411. *Nucleotides. Part VII.* Analogues of Adenosine-5′ Phosphate and Uridine-5′ Phosphate containing Phenylphosphonate and Ethylphosphonate Groups.*

By Nity Anand and A. R. Todd.

Synthesis of the following phosphonate analogues of muscle adenylic acid and uridine-5′ phosphate is described: adenosine-5′ phenylphosphonate, adenosine-5′ ethylphosphonate, uridine-5′ phenylphosphonate, uridine-5′ ethylphosphonate. All of these compounds were prepared by the action of the appropriate benzyl aryl(or alkyl)chlorophosphinate (VI) on the 2′ : 3′-*iso*propylidene derivative of adenosine or uridine, followed by removal of the *iso*propylidene and benzyl groups. Uridine-5′ ethylphosphonate was also prepared by phosphonylation using ethylphosphonyl dichloride.

Adenosine-5′ phenylphosphonate and adenosine-5′ ethylphosphonate appeared to exert no inhibitory action on several of the enzyme systems involving muscle adenylic acid derivatives.

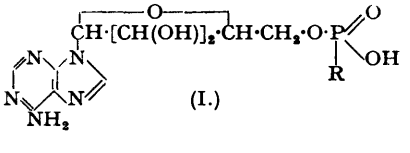
Derivatives of muscle adenylic acid (adenosine-5′ phosphate) (I; R = OH) perform a number of vitally important functions in biological systems. Adenosine-5′ diphosphate (ADP) and adenosine-5′ triphosphate (ATP) are coenzymes of phosphate transfer, and more complex esters containing an adenylic acid residue as an integral part of their molecule are found in the nucleotide coenzymes cozymase (DPN), coenzyme II (TPN), flavin-adenine-dinucleotide (FAD), and apparently also in Lipmann's coenzyme A (Lipmann, Kaplan, Novelli, Tuttle, and Guirard,

* Part VI, *J.*, 1950, 3299.

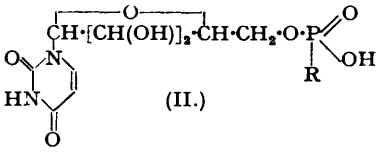
J. Biol. Chem., 1950, **186**, 235). The coenzyme (UDPG) of the system which brings about conversion of galactose-6 phosphate into glucose-6 phosphate (galactowaldenase) has recently been shown by Caputto, Leloir, Cardini, and Paladini (*J. Biol. Chem.*, 1950, **184**, 133) to be a derivative of uridine-5′ phosphate (II; R = OH); on the evidence presented by these workers, the coenzyme is almost certainly the mixed anhydride (pyrophosphate) of uridine-5′ phosphate and glucose-1 phosphate. Whether phosphates of any other natural nucleosides are components of further coenzymes is as yet unknown.

In view of these facts, it seemed possible that replacement of phosphate residues in nucleoside phosphates and polyphosphates by alkyl- or aryl-phosphonate residues would yield compounds which might act as biological antagonists to the natural analogues or might produce unusual or toxic effects in living organisms through the introduction of alkyl- or aryl-phosphonate residues in place of phosphate groups in the normal processes involving phosphate transfer. This possibility acquires added interest through the recent intensification of studies on phosphates, phosphonates, and pyrophosphates as inhibitors of cholinesterase and their use as insecticidal agents. It is natural to enquire whether the toxic action of some of these compounds is bound up with the substitution through their agency of phosphonate or esterified phosphate residues for the normal phosphates in vital processes. The present paper describes some initial studies along these lines in which we have undertaken the synthesis of some analogues of the simple mononucleotides, adenosine-5′ phosphate and uridine-5′ phosphate, in which the phosphate group is replaced by an alkyl- or aryl-phosphonate residue.

