Chemical and Spectroscopic Properties of the Binuclear Copper Active Site in *Rhus* Laccase: Direct Confirmation of a Reduced Binuclear Type 3 Copper Site in Type 2 Depleted Laccase and Intramolecular Coupling of the Type 3 to the Type 1 and Type 2 Copper Sites

C. D. LuBien,[1a] M. E. Winkler,[1a] T. J. Thamann,[1a] R. A. Scott,[1b] M. S. Co,[1b] K. O. Hodgson,[1b] and E. I. Solomon*[1a]

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
and Department of Chemistry, Stanford University
Stanford, California 94305

Received June 1, 1981

Rhus vernicifera laccase[2] contains one type 1 (blue), one type 2 (normal), and one type 3 (coupled binuclear) copper site. As the coupled binuclear site in hemocyanin[3] and tyrosinase[3a,4] has become well-defined, it is important to determine how this relates to the type 3 site in the oxidases. In native laccase the type 3 site has been associated with a 330-nm ($\epsilon \sim 2800\ M^{-1}\ cm^{-1}$) absorption band which reduces as a two-electron acceptor[5] and exhibits no additional spectral features down to 5000 cm^{-1}.[6] In azide binding studies of native laccase, $N_3^- \rightarrow$ Cu(II) charge-transfer (CT) bands[7] are observed at ~400 and 500 nm, with the 400-nm band deriving from type 2 binding at high $[N_3^-]$. Type 3 copper has been suggested to be the binding site at low $[N_3^-]$. Recently, a simplification has been achieved through reversible preparation of a type 2 depleted (T2D) derivative, first reported by Graziani et al.[8] This form is invaluable in understanding the role of type 2 copper in the mechanism and active site structure of laccase.[9-11a] This T2D derivative, however, is reported not to exhibit a 330-nm band[9] nor any azide binding[9] to type 3 copper. Anaerobic ferrocyanide titrations[10] suggested that the T2D laccase contains an oxidized type 3 site which does not show significant absorption at 330 nm. This problem is complicated by the results of Reinhammar et al.,[11a] who produce at pH 7.4 a T2D laccase by the previously reported method[8] which exhibits a 330-nm band of the same magnitude as in native laccase.[11b] We report here results which indicate that the type 3 site is, in fact, *reduced* in T2D laccase but can be oxidized, resulting in spectral features associated with the oxidized type 3 site. Further, both the type 3 and type 2 sites together are found to be involved in binding small molecules to native laccase, and changes in the oxidation state of the type 3 center produce structural changes at the type 1 site.

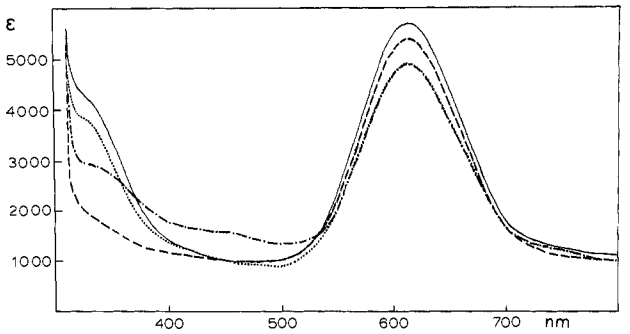

Figure 1. Room temperature absorption spectra of native and T2D laccase: (—) native; (---) T2D and T2D + 100× N_3^-; (···) T2D + 30-fold excess H_2O_2, dialyzed; (-·-) T2D + H_2O_2, dialyzed, + 100 × N_3^-. [Protein] ~0.15 mM in 0.1 M potassium phosphate buffer, pH 6.0. The ϵ values are given per mole of protein.

