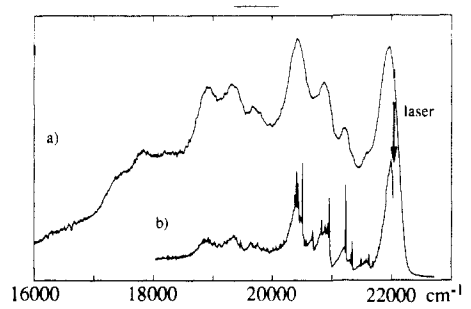

Figure 1. Luminescence spectra of $[Rh(phpy)_2bpy]PF_6$ in a nitrile glass at 6 K, using (a) 337-nm and (b) 454.5-nm excitation, respectively.

it with chemical variation we can gain new insight into the nature of the emitting state.

Low-temperature luminescence spectra of Rh^{3+} and Ir^{3+} complexes with π-accepting ligands in glassy matrices are characterized by a structured band in the visible part of the spectrum.[1,3] Figure 1a shows, as an example, the 6 K luminescence spectrum of $[Rh(phpy)_2bpy]^+$ in nitrile glass (phpyH = phenylpyridine). The luminescence lines are 100–200 cm^{-1} wide; this width is inhomogeneous, resulting from a very broad distribution of sites in the glass.[2] A small subset of complexes can be excited by choosing a narrow laser line within the inhomogeneously broadened profile of the first excitation origin. In the absence of energy transfer and thermal reorganization of the matrix during the lifetime of the excited state, only the subset of complexes directly excited by the narrow laser will luminesce, resulting in a sharp-line spectrum. The result of such an experiment with $[Rh(phpy)_2bpy]^+$ in a nitrile glass is shown in Figure 1b. The narrowing effect, compared to the unselectively excited spectrum (Figure 1a), is dramatic. The sharp lines have widths of 4 cm^{-1} at 6 K, very close to our instrumental resolution. We notice that part of the luminescence intensity is narrowed to a lesser degree, each sharp line being accompanied by a broader sideband at lower energy. This is a direct result of the electronic splitting of the first excited state.[4] For the present discussion we concentrate on the sharp-line spectrum; it represents a distinctive fingerprint of the complexes, even without specific assignments of the vibrations.

In Figure 2 we compare LLN spectra of $[Rh(phpy)_2bpy]^+$, $[Rh(phpy)_2en]^+$, and $[Rh(thpy)_2bpy]^+$, all in a nitrile glass or in Plexiglass at comparable temperature (thpyH = thienylpyridine). Substitution of bpy by en in $[Rh(phpy)_2bpy]^+$ has virtually no effect on the sharp-line pattern (Figure 2a,b). It follows immediately that the $phpy^-$ ligand and not the bpy ligand is involved in the lowest energy excited state of $[Rh(phpy)_2bpy]^+$. This is fully supported by the result obtained by substituting $phpy^-$ with $thpy^-$ (Figure 1c). This pattern is distinctly different from the pattern in Figure 2a, and it is clear that the active ligand is $thpy^-$ and not bpy. In agreement with this, the spectrum of $[Rh(thpy)_2en]^+$ shows the same LLN pattern as $[Rh(thpy)_2bpy]^+$ in Figure 2c.

For an understanding of the nature of the first excited state in mixed-chelate d^6 complexes, it is highly relevant to determine which ligand is involved in the excitation process. We have performed Extended-Hückel calculations on model complexes of $[Rh(phpy)_2bpy]^+$ and $[Rh(thpy)_2bpy]^+$.[5] The lowest energy unoccupied orbital turned out to be essentially a bpy orbital in these calculations. In apparent contrast, the lowest excited state in both complexes has been assigned on the basis of general chemical, spectroscopic, and electrochemical arguments as $\pi-\pi^*$ on the cyclometalated ligand.[1b,c] Our LLN experiments demonstrate very clearly that the cyclometalated ligand and not bpy is involved in the first excited state. One-electron models are obviously of very limited value in predicting the energy order of excited states in mixed-ligand complexes. It is essential to obtain

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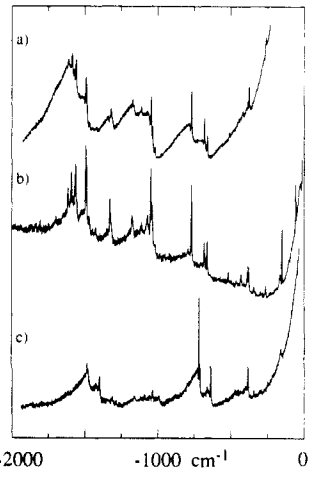

Figure 2. LLN spectra at 6 K relative to the exciting laser line of (a) $[Rh(phpy)_2en]PF_6$ in a Plexiglass matrix, excitation at 465.8 nm; (b) $[Rh(phpy)_2bpy]PF_6$ in a nitrile glass, excitation at 457.9 nm; and (c) $[Rh(thpy)_2bpy]PF_6$ in Plexiglass, excitation at 530.9 nm.

this information from experiment, and we have shown that LLN can provide it in the most direct and straightforward way. It is a technique that has not been used so far in this area of research but that has a great potential for providing relevant information about the nature of the emitting state. It is easily applied to diluted samples, and the problems connected with crystal quality, energy transfer, and impurities in concentrated crystalline samples can be overcome.

