Biosynthesis of Vitamin B_{12}: Preparation of Specifically Deuteriated Heptamethyl Dicyanocobyrinate for Study by ^2H N.M.R. Spectroscopy

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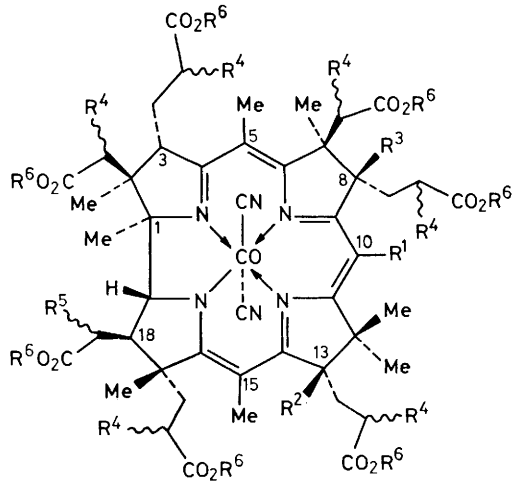
Methods have been developed for deuteriation of heptamethyl dicyanocobyrinate(cobester) (**1**) specifically at different sites; these products show broad signals in their ^2H n.m.r. spectra.

Direct detection of deuterium by n.m.r. spectroscopy has proved to be a useful method for biosynthetic studies.[1] It was important to explore the potential of ^2H n.m.r. studies for our work on the biosynthesis of the large, complex molecule of vitamin B_{12}[2] and we therefore required a set of specifically deuteriated samples of cobester (**1**).

Exchange of the hydrogen at C-10 of metallocorrins, and attack by electrophiles at that carbon atom are well known.[3] Thus, [10-^2H]cobester (**1a**) was conveniently prepared by heating (**1**) under reflux for 18 h in CH_3OD containing 5% sulphuric acid; these conditions left H-8 and H-13 essentially unaffected. In contrast, the reaction of (**1**) with deuteriotrifluoroacetic acid (19 h, ambient) exchanged H-10 (*ca.* 50%) and H-13 (*ca.* 40%) and yielded (**1b**). Interestingly, no 13-epi-cobester[4] [as in (**1**), inverted at C-13] could be detected in the crude product by comparing its ^1H n.m.r. spectrum with that of authentic 13-epi-cobester kindly provided by Professor R. Bonnett. The deuterium at C-10 of (**1b**) was then replaced by hydrogen under the conditions used to prepare (**1a**), except that CH_3OH was the organic solvent; this afforded (**1c**).

The exchange at C-13 is presumably initiated by deuteriation at one of the bridge carbon atoms (C-5, C-10, or C-15) of the corrin with loss of the proton from C-13, deuteriation at C-13, and then loss of the originally added deuteron (or original proton in the case of C-10).

Turning to base-catalysed conditions, we have found that the cyanide ion is sufficiently basic to deprotonate C-8 (in preference to C-13). Thus, heating cobester (**1**) under reflux with 1.3 mol. equiv. of KCN in CD_3OD for 14 h not only exchanged H-8 (*ca.* 70%) but also caused considerable exchange of the methylene groups adjacent to the ester carbonyls (1—2 deuterium atoms on each methylene carbon atom), and provided (**1d**). The concomitant conversion of the methyl esters into trideuteriomethyl esters was expected.[5] Under milder



Compound	R^1	R^2	R^3	R^4	R^5	R^6	Yield/%
(**1**)	H	H	H	H	H	CH_3	—
(**1a**)	D	H	H	H	H	CH_3	90
(**1b**)	D	D	H	H	H	CH_3	60
(**1c**)	H	D	H	H	H	CH_3	83
(**1d**)	H	H	D	H,D	D	CD_3	65
(**1e**)	H	H	H	H	H,D	CD_3	72

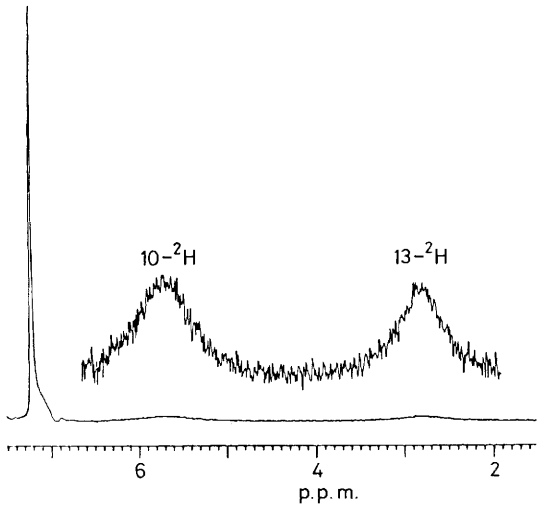


Figure 1. ^{2}H N.m.r. spectrum of (**1b**) measured at 61.4 MHz; 50 mg of (**1b**), prepared as in text, in 10% C_6F_6–C_6H_6 using C_6D_6 as an internal reference (δ = 7.2), and a ^{19}F lock. Acquisition time of 1.024 s, sweep width 1000 Hz, 2000 data points, 1152 transients.

conditions (1.3 mol. equiv. of KCN, CD_3OD, 23 h, ambient) there was selectivity for the C-18 acetate residue, as well as complete ester exchange, to afford (**1e**). It seems probable that exchange of the methylene hydrogens in the last two examples occurs *via* the acyl cyanide (RCH_2COCN) which is thought to be the intermediate for ester exchange.[5]

The location of the deuterium atoms in all the products (**1a**—**e**) was established by n.m.r. spectroscopy, based upon those signals from (**1**) which were absent or greatly diminished in their 400 MHz ^{1}H spectra and 100.6 MHz ^{13}C spectra; we have rigorously assigned all the important signals in both the ^{1}H and ^{13}C n m r. spectra of (**1**).[6]

The ^{2}H n.m.r. spectra of (**1a**—**c**) at 61.4 MHz showed that deuterium atoms attached directly to the corrin nucleus give signals with line widths at half-height, $W_{\frac{1}{2}}$, of 35—40 Hz (Figure 1). For (**1d**) and (**1e**) the signals due to the deuteriated methylenes are completely unresolved and appear as a broad peak centred at δ 2.3 p.p.m.; those of the trideuteriomethyl esters are sharpest ($W_{\frac{1}{2}}$ *ca.* 6 Hz), but are only partially resolved, even after resolution enhancement of the original free induction decay.

The n.m.r. linewidth of a nucleus for which quadrupolar relaxation is the dominant relaxation mechanism, as in the case of deuterium attached to a rigid molecule, is given by equation (1),[7] where a is the radius of the macromolecule,

$$W_{\frac{1}{2}} \propto \tfrac{4}{3}\pi\eta a^3/kT \quad (1)$$

η is the viscosity of the solvent, and k is Boltzmann's constant. Hence it is clear that the linewidth of deuterium signals will increase markedly with molecular dimensions.[7,8] The resulting poor dispersion and low sensitivity will impose limitations on the use of existing ^{2}H n.m.r. methodology for biosynthetic studies of large, rigid molecules such as corrins.

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