704. *Nucleotides. Part XVI.* Ribonucleoside-5′ Phosphites. A New Method for the Preparation of Mixed Secondary Phosphites.*

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Solutions of *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (VII), prepared by the interaction of a salt of monobenzyl phosphite and diphenyl chlorophosphonate or tetraphenyl pyrophosphate, react with alcohols in the presence of base, yielding alkyl benzyl phosphites. The constitutions of 2′ : 3′-*iso*propylidene uridine-5′ and 2′ : 3′-*iso*propylidene adenosine-5′ benzyl phosphites, prepared in this way, have been demonstrated by conversion of these esters into the corresponding *p*-bromobenzylaminophosphonates and benzyl hydrogen phosphates.

The key to successful synthesis in the polynucleotide and the nucleotide coenzyme fields clearly lies in the development of methods for linking together dissimilar molecules (in particular nucleoside molecules) through phosphate or pyrophosphate residues, and such methods have been sought in an extensive series of investigations in this laboratory during recent years. In many respects the two problems overlap and it seems probable that any method suitable for one might, with certain modifications, be applied to the other. In considering the problem of the synthesis of dinucleoside pyrophosphates, a solution to which would give a route to the nucleotide coenzymes (*e.g.*, cozymase, flavin adenine dinucleotide), several possibilities are apparent. One of them involves exchange reactions between

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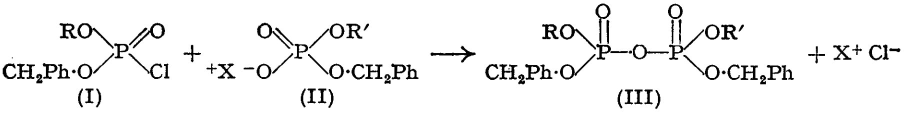
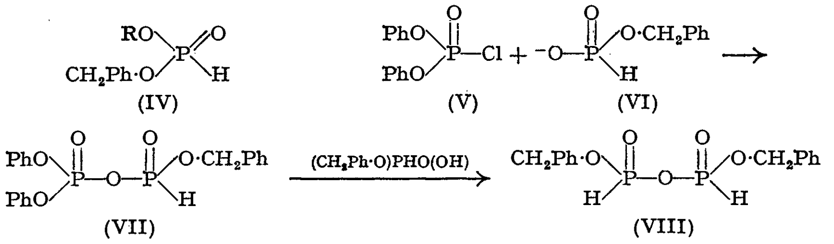
* Part XV, preceding paper.

a nucleoside pyrophosphate, or a mixed anhydride of a nucleotide with some stronger acid, and the salt of a monoester of a nucleotide, and model experiments on the preparation of simple pyrophosphates by this method have already been reported (Corby, Kenner, and Todd, *J.*, 1952, 1234). A second route might utilise the reaction between a 5′-halogeno-5′-deoxynucleoside and a nucleoside dibenzyl pyrophosphate. A model synthesis indicating the possibility of realising such a reaction exists in the synthesis of uridine-5′ pyrophosphate from 5′-iodo-5′-deoxyuridine already described (Part XV, Anand, Clark, Hall, and Todd, preceding paper); this route would, of course, only be applicable in a limited number of cases since adenosine and cytidine undergo conversion into *cyclo*nucleoside derivatives when attempts are made to prepare from them the corresponding 5′-iodo-compounds (Part VIII, Clark, Todd, and Zussman, *J.*, 1951, 2952). The third and most obvious method would be to utilise the well-known and almost universally applied route to pyrophosphates in which a dialkyl (or diaryl) chlorophosphonate is treated with the salt of a diester of phosphoric acid. In practice this would mean reaction of a nucleoside benzyl chlorophosphonate (I; R = nucleoside residue) with the salt of a nucleoside benzyl hydrogen phosphate (II; R′ = nucleoside residue) to give a tetraester (III) of pyrophosphoric acid, from which the protecting benzyl groups could subsequently be removed. Hitherto this method could not be applied since it was not possible to prepare nucleoside benzyl chlorophosphonates (cf. Mason and Todd, *J.*, 1951, 2267). Experiments directed to the solution of the latter problem form the subject of the present communication.



