NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CXLII.*

PREPARATION OF 3-(β-D-RIBOFURANOSYL)URACIL 2',3'-CYCLIC PHOSPHATE AND RELATED COMPOUNDS AND THEIR BEHAVIOUR TOWARDS PANCREATIC RIBONUCLEASE AND RIBONUCLEASE T2

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1-(β-D-Ribofuranosyl)-4-methyl-2-pyrimidinone (*IV*) was prepared from the mercuric salt of base *I* and the halogenose *II* and methanolysis of the resulting tribenzoate *III*. Treatment of compound *IV* with phosphoric acid in the presence of trichloroacetonitrile afforded the 2',3'-cyclic phosphate *V* which represents a good substrate for both the title enzymes. The 2',3'-cyclic phosphates of 3-(β-D-ribofuranosyl)uracil (*XIa*), of its 6-methyl derivative *XIb*, and of its 5-bromo-6-methyl derivative *XIc* were obtained from the corresponding nucleosides *IX* by successive reactions with triethyl phosphite and hexachloroacetone. The derivatives *XI* represent very bad substrates for both the title enzymes. Methylation of the nucleoside *XIb* with diazomethane afforded the N^1,6-dimethyl derivative *XIII*. The 5-bromo derivative *IXc* was obtained by bromination of the nucleoside *XIb* with bromine water. The ultraviolet absorption spectra of uracil 3-ribofuranosyl derivatives and the corresponding N^1-methyl derivatives are discussed.

In an earlier paper[1] of this Series, we have reported the behaviour of uridine 2',3'-cyclic phosphate derivatives, substituted on the pyrimidine moiety, towards pancreactic ribonuclease. Our results and the earlier observations[2]) may be summarised as follows: *a*) substitution at position 5 of the pyrimidine ring does exert any qualitative influence on the pancreatic ribonuclease degradation; *b*) substitution at position 6 of the uracil ring, when resulting in a change of conformation of the nucleoside moiety, causes resistance towards enzymatic hydrolysis; *c*) substitution by a methyl group[1,2] or 2-hydroxyethyl group[3] at position $N_{(3)}$ of the pyrimidine or 6-azauracil moiety, as well as substitution by a methyl group[2] at position $C_{(3)}$ of the 2-pyridinone derivatives also causes resistance of the corresponding 2',3'-cyclic phosphates towards pancreatic ribonuclease degradation. The reason of the latter effect is obviously of the steric nature. As shown earlier[4] by our investigation on the behaviour of L-nucleotides towards pancreatic ribonuclease, the formation of a complex between the enzyme and the substrate depends on the inter-

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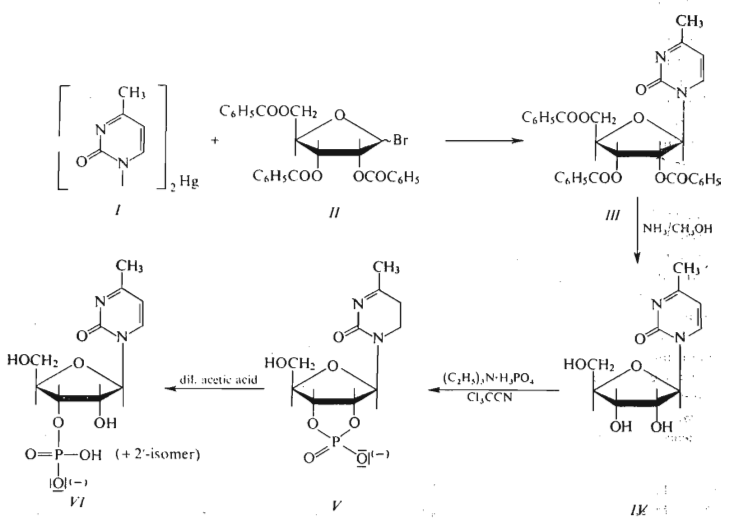
action between the pyrimidine base of the nucleotide derivative and the enzyme. Conclusively, the above mentioned findings on the influence of substituents at different positions of the pyrimidine ring indicate the critical importance of substitution at that portion of the uracil ring which contains the carbonyl function responsible for the catalysis in the hydrolytical step[2] while the substitution in the opposite portion of the uracil nucleus does not interfere in the interaction between the enzyme and the base unless this substitution is connected with changes in conformation of the nucleoside.

