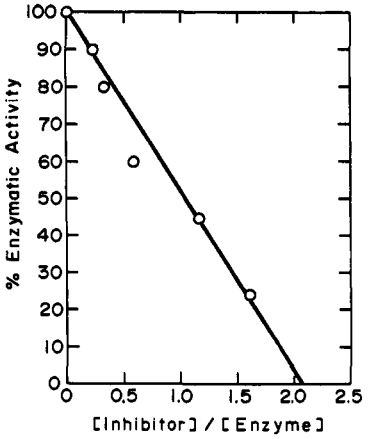

Figure 1. The activity of arylsulfate sulfohydrolase II inhibited by varying amounts of *o*-nitrophenyl oxalate as a function of the ratio of inhibitor per enzyme. The protein[5] (homogenous by disc gel electrophoresis and equilibrium ultracentrifugation criterion, specific activity = 0.7 unit/mg) was incubated with varying amounts of the oxalate ester for 20 hr at 4°.

activity as a variation of the concentration of the phosphate ester is characteristic of the minimal scheme

$$EI_2 \underset{k_{I_2}}{\overset{I_2}{\longleftarrow}} E + I_1 \overset{K_S}{\rightleftharpoons} EI_1 \qquad (1)$$

where I_1 represents *p*-nitrophenyl phosphate, I_2, *o*-nitrophenyl oxalate, and E, arylsulfate sulfohydrolase. Solution of eq 1 gives

$$\ln\left(1 - \frac{[EI_2]}{[E_0]}\right) = \frac{-k_{I_2}[I_2]t}{1 + [I]/K_s} \qquad (2)$$

where $[E_0] \cong$ initial enzyme concentration, K_s is the dissociation constant for EI_1, and k_{I_2} is the second-order rate constant for oxalate inhibition. Values of $k_{I_2} = 4.3 \times 10^2\ M^{-1}\ \text{min}^{-1}$ and $K_s = 3.5 \times 10^{-4}\ M$ were employed to calculate the solid lines of Figure 2. The increased protection afforded by higher concentrations of I_1 at a given time is in accord with *o*-nitrophenyl oxalate reacting at the active site. Similar results were obtained in the presence of excess substrate, *p*-nitrophenyl sulfate.

The reaction of the oxalate ester with the enzyme occurs with an initial rapid exponential release of *o*-nitrophenol followed by its slower liberation, the latter apparently due to both spontaneous hydrolysis and unspecified reaction with the protein. Since the difference in the two rate processes is a factor of *ca.* 16-fold at pH 4.8, the measurement of the burst may be accomplished by extrapolation of the initial OD values. The burst height (π) is directly proportional to enzyme concentration.[5,6] The results, listed in Table I, are in agreement with the data of Figure 1 and collectively imply but do not mandate an active site per subunit. It is noteworthy that k_{I_2} calculated from the initial phase of *o*-nitrophenol release is $2.0 \times 10^2\ M^{-1}\ \text{min}^{-1}$, a value *ca.* 50% less than that deduced in the above experiments at a lower I_2 concentration. This discrepancy provides minimal evidence that eq 1 be expanded to include an EI_2 complex formed prior to inactivation. The corresponding solution has a $K_{I_2}[I_2]$ term in both numerator and denominator so that the nonlinear dependency of the observed rate constant on I_2 concentration is described.

(5) Protein concentration determined by the method of Lowry (O. H. Lowry, *et al.*, *J. Biol. Chem.*, 193, 265 (1951).

(6) M. L. Bender and T. H. Marshall, *J. Amer. Chem. Soc.*, 90, 201 (1968).

