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## Dialdehydes Derived from Adenine Nucleosides as Substrates and Inhibitors of Adenosine Aminohydrolase<sup>†</sup>

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**ABSTRACT:** A series of nucleoside dialdehydes have been obtained as powders after treatment of various adenine nucleosides with paraperiodic acid. Thus, oxidation gave dialdehydes derived from adenosine (1), 9- $\alpha$ -D-mannopyranosyladenine (2), 9-(5-deoxy- $\alpha$ -D-arabinofuranosyl)adenine (3), 9- $\alpha$ -L-rhamnopyranosyladenine (4), 9- $\beta$ -L-fucopyranosyladenine (5), 9- $\beta$ -D-fucopyranosyladenine (6), 9- $\alpha$ -D-arabinopyranosyladenine (7), 9- $\beta$ -D-ribosepyranosyladenine (8), and 9-(5-deoxy- $\beta$ -D-erythro-pent-4-enofuranosyl)adenine (9). Nucleoside dialdehydes 1-3 and 6-8 were weak substrates for adenosine aminohydrolase from calf intestinal mucosa. Di-

aldehyde 8 had the strongest affinity, but 1 had the highest  $V_{max}$ . All of the dialdehydes except 5 were inhibitors of the enzyme. The best inhibitors were 9 ( $K_i = 4 \mu\text{M}$ ) and 4 ( $K_i = 28 \mu\text{M}$ ), and neither were substrates. The inhibitors did not exhibit time-dependent inhibition and did not appear to form covalent bonds with the protein. The data strongly suggest that the active form of the dialdehydes is as the open-chain dihydrates. The alcohol obtained by reduction of 9 (compound 10) was the strongest inhibitor ( $K_i = 0.9 \mu\text{M}$ ) among the related alcohols and the nucleoside dialdehydes.

Periodate oxidation of nucleosides yields compounds referred to as nucleoside dialdehydes. It is generally recognized that these compounds are actually equilibrium mixtures of various cyclic and acyclic hemiacetals and hydrates (Guthrie, 1961; Khym & Cohn, 1960; Jones et al., 1976; Hansske & Cramer, 1977). The nomenclature and structural representation as the aldehyde form used in publication are a matter of convenience to simplify discussion, and since they react as typical aldehydes, the different structures could usually be ignored. The closest analogy to this chemistry would be the free sugars which, in solution, exist mainly as cyclic hemiacetals rather than as straight-chain aldehydes. In recent years, the nucleoside dialdehydes have been shown to bind to a number of enzymes. Ribonucleotide reductase from Ehrlich ascites tumor cells was inhibited by the periodate oxidation products of adenosine (Cory et al., 1976) and inosine (Cory & Mansell, 1975). S-Methylthioinosine dialdehyde inhibited enzymes as varied as thymidylate kinase and DNA polymerase (Kimball et al., 1968), bacterial RNA polymerase (Nixon et al., 1972), and pancreatic ribonuclease (Spoor et al., 1973). Inosine dialdehyde formed stable complexes with lysine, glycine, histidine, and bovine serum albumin. The latter was shown to

be cross-linked by the periodate oxidation products of inosine, adenosine, cytidine, and methyl  $\beta$ -D-ribofuranoside (Cysyk & Adamson, 1976). This cross-linking property was consistent with the time-dependent nature of enzyme inhibition, macromolecular synthesis, and cell proliferation (Cory et al., 1976). The finding that other aldehydes have similar effects on the same systems has led some workers to believe that cross-linking, either by Schiff base or carbinolamine formation, is the major mode of interaction of nucleoside dialdehydes and proteins (Cysyk & Adamson, 1976; Cory et al., 1976; Plagemann et al., 1977). In either case the mechanism of formation of the covalent bonds requires the hydrate form of the aldehydes (Milch, 1964). It became necessary to see how changes in structure of the nucleoside dialdehydes would affect this process and to better ascertain the range of other mechanistic possibilities. We have begun by investigating the binding of a number of analogues of adenosine dialdehyde to adenosine aminohydrolase (EC 3.5.4.4), an enzyme that does not react to any great extent with small aldehydes despite the availability of lysine residues.

