

THE OCCURRENCE AND DETERMINATION OF δ -AMINOLEVULINIC ACID AND PORPHOBILINOGEN IN URINE

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A number of recent studies have shown that δ -aminolevulinic acid (δ AL) and porphobilinogen (PBG) are early intermediates in the biosynthesis of porphyrins (1-4). Enzyme studies have also shown that PBG is formed by the condensation of 2 molecules of δ AL (5-7).

The occurrence of δ AL in biological material has not been reported primarily because a method for its detection has not been available. In this paper, a sensitive colorimetric method for the quantitative determination of δ AL is described. The analysis of δ AL in urine is also described. By means of this method, δ AL has been found in urine of a patient with acute porphyria (8) and also in normal urine.

The determination of PBG is based on its reaction with *p*-dimethylaminobenzaldehyde (DMAB) (Ehrlich's reagent) in acid solution to form a red compound. Determinations of PBG in urine are interfered with by urea, various pigments, and oxidizing and reducing agents (9). Various indole derivatives also give color tests with this reagent (10). Urobilinogen does not interfere at the acidities which are used (11). Because of the high concentration of PBG in the urine of patients with acute porphyria, Vahlquist (12) found that a semiquantitative determination could be made if the urine was diluted greatly, thus diminishing the effect of interfering substances. However, no method has been available for determining low or intermediate concentrations of PBG in urine. In this paper, methods are described for the quantitative determination of PBG in urine.

EXPERIMENTAL

Outline of Method—Urine containing PBG and δ AL is passed through a column of Dowex 2 resin in the acetate form. PBG is held on the column (13) while urea and δ AL are washed from the Dowex 2. PBG is then eluted from the column with acetic acid and determined colorimetrically with Ehrlich's reagent.

The washings from the first column are passed through a column of Dowex 50 resin in the acid form. Urea is washed from the column, the δ AL is then eluted with sodium acetate and allowed to react with acetyl-

acetone at pH 4.6, and the resulting pyrrole is determined colorimetrically with a modified Ehrlich's reagent.

Colorimetric Determinations and Reagents—Colorimetric measurements were made at room temperature ($23^{\circ} \pm 2^{\circ}$) with a Beckman DU spectrophotometer in cells of 1 cm. light path. Molar extinction coefficient $\epsilon = \log_{10} (I_0/I) \times 1/(\text{cm.} \times \text{mole per liter})$. Regular Ehrlich's reagent is the usual 2 per cent (weight per volume) DMAB in 6 N HCl. Modified Ehrlich's reagent is as follows: For the reagent 2 N with respect to perchloric acid, 1 gm. of DMAB is dissolved in about 30 ml. of glacial acetic acid, 8.0 ml. of 70 per cent perchloric acid are added, and the solution is diluted to 50.0 ml. with acetic acid. This reagent is somewhat unstable and should be used on the day it is made. Any remainder is discarded. It is not advisable to prepare a reagent greater than 4 N with respect to perchloric acid because of the possible danger of spontaneous decomposition. The advantage of the reagent containing perchloric acid is that the color which is developed with the pyrrole is both more intense and more stable. On mixing this reagent with an equal volume of a dilute pyrrole solution, a 3 per cent reduction in total volume occurs. This has not been corrected for in calculating apparent molar extinction coefficients. Porphobilinogen was obtained from urine from patients with acute porphyria and purified by the method of Cookson and Rimington (14). δ -Amino-levulinic acid was prepared by a phthalimide synthesis according to the procedure of Dr. J. Dice.¹ Generous samples were also obtained from Parke, Davis, and Company. *p*-Dimethylaminobenzaldehyde was recrystallized from aqueous methanol.

Chromatography—Paper chromatograms were made by the ascending method at room temperature. The upper phases of the following two mixtures were used as solvents: (1) butanol-acetic acid contains 4 volumes of *n*-butanol, 1 volume of glacial acetic acid, and 5 volumes of water. (2) Butanol-ammonia contains equal volumes of *n*-butanol and 1.5 M aqueous ammonia.

