4. V. B. Prozorovskii, M. P. Prozorovskaya, and V. M. Demchenko, Farmakol. Toksikol., No. 6, 733-736 (1980).
5. Yu. M. Yutilov, A. G. Ignatenko, and L. E. Mikhailova, USSR Inventor's Certificate No. 910,635; Otkrytiya, No. 9, 86 (1982).
6. Yu. M. Yutilov, A. G. Ignatenko, and L. E. Mikhailova, USSR Inventor's Certificate No. 813,921; Otkrytiya, No. 47, 303 (1986).
7. Yu. M. Yutilov, A. G. Ignatenko, L. E. Mikhailova, and V. V. Kirichenko, USSR Inventor's Certificate No. 1,048,742; Otkrytiya, No. 47, 303 (1986).
8. United Kingdom Patent No. 999,874.
9. US Patent No. 2,706,193.
10. US Patent No. 2,511,222.
11. D. O. Holland and J. H. C. Noyler, J. Chem. Soc., 1657-1662 (1955).

SYNTHESIS AND ANTIVIRAL ACTIVITY OF 1-(β-D-ARABINOFURANOSYL)THYMINE

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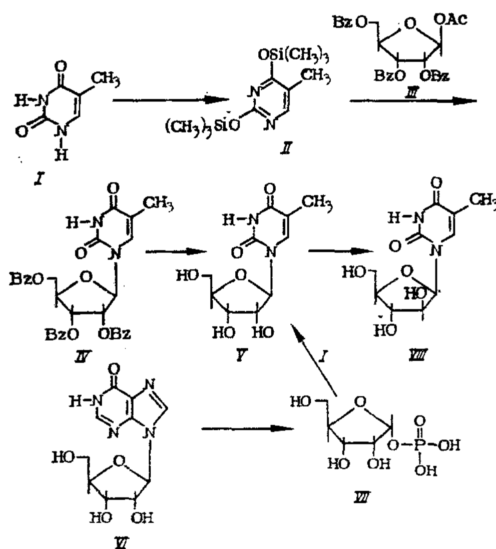
UDC 615.281.8:547.854.4].012.1

The arabinofuranosides of natural heterocyclic bases and their analogs are of considerable interest as antiviral and antitumor agents [4, 14]. Among the indicated compounds, detailed studies have been made of the biological effects and pharmacological properties of cytosine (ara-C) and adenine (ara-A) which have been employed in medicine [14]. The other representatives of this class of compounds have been studied to a much lesser degree. In our view, primary attention among those compounds is warranted by the antiherpes activity of 1-(β-D-arabinofuranosyl)thymine (ara-T, VIII) [3, 6, 8, 9, 12, 14]. The activity of ara-T is associated with its conversion to the 5'-triphosphate (ara-TTP) in which the first stage, the conversion of the nucleoside to 5'-monophosphate, is activated by the virus-induced desoxythymine kinase [5, 14]. At the same time, the conversion of ara-T to 5'-triphosphate in some cell cultures is activated by cellular enzymes that are not infected by a virus [4, 10]. One should also note that ara-TTP is a competitive inhibitor of the α and β cellular DNA-polymerases [10, 11, 14]. These data, as well as the high level of antiherpes activity exhibited by ara-T [14], would seem to warrant a detailed examination of its biological properties, in our view.



Institute of Bioorganic Chemistry, Belorussian SSR Academy of Sciences. Institute of Microbiology, Belorussian Academy of Sciences, Minsk. Moscow Scientific-Research Institute of Viral Preparations, USSR Ministry of Health. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 6, pp. 699-702, June, 1989. Original article submitted May 27, 1988.

In the present work we have described the synthesis of ara-T and studied its activity against herpes simplex 1 type viruses (HSV-1) and vaccine. The selected starting compound for the synthesis of ara-T was 1-(β-D-ribofuranosyl)thymine (ribo-T, V) which was synthesized by two methods; chemically, by the condensation of bis(trimethylsilyl)thymine (II) and 1-oxacetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (III) followed by the debenzoylation of benzoate IV [13], and microbiologically, by the transglycolization of thymine (I) and the use of inosine (VI) as the donor of the ribofuranose fragment [2].