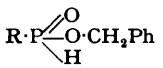
In previous papers of this series, the phosphorylating agent of choice for the preparation of nucleotides has usually been dibenzyl chlorophosphonate (Atherton, Openshaw, and Todd, *J.*, 1945, 382), not only because it is mild in action and the protecting benzyl groups are readily removed from the initial reaction products, but also because selective removal of one benzyl group can be achieved in a variety of ways (cf. Atherton, Openshaw, and Todd, *loc. cit.*; Baddiley and Todd, *J.*, 1947, 648; Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815; Clark and Todd, *J.*, 1950, 2023, 2030), yielding substances of the type RO·P(O)(O·CH_2Ph)·OH which are suitable starting materials for polyphosphate synthesis. For the purpose of alkyl- or aryl-phosphonylation, the analogous reagent would be a benzyl alkyl(or aryl)chlorophosphinate * (VI); compounds of this type have now been prepared and used successfully. Benzyl phenylphosphinate (III; R = Ph) was obtained as a colourless liquid by treatment of phenyldichlorophosphine (Ph·PCl_2) with a mixture of benzyl alcohol (1 mol.) and dimethylaniline (1 mol.), followed by addition of a further amount (1 mol.) of benzyl alcohol (cf. preparation of dibenzyl phosphite by Atherton, Openshaw, and Todd, *loc. cit.*). By allowing phenyldichlorophosphine to react with a solution of benzyl alcohol (3 mols.) in pyridine (3 mols.), dibenzyl phenyl-



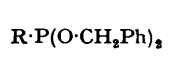
(I.)



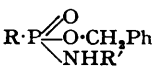
(II.)



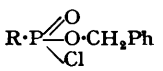
(III.)



(IV.)



(V.)



(VI.)

phosphonite (IV; R = Ph) was obtained in good yield; this ester readily yielded benzyl phenylphosphinate (III; R = Ph) on treatment with lithium chloride (cf. Clark and Todd, *J.*, 1950, 2030), but the direct preparation of (III; R = Ph) from phenyldichlorophosphine was more convenient in practice as the product could be readily purified by distillation. Benzyl

* As a result of discussions between nomenclature committees of the American Chemical Society and our Society, it has been decided that, in our Society's publications (as already in *Chemical Abstracts*), the nomenclature of organic acids of phosphorus shall be such that the terminations " ic " and " ous " denote quinque- and ter-valent phosphorus respectively, the syllable " on " denoting the larger and " in " the smaller number of hydroxyl groups. (Similar rules apply to arsenic and antimony compounds.) Thus Et·$PO(OH)_2$ is ethylphosphonic acid, Et·PHO·OH is ethylphosphinic acid, Et·$P(OH)_2$ is ethylphosphonous acid, and Et·PH·OH is ethylphosphinous acid.

In this paper, compounds of type (V) and (VI) are considered, in conformity with current British practice, to be derived by replacement of hydrogen (not hydroxyl) by NHR′ or Cl, *i.e.*, to be derived from R·PH(O)·O·CH_2Ph, so that, *e.g.*, Et·PCl(O)·OH is derived from Et·PH(O)·OH and is named ethylchlorophosphinic acid; other examples are in the text. Ed.

phenylphosphinate reacted readily with carbon tetrachloride in the presence of *cyclo*hexylamine, yielding benzyl *cyclo*hexylaminophenylphosphinate (V; R = Ph, R′ = *cyclo*hexyl), a reaction strictly analogous to that undergone by dibenzyl phosphite (dibenzyl phosphonate) under similar conditions (Atherton, Openshaw, and Todd, *J.*, 1945, 660). Benzyl phenylchlorophosphinate (VI; R = Ph) was prepared in solution by the action of a solution of chlorine in carbon tetrachloride on benzyl phenylphosphinate at low temperature. The chlorophosphinate, like dibenzyl chlorophosphonate, could not be distilled without decomposition; it was used in freshly prepared solution without isolation, its purity being assayed by treating a portion of the solution with *cyclo*hexylamine and isolating the crystalline benzyl *cyclo*hexylaminophenylphosphinate (V; R = Ph, R′ = *cyclo*hexyl).

