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.

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(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

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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).

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(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.
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(6) Filby, W. G.; Gunther, K.; Penshorn, R. D. *J. Org. Chem.* **1973**, *38*, 4070.
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(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**, $Me_3CS(O)$-, 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.

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Table I. Oxidation of 2-Methyl-2-propanethiol (**1**) by 2-Benzenesulfonyl-3-phenyloxaziridine (**2**) at 25 °C for 36 h

entry	mole ratio of 1/2	conditions	product yield,[a] % RS(O)CH=CHCO$_2$Me (4)	RS(O)SR (5)	RSSR (6)	PhCH(SO$_2$R)SR	PhCH(SR)$_2$ (11)	PhSO$_2$N=CHPh (8)	PhCHO[f]
1	1:1	$CDCl_3$ fast[b]		5		35	2	24	18
2	2:1	$CDCl_3$ fast[b]		6		35	40		7
3	10:1	$CDCl_3$ fast[b]		8		35	40		
4	2:1	$CDCl_3$ slow[c]		18		27	20	15	27
5	10:1	$CDCl_3$ slow[c]		18	3	35	50		
6	10:1	benzene slow		20	9	30	60		
7	10:1	benzene slow[d]		15	16	81			
8	1:1	$CDCl_3$ slow[e]	25	24				29	31
9	1:1	Et_2O slow[e]	25	25				50	17
10	1:1	benzene slow[e]	47	44				50	30

[a] Yields are based on **2** and calculated from the NMR data, see ref 8. [b] Fast addition of **2** to the solution of **1** in $CDCl_3$. [c] Slow addition of **2** to a solution of **1**. See ref 18. [d] 1 equiv of 2-methyl-2-propanesulfinic acid (**7**) present. [e] Slow addition of **2** to a methyl propiolate solution of **1**. [f] See ref 27.

One source of thiosulfinate **5**, observed in the oxidation of **1** by **2**, is sulfenic acid dehydration (reaction 3) the principal reaction

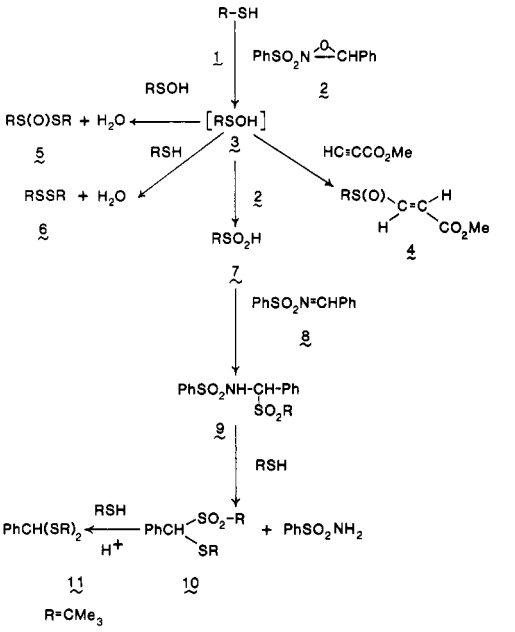
$$2RSOH \rightarrow RS(O)SR + H_2O \quad (3)$$

of simple sulfenic acids.[1,16,19,20] Alternatively, oxidation of disulfide **6**, formed as shown in reaction **2**,[2,21] would also afford thiosulfinate **5**. Disulfide **6**, observed under conditions where thiol **1** is in large excess, is undoubtedly formed in this manner (entries 5–7). Furthermore, a competitive experiment, carried out by oxidizing equivalent amounts of **1** and **6** with **2** revealed that di-*tert*-butyl disulfide (**6**) is actually oxidized to the thiosulfinate **5** four times faster than is **1**.

