BIOORGANIC CHEMISTRY 2, 155–162 (1973)

On Corrin Biosynthesis

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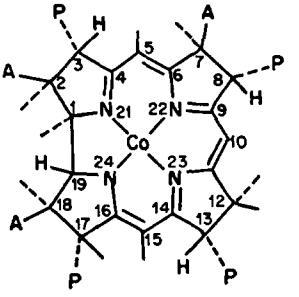
Received June 26, 1972

A scheme is proposed for the biosynthesis of vitamin B_{12} which proceeds via the intermediary of a cobalt porphyrin.

While the occurrence of type-III porphyrins in nature has prompted much speculation as to their biosynthetic origins (*1–4*), the biosynthesis of the closely related macrocyclic corrin chromophore of vitamin B_{12}, cobyrinic acid **1**, has received considerably less attention. Matthewson and Corwin (*3*) have proposed that both type-III prophyrins, and corrins (which also have the type-III arrangement of peripheral substituents)



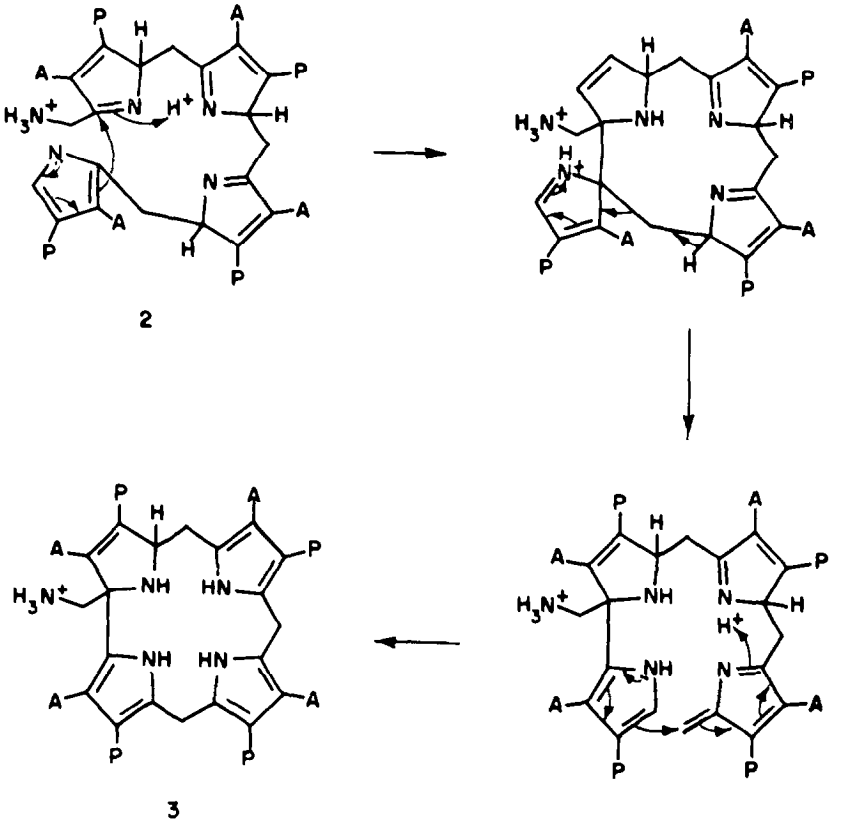
A = $CH_2\ CO_2H$

P = $CH_2\ CH_2\ CO_2H$

1

may have a common precursor in the form of a linear tetrapyrrole **2** which can cyclize to give either uroporphyrinogen III or a corrin-like macrocycle **3** (Scheme 1). These hypotheses have been criticized (*4*) on the basis of the supposed instability of the α-pyrrolenine units in the above intermediates. However a more fundamental criticism may be raised, for while it is unclear why nature uses only tetrapyrroles having the type-III arrangements of peripheral substituents, it seems most unlikely that two separate biosynthetic pathways would have been developed, one to give type-III porphyrins and the other type-III corrins, if a cyclic type-III intermediate could be found which is common to both porphyrins and corrins.

