

Figure 2. (a) Lineweaver-Burk plots ($[CA] = 3.75 \times 10^{-6} M$) as a function of pH and inhibitor: \square , pH 7.00; \bullet , pH 7.55; Δ , pH 7.62; \blacksquare , pH 8.45 with $5 \times 10^{-7} M$ acetazolamide; \circ , pH 8.45. (b) Lineweaver-Burk plots ($[CA] = 3.75 \times 10^{-6} M$) at pH 7.55 in the presence of 0.09 M salt: \square , NaI; \circ , NaNO₃; Δ , NaBr; \blacksquare , NaOAc; \bullet , NaCl; \blacktriangle , NaF.

catalytic activity,⁵ and we have shown that while catalysis by zinc ion in Tris or malonate buffers is very mild, marked enhancement is observed in imidazole buffers well in excess of that accorded by imid-

(5) S. Lindskog and B. G. Malmström, *J. Biol. Chem.*, **237**, 1129 (1962).

azole alone: $k_{HI_m^+}$, very small; $k_{Im} = 15 \text{ min.}^{-1} M^{-1}$ (similar k_{Im} is reported by others⁶); $k_{Zn^{2+}}$ (in imidazole buffers⁷; pH 6.9–7.2; $[Zn^{2+}]$ up to $5 \times 10^{-3} M$; $([Im] + [HI_m^+]) = 0.036 M \approx 40 \text{ min.}^{-1} M^{-1}$).

We find that the dependence of the enzymatic rate on substrate concentration follows the classical Michaelis-Menten relationship. Lineweaver-Burk plots at constant enzyme concentration give values of K_M and V_M which increase with pH (Figure 2a). The observed variation of K_M is a linear function of V_M , indicating that the apparent binding constant, k_1/k_{-1} , is invariant in the pH range 6–10.5. This implies that the inflection point in the pH-rate profile refers to the titration of the hydrolyzing rather than the binding site. These observations accord with the hypothesis that the binding site must be composed of a group or groups whose pK_a lies outside the region 6–10.5, while the hydrating site has a pK_a of 7.5, as would accord with the suggestion that unprotonated imidazole and water coordinated to zinc participate in hydrolysis.

Salt (anionic) inhibition follows Michaelis-Menten kinetics and is noncompetitive, the order of inhibition being⁸ $F^- < Cl^- < AcO^- < Br^- < NO_3^- < I^- < CNS^-$ (Figure 2b). Combining the pH and salt inhibition data with the equation⁹ $K_M = a + bV_M$, it is found that $a = 2.5 \times 10^{-4} M$ and $b = 13.4 \text{ min.}$, leading to an apparent $k_1 = 2.0 \times 10^4 M^{-1} \text{ min.}^{-1}$ and an apparent $k_{-1} = 4.9 \text{ min.}^{-1}$. Acetazolamide,¹⁰ one of the most powerful noncompetitive inhibitors of the hydase activity of CA, is also found to inhibit noncompetitively its esterase activity with respect to *p*-nitrophenyl acetate hydrolysis, with an inhibition constant $K_i = 3.7 \times 10^{-6} M$ at pH 8.45. The totality of these observations, particularly the effect of pH, as well as the inhibitory effects of anions and of acetazolamide on k_2 for both hydase and esterase activity suggest that the underlying mechanisms of CA-catalyzed hydration and hydrolysis are similar.

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Hydrogen Exchange in Benzyl Methyl Sulfoxide. Kinetic and Spectroscopic Nonequivalence of Methylene Protons

Sir:

Although measurement of Hammett substituent constants for the methylsulfonyl and methylsulfinyl groups¹

