International Journal of Pharmaceutics, 60 (1990) 53–60
Elsevier

IJP 02027

Stability and solubility of 2-chloro-2′,3′-dideoxyadenosine

L.A. Al-Razzak and V.J. Stella

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 30 May 1989)
(Modified version received 19 October 1989)
(Accepted 6 November 1989)

Key words: Chloro-2′,3′-dideoxyadenosine,2-; Chloroadenine,2-; Dideoxyribose, 2′,3′-; pK_a; Cytotoxicity; Nucleoside; Hydrolysis; Solubility; Stability; AIDS

Summary

2-Chloro-2′,3′-dideoxyadenosine (2-ClDDA) is an experimental antiviral and cytotoxic agent. Since little was known about the chemical stability and physical properties of this experimental agent and its degradation products, the purpose of this study was to determine its stability and solubility and to develop prototype parenteral dosage forms. The hydrolysis of 2-ClDDA in various aqueous buffered solutions was determined. The variables studied were pH, temperature, buffer type and concentration. The pH-rate profile was analyzed and rate constants capable of describing the profile were calculated. In the pH range 1–10.5, 2-chloroadenine was the principal degradation product detected by HPLC. Presumably, 2,3-dideoxyribose was also formed but its presence was not determined. The solubilities of 2-ClDDA and 2-chloroadenine were determined in various solvents. A mixture of propylene glycol, ethanol, water at several different ratios provided the desired solubility of > 5.0 mg/ml. 2-ClDDA had an apparant pK_a of 2.2, as determined spectrophotometrically. The pK_a was assigned to protonation of the N_1 group, however protonation of the amino group at the C_6 position cannot be ruled out. Prototype solution formulations having projected shelf-lives in excess of 2 years at room temperature were developed. The shelf-lives were limited by the time for precipitation of the 2-chloroadenine rather than the time for 10 % degradation of 2-ClDDA.

Introduction

2-Chloro-2′,3′-dideoxyadenosine (2-ClDDA, Rosowsky et al., 1989) is a member of the family of 2′,3′-dideoxyadenosine nucleoside analogs which exhibit potent inhibition of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired imunodeficiency syndrome, AIDS (DeVita et al., 1987). Based on personal communication with the National Cancer Institute it has also shown cytotoxic activity. Since little is known about the chemical stability and physical properties of this experimental agent and its degradation products, this study was undertaken with the purposes of determining its stability and solubility and developing some prototype parenteral dosage forms.

Correspondence: V.J. Stella, 3006 Malott Hall, Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045, U.S.A.

Experimental:

Material and methods

2-ClDDA (NSC-619531) was supplied by the National Cancer Institute (Bethesda, MD) (Lot no. HLR-02-54). All other chemicals including

0378-5173/90/$03.50 © 1990 Elsevier Science Publishers B.V. (Biomedical Division)

buffer components were reagent grade obtained from commercial sources and were used without further purification. All organic solvents were HPLC grade. Aqueous solutions and buffers were prepared with deionized and glass distilled water (Mega-Pure System, Model MP-1, Corning). All pH measurements were conducted at 25 °C with a digital pH meter (Model 155, Corning, Medfield, MA). ^{1}H- and ^{13}C-NMR spectroscopy was performed at 300 MHz with a Varian XL-300 spectrometer using (dimethyl sulfoxide)-d_6 as solvent (Aldrich, 99.9%). Chemical shifts (δ, ppm) are reported for band centers relative to tetramethylsilane (δ0.00 ppm). Ultraviolet spectra were measured using a Shimadzu-260 recording spectrophotometer. Mass spectra were obtained from a Varian CH_5 or Ribermg R-10-10 quadrapole mass spectrometer.

High-performance liquid chromatography (HPLC) was performed using a system consisting of a Kratos Spectroflow 400 solvent delivery system linked to a Kratos spectroflow 480 injector fitted with a 20 μl loop. The detector was a variable-wavelength Kratos spectroflow 783 operated at 254 nm with peak areas determined via a Nelson Analytical integrator. The HPLC studies were conducted using a reverse-phase, C_{18} (Shandon 150 × 4.6 mm ODS Hypersil, 5 μm) column. The mobile phase contained 1.0 part acetonitrile and 9.0 parts 0.01 M phosphate buffer (pH 7.0), and the flow rate was 2 ml/min. The retention volume for 2-CIDDA was 16.4 ml. The major acidic degradation product observed, 2-chloroadenine, had a retention volume of 4.1 ml. In the alkaline region (pH > 11.0) several degradation products eluted near the solvent front. Calibration curves for 2-CIDDA of 0.001–0.04 mg/ml were constructed from linear plots of peak area vs concentration.

