Tetrahedron. Vol. 25, pp. 477 to 484. Pergamon Press 1969. Printed in Great Britain

THE REACTION OF PHENYLBORONIC ACID WITH NUCLEOSIDES AND MONONUCLEOTIDES

A. M. YURKEVICH, I. I. KOLODKINA, L. S. VARSHAVSKAYA,
V. I. BORODULINA-SHVETZ, I. P. RUDAKOVA and N. A. PREOBRAZHENSKI
The All-Union Research Vitamin Institute, Moscow, USSR

(*Received in the UK* 4 *August* 1967; *Accepted for publication* 31 *July* 1968)

Abstract—2',3'-O-Phenylboronates of adenosine (I), uridine (II), inosine (III) and cytidine (IV) have been prepared from corresponding nucleosides and phenylboronic acid (PBA). The protecting group can be removed with propane-1,3-diol under mild conditions in anhydrous medium. On heating 2'(3')-monophosphates of adenosine and cytidine with PBA a specific dephosphorylation was achieved leading to 2',3'-phenylboronates (I, IV). The synthetic aspects of the application of nucleoside phenylboronic esters (tritylation, tosylation and phosphorylation) have been studied.

IN A previous paper we showed that phenylboronic acid (PBA) can be used for protection of 2',3'-*cis*-glycol groups in the phosphorylation of adenosine.[1] The present paper deals with a synthesis as well as with some properties and reactions of phenylboronates of adenosine (I), uridine (II), inosine (III) and cytidine (IV).

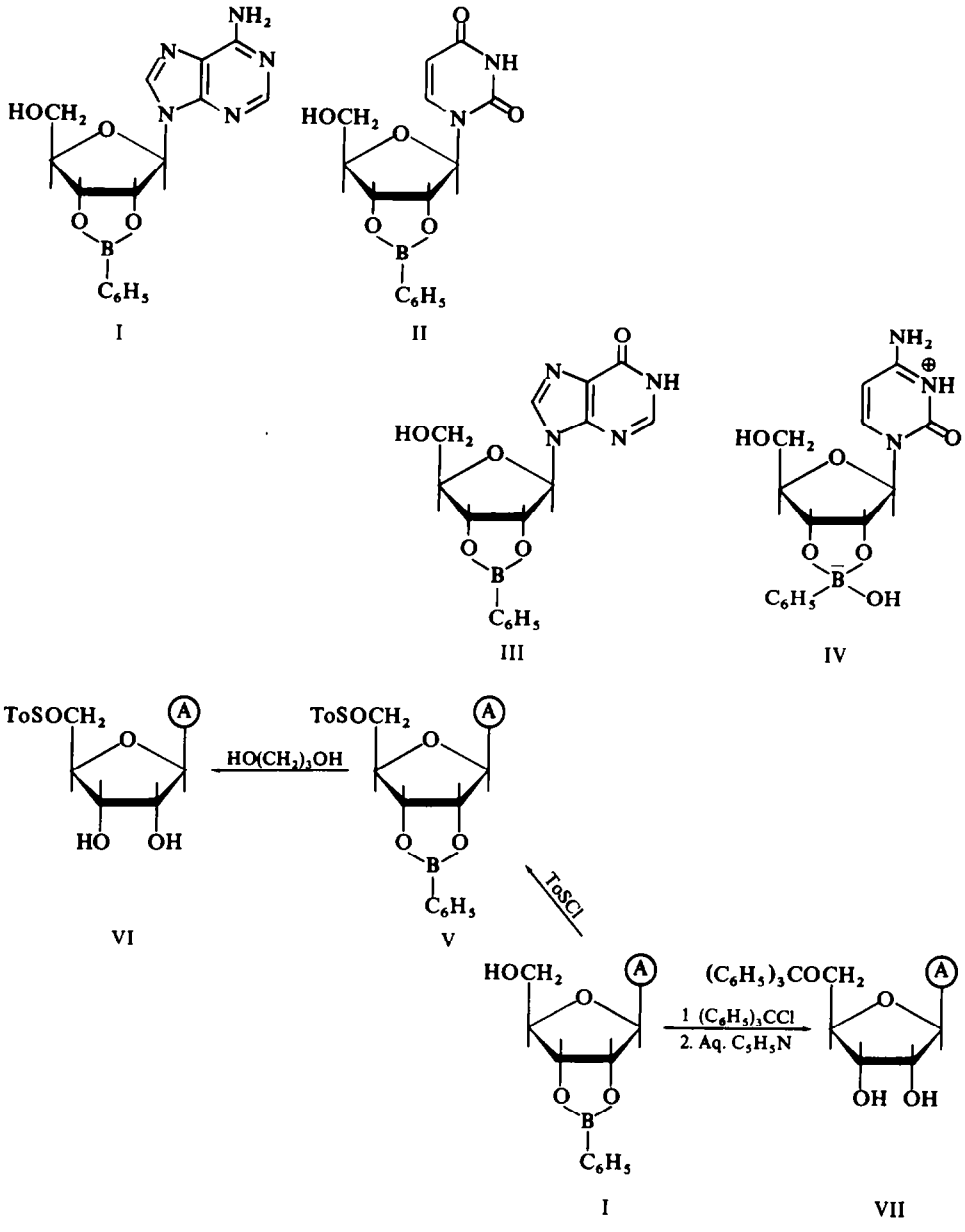
We have prepared 2',3'-O-phenylboronic esters of nucleosides from PBA and nucleosides in pyridine solution. The reaction afforded the crystalline solids, stable in air, soluble in acetone, pyridine, dioxan and dimethylformamide.

