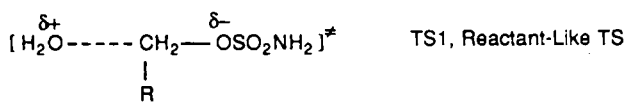
Mechanistic Investigation of the Degradation of Sulfamic Acid 1,7-Heptanediyl Ester, an Experimental Cytotoxic Agent, in Water and ^{18}Oxygen-Enriched Water

MEHDI PABORJI*, WANDA N. WAUGH, AND VALENTINO J. STELLA[x]

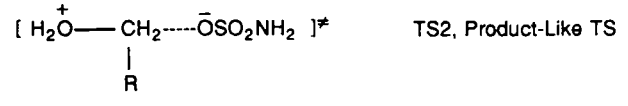
Received September 17, 1986, from the *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.* Accepted for publication December 8, 1986. Present address: *Bristol-Myers, Syracuse, NY 13221.

Abstract □ The hydrolytic degradation of sulfamic acid 1,7-heptanediyl ester (**1**) was carried out in water and ^{18}O-enriched water at 47 °C. The degradation of **1** was also studied at various pH values in the range of 2.5 to 8.0 at constant ionic strength (0.15 M) and temperature (25 °C). The hydrolysis was first order and independent of pH with a mean (±SD) observed rate constant (k_{obs}) of 2.38 ± 0.6 × 10^{-3} h^{-1}. No significant buffer catalysis was observed. From TLC, HPLC, and mass spectral studies, **1** initially degraded to sulfamic acid 1,7-heptanemonoyl ester (**2**) and subsequently to 1,7-heptanediol (**3**). The site of bond cleavage was assessed by mass spectrometry of the ^{18}O-enriched water reaction mixtures. Exclusive C—O bond fission was observed. Several mechanistic pathways for the degradation of **1** could be postulated. The results from ^{18}O-labeling studies, the pH-rate profile and buffer studies, and kinetic solvent isotope effect (KSIE) studies were consistent with an S_N2 mechanism with an early transition state (reactant-like transition state) where no appreciable bond had developed between the incoming nucleophile, water, and the carbon atom of **1**. Although an S_N1 mechanism was unlikely, based on the need to postulate the formation of a primary carbocation, this mechanism could not be totally ruled out.

The purpose of the present study was to investigate the mechanism of aqueous degradation of sulfamic acid 1,7-heptanediyl ester (**1**; NSC-329680), a weak alkylating agent with significant cytotoxicity.[1] For stability reasons, **1** was formulated as a sterile freeze-dried powder. On reconstitution with a solvent consisting of 10% ethanol, 40% propylene glycol, and 50% 0.05 M aqueous phosphate buffer of pH 7.4 (this solvent being necessary to achieve the desired solubility), **1** had an effective shelf-life ($t_{90\%}$) of 9.3 d. The hydrolysis of a similar bifunctional alkylating agent, busulfan (1,4-butanediol dimethane sulfonate) has been investigated previously.[2,3] In addition, the hydrolysis of aliphatic sulfonic acid esters has been studied by Hartman et al.,[4] Barnard et al.,[5] Mori et al.,[6] and others.[7] To the best of our knowledge, the mechanism of hydrolysis of straight-chain alkyl sulfonic acids and sulfamic esters has not been extensively studied in the past and no attempts have been made to explore whether the hydrolysis of straight-chain alkyl sulfamic acid esters occurs via S—O or C—O bond cleavage. Thus, the present studies were aimed at determining the mechanism of degradation of **1** and, perhaps indirectly, also providing further insight into the mechanism of action of **1** as an alkylating agent.



