blotting (using anti-P protein antibodies) matched those predicted from the DNA sequences.

With reliable sources of the fusion proteins in hand, we turned our attention to isolating the 109 amino acid mutase fragment. Following the procedure of Germino et al., the fusion protein (Figure 2, lanes 2 and 3) was purified from a crude extract of KB357(pJS32) in one step with β-galactosidase affinity chromatography (8 mg/L of culture, ca. 2% of total soluble protein).[16] The collagen segment of this tripartite protein contained six sites for collagenase digestion. Clostridiopeptidase A[17] specifically digested the collagenase linker region to produce β-galactosidase and the desired P protein fragment (Figure 2, lanes 4 and 5). Gel filtration chromatography afforded the pure truncated mutase (Figure 2, lane 6).

On a weight-adjusted basis, the N-terminal 109 amino acids of the P protein possessed the same chorismate mutase specific activity (200 U/mg of enzyme) as the wild-type P protein (60 U/mg of enzyme), but without any prephenate dehydratase activity. Moreover, the truncated enzyme followed straightforward Michaelis-Menten kinetics (K_M for chorismate = 290 μM). This small, kinetically simple chorismate mutase thus becomes amenable to further physical studies, which will be reported in due course.

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(17) Clostridiopeptidase A, type VII (high purity), obtained from Sigma Chemical Co.

(*Z,Z*)-*d,l*-2,3-Dimethyl-1,4-butanedithial *S,S'*-Dioxide: A Novel Biologically Active Organosulfur Compound from Onion. Formation of *vic*-Disulfoxides in Onion Extracts[1]

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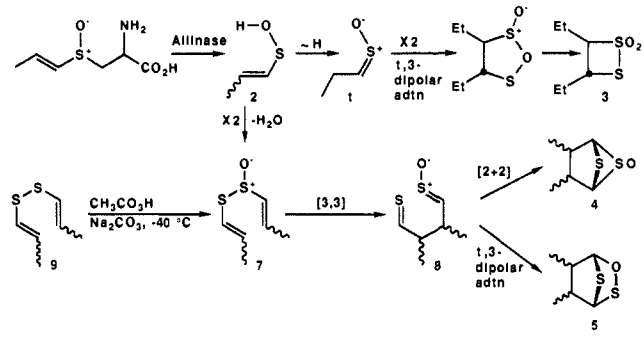
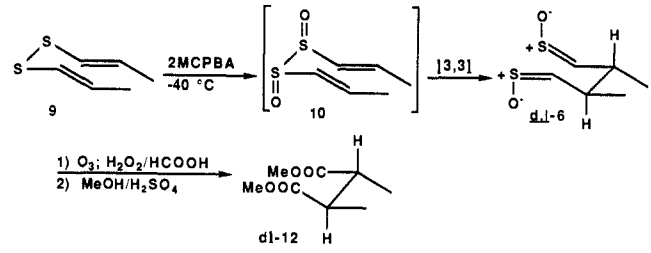
(*Z*)-Propanethial *S*-oxide (**1**), the lachrymatory factor (LF) of the onion (*Allium cepa*),[2] is unique as the only known example of a naturally occurring thiocarbonyl *S*-oxide (sulfine). It is formed in onion extracts from the stable precursor *trans*-(+)-*S*-1-propenyl-L-cysteine sulfoxide by way of 1-propenesulfenic acid (**2**).[2] Compounds **1** and **2**, through dimerization or self-condensation, lead to unusual heterocycles **3**–**5**[3] by the routes shown in Scheme I. We now report the isolation from onion extracts of a remarkable new, biologically active dimer, (*Z,Z*)-*d,l*-2,3-dimethyl-1,4-butanedithial *S,S'*-dioxide (O⁻S⁺=CHCHMeCHMeCH=S⁺O⁻, *d,l*-**6**), the first bis(thial *S*-oxide).[4] It is noteworthy that compound **6** bears a close relationship to proposed intermediate **8** in Scheme I. We propose that **6** is formed via a novel double sulfoxide-accelerated dithio-Claisen rearrangement[5] of a *vic*-disulfoxide[6] (Scheme II), which in turn results from a process initiated by addition of **2** to **1** (Scheme III). On the basis of our proposal, we have developed a simple, stereoselective synthesis of *d,l*-**6** (Scheme II).

