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Reactive sulfur species: hydrolysis of β-sulfinyl esters to give a sulfenic acid in aqueous solution

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This paper is dedicated to Professor Juzo Nakayama on the occasion of his 65th birthday and retirement.

An efficient method for synthesizing the sulfenic acid derivative of 5-mercapto-2-nitrobenzoic acid in aqueous medium is described. The electronic spectrum is reported ($\lambda_{max} = 490$ nm, $\varepsilon = 11,600\,M^{-1}\,cm^{-1}$).

Keywords: sulfenic acid; sulfenate; Ellman's reagent; UV-vis spectroscopy; hydrolysis

1. Introduction

Functional sulfenic acid moieties have been found in native proteins, *e.g.* NADH peroxidase (*1*), NADH oxidase (*2*), nitrile hydratase (*3*), and certain peroxiredoxins (*4–8*). In addition, sulfenic acid derivatives have been implicated in the redox status regulation of certain cellular functions. However, in contrast to the relatively stable sulfenic derivatives of cysteine that have been identified in proteins, small molecular sulfenic acids are considerably less stable (*9*, *10*). Despite the fact that small molecular derivatives are often proposed or implicated, we are aware of few examples of sulfenates being observed in an aqueous environment. Nonetheless, sulfenic acids are likely key intermediates in the interconversion of many thiol redox derivatives. For example, of the 16 possible two-step and three-step mechanisms for the hydrolysis of RS(=O)SR to give a 1:1 mixture of RSO_2H:RSSR, 15 involve RSOH (*11*). Furthermore, since the first step of the hydrolysis reaction is usually rate-limiting (reaction of RS(=O)SR with OH^-), it is not possible to differentiate between these possible mechanisms on the basis of the rate law alone (*11*).

A "red complex" is produced from alkaline solutions of 5,5'-dithiobis(2-nitrobenzoate) (DTNB, Ellman's reagent) that has been previously attributed to the sulfenate ($TNBO^{2-}$)[1] (*12*). Unfortunately, TNBO cannot be prepared cleanly by hydrolysis of DTNB, because DTNB and TNBO react to give 5-mercapto-2-nitrobenzoic acid (TNB) and the corresponding sulfinate ($TNBO_2$) (*12*). The latter reaction limits the concentration of TNBO that can be prepared by hydrolysis of DTNB (because higher concentrations of DTNB yield less TNBO and more $TNBO_2$). Lower yields of TNBO are also obtained at lower pH (because the hydrolysis reaction is slower, giving more

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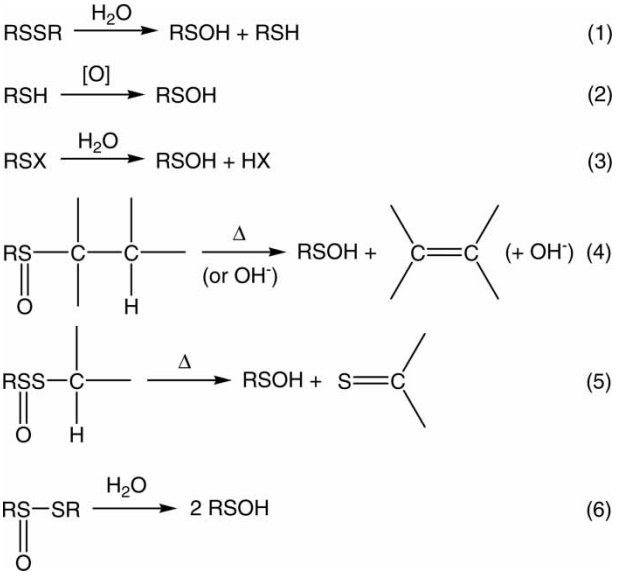
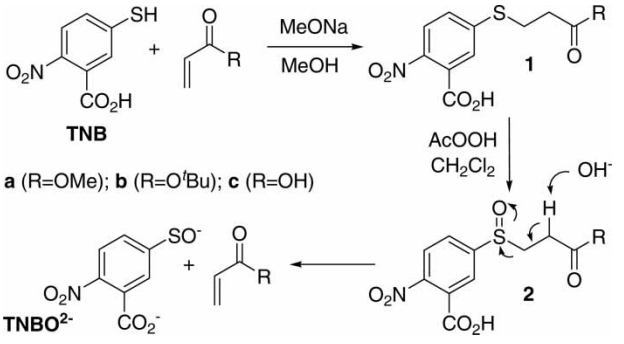
time for TNBO to react with DTNB). A further complication is that TNBO is oxygen-sensitive, oxidizing to the sulfinic acid ($TNBO_2$) in air (*12*). Thus, in addition to one (theoretical) molar equivalent of TNB, hydrolysis also produces $TNBO_2$ (via O_2 oxidation of TNBO and through the aforementioned reaction of DTNB with TNBO). Besides the complication of low chemical yields of TNBO (which results in a UV-vis spectrum that is dominated by TNB), in many cases these TNB-derived side products will interfere with mechanistic investigations. Because the aqueous chemistry of sulfenic acids is poorly understood, we have sought an effective means of synthesizing TNBO. We reason that TNB derivatives are likely to exhibit signature absorption spectra that may facilitate the study of their reaction mechanisms. We report here the efficient generation of TNBO *in situ* by hydrolysis of sulfoxide derivatives of TNB that bear electron-withdrawing substituents in the β position.

