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Registry No. 4, 84352-42-1; **5**, 87340-59-8; $[Ru(bpy)_3]Cl_2$, 14323-06-9; 9-fluorenone, 486-25-9; thioxanthone, 492-22-8; biacetyl, 431-03-8.

Carbon-13 and Oxygen-18 Kinetic Isotope Effects on Methanolysis of *p*-Nitrostyrene Oxide

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Abstract: Kinetic isotope effects for the acid- and base-catalyzed methanolysis of [*epoxide*-^{18}O]- and [8-^{13}C]-*p*-nitrostyrene oxide have been measured at 30.0°. In acid 94.7% of the reaction occurs at the benzylic carbon, while in base 83.5% occurs at the primary carbon (C(8)). In base the isotope effects k_{16}/k_{18} and k_{12}/k_{13} were 1.035 ± 0.013 and 1.082 ± 0.012, while in acid they were 1.012 ± 0.011 and 0.995 ± 0.012, respectively. These data complement previously determined deuterium isotope effects for the reaction in base. They suggest a late transition state in base with considerable ring opening via an S_N2 mechanism. However, in acid, the data suggest a somewhat earlier transition state with less ring opening and weaker bonding to the nucleophile than in base.

Epoxides are important intermediates in many synthetic and biosynthetic pathways, and they are potentially toxic intermediates in the biotransformation of aromatic and olefinic compounds. The chemical reactivity of epoxides arises from the strained three-membered ring. Because of their chemical reactivity and widespread involvement, epoxides and their reactions have been the subject of considerable interest.

The mechanisms of ring-opening reactions of epoxides with nucleophiles have been widely studied.[1,2] Many previous studies of epoxide ring-opening reactions have centered on the questions of degree of carbonium ion formation vs. requirement for nucleophilic participation under acidic conditions. These considerations are also relevant to current interest in the relationship between the chemical properties and the biological effects of epoxides.

Recently we utilized secondary kinetic deuterium isotope effects to characterize transition-state structures for the acid- and base-catalyzed methanolysis of *p*-nitrosytrene oxide (PNSO, **1**).[3a] We now report complementary ^{13}C and ^{18}O kinetic isotope effects which define further the transition states for these reactions.

Experimental Section

A. Instrumentation. Melting points were determined in open capillary tubes with a Thomas-Hoover Uni-melt melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on Varian FT-80, T-60, or EM-360 spectrometers with tetramethylsilane as an internal standard. Mass spectra and selected ion monitoring data were obtained on a Nermag R10-10 quadrupole mass spectrometer by electron impact. HPLC analyses were performed with a Waters liquid chromatograph with an Altex 153 UV detector at 254 nm and a Varian CDS 111 digital electronic integrator. Kinetic studies were performed on a Cary 118C UV/vis recording spectrophotometer interfaced to a Cromemco Z-2 microcomputer for data collection.

B. Synthesis. *p*-Nitrostyrene (2a). (*p*-Nitrobenzyl)triphenylphosphonium bromide (50.3 g, 0.105 mol)[4] was dissolved in a mixture of 100 mL of ethanol and 100 mL of aqueous formaldehyde (37%, 2.77 mol). Aqueous sodium carbonate (30%, 100 mL) was added in 2-mL portions over a 2-h, period when the intermediate phosphorane (red color) faded to yellow. The solution was stirred for an additional 60 min and the white precipitate (triphenylphosphine oxide) which formed was filtered off. The product was extracted with twelve 50-mL portions of hexane. The crude product (21 g) was chromatographed on 350 g of silica gel with 10% ether in hexane, which yielded 13.5 g (86%) of pure **2a** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 5.44 (d, 1, J = 11 Hz, trans C8-H), 5.85 (d, 1, J = 18 Hz, cis C8-H), 6.78 (dd, J = 11, 18 Hz, C7-H), 7.50 (d, 2, J = 9 Hz, Ar), 8.18 (d, 2, J = 9 Hz, Ar).

