NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CL.* PREPARATION AND PROPERTIES OF SOME NUCLEOSIDE HYDROXYALKANEPHOSPHONATES**

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Nucleoside 5'-O-hydroxymethanephosphonates *I* and *XIV* were prepared by reaction of hydroxymethanephosphonic acid (*II*) with ribonucleosides, 2'-deoxyribonucleosides or ribonucleoside 2',3'-O-ethoxymethylene derivative *IX* in the presence of N,N'-carbonyldiimidazole and the subsequent deblocking in acidic media. Uridine 2'(3')-O-hydroxymethanephosphonate (*XII*) was prepared similarly from 5'-O-benzoyluridine (the deblocking was performed in alkaline media). Uridine 5'-O-(2-hydroxyethane)phosphonate (*XVI*) and uridine 5'-(1-hydroxyethane)phosphonate (*XVII*) were obtained analogously from the uridine derivative *IXa*. The preparation of uridine 5'-O-methanephosphonate (*XIXa*) and adenosine 5'-O-methanephosphonate (*XIXb*) was effected by reaction of methanephosphonic acid pyridinium salt with the 2',3'-O-ethoxymethylene derivative *IX* in the presence of N,N'-dicyclohexylcarbodiimide and acidic hydrolysis.

In contrast to the methanephosphonates *XIX*, the hydroxymethanephosphonates *I* and *XIV* are good substrates for the snake venom 5'-nucleotidase; the homologous derivatives *XVI* and *XVII* are not cleaved by this enzyme. 5'-Nucleotidase requires for the complex formation with substrate the presence of hydrophilic substituents at the phosphorus atom and in its close vicinity.

In investigations on analogues of nucleotide derivatives, relatively little attention has been paid to the modification of the ester residue of phosphoric acid. Thus, in the earlier reported[2,3] nucleoside alkanephosphonates, the alkyl group is attached directly to the phosphorus atom. These analogues are of interest in view of enzyme specificity in nucleic acid metabolism, particularly of nucleolytic enzymes. In the present paper, we wish to report the preparation and properties of some nucleoside alkanephosphonates substituted by the hydrophilic hydroxyalkyl group which is attached to the phosphorus atom through a C—P bond. The simplest representatives of this series are derivatives of hydroxymethanephosphonic acid *I*. The attempted preparation of these compounds by condensation of hydroxymethanephosphonic acid (*II*) pyridinium salt[4] with 2',3'-O-ethoxymethyleneadenosine[5] (*III*) in the presence of N,N'-dicyclohexylcarbodiimide as activating agent failed. After removal

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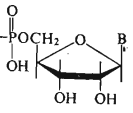
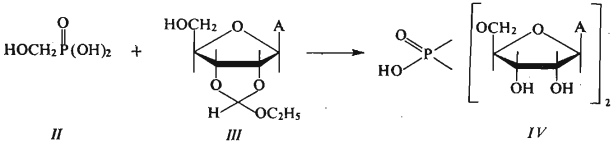
** Some results given in the present paper have appeared in a preliminary communication[1].

of protecting groups, adenylyl(5′→5′)-adenosine (*IV*) has been isolated in a small yield from the reaction mixture as the sole product (Scheme 1).



I

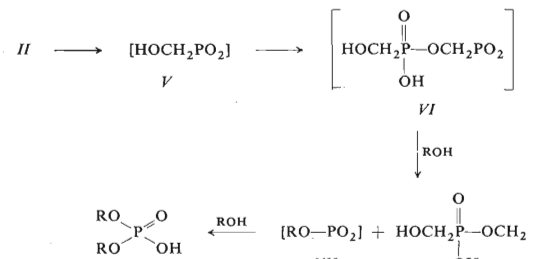
Ia B = uracil, *b* B = cytosine, *c* B = adenine, *d* B = guanine



