644. *Nucleotides. Part XLI.* Mixed Anhydrides as Intermediates in the Synthesis of Dinucleoside Phosphates.*

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Adenosine-5′ uridine-5′ phosphate (II) was chosen as a model for an investigation of methods suitable for the synthesis of dinucleoside phosphates. Reactions involving condensation of nucleoside benzyl phosphorochloridates with appropriately protected nucleoside derivatives gave low yields (*ca.* 20%) whereas reaction of the phosphorochloridates with 2 : 6-lutidine diphenyl phosphate or trifluoroacetate gave the mixed anhydrides (*e.g.*, IV) which gave excellent yields (70%) of the phosphate (II). Similar mixed anhydrides (V) of nucleoside phosphites and diphenyl hydrogen phosphate were used to prepare the dinucleoside phosphites which were converted *via* the phosphorochloridate into the phosphate (II). The methods utilising mixed anhydrides are likely to be useful in the synthesis of polynucleotides.

The formulation of the natural nucleic acids as 3′ : 5′-linked polynucleotides is now generally accepted,[1] and as a result the synthesis of model compounds containing this type of structure is of particular interest. Not only can the study of such synthetic compounds confirm the interpretation of existing degradative information but it may also help to clarify such unresolved questions as the possibility of chain-branching in ribonucleic acids and provide models for an approach to the problem of nucleic acid function. In earlier papers of this series [2,3] the preparation of three such compounds, dithymidine dinucleotide, thymidine-3′ thymidine-5′ phosphate, and adenosine-2′ uridine-5′ phosphate, has been described. The method employed in each case was essentially the base-catalysed reaction of a suitably protected nucleoside benzyl phosphorochloridate (I; R = nucleoside residue) with an alcoholic hydroxyl group in another, appropriately protected nucleoside, followed by removal of all protecting groups. The yields obtained in these syntheses were rather low (8—20%), although high yields of pyrophosphates were produced by the reaction of the same phosphorochloridates with salts of dialkyl phosphates.[4] This drawback was early apparent in model experiments and the present paper records the results of some studies, begun in 1952, on alternative methods, particularly those employing mixed anhydrides, together with some more recent work which provides a route of considerable general interest and applicability.

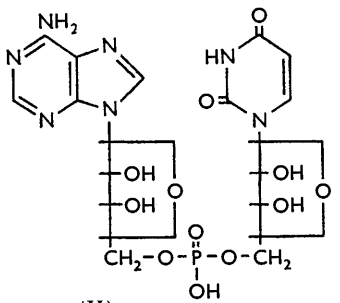
Adenosine-5′ uridine-5′ phosphate (II) provided a convenient model dinucleoside

* Part XL, *J.*, 1957, 868.

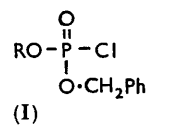
[1] Brown and Todd, *Ann. Rev. Biochem.*, 1955, **24**, 311.
[2] Michelson and Todd, *J.*, 1955, 2632.
[3] Michelson, Szabo, and Todd, *J.*, 1956, 1546.
[4] Kenner, Todd, Webb, and Weymouth, *J.*, 1954, 2288.

phosphate for the study of possible synthetic methods since it had previously been prepared in these laboratories [5] and its availability simplified its assay in reaction products by ion-exchange and paper chromatography. As a preliminary more quantitative information on the utility of the simple phosphorochloridate route was sought. To this end the reaction of benzyl 2′ : 3′-*O*-*iso*propylideneuridine-5′ phosphorochloridate [6] (I; R = 2′ : 3′-*O*-*iso*propylideneuridine-5′) with 2′ : 3′-*O*-*iso*propylideneadenosine was investigated in different solvents, the proportion and nature of the tertiary base employed being varied. Under optimum conditions for the condensation of equimolecular proportions of the two nucleoside derivatives, *viz.*, 48 hours in a mixture of benzene and methyl cyanide with 2 : 6-lutidine (5 mols.), the yield of phosphate (II) obtained after removal of protecting groups was of the order of 20%. A similar results was obtained when benzyl 2′ : 3′-*O*-*iso*propylideneadenosine-5′ phosphorochloridate and 2′ : 3′-*O*-*iso*propylideneuridine were employed; yields were somewhat higher when the 5′-sodio-derivatives of the 2′ : 3′-protected nucleosides were employed.

