398. *Synthesis of Nucleoside-5′ Pyrophosphates.*

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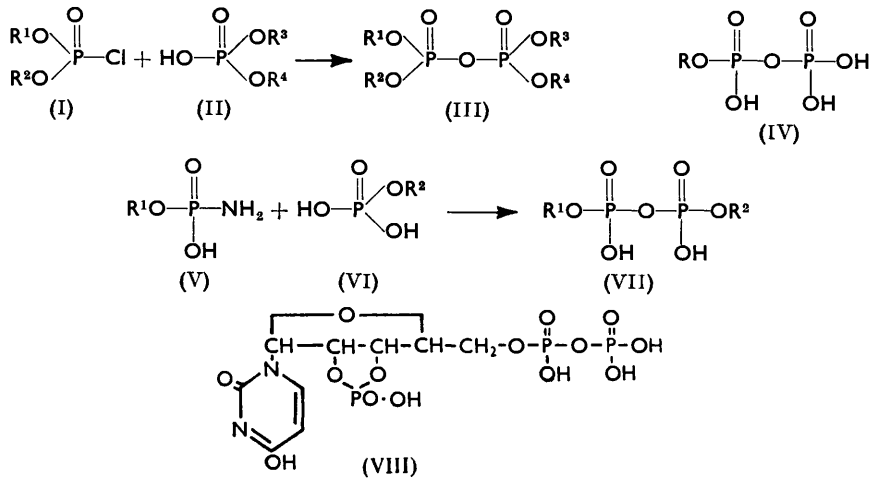
Improved syntheses of uridine-5′ phosphate, cytidine-5′ phosphate, and uridine-2′(3′) : 5′ diphosphate are described. Adenosine-5′ pyrophosphate, uridine-5′ pyrophosphate, thymidine-5′ pyrophosphate, and uridine 2′ : 3′-(cyclic phosphate) 5′-pyrophosphate have been prepared in very high yield by treatment of the appropriate nucleotide with dibenzyl phosphorochloridate, followed by removal of benzyl groups. Uridine-5′ phosphate has been brominated to 5-bromouridine-5′ phosphate.

The biological importance of nucleoside-5′ pyrophosphates and triphosphates is now well established, both for their direct function in cellular metabolism and as components of various coenzymes. Methods for the synthesis of such nucleoside derivatives fall roughly into three groups: (*A*) Reaction of a phosphorochloridate with a phosphate, followed by removal of protecting groups. Examples of the variations of this method are the synthesis of adenosine-5′ pyrophosphate [1] (I + II; $R^1 = R^2 = R^3 =$ benzyl, $R^4 =$ nucleoside),

[1] Baddiley and Todd, *J.*, 1947, 648.

of uridine-5′ pyrophosphate [2] (I + II; $R^1 = R^3 = R^4$ = benzyl, R^2 = nucleoside), and of thymidine-5′ pyrophosphate and uridine-5′ pyrophosphate [3,4] (I + II; $R^1 = R^3$ = benzyl, R^2 = nucleoside, R^4 = H). (*B*) Treatment of the 5′-nucleotide with phosphoric acid and a suitable reagent such as di*cyclo*hexylcarbodi-imide [5] or *cyclo*pentanone oxime sulphonate.[6] (*C*) Reaction of phosphoramidates with phosphates; Todd and his co-workers [7] have thus synthesised adenosine-5′ pyrophosphate *via* (V; R^1 = benzyl) and



(VI; R^2 = nucleoside). The same compound has been prepared by Khorana and his colleagues [8] with the somewhat more useful nucleoside phosphoramidate (V; R^1 = nucleoside) and (VI; R^2 = H or benzyl). Treatment of a 5′-deoxy-5′-halogeno-nucleoside with a salt of tribenzyl pyrophosphate has also been used,[9,10] but owing to cyclisation [11] and other difficulties this method is of very limited application.

