705. *Nucleotides. Part XVII.** N-*Chloroamides as Reagents for the Chlorination of Diesters of Phosphorous Acid. A New Synthesis of Uridine-5′ Pyrophosphate.*

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N-Chlorosuccinimide and *N* : 2 : 4-trichloroacetanilide are shown to be satisfactory reagents for the preparation of dibenzyl and dialkyl chlorophosphonates from dibenzyl and dialkyl phosphites. 2′ : 3′-*iso*Propylideneuridine-5′ benzyl phosphite has been chlorinated with *N*-chlorosuccinimide and from the product, by reaction with triethylammonium dibenzyl hydrogen phosphate and subsequent removal of protecting groups, uridine-5′ pyrophosphate has been synthesised. The pyrophosphate isolated as its barium salt has been shown to be identical with the major component of " barium uridine diphosphate " obtained from natural sources by direct comparison on paper chromatograms using several different solvent systems.

AMONG the theoretically possible methods for the synthesis of polynucleotides and nucleotide coenzymes which can be represented by the formulæ RO•PO(OH)•OR′ and RO•PO(OH)–O–PO(OH)•OR′ respectively, in which at least one of the groups R and R′ is a purine or pyrimidine nucleoside residue, the most obvious are those which employ nucleoside benzyl chlorophosphonates as intermediates. From the latter compounds the

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* Part XVI, preceding paper.

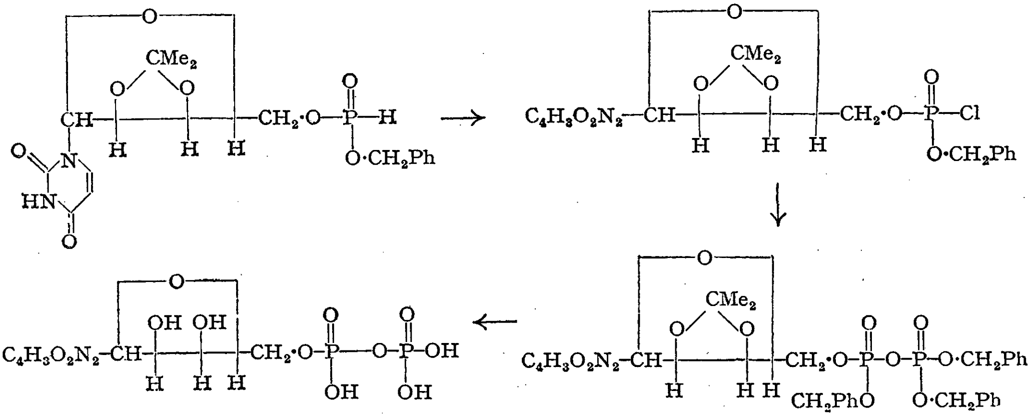
polynucleotides or nucleotide coenzymes might be synthesised by well-established methods of phosphate and pyrophosphate preparation developed over a period of years in this laboratory. It was with this end in view that a study of methods for preparing nucleoside benzyl phosphites was undertaken; the development of a successful method employing mixed anhydrides of phosphorous and phosphoric acid derivatives has been reported in Part XVI.* Concurrently it was necessary to seek methods for the conversion of secondary phosphites into chlorophosphonates under conditions which would be applicable to the nucleoside benzyl phosphites. The present paper describes work leading to the discovery of such a method and the practical application of a nucleoside benzyl chlorophosphonate in the synthesis of uridine-5′ pyrophosphate.

