Biological Activity of Furanoses I: Tetrofuranosyl Adenines

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Abstract □ A number of aldofuranosyl adenines, including all eight possible tetrofuranosyl adenines, were tested for biological activity with adenosine deaminase and with a strain of *Streptococcus faecalis*, ATCC 8043, in which adenosine deaminase activity is undetectable. Ten compounds were either substrates or inhibitors of the adenosine deaminase, and, of these, nine showed significant capacity to inhibit the growth of the *Streptococcus faecalis*. Eleven compounds showed no capacity either to interact with adenosine deaminase or to inhibit the cells. In view of this parallelism of activity in 20 of 21 cases, it is suggested that adenosine deaminase may be a useful model for testing the likelihood that fraudulent adenine nucleosides may have growth-inhibitory capacity against whole cells.

Keyphrases □ Tetrofuranosyl adenines—biological activity □ Antimicrobial activity—tetrofuranosyl adenines □ Adenosine deaminase inhibition—tetrofuranosyl adenines

A limitation of the capacity of certain fraudulent adenine nucleosides to inhibit cell growth has been their ease of deamination by deaminases (1–3). Protection against such deamination has been achieved through concurrent use of adenosine deaminase inhibitors, for example, β-D-arabinofuranosyl-6-methylaminopurine (2). Recent studies have shown that the presence of a 5′-OH is of major importance for enzymatic deamination of adenine nucleosides (4). These findings suggest that deletion of the 5′-OH group of an appropriate sugar moiety in such a compound should give rise to a nucleoside with limited or no substrate activity toward adenosine deaminase.

The tetrosyl adenines are a group of compounds in which, since they lack a 5′-OH, deaminase substrate activity should be so minimized. Within this group, by analogy with the sugar moieties of the known inhibitory nucleosides, β-D-xylofuranosyl adenine (3) and spongoadenosine (1), α-L-threofuranosyl adenine and β-D-threofuranosyl adenine might be expected to be significant inhibitors of cell growth. Compounds of this type, lacking a C_4′-hydroxymethyl group, are not amenable to 5′-nucleotide formation, and should not exert an inhibitory activity as nucleosides. This is a particularly interesting class of inhibitors to study, in view of the fact that the majority of known inhibitory nucleosides, such as 6-mercaptopurine riboside, spongoadenosine, cordycepin, and xylofuranosyl adenine, are considered to exert their inhibitory activity after conversion to the nucleotide.

Interest in the tetrosyl adenines relates as well to a more general postulate, which takes cognizance of the structural features common to a number of metabolic intermediates of nucleic acid pathways. The fact that a variety of compounds containing adenine-furanose moieties are substrates, or inhibitors, of one or more enzymes of these pathways (5), and that there is reasonable indication, at least in some cases, that the carbohydrate moiety is an important participant in the interaction of such compounds with such enzymes, suggests that the portions of the active sites of the enzymes which accommodate the sugar moiety resemble each other, at least partially. Hence, it would appear reasonable that a fraudulent adenine nucleoside whose sugar moiety is found to contribute significantly to the interaction with an isolated such enzyme might be capable of also interacting with, and, in some instances of inhibiting other enzymes in nucleic acid pathways. In intact cells, such inhibition might be detectable as a restriction of cell growth.

With this possibility in mind, adenosine deaminase has been considered as a model enzyme for testing the likelihood that fraudulent adenine nucleosides may inhibit other enzymes whose substrates also contain adenine-furanose moieties. On this basis, one would anticipate that a fraudulent nucleoside which can interact with adenosine deaminase might also be capable of inhibiting the growth of a cell type, such as *Streptococcus faecalis* (ATCC 8043), in which this enzyme activity is low or absent. Results of tests to date of this anticipated correlation are reported here.

