for this compound; Virazole is the ICN Pharmaceuticals, Inc., trademark.
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Synthesis and Anti-DNA Virus Activity of the 5′-Monophosphate and the Cyclic 3′,5′-Monophosphate of 9-(β-D-Xylofuranosyl)guanine

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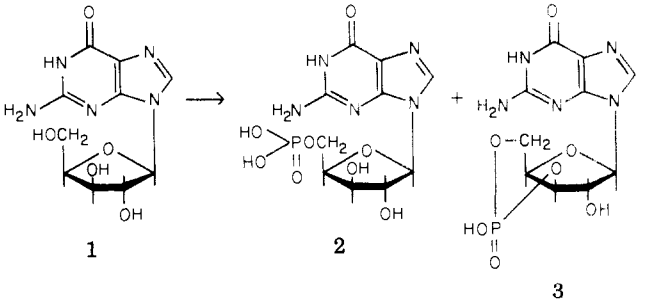
9-(β-D-Xylofuranosyl)guanine (xylo-G) was converted chemically to the 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (xylo-GMP) and 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (c-xylo-GMP). These compounds were tested against a variety of DNA viruses in tissue culture in parallel with 9-(β-D-arabinofuranosyl)adenine (ara-A). This evaluation revealed that xylo-G, xylo-GMP, and c-xylo-GMP were all moderately active but less effective than ara-A. When the four compounds were administered intracerebrally as a treatment for herpes virus, type 1 induced encephalitis in mice, c-xylo-GMP exhibited superior activity to that shown by the other three. When administered intraperitoneally, c-xylo-GMP was found to have a therapeutic index of about 4, which is less than that for ara-A (~30) in the same system.

Nucleosides possessing either D-arabinofuranose[1] or D-xylofuranose[2] in place of D-ribofuranose moieties have received increasing attention in recent years as antimetabolites. Of particular importance in antiviral and antitumor studies of such nucleoside analogues are 1-(β-D-arabinofuranosyl)cytosine (ara-C),[3] 9-(β-D-arabinofuranosyl)adenine (ara-A),[4] and 9-(β-D-xylofuranosyl)-adenine.[5] The observation that most of the nucleoside analogues must be converted to nucleotides[1,6] before they are biologically active, coupled with the increased water solubility of such nucleotides over the corresponding nucleosides,[7] has prompted considerable activity toward the synthesis and biological evaluation of phosphorylated compounds of the above class.[8]

The importance of guanine nucleotide metabolism in a variety of microbiological and mammalian systems has been comprehensively reviewed.[9] Antimetabolites have proved to be unique biochemical tools in probing enzymatic transformations. The biological resistance to purine and pyrimidine antimetabolites is ascribed to high levels of a deaminase[4b] or lack of enzymatic phosphorylation of the nucleosides.[10] This problem could be overcome by using 5′-monophosphates of the nucleosides. However, the free nucleotides at physiological pH carry two negative charges and in general[11] penetrate the cell as an intact nucleotide in very small amounts. The exogenous adenosine cyclic 3′,5′-monophosphate (c-AMP)[12a] and guanosine cyclic 3′,5′-monophosphate (c-GMP)[13] may exert specific biological effects of c-AMP or c-GMP, respectively, on the cell membrane[12b] and the metabolic pathways inside the cell. The interesting biological activity reported for 9-(β-D-xylofuranosyl)purines[14] suggested the synthesis of 9-(β-D-xylofuranosyl)guanine 5′-monophosphate and the cyclic 3′,5′-monophosphate as potential antiviral agents.



1 2 3

For the synthesis of 9-(β-D-xylofuranosyl)guanine (1), the method reported by Lee and co-workers[15] via the

Table I. Comparative in Vitro Antiviral Activity of Ara-A, Xylo-G, Xylo-GMP, and c-Xylo-GMP

Compd	Virus ratings[a]			Min KB cell cytotoxic dose, μg/ml
	Herpes virus[b] type 1	Herpes virus type 2	Vaccinia virus	
Ara-A	0.9	0.8	0.8	3.2
Xylo-G	0.6	0.2	0.5	10.0
Xylo-GMP	0.6	c	0.4	3.2
c-Xylo-GMP	0.5	0.4	0.5	32.0

[a] The virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0-4) assigned to T for each drug level was subtracted from that of C, and the differences (C − T) were totaled. If partial toxicity was evident at any drug level, the C − T of that level was divided by 2. The sum of all C − T values was then divided by ten times the number of test cups used per drug level. [b] Minimum inhibitory concentrations for compounds in this experiment are as follows: ara-A, 3.2 μg/ml; xylo-G, 10 μg/ml; xylo-GMP, 10 μg/ml; and c-xylo-GMP, 32 μg/ml. [c] Not determined.

