evaporated, and the pentane extracts of the residue had an esr spectrum indistinguishable from that formed upon heating **10** in poorly degassed sample tubes.

**11 → 12 Kinetics.** Samples of **10** were weighed into esr tubes, fixed to the vacuum line (diffusion pump), and filled to a precalibrated mark by bulb-to-bulb distillation of methylcyclohexane from a sample previously freeze–pump–thaw cycle degassed. After 4–6 further freeze–pump–thaw cycles, the tubes were sealed under vacuum. A series of seven di-*tert*-butyl nitroxide samples (vpc pure) were prepared by successive dilutions in volumetric glassware, degassed before sealing, and stored at −10°. The appropriate instrument settings for best display of the initial radical concentration were determined; a sample of standard was placed in the spectrometer and the low-field line was run twice and the peak-to-peak height measured. The low-field peak of the sample of **11** was then run with the same instrument settings; the concentration of **11** was determined by use of peak-to-peak height and the proper intensity factors (1:3 for the nitroxide standard, 1:24 for **11**). Separate control calibrations demonstrated that <5% error was introduced by measuring only the low-field peaks, and that peak-to-peak heights were linear with area under integrated (absorption) peaks for **11**. Comparison of concentration obtained by use of peak-to-peak heights and areas under integrated (absorption) curves showed that use of peak-to-peak heights introduced <10% difference, with no systematic deviations detected as a function of temperature. Corrections to the standard concentration because of volume changes with temperature were obtained from the literature.[19] All concentration measurements had both sample and standard at the same temperature. A single cavity esr probe was employed, alternating standard and sample tubes. No provision was made for the effects of minor variations of field strength, but this is not felt to significantly affect our concentration measurements.

**Acknowledgment.** We thank the National Science Foundation and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for financial support of this work, as well as the Major Instrument Program of the National Science Foundation for funds used in purchasing spectrometers employed.

(19) J. Timmerman, "Physicochemical Constants of Pure Organic Compounds," Vol. I, Elsevier, New York, N. Y., 1950, p 197.

# Reaction of Hydrogen Peroxide with Alkyl Sulfonate Esters[1,2]

**Nellie A. Radomsky and Morton J. Gibian***

*Contribution from the Department of Chemistry, University of California, Riverside, California 92502.* *Received August 7, 1973*

**Abstract:** The chemistry between a typical sulfonate ester, *n*-butyl methanesulfonate, and hydrogen peroxide in neutral and basic aqueous solutions was examined. Hydrogen peroxide itself at 0.64 *M* has no effect on the ester; *n*-butyl alcohol, the sole product below pH 10, is formed at the same rate as in the absence of peroxide. Above pH 11 *n*-butyl hydroperoxide is formed quite rapidly as the sole product, arising from attack by $HO_2^-$. The second-order rate constant for $HO_2^-$ attack at 25° in water is $96 \pm 13 \times 10^{-5}$ $M^{-1}$ $sec^{-1}$, some 55 times greater than the $HO^-$ rate constant. This, combined with the much lower $pK_a$ (11.57) of $H_2O_2$ than water, produces hydroperoxide quantitatively. The reaction proceeds solely by alkyl–oxygen, and not sulfur–oxygen, scission. An attempt to observe S–O scission was made by examining neopentyl methanesulfonate in 40% *tert*-butyl alcohol at a nominal pH of 12.5 with about 0.3 *M* $H_2O_2$. Neither loss of ester nor formation of product was observed in 21 days.

A previous publication[3] and the accompanying paper describe the reaction of hydrogen peroxide with serine-195 sulfonated chymotrypsin. Because of the possibility of attack at sulfur by peroxide in that reaction, we undertook an examination of the chemistry of primary organic sulfonates with hydrogen peroxide especially under conditions analogous to these used in the protein study. From previously known chemistry and established synthetic routes,[4,5] it was anticipated that the major path would be SN2 attack at carbon to produce alkyl hydroperoxide (eq 1), but another possibility was attack at sulfur (eq 2) to produce alcohol and persulfonate (which would rapidly decompose). That the alkyl hydroperoxide should be reasonably stable under the reaction conditions was predicted from earlier studies and confirmed.

$$RCH_2O\overset{\overset{O}{\uparrow}}{\underset{\underset{O}{\downarrow}}{S}}R' \xrightarrow{H_2O_2} RCH_2OOH + R'SO_2O^- \quad (1)$$

$$RCH_2O\overset{\overset{O}{\uparrow}}{\underset{\underset{O}{\downarrow}}{S}}R' \xrightarrow{H_2O_2} RCH_2OH + R'SO_2OO^- \quad (2)$$

