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Preformulation solubility and kinetic studies of 2′,3′-dideoxypurine nucleosides: potential anti-AIDS agents

Bradley D. Anderson [1], Michele B. Wygant [1], Tian-Xiang Xiang [1], Wanda A. Waugh [2] and Valentino J. Stella [2]

[1] *Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84108 (U.S.A.)* and [2] *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045 (U.S.A.)*

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Summary

Prior to the development of parenteral formulations of 2′,3′-dideoxyadenosine (DDA) and 2′,3′-dideoxyinosine (DDI) for preclinical and clinical testing for possible treatment of HIV infections, studies of the physicochemical properties and solution stability of these compounds were conducted. Apparent pK_a values at 25 °C, determined by titration, are 3.77 for DDA and 9.12 for DDI. Intrinsic solubilities in water at 25 °C are 43 mg/ml and 27 mg/ml for DDA and DDI, respectively. The pH–solubility profile of DDI was found to be in close agreement with that predicted for a weak acid with a pK_a of approx. 9. Adenine and hypoxanthine, the principle products of hydrolysis of DDA and DDI, respectively, exhibit much lower solubilities in water than their corresponding dideoxynucleosides but are solubilized in concentrated formulations of the nucleosides. The rates of degradation of DDA and DDI were determined as a function of pH, temperature, and drug concentration. Consistent with previous reports, acid-catalyzed hydrolysis rates increase dramatically (nearly 10^5-fold) upon removal of the 2′- and 3′-hydroxyls from adenosine and inosine. Entropies of activation are significantly more positive for the hydrolysis of DDA and DDI compared to adenosine and inosine, consistent with a carbonium ion mechanism in which the C–N bond cleavage is relatively further advanced in the transition state. Prototype solution formulations of both compounds having projected shelf-lives well in excess of 2 years at room temperature have been prepared.

Introduction

2′,3′-Dideoxyadenosine (DDA, **I**) and 2′,3′-dideoxyinosine (DDI, **II**), shown in Scheme I, are members of a broad family of 2′,3′-dideoxynucleoside analogues which are known to be potent inhibitors of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS) (DeVita et al., 1987). Upon phosphorylation of dideoxynucleosides inside a target cell to the 5′-triphosphates they become analogues of the 2′-deoxynucleotides that are the natural substrates for cellular DNA polymerases and viral DNA polymerase (reverse transcriptase). Their activity appears to lie in the fact that they bring about a selective chain termination during replication because normal 5′–3′ phosphodiester linkages cannot be completed (Yarchoan and Broder,

Correspondence: B.D. Anderson, Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84108, U.S.A.

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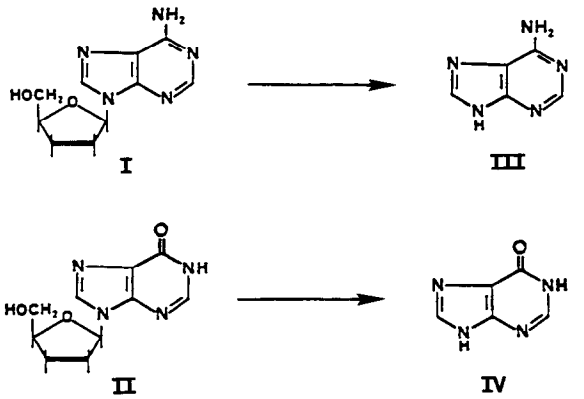

Scheme I. Structure of 2′,3′-dideoxypurine nucleosides and their major degradation products.

1986). Dideoxyadenosine is currently under development for use in HIV infections by the National Institutes of Health, through the Developmental Therapeutics Program of the NCI. The development of dideoxyinosine is also under consideration.

Prior to the development of parenteral formulations for preclinical and clinical testing of DDA and DDI, information on the physicochemical properties of these compounds, including solution stability data were needed. The results of these preformulation studies and the properties of subsequent prototype formulations are the focus of this report.

Materials and Methods

2′,3′-Dideoxyinosine (DDI) and 2′,3′-dideoxyadenosine (DDA) with reported purities of 99% were supplied by the National Cancer Institute. Adenine (**III**) and hypoxanthine (**IV**) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other compounds were reagent grade obtained from commercial sources and were used without further purification.

High-performance liquid chromatography (HPLC) was performed using one of two modular systems. One consisted of 2 Beckman model 110B pumps linked to a model 421A controller (Beckman Instruments, San Ramon, CA), a Waters model 480 Variable Wavelength Detector (Waters Associates, Milford, MA) operated at 254 nm, and a Waters Data Module (model 730) integrator. The second system consisted of a Waters model 6000A pump, a Kratos Spectroflow 757 detector operated at 254 nm, and an Omni Scribe Recorder. pH measurements were taken with a PHM82 standard pH meter (Radiometer America, Cleveland, OH) using a Ross combination pH electrode (Orion Research, Boston, MA).

