NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLIV.*

NUCLEOSIDE AND NUCLEOTIDE DERIVATIVES OF α-URIDINE, 2'-DEOXY-α-URIDINE AND 2'-DEOXY-α-CYTIDINE, AND THEIR AFFINITY TOWARDS NUCLEOLYTIC ENZYMES

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Received February 29th, 1972

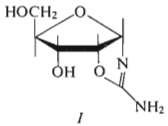
$O^{2,2'}$-Anhydro-α-uridine (*II*) was prepared by reaction of the oxazoline *I* with methyl propiolate. Alkaline hydrolysis of *II* afforded α-uridine (*III*) which was converted by reaction with triethyl orthoformate into the 2',3'-O-ethoxymethylene derivative *IV*. Reaction of compound *II* with benzoyl cyanide afforded the 3',5'-dibenzoate *VII* which was converted on treatment with anhydrous hydrogen chloride in dimethylformamide into the 2'-deoxy-2'-chloro derivative *VIII*. The tri-n-butyltin hydride reduction of compound *VIII* and the subsequent alkaline hydrolysis afforded 2'-deoxy-α-uridine (*X*). 2'-Deoxy-α-cytidine (*XI*) was prepared from compound *IX* by the thiation with phosphorus pentasulfide and the subsequent ammonolysis. Phosphorylation of compounds *III*, *X*, and *XI* with phosphorus oxychloride led to 5'-nucleotides *XII* which are substrates for the snake venom 5'-nucleotidase. α-Uridine 5'-diphosphate (*XIII*) was prepared from the monophosphate *XIIa* by reaction with diphenylphosphoryl chloride and bis-tri-n-butylammonium phosphate. α-Uridine 2',3'-cyclic phosphate (*XIV*) was prepared by reaction of compound *III* with triethyl phosphite and hexachloroacetone. Compound *XIV* is resistant to pancreatic ribonuclease or ribonuclease T 2 degradation. Condensation of 5'-O-acetyl-2'-O-tetrahydropyranyluridine 3'-phosphate (*XVIIa*) or N^2,$O^{5'}$-diacetyl-2'-O-tetrahydropyranylguanosine 3'-phosphate (*XVIIb*) with compound *IV* in the presence of N,N'-dicyclohexylcarbodiimide and the subsequent both alkaline and acidic deblocking afforded uridylyl-(3' → 5')-α-uridine (*XIXa*) and guanylyl-(3' → 5')-α-uridine (*XIXb*), resp. Compounds *XIX* are not substrates for the snake venom phosphodiesterase.

Investigations on the specificity of certain nucleolytic enzymes reported in some earlier papers of this Series comprise nucleotide derivatives modified both in the heterocyclic[1-5] and the sugar[6-11] moiety of the corresponding nucleosides. Most representatives of the latter group of analogues are deduced from the naturally occurring β-anomers of nucleosides by changing the conformation of one hydroxylic function or by changing the chirality of the whole moledule. In connection with the ready accessibility of α-uridine and α-cytidine by the recent procedure of Sanchez and Orgel[12] it appeared of interest to prepare some nucleotide derivatives of α-uridine and study their behaviour from the standpoint of the enzyme specificity.

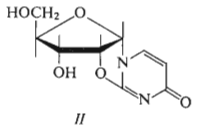
* Part CLIII: This Journal *37*, 4072 (1972).

The original method[12] starts from D-ribose which is treated with cyanamide to give the 2'-amino-1,2-oxazoline *I*. The high yield of this reaction and the negligible solubility of the product *I* make possible a modification consisting in the use of dilute aqueous cyanamide obtained from the sodium salt by ion exchange. On treatment with methyl propiolate in aqueous ethanol, compound *I* is converted into $O^{2,2'}$-anhydro-α-uridine (*II*). The modified conditions[13] lead to a higher yield than the original ones[12]. The constitution of compound *II* was confirmed by analysis, ultraviolet spectra and NMR spectra. In comparison with stereoisomeric $O^{2,2'}$-anhydrouridine, the hydrolysis of compound *II* in aqueous alkali is unusually slow. The resulting α-uridine (*III*) is then converted either to the 2',3'-O-ethoxymethylene derivative *IV* (on treatment with triethyl orthoformate[14]) or to the 2',3'-O-isopropylidene derivative *V* (on treatment with a mixture of triethyl orthoformate and acetone[15]). The structure of compound *III* is in accordance with analytical data, chromatographic and spectroscopic behaviour, and NMR spectra of both *III* and the 2',3'-O-isopropylidene derivative *V*. The structure of the pyridine moiety was confirmed by reaction of the derivative *V* with dimethylformamide dimethylacetal[16,17] to give N^3-methyl-2',3'-O-isopropylidene derivative *VI* (methylation of the heterocyclic ring).

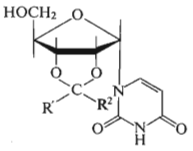
In a paper[13], we have described conversion of pyrimidine ribonucleosides into 2'-deoxyribonucleosides. This novel method comprises reaction of 3',5'-di-O-benzoyl-$O^{2,2'}$-anhydroribonucleosides with hydrogen chloride or lithium iodide in the presence of hydrogen chloride and the subsequent dehalogenation of the resulting 2'-deoxy-2'-halo derivative by the action of tri-n-butyltin hydride. Application of this method to the preparation of 2'-deoxy-α-uridine was accompanied by minor complications, as it may be seen from the following text.