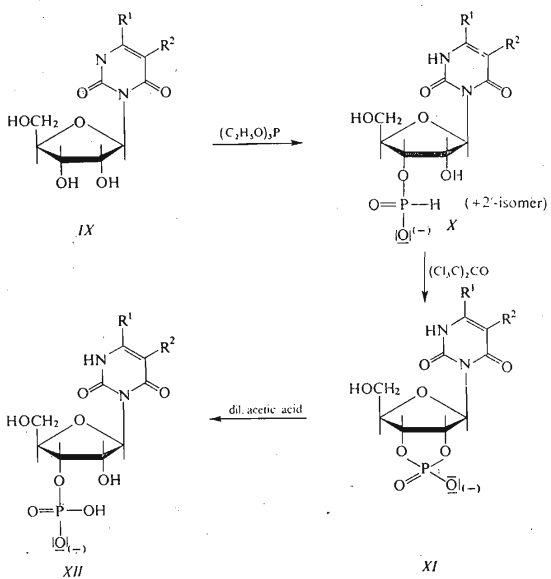
There is little known on the influence of the substituent at position $C_{(4)}$ of the pyrimidine system. Thus the pancreatic ribonuclease degradation of the 2',3'-cyclic phosphates of uridine, cytidine, N^4-dimethylcytidine, and N^4-acetylcytidine[5] was reported to afford the corresponding 3'-nucleotides. The steric requirements of the amino group, however, are low and the influence of the N-alkyl or N-acyl substituent may be partially weakened by deviation of the bulky substituent from the plane of the pyrimidine ring. For this reason, we have been interested in the preparation and properties of compounds bearing a methyl group at position $C_{(4)}$ of the pyrimidine nucleus. The simplest model is represented by 4-methyl-2-pyrimidinone derivatives.



SCHEME 1

The starting nucleoside *IV* was prepared* by reaction of 4-methyl-2-pyrimidinone mercuric salt (*I*) with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (*II*) under conditions analogous to those in the synthesis of 1-(β-D-ribofuranosyl)-2-pyrimidinone[6] and the subsequent removal of benzoyl groups from the tribenzoate *III*. In view of the low stability of the nucleoside *IV*, the 2′,3′-*cis*-diol system was phosphorylated by reaction with a salt of phosphoric acid in the presence of trichloroacetonitrile[8] (Scheme 1). The 2′,3′-cyclic phosphate *V* was identified on cleavage with 50% aqueous acetic acid to the 2′(3′)-phosphate *VI*, the bacterial phosphatase degradation of which afforded quantitatively the starting nucleoside *IV*.

Compound *V* is a good substrate for pancreatic ribonuclease (degradation to the 3′-isomer *VI*). The ribonuclease T2 degradation is also quantitative. This observa-



In formulae *IX*–*XII*: *a*, $R^1 = R^2 = H$; *b*, $R^1 = CH_3$, $R^2 = H$; *c*, $R^1 = CH_3$, $R^2 = Br$.

SCHEME 2

* When this preparation was finished, Fox and coworkers published a paper on the synthesis of compound *IV* by another route[7].

tion represents a direct proof on the low influence of a substituent at position $C_{(4)}$ on the formation of complex between the enzyme and the substrate in the case of both enzymes. Since the pyrimidine moiety of compound *V* lacks proton at position $N_{(3)}$, our observations bring a further argument against the theory of a linkage of the pyrimidine base to the enzyme by means of the $N_{(3)}$–H group[2,8,9]. The presence of a suitably orientated 2-carbonyl group on the heterocyclic moiety of compound *V* fulfils conditions required by the enzyme specificity with respect to the complex formation as well as the catalysis of the hydrolytical process.

As shown earlier[8], the pancreatic ribonuclease degradation proceeds well in the case of the 1-(β-D-ribofuranosyl)-2-pyrimidinone derivative *VII* and somewhat poorly with the isomeric derivative *VIII*. We have now extended the investigations on the substrate specificity of pancreatic ribonuclease on compounds *XI* the heterocyclic moiety of which bears the carbonyl function formally at both α-positions with respect to the nucleoside linkage. Compounds *XI* belong to the series of uracil 3-(β-D-ribofuranosyl) derivatives and are prepared[13] according to the Scheme 2 from the nucleosides *IX*, reported earlier[10–12]. (The preparation of compound *IXa* by reaction of 4-methoxy-2-pyrimidinone with the halogenose *II* in acetonitrile as solvent and in the presence of molecular sieves was accompanied by the formation of uracil and 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-2-pyrimidinone as by-products).

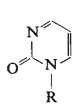
The 2′,3′-cyclic phosphates *XI* were identified by cleavage with 50% aqueous acetic acid to compounds *XII* which were dephosphorylated to the starting nucleosides *X* by the action of alkaline phosphatase *E. coli*. Unexpectedly, compounds *XI* are very poor substrates for pancreatic ribonuclease under standard conditions as well as at a high enzyme concentration. The ribonuclease T2 degradation proceeds also very sluggishly. Another derivative (*XIc*) of this type was prepared analogously from the 5-bromo derivative *IXc* (obtained in turn from compound *IXb* by the action of bromine water). Neither compound *XIc* is a good substrate for pancreatic ribonuclease.