TS1, Reactant-Like TS



TS2, Product-Like TS

Experimental Section

Materials—Sulfamic acid 1,7-heptanediyl ester (**1**) (lot number CEB-Q-147) was obtained from the National Cancer Institute, Bethesda, MD, and was used without any further purification. All chemicals, including buffer components, were ACS reagent grade and were used without further purification. 1,7-Heptanediol (**3**) was purchased from Aldrich Chemicals. Organic solvents were HPLC grade. The water used was deionized and glass distilled (Mega-Pure system model MP-1, Corning). Enriched ^{18}O-water was purchased from MSD Isotopes (Montreal, Canada) and was 97.2 atom%. Deuterium oxide (D_2O) was obtained from Stohler Isotopes Chemicals (Waltham, MA) and was 99.8%. All pH measurements were made at 25 °C with a digital pH meter (Corning model 155; Medfield, MA).

High-Performance Liquid Chromatographic Analysis—Analysis of **1–3** by HPLC was accomplished with a Waters M-6000A pump, an Altex 156 refractive index detector, an Altex 210 injector fitted with a 50-μL loop, and a 150 mm × 5 mm column packed with 5 μm ODS-Hypersil (Shandon). Calibration curves were constructed from peak area (Varian CDS 111 integrator) versus concentration plots. The mobile phase consisted of 0.1 M acetate buffer (pH 4.5):methanol (77:23 v/v) and the flow rate was 2 mL/min. Chart speed (Omni Scribe, Industrial Scientific, Inc.) was 0.2 inch/min and the retention volumes were 12.8, 10.8, and 9.2 mL for **1**, the first degradation product and the second degradation product, respectively. The Altex refractive index detector was set at range 8. Sample concentration was 1–4 mg/mL. A typical chromatogram of a partially degraded sample of **1** is presented in Figure 1. A plot of the disappearance of **1** and the appearance of the first degradation product, **2**, and the second degradation product, **3**, as a function of time, is shown in Figure 2.

Thin-Layer Chromatographic Analysis—The degradation of **1** was also monitored by TLC (EM Reagents, Silanized Silica Gel 60 F_{254}, Darmstadt, W. Germany). The solvent system consisted of chloroform:ethyl acetate (70:30). Spots were visualized with sulfuric acid followed by charring. The R_f values for **1**, and presumably **2** and **3**, were 0.76, 0.62, and 0.44, respectively. Component **3** could be directly confirmed by comparison with an authentic sample.

^{18}O Labeling Mass Spectrometry Studies—Low and high resolution mass spectral analyses were carried out with a Nermag R10-10 Quadrapole mass spectrometer. Chemical ionization (CIMS) and electron impact mass spectrometry (EIMS) were performed by direct probe injection; CIMS utilized an ammonia probe and EIMS was at 70 mV.

Into two ampules, each containing 0.6 mg of **1**, were placed 200 μL of ^{18}O-enriched water and 200 μL of unlabeled water. The ampules were then sealed and incubated at 47 °C for 30 h in a water bath. Degradation of **1** was checked by TLC and HPLC prior to mass spectral analysis. Direct mass spectrometry of the mixtures was used to identify the presence of various degradation products. Direct mass spectrometry of pure **1** and **3** were used as controls.

pH–Rate Profile—Apparent first-order rate constants for the degradation of **1** at 25 °C were determined in aqueous buffer solutions at various pH values. The ionic strength was adjusted to 0.15 M by the addition of KCl. The following buffer systems were employed, as potassium salts: HCl (pH 2.5), acetate (pH 3.5–5.0), and phosphate (pH 7–8). At all pH values, the degradation rates of **1** were followed using the reversed-phase HPLC procedure reported earlier. Aliquots were withdrawn periodically and injected onto the HPLC. Quantification was performed by peak area analysis in relation to standard calibration curves. Observed first-order rate constants were calculated from the slopes of linear plots of log C versus time, where C is the

0022-3549/87/0200-0161$01.00/0

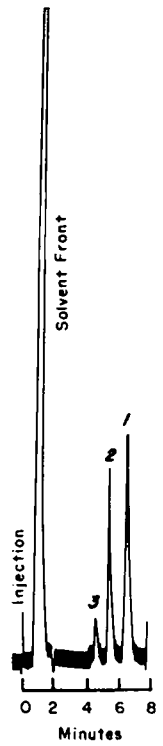


Figure 1—*A typical chromatogram of a partially degraded sample of 1, showing the formation of the first degradation product, 2, and the second degradation product, 3.*