pK_a determination

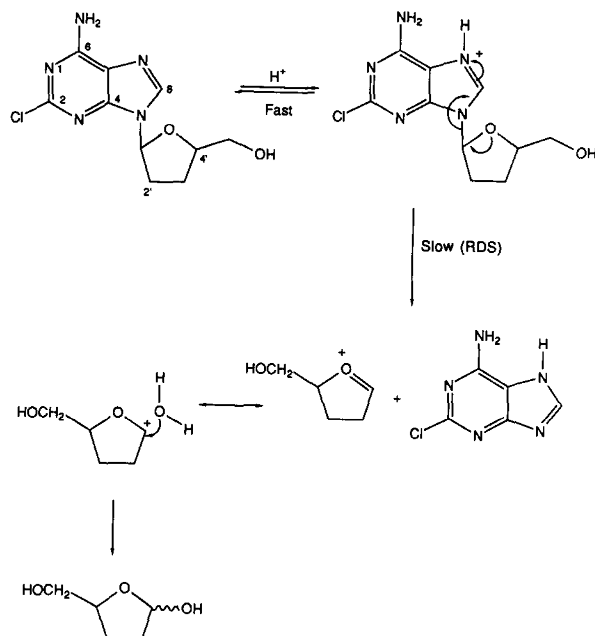
The pK_a value of 2-CIDDA was determined using UV spectroscopy. Aliquots (10 μl each) of 2×10^{-2} M 2-CIDDA in 90% ethanol were diluted with 1.0 ml of 0.05 M buffer solutions (μ = 0.15 M with NaCl) at various pH values (range: pH 1.0–5.0). Samples of the resulting solutions were placed into a cuvette thermostated at 25 °C. Absorbance values were monitored at 267 nm. A solution of 2-CIDDA in 0.1 N NaOH was used to determine the absorbance of the neutral 2-CIDDA, which was consistent with the value obtained with 0.05 M phosphate buffer at pH 7.0. A solution of 2-CIDDA in HCl (0.1 N) was used to determine the approximate absorbance of the fully protonated 2-CIDDA. In the case of low pH solutions (pH < 3.0), it was necessary to extrapolate the absorbance measurement to zero time, since the compound readily undergoes degradation under acidic conditions.

Solubility of 2-chloro-2′,3′-dideoxyadenosine and 2-chloroadenine

The equilibrium solubilities of 2-CIDDA and 2-chloroadenine in different solvents were determined in duplicate by placing an excess of 2-CIDDA or 2-chloroadenine (1.5–2.0-fold excess) in teflon-lined screw-capped vials. The resulting suspensions were placed in a thermostated shaking water bath at 25.0 ± 0.1 °C. After establishment of equilibrium (see data in Tables 4 and 5) each suspension was filtered through a 0.2 μm filter (ACRO LC13, disposable filter, Gelman Sciences). A 250 μl aliquot of the filtrate was diluted with water and analyzed by HPLC. No degradation of 2-CIDDA was observed in any of the solvents during the time needed to establish the equilibrium solubility.

Isolation of a major degradation product of 2-CIDDA in aqueous buffered solution in the pH range 1.0–10.5

A 1 mg/ml solution of 2-CIDDA in water was adjusted to pH 1 with conc. HCl. The hydrolysis of 2-CIDDA was followed to completion by HPLC (defined as complete disappearance of the starting material). The hydrolysis product, which precipitated from solution on pH adjustment to neutrality, was isolated by filtration and recrystallization from an ethanol/water mixture. The electron impact mass spectrum of the product showed a molecular ion m/z of 169 and a fragment at m/z 134 (M − Cl), consistent with the expected product, 2-chloroadenine (see Scheme 1.) ^{1}H-NMR data for 2-chloroadenine (d_6-DMSO): δ 2.50–2.505 (d, 1H,- NH-, $J = 1.5$ Hz); δ 7.636 (s, 2H,



Scheme 1. Proposed mechanism for the hydrolysis of 2-CIDDA in acidic aqueous solutions.

NH_2); δ 8.136 (s, 1H, C_8). ^{13}C-NMR (d_6-DMSO) data: δ 140.225 (C_8); δ 152.75 (C_6); δ 156.8 (C_2). The isolated, purified and identified 2-chloroadenine decomposed at 300 °C without melting and co-chromatographed with the major degradation peak seen during the degradation of 2-CIDDA over the pH range 1–10.5.