2. Results and discussion

Scheme 1 summarizes the principal methods that have been previously employed to synthesize sulfenic acids (*13*). We have investigated several of these methods for synthesizing TNBO. The problems associated with hydrolysis of DTNB to give TNBO (Scheme 1, method 1) were discussed in the introduction. Oxidation of TNB (Scheme 1, method 2) has proven to be ineffective as well, either because the reaction proceeds via one-electron pathways and presumably DTNB (*e.g.* H_2O_2) or because the O-atom transfer (two-electron) reaction "over-oxidizes" TNB (*e.g.* by HOCl and HOBr) (unpublished results). Hydrolysis of sulfenyl derivatives (Scheme 1, method 3; *e.g.* the sulfenyl chloride) and hydrolysis of the corresponding thiosulfinate ester (Scheme 1, method 6) yield TNBO, but the precursors are relatively difficult to synthesize, and poor yields are observed (unpublished results). Thermolysis of sulfoxides and thiosulfinate esters (Scheme 1, methods 4 and 5, respectively) has proven to be effective for the non-aqueous synthesis of sulfenic acids (*14*, *15*), but this method is presumably not appropriate for synthesis in water, as sulfenic acids are thermally labile in aqueous solutions. However, we report here the efficient generation of TNBO *in situ* by hydrolysis of sulfoxide derivatives of TNB (Scheme 1, method 4 with OH^-). The synthetic approach that we employed is summarized in Scheme 2.



Scheme 1.



Scheme 2.

We synthesized the thioethers **1a–c** by nucleophilic addition of TNB^{2-} to the corresponding acrylic acid derivative. We synthesized the corresponding sulfoxide derivatives **2a–c** by oxidation with AcOOH. The Me derivative **2a** did not give good yields of TNBO because the ester moiety hydrolyzed with a comparable rate to give the corresponding carboxylic acid **2c**. We independently determined that **2c** also gives low yields of TNBO (as a consequence of the fact that the carboxylate is a poor electron withdrawing functional group). However, the ^{t}Bu ester was found to be less susceptible to hydrolysis, and the corresponding sulfoxide derivative **2b** proved to be an effective synthetic precursor for TNBO. Higher yields of TNBO were observed for more basic solutions, and near quantitative yield of TNBO was obtained for the hydrolysis of **2b** in 2 M NaOH. Under the alkaline conditions of the hydrolysis reaction, the α,β-unsaturated ester that is presumably produced (Scheme 2) is also hydrolyzed (yielding non-electrophilic propenoic acid that is not likely to interfere in the reactions of interest). This outcome is fortunate, as electronic deficient alkenes and alkynes have been previously used to trap sulfenic acids (*16*, *17*). We note that TNBO is air-sensitive, and better yields are obtained under anaerobic *vs.* aerobic conditions. Thus, only 73% yield of TNBO was obtained under aerobic conditions at pH 13, but the yield improved to 90% under anaerobic conditions. Under aerobic conditions, we observed TNBO is less prone to air oxidation under more alkaline conditions.

