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Thiiranium Ion Intermediates in the Formation and Reactions of S-(2-Haloethyl)-L-cysteines

DAVID R. DOHN AND JOHN E. CASIDA

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720

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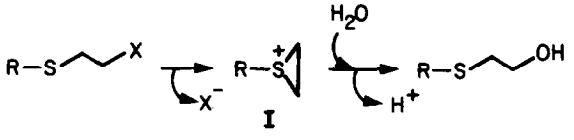
Thiiranium ions formed from the cysteine or glutathione conjugates of 1,2-dihaloethanes are believed to be responsible for the genotoxicity of the parent alkyl halides. The conversions of specifically deuterated β-hydroxyethyl sulfides to the corresponding β-haloethyl sulfides are studied to provide direct evidence for the involvement of thiiranium ions in the reactions of the cysteine conjugates of 1,2-dihaloethanes. *S*-(2-Hydroxyethyl-1,1-d_2)-L-cysteine is converted to an equal mixture of the 1,1-d_2 and 2,2-d_2 isomers of the corresponding *S*-(2-haloethyl)-L-cysteines in concentrated hydrochloric, hydrobromic, or hydroiodic acids without detectable formation of the 2,2-d_2 isomer of the parent hydroxyethyl derivative. Dissolution of *S*-(2-hydroxyethyl)-L-cysteine in trifluoromethanesulfonic acid yields a compound with the NMR spectral properties of *S*-(L-cysteinyl)ethyl thiiranium trifluoromethanesulfonate. The organosoluble *S*-(2-hydroxyethyl-1,1-d_2) benzyl sulfide is converted to an equal mixture of the 1,1-d_2 and 2,2-d_2 isomers of *S*-(2-chloroethyl) benzyl sulfide by thionyl chloride or triphenylphosphine : carbon tetrachloride. These results demonstrate the involvement of thiiranium ion intermediates in the conversion of 2-hydroxyethyl sulfides to 2-haloethyl sulfides in halogen acids and a similar symmetrical intermediate in the chlorination reactions effected by thionyl chloride or triphenylphosphine : carbon tetrachloride.

INTRODUCTION

β-Haloethylsulfides ("sulfur mustards") are unusually reactive toward nucleophilic displacement of the halide. The hydrolysis of these alkyl halides shows first-order kinetics and is insensitive to pH (*1–3*), unusual features that led to the postulation of halide displacement via thiiranium ion intermediate **I** (*3*).



S-(2-Chloroethyl)-L-cysteine and *S*-(2-bromoethyl)-L-cysteine, putative metabolites of 1,2-dichloroethane and 1,2-dibromoethane, are sulfur mustards of current interest. These alkylating agents (*4*), or the corresponding glutathione conjugates, are believed to mediate the genotoxic action of the parent dihaloethanes. Thiira-

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nium ions are presumed to be the electrophilic intermediates actually responsible for covalent modification of DNA effected by 1,2-dihaloethanes (*5–8*).

The involvement of thiiranium ions (or other symmetrical intermediates) in solvolysis can, in principle, be detected by deuterating one of the methylene groups of the ethyl moiety and following the fate of the deuteriums (or, more conveniently, the remaining hydrogens) by NMR spectroscopy. This approach has been used to detect symmetrical intermediates in similar reactions (*9, 10*). This report describes the preparation of *S*-(2-hydroxyethyl-1,1-d_2)-L-cysteine (HEC-1,1-d_2)[1] and the corresponding hydroxyethyl-1,1-d_2 benzyl sulfide (HEBS-1,1-d_2) and attempts to convert these compounds to the corresponding alkyl halides without randomization of the methylene units of the ethyl group. The results demonstrate the involvement of thiiranium ions in the facile conversion of these alcohols to the alkyl halides and also document the direct spectroscopic observation of the *S*-(cysteinyl)ethyl thiiranium ion in super acid solution.

