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Chemistry and Pharmacology of Apiose. 7.† Susceptibility of Some Branched-Chain Sugar Nucleosides to Enzymatic Deamination

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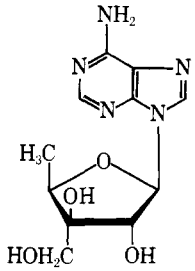
The synthesis of a new branched-chain sugar nucleoside 9-(5-deoxy-3-*C*-hydroxymethyl-β-D-xylofuranosyl)adenine (1) is described. This compound and 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenine (2) are substrates of calf adenosine deaminase whereas the 3′ epimer of 2 (3) is not deaminated. This shows that a C-3′ carbon ramification on a nucleoside does not always abolish susceptibility to enzymatic deamination and that the configuration at the branching point plays an important role in substrate specificity.

The therapeutic value of numerous adenosine analogs is limited by their facile degradation into inosine derivatives by adenosine deaminase. Hence, it is of interest to study the effect of the chemical manipulation of nucleosides on their susceptibility to deamination by this enzyme. A number of structural analogs of adenosine modified in the base or in the sugar have been studied as substrates of adenosine deaminase. In 1966, Walton, *et al.*,[2] reported that a biologically active nucleoside of a new type, containing a branched-chain sugar, 3′-*C*-methyladenosine, was not deaminated by the enzyme.

This paper describes the synthesis of a new branched-chain sugar nucleoside, the 5′-deoxy-3′-*C*-hydroxymethyl-β-D-xylofuranosyladenine (1), and the susceptibility to enzymatic deamination of this compound along with that of two other adenosine analogs we have previously described,[1,3,4] namely, 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenine (2) and 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenine (3).



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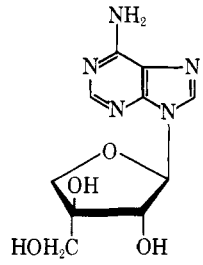

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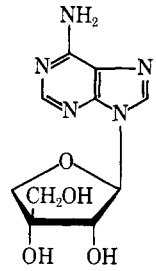

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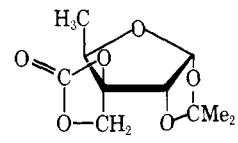
Chemistry. Treatment with phosgene of 5-deoxy-3-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose[5] gave 4 whose acetolysis in the usual manner[6] afforded a mixture of the α and β anomers of 5. Assignment of the anomeric configuration was made by nmr, mainly on the basis of the $J_{1,2}$ coupling constant (α 4.5 Hz and β <0.5 Hz). Reaction of 5 with chloromercuri-*N*-benzoyladenine in the presence[7] of $TiCl_4$ produced the acylated nucleoside 6 which was purified by tlc. The expected (trans rule[8]) β configuration of 6 was supported by nmr ($J_{1',2'}$ = 2.5 Hz). The α anomer was not formed. Removal of the acyl and carbonyl groups by transesterification in methanolic sodium methoxide yielded the branched-chain sugar nucleoside 1 which was purified by crystallization from methanol and water and also by conversion to its picrate followed by regeneration to the free nucleoside.

Enzymatic Deamination Studies. The activity of calf intestine adenosine deaminase on 1, 2, and 3 was compared with that observed with adenosine. The assays were carried out as described by Bloch, Robins, and McCarthy.[9] With 0.06 unit of enzyme per milliliter of assay mixture (1 unit being defined[10] as that amount of enzyme which causes the deamination of 1 μmol of adenosine to inosine per minute at pH 7.5 and 25°), it was found that only 1 and 2 were subject to deamination with an initial velocity relative to adenosine of 0.003 for 1 and 0.012 for 2. Nucleoside 3 was not deaminated even in the presence of 30 times this amount of enzyme.