HPLC analyses. HPLC studies were conducted using reverse-phase C_8 (Shandon 5 μm MOS Hypersil) or C_{18} (μBondapak, 4.6 mm $\times$ 250 mm, 10 μm) columns with acetonitrile/water mobile phases. For DDA, the mobile phase contained 10% acetonitrile with pH 7 phosphate buffer. Retention volumes (μBondapak column) were: adenine 5.5 ml; dideoxyadenosine 10.4 ml. For DDI, 5% acetonitrile was employed with the same pH 7 phosphate buffer. Retention volumes were: hypoxanthine 4.7 ml; dideoxyinosine 9.4 ml.

Solubility studies. Solubilities were determined by adding an amount of compound well in excess of its saturation solubility to the solvent of interest. NaOH was added to adjust pH of unbuffered systems as needed. Samples were rotated in a 4°C controlled-temperature chamber or 25°C shaking water bath and allowed to equilibrate for at least 24 h. pHs were recorded and the samples were filtered (0.45 μm, Gelman, ACRO LC3A), diluted, and analyzed by HPLC.

pK_a determinations. The pK_a of DDA was determined in water and in 0.15 M NaCl by titrating a 0.05 M solution of drug with a standardized solution of 0.1 N HCl. Similarly, a 0.01 M solution of DDI in water was titrated with 0.1 N NaOH to determine its pK_a. No corrections were made for ionic strength in these studies.

Kinetic studies. Degradation of the dideoxynucleosides was studied over a pH range of 1–13 and a temperature range of 4–80°C. For these studies, a weighed quantity of drug was dissolved in buffer to give a final concentration of approximately 1.0×10^{-4} M. Buffers selected varied in buffer concentration and ionic strength. 50 mg/ml (pH 9.5), 25 mg/ml (pH 9.5), and 20 mg/ml (pH 7.4) concentrations of DDI in water were also monitored for stability, as well as 25 mg/ml solutions of DDA in 0.1 M tris buffer (pH

8.40) or 0.1 M NaCl (pH 7.56). Samples were stored in water baths or controlled temperature chambers and aliquots were taken out at appropriate intervals for analysis by HPLC. In the HPLC analyses both disappearance of reactant and appearance of either adenine or hypoxanthine were monitored. Rate constants were calculated from first-order plots of disappearance of the starting material or from the initial rates of product formation.

Studies of the stability of DDA and DDI after diluting reconstituted lyophilized DDA and DDI formulations in various quantities of normal saline or 5% dextrose in water (D5W) were also conducted by storing the diluted samples at room temperature and monitoring the % of initial concentration remaining over a 48 h period. The reconstituted DDA formulations used for this study contained 25 mg/ml of 2′,3′-dideoxyadenosine and 25 mg/ml of mannitol. The reconstituted DDI preparations contained either 20 mg/ml 2′,3′-dideoxyinosine with pH adjusted to 7.4 with sodium hydroxide or 50 mg/ml 2′,3′-dideoxyinosine with pH adjusted to 9.5 with sodium hydroxide.

Results and Discussion

pK_a values and aqueous solubilities

Apparent pK_a values of DDA and DDI in water, uncorrected for activity coefficients, were obtained by titration with standard HCl (DDA) or NaOH (DDI) at room temperature. The apparent pK_a of DDA was found to be 3.77 ± 0.11 (mean $\pm$ S.D. of the results obtained in two laboratories). This value is comparable to the pK_a values at 25 °C reported for structurally related analogues including adenosine (3.45–3.63), 2′-deoxyadenosine (3.80), 3′-deoxyadenosine (3.71), and adenine (4.1–4.22) (Garrett and Mehta, 1972; Christensen et al., 1970). The apparent pK_a of dideoxyinosine was found to be 9.12 ± 0.02 ($\pm$ S.D., $n = 2$), in comparison to the pK_a of 8.96 reported for inosine and 8.91 for hypoxanthine (Christensen et al., 1970).

The site of protonation in adenine and adenine nucleosides has been assigned to both the N_1H^+ (Christensen and Izatt, 1962; Cochran, 1951; Jardetsky and Jardetsky, 1960; Zubay, 1958) and the $C_6NH_3^+$ groups (Alberty et al., 1951; Beers and Steiner, 1958; Cheney et al., 1959; Levene and Simms, 1925; Taylor, 1948). The site of proton removal corresponding to the pK_a values of inosine and hypoxanthine reported above is the enol form 6-hydroxyl (Albert, 1953).

Due to the relatively high doses anticipated, solution formulations containing $\geqq 25$ mg/ml DDA or DDI were desired. Reliable solubility data were therefore essential to determine the feasibility of parenteral formulations at these concentrations.