TNBO has not yet been isolated, although it has been characterized previously by its unique UV-vis spectrum (*12*). Our efforts to isolate TNBO have not as yet proven to be successful. We have based our analysis of yields *in situ* on a previously reported (*12*) extinction coefficient for TNBO, 11,600 $M^{-1}\,cm^{-1}$ at 490 nm,[2] and we believe that this value is correct based upon convergence of the maximum absorbance we observed while optimizing the yield. In addition,

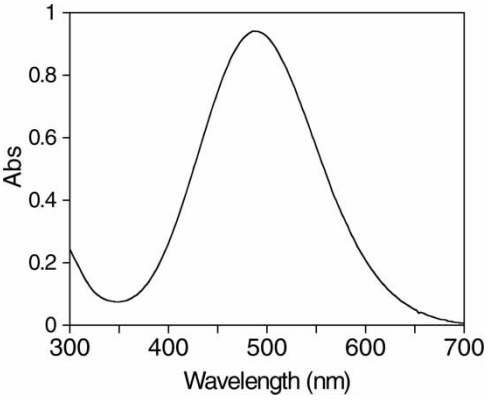


Figure 1. UV-vis spectrum of TNBO (84 μM) at pH 14.

near quantitative yields of TNBO in aqueous solution are indicated by the clean UV-vis spectra that are observed upon hydrolysis of **2b** under the optimized conditions (Figure 1), which show no indication of the presence of other derivatives of TNB that generally exhibit unique UV-vis spectral signatures (*e.g.* TNB, DTNB, $TNBO_2$, etc.). Now that an efficient synthesis has been devised, we intend to investigate the mechanisms of reactions that likely involve TNBO.

3. Experimental

All chemicals were ACS certified grade or better. A literature procedure was used to synthesize TNB (*18*). Water was doubly-distilled in glass. Solutions of NaOH, mostly free of CO_2 contamination, were quantified by titration with a standardized HCl solution using phenolphthalein as an indicator. Electronic spectra were measured using a HP 8452A diode array spectrophotometer. ^{1}H and ^{13}C NMR measurements were made with a Varian Inova 300 MHz NMR spectrometer using a Varian 4-nuclei switchable 5 mm probe, while employing deuterated solvent as a frequency lock. Chemical shifts were referenced to internal TMS. A Micromass Q-TOF was used to measure the time-of-flight electrospray mass spectra in negative ion mode (TOF MS ES$^-$).

3.1. *5-[(2-methyloxycarbonyl)ethylthio]-2-nitrobenzoic acid (1a)*

To a solution containing TNB (0.30 g, 1.5 mmol), methyl acrylate (0.41 ml, 4.5 mmol) and 5 ml of MeOH, MeONa (0.24 g, 4.5 mmol) was added at 0 °C under N_2 atmosphere. The reaction mixture was stirred and allowed to warm to rt overnight, 1 *N* HCl (5 ml) was added, and the organic solvents were removed under vacuum. Note that if excess HCl is added when the reaction mixture is neutralized, the ester moiety is also hydrolyzed. To the residue from evaporation was added AcOEt (20 ml) and water (20 ml), and the organic layer was extracted with AcOEt (3 × 20 ml). The combined organic extractions were dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated to give **1a** in quantitative yield (>95% purity). ^{1}H NMR in CD_3OD δ 2.73 (2 H, t, $J = 7.0$ Hz), 3.33 (2 H, t, $J = 7.0$ Hz), 3.67 (3 H, s), 7.48 (1 H, dd, $J = 8.5$, 2.1 Hz), 7.52 (1 H, d, $J = 2.4$ Hz), 7.89 (1 H, d, $J = 8.5$ Hz). ^{13}C NMR δ 28.0, 34.4, 52.6, 125.8, 127.7, 129.1, 132.6, 145.7, 146.5, 169.6, 173.5. MS: M–(H), 84. mp: 111.2 °C. HRMS (TOF) [M–H]$^-$ calcd for $[C_{11}H_{11}N_1O_6S_1–H]^-$: 284.0299, found: 284.0225.

