NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLXII.*

PREPARATION OF 6-METHYL-2′-DEOXYURIDINE AND RELATED COMPOUNDS**

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2-Amino-β-D-arabinofuro[1′,2′:4,5]oxazoline (*I*) reacts with ethyl tetrolate under the formation of 6-methyl-$O^{2,2'}$-anhydrouridine (*IIa*) which is converted *in situ* by benzoylation into the derivative *IIb*. On treatment with hydrogen chloride in dimethylformamide, compound *IIb* is converted into the 2′-chloro-2′-deoxy derivative *III*. The tri-n-butyltin hydride reduction of compound *III* and the subsequent methanolysis of benzoyl groups affords 6-methyl-2′-deoxyuridine (*IVa*). 1-(β-D-Arabinofuranosyl)-6-methyluracil (*V*) is obtained by the alkaline hydrolysis of the anhydro derivative *IIa*. Benzoylation of the oxazoline *I* with benzoyl cyanide affords the N^1,$O^{3',5'}$--tribenzoyl derivative *VI*. The acidic hydrolysis of compound *VI* affords 1,3′,5′-tribenzoyl--β-D-arabinofuro[1′,2′:4,5]oxazolin-2-one (*VII*) while the treatment of *VI* with boron trifluoride etherate in methanol leads to the analogous 3′,5′-di-O-benzoyl derivative *VIII*. An analogous sequence of reactions served in the conversion of 2-amino-α-D-ribofuro[1′,2′:4,5]oxazoline (*IX*) into $O^{2,2'}$-anhydro-6-methyl-α-uridine (*Xa*), 6-methyl-2′-deoxy-α-uridine (*XIIa*), and 6-methyl--α-uridine (*XIII*). 2′-Amino-α-D-xylofuro[1′,2′:4,5]oxazoline (*XIV*) is converted to the $O^{2,2'}$--anhydro derivative *XVa* which affords 1-(2-deoxy-α-D-lyxofuranosyl)-6-methyluracil (*XVIIa*) and 1-(α-D-xylofuranosyl)-6-methyluracil (*XVIII*). Phosphorylation of 6-methyl-2′-deoxyuridine (*IVa*) with phosphorus oxychloride in triethyl phosphate affords 6-methyl-2′-deoxyuridine 5′-phosphate (*XXIa*).

One of the most promising recent methods in the synthesis of pyrimidine nucleosides consists in the approach, the principle of which was devised by Sanchez and Orgel[2]. The approach is based on the addition of suitable acetylenecarboxylic acid derivatives to 2-aminooxazoline derivatives, readily accessible by the reaction of some free sugars with cyanamide (*cf.* also Shannahoff and Sanchez[3]). In this Laboratory, we have paid attention to the extension of the Sanchez reaction to other sugar derivatives[4–6] as well as to the synthetic application of the intermediary $O^{2,2'}$-anhydro nucleosides, obtained by this route. The latter group of investigations includes the synthesis of 2′-deoxyribonucleosides[4,5] and 2′-deoxylyxofuranosides[6] and a new

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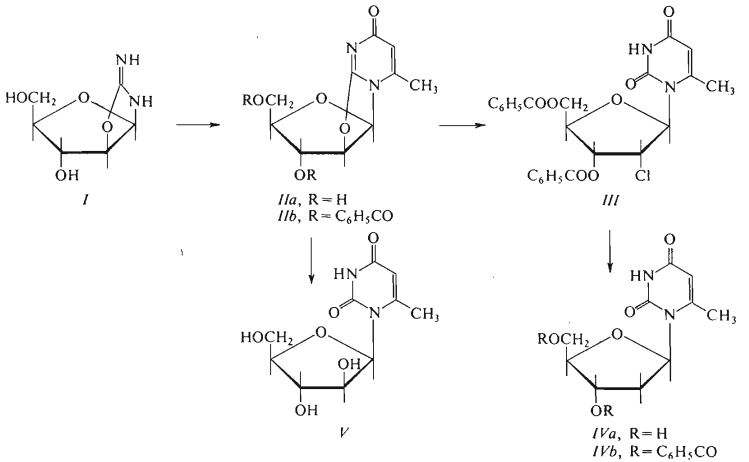
** Some results have been reported in a preliminary form elsewhere[1].

conversion of the above mentioned intermediates to ribonucleosides and lyxofuranosides of the pyrimidine series[7]. The scope of the Sanchez reaction could be obviously extended also with respect to the second reaction component, namely, the acetylenecarboxylic acid derivative. The mechanism of the reaction excludes the preparation of 5-substituted uracil or cytosine derivatives while, on the other hand, the homologues of propiolic acid should afford uracil or cytosine derivatives substituted at position 6 of the pyrimidine ring.

The simplest and the most attractive reaction component of this type is the diester of acetylenedicarboxylic acid which should afford derivatives of orotic acid (6-uracilcarboxylic acid) nucleosides. Our results in this respect will be discussed in a limited extent only, as far as they concern the main problems of the present paper since some results from this field have been reported by another team of authors[8] prior to accomplishment of our investigations.

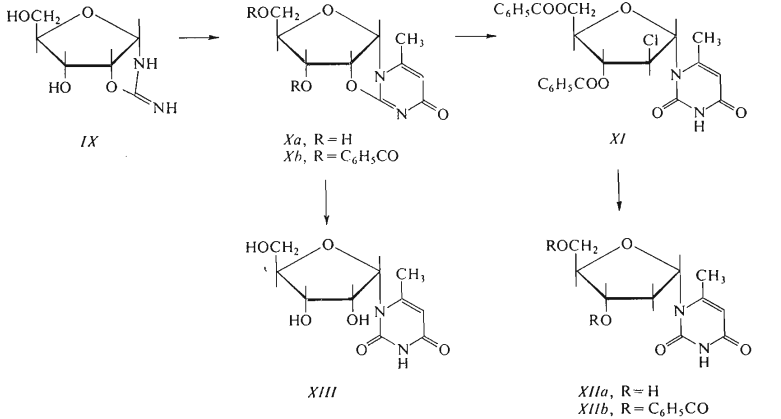
The main attention has been paid to the preparation and properties of 6-methyluracil nucleosides glycosylated at position 1. With the use of the Hilbert–Johnson reaction as well as the mercuri process, the ribosylation of 6-methyluracil occurs mainly at position N^3 and the minor N^1-ribosylated product has to be separated from the reaction mixture[9,10]. The recently[11] reported preparation of 6-methylcytidine by condensation of the N^4-acetylated base with the protected ribosyl halide in the presence of mercuric cyanide appears uniform but the yield is not high and



SCHEME 1

some loss is encountered in the deamination step to the uracil derivative. Especially the classical preparation of the corresponding 2'-deoxyribonucleosides is difficult because of the formation of the two possible anomers which have to be separated[11]. One of the aims of the present paper was a suitable preparation of 6-methyl-2'-deoxyuridine, *i.e.*, the position isomer of the naturally occurring 2'-deoxythymidine and the preparation of some of its derivatives for further biochemical and physicochemical investigations.