The most satisfactory method for the preparation of dialkyl or diaralkyl chlorophosphonates is the chlorination of diesters of phosphorous acid, but before this method could be explored in the present instance it was necessary to have available nucleoside benzyl phosphites (IV; R = nucleoside residue). Such compounds have not hitherto been prepared and, indeed, very little is known about mixed secondary phosphites of any type (cf. Kosolapoff, *J. Amer. Chem. Soc.*, 1951, **73**, 4989); we therefore sought a general method for their preparation. In experiments bearing on the exchange reactions of mixed anhydrides of diesters of phosphoric acid (Corby, Kenner, and Todd, *loc. cit.*) it had been consistently observed that, in reaction with an amine, these mixed anhydrides always gave the aminophosphonate corresponding to the weaker of the two parent acids from which the anhydride was derived; thus, dibenzyl diphenyl pyrophosphate always yielded with ammonia dibenzyl aminophosphonate. It therefore seemed to us probable that a mixed anhydride (VII) of monobenzyl phosphite and diphenyl phosphate would prove a suitable reagent for the preparation of mixed secondary phosphites since it should, by analogy with



the pyrophosphates, react with an alcohol (ROH) in presence of a tertiary base to form the ester (IV) of the weaker acid (phosphorous acid). It may be mentioned that in the absence of a systematic nomenclature for polyacids of the phosphorus series the naming of (VII) presents some difficulty. For the present at any rate it would seem simplest to use a "mixed anhydride" form of nomenclature for compounds of this and similar types; (VII) would thus be named *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride.

The mixed anhydride (VII) was first prepared by allowing diphenyl chlorophosphonate

(V) to react with monobenzyl phosphite (VI) (Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815). The product was a thick resin which, like many of the anhydrides of oxy-acids of phosphorus, was unstable to moisture or heat, or to ammonia and amines generally. Purification by distillation was impossible and so the product was always used directly in the crude state. It was not analysed since in any case elementary analysis of mixed anhydrides has little value in view of their marked tendency to disproportionate, giving mixtures of the corresponding symmetrical substances (cf. Corby, Kenner, and Todd, *loc. cit.*). Indeed the only satisfactory evidence for the identity of any such anhydride is the demonstration that it can be converted in good yield into a product whose structure is known and which could only have been produced from the anhydride in question. This method was employed in the present instance. The anhydride (VII) reacted with benzyl alcohol in presence of one equivalent of 2 : 6-lutidine, giving the expected dibenzyl phosphite, easily identified by conversion into the crystalline dibenzyl aminophosphonate (Atherton and Todd, *J.*, 1947, 674). The yield of dibenzyl phosphite was some 61%, calculated on the assumption that the starting material was pure *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (VII). The mixed anhydride (VII) could also be prepared by allowing tetraphenyl pyrophosphate to react with a salt of monobenzyl phosphite, but the purity of the product was evidently lower since the yield of dibenzyl phosphite obtainable from it by reaction with benzyl alcohol was only 46%, calculated on the above basis.