Benzyl phenylchlorophosphinate (VI; R = Ph) reacted with 2′ : 3′-*iso*propylidene adenosine in pyridine solution to give 2′ : 3′-*iso*propylidene adenosine-5′ benzyl phenylphosphonate, converted by catalytic hydrogenation into 2′ : 3′-*iso*propylidene adenosine-5′ phenylphosphonate. From the latter, adenosine-5′ phenylphosphonate (I; R = Ph) was obtained by mild acid hydrolysis. When a similar procedure was applied to 2′ : 3′-*iso*propylidene uridine the initial reaction product did not crystallise; it was not isolated in a pure state but was converted directly into uridine-5′ phenylphosphonate (II; R = Ph), an amorphous powder yielding an amorphous barium salt. For the synthesis of the corresponding ethylphosphonates of adenosine and uridine, similar methods were employed. Ethyl dichlorophosphine (Et·PCl_2), readily available from tetraethyl-lead and phosphorus trichloride by the method of Kharasch, Jensen, and Weinhouse (*J. Org. Chem.*, 1949, **14**, 429), was converted into benzyl ethylphosphinate (III; R = Et) by reaction with benzyl alcohol (2 mols.) and a tertiary base (1 mol.) in the usual way; chlorination of this ester in carbon tetrachloride furnished a solution of benzyl ethylchlorophosphinate (VI; R = Et) which was used directly. Treated with benzyl ethylchlorophosphinate, 2′ : 3′-*iso*propylidene adenosine yielded 2′ : 3′-*iso*propylidene adenosine-5′ benzyl ethylphosphonate; removal of the benzyl group by hydrogenation, followed by hydrolysis of the *iso*propylidene moiety, gave adenosine-5′ ethylphosphonate (I; R = Et) as an amorphous white powder yielding an amorphous barium salt. In similar manner, uridine-5′ ethylphosphonate (II; R = Et) was prepared from 2′ : 3′-*iso*propylidene uridine. In this case, as in that of the corresponding uridine-5′ phenylphosphonate preparation, the *iso*propylidene group was apparently eliminated either during hydrogenolysis of the benzyl ester or during the working up of the debenzylated product. That the *iso*propylidene residue had in fact been lost was in each case demonstrated by the paper chromatographic method for detection of nucleosides and nucleotides containing free hydroxyls at $C_{(2')}$ and $C_{(3')}$ in the ribofuranose residue (Buchanan, Dekker, and Long, *J.*, 1950, 3162).

2′ : 3′-*iso*Propylidene uridine-5′ ethylphosphonate was prepared by treating 2′ : 3′-*iso*propylidene uridine with ethylphosphonyl dichloride (Et·$POCl_2$) in pyridine solution; on mild hydrolysis it yielded uridine-5′ ethylphosphonate (II; R = Et), identical with the product obtained by using benzyl ethylchlorophosphinate as phosphonylating agent. 2′ : 3′-*iso*-Propylidene adenosine reacted readily enough with ethylphosphonyl dichloride but the product was a mixture, evidently containing *N*-phosphonylated material, since analysis indicated a high phosphorus content. No homogeneous product could be isolated from the reaction; it may be recalled that Levene and Tipson (*J. Biol. Chem.*, 1937, **121**, 131) had a similar experience in attempting to phosphorylate 2′ : 3′-*iso*propylidene adenosine with phosphoryl chloride.

Although it was not expected that these simple nucleotide analogues would display any marked action through direct transfer of their phosphonate residues in biological systems, it was thought that the adenosine derivative might inhibit some of the enzyme systems involving muscle adenylic acid or its derivatives. No evidence of any inhibition was obtained, however, when adenosine-5′ phenylphosphonate or ethylphosphonate was introduced into the following enzyme systems: phosphofructokinase, 5-nucleotidase, and adenylic acid deaminase.

We should like to express our gratitude to Dr. T. Mann of the Molteno Institute, Cambridge, for his kindness in carrying out these enzymic experiments and for allowing us to record his results.

Experimental.

