Hydrolysis and Solvent-Dependent 2′→5′ and 3′→5′ Acyl Migration in Prodrugs of 9-β-D-Arabinofuranosyladenine

BRADLEY D. ANDERSON*x, MAN-CHEONG FUNG*, SHIV D. KUMAR‡, AND DAVID C. BAKER‡

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Abstract □ As a prerequisite to quantitative in vivo studies to further explore the promising topical activity of the 2′,3′-di-*O*-acetyl derivative of 9-β-D-arabinofuranosyladenine (ara-A) against herpes virus infections, the kinetics of solution degradation of the 2′,3′-di-*O*-acetyl derivative and the 2′-, 3′-, and 5′-monoacetates were investigated. The rates of aqueous solution hydrolysis were found to be consistent with rank order predictions based on a consideration of substituent effects. Preliminary in vivo hydrolysis data, however, do not correlate with such predictions, indicating a need for more systematic studies of the effect of molecular structure on enzyme-catalyzed hydrolysis. An important reaction of the 2′,3′-diester and the 3′-monoester in aqueous solution, in addition to ester hydrolysis, is 3′→5′ acyl migration. 2′→5′ Acyl migration does not occur in water but is the predominant migration pathway in organic solvents, as verified by studies in acetonitrile. ^{1}H NMR spectroscopy was employed to study the dependence of the conformation of the sugar ring on the solvent environment. Although a change in the equilibrium between the C(2′)endo and C(3′)endo conformational states does occur, it is not a dramatic change and cannot explain the solvent selectivity observed in the acyl migration kinetics.

9-β-D-Arabinofuranosyladenine (ara-A, VIRA-A, vidarabine; 6) is a potent, relatively noncytotoxic nucleoside which has been shown to have a broad spectrum of activity against DNA viruses in cell culture and in vivo.[1] Of significant interest is the activity of ara-A toward herpes viruses, which has been demonstrated in cell culture,[2,3] in laboratory animals,[4–12] and in humans.[1–13]

Topical treatment of human oral or genital herpes virus infections by ara-A, on the other hand, has proven totally ineffective.[14–17] The use of ara-A topically is largely precluded both by its low aqueous solubility and lipophilicity, which limit the rate at which the compound can diffuse through membranes, and by its susceptibility to deamination by adenosine deaminase, resulting in the formation of the less potent 9-β-D-arabinofuranosylhypoxanthine (ara-hypoxanthine, ara-H).[18,19] In an attempt to overcome these problems, several mono- and diester prodrugs of ara-A having lower melting points and increased lipophilicity have been synthesized and evaluated.[18,20–22]

Particularly noteworthy among these comounds is 9-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)adenine (1) which has been shown to be at least as effective topically as acyclovir in the treatment of genital herpes in a guinea pig model.[20,21] Furthermore, the effectiveness of 1 was found to be enhanced in the treatment of herpes simplex virus type 1 infections in hairless mice when used in conjunction with the penetration enhancer 1-dodecylhexahydro-2*H*-azepin-2-one (Azone).[23,24] Additional studies are now underway to develop a detailed quantitative description of the steady-state blood and tissue profiles, assess the relative potencies of the various chemical species, and determine their site of action so that the prodrug properties and its delivery system can be optimized for maximum topical antiviral activity.

As a prerequisite for the transport and in vivo studies, a detailed quantitative understanding of the kinetics of the degradation of the 2′,3′-diacetate 1 in vitro is necessary. This is a report on the kinetics of hydrolysis and acyl migration reactions observed for the 2′,3′-diacetate 1 and its degradation products.

Results and Discussion

Chemistry—In examining Scheme I, some of the possible products of ester hydrolysis and in vivo ester cleavage of 9-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)adenine (1) include the 2′-,[22] 3′-,[20] and 5′-*O*-acetyl[18] derivatives of ara-A, 4, 2, and 5, respectively. The latter possibly arises via hydrolysis of 1 at either the 2′- or 3′-position, with concomitant acetyl migration to the 5′-position.

While adequate synthetic methods are available for the 2′-[22] and 5′-*O*-acetyl[18] compounds 4 and 5, the 3′-*O*-acetyl compound 2[20] had been isolated as a presumed hydrolysis byproduct in the multigram-scale production of 1; thus, a more practical route for the synthesis of 2 was necessary. To this end ara-A was converted to the 2′,5′-bis-*O*-(*tert*-butyldimethylsilyl) derivative 7 (Scheme II) using *tert*-butylchlorodimethylsilane in *N*,*N*-dimethylformamide with imidazole as base. These conditions have been found[25] to be the most effective in producing the 2′,5′-bis-*O*-protective derivative 7 in respect to the 3′,5′-bis-*O*(*tert*-butyldimethylsilyl) compound 8. Acetylation of 7 with acetic anhydride:pyridine then gave 9-[3-*O*-acetyl-2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (11), the structure of which was confirmed by the ^{1}H NMR spectrum that showed, most importantly, the signal for H-3′ at δ 5.36 (compare with that for 7, in which H-3′ resonates at approximately δ 4.4[22,26]). Removal of the *tert*-butyldimethylsilyl protecting groups from 11 was effected under conditions[20] that suppress acetate ester hydrolysis and/or migration to give the 3′-*O*-acetyl derivative 2 in 92% yield. Identification of 2 was made via ^{1}H NMR spectroscopy, wherein the H-3′ signal was shifted downfield to δ 5.20 (compare H-2′ for 2 at δ 4.31), identical with that reported earlier.[22]

