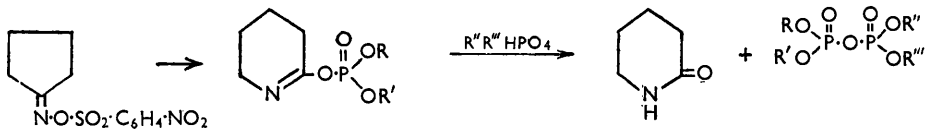
278. *Nucleotides. Part XXXV.** cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate as an Intermediate in the Synthesis of Nucleotide Derivatives: an Alternative Synthesis of Adenosine-5′ Triphosphate.*

By B. H. Chase, G. W. Kenner, Sir Alexander R. Todd, and R. F. Webb.

The recently discovered imidoyl phosphate route to esters of pyrophosphoric acid [1,2] has been successfully applied to the synthesis of tribenzyl 2′ : 3′-O-*iso*propylideneuridine-5′ pyrophosphate and hence of uridine-5′ pyrophosphate. Simultaneous production of the related symmetrical pyrophosphates could only be avoided when the initial condensation was carried out in non-polar solvents. The problems associated with the application of the method in the nucleotide field are discussed and the use of unprotected nucleotides is exemplified by syntheses of P^1-benzyl P^2-adenosine-5′ pyrophosphate and adenosine-5′ triphosphate in which the value of long-chain alkylamines for bringing nucleotides into solution has also been demonstrated.

The major nucleotide coenzymes are unsymmetrical P^1P^2-diesters of pyrophosphoric acid and we have therefore in recent years devoted considerable attention to the development of methods for pyrophosphate synthesis. In Part XIII [2] of our related series "Studies on Phosphorylation" available methods were reviewed and a new method of potential value in the coenzyme field was presented. This method depended on the Beckmann rearrangement of an oxime ester, preferably *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (I) in presence of a salt of a dialkyl phosphate, to give an imidoyl phosphate (II). The intermediate (II) was not isolated but was directly subjected to phosphorolysis producing a tetra-alkyl pyrophosphate. The method was tested in the synthesis of tetrabenzyl pyrophosphate and dibenzyl diphenyl pyrophosphate under various conditions. The present



(I) (II) (III)

paper presents the results of experiments in which the method has been applied in the nucleotide field.

The synthesis of uridine-5′ pyrophosphate, which has been thoroughly explored by using

* Part XXXIV, *J.*, 1955, 4396.

[1] Atherton, Morrison, Cremlyn, Kenner, Todd, and Webb, *Chem. and Ind.*, 1955, 1183.
[2] Kenner, Todd, and Webb, *J.*, 1956, 1231.

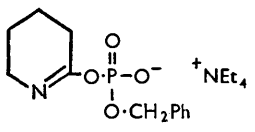
the phosphorochloridate route,[3,4] provided a convenient starting point. Benzyl 2′ : 3′-*O*-*iso*propylideneuridine-5′ phosphate [5] was converted into its tetra-*n*-butylammonium salt and then brought into reaction with *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (I). The intermediate (II; R = 2′ : 3′-O-*iso*propylideneuridine-5′; R′ = benzyl) was treated directly with dibenzyl hydrogen phosphate in benzene solution, and the protecting groups were removed from the product (III; R = 2′ : 3′-O-*iso*propylideneuridine-5′, R′ = R″ = R‴ = benzyl) by treatment with *m*-cresol [4,6] followed by hydrogenolysis in acidic solution. The final product was examined by paper electrophoresis both in disodium hydrogen phosphate and in potassium dihydrogen phosphate solution, since neither paper nor ion-exchange chromatography resolved satisfactorily mixtures of uridine-5′ pyrophosphate and P^1P^2-di(uridine-5′) pyrophosphate. It was thus established that the product contained uridine-5′ pyrophosphate (yield, 40%) and uridine-5′ phosphate as the sole nucleotidic constituents; no trace of the diuridine pyrophosphate could be detected. When, however, the same sequence of operations was performed with triethylammonium or tetraethylammonium salts of the nucleotide derivative in nitromethane the product contained both uridine-5′ pyrophosphate and P^1P^2-di(uridine-5′) pyrophosphate, the latter being the major nucleotidic component. These results confirm our earlier conclusion [2] that the oxime sulphonate route is satisfactory for the synthesis of unsymmetrical pyrophosphates provided that non-polar solvents are employed: in polar solvents exchange reactions between the imidoyl phosphate and phosphate anions (as well as similar reactions involving the unsymmetrical pyrophosphate) are favoured and the unsymmetrical pyrophosphate is obtained in a complex mixture with the two related symmetrical esters.