Use of method (*A*) requires protected nucleotide derivatives, usually involving a series of intermediates from the free nucleoside, while method (*B*) generally gives complex mixtures of phosphates, requiring tedious purification with low yields of the desired compound. Method (*C*) effected some improvement in both yield and specificity but, again, purification of the product by ion-exchange is desirable. Since an easy high-yielding synthesis of nucleoside-5′ pyrophosphates would be valuable, the classical method was re-investigated, by techniques not available at the time of the original syntheses of adenosine-5′ pyrophosphate [1] and triphosphate.[12,13] It was found that quantitative yields of nucleoside-5′ pyrophosphates (IV; R = nucleoside) were obtained by treating the unprotected 5′-mononucleotide (II; R^3 = nucleoside, R^4 = H) directly with dibenzyl phosphorochloridate [14] (I; $R^1 = R^2$ = benzyl) to give the P^1-nucleoside P^2-dibenzyl hydrogen pyrophosphate (III; $R^1 = R^2$ = benzyl, R^3 = nucleoside, R^4 = H) from which the benzyl groups were readily removed by catalytic hydrogenation. Under the conditions employed, triphosphate was not formed and the crude pyrophosphates were approximately

[2] Kenner, Todd, and Weymouth, *J.*, 1952, 3675.
[3] Griffin and Todd, *J.*, 1958, 1389.
[4] Michelson and Todd, *J.*, 1956, 3459.
[5] Khorana, *J. Amer. Chem. Soc.*, 1954, **76**, 3517.
[6] Chase, Kenner, Todd, and Webb, *J.*, 1956, 1371.
[7] Clark, Kirby, and Todd, *J.*, 1957, 1497.
[8] Chambers, Moffatt, and Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 4240.
[9] Anand, Clark, Hall, and Todd, *J.*, 1952, 3665.
[10] Michelson, unpublished work.
[11] Clark, Todd, and Zussman, *J.*, 1951, 2952.
[12] Baddiley, Michelson, and Todd, *J.*, 1949, 582.
[13] Michelson and Todd, *J.*, 1949, 2487.
[14] Atherton, Openshaw, and Todd, *J.*, 1945, 382.

95% pure and could be further purified by precipitation of the calcium salt from acid solution by the addition of ethanol. Purification by the elegant but somewhat tedious ion-exchange chromatography was not necessary, the synthetic compounds being homogeneous on paper chromatography, paper electrophoresis, and analytical ion-exchange chromatography and identical in such behaviour with authentic specimens. Thymidine-5′ and uridine-5′ pyrophosphate were hydrolysed by acid to the monophosphates only, under conditions in which the symmetrical di(nucleoside-5′) pyrophosphates are stable.

Uridine-5′ phosphate was prepared from 2′ : 3′-*O*-*iso*propylideneuridine [15] by a modification of the " polyphosphoric acid " method used by Hall and Khorana,[16] whereby the necessity for ion-exchange purification of the product was eliminated. The protected nucleoside was treated with a mixture of phosphoric acid and phosphoric oxide, and the resultant polyphosphates were hydrolysed. Treatment of the aqueous solution with baryta to pH 6·5 precipitated orthophosphate which was removed, and the uridine-5′ phosphate in the filtrate was isolated either as the barium salt or the free nucleotide in 70—80% yield. Traces of uridine and uridine-2′(3′) : 5′ diphosphate are satisfactorily removed by this process. A similar treatment of 2′ : 3′-*O*-benzylidenecytidine [17] gave crystalline cytidine-5′ phosphate in good yield.