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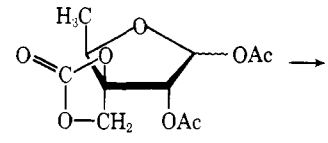

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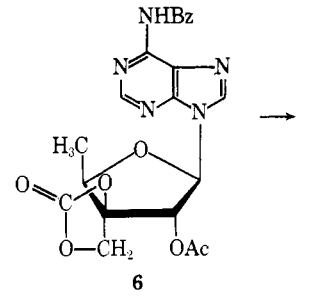

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These results indicate that a carbon ramification at C-3′ of an adenosine analog does not always abolish its susceptibility to deamination and that the stereochemistry at that position plays a very important role in establishing substrate activity. Thus, the only C-3′ branched-chain sugar nucleosides susceptible to deamination so far described (1 and 2) bear their ramification in a position trans to the base. We have shown[1,3,4] that the configuration at C-3′ controls the conformation of the furanose ring of these molecules, the carbon ramification adopting an equatorial position. However, it does not seem that the conformation of the furanose ring as such is an important factor for substrate activity since the conformation of the inactive compound 3 ($J_{1',2'}$ = 7.3 Hz) is closer to that of adenosine[11] ($J_{1',2'}$ = 6.0 Hz) than those of 1 ($J_{1',2'}$ = 1.7 Hz) and 2 ($J_{1',2'}$ = 2.9 Hz).

It is firmly established[9,12,13] that the binding to the enzyme of a substrate or inhibitor requires the presence of a hydroxy group on a carbon chain borne by the N-9 of adenine. In the case of adenosine it is the 5′-hydroxy group which binds to the enzyme but in nucleoside analogs where this group is lacking, a 3′-hydroxy group cis to the base can substitute for it.[9,12] When Bloch, *et al.*,[9] wrote their paper the only exception known to that rule was the 9-(α-L-erythrofuranosyl)adenine which is not deaminated. One should *a priori* expect 3′-*C*-methyladenosine, α-L-erythrofuranosyladenine, and compounds 1, 2, and 3 to act as substrates of the enzyme since all these compounds bear a hydroxy group which, at first sight, seems to be able to occupy a position similar to that of the 5′-hydroxy

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group of adenosine. The fact that three of these compounds are not deaminated indicates that the spatial position of this hydroxy group is very critical. The following hypothesis could explain the observed facts. The hydrogen of the hydroxy group should be positioned on a line almost perpendicular to the plane of the furanose ring and passing through C-3′; this position would be hindered by the methyl group of the 3′-*C*-methyladenosine and could be taken by a 3′-hydroxy group only if axial as in **1**, **2**, and probably 9-(α-L-threofuranosyl)adenine. Clearly, this hypothesis requires confirmation and one cannot rule out the possibility that a C-3′ carbon chain cis to the base could hinder the binding to the enzyme by a mechanism other than a mere conformational change around the C-4′–C-5′ bond.

Experimental Section

Physical properties of these compounds were determined with the following instruments: Mettler FP 52 and FP 5 (melting point); Schmidt & Haensch polarimeter (specific rotations); Unicam SP 800 uv spectrometer (uv spectra); Perkin-Elmer 157 ir spectrometer (ir spectra); Varian XL 100 or Perkin-Elmer R 12 nmr spectrometers (nmr spectra); Varian SM 1 B mass spectrometer (mass spectra, 70 eV). Chromatographic separations have been effected as previously described.[1] Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.3% of the theoretical values. Adenosine deaminase from calf intestine was obtained from Boehringer.