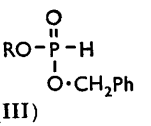
The nucleoside benzyl phosphites (III) used to prepare the corresponding phosphorochloridates were themselves obtained in high yield from the appropriately protected nucleosides by treatment with the mixed anhydride of benzyl hydrogen phosphite and diphenyl hydrogen phosphate. Clearly a similar mixed anhydride derived from a nucleoside benzyl phosphate and a stronger acid (*e.g.*, diphenyl hydrogen phosphate, trifluoroacetic or toluene-*p*-sulphonic acid) could similarly be used to prepare triesters of phosphoric acid. Mixed anhydrides of this type were prepared by Corby, Kenner, and Todd [7] by the action of tetraphenyl pyrophosphate, trifluoroacetic anhydride, or toluene-*p*-sulphonyl chloride on salts of dialkyl phosphates; the work was aimed primarily at the production of tetra-alkyl pyrophosphates by exchange reactions. For our present purpose—phosphorylation of an alcoholic hydroxyl group by the mixed anhydride—it seemed desirable therefore to start with a nucleoside benzyl phosphorochloridate and the salt of a strong acid rather than the reverse procedure, so as to minimise formation of dinucleoside pyrophosphate. When benzyl 2′ : 3′-*O*-*iso*propylideneadenosine-5′ phosphorochloridate (I; R = 2′ : 3′-*O*-*iso*propylideneadenosine-5′) was brought into reaction with diphenyl hydrogen phosphate (1 mol.), 2 : 6-lutidine (2 mols.), and 2′ : 3′-*O*-*iso*propylideneuridine (1 mol.) in a suitable solvent a product was obtained which on removal



(II)



(I)



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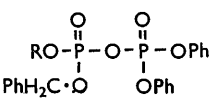

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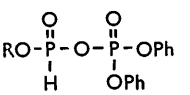

(V)

of benzyl and *iso*propylidene groups furnished adenosine-5′ uridine-5′ phosphate (II) in a yield of 70%; by increasing the proportion of uridine derivative to 2 mols. the yield rose to 87% based on phosphorochloridate. These experiments indicate that the mixed anhydride (IV; R = 2′ : 3′-*O*-*iso*propylideneadenosine-5′) reacts more smoothly than does the corresponding phosphorochloridate and that removal of the protecting groups is not a major source of loss. It was also found that the unprotected nucleoside could be used in place of the 2′ : 3′-*O*-*iso*propylidene derivative in the above reaction, the

[5] Elmore and Todd, *J.*, 1952, 3681.
[6] Kenner, Todd, and Weymouth, *J.*, 1952, 3675.
[7] Corby, Kenner, and Todd, *J.*, 1952, 3669.

primary 5′-hydroxyl group being preferentially attacked. The mixed anhydride of benzyl 2′ : 3′-*O*-*iso*propylideneuridine-5′ phosphate and trifluoroacetic acid proved less effective but this we attribute mainly to the difficulty of excluding moisture when adding the hygroscopic trifluoroacetic acid. Apart from this purely technical problem there is no obvious reason why trifluoroacetic or another strong acid should not function as well as diphenyl hydrogen phosphate; indeed the acid of choice may well vary from case to case, and Khorana, Tener, Moffatt, and Po [8] recently reported good results with toluene-*p*-sulphonic acid.