Dialkyl chlorophosphonates were first prepared from dialkyl phosphites by direct action of chlorine at room temperature, the hydrogen chloride evolved being removed by blowing air through the reaction mixture or by treatment with lead carbonate (McCombie, Saunders, and Stacey, *J.*, 1945, 380). The same procedure was used for preparing the less stable dibenzyl chlorophosphonate (Atherton, Openshaw, and Todd, *J.*, 1945, 382), although here it was necessary to work at a low temperature and to use an inert diluent. A somewhat milder reagent was later found in sulphuryl chloride which chlorinates dibenzyl phosphite readily at room temperature (Atherton, Howard, and Todd, *J.*, 1948, 1106). Neither of these methods seemed suitable for our purposes, however, since large amounts of hydrogen chloride are produced in each and nucleoside benzyl phosphites are extremely labile to acids (Corby, Kenner, and Todd, Part XVI). The reaction between dialkyl phosphites and carbon tetrachloride in presence of bases (Atherton, Openshaw, and Todd, *J.*, 1945, 660; Atherton and Todd, *J.*, 1947, 674) yields dialkyl chlorophosphonates as initial products and Steinberg (*J. Org. Chem.*, 1950, **15**, 637) has adapted this method to their preparation and isolation by allowing simple dialkyl phosphites to react with carbon tetrachloride in presence of only a small amount of a tertiary base. When applied to 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite (Part XVI, *loc. cit.*), this procedure was disappointing owing to extensive side reactions (cf. Atherton, Openshaw, and Todd, *loc. cit.*).

Some years ago Dr. F. R. Atherton in this laboratory observed that when a solution of dibenzyl phosphite in an inert solvent was treated with *N*-bromosuccinimide and then with an amine good yields of the corresponding dibenzyl aminophosphonate were obtained, clearly as a result of the formation of dibenzyl bromophosphonate in the initial reaction. The greater instability of bromophosphonates compared with chlorophosphonates, and the readiness with which *N*-bromosuccinimide attacks pyrimidine nucleosides (uridine being brominated readily at room temperature), made this reagent unattractive and attention was directed to the less reactive *N*-chloroamides, a number of which have been examined by Ziegler (*Annalen*, 1942, **551**, 80) as potential chlorinating agents in other fields. For convenience in use a stable crystalline reagent was desirable and *N*-chlorosuccinimide (Tscherniac, *Ber.*, 1901, **34**, 4213) and *N* : 2 : 4-trichloroacetanilide (Chattaway and Orton, *J.*, 1899, **75**, 1046) were chosen for study. In the absence of diluent both compounds reacted violently with dibenzyl phosphite, considerable heat being evolved, but in suitable inert solvents (*e.g.*, carbon tetrachloride, benzene, chloroform, ether or methyl cyanide) reaction proceeded smoothly with little rise in temperature. When using *N*-chlorosuccinimide it was found desirable to use about 20 volumes of solvent and the progress of the reaction could be followed by the separation of fine crystals of succinimide which replaced the denser *N*-chlorosuccinimide. With dibenzyl phosphite reaction was complete in about five minutes although in practice a longer reaction time was allowed; the yield of chlorophosphonate, determined by the usual assay methods, was almost quantitative. With diethyl phosphite the reaction was slower, but could be accelerated by halving the volume of solvent used, whereupon the mixture warmed spontaneously to about 50°. When *N* : 2 : 4-trichloroacetanilide was used as chlorinating agent more concentrated solutions were required (5 vols. of solvent) and in ether or benzene the 2 : 4-dichloroacetanilide produced separated quantitatively during the reaction. Although less reactive than *N*-chlorosuccinimide, *N* : 2 : 4-trichloroacetanilide may be a valuable reagent in the preparation of water-soluble phosphates or pyrophosphates from phosphites, since both it and the 2 : 4-dichloroacetanilide produced from it are virtually insoluble in water and can thus be readily separated from the desired reaction products. The application of *N*-chloroamides in the preparation of a number of model substances from diesters of phosphorous acid is described in the Experimental section.

When applied to nucleoside benzyl phosphites the chlorination method using *N*-chlorosuccinimide gave excellent results, very little in the way of undesirable by-products being obtained (Part XVI, *loc. cit.*). *N*-Chlorosuccinimide does attack some nucleosides slowly, however, and the use of excess of chlorinating agent for prolonged periods is inadvisable. When a solution of 2′ : 3′-*iso*propylidene uridine in methyl cyanide was kept at room temperature for 7 days with *N*-chlorosuccinimide (1·5 mols.) or for 2 days with the same reagent in presence of triethylamine, a considerable amount of a new substance, presumably 2′ : 3′-*iso*propylidene 5-chlorouridine, was produced.