Another aspect of the work was to investigate the reality and magnitude of the enhanced nucleophilicity of hydroperoxide ion in SN2 attack at the saturated carbon, since at the outset of our work there had been only one report concerning this phenomenon.[6]

## Experimental Section

**Materials.** *n*-Butyl alcohol and 30% hydrogen peroxide were Mallinckrodt reagents. *n*-Butyl hydroperoxide, *n*-butyl methanesulfonate, and neopentyl methanesulfonate were prepared by the procedure of Williams and Mosher.[7] Buffer solutions were made with double distilled water and analytical grade reagents. All

(1) (a) This research was supported by the Research Corporation and the Intramural Fund of the University of California. (b) Abstracted in part from the Ph.D. Thesis of N. A. R., March 1973.

(2) Presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, Abstract, Biol-51.

(3) M. J. Gibian, D. L. Elliott, and W. R. Hardy, *J. Amer. Chem. Soc.*, 91, 7528 (1969).

(4) A. G. Davies, "Organic Peroxides," Butterworths, London, 1961, Chapter 9.

(5) D. Swern, Ed., "Organic Peroxides," Vol. 1, Wiley-Interscience, New York, N. Y., 1970, Chapter 1 (O. L. Magelli and C. S. Sheppard) and Chapter 4 (R. Curci and J. O. Edwards).

(6) R. G. Pearson and D. N. Edgington, *J. Amer. Chem. Soc.*, **84**, 4607 (1962).

(7) H. R. Williams and H. S. Mosher, *ibid.*, **76**, 2984 (1954).

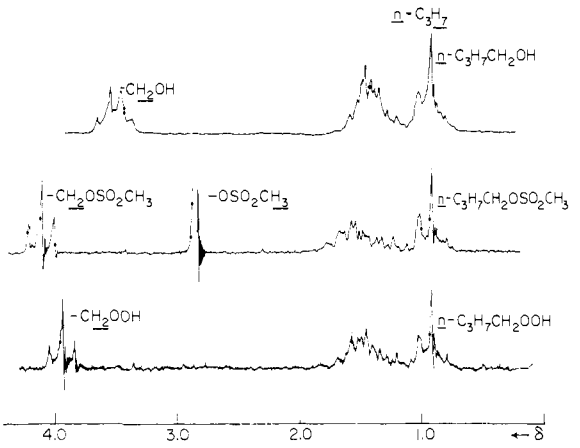


Figure 1. Nmr spectra (Varian A60D) of *n*-butyl alcohol, methanesulfonate, and hydroperoxide in chloroform.

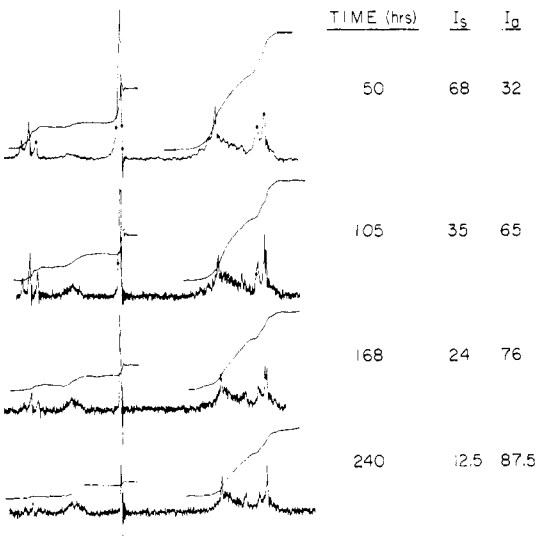


Figure 2. Nmr spectra and normalized integrated areas ($I_s$ is the $CH_2$ of the sulfonate and $I_o$ that of alcohol) *vs.* time for hydrolysis of *n*-butyl methanesulfonate (0.05 *M*) in 0.2 *M* phosphate buffer, pH 7.0, 25°.

other solvents were freshly distilled. Hydrogen peroxide concentrations were determined by a standard iodometric method.