That the reagent (VII) could be used satisfactorily for the preparation of mixed secondary phosphites was demonstrated by treating it with *p*-nitrobenzyl alcohol in presence of one equivalent of 2 : 6-lutidine. The product, benzyl *p*-nitrobenzyl phosphite (IV; R = $NO_2{\cdot}C_6H_4{\cdot}CH_2$), was not isolated as such but was chlorinated directly and the crude chlorophosphonate produced treated directly (*a*) with aqueous sodium hydrogen carbonate, yielding benzyl *p*-nitrobenzyl hydrogen phosphate, and (*b*) with *cyclo*hexylamine, giving benzyl *p*-nitrobenzyl *cyclo*hexylaminophosphonate. Attention was next turned to the preparation of nucleoside benzyl phosphites. From the reaction between the mixed anhydride (VII) and 2′ : 3′-*iso*propylidene uridine in acetonitrile solution in presence of base, a resinous product was obtained. This appeared homogeneous on inspection of its paper chromatograms in ultra-violet light, but the molybdate reagent (Hanes and Isherwood, *Nature*, 1949, **164**, 1107) revealed the presence of a second more mobile phosphorus-containing substance. Owing to contamination with this impurity, the nature of which is discussed below, the nitrogen analysis of the resin was lower than that calculated for 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite (IV; R = 2′ : 3′-*iso*propylidene uridine-5′ residue), but the evidence of its reactions left no doubt that it was essentially this substance. It appeared to be stable in cold chloroform solution towards water and saturated aqueous potassium hydrogen sulphate or sodium hydrogen carbonate; it was, however, very unstable towards dilute acids or alcoholic ammonia, the phosphite group undergoing hydrolysis. For identification it was brought into reaction with carbon tetrachloride and *p*-bromobenzylamine, a compound having the composition and behaviour of 2′ : 3′-*iso*propylidene uridine-5′ benzyl *p*-bromobenzylaminophosphonate being obtained in good yield. When equimolecular proportions of nucleoside, base, and mixed anhydride were used in its preparation the yield of 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite was only moderate. A series of experiments was made, varying the quantities of the individual reactants, and it was finally established that optimum yields were obtained when the proportion nucleoside : base : mixed anhydride was 1 : 2 : 2. Reaction was usually complete in 2—3 hours but no drop in yield was noticed if the reaction mixture was kept overnight. When the same reaction was carried out with 2′ : 3′-*iso*propylidene adenosine in place of the corresponding uridine derivative, 2′ : 3′-*iso*propylidene adenosine-5′ benzyl phosphite (IV; R = 2′ : 3′-*iso*propylidene adenosine-5′ residue) was obtained as a hygroscopic glass very similar in properties to the uridine analogue; it could, however, be freed from the non-nitrogenous impurity by precipitation from benzene with *cyclo*hexane, being obtained as an amorphous solid of the correct composition. Treated with carbon tetrachloride and *p*-bromobenzylamine it gave the expected 2′ : 3′-*iso*propylidene adenosine-5′ benzyl *p*-bromobenzylaminophosphonate.

These results were encouraging but, in order to establish that the nucleoside derivatives obtained really were phosphites of type (IV), it was necessary to show that they could be converted into the related phosphates accessible by well-established procedures. This entailed the preparation from the phosphites of the corresponding chlorophosphonates, products which would be in any case required if the ultimate object of the investigation—the preparation of dinucleoside pyrophosphates or dinucleoside phosphates—was to be achieved. A satisfactory procedure for this purpose was devised by Mr. F. J. Weymouth (see Kenner, Todd, and Weymouth, succeeding paper) in which chlorination of a secondary phosphite is carried out in neutral solution with *N*-chlorosuccinimide. This reagent evidently converted 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite into 2′ : 3′-*iso*propylidene uridine-5′ benzyl chlorophosphonate, since, when it was shaken with aqueous sodium hydrogen carbonate solution and the aqueous solution was acidified, the product gave 2′ : 3′-*iso*propylidene uridine-5′ benzyl hydrogen phosphate, an amorphous solid with the same R_F value as material prepared by Mr. S. M. H. Christie from 2′ : 3′-*iso*propylidene uridine-5′ dibenzyl phosphate (Michelson and Todd, *J.*, 1949, 2476) by debenzylation with 4-methylmorpholine (Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815). On further treatment with acid this material gave crystalline uridine-5′ benzyl hydrogen phosphate having the correct composition and consumption of periodate. In a similar manner amorphous 2′ : 3′-*iso*propylidene adenosine-5′ benzyl hydrogen phosphate and crystalline adenosine-5′ benzyl hydrogen phosphate were prepared from the correspondng phosphite and compared directly with authentic specimens.

The phosphorus-containing impurity, which did not absorb ultra-violet light, evidently did not interfere greatly with use of the nucleoside phosphites, but nevertheless its nature was of interest. A plausible hypothesis was that it was dibenzyl pyrophosphite (VIII) produced by attack of the anion of monobenzyl phosphite on the mixed anhydride (VII), during its preparation. This symmetrical anhydride (VIII) might have sufficiently low reactivity to survive washing with aqueous solutions of potassium hydrogen sulphate and sodium hydrogen carbonate. The reaction between two equivalents of monobenzyl triethylammonium phosphite and one of diphenyl chlorophosphonate should have led to (VIII) and, in fact, a small amount of oil remained after washing of the benzene solution with water and aqueous sodium hydrogen carbonate solution. This oil was, however, dibenzyl phosphite (IV; $R = CH_2Ph$). Moreover, when the washing with sodium hydrogen carbonate solution was omitted, a larger quantity of neutral oil was obtained, which developed acidity when kept at room temperature. We therefore conclude that dibenzyl pyrophosphite (VIII) is slowly transformed into dibenzyl phosphite and, presumably, metaphosphorous acid. Mixed secondary phosphites (IV) prepared by the method described here will thus always tend to be contaminated with dibenzyl phosphite, since the production of some (VIII) in the preparation of (VII) cannot be avoided. Likewise the reaction mixture in the preparation of (VIII) probably contained some (VII) which was destroyed by washing with water.

