1268. *Purines, Pyrimidines, and Imidazoles. Part XXIII.* The Use of 5-Phospho-β-D-ribosyl Azide in a New Direct Synthesis of Nucleotides*

By R. Carrington, G. Shaw, and D. V. Wilson

2,3-*O*-Isopropylidene-β-D-ribosyl azide 5-*O*-phosphate has been prepared from β-D-ribofuranosyl azide by acetonation followed by phosphorylation with either pyrophosphoryl chloride or 2-cyanoethyl phosphate and dicyclohexylcarbodi-imide. Hydrogenation of the tri-n-octylammonium salt of the azide phosphate and condensation of the resulting ribosylamine phosphate with ethyl *N*-(carbamoylcyanomethyl)formimidate gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxyamide 5′-phosphate, with ethyl *N*-(methoxycarbonylcyanomethyl)formimidate the corresponding methyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylate 5′-phosphate, and with α-acetyl-β-ethoxy-*N*-ethoxycarbonyl acrylamide, 5-acetyluridylic acid. Also acylation of the ribosylamine phosphate with formylglycyl chloride gave *N*-(*N*-formylglycyl)-D-ribofuranosylamine 5-phosphate, and the structure of the ribosylamine phosphate was further confirmed by its conversion into a hexosiminic acid phosphate.

The synthesis of pyrimidine and imidazole (and hence purine) nucleosides with a *trans* arrangement between the base and the 2′-hydroxyl group of the sugar by condensation of glycosylamines, including D-ribofuranosylamine derivatives, with a variety of linear precursors of these heterocyclic systems has been recorded in previous Papers in this Series.[1] The subsequent conversion of nucleosides into corresponding 5′-*O*-phosphates, which normally requires protection of the 2′,3′-hydroxyl groups by acetonation, phosphorylation, and finally removal of protecting groups, is not always simple, and may be difficult to carry to completion satisfactorily especially with labile linear groups in the glycosidic position. Such compounds of special interest to us include the formylglycyl derivative (I) and the corresponding hydrolysis product (II) both of which are important intermediates in the biosynthesis *de novo* of purine nucleotides.[2] For these and other reasons we have been interested in extending the range of our synthetic methods to include a direct synthesis of nucleotides in which the sugar phosphate is introduced as a single unit.

For this purpose we required the 5-phosphoribosylamine (PRA) (III) or a simple derivative thereof. This compound, which has never been isolated in a solid state from natural sources, is generally accepted as the first nitrogen-containing intermediate in the biosynthesis of purine nucleotides.[2] Solutions containing unstable material claimed to be (III) have been formed biochemically by the reaction of 5-phospho-α-D-ribosyl pyrophosphate (PRPP) with a suitable nitrogen donor in the presence of enzyme fractions from pigeon liver [3] (donor L-glutamine) and wheat embryos [4] (donors L-asparagine, carbamoyl phosphate, and ammonia). Recently [5] it was shown that solutions of ribose 5-phosphate and ammonia, after incubation at 37° in the absence of enzymes, contain material, presumably PRA (III), which is converted into GAR (II) in the presence of glycine, adenosine triphosphate, and a GAR synthetase. In the concentration range 10^{-2} to 10^{-3}M only about 0·1% of the ribose phosphate is converted into PRA at equilibrium. This presumed PRA was shown to be very unstable at pH < 10, its half-life in water at pH $< 8{\cdot}5$ being less

* Part XXII, J. M. Carpenter and G. Shaw, *J.*, 1965, 3987.

[1] R. K. Ralph and G. Shaw, *J.*, 1956, 1877; G. Shaw, R. N. Warrener, H. M. Maguire, and R. K. Ralph, *J.*, 1958, 2294; G. Shaw and R. N. Warrener, *J.*, 1959, 50; G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J.*, 1959, 1648; G. Shaw and D. V. Wilson, *J.*, 1962, 2937; 1963, 1077.

