NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLVII.*
5′-TRIPHOSPHATES OF 3′-DEOXYRIBONUCLEOSIDES

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The synthesis of four 3′-deoxyribonucleosides derived from U, C, A, and G as well as of the corresponding 3′-deoxyribonucleotides and 5′-triphosphates has been reported.

Cordycepin 5′-triphosphate (*I*) inhibits specifically the final step of RNA biosynthesis[1-3]; the mechanism of its action consists in termination of the growing polyribonucleotide chain due to the absence of the 3′-OH group. We have now prepared the 5′-triphosphates of additional 3′-deoxyribonucleosides since it is of interest if these substances, especially the pyrimidine derivatives, exhibit analogous effects.

The 3′-deoxyribonucleosides *II*–*IV* derived from the naturally occurring heterocyclic bases are known[4-10] but the methods of their preparation are not usable for our purpose. As it is advantageous to start the syntheses of all required nucleosides from a single simple parent compound, we have modified those procedures where the sugar component to be introduced possesses the 3-deoxy-*erythro*-D-pentofuranosyl (3-deoxyribofuranosyl) structure and where no additional treatments of the sugar moiety are necessary. The nucleosidation was performed with the use of the protected 3-deoxy-*erythro*-D-pentofuranosyl bromide[8-10] *VI* which was prepared from 1,2-isopropylidene-D-xylofuranose by a reported procedure[8,11]. The existing methods were modified as follows. *1*) The *p*-toluyl residue was used to protect the hydroxylic functions of the sugar moiety because of the known good crystallisation ability of *p*-toluates. *2*) In the course of the synthesis of the sugar component, both the anomeric methyl 3-deoxy-*erythro*-D-pentofuranosides were isolated. In comparison with the known[8] β-anomer, the rotation of the α-anomer (reported in the present paper) both of free (*VII*) and *p*-toluylated (*VIII*) is shifted to more positive values in accordance with our expectations. Both anomers are equally suitable for the preparation of the reactive halogenose. *3*) Bis(trimethylsilyl)cytosine (*IX*), bis(trimethylsilyl)-N-benzoyladenine (*X*) and tris(trimethylsilyl)-N-acetylguanine (*XI*) were used as heterocyclic components of the nucleosidation since the reaction with these silyl

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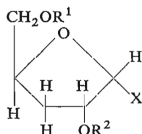
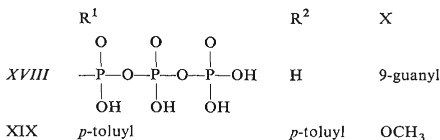
derivatives is smooth and free of by-products. The resulting protected nucleosides may be deblocked without any previous isolation. The guanine derivative mentioned was especially suitable from the standpoint of a facile preparation. Moreover, only the 9-isomer isomer is formed in the silyl process in contrast to the mercuri process which leads to a mixture of 7- and 9-isomers[10]. *4*) 3'-Deoxyuridine (*III*) was prepared by deamination of 3'-deoxycytidine (*II*) with sodium bisulfite[12,13].

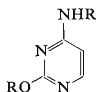
The 3'-deoxyribonucleosides *II*–*V* were phosphorylated at position 5' with phosphorus oxychloride in triethyl phosphate according to Yoshikawa and coworkers[14]. The main reaction products *XII*–*XV* were always accompanied by lesser amounts of a substance, probably a 2',5'-diphosphate as suggested by the chromatographical and electrophoretical behaviour.



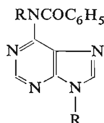
	R^1	R^2	X
I	$-P(O)(OH)-O-P(O)(OH)-O-P(O)(OH)-OH$	H	9-adenyl
II	H	H	1-cytosyl
III	H	H	1-uracilyl
IV	H	H	9-adenyl
V	H	H	9-guanyl
VI	*p*-toluyl	*p*-toluyl	Br
VII	H	H	OCH_3
VIII	*p*-toluyl	*p*-toluyl	OCH_3
XII	$PO(OH)_2$	H	1-cytosyl
XIII	$PO(OH)_2$	H	1-uracilyl
XIV	$PO(OH)_2$	H	9-adenyl
XV	$PO(OH)_2$	H	9-guanyl
XVI	$-P(O)(OH)-O-P(O)(OH)-O-P(O)(OH)-OH$	H	1-cytosyl
XVII	$-P(O)(OH)-O-P(O)(OH)-O-P(O)(OH)-OH$	H	1-uracilyl



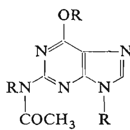
With compound *VI* the position of substituent X has not been determined, with *VII* and *VIII* it is in position α, and with all other compounds in position β.