[8-^{13}C]-*p*-Nitrostyrene (2b). (*p*-Nitrobenzyl)triphenylphosphonium bromide (7.96 g, 16.7 mmol) was dissolved in a mixture of 30 mL of ethanol and 10 mL of water. ^{13}C-Enriched formaldehyde (200 mg, 6.47 mmol, 90% ^{13}C, KOR Isotopes Lot DM-1-123) was added to the solution. Aqueous sodium carbonate (30%, 16 mL) was added dropwise over a 3-day period. The white precipitate which formed (triphenylphosphine oxide) was filtered off, and the product was extracted with three 30-mL portions of hexane. The hexane was removed, and the crude product (2.39 g) was chromatographed on 30 g of silica gel with 6% ether in hexane which yielded 1.77 g of a mixture consisting of ca. 55% *p*-nitrostyrene (100% yield) and 45% *p*-nitrotoluene; this mixture was used directly for bromohydrin formation.

2-Bromo-1-(4-nitrophenyl)ethanol (3a). The styrene **2a** (4.85 g, 32.5 mmol) was dissolved in 75 mL of acetonitrile, 50 mL of water and 6.27 g of *N*-bromosuccinimide (35.2 mmol) were added, and the mixture was stirred for 90 min. The acetonitrile was removed under reduced pressure, and the product was extracted with four 40-mL portions of ether. The ether was dried over Na_2SO_4, filtered, and evaporated to obtain the crude product (8.68 g). The latter was purified on 175 g of silica gel with 30-40% ether in hexane, yielding 7.25 g (91%) of pure product. Mp 83-84 °C; ^{1}H NMR ($CDCl_3$) δ 2.84 (d, 1, J = 4 Hz, OH), 3.53 (d, 1, J = 8 Hz, CH_2Br), 3.57 (d, 1, J = 4 Hz, CH_2Br), 5.00 (m, 1, C7-H), 7.54 (d, 2, J = 9 Hz, Ar), 8.21 (d, 2, J = 9 Hz, Ar).

[2-^{13}C]-2-Bromo-1-(4-nitrophenyl)ethanol (3b). This bromohydrin was prepared as described for **3a** with crude **2b** (contaminated with *p*-nitrotoluene, see above). From 1.06 g of crude **2b** (corresponding to *ca.* 596 mg of pure **2b**) a total of 997 mg of pure **3b** (102%) was obtained. ^{1}H NMR ($CDCl_3$) δ 2.28 (d, 0.5, J = 8 Hz, $J(^{13}C-H)$ = 76 Hz, CH_2Br), 2.33 (d, 0.5, J = 4 Hz, $J(^{13}C-H)$ = 76 Hz, CH_2Br), 3.17 (br s, 1, OH), 5.00 (m, 2, CH_2Br, C7-H), 7.54 (d, 2, J = 9 Hz, Ar), 8.19 (d, 2, J = 9 Hz, Ar).

[^{18}O]-2-Bromo-1-(4-nitrophenyl)ethanol (3c). The styrene **2a** (304 mg, 2.01 mmol) was dissolved in 6 mL of dry acetonitrile, 450 mg of *N*-bromosuccinimide (2.53 mmol) and 1 mL of [^{18}O]-enriched water (97 atom %, KOR Isotopes Lot YE-I-11) were added, and the mixture was stirred for 6 h. The acetonitrile and [^{18}O]-water were removed by

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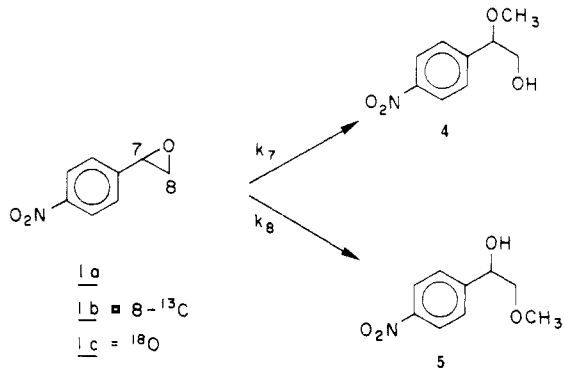
Scheme I



bulb-to-bulb distillation and saved for reuse. Ether (25 mL) and water (15 mL) were added to dissolve the solid residue, the product was extracted with three 10-mL portions of ether, and the ether was dried over Na_2SO_4. The solvent was removed and the crude product (476 mg) was purified on 15 g of silica gel with 15–30% ether in hexane. ^1H NMR as for **3a**.