II *III* *IV*

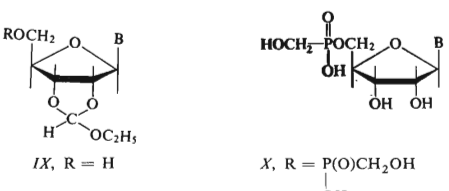
SCHEME 1

The structure of compound *IV* was established as follows: its chromatographic and electrophoretic behaviour is very similar to the authentic (3′ → 5′)-isomer[6]. The analysis has shown the presence of two adenosine residues per one atom of phosphorus. Compound *IV* is resistant towards the action of phosphomonoesterases and T2-ribonuclease (the possibility of a (3′ → 5′)-isomer is thus excluded) and the phosphodiesterase degradation leads quantitatively to adenosine and adenosine 5′-phosphate, both identical with authentic samples on chromatography and electrophoresis. The degradation products are in an equimolar ratio and the snake venom 5′-nucleotidase degradation of the latter affords quantitatively adenosine (proof of a 5′-nucleotide[7]). This unexpected course leading under conditions of a carbodiimide reaction to cleavage of the C—P bond of hydroxymethanephosphonic acid and formation of a phosphoric acid diester might be explained on the basis of an analogy with the reaction of aminomethanephosphonic acid and ninhydrin[8] which is accompanied by an intermediary formation of a metaphosphate particle. With compound *II*, the carbodiimide activation may lead to the formation of compound *II* which undergoes an intermolecular reaction to compound *VI*. Under the influence of the ester-bonded phosphonate residue an electron shift occurs and the attack with an alcohol (a nucleoside) is followed by cleavage of the C—P bond under the formation of the methylester of hydroxymethanephosphonic acid and the metaphosphate particle *VII* which may react with another molecule of the nucleoside to give *VIII*. Such a reaction may be of course only of a limited extent in view of a greater possibility of mutual intermolecular reactions of *V* and *VI* which lead to complex products (Scheme 2). It may thus be expected that none of activating agents which are being used for activation of phosphomonoesters (formation of intermediates of the metaphosphate type) will be suitable in preparation of hydroxymethanephosphonates of type *I*. In accordance with these conclusions, replacement of N,N′-dicyclohexylcarbodiimide by 2,4,6-triisopropylbenzenesulfonyl chloride did not improve the reaction course. We have therefore tried to exclude the undesirable presence of the free primary hydroxylic function in compound *II* by reaction with ethyl vinyl ether[9]. However, the corresponding 1-ethoxyethyl derivative is not formed.



SCHEME 2

Another possibility to activate hydroxymethanephosphonic acid (*II*) consists in the formation of an imidazolide analogously to activation of phosphoric acid esters[10,11]. Thus, the reaction of the acid *II* and the protected nucleoside *IX* in dimethylformamide in the presence of N,N'-carbonyldiimidazole led to the 5'-O-hydroxymethanephosphonate *X* and, after removal of the protecting group in acidic media, the final product *I*. This method was used to prepare the derivatives of uridine, cytidine,



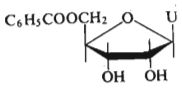
IX, R = H

X, R = P(O)CH_2OH

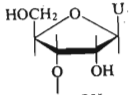
In formulae *IX*, *X*: *a* B = uracil, *b* B = cytosine, *c* B = adenine, *d* B = guanine.

adenosine, and guanosine *Ia*–*Id*. The attempted preparation of the isomeric 2'(3')-O-hydroxymethanephosphonate of uridine (*XII*) from 5'-O-benzoyluridine[12] (*XI*) resulted in the formation of about 2% of compound *XII* which differs from the 5'-isomer in the chromatographic behaviour, absence of the *cis*-diol grouping, and enzymatic reactions. The low yield cannot be due to the alkali-lability of the product which is quite stable under conditions necessary for removal of the protecting benzoyl group. The reaction of 5'-O-bis(*p*-methoxyphenyl)phenylmethyl-2'-deoxythymidine[13] which contains only an isolated secondary alcoholic function at position 3', does not occur at all. The reaction of 3'-O-benzoyl-2'-deoxythymidine[12] (*XIII*) with hydroxy-

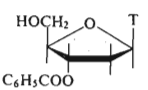
methanephosphonic acid in the presence of N,N'-carbonyldiimidazole and the subsequent removal of the benzoyl group in alkaline medium afforded compound *XIV* *i.e.* product of the reaction at the primary alcoholic function in position 5'.



