$-1.8°$ in ethanol,[15] thus leaving 300 mg in the cyclodextrin. Since we measured for optically pure (*R*)-(+)-3-methylcyclohexanone $[\alpha]_D$ +13.4°, in the same solvent[16]

$$\gamma = \tfrac{1}{2}[1 + 1.8/13.4(550/300 - 1)] = 0.56$$

and $\rho' = 1.7 \pm 0.25$ at 20 °C. This result can be compared to the value determined by ESR for the related biradical.

Conclusion

Evidence has been given by ESR for the selective inclusion in β-cyclodextrin of one of the two enantiomers of an optically active paramagnetic molecule. The two association constants have been determined. For paramagnetic molecules this method is more rapid and requires a smaller amount of material than the same determination by precipitation. The selectivity in the complexation is the same order of magnitude for racemic 3-methylcyclohexanone and the cognate racemic biradical. This is consistent with a chiral recognition based on the 3-methylcyclohexane residue in both compounds.

Acknowledgment. We thank Mrs. Joelle Martinie-Hombroück for some preliminary work.

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Role of Buffers in a Methylase Model Reaction. General Base Catalysis by Oxyanions vs. Nucleophilic Dealkylation by Amines

Jay O. Knipe and James K. Coward*

Contribution from the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received December 26, 1978*

Abstract: The *cis*-cyclopentanol derivative, **1**, was synthesized as a model for the *O*-methylation of ribose of tRNA, as catalyzed by tRNA 2′-*O*-methyltransferase. The decomposition of **1** in oxyanion buffers was studied over a wide pH range, at 25 and 40 °C. This reaction exhibits a plateau rate at low pH (k_{ROH}) and a hydroxide dependence at higher pH, associated with k_{RO^-}. Thermodynamic data gathered from a study of k_{ROH} and k_{RO^-} at five temperatures (25–40 °C) gave $\Delta S^{\ddagger} = -15$ eu for the k_{ROH} reaction and −3.7 eu for the k_{RO^-} reaction. Kinetic studies and product analysis data for the reaction of **1** lead to the conclusion that **1** cyclizes in an intramolecular fashion to give *cis*-2-oxabicyclo[3.3.0]octane (**3**) and *p*-nitrothioanisole (**12**) in a reaction which is catalyzed by the added buffer base. The fact that the trans analogue, **2**, which cannot undergo intramolecular alkylation, is inert in oxyanion buffers rules out any participation by buffer base in an intermolecular reaction. When the reaction of **1** is carried out in amine buffers, however, both *p*-nitrothioanisole and (2-*cis*-hydroxycyclopentyl)ethyl *p*-nitrophenyl sulfide (**4**) are detected in the reaction mixture by high-pressure liquid chromatographic (LC) analysis. The amount of each compound formed is dependent upon the amine used, the total buffer concentration, and the ratio of basic to acidic buffer species. When **2** reacts in amine buffers, *p*-nitrothioanisole and (2-*trans*-hydroxycyclopentyl) ethyl *p*-nitrophenyl sulfide (**5**) are formed, but the product ratios (**5**:**12** = ca. 9:1) are not dependent on buffer concentration, as with the reaction of **1**. Such product analysis data, as well as a comparison of the rates of reaction of **1** with those of other sulfonium compounds, indicate that amine buffers preferentially effect nucleophilic demethylation rather than function as general base catalysts, as do the oxyanions. A β value of 0.27 is obtained for all oxyanions and amines studied, with the exception of imidazole. No nucleophilic catalysis by imidazole of the demethylation of **1**, **2**, or dimethyl-*p*-nitrophenylsulfonium perchlorate (**6a**) could be demonstrated.

Introduction

The transfer of an intact methyl group from a suitable donor to a suitable acceptor is a vitally important biological process. With the exception of the role played by 5-methyltetrahydrofolic acid in methionine biosynthesis,[1] the universal donor of intact methyl groups is *S*-adenosylmethionine (AdoMet).[2] AdoMet is an important cofactor for enzymes that methylate a diverse array of nucleophilic acceptors within the cell (i.e., DNA, RNA, proteins, biogenic amines, etc.)[3] and has been implicated, by the fact that it may promote elevated levels of potentially psychotogenic methylated amines, as a potential causative agent in schizophrenia.[4]

Following a series of kinetic studies on catechol *O*-methyltransferase (COMT, EC 2.1.1.6), Coward and co-workers postulated that one of the two hydroxyl groups of a suitable catechol substrate could function as an intramolecular general base catalyst to facilitate methylation by AdoMet of the other hydroxyl group.[5] The proposed mechanism is shown below for this methylation. Also shown below is a possible mechanism involving intramolecular general base catalysis in the meth-

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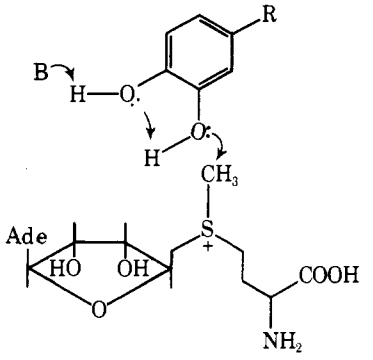
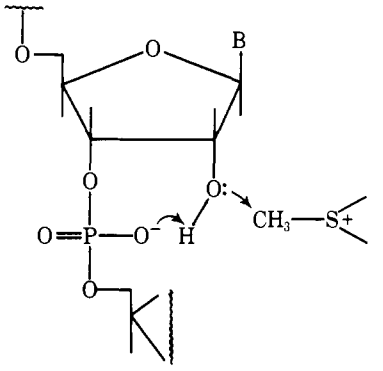
ylation of the 2′-hydroxyl of tRNA by tRNA methyltransferase (tRNA-MT, e.g., EC 2.1.1.34).

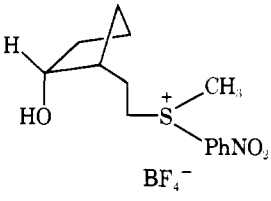


COMT

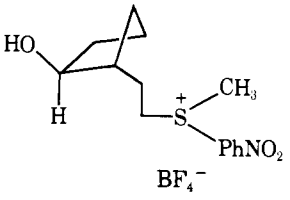


tRNA-MT

In order to test the feasibility of such a proposal, a series of compounds has been developed as models for both inter- and intramolecular transalkylations from a sulfonium to a nucleophile.[6] The goal of such studies has been to obtain a model compound containing both a sulfonium moiety and a nucleophilic (hydroxyl) group suitably positioned in relation to one another so that catalysis by added general bases would create a situation leading to a facile intramolecular alkylation of the nucleophile. Compound **1** has been synthesized (Scheme I) as a model for tRNA-MT, and the reaction of **1** and its trans isomer **2** with added bases has been studied.[7]



1



2

In this paper, we describe our findings on the different modes of buffer catalysis at sp^3 carbon, wherein oxyanion buffers catalyze the expected ring closure reaction of **1** to form *p*-nitrothioanisole and the bicyclic ether **3**, whereas amine buffers effect an intermolecular dealkylation to give primarily the thioether **4** (Scheme II). For comparative purposes, the kinetics of the intermolecular reactions of amines with three *p*-nitrophenylsulfonium compounds, **6**, has also been studied. The synthesis of a phenolic analogue as a model for COMT has recently been completed, and kinetic studies of its reactions will be the subject of a future publication.

Experimental Section

Preparation of Sulfonium Salts (6). The dimethyl- and diethyl-*p*-nitrophenylsulfonium salts (**6a**[8] and **6c**, respectively) were prepared by alkylation of the appropriate thioether precursor (obtained by treating 4-nitrothiophenol in NaOEt with either CH_3I or C_2H_5I) using methyl or ethyl iodide and $AgClO_4$, according to the procedure of Coward and Sweet.[8] **6c,** Anal. Calcd for $C_{10}H_{14}NO_6SCl$: C, 38.53; H, 4.53; N, 4.49; Cl, 11.37. Found: C, 38.50; H, 4.62; N, 4.42; Cl, 12.23; mp 81–82 °C.

The ethylmethyl-*p*-nitrophenylsulfonium salt **6b** was prepared by alkylating the thioether precursor (*p*-nitrothioanisole) with triethyloxonium tetrafluoroborate (Aldrich Chemical Co.) in methylene chloride according to the method of Bohme and Krack.[9] **6b,** Anal. Calcd for $C_9H_{12}NO_2SBF_4$: C, 37.92; H, 4.24; N, 4.93. Found: C, 38.40; H, 4.33; N, 4.83; mp 81–82 °C.

