Intact Transfer of Methyl Groups in the Biosynthesis of Vitamin B_{12}

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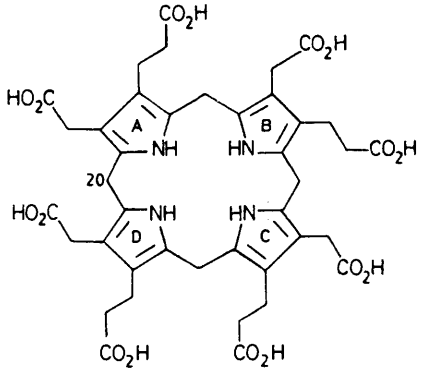
Summary The incorporation of label from [*methyl*-$^{13}CD_3$]-methionine into vitamin B_{12} is shown by n.m.r. techniques to occur by the intact transfer of seven methyl groups.

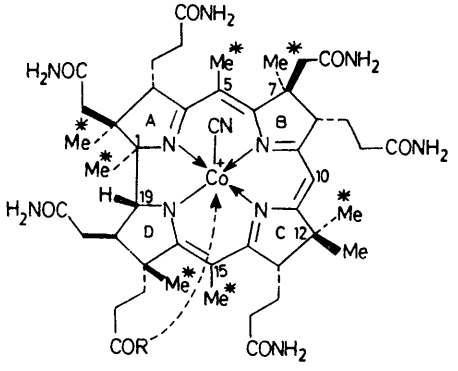
BIOSYNTHESIS of vitamin B_{12} (**2**) in *Propionibacterium shermanii* is known to proceed through uro'gen III (**1**)[1-3] in a complex sequence of steps involving, among others, loss of C-20 of the precursor and incorporation of seven methyl groups (atoms marked with asterisk in **2**) from methionine.[1,4,5] The availability of degradation products corresponding to rings B[6] and C[6,7] of the vitamin has been exploited in experiments with [*methyl*-CD_3]methionine to show that the methyl groups at C-7 and C-12 have been transferred intact.[8] Attempts to detect a similar intact transfer for the remaining five methyl groups have been thwarted so far by ambiguities in the interpretation of the mass spectra of the derivatives of (**2**) having an intact corrin ring system[8] as well as by the lack of degradative procedures allowing isolation of suitable fragments of the molecule. The question of loss or retention of the protons is of particular importance for the C-1 methyl group of the vitamin as it bears critically on current theories concerning the unsettled mechanism for the formation of the C-1 to C-19 bond from a hypothetical seco-corrin precursor.[9]

In connection with other studies on biological methylation a solution has now been provided to the problem with the following experiment. A specimen of [*methyl*-$^{13}CD_3$]-methionine (90 atom % ^{13}C, 98 atom % ^{2}H), prepared from $^{13}CD_3I$ and benzylhomocysteine by known methods,[10] was fed, in the amount of 100 mg/l, to a growing culture of *P. shermanii*.[4] Isolation of the vitamin[11] gave a sample strongly enriched in ^{13}C.† The off-resonance proton-decoupled ^{13}C-Fourier transform n.m.r. spectrum of the derived dicyanocobalamin (**3**) [same as (**2**) but internal axial ligand on cobalt replaced by CN] displayed in the methyl region only a diffuse manifold of overlapping signals with intensities not affected by variation of the pulse delay between 0 and 100 s; essentially the same signal pattern was observed in the proton-noise decoupled spectrum of the substance. These data were taken as a first indication that no new ^{13}C–^{1}H bond had been formed during the incorporation. Direct confirmation of this point was provided by the deuterium-noise decoupled spectrum of (**3**)

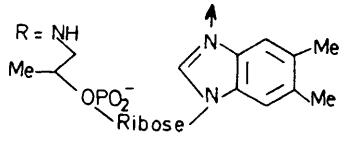
shown in the Figure. The signals of the seven methyl groups are displaced slightly toward higher field (0·7—0·9 p.p.m.) from their normal values as a consequence of the deuterium substitution.[12] The broadening observed for



(1)



(2)



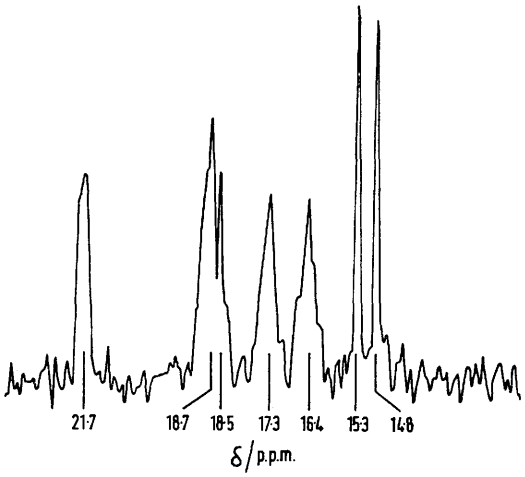


FIGURE. Deuterium noise-decoupled ^{13}C-Fourier transform n.m.r. sprectum of [*methyl*-$^{13}CD_3$]methionine enriched dicyanocobalamin (**3**) (0·1 M-KCN in D_2O, H_2O; 10 K points). Recorded with a Bruker HX-90R instrument (22·6 MHz). Chemical shifts are downfield from Me_4Si, ± 0·1 p.p.m.

four of the singlets at lower field is undoubtedly due to long-range (possibly γ) coupling between ^{13}C and ^{1}H. The sharpness of the two resonances at high field, previously assigned[1,5] to the methyl groups at C-5 and C-15, is in agreement with the expected lack of such coupling partners. The most significant feature of the spectrum is the fact that none of the signals of the enriched carbon atoms displays the large coupling (*J ca.* 125 Hz) anticipated for ^{13}C–^{1}H couplings. Thus, it can be concluded that all seven methyl groups have been transferred intact in the course of the biosynthesis. Specifically, this result provides a strong argument against the formation of intermediates in which the methyl group which finally becomes attached at C-1 of the vitamin has been transformed transiently into a terminal methylene group.

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† Under similar conditions a specific incorporation of 34% was observed with [*methyl*-^{14}C]methionine.

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