It was pointed out earlier that the removal of protecting groups without disrupting the molecule may be, at times, a formidable difficulty in the last stages of coenzyme synthesis, and it was therefore desirable to explore the application of the oxime sulphonate route to less fully protected nucleotides. True, the starting materials in such applications would almost certainly be insoluble in non-polar media and, indeed, special methods would have to be employed to get them into solution, even in solvents such as dimethylformamide or nitromethane. On the other hand, if free nucleotides rather than their monoesters were employed, the disadvantages of polar media might be offset to some extent by the reduced tendency to exchange reactions shown by diesters as compared with tetraesters of pyrophosphoric acid. It was found after a number of trials that tri-*n*-octylamine can be used to bring many nucleotides into solution in dimethylformamide. Benzyl adenosine-5′ phosphate,[7] an extremely insoluble nucleotide derivative, is only slightly soluble in a mixture of tri-*n*-octylamine and dimethylformamide but a suspension in this medium was treated with our reagent (I). P^1P^2-Dibenzyl P^1P^2-di(adenosine-5′) pyrophosphate was produced since P^1P^2-di(adenosine-5′) pyrophosphate [8] was obtained after debenzylation with sodium iodide.[9] The yield from this reaction was low (18%), doubtless because of the low solubility of the benzyl adenosine-5′ phosphate and the consequent rearrangement of the oxime ester (I) to the imidoyl sulphonate; it has already been shown that if the Beckmann rearrangement of the oxime ester is allowed to occur before any phosphate is added the yield of pyrophosphate is reduced.[2]

Adenosine-5′ phosphate dissolves completely in dimethylformamide containing tri-*n*-octylamine. When allowed to react in this medium with the ester (I) in presence of benzyl dihydrogen phosphate it yielded P^1-benzyl P^2-adenosine-5′ pyrophosphate (III; R = benzyl, R′ = R″ = H, R‴ = adenosine-5′) which furnished adenosine-5′ pyrophosphate on hydrogenolysis. The two symmetrical pyrophosphates were also produced in the reaction, but in a series of experiments the yield of benzyl adenosine-5′ pyrophosphate (30—40%) was rather higher than that of di(adenosine-5′) pyrophosphate (30%); a substantial amount of adenosine-5′ phosphate (30%) was recovered unchanged. It seemed

[3] Kenner, Todd, and Weymouth, *J.*, 1952, 3675.
[4] Kenner, Todd, Webb, and Weymouth, *J.*, 1954, 2288.
[5] Corby, Kenner, and Todd, *J.*, 1952, 3669.
[6] Curry, Ph.D. Thesis, Cambridge, 1952; Kenner and Mather, to be published.
[7] Baddiley and Todd, *J.*, 1947, 648.
[8] Christie, Elmore, Kenner, Todd, and Weymouth, *J.*, 1953, 2947.
[9] Cremlyn, Kenner, Mather, and Todd, to be published.

possible that formation of the symmetrical products might be greatly reduced by conducting the reaction in two stages and using tetra-alkylammonium rather than trialkylammonium salts; for, although polar solvents would be employed, an imidoyl phosphate such as (IV) would be unlikely to undergo exchange of phosphate anions in presence of another phosphate. However we were unable to detect any reaction between the salt (IV) [prepared from the ester (I) and bistetraethylammonium benzyl phosphate] and uridine-5′ phosphate. From these results we conclude that the scope of the imidoyl phosphate method in effecting the condensation of trialkylammonium salts in dimethylformamide solution is generally similar to the earlier carbodi-imide method for pyridinium salts in pyridine solution.[10]



(IV)

In any given instance one or other method may be preferred, but neither provides a direct logical synthesis of unsymmetrical P^1P^2-diesters of pyrophosphoric acid unaccompanied by symmetrical esters. The provision of such a synthetic route of general application would render the preparation of nucleotide coenzymes in quantity comparatively easy, and further experiments to this end will be reported later.