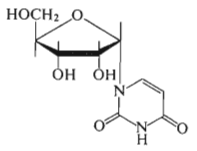
I



II



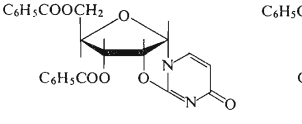
IV, $R^1 = H$, $R^2 = OC_2H_5$
V, $R^1 = R^2 = CH_3$



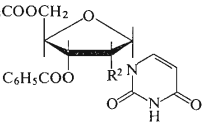
III

Compound *II* was converted on treatment with benzoyl cyanide[18] to the 3′,5′-dibenzoate *VII*, the reaction of which with hydrogen chloride in dimethylformamide afforded a quantitative yield of the 2′-deoxy-2′-chloro derivative *VIII* in the form of the pure *arabo* isomer *VIIIa* (as shown by NMR spectrum). When treated, however, with lithium iodide in the presence of hydrogen chloride[13], the dibenzoate *VII* gave instead of the expected 2′-deoxy-2′-iodo derivative a low yield of the 2′-deoxy-2′-chloro compound *VIII* in the form of a mixture of the *arabo* (*VIIIa*) and *ribo* (*VIIIb*) isomer in the ratio 85 : 15; the reaction is accompanied by the formation of hydrolytical by-products, particularly of α-uridine dibenzoate. The different behaviour of the β-*arabo* and α-*ribo* isomers of the $O^{2,2'}$-anhydro derivative might be ascribed to participation of the 3′-benzoyl group of the α-*ribo* isomer in the reaction with the protonated form of compound *VII*. In view of this participation, an intermediate of the orthobenzoate type is formed instead of the nucleophilic substitution at the $C_{(2')}$ carbon atom. When the reaction mixture is then processed with water, an opening of this intermediate occurs under the preferential formation of a hydroxy derivative with the *ribo* configuration at the $C_{(2')}$ carbon atom.

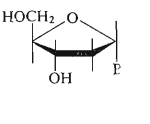
On treatment with tri-n-butyltin hydride, compound *VIII* is quantitatively converted to 3′,5′-di-O-benzoyl-2′-deoxy-α-uridine (*IX*) which is then debenzoylated to give 2′-deoxy-α-uridine (*X*). Reaction of compound *IX* with phosphorus pentasulfide afforded the 4-thio derivative which was not isolated but converted by means of ammonolysis into 2′-deoxy-α-cytidine (*XI*).



VII



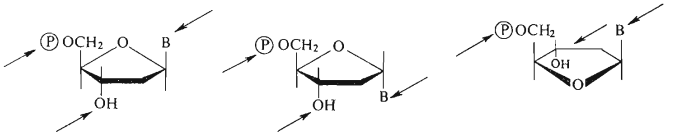
VIIIa; $R^1 = Cl$, $R^2 = H$
VIIIb; $R^1 = H$, $R^2 = Cl$
IX; $R^1 = R^2 = H$



X; B = uracil residue
XI; B = cytosine residue

Phosphorylation of nucleosides *III*, *X*, and *XI* with phosphorus oxychloride in triethyl phosphate[19] affords selectively the 5′-nucleotides *XII*, homogeneous on paper chromatography and electrophoresis; compounds *XII* are degraded quantitatively with nonspecific phosphomonoesterases (*E. coli*, intestinal alkaline phosphatase) as well as with the snake venom 5′-nucleotidase. The course of the latter degradation may be regarded as a proof of the isomeric purity of the reaction product. Concerning the structural requirements of the snake venom 5′-nucleotidase, it has been known that this enzyme requires an interaction with the heterocyclic base of the substrate[4,11], the presence of a single negative charge at the phosphorus atom, and the presence of two hydrophilic centers suitably sterically situated (in the nearest neighbourhood

of the phosphorus atom[20] and on the sugar moiety at position 3′ with the *ribo* configuration). The affinity of the α-D-*ribo* derivatives on the one hand and the resistance of the β-L-*ribo* derivatives on the other hand may be explained by the assumption that the interaction with the heterocyclic base is of a cooperative character; the active center region which is responsible for this interaction, is either large or of a less rigid tertiary structure. Accordingly, the presence of the heterocyclic base is more important than the character of the base. The mutual orientation of the phosphate group and the 3′-hydroxylic function in compounds *XII* is the same as in the naturally occurring β-D-*ribo* and 2′-deoxy-β-D-*ribo* derivatives; on the other hand, with β-L-*ribo* derivatives, the orientation of the substrate molecule to the enzyme cannot be the same at all three sites (as with compounds *XII*), see Scheme 1.



β-D-*Ribo*- α-D-*Ribo* β-L-*Ribo*

(*XII*)

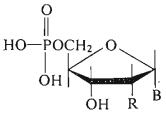
Ⓟ = phosphoric acid ester residue, B = heterocyclic base residue

SCHEME 1

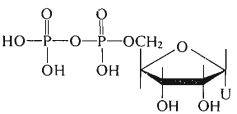
In this connection we have also prepared α-uridine 5′-diphosphate (*XIII*) by reaction of compound *XIIa* with diphenylphosphoryl chloride in the presence of an orthophosphate[21]. As shown by preliminary results[22], the 5′-diphosphate *XIII* is not a substrate for polynucleotide phosphorylase *E. coli* in polymerisation, exchange, or phosphorolytic reactions.

The reported[23] degradation of α-cytidine 3′-phosphate esters by the action of ribonuclease T 2 is at variance with the present knowledge on this enzyme, *cf.* the necessity of a cooperative interaction with the heterocyclic base of the substrate [11] (though the steric requirements are not as rigorous as *e.g.* with pancreatic ribonuclease[10]). In this connection, we have reinvestigated this problem with the use of the simplest substrate type, namely, α-uridine 2′,3′-cyclic phosphate (*XIV*). Compound *XIV* was prepared according to the procedure for the synthesis of cyclic phosphates from nucleosides which contain the *cis*-diol system, *i.e.*, by the successive treatment of α-uridine with triethyl phosphite and then hexachloroacetone[2,24,25]. The constitution of *XIV* was confirmed by acidic hydrolysis to give a mixture of 2′- and 3′-monophosphates *XV*, the chromatographic behaviour of which is different from that of compound *XXIa* and the intestinal alkaline phosphatase degradation of which affords α-uridine (*III*) as the single product. In comparison with the β-series, the yield

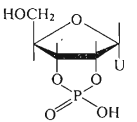
of compound *XIV* is considerably lower, obviously because of different steric requirements for the formation of cyclic intermediates[24]. As expected, compound *XIV* is completely resistant towards the action of pancreatic ribonuclease (the β-L-lyxofuranosyl analogue *XVI* which differs from compound *XIV* by configuration at the $C_{(4')}$ carbon atom is also resistant in this respect[7]). Compound *XIV* is also resistant to ribonuclease T 2 under standard conditions (the β-anomers are degraded quantitatively under these conditions) as well as with the use of a higher concentration of the enzyme or of a longer period of time. Since the resistance of the α-nucleotide derivative *XIV* towards the action of ribonuclease T 2 is in accordance with other observations on the specificity of this enzyme, the structure of the reported[23] non-resistant substance (isolated from a natural material) is probably in error.



