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# Synthesis and Relative Stereochemistry of the Benzylic Thioether Diastereoisomers Formed from Glutathione and Styrene Oxide

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The chemical reaction between (±) styrene oxide and glutathione produces both the benzylic and primary thioether positional isomers as a mixture of diastereoisomers (**2, 5** and **3, 6**), with a preference for the benzylic thioether isomers (66 : 34). Synthesis of the styrene oxide–glutathione conjugates from either (+)– or (–)– styrene oxide produces both positional isomers as single diastereoisomers. The benzylic thioether isomers (**2** and **5**) were prepared from protected 2-bromo-2-phenylethanol (**8**) and glutathione and were separated using hplc. The relative stereochemistry of the benzylic thioether isomers was assigned on the basis of the established chemical correlation between the optically pure styrene oxides and their precursors, the mandelic acids, as well as considerations of the mechanism of ring opening of epoxides by sulfur nucleophiles. The availability of the single diastereoisomers of the benzylic thioether isomers and the styrene oxideglutathione conjugates enables investigations concerned with the influence of chirality on the biotransformation and excretion of these conjugates.

## INTRODUCTION

Styrene and styrene oxide are widespread environmental pollutants as a result of their use in the manufacture of several commercial products. Several million tons are produced annually in the United States (*1, 2*). The metabolism of a wide variety of aromatic and aliphatic hydrocarbons is mediated by the cytochrome *P*-450-dependent monooxygenase system (*3*). *In vivo,* the oxidative metabolism of styrene proceeds (*4*) to produce styrene oxide, which is known to be a skin carcinogen (*5*) and a bacterial mutagen (*6–8*). The toxicity of styrene oxide is due primarily to its electrophilic attack on nucleophilic sites in tissue macromolecules, including nucleic acids (*6*). Since styrene has been found as a contaminant in drinking water (*9*) and in cigarette smoke, and is a premutagen (*6*), a comprehensive understanding of its metabolism and toxicity is desirable.

The major detoxication pathways for styrene oxide include conjugation with glutathione (GSH) (*10*), catalyzed by cytosolic glutathione transferases (EC 25118) (*11*), and conversion to styrene glycol by microsomal epoxide hydrolases (EC 3322) (*12*). It has been shown by us (*13, 14*) and others (*15*) that with (±)-styrene oxide, both the chemically (*14*) and enzymatically (*14, 15*) produced GSH



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conjugates occur as a mixture of diastereoisomers of both the benzylic and primary thioether conjugates. With both the chemical (66:34) and enzymatic (90:10) conjugates, the benzylic thioether derivatives are the predominant reaction products (*14*). It is well documented that the intermediary GSH conjugates of styrene oxide are metabolized further to mercapturic acids (*N*-acetylcysteine derivatives) (*16, 17*).

To date, the chemical characterization of the GSH conjugates of styrene oxide and the corresponding intermediary metabolites is incomplete. Our interest in the stereochemistry of the metabolism of styrene oxide was stimulated by an earlier report (*17*) which showed that, after ip administration of styrene to rats, (a) the major urinary metabolites were the two positional isomers of the mercapturic acids; and (b) the ratio of one of the benzylic thioether diastereoisomers was present in three times the amount of the other. These results suggest that the metabolism and/or excretion of the metabolites of styrene oxide is stereoselective. The stereoselective metabolism and excretion of other xenobiotics is well documented (*18, 19*). This aspect of the metabolism of styrene oxide is important since this epoxide has been used as a common substrate to assay for glutathione transferase activity.

To more fully investigate the influence of stereochemistry on the major pathways of metabolism and elimination of styrene oxide, we required the optically pure diastereoisomers of the benzylic thioether GSH conjugates of styrene oxide. We report here the synthesis, separation, purification, and configurational assignment of the individual benzylic thioether glutathione conjugates of styrene oxide.