**Kinetics and Products.** An nmr method had to be employed to follow the reaction, and it was found that *n*-butyl methanesulfonate (after examining a variety of aliphatic sulfonate esters) would maximize the ease and accuracy of analysis in terms of its water solubility ($\sim 5 \times 10^{-2}$ *M*), the extractability of it and its products of reaction into organic solvent, and the well resolved and separated nmr spectra that resulted. Figure 1 shows separate nmr spectra in chloroform of the ester, alcohol, and hydroperoxide. The $\alpha$-$CH_2$ protons of the alcohol are separated from those of the sulfonate and the hydroperoxide, and the $CH_3$ of the methanesulfonate is separated from all other resonances. While it is possible to discern the presence of hydroperoxide from its $\alpha$-$CH_2$ resonance in the presence of ester, the peaks are not sufficiently shifted to integrate each separately. All hydrolyzed methanesulfonate ion was removed by extracting unreacted ester, product alcohol, and hydroperoxide from aqueous solution into chloroform. Integration of the chloroform extract nmr spectrum gives relative amounts of alcohol $\alpha$-$CH_2$, ester-$CH_3$, and total $\alpha$-$CH_2$ of ester and hydroperoxide; hydroperoxide may then be calculated and all values normalized.

A series of experiments with standard solutions in chloroform showed that the $\alpha$-$CH_2$ integrations treated in this manner gave excellent agreement with the actual molarities. However, in the extraction of the three compounds into chloroform from various buffers (always adjusted to pH 7 with concentrated HCl or NaOH before extraction), it was found that two extractions from 25 ml of aqueous solution into 2.5 ml of chloroform did not give quantitative amounts of each compound. Since it was necessary to use approximately these quantities of solvent (too much chloroform would produce a weak nmr signal), a standardization plot for the three (known concentrations in water *vs.* chloroform extracted integrations) was determined under rigidly fixed conditions that were used throughout this work. Excellent linear plots were obtained, and the slopes were used as correction factors for the kinetic runs. The correction factor for sulfonate/alcohol was 2.3 ± 0.3 and for the sulfonate/peroxide 2.1 ± 0.1. For kinetic runs, semilog plots of the corrected integrated sulfonate peak *vs.* time were linear to 3 or more half-lives, beyond which the nmr method became quite inaccurate.

All kinetic runs contained 230 ml of aqueous 0.2 *M* (total) buffer solution with 1.50 ml of *n*-butyl methanesulfonate (0.0495 *M*), and for the peroxide runs 15.0 ml of 30% hydrogen peroxide was included in the 230 ml ($\sim$0.6 *M*). The pH was determined on a Corning Model 12 expanded scale meter, and at various times during the reaction, if necessary, the pH was adjusted back to the original value (this was only called for in the high pH runs after significant reaction). At various time intervals, 25.0-ml aliquots were removed and the pH of the aliquot was adjusted to pH 7.0, and then the mixture was thoroughly shaken twice in a separatory funnel with 2.50 ml of chloroform. The two combined chloroform extracts were then used for nmr analysis as described above.

For neopentyl methanesulfonate a similar procedure was used, but the conditions were 3.0 ml of ester (0.15 *M*), 10.0 ml of 30% $H_2O_2$ (0.3 *M*), and 3.2 g of NaOH (0.3 *M*) in a solvent consisting of 100 ml of *tert*-butyl alcohol and 150 ml of $H_2O$. Aliquots (10.0 ml) of the reaction mixture were extracted with 2.0 ml of $CHCl_3$.

## Results

The only reactions observable under the conditions of this study were nucleophilic displacement reactions. At all pH values (pH 3 to 13), and up to 1.0 *M* hydrogen peroxide, gas chromatographic analysis and nmr showed that reaction 1, accompanied by water and hydroxide hydrolyses to give alcohol, was the sole reaction for *n*-butyl methanesulfonate and hydrogen peroxide. Preliminary experiments at about neutrality and 0.5 *M* hydrogen peroxide with *n*-propyl tosylate gave solely *n*-propyl alcohol, while at pH 12 only *n*-propyl hydroperoxide was obtained. The concentration of hydrogen peroxide necessary to compete effectively with water and hydroxide precluded the use of pH titrimetric methods for following the rate, and the nmr method described in the Experimental Section was developed. For *n*-butyl methanesulfonate it was possible to determine simultaneously the overall rate of loss of ester and the production of both *n*-butyl alcohol and *n*-butyl hydroperoxide.