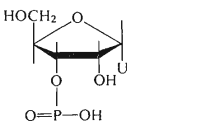
XIIa; B = uracil, R = OH
XIIb; B = uracil, R = H
XIIc; B = cytosine, R = H



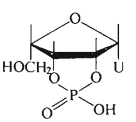
XIII



XIV



XV



XVI

In formulae *XIII*—*XVI* U = uracil residue.

The specificity of the snake venom phosphodiesterase was studied with the use of the diribonucleoside phosphates *XIX* which contain α-uridine at the 3'-end. Compounds *XIX* were prepared by condensation of the 2',3'-O-ethoxymethylene derivative *IV* with 5'-O-acetyl-2'-O-tetrahydropyranyluridine 3'-phosphate[26] (*XVIIa*) or N^2,$O^{5'}$-diacetyl-$O^{2'}$-tetrahydropyranylguanosine 3'-phosphate[27] (*XVIIb*) in the presence of N,N'-dicyclohexylcarbodiimide[27] and the alkaline work-up of the reaction mixture to give the protected derivatives *XV*; the acidic removal of protecting groups led to the final uridylyl-(3' → 5')-α-uridine (*XIXa*) and guanylyl-(3' → 5')-α-uridine (*XIXb*), resp.

$$\underset{XVIIa}{AcUp^{THP}} + \underset{IV}{\alpha\text{-U} > EM} \rightarrow \underset{XVIIIa}{Up^{THP}\,\alpha\text{-U} > EM} \xrightarrow{H^+} \underset{XIXa}{Up\alpha\text{-U}}$$

$$\underset{XVIIb}{\overset{Ac}{\overset{|}{AcGp^{THP}}}} + \underset{IV}{\alpha\text{-U} > EM} \rightarrow \underset{XVIIIb}{Gp^{THP}\,\alpha\text{-U} > EM} \xrightarrow{H^+} \underset{XIXb}{Gp\alpha\text{-U}}$$

α-U, α-uridine (*III*); for other abbreviations see ref.[27]

Pancreatic ribonuclease or ribonuclease T 1 degradation (standard conditions) of compounds *XIXa* and *XIXb* affords quantitatively a mixture of α-uridine (*III*) with uridine 3′-phosphate and guanosine 3′-phosphate, resp., in an equimolar ratio. The contamination with the (2′ → 5′)-isomer is lower than 2%. On the other hand, compounds *XIX* are resistant towards the snake venom phosphodiesterase; this enzyme (similarly to 5′-nucleotidase) requires an interaction with the heterocyclic base of the nucleoside for the formation of a complex with the substrate[7]. With the α-anomeric nucleotide, the requirement of a simultaneous suitable orientation of the phosphate to the heterocyclic base cannot be fulfilled only in that case when the mutual position of the enzyme and the substrate is determined by at least three sites on the substrate molecule as exemplified, *e.g.*, in the case of phosphodiesterase by the loss of affinity towards the L-enantiomers of the β-ribonucleotide derivatives[11].

Additional applications of the dinucleoside phosphates *XIX* in investigations on the mutual interactions of heterocyclic bases in nucleic acid chains have been published elsewhere[28,29].

EXPERIMENTAL

Methods. Solutions were taken down on a rotatory evaporator at 40°C/15 Torr. Substances were dried over P_2O_5 at 0·1 Torr. Descending paper chromatography was performed on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM) in the solvent systems S_1, 2-propanol–conc. NH_4OH–water (7 : 1 : 2), and S_2, ethanol–1M ammonium acetate (5 : 2). Paper electrophoresis was performed by the technique of Markham and Smith[30] on paper Whatman No 3 MM in the buffer solution E_1, 0·1M triethylammonium hydrogen carbonate, and E_2, 0·2M triethylammonium borate (pH 7·5) for 1 h. The detection was performed by viewing in ultraviolet light (Chromatolite).

Thin-layer chromatography on silica gel was performed on ready-for-use Silufol UV_{254} plates containing a fluorescent indicator (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent system S_4, ethanol–chloroform (5 : 95), and S_5, ethyl acetate–benzene (40 : 60). Preparative thin-layer chromatography was performed on loose layers (60 × 16 × 0·3 cm) of fluorescent silica gel (produced by Service Laboratories of our Institute). The elution was performed with the use of methanol. Preparative separations on DEAE-cellulose were performed on a 80 × 4 cm column of Cellex D (standard capacity, Calbiochem) in the bicarbonate cycle with the use of a linear gradient elution (2 l of water in the mixing chamber and 2 l of 0·3M buffer solution in the reservoir); elution rate, 3 ml per min. The fractions were withdrawn in 10 min intervals. The course of elution was checked by means of the Uvicord apparatus. The effluent was taken down

under diminished pressure and the residue coevaporated twice with methanol to remove the volatile buffer.

Enzymatic degradations were performed with 1—2 μmol of the substrate in 100 μl of 0·05M Tris buffer solution and *a*) pancreatic ribonuclease at pH 8·0 (20 μg of the enzyme, Calbiochem, A grade); *b*) ribonuclease T1 at pH 7·0 (20 e.u. of the enzyme, Sankyo); *c*) ribonuclease T 2 at pH 6·5 in 0·1M γ,γ-dimethylglutarate (20 or 50 μg of the enzyme prepared by Prof. Dr H. Witzel, Münster, GFR); *d*) alkaline phosphatase *E. coli* at pH 9·0 (10 μl of the suspension, Worthington, A grade); *e*) alkaline intestinal phosphatase at pH 9·0 (20 μg of the enzyme, Reanal); *f*) snake venom (*Crotalus terr. terr.*) phosphodiesterase at pH 9·0 (10 or 20 μg of the enzyme, Boehringer, Mannheim, Germany); *g*) snake venom (*Crotalus adamanteus*) 5′-nucleotidase at pH 9·0 in 0·005M-$MgSO_4$ (20 μg of the enzyme, Worthington). Incubation, 4 h (12 h) at 37°C.