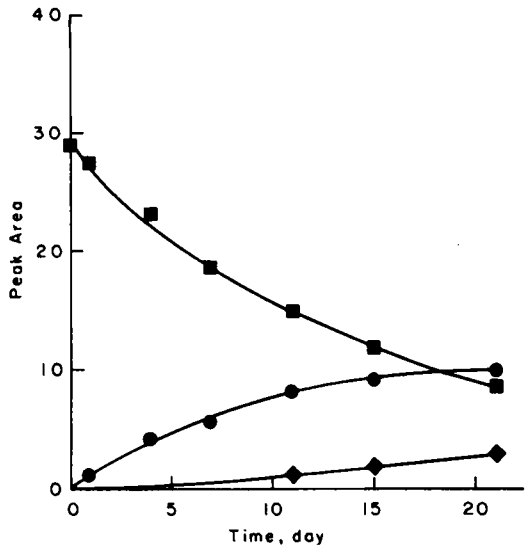


Figure 2—*Plot showing the loss of peak area for 1 (■), the appearance of 2 (●), and the eventual formation of 3 (◆), as a function of time in phosphate buffer (0.05 M) at pH 7 at 25 °C over a period of 21 d.*

concentration of the remaining intact 1 at time t. The preliminary data indicated that the degradation of 1 was not subject to significant catalysis by acetate or phosphate buffers; thus, the observed rate constants in 0.05 M buffer were used to construct the pH–rate profile.

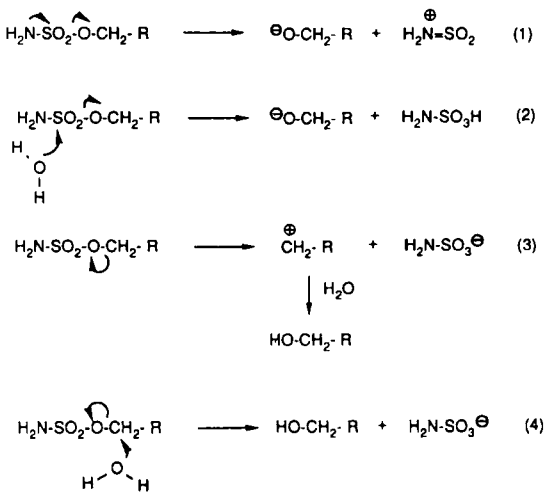
Kinetic Solvent Isotope Effect—The kinetic solvent isotope effect for the degradation of 1 was determined by measuring the degradation rates of 1 in deuterium oxide (D_2O) and water at 25 °C under identical conditions. The ionic strength was maintained constant at 0.15 M with KCl. No buffers were employed as the degradation of 1 was shown to be pH independent.

Results and Discussion

^{18}O-Labeling Mass Spectrometry—Several mechanisms could be postulated for the hydrolysis of 1 as illustrated in Scheme I. Various mechanistic tools can be employed to differentiate these mechanisms. Note, an S—O bond is cleaved in mechanisms 1 and 2, whereas C—O bond fission occurs in mechanisms 3 and 4. A mass spectrometry technique was therefore utilized to probe the site of bond cleavage during the hydrolysis of 1 in ^{18}O-enriched water (97.2 atom% ^{18}O) and unlabeled water at 47 °C. The extent of the reaction was monitored by TLC. Three spots which corresponded to 1, the intermediate product, presumably sulfamic acid 1,7-heptanemonoyl ester, 2 (subsequently confirmed by the mass spectral data), and 1,7-heptanediol, 3, were detected on a TLC plate. The formation of 3 as the second degradation product was confirmed by TLC, HPLC, and mass spectrometry by comparison with an authentic sample. Chemical ionization mass spectrometry (CIMS-NH_3) of the water reaction mixture indicated mixture components consistent with unreacted 1 [291 (M + 1); 308 (M + 18)], the intermediate, 2 [212 (M + 1); 229 (M + 18)], and 3 [133 (M + 1); 150 (M + 18)]. Chemical ionization mass spectrometry of the ^{18}O-enriched water reaction mixture indicated little detectable ^{18}O in unreacted 1, one ^{18}O-labeled atom incorporated into the intermediate, 2 [214 (M + 1); 231 (M + 18)] and, two ^{18}O-labeled atoms incorporated into the final degradation product, 3 [137 (M + 1); 154 (M + 18)].

