SYNTHESIS OF O^2,2'-CYCLO-β-D-ARABINOFURANOSYL- and β-D-ARABINOFURANOSYL-5-FLUOROCYTOSINE

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Methods for the synthesis of 5-fluorocytosine by direct fluorination of cytidine tetraacetate with elementary fluorine in acetic acid and by amination of 5-fluorouridine triacetate by the action of sodium hydride and p-toluenesulfonyl chloride and subsequent ammonolysis were studied. 5-Fluorocytidine was converted to O^2,2'-cyclo-β-D-arabinofuranosyl-5-fluorocytosine by the successive action of acetylsalicylyl chloride and acetyl chloride in methanol to remove the protective groups. Removal of the protective groups by means of a methanol solution of ammonia gave β-D-arabinofuranosyl-5-fluorocytosine. The latter was also obtained by amination of β-D-arabinofuranosyl-5-fluorouracil tribenzoate.

The interest in the synthesis of O^2,2'-cyclo-β-D-arabinofuranosyl-5-fluorocytosine (AAFC) (I) and β-D-arabinofuranosyl-5-fluorocytosine (AFC) (II) and their biological properties is due to the bilateral mechanism of their biological action [1]. Compound II (AFC), which is formed as a result of hydrolytic cleavage of the O^2,2'-anhydro ring of AAFC, displays a cytostatic effect by disrupting DNA synthesis. The product of deamination of AFC, viz., β-D-arabinofuranosyl-5-fluorouracil (AFU) (IIIa), displays the biological activity that is characteristic for 5-fluorouracil and its derivatives. In the present research we studied new approaches to the synthesis of AAFC and AFC.

The direct fluorination of the tetracetyl derivative (IVb) of cytidine by the action of elementary fluorine in acetic acid at 20°C with subsequent removal of the protective groups leads to the formation of 5-fluorocytidine (IVc) in 50% yield. The yield of 5-fluorocytidine (IVc) depends to a pronounced degree on the purity of all of the reagents, particularly the gaseous fluorine. We found that column chromatography on Dowex 1 × 8 ion-exchange resin (in the AcO^- form) makes it possible to simply separate the mixture of cytidine (IVa) and its 5-fluoro derivative (IVc). Treatment of 5-fluorocytidine (IVc) successively with acetylsalicylyl chloride [2, 3] in liquid sulfur dioxide and with acetyl chloride in methanol gave AAFC (I) in 70% yield. The use of a methanol solution of ammonia instead of acetyl chloride to remove the acyl protective groups [2] and to simultaneously open the O^2,2'-anhydro ring leads to the formation of AFC (II) in 50% yield.

We also studied other methods for the synthesis of AAFC and AFC. The formation of two new reaction products — probably N^3- and O^4-monotolysulfonyl derivatives of starting nucleoside IVd — was observed [by thin-layer chromatography (TLC)] when 2',3',5'-tri-O-acetyl-5-fluorouridine (IVd) [4] in anhydrous dioxane was treated successively with sodium hydride and freshly crystallized p-toluenesulfonyl chloride in accordance with patent data [5]. Attempts to isolate them in the individual state were unsuccessful. 5-Fluorocytidine (IVc) and 5-fluorouridine (IVe) were obtained by treatment of the reaction mixture with gaseous ammonia and a methanol solution of ammonia and subsequent ion-exchange (Dowex 50 × 8 in the H^+ form) and adsorption (silica gel) chromatography. The yield of 5-fluorocytidine (IVc) based on the converted starting acetate IVd was 28.7%. A similar procedure was used to obtain AFC (II) (48%) and AFU (IIIa) from 5-fluoro-(2,3,5-tri-O-benzoyl-β-D-arabinofuranosyl)uracil (IIIb) (the degree of conversion was 65%). Compound IIIb was obtained by fluorination [4] of tri-O-benzoyl derivative IIIc of β-D-arabinofuranosyluracil (IIId) in 92% yield.

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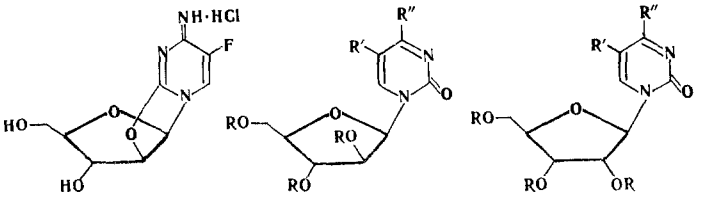

I II, III a-d IVa-e

II R=H, R′=F, R″=NH_2; III a R=H, R′=F, R″=OH; b R=Bz, R′=F, R″=OH; c R=Bz, R′=H, R″=OH; d R=R′=H, R″=OH; IV a R=R′=H, R″=NH_2; b R=Ac, R′=H, R″=NHAc; c R=H, R′=F; R″=NH_2; d R=Ac, R′=F, R″=OH; e R=H, R′=F, R″=OH

The structures of the synthesized compounds were proved by a combination of spectral methods and also by comparison with the literature data.