3.2. *5-[(2-tert-butyloxycarbonyl)ethylthio]-2-nitrobenzoic acid (1b)*

To a flask containing TNB (0.57 g, 2.9 mmol) and *tert*-butyl acrylate (1.3 ml, 8.7 mmol) in 10 ml MeOH, MeONa (1.27 g, 8.7 mmol) was added at 0 °C under a N_2 atmosphere. After stirring the mixture for 24 h, 1 *N* HCl (9 ml) was added to acidify and the solvents were evaporated under vacuum. The product was worked up as for **1a**. **1b** was obtained in 96% yield (>95% purity). ^{1}H NMR in CD_3OD δ 1.46 (9 H, s), 2.64 (2 H, dd, $J = 7.0$, 6.7 Hz), 3.32 (2 H, dd, $J = 7.0$, 6.7 Hz), 7.56 (1 H, dd, $J = 8.5$, 2.3 Hz), 7.59 (1 H, d, $J = 1.8$ Hz), 7.92 (1 H, d, $J = 8.2$ Hz). ^{13}C NMR δ 28.3, 28.4, 35.7, 82.5, 125.8, 127.8, 129.6, 130.8, 146.0, 146.7, 168.4, 172.3. MS: M – (H), 100. HRMS (TOF) [M–H]$^-$ calcd for $[C_{14}H_{17}N_1O_6S_1–H]^-$: 326.0698, found: 326.0688.

3.3. *5-(2-carboxyethylthio)-2-nitrobenzoic acid (1c)*

To a solution containing TNB (1.00 g, 5.0 mmol), acrylic acid (1.0 ml, 15 mmol) and 15 ml of MeOH, MeONa (0.82 g, 15 mmol) was added at 0 °C under a N_2 atmosphere. The reaction was worked up as for **1a**. **1c** was obtained in quantitative yield (>90% purity). ^{1}H NMR in CD_3OD

δ 2.71 (2 H, t, $J = 7.0$ Hz), 3.34 (2 H, t, $J = 7.0$ Hz), 7.56 (1 H, dd, $J = 8.5$, 2.0 Hz), 7.59 (1 H, d, $J = 1.8$ Hz), 7.92 (1 H, d, $J = 8.5$ Hz). ^{13}C NMR δ 28.2, 34.4, 125.9, 127.9, 129.7, 131.0, 146.2, 146.9, 168.6, 174.9. MS: 2 M–(H), 84; M–(H), 26.

3.4. *5-[(2-methyloxycarbonyl)ethylsulfinyl]-2-nitrobenzoic acid (2a)*

To a solution of compound **1a** (0.44 g, 1.6 mmol) in 10 ml of CH_2Cl_2, AcOOH (320 µl of 6.85 M, 2.2 mmol) was added at 0 °C under N_2 atmosphere. The reaction mixture was stirred at 0 °C for 30 min, Na_2SO_3 aq was added (10 ml of a saturated solution), 1 *N* HCl (to a pH of 3–4) was added to the aqueous layer for acidification, and the product was extracted with AcOEt (3 × 20 ml). Too much amount of acid could cause the hydrolysis of the ester. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed with a rotary evaporator. After purification by silica gel column ($MeOH/CH_2Cl_2 = 1/8$), **2a** was obtained in 89%. ^{1}H NMR in CD_3OD δ 2.61 (1 H, ddd, $J = 17.3$, 7.3, 6.5 Hz), 2.82 (1 H, dt, $J = 17.3$, 7.3 Hz), 3.15 (1 H, ddd, $J = 13.8$, 7.3, 6.5 Hz), 3.46 (1 H, dt, $J = 13.8$, 7.3 Hz), 3.64 (3 H, s), 7.90 (1 H, dd, $J = 8.5$, 2.1 Hz), 8.02 (1 H, d, $J = 1.8$ Hz), 8.07 (1 H, d, $J = 8.5$ Hz). ^{13}C NMR δ 26.8, 51.7, 52.8, 125.9, 126.7, 127.7, 134.4, 149.1, 151.1, 169.4, 173.1. MS: M–(H), 86; 2 M, 78. HRMS (TOF) $[M–H]^-$ calcd for $[C_{11}H_{11}N_1O_7S_1–H]^-$: 300.0178, found: 300.0187. UV-vis in H_2O, 1% MeOH = 282 nm; $\varepsilon = 5900$ ($\pm$500) M^{-1} cm^{-1}.