**Hydrolysis in the Absence of Peroxide.** Eighteen kinetic runs were made in buffered solutions at pH values ranging from pH 7 to 13.9. Figure 2 is a series of nmr spectra for a typical run. In every reaction the loss of sulfonate was experimentally first order, the rate expression in eq 3 being obtained ([S] = sulfonate

$$-\mathrm{d[S]}/\mathrm{d}t = (k_1 + k_2[\mathrm{HO^-}])[\mathrm{S}] \qquad (3)$$

concentration) over the entire pH range for buffered media. Table I gives a summary of the pseudo-first-order rate constants, $k'$ (where $k' = k_1 + k_2[\mathrm{HO^-}]$). The value for $k_1$, the water reaction, was taken as 2.5 ± 0.3 × $10^{-6}$ $\mathrm{sec^{-1}}$, an average of $k'$ from pH 7 to 11.[8] A plot of $k'$ *vs.* [$HO^-$], Figure 3, yielded a value for $k_2$ of 17.6 ± 3.1 × $10^{-6}$ $M^{-1}$ $\mathrm{sec^{-1}}$ and $k_1 = 2.50 \pm 1.43 \times 10^{-6}$ $\mathrm{sec^{-1}}$. A log–log plot of [$HO^-$] against calculated values of $k' - k_1$ has a slope of 1.02 ± 0.12, showing that the form of eq 3 is being followed.

(8) The experimental $k'$ value at pH 11 is within 10% of that at pH 7, and the calculated value of $k_2[\mathrm{HO^-}]$, see below, is less than 10% of $k_1$ at pH 11.

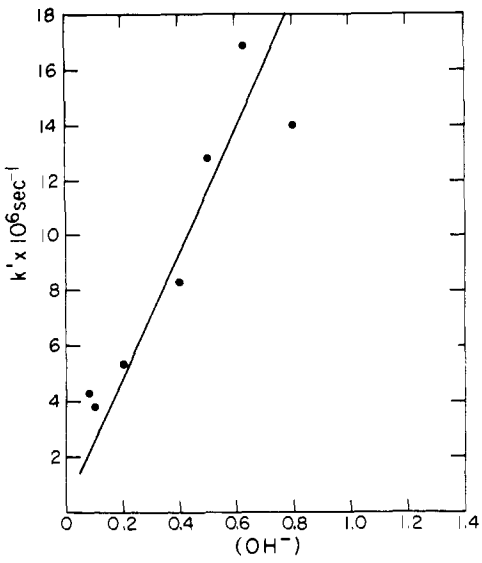


Figure 3. Plot of pseudo-first-order rate constants ($k'$) for the hydrolysis of *n*-butyl methanesulfonate *vs.* [HO⁻]. Data are from Table I.

**Table I.** Experimental First-Order Rate Constants for the Hydrolysis of *n*-Butyl Methanesulfonate[a]

| pH (buffer) | $10^6k'$, sec$^{-1}$ |
|---|---|
| 7.00 (Phos) | 2.1 ± 0.2 |
| 8.00 (Phos) | 2.6 ± 0.5 |
| 9.00 (Carb) | 2.3 ± 0.5 |
| 10.00 (Carb) | 2.7 ± 0.5 |
| 11.00 (Carb) | 3.1 ± 0.2 |
| 11.00 (Phos) | 2.7 ± 0.4 |
| 12.00 (Phos) | 3.2 ± 0.3 |
| 12.40 (Phos) | 3.6 ± 0.2 |
| 12.90 (Phos) | 4.3 ± 0.3 |
| 13.00 (NaOH) | 3.8 ± 0.2 |
| 13.30 (NaOH) | 5.3 ± 0.5 |
| 13.60 (NaOH) | 8.3 ± 1.0 |
| 13.70 (NaOH) | 12.8 ± 1.0 |
| 13.80 (NaOH) | 16.8 ± 1.0 |
| 13.90 (NaOH) | 14.0 ± 1.0 |

[a] 25°, aqueous 0.20 *M* buffers except for pH >13, which was just NaOH.

**Reaction with Hydrogen Peroxide.** Twelve kinetic runs were made with *n*-butyl methanesulfonate and hydrogen peroxide between pH 7 and 12. From pH 7 to about 10 the sole product was *n*-butyl alcohol, mixtures of alcohol and *n*-butyl hydroperoxide were obtained between pH 10 and 11, and solely hydroperoxide was obtained above that.

The self-decomposition of hydrogen peroxide is strongly pH dependent in the basic region and exhibits a maximum rate at about pH 11. The rate law for this reaction,[9] $-d[H_2O_2]/dt = k[H_2O_2][HO_2^-]$, was confirmed by us. We were able to study the ester reaction kinetically below pH 10 and above pH 11, since for the former conditions the peroxide is stable over the time course of our studies, and for the latter conditions the reaction rate for ester disappearance is sufficiently more rapid than peroxide decomposition. Indeed, alkyl hydroperoxide is also less stable at pH 10.5 than at 12.