RESULTS AND DISCUSSION

Reactions of HEC-1,1-d_2 with Strong Halogen Acids

HEC-d_2 (a mixture of 90% 1,1-d_2 and 10% 2,2-d_2, see Experimental) is cleanly converted to *equal* amounts of the alkyl chlorides CEC-1,1-d_2 and CEC-2,2-d_2 in 37% (w/w) deuterium chloride with 50% conversion in 15 min at 50°C (Fig. 1) and 18 h at 22°C. Dissolution of HEC-1,1-d_2 in 47% (w/w) deuterium bromide yields equal amounts of the 1,1-d_2 and 2,2-d_2 isomers of the alkyl bromide (BEC-d_2) with half-times of 50 min at 50°C and 29 h at 22°C. Likewise, equal amounts of the two alkyl iodides (IEC-d_2) form from HEC-d_2 in 53% (w/w) deuterium iodide (containing 1.5% deuterated hypophosphoric acid) at 50°C (half-time 30 min). In all reactions the ratio of the 1,1-d_2 and 2,2-d_2 isomers of the alkyl halide is unity at all times, and the isomeric ratio of the parent alcohol remains at the starting value of 0.9 throughout the reaction.

The conversion of HEC-1,1-d_2 to the corresponding chloride, bromide, or iodide in strong aqueous acids, proceeding with randomization of the deuterium label, clearly indicates the existence of an intermediate in which the two methylene units of the ethyl group are equivalent (i.e., a thiiranium ion). These experiments do not distinguish between the formation of a symmetrical intermediate during the halogenation process or a rapid scrambling of the label via such an intermediate after formation of the carbon–halogen bond. However, the extreme ease with which β-hydroxyethylsulfides, as compared to other primary alcohols, undergo conversion to β-haloethylsulfides under acidic conditions (*11*) and the

[1] Abbreviations and notation used: BEC, *S*-(2-bromoethyl)-L-cysteine; CEBS, *S*-(2-chloroethyl) benzyl sulfide; CEC, *S*-(2-chloroethyl)-L-cysteine; HEBS, *S*-(2-hydroxyethyl) benzyl sulfide; HEC, *S*-(2-hydroxyethyl)-L-cysteine; IEC, *S*-(2-iodoethyl)-L-cysteine; TFA, trifluoroacetic acid; TFAEC, *S*-[2-(trifluoroacetyl)ethyl]-L-cysteine. The designations 1,1-d_2 and 2,2-d_2 indicate compounds deuterated on carbons 1 or 2 of the ethyl group, respectively. The designation d_2, without specifying carbon 1 or 2, indicates a mixture of the two deuterated isomers.

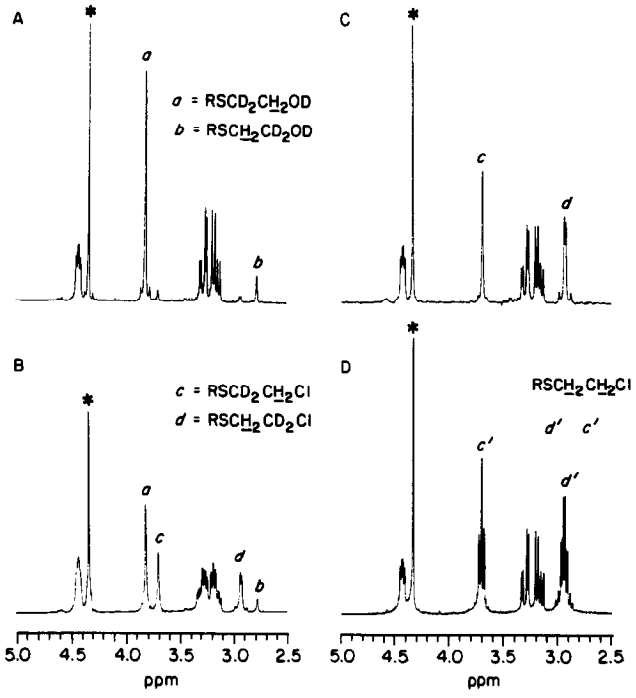


FIG. 1. ^{1}H NMR spectra of HEC-1,1-d_2 (90%) and HEC-2,2-d_2 (10%) in 37% (w/w) DCl:D_2O at various times after dissolution. (A) Initial spectrum. (B) 15 min at 50°C. (C) 90 min at 50°C. (D) Authentic CEC (non-deuterated) in 37% DCl:D_2O. *, nitromethane reference. R = DO_2C $CH(ND_3{+})CH_2$–.

spectral observation of the corresponding thiiranium ion in super acid (see below) establish the involvement of these ions in the product-forming reactions.