Kinetic studies

The hydrolysis of 2-CIDDA was studied over the pH ranges 1.0–5.5, 5.0–12.0 and 6.22–7.51 at 25, 70 and 90 ± 0.1 °C, respectively. Buffer systems employed for the kinetic studies were: 0.1 N HCl (pH 1.0); 0.01 N HCl (pH 2.0); formate buffer (pH 3.0–4.0); acetate buffer (pH 4.5–5.5); phosphate buffer (pH 7.0–8.0 and 11.0–12.0); and NaOH. The effect of buffer concentration changes (0.025–0.1 M) were studied with formate buffer at pH 3.0 and acetate buffer at pH 5.0. The ionic strength was maintained at 0.15 M with NaCl. For these studies, a weighed quantity of 2-CIDDA was dissolved in a buffered solution to give a final concentration of approx. of 1×10^{-4} M. For the kinetics at 70 and 90 °C, buffered solutions were sealed in 1 ml ampoules and stored in constant-temperature ovens. In the studies at elevated temperature, pH measurements were made at the appropriate temperatures. The degradation of 2-CIDDA was followed by HPLC by measuring the peak area of the remaining 2-CIDDA as a function of time. Observed first-order rate constants were calculated from the slope of linear plots of log C vs time, where C is the concentration of the remaining intact 2-CIDDA. The data are presented in Tables 1–3. The pH-rate profiles for the

TABLE 1

Apparent first-order rate constants for the degradation of 2-CIDDA in aqueous buffered solutions at 25 °C, and 0.15 ionic strength (NaCl)

Buffer		pH	k_{obs} (min^{-1})	t_{50} (min)	r^2
HCl	0.1 N	1.0	1.52×10^{-1}	4.6	0.994
	0.001 N	2.0	3.05×10^{-2}	22.7	0.999
Formate [a]		3.0	3.58×10^{-3}	193.2	0.999
	0.05 M	4.0	3.88×10^{-4}	1783.3	0.998
Acetate	0.05 M	4.5	1.20×10^{-4}	5754.0	0.999
	[a]	5.0	3.51×10^{-5}	19743.6	0.998
	0.05 M	5.5	9.60×10^{-6}	72187.5	0.985

[a] Average of k_{obs} at various buffer concentrations (0.025, 0.05, 0.075 and 0.1 M) since no buffer dependence was observed.

TABLE 2

Apparent first-order rate constants for the degradation of 2-CIDDA in aqueous buffered solutions at 70 °C, and 0.15 ionic strength (NaCl)

Buffer		pH	k_{obs} (h^{-1})	t_{50} (h)	r^2
Acetate	0.05 M	5.0	3.60×10^{-1}	1.9	0.998
Phosphate	0.05 M	6.0	5.60×10^{-2}	12.4	0.999
		6.5	2.22×10^{-2}	31.2	0.994
		7.0	9.28×10^{-3}	74.7	0.997
		7.5	5.23×10^{-3}	132.6	0.999
		8.0	3.25×10^{-3}	213.2	0.999
		8.5	3.45×10^{-3}	200.9	0.997
		10.5	2.89×10^{-3}	239.8	0.998
	0.02 M	11.0	3.08×10^{-3}	225.0	0.999
		11.5	3.19×10^{-3}	217.2	0.999
		12.0	4.49×10^{-3}	154.3	0.973
NaOH	0.1 N	N.D. [a]	4.54×10^{-2}	15.3	0.998
	1 N	N.D. [a]	3.78×10^{-1}	1.8	0.985

[a] Not determined.

TABLE 3

Apparent first-order rate constants for the degradation of 2-CIDDA in aqueous buffered solutions at 90°C, and 0.15 ionic strength (NaCl) (buffer: 0.01 M Tris in each case)

pH	k_{obs} (h^{-1})	t_{50} (h)
6.22	2.14×10^{-1}	4.67
6.39	1.41×10^{-1}	4.92
6.84	7.41×10^{-2}	9.35
7.29	4.36×10^{-2}	22.9
7.51	3.69×10^{-2}	18.8

degradation of 2-CIDDA at 25, 70 and 90°C are shown in Fig. 1.

The stability of 2-CIDDA at 10 mg/ml in 60% propylene glycol, 10% ethanol, 30% phosphate buffer (0.05 M, pH 8.0) and at 5 mg/ml in 40% propylene glycol, 10% ethanol, 50% phosphate buffer at 5 mg/ml was studied at 37, 50, 70 and 90°C.