Ultraviolet absorption spectra were measured on a Beckman DU apparatus in methanol or 0·01M-HCl. NMR spectra were taken on a Varian HA-100 apparatus in hexadeuteriodimethyl sulfoxide (hexamethyldisiloxane as internal standard). CD spectra were recorded on a Jouan Model CD-185 Dichrograph in water at 20°C.

Reaction of D-Ribose with Cyanamide

A solution of the sodium salt of cyanamide (38·5 g; 0·6 mol) in water (600 ml) was adjusted to pH 6 by the addition of the H^+ form of Dowex 50 ion exchange resin. The resin was filtered off without delay, washed with water (500 ml), the filtrate and washings combined, diluted with conc. NH_4OH (70 ml), treated with D-ribose (75 g; 0·5 mol) and the whole heated with stirring for 2 h at 60°C. The mixture was then cooled down, the crystals collected with suction, washed

TABLE I

Paper Chromatography and Electrophoresis

Compound	R_F		E_1[a]	Compound	R_F		E_1[a]
	S_1	S_2			S_1	S_2	
Uridine	0·50	0·73	0	*XVIIIb*	0·55	—	0·40
III	0·50	0·73	0	*XIXa*	0·25	0·50	0·48
IV	0·78	—	—	*XIXb*	0·11	0·37	0·44
V	0·75	—	—	Up	0·14	0·29	1·0
VI	0·88	—	—	pU	0·14	0·27	1·00
X	0·64	—	—	ppU	0·05	0·13	1·24
XI	0·66	—	—	pdU	0·10	0·37	1·00
XIIa	0·14	0·27	1·00	pdC	0·12	0·40	0·87
XIIb	0·09	0·37	1·00	UpU	0·27	0·56	0·48
XIIc	0·10	0·39	0·89	GpU	0·11	0·35	0·44
XIII	0·05	0·13	1·24	U > p	0·37	0·62	0·63
XIV	0·47	0·65	0·60	dU	0·64	—	0
XV	0·10	0·26	1·00	dC	0·66	—	0
XVIIIa	0·70	—	0·45				

[a]The electrophoretical mobility refers to uridine 3′-phosphate.

with water and ethanol, and dried. Yield, 65 g (75%) of the pure oxazoline *I*, identical with a specimen prepared by a reported[12] procedure. For $C_6H_{10}N_2O_4$ (174·2) calculated: 41·36% C, 5·78% H, 16·08% N; found: 41·43% C, 5·80% H, 16·03% N.

$O^{2,2'}$-Anhydro-α-uridine (*II*)

A mixture of the oxazoline *I* (20 g; 115 mmol), methyl propiolate (50 ml), and 50% aqueous ethanol was refluxed for 5 h, cooled, evaporated to dryness, the residue coevaporated with two 50 ml portions of ethanol, and the final residue crystallised from hot ethanol (25 ml) by the addition of acetone (300 ml). The solid was collected with suction, washed with acetone and ether, and dried. Yield, 18·6 g (72%) of compound *II* which does not melt up to 250°C. The analytical sample was crystallised from ethanol–acetone (1 : 2) and was homogeneous on chromatography and electrophoresis. Optical rotation: $[\alpha]_D^{25}$ −22·4° (*c* 0·5, water). UV spectrum (methanol): λ_{max} 248 nm. For $C_9H_{10}N_2O_5$ (226·2) calculated: 47·78% C, 4·45% H, 12·38% N; found: 47·35% C, 4·44% H, 12·16% N.

α-Uridine (*III*)

A solution of compound *II* (4·5 g; 20 mmol), 50% aqueous ethanol (50 ml), and triethylamine (25 ml) was refluxed for 6 h (after this period of time the hydrolysis was quantitative, as shown by electrophoresis in the buffer solution E_2). The reaction mixture was evaporated under diminished pressure, the residue coevaporated with three 100 ml portions of ethanol, and the final residue kept for 2 days at room temperature under a mixture (100 ml) of acetone and ether (1 : 1). The solid was collected with suction, washed with 100 ml of the above solvent mixture, and recrystallised. Yield, 3·4 g (70%) of α-uridine (*III*), m.p. 228°C. For $C_9H_{12}N_2O_6$ (244·2) calculated: 44·26% C, 4·95% H, 11·47% N; found: 44·50% C, 4·80% H, 11·52% N.

2′,3′-O-Isopropylidene-α-uridine (*V*)

A mixture of compound *III* (2·45 g; 10 mmol), dimethylformamide (20 ml), triethyl orthoformate (10 ml), acetone (4 ml), and 6M-HCl in dimethylformamide (2 ml) was shaken until the solid dissolved and then kept at room temperature overnight. Triethylamine was added (5 ml), the solid filtered off, the filtrate evaporated to dryness at 40°C/0·1 Torr, and the residue coevaporated under the same conditions with 50 ml of pyridine and then with 50 ml of toluene. The final residue was dissolved in chloroform and chromatographed on two layers of silica gel in ethanol–chloroform (5 : 95). The band corresponding to compound *V* (R_F 0·35—0·45) was eluted with methanol (200 ml), the eluate evaporated under diminished pressure, the residue dissolved in hot ethanol (10 ml), and the solution treated with cyclohexane until turbid. The mixture was kept at 4°C for 2 days to deposit crystals which were collected with suction, washed with cyclohexane, and dried under diminished pressure. Yield, 2·13 g (75%) of the chromatographically homogeneous compound *V*, m.p. 206—208°C. Optical rotation: $[\alpha]_D^{25}$ −180·9° (*c* 0·5, ethanol). For $C_{12}H_{16}N_2O_5$ (284·2) calculated: 50·69% C, 5·67% H, 9·85% N; found: 50·49% C, 5·59% H, 9·93% N.