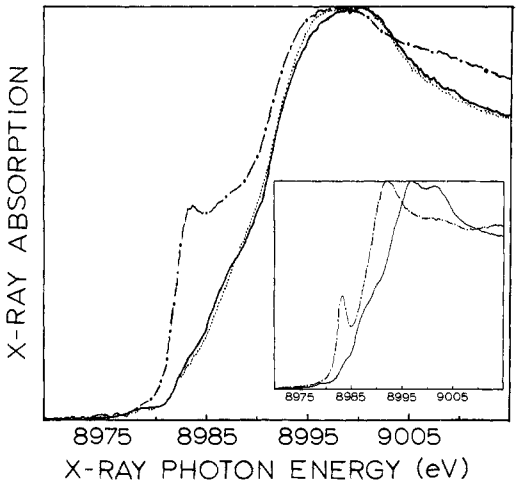

Figure 2. X-ray absorption edges for native and T2D laccase: (—) native; (---) T2D; (···) T2D, + 30× H_2O_2, dialyzed. [Cu]: native, ~5 mM; T2D and T2D + H_2O_2, dialyzed, ~6.4 mM in 0.1 M potassium phosphate, pH 6.0. Insert: X-ray absorption edges for imidazole complexes: (—) Cu(II) imidazole; (---) Cu(I) imidazole. Spectra were recorded (at -60 °C) at the Stanford Synchrotron Radiation Laboratory (SSRL) on the wiggler beam line (IV-2) using Si[220] monochromator crystals. All data were recorded as fluorescence excitation spectra by using an array of 20 NaI(Tl) scintillation counters with Ni filters.[14] The energy scale was calibrated relative to a Cu foil, with the first preedge inflection of the Cu K-absorption edge being assigned to 8980.3 eV.

The T2D protein we obtain[12] is optically similar to that of Morpurgo et al.,[9,11b] exhibiting essentially no absorption at 330 nm (Figure 1). From EPR and optical spectra it is clear that the type 1 copper is oxidized after preparation of T2D laccase. T2D protein which is treated with 30 × [protein] of H_2O_2 shows the immediate appearance of absorption bands at 330, ~400, and 745 nm without significant perturbation of the 614-nm band. In <30 min the spectral features have maximized ($\Delta\epsilon_{330} \sim 2000\ M^{-1}$

