partial structure A. In this unit the olefinic proton is found at τ 3.45 and the methyl groups at τ 7.85 and 8.06. A decision in favor of placing the acetyl group at the terminal oxygen of the unit A is reached from consideration of the mass spectrum of streptovarone, which shows the fragmentation sequence indicated. The structure of streptovarone is then assigned as IV.

The nmr spectrum of prestreptovarone, mp 194–197° [$C_{29}H_{29}NO_9$. *Anal.* Found: C, 65.07; H, 5.55; N, 2.65; mol wt, 535 (mass spectroscopy)], indicates that it has structure V and, in particular, that it differs from streptovarone in having the amide side chain shown in V in place of the pyruvamide group.

Future reports in this series will deal with the side chains and structures of the individual streptovaricin components, as well as their obvious relationship to rifamycin S.[9]

Acknowledgment. This investigation was supported in part by Public Health Service Research Grants No. AI-01278 and AI-04769 from the National Institute of Allergy and Infectious Diseases. We also thank The Upjohn Co. for generous samples of streptovaricin.

(12) Holder of University of Illinois and E. I. du Pont Teaching Fellowships and a National Science Foundation Summer Fellowship.

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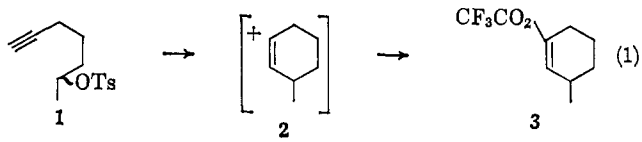
Triple Bond Participation and a Bent Vinyl Cation in the Trifluoroacetolysis of 6-Heptyn-2-yl *p*-Toluenesulfonate[1]

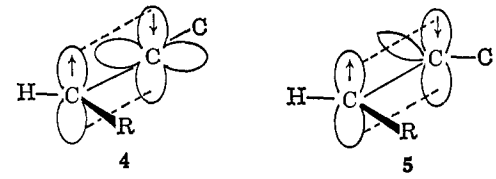
Sir:

We wish to report that the solvolysis of 6-heptyn-2-yl tosylate (**1**) in trifluoroacetic acid (eq 1) occurs with triple bond participation to give predominantly the cyclic product 3-methylcyclohexenyl trifluoroacetate (**3**).[2] In addition to the synthetic interest attached to



1 2 3

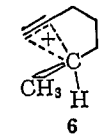
this new cyclization reaction there are novel mechanistic implications concerning the hybridization of the cationic intermediate or transition state. In general, vinyl cations obtained by addition of a positive R group to a terminal triple bond might retain the hybridization and linear geometry of the alkyne at C-2 (cation **4**, below)[3] or might undergo rehybridization to sp^2 at



4 5

C-2 to give a bent cation, **5**. The ion **4** is favored, based on the considerations of maximum bond strength and maximum s character for the occupied orbitals which also lead to the familiar prediction of a planar structure for carbonium ions of the type R_3C^+.

The most striking evidence for the planar structure of ordinary carbonium ions is the unreactivity of bridgehead leaving groups, attributable to restraint upon the achievement of planarity. Clearly the ion **2** is constrained to be nonlinear; yet it seems to be formed readily in competition with ordinary substitution and elimination reactions available to **1**. Furthermore the cyclization appears to occur during the rate-determining step, as indicated by the rate constant for solvolysis of **1** at 25.0°, 26.6×10^{-5} sec^{-1}, which is as large as that for solvolysis of 2-heptyl tosylate (24.9×10^{-5}),[4] despite the expected 17.5-fold rate-retarding inductive effect of the triple bond.[5] The postulation of a bridged transition state or intermediate **6** may avoid the neces-



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sity for bending at the cationic carbon in the rate-determining step, but, in view of the ultimate formation of **3**, a bridged ion postulate merely defers the formation of a transition state resembling **2**.

The apparent formation of the unfavored bent vinyl cation or a similar species in our study is, as an afterthought, not difficult to rationalize. The energetically favored formation of a new C–C σ bond in **2** from a C–C π bond in **1** can be invoked to supply the "driving force." The discovery of a reaction which proceeds *via* the unfavored type of vinyl cation, however, opens the way to many interesting studies, including comparison with alkene cyclizations *where there is no comparable unfavorable geometrical constraint*, comparison of the ease of formation of various ring sizes having *differing extents* of bending in the transition state,[6] and solvolyses in solvents of differing nucleophilicities which may compete to differing extents

(1) This work was supported by the Petroleum Research Fund of the American Chemical Society through Grant No. 790A-4.

