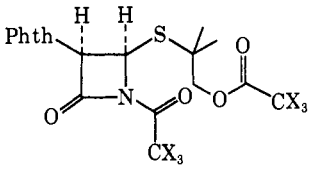
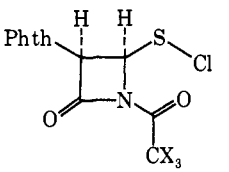
3.71 ppm downfield from external trifluoroacetic acid. While rearrangements are possible in the thioethyl side chain, the chemical shift of the methylene protons in **3a** (an AB system centered at δ 4.3 (J = 11 Hz)) indicates an unrearranged product. The *gem*-dimethyl groups appear at δ 1.45 and 1.35.

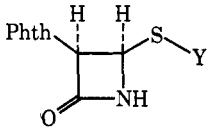
In contrast to **1**, treatment of **3a** with 2 equiv of



3a, X = F
b, X = H



4a, X = F
b, X = H

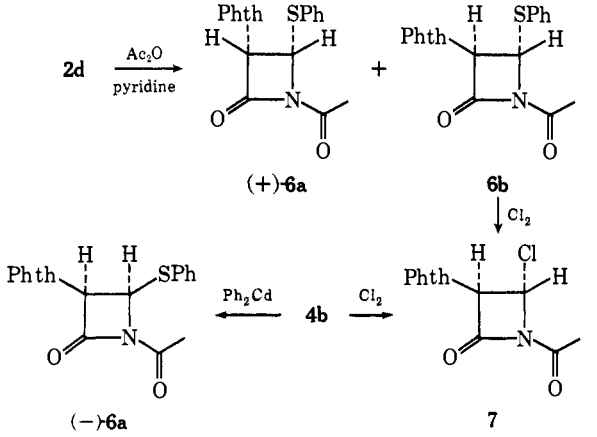


5a, Y = SPh
b, Y = 2-chlorocyclohexyl

chlorine in CH_2Cl_2 solution at room temperature produced a moderately stable sulfenyl chloride, **4a**. Apparently the *N*-acyl group inhibits participation of the nitrogen lone-pair electrons in cleavage of the azetidine C–S bond and makes cleavage of the tertiary C–S bond the favored pathway. The sulfenyl chloride was not obtained completely free of other cleavage products due to difficulty in crystallization and instability during chromatography. The ir spectrum of the crude product shows a high-frequency β-lactam carbonyl, indicative of the imidic system.[6] The ring protons appear as a singlet at δ 6.1 in $CDCl_3$ but become an AB system centered at δ 5.25 in C_6D_6. The yellow oil liberated iodine from sodium iodide in acetic acid and reacted rapidly at room temperature with thiophenol or cyclohexene, reactions typical of a sulfenyl chloride.[7] When the products of the latter two reactions were chromatographed on Florisil, the aliphatic cleavage products eluted rapidly with CH_2Cl_2. The products **5a** and **5b**, respectively, came off the column with CH_2Cl_2–ethyl acetate, 9:1. Apparently, the Florisil catalyzes the removal of the highly labile trifluoroacetyl group by traces of moisture or ethanol. The disulfide **5a**, mp 104–107°, $[\alpha]D$ −76°, was obtained in 75% yield and shows a normal β-lactam carbonyl absorption and an AB system characteristic of a cis-3,4 disubstituted ring. Similarly, **5b**, mp 164–166°, was obtained in 85% yield. The absorption of the C_4 proton in the nmr spectrum of **5b** appears as two overlapping signals (each a doublet of doublets) indicating that the compound is a mixture of the two possible isomers with chlorine cis or trans to sulfur on the cyclohexane ring. An excess of chlorine converts **5a** to **2a** in 70% yield.

A similar series of reactions has been performed utilizing the diacetyl analog **3b**, prepared by treating the alcohol with acetic anhydride–pyridine at 50° after the method of Heusler.[6] This compound was obtained as an oil, $[\alpha]D$ −141°, which shows an AB system centered at δ 4.0 (J = 11 Hz) and four three-proton singlets at δ 2.5, 1.9, 1.6, and 1.2 as well as the absorptions listed in Table I. Two equivalents of chlorine reacted with **3b** to give **4b**, an oil which resisted purification and which also shows an anomalous two-proton singlet for the azetidine ring protons, both in $CDCl_3$ and C_6D_6. Reactions of **4b** with thiophenol and cyclohexene appear to parallel those of **4a**, except that the *N*-acetyl group remains intact during chromatography. The products are oils which have not been obtained analytically pure.