(1) (a) Massachusetts Institute of Technology. (b) Stanford University.
(2) (a) Fee, J. A. *Struct. Bonding (Berlin)* **1975**, *23*, 1-60. (b) Malkin, R.; Malmström, B. G. *Adv. Enzymol.* **1970**, *33*, 177-244.
(3) (a) Solomon, E. I. In "Copper Proteins"; Spiro, T. G., Ed.; Wiley: New York, in press. (b) Eickman, N. C.; Himmelwright, R. S.; Solomon, E. I. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2094-2098. (c) Himmelwright, R. S.; Eickman, N. C.; LuBien, C. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1980**, *102*, 5378-5388 and references therein.
(4) (a) Himmelwright, R. S.; Eickman, N. C.; LuBien, C. D.; Lerch, K.; Solomon, E. I. *J. Am. Chem. Soc.* **1980**, *102*, 7339-7344. (b) Winkler, M. E.; Lerch, K.; Solomon, E. I. *J. Am. Chem. Soc.*, in press.
(5) Reinhammar, B. *Biochim. Biophys. Acta* **1972**, *275*, 245-259.
(6) Dooley, D. M.; Rawlings, J.; Dawson, J. H.; Stephens, P. J.; Andreasson, L.-E.; Malmström, B. G.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5038-5046.
(7) Morpurgo, L.; Rotilio, G.; Finazzi-Agro, A.; Mondovi, B. *Biochim. Biophys. Acta* **1974**, *336*, 324-328.
(8) Graziani, M. T.; Morpurgo, L.; Rotilio, G.; Mondovi, B. *FEBS Lett.* **1976**, *70*, 87-90.
(9) Morpurgo, L.; Graziani, M. T.; Finazzi-Agro, A.; Rotilio, G.; Mondovi, B. *Biochem. J.* **1980**, *187*, 361-366.
(10) Morpurgo, L.; Graziani, M. T.; Desideri, A.; Rotilio, G. *Biochem. J.* **1980**, *187*, 367-370.
(11) (a) Reinhammar, B.; Oda, Y. *J. Inorg. Biochem.* **1979**, *11*, 115-127. (b) It is not clear how the T2D protein we prepare (which exhibits chemical and spectral properties parallel to those of Graziani[8,9]) relates to that of Reinhammar and Oda in ref 12a. We have been unsuccessful in directly producing T2D laccase with a 330-nm band of the same magnitude as in native protein. Further, our H_2O_2-treated T2D protein (vide infra) does not show the same EPR parameters as reported in ref 11a ($A_\parallel$ = 35 G, whereas we observe $A_\parallel$ = 40 G). Also, the 330-nm band reduces within 5 min after addition of 3 equiv of ascorbate mediated with ferrocyanide at pH 7.4 ($\Delta\epsilon$ 1500 $M^{-1}\ cm^{-1}$), again differing significantly from the reduction kinetics reported in ref 11a (which require >150 min).
(12) Native enzyme from Japanese lacquer (Saito & Co., Osaka, Japan) was purified to a ratio A_{280}/A_{614} of 14.0-16.5 as in: Reinhammar, B. *Biochim. Biophys. Acta* **1970**, *205*, 35-47; ref 11a. T2D protein was prepared by the modified method of Graziani.[8,9] Atomic absorption results for total Cu in mol of Cu/mol of protein for two preparations were native: 4.04, 4.10. T2D laccase: 3.06, 2.82. Total integrated EPR intensity was native: 1.98 ± 0.2, 2.08 ± 0.2. T2D laccase: 0.73 ± 0.1, 1.07 ± 0.1. Type 2 copper was not detected in the T2D samples based on the lack of EPR parallel hyperfine features except at high gains but could contribute weakly to the total EPR intensity. An ϵ_{614} of ≥5400 $M^{-1}\ cm^{-1}$ is estimated by EPR intensity and AA for T2D laccase.

0002-7863/81/1503-7014$01.25/0

cm^{-1}; $\Delta\epsilon_{400} \sim 175$; $\Delta\epsilon_{745} \sim 150$) while some decrease is observed at 500 and 614 nm ($\Delta\epsilon_{614} \geq -300\ M^{-1}\ cm^{-1}$, as the integrated EPR intensity remains constant). Dialysis for 24 h does not perturb this final spectrum (Figure 1, ···). The difference at 330 nm between native and H_2O_2-treated T2D protein suggests that type 2 copper may also contribute weakly ($\epsilon \sim 400\ M^{-1}\ cm^{-1}$). These results and the appearance of $N_3^- \rightarrow$ Cu(II) CT transitions, vide infra, suggest that type 3 copper is a reduced binuclear cuprous site after T2D preparation with oxidation by H_2O_2 regenerating the type 3 cupric spectral features.

An X-ray absorption edge comparison of Cu(I) and Cu(II) imidazole solutions[13a] to native, T2D and H_2O_2-treated T2D laccase unambiguously confirms these oxidation states. For a range of inorganic Cu(I) and Cu(II) complexes,[13b,15,16] the K edges appear similar in position and shape to those shown for Cu(I) and Cu(II) imidazole solutions (Figure 2, insert). The edge for native laccase (Figure 2) shows that the copper atoms are all oxidized, exhibiting only the preedge transition (1s → 3d) at 8979 eV smoothly rising to the maximum at 9000 eV characteristic of Cu(II). T2D laccase has an edge strikingly different from that of native laccase, resembling that of Cu(I) imidazole. The presence of a strong transition (1s → 4s)[13b,15,17] at 8983 eV and a maximum at 8992 eV is definitive evidence for a significant amount of Cu(I). H_2O_2-treated T2D laccase shows a loss of the 8983-eV transition and gives an edge superimposable upon that of native laccase, indicative of Cu(II). Since the EPR spectrum, reflecting only type 1 and residual type 2 copper, does not change integrated intensity, we conclude that T2D laccase contains a significant amount of type 3 Cu(I) which is oxidized upon treatment with H_2O_2. Anaerobic ferrocyanide titrations of repeatedly deoxygenated laccase provide further evidence for these oxidation states. T2D and H_2O_2-treated T2D laccase generate, respectively, 1.5 and 3.3 equiv of ferricyanide with 90% reduction of type 1 copper. As residual oxygen contributes to ferricyanide production, these numbers are upper limits on the oxidized copper. After dialysis, the 330-nm band is reduced in both derivatives but can be restored by H_2O_2 addition.

