which are far less reactive to electrophilic attack. This oxidation could occur either by hydrogen transfer or by condensation to give a dipyrrylethylene with subsequent tautomerization to a meso-substituted dipyrrylmethene of the type studied by Treibs, *et al.*[22] The observation (expt. 9) that the yield of recovered coproporphyrin is but one-third (30%) that of uroporphyrin when coproporphyrinogen is isomerized under identical conditions, coupled with the far larger preoxidation, can be interpreted as a measure of the importance of this lactone. The formation of this lactone is not essential to the rearrangement, since the isomerization proceeds as readily with coproporphyrinogen. Its presence reduces the concentration of free formaldehyde at equilibrium and so decreases the observed side reactions.

The isomerization of porphyrinogens weakens the arguments based on the formation of isomer "III" of coproporphryin[23] and of uroporphyrin[24] when appropriately substituted α-acetoxymethyl or α-aminomethyl pyrroles are condensed in hot acid solution.

The incorporation of formaldehyde into a porphyrinogen may be used to prepare uroporphyrin or coproporphyrin highly and specifically labeled in the methine groups.

Acknowledgment.—I wish to thank Dr. S. Granick for his continual interest and advice, and Mr. W. Cumming for able assistance. I am grateful to Dr. S. F. MacDonald for the generous gift of several chemicals. The fraction of diphtherea broth was obtained through the kind courtesy of Dr. P. H. Clarke of Lederle Laboratories. This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service, R.G. 4922.

(22) A. Treibs, E. Hermann, E. Meissner and A Kuhn, *Ann. Chem.*, **602**, 170 (1957).

(23) E. Bullock, A. W. Johnson, E. Markham and K. B. Shaw, *J. Chem. Soc.*, 1430 (1958).

(24) A. Treibs and W. Ott, *Ann. Chem.*, **615**, 137 (1958).

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE, NEW YORK, N. Y.]

The Condensation of Porphobilinogen to Uroporphyrinogen[1]

BY D. MAUZERALL

RECEIVED OCTOBER 12, 1959

The condensation of porphobilinogen gives high yields of uroporphyrinogen. The isomer and yield of uroporphyrinogen and the incorporation of formaldehyde when the condensation is carried out under neutral or alkaline conditions support a mechanism involving attack by pyrryl-$CH_2NH_3^+$ (or an equivalent lactone) with the elimination of either H^+ or $CH_2{=}NH_2^+$ from the second pyrrole. The reaction in hot acid solution is complicated by the isomerization and reactivity of the macrocyclic product.

The isolation of porphobilinogen[2] and the proof of its structure[3] were major steps in the identification of the intermediates in porphyrin biosynthesis.[4] This pyrrole forms uroporphyrin in high yields under mild experimental conditions. Early work indicated that the product was largely the naturally occurring isomer III[1b] showing that a rearrangement had taken place. A very reasonable mechanism was proposed by Cookson and Rimington to explain these facts.[3] This paper clarifies the question of the identity of the isomers and the reduction level of the product, and gives some evidence for the mechanism of the condensation.

Experimental

Materials.—The porphobilinogen was isolated from urine of porphyric patients by the method of Cookson and Rimington,[3] and purified just prior to this series of experiments by crystallizing the HCl salt. This pyrrole was kept at −20°. It was analyzed as PBG·H_2O. Calcd. for $C_{10}H_{16}O_5N_2$: C, 49.14; H, 6.60. Found: C, 48.77; H, 6.84. The source and method of purification of the porphyrins and other materials are given in the preceding paper.[5]

Analytical Methods.—Porphobilinogen was determined colorimetrically with a modified Ehrlich reagent.[6] The method of isomer analysis and other procedures are given in the preceding paper.[5] Due to the inherent difficulties of determining radioactivity by the "infinitely thin" technique, several products were checked by counting in solution.[7] This is a convenient and highly reproducible method of obtaining homogeneous infinitely thick counting with very small amounts of material. However, accuracy requires a high specific activity. Formamide, di-*n*-butyl phthalate, diphenyl ether and mixtures of these compounds are useful as non-volatile solvents. The comparison with the plating technique is good (Table I) and serves to justify this simpler, more sensitive technique. The counting error was easily held to less than 1% except for the very weakly labeled products. As a result of errors in determining the concentration and activity of added highly labeled formaldehyde, these ratios are only accurate to ±10%. About one-half of the uroporphyrin ester samples were rechromatographed on silica gel and re-assayed to check radioactive purity. The molecular activities were constant to ±5%. Various fractions from the band of the chromatographic column also had roughly constant activities. One sample was crystallized twice between chromatographs and also had constant activity. In addition, two uroporphyrin samples were chemically decarboxylated[8] and the molar activity of the coproporphyrin was also found to be constant (Table I).