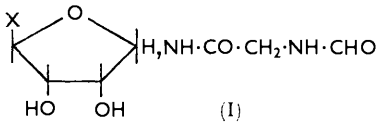
[2] J. M. Buchanan in "The Nucleic Acids," ed. E. Chargaff and J. N. Davidson, vol. III, Academic Press, New York, 1960.

[3] D. A. Goldthwait, *J. Biol. Chem.*, 1956, **222**, 1051.

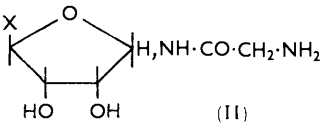
[4] M. Kapoor and E. R. Waygood, *Biochem. Biophys. Res. Comm.*, 1962, **9**, 7.

[5] D. P. Nierlich and B. Magasanik *J. Biol. Chem.*, 1965, **240**, 366.

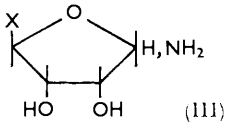
than 1 min. at 37°. An earlier report [3] claimed a preparation of PRA by the reaction of ribose 5-phosphate with liquid ammonia. The product was said to be unstable in acid but stable in dilute potassium hydroxide at 0°. In the pH range 7—8 only 4% was claimed to be lost during 30 min. at 25°. These results are at variance with those recorded by Nierlich and Magasanik,[5] although conditions are not completely comparable in the two series of experiments. The identity of the substance, which is accompanied by a more stable nitrogen-containing material, was further confirmed by its conversion into a hexosiminic acid with hydrogen cyanide followed by hydrolysis.[3] After enzymic dephosphorylation the hexosiminic acid obtained was identical on paper chromatograms with material prepared in a similar manner from D-ribopyranosylamine.[3] In our hands this last synthesis of PRA gave material which was not readily adaptable to further chemical reactions.



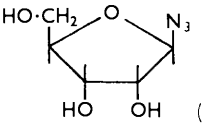
(I)



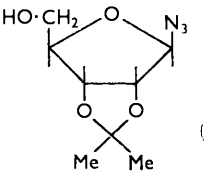
(II)



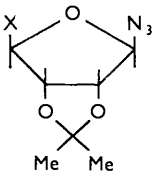
(III)



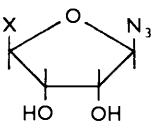
(IV)



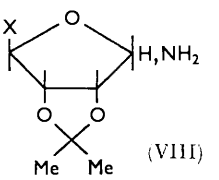
(V)



(VI)



(VII)

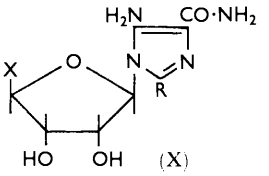


(VIII)

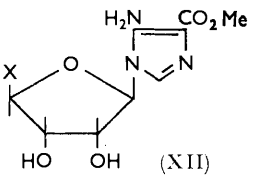
EtO·CR:N·CH(CN)·CONH₂
(IX)

EtOCH:N·CH(CN)·CO₂Me
(XI)

[X = CH₂·O·OP(OH)₂]



(X)



(XII)

We now record some results of an initial investigation into the preparation of derivatives of PRA (III) more suitable for further chemical reactions; a preliminary account of the work has been published.[6]

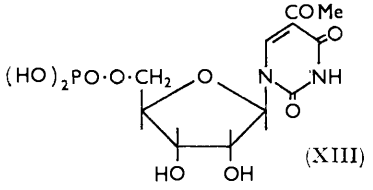
Reaction of β-D-ribofuranosyl azide (IV), prepared by improvements in the recorded synthesis, with acetone and toluene-*p*-sulponic acid or better with 2,2-dimethoxypropane and Zeo-Karb 225 resin in the H^+ form, gave almost quantitatively the isopropylidene derivative (V) as a colourless oil. Phosphorylation of this with 2-cyanoethyl phosphate and dicyclohexylcarbodi-imide in pyridine, or better with pyrophosphoryl chloride in methyl cyanide and pyridine, gave an excellent yield of the azide phosphate (VI) isolated as lithium or barium salts with a well defined azide maximum at 2120 $cm.^{-1}$ and also a peak at 1168 $cm.^{-1}$ and a doublet at 1380 $cm.^{-1}$ characteristic of the *gem*-dimethyl group. In a separate experiment hydrolysis of the material with dilute acetic acid after phosphorylation similarly gave the unprotected azide phosphate (VII).