Several factors must be taken into consideration in attempts at explanation of the above effect. Thus, the influence of the methyl group in compound *XIb* may be excluded; as shown in the case of compound *V*, the substituent at position $C_{(4)}$ does not interfere with the enzyme activity. Neither of the isomeric pyrimidinone derivatives *VII* and *VIII* exhibited a full resistance towards the enzyme action[8]. For this reason, the resistance of compounds *XI* must be due to different properties of the pyrimidine base or the whole nucleoside in derivatives of the type *IX* and *XI*. As shown in earlier papers[2,8], the carbonyl function at the α-position in respect to the nucleosidic bond clearly participates on catalysis of the hydrolytical process. This participation may be influenced by a change in conformation of the whole nucleoside (*e.g.*, by introduction of a methyl group into position $C_{(6)}$ of the uracil ring) resulting in a greater distance between the carbonyl function and the $C_{(2')}$

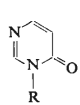
carbon atom, or by a change in the polarisibility. Consequently, the pancreatic ribonuclease degradation of the derivative[8] *VIII* and of 2-thiouracil and 2-thio-6-aza-uracil derivatives[1] proceeds much worse than that of compound *VII* and uridine derivatives. In the case of uracil 3-ribofuranosyl derivatives *IX* and *XI*, one of these effects or their combination may be involved. Conformation of systems of this type has not been, however, investigated. Ultraviolet spectra of compounds *IX* indicate a greater polarisability of carbonyl functions if compared with 1-ribofuranosyl derivatives (uridine, 6-methyluridine). The great bathochromic shift of compounds *IX* accompanying the change from acid (or neutral) media into alkaline media (*IXa*, λ_{max} 263 nm → 292 nm, *cf.*[10], and *IXb*, λ_{max} 265 nm → 294 nm, *cf.*[11]) suggests the formation of an anion with a wide stabilisation by mesomerism.

Compounds of the type *IX* could possess the structure corresponding to 6-hydroxy-2-pyrimidinone (*XIV*) derivatives. To exclude this alternative, two routes were used in the preparation of 3-(β-D-ribofuranosyl)-N[1],6-dimethyluracil (*XIII*), namely, methylation of compound *IXb* with diazomethane as well as methylation of the tribenzoate of compound *IXb* with alkali-free diazomethane followed by debenzoylation, since there is possibility of an irreversible change of structure during the deblocking process. It has been found that the methyl derivatives obtained by both routes are identical on chromatography as well as ultraviolet and nuclear magnetic resonance spectroscopy. The NMR spectrum confirms the presence of a N[1]-methyl group corresponding to the structure *XIII*. The absence of the methoxyl group was shown by analysis. Consequently, the hypothetical structure *XIV* seems highly improbable.

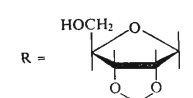
Interpretation of ultraviolet spectra of the N[1]-methyl derivative *XIII* and related compounds is rather difficult. The absorption maximum of compound *XIII* (272 nm) corresponds to that of the methyl derivative obtained from 3-(β-D-glucopyranosyl)-6-methyluracil[11], *i.e.*, it shows

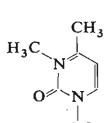


VII

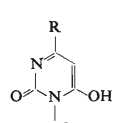


VIII

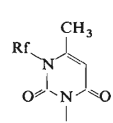




XIII



XIV



XV

In formulae *XIII*—*XV* R = H, CH_3; Rf = β-D-ribofuranosyl residue

a bathochromic shift 10 nm when compared with λ_{max} (262 nm) of compound *IXb* or N^1,N^3-di-(β-D-ribofuranosyl)-6-methyluracil (*XV*) the structure of which may be assumed as unequivocally established. A similar shift may be observed in the case of compound *IXa* (λ_{max} 263 nm; reported[10], 261 nm) and its N^1-methyl derivative (λ_{max} 272 nm; reported[10], 270 nm). The following conclusions may be drawn from spectral data of some methyl and ribofuranosyl derivatives of uracil and 6-methyluracil: *1.* in the series of N^1-substituted uracil derivatives (R^1 = methyl or β-D-ribofuranosyl), the absorption maxima are hardly dependent on an additional substitution of the molecule at positions N^3 or $C_{(6)}$ by a methyl or β-D-ribofuranosyl group[2,11,14,15,22] (when compared with the N^1-methyl derivatives, the N^1-glycosyl derivatives show a shift by 6 nm); *2.* on the other hand, in the case of uracil and 6-methyluracil derivatives substituted at position N^3 by a methyl group or by a sugar residue, the introduction of a methyl group into position N^1 results in a bathochromic shift of the absorption maximum by 6–7 nm (*cf.*[2,10–12,15]). With compounds of this type, replacement of the methyl group at position N^3 by a sugar residue leads to a bathochromic shift by 4–6 nm. The opposite direction of this effect with N^1- and N^3-substituted derivatives may be ascribed to the hydrophilic character of the sugar substituent and its interaction with the heterocyclic moiety, *e.g.*, by means of the hydrogen bonding. In the case of the more polarisable system of uracil-N^3-substituted derivatives this effect is connected with a shift on behalf of the anion type *XIV* and bathochromic shift of the maximum. With the diribofuranosyl derivative *XV*, a combination of both effects mentioned may be involved.