In Part XVI of this series (*loc. cit.*) examples were given of the synthesis of some simple



nucleotides *via* the *iso*propylidene nucleoside benzyl phosphites. As a further development of the use of these compounds it was desirable to synthesise a nucleoside pyrophosphate. As an example uridine-5′ pyrophosphate (uridine diphosphate, UDP) was selected in view of its importance as a breakdown product of the coenzyme uridine-diphosphate-glucose (UDPG) concerned in the conversion of galactose-1 phosphate into glucose-1 phosphate (Caputto, Leloir, Cardini, and Paladini, *J. Biol. Chem.*, 1950, **184**, 333). Moreover the original synthesis of this compound (Part XV; Anand, Clark, Hall, and Todd, *J.*, 1952, 3665), although not very convenient for preparation of the pyrophosphate in quantity, had provided the necessary data which would permit ready identification of intermediate and final products by paper-chromatographic methods. Crude 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite prepared by a slight modification of the previous procedure (Part XVI, *loc. cit.*) was treated with *N*-chlorosuccinimide, and the product brought into reaction with triethylammonium dibenzyl phosphate. The resinous 2′ : 3′-*iso*propylidene uridine-5′ tribenzyl pyrophosphate so obtained was treated with lithium chloride in 2-ethoxyethanol to effect partial debenzylation. The product, which appeared to consist mainly of the lithium salt of 2′ : 3′-*iso*propylidene uridine-5′ benzyl pyrophosphate contaminated with lithium uridine-5′ benzyl pyrophosphate and some non-nucleosidic impurities derived from the original phosphite preparation, was hydrogenated to remove the remaining benzyl group (cf. Anand, Clark, Hall, and Todd, *loc. cit.*), hydrolysed with acid to remove the *iso*propylidene residue, and converted into its barium salt. Removal of barium phosphate and barium uridine-5′ phosphate from this material by re-precipitation yielded the pure barium uridine-5′ pyrophosphate, from which lithium uridine-5′ pyrophosphate was also prepared. The purified barium salt was shown to be identical with the synthetic product described by Anand *et al.* (*loc. cit.*) and with the main component of a sample of " barium uridine diphosphate " prepared from natural UDPG and kindly supplied by Dr. Leloir. The new synthesis was relatively efficient, for the purified barium uridine-5′ pyrophosphate was obtained in 25% overall yield from uridine.

EXPERIMENTAL

M. p.s are corrected.

Dibenzyl cyclo*Hexylaminophosphonate.*—Pure dibenzyl phosphite (1·3 g., 1 mol.) was dissolved in dry benzene (20 c.c.), and *N*-chlorosuccinimide (0·67 g., 1 mol.; Tscherniac, *loc. cit.*) was added. The solution became warm and succinimide separated rapidly. After 2 hours at room temperature the succinimide was filtered off through a dry sintered-glass funnel, and *cyclo*hexylamine (1·28 c.c., 2·1 mols.) was added to the filtrate. The solution was set aside for a further hour, *cyclo*hexylamine hydrochloride filtered off (0·61 g., 91%), and the filtrate washed with water (50 c.c.), saturated sodium hydrogen sulphate (50 c.c.) solution, and water (50 c.c.). The solution was dried and evaporated, giving a crystalline mass of dibenzyl *cyclo*hexylaminophosphonate (1·64 g.; m. p. 69—75°). Recrystallisation from *n*-hexane gave fine colourless needles (1·42 g., 80%), m. p. 77—79°, undepressed on admixture with an authentic specimen (m. p. 79—80°; Atherton, Openshaw, and Todd, *loc. cit.*).

Tetrabenzyl Pyrophosphate.—Pure dibenzyl phosphite (1·95 g., 1 mol.) was dissolved in dry benzene (30 c.c.), and *N*-chlorosuccinimide (0·99 g., 1 mol.) was added. The solution was set aside for 2 hours at room temperature, moisture being excluded. Dibenzyl hydrogen phosphate (2·07 g., 1 mol.) was next added, followed by triethylamine (1·04 c.c., 1 mol.). After a further 2 hours the precipitated succinimide and triethylamine hydrochloride were filtered off and washed with benzene. The combined filtrate and washings (50 c.c.) were washed with water (100 c.c.), saturated sodium hydrogen carbonate solution (100 c.c.), and water (100 c.c.), and dried (Na_2SO_4). Evaporation gave a mobile oil (3·72 g.) which solidified when shaken with water (20 c.c.) and seeded. After drying over phosphoric oxide a colourless crystalline product was obtained (3·6 g., 90%), m. p. 59—62°. Recrystallisation from ether–*cyclo*hexane gave colourless needles, m. p. 61—63°, undepressed on admixture with an authentic specimen of tetrabenzyl pyrophosphate (m. p. 60—61°; Atherton and Todd, *J.*, 1947, 674).

