

---

 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<sup>1</sup> and by Kennard<sup>2</sup> on the structure of this compound have prompted us to report on some of our own current studies.

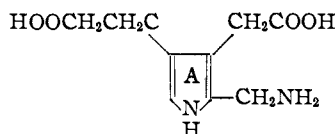
Porphobilinogen was isolated by the method of Westall.<sup>3</sup> Because of its lability and solubility properties the customary methods cannot be used for the determination of its molecular weight. To obtain evidence whether the compound is a monopyrrole or a dipyrromethane<sup>4</sup> the iodo derivative of porphobilinogen was prepared. Twenty mg. of crystalline porphobilinogen was suspended in 1.0 cc. of 1 *M* acetate buffer of *pH* 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 disappears per pyrrole. (The *pH* at which the reaction is run is important; at a lower *pH* the reaction is too slow. At a *pH* 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 *pH* 4. On the basis of a mono-iodo-porphobilinogen of the composition C<sub>10</sub>H<sub>13</sub>O<sub>4</sub>N<sub>2</sub>I the calculated C = 34.00, I = 36.0. Found: C = 34.19, I = 34.0. For a dipyrromethane the calculated iodine would be 21.9%. The percentage iodine which was found rules out the possibility of a dipyrromethane 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  $\alpha$  position indicate that porphobilinogen is more reactive with aq. KI<sub>3</sub> at *pH* 4 than are the other pyrroles. Pyrroles substituted in both  $\alpha, \alpha'$  and  $\beta, \beta'$  positions, *i.e.*, tetra-substituted pyrroles, do not react. The absorption maximum of porphobilinogen as measured in a Cary Spectrophotometer is 212 *m* $\mu$  with  $\epsilon$ 6770 indicating that no resonating groups are attached directly to the pyrrole ring. The iodo-porphobilinogen has a maximum at 230 *m* $\mu$  with  $\epsilon$ 10,200. On paper chromatography, the iodo-porphobilinogen formed only one spot with an *R<sub>f</sub>* of 0.71 as compared to an *R<sub>f</sub>* 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<sup>-</sup> groups is in the neighborhood of an -NH<sub>3</sub><sup>+</sup>. 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<sup>1</sup> that porphobilinogen is a monopyrrole. The *pK'* value of 3.7 is low, although possibly compatible with structure A which they propose.



ROCKEFELLER INSTITUTE  
FOR MEDICAL RESEARCH  
NEW YORK 21, N. Y.

S. GRANICK  
L. BOGORAD

RECEIVED JUNE 4, 1953

---

 ENZYMIC SYNTHESIS OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE RIBOSIDE FROM 4-AMINO-5-IMIDAZOLECARBOXAMIDE AND RIBOSIDE-1-PHOSPHATE<sup>1</sup>

Sirs:

Mammalian purine nucleoside phosphorylase has been shown to catalyze the synthesis of inosine,<sup>2</sup> guanosine,<sup>2</sup> xanthosine,<sup>3</sup> 8-azaguanine riboside<sup>4</sup> and nicotinamide riboside<sup>5</sup> 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  $\mu$ M), riboside-1-phosphate (1.0  $\mu$ M of the crystalline cyclohexylamine salt), purified beef liver nucleoside phosphorylase<sup>6,7</sup> (0.25 mg.) and glycylglycine buffer (*pH* 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

(1) Supported by grants from National Cancer Institute, National Institutes of Health, United States Public Health Service, and the Damon Runyon Memorial Fund for Cancer Research, Inc. One investigator (E. D. K.) is a research fellow of the Damon Runyon Memorial Fund.

(2) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(3) M. Friedkin, *THIS JOURNAL*, **74**, 112 (1952).

(4) M. Friedkin, *Federation Proc.*, **11**, 216 (1952).

(5) J. W. Rowen and A. Kornberg, *J. Biol. Chem.*, **198**, 497 (1951).

(6) J. M. Buchanan in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Baltimore, Md., 1952, Vol. II, **2**, p. 406.