(1) (a) Part of this work was presented at the IVth International Congress of Biochemistry, Vienna, Sept., 1958; abstracts, p. 4. (b) Formulas of the various compounds and isomers mentioned are to be found in the preceding paper.[5]

(2) P. G. Westall, *Nature*, **170**, 614 (1952).

(3) G. H. Cookson and C. Rimington, *Biochem. J.*, **57**, 476 (1954).

(4) D. Shemin, "Harvey Lectures," 1954–1955, Academic Press, Inc., New York, N. Y., 1956, p. 258; S. Granick and D. Mauzerall, *J. Biol. Chem.*, **232**, 1119 (1958); C. Rimington, *Brit. Med. Bull.*, **15**, 19 (1959).

(5) D. Mauzerall, THIS JOURNAL, **82**, 2601 (1960).

(6) D. Mauzerall and S. Granick, *J. Biol. Chem.*, **219**, 435 (1956).

(7) A. Schwebel, H. S. Isbell and J. D. Mayer, *J. Research Natl. Bur. Standards*, **53**, 221 (1954).

(8) P. R. Edmondson and S. Schwartz, *J. Biol. Chem.*, **205**, 605 (1953).

TABLE I

COMPARISON OF PLATING AND SOLUTION COUNTING TECHNIQUES[a]

Expt.	HCHO Activity[b]	URO Plate	URO Soln.	COPRO Soln.	HCHO reisolated Plate	HCHO reisolated Soln.
15	570	0.22	0.24	..	...	...
16	49	.51	.47	0.43	...	...
17	120	.88	..	..	0.057	0.059
18	115	.95	.88	.85	.19	.24
20	2.4	1.8	..	..	.47	.48

[a] The data, except for the first column are given as the ratio of the molar activity of the compound isolated to that of the original formaldehyde added to the reaction mixture; error: ±10%. [b] Activity in millicuries per mole of the original formaldehyde, measured as the dimedon derivative.

Experimental Procedures.—The solutions of porphobilinogen were prepared just prior to use. One to ten milligrams of the porphobilinogen was used in each experiment. The preparation of the tubes and the work-up were as described previously.[5] Porphobilinogen was determined on separate aliquots. In expt. 17 (Table III) the reaction was stopped following 0.5 hour of heating and the porphobilinogen was determined colorimetrically. The pyrrole was isolated by chromatography on Dowex-1. It was purified by repeated chromatography on Dowex-1[6] and on paper using acetic acid–butanol as the solvent. The purity was also checked by paper electrophoresis at *p*H 7.5. Separation from a highly active, Ehrlich-negative material was thus achieved. The activity of the porphobilinogen was determined by plating from an aqueous solution of known concentration.

Except where otherwise specified, three experimental conditions were used: (1) acid: 1.0 *M* HCl, 0.5 hour at 98°; (2) neutral: 0.25 *M* sodium phosphate, *p*H 7.6, 21 hours at 60°; (3) alkaline: 0.10 *M* NaOH, 2 hours at 98°; all in dearated solutions in sealed tubes. The *p*H's mentioned in this paper refer to the solution at 25°. An equivalent amount of ammonia was always added with the formaldehyde to approximate the amino-methyl group of porphobilinogen. The omission of ammonia under alkaline conditions where its polymerization with formaldehyde is favored (*e.g.*, hexamethylenetetramine) did not greatly increase the yield of uroporphyrin. However, the addition of two equivalents of ammonia per mole of formaldehyde caused the yields to fall from 30% to ~5%.

