[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE 18, MD.]

Biosynthesis of Pyrrole Pigments: A Mechanism for Porphobilinogen Polymerization[1]

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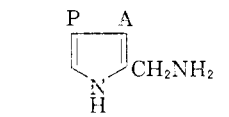
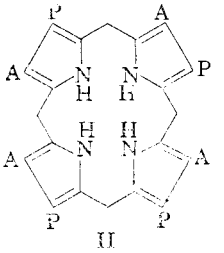
RECEIVED JULY 13, 1960

A key reaction on the pathway for the biosynthesis of porphyrins is the polymerization of porphobilinogen to uroporphyrinogen 3. The corrin moiety of cobalamine has the "type 3" order of substituents and is also derived from porphobilinogen. A new mechanism based on existing data and studies of Stuart models is proposed to explain the formation of the type 3 macrocycles. Polymerization of porphobilinogen yields a flexible protonated tetramer that serves as a common intermediate for uroporphyrinogen 1, uroporphyrinogen 3 and a cobalamine precursor. The tetramer can cyclize at different sites, then open and recyclize to the required products.

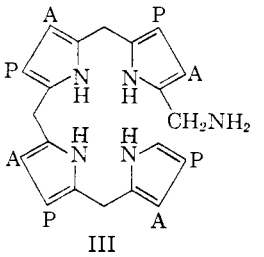
This paper offers a mechanism to explain the establishment of the order of substituents on the naturally occurring pyrrole pigments. The biosynthetic reaction which determines this order in the case of porphyrins is the polymerization of porphobilinogen (I) to uroporphyrinogen 3 (II).[2-7] The naturally occurring porphyrins and chlorins are derived from II. The corrin element in cobalamine has the type 3 order of substituents and is also derived from porphobilinogen.[8]



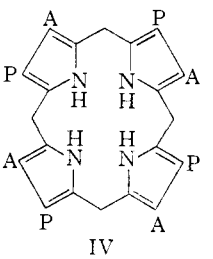
I, P = CH_2CH_2COOH
A = CH_2COOH



II



III



IV

Successive substitutions of α-aminomethyl groups at α-free positions in porphobilinogen would be expected to yield a tetramer (III) that could cyclize directly to uroporphyrinogen 1 (IV). Obviously the formation of uroporphyrinogen 3 requires a more complex reaction sequence. It has been shown that at least two enzymes—a deaminase and an isomerase—are involved and that incomplete systems produce uroporphyrinogen 1.[6,7] No type 2 or 4 isomers have been isolated from natural sources.

Non-enzymatic self-condensations of porphobilinogen occur readily and yield mixtures of uroporphyrinogen isomers, the compositions of which vary with *p*H.[4,9] The product from acid polymerization is apparently an equilibrium mixture of all four isomers ($^1/_2$ 3, $^1/_4$ 4, $^1/_8$ 1, $^1/_8$ 2); under alkaline conditions the product contains higher yields of isomer 1 but no isomer 2. The presently available techniques for analyzing mixtures of uroporphyrinogen isomers are imprecise and do not distinguish between types 3 and 4. Furthermore, uroporphyrinogens isomerize in acid, the best condition for porphobilinogen polymerization.[10] Thus no final interpretation can be made of the non-enzymatic polymerizations, although they do indicate that the type 3 structure does not have to be determined by an enzyme.

A variety of mechanisms have been offered to rationalize porphobilinogen polymerization reactions,[4,9,11-15] reflecting the large number of pathways that are possible. No detailed mechanism has been offered for the formation of the cobalamine corrin. The mechanism proposed in this paper is based on the existing data concerning the polymerization, on the literature of pyrrole chemistry and on studies of Stuart models. Two variations of the mechanism are presented: scheme 1, for the formation of uroporphyrinogens 1 and 3, either enzymatically or non-enzymatically; and scheme 2 for the enzymatic formation of a postulated cobalamine precursor.

