NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CXXXVII.*

PREPARATION AND PROPERTIES OF SOME N-(2-HYDROXYETHYL) DERIVATIVES OF RIBONUCLEOSIDES AND NUCLEOTIDES

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Heating of the corresponding nucleoside with 2-dimethylamino-1,3-dioxolane (*I*) affords N^3-(2-hydroxyethyl)uridine (*IIIa*), N^3-(2-hydroxyethyl)-6-azauridine (*Va*), and N^1-(2-hydroxyethyl)-inosine (*VIIa*). From 2′,3′-isopropylidene derivatives of uridine (*IIb*) and inosine (*VIb*), the N-(2-hydroxyethyl) derivatives *IIIb* and *VIIb* are obtained. The analogous reaction of 5′-nucleotides *XIII* and *XVII* with compound *I* affords as the principal product the 2-hydroxyethyl esters *XV* and *XVIII*, resp. 2-Hydroxyethyl esters of uridine 5′-phosphate (*XV*), adenosine 5′-phosphate (*XVIII*), and guanosine 5′-phosphate (*XX*) are prepared by condensation of the pyridinium salt of the corresponding 5′-nucleotide with ethylene glycol in the presence of N,N′-dicyclohexylcarbodiimide. Treatment of compound *IIIa* with triethyl phosphite affords the 2′(3′)-phosphite *XXII* the reaction of which with hexachloroacetone leads to N^3-(2-hydroxyethyl)uridine 2′,3′-cyclic phosphate (*XXIII*). The latter compound is resistant towards pancreatic ribonuclease but is cleaved by ribonuclease T2. The reaction of compound *IIIa* with guanosine-2′,3′-cyclic phosphate in the presence of ribonuclease T1 occurs exclusively on the hydroxylic function at position 5′ under the formation of the derivative *XXVII*. The latter compound was also prepared by reaction of the protected GpU derivative *XXV* with compound *I* and subsequent removal of protecting groups in acidic and alkaline media. N^3-(2-Hydroxyethyl)uridine 5′- phosphate *XIV* is formed from the compound *XXVII* by cleavage with the snake venom phosphodiesterase.

In connection with investigations on modified nucleosides and nucleotides, we have been engaged in the preparation of compounds substituted on the heterocyclic moiety by hydrophilic substituents. In view of a study on the specificity of some nucleolytic enzymes, we have focussed our attention mainly on the preparation and properties of such hydroxyalkyl derivatives, the hydrophilic substituent of which replaces the N–H function of the heterocyclic moiety. Because of the instability of

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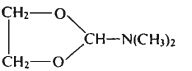
nucleoside N-hydroxymethyl derivatives, the nucleoside and nucleotide N-(2-hydroxyethyl) derivatives have been elected as model representatives of the above mentioned group of compounds.

2-Hydroxyethylation of adenosine and its nucleotides into the position N^1 of the adenine moiety by reaction with ethylene oxide has been reported some time ago[1]. As shown in some earlier papers of this Laboratory, several dimethylaminodialkoxymethanes – dimethylformamide acetals – represent suitable reagent for alkylation of some nucleosides[2–4], nucleotides[5], and dinucleoside phosphates[6], the heterocyclic moiety of which is formed by uracil, thymine, 6-azauracil, hypoxanthine, and xanthine. In this connection, we have been interested in the use of an analogous alkylating agent, namely, 2-dimethylamino-1,3-dioxolane (dimethylformamide ethyleneacetal, *I*) in the preparation of N-(2-hydroxyethyl) derivatives of the nucleosides and nucleotides mentioned.



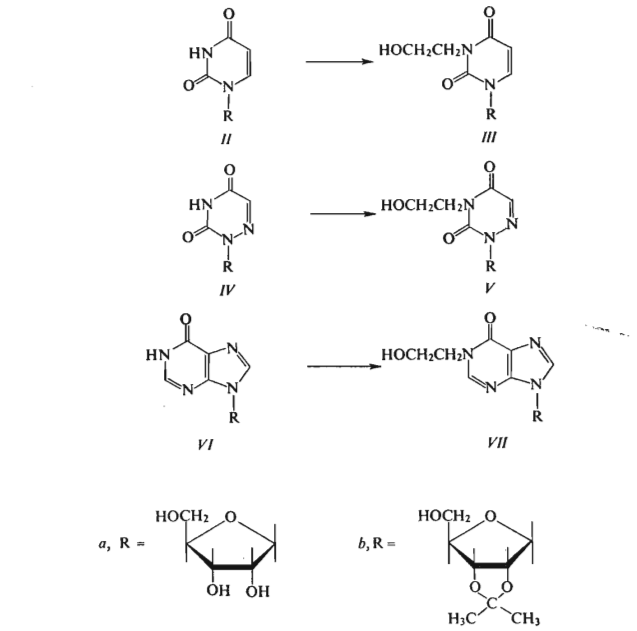
I

On heating a nucleoside with the agent *I* in dimethylformamide, *i.e.* under conditions usual with the dimethylformamide acetal alkylations, there are readily obtained the 2-hydroxyethyl derivatives of uridine (*IIIa*), 6-azauridine (*Va*), and inosine (*VIIa*). The 2′,3′-isopropylidene derivatives of N^3-(2-hydroxyethyl)uridine (*IIIb*) and N^1-(2-hydroxyethyl)inosine (*VIIb*) were prepared analogously, see Scheme 1. Reaction products were isolated by preparative thin-layer chromatography on silica gel or paper chromatography, and their homogeneity was confirmed by elemental analysis, ultraviolet spectra, paper chromatography and electrophoresis.

Absence of the bathochromic shift and hyperchromicity in ultraviolet spectra of N-(2-hydroxyethyl) derivatives *III*, *V*, and *VII* during the transition from an acidic into an alkaline medium are in accordance with properties of N-substituted derivatives. In addition to signals corresponding to the fundamental framework of the nucleoside, the NMR spectra of compounds examined exhibit multiplets (2-protons) in the 3·30–3·70 p.p.m. region attributable to —CH_2N— groups and in the 3·70 to 4·10 p.p.m. region (2 protons) indicative of the —CH_2O— groups. Signals corresponding to the —NH— group disappeared in accordance with the structure proposed.

Infrared spectrum of the 2′,3′-O-isopropylidene derivative *IIIb* (measured in chloroform) is also in accordance with the structure of a N^3-(2-hydroxyethyl) derivative. Thus, band of the NH group is absent, wavenumbers of carbonyl bands ($\nu_{(CO)}$ 1709, 1666 cm^{-1}) correspond to those of 1,3-dimethyluracil ($\nu_{(CO)}$ 1710, 1664 cm^{-1}), *cf.* ref.[7], but differ from those of 2′,3′-O-isopropylideneuridine (*IIa*, $\nu_{(CO)}$ 1709 cm^{-1} inflex, 1693 cm^{-1}). Comparison of the $\nu_{(OH\ bonded)}$ and $\nu_{(CO)}$ values of compound

IIIb and those of N^3-methyl-2′,3′-O-isopropylideneuridine[3] indicates that probably in both compounds an identical type of a hydrogen bond is involved between the hydroxylic function at position 5′ and the carbonyl group at position 2 of the heterocyclic moiety. The presence of a hydrogen bond between the carbonyl group at position 4 of uracil and hydroxylic function of the N^3-(2-hydroxyethyl) group in compound *IIIb* would result in a decreased wavenumber value of the carbonyl group. Notwithstanding, the formation of an analogous hydrogen bond between the N^3-substituent and the carbonyl group at position 2 cannot be excluded; its effect might be analogous to that of the intramolecular hydrogen bond 5′-2, known in the case of the N^3-methyl derivative mentioned above.