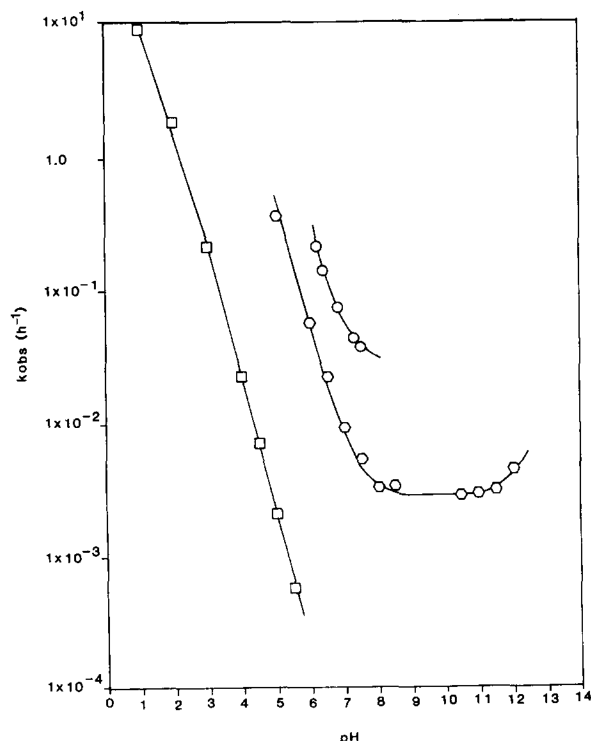


Fig. 1. Plot of ln k_{obs} vs pH for the hydrolysis of 2-CIDDA at 25°C (□), 70°C (○), and 90°C (○) ($\mu \approx 0.15$ M NaCl).

Results and Discussion

The apparent pK_a of 2-CIDDA determined spectrophotometrically in water, at an ionic strength of 0.15 M and at a temperature of 25.0 ± 0.1°C was found to be 2.2 (uncorrected for activity coefficients). This pK_a value is lower than that of the dehalogenated analog, 2′,3′-dideoxyadenosine, which was reported to have a pK_a of 3.7 ± 0.11 (Anderson et al., 1988). The results, however, are in agreement with the pK_a value obtained for similar compounds with or without the presence of an electron-withdrawing substituent at the 2-position of the purine moiety. 6-Aminopurine, for example, has a pK_a of 4.12 (Taylor, 1948), and that of 2-aminopurine is 3.73 (Albert and Brown, 1954), while 2-amino-6-trifluromethylpurine showed a pK_a of 1.85 (Giner-Sorolla and Bendich, 1958), which indicates that the presence of an electron-withdrawing group can lower the pK_a by up to 2 units. Based on structural similarities to other purines, the site of protonation of 2-CIDDA was assigned to the N_1 position (Zubay, 1958; Jardetzky and Jardetzky, 1960; Christensen et al., 1962, 1970; Izatt et al., 1971) or the amino group at the C_6 (Levene and Simms, 1925; Taylor, 1948; Alberty et al., 1951; Beers and Steiner, 1958; Cheney et al., 1959). Protonation at the N_7 position is unlikely but cannot be ruled out (Izatt et al., 1971).

2-CIDDA was determined to have a relatively low solubility of 1.01 mg/ml in water (Table 4). The desired solubility for formulation purposes (personal communications from the National Cancer Institute) was > 5 mg/ml. Due to the low apparent pK_a value for 2-CIDDA and the instability of this compound in acidic aqueous solution (at pH 2.0, $t_{1/2} = 23$ min, Table 1), it was impossible to improve the aqueous solubility by formulating the compound at a pH lower than the pK_a.

The solubility data for 2-CIDDA in mixed solvents (see Table 4) suggested the use of a solvent consisting of 40% propylene glycol, 10% ethanol, 50% 0.05 M aqueous phosphate buffer (pH 8.0) for a solution formulation of 5 mg/ml (pH 8.0 was chosen, because this corresponds to a pH value close to that of maximum stability). Ample supportive evidence is available in the liter-

TABLE 4

Solubility of 2-CIDDA in various solvents at 25 °C

Solvent	Solubility (mg/ml)	Equilibration time (h)
H_2O	1.01	96
t-BuOH	2.91	96
40% *t*-BuOH	8.17	96
50% *t*-BuOH	9.27	72
60% *t*-BuOH	12.56	72
95% EtOH	10.05	96
100% EtOH	2.71	96
10% EtOH/30% PG/60% H_2O	5.09	96
10% EtOH/40% PG/50% H_2O	6.87	96
10% EtOH/50% PG/40% H_2O	9.00	96
10% EtOH/60% PG/30% H_2O	10.32	72
Normal saline (NS)	0.98	72
5% Dextrose (D5W)	1.02	72

t-BuOH, *t*-butanol; PG, propylene glycol; EtOH, ethanol.

ature for the use of this and similar propylene glycol/ ethanol/ water mixtures in parenteral products (Yalkowsky and Roseman, 1981 and references therein). A 10 mg/ml solution could be obtained with a 60% propylene glycol, 10% ethanol, 30% 0.05 M aqueous phosphate buffer (pH 8.0) solution. It should be noted that these vehicles, especially the latter, are likely to be irritating if directly administered to patients. It was anticipated, however, that the contents of these ampoules would first be diluted with normal saline or other large volume parenterals and infused as the diluted solutions. Since the solubility of 2-CIDDA in water is at least 1 mg/ml, greater than 1 : 10 dilutions of these concentrates should not result in the precipitation of 2-CIDDA. Various aqueous ethanol solvents could have been used to obtain the desired solubility goals, however, the decision to use the propylene glycol/ethanol/ water mixture was based on its use in other parenteral products (Yalkowsky and Roseman, 1981).