(2) From 1 (6.0 g, 0.022 mole), which was allowed to react for 10 hr at 25° in 100 ml of trifluoroacetic acid containing 0.025 mole of sodium trifluoroacetate, 2.8 g of distillate was obtained (60% calculated as **3**). Gas chromatographic analysis of the distillate indicated the presence of 9% 3-methylcyclohexanone, 3% 6-heptyn-2-yl trifluoroacetate, and ~4% incompletely resolved peak, possibly 5-methylcyclohexenyl trifluoroacetate, in addition to **3**. A 150-ft diethylene glycol succinate (LAC) capillary column at 55° was used. Areas based on the uncorrected flame ionization detector response are reported here. Identification of **3** is based on the C and H analyses, infrared and nmr data, and hydrolysis to 3-methylcyclohexanone. In another experiment an 80.5% yield of the crude 2,4-dinitrophenylhydrazone of 3-methylcyclohexanone was obtained, based on the weight of tosylate **1** employed. Under the same conditions authentic 3-methylcyclohexanone gave a 90% yield.

(3) See D. S. Noyce, M. A. Matesich, O. P., M. D. Schiavelli, and P. E. Peterson, *J. Am. Chem. Soc.*, 87, 2295 (1965), for a reaction proposed to involve an ion of type **4** and for leading references to recent studies of vinyl cations.

(4) P. E. Peterson, R. E. Kelley, Jr., R. Belloli, and K. A. Sipp, *ibid.*, 87, 5169 (1965).

(5) Our estimate is based on the σ^* value of 1.3 previously used by M. M. Kreevoy, *ibid.*, **81**, 1608 (1959). Using an attenuation factor of 0.65 per methylene group to obtain a σ value for the H—C≡C—CH_2 group in $H—C{\equiv}C—CH_2—CH_2CH_2C^+HCH_3$, applying the relationship $\sigma 1 = 0.45\sigma^*$, and using a $\rho 1$ value of 3.27 applicable to solvolysis of substituted tosylates proceeding *via* the ion $X—CH_2—CH_2CH_2C^+HCH_3$ (P.E. Peterson and D. M. Chevli, unpublished results) gives the factor 17.5. Although the magnitude of this estimate is not very reliable, a very appreciable inductive effect of the remote triple bond may be predicted with confidence, based on our previous extensive studies of the remarkably large substituent effects found in trifluoroacetic acid. *Cf.* P. E. Peterson, C. Casey, E. V. P. Tao, A. Agtarap, and G. Thompson, *ibid.*, 87, 5163 (1965).

(6) Formolysis of a 3-pentyn-1-yl sulfonate to give products possibly arising *via* triple bond participation has very recently been reported by M. Hanack, J. Haeffner, and I. Herterich, *Tetrahedron Letters*, 875 (1965).

with the alkyne group in bond formation at the cationic carbon to which the leaving group is initially attached.

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A New Enzyme Containing a Synthetically Formed Active Site. Thiol-Subtilisin[1]

Sir:

We wish to report the synthesis of a new, active enzyme containing the backbone of subtilisin,[2] but containing a thiol group rather than a hydroxyl group as the essential nucleophile of the active site. We call this synthetic enzyme thiol-subtilisin.

The alteration of the active site of an enzyme may give mechanistically meaningful results. To this end, we have investigated the alteration of the active site of subtilisin in order to convert it from a "serine enzyme" to a "cysteine enzyme." Subtilisin contains a serine hydroxyl group at its active site; this group serves as a nucleophile during enzymatic catalysis, with the intermediate formation of an acyl-enzyme in which the acyl group is attached to this serine hydroxyl group. Recent investigations on small serine peptides showed the possibility of changing a serine to a cysteine residue without racemization: the O-tosyl derivative of a serine peptide was treated with either thiolacetate or thiolbenzoate ion in various organic solvents, yielding the thiol ester of the cysteine residue. The thiol ester could easily be decomposed to the mercaptan and acid.[3,4] By using this synthetic procedure subtilisin has been changed to a synthetic enzyme bearing a thiol group.

The transformation of subtilisin to thiol-subtilisin was carried out in three steps according to eq 1. The first step involves the almost instantaneous stoichiometric reaction of phenylmethanesulfonyl fluoride-^{14}C

$$\text{subtilisin-OH} \xrightarrow[-\text{HF}]{+\text{C}_6\text{H}_5\text{CH}_2\text{SO}_2\text{F}} \text{subtilisin-OSO}_2\text{CH}_2\text{C}_6\text{H}_5 \xrightarrow[-\text{C}_6\text{H}_5\text{CH}_2\text{SO}_3^-]{+\text{CH}_3\overset{\overset{\text{O}}{\|}}{\text{C}}\text{S}^-} \text{subtilisin-S}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{CH}_3 \xrightarrow[-\text{CH}_3\text{CO}_2^-]{+\text{H}_2\text{O}} \text{subtilisin-SH} \quad (1)$$

