Biosynthesis of Corrins: the C-Methylation Steps

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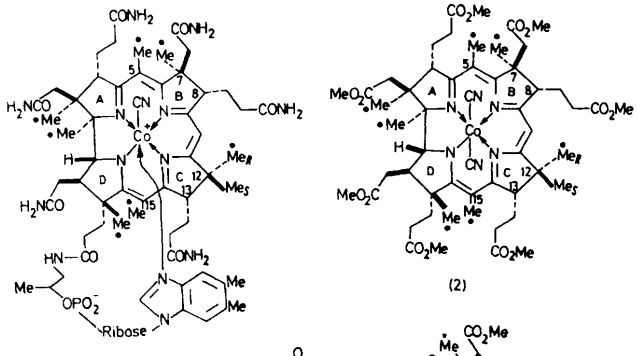
Summary Biological derivation of the *pro-R* methyl group at C-12 of vitamin B_{12} from methionine is confirmed by both ^{13}C and ^{2}H labelling and the methyl groups at C-7 and C-12 are shown to be incorporated intact.

ONE of the geminal methyl groups at C-12 on ring C of vitamin B_{12} (**1**) is biochemically derived from methionine and the other by decarboxylation of an acetic acid residue.[1] Our earlier work[2] using [methyl-^{13}C]methionine as precursor showed that it is the C-12 *pro-R* methyl group which arises from methionine. The work of Scott and his co-workers[3] was in agreement but recently Shemin's group,[4] by a method which involves correlation of ^{13}C-resonances with the assigned proton signals,[5] have reached the opposite conclusion, *viz.* that the *pro-S* methyl at C-12 is inserted from methionine.

Our previous conclusion[2] was based upon degradation of the labelled vitamin (**1**) *via* heptamethyl cobyrinate (**2**) to the imide (**3**).† The conclusion could only be wrong if virtually complete inversion (85% or more) had occurred at position-13 (ring C) during conversion of the small quantity of ^{13}C- labelled vitamin B_{12} (**1**) into ester (**2**); inversion was shown not to occur on a larger scale with unlabelled material.[2] This remote possibility has now been eliminated. The seven n.m.r. signals from the ^{13}C-enriched methyl groups of the labelled sample of ester (**2**) which we had degraded[2] to the imide (**3**) coincided exactly

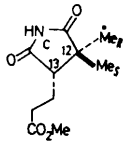
† Ozonolysis and isolation of crystalline ring C imide and amorphous ring B imide by method of T. L. Bogard and A. Eschenmoser unpublished work; see also ref. 7.

with the corresponding signals for authentic normal ester (**2**).‡ These signals differed in chemical shift from those shown by heptamethyl 13-*epi*-cobyrinate[6]‡§ (**2**, epimeric at C-13). Our original conclusion is thus confirmed.

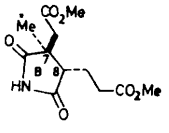


(1)

(2)



(3)



(4)

Experiments with [methyl-$^2H_3$]methionine were used to see whether intact methyl transfer occurs from methionine to the seven sites of *C*-methylation in vitamin B_{12}. *Propionibacterium shermanii* was grown in the presence of [methyl-$^2H_3$]methionine (72 mg l^{-1}) to afford deuteriated vitamin B_{12} (**1**) which was converted by methanol and sulphuric acid[7] into the hepta-ester (**2**). Part was reserved for mass spectrometry and the rest was ozonized† to yield the imides (**3**) and (**4**), corresponding to rings C and B of (**2**); the imides were further characterised by accurate mass determinations. The mass spectrum of the ring-C imide (**3**) showed a new parent ion three mass units higher than that corresponding to undeuteriated imide and of intensity equivalent to 26 ± 1% of the trideuterioimide (**3**) being present. No significant amounts of mono-, or di-deuterioimide (**3**) were detected. The spectrum of the ring-B imide (**4**) showed the same isotopic shift of three units and the absence of $^2H_1$ and $^2H_2$ species. Thus, *C*-methylation at C-7 and C-12 of vitamin B_{12} occurs by intact transfer of the *S*-methyl group from methionine.

A parent ion is not seen in the mass spectrum of unlabelled ester (**2**) but a strong base peak X^+ appears[6] at *m/e* 962, corresponding to $M^+ - (HCN + CN + CH_2CO_2Me)$. The mass spectrum of the deuteriated hepta-ester (**2**) showed that a mixture of labelled species was present as expected. Clear peaks which stood out from the adjacent ones appeared at X^+, $X^+ + 3$, $X^+ + 6$ and $X^+ + 9$ ($X^+ + 3n$) and smaller peaks appeared up to and including $X^+ + 21$. However, significant peaks also appeared between the ($X^+ + 3n$) series of peaks and these could be biosynthetically important or the trivial result of possible exchange from the C-5 and C-15 methyl groups during methanolysis. Further work is in progress.

The ring-C imide (**3**) obtained above (26% of the molecules fully deuteriated at the *C*-methyl group derived from methionine) was also examined by ^1H n.m.r. The signal rigorously assigned[7] to the *pro-R* methyl group was reduced in intensity as a result of the isotopic substitution; our coclusion reached by the ^{13}C approach is thus independently supported.

The results (a) confirm the origin of the C-12 *pro-R* methyl group from methionine (b) establish intact incorporation of the *C*-methyl groups at C-7 and C-12 (c) show heavy deuterium labelling at other sites of vitamin B_{12} biosynthesised from deuteriomethionine.

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‡ Measured at natural abundance of ^{13}C in C_6D_6.

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