The carbodi-imide method has been applied with considerable success to the preparation of adenosine-5′ triphosphate[11] and uridine-5′ triphosphate.[12] It seemed worthwhile trying the oxime sulphonate method in the same kind of procedure, *i.e.*, condensation of adenosine-5′ phosphate with excess of orthophosphoric acid. Anion-exchange chromatography[13] showed that a 40% yield of adenosine-5′ triphosphate was produced by the reaction between adenosine-5′ phosphate (1 mol.), orthophosphoric acid (10 mols.), tri-*n*-octylamine (24 mols.), and *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (I) (7 mols.). The main difficulty in preparations of this general type lies in the separation of the desired product from the large quantities of inorganic polyphosphates produced; selective precipitation of the mercuric salts according to the classical method of Lohmann[14] leads to considerable losses. A convenient solution to the problem has been found in extraction with ethyl acetate containing tri-*n*-decylamine, the nucleotidic materials being then preferentially retained in the aqueous layer. After a few stages of countercurrent extraction, the nucleotides can be separated by anion-exchange chromatography in the usual way and the adenosine-5′ triphosphate isolated as its barium salt.

Experimental

Tetra-n-*butylammonium Benzyl* 2′ : 3′-O-iso*Propylideneuridine*-5′ *Phosphate*.—Benzyl 2′ : 3′-O-*iso*propylideneuridine-5′ phosphate[5] (0·614 g.) was added in small portions to a vigorously stirred suspension of silver carbonate (0·6 g.) in water (5 c.c.). The mixture was stirred for 15 min., then filtered, and the excess of silver carbonate was washed with water (5 c.c.) and acetone (5 c.c.). The combined filtrate and washings were refluxed for 20 min. with tetra-*n*-butylammonium iodide[15] (0·369 g., 1 mol.), the mixture was filtered, and the precipitated silver iodide washed with acetone (10 c.c.) and water (10 c.c.). The combined filtrate and washings were evaporated under reduced pressure and finally dried *in vacuo* over phosphoric oxide, yielding *tetra*-n-*butylammonium benzyl* 2′ : 3′-O-iso*propylideneuridine*-5′ *phosphate* (0·81 g., 94%) as a colourless gum slightly soluble in benzene and readily so in chloroform and ethyl methyl ketone (Found : N, 6·0; P, 4·3. $C_{35}H_{58}O_9N_3P$ requires N, 6·0; P, 4·5%).

Uridine-5′ *Pyrophosphate by Use of Tetra*-n-*butylammonium Benzyl* 2′ : 3′-O-iso*Propylideneuridine*-5′ *Phosphate*.—The above tetra-*n*-butylammonium salt (0·213 g.) in ethyl methyl ketone (1 c.c.) and benzene (1 c.c.) was treated with *cyclo*pentanone oxime *p*-nitrobenzenesulphonate[2] (0·072 g., 1 mol.), and the solution refluxed for 2 hr. and then evaporated under reduced pressure. A solution of dibenzyl hydrogen phosphate (0·069 g., 1 mol.) in benzene (2 c.c.) was then added, and the mixture refluxed for 1½ hr. and then set aside at room temperature

[10] Khorana and Todd, *J.*, 1953, 2257; Kenner, Todd, and Webb, *J.*, 1954, 2843.
[11] Khorana, *J. Amer. Chem. Soc.*, 1954, **76**, 3517.
[12] Hall and Khorana, *ibid.*, p. 5056.
[13] Cohn and Carter, *ibid.*, 1950, **72**, 4273.
[14] Lohmann, *Biochem. Z.*, 1931, **233**, 460.
[15] Hager and Manel, *J. Amer. Chem. Soc.*, 1926, **48**, 2697.

