Carbocyclic Analogues of Xylofuranosylpurine Nucleosides. Synthesis and Antitumor Activity

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(±)-4α-Amino-2α,3β-dihydroxy-1α-cyclopentanemethanol (6), the carbocyclic analogue of xylofuranosylamine, was synthesized from the previously reported 4α-acetamido-2α,3α-epoxycyclopentane-1α-methanol. Amine 6 was converted to (±)-4α-[(5-amino-6-chloro-4-pyrimidinyl)amino]-2α,3β-dihydroxy-1α-cyclopentanemethanol (7) by condensation with 5-amino-4,6-dichloropyrimidine. From 7, the carbocyclic analogues of xylofuranosyladenine and xylofuranosyl-8-azaadenine were prepared. In contrast to 9-β-D-xylofuranosyladenine and its 8-aza analogue, the corresponding carbocyclic nucleosides were resistant to deamination by adenosine deaminase. The carbocyclic 8-aza derivative 10 exhibited significant in vivo antitumor activity which varied according to treatment schedule.

The significant antitumor activity of 9-β-D-xylofuranosyladenine,[1,2] and its enhanced activity in mice pretreated with the adenosine deaminase inhibitor 2-deoxycoformycin strongly suggests that its in vivo activity is presently limited by rapid metabolic deamination.[3] The nucleoside analogue 8-aza-9-β-D-ribofuranosyladenine is also cytotoxic to tumor cells[4] and is a substrate for adenosine deaminase.[5] These results, coupled with our observed antiviral and antitumor activities exhibited by the adenosine deaminase resistant carbocyclic analogues of arabinofuranosylpurine nucleosides[6,7] and aminonucleosides,[8] provided an excellent rationale for the synthesis of carbocyclic xylofuranosylpurine nucleosides.

Chemistry. Hydrolysis of the easily synthesized epoxide 1[9] with 2% sulfuric acid and subsequent acetylation gave a mixture of 2 and 3 (Scheme I). The major isomer, (±)-4α-acetamido-2β,3α-diacetoxy-1α-cyclopentanemethylacetate (2), was separated from the mixture with one crystallization as colorless prisms (53%). When 2 was subjected to mild acid hydrolysis, amine 4 was formed, since acyl migration to the adjacent cis hydroxyl facilitates hydrolysis of the acetamide.[9] Subjection of a mixture of 2 and 3 to the same hydrolysis conditions gave a mixture of 4 and acetamide 5. This mixture was separated by passage through an IRA-120 (H^+) resin and 5 was recovered as a colorless syrup (17% from 1). Reacetylation of 5 gave pure 3 as a colorless syrup. Amine 6 was prepared by hydrolysis of 5 in refluxingg hydrochloric acid (Scheme II). Condensation of 6 with 5-amino-4,6-dichloropyrimidine gave (±)-4α-[(5-amino-6-chloro-4-pyrimidinyl)amino]-2α,3β-dihydroxy-1α-cyclopentanemethanol (7) in 84% yield. Ring closure of 7 with triethyl orthoformate gave the 6-chloropurine (not isolated). Treatment of 8 with liquid ammonia gave the desired (±)-4α-adenin-9-yl-2α,3β-dihydroxy-1α-cyclopentanemethanol (C-*xylo*-A; 9; 77% from 7). The 8-azapurine analogue was obtained by ring closure of 7 with sodium nitrite and hydrochloric acid followed by treatment with ammonium hydroxide. Thus, (±)-4α-(7-amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2α,3β-dihydroxy-1α-cyclopentanemethanol (10) was obtained in 86% yield.

Table I. Evaluation of Carbocyclic Xylofuranosyl 8-Azaadenine against P388 Leukemia[a]

dose, mg/kg	treatment schedule, days	survival time, days	saline control	T/C, %
1	1–9	13.9	10.9	128
2	1–9	16.1	10.9	149
5	1–6	17.9	10.3	173
5	1–5	18.4	10.5	175
50	1 & 5	19.5	8.6	226

[a] Each group contained eight BDF1 male mice intraperitoneally implanted with 10^6 P388 cells. All drugs were contained in 0.5 mL of saline and injected ip.

Results and Discussion

In contrast to 9-β-D-xylofuranosyladenine and its 8-aza analogue, the corresponding carbocyclic analogues 9 and 10 were completely resistant to deamination by adenosine deaminase (calf intestinal, type III, Sigma). Additional enzymatic analysis demonstrated that 9 is an effective inhibitor of adenosine deaminase, while 10 did not inhibit the deamination of adenosine. The double-reciprocal plot presented in Figure 1 indicates that 9 is a competitive inhibitor with $K_i = 9.9 \times 10^{-6}$ M. The K_m for adenosine in this assay is 7.8×10^{-6} M.