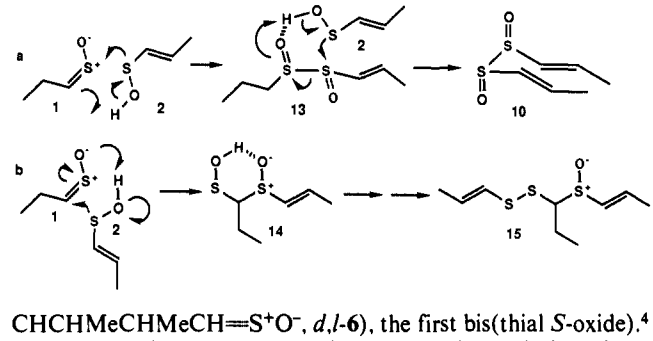
Scheme I



Scheme II



Scheme III



Onion bulbs were peeled, homogenized at 25 °C, chilled to 4 °C and at this temperature rapidly filtered through cheesecloth, extracted with CH_2Cl_2, centrifuged, dried ($MgSO_4$), and concentrated in vacuo. The concentrate was subjected to column chromatography (silica gel, 100:1 methylene chloride–acetone), affording a mixture of **6** and **4a**.[3c] Although **6** is not readily separated from **4a**, on the basis of its NMR spectra (^{1}H NMR ($CDCl_3$) δ 8.09 (d, J = 9.6 Hz, 2 H), 3.73 (m, 2 H), 1.24 (d, J = 6.5 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 179.6 (CH), 36.11 (CH), 17.3 (CH_3); see also below), **6** can be characterized as an isomer of 2,3-dimethyl-1,4-butanedithial *S,S'*-dioxide.

We developed a simple stereoselective synthesis of *d,l*-**6** via [3,3]-sigmatropic rearrangement of bis((*E*)-propenyl) *vic*-disulfoxide (**10**, see Scheme II). Addition of a chilled CH_2Cl_2 solution of (*E,E*)-**9**[3b] to a solution of 2.2 equiv of MCPBA in

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(4) A single example of a bis(thione *S*-oxide), 2,2,4,4-tetramethylcyclobutane-1,3-dithione *S,S'*-dioxide, has been reported: Zwanenburg, B.; Wagenaar, A.; Thijs, L.; Strating, J. *J. Chem. Soc., Perkin Trans. 1* **1973**, 73.
(5) (a) Block, E.; Ahmad, S. *J. Am. Chem. Soc.* **1985**, *107*, 6731. Block, E.; Ahmad, S.; Catalfamo, J.; Jain, M. K.; Apitz-Castro, R. *J. Am. Chem. Soc.* **1986**, *108*, 7045. (b) Hwu, J. R.; Anderson, D. A. *Tetrahedron Lett.* **1986**, *27*, 4965. (c) The dithio-Claisen rearrangement of bis(1-propenyl) disulfide has also been described: Block, E.; Zhao, S. H. *Tetrahedron Lett.*, in press.
(6) (a) Freeman, F. *Chem. Rev.* **1984**, *84*, 117. (b) Freeman, F.; Angeletakis, C. N.; Pietro, W. J.; Hehre, W. J. *J. Am. Chem. Soc.* **1982**, *104*, 1161.

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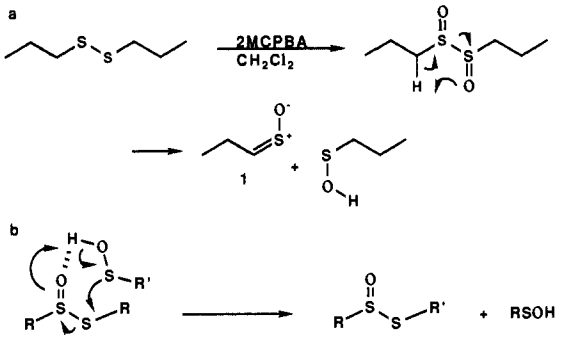
Scheme IV