This explanation is in accordance with the observed resistance of derivatives *XI* towards the action of pancreatic ribonuclease which catalyses the hydrolytical process under participation of the carbonyl group at the α-position of the uracil system (this participation decreases with the increasing polarisability of the carbonyl group). A simultaneous change in conformation of the nucleoside cannot, however, be excluded. This change would interfere with the necessary interaction of the heterocyclic base with the enzyme. The resistance of compounds *XI* towards ribonuclease T2 might be explained similarly. Ribonuclease T2 is highly unspecific to the nature of the base provided that there exists an interaction with the enzyme molecule. Consequently, the lack of activity with the latter enzyme can be hardly ascribed to the changed polarisability of the carbonyl function only.

EXPERIMENTAL

Methods

Paper chromatography was performed by the descending technique on paper Whatman No 1 (preparative runs on paper Whatman No 3 MM) in the following solvent systems: S_1, 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2), and S_2, ethanol–1M sodium acetate (5 : 2).

Paper electrophoresis was performed on the same paper by the technique according to Markham and Smith[16] at 20 Volt per cm (preparative runs at 10 Volt per cm) for one hour in the buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), and E_2, 0·1M triethylammonium borate (pH 7·5). Spots were detected under ultraviolet light (Chromatolite). The phosphorus-containing compounds were detected by the reagent according to Isherwood[17]. The R_F values and electrophoretical mobilities are listed in Table I.

TABLE I

Paper Chromatography and Electrophoresis

R_F values and electrophoretic mobilities of compounds *X* are identical with those of compounds *XI*.

Compound	R_F		E_1[a]	E_2[b]
	S_1	S_2		
Uridine	0·43	0·70	0	1·00
I	0·56	0·77	0	—
IV	0·64	0·77	—	0·91
Uridine-3′-phosphate	0·12	0·30	1·00	2·10
Uridine 2′,3′-cyclic phosphate	0·36	0·68	0·65	—
V	0·49	—	0·59	—
VI	0·24	0·45	0·95	—
IXa	0·53	0·72	0	1·00
XIa	0·43	0·68	0·59	—
XIIa	0·13	0·32	0·99	—
IXb	0·60	—	0	1·00
XIb	0·47	0·70	0·47	—
XIIb	0·16	0·35	1·00	—
IXc[c]	0·65	—	0	0·95
XIc	0·50	—	0·70	—
XIIc	0·20	—	0·94	—
XIII	0·60	0·78	0	0·95

[a] Referred to uridine 3′-phosphate; [b] referred to uridine; [c] butanol saturated with water, R_F values: 0·20 (uridine), 0·41 (*IXb*), 0·54 (*IXc*).

Ultraviolet spectra were taken on a Beckman DU apparatus in 1 cm cells. One optical density unit ($1A_{260}$) is defined as that amount of the ultraviolet-absorbing material which dissolved in 1 ml of a solution causes at 260 nm the absorbancy equal to one.

Nuclear magnetic resonance spectra were measured on a Varian HA-100 spectrometer in deuteriochloroform using tetramethylsilane as internal reference.

Preparative chromatography was performed on a 100 × 4 cm column of DEAE-cellulose Cellex D (standard capacity, purchased from Calbiochem Ltd., Los Angeles, U.S.A.) with the use of a linear gradient of triethylammonium hydrogen carbonate (pH 7·5), elution rate 3 ml per min. The elution was monitored by the continuous absorption measurement on a Uvicord apparatus (LKB, Upsalla, Sweden), the fractions being collected in 10 min intervals. The corresponding fractions were pooled, evaporated at 35°C/15 Torr, the residue coevaporated under the same conditions with methanol, and the yield determined spectrophotometrically.

Enzymatic degradations were performed with 2 μmol of the substrate dissolved in 100 μl of a 0·05M-Tris buffer solution with the use of *a*) pancreatic ribonuclease (pH 8·0), 100μg (with re-

sistant compounds, 500μg), A grade (purchased from Calbiochem); *b*) ribonuclease T2 (pH 6·5), 50μg, a preparation of Professor H. Witzel, Marburg, Germany; *c*) alkaline phosphatase *E. coli* (pH 8·0), 10 μl of the enzyme suspension in ammonium sulfate (A grade, purchased from Worthington). Incubation, 4 hours at 37°C (pancreatic ribonuclease degradation of resistant substrates, at 37°C overnight). The blanks were performed similarly, but without the addition of the enzyme. The value obtained by non-enzymatic hydrolysis was then subtracted.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-4-methyl-2-pyrimidinone (*III*)

4-*Methyl-2-pyrimidinone* (I). This compound was prepared from 2-oxobutyraldehyde dimethylacetal[18] as reported[19]. Ultraviolet spectrum (pH 2): λ_{max} 305 nm, λ_{min} 236 nm; at pH 12: λ_{max} 290 nm, λ_{min} 246 nm.