overnight. The solution was washed successively with water (2 c.c.), with 2% sodium hydrogen carbonate solution (2 c.c.), and with water (2 c.c.), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in *m*-cresol (2 c.c.), and the solution heated at 40° for 7 min. to effect partial debenzylation. Water (2 c.c.), ethanol (1 c.c.), palladium oxide (*ca.* 5 mg.), and palladised charcoal (*ca.* 5 mg. of 10%) were added and the mixture shaken overnight in an atmosphere of hydrogen. The filtered solution was extracted with ether (4 × 5 c.c.) to remove *m*-cresol, and the aqueous solution neutralised (to pH 7) with aqueous lithium hydroxide.

Examination of the neutralised solution by paper electrophoresis in 0·1M-disodium hydrogen phosphate and in 0·1M-potassium dihydrogen phosphate buffers (see below) showed that uridine-5′ phosphate and uridine-5′ pyrophosphate were the only nucleotidic materials present. Elution of the appropriate areas of the chromatograms with 0·1N-hydrochloric acid and determination of the optical densities of the solutions at 260 mμ showed the ratio of the nucleotides to be : uridine-5′ phosphate 53, uridine-5′ pyrophosphate 47%. The above neutralised solution was evaporated under reduced pressure to 1 c.c., a solution of barium acetate (0·3 g.) in water (1 c.c.) added, and the precipitate collected by centrifugation, washed successively with water (2 c.c.), ethanol (2 × 2 c.c.), and ether (2 c.c.), and dried *in vacuo* over phosphoric oxide. The product (0·16 g.) was shown by paper electrophoresis in the above-mentioned buffers to contain uridine-5′ pyrophosphate as the only nucleotidic material present. Paper chromatography in *iso*propyl alcohol–1% ammonium sulphate solution (3 : 2) confirmed the above finding and showed also the presence of inorganic pyrophosphate after spraying with the perchloric acid–ammonium molybdate reagent.[16] Determination of the ultraviolet absorption of a dilute solution of the product showed that uridine-5′ pyrophosphate accounted for 37% of the weight of the crude material : the yield of barium uridine-5′ pyrophosphate was thus 0·06 g. (40 %). The material behaved exactly as an authentic sample 4 when examined by anion-exchange, paper-chromatographic, and paper-electrophoretic methods.

Other Syntheses of Uridine-5′ Pyrophosphate using cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate.*—(1) To a solution of dibenzyl hydrogen phosphate (0·139 g.) and triethylamine (0·07 c.c., 1 mol.) in nitromethane (2·5 c.c.) *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·142 g., 1 mol.) was added and the solution kept at room temperature for 2 hr. Benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphate (0·227 g., 1 mol.) was added, the solution kept at 22° for 17 hr., and the nucleotide fraction isolated and examined by paper electrophoresis as described above; the nucleotide material had the following composition : uridine-5′ phosphate 25, uridine-5′ pyrophosphate 10, P^1P^2-di(uridine-5′) pyrophosphate 65%; all products were identified by comparison with authentic samples.

(2) A solution of dibenzyl hydrogen phosphate (0·278 g.) in water (5 c.c.) and acetone (5 c.c.) was titrated to pH 7 with tetraethylammonium hydroxide solution, then evaporated under reduced pressure, and the residue dried by repeated evaporation with benzene and nitromethane. The dry gum was dissolved in nitromethane (5 c.c.), *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·284 g., 1 mol.) added, and the solution was kept at 25° for 3 hr. Benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphate (0·454 g., 1 mol.) was now added and the solution kept at room temperature for 16 hr. The nucleotidic fraction was isolated and examined in the usual manner and shown to have the following nucleotide content : uridine-5′ phosphate 34, uridine-5′ pyrophosphate 12, P^1P^2-di(uridine-5′) pyrophosphate 44%.