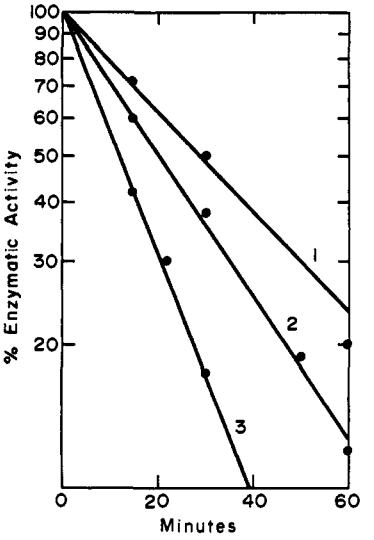

Figure 2. Protection of arylsulfate sulfohydrolase II against inactivation by *o*-nitrophenyl oxalate with *p*-nitrophenyl phosphate. *o*-Nitrophenyl oxalate, $3.3 \times 10^{-4}\ M$; *p*-nitrophenyl phosphate, plot 1, $2.0 \times 10^{-2}\ M$; 2, $9.5 \times 10^{-3}\ M$; 3, $5.1 \times 10^{-3}\ M$. All solutions are in 0.4 *M* acetate buffer, pH 4.8, 25°; enzyme specific activity $\cong$ 0.5 unit/mg.[1]

Table I. Effect of Enzyme Concentration on the Reaction with *o*-Nitrophenyl Oxalate[a]

[Enzyme] $\times 10^5\ M$	π/[enzyme]
2.6	1.8
1.1	1.8

[a] [Inhibitor] = $1.1 \times 10^{-3}\ M$; [enzyme], specific activity = 0.7 unit/mg; 0.2 *M* acetate, pH 4.8, 25°.

The discovery of this active-site titrant should expedite quantitative studies of the mechanism of action and active-site characterization of the arylsulfate sulfohydrolases.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM 13306. A comparison sample of *o*-nitrophenyl oxalate was the generous gift of Dr. T. C. Bruice.

(7) Career Development Awardee of the National Institutes of Health.

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Chemistry of α-Alkoxy Sulfoxides. Formation of Methylene Acetals from Dimethyl Sulfoxide and Alcohols

Sir:

Among the large number of reactions undergone by DMSO[1-3] is its thermal decomposition, alone or cat-

(1) T. Durst, *Advan. Org. Chem.*, 6, 285 (1969).

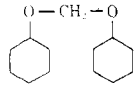
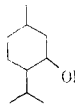
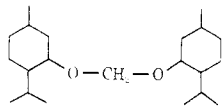
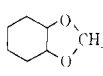
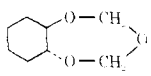
(2) H. Szmant in "Dimethyl Sulfoxide," S. W. Jacob, E. E. Rosenbaum, and D. C. Wood, Ed., Vol. 1, Marcel Dekker, New York, N. Y., 1971, p 1.

(3) J. C. Bloch, *Ann. Chim.*, 419 (1965).

alyzed by a strong acid to yield formaldehyde, in addition to other products. The few reports that deal with the preparative aspect of this reaction include the preparation of methylene acetals of ethylene glycol and propane-1,3-diol when these are heated in DMSO at reflux for 3 days.[4] Methylene bisamides[4,5] and dialkoxymethanes[6] are formed from amides and alcohols, respectively, in the presence of polyphosphoric acid and in DMSO at elevated temperatures.

We wish to report in this communication on the facile formation of methylene acetals from the reaction of alcohols and diols with DMSO and *N*-halosuccinimides. The reaction has both synthetic and mechanistic significance. In a typical example, cyclohexanol was added to a solution containing 1–2 molar equiv of *N*-bromosuccinimide (NBS) in dry DMSO. The solution was heated with stirring at 50° overnight, the reaction mixture was neutralized with aqueous sodium bicarbonate, and the product was extracted with diisopropyl ether and isolated in a pure state by distillation or chromatography over silica gel to give dicyclohexyloxymethane,[6] bp 75–76° (0.25 mm) in 86% yield.[7] The reaction can also be performed at room temperature although it is slower. The same results were obtained with *N*-chlorosuccinimide. Additional examples, including the preparation of cyclic methylene acetals, are given in Table I. Applications in the area of polyhydroxy compounds such as carbohydrates are possible and various acid-sensitive groups such as acetals, aglycons, and esters are unaffected; phenol and catechol, however, did not give the respective acetals under the conditions of the reaction.