2-Oxocyclopentylethanol Ethylene Ketal (8) and Tosylate (9). Ethyl-2-oxocyclopentylacetate ethylene ketal (**7**) was reduced with $LiAlH_4$ in ether as previously described.[10] The alcohol **8** had the following characteristics: bp 101–105 °C (0.5 mm); NMR ($CDCl_3$) δ 3.88 (4 H, singlet, ketal protons), 3.60 (2 H, triplet, methylene protons adjacent to OH), 2.94 (1 H, broad singlet, OH), 1.8–1.2 (9 H, broad multiplet, ring protons and other methylene protons of side chain). The conversion of **8** to the tosylate **9** was carried out using the procedure of Lok and Coward.[12] The ketal tosylate obtained as a clear oil after high-vacuum drying had the following NMR ($CDCl_3$) characteristics: δ 7.70, 7.22 (4 H, 2 sets of doublets, aromatic protons), 4.04 (2 H, triplet, methylene protons adjacent to oxygen; note shift compared to pattern observed with **8**), 3.79 (4 H, singlet ketal protons), 2.41 (3 H, singlet, *p*-CH_3), 1.9–1.3 (9 H, broad multiplet, ring protons and other methylene protons of side chain).

1-(2-Oxocyclopentyl)-2-*p*-nitrothiophenylethane Ethylene Ketal (10). The ketal tosylate **9** was reacted with 4-nitrothiophenol in sodium ethoxide according to the general procedure of Waldron and Reid.[11] The resulting ketal thioether **10** was purified by preparative TLC (silica gel; hexane–EtOAc, 3:1). The oily material eluted ($CHCl_3$) from the TLC plate had the following NMR ($CDCl_3$) characteristics: δ 8.02, 7.20 (4 H, 2 sets of doublets, aromatic protons), 3.81 (4 H, singlet, ketal protons), 3.00 (2 H, triplet, methylene protons adjacent to sulfur; compare with **8** and **9**), 2.0–1.3 (9 H, multiplet, ring protons and other methylene protons of side chain).

1-(2-Oxocyclopentyl)-2-*p*-nitrothiophenylethane (11). The ketal sulfide **10** was converted to the keto sulfide **11** by the procedure of Lok and Coward[12] to give a yellow oil which solidified upon standing. The NMR ($CDCl_3$) had the following characteristics: δ 8.02, 7.21 (4 H, 2 sets of doublets, aromatic protons), 3.08 (2 H, triplet, methylene adjacent to sulfur), 2.4–1.4 (9 H, broad multiplet, ring protons and other methylene protons of side chain). Anal. Calcd for $C_{13}H_{15}NO_3S$: C, 58.92; H, 5.73; N, 5.30; S, 12.15. Found: C, 58.71; H, 5.78; N, 5.31; S, 11.95.

Reduction of 11. The preparation of the desired alcohols **4** and **5** was accomplished by reducing the keto sulfide **11** with either $NaBH_4$, as described by Lok and Coward,[12] or by using aluminum isopropoxide (Meerwein–Ponndorf reaction), as described by Macbeth and Mills.[13] Sodium borohydride reduction of **11** yielded predominantly the trans isomer **5,** as determined by recovery of the products from preparative TLC plates (trans:cis = 4:1), while isopropoxide reduction gave largely the cis isomer **4** (cis:trans = 3.5:1). The NMR ($CDCl_3$) characteristics of **4** (cis) are: δ 8.08, 7.28 (4 H, 2 sets of doublets, aromatic protons), 4.21 (1 H, singlet CH), 3.09 (2 H, triplet, methylene adjacent to sulfur), 2.1–1.1 (10 H, broad multiplet, ring protons and other methylene groups of side chain). The NMR spectrum of the trans alcohol **5** is identical with that of **4** with the exception that the singlet for the methine proton is at 3.83 instead of 4.21.[14] Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.40; H, 6.41; N, 5.24; S, 11.99. **4,** Found: C, 58.26; H, 6.16; N, 5.31; S, 12.16. **5,** Found: C, 58.62; H, 6.54; N, 5.18.

Preparation of Sulfonium Salts 1a,b, and 2a,b. Methylation of **4** and **5** to give **1a** and **2a**, respectively, was carried out using CH_3I and $AgBF_4$ in toluene and methylene chloride, as described by Lok and Coward.[6] Ethylation of **4** and **5** to give **1b** and **2b**, respectively, was performed according to the procedure of Bohme and Krack[9] using triethyloxonium tetrafluoroborate in methylene chloride. These sulfonium salts were not isolated but stored in aqueous solution in the cold. For kinetic experiments an aliquot of one of these solutions, sufficient to give a final substrate concentration of ca. 10^{-4} M in the buffer medium, was used. The concentration was estimated from the extinction at the λ_{max} of the sulfonium salt (ca. 250 nm) in water.[8]

***cis*-2-Oxabicyclo[3.3.0]octane (3).** The reduction of 2-cyclopentene-1-acetic acid (Aldrich Chemical Co., 98%, used without further purification) to the corresponding alcohol was carried out using $LiAlH_4$ in ether as previously described.[15] This alcohol was then converted to **3** using the method of Moon and Waxman.[16] The product was isolated by distillation (bp 55–61 °C (33–36 mm); lit. bp 49–50

°C (28 mm)) and shown to be pure by GC/MS analysis.

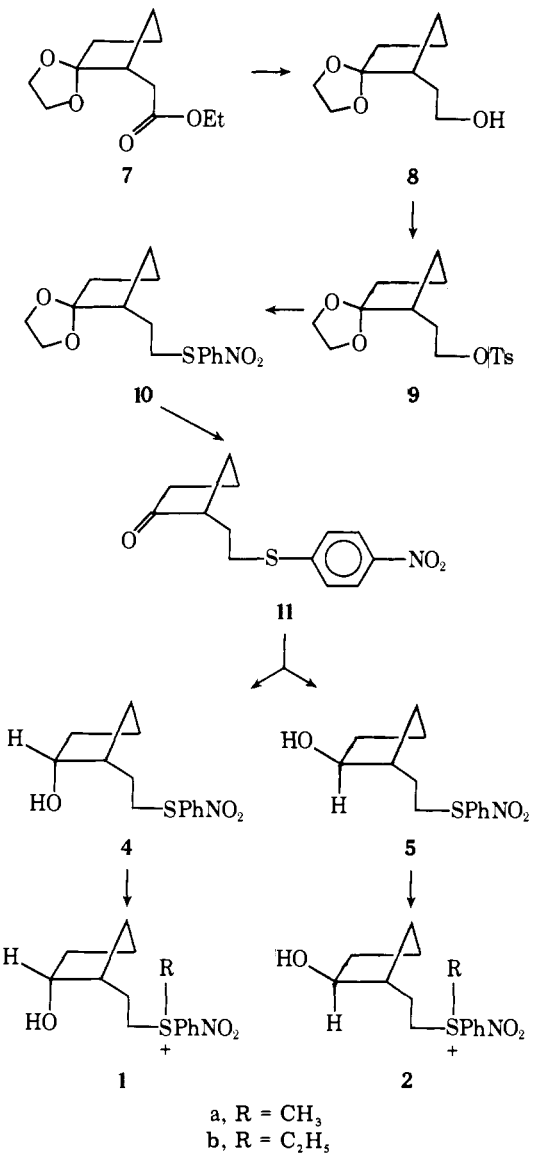
Cis (14) and Trans (15) Isomers of 1-(2-Methoxycyclopentyl)-2-*p*-nitrothiophenylethane. The keto sulfide **11** was reduced in TFA/methanol (3:1 mole ratio) using triethylsilane (Pflatz and Bauer) according to the procedure given by Doyle et al.[17] Following workup, the products were partially purified using preparative silica gel TLC (hexane-EtOAc, 3:1). Using this system, however, complete resolution of two bands (R_f 0.60 and 0.68) could not be achieved. The NMR ($CDCl_3$) spectrum of the upper band had the following characteristics: δ 8.10, 7.24 (4 H, 2 sets of doublets, aromatic protons), 3.65 (1 H, singlet, CH), 3.23 (3 H, singlet, OCH_3), 3.03 (2 H, broad triplet, CH_2 adjacent to S), 2.15-1.20 (9 H, broad multiplet, ring protons and other CH_2 of side chain). The NMR of the material eluted from the lower band had essentially the same characteristics, with the exception that a broad multiplet (integrating for 2 H) was present at δ 3.70 instead of the singlet at δ 3.65 seen in the spectrum of the sample from the upper band. Therefore, it is not possible to differentiate the cis (**14**) from the trans (**15**) isomer by NMR. High-pressure liquid chromatographic (LC) analysis (ODS, see below) indicated that the upper band was pure, the only peak having a retention time of 85 min (50% $MeOH/H_2O$ mobile phase; flow = 0.6 mL/min). The lower band appeared to be ca. 75% pure, its chromatogram containing a predominant peak having a retention time of 78 min and 2 smaller peaks having retention times of 45 and 50 min. The latter values approximately correspond to those obtained for the cis and trans alcohols, **4** and **5**. As noted previously,[17] alcohols are often byproducts of this type of synthesis. Anal. Calcd for $C_{14}H_{19}NO_3S$: C, 59.76; H, 6.81; N, 4.98; S, 11.40. Upper band, Found: C, 59.69; H, 6.96; N, 5.08; S, 11.15.