The solubility measurements in *t*-butanol and *t*-butanol/water mixtures were performed in case freeze-dried preparation of 2-CIDDA was needed. These have recently been suggested as acceptable solvents for freeze drying for those agents exhibiting inadequate water solubility (Seager et al., 1985; Stella et al., 1988).

The major degradation product obtained in the solution formulation of 2-CIDDA was 2-chloroadenine (Scheme 1). The solubility studies of 2-chloroadenine in various solvents suggested that it is significantly less soluble than the corresponding nucleoside (Table 5). These results are in agreement with the data obtained with similar compounds (Anderson et al., 1988). Although adenine solubility was significantly enhanced in the presence of dideoxyadenosine, which was explained on the basis of self-association of purine nucleosides through stacking interactions (Broom and Schweizes, 1967), there was only a slight increase in the solubility of 2-chloroadenine in the presence of 2-CIDDA (Table 5) in the mixed solvent systems.

The poor solubility of 2-chloroadenine could potentially limit the shelf-life of a liquid formulation of 2-CIDDA. For example, the solubility of 2-chloroadenine in a 5 mg/ml solution of 2-CIDDA in the propylene glycol/ethanol/water mixture is 0.105 mg/ml, suggesting that 2% degradation of such a 2-CIDDA solution would produce sufficient 2-chloroadenine for it potentially to precipitate from solution. Therefore, the shelf-lives of the prototype formulations of 2-CIDDA will be reported as $t_{98\%}$ values, i.e., the time for 2% decomposition of 2-CIDDA.

TABLE 5

Equilibrium solubility of 2-chloroadenine in various solvents at 25 °C

Solvent	Solubility (mg/ml)
H_2O	0.0083
40% PG/10% EtOH/50% aqueous phosphate (pH 8.0)	0.084
40% PG/10% EtOH/50% aqueous phosphate (pH 8.0) (containing 5 mg/ml of 2-CIDDA)	0.105
60% PG/10% EtOH/30% aqueous phosphate (pH 8.0)	0.23
60% PG/10% EtOH/30% aqueous phosphate (pH 8.0) (containing 10 mg/ml of 2-CIDDA)	0.27

Hydrolysis of 2-chloro-2′,3′-dideoxyadenosine

The major degradation products of 2-ClDDA in aqueous solution in the pH range 1.0–10.5 were 2-chloroadenine and 2,3-dideoxyribose (Scheme 1). The hydrolysis of 2-ClDDA was studied as a function of pH at 25 °C, in the pH range 1.0–5.5. The pseudo-first order rate constants at various pH values were calculated from a plot of log C vs time, where C is the concentration of the remaining 2-ClDDA in solution. When the rate of hydrolysis of 2-ClDDA was studied in formate buffer (pH 3.0) and acetate buffer (pH 5.0) at various buffer concentrations (0.025, 0.05, 0.075 and 0.1 M), no buffer catalysis was observed. The absence of buffer catalysis has been noted for other nucleosides (Garret et al., 1965, 1972a,b; Anderson et al., 1988; and Stella et al. unpublished work on the nucleoside, pentostatin). The cleavage of the glycosidic bond was explained by a rate-limiting unimolecular decomposition, or A-1 mechanism (Zoltewicz and Clark, 1970, 1972; Garrett and Mehta, 1972a; Hevesi et al., 1972; Panzica et al., 1972; Romero et al., 1978; York, 1981) of the mono- and diprotonated nucleoside to the glycosyl carbonium ion and the corresponding base.

At 70 and 90 °C the hydrolysis of 2-ClDDA was studied in aqueous buffered solutions at pH 5.0–12.0 (and in 0.1 and 1 N NaOH) and 6.22–7.51 ($\mu = 0.15$ NaCl), respectively. The pH-rate data obtained at various temperatures are listed in Tables 1–3 and the pH-rate profiles are plotted in Fig. 1.

The observed first-order rate constants for the degradation of 2-ClDDA were analyzed according to Eqn 1:

$$k_{obs} = k_H[H^+]f_{AH} + k'_H[H^+]f_A + k'_0 f_A + k'_{OH}[OH^-]f_A \quad (1)$$

where $f_{AH} = [H^+]/([H^+] + K_a)$ and $f_A = K_a/(K_a + [H^+])$ denote the fraction of 2-ClDDA in the monoprotonated and neutral form, respectively.

The rate constant, k_H is the bimolecular rate constant for hydrogen ion-catalyzed hydrolysis of monoprotonated 2-ClDDA which was estimated to be 8.5×10^1 M^{-1} h^{-1} at 25.0 °C. This value could only be estimated because of insufficient data at pH values lower than the pK_a. The term $k_H[H^+]f_{AH}$, is kinetically equivalent to the spontaneous hydrolysis of a diprotonated form of 2-ClDDA.