Preparation of Resins and Columns—The resins were commercial samples of 200 to 400 mesh. They were placed in water and decanted until the supernatant fluid was clear. The Dowex 2-X8 resin was converted to the acetate form by washing the resin on a column with 3 N sodium acetate until the eluate was chloride-free. It was then washed with water until the eluate was free of sodium acetate. The Dowex 50-X8 resin was converted first to the sodium form by allowing it to stand overnight with 2 N NaOH; it was then washed until neutral and reconverted to the acid form by treating it with about 1 volume of 4 N HCl, then in turn with 6 volumes of 2 N HCl, 1 N HCl, and water. The columns were 0.7×30 cm. tubes with indentations at a level 10 cm. from the lower end. This lower end

¹ Personal communication.

was made water-repellent with Beckman Desicote. A glass wool plug was placed above the indentations and a slurry of resin sufficient to give 2.0 ± 0.1 cm. of settled material was added. A glass wool plug on the top completed the columns. The flow rate of the completed columns was about 3 ml. per 10 minutes.

Buffers—Acetate buffer of pH 4.6 was made by adding 57 ml. of glacial acetic acid (1 mole) to 136 gm. of sodium acetate trihydrate (1 mole) and diluting to 1 liter.

Phosphate buffer of pH 6.8 was made by mixing equal volumes of 0.5 M NaH_2PO_4 and 0.5 M Na_2HPO_4 .

Colorimetric Determination of PBG—A solution containing PBG was diluted with an equal volume of regular Ehrlich's reagent. The density of the colored solution was read at $555 \text{ m}\mu$ exactly 5 minutes after mixing, since the color fades with time. Equal volumes of Ehrlich's reagent and water were used in the blank cell. A plot of this optical density *versus* concentration of PBG is given in Fig. 1, Curve B. The relation is approximately linear below a density of 0.2, at which the apparent molar extinction coefficient is 3.6×10^4 .

If the modified Ehrlich reagent containing 4 N perchloric acid is mixed with an equal volume of PBG solution, the density readings 5 minutes after mixing are directly proportional to the PBG concentration over the density range 0.1 to 0.7 (Fig. 1, Curve A). The apparent molar extinction coefficient is 6.2×10^4 . If the modified Ehrlich reagent containing 2 N perchloric acid is used, 15 minutes must be allowed for the color to develop, after which time it is stable for about 10 minutes. The apparent molar extinction coefficient in this case is 6.1×10^4 .

Colorimetric Determination of δAL —When δAL is condensed with either acetylacetone or ethyl acetoacetate, a pyrrole is formed which has a free α position and which can react with Ehrlich's reagent to form a colored compound. Since this is a general procedure for amino ketones, an independent method must be used to distinguish δAL from other amino ketones.

The condensation with acetylacetone is carried out at pH 4.6 and is described in the section on the determination of δAL in urine. This method is less sensitive than the ethyl acetoacetate procedure to color interference with high concentrations of amino acids, ammonia, or glucosamine. At this pH a concentration of about 0.3 mg. per ml. of glucosamine or 2 mg. per ml. of ammonia or 2 mg. per ml. of glycine in the sample is required to give an optical density of 0.01. A plot of the optical density *versus* concentration of δAL determined by this method is given in Fig. 2, Curve B. The curve for density *versus* concentration of the purified pyrrole is also given (Curve A). The condensation is seen to be about 95 per cent complete.

The condensation with ethyl acetoacetate is carried out at pH 6.8. Into

a 10 ml. volumetric flask containing δ AL is added 0.2 ml. of ethyl acetoacetate; the solution is brought to the mark with phosphate buffer at pH 6.8, stoppered, and heated in a boiling water bath for 10 minutes. When cooled, an aliquot is mixed with an equal volume of the modified Ehrlich's reagent containing 2 N perchloric acid. The density is read at 553 $m\mu$ 5 minutes after mixing. The density *versus* concentration is linear between 0.05 and 0.6 and corresponds to an apparent molar extinction of 7.2×10^4 . The reaction is quantitative to within the experimental error.