***p*-Nitrostyrene Oxides (1a–c).** The appropriate bromohydrin (e.g., **3a**: 200 mg, 0.81 mmol) was dissolved in 30 mL of methanol and 100 mg of potassium carbonate was added. After the mixture was stirred for 60 min, 25 mL of water was added, and the methanol was removed under reduced pressure. The product was extracted with four 20-mL portions of ether and dried over Na_2SO_4. The solvent was removed and the crude product (425 mg) was purified on 30 g of silica gel with 20% ether in hexane, yielding 112 mg (83%) of pure **1a**. For **1a** and **1c**: mp 83.5–84 °C; ^1H NMR ($CDCl_3$) δ 2.79 (dd, 1, J = 2.5, 6 Hz, cis C8–H), 3.23 (dd, 1, J = 4, 6 Hz, trans C8–H), 3.99 (dd, 1, J = 2.5, 4 Hz, C7–H), 7.48 (d, 2, J = 9 Hz, Ar), 8.24 (d, 2, J = 9 Hz, Ar). For **1b**: ^1H NMR ($CDCl_3$) δ 1.65 (dd, 0.5, J = 2.5, 5.6 Hz, $J(^{13}C–H)$ = 88 Hz, cis C8–H), 2.10 (dd, 0.5, J = 4.1, 5.6 Hz, $J(^{13}C–H)$ = 88 Hz, trans C8–H), 3.92 (m, 1.5, C7–H, cis C8–H), 4.34 (dd, 0.5, J = 4.1, 5.6 Hz, $J(^{13}C–H)$ = 88 Hz, trans C8–H), 7.45 (d, 2, J = 9 Hz, Ar), 8.21 (d, 2, J = 9 Hz, Ar).

C. Determination of Isotopic Enrichment. The isotopic enrichment of epoxides **1a–c** was determined by selected ion monitoring (SIM) of the M^+, $M^+ - 17$, and $M^+ - 47$ ion clusters. The relative abundance of each ion in the cluster was normalized and the data processed via a matrix algebra least-squares technique[5] to determine the mole fraction of isotopic enrichment. For epoxides **1a–c**, analysis of the M^+, $M^+ - 17$, and $M^+ - 47$ clusters yielded essentially the same isotopic enrichment.

D. Methanolysis Studies. Kinetic Studies. Heavy-atom isotope effects are commonly measured by competitive methods in which reactant or product isotope ratios are followed as a function of time by isotope ratio mass spectrometry (or by scintillation counting, where applicable). They are less commonly determined by direct kinetic methods because of the greater intrinsic precision of the competitive methods. Nevertheless, in favorable circumstances direct kinetic methods can yield rate constants precise enough to determine small but statistically significant isotope effects. For the present study we have utilized a direct (photometric) method because of our favorable experience with its use for determining small secondary deuterium isotope effects in both chemical[3a] and enzymic systems.[3b]

The reactions of **1a–c** in 0.25 M H_2SO_4/CH_3OH were followed by monitoring the decrease in absorbance at 290 nm for 8 half-lives with a Cary 118C spectrophotometer interfaced to a microcomputer, essentially as reported previously.[3b] The overall absorbance change for each of these reactions was ca. 0.31 au, and 600–750 data points were collected for each run. Each run was initiated by the addition of 15 μL of an approximately 0.020 M solution of epoxide in acetonitrile to a temperature-equilibrated cuvette containing 2.0 mL of the stock acid solution. The stock acid solution was kept in a water bath at 30.0 °C, 2.0 mL of the stock solution was transferred to a cuvette, and the cuvette was allowed to equilibrate in the instrument for 3 min before the addition of the epoxide solution.

The reactions of **1a–c** in 1.0 M $NaOCH_3/CH_3OH$ were performed as described above except that (1) the much slower decrease in absorbance was monitored for 3 half-lives, (2) 800–1000 absorbance points were collected for each run, (3) ΔA for these reactions was ca. 0.17 au, and (4) the 2.0 mL of stock base solution was equilibrated for 15 min in the cuvette before the addition of the epoxide solution, because the stock solution of methoxide was kept at ambient temperature.