By boiling I in hexamethyldisilasane we obtained its bistrimethylsilyl derivative II whose condensation with compound III [7] in the presence of stannic tetrachloride as the condensing agent resulted in the formation of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)thymine (IV). The use of dichloroethane as the solvent in this reaction enabled us to obtain tribenzoate IV at a 93% yield. In a previously described synthesis in which the reaction took place in acetonitrile, the yield of compound IV was 68% [12]. By treating tribenzoate IV with a solution of sodium methylate we obtained riboside V at an 80% yield.

E. coli BM-11 cells from the collection at the Institute of Microbiology of the Academy of Sciences of the BSSR were used for the microbiological transglycolization of thymine I [2]. An equilibrium phosphorolysis of inosine VI activated by purine nucleoside phosphoridase from *E. coli* cells resulted in the formation of hypoxanthine and α-D-ribofuranoso-1-phosphate (RP, VII). The latter acts as the substrate of another equilibrium reaction that is catalyzed by pyrimidine nucleoside phosphorylase which results in the formation of ribo-T (V) from compounds I and VII and is separated by chromatography on silica gel at a 45% yield.

By reacting riboside V with acetyl salicylic chloroanhydride [1] followed by treating the reaction mixture with ether, we obtained the derivative 1-(O^2, 2'-anhydro-β-D-arabinofuranosyl)thymine [1], which was treated with a 0.5 N HCl solution without separation in a water–dioxane mixture (9:1). After neutralization, dry evaporation, and crystallization of the residue we obtained ara-T VIII at a 55% yield. An additional quantity of ara-T was obtained by silica gel chromatography of the crystallized residue whose yield was brought up to 70%.

The structure of compounds IV, V, and VIII was confirmed by spectral methods and element analysis.

EXPERIMENTAL (CHEMISTRY AND MICROBIOLOGY)

UV spectra were recorded on a Specord UV-VIS spectrophotometer (GDR), PMR spectra of compounds V and VIII were recorded on a JNM-PS-100 Jeol spectrometer (Japan), and the spectra of compound IV were recorded on a WM-360 Bruker spectrometer (FRG); solvents were in deuterodimethylsulfoxide, internal standard was tetramethylsilane. TLC was performed on silica gel Silufol plates (Czechoslovakia) in hexane–ethylacetate, 7:5 (A), and chloroform–methanol, 4:1 (B) systems. Column chromatography was performed on silical gel L 40/100 μ (Czechoslovakia). BM-11 *E. coli* cells from the Institute of Microbiology, Academy of Sciences of the BSSR, were preliminarily grown by submersion cultivation at 37°C in 1-liter Ehrlenmeyer flasks on a rotary rocker (180 rpm). Each flask contained 125 ml of beef extract with 0.5% yeast extract. The biomass was collected by centrifuging at 4500g for 10 min.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)thymine (IV). A suspension of 5 g (39 mmole) of thymine (IV) in 50 ml of hexamethyldisilasane was boiled in the presence of 50 mg of ammonium sulfate for 2 h. The solution was evaporated and the residue was dissolved in 150 ml of dichloroethane to which 25.5 g (50 mmole) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (III) was added. A 22.3-g portion (86 mmole) of $SnCl_4$ in 50 ml of dichloroethane was added to the mixture with stirring over a period of 30 min. The mixture was kept for 2 days at 20°C after which 300 ml of chloroform was added. The mixture was extracted with a saturated solution of sodium bicarbonate (2 × 150 ml). The organic layer was separated, dried with anhydrous Na_2SO_4, and evaporated. The residue was crystallized from alcohol to yield 14 g of the tribenzoate IV. The residue from the crystallization was chromatographed on a silica gel column (700 cm³) and eluted with a 1:1 hexane–ethylacetate mixture to yield an additional 7 g of the compound for a total yield of 21 g (93%), mp 167-168°C; UV spectrum, ν_{max}, nm ($\varepsilon \cdot 10^{-3}$) ($CH_3OH$): 231 (39.6), 265 (12.4). R_f 0.45 (A). PMR spectrum, δ, ppm: 8.08-7.38 (15H, aromatic), 7.69 s (1H, H-6), 6.19 d (1H, H-1', $J_{1',2'}$, 4.2 Hz), 6.00 m (2H, H-2', 3'), 4.81 m (2H, H-4', 5'), 4.71 d.d (1H, H-5", $J_{5'',4''}$ 6.0 Hz, $J_{5'',5'}$ 12.6 Hz), 1.73 s (3H, CH_3), $C_{31}H_{26}N_2O_9$.