The second-order acid-catalyzed rate constant operating on neutral 2-ClDDA, k'_H, was determined by plotting k_{obs} vs H^+ concentration over the pH range 4.0–7.0. In this pH range, a value of unity was assumed for f_A and the contributions of the other rate constants to the profile can be neglected. Values of 2.35×10^2, 5.21×10^4 and 3.01×10^5 M^{-1} h^{-1} at 25, 0 and 90 °C, respectively, for k'_H were found to describe the data adequately. The value of 2.35×10^2 M^{-1} h^{-1} at 25 °C for k'_H can be compared to 1.68×10^2 M^{-1} h^{-1} for 2′,3′-dideoxyadenosine hydrolysis. The 2-chloro substituent has a slight accelerating effect on the hydrolysis. This effect was similar in magnitude to that noted by Garrett and Mehta (1972a) in their study at 80 °C in 0.01 M HCl when 2-ClDDA was compared to 2′,3′-dideoxyadenosine. The term, $k'_H[H^+]f_A$, is kinetically equivalent to the spontaneous hydrolysis of monoprotonated 2-ClDDA, $k_0 f_{AH}$.

The rate constant k'_0 is the pH-independent hydrolysis rate constant operating on the neutral form of 2-ClDDA. This constant was determined from the observed rate constants in the pH range 8.0–10.5 where a value of unity was again assumed for f_A. A value of 2.89×10^{-3} h^{-1} at 70 °C was estimated for k'_0. The fourth term in Eqn 1 accounts for the OH^--catalyzed second-order rate constant operating on the neutral form of 2-ClDDA, k'_{OH}. This term was determined from the slope of a linear plot of the observed rate constants vs OH^--concentration over the pH range 11–12; k'_{OH} was determined to be 1.3×10^{-2} M^{-1} h^{-1} at 70 °C.

The solid line drawn in Fig. 1 was generated using Eqn 1 and the values of the rate constants estimated from the various regions of the pH-rate profile (Table 6). There was no obvious break in the pH-rate profile around the pK_a of 2.2 consistent with the findings of 2′,3′-dideoxyadenosine (Anderson et al., 1988).

The possible mechanism for degradation of 2-ClDDA over the pH range of 1.0–10.5 is illustrated in Scheme 1. Although protonation is depicted as being on the N_7 position, as suggested

TABLE 6

Summary of rate constants for the hydrolysis of 2-CIDDA at 25, 70 and 90°C

T (°C)	k'_H ($M^{-1} h^{-1}$)	k'_0 (h^{-1})	k'_{OH} ($M^{-1} h^{-1}$)
25	2.35×10^2		
70	5.21×10^4	2.89×10^{-3}	1.08×10^{-2}
90	3.01×10^5	2.75×10^{-2}	

by others (Zoltewicz et al., 1970; Zoltewicz and Clark, 1972), a similar mechanism involving protonation at N_1 could be suggested. Nucleoside hydrolysis is explained by an initial reversible protonation at the purine ring followed by a rate-determining fragmentation to give the purine and a cyclic carbonium ion form of the sugar. This carbonium ion on reaction with water gives 2,3-dideoxyribose. The enthalpy of activation, $\Delta H^{\ddagger}$ (23.2 ± 0.6 kcal/mol) and entropy of activation, $\Delta S^{\ddagger}$ (13.9 ± 1.8 e.u.), for the hydrolysis of 2-CIDDA in the acid-catalyzed region were calculated from an Eyring plot (Fig. 2). This data supports the A-1 mechanism and is in agreement with previous data (Anderson et al., 1988; and Stella et al. unpublished work on the nucleoside, pentostatin).

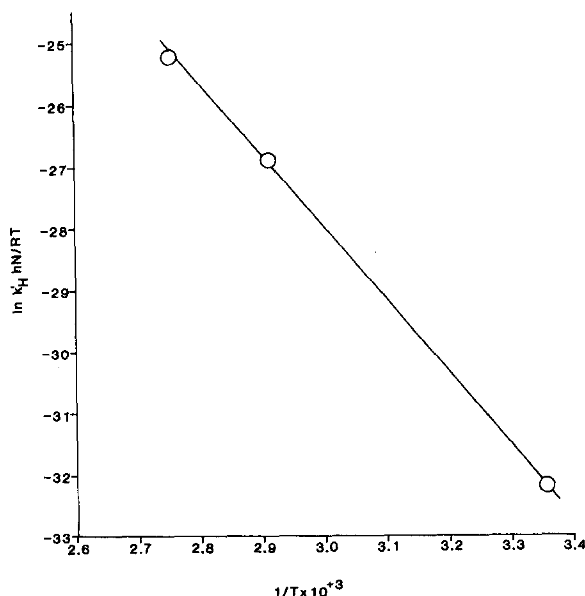


Fig. 2. Eyring plot for k'_H for the hydrolysis of 2-CIDDA.