The propagation of cells of Erlich's ascitic carcinoma in cultures *in vitro* was inhibited 87, 100, and 100% and 80, 93, and 100%, respectively, by AAFC (I) and AFC (II) in concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M.

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EXPERIMENTAL

The UV spectra were recorded with a Specord UV-vis spectrophotometer. The PMR spectra were obtained with a Varian HA-60 spectrometer. The mass spectra were recorded with an AEI MS 902S spectrometer with direct introduction of the samples into the source at 180°C at an ionizing-electron energy of 70 eV. The melting points were determined with a Boetius microheating stage (German Democratic Republic). Silica gel L (40/100 μ) (Czechoslovakia) was used in the research.

5-Fluorocytidine (IVc). A) A solution of 2.5 g (6.09 mmole) of cytidine tetraacetate (IVb) in 200 ml of glacial acetic acid containing 7-8 mmole of fluorine was stirred at 20°C for 1 h, after which the solvent was evaporated to dryness *in vacuo*, and the residue was evaporated with glacial acetic acid (two 50-ml portions) and anhydrous ethanol (two 100-ml portions). The residue was chromatographed with a column filled with silica gel (150 cm^3) by elution with chloroform–methanol (50:1). The fractions containing starting tetraacetate IVb and its 5-fluoro derivative were evaporated, and the residues were treated with a 5% solution of sodium methoxide in methanol (25 ml). The solution was neutralized to pH ∿ 8 with Dowex 50 × 2 resin (in the H^+ form), the resin was removed by filtration, and the filtrate was evaporated. The residue was dissolved in a small volume of methanol, and the solution was applied to a 2 by 50 cm column filled with Dowex 1 × 8 resin (in the AcO^- form) that had been equilibrated in methanol, and the products were eluted with methanol. The fractions containing 5-fluorocytidine (IVc) were combined and evaporated, and the residue was crystallized from ethanol to give 0.8 g (50%) of IVc with mp 190-192°C. UV spectrum (in water), λ_{max} (log ε): 238.5 (3.92) and 282 nm (3.92); λ_{min}: 227 and 261 nm. According to the data in [6], this compound had mp 193-193.5°C (from ethanol). UV spectrum (in water), λ_{max}: 237 (3.91) and 281 nm (3.906); λ_{min}: 226 and 259 nm. Mass spectrum, m/z (relative intensity, %): M^+ 261 (7), $M^+ - H_2O$ 243 (3), $M^+ - 5'\text{-}CH_2O$ 231 (5), B (heterocyclic base) + 60.188 (6), B + 44.172 (8), $[C_6H_4FN_3O_2]^+$ 169 (18), B + 30.158 (33), B + 2H 130 (100), and B + H 129 (73).

B) A mixture of 5.7 g (14.69 mmole) of 2′,3′,5′-tri-O-acetyl-5-fluorouridine (IVd) [4] and 0.9 g (37.5 mmole) of sodium hydride in 180 ml of anhydrous dioxane was stirred at 20°C without access to moisture for 4 h, after which 14.3 g (75 mmole) of freshly crystallized p-toluenesulfonyl chloride was added, and stirring was continued for 10 h. The reaction mixture was cooled to 0°C, and gaseous ammonia was bubbled through it slowly for 2 h, during which the temperature of the mixture gradually reached 20°C. The mixture was then evaporated to dryness *in vacuo*. The residue was dissolved in 200 ml of methanol, and the solution was saturated with gaseous ammonia at 0°C and maintained at 20°C for 3 h. It was then

evaporated to dryness, and the residue was extracted with ether (three 200-ml portions). The combined extracts were evaporated to dryness *in vacuo*, and the residue was dissolved in 200 ml of methanol–water (3:1). The solution was applied to a column (2 by 50 cm) filled with Dowex 50 × 8 ion-exchange resin (H^+ form) and eluted with methanol–water (3:1) until absorption in UV light was absent. The fractions containing 5-fluorouridine were combined and evaporated *in vacuo*, and the residue was applied to a column (3 by 60 cm) filled with silica gel and eluted with chloroform–methanol (9:1) to give 2.8 g [10.69 mmole (72.7%)] of 5-fluorouridine (IVe) [4] with mp 180-181°C (from ethanol).