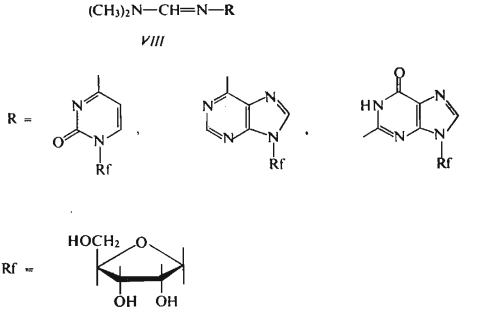


SCHEME 1

In all alkylations with the agent *I*, the resulting N-(2-hydroxyethyl) derivatives were accompanied by a lesser amount (even 20% in some cases) of a by-product which was separated by paper chromatography or chromatography on Dowex 50 (NH_4^+) ion exchange resin. Investigations

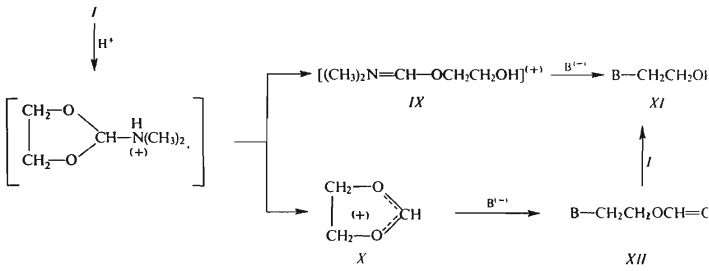
on properties of the by-product accompanying the reaction of the uridine derivative *IIa* and the agent *I* indicate that a compound of a basic character (pK_a 8·2, titrimetrically) is at stake, the ultraviolet characteristics of which correspond to those of a N^3-substituted uridine derivative. The possibility that the by-product might be formed by reaction at the *cis*-diol system of the nucleoside can be excluded, since similar derivatives have been observed in alkylations of compounds *IIb* and *VIb* (the *cis*-diol system of which is protected by the isopropylidene group) and of N^3-methyluracil with the agent *I*. The by-product is unstable and is partly converted to the derivative *IIIa* in the course of chromatography on silica gel. Acetylation of the by-product with acetic anhydride in pyridine affords the tetraacetate of compound *IIIa*, identical with an authentic specimen, as shown by thin-layer chromatography and NMR spectra. The NMR spectrum of the by-product does not exhibit any additional signals when compared with that of compound *IIIa*, characteristic signals of the sugar and heterocyclic moiety remaining unchanged. On the basis of a decreased hydrophilic character (in comparison with compound *IIIa*) it may be concluded that the by-product represents a derivative of compound *IIIa* substituted at position 5′ of the sugar moiety or, more probably, at the hydroxylic function of the N^3-substituent.

Analogously to other dimethylformamide acetals, the agent *I* does not alkylate the heterocyclic moiety of adenosine, guanosine, and cytidine, the N-dimethylaminomethylene derivatives *VIII* being exclusively formed (*cf.* ref.[2,8,9]). Compounds *VIII* (characterised by absorption spectra) are hydrolysed in alkaline media to the starting nucleoside as the sole reaction product.



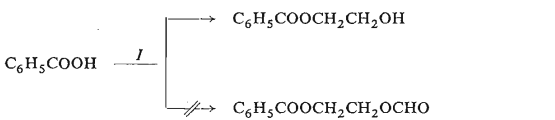
Our findings are in accordance with the mechanism proposed by Žemlička[3] for alkylations with dimethylformamide acetals. According to this proposal, the agent *I* is protonated with the proton of the —NHCO-grouping and this protonated form is cleaved under the formation of the active intermediate *IX* or *X*. In the next stage, an attack by the base anion occurs under the formation of the N-alkylated product. In contrast to alkylations with non-cyclic dimethylformamide dialkylacetals, the alkylations with the agent *I* may lead to different products depending on the nature

of the active particle. Thus, the dimethylalkoxymethyleneammonium cation *IX* affords directly the 2-hydroxyethyl derivative *XI*. On the other hand, the dialkoxycarbonium cation *X* should afford primarily the 2-formyloxyethyl derivative *XII* which could be also converted to the final 2-hydroxyethyl derivative *XI* owing to the reaction conditions, namely, to the presence of a strongly basic agent *I* (see Scheme 2).*



SCHEME 2

In connection with this hypothesis, we have studied the esterification of benzoic acid with the agent *I* in refluxing benzene under conditions similar to those of Eschenmoser[11] in esterifications with dimethylformamide dialkylacetals. We have isolated as the main product benzoic acid 2-hydroxyethyl ester, identical with an authentic specimen[12], as shown by thin-layer chromatography and infrared spectra. Benzoic acid 2-formyloxyethyl ester has not been found. An authentic specimen of this formyl derivative has been prepared by reaction of benzoic acid 2-hydroxyethyl ester with the mixed formic acetic anhydride. This specimen was considerably stable under conditions similar to those of alkylation with the agent *I*; only a negligible degradation to the starting 2-hydroxyethyl ester has been observed. Consequently, the route *via* the intermediate *X* appears improbable, at least under conditions similar to those of alkylations of a carboxylic acid by the agent *I*.



* In experiments on the preparation of the corresponding N^6-dimethylaminomethylene derivative by reaction of 2',3'-di-O-acetyladenosine with dimethylformamide dimethylacetal, a partial deacetylation has been observed even at room temperature[10].

The use of the agent *I* in N-alkylations of uridine nucleotides did not meet with success. Thus, reaction (under the above mentioned conditions, *cf.* ref.[5]) of uridine 5'-phosphate (*XIII*) and the agent *I* afforded a complex mixture containing only a small amount of the desired compound *XIV* (see Scheme 3). The main components of the mixture are formed by the 2-hydroxyethyl esters *XV* and *XVI*. Furthermore, a lesser amount of nucleosides *IIa* and *IIIa* is present, arisen on dephosphorylation of compounds *XIII* and *XIV* (*cf.* ref.[13]).

