COMMUNICATIONS TO THE EDITOR

PORPHOBILINOGEN A MONOPYRROLE

Sir:

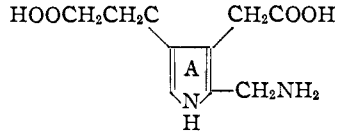
Porphobilinogen, a compound which is excreted in the urine of patients with acute prophyria, is of interest because it may represent one of the early precursor steps in the biosynthetic chain of the porphyrins. Two recent notes by Cookson and Rimington[1] and by Kennard[2] on the structure of this compound have prompted us to report on some of our own current studies.

Porphobilinogen was isolated by the method of Westall.[3] Because of its lability and solubility properties the customary methods cannot be used for the determination of its molecular weight. To obtain evidence whether the compound is a monopyrrole or a dipyrrylmethane[4] the iodo derivative of porphobilinogen was prepared. Twenty mg. of crystalline porphobilinogen was suspended in 1.0 cc. of 1 *M* acetate buffer of *p*H 4.6 and 0.35 cc. of 0.50 *N* iodine in aqueous potassium iodide solution was added dropwise over a 10-minute period at room temperature. The reaction is quantitative, the disappearance of iodine color indicating that one molecule of iodine dissappears per pyrrole. (The *p*H at which the reaction is run is important; at a lower *p*H the reaction is too slow. At a *p*H of 7 further reactions occur so that no crystalline product has been isolated.) The faintly yellowish needle shaped crystals obtained in this reaction in a yield of 66% were recrystallized by dissolving them in 0.8 cc. of 0.3 *N* HCl, filtering and adding 3 *M* sodium acetate to *p*H 4. On the basis of a monoiodo-porphobilinogen of the composition $C_{10}H_{13}O_4N_2I$ the calculated C = 34.00, I = 36.0. Found: C = 34.19, I = 34.0. For a dipyrrylmethane the calculated iodine would be 21.9%. The percentage iodine which was found rules out the possibility of a dipyrrylmethane structure. The somewhat low iodine value may have been due to a loss of iodine from the pyrrole when the iodo-porphobilinogen was dissolved in acid.

Studies of the reaction of iodine with other monopyrroles having a free α position indicate that porphobilinogen is more reactive with aq. KI_3 at *p*H 4 than are the other pyrroles. Pyrroles substituted in both α,α' and β,β' positions, *i.e.*, tetra-substituted pyrroles, do not react. The absorption maximum of porphobilinogen as measured in a Cary Spectrophotometer is 212 mμ with ϵ6770 indicating that no resonating groups are attached directly to the pyrrole ring. The iodo-porphobilinogen has a maximum at 230 mμ with ϵ10,200. On paper chromatography, the iodo-porphobilinogen formed only one spot with an R_f of 0.71 as compared to an R_f of 0.56 for porphobilinogen itself. The solvent system used for the paper chromatography was the upper phase of a mixture of 4 parts *n*-butanol: 1 part glacial acetic: 5 parts water.

Titration of 0.01 *M* porphobilinogen solution reveals three ionizable groups with pK' 3.70, 4.95 and 10.1 and an isoelectric point pI' of 4.3. To account for the pK' of 3.70 it is necessary to assume that one of the $-COO^-$ groups is in the neighborhood of an $-NH_3^+$. The pK' of 4.95 would represent the ionization constant of the other carboxyl group and pK' 10.1 that of the amino group.

These data support the suggestion of Cookson and Rimington[1] that porphobilinogen is a monopyrrole. The pK' value of 3.7 is low, although possibly compatible with structure A which they propose.



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ENZYMATIC SYNTHESIS OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE RIBOSIDE FROM 4-AMINO-5-IMIDAZOLECARBOXAMIDE AND RIBOSIDE-1-PHOSPHATE[1]

Sirs:

Mammalian purine nucleoside phosphorylase has been shown to catalyze the synthesis of inosine,[2] guanosine,[2] xanthosine,[3] 8-azaguanine riboside[4] and nicotinamide riboside[5] from their respective bases and ribose-1-phosphate. A similar synthesis of 4-amino-5-imidazolecarboxamide riboside has now been demonstrated. The incubation mixture contained: 4-amino-5-imidazolecarboxamide (0.5 μM), riboside-1-phosphate (1.0 μM of the crystalline cyclohexylamine salt), purified beef liver nucleoside phosphorylase[6,7] (0.25 mg.) and glycylglycine buffer (*p*H 8, 0.05 *M*) in a total volume of 0.5 ml. The incubation was carried out at 38° for 30 minutes and the enzymatic reaction stopped by placing the vessel in a boiling water-bath. As expected inorganic phosphate was liberated during the course of the reaction. The mixture was then chromatographed on paper for 12 hours in a

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