[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis of 9-β-D-Ribofuranosylpurine-5′-phosphate and its Behavior Toward Aqueous Alkali[1]

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The synthesis of 9-β-D-ribofuranosylpurine-5′-phosphate is reported. Two substances formed by the action of dilute aqueous alkali on the nucleotide, and also isolated as by-products during its synthesis, have been tentatively identified as 5-amino and 5-formamido derivatives of 4-(5′-phosphoribofuranosylamino)-pyrimidine.

In studies of potential tumor-inhibiting agents, 9-β-D-ribofuranosylpurine (I) was synthesized in this Laboratory and shown[3,4] to be identical with nebularine, a toxic, naturally occurring material.[4-6] The metabolism[7] of this compound led to three derivatives of purine-C^{14} in the soluble nucleotide fraction from the liver. From their behavior on the ion-exchange resin, these were postulated to be the 5′-mono-, di- and triphosphates of the administered ribosylpurine. Since it was desirable to have an authentic specimen for comparison and for investigation of potential pharmacodynamic properties, the synthesis of 9-β-D-ribofuranosylpurine-5′-phosphate (II) was undertaken.

2′,3′-*O*-Isopropylidene-9-β-D-ribofuranosylpurine[8] was treated with dibenzylphosphorochloridate in pyridine solution to give 2′,3′-*O*-isopropylidene-9-β-D-ribofuranosylpurine-5′-dibenzylphosphate. Hydrogenation effected smooth removal of both benzyl groups, and the isopropylidene group could then be removed from the product by allowing the free acid to stand in aqueous solution.[9]

Examination of this solution by paper chromatography revealed the main product and two other phosphorus-containing, ultraviolet light-absorbing materials, as well as a small amount of free ribosylpurine. The contaminants were separated from the main product by chromatography over a column of powdered cellulose. The main product contained organically-bound phosphorus and a *cis*-glycol[10] system and, in several paper chromatographic systems, gave only one ultraviolet light-absorbing spot with R_f values of the order to be expected of a mononucleotide. Further purification over Dowex-50 resin furnished the free acid as a pale cream-colored solid. Its ultraviolet spectrum in acid, alkali and water was almost identical with that of 9-β-D-ribofuranosylpurine,[3] and analysis indicated it to be a monohydrate of the 5′-monophosphate. It was eluted as a single, sharp peak from a Dowex-2 (formate) ion-exchange column at a position on the elution diagram consistent with its being a mononucleotide.

9-β-D-Ribofuranosylpurine-5′-phosphate was not unstable in 0.1 *N* acid at room temperature but in alkali was decomposed readily to give a mixture of two substances similar to the by-products formed during its synthesis.[12] A mixture of the latter, recovered as a cream-colored powder from the cellulose column, yielded, upon acid hydrolysis, a number of products. The main ultraviolet light-absorbing component had R_f values and spectral characteristics the same as 4,5-diaminopyrimidine. Examination of chromatograms showed a pattern of phosphorus and aniline phthalate-positive (*i.e.*, reducing carbohydrate[13]) spots identical with that obtained with a similar hydrolysate of adenosine-5′-phosphate: namely, two aniline phthalate-positive spots, one faint and having R_f values similar to D-ribose, the other, present in considerable amount, containing phosphorus and having R_f values similar to D-ribose-5-phosphate. Inorganic phosphate was distinguished from the sugar phosphate on chromatograms run in acetone–water–acetic acid.[14]

The two by-products were separated on a small scale by chromatography on paper. Both contained organically bound phosphate and a *cis*-glycol system and migrated on paper chromatography and electrophoresis as typical mononucleotides. The chromatographically faster-moving by-product could be converted into the slower-moving component by further alkaline hydrolysis.

The slower-moving component appeared as a fluorescent blue spot under ultraviolet light, gave positive reactions in the alkaline phosphomolybdate test[15] and the Bratton–Marshall test[16] and had a spectrum in acid, alkali and water generally similar to that of 4,5-diaminopyrimidine (Fig. 1). Its pK_a, determined spectrophotometrically, was 5.7 (4,5-diaminopyrimidine, 6.03).[17] The chromatographically faster-moving by-product gave a negative reaction in both the above color tests. The

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(3) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(4) N. Löfgren, B. Lüning and H. Hedström, *Acta Chem. Scand.*, **8**, 670 (1954).