Mercuric salt of compound I. A solution of mercuric chloride (5·4 g) in ethanol (120 ml) was added dropwise under stirring to another solution obtained by dissolving compound *I* (2·2 g; 20 mmol) in aqueous (30 ml) sodium hydroxide (0·8 g; 20 mmol) and the whole mixture was heated briefly to the boiling point. After cooling, the precipitate was collected with suction, washed with cold water (to remove the chloride ions), then with ethanol and ether, and dried at 0·1 Torr over phosphorus pentoxide. Yield, 6 g (87%) of the mercuric salt of compound *I*.

A suspension of finely powdered mercuric salt of compound *I* (4·2 g; 12 mmol) in toluene (250 ml) was concentrated at the ordinary pressure (about 100 ml of toluene). The concentrate was cooled to about 80°C and treated under vigorous stirring with a solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide[20] (10 mmol) in toluene (50 ml). Mercuric bromide (4·0 g) was then added and the whole suspension refluxed for 90 min. After cooling, the mixture was filtered, the filtrate evaporated to dryness at 35°C/15 Torr, the residue dissolved in chloroform (100 ml) and this solution washed successively with 40% aqueous potassium iodide, 10% aqueous sodium thiosulfate, and water (50 ml each). The organic phase was dried over magnesium sulfate, evaporated at 35°C/15 Torr, and the residue chromatographed on four 40 × 16 × 0·3 cm plates of silica gel in benzene–ethyl acetate (7 : 3). Bands of the product (R_F value, 0·12) were eluted with chloroform, the eluate concentrated, and the concentrate precipitated with light petroleum (200 ml). The precipitate was collected with suction, washed with light petroleum, and dried over phosphorus pentoxide at 0·1 Torr. Yield, 2·18 g (39%) of compound *III*. For $C_{31}H_{26}N_2O_8$ (554·5) calculated: 67·14% C, 4·72% H, 5·05% N; found: 67·38% C, 5·04% H, 5·24% N.

1-(β-D-Ribofuranosyl)-4-methyl-2-pyrimidinone (*IV*)

A solution of the tribenzoate *III* (1·67 g; 3 mmol) in 30% methanolic ammonia (50 ml) was kept at room temperature overnight and then evaporated at 35°C/15 Torr. The residue was dissolved in water (50 ml), the solution washed with three 20 ml portions of ether, and evaporated to dryness at 35°C/15 Torr. This residue was coevaporated under the same conditions with ethanol (20 ml) and then chromatographed as above on silica gel plates with the use of chloroform containing 20% of methanol as eluant. The product (R_F value, 0·43) was eluted with methanol, the eluate concentrated under diminished pressure, the concentrate precipitated with ether (100 ml), and the precipitate dried over phosphorus pentoxide at 0·1 Torr. Yield, 580 mg (80%) of compound *IV*, chromatographically (solvent systems S_1 and S_2) and electrophoretically (buffer solution E_2) homogeneous. For $C_{10}H_{14}N_2O_5$ (242·2) calculated: 49·58% C, 5·82% H, 11·56% N; found: 49·35% C, 5·82% H, 11·04% N. Optical rotation: $[\alpha]_D^{25} + 10·0°$ (*c* 0·5, water). Ultraviolet spectrum, pH 2: λ_{max} 309 nm, λ_{min} 258 nm; $A_{250/260}$ 0·92, $A_{280/260}$ 4·25; pH 7: λ_{max} 298 nm, λ_{min} 253 nm.

1-(β-D-Ribofuranosyl)-4-methyl-2-pyrimidinone 2′,3′-Cyclic Phosphate (*V*)

A solution (0·5 ml) of triethylammonium phosphate (1 mmol) in dimethylformamide, triethylamine (0·2 ml), and trichloroacetonitrile (0·375 μl) was added successively to a solution of compound *IV* (60·5 mg; 0·25 mmol) in dimethylformamide (0·5 ml), the whole mixture incubated at 40°C for 30 minutes, diluted with 40 ml of 0·4M triethylammonium hydrogen carbonate (pH 7·5), washed with three 20 ml portions of ether, and evaporated at 35°C/15 Torr. The residue was chromatographed on 2 sheets of paper Whatman No 3 MM overnight in the solvent system S_1. Bands of the product (R_F value, 0·49) were eluted with water (20 ml) and the eluate evaporated at 30°C/15 Torr. The residue was purified by preparative electrophoresis on a 16 cm wide strip of paper Whatman No 3 MM in the buffer solution E_1 under standard conditions. The product was eluted with water (the yield was determined spectrophotometrically) and the eluate freeze-dried to afford $165 A_{307}^{pH2}$ of compound *V*, which was chromatographically (solvent systems S_1 and S_2) and electrophoretically (buffer solution E_1) homogeneous. The spectral data of compound *V* correspond to those of compound *IV* (pH 2). Pancreatic ribonuclease and ribonuclease T2 degradation (standard conditions) of compound *V* affords quantitatively compound *VI* which, in turn, is converted to the nucleoside *IV* by the action of alkaline phosphatase *E. coli* (standard conditions). The nucleoside *IV* was identified by comparison with an authentic specimen in solvent systems S_1 and S_2 as well as in the buffer solution E_2.