3.5. *5-[(2-tert-butyloxycarbonyl)ethylsulfinyl]-2-nitrobenzoic acid (2b)*

To a flask containing the thioether **1b** (1.6 g, 5.0 mmol) in 20 ml CH_2Cl_2, AcOOH (730 µl of 6.85 M, 5.0 mmol) was added at 0 °C under N_2 atmosphere. After stirring the mixture for 3 h, the reaction was worked up as for **2a**. After filtration, evaporation of the solvents, and silica gel chromatography, the sulfoxide **2b** was obtained in 75% yield. ^{1}H NMR in CD_3OD δ 1.41 (9 H, s), 2.54 (1 H, m), 2.70 (1 H, m), 3.14 (1 H, m), 3.40 (1 H, m), 8.00 (1 H, dd, $J = 8.5$, 1.8 Hz), 8.07 (1 H, d, $J = 8.5$ Hz), 8.15 (1 H, d, $J = 1.8$ Hz). ^{13}C NMR δ 27.7, 28.3, 51.5, 82.6, 125.9, 127.2, 129.2, 129.8, 148.9, 151.5, 166.7, 171.5. UV-vis in H_2O, 1% MeOH = 282 nm; $\varepsilon = 5400$ ($\pm$100) M^{-1} cm^{-1}. MS: M–(H), 100. HRMS (TOF) $[M–H]^-$ calcd for $[C_{14}H_{17}N_1O_7S_1–H]^-$: 342.0648, found: 342.0639.

3.6. *5-(2-carboxyethylsulfinyl)-2-nitrobenzoic acid (2c)*

To a solution of compound **1c** (5.02 mmol) in 30 ml of CH_2Cl_2, 6.85 M AcOOH (1.03 ml of 8.85 M, 7.03 mmol) was added at 0 °C under N_2 atmosphere. After stirring the mixture for 60 min, the reaction was worked up as for **2a**. After filtration, evaporation of the solvents, and silica gel chromatography, the sulfoxide **2c** was obtained in 36% yield. ^{1}H NMR in CD_3OD δ 2.59 (1 H, ddd, $J = 17.6$, 7.3, 6.2 Hz), 2.80 (1 H, dt, $J = 17.3$, 7.3 Hz), 3.14 (1 H, ddd, $J = 13.5$, 7.3, 6.2 Hz), 3.44 (1 H, dt, $J = 14.0$, 7.3 Hz), 8.02 (1 H, dd, $J = 8.5$, 2.1 Hz), 8.09 (1 H, d, $J = 8.2$ Hz), 8.17 (1 H, d, $J = 1.8$ Hz). ^{13}C NMR δ 27.1, 52.3, 125.9, 126.5, 126.9, 135.9, 149.1, 150.9, 170.8, 174.7. MS: 2 M–(H), 100; M–(H), 23.

3.7. *Synthesis of $TNBO^{2-}$ from 2a with OH^-*

To a solution of **2a** (108.85 µM in 0.5 ml MeOH and 49.5 ml H_2O) was added 2.324 M NaOH in 1:1 ratio, and mixed well. Then, UV-vis was taken immediately (<30 s from the addition of the base). Based on molar absorption coefficient of TNBO ($\varepsilon_{490} = 11,600$), the yield of TNBO was calculated (76%).

3.8. *Synthesis of $TNBO^{2-}$ from 2b with OH^-*

To a solution of **2b** (169 μM in 0.5 ml MeOH and 49.5 ml H_2O) was added 2.324 M NaOH in 1:1 ratio, and mixed well. Then, UV-vis was taken immediately (<30 s from the addition of the base). Based on molar absorption coefficient of $\varepsilon_{490} = 11,600$, the yield of TNBO was calculated (96%). The $[OH^-]$ has a marked effect on the yield: 73–99% for 0.1–2.0 M. Also, solutions of TNBO that are prepared aerobically decompose rapidly due to air oxidation to $TNBO_2$. In contrast, samples of TNBO that were prepared in a N_2 atmosphere were found to be much more stable (*e.g.* about 11% decomposition of TNBO was observed for a 5 μM solution of TNBO at pH 13 under an N_2 atmosphere over a period of 16 h).