### Experimental Section

**General Materials and Methods.** Adenosine, DL-glycerinaldehyde, pyridoxal, and pyridoxal phosphate were purchased from Sigma Chemical Co. Adenosine was recrystallized three times from water and dried under high vacuum at 80 °C over phosphorus pentoxide. Formaldehyde (37.2% in water) was purchased from Fisher Scientific Co., and glutaraldehyde

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(Puriss, 99%) was purchased from Tridom Chemical Co. (Fluka, A.G.). Dialcohol 10 was a gift from Dr. P. T. Gilham of Purdue University. The nucleosides used as starting materials for the preparation of the nucleoside dialdehydes were synthesized according to the directions given in the references stated below after each compound.

Elemental analyses were performed by the Baron Consulting Co., Orange, CT. Ultraviolet spectra were obtained with a Beckman DK-2 spectrophotometer in standard 1-cm silica cells by using 0.05 M sodium phosphate buffer, pH 7.0. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer from solid samples pressed into potassium bromide pellets. Paper chromatography was performed on Whatman No. 1 paper by a descending technique, and mobilities were recorded as  $R_f$  values. Spots were located with a Mineralight lamp that produced ultraviolet radiation at 254 nm. Solvent A is water and solvent B is 5:1:4 v/v 1-butanol-ethanol-water, upper phase.

**General Procedure for Preparation of Nucleoside Dialdehydes.** The procedure described by Khym & Cohn (1960) was followed. The nucleosides were oxidized with the theoretical amount of paraperiodic acid ( $\text{H}_5\text{IO}_6$ ) at room temperature, protected from light. The solution was passed through a column of Bio-Rad AG1-X8 (acetate form, 100–200 mesh) anion-exchange resin to remove iodate ion. The eluates were checked for the latter by a starch-iodide test (Welcher, 1963). The solutions were evaporated under high vacuum (oil pump) at room temperature on a rotary evaporator to a small volume and lyophilized. The white powders obtained were dried further under high vacuum over phosphorus pentoxide at room temperature and then characterized. Paper chromatography revealed that no starting nucleosides were present.

**Nucleoside Dialdehydes.** Oxidation of adenosine (0.7 g, 2.6 mmol) with paraperiodic acid (0.6 g, 2.6 mmol) for 1.5 h gave 0.68 g (89%) of dialdehyde 1:  $R_f$  0.54 (A), 0.61 (B); UV max 259 nm ( $\epsilon$  14 600). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_4 \cdot 1.5\text{H}_2\text{O}$ : C, 41.09; H, 4.83; N, 23.97. Found: C, 41.36; H, 4.57; N, 23.73.

9- $\alpha$ -D-Mannopyranosyladenine (Lerner & Kohn, 1964) (2.6 g, 8.6 mmol) was treated with paraperiodic acid (4.2 g, 18.4 mmol) for 24 h to yield 2.43 g (93%) of dialdehyde 2:  $R_f$  0.56 (A), 0.64 (B); UV max 258 nm ( $\epsilon$  15 000). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_4 \cdot 2\text{H}_2\text{O}$ : C, 39.87; H, 5.02; N, 23.25. Found: C, 40.46; H, 4.71; N, 22.74.

Treatment of 9-(5-deoxy- $\alpha$ -D-arabinofuranosyl)adenine (Lerner, 1978) (0.12 g, 0.5 mmol) with paraperiodic acid (0.15 g, 0.65 mmol) for 7 days gave 93 mg (67%) of dialdehyde 3:  $R_f$  0.63 (A), 0.80 (B); UV max 259 nm ( $\epsilon$  15 300). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_3 \cdot 2.5\text{H}_2\text{O}$ : C, 40.81; H, 5.48; N, 23.80. Found: C, 40.79; H, 5.25; N, 21.70.