Subsequent elution with a 1% solution of ammonia in methanol–water (3:1) gave 0.3 g (mp 191-193°C, from ethanol) of 5-fluorocytidine (IVc) (the yield was 28.7% based on the converted starting acetate IVd).

O^2,2'-Cyclo-β-D-arabinofuranosyl-5-fluorocytosine (I). A mixture of 0.4 g (1.53 mmole) of 5-fluorocytidine (IVc) and 1.3 g (6.5 mmole) of acetylsalicylyl chloride in 25 ml of liquid sulfur dioxide was stirred without access to moisture at –10 to –20°C for 1 h. The sulfur dioxide was evaporated, and the residue was treated with ether. The crystalline residue was removed by filtration and washed with ether. The crystalline residue was then dissolved in a mixture of 80 ml of methanol with 4 ml of acetyl chloride, and the solution was maintained at 20°C for 10 h and evaporated *in vacuo*. The residue was treated with ether–acetone, and the crystalline substance was removed by filtration and crystallized from alcohol to give 0.30 g (70%) of AAFC (I) in the form of the hydrochloride with mp 265-270°C. UV spectrum (in water), λ_{max} (log ε): 230 (3.95) and 269 nm (4.05). According to the data in [3], this compound had mp 260-275°C (dec.). The data from UV and PMR spectroscopy were in good agreement with the published data [8, 9].

1-(2,3,5-Tri-O-benzoyl-β-D-arabinofuranosyl)-5-fluorouracil (IIIb). A solution of 6.7 g (12.05 mmole) of tribenzoate IIIc (mp 205-206°C) was obtained by benzoylation (BzCl/Py) of arabinofuranosyluracil, which was obtained in turn from O^2,2'-cyclo-β-D-arabinofuranosyluracil [7] in 125 ml of glacial acetic acid containing 13-14 mmole of fluorine by stirring at 20°C for 1 h. The solvent was removed by evaporation *in vacuo*, and the residue was evaporated with glacial acetic acid (two 20-ml portions) and ethanol (four 50-ml portions) and crystallized from ethanol to give 5.7 g of benzoate IIIb. The filtrate after crystallization was evaporated, and the residue was dissolved in chloroform. The solution was applied to a column (2 by 30 cm) filled with silica gel and eluted with chloroform to give an additional 0.7 g of benzoate IIIb, the overall yield of which was 92%. The product had mp 215-217°C. UV spectrum (in methanol), λ_{max} (log ε): 270 (4.13) and 233 nm (4.70). Found: C 62.5; H 4.08; N 4.7%. $C_{30}H_{23}FN_2O_9$. Calculated: C 62.72; H 4.03; N 4.88%.

1-(β-D-Arabinofuranosyl)-5-fluorocytosine (II). A) A mixture of 0.4 g (1.53 mmole) of 5-fluorocytidine (IVc) and 1.3 g (6.5 mmole) of acetylsalicylyl chloride in 20 ml of liquid sulfur dioxide was stirred at –10 to –20°C for 1 h, after which the solvent was evaporated, and the residue was treated with ether. The crystalline substance was removed by filtration, washed with ether, and dissolved in 50 ml of methanol saturated at 0°C with ammonia. The mixture was maintained at 20°C for 5-10 h, after which it was evaporated *in vacuo*. The residue was dissolved in methanol, and the solution was treated with 10 ml of Dowex 1 × 2 ion-exchange resin (OH^- form). The resin was removed by filtration and washed with water. The filtrate and wash waters were combined and concentrated to a small volume *in vacuo*. The concentrate was applied to a column (2 by 15 cm) filled with Dowex 1 × 2 resin (OH^- form) and eluted with water to give 0.2 g (50%) of AFC (II) with mp 228-231°C (from aqueous methanol). UV spectrum (in water), λ_{max} (log ε): 238 (3.95) and 282 nm (3.97). According to the data in [10], this compound had mp 230-232°C.

B) A 0.9-g (48% based on the converted IIIb) sample of AFC (II) and 1.0 g (35%) of AFU (IIIa), with mp 187-188°C (from ethanol), were obtained as described above in the case of 5-fluorocytidine (IVc) starting from 6.3 g (10.95 mmole) of benzoate IIIb, 0.7 g (29.1 mmole) of sodium hydride, and 10.5 g (55.2 mmole) of p-toluenesulfonyl chloride in 150 ml of anhydrous dioxane. UV spectrum (in water), λ_{max} (log ε): 271 nm (3.99). According to the literature, this compound had mp 187-188°C [10] and 187-189°C [11] and its UV spectrum (in water) contained a λ_{max} band at 270 nm (log ε 3.95) [11].

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