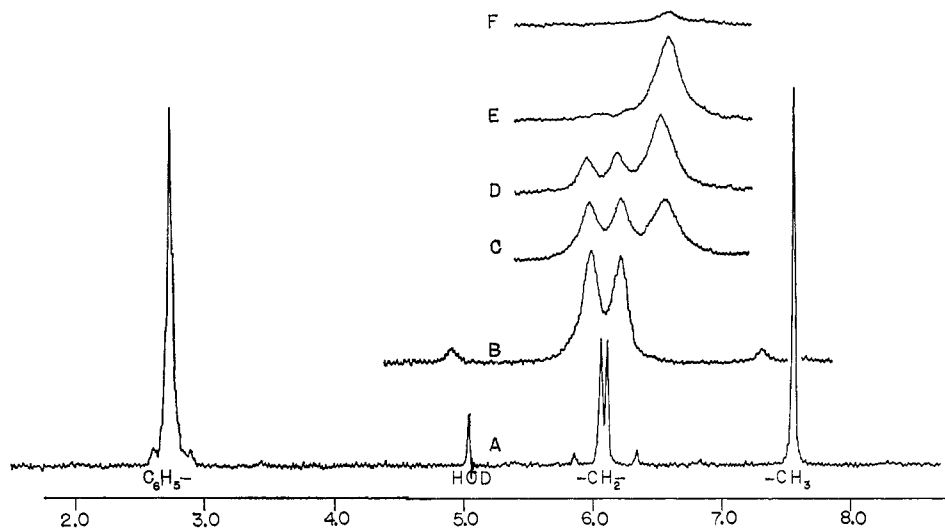


Figure 1. Illustrative n.m.r. spectra for the exchange of the methylene hydrogens of benzyl methyl sulfoxide (I). The scale refers to spectrum A only; other spectra have the scale expanded five times. All spectra were determined at 5°. A, I in D₂O; B, I in D₂O on magnified scale; C, I in 1 M NaOD-D₂O after allowing exchange (~25%) to take place; D-F are similar to C but represent, respectively, 40, 50, and 95% exchange. To make comparison easier, spectra C, D, E and F have been shifted slightly to compensate for the change in chemical shift caused by the addition of base.

would lead to the prediction that -SO₂- and -SO- should have similar conjugative ability with adjacent carbanionic centers, previous experience indicated that a sulfinyl²⁻⁴ group was much less effective than a sulfonyl⁵ group in facilitating hydrogen-deuterium exchange at an α carbon. Therefore the recently reported⁶ facile hydrogen exchange of dimethyl sulfoxide in the presence of deuterioxide might have been a consequence not of the conjugative effect of the -SO- group but of the generally enhanced reactivity of bases in dimethyl sulfoxide media.⁷ It was anticipated that a study of hydrogen exchange of benzyl methyl sulfoxide in a medium of deuterium oxide would throw light on both of these factors, since this substrate has two sites, one (methyl) activated only by -SO-, the other (methylene) activated also by phenyl. The two sites did, in fact, undergo exchange at different rates. However, the most interesting result of this study was the unexpected difference in the rates of exchange of the two methylene hydrogens.

In Figure 1 is shown the n.m.r. spectrum at 5° of benzyl methyl sulfoxide in D₂O with tetramethylsilane as an external standard. The methyl hydrogens appear as a single peak, as expected, but the methylene hydrogens appear as a quartet, $|J_{AB}| = 13.0$ c.p.s., τ_A 6.07 p.p.m., τ_B 6.21 p.p.m.⁸ The quartet collapses to a single peak at 65°.¹¹

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When a solution of benzyl methyl sulfoxide in 1 M NaOD-D₂O is observed as a function of time the signals due to the methylene group undergo changes which are shown in Figure 1 (inset). The quartet gradually disappears and is replaced by a broad peak centered at τ 6.21. This broad peak then slowly disappears. Since the broad peak collapses to a sharp singlet at τ 6.21 upon deuterium decoupling,¹⁴ it follows that the methylene hydrogen at τ 6.07 is exchanged faster than its twin. That these observations are not merely the result of an isotope effect was shown by the conversion of PhCD₂SOCD₃ to PhCHDSOCD₃ with OH⁻-H₂O. The product of this reaction shows a broad peak at τ 6.07.

The rate of exchange of each methylene hydrogen in 1 M NaOD-D₂O can be determined by integration of spectra such as those shown in Figure 1. At 15.0° the rate constants are¹⁵: $k_A = 59 \times 10^{-5}$ l. M⁻¹ sec.⁻¹, $k_B = 4.3 \times 10^{-5}$ l. M⁻¹ sec.⁻¹. Rate data for the exchange of the methyl hydrogens in this medium were obtained at 60.0 and 75.1°; from the activation

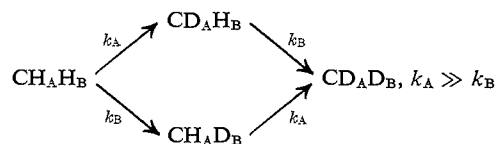
(11) Temperature-dependent spectra have also been observed in CDCl₃ and CD₃COCD₃. In CDCl₃ solutions the coalescence temperature was 50°; interestingly, coalescence was also observed below -10° in deuteriochloroform. This result, which suggests the formation of a sulfoxide dimer, is in accord with the recent reports by Watson and Eastham¹² and Mislow and co-workers.¹³ In CCl₄ and in pyridine containing less than 75% water the methylene protons were equivalent at all temperatures examined. The solvent effect studies together with rotational energy barriers will be reported in a subsequent communication.

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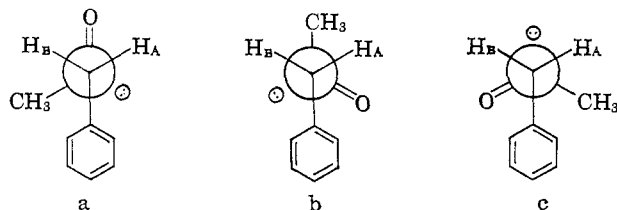
(14) This experiment was performed by Dr. R. R. Fraser at the University of Ottawa.