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A Genetically Engineered Monofunctional Chorismate Mutase

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The Claisen rearrangement of chorismic acid **1** to prephenic acid **2** (eq 1) catalyzed by the enzyme chorismate mutase plays a central role in the biosynthesis of phenylalanine and tyrosine. This remarkable transformation is the only documented example of an enzyme-catalyzed pericyclic reaction primary metabolism.[1] To understand the nature of catalysis, we[2] and others[3] have probed the influence of solvent and substituents, as well as conformational and isotope effects on both the enzymic and non-enzymic processes. Several plausible suggestions for catalysis have been put forth; however, the actual role of the enzyme remains obscure.[4] Clearly,

[†] Department of Chemistry, Baker Laboratory.
[‡] Section of Biochemistry, Molecular and Cell Biology.

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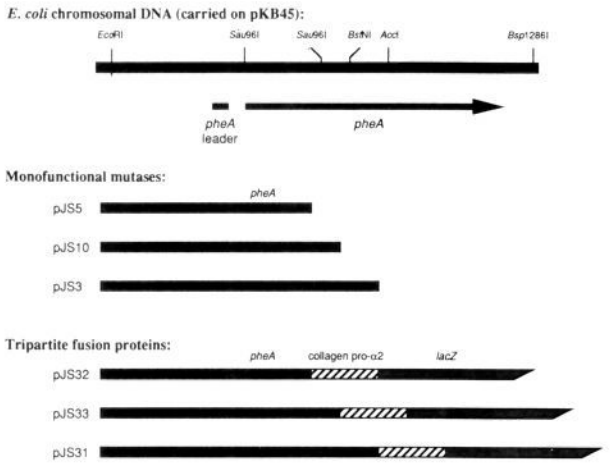


Figure 1. Maps of *pheA* Inserts.

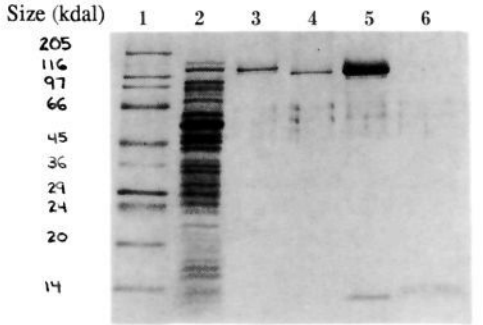
further progress in this area requires an enzyme amenable to physical studies.

(1)

1 2

While several mono-[5] and bifunctional[6] chorismate mutases have been identified, the two enzymes produced by the bacterium *Escherichia coli* have been by far the best studied.[7] In this organism, chorismate mutase is fused either to prephenate dehydratase (P protein; subunit M_r = 43.1 kdal) or to prephenate dehydrogenase (T protein; subunit M_r = 42.0 kdal). Unfortunately both enzymes are catalytically active only as oligomers that are too large for NMR structural analysis. Moreover both present complex product and dead-end inhibition patterns that interfere with kinetic investigations of the rearrangement mechanism. Here we describe the use of recombinant DNA techniques to engineer a low molecular weight, monofunctional chorismate mutase that contains only 109 amino acids (subunit $M_r \approx 13.0$ kdal). This protein represents the smallest known chorismate mutase.

We elected to use the *E. coli* P protein (386 amino acids) as our starting material, since earlier studies indicated that this enzyme contained distinct mutase and dehydratase catalytic sites.[8] These further suggested that the P protein's mutase active site lies within the N-terminal one-third of the protein, corresponding to the first third of the P protein gene (*pheA*). Both the *E. coli* P and T proteins have been cloned and sequenced, and the deduced protein sequences show sequence similarity only in their N-termini.[8a] We planned to produce a monofunctional enzyme by cloning the mutase domain of the P protein. Maruya et al. previously used a similar strategy to show that suitable fragments



SDS polyacrylamide gradient gel (8.5% to 20%)
Lane 1: Molecular weight standards;
Lane 2: KB357(pJS32) crude extract, 10 μg;
Lane 3: Purified tripartite protein, 0.5 μg;
Lane 4: After collagenase digestion, 0.5 μg;
Lane 5: Same as lane 4, 5.0 μg;
Lane 6: Purified mutase fragment, 0.5 μg

Figure 2. Purification of the P protein fragment.

of the *E. coli* T protein apparently function as monofunctional chorismate mutases, although none of these protein fragments was isolated.[9]