The esters are easily hydrolysed in water. The spectrophotometric measurements of the quantity of phenylboronic acid formed during the hydrolysis showed that the hydrolysis of the esters at pH 6·5 was complete within 10–15 min. The protecting phenylboronic group could be also removed in organic solvents in the presence of propan-1,3-diol.

Table 1 lists some properties of the compounds prepared. IR spectra of all the phenylboronates obtained exhibit absorption bands at 1370 and 1440 cm^{-1} which could be due to B—O and B—C_{aryl}-vibrations.[2] Phenylboronate of cytidine also provides a band at 1725 cm^{-1} ($\delta = \overset{+}{N}_1$—H).[3] The same band was observed in spectra of three cytidyl acids existing in the form of bipolar ions. Moreover, hydroxy vibrations caused an absorption at 3630 cm^{-1}.[4] Considering the above data, phenylboronate of cytidine may be assigned the structure IV:

2'3'-O-Phenylboronic esters of nucleosides were shown to be convenient intermediate compounds for the synthesis of 5'-O-derivatives of nucleosides. Thus, in the reaction of adenosine-2',3'-O-phenylboronate with toluene *p*-sulphonyl chloride the totally protected nucleoside (V) was obtained, which after treatment with propane-1,3-diol gave 5'-O-tosyladenosine (VI); on the other hand, the reaction of I with triphenylchloromethane and further work up with aqueous pyridine afforded 5'-trityladenosine (VII).

We have also studied the possibility of using phenylboronates of nucleosides in a synthesis of nucleoside-5'-mono-di- and triphosphates.



Phenylboronates were phosphorylated with P^1-diphenyl-P^2-morpholidopyrophosphochloride, as well with morpholidophosphodichloride[5] or with β-cyanoethyl phosphate in the presence of N,N′-dicyclohexylcarbodiimide[6] and corresponding treatment led to 5′-monophosphates (IX–XI; cf. Table 2).

Mild hydrolysis of intermediate morpholidophosphochlorides of 2′,3′-O-phenylboronates of nucleosides (VIII) and subsequent removal of phenylboronic protection with propane-1,3-diol yielded the 5′-phosphormorpholidates of nucleosides (XII,

TABLE 1. PROPERTIES OF NUCLEOSIDE PHENYLBORONATES PREPARED

Compound	Yield %	M.p. (solv)	$[\alpha]_D^{20}$ soluble conc.	IR spectrum				Found %			Analysis			
				ν_{O-H}	ν_{B-O}	$\nu_{B-C_{aryl}}$	$\delta = \overset{+}{N}-H$	C	H	N	Formula	Calc. % C	H	N
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2',3'-O-Phenylboronate of adenosine (I)	80·0	223·5–224° (dioxan–ether)	−93° pyridine 0·6	3400	1350	1400	—	54·26	4·68	19·95	$C_{16}H_{16}BN_5O_4$	54·42	4·57	19·83
2',3'-O-Phenylboronate of uridine (II)	72·0	221·5–222° (acetone)	−57·2° pyridine 0·45	3400	1360	1440	—	55·04	4·65	8·51	$C_{15}H_{15}BN_2O_6$	55·38	4·49	8·33
2',3'-O-Phenylboronate of inosine (III)	57·0	178–179° (pyridine)	—	3350	1370	1440	—	54·54	4·82	16·07	$C_{16}H_{15}BN_4O_5$	54·25	4·48	15·82
2',3'-O-Phenylboronate of cytidine (IV)	66·0	140° (acetone)	−65° pyridine 0·45	3630 3350	1370	1440	1725	48·97	5·40	—	$C_{15}H_{16}BN_3O_5 \cdot 2H_2O$	49·31	5·75	—