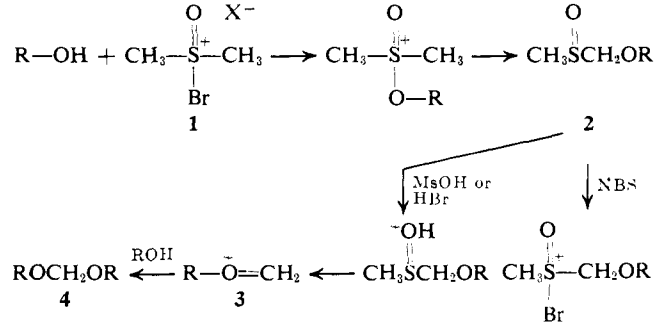
Table I. Products from the Reaction of Alcohols and Diols with DMSO and NBS (2 equiv) at 50°

Reactant	Product	Yield, %	Ref
CH_3OH	$CH_3OCH_2OCH_3$	Quant	*a*
		86	6
		77	6
		62	*b, c*
		70	*b–d*

[a] A product of Aldrich Chemical Co., Inc. [b] F. S. Head, *J. Chem. Soc.*, 1778 (1960). [c] J. S. Brimacombe, A. B. Foster, B. D. Jones, and J. J. Willard, *J. Chem. Soc. C*, 2404 (1967). [d] P. V. Bonsignore and M. D. Hurwitz, *J. Org. Chem.*, **28**, 3535 (1963).

Although the formation of methylene acetals under the mild conditions of the reaction can be attributed to a sustained generation of formaldehyde from a reactive intermediate,[8] alternate or concurrent pathways are also possible. Experiments were designed to prepare some possible reaction intermediates and to simulate the actual reaction conditions. That both hydrogen atoms in the methylene group were originating from DMSO was firmly established by using DMSO-d_6 as the solvent. The corresponding acetals were shown to have a CD_2 unit.[9] The possibility of a Pummerer-type rearrangement of an initially formed bromosulfoxonium ion[10] **1** leading to an α-alkoxy sulfoxide intermediate **2**, followed by an acid or NBS-catalyzed formation of an oxonium intermediate **3**, seemed attractive (Scheme I). In a model experiment, cyclohexyloxymethyl thiomethyl ether (**5**) (bp 117–119° (30 mm)) was prepared from the sodium salt of cyclohexanol and chloromethyl methyl sulfide. The product was oxidized with sodium metaperiodate in aqueous acetone to give the sulfoxide derivative **2** (R = cyclohexyl). Treatment of **2** with NBS in cyclohexanol gave dicyclohexyloxymethane (**4**, R = cyclohexyl) as the sole product. The versatility and reactivity of the α-alkoxy sulfoxide **2** was interestingly shown by its transformation into the acetal **4** in the presence of 1 equiv of methanesulfonic acid in DMSO (room temperature, 18 hr). Furthermore, the same acetal **4** was the sole product when the cyclohexyloxymethyl thiomethyl ether was treated with periodic acid in anhydrous ether (−10°, 1 hr; room temperature, 1 hr).[11] Finally, it was shown that, as expected, mixed acetals could be obtained from two different alcohols. Treatment of cyclohexanol and methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside with DMSO and NBS (2 equiv) at 50° gave the three possible acetals in the ratio of 2:1:1.[12] When a mixture of the thiomethyl ether derivative and the above-mentioned sugar derivative was treated with periodic acid in ether, the major products were the acetal **4** and the mixed acetal **6** (*m/e* 301, $M^{\cdot+}$ − 15), with little if any of the symmetrical acetal corresponding to the sugar derivative (Scheme II). Since there was no scrambling of

Scheme I



(4) V. J. Traynelis and W. L. Hergenrother, *J. Org. Chem.*, **29**, 221 (1964).

(5) A. Sekera and P. Rumpf, *C. R. Acad. Sci.*, 2252 (1965).

(6) T. Sato, Y. Saito, M. Kainosho, and K. Hata, *Bull. Chem. Soc. Jap.*, 40, 391 (1967).

(7) The products reported herein were characterized by comparison with known samples wherever possible. Their purity was checked by vapor phase chromatography (Carbowax 20M, 15%) and they gave the expected spectral (nmr, mass) characteristics. Mass spectra were obtained on an MS-902 high-resolution mass spectrometer.