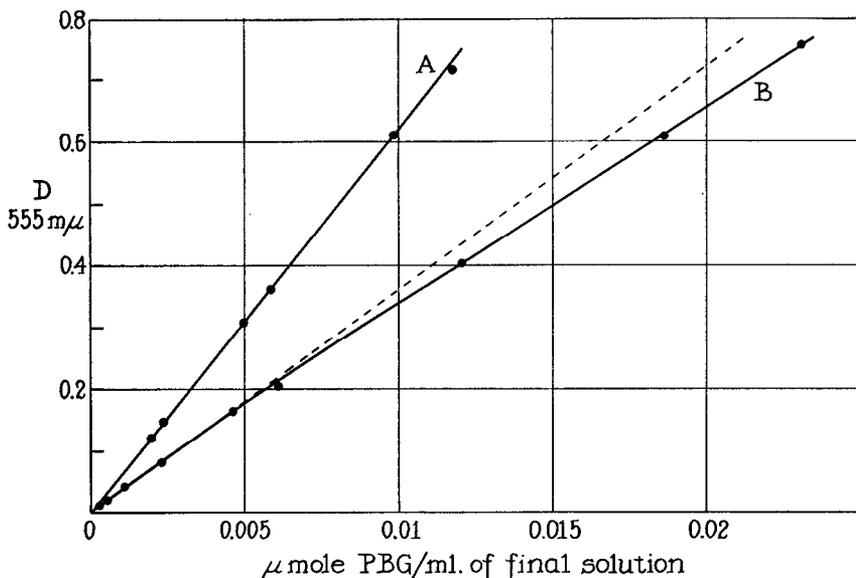


FIG. 1. Curve A, PBG solution plus equal volume of modified Ehrlich's reagent, 4 N in HClO_4 , read at 553 $m\mu$ 5 minutes after mixing. Curve B, PBG solution plus equal volume of regular Ehrlich's reagent, read at 555 $m\mu$ 5 minutes after mixing. Deviation from a straight line is seen by comparison with the straight dash line.

Under these conditions the color formed is stable for at least 30 minutes after mixing.

Determination of PBG and δ AL in Urine—Adsorption and elution from Dowex resins are used to remove interfering substances. For example, mesobilirubinogen is adsorbed on the Dowex 2, but is not eluted with the PBG. The recovery of PBG from Dowex 2 is quantitative within experimental error (less than 5 per cent). The recovery of δ AL from Dowex 50 is 90 ± 2 per cent (see Table I).

A 1.0 ml. aliquot of urine at pH 5 to 7 is placed on the column of Dowex 2. Two 2 ml. portions of water are then added. The combined eluate is quantitatively transferred to the Dowex 50 column. The PBG is eluted from the Dowex 2 column by adding 2 ml. of 1 M acetic acid, allowing it to

drain, and then adding 2 ml. of 0.2 M acetic acid. The combined eluates are quantitatively transferred to a 10 ml. volumetric flask and diluted to the mark with water. 2 ml. of regular Ehrlich's reagent are added to a 2.0 ml. aliquot of the PBG solution. The mixture is placed in a cuvette of 1