The excellent yields of phosphate obtained in the above reactions together with the well-nigh quantitative yields of phosphoramidates [9] and of tetra-alkyl pyrophosphates [6] from benzylphosphorochloridates suggest an additional reason for the losses involved in direct phosphorylation of alcohols by the latter reagents. Hitherto we had assumed that the main reason for the low yields observed in some cases was debenzylation of the phosphorochloridate by the tertiary base employed in the reaction.[10] It has been shown by Hall [11] that tetraethyl pyrophosphate and ethyl chloride are formed by the action of diethyl phosphorochloridate on triethyl phosphate. The initial product formed in phosphorylation with phosphorochloridates is a phosphotriester and benzyl groups are more readily attacked by incipient chloride ion than are ethyl groups.[12] Debenzylation of the first-formed triester by phosphorochloridate in the reaction mixture would lead to production of pyrophosphate even in absence of moisture, as has been observed.[3] This type of reaction would be less marked with mixed anhydrides of dialkyl phosphates and stronger oxy-acids, and more in evidence with phosphorobromidates.

An alternative route to dinucleoside phosphates using mixed anhydrides in which dinucleoside phosphites are intermediate products has been devised. Anionic debenzylation of protected nucleoside-5′ benzyl phosphites with 4-methylmorpholinium thiocyanate readily yielded the corresponding salts of nucleoside phosphites. These salts reacted smoothly with diphenyl phosphorochloridate, forming mixed anhydrides (V; R = protected nucleoside residue) which were not isolated but directly brought into reaction with a suitably protected nucleoside derivative, giving dinucleoside phosphites in high yield. 2′ : 3′-*O*-*iso*Propylideneadenosine-5′ 2′ : 3′-*O*-*iso*propylideneuridine-5′ phosphite prepared in this way was converted into the dinucleoside phosphate (II) by treatment with *N*-chlorosuccinimide followed by hydrolysis; more direct methods for phosphite–phosphate conversion are being studied.

Attempts have been made at various times in these laboratories to remove acetyl or *iso*propylidene groups from protected nucleoside benzyl phosphites but in every case preferential removal of the phosphite group occurred. This lability of the phosphite group can apparently be overcome by preliminary debenzylation; removal of the *iso*propylidene group from 4-methylmorpholinium 2′ : 3′-*O*-*iso*propylideneuridine-5′ phosphite with 80% acetic acid was accompanied by some decomposition but there appeared to be little loss of phosphorous acid when the corresponding 2′ : 3′-di-*O*-acetyl compound was deacetylated with methanolic ammonia. The use of such unprotected phosphites for the preparation of both polynucleotides and their phosphite analogues is under investigation.

Experimental

Formation and Assay of Adenosine-5′ Uridine-5′ Phosphate from Nucleoside Phosphorochloridates.—(*a*) A solution of benzyl 2′ : 3′-*O*-*iso*propylideneadenosine-5′ phosphite (0·924 g.) and *N*-chlorosuccinimide (0·266 g., 1 mol.) in benzene (8 c.c.) and methyl cyanide (7 c.c.) was kept at room temperature for 3 hr. and then 2′ : 3′-*O*-*iso*propylideneuridine (0·57 g., 1 mol.)

[8] Khorana, Tener, Moffatt, and Po, *Chem. and Ind.*, 1956, 1523.
[9] Atherton, Openshaw, and Todd, *J.*, 1945, 382.
[10] Atherton and Todd, *J.*, 1947, 676.
[11] Hall, *Ind. Eng. Chem.*, 1948, **40**, 694.
[12] Lecoq and Todd, *J.*, 1954, 2381.

and 2 : 6-lutidine (1 c.c., 5 mols.) were added. The solution was set aside for 48 hr., then evaporated, and the residue dissolved in a mixture of ethanol (5 c.c.) and N/50-sulphuric acid (35 c.c.). The solution was boiled under reflux for 1½ hr., cooled, and neutralised with the calculated quantity of barium hydroxide solution. The mixture was filtered through " Hyflo-Supercel," and the combined filtrate and washings (total 70 c.c.) were adjusted to pH 8 with dilute ammonia solution and run on to a column (12·5 cm. × 10 sq. cm.) of Dowex-2 resin (250—500 mesh; formate cycle). The column was washed with water (200 c.c.) and then eluted with 0·02N- (540 c.c.) and 0·05N-formic acid (680 c.c.). Adenosine-5′ uridine-5′ phosphate was eluted by 0·05N-acid. Fractions having an optical density at 260 mμ greater than 0·2 × max. optical density were combined, concentrated under reduced pressure, and finally freeze-dried to a hygroscopic fluffy powder (0·214 g., 18·5%). The product was identical with an authentic specimen of adenosine-5′ uridine-5′ phosphate, having the same optical density ratio D_{280}/D_{260} = 0·3 and identical paper chromatographic behaviour (see Table).