The reaction of 2-amino-β-D-arabinofuro[1',2' : 3,5]oxazoline[2,4] with ethyl tetrolate did not take place under the conditions which had been earlier used in the case of ethyl propiolate, in ethanol, dioxane or dimethylformamide at room temperature or at an elevated temperature. It has been found, however, that the desired $O^{2,2'}$-anhydro-6-methyluridine (*II*) is formed when the reaction is performed in the presence of triethylamine and at room temperature. In order to exclude the possibility of a subsequent opening of the anhydro bond, the reaction was carried out in an aprotic solvent, namely, in dimethylformamide. The reaction is relatively slow and, after three days, the yield of compound *II* does not increase. The reaction product *IIa* was not isolated directly but (to simplify the further reaction steps) was *in situ* converted to the 3',5'-di-O-benzoyl derivative *IIb* by reaction with benzoyl cyanide[4,12] Pure compound *IIa* may be recovered from the dibenzoate *IIb* by methanolysis. Analyses and NMR spectra of compounds *IIa* and *IIb* were in agreement with the



SCHEME 2

structure proposed. The ultraviolet absorption spectrum of compound *IIa* exhibits as expected a hypsochromic shift of the absorption maximum when compared with the spectrum of 6-methyluridine[9,10].

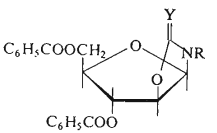
By the action of hydrogen chloride in dimethylformamide[4], compound *IIb* was quantitatively converted to the 2'-deoxy-2'-chloro derivative (*III*) of the *ribo* configuration. The structure of this derivative was also verified by NMR spectrum (a single doublet of the $H_{1'}$ proton). The tri-n-butyltin hydride reduction of compound *III* afforded 3',5'-di-O-benzoyl-2'-deoxy-6-methyluridine (*IVb*) which was methanolysed to the free 2'-deoxy-6-methyluridine (*IVa*) (Scheme 1). The structure of the final products *IV* was again confirmed by analysis and NMR spectra (doublet of the doublets with compound *IVb* – coupling constants $J_{1',2'} = 4{\cdot}5$ and $J_{1',2} = 7{\cdot}7$ – which is converted to a pseudotriplet with compound *IVa* – coupling constant 6·8, in accordance with the behaviour of the $H_{1'}$ proton with 2'-deoxy-β-D-ribonucleosides). Also the UV-spectrum of compound *IVa* is identical with that of a ribonucleoside[9,10]. Similarly to the reaction with esters of propiolic acid[4], neither the reaction mechanism (starting from compound *I* with a preformed β-D-*-arabino* configuration) nor the stereospecific S_N2 anhydro ring opening of compound *IIb* admit another possibility than the β-configuration of the product *IV*. 6-Methyl-2'-deoxyuridine (*IVa*) has been mentioned in the literature as a by-product obtained in the methylation of 2'-deoxyuridine 5-organolithium derivative with methyl iodide[13]; the reported constants are in agreement with data of the specimen prepared by the novel method.

The *in situ* benzoylation of the crude compound *IIa* is accompanied by the formation of compound *VI* from the unreacted oxazoline *I*. An identical product was obtained in a high yield by benzoylation of the pure *I* with benzoyl cyanide. As shown by elemental analysis, compound *VI* is a tribenzoate. It was therefore necessary to determine whether the nitrogen atom at position 1 or on the substituent at position 2 of the oxazoline ring is involved in the benzoylation. The attachment of the N-benzoyl group was determined on the basis of the IR spectrum (the presence of a band due to the bound exo-imino group, $\nu(\text{N—H})_{\text{bound}}$ 3 320 cm^{-1}, and a band $\nu(\text{C=N})$ 1 650 cm^{-1}) and NMR spectrum (absence of the signal due to the proton type C_6H_5CONH—, presence of the signal due to the proton type =N—H at 10·26 p.p.m.). The structure of compound *VI* was also confirmed by reactions performed with both its enantiomers, namely, the acidic hydrolysis (sulfuric acid in acetic anhydride or hydrogen chloride in dioxane) leading in both cases to the N^1-benzoyl derivative *VII*) and methanolysis in the presence of boron trifluoride etherate leading to the dibenzoate *VIII*.

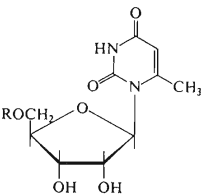
The alkali-catalysed hydrolysis of the anhydronucleoside *IIa* afforded 1-(β-D-arabinofuranosyl)-6-methyluracil (*V*) which was characterised by elemental analysis, UV spectrum and NMR spectrum.

The synthesis of the α-anomer of 6-methyl-2'-deoxyuridine (*XIIa*) was effected from 2-amino-α-D-ribofuro[1',2' : 4,5]oxazoline[2] (*IX*) by the reaction with ethyl tetrolate in dimethylformamide in the presence of triethylamine, benzoylation of the intermediary anhydro nucleoside *Xa* with benzoyl cyanide, and conversion

of the dibenzoate *Xb* into the 2'-deoxy-2'-chloro derivative *XI* of the *arabino* configuration. The low yields of compounds *Xa* and *Xb* are due to the extraordinarily low solubility of the starting compound *IX* which is to a great extent recovered unchanged from the reaction mixture and even does not afford the corresponding tribenzoate (*cf. VI*). The 2'-deoxy-2'-chloro derivative *XI* was then reduced with tri-n-butyltin hydride to 3',5'-di-O-benzoyl-6-methyl-2'-deoxy-α-uridine (*XIIb*), the methanolysis of which afforded the final 6-methyl-2'-deoxy-α-uridine (*XIIa*). The alkali-catalysed hydrolysis of the anhydro nucleoside *Xa* led to 6-methyl-α-uridine (*XIII*) (the hydrolysis of $O^{2,2'}$-anhydro nucleosides proceeds by an attack of hydroxylic ions at position 2 of the heterocyclic nucleus; the mutual orientation of the base and the hydroxylic function at position 2' must be therefore always *cis*, *cf.*[14]). For the sequence of reactions see Scheme 2.



VI, R = COC_6H_5, Y = NH
VII, R = COC_6H_5, Y = O
VIII, R = H, Y = O

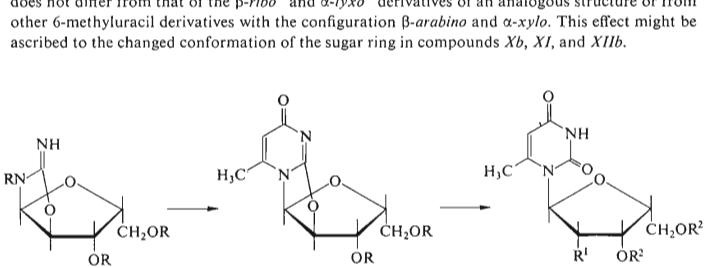


XX, R = H
XXIa, R = $-P(O)(OH)_2$

2-Amino-α-D-xylofuro[1',2' : 4,5]oxazoline[6] (*XIV*) was processed analogously. Thus, the reaction with ethyl tetrolate afforded the anhydro nucleoside *XVa* and the treatment of the dibenzoate *XVb* with hydrogen chloride in dimethylformamide led to the 2'-deoxy-2'-chloro derivative *XVI* of the D-*lyxo* configuration. The tri-n-butyltin hydride reduction of compound *XVI* and the subsequent methanolysis afforded 1-(2-deoxy-α-D-lyxofuranosyl)-6-methyluracil (*XVIIa*). In this case, there was again isolated and characterised as by-product of the first reaction step the tribenzoate *XIX* of the starting oxazoline *XIV*. As expected, the alkali-catalysed hydrolysis of the anhydro nucleoside *XVa* afforded 1-(α-D-xylofuranosyl)-6-methyluracil (*XVIII*).