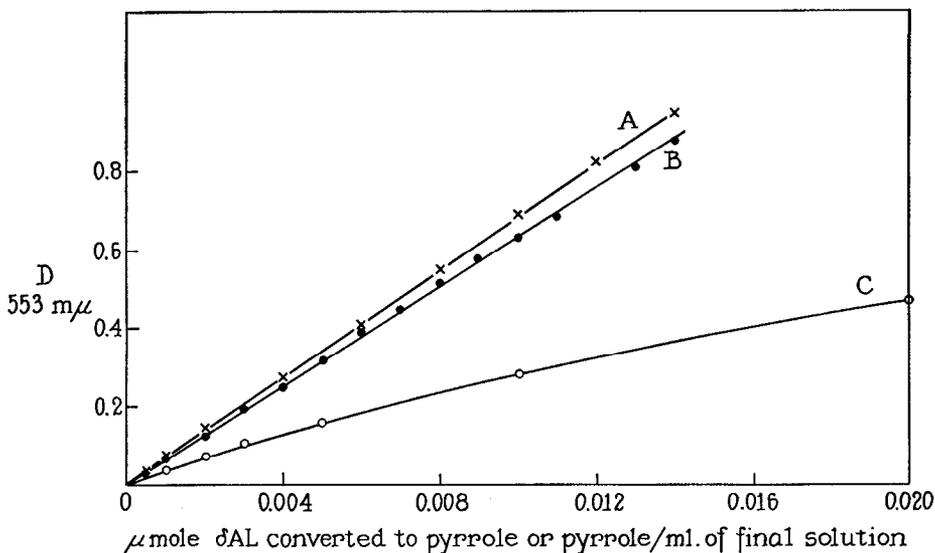


FIG. 2. Curve A, 2-methyl-3-acetyl-4-(3-propionic acid)pyrrole; Curve B, δAL converted to same pyrrole. The pyrrole solution is treated with an equal volume of modified Ehrlich's reagent, 2 N in HClO₄, read at 553 mμ 15 minutes after mixing. Curve C, above pyrrole plus equal volume of regular Ehrlich's reagent, read at 555 mμ 5 minutes after mixing.

TABLE I
Determination of PBG and δAL in Urine

The specified amounts of PBG and δAL were added to 1 ml. of normal urine and the mixture was analyzed.

Experiment No.	PBG μmoles × 10 ³		δAL μmoles × 10 ³	
	Added	Found*	Added	Found†
1	46	42	32	29
2	93	93	60	53
3	278	270	140	128
4	371	365	200	185
5	464	445	260	235

* Corrected for the presence of 4×10^{-3} μmole of PBG in this 1 ml. sample of urine. See under "Identification of PBG (a) and δAL (b) in urine."

† Corrected for the presence of 18×10^{-3} μmole of δAL in this 1 ml. sample of urine. See under "Identification of PBG (a) and δAL (b) in urine."

cm. path and the optical density at 555 $m\mu$ is determined 5 minutes after mixing. A solution of equal volumes of Ehrlich's reagent and water is used as a blank. The concentration of PBG corresponding to the observed optical density is read from the graph (Fig. 1). Multiplication by 20 gives the value in micromoles of PBG per ml. of urine; the limit of detection (optical density reading of 0.01) is about 0.006 μ mole of PBG per ml. If the modified Ehrlich reagent containing 4 N HClO_4 is used, the optical density at 553 $m\mu$ is determined 5 minutes after mixing. In this case, multiplication of the observed optical density by 0.32 gives the value of PBG in micromoles per ml. of urine. If the modified reagent 2 N in HClO_4 is employed, the reading is taken 15 minutes after mixing and the factor is 0.33. The limit of detection is then about 0.003 μ mole of PBG per ml.