Although ^{18}O incorporation into 2 and 3 by alternative mechanisms could be proposed (i.e., exchange of the primary hydroxyl groups with $H_2^{18}O$ subsequent to the bond cleavage), this possibility was ruled out on the basis of findings of Indelicato et al.[8] who demonstrated no appreciable ^{18}O exchange even when allylic alcohol was placed in $H_2^{18}O$. Furthermore, for 2, it was unlikely that the ^{18}O was incorporated into the sulfamate group since it was demonstrated that little ^{18}O was incorporated into unreacted 1. Others have also shown that the sulfonate group itself was not subjected to ^{18}O-exchange when placed in ^{18}O-labeled water.[9,10] Similarly, no ^{18}O label was detected in the mass spectrum of the sulfonate degradation product of 2-chloroethyl(methylsulfonyl)methane sulfonate, another cytotoxic agent whose stability in ^{18}O-labeled water was investigated in our laboratory.[11]

The CIMS-NH_3 findings were confirmed by electron impact mass spectrometry (EIMS) of the reaction mixtures. The fragmentation pattern of the final degradation product, 3,



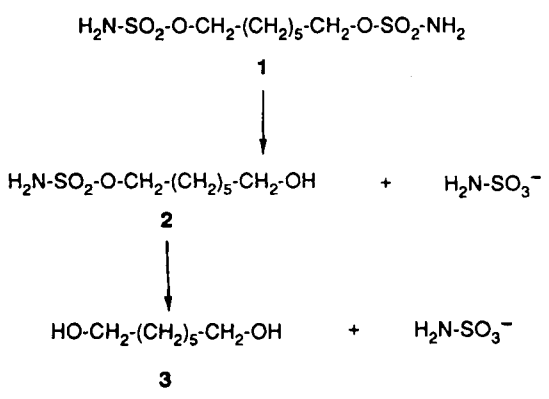
Scheme I

suggested that there is a peak at *m/z* 31 which presumably corresponds to a $-CH_2OH$ fragment. If the hydrolytic degradation of 1 occurs via C—O bond fission as opposed to a S—O bond cleavage, one would expect to find a peak at *m/z* 33 which would correspond to a $-CH_2^{18}OH$ fragment. Inspection of the EIMS analysis of the reaction mixtures carried out in ^{18}O-enriched water clearly showed a peak at *m/z* 33 and no detectable peak at *m/z* 31.

Thus, the CIMS-NH_3 as well as the EIMS results strongly indicated that the hydrolytic degradation of 1 occurred in a stepwise manner, where the first step involves hydrolysis of 1 via C—O bond cleavage to yield 2 with the ^{18}O-labeled atom of the water molecule incorporated into the alcohol moiety. In the second step, 2 underwent further hydrolysis, through the same pathway via C—O bond fission, to give 3 with ^{18}O-labeled atoms incorporated into both alcohol moieties. Scheme II illustrates these reaction sequences. A similar stepwise mechanism for the degradation of busulfan has also been proposed. In the first step, busulfan undergoes a nucleophilic displacement by water to give 4-methanesulfonyloxybutanol which subsequently undergoes an intramolecular displacement to render the final degradation products, tetrahydrofuran and methanesulfonic acid. No indication of a similar intramolecular mechanism was observed in the present study. In the busulfan study, cleavage of the C—O bond versus S—O bond was not confirmed.[2]

