and 2-isopropylcyclopentadienes were isolated as the only products of the reaction. Similarly, **5b**[13] gave 1- and 2-isobutyl- and 1- and 2-*t*-butylcyclopentadienes as the major products. Since the rigid spiro[2.4]hepta-4,6-diene structure provides no conformational advantage to either pathway a or b, the moderate preference for the latter ($k_b/k_a = 4.8 \pm 0.3$ for **5a** and 10.1 ± 0.4 for **5b**) indicates that only a small amount of excess negative charge accumulates on the methyl-substituted carbon in the activated complex for pathway b relative to the same carbon in pathway a.[14,15] This is consistent with a description of the activated complex in which the transition state occurs early on the reaction coordinate and the negative charge is largely localized on the incipient cyclopentadienyl ring.[16]

Both radical-anion[2e,5,14,17,18] and dianion[2b] mechanisms have been suggested for reductive cleavage reactions of this type, but there is very little evidence in support of either. Although we cannot distinguish between these mechanisms (or a combination thereof)[19] on the basis of the present data, it should be noted that the above conclusions are independent of this point.

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(15) The cleavage of **5b** also produced *ca.* 1% of 1- and 2-methylallylcyclopentadiene, possibly *via* a radical-anion cleavage. These products were taken into account in the calculation of k_b/k_a.
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Modification of α-Chymotrypsin by Methyl *p*-Nitrobenzenesulfonate[1]

Sir:

We wish to report a new active-site modifying reagent, methyl *p*-nitrobenzenesulfonate, which methylates specifically histidine-57 of α-chymotrypsin. However, this methyl ester does not modify trypsin or subtilisin.

Previously L-1-tosylamido-2-phenylethyl chloromethyl ketone,[2] phenoxymethyl chloromethyl ketone,[3] and 2-phenyl-1,4-dibromoacetoin[4] have been reported as modifying reagents for this histidine of α-chymotrypsin. These compounds are structurally similar to the specific substrate for α-chymotrypsin; however, methyl *p*-nitrobenzenesulfonate has a structure analogous to nonspecific substrates for α-chymotrypsin. Therefore methyl *p*-nitrobenzenesulfonate is a member of a new class of active-site-specific reagents.

The methyl ester was prepared by the method of Morgan and Cretcher.[5] The product was identified by using ir, nmr, and elemental analyses.[6] *Anal.* Calcd for $C_7H_7O_5NS$: C, 38.7; H, 3.2; N, 6.4; S, 14.7. Found: C, 39.3; H, 3.4; N, 6.6; S, 14.4. A radioactive methyl ester was obtained by using methanol-^{14}C (2 mCi/mmol) in the preparation.

When this methyl ester was incubated with purified α-chymotrypsin in 0.1 *M* sodium phosphate buffer, pH 7.93, the enzyme activity decreased with incubation time. For this experiment, the activity of the enzyme was determined by titration with N-*trans*-cinnamoylimidazole[7a] and by rate assay with N-acetyl-L-tryptophan methyl ester.[7b] The results are shown in Figure 1. Inhibition of the enzymatic activity by the methyl ester was prevented by adding a competitive inhibitor, β-phenylpropionic acid. The pH profile of inhibition was a bell-shaped curve, and p*K* values were obtained by a graphical method.[8] The values are as follows: $pK_1 = 6.73$ and $pK_2 = 9.14$. These results indicate that methylation occurred at an active site of α-chymotrypsin.

The uv absorption spectra of native and modified α-chymotrypsin were almost identical (λ_{max} 281 mμ). There was no spectral shift after the modified enzyme had been denatured by 8 *M* urea.[9] This indicates that the *p*-nitrobenzenesulfonyl group is not involved in the modification.

By incubating the enzyme with methyl-^{14}C *p*-nitrobenzenesulfonate (sp act. 6.6×10^7 cpm/mol) in a sodium phosphate buffer solution, pH 7.9, methyl-^{14}C-α-chymotrypsin was prepared. The radioactivity was found almost stoichiometrically in the modified enzyme. In addition, this radioactivity was distributed principally in the B chain of α-chymotrypsin after the B and C chains had been separated by column chromatography.[10] A diagonal paper electropherogram[11] indicated that histidine-57 was modified, and amino acid analyses revealed that 1-amino-2-(1-methyl-4-imidazolyl)propanoic acid (3-methylhistidine) was formed with a loss of a corresponding amount of histidine in the modified enzyme.