All the above mentioned compounds were characterised by elemental analysis, paper chromatography, paper electrophoresis, UV spectra, and NMR spectra. The thus-obtained data were in accordance with the structure proposed. The presence (at room temperature) of a complete

double system of all signals in the NMR spectrum (Table I) of the 2'-deoxy-α-D-ribonucleoside derivative *XIIa* indicates the existence of two rotational isomers or a high energy barrier of the rotation about the nucleosidic linkage; $H_{1'}$ represents the single undoubled signal, the triplet character of which suggests the anomeric purity of the compound. The values of the particular chemical shifts and coupling constants do not almost differ from those of the derivatives of the unsubstituted uracil[4-6]. Of a special interest, however, is the high (7·5–9·0) $J_{3',4'}$ coupling constant value of compounds *Xb*, *XI*, and *XIIb* with the α-*ribo* configuration; this value considerably differs from those of all the remaining members of the present set of compounds. In the corresponding uracil derivatives with the α-*ribo* configuration[5] the $J_{3',4'}$ value is 2·5–2·8 and does not differ from that of the β-*ribo*[4] and α-*lyxo*[6] derivatives of an analogous structure or from other 6-methyluracil derivatives with the configuration β-*arabino* and α-*xylo*. This effect might be ascribed to the changed conformation of the sugar ring in compounds *Xb*, *XI*, and *XIIb*.



XIV, R = H
XIX, R' = COC_6H_5

XVa, R = H
XVb, R = COC_6H_5

XVI, R^1 = Cl, R^2 = COC_6H_5
XVIIa, R^1 = R^2 = H
XVIIb, R^1 = H, R^2 = COC_6H_5
XVIII, R^1 = OH, R^2 = H

In connection with the synthetic work, the CD spectra of the present nucleoside derivatives of 6-methyluracil have been examined (Table II). The first group of compounds (*IIa*, *Xa*, and *XVa*) includes $O^{2,2'}$-anhydro nucleosides, the spectral data of which do not qualitatively or quantitatively differ from those reported[15] in the case of $O^{2,2'}$-anhydrouridine (the negative sign of the long-wavelength Cotton effect of compounds *Xa* and *XVa* is of course due to their α-anomeric configuration). This observation is in accordance with the rigid *anti*-conformation of $O^{2,2'}$-anhydro derivatives due to the introduction of a third ring into the molecule. The second group of nucleoside derivatives, including the β-arabinoside *V*, the α-D-riboside *XIII*, and the α-D-xylofuranoside *XVIII*, is characterised by an extreme increase of molar ellipticities which is especially striking with compound *V* of the β-*arabino* configuration. In comparison with the reported[16] increased extremum values (with respect to ribosides) in CD and ORD spectra of some arabinosides, the observed data of our group of nucleoside derivatives mentioned above are extraordinarily high, obviously because of a marked restriction of the rotation about the nucleosidic linkage in these compounds where the 2'-hydroxylic function is orientated *cis* in respect to the heterocyclic base bearing a bulky substituent at position 6. In all the

TABLE I

NMR-Spectra Chemical Shifts in ppm, Coupling Constants in Hz)

Compound	$H_{1'}$ ($J_{1',2'}$; $J_{1',2''}$)	$H_{2'}$ ($J_{2',3'}$; $J_{2''3'}$)	$H_{3'}$ ($J_{4',4'}$)	$H_{4'}$ ($J_{3',5'}$; $J_{4',5''}$)	$H_{5'}$ ($J_{5',5''}$)	H_5	6-CH_3	NH	OH
IIa[a]	6·42 d (5·8)	5·13 d (<1·0)	4·37 m (1·6)	4·05 m	3·25 m	5·64 d	2·24 d (1·0)	—	5′: 4·85 t 3′: 5·77 d
XVa[a]	6·48 d (5·5)	5·12 d	4·33 d (2·5)	3·82 m (6·5; 3·3′)	3·45—3·85 m (12·5)	5·76 br s	2·28 s	—	4·55 (br s)
IIb[b]	6·64 d (5·7)	5·60—5·80 compl. m.	5·60—5·80 compl. m.	4·64 m	4·40 m	5·67 s	2·28 s	—	[c]
Xb[b]	6·46 d (5·5)	5·68 t (5·5)	5·35 q (9·0)	4·30—4·50 m (4·0; 4·0)	4·72 q 4·52 q (13·0)	5·82 br s	2·28 s	—	[c]
XVb[b]	6·62 d (5·2)	5·55 d (<1·0)	5·86 d (2·6)	4·40—4·70	compl. m.	5·76 s	2·32 s	—	[c]
III[b]	5·88 d (3·5)	5·49 dd (6·5)	5·90 t	4·35—4·80	compl. m.	5·58 s	2·27 s	9·80 br s	[c]
XI[b]	5·92 d (6·5)	5·66 t (7·0)	5·90 q (7·8)	4·97 m (3·6; 4·5)	4·70 q 4·48 q (12·5)	5·62 br s	2·30 s	9·80 br s	[c]
XVI[b]	5·97 d (6·8)	5·68 q (5·0)	6·13 q (3·5)	5·25 m (6·5; 5·5)	4·67 q 4·49 q (11·5)	5·62 br s	2·35 s	9·70 br s	[c]
IVa[a]	6·02 t (6·8; 6·8)	1·85—2·30 m	4·10—4·50	compl. m.	3·62 m	5·49 s	2·25 s	11·08 s	—

Compound	$H_{1'}$ ($J_{1',2'}$; $J_{1',2''}$)	$H_{2'}$ ($J_{2',3'}$; $J_{2''3'}$)	$H_{3'}$ ($J_{3',4'}$)	$H_{4'}$ ($J_{4',5'}$; $J_{4',5''}$)	$H_{5'}$ ($J_{5',5''}$)	H_5	6-CH_3	NH	OH
XIIa[a]	6·0 t	[g]	[g]	[g]	[g]	[g]	[g]	11·10 s	—
XVIIa[a]	6·08 t (7·0; 7·0)	2·88 pent[d] (5·7; 1·0)	4·30—4·55 m (5·0; 5·0)		3·63 m	5·38 s	2·25 s	9·40 br s	4·79 br d 4·40 br d
IVb[b]	6·15 dd (4·5; 7·7)	3·30 m[e]	5·84 m	4·49 m	4·72 m	5·57 s	2·33 s	9·55 br s	[c]
XIIb[b]	6·13 t (7·5; 7·5)	2·99 t (7·5)	5·48 t (7·5)	5·03 m (3·5; 5·0)	4·65 q 4·48 q (12·0)	5·56 br s	2·32 s	9·23 br s	[c]
XVIIb[b]	6·13 q (5·8; 7·8)	3·40 sext[f] (6·4; 2·0)	6·00 m (4·4)	5·17 q (5·6; 5·6)	4·62 m	5·56 br s	2·33 s	9·40 br s	[c]
V[a]	5·86 d (4·5)	4·59 q (4·5) (2·0)	4·05 q (6·0)	3·40—3·80 m		—	1·51 s	10·43 br s	5·38 br s 3·50 br s 3·25 br s

[a] Hexadeuterio dimethylsulfoxide, [b] deuteriochloroform, [c] aromatic protons at 7·20—8·20 ppm [d] $H_{2''}$ 2·11 q ($J_{2',2''}$ = 13·3) [e] $H_{2''}$ 2·45 m, [f] $H_{2''}$ 2·46 okt ($J_{2',2''}$ = 14·5) [g] double signal.

three cases, the sign of the Cotton effect is opposite to that of compounds unsubstituted by a methyl group as well as of the corresponding $O^{2,2'}$-anhydro nucleosides (*IIa*, *Xa*, *XVa*) and is indicative of a *syn*-conformation. It should be mentioned, however, that the extremum wavelength (λ_{II}) suggests overlapping of the bands corresponding to the two transitions which are partially resolved in the α-ribonucleoside *XIII*.