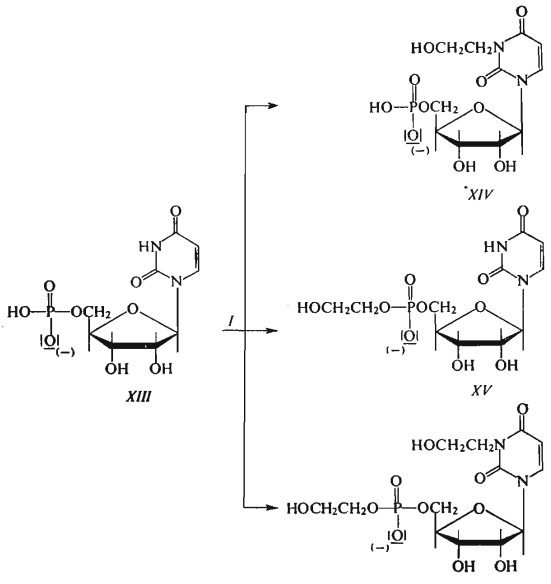
Compound *XV* was identified on comparison with an authentic sample obtained by reaction of compound *XIII* and ethylene glycol in the presence of N,N'-dicyclohexylcarbodiimide (see Scheme 4). This reaction was also used in the preparation of the 2-hydroxyethyl esters of adenosine 5'-phosphate (*XVIII*) and guanosine 5'-phosphate (*XX*) from the corresponding nucleotides *XVII* and *XIX*.*

Another proof of the constitution of compound *XV* consists in the snake venom phosphodiesterase degradation leading to uridine 5'-phosphate (*XIII*) as the sole ultraviolet-absorbing product (criterion for an ester of 5'-nucleotide). An analogous degradation of compound *XVI* afforded N^3-(2-hydroxyethyl)uridine 5'-phosphate (*XIV*), identified on comparison with an authentic specimen (*vide infra*). In a later stage of our investigations on esterifications of 5'-nucleotides with the agent *I*, we have replaced uridine 5'-phosphate (*XIII*) by adenosine 5'-phosphate (*XVII*), the heterocyclic moiety of which cannot undergo the alkylation. After an alkaline work-up (in order to hydrolyse the N^6-dimethylaminomethylene derivative), the 2-hydroxyethyl ester of adenosine 5'-phosphate (*XVIII*) was obtained almost exclusively. Compound *XVIII* was identified similarly to compound *XV*, namely, on comparison with an authentic specimen prepared according to Scheme 4 and by the snake venom phosphodiesterase degradation (formation of compound *XVII* as the sole ultraviolet-absorbing product).

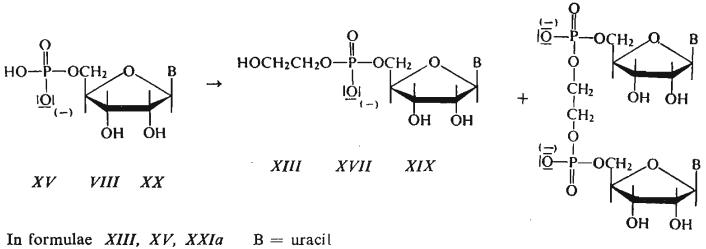
As shown by the above mentioned results, the esterification ability of the agent *I* in respect to nucleotides considerably exceeds the activity of dimethylformamide dimethyl acetal (*cf.* ref.[5]). The resulting 5'-nucleotide 2-hydroxyethyl esters are of some interest in connection with the template activity of oligonucleotides[14]. For two independent methods of preparation of these compounds see Scheme 3 and 4.

It has been found in the course of investigations on the specificity of pancreatic ribonuclease that N^3-methyl uridine 2',3'-cyclic phosphate does not represent suitable substrate for this enzyme.[15] This failure is ascribed to steric hindrance of an interaction of the heterocyclic moiety with the appropriate enzyme region due to the bulky methyl

* Some experiments were performed with the use of ethylene glycol monobenzyl ether to exclude the formation of products *XXI* arisen by a double phosphorylation of ethylene glycol. Under the conditions of a carbodiimide condensation, however, a quantitative cleavage of the benzyl ether bond occurred. For this reason, we have discontinued investigations on the preparation of nucleotide 2-hydroxyethyl esters by this route.



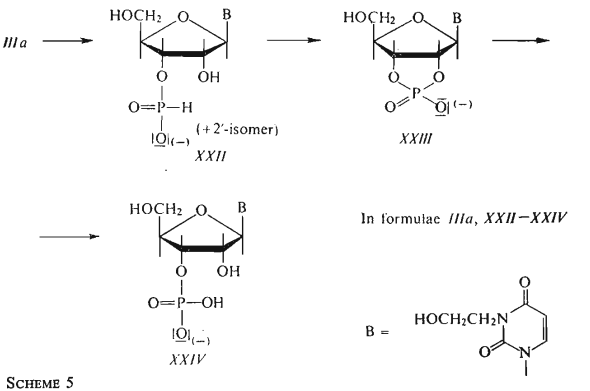
SCHEME 3



In formulae *XIII, XV, XXIa* B = uracil
XVII, XVIII, XXIb B = adenine
XIX, XX, XXIc B = guanine

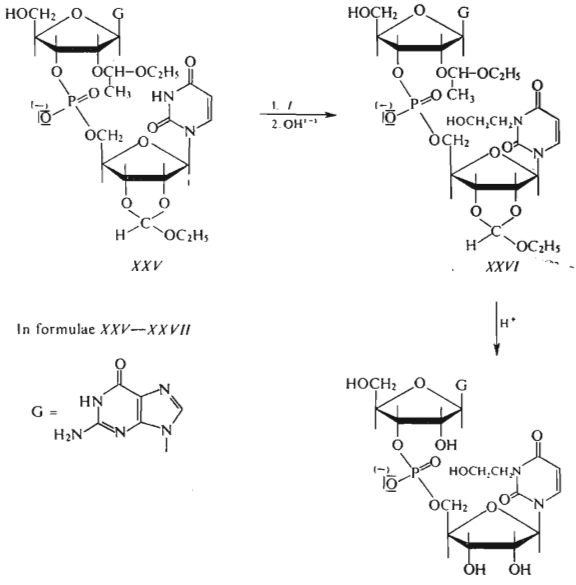
SCHEME 4

group at position N^3 of the heterocyclic ring. In addition to the steric effect of the methyl group, a considerable hydrophobic effect has to be taken into account. In this connection, N^3-(2-hydroxyethyl)uridine 2',3'-cyclic phosphate (*XXIII*) has been prepared and its behaviour towards pancreatic ribonuclease investigated. Alkylation of uridine 2',3'-cyclic phosphate with the agent *I* led, however, to a complex reaction mixture similar to alkylations of 5'-nucleotides (*vide supra*). For this reason, compound *XXIII* was prepared by the usual phosphorylation of the nucleoside *IIIa via* the phosphite intermediate *XXII* (*cf.* ref.[16]). Selectivity of the first reaction step (reesterification with triethyl phosphite occurring with participation of the *cis*-diol system[17]) makes possible to use the unprotected nucleoside *IIIa* as the starting material. Compound *XXIII* obtained by this method without any difficulty, does not undergo the pancreatic ribonuclease degradation even with the use of a greater amount of the enzyme and prolonged incubation periods of time. On the other hand, compound *XXIII* is cleaved by ribonuclease T 2 quantitatively to the 3'-phosphate *XXIV* identical according to paper chromatography and electrophoresis with a mixture of the 2'- and 3'-isomer, obtained by acidic hydrolysis of the phosphodiester *XXIII* and affording by the alkaline phosphatase *E.coli* degradation compound *IIIa* as the sole product (see Scheme 5). The present findings are not at variance with the original idea on the steric influence of the N^3-methyl group on the substrate activity towards pancreatic ribonuclease[15]. Also N^3-methyluridine 2',3'-cyclic phosphate[18] is cleaved with ribonuclease T 2. Consequently, the steric requirements of ribonuclease T 2 towards the substrate are different from those of pancreatic ribonuclease.