On the basis of the ^{18}O experiments, mechanisms 1 and 2 in Scheme I can be ruled out for the hydrolysis of 1.

pH–Rate Profile—The kinetics of the hydrolytic degradation of 1 were investigated in aqueous solution at various pH values at 25 °C (μ = 0.15 M with KCl). The apparent first-order rate constants were used to construct the pH–rate profile shown in Figure 3. Furthermore, the preliminary results suggested that the degradation of 1 was not buffer catalyzed in the pH range studied. The observed rate constants were independent of the pH in the range of 2.5 to 8.0 giving a mean (±SD) observed rate constant (k_{obs}) of 2.38 ± 0.6 × 10^{-3} h^{-1}. The lack of pH dependency for the hydrolysis of 1 is consistent with the observations of Mori et al.[6] who studied the hydrolysis of a related compound, ethyl ethanesulfonate. Mori et al.[6] also studied the hydrolysis of ethyl ethanesulfonate in ^{18}O-labeled water. Their conclusions, that the hydrolysis of ethyl ethanesulfonate occurred via an S_N2 mechanism with exclusive C—O bond cleavage, are in good agreement with our observations. Furthermore, our results were also consistent with the findings of Hassan and Ehrsson[2] who studied the hydrolysis of busulfan within the pH range of 1.5 to 13.5. No significant buffer catalysis was observed by Hassan and Ehrsson in the pH range 1.5–6. Some catalysis, attributed to the phosphate dianion (HPO_4^{-2}), was detected at higher pH values.



Scheme II

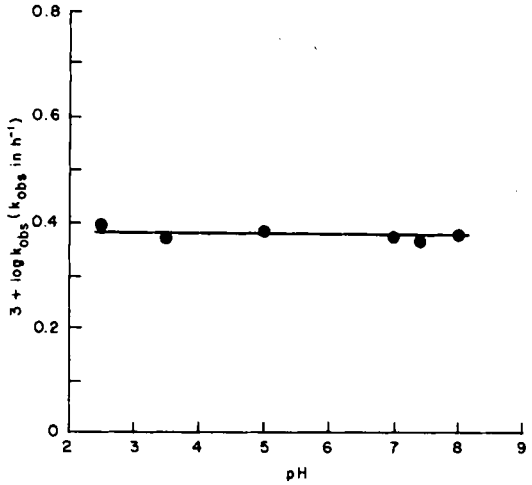


Figure 3—*pH-Rate profile for the degradation of 1 in aqueous solutions at 25 °C, μ = 0.15 M (KCl).*

The independency of the observed rate constants on pH and buffer species in the present study is consistent with either mechanisms 3 or 4 in Scheme I. Nucleophilic substitution by water (mechanism 4) at a saturated carbon or an S_N1 mechanism (mechanism 3) are not usually specific or general acid or base catalyzed.[12]

Kinetic Solvent Isotope Effect—Kinetic and equilibrium solvent isotope effects have been the subject of several reviews.[13,14] The kinetic solvent isotope effect (KSIE) can be utilized to characterize the structural features of the transition state(s) of a particular reaction under investigation. The observed KSIE is the result of contributions from a number of processes. Changing the solvent from water (H_2O) to deuterium oxide (D_2O) will lead to rate differences if any of the following changes occur in going from the effective reactant state to the effective transition state: (*a*) differences in bulk solvent properties; (*b*) variations in solute–solvent interactions; (*c*) discrepancies in zero-point energy of O–L bonds (where L refers to either H or D) of reacting solvent water molecules; or (*d*) differences in zero-point energy of solute bonds to exchangeable hydrogens which have undergone rapid exchange with solvent.

The KSIE for a particular reaction can be estimated by the fractionation-factor approach.[15] The KSIE for substrates with multiple exchangeable sites is given by the ratio of the reactant-state to the transition-state fractionation factors;

$$k_{HOH}/k_{DOD} = \prod_i \Phi_i^R / \prod_j \Phi_j^T \qquad (1)$$

where Φ is the isotopic fractionation factor of a particular site and is the preference of deuterium over protium relative to the preference of the protonic site of the bulk solvent for deuterium over protium.