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(6) L. Ehrenberg, H. Hedström, N. Löfgren and B. Takman, *Svensk. Kem. Tidskr.*, **58**, 269 (1946).

(7) M. P. Gordon and G. B. Brown, *J. Biol. Chem.*, **220**, 927 (1956).

(8) A. Hampton and D. I. Magrath, THIS JOURNAL, **79**, 3250 (1957).

(9) Attempts to remove simultaneously the isopropylidene and one benzyl group by acid hydrolysis led to considerable hydrolysis of the glycosyl linkage.

(10) See footnote 26 of ref. 11.

(11) M. P. Gordon, V. S. Weliky and G. B. Brown, THIS JOURNAL, **79**, 3245 (1957).

(12) It was also found that extensive degradation occurred during an attempt to prepare the dimethyl ester by reaction with diazomethane.

(13) S. M. Partridge, *Nature*, **164**, 443 (1949).

(14) S. Burrows, F. S. M. Grylls and J. S. Harrison, *ibid.*, **170**, 800 (1952).

(15) A. Bendich and G. C. Clements, *Biochim. et Biophys. Acta*, **12**, 462 (1953).

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

(17) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

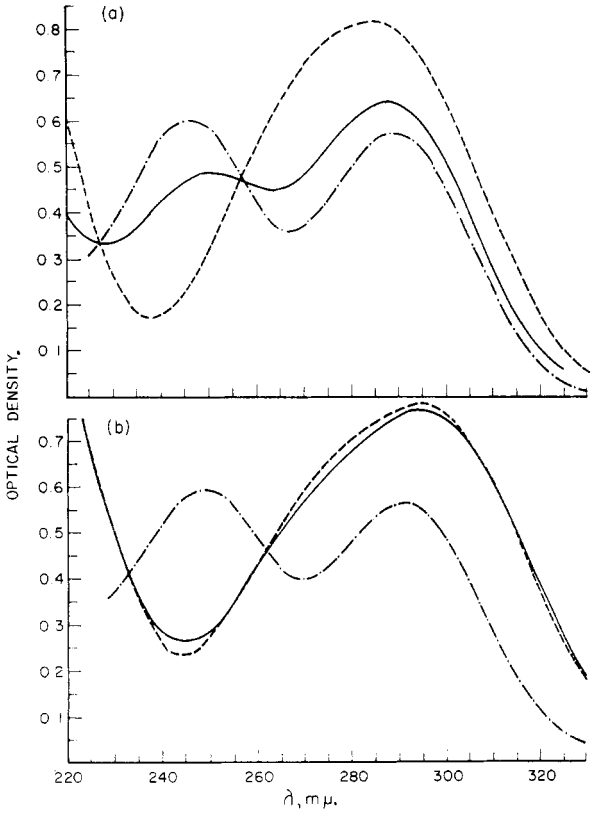


Fig. 1.—Ultraviolet spectra of: (a) 4,5-diaminopyrimidine; (b) chromatographically slower-moving by-product. In 0.1 *N* HCl (.....), 0.1 *N* NaOH (—·—·) and H_2O (———, *p*H 6.48 and 4.51, respectively).

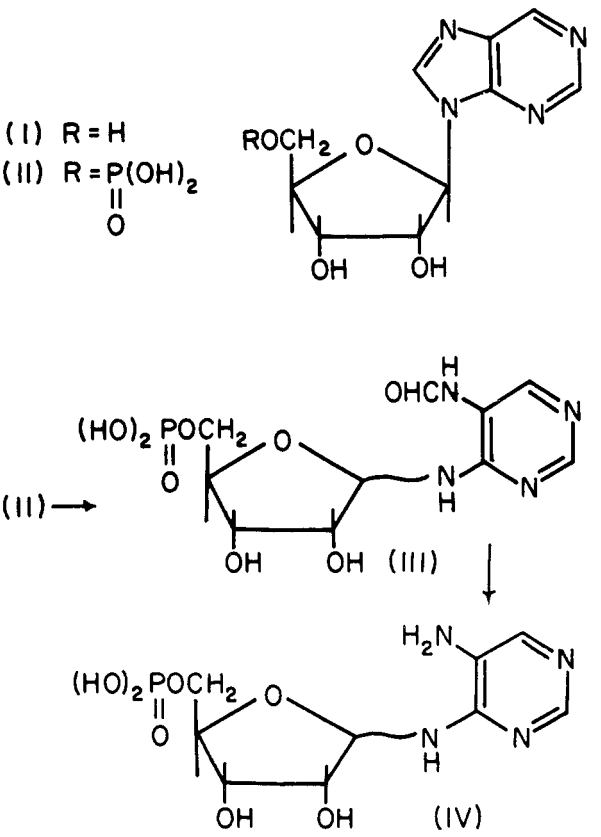
similarity of its ultraviolet spectrum with that of 4-amino-5-formamidopyrimidine[18] is apparent (Fig. 2). The latter was found to possess a pK_a value of 11.40, which may be attributed to the 5-formamido group, and a value of 11.43 was found for the by-product.