SCHEME 5

Another interesting problem was stimulated by investigations on the biological activity of N^3-(2-hydroxyalkyl) derivatives of nucleosides (*IIIa* and *Va*). Thus, compound *IIIa* exbits a significant bacteriostatic activity on the growth of *Escherichia coli* strain B (synthetic medium): no inhibition at 1γ/ml, 14% at 10γ/ml, 29% at 100γ/ml, and 100% at 1000γ/ml*. On the other hand, the derivative *Va* did not show any effect on mouse leukaemia type AK (*cf.* ref.[19]). One of the explanations of the growth-inhibiting effects of compound *III* may be based on a competitive inhibition in those metabolic processes comprising enzymatic phosphorylation reactions caused by the presence of a further primary alcoholic function in compound *IIIa* in addition to the 5'-hydroxylic group of the ribofuranose moiety. Evaluation of this possibility was performed with the use of a model reaction consisting in an enzymatic transfer of the 3'-guanylic acid residue to the primary hydroxylic function of the nucleoside



XXV *XXVI* *XXVII*

SCHEME 6

* These assays were performed by Dr I. Votruba of this Institute.

IIIa under catalysis of ribonuclease T 1. This reaction is known[20] to occur at any primary alcoholic function. The ratio of reaction products at the 5'- and 2-hydroxyethyl group would indicate competition of both groups in respect to the enzymatically catalysed transfer reaction. The snake venom phosphodiesterase degradation should take place exclusively in the case of compound *XXVII*. In this connection, we have prepared the authentic specimen of compound *XXVII* by reaction of the protected derivative *XXV* (*cf.* ref.[21]) with the agent *I* followed by removal of protecting groups from the resulting compound *XXVI* by acidic hydrolysis. The structure of compound *XXVII* was confirmed by ribonuclease T 2 degradation affording quantitatively guanosine 3'-phosphate and compound *IIIa* in the ratio close to one. On the other hand, the snake venom phosphodiesterase degradation affords guanosine and N^3-(2-hydroxyethyl)uridine 5'-phosphate (*XIV*); this procedure was used in the preparation of an authentic specimen of compound *XIV* (*vide supra*). The alkaline phosphatase *E. coli* degradation transforms compound *XIV* to the nucleoside *IIIa*.

The enzymatically catalysed reaction of guanosine 2',3'-cyclic phosphate and compound *IIIa* was performed under standard conditions (*cf.* ref.[22]). The dinucleoside phosphate isolated from this reaction was completely homogeneous and identical with compound *XXVII*, as shown by all analytical criteria used. The quantitative course of the snake venom phosphodiesterase degradation of compound *XXVII* (to guanosine and compound *XIV*) indicates that the transfer of the 3'-guanylyl residue to compound *IIIa* catalysed by ribonuclease T 1 occurs under the conditions stated specifically at the 5'-hydroxylic function of the sugar moiety of the reaction component *IIIa* while the presence of another primary hydroxylic function as the substituent of the heterocyclic moiety does not interfere in this reaction. Such an interfering effect cannot be, however, excluded in the case of other phosphorylating enzymes; experiments in this direction are in progress.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected.

Methods

Infrared spectra were taken on a Zeiss UR-10 apparatus in chloroform. NMR spectra were measured in hexadeuteriodimethyl sulfoxide on a Varian 100 apparatus with the addition od deuteriochloroform (hexamethyldisiloxane as internal standard). Ultraviolet absorption spectra were recorded in 0·01M-HCl on a Beckman DU apparatus. Quantitative determinations were performed with the use of the following extinction coefficients[23]: uridine, ε_{260} 10000; adenosine, ε_{260} 14200; guanosine, ε_{260} 11800; inosine, ε_{260} 7500; 6-azauridine, ε_{260} 6000; GpU, ε_{260} 21800. One optical density unit (A_{260}) is defined as that amount of the substance in 1 ml of the solution which shows in 1 cm cell at 260 nm the absorbancy equal to one.

Paper chromatography was performed on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM) by the descending technique in the solvent systems S_1, 2-propanol–concen-

TABLE I

Chemical Shifts δ (p.p.m.) and Coupling Constants $J_{1'2'}$ (c.p.s.) (Hexadeuteriodimethyl sulfoxide — deuteriochloroform as solvent, hexamethyldisiloxane as internal reference)

Formula	H_5	H_6	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$-CH_2O$
IIIa	5·68 d	7·91 d	5·78 $J_{1'2'} = 4{\cdot}0$ d		3·75—4·10 m		
IIIb	5·65 d	7·76 d	5·81 $J_{1'2'} = 2{\cdot}0$ d	4·65—4·90 m		4·09 q	3·86 t
VIIa[a]	—	—	5·85 $J_{1'2'} = 5{\cdot}0$ d	4·47 $J_{2'3'} = 5{\cdot}0$ t	3·89—4·23 m		

[a] H_2 8·25 s, H_8 8·20 s.

trated aqueous ammonia–water (7 : 1 : 2), and S_2, ethanol–1M ammonium acetate (5 : 2). Paper electrophoresis was performed by the technique of Markham and Smith[24] on paper Whatman No 1 (preparative runs on 16 cm wide strips of Whatman paper No 3 MM) in the following buffer solutions (25 volt/cm, 1 hour): E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5); 0·05M sodium hydrogen citrate (pH 3·5); E_3, 0·2M triethylammonium borate (pH 7·5). For R_F values and electrophoretical mobilities see Table II. Thin-layer chromatography was performed on ready-to-use Silufol U_{254} plates precoated with silica gel (produced by Glasswork Kavalier, Votice, Czechoslovakia) in the solvent system S_3, methanol–chloroform (1 : 9). Preparative runs were performed on a loose layer of silica gel with incorporated luminiscent indicator (particle size, 30—50 microns; produced by Service Laboratories of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague - Suchdol).

Enzymatic degradations were performed in solutions containing 2—3 μmol of the substance in 50 μl of 0·05M-Tris-HCl buffer (incubated for 4 hours at 37°C) with the use of *a*) pancreatic ribonuclease, pH 8·0, 50 γ of the enzyme (Calbiochem, A grade, fivefold recrystd.), *b*) ribonuclease T1, pH 7·00, 25 e.u. of the enzyme (Sankyo Co., Ltd.), *c*) ribonuclease T2, 0·2M sodium γ,γ-dimethylglutarate, 0·1M-NaCl, pH 6·5, 20 γ of the enzyme (a gift of Professor Dr H. Witzel, Marburg, Germany), *d*) snake venom phosphodiesterase, pH 9·0, 20 γ of the enzyme (*Crotalus terr. terr.* 0·1% in 50% aqueous glycerine, purchased from Boehringer, Mannheim, Germany), and *e*) alkaline phosphatase *E. coli*, pH 9·0, 10 γ of the enzyme, purchased from Worthington, U.S.A., 0·1% solution in 30% aqueous ammonium sulfate.