Reactions of HEC-1,1-d_2 in Trifluoroacetic Acid-d (TFA-d)

Dissolution of HEC-1,1-d_2 in TFA-*d* at 50°C results in the quantitative formation over several hours of a new compound with ^{1}H and ^{13}C NMR spectra consistent with *S*-[2-(trifluoroacetyl)ethyl-1,1-d_2]-L-cysteine (TFAEC-1,1-d_2). The resonances assigned to the protons of the ethyl group undergo pronounced changes. The doublet of doublets (3.98 ppm) from the $-CD_2CH_2OD$ protons disappears and is replaced by a singlet at 4.68 ppm (which overlaps the α H resonance at 4.65 ppm), while the signal ascribed to the $-CH_2CD_2OD$ protons changes from a doublet of doublets at 2.89 ppm to a singlet at 3.07 ppm. The signal from the major non-deuterated carbon in the ethyl group shifts downfield from 64.1 to 69.8 ppm. There are only slight changes in the chemical shifts of ^{1}H and ^{13}C resonances originating from the cysteinyl portion of the molecule. These alterations in the NMR spectra are consistent with the formation of a trifluoroacetyl ester, but not with trifluoroacetylation of the amino group. Integration of the proton resonance at 3.07 ppm in the product indicates that no significant randomization of the deuterium label occurs.

All attempts to isolate TFAEC-1,1-d_2 resulted in hydrolysis to the starting alcohol. For example, removal of the TFA-*d* under high vacuum and dissolution in CD_3OD gives a ^1H NMR spectrum consistent with a mixture of 67% TFAEC-1,1-d_2 and 33% starting alcohol. However, after several hours at room temperature, all of the putative ester is transformed to alcohol, the identity of which was confirmed by mass spectrometry. The isomer ratio of the alcohol obtained is identical to the starting material.

Solutions of HEC-1,1-d_2 (100 mM) and either tetra-*n*-butylammonium bromide or the corresponding chloride (400 mM) in TFA-*d* quantitatively yield TFAEC-1,1-d_2 with no rearrangement of label at room temperature. No alkyl halide is detected after 3 days at room temperature. Heating of these solutions at 45–60°C for 18 h produces mixtures of the trifluoroacetate and the bromide or chloride in which the label is completely randomized in *all* products.

Formation of unrearranged TFAEC-d_2 in neat TFA indicates that this reaction is a normal acid-catalyzed esterification. It is possible that TFA does not possess sufficient acid strength and/or polarity to effect the formation of the thiiranium ion intermediate. The addition of bromide or chloride (as tetra-*n*-butylammonium salts) leads to the formation of both rearranged alkyl halide and trifluoroacetate. The rearranged ester may arise by direct formation of the thiiranium ion from unrearranged ester, or by solvolysis of the thiiranium ion formed from the alkyl halide. Formation of the thiiranium ion from the chloro or bromo derivative under conditions (solvent and temperature) where the trifluoroacetate does not yield this ion may be explained by the greater propensity of the halide ions to leave or by the increased polarity of the reaction media due to the presence of the amine salts. The lack of rearrangement of the trifluoroacetate during solvolysis in methanol also demonstrates the importance of solvent polarity for thiiranium ion formation and is consistent with the fact that CEC is stable and does not solvolyze in methanol (no solvolysis was detectable by ^1H NMR after 30 days at room temperature).

Reactions of HEC and HEC-d_2 in Super Acid

Careful dissolution of HEC in cold trifluoromethanesulfonic acid diluted with sulfur dioxide, followed by slow warming to room temperature, yields the *S*-(cysteinyl)ethyl thiiranium ion, identified by its ^1H and ^{13}C NMR spectra (Fig. 2A). This ion is stable for several hours at room temperature. Irradiation of the broad singlet at 6.37 ppm (ascribed to the $-NH_3+$ protons) causes the multiplet at 4.26 ppm to collapse to a doublet of doublets with coupling constants of 4.3 and 9.3 Hz, values matching couplings observed in the resonances assigned to the β-hydrogens at 3.02 and 2.70 ppm, results which unambiguously identify these protons as derived from the cysteinyl portion of the molecule. The ring protons appear as a broad singlet at 3.33 ppm. The ^{13}C NMR spectrum also supports the assigned structure; there are two closely spaced signals for the ring carbons at 41.1 and 40.7 ppm, a consequence of the asymmetric center in the molecule which makes the ring carbons diastereotopic. The chemical shifts of the ring carbons indicate that they carry little or no positive charge and are consistent with the ^{13}C