In an attempt to establish more definitely the structure of **4b**, treatment with diphenylcadmium in tetra-



(−)-**6a** 7

hydrofuran gave **6a**, mp 149.5–152°, $[\alpha]D$ −199°, in low yield (*ca.* 3%). The enantiomer of **6a**, mp 150–153°, $[\alpha]D$ 174°, was formed by acetylation of **2d**, followed by chromatographic separation of the two isomers. Apparently, the major portion of the cis isomer from this reaction arises from epimerization at C_3 with a small amount due to the fact that **2d** contains some cis isomer. Compound **6b** can be observed to epimerize in pyridine at 50°, but epimerization was not observed for **2d** or **3b**. The fact that both reaction pathways lead to optically active products rules out any intermediate (such as a 3,4-dehydroazetidin-2-one) in which both asymmetric centers are destroyed. Compound **4b** reacts with excess chlorine to give **7**, mp 167–168°, also obtainable by chlorinolysis of **6b**.

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Solvent Isotope Effects and the Mechanism of Chymotrypsin Action[1]

Sir:

A recent communication[2] reported that the solvent isotope effect on the deacetylation of acetyl-α-chymo-

(1) This research was supported by the National Science Foundation through Grant No. GP-36004X.
(2) E. Pollock, J. L. Hogg, and R. L. Schowen, *J. Amer. Chem. Soc.*, **95**, 968 (1973).

trypsin in H_2O–D_2O mixtures is a linear function of solvent deuterium content. It inferred from this that only one proton is being transferred in the rate-determining step of this reaction, and then concluded that the charge-relay mechanism,[3] in which two protons are presumably moving at the same time, is thereby ruled out. Unfortunately, this conclusion is unjustified, for a linear relationship between isotope effect and solvent deuterium content does not necessarily require a one-proton transfer mechanism.

Solvent isotope effects in H_2O–D_2O mixtures, k_n/k_H, may be related to the atom fraction of deuterium in the solvent, n, to an approximation sufficient for the present purposes, by the general expression of eq 1.[4] In this

$$\frac{k}{k_H} = \frac{\Pi_i(1 - n + n\phi_i^{\pm})}{\Pi_j(1 - n + n\phi_j)} \quad (1)$$

relation, ϕ represents a fractionation factor, the D/H ratio at a particular exchangeable hydrogenic site relative to the D/H ratio of the solvent, with $\phi^{\pm}$ referring to transition-state sites and ϕ to initial-state sites. The numerator of this expression includes terms for all i transition-state sites and the denominator likewise covers all j initial-state sites; however, only those sites which undergo a change in fractionation factor between initial state and transition state will contribute to the isotope effect, and all others may be excluded.

For a simple system with only one hydrogen undergoing a change in fractionation factor from an initial-state value of unity, the denominator of this expression will be one and the isotope effect, a linear function of solvent deuterium content: $k_n/k_H = 1 - n + n\phi^{\pm} = 1 + (\phi^{\pm} - 1)n$. If in this case the limiting, *i.e.*, pure D_2O, isotope effect is fairly strong and therefore primary, $\phi^{\pm}$ will have a value considerably less than unity and will refer to a proton in transit. Addition of another transferring proton destroys the linear relationship, for now $k_n/k_H = (1 - n + n\phi_1^{\pm})(1 - n + n\phi_2^{\pm}) = 1 + (\phi_1^{\pm} + \phi_2^{\pm} - 2)n + (\phi_1^{\pm} - 1)(\phi_2^{\pm} - 1)n^2$, which is a quadratic expression in n. Still more transferring protons introduce further curvature. It does not follow, however, that the relationship will be effectively linear *only* when one proton is being transferred, for introduction of still further terms with appropriate fractionation factors can easily straighten out the curvature produced by several protons in transit.

This is illustrated by the calculations for some two-proton transfer systems summarized in Table I. In each of these models the limiting isotope effect is set equal to the value observed for the chymotrypsin reaction, $k_{D_2O}/k_{H_2O} = 1/2.4$,[2] and the fractionation factors for the two protons in transit are arbitrarily taken to be equal. It may be seen that the introduction of ten initial-state sites with $\phi = 0.96$ (model A) gives a relationship between isotope effect and solvent deuterium content which is very nearly linear; the same is true for introduction of ten initial-state sites with $\phi = 0.90$ which change to transition-state sites with $\phi^{\pm} = 0.94$ (model B), or five initial-state sites with $\phi = 0.90$ which change to sites with $\phi^{\pm} = 0.97$ (model C), or one initial-state site with $\phi = 0.75$ and five with $\phi = 0.96$ (model D), or one initial state site with $\phi = 0.75$ and five with $\phi = 0.95$ three of which change to $\phi^{\pm} = 0.95$ (model E). Many more linear models can be constructed. For example, in the systems with ten sites changing from $\phi = 0.90$ to $\phi^{\pm} = 0.94$ (model B), it is only the difference between ϕ and $\phi^{\pm}$ and not their absolute values which is important in maintaining linearity; similar results are obtained with $\phi = 0.92$, $\phi^{\pm} = 0.96$; $\phi = 0.94$, $\phi^{\pm} = 0.98$; $\phi = 0.96$, $\phi^{\pm} = 1.00$ (model A); $\phi = 0.98$, $\phi^{\pm} = 1.02$; $\phi = 1.00$, $\phi^{\pm} = 1.04$; $\phi = 0.76$, $\phi^{\pm} = 0.80$; etc. The same is true of the five-site systems, and indeed linear models can be built with any number of sites. The fractionation factors for the two protons in transit, moreover, need not be equal.[5] It would seem, therefore, that a limitless number of linear, multiple-proton transfer models duplicating the chymotrypsin data can be constructed.

