Biosynthesis of Vitamin B_{12}: Experiments on Loss of C-20 from the Precursor Macrocycle

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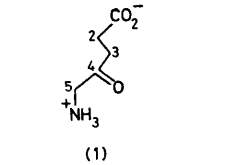
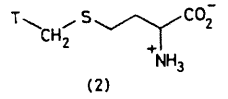
Summary N.m.r. studies on ^{13}C-labelled trimethylisobacteriochlorin from the B_{12}-producer *P. shermanii* confirm *C*-methylation at C-20 and it is shown by ^{3}H–^{14}C double labelling that this C-20 methyl group is lost during contraction of the macrocycle to the corrin system.

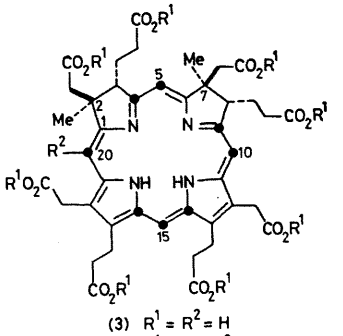
THE surprising structure (as **5**) recently elucidated[1] for the trimethylisobacteriochlorin isolated[2,3] from *Propionibacterium shermanii*, a producer of vitamin B_{12}, raised two fascinating possibilities for the biosynthesis of cobyrinic acid (**11**), the precursor of the vitamin itself: (a) the necessary loss of C-20 from the precursor macrocycle might occur as a C_2-unit or as two C_1-units (C-20 is reported[4] to be lost



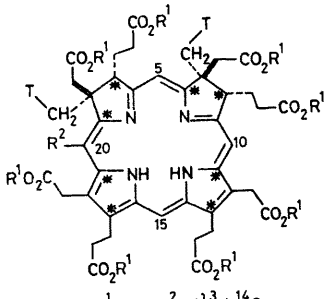
(1)



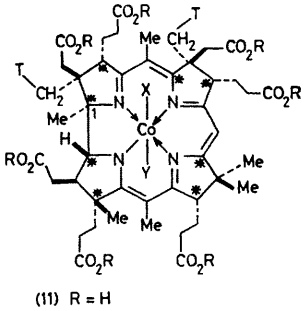
(2)



(3) $R^1 = R^2 = H$
(4) $R^1 = Me$, $R^2 = H$
(5) $R^1 = H$, $R^2 = Me$
(6) $R^1 = R^2 = Me$



(7) $R^1 = Me$, $R^2 = H$ } ^{3}H:^{14}C
(8) $R^1 = R^2 = H$ } Ratio 1·26
(9) $R^1 = Me$, $R^2 = CH_2T$ } ^{3}H:^{14}C
(10) $R^1 = H$, $R^2 = CH_2T$ } Ratio 1·78



(11) R = H
(12) R = Me, X = Y = CN
^{3}H: ^{14}C Ratio from (8) 1·18
^{3}H: ^{14}C Ratio from (10) 1·26