Kinetics. Inorganic salts were of reagent grade and used without further purification. The preparation of *tert*-butylphosphonic acid was carried out according to the procedure of Crofts and Kosolapoff.[18] Amines were distilled over barium oxide before use, or, when amine hydrochlorides were used, recrystallized before use. All buffers used in the course of these kinetic experiments were prepared in double distilled water and the ionic strength of all buffers was maintained at 1.0 M with potassium chloride. All kinetic experiments were performed in aqueous media at temperatures ranging from 25 to 40 °C. Under these conditions all buffers were stable and showed no pH change at a particular temperature, as would be expected from evaporation of volatile buffer species (e.g., amines). Kinetic experiments were carried out on a Gilford Model 25 spectrophotometer equipped with a thermostated sample chamber which was maintained at desired temperature by means of a circulating water bath. The pH and temperature of each buffer were determined at the beginning and end of each kinetic run. Spectral scans of the products of a kinetic experiment were performed on a Cary Model 15 spectrophotometer. A kinetic experiment was defined as complete after it had proceeded ca. 10 half-lives.

Kinetic experiments were performed in 3-mL quartz cuvettes and initiated by the rapid addition of the substrate (sulfonium salt) to a preequilibrated buffer solution at the desired temperature. Each experiment was carried out using a fixed ratio of basic to acidic buffer species, but with three different total buffer concentrations (μ = 1.0 M with KCl). Typically, the concentration of the substrate in the buffer solution was ca. 10^{-4} M. Reactions were followed by monitoring the increase in optical density at 350 nm, indicative of formation of a thioether from the corresponding sulfonium compound (λ_{max} ca. 250 nm). Under the pseudo-first-order conditions of these experiments, a plot of ln $(A_\infty - A_t)$ vs. time was linear over at least 3 half-lives, giving k_{obsd} (s^{-1}) as the slope of the line. A secondary graphing of k_{obsd} vs. total buffer concentration gave, as y intercept k_0 (s^{-1}) and, as the slope, k_{B_T} (s^{-1} M^{-1}), indicating the degree of catalysis by both the acidic and basic components of the buffer mixture. Further analysis of the kinetic data yielded the necessary data to construct Brønsted and Arrhenius plots.[19]

The analysis of products of a kinetic experiment was performed using high-pressure liquid chromatography (LC). All LC analyses were carried out using a system comprised of the following components: Milton Roy Mini-Pump with a maximum pressure of 5000 psig, an Altex analytical UV detector, an Altex analytical optical unit equipped with a 340-nm filter, and a Linear recorder. A Whatman Partisil PXS 10/25 ODS reverse phase column was used for all experiments. For elution, 50% methanol-water was found to give the best separation of the required compounds. The products formed during a kinetic experiment were identified by comparing their retention times with those of known compounds, under identical conditions. Aliquots from a kinetic experiment (ca. 10-20 μL) were injected directly onto the LC column.

Scheme I



Results

The syntheses of **1** and **2** were effected by the reactions shown in Scheme I and are described in detail in the Experimental Section.

The decomposition of **1a** in aqueous oxyanion buffers (acetate, phosphate, borate, *tert*-butylphosphonate, hexafluoro-2-propanol, carbonate, and hydroxide) was studied over a wide range of pH at various temperatures. This buffer-catalyzed decomposition proceeds according to Scheme II (k_{GB}). In contrast to the behavior of the cis alcohol **1a**, the trans alcohol **2a** is completely inert under the same reaction conditions. Figure 1 shows the pH-rate profiles for the decomposition of **1a** at 25 and 40 °C in aqueous oxyanion buffers. These profiles fit a general rate law (eq 1):

$$k_0 = k_{H_2O} + k_{OH^-}[OH^-] \qquad (1)$$

where k_0 is the observed rate constant (k_{obsd}) extrapolated to zero buffer concentration, which is pH independent from pH 4.7 to ca. 9-10. In this latter region, a transition occurs from the pH-independent rate to a strongly pH-dependent one (slope = 1.0) associated with the k_{OH^-} term in eq 1. The apparent k_{H_2O} and k_{OH^-} terms in eq 1 are, however, due to the presence

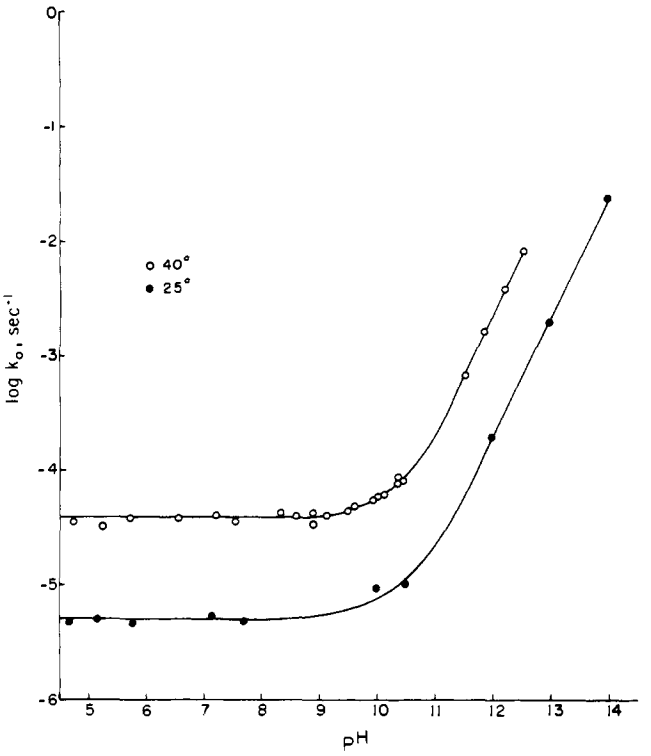
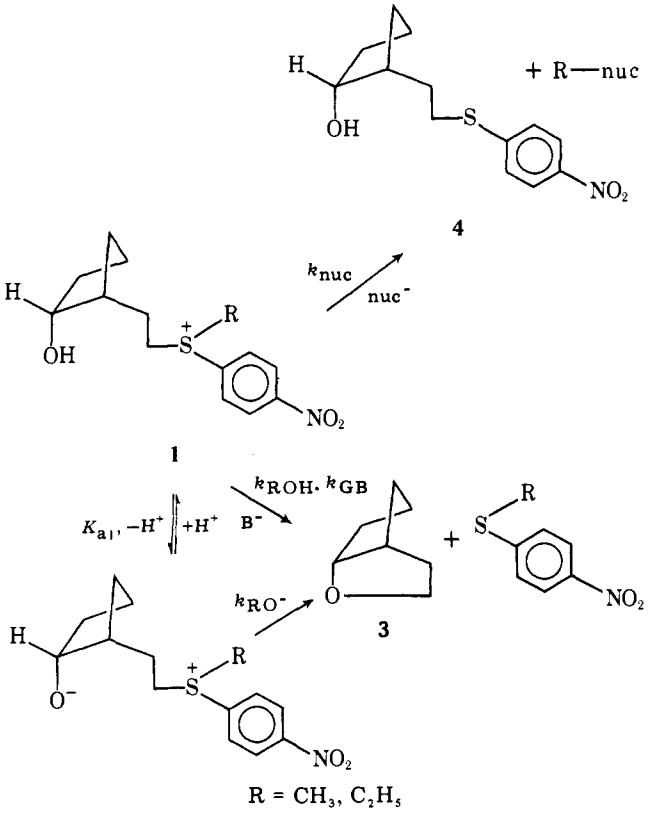


Figure 1. pH-rate profiles for the decomposition of **1a** in aqueous oxyanion buffer solutions at 40 and 25 °C. At pH greater than 11, pH = pK_w + log [OH$^-$].