In addition it was deemed worthwhile to examine 9-(2,5-di-*O*-acetyl-β-D-arabinofuranosyl)adenine (3) as a possible intermediate that could arise via 3′→5′ acetyl migration on 1. Its synthesis was developed as follows. 9-[3,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (8)[22] was subjected to selective hydrolysis in acetic acid to remove the 5′-*O*-(*tert*-butyldimethylsilyl) group,[27] giving the 3′-monoprotected compound 12. Acetylation of 12 then gave its 2′,5′-diacetate 13 which was deprotected in same manner as 11 to give 2′,5′-di-*O*-acetyl-ara-A (3). Inspection of the ^{1}H NMR spectrum of 3 (see *Experimental Section*) revealed marked downfield shifts for both H-5′,5′a (of ~0.5 ppm) and H-2′ (of ~1 ppm) relative to those of the unacetylated precursor 12. Such shifts are in line with those that have been observed for acetylation of methylene and methine hydroxyl groups, respectively, in similar compounds.[18,20,22]

Ester Hydrolysis Kinetics—Semilogarithmic plots of the pseudo-first-order rate constants for the hydrolysis of ara-A derivatives 4, 5, and 2 versus pH are shown in Fig. 1 superimposed (for reference purposes) on the pH–hydrolysis rate profile of ethyl acetate.[28] Similar plots for the hydrolysis of the 2′,3′-di-*O*-acetyl derivative 1 at the 2′- and the 3′-

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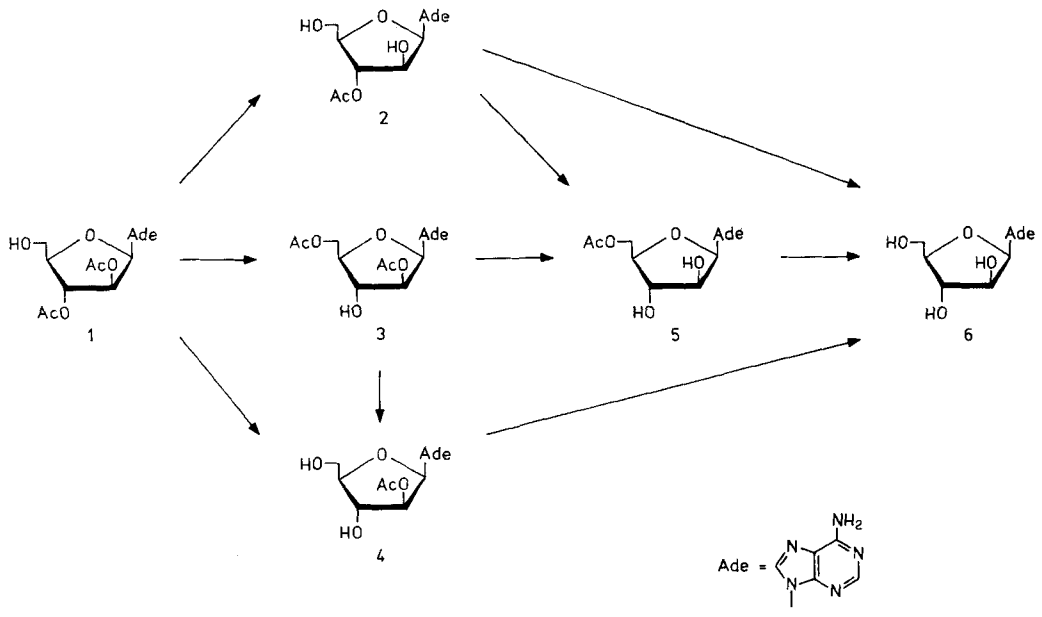
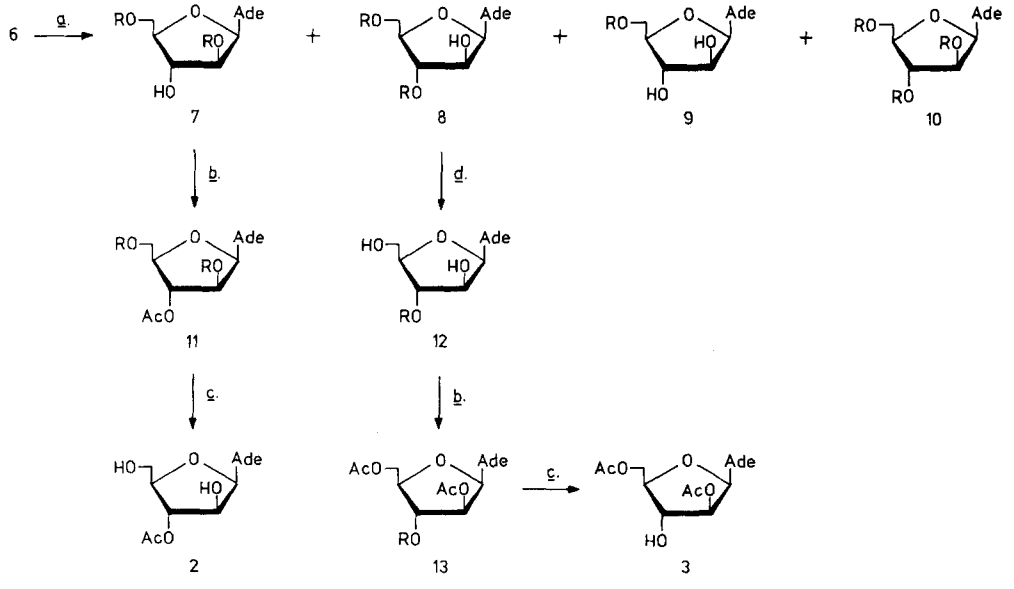