## MATERIALS AND METHODS

### *Thin-Layer-Chromatography (tlc)*

Chromatograms on silica gel/60/F-254 (Merck and Co.) were developed with solvent A, *n*-butanol/ethanol/concentrated $NH_4OH$/water (4:1:1:1,v/v) and solvent B, *n*-butanol/acetic acid/water (4:1:1,v/v). Spots were detected by viewing the plates under short wavelength uv, spraying with ninhydrin solution, followed by heating at 120°C for 2 min.

### *High-Pressure Liquid Chromatography (hplc)*

The diastereoisomers of the styrene oxide–glutathione conjugates **2** and **5** were separated on a Waters Model ALC 244 hplc instrument using system (a): Whatman Partisil M9 10/50 ODS column eluted with 1.0 g/liter $NH_4OAc$ (buffered with acetic acid to pH 4.1), 15% MeOH, with a flow rate of 2 ml/min. Analysis of the reaction mixtures of styrene oxide and glutathione was performed with hplc system (b): $\mu$Bondapack $C_{18}$, 0.39 × 30 cm with 0.05 *N* $H_3PO_4$ (buffered with triethylamine to pH 3.1), 15% MeOH, and a flow rate of 1 ml/min. The 2-bromo-2-phenylethanol (**7**) and its ether derivative (**8**) were purified on a Waters prep LC/system 500, using silica gel prep Pak 500 cartridges.

*Mass Spectra*

Chemical ionization (CI) mass spectra were obtained with a VG Micromass 70/70 mass spectrometer using methane or *iso*butane as the reagent gas. The mass spectra of **7** and **8** were obtained by the electron impact technique.

*Nuclear Magnetic Resonance (nmr)*

Natural abundance, proton-decoupled $^{13}C$ nmr spectra were obtained in the Fourier transform mode on a Varian Associates XL-100-12 spectrometer equipped with the 620-L disk data system. Parameters used in acquiring the nmr spectra were as follows: spectral width 5000 Hz; pulse angle ~30°; repetition time between pulses 2.8 sec; data points 8K; exponentional broadening −0.5; probe temperature 30°; and 100-Hz square-wave, modulated proton decoupling. Single frequency, off-resonance, proton-decoupled (sford) spectra were obtained by offsetting the decoupler frequency 1000 cps upfield from the center of the proton absorptions. Samples for nmr analysis were prepared in 5-mm sample tubes using 400 $\mu$l of $D_2O$ as solvent. A small amount of *p*-dioxane was added as internal reference and chemical shifts were converted to the tetramethylsilane (TMS) scale using the relationship $^{\delta}TMS = {}^{\delta}dioxane + 67.4$ ppm (*20*).

*Synthesis*

The following reactions were conducted under a nitrogen atmosphere.

*2-Bromo-2-phenylethanol* (*7*). To a stirred solution of (±)-styrene oxide (10 g) in tetrahydrofuran (100 ml) cooled to 0°C was added 49% HBr (10 ml) (Fisher) dropwise over a period of 30 min followed by stirring an additional 30 min at room temperature. Ether (100 ml) was added and the solution washed with brine (100 ml), sodium bicarbonate (100 ml), and brine (100 ml). After drying the ether solution with anhydrous $MgSO_4$, the solvent was removed *in vacuo*. The residual oil was purified on a Waters Model 500 preparative hplc using 20% ethyl acetate in hexane as the solvent. Removal of the solvent yielded a clear oil proving to be the bromohydrin **7** (yield 85%) (*21*). High-resolution mass spectrum: Found: $M^+$, 201.9821; $C_8H_9O^{81}Br$ requires 201.9817. $^1H$ nmr spectrum (T-60 in $CDCl_3$): 2.53 (s,OH); 3.97 (d,d,$CH_2$); 5.03 (t,CH); and 7.33 ppm (s,phenyl).

