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Chemistry

Biosynthesis of vitamin B_{12}: Mode of incorporation of factor III into cobyrinic acid

(cobyrinic acid heptamethyl ester degradation/corrins/isobacteriochlorins)

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ABSTRACT **Extensive chemical degradation of cobyrinic acid biosynthesized from a sample of factor III radiochemically labeled in its three methyl groups demonstrates that label is retained exclusively in the two methyl groups at positions C-2 and C-7 and confirms that the C-20 methyl group of the precursor is lost during formation of the corrin ring system.**

The structure of factor III, a trimethylisobacteriochlorin isolated in minor amounts from the vitamin B_{12}-producing organism *Propionibacterium shermanii* (1, 2), has been assigned recently as in **1** (see Fig. 1) by analogy with the well-proven (3, 4) structure **2** of sirohydrochlorin, with which it occurs, and on the basis of extensive spectroscopic and biosynthetic evidence (5, 6). Biochemical conversion of **1** into cobyrinic acid, **3**, has been demonstrated with cell-free systems from *P. shermanii* (7) and *Clostridium tetanomorphum* (1, 5). It is known from previous work that formation of **3** occurs with extrusion of the carbon atom C-20 from the macrocyclic ring, and the results of two independent sets of double-labeling experiments are consistent with the additional loss of a methyl group during formation of **3** from **1** (5, 7).

To rule out conclusively an alternative biosynthetic path in which the extra methyl group of **1** serves as the source of the C-1 methyl group of **3**, the following experiments have been carried out.

A sample of factor III specifically labeled in its three methyl groups as in **1** was prepared from [methyl-^{14}C]methionine in cobalt-free incubations of *P. shermanii* (1) and converted biochemically into cobyrinic acid (**3**) by using the *C. tetanomorphum* system (8). The labeling pattern of the derived radioactive cobyrinic acid heptamethyl ester (cobester) **4** was established through chemical degradation.

Ozonolysis of **4** in methanol solution followed by oxidative work-up with H_2O_2 gave acetic acid (from C-5 and the attached methyl group) and a mixture that could be resolved by thin-layer chromatography into three compounds, **5**, **6**, and **7**, corresponding to ring B, ring C, and the A-D moiety of the starting material, respectively. Photolysis of the homogeneous *N*-nitrosolactam **8**, derived from **7** by the action of N_2O_4, afforded *inter alia* the labile enaminolactam **9**, which could be trapped as the crystalline cyanolactam **10**. The structure of this compound was established by comparison with an authentic sample obtained by total synthesis (9). Alternatively, ozonolysis of the crude photolysis mixture provided an entry into the A succinimide **11**, identical in all respects (including optical rotation) with the ring B derivative **5**.

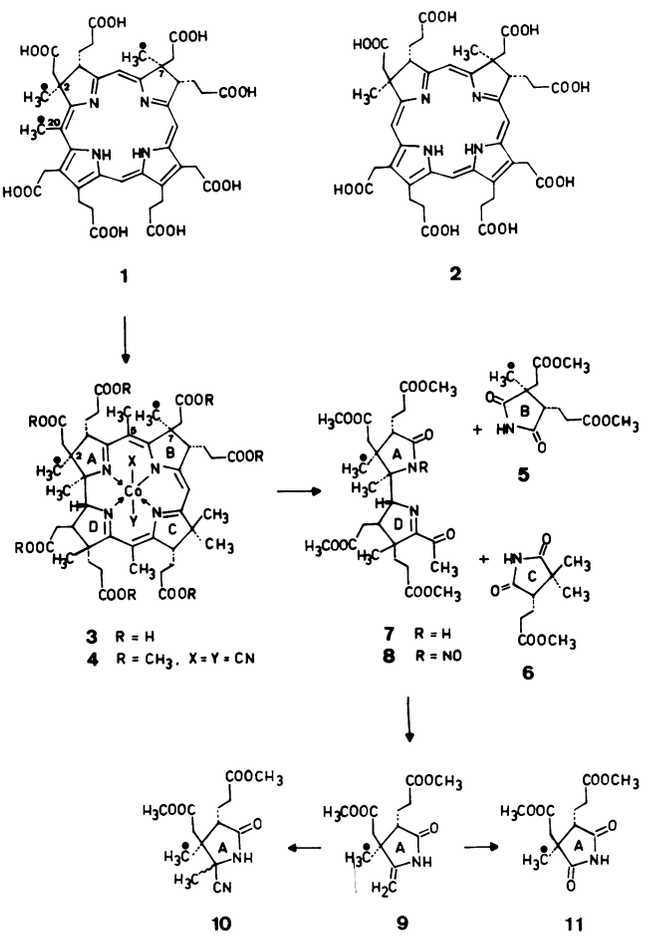

FIG. 1. Structures and degradation scheme. ●, ^{14}C.

Radioactivity data for the different fragments obtained in the degradation of the labeled cobester are collected in Table 1. These results, besides providing welcome independent verification for the correctness of the structure assigned to factor III, make it clear that the cobyrinic acid **3** derived from the triply labeled precursor **1** carries radioactivity exclusively in two positions, namely the two methyl groups attached at C-2 and C-7.

It is thus confirmed in an unequivocal manner that the C-20 methyl group of factor III does not rearrange to C-1 but rather is lost from the periphery of the macrocycle at some stage in the formation of the corrin ring system.

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Table 1. Degradation of labeled cobester 4

Compound	Relative molar radioactivity
Cobester 4	1.00 ± 0.04
Acetic acid (from C-5)	0.02
Ring B imide 5	0.50
Ring C imide 6	0.50
Rings A-D fragment 7	0.50
Ring A cyanolactam 10	0.46
Ring A imide 11	0.47

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