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Mechanism of Alkaline Hydrolysis of *S*-Adenosyl-L-methionine and Related Sulfonium Nucleosides

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Abstract: Sulfonium nucleosides such as *S*-adenosylmethionine (SAM) and 5′-deoxy-5′-dimethylthioadenosine (DMTA) are very labile to mild alkaline conditions resulting in the cleavage of the glycosidic bond. This glycoside cleavage results from an elimination reaction where proton abstraction occurs at the carbon atom (5′ position) adjacent to the sulfonium center with subsequent elimination to form a 4′,5′ double bond. Cleavage of the glycosidic bond can be envisioned as a concerted part of the elimination reaction or via the formation of a "hemiacetal" intermediate which rapidly breaks down. The rate of glycosidic cleavage of SAM (DMTA) was found not to be linearly dependent on hydroxide ion concentration. This nonlinearity resulted because of the existence of two reacting species. SAM^- (or $DMTA^-$), which has the 2′- (or 3′-) hydroxyl group ionized (pK_a = 12.1), undergoes hydrolysis at a substantially slower rate (k_1 = 0.0363 M^{-1} s^{-1}) than the nonionized species (k_1 = 0.790 M^{-1} s^{-1}). The hydrolysis of 3′-deoxy-SAM, which does not have an acidic functionality in the pH range studied, exhibits a linear dependence on hydroxide ion concentration (k_1 = 0.488 M^{-1} s^{-1}). NMR experiments using DMTA in $NaOD/D_2O$ revealed that during the hydrolysis only a single hydrogen atom was exchanged with deuterium at the 5′ position. The small primary deuterium isotope effects (k_H/k_D = ~1.4) observed for the hydrolysis of both DMTA and $DMTA^-$ suggest asymmetric transition states for these proton abstractions. For the hydrolysis of the nonionized DMTA a significant solvent isotope effect was observed ($k_1(H_2O)/k_1(D_2O)$ = 0.524) suggesting a transition state with substantial bond making to the hydrogen acceptor. In contrast, for the hydrolysis of $DMTA^-$ the lack of a solvent isotope effect ($k_2(H_2O)/k_2(D_2O)$ = 1.02) suggested a transition state with little bond breaking in the reactant.

The role of *S*-adenosyl-L-methionine (SAM) in enzymatically catalyzed group transfer reactions is now well recognized.[1] In addition to SAM's unique properties as a biological methyl or aminopropyl donor, the molecule also exhibits some interesting chemical properties, many of which are inherent in its sulfonium nucleoside structure.[2] For example, SAM has been shown to be very labile to mild alkaline conditions resulting in the cleavage of the glycoside bond.[3] In sharp contrast, *S*-adenosylhomocysteine is stable under similar alkaline conditions, suggesting that the labilization of the glycosidic bond of SAM results from the presence of the sulfonium center. This sensitivity to alkaline hydrolysis appears to be a general characteristic of sulfonium nucleosides, since various purine and pyrimidine 5′-deoxy-5′-dimethylsulfonium nucleosides have been shown to undergo similar hydrolytic reactions.[3b,d] Using 5′-deoxy-5′-dimethylthioadenosine and methyl (5-deoxy-5-dimethylthio)-β-D-ribofuranoside as model compounds, Baddiley et al.[3d] and Frank et al.[3b] showed that

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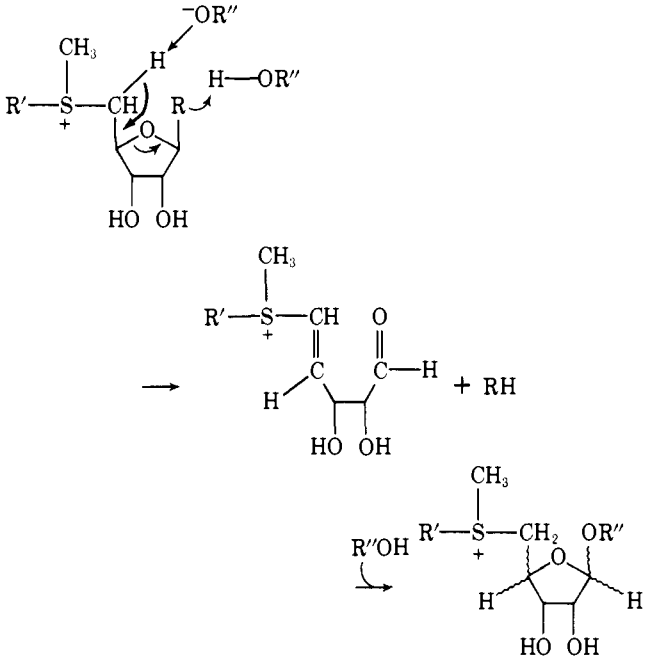
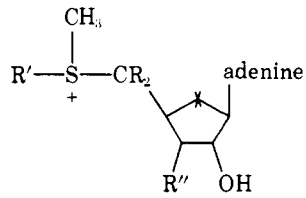
Scheme I