Noteworthy is also the CD spectral analysis of 2'-deoxynucleosides *IVa*, *XIIa*, and *XVIIa*. In the literature[10], the both anomers of 6-methyl-2'-deoxycytidine are reported to correspond to the empirical rules devised for the correlation of CD spectra and conformation of the nucleoside molecule, *i.e.*, the Cotton effect of the β-anomer is positive and of the α-anomer negative that is of the opposite sign when compared with anomers of 2'-deoxycytidine. On the basis of this observation, the *syn*-conformation of the both 2'-deoxycytidine anomers has been proposed. On the other hand, the 2'-deoxynucleosides of 6-methyluracil exhibit a single Cotton effect of the same sign as do the corresponding uracil derivatives[4-6]. The structure and the isomeric as well as anomeric purity of compounds *IV*, *XIIa*, and *XVIIa* is beyond any doubt (*cf.* NMR spectra) and also follows from the stereochemically unequivocal synthetic route. As suggested, however, by the presence of a shoulder at 246 nm with compound *IVa*, the Cotton effect even in this group of compounds is a result of overlapping of two bands corresponding to different transitions (*cf.* the absence of the extremum at 235 nm observed with the both anomers of 2'-deoxyuridine[4,5] and 2-deoxylyxofuranosides[6]). As suggested by some other observations (*e.g.*, NMR spectrum of compound *XIIa*), the restricted rotation about the nucleosidic linkage can be of such a character that in the solution both the rotational isomers can exist in the presence of each other. The data shown in Table II can be therefore hardly used to assign unambiguously the *syn* or *anti* conformation to the 6-methyluracil 2'-deoxynucleosides.

In an earlier paper of this series[7] a reaction has been reported leading to an anomalous opening of $O^{2,2'}$-anhydro nucleosides of the uracil series, substituted at position 3' with a benzoyloxy group in trans configuration in respect to the anhydro bond. This reaction which proceeds quantitatively by the action of boron trifluoride etherate in methanol, consists undoubtedly in participation of the 3'-benzoyl group by the S_N2 mechanism on the carbon atom at position 2' of the anhydro bond activated by a Lewis acid. The reaction of this type has not been, however, observed with the present 6-methyluracil anhydronucleoside derivatives *IIb* and *XVb* even with the use of a greater excess of the Lewis acid or when the treatment was effected for a longer period of time. This behaviour is not limited to 6-methyluracil derivatives only (the failure of the anomalous ring opening has been also observed with 3',5'-di-O-benzoyl-6-methoxycarbonyl-$O^{2,2'}$-anhydrouridine obtained by an independent route from the oxazoline *I* by the action of dimethyl acetylenedicarboxylate and the subsequent benzoylation of $O^{2,2'}$-anhydroorotidine methyl ester[18]). Thus, only a trace amount of 6-methyluridine (*XX*) has been isolated from compound *IIb*.

Neither 6-methyl-2'-deoxyuridine (*IVa*) nor the related compounds mentioned above show any bacteriostatic activity on the growth of *E. coli* B in synthetic media

TABLE II

Ultraviolet and Circular Dichroism Spectra in Water (wavelengths in nm; molar ellipticities Φ in parentheses)

Compound	Ultraviolet spectra					CD-Spectra			
	λ_{max}	$\varepsilon_{max}.10^{-3}$	λ_{min}	A_{260}^{250}	A_{260}^{280}	λ_I	λ_{II}	$\lambda_{\Phi=0}$ [a]	Φ_{200} [b]
IIa	251	10	235	1·18	0·15	215·5 (−6 300)	241·5 (+17 400)	224	− 5 000
Xa	250	10	235	1·17	0·16	216 s (+8 500)	247 (−13 600)	222·5	+15 300
XVa	250	10	235	1·15	0·14	216·5 (+6 750)	246 (−14 350)	225	+ 850
IVa	262	12	235	0·78	0·56	−[c]	261·5 (+5 700)	229	− 1 600
XIIa	262	12	236	0·81	0·57	—	261·5 (−2 200)	237·5	+ 1 000
XVIIa	262	12	235	0·80	0·55	—	262 (−4 550)	233	+ 3 650
V	263	12	241	0·80	0·57	208 (+330 000)	240 (−84 400)	228	—
XIII	262	12	242	0·80	0·57	209 (−84 000)	243·5[d] (+13 300)	252·5 258	—
XVIII	262	12	238	0·76	0·54	210·5 (−116 500)	248·5 (+14 00)	236	—
XXIa	262	11·5	235	0·78	0·55	213	258·5	227	—

[a] Crossover wavelength; [b] molar ellipticity at 200 nm; [c] 246 s (+4 500); [d] 270·5 (−7 500).

with glucose under standard conditions[19]. Notwithstanding, some nucleotide derivatives of compound *IVa* have been prepared and their enzymatic reaction *in vitro* investigated. Thus, phosphorylation or compound *IVa* with phosphorus oxychloride in triethyl phosphate[20] afforded 6-methyl-2′-deoxyuridine 5′-phosphate (*XXIa*), the CD spectrum of which does not qualitatively differ from that of the starting nucleoside *IVa*. Consequently, substitution at position 5′ does not exert any influence on the original conformation of the base. The 5′-nucleotide *XXIa* is a good substrate for the alkaline phosphatase *E. coli* (degradation to compound *IVa*) but a very bad substrate (similarly to the corresponding ribonucleotide, namely, 6-methyluridine 5′-phosphate) for the snake venom 5′-nucleotidase[21]. Since the problem of the conformation of the 2′-deoxynucleoside *IVa* cannot be solved on the basis of the CD

TABLE III
Chromatography and Electrophoresis

Compound	R_F					$E2^a$
	S1	S3	S4	S5	S7	
Uridine	0·50	0·26				1·00
$O^{2,2'}$-Anhydro-uridine	0·63					0
IIa	0·66		—	—		0
IIb			0·12	0·12		—
III			0·70	0·80	0·30	—
IVa	0·65	0·45			—	0
IVb	—				0·10	—
V	0·62					0
VI			0·80	0·96	—	—
VII			0·44	0·47		—
VIII			0·42			—
Xa	0·67					
Xb					0·17	—
XI				0·70	0·40	—
XIIa	0·60	0·40			—	
XIIb		—		0·1′	0·15	—
XIII	0·56			—	—	1·00
XVa	0·55	0·33				0·76
XVb		—		0·18		—
XVI				0·80	0·37	—
XVIIa	0·60	0·43			—	0·42
XVIIb		—			0·15	—
XVIII						
XIX			0·90	—	—	
XX	0·56					1·00
XXIa	0·24	0·31[c]				1·00[b]
XXIb	0·08	0·25[c]				1·10[b]
2′-Deoxyuridine 5′-phosphate	0·18	0·25[c]				1·00[b]
2′-Deoxythymidine 5′-phosphate	0·24	0·31[c]				1·00[b]
2′-Deoxythymidine	0·70					0
2′-Deoxyuridine	0·60	0·45				

[a] Referred to uridine; [b] referred to Up in *E*1; [c] in S2.

spectral analysis, an additional evidence should be required for the interpretation of the low affinity of 6-methyluracil nucleotides towards 5'-nucleotidase, based[21] on the assumed *syn*-conformation of the nucleoside moiety of the substrate. In the interpretation of experimental data, the sterical influence of the substituent at position 6 on the necessary interaction of the heterocyclic base with the corresponding region of the enzyme should be of at least the equal importance as the conformational factors.