Table I. Rate Constants, Regiospecificity, and Isotope Effects on Acid- and Base-Catalyzed Methanolysis of *p*-Nitrostyrene Oxide

reaction	$k_{obsd} \times 10^4$, s^{-1}	regiospecificity[a]	isotope effect[b]
acid		f_7	k_7/k_7^*
1a	91.25 ± 0.71	0.947 ± 0.002	
1b	91.86 ± 0.70	0.946 ± 0.002	0.995 ± 0.012
1c	90.53 ± 0.47	0.944 ± 0.002	1.012 ± 0.011
base		f_8	k_8/k_8^*
1a	0.8796 ± 0.0056	0.838 ± 0.003	
1b	0.8329 ± 0.0060	0.833 ± 0.003	1.082 ± 0.012
1c	0.8548 ± 0.0069	0.835 ± 0.005	1.035 ± 0.013

[a] Defined by $f_7 = [4]/[4 + 5]$ and $f_8 = [5]/[4 + 5]$. [b] Corrected for regiospecificity and isotopic enrichment (**1b** = 92.5 mol % ^{13}C, **1c** = 92.7 mol% ^{18}O).

Product Analysis. The product distributions for the methanolysis reactions of **1a–c** were determined by HPLC separation and quantitation of the two isomeric methyl ether products **4** and **5** (Scheme I). At least 3 separate runs in acid and base were analyzed. Base-catalyzed solvolysis reactions were initiated by adding epoxide (125 μL of 0.020 M solution in acetonitrile) to 10 mL of 1.0 M sodium methoxide in methanol. The reactions were maintained at 30.0 °C in screw-cap culture tubes. After the completion of the reaction the methanol solution was neutralized with 10 mL of 1 M acetic acid and the methanol was removed under reduced pressure. The aqueous solution was extracted twice with 10 mL of ethyl acetate. The extract was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated. The residue was dissolved in 10 mL of ether and 30 μL of this solution was injected onto a 5 μm cyano bonded phase HPLC column (Alltech, 250 × 4.6 mm) eluted with 4% 2-propanol in hexane flowing at 2.0 mL/min. Under these conditions the isomeric products were resolved to the base line. The peak areas of **4** (R_t = 15.32 min) and **5** (R_t = 12.40 min) were determined by electronic integration of the output of a UV detector operating at 254 nm with a sensitivity of 0.02 au full scale.

Acid-catalyzed solvolysis reactions were initiated by adding epoxide (125 μL of 0.020 M solution in acetonitrile) to 10 mL of 0.25 M sulfuric acid in methanol. These reactions were maintained at 30.0 °C for 30 min in screw-cap culture tubes. After this time the methanol solutions were neutralized with 2.5 mL of 1 M sodium methoxide in methanol and the methanol was removed under reduced pressure. The residue was taken up in 10 mL of water and extracted with two 10-mL portions of ethyl acetate. The extract was dried over Na_2SO_4, filtered, and evaporated. The product was dissolved in 10 mL of ether, and 30 μL of this solution was analyzed on HPLC as described above.

E. Method of Calculation. A linear regression program was used to calculate k_{obsd} from the equation $\ln (A - A_f) = k_{obsd}t$. The same time window was used to calculate k_{obsd} for each data file (one data file per progress curve). The time window began 30 s after the reaction was initiated in acid (60–120 s afterwards in base) and continued to the end of 4 half-lives (3 half-lives in base). Data from 4–10 individual runs were used to calculate the mean and standard deviation of k_{obsd} for each isotopic variant of **1**.

As the data in Table I show, epoxides are incompletely enriched in the heavy isotope at the desired position, and the overall regiospecificity is less than 100% in both acid and base. As indicated in Scheme I, k_7 and k_8 for the unlabeled epoxide are related to k_{obsd} through the regiospecificity index, f, determined by HPLC; i.e., $k_7 = f_7 k_{obsd}$ and $k_8 = f_8 k_{obsd}$, where $f_7 = [4]/[4 + 5]$ and $f_8 = [5]/[4 + 5]$.[3a] For the labeled epoxides the specific rates k_7^* and k_8^* were determined by solving eq 1 and 2, where χ represents the mole fraction enrichment in either ^{13}C or ^{18}O (indicated by *). Values of f_7^* and f_8^* were determined in each case (Table I) but did not differ significantly from those of f_7 and f_8 for the natural abundance epoxide. The standard deviations of the resultant

$$k_{obsd}^* f_7^* = (\chi)k_7^* + (1 - \chi)k_7 \quad (1)$$

$$k_{obsd}^* f_8^* = (\chi)k_8^* + (1 - \chi)k_8 \quad (2)$$

isotope effects (k/k^*) were calculated with propagation of error techniques.[6]