In the absence of acid the rate of reaction 2 is negligable compared to reaction 3. This was demonstrated by the treatment of 2-methyl-2-propanesulfenic acid (**3**) with a ca. 50 molar excess of **1**.[23] Under these conditions a 60% yield of **5** was obtained with less than 1% disulfide being detected. When the oxidation of **1** by **2** was carried out in the presence of 1 equiv of sulfinic acid **7**, an increase in disulfide **6** was noted. However, the increase in thiosulfinate **5** was negligable (compare entries 6 and 7). In the presence of acid a protonated sulfenic acid ($RSOH_2^+$) should be a highly electrophilic species, facilitating disulfide formation (reaction 2).[24] These results suggest, under our conditions, that the rate of sulfenic acid dehydration (reaction 3) is faster than sulfenic acid disulfide formation (reaction 2) and that both of these reactions are slow compared to the oxidation of the sulfenic acid to the sulfinic acid (reaction 1).

It has frequently been necessary, in order to interpret many reactions where sulfenic acids are proposed intermediates, to attribute unusually high nucleophilicity to them.[24,25] Kice and Cleveland have suggested that sulfenic acids may be α-effect nucleophiles and have reported that benzenesulfenic acid (PhSOH) is some 10^4–10^5 times more nucleophilic toward sulfenyl sulfur (RS–X) than is water.[25a] However, both quantitative and qualitative comparisions with other nucleophiles such as thiols was not possible. The preferential oxidation of 2-methyl-2-propanesulfenic acid (**3**), even in the presence of a large excess of thiol **1**, clearly demonstrates the much greater nucleophilicity of the sulfenic acid over the corresponding thiol. This property of sulfenic acids is undoubtedly responsible for the inability to detect or isolate sulfenic acids in the oxidation of thiols[2] as well as the fact, discussed by Kice et al., that hydrolysis of sulfenyl derivatives is not a feasible route to sulfenic acids.[25a]

Scheme I



In summary, our results provide the first unambigous evidence for the involvement of sulfenic acids in the oxidation of thiols to higher sulfur oxides (reaction 1). For simple sulfenic acids, which are dual nucleophiles/electrophiles (reaction 1/reaction 2),[19] the nucleophilic component predominates. In the presence of acids, where a protonated sulfenic acid ($RSOH_2^+$) may be involved, sulfenic acid electrophilicity becomes somewhat more important. Although the oxidation of **1** to 2-methyl-2-propanesulfenic acid (**3**) can be considered a special case because of the relative stability of this sulfenic acid, we believe that our results are relevant to the oxidation of thiols in general. Indeed, oxidation of aromatic thiols by **2** gave similar products (Scheme I).[26] Furthermore, our results suggest that the oxidation of thiols by neutral, aprotic

(19) For recent reviews of the chemistry of sulfenic acids see: Kice, J. L. *Adv. Phys. Org. Chem.* **1980**, *17*, 65. Hogg, D. R. in *Compr. Org. Chem.* **1979**, *4*, 261.

(20) Davis, F. A.; Friedman, A. J.; Nadir, U. K. *J. Am. Chem. Soc.* **1978**, *100*, 2844.

(21) The reaction $RSO_2H + RSH \rightarrow RS(O)SR + H_2O$ has been proposed.[22] However, when R = CMe_3 this reaction does not occur.

(22) Cipris, D.; Pouli, D. *Synth. Commun.* **1979**, *9*, 207.

(23) 2-Methyl-2-propanesulfenic acid (**3**) was prepared by FVP of di-*tert*-butyl sulfoxide at 350 °C[1] and deposited on a cold finger along with a 10% toluene solution of 2-methyl-2-propanethiol (**1**).

(24) Ciuffarin, E.; Gambarotta, S.; Isola, M.; Senatore, L. *J. Chem. Soc. Perkin Trans. 2* **1978**, 554.

(25) For discussions of the α effect, as related to sulfenic acids, see: (a) Kice, J. L.; Cleveland, J. P. *J. Am. Chem. Soc.* **1973**, *95*, 104. (b) Davis, F. A.; Rizvi, S. Q. A.; Ardecky, R.; Gosciniak, D. J. *J. Org. Chem.* **1980**, *45*, 1650 and references cited therein.