We consider that these experiments establish that derivatives of the ribonucleoside-5′ benzyl phosphites can be prepared through the agency of *OO*-diphenylphosphoric *O*-benzylphosphorous anhydride, and that by chlorination with *N*-chlorosuccinimide the corresponding nucleoside benzyl chlorophosphonates are produced from them. The accessibility of such chlorophosphonates should open up a number of routes to both phosphates and polyphosphates containing one or more nucleoside residues, and experiments designed to realise such routes will be reported in later papers.

EXPERIMENTAL

All m. p.s are corrected. Monobenzyl phosphite was prepared as a pale yellow thick oil by acidifying cold aqueous solutions of its ammonium salt (Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815) with dilute hydrochloric acid, extracting the mixture with chloroform, and evaporating the dried extract. It is stable in a nitrogen atmosphere but slowly decomposes when exposed to air for several days.

O-*Benzylphosphorous* OO-*Diphenylphosphoric Anhydride* (VII).—*Method* 1. Monobenzyl phosphite (0·195 g., 1 mol.) and diphenyl chlorophosphonate (0·24 c.c., 1 mol.) were dissolved in dry benzene (15 c.c.). The solution, from which air and moisture were excluded, was stirred magnetically and a solution of anhydrous pyridine (0·09 c.c., 1 mol.) in dry benzene (5 c.c.) was gradually added. Pyridine hydrochloride began to separate after 2—3 minutes and after being stirred for 3 hours the mixture was filtered. No attempt was made to isolate the mixed anhydride (in other experiments it was obtained as an unstable resin by evaporating the solution) but its presence was demonstrated in the following way. Benzyl alcohol (0·12 c.c., 1 mol.) and 2 : 6-lutidine (0·13 c.c., 1 mol.) were added to the solution which was set aside at room temperature overnight, then washed with water, saturated aqueous sodium hydrogen carbonate, and saturated aqueous potassium hydrogen sulphate solutions, and finally dried (Na_2SO_4) and evaporated. The residue was dissolved in dry carbon tetrachloride, and gaseous ammonia passed through the solution for 20 minutes. Ammonium chloride (60 mg.) was removed by filtration and the filtrate concentrated to small bulk, whereupon dibenzyl aminophosphonate separated, having m. p. and mixed m. p. 101—103° (0·191 g., 61%).

Method 2. Monobenzyl phosphite (0·174 g., 1 mol.) was dissolved in dry methyl cyanide (10 c.c.), and 2 : 6-lutidine (0·115 c.c., 1 mol.) was added, followed by tetraphenyl pyrophosphate (0·518 g., 1 mol.; Corby, Kenner, and Todd, *loc. cit.*) in methyl cyanide (3 c.c.). After 1 hour the solution was evaporated and the residue dissolved in dry benzene (10 c.c.). To this solution, containing the mixed anhydride (VII), benzyl alcohol (0·11 c.c., 1 mol.) and 2 : 6-lutidine (0·115 c.c., 1 mol.) were added and the whole was set aside overnight. The resulting solution was then treated as in method 1 above and yielded dibenzyl aminophosphonate, m. p. and mixed m. p. 101—103° (0·125 g., 46%). When the amount of tertiary base used in the initial reaction with monobenzyl phosphite was increased to 2 mols. the yield of dibenzyl aminophosphonate fell to 26%.

Attempted Preparation of Dibenzyl Pyrophosphite (VIII).—Monobenzyl phosphite (1·54 g., 2 mols.) and diphenyl chlorophosphonate (0·93 c.c., 1 mol.) were dissolved in dry benzene (20 c.c.), and triethylamine (1·26 c.c., 2 mols.) was added gradually. After 6 hours triethylamine hydrochloride was filtered off and the liquors were washed with water (4 × 10 c.c.) before division into two equal parts.