A spectral correlation of type 3 copper in laccase to met[18] hemocyanin and tyrosinase[4] shows the 745-nm band is similar in these proteins and has been assigned[3] as d–d transitions for two tetragonal cupric sites. The 330-nm region is broader and weaker in met hemocyanin than in laccase ($\epsilon_{330} < 1500\ M^{-1}\ cm^{-1}$) with a shoulder at 420 nm ($\epsilon \sim 250\ M^{-1}\ cm^{-1}$). In hemocyanin and tyrosinase the 300–350-nm region is associated with imidazole → Cu(II) charge transfer,[3,19a] while the 420-nm band has been assigned[3,20] as a $RO^- \rightarrow$ Cu(II) CT transition, probably from a bridging phenolate ligand. A strong S–Cu bond in a tetragonal cupric site can also contribute CT intensity[19b,20] in this region. While no strong sulfur ligand is present in hemocyanin,[3a] this has not yet been determined for type 3 copper in laccase.

In azide titrations of native laccase at pH 6.0, separate binding constants can be determined for the ~400- and 500-nm bands.[21]

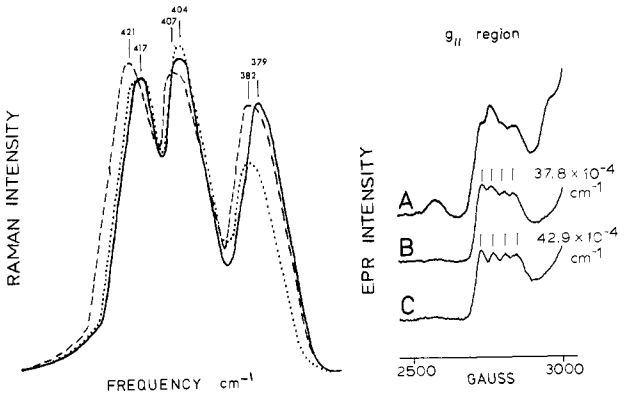

Figure 3. Resonance Raman spectra (base line corrected) of *Rhus* laccase: (—) native; (···) T2D; (---) T2D + excess H_2O_2, dialyzed. Laser irradiation was 100 mW (measured at the sample) of 568.2-nm excitation, spectral slit width 3 cm^{-1}, and the scan rate 0.2 $cm^{-1}\ sec^{-1}$. Samples were run at room temp. in a spinning cell. EPR spectra of *Rhus* laccase at 77 K: (A) native; (B) T2D; and (C) T2D + H_2O_2, dialyzed. Microwave frequency was 9.084 GHz and microwave power was 10 mW. $A_\parallel$ splittings are indicated for T2D and T2D + H_2O_2 laccase. $A_\parallel$ for native type 1 copper from EPR simulations is $43 \times 10^{-4}\ cm^{-1}$.[25] [Protein] ≥0.4 mM for resonance Raman samples, ~0.17 mM for EPR samples in 0.1 M potassium phosphate buffer, pH 6.0.