A solution of anhydrous tri-*n*-octylammonium uridine-5′ hydrogen phosphate in dioxan was treated with dibenzyl phosphorochloridate (1·5—4 mols.) and tri-*n*-butylamine at room temperature for three hours. Paper chromatography showed complete conversion into P^1-uridine-5′ P^2P^2-dibenzyl hydrogen pyrophosphate (III; R^1 = uridine, R^2 = H, R^3 = R^4 = benzyl) which was precipitated from solution by ether. Hydrogenation in 70% ethanol was complete in 5—20 min. at a palladium catalyst, and the pyrophosphate (III; R^1 = uridine, R^2 = R^3 = R^4 = H) was isolated as its acid calcium salt in 90% yield. Analogous reactions with tri-*n*-octylammonium thymidine-5′ and adenosine-5′ hydrogen phosphate gave 80—90% yields of the corresponding pyrophosphates. In one preparation precipitation of the benzylated intermediate by ether was omitted; hydrogenation was then very slow owing to catalyst poisons and a good yield of P^1-adenosine-5′ P^2-benzyl dihydrogen pyrophosphate (VII; R^1 = adenosine, R^2 = benzyl) was obtained. When a mixture of uridine-2′ : 5′ diphosphate and uridine-3′ : 5′ diphosphate (prepared by an improvement of the " polyphosphoric acid " method [16] utilising the reverse temperature-solubility properties of the barium salts of such phosphates [18]) was treated with excess of dibenzyl phosphorochloridate, it gave after hydrogenolysis of the benzyl groups a good yield of uridine 2′ : 3′-(cyclic phosphate) 5′-pyrophosphate (VIII). Mild acid hydrolysis of this derivative broke both the cyclic phosphate and the pyrophosphate linkage to give the original mixture of uridine-2′(3′) : 5′ diphosphates. The compound is of some interest as a possible chain-terminating nucleotide in the enzymic synthesis of polynucleotides from pyrophosphates, described by Ochoa and his co-workers.[19] Its preparation serves to demonstrate the mildness of the method, which could readily be extended to the synthesis of such unstable derivatives as deoxyadenosine-5′ pyrophosphate, since at no time are strongly acidic conditions employed.

An alternative approach to pyrophosphate derivatives lies in the treatment of the mononucleotides with tetraesters of pyrophosphoric acid, utilising the well-known disproportionation reaction.[20] When uridine-5′ phosphate was treated with tetraphenyl pyrophosphate [21] at room temperature, quantitative formation of P^1-uridine-5′ P^2P^2-diphenyl hydrogen pyrophosphate (III; R^1 = uridine, R^2 = H, R^3 = R^4 = phenyl) was observed. At neutrality and room temperature this derivative was relatively stable, and did not

[15] Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113.
[16] Hall and Khorana, *J. Amer. Chem. Soc.*, 1955, **77**, 1871.
[17] Gulland and Smith, *J.*, 1948, 1527.
[18] Dekker, Michelson, and Todd, *J.*, 1953, 947.
[19] Grunberg-Manago and Ochoa, *J. Amer. Chem. Soc.*, 1955, **77**, 3165.
[20] Corby, Kenner, and Todd, *J.*, 1952, 1234.
[21] Khorana and Todd, *J.*, 1953, 2257.

give rise to diuridine pyrophosphate through further disproportionation. In view of the easy syntheses described above, this modification was not elaborated to preparative work.

Kenner, Todd, and Weymouth [22] reported the chlorination of 2′ : 3′-*O*-*iso*propylideneuridine to 5-chloro-2′ : 3′-*O*-*iso*propylideneuridine by *N*-chlorosuccinimide. When a dioxan solution of tri-*n*-octylammonium uridine-5′ hydrogen phosphate was treated with *N*-bromosuccinimide at room temperature for one week, 5-bromouridine-5′ phosphate (isolated as its calcium salt) was produced.

Experimental

Cytidine-5′ Phosphate.—Anhydrous 2′ : 3′-*O*-benzylidenecytidine [17] (8 g.) was stirred into a solution of phosphoric oxide (15 g.) in 90% phosphoric acid (20 g.), and the mixture kept at 60° for 2 hr., under anhydrous conditions, with occasional stirring. Water (200 c.c.) was added and the solution placed on a boiling-water bath for 30 min. Aqueous barium hydroxide was then added to pH 6·5, and the hot solution filtered, the barium phosphate precipitate being washed well with hot water, and the filtrates evaporated to small volume under reduced pressure. The pH was adjusted to 7·1 with barium hydroxide, and two volumes of ethanol were added. The precipitated barium cytidine-5′ phosphate was washed with ethanol and ether and dried (7·0 g.). This product was dissolved in water (100 c.c.), and N-sulphuric acid (*ca.* 20 c.c.) was added to remove barium (rhodizonic acid used as indicator). The boiling solution was filtered through Hyflo Supercel and concentrated to *ca.* 30 c.c. under reduced pressure, and the hot solution was filtered. Boiling ethanol (*ca.* 100 c.c.) was added slowly and, after cooling, the deposited crystalline cytidine-5′ phosphate was collected (3·75 g.); it softened at 215°, decomposed at 232° (Found: N, 13·0; P, 9·7. Calc. for $C_9H_{14}O_8N_3P$: N, 13·0; P, 9·6%).