The cytotoxicities of the carbocyclic xylonucleosides were evaluated by growing P388 mouse lymphoid leukemia cells in the presence of 9 or 10 by use of a method previously described.[10] Both analogues exhibited significant cytotoxicities in this assay. Thus, carbocyclic xylofuranosyladenine 9 and the corresponding 8-aza derivative 10 exhibited ED_{50} concentrations of 67 and 0.38 μM, respectively. Compounds 9 and 10 were tested in mice innoculated with leukemia P388. There was no evidence of antileukemic activity or toxicity with 9 at a maximum dose of 100 mg/kg/day (qd 1–9). However, as shown in Table I, the 8-aza derivative 10 demonstrated significant activity which varied according to treatment schedule.

The desirable features such as hydrolytic stability, adenosine deaminase resistance, and significant antitumor activity makes the carboxylic nucleoside analogue 10 an excellent candidate for detailed evaluation as a chemotherapeutic agent. Additional studies are continuing in this laboratory and elsewhere to study the metabolism and antitumor spectrum of carbocyclic 8-azaxylofuranosyladenine in vivo.

Experimental Section

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra

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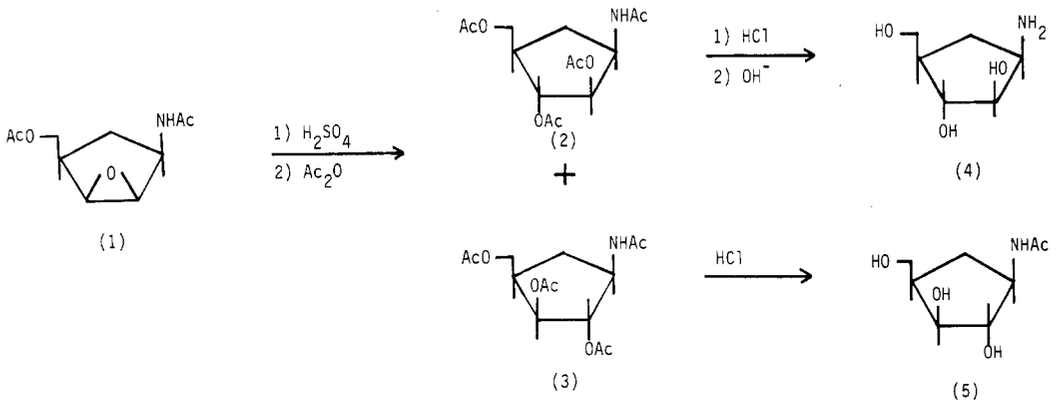
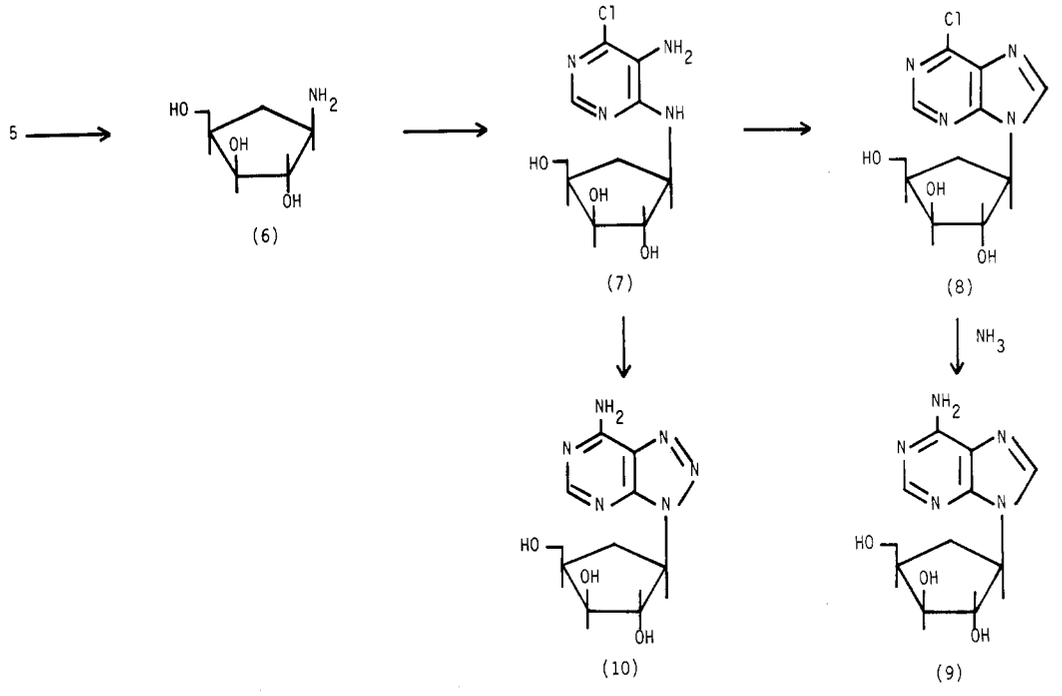
Scheme I