(*b*) A solution of benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite (0·342 g.) and *N*-chlorosuccinimide (0·104 g., 1 mol.) in benzene (10 c.c.) was kept for 2 hr., then evaporated *in vacuo*, and the residue, dissolved in pyridine (3 c.c.), was added to a solution of 2′ : 3′-*O-iso*propylideneadenosine (0·25 g., 1 mol.), in pyridine (4 c.c.) previously cooled to −30°. The clear solution was kept just above its m. p. (*ca.* −30°) for 12 hr., then at room temperature for 48 hr., evaporated *in vacuo*, and dissolved in ethanol (15 c.c.) and N/50-sulphuric acid (50 c.c.). The solution was then treated as described in (*a*). The yield of adenosine-5′ uridine-5′ phosphate was shown to be approx. 15% : P^1P^2-di(uridine-5′) pyrophosphate (12%) was also produced in this reaction.

(*c*) A solution of 2′ : 3′-*O-iso*propylideneadenosine (1 g.) in redistilled liquid ammonia (15 c.c.) was treated with sodium (0·075 g., 1 mol.), the solvent evaporated, and the residual 5′-sodio-derivative collected and stored *in vacuo* over phosphoric oxide. To a solution of this sodio-derivative (0·28 g.) in dimethylformamide (5 c.c.) was added a solution of benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphorochloridate (1 mol.) in methyl cyanide (2 c.c.) prepared as described in (*b*) above from benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphate (0·37 g., 1 mol.) and *N*-chlorosuccinimide (0·135 g., 1 mol.). After 48 hr. the solution was worked up as previously described and gave a 25% yield of adenosine-5′ uridine-5′ phosphate.

Formation and Assay of Adenosine-5′ Uridine-5′ Phosphate by the " Mixed Anhydride " Route.—(a) *Mixed anhydride with diphenyl hydrogen phosphate.* (i) A solution of benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphorochloridate in benzene (3 c.c.), prepared as described above from benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite (0·105 g.) and *N*-chlorosuccinimide (0·034 g., 1·05 mol.), was treated with 2′ : 3′-*O-iso*propylideneadenosine (0·147 g., 2 mols.), 2 : 6-lutidine (0·055 c.c., 2 mols.), diphenyl hydrogen phosphate (0·060 g., 1 mol.), and dimethylformamide (2 c.c.). The solution was kept at 37° for 24 hr., then evaporated, and the residue refluxed for 1½ hr. with ethanol (7 c.c.) and N/50-sulphuric acid (25 c.c.). After cooling and neutralisation with barium hydroxide solution the mixture was filtered and the filtrate shown by paper chromatography to contain adenosine-5′ uridine-5′ phosphate, uridine-5′ phosphate, and adenosine. Separation was achieved by ion-exchange chromatography as described previously and the adenosine-5′ uridine-5′ phosphate isolated as a fluffy hygroscopic powder (0·117 g., 87% based on benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite).

(ii) In a similar experiment, reaction of benzyl 2′ : 3′-*O-iso*propylideneadenosine-5′ phosphorochloridate [from the corresponding phosphite (0·258 g.) and *N*-chlorosuccinimide (0·076 g., 1 mol.)] with 2′ : 3′-*O-iso*propylideneuridine (0·16 g., 1 mol.), diphenyl hydrogen phosphate (0·14 g., 1 mol.), and 2 : 6-lutidine (0·12 c.c., 2 mols.) in benzene (5 c.c.) and methyl cyanide (2·8 c.c.), gave a yield of adenosine-5′ uridine-5′ phosphate shown by paper chromatography to be 70%.

