SYNTHESIS AND ANTIVIRAL PROPERTIES OF ANOMERIC 5-ISOPROPOXY-METHYL AND 5-(3-HYDROXYPROPOXYNETHYL)-2'-DESOXYURUDINES

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In continuation of our investigations of modified pyrimidine nucleosides and antimetabolites with potential antitumorigenic or antiviral properties [2, 4-6], we synthesized 2'-desoxyuridines containing a 3-hydroxypropoxymethyl or isopropoxymethyl substituent at the 5-position of the pyrimidine ring.

The preparation of 5-(3-hydroxypropoxymethyl)uracil (II) from 5-hydroxymethyluracil (I) and 1,3-propanediol was previously described in [3]. 5-Isopropoxymethyluracil (III) was synthesized by the reaction of I with isopropanol in the presence of hydrochloric acid. The 5-substituted uracils II and III were converted into the corresponding 2,4-bis(O-trimethylsilyl) derivatives which were glycosylated by 2-desoxy-3,5-di(O-p-toluyl)-α-D-ribofuranosyl chloride (IV) in dichloroethane without a catalyst or in acetonitrile, in the presence of $SnCl_4$. The thus formed 1-[2-desoxy-3,5-di(O-p-toluyl)-α-D-ribofuranosyl]-5-(3-hydroxypropoxymethyl)uracil (V) was isolated by column chromatography as a mixture of anomers α:β = 1:1. Attempts to separate the mixture into the individual anomers by crystallization, and also by column or preparative TLC were unsuccessful. Deacylation of compound V by sodium methylate in methanol gave the anomeric 1-(2-desoxy-D-ribofuranosyl)-5-(3-hydroxypropoxymethyl)uracils (VI), which also could not be separated by multiply repeated chromatography on plates with silica gel.

The reaction mixture obtained from uracil III and halogenose IV after treatment was crystallized from acetonitrile and 15.4% of a chromatographically homogeneous 1-[2-desoxy-3,5-di(O-p-toluyl)-β-D-ribofuranosyl]-5-isopropoxymethyluracil (VII) was obtained. From the filtrate, by column chromatography, a mixture of anomeric O-protected nucleosides VII and IX was isolated, which was deacylated with sodium methylate in methanol, and the pure α-nucleoside X was isolated by chromatography on plates. Deacylation of compound VII led to 2-desoxy-5-isopropoxymethyluridine (VIII).

The structure of the synthesized desoxynucleosides V, VI, VIII and X was confirmed by the UV, IR and ^1H NMR spectral data. The position of the 2-desoxy-D-ribose residue at the N(1) nitrogen atom of the pyrimidine ring was proved by the retention of the absorption maximum in the UV spectra of nucleosides VI, VIII, and X on transition from pH 7.0 to pH 11.0. In the ^1H NMR spectra of compounds V and VI there is a double set of signals with an integral intensities ratio of 1:1, indicating that each of these nucleosides is a mixture of α- and β-anomers. In the ^1H NMR spectra of compounds VI, VIII and X features were observed, which are characteristic for anomers of 5-substituted 2'-desoxyuridines [2]: a signal of the anomeric proton in the form of a doublet of doublets for α- (VI-α) and X and a pseudotriplet for β-anomers (VI-β, VIII), the values of the chemical shifts of protons H2'a and H2'b, and also the 0.4 ppm shift into the weak field of the H4' proton in α- (VI-α, X), compared with the β-anomers (VI-β, VIII).

The study of the antiviral properties of the synthesized desoxynucleosides showed that compound VI in a concentration of 60 μg/ml does not influence the reproduction of the smallpox vaccine (VV) and herpes simplex type 1 (HSV-1) viruses multiply infected with 0.1 MCD_{50} [maximum cytotoxic dose]. Higher concentrations of this compound were found to be toxic for the cells not infected by the virus. In a pair of anomeric 2'-desoxy-5-isopropoxymethyluridines VIII and X, the α-nucleoside X displayed activity with respect to HSV-1 and VV: in a concentration of 250 mg/ml, it inhibits the reproduction of both types of viruses by 3 log MCD_{50}. The β-nucleoside VIII in a concentration of 250 μg/ml exhibits a moderate activity with respect to VV (2 log MCD_{50}) and does not influence the reproduction of HSV-1.