Azide addition to untreated T2D laccase produces no spectral features (Figure 1). Azide addition to a H_2O_2-treated, dialyzed T2D sample generates an $N_3^- \rightarrow$ Cu(II) CT band peaking at 450 nm ($\epsilon\ 500\ M^{-1}\ cm^{-1}$) with a low binding constant ($K_1 = 200\ M^{-1}$). Dialysis for 24 h restores the H_2O_2-treated T2D features. Thus, azide binds to oxidized type 3 copper, but the altered $N_3^- \rightarrow$ Cu(II) CT peak position and lower binding constant[21] indicate that an interaction with type 2 copper is required for the high azide affinity of native laccase. Further, although H_2O_2 is an oxidant, it is not a high-affinity ligand since (1) the spectral features of H_2O_2-oxidized T2D laccase are not affected by dialysis, (2) these spectral features are also present after addition of 100× N_3^- and subsequent dialysis, and (3) H_2O_2 addition to oxidized T2D protein previously treated with 100× N_3^- does not affect the N_3^- CT band, demonstrating that O_2^{2-} is not displacing N_3^-.[22] Therefore, unlike hemocyanin and tyrosinase, type 2 copper is also required for the high affinity of peroxide binding[23] to type 3 copper in native laccase. This type 3-type 2 involvement in binding small molecules may be associated[24] with the irreversibility of peroxide binding in its reduction to water at the type 3 site in laccase, in contrast to the reversible oxygen binding to hemocyanin and tyrosinase.

Finally, the spectral features of type 1 copper are found to change on oxidation of type 3 copper in T2D laccase. Laser excitation at 568.2 nm into the blue copper CT band for T2D laccase shows a significant decrease (~40%) in the resonance Raman intensity of the 384-cm^{-1} vibration as compared to native laccase. However, a spectrum similar to that of native laccase is obtained for oxidized T2D protein (Figure 3). In the EPR spectra, $A_\parallel$ increases from 37.8 to $42.9 \times 10^{-4}\ cm^{-1}$ upon oxidation of the T2D derivative. This is accompanied by the decrease in $\epsilon_{614} \geq -600\ M^{-1}\ cm^{-1}$ (Figure 1). These spectral changes indicate a structural change of type 1 copper upon type 3 copper oxidation. Since deuteration shifts of the 384-cm^{-1} Raman vibration suggest

(13) (a) Aqueous Cu(II) imidazole solution was prepared by dissolving crystals of $Cu(Im)_4SO_4$ in deionized water. Cu(I) imidazole solution was obtained by adding excess dithionite to the Cu(II) imidazole solution until the solution turned colorless and was immediately frozen in liquid N_2 to prevent disproportionation of copper. (b) Brown, J. M.; Powers, L.; Kincaid, B.; Larrabee, J. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4210–4216. (Note that the photon energy scale in Table I of this reference is shifted by −10 eV.)

(14) Cramer, S. P.; Scott, R. A. *Rev. Sci. Instrum.* **1981**, *52*, 395–399.

(15) Hu, V. W.; Chan, S. I.; Brown, G. S. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3821–3825.

(16) Eccles, T. K. PhD dissertation, Stanford University, Stanford, CA, 1977.

(17) Recent SCF-Xα molecular orbital calculations dictate that such assignments for complexes with appreciable covalency are not adequate descriptions (to be published).

(18) The met hemocyanin derivative[3] contains a binuclear site of strongly antiferromagnetically coupled Cu(II) ions and is therefore analogous to the oxidized type 3 site in laccase. See ref 3(a).

(19) (a) Fawcett, T. G.; Bernarducci, E. E.; Krogh-Jespersen, J.; Schugar, H. J. *J. Am. Chem. Soc.* **1980**, *102*, 2598–2604. (b) Miskowski, V. M.; Thich, J. A.; Solomon, R.; Schugar, H. J. *J. Am. Chem. Soc.* **1976**, *98*, 8344–8350.

(20) Amundsen, A. R.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6730–6739.