TABLE 2. PHOSPHORYLATION OF 2',3'-O-PHENYLBORONATES OF NUCLEOSIDES

2',3'-Phenylboronic ester of nucleoside	Phosphorylating agent	Yield %				
		Initial nucleoside	5'-Monophosphate	2'(3')-Monophosphate	2'(3')-5'-diphosphate	Unidentified
Adenosine	Morpholidophosphodichloride	—	57·5	18·0	23·0	—
Adenosine	β-Cyanoethyl phosphate	6·9	61·0	20·0	30·0	—
Adenosine	P^1-Diphenyl-P^2-Morpholidopyrophosphochloride	2·8	77·5	1·5	13·0	3·1
Uridine	P^1-Diphenyl-P^2-Morpholidopyrophosphochloride	—	82·0	6·4	8·1	3·0
Cytidine	P^1-Diphenyl-P^2-Morpholidopyrophosphochloride	—	80·0	—	18·6	0·8

XIII) in the form of their salts with strong amines which were used in reaction with inorganic phosphate and pyrophosphate for the synthesis of di- and triphosphates.[7]

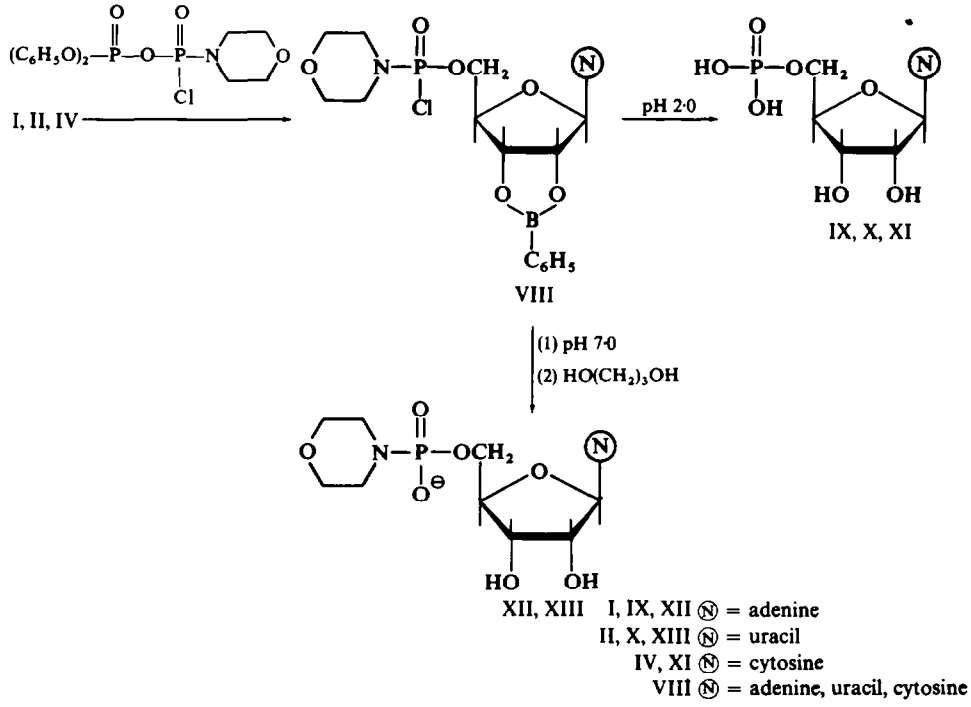


TABLE 3. SPLITTING OF 2′(3′)-MONOPHOSPHATES OF ADENOSINE AND CYTIDINE IN THE PRESENCE OF PBA

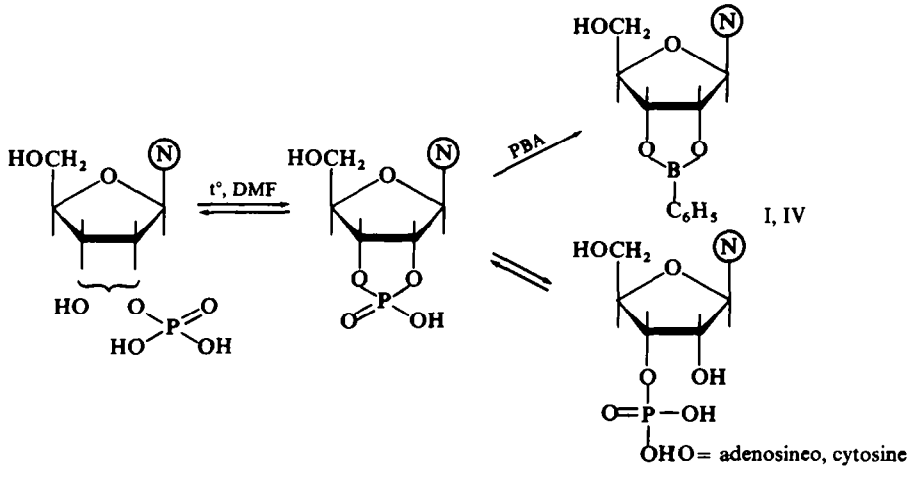
NN	Compound Initial	Obtained	Temperature								
			100° Duration (hr)			120° Duration (hr)			140° Duration (hr)		
			1	2	3	1	2	3	1	2	3
1	2	3	4	5	6	7	8	9	10	11	12
1.	Adenosine-2′(3′)-monophosphate and PBA	Adenosine-3′-monophosphate (%)	29·3	40·3	48·8	23·6	21·6	16·0	6·9	5·8	5·7
		Adenosine-2′-monophosphate (%)	35·9	21·1	10·0	—	—	—	—	—	—
		Adenosine 2′,3′-cyclophosphate (%)	22·9	28·7	29·3	48·8	44·1	39·7	30·7	28·7	24·0
		2′,3′-O-phenylboronate of adenosine (%)	8·9	9·9	11·9	27·6	34·4	44·3	62·4	65·5	70·3
2.	Adenosine-2′(3′)-monophosphate	Adenosine-2′(3′)-momophosphate* (%)	69·0	59·0	51·6	—	—	15·0	11·4	8·2	2·7
		Adenosine-2′,3′-cyclophosphate (%)	23·2	32·4	37·5	—	—	65·0	57·3	59·4	75·7
		Adenosine (%)	7·8	8·6	10·9	—	—	20·0	31·3	32·4	21·6