The fractionation-factor approach can be applied to proposed transition-state (TS) models and a reasonable TS structure can be deduced by comparison of the experimentally determined solvent isotope effect with the theoretically expected solvent isotope effect. The validity of the accepted TS model can be further examined by other mechanistic tools.

The KSIE for the hydrolytic degradation of 1 was determined by measurement of the hydrolytic rates of 1 in D_2O and H_2O at 25 °C under identical conditions. The results are presented in Table I. The magnitude of the KSIE is statistically equivalent to unity. An isotope effect of unity ($k_{HOH}/k_{DOD} \approx 1$) has been observed for a nucleophilic displacement

Table I—Kinetic Solvent Isotope Effect for the Degradation of Sulfamic Acid 1,7-Heptanediyl Ester (1)[a]

Solvent	$10^3 k_{obs} \pm$ SEE, h^{-1} [b]	$k_{HOH}/k_{DOD} \pm$ SD[c]
H_2O	2.44 ± 0.09	0.96 ± 0.05
D_2O	2.53 ± 0.08	

[a] Temperature maintained at 25 °C, μ = 0.15 M (KCl), concentration of 1 was 3 mg/mL. [b] Average of two determinations of the observed rate constant; SEE, standard error of estimate. [c] SD, standard deviation.

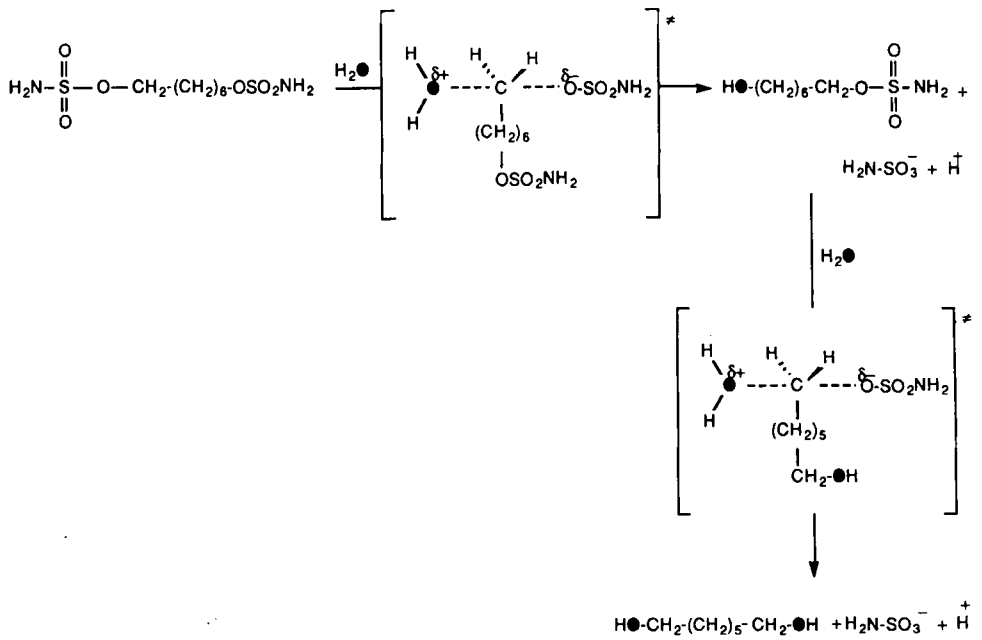
at a saturated carbon center.[16] The fractionation-factor approach can be employed to test various possible transition states at extreme limits (i.e., an early TS or reactant-like TS, and a late TS or product-like TS). Consider the following TS structures for the hydrolytic degradation of **1**.