(7) E. D. Korn and J. M. Buchanan, *Federation Proc.*, **12**, 233 (1953).

(1) G. H. Cookson and C. Rimington, *Nature*, **171**, 875 (1953).

(2) O. Kennard, *Nature*, **171**, 876 (1953).

(3) R. G. Westall, *Nature*, **170**, 614 (1952).

(4) J. Waldenström and B. Vahlquist, *Z. physiol. Chem.*, **260**, 189 (1939).

solvent containing 80% *n*-propanol and 20% water. Two bands were observed under an ultraviolet lamp. Their *R<sub>f</sub>*'s, 0.54 and 0.39, correspond with those of 4-amino-5-imidazolecarboxamide and its riboside, respectively.<sup>8</sup> Ribose and ribose phosphate do not migrate from the origin in this solvent. The ultraviolet absorption curve of the riboside had a maximum at 267  $\mu$ . Analysis of the eluted compound by ultraviolet absorption (using  $E_{267}^1 \mu = 1.27 \times 10^4$ ), diazotizable amine,<sup>9</sup> and the orcinol reaction<sup>10</sup> gave a 1:1:1 ratio. Approximately 0.08  $\mu$ M of the riboside was recovered from the paper chromatogram. When either *D*-ribose or ribose-5-phosphate was substituted for ribose-1-phosphate in the incubation medium only the 4-amino-5-imidazolecarboxamide band appeared.

The 4-amino-5-imidazolecarboxamide riboside isolated in these experiments has the same properties as that isolated by Greenberg<sup>8</sup> from a culture of *E. coli* whose growth was inhibited by sulfonamides. The synthesis of 4-amino-5-imidazolecarboxamide deoxyriboside has been demonstrated in suspensions of *E. coli* B,<sup>11</sup> and by a *trans*-N-glycosidase reaction in an enzyme preparation from *L. helveticus*.<sup>12</sup>

Greenberg<sup>13</sup> has reported that 4-amino-5-imidazolecarboxamide riboside may be converted to its ribotide by the action of adenosine triphosphate in the presence of pigeon liver extract. The present experiments, when coupled with those of Greenberg provide analogy for a metabolic pathway for the conversion of the carboxamide to its ribotide and to inosinic acid in pigeon liver, a series of reactions which were previously postulated on the basis of experiments with radioactive carboxamide<sup>14</sup> and experiments with inosinic acid and radioactive formate.<sup>15</sup>

When adenine and ribose-1-phosphate were incubated with the nucleoside phosphorylase, a rapid formation of inosine was noted. The enzyme preparation completely converted adenosine to inosine or to inosine and hypoxanthine in the presence of inorganic phosphate. As the formation of hypoxanthine from adenine (in the absence of ribose-1-phosphate) could not be detected, it seems probable that adenine and ribose-1-phosphate had reacted to form adenosine which was then deaminated to inosine. These results are in contrast to those of Kalckar,<sup>16</sup> who was unable to find any reaction between adenine and ribose-1-phosphate in the presence of his preparation of rat liver nucleoside phosphorylase.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY  
SCHOOL OF MEDICINE EDWARD D. KORN  
UNIVERSITY OF PENNSYLVANIA FRIXOS C. CHARALAMPOUS  
PHILADELPHIA 4, PA. JOHN M. BUCHANAN

RECEIVED JUNE 1, 1953

- (8) G. R. Greenberg, *THIS JOURNAL*, **74**, 6307 (1952).  
(9) J. M. Ravel, R. E. Eakin and W. Shive, *J. Biol. Chem.*, **172**, 67 (1948).  
(10) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).  
(11) Ben Ishai, Bergmann and Volcani, *Nature*, **168**, 1124 (1951).  
(12) MacNutt, *Biochem. J.*, **50**, 384 (1952).  
(13) G. R. Greenberg, *Federation Proc.*, **12**, 211 (1953).  
(14) M. P. Schulman and J. M. Buchanan, *J. Biol. Chem.*, **196**, 513 (1952).  
(15) J. M. Buchanan and M. P. Schulman, *ibid.*, **202**, 241 (1953).  
(16) Kalckar, Harvey Lectures, Series XLV, 11 (1949-1950).