9- $\alpha$ -L-Rhamnopyranosyladenine (Baker & Hewson, 1957) (1.5 g, 5.3 mmol) was oxidized with paraperiodic acid (2.6 g, 11.6 mmol) for 24 h to yield 1.25 g (83%) of dialdehyde 4:  $R_f$  0.62 (A), 0.79 (B); UV max 259 nm ( $\epsilon$  14 100). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_3 \cdot 2\text{H}_2\text{O}$ : C, 42.10; H, 5.30; N, 24.55. Found: C, 42.67; H, 5.11; N, 24.03.

Dialdehyde 5 (2 g, 92%) was prepared by oxidation of 9- $\beta$ -L-fucopyranosyladenine (Lerner & Rossi, 1972) (2.2 g, 7.6 mmol) with paraperiodic acid (4 g, 17.5 mmol) for 24 h:  $R_f$  0.64 (A), 0.82 (B); UV max 258 nm ( $\epsilon$  14 800). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_3 \cdot 2\text{H}_2\text{O}$ : C, 42.10; H, 5.30; N, 24.55. Found: C, 42.76; H, 5.12; N, 23.31.

9- $\beta$ -D-Fucopyranosyladenine (Lerner, 1971) (0.11 g, 0.4 mmol) was treated with paraperiodic acid (0.19 g, 0.84 mmol) for 24 h to yield 96 mg (87%) of dialdehyde 6:  $R_f$  0.62 (A),

0.79 (B); UV max 258 nm ( $\epsilon$  15 000). Anal. Calcd for  $\text{C}_{10}\text{H}_{11}\text{N}_5\text{O}_3 \cdot \text{H}_2\text{O}$ : C, 44.94; H, 4.90; N, 26.21. Found: C, 45.58; H, 4.81; N, 26.60.

Oxidation of 9- $\alpha$ -D-arabinopyranosyladenine (Martinez et al., 1969) (1.6 g, 5.8 mmol) for 24 h with paraperiodic acid (3 g, 13.2 mmol) gave 1.3 g (93%) of dialdehyde 7:  $R_f$  0.55 (A), 0.70 (B); UV max 259 nm ( $\epsilon$  14 500). Anal. Calcd for  $\text{C}_9\text{H}_9\text{N}_5\text{O}_3 \cdot 0.5\text{H}_2\text{O}$ : C, 45.76; H, 4.27; N, 29.65. Found: C, 45.59; H, 4.18; N, 29.10.

9- $\beta$ -D-Ribopyranosyladenine (Davoll & Lowy, 1952) (1.9 g, 6.9 mmol) was oxidized with paraperiodic acid (3.3 g, 14.6 mmol) for 24 h to yield 1.35 g (75%) of dialdehyde 8:  $R_f$  0.54 (A), 0.68 (B); UV max 259 nm ( $\epsilon$  15 100). Anal. Calcd for  $\text{C}_9\text{H}_9\text{N}_5\text{O}_3 \cdot 2.5\text{H}_2\text{O}$ : C, 38.57; H, 5.04; N, 24.99. Found: C, 39.48; H, 4.44; N, 24.60.

9-(5-Deoxy- $\beta$ -D-erythro-pent-4-enofuranosyl)adenine (Lerner, 1977) (0.1 g, 0.41 mmol) was oxidized with paraperiodic acid (0.1 g, 0.45 mmol) for 1.5 h to yield the product 9 (86 mg, 77% yield):  $R_f$  0.57 (A), 0.75 (B); UV max 260 nm ( $\epsilon$  13 400). Anal. Calcd for  $\text{C}_{10}\text{H}_9\text{N}_5\text{O}_3 \cdot 2\text{H}_2\text{O}$ : C, 42.40; H, 4.63; N, 24.73. Found: C, 44.70; H, 4.74; N, 24.61.