Diethyl Anilinophosphonate.—Diethyl phosphite (2·25 g., 1 mol.) was dissolved in benzene (20 c.c.), and *N*-chlorosuccinimide (2·18 g., 1 mol.) was added. The temperature slowly rose to about 50°, and succinimide separated. Reaction was slower than with dibenzyl phosphite, about 10 minutes being required for disappearance of the *N*-chlorosuccinimide. After 2 hours the precipitated succinimide was filtered off through a dry sintered-glass funnel, and aniline (3·13 c.c., 2·1 mols.) was added to the filtrate. Aniline hydrochloride separated rapidly and after a further 2 hours it was filtered off (1·8 g., 86%) and washed with benzene. The benzene filtrate and washings (50 c.c.) were washed with water (30 c.c.), N-hydrochloric acid (30 c.c.), saturated sodium hydrogen carbonate solution (30 c.c.), and water (30 c.c.), and dried (Na_2SO_4). Evaporation of the filtered solution gave a crystalline residue (3·27 g., 87%), m. p. 90—94°, which, recrystallised from aqueous ethanol, gave fine needles, m. p. 95—96°, undepressed on admixture with an authentic specimen of diethyl anilinophosphonate (m. p. 96·5°; McCombie, Saunders, and Stacey, *loc. cit.*).

Dibenzyl Hydrogen Phosphate.—Dibenzyl phosphite (2·22 g., 1 mol.) was dissolved in benzene (10 c.c.), and *N* : 2 : 4-trichloroacetanilide (2·66 g., 1·3 mols.; Chattaway and Orton, *loc. cit.*) was added. Warming to about 40° initiated a vigorous reaction and 2 : 4-dichloroacetanilide crystallised rapidly. The solution was set aside at room temperature for 2 hours, and the 2 : 4-dichloroacetanilide (1·79 g.) was filtered off and washed with benzene. Water (6 c.c.) and 2 : 6-lutidine (20 c.c.) were added to the combined filtrate and washings, and the mixture was set aside for 3 days at room temperature. Removal of solvents under reduced pressure gave a brown oil which was diluted with water (50 c.c.) and neutralised (phenolphthalein) with aqueous sodium hydroxide. The solution was extracted with chloroform (2 × 40 c.c.) and acidified with hydrochloric acid, producing a heavy oily emulsion which was extracted with chloroform (3 × 20 c.c.). The chloroform extract was dried (Na_2SO_4) and evaporated under reduced pressure, to give a pale yellow syrup which on being seeded with dibenzyl hydrogen phosphate set to a mass of crystals (2 g., 83%; m. p. 75—78°). Recrystallisation from chloroform–pentane gave pure dibenzyl hydrogen phosphate (1·83 g., 76%), m. p. 79—81°, undepressed on admixture with an authentic sample (m. p. 78—79°; Lossen and Köhler, *Annalen*, 1891, **262**, 209).

2′ : 3′-iso*Propylidene Uridine-5′ Benzyl Phosphite.*—A solution of ammonium monobenzyl phosphite (3·5 g.; Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815) in water (30 c.c.) was acidified with hydrochloric acid (10 c.c.; 3N) and extracted rapidly with chloroform (2 × 25 c.c.). The chloroform extract was dried (Na_2SO_4) and evaporated, to give monobenzyl hydrogen phosphite (2·43 g.) as a mobile oil which was dried overnight at 0·1 mm. over phosphoric oxide.