The present method of preparing the 6-methyluracil (and also the 6-methylcytosine) derivatives and especially of the corresponding 2-deoxynucleosides of an anometic purity is evidently more advantageous than the known procedures. The preparation of some other 6-alkylnucleoside derivatives by this method might be limited by the difficult accessibility of the corresponding acetylenecarboxylic acids.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected. Unless stated otherwise, the solutions were taken down on a rotatory evaporator at 35°C/15 Torr and the substances were dried over phosphorus pentoxide at 0.05 Torr.

Methods

Paper chromatography (descending technique) was performed on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2); S_2, ethanol–1M ammonium acetate (5 : 2); and S_3, 1-butanol–ethanol–water (4 : 1 : 2). Paper electrophoresis was carried out by the technique of Markham and Smith[22] on paper Whatman No 3 MM at 20 V/cm for 1 h in the buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), and E_2, 0·1M triethylammonium borate (pH 7·5). For the R_F values and electrophoretical mobilities see Table III. Thin-layer chromatography was effected on ready-for-use fluorescent silica gel foils Silufol UV_{254} (produced by Kavalier Glassworks, Votice, Czechoslovakia) in the following solvent systems: S_4, chloroform–ethanol (97 : 3); S_5, chloroform–ethanol (95 : 5); S_6, chloroform–ethanol (9 : 1); and S_7, benzene–ethyl acetate (6 : 4). Preparative runs were performed on 40 × 16 × 0·5 cm layers of loose fluorescent-indicator-containing silica gel (produced by Service Laboratories of this Institute); the product was eluted with methanol. Column chromatography was performed on wide-pore silica gel according to Pitra (produced by Service Laboratories); the columns were packed with a suspension of silica gel (400 g) in chloroform. Preparative separations on DEAE-cellulose were performed on a 70 × 4 cm column of Cellex D (standard capacity, Calbiochem, U.S.A.) with a linear gradient of triethylammonium borate (pH 7·5); the course of the elution was checked on a Uvicord apparatus. Elution rate, 3 ml per min; the fractions were withdrawn in 10 min intervals. Ultraviolet absorption spectra were taken on a Specord Zeiss apparatus in aqueous solutions. CD spectra were recorded on a Jouan Dichrograph apparatus in aqueous solutions. NMR spectra (Table I) were measured on a Varian 100 apparatus in deuteriochloroform or hexadeuteriodimethyl sulfoxide (hexamethyldisilazane as internal standard).

Enzymatical assays. Compound *XXI* (2 μmol) was incubated in a 0·05M-Tris 8 buffer solution (50 μl) containing 10 μg of the alkaline bacterial phosphatase (Worthington) or snake venom *Crotalus adamanteus* 5'-nucleotidase (Worthington) at 37°C. Samples were analyzed after 3 and

24 h in the solvent system S_1. The spots of the starting compound and of the product were eluted with 0·01M-HCl and their ratio was determined by extinction measurements at 267 nm.

3′,5′-Di-O-benzoyl-6-methyl-O$^{2,2'}$-anhydrouridine (*IIb*)

A mixture of the oxazoline[4] *I* (5·2 g; 30 mmol), dimethylformamide (25 ml), ethyl tetrolate (10 ml), and triethylamine (3 ml) was stirred at room temperature for 3 days, evaporated at 40°C/0·05 Torr, and the residue coevaporated with four 20 ml portions of toluene to remove dimethylformamide. To a suspension of the final residue in acetonitrile (100 ml), there was added with stirring benzoyl cyanide (13 g; 0·1 mol) and then triethylamine (5 ml) until an exothermic reaction set in. The whole mixture was stirred for additional 30 min, evaporated under diminished pressure, the residue dissolved in chloroform (50 ml), and the solution applied to a column of silica gel. Elution with chloroform (fraction, 100 ml) afforded 3·5 g (24%) of compound *VI*, m.p. 146—147°C, undepressed on admixture with an authentic specimen. After the elution with chloroform (2 l total), the column was eluted with 95 : 5 chloroform–ethanol (3 l), and the corresponding fractions were evaporated under diminished pressure. Recrystallisation of the residue from ethanol afforded 7·4 g (55%) of compound *IIb*, m.p. 223—224°C, $[\alpha]_D^{25}$ −47·9° (*c* 1, dimethylformamide). For $C_{24}H_{20}N_2O_7$ (448·4) calculated: 64·28% C, 4·49% H, 6·25% N; found: 64·03% C, 4·46% H, 6·30% N.

6-Methyl-O$^{2,2'}$-anhydrouridine (*IIa*)

A suspension of compound *IIb* (1·5 g; 3·3 mmol) in methanol (20 ml) was adjusted under stirring to pH 9 (moistened pH-paper) by a dropwise addition of 1M methanolic sodium methoxide (0·2 ml), the mixture stirred at room temperature overnight, neutralised with dry Dowex 50 (H^+) ion exchange resin, filtered, and the filtrate evaporated under diminished pressure. The residue was dissolved in water (50 ml), the aqueous solution washed with two 20 ml portions of ether, the aqueous phase evaporated under diminished pressure, the residue coevaporated with two 25 ml portions of ethanol, and crystallised from a 9 : 1 mixture of acetonitrile and ethanol (10 ml) to afford 0·64 g (80%) of the chromatographically homogeneous (in solvent systems S_1 and S_4) compound *IIa*, m.p. 230—231°C, $[\alpha]_D^{25}$ −39·4° (*c* 1, water). For $C_{10}H_{12}N_2O_5$ (240·2) calculated: 50·00% C, 5·03% H, 11·66% N; found: 50·26% C, 5·03% H, 11·57% N.

3′,5′-Di-O-benzoyl-2′-chloro-2′-deoxy-6-methyluridine (*III*)

A solution of compound *IIb* (3·5 g; 7·8 mmol) in 3M hydrogen chloride in dimethylformamide (30 ml) was heated at 100°C under an air reflux condenser for 2 h under exclusion of atmospheric moisture (calcium chloride tube) and poured into water (500 ml). The precipitate was collected with suction, washed with water until neutral, dissolved in chloroform (50 ml), the solution dried over anhydrous magnesium sulfate, filtered, and the filtrate evaporated under diminished pressure. The crude residue was purified on 3 silica gel layers by chromatography. Bands of the products (R_F value 0·56 in the solvent system S_5) were eluted with methanol. Crystallisation from a mixture of ethanol (100 ml) and cyclohexane (100 ml) afforded 2·5 g (66%) of compound *III*, m.p. 178 to 179°C, $[\alpha]_D^{25}$ −52·6° (*c* 1, dimethylformamide). For $C_{24}H_{21}ClN_2O_7$ (484·9) calculated: 59·44% C, 4·36% H, 7·31% Cl, 5·77% N; found: 59·61% C, 4·43% H, 7·04% Cl, 5·90% N.

3′,5′-Di-O-benzoyl-2′-deoxy-6-methyluridine (*IVb*)

A mixture of compound *III* (1·8 g; 3·7 mmol), tri-n-butyltin hydride[23] (3·6 g), benzene (18 ml), and α,α′-azodiisobutyronitrile (0·05 g) was refluxed for 30 min, evaporated under diminished

pressure, the residue triturated with light petroleum (100 ml), the precipitate collected with suction, washed with light petroleum, and crystallised from ethanol to afford 1·2 g (72%) of compound *IVb*, m.p. 151–152°C, $[\alpha]_D^{25}$ −24·1° (*c* 1, dimethylformamide). For $C_{24}H_{22}N_2O_6$ (450·4) calculated: 64·00% C, 4·92% H, 5·22% B; found: 64·50% C, 5·19% H, 6·14% N.