Table I—Activity of Some Pento- and Hexofuranosyl Adenines toward Adenosine Deaminase and *Streptococcus faecalis*

Carbohydrate Moiety of Adenine Nucleoside	Activity toward Adenosine Deaminase: Substrate [Initial Velocity Unit of Enzyme Relative to Adenosine]	Activity toward Adenosine Deaminase: Inhibitor $[I/S]_{0.5}$	Inhibitory Activity toward *Streptococcus faecalis* (ATTC 8043) [Molar Concn. for 50% Inhibition of Growth]
β-D-5′-Deoxyxylofuranosyl-[a]	0.04[a]	3.2[i]	4×10^{-5}
β-D-Arabinofuranosyl-[a]	0.20[a]		5×10^{-8}
α-D-Arabinofuranosyl-[b]	0[i]	0	0
α-L-Arabinofuranosyl-[c]	0	0	0
β-D-3′-Amino-3′-deoxyfuranosyl-[a]	0.45[a]		4×10^{-6}
β-D-3′-Deoxyribofuranosyl-[a]	0.71[a]		2×10^{-5}
β-D-3′-Deoxyarabinofuranosyl-[d]	0	0	0
β-D-Glucofuranosyl-[e]	0	0	0
β-D-3′-Deoxyglucofuranosyl-[f]	0	0	0
α-D-3′-Deoxymannofuranosyl-[g]	0	0	0
β-D-3′-Deoxygalactofuranosyl-[h]	0	0	0
β-D-2′,3′-Dideoxyribofuranosyl-[a]	0.60[a]		8×10^{-7}
β-D-5′-Amino-5′-deoxyribofuranosyl-[a]	0.004[a]		8×10^{-4}

[a] Reported in *Reference* 4. [b] *Reference* 8. [c] *Reference* 9. [d] Isolated by chromatography of the filtrate obtained in the preparation of 9-(3-deoxy-α-D-arabinofuranosyl) adenine, *Reference* 10. [e] *Reference* 11. [f] *Reference* 12. [g] *Reference* 10. [h] *Reference* 13. [i] Reported in *Reference* 6. [j] Designations of 0 in the several columns of the above Table I and the following Table II signify respectively, no detectable substrate activity, $[I/S]_{0.5} > 10$, or inhibitory activity toward *Streptococcus faecalis* $> 5 \times 10^{-3}$ *M*.

Table II—Activity of Tetrofuranosyladenines toward Adenosine Deaminase and *Streptococcus faecalis*[a]

Carbohydrate Moiety of Adenine Nucleoside	Activity toward Adenosine Deaminase Substrate [Initial Velocity/Unit of Enzyme Relative to Adenosine]	Activity toward Adenosine Deaminase Inhibitor $[I/S]_{0.5}$	Inhibitory Activity toward *Streptococcus faecalis* (ATTC 8043) [Molar Concn. for 50% Inhibition of Growth]
β-D-Erythrofuranosyl-[b]	0[c]	7.8	5×10^{-4}
α-D-Erythrofuranosyl-[b]	0	>10	0
β-L-Erythrofuranosyl-[b]	0	10 ± 2	0
α-L-Erythrofuranosyl-[b]	0	0.15	0
β-D-Threofuranosyl-[b]	0	1.8	3×10^{-5}
α-D-Threofuranosyl-[b]	0	6.8	0
β-L-Threofuranosyl-[b]	0	8.3	0
α-L-Threofuranosyl-[b]	0.01[d]	1.7	2×10^{-5}

[a] See footnote to Table I. [b] *Reference* 14. [c] See footnote *i* in Table I. [d] Reported in *Reference* 4.

MATERIALS

The sources of the nucleosides tested are listed in the footnotes in Tables I and II. Furthermore α-D-arabinofuranosyl-, α-L-arabinofuranosyl-, 3′-deoxy-β-D-arabinofuranosyl-, and β-D-glucofuranosyladenine were prepared in the authors' laboratory.

EXPERIMENTAL

Data on inhibitory or substrate activity of a number of adenine nucleosides were as reported in the literature, or were determined by procedures reported by Schaeffer (6) or Bloch (4), as referenced in Tables I and II.