Catalytic hydrogenation of (VI) with platinic oxide in aqueous solution at pH 9—10 at room temperature or below, followed by reaction of the product, presumably (VIII),

[6] R. Carrington, G. Shaw, and D. V. Wilson, *Tetrahedron Letters*, 1964, 2861.

with the imidate (IX; R = H) and then treatment with acid to remove the isopropylidene group, gave a small amount of diazotisable amine which after concentration was shown to correspond on paper chromatograms to 5-amino-1-β-D-ribofuransoylimidazole-4-carboxyamide 5'-phosphate (X; R = H) (AICAR). A similar diazotisable amine, presumably (X; R = Me), was also formed in very low yield from (VIII) and the imidate (IX; R = Me).

However, hydrogenation of the tri-n-octylammonium salt of (VI) in methanol under anhydrous conditions resulted in loss of the azide maximum and formation of a basic compound, again presumably (VIII) since with the linear imidate (IX; R = H) (prepared by an improvement of the earlier method) it readily gave an isopropylidene derivative from which AICAR (X; R = H) was obtained by mild acid hydrolysis in about 20% overall yield. The structure of the last compound was confirmed by direct comparison with an authentic sample and by its enzymic conversion into *N*-(5-amino-1-β-D-ribofuransoyl-imidazole-4-carbonyl)-L-aspartic acid 5'-phosphate (SAICAR).[2,7] A similar reaction of the reduced azide phosphate with the imidate (XI) gave the imidazole ester phosphate (XII) identical with material prepared by phosphorylation of the corresponding isopropylidene



(XIII)

$$MeCO{\cdot}C(:CHOEt){\cdot}CO{\cdot}NH{\cdot}CO_2Et$$

(XIV)

nucleoside and recorded earlier.[7] Further evidence for the structure of (VIII) came from its reaction with hydrogen cyanide followed by acid hydrolysis; a hexosiminic acid phosphate was obtained with the same paper chromatographic behaviour as the hexosiminic acid phosphate obtained by Goldthwait[3] from his material which is presumably PRA (III). We have also applied the general synthesis to the preparation of a pyrimidine nucleotide, 5-acetyluridylic acid (XIII), by reaction of (VIII) with the urethane (XIV) and hydrolysis. The structure of the uracil followed from its ultraviolet absorption spectrum which is almost identical with that of 5-acetyl-1-methyluracil,[8] and from its paper-chromatographic behaviour when it formed a spot which gave positive tests for phosphate and ketone groups.

The synthesis may be particularly useful for the preparation of linear ribonucleotides, and in preliminary experiments we have applied it to the synthesis of the formylglycinamide ribonucleotide (I) (FGAR) by acylation of (VIII) with formylglycyl chloride in dimethylformamide, and mild hydrolysis of the product. The compound had the same paper chromatographic behaviour as a sample of the naturally occurring material and was also converted into 5-amino-1-β-D-ribofuranosylimidazole 5'-phosphate (AIR) in the presence of L-glutamine, adenosine triphosphate, and an enzyme preparation containing formylglycinamide ribotide amidotransferase and formylglycinamidine ribotide kinocyclodehydrase. This is the first recorded chemical synthesis of FGAR, and since it has been recorded that the formyl group may be removed by mild acid hydrolysis[9] this also represents a synthesis of glycinamide ribotide (II) (GAR).

Experimental

Unless otherwise stated, evaporations were carried out in a Buchi rotary evaporator, under water-pump vacuum, with a flask temperature of 40° or less. Paper chromatograms were run on Whatman No. 1 paper by the ascending technique in the solvents (A) n-butanol–acetic acid–water (12 : 3 : 5), (B) propan-2-ol–water (7 : 3), or (C) propan-2-ol–water–ammonia (*d* 0·88)

[7] G. Shaw, D. V. Wilson, and C. P. Green, *J.*, 1964, 2650.
[8] J. H. Dewar and G. Shaw, *J.*, 1961, 3254.
[9] "Methods in Enzymology," ed. S. P. Colowick and N. O. Kaplan, vol. VI, Academic Press, New York and London, 1963, p. 676.