IX



X



XI

$R = (CH_3)_3Si$

The 5'-monophosphates *XII*–*XV* were converted into the 5'-triphosphates *XVI* to *XVIII* by reaction with tri-n-butylammonium pyrophosphate according to Hoard and Ott[15]. The reaction mixture was separated by preparative paper chromatography in contrast to the original method where DEAE-cellulose was used. The modified preparation of 3'-deoxy compounds was also accompanied by the formation of numerous by-products. As indicated by chromatography and electrophoresis, these by-products may be analogous to those reported in the original paper[15]. We have isolated only the triphosphates which were purified after elution of the paper by adsorption on active charcoal. 3'-Deoxyuridine 5'-triphosphate (*XVI*) and 3'deoxyadenosine 5'-triphosphate (*I*) were not obtained entirely free of the starting monophosphates. The results of biological assays with the 5'-triphosphates mentioned above will be reported elsewhere.

EXPERIMENTAL

Methyl 3-Deoxy-2,5-di-O-*p*-toluyl-*erythro*-β-D-pentofuranoside (*XIX*)

A solution of methyl 3-deoxy-*erythro*-β-D-pentofuranoside[8,11] (15·8 g; 0·1 mol) in pyridine (200 ml) was treated dropwise under ice-cooling and stirring with *p*-toluyl chloride (34 g; 0·22 mol). The reaction mixture was kept at room temperature overnight, treated with methanol (5 ml), evaporated under diminished pressure, and the residue processed as usual to afford 35 g (88%) of the furanoside *XIX*, m.p. 85–87°C (methanol), $[\alpha]_D^{25}$ −27·7° (*c* 1, chloroform). For $C_{22}H_{24}O_6$ (384·4) calculated: 68·73% C, 6·29% H; found: 68·75% C, 6·31% H.

Methyl 3-Deoxy-*erythro*-α-D-pentofuranoside (*VII*)

The methyl 2,3-anhydro-D-ribofuranosides were distilled and the fraction (7 g; 0·05 mol) containing mostly the α-anomer[11] was hydrogenated in ethanol (300 ml) over Raney nickel at 10 atm

and 80°C analogously to the β-anomer[8]. The product was *p*-toluylated without any previous purification. The free sirupous α-furanoside *VII*, $[\alpha]_D^{20}$ +14·36° (*c* 0·6, water), was obtained by removal of the protecting groups trom the crystalline α-furanoside *VIII* (*vide infra*) with the use of alkaline hydrolysis. For $C_6H_{12}O_4$ (148·2) calculated: 48·64% C, 8·16% H; found: 48·01% C, 8·13% H.

Methyl 3-Deoxy-2,5-di-O-*p*-toluyl-*erythro*-α-D-pentofuranoside (*VIII*)

The crude product of the above hydrogenation (7·0 g; 0·48 mol) was *p*-toluylated analogously to the β-anomer to afford 16·5 g (91%) of the crude sirupous *p*-toluate, a portion (1·0 g) of which was chromatographed on a column (3·5 × 55 cm) of 200 ml of partially deactivated (20% of water) silica gel in benzene, 100 ml-fractions being taken. Fractions 5 and 6 contained 0·15 g of the protected β-furanoside *XIX* (m.p. 84—86°C). Fractions 8—11 afforded 0·65 g of the α-anomer *VIII*, m.p. 79—80°C (methanol), $[\alpha]_D^{20}$ +262·7° (*c* 0·26, chloroform). For $C_{22}H_{24}O_6$ (384·4) calculated: 68·73% C, 6·29% H; found: 68·73% C, 6·29% H; found: 69·15% C, 6·34% H.

Tris(trimethylsilyl)-N-acetylguanine (*XI*)

A stirred mixture of acetic anhydride (20 ml) and N,N-dimethylacetamide (100 ml) was treated at 130°C with guanine (10 g; 66 mmol) and heated at the same temperature until homogeneous. A portion of the solvent was removed under diminished pressure and the residue cooled down to deposit 9·5 g (73%) of N-acetylguanine, m.p. 260°C (decomp.); *cf.*[16,17]. Triethylamine (7·6 g; 75 mmol) was added dropwise under stirring to a suspension of N-acetylguanine (4·8 g; 22 mmol) and trimethylchlorosilane (8·1 g; 70 mmol) in toluene (50 ml). The mixture was heated at 50°C for 60 h, filtered (to remove triethylamine hydrochloride), and the filtrate[12] evaporated under diminished pressure. The residue (9·1 g; 89%) was used in the subsequent step without any additional purification.

Preparation of 3′-Deoxyribonucleosides

The protected furanoside *VIII* or *XIX* (3·8 g; 10 mmol) was converted to the corresponding bromide *VI* according to the literature[8]. The bromide *VI* was coevaporated under diminished pressure with three 50 ml portions of toluene to remove any excess of hydrogen bromide. The residue was then dissolved in acetonitrile (30 ml) and the solution was added dropwise to a mixture containing 11 mmol of the silylated base *IX*—*XI*, mercuric acetate (0·1 g), and acetonitrile (30 ml). The whole mixture was stirred at 20°C for 12—24 h until the alkylating agent disappeared (negative reaction with *p*-nitrobenzylpyridine). The acetonitrile was evaporated under diminished pressure, the residue was dissolved in chloroform, the solution washed with water and filtered to remove the precipitate. The filtrate was dried over magnesium sulfate, evaporated, and the residue subjected to alcoholysis under catalysis of barium methoxide to remove the *p*-toluyl groups in the usual manner. For the results see Table I.