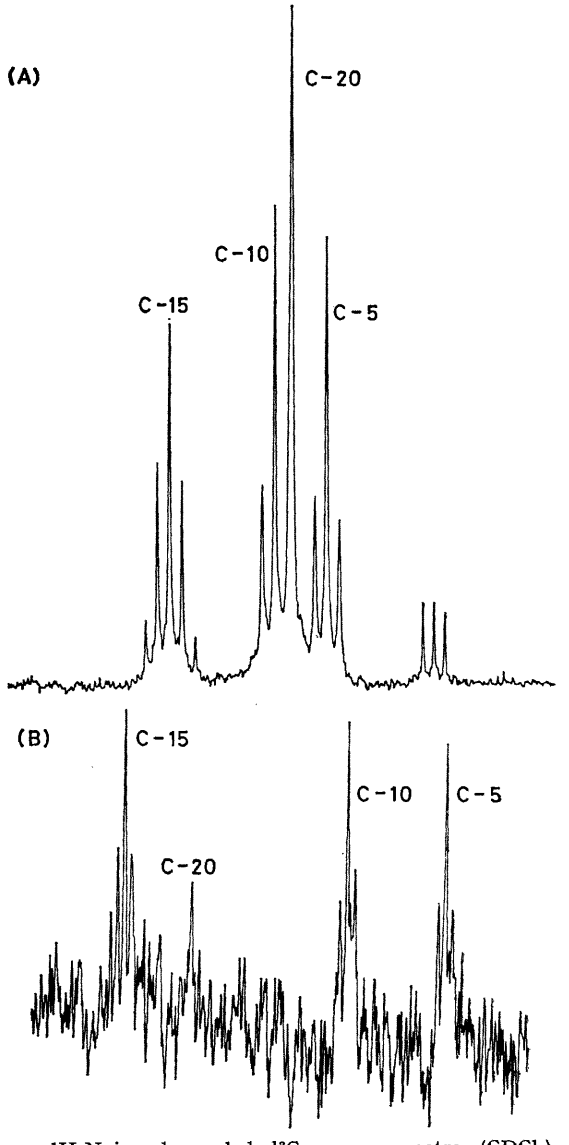


FIGURE. ^{1}H-Noise decoupled ^{13}C n.m.r. spectra ($CDCl_3$) of isobacteriochlorins biosynthesised from [5-^{13}C]ALA (as **1**). (A) Sirohydrochlorin octamethyl ester (**4**) at 25·2 MHz (*cf.* ref. 6). (B) Trimethylisobacteriochlorin octamethyl ester (**6**) at 90·5 MHz.

as formaldehyde); (b) the methyl group at C-1 of cobyrinic acid (**11**) may arise by migration from C-20.

Firstly, [5-^{13}C]δ-aminolaevulinic acid (as **1**), ALA, was incorporated into the dimethylisobacteriochlorin (sirohydrochlorin) (**3**) and into the trimethyl relative (**5**) by resting cells of *P. shermanii*;[5] the pigments were isolated as their octamethyl esters (**4**) and (**6**), respectively. The ^{13}C-signal from C-20 in the ^{1}H-noise decoupled spectrum of sirohydrochlorin ester (**4**) was unambiguously assignable (*cf.* ref. 6) since it was the only singlet and the tallest signal (δ 93·4 p.p.m.), see Figure (A). The signal for C-15 is also directly assignable by having 5 lines and C-5 and C-10 were distinguished by off-resonance decoupling.[6] *C*-Methylation at C-20 should, by comparison of benzene and toluene,[7] move the singlet *ca.* 9—10 p.p.m. downfield and should greatly reduce the size of the signal owing to loss of Overhauser enhancement. In fact, the ^{13}C-singlet for the trimethylisobacteriochlorin (therefore certainly from C-20) appeared as the smallest signal at δ 104·3 p.p.m., a shift of 10·9 p.p.m., see Figure (B). The structure (as **5**) for the trimethylisobacteriochlorin was thus rigorously confirmed.†

Sirohydrochlorin (**8**) and the trimethyl system (**10**) were prepared biosynthetically in doubly-labelled form by adding both [*methyl*-^{3}H]methionine (**2**) and [4-^{14}C]ALA (as **1**) to the medium containing the *P. shermanii* cells. The two isobacteriochlorins were carefully purified as their octamethyl esters (**7**) and (**9**) for determination of ^{3}H:^{14}C ratios; the values given under structures (**7**) and (**9**) show a 2:3 relationship. The corresponding doubly-labelled acids (**8**) and (**10**) were then separately incorporated into cobyrinic acid (**11**) by the cell-free system[8] from *P. shermanii*; isolation of the product as the crystalline cobester (**12**) allowed accurate assay of ^{3}H:^{14}C ratios (see under structure).

No ^{14}C-labelled carbon is lost during conversion of (**8**) or (**10**) into cobyrinic acid (**11**). Nor was any loss of tritium expected during the conversion of sirohydrochlorin (**8**) *via* a dihydro-derivative‡ into cobyrinic acid (**11**) because of earlier ^{14}C-labelling experiments;[9] the values found for (**8**) and for (**12**) derived from (**8**) matched this expectation and independent work[6] is in agreement. This result acts as a standard for incorporation experiments with the trimethylisobacteriochlorin (**10**) for which almost exactly one third of the ^{3}H-activity was lost during the conversion (**10**) → (**11**), isolated as (**12**); see ^{3}H:^{14}C ratios under structures.§ Thus, the *C*-methyl group at C-20 of the trimethyl macrocycle (**10**) is *not* transferred to C-1 and is lost at some stage during contraction of the macrocycle to produce the corrin system (**11**).

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† Also confirmed by Professor A. I. Scott in a similar way, Zürich, March 1979.

‡ It had been generally recognised that a dihydroisobacteriochlorin is the probable form on the biosynthetic pathway since two *C*-methylations of uro'gen-III should afford such a dihydro system. Dr. G. Müller (Stuttgart) reported fluorescence measurements (B_{12} Symposium, Zürich, March 1979) indicating that this is almost certainly so.

§ Exactly parallel results were reported in Zürich, March 1979 by Dr. G. Müller (Stuttgart) using the same approach.

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