Solubility data obtained for dideoxyadenosine, dideoxyinosine, adenine, and hypoxanthine under various conditions (one determination per condition) are reported in Table 1. Because of the relatively high water solubility of dideoxyadenosine, pH–solubility studies were not necessary. Such data were generated for dideoxyinosine, however, which exhibits a somewhat lower intrinsic solubility, to identify a pH range at which higher solubility could be achieved. Shown in Fig. 1 is the pH solubility profile found for DDI at 25 °C. The solid line in Fig. 1 represents the theoretical curve obtained by non-linear least-squares regression (MINSQ, MicroMath, Salt Lake City, UT) for the solubility of a weak acid with an intrinsic solubility of 27 mg/ml and a pK_a of 8.92 – in close agreement with the pK_a values reported above. Evident from this pH–solubility profile, concentrated solution formulations (50 mg/ml) of DDI required a pH of > 9.0.

The major degradation products formed in solution formulations of dideoxyadenosine and dideoxyinosine, as depicted in Scheme I are the corresponding purines, adenine (**III**) and hypoxanthine (**IV**), respectively (see following section). Preliminary solubility studies suggested that the purines are significantly less soluble in water than their corresponding dideoxynucleosides (Table 1). Such solubility differences between the starting compound and its degradation product(s) can potentially limit the shelf-life of solution formulations if the saturation solubility of the degradation product is exceeded before 10% degradation occurs. However, solubility studies of adenine and

TABLE 1

Solubility of 2′,3′-dideoxynucleosides and their hydrolysis products in various solvent environments at 4 and 25 °C

Compound	Solvent	Temp. (°C)	pH	Solubility (mg/ml)
DDA	H_2O	4	–	28.9
	H_2O	25	7.9	43.2
	D5W [a]	25	7.6	45.0
	0.9% NaCl	25	7.54	38.1
Adenine	H_2O	25	–	0.89
	H_2O (w/25 mg/ml DDA)	25	–	2.52
	0.1 M Tris	25	8.40	0.97
	0.1 M Tris (w/25 mg/ml DDA)	25	8.40	2.64
DDI	H_2O [b]	4	7.02	10.9
	H_2O [c]	4	9.28	42.9
	H_2O [d]	25	6.21	27.3
	H_2O [d]	25	8.06	31.1
	H_2O [d]	25	8.60	38.2
	H_2O [d]	25	9.06	67.6
	H_2O [d]	25	9.64	188
	H_2O [d]	25	9.71	224
	H_2O [d]	25	10.01	429
	H_2O [d]	25	10.18	460
Hypoxanthine	H_2O [d]	25	7.4	0.72
	H_2O (w/20 mg/ml DDI) [d]	25	7.4	0.99
	H_2O [d]	25	9.5	2.09
	H_2O (w/50 mg/ml DDI) [d]	25	9.5	3.75

[a] 5% Dextrose Injection U.S.P.
[b] 0.01 μ phosphate buffer.
[c] 0.01 μ borate buffer.
[d] pH adjusted with NaOH.

hypoxanthine in concentrated formulations of dideoxyadenosine and dideoxyinosine, respectively, showed that both purines are solubilized by their corresponding nucleosides.

The solubilization of adenine and hypoxanthine in solution formulations of dideoxyadenosine and dideoxyinosine is consistent with the known self-association of purine nucleosides through stacking interactions (Broom et al., 1967). In agreement with the order of the association tendencies reported previously (adenosine and deoxyadenosine > inosine) (Broom et al., 1967), adenine is solubilized more effectively by dideoxyadenosine than hypoxanthine is solubilized by dideoxyinosine (Table 1).

Based on the literature for related compounds and the above solubilization data, one would expect that the self-association of DDI is likely to be weak but not negligible. In the pH–solubility profile for DDI (Fig. 1) discussed previously, a positive deviation from the theoretical curve would be expected at higher concentration if significant self-association occurs. Such deviation is not observed, suggesting that the ionized form of the purine base does not self-associate to any significant extent. This is not in conflict with prior findings, since it is intuitively reasonable that charge repulsion would counteract the relatively weak attractive forces exhibited between neutral hypoxanthine rings.

Hydrolysis of dideoxynucleosides

The degradation of dideoxyadenosine and dideoxyinosine in dilute aqueous solutions (approx. 1×10^{-4} M) was monitored as a function of temperature and pH. The kinetic data obtained (one experiment per condition) are shown in Tables 2 and 3 for dideoxyadenosine and dideoxyinosine, respectively.

For dideoxyadenosine, rate constants were obtained at low (0.01 μ) and at high (0.15 μ) ionic strengths and as a function of acetate buffer con-

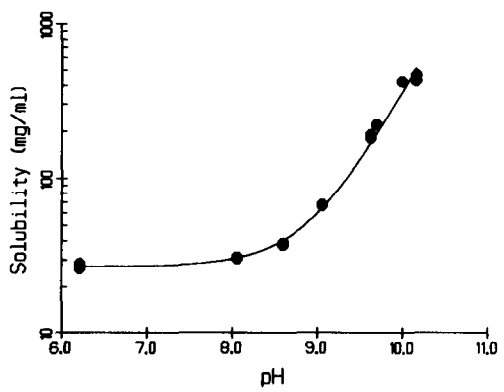

Fig. 1. 2′,3′-Dideoxyinosine solubility versus pH at 25 °C. Solid line represents the theoretical curve obtained by non-linear least-squares regression for a weak acid with an intrinsic solubility of 27 mg/ml and a pK_a of 8.92.