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TABLE 1. ^1H NMR Spectra of Synthesized Compounds

Compound	Anomer	Chemical shift, δ, ppm									Spin-spin coupling constants, S, Hz								
		H6	H1′	H2′a	H2′b	H3′	H4′	H5′a	H5′b	other protons	1′2′a	1′2′b	2′a2′b	2′a3′	2′b3′	3′4′	4′5′a	4′5′b	Solvent
V	β	7,63	6,42	2,94	2,74	5,59	4,92	4,56	4,50	7,75—8,0, 7,20—7,25 (16H, 2Ph=α+2Ph==β), 2,40, 2,41, 2,42	8,5	5,8	...	...	...	...	...	...	$CDCl_3$
	α	7,58	6,32	3,55	2,52	5,59	4,54	4,71	4,66	2,43 (12H, 2Me-α+2Me-β), 4,23 (1H, H5-α), 4,12 (1H, H5б-α), 4,01 (2H, H5a-β+H5б-β), 3,62—3,74, 3,48—3,53, 1,68—1,78 (12H, $^3CH_2$-α+$3CH_2$-β)	6,8	1,7	...	...	...	...	...	...	
VI	β	7,91	6,18	2,00—2,19		4,17	3,80	3,61	3,56	4,12—4,08 (4H, 2H5-α+2H5-β), 3,40—3,50,	6,9	6,9	...	...	...	...	...	...	DMSO-d_6+CD_3OD
	α	7,89	6,13	2,58	1,92	4,25	4,25	3,41	3,41	1,6—1,72 (12H, $^3CH_2$-α+$^3CH_2$-β)	7,6	2,8	14,2	6,3	6,3	...	...	...	
VIII	β	8,01	6,28	2,29	2,21	4,40	3,92	3,78	3,73	4,23, 4,20 (2H, $5CH_2$), 3,71 (1H, OCH), 1,18 (6H, $2CH_3$)	6,3	6,3	13,5	3,7	7,2	6,8	3,1	3,1	CD_3OD
X	α	7,98	6,21	2,67	2,06	4,36	4,29	3,60	3,56	4,23 (2H, $5CH_2$), 3,71 (1H, OCH), 1,18 (6H, $2CH_3$)	7,5	2,4	14,5	6,1	2,2	2,0	4,5	4,5	CD_3OD

EXPERIMENTAL

The ^1H NMR spectra of the synthesized compounds were recorded ion a "Bruker WH-306" spectrometer (GFR), using tetramethylsilane as internal standard. The UV spectra were obtained on a "Specord UV-VIS" spectrophotometer (GFR) with 1 cm length of the optical path, using ethanol as a solvent. The IR spectra were recorded on an "Perkin-Elmer 283" spectrophotometer USA, using KBr tablets. Silufol UV-254 ("Kavalier", CSFR) was used for the TLC. The preparative chromatography was carried out on plates (20 × 20 cm), using silica gel LSL_{254} 5-40 μm ("Chemapol", CSFR) with a layer thickness of 1 mm. The elemental analysis data corresponded to the calculated values. The antiviral activity was studied on a culture of chicken fibroblasts, infected with VV or HSV-1 by a method described in [1].

5-Isopropoxymethyluracil (III). A mixture consisting of 5 g (35.2 mmoles) of 5-hydroxymethyluracil, 130 ml of isopropanol and 2 ml of HCl was boiled with stirring for 4 h. After cooling the reaction mixture, the precipitate was separated and crystallized from an isopropanol–water (7:3) mixture. Yield, 5.73 g (93.5%). The compound was chromatographed in a chloroform–methanol (4:1) mixture and a homogeneous material was obtained. UV spectrum: λ_{max} 260 nm. ^1H NMR spectrum (d_6-DMSO), δ, ppm: 7.32 (s, 1H, H6), 4.05 (s, 2H, $-CH_2O$), 3.60 (m, 1H, OCH), 1.09 [d, 6H, $(CH_3)_2$]. $C_8H_{12}N_2O_3$.

Anomeric 2'desoxy-5-(3-hydroxypropoxymethyl)uridines (VI). A mixture consisting of 1.7 g (8.5 mmoles) of 5-(3-hydroxypropoxymethyl)-uracil II, 1.7 mg of $(NH_4)_2SO_4$ and 10 ml of hexamethyldisilazane was boiled for 14 h. The excess of hexamethyl-disilazane was distilled off in vacuo, the residue was dissolved in 10 ml of anhydrous dichloroethane and 2.69 g (6.9 mmoles) of 2-desoxy-3,5-di(O-p-toluyl)-α-D-ribofuranosyl chloride IV in 10 ml of the same solvent was added. The reaction mixture was stirred for 4 h at 20-22°C, and then was successively washed with a saturated $NaHCO_3$ solution and water. The solvent was distilled off in vacuo and the residue (3.45 g) was chromatographed on a column with silica gel. Elution with a chloroform-methanol (20:1) mixture gave 2.76 g (58%) of anomeric 2'-desoxy-3',5'-di(O-p-toluyl)-5-(3-hydroxypropoxymethyl)uridines (V). A solution of 0.75 g of compound V in 25 ml of a 0.1 N sodium methylate in methanol was neutralized in the course of 3 h at 20-22°C with Dowex 50(H^+) to pH 7.0 according to a universal indicator. The resin was separated and the filtrate was evaporated to dryness. The residue (0.39 g) was chromatographed on plates with silica gel in an ethyl acetate–methanol (10:1) system, passing the solvent three times through the plates. Yield, 0.27 g (61.4%) of anomeric 2'-desoxy-5-(3-hydropropoxymethyl)-uridines VI, mp 129-130°C, $C_{13}H_{20}N_2O_7 \cdot 0.5\ H_2O$. UV spectrum: λ_{max} 265 nm, ε 9600. IR spectrum (ν, cm^{-1}): 3400, 3040, 1680, 1650.