2′,3′-O-Isopropylidene-N^3-methyl-α-uridine (*VI*)

A mixture of compound *V* (300 mg; 1·05 mmol), dimethylformamide (3 ml), and dimethylformamide dimethylacetal[31] (1 ml) was heated at 100°C for 2 h, evaporated to dryness at 40°C : : 0·1 Torr, and the crude residue coevaporated with 50% aqueous pyridine and chromatographed

on a loose layer of silica gel in the solvent system S_4. The ultraviolet-absorbing band was eluted with methanol, the eluate evaporated, and the residue crystallised from a mixture of ethanol and cyclohexane to afford 300 mg (96%) of compound *VI*, m.p. 115—116°C. For $C_{13}H_{18}N_2O_6$ (298·3) calculated: 52·34% C, 6·08% H, 9·39% N; found: 52·04% C, 6·03% H, 8·93% N.

2′,3′-O-Ethoxymethylene-α-uridine (*IV*)

The crude α-uridine (*III*; prepared from 20 mmol of compound *II* according to the above procedure) was coevaporated with two 100 ml portions of ethanol, the residue kept over P_2O_5 at 0·1 Torr overnight and the next day a mixture of dimethylformamide (40 ml), triethyl orthoformate (20 ml), and 6M-HCl in dimethylformamide (1 ml) was added. The whole mixture was kept at room temperature overnight, made alkaline by the addition of triethylamine (2 ml), and evaporated at 40°C/0·1 Torr. The residue was chromatographed on two layers of loose silica gel in a mixture of ethanol and chloroform (5 : 95) containing 0·1% of triethylamine. Bands of the product were eluted with methanol (300 ml), the eluate evaporated, and the residue dried under diminished pressure to afford 4·07 g (68%) of the amorphous, chromatographically homogeneous product *IV*. For $C_{12}H_{16}N_2O_7$ (300·2) calculated: 48·04% C, 5·33% H, 9·34% N; found: 48·52% C, 5·37% H, 9·18% N.

3′,5′-Di-O-benzoyl-$O^{2,2'}$-anhydro-α-uridine (*VII*)

A suspension of compound *II* (11·3 g; 50 mmol), benzoyl cyanide (14·4 g; 110 mmol), and acetonitrile (80 ml) was treated under stirring with triethylamine (3 ml). The temperature rose spontaneously under the formation of a red solution which was stirred for additional 2 h, diluted with ethanol (20 ml), and evaporated under diminished pressure. The residue was chromatographed in chloroform on a column of Pitra silica gel (250 g; particle size, 60—120 μ). The column was washed with chloroform (1 000 ml) and then eluted with a mixture of ethanol and chloroform (5 : 95). The eluates of compound *VII* were combined, evaporated under diminished pressure, the residue dissolved in ethanol (50 ml), and this solution added dropwise with stirring into ether (1 000 ml). The precipitate was collected with suction, washed with ether (500 ml) and light petroleum (500 ml), and dried under diminished pressure. Yield, 16·9 g (78%) of compound *VII*, m.p. 107—108°C; R_F 0·12 (in the solvent system S_4). For $C_{23}H_{18}N_2O_7$ (434·4) calculated: 63·59% C, 4·17% H, 6·45% N; found: 63·52% C, 4·42% H, 6·84% N.

1-(3,5-Di-O-benzoyl-2-chloro-2-deoxy-α-D-arabinofuranosyl)uracil (*VIIIa*)

A mixture of compound *VII* (8·7 g; 20 mmol), dimethylformamide (70 ml), and 6M-HCl in dimethylformamide (15 ml) was heated at 100°C for 2 h, poured into 500 ml of water, the precipitate collected with suction, washed with water (500 ml), and recrystallised from ethanol (180 ml). As shown by thin-layer chromatography in the solvent system S_4, the product (R_F 0·64) was entirely pure and the mother liquors did not contain any by-products. Yield, 7·0 g (74·5%) of compound *VIIIa*, m.p. 163°C. Optical rotation: $[\alpha]_D^{25}$ −42·6° (*c* 0·5, dimethylformamide). NMR spectrum: $H_{1'}$ (d) 6·12 ($J_{1',2'}$ 3·0); $H_{4'} + H_{2'} + 2\,H_{5'}$ (m) 4·6—5·0 ($J_{5',4'}$ 4·5); $H_{3'}$ (t) 5·7 ($J_{2',3'}$ 2·8, $J_{3',4'}$ 2·8); H_5 (d) 5·78 ($J_{5,6}$ 8·1); arom. + H_6(m) 7·3—7·7; arom. (m) 7·8—8·2; NH (br s) 9·88. Thin layer chromatography, R_F: 0·64 (in S_4) and 0·50 (in S_5). For $C_{23}H_{19}ClN_2O_7$ (470·9) calculated: 58·67% C, 4·06% H, 5·95% N, 7·53% Cl; found: 58·22% C, 4·11% H, 5·87% N, 7·56% Cl.