Scheme I



Scheme II—(a) *tert*-$BuMe_2SiCl$, imidazole, DMF; (b) Ac_2O-pyridine; (c) *n*-Bu_4NF, HOAc (1 equivalent); (d) 80% HOAc, 80 °C; R = — $SiMe_2$-*tert*-Bu.

position are shown in Fig. 2. Each of these curves exhibit the typical U-shape consistent with terms in the rate equation for acid catalysis, hydroxide-ion attack, and neutral water attack on the ester as expressed:

$$k_{obs} = k_{H^+}[H^+] + k_{H_2O} + k_{OH^-}[OH^-] \qquad (1)$$

where k_{obs} is the observed pseudo-first-order rate constant at any pH. Values of k_{H^+}, k_{H_2O}, and k_{OH^-} for the hydrolytic reactions (see Scheme I) are reported in Table I.

Although the limited number of compounds in this series and the precision of the data preclude a quantitative assessment of the effect of structure on lability of ara-A esters in aqueous solution, the kinetic data are qualitatively consistent with the modified Taft equation[29] for the effect of substituents on the reactivity of aliphatic esters:

$$\log (k/k_0) = \rho \cdot \sigma^* + \delta \cdot E_s \qquad (2)$$

Acid-catalyzed hydrolysis is a function only of steric effects, while both polar and steric effects influence base-catalyzed rates.[30] As is evident from Fig. 1 and Table I, the order of ester reactivity in acid is that predicted from a consideration of steric effects: ethyl acetate > 5′-*O*-acetyl > 2′-*O*-acetyl = 3′-*O*-acetyl. In base, the rank order is reversed (2′-*O*-acetyl > 3′-*O*-acetyl > 5′-*O*-acetyl > ethyl acetate) and in fact parallels the acidities of the free hydroxyl leaving groups.[31] Thus the electron-withdrawing effect of substituents on the arabinofuranosyl ring appears to be the predominating factor influencing the rates in the base-catalyzed hydrolysis. As

seen from a comparison of the rate constants in Table I, hydrolysis rates of neither the 2′-acetate 4 nor the 3′-acetate 2 are significantly affected by esterification of the adjacent hydroxyl group.

It is of interest in studies of prodrugs to relate the effect of structure not only to reactivity in vitro but to in vivo lability as well. Although data of this type are limited, in a few cases linear free energy relationships have been developed for hydrolysis by esterases using substituent constants obtained from aqueous solution data.[32]

There are a number of enzymes which are capable of hydrolyzing esters, but it is not known which are specifically involved in the hydrolysis of esters of ara-A. However, from the similarity of the reactivities of the 2′- and 3′-acetates in both acid and base, one might intuitively expect hydrolysis of esters at these positions to also proceed at similar rates in vivo. Pharmacokinetic studies of the fate of the 2′,3′-di-*O*-acetyl derivative 1 of ara-A on intraperitoneal administration in mice[33] and intravenous administration in guinea pigs[34] indicate that bioconversion proceeds entirely via initial ester hydrolysis at the 3′-position leading to the formation of the 2′-*O*-acetyl intermediate 4. One factor which may contribute to this dramatic difference in relative rates of conversion is the presence of a bulky adenine ring cis to the 2′-*O*-acyl function. This group appears to be at too great a distance to substantially hinder the attack of a small molecule, such as water, at the 2′-*O*-acetyl carbonyl group, but it may exert a long-range steric effect in preventing close contact between the 2′-*O*-acyl group and the catalytic site of a large enzyme. Such observations suggest that linear free energy relationships for enzyme-catalyzed hydrolyses may require different substituent constants than those obtained from aqueous solution hydrolysis experiments.