Scheme II



were obtained with a Varian T-60A spectrometer or JEOL FX 90QFT (89.55 MHz), infrared spectra with a Perkin-Elmer 237B spectrometer, and ultraviolet spectra with a Beckman 25 recording spectrometer. Thin-layer chromatography (TLC) was done with use of 0.25-mm layers of Merck silica gel 60F-254 and column chromatography on Merck slica gel 60. Mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer. Low-resolution mass spectra were run on all compounds, and the molecular ion and fragmentation paterns were reasonable.

(±)-4α-Acetamido-2β,3α-diacetoxy-1α-cyclopentanemethyl Acetate (2) and (±)-4α-Acetamido-2α,3β-diacetoxy-1α-cyclopentanemethyl Acetate (3). A solution of crude **1** (7.42 g, 34.8 mmol) in 2% sulfuric acid (450 mL) was warmed (steam bath) for 1 h. A small amount of gummy solid (mostly *m*-chlorobenzoic acid contaminating **1**) was removed by filtration. The pH of the cooled filtrate was adjusted to 7 (indicator paper) with 6 N sodium hydroxide. The water was removed in vacuo and the residue was dissolved in pyridine (2 × 200 mL) and evaporated. The residual syrup was dissolved in acetic anhydride (100 mL) and pyridine (200 mL) and stirred at room temperature overnight. The volatile materials were removed by evaporation, and the residue was dissolved in methylene chloride (250 mL), extracted with saturated sodium bicarbonate (25 mL), and dried (calcium sulfate). The solvent was removed by evaporation in vacuo followed by azeotroping with toluene to remove pyridine. The brown syrup residue (9.61 g) was crystallized from ethyl acetate and gave **2** as white prisms: yield 5.77 g (53%), mp 137–138 °C; IR (KBr) 3290, 3080 (NH), 1740, 1730 (OAc), 1647 (amide 1), 1560 cm^{-1} (amide 2); NMR ($CDCl_3$) δ 5.87 (d, J = 8.0 Hz, 1.0, NHC=O), 4.92 (m, 2.0, 2 CHO), 4.7–4.2 (m, 1.0, CHN), 4.05 (d, J = 6.0 Hz, 2.0 OCH_2), 2.06, 2.00, and 1.93 (all s) overlapped by 2.6–1.5 (m, 15.0 4 CH_3C=O, CH_2, and CH). Anal. ($C_{14}H_{21}NO_7$) C, H, N.

The mother liquors from crystallization of **2** contained an approximately 1:1 mixture of **2** and **3** (from NH resonances in the NMR spectrum). Although some slight separation appeared on TLC (5% methanol–chloroform), column chromatography of the mother liquor contents on silica gel (250 g) with elution by 1% methanol–chloroform gave only a slight enrichment of the early fractions in the minor isomer. The mixture of **2** and **3** (3.30 g, 10.5 mmol) was dissolved in 2 N hydrochloric acid (100 mL) and maintained at 70 °C (oil bath) for 1 h. The solution was evaporated to dryness. The residue was dissolved in H_2O and the solution was stirred briefly with IRA-400 (OH^-) resin (30 mL). The solution was passed through a column of IRA-120 (H^+) resin (60 mL). Elution of the column with H_2O (250 mL) gave, after evaporation of H_2O and azeotroping with absolute ethanol, **5** as a colorless syrup (1.13 g, 17% from **1**): IR (neat) 3400–3100 (OH, NH), 1640 (amide 1), 1550 cm^{-1} (amide 2); NMR ($CDCl_3$) δ 8.05 (br d, j = 7.0 Hz, slow to exchange, NHC=O), 2.00 (s, 1 CH_3C=O). The syrup was reacetylated in acetic anhydride–pyridine (as above) and gave **3** as a colorless syrup (1.58 g, 14% from **1**): NMR identical with that of an analytical sample. All attempts to crystallize **3** were unsuccessful. An analytical sample was prepared by chromatography of a sample of **3** on a silica gel preparative plate developed in 10% methanol–chloroform, giving a colorless syrup, almost in quantitative recovery from the plate and very

