The Methylation Process in Corrin Biosynthesis. Application of $^1H\{^{13}C\}$ Nuclear Magnetic Resonance Difference Spectroscopy to a Biochemical Problem

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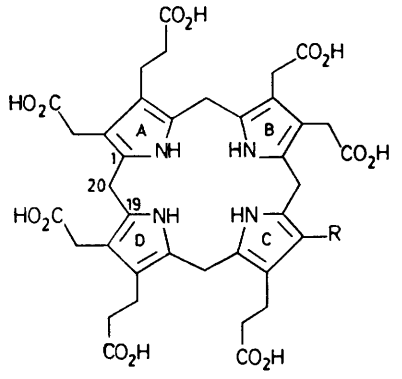
Summary $^1H\{^{13}C\}$ n.m.r. difference spectroscopy has been used to determine the intact incorporation of [*methyl*-$^{13}CD_3$]methionine into the C-1- and C-12α-methyl groups of vitamin B_{12}.

RECENT work has delineated the pathway of corrin biosynthesis as far as the intermediacy of uro'gen III (**1**) and the ring c decarboxylated uro'gen (**2**).[1] Several mechanistic proposals[2,3] have been made regarding the subsequent steps whereby the C-20 (δ-*meso*) carbon of (**2**) is lost and its place taken by a methionine-derived methyl group at C-1 in vitamin B_{12} (**3**). Since the above hypotheses involve seco-corrin systems (as **4**) in which enzymatic and/or chemical exchange (**4** ⇌ **4a**) of the C-1 methyl protons could occur, it became of interest to examine various prototropic possibilities at this methyl group and at C-19, the other terminus of the seco-corrin → corrin cyclization, where knowledge of the oxidation level and timing of the loss of the departing 'C-1 unit' is lacking. We now report on the fate of the C-1 methyl protons during biosynthesis of vitamin B_{12} in *Propionibacterium shermanii*.

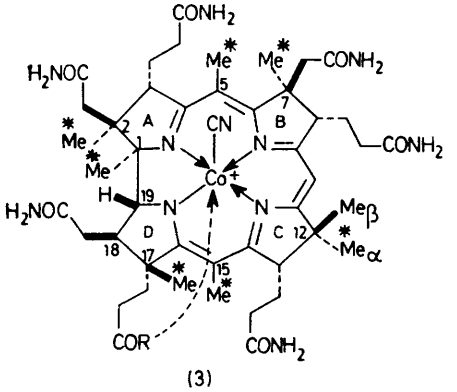
Previous independent studies at Yale[4] and at Cambridge[5] indicated that virtually intact methyl transfer from *S*-adenosylmethionine was taking place and it could be shown[5] that this was indeed the case at C-7 (ring B) and C-12α (ring c). However, mass spectrometric[5] and ^3H–^{14}C results[4] indicated that some minor exchange, possibly at C-5 or C-15, or even at C-1, might be occurring. In order to achieve maximum sensitivity in a double labelled (^{13}CD) experiment, a sample (0·36 g) of [*methyl*-$^{13}CD_3$]methionine (90% in ^{13}C; 98% in D) was administered to resting whole cells of *P. shermanii* (583 g; wet cells) and the resultant purified cyanocobalamin (32 mg) (**3**) was examined by the following technique.

First, ^{13}C and ^2H Fourier transform (F.T.) n.m.r. data indicated that (as shown in a simultaneous experiment with [*methyl*-$^{14}CH_3$]methionine) good incorporation (20—25%) of the doubly labelled methionine had been achieved, with equal distribution of ^{13}C label to the seven 'extra' methyl groups (at C-1, C-2, C-5, C-7, C-12α, C-15, and C-17) of (**3**). As shown in Figure 1(a), the undecoupled ^1H-F.T. n.m.r. spectrum of the enriched sample does not reveal any

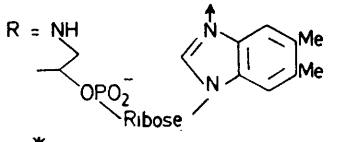
unusual ^{1}H–^{13}C coupling of the methyl signal centred at 0·47 p.p.m., which has been unambiguously assigned to



(1) R = CH_2CO_2H
(2) R = Me

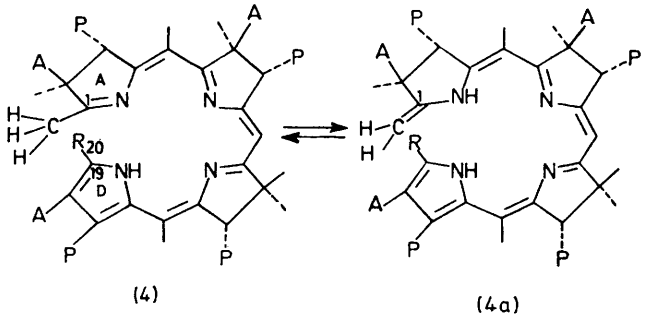


(3)



* Denotes enrichment from [^{13}C]methionine

C-1.[6,7] However, quantitative confirmation that $^{1}H\{^{13}C\}$ coupling was present and *not* due to any exchange of the enriched C-1-methyl carbon (→$^{13}CD_2H$, $^{13}CDH_2$, $^{13}CH_3$) was