R = adenine, uracil, hypoxanthine, $-OCH_3$
R′ = $-CH_3$, $-CH_2CH_2CH(NH_2)CO_2H$
R″ = H, CH_3

cleavage of the glycoside bond occurred with loss of asymmetry at position 4 of the sugar and addition of the nucleophilic ion (^-OH or $^-OCH_3$) at position 1. These results are consistent with the mechanism outlined in Scheme I.[3b,d] Further evidence in support of this mechanism was reported by Schlenk and Dainko,[3c] who studied the alkaline hydrolysis of SAM in tritiated water. Their results[3c] showed that upon hydrolysis of SAM one tritium atom per molecule was incorporated into the "*S*-pentosylmethionine". These data are consistent with either a totally concerted E_2-type mechanism or a mechanism involving a rate-limiting E_2 type elimination to yield a "hemiacetal"-type intermediate followed by its subsequent rapid breakdown. The data would also be consistent with the E1cB mechanism in which the proton is removed fast and reversibly, but the oxy anion is expelled faster than proton exchange with the medium.

In an effort to further characterize the mechanism of alkaline hydrolysis of sulfonium nucleosides, we report here the kinetic order, the primary deuterium isotope effect, and the solvent deuterium isotope effect for this reaction. To determine the kinetic order for this reaction, we have studied the alkaline hydrolysis of SAM, *S*-3′-deoxyadenosyl-L-methionine (3′-deoxy-SAM), and 5′-deoxy-5′-dimethylthioadenosine (DMTA). The primary deuterium isotope effect was determined by comparing the hydrolysis rates for DMTA and DMTA-*5′,5′-d*$_2$, whereas the solvent isotope effect was determined by measuring the rates of hydrolysis of DMTA in



compd	R	R′	R″	X
SAM	H	$-CH_2CH_2CH(NH_2)CO_2H$	OH	O
3′-deoxy-SAM	H	$-CH_2CH_2CH(NH_2)CO_2H$	H	O
DMTA	H	$-CH_3$	OH	O
DMTA-*5′,5′-d*$_2$	D	$-CH_3$	OH	O
SAmM	H	$-CH_2CH_2CH(NH_2)CO_2H$	OH	CH_2

alkaline H_2O and D_2O. NMR experiments were also carried out to determine the rate of proton loss at the 5′ position of DMTA relative to the rate of hydrolysis. The hydrolytic properties of *S*-aristeromycinyl-L-methionine (SAmH), a carbocyclic analogue of SAM, were also investigated, since, according to the mechanism outlined in Scheme I, SAmH should not be labile to alkaline conditions.

Experimental Section

Materials. SAM chloride (Sigma) was purified by chromatography on Amberlite IRC-50 ion-exchange resin. 3′-Deoxy-SAM iodide and *S*-aristeromycinyl-L-methionine iodide (SAmM) were synthesized by methylation of *S*-3′-deoxyadenosyl-L-homocysteine[4a,b] and *S*-aristeromycinyl-L-homocysteine,[4c] respectively, with methyl iodide in acetic acid and formic acid.[5] These sulfonium nucleosides were purified as described earlier.[4a,b] Samples used for kinetic determinations were analytically pure as determined by spectral (NMR) and elemental analysis.[4a,b]

5′-Deoxy-5′-dimethylthioadenosine Iodide (DMTA). To a 0.1 N sodium methoxide in methanol solution (50 mL) saturated with methyl mercaptan and maintained at 0°C was added 460 mg (1 mmol) of 2′,3′-*O*-isopropylidene 5′-*O*-toluene-*p*-sulfonyladenosine.[6] The reaction mixture was slowly brought to reflux over a 90-min period. The reaction mixture was maintained at reflux for 16 h after which it was neutralized with acetic acid and evaporated to dryness in vacuo. A solution of the residue in $CHCl_3$ was washed with water, dried over magnesium sulfate, and evaporated to dryness in vacuo, yielding 155 mg of 5′-deoxy-2′,3′-isopropylidine-5′-methylthioadenosine as a colorless oil: NMR ($CDCl_3$) δ 1.43 (s, 3 H), 1.56 (s, 3 H), 2.11 (s, 3 H), 3.81 (d, 2 H), 4.45 (m, 1 H), 5.09 (m, 1 H), 5.58 (m, 1 H), 6.12 (d, 1 H), 7.98, 8.36 (2 s, 2 H).