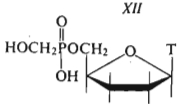
XI



XII



XIII



XIV

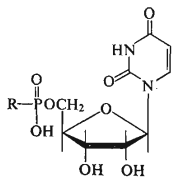
In formulae *XI* and *XII* U = uracil In formulae *XIII* and *XIV* T = thymine

Since the reaction occurs exclusively at the primary alcoholic function, we have used in another experiment the unprotected uridine (*XVa*) and 2'-deoxythymidine (*XVb*) as the starting compounds under otherwise the same conditions and obtained the corresponding 5'-O-hydroxymethanephosphonates *Ia* and *XIV* as the single reaction products, identical with authentic specimens obtained by the above mentioned routes. The preparation of hydroxymethanephosphonates on the primary alcoholic function of nucleosides does not consequently require protection of other alcoholic functions in the molecule.

Uridine 5'-O-2-hydroxyethanephosphonate *XVI* and uridine 5'-O-1-hydroxyethanephosphonate *XVII* were prepared similarly by activation of the corresponding phosphonic acid[14,15] with N,N'-carbonyldiimidazole and reaction with compound *IXa* or 2',3'-isopropylideneuridine. The yields, however, were somewhat lower than in the case of hydroxymethanephosphonic acid. Compound *XVI* was also prepared by condensation of 2-hydroxyethanephosphonic acid pyridinium salt with 2',3'-isopropylideneuridine in the presence of N,N'-dicyclohexylcarbodimide and the subsequent acidic removal of the protecting group.

Compounds *I*, *XVI*, and *XVII* were isolated as ammonium salts. Their electrophoretic properties correspond to the presence of one dissociable group. The analytical data confirmed the presence of one phosphorus atom per the heterocyclic base. Titration with periodic acid gave values corresponding to one *cis*-diol grouping in the molecule. Compounds *XII* and *XIV* were characterised similarly except for the periodic acid titration which gave negative results with both compounds in accordance with the structure proposed.

Tests* on the bacteriostatic activity of compounds *I* on the growth of *Escherichia coli* in a synthetic medium were negative up to the concentration of 1·0 mg/ml. As shown by enzymatic reactions performed under standard conditions or with an excess of the enzyme or with a prolonged incubation period, compounds *I* are (similar to nucleoside methanephosphonates[3]) quite resistant to the action of non-specific phosphomonoesterases such as alkaline phosphatase *E. coli*, intestinal alkaline phosphatase, and wheat germ acid phosphatase. These phosphomonoesterases require the presence of two dissociable groups in the substrate since neither nucleoside phosphites *XVIII* nor methanephosphonates *XIX* are degraded by their action. The negative results obtained with the hydroxymethanephosphonates eliminate the assumption that the failure of enzymatic reactions might be caused by the hydrophobic character of the substituent on the phosphorus atom. Steric effect of this substitution cannot also be taken into account in view of the aforementioned resistance of nucleoside phosphites *XVIII* towards the above enzymes, since in the latter phosphites the phosphorus atom is substituted by hydrogen. Compounds *I*, *XII*, *XIV*, *XVI*, and *XVII* are quite resistant to the action of the snake venom phosphodiesterase and compound *XII* is also resistant to pancreatic ribonuclease and ribonuclease T2 degradation. Noteworthy is however the snake venom (*Crotalus adamanteus*) 5′-nucleotidase degradation of nucleoside hydroxyalkanephosphonates.



XVI, R = $HOCH_2CH_2$
XVII, R = CH_3CHOH

XVIII, R = H
XIX, R = CH_3
XX, R = $HOCH_2CH_2O$

In formulae *XVIII*—*XX*, a B = uracil, b B adenine

5′-Nucleotidase hydrolyses the phosphomonoester linkage of 5′-ribonucleotides and 2′-deoxyriboside 5′-phosphates[7] under the formation of nucleosides and inorganic phosphate. This enzyme requires the presence of a free 3′-hydroxylic function which must be in the *ribo* configuration[16]. In contrast to nonspecific phosphomonoesterases which hydrolyse whatever phosphomonoester, only nucleotide derivatives are hydrolysed by 5′-nucleotidase. Interaction between the enzyme and the heterocyclic moiety of the nucleotide molecule appears as the necessary