Results and Conclusions

Conditions for and Product of the Condensation of Porphobilinogen.—Previous work has shown that the optimum condition for the formation of uroporphyrin from porphobilinogen is heating for a short time in dilute acid.[2,3,9] It was observed that the condensation in neutral solution yielded no porphyrin but only other pigments ("porphobilin"). Preliminary experiments showed this to be due to the presence of air, and that this effect was more serious when more dilute solutions of porphobilinogen were used. For this reason all experiments, except where stated otherwise, were carried out in thoroughly deaerated solutions in sealed tubes and with a rather high concentration of porphobilinogen (10^{-2} *M*). The small amount of porphobilinogen available precluded a detailed kinetic study, but preliminary experiments showed this pyrrole to be most stable (at 38 to 60°) in acid or alkaline solution. The rate of condensation increased markedly over the *p*H ranges of 3 to 5 and of 12 to 10. Varying the initial porphobilinogen concentration in neutral solution gave half-lives of Ehrlich color disappearance varying very roughly as the square root of the initial concentration. Uroporphyrinogen does not react with the Ehrlich reagent under the conditions used.

Less than 1% of the recovered uroporphyrin was in the oxidized or porphyrin form at the end of the reactions (Table II). The amount of porphomethene (the di and tetrahydro reduction levels between a porphyrin and a porphyrinogen[10]) could not be measured as accurately due to interference by other colored products, but it was less than 5% of the recovered uroporphyrin in acid and neutral solution, and still less in alkaline solution. The colorless condensation product was identified as a porphyrinogen by the formation of the characteristic porphomethene intermediates during oxidation with iodine, or with oxygen in the presence of light.[10] The immediate formation of the porphyrin bands on adding an equivalent amount of iodine showed that the macrocycle was preformed. When the crude product formed in acid solution was titrated with iodine it was found to contain 6.3 hydrogens.

Most of the color of these solutions at the end of the reaction was attributable to by-products having absorption bands (in 1 *M* HCl) between 460 and 490 mμ. A part of these by-products were in the reduced, colorless state. At least some of these compounds were shown to have dipyrrylmethene-like structures since the bands shifted with *p*H near neutrality, and partly vanished on adding sulfite ion at *p*H 7.[10] Assuming an extinction coefficient of 10^5, these by-products would account for 5% (acid) to 15% (alkaline) of the porphobilinogen added. These products very likely form part of the "porphobilin" mentioned in the early work on porphobilinogen[9] and present in the urine of patients having acute porphyria. As mentioned above, they are the major product when dilute solutions of porphobilinogen are condensed in the presence of air at neutral or alkaline *p*H.

The Condensation in Acid Solution.—The condensation of porphobilinogen in acid solution gives a high yield of uroporphyrinogen which is found, following oxidation and decarboxylation, to contain a ratio of isomers corresponding to that expected for a random mixture: 1/2 III, 1/4 IV, 1/8 I and 1/8 II[3] (Table II, expt. 10). Isomers

TABLE II

UROPORPHYRIN FORMED BY CONDENSING PORPHOBILINOGEN UNDER VARIOUS CONDITIONS

Expt.	Condition[a]	URO % yield	URO % pre-oxidized	Isomer[b] I	Isomer[b] II
10	Acid	78	0.1	1/8	1/8
11	Neutral	55	0.6	1/2	0
12	Neutral + dimedon[c]	10	..	1/4[d]	..
13	*p*H 10.0[e]	70	0.45	5/8	0
14	Alkaline	40	1.1	3/4	0

[a] The concentration of porphobilinogen was 10^{-2} *M*; see Experimental section. [b] Determined as coproporphyrin; error ± 1/8 except expt. 10, ± 1/16. [c] The solution contained 11 moles of dimedon per mole of porphobilinogen. [d] Isomers determined on small amount of uroporphyrin esters only; sum of isomers I + II, error ± 1/4. [e] In a solution of 0.20 *M* sodium carbonate, *p*H 10.0; heated at 60° for 20 hours.

(9) J. Waldenström and B. Vahlquist, *Z. physiol. Chem., Hoppe-Seyler's*, **260**, 189 (1939); P. E. Brockman and C. H. Gray, *Biochem. J.*, **54**, 22 (1953); F. K. Herbert, *ibid.*, **52**, XII (1952).

(10) D. Mauzerall and S. Granick, *J. Biol. Chem.*, **232**, 1141 (1958).