The Dowex 50 column, containing δ AL and urea, is washed with 16 ml. of water to remove the urea. (Removal of urea may be followed on a drop plate by testing the eluate with Ehrlich's reagent which gives a yellow color with urea.) Then 3 ml. of 0.5 M sodium acetate are added. (The color of the resin should become lighter three-fourths of the way down the resin but not all the way down.) After draining, the δ AL is eluted by the addition to the column of 7 ml. of 0.5 M sodium acetate. This eluate is collected in a 10 ml. volumetric flask, 0.2 ml. of acetylacetone is added, and the solution is diluted to the mark with acetate buffer at pH 4.6. The stoppered flask is placed in boiling water for 10 minutes, then cooled to room temperature. To 2.0 ml. of this solution are added 2.0 ml. of the modified Ehrlich reagent containing 2 N perchloric acid; the solution is mixed and is placed in a cuvette of 1 cm. length. After 15 minutes the optical density at 553 $m\mu$ is read against a blank. The blank consists of water treated in the same manner as the Dowex 50 eluate. Multiplication of this optical density by 0.35 gives the value of δ AL in micromoles of δ AL per ml. of urine, or multiplication of the optical density by 47 gives the value of δ AL in micrograms per ml. of urine. Under these conditions the color produced is stable for at least 15 minutes after reaching its maximal intensity (about 10 to 15 minutes after mixing). The limit of detection of δ AL (optical density reading of 0.01) is about 0.003 μ mole per ml. On the basis of this method the daily excretion of PBG in the normal urine of ten adults was below 4 μ moles (1 mg.) and that of δ AL was equivalent to about 20 μ moles (2.5 mg.). However, see the paragraph on the identification of PBG and δ AL. A patient with acute porphyria was found to excrete 260 to 410 μ moles (35 to 55 mg.) of δ AL and 310 to 440 μ moles (70 to 100 mg.) of PBG per day for a period of 1 month. Single daily samples from another patient contained as much as 180 mg. of δ AL and 170 mg. of PBG.

Rapid Method for PBG Determination in Urine—As found by Vahlquist

(12), urine containing a high concentration of PBG can be diluted sufficiently so that interference by urea and by other constituents may be negligible. To check the validity of this rapid determination of PBG in urine from a patient with acute porphyria, this procedure was compared with the resin method described above. The results are given in Table II. Values obtained with the rapid method average about 5 per cent higher than those obtained with the resin method but show considerable scatter.

The rapid method is as follows: 0.1 ml. of urine from a patient with acute porphyria is pipetted into a 10 ml. volumetric flask. This is diluted to 5.0 ml. and 5 ml. of regular Ehrlich's reagent are added. The optical

TABLE II
*Comparison of Direct Method and Resin Method for PBG Determination
in Urine of Patients with Acute Porphyria*

Sample No.	PBG, mg. per day	
	Direct method	Resin method
1	90	90
2	90	88
3	89	78
4	78	76
5	98	88
6	108	99
7	96	82
8	87	80
9	93	84
10	87	93

density at $555\text{ m}\mu$ in a cell of 1 cm. path is read exactly 5 minutes after the mixing. (The optical density was usually below 0.15 even for urines rich in PBG.) The density $\times 2.8 =$ micromoles of PBG per ml. of urine, or the density $\times 630 =$ micrograms per ml. of urine.

Identification of PBG (a) and δAL (b) in Urine—(a) When urine from a patient with acute porphyria is adsorbed on Dowex 2, the component eluted from the resin with 1 M acetic acid has the same R_F value (0.5) on paper chromatography with butanol-acetic acid as does PBG. This component and PBG migrate similarly on paper electrophoresis at pH 5.0. The Ehrlich reaction product of both this component and PBG have a maximal absorption density at $555\text{ m}\mu$ and a shoulder at $525\text{ m}\mu$, the ratio of the latter to the former being 0.85. On a large scale run, crystalline PBG was isolated from this Dowex 2 eluate. No other Ehrlich positive component was observed in the eluate. With larger samples of normal