Results and Discussion

Our purpose was to determine kinetic isotope effects for the acid- and base-catalyzed methanolysis of **1** and from these to

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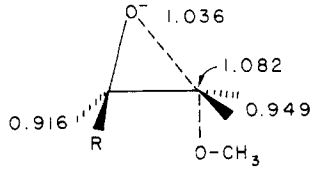
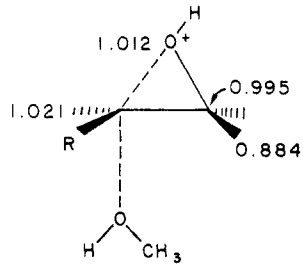
Scheme II



Scheme III



deduce the structures of transition states for these processes. The observed rate constants we determined (k_{obsd}) and the corresponding kinetic isotope effects are presented in Figure 1 and Table I, respectively. The ^{13}C- and ^{18}O-isotope effects determined in the present study should be considered together with deuterium isotope effects on these reactions measured previously in this laboratory.[3a] For convenience in reference and discussion both the deuterium and heavy-atom isotope effects are reiterated in Schemes II and III, which represent the base- and acid-catalyzed methanolysis of PNSO, respectively.

In the base-catalyzed methanolysis of PNSO the major reaction occurs at the least hindered carbon, C(8) (Scheme II). The large inverse deuterium isotope effect at C(7) (0.916 ± 0.022) implies substantial rehybridization of C(7) from the initial $sp^{2.22}$ state[7] toward a more sp^3-like transition state, which in turn implies a considerable degree of ring opening via C(8)–O bond cleavage (i.e., an "α-effect at the β carbon"). Because the C(7)–H bond is essentially orthogonal to the C(8)–O bond this isotope effect cannot arise from hyperconjugative interactions. The relatively smaller but still inverse deuterium isotope effect at C(8) (0.949 ± 0.024, or about 2% per deuterium) suggests an S_N2-type transition state in which out-of-plane C–H bending is more restricted than in the ground state, presumably because of close approach of the nucleophile.

Although ^{13}C- and ^{18}O-isotope effects are not as widely studied as deuterium isotope effects, heavy-atom isotope effects have been used to probe both chemical and enzymatic reaction mechanism.[8-12] For example, in aliphatic nucleophilic substitution reactions the carbon isotope effect at the reacting carbon is generally observed to be large in S_N2 reactions and small in S_N1 reactions.[9] Maximum carbon isotope effects would be expected in a transition state in which the bonds to the entering group and leaving group are of comparable strength,[13] and a k_{12}/k_{13} effect of 1.09 has been reported for methyl transfer catalyzed by catechol *O*-methyltransferase.[8] A k_{12}/k_{14} effect of 1.160 (corresponding to $k_{12}/k_{13} = 1.084$)[8] for the reaction of benzyl benzenesulfonate with *N,N*-dimethyltoluidine in acetone[14] and a calculated k_{12}/k_{13} of 1.075 for the reaction of iodide ion with methyl chloride[15] have also been reported. A few secondary carbon isotope effects have been measured but the reported effects are either quite small or the standard deviation is greater than the effect.[9,16] Oxygen isotope effects are usually considered to reflect the amount of C–O bond cleavage in the transition state. The largest ^{18}O kinetic isotope effects reported have been in the range of 1.04–1.06 for the hydrolysis of *p*-nitrophenyl galactoside[10] and for the hydrazinolysis of methyl benzoate[11] and methyl formate.[12]