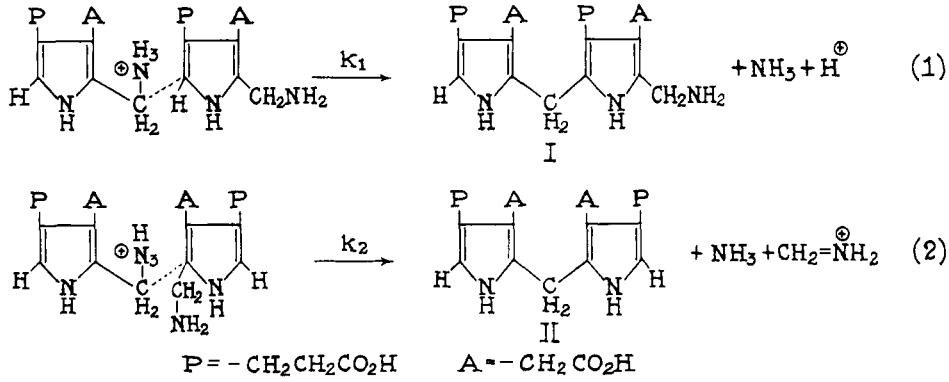


Fig. 1.

III and IV cannot be distinguished at the present time. The ratio of isomers remains the same in the presence of formaldehyde and considerable incorporation of formaldehyde takes place although the yield is strongly reduced (Table III, expt. 15, 16). Since the yield of porphyrin is low under these conditions, the ratio of molar activities calculated on the basis of complete exchange of the formaldehyde and methylene bridges can only be an approximation. The decrease in yield of uroporphyrin is accompanied by an increase in pyrrylmethene-like by-products absorbing at about 480 mμ. When uroporphyrinogen is isomerized under the same conditions, similar results are obtained.[5] The reaction reaches the same equilibrium, whether the starting material is the pyrrole or the cyclized prophyrinogen. Thus, isomer ratios and formaldehyde incorporation cannot be used to explain the mechanism of the condensation of porphobilinogen in acid solution.

TABLE III

UROPORPHYRIN FORMED BY CONDENSING PORPHOBILINOGEN UNDER VARIOUS CONDITIONS IN THE PRESENCE OF FORMALDEHYDE-C^{14}

Expt	Conditions[a]	Molar ratio HCHO/PBG	URO % yield	Isomer I	Isomer II[b]	Ratio of molar activities URO/HCHO Found[c]	Calcd.[d]
15	Acid	0.049	19	0[e]	..	0.22	0.19
16	Acid	.27	33	1/8	1/8	.51	0.85
17	Neutral[f]	.23	20	0[e]	..	.88	1.44
18	Neutral	.25	70	0[e]	..	.95	0.80
19	Neutral	.98	48	1/8	1/8	1.7	1.98
20	Neutral	5.6	12	0[e]	..	1.8	3.4
21	Alkaline	0.28	34	1/8	1/8	0.8	0.88

[a] The concentration of porphobilinogen (PBG) was 10^{-2} *M*; see experimental section for details and Table I for specific activity of the formaldehyde. [b] Determined as coproporphyrin, error $\pm 1/16$. [c] These ratios are accurate to $\pm 10\%$ only. [d] Calculated on the assumption of complete exchange between formaldehyde and the aminomethyl group of porphobilinogen. [e] Sum of isomers I + II; analyzed as uroporphyrin (URO) esters, error $\pm 1/4$. [f] The reaction was stopped after 55% of the porphobilinogen had reacted. The uroporphyrin yield was calculated on the porphobilinogen reacted. The ratio of molar activities of the reisolated porphobilinogen to the added $HC^{14}HO$ was <0.01.

The Condensation in Neutral and Alkaline Solution.—Since uroporphyrinogen neither isomerizes nor incorporates formaldehyde in neutral or alkaline solution,[5] the results of condensing porphobilinogen under these conditions are more easily interpretable in terms of the structure of this pyrrole. The observations are explained by a general mechanism first proposed by Cookson and Rimington[3] (Fig. 1). It will be seen that the data are accommodated with only a slight modification of this scheme.