TABLE 2

Apparent first-order rate constants for the degradation of 2′,3′-dideoxyadenosine in dilute aqueous solutions

Temp. (°C)	Buffer	pH	Ionic strength	k_{obs} (h^{-1})
25	HCl	1.0	0.15	18.5
		1.0	0.15	17.9
		1.74	0.018	2.61
		1.9	0.15	1.75
		2.5	0.15	4.48×10^{-1}
		2.5	0.15	4.48×10^{-1}
	Chloroacetate [a]	2.32	0.01	6.15×10^{-1}
	Formate [a]	3.32	0.01	6.05×10^{-2}
	Acetate, 0.10 M	3.5	0.15	6.87×10^{-2}
	Acetate, 0.05 M	3.5	0.15	6.86×10^{-2}
	Acetate, 0.025 M	3.5	0.15	6.24×10^{-2}
	Acetate [a]	4.38	0.01	5.61×10^{-3}
	Acetate, 0.05 M	4.5	0.15	6.62×10^{-3}
		5.4	0.15	5.70×10^{-4} [c]
	Phosphate [a]	6.57	0.01	6.74×10^{-5}
	Tris [a]	8.46	0.01	1.90×10^{-6} [b]
	NaOH	12.85	0.10	1.50×10^{-4} [c]
50	Acetate, 0.05 M	5.4	0.15	2.50×10^{-2}
	Phosphate, 0.05 M	7.0	0.15	6.42×10^{-4}
	Phosphate [a]	7.05	0.01	5.28×10^{-4} [b]
	Phosphate, 0.05 M	8.0	0.15	1.10×10^{-4} [c]
	Borate [a]	9.0	0.01	1.08×10^{-4} [b]
	Carbonate, 0.05 M	9.25	0.15	6.60×10^{-5} [c]
		10.4	0.15	8.90×10^{-5} [c]
	NaOH	12.0	0.15	1.25×10^{-3}
65	Phosphate [a]	7.05	0.01	1.57×10^{-3}
	Borate [a]	8.99	0.01	3.24×10^{-4} [b]
	KOH	12.75	0.10	3.85×10^{-3}
80	Phosphate [a]	7.09	0.01	9.55×10^{-3}
	Borate [a]	9.02	0.01	2.27×10^{-3}
	KOH	12.8	0.10	1.20×10^{-2}

[a] (Perrin and Dempsey, 1974).
[b] Calculated from initial rate of adenine formation.
[c] Based on disappearance data over less than one half-life.

centration. Neither ionic strength nor acetate buffer concentration had a significant effect on the acid-catalyzed rate constant. Thus, all the data were pooled in further analyses. The absence of general acid–base catalysis by acetate buffers has been reported previously in the solvolysis of 2′-deoxyadenosine (Garrett and Mehta, 1972a), psicofuranine (Garrett, 1960), and 5-iodo-2′-deoxyuridine (Garrett et al., 1965). No systematic studies of ionic strength or buffer effects were conducted for dideoxyinosine.

Generally, when reaction rates were sufficiently rapid, apparent first-order rate constants were obtained from the disappearance of nucleoside versus time. Except at higher pH (> 10 in our studies), mass balance calculations on the adenine and dideoxyadenosine or hypoxanthine and dideoxyinosine concentrations in partially degraded samples indicated that the only reaction occurring is hydrolysis of the glycosyl–purine bond. Therefore, at low temperatures near the pH–rate minimum apparent first-order rate constants were calculated from the initial rates of formation of

TABLE 3

Apparent first-order rate constants for the degradation of 2′,3′-dideoxyinosine in dilute aqueous solutions

Temp. (°C)	Buffer [a]	pH	k_{obs} (h^{-1})
25	Chloroacetate	2.34	3.04
	Formate	3.35	0.298
	Acetate	4.43	2.68×10^{-2}
	Phosphate	6.63	2.34×10^{-4}
	Tris	8.49	1.60×10^{-5} [b]
		8.50	8.33×10^{-6} [b]
	Borate	9.00	4.21×10^{-6} [b]
		9.37	2.58×10^{-6} [b]
	Carbonate	9.80	1.65×10^{-6} [b]
	KOH	12.8	5.0×10^{-7} [c]
50	Phosphate	7.05	2.22×10^{-3}
			2.58×10^{-3}
	Borate	8.95	1.53×10^{-4} [b]
			2.07×10^{-4}
	Carbonate	9.78	4.70×10^{-5}
	KOH	12.8	2.40×10^{-5}
65	Phosphate	7.05	1.11×10^{-2}
			1.07×10^{-2}
	Borate	8.95	1.32×10^{-3} [b]
			1.34×10^{-3}
	Carbonate	9.69	3.00×10^{-4}
	KOH	12.8	2.88×10^{-4}
80	Phosphate	7.06	4.91×10^{-2}
			4.75×10^{-2}
	Borate	8.95	5.1×10^{-3}
			6.05×10^{-3}
	Carbonate	9.76	2.94×10^{-3}
	KOH	12.8	1.50×10^{-3}

[a] All buffers were 0.01 μ ionic strength (Perrin and Dempsey, 1974).
[b] Determined from initial rate of hypoxanthine formation.
[c] Estimated from data at higher temperatures.