**Enzyme Assay and Kinetic Measurements.** Calf intestinal adenosine aminohydrolase (adenosine deaminase, EC 3.5.4.4) was purchased from Sigma Chemical Co. (Type 1). It had a specific activity of 220 units/mg of protein. One unit is defined as the amount of enzyme that will deaminate 1  $\mu\text{mol}$  of adenosine to inosine per minute at pH 7.5 and 25  $^\circ\text{C}$ . The assay method was based upon that of Kalckar (1947) and reported in a modified version by Kaplan (1955). The rate of change in absorbance at 265 nm in 0.05 M sodium phosphate buffer (pH 7.0) at 25  $^\circ\text{C}$  was measured on a Beckman DK-2 spectrophotometer. Substrate activity was determined by addition of 60 units of adenosine aminohydrolase to 3 mL of a solution of the nucleoside dialdehyde and allowing the solution to stand for up to 24 h. A shift in the absorption maximum from  $\sim 260$  to  $\sim 249$  nm was indicative of transformation of an  $\text{N}^9$ -substituted adenine to an  $\text{N}^9$ -substituted hypoxanthine. Compounds that did not appear to be deaminated were treated with another dose of enzyme for an additional 24 h to be certain that they were not substrates. The values of  $K_m$  and  $V_{\max}$  for substrates were determined by the reciprocal plot method of Lineweaver & Burk (1934) by using the weighted least-squares analysis of Wilkinson (1961). Initial rates of reaction were measured by using concentrations of dialdehydes from 30 to 200  $\mu\text{M}$ . The amount of enzyme added to 3 mL of solution ranged from 5 to 50 units, depending upon the activity of the compound. The  $K_m$  for adenosine was 50  $\mu\text{M}$ , and the  $V_{\max}$  was 220 ( $\mu\text{mol}/\text{min}$ )/mg of protein.

Inhibitor constants were determined by adding 0.1 mL of enzyme solution containing 0.25 unit to 3 mL of buffer solution containing adenosine and the nucleoside dialdehyde. The concentration of adenosine ranged from 30 to 70  $\mu\text{M}$  and the concentration of dialdehyde ranged from 3 to 190  $\mu\text{M}$ , depending upon the activity of the compound. At least five substrate concentrations and two inhibitor concentrations were used for each assay. The  $K_i$  values were determined by the same reciprocal plot method. The slopes of inhibition lines were statistically different from the line containing no inhibitor, and the intercepts of all lines on the  $1/V$  axis were the same. Experiments performed to determine the percent inhibition contained 70  $\mu\text{M}$  adenosine or 1, 100  $\mu\text{M}$  nucleoside dialdehyde, and 0.25 unit of enzyme. For experiments with small nonnucleosidic aldehydes, concentrations ranged up to 100 mM. Assays of substrate and inhibitor activity of the parent

nucleosides were carried out in the same manner.