It appears that the two substances produced by the alkaline hydrolysis of 9-β-D-ribofuranosylpurine-5′-phosphate, and also found as by-products during its synthesis, are 5′-phosphoribofuranosyl derivatives of 4,5-diaminopyrimidine (III and IV), one (III) still carrying the 8-carbon atom of the original molecule as a formyl group attached to the primary[19] amino group of IV. The presence of the 5′-phosphate group precludes furanose–pyranose isomerization, although mutarotation at the glycosyl linkage is still possible for either compound.

The initial step in the alkaline degradation of the purine nucleoside[11] and of this nucleotide probably involves a nucleophilic attack on the 8-carbon atom by hydroxyl ion; the subsequent ring opening is perhaps facilitated by conjugation of the resulting ionized formamido group with the pyrimidine ring.

(18) Authentic samples of this and related compounds were kindly sent us by Professor Adrien Albert and Dr. D. J. Brown, Department of Medical Chemistry, the Australian National University. For proof of the position of the formyl group see D. J. Brown, *J. Applied Chem.*, **5**, 358 (1955).

(19) A formyl group attached to the ribosylamino group of IV should not give rise to the pK_a value of 11.4 observed for the faster-moving by-product.



The alkali degradation products of 9-β-D-ribofuranosylpurine are thought[11] to be ribofuranosyl and -pyranosyl derivatives of 4,5-diaminopyrimidine, which had resulted from the loss of the 8-carbon atom. The degradation was postulated as proceeding by way of the intermediate formation of the 5-formamido compound, although there was

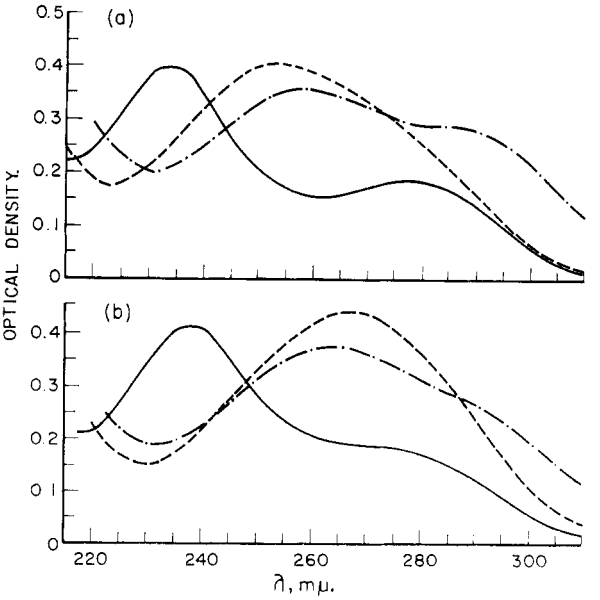


Fig. 2.—Ultraviolet spectra of: (a) 4-amino-5-formamidopyrimidine; (b) chromatographically faster-moving by-product, in 0.1 *N* HCl (.....), 0.1 *N* NaOH (—·—·) and H_2O (———, *p*H 5.88 and 5.23, respectively).

no conclusive evidence for its presence in that hydrolysis mixture. The reason for the increased stability of the 5-formamido group toward alkali with the presence of the 5′-phosphate is not obvious.

A comparison has been made[20] of the synthetic purine mononucleotide with the relevant metabolite of 9-β-D-ribofuranosylpurine isolated from rats.