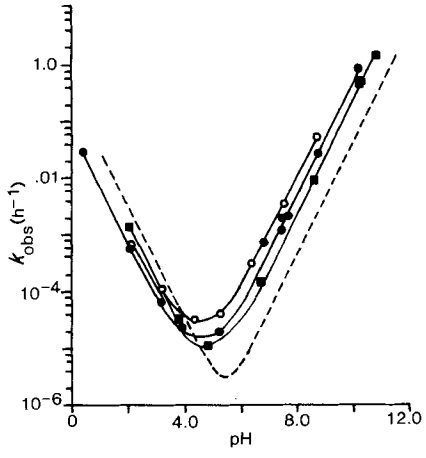


Figure 1—*Semilogarithmic plots of the pseudo-first-order rate constants at 25 °C for hydrolysis of the 2′- (○), 3′- (●), and 5′-O-acetyl (■) derivatives of ara-A versus pH, superimposed (for reference purposes) on the pH–hydrolysis rate profile of ethyl acetate.*

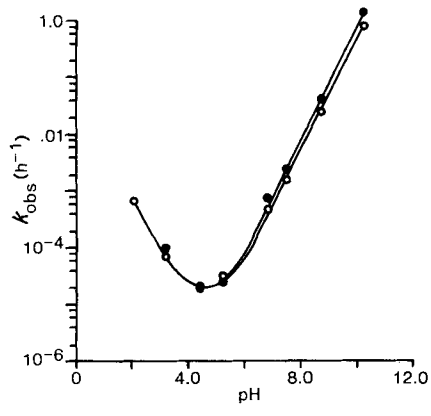


Figure 2—*Semilogarithmic plots of the pseudo-first-order rate constants at 25 °C for hydrolysis of the 2′- (●) and 3′-acetates (○) in the 2′,3′-di-O-acetyl derivative of ara-A.*

Acyl Migration Kinetics—In a previous report on the preparation of 2′,3′-di-*O*-acyl derivatives of ara-A, a 2′→5′-*O*-acyl migration was observed to occur in tetrahydrofuran during a deprotection step in the synthesis.[20] One of the initial objectives of this study was to further investigate this reaction in aqueous solution. The kinetic data generated indicate that this reaction is negligible in water relative to other degradative processes. Thus, the predominant route of degradation of the 2′-*O*-acetyl derivative 4 in aqueous solution is hydrolysis to give ara-A (6).

Base-catalyzed degradation of the 3′-*O*-acetyl 2 and the 2′,3′-di-*O*-acetyl 1 in aqueous solutions results in significant concentrations of the 5′-*O*-acetyl 5 and 2′,5′-di-*O*-acetyl 3, respectively, due to 3′→5′ acyl migration. This reaction has not to the authors' knowledge previously been reported in esters of D-arabinofuranosyl (or for that matter, in D-ribofuranosyl) nucleosides.

Those reactions which are shown occur to a significant extent in aqueous solutions are depicted in Scheme I. All products were identified by comparison with the authentic compounds by HPLC. Species concentration versus time profiles for the degradation of the 3′- and 2′,3′-derivatives, 2 and 1, are shown in Figs. 3 and 4, respectively. The solid curves drawn in Figs. 3 and 4 are theoretical profiles obtained by curve fitting the experimental data to the kinetic model proposed in Scheme I. The excellent agreement between the theoretical curves and the experimental data support the assumptions that (*a*) all reactions in Scheme I are first order in reactant concentration and (*b*) other possible reactions which may be expected to occur to some extent (such as 5′→3′ migration) need not be included to satisfactorily describe the species concentration versus time profiles.

The 3′→5′ acyl migration in the 3′-acetate 2 and 2′,3′-diacetate 1 is primarily base catalyzed above pH 6, as demonstrated in the pH profiles shown in Fig. 5. 3′→5′ Acyl

Table I—Rate Constants for Hydrolysis and Acyl Migration of Mono- and Diacetates of 9-β-D-Arabinofuranosyladenine

	Degradation Pathways (by Compound Numbers)								
	1→2	**1→3**	**1→4**	**2→5**	**2→6**	**3→4**	**3→5**	**4→6**	**5→6**
k_{H^+} ($L \cdot mol^{-1} \cdot h^{-1}$)	0.094 (±35%)[a]	ND[b]	0.086 (±60%)	ND	0.072 (±33%)	ND	ND	0.10 (±31%)	0.16 (±77%)
k_{H_2O} (h^{-1})	1.6×10^{-5} (±48%)	ND	1.8×10^{-5} (±67%)	ND	1.2×10^{-5} (±66%)	ND	ND	2.5×10^{-5} (±38%)	7.6×10^{-6} (±127%)
k_{OH^-} ($L \cdot mol^{-1} \cdot h^{-1}$)	6.6×10^3 (±22%)	3.1×10^3 (±17%)	4.5×10^3 (±37%)	2.1×10^3 (±14%)	4.5×10^3 (±23%)	1.1×10^3 —[c]	1.2×10^4 —[c]	9.6×10^3 (±22%)	2.1×10^3 (±54%)

[a] 95% Confidence limit from nonlinear least-squares regression analysis. [b] Not determined. [c] Single pH determination.

migration occurs at a slightly faster rate in the 2',3'-diacetate 1. Rate constants for these reactions are listed in Table I.