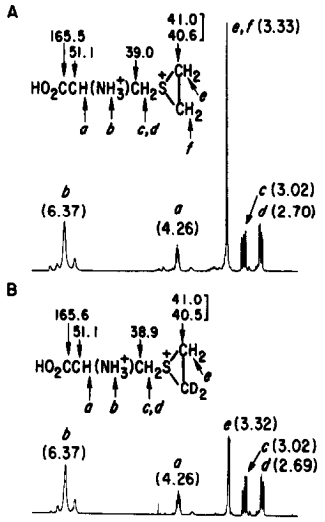


FIG. 2. ^{1}H NMR spectra of thiiranium ions derived from (A) HEC and (B) HEC-d_2 in trifluoromethanesulfonic acid : sulfur dioxide at 22°C. The ^{13}C chemical shifts are given with the structural formulae.

chemical shifts reported for thiiranium ions with a methyl substituent on the ring (*12*) when the effect of a β-methyl group is taken into account.

Dissolution of HEC-1,1-d_2 in trifluoromethanesulfonic acid : sulfur dioxide as above results in a similar ^{1}H NMR spectrum (Fig. 2B), except that the signal ascribed to the ring protons appears as two singlets at 3.29 and 3.31 ppm and is reduced in area by one-half. The ^{13}C NMR spectrum is also similar to that observed for the product obtained from HEC, except that the signals assigned to the ring carbons are of lesser intensity.

Other reasonable reaction pathways for the parent compound in strong acid include dehydration to the olefin, which would rapidly polymerize in super acid (the ^{1}H NMR spectra showed evidence for a small amount of polymeric material) and formation of a protonated sulfide. The ^{1}H NMR of the latter would be distinctive, showing additional couplings in the resonances from the protons adjacent to the sulfur (*13*), and no evidence for such a species was obtained.

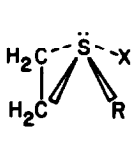
Chlorination of HEBS-1,1-d_2

HEBS-1,1-d_2 was prepared to determine if an organosoluble β-hydroxyethylsulfide could be converted to the corresponding chloride without randomization of the deuterium label. Reaction of HEBS-1,1-d_2 with a 10% molar excess of thionyl chloride in $CDCl_3$ at room temperature produces a 50 : 50 mixture of chlorides CEBS-1,1-d_2 and CEBS-2,2-d_2. The reaction is complete in less than 5 min and no NMR signals attributable to the intermediate chlorosulfite ester are observed. Identical results are obtained when the reaction is conducted in the presence of a 10% molar excess of pyridine-d_5. This extremely rapid reaction suggests

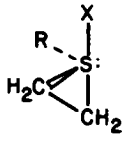
the involvement of a thiiranium ion in product formation. Reaction of HEBS-d_2 with 1 equivalent of triphenylphosphine and 10 equivalents of carbon tetrachloride in $CDCl_3$ at room temperature produces equal amounts of the two CEBS-d_2 isomers over a period of several days, indicating that a symmetrical intermediate is involved in the product-forming step or that randomization of the product via such an intermediate is rapid compared to product formation. Similar results are obtained with tri-*n*-octylphosphine or tri-*n*-butylphosphine. An earlier inability to synthesize specifically deuterated 2-chloroethyl methyl sulfide by "a variety of procedures" (not specified) has been reported (*10*).

Thiiranium Ion vs Covalent Sulfurane

Tetravalent sulfur species have been suggested as alternatives to thiiranium ions as intermediates in the addition of sulfenyl halides to olefins (*14*, *15*). These intermediates could also be involved in the solvolysis of β-haloethylsulfides. However, the square pyramidal structure **II** seems an unlikely candidate as it does not allow for exchange of the methylene groups by an intramolecular reaction. The trigonal bipyramidal structure **III** has the requisite features and is consistent with the known geometry of several isolated tetravalent sulfur compounds (*16*). However, the effects of solvent polarity on the reactions of the cysteine derivatives presented here support earlier studies on the solvolysis of other sulfur mustards (*3*, *4*) and indicate that the hydrolysis and alkylation reactions proceed through a solvent-separated ion pair.



II



III

It is noteworthy that hydrolysis of specifically deuterated 1-(methylthio)ethyl-2-dinitrophenolate in 50% water : acetone or 50% water : acetonitrile is reported to occur with complete scrambling of the label, and that "ion pair return is a facile process" (*10*). This presumably means that randomization of the deuterium label occurred in the starting material, which is surprising if a thiiranium ion is involved, as these ions are powerful electrophiles and should rapidly react with water (*15*). It therefore appears that an intermediate less electrophilic than a thiiranium ion (e.g., a covalent sulfurane) is involved in the randomization of the deuterium label in this case. Such an intermediate may explain the inability to prepare *specifically* deuterated β-haloethylsulfides in organic solvents.