The stability of 2-CIDDA at 10 mg/ml in 60% propylene glycol, 10% ethanol, 30% aqueous phosphate buffer (pH 8.0), and at 5 mg/ml in 40% propylene glycol, 10% ethanol, 50% aqueous phosphate buffer (pH 8.0), was estimated from studies at elevated temperature. The loss of 2-CIDDA followed apparent first-order kinetics in the mixed solvents. The energy of activation, 30.7 kcal/mol, for the hydrolysis of 2-CIDDA in 60% propylene glycol, 10% ethanol, 30% aqueous phosphate buffer (pH 8.0) was estimated from the degradation data for 2-CIDDA at 70°C (k_{obs} of 7.048×10^{-3} day^{-1}) based on data collected over 59 days) and 90°C (k_{obs} of 9.168×10^{-2} day^{-1}, based on data collected over three half-lives). No degradation of 2-CIDDA was noted at 37 and 50°C over 87 days. Based on this estimated value of the activation energy, the $t_{1/2}$ value of the hydrolysis of 2-CIDDA in this solvent at room temperature was estimated to be ≈ 226 years, and the shelf-life, $t_{98\%}$, ≈ 6.6 years.

The energy of activation for the hydrolysis of 2-CIDDA in the 40% propylene glycol, 10% ethanol, 50% aqueous phosphate buffer (pH 8.0) solvent was estimated to be 29.3 kcal/mol based on the data obtained at 70°C (k_{obs} of 1.561×10^{-2} day^{-1}, based on data collected over 87 days) and 90°C (k_{obs} of 1.67×10^{-1} day^{-1}, based on data collected over three half-lives). The $t_{1/2}$ at 25°C was estimated to be 82 years. Again no degradation was noted at 37°C and ≈ 5% degradation was noted at 50°C over 87 days. A shelf-life of greater than 2 years, $t_{98\%}$ of ≈ 2.4 years, can be estimated. Data collection for the 37 and 50°C studies is ongoing and will provide more definitive information on the long-term stability of these prototype dosage forms.

In summary, 2-CIDDA appears to degrade via an identical mechanism and at comparable rates to 2′,3′-dideoxyadenosine. Prototype formulations, using a mixed solvent approach, are estimated to have shelf-lives of greater than 2 years, the shelf-lives being determined by the expected precipitation times of 2-chloroadenine, the principle degradation product of 2-CIDDA in the pH range 1–10.5 rather than the time for 10% degradation of 2-CIDDA.

Acknowledgements

This work was supported by the National Cancer Institute, Contract Nos. N01-CM-67912 and N01-CM-97576.

References

Albert, A. and Brown, D.J., Purine studies. 1. Stability to acid and alkali. Solubility. Ionization. Comparison with pterdines., *J. Chem. Soc.*, (1954) 2060–2071.

Alberty, R.A., Smith, R.M. and Bock, R.M., The apparent ionization constants of adenosine phosphates and related compounds. *J. Biol. Chem.*, 193 (1951) 425–434.

Anderson, B.D., Wygant, M.B., Xiang, T.-X.; Waugh, W. and Stella, V.J., Preformulation solubility and kinetic studies of 2′,3′-dideoxypurine nucleosides: potential anti-AIDS agents. *Int. J. of Pharm.*, 45 (1988) 27–37.

Beers, R.F. and Steiner, R.F., Spectrophotometric, light scattering, and titration studies upon interaction of polyadenylic and polyuridylic acids. *Nature*, 181 (1958) 30–32.

Broom, A.D., Schweizes, M.P. and Ts'o, P.O.P., Interaction and association of base and nucleosides in aqueous solutions. V. Studies of the association of purine nucleosides by vapor pressure osmometry and by proton magnetic resonance. *J. Am. Chem. Soc.*, 89 (1967) 3611–3621.

Cheney, G.E., Feiser, H. and Fernando, Q., Metal complexes of purine and some of its derivatives. *J. Am. Chem. Soc.*, 81 (1959) 2611–2615.

Christensen, J.J. and Izatt, R.M., Thermodynamics of proton dissociation in dilute aqueous solution. II. Heats of proton dissociation from ribonucleotides and related compounds determined by a thermometric titration procedure. *J. Phys. Chem.*, 66 (1962) 1030–1043.

Christensen, J.J., Rytting, J.H. and Izatt, R.M., Thermodynamic pK, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ values for proton dissociation from several purines and their nucleosides in aqueous solution. *Biochemistry*, 9 (1970) 4907–4913.