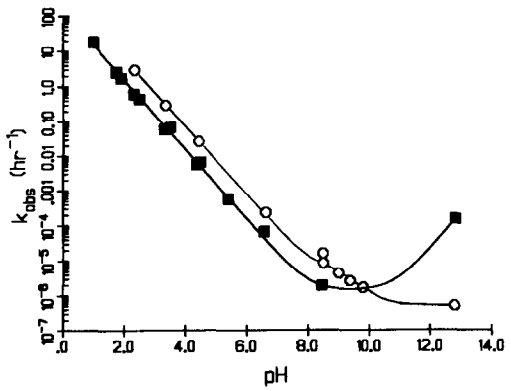

Fig. 2. Apparent first-order degradation rate constants versus pH at 25 °C for 2′,3′-dideoxyadenosine (■) and 2′,3′-dideoxyinosine (○). The solid lines are the theoretical curves obtained using Eqns. 1 and 2 and the parameter values listed in Table 4.

adenine or hypoxanthine. Rate constants obtained under the same conditions by the two methods were identical within experimental error.

The pH–degradation rate data at 25 °C for dideoxyadenosine and dideoxyinosine are plotted in Fig. 2. The observed first-order rate constants for the degradation of dideoxyadenosine were analyzed by non-linear least-squares regression according to Eqn. 1 to obtain estimates of the bimolecular rate constants for hydrogen ion-catalyzed hydrolysis of both the protonated (ADH^+) and neutral (AD) species of dideoxyadenosine, k_{H^+} and k'_{H^+}, uncatalyzed hydrolysis of the neutral species, k_{H_2O} and alkaline hydrolysis, k_{OH}.

$$k_{obs} = k_{H^+}[H^+] \cdot f_{ADH^+} + k'_{H^+}[H^+] \cdot f_{AD} + k_{H_2O}[H_2O] \cdot f_{AD} + k_{OH}[OH^-] \quad (1)$$

where $f_{ADH^+} = H^+/(H^+ + K_a)$ and $f_{AD} = K_a/(H^+ + K_a)$ and $K_a = 1.70 \times 10^{-4}$. The parameter values obtained from the regression analysis are listed in Table 4. Previous kinetic studies of the hydrolysis of 2′-deoxyadenosine and adenosine found large differences of 2–3-fold in the reactivities of the protonated and neutral species toward acid-catalyzed hydrolysis (Garrett and Mehta, 1972a). Although the fit was slightly improved as measured by the sum of squares of the deviations between calculated and observed values when terms representing the hydrogen ion-catalyzed hydrolysis of both the protonated and neutral forms were included in the model, as compared to the fit when ionization was neglected, the differences in the two rate constants were small (< 15%) and not significant. Since the better fit obtained by the model which takes into account the ionization of DDA is at the expense of an additional parameter, the Akaike Information Criterion was applied to compare the two models (Akaike, 1973, 1976). The AIC requires the model with more parameters to not only have a better coefficient of determination but quantifies how much better it must be for the

TABLE 4

Bimolecular rate constants and thermodynamic parameters for the hydrolysis of 2′,3′-dideoxyadenosine (DDA) and 2′,3′-dideoxyinosine (DDI)

Compound	Rate const.	k ($l \cdot mol^{-1} \cdot h^{-1}$)				Activation parameters	
		25 °C	50 °C	65 °C	80 °C	$\Delta H^{\neq}$ (kcal · mol^{-1})	$\Delta S^{\neq}$ (eu)
DDA	k_{H^+} [a]	147					
	k'_{H^+} [a]	168	5.6×10^3	1.42×10^4	9.1×10^4	22.8	+12.2
	k_{H_2O} [a]	1.4×10^{-6}	6.6×10^{-5}	3.09×10^{-4}	2.18×10^{-3}	27.0	−18.8
	k_{OH} [a]	2.1×10^{-3}	0.021	0.063	0.156	15.8	−33.8
DDI	k_{H^+} [b]	733	2.41×10^4	1.03×10^5	4.53×10^5	23.9	+18.9
	k'_{H^+} [b]	9.4×10^3	2.81×10^5	1.60×10^6	1.05×10^7	25.8	+21.9
	k_{H_2O} [b]	4.9×10^{-7}	2.21×10^{-5}	2.12×10^{-4}	1.54×10^{-3}	31.4	+0.9

[a] Eqn. 1.
[b] Eqn. 2.

model to be more appropriate. Based on this criterion, the two models are nearly equally appropriate. We therefore chose the model represented by Eqn. 1 which, though more complex, is more consistent with the literature for structurally related compounds.

