also eliminated on the basis of detailed comparison of $F_{calcd.}$ with the lower limit of observation on our films for those reflections for which $h + k + l$ is odd.

TABLE I

SPACING AND INTENSITY DATA FOR H_3NBH_3

hkl	$\sin^2\theta$ obs.	$\sin^2\theta$ calcd.	*F* obs.	*F* calcd.
110	0.0439	0.0434	7.66	7.02
101	unresolved	.0452	3.24	3.79
200	0.0868	.0868	4.56	4.56
002	.0941	.0941	4.18	4.31
121	.1317	.1320	2.34	2.30
112	.1377	.1374	3.67	2.39
220	.1732	.1735	2.57	2.76
202	.1809	.1808	2.72	1.54
130	.2172	.2169	2.73	2.50
301		.2188		2.35
103	.2334	.2333	2.96	3.19
222	.2671	.2676	0.69	1.00
231	.3045	.3055	1.17	1.29
132		.3110		1.16
123	.3198	.3201	1.82	2.37

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SCHOOL OF CHEMISTRY
UNIVERSITY OF MINNESOTA ERNEST L. LIPPERT
MINNEAPOLIS 14, MINNESOTA WILLIAM N. LIPSCOMB

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FORMYLGLYCINAMIDINE RIBOTIDE AND 5-AMINOIMIDAZOLE RIBOTIDE—INTERMEDIATES IN THE BIOSYNTHESIS OF INOSINIC ACID *DE NOVO*[1]

Sir:

In preliminary experiments from this Laboratory,[2] it has been reported that (α-N-formyl)-glycinamide ribotide (FGAR) reacted with glutamine in ethanol-precipitated extracts of pigeon liver to yield a new arylamine ribotide. Upon incubation of this arylamine with aspartic acid, 5-amino-4-imidazolecarboxamide ribotide was formed. We wish to report further studies upon the characterization of the enzymatic systems involved in the above process, as well as the identification by chemical analysis of the arylamine as 5-aminoimidazole ribotide (AIR). Furthermore, by fractionation of the enzymatic components, a second compound, which is an intermediate in the conversion of FGAR to AIR, has been isolated, purified and identified as (α-N-formyl)-glycinamidine ribotide (FGAM).

The enzymatic system responsible for the conversion of FGAR to AIR was found in a fraction of pigeon liver extract precipitating between 13 and 33% ethanol concentration. Reaction took place only upon the addition of glutamine and adenosine triphosphate (ATP). The conversion was conveniently followed by measurement of the formation of a chromophore with an absorption maximum at 500 mμ when the arylamine was treated with the Bratton-Marshall reagents.[3]

AIR behaves as a typical mononucleotide in regard to its properties on ion exchange resins. It has been isolated from deproteinized incubation mixtures by chromatography on Dowex 1 acetate with 0.04 *M* ammonium acetate buffer, *p*H 5.3. The compound was precipitated from 85% ethanol in crude form as the barium salt. To remove certain impurities, chiefly glutamine, a solution of this water-soluble barium salt was acidified (*p*H 2) and passed through a Dowex 50 sodium column. The eluate was then rechromatographed on Dowex 1 acetate. A barium salt was again formed, reprecipitated from water–ethanol and dried *in vacuo* over phosphorus pentoxide. All of the above chromatographic operations were performed at 3° because of the considerable instability of the arylamine.

Analysis of the compound by methods previously described[4] is shown in Table I.