The monobenzyl phosphite was taken up in dry benzene (15 c.c.), and diphenyl chlorophosphonate (2·9 c.c., 1 mol.) was added. This solution was stirred magnetically while a solution of triethylamine (1·98 c.c., 1 mol.) in benzene (10 c.c.) was added dropwise during 10 minutes. After a further hour's stirring the resulting solution of *OO*-diphenylphosphoric *O*-benzylphosphorous anhydride was freed from triethylamine hydrochloride by filtration through a dry sintered-glass funnel and 2′ : 3′-*iso*propylidene uridine (2·02 g., 0·5 mol.; Levene, and Tipson, *J. Biol. Chem.*, 1934, **106**, 131), 2 : 6-lutidine (1·65 c.c., 1 mol.) and methyl cyanide (10 c.c.) were added. The 2′ : 3′-*iso*propylidene uridine dissolved rapidly. The solution was set aside at room temperature for 30 minutes, then evaporated, to yield a viscous syrup which was dissolved in chloroform (70 c.c.) and washed with water (70 c.c.), saturated aqueous sodium hydrogen carbonate (70 c.c.), saturated aqueous potassium hydrogen sulphate (70 c.c.), and water (2 × 70 c.c.). The dried chloroform solution was evaporated under reduced pressure, to give crude 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite as a pale yellow syrup (3·34 g.).

Paper chromatography in *n*-propanol–water (85 : 15) showed the only component showing selective ultra-violet absorption to be the required phosphite (R_F 0·84), although spraying for phosphate (Hanes and Isherwood, *Nature*, 1949, **164**, 1107) revealed an additional phosphorus-containing component (R_F 0·90) (cf. Part XVI, *loc. cit.*).

Dilithium 2′ : 3′-iso*Propylidene Uridine*-5′ *Benzyl Pyrophosphate*.—Crude 2′ : 3′-*iso*propylidene uridine-5′ benzyl phosphite (3·34 g.; prepared as above) and *N*-chlorosuccinimide (1·02 g., 1 mol.) were dissolved in a mixture of dry benzene (50 c.c.) and methyl cyanide (10 c.c.) and set aside for 2 hours. Dibenzyl hydrogen phosphate (2·11 g., 1 mol.) and triethylamine (1·07 c.c., 1 mol.) were then added. There was immediate precipitation of triethylamine hydrochloride and after 2 hours at room temperature the solution was washed with water (100 c.c.), saturated aqueous sodium hydrogen carbonate (70 c.c.), hydrochloric acid (70 c.c.; N/50), and water (70 c.c.), and dried (Na_2SO_4). Evaporation of the solution at reduced pressure gave a pale yellow resin (4·41 g.), similar to the uridine-5′ tribenzyl pyrophosphate prepared by Anand, Clark, Hall, and Todd (*loc. cit.*). In each case paper chromatography in *n*-propanol–water (85 : 15) gave a single ultra-violet light absorbing spot of R_F 0·87, trailing to R_F 0·55, the lower R_F value corresponding to that of 2′ : 3′-*iso*propylidene uridine-5′ benzyl hydrogen phosphate formed by hydrolysis of the pyrophosphate. This resin was dissolved in a solution of freshly fused lithium chloride (1·28 g., 5 mols.) in dry 2-ethoxyethanol (25 c.c.) and heated at 100° for 2 hours to effect debenzylation. When cool, the solution was diluted with dry ethanol (20 c.c.) and poured into dry ether (80 c.c.), producing a flocculent white precipitate, which was centrifuged off, washed with dry acetone (2 × 80 c.c.) and dry ether (70 c.c.), and finally dried under reduced pressure at 60°; crude dilithium 2′ : 3′-*iso*propylidene uridine-5′ benzyl pyrophosphate (2·7 g.) was thus obtained as a hygroscopic white powder.

Paper chromatography of the product in butanol–acetic acid–water (50 : 10 : 40; upper layer) gave two main spots which absorbed ultra-violet light and contained phosphorus (R_F 0·17 and 0·40). When sprayed with neutral sodium periodate followed by Schiff's reagent (Buchanan, Dekker, and Long, *J.*, 1950, 3162), only the slower of these gave a positive reaction, indicating that the substance responsible for it contained a free α-glycol system, *i.e.*, that it had lost the 2′ : 3′-*iso*propylidene group. Very small amounts of slower-moving materials were also present, together with a considerable amount of faster-running phosphate impurity (R_F 0·55), possibly derived from the unknown contaminant of the uridine phosphite (see above). In 95% ethanol–ammonia (*d* 0·88) (2·5 : 1) the main products ran on paper chromatograms with R_F 0·57 and 0·64 respectively.