Scheme II



of the secondary hydroxyl group of **1a.** While intramolecular participation of the hydroxyl group of the trans alcohol **2a** is impossible, **2a** could undergo the usual intermolecular lyate reactions normally associated with the k_{H_2O} and k_{OH^-} terms. The fact that **2a** is inert under these conditions is consistent with this interpretation of the apparent k_{H_2O} and k_{OH^-} terms.

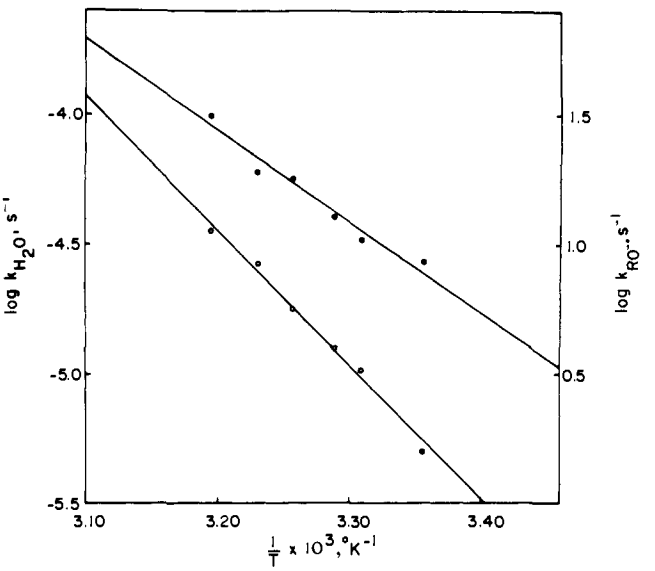


Figure 2. Arrhenius plot constructed from the data given in Table I: open circles, k_{ROH}; closed circles, k_{RO^-}.

Additionally, the k_{OH^-} terms obtained for the reaction of **1a,** at temperatures ranging from 25 to 40 °C (see Table I), are much larger than the k_{OH^-} values obtained previously with other sulfonium compounds at higher temperatures.[8] These k_{OH^-} values are also about 10^2 higher than that which would be predicted from a Brønsted plot if hydroxide were acting as a general base (see below).

These data are consistent with a rate law of the type given in eq 2:

$$k_0 = k_{ROH}\left(\frac{a_H}{K_a + a_H}\right) + k_{RO^-}\left(\frac{K_a}{K_a + a_H}\right) \qquad (2)$$

where K_a refers to the dissociation constant of the secondary alcohol of **1a.** It has been difficult to estimate a pK_a for this alcohol at a temperature other than 25 °C due to a lack of data in the literature dealing with the variation of pK_a with temperature for secondary alcohols. Data given by Cohen and co-workers[20] indicate a pK_a of 16.57 for 2-propanol at 25 °C, obtained by an evaluation of the carbonyl stretching frequency of its 3-phenylpropionate ester. Earlier, Murto[21] presented a pK_a of 18.1, based on kinetic measurements. Thermodynamic data (ΔH values) have been given by Briere and colleagues[22] for the pK_a change of methanol between 18 and 37 °C and for that of ethanol between 20 and 25 °C. Using the pK_a value given by Cohen et al. (16.57 at 25 °C) and the ΔH values of Briere et al., a pK_a of ca. 16.2 has been calculated for 2-propanol at 40 °C. A linear relationship was assumed to hold between these temperatures, thus giving the values at other temperatures (Table I). These values were applied to the hydroxyl group of **1a** and used to calculate k_{RO^-} at each temperature studied, according to eq 2. In Table I are listed the values of k_{ROH} (s^{-1}), apparent k_{OH^-} (s^{-1} M^{-1}), and k_{RO^-} (s^{-1}) evaluated for the reaction of **1a** with oxyanion buffers at various temperatures from 25 to 40 °C. An Arrhenius plot of these data (k_{ROH} and k_{RO^-}) is shown in Figure 2. Such a plot allows the calculation of E_a for both the alcohol rate (k_{ROH}) and alkoxide rate (k_{RO^-}). From E_a, values for $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ may be obtained. These thermodynamic parameters are given in Table II.

In addition to the oxyanion buffers mentioned above, the decomposition of **1a** was studied in the presence of various amine buffers at 40 °C. A comparison of kinetic constants obtained for the reaction of **1a** and several related compounds in the presence of a representative oxyanion (phosphate) and amine (*N*-methylmorpholine) buffer at 40 °C is shown in

Table I. Rate Constants Obtained for the Decomposition of **1a** at Various Temperatures in Aqueous Solution[a]

T, °C	pK_a[b]	$10^5 k_{ROH}$, s^{-1}	$10^2 k_{OH^-}$, s^{-1}, M^{-1}	k_{RO^-}, s^{-1}
25	16.57	0.50	2.32	8.63
29	16.45	1.05	2.73	10.50
31	16.39	1.27	3.51	12.88
34	16.30	1.80	4.68	17.93
36.5	16.23	2.64	4.86	18.90
40	16.13	3.59	8.13	31.23

[a] All values are an average of at least three determinations. The 25 and 40 °C values were obtained by a "best-fit" to either eq 1 or 2, over the entire pH profile. At other temperatures, k_{ROH} values were obtained from the plateau rates observed in phosphate buffer, at three different buffer ratios (pH 6.60, 7.20, and 7.60), while k_{RO^-} and k_{OH^-} were calculated from eq 1 and 2 from rates obtained in hydroxide solution (5×10^{-3}–10^{-1} M). [b] Values of pK_a at the various temperatures were calculated as described in the text.

Table II. Thermodynamic Parameters Calculated for the Decomposition of **1a** in Aqueous Solution[a]

parameter	alcohol reaction, k_{ROH}, kcal/mol	alkoxide reaction, k_{RO^-}, kcal/mol
E_a	23.11	15.67
$\Delta H^{\ddagger}$	22.52	15.13
$\Delta G^{\ddagger}$	26.99	16.13
$\Delta S^{\ddagger}$[b]	−15	−3.7

[a] Calculated from Arrhenius plots (Figure 2). [b] $\Delta S^{\ddagger}$ given in cal/deg-mol.

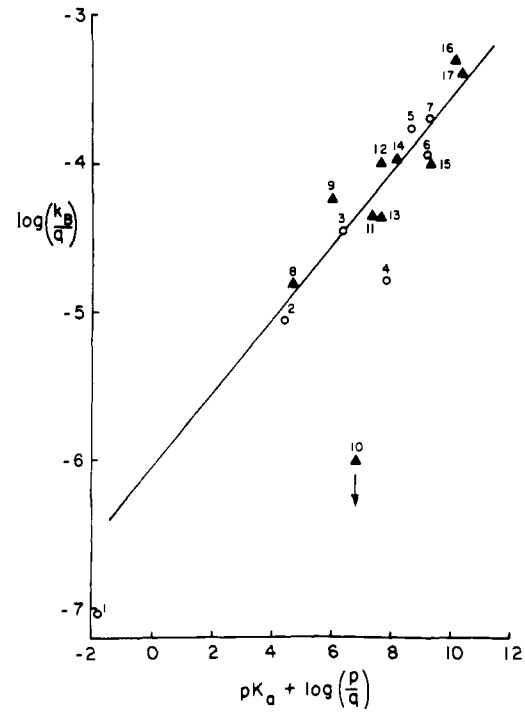
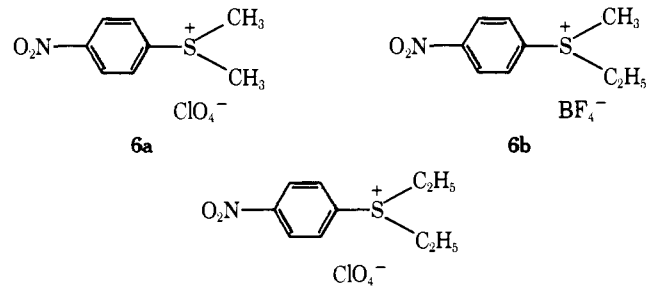


Figure 3. Brønsted plot constructed from the data given in Table IV. Open circles are points for oxyanion buffers; closed triangles are points for amine buffers. The line drawn ($\beta = 0.27$) is the best fit of the data with points 1, 6, and 10 omitted. Statistical corrections, according to the procedure of Bell,[39] have been applied to the values given in Table IV for buffers 2, 3, 4, 7, 11, and 13.