CONCLUSIONS

Conversion of *S*-(2-hydroxyethyl)-L-cysteine to the corresponding chloride, bromide, or iodide in strong acid proceeds via a thiiranium ion intermediate. The *S*-(cysteinyl)ethyl thiiranium ion exists as a stable entity at room temperature in

super acid. This is the first report of a thiiranium ion with no ring substituents and one of biochemical and toxicological relevance. These findings provide additional evidence for the hypothesis that thiiranium ions are responsible for the unusual reactivity of β-haloethylsulfides in biological systems. The facile exchange of the methylene units of β-haloethyl benzyl sulfide in organic solvents suggests that a non-thiiranium ion pathway is involved in the rearrangement of such compounds.

EXPERIMENTAL

Spectroscopy

NMR spectra were recorded at 300 MHz (^{1}H), 75 MHz (^{13}C), or 46 MHz (^{2}H). ^{1}H and ^{13}C spectra were referenced to internal tetramethylsilane (samples in organic solvents), 3-(trimethylsilyl)propanoic acid-2,2,3,3-d_4 (samples in D_2O), or nitromethane (samples in strong acids, ^{1}H resonance assigned 4.33 ppm). ^{2}H spectra were referenced to internal $CDCl_3$ (7.24 ppm, samples in organic solvents) or internal 0.5% CD_3CN (2.06 ppm, samples in H_2O). Mass spectra were obtained with a Hewlett–Packard 5985 quadrupole mass spectrometer operating in the chemical ionization mode with methane at 1 Torr.

Synthesis of Bromoethanol-2,2-d_2

Bromine (123 g, 0.77 mol) was added to acetic acid-d_4 (44.8 g, 0.70 mol) and phosphorous trichloride (1.9 g, 0.014 mol) and the solution was heated at 90°C for 6 h. After cooling, the resulting material was kept *in vacuo* for 2 h to remove residual bromine, giving 90.2 g of colorless solid (93%). NMR: ^{13}C, 173.3 (s), 24.8 (quintet, J = 23 Hz); ^{2}H, 10.3 (broad s), 3.85 (s). The ^{2}H NMR spectrum also showed a small peak at 2.07 ppm from residual acetic acid-d_4; integration indicated that the sample was 98 mole% bromoacetic acid-d_3.

A solution of 1.0 M BH_3 in tetrahydrofuran (360 ml) was added over 1 h to 42.2 g (0.30 mol) of bromoacetic acid-d_3 in 120 ml of tetrahydrofuran under an argon atmosphere. The solution was stirred for 4 h and the solvent was removed, giving a waxy solid which was slurried with dichloromethane and the solvent was evaporated again. The boroxine intermediate was hydrolyzed by stirring with 60 ml of 0.5 M Na_2CO_3 for 30 min. The thick sludge was diluted with water, saturated with NaCl, and extracted with dichloromethane. The organic fraction was dried ($MgSO_4$) and distilled, giving 26.2 g (69%) of bromoethanol-2,2-d_2 (bp 26–27°C at 2 mm, lit. bp 63–64.5°C at 30 mm, *17*). NMR: ^{1}H, 3.94 (broad s), 3.90 (s, overlapping the OH signal); ^{13}C, 62.4 (s), 34.5 (quintet, J = 23 Hz); ^{2}H, 3.49 (s).

The overall yield of bromoethanol-2,2-d_2, starting from acetic acid-d_4 is 63%. A published synthesis of bromoethanol-2,2-d_2 from the same precursor by a different procedure gave the product in 21% overall yield (*17*).

Synthesis of Cysteine Conjugates

HEC was prepared as described (*18*). NMR (0.4 N DCl in D_2O): ^{1}H, 4.35 (dd, J's = 4.5 and 7.4 Hz, 1.0 H), 3.77 (m, 2.0 H), 3.27 (dd, J's = 4.5 and 15.0 Hz, 1.0 H),

3.15 (dd, J's = 7.4 and 15.0 Hz, 1.0 H), 2.80 (m, 2.0 H); ^{13}C, 173.3, 63.1, 55.3, 37.0, 34.3. Mass spectrum: 166, $M + H^+$, 42%; 149, $M + H^+-NH_3$, 100%.