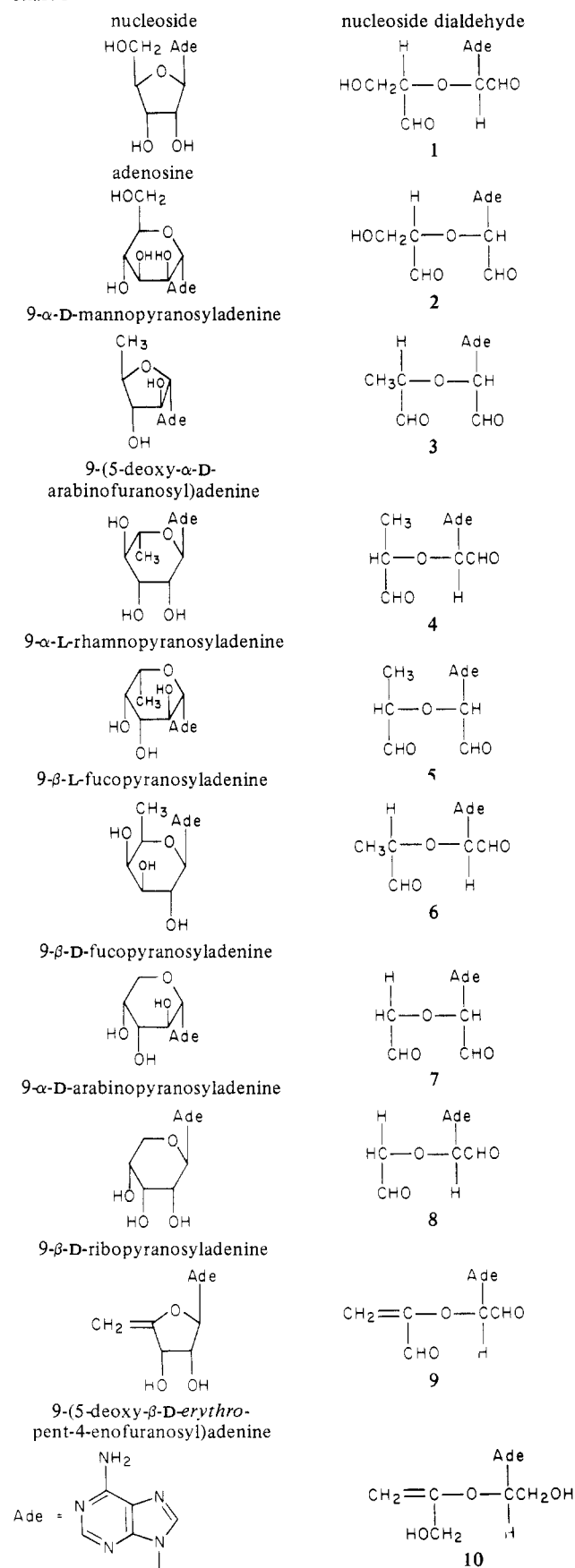
## Results

The structures of the nucleosides and their dialdehyde products **1–9** are illustrated in Chart I. The dialdehydes are represented as Fischer projection formulas. It must be realized that these are not the forms in which the dialdehydes exist, except in trace amounts, and that writing of the formulas in this way is equivalent to writing the structure of a monosaccharide as a straight-chain poly(hydroxyaldehyde) rather than as a mixture of cyclic hemiacetals. The nucleoside dialdehydes were prepared from nucleosides with paraperiodic acid so as not to introduce salts into the reaction mixtures. It should be realized that the particular nucleosides used as starting materials were chosen for convenience and that a given dialdehyde could be prepared from more than one nucleoside. For example, dialdehyde **8**, which was prepared by oxidation of 9- $\beta$ -D-ribofuranosyladenine, could have been prepared from 9- $\beta$ -D-xylopyranosyladenine as well. The nucleoside dialdehydes were obtained as white solids, free of contamination with salts or starting nucleosides, and were never exposed to either alcohols or heat. The solids contained water of hydration, considered to be mostly covalent water. Further drying under high vacuum over phosphorus pentoxide at room temperature did not alter the elemental analyses, which were calculated on the basis of the aldehyde formulas and adjusted to account for water. The elemental analyses were in best agreement with formulas having the approximate amounts of covalently bound water shown in each case. The UV spectra were nearly identical with the parent nucleosides. The infrared spectra verified the lack of carbonyl absorption peaks but had the peaks characteristic for these compounds at 3.0–3.1 (NH, OH), and 6.0–7.0  $\mu$ m (NH and purine ring) and a very broad peak at 9.0–9.6 (C–O–C, C–O–H)  $\mu$ m. These findings agree with reports of others who were not able to find an aldehyde proton by NMR spectroscopy either (Jones et al., 1976; Hansske & Cramer, 1977).