Table III. Within this table a number of interesting comparisons should be noted. While the trans isomer **2a** is inert to phosphate, it reacts with *N*-methylmorpholine with a second-order rate constant, k_B, which is similar to that for the reaction of *N*-methylmorpholine with the cis isomer **1a.** The *cis*-ethylsulfonium compound **1b** is similarly decomposed in phosphate buffer (but with a lower k_{ROH} and k_B than the *cis*-methylsulfonium **1a;** see Discussion). The ethyl analogue, **1b,** shows a lesser sensitivity to *N*-methylmorpholine concentration (lower k_B) than does **1a.** Note that the pH-independent rates (k_{ROH}) for the cis isomers, **1a** (3.6×10^{-5} s^{-1}, 3.4×10^{-5} s^{-1}) and **1b** (2.1×10^{-5} s^{-1}, 2.2×10^{-5} s^{-1}), are essentially the same in both the amine and oxyanion buffers. The k_B for the reaction of *N*-methylmorpholine with the trans isomer **2b** is similar to that for the reaction of the cis isomer **1b** and, as seen with **2a, 2b** is inert to phosphate buffer. Compounds **6a, 6b,** and **6c,** which do not react in phosphate buffer, are interesting in that they allow one to compare the ability of *N*-methylmorpholine to attack a carbon of a methyl or ethyl group adjacent to a sulfonium pole (compare k_Bs). This difference should be noted and compared to the k_B differences



shown for the reaction of the ethyl series (**1b, 2b**) vs. the methyl series (**1a, 2a**) in *N*-methylmorpholine.

Table IV lists the oxyanions and amines employed in this study and the k_B values obtained for the decomposition of **1a** in each buffer. From these data the Brønsted plot shown in Figure 3 ($\beta = 0.27$) for both oxyanions and amines was constructed.

The fact that the trans alcohol **2a,** as well as the sulfonium compounds **6a, 6b,** and **6c,** reacted with amine buffers indi-

Table III. Kinetic Constants for the Reaction of Selected Compounds with Either Phosphate ($pK_a = 6.59$) or *N*-Methylmorpholine ($pK_a = 7.62$) Buffers at 40 °C (1.0 M Ionic Strength)

	phosphate			*N*-methylmorpholine		
compd[a]	$10^5 k_{ROH}$, s^{-1}	$10^5 k_B$, s^{-1} M^{-1}	type of catal[b]	$10^5 k_{ROH}$, s^{-1}	$10^5 k_B$, s^{-1} M^{-1}	type of catal[b]
1a	3.55	10.74	G.B.	3.38	10.50	nuc
2a	~0.02	<0.01		0.01	7.52	nuc
1b	2.12	3.98	G.B.	2.21	0.87	nuc
2b	~0.01	<0.01		0.02	0.89	nuc
6a	~0.005	<0.01		0.01	17.36	nuc
6b	~0.02	0.001		0.03	4.83	nuc
6c	~0.01	0.01		0.005	0.68	nuc

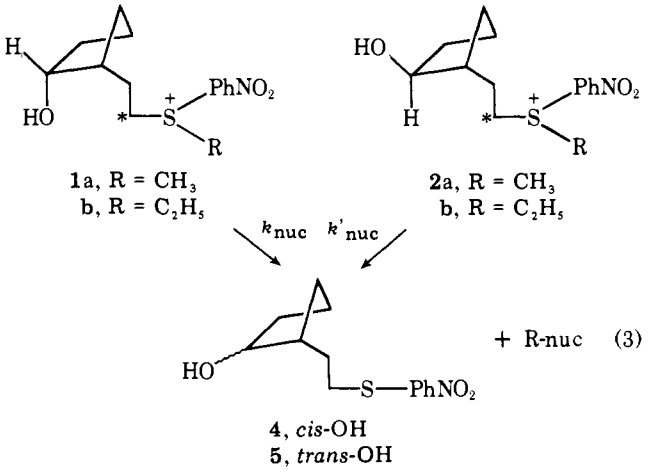
[a] Refer to eq 3 for the structures of **1a, 1b, 2a,** and **2b** and to the Discussion for the structures of **6a, 6b,** and **6c.** [b] G.B., general base catalysis; nuc, nucleophilic catalysis.

Table IV. Second-Order Rate Constants (k_B) for the Decomposition of **1a** in Various Buffers at 40 °C

no.[a]	buffer	pK_a[b]	k_B, s^{-1} M^{-1}
1	water	−1.74	9.01×10^{-8}
2	acetate	4.77	1.78×10^{-5}
3	phosphate	6.59	1.07×10^{-4}
4	*tert*-butylphosphonate	8.35	5.07×10^{-5}
5	borate	8.63	1.72×10^{-4}
6	hexafluoro-2-propanol	9.18	1.16×10^{-4}
7	carbonate	9.75	5.90×10^{-4} [c]
8	*N,O*-dimethylhydroxylamine	4.73	1.57×10^{-5}
9	*N*-methylhydroxylamine	6.02	5.89×10^{-5}
10	imidazole	6.85	$<10^{-6}$
11	ethylenediamine	7.06	4.56×10^{-5}
12	*N*-methylmorpholine	7.62	1.05×10^{-4}
13	hydrazine	7.95	8.85×10^{-5}
14	morpholine	8.16	1.10×10^{-4}
15	ethanolamine	9.32	9.94×10^{-5}
16	cyclohexylamine	10.10	4.84×10^{-4}
17	*n*-butylamine	10.34	4.02×10^{-4}

[a] Numbers refer to the Brønsted plot, Figure 3. [b] The pH of a solution containing a 1:1 ratio of acidic to basic buffer species, at 40 °C (1.0 M ionic strength). [c] pH changes of up to 0.4 unit were observed upon dilution of this buffer with KCl, leading to some uncertainty in the value of the k_B obtained.

cated that the decomposition of **1a** in those buffers was probably not as simple as originally described for oxyanions.[7] In light of the facts that k_B for the reaction of **6c** with *N*-methylmorpholine is lower (ca. 25-fold) than that for the reaction of **6a** with *N*-methylmorpholine and that k_B for the reaction of **1b** with *N*-methylmorpholine is lower (ca. tenfold) than that for the reaction of **1a** with *N*-methylmorpholine, the reactions shown in eq 3 were considered. This equation omits the possible nucleophilic attack at the methylene carbon (*) adjacent to the sulfonium pole, which, in view of the above considerations, is thought to participate only to a minor extent, if at all. This question was further addressed with the aid of product analysis as described below.



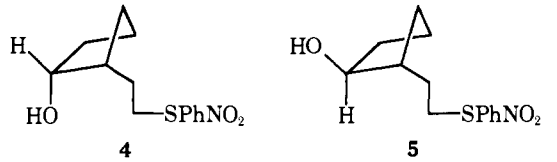
1a, R = CH_3; **b**, R = C_2H_5

2a, R = CH_3; **b**, R = C_2H_5

4, *cis*-OH

5, *trans*-OH

In order to further clarify the mechanisms of the reactions studied, product analyses were carried out. It was necessary to develop a system that could separate the following four thioethers (**4, 5, 12,** and **13**). This could not be done using a



4 $O_2NPhSCH_3$ **12**

5 $O_2NPhSC_2H_5$ **13**

spectral method as all four compounds possess the same λ_{max} (ca. 350 nm). The method of choice proved to be high-pressure liquid chromatography (LC) using a reverse-phase column coupled to a 340-nm filter in the detector, with elution carried out using 50% aqueous methanol, as described in the Experimental Section. All LC analyses were performed on the reaction mixtures of kinetic runs which had been carried out at 40 °C. In such a system, the sulfonium precursors of the above thioethers were not retained on the column but were eluted in the void volume. The thioethers had the following retention times and were thus easily differentiated: **4,** 52–53 min; **5,** 48–49 min; **12,** 23–24 min; **13,** 31–32 min. In order to determine the relative amount of each product formed in a kinetic reaction, the areas under the peaks on the chromatogram corresponding to the retention times of the standards were manually integrated. Standards were run on the same day and under the same conditions as those used for the analysis of the kinetic reaction mixture.