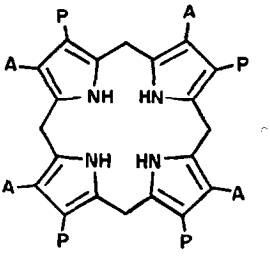
The earliest type-III intermediate in porphyrin biosynthesis from porphobilinogen is urophyrinogen III **4** (*5*, *6*), and Scott has recently proposed a scheme for the biochemical conversion of uroporphyrinogen III into cobyrinic acid **1** (*7*). On the other hand the economy of nature is impressive; and we suggest that the divergence of the



Scheme 1

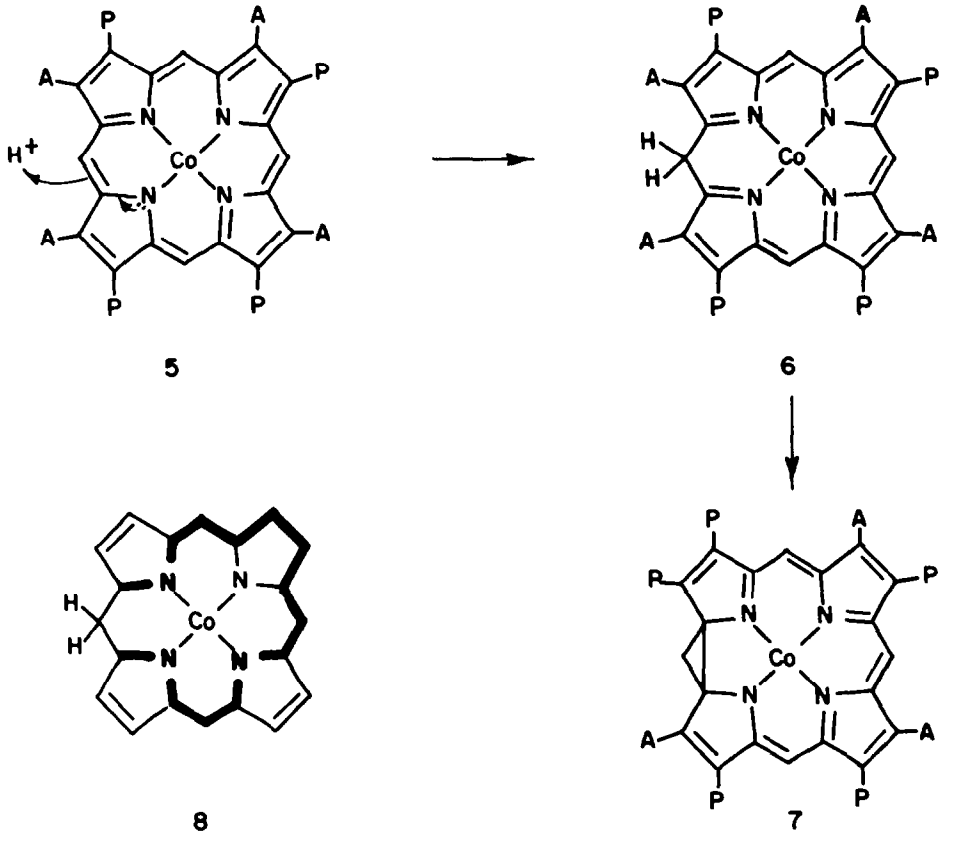
porphyrin and corrin biosynthetic pathways occurs much later than the early porphyrinogen stage, and that corrins may be derived biosynthetically from cobalt porphyrins.