The crude 5′-deoxy-2′,3′-isopropylidene-5′-methylthioadenosine was hydrolyzed to 5′-deoxy-5′-methylthioadenosine using 0.5 N sulfuric acid at ambient temperature for 16 h. The reaction mixture was neutralized with lead carbonate to pH 5.0 and filtered and the filtrate was lyophilized. The 5′-deoxy-5′-methylthioadenosine was recrystallized (H_2O) to yield 105 mg (35%), mp 208–209°C (lit.[7] mp 205°C), *m/e* 297 (M^+).

5′-Deoxy-5′-methylthioadenosine (100 mg, 0.34 mmol) was methylated to the desired DMTA using methyl iodide (1.0 mL) in formic acid (2.0 mL) and acetic acid (2.0 mL).[5] The reaction mixture was kept at ambient temperature for 5 days, then diluted with water (10 mL), and the aqueous phase was extracted three times with ethyl ether. The aqueous layer was lyophilized and the residue was precipitated from methanol-ethyl ether to yield 97 mg (65%) of the desired DMTA (mp 118–120°C): NMR (D_2O) δ 2.98 (s, 6 H), 3.95 (d, 2 H), 4.45–5.10 (m, 3 H), 6.10 (d, 1 H), 8.10, 8.20 (2 s, 2 H). Anal. Calcd for $C_{12}H_{18}IN_5O_3S$: C, 32.81; H, 4.13; N, 15.95. Found: C, 33.01; H, 4.11; N, 16.07. *m/e* 312 (M^+), 297 ($M^+ - CH_3$).

5′-Deoxy-5′-dimethylthioadenosine-*5′,5′-d*$_2$ Iodide (DMTA-*5′,5′-d*$_2$). 2′,3′-Isopropylideneadenosine-*5′,5′-d*$_2$ was prepared from 2′,3′-isopropylideneadenosine by oxidation at the 5′ position followed by reduction with sodium borodeuteride.[8,9] The 2′,3′-isopropylideneadenosine-*5′,5′-d*$_2$ was converted to the corresponding tosylate with *p*-toluenesulfonyl chloride and pyridine.[6] Using the procedures described above for the preparation of DMTA, 2′,3′-isopropylidene-5′-*O*-toluene-*p*-sulfonyladenosine-*5′,5′-d*$_2$ was reacted with 0.1 N sodium methoxide in methanol which was saturated with methyl mercaptan to yield 5′-deoxy-2′,3′-isopropylidene-5′-methylthioadenosine-*5′,5′-d*$_2$. The isopropylidene derivative was hydrolyzed to 5′-deoxy-5′-methylthioadenosine-*5′,5′-d*$_2$ [M^+ *m/e* (rel intensity) 299 (98), 297 (2)].

5′-Deoxy-5′-methylthioadenosine-*5′,5′-d*$_2$ was methylated using methyl iodide in formic and acetic acid to yield (72%) DMTA-*5′,5′,d*$_2$ (mp 119–121°C): NMR (D_2O) δ 2.90 (s, 6 H), 4.55 (d, 1 H), 4.60–5.10 (m, 2 H), 6.08 (s, 1 H), 8.10, 8.20 (2 s, 2 H); *m/e* (rel intensity) M^+ 314 (98), 312 (2), $M^+ - CH_3$ 299 (98), 297 (2).

Kinetic Measurement. The hydrolyses of SAM, 3′-deoxy-SAM, DMTA, and DMTA-*5′,5′-d*$_2$ were followed at 256 nm on a Gilford 240 spectrophotometer equipped with a thermostated cell compartment. All kinetic measurements were carried out at 37 °C. The reaction was initiated by the addition of 5–10 μL of stock solution of the substrate in water to 2 mL of thermoequilibrated alkaline solutions, followed by thorough mixing of the solution. The infinite absorbance values were obtained after at least 8 half-lives. All the reactions fol-

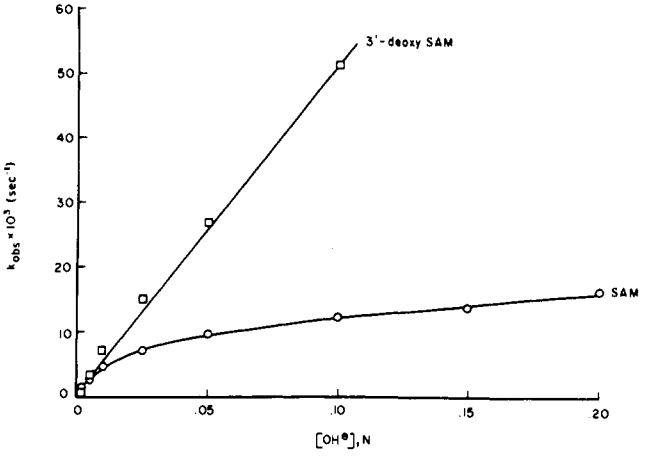


Figure 1. Plots of k_{obsd} vs. hydroxide concentration for the hydrolysis of SAM and 3′-deoxy-SAM at 37 °C.