3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)uracil (*cf.*[10])

A mixture of 4-methoxy-2-pyrimidinone[21] (1·26 g; 10 mmol), the halogenose *II* (10 mmol; *cf.*[20]), acetonitrile (100 ml), and molecular sieve Potassit 3 (10 g) was refluxed for 90 minutes under exclusion of atmospheric moisture, cooled, and filtered. The filtrate was evaporated to dryness under diminished pressure, the residue dissolved in benzene (20 ml), and the solution chromatographed on a column of silica gel (according to Pitra; 60—120 micron; 200 g) with the use of benzene containing 10% of ethyl acetate as eluant. The fractions (100 ml each) were tested chromatographically on a thin layer of silica gel in benzene containing 40% of ethyl acetate. The fractions containing the nucleoside derivatives were pooled and purified by preparative chromatography on loose silica gel layers (16 × 40 × 0·3 cm; 60—100 micron particles containing fluorescent indicator) in benzene containing 40% of ethyl acetate. The ultraviolet-absorbing bands were separated, eluted with chloroform, the eluates evaporated, and the residues dried over phosphorus pentoxide at 0·1 Torr. Yield, 750 mg (13%) of 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)uracil (from the band possessing the R_F value of 0·36) and (after crystallisation from ethanol) 1·12 g (20%) of 1-(2,3,5-tri-O-benzoyl-3-D-ribofuranosyl)-4-methoxy-2-pyrimidinone (from the band possessing the R_F value of 0·64), identical with an authentic specimen[11].

3-(β-D-Ribofuranosyl)uracil (*IXa*)

A solution of 3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)uracil (750 mg; 1·3 mmol) in 1M methanolic sodium methoxide (5 ml) was kept at room temperature overnight, neutralised with dry Dowex 50 (H^+) ion exchange resin, and filtered. The solid was washed with methanol, the filtrate and washings combined, and evaporated to dryness at 35°C/15 Torr. The residue was dissolved in water (50 ml), the aqueous solution washed with three 10 ml portions of ether, and reevaporated to dryness at 35°C/15 Torr. The residue was coevaporated with three 20 ml portions of ethanol, the residual foamy material triturated with acetone, and kept at room temperature overnight to deposit crystals which were collected with suction, washed with acetone and ether, and dried over phosphorus pentoxide at 0·1 Torr. Yield, 310 mg (97%) of compound *IXa*, chromatographically homogeneous (solvent systems S_1 and S_2). For $C_9H_{12}N_2O_6$ (244·2) calculated: 44·26% C,

4·95% H, 11·47% N; found: 44·13% C, 5·01% H, 11·32% N. Ultraviolet spectrum, pH 2: λ_{max} 263 nm, λ_{min} 232 nm, ε_{max} 7200, $A_{250/260}$ 0·70, $A_{280/260}$ 0·40; pH 12: λ_{max} 292 nm, λ_{min} 250 nm, $A_{250/260}$ 0·68, $A_{280/260}$ 3·8.

3-(β-D-Ribofuranosyl)uracil 2',3'-Cyclic Phosphate (*XIa*)

A mixture of compound *IXa* (0·35 mmol), dimethylformamide (3 ml), triethyl phosphite (1·5 ml), and 6M hydrogen chloride in dimethylformamide (0·2 ml) was allowed to stand at room temperature overnight. Triethylammonium hydrogen carbonate (0·4M, 50 ml; pH 7·5) was then added, the mixture evaporated to dryness at 40°C/15 Torr, the residue dissolved in water (10 ml), and the aqueous solution applied to a column (20 × 4 cm) of DEAE-cellulose. The unreacted nucleoside *IXa* was eluted with water. The column was then eluted under standard conditions with 0·2M triethylammonium hydrogen carbonate. The fraction which contained the 2'(3')-phosphite *Xa* ($2000A^{pH_2}_{263}$, 80%) was evaporated to dryness at 35°C/15 Torr, the residue coevaporated with three 50 ml portions of ethanol, and dried over phosphorus pentoxide at 0·1 Torr overnight. A mixture of this residue, dimethylformamide (2 ml), and hexachloroacetone (1 ml) was allowed to stand at room temperature for two days, evaporated to dryness at 40°C/0·1 Torr, the residue coevaporated under the same conditions with dimethylformamide, and finally dissolved in 25 ml of 0·4M triethylammonium hydrogen carbonate (pH 7·5). This solution was washed with two 10 ml portions of ether, evaporated to dryness under diminished pressure, and the residue applied to a column of DEAE-cellulose. The chromatography was performed as usual (linear gradient of 0—0·2M buffer solution). Fractions containing compound *XIa* were evaporated under diminished pressure to dryness and the residue chromatographed on two sheets of paper Whatman No 3 MM in the solvent system S_1. Bands of the product *XIa* were eluted with dilute (1 : 100) aqueous ammonia (50 ml) and the eluate freeze-dried. Yield, 77 μmol (22%, referred to compound *IXa*) of the ammonium salt of compound *XIa*, chromatographically (solvent systems S_1 and S_2) and electrophoretically homogeneous (buffer solution E_1). Ultraviolet spectrum of compound *XIa* at pH 2 corresponds to that of compound *IXa*. Pancreatic ribonuclease degradation of compound *XIa*: 1·2% (100μg of the enzyme); 13% (500μg). Ribonuclease T2 degradation: 4%.