TABLE I

ANALYSES OF 5-AMINOIMIDAZOLE RIBOTIDE AND (α-N-FORMYL)-GLYCINAMIDINE RIBOTIDE

	Molar ratio (glycine = 1.00) AIR	$FGAM_1$	$FGAM_2$
Glycine	1.00	1.00	1.00
Formic acid	1.10	0.93	1.03
Acid-labile N	2.27	1.73	1.80
Total N	3.28	...	2.74
Pentose	1.00	0.99	0.90
Organic P	1.26	0.99	0.93

The results show that glycine, formic acid, acid-labile N, total N, pentose and organic phosphorus were liberated from AIR in the approximate molar ratios of 1:1:2:3:1:1. It is known that, upon hydrolysis, glycine, formic acid and ammonia are produced from the free base, 5-aminoimidazole, in the ratios of 1:1:2.[5]

The chemical constitution of the heterocyclic ring system of AIR was established by conversion of AIR-2-C^{14} to the ureido derivative, followed by hydrolysis to the free base and admixture with unlabeled 5-ureidoimidazole[6] as carrier. From the mixture, a picrate of 5-ureidoimidazole was isolated, which could be recrystallized to constant specific activity. The structure of the new arylamine ribotide, which is consistent with all of the above data, is presented below.[7] On the basis of the molecular weight calculated for this structure (430), the sample obtained for the above analysis was approximately 60 per cent. pure.

(1) This work was supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, United States Public Health Service, the Damon Runyon Memorial Fund for Cancer Research, Inc., and the National Science Foundation.

(2) B. Levenberg, S. C. Hartman and J. M. Buchanan, *Federation Proc.*, **14**, 243 (1955).

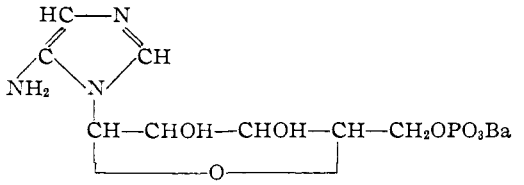
(3) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

(4) S. C. Hartman, B. Levenberg and J. M. Buchanan, THIS JOURNAL, **77**, 501 (1955).

(5) G. Hunter and J. A. Nelson, *Canadian J. Research*, **19B**, 296 (1941).

(6) G. Hunter and I. Hlynka, *Biochem. J.*, **31**, 488 (1937).

(7) The compound isolated from bacterial sources by S. H. Love and J. S. Gots, *J. Biol. Chem.*, **212**, 647 (1955), has recently been identified in this laboratory, by similar procedures, as 5-aminoimidazole riboside (S. H. Love and B. Levenberg unpublished data). It is of interest that the heterocyclic ring structure of 5-aminoimidazole represents an aglycone common to the pathways of purine *de novo* synthesis in avian and bacterial systems as well as to the reactions of purine catabolism in microorganisms (J. C. Rabinowitz and W. E. Pricer, Jr., *Federation Proc.*, **14**, 266 (1955)).

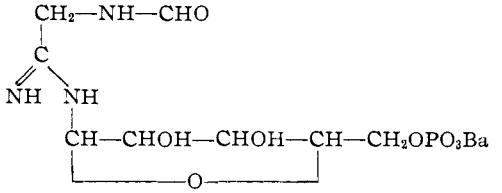


5-Aminoimidazole ribotide, barium salt

In order to demonstrate the accumulation of (α-N-formyl)-glycinamidine ribotide, enzymes of pigeon liver extract precipitating between a concentration of 13 and 33% ethanol were further fractionated with ammonium sulfate. Two fractions were obtained, one precipitating between 0 and 35% saturation (Fraction I) and the other between 45 and 60% saturation (Fraction II). Neither fraction separately could effect the synthesis of AIR from FGAR in the presence of glutamine and ATP. When, however, Fraction I was incubated with all three substrates (*i.e.*, glutamine, ATP and FGAR), a new compound (FGAM) was formed. In the presence of ATP and Fraction II, partially purified samples of FGAM were converted to AIR.

FGAM was isolated by essentially the same procedures described above for AIR. Analyses of the barium salts of two highly purified samples of FGAM are reported in Table I. It is seen that glycine, formic acid, acid-labile N, total N, pentose and organic phosphorus were liberated from FGAM in the approximate molar ratios of 1:1:2:3:1:1.