(21) The 410-nm band is associated with a high and a low binding constant ($K_1 \sim 15\,000\ M^{-1}$, ϵ_1 630 $M^{-1}\ cm^{-1}$, $K_2 = 60\ M^{-1}$, ϵ_2 1900 $M^{-1}\ cm^{-1}$) while the 500-nm band relates only to the high binding constant form ($K_1 \sim 31\,000\ M^{-1}$, ϵ_1 500 $M^{-1}\ cm^{-1}$). Only K_2 is associated with changes in the type 2 EPR signal, indicating binding to type 2 copper. [Protein] was 1.57×10^{-4} M in 0.1 M potassium phosphate buffer, pH 6.0.

(22) In native laccase, bound peroxide is removed by dialysis at pH 6.0 and azide and peroxide compete for the same binding site. LuBien, C. D.; Solomon, E. I., unpublished results.

(23) Farver, O.; Goldberg, M.; Pecht, I.; *Eur. J. Biochem.* **1980**, *104*, 71–77.

(24) Hoffman, A. B.; Taube, H. *Inorg. Chem.* **1968**, *7*, 1971–1976.

(25) Malmström, B. G.; Reinhammar, B.; Vänngård, T. *Biochim. Biophys. Acta* **1970**, *205*, 48–57.

substantial contribution of $\nu(Cu-N_{His})$,[26] this distortion would appear to involve a shift of an imidazole away from the $d_{x^2-y^2}$ plane of the elongated C_{3v} blue copper site[27] upon reduction of type 3 copper. This intersite structural interaction may relate to the electron-transfer pathway from type 1 to type 3 copper. Further studies on the effects of these intersite interactions are presently under way.

Acknowledgment. We thank the National Science Foundation [E.I.S. (Grant PCM 78-13714) and K.O.H. (Grant PCM 79-04915)] and the National Institutes of Health [E.I.S. (Grant AM 20406) for support. M.E.W. acknowledges the N.I.H. for a postdoctoral fellowship. We also thank the Stanford Synchrotron Radiation Laboratory (supported by NSF contract DMR 77-27489) for beam time and the M.I.T. Regional Laser Center, which is an NSF Regional Instrumentation Facility. Finally, we thank Darlene Spira for useful discussions.

(26) Larrabee, J. A.; Woolery, G.; Reinhammar, B.; Spiro, T. G. *Biochemistry*, in press.
(27) Penfield, K. W.; Gay, R. R.; Himmelwright, R. S.; Eickman, N. C.; Norris, V. A.; Freeman, H. C.; Solomon, E. I. *J. Am. Chem. Soc.* **1981**, *103*, 4382-4388.

Chemistry of Sulfenic Acids. 4.[1] The First Direct Evidence for the Involvement of Sulfenic Acids in the Oxidation of Thiols

Franklin A. Davis* and Robert L. Billmers

Department of Chemistry, Drexel University
Philadelphia, Pennsylvania 19104

Received June 26, 1981

The biological function of thiols depends, to a large extent, on the facility with which the SH group is oxidized to higher sulfur oxides (RSO_xH) and disulfides (RSSR).[2] It is generally assumed that sulfenic acids (RSOH) are transient intermediates in the former reaction, sulfur oxide formation (reaction 1).[2c] Recent

$$RSH \rightarrow RSOH \rightarrow RSO_2H \quad (1)$$

reports suggest that they may be involved in disulfide formation as well (reaction 2).[3] However, the evidence is primarily circumstantial. For example, the view that the catalytically active thiols in certain enzymes are oxidized to stable sulfenic acids rest on the following: oxidation does not produce sulfinic acids (RSO_2H) and disulfides; the oxidation product is reduced by mild reducing agents back to the active thiol; they react with nucleophiles that do not react with disulfides.[4,5] On the other hand, simple thiols,[2,6] including cysteine and derivatives[7], are oxidized by peracids or hydrogen peroxide to sulfinic acids and/or disulfides. The evidence for oxidation of 2-mercaptopyridine to 2-pyridinesulfenic acid by the neutral, aprotic oxidizing reagent (2-(benzenesulfonyl)-3-phenyloxaziridine (**2**) rests on the similarity in properties of this sulfenic acid and that prepared by flash vacuum pyrolysis (FVP) of the corresponding sulfoxide.[3a] In these examples, the sulfenic acids were not actually isolated or detected.