(7 : 3 : 1). Spots were detected by examination under a u.v. lamp, by the modified Bratton–Marshall spray reagents,[10] or by the ammonium molybdate reagent.[11] Barium or lithium salts, were treated with a slight excess of Zeo-Karb 225 H^+ resin before chromatography.

Ion-exchange separations were performed in an apparatus, all Teflon or glass, equipped with a Buchler micropump for accurate control of flow rates, and an LKB Uvicord 4701A ultraviolet absorptiometer with a flow cell of 3-mm. light path for continuous recording of the column eluates at 253·7 mμ or a Vanguard model 1056 double-beam automatic ultraviolet analyser with continuous recording at a variety of wavelengths between 200 and 400 mμ. All resins used for ion-exchange chromatography were an analytical grade of Dowex prepared and marketed by Bio-Rad Laboratories, Richmond, California.

Ultraviolet spectra were measured on a Perkin-Elmer 137UV recording spectrophotometer, and infrared spectra on a Perkin-Elmer 237 spectrophotometer for potassium bromide discs

2,3,5-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl Azide*.—We find that the following variation of the published method [12] results in a considerable saving of time. Dry hydrogen chloride was passed through a suspension of finely powdered and dried 1-*O*-acetyl-2,3,5-tri-*O*-benzoylribose [13] (20 g.) in ether (300 ml.) at 0° until a clear solution was obtained (2—3 hr.). The mixture was then set aside at 0° overnight. The solvent was removed and the residual gum evaporated successively with dry benzene (2 × 25 ml.) and toluene (25 ml.). The residue in methyl cyanide (250 ml.) was boiled under reflux with sodium azide (20 g.) for 2 hr. The filtered solution was evaporated and the residual azide (15—18 g.) crystallised from methanol as needles, m. p. 70°.

2,3-O-*Isopropylidene*-β-D-*ribofuranosyl Azide*.—(*a*) A solution of β-D-ribofuranosyl azide [12] (7·15 g.) in acetone (200 ml.) was shaken for 2 hr. with 2,2-dimethoxypropane (50 ml.) and Zeo-Karb 225 resin (H^+ form) (5 ml. methanol suspension) for 40 min. The resin was removed and washed thoroughly with acetone, and the combined filtrate and washings were evaporated to a viscous oil (8·0 g.). The product was distilled and had b. p. 90—94°/0·001 mm. (Found: C, 45·45; H, 6·25; N, 17·75. $C_8H_{13}N_3O_4$ requires C, 44·65; H, 6·1; N, 19·55%). The analysis corresponds to a product about 90% pure; it had an infrared band at 2120 $cm.^{-1}$.

(*b*) A solution of β-D-ribofuranosyl azide (1·9 g.) in acetone (200 ml.) at room temperature was shaken with toluene-*p*-sulphonic acid monohydrate (20 g.; dried over phosphoric oxide and sodium hydroxide) for 2½ hr. The filtered solution was added to a vigorously stirred suspension of sodium hydrogen carbonate (15 g.) in a slurry of ice and water (300 ml.). The mixture was evaporated to dryness and the residue further evaporated with benzene. The dried residue was extracted with boiling chloroform (3 × 50 ml.), allowing 10 min. per extraction. The combined, dried (sodium sulphate) extracts were evaporated and the residue was dissolved in benzene, filtered, and evaporated to a viscous straw coloured oil from which traces of solvent were removed at 0·001 mm. (yield 2·1 g.) (Found: C,45·45; H, 6·45; N, 16·0%); the analysis corresponds to a product about 82% pure. Even after several distillations it was difficult to improve the purity of the compound more than that recorded under (*a*), and for most purposes the crude material was adequate. The products obtained by methods (*a*) and (*b*) generally had almost identical infrared spectra, very minor differences with different samples being due undoubtedly to impurities.