Solvent Dependence of Acyl Migration—The finding in this study that the predominant migration in aqueous solution is 3'→5' and that the 2'→5' acyl migration is negligible under these conditions was unexpected because only the 2'→5' migration had been reported previously.[20] Since 2'→5' migration was found[20] to occur in a nonaqueous solvent (tetrahydrofuran), the influence of solvent on the relative rates of 2'→5' versus 3'→5' acyl migration was investigated. The dipolar, aprotic solvent acetonitrile was chosen for this comparison. Studies of the degradation of the 2',3'-diacetate 1 in acetonitrile required the use of tetra-*n*-butylammonium hydroxide as a base catalyst rather than sodium hydroxide due to the poor solubility of the latter in acetonitrile. Similar studies conducted in water using tetra-*n*-butylammonium hydroxide as the base did not change the conclusion that 3'→5' migration predominates in water. The relative percentages of 2'→5' and 3'→5' acetyl migration in solvents varying in acetonitrile:water composition are shown in Table II.

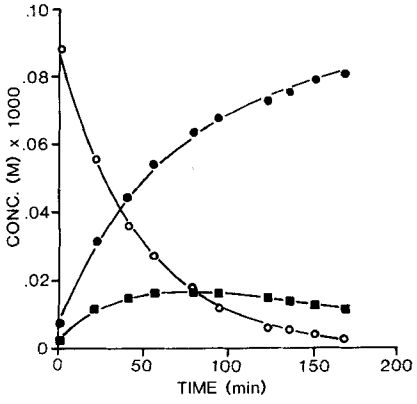


Figure 3—*Concentration versus time profiles for the degradation of the 3'-O-acetyl derivative of ara-A (○) at 25 °C in aqueous solution (pH 10.24). Products formed are the 5'-O-acetyl intermediate (■) and ara-A (●). The solid lines are based on the kinetic model for 3'-monoester degradation shown as a part of Scheme I.*

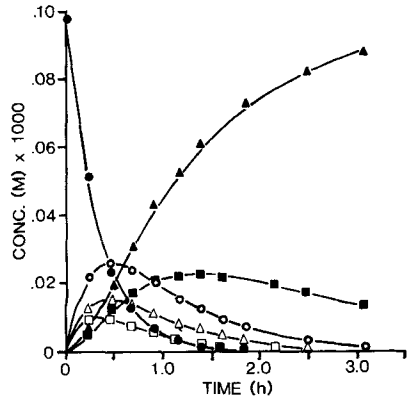


Figure 4—*Concentration versus time profiles for the degradation of the 2',3'-di-O-acetyl derivative of ara-A (●) at 25 °C in aqueous solution (pH 10.30). Products formed are the 2',5'-di-O-acetyl derivative (□), the 2'- (△), 3'- (○), and 5'- (■) monoesters, and ara-A (▲). The solid lines are based on the kinetic model depicted in Scheme I.*

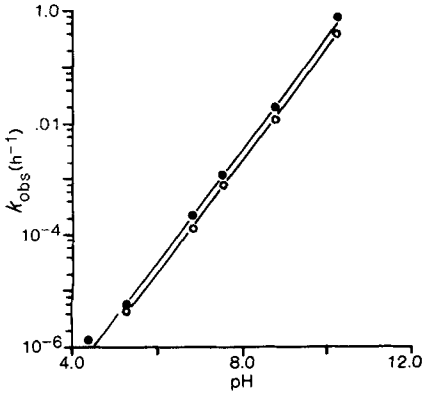


Figure 5—*Semilogarithmic plots of the pseudo-first-order rate constants at 25 °C for 3'→5' acetyl migration in the 3'-monoacetate (○) and 2',3'-diacetate (●) esters of ara-A.*

Table II—Relative Percentages of 2'→5' and 3'→5' Acyl Migration in 9-[2,3-Di-*O*-acetyl-β-D-arabinofuranosyl]adenine in Alkaline Acetonitrile:Water Solvent Systems

Solvent Composition	Percent of Total Initial Migration Rate	
	2'→5'	3'→5'
100% H_2O	—	>90
90% CH_3CN:10% H_2O	20%	80%
99% CH_3CN:1% H_2O	70%	30%
100% CH_3CN	>90%	—

One possibility considered to explain the striking solvent selectivity illustrated in Table II is a solvent-dependent change in average conformation of the arabinose ring. The conformation of the 5-membered sugar (furanose) ring in solutions has been described most simply as an equilibrium between two conformational states, referred to as C(2')endo and C(3')endo or ^2E and ^3E.[35,36] In the C(2')endo conformation, the 2'-carbon is puckered up and out of the plane of the sugar ring, while in the C(3')endo conformation, the 3'-carbon is above the plane formed by the other four atoms in the ring. 3'→5' Acyl migration can proceed only through the C(3')endo conformation, since only in this conformation can the 5'-OH approach a carbonyl group attached to the 3'-oxy group. Similarly, the 2'→5' migration can only proceed through the C(2')endo conformation in which the 2'-*O*-acetyl group would be axial and readily accessible to attack by the 5'-OH. The ara-A is predominantly in the C(3')endo conformation in aqueous solution,[36] as determined experimentally by an analysis of vicinal coupling constants in the ^1H NMR spectrum.