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (2)$$

In this communication we report the first direct evidence for the oxidation of thiols to sulfenic acids. We also propose that the sulfenic acid "α-effect" nucleophilicity is a significant contributor to the overall properties of sulfenic acids.

Fast addition of 1.0 equiv of 2-(benzenesulfonyl)-3-phenyloxaziridine (**2**) to 2 equiv of 2-methyl-2-propanethiol (**1**) in an NMR tube, with diphenylethane standard, resulted in an immediate, mildly exothermic, reaction. As indicated by NMR spectroscopy, within less than a minute, **2** was completely consumed with formation of sulfonimine **8** (>90%), sulfinic acid **7** (80%), and thiosulfinate **5** (6%).[8] Over the next 36 h benzenesulfonamide ($PhSO_2NH_2$) precipitated from solution, **7** and **8** gradually disappeared with buildup, and the disappearance of **9** (the adduct of **7** and **8**).[10] The loss of **9** corresponded to the formation of dithioacetal monosulfone **10**[12,13] and dithioacetal **11**.[14] The latter is formed from **10**, via an acid-catalyzed reaction with thiol **1**.[15] Note that when the thiol is limited the major product is the dithioacetal monosulfone **10** (compare entries 1 with 2).

2-Methyl-2-propanesulfenic acid (**3**) is one of only a very few special examples of sulfenic acids that have some stability.[1,16] The stability of **3** has generally been attributed to steric inhibition of substituent attack at the SOH group.[1] Attempts to stabilize arenesulfenic acids by steric hindrance, however, proved unsuccessful.[1] That **3** is an intermediate in the oxidation of **1** by **2** was clearly demonstrated by trapping with methyl propiolate. Slow addition of **2**, using high dilution techniques,[18] to a solution of **1** and methyl propiolate gave the vinyl sulfoxide **4** in good yield (entries 6, 7, and 10)[1,17] The stabilization of sulfenic acids by aromatic solvent has been reported,[1,16a] and the highest yield of **4** was realized in benzene (entry 10).