3.9. *Reaction of 2c with OH^-*

The formation of TNBO from **2c** is slow compared with the reaction of **2a** and **2b**. After 1 h, the yield of TNBO was only 9%, and its formation was accompanied by significant decomposition.

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Notes

1. Note that references to compounds without charges are inclusive of all acid/base derivatives (*e.g.* TNBO), whereas specific acid/base derivatives are referred to by adding charges and/or "H" to the root names (*e.g.* TNBOH and $TNBO^{2-}$ is the conjugate acid and base, respectively).
2. Note that the value of 11,600 M^{-1} cm^{-1} is reported in the text, but a different value is reported in the abstract of (*12*).

References

(1) Yeh, J.I.; Claiborne, A. *Meth Enzymol*. **2002,** *353*, 44–54.
(2) Mallett, T.C.; Parsonage, D.; Claiborne, A. *Biochemistry* **1999,** *38*, 3000–3011.
(3) Endo, I.; Nojiri, M.; Tsujimura, M.; Nakasako, M.; Nagashima, S.; Yohda, M.; Odaka, M. *J. Inorg. Biochem.* **2001,** *83*, 247–253.
(4) Claiborne, A.; Yeh, J.I.; Mallett, T.C.; Luba, J.; Crane, E.J., III; Charrier, V.; Parsonage, D. *Biochemistry* **1999,** *38*, 15407–15416.
(5) Claiborne, A.; Miller, H.; Parsonage, D.; Ross, R.P. *FASEB J.* **1993,** *7*, 1483–1490.
(6) Claiborne, A.; Ross, R.P.; Parsonage, D. *Trends Biochem. Sci.* **1992,** *17*, 183–186.
(7) Claiborne, A.; Mallett, T.C.; Yeh, J.I.; Luba, J.; Parsonage, D. *Adv. Protein Chem.* **2001,** *58*, 215–276.
(8) Van den Broek, L.A.G.M.; Delbressine, L.P.C.; Ottenheijm, H.C.J. In *The Chemistry of Sulphenic Acids and Their Derivatives*; Patai, S., Ed.; John Wiley: New York, 1990, 701–721.
(9) Nagy, P.; Lemma, K.; Ashby, M.T. *J. Org. Chem.* **2007,** *72*, 8838–8846.
(10) Nagy, P.; Ashby, M.T. *J. Am. Chem. Soc.* **2007,** *129*, 14082–14091.
(11) Nagy, P.; Ashby, M.T. *Chem. Res. Tox.* **2007,** *20*, 1364–1372.
(12) Blakeley, R.L.; Riddles, P.W.; Zerner, B. *Phosphorus Sulfur Silicon Related Elem.* **1980,** *9*, 127–135.
(13) Drabowicz, J.; Lyzwa, P.; Mikolajczyk, M. In *The Chemistry of Sulphenic Acids and Their Derivatives*; Patai, S., Ed.; John Wiley: New York, 1990, 187–220.
(14) Adams, H.; Anderson, J.C.; Bell, R.; Neville Jones, D.; Peel, M.R.; Tomkinson, N.C.O. *J. Chem. Soc. Perkin Trans. I* **1998,** 3967–3974.
(15) Janssen, J.W.A.M.; Kwart, H. *J. Org. Chem.* **1977,** *42*, 1530–1533.
(16) Heckel, A.; Pfleiderer, W. *Helv. Chim. Acta* **1986,** *69*, 1095–1106.
(17) Shelton, J.R.; Davis, K.E. *J. Am. Chem. Soc.* **1967,** *89*, 718–719.
(18) Luyt, L.G.; Katzenellenbogen, J.A. *Bioconj. Chem.* **2002,** *13*, 1140–1145.