Reaction with LiI. Compound *VII* (17·4 g; 40 mmol) was added with stirring at 100°C to a solution of freshly fused LiI (35 g) in dimethylformamide (150 ml), the mixture stirred at 100°C 10 min,

treated with 6M-HCl in dimethylformamide (10 ml), stirred under exclusion of atmospheric moisture at 100°C for 1 h, treated with additional 6M-HCl in dimethylformamide (5 ml), and heated at 100°C for 30 min more. The resulting reaction mixture was then rapidly cooled down, poured into 1 l of water, extracted with three 100 ml portions of chloroform, the extract washed with two 100 ml portions of 10% aqueous sodium thiosulfate and 100 ml of water, dried over magnesium sulfate, and evaporated under diminished pressure. The residual oil was coevaporated with five 20 ml portions of toluene at 40°C/0·1 Torr to remove dimethylformamide. The final residue was dissolved in chloroform (50 ml) and chromatographed on a column of Pitra silica gel (250 g; particle size, 60—120 μ). The column was eluted with chloroform, the fractions of compound *VIII* evaporated, the residue dissolved in ethanol (100 ml), the solution seeded, and kept in a refrigerator for 2 h to deposit crystals which were collected with suction, washed with light petroleum, and dried under diminished pressure. Yield, 6·5 g of compound *VIII*, m.p. 162—163°C. The mother liquor was purified by thin-layer chromatography on silica gel in chloroform. The elution was performed with methanol, the eluate evaporated, and the residue crystallised from ethanol to afford an additional crop (0·7 g) of compound *VIII*. Overall yield, 7·2 g (38%) of compound *VIII*. Optical rotation: $[\alpha]_D^{25}$ −46·0° (*c* 0·5, dimethylformamide). For $C_{23}H_{19}.ClN_2O_7$ (470·9) calculated: 58·67% C, 4·06% H, 7·53% Cl, 5·95% N; found: 59·18% C, 4·32% H, 7·13% Cl, 6·00% N (the iodo atom was absent). As shown by NMR spectrum, the product *VIII* is a mixture of the *arabo* (*VIIIa*) and *ribo* (*VIIIb*) isomers in the ratio 85 : 15. *VIIIa*: $H_{1'}$(d) 6·12 ($J_{1',2'}$ 2·7); *VIIIb*: $H_{1'}$(d) 6·31 ($J_{1',2'}$ 3·8). The other signals are identical with those of compound *VIIIa* (*vide supra*).

3′,5′-Di-O-benzoyl-2′-deoxy-α-uridine (*IX*)

A mixture of compound *VIII* (7·0 g; 15 mmol), tri-n-butyltin hydride (14 g), and 120 ml of benzene was treated with azabisisobutyronitrile (0·2 g), the whole refluxed for 1 h, evaporated under diminished pressure, and the residue triturated with light petroleum (200 ml) to deposit a solid which was collected with suction, washed with light petroleum (500 ml), and recrystallised from ethanol (200 ml). Yield, 4·5 g (67%) of compound *IX*, homogeneous on chromatography (S_5); m.p. 154—155°C. Optical rotation: $[\alpha]_D^{25}$ −24·0° (*c* 0·5, dimethylformamide). NMR spectrum: $H_{2'} + H_{2''}$(m) 2·56, 3·01; 2 × $H_{5'}$(d) 4·57 ($J_{4',5'}$ 4·5); $H_{4'}$ (m) + $H_{3'}$(m) 4·87, 5·63; H_5(d) 5·74 ($J_{5,6}$ 8·1); $H_{1'} + H_6$ (2 × d) 6·33; arom. (m) 7·15—7·7; arom. (m) 7·7—8·2; NH (br s) 9·40. Thin-layer chromatography, R_F: 0·35 (in S_5). For $C_{23}H_{30}N_2O_7$ (436·4) calculated: 63·29% C, 4·62% H, 6·42% N; found: 63·57% C, 4·57% H, 6·53% N.

2′-Deoxy-α-uridine (*X*)

A solution of compound *IX* (1·5 g; 3·45 mmol) in a mixture of methanol (50 ml) and 1M methanolic sodium methoxide (5 ml) was kept at room temperature overnight, evaporated under diminished pressure, the residue diluted with water (100 ml), treated with the H^+ form of Dowex 50 ion exchange resin to bring the pH to the value of 6·5—7·0, the resin filtered off, and washed with water (50 ml). The filtrate and washings were combined and shaken with two 50 ml portions of ether. The aqueous phase was evaporated under diminished pressure and the residue coevaporated with three 20 ml portions of ethanol. The noncrystalline residue was dissolved in ethanol (3 ml) and the solution added dropwise into ether (300 ml). The microcrystalline precipitate was collected with suction, washed with ether, and dried. Yield, 0·6 g (76%) of compound *X*, m.p. 128°C. CD spectrum: 266·5 (−12300); 245·5 (0); 235 (+3300), 233·5 min. (+2100); 217·5 (+2300), 210 (0). For $C_9H_{22}N_2O_5$ (228·2) calculated: 47·36% C, 5·30% H, 12·27% N; found: 47·23% C, 5·80% H, 11·95% N.

2′-Deoxy-α-cytidine (*XI*)

A mixture of compound *IX* (2·2 g; 5 mmol), P_2S_5 (1·3 g), and dioxane (120 ml) was refluxed for 30 min, treated with additional 1·3 g of P_2S_5, and refluxed for 30 min more. The mixture was filtered while hot, the material on the filter washed with dioxane, the filtrates combined and evaporated under diminished pressure. The residue was dissolved in chloroform (100 ml), the solution washed with two 25 ml portions of saturated aqueous $NaHCO_3$ and one portion of water (25 ml), dried over magnesium sulfate, and evaporated under diminished pressure. The residue was dried over P_2O_5 under diminished pressure, dissolved in methanol (25 ml), the solution treated with 30% methanolic ammonia (150 ml), the whole sealed into a glass ampoule, and heated in an autoclave at 100—110°C for 10 h. The reaction mixture was evaporated under diminished pressure, the residue dissolved in water (100 ml), the aqueous solution washed with two 25 ml portions of ether, the aqueous phase concentrated under diminished pressure to the volume of about 25 ml, the pH adjusted by the addition of conc. HCl to the value of 3·0—3·5, and the mixture applied to a column (100 ml) of the H^+ form of Dowex 50 X 8 ion exchange resin. The column was eluted with water until the ultraviolet absorption decreased and then with dilute (1 : 3) aqueous ammonia. The ultraviolet-absorbing ammoniacal eluate was evaporated under diminished pressure, the residue coevaporated with three 25 ml portions of ethanol, and the final residue purified by precipitation from ethanol (10 ml) by a dropwise addition into ether (100 ml). The chromatographically homogeneous (solvent systems S_1 and S_2) microcrystals were collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·1 g (97%, based on the starting compound *IX*) of compound *XI*, m.p. 138—149°C. CD spectrum: 272 (−15700), 236 (0), 233·5 (sh, +200), 216 (+5050), 207 (0). For $C_9H_{13}N_3O_4$ (227·2) calculated: 47·57% C, 5·76% H, 18·49% N; found: 47·67% C, 5·52% H, 18·13% N.