Scheme 1.—Porphobilinogen (Ia) is assumed to be protonated on the amine group in all but strongly alkaline solutions.[16] Elimination of ammonia creates a positive carbon which substitutes at the α-free position of another molecule of Ia. The product is a dipyrrylmethane with the second ring in a protonated pyrrolenine form (V). In all previous mechanisms the α-proton on the second ring in V is automatically assumed to be eliminated to regenerate the aromatic pyrrole ring. However, this proton loss is reversible,[17,18] and in some cases a stable pyrrole acid salt can be isolated.[19]

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(7) L. Bogorad, *ibid.*, **233**, 501 (1958); **233**, 510 (1958); **233**, 516 (1958).

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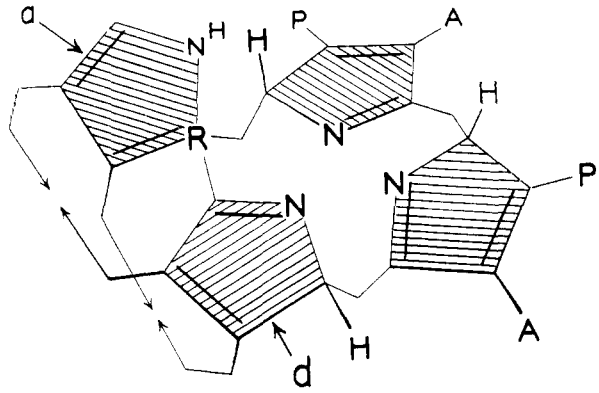
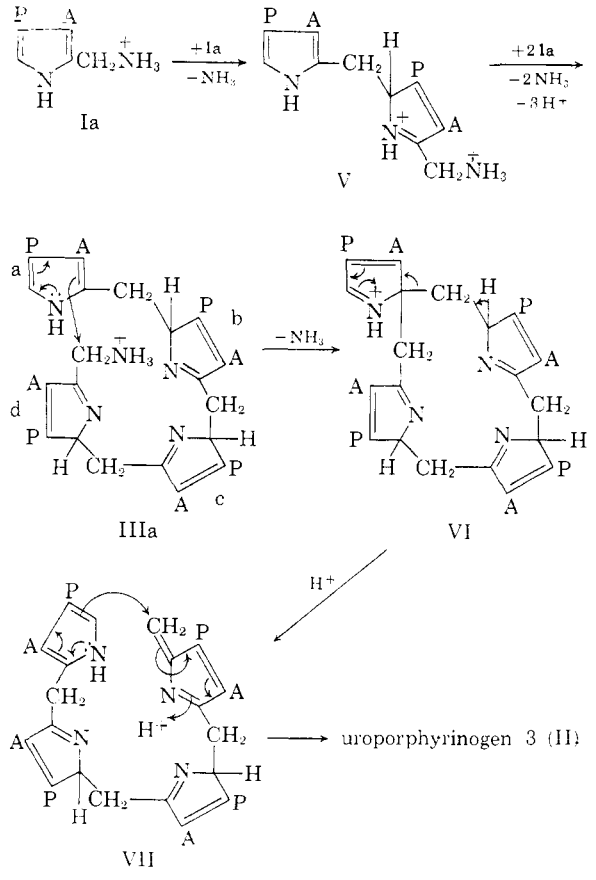


Fig. 1.—A = CH_2COOH, P = CH_2CH_2COOH,

R = $CH_2NH_3^+$, $\longrightarrow$ $\longleftarrow$ = —C(=O...HO)(OH...O)C—

Recent studies have confirmed that pyrrole rings exist in the protonated pyrrolenine form in acid solutions.[20] Under special conditions in alkaline solution it is possible to force the loss of the proton on the heterocyclic nitrogen leaving a stable pyrrolenine.[21]

SCHEME 1



If substitution at free positions continues with retention of α-hydrogens and loss of imine hydrogens, a flexible tetramer (IIIa) results. Because of the tetrahedral carbons at the α-positions in rings b, c and d, IIIa can easily fold so that the terminal α-aminomethyl group on ring d lies immediately over the *inside* α position of ring a (see Fig. 1). The juxtaposition of the terminal substituting group with the still aromatic ring a favors cyclization to VI. A Stuart model of VI is strainless. The intermediate VI can reopen to give the tetramer VII with one inverted ring. Resubstitution of the terminal methylene at the outside α-free position and elimination of α-hydrogens yields uroporphyrinogen 3 (II).