LC product analyses of the decomposition of **1** in hydroxide and some oxyanion buffers showed that the thioethers **12** and **13** (from **1a** and **1b,** respectively) were the only compounds detected (data not shown), indicating that the decomposition of these compounds in oxyanion buffers proceeds as in Scheme II (k_{GB} or k_{RO^-}). It should be mentioned that the other product of this reaction, the bicyclic ether **3,** lacks a chromophore and therefore could not be detected using this assay system. An attempt was made to establish its presence using a GC/MS technique, but this could not be done unequivocably, owing to the volatility of **3** and interference by volatile materials (buffer species, impurities) in the mixture extracted from the kinetic runs. The fact that the trans alcohols **2a** and **2b** are inert in oxyanion buffers rules out the possibility of intermolecular nucleophilic attack by the oxyanion on one of the carbons adjacent to the sulfonium pole, and limits the decomposition of **1a** and **1b** in those buffers to the intramolecular cyclization (Scheme II).

Product analysis was performed on the mixture following completion of the reaction of **1a** in several amine buffers and the results of these analyses are shown in Table V. Varying amounts of each product were detected in the reaction mixtures of all amines, save one. The product proportions were dependent upon the total buffer concentration ($[B]_T$) and the ratio of basic to acidic buffer species ([B]/[BH]). The notable exception among these amines is imidazole, in which case no evidence of intermolecular nucleophilic attack (i.e., the presence of the *cis*-thioether **4**) could be detected using the LC system. This observation of no (or very little) nucleophilic attack is consistent with the unexpected position of imidazole on the Brønsted plot (Figure 3) of amine nucleophiles. LC analyses of the products formed from the reaction of the trans alcohol **2a** with three amines are shown in Table VI. The data indicate that, with hydrazine and *N*-methylmorpholine, nucleophilic attack is predominantly at the methyl carbon (ca. 90%), and not at the methylene carbon. This agrees with predictions based upon kinetic data, discussed above.

When the reaction products of the *cis*-ethylsulfonium **1b** with *N*-methylmorpholine were analyzed (data not shown), it was observed that, as expected, the predominant reaction was intramolecular. Only about 5% of the material in the reaction mixture was **4,** the thioether expected from intermolecular nucleophilic attack on the ethyl group. This observation is consistent with the low k_B of *N*-methylmorpholine in its reaction with **1b,** relative to that in its reaction with the *cis*-methylsulfonium **1a** (Table III). LC analysis of the reaction mixture following reaction of the *trans*-ethylsulfonium **2b** with morpholine and *N*-methylmorpholine is shown in Table VI. These data allow a comparison to be made between the rate of nucleophilic attack at an ethyl group with that of attack at a substituted ethyl group (in this case at the end of a longer

Table V. High-Pressure Liquid Chromatographic Analyses of the Reaction Mixture Following the Reaction of **1a** with Amine Buffers at 40 °C (Ionic Strength 1.0 M)

buffer	pK_a	[B]/[BH][b]	$[B]_T$, M[b]	% [a] 12	% [a] 4
N,O-dimethylhydroxylamine	4.73	1/1	1.0	88	12
			0.5	87	13
			0.2	97	3
		3/1	1.0	81	19
			0.5	93	7
			0.2	97	3
		9/1	1.0	85	15
			0.5	79	21
			0.2	81	19
imidazole	6.85	1/1	1.0	100	N.D.
			0.5	100	N.D.
			0.2	100	N.D.
		3/1	1.0	100	N.D.
			0.5	100	N.D.
			0.2	100	N.D.
		9/1	1.0	100	N.D.
			0.5	100	N.D.
			0.2	100	N.D.
N-methylmorpholine	7.62	1/1	1.0	76	24
			0.5	87	13
			0.2	95	5
		3/1	1.0	86	14
			0.5	89	11
			0.2	94	6
		9/1	1.0	85	15
			0.5	89	11
			0.2	93	7
hydrazine	7.95	1/1	1.0	89	11
			0.5	92	8
			0.2	97	3
		3/1	1.0	79	21
			0.5	85	15
			0.2	92	8
		9/1	1.0	58	42
			0.5	77	23
			0.2	88	12
morpholine	8.16	2/1	1.0	39	61
			0.5	52	48
			0.2	47	53
		9/1	1.0	29	71
			0.5	50	50
			0.2	71	29
ethanolamine	9.32	1/1	1.0	68	32
			0.5	84	16
			0.2	70	30
		3/1	1.0	65	35
			0.5	66	34
			0.2	84	16
		9/1	1.0	58	42
			0.5	74	26
			0.2	68	32
cyclohexylamine	10.10	1/1	1.0	85	15
			0.5	90	10
			0.2	95	5
		3/1	1.0	84	16
			0.5	94	6
			0.2	97	3
		9/1	1.0	86	14
			0.5	95	5
			0.2	98	2
n-butylamine	10.34	3/1	1.0	71	29
			0.5	78	22
			0.2	95	5
		9/1	1.0	83	17
			0.5	84	16
			0.2	97	3

[a] Products detected. These columns indicate the relative percentage of each compound detected by LC, as determined by measuring the area beneath the peak having a retention time equal to that of an authentic sample. [b] These columns indicate the ratio of basic to acidic buffer species ([B]/[BH]) and the total buffer concentration $[B]_T$.

Table VI. High-Pressure Liquid Chromatographic Analyses of the Product Mixtures following Reaction of **2a** and **2b** in Amine Buffers at 40 °C (Ionic Strength 1.0 M)

substrate	buffer	pK_a	[B]/[BH][a]	$[B]_T$, M[a]	products detected,[a] % 12	13	5
2a	*N*-methylmorpholine	7.62	9/1	1.0	10		90
				0.5	11		89
				0.2	9		91
2a	hydrazine	7.95	9/1	1.0	10		90
				0.5	9		91
				0.2	12		88
2a	imidazole	6.85	9/1	1.0	14		86
				0.5	20		80
				0.2	21		79
2b	*N*-methylmorpholine	7.62	9/1	1.0		10	90
				0.5		7	93
				0.2		9	91
2b	morpholine	8.16	9/1	1.0		12	88
				0.5		20	80
				0.2		17	83

[a] For an explanation of these columns, see Table V.

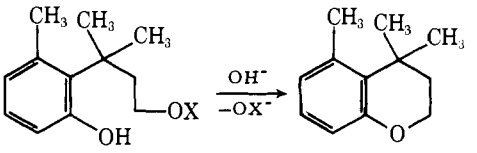
chain which includes the cyclopentyl ring). If the two "ethyl" groups were equivalent, one would expect an equal mixture of the two possible products to be formed. The results obtained (80–90% of the product formed arose from abstraction of the unsubstituted ethyl group) indicate that these carbons are not equivalent. Similar LC analyses of the products (data not shown) were obtained from reactions of the sulfonium salts **6a**, **6b**, and **6c** with *N*-methylmorpholine. The results from the reaction of **6b** confirm the previous observations that nucleophilic attack on a methyl group adjacent to a sulfonium pole is favored over such attack on an ethyl group.

Discussion

The kinetic data as well as the product analyses presented above are consistent with the proposal that, in the presence of oxyanion buffers in aqueous solution, the decomposition of **1**

is the subject to general base catalysis. As previously indicated, the slopes of plots of k_{obsd} vs. total buffer concentration are quite sensitive to the concentration of the basic buffer species. A solvent isotope effect for the decomposition of **1a** at 25 °C in oxyanion buffers was found to be close to unity, $k_{H_2O}/k_{D_2O} = 1.37$.[7] These findings plus the product analysis data, which give no indication of nucleophilic attack (i.e., neither methyl abstraction from **1a** nor ethyl abstraction from **1b**), suggest that the oxyanions are acting as general base catalysts to promote the intramolecular S_N2 attack as depicted in Scheme II (k_{GB}). The same analysis and conclusion hold for the ethyl analogue **1b**. Under conditions (oxyanion buffers, 40 °C) where **1a** and **1b** are readily decomposed to the sulfides **12** and **13**, the trans alcohols **2a** and **2b**, as well as the simpler sulfonium salts **6a, 6b,** and **6c,** are all inert (no decomposition detected by LC analysis of the reaction mixture). This contrasting behavior is further emphasized by the fact that the latter five compounds are not unreactive species; they are quite readily decomposed in aqueous solution by amine buffers (see below).