coupling of the trimethylsilyl derivative of 2-amino-6-chloropurine with 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide followed by the conversion of the condensed product, 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)purine, with mercaptoethanol in the presence of sodium methoxide in methanol was used. The enzymatic phosphorylation of **1** using nucleoside phosphotransferase (from *Pseudomonas trifolii*[16]) and *p*-nitrophenyl phosphate as the phosphate donor has been reported[17] to yield 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (**2**), isolated as the ammonium salt. However, the direct chemical phosphorylation[8c,18] of **1** using phosphoryl chloride in trimethyl phosphate at −10° for 2 h followed by hydrolysis furnished a mixture of **2** and 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (**3**), which was separated by ion-exchange chromatography (Dowex 1) and isolated in free acid form. The purity of **2** and **3** was assured by the homogeneity in several thin-layer systems and on paper electrophoresis (phosphate buffer, pH 7.2, and borate buffer, pH 9.2).

Antiviral Evaluation. Inhibition of the virus-induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed in human carcinoma of the nasopharynx (KB) cells after infection with type 1 (HV/1) or type 2 (HV/2) herpes virus or vaccinia virus (VV). In this system, monolayers (18–24 h) of cells were exposed to 320 $CCID_{50}$ of virus and concentrations of each compound ranging in one-half log dilutions from 1000 to 1 μg/ml were added within 15 min. The degree of CPE inhibition and compound cytotoxicity were observed microscopically after 72 h of incubation at 37° and scored numerically in order to calculate a virus rating (VR) as previously described.[19] Significance of antiviral activity in terms of VR's has been assigned as follows: <0.5, slight or no activity; 0.5–0.9, moderate activity; and ≥1.0, marked activity. The results of a single experiment in parallel with ara-A are shown in Table I. Of the compounds tested, ara-A possessed the best activity against all viruses with the xylopurines having comparable slight to moderate antiviral activity against HV/1 and VV but none had appreciable activity against HV/2.

The moderate in vitro anti-DNA virus activity observed for this class of synthetic compounds led us to the evaluation of their efficacy as antiviral agents in vivo. Encephalitis was induced in 18–20-g Swiss mice by intracerebral (ic) inoculation of an LD_{75} of HV/1, strain 123.

Table II. Effect of Intracerebrally Administered Xylo-G, Xylo-GMP, c-Xylo-GMP, or Ara-A on Herpes Virus Type 1 Induced Encephalitis in Mice

Compd	Dosage, mg/kg	Survivors/total
Experiment 1		
Saline	0.03[c]	6/24
Xylo-G	5.0	4/10
Xylo-GMP	1.25	4/10
c-Xylo-GMP	1.25	9/10[a]
Ara-A	3.2	5/9
Experiment 2		
Saline		3/20
c-Xylo-GMP	1.25	6/10[b]
	0.31	5/10[b]

[a] Probability <0.01, Fisher's exact test. [b] Probability <0.05, Fisher's exact test. [c] ml.

Table III. Effect of Intraperitoneally[a] Administered c-Xylo-GMP on Herpes Virus Type 1 Induced Encephalitis in Mice

Drug dosage, mg/kg/day	Toxicity controls (survivors/total)	Infected (survivors/total)
Saline		2/21
104	4/5	2/10
52	5/5	5/10[b]
26	c	3/9[d]

[a] b.i.d. × 9, starting 4 h postvirus inoculation. [b] Probability (p) < 0.02, Fisher's exact test. [c] Not determined. [d] This dose extended survival by 2.4 days ($p < 0.02$, t test).

A single treatment with the maximum tolerated dose (MTD) of each drug was administered ic at 6 h postvirus inoculation.[8d] c-Xylo-GMP increased the number of surviving animals to the greatest extent with xylo-G, xylo-GMP, and ara-A being similar (see Table II, experiment 1). These observations suggest that the cyclic nucleotide could be the active form, since xylo-G and xylo-GMP would have the same potential for conversion to other nucleotides (a di- or triphosphate). The c-xylo-GMP activity was further verified in a second experiment with the compound being active at its MTD and MTD/4 (0.31 mg/kg). Thus, in this experiment c-xylo-GMP demonstrated a marked ability to protect mice from HV/1 encephalitic deaths with the compound having a therapeutic index (TI = MTD/minimum inhibitory dose) of ≥4. This activity stimulated an additional experiment to determine the effect of intraperitoneal administration of c-xylo-GMP on the encephalitic deaths. c-Xylo-GMP was found to have a narrow range of effectiveness (Table III). The only dose (52 mg/kg/day) which increased survivors caused a marked weight loss in both toxicity and infected animals. In addition, the lowest dose significantly increased survival time. The TI of about 4, observed for c-xylo-GMP, was much lower than that usually found for ara-A (~30)[8e] and probably resulted from the greater toxicity. Since c-xylo-GMP was more toxic and comparatively less active than other potential anti-DNA virus agents,[8c] no further studies have been planned.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Nuclear magnetic resonance (^1H NMR) spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 as well as in D_2O–NaOD using DSS as an internal standard. Ultraviolet spectra (uv, s = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by