^1H NMR was employed to study the solvent dependence of the solution conformations of the 2',3'-di-*O*-acetyl derivative 1 of ara-A to determine if the solvent selectivity in acyl migrations can be attributed to conformational differences. Chemical shift data and coupling constants of the pentose protons in D_2O and CD_3CN are presented in Tables III and IV, respectively.

Determination of the fractional populations of the two conformational states is based on the experimental values of $J_{2',3'}$ and $J_{3',4'}$, assuming that these are 0–1 and 0–1 Hz, and 6–8 and 8–10 Hz, respectively, for the C(2')endo and C(3')endo conformations.[37] From these coupling constants it is estimated that the 2',3'-di-*O*-acetyl derivative 1 is ~45% C(3')endo in water and 64% C(3')endo in acetonitrile. This is a relatively minor difference and in the wrong direction to account for the solvent selectivity in the kinetics.

It is apparent, however, that in either solvent a significant percentage of the D-arabinose rings exist in that conforma-

Table III—Chemical Shifts (ppm) at 25°C of the Arabinose and Base Protons of 9-β-D-Arabinofuranosyladenine (ara-A) and Its 2',3'-Di-*O*-acetyl Derivative in D_2O and CD_3CN

Compound	Solvent	H (1')	H (2')	H (3')	H (4')	H (5')	H (5')	H (2)	H (8)
ara-A[a]	D_2O	6.43	4.56	4.36	4.08	3.97	3.89	8.22	8.35
2',3'-Di-*O*-acetyl derivative	D_2O	6.61	5.61	5.47	4.33	4.00	3.91	8.22	8.35
	CD_3CN	6.50	5.51	5.64	4.14	3.86	3.75	8.02	8.21

[a] The ara-A values are taken from ref. 36.

Table IV—Coupling Constants Between Vicinal Protons in 9-β-D-Arabinofuranosyladenine (ara-A) and Its 2',3'-Di-*O*-acetyl Derivative in D_2O and CD_3CN at 25°C

Compound	Solvent	$J_{1',2'}$ Hz	$J_{2',3'}$ Hz	$J_{3',4'}$ Hz	$J_{4',5'}$ Hz	$J_{4',5'}$ Hz
ara-A[a]	D_2O	5.5	5.5	6.3	2.8	4.6
2',3'-Di-*O*-acetyl	D_2O	5.1	2.9	4.4	2.9	5.1
derivative	CD_3CN	5.1	4.4	5.9	ND[b]	ND

[a] The ara-A values are taken from ref. 36. [b] Not determined.

tion which is considered a prerequisite for the observed acyl migration. The pronounced solvent selectivity in the migration may be due to a large shift in the relative concentrations of two or more conformational subpopulations directly involved in the reaction, or it may simply be due to differences in solvent stabilization of the transition states for the two reactions.

Experimental Section

Materials—All reagents were of the best quality commercially available and were used without further purification. Compound 6 (crystalline monohydrate) was provided by the Warner-Lambert Co., Ann Arbor, MI. *N,N*-Dimethylformamide (DMF) and tetrahydrofuran (THF) were rigorously dried by distillation from calcium hydride and potassium: benzophenone ketyl, respectively.

Analytical Procedures—HPLC was carried out in the reversed-phase mode using an octadecylsilyl-derivatized column (μ-Bondapak C_{18}, 4 × 300 mm; Waters Associates, Milford, MA) with mobile phases consisting of 15–25% methanol in water containing 1 mL/L of acetic acid, with sodium hydroxide added to adjust to pH 4.5. The instrument used for the HPLC analyses consisted of a Wisp 710B autosampler, M-45 solvent delivery system, Lambda-Max model 480 LC spectrophotometer (at 258 nm), model 720 system controller, and a data module (model 730) all from Waters Associates. Precision in these analyses was ±1–2% of analyte concentration for all species of interest. TLC was carried out using E. Merck aluminum-backed silica gel plates (0.2 mm thickness, "DC Alufoil F_{254}," cat. no. 5554). Visualization was by UV light at 254 nm.

Fourier transform ^1H NMR spectra were recorded on a JEOL JNM-FX-270 (270 MHz) or on a Nicolet NT-200 (200 MHz) instrument. Spectra were run at 24°C on solutions in D_2O (99.9 mol %), in CD_3CN (99.9 mol %) or in Me_2SO-d_6 at a concentration of 0.0067 M with sodium 3-(trimethylsilyl)-1-propanesulfonate (DDS) (for D_2O) or Me_4Si as internal standards. An asterisk indicates that the proton was shown to exchange with D_2O. Chemical shifts and spin–spin couplings are apparent, first-order values. Multiplicities are indicated: (s) singlet; (d) doublet; (dd) doublet of doublets; (ψt) pseudo triplet (i.e., a dd where *J* values are approximately equivalent); (m) multiplet; (br s) broad singlet. Identification of the H-2' and H-3' signals of the D-arabinose ring was accomplished using decoupling by irradiation of H-1' and/or H-4'. Elemental analyses are ±0.3% of the theoretical value for the elements listed.