(26) For example, oxidation of 2 equiv of benzenethiol (**1**, R = Ph) by **2** gave after 36 h **10** (13%), **11** (40%), PhCHO (35%), **6** (45%), and $PhSO_2SPh$ (10%). Adduct **9** can be observed at the beginning of the reaction and isolated. Reaction of **9** (R = Ph) with benzenethiol affords **10** and **11**. See also ref 10. Interpretation of the oxidation of aromatic thiols by **2** is complicated because of the instability of aromatic thiosulfinates **5** and the facile reaction of aromatic thiols with methyl propiolate and with arenesulfinic acids.[21] An additional complication is the difficulty in monitoring the course of the reaction by NMR spectroscopy.

(27) Benzaldehyde is formed by the acid-catalyzed hydrolysis of sulfonimine **8**. Water results from sulfenic acid–disulfide formation (reaction 2) and from sulfenic acid dehydration (reaction 3).

reagents such as **2** will be of use in preparing and elucidating the chemistry of sulfenic acids.

Finally, the possibility that the nucleophilic catalytic function of certain sulfhydryl enzymes actually involve transient sulfenic acids needs to be seriously considered. The acyl phosphatase activity of glyceraldehyde-3-phosphate dehydrogenase has, for example, been suggested by Allison[4,5a] and others[5b-d] to involve a stable sulfenic acid.

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Oxygen Chiral Phosphodiesters. 5. Stereochemical Course of the Hydrolysis of Thymidine 3′-[(4-Nitrophenyl)[^{17}O,^{18}O]phosphate] in $H_2{}^{16}O$ Catalyzed by the Exonuclease from Bovine Spleen

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Determination of the stereochemical course of nucleophilic displacement reactions at phosphorus catalyzed by enzymes is now considered to be the most direct experimental method for determining whether the enzyme-catalyzed reaction involves the formation of a covalent adduct between the enzyme and substrate. Although the use of chiral phosphorothioate substrate analogues to solve these stereochemical problems is often the experimentally easiest approach,[1,2] fears that the results obtained from this approach are mechanistically ambiguous due to the low rates at which these analogues are processed by enzymes has led to the development of methodology for the synthesis and configurational analysis of phosphate mono- and diesters which are chiral by virtue of the three stable isotopes of oxygen.[3-6] The stereochemical consequences of the reactions catalyzed by seven enzymes have now been determined with both oxygen chiral and phosphorothioate substrates;[7] in each case, the stereochemical outcomes obtained from the two approaches were identical, suggesting that sulfur substitution is not expected to alter the stereochemical course of enzyme-catalyzed displacement reactions at phosphorus. In this communication we report the stereochemical course of the hydrolysis reaction catalyzed by the exonuclease from bovine spleen using one of the diastereomers of thymidine 3′-[(4-nitrophenyl)[^{17}O,^{18}O]phosphate]([^{17}O,^{18}O]-TpNP) as substrate. With this enzyme, stereochemical studies of the hydrolysis reaction using the phosphorothioate analogue of TpNP, Tp(S)NP, do not appear to be feasible, since the enzyme does not readily catalyze the hydrolysis of either diastereomer of Tp(S)NP; instead, the enzyme catalyzes a transphosphorylation reaction to yield oligonucleotides as products.[8] Both the retention of the configuration observed for the hydrolysis of [^{17}O,^{18}O]-TpNP and the transphosphorylation reaction observed with the separate diastereomers of Tp(S)NP are in accord with the formation of a nucleotidylated enzyme intermediate during the course of the reaction.