The reactant state consists of bulk water and **1** with no exchangeable methylene hydrogen atoms. Thus, the fractionation factor for the reactant state is unity (Φ^R). The fractionation factor for an exchangeable hydrogen in the TS(Φ^T) can be estimated by assuming various TS structures. In TS1, the Φ^T for those hydrogen atoms attached to the water nucleophile is about unity because not much bond has been developed between the oxygen atom of the nucleophile and the carbon atom (i.e., the property of these hydrogen atoms is not significantly different from bulk solvent). However, in TS2, a full bond has been formed between the oxygen and the carbon atom. In other words, the nucleophilic oxygen bears a full positive charge in the TS which causes these hydrogen atoms to be loosely bonded. Therefore, the hydrogen atoms attached to the oxygen are different from those of the bulk solvent (reactant state). The fractionation factors for exchangeable hydrogen atoms in a H_3O^+ molecule have been determined to be 0.69.[17,18] Therefore, the fractionation factor (Φ^T) for those exchangeable hydrogen atoms in TS2 is estimated to be ~0.7. In other words, the fractionation factors of a functional group with exchangeable hydrogen atoms will be the same regardless of the molecule in which the group is attached.[17]

The KSIE was estimated to be 1 (k_{HOH}/k_{DOD} = 1) and 2.1 (k_{HOH}/k_{DOD} = $1/\Phi^2$ = $1/0.69^2$ = 2.1) for TS1 and TS2, respectively. The observed KSIE of unity is consistent with an S_N2 transition state (TS) where there is not much interaction between the incoming nucleophile and the carbon center (i.e., an early TS with no appreciable bond formation). The KSIE of unity can also be explained if solvent reorganization is partially or fully the rate-limiting step on going from the reactant state to the TS. Also consistent with a KSIE of unity is an S_N1 mechanism, mechanism 3 in Scheme I, where the C—O bond cleavage occurs in the rate-limiting step.[19]

Arguments Against an S_N1 Mechanism—There are two principle arguments against an S_N1 mechanism. First, an S_N1 mechanism would require the formation of an energetically unfavorable primary carbocation. Second, the results from Hartman et al.[4] on the hydrolysis of methyl methanesulfonate ($CH_3SO_2OCH_3$), a molecule structurally similar to **1**, argues against an S_N1 mechanism. Hartman et al.[4] found that the ratio k_{OH}/k_{HOH} for the hydrolysis of methyl methanesulfonate was ~76 at 30 °C. A secondary deuterium kinetic isotope effect (SDKIE) for the fully deuterated methyl ester ($CH_3SO_2OCD_3$) was also determined. An inverse α-SDKIE of 0.96 indicated some degree of nucleophilic interaction at the carbon atom in the TS.[20] In other words, the carbon–hydrogen bonds of the methyl group experienced a tighter environment in the TS than that in the reactant state. This observation was perfectly consistent with an S_N2 transition state (TS) where the interaction of the incoming nucleophile and the leaving group ($CH_3SO_2O^-$) lead to crowding in the TS. In addition, the ratio $k_{OH}/k_{HOH} > 1$ also indicated some degree of nucleophilic interaction in the TS. A ratio of $k_{OH}/k_{HOH} \approx 1$ is expected for an S_N1 mechanism where the rate-limiting step is the C—O bond fission without any nucleophilic assistance from the solvent. Furthermore, there was a correlation between the observed α-SDKIE ratios of k_H/k_D and k_{OH}/k_{HOH} for methyl methanesulfonate, benzenesulfonate, and methoxysulfonate, and methyl chloride, bromide, and iodide, respectively. The mechanism by which the methyl halides undergo solvolysis is believed to be a bimolecular nucleophilic substitution (S_N2) at the carbon atom. Thus, the existence of a correlation between the observed α-SDKIE and k_{OH}/k_{HOH} ratios of a series of methyl esters of sulfonate and methyl halides allows one to draw the same mechanistic conclusion about the mechanism of degradation of **1**, which is structurally analogous to methyl methanesulfonate. That is, the hydrolysis of **1** probably occurs via an S_N2 mechanism.