(*a*) Evaporation of the solvent after drying (Na_2SO_4) gave an initially neutral oil (0·38 g.), which became strongly acid overnight.

(*b*) After washing with sodium hydrogen carbonate solution (2 × 15 c.c.), drying (Na_2SO_4), and evaporation there remained an oil (0·13 g.), which was neutral and did not develop acidity. This was identified as dibenzyl phosphite by conversion in 75% yield by means of *cyclo*hexylamine and carbon tetrachloride into dibenzyl *cyclo*hexylaminophosphonate, m. p. 79—81°, and by elementary analysis (Found: C, 63·6; H, 5·5; P, 11·9. Calc. for $C_{14}H_{15}O_3P$: C, 64·2; H, 5·7; P, 11·9%).

Inspection of paper chromatograms failed to reveal any reaction of either of the oils described in (*a*) and (*b*) above with 2′ : 3′-*iso*propylidene uridine in presence of 2 : 6-lutidine.

Dibenzyl p-*Bromobenzylaminophosphonate*.—This compound, required as a reference substance, was prepared by adding *p*-bromobenzylamine (1·1 g.; cf. Nystrom and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3738) to a solution of dibenzyl phosphite (0·77 g.) in carbon tetrachloride (15 c.c.). Heat was evolved on mixing and after 1½ hours the mixture was heated to boiling and filtered hot, and the filtrate evaporated. The residue was recrystallised from light petroleum (b. p. 60—80°), giving *dibenzyl* p-*bromobenzylaminophosphonate* (1·2 g.) as fine colourless needles, m. p. 98—100° (Found: C, 56·6; H, 4·3; N, 3·2. $C_{21}H_{21}O_3NBrP$ requires C, 56·5; H, 4·7; N, 3·1%).

Benzyl p-*Nitrobenzyl Phosphite*.—Crude *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (VII) (prepared from 1·76 g., 2 mols., of monobenzyl phosphite by method 1 above), dissolved in benzene (20 c.c.)–methyl cyanide (10 c.c.), was treated with *p*-nitrobenzyl alcohol (0·8 g., 1 mol.) and 2 : 6-lutidine (1·17 c.c., 2 mols.) at room temperature. After 45 minutes the mixture was evaporated and the residue dissolved in chloroform (30 c.c.). The solution was washed successively with water, saturated aqueous sodium hydrogen carbonate, saturated aqueous potassium hydrogen sulphate, and water, dried (Na_2SO_4), and evaporated, giving crude benzyl *p*-nitrobenzyl phosphite (1·43 g.) as a thick yellowish oil with an odour very similar to that of dibenzyl phosphite. Since it decomposed on attempted distillation it was not analysed but its identity was demonstrated as follows. (*a*) One half of the above product was dissolved in benzene (7 c.c.) and methyl cyanide (3 c.c.), and *N*-chlorosuccinimide (0·31 g.) added. After 2 hours excess of aqueous sodium hydrogen carbonate was added to the chlorophosphonate solution produced, and the whole stirred vigorously for 8 hours, then left overnight. The mixture was extracted with chloroform (10 c.c.), and the aqueous layer was acidified and extracted with chloroform (3 × 20 c.c.). The chloroform extract was dried (Na_2SO_4) and evaporated, giving a pale yellow oil which gradually solidified. Recrystallised from chloroform–light petroleum (b. p. 60—80°), *benzyl* p-*nitrobenzyl hydrogen phosphate* was obtained as colourless needles (54%), m. p. 108—110° (Found: C, 52·0; H, 4·6; N, 4·6. $C_{14}H_{14}O_6NP$ requires C, 52·1; H, 4·3; N, 4·3%).

(*b*) The other half of the crude phosphite was treated as above with *N*-chlorosuccinimide and the product allowed to react with *cyclo*hexylamine (0·57 c.c., 2 mols.). Worked up in the usual way the reaction mixture gave *benzyl* p-*nitrobenzyl* cyclo*hexylaminophosphonate*, which crystallised from light petroleum (b. p. 60—80°) as colourless needles, m. p. 90—92° (Found: C, 59·5; H, 5·9; N, 7·1. $C_{20}H_{25}O_3N_2P$ requires C, 59·5; H, 6·2; N, 7·0%).