α-Uridine 5′-Phosphate (*XIIa*)

Phosphorus oxychloride (1 ml) was added under cooling with ice and stirring to a mixture of the crude α-uridine (*III*; prepared from 5 mmol of compound *II* and dried under diminished pressure) and triethyl phosphate (10 ml). The reaction mixture was then stirred at 0°C for 5 h, diluted with ether (250 ml), the precipitate collected with suction, washed with ether, dissolved in water (200 ml), and the solution adjusted to pH 8·5 by the addition of 10% LiOH. The solution was heated at 70°C for 30 min, evaporated under diminished pressure, the residue dissolved in water (20 ml), and the aqueous solution applied to a column (100 ml) of pyridinium Dowex 50 ion exchange resin. The column was eluted with 20% aqueous pyridine (500 ml), the eluate adjusted to pH 8·5 by the addition of triethylamine, and evaporated to dryness under diminished pressure. As shown by electrophoresis in the buffer solution E_1, the product contains a small amount of compound *III*. The residue was dissolved in water (50 ml) and the aqueous solution applied to a column of DEAE-cellulose. The product *XIIa* was eluted with 0·3M buffer solution and the eluate processed as above to afford the triethylammonium salt *XIIa* which was purified by precipitation, *i.e.* the solution of the salt in ethanol (20 ml) was added dropwise into ether (300 ml), the precipitate collected by centrifugation, washed with ether, and dried under diminished pressure. Yield, 785 mg (37%, based on compound *II*) of the mono-triethylammonium salt of compound *XXIa* (content, c. 95%, as determined spectrophotometrically), homogeneous in systems S_1, S_2, and E_1. For $C_{15}H_{28}N_3O_9P$ (425·4) calculated: 9·88% N, 7·29% P; found: 10·15% N, 1·00% P. The alkaline intestinal and bacterial phosphatase degradation affords compound *III*.

2'-Deoxy-α-uridine 5'-Phosphate (*XIIb*)

A solution of compound *X* (0·25 mmol) in triethyl phosphate (1 ml) was treated under cooling (ice) with phosphorus oxychloride (70 µl), the mixture stirred at 0°C for 2 h, diluted with water (5 ml), the solution adjusted to pH 9·0 by the addition of 30% NaOH and heated to the boiling point. After cooling, the solution was applied to two sheets of paper Whatman No 3 MM and chromatographed for two days in the solvent system S_1. Bands of compound *XIIb* were eluted with dilute (1 : 100) aqueous ammonia and the eluate (25 ml) freeze-dried. Yield, 70 mg (86%) of the ammonium salt of compound *XIIb* (content, >95%), chromatographically homogeneous.

2'-Deoxy-α-cytidine 5'-Phosphate (*XIIc*)

This preparation was analogous to that of compound *XIIb*. The nucleoside *XI* (0·5 mmol) was used as the starting material. The isolation was performed by chromatography on four sheets of paper Whatman No 3 MM in the solvent system S_1. Yield, 74% of the ammonium salt of compound *XIIc* (content, >97%, as determined spectrophotometrically), homogeneous on chromatography and electrophoresis.

α-Uridine 5'-Diphosphate (*XIII*)

The triethylammonium salt of compound *XIIa* (0·5 mmol) was converted to the pyridinium salt by means of a passage through a 10 ml column of pyridinium Dowex 50 ion exchange resin and elution with 20% aqueous pyridine (50 ml). The eluate was treated with tri-n-butylamine (0·5 ml), evaporated to dryness under diminished pressure, the residue coevaporated with three 50 ml portions of ethanol, and dried over P_2O_5 at 0·1 Torr overnight. To the residual tri-n-butylammonium salt of compound *XIIa* there was added dioxane (3·5 ml), tri-n-butylamine (0·45 ml), and diphenylphosphoryl chloride (0·30 ml). The resulting solution was kept at room temperature for 3 h and evaporated at 30°C/0·1 Torr. The residue was decanted with two 50 ml portions of ether and dried at 0·1 Torr for 10 min. A solution of the bis-tri-n-butylammonium salt of phosphoric acid (2 mmol) in pyridine (5 ml) was then added to the residue and the whole kept at room temperature overnight under exclusion of atmospheric moisture. The mixture was evaporated under diminished pressure, the residue dissolved in water (10 ml), the solution adjusted to pH 3·0—3·5 by the addition of conc. HCl, kept at room temperature for 1 h, adjusted to pH 9·0 by the addition of conc. NH_4OH, and evaporated under diminished pressure. The residue was applied to 3 sheets of paper Whatman No 3 MM and chromatographed for 3 days in the solvent system S_1. Bands of the product were eluted with dilute (1 : 100) aqueous ammonia (50 ml) and the eluate freeze-dried. Yield, 30·4% of the ammonium salt of compound *XIII*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1). The alkaline bacterial and intestinal phosphatase degradation affords compound *III* as the single ultraviolet-absorbing product.

