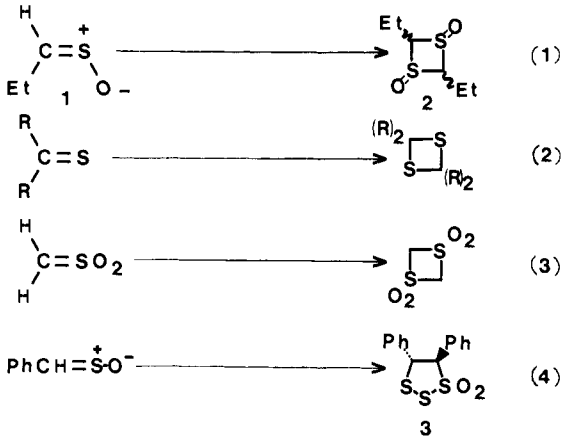
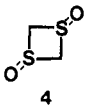
Dimer of the Onion Lachrymatory Factor: The First Stable 1,2-Dithietane Derivative[1]

Sir:

The lachrymatory factor (LF) of the onion (*Allium cepa*) was characterized as the sulfine propanethial *S*-oxide (**1**) by Wilkens in 1961[2] and was recently found by us to possess *Z* stereochemistry.[1c] In the earlier report by Wilkens[2] it was also suggested that the LF **1** undergoes self-condensation to afford 2,4-diethyl-1,3-dithietane 1,3-dioxide (**2**, eq 1). While the



formation of **2** would seem to be consistent with the known cyclodimerization of other thiocarbonyl compounds to 1,3-dithietane derivatives (see eq 2[3a] and 3[3b]), the conversion of **1** into **2** is predicted to be unfavorable on molecular orbital grounds.[4] Furthermore, it is mechanistically very difficult to reconcile the formation of **2** from **1** with the reported formation of **3** from self-condensation of thiobenzaldehyde *S*-oxide (eq

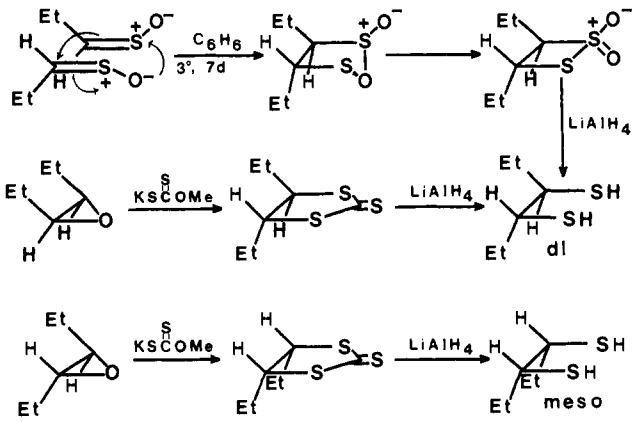


4).[5] Finally, the properties attributed to **2** seem inconsistent with those determined by us for *cis*- and *trans*-1,3-dithietane 1,3-dioxide (**4**).[6] We have, therefore, reexamined the purported dimerization of the LF **1** and report our novel findings herein.

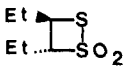
A sample of **1** from natural[7a] or synthetic[7b] sources was purified by trap to trap distillation at −30 °C, dissolved in about twice its volume of freshly dried benzene, and kept in the dark at 3 °C for 7 days. The slightly yellow solution, now devoid of lachrymatory properties, was concentrated in vacuo and the residue subjected to molecular distillation (50 °C, 10^{-3} mm) affording a practically colorless, clear liquid with a strong onion-like odor. Analysis by GLC indicated a single major product with a retention time slightly longer than that of *n*-propyl propanethiosulfonate (9.45 and 7.28 min, respectively, at 140 °C on a 1/8 in. × 4 ft 10% Apiezon L/Chromosorb W column). High resolution mass spectrometry confirmed the formula $C_6H_{12}S_2O_2$ for the LF dimer.[8] The IR spectrum showing bands at 1139 and 1333 cm^{-1} ($-SO_2-$) was very similar to that published by Wilkens.[2] The ^1H FT NMR (C_6D_6) showed δ 0.50 (t, 3 H, J = 7.3 Hz), 0.71 (t, 3 H, J = 7.3 Hz), 1.36 (m, 2 H), 1.91 (m, 2 H), 2.73 (d of t, 1 H, J = 6.10 (d) and 7.82 (t) Hz), and 4.35 (d of t, 1 H, J = 6.10 (d) and 9.04 (t) Hz) while the ^{13}C FT NMR (C_6D_6) showed δ_C 11.26 (q), 12.72 (q), 24.32 (t), 29.49 (t), 39.18 (d), and 97.91 (d).[9] The LF dimer gave a positive thiosulfonate test[10] and had UV λ_{max} (EtOH or hexane) at ~280 (ε 100, sh).[11]

The above data is most consistent with 3,4-diethyl-1,2-dithietane 1,1-dioxide (**5**) as the structure for the LF dimer.[12] The stereochemistry of **5** was established as trans by $LiAlH_4$ reduction to *dl*-hexane-3,4-dithiol, shown by FT NMR to be