Similarly, the apparent first-order rate constants for the degradation of dideoxyinosine were fitted to Eqn. 2 to obtain estimates of the bimolecular rate constants for hydrogen ion-catalyzed hydrolysis of both the neutral (INH) and anionic (IN^-) species of dideoxyinosine, k_{H^+} and k'_{H^+}, and uncatalyzed hydrolysis of the anion, k_{H_2O}.

$$k_{obs} = k_{H^+}[H^+] \cdot f_{INH} + k'_{H^+}[H^+] \cdot f_{IN^-} + k_{H_2O}[H_2O] \cdot f_{IN^-} \quad (2)$$

where $f_{INH} = H^+/(H^+ + K_a)$ and $f_{IN^-} = K_a/(H^+ + K_a)$ and $K_a = 7.4 \times 10^{-10}$. The parameter values obtained are also listed in Table 4. Again the necessity of including separate terms for the reactivity of the neutral and ionized species toward acid-catalyzed hydrolysis was evaluated. The model described by Eqn. 2 was shown by application of the Akaike Information Criterion to be statistically superior to a two parameter equation which did not take into account the ionization of dideoxyinosine.

Activation enthalpies and entropies for the various specific rate constants were derived from the slopes of Eyring plots (Eyring, 1935) of the bimolecular rate constants as a function of temperature. The plots generated for these data are displayed in Figs. 3 and 4. The activation parameters are included in Table 4.

From a practical standpoint, the acid-catalyzed hydrolysis rates of dideoxypurine nucleosides are most important, as these compounds are unstable in even mildly acidic solutions. For example, the times estimated for 10% decomposition at 37 °C and pH < 3, conditions likely to be encountered upon oral administration, are < 10 min and < 2 min for DDA and DDI, respectively. As illustrated in a later section of this paper, dilution of relatively stable solution formulations in mildly acidic diluents (e.g. D5W) may also result in unexpectedly rapid degradation.

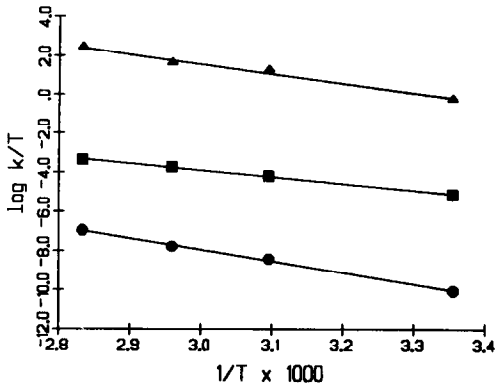

Fig. 3. Eyring plots for the bimolecular rate constants for degradation of 2′,3′-dideoxyadenosine as a function of temperature. Key: ▲, k'_{H^+}; ●, k_{H_2O}; ■, k_{OH}.

Several pieces of evidence suggest that the acid-catalyzed hydrolysis of purine nucleosides occurs via rate-limiting unimolecular decomposition of protonated and diprotonated forms of the substrate to a glycosyl carbonium ion and the free purine (Garrett and Mehta, 1972a; York, 1981; Zoltewicz et al., 1970; Zoltewicz and Clark, 1972; Hevesi et al., 1972; Panzica et al., 1972; Romero et al., 1978). The alternative mechanism involving sugar ring opening to form a Schiff base intermediate (Kenner, 1957) is now considered unlikely.

Our results are also consistent with the carbonium ion pathway. First, removal of the 2′- and 3′-hydroxyl groups results in dramatic in-

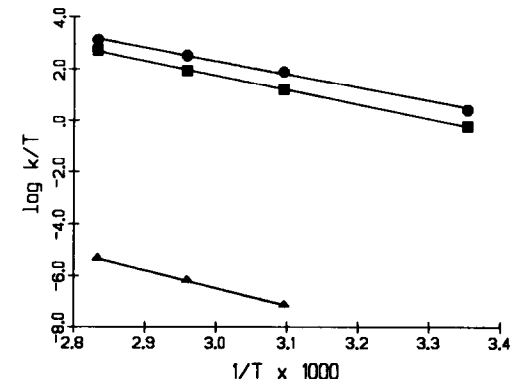

Fig. 4. Eyring plots for the bimolecular rate constants for degradation of 2′,3′-dideoxyinosine as a function of temperature. Key: ●, k_{H^+}; (●), k'_{H^+}; (▲), k_{H_2O}.

creases in the acid-catalyzed hydrolysis rates in both dideoxyadenosine and dideoxyinosine, in agreement with the reports of others (Venner, 1964; Garrett and Mehta, 1972a; York, 1981). Compared to estimates from previously reported data for the rate constants at 25 °C for the hydrogen ion-catalyzed hydrolysis of the neutral species of adenosine (Garrett and Mehta, 1972a) and inosine (Suzuki, 1974), removal of the 2′- and 3′-hydroxyls increases the rate by 8×10^4 (DDA vs adenosine) and 6×10^4 (DDI vs inosine). The increase estimated in this study compares favorably with the 4×10^4-fold increase reported by York for DDA vs adenosine at 40 °C (York, 1981). The 2′- and 3′-hydroxyls are believed to decrease the lability of the C–N bond by destabilizing the developing carbonium ion through an inductive effect. Changing the purine portion of the molecule from adenine to hypoxanthine increases reactivity by 5–6-fold regardless of the number of hydroxyls in the glycoside portion.

