Vitamin B_{12}: After 25 Years, the First Synthesis

Vitamin B_{12}, first isolated in 1948, is the largest, most complicated vitamin yet discovered. Although its biochemical role has not been completely elucidated, vitamin B_{12}—also known as cyanocobalamin—has been shown to be required for normal blood formation, maintenance of neural function, normal growth, and for some metabolic processes, in which the vitamin acts as a coenzyme (*1*). Now, 25 years after its isolation, the first synthesis of cyanocobalamin has been completed.

The synthesis, a joint effort directed by R. B. Woodward of Harvard University, Cambridge, Massachusetts, and Albert Eschenmoser of the Eidgenössische Technische Hochschule, Zurich, Switzerland, required the efforts of 99 workers from 19 countries during an 11-year period. The conclusion of the synthesis was recently described by Woodward at Wesleyan University's first Peter A. Leermakers Symposium in Middletown, Connecticut.

Vitamin B_{12} is required by all higher animals, but it has not been found in higher plants. The principal dietary sources of the vitamin for humans are animal tissues and liver, but the vitamin is not synthesized by animals. Rather, it is produced by bacterial or fungal fermentation in the rumen and absorbed and concentrated during metabolism. This exclusive microbial synthesis is unique among the known vitamins.

Deficiency of vitamin B_{12} produces pernicious anemia, a severe and eventually fatal anemia characterized by an abundance of large oval red corpuscles, well filled with hemoglobin, and by a paucity of the other cellular products of normal bone marrow activity: young red cells, white cells, and platelets. Pernicious anemia is not generally caused by a dietary deficiency of the vitamin, however, but by the absence from the gastric juices of a glycoprotein—normally termed "gastric intrinsic factor"—which facilitates absorption of the vitamin by the intestine. The condition can thus be controlled either by injection of small amounts of vitamin B_{12} into the bloodstream or by oral administration of intrinsic factor, either alone or in conjunction with the vitamin.

The precise biochemical nature of pernicious anemia is not yet known, but it seems clear that lack of the vitamin inhibits the synthesis of DNA in proliferating cells. It has been shown, for example, that one coenzyme form of the vitamin, methylcobalamin, is the source of the methyl group that is added to deoxyuridylic acid in the synthesis of thymidylic acid.

Methylcobalamin has also been shown to participate in the synthesis of choline and methionine. (In mud-dwelling anaerobic bacteria, methylcobalamin has been implicated in the conversion of mercury to toxic methylmercury.) Another coenzyme form, adenosylcobalamin, functions as a cofactor in a variety of metabolic reactions, particularly isomerizations and transformations of amino acids and their homologs.

Cobalt Is Unique

Cyanocobalamin is unique in that it is the only known vitamin containing a metal ion, in this case cobalt. The cobalt is surrounded by a macrocyclic corrin ring consisting of four nitrogen-containing five-membered rings joined through three methylene bridges. The corrin ring is similar to the dihydroporphyrin (chlorin) ring of chlorophyll—for the synthesis of which, among many other naturally occurring products, Woodward won the Nobel prize in 1965—but the dihydroporphyrin contains only two chiral (asymmetric) centers, whereas the corrin contains nine chiral centers around its periphery.

The final stages of the synthesis begin with β-corrnorsterone (**I**), so named by Woodward because it is the "cornerstone" of the plan for completion of the construction of the cyanocobalamin molecule. Produced by a previously described (*2*) 37-step synthesis, **I** is a vital building block because it contains six contiguous chiral centers in configurations corresponding to those in cyanocobalamin. It was during the synthesis of this molecule, incidentally, that Woodward and Roald Hoffman, now at Cornell University, Ithaca, New York, developed the well-known Woodward-Hoffman rules of orbital symmetry for predicting the feasibility, products, and stereochemistry of concerted reactions.