Using standard cloning techniques, we prepared plasmids that encoded N-terminal fragments of the P protein (Figure 1).[10] The complete *pheA* gene resides on a 1.9 kilobase (kb) DNA segment (*Eco*RI to *Bsp*1286I) present in plasmid pKB45.[11] Subclones derived from this region were used to clone monofunctional chorismate mutases. For example, the 1.2 kb *Eco*RI to *Acc*I segment of pKB45 was subcloned into the polylinker region of plasmid pUC18.[12] This plasmid, designated pJS3, encoded the N-terminal 205 amino acids of the P protein. Plasmids encoding the N-terminal 109 and 152 amino acids of the P protein (pJS5 and pJS10, respectively) were similarly prepared. Plasmids pJS3, pJS5, and pJS10 were used to transform *E. coli* strain KB357 (relevant genotype Δ[*pheA-tyrA*]).[9] With use of the growth assay described by Backman et al., each of these strains produced an active, monofunctional chorismate mutase.[13] Unfortunately these truncated mutases were rapidly degraded by bacterial proteases and could not be isolated from the recombinant strains.[14]

The mutase fragments were, however, resistant to degradation when expressed as part of a tripartite fusion protein composed of the P protein fragment, a short stretch of collagen, and *E. coli* β-galactosidase.[15] Plasmids pJS32, pJS33, and pJS31 were constructed by joining the inserts of pJS3, pJS10, and pJS5, respectively, with the DNA coding for 60 amino acids of collagen pro-α2 and β-galactosidase. After construction, plasmids pJS31, pJS32, and pJS33 were moved into KB357. Crude extracts of KB357(pJS31), KB357(pJS32) and KB357(pJS33) all showed good mutase and β-galactosidase activities. In addition, the molecular weights of the fusion proteins determined by Western

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(13) This assay used the cloned enzyme to complement the *pheA* deletion of KB357. A monofunctional chorismate mutase permitted growth of the KB357 derivative in a medium containing tyrosine and thiamine at a low pH (where prephenate was chemically converted to phenylpyruvate by acid). The same strain was unable to grow at neutral pH since the conversion of prephenate to phenylpyruvate was blocked.

(14) We found one exception to this result. Plasmid pJS3 was used to transform *E. coli* strain KS474 (relevant genotype: Δ*deg*P). A crude extract of KS474(pJS3) showed high chorismate mutase activity and very low prephenate dehydratase activity. We did not attempt to isolate the P protein fragment from this extract. The *deg*P gene products has recently been shown to be a periplasmic protease: Strauch, K. L.; Johnson, K.; Beckwith, J. *J. Bacteriol.* **1989**, *171*, 2689.

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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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(*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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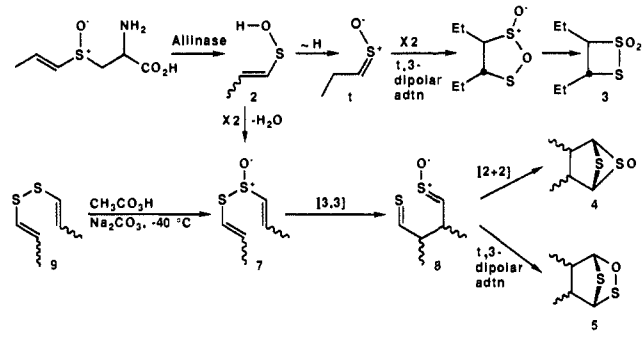
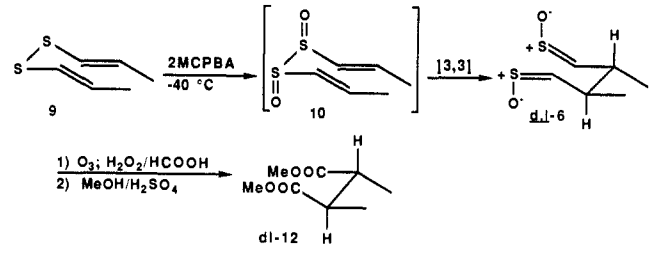
(*Z*)-Propanethial *S*-oxide (**1**), the lachrymatory factor (LF) of the onion (*Allium cepa*),[2] is unique as the only known example of a naturally occurring thiocarbonyl *S*-oxide (sulfine). It is formed in onion extracts from the stable precursor *trans*-(+)-*S*-1-propenyl-L-cysteine sulfoxide by 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).

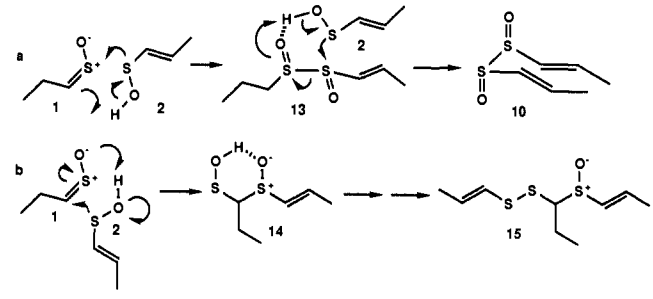
Scheme I



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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