Barium Uridine-5′ *Pyrophosphate*.—The above crude lithium salt (1·6 g.) was dissolved in water (114 c.c.), acidified with hydrochloric acid (6 c.c. of N/10, giving a final concentration of N/200), and hydrogenated overnight at atmospheric pressure with a mixture of palladous oxide (Adams's catalyst, 0·2 g.) and palladised charcoal (0·2 g.; 10%). Catalyst was removed by filtration and washed thoroughly with water, and the combined filtrate and washings (190 c.c.) were treated with hydrochloric acid (6 c.c. of 3N, giving a final concentration of N/10) and set aside at room temperature overnight. After the solution had been brought to pH 7·1 with lithium hydroxide, barium bromide (1·31 g.) in water (5 c.c.) was added, the gelatinous precipitate of barium phosphate produced was filtered off through " Hyflo Supercel," and the filtrate evaporated to 80 c.c. under reduced pressure. A slight precipitate was centrifuged off and, after addition of more barium bromide (0·6 g.), the crude barium uridine-5′ pyrophosphate was precipitated by ethanol (2 vols). The gelatinous precipitate was centrifuged off, washed with acetone (100 c.c.) and ether (100 c.c.), and dried at 60° under reduced pressure, to give a fine white powder (1 g.). Paper chromatography in *iso*propyl alcohol–1% ammonium sulphate solution (60 : 40) showed that this material contained, in addition to uridine-5′ pyrophosphate, small amounts of uridine-5′ phosphate (R_F 0·47) and of 2′ : 3′-*iso*propylidene uridine pyrophosphate (R_F 0·64). Optical-density measurements at 260 mμ on eluted spots showed the approximate composition to be uridine-5′ pyrophosphate 83%, uridine-5′ phosphate 7%, and 2′ : 3′-*iso*propylidene uridine-5′ pyrophosphate 10%.

The above crude barium salt (0·98 g.) was dissolved in 0·1N-hydrochloric acid (22 c.c.) and set aside at room temperature overnight. The filtered solution was then brought to pH 8 by addition of saturated aqueous barium hydroxide, and precipitation was completed by adding two volumes of ethanol. The precipitate was centrifuged off and washed with aqueous ethanol (45 c.c. of 70%), acetone (45 c.c.), and ether (45 c.c.). Drying at 60°/0·1 mm. gave a fine hygroscopic powder (0·96 g.) which rapidly reached constant weight on exposure to the atmosphere. Paper chromatography in *iso*propyl alcohol–1% aqueous ammonium sulphate (60 : 40) showed the 2′ : 3′-*iso*propylidene uridine-5′ pyrophosphate to have been completely removed, only a faint trace of uridine-5′ phosphate remaining. Phosphate analysis by Allen's method (*Biochem. J.*, 1940, **34**, 858), however, revealed 6% of the total phosphorus to be present as orthophosphate. To remove this the barium salt (1·71 g.) was dissolved in a minimum of 0·2N-hydrochloric acid and reprecipitated by ethanol (3 vols). The precipitate was collected, washed with acetone and ether, and dried at 60°/0·1 mm., giving monobarium uridine-5′ pyrophosphate as a fine white powder (1·08 g., 24% based on 2′ : 3′-*iso*propylidene uridine or 22% on uridine) (Found, in material exposed to the atmosphere for 24 hours: C, 17·6; H, 3·1; N, 4·4; P, 10·2%; total P/acid-labile P, 1·99 : 1. Calc. for $C_9H_{12}O_{12}N_2P_2Ba,4H_2O$: C, 17·7; H, 3·3; N, 4·6; P, 10·1. Calc. for $C_9H_{12}O_{12}N_2P_2Ba,3H_2O$: C, 18·2; H, 3·0; N, 4·7; P, 10·4%; total P/acid-labile P, 2 : 1). Concentration of the mother-liquors followed by precipitation with ethanol at pH 1 gave a further quantity of slightly less pure barium salt (0·21 g.). Inorganic phosphate amounted to less than 1% of the total phosphorus, and elution of the uridine-5′ phosphate and uridine-5′ pyrophosphate spots from a paper chromatogram (*iso*propyl alcohol–1% ammonium sulphate) with hydrochloric acid (N/100) and determination of the optical densities of the eluates at 260 mμ showed that the uridine-5′ phosphate present did not exceed 2% by weight. Ultra-violet absorption in N/100-HCl: max. 262—263 mμ (ε, 9070); min. 231 mμ (ε, 1910) (based on $C_9H_{12}O_{12}N_2P_2Ba,4H_2O$). The purified barium salt was identical with the barium uridine-5′ pyrophosphate of Anand, Clark, Hall, and Todd (*loc. cit.*) in paper-chromatographic behaviour and in infra-red spectrum.