urine the maximal concentration of PBG as estimated by the Ehrlich color reaction is found to be no more than 0.8 μ mole per liter of urine. (b) With the urine from a patient with acute porphyria, the component in the Dowex 50 eluate has the same R_F value (0.3) on paper chromatography in butanol-acetic acid as does the eluate plus added δ AL. Owing to the presence of amino acids, this R_F value is lower than that of pure δ AL (0.35). Concentrated eluates are qualitatively transformed to PBG by the δ AL-condensing enzyme of Granick (5). The δ AL in the eluates of a large scale run was converted to 2-methyl-3-acetyl-4-(3-propionic acid)pyrrole by the method given in the section on δ AL analysis in urine. This compound was purified by vacuum sublimation; m.p. 193–195°, mixed m.p. 192–194°. Calculated, N 7.18 per cent; found, N 7.37 per cent. Both this product and the known pyrrole have the same R_F values in butanol-acetic acid (0.9) and in butanol-ammonia (0.2). The ultraviolet spectra and the Ehrlich reaction product spectra of this compound and of the known pyrrole are quantitatively identical. Their infra-red spectra, determined when they were imbedded in KBr, are also identical. KBr is transparent in the 2 to 15 $m\mu$ region. By means of paper electrophoresis of the urine samples and of paper chromatography of the pyrrole resulting from reaction with acetylacetone, no other component related to δ AL, such as amino acetone, was found in any relatively significant amount. With similar chromatographic and enzymic methods, δ AL has been qualitatively identified in normal urine. Further studies on larger samples of normal urine have been carried out by condensing the δ AL fraction of the Dowex 50 eluates with acetylacetone at pH 4.6. The resulting Ehrlich positive products were separated by extraction with ether at various pH values and chromatographed on paper with butanol-ammonia. Only 10 to 20 per cent of these products is the pyrrole corresponding to free δ AL (R_F of 0.2). Another 20 to 40 per cent behaves as a pyrrole corresponding to an amino ketone without a free carboxyl group (e.g. amino acetone, R_F of 0.9). The remainder is organic solvent-insoluble material, such as might be formed from glucosamine.

Preparation of 2-Methyl-3-acetyl-4-(3-propionic acid)pyrrole—A solution of 174 mg. of δ AL-HCl and 0.5 ml. of acetylacetone in 50 ml. of buffer (0.1 M disodium tartrate adjusted to pH 4.4 with hydrochloric acid) was heated under reflux. The reaction was complete after 15 minutes. The pH of the cooled solution was increased to 7, the excess acetylacetone extracted with chloroform, the aqueous layer aerated to remove the chloroform, and the pH lowered to about 1. After cooling, white crystals formed. They were recrystallized from methanol-water and dried over P_2O_5 at 2 mm., yielding 90 mg. of the pyrrole; m.p. 194–195°. The compound may be further purified by vacuum sublimation. Calculated for $C_{10}H_{13}NO_3$,

C 61.5 per cent, H 6.72 per cent, N 7.18 per cent; found, C 61.74 per cent, H 6.67 per cent, N 7.26 per cent. The ultraviolet spectrum of this compound showed a maximum at $253\text{ m}\mu$, $\epsilon = 9.0 \times 10^3$, a shoulder at 280 to $285\text{ m}\mu$, $\epsilon = 4.8 \times 10^3$, and a minimum at $224\text{ m}\mu$, $\epsilon = 2.4 \times 10^3$. This spectrum is closely similar to that of 2,4-dimethyl-3-acetylpyrrole (15). The R_F is 0.90 in butanol-acetic acid and 0.20 in butanol-ammonia. Reaction of this pyrrole with the modified Ehrlich reagent (2 N in HClO_4) gave a pink solution showing a maximum at $552\text{ m}\mu$, with a shoulder at $525\text{ m}\mu$, the ratio of the latter to the former being 0.69. The quantitative data of this Ehrlich reaction are shown in Fig. 2, Curve A, the apparent extinction coefficient being 6.8×10^4 .