Dibenzyl Phenylphosphonite (IV; R = Ph).—Freshly distilled phenyldichlorophosphine (9 g., 1 mol.) in ether (25 c.c.) was added slowly during 30 minutes to a stirred, ice-cold mixture of pyridine (12 g., 3 mols.) and benzyl alcohol (16 g., 3 mols.). The solution was stirred at 0° for a further 2 hours, then left overnight at room temperature. Pyridine hydrochloride was filtered off and the filtrate evaporated in a nitrogen atmosphere under reduced pressure at 50°. The colourless residue (12 g.) was distilled in a molecular still; *dibenzyl phenylphosphonite* distilled as a colourless liquid at 65—70°/0·1 mm. (Found: C, 73·9; H, 6·1. $C_{20}H_{19}O_3P$ requires C, 74·5; H, 5·9%).

Benzyl Phenylphosphinate (III; R = Ph).—(*a*) A solution of benzyl alcohol (4·2 g., 1 mol.) in freshly distilled dimethylaniline (4·7 g., 1 mol.) was added gradually, with stirring, during 1½ hours to an ice-cold solution of phenyldichlorophosphine (7 g., 1 mol.) in benzene (25 c.c.). When all had been added a further quantity (4·2 g., 1 mol.) of benzyl alcohol was run in gradually during 30 minutes and stirring was continued for a further 4 hours before the mixture was allowed to come to room temperature and stand overnight. The mixture was diluted with benzene (*ca.* 25 c.c.), washed first with dilute hydrochloric acid, then with aqueous sodium carbonate, and finally with water, dried (Na_2SO_4), and freed from all solvents by evaporation for 4—5 hours at 50°/*ca.* 0·1 mm. in a nitrogen atmosphere. The residue (10·2 g.) was distilled in a " molecular " still. *Benzyl phenylphosphinate*, a colourless liquid, distilled at 90—95°/0·05 mm. (Found: C, 66·9; H, 6·0. $C_{13}H_{13}O_2P$ requires C, 67·3; H, 5·6%).

(*b*) Dibenzyl phenylphosphonite (IV; R = Ph) (3·2 g.; prepared as described above) was dissolved in 2-ethoxyethanol (30 c.c.), lithium chloride (1·27 g.) added, and the mixture heated on the steam-bath for 3 hours. Solvent was removed under reduced pressure in a nitrogen atmosphere, and the residue dissolved in water. The solution was acidified, then extracted with chloroform, and the extract was dried and evaporated. The colourless oil so obtained was identified as benzyl phenylphosphinate by reaction with *cyclo*hexylamine in carbon tetrachloride, whereby benzyl *cyclo*hexylaminophenylphosphinate (m. p. and mixed m. p. 119—120°) was produced.

Benzyl cyclo*Hexylaminophenylphosphinate* (V; R = Ph, R′ = *cyclo*hexyl).—Crude (*i.e.*, undistilled) benzyl phenylphosphinate (1·1 g.) was dissolved in carbon tetrachloride (4 c.c.), and *cyclo*hexylamine (2·5 c.c.) added. There was immediate reaction with separation of *cyclo*hexylamine hydrochloride. After 2 hours the mixture was filtered, the filtrate evaporated, and the residue recrystallised from benzene–light petroleum (b. p. 40—60°); *benzyl* cyclo*hexylaminophenylphosphinate* (1·6 g., 95%) formed colourless needles, m. p. 119—120° (Found: C, 69·4; H, 7·6; N, 4·0. $C_{19}H_{24}O_2NP$ requires C, 69·3; H, 7·3; N, 4·2%).

Benzyl Phenylchlorophosphinate (VI; R = Ph) —A half-saturated solution of chlorine in carbon tetrachloride (*ca.* 8 c.c.) was added gradually to benzyl phenylphosphinate (1 g.), dissolved in carbon tetrachloride (5 c.c.), with exclusion of moisture. Excess of chlorine and hydrogen chloride was removed by passing a rapid stream of nitrogen through the solution for 1 hour and the carbon tetrachloride was then evaporated under reduced pressure at room temperature. Benzyl phenylchlorophosphinate remained as a colourless oil which decomposed when kept or on attempted distillation; it was evidently very nearly pure, for on dissolution in benzene and addition of *cyclo*hexylamine it gave benzyl *cyclo*hexylaminophenylphosphinate (m. p. and mixed m. p. 119°) in almost quantitative yield.