2′ : 3′-iso*Propylidene Uridine-5′ Benzyl Phosphite*.—*O*-Benzylphosphorous *OO*-diphenylphosphoric anhydride (VII) (prepared from 0·618 g., 2 mols., of monobenzyl phosphite by method 1 above and evaporation of solvent in the frozen state) was mixed with a solution of 2′ : 3′-*iso*propylidene uridine (0·52 g., 1 mol.; dried at 70°/0·1 mm. for 12 hours over phosphoric oxide) and 2 : 6-lutidine (0·42 c.c., 2 mols.) in methyl cyanide (25 c.c.), and the mixture was set aside for 3 hours. Solvent was removed by evaporation, the residue taken up in chloroform (25 c.c.), and the solution washed with water, saturated aqueous sodium hydrogen carbonate, potassium hydrogen sulphate, and again water, dried (Na_2SO_4), and evaporated; the crude phosphite so obtained (0·68 g.) was an almost colourless resin. On chromatography on Whatman No. 1 paper in *n*-butanol saturated with water, this showed a single spot absorbing ultra-violet radiation, containing phosphorus, and having R_F 0·8. A second minor spot contained phosphorus, but was not detected by ultra-violet light, and had the same R_F value, 0·9, as dibenzyl phosphite.

2′ : 3′-iso*Propylidene Uridine-5′ Benzyl* p-*Bromobenzylaminophosphonate*.—The phosphite prepared in the preceding experiment was dissolved in carbon tetrachloride containing 5% of methyl cyanide. On addition of *p*-bromobenzylamine (2 mols.), heat was evolved and *p*-bromobenzylamine hydrochloride separated immediately. After 3 hours the solution was filtered, washed with water, dried (Na_2SO_4), and evaporated. The p-*bromobenzylaminophosphonate* remained as a stable glass, containing bromine and having R_F 0·91 on Whatman No. 1 paper in *n*-butanol saturated with water (Found: C, 49·9; H, 4·5; N, 6·6; P, 5·3. $C_{26}H_{29}O_8N_3BrP$ requires C, 50·1; H, 4·7; N, 6·8; P, 5·0%).

2′ : 3′-iso*Propylidene Uridine-5′ Benzyl Hydrogen Phosphate*.—*N*-Chlorosuccinimide (0·335 g., 1 mol.; Tscherniac, *Ber.*, 1901, **34**, 4213) was added to a solution of 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite (1·02 g., 1 mol.) in methyl cyanide (25 c.c.). After 2 hours an equal volume of a saturated aqueous solution of sodium hydrogen carbonate was added and the mixture stirred vigorously during 6 hours and then kept overnight. Methyl cyanide was distilled under reduced pressure from the liquors, which were acidified (Congo-red) with hydrochloric acid and extracted with chloroform (5 × 10 c.c.). Evaporation of the dried (Na_2SO_4) extracts left a hygroscopic glass, which was transformed into a colourless amorphous solid (0·87 g., 83% from 2′ : 3′-*iso*propylidene uridine) by pouring a concentrated chloroform solution into a large volume of dry ether. Like the 2′ : 3′-*iso*propylidene uridine-5′ benzyl hydrogen phosphate prepared by Mr. S. M. H. Christie, this had R_F 0·75 in *n*-butanol (5 volumes)–water (3 volumes)–acetic acid (1 volume) on Whatman No. 4 paper.

Uridine-5′ Benzyl Hydrogen Phosphate.—The foregoing *iso*propylidene derivative (0·100 g.) was kept during 15 hours with a 5% solution (1 c.c.) of hydrogen chloride in methanol. When the mixture was poured into dry ether (50 c.c.) turbidity developed and small rosettes of crystals (0·031 g.) were deposited. The *uridine-5′ benzyl hydrogen phosphate* had m. p. 129—132°, R_F 0·61 in *n*-butanol (5 volumes)–water (3 volumes)–acetic acid (2 volumes), and consumed 0·94 mol. of periodate ion per mol. during 24 hours at room temperature (Found: C, 46·5; H, 4·7; N, 6·8; P, 7·5. $C_{16}H_{19}O_9N_2P$ requires C, 46·4; H, 4·6; N, 6·8; P, 7·5%).