Preparation of 2-Methyl-3-carbethoxy-4-(3-propionic acid)pyrrole—A solution of 172 mg. of δ AL-HCl and 1.0 ml. of ethyl acetoacetate in 50 ml. of buffer (0.25 M phosphate at pH 6.6) was refluxed for 15 minutes. The pyrrole was isolated in a manner similar to that employed for 3-acetylpyrrole, yielding 101 mg. of white crystals; m.p. $164\text{--}165^\circ$. Calculated, for $\text{C}_{11}\text{H}_{15}\text{NO}_4$, C 58.6 per cent, H 6.74 per cent, N 6.22 per cent; found, C 58.66 per cent, H 6.54 per cent, N 6.26 per cent. The ultraviolet spectrum of this compound showed a maximum at $233\text{ m}\mu$, $\epsilon = 8.2 \times 10^3$, and a shoulder at 250 to $260\text{ m}\mu$, $\epsilon = 5.0 \times 10^3$. The spectrum is closely similar to that of 2,4-dimethyl-3-carbethoxypyrrole (15). The R_F is 0.95 in butanol-acetic acid and 0.40 in butanol-ammonia. Reaction of this pyrrole with the modified Ehrlich reagent (4 N in HClO_4) gave a pink solution showing a maximum at $552\text{ m}\mu$, with a shoulder at about $525\text{ m}\mu$, the ratio of the latter to the former being 0.66. The apparent extinction coefficient is 7.2×10^4 .

Factors Influencing Ehrlich Reaction—In the usual colorimetric procedure of the Ehrlich reaction a pyrrole, P (which usually has an unsubstituted α position), condenses in acid solution with DMAB (present in large excess) to form a colored condensation product, E (Reaction I) (see Fig. 3). The intensity of color is observed to increase rapidly and to diminish more slowly. Treibs and Herrmann (16) have published a systematic study of the Ehrlich reaction and have shown that the color salt E can further react with another molecule of P to form a colorless dipyrrolylphenylmethane, M (Reaction II). Under the usual conditions of acidity (about 3 M HCl) and excess DMAB, Reaction I is observed to be very fast compared to Reaction II; the initial rate of decay of E should then be second order. When dilute solutions (5×10^{-5} M) of either PBG or 2-methyl-3-acetyl-4-(3-propionic acid)pyrrole were mixed with an equal volume of regular Ehrlich's reagent, the initial rate of decay of the observed color, assuming Beer's law to hold, was found to follow second order kinetics. These results support the assumption of the two consecutive reactions as

written. They also explain in part the rapid flattening of the curve of optical density *versus* pyrrole concentration (Fig. 2, Curve C) when the regular Ehrlich reagent is used.

Some of the factors which influence the concentration of *E* in the steady state are the substituents in the pyrrole nucleus, the concentration of acid, and the solvent medium. These factors are discussed in turn. The temperature is assumed constant at about 20°.

Substituents in the pyrrole nucleus will affect the formation of the color salt through both resonance and inductive effects. In general electron-donating groups (*e.g.* alkyl) favor the formation of the color salt while electron withdrawal groups (*e.g.* acetyl, carbethoxy, nitro) have the opposite effect (16). These results are in agreement with the expected stabili-