6-Methyl-2′-deoxyuridine (*IVa*)

A solution of compound *IVb* (0·90 g; 2 mmol) in 0·1M methanolic sodium methoxide (50 ml) was kept at room temperature overnight, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin, filtered, and the filtrate evaporated under diminished pressure. The residue was dissolved in water (50 ml), the aqueous solution washed with two 20 ml portions of ether, the aqueous phase evaporated to dryness under diminished pressure, the residue coevaporated with two 20 ml portions of ethanol, and crystallised from acetonitrile (20 ml) and such an amount of ethanol to dissolve the solid. Yield, 0·45 g (93%) of the chromatographically pure (solvent systems S_1 to S_3) compound *IVa*, m.p. 168–169°C, $[\alpha]_D^{25}$ +24·7° (*c* 1, water); reported[13], +22·0°. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·28% H, 11·56% N; found: 49·76% C, 5·81% H, 11·60% N.

1-(β-D-Arabinofuranosyl)-6-methyluracil (*V*)

A solution of compound *IIa* (0·20 g; 0·83 mmol) in 1% aqueous lithium hydroxide (5 ml) was kept at room temperature overnight, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin, filtered, and the filtrate evaporated under diminished pressure. The residue was coevaporated with ethanol (20 ml), dissolved in hot ethanol (5 ml), the solution treated with ether until faintly turbid, and kept in a refrigerator overnight to deposit crystals which were collected with suction, washed with ethanol and ether, and dried. Yield, 0·15 g (70%) of the chromatographically (solvent systems S_1 to S_3) and electrophoretically (buffer solution E_2) homogeneous compound *V*, m.p. 185–186°C. For $C_{10}H_{14}N_2O_6$ (258·2) calculated: 46·51% C, 5·46% H, 10·58% N; found: 46·25% C, 5·75% H, 10·49% N.

3′,5′-Di-O-benzoyl-6-methyl-O$^{2,2'}$-anhydro-α-uridine (*Xb*)

The oxazoline[2] *IX* was reacted with ethyl tetrolate and then benzoyl cyanide analogously to the preparation of compound *IIb* (*vide supra*). The reaction mixture was evaporated under diminished pressure, the residue diluted with chloroform (100 ml), the unreacted compound *IX* collected with suction, washed with chloroform (50 ml), and dried. Recovery, 2·6 g (50%) of the starting material *IX*. The chloroform filtrate and washings were combined, concentrated to the volume of about 50 ml, and the concentrate chromatographed on a column of silica gel analogously to compound *IIb*. Elution with chloroform afforded an amorphous product which was dried under diminished pressure. Yield, 3·0 g (22%) of the chromatographically homogeneous (solvent system S_5) compound *Xb*, $[\alpha]_D^{25}$ +140·8° (*c* 1, dimethylformamide). For $C_{24}H_2\ N_2O_7$ (448·4) calculated: 64·28% C, 4·49% H, 6·25% N; found: 64·39% C, 4·43% H, 5·75% N.

6-Methyl-O$^{2,2'}$-anhydro-α-uridine (*Xa*)

Compound *Xa* was prepared from compound *Xb* (0·5 g; 1·1 mmol) analogously to compound *IIa* and purified by crystallisation from ethanol (2 ml), the ether being added until the solution was faintly turbid. Yield, 0·20 g (76%) of compound *Xa*, m.p. 252°C. For $C_{10}H_{12}N_2O_5$ (240·2) calculated: 50·00% C, 5·03% H, 11·66% N; found: 50·21% C, 5·17% H, 12·00% N.

1-(3,5-Di-O-benzoyl-2-chloro-2-deoxy-α-D-arabinofuranosyl)-6-methyluracil (*XI*)

A solution of compound *Xb* (1·0 g; 2·2 mmol) in 3M hydrogen chloride in dimethylformamide (15 ml) was heated at 100°C for 2 h, the reaction mixture poured into water (200 ml), the solid collected with suction, washed with water, and dissolved in chloroform (50 ml). The solution was dried over magnesium sulfate, filtered, the filtrate concentrated under diminished pressure, and the concentrate chromatographed on a layer of loose silica gel in the solvent system S_4. The band of the product (R_F value 0·80 in S_5) was eluted with methanol, the eluate evaporated, and the residue dried to afford 0·86 g (62%) of compound *XI* as an amorphous foam, $[\alpha]_D^{25}$ +36·6° (*c* 1, dimethylformamide). For $C_{24}H_{21}ClN_2O_7$ (484·9) calculated: 59·44% C, 4·36% H, 1·31% Cl, 5·77% N; found: 58·97% C, 4·54% H, 7·03% Cl, 6·13% N.

1-(3,5-Di-O-benzoyl-2-deoxy-α-D-ribofuranosyl)-6-methyluracil (*XIIb*)

A mixture of compound *XI* (0·52 g (1·07 mmol), tri-n-butyltin hydride[23] (1·2 g), benzene (12 ml), and α,α'-azodiisobutyronitrile (50 mg) was refluxed for 90 min (the reaction is finished after 80 min while only 30 min are required in the preparation of compound *IVb*) and evaporated under diminished pressure. The residue was chromatographed on a layer of loose silica gel in the solvent system S_7 and the product was eluted from the corresponding band with methanol. Yield, 0·33 g (70%) of the amorphous compound *XIIb*, $[\alpha]_D^{25}$ +30·9° (*c* 0·5, dimethylformamide). For $C_{24}H_{22}N_2O_7$ (450·4) calculated: 64·00% C, 4·92% H, 6·22%; found: 63·80% C, 4·89% H, 5·86% N.

1-(2-Deoxy-α-D-ribofuranosyl)-6-methyluracil (*XIIa*)

A solution of compound *XIIb* (0·275 g; 0·61 mmol) in 0·1M methanolic sodium methoxide was kept at room temperature overnight and processed analogously to the preparation of compound *IVa*. The residue was coevaporated with ethanol and finally precipitated from ethanol (2 ml) with ether. Yield, 153 mg (quantitative) of the hygroscopic amorphous compound *XIIa*. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H,11·56% N; found: 49·86% C, 5·86% H, 11·90% N.

1-(α-D-Ribofuranosyl)-6-methyluracil (*XIII*)

A solution of compound *Xa* (0·10 g; 0·41 mmol) in 5% aqueous lithium hydroxide (5 ml) was processed analogously to the preparation of compound *V*. The residue was coevaporated with ethanol and finally, precipitated from ethanol (1 ml) with ether (50 ml). The precipitate was collected by centrifugation, washed with ether, and dried to afford 62 mg (58·5%) of the chromatographically (solvent systems S_1 and S_2) and electrophoretically (buffer solution E_2) amorphous compound *XIII*. For $C_{10}H_{14}N_2O_6$ (258·2) calculated: 46·51% C, 5·46% H, 10·85% N; found: 46·04% C, 5·28% H, 11·14% N.

$O^{2,2'}$-Anhydro-1-(3,5-di-O-benzoyl-α-D-xylofuranosyl)-6-methyluracil (*XVb*)

The oxazoline[6] *XIV* was reacted with ethyl tetrolate and then benzoyl cyanide analogously to the preparation of compound *IIb*. Elution of the silica gel column with chloroform afforded 4·9 g (33%) of compound *XIX*, m.p. 197—198°C (ethanol), $[\alpha]_D^{25}$ +67·0° (*c* 0·5, dimethylformamide). For $C_{27}H_{22}N_2O_7$ (486·5) calculated: 66·65% C, 4·56% H, 5·76% N; found: 66·61% C, 4·45% H, 5·58% N.