Materials and Reagents

Uridine, inosine, and 5′-nucleotides were purchased from Calbiochem, Los Angeles, California, U.S.A. 6-Azauridine was the product of Spofa, United Pharmaceutical Works, Prague, Czechoslovakia. Isopropylideneuridine and isopropylideneinosine were prepared by known procedures[25] 2-Dimethylamino-1,3-dioxolane was the product of Fluka, Buchs, Switzerland; another specimen

TABLE I
(*continued*)

—CH_2N, 2 × H_5·	CH_3	OH	Formula
3·30—3·65 m	—	4·50—5·50 m	*IIIa*
3·35—3·70 m	1·23 s 1·43 s	4·98 t 4·64 m	*IIIb*
3·35—3·73 m	—	4·50—5·50 m	*VIIa*[a]

was prepared by transacetalisation of dimethylformamide dimethylacetal with ethylene glycol (Reanal, Budapest, Hungary) according to a general method[26]; both specimens were identical and homogeneous, as shown by gas–liquid chromatography; b.p. 78°C/80 Torr. Dimethylformamide was dried by distillation from phosphorus pentoxide and stored over molecular sieves (Potassit 3, in rotulis, diameter 2—3 mm).

N^3-(2-Hydroxyethyl)uridine (*IIIa*)

A mixture of uridine (0·98 g; 4 mmol), dimethylformamide (4 ml), and the agent *I* (2 ml) was heated at 100°C for 20 hours and then chromatographed on 6 sheets of paper Whatman No 3 MM in the solvent system S_1. Ultraviolet-absorbing bands of compound *IIIa* (R_F = 0·70) and the by-product (R_F = 0·83) were eluted with water (100 ml each), the eluates evaporated at 35°C/15 Torr, and the yield determined spectrophotometrically at pH 2 and 260 nm. The yield of compound *IIIa* was 72% (2·9 mmol). The yield of the by-product (R_F = 0·83) was 11% (0·45 mmol, 4 500 A_{260}). The fractions containing compound *IIIa* contaminated with a small amount of ethylene glycol, were rechromatographed on a loose layer of silica gel (40 × 18 × 3 mm) in the solvent system S_3. The ultraviolet absorbing band was eluted with methanol (300 ml), the eluate evaporated, and the residue crystallised from a 2 : 1 mixture of acetonitrile and ethanol under the addition of ether till turbid. The crystals were collected with suction, washed with ether, and dried over phosphorus pentoxide at 20°C/0·1 Torr. Yield, 634 mg (55%) of compound *IIIa*. Optical rotation: $[\alpha]_D^{25}$ +20·7° (*c* 0·42, water). Mass spectrum: *m/e* 288 (mol peak), *m/e* 157 (BH_2), *m/e* 139 (BH_2-18), *m/e* 133 (S), *m/e* 113 (BH_2-44*, intens.). Ultraviolet spectrum (pH 2): λ_{max} 261 nm (ε_{260} 10100), λ_{min} 235 nm; $A_{250/260}$ 0·72, $A_{280/260}$ 0·41, $A_{290/260}$ 0·08; pH 12: λ_{max} 262 nm (ε_{260} 10000). For $C_{11}H_{16}N_2O_7$ (288·2) calculated: 45·80% C, 5·59% H, 9·72% N; found: 46·02% C, 5·70% H, 10·05% N.

* *m/e* = 44, ethylene oxide.

N^3-(2-Hydroxyethyl)-6-azauridine (*V*)

A mixture of 6-azauridine (*IV*, 2·45 g; 10 mmol), dimethylformamide (15 ml), and the agent *I* (5 ml) was heated at 100°C for 12 hours. After this period of time, the composition of reaction mixture did not change, as shown by chromatography in the solvent system S_1. The mixture was evaporated at 40°C/15 Torr, the residue coevaporated with two 20 ml portions of pyridine and with toluene (20 ml), the final residue dissolved in 50% aqueous ethanol (50 ml), the solution treated with about 1 g of Dry Ice to keep the reaction neutral, and allowed to stand at room temperature for 1 hour. The solution was concentrated at 35°C/15 Torr and the concentrate applied to a 80 × 4 cm column of DEAE-cellulose Cellex D, standard capacity, HCO_3^-. The column was eluted with water with the use of the Uvicord apparatus at the rate of 3 ml per min, the fractions being taken in 10 min intervals. The neutral ultraviolet-absorbing fraction was evaporated at 35°C/15 Torr, the residue coevaporated with ethanol (twice), and reprecipitated from ethanol (20 ml) with ether (300 ml). The precipitate was collected with suction, washed with ether, and dried at 0·1 Torr over phosphorus pentoxide. Yield, 1·80 g (62%) of light-yellow hygroscopic compound *V*, weakly contaminated with a by-product possessing the R_F value 0·82 (S_1), Ultraviolet spectrum (pH 2): λ_{max} 260 nm; at pH 12: λ_{max} 260 nm, no hyperchromicity. For $C_{10}H_{15}N_3O_7$ (289·3) calculated: 41·51% C, 5·22% H, 14·52% N; found: 41·32% C, 5·08% H, 14·80% N.

N^1-(2-Hydroxyethyl)inosine (*VIIa*)

A mixture of inosine (1·08 g; 4 mmol), dimethylformamide (4 ml), and the agent *I* (2 ml) was heated at 110°C for 50 hours, evaporated to dryness at 40°C/0·1 Torr, the residue coevaporated with dimethylformamide (20 ml), dissolved in 50% aqueous dioxane (25 ml) under the addition of Dry Ice, the whole kept at room remperature for 30 min, and evaporated to dryness under diminished pressure. The residue was chromatographed on 15 sheets of paper Whatman No 3 MM in the solvent system S_1. Bands of the product *VIIa* were eluted with water (50 ml), the eluates evaporated at 35°C/15 Torr, the residue coevaporated with three 20 ml portions of ethanol, and reprecipitated from ethanol (5 ml) with ether (200 ml). The precipitate was collected with suction and dried at 20°C/0,1 Torr over phosphorus pentoxide. Yield, 520 mg (42%) of compound *VIIa*, chromatographically homogeneous (S_1, S_2). Ultraviolet spectrum at pH 2, 7, and 11: λ_{max} 250 nm, λ_{min} 226 nm, without any change. For $C_{12}H_{16}N_4O_6$ (312·3) calculated: 46·14% C, 5·16% H, 17·94% N; found: 46·45% C, 5·20% H, 18·15% N.

Bands possessing the R_F value 0·62 (S_1) were processed similarly to afford 180 mg of a by-product, the ultraviolet and mass spectrum of which is almost identical with those of the main product *VIIa*.