In conclusion, based on the results of ^{18}O-labeling studies,



SCHEME III

kinetic solvent isotope effect studies, and the pH–rate profile and buffer data, it is proposed that the hydrolysis of 1 occurs via an S_N2-type synchronous mechanism where the nucleophile (water) and the leaving group are aligned in an early TS with no appreciable bond formation between the oxygen of the water molecule and the carbon atom of 1. The results, however, do not conclusively rule out an S_N1 mechanism. The same conclusion is also reasonable for the degradation of the intermediate, 2, to the final degradation product, 3. The proposed S_N2 mechanism is shown in Scheme III.

References and Notes

1. The present formulation of sulfamic acid 1,7-heptanediyl ester in 10% ethanol, 40% propylene glycol, 50% 0.05 M aqueous phosphate buffer of pH 7.4, has been shown to exhibit activity against a P388 tumor test system. Personal communication from the National Cancer Institute.
2. Hassan, M.; Ehrsson, H. *J. Pharm. Biomed. Anal.* **1986**, *4*, 95–101.
3. Feit, P. W.; Anderson, N. R. *J. Pharm. Sci.* **1973**, *62*, 1007–1008.
4. Hartman, S.; Robertson, R. E. *Can. J. Chem.* **1960**, *38*, 2033–2038.
5. Barnard, P. W. C.; Robertson, R. E. *Can. J. Chem.* **1961**, *39*, 881–888.
6. Mori, A.; Nagayama, M.; Mandai, H. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1669–1672.
7. (*a*) Williams, A.; Douglas, K. T. *J. Chem. Soc., Perkin Trans. II.* **1974**, 1727–1732. (*b*) Thea, S.; Guanti, G.; Hopkins, A. R.; Williams, A. *J. Org. Chem.* **1985**, *50*, 3336–3341. (*c*) Thea, S.; Guanti, G.; Hopkins, A. R.; Williams, A. *J. Org. Chem.* **1985**, *50*, 5592–5597. (*d*) Konasiewicz, A. K.; Sammy, G. M.; Maccoll, A. *J. Chem. Soc. B* **1968**, 1364–1369.
8. Indelicato, J. M.; Engel, J. M.; Occolowitz, J. L. *J. Pharm. Sci.* **1985**, *74*, 1162–1166.
9. Christman, D. R.; Oae, S. *Chem. Ind.* **1959**, 1251–1252.
10. Bunton, C. A.; Welch, V. A. *J. Am. Chem. Soc.* **1956**, *78*, 3240–3242.
11. Stella, V. J.; Kennedy, P. E., unpublished results.
12. Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 169.
13. (*a*) Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87c*, 551–606. (*b*) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275–332.
14. Kresge, A. J. *Pure Appl. Chem.* **1964**, *8*, 243–258.
15. (*a*) Melander, L.; Saunders, Jr., W. H. *Reaction Rates of Isotopic Molecules*; Wiley-Interscience: New York, 1972; Chapter 7. (*b*) Schowen, K. B. In *Transition States of Biochemical Process*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 6.
16. Kurz, J. L.; Lee, J. *J. Am. Chem. Soc.* **1980**, *102*, 5427–5429.
17. Venkatasubban, K. S.; Schowen, R. L. *CRC Critical Rev. Biochem.* **1984**, *17*, 1–44.
18. Gold, V. *Proc. Chem. Soc. London* **1963**, 141–143.
19. Laughton, P. M.; Robertson, R. E. *Can. J. Chem.* **1956**, *34*, 1714–1718.
20. do Amaral, L.; Bastos, M. P.; Bull, H. C.; Ortiz, J. J.; Cordes, E. H. *J. Am. Chem. Soc.* **1979**, *101*, 169–173.

Acknowledgments

The authors would like to express their thanks to Dr. Charles Judson for performing the mass spectral work reported here. This work was presented at the 39th National Meeting of the American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Minneapolis, October 20–24, 1985, and was supported by the National Cancer Institute, Contracts NO1-CM-37562 and NO1-CM-67912.