Biosynthesis of Vitamin B_{12} : the Macrocycle and Studies on the Geminal Methyl Groups of Ring c

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Summary The biosynthesis of vitamin B_{12} is studied by ^{13}C-spectroscopy on the derived heptamethyl ester (**4**); work with amino[5-^{13}C]laevulinic acid and [*Me*-^{13}C]-methionine confirms that seven peripheral methyl groups are derived from methionine and proves that one of these is the *pro-R* methyl group on ring c.

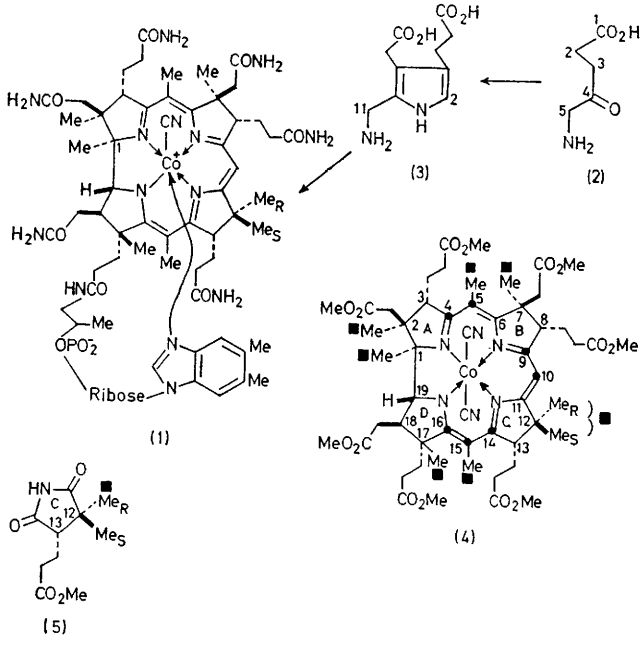
THE corrin macrocycle of vitamin B_{12} (**1**) is biosynthesised from δ-aminolaevulinic acid[1] (**2**) *via* porphobilinogen[2] (**3**). At least six of the *C*-methyl groups were proved to be derived from methionine.[1] Recently, Shemin and Scott have shown that the C-1 methyl group [see (**1**)] is derived not from C-5 of (**2**)[3,4] but from methionine.[4]

^{13}C-*Chemical shifts for the ester* (**4**) *with proton decoupling and FT at* 25·2 MHz *in* C_6D_6 (δ *from* Me_4Si).

Sites	δ
7 Ester CO, C-6 and -11[a] ..	176·0, 173·1, 172·6, 172·3 171·3, 170·2, 162·7
C-4, -9, -14, and -16	175·1[b], 174·9,[b] 171·8,[b] 163·1[b]
C-5, -15	103·8,[b] 102· [b], respectively[c]
C-10, -1, and -19	91·2,[b] 82·8. 75·0, respectively
C-3, -8, and -13	57·1, 54·5, 53·8
7 OMe, overlapped	51·7, 51·3, 51·1
C-2, -7, -12, and -17	58·5, 49·0, 46·9 46·1
11 CH_2 groups and C-18 ..	42·4, 41·7, 39·8, 33·9, 33·2, 31·9, 31·4, 30·9, or 30·7, 30·0, 26·9, 26·0, 25·1
C-Methyl at C-1, -2, -7, -12 (*pro-R*), and -17	22·3,[d] 19·6,[d] 19·3,[d] 18·3,[d] 17·0[d]
C-Methyl at C-5 and -15 ..	16·0,[d] 15·6[d]
C-Methyl at C-12 (*pro-S*) ..	30·9 or 30·7

[a] Three signals overlap at δ 171·3. [b] Only these seven signals observed for (**4**) in experiment with [5-^{13}C](**2**). [c] Respective assignment by analogy[3] with vitamin B_{12} and will be confirmed. [d] Only these seven signals observed for (**4**) in experiment with [*Me*-^{13}C]methionine.

For our concurrent ^{13}C-studies on vitamin B_{12}, the spectra were measured on the derived heptamethyl ester[5] (**4**). This is benzene soluble and with 45 skeletal carbons,



contains 17 fewer and three *C*-methyl groups less than the parent vitamin. The ^{13}C-spectrum of (**4**) shows 43 sharp signals largely assignable (Table) using Doddrell and Allerhand's work on other corrins[6] together with our own measurements.

The compound [5-^{13}C](**2**), synthesised in 57% overall yield from K^{13}CN (90% enrichment) by a new method,[7] was diluted† with unlabelled (**2**) and incorporated into vitamin B_{12} by *Propionibacterium shermanii*. [*Me*-^{13}C]Methionine was incorporated in a separate experiment. The ^{13}C-n.m.r. spectra of the derived labelled esters (**4**) each showed seven singlets (Table) corresponding to the carbon atoms marked ● from (**2**) and ■ from methionine. These results confirm the findings of Shemin and Scott and they add strength by being obtained from the ^{13}C-spectra of a different corrin.

The geminal methyl groups on ring c are derived[1] one from methionine and one from C-2 of (**2**). Degradation‡ of the ester (**4**) from the ^{13}C-methionine experiment gave the imide (**5**) with 12% ^{13}C-enrichment; a larger sample of ^{12}C-imide prepared under identical conditions showed $[\alpha]_D$ — 48·6° ($CHCl_3$) corresponding to 11% inversion at C-13. The 3H singlet, τ 8·75, in the proton spectrum ($CDCl_3$) of the ^{12}C-imide (**5**) corresponds[8] to the 12-*pro*-*R*-methyl group; that for the *pro*-*S* group is at τ 8·64. The ^{13}C-labelled imide gave an identical spectrum save that two new sharp peaks (J 128 Hz) were centred on the signal at τ 8·75 which was of diminished intensity. When the spectrum was recorded in C_6D_6, the *C*-methyl signals moved to τ 9·23 and 9·06, the former again being of lowered intensity and, importantly, still centred on ^{13}C-satellites (J 128 Hz). It is thereby established that in the biosynthesis of vitamin B_{12}, the C-12 *pro*-*R* methyl group is the one derived from methionine. Thus the overall methylation process for ring c involves the formal *trans*-addition of Me and H as is the case for rings A, B, and D.

We thank Prof. A. Eschenmoser for a sample of the imide (**5**) and for unpublished information, Dr. B. A. Hems and Dr. W. F. J. Cuthbertson and their colleagues (Glaxo Research Limited) for a gift of vitamin B_{12} and for help with isolation methods, and the Nuffield Foundation and S.R.C. for financial support.

(*Received*, 11*th April* 1973; *Com.* 518.)

† Lowered ^{13}C-enrichment to avoid ^{13}C–^{13}C coupling.

‡ Ozonolysis and isolation of crystalline ring c imide: T. L. Bogard and A. Eschenmoser, unpublished work.

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