Uridine-5′ Phosphate.—A solution of 2′ : 3′-*O*-*iso*propylideneuridine [15] (2·75 g.) in 85% phosphoric acid (13 g.) and phosphoric oxide (10 g.) was kept at 60° for 2 hr. Water (100 c.c.) was then added and the mixture heated at 100° for ½ hr. Barium hydroxide solution was added to pH 6·5 and the boiling suspension (total vol. *ca.* 1 l.) filtered, the precipitated barium phosphates being well washed with hot water. The filtrates were taken to small volume (*ca.* 40 c.c.) under reduced pressure and adjusted to pH 7·4 with barium hydroxide solution, and two volumes of ethanol were added. The precipitated barium uridine-5′ phosphate was collected at a centrifuge, washed with ethanol and ether, and dried (3·41 g.). A solution of this salt in water (100 c.c.) was passed through a column of IR-120 (H^+ form; 2·5 × 6 cm.), and the eluate concentrated to small volume under reduced pressure. Ethanol and benzene were added and the residual water was removed by azeotropic distillation; the residue was dissolved in ethanol (15 c.c.) and filtered into dry ether (200 c.c.) with stirring. The precipitated uridine-5′ phosphate was collected, washed with ether, and dried, to a white powder (2·19 g., 70%) (Found, in material dried at 100°/10^{-3} mm. for 6 hr.: N, 8·5; P, 9·7. Calc. for $C_9H_{13}O_9N_2P$: N, 8·6; P, 9·6%). A similar run from 7 g. of *iso*propylideneuridine gave 8·95 g. (80%) of the pure barium salt of uridine-5′ phosphate.[23]

Uridine-5′ Pyrophosphate.—Tri-*n*-octylamine (0·545 g., 1 mol.) was added to a solution of uridine-5′ phosphate (0·5 g.) in ethanol (10 c.c.), and the mixture evaporated to dryness under reduced pressure. The residual monotri-*n*-octylammonium uridine-5′ phosphate was a glass which was dried by evaporating its solution in dioxan, benzene, and toluene to dryness several times. To the product in dry dioxan (10 c.c.) and benzene (5 c.c.) was added dibenzyl phosphorochloridate [from 0·61 g. of dibenzyl phosphite and 0·315 g. of *N*-chlorosuccinimide in 10 c.c. of dioxan and 5 c.c. of benzene at room temperature (2 hr.)], followed immediately by tri-*n*-butylamine (0·428 g., 1·5 mol.) in dioxan (15 c.c.) and benzene (5 c.c.) dropwise during 10 min. The mixture was kept at room temperature for a further 3 hr., then solvent was removed under reduced pressure and the residue shaken with ether (50 c.c.) and *n*-heptane (50 c.c.). The gummy precipitate was hydrogenated in 70% ethanol (50 c.c.) at atmospheric temperature and pressure over palladium (PdO) and palladised-charcoal. Uptake was complete in 10 min. Catalyst was removed and the filtrate neutralised to pH 7·4 with triethylamine, then taken to small volume (*ca.* 10 c.c.), ethanol (50 c.c.) was added, and the solution filtered. To this filtrate

[22] Kenner, Todd, and Weymouth, *J.*, 1952, 3675.
[23] Michelson and Todd, *J.*, 1949, 2476.

was added calcium chloride (0·75 g.) in 90% ethanol (10 c.c.). The precipitated calcium salt was collected by centrifugation, washed with ethanol and ether, and dried. Water (10 c.c.) was added to this salt, then N-hydrochloric acid to pH 3·6. The solution was filtered and ethanol (25 c.c.) added to the filtrate, to give calcium uridine-5′ pyrophosphate as a white powder, which was collected, washed with ethanol and ether, and dried (0·605 g.). Paper chromatography showed only traces of uridine-5′ phosphate and inorganic phosphate. A similar run with 4 mols. of dibenzyl phosphorochloridate and 0·5 g. of uridine-5′ phosphate gave 0·710 g. of a less pure calcium salt of uridine-5′ pyrophosphate. The two preparations were combined (1·315 g.) and water (10 c.c.) was added. The pH was adjusted to 4·2, and the solution clarified by centrifugation. Hydrochloric acid was then added to pH 2·5, followed by ethanol (30 c.c.). The precipitated calcium hydrogen uridine-5′ pyrophosphate was collected, washed with ethanol and ether, and dried (0·95 g.). Paper chromatography and electrophoresis showed that this product was homogeneous and contained only uridine-5′ pyrophosphate as organic constituent (Found, in material dried at 50°/10^{-3} mm. for 12 hr.: N, 6·0; P, 14·2. Calc. for $C_9H_{12}O_{12}N_2P_2Ca$: N, 6·3; P, 14·0%).