* The tests were performed by Dr I. Votruba, Department of Molecular Biology of this Institute.

condition of the enzyme activity[17]. The optimum pH value (8—8·5) of this enzyme lies in a region where a full dissociation of the phosphomonoester group in nucleotide derivatives may be assumed. It has been however observed that the snake venom 5'-nucleotidase hydrolyses the ester linkage of nucleoside 5'-phosphites *XVIII* under the formation of the nucleoside and phosphorous acid[18]. Since compounds *XVIII* can form only monoanions under the conditions given, a monoanion of the nucleotide derivative is obviously sufficient for the enzymatic reaction. It is not however clear why no degradation of 5'-nucleotide esters or even of the internucleotide linkage occurs while the corresponding compounds are capable of dissociation under the formation of monoanions.

The resistance of 6-azauridine 5'-methanephosphonate towards 5'-nucleotidase has not been explained so far[3]. We have therefore reinvestigated this observation with the use of two additional 5'-O-methanephosphonates *XIXa,b* derived from uridine and adenosine. Compounds *XIXa,b* were prepared by a modification of the reported method[3], namely, by condensation of methanephosphonic acid pyridinium salt with the protected nucleoside derivative *IX* and the subsequent removal of the protecting group in acidic media. We have now observed that compounds *XIXa,b* are degraded to a small extent when a great excess of the enzyme is used. The corresponding nucleoside and methanephosphonic acid (both identical with authentic specimens on paper chromatography) are formed as degradation products.

In contrast to the resistant 5'-O-methanephosphonates *XIX*, the 5'-O-hydroxymethanephosphonates *I* and *XIV* are very good substrates for the snake venom 5'-nucleotidase (Table I). Nucleosides and hydroxymethanephosphonic acid (*II*) which are obtained as degradation products, confirm also the structure of the substrate. It is evident

TABLE I

Degradation of Uridine 5'-Phosphate Analogues of the General Formula RO—P(O)OH (R = 5'-uridyl) with the Snake Venom 5'-Nucleotidase (*Crotalus adamanteus*)
|
X

Compound	X	k^a	Degradation[b], %
UMP	OH	1·00	80·0
XIXa	H	0·49	76·0
XXa	CH_3	[c]	8·0
Ia	CH_2OH	0·39	70·0
XVII	CH_2CH_2OH	[c]	5[d]
XVIII	$CH(OH)CH_3$	[c]	2[e]
XXIa	OCH_2CH_2OH	[c]	0

[a] Catalytic constant (rel. to UMP); [s] = $2 \cdot 10^{-2}$M, [e] = $3 \cdot 0 - 7 \cdot 0 \cdot 10^{-7}$M (empirical for $M = 1 \cdot 10^5$), 0·05M-Tris, pH 9·0; for uridine 5'-phosphate, $k = 202$; [b] stationary experiment, [e] = $2 \cdot 10^{-6}$M; other data see note *a*; incubation at 37°C for 4 h; [c] cannot be measured; [d] 8% degradation at [e] = $5 \cdot 10^{-6}$M; [e] 5% degradation at [e] = 10^{-5}M.

that the methyl group at the phosphorus atom of compounds *XIX* may interfere with the interaction between the enzyme and the substrate because of the steric as well as hydrophobic effect. The behaviour of 5'-hydroxymethanephosphonates *I* and *XIV* excludes any steric effect and indicates that it is the hydrophilic character of groups in the neighbourhood of the active centre of the enzyme which is the decisive factor for the complex formation between the substrate and the enzyme. Consequently, the methyl esters of 5'-nucleotides and 5'-O-methanephosphonates *XIX* are not degraded by 5'-nucleotidase while the 5'-O-phosphites *XVIII* and 5'-O-hydroxymethanephosphonates *I* and *XIV* are substrates for this enzyme. The 5'-nucleotide 2-hydroxyethyl esters *XIX* are quite resistant to the action of the snake venom 5'-nucleotidase. Compounds *XVI* and *XVII* are also practically resistant. Consequently, the hydrophilic group must be located in the α-position in respect to the phosphorus atom and cannot be secondary. The affinity of the 2-hydroxyethyl ester *XX* must be even lower than that of the otherwise poor substrate 5'-O-2-hydroxyethanephosphonate *XVI*, since the hydrophilic hydroxylic function in compound *XX* is more distant from the phosphorus atom than in compound *XVI*. With (3'→5')-dinucleoside phosphates, the secondary hydroxylic function is equally distant from the phosphorus atom as with 2-hydroxyethyl esters *XX*; hence a similar resistance of (3'→5')-dinucleoside phosphates to the action of 5'-nucleotidase. The requirement of a hydrophilic character of the substrate near the active site of 5'-nucleotidase is obviously accompanied by another postulate of the presence of a free 3'-hydroxylic group in the *ribo* configuration as stated earlier.