Figure 4 presents nmr spectra and data for a typical run in which only peroxide was formed. Table II gives a summary of pseudo-first-order rate constants, $k''$ of eq 4, obtained as described in the Experimental Section.

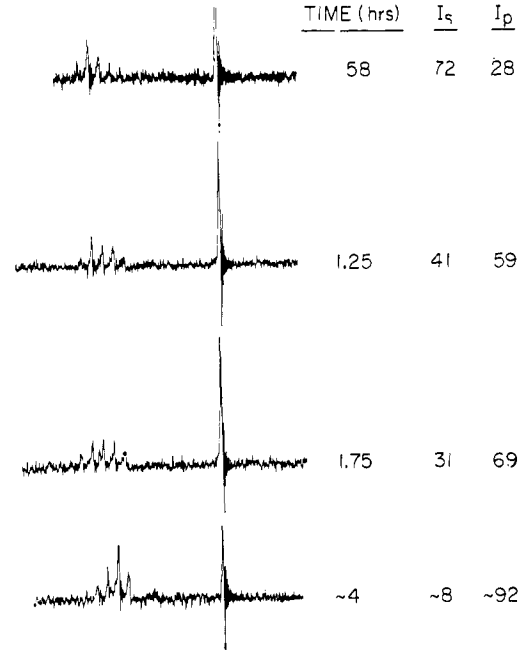


Figure 4. Nmr spectra and normalized integrated areas ($I_s$ is the $CH_2$ of the sulfonate and $I_p$ that of the peroxide) *vs.* time for the reaction of *n*-butyl methanesulfonate (0.05 *M*) with hydrogen peroxide (0.55 *M*) in 0.2 *M* phosphate buffer, pH 11.3, 25°.

**Table II.** Pseudo-First-Order Rate Constants for the Hydrolysis of *n*-Butyl Methanesulfonate in Aqueous Hydrogen Peroxide[a]

| pH (buffer) | $10^6k''$, sec$^{-1}$ | Product[b] |
|---|---|---|
| 7.00 (Phos) | 2.0 ± 0.2 | A |
| 8.00 (Phos) | 2.5 ± 0.2 | A |
| 9.00 (Carb) | 2.1 ± 0.1 | A |
| 10.2 (Carb) | (4.4)[c] | A, P |
| 10.5 (Carb) | (3.6)[c] | A, P |
| 10.8 (Carb) | (6.4)[c] | A, P |
| 11.10 (Phos) | 111 ± 10 | P |
| 11.35 (Phos) | 202 ± 22 | P |
| 11.50 (Phos) | 274 ± 5 | P |
| 11.70 (Phos) | 301 ± 20 | P |
| 11.90 (Phos) | 377 ± 51 | P |

[a] 25°, 0.2 *M* aqueous buffers, approximately 0.6 *M* $H_2O_2$ (titrated before and after each run). [b] A = *n*-butyl alcohol; P = *n*-butyl hydroperoxide. [c] Approximate, $H_2O_2$ decomposing at a rate comparable to reaction.

$$-d[S]/dt = (k_1 + k_2[HO^-] + k_3[HO_2^-])[S]$$
$$k'' = k_1 + k_2[HO^-] + k_3[HO_2^-] \quad (4)$$

Taking the $pK_a$ of $H_2O_2$ as 11.57,[10] the overall rate law was found to follow eq 4 with no term in $H_2O_2$ itself. Plotting $(k'' - k_2[HO^-])$ *vs.* $[HO_2^-]$, Figure 5, leads to a value for $k_3$, the slope, of $96 \pm 13 \times 10^{-5}$ $M^{-1}$ sec$^{-1}$.

That the form of the equation is correct was shown by a plot of log $[HO_2^-]$ against log $(k'' - k_1 - k_2[HO^-])$, which had a slope of 1.06 ± 0.13. That there is no term in $[H_2O_2]$ was in accord with the product data in which it was found that alkyl hydroperoxide is obtained only from hydroperoxide ion. The rate of sulfonate ester disappearance below pH 10 is equivalent in the presence and absence of as high as 1.0 *M* hydrogen

(9) F. Duke and T. Haas, *J. Phys. Chem.*, **65**, 304 (1961).