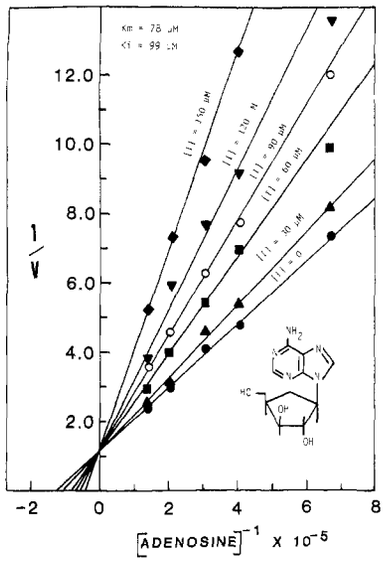


Figure 1. Double-reciprocal plot of inhibition of calf intestinal mucosa adenosine deaminase by carbocyclic xylofuranosyladenine **9**. The deamination reaction was followed spectrophotometrically by decrease in absorbance at 265 nm with a Beckman Model 25 recording spectrophotometer at 25 °C. Reaction mixtures of adenosine and appropriate concentrations of **9** were contained in 1 mL of 0.05 M phosphate buffer at pH 7.5. Reactions were initiated by addition of 20 milliunits of enzyme in 5 μL of buffer. The kinetic parameters were determined by using a Wilkinson analysis.[11] All plotted points represent an average of triplicate determinations. The standard deviation of the obtained values averaged ±5%.

difficult to free of solvents, which was dried at 0.001 mm for 2 days: IR (neat) 3360 (sh), 3280 (NH), 1735 (br, OAc), 1640 (amide 1), 1525 cm^{-1} (amide 2); NMR ($CDCl_3$) δ 6.25 (d, J = 7.5 Hz, 0.9, NHC=O), 5.10 (m, 2.1, 2 CHO), 4.5–3.9 (m) overlapping 4.05 (d, J = 6.0 Hz, 3.0, CHN and OCH_2), 2.11, 2.10, 2.07, 1.99 (all s) overlapped by 3.0–1.2 (m, 15.0, 4 CH_3CO, CH_2, and CH). Anal. ($C_{14}H_{21}NO_7$) C, H, N.

(±)-4α-Acetamido-2α,3β-dihydroxy-1α-cyclopentanemethanol (5). The crude **5** eluted from the acidic resin above was washed through a silica gel column with 15% methanol–methylene chloride and gave a colorless syrup which crystallized from ethanol to white granules (10–17% from **1**): mp 109–110 °C; IR (KBr) 3300 (br, OH, NH), 1655 (amide 1), 1540 (amide 2); NMR (Me_2SO-d_6) δ 7.73 (d, J = 7.0 Hz, 1.0, NHC=O), 4.80 and 4.67 (both d, J = 4.0 Hz, 2.1, 2 CHO*H*), 4.19 (t, J = 5.0 Hz, 1.1, CH_2O*H*), 4.0–2.8 (m, 5.0, 2 CHO, CHN, CH_2O), 1.80 (s) overlapped by 2.4–0.8 (m, 5.8, CH_3C=O, CH_2, CH), addition of D_2O confirmed the assignment of the OH and NH resonances. Anal. ($C_8H_{15}NO_4$) C, H, N.

(±)-4α-Amino-2β,3α-dihydroxy-1α-cyclopentanemethanol (4). A solution of **2** (3.37 g, 10.7 mmol) in 2 N hydrochloric acid (110 mL) was maintained at 70 °C (oil bath) for 1 h. The solution was evaporated to dryness and the residue was dissolved in methanol (100 mL) and stirred briefly with IRA-400 (OH^-) resin (25 mL). Evaporation left **4** as a viscous syrup which could not be solidified and turned yellow on standing: IR (neat) 3500–3000 (OH, NH_2), 1587 cm^{-1} (NH_2). Since **4** appeared to carbonate on exposure to air, it was used immediately for the preparation of carbocyclic arabinofuranosylpurine nucleosides.[6]

(±)-4α-Amino-2α,3β-dihydroxy-1α-cyclopentanemethanol (6). A solution of **5** (3.75 g, 19.8 mmol) in 2 N hydrochloric acid (600 mL) was heated under reflux for 3 h and evaporated to dryness, and the residue was dried by evaporation with several portions of absolute ethanol. The residue was dissolved in methanol (200 mL) and stirred with Dowex 1X8-50 resin (OH^- form, 50 mL). Evaporation left a colorless syrup (2.73 g, 94%), chromatographically homogeneous on silica gel (20% methanol–methylene chloride) with a lower R_f value than that of **5**. The syrup solidified slowly on standing and was sufficiently pure for use. An analytical sample was prepared by crystallization from absolute ethanol and gave white crystals: mp 111.5–112.5 °C; IR (KBr) 3300 (br), 3150 (br, OH, NH_2), 1595 cm^{-1} (NH_2). Anal. ($C_6H_{13}NO_3$) C, H, N.