Experimental

Paper Chromatography.—The following solvent systems were used: A, *n*-butyl alcohol saturated with water; B, *n*-butyl alcohol–ethyl alcohol–water (45:30:40); C, *n*-butyl alcohol–acetic acid–water (50:20:35); D, 1% aqueous ammonium sulfate–isopropyl alcohol (1:2)[21]; E, acetone–30% acetic acid (1:1)[14]; F, isopropyl alcohol–1% aqueous boric acid–10% ammonia (50:25:1)[22]; G, pyridine–ethyl acetate–water (1:2:2).[23] Solvent D was used with papers previously soaked in 1% aqueous ammonium sulfate and dried. R_f values quoted are for ascending chromatograms run on Whatman No. 1 paper in freshly made up solvent. Ultraviolet light-absorbing materials were located on the chromatograms by visual examination and photography of the dried papers under ultraviolet light (Corning No. 9863 filter), reducing carbohydrates by the aniline hydrogen phthalate spray reagent,[13] *cis*-glycol systems by the periodate spray technique[10,24] and phosphates by Hanes and Isherwood reagent.[25] The spectra were determined with a Beckman spectrophotometer, model DU, with 1-cm. matched cells.

9-β-D-Ribofuranosylpurine-5′-phosphate.—2,3-*O*-Isopropylidene-9-β-D-ribofuranosylpurine (1 g., dried *in vacuo* over phosphorus pentoxide for 3 days) was dissolved in dry pyridine (5 cc.) contained in a 25-cc. flask, the solution cooled to near freezing (f.p. pyridine −42°) in a Dry Ice–acetone bath and dibenzylphosphorochloridate (from 2.17 g. of dibenzyl phosphite and 1.44 g. of N-chlorosuccinimide)[26] added and the residual reagent washed from the container with *ca.* 3 cc. of cold pyridine. The flask was stoppered and the reaction mixture kept in a semi-frozen condition for 5 hr., then left in the cooling bath overnight. After removal from the cooling bath, a solution of sodium acetate (4 g. anhydrous) in water (10 cc.) was added and the mixture shaken vigorously for 1.5 hr., then evaporated to dryness under reduced pressure (bath temperature <30°). The crystalline residue was thoroughly triturated with chloroform and the solid collected and washed with more solvent (total filtrate *ca.* 100 cc.). The filtrate was shaken with ice-cold sodium bicarbonate solution (5%; 2 portions, 25 cc. and 10 cc., respectively), then with water (three 25-cc. portions) and finally dried over anhydrous sodium sulfate. After filtration, the solvent was removed *in vacuo* and the pale yellow, oily residue was evaporated down with three 10-cc. portions of ethyl alcohol, then dissolved in a little methyl alcohol and the solution clarified by filtration. Removal of solvent under reduced pressure gave crude 2′,3′-*O*-isopropylidene-9-β-D-ribofuranosylpurine-5′-dibenzylphosphate as a thick, pale yellow oil which, after keeping at 0.22 mm. and 30° for 2 hr., weighed 1.69 g. Paper chromatography in solvent A showed a major ultraviolet light-absorbing component (R_f 0.89) which contained organically-bound phosphorus, and a minor spot with the same R_f (0.79) as the starting material.

The crude dibenzyl ester was dissolved in aqueous ethyl alcohol (60%, 50 cc.) and the solution hydrogenated at room temperature and pressure in the presence of 10% Pd–C catalyst (160 mg.). After uptake of hydrogen had ceased (*ca.* 50 minutes), the catalyst was separated and the filtrate concentrated to *ca.* 5 cc. under reduced pressure. Examination of this solution by paper chromatography (solvents A, B and C) indicated a major ultraviolet light-absorbing component (R_f values 0.14, 0.60 and 0.61, respectively) which contained phosphorus but no *cis*-glycol system. There were also traces of isopropylideneribosylpurine and a substance with R_f values of 0.02, 0.40 and 0.36, respectively, and the latter contained phosphorus and a *cis*-glycol system.