In a comparison of adenosine and 2′-deoxyadenosine, the number of hydroxyl groups in the sugar moiety did not appear to have a highly significant effect on rates of alkaline hydrolyses (Garrett and Mehta, 1972b). Our results support this conclusion and extend it to the dideoxynucleosides. In 0.1 M NaOH or KOH and 80 °C the apparent first-order rate constants are 0.026 h^{-1}, 0.043 h^{-1}, and 0.012 h^{-1} for adenosine (Garrett and Mehta, 1972b), 2′-deoxyadenosine (Garrett and Mehta, 1972b), and 2′,3′-dideoxyadenosine, respectively. The degradation rates of inosine and dideoxyinosine in 0.1 M NaOH or KOH appear to be pH independent although hydroxyl ion catalysis has been reported at still higher pH (Suzuki, 1974). In 0.1 M base and 80 °C the apparent first-order rate constant for inosine degradation, estimated from data at higher temperatures (Suzuki, 1974), is 2.75×10^{-3} h^{-1} compared to 1.5×10^{-3} h^{-1} obtained for dideoxyinosine. Thus, the number of hydroxyl groups in the sugar moiety has little influence on degradation rates in the alkaline region.

A second observation supporting the carbonium ion pathway in the acid-catalyzed hydrolysis is the fact that the entropies of activation for these reactions are usually only slightly less than zero or positive (Zoltewicz and Clark, 1972; Garrett and Mehta, 1972a; York, 1981). For example, the values of $\Delta S^{\neq}$ reported for adenosine, 3′-deoxyadenosine and 2′-deoxyadenosine are −3.8 eu, −8.3 eu, and +1.2 eu, respectively (Garrett and Mehta, 1972a). Bimolecular reactions are characterized by large negative $\Delta S^{\neq}$ values – about 20 eu more negative than observed for unimolecular mechanisms (Long and Paul, 1957). Our results show that whereas the enthalpies of activation are not changed significantly with a decrease in the number of hydroxyl groups in the sugar moiety ($\Delta H^{\neq}$ values reported for adenosine, 3′-deoxyadenosine, and 2′-deoxyadenosine are 24.8, 21.8, and 23.2, respectively (Garrett and Mehta, 1972a)), the $\Delta S^{\neq}$ values obtained for the hydrogen ion-catalyzed hydrolysis become significantly more positive with removal of both the 2′- and 3′-hydroxyl groups (Table 4). This is consistent with a carbonium ion mechanism in which C–N bond cleavage is relatively advanced in the transition state of the dideoxynucleosides compared to their hydroxy containing analogues.

Formulation stability studies

Both dideoxyadenosine and dideoxyinosine can be formulated acceptably as lyophilized products. However, ready-made sterile solutions would offer several advantages. Dilute solution kinetic studies suggested that at or near their pH–degradation rate minima, both DDA and DDI are sufficiently stable to allow formulation as sterile solutions. However, in view of the fact that self-association has been well-documented in solutions of purine-containing nucleosides, the solution stability of higher concentrations of solute may differ from that in dilute solutions. Concentrated solution formulations of DDA (25 mg/ml in 0.1 M Tris (pH 8.4) and 25 mg/ml in 0.1 M NaCl (pH 7.56)) and DDI (20 mg/ml in water (pH adjusted to 7.4 with NaOH) and 50 mg/ml in water (pH adjusted to 9.5 with NaOH)) stored at various temperatures were monitored for disappearance of DDA or DDI and appearance of adenine and hypoxanthine with time. The DDA results represent the repeated analyses of a single sample. The DDI data are averages of 4 samples for each condition, two inverted and two upright. No differences in

TABLE 5

Apparent first-order rate constants for hydrolysis and projected shelf-lives of 2',3'-dideoxyadenosine and 2',3'-dideoxyinosine in concentrated solution formulations at various temperatures

Compound	Conc. (mg/ml)	Diluent	pH	Temp. (°C)	k_{obs} (h^{-1})	Projected shelf-life (days)
DDA	25	0.1 M NaCl	7.56	25	9.0×10^{-6} [a]	486 [a]
				37	5.6×10^{-5} [b]	78
				50	5.6×10^{-4} [b]	7.8
				70	6.7×10^{-3} [b]	1.3
DDA	25	0.1 M Tris	8.40	25	8.5×10^{-7} [a]	5150
				37	7.8×10^{-6} [b]	561
				50	1.2×10^{-4} [b]	36
				70	3.53×10^{-3}	1.2
DDI	20	H_2O w/NaOH to adjust pH	7.40	25	2.2×10^{-5} [c]	202
				37	2.8×10^{-4} [c]	16
				50	2.9×10^{-3}	1.5
DDI	50	H_2O w/NaOH to adjust pH	9.50	25	8.3×10^{-7} [a]	5250
				37	8.6×10^{-6} [c]	509
				50	9.7×10^{-5} [c]	45