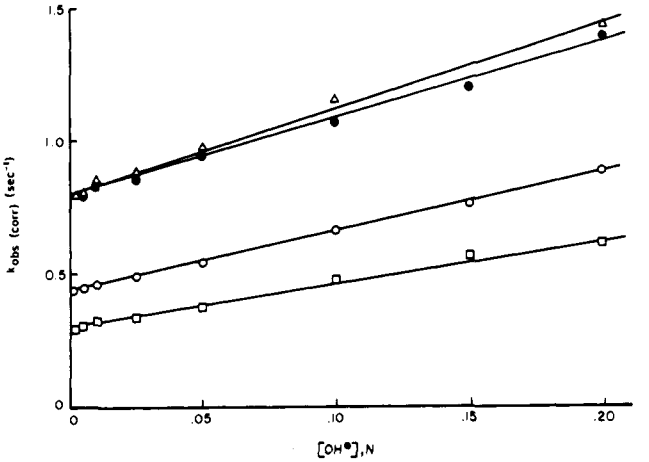


Figure 2. Plots of $k_{obsd(cor)}$ vs. hydroxide concentration for the hydrolysis of SAM (●—●), DMTA (○—○), and DMTA-5′,5′-d_2 (□—□) in H_2O and DMTA (△—△) in D_2O at 37 °C. $k_{obsd(cor)}$'s were calculated by multiplying the k_{obsd}'s by the following correction factor: $(1 + (K_a/K_w)\cdot[OH^-])/[OH^-]$. For DMTA, DMTA-5′,5′-$d_2$, and SAM a p$K_a$ = 12.1 was assumed. For ionization of DMTA to DMTA$^-$ in D_2O a pK_a = 12.90 was assumed.

lowed first-order kinetics. The ionic strength was maintained at 0.2 M by use of potassium chloride.

With DMTA and DMTA-5′,5′-d_2, the only reaction which occurred under the conditions used for the kinetic experiments was hydrolysis to yield adenine and 5-deoxy-5-dimethylthiopentose. The reaction products were separated by paper chromatography (Whatman no. 1) eluting with ethanol–water–acetic acid (65/34/1) and identified by comparison with the appropriate standards. Under strong alkaline conditions ([OH$^-$] > 0.05 M), SAM and 3′-deoxy-SAM also hydrolyzed to yield exclusively adenine and pentosylmethionine (or 3′-deoxypentosylmethionine), which were identified by paper chromatography. However, at alkaline concentrations below 0.05 M, a competing hydrolytic reaction occurred resulting in the formation of homocysteine lactone and methylthioadenosine.[2] This competing hydrolytic reaction did not result in a spectral change at 256 nm, and therefore did not interfere with the determination of the rates of glycoside cleavage.

Results

Synthesis. The sulfonium compounds used in this study were synthesized by methylation (methyl iodide–formic acid–acetic acid) of the appropriate alkylthioadenosine.[4,5] For the synthesis of DMTA-5′,5′-d_2, the intermediate 2′,3′-isopropylideneadenosine-5′,5′-d_2 was prepared from 2′,3′-isopropylideneadenosine by oxidation with potassium permanganate to form 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid.[8,9] This adenosine-5′-carboxylic acid derivative was subsequently reduced with sodium borodeuteride to form the desired 2′,3′-isopropylideneadenosine-5′,5′-d_2.[8]

2′,3′-Isopropylideneadenosine-5′,5′-d_2 was converted to the 5′-tosylate, which was reacted with methyl mercaptan in sodium methoxide in methanol to afford the desired 5′-deoxy-5′-methylthioadenosine-5′,5′-d_2. Mass spectral analysis of 5′-deoxy-5′-methylthioadenosine-5′,5′-d_2 revealed a molecular ion at *m/e* 299 with a fragmentation pattern consistent with that reported earlier for the protonated compound.[14] By analysis of the mass spectral data, the extent of deuteration was determined to be >98%.

Kinetic Experiments. The dependency of the rate (k_{obsd}) of glycosidic cleavage of SAM on hydroxide concentration is shown in Figure 1. The observed nonlinear dependence on hydroxide ion concentration suggests a possible change in the reacting species with increasing hydroxide concentration. Since the 2′- (or 3′-) hydroxyl group in adenosine and related nucleosides is known to be fairly acidic (pK_a = 12.3–12.5),[10] we considered the possibility that this deviation from linearity resulted from a change in the reacting species caused by an ionization. The proposed model system is outlined in Scheme II. According to this model at low hydroxide ion concentrations, SAM would react with a rate constant = k_1, whereas at higher hydroxide ion concentrations, the ionized species SAM$^-$ would react more slowly with a rate constant = k_2. From the mechanism shown in Scheme II, the rate equation 1 was derived, which can be rearranged to eq 2. The left side of eq 2 was defined as $k_{obsd(cor)}$ yielding eq 3. According to eq 3 a plot of