(iii) When a solution of benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphorochloridate [from the corresponding phosphite (0·102 g.) and *N*-chlorosuccinimide (0·033 g., 1 mol.)] in benzene (3 c.c.) was similarly treated with adenosine (0·125 g., 2 mols.), diphenyl hydrogen phosphate (0·0582 g., 1 mol.), 2 : 6-lutidine (0·056 c.c., 2 mols.), and dimethylformamide (3 c.c.) the yield of the di(nucleoside-5′) phosphate was 75%.

(b) *Mixed anhydride with trifluoroacetic acid.* When benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphorochloridate [from benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite (0·192 g.) and *N*-chlorosuccinimide (0·06 g., 1 mol.)] in benzene (5 c.c.) was treated with 2′ : 3′-*O-iso*propylideneadenosine (0·162 g., 1 mol.), 2 : 6-lutidine (0·1 c.c., 2 mols.), trifluoroacetic acid (0·032 c.c.,

1 mol.), and methyl cyanide (2 c.c.) the product was shown to contain adenosine-5′ uridine-5′ phosphate (32%), adenosine, and an unidentified nucleotide.

4-Methylmorpholinium Thiocyanate (with Dr. F. J. WEYMOUTH).—A solution of 4-methylmorpholine (26 c.c.) and ammonium thiocyanate (13 g.) in water (130 c.c.) was refluxed until no more ammonia was evolved (6 hr.). On cooling, the crystalline product (13·4 g., 49%) was filtered off, dried, and recrystallised from ethyl methyl ketone, giving *4-methylmorpholinium thiocyanate* as colourless plates, m. p. 103° (Found, in material dried at 50°/1 mm. over P_2O_5: C, 45·1; H, 7·4; N, 17·4. $C_6H_{12}ON_2S$ requires C, 45·0; H, 7·5; N, 17·5%).

*4-Methylmorpholinium 2′ : 3′-O-iso*Propylideneadenosine-5′ *Phosphite.*—A solution of benzyl 2′ : 3′-*O-iso*propylideneadenosine-5′ phosphite (0·6 g.) and 4-methylmorpholinium thiocyanate (0·2 g., 1·1 mols.) in ethyl methyl ketone (5 c.c.) was refluxed for 2 hr., then cooled, and the supernatant liquid decanted from the yellow gum produced. The gum was heated with methyl cyanide (2 c.c.) and ethyl methyl ketone (2 c.c.), then cooled, benzene (2 c.c.) added, and the semi-solid precipitate separated by decantation, washed with benzene (8 c.c.), and dried *in vacuo* over phosphoric oxide, affording *4-methylmorpholinium 2′ : 3′-O-iso*propylideneadenosine-5′ *phosphite* as a white hygroscopic powder (Found: C, 45·7; H, 5·5; P, 6·9. $C_{18}H_{29}O_7N_6P$ requires C, 45·8; H, 6·1; P, 6·6%). Paper chromatography in butan-1-ol–water–acetic acid (5 : 3 : 2) showed the only component with selective ultraviolet absorption to be the required phosphite (R_F 0·70), although traces of monobenzyl phosphite (R_F 0·85—0·95) were present. Similar results were obtained by using the system propan-2-ol—1% ammonium sulphate (3 : 2) on Whatman No. 1 paper previously soaked in 1% ammonium sulphate solution and dried; the R_F values were: nucleoside phosphite, 0·90; monobenzyl phosphite, 0·95. Anionic debenzylation of benzyl 2′ : 3′-di-*O*-acetyladenosine-5′ phosphite with 4-methylmorpholinium thiocyanate gave a glass with similar behaviour on paper chromatography.