2′ : 3′-iso*Propylidene Adenosine-5′ Benzyl Phenylphosphonate*.—2′ : 3′-*iso*Propylidene adenosine (1 g.; dried at 110°/0·1 mm. for 12 hours) was dissolved in pyridine (15 c.c.) by gentle warming. The solution was cooled rapidly to −40° and benzyl phenylchlorophosphinate (from 2 g. of benzyl phenylphosphinate) added. The mixture was kept just above its m. p. for 3 hours, then set aside overnight. Sodium carbonate (2 g.) and water (5 c.c.) were added, the mixture was evaporated to dryness under reduced pressure, and the residue extracted with chloroform. The chloroform extract was washed with aqueous sodium hydrogen carbonate, dilute sulphuric acid, and water, dried and evaporated. The yellowish gum (1·9 g.) so obtained was dissolved in a minimum of hot ethanol, and the solution set aside to cool; 2′ : 3′-iso*propylidene adenosine-5′ benzyl phenylphosphonate* separated as colourless rods (1·1 g.), m. p. 188—189° (Found, in material dried at 100°/0·1 mm.: C, 57·9; H, 5·1; N, 12·9. $C_{26}H_{28}O_6N_5P$ requires C, 58·1; H, 5·3; N, 13·0%).

2′ : 3′-iso*Propylidene Adenosine-5′ Phenylphosphonate*.—The above benzyl ester (0·5 g.) was dissolved in aqueous ethanol (50 c.c. of 80%) and hydrogenated at atmospheric pressure, the catalyst being a mixture of palladous oxide and palladised charcoal. The theoretical amount of hydrogen for removal of the benzyl residue was taken up in 1½ hours after which absorption ceased. The filtered solution was evaporated under reduced pressure, leaving 2′ : 3′-iso*propylidene adenosine-5′ phenylphosphonate* as a resin which could not be crystallised; it was purified by dissolution in ethanol and reprecipitation by ether, then forming a white amorphous powder (Found, in material dried at room temperature/0·1 mm.: C, 50·5; H, 4·9; N, 15·2. $C_{19}H_{22}O_6N_5P$ requires C, 51·0; H, 4·9; N, 15·6%). The phosphonate, treated with *cyclo*hexylamine in ethanol, gave an amorphous cyclo*hexylamine* salt, purified by reprecipitation from ethanol solution with ether (Found, in material dried at room temperature/0·1 mm. C, 53·6; H, 6·1; N, 14·8. $C_{25}H_{35}O_6N_6P$ requires C, 55·0; H, 6·4; N, 15·4. $C_{25}H_{35}O_6N_6P,H_2O$ requires C, 53·2; H, 6·6; N, 14·9%).

Adenosine-5′ Phenylphosphonate (I; R = Ph).—The above *iso*propylidene derivative (0·2 g.) was dissolved in dilute sulphuric acid (50 c.c. of 0·1N.) and set aside for 2 days. The sulphuric acid was then exactly neutralised with barium hydroxide, barium sulphate centrifuged off, and the solution evaporated to dryness. The residue of *adenosine-5′ phenylphosphonate* separated from warm ethanol as a white powder of doubtful crystallinity. In this form the phosphonate appears to be hydrated and when heated softens at 80° and melts at 95° (Found, in material dried at 50°/0·1 mm. for 24 hours: C, 45·6; H, 5·0; N, 16·4. $C_{16}H_{18}O_6N_5P,H_2O$ requires C, 45·2; H, 4·7; N, 16·4%). The phosphonate had $[\alpha]_D^{15} = -40° \pm 2°$ (*c*, 0·15 in water) and was chromatographically homogeneous on paper, showing $R_F = 0·31$ in the system ethyl acetate–pyridine–water (10 : 45 : 10). The brucine salt prepared in the usual manner with brucine in ethanol could not be crystallised.