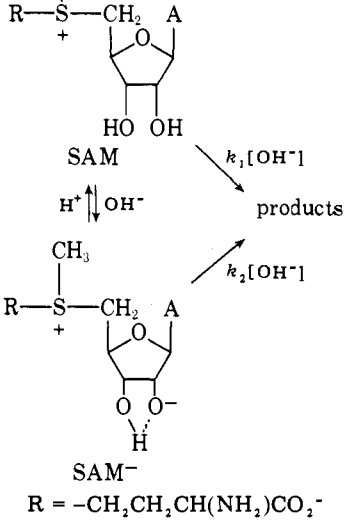
$$k_{obsd} = \frac{k_1[OH^-] + k_2\frac{K_a}{K_w}[OH^-]^2}{1 + \frac{K_a}{K_w}[OH^-]} \quad (1)$$

$$\frac{k_{obsd}\left(1 + \frac{K_a}{K_w}[OH^-]\right)}{[OH^-]} = k_1 + \frac{k_2K_a}{K_w}[OH^-] \quad (2)$$

$$k_{obsd(cor)} = k_1 + \frac{k_2K_a}{K_w}[OH^-] \quad (3)$$

$k_{obsd(cor)}$ vs. hydroxide ion concentration should yield a straight line with the intercept = k_1 and the slope = k_2K_a/K_w. Since the exact pK_a value for the ionization of SAM to SAM$^-$ is unknown, various pK_a values ranging from 11.5 to 12.5 were

Scheme II



R = $-CH_2CH_2CH(NH_2)CO_2^-$

Table I. k_1 and k_2 Values for the Hydrolysis of SAM, 3′-Deoxy-SAM, DMTA, and DMTA-5′,5′-d_2[a]

substrate	solvent	k_1, M^{-1} s^{-1}	solvent or primary isotope effect	k_2, M^{-1} s^{-1}	solvent or primary isotope effect
SAM	H_2O	0.790 ± 0.031		0.0363 ± 0.0015	
3′-deoxy-SAM	H_2O	0.488 ± 0.021			
DMTA[b]	H_2O	0.424 ± 0.044		0.0286 ± 0.0005	
DMTA[c]	D_2O	0.809 ± 0.021	$k_1(H_2O)/k_1(D_2O)$ = 0.524	0.0277 ± 0.0019	$k_2(H_2O)/k_2(D_2O)$ = 1.03
DMTA-5′,5′-d_2[b]	H_2O	0.290 ± 0.015	k_1(DMTA)/k_1(DMTA-5′,5′-d_2) = 1.46	0.0210 ± 0.0006	k_2(DMTA)/k_2(DMTA-5′,5′-d_2) = 1.36

[a]Kinetic determinations were made at 37°C and ionic strength was maintained at 0.2 M by use of KCl. Rate constants (k_1 and k_2) were calculated by linear regression analysis using eq 3 and the data shown in Figures 1 and 2. Correlation coefficients were >0.995. [b]The pK_a value for the ionization of DMTA to DMTA$^-$ (or DMTA-5′,5′-d_2 to DMTA-5′,5′-d_2^-) was assumed to be 12.10 in H_2O. [c]The pK_a value for the ionization of DMTA to DMTA$^-$ was assumed to be 12.90 in D_2O.

assumed and the data fitted to eq 3. The best fit of the data to eq 3 resulted when a pK_a = 12.1 was assumed. The resulting linear plot of $k_{obsd(cor)}$ vs. hydroxide ion concentration is shown in Figure 2. The rate constants, k_1 and k_2, which were determined by linear regression analysis of the data shown in Figure 2, are listed in Table I. The pK_a value of 12.1, which provided the best fit of the data, is in good agreement with the pK_a value (pK_a = 12.35[3c]) reported for the similar ionization of adenosine.

Further evidence in support of the model system shown in Scheme II was obtained by studying the rate of glycosidic cleavage of 3′-deoxy-SAM. A plot of k_{obsd} for the hydrolysis of 3′-deoxy-SAM vs. hydroxide concentration is shown in Figure 1. 3′-Deoxy-SAM, unlike SAM, has no acidic protons within the pH range studied.[10] Therefore, as would be predicted, the rate of hydrolysis of 3′-deoxy-SAM is linearly dependent upon the hydroxide concentration as shown in Figure 1. The calculated rate constant (k_1) for hydrolysis of 3′-deoxy-SAM is listed in Table I.