2,3-O-*Isopropylidene*-β-D-*ribofuranosyl Azide* 5-O-*Phosphate*.—(*a*) 2,3-*O*-Isopropylidene-β-D-ribofuranosyl azide (2·5 g.; crude material) was dissolved in methyl cyanide (100 ml.) and pyridine (1·5 g.), cooled to 0°, treated with pyrophosphoryl chloride (5·35 g.) in methyl cyanide (15 ml.), and set aside at room temperature for 3 hr. The mixture was then poured slowly with vigorous stirring into a solution of lithium acetate (7·5 g.) in water (25 ml.). The mixture was evaporated to remove the organic phase and the remaining aqueous solution was adjusted to pH 10 with lithium hydroxide solution, and the precipitated trilithium phosphate removed by centrifugation. The supernatant solution was carefully adjusted to pH 8 with N-hydrochloric acid and evaporated to dryness. The residue was washed several times with dry methanol and finally ether, collected by centrifugation, and dried *in vacuo* at room temperature over phosphoric oxide. The *lithium salt* of the *azide phosphate hydrate* was obtained as a white powder (1·68 g.) which retained a little lithium chloride (Found: C, 29·05; H, 3·85; Li, 5·05; N, 11·85; P, 9·7. $C_8H_{12}Li_2N_3O_7P,H_2O,\frac{1}{2}LiCl$ requires C, 28·6; H, 4·2; Li, 4·65; N, 12·5; P, 9·25%).

[10] J. Baddiley, J. G. Buchanan F. E. Hardy and J. Stewart, *J.*, 1959, 2893.
[11] S. Burrows, F. S. M. Grylls, and J. S. Harrison, *Nature*, 1952, **170**, 800.
[12] J. Baddiley, J. G. Buchanan, L. Hodges, and J. F. Prescott, *J.*, 1957, 4769.
[13] E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, 1959, **42**, 1171.

(*b*) The mono-2-cyanoethyl phosphate–pyridine reagent [14] (6 ml. containing 6 mmoles) and 2,3-*O*-isopropylidene-β-D-ribofuranosyl azide (0·6 g.) were mixed and the solution evaporated to a syrup which was further evaporated with pyridine (3 × 10 ml.). The residue in anhydrous pyridine (10 ml.) with dicyclohexylcarbodi-imide (3·6 g.) was kept at room temperature with occasional shaking for 7 days. Water (10 ml.) was added to the mixture which was set aside at room temperature overnight, heated for 90 min. with aqueous ammonia (10 ml.; *d* 0·88) at 60°, and filtered; the filtrate and washings were evaporated to a gum which was extracted with small portions of water (total 15 ml.), the filtered extract treated with M-barium bromide solution (8 ml.), the solution adjusted to pH 8·2 with saturated barium hydroxide solution, and set aside overnight at 0°. The precipitate was spun off and the supernatant solution treated with 1½ volumes of ethanol, to give a precipitate which after 1 hr. was collected by centrifugation, washed with 80% aqueous ethanol (20 ml.), absolute ethanol (2 × 10 ml.), and ether (10 ml.), and dried in air. The *barium salt* of the *azide phosphate* was obtained as a white solid (0·51 g.) (Found: N, 6·35; P, 7·3. $C_8H_{12}BaN_3O_7P$ requires N, 9·75; P, 7·2%) which from the nitrogen analysis was about 65% pure. Both products obtained under (*a*) and (*b*) had strong azide maxima at 2120 cm.$^{-1}$ in their infrared spectra. The experiment (*b*) was repeated using isopropylidene azide (1 g.) and the cyanoethyl phosphate–pyridine reagent (10 ml. containing 10 mmoles) except that after the phosphorylation step the product was suspended in 20% aqueous acetic acid (60 ml.) and kept at 100° for 1 hr., and evaporated to dryness before proceeding with the treatment with ammonia. The product was then worked up in the same way, to give the crude β-D-*ribofuranosyl azide* 5-*phosphate barium salt* (1·57 g.) (Found: N, 7·2; P, 8·55. $C_5H_8BaN_3O_7$ requires N, 10·75; P, 7·95%) about 67% pure on the nitrogen analysis. This compound also had a strong maximum at 2120 cm.$^{-1}$ in its infrared spectrum.