This same sequence of reactions can be carried through if one or two α-hydrogens are eliminated from IIIa; *i.e.*, the tetramer can have fewer pyrrolenine rings and still retain sufficient flexibility for substitution of the terminal aminomethyl group at the inside carbon atom of ring a. The Stuart model of the intermediate arising in this way from III (VI with pyrrole instead of pyrrolenine rings) is strained, making it very probable that III would cyclize only to uroporphyrinogen 1. Under progressively more alkaline conditions there would be fewer α-hydrogens, increasing concentrations of III, and higher proportions of uroporphyrinogen 1 in the product. If the imine hydrogens are lost in preference to α-hydrogens and the rate of cyclization is high, alkaline conditions would still not prevent formation of uroporphyrinogen 3. Under very acid conditions even ring a would become protonated halting cyclization altogether.

The formation of IIIa from Ia is assumed to correspond with the "porphobilinogen deaminase" step in the enzymatic process, and the cyclization, reversal of ring a and reclosure with the "isomerase" step.[6,7] It may not be meaningful to consider the effect of *p*H on the enzymatic polymerization simply in terms of bulk hydrogen ion concentration; a pyrrolenine form of a pyrrole ring as in IIIa may be stable in a protein complex due to the relative lack of properly oriented proton acceptors.

Each of the three tetrahedral α-carbon atoms in IIIa is asymmetric, giving eight possible configurations for the tetramer chain. Since the removal of the α-proton after substitution is reversible, the molecule can readily assume the most favorable configuration. Stuart models show that for each configuration of α-carbons the tetramer can assume a conformation favoring cyclization according to Scheme 1. The configuration of α-carbons might be determined by the enzyme structure in enzymatic condensations.

In the discussion so far it has been assumed that some directive influence favors cyclization of IIIa in accordance with Scheme 1. In the absence of any directive influence, random folding would lead to substitution at both the outside and inside α-positions on ring a, giving a mixture of uroporphyrinogens 1 and 3. An enzyme could presumably force exclusive substitution at the inside position. Electronic substituent effects might affect the course of substitution if the terminal aminomethyl group were constrained to the vicinity of the first ring. Intramolecular hydrogen bonding could contribute to the required folding of the tetramer in non-enzymatic cyclizations. Stuart models of

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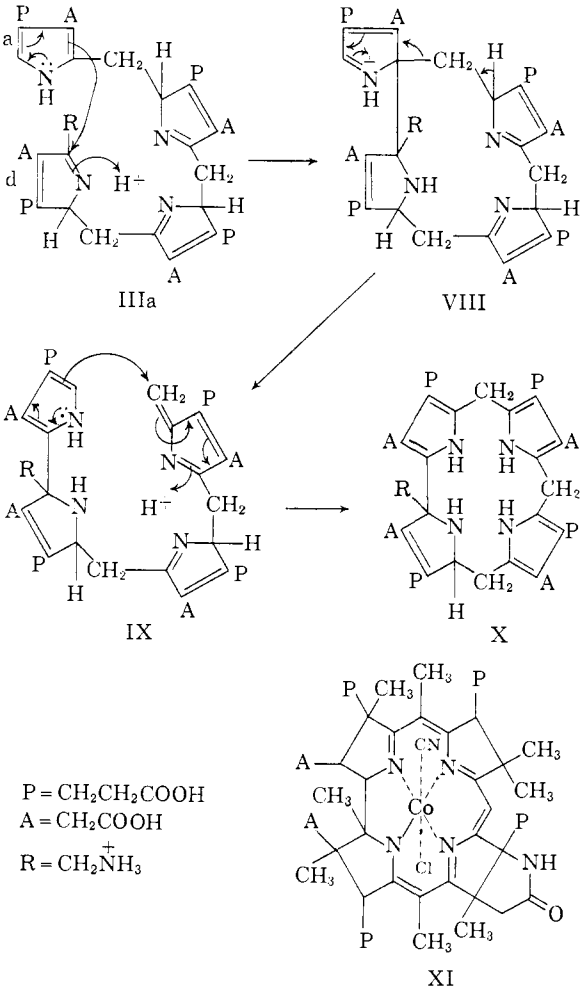
SCHEME 2