Reaction 2 is followed by condensation of the formaldehyde (or formaldehyde–ammonia, depending on the *p*H) at the α position of the dipyrrylmethane. In general, the dipyrrylmethanes will not dimerize to porphyrinogens because the porphobilinogen is present in excess during most of the reaction and the amount of intermediates formed during the reaction (20% at *p*H 7.6, 60°) indicates that the self-condensation of porphobilinogen is the slowest step in the sequence. The reaction of dipyrrylmethane with porphobilinogen to form a linear tripyrrylmethane is thus favored. This cannot cyclize due to strain in the ring thus formed. However, further condensation with porphobilinogen produces a linear tetrapyrrylmethane which can cyclize to a strain-less ring. The cyclization is favored because the probability of the ends meeting is very high. The maximum end to end distance is ~18 Å., equivalent to a concentration of ~0.5 *M*. The mean distance, using Kuhn's approximation,[11] is about 9 Å. or about 4 *M* in terms of concentration. The situation is similar to that in polymer initiation *via* biradicals in that both ends of the chain are reactive. Such biradicals are ineffective in initiating polymerization reactions.[12] This is the usual argument of kinetic control over the cyclization and it is supported by the rather high yield of porphyrinogen and by the low reactivity of this macrocycle under these conditions. The lack of dimerization of the dipyrrylmethanes is borne out by the absence of isomer II when porphobilinogen is condensed in neutral or alkaline solution (Table II). It is easily formed by such dimerizations as can be seen by writing out the various possible schemes (*e.g.*, Fig. 3). However, only three times as much uroporphyrin need be formed by linear addition as by dimerization to reduce the content of isomer II below the limit of detection.

As seen in the initial reaction of Fig. 1, the $-CH_2NH_3^+$ group will resist elimination by re-

(11) W. Kuhn, *Kolloid. Z.*, **68**, 2 (1934).

(12) C. Walling, "Free Radicals in Solution," J. Wiley and Sons, Inc., New York, N. Y., 1957, p. 182.

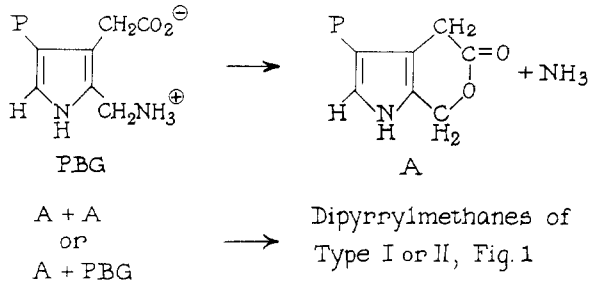


Fig. 2.

action 2, and, conversely, attack by a $-CH_2NH_2$ group will also be slow due to the difficulty in eliminating the NH_2^- ion. Reaction 1 may also be slowed by the electron-withdrawing inductive effect of a $CH_2NH_3^+$ group. We thus expect these reactions to have a maximum rate at the pK of the aminomethyl group of porphobilinogen, *i.e.*, pH 10.[13] This explains the stability of porphobilinogen in alkaline or acid solution, and could account for the break in rate of reaction near pH 11, but does not account for the break near pH 4. The scheme shown in Fig. 2 allows for this change in rate and could explain other observations, such as the effect of porphobilinogen concentration on the rate.

Essentially this is a way of stabilizing the "carbonium" ion formed on loss of ammonia, and would be disfavored by protonating the carboxyl group (pK 3.7).[13]

The isomers formed in neutral and alkaline solution can be explained by a combination of the two reactions of Fig. 1. If k_1 were the only reaction, clearly only isomer I would be formed. If k_2 were the only reaction (followed by rapid condensation of formaldehyde with the polypyrrylmethanes), then equal amounts of isomers III and IV would be formed (Fig. 3). If the formaldehyde (or its equivalent) is also allowed to condense with the remaining porphobilinogen, a much greater number of intermediates are involved, and isomers II and I may form. Approximate calculations, using stepwise reactions, give the order of the ratio of isomers as III > IV > II > I. If both reactions k_1 and k_2 are allowed, the ratio of isomers observed (Table II) can be explained if $k_1 \cong 2 - 4k_2$. The calculations show that the fraction of isomer II would be at or below the limit of detection ($\sim$0.03).

The change in isomer ratio and the incorporation of formaldehyde on adding this compound to the reaction mixture is in accord with this type of elimination mechanism. Previous workers have detected formaldehyde under conditions that caused porphobilinogen to condense to uroporphyrin.[14,15] The condensation of formaldehyde with porphobilinogen is fairly rapid in neutral solution: the second-order rate constant for the disappearance of Ehrlich color in this reaction at pH 7.6 (0.25 M sodium phosphate) and 60° is about 15 M^{-1} min.$^{-1}$. The half-life of a 10^{-2} M solution of both reactants

(13) S. Granick and L. Bogorad, THIS JOURNAL, 75, 3610 (1953).

(14) D. Shemin, C. Russell and T. Abramsky, *J. Biol. Chem.*, 215, 613 (1955).

(15) L. Bogorad and G. Marks, unpublished observations.