TABLE 1. Antiviral Activity of ara-T against HSV-1 and Vaccine Virus in CEF Cultures

Concentration of ara-T, μm/ml	Test virus titer, CPD_{50}		Reduction in virus yield, log CPD_{50}	
	herpes	vaccine	herpes	vaccine
2000	$10^{0,5}$	10^3	5,5	3
1500	$10^{0,5}$	10^4	5,5	2
1000	$10^{0,5}$	10^4	5,5	2
500	10	10^5	5,0	1
250	$10^{2,5}$	10^6	3,5	0
125	10^3	10^6	3,0	0
62	10^4	10^6	2,0	0
3_1	10^5	10^6	1,0	0
1_5	10^6	10^6	0	0

1-(β-D-Ribofuranosyl)thymine (V). A. A 3.9 g (6.8 mmole) portion of tribenzoate IV in 100 ml of a 1 N solution of sodium methylate was dissolved in methanol and kept for 3 h at 20°C. The solution was neutralized by the addition of a Dowex 50 × 2 ion exchange resin in a H^+ form. The resin was filtered off and washed on a 100-ml alcohol filter. The combined solutions were evaporated to dryness and the residue was crystallized from the alcohol. Yield was 1.5 g (80%) of compound V, mp 182-183°C; UV spectrum, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$) ($H_2O$, pH 7.0): 268 (8.8). R_f 0.55 (B). PMR spectrum, δ, ppm: 7.84 s (1H, H-6), 5.81 d (1H, H-1', $J_{1',2''}$ 5.0 Hz), 4.02 m (2H, H-2', 3'), 3.83 m (1H, H-4), 3.60 m (2H, H-5', 5''), 1.77 s (3H, CH_3). $C_{10}H_{14}N_2O_6$.

B. A 1-liter quantity of the reaction mixture containing 7.5 g (48 mmole) of I, 80 g (240 mmole) of VI, and 10 g of BM-11 E. coli cells (calculated per dry weight) was cultivated in 0.015 M potassium phosphate buffer (pH 7.25) with stirring for 2 h at 60°C. The cells were removed from the reaction medium by centrifuging and the packing liquid was kept for 18 h at 4°C. The resultant hypoxanthine precipitate was filtered off. A 100-cm^3 portion of silica gel was added to the filtrate and the suspension was evaporated to dryness. The silica gel was placed on a silica gel column (900 cm^3) and eluted with a chloroform—methanol mixture in a methanol concentration gradient of from 10 to 25% (volumes). The fractions containing compound V were combined and evaporated. The residue was crystallized from alcohol. Yield was 5.57 g (45%) of compound V, which was identical to the sample synthesized by chemical means.

1-(β-D-Arabinofuranosyl)thymine (VIII). A mixture of 2 g (7.7 mmole) of V and 4 g (25.6 mmole) of acetylsalicylic chloroanhydride in 15 ml of dry nitromethane was stirred for 15 h at 20°C. The solution was added upon stirring to 700 ml of ether, and the resultant precipitate was filtered off, washed on an ether filter (50 ml), and dissolved in 50 ml of a 0.5 N solution of HCl in a 9:1 water—dioxane mixture and kept on a boiling water bath for 2.5 h. The reaction was controlled by TLC. The solution was cooled to room temperature, neutralized with a Dowex 1 × 8 ion-exchange resin in a OH^- form, and the resin was filtered off and washed with 80% aqueous methanol (100 ml). The combined filtrates were evaporated to dryness and the residue was crystallized from alcohol. Yield was 1.1 g of compound VIII. The mother liquor was chromatographed after crystallization on a silica gel (100 cm^3) column. The column was eluted with a chloroform—methanol mixture in a methanol concentration gradient of from 2 to 25% (volumes). An additional 0.3 g of compound VIII was obtained, bringing the total yield to 1.4 g (70%); mp 246-247°C; UV spectrum, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$) ($H_2O$, pH 7.0): 268 (10.3). R_f 0.73 (B). PMR spectrum, δ, ppm: 7.5 s (1H, H-6), 5.94 d (1H, H-1', $J_{1'',2'}$ 4.3 Hz), 3.96 m (2H, H-2', 3'), 3.63 m (3H, H-4', 5', 5''), 1.77 s (3H, CH_3). $C_{10}H_{14}N_2O_6$.