Contrary to previous findings (Cory et al., 1974), we have found that **1** is a substrate for adenosine aminohydrolase, although larger quantities of enzyme must be used to obtain the  $K_m$ . It is also an inhibitor, albeit a weak one. In fact, all of the adenine nucleoside dialdehydes tested here except **4**, **5**, and **9** were substrates, and all but **5** were competitive inhibitors of the enzyme. Table I shows the values of the kinetic constants. Tritsch (1974) has demonstrated that the  $K_m$  is approximately equal to the dissociation constant, which makes comparisons between  $K_m$  and the inhibitor constant,  $K_i$ , valid. As seen from the data in Table I, there is a fairly good correspondence between the values of  $K_m$  and  $K_i$ . Dialdehyde **1** had a very poor affinity for adenosine aminohydrolase, and yet it displayed the highest  $V_{max}$ , indicating a favorable breakdown of the enzyme–substrate complex to products. On the other hand, the substrate that had the best affinity for the enzyme was **8**; however, its  $V_{max}$  was very low. The best inhibitors among this group of dialdehydes were not substrates, namely, **4** and the  $\alpha,\beta$ -unsaturated dialdehyde **9**. The latter was the best inhibitor in the group with a  $K_i$  of 4  $\mu$ M.

Preincubation of the nucleoside dialdehydes up to 1 h with adenosine aminohydrolase resulted in no change in the degree of inhibition; the  $K_i$  values were virtually identical with those listed in Table I. Because past experiments with nucleoside or nucleotide dialdehydes have indicated a considerable amount of indiscriminate covalent binding, a few experiments were performed with small, nonnucleosidic aldehydes to see if they would bind to amino groups of the enzyme. Formaldehyde, DL-glyceraldehyde, glycolaldehyde, and pyridoxal phosphate

Chart I



failed to cause inhibition at concentrations up to 100 mM, even after preincubation with the enzyme. Pyridoxal and glutaraldehyde did weakly inhibit, but only after preincubation

Table I: Kinetic Constants for Nucleoside Dialdehydes with Adenosine Aminohydrolase and Comparison of the Inhibitor Constants with the Related Alcohols

nu- cleo- side di- alde- hyde	configuration		$K_m$ ( $\mu$ M)	$V_{max}$ [(mol/ min)/ mg]	$K_i$ ( $\mu$ M)	related <sup>a</sup> alcohol $K_i$ ( $\mu$ M)
	prox- imal car- bon	distal car- bon				
1	R	R	600	6.2	520	170
2	S	R	310	0.01	310	b
3	S	R	350	0.03	100	c
4	R	S	d	d	28	4
5	S	S	d	d	b	140
6	R	R	98	0.20	160	68
7	S		820	0.02	940	47
8	R		65	0.08	46	12
9	R		d	d	4	0.9 <sup>e</sup>

<sup>a</sup> Results from Lerner & Rossi (1972). The structures of the alcohols in Fischer projection formulas would be identical with the formulas of the dialdehydes depicted in Chart I, except that the aldehyde groups should be replaced with hydroxymethyl groups. <sup>b</sup> Not an inhibitor. <sup>c</sup> This alcohol was never prepared. <sup>d</sup> Not a substrate. <sup>e</sup> This value is for dialcohol 10 and was determined as part of the present work.

Table II: Percent Inhibition of Adenosine Aminohydrolase Using Two Different Substrates

inhibitor <sup>a</sup>	% inhibition <sup>b</sup>	
	adenosine	1
2	10	11
4	66	71
6	24	29
8	53	48

<sup>a</sup> Inhibitor concentration was 100  $\mu$ M. <sup>b</sup> Percent inhibition is the rate of deamination in the presence of an inhibitor over the rate when no inhibitor was present ( $\times 100$ ). Adenosine and 1 were the substrates at a concentration of 70  $\mu$ M.