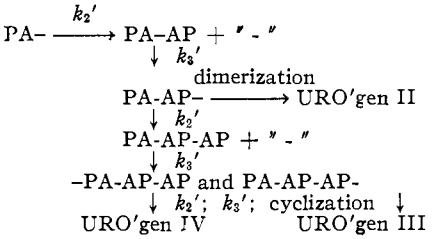


Fig. 3.—Schematic chart for the condensation of PBG to URO'gen III + IV; PA- = PBG or lactone A, Fig. 2; $k_2' = k_2$(PA-) and $k_3' = k_3$(formaldehyde); assumptions: $k_2 \gg k_1$ and $k_3' \gg k_2'$, where k_1 and k_2 refer to Fig. 1.

in thus about 7 minutes, compared to about an hour for the reaction of porphobilinogen with itself.

The incorporation of formaldehyde into the product uroporphyrinogen is not due to simple exchange of the aminomethyl group of porphobilinogen with the added formaldehyde–ammonia, since on re-isolating the porphobilinogen after 55% reaction, it had less than 1% of the possible label, whereas the uroporphyrin formed was already highly labeled (expt. 17).

The formaldehyde condenses with the α-free positions of porphobilinogen or poly-pyrrylmethanes and randomizes the positions adjacent to P or A (Fig. 1). The approximately random mixture of isomers produced on adding one or more moles of formaldehyde per mole of porphobilinogen is thus explained (Tqble III). The fact that 0.25 mole of formaldehyde per mole of porphobilinogen also forms this same mixture argues that the formaldehyde is recycled after being eliminated in a condensation of type 2 (Fig. 1). The high incorporation of formaldehyde also supports the idea of recycling. In addition, the formaldehyde reisolated at the end of a reaction at pH 7.6 was still quite highly labeled (ratio to original activity 0.2), but that isolated after only 55% of the porphobilinogen had reacted was low in activity (ratio 0.06) (Table I, expt. 17 and 18).

The quantitative amount of incorporation of formaldehyde can be estimated by noting that the above schemes require a limit of $4P$ moles of formaldehyde to be incorporated per mole of uroporphyrin where P is the probability of incorporating the labeled formaldehyde residue at each step. If this probability is one-half, *i.e.*, if the aminomethyl group of porphobilinogen and the added formaldehyde–ammonia become equivalent, a limit of two is set, and this is in approximate agreement with observation (Table III, expt. 19 and 20). The ratios calculated on the basis of complete exchange are in error when the condensation is carried out in the presence of a large excess of formaldehyde (expt. 20) and also at the point of 50% reaction of porphobilinogen (expt. 17). At a ratio of 0.25 mole of formaldehyde per mole of porphobilinogen, the incorporation should be greater than 0.5 due to recycling and less than 1.0 due to dilution with unlabeled formaldehyde from the porphobilinogen. Approximate calculations, assuming stepwise reactions, gives values of about 0.8, in rough agreement with observation.

The Effect of Dimedon on the Condensation of Porphobilinogen in Neutral Solution.—In an attempt to trap the formaldehyde produced in a reaction such as 2 in Fig. 1, dimedon was added (11 moles per mole of porphobilinogen) to the usual neutral reaction mixture. The yield of uroporphyrin fell from 55 to 10%, but the isomer ratio was uncertain since only a small amount of material was available (Table II, expt. 12). In addition, there was some Ehrlich-positive material remaining after 21 hours at 60°. Had the dimedon attacked the porphobilinogen directly, removing the aminomethyl group, the resulting pyrrole would have been opsopyrroledicarboxylic acid. This pyrrole was shown to be stable under the reaction conditions: less than 3% decomposed in 20 hours. Although the Ehrlich color band was at the correct position (560 mμ) for this pyrrole, the absorption faded much too rapidly. Moreover, as this 560 mμ band faded, a band at 490 mμ appeared. This suggested that the unknown material was a dipyrrylmethane with an α-hydrogen which reacted normally with the Ehrlich reagent (*p*-dimethylaminobenzaldehyde), then underwent hydrogen transfer to the corresponding dipyrrylmethene. Similar observations have been made on known dipyrrylmethanes by Bogorad and Marks.[15] Paper chromatography also gave evidence of dipyrrylmethenes (oxidation) but not of opsopyrroledicarboxylic acid. This is direct evidence for an elimination reaction of type 2, Fig. 1.