A resonance energy of greater than 400 kcal mole^{-1} for the porphyrin macrocycle (*8*) suggests a somewhat limited chemistry [a chemistry which is in fact dominated by electrophilic substitution (*9*)], and at first sight a rearrangement of the porphyrin skeleton may seem unlikely. Metal free porphyrins most readily undergo electrophilic



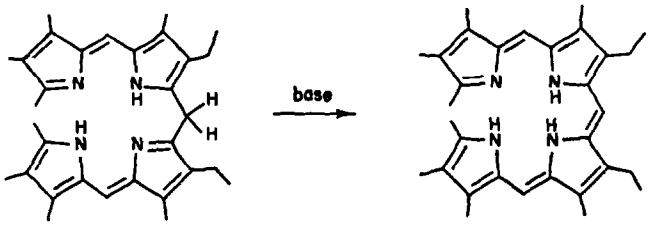
(4)

attack at nitrogen, and *N*-protonation in turn deactivates the porphyrin periphery to further electrophilic attack (*10*). In contrast to this, metalloporphyrins are not easily protonated at nitrogen, and in the case when the β-pyrrolic carbon atoms are substituted, rapid electrophilic attack at the *meso*-carbon atom is observed. Moreover, the fastest rate is observed with cobalt porphyrins (*10*). Protonation of cobalt uroporphyrin III **5** at the meso-position could lead to an isoporphyrin **6** (*11*). Such an isoporphyrin would have a geometry ideally suited for an electrocyclic ring closure **6** → **7**. It must be noted however, that if one considers the isoporphyrin as a 16π electron system **8**, a concerted electrocyclic ring closure must proceed in a conrotatory fashion (*12*), which would result in the biologically improbable transfused cyclopropane. Such



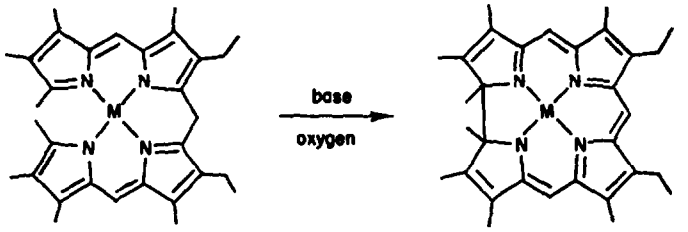
considerations make no allowances, however, for the effect of the cross-conjugated double bonds, and more importantly allow a passive role for the metal, a situation which may not obtain experimentally (*13*).

In the presence of base, the 1,19-dideoxybiladiene-ac **9** has been shown to tautomerize to the corresponding bilatriene **10** (*14*). Such bilatrienes are remarkably stable and their only chemistry is that with acid to give the diprotonated salt of the biladiene. Biladienes form metal complexes **11** with a variety of transition metals. When such metallocomplexes are treated with base and oxygen, their chemistry is governed by the central



9 10

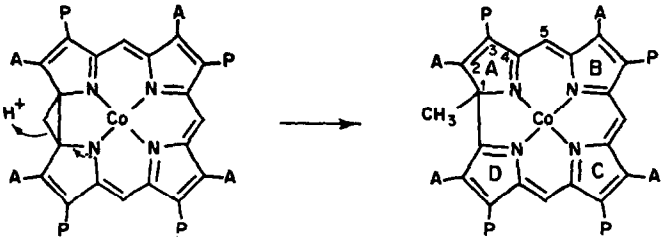
metal atom. Thus the zinc complex remains unchanged, the nickelous complex is slowly converted to the corresponding 1,19-dimethyltetradehydrocorrin (**12**; M = Ni) while the cobaltous complex (**11**; M = Co) is rapidly converted to the cyclized



11 12

cobaltous complex (**12**; M = Co) (*15*). Cyclization of the oxidized complex **11** proceeds to give **12**, which has a chromophore identical to our proposed cyclopropane intermediate **7**. Thus the reaction **11** → **12** would appear to be a good experimentally observed model for the proposed reaction **6** → **7**. Furthermore, the difference in rates of cyclization between the nickel and cobalt complexes is indicative that these reactions cannot be considered as simple concerted electrocyclic ring closures, and we note once again a difference in rate between the cobalt and other metal complexes.