3.	Cytidine-2′(3′)-monophosphate	Cytidine-3′-monophosphate (%)	65·0	61·8	58·2	42·2	15·6	15·5	7·6	5·6	5·3
		Cytidine-2′,3′-cyclophosphate (%)	24·3	27·2	28·9	22·3	32·7	32·5	15·8	8·8	7·5
		Phenylboronic ester of cytidine (%)	10·7	11·0	12·9	35·5	51·7	52·0	65·0	76·6	74·6
		Cytosine	–	—	—	—	—	—	11·6	9·0	12·6

* The percentage of monophosphate mixture corresponds to the initial one: adenosine-2′-monophosphate 60%, adenosine-3′-monophosphate 40%.

The reaction of isomeric 2′(3′)-monophosphates of adenosine and cytidine with PBA proceeds via dephosphorylation and provides nucleoside phenylboronates (I and IV). The reaction was carried out in dimethylformamide at various temperatures (100, 120, 140°). The results are shown in Table 3.

The data of this Table show that the heating of nucleoside 2′(3′)-monophosphates with PBA in dimethylformamide at 100° led to a cyclophosphate and to isomerization of 2′-monophosphate into 3′-monophosphate of adenosine. Increase in temperature (120°) caused a considerable decrease in yield of monophosphates, while the content of nucleoside phenylboronates (I, IV) increased. Thus, phenylboronate of cytidine (IV) was obtained in 74·6% yield.

The same method provides 70·3% of adenosine from phenylboronic ester (I; spectrophotometric determination). In the control runs in absence of PBA the heating of 2′(3′)-mononucleotides in dimethylformamide yielded adenosine-2′,3′-cyclophosphate showing a notable increase in yield with temperature (75%, 140°, 3 hr). 2′,3′-Cyclophosphates of nucleosides should be the intermediates in the reaction of nucleoside-2′(3′)-phosphates with PBA. The elucidation of the reaction mechanism is now in progress.