Thymidine-5′ Pyrophosphate.—A solution of diammonium thymidylate dihydrate (0·5 g.) in water (100 c.c.) was passed through a column of IR-120 (H^+ form; 1 × 8 cm.) and the eluate evaporated to *ca.* 20 c.c. under reduced pressure. Tri-*n*-octylamine (0·45 g., 1 mol.) was then added, and the mixture evaporated to dryness several times with dioxan, benzene, and toluene to give the anhydrous tri-*n*-octylamine salt of thymidine-5′ phosphate as needles. To this in dry dioxan (10 c.c.) and anhydrous benzene (5 c.c.), dibenzyl phosphorochloridate (from 0·50 g. of dibenzyl phosphite, etc.) was added. Tri-*n*-butylamine (0·354 g., 1·5 mol.) in dioxan (10 c.c.) was then run in during 10 min. The mixture was kept at room temperature for a further 3 hr. and worked up as described for uridine-5′ pyrophosphate. Hydrogenation was complete in 20 min. The crude calcium salt obtained by addition of calcium chloride to an alcoholic solution of the amine salts was dissolved in water (10 c.c.) at pH 3·5 with hydrochloric acid, and after filtration ethanol (40 c.c.) was added. The precipitated calcium hydrogen thymidine-5′ pyrophosphate was washed with ethanol and ether and dried (0·515 g.). Spectroscopic and paper-chromatographic examination showed that this product was 92% pure on a weight basis, representing an actual yield of 85% of thymidine-5′ pyrophosphate. Traces of thymidine-5′ phosphate and inorganic phosphate were present. A similar run with 4 mols. of dibenzyl phosphorochloridate gave a 95% yield. The preparations (total 1·215 g.) were combined, dissolved in water (10 c.c.) at pH 4·2, and clarified by centrifugation, and to the supernatant liquid and washings were added 4 volumes of ethanol. The precipitated pure calcium hydrogen thymidine-5′ pyrophosphate was collected, washed with ethanol and ether, and dried (0·836 g.). Paper chromatography and electrophoresis showed that the compound was homogeneous and contained thymidine-5′ pyrophosphate as sole organic ingredient (Found, in material dried at 50°/10^{-3} mm. for 12 hr.: N, 6·2; P, 14·0. $C_{10}H_{14}O_{11}N_2P_2Ca$ requires N, 6·4; P, 14·1%).

Adenosine-5′ Pyrophosphate.—Tri-*n*-decylamine (0·95 g., 1·5 mols.) was added to a suspension of adenosine-5′ phosphate (0·5 g.) in methanol (35 c.c.) and ethanol (35 c.c.), and the mixture boiled under reflux until a clear solution was obtained. Solvent was then removed under reduced pressure, and the residue dried in the usual manner and finally dissolved in anhydrous dioxan (10 c.c.). A solution of dibenzyl phosphorochloridate (from 0·567 g. of dibenzyl phosphite and 0·293 g. of *N*-chlorosuccinimide in 10 c.c. of dioxan at room temperature for 1 hr.) was added, followed dropwise by tri-*n*-butylamine (0·4 g., 1·5 mols.) in dioxan (5 c.c.). The mixture was kept at room temperature for a further 2 hr. with stirring, then evaporated to *ca.* 10 c.c. under reduced pressure, and stirring was continued for a further hour. Solvent was removed under reduced pressure and the residual syrup shaken vigorously with 1 : 1 ether–*n*-heptane (2 × 50 c.c.). The gummy precipitate was hydrogenated in 70% ethanol in the usual way, and worked up as for uridine-5′ pyrophosphate. The crude calcium salt was dissolved in N/25-hydrochloric acid, two volumes of ethanol were added, and the precipitated calcium hydrogen adenosine-5′ pyrophosphate was washed with alcohol and ether and dried (0·530 g., 80%) (Found, in material dried at 50°/10^{-3} mm. for 12 hr.: N, 14·6; P, 13·1. Labile P : acid stable P, 1·01. Calc. for $C_{10}H_{13}O_{10}N_5P_2Ca$: N, 15·0; P, 13·3%).

