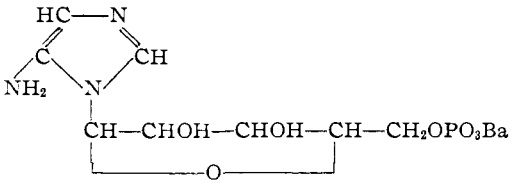
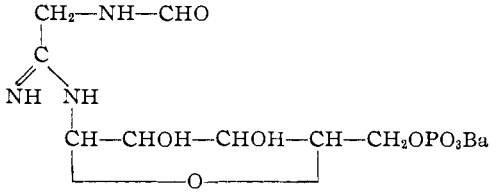


5-Aminoimidazole ribotide, barium salt

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.

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

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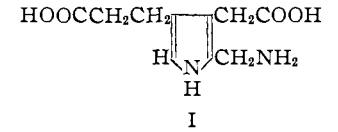
A SYNTHESIS OF PORPHOBILINOGEN[1]

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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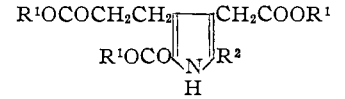
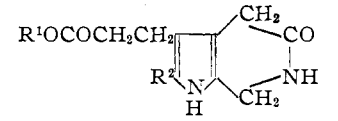


II, R^1 = Et { a) R^2 = CH_3; b) R^2 = CHO
III, R^1 = H { c) R^2 = CH=NOH; d) R^2 = CH_2NH_2



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).

We have reported the conversion of IIa through IIb of m.p. 81° (67°[5]) and IIIb into IIIc, which took up two moles of hydrogen in dilute ammonia over palladium.[7] The reduction product, IIId, was not reported because the analyses were variable; from dilute ammonia with acetic acid, it formed stable colorless needles[5] (82%), m.p. *ca.* 233° (dec.) (*Anal.* Calcd. for $C_{11}H_{14}N_2O_6$: C, 48.89; H, 5.22; N, 10.36. Found: *e.g.*, C, 48.21, 47.72; H, 5.92, 5.83; N, 10.28).

The same product, IIId, has now been obtained from IIb by these reactions in different orders: through IIc (85%), colorless needles, m.p. 86° (58°[5]) (*Anal.* Calcd. for $C_{17}H_{24}N_2O_7$: C, 55.42; H, 6.56; N, 7.61. Found: C, 55.47; H, 6.33; N, 7.26), and either IIIc,[7] the *hydrochloride* of IId, or the lactam IVa (80%). We have not encountered the free base IId.[5] The oxime IIc is reduced over palladium in ethanolic hydrochloric acid to the hydrochloride of IId (90%), colorless needles, m.p. 195–196° (*Anal.* Calcd. for $C_{17}H_{28}N_2O_6 \cdot HCl$: C, 52.37; H, 6.72; Cl, 9.07; N, 7.18. Found: C, 52.36; H, 6.90; Cl, 8.97; N, 7.41). In ethanolic ammonia IIc is similarly reduced to the lactam IVa (80%), colorless needles, m.p. 235–236° (decomp.), also obtained from the above hydrochloride with alkali (*Anal.* Calcd. for $C_{15}H_{20}N_2O_5$: C, 58.41; H, 6.54; N, 9.09; OEt, 29.20. Found: C, 58.76; H, 6.57; N, 9.33; OEt, 28.92).

However synthesized, the infrared spectra of IIId in Nujol mull showed inconsistent and minor differences, and the analyses were variable, though better on the monohydrate. Its nature and purity are no longer doubted because all specimens show identical X-ray powder photographs and chromatographic behavior. Also with aqueous pyridine and acetic anhydride, they give the lactam IVb (60%), colorless plates decomposing above 325° (*Anal.* Calcd. for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.10; H, 4.65; N, 11.00).