(10) Reference 5, p 206.

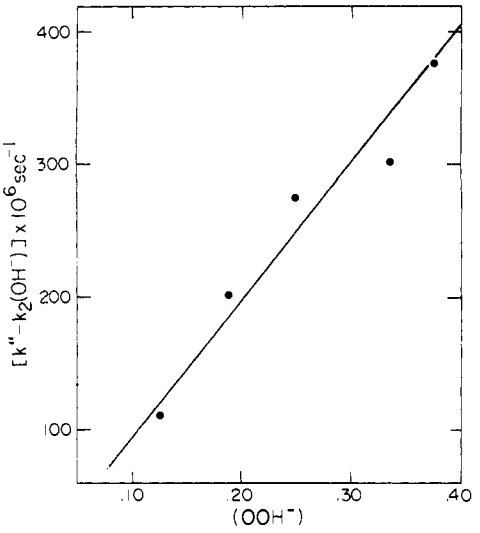


Figure 5. Plot of $(k'' - k_2[HO^-])$ *vs.* $[HOO^-]$ for the reaction of *n*-butyl methanesulfonate with hydrogen peroxide (see eq 4). Data are from Table II.

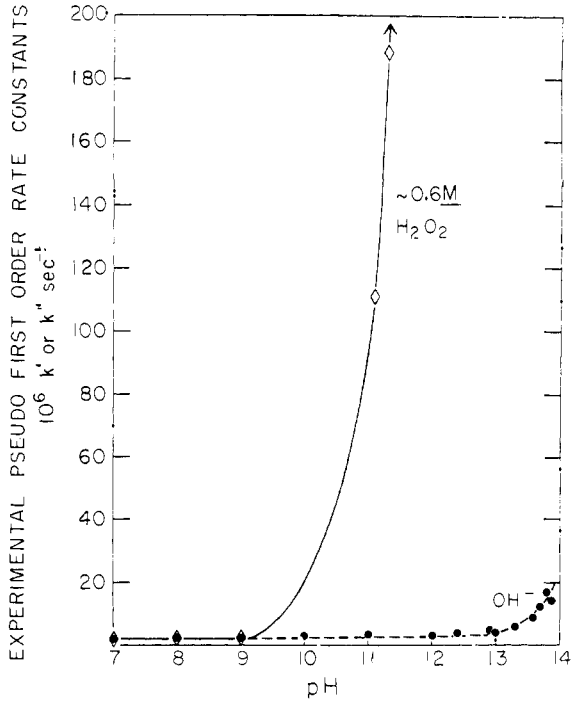


Figure 6. Pseudo-first-order rate constants in the absence ($k'$) and presence ($k''$) of 0.6 *M* hydrogen peroxide as a function of pH.

peroxide, shows an increase for the peroxide runs over the buffer alone between pH 10 and 11, and becomes extremely fast for the peroxide reaction above pH 11. Figure 6 depicts the pH dependence of the data with and without a constant $[H_2O_2]_{total}$ and shows the dramatic effect of hydrogen peroxide near and above its $pK_a$.

Neopentyl methanesulfonate, in 21 days at pH 12.5 with 0.3 *M* $H_2O_2$ in 40% *tert*-butyl alcohol, could not be detected to react or give any product. The $H_2O_2$, which decomposed during this experiment, was replenished each day by adding sufficient 30% $H_2O_2$ to maintain the original concentration as determined by titrating an aliquot. Higher temperatures and/or $H_2O_2$ concentrations caused very rapid $H_2O_2$ decompositions with no evidence of attack on ester.

## Discussion

Figure 6 and Tables I and II reveal that at pH values below 10 the first-order experimental rate constants for *n*-butyl sulfonate ester hydrolysis are identical with and without 0.64 *M* hydrogen peroxide; only *n*-butyl alcohol is obtained. In order for even a 10% increase in rate (or 10% $H_2O_2$ derived product), the rate constant for $H_2O_2$ reaction would have to be about ninefold that of the water reaction (0.6 *M* $H_2O_2$ *vs.* 55 *M* $H_2O$).

