 8) as eluant. The title compound was isolated as a colourless, viscous syrup: 2.82 g (77%); $[\alpha]_D$ −67.8° (*c* 3.245%, $CHCl_3$) (lit. value −68.6° (*c* 2, $CHCl_3$) [25]).

TLC R_f 0.42 (ether/petroleum, 1 : 8). δ_H (200 MHz; $CDCl_3$): 5.05 (1H, s, H-1); 4.77 (1H, dd, H-3, $J_{3,4} = 0.7$ and $J_{3,2} = 5.9$ Hz); 4.63 (1H, d, H-2, $J_{2,3} = 5.9$ Hz);

4.44 (1H, qd, H-4, $J_{4,3} = 0.7$ Hz, $J_{4,5} = 6.1$ Hz, and $J_{4,5} = 10.0$ Hz); 3.37 (3H, s, OMe); 3.30 (1H, dd, H5, $J_{5,4} = 6.1$ Hz and $J_{5,5'} = 9.9$ Hz); 3.16 (1H, dd, H-5', $J_{5',5} = 9.9$ Hz and $J_{5',4} = 10.0$ Hz); 1.48 (3H, s, Me); 1.33 (3H, s, Me). δ_C (50 MHz; $CDCl_3$): 112.4 (s, CMe_2); 109.5 (d, C-1); 87.2 (d); 85.2 (d); 82.9 (d); 55.1 (q, OMe); 26.4 (q, Me); 25.0 (q, Me); 1.33 (t, C-5). EI MS: m/z 315 $(MH)^+$, 299 $(M - Me)^+$, 283 $(M - OMe)^+$. Anal. Found: C, 34.92; H, 4.75. $C_9H_{15}IO_4$ calc.: C, 34.41; H, 4.81%.

Methyl 5-deoxy-5-iodo-β-D-ribofuranoside

A solution of methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranoside (1.01 g, 3.2 mmol) in dry methanol (70 ml), to which acetyl chloride (1.5 ml) had been added, was heated at reflux for 3 h. The solution was neutralized with anhydrous sodium carbonate and the solvent was removed. The residue was purified by column chromatography on silica (50 g) using ether as eluant. Unchanged starting material was recovered first (0.27 g), followed by methyl 5-deoxy-5-iodo-β-D-ribofuranoside as a white solid: 0.48 g (75%), m.p. 68–70°C, $[\alpha]_D$ −78.3° (*c* 1.05%, 22°C, $CHCl_3$).

TLC R_f 0.4 (ether). δ_H (200 MHz; $CDCl_3$): 4.87 (1H, s, H-1); 4.22 (1H, br m, H-3, $J_{3,2} = 5.2$ Hz and $J_{3,4} = 6.0$ Hz); 4.12 (1H, br m, H-2, $J_{2,3} = 4.9$ Hz); 4.04 (1H, br m, H-4, $J_{4,3} = 6.0$ Hz, $J_{4,5} = 6.0$ Hz, and $J_{4,5'} = 6.9$ Hz); 3.37 (3H, s, OMe); 3.33 (2H, m, H-5 and H-5', $J_{5,4} = 5.9$ Hz and $J_{5',4} = 7.0$ Hz). δ_C (50 MHz; $CDCl_3$): 108.4 (d, C-1); 83.0 (d); 75.9 (d); 75.8 (d); 55.5 (q, OMe); 7.9 (t, C-5). EI MS: m/z 274 M^+, 243 $(M - OMe)^+$, 115 $(M - OMe-I)^+$. ν_{max} (disc) 3215–3487br, 2930–2990, 1017 cm^{-1}. Anal. Found: C, 26.10; H, 3.85. $C_5H_{11}IO_4$ calc.: C, 26.30; H, 4.05%.

Methyl 5-deoxy-β-D-ribofuranos-5-ylcobalamin

A solution of hydroxocobalamin (148 mg, 7.8×10^{-5} mol) and a catalytic quantity of cobalt(II) nitrate (1–3 mg) in 10 ml of water/ethanol (8:2) was degassed by pumping briefly (*ca.* 1 mmHg) and flushing with nitrogen. This operation was repeated three times and finally the mixture was flushed with nitrogen for 15–30 min. Sodium borohydride (29 mg, 7.6×10^{-4} mol) in water (1 ml) was added *via* syringe over 1 min and the resulting solution was stirred under a nitrogen atmosphere for 10 min, during which time the cob(III)alamin was reduced to cob(I)alamin with evolution of hydrogen. The colour changed from red to brown and finally to blackish green. All further operations were carried out in a dark room under a red safe-light. Methyl 5-deoxy-5-iodo-β-D-ribofuranoside (80 mg, 2.9×10^{-4} mol) in ethanol (1 ml) was added and the solution was stirred under a nitrogen atmosphere for 15 min, before acetone (0.5 ml) was added. The resulting solution was exposed to air and the cobalamin was extracted [29] into portions (5–10 ml) of phenol-dichloromethane (1:1, w/v; **CARE: extremely toxic by skin absorption**). The combined organic extracts were washed with water (equal volume to organic layer) before being diluted with dichloromethane to 10 times the original volume. The cobalamin was re-extracted into several small aliquots of water, until the extract was colourless. The aqueous extracts were combined and washed with several portions (3 × volume of aqueous layer) of dichloromethane. The water was removed under high vacuum (0.1 mmHg) to yield a red solid. The residue was purified by column chromatography on silica (10 g) using propanol/water/ammonia (100:99:1) as eluant. The alkylcobalamin was collected and the

solvent removed under high vacuum. The residue was recrystallized from aqueous acetone yielding red crystals of methyl 5-deoxy-β-D-ribofuranos-5-ylcobalamin; 56 mg (50%).

TLC (propan-1-ol/water/ammonia (100:99:1)) R_f 0.5. FAB MS: m/z 1476 (MH^+, 1.4%), 1329 (MH – alkyl$^+$, 5%). See text for ^{1}H NMR and assignments.

Acknowledgements

We thank SERC for a studentship (RMD), SERC and British Petroleum for a CASE studentship (RJA) and Glaxo for generous gifts of vitamin B_{12}.

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