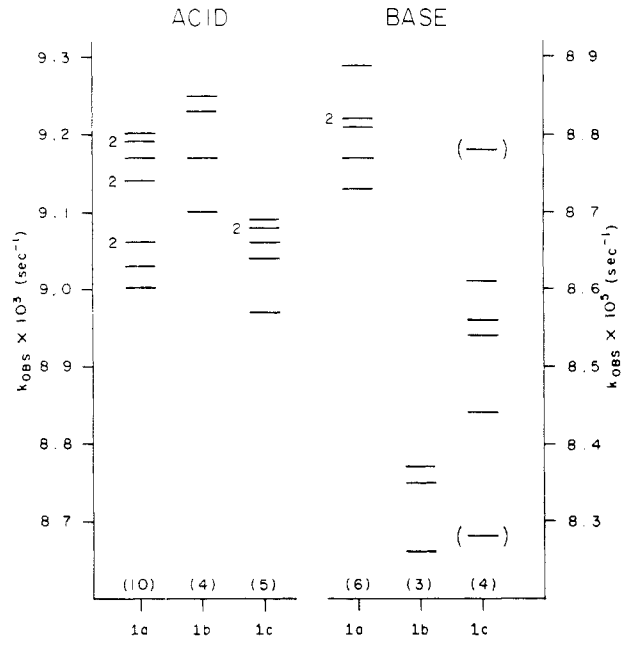


Figure 1. One-dimensional plots of k_{obsd} for the acid- and base-catalyzed methanolysis of natural abundance-, 8-^{13}C-, and ^{18}O-PNSO (**1a**, **1b**, and **1c**, respectively).[28]

For the base-catalyzed methanolysis of PNSO, the ^{13}C effect at C(8) (1.082 ± 0.012) is quite large, indicating that this reaction is strongly S_N2 in character. This is in agreement with the inverse isotope effect observed when deuterium is substituted at the reacting carbon (C(8)). The ^{18}O leaving group effect (1.035 ± 0.013) is also very large, although probably not maximal, suggesting that in the transition state ring opening has occurred to a significant extent. This conclusion is supported by the large inverse isotope effect observed for deuterium substitution at the non-reacting carbon (C(7)). The fact that the ^{18}O leaving group effect is approximately half-maximal agrees with the prediction that ^{13}C effects are maximal when bonds to the entering and leaving groups are of comparable strengths. Thus overall, information from all of the isotope effects blends into a coherent picture of a relatively late S_N2 transition state with significant nucleophilic involvement of methoxide and a substantial degree of ring opening.

In acidic media ring-opening reactions of epoxides occur at the carbon best able to accommodate the development of a positive charge[17,18] and with the exception of arene oxides[19] occur via a nucleophilic attack on the protonated epoxide rather than through the intermediacy of a carbonium ion.[20,21] Reactions of *p*-nitrostyrene oxides in acidic methanol show a high degree of regiospecificity for attack at the benzylic carbon, C(7) (Scheme III).[3]

In the present study the secondary ^{13}C-isotope effect at the "nonreacting" carbon is essentially nil (0.995 ± 0.012), as could be expected from previously reported secondary carbon isotope effects.[9,16] Furthermore, with use of the ^{13}C-fractionation factors

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for ethylene (1.0162) and ethane (1.0175) given by Hartshorn and Shiner,[22] the ^{13}C effect for converting a trigonal carbon to a tetrahedral carbon would be very small (1.0175/1.0162 = 1.001). ^{13}C-Fractionation factors are not available for oxirane and ethanol, but if they may be approximated by those for cyclopropane (1.0163) and propane (1.0286) the ^{13}C effect estimated for a product-like transition state would be 1.0286/1.0163 = 1.012; for an early transition state the effect might be even smaller.

The other isotope effects observed in acid are in principle the product of an equilibrium isotope effect (EIE) for protonation of the labeled epoxides times the kinetic isotope effect on the ring-opening reaction. For example, in the case of ^{18}O, the EIE is expected to favor protonation of the ^{18}O oxirane, as observed recently for formate protonation (^{18}O-EIE ca. 1.014).[23] To the extent that this occurs, the observed KIE will be less than the true KIE arising from ring opening (C–O bond breaking). In the present case the observed ^{18}O-KIE is 1.012 ± 0.011. If the ^{18}O-EIE for oxirane protonation is similar to that for formate protonation, then the true ^{18}O-KIE would be ca. 1.026, which is slightly less than that for the reaction of PNSO with methoxide, and definitely less than the maximum value of ^{18}O leaving group effects (ca. 1.04–1.06, see above). Thus one might conclude that the transition state for acid methanolysis is neither very early nor very late but intermediate in terms of ring opening.