Table I. Solvent Isotope Effects for Some Two-Proton Transfer Systems

Model	k_n/k_H, n = 0.0	0.2	0.4	0.6	0.8	1.0
Linear	1.000	0.883	0.767	0.650	0.533	0.417
A	1.000	0.888	0.772	0.653	0.534	0.417
B	1.000	0.886	0.770	0.652	0.533	0.417
C	1.000	0.883	0.765	0.647	0.530	0.417
D	1.000	0.889	0.775	0.656	0.536	0.417
E	1.000	0.886	0.769	0.651	0.533	0.417

A. $(1 - n + n\phi^{\pm})^2/(1 - n + n\phi)^{10}$; $\phi^{\pm} = 0.526, \phi = 0.960$
B. $(1 - n + n\phi_1^{\pm})^2(1 - n + n\phi_2^{\pm})^{10}/(1 - n + n\phi)^{10}$; $\phi_1^{\pm} = 0.519, \phi_2^{\pm} = 0.940, \phi = 0.900$
C. $(1 - n + n\phi_1^{\pm})^2(1 - n + n\phi_2^{\pm})^5/(1 - n + n\phi)^5$; $\phi_1^{\pm} = 0.527, \phi_2^{\pm} = 0.970, \phi = 0.900$
D. $(1 - n + n\phi^{\pm})^2/(1 - n + n\phi_1)(1 - n + n\phi_2)^5$; $\phi^{\pm} = 0.505, \phi_1 = 0.750, \phi_2 = 0.960$
E. $(1 - n + n\phi_1^{\pm})^2(1 - n + n\phi_2^{\pm})^3/(1 - n + n\phi_1)(1 - n + n\phi_2)^5$; $\phi_1^{\pm} = 0.517, \phi_2^{\pm} = 0.950, \phi_1 = 0.750, \phi_2 = 0.940$

All of these models require the system to be complex enough to have a number of exchangeable hydrogens in addition to the ones undergoing transfer. The chymotrypsin reaction meets this requirement easily, for the enzyme itself consists of nearly 250 amino acid residues with some 400 exchangeable sites, and many more exist in the associated solvating water molecules. It is not unlikely, moreover, that the conformational and other changes which occur during the enzymatic reaction will alter the fractionation factors at some of these sites, thus generating the secondary isotope effects upon which these multiple-proton transfer models depend for their linearity.

Such secondary isotope effects might, of course, operate to increase rather than to reduce the curvature of a two-proton transfer model; there is unfortunately no way, on the basis of presently available information, to estimate their magnitude and direction and thus to make a realistic prediction. The fact remains, however, that linear behavior does not exclude a multiple-proton transfer model, and this severely limits the utility of solvent isotope effects in H_2O–D_2O mixtures as a means of deducing the number of protons being transferred in the rate-determining step of the deacetylation of acetyl-α-chymotrypsin. Indeed, the fact that secondary isotope effects in the solvent should be taken

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(4) A. J. Kresge, *Pure Appl. Chem.*, 8, 243 (1964).
(5) They will, in fact, be equal only by unlikely coincidence. It is possible, moreover, that one of these fractionation factors would differ little from that for the corresponding initial-state site, in which case the two-proton transfer model would give behavior similar to that of a one-proton transfer model. This might happen, for example, if transfer of one proton lagged substantially behind transfer of the other.

into account in any case could well obscure the mechanistic interpretation of similar data for even fairly simple nonenzymatic reactions.

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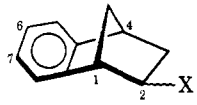
Bridgehead Oxygen Substitution as a Potent Probing Tool for Evaluating Exo/Endo Rate Ratios and σ–π Participation. Acetolysis of the Epimeric Benzo-7-oxabicyclo[2.2.1]hepten-2-yl Brosylates[1]

Sir:

Solvolysis reactions of benzobicyclo[2.2.1]heptene derivatives, rich as they are in important mechanistic information, remain as continuing sources of controversy. In this connection, the acetolysis of the epimeric benzo-7-oxabicyclo[2.2.1]hepten-2-yl brosylates (**4**) has now been examined and the observed exo/endo rate ratio at 25° (5700) has been shown to be of the same order of magnitude as that derived for the parent system (15,000).[2] Since the O-7 atom provides a sensitive probe for the delocalization of charge to the 1 position, it is concluded that the rate-determining ionization of *exo*-**4** cannot proceed through σ-delocalized cation **7**, corresponding to the related ion **3** proposed for the parent system.[3,4]