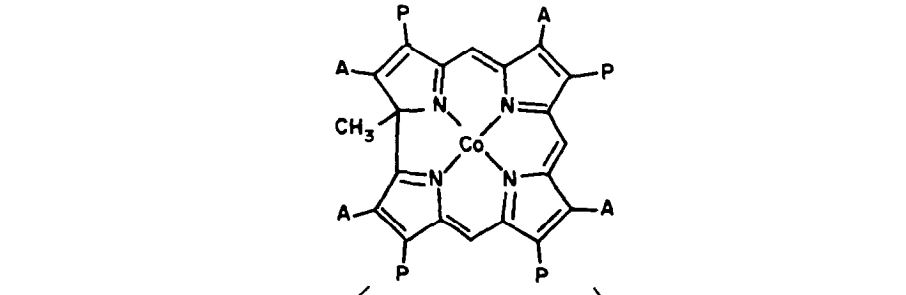
The acid-catalyzed ring opening of cyclopropane derivatives is well established (*16*). Acid-catalyzed ring opening (assisted in this case by nitrogen) of **7** could give the corrin-like macrocycle **13**.



7 13

Is the proposed macrocycle **13** a plausible intermediate for the further elaborations required to generate cobyrinic acid **1**? Shemin (*17*) has shown that the biosynthetic

origin of the methyl groups at C_2, C_5, C_7, C_{12}, C_{15}, and C_{17} most probably arises from methionine, which in turn acts as a methylating agent via its *S*-adenosyl derivative. Such methylations of **13** will clearly generate some carbonium ion character in the transition state, and it is apparent that methylation of ring A at C_2 (**13** → **14**) would be far more favorable than at C_3 (**13** → **15**). If one assumes that it is the same enzyme which alkylates



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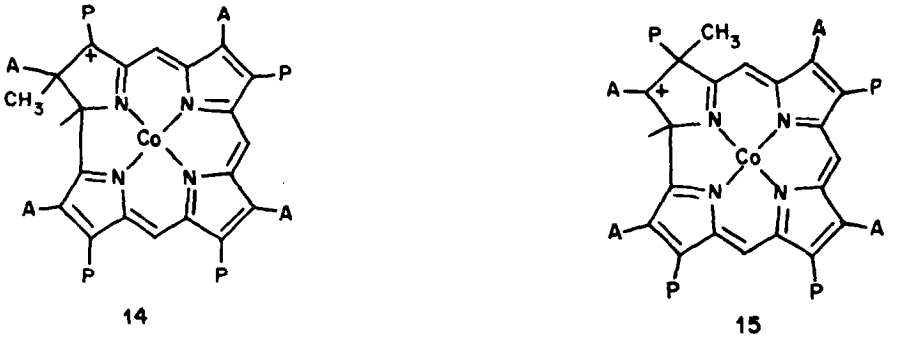
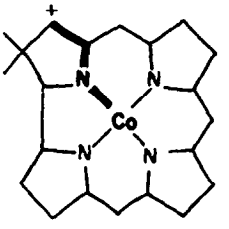


14 15



16

each of the four rings, and if electronic considerations require alkylation at C_2 rather than C_3 in ring A, where might such an enzyme alkylate rings B, C, and D? Stabilization of any positive charge which develops at C_3 during methylation at C_2 can occur via the conjugated system, including the cobalt atom, shown in **16**. If we use the same enzyme to alkylate each ring, one can ask, is *the same* delocalized system of $\overset{+}{C}$—C=N—Co

present upon alkylation of rings B, C, and D? Alkylation of ring B at C_7, ring C at C_{12}, and ring D at C_{17} does, indeed, satisfy this requirement. Thus electronic consideration, coupled with the assumption that only one enzyme is responsible for the peripheral alkylations, suggests that methyl groups appear at C_2, C_7, C_{12} and C_{17}. This is the arrangement of the methyl groups in cobyrinic acid **1**.