Acid cleavage of compound XIa. A solution of compound *XIa* (20 μmol) in 50% aqueous acetic acid (200 μl) was heated at 50°C for five hours and chromatographed in the solvent system S_1. The band of compound *XIIa* was eluted and the eluate freeze-dried under usual conditions. Yield, 15 μmol (75%) of the ammonium salt of compound *XIIa*, homogeneous in the solvent systems S_1 and S_2 as well as the buffer solution E_1. Alkaline phosphatase *E. coli* degradation (standard conditions) afforded the starting nucleoside *IXa*, identical with an authentic specimen on chromatography (solvent systems S_1 and S_2) and ultraviolet spectra (pH 2 and 12).

3-(β-D-Ribofuranosyl)-6-methyluracil 2',3'-Cyclic Phosphate (*XIb*)

A mixture of the nucleoside *IXb* (2 mmol; *cf.*[11]), dimethylformamide (5 ml), triethyl phosphite (2·5 ml), and 6M hydrogen chloride in dimethylformamide (0·5 ml) was allowed to stand at room temperature overnight and processed analogously to preparation of compound *XIa*. The residue of compound *Xb* (1·6 mmol; 80%) was dried analogously to compound *Xa* and then dissolved in a mixture of dimethylformamide (5 ml) and hexachloroacetone (2·5 ml). This mixture was processed analogously to the preparation of compound *XIa*. Chromatography on DEAE-cellulose afforded a fraction of compound *XIb*. This fraction was purified by chromatography on 6 sheets of paper Whatman No 3 MM in the solvent system S_1. Bands of the product were eluted and the eluates freeze-dried to afford 0·96 mmol (48%, referred to compound *IXb*) of the ammonium salt of compound *XIb*, chromatographically (solvent systems S_1 and S_2) and electrophoretically

(buffer solution E_1) homogeneous. Ultraviolet spectrum, pH 2: λ_{max} 265 nm, λ_{min} 233 nm, ε_{max} 8600. It was obtained also 0·16 mmol (8%) of the ammonium salt of compound *XIIb*, identical with an authentic specimen prepared on hydrolysis of compound *XIb* (*vide infra*).

Hydrolysis of compound XIb. With pancreatic ribonuclease: 0 (100γ), 10% (500μg), with ribonuclease T2: 8%. The hydrolysis in 50% aqueous acetic acid was performed analogously to hydrolysis of compound *XIa*. The resulting compound *XIIb* affords quantitatively the nucleoside *IXb* by the action of alkaline phosphatase *E. coli*. The nucleoside *IXb* was identical with an authentic specimen on chromatography in the solvent systems S_1 and S_2.

3-(β-D-Ribofuranosyl)-5-bromo-6-methyluracil (*IXc*)

A solution of the nucleoside[11] *IXb* (5 mmol) in water (100 ml) was treated portionwise under stirring at room temperature with bromine water until the yellow colour of the reaction mixture persisted. The mixture was then evaporated to dryness at 35°C/15 Torr, the residue coevaporated with two 50 ml portions of toluene and two 50 ml portions of ethanol at 35°C/15 Torr, and then chromatographed on a silica gel plate (40 × 16 × 0·3 cm) in chloroform containing 20% of methanol. Fraction of the product (R_F value, 0·45) was eluted with methanol (100 ml), the eluate evaporated to dryness at 35°C/15 Torr and the residue recrystallised from water. Yield, 1·35 g (72%) of compound *IXc*. For $C_{10}H_{13}BrN_2O_6$ (337·2) calculated: 35·61% C, 3·88% H, 8·30% N, 23·71% Br; found: 35·76% C, 4·8% H, 7·62% N, 23·49% Br. Ultraviolet spectrum (pH 2): λ_{max} 280 nm, λ_{min} 245 nm, $A_{250/260}$ 0·50, $A_{280/260}$ 2·12; at pH 12: λ_{max} 302 nm, λ_{min} 258 nm, $A_{250/260}$ 1·95, $A_{280/260}$ 5·8.

3-(β-D-Ribofuranosyl)-5-bromo-6-methyluracil 2′,3′-Cyclic Phosphate (*XIc*)

The title compound was prepared from the nucleoside *IXc* analogously to the synthesis of compound *XIa*. Yield of the intermediary 2′(3′)-phosphite *Xc*, 36%. The oxidation of compound *Xc* with hexachloroacetone and work-up of the reaction mixture was performed again analogously to the synthesis of compound *XIa*. The product was purified by chromatography on DEAE-cellulose and on paper in the solvent system S_1. Yield, 28% of the ammonium salt of compound *XIc* (referred to compound *IXc*), homogeneous in the solvent systems S_1 and S_2 as well as in the buffer solution E_1. Ultraviolet spectrum (at pH 2 and 12) corresponds to that of the nucleoside *IXc*.