Anomeric 5-isopropoxymethyl-2-desoxyuridines (VIII) and (X). A mixture consisting of 3 g (16.3 mmoles) of 5-isopropoxymethyluracil III, 1.5 mg of ammonium sulfate, and 5-isopropoxymethyluracil III, 1.5 mg of ammonium sulfate, and 50 ml of hexamethyldisilazane was boiled for 16 h, the excess of the silylating agent was distilled off in vacuo. The residue was dissolved in 20 ml of acetonitrile, and 5.68 g (14.7 mmoles) of halogenose IV and 0.5 ml of $SnCl_4$ in 20 ml of acetonitrile were added. The reaction mixture was stirred for 4 h, the solvent was distilled off, and the residue was dissolved in chloroform, and washed successively with a saturated solution of $NaHCO_3$ and water. The solvent was distilled off under vacuum, and from the residue, by crystallization in acetonitrile, 1.21 g (15.4%) of chromatographically homogeneous 1-[2-desoxy-3,5-di(O-p-toluyl)-β-D-ribofuranosyl]-5-isopropoxymethyluracil (VII) was isolated. The filtrate was evaporated to dryness and the residue was deposited on a column of silica gel. The carbohydrate impurities were eluted with a benzene–ethyl acetate (5:1) mixture, and 2.5 g (31.7%) of a mixture of the acylated nucleosides VII and IX was eluted by a benzene–ethyl acetate (7:3) mixture. A solution of 1.21 g (2.26 mmoles) of β-nucleoside VII in 40 ml of 0.1 N sodium methylate in methanol was allowed to stand for 4 h at 20-22°C. The reaction mixture was neutralized with Dowex 50(H^+) to pH 7.0 according to a universal indicator, the resin was separated, washed with methanol and the combined filtrates were evaporated in vacuo. Yield, 0.61 g (89.7%) of 1-(2-desoxy-β-D-ribofuranosyl)-5-isopropoxymethyluracil (VIII), UV spectrum: λ_{max} 266 nm, ε 9600. $C_{13}H_{20}N_2O_6 \cdot 1/2\ H_2O$.

In a similar way from, 2.5 g of a mixture of acylated nucleosides VII and IX in 70 ml of 0.1 N sodium methylate in methanol, 1.1 g (79%) of a mixture of anomeric 2'-desoxynucleosides VIII and IX was obtained, which was chromatographed on plates with silica gel in a methylene chloride–ethyl acetate (10:1) system. The zone absorbing in the UV light was divided in two. From the lower part, a fraction enriched with the α-anomer X, was isolated,

and chromatographed in the same system, passing the solvents six times through the plate. From the lower zone, 0.1 g (7.2%) of 1-(2-desoxy-α-D-ribofuranosyl)-5-isopropoxy-methyluracil (X) was isolated. UV spectrum; λ_{max} 266 nm, ε 10000. $C_{13}H_0N_2O_6 \cdot 3H_2O$.

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ANTIVIRAL ACTIVITY OF PYRIMIDIN-2-YLTHIOACETIC ACID DERIVATIVES

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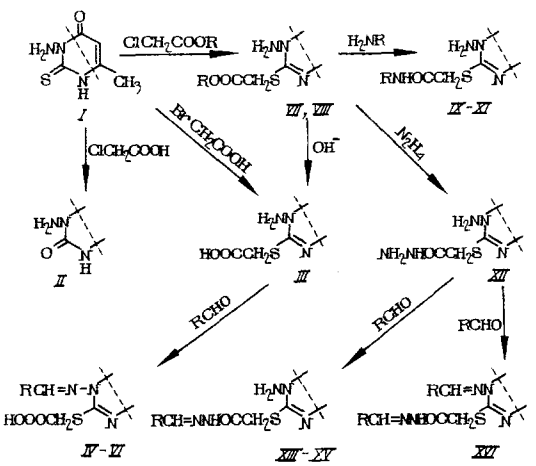
Pyrimidinylthioacetic acids have a wide spectrum of biological activity, including the antiviral activity [1, 2, 5, 6]. To study their anti-influenza activity, we prepared the derivatives of 1-amino-4-methyl-6-oxopyrimidin-2-ylthioacetic acid (III) — compounds IV-XVI (Table 1).



R=H(IX), CH_3(VII, X), C_2H_5(VIII), CH_2CH_2OH(XI), $C_6H_4NO_2$-*n*(IV, XIII), C_6H_5(V, XIV), C_6H_4OH-*o*(VI, XV, XVI).

One of the main methods of synthesis of heterylthioacetic acids is alkylation of mercapto derivatives with haloacetic acids. The starting 1-amino-4-methyl-6-oxo-2(3H)-pyrimidinethione (I) did not react with chloroacetic acid at room temperature in a 1 N KOH solution, while with increase in the temperature, the main reaction product was pyrimidinone

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