3′-Deoxyuridine (*III*)

A mixture of 3′-deoxycytidine (*II*; 0·5 g; 2·2 mmol) and 1M-$NaHSO_3$ (20 ml) was stirred at 20°C for 12 h. The excess sodium bisulfite was precipitated by the addition of 0·7M methanolic barium methoxide (30 ml). The precipitate was filtered off and the filtrate passed through a column of Dowex 50 (H^+) ion exchange resin (30 ml). The deionised effluent was evaporated under diminished pressure to afford 0·4 g (80%) of the nucleoside *III*, m.p. 174°C, $[\alpha]_D^{20}$ +22·4° (*c* 0·2, water) (*cf.*[8,12]).

TABLE I

Preparation of 3′-Deoxyribonucleosides

Starting silyl derivative	Product	Yield %	M.p.	$[\alpha]_D^{20}$ water	Note
IX (*cf.*[18])	*II*	53	222°C	+44·0° (*cf.*[9])	[a]
X (*cf.*[19])	*IV*	48	224°C	−45·0° (*cf.*[4−8])	[b]
XI (*cf.*[12])	*V*	34	300°C	−40·1° (*cf.*[10])	[c]

[a] Purified through the picrate, m.p. 205—206°C (ethanol). For $C_{15}H_{16}N_6O_{11}$ (456·3) calculated: 39·48% C, 3·54% H, 18·42% N; found: 39·54% C, 3·69% H, 18·25% N. The nucleoside was liberated with Dowex 1 (HCO_3^-) ion exchange resin. [b] Purified on a column (20 ml) of ammonium Dowex 50 ion exchange resin. The column was washed with water and eluted with 5% aqueous ammonia. [c] Purified on a column (3·5 × 55 cm) of silica gel (200 g) in n-butyl alcohol saturated with water. Ultraviolet spectrum: $\lambda_{max}^{H_2O}$ data are in accordance with those reported in the literature[10].

3′-Deoxynucleotides *XII*—*XV*

A mixture of the corresponding nucleoside *II*—*V* (2·5 mmol), phosphorus oxychloride (625 μl) and triethyl phosphate (5·2 ml) was processed according to the reported method[14]. The crude product was chromatographed on 6 sheets of Whatman paper No 3 MM in 7:1:2 2-propanol–concentrated aqueous ammonia–water for 3 days. The ultraviolet-absorbing bands were eluted with 5% aqueous ammonia and the monophosphate-containing (as shown by electrophoresis) eluate was freeze-dried. The content of the 3′-deoxynucleotide was determined by ultraviolet absorption at λ_{max}. A portion of the lyophilisate was neutralised with an equivalent amount of barium methoxide, precipitated with excess methanol, and the precipitate analysed. For the results of phosphorylation see Table II.

TABLE II

Phosphorylation of 3′-Deoxyribonucleosides

Nucleoside	Yield[a] %	Ratio[b] N/P	Product
3′-Deoxyuridine (*III*)	64	1·95	3′-dUMP (*XIII*)
3′-Deoxycytidine (*II*)	73	3·15	3′-dCMP (*XII*)
3′-Deoxyadenosine (*IV*)	72	5·2	3′-dAMP (*XIV*)
3′-Deoxyguanosine (*V*)	54	4·8	3′-dGMP (*XV*)

[a] Based on absorption at λ_{max}; [b] in the barium salt.

5′-Triphosphates of 3′-Deoxyribonucleosides (*I* and *XVI—XVIII*)

The monophosphate (0·5 mmol) from the preceding process was passed through a column of pyridinium Dowex 50 ion exchange resin (5 ml), the effluent freeze-dried, the residue converted to the tri-n-butylammonium salt, and processed according to the method of Hoard and Ott[15]. Per 0·5 mmol of the monophosphate there was used 400 mg (2·5 mmol) of carbonyldiimidazole in 5 ml of dimethylformamide, 165 μl of methanol, and 25 ml of 0·1M tri-n-butylammonium pyrophosphate in dimethylformamide. The crystalline precipitate was removed by centrifugation. The dimethylformamide was removed by repeated coevaporations with methanol under diminished pressure. The crude residue was chromatographed on four sheets of paper Whatman No 3 MM in 7:1:2 2-propanol–concentrated aqueous ammonia–water for 7 days. The analytical chromatography on paper Whatman No 1 in the same solvent system and electrophoresis in citrate buffer solution was used to determine the triphosphate band (usually the broadest one and the least distant from the start). The triphosphate band was eluted with 5% aqueous ammonia and freeze-dried. The lyophilysate was purified on a column of active charcoal, the column washed with water, and the product eluted with 5% ethanolic ammonia. The content of phosphorylated 3′-deoxynucleosides was determined by ultraviolet absorption at λ_{max}; the phosphorus determination was performed according to Lowry[20]. Results (content of phosphorylated nucleosides and the P/base ratio given): 3′-dCTP (74·5%, 3·0), 3′-dUTP (74·4%, 2·05), and 3′-dATP (100%, 2·15).

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