EXPERIMENTAL

Unless stated otherwise, the solutions were taken down on a rotatory evaporator at 35°C/15 Torr. Substances were dried over phosphorus pentoxide at room temperature and 0·1 Torr.

Methods

Ultraviolet spectra were measured on a Beckman DU apparatus in 0·01M-HCl. Quantitative determinations were performed with the use of extinction coefficients[19]. Paper chromatography was performed by the descending technique on Whatman No 1 (preparative runs on Whatman No 3 MM) in the solvent system S_1, 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2), and S_2, ethanol–1M ammonium acetate (5 : 2). Paper electrophoresis was performed by the Markham and Smith[20] technique on Whatman No 3 MM (preparative runs on 16 cm wide strips of Whatman No 3MM) in the buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), and E_2, 0·05M sodium hydrogen citrate (pH 3·5). For R_F values and electrophoretic mobilities see Table II. DEAE-Cellulose (Cellex D, standard capacity, Calbiochem, Los Angeles, U.S.A.) column chromatography was performed in the HCO_3^- cycle on a 100 × 4 cm column with the use of a linear gradient of triethylammonium hydrogen carbonate pH 7·5 (2 l of water in the mixing chamber and 2 l of the buffer of the corresponding final concentration in the reservoir) at the rate of 3 ml per min, the fractions being taken in 10 min intervals. The course of elution was continuously determined on a Uvicord apparatus. The appropriate fractions were pooled, evaporated, the residue coevaporated with two 50 ml portions of methanol, and the content determined spectrophotometrically in methanol as solvent.

Enzymatic degradations. The substance (2—3 μmol) in 50 μl of 0·05M-Tris-HCl was incubated at 37°C for 4 h (corrections for blanc values) with *a*) ribonuclease T2, pH 7·0, 20 μg of the enzyme (a gift of Professor H. Witzel, University Marburg, Germany), *b*) pancreatic ribonuclease, pH 8·0, 50 μg of the enzyme (Calbiochem, Los Angeles, U.S.A.), *c*) snake venom phosphodiesterase, pH 9·0, 20 μg of the enzyme from *Crotalus terr. terr.* (0·1% in 50% aq. glycerol; Boehringer, Mannheim, Germany), *d*) alkaline phosphatase *Escherichia coli*, pH 9·0, 10 μg of the enzyme (0·1% solution in 30% aq. ammonium sulfate: Worthington, U.S.A.), and *e*) 5′-nucleotidase (*Crotalus adamanteus*), pH 9·0, 0·005M magnesium sulfate, 100 μg of the protein (Worthington, U.S.A.).

Reaction of 2′,3′-O-Ethoxymethyleneadenosine (*III*) and Hydroxymethanephosphonic acid (*II*)