EXPERIMENTAL

Whatman No. 1 paper and the following solvents systems were used in chromatography: (1) propan-2-ol–conc NH_4OH–water (7:1:2); (2) EtOH–0·5N NH_4OAc, saturated with sodium tetraborate (5:2); (3) EtOH–1M NH_4OAc, pH 7·5 (5:2); (4) BuOH–AcOH–water (4:1:5); (5) propan-1-ol–conc NH_4OH–water (6:3:1); (6) conc $(NH_4)_2SO_4$–1M NaOAc–propan-2-ol (80:12:2); (7) BuOH–HCO_2H–water (77:20:3).

Paper electrophoresis was carried out at a voltage gradient 16–20 v/cm for 1 hr in 0·05 M triethylammonium bicarbonate soln (pH 7·5). Inorganic phosphorus was detected by molybdate method;[8] PBA was defined by a qualitative reaction with diphenylcarbazone,[9] and the sec. OH groups—by the Dekker's method.[10] Electrophoretic mobilities of the compounds were calculated relative to those of nucleoside-5′-monophosphates.

Nucleoside phenylboronates (I–IV). To a stirred 2·0 mM soln of nucleoside in 100 ml pyridine, 2·0 mM PBA in 50 ml of the same solvent was added while heating under reflux for 2 hr. The mixture was evaporated *in vacuo*, the residue was washed with ether and recrystallized (Table 1).

Determination of the stability of 2′,3′-phenylboronic esters of nucleosides. Aliquot parts of aqueous solns of phenylboronates of nucleosides (30 μg/ml) were shaken with equal volumes of ester (20 sec) in 10, 30

and 60 min and the optical density of the solns was measured at 217 mμ. A quantity of PBA, formed during the hydrolysis was determined by comparing the extinctions of the solns with the extinction of the control ester solution of PBA, prepared in the same manner.

During the first 10 min more than 90% of phenylboronic acid was extracted from aqueous solns of phenylboronates of nucleosides.

5'-O-*Tosyl adenosine-2',3'-O-phenylboronate* (V). To a stirred soln of 1·76 g of I in 40 ml pyridine, 0·94 g toluene *p*-sulphonylchloride was added at 0°, and the mixture was left for 18 hr at the same temp. The cold mixture was neutralized and extracted with chloroform. After evaporation *in vacuo*, the ether was added yielding 1·26 g of V (49·5%); R_f 0·72 (system I); 0·78 (system 4); UV spectrum (in MeOH): λ_{max} 260 mμ log ε 4·16. (Found: C, 54·22; H, 4·64. Calc. for $C_{23}H_{22}BN_5O_6S$: C, 54·46; H, 4·37%).

5'-O-*Tosyl adenosine* (VI). To a soln of 0·3 g of amorphous V in 10 ml $CHCl_3$, 0·08 ml propane-1,3-diol was added dropwise. The solvent was removed and the residue washed with petroleum ether. 0·22 g (96·5%) of VI was obtained (dec at 155–156°). UV spectrum (in MeOH): λ_{max} 260 mμ, log ε 4·11. (Found: C, 48·50; H, 5·01. Calc. for $C_{17}H_{19}N_5O_6S$: C, 48·45; H, 5·02%).

5'-*Trityl adenosine* (VII). 1·0 g of I and 1·15 g of triphenylchloromethane was heated at 100° in 30 ml pyridine and left at 18–20° for 20 hr. 200 ml of ice water was added (10 hr, 0°) and the solid was separated, yield of VII was 0·67 g (46%), m.p. 254° (pyridine–alcohol), $[\alpha]_D^{20} = -22°$ (pyridine, 1·0) lit. m.p. 250°.[11] (Found: C, 68·63; H, 5·36; N, 13·4. Calc for $C_{29}H_{27}N_5O_4$: C, 68·35; H, 5·34; N, 13·7%).