(±)-4α-[(5-Amino-6-chloro-4-pyrimidinyl)amino]-2α,3β-dihydroxy-1α-cyclopentanemethanol (7). A solution of **6** (2.27 g, 15.4 mmol), 5-amino-4,6-dichloropyrimidine (2.62 g, 16.0 mmol), and triethylamine (7.5 mL, 53.5 mmol) in 1-butanol (50 mL) was refluxed under N_2 for 24 h. The solution was evaporated to dryness and the residue was partitioned between H_2O (80 mL) and chloroform (40 mL). The aqueous layer was extracted with additional chloroform (3 × 10 mL). The aqueous layer was stirred briefly with IRA-400 (OH^-) resin (18 mL) and evaporated in vacuo to an off-white solid foam (4.11 g) which solidified to off-white powder from methanol: yield 3.56 g (84%), mp 207–209 °C; IR (KBr) 3460, 3445, 3250 (OH, NH), 1650 (NH), 1585 cm^{-1} (br, C=C, C=N). Anal. ($C_{10}H_{15}ClN_4O_3$) C, H, N, Cl.

(±)-4α-Adenin-9-yl-2α,3β-dihydroxy-1α-cyclopentanemethanol (9). A mixture of **7** (1.00 g, 3.64 mmol), triethyl orthoformate (50 mL), and concentrated hydrochloric acid (0.5 mL) was stirred at room temperature overnight. The resulting solution was evaporated to dryness and reacted with liquid ammonia (50 mL) in a stainless steel bomb overnight. The ammonia was allowed to evaporate and the residue was dissolved in 1 N hydrochloric acid (100 mL) and stirred at 60 °C for 45 min. The solution was evaporated to dryness, dissolved in methanol, and passed through a column of IRA-400 (OH^-) resin (20 mL). The methanol eluent (250 mL) was evaporated and the residue was triturated with absolute ethanol. The white powder product (743 mg, 77%, mp 224–226 °C) was crystallized from water and gave pure **9**: mp 225–226 °C; IR (KBr) 3320, 3160 (OH, NH_2), 1660, 1607, 1560 cm^{-1} (adenine); mass spectrum (20 eV), 200 °C), m/e (relative intensity) 265 (8.7, M^+), 247 (5.4, $M^+ - H_2O$), 234 (6.0, $M^+ - CH_2OH$), 216 (22.4, $M^+ - CH_2OH - H_2O$), 190 (9.8, B^+56), 178 (5.1, B^+44), 162 (27.4, B^+28), 149 (7.1, B^+15), 136 (100, BH_2^+), 135 (61.8, BH^+), 10, (13.4, $BH^+ - HCN$), 81 (11.2, $BH^+ - 2HCN$). Anal. ($C_{11}H_{15}N_5O_3$) C, H, N.

(±)-4α-(7-Amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2α,3β-dihydroxy-1α-cyclopentanemethanol (10). To a cooled (ice bath) solution of **7** (1.26 g, 4.58 mmol) in 1 N hydrochloric acid (10 mL) was added sodium nitrite (379 mg, 5.49 mmol). After 15 min, concentrated ammonium hydroxide (20 mL) was added, the ice bath was removed, and the solution was heated to reflux for 5 min. The mixture was cooled and a copious precipitate was removed by filtration and washed with H_2O. The white product (1.20 g, mp 250–252 °C dec) was recrystallized from H_2O and gave pure **10** as a white powder; yield 1.04 g (86%), mp 254–256 °C dec; IR (KBr) 3400–2500 (OH, NH_2), 1697, 1623, 1579 cm^{-1} (C=C, N=N); NMR (Me_2SO-d_6) δ 8.28 (s) overlapped by 8.23 (br s, 3.0, 8-azapurine CH and NH_2), 5.8–3.3 (m, 8.5, 3 OH, 2 CHO, CHN, CH_2O, H_2O in solvent), 2.6–2.0 (m, 3.0, CH_2, CH); mass spectrum (70 eV, 150 °C), m/e (relative intensity), 267 (0.4, M^+1), 237 (1.0, $M^+ - HCHO$), 191 (1.1, BCH=CHCH=OH^+), 179 (14.4, $^+$BHCH=CHOH), 137 (100, BH_2^+), 136 (8.6, BH^+), 135 (12.4), 111 (9.7). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

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