Elution of the silica gel column with a mixture (3 l) of chloroform and ethanol (95 : 5) afforded the crude *XVb* which was rechromatographed on 3 layers of silica gel in the solvent system S_5. Bands of the product were eluted with methanol, the eluates evaporated, and the residue dried under diminished pressure to afford an amorphous foam (2·7 g; 20%) of compound *XVb*, $[\alpha]_D^{25}$ +21·9° (*c* 0·5, dimethylformamide). For $C_{24}H_{20}N_2O_7$ (448·4) calculated: 64·28% C, 4·49% H, 6·25% N; found: 63·78% C, 4·63% H, 6·69% N.

$O^{2,2'}$-Anhydro-1-(α-D-xylofuranosyl)-6-methyluracil (*XVa*)

The title compound was obtained from compound *XVb* (0·5 g; 1·11 mmol) analogously to the preparation of compound *Xa*. Yield, 0·24 g (90%) of compound *XVa*, m.p. 251–252°C. For $C_{10}H_{12}N_2O_5$ (240·2) calculated: 50·00% C, 5·03% H, 11·66% N; found: 50·47% C, 4·85% H, 11·20% N.

1-(3,5-Di-O-benzoyl-2-chloro-2-deoxy-α-D-lyxofuranosyl)-6-methyluracil (*XVI*)

A solution of compound *XVb* (1·5 g; 3·4 mmol) in 3M hydrogen chloride in dimethylformamide (18 ml) was heated under reflux at 100°C under exclusion of atmospheric moisture (calcium chloride tube) for 2 h, cooled down, and poured into water (200 ml). The solid was collected with suction, washed with water, and dissolved in chloroform (50 ml). The solution was dried over magnesium sulfate, concentrated under diminished pressure, and the concentrate chromatographed on two layers of silica gel in the solvent system S_4. Bands of the product were eluted with methanol, the eluates evaporated, and the residue dried under diminished pressure. Yield, 0·99 g (66%) of the amorphous compound *XVI*, $[\alpha]_D^{25}$ +39·5° (*c* 0·5, dimethylformamide). For $C_{24}H_{21}ClN_2O_7$ (484·9) calculated: 59·44% C, 4·36% H, 7·31% Cl, 5·77% N; found: 59·37% C, 4·49% H, 7·29% Cl, 6·08% N.

1-(3,5-Di-O-benzoyl-2-deoxy-α-D-lyxofuranosyl)-6-methyluracil (*XVIIb*)

A mixture of compound *XVI* (0·82 g; 1·7 mmol), tri-n-butyltin hydride[23] (1·6 g), benzene (16 ml), and α,α'-azodiisobutyronitrile (50 mg) was refluxed for 30 min (as shown by chromatography in the solvent system S_7, the reaction is quantitative after 20 min), evaporated, and the residue chromatographed on a layer of loose silica gel in S_7. The corresponding band was eluted with methanol, the eluate evaporated, and the residue dried under diminished pressure. Yield, 0·61 g (80%) of the chromatographically homogeneous (solvent systems S_5 and S_7) compound *XVIIb*, $[\alpha]_D^{25}$ +48·0° (*c* 0·5, dimethylformamide). For $C_{24}H_{22}N_2O_7$ (450·4) calculated: 64·00% C, 4·92% H, 6·22% N; found: 64·39% C, 4·94% H, 5·94% N.

1-(2-Deoxy-α-D-lyxofuranosyl)-6-methyluracil (*XVIIa*)

A solution of compound *XVIIb* (0·534 g; 1·18 mmol) in 0·1M methanolic sodium methoxide (5 ml) was kept at room temperature overnight, neutralised with dry Dowex 50 X 8 ion exchange resin in the H^+ cycle, filtered, and the resin washed with methanol. The filtrate and washings were combined, evaporated under diminished pressure, and the residue dissolved in water (50 ml). The aqueous solution was washed with two 20 ml portions of ether, evaporated, the residue coevaporated with two 20 ml portions of ethanol, and finally crystallised from ethanol. Yield, 0·203 g (71%) of compound *XVIIa*, chromatographically (solvent systems S_1 to S_3) and electrophoretically (buffer solution E_2) homogeneous. M.p. 151–153°C. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·56% N; found: 49·69% C, 5·86% H, 11·48% N.

1-(α-D-Xylofuranosyl)-6-methyluracil (*XVIII*)

A solution of compound *XVa* (0·20 g; 0·8 mmol) in 1% aqueous lithium hydroxide (5 ml) was kept at room temperature for 24 h, neutralised with Dowex 50 X 8 (H^+) ion exchange resin, filtered, and the resin washed with water (20 ml). The filtrate and washings were combined, evaporated under diminished pressure, the residue coevaporated with two 20 ml portions of ethanol, and finally crystallised from hot ethanol (2 ml) and ether which was added until the solution was turbid. Yield, 0·15 g (71%) of compound *XVIII*, homogeneous on chromatography in the solvent systems S_1 to S_3 and electrophoresis in the buffer solution E_2. For $C_{10}H_{14}N_2O_6$ (258·2) calculated: 46·51% C, 5·46% H, 10·85% N; found: 46·08% C, 5·38% H, 11·22% N.

Reaction of Compound *IIb* with Boron Trifluoride Etherate in Methanol (*cf.*[7])

A mixture of compound *IIb* (2·0 g; 4·5 mmol), methanol (100 ml), and boron trifluoride etherate (4 ml) was refluxed under exclusion of atmospheric moisture (calcium chloride tube) for 7 h, evaporated to dryness under diminished pressure, and the residue dissolved in chloroform (100 m). The solution was washed with saturated aqueous sodium hydrogen carbonate (three 100 ml portions) and water (100 ml), dried over magnesium sulfate, and evaporated under diminished pressure. The residue was kept in 0·1M methanolic sodium methoxide at room temperature overnight, the mixture neutralised with dry Dowex 50 X 8 ion exchange resin in the H^+ cycle, filtered, and the resin washed with methanol (50 ml). The filtrate and washings were combined and evaporated under diminished pressure to dryness. The residue was dissolved in water (100 ml), the solution washed with two 50 ml portions of ether, the aqueous phase concentrated under diminished pressure to the volume of about 50 ml, the concentrate adjusted to pH 9 by the addition of conc. aqueous ammonia, diluted with 2M triethylammonium borate pH 7·5 (2·5 ml), and applied to a 70 × 3·5 cm column of Cellex D (standard capacity) in the borate cycle. The column was eluted (rate, 3 ml per min; fractions were taken in 10 min intervals) first with water to the drop of absorption (fraction I) and then with a gradient of 0—0·2M triethylammonium borate pH 7·5 (2 l of water in the mixing chamber and 2 l of the buffer solution in the reservoir). The 0·10—0·15M peak was evaporated under diminished pressure and the residue was coevaporated with six 50 ml portions of methanol to remove the buffer substance (fraction II). The fractions were deionised on a 20 ml column of Dowex 50 X 8 (H^+) ion exchange resin and then on a 50 ml column of Amberlite IR 4B (acetate) ion exchange resin (the elution was checked by means of a Uvicord apparatus) and the eluates were evaporated to dryness under diminished pressure. Fraction I afforded 0·60 g (55·5%) of compound *IIa* identical with an authentic specimen on chromatography in the solvent systems S_1 to S_3, electrophoresis in buffer solution E_2, UV spectrum, and alkaline hydrolysis with 1% aqueous lithium hydroxide under the formation of compound *V*. Fraction II afforded 0·26 mmol (determined spectrophotometrically) *i.e.* 5·8% of 6-methyluridine (*XX*), identical with an authentic specimen in S_1—S_3, E_2 and according to UV spectra.