The above solution was diluted to 30 cc. with water and allowed to stand at 24° for 48 hr. Paper chromatography (solvents B and C) showed a major ultraviolet light-absorbing component (R_f values 0.40 and 0.36, respectively) which contained both phosphorus and a *cis*-glycol system, and the spectra of which in water, acid and alkali closely resembled those of 9-β-D-ribofuranosylpurine. In addition to a trace of the latter, two other ultraviolet light-absorbing spots also were observed on chromatograms run in solvent B (R_f values 0.34 and 0.26, respectively); they ran as one spot in solvent C (R_f 0.26). Both contained phosphorus and a *cis*-glycol system. The solution was adjusted to *ca.* *p*H 7 with ammonium hydroxide and then evaporated to dryness under reduced pressure. The thick, brown, oily residue was evaporated three times with small portions of ethyl alcohol and left in a vacuum desiccator (P_2O_5) for 2 days. It weighed 1.126 g.

A column of powdered cellulose (350 g., 6.5 cm. diam.) was prepared by firmly pressing down successive additions (*ca.* 50 g.) of the dry powder, and then washed with solvent C until the eluate was colorless (*ca.* 1300 cc.). The crude product above was dissolved in 10 cc. of solvent C, the solution carefully applied to the top of the column, the container rinsed out with a little more solvent and the column developed with the same solvent. The first 230 cc. of eluate was discarded and the remainder collected in fractions of *ca.* 18 cc. at 7–10 minute intervals until ultraviolet light-absorbing material ceased to be eluted (*ca.* fraction 60). The small amount of ribosylpurine present appeared in fractions 19–31, the bulk of the major ultraviolet light-absorbing component in fractions 34–41 and a mixture of the two by-products in fractions 49–58.

Fractions 34–41 were combined and evaporated to dryness *in vacuo;* small white crystals separated during the latter stages of the evaporation. The pale brown residual sirup was evaporated down once with methyl alcohol and then left in a vacuum desiccator (P_2O_5) overnight, when it became converted to a hygroscopic frothy solid, weight 0.61 g. Paper chromatograms of this material, run in solvent C, showed only one ultraviolet light-absorbing spot (R_f 0.36), giving tests for both phosphorus and a *cis*-glycol system. The molybdate spray reagent, however, revealed the presence of a small amount of inorganic phosphate[27] traveling just ahead of the ultraviolet light-absorbing compound. A further 61 mg. of almost chromatographically pure material was recovered from fractions 42–44.

The solid (0.60 g.) was dissolved in water (10 cc.), the *p*H adjusted to *ca.* 7 with dilute ammonium hydroxide and the solution applied to a column (1 cm. diam.) of Dowex-50 ion-exchange resin (H^+; 5 cc. wet resin).[28] The column was then washed with water until ultraviolet light-absorbing material ceased to be eluted and early fractions of eluate, containing inorganic phosphate[29] but no ultraviolet light-absorbing material, were discarded. Vacuum evaporation of the eluate (bath temperature <25°) gave a semi-solid residue which was redissolved in water (10 cc.) and the ice-cooled solution brought to *p*H 8–9 by the dropwise addition of 7% aqueous barium hydroxide (*ca.* 5 cc.), with any excess being removed quickly by passing in carbon dioxide. Only a very slight precipitate of barium phosphate formed. The solution was concentrated somewhat under reduced pressure, then clarified by filtration and the filtrate concentrated to 5–10 cc. This solution was applied to a second column of Dowex-50 resin (H^+; 5.5 cc. of wet resin), which was then washed with water and the eluate collected and evaporated as before. The gummy residue was evaporated

(20) M. P. Gordon, D. I. Magrath and G. B. Brown, THIS JOURNAL, **79**, 3256 (1957).

(21) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).

(22) F. Baron and D. M. Brown, *J. Chem. Soc.*, 2855 (1955).

(23) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(24) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(25) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(26) G. W. Kenner, A. R. Todd and F. J. Weymouth, *J. Chem. Soc.*, 3675 (1952).

(27) In an early experiment most of the inorganic phosphate was removed at this stage by precipitation as barium phosphate.

(28) The use of a larger quantity of resin should permit the complete removal of inorganic phosphate at this stage and thus make unnecessary the further steps of purification described.