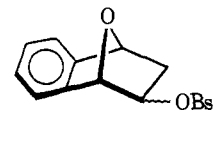


1

2

3

The benzo-7-oxabicyclo[2.2.1]hepten-2-yl system (**4**) offers a unique opportunity to evaluate the importance and nature (σ or π) of carbon participation (if any) in a *secondary* derivative free of added steric encumbrances. Anchimeric assistance to ionization in *endo*-**4** by direct oxygen involvement is not anticipated by analogy to the behavior of *endo*-**5**.[5] However, if significant buildup of positive charge on C_1 were to operate during rate-determining ionization of *exo*-**4**, an appreciable increase in the exo/endo rate ratio might be expected based upon **6** as an extraannular model (–OBs, 9800;[6a] –OTs, 6200[6b]), providing that here, as in other strained heterobicyclics, Bredt's rule considerations do not apply.[7]



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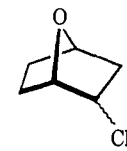

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6

On the basis of a high exo/endo rate ratio (15,000 at 25°) and clean retention of stereochemistry during acetolysis of *exo*-**1**-OBs, Bartlett and Giddings originally proposed intervention of the unsymmetrical π-delocalized homobenzylic cation **2**.[2] The subsequent finding that acetolysis of optically active *exo*-**1**-OBs gives racemic *exo*-**1**-OAc led to acceptance of the symmetrical σ-delocalized intermediate **3** as the species most compatible with the data.[3] 6-Methoxyl substitution of *exo*-**1**-OBs eventuates in a 178-fold rate acceleration at 77.6° (exo/endo = 310,000[8]), whereas the 7-CH_3O derivative ionizes somewhat more slowly ($k_{rel}^{77.6°}$ = 0.72, exo/endo = 2800[8]) but still with full retention of stereochemistry.[9] In contrast, the *exo*-6,7-$(NO_2)_2$ derivative is strongly deactivated ($k_{rel}^{77.6°} = 1.1 \times 10^{-5}$) and reacts to a significant extent by SN2 displacement at C_2 (exo/endo = 3.7).[9d,e]

Although Winstein[9a] and Tanida[9b,d,e] have cited such evidence as supportive of intermediate **3**, Brown has advanced an alternative steric explanation[9c,10] in which the space-filling requirements of the aromatic ring presumably effect destabilization of the endo transition state by impeding departure of the leaving group. Support for the steric argument was gained by examining the exo/endo rate ratios of tertiary systems such as the 2-methyl- (65),[11a] 2-phenyl- (4300),[11a] and 2-*p*-anisyl-2-OPNB's (3000).[11b] If it is granted that participation is not operative in the last derivative, then the exo/endo ratio for **1** could result from a factor of up to 20 attributable to participation and 3000 due to steric effects.

Reaction of benzo-7-oxabicyclo[2.2.1]heptadiene[12] with *m*-chloroperbenzoic acid in buffered (Na_2CO_3) methylene chloride proceeded stereoselectively to afford only exo epoxide (70–77%), which proved unusually resistant to lithium aluminum hydride reduction (large excess of $LiAlH_4$, refluxing THF, 5 days, 81%). The resulting alcohol was converted directly to *exo*-**4** and also oxidized to the corresponding ketone with dipyridinechromium(VI) oxide[13] in dichloromethane (65%). Hydride reduction and reaction with *p*-bromobenzenesulfonyl chloride in pyridine completed the sequence to *endo*-**4**.[14]

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(2) P. D. Bartlett and W. P. Giddings, *ibid.*, 82, 1240 (1960). The original k_{exo}/k_{endo} value has been corrected from 7500 to 15,000: H. Tanida, K. Tori, and K. Kitahonoki, *ibid.*, **89**, 3212 (1967), ref 27.

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(11) (a) H. C. Brown and G. L. Tritle, *J. Amer. Chem. Soc.*, 88, 1320 (1966); (b) H. C. Brown, personal communication.

(12) L. F. Fieser, private communication; G. Wittig and L. Pohmer, *Chem. Ber.*, **89**, 1334 (1956); E. Wolthuis, *J. Org. Chem.*, **26**, 2215 (1961); R. Gompper, G. Seybold, and B. Schmolke, *Angew. Chem., Int. Ed. Engl.*, 7, 389 (1965); G. R. Ziegler, *J. Amer. Chem. Soc.*, **91**, 446 (1969).

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(14) Analytical and spectral data for all new compounds were fully compatible with the given assignments.