This observation of general base catalysis is significant because the reaction in question is an intramolecular nucleophilic (S_N2) displacement at an sp^3 carbon. Many data have been obtained for general base catalysis of displacement at sp^2 carbon, but little is available concerning this phenomenon at sp^3 carbon. Swain and colleagues found that the cyclization of 4-chlorobutanol was sensitive to general base catalysis by borate ion in water at 50 °C.[23] The Brønsted β value obtained by them from their limited data, 0.25, agrees with that found in the present work (0.27) (Figure 3). Of the oxyanions studied by us, only *tert*-butylphosphonate deviates significantly from the best-fit line, presumably due to steric hindrance. It is interesting to note that, in a related ring closure reaction depicted in eq 4, Borchardt and Cohen did not find any sensitivity to

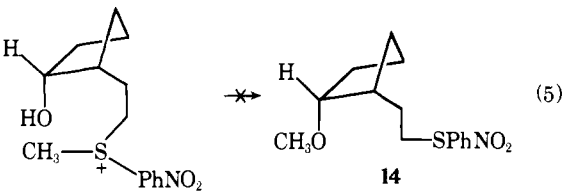


(4)

X = $-SO_2CH_3$, $-PO(OEt)_2$, $-COCCl_3$

buffer catalysis (using carbonate, Tris, phosphate, and acetate).[24] However, the fact that these studies were performed in 40% dioxane–60% H_2O may make comparison with the data presented herein (100% H_2O) difficult. It should also be noted that the starting materials for the above cyclization reaction have highly strained ground states and experience a relief of strain upon attaining the transition state.[25]

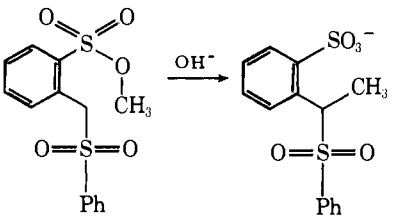
The ring closure reaction depicted in Scheme II may be classified as an exocyclic process, using the terminology of Eschenmoser and co-workers.[26] These workers found that, in keeping with many other studies,[27] there is a fairly strict requirement for colinearity of nucleophile, central carbon atom, and leaving group in the transition state for an S_N2 reaction. Previous work by Lok and Coward also indicated that a strict colinearity was required in the transition state for nucleophilic attack on a methyl group.[6] Inspection of a molecular model of **1a** indicates that the deviation from colinearity in this compound is about 5° (for attack on the methylene carbon adjacent to the sulfonium pole, *not* on the methyl carbon). It is interesting to note that Martin and Basalay found that a deviation of ca. 17° was permitted for S_N2 attack on a methylene adjacent to a sulfonium.[28] Results with amine buffers, discussed below, indicate that intermolecular abstraction of the methyl group (nucleophilic attack by amine) of the sulfonium moiety of **1a** and related compounds competes with intramolecular attack by the hydroxyl group at the methylene position adjacent to sulfur. The corresponding situation for a



1a **14** (5)

general base catalyzed intramolecular methyl abstraction for **1a** is shown in eq. 5. Inspection of molecular models indicates that a reaction of this sort, which would proceed through a seven-membered intermediate, would have to deviate greatly from a linear transition state in order to occur. The postulated product (**14**) of this reaction was prepared (see Experimental Section) for LC comparison in product analyses; no such *O*-methyl ether was observed, indicating that the reaction shown in eq 5 does not occur under our conditions. It should also be pointed out that the trans alcohol **2a** is inert in oxyanion buffers, which would not be the case if a facile intermolecular methyl abstraction were occurring.

The reaction studied by Eschenmoser and colleagues, eq 6, was found to occur via an intermolecular, not an intramolecular

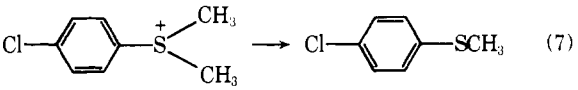


(6)

process. An alkylation of this type would be classified according to Baldwin as a 6-*endo*-Tet process, which is disfavored.[29] The decomposition of **1a** according to eq 5 is an example of a 7-*endo*-Tet process, and, while not discussed by Baldwin, apparently is also disfavored. The intramolecular decomposition of **1a** according to Scheme II, on the other hand, is a 5-*exo*-Tet process, which is favored. In their system (eq 6), Eschenmoser and co-workers used a d_3-labeled methyl group to demonstrate the intermolecular nature of the alkylation. In our system (Scheme II), no intermolecular alkylation (methylation) reaction was detected by product analyses when the reaction was carried out in oxyanion buffers.

The thermodynamic parameters, calculated from Arrhenius plots (Figure 2), for both the pH-independent (k_{ROH}) and the pH-dependent (k_{RO^-}) rates are given in Table II. As mentioned earlier, the parameters for k_{RO^-} must be taken as only approximate owing to the uncertainty with which the pK_a of **1a** is known as a function of temperature. Upon formation of the alkoxide anion, there is a significant decrease in E_a and $\Delta G^{\ddagger}$, compared to the values obtained for k_{ROH}. The favorable decrease in $\Delta G^{\ddagger}$ is reflected by both favorable enthalpy and entropy changes. Since there is a significant increase (ca. 12 eu) in $\Delta S^{\ddagger}$ when k_{ROH} is compared to k_{RO^-}, one may say qualitatively that, in the pH-independent reaction, gross atomic rearrangements leading to a highly ordered transition state are involved. In the alkoxide reaction, with its $\Delta S^{\ddagger}$ near 0, much less severe atomic rearrangements are involved leading to the transition state. Further data will be presented[31] that also indicate a progressive tightening of the transition state in the series k_{ROH}, k_{GB}, and k_{RO^-}, based on secondary isotope effects.

As earlier determined, the $\Delta S^{\ddagger}$ for the reaction shown in eq 7 is −5.0 eu,[8] which is within the range for many bimolecular reactions in solution.[30] E_a and $\Delta G^{\ddagger}$ for the pH-independent composition of **1a** (k_{ROH}; Table II) are similar to those



(7)

obtained for the bimolecular reaction of eq 7. Qualitatively, these similarities indicate that the intramolecular alcohol reaction of **1a** is more akin to a bimolecular process than is the intramolecular alkoxide reaction. The difference in $\Delta S^{\ddagger}$ between the intermolecular reaction of eq 7 and the pH-independent intramolecular cyclization of **1a** (k_{ROH}) (−5 eu vs. −15 eu, respectively) may be attributable to solvation effects on the nucleophile (OH^- vs. ROH) as has been discussed for a series of lactonization reactions by Mandolini.[32] He found that $\Delta S^{\ddagger}$ for the lactonization of 4-bromobutanoic acid (in 99% Me_2SO) was −5.6 eu, while that for the corresponding intermolecular reaction (potassium butanoate and butyl bromide) was −18.4 eu. Such an entropy difference of ca. 12 eu is comparable to that observed in our system.

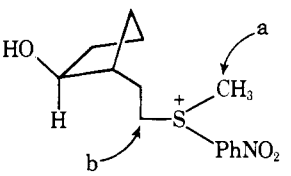
Page has recently presented an interesting series of correlations between the relative equilibrium constants for anhydride formation from dicarboxylic acids and those for the cyclization of the corresponding hydrocarbons (the same analysis has been applied to the relative rate constants for lactonization of hydroxy acids vs. the rate constants for cyclization of the corresponding hydrocarbons).[33] Such correlations demonstrate that variations in the relative rates or equilibria of a series of intramolecular reactions may be explained by the relative difference in strain energies of reactants vs. products, and the difference in entropy changes due to the loss of internal rotations upon cyclization. The change from a bimolecular to an intramolecular reaction in the case of **1a** might be expected to lead to a rate enhancement (expressed as effective molarity) of about 10^5–10^8 M attributable entirely to such a procedure.[33,34] The effective molarity calculated previously for the decomposition of **1a** relative to the reaction occurring in eq 7 (para nitro substituent instead of para Cl) of ca. 5×10^5 M[7] is well within this range.

When the decomposition of **1a** or **1b** was carried out in amine buffers at 40 °C, some significant differences from the reaction with oxyanions were noted. As indicated previously, all other sulfonium compounds studied, which are inert in oxyanion buffer, reacted readily in the presence of amines. From these initial observations it appeared that amine buffers were acting as nucleophiles by attacking an alkyl group, methyl or methylene, adjacent to the sulfonium pole. The kinetic experiments and product analyses described for the sulfonium salts, **6,** which are inert in oxyanion buffers, were performed in order to have a better understanding of the possible reactions when the decomposition of the cis alcohols **1a** and **1b** were studied in amine buffers.

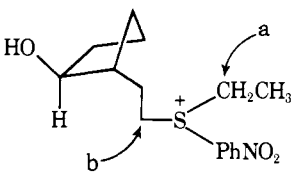
The dimethyl derivative **6a** reacts much faster and shows a much greater dependence on amine concentration than do either **6b** or **6c** (**6b,** in turn, displays a greater rate and a greater buffer dependence than does **6c**). The k_B for the reaction of each of these compounds, given in Table III, indicates these differences in buffer concentration dependence. These kinetic data, in addition to LC analysis of the products formed when each of these compounds reacts with *N*-methylmorpholine buffer, demonstrate that methyl abstraction is greatly favored (88% in **6b**) over abstraction of an ethyl group.