Uridine-5′ Phenylphosphonate (II; R = Ph).—Benzyl phenylchlorophosphinate (from 2·5 g. of benzyl phenylphosphinate) was added to a solution of 2′ : 3′-*iso*propylidene uridine (1·2 g., dried at 110°/0·1 mm. for 2 hours; Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113) in dry pyridine (10 c.c.) at −40° and the mixture kept just above its m. p. for 2 hours, then allowed to warm to room temperature and set aside overnight. Water (5 c.c.) and sodium carbonate (2 g.) were added, and the mixture was evaporated under reduced pressure below 35°. The residue was extracted with chloroform, and the extract washed with sodium hydrogen carbonate, dilute sulphuric acid and water, dried, and

evaporated. The residual gum (2 g.)—presumably mainly 2′ : 3′-*iso*propylidene uridine-5′ benzyl phenylphosphonate—could not be crystallised. It was accordingly dissolved in aqueous ethanol (15 c.c. of 80%) and hydrogenated at room temperature and atmospheric pressure, a mixture of palladous oxide and palladised charcoal catalysts being used. Hydrogenation ceased after *ca.* 1 hour, when an amount of hydrogen corresponding to the removal of one benzyl group had been absorbed. Evaporation of the filtered solution gave a colourless glass which was very soluble in water and ethanol and had evidently lost the *iso*propylidene group since it gave a positive reaction with periodate. It was purified by dissolution in ethanol and reprecipitation with ether; the glassy *uridine-5′ phenylphosphonate* so obtained gradually fell to a white powder when kept in a desiccator (Found, in material dried at room temperature/0·1 mm. for 24 hours: C, 47·1; H, 4·5; N, 6·8. $C_{15}H_{17}O_8N_2P$ requires C, 46·9; H, 4·4; N, 7·2%).

The *barium* salt of the phosphonate formed a white amorphous powder when precipitated from its aqueous solution by an equal volume of ethanol [Found, in material dried at 110°/0·1 mm. for 8 hours: C, 39·0; H, 3·6; N, 6·3. $(C_{15}H_{16}O_8N_2P)_2Ba$ requires C, 39·8; H, 3·5; N, 6·2%].

Benzyl Ethylphosphinate (III; R = Et).—Benzyl alcohol (8·3 g., 1 mol.) in dimethylaniline (9·4 g., 1 mol.) was added dropwise to an ice-cold stirred solution of ethyldichlorophosphine (10 g.; Kharasch, Jensen, and Weinhouse, *loc. cit.*) in benzene (20 c.c.) during 1 hour. A further quantity (8·3 g., 1 mol.) of benzyl alcohol was then added, the mixture set aside overnight at room temperature, and the product worked up as in the case of benzyl phenylphosphinate (see above). *Benzyl ethylphosphinate* (12 g.), a colourless liquid with an unpleasant odour, is usually pure enough for use without distillation. In a " molecular " still it distilled at *ca.* 90—95°/0·1 mm. (Found: C, 57·8; H, 7·1. $C_9H_{13}O_2P$ requires C, 58·7; H, 7·1%). The ester reacted at once with carbon tetrachloride and *cyclo*hexylamine, yielding *benzyl ethyl*-cyclo*hexylaminophosphinate* (V; R = Et, R′ = *cyclo*hexyl) which crystallised from *n*-hexane in colourless needles, m. p. 60—61° (Found: C, 64·1; H, 8·6; N, 5·1. $C_{15}H_{24}O_2NP$ requires C, 64·2; H, 8·2; N, 5·0%). Treated with a half-saturated solution of chlorine in carbon tetrachloride in the usual way, benzyl ethylphosphinate yielded benzyl ethylchlorophosphinate (VI; R = Et) as a colourless unstable oil which could not be purified by distillation and was used directly; it reacted at once with *cyclo*hexylamine, to give benzyl ethyl-*cyclo*hexylaminophosphinate (m. p. and mixed m. p. 60—61°) in quantitative yield.

2′ : 3′-iso*Propylidene Adenosine-5′ Benzyl Ethylphosphonate.*—Benzyl ethylchlorophosphinate (from 0·6 g. of benzyl ethylphosphinate) was added to a solution of 2′ : 3′-*iso*propylidene adenosine (0·4 g.; dried at 110° for 12 hours) in dry pyridine (10 c.c.) at −40° and the mixture kept just above its m. p. for 2 hours then left overnight at room temperature. When the mixture was worked up as described for the corresponding phenylphosphonate, 2′ : 3′-iso*propylidene adenosine-5′ benzyl ethylphosphonate* (0·65 g.) was obtained as a white amorphous powder by precipitation from its ethanol solution with ether (Found, in material dried at room temperature/0·1 mm. for 24 hours: C, 54·4; H, 6·2; N, 14·0. $C_{22}H_{28}O_6N_5P$ requires C, 54·0; H, 5·7; N, 14·3%).