N^3-(2-Hydroxyethyl)-2′,3′-O-isopropylideneuridine (*IIIb*)

A mixture of compound *IIb* (200 mg; 0·7 mmol), dimethylformamide (2·5 ml), and the acetal *I* (1 ml) was heated at 110°C for 6 hours, evaporated at 40°C/0·1 Torr, the residue coevaporated with dimethylformamide (20 ml), dissolved in 50% aqueous dioxane (25 ml) under the addition of Dry Ice, the whole kept at room temperature for 30 min, and evaporated at 35°C/15 Torr. The residue was coevaporated with three 20 ml portions of ethanol and purified by reprecipitation from chloroform (5 ml) with light petroleum (200 ml). The precipitate was collected with suction and dried at 20°C/0·1 Torr over phosphorus pentoxide. Yield, 166 mg (72%) of compound *IIIb*, chromatographically homogeneous (thin-layer chromatography in 5 : 95 methanol–ethyl acetate:

TABLE II

Paper Chromatography and Electrophoresis

Compound	R_F S_1	R_F S_2	E_1[a]
Uridine (*IIa*)	0·45	0·70	- -
IIb	0·75	—	- -
IIIa	0·68	—	- -
IIIb	0·85	—	—
IVa	0·45	—	0·46
Va	0·65	—	—
VIa	0·45	0·62	—
VIb	0·70	—	- -
VIIa	0·56	0·74	—
VIIb	0·80	—	—
Uridine 2′(3′)-phosphate	0·12	0·25	1·00
XIII	0·12	0·25	1·00
XIV	0·13	0·37	1·00
XV	0·39	0·54	0·58
XVI	0·28	0·62	0·55
XVII	0·06	0·21	0·85
XVIII	0·33	0·52	0·30
XIX	0·03	0·21	0·80
XX	0·23	0·48	0·45
XXIc	0·02	0·02	0·52
XXII	0·51	—	0·61
XXIII	0·51	—	0·61
XXIV	0·21	—	0·95
Uridine 2′,3′-cyclic phosphate	0·36	0·60	0·67
XXV	0·41	—	0·42
XXVI	0·61	—	0·40
XXVII	0·19	0·43	0·40
Guanylyl-(3′→5′)-uridine	0·10	0·29	0·42
Guanosine 2′,3′-cyclic phosphate	0·29	0·48	0·48

[a] Electrophoretical mobility referred to uridine 2′(3′)-phosphate.

compound *IIb*, R_F 0·57; compound *IIIb*, R_F 0·41). Ultraviolet spectrum at pH 2, 7, and 12: λ_{max} 262 nm, unchanged. Infrared spectrum (in chloroform): compound *IIa*, ν(CO) 1 709 (inflex), 1 693 cm^{-1}, $\nu(NH)_{free}$ 3 388 cm^{-1}, $\nu(NH)_{bonded}$ 3 195 cm^{-1}, $\nu(OH)_{free}$ 3 626 cm^{-1}, $\nu(OH)_{bonded}$ 3 469 cm^{-1}; compound *IIIa*, ν(CO) 1 709, 1 666 cm^{-1}, $\nu(OH)_{free}$ 3 620 cm^{-1}, $\nu(OH)_{bonded}$ 3 472 cm^{-1}; N^3-methyl-2′,3′-O-isopropylideneuridine, ν(CO) 1 712, 1 670 cm^{-1}, $\nu(OH)_{free}$

3625 cm^{-1}, $\nu(OH)_{bonded}$ 3462 cm^{-1}. For $C_{14}H_{20}N_2O_7$ (328·3) calculated: 51·22% C, 6·10% H, 8·53% N; found: 51·21% C, 6·34% H, 8·21% N.

N^1-(2-Hydroxyethyl)-2',3'-O-isopropylideneinosine (*VIIb*)

A mixture of compound *VIb* (0·50 g; 1·6 mmol), dimethylformamide (3 ml) and the agent *I* (1 ml) was heated at 100°C for 20 hours, evaporated at 40°C/0·1 Torr, the residue coevaporated with dimethylformamide (20 ml), and dissolved in 50% aqueous ethanol. Dry Ice (about 1 g) was added, the solution allowed to stand at room temperature for one hour, evaporated to dryness at 35°C/15 Torr, and the residue chromatographed on a loose layer (400 × 180 × 3 mm) of silica gel in the solvent mixture S_3. The band of product *VIIb* (R_F 0·40) was eluted with methanol (200 ml), the eluate evaporated to dryness at 35°C/15 Torr, the residue dissolved in ethanol (5 ml), and the solution precipitated with ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and air-dried. As shown by chromatography in the solvent system S_1, product *VIIb* (R_F 0·83) is contaminated by a small amount of a by-product (R_F 0·92). The crude product was chromatographed on 6 sheets of paper Whatman No 3 MM in the solvent system S_1. The corresponding bands of compound *VIIb* were eluted with water (100 ml), the eluates evaporated to dryness, the residue coevaporated with two 50 ml portions of ethanol, and reprecipitated as above. The precipitate was washed with ether and dried at 20°C/0·1 Torr over phosphorus pentoxide. Yield, 320 mg (55%) of compound *VIIb*, chromatographically homogeneous (S_1, S_3). Mass spectrum: *m/e* 352 (mol. peak), *m/e* 209 (B + 30), *m/e* 180 (BH), *m/e* 181 (BH_2), *m/e* 173 (S-low peak), *m/e* 163 (BH_2-18), *m/e* 137 (BH_2-44). For $C_{15}H_{20}N_4O_6$ (352·3) calculated: 51·13%C, 5·72% H, 15·90% N; found: 49·61% C, 5·83% H, 14·76% N.

N^3-(2-Hydroethyl)uridine 2',3'-Cyclic Phosphate (*XXIII*)

A mixture of compound *IIIa* (1 mmol; dried at 20°C/0·1 Torr over phosphorus pentoxide), dimethylformamide (5 ml), and triethyl phosphite (2·5 ml) was treated dropwise with 6M-HCl in dimethylformamide until the reaction was acid to moistened pH paper (0·2 ml), and the whole allowed to stand overnight at room temperature. There was added 50 ml of 0·4M triethylammonium hydrogen carbonate (pH 7·5), the mixture evaporated at 40°C/15 Torr, the residue coevaporated under the same conditions with water, and applied (in 20 ml of water) to a column (20 × 4 cm) of DEAE-cellulose (HCO_3^-). With the use of an Uvicord apparatus, the column was washed first with water and then (after drop of the ultraviolet absorbancy) eluted with a 0·2M buffer solution. The ultraviolet-absorbing fraction was evaporated under the above mentioned conditions, the residue coevaporated with three 50 ml portions of ethanol, and finally dried at 50°C/0·1 Torr over phosphorus pentoxide. The residual triethylammonium salt *XXII* (homogeneous in S_1 and E_1) was shaken with a mixture of dimethylformamide (5 ml) and hexachloroacetone (2·5 ml) until a solution was obtained. The solution was allowed to stand at room temperature overnight, treated with 25 ml of 0·4M triethylammonium hydrogen carbonate (pH 7·5), the whole stirred for one hour, diluted with water (100 ml), and washed with two 25 ml portions of ether. The aqueous phase was evaporated to dryness as above, the residue dissolved in water (50 ml), the solution filtered through Cellit, and the filtrate applied to a column (100 × 4 cm) of DEAE-cellulose (*vide supra*). The elution was performed with the use of a linear gradient of triethylammonium hydrogen carbonate, pH 7·5 (2 liter of water in the mixing chamber, 2 liter of 0·2M buffer solution in the reservoir). The elution was checked with the use of the Uvicord apparatus (Uppsala, Sweden); elution rate, 3 ml per min; the fractions were taken in 10 min intervals. The fractions containing compound *XXIII* were combined, evaporated at 35°C/15 Torr, the residue coevaporated with ethanol (50 ml), and chromatographed on 4 sheets of paper What-

man No 3 MM in the solvent system S_1. Bands of the product *XXIII* were eluted with dilute (1 : 100) aqueous ammonia (50 ml), and the eluate freeze-dried. Yield, 208 mg (58%) of the ammonium salt of compound *XXIII*, homogeneous in S_1 and E_1.