EXPERIMENTAL (BIOLOGY)

The antiviral activity of ara-T against vaccine virus and HSV-1 was studied in vitro in a monolayer L-68 human lung embryo cells and in a culture of chick embryo fibroblasts (CEF). The monolayer L-68 culture was inoculated 48 h after cultivation with vaccine virus (strain L-IVP) and HSV-1 (strain VR-3) at a multiple of 0.1 CPD_{50}/cell. The virus titer in all of the experiments in the control was 10^6 CPD_{50}.

At a concentration of 250 μg/ml ara-T inhibited vaccine virus reproduction in the L-68 cell culture by 3 log CPD_{50}, and HSV-1 reproduction was inhibited by 4-5 log CPD_{50} in comparison to the control.

In the CEF culture we found the 50% toxic concentration of ara-T was 2000 μg/ml.

We measured the effect of various ara-T concentrations on viral reproduction by washing monolayer cultures 1 h after inoculation with a Hanks solution and introduced the ara-T preparation at concentrations of from 15 to 2000 μg/ml. The virus titer was measured by titration in a CEF culture in both test and control samples. Cell cultures inoculated with the virus but not treated with the preparation were used as the control.

The experimental results are given in Table 1.

The ratio of the 50% toxic concentration of ara-T to the concentration at which herpes virus reproduction was suppressed by 2 log CPD_{50} yields a therapeutic index of 32 for ara-T.

LITERATURE CITED

1. A. A. Akhrem, G. V. Zaitseva, E. N. Kalinenko, et al., Bioorg. Khim., 2, 1325-1327 (1976).
2. L. A. Eroshevskaya, V. N. Barai, A. I. Zinchenko, et al., Antibiotiki, 31, 173-178 (1986).
3. L. B. Allen, H. C. Schroder, H.-G. Löbering, et al., Chemotherapy, 33, 272-277 (1987).
4. L. Carrasco and D. Vazquez, Med. Res. Rev., 4, 471-512 (1984).
5. E. DeClercq, E. Krajewska, J. Descamps, et al., Mol. Pharmacol., 13, 980-984 (1977).
6. G. A. Gentry and J. F. Aswell, Virology, 65, 294-296 (1975).
7. H. M. Kissman, C. Pidacks, and B. R. Baker, J. Am. Chem. Soc., 77, 18-25 (1955).
8. H. Machida, S. Sakata, A. Kuninaka, et al., Antimicrob. Agents. Chemother., 16, 158-163 (1979).
9. R. L. Miller, J. P. Iltis, and F. Rapp, J. Virol., 23, 679-684 (1977).
10. W. E. G. Müller, R. K. Zahn, A. Maidhof, et al., Chem.-Biol. Interact., 23, 141-150 (1978).
11. W. E. G. Müller, R. K. Zahn, and J. Arendes, Chem.-Biol. Interact., 23, 151-158 (1978).
12. C. Nakayama, H. Machida, and M. Saneyoshi, J. Carbohydr. Nucleosid. Nucleotid., 6, 295-308 (1979).
13. U. Niedballa and H. Vorbruggen, J. Org. Chem., 39, 3654-3660 (1974).
14. T. W. North and S. S. Cohen, International Encyclopedia of Pharmacology and Therapeutics. Sec. III. Viral Chemotherapy, Vol. 1, Oxford (1984), pp. 303-340.