5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carboxyamide* 5′-O-*Phosphate*.—The lithium salt of 2,3-*O*-isopropylidene-β-D-ribofuranosyl azide 5-*O*-phosphate (0·4 g.) in water was shaken with Zeo-Karb 225 resin (5 ml.; H^+ form) for 40 min. and the solution filtered, then shaken with tri-n-octylamine (1·5 g.) in ethanol (20 ml.) for 2 hr. The solution was extracted with ether (3 × 20 ml.) and evaporated to dryness. The residue, in anhydrous methanol (100 ml.), was hydrogenated over platinic oxide with dry hydrogen for 2½ hr., treated with ethyl *N*-(carbamoylcyanomethyl)formimidate (0·3 g.), and set aside for 30 min. with shaking. The filtered solution was evaporated to a gum which was dissolved in water (10 ml.) and heated at 100° with 10% aqueous acetic acid (10 ml.) for 90 min. The solution was evaporated to dryness, the residue in water (200 ml.) adjusted to pH 8·5—9, and the solution placed on a column (1 × 13 cm.) of Dowex analytical grade 1 × 8 (200—400 mesh) resin in the Cl^- form. After washing with water, products were eluted with 0·005N-hydrochloric acid at about 60 ml./hr. until all ultraviolet absorbing material had been recovered. The aminoimidazole phosphate (AICAR) appeared after the first 220 ml. of eluate and was eluted in the next 320 ml. The fraction (220—420 ml.) contained an impurity and was rechromatographed on a larger column (1 × 21·5 cm.), when separation was achieved. The combined AICAR fractions were adjusted to pH 7 with lithium hydroxide and evaporated to a small volume. The solution was adjusted to pH 8—8·5 with lithium hydroxide, filtered, and evaporated to dryness. The dry residue was extracted with dry methanol until free from chloride, then with ether, and finally dried. The aminoimidazole phosphate was obtained as a white powder (0·051 g.) which gave a single spot [R_F 0·12 in solvent (A), 0·20 in solvent (B), and 0·23 in solvent (C)] on paper chromatograms and was identical with material prepared by several other methods and described elsewhere.[7] The compound was readily converted into 5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl-L-aspartic acid 5′-phosphate (SAICAR) in the presence of fumaric acid and a preparation of adenylosuccinate AMP-lyase from yeast in a phosphate buffer.[2,7]

Methyl 5-*Amino*-1-β-D-*ribofuranosylmidazole*-4-*carboxylate* 5′-*Phosphate*.—A solution of the tri-n-octylammonium salt of 2,3-*O*-isopropylidene-β-D-ribofuranosyl azide 5-phosphate (from 0·5 g. of the isopropylidene azide) in methanol (100 ml.) was prepared as above. This was hydrogenated for 2½ hr. over Adams catalyst prepared by hydrogenation of platinic oxide (0·25 g.) in methanol, evaporation of the mixture to dryness, and suspension of the reduced catalyst in anhydrous methanol. The solution was treated with the solution obtained by filtration of a mixture of methyl α-amino-α-cyanoacetate (from 1 g. of methyl α-hydroxyimino-α-cyanoacetate) and ethyl formimidate hydrochloride (0·5 g.) in methyl cyanide (20 ml.). The mixture was warmed to 50° for 15 min. then set aside overnight. The filtered solution was evaporated to

[14] G. M. Tener, *J. Amer. Chem. Soc.*, 1961, **83**, 159.