Pancreatic ribonuclease degradation of compound *XXIII* does not occur even in a tenfold excess of the enzyme. Ribonuclease T2 degredation of compound *XXIII* (standard conditions) affords compound *XXIV*. On heating (50°C for 4 hours) in 50% aqueous acetic acid, compound *XXIII* is quantitatively converted to the nucleotide *XXIV*.

Reaction of Uridine 2',3'-Cyclic Phosphate with the Agent *I*

A mixture of uridine 2',3'-cyclic phosphate triethylammonium salt (2 mmol), dimethylformamide (4 ml), and the agent *I* (1·5 ml) was heated at 110°C for 6 hours, evaporated at 40°C/0·1 Torr, the residue coevaporated under identical conditions with dimethylformamide (20 ml), and finally dissolved in 50% aqueous dioxane with the addition of Dry Ice. The solution was allowed to stand at room temperature for 30 min, evaporated at 35°C/15 Torr, and the residue chromatographed on 8 sheets of paper Whatman No 3 MM in the solvent system S_1. The elution was performed with dilute (1 : 100) aqueous ammonia and the eluates freeze-dried to afford 4 main fractions: *a*) R_F 0·36, content, 32% of the starting uridine 2',3'-cyclic phosphate, identified on comparison with an authentic specimen in solvent system S_1 and buffer solution E_1, as well as by pancreatic ribonuclease degradation leading quantitatively to uridine 3'-phosphate; *b*) R_F 0·47; content, 0·28 mmol (based on the monomer) of an unidentified material, probably a phosphodiester; *c*) R_F 0·55; content, 0·28 mmol (14%) of compound *XXIII*, identified on comparison with an authentic specimen (*vide supra*) in S_1 and E_1, as well as on cleavage in acetic acid (*vide supra*); and *d*) R_F 0·66; content, 0·26 mmol (based on the monomer) of an unknown substance of a 2',3'-cyclic phosphate character (R_F 0·65 in S_1; E_{Up} 0·54 in E_1), the cleavage of which in acetic acid affords a substance of a phosphomonoester character (R_F 0·34 in S_1); its structure appears to correspond to that of the by-product obtained in the reaction of uridine with the agent *I*.

Reaction of Uridine 5'-phosphate (*XIII*) with the Agent *I*

Sodium salt of compound *XIII* (1 mmol) was converted to the triethylammonium salt on a column (15 ml) of pyridinium Dowex 50 X 8 ion exchange resin (the elution was performed with 100 ml of 20% aqueous pyridine, the eluate was treated with 2 ml of triethylamine, the whole evaporated at 35°C/15 Torr, the residue coevaporated with three 50 ml portions of pyridine, and finally dried at 50°C/0·1 Torr over phosphorus pentoxide). The residual triethylammonium salt of compound *XIII* was heated for 8 hours at 100°C in a mixture of dimethylformamide (5 ml) and the agent *I* (1 ml), the whole evaporated at 40°C/15 Torr, the residue kept at room temperature for one hour in 50% aqueous ethanol (10 ml), and the solution evaporated to dryness. The residue was applied (in 10 ml of water) to a column (25 × 4 cm) of DEAE-cellulose (Cellex, D, standard capacity, HCO_3^-) and the column eluted with water (3 ml per min to the drop of ultraviolet absorption). As shown by chromatography in the solvent system S_1, this neutral fraction contains 950 A_{260} (9·5%) of a mixture consisting of compounds *IIa* and *IIIa* in the ratio 35 : 65. The elution was continued under analogous conditions with the use of 0·5M triethylammonium hydrogen carbonate (pH 7·5). This acid fraction was evaporated and the residue chromatographed on 6 sheets of paper Whatman No 3 MM in the solvent system S_1. Bands of four main ultraviolet-absorbing products were eluted with dilute (1 : 100) aqueous ammonia (100 ml each), the eluates evaporated, and the content determined spectrophotometrically. It was obtained 9% of P^1, P^2-uridine 5'-pyrophosphate, 12% of the starting uridine 5'-phosphate (*XIII*), 10% of compound *XIV* (identified on comparison with an authentic specimen and alkaline phosphatase *E. coli*

degradation to compound *IIIa*), 24% of compound *XV* (identified on comparison with an authentic specimen and snake venom phosphodiesterase degradation to compound *XIII*), and 26% of compound *XVI* which is converted to compound *XIV* by the action of snake venom phosphodiesterase.

Preparation of 2-Hydroxyethyl Esters of 5'-Nucleotides *XV*, *XVIII*, and *XX*

A solution of the sodium salt of the 5'-nucleotide *XIII* or *XIX* (2 mmol) in water (10 ml) was applied to a column (20 ml) of pyridinium Dowex 50 X 8 ion exchange resin and the column eluted with 30% aqueous pyridine (100 ml). The eluate was evaporated at 35°C/15 Torr and the residue dissolved in pyridine (50 ml). Compound *XVII* (free acid) was dissolved directly in 50 ml of pyridine. Solution of the pyridinium salt of a 5'-nucleotide was dried by repeated coevaporations with pyridine (five 25 ml portions) at 30°C/0·1 Torr, the residue dissolved in pyridine (20 ml), ethylene glycol (2 ml) and N,N'-dicyclohexylcarbodiimide (2 g) added, and the whole allowed to stand at room temperature for 4 days. The nixture was decomposed with water (10 ml), allowed to stand at room temperature for one hour, diluted with additional water (100 ml), washed with three 25 ml portions of ether, the aqueous phase made alkaline with aqueous ammonia, and evaporated at 35°C/15 Torr. The residue was dissolved in water (50 ml), the solution filtered through Cellit, and the filtrate applied to a column (80 × 4 cm) of DEAE-cellulose (Cellex D, standard capacity, HCO_3^-). The elution was performed with the use of a linear gradient of triethylammonium hydrogen carbonate, pH 7·5 (2 liter of water in the mixing chamber, 2 liter of 0·4M buffer solution in the reservoir); rate, 3 ml per min; checked by absorption measurements on an Uvicord apparatus. The 0·20—0·30M fraction contained the product (*XV*, *XVIII*, or *XX*), the 0·30—0·35M fraction consisted of the starting 5'-nucleotide, and the 0·35—0·40M fraction contained compound *XXI*. The fractions were evaporated at 35°C/15 Torr, the residues coevaporated with two 50 ml portions of ethanol, and reprecipitated from ethanol (20 ml) with ether (300 ml). The precipitates were collected by centrifugation, washed with ether, and dried at 20°C/0·1 Torr over phosphorus pentoxide. All products examined (*XV*, *XVIII*, *XX*, and *XXI*) were resistant towards alkaline phosphatase *E. coli*, contained 1 equivalent of the cis-diol system per the heterocyclic moiety, and were cleaved quantitatively to the corresponding starting 5'-nucleotide by the action of snake venom phosphodiesterase. The following compounds were prepared: *XV* (triethylammonium salt; yield, 22%); *XVIII* (triethylammonium salt; yield, 35%); *XX* (triethylammonium salt; yield, 25%). In all cases, the purity was higher than 95%, as determined spectrophotometrically. The bis-triethylammonium salts of compounds *XXI* were obtained in the following yields: 7% (*XXIa*), 11% (*XXIb*), and 10% (*XXIc*). Their analytical data were in accordance with calculated values and their purity was higher than 90%.