The primary deuterium isotope effect for this glycosidic cleavage was determined using DMTA and DMTA-5′,5′-d_2 as model compounds. Similar to the results reported above for the hydrolysis of SAM, the observed rates (k_{obsd}) of hydrolysis of DMTA and DMTA-5′,5′-d_2 are not linearly dependent on hydroxide concentration. This nonlinear dependence on hydroxide concentration apparently results from the ionization of DMTA to DMTA$^-$ (and DMTA-5′,5′-d_2 to DMTA-5′,5′-d_2^-) with an apparent pK_a = 12.1. The ionized species (DMTA$^-$ or DMTA-5′,5′-d_2^-) undergo hydrolysis at substantially slower rates than the nonionized species (DMTA or DMTA-5′,5′-d_2). When the observed rate constants were corrected for this ionization and plots of $k_{obsd(cor)}$ vs. hydroxide concentration were made, linear relationships were observed as shown in Figure 2. The rate constants k_1 and k_2 for the hydrolysis of DMTA and DMTA-5′,5′-d_2, which were determined by linear regression analysis of the data shown in Figure 2, and the primary deuterium isotope effects are listed in Table I.

The solvent isotope effects for this glycosidic cleavage were determined by studying the rates of hydrolysis of DMTA in H_2O and D_2O. For the hydrolysis of DMTA in D_2O, the best fit to the data was obtained when a pK_a = 12.90 was assumed. The increase in pK_a from 12.1 in H_2O to 12.9 in D_2O is consistent with the solvent isotope effect seen for ionization of other acids.[12a,b] When the observed rate constants for hydrolysis of DMTA in alkaline D_2O were corrected for ionization of the substrate and plots of $k_{obsd(cor)}$ vs. hydroxide concentration were made, a linear relationship was observed as shown in Figure 2. The rate constants for hydrolysis of DMTA in D_2O were then calculated using eq 3 and the results are listed in Table I.

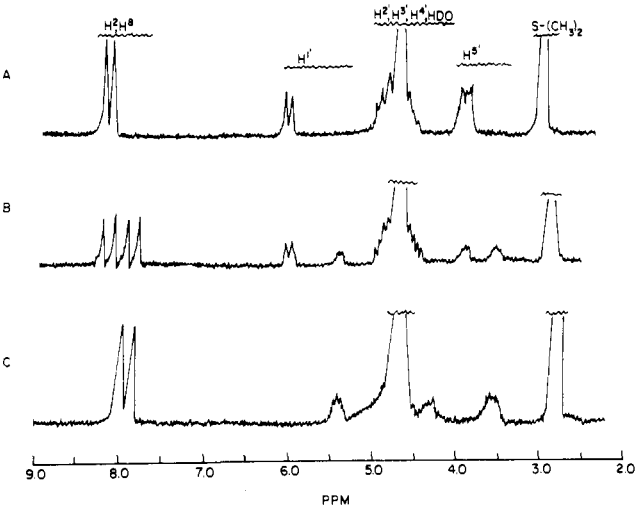


Figure 3. ^{1}H NMR spectra of DMTA and the alkaline hydrolysis products. Panel A is the spectrum of DMTA in D_2O. Panel B is the spectrum of DMTA in D_2O approximately 1 min after addition of NaOD (final OD^- concentration = 0.25 N). Panel C is the spectrum of DMTA in D_2O approximately 20 min after addition of NaOD.

NMR Experiments. The hydrolysis of DMTA could also be followed by NMR. As shown in Figure 3, the cleavage of the glycoside bond could be monitored by following the upfield shift of the C-2 and C-8 adenine protons. Characteristic changes were also observed in the signals for the C-1′ and C-5′ protons. After hydrolysis was complete, the signal for the C-5′ position integrated for approximately one proton, suggesting loss of a proton during the hydrolysis. These results are consistent with the exchange experiments reported earlier by Schlenk and Dainko.[3c] Under the conditions of the NMR experiment (DMTA concentration 0.02 M, 0.25 N NaOD–D_2O, ~30°C) the rate of hydrolysis (0.53 ± 0.05 min^{-1}), as determined by the upfield shift of the C-2 and C-8 protons, was approximately equal to the rate of loss (0.48 ± 0.07 min^{-1}) of the C-5′ proton. These results are consistent with an E1cB mechanism where elimination of the adenine base occurs much faster than equilibration of the removed proton with the solvent.

Hydrolytic Properties of SAmH. SAmH, a carbocyclic analogue of SAM, was synthesized[4,5] and its sensitivity to alkaline conditions determined. Under conditions (0.2 N NaOH, 37°C) in which the glycosidic bond of SAM is rapidly hydrolyzed ($T_{1/2}$ = 42 s), SAmM showed no evidence of glycosidic bond hydrolyses even after prolonged periods (3–4 h).