Uridine-2′(3′) : 5′-Diphosphate.—Anhydrous uridine (1 g.) in phosphoric acid (6 g.) containing phosphoric oxide (4 g.) was kept at 60° for 2½ hr. Water (60 c.c.) was added, and the solution kept at 100° for 30 min., then the hot solution was neutralised to pH 9 with hot concentrated

lithium hydroxide solution. After cooling, the lithium phosphate was removed, the filtrate and washings were run through a column of IR-120(H^+ form), and the effluent was neutralised to pH 7·4 with barium hydroxide solution. The precipitate was removed by centrifugation and the supernatant liquid and washings were concentrated to small volume under reduced pressure, heated to boiling to complete precipitation of the diphosphate, and filtered hot (washing being with a little hot water), and the collected dibarium uridine-2′(3′) : 5′ diphosphate was dried (2·45 g.) (Found, in air-equilibriated material: N, 3·2; P, 7·9. Calc. for $C_9H_{10}O_{12}N_2P_2Ba_2,8H_2O$: N, 3·4; P, 7·6%).

Bistrioctylamine Salt of Uridine-2′(3′) : 5′ Diphosphate.—The dibarium salt (1·7 g.) was shaken in water (500 c.c.) till dissolved, then passed through a small column of IR-120(H^+ form) to remove barium, and the eluate was evaporated to small volume under reduced pressure. Tri-*n*-octylamine (1·465 g., 2 mols.) and ethanol (50 c.c.) were then added and the solution was evaporated to dryness; the residue was evaporated with dioxan and dry toluene to remove traces of moisture and stored *in vacuo* over phosphoric oxide.

Uridine (2′ : 3′-Cyclic Phosphate) 5′-Pyrophosphate.—Dibenzyl phosphorochloridate (from 0·96 g. of dibenzyl phosphite in 5 c.c. of dioxan and 5 c.c. of benzene plus 0·54 g. of *N*-chlorosuccinimide at room temperature for 1 hr.) was added to a solution of the bistri-*n*-octylamine salt of uridine-2′(3′) : 5′ diphosphate (from 1 g. of the dibarium salt) in dioxan (5 c.c.) and benzene (5 c.c.). A solution of tri-*n*-butylamine (0·905 g., 4 mols.) in dioxan (3 c.c.) and benzene (3 c.c.) was then added dropwise and the clear solution kept at room temperature for 3 hr. Solvent was removed under reduced pressure and ether (40 c.c.) and *cyclo*hexane (60 c.c.) were added to the residue. The precipitated gum was washed by decantation, then hydrogenated in 70% ethanol (50 c.c.) (20 min.), and the product was worked up in the usual way, ethanolic calcium chloride (250 mg.) being used to afford the calcium salt. The crude product was dissolved in 0·05N-hydrochloric acid (6 c.c.), and clarified at a centrifuge, and 3 volumes of ethanol were added. The precipitated *calcium hydrogen uridine (2′ : 3′-cyclic phosphate) 5′-pyrophosphate* was washed with ethanol and ether and dried (0·465 g.) [Found, in material dried at 50°/10^{-3} mm. for 12 hr.: N, 5·0; P, 17·7. $(C_9H_{10}O_{14}N_2P_3)_2Ca_3$ requires N, 5·3; P, 17·8%].

Attempted Synthesis of Uridine-5′ Triphosphate from the Diphosphate.—A solution of calcium uridine diphosphate (300 mg.) in water (10 c.c.) was passed through a column of IR-120 (H^+ form; 1 × 8 cm.), washing being with water. The eluate was treated with tri-*n*-octylamine (0·48 g., 2 mols.) and ethanol, and the anhydrous salt prepared in the usual way. A solution of dibenzyl phosphorochloridate (from 0·355 g. of dibenzyl phosphite as above) was added, followed by tri-*n*-butylamine (0·252 g., 2 mols.) in benzene (5 c.c.) and dioxan (10 c.c.), and the mixture kept at room temperature for 3 hr. Working up and hydrogenating gave an acid calcium salt (277 mg.) which streaked on paper chromatography and was shown by paper electrophoresis to contain little if any triphosphate. Acid hydrolysis (N-acid at 100° for 10 min.) gave largely uridine-5′ phosphate, though a small spot was observed near the origin on chromatography in ethanol–M-ammonium acetate (5 : 2).