Hydrolysis. Pancreatic ribonuclease degradation: 3% (100μg), 22% (500μg). Ribonuclease T2 degradation: 5%. Acidic cleavage of compound *XIc* (for conditions see compound *XIa*) afforded 2′(3′)-phosphate *XIIc* which was quantitatively dephosphorylated with alkaline phosphatase *E. coli* (standard conditions) to the nucleoside *IXc*, identical with an authentic specimen on chromatography in the solvent systems S_1 and S_2, and according to ultraviolet spectra at pH 2.

3-(β-D-Ribofuranosyl)-N^1,6-dimethyluracil (*XIII*)

a) *On methylation of compound* IXb with diazomethane. Ethereal diazomethane was added portionwise at room temperature to a stirred solution of compound *IXb* (1 mmol; *cf.*[11]) in water (10 ml) until the yellow colour persisted. The reaction mixture was then evaporated to dryness at 35°C/15 Torr, the residue coevaporated with two 25 ml portions of ethanol under analogous conditions and finaly chromatographed on a silica gel plate (40 × 16 × 0·3 cm, *vide supra*) in chloroform containing 10% of methanol. The band corresponding to the product was eluted with methanol, the eluate evaporated under diminished pressure, and the residue dried over phosphorus pentoxide at 60°C/0·1 Torr. Yield, 80% of compound *XIII*, chromato-

graphically homogeneous in the solvent systems S_1 and S_2. For $C_{11}H_{16}N_2O_6$ (272·3) calculated: 48·51% C, 5·96% H, 10·29% N; found: 48·20% C, 6·13% H, 9·95% N, 0·15% OCH_3. Ultraviolet spectrum, pH 2: λ_{max} 272 nm.

b) *On methylation of the tribenzoate of compound* IXb with diazomethane. Freshly distilled ethereal diazomethane was added portionwise at room temperature to a stirred solution of the tribenzoate[11] of compound *IXb* (1 mmol) in dimethylformamide (10 ml) until the yellow colour persisted. The reaction mixture was evaporated to dryness at 40°C/0·1 Torr and the residue co-evaporated with three 20 ml portions of toluene to remove dimethylformamide. The final residue was purified by chromatography (*vide supra*) on one silica gel plate in chloroform containing 10% of ethanol. The ultraviolet-absorbing band of the tribenzoate of compound *XIII* was eluted with methanol, the eluate evaporated, and the residue dried over phosphorus pentoxide at 60°C/0·1 Torr. Yield, 85% of 3-(2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)-N^1,6-*dimethyluracil*. For $C_{32}H_{28}N_2O_9$ (584·6) calculated: 65·74% C, 4·82% H, 4·78% N; found: 65·02% C, 4·90% H, 4·78% N, 0·2% OCH_3. This tribenzoate (300 mg) was dissolved in methanol (10 ml) and the solution kept at room temperature overnight with 1 ml of 1M methanolic sodium methoxide. The reaction mixture was then neutralised with dry Dowex 50 (H^+) ion exchange resin, filtered, the resin washed with methanol, the filtrate and washings combined, evaporated at 35°C/15 Torr, and the residue chromatographed (*vide supra*) on one silica gel plate in chloroform containing 10% of methanol. Yield, 70% of compound *XIII*, identical with the specimen obtained by procedure *a*) as shown by thin-layer chromatography on silica gel in chloroform containing 10% of methanol, and by paper chromatography in the solvent systems S_1 and S_2.

NMR Spectra

Tribenzoate of compound IXb: δ 2·15 (s, 3, C_6—CH_3, $J_{CH_3-H_5} < 1{\cdot}0$), 4·75 (m, 3, $H_{4'}$ and 2 $H_{5'}$), 5·55 (br s, 1, H_5, $J_{H_5-CH_3} < 1{\cdot}0$), 6·05—6·20 (m, 2, $H_{2'}$ and $H_{3'}$), 6·69 (br s, 1, $H_{1'}$, $J_{1'2'} \sim 1{\cdot}0$), 7·15—7·60 (m, 9, aromatic H), 7·85—8·10 (m, 6, aromatic H), 10·59 (br, 1, N_1—H).

Tribenzoate of compound XIII: δ 2·22 (s, 3, C_6—CH_3, $J_{CH_3-H_5'} < 1{\cdot}0$), 3·37 (s, 3, N_1—CH_3), 4·70 (m, 3, $H_{4'}$ and 2 $H_{5'}$), 5·60 (br s, 1, H_5, $J_{H_5-CH_3} < 1{\cdot}0$), 6·15—6·25 (m, 2, $H_{2'}$, $H_{3'}$), 6·68 (d, 1, $H_{1'}$, $J_{1'2'} = 1{\cdot}0$), 7·20—7·60 (m, 9, aromatic H), 7·85—8.15 (m, 6, aromatic H).

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