As a model system, methyl *p*-nitrobenzenesulfonate was incubated with individual proposed functional amino groups of α-chymotrypsin to see the effects of these amino acid residues on the methyl ester in solution. To determine the effect of these amino acids, the disappearance of methyl *p*-nitrobenzenesulfonate was observed at 253 mμ at 25°. The results are shown in Table I. The spon-

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Table I. Effect of Functional Amino Groups on the Spontaneous Hydrolysis of Methyl *p*-Nitrobenzenesulfonate[a]

Methyl *p*-nitrobenzenesulfonate, *M*	Amino acid (concn, *M*)	$k_{exptl} \times 10^4$ sec^{-1}
4.17×10^{-5}	None	1.77
5.16×10^{-5}	Isoleucinamide (8.01×10^{-4})	1.61
4.14×10^{-5}	N-Acetyl-L-methioninamide (5.44×10^{-4})	1.73
4.14×10^{-5}	N-Acetyl-L-serinamide (1.74×10^{-3})	1.67
4.14×10^{-5}	Imidazole (5.00×10^{-2})	2.88

[a] The buffer used was 0.1 *M* sodium phosphate, pH 7.94. Methyl *p*-nitrobenzenesulfonate was dissolved in CH_3CN. The amino acid derivatives were in aqueous solutions. The decomposition of the ester was measured at 253 mμ at 25°.

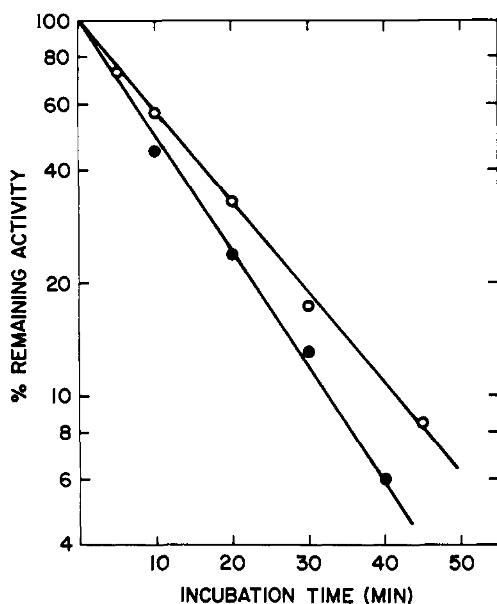

Figure 1. Loss of α-chymotrypsin activity upon incubation with methyl *p*-nitrobenzenesulfonate. Purified α-chymotrypsin in 0.1 *M* sodium phosphate buffer, pH 7.93 (3.65×10^{-4} *M*), was incubated with methyl *p*-nitrobenzenesulfonate (2.86×10^{-3} *M*) at 25°. The concentration of CH_3CN was 9.9%. The remaining activity was measured by N-*trans*-cinnamoylimidazole titration (full circles) and by a rate assay method with N-acetyl-L-tryptophan methyl ester (2.48×10^{-4} *M*, open circles).

taneous hydrolysis rate of the methyl ester with imidazole at pH 7.94 was 2.2×10^{-3} M^{-1} sec^{-1}. The highest inhibition rate of α-chymotrypsin at the same pH was 41.5×10^{-2} M^{-1} sec^{-1}. Thus the inhibition rate of α-chymotrypsin is at least 190-fold faster than the spontaneous hydrolysis of the ester with imidazole. When histidine was incubated with the methyl ester at pH 7.9 for 48 hr, most of the histidine was unattacked, and a small amount of 1-amino-2-(1-methyl-5-imidazolyl)propanoic acid (1-methylhistidine) and a trace amount of 1-amino-2-(1-methyl-4-imidazolyl)propanoic acid were detected by an amino acid analysis.

The results lead us to conclude that the methyl ester in order to inhibit the enzymatic activity must be oriented in a position similar to that of a substrate on the surface of the enzyme. From the experimental data the enzyme truly catalyzed the reaction of this ester at N-3 of histidine-57. Histidine-57 is known to be present in the active site of α-chymotrypsin from X-ray crystallographic data.[12] It thus appears from the loss of activity found in the rate assay (Figure 1) that the hydrogen atom at position 3 of this histidine is essential to the mechanism of hydrolysis of substrates by the enzyme.

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The Structure of an α-Sulfonyl Carbanion

Sir:

Conformationally unconstrained α-sulfonyl carbanions are virtually unique among the reactive intermediates of organic chemistry in their ability to retain asymmetry under a wide variety of experimental conditions.[1] This fact, discovered independently by three groups of workers about 7 years ago,[2-4] has since stimulated much interest in the structures and stereochemical capabilities[5] of these species. The structural discussion has, from the beginning, followed two main lines: (1) the carbanion carbon is *pyramidal*, *i.e.*, **1**, **2**, or **3**, and there is a high barrier to inversion of the *stable* structure; (2) the carbanion carbon is *planar*, and there is a high barrier to rotation. If the latter were correct, then the observed asymmetry would require that the structure be **4** rather than **5** and, further, that proton transfer to **4** (or its microscopic reverse) must occur largely from one face of

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