### THE OCCURRENCE OF A NEW FLAVIN DINUCLEOTIDE (FAD-X)

Sir:

During a recent investigation<sup>1,2</sup> concerned with the isolation of flavin-adenine-dinucleotide (FAD) from animal tissues, the existence of a new flavin nucleotide, (FAD-X), not identical with any of the known flavins, was noted. Specifically, when the flavin concentrates were subjected to partition chromatography between phenol-butanol and water, or adsorption chromatography on dicalcium phosphate, FAD-X, FAD and flavin mononucleotide (FMN) were separated. Each of these flavins could be rechromatographed on either column as a single band. The relative amount of FAD-X, compared to FAD, ranged approximately from 5 to 15% for a large number of preparations.

Using paper chromatography, a comparison of FAD-X with other flavins is given in Table I. In many solvent systems FAD-X is indistinguishable from FAD; however, in systems such as 3 and 4, the two are readily separated.

Analysis of FAD-X indicates a flavin:phosphate:adenine ratio of 1:2:1. FAD-X is similar to FAD in several other respects: absorption spectrum, dependence of fluorescence upon  $pH^3$  and the formation of insoluble complexes with heavy metals. It has no coenzyme activity, however, with the *D*-amino acid apo-oxidase.<sup>4</sup>

Enzymatic cleavage of FAD-X with nucleotide pyrophosphatase,<sup>5</sup> kindly supplied by Dr. A. Kornberg, yields a flavin mononucleotide FMN-X and adenosine-5'-phosphate (AMP); upon acid hydrolysis a mixture of FMN and FMN-X are produced. FMN and FMN-X may be separated by paper chromatography, as shown in Table I. As judged by paper chromatography, FMN-X is identical with *cyclic* riboflavin-4',5'-phosphate (cyc-FMN), kindly supplied by Dr. A. R. Todd.<sup>6</sup> Exposed to ammonia, FAD is converted to cyc-

TABLE I

*R<sub>f</sub>* VALUES FOR ASCENDING PAPER CHROMATOGRAMS<sup>a</sup>

Col. (1) 5% Na<sub>2</sub>HPO<sub>4</sub> in H<sub>2</sub>O; (2) 4/1/5 *n*-butanol/acetic acid/water (top phase); (3) 160 g. phenol/30 ml. *n*-butanol/100 ml. water (lower phase); (4) collidine saturated with water.

	1	2	3	4
FAD	0.40	0.05	0.23	0.17
FAD-X	.40	.05	.47	.30
FMN	.54	.10	.17	.04
FMN-X	.54	.13	.50	.15
Rb	.30	.30	.79	.69
Lyxoflavin <sup>b</sup>	.29	.32	.77	.66
Riboflavinyl glucoside <sup>c</sup>	.40	.22	.60	.50
Lumichrome	.07	.68	.88	.72
Lumiflavin	.18	.48	.94	.68

<sup>a</sup> Whatman No. 1 paper. <sup>b</sup> Kindly supplied by Dr. Karl Folkers. <sup>c</sup> Kindly supplied by Dr. L. G. Whitby.

- (1) D. R. Sanadi and F. M. Huenekens, 117th Meeting of the American Chemical Society, Abstracts of Papers, p. 60C (1950).  
(2) E. Dimant, D. R. Sanadi and F. M. Huenekens, *THIS JOURNAL*, **74**, 5440 (1952).  
(3) O. Bessey, O. H. Lowry and R. H. Love, *J. Biol. Chem.*, **180**, 755 (1949).  
(4) H. A. Krebs, *Biochem. J.*, **29**, 1620 (1935).  
(5) A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.*, **182**, 763 (1950).  
(6) H. S. Forrest and A. R. Todd, *J. Chem. Soc.*, 3295 (1950).