CH_2Cl_2 at −60 °C followed by warming up to −40 °C during 1 h, rapid workup with ice-cold $NaHCO_3$ solution, drying over K_2CO_3, and concentration in vacuo at 0 °C gave **6** in 34% yield. Compound **6** is a colorless solid of formula $C_6H_{10}S_2O_2$ (decomposition point 48 °C; high-resolution EI-Ms 178.0121, calcd 178.0122; FD-MS also indicates the parent ion at 178) with IR bands at 1103 and 1120 cm^{-1}. The ^1H and ^{13}C NMR spectra in $CDCl_3$ are identical with those of **6** isolated from onion. Upon changing of the solvent to C_6D_6, the ^1H NMR spectrum of **6** showed peaks at δ 7.06 (d, J = 9.6 Hz, 2 H), 3.3 (m, 2 H), and 0.60 (d, 6.3 Hz, 6 H). The ^{13}C and ^1H NMR data for **6** in $CDCl_3$ and C_6D_6 are in good agreement with analogous data for **1**[2b] and are consistent with **6** having *all-Z* stereochemistry at the C=S bonds. Sequential treatment of synthetic **6** with ozone/−50 °C, H_2O_2–HCOOH, and MeOH–H_2SO_4 gave in 88% yield a compound identical by GC-MS and ^{13}C NMR with authentic *d,l*-dimethyl 2,3-dimethylsuccinate (*d,l*-**12**) and different from authentic *meso*-**12**,[7] thereby establishing synthetic/natural **6** as (*Z,Z*)-*d,l*-2,3-dimethyl-1,4-butanedithial *S,S'*-dioxide (*d,l*-**6**). Compound **6** shows moderate in vitro inhibition of 5-lipoxygenase in porcine leucocytes.[8]

We suggest that in the presence of excess oxidant (*E,E*)-**9** is converted into **10**. The anti conformation of the oxygen atoms depicted in **10**, which correlates upon rearrangement with *Z* CSO geometry, follows theoretical predictions that this conformation represents an energy minimum.[6b] Compound **10** should undergo a particularly facile [3,3]-sigmatropic rearrangement due to the weakness of the S–S bond (ca. 36 kcal)[6b] and the rate-enhancing effect of the two zwitterionic sulfinyl functions.[5b] The exclusive formation of *d,l*-**6** from dioxidation of (*E,E*)-**9** is consistent with concerted [3,3]-sigmatropic rearrangement of **10** and with previous observations on the stereospecificity of products from *monooxidation* of isomers of **9**.[3b,9] Unexpectedly, dioxidation of (*E,Z*)- and (*Z,Z*)-**9** gives mixtures of *d,l*- and *meso*-**6**,[10] suggesting a change to a stepwise mechanism when the disulfide oxidation and/or [3,3]-process is retarded by *Z* C=C stereochemistry.[10]

How is bis(sulfine) **6** formed in onion extracts? An attractive possibility would involve "thiophilic" addition of **2** to **1** (see Scheme III, path a) followed by nucleophilic attack of a second molecule of **2** on α-disulfoxide intermediate **13**. The formation of (*E*)-1-propenyl propyl *vic*-disulfoxide (**13**) illustrated in Scheme III, path a, is the reverse of the reaction observed in the decomposition of aliphatic *vic*-disulfoxides (Scheme IV, path a)[6a,11] while the reaction of **13** with **2** is analogous to the reaction of sulfenic acids with thiosulfinates (Scheme IV, path b).[12] The alternative "carbophilic" mode of addition of **2** to **1** (Scheme III, path b) would generate an α-(alkenylsulfinyl)propanesulfenic acid **14**, a likely intermediate in the formation of "cepaene" **15**.[13,14]

Compound **6** could also originate via homolytic decomposition of **13** into *n*-propanesulfinyl and (*E*)-1-propenesulfinyl radicals followed by self-coupling of the latter. However, the likelihood of the occurrence of such radical recombination on a significant scale in onion extracts is small. Furthermore, model studies[10] involving **13** generated by oxidation of 1-propenyl propyl disulfide indicate formation of characteristic products in addition to **6** and in amounts comparable to **6**, which we have been unable to detect in onion extracts. Efforts to establish the mechanisms of formation, determine biological properties and reactions, and achieve syntheses of the remarkable organosulfur compounds found in onion extracts are continuing.

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(9) Monoxidation of (*E,E*)-**9**, (*Z,Z*)-**9**, and (*E,Z*)-**9** affords, respectively, *trans*-**5**, **4b**–*trans*-**5**, and **4a**–*cis*-**5**, by processes that must involve cyclization by two competitive concerted mechanisms (a modification of our earlier mechanism[3b]).[10]

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Insulin Stabilizes Copper(II)–Thiolate Ligation That Models Blue Copper Proteins

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Blue (or type 1) copper proteins[1] are characterized by two unique spectroscopic properties,[2,3] an intense absorption envelope

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