Adenosine-5'-monophosphate (IX)

(a) *With* P^1-*diphenyl*-P^2-*morpholidopyrophosphochloride.* To a soln of 0·04 g morpholidophosphodichloride and 0·05 g diphenylphosphoric acid in 0·5 ml dioxan, 0·06 ml 2,6-lutidine was added and in 15 min 0·035 g phenylboronic ester of adenosine. The mixture was left for 48 hr and then hydrolysed with 100 ml water at pH 2·0. After 2 days standing, the mixture was worked up with chloroform (for removing phenylboronic and diphenylphosphonic acids), mixed with 5% NH_4OH to pH 8·0, extracted with ether (amines) and chromatographed over the "Dowex 1 × 4", 200–400 mesh ($HCOO^-$). The column was washed with water. The compounds were eluated, using the concentration gradient of formic acid. The eluting solutions were: (1) 100 ml water–100 ml 0·125 N formic acid; (2) 50 ml 0·125 N acid–50 ml 0·5 N acid; (3) 50 ml 0·5 N acid–50 ml 1 N acid; (4) 5 N acid. Fraction volumes were 5 ml. Fractions 11–17 gave adenosine: R_f 0·64 (system 1), E_{AMP} 0·12. Fractions 21–35 gave adenosine-5'-monophosphate (IX): R_f values were: 0·12 (system 1), 0·02 (system 2), 0·2 (system 3), 0·22 (system 5), E_{AMP} 1·0.

Fractions 41–44 gave adenosine-2'(3')-monophosphate: R_f values were 0·11 (system 1), 0·15 (system 2), 0·26 (system 3) : E_{AMP} 1·0 Fractions 83–88 gave adenosine-2'(3'),5'-diphosphate E_{AMP} 1·32.

The compounds from the 58–63 and 73–78 fractions were not identified. The results of the chromatographic resolution are given in Table 2.

(b) *With morpholidophosphodichloride.* To a soln of 0·051 g morpholidophosphodichloride in 1·0 ml dioxan, 0·044 g of I and 0·016 ml 2,6-lutidine was added. The reaction mixture was stirred at 80° for 3 hr, left at 18–20° for 20 hr, then hydrolysed with 25 ml water (pH 2·0), worked up with chloroform, 5% NH_4OH and ester in succession and chromatographed over the "Dowex-1" as it is described in A.

(c) *With β-cyanoethyl phosphate.* A soln of 0·035 g of I in 2·0 ml dioxan was mixed with 0·3 ml of a standard soln of β-cyanoethyl phosphate and the reaction mixture was dried by azeotropic distillation with pyridine. The syrup was treated with a soln of 0·18 g N,N'-dicyclohexylcarbodiimide in 1·0 ml pyridine and left standing at 18–20° for 2 days. Then 1 ml water was added and after standing 30 min, the solvent was removed; 2 ml water was added to the residue and N,N'-dicyclohexylurea was filtered off; 4 ml water, 1 ml 25% NH_4OH was added to the filtrate, the mixture was heated at 60° for 1 hr and evaporated *in vacuo*. The oil residue was mixed with 2 ml EtOH, worked up with propane-1,3-diol and chromatographed in the same manner as before.

Triethylammonium salt of adenosine-5'-phosphomorpholide (XII)

To a soln of VII, prepared from 0·5 g adenosine 2',3'-O-phenylboronic ester, as described 40 ml 0·05 M triethylammonium bicarbonate and 0·70 ml tri-n-butyl amine and evaporated *in vacuo*. The solid was dissolved in dimethylformamide, treated with 0·5 ml propane-1,3-diol, evaporated *in vacuo* and washed thoroughly with ether.

The residue was dissolved in 500 ml water, mixed with 3% NH_4OH, to make the pH 8·0, after which it was passed through the column containing 40 cc of "Dowex 1 × 4", 200–400 mesh (HCO_3^-). Phosphomorpholide (XII) was eluted with 0·1 M soln of triethylammonium bicarbonate, and concentrated

in vacuo. The residue was dissolved in 10 ml MeOH and precipitated with ether; 0·3 g (41%) of XII was obtained, R_f 0·45 (system 1), 0·48 (system 3), 0·43 (system 5), E_{AMP} 0·6.

Uridine-5'-phosphate (X). To a soln of 0·04 g of morpholidophosphodichloride and 0·05 g diphenylphosphoric acid in 0·5 ml dioxan, 0·07 ml 2,6-lutidine was added and in 15 min 0·03 g phenylboronic ester of uridine (II). The mixture was left for 48 hr at 16–18° and the ppt of lutidine hydrochloride was filtered off. Water (100 ml) was added to the filtrate and the soln was acidified with 0·1N HCl to pH 2·0. After 2 days standing the mixture was worked up with chloroform, mixed with 5% NH_4OH to pH 8·5, extracted with ether (amines) and chromatographed over the "Dowex 1 × 4", 200–400 mesh ($HCOO^-$). The column was washed with water. The compounds were eluted, using the linear concentration gradient of ammonium formiate and formic acid. The concentrations were chosen from 0·1N ammonium formiate in water to 0·5N ammonium formate in 0·01N soln of formic acid.