The cyclohexylammonium salt[4] of the acid *II* (5 mmol) was converted into the pyridinium salt on a column (25 ml) of pyridinium Dowex 50 X 8 ion exchange resin (the elution was performed with 200 ml of 30% aqueous pyridine). The effluent was concentrated to a small volume, the concentrate treated with 2 mmol of compound[5] *III*, and the mixture dried by coevaporation with five 25 ml portions of pyridine at 30°C/0·1 Torr. The residue was then dissolved in pyridine (20 ml) and N,N′-dicyclohexylcarbodiimide (5 g; 25 mmol) was added. The mixture was kept at room temperature for 6 days. Water (5 ml) and triethylamine (2 ml) were than added, the mixture kept at room temperature for one hour, diluted with water, washed with two 25 ml portions of ether, the aqueous phase concentrated, the concentrate diluted with 50% aqueous acetic acid (25 ml), and the resulting mixture heated at 50°C for 30 min. The mixture was evaporated, the residue coevaporated with three 25 ml portions of water, the final residue dissolved in water (50 ml), the solution adjusted to pH 7—7·5 by the addition of aqueous ammonia, filtered through Celit, and the filtrate applied to a column of DEAE-cellulose. The elution was performed under standard conditions (2 l of 0·4M buffer solution in the reservoir). The 0·12—0·14M fraction was rechromatographed on Whatman No 3 MM in the solvent system S_1. The ultraviolet-absorbing bands of compound *IV* were eluted with dilute (1 : 100) aqueous ammonia (50 ml). The eluate was concentrated and the concentrate freeze-dried. Yield, 1960 A_{260} (0·07 mmol; 7%) of the am-

TABLE II

Paper Chromatography (R_F values) and Electrophoresis (mobility related to uridine 2′(3′)-phosphate)

Compound	R_F				Compound	R_F			
	S_1	S_2	E_1	E_2		S_1	S_2	E_1	E_2
Uridine 2′(3′)-phosphate	0·12	0·25	1·00	1·00	*Ic*	0·42	0·48	0·34	0·80
IV	0·21	0·36	0·32	0·56	*Id*	0·51	0·27	0·56	0·90
II	0·18	—	—	—	*XI*	0·44	0·56	0·56	1·00
IXa	0·76	—	—	—	*XIV*	0·49	0·63	0·41	1·00
IXb	0·75	—	—	—	*XVa*	0·50	—	—	—
IXc	0·78	—	—	—	*XVb*	0·67	—	—	—
IXd	0·70	—	—	—	*XIXa*	0·46	0·58	0·56	1·00
Ia	0·44	0·56	0·56	1·00	*XIXb*	0·45	0·45	0·34	0·80
Ib	0·35	0·48	0·57	0·25	*XVI*	0·24	0·47	0·54	—
					XVII	0·25	0·50	0·35	0·82

monium salt of compound *IV*, homogeneous on chromatography in solvent systems S_1 and S_2 and electrophoresis in buffer solutions E_1 and E_2; content (spectrophotometrically), 96%. For N/P calculated: 11·0; found: 10·8. For adenosine/phosphorus calculated: 2·0; found (spectrophotometrically): 2·12.

Ribonucleoside 5′-O-Hydroxymethanephosphonates *I*

1,1′-Carbonyldiimidazole (6 mmol) was added to a solution of anhydrous acid[4] *II* (3 mmol) in dimethylformamide (7 ml), the mixture stirred at room temperature for 2 hours, and then treated with 2 mmol of the 2′,3′-O-ethoxymethylene ribonucleoside derivative[5] *IX* (with basic ribonucleosides, it is not necessary to use derivatives with a protected amino group on the heterocyclic moiety). The whole was stirred at room temperature for 5 days, evaporated, the residue heated in 50% aqueous acetic acid (20 ml) at 50°C for 30 min, the solution evaporated, the residue coevaporated with three 25 ml portions of water, the final residue dissolved in water (50 ml), the solution adjusted to pH 7·0—7·5 by the addition of aqueous ammonia, filtered through Celite, and the filtrate applied to a column of DEAE-cellulose. The elution was performed under standard conditions (2 l of 0·2M buffer solution in the reservoir). The fraction of product *I* was processed as usual and the residue chromatographed on Whatman No 3 MM (50—60 mg per one sheet) in the solvent system S_1. Bands of the product *I* were eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate concentrated, and the concentrate freeze-dried. For yields and characteristics of compounds *I* see Table III.