α-Uridine 2',3'-Cyclic Phosphate (*XIV*)

The residual crude α-uridine (*III*; prepared from 5·0 mmol of compound *II* as above) was shaken with a mixture of dimethylformamide (10 ml), triethyl phosphite (5 ml), and 6M-HCl in dimethylformamide (1 ml). The resulting solution was kept at room temperature overnight under exclusion of atmospheric moisture, diluted with 0·4M triethylammonium hydrogen carbonate pH 7·5 (50 ml), and evaporated under diminished pressure. The residue was chromatographed on a column of DEAE-cellulose under standard conditions. The 2'(3')-phosphite peak (0·10—0·15M buffer solution) was evaporated under diminished pressure, the residue coevaporated with ethanol,

and dried over phosphorus pentoxide at 0·1 Torr overnight. The dry residue was then shaken with a mixture of dimethylformamide (10 ml) and hexachloroacetone (5 ml) until a solution was obtained and then kept at room temperature for 20 h; 0·4M triethylammonium hydrogen carbonate pH 7·5 (100 ml) was then added, the mixture stirred for 30 min, washed with two 25 ml portions of ether, and the aqueous phase evaporated to dryness under diminished pressure. The residue was chromatographed on DEAE-cellulose under standard conditions. The peak (0·10—0·12M buffer solution) of the product was evaporated and rechromatographed on Whatman No 3 MM in the solvent system S_1. Compound *XIV* was eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate evaporated to dryness under diminished pressure, and the residue purified by adding the ethanolic solution (10 ml) into ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and dried under diminished pressure. Yield, 340 mg (20%) of the ammonium salt of compound *XIV*, content >95%, as determined spectrophotometrically. For $C_9H_{16}N_3O_9P$ (341·4) calculated: 12·31% N, 9·12% P; found: 12·70% N, 9·00% P.

α-Uridine 2′(3′)-Phosphate (*XV*)

A solution of the ammonium salt of compound *XIV* (100 mg; 294 μmol) in 50% aqueous acetic acid (3 ml) was heated at 50°C for 3 h and chromatographed on Whatman No 3 MM in the solvent system S_1. Bands of the product *XV* were eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate evaporated under diminished pressure, and the residue freeze-dried. Yield (as determined spectrophotometrically), 68% of the ammonium salt of compound *XV*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1). The chromatographic behaviour of compound *XV* is different from that of the 5′-isomer *XII*. The *cis*-diol test was negative and the alkaline bacterial phosphatase degradation afforded compound *III* as the single ultraviolet absorbing product.

Uridylyl-(3′ → 5′)-α-uridine (*XIXa*)

A solution of the pyridinium salt of 5′-O-acetyl-2′-O-tetrahydropyranyluridine 3′-phosphate[26] (*XVIIa*; 1·4 mmol) and of the pyridinium salt of compound *IV* (3 mmol) in pyridine (50 ml) was evaporated and the residue dried by repeated coevaporations with pyridine (five 20 ml portions) at 30°C/0·1 Torr. The final residue was dissolved in pyridine (15 ml) and the solution shaken with N,N′-dicyclohexylcarbodiimide (2 g) for 30 min. The mixture was kept at room temperature under exclusion of atmospheric moisture for 6 days, treated with 5 ml of water and 1 ml of triethylamine, kept for additional 2 h at room temperature, diluted with water (100 ml), and washed with two 25 ml portions of ether. The aqueous phase was concentrated under diminished pressure to the volume of about 10 ml and the concentrate heated with conc. NH_4OH (10 ml) for 2 h at 50°C. The mixture was evaporated to dryness under diminished pressure, the residue dissolved in water (50 ml), the solution filtered through a layer of Cellite, and the filtrate chromatographed on a column of DEAE-cellulose. The 0·08—0·12M buffer fraction was evaporated under diminished pressure and the residue chromatographed on Whatman No 3 MM in the solvent system S1. Bands of compound *XVIIIa* were eluted with dilute (1 : 100) aqueous ammonia (50 ml), the eluate evaporated under diminished pressure, and the residue precipitated from ethanol (10 ml) by the addition into ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and dried *in vacuo*. Yield, 336 mg (30%) of the ammonium salt of compound *XVIIIa*, chromatographically (S_1) and electrophoretically (E_1) homogeneous. UV spectrum (pH 2): λ_{max} 261 nm, $A_{250/260}$ 0·76, $A_{280/260}$ 0·38, $A_{290/260}$ 0·05. For $C_{26}H_{38}N_5O_{16}P$ (707·6) calculated: 9·89% N, 4·38% P; found: 9·70% N, 3·80% P. A solution of the ammonium salt of compound *XVIIIa* (200 mg; 268 μmol) in 50% aqueous acetic acid (2 ml) was heated at 50°C

for 30 min and then chromatographed on Whatman No 3 MM in the solvent system S_1. Bands of the product *XIXa* were eluted with dilute (1 : 100) aqueous ammonia and the eluate freeze-dried. Yield (as determined spectrophotometrically) 70% of the ammonium salt of compound *XIXa*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_2). Pancreatic ribonuclease degradation of *XIXa* affords quantitatively a mixture of uridine 2′-phosphate and α-uridine (*II*) in the ratio 1·0 : 0·95. Compound *XIXa* is resistant towards the snake venom phosphodiesterase under standard conditions.

Guanylyl-(3′ → 5′)-α-uridine (*XIXb*)

The title compound was prepared analogously to compound *XIXa* from 1·5 mmol of N^2,$O^{5'}$-diacetyl-2′-O-tetrahydropyranylguanosine 3′-phosphate[27] (*XVIIb*) and 3 mmol of compound *IV*. Yield, 32% of the ammonium salt of compound *XVIIIb* (content > 95%, determined spectrophotometrically), chromatographically (S_1) and electrophoretically (E_1) homogeneous. Deblocking of compound *XVIIIb* with aqueous acetic acid (for the conditions see compound *XVIIIa*) and the subsequent chromatography in the solvent system S_1 (2 days) afforded 65% of the ammonium salt of compound *XIXb*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_1). The ribonuclease T 1 degradation of compound *XIXb* (standard conditions) affords quantitatively a mixture of guanosine 3′-phosphate and α-uridine (*III*) in the ratio 1 : 1·08. On the other hand, compound *XIXb* is resistant towards the snake venom phosphodiesterase. UV spectrum (pH 2): λ_{max} 258 nm, $A_{250/260}$ 0·92, $A_{280/260}$ 0·67, $A_{290/260}$ 0·46.

The author wishes to thank Professor F. Šorm for the interest and encouragement, Dr I. Frič for measurements of CD spectra, Dr M. Masojídková and Dr M. Budéšínský for measurement and interpretation of NMR *spectra. The excellent technical assistance of Mrs B. Kopecká is gratefully acknowledged.*

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Translated by J. Pliml.