(15) Results were calculated by application of standard kinetic expressions to the following simplified model of the reaction, isotope effects being ignored



energy ($E_a = 28.1$ kcal./mole) the rate constant at 15.0° is calculated to be 9.3×10^{-8} l. M^{-1} sec. $^{-1}$ for a single hydrogen. The much smaller reactivity of the methyl hydrogens is evident.

Due to the pyramidal configuration of the sulfoxide group,¹⁶ benzyl methyl sulfoxide is asymmetric and, indeed, the optically active compound can be prepared in a state of high optical purity.¹⁷ The nonequivalence of the methylene hydrogens in an asymmetric acyclic compound has been shown to be mainly due to conformational preference.¹⁸ Therefore, considering only one enantiomer of benzyl methyl sulfoxide, the n.m.r. spectrum indicates that the conformations a, b, and c are unequally populated under the conditions of exchange on the methylene group.



In an attempt to derive the principal conformation the dipole moment of benzyl methyl sulfoxide has been determined. The measured¹⁹ value, 3.86 D. (benzene), is unfortunately too close to that of the sulfoxide group itself (3.90 D.²⁰) for inference to be made. *p*-Chlorobenzyl methyl sulfoxide has a dipole moment of 3.66 D.¹⁹

It must be concluded from the n.m.r. and rate data that the hydrogen exchange process is stereospecific. It follows then that the carbanion must be protonated from the side of the departing proton.²¹ Yet the carbanionic center must be nearly flat because of the observed effect of phenyl on the rates.

Which of the methylene hydrogens is preferentially exchanged should become known from studies, now in progress, with benzyl methyl sulfoxide of known absolute configuration.^{17,24} An intriguing speculation is that a hydrogen to be exchanged must be *trans* to the unshared pair on sulfur.²⁵ It will also be of interest to examine similarly optically active α -deuterio-

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(19) We thank Mr. Brian F. Scott, University of Toronto, for this measurement.

(20) L. E. Sutton in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press Inc., New York, N. Y., 1965, p. 395.

(21) Similar conclusions have been drawn regarding protonation of sulfonyl anions.^{22,23}

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(25) It has been suggested²² that the most stable conformation of sulfonyl and sulfinyl carbanions would have the anionic charge oriented *trans* to the sulfur oxygen(s). While this situation is plausible for the sulfonyl case (see however ref. 23, for an opposing view), the conformational preference of oxygen of thiane oxides to the axial position²⁶ as well as other evidence²⁶ suggests that in the sulfoxide case it is the unshared electron pair that may have dominating influence.

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benzyl methyl sulfone, in view of the evidence²² that sulfonyl anions are superior to sulfinyl and phosphinoxy in configuration-holding capability.

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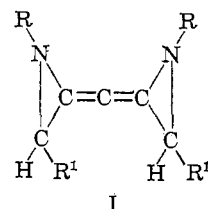
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Reaction of Carbon Vapor with Imines

Sir:

Recent investigations of the reactivity of carbon vapor has involved reaction of monatomic and triatomic carbon vapor, produced in a carbon arc, with alkenes,¹⁻³ monatomic carbon, produced by nuclear techniques, with alkenes,^{4,5} and thermally produced carbon atoms with benzene.⁶ Skell and co-workers report the addition of C_3 to alkenes producing substituted bisethanoallenes.^{1,2} The stereospecific nature of the addition of C_3 to olefins has indicated that the ground state of C_3 is singlet,² a conclusion consistent with analysis of spectra of carbon vapor.⁷ Wolfgang and co-workers have reported insertion reactions of monatomic carbon into alkenes producing allenes⁴ as well as many other products.⁵

We report here the reaction, occurring on a surface cooled to -196° , of carbon vapor with imines, producing previously unknown compounds having what we believe to be structure I.



The apparatus employed, shown in Figure 1, was similar to that used by Skell, *et al.*² Graphite electrodes were threaded into brass rods which were sealed into the vacuum system by rubber O rings. Alternating current was supplied by a transformer (output 17 v., 100 amp. maximum; input 230 v.). Graphite rods, $5/16$ in. diameter, were National Carbon Co. spectroscopic quality carbons. The carbons were outgassed at 800° in hydrogen atmosphere prior to use. The apparatus shown in Figure 1 was connected directly to an oil diffusion pump by 32-mm. glass tubing. The vacuum system was capable of pumping to 10^{-6} mm. pressure. In a typical experiment, 2-3 ml. of imine in the lower flask was cooled with Dry Ice. The apparatus was evacuated and the cold finger cooled with liquid N_2 . The arc was operated on a 3 sec. on-27 sec. off

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