Galbraith Laboratories, Inc., Knoxville, Tenn., and the results are within ±0.4% of the theoretical values. Thin-layer chromatography was run on a silica gel F-254 (EM Reagents) plates and developed with either solvent system: A, 2-propanol–concentrated ammonium hydroxide–water (7:1:2, v/v); B, 2-propanol–concentrated ammonium hydroxide–water (5.5:1.0:3.5, v/v); C, 1-butanol–acetic acid–water (5:2:3, v/v); and D, acetonitrile–0.2 M aqueous ammonium chloride (7:3, v/v). Evaporations were carried out under reduced pressure with bath temperature below 30°.

9-(β-D-Xylofuranosyl)guanine 5′-Monophosphate (2) and 9-(β-D-Xylofuranosyl)guanine Cyclic 3′,5′-Monophosphate (3). To a solution of phosphorus oxychloride (2.9 ml) in freshly distilled trimethyl phosphate (20.0 ml) cooled in an ice–salt bath (−10°) was added 9-(β-D-xylofuranosyl)guanine[15] (1, 3.0 g, 0.0105 mol, dried at 80° for 15 h over P_2O_5 under vacuum). The contents of the stoppered flask was stirred at −10°. Within 25 min a clear solution was obtained. Stirring was continued for 2 h before the resulting, slightly orange-colored solution was poured into ice–water (150 ml) containing sodium hydrogen carbonate (2.5 g) with stirring and external cooling. The mixture was occasionally stirred in an ice bath for 1 h, and the pH was monitored at 5–6 by adding solid sodium hydrogen carbonate when needed. The pH stabilized solution was extracted with ether (3 × 50 ml) and the aqueous phase was concentrated in vacuo until salts began to crystallize. Enough water was added to achieve solution; the pH was adjusted to 6–7, before it was applied to a column containing Dowex 1 X2 (100–200 mesh, formate form, 100 ml). The resin was washed with water (3 l.) to remove unreacted 1 and the inorganic salts before using gradient elution (0.5 M formic acid to water). The eluent containing the product was pooled, frozen, and lyophilized to yield 2.95 g of a cream-colored solid which was found to be a mixture of **2** and **3**.

The above mixture was dissolved in water (25 ml) and the pH was checked (6–7) before it was rechromatographed on a freshly generated Dowex 1 X2 resin column (100–200 mesh, formate form, 100 ml). The column was first washed with water (2 l.) before the nucleotides were eluted using a gradient (0.25 M formic acid to water). The first fraction was pooled and concentrated to ~15 ml. Ethanol (50 ml) was added and refrigerated overnight. The white solid that separated was collected, washed with ethanol, and dried over P_2O_5. It was crystallized from aqueous ethanol as needles to yield 2.45 g (63.7%) of 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (**3**), mp >235° dec, which was homogeneous on paper electrophoresis and on paper chromatography (solvent A): $[\alpha]^{25}D$ −48.5° (c 1.0, water); ^1H NMR (D_2O) δ 7.98 (s, C_8H), 5.99 (s, $C_1'H$); uv λ max (pH 1) 255 nm (ε 13 460), 275 s (9400); λ max (pH 7) 252 nm (ε 15 140), 269 s (11 100); λ max (pH 11) 256–268 nm (ε 12 800). Anal. ($C_{10}H_{12}N_5O_7P\cdot H_2O$, 363.22) C, H, N.

The second fraction containing the homogeneous product was pooled and concentrated to ~3 ml. Ethanol (25 ml) was added and refrigerated overnight. The white amorphous solid that separated was collected, washed with ethanol, and dried over P_2O_5 at 60° to yield 0.35 g (8.0%) of 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (**2**), mp >250° dec, which was homogeneous on paper chromatography (solvent A): $[\alpha]^{25}D$ −40.3° (c 1.0, water); ^1H NMR (Me_2SO-d_6–D_2O) δ 7.90 (s, C_8H), 5.85 (d, J = 2.5 Hz, $C_1'H$); uv λ max (pH 1) 255 nm (ε 12 700), 274 s (8600); λ max (pH 7) 252 nm (ε 13 800), 270 s (9700); λ max (pH 11) 256–267 nm (ε 11 960). Anal. ($C_{10}H_{14}N_5O_8P\cdot 3H_2O$, 417.26) C, H, N.

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