various intramolecularly hydrogen bonded configurations and conformations of IIIa utilizing carboxyl-to-imine nitrogen and carboxyl-to-carboxyl hydrogen bonds were studied. Both the acetic acid and propionic acid substituents on the end ring of IIIa can bond either in pairs or with all four carboxyls at once (Fig. 1). This multiple hydrogen bonding would favor substitution in accordance with Scheme 1, and thus increase the proportion of uroporphyrinogen 3 in the product.

Scheme 2.—The tetrameric intermediate discussed above (IIIa) can be folded more tightly than required for substitution of the terminal aminomethyl group onto ring a. The carbon holding the aminomethyl group—the outside α-position on ring d—can be brought immediately over the inside α-position of ring a (see Fig. 2). Because the terminal α-position on ring d is an imine carbon it

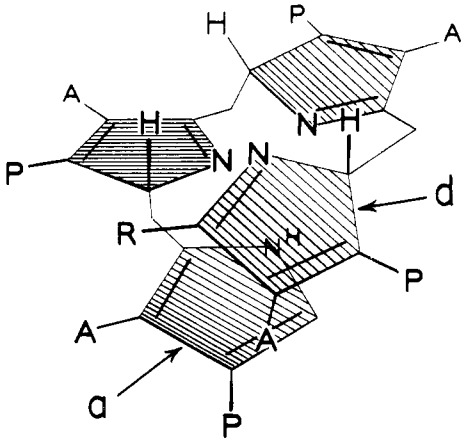


Fig. 2.—A = CH_2COOH, P = CH_2CH_2COOH, R = $CH_2NH_3^+$.

is electron deficient and can act as a substituting agent at the α-carbon on ring a. A cyclization gives rise to the intermediate VIII (Scheme 2). A Stuart model of this intermediate is strainless although rigid. Reopening can proceed as in Scheme 1, again inverting ring a (IX). Reclosure yields an intermediate (X) that appears to be closely related to the cobalamine corrin hexacarboxylic acid (XI).[22] Reasonable mechanisms can be formulated for the methylation of β-positions and bridges.[23] The reduction of the angular aminomethyl group to a methyl group is the only change in oxidation level required.

Conclusions.—The most important point in the mechanism proposed here is the retention of α-hydrogens after successive self-condensations of porphobilinogen. This allows sufficient flexibility of the resulting tetramer (IIIa) for the cyclizations to uroporphyrinogen 3 and the corrin precursor. The required folding of the tetramer in each case is presumably achieved by enzymes. In the absence of enzymes, random folding of IIIa should lead to uroporphyrinogens 1 and 3. Since the desmethylcorrin (X) could also arise from random cyclization, a compound related to X might be found in the products from a non-enzymatic polymerization of porphobilinogen or its analogs. The mechanism predicts that IIIa is an intermediate in the biosynthesis of uroporphyrin 3 and that IIIa and X are intermediates in the biosynthesis of cobalamine.

Acknowledgment.—This work was supported by a grant (RG-6691) from the National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.

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