Microbial Assay Procedure—All growth experiments were carried out in the basal medium of Flynn *et al.* (7), from which uracil and the purines had been omitted, and to which 1 mcg./ml. of folic acid was added. Portions (1 ml.) of this double-strength medium were placed into 13 × 100-mm. Pyrex culture tubes and 1 ml. of water or 1 ml. of the solution containing the test compound was then added to each tube. Sterilization of the medium was carried out by autoclaving for 6 min. at 116–121°. The inocula were prepared from cultures of *S. faecalis* grown in 5 ml. of the basal medium for 20 hr. at 37°. Following centrifugation and washing twice with isotonic saline, the cells were resuspended in enough saline to give an optical density of 0.30 as measured in a spectrophotometer (Beckman model B) at 470 mμ. A 1-ml. portion of this suspension containing approximately 1.5×10^7 cells was diluted 1:10 in saline, and 1 drop of this final dilution was placed in each assay tube. Incubation proceeded for 20 hr. at 37°, at which time the control culture is still in log phase. The extent of growth was determined by means of a photoelectric colorimeter (Klett-Summerson), using a red filter (640–700 mμ). To determine their potency as inhibitors of growth, the adenine nucleosides were added to the basal medium in concentrations ranging from 10^{-3}–10^{-7} *M*.

RESULTS AND DISCUSSION

The data on a number of available adenine nucleosides tested are given in Tables I and II. Table I summarizes tests of the postulated correlation with a series of pento- and hexo-furanosyl adenines. All of the compounds which are significantly active as a substrate or inhibitor of adenosine deaminase are active also as inhibitors of the growth of the intact cells in which this enzyme activity is low or absent. Further, all of the compounds which are inactive toward the isolated enzyme are likewise inactive against these cells.

Table II is a summary of the results of similar tests with eight tetrofuranosyl adenines. Analogy with the sugar moieties of β-D-xylofuranosyl adenine and spongoadenosine suggested *a priori* that α-L-threofuranosyl adenine and β-D-threofuranosyl adenine might have inhibitory activity toward both adenosine deaminase and *Streptococcus faecalis*. As shown, this prediction was borne out. Limited substrate activity for adenosine deaminase was observed with the α-L-compound as well, but not for the β-D. These findings are in accord with the apparent rather general requirement for substrate activity with this enzyme that there be a C_4'-hydroxymethyl group present (4), or a C_3'-OH so positioned as feasibly to permit interaction with the same site as will normally accommodate a C_5'-OH (6). For example, 5′-deoxyxylofuranosyl adenine is a significant substrate for adenosine deaminase (4, 15), but 5′-deoxyarabinofuranosyl adenine and 5′-deoxyadenosine are not (4).

Of the erythrosyl adenines, the β-D-compound showed limited inhibitory activity toward both the isolated enzyme and the whole cell system, in further accord with the general hypothesis. Somewhat surprisingly, α-L-erythrosyl adenine was a potent inhibitor of the deaminase but not detectably inhibitory to the whole cell system. The authors have no current explanation of this discrepancy, except to note that the hypothesis of partial resemblance of enzyme sugar-moiety binding sites does not exclude the probability of substantial specificity in some instances.

The anomers of the foregoing four compounds were expected to show no significant inhibitory activity, because one anomer can occupy a site, customarily accommodating the other, only at the expense of a significant skewing of the molecule, which would usually be expected to render it inactive. On occasion, however, an enzyme may accept both anomers. For example, nucleoside phosphorylase is active on both α- and β-anomers of D-2′-deoxyribosyl-6-mercaptopurine, and α-D-xylosyl adenine limitedly inhibits the deamination of β-D-xylosyl adenine (16). Such observations of biological activity by both α- and β-anomers are, however, rare.

The foregoing considerations suggested that α-D-threofuranosyl adenine and β-L-threofuranosyl adenine would have little or no activity in the test systems employed in the present study, as found. They were inactive toward *Streptococcus faecalis*, and showed only a limited capacity to inhibit adenosine deaminase. Similarly, α-D-erythrofuranosyl adenine and β-L-erythrofuranosyl adenine would be expected to be essentially inactive, as found.

The composite data of Table I and II support the general hypothesis in 20 of the 21 cases. This correlation suggests the possibility that studies with adenosine deaminase may allow a good measure of predictability of inhibitory action of adenine nucleosides against cell populations. Where such cells have themselves significant deaminase activity, the active cellular inhibitor may be protected from deamination by a deaminase inhibitor, as has been reported (2).

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