Above pH 11 (Table II, Figure 6) there is a dramatic increase in the rate of reaction in the peroxide system, and product analyses show increasing amounts of *n*-butyl hydroperoxide above pH 10. Above about pH 11.1 only alkyl hydroperoxide is formed. Since the $pK_a$ of hydrogen peroxide is 11.57, we ascribe this increase in rate and the change in product to a reaction of the hydroperoxide anion ($HO_2^-$) and using this value for the $pK_a$ obtain excellent agreement with eq 4 (Figure 5). At high pH in the purely water–buffer systems the rate constant of second-order hydroxide displacement of sulfonate is $1.76 \times 10^{-5}$ $M^{-1}$ $sec^{-1}$, which we may compare to that of second-order displacement by hydroperoxide ($96 \times 10^{-5}$ $M^{-1}$ $sec^{-1}$). The ratio is 55 ± 8.

Product studies gave no evidence of other than $HO_2^-$ nucleophilic attack at the primary carbon to produce alkyl hydroperoxide and sulfonate. Neopentyl methanesulfonate, being very inert (typically $10^{-5}$ normal primary alkyl) in displacement reactions, was chosen to look further for S–O scission. No such process was found.

Organic sulfonate esters usually react entirely *via* carbon–oxygen scission under nucleophilic (basic) conditions, the exceptions being for certain nucleophiles (hard bases) with aromatic and neopentyl esters. Bunnett[11] found that polarizable nucleophiles ($C_6H_5S^-$, piperidine) gave high ratios of C–O scission for nitro-substituted phenyl tosylates, while methoxide gave predominantly S–O scission. Parker[12] observed S–O scission with dimsyl anion and phenyl tosylate in DMSO, although with alkyl tosylates alkyl–oxygen scission was vastly predominant. Earlier, Bordwell[13] had observed S–O scission of neopentyl tosylate with methoxide but C–O (SN2, unrearranged product) with thiophenoxide. In hexamethylphosphoramide, however, neopentyl tosylate gave SN2 inversion displacements at carbon with a wide variety of nucleophiles (including alkoxides).[14] This solvent, of course, gives small anions a very high reactivity. The cyclic sulfonate esters derived from 2-hydroxy-α-toluenesulfonic acid undergo S–O scission by $OH^-$ in aqueous systems.[15] These are highly reactive intramolecular reactions, however. Organic sulfates undergo either kind of scission.[16]

Most recently, Kice[17] examined attack upon $PhSO_2$-

(11) J. F. Bunnett and J. Bassett, Jr., *J. Amer. Chem. Soc.*, **81**, 2104 (1959).
(12) T. Broxton, Y. Mac, A. J. Parker, and M. Ruane, *Aust. J. Chem.*, **19**, 521 (1966).
(13) F. Bordwell, B. Pitt, and M. Knell, *J. Amer. Chem. Soc.*, **73**, 5004 (1951).
(14) B. Stephenson, G. Solladié, and H. S. Mosher, *ibid.*, **94**, 4184 (1972).
(15) (a) E. T. Kaiser and O. R. Zaborsky, *ibid.*, **90**, 4626 (1968); (b) O. R. Zaborsky and E. T. Kaiser, *ibid.*, **92**, 860 (1970).
(16) E. Buncel, A. Raoult, and J. F. Wiltshire, *ibid.*, **95**, 799 (1973).
(17) J. L. Kice and E. Legan, *ibid.*, **95**, 3912 (1973).

$SO_2Ph$ by a wide variety of nucleophiles in 60% dioxane. The $pK_a$ of the leaving group $PhSO_2^-$ is about 1.5, the sulfur is highly polarized, and the system is very reactive. The rate constants for attack at sulfonyl sulfur by 20 nucleophiles closely paralleled those for attack at carbonyl carbon of acetate esters with very good leaving groups. In neither Kaiser's work on sultones nor Kice's work could the question of direct displacement on sulfur *vs.* pentacovalent intermediates be resolved. For the α-disulfones, either attack is rate limiting or there is no intermediate. Kice found an α effect of 72 for $HO_2^-/HO^-$. Our data show that hydroperoxide ion acts toward primary alkyl sulfonates like the vast bulk of nucleophiles, attacking entirely at carbon. However, the possibility of sulfonyl sulfur attack with some very hard nucleophiles or with favorable geometric alignments is by no means excluded. From the neopentyl methanesulfonate result, however, it is clear that the leaving group must be rather good for reaction to be of reasonable rate.