2′ : 3′-iso*Propylidene Adenosine-5′ Benzyl Phosphite*.—A solution of *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (2 mols.) in benzene (30 c.c.) was prepared from monobenzyl phosphite (2·01 g.), diphenyl chlorophosphonate (2·42 c.c.), and triethylamine (1·65 c.c.). To this was added directly 2′ : 3′-*iso*propylidene adenosine (1·85 g., 1 mol.; dried during 12 hours at 90—100°/0·1 mm.), 2 : 6-lutidine (1·3 c.c., 2 mols.), and methyl cyanide (3 c.c.). When shaken at room temperature the mixture became homogeneous in about 10 minutes. After a further 20 minutes the solvents were evaporated and the residual oil was taken up in chloroform (30 c.c.) and washed twice with an equal volume of water, twice with 30 c.c. portions of saturated potassium hydrogen sulphate solution, and once with saturated sodium hydrogen carbonate solution. Evaporation of the dried (Na_2SO_4) solvent left the product (2·36 g.) as a stiff gum, the main component of which had R_F 0·77 in *n*-butanol saturated with water on Whatman No. 1 paper and had an ultra-violet absorption maximum at 260 mμ. The minor component resembled dibenzyl phosphite and had R_F 0·9. The gum was taken up in dry benzene (15 c.c.) and poured into dry *cyclo*hexane (200 c.c.). The pure *phosphite* was separated by decantation as a heavy amorphous solid (1·9 g., 68%) (Found, in material dried at room temperature: C, 51·0; H, 5·2; N, 14·8; P, 6·7. $C_{20}H_{24}O_6N_5P,\frac{1}{2}H_2O$ requires C, 51·1; H, 5·3; N, 14·9; P, 6·6%).

2′ : 3′-iso*Propylidene Adenosine-5′ Benzyl* p-*Bromobenzylaminophosphonate*.—*p*-Bromobenzylamine (0·635 g., 2·5 mols.) was added to a solution of 2′ : 3′-*iso*propylidene adenosine-5′ benzyl phosphite (0·64 g., 1 mol.) in carbon tetrachloride (6 c.c.). The *p*-bromobenzylamine hydrochloride, which was rapidly precipitated, was removed by filtration after 2 hours and the filtrate was evaporated. The residual gum was dissolved in chloroform, which was washed with water, dilute hydrochloric acid, and saturated sodium hydrogen carbonate solution before drying (Na_2SO_4). The pale yellow stable glass (0·69 g.) obtained on evaporation was converted into a white amorphous *product* (0·505 g., 46% from 2′ : 3′-*iso*propylidene adenosine) by pouring a solution in chloroform (1 c.c.) into ether (50 c.c.) and then pouring a solution of the precipitated solid in benzene (1 c.c.) into *cyclo*hexane (50 c.c.) (Found: C, 50·3; H, 5·2; N, 12·9; P, 5·2. $C_{27}H_{30}O_6N_6BrP$ requires C, 50·3; H, 4·7; N, 13·0; P, 4·8%).

2′ : 3′-iso*Propylidene Adenosine-5′ Benzyl Hydrogen Phosphate*.—This was prepared from 2′ : 3′-*iso*propylidene adenosine-5′ benzyl phosphite in the same way as the uridine analogue as an amorphous solid (54% from 2′ : 3′-*iso*propylidene adenosine), having R_F 0·64 in *n*-butanol (4 volumes)–water (5 volumes)–acetic acid (1 volume) on Whatman No. 4 paper like an authentic specimen prepared by Mr. S. M. H. Christie from 2′ : 3′-*iso*propylidene adenosine-5′ dibenzyl phosphate (Baddiley and Todd, *J.*, 1947, 648).

Adenosine-5′ Benzyl Hydrogen Phosphate.—A solution of the *iso*propylidene derivative (0·14 g.) from the preceding preparation in ethanol (3 c.c.) was boiled during 1 hour with 0·02N-sulphuric acid (10 c.c.). When the solution cooled, adenosine-5′ benzyl hydrogen phosphate (0·08 g.) separated, having m. p. 228—230° alone or mixed with an authentic sample (Baddiley and Todd, *loc. cit.*).

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