Scheme I



identical with authentic *dl*-hexane-3,4-dithiol (prepared stereospecifically[16] from *cis*-3,4-epoxyhexane) and different from authentic *meso*-hexane-3,4-dithiol (prepared from *trans*-3,4-epoxyhexane) as summarized in Scheme I.[17] Scheme I also rationalizes the stereospecific formation of *trans*-**5** from **1** via



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a [4 + 2] cycloaddition sequence, in which sulfine **1** functions as both a 1,3 dipole and a dipolarophile,[18] followed by rearrangement of the unstable cyclic sulfenyl sulfinate ester.[19]

Also detected in the self-condensation of LF **1** was ~5% (by GC) of a heavier minor component indicated by high resolution mass measurements to have the composition $C_6H_{12}S_3O_2$. While other spectroscopic information is not yet available for this trace product, we suspect that it may be a 1,2,3-trithiolane 1,1-dioxide, analogous to that shown in eq 4. This product may arise through insertion of sulfur (formed along with aldehyde, which we also detect, by an alternative decomposition of the sulfine via an oxathiirane[20]) into **5** or its sulfenyl sulfinate precursor (see Scheme I).

Compound **5** is notable as the first example of an isolable 1,2-dithietane derivative.[21] We are actively exploring the reactions of this interesting new compound.

Acknowledgment. Support for this research from the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the University of Missouri—St. Louis is gratefully acknowledged. The FT NMR spectrometer was purchased through NSF Grant CHE 77-02068.

References and Notes

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(7) (a) Quartered and frozen white globe onions were converted into a powder in a Waring Blendor and extracted with trichlorofluoromethane, which in turn was dried ($MgSO_4$) and concentrated in vacuo at −78 °C. (b) From propanesulfinyl chloride and triethylamine at −20 °C.
(8) Found: *m/e* 180.02776 (calcd for $C_6H_{12}S_2O_2$: *m/e* 180.02786).

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(9) Assignments established by multiple resonance or off-resonance techniques.
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(12) The ^1H and ^{13}C NMR data clearly indicate the presence of two substantially different, coupled "CHEt" groups, e.g., $-SO_2CHEt$ and $-SCHEt$. The striking difference in δ_C of the two methine carbons can be interpreted in terms of an unusual deshielding effect characteristic of four-membered-ring sulfones,[13] a further deshielding effect seen for the α-sulfonyl carbons of thiosulfonates compared with that for sulfones,[14] and a vicinal deshielding effect due to the trans ethyl groups (vide infra)[15] giving the α-sulfonyl δ_C of 97.9 which contrasts to the α-sulfenyl δ_C of 39.2.
(13) Thietane 1,1-dioxide shows the α-sulfonyl carbon at δ_C 65.6 compared with the α-sulfonyl carbon shift of δ_C 51.1 for thiolane 1,1-dioxide: Block, E.; Bazzi, A. A.; Lambert, J. B.; Wharry, S. M.; Andersen, K. K.; Dittmer, D. C.; Patwardhan, B. H.; Smith, D. J. H., manuscript submitted for publication.
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(17) The dithiol prepared from **5** shows δ_C 19.98, 38.16, and 56.58, authentic *dl*-dithiol shows δ_C 20.08, 38.21, and 56.63, and authentic *meso*-dithiol shows δ_C 19.88, 35.68, and 57.80. The ^1H FT NMR of the dithiol from **5** is more closely similar to that of *dl*- than that of *meso*-hexane-3,4-dithiol.
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(21) (a) For a review of four-membered cyclic disulfides, see Vasil'eva, T. P.; Lin'kova, M. G.; Kil'disheva, O. V. *Russ. Chem. Rev.* **1976**, *45*, 639–659. (b) The instability of 1,2-dithetanes is attributed to strong repulsion of the p-π electrons of the neighboring sulfur atoms[21a] (note, however, that 1,2-dioxetanes are isolable[22]). Replacement of the lone pairs on one sulfur would remove this destabilizing influence; a similar situation exists with the unstable 1,2-dithiolane which is more stable as the 1,2-dithiolane 1,1-dioxide.[11] Various 1,2-dithietenes (dithietes) are known and in some cases are stable aromatic systems.[21a,23]
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Kinetic α-Deuterium Isotope Effects for Acylation of Chymotrypsin by 4-Methoxyphenyl Formate and for Deacylation of Formylchymotrypsin

Sir:

The kinetic α-deuterium isotope effect, $(V_{max}/K_m)_D/(V_{max}/K_m)_H$, for acylation of α-chymotrypsin by 4-methoxyphenyl formate in aqueous solution over the pH range 5.8–6.8 is 1.18 ± 0.02. For deacylation of the formylchymotrypsin generated in this reaction, the kinetic α-deuterium isotope effect, $(V_{max})_D/(V_{max})_H$, is 1.15 ± 0.03 over the pH range 6.2–6.8. These values strongly suggest that acylation of chymotrypsin by this reagent occurs at serine and that ≻C-O-Enz bond formation and cleavage in the transition states for acylation and deacylation reactions, respectively, are well advanced but not complete.