(3) When benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphate (1 mol.) was converted into its tetraethylammonium salt and the latter allowed to react in nitromethane first with *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (1 mol.) and then with dibenzyl hydrogen phosphate (1 mol.) the product had the following nucleotide content : uridine-5′ phosphate 42, uridine-5′ pyrophosphate 22, P^1P^2-di(uridine-5′) pyrophosphate 36%.

Reaction of Benzyl Adenosine-5′ Phosphate with cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate.*—Benzyl adenosine-5′ phosphate[7] (0·22 g.) was suspended in a mixture of tri-*n*-octylamine (0·23 c.c.) and dimethylformamide (2·5 c.c.). *cyclo*Pentanone oxime *p*-nitrobenzenesulphonate (0·124 g., 1 mol.) was added and the suspension vigorously shaken for 16 hr., after which the undissolved benzyl adenosine-5′ phosphate (0·1 g.) was filtered off and the filtrate evaporated under reduced pressure. The residue was dissolved in ethyl methyl ketone (10 c.c.), sodium iodide (0·3 g.) added, and the solution heated to reflux : a precipitate was rapidly formed. The solid was collected, washed with acetone (10 c.c.), and dried *in vacuo* over phosphoric oxide. The solid (0·224 g.), when examined by paper chromatography with the solvent systems *iso*propyl alcohol–1% ammonium sulphate (3 : 2) and *n*-butanol–water–acetic acid (5 : 3 : 2), was

[16] Hanes and Isherwood, *Nature*, 1949, **164**, 1107.

found to contain P^1P^2-di(adenosine-5′) pyrophosphate (yield 18%) and unchanged adenosine-5′ benzyl phosphate.

Benzyl Dihydrogen Phosphate (with Dr. A. S. CURRY).—Dibenzyl hydrogen phosphate (8·0 g.) in water (125 c.c.) and ethanol (125 c.c.) was shaken in an atmosphere of hydrogen with palladium oxide (0·4 g.). The rapid uptake of hydrogen was interrupted after 700 c.c. had been absorbed. Catalyst was removed and the solution extracted with chloroform (3 × 250 c.c.). The dried (Na_2SO_4) extract was evaporated under reduced pressure, the residue dissolved in chloroform (50 c.c.), *cyclo*hexylamine (4·35 g.) added, the solution cooled to 5°, and the precipitate (7·2 g.) collected and washed with chloroform. Recrystallisation from aqueous acetone afforded colourless needles (6·4 g., 55%), m. p. 233—235°. When dried *in vacuo* at room temperature ($CaCl_2$) *bis*cyclo*hexylammonium benzyl phosphate* was obtained as the *monohydrate* (Found: C, 56·2; H, 9·1; N, 6·4. $C_{19}H_{35}O_4N_2P,H_2O$ requires C, 56·4; H, 9·2; N, 6·9%). Prolonged "drying" at 90°/1 mm. afforded cyclo*hexylammonium benzyl hydrogen phosphate*, m. p. 233—235° undepressed on admixture with the bis*cyclo*hexylammonium salt (Found: C, 54·4; H, 8·0; N, 4·7. $C_{13}H_{22}O_4NP$ requires C, 54·3; H, 7·7; N, 4·9%). The *benzyl dihydrogen ester* when regenerated from the *cyclo*hexylammonium salt, crystallised from ethanol–benzene in colourless prisms, whose m. p., though sharp, varied between 85° and 118° according to the rate of heating (Found, in material dried *in vacuo* over P_2O_5 at room temperature: C, 45·0; H, 4·7. $C_7H_9O_4P$ requires C, 44·7; H, 4·8%). When the benzyl dihydrogen ester was directly isolated from the hydrogenation of dibenzyl hydrogen phosphate without the intermediate formation of the *cyclo*hexylammonium salt it was invariably accompanied by a second substance which separated from water in colourless prisms, m. p. 188—189° and appeared from its properties to be the "quarter sodium salt" [18] of benzyl dihydrogen phosphate (Found: C, 42·3; H, 4·1. $C_{14}H_{17}O_8P_2Na$ requires C, 42·2; H, 4·3%).