(1.2×10^{-3} *M*) with subtilisin (1×10^{-3} *M*) at pH 7.0, 0.1 *M* phosphate buffer. The resultant phenylmethanesulfonyl-subtilisin contained 0.9 equiv of carbon-14 per mole of enzyme,[5] and its enzymatic activity, tested with *p*-nitrophenyl acetate, was less than 1% of the original activity. The second step of the transformation involves the displacement of the phenylmethanesulfonyl group by thiolacetate ion in an SN2 displacement reaction.[6] The reaction was carried out in 0.7 *M* thiolacetate ion at pH 5.25 and 25° for 48 hr. During this period, more than 99% of the phenylmethanesulfonyl groups were removed from the enzyme as monitored by the loss of protein-bound carbon-14. The third step of the transformation involves the spontaneous hydrolysis of the acetyl group from the thiol ester, presumably assisted by the enzyme.

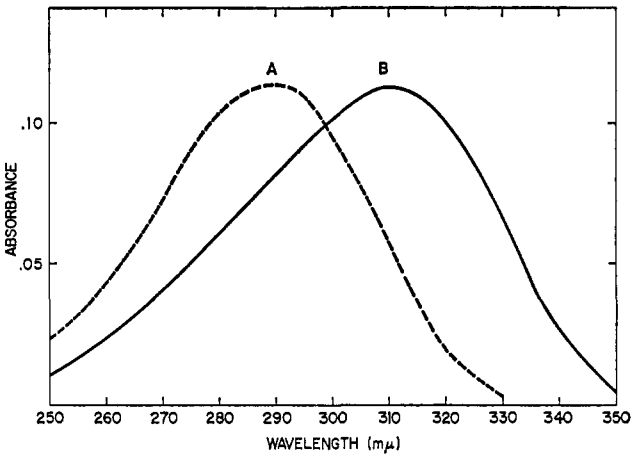

Figure 1. The difference spectra of *trans*-cinnamoyl-subtilisin *vs.* subtilisin (A) and *trans*-cinnamoyl-thiol-subtilisin *vs.* thiol-subtilisin (B) at pH 5, 0.1 *M* acetate buffer. *N-trans*-Cinnamoylimidazole (5.3×10^{-6} *M*) was treated with excess enzyme (9.2×10^{-6} *M*) to transform completely the cinnamoyl group to cinnamoyl-enzyme.

Table I shows the results of three different analytical procedures used to characterize subtilisin before and after the treatment described above. (1) Spectrophotometric titration of thiol groups using *p*-chloromercuribenzoate ion[7] indicates the appearance of 0.7–0.8 equiv of thiol group/mole in the modified enzyme. (2) Amino acid analysis of the hydrolysate of the performic acid oxidized proteins using a Spinco amino acid analyzer shows that the thiol group of the synthetic enzyme exists in a cysteine residue and, moreover, that the amount of cysteine per mole is comparable to the amount of thiol groups per mole found in (1). (3) The wavelength of maximum absorption of an O-cinnamoyl compound is significantly lower than that of an S-cinnamoyl compound.[8] The difference spectra of O-cinnamoyl-subtilisin *vs.* subtilisin and S-cinnamoyl-subtilisin *vs.* thiol-subtilisin show the same relationship, as seen in Figure 1. Furthermore, in the S-cinnamoyl-

Table I. Comparison of Subtilisin and Thiol-Subtilisin

Determination	Subtilisin	Thiol-subtilisin
Thiol groups/molecule using titration with *p*-chloromercuribenzoate ion	<1.0	0.80
Cysteic acid/molecule from amino acid analyses of the hydrolysate from the performic acid oxidized protein	0.096	0.78
λ_{max} of (cinnamoyl-enzyme) *vs.* (enzyme)	289	~310

(1) This research was supported by grants from the National Institutes of Health.
(2) Bacterial Proteinase Novo, Novo Pharmaceutical Co., Copenhagen, Denmark.
(3) I. Photaki and V. Bradakos, *J. Am. Chem. Soc.*, **87**, 3489 (1965).
(4) C. Zioudrou, M. Wilchek, and A. Patchornik, *Biochemistry*, **4**, 1811 (1965).
(5) Based on a molecular weight of the enzyme of 27,600 and $A^{1\%}_{278}$ = 11.7: H. Matsubara, C. B. Casper, D. M. Brown, and E. L. Smith, *J. Biol. Chem.*, 240, 1125 (1965).
(6) The alkanesulfonyl group is an excellent leaving group in such a reaction; the *p*-toluenesulfonyl group used for small peptides[3,4] was replaced by the phenylmethanesulfonyl group here because the first step of the transformation was much more rapid with the latter group.
(7) P. D. Boyer, *J. Am. Chem. Soc.*, **76**, 4331 (1954).
(8) M. L. Bender and F. J. Kézdy, *Ann. Rev. Biochem.*, **34**, 49 (1965).