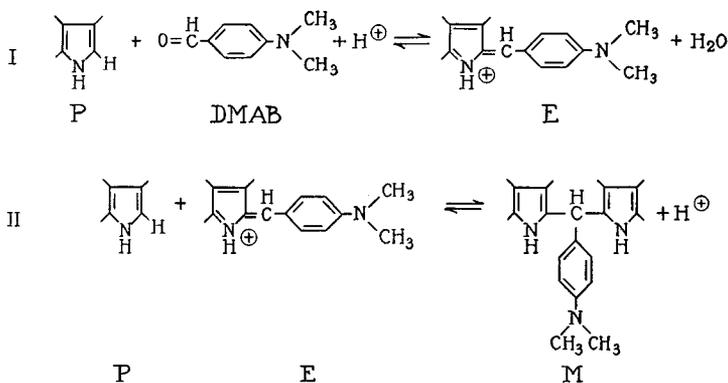


FIG. 3. Steps for Reactions I and II

zation of the pyrrole structure in *P* or *M* by electron withdrawal groups (17). More specifically, pyrroles which have α' , β , β' substituents will form *E* most readily when the three substituents are alkyl groups and less readily when two are alkyl and one is an electron withdrawal group. Two electron withdrawal groups in the α' , β positions (*e.g.* α' -carbethoxy- β -nitropyrrole (16) and α' , β -dicarbethoxy- β' -methylpyrrole) will deactivate the α position sufficiently to prevent the Ehrlich reaction. A free β position reacts similarly to a free α position, although more slowly and with a shift of the main absorption band of the colored product to about 520 m μ in the example available: α , β' -dimethyl- α' -carbethoxypyrrole. The slower rate in this example may be due to steric effects. Pyrroles with only one or two substituents, especially if they are alkyl groups, may be expected to undergo increasingly severe decomposition in acid. However, β -methyl- β' -carbethoxypyrrole reacted normally, owing to stabilization by the carbethoxy group. α , α' , β , β' -Tetramethylpyrrole reacts with Ehrlich's reagent

by splitting out methanol (16). Treibs and Herrmann use this splitting reaction to explain the positive Ehrlich reaction of urobilinogen and stereobilinogen.

The choice of acid concentration is governed by the rates of Reactions I and II and by the position of the numerous acid-base equilibria involved. At very low acid concentrations, E forms too slowly. At high acid concentrations, E forms rapidly, but the rate of fading becomes significant. Furthermore, the observed intensity of E at high acid concentrations decreases, owing to the formation of the relatively colorless diprotonated color salt. For pyrroles with single electron withdrawal groups an acid concentration of about 1 M was found to be optimal for readings within 15 minutes. Owing to the electron-donating groups, purely alkyl pyrroles (*e.g.* cryptopyrrole) are more basic. They therefore not only react with DMAB more readily, but also the color salt E picks up a 2nd proton more easily. A lower acid concentration is thus required. Alternatively, the condensation may be carried out in 1 to 2 M acid, followed by the addition of sodium acetate to free the color salt E .

The solvent will also influence the steady state of E . A change in solvent will affect the measured value of E by changing both the rates of Reactions I and II and the relative base strengths of all the species involved. The use of 50 per cent (volume per volume) acetic acid in place of water is observed to slow the rates of Reactions I and II and to increase the intensity of E . The intensity of the color is somewhat greater when perchloric rather than hydrochloric acid is used in this solvent. With increasing concentration of acetic acid, this increase in E is found to pass through a maximum at about 70 per cent (volume per volume) acetic acid and so can be due only in part to the lowering of the water concentration. This maximum may be due to an increase in relative base strength of E . The shifting of Reaction I to the right would cause an increase in E , while the formation of the diprotonated form of E would decrease the observed value of E .

An analysis of these factors in a qualitative manner has permitted the selection of conditions such that the apparent molar extinction coefficients of the Ehrlich color salts are about 60,000 as compared to 30,000 with the regular Ehrlich reagent. The stability of the Ehrlich color salt is increased so that the relation between concentration of pyrrole and color production is linear (compare Curve A with Curve B of Fig. 1 and Curve B with Curve C of Fig. 2).

SUMMARY

1. Methods including the use of ion exchange resins have been developed for the quantitative determination of porphobilinogen (PBG) and δ -amino-

levulinic acid (δ -AL) in urine. PBG is determined colorimetrically with Ehrlich's reagent. δ AL is determined by condensing it with acetylacetone to form a pyrrole which can react with a modified Ehrlich reagent.

2. The error of the method is less than 5 per cent and the limit of detection (optical density of 0.01 for a 1 cm. light path) in a 1 ml. sample of urine is 1 γ of PBG and 0.5 γ of δ AL.

3. δ AL has been found in high concentration in the urine of patients with acute porphyria and has also been detected in normal urine.

4. Some of the factors influencing the selection of optimal conditions for the Ehrlich reaction are discussed.

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