Column chromatography was conducted at 1 atm. using E. Merck silica gel-60 (70–230 mesh ASTM, cat. no. 7734) with a ratio of 60–100:1 [silica gel:product (w/w)]. Product detection was by either TLC or continuous monitoring at 254 nm with an ISCO UA-5 detector.

Kinetics Procedures—Aqueous solution reaction kinetics were generally monitored over a pH range of ~2–10 in 0.01 ionic strength buffers.[38] Samples were prepared by dissolving an accurately weighed amount of reactant in an appropriate volume of buffer. These solutions were then placed in a water bath at 25°C prior to taking the first aliquot (representing time zero). Aliquots of samples taken at various time intervals were diluted 1:5 in a 1 M pH 4.5 acetate buffer and stored at −20°C prior to analysis by HPLC.

Slow reactions were studied by monitoring the initial rates of product formation (up to 3% total degradation) in solutions containing ~5×10^{-4} M reactant. First-order rate constants were calculated by dividing the slopes of plots of product concentration versus time obtained from linear regression by the initial ester or diester concentration. When reactions were more rapid, both reactant disappearance and product formation were monitored over several half-lives. First-order rate constants in these experiments were calculated using a nonlinear least-squares curve-fitting routine[39] applied simultaneously to the experimental species concentration versus time curves.

Initial rate determinations of the relative rates of 2'→5' versus 3'→5' acyl migration were also carried out in solvents varying in composition from 90 to 100% acetonitrile:water. In 100% acetonitrile, the more soluble tetra-*n*-butylammonium hydroxide (at a concentration of 8.27×10^{-3}) was used as a base catalyst rather than sodium hydroxide. A similar study was carried out in water using tetra-*n*-butylammonium hydroxide to determine the effect of the catalyst. Rate constants are tabulated in Table I. Both kinetics and product composition are shown in Figs. 1–5.

Preparation of 9-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (7)—To a stirred suspension of 2.66 g (10 mmol) of dry 9-(β-D-arabinofuranosyl)adenine (6) in 50 mL of dry DMF was added 3.40 g (5 equivalents) of imidazole, followed by 3.77 g (2.5 equivalents) of *tert*-butylchlorodimethylsilane (Petrarch). The mixture was stirred under nitrogen at room temperature for 48 h, at the end of which time the solvent was removed under reduced pressure at ~60°C. The syrupy residue was dissolved in ethyl acetate and washed with water, and the aqueous layer was extracted with 2 × 50 mL of ethyl acetate. The combined, dried ($MgSO_4$) organic layers were evaporated to give a crude syrup which was purified by silica gel chromatography (96:4 chloroform:ethanol as eluant) to give 1.44 g (29%; R_f 0.27, 93:7 chloroform:ethanol) of pure 7, identical to that described earlier.[26] Along with 7 was obtained 2.35 g (47.5%; R_f 0.30) of 9- [3,5-bis-*O*-(*tert*-butyldimethylsilyl)- β-D-arabinofuranosyl] adenine (8), 0.75 g (19.6%, R_f 0.10) of 5'-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (9), and 0.275 g (4.5%; R_f 0.42) of 9-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (10) which were isolated from the column and identified by ^1H NMR spectroscopy, as described previously.[22]

9-[3-*O*-Acetyl-2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (11)—To a stirred solution of 4.95 g (10 mmol) of 9-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (7) in 50 mL of dry pyridine cooled to 0°C was added 1.32 mL (12 mmol) of acetic anhydride; the mixture was stirred under a nitrogen atmosphere at 0–5°C for 48 h, at the end of which time the reaction was terminated by the addition of a few milliliters of water. The solvent was evaporated, and the residue was dissolved in ethyl acetate, washed with aqueous sodium bicarbonate and then with water, dried ($MgSO_4$), and evaporated to give a syrup. Silica gel column chromatography of the crude product with 95:5 chloroform:ethanol gave 3.16 g (59%) of pure 11: R_f 0.33 (93:7 chloroform:ethanol); ^1H NMR (200 MHz, Me_2SO-d_6): δ -0.4 (s, 3, SiMe), −0.05 (s, 3, SiMe), 0.07 (s, 6, $SiMe_2$), 0.60 (s, 9, *tert*-Bu), 0.9 (s, 9, *tert*-Bu), 2.12 (s, 3, Ac), 3.88 (m, 2, H-5',5'$_a$), 4.05 (m, 1, H-4'), 4.6 (m, 1, H-2'), 5.36 (m, 1, H-3'), 6.34 (d, 1, H-1'), 7.31 (br s, 2, NH_2*), and 8.11 and 8.15 ppm (2 s, 2, H-2, H-8). Anal.: C, H, N.