The deuterium KIE at the nonreacting carbon (C(8)) is 0.940 per deuterium. If this were attributed entirely to changes in hybridization one might conclude that this reaction involved a relatively late transition state with substantial ring opening. However, since the EIE on protonation is expected to favor protonation of the deuterated epoxide,[24] the observed D-KIE probably overestimates the degree of ring opening, just as the observed ^{18}O-KIE underestimates it. Similarly, the observed D-KIE for reaction at C(7) could be reduced somewhat by the influence of an EIE, although the effect would be smaller in this case than the preceding case since only a single deuterium is involved. Thus the true KIE for deuteration at C(7) could be slightly larger than the observed[3a] effect of 1.021 ± 0.032. The fact that this effect is slightly *normal* suggests that bonding at C(7) is slightly looser in the transition state than the ground state but not loose enough to imply a carbonium ion-like structure (i.e., the nucleophile participates in ring opening).[3a] Collectively, then, the KIEs for acid methanolysis of PNSO suggest a transition state with an intermediate degree of ring opening (but probably less than in the PNSO + methoxide reaction), along with some nucleophilic participation at C(7) by solvent methanol.

Recently quantum mechanical studies of the energies and geometries of gas-phase transition states for the ring-opening reaction of fluoride ion with oxirane have been reported in brief.[27] One important conclusion of this work is that the transition state for attack with inversion of configuration is of considerably lower energy than that for attack with retention of configuration at the reacting carbon. Comparison of the calculated transition-state geometry[27] to the ground-state geometry of oxirane (calculated from the microwave absorption spectrum)[7] shows that the reacting C–O bond has lengthened from 1.436 to 1.630 Å, while the O–C–C angle around the β or "nonreacting" carbon has increased from 61.4° to 68.1°, indicating that some geometrical reorganization in the direction of product has occurred at the transition state. These results are not inconsistent with the structure for the transition state of the solution-phase reaction of methoxide with PNSO depicted in Scheme II.

Conclusions

Ring-opening reactions of *p*-nitrostyrene oxide in acidic and basic methanol have been shown to be sensitive to ^{13}C, ^{18}O, and secondary deuterium kinetic isotope effects. Collectively these observations give considerable insight to the structure of the transition state for these reactions.

Inverse deuterium isotope effects at the nonreacting carbon are observed in acid and base. In the base-catalyzed reaction the magnitude of this effect is logically associated with the degree of epoxide ring opening in the transition state; this is supported by the moderately large ^{18}O leaving group effect. The ^{13}C-KIE for this reaction is quite large, consistent with its strongly S_N2 character.

In acid, the ^{13}C-KIE at the nonreacting carbon is expected to be quite small, and indeed none could be observed. The ^{18}O and deuterium isotope effects observed are all the product of KIEs for ring opening and EIEs for epoxide protonation and thus do not give direct measures of ring opening in the transition state. Nevertheless, the data indicate that the transition state is neither very early nor very late and that the reaction involves some degree of nucleophilic attack by solvent on the protonated epoxide rather than trapping of a carbonium ion intermediate.

In addition to helping define transition-state structures for chemical ring-opening reactions of epoxides, these patterns of kinetic isotope effects offer a means for probing mechanistic details of enzyme-catalyzed reactions of epoxides. Such studies are presently under way in our laboratory.

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(24) This effect must be *inductive* in nature, since hyperconjugative effects are ruled out by reasons of geometry. Inductive deuterium isotope effects are well-established phenomena. For example, the equilibrium constant K_b for the protonation of mono- and dimethylamine is smaller for unsubstituted amines than for the fully deuterated analogues. The inverse isotope effects (K_H/K_D) range from 0.75 to 0.89 depending on the temperature and on whether one or two methyl groups are attached to the nitrogen.[25] In the ^{13}C NMR of various alcohols, the substitution of deuterium for protium increases the electron density at the α-carbon and to a lesser extent at the β-carbon, thus leading to increased shieldings of those carbon atoms; upfield shifts of ca. –0.9 ppm for the α-carbon and –0.1 ppm for the β-carbon were observed.[26]
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(28) The number 2 next to a line indicates duplicate observations. The total number of observations used to calculate kinetic isotope effects is given in parentheses at the botom of each column. For the first 5 data sets the relative standard deviation is ca. 1.0%. For the data for **1c** in base the RSD (n = 6) is ca. 2.5%. However, taking the two values in parentheses to be outliers and omitting them from calculations reduces the RSD to the 1% level characteristic of the other 5 data sets without significantly affecting the mean (see Table I).