(8) D. Martin, A. Berger, and R. Peschel, *J. Prakt. Chem.*, **312**, 683 (1970).

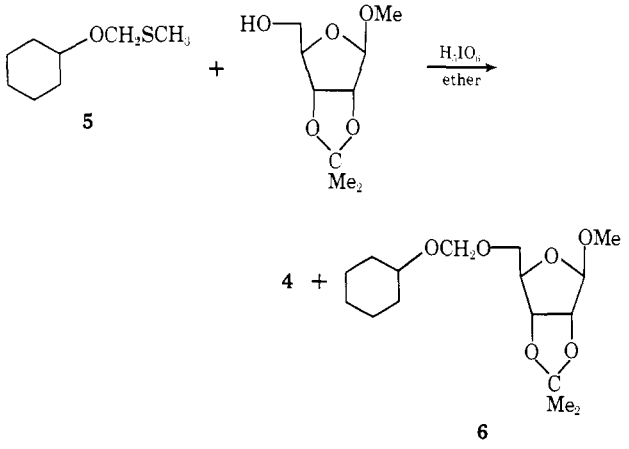
(9) For example, *cis*-cyclohexane-1,2-diol gave the corresponding dideuteriomethylene acetal ($M^{\cdot+}$ 130, *m/e* 129 (M − 1), 100 (M − CD_2O), etc.) and the trans diol gave the tetradeuteriotrioxepan derivative ($M^{\cdot+}$ 162; *m/e* 130 (M − CD_2O), 98 (M − $2CD_2O$), etc.).

(10) For a related formation of chlorosulfoxonium ions, see: T. Durst and K-C. Tin, *Can. J. Chem.*, **49**, 2374 (1971).

(11) The formation of the acetal **4** in these experiments can be rationalized in terms of an acid-catalyzed disproportionation of the sulfoxide **2**, or a related mechanism. These possibilities are presently under study.

(12) In a model experiment, cyclohexanol was treated with DMSO containing 1 equiv of anhydrous hydrogen bromide in dichloromethane. There was no formation of acetal.

Scheme II



products in this experiment,[13] one can presume that in those cases where the sulfoxide **2** is one of the intermediates in the reaction of an alcohol with DMSO and NBS, it could also be the precursor of the acetal.[14] If the highly reactive oxonium compounds such as **3** are indeed formed during the acid-catalyzed decomposition of α-alkoxy sulfoxides, then a wide variety of synthetic transformations are possible with this little explored class of sulfoxides. Efforts in this direction are in progress in this laboratory.

Acknowledgment. The authors thank the National Research Council of Canada for financial support and Professor C. R. Johnson for helpful discussions.

(13) Based on the nature of the products, the formation of formaldehyde in significant amounts can be ruled out in these reactions.

(14) The formation of cyclic methylene acetals from *cis*-diols can also be explained by invoking α-alkoxy sulfoxide and oxonium intermediates. The situation is somewhat more complicated in the case of the trioxepan derivative arising from *trans*-cyclohexane-1,2-diol, and a definitive mechanism must await further studies.

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Protonated Cyclopropanes. VII. Evidence for Edge Protonation

Sir:

According to the steady-state treatment of Collins,[1] in reactions with 1-propyl-*1*-^{14}C systems, in which secondary isotope effects should be negligible, the 1-propyl product *can* show more isotopic scrambling to C-3 than C-2 if the mechanism were to involve equilibrating edge-protonated cyclopropane intermediates. On the other hand, processes involving corner-protonated species *can never* give more scrambling to C-3 than C-2. Most of our previous studies on such systems gave 1-propyl products showing about equal amounts of rearrangement of the ^{14}C label from C-1 to C-2 and to C-3.[2] In the treatment of 1-chloropropane-*1*-^{14}C with $AlCl_3$,[3] more scrambling to C-3 than C-2 was observed in the recovered 1-chloropropane, but in this case, reversible isomerization between 1- and 2-chloropropanes rendered these results inapplicable to the conclusions of Collins. We now report that the trifluoroacetolysis of 1-propyl-*1*-^{14}C-mercuric perchlorate (**1**-$HgClO_4$-*1*-^{14}C) indeed gave a 1-propyl product with more scrambling to C-3 than C-2.