DeVita, V.T., Broder, S., Fauci, A.S., Kovacs, J.A. and Chabner, B.A., Developmental therapeutics and the acquired immunodeficiency syndrome. *Ann. Intern. Med.*, 106 (1987) 568–581.

Giner-Sorolla, A. and Bendich, A., Synthesis and properties of some substituted purines. *J. Am. Chem. Soc.*, 80 (1958) 3932–3937.

Garrett, E.R., Chemburkar, P.B. and Suzuki, T., Prediction of stability in pharmaceutical preparations. XIV. The complete pH dependent solvolytic degradations of an iodinated nucleoside, the antiviral 5-iodo-2′-deoxyuridine. *Chem Pharm. Bull.*, 13 (1965) 1113–1130.

Garrett, E.R. and Mehta, P.J., Solvolysis of adenine nucleosides. I. Effects of sugars and adenine substituents on acid solvolysis. *J. Am. Chem. Soc.*, 94 (1972a) 8532–8541.

Garrett, E.R. and Mehta, P.J., Solvolysis of adenine nucleosides. II. Effects of sugars and adenine substituents on alkaline solvolysis. *J. Am. Chem. Soc.*, 94 (1972b) 8542–8547.

Hevasi, L., Wolfson-Davidson, E., Nagy, J.B., Nagy, O.B. and Bruylants, A., Contribution to the mechanism of the acid-catalyzed hydrolysis of purine nucleosides. *J. Am. Chem. Soc.*, 94 (1972) 4715–4720.

Izatt, R.M., Christensen, J.J. and Rytting, J.H., Sites and thermodynamic quantities associated with proton and metal ion interaction with ribonucleic acid, deoxyribonucleic acid, and their constituent bases, nucleosides, and nucleotides. *Chem. Rev.*, 71 (1971) 439–481.

Jardetzky, C.D. and Jardetzky, O., Investigation of the structure of purines, pyrimidines, ribose nucleosides and nucleotides by proton magnetic resonance. II. *J. Am. Chem. Soc.*, 82 (1960) 222–229.

Levene, P.A. and Simms, H.S., Dissociation constants of plant nucleotides and nucleosides and their relation to nucleic acid structure. *J. Biol. Chem.*, 65 (1925) 519–534.

Panzica, R.D., Rousseau, R.J., Robins, R.K. and Townsend, L.B., Relative stability and a quantitative approach to the reaction mechanism of the acid-catalyzed hydrolysis of 7- and 9-β-*O*-ribofuranosylpurines. *J. Am. Chem. Soc.*, 94 (1972) 4708–4714.

Romero, R., Stein, R., Bull., H.G and Cordes, E.H., Secondary deuterium isotope effects for acid-catalyzed hydrolysis of inosine and adenosine. *J. Am. Chem. Soc.*, 100 (1978) 7620–7624.

Rosowsky, A., Solan, V.C., Sodroski, J.G. and Ruprecht, R.M., Synthesis of the 2-chloro analogues of 3′-deoxyadenosine, 2′,3′-dideoxyadenosine, and 2′,3′-didehydro-2′,3′-dideoxyadenosine as potential antiviral agents. *J. Med. Chem.*, 32 (1989) 1135–1140.

Seager, H., Taskis, C.B., Syrop, M. and Lee, T.J., Structure of products prepared by freeze-drying solutions containing organic solvents. *J. Parenteral Sci. Tech.*, 39 (1985) 161–179.

Stella, V.J., Umprayn, K. and Waugh, W.N., Development of parenteral formulations of experimental cytotoxic agent. I. Rhizoxin (NSC-332598). *Int. J. Pharm.*, 43 (1988) 191–199.

Stella, V.J., Benedetti, A., Waugh, W. and Al-Razzak, L.A., Chemical stability of pentostatin (NSC-21831), a cytotoxic and immunosuppressant agent. *Pharm. Res.*, (1989) in press.

Taylor, H.F.W., The dissociation constants of benziminazole and certain purine derivatives. *J. Chem. Soc.*, (1948) 765–766.

Yalkowsky, S.H. and Roseman, T.J., Solubilization of drugs by co-solvents. In Yalkowsky, S. (Ed.), *Techniques of Solubilization of drugs*, Dekker, New York, 1981, pp. 91–135.

Zoltewicz, J.A., Clark, D.F., Sharpless, T.W. and Grahe G., Kinetics and mechanism of hydrolysis of the acid-catalyzed hydrolysis of some purine nucleosides. *J. Am. Chem. Soc.*, 92 (1970) 1741–1750.

Zoltewicz, J.A. and Clark, D.F., Kinetics and mechanism of hydrolysis of guanosine and 7-methylguanosine in perchloric acid. *J. Org. Chem.*, 37 (1972) 1193–1197.

Zubay, G., Mechanism of mild acid denaturation of deoxyribonucleic acid. *Biochim. Biophys. Acta*, 28 (1958) 644–645.