Discussion

SAM and related sulfonium nucleosides are extremely sensitive to alkaline conditions resulting in the rapid cleavage of the glycoside bond.[2] Certain aspects of the mechanism of this hydrolytic reaction have been elucidated and these are summarized in Scheme I.[3] This glycosidic cleavage results from an unusual elimination reaction where proton abstraction occurs at the carbon atom (5′ position) adjacent to the sulfonium center with subsequent elimination to form a 4′,5′ double bond. Cleavage of the glycosidic bond can be envisioned as occurring as a concerted part of the elimination reaction (as shown in Scheme I) or through the formation of a "hemiacetal" intermediate which rapidly breaks down to give the aldehyde. The observation made in this study that SAmM, a carbocyclic analogue of SAM, is stable to alkaline conditions provides evidence in support of this overall elimination mechanism (Scheme I).

Examination of the dependency of the rates of glycosidic cleavage of SAM and related sulfonium nucleosides (e.g., DMTA) on hydroxide concentration revealed that two reacting species exist, which undergo hydrolysis at differing rates (Scheme II). These reacting species result because of the acidic nature of the 2′- (or 3′-) hydroxyl group on adenosine-type compounds (pK_a = 12.0–12.5). The ionized species (e.g., SAM^-, $DMTA^-$, Scheme II) undergo hydrolysis at a substantially slower rate than the nonionized species (e.g., SAM, DMTA, Scheme II) (Table I). For nucleoside sulfonium compounds which do not have this acidic functionality (e.g., 3′-deoxy-SAM), the rate of hydrolysis is linearly dependent on hydroxide concentration.

The NMR experiments using DMTA, which were described in this study, confirm the results reported earlier by Schlenk and Dainko[3c] that a single hydrogen atom at the C-5′ position is lost in this hydrolytic reaction. In addition, the NMR experiments indicate that the rate of exchange appears to parallel the rate of cleavage of the glycoside bond. These observations would tend to rule out a E1cB mechanism where the resulting carbanion rapidly equilibrates with the starting material. Mamalis and Ryden,[11] while studying the alkaline-catalyzed elimination of aromatic acids from 2-aroyloxyethyldimethylsulfonium iodides, proposed such a carbanion intermediate, which they felt would be stabilized by delocalization through the sulfonium center. The results reported here and those reported earlier by Schlenk and Dainko[3c] would suggest that if these elimination reactions occur via a carbanion intermediate (E1cB mechanism) then the resulting carbanion must go to product much faster than it reverts to starting material (Scheme III, $k_{2'} \gg k_{-1'}$).

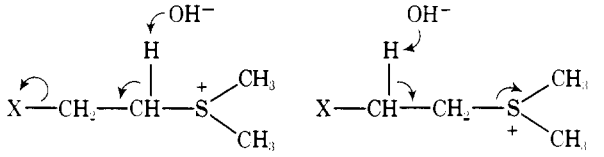
Scheme III

$$BH \underset{k_{-1'}}{\overset{k_{1'}}{\rightleftharpoons}} B \xrightarrow{-k_{2'}} \text{products}$$

To our knowledge no deuterium isotope effect studies have been reported for elimination reactions of the type observed in SAM, where proton abstraction occurs on the carbon adjacent to the sulfonium center (Scheme IV, type (a) elimination). Primary deuterium isotope effects have been reported, however, for Hofmann-type elimination reactions from 2-phenylethyldimethylsulfonium-*2,2-d*$_2$,[13] where proton abstraction occurs one carbon removed from the sulfonium center and dimethyl sulfide is the leaving group (Scheme IV, type (b) elimination). The deuterium isotope effect for the type (b) elimination (Scheme IV) is approximately 5,[13] which is well within the magnitude expected for rate-limiting proton abstraction.[12a]

The small primary deuterium isotope effects (Table I) observed for the reaction of both the nonionized and ionized forms of DMTA-*5′,5′-d*$_2$ suggest that in the transition state for these

Scheme IV



type (a) elimination type (b) elimination

proton transfer reactions there is either a small amount of bond breaking in the reactant or a large amount of bond making to the hydrogen acceptor. The latter possibility would imply considerable carbanion character in the transition state with the extreme being formation of a discrete carbanion and the reaction proceeding by a E1cB mechanism. However, it should again be emphasized that exchange experiments suggest that if a carbanion is formed it goes to product much faster than it reverts to starting material. The primary deuterium isotope studies alone do not permit differentiation of the possible asymmetric transition states. It can be concluded, however, that a symmetric bond-breaking and bond-making process is probably not occurring in the transition state, since a more substantial isotope effect of 3–5 would then be expected.

Possible Asymmetric Transition States. The solvent isotope effects reported in Table I provide some additional insight into the possible nature of the transition states for these reactions.