2′(3′)-O-*Hydroxymethanephosphonyluridine* (XII) was prepared from 5′-O-benzoyluridine[12] (*XI*; 2 mmol) analogously to compounds *I*. The dimethylformamide was evaporated, the residue heated at 50°C for 2 hours with a mixture (1 : 1; 50 ml) of 30% methanolic ammonia and water, the solution evaporated, the residue redissolved in water (50 ml), and the solution washed with ether (20 ml). The aqueous phase was concentrated and the concentrate processed by chromatography on DEAE-cellulose as above. Yield, 0·04 mmol (2%) of compound *XII*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1).

The attempted preparation of 3′-O-*hydroxymethanephosphonyl-2′-deoxythymidine* was performed similarly with the use of 5′-O-di(*p*-methoxyphenyl)phenylmethyl-2′-deoxythymidine[13] (2 mmol). The reaction mixture was processed after 5 days at room temperature analogously to compounds *I*. Only 2′-deoxythymidine (*XVb*) was then detected by chromatography in the solvent systems S_1 and S_2 and electrophoresis in the buffer solution E_1. The same result was obtained when the reaction mixture was kept at room temperature for 5 days and then heated at 60°C for 2 days.

TABLE III

Nucleoside 5′-O-Hydroxymethanephosphonates *I* (Ammonium Salts)

Compound	Yield %	Formula	M.w.	Calculated		Found	
				% N	% P	% N	% P
Ia[a]	26	$C_{10}H_{18}N_3O_9P$	355·3	11·8	8·72	10·00	9·05
Ib	19	$C_{10}H_{19}N_4O_8P$	354·3	15·8	8·70	14·40	7·02
Ic	13	$C_{10}H_{19}N_6O_7P$	378·2	22·2	8·2	18·4	7·7
Id	40	$C_{11}H_{19}N_6O_8P$	394·2	21·3	7·86	20·5	7·6

[a] Prepared also from the 2′,3′-O-isopropylidene derivative. The protecting group was removed on refluxing in 80% aqueous acetic acid for 30 min. Yield 46%. Found: 12·05% N, 9·02% P.

5′-O-Hydroxymethanephosphonyl-2′-deoxythymidine (*XIV*)

A. The reaction was performed from 2 mmol of 3′-O-benzoyl-2′-deoxythymidine[12] (*XIII*) analogously to the preparation of compound *XII*. The chromatography was carried our on DEAE-cellulose and then on Whatman No 3 MM in the solvent system S_1. Yield, 10% of the ammonium salt of compound *XIV*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1); content (by spectrophotometry), 95%. For $C_{11}H_{20}N_3O_8P$ (353·2) calculated: 11·9% N, 8·78% P; found: 10·9% N, 8·10% P.

B. The reaction was performed from 2 mmol of 2′-deoxythymidine (*XVb*) analogously to the preparation of compounds *I*. After 5 days at room temperature, the mixture was diluted with water (50 ml) and directly applied to a column of DEAE-cellulose. The eluates were processed similarly to those of compounds *I*. Yield, 0·64 mmol (32%) of compound *XIV*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1), and identical with the specimen prepared by procedure *A*.

Uridine and Adenosine 5′-O-methanephosphonates (*XIXa,b*)

The barium salt[3] of methanephosphonic acid (10 mmol) was converted into the pyridinium salt on a column (50 ml) of pyridinium Dowex 50 X 8 (the elution was performed with 200 ml of 30% aqueous pyridine). The eluate was evaporated, the residue dissolved in pyridine (50 ml) and then the 2′,3′-O-ethoxymethylene ribonucleoside derivative[5] *IX* (5 mmol) was added. The mixture was dried by coevaporation with five 25 ml portions of pyridine and the residue was dissolved in 50 ml of pyridine. N,N′-Dicyclohexylcarbodiimide (10 g) was then added and the whole kept at room temperature for 6 days under exclusion of atmospheric moisture. Water (20 ml) was added, the mixture kept at room temperature for 1 h, diluted with additional water (200 ml), and washed with two 50 ml portions of ether. The aqueous phase was evaporated, the residue dissolved in 50% aqueous acetic acid (50 ml), the solution heated at 50°C for 30 min, and evaporated with three 50 ml portions of water, the final residue dissolved in water (100 ml), the solution adjusted to pH 7·5 by the addition of aqueous ammonia, filtered through Celit, and the filtrate applied to a column of DEAE-cellulose. The elution was performed under standard conditions (2 l of 0·2M buffer solution in the reservoir). The 0·08—0·12M fraction was purified by chromatography on Whatman No 3 MM in the solvent system S_1. The elution was performed with 50 ml of dilute (1 : 100) aqueous ammonia. Concentration and freeze-drying afforded the ammonium salt of compounds *XIX*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1). Yield, 60% of 5′-O-methanephosphonyluridine (*XIXa*); content 92%. For N/P calculated: 3·0; found: 3·1. Yield, 48% of 5′-O-methanephosphonyladenosine (*XIXb*); content 97%. For N/P calculated: 6·0; found: 6·2.