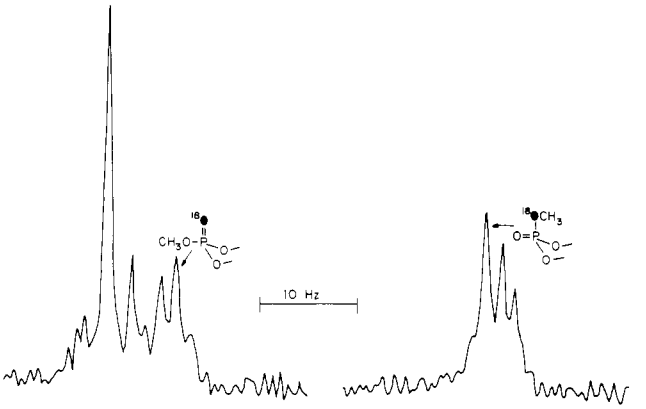
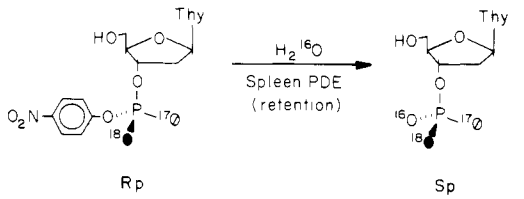


Figure 1. Proton-decoupled ^{31}P NMR spectrum at 81 MHz of the methyl esters of isotopically labeled cTMP derived from enzymatic hydrolysis of the R_P diastereomer of [^{17}O,^{18}O]TpNP in $H_2{}^{16}O$ followed by chemical activation and cyclization. The spectrum was obtained with a 1600-Hz sweep width and a 2-s acquisition time; 400 transients were obtained prior to application of a 0.1-Hz line broadening and Fourier transformation. The approximate chemical shift of the diester and the equatorial methyl ester is −2.7 ppm and that of the axial ester is −3.5 ppm (upfield, relative to an external capillary of 85% H_3PO_4).

Scheme I



The diastereomers of [^{17}O,^{18}O]-TpNP were prepared by reaction of the sodium salts of the diastereomerically pure [^{17}O]-enriched *P*-anilidates of 5′-(monomethoxytrityl)thymidine 3′-[(4-nitrophenyl)phosphate] with $C^{18}O_2$.[9,10] After removal of the trityl groups, the ^{17}O,^{18}O-chiral diesters were purified by chromatography on Amberlite XAD-2. The products which were obtained were identical with an authentic sample of TpNP (Sigma) using the criteria of TLC and ^{1}H NMR spectroscopy at 270 MHz; the ^{31}P NMR spectra at 32 and 81 MHz revealed the expected ratio of ^{16}O,^{18}O and ^{18}O,^{18}O resonances.[11] Instead of assuming that the stereochemical outcome of the reaction of the acyclic

† NIH Research Career Development Awardee (CA-00499), 1978-1983; Fellow of the Alfred P. Sloan Foundation, 1981-1983.

(1) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.
(2) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.
(3) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Bockhoff, F. M.; McLafferty, F. W.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 4323.
(4) Cullis, P. M.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1978**, 512.
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(6) Baraniak, J.; Lesiak, K.; Sochacki, M.; Stec, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 4533.
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(8) At pH 5.7, the rate of release of 4-nitrophenol from either diastereomer of Tp(S)NP is approximately 50-fold less than that observed with TpNP.

(9) Gerlt, J. A., Mehdi, S.; Coderre, J. A.; Rogers, W. O. *Tetrahedron Lett.* **1980**, *21*, 2385.

(10) The isotopic composition of the [^{17}O]$POCl_3$ used to prepare the *P*-anilidates was determined by reaction of an aliquot with excess methanol followed by gas chromatography/mass spectral analysis of the resulting trimethyl phosphate: ^{16}O, 18.8%; ^{17}O, 49.2%, ^{18}O, 32.0%. The isotopic composition of the $C^{18}O_2$ specified by the supplier (Prochem) was 99%.

(11) Since the carbon dioxide used to prepare the chiral diesters was 99% enriched we only observe resonances arising from the ^{16}O and ^{18}O present in the ^{17}O-enriched position.

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