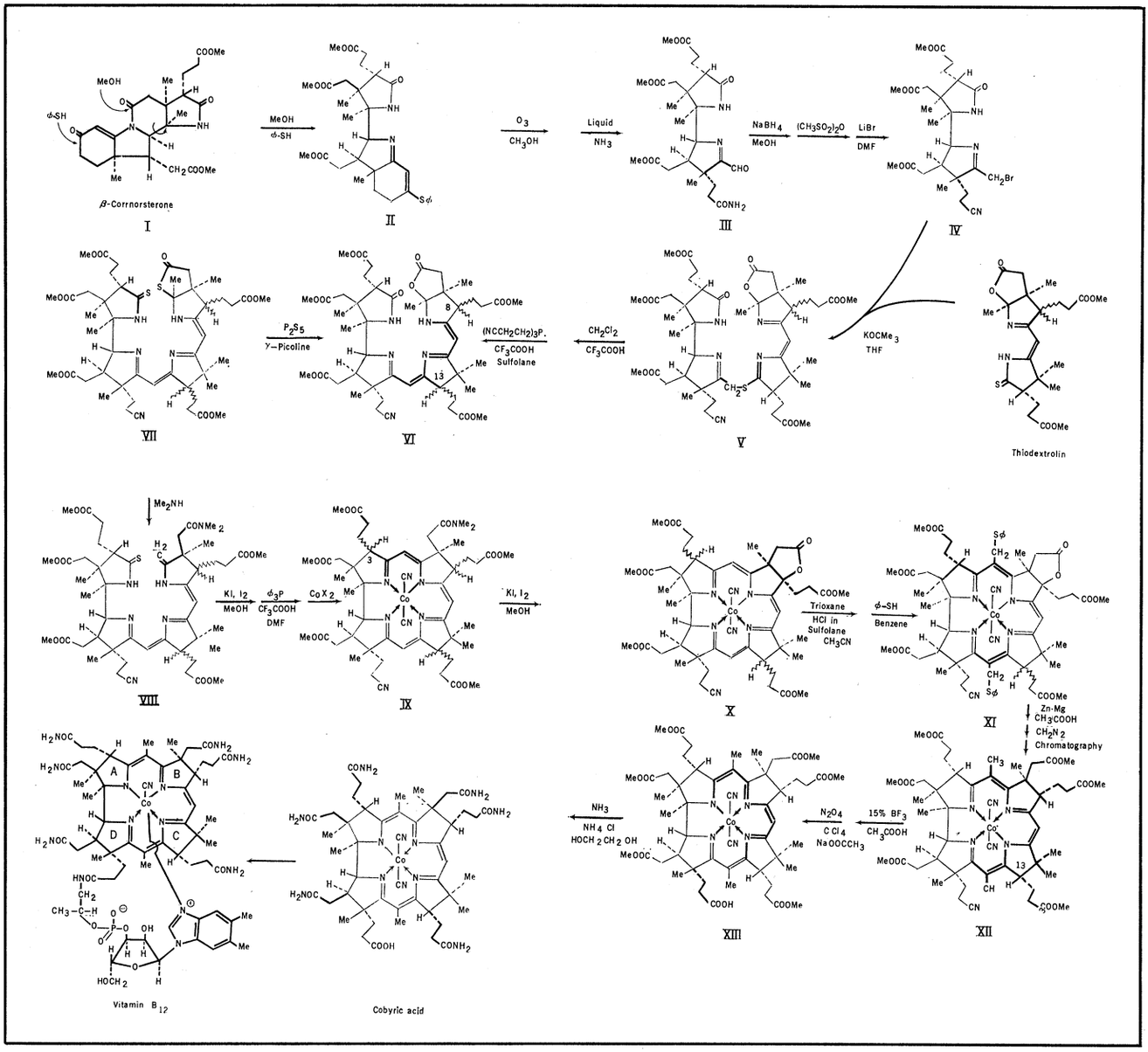
Methanolysis of **I** cleaves the fused lactam ring, allowing the A and D rings of the target cobalamin to assume the proper orientation. Concomitant with methanolysis, thiophenol converts the conjugated ketone to a thioether, thereby permitting differentiation of the carboxylate moiety formed in the subsequent ozonolysis of **II**. Ozonolysis thus yields a thioester side chain, which is converted to an amide in liquid ammonia. It also produces what Woodward describes as the only known example of a β-iminoaldehyde. He attributes the stability of this theoretically highly reactive group to steric hindrance around the carbon-nitrogen double bond.

The aldehyde **III** is then reduced to an alcohol with sodium borohydride and esterified with methane sulfonyl anhydride. The anhydride simultaneously dehydrates the amide to a nitrile. Displacement of methane sulfonate with lithium bromide then gives the cyanobromide **IV**.

The second major building block is thiodextrolin (*3*), the source of the B and C rings. The cyanobromide is linked to thiodextrolin by a base-catalyzed displacement of bromine to form the methylene thioether **V**, which holds the two segments in position for formation of a carbon-carbon bond. Sulfur is then extruded from this link with tris(β-cyanoethyl)phosphine, which also destroys the chirality at C-13 of the corrin ring (**VI**).

The first step in linking the A and B rings is exchange of sulfur for oxygens in the lactam and lactone rings (**VII**). Aminolysis with dimethylamine then cleaves the thiolactone, producing a dimethylamide side chain and a methylene group in proximity to the thiolactam (**VIII**). These two groups are held in position by coordination of the four nitrogens with a zinc halide and linked by iodine oxidation. After removal of the zinc, the sulfur is extruded with triphenylphosphine, which also destroys the chirality at C-3.

At this point, the product is a mixture of eight diastereomers. Separation of the diastereomers by high pressure liquid chromatography shows that only



36 percent of the mixture has the correct configuration at all three chiral centers. More than 78 percent of the mixture has the proper configuration at C-3, however, and that proves crucial because the bridgehead methyl group at C-5 can be introduced only when the proper configuration exists at C-3.

Iodine oxidation of **IX** converts the dimethylamide to a lactone (**X**) linked to C-8; because the fused lactone is more stable in the *cis*-configuration, this reaction regenerates the required chirality at that position. Lactone formation also protects the bridgehead C-10 from substitution in the next step, which is introduction of methyl groups at the C-5 and C-15 bridgeheads.

Substitution at the bridgeheads is accomplished by hydroxymethylation with trioxane, followed by reaction with thiophenol to form the thioether **XI**. As was mentioned above, substitution can occur at C-5 only in molecules with the proper chirality at C-3; in disubstituted rings, therefore, the only remaining stereochemical problem is at C-13.

Reduction of **XI** with zinc and a catalytic amount of magnesium desulfurizes the molecule and opens the lactone ring, introducing a hydrogen at C-8. The carboxymethyl group thus formed is then esterified with diazomethane. The two resulting substances, differing only in configuration at C-13, can now be separated by high pressure liquid chromatography; one of the resulting products is stereochemically pure **XII**.

Treatment of **XII** with boron trifluoride in acetic acid converts the nitrile to an amide, which is then deaminated with dinitrogen tetroxide and a catalytic amount of sodium acetate to yield the free acid **XIII**. Ammonolysis of **XIII** in ethylene glycol, catalyzed by ammonium ion, converts the six ester side chains to amides, producing cobyric acid. Since cobyric acid has previously been condensed with the 3′-ribazoleaminoalkylsodium phosphate (*4*), the synthesis of vitamin B_{12} is thus complete.—THOMAS H. MAUGH II

References

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