Discussion.—The results presented here support the mechanism shown in Figs. 1 and 2, but further work is needed to provide detailed evidence. Treibs and Fritz[16] have used a similar mechanism to explain the acid-catalyzed "exchange" reactions of pyrroles and dipyrrylmethanes. This type of reaction has many analogies in the phenol–formaldehyde condensations,[17] and is mechanistically related to the Mannich reaction.[18]

(16) A. Treibs and G. Fritz, *Ann. Chem.*, **611**, 162 (1958).

Robinson[19] has proposed a scheme for the enzymatic condensation of porphobilinogen which allows the exclusive formation of isomer III uroporphyrin. Bullock, *et al.*,[20] have elaborated the aspect of intramolecular migration of the "formaldehyde" residue to explain the formation of a coproporphyrin which they claimed to be coproporphyrin III from a suitably substituted α-acetoxymethylpyrrole. The evidence presented in this paper does not strictly exclude this intramolecular migration. A real distinction awaits a method capable of differentiating isomer III from isomer IV.

When a sample of one of the coproporphyrin mixtures (expt. 18) was reduced to coproporphyrinogen and incubated with frozen-thawed *Euglena* cells, it gave a 35% yield of a labeled protoporphryin. Such a porphyrin would be useful in the study of bile pigment formation since labeled carbon monoxide would be evolved.[21] It is possible that the structural specificity of this enzymatic oxidation would allow the determination of the relative labeling of the methine carbons. This would lead to a more detailed understanding of both the chemical and the enzymatic condensation of porphobilinogen.

The high yields of uroporphyrinogen obtainable from porphobilinogen in neutral solution under anerobic conditions are of interest in connection with current views on the evolution of photosynthesis.[22]

Acknowledgment.—I wish to thank Dr. S. Granick for his continual interest and advice, and Mr. W. Cumming for able assistance.

(17) J. F. Walker, "Formaldehyde," second ed., A. C. S. Monograph No. 120, Reinhold Publ. Corp., New York, N. Y., 1953, Chapter 12.
(18) H. Hellmann and G. Opitz, *Angew. Chem.*, **68**, 265 (1956).
(19) R. Robinson, "The Structural Relations of Natural Products," Oxford University Press, New York, N. Y., 1955, p. 24.
(20) E. Bullock, A. W. Johnson, E. Markham and K. B. Shaw, *J. Chem. Soc.*, 1430 (1958).
(21) T. Sjostrand, *Nature*, **168**, 1118 (1951).
(22) S. Granick, *Ann. N. Y. Acad. Sci.*, **69**, 292 (1957).

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Conidine—Synthesis, Polymerization and Derivatives

BY EDWARD R. LAVAGNINO, ROBERT R. CHAUVETTE, WILLIAM N. CANNON AND EDMUND C. KORNFELD

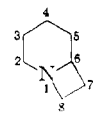
RECEIVED SEPTEMBER 10, 1959

Conidine (IV) has been prepared in a three-step synthesis starting with 2-(β-hydroxyethyl)-pyridine. Treatment of IV with boron trifluoride-etherate or methyl iodide yields a polymer. Several derivatives of IV were prepared and studied. Octahydropyrrocoline was formed by the direct intramolecular cyclic alkylation of 2-(γ-hydroxypropyl)-piperidine at atmospheric pressure; however, attempts to cyclize 2-(β-hydroxyethyl)-piperidine to IV yielded only α-pipecoline.

As an extension of work in the field of polycyclic amines related to quinuclidine, the bicyclic base IV[1] was prepared and studied. This compound has not been reported previously, although substituted derivatives have been synthesized. The trivial name "conidine" was assigned to the ring system[2] when these derivatives were made.

Löffler and co-workers[3] prepared ε-coniceine, which has a methyl group at position 2. This was separated into two isomers termed 2-methyl-

(1) The numbering system is that used by the earlier workers and is used here to avoid confusion. The Ring Index suggested numbering is indicated at the right, and the systematic name is 1-azabicyclo[4.2.0]octane.



(2) K. Löffler and P. Plöcker, *Ber.*, **40**, 1310 (1907). G. R. Clemo and G. R. Ramage [*J. Chem. Soc.*, 2969 (1932)] have attempted without success to prepare conidine by cyclization of ethyl 2-carbethoxypiperidine-1-acetate.
(3) K. Löffler, *Ber.*, **42**, 948 (1909).