a gum which was dissolved in water (20 ml.) and heated under reflux with 10% aqueous acetic (30 ml.) for 90 min. The solution was evaporated to a gum which was dissolved in water (40 ml.), the pH adjusted to 7·6 with aqueous ammonia, and the filtered solution applied to a column (2 × 13 cm.) of Dowex AG 1 × 2 (200—400 mesh) resin in the Br^- form, and after washing with water products were eluted with 0·008N-hydrobromic acid (rate of 60 ml./hr.) with the ultraviolet scan set at 267 mμ. A single sharp peak was eluted between 300 and 600 ml. The fractions were bulked, adjusted to pH 7—8 with barium hydroxide, and evaporated to about 10 ml. The pH was carefully adjusted with barium hydroxide solution to 8·5 and the clarified solution treated with M-barium bromide (0·5 ml.) followed by ethanol (80 ml.), to give a precipitate. This was spun off, washed with ethanol and ether, and dried. The imidazole phosphate was obtained as a white powder (0·096 g.) which gave a single spot [R_F 0·36 in solvent (A), 0·23 in solvent (B), and 0·33 in solvent (C)] on paper chromatograms and was identical with material prepared earlier.[7]

Formation of a Hexosiminic Acid Phosphate from 2,3-O-*Isopropylidene*-β-D-*ribofuranosyl Azide* 5-*Phosphate*.—The barium salt of the azide phosphate (0·15 g.) was converted into its tri-n-octylammonium salt as above and hydrogenated in methanol in the usual way. The clarified solution was treated with calcium chloride (0·2 g.) and sodium cyanide (0·163 g.) in water (5 ml.). A control experiment using ribose-5-phosphate (0·06 g.), ammonium chloride (0·0083 g.), and N-sodium hydroxide (0·15 ml.), together with calcium cyanide as before, was run at the same time. The solutions were set aside at 0° for 3 days then worked up using Goldthwait's procedure.[3] A negative result was obtained with the control but the primary experiment gave a product with a positive ninhydrin reaction and a positive phosphate test, and which ran as a single spot on paper chromatograms in a solvent system composed of butanol–acetic acid–water (12 : 3 : 5); R_F 0·1 corresponding to the hexosiminic acid phosphate obtained by Goldthwait (R_F 0·1).

5-*Acetyluridylic Acid*.—A solution of the tri-n-octylammonium salt of the foregoing isopropylidene azide phosphate (from 0·26 g. of lithium salt) was hydrogenated in dry methanol (60 ml.) as above. The solution was shaken for 30 min. with α-acetyl-β-ethoxy-*N*-ethoxycarbonylacrylamide [8] (0·4 g.) and a few drops of tri-n-octylamine, and set aside overnight. The mixture was boiled under reflux for 30 min., filtered, and evaporated to a gum which was dissolved in water (10 ml.) and heated to 100° for 90 min. with 10% acetic acid (10 ml.) and evaporated to a gum. This was dissolved in water (15 ml.) and the solution adjusted to pH 8 with lithium hydroxide solution, filtered, and evaporated to dryness. The residual solid was washed with methanol until free from Cl^-, dissolved in water (200 ml.), and the solution adjusted to pH 8·5 with lithium hydroxide solution and applied to a column (1 × 30 cm.) of Dowex AG 1 × 8 (200—400 mesh) resin in the Cl^- form. After washing with water, the column was eluted with 0·005N-hydrochloric acid with the ultraviolet scan set at 283 mμ, and, after 1650 ml. had been collected, with 0·01N-hydrochloric acid. Absorbing material was collected between 40 and 250 ml. The solution was adjusted to pH 7—8 with lithium hydroxide, evaporated to dryness, and lithium chloride removed by washing the residue with dry methanol. The remaining 5-*acetyluridylic acid lithium salt* (0·018 g.) was obtained as a white powder (Found: N, 5·3; P, 5·2. $C_{11}H_{13}Li_2N_2O_{10}P,9H_2O$ requires N, 5·35; P, 5·95%). The compound ran as a single discrete ultraviolet absorbing spot (R_F 0·27) in butanol–acetic acid–water (12 : 3 : 5) and the spot also gave positive tests for phosphate with the ammonium molybdate reagent,[11] and for ketones with the 2,4-dinitrophenylhydrazine–sodium hydroxide reagent. In addition the nucleotide had $\lambda_{max.1}$ 230, $\lambda_{min.}$ 253, and $\lambda_{max.2}$ 283—284 mμ in water; $\lambda_{max.1}$ 229—230, $\lambda_{min.}$ 253—254, and $\lambda_{max.2}$ 283—284 mμ in 0·1N-hydrochloric acid; λ(infl.) 232—234, $\lambda_{min.}$ 263, and $\lambda_{max.}$ 285—286 mμ in 0·1N-sodium hydroxide. By contrast, 5-acetyl-1-methyluracil [8] with an almost identically shaped ultraviolet spectral curve had $\lambda_{max.1}$ 231—232, $\lambda_{min.}$ 252—253, and $\lambda_{max.2}$ 286—287 mμ in water; $\lambda_{max.1}$ 231—232, $\lambda_{min.}$ 253—254, and $\lambda_{max.2}$ 286—287 mμ in 0·1N-hydrochloric acid; λ(infl.) 231—234, $\lambda_{min.}$ 262—263, and $\lambda_{max.}$ 287—288 mμ in 0·1N-sodium hydroxide.