9-[3-*O*-Acetyl-β-D-arabinofuranosyl]adenine (2)—To a stirred solution of 1 g (1.86 mmol) of 9-[3-*O*-acetyl-2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (11) in 20 mL of dry THF was added 0.27 mL (0.28 g, 4.7 mmol) of glacial acetic acid, followed by

3.74 g (14.9 mmol) of tetra-*n*-butylammonium fluoride,[22] and the reaction was monitored by TLC. After TLC indicated complete removal of the protecting groups, the solution was passed through a bed of 100 g of silica gel and eluted with THF to give the crude product. Silica gel column chromatography of the crude product with 90:10 chloroform:ethanol gave 0.524 g (92.1%) of pure **2**, mp 223–224°C; R_f 0.22 (80:20 chloroform:ethanol); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 2.12 (s, 3, Ac), 3.69 (m, 2, H-5′,5′$_a$), 3.99 (m, 1, H-4′), 4.31 (m, 1, H-2′), 5.20 (m, 2, H-3′, OH*), 6.00 (m, 1, OH*), 6.27 (d, 1, H-1′), 7.30 (br s, 2, NH_2*), and 8.14 and 8.22 ppm (2 s, 2, H-2, H-8). Anal.: C, H, N.

9-[3-*O*-(*tert*-Butyldimethylsilyl)-β-D-arabinofuranosyl] adenine (12)—A suspension of 6.00 g (12.1 mmol) of 9-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (**8**) in 100 mL of 80% glacial acetic acid was heated at 80°C on a water bath for 8 h, after which time the glacial acetic acid was evaporated, and the residue was chromatographed over silica using 85:15 chloroform:ethanol to give 2.35 g (50.9%) of **12**, mp 237–238°C; R_f 0.43 (80:20, chloroform:methanol); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 0.13 (s, 6, $SiMe_2$), 0.9 (s, 9, *tert*-Bu), 3.58–3.79 (m, 3, H-4′, H-5′), 4.14 (m, 1, H-3′), 4.32 (br, 1, H-2′), 5.14 (br, 1, OH*), 5.75 (d, 1, OH*), 6.26 (d, 1, H-1′), 7.3 (br s, 2, NH_2*), and 8.15 and 8.2 ppm (2 s, 2, H-2, H-8).

9-[2,5-Di-*O*-acetyl-3-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (13)—To a stirred solution of 1.4 g (3.67 mmol) of 9-[3-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (**12**) in 20 mL of dry pyridine cooled to 0°C was added 0.83 mL (0.90 g, 8.81 mmol) of acetic anhydride; the mixture was stirred under a nitrogen atmosphere at 0–5°C for 48 h, at the end of which time the reaction was terminated by the addition of 5 mL of water. The solvents were evaporated, and the residue was dissolved in ethyl acetate, washed with aqueous sodium bicarbonate and then with water, dried ($MgSO_4$), and evaporated to give a syrup. Column chromatography of the syrup on silica gel using 90:10 chloroform:ethanol gave 1.51 g (88.4%) of **13**, mp 206–208°C; R_f 0.57 (80:20 chloroform:methanol); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 0.11 and 0.14 (2 s, 6, $SiMe_2$), 0.89 (s, 9, *tert*-Bu), 1.74 (s, 3, Ac), 2.05 (s, 3, Ac), 4.04–4.12 (m, 1, H-5′), 4.23–4.37 (m, 2, H-4′, H-5′), 4.78 (ψt, 1, H-3′), 5.30 (ψt, 1, H-2′), 6.5 (d, 1, H-1′), 7.33 (s, 2, NH_2*), and 8.15 and 8.25 ppm (2 s, 2, H-2, H-8). Anal.: C, H, N.

9-[2,5-Di-*O*-acetyl-β-D-arabinofuranosyl]adenine (3)—To a stirred solution of 1.32 g (2.83 mmol) of 9-[2,5-di-*O*-acetyl-3-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl] adenine (**13**) in 50 mL of dry THF was added 0.20 mL (3.3 mmol) of glacial acetic acid, followed by 2.13 g (8.47 mmol) of tetra-*n*-butylammonium fluoride,[22] and the reaction was monitored by TLC. After TLC indicated complete removal of the protecting group, the mixture was passed through a bed of 100 g of silica gel and was eluted with THF to give a crude product which was chromatographed on silica gel with 85:15 chloroform:ethanol to give 932 mg (93.6%) of **3** as a glass: R_f 0.42 (80:20 chloroform:methanol); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 1.74 (s, 3, Ac), 2.05 (s, 3, Ac), 4.03–4.09 (m, 1, H-5′), 4.32–4.42 (m, 2, H-4′, H-5′$_a$), 4.55–4.60 (m, 1, H-3′), 5.29 (ψt, 1, H-2′), 6.04 (br s, 1, OH*), 6.50 (d, 1, H-1′), 7.33 (s, 2, NH_2*), and 8.16 and 8.19 ppm (2 s, 2, H-2, H-8). Anal.: C, H, N.

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