HEC-1,1-d_2 (90%), along with HEC-2,2-d_2 (10%), was prepared in an analogous fashion by reaction of bromoethanol-2,2-d_2 with cysteine. NMR (0.4 N DCl in D_2O): ^{1}H, 4.35 (dd, J's = 4.5 and 7.4 Hz, 1.0 H), 3.78 and 3.74 (two d's, J = 11.7 Hz, 1.8 H), 3.27 (dd, J's = 4.4 and 15.0 Hz, 1.0 H), 3.14 (dd, J's = 7.5 and 15.0 Hz, 1.0 H), 2.78 (broad s, 0.2 H); ^{13}C (major isomer), 173.3, 63.0, 55.3, 36.4 (quintet, J = 21 Hz), 34.2. ^{2}H NMR (0.4 N HCl), 3.78 (s, 0.2 D), 2.81 (s, 1.8 D). Mass spectrum: 168, $M + H^+$, 40%; 151, $M + H^+-NH_3$, 100%.

The 90 : 10 isomeric composition of HEC-d_2 dissolved in 0.1 N DCl or in neutral D_2O does not change over a period of weeks, indicating that the formation of the 2,2-d_2 isomer occurs during synthesis and not during purification. The most likely explanation for the formation of the 2,2-d_2 isomer is that 20% of the bromoethanol is initially converted to ethylene oxide in the liquid ammonia solution, which reacts with cysteine to yield equal amounts of the two HEC isomers; the remaining 80% of the product forms by direct reaction of cysteine with bromoethanol.

CEC was prepared as described (*18*). NMR (CD_3OD): ^{1}H, 4.24 (dd, J's = 4.4 and 7.2 Hz, 1.0 H), 3.73 (t, J = 7.4 Hz, 2.0 H), 3.25 (dd, J's = 4.4 and 14.9 Hz, overlapping the solvent peak), 3.12 (dd, J's = 7.3 and 14.9 Hz, 1.1 H), 2.98 (t, J = 7.4 Hz, 2.0 H); ^{13}C, 170.2, 53.6, 44.0, 35.5, 33.0. Mass spectrum: 184, $M + H^+$, 15%; 167, $M + H^+-NH_3$, 40%; 148, $M + H^+$–HCl, 100%; 102, $M + H^+$–HCl–HCOOH, 75%.

Reactions of HEC-d_2 with Neat Acids

HEC-d_2 (90 μmol) and nitromethane (1.5 μl) were dissolved in 600 μl of deuterated acid at room temperature and initial ^{1}H NMR spectra recorded. The samples were heated to 50°C and ^{1}H NMR spectra recorded periodically (with solvent presaturation and a relaxation delay of 4 s to ensure accurate integration). The reactions proceeded to a negligible extent during the brief periods at room temperature for NMR. On completion, the samples were reduced to dryness and dissolved in CD_3OD for ^{1}H and ^{13}C NMR and mass spectrometry to confirm the identity of the products.

Reaction of HEC and HEC-d_2 with Trifluoromethanesulfonic Acid

Solid HEC or HEC-d_2 (50 μmol) was added over 5 min to 0.5 ml of redistilled trifluoromethanesulfonic acid and 10 ml of sulfur dioxide at −70°C. The amino acids dissolved after a few minutes of stirring and the solution was warmed to room temperature; most of the sulfur dioxide boiled away leaving approximately 1 ml of pale yellow solution, which was transferred to a 5-mm NMR tube for spectroscopy. The spectra were referenced to external tetramethylsilane in acetone-d_6. After obtaining values for spectral reference the spectrometer lock was deactivated prior to acquiring spectra of the ions.