The enhanced nucleophilicity of $HO_2^-$ (*vs.* other oxygen nucleophiles) toward $sp^2$ (carbonyl and olefinic) carbon is well known and along with other examples of the α effect has been discussed recently.[17,18] At the outset of our research project, there was only one unambiguous example of enhanced nucleophilicity by an α-effect nucleophile toward $sp^3$ carbon, that of $HO_2^-$ toward benzyl bromide, in which Pearson reported a ratio for $k_{HO_2^-}/k_{HO^-}$ of 35.[6] Several recent reports with other nucleophiles have shown that amine nucleophiles tend not to give enhanced reactivity toward $sp^3$ carbon but that oxygen nucleophiles do.[18,19] Our study confirms these observations and provides another value in agreement with those previously observed.

The lack of reactivity of the neopentyl system puts an upper limit upon the reactivity of sulfonate sulfur toward hydroperoxide ion for normal sulfonates ($k_2 < 5 \times 10^{-7}\ M^{-1}\ sec^{-1}$). The cyclic sulfonates are thus attacked at sulfur by $HO^-$ at $10^8$ times this rate and the α-disulfones attacked by $HOO^-$ about $10^{10}$ times as fast.

(18) J. E. Dixon and T. C. Bruice, *J. Amer. Chem. Soc.*, **94**, 2052 (1972).
(19) J. E. Dixon and T. C. Bruice, *ibid.*, **93**, 6592 (1971); W. Richardson and V. Hodge, *ibid.*, **93**, 3996 (1971).

# Reaction of Phenylmethanesulfonyl-α-chymotrypsin with Hydrogen Peroxide and Other Nucleophiles[1,2]

**Nellie A. Radomsky, Morton J. Gibian,* and D. Lauriston Elliott**

*Contribution from the Department of Chemistry, University of California, Riverside, California 92502.* *Received August 7, 1973*

**Abstract:** Phenylmethanesulfonyl-chymotrypsin (PMS-CT), a stable catalytically inactive sulfonate ester of the active site serine of the enzyme, is desulfonated and reactivated by hydrogen peroxide. The reactivated material was isolated and subjected to a variety of analyses. Previously reported data indicating the presence of a hydroperoxy group at the site have now been compared to data from the same experiments using chymotrypsin incubated with hydrogen peroxide as a control, and there is now clear evidence that the reactivated material is normal hydroxy enzyme oxidized to the sulfoxide at methionine-192. The catalytic behavior and its pH dependence, the reactions with active site serine and histidine reagents, and amino acid analyses of reactivated and hydrogen peroxide oxidized chymotrypsin are identical. Preincubation of α-chymotrypsin with hydrogen peroxide (generation of methionine-192 sulfoxide) neither retards sulfonation nor alters the stability of the sulfonyl enzyme in buffer alone. The rate of reactivation with peroxide is the same for preoxidized and native sulfonyl enzymes. Thus, hydrogen peroxide is reacting with the sulfonate group directly. The reactivation reaction itself shows dependence on a basic group of $pK_a \sim 7$, is overall first order (at high peroxide concentrations) in PMS-CT, and gives one $^{14}C$ loss for each active site appearing for [7-$^{14}$C]PMS-CT. A range of other nucleophiles including hydrazine, hydroxylamine, *N*-phenylbenzohydroxamic acid, phenol, and pyridine-2-aldoxime methiodide gave no loss of sulfonate. Hydrogen peroxide did not reactivate diisopropylphosphoryl-chymotrypsin. Methyl hydroperoxide, however, very slowly but quantitatively reactivates PMS-CT leading to one active site per sulfonate lost (by isotope labeling). This is shown to not be due to contamination by, or formation of, hydrogen peroxide. As shown by an isotope dilution and recovery experiment, phenyl methanesulfonate is the exclusive (and quantitative) other product in the hydrogen peroxide reaction. No persulfonate could be detected. These data, along with rates of reaction in comparison with model systems, show that the reactivation reaction involves direct attack on the sulfonate ester group to give ultimately serine enzyme and sulfonate ion, that the reaction is specific for hydrogen peroxide, and that the reaction is enzyme catalyzed. Possible pathways are discussed.

An earlier communication from this laboratory[3] described the reaction of catalytically inactive serine-195 sulfonated chymotrypsin (phenylmethanesulfonyl-chymotrypsin, PMS-CT) with hydrogen peroxide to produce a reactivated enzyme (react-CT). On the basis of organic primary sulfonate chemistry along with various aspects of the nature of the reactivation reaction, the loss of $^{14}C$ label from radioactive PMS-CT,

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(2) Presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, Abstract Biol-51. The current conclusions are described in this abstract.

(3) M. J. Gibian, D. L. Elliott, and W. R. Hardy, *J. Amer. Chem. Soc.*, 91, 7528 (1969).