The abstraction of an alkyl group from these substrates is presumably an S_N2 process, as has been demonstrated for the reaction of trimethylamine with the trimethylsulfonium cation.[35] The S_N2 displacement of a methyl group is greatly favored over that of an ethyl group, when the substrates compared are alkyl halides, presumably due to increased steric crowding in the transition state.[27] Recent data by a Japanese group have indicated that, with sulfoniums similar to **6,** the relative reactivities of alkyl groups attached to sulfur are methyl 1.0, ethyl 0.20, *n*-propyl 0.16, and isopropyl 0.05.[36] These data agree with those obtained for the decomposition of **6,** i.e., preferential nucleophilic attack at methyl over ethyl.

When the reaction of the trans alcohol **2a** was studied in amine buffers, a situation similar to that noted for the above sulfoniums (i.e., alkyl abstraction) was observed. This compound, which cannot undergo a ring closure reaction as can **1a,** can be attacked by added amine at the methyl carbon (path a) or at the methylene carbon (path b). Product analysis of the reaction mixture following reaction of **2a** (Table VI) indicated that the preferred mode of attack was one leading to methyl abstraction, giving the sulfide **5.** It should be noted that an intermolecular demethylation, leading to the *trans-O*-methyl ether **15,** is unlikely (see oxyanion discussion, eq 5). The reaction of **2b** was studied in a similar manner. The k_B for the reaction of this compound with *N*-methylmorpholine is much lower than that for **2a** (compare k_B for **6c** vs. **6b,** Table III). Product analysis indicates that **2b,** like **2a,** is susceptible to nucleophilic attack at two positions (Table VI). Path a is



2a



2b

preferred over path b, which indicates that the (nonethyl) methylene is probably closer to a larger alkyl group (e.g., *sec*-butyl or isobutyl), in terms of bulk, than to an ethyl group.

The rate extrapolated to zero buffer concentration (k_{ROH}) for the cis compounds **1a** or **1b** is independent of the buffer used (amine or oxyanion). However, a slight difference in k_{ROH} of 3.5×10^{-5} s^{-1} for **1a** vs. 2.2×10^{-5} s^{-1} for **1b** has been noted repeatedly (Table III). In addition, phosphate ion catalyzes the cyclization of **1a,** with k_B ca. 2.5 times that observed with **1b.** To explain these observations, it is postulated that the transition state leading to ring closure of **1a** is less sterically hindered than is the transition state leading to ring closure of **1b,** with its bulkier ethyl group. It can also be seen from Table III that the k_B for the intermolecular reaction of **1a,** the *cis*-methylsulfonium, with *N*-methylmorpholine is very similar to that for the reaction of the *trans*-methylsulfonium **2a** with *N*-methylmorpholine. The same similarity of k_Bs was found for the corresponding ethyl derivatives, **1b** and **2b.**

Table IV gives the k_B values for the reaction of **1a** with a series of amines, and Figure 3 is a Brønsted plot constructed from these data. The β value is 0.27, the same as that seen with oxyanion buffers. The deviation of imidazole from this plot is discussed below. Three α-effect nucleophiles (hydrazine, *N*-methylhydroxylamine, and *N,O*-dimethylhydroxylamine) are included in this study; however, no α effect was observed. Previously, amines such as these have been found to show a large positive deviation from the Brønsted plot (α effect) when acting at an sp^2 center, but not at an sp^3 carbon.[37]

The hypothesis developed after obtaining the kinetic data given above was that, in the systems studied (i.e., decomposition of **1a** or **1b**), added amines are acting predominantly, if not entirely, as nucleophiles. In contrast, oxyanions act as general bases. The mechanism of the amine reaction was further elucidated by LC analysis of the product mixtures. These data are given in Table V. In general, as the buffer concentration ($[B]_T$) increases the extent of nucleophilic reaction (demethylation), as indicated by the proportion of **4** in the reaction mixture, also increases, at the expense of the ring closure reaction. Such an increase in the product of the nucleophilic reaction would not be expected if the amine were acting in a competing general base catalyzed ring closure reaction. In the case of amines with high pK_a (cyclohexylamine and *n*-butylamine) the predominant reaction is the ring closure, which would be expected at the pH of these kinetic runs, as

they lie in the ascending, pH-dependent limb of the pH profile associated with k_{RO^-} (Figure 1, 40° profile). A similar analysis may be applied to amines of lower pK_a, such as *N*-methylmorpholine and *N,O*-dimethylhydroxylamine, which buffer in the pH range (4.7–8.6) where a pH-independent cyclization reaction (k_{ROH}) is occurring. Added amine (an increase in $[B]_T$), if it were acting as a general base, would not be expected to increase the amount of **4** formed (nucleophilic reaction) at the expense of the amount of **12** formed (ring closure reaction). The fact that the demethylation reaction never becomes predominant with *N,O*-dimethylhydroxylamine is consistent with the position of that base on the Brønsted plot (a relatively poor nucleophile). Amines of intermediate pK_a (hydrazine, morpholine, and ethanolamine) give similar results in that the demethylation reaction, expecially at a [B]/[BH] ratio of 9/1, is increased at the expense of cyclization. All of the data collected for morpholine and hydrazine fall on the pH-independent portion of the profile. Again, a large increase in the amount of **4** is observed, indicating a greater proportion of demethylation. The situation is similar with ethanolamine, except that k_0 is increasing because of the higher pH of the medium; general base catalysis would predict more ring closure. Instead, more demethylation is occurring as $[B]_T$ is raised at each [B]/[BH] ratio. As mentioned above, in reference to compound **2a,** amines may attack at either the methyl (path a) or methylene carbon (path b) adjacent to the sulfonium pole. Methylene attack apparently occurs at a rate of 10–12% of that at the methyl carbon, when *N*-methylmorpholine and hydrazine are the amines studied with **2a** (Table V). Therefore, a small portion of **12** formed during the decomposition of **1a** in amine buffers might be due to an intermolecular nucleophilic reaction (path b), and not to an intramolecular cyclization.

As mentioned earlier, imidazole displays a significant negative deviation from the Brønsted plot. The k_B value given in Table II is an upper limit, as a dependence of rate on buffer concentration cannot be clearly demonstrated, even at 1.0 M B_T, (plots of k_{obsd} vs. $[B]_T$ have zero slope). Consequently, the cis-alcohol **1a** decomposes in imidazole buffer at essentially the pH-independent rate, k_{ROH}, which is reflected in the product analysis data shown in Table V (no demethylated material, **4,** is detected). These data indicate that the only reaction occurring in this case is the buffer-independent intramolecular cyclization of **1a.** Imidazole is, however, capable of acting as a nucleophile, as shown by the product analysis of the decomposition of the trans isomer **2a** (Table V). Methyl abstraction (path a) predominates over methylene abstraction (path b), as with other amines. However, since this overall nucleophilic reaction occurs at a rate of ca. 10% of the k_{ROH} rate for **1a** decomposition (k_B for **2a** = 1.55×10^{-6} s^{-1} M^{-1}; k_B for **6a** = 6.20×10^{-6} s^{-1} M^{-1}), imidazole is not very effective as an intermolecular nucleophile for attack at sp^3 carbon, as compared with the intramolecular cyclization reaction ($k_{ROH} = 3.55 \times 10^{-5}$ s^{-1}). This finding is quite surprising in light of the fact that imidazole is a very effective nucleophile and general base catalyst for reactions occurring at sp^2 carbon.[22] Further studies are planned to investigate more fully this interesting observation.

In any discussion of possible mechanisms of enzyme-catalyzed reactions at sp^3 carbon, this demonstration of different modes of catalysis by oxyanion vs. amine buffers should be considered. Specifically, it may be possible for enzyme glutamyl and aspartyl residues to catalyze proton transfer, analogous to that involved in the conversion of **1** to **3,** whereas amine residues (e.g., lysyl) would be unable to effect this type of catalysis. Especially unusual in this regard is the result for imidazole, which has little or no effect on the reactions studied in this work.

It is not yet possible to compare the rate data obtained in this work with corresponding data for a purified RNA 2′-*O*-methyltransferase. Recent work[38] has resulted in the purification of such an enzyme, but kinetic data required for this comparison were not presented.

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