Adenosine-5′ Pyrophosphate from Adenosine-5′ Phosphate, Benzyl Dihydrogen Phosphate and cyclo*Pentanone Oxime* p-*Nitrobenzenesulphonate*.—A mixture of adenosine-5′ phosphate[7] (0·035 g.,), benzyl dihydrogen phosphate (0·095 g., 5 mols.), tri-*n*-octylamine (0·212 g., 6 mols.), dry benzene (5 c.c.), and dry dimethylformamide (20 c.c.) was concentrated to 4 c.c. at atmospheric pressure, then cooled to room temperature, and *cyclo*pentanone oxime *p*-nitrobenzenesulphonate (0·12 g., 4 mols.) was added. The mixture was kept at room temperature for 16 hr. and then examined by paper chromatography on Whatman No. 1 paper, using *n*-butanol–acetic acid–water (5 : 2 : 3). Five ultraviolet absorbing areas were present in the developed chromatogram which were identified by comparison with authentic specimens as (*a*) P^1P^2-di(adenosine-5′) pyrophosphate (R_F, 0·10), (*b*) adenosine-5′ phosphate (R_F, 0·30), (*c*) *p*-nitrobenzenesulphonic acid (R_F, 0·64), and (*d*) benzyl dihydrogen phosphate and P^1 : P^2-dibenzyl pyrophosphate, the fifth area (*e*) (R_F, 0·38) being tentatively assumed to represent the hitherto unknown P^1-benzyl P^2-adenosine-5′ pyrophosphate. The reaction mixture was then run as bands on sheets of Whatman No. 1 paper in the above solvent system, and the strips (R_F, 0·38) corresponding to (*e*) above were cut out and eluted with 10% aqueous acetic acid. The extract, after concentration under reduced pressure (to 50 c.c.), was shaken with palladium oxide (20 mg.) in an atmosphere of hydrogen for 18 hr., filtered, and concentrated under reduced pressure. The concentrate was shown by paper electrophoresis in two buffer systems and by paper chromatography (see below) to contain only adenosine-5′ pyrophosphate and a trace of adenosine-5′ phosphate. The yield of P^1-benzyl P^2-adenosine-5′ pyrophosphate (and also of the adenosine-5′ pyrophosphate formed from it) was estimated in the usual way by elution of the appropriate areas of chromatograms with dilute acid and determination of the optical density of the eluates at 260 mμ. In a number of experiments the yield of P^1-adenosine-5′ P^2-benzyl pyrophosphate was 30—40% and that of the accompanying P^1P^2-di(adenosine-5′) pyrophosphate and unchanged adenosine-5′ phosphate were each *ca.* 30%.

Adenosine-5′ Triphosphate.—An anhydrous solution of tri-*n*-octylamine (6 c.c., 24 mols.) and phosphoric acid (from 1·0 g. of "88%" syrupy phosphoric acid; 10 mols.) in dimethylformamide (20 c.c.) was prepared by repeated addition and evaporation of dimethylformamide. Adenosine-5′ phosphate (0·33 g.) was then added and the solution evaporated to 12 c.c. *cyclo*Pentanone oxime *p*-nitrobenzenesulphonate (1·99 g., 7 mols.) was added to the pale yellow syrupy liquid, and the mixture was shaken until homogeneous (30 min.); the solution was kept at room temperature for 15 hr., then diluted with water (20 c.c.), and the mixture evaporated under reduced pressure to 20 c.c. A solution of barium acetate (3·0 g.) in water (28 c.c.) was added, and the precipitate collected by centrifugation, and washed successively with ethanol (20 c.c.), water (20 c.c.), aqueous ethanol (1 : 1; 20 c.c.), ethanol (20 c.c.), and ether (20 c.c.). The crude barium salt so obtained was dried *in vacuo* over phosphoric oxide

at 60° for 2 hr., giving a white powder (1·8 g.). Anion-exchange analysis of this barium salt on Dowex-2 resin (chloride form) showed that the adenosine-5′ triphosphate eluted with 0·1N-hydrochloric acid accounted for 70% of the total nucleotides present.