Since it was possible to differentiate the rates of glycosidic cleavage for the nonionized species DMTA (k_1) and the ionized species $DMTA^-$ (k_2), we were able to determine the solvent isotope effect on both reaction rates. For the reaction of the nonionized species, DMTA, a substantial solvent isotope effect was observed ($k_1(H_2O)/k_1(D_2O)$ = 0.524) suggesting a transition state where considerable bond making to the hydrogen acceptor had occurred. In sharp contrast, for the reaction of the ionized species, $DMTA^-$, no solvent isotope effect was observed suggesting a transition state where little bond breaking in the reactant and little bond making with the hydrogen acceptor had occurred. This apparent lack of carbanion character in the transition state for the reaction of $DMTA^-$ may be due to the anion character already present in this reacting species. The results from the solvent isotope studies suggest different asymmetric transition states for the elimination reactions from the ionized and nonionized forms of DMTA.

In conclusion, we have provided in this study additional insight into the general mechanism (Scheme I) by which the glycoside bonds in sulfonium nucleosides are cleaved under alkaline conditions. This glycosidic cleavage results from an unusual elimination reaction, where proton abstraction occurs from the carbon adjacent to a sulfonium center. The accessibility of such sulfonium nucleosides has permitted a detailed study of the nature of the transition state for such elimination reactions.

Acknowledgments. This work was supported by grants from the National Institutes of Health (NS-10198 and GM-22357) and by the American Heart Association (Established Investigatorship). The many helpful comments and suggestions provided by Dr. R. Schowen are gratefully acknowledged.

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Variation of the Linear Polarization Across the Emission Band of Nicotinamide 1,N^6-Ethenoadenine Dinucleotide Bound to Dehydrogenases

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Abstract: The linear polarization of the fluorescence of nicotinamide 1,N^6-ethenoadenine dinucleotide bound to several dehydrogenases studied varies significantly with emission wavelength. This spectral behavior is different for the different enzymes. No dependence of the linear polarization on the emission wavelength was observed for the free dinucleotide in glycerol solution. Similarly, the polarization spectrum of the excitation light of the complexes studied depends on the wavelength of the fluorescence selected for the measurements. The above findings are attributed to the fact that the transition responsible for the emission of the 1,N^6-ethenoadenine chromophore is weak. This transition thus "borrows" intensity from transitions involving higher electronic levels by vibronic coupling, thus conferring different polarization behavior to different vibronic transitions in the emission band. It is suggested that the sensitivity of the spectrum of the linear polarization across emission bands to the environment of the fluorophore in the case of weak transitions may be applied to the study of binding sites of biopolymers and other biological structures.

Introduction

1,N^6-Ethenoadenine and its derivatives are strongly fluorescent and have therefore been introduced as fluorescent probes in the study of nucleic acids and enzymes which require derivatives of adenine as substrates, cofactors, or effectors.[1-5] The 1,N^6-ethenoadenine derivatives retain a considerable fraction of the biochemical activity of the parent compounds in most of the cases studied;[1,2,4,5] the fluorescence properties of the etheno compounds are thus relevant to the specific binding sites of the macromolecules. It has been pointed out[5] that one of the important possibilities offered by these fluorescent compounds is the study of the linear polarization of the emitted light, which may be applied to the study of the rotational relaxation times of macromolecules of high molecular weight, owing to the relatively long fluorescence lifetime.

While a long lifetime of a fluorophore may permit wider application of its linear polarization in the study of macromolecules, it may, on the other hand, lead to serious complications of a fundamental nature in the behavior of the polarization of the fluorescence. The reason for this is that a long fluorescence lifetime of a fluorophore demonstrates that the electronic transition responsible for the emission is weak. In transitions of this kind the polarization properties in light absorption or emission depend not only on the electronic levels between which the transition takes place, but also on the particular vibrational levels of the ground and excited states which are involved in the absorption or emission at a specified region of the spectrum.[6-8] The polarization may consequently change across a spectral band which corresponds to a single electronic transition. Furthermore, the polarization may be sensitive to perturbations by the environment of the chromophore. Such behavior may be particularly pronounced if there is a strong electronic band located in the spectrum near the band under consideration.[6] It is thus obvious that the simplifications in the measurement and the interpretation of linear polarization which are permitted when the polarization is constant for every electronic transition cannot be utilized in the case of weak transitions, and one should be alert to possible complications which may arise because of the peculiarities of such transitions.

In recent studies of the *circular* polarization of the fluorescence of nicotinamide 1,N^6-ethenoadenine dinucleotide (ϵ-NAD^+) bound to glyceraldehyde 3-phosphate dehydrogenase (GPDH), an anomaly was noted in the spectral behavior of the circular polarization across the emission band.[9] This anomaly was attributed to the fact that the transition involved is a weak one. It has thus become of interest to test whether the "weakness" of the transition is also manifested in the spectral behavior of the linear polarization of the transition. In the following we present the results of this study. As will be shown below, the linear polarization of the fluorescence of ϵ-NAD^+ may indeed vary significantly across the emission band and the spectrum of the linear polarization is affected significantly by the environment of the fluorophore. The implications and possible applications of these findings will be discussed.

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