5′-O-(2-Hydroxyethane)phosphonyluridine (*XVI*)

A. The barium salt of 2-hydroxyethanephosphonic acid[14] (3 mmol) was converted into the pyridinium salt on a column (20 ml) of pyridinium Dowex 50 X 8 ion exchange resin. The column was eluted with 30% aqueous pyridine (100 ml), the eluate evaporated to dryness, the residue dried by coevaporation with five 25 ml portions of pyridine at 30°C/0·1 Torr and the pyridine removed by coevaporation with four 20 ml portions of dimethylformamide at 40°C/0·1 Torr. The final residue was dissolved in dimethylformamide (7 ml) and N,N′-carbonyldiimidazole (6 mmol) was added to the solution. The resulting mixture was stirred at room temperature for 2 h, treated with 2′,3′-O-isopropylideneuridine (2 mmol), the stirring continued for additional 5 days at room temperature, the mixture diluted with water (50 ml), and applied to a column of DEAE-cellulose. The elution was performed under standard conditions (2 l of 0·2M buffer solution in the reservoir). The 0·10—0·12M fraction was evaporated, the residue refluxed in 80%

aqueous acetic acid (50 ml) for 30 min, the solution evaporated, the residue coevaporated with water (20 ml), and the final residue chromatographed on Whatman No 3 MM in the solvent system S_1. Bands of the product *XVI* were eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate concentrated, and the concentrate freeze-dried. Yield (by spectrophotometry), 30% of the ammonium salt of compound *XVI*, homogeneous on chromatography (S_1, S_2) and electrophoresis (E_1); content 60% (salts as contaminants). For N/P calculated: 3·0; found: 3·3.

B. A solution of 2-hydroxyethanephosphonic acid imidazolide (prepared from 3 mmol of the free acid, see paragraph *A*) in dimethylformamide (7 ml) was treated with 2 mmol of 2′,3′-O-ethoxymethyleneuridine[5] (*IXa*), the mixture stirred at room temperature for 5 days, and processed analogously to compounds *I*. Yield 0·2 mmol (10%) of the ammonium salt of compound *XVI*, identical with the specimen obtained by procedure *A*.

C. The pyridinium salt (3 mmol) of 2-hydroxyethanephosphonic acid (for the preparation see paragraph *A*) was dissolved in pyridine (10 ml). The nucleoside derivative[5] *IXa* (2 mmol) was then added to the solution and the mixture dried by repeated coevaporations with five 20 ml portions of pyridine at 30°C/0·1 Torr. The final residue was dissolved in pyridine (15 ml) and N,N′-dicyclohexylcarbodiimide (3 g) was added to the solution. The mixture was kept at room temperature for 5 days under exclusion of atmospheric moisture and processed analogously to compounds *XIV*. Yield 18% of the ammonium salt of compound *XVI*, identical with specimens obtained by procedures *A* and *B*.

5′-O-(1-*Hydroxyethane*)*phosphonyluridine* (XVII) was prepared by analogy to the above procedure *A* (reaction period 5 days) from 2 mmol of 2′,3′-O-isopropylideneuridine and 3 mmol of 1-hydroxyethanephosphonic acid imidazolide in dimethylformamide (7 ml). Yield, 25% of the ammonium salt of compound *XVI*, homogeneous on chromatography (S_1, S_2) and electrophoresis (E_1); content 90%. For N/P calculated: 3·0; found: 3·2.

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