5-Bromouridine-5′ Phosphate.—Tri-*n*-octylamine (0·267 g., 1 mol.) in ethanol (10 c.c.) was added to uridine-5′ phosphate (0·245 g., 1 mol.) and the mixture evaporated to dryness and dried as previously described. The residue was dissolved in anhydrous dioxan (20 c.c.), *N*-bromosuccinimide (540 mg., 4 mol.) added with shaking, and the mixture kept at room temperature for 1 week, with occasional shaking, in a stoppered flask. Solvent was then removed under reduced pressure, the residue dissolved in ethanol and neutralised to pH 8 with triethylamine, and excess of alcoholic calcium chloride added. The precipitated calcium salt was dried, dissolved in water (7 c.c.) at pH 8 (a small amount of N-hydrochloric acid was added to effect dissolution), and filtered. Two volumes of ethanol were added and the *calcium 5-bromouridine-5′ phosphate* collected by centrifugation, washed with ethanol and ether, and dried (0·251 g., 75%), $\lambda_{max.}$ 275 mμ in N/50-HCl, density ratios 280/260 mμ = 1·41, 260/250 mμ = 1·64 (Found, in material dried at 50°/10^{-3} mm. for 12 hr.: N, 5·8; P, 6·9. $C_9H_{10}O_9N_2PBrCa$ requires N, 6·3; P, 7·0%).

Paper Chromatography.—R_F values are as follows for ascending chromatograms on Whatman No. 1 paper, the solvent being 95% ethanol–M-ammonium acetate (5 : 2): Uridine 0·64. Uridine-5′ phosphate 0·20. Uridine-5′ pyrophosphate 0·09. Uridine-2′(3′) : 5′ diphosphate 0·04. Uridine-2′ : 3′ cyclic phosphate 0·59. Uridine 2′ : 3′-(cyclic phosphate) 5′-pyrophosphate 0·05. P^1P^2-Di(uridine-5′) pyrophosphate 0·27. P^1-Uridine-5′ P^2P^2-diphenyl pyrophosphate

0·83. Uridine 2′ : 3′-(cyclic phosphate) 5′-(P^2P^2-dibenzyl pyrophosphate) 0·71. Thymidine-5′ phosphate 0·31. Thymidine-5′ pyrophosphate 0·18. Thymidine-3′ : 5′ diphosphate 0·05. Thymidine-3′ thymidine-5′ phosphate 0·65. Dithymidine dinucleotide 0·24. Adenosine 0·58. Adenosine-5′ phosphate 0·13. Adenosine-5′ pyrophosphate 0·05. Adenosine-2′(3′) : 5′ diphosphate 0·02. P^1-Adenosine-5′ P^2P^2-dibenzyl pyrophosphate 0·77. P^1-Adenosine-5′ P^2P^2-benzyl pyrophosphate 0·48.

Paper Electrophoresis.—Movement towards the anode is tabulated, for Whatman No. 1 paper, 30 cm. in length, with (I) M/50-KH_2PO_4, 750 v for 75 min.; (II) M/50-Na_2HPO_4, 600 v for 60 min.; (III) 0·05M-$H{\cdot}CO_2NH_4$ at pH 3·5, 400 v for 60 min.

	Movement (cm.)		
	I	II	III
Uridine-5′ phosphate	9·9	10·0	7·1
Uridine-5′ pyrophosphate	11·8	10·0	8·2
Uridine-2′(3′) : 5′ diphosphate	12·2	12·2	8·5
Uridine 2′ : 3′-(cyclic phosphate) 5′-pyrophosphate	14·8	11·9	9·3
Adenosine-5′ phosphate	9·2	9·6	5·2
Adenosine-5′ pyrophosphate	11·3	9·8	6·4
Adenosine-2′(3′) : 5′ diphosphate	12·7	11·1	7·0

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