(4) (4a)

A = CH_2CO_2H
P = $CH_2CH_2CO_2H$
R = H or CH_2OH

forthcoming by simultaneous subtraction of the ^{13}C-*decoupled* spectrum to give the difference spectrum shown in Figure 1(b). The latter technique reveals that (i) the resonances marked x in Figure 1 (a) arise from foldovers, (ii) the ratio of ^{13}C-satellite to ^{1}H resonance intensity at

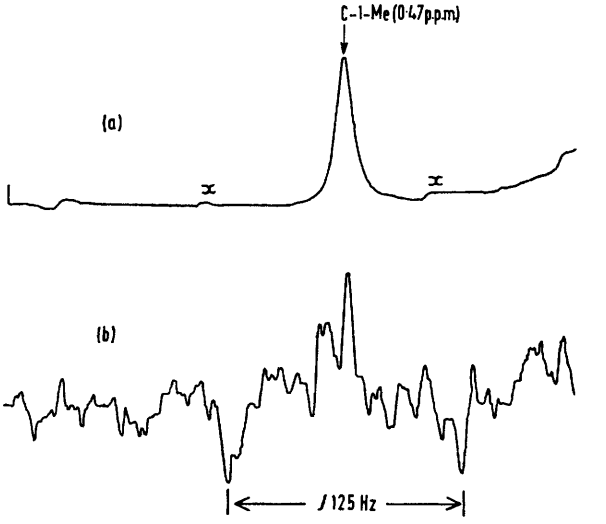


FIGURE 1. (a) F.T.-^{1}H n.m.r. spectrum of ^{13}CD-enriched cyanocobalamin (**3**) (0·024 M D_2O) showing the C-1-Me resonance (0·47 p.p.m.); (b) the difference spectrum of (a) and its ^{13}C decoupled spectrum. The two spectra were collected simultaneously by gating the ^{13}C decoupling frequency (noise modulated, 1 W) on for every other block of 4 transients. Each spectrum was stored automatically in separate 8K blocks of memory. The F_1R.F. frequency was positioned to the high field side of C-1 and a 1000 Hz SW was taken. This minimized foldovers [marked x in (a)] and the spectrum as displayed appears in reverse order.

the C-1-methyl group (1 : 200 for each satellite) [Figure 1(b)] is within experimental error (10%) identical with natural abundance (1·1%) [$J(^{1}H$–$^{13}C)$ 125 Hz], (iii) the analysis of

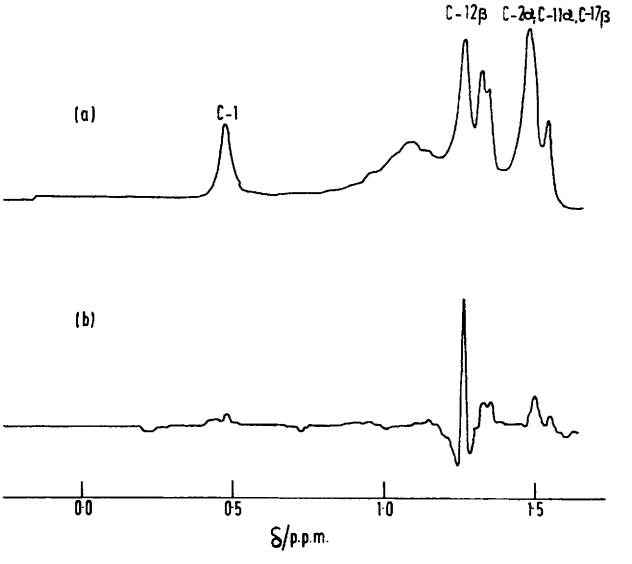


FIGURE 2. (a) F.T.-^{1}H n.m.r. spectrum of ^{13}CD-enriched cyanocobalamin (**3**) in the region 0—1·50 p.p.m. downfield from Me_4Si; (b) the F.T. difference spectrum of (a) and its ^{13}C-decoupled spectrum showing ^{13}C–H satellites for C-1 and $^{3}J_{CH}$ coupling into 12β of 4 Hz.†

the difference spectrum corresponding to the chemical shifts for the C-2α, C-12α, and C-17β methyl groups[7] [Figure 2(b)] shows no exchange of these $^{13}CD_3$-groups (natural abundance satellites only) thus confirming the earlier conclusion,[5] and (iv) a strong 3-bond coupling (J 4 Hz)† centred at 1·26 p.p.m. due to $^{13}C(12\alpha)$–$^{1}H(12\beta CH_3)$ is clearly evident in Figure 2(b) corresponding to a 20-fold enhancement of the ^{13}C-12α-methyl signal which also is an internal standard. These results require that the seco-corrin → corrin cyclisation proceeds with *retention* of the C-1-methyl protons. Further studies are in progress to examine in greater detail the possibility of *minor* exchange at the C-5- and C-15-methyl groups and the proton mobility at C-18 and C-19, as well as the nature of the liberated C-20 carbon.

Using different techniques, Professors Arigoni and Battersby have reached identical conclusions regarding the intact nature of C-1 methyl group in corrin biosynthesis as described in the accompanying communications.[8]

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† This value for ^{3}J(C–H) was obtained by computer simulation.

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