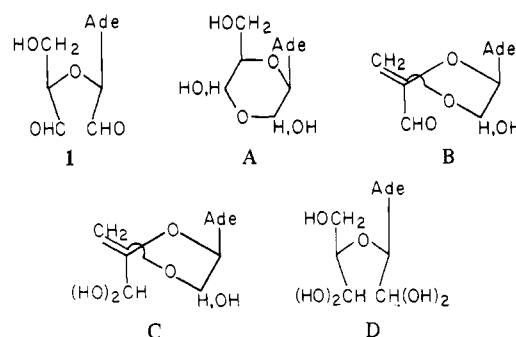
for 25 min. The percent inhibition was 6% for pyridoxal and 3% for glutaraldehyde at a concentration of 100  $\mu$ M. This time-dependent inhibition probably signifies that covalent bonds were forming to some extent.

It is possible that deamination of the nucleoside dialdehydes was caused by a contaminating protein, and not by adenosine aminohydrolase. Since these compounds are competitive inhibitors, the degree of inhibition depends on the stability of the enzyme-inhibitor complex. The nature of the substrate and its rate of reaction with the enzyme should not affect the stability of the enzyme-inhibitor complex. Therefore, the same degree of inhibition should be observed irrespective of what the substrate is. Table II shows the results of a determination of the percent inhibition of adenosine aminohydrolase by using several inhibitors and two different substrates. The substrates were adenosine and 1, and the inhibitors were compounds 2, 4, 6, and 8. The results show that there was no real significant difference in the percentage of inhibition between the two substrates. Furthermore, 4, which is not a substrate, had a  $K_i$  of 28  $\mu$ M when adenosine was the substrate, 24  $\mu$ M when 1 was the substrate, 30  $\mu$ M when 6 was the substrate, and 37  $\mu$ M when 8 was the substrate. These values are very close and represent strong evidence that the protein responsible for catalyzing the deamination was adenosine aminohydrolase.

## Discussion

Chart II is a representation of the equilibrium forms (A-D) in which a nucleoside dialdehyde, in this case adenosine dialdehyde (1), can exist. It should be realized that a structure

Chart II



like A represents four different compounds because the configurations of the hydroxyl groups can both be drawn up, both down, or on opposite sides of the ring. Similarly, structures B and C each represent two compounds. Because the aldehyde group has not been detected, structure C is probably a better representation of this hemiacetal form. In addition, a large part of the solid dialdehyde appears to be in a polymeric form (Hansske & Cramer, 1977). Although the latter is thought to be hydrolyzed upon dissolution in water, this hydrolysis may not go to completion in each individual case, and the rate of hydrolysis may differ for each compound. It appeared unlikely that the open-chain aldehyde form was the active compound because, as already discussed above, physical and chemical studies have demonstrated that no detectable free aldehyde is present except as an intermediate for the transformation of the molecules from one form to another. It did not seem likely that trace amounts of aldehydes could be such powerful inhibitors. Structural forms B and C were not considered as serious contenders because they appear to be present in high concentration only at acid pH (Khym & Cohn, 1960; Jones et al., 1976). Moreover, compounds 3-9 cannot form the internal hemiacetal ring of structures B or C because no hydroxymethyl group is available. The four forms represented by structure A are thought to be present in a large proportion. The problem here is that they contain rings with six atoms, and no nucleosides having pyranose rings have ever been shown to be substrates or inhibitors of the enzyme from calf intestinal mucosa. To test this notion further, we assayed all of the parent nucleosides with the enzyme. It was found that none of the pyranose nucleosides were substrates or inhibitors. On the other hand, the furanose nucleoside 9-(5-deoxy- $\alpha$ -D-arabinofuranosyl)adenine was a weak substrate with a  $K_m$  of 350  $\mu$ M and  $V_{max}$  of 0.26 ( $\mu$ mol/min)/mg. 9-(5-Deoxy- $\beta$ -D-erythro-pent-4-enofuranosyl)adenine has already been reported to be a substrate (Grant & Lerner, 1978). Another species that is present is structure D (Jones et al., 1976). Of all of the structures that the dialdehydes can assume, this one appears to be the most reasonable one as the form interacting with adenosine aminohydrolase. It is present in fairly large amounts (Jones et al., 1976), and it has a close resemblance to a series of inhibitors previously reported from this laboratory (Lerner & Rossi, 1972). The inhibitor data for the aldehydes bear a striking resemblance to the inhibitor data for the corresponding alcohols in both value and order of inhibitory activity as illustrated in Table I. The structures of the hydrates and the alcohols are the same as those shown for the Fischer projection formulas of the aldehyde forms in Chart I. The aldehyde groups should be replaced with the hydrated forms as shown for 1 (D) in Chart II or with hydroxymethyl groups to represent the corresponding alcohols. Although 5 and the alcohol related to 2 were not inhibitors, and the  $K_i$  values for 7 and