The fraction volumes were 5 ml. Fractions 26–40 gave X; R_f values were 0·08 (system 1). 0·04 (system 2), 0·22 (system 3); E_{UMP} 1·0. Fractions 42–45 gave uridine-2'(3')-monophosphate, R_f were 0·23 (system 2), 0·22 (system 3); E_{UMP} 1·0.

Fractions 52–53 gave uridine-2'(3'),5'-diphosphate, R_f were 0·03 (system 1), 0·12 (system 3); E_{UMP} 1·35.

Fractions 20–22 and 47–50 were not identified.

Cytidine-5'-phosphate (XI) was prepared by phosporylation of IV using the procedure described. The hydrolysis was carried out for 4–5 days at pH 2·0. The mixture was treated as described above and chromatographed over the "Dowex-1" ($HCOO^-$). The nucleotides were eluted with formic acid. The eluting solutions were:

(1) 100 ml water–100 ml of 0·02N formic acid;
(2) 30 ml 0·02N formic acid–30 ml of 0·1N formic acid:
(3) 70 ml 0·1N ammonium formiate in 0·1N formic acid.

Fractions 29–38 gave 80% of XI; R_f values were 0·06 (system 1), 0·05 (system 2), 0·17 (system 3); E_{CMP} 1·0.

Fractions 53–60 and 64–74 gave—cytidine 2'(3'),5'-diphosphate; R_f values were 0·03 (system 1), 0·09 (system 3); E_{CMP} 1·34.

Fraction 62 was not identified.

Triethylammonium salt of uridine-5'-phosphomorpholide- (XIII)

To a soln of 0·280 g morpholidophosphodichloride and 0·72 g diphenylphosphoric acid in 4 ml dioxan. 0·49 ml 2,6-lutidine was added and in 15 min 0·23 g of II. The mixture was left for 48 hr. After treatment as above for adenosine-5'-phosphomorpholide, the residue was crystallized by addition of ether. The yield of XIII was 0·115 g (40%), R_f 0·32 (system 1), 0·58 (system 3), E_{UMP} 0·6.

Dephosphorylation of adenosine-2'(3')-monophosphate, cytidine-2'(3')-monophosphate and adenosine-2',3'-cyclophosphate

To a stirred soln of 0·02 g phosphate (2'[3']-AMP, 2'[3']-CMP or 2':3'-AMP) in 4 ml dimethylformamide, 0·01 g PBA was added at 100, 120 and 140°. The periodic tests were withdrawn and analysed by chromatography in systems 1, 6, 7 and by electrophoresis. The quantitative composition of the mixture was detected by spectrophotometry. The analogous control runs were performed involving no PBA. The results are shown in Table 3.

REFERENCES

[1] A. M. Yurkevich, I. I. Kolodkina, and N. A. Preobrazhenski, *Dokl. Akad. Nauk SSSR* **164**, 828 (1965).
[2] R. J. Ferrier, *J. Chem. Soc.* 2325 (1961).
[3] C. Angell, *Ibid.* 504 (1961).
[4] J. D. Morrison and R. L. Letsinger, *J. Org. Chem.* **29**, 11, 3405 (1964).
[5] M. Ikehara and E. Ohtsuka, *Chem. and Pharm. Bull.* **11**, 435, 961 (1963).
[6] J. M. Tener, *J. Am. Chem. Soc.* **83**, 159 (1961).
[7] I. I. Kolodkina, L. S. Varshavskaya, A. M. Yurkevich, N. A. Preobrazhenski, *Zh. Obshch. Khim* **37**, 1996 (1967).
[8] C. S. Hanes and F. A. Isherwood, *Nature, Lond.* **164**, 1107 (1949).
[9] F. J. Welcher, *Org. Anal. Reagents* **3**, 456 (1947).
[10] G. Buchanan, C. Dekker and A. Long, *J. Chem. Soc.* 3162 (1950).
[11] P. H. Levene and R. S. Tipson, *J. Biol. Chem.* **121**, 131 (1937).