A solution of **1**-$HgClO_4$-*1*-^{14}C[2c] (about 0.5 *M*) in F_3CCOOH was heated under reflux for 8 hr. The products, analyzed by vpc, consisted of 7 and 93%, respectively, of the 1- and 2-propyl trifluoroacetates (**1**-$OAcF_3$-^{14}C and **2**-$OAcF_3$-^{14}C) (total yield was 92–95% as measured by isotope dilution). Degradation[2c] of the vpc purified **1**-OH-^{14}C derived from the **1**-$OAcF_3$-^{14}C gave the results summarized in Table I.

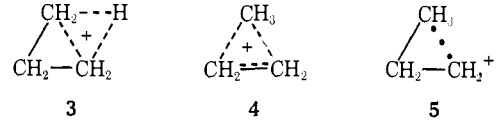
Table I. Isotopic Scrambling Data from Degradation of 1-Propanol-^{14}C Derived from 1-Propyl-^{14}C Trifluoroacetate

	Specific activity, cpm/mmol			^{14}C distribution, %		
Expt	CH_3CH_2-CH_2OH[a]	CH_3-$COOH$[b]	CH_3NH_2[c]	C-1	C-2	C-3
1	97,400	24,500	14,500	74.8	10.3	14.9
2	83,100	21,000	12,300	74.7	10.5	14.8
3	108,000	28,200	15,500	73.9	11.7	14.4
4	107,000	26,100	15,600	75.6	9.8	14.6

[a] Assayed as the α-naphthylurethane. [b] Assayed as the *p*-toluidide. [c] Assayed as the *p*-toluenesulfonamide.

The data in Table I were obtained from two sets of duplicate experiments carried out by different workers at different times. Hence the finding of more scrambling to C-3 than C-2 has in effect been independently verified. Under the conditions of the experiments, 2-$OAcF_3$ did not give any 1-$OAcF_3$ according to nmr analysis, thus also eliminating any complication from reversible 1,2-hydride shifts. These results, therefore, constitute evidence in support of equilibrating edge-protonated cyclopropane intermediates as the mechanism for the isotopic scrambling in the **1**-$OAcF_3$-^{14}C obtained from the trifluoroacetolysis of **1**-$HgClO_4$-*1*-^{14}C.

Recently, however, Radom, *et al.*,[4] have carried out *ab initio* calculations with optimization of geometry on the $C_3H_7^+$ ions and have obtained results indicating that edge-protonated cyclopropane **3** is less stable than the corner-protonated species **4** by about 10 kcal/mol. It was also concluded that besides the 2-propyl cation, the only stable intermediate may be the methyl-eclipsed 1-propyl cation or distorted corner-protonated cyclopropane **5**, with **3** and **4** as transition states for the 1,3-



hydride and 1,2-methyl shifts, respectively. These calculations predict that there should be more isotopic scrambling from C-1 to C-2 than to C-3 for reactions *via* protonated cyclopropanes from C-1 labeled 1-propyl substrates. It was suggested[4] that discrepancies between this prediction and data obtained from reactions in solution might be the result of a solvent assistance for the 1,3-hydride shift, or a specific hydrogen bonding of the edge proton in transition state **3** for the 1,3 shift.

(1) C. J. Collins, *Chem. Rev.*, **69**, 543 (1969).

(2) (a) C. C. Lee and K. M. Wan, *J. Amer. Chem. Soc.*, **91**, 6416 (1969); (b) C. C. Lee and W. K. Y. Chwang, *Can. J. Chem.*, **48**, 1025 (1970); (c) C. C. Lee and J. Law, *ibid.*, **49**, 2746 (1971).

(3) C. C. Lee and D. J. Woodcock, *J. Amer. Chem. Soc.*, **92**, 5992 (1970).

(4) L. Radom, J. A. Pople, V. Buss, and P. v. R. Schleyer, *ibid.*, **94**, 311 (1972).