(29) Reducing phosphorylated carbohydrate was also present, indicating that some hydrolysis of the glycosyl linkage had occurred on the resin. This could be minimized by jacketing the column with circulating ice-water.

down once with a little methyl alcohol and twice with ethyl alcohol, giving 9-β-D-ribofuranosylpurine-5′-phosphate as a pale cream-colored solid, melting with decomposition at 140–150°. After drying in a vacuum desiccator (P_2O_5) for 3 days, it weighed 0.32 g. (26.5%, based on the isopropylidenepurineriboside used). *Anal.*[30] Calcd. for $C_{10}H_{13}O_7N_4P\cdot H_2O$: C, 34.30; H, 4.32; N, 16.00; P, 8.85. Found: C, 34.16; H, 4.57; N, 15.92; P, 8.75. Light absorption in water at *p*H 3.9, 0.1 *N* hydrochloric acid and 0.1 *N* sodium hydroxide: λ_{max} 263 mμ (ε 7350, 5900 and 7500, respectively); λ_{min} 223.5, 235.5 and 227.5 mμ, respectively. The substance was freely soluble in water and gave a single ultraviolet light-absorbing spot on paper chromatograms run in solvents A, B, C and D (R_f values, 0.02, 0.40, 0.36 and 0.47, respectively), which gave a positive reaction in the molybdate spray test for phosphorus and in the periodate spray test for *cis*-glycols. It was free of inorganic phosphate and was eluted from a column (11 × 1 cm.) of Dowex-2 (formate), using 0.1 *M* formic acid which was 0.02 *M* with respect to sodium formate as eluent, as a single, symmetrical peak.[31] Fractions of 11 cc. were collected every 18–20 minutes, the highest O.D. at 260 mμ occurring at fraction 71. When subjected to electrophoresis in sodium phosphate–citric acid buffer (*p*H 8.25), on an 18.8-cm. wide strip of Whatman No. 3 mm. paper, at 860 volts and 90 milliamp. for 45 minutes, it migrated 10 cm. toward the anode (adenosine-5′-phosphate, 9.3 cm.; ribosylpurine moved 1.4 cm. toward the cathode).

Action of Acid and Alkali.—Hydrochloric acid (0.1 *N*) for 48 hr. at 24° had no appreciable effect. Sodium hydroxide (0.1–0.2 *N*) for 24 hr. at 24° caused extensive decomposition to a mixture of two substances having the same chromatographic, electrophoretic and ultraviolet spectral characteristics and giving the same color reactions as the two by-products formed in the synthesis. To provide material for the spectral measurements and color tests, 2-mg. lots of the 5′-phosphate were dissolved in *ca.* 0.2 *N* sodium hydroxide (0.23 cc.), and the solution was allowed to stand at 24° for 30 hr. After neutralization with dilute hydrochloric acid, the solution was applied as a band to a 19 cm. wide strip of Whatman No. 1 paper (previously treated with 1% aqueous ammonium sulfate[21]), the chromatogram developed with solvent D and the various bands eluted with water. Determination of the O.D. at 263 mμ (OH^-) of the eluate containing the residual purine nucleotide indicated that *ca.* 60% had been decomposed.

Isolation and Nature of the By-products Formed in the Synthesis.—Fractions 49–58 from the cellulose column experiment were bulked and worked up as described for the ribosylpurine-5′-phosphate. A cream-colored powder (0.079 g.) was obtained, which on chromatography in solvents B and D gave two ultraviolet light-absorbing spots, one (R_f values, 0.34 and 0.29, respectively) dark and the other (R_f values, 0.26 and 0.15, respectively) fluorescent blue; like 4,5-diaminopyrimidine, the latter tended to become brown on the dried chromatogram. Both compounds contained phosphorus and a *cis*-glycol system, and both were eluted from a column of Dowex-50 resin (H^+) with water. On electrophoresis they migrated 9.6 cm. toward the anode, as one spot.