Trilithium Uridine-5′ Pyrophosphate.—An aqueous solution of crude barium uridine-5′ pyrophosphate (0·1 g.) was passed slowly down an ion-exchange column (I.R.100 resin; 6 c.c.) saturated with lithium ions. To the eluate (15 c.c.) ethanol (40 c.c.) was added, producing a flocculent white precipitate which was centrifuged off, washed with ether, and dried at 60°/0·1 mm. *Trilithium uridine-5′ pyrophosphate* was thus obtained as a hygroscopic white powder (Found, in material dried over phosphoric oxide at 100°/1 mm.: C, 23·7; H, 3·2; N, 5·8. $C_9H_{11}O_{12}N_2P_2Li_3,2H_2O$ requires C, 23·6; H, 3·3; N, 6·1%). The acridine salt of uridine-5′ pyrophosphate proved to be too soluble in water to be useful for purification (solubility *ca.* 0·5 g./c.c.).

Identification of the Synthetic Barium Uridine-5′ Pyrophosphate with a Specimen of "Barium Uridine Diphosphate" isolated by Acid Hydrolysis of Natural UDPG.—The synthetic barium uridine-5′ pyrophosphate and the sample prepared from natural UDPG by Dr. L. Leloir were run side by side on paper chromatograms, using a number of solvent systems. Only acidic systems, or neutral systems containing ammonium sulphate, gave chromatograms free from trailing. In basic solvent systems the bulk of the barium salts failed to move from the starting line. The most satisfactory systems found were the four quoted below. No precautions were taken to standardise R_F values, which, particularly in the case of solvent *A*, varied with minor changes in the experimental conditions.

	R_F in solvent system			
Substance	*A*	*B*	*C*	*D*
Natural barium uridine diphosphate:				
(*a*) main component	0·47	0·43	0·14	0·24
(*b*) minor component (uridine-5′ phosphate)	0·64	0·59	0·25	0·44
Synthetic barium uridine-5′ pyrophosphate	0·47	0·43	0·16	0·25
Ammonium uridine-5′ phosphate	0·64	0·60	0·28	0·45

Solvent systems: *A*, *iso*propyl alcohol (60)–1% ammonium sulphate solution (40); *B*, *n*-propanol (45)–1% ammonium sulphate solution (35)–acetic acid (20); *C*, *n*-butanol (50)–acetic acid (20)–water (30); *D*, methanol (80)–98% formic acid (10)–1% ammonium sulphate solution (10). All solvent systems containing ammonium sulphate were run on Whatman No. 1 paper previously soaked in 1% ammonium sulphate solution and dried.

The relative molecular proportion of uridine-5′ pyrophosphate and uridine-5′ phosphate in the sample of " barium uridine diphosphate " of natural origin was found to be 9 : 1 by eluting the spots from a paper chromatogram (solvent system *A* above) with N/100-hydrochloric acid and measuring the relative optical densities (1·50 and 0·166 respectively) at 260 mμ with a Beckmann quartz spectrophotometer; elution of the spots was practically quantitative. Determination of orthophosphate by Allen's method (*loc. cit.*) showed that the same sample of " barium uridine diphosphate " contained 5·2% by weight of orthophosphate, assumed to be present as $BaHPO_4$.

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