Preparation and Reactions of HEBS-1,1-d_2

HEBS was obtained from Alfa Products (Danvers, MA) or was synthesized from benzyl chloride and 2-mercaptoethanol. ^{1}H NMR as described (*19*). ^{13}C NMR ($CDCl_3$), 138.0, 128.8, 128.5, 127.1, 60.3, 35.8, 34.3. HEBS-1,1-d_2 was prepared by addition of bromoethanol-d_2 (40 mmol) in 10 ml of dichloromethane to benzyl mercaptan (40 mmol) and triethylamine (40 mmol) in 40 ml of dichloromethane. After 5 days at room temperature and 12 h at reflux, the solution was extracted with water, dried, and distilled (bp 112°C at 1.2 mm, lit. 161°C at 4.2 mm, (*19*)). The distillate was freed of benzyl mercaptan by silica column chromatography (elution of benzyl mercaptan with dichloromethane, followed by elution of product with 50 : 50 dichloromethane : ethyl acetate). Removal of solvent afforded 3.53 g (52%) of HEBS-1,1-d_2 (100% isomeric purity). NMR ($CDCl_3$): ^{1}H, 7.2–7.3 (m, 5.0 H), 3.71 (s, 2.0 H), 3.65 (s, 2.0 H), 2.63 (s, 1.0 H); ^{13}C, 138.0, 128.8, 128.5, 127.1, 60.1, 35.7, 33.3 (quintet, $J \doteq 21$ Hz). On replacing dichloromethane and triethylamine with ethanol and sodium hydroxide an equal mixture of HEBS-1,1-d_2 and HEBS-2,2-d_2 was obtained.

NMR studies on the conversion of HEBS or HEBS-1,1-d_2 to the corresponding CEBS involved 100–200 μmol of HEBS derivative and the reagents under study in 400–800 μl of $CDCl_3$. Product identities were confirmed by mass spectrometry.

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REFERENCES

1. Peters, R. A., and Walker, E. (1923) *Biochem. J.* **17,** 260–276.
2. Ogston, A. G., Holiday, E. R., Philpot, J. St. L., and Stocken, L. A. (1948) *Faraday Soc. Trans.* **44,** 45–52.
3. Bartlett, P. D., and Swain, C. G. (1949) *J. Amer. Chem. Soc.* **71,** 1406–1415.
4. Schasteen, C. S., and Reed, D. J. (1983) *Toxicol. Appl. Pharmacol.* **70,** 423–432.
5. Rannug, U., Sundvall, A., and Ramel, C. (1978) *Chem. Biol. Interact.* **20,** 1–16.
6. Guengerich, F. P., Crawford, W. M., Jr., Domoradzki, J. Y., MacDonald, T. L., and Watanabe, P. G. (1980) *Toxicol. Appl. Pharmacol.* **55,** 303–317.
7. Shih, T.-W., and Hill, D. L. (1981) *Res. Commun. Chem. Pathol. Pharmacol.* **33,** 449–461.
8. Koga, N., Inskeep, P. B., Harris, T. M., and Guengerich, F. P. (1986) *Biochemistry* **25,** 2192–2198.
9. Eliel, E. L., Pilato, L. A., and Badding, V. G. (1962) *J. Amer. Chem. Soc.* **84,** 2377–2384.
10. McManus, S. P., Neamati-Mazraeh, N., Hovanes, B. A., Paley, M. S., and Harris, J. M. (1985) *J. Amer. Chem. Soc.* **107,** 3393–3395.
11. Bennett, G. M., and Hock, A. L. (1925) *J. Chem. Soc.* **127,** 2671–2677.
12. Smit, W. A., Gybin, A. S., Bogdanov, V. S., Krimer, M. Z., and Vorobieva, E. A. (1978) *Tetrahedron Lett.*, p. 1085–1088.
13. Olah, G. A., O'Brien, D. H., and Pittman, C. U., Jr. (1967) *J. Amer. Chem. Soc.* **89,** 2996–3001.

14. OWSLEY, D. C., HELMKAMP, G. K., AND RETTIG, M. F. (1969) *J. Amer. Chem. Soc.* **91,** 5239–5242.
15. SMIT, W. A., ZEFIROV, N. S., BODRIKOV, I. V., AND KRIMER, M. Z. (1979) *Acc. Chem. Res.* **12,** 282–288.
16. CSIZMADIA, V. M., SCHMID, G. H., MEZEY, P. G., AND CSIZMADIA, I. G. (1977) *J. Chem. Soc. Perkins Trans. II,* p. 1019–1024.
17. HOGG, J. L., AND SCHOWEN, R. L. (1974) *J. Pharm. Sci.* **63,** 1620–1623.
18. CARSON, J. F., AND WONG, F. F. (1964) *J. Org. Chem.* **29,** 2203–2205.
19. FONG, H. O., HARDSTAFF, W. R., KAY, D. G., LANGLER, R. F., MORSE, R. H., AND SANDOVAL, D.-N. (1979) *Canad. J. Chem.* **57,** 1206–1213.