The above barium salt (1·7 g.) was suspended in ice-water (40 c.c.), and barium precipitated as sulphate by dropwise addition of 0·5N-sulphuric acid. The precipitate was centrifuged off and lithium chloride (1·2 g.) added to the supernatant solution which was then diluted with water to 60 c.c. The solution was used as the lower phase of tube 0 in an eight-stage manually operated counter-current separation where the phases (60 c.c. each) had been previously prepared by equilibrating a solution of tri-*n*-decylamine (8 c.c.) in ethyl acetate (100 c.c.) with a 2% aqueous solution of lithium chloride: the contents of tubes 0—4 were combined, ammonia (4 c.c.; *d* 0·88) added, and the mixture shaken for 30 min. The aqueous layer was separated and put on a column of Dowex-2 resin (chloride form). The column was first eluted with 0·05N-hydrochloric acid (2·8 l.), the eluate being discarded, and then with ice-cold 0·1N-hydrochloric acid, the eluate being collected in a flask cooled to 0°. The first 150 c.c. of the 0·1N-hydrochloric acid eluate were discarded, and the next 1800 c.c. collected and extracted twice with a solution of tri-*n*-decylamine (35 c.c.) in ethyl acetate (500 c.c.). The aqueous solution was then adjusted to pH 8 with lithium hydroxide solution, washed with ethyl acetate (200 c.c.), neutralised (pH 7) with dilute hydrochloric acid, and evaporated, *in vacuo*, to 25 c.c. Addition of barium acetate (2·0 g.) in water (20 c.c.) gave a white precipitate which was collected by centrifugation, washed successively with water, aqueous ethanol, 95% ethanol, and ether, and dried *in vacuo* over phosphoric oxide. The barium adenosine-5′ triphosphate obtained (0·108 g., 33%) was shown by paper chromatography on Whatman No. 1 paper with *iso*propyl alcohol–1% ammonium sulphate (3 : 2) to be the only nucleotidic material present. Determination of orthophosphate[17] showed negligible (0·2%) contamination (Found: N, 7·7; P total, 10·4%; P total/P labile, 1·46. Calc. for $C_{10}H_{12}N_5O_{13}P_3Ba_2,6H_2O$: N, 7·9; P total, 10·5%; P total/P labile, 1·5).

Paper-electrophoretic Data.—All migrations towards the negative pole. Buffer systems: *A*, 0·1M-potassium dihydrogen phosphate; *B*, 0·1M-disodium hydrogen phosphate. Whatman No. 4 paper was used throughout. Uridine derivatives were run for 9 hr. at 200 v: adenosine derivatives for 16 hr. at 150 v. The results are tabulated.

	Migration (cm.) in buffer:		R_F in system:	
	A	*B*	*C*	*D*
Uridine-5′ phosphate	3·7	5·1	0·64	0·29
Uridine-5′ pyrophosphate	5·9	6·0	0·47	0·16
P^1P^2-Di(uridine-5′) pyrophosphate	4·7	3·6	0·45	0·14
Uridine	0	0	—	—
Adenosine-5′ phosphate	5·1	7·8	0·43	0·30
Adenosine-5′ pyrophosphate	8·8	9·3	0·35	—
P^1P^2-Di(adenosine-5′) pyrophosphate	6·6	6·1	0·26	0·09
Adenosine-5′ benzyl phosphate	—	—	0·68	0·61
P^1-Adenosine-5′ P^2-benzylpyrophosphate	—	—	—	0·38
Adenosine-5′ triphosphate	—	—	0·20	—
p-Nitrobenzenesulphonic acid	—	—	0·83	0·66

Paper-chromatographic Data.—Solvent systems: *C*, *iso*propyl alcohol–1% ammonium sulphate (3 : 2) run on Whatman No. 1 paper previously soaked in 1% ammonium sulphate solution and dried; *D*, butan-1-ol–acetic acid–water (5 : 3 : 3) on Whatman No. 1 paper. Results are tabulated.

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[17] Allen, *Biochem. J.*, 1940, **34**, 858.
[18] Brown, Malkin, and Maliphant, *J.*, 1955, 1584.