3,3′-*O*-Carbonyl-5-deoxy-3-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose (4). A stream of $COCl_2$ was passed through a solution of 400 mg (1.95 mmol) of 5-deoxy-3-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose[6] in 20 ml of pyridine for 0.5 hr at 0° and then 0.5 hr at room temperature. The excess of $COCl_2$ was removed (N_2) and, after addition of 100 g of ice, the mixture extracted with $CHCl_3$ (3 × 50 ml). The extracts were washed (10% HCl and then 5% $NaHCO_3$), dried ($MgSO_4$), concentrated, and then chromatographed on silica gel (Et_2O). Recrystallization (AcOEt–hexane) gave 270 mg (61%) of **4**: mp 75–76°; $[\alpha]^{23}D$ +68.3° (*c* 0.4, $CHCl_3$); nmr ($CDCl_3$) τ 3.94 (d, 1, $J_{1,2}$ = 4.2 Hz, H-1), 5.20 (d, , $J_{3'a,3'b}$ = 9.3 Hz, H_a-3′), 5.31 (d, 1, H-2), 5.82 (d, 1, H_b-3′), 5.78 (q, 1, $J_{4,5}$ = 6.4 Hz, H-4), 8.50 and 8.64 (2 s, 2 × 3, CMe_2), 8.65 (d, 3, 5 CH_3). *Anal.* ($C_{10}H_{14}O_6$) C, H.

1,2-Di-*O*-acetyl-3,3′-*O*-carbonyl-5-deoxy-3-*C*-hydroxymethyl-α- and -β-D-xylofuranose (5). To a stirred solution of 590 mg (2.57 mmol) of **4** in 20 ml of glacial acetic acid, 0.6 ml of concentrated sulfuric acid was slowly added, the temperature being kept below 20°. The solution was left overnight at room temperature and then poured into 100 g of ice. The mixture was extracted with $CHCl_3$ (5 × 50 ml) and the combined extracts were washed (5% $NaHCO_3$ and then H_2O) and dried ($MgSO_4$). Evaporation of the solvent afforded 0.615 g (87%) of crude **5** as a yellow syrup which was purified by tlc on silica gel (Et_2O) and gave pure **5** as a solid: mp 87–89°; glc V_{RR} (180°) 5.53 (α anomer, 61%), V_{RR} (180°) 6.60 (β anomer, 39%); nmr ($CDCl_3$) for α-**5**, τ 3.53 (d, 1, $J_{1,2}$ = 4.5 Hz, H-1), 4.63 (d, 1, H-2), 5.19 (d, 1, $J_{3'a,3'b}$ = 9.5 Hz, H_a-3′), 5.72 (q, 1, $J_{4,5}$ = 6.5 Hz), 5.77 (d, 1, H_b-3′), 7.86 and 7.94 (2 s, 2 × 3, Ac), 8.61 (d, 3, 5 CH_3); nmr for β-**5** τ 3.92 (s, 1, $J_{1,2}$ < 0.5 Hz, H-1), 4.66 (s, 1, H-2), 5.43 (d, 1, $J_{3'a,3'b}$ = 9.5 Hz, H_a-3′), 5.72 (q, 1, $J_{4,5}$ = 6.5 Hz, H-4), 5.83 (d, 1, H_b-3′), 7.84 and 7.88 (2 s, 2 × 3, Ac), 8.57 (d, 3, 5 CH_3). *Anal.* ($C_{11}H_{14}O_8$) C, H.