Adenosine-5′ Ethylphosphonate (I; R = Et).—The above benzyl ester (0·6 g.) was hydrogenated in aqueous ethanol (20 c.c.; 80%), a mixture of palladous oxide and palladised charcoal catalysts being used at room temperature and atmospheric pressure, and the glassy product was set aside for 2 days in dilute sulphuric acid (100 c.c.; 0·1N.) to remove the *iso*propylidene residue. Sulphuric acid was exactly neutralised with barium hydroxide, barium sulphate centrifuged off, and the clear supernatant liquid evaporated to dryness under reduced pressure. The colourless residue separated from warm ethanol as a white, apparently amorphous powder, which softened at *ca.* 75° and gradually melted above this temperature. In this state the *adenosine-5′ ethylphosphonate* appeared to be hydrated (Found, in material dried at room temperature/0·1 mm. for 24 hours: C, 33·9; H, 6·2; N, 16·3. $C_{12}H_{18}O_6N_5P,4H_2O$ requires C, 33·4; H, 6·0; N, 16·2%). Part of the water could be removed by drying at 80° (Loss, 10·1. Calc. for loss of 2·5 mols. of H_2O: 10·6%) but above this temperature decomposition gradually set in. On paper chromatography with *tert.*-butyl alcohol–pyridine–water (65 : 25 : 15) the phosphonate had $R_P = 0·3$ but appeared to be contaminated by a small amount of some fast-moving impurity. It was therefore purified by chromatography on a cellulose column, the above *tert.*-alcohol–pyridine–water mixture being used to wash the material through the column. The nucleotide-containing fractions were combined and evaporated under reduced pressure. The residue was dissolved in water and converted into the *barium* salt which was obtained as a white powder by addition of a small amount of ether to its solution in aqueous ethanol [Found, in material dried at 110°/0·1 mm. for 8 hours: N, 15·7; P, 7·0. $(C_{12}H_{17}O_6N_5P)_2Ba,2H_2O$ requires N, 15·8; P, 7·0%].

2′ : 3′-iso*Propylidene Uridine-5′ Benzyl Ethylphosphonate.*—Benzyl ethylchlorophosphinate (from 2 g. of benzyl ethylphosphinate) was added to a solution of 2′ : 3′-*iso*propylidene uridine (1 g.; dried at 110°/0·1 mm. for 12 hours) in dry pyridine (10 c.c.) at −40°, and the solution kept just above its m. p. for 2 hours and then left overnight at room temperature. Working up as described for the corresponding adenosine derivative gave a yellowish glass (1·2 g.) (Found, in material dried at room temperature/0·1 mm. for 24 hours: C, 53·9; H, 6·2; N, 5·9. $C_{21}H_{27}O_8N_2P$ requires C, 54·1; H, 5·9; N, 6·0%).

Uridine-5′ Ethylphosphonate (II; R = Et).—The above benzyl ester was hydrogenated in aqueous ethanol solution, a mixture of palladous oxide and palladised charcoal catalysts being used. Hydrogenation was complete in 1 hour. When isolated in the usual way, the product was found by the periodate test to have lost both the benzyl and the *iso*propylidene residues; it was a colourless glass which fell to a white amorphous powder on trituration with ethanol. *Uridine-5′ ethylphosphonate* was very hygroscopic (Found, in material dried at room temperature/0·1 mm. for 24 hours: C, 40·1; H, 5·1; N, 8·2; P, 8·8. $C_{11}H_{17}O_8N_2P$ requires C, 39·3; H, 5·1; N, 8·3; P, 9·2%). It had $[\alpha]_D^{15} = +3·5° \pm 0·5$ (*c*, 2·9 in water) and $R_P = 0·68$ on paper with an ethyl acetate–pyridine–water (100 : 45 : 100) system. The *acridine* salt, prepared by addition of 1 molecular proportion of acridine in ethanol to an aqueous solution of the phosphonate, was a yellow amorphous powder (Found: C, 53·7; H, 5·4; N, 7·9. $C_{11}H_{17}O_8N_2P,C_{13}H_9N,H_2O$ requires C, 54·1; H, 5·3; N, 7·9%).