An electrometric titration of FGAM (sample 2) revealed the presence of two titratable groups (*i.e.*, a secondary phosphate dissociation, pK 6.0, and an imino dissociation, pK 9.2) between pH 3.5 and pH 10. Based upon the glycine released on hydrolysis both samples of FGAM were approximately 85% pure. These data are in agreement with the formulation of the structure of this compound as shown below



(α-N-Formyl)-glycinamidine ribotide, barium salt

In contrast to AIR, FGAM reacts neither in the Pauly test for imidazoles[8] nor in the Bratton–Marshall reaction. FGAM exhibits only weak end-absorption (below 240 mμ) in the ultraviolet region. AIR, although possessing a definite, general absorption in this area, likewise has no specific absorption band between 215 and 300 mμ. Upon hydrolysis in a sealed tube with 0.2 N HCl, each compound liberated glycine as the only ninhydrin-reactive substance detectable on paper chromatograms.

Both ribotides were readily converted into inosinic acid (IMP) in the presence of pigeon liver enzymes supplemented with bicarbonate, aspartic acid, ATP and formate. $C^{14}O_2$ was fixed by these ribotides in stoichiometric amounts during this reaction, a finding which provides evidence that both intermediates lack a carbon atom which ultimately could become carbon 6 of the purine ring.

In a previous communication,[4] the site of inhibition of purine *de novo* synthesis by L-azaserine was shown to be confined to a reaction subsequent to the formation of FGAR. In the present study, it has been found that the antibiotic exerts a powerful inhibitory action on the formation of FGAM from FGAR, and at the same time is without significant effect on the conversion of AIR to 5-amino-4-imidazolecarboxamide ribotide and IMP.

DIVISION OF BIOCHEMISTRY
DEPARTMENT OF BIOLOGY BRUCE LEVENBERG[9]
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS JOHN M. BUCHANAN

RECEIVED DECEMBER 14, 1955

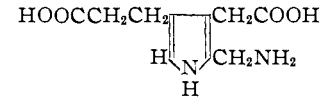
(8) K. K. Koessler and M. T. Hanke, *J. Biol. Chem.*, **39**, 497 (1919).

(9) United States Public Health Service Research Fellow of the National Institute of Neurological Diseases and Blindness (1954–1955).

A SYNTHESIS OF PORPHOBILINOGEN[1]

Sir:

Porphobilinogen, I,[2] is an intermediate in the biosynthesis of hemin which occurs in the urine of patients with acute porphyria. It has been synthesized from δ-aminolevulinic acid by a purely chemical method[3] as well as enzymically. Further, although porphobilinogen was not isolated, paper chromatography indicated its formation when IIId was decarboxylated.[4] The latter, reported as unstable, was synthesized[5] from IIa[6] through II (R^2 = CH_2OH), IIb, IIc, and IId.



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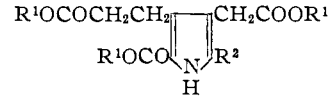
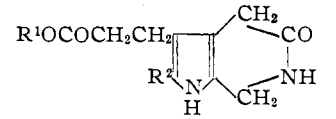


II, R^1 = Et; III, R^1 = H; a) R^2 = CH_3; b) R^2 = CHO; c) R^2 = CH=NOH; d) R^2 = CH_2NH_2



IVa, R^1 = Et; R^2 = COOEt
b, R^1 = H; R^2 = COOH
c, R^1 = H; R^2 = H

(1) Issued as N.R.C. Contribution No. 3806.

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

(3) J. J. Scott, private communication; to be reported to the Biochemical Society, London, Nov. 19, 1955, and later abstracted in the *Biochem. J.*

(4) C. Rimington and S. Krol, *Nature*, **175**, 630 (1955).

(5) K. S. N. Prasad and R. Raper, *ibid.*, **175**, 629 (1955).

(6) S. F. MacDonald and R. J. Stedman, *Canad. J. Chem.*, **33**, 458 (1955).