[a] Estimated from Arrhenius plots of higher temperature data.
[b] Rate constants obtained from disappearance over less than one half-life.
[c] Rate constants obtained from initial rates of product formation.

the hydrolysis kinetics were observed between the samples stored upright and inverted. From these results estimated rate constants at 25 °C were obtained to determine the feasibility of formulating these products as sterile solutions. The observed rate constants found at various temperatures are listed in Table 5 along with the projected shelf-lives assuming 10% degradation can occur. No dramatic differences were observed between the rate constants in dilute solutions and the rate constants in concentrated formulations.

Solubilization of the purine degradation products by the dideoxynucleosides plays an important role in determining the shelf-life of concentrated formulations. In the absence of solubilization less than 10% degradation in concentrated formulations would lead to supersaturation with respect to the corresponding purines, as evident in the solubility data in Table 1. With solubilization, which presumably results from stacking interactions between the purine rings, supersaturation of concentrated formulations prior to 10% degradation should not occur.

These preliminary stability and solubility data suggest that it should be possible to formulate dideoxyadenosine at 25 mg/ml in 0.1 M Tris buffer at pH 8.4 and dideoxyinosine at 50 mg/ml and pH 9.5 as ready-made solutions with greater than 2 year shelf-lives at 25 °C.

Stability in i.v. fluids

To assess the stability of DDA and DDI after dilution for use in infusion sets lyophilized formulations of dideoxyadenosine and dideoxyinosine which were reconstituted with sterile water for injection were diluted to various concentrations in either normal saline or 5% dextrose in water, maintained at room temperature, and analyzed for drug content over a 48 h period. The formulations evaluated, the drug concentration after dilution, the diluent, container, solution pH after the dilution, and drug concentration remaining are shown in Table 6. Results represent means ± S.D. of 2 samples per condition.

2',3'-Dideoxyadenosine appears to be stable for > 24 h when diluted with D5W or normal saline. The stability can be compromised in acidic D5W solutions, but even under the worst pH conditions

TABLE 6

Percent of DDA or DDI remaining at various times after dilution of reconstituted formulations in i.v. infusion fluids at room temperature

Drug (formulation)	Conc. (mg/ml)	Diluent	Container	pH after dilution	Time (h)	% Drug remaining
DDA (25 mg/ml w/mannitol)	2	D5W	glass	5.73 ± 0.03	24	97.9 ± 0.2
					48	100.0 ± 1.4
		NS		6.69 ± 0.03	24	100.5 ± 0.5
					48	99.0 ± 1.9
	0.5	D5W		5.06 ± 0.04	24	99.3 ± 0.0
					48	93.6 ± 5.6
		NS		6.23 ± 0.01	24	100.9 ± 0.0
					48	100.0 ± 0.9
DDI (50 mg/ml, pH 9.5)	0.5	D5W	glass [a]	9.26 [c]	8	99.7 ± 1.9
					24	95.8 ± 0.5
			PVC [b]	9.28 [c]	8	99.5 ± 0.5
					24	99.7 ± 1.3
		NS	glass [a]	9.25 [c]	8	98.5 ± 3.0
					24	95.7 ± 1.3
			PVC [b]	9.31 [c]	8	98.9 ± 0.5
					24	97.1 ± 1.8
DDI (20 mg/ml, pH 7.4)	0.2	D5W	glass [a]	6.56 ± 0.22	8	100.1 ± 1.8
					24	97.5 ± 0.7
					48	94.4 ± 0.7
			PVC [b]	5.74 ± 0.16	8	94.6 ± 1.7
					24	83.9 ± 1.9
					48	70.0 ± 0.7
		NS	glass [a]	6.27 ± 0.02	5	99.1 ± 0.9
					48	98.2 ± 1.3
			PVC [b]	6.49 ± 0.06	5	100.6 ± 2.6
					48	97.9 ± 0.4

[a] Kendall McGaw, 100 ml partial fill glass bottles.
[b] Abbott, 250 ml PVC bags.
[c] pH measured in a separate experiment.

in D5W, 24 h stability is maintained. Two formulations of dideoxyinosine were evaluated – one containing 20 mg/ml at a pH of 7.4 and a second containing 50 mg/ml of drug at a pH of 9.5. Due to the higher buffer capacity of the latter formulation and the resulting higher pH after a 100-fold dilution, no instability was observed over the period of study. The pH 7.4 formulations had very low buffer capacity, however, as evident in the significantly lower pH values obtained after a 100-fold dilution. Dilutions of pH 7.4 formulations in D5W, particularly, are likely to exhibit variable stability, depending on the initial pH and buffer capacity of the diluent and the extent of dilution. Under the conditions examined in this study, storage of 100-fold dilutions in D5W of the pH 7.4 formulations for a period of > 8 hours would not be recommended.

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