*4-Methylmorpholinium 2′ : 3′-O-iso*Propylideneuridine-5′ *Phosphite.*—A solution of benzyl 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite (18 g.) and 4-methylmorpholinium thiocyanate (6·2 g., 1·1 mols.) in ethyl methyl ketone (150 c.c.) was refluxed for 4 hr., the cooled, cloudy solution diluted with benzene (100 c.c.), and the supernatant liquid decanted from the oily precipitate. The residue was heated with ethyl methyl ketone (50 c.c.), then cooled, and the supernatant layer discarded. The gummy product was dried *in vacuo* over phosphoric oxide, giving a colourless foam (6·67 g.) free from thiocyanate. Paper chromatography in butan-1-ol–water–acetic acid (5 : 3 : 2) showed one component with selective ultraviolet absorption (R_F 0·66), while spraying for phosphate showed a trace of monobenzyl hydrogen phosphite. In the system propan-2-ol–1% ammonium sulphate (3 : 2) the major component had R_F 0·95. Unsatisfactory analytical values were obtained on the hygroscopic glass but the product was shown to be essentially pure 4-methylmorpholinium 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite by its conversion in high yield into 2′ : 3′-*O-iso*propylideneadenosine-5′ 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite as outlined below. Benzyl 2′ : 3′-di-*O*-acetyluridine-5′ phosphite on treatment with 4-methylmorpholinium thiocyanate gave a similar hygroscopic gum which is considered to be 4-methylmorpholinium 2′ : 3′-di-*O*-acetyluridine-5′ phosphite.

Removal of Protecting Groups from Nucleoside Phosphite Salts.—4-Methylmorpholinium 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite was heated with 80% acetic acid for 30 min.; the solution was evaporated *in vacuo* and examined by paper chromatography in the system butan-1-ol–water–acetic acid (5 : 3 : 1), on Whatman No. 1 paper. In addition to uridine (R_F 0·5) and phosphorous acid (R_F 0·59) a component was obtained (R_F 0·32) which showed selective ultraviolet absorption, contained phosphorus, and gave a positive test for an α-glycol grouping with the periodate spray reagent. This component is considered to be uridine-5′ phosphite. Elution of the spots from the chromatogram and comparison of the intensities of ultraviolet absorption at 260 mμ indicated a uridine : uridine phosphite ratio of 1 : 1. Treatment of 4-methylmorpholinium 2′ : 3′-di-*O*-acetyluridine-5′ phosphite with saturated methanolic ammonia followed by evaporation and paper chromatography showed that deacetylation occurred; the product with R_F 0·32 in the system used above was now the major product (uridine : uridine phosphite ratio 1 : 9).

2′ : 3′-O-iso*Propylideneadenosine-5′ 2′ : 3′-O-iso*Propylideneuridine-5′ *Phosphite.*—The crude 4-methylmorpholinium 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite obtained as described above (1·34 g.) was dissolved in methyl cyanide (20 c.c.) and rapidly added to a stirred solution of diphenyl phosphorochloridate (0·52 c.c., 1 mol.) in methyl cyanide (5 c.c.). After 1 hr., 2 : 6-lutidine (0·33 c.c., 1 mol.) and 2′ : 3′-*O-iso*propylideneadenosine (0·42 g., 0·5 mol.) were

added, and the solution was stirred for 2 hr. and then evaporated. The residue was dissolved in chloroform (20 c.c.), washed with water, saturated sodium hydrogen carbonate solution, saturated potassium hydrogen sulphate solution, and again with water, and dried (Na_2SO_4). The chloroform solution was evaporated to *ca.* 2 c.c. and the solution poured into *n*-pentane (100 c.c.). The white precipitate was redissolved in chloroform (2 c.c.) and again precipitated by *n*-pentane (80 c.c.), collected by centrifugation, and dried *in vacuo* over phosphoric oxide, giving 2′ : 3′-O-iso*propylideneadenosine*-5′ 2′ : 3′-O-iso*propylideneuridine*-5′ *phosphite* (0·81 g.) as a white powder (Found : C, 47·2, H, 5·0; N, 14·2. $C_{25}H_{32}O_{11}N_7P$ requires C, 47·1; H, 5·0; N, 15·0%). Ultraviolet absorption in EtOH : $\lambda_{max.}$ 260 mμ; $\lambda_{min.}$ 235 mμ. Paper chromatography in propan-2-ol–1% ammonium sulphate solution (3 : 2) showed only one component (R_F 0·87) to be present.