Any hypothesis concerning the biosynthesis of B_{12} must account not only for the positioning of the peripheral methyl groups but for their stereochemistry also. In cobyrinic acid **1** the methyl groups on rings A, B, and C (assuming that the proximal methyl group on ring C derives from a decarboxylated acetic acid side chain) are below

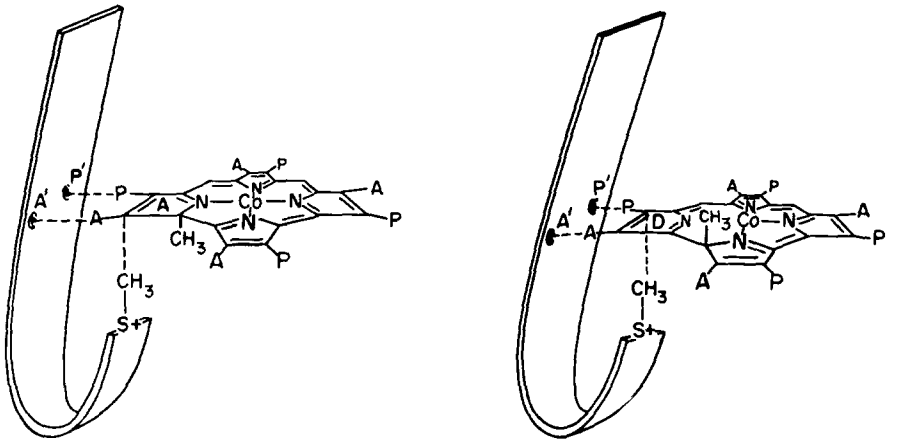
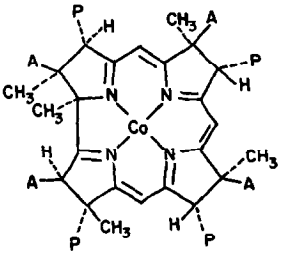


FIG. 1

the plane of the corrin ring and at carbon atoms bearing an acetic acid side chain, while the methyl group on ring D is above the plane of the corrin ring and at a carbon atom bearing a propionic acid side chain. This spatial arrangement of methyl groups is readily explained on the assumption (*vide supra*) that only one enzyme is responsible for the alkylations of an intermediate such as **13**. An enzyme which has binding sites for both an acetic and propionic acid side chain of the ring to be alkylated, and which is capable of delivering the methyl group from only one side of the corrin ring, will alkylate rings A, B, and C in the same manner (Fig. 1). Ring D, which has the order of its two side chains reversed compared to rings A, B, and C, when bound, would have the methyl group delivered on the opposite side of the ring (compared to the other three rings). Thus following the concepts outlined above, the successive methylation of the four rings of **13** would yield **17**. Methylation at C_5 and C_{15} [these two positions are very susceptible to electrophilic attack (*18*)], decarboxylation of the ring C acetic acid side chain, and one final reduction of the $C_{19}{=}N$ (to give the sterically more favorable *trans*-substitution at the direct link) gives cobyrinic acid **1**.

Throughout the above discussion we have assumed a special and unique role for the cobalt atom. It facilitates protonation of the *meso*-carbon atoms of porphyrins (*10*). Ring closures to give a direct link between the two termini of tetrapyrroles are fastest for cobalt complexes (*15*) and the transition state during alkylation will be stabilized by



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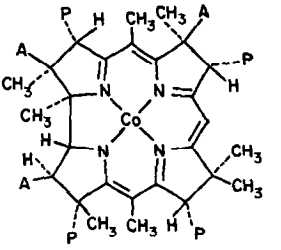

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the cobalt, particularly when it is in a low oxidation state. We have chosen to insert the cobalt atom, which occurs in B_{12}, at an early stage in the biosynthetic pathway in order to take advantage of these properties of cobalt. However the isolation (*19*) of cobalt-free corrins might suggest that the insertion of cobalt is the last step in the biosynthesis of B_{12}. The trace amounts of the metal-free corrins that are produced, coupled with the small number of microorganisms that produce them, suggests to us that they are not true intermediates in the biosynthesis, but are artifacts derived by removal of cobalt from some stage in the normal biosynthetic pathway.

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