Kinetic α-deuterium isotope effects have proved to be useful criteria of mechanism for a growing number of enzymatic reactions.[2,3] We report here two such isotope effects, measured in an effort to obtain novel information concerning the transition state structure for α-chymotrypsin-catalyzed hydrolysis of esters. The nature of the measurements requires the utilization of formates, esters not previously established to be chymotrypsin substrates. 4-Methoxyphenyl formate, 4-methoxyphenyl [^2H]formate, 4-methoxyphenyl [^3H]formate (0.1 mCi/mmol), and 4-methoxyphenyl [^{14}C]formate (0.1 mCi/mmol) were synthesized from the appropriate substrates as described.[4] Titrimetric assay of reaction mixtures containing 4-methoxyphenyl formate and bovine pancreatic chymotrypsin (crystalline, Schwarz-Mann, 1220 U/mg) revealed that the rate of proton liberation increased with increasing enzyme concentration. Replacement of chymotrypsin by chymotrypsinogen abolished this effect. Employing a spectrophotometric assay, 4-nitrophenyl formate was also found to be a chymotrypsin substrate. At pH 5.5, this compound exhibited an "initial burst" of color at 320 nm, strongly suggesting rate-determining enzyme deformylation.[5] This conclusion was confirmed by the observation that the chymotrypsin-catalyzed hydrolysis of the 4-nitro- and 4-methoxyphenyl formates occurs at comparable rates under the same conditions. For isotope effect measurements, the 4-methoxyphenyl compound was chosen; the 18-min half-life of 4-nitrophenyl formate in water at 25 °C[6] results in a large nonenzymatic hydrolysis of this ester under the conditions of our measurements. The corresponding value for the methoxy ester is 630 minutes,[7] minimizing the contribution from nonenzymatic routes. A thorough spectrophotometric study (see eq 1) of the kinetics

$$E + S \underset{}{\overset{k_s}{\rightleftharpoons}} ES \xrightarrow{k_2} EP_2 \xrightarrow{k_3} E + EP_2 \qquad (1)$$
$$\downarrow$$
$$P_1$$

of chymotrypsin-catalyzed hydrolysis of isotopically normal 4-methoxyphenyl formate revealed a good fit to Michaelis-Menten kinetics: at pH 6.8, V_{max} is (11.3 mol of substrate/min)/mol of enzyme or $k_3 = 11.3\ min^{-1}$ and K_m is 4.3×10^{-4} M. The value of K_m is smaller than that for 4-nitrophenyl acetate and chymotrypsin at pH 7.8, 1.9×10^{-3} M, and the value of V_{max} (or k_3) is considerably greater than that for deacetylation of acetylchymotrypsin at pH 7.8, 0.32 min^{-1}[7]. Thus, 4-methoxyphenyl formate is at least as suitable a substrate for chymotrypsin as is the much-employed 4-nitrophenyl acetate.

The α-tritium isotope effect on V_{max}/K_m for chymotrypsin acylation was measured by a competitive method in which 0.001 M 4-methoxyphenyl [^3H]formate and 0.001 M 4-methoxyphenyl [^{14}C]formate were simultaneously incubated with 6×10^{-6} M chymotrypsin in dilute citrate-phosphate buffers. After an appropriate time interval, unreacted ester was extracted into ether, and an aliquot of the aqueous phase, containing the isotopically labeled formates, was removed, added to a small column of Dowex 1-X4 (chloride form), and washed with distilled water, followed by elution of the formate with 0.01 M HCl. Four aliquots of the formate eluant were counted in a Beckman LS-230 liquid scintillation spectrometer employing a modified Bray's solution. The isotope effect was calculated by comparing the ratio of ^{14}C to ^3H activity in reactant ester and product formate, as previously described.[8,9] Isotope effect measurements were made at three values of pH, 5.8, 6.3, and 6.8, and at several extents of ester consumption. The values are plotted as a function of extent of reaction in Figure 1; solid lines in this figure are theoretical plots for tritium isotope effects of 1.25, 1.27, and 1.29. We conclude that the isotope effect is 1.27 ± 0.02. The indicated error limit is the standard deviation from the mean of all measurements. This corresponds to an α-deuterium isotope effect of $(1.27)^{1/1.442}$ or 1.18 ± 0.02.[10,11] This isotope effect refers specifically to the acylation reaction, although enzyme deacylation is the rate-determining step, since isotopic discrimination can occur upon acylation only; the formyl enzyme is committed to yield products.

The α-deuterium secondary isotope effect on V_{max} (and therefore on the hydrolysis of formylchymotrypsin) was measured by a direct spectrophotometric assay. Reactions were monitored using an HP2100A minicomputer interfaced to a Cary spectrophotometer by following formation of 4-methoxyphenol with time at 295 nm. Reactions were initiated at values of $[S]_0$ of four-five times the K_m values of the isotopic substrates and were followed until substrate was depleted.

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