2′ : 3′-Di-*O*-acetyladenosine-5′ 2′ : 3′-di-*O*-acetyluridine-5′ phosphite was prepared in an analogous way from 4-methylmorpholinium 2′ : 3′-di-*O*-acetyluridine-5′ phosphite and 2′ : 3′-di-*O*-acetyladenosine.

Adenosine-5′ Uridine-5′ Phosphate.—*N*-Chlorosuccinimide (0·133 g.) was added to a solution of 2′ : 3′-*O-iso*propylideneadenosine-5′ 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite (0·636 g., 1 mol.) in methyl cyanide (20 c.c.). After 6 hr. a solution of sodium hydrogen sulphite (1 g.) in water (20 c.c.) was added together with methyl cyanide (30 c.c.), and the solution stirred for 12 hr. The solution was evaporated to remove the organic solvent, water (to 50 c.c.) added, and the pH adjusted to 1 with 3N-hydrochloric acid. The solution was rapidly extracted with chloroform (3 × 50 c.c.), the combined chloroform extracts were evaporated and the residue was refluxed for 1½ hr. with ethanol (10 c.c.) and N/50-sulphuric acid (35 c.c.). Sulphate was removed by addition of the calculated quantity of barium hydroxide solution, the barium sulphate centrifuged off, and the supernatant liquid adjusted to pH 8·5 with aqueous ammonia and run on to a column (6 cm. × 3 sq. cm.) of Dowex-2 resin (200—400 mesh; formate cycle). Elution as previously described with 0·08N-formic acid, followed by concentration and freeze-drying of fractions with optical density greater than 0·2 × max. and with optical density ratio $D_{280}/D_{260} = 0·3 \pm 0·02$, gave a white solid (0·246 g.) (Found, in material dried at 100°/0·1 mm. for 16 hr.: C, 39·5; H, 3·7; N, 16·9. Calc. for $C_{19}H_{24}O_{12}N_7P$: C, 39·8; H, 4·0; N, 17·0%). The material was directly compared with an authentic specimen of adenosine-5′ uridine-5′ phosphate by paper chromatography (see Table). The ultraviolet absorption spectrum was identical with that previously described having $\lambda_{max.}$ 260 mμ, $\lambda_{min.}$ 231 mμ in N/100-sulphuric acid, and $\lambda_{max.}$ 260 mμ, $\lambda_{min.}$ 234 mμ in N/100 sodium hydroxide. The dinucleoside phosphate was prepared in a similar way from 2′ : 3′-di-*O*-acetyladenosine-5′ 2′ : 3′-di-*O*-acetyluridine-5′ phosphite and *N*-chlorosuccinimide, followed by hydrolysis of the phosphorochloridate and removal of the acetyl groups with saturated methanolic ammonia.

Paper Chromatography of Nucleotide Derivatives.—Ascending chromatograms on Whatman No. 1 paper. Solvent systems : I, butan-1-ol–acetic acid–water (5 : 2 : 3); II, propan-2-ol–1% ammonium sulphate (3 : 2); III, butan-1-ol–acetic acid–water (5 : 1 : 3). Results are tabulated.

	R_F in solvent system		
	I	II	III
Uridine-5′ phosphate	0·28	0·64	
Adenosine-5′ phosphate	0·32	0·43	
Adenosine-5′ uridine-5′ phosphate	0·22	0·57	
P^1P^2-Di(uridine-5′) pyrophosphate	0·15	0·47	
Uridine-5′ phosphite	—	—	0·32
2′ : 3′-*O-iso*Propylideneadenosine-5′ phosphite	0·70	0·90	
2′ : 3′-*O-iso*Propylideneuridine-5′ phosphite	0·66	0·95	
2′ : 3′-*O-iso*Propylideneadenosine-5′ 2′ : 3′-*O-iso*propylideneuridine-5′ phosphite	0·89	0·87	
Benzyl hydrogen phosphite	0·85—0·95	0·95	

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