(1) Part 3: Davis, F. A.; Jenkins, R. H., Jr.; Rizvi, S. Q. A.; Yocklovich, S. G. *J. Org. Chem.* **1981**, *46*, 3467.
(2) (a) Kharasch, N.; Arora, A. S. *Phosphorus Sulfur* **1976**, *2*, 1. (b) Jocelyn, P. C. "Biochemistry of the SH Group"; Academic Press: New York, 1972. (c) Capossi, G.; Modena, G. "Chemistry of the Thiol Group"; Wiley: Chichester, England, 1974; Vol. 2, p 367.
(3) (a) Davis, F. A.; Jenkins, R. H.; Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7967 and references cited therein. (b) Allan, R. D.; Barton, D. H. R.; Girijavallabhan, M.; Sammes, P. G.; Taylor, M. V. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1182.
(4) Allison, W. S. *Acc. Chem. Res.* **1976**, *9*, 293.
(5) (a) Allison, W. S.; Conners, M. J. *Arch. Biochem. Biophys.* **1970**, *136*, 383. (b) Little, C.; O'Brien, P. J. *Ibid.* **1967**, *122*, 406. (c) Lin, W. S.; Armstrong, D. A.; Gaucher, G. M. *Can. J. Biochem.* **1975**, *53*, 298. (d) Glazer, A. N.; Smith, E. L. *Enzymes* **1971**, *3*, 502.
(6) Filby, W. G.; Gunther, K.; Penshorn, R. D. *J. Org. Chem.* **1973**, *38*, 4070.
(7) Oxidation of cysteine to cystine by H_2O_2: Toennies, G.; Bennett, M. A. *J. Biol. Chem.* **1936**, *112*, 497. Reid, E. E. "Organic Chemistry of Bivalent Sulfur"; Chemical Publishing Co.: New York, 1958; Vol. 1, p 460. Metabolic oxidation of cysteine to cysteinesulfinic acid: Singer, T. P. In *Metab. Pathways, 3rd Ed.* **1975**, *7*, 535. *m*-Chloroperbenzoic acid oxidation of penicillamine to penicillaminesulfinic acid: Kollonitsch, J.; Marburg, S.; Perkins, L. M. *J. Org. Chem.* **1976**, *41*, 3107.
(8) The various products (Scheme I) were determined by integration of the appropriate absorption in the NMR spectrum relative to an internal standard. Proton NMR absorptions that were monitored are as follows: **8**, N=CH, δ 9.0; **5**, $Me_3CS(0)$-, δ 1.55; Me_3CS, δ 1.36; **7**, Me_3CSO_2H, δ 1.2; **9**, SO_2NH-*CH*-Ph, δ 6.8-6.3 (m); **10**, Ph*CH*-($SCMe_3SO_2CMe_3$), δ 5.1; **11**, Ph*CH*($SCMe_3$)$_2$, δ 5.05; **4**, RS(O)*CH*=$CHCO_2Me$, 6.4 (d, J = 15 Hz). The yield of disulfide **6** was determined by GLC using a 6-ft 3% OV-17 on Anakorm Q 90/100-mesh column. 2-Methyl-2-propanesulfinic acid (**7**) was prepared according to the procedure of Pinnick and Reynolds.[9]
(9) Pinnick, H. W.; Reynolds, M. A. *J. Org. Chem.* **1979**, *44*.
(10) Attempts to isolate compound **9**, the adduct of **7** and **8**, were unsuccessful. The structure of **9** is based on the nearly identical pattern of the $PhSO_2NH$-*CH*(SO_2R)Ph protons for **9** (R = CMe_3 and R = Ph). Compound **9** (R = Ph) can be isolated and has been reported.[11] Under the reaction conditions the thiol **1** does not react with the sulfonimine **8**.
(11) Messinger, P. *Arch. Pharmacd.* **1974**, *307*, 348.
(12) Compound **10** has the following properties: mp 114-115 °C; IR (KBr) 1300 and 1120 cm^{-1} (SO_2); NMR ($CDCl_3$) δ 1.3 ($SCMe_3$), 1.38 (SO_2CMe_3), 5.1 (1 H, PhCH), and 7.3 (5 H, Ph).
(13) Satisfactory elemental analyses were obtained.
(14) Le Foc'h, Y.; Brault, A.; Kerfunto, M. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1969**, *268*, 1718.
(15) In the absences of sulfinic acid **7**, dithioacetal monosulfone **10** is stable in the presence of thiol **1**. Addition of a trace of **7** quantitatively and irreversibly transforms **10** into **11**. The mechanism for formation of **10** from **9** and **11** from **10** apparently does not involve a carbene (Ph(R)C:, R=$PhSO_2$— or RS—) formed by elimination of $PhSO_2NH_2$ and $PhSO_2$, respectively. There was no incorporation of deuterium into **10** or **11** when the oxidation of **1** by **2** was carried out with Me_3CSD.
(16) (a) Davis, K. E.; Shelton, J. R. *Int. J. Sulfur Chem.* **1973**, *8*, 197. (b) Block, E.; O'Conner, J. *J. Am. Chem. Soc.* **1974**, *96*, 3921, 3929.
(17) Under these reaction conditions 2-methyl-2-propanethiol (**1**) does not add to methyl propiolate.
(18) A Sage Model 341 syringe pump set at 0.17 mL/h and using a 5-mL syringe delivers 0.013 mmol/h.

0002-7863/81/1503-7016$01.25/0