N-(N-*Formylglycyl*)-D-*ribofuranosylamine* 5-*Phosphate*.—A solution of the tri-n-octylammonium salt of the foregoing isopropylidene azide phosphate (from 0·5 g. of lithium salt) was hydrogenated in dry methanol (100 ml.) as before. Dry *NN*-dimethylformamide (40 ml.) was added to the reduced solution which was evaporated at room temperature and 0·001 mm. to remove methanol. To the solution was added formylglycyl chloride [15] (0·6 g.) and tri-n-octylamine (0·4 g.), and the mixture then set aside overnight. The solvent was removed at 40°/

[15] J. Max, *Annalen*, 1909, **369**, 285.

0·001 mm. and the residue treated with N-lithium hydroxide (6 ml.) and water (15 ml.). The solution was adjusted to pH 7—8 with N-hydrochloric acid, filtered, and evaporated to dryness. The residue was dissolved in water, treated with M-barium bromide, the precipitate spun off, and the supernatant liquid treated with ethanol, to give a solid precipitate. This (0·047 g.) was found by paper chromatographic comparisons with authentic specimens to consist largely of FGAR with a smaller amount of ribose 5-phosphate. The solid, in water (150 ml.), was treated with a little Zeo-Karb 225 resin (H^+ form) to remove barium, the pH adjusted to 8·5—9·0 with aqueous ammonia, and the solution applied to a column (1 × 8·5 cm.) of Dowex AG 1 × 2 (200—400 mesh) resin in the formate form, and, after washing with water, products were eluted with 0·05M-ammonium formate–formic acid buffer (pH 5·0). The product was eluted between 300 and 450 ml. and located by phosphate tests and enzyme assays. The solution was lyophilised to give FGAR as a white powder (10 mg.) The compound gave a single discrete spot [R_F 0·22 in solvent (A), 0·25 in solvent (B), and 0·25 in solvent (C)] (positive reactions with the ammonium molybdate and chlorine–starch iodide reagents) on paper chromatograms, identical with that of an authentic specimen which was run at the same time. The compound gave a negative ninhydrin reaction but ninhydrin-positive material corresponding to glycine was obtained after acid hydrolysis. The compound was also readily converted into 5-amino-1-β-D-ribofuranosylimidazole 5′-phosphate (AIR) in the presence of L-glutamine, adenosine triphosphate, and a mixture of preparations of the enzymes, formylglycinamide ribotide amidotransferase and formylglycineamidine ribotide kinocyclodehydrase.

We thank Professor J. M. Buchanan, Division of Biochemistry, Massachusetts Institute of Technology, for a sample of a solution of FGAR, and the Medical Research Council for financial assistance.

INSTITUTE OF TECHNOLOGY, BRADFORD 7. [*Received, June 15th,* 1965.]