A solution of this material (1–2 mg.) in 0.5 *N* sulfuric acid (0.2 cc.) was heated in a stoppered tube at 100° for 2.5 hr. The dark solution was cooled, neutralized with ammonium hydroxide and examined for ultraviolet light-absorbing and reducing-carbohydrate components by paper chromatography. Adenosine-5′-phosphate (1–2 mg.) was treated similarly and the resulting solution used as a reference material on the chromatograms. Commercial samples of D-ribose-5-phosphate and D-ribose were also included as standards. The major ultraviolet light-absorbing spot present in the hydrolysate from the by-products had R_f values identical with those of 4,5-diaminopyrimidine. It was eluted from chromatograms run in solvents C and D, and its ultraviolet spectra in 0.1 *N* acid and 0.1 *N* alkali were determined: λ_{max} 283–284 mμ and 245–246, 288–289 mμ, respectively (4,5-diaminopyrimidine, λ_{max} 284–285 mμ and 246–247, 289–290 mμ, respectively). Chromatograms run in solvents C, D, E, F and G were examined for reducing carbohydrate and phosphorus by spraying with the aniline hydrogen phthalate and acid molybdate reagents, respectively. In every case, both the hydrolysate from the mixed by-products and that from adenosine-5′-phosphate gave a faint aniline phthalate-positive spot, the R_f values of which were similar to those of D-ribose, and a much more intense spot with R_f values similar to those of D-ribose-5-phosphate. Inorganic phosphate was also detected in both hydrolysates and separated from carbohydrate phosphates on chromatograms run in solvent E or, less efficiently, in solvent F.

The two by-products were separated in mg. quantities by chromatographing as bands on Whatman No. 1 or 3 MM. paper as described above. Where the presence of ammonium sulfate in the product was undesirable, most was removed by evaporating the eluate and extracting the organic material from the residue with methyl alcohol. The slower-moving component (*i.e.*, R_f 0.15 in solvent D) which had a blue fluorescence in ultraviolet light, gave a blue coloration with alkaline phosphomolybdate[15] and a positive reaction in the Bratton–Marshall test for diazotizable amino groups[16]; 4,5-diaminopyrimidine also reacted positively in both tests; light absorption in 0.1 *N* HCl, λ_{max} 294–294.5 mμ; in water (*p*H 4.51), 294–294.5 mμ; in 0.1 *N* NaOH, 249–249.5 and 294–294.5 mμ (Fig. 1b); pK_a value (determined spectrophotometrically), 5.70 ± 0.05 (4,5-diaminopyrimidine, 6.03[17]). The values for λ_{max} of 4,5-diaminopyrimidine, the spectrum of which is shown in Fig. 1a, are given by Mason.[17]

The faster-moving component (*i.e.*, R_f 0.29 in solvent D), which appeared as a dark spot on chromatograms viewed in ultraviolet light, gave a negative reaction in both the above tests; light absorption in 0.1 *N* HCl, λ_{max} 267.5 mμ; in water (*p*H 5.23), 238 mμ with a pronounced shoulder at about 275 mμ, in 0.1 *N* NaOH 264 mμ with a shoulder at about 290 mμ (Fig. 2b). The spectrum of 4-amino-5-formamidopyrimidine is shown in Fig. 2a: in 0.1 *N* HCl, λ_{max} 253 mμ; in water at *p*H 5.88, at both 234 mμ and 278.5 mμ; in 0.1 *N* NaOH, 258.5 mμ with a shoulder at 285 mμ; pK_a values, spectrophotometric, 3.82 ± 0.05, 11.40 ± 0.1 (4-amino-5-formamidopyrimidine, 4.42 ± 0.05, 11.43 ± 0.1). A sample of the material was dissolved in 0.2 *N* NaOH and the solution set aside at 24°. At 24 and 48 hr. the solution was chromatographed in solvent D; the spots applied to the origin were dried in a stream of carbon dioxide. Examination of the chromatograms in ultraviolet light showed that the material was being converted gradually (*ca.* one-third after 24 hr.) to a phosphorus-containing, blue-fluorescent substance having the same R_f as the slower-moving by-product. The substance was eluted, and its ultraviolet spectra in 0.1 *N* acid, 0.1 *N* alkali and water were determined and found to be identical to those of the slower-moving by-product. After 48 hr., a small amount of material with the same R_f as 4,5-diaminopyrimidine was also present in the hydrolysate.

Acknowledgment.—The authors wish to acknowledge the capable assistance of Mrs. Elizabeth Carroll.

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(30) C, H and N analyses by J. F. Alicino, Metuchen, N. J. Phosphorus was determined according to R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940).

(31) Slow decomposition tended to occur on this resin also. Quantitative recovery was not achieved, and end-absorbing material trailed the major peak.