When the above reaction was performed with ethylene glycol monobenzyl ether (2·5 g) instead of ethylene glycol under otherwise identical conditions, a mixture of compounds *XIX*, *XX*, and *XXIc* was obtained with the use of compound *XIX* as the starting substance.

Guanylyl-(3'→5')-N^3-(2-hydroxyethyl)uridine (*XXVII*)

A solution of the triethylammonium salt of compound[21] *XXV* (100 mg; 113 μmol) in a mixture of dimethylformamide (1 ml) and the agent *I* (0·5 ml) was heated at 100°C for 12 hours and then chromatographed directly on 2 sheets of paper Whatman No 3 MM in the solvent system S_1. Band of the product *XXVI* (R_F 0·61) was eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate evaporated to dryness at 30°C/15 Torr, and the yield determined spectrophotometrically (64%). The residue of compound *XXVI* was dissolved in 2 ml of 50% acetic acid, the solution allowed to stand at 50°C for 30 min, and then chromatographed on 2 sheets of paper Whatman

No 3 MM in the solvent system S_1. The slowest (principal) ultraviolet-absorbing band was eluted as above (content as determined spectrophotometrically, 54%, based on compound *XXV*), and the eluate freeze-dried. The resulting ammonium salt of compound *XXVII* is homogeneous in S_1, S_2, and E_1; ribonuclease T2 degradation affords quantitatively Gp and compound *IIIa*; snake venom phosphodiesterase degradation affords guanosine and compound *XIV* (1 : 1·10). Ultraviolet spectrum: λ_{max} 260 nm, λ_{min} 230 nm, $A_{250/260}$ 0·88, $A_{280/260}$ 0·56, $A_{290/260}$ 0·27.

N^3-(2-Hydroxyethyl)uridine 5'-Phosphate (*XIV*)

The ammonium salt of compound *XXVII* (870 A_{260}; 40 μmol) was incubated in 2 ml of 0·1M triethylammonium hydrogen carbonate (pH 7·5) with 50 γ of snake venom phosphodiesterase (16 hours at 37°C). The mixture was then applied to a 16 cm wide strip of paper Whatman No 3 MM and subjected to electrophoresis in the buffer solution E_1. Band of the product *XIV* was eluted with water (10 ml) and the eluate (triethylammonium salt) freeze-dried. Yield, 84%, as determined spectrophotometrically. The product is homogeneous in systems S_1, S_2, and E_1. Alkaline phosphatase *E. coli* degradation (standard conditions, *vide supra*) of compound *XIV* affords quantitatively compound *IIIa*. The ratio phosphorus — ultraviolet absorption, 1 : 0·96.

Enzymatic Reaction of Guanosine 2',3'-Cyclic Phosphate with Compound *IIIa*

A solution of the triethylammonium salt of guanosine 2',3'-cyclic phosphate (100 μmol) and compound *IIIa* (500 μmol) in 0·50 ml of 0·05M-TRIS-HCl buffer (pH 7·0) was incubated with 100 e.u. of ribonuclease T1 (at 0°C overnight) and then applied quickly to 2 sheets of paper Whatman No 3 MM. After chromatography in the solvent system S_1, the bands near the start line were eluted with dilute (1 : 100) aqueous ammonia (25 ml), the eluate evaporated under diminished pressure at 30°C, and the residual mixture purified by electrophoresis on a 16 cm wide strip of paper Whatman No 3 MM in the buffer solution E_1 (10 volt/cm, 2 hours). Band corresponding to dinucleoside phosphate was eluted with water (10 ml). Yield, 810 A_{260} (37% for GpU), as determined spectrophotometrically. Ultraviolet spectrum: λ_{max} 260 nm, λ_{min} 230 nm, $A_{250/260}$ 0·89, $A_{280/260}$ 0·57, $A_{290/260}$ 0·29 (pH 2).

Snake venom phosphodiesterase degradation of this product (for conditions see the degradation of compound *XXVII* to compound *XIV*) afforded after 6 hours quantitatively compound *XIV* which was then isolated in 78% yield.

Reaction of Benzoic Acid with the Agent *I*

A mixture of benzoic acid (1·5 g; 12 mmol), benzene (30 ml), and the agent *I* (2 ml) was refluxed for 4 hours, evaporated at 35°C/15 Torr, and the residue diluted with water (50 ml). After the addition of dry ice (neutralisation), the mixture was allowed to stand at room temperature for 15 min and then extracted with two 50 ml portions of ether. The extract was dried over magnesium sulfate, the ether evaporated, and the residue chromatographed on a loose layer of silica gel (400 × 180 × 3 mm) in chloroform. Yield, 74% of 2-hydroxyethyl benzoate, identified by infrared spectroscopy on comparison with an authentic specimen (the analytical sample was obtained by thin-layer chromatography, elution with chloroform, and rectification). No 2-formyloxyethyl ester of benzoic acid was present. Heating of an authentic sample of 2-formyloxyethyl benzoate with the agent *I* in benzene for 4 hours led only to a negligible deformylation, as determined by thin-layer chromatography in chloroform.

2-Hydroxyethyl benzoate. Prepared according to the ref.[11]. B.p. 112°C/0·2 Torr, n_D^{22} 1·5302. Thin-layer chromatography on silica gel in chloroform: R_F 0·16.

2-*Formyloxyethyl benzoate.* Formic acid (100%; 15 ml) was added to acetic anhydride (30 ml) precooled to 0°C, the mixture kept 30 min at 0°C and 15 min at 50°C, cooled down to −40°C, and treated with pyridine (50 ml) and 2-hydroxyethyl benzoate (10 g; 60 mmol). The reaction mixture was allowed to stand at 0°C overnight, cooled down again to −50°C, and treated with methanol (50 ml). After 2 hours at room temperature, the mixture was poured into 500 ml of iced water, and extracted with three 100 ml portions of ether. The extracts were combined, washed with two 100 ml portions of 5% ice-cool aqueous sodium hydrogen carbonate and two 100 ml portions of water, dried over magnesium sulfate, and evaporated. The residue was applied to a column of silica gel (300 g; packed in chloroform). The column was eluted with chloroform, 200 ml fractions being taken. Fractions 4 and 5 were combined, evaporated at 30°C/15 Torr, and the residue rectified under diminished pressure. B.p. 125°C/0·3 Torr, n_D^{22} 1·5123. Yield, 6·5 g (60%). Thin-layer chromatography on silica gel in chloroform: R_F 0·60. For $C_{10}H_{10}O_4$ (194·2) calculated: 61·84% C, 5·18% H; found: 91·69% C, 5·11% H.

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