The lactam IVb is much more easily decarboxylated than is IIId. We have not yet been able to isolate porphobilinogen after heating IIId, either in 2*N* hydrochloric acid or better in aqueous pyridine with copper acetate[4], although paper chromatography does show its presence. On boiling with water, IVb gave porphobilinogen lactam, IVc, (80%), colorless plates, m.p. 282–284° (dec.) (*Anal.* Calcd. for $C_{10}H_{12}O_3N_2$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.55; H, 5.80; N, 13.19). Its identity with material of natural origin[2] was confirmed by mixed m.p., paper chromatography, infrared spectra in Nujol mull, X-ray powder photographs, and by the m.p., 248–250°,[2] and analysis of its methyl ester.

Porphobilinogen, I, was obtained from IVc with a slight excess of 2*N* sodium hydroxide at 100°. The removal of about 10% of unchanged IVc by repeated treatment with lead and mercuric acetates then recrystallization,[2] reduced the yield from 80 to 25%. It formed slightly pink micro-prisms, m.p. 172–175° (dec.) (*Anal.* Calcd. for $C_{10}H_{14}N_2O_4 \cdot H_2O$: C, 49.17; H, 6.60; N, 11.47. Found: C, 49.29, 49.15; H, 6.63, 6.56; N, 11.23). Its purity and identity were established by paper chromatography, and by the infrared spectra in Nujol mull and the X-ray powder photographs, of the natural and synthetic porphobilinogens and also of their hydrochlorides.

(7) D. M. MacDonald and S. F. MacDonald, *Canad. J. Chem.*, **33**, 573 (1955).

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A SYNTHESIS OF α-D-RIBOFURANOSE-1-PHOSPHATE

Sir:

The synthesis of β-D-ribofuranose-1-phosphate recently has been reported from this laboratory.[1] The purpose of the present communication is to record the synthesis of the anomeric compound (I) which is identical in chemical properties with the enzymatically-prepared samples of ribose-1-phosphate[2,3] and is fully active as a substrate for the fish muscle purine nucleoside phosphorylase.[3]

The importance of "neighbouring group participation" in the synthesis of nucleosides,[4] as well as β-D-ribofuranose-1-phosphate,[1] using acylfuranoside-1-halides has become clear. Thus, in most of the syntheses reported products having a C_1-C_2 *trans*-configuration are obtained.[4] Our basic aim, therefore, in approaching the problem of the synthesis of α-D-ribofuranose-1-phosphate was to prepare a suitably protected ribofuranosyl-1-halide in which the blocking group at C_2 would not exercise the important neighboring group influence in the replacement reaction at C_1, and which would, at the same time, be readily removed at a later step in the synthesis. The synthesis of a D-ribofuranose 2,3-cyclic carbonate, which meets the above requirements, was undertaken.

Methyl-2,3-isopropylidene-5-benzyl-D-ribofuranoside[5] (II) was converted quantitatively to the oily methyl 5-benzyl-D-ribofuranoside (III) (b.p. 140° (0.02 mm.)) by treatment with aqueous methanolic sulfuric acid.[6]

The glycol (III) was then brought into reaction with phosgene in a mixture of pyridine and dioxane, and the product, methyl 5-benzyl-ribofuranoside 2,3-cyclic carbonate (IV), was obtained, again in excellent yield, as an oil distilling at bath temperature up to 190° (0.01 mm.). This product gave no reaction with periodic acid. On storage at low temperature, an aqueous methanolic solution of the product, which presumably is a mixture of the α- and β-anomers, deposited crystals in 40% yield; m.p., after recrystallization from ether–petroleum

(1) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955), and in press.
(2) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).
(3) H. L. A. Tarr, *Fed. Proc.*, **14**, 291 (1955). We are grateful to Dr. Tarr for the enzymatic tests on the synthetic sample of D-ribose-1-phosphate.
(4) See *e.g.* B. R. Baker, *et al.*, *J. Org. Chem.*, **18**, 1786 (1954).
(5) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, **104**, 299 (1934); G. W. Kenner, C. W. Taylor and A. R. Todd, *J. Chem. Soc.*, 1620 (1949).
(6) *Cf.* C. H. Shunk, J. B. Lavigne and K. Folkers, THIS JOURNAL, **77**, 2210 (1955).