9-(2-*O*-Acetyl-3,3′-*O*-carbonyl-5-deoxy-3-*C*-hydroxymethyl-β-D-xylofuranosyl)-*N*-benzoyladenine (6). A mixture of 0.5 g (1.83 mmol) of **5**, 1.1 g (2.33 mmol) of chloromercuri-*N*-benzoyladenine,[14] 1.3 g of Celite, and 150 ml of 1,2-dichloroethane was distilled until 80 ml of distillate had been collected. To the somewhat cooled mixture was added 3 g of molecular sieves (4A) and then, dropwise, a solution of 0.26 ml (2.33 mmol) of $TiCl_4$ in 10 ml of 1,2-dichloroethane, and the mixture was refluxed under dry nitrogen for 48 hr. While the mixture was still warm, 50 ml of saturated, aqueous $NaHCO_3$ was added and vigorous stirring maintained for 2 hr. The mixture was then filtered through Celite, the cake washed with hot $CHCl_3$ (6 × 50 ml), and the organic layer of the filtrate separated and evaporated to dryness. A solution of the residue in 150 ml of $CHCl_3$ was washed successively with 50 ml of 30% aqueous KI and 50 ml of H_2O, dried ($MgSO_4$), and evaporated to dryness to give 0.68 g (82%) of a yellow glass which was submitted to preparative tlc (SiO_2, AcOEt–MeOH 9:1) which afforded 63 mg (7.6%) of a minor product (R_f 0.63) (not the α anomer) and 370 mg (44.5%) of **4** (R_f 0.47) which was recrystallized (AcOEt–hexane): mp 109.9–111.0°; $[\alpha]^{22}D$ +21.1° (*c* 0.6, $CHCl_3$); uv_{max} (EtOH) 280 nm (ε 10,700); nmr ($CDCl_3$) τ 0.48 (s, 1, NH), 1.04 and 1.40 (2 s, 2 × 1, H-2, H-8), 1.7–1.9 (m, 2, H_{ortho}-Bz), 2.10–2.40 (m, 3, H_{meta}, H_{para}-Bz), 3.56 (d, 1, $J_{1',2'}$ = 2.5 Hz, H-1′), 4.20 (d, 1, H-2′), 5.24 (d, 1, $J_{3'a,3'b}$ = 10.3 Hz, H_a-3″), 5.60 (q, 1, $J_{4',5'}$ = 6.7 Hz, H-4′), 5.62 (d, 1 p, H_b-3″), 7.73 (s, 3, Ac), 8.43 (d, 3, 5′ CH_3). *Anal.* ($C_{21}H_{19}N_5O_7$) C, H, N.

9-(5-Deoxy-3-*C*-hydroxymethyl-β-D-xylofuranosyl)adenine (1). To a solution of 226.5 mg (0.5 mmol) of **6** in 2 ml of dry MeOH was added 0.5 g of molecular sieves (4A) and 5 ml of a 0.1 *M* methanolic solution of MeONa. The reaction mixture was refluxed 1 hr with exclusion of moisture and then concentrated at 30° under vacuum. The residue was dissolved in 10 ml of H_2O and neutralized with 10% AcOH. On concentration of the solution 109 mg (76%) of **1** crystallized. The analytical sample was obtained by recrystallization (MeOH–H_2O) and thorough drying or by regeneration of **1** from its picrate (see below): mp 181–184°; $[\alpha]^{20}D$ −28.2° (*c* 0.3, H_2O); uv_{max} (H_2O) 260 nm (ε 11,950); uv_{max} (phosphate buffer pH 7.5) 260 nm (ε 11,500); nmr (D_2O) τ 1.78 and 1.92 (2 s, 2 × 1, H-2, H-8), 4.11 (d, 1, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.51 (d, 1, H-2′), 5.70 (q, 1, $J_{4',5'}$ = 6.3 Hz, H-4′), 6.09 (d, 1, $J_{3'a,3'b}$ = 12.0 Hz, H_a-3″), 6.39 (d, 1, H_b-3″), 8.68 (d, 3, 5′ CH_3); mass spectrum 281 ($M^{\cdot+}$), 135 (adenine + H), 136. *Anal.* ($C_{11}H_{15}N_5O_4$) C, H, N.

Picrate of 1. To a solution of crude **1** (70 mg, 0.24 mmol) a solution of 55 mg (0.24 mmol) of picric acid in 3 ml of EtOH was added and the mixture boiled for 1 min and then cooled to 0°. The yellow precipitate which formed (40 mg, 32%) was removed by filtration: mp 197° dec. *Anal.* ($C_{17}H_{18}N_8O_{11}$) C, H, N. To generate **1** the picrate was suspended in 10 ml of H_2O and treated with 2 ml of Dowex 1 (CO_3^{2-}) for 2 hr at 60°. The mixture was filtered and the filtrate evaporated to dryness gave **1**.

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