6-Methyl-2′-deoxyuridine 5′-Phosphate (*XXIa*)

A suspension of 6-methyl-2′-deoxyuridine (*IVa*; 103 mg; 0·425 mmol) in triethyl phosphate (1 ml) was treated dropwise at 0°C under stirring with phosphorus oxychloride (0·10 ml; 167·5 mg; 1·09 mmol) and the stirring was continued for additional 4 h at 0°C. Water (5 ml) and triethylamine (2 ml) were then added, the whole mixture stirred at room temperature for 2 h, concentrated under diminished pressure, and the concentrate applied to 2 sheets of paper Whatman No 3 MM and chromatographed in the solvent system S_1. Bands of the product were eluted with dilute (1 : 100) aqueous ammonia (25 ml), the eluates evaporated under diminished pressure, and the

content of the residue determined spectrophotometrically. Yield, 0·30 mmol (71%) of compound *XXIIa*. The residue was then dried by coevaporation with ethanol (20 ml) and purified by precipitation from methanol (3 ml) with ether (100 ml). The precipitate was collected by centrifugation, washed with ether, and dried under diminished pressure to afford 100 mg of the ammonium salt of compound *XXIIa*. $C_{10}H_{15}N_2O_8P.NH_3$ (339·2) calculated: 12·38% N, 9·15% P; found: 12·82% N, 9·02% P. The alkaline phosphatase *E. coli* degradation of compound *XXIa* (3 h) affords quantitatively compound *IVa*. On the other hand, only 5% of compound *IVa* is formed by the snake venom 5'-nucleotidase degradation after 24 h.

6-Methyl-2'-deoxyuridine 5'-Triphosphate (*XXIb*)

A suspension of the ammonium salt of compound *XXIa* (37·2 mg; 0·11 mmol) in dimethylformamide (1·5 ml) was treated with N,N'-carbonyldiimidazole (0·2 g; 1·25 mmol) and the whole was stirred at room temperature overnight. Methanol (0·1 ml) was then added, the mixture kept for additional 2 h and treated with a solution of tri-n-butylammonium pyrophosphate (0·8 mmol) in dimethylformamide (1 ml). The reaction mixture was stirred at room temperature overnight, diluted with water (10 ml), kept for 1 h, adjusted to pH 4 by the addition of Dowex 50 X 8 (H^+) ion exchange resin, and filtered. The resin was washed with water (50 ml), the filtrate and washings combined, neutralised with aqueous ammonia, and evaporated under diminished pressure. The residue was chromatographed on 2 sheets of paper Whatman No 3 MM in the solvent system S_1 for 4 days to separate the product from other components of the reaction mixture of higher R_F values. Bands of the product *XXIb* were eluted with 50 ml of water (previously adjusted to pH 8·5—9·0 by the addition of aqueous ammonia) and the eluates were concentrated at 30°C : : 15 Torr to a small volume. The concentrate (its content was determined spectrophotometrically) was freeze-dried over phosphorus pentoxide to afford 60 μmol (54·5%) of the ammonium salt of compound *XXIb*, homogeneous on chromatography in the solvent systems S_1 and S_2 and electrophoresis in the buffer solution E_1. The alkaline bacterial phosphatase degradation of compound *XXIb* affords compound *IVa* as the single product.

2-Imino-N^1,$O^{3',5'}$-tribenzoyl-β-D-arabinofuro[1',2':4,5]oxazoline (*VI*)

A mixture of compound *I* (8·7 g; 50 mmol), benzoyl cyanide (21 g; 160 mmol), and acetonitrile (70 ml) was treated dropwise under stirring with triethylamine (6 ml) until the exothermic reaction set in. The resulting solution was stirred at room temperature for 2 h and evaporated under diminished pressure. Crystallisation of the residue from ethanol afforded 10·6 g (44%) of the oxazoline *VI*, m.p. 146—147°C, $[\alpha]_D^{25}$ −113·3° (*c* 0·5, dimethylformamide). For $C_{27}H_{22}N_2O_7$ (486·5) calculated: 66·65% C, 4·56% H, 5·76% N; found: 66·82% C, 4·61% H, 5·72% N. NMR spectrum ($CDCl_3$): 4·35—4·75 (complex multiplet, 3 H), $H_{4'}$ + 2 $H_{5'}$, 5·26 (d, 1 H), $H_{2'}$ ($J_{2',1'}$ = 5·4), 5·77 (m, 1 H) $H_{3'}$, 6·16 (d, 1 H), $H_{2'}$ ($J_{1',2'}$ = 5·4), aromatic protons 7·20—7·60, 7·90—8·30. IR spectrum ($CHCl_3$): ν(C=O) 1 726 cm^{-1}, ν(C=N) 1 650 cm^{-1}, $\nu(N—H)_{bonded}$ 3 320 cm^{-1}. The L-enantiomer of compound *VI* was prepared analogously in 45% yield. M.p. 149—150°C, $[\alpha]_D^{25}$ +117·0° (*c* 1, dimethylformamide). Found: 66·55% C, 4·53% H, 5·87% N.

Reaction of Compound *VI* with Hydrogen Chloride in Dioxane

A solution of compound *VI* (1·0 g; 2·06 mmol) in a mixture of dioxane (9 ml) and concd. hydrochloric acid (1 ml) was heated at 50°C for 1 h, diluted with water (50 ml), and extracted with two 25 ml portions of chloroform. The extract was washed with two 50 ml portions of saturated aqueous sodium hydrogen carbonate and water (25 ml), dried over magnesium sulfate, con-

centrated under diminished pressure, and the concentrate chromatographed on a layer of loose silica gel in the solvent system S_4. The band of the product (in S_3, R_F 0·27; R_F of $VI = 0·90$) was eluted with methanol, the eluate evaporated, and the residue dried under diminished pressure to afford 0·50 g (50%) of the amorphous product *VII*, $[\alpha]_D^{25}$ +25·8° (*c* 1, dimethylformamide). IR spectrum ($CHCl_3$): five-membered lactone ν(C=O) 1 800 cm^{-1}, the free hydroxyl absent. For $C_{27}H_{21}NO_8$ (487·5) calculated: 66·52% C, 4·34% H, 2·87% N; found: 66·80% C, 4·25% H, 3·01% N.

3′,5′-Di-O-benzoyl-β-D-arabinofuro[1′,2′:4,5]oxazolin-2(*H*)-one (*VIII*)

A mixture of compound *VI* (9·0 g; 18·5 mmol), methanol (180 ml), and boron trifluoride etherate (18 ml) was refluxed for 2 h under exclusion of atmospheric moisture (calcium chloride tube) and evaporated under diminished pressure. The residue was dissolved in chloroform (200 ml), the solution washed with three 100 ml portions of saturated aqueous sodium hydrogen carbonate and water (100 ml), dried over magnesium sulfate, and evaporated under diminished pressure. Crystallisation of the residue from ethanol afforded 7·2 g (99%) of compound *VIII*, m.p. 149 to 150°C, $[\alpha]_D^{25}$ −14·1° (*c* 1, dimethylformamide). For $C_{20}H_{17}NO_7$ (383·4) calculated: 62·65% C, 4·47% H, 3·65% N; found: 63·01% C, 4·58% H, 3·49% N. NMR spectrum ($CDCl_3$): 4·30—4·70 complex multiplet, 3 H ($H_{4'}$ + 2 $H_{5'}$), 5·17 (d, 1 H) $H_{2'}$ ($J_{2',1'} = 5·7$), 5·62 (m, 1 H) $H_{3'}$, 5·88 (d, 1 H) $H_{1'}$ ($J_{1',2'} = 5·7$), 7·12 (br s, 1 H) NH, aromatic protons (10 H) 7·20—7·70, 7·90 to 8·15.

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