its related alcohol are not in good agreement, there appears to be a rather good agreement in the order of inhibitor constants. The sequence of compounds in order of decreasing  $K_i$  value is  $1 > 6 > 8 > 4 > 9$  for both the dialdehydes and the related alcohols.

Because six of the dialdehydes were substrates, it was not unreasonable to expect that some of the related alcohols would be substrates also. However, it had been reported earlier that they were not. Reinvestigation with larger quantities of enzyme revealed that at least one of the alcohols, 2-*O*-[1-(*R*)-(9-adenyl)-2-hydroxyethyl]ethanediol, the alcohol related to **8**, was a substrate with a  $K_m$  of 17  $\mu$ M and  $V_{max}$  of 0.01 ( $\mu$ mol/min)/mg. Although this was the only alcohol that acted as a substrate, the fact that it was one demonstrates that compounds with this structure are capable of acting as substrates.

In the following discussion the carbon atom bonded to N<sup>9</sup> of the purine will be termed the *proximal* carbon and the one attached to the oxygen bridge will be termed the *distal* carbon. The hydrates and aldehydes have the exact same configurational designations in the Cahn-Ingold-Prelog system. The configurations at the proximal and distal carbon atoms are shown in Table I. In general, there is a preference for the *R* configuration at the proximal carbon for binding of the compounds to the enzyme as substrates or inhibitors. If the distal carbon is a chiral center, then the *R* configuration at this center appears to favor substrate activity, whereas the limited data here and previously reported for the alcohols favor the *S* configuration for inhibitory activity. A comparison of the configurations at the proximal carbon with values for  $V_{max}$  shows that the dialdehydes having the *R* configuration had higher rates of deamination than those with the *S* configuration. It appears that the rate of deamination as determined by the rate of breakdown of the enzyme-substrate complex to form products is controlled from this position. From other experiments it would be expected that a hydroxymethyl group at the distal carbon atom would favor deamination (Grant & Lerner, 1978, and references cited therein). Substrate activity could perhaps be explained by the presence of the CH(OH)<sub>2</sub> group which can be utilized by the enzyme in place of the CH<sub>2</sub>OH group as a binding site when the latter group is otherwise not present. In this context, it should be noted that **1**, which was the CH<sub>2</sub>OH group, was the best substrate.

Some workers (Sporer et al., 1973; Dollocchio et al., 1975) have demonstrated that nucleoside and nucleotide dialdehydes can inhibit enzyme activity and bind proteins covalently, presumably through the formation of Schiff bases or carbinolamines, and evidence has been presented supporting the formation of cross-linked proteins (Cysyk & Adamson, 1976). One characteristic of these interactions is a time-dependent increased inhibition. The present work shows that no time-dependent interaction is operable and was further supported by the fact that six of the compounds were substrates. This was of interest because adenosine aminohydrolase was shown to have a lysine residue at or near the active site (Ronca et al., 1970). The present work shows that very slight alterations in structure of the carbon-chain aldehyde portion of these molecules can result in different activity and that the nature of the configuration at the two chiral centers is very important. It will be of interest to determine if a protein that reacts with nucleoside dialdehydes by covalent bonding would be as

sensitive to structural changes. It has been noted already that biological activity does differ somewhat depending upon the nature of the base (Dvornich et al., 1966; Cory & Mansell, 1976).

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