2′ : 3′-iso*Propylidene Uridine-5′ Ethylphosphonate.*—A solution of 2′ : 3′-*iso*propylidene uridine (1·42 g.; dried at 110°/0·1 mm. for 12 hours) in pyridine (5 c.c.) at −20° was added in three portions to a solution of ethylphosphonyl dichloride (0·8 g., 1·1 mols.; b. p. 67—69°/14 mm.) in pyridine (3 c.c.) at −20°. The mixture was kept in an ice–salt bath for 2 hours, left overnight at 0°, then cooled again to −20°, and aqueous pyridine (2 c.c.) was added, followed by excess of ice-water (20 c.c.). The ice-cold solution was then made alkaline with barium hydroxide, whereupon its colour changed from orange to yellow. The solution was decolorised with charcoal (norite) and evaporated to dryness under reduced pressure. The residue was dissolved in water (*ca.* 40 c.c.), and acetone (400 c.c.) was added. The precipitate so obtained (0·9 g.) consisted mainly of *barium ethyl phosphonate* which was recrystallised from water and formed glittering plates (Found, in material dried at 100°/0·1 mm. for 8 hours: C, 9·5; H, 3·0. $C_2H_5O_3PBa,H_2O$ requires C, 9·1; H, 2·6%). The filtrate, evaporated to dryness, gave a residue (1·4 g.) which was dissolved in water (5 c.c.), treated with charcoal, and filtered, and ethanol (50 c.c.) was added. A small amount of precipitated material was centrifuged off and the clear solution evaporated to dryness. The same procedure was repeated once more, giving *barium* 2′ : 3′-iso*propylidene uridine-5′ ethylphosphonate* as a white amorphous powder [Found, in material dried at 115°/0·1 mm. for 12 hours: C, 37·6; H, 4·6; N, 6·3. $(C_{14}H_{20}O_8N_2P)_2Ba$ requires C, 37·8; H, 4·5; N, 6·3%]. The *cyclo*hexylamine salt was prepared from the barium salt by decomposing the latter with the calculated amount of sulphuric acid, removing barium sulphate, and adding one molecular proportion of *cyclo*hexylamine. It was extremely soluble in water and was obtained as a white amorphous powder, softening between 85° and 90°, by precipitation from concentrated aqueous solution with ethanol (Found, in material dried at room temperature/0·1 mm. for 24 hours: N, 9·3. $C_{20}H_{32}O_8N_3P$ requires N, 8·9%).

Uridine-5′ Ethylphosphonate (II; R = Et) *from the above* iso*Propylidene Derivative.*—A solution of 2′ : 3′-*iso*propylidene uridine-5′ ethylphosphonate in N-sulphuric acid was warmed to 75° for 1½ hours, then neutralised with barium hydroxide, filtered, and evaporated to dryness. The barium salt was dissolved in water and barium was removed by addition of the calculated amount of sulphuric acid (end-point checked with rhodizonic acid). Barium sulphate was centrifuged off and the clear supernatant liquid evaporated. Uridine-5′ ethylphosphonate remained as a yellowish glass which was very soluble in water. Paper chromatography, with ethyl acetate–pyridine–water (100 : 45 : 100), showed that it had $R_F = 0·68$ but that it was accompanied by a trace of some phosphorus-free material. The substance was therefore chromatographed on broad sheets of filter paper, the above solvent mixture being used. The bands carrying the phosphonate were cut out and eluted with water, and the eluate evaporated under reduced pressure. The residue was identical in its properties with uridine-5′ ethylphosphonate prepared by using benzyl ethylchlorophosphinate as phosphonylating reagent.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, March 21st,* 1951.]