*2-Bromo-2-phenylethanol α-ethoxyethyl ether* (*8*). A mixture of the bromohydrin **7** (1.5 g) in methylene chloride (10 ml), ethylvinyl ether (20 ml), and concentrated hydrochloric acid (0.5 ml) was stirred at 0°C for 16 hr, followed by stirring at room temperature for 4 hr. The workup of the product was done as above. The final purification of the product was accomplished by preparative hplc using hexane–methylene chloride (1 : 1) as the solvent. The protected bromohydrin **8** was obtained in 76% yield. High resolution mass spectra: Found: $M^+-C_2H_5O$, 229.0037; $C_{10}H_{12}O^{81}Br$ requires $M-C_2H_5O$, 229.0049. $^1H$ nmr spectrum (T-60,$CDCl_3$): 1.15 (t,$CH_3$); 1.25 (d,d,$CH_3$); 3.45 (m,O$CH_2CH_3$); 4.05 (d,–CH$CH_2$O–); 4.75 (m,$CH$$CH_3$); 5.05 (t,–$CH$Br$CH_2$–); and 7.35 ppm (s,phenyl).

*(+)- and (−)-styrene oxide.* The mandelic acids used in this investigation were of commercial origin and were used as received [(*S*)-(+)-mandelic acid, $[\alpha]_D^{23}$157, Chemical Dyanamics Corp. South Plainfield, N.J.; (*R*)-(−)-mandelic acid, $[\alpha]_D^{23}$− 154.4, Aldrich Chemical Co., Milwaukee, Wisc.]. Optically pure (*S*)-(+)- and (*R*)-(−)-styrene oxides were prepared form the (*S*)-(+)- and (*R*)-(−)-mandelic acids, respectively, employing the procedure of Dupin and Dupin (*22*). The specific rotation obtained for (−)-styrene oxide was $[\alpha]_D^{23}$ − 27.45 (c0.5 in $CHCl_3$) (lit. value, $[\alpha]_D^{23}$ − 24.6, cl.37 in $CHCl_3$) and for (+)-styrene oxide was $[\alpha]_D^{23}$ + 23.8 (c0.53 in $CHCl_3$) (lit. value $[\alpha]_D^{23}$ + 24.4, cl.37 in $CHCl_3$).

*(1S)-S-(1-phenyl-2-hydroxyethyl)glutathione* (*2*) *and (2R)-S-(2-phenyl-2-hydroxyethyl)glutathione* (*3*). A solution of (−)-styrene oxide (150 mg) in dimethylsulfoxide (0.06 ml) was added to GSH (160 mg) (Sigma Chemical Co.) in 60 ml of 0.01 *M* potassium phosphate buffer, pH 7.2, and sonicated for 2 min. The suspension was stirred at room temperature for 24 hr. The reaction mixture was extracted (4 × 15 ml) with ethyl acetate and the organic fractions discarded. Water was removed from the aqueous fraction *in vacuo*. The oily residue was extracted (3 × 25 ml) with methanol. The solid remaining after methanol extraction did not contain glutathione conjugates as established by hplc analysis (system b).

After evaporation of the methanol, the product was purified by column chromatography on polystyrene resin, Amberlite XAD-2 (Mallinckrodt). The resin was prepared for chromatography by washing with chloroform–methanol (1 : 3), methanol, and water. A slurry of the resin in water was poured into a glass column to give resin beds of 4 × 90 cm. The sample was applied to the column in water buffered to pH 3.1 with $H_3PO_4$. Elution was carried out with water followed by 20% methanol : water. The column eluent was monitored by tlc (solvent A). Fractions of 25 ml were collected at a flow rate of 2 ml/min. Fractions containing styrene oxide–glutathione conjugates were combined. High-pressure liquid chromatographic analysis of the product (system b) (Fig. 2B) resulted in two peaks, corresponding to **2** (first eluting peak) and **3** (later eluting peak). These compounds were present in a ratio of 66 : 34 as determined by integration of their $^{13}C$ nmr spectra (Table 2) (Fig. 1). The yield of **2** and **3** was 78.4% based on styrene oxide. A sample of the conjugates was derivatized using methanol and hydrogen chloride (24 hr, room temperature). The product, the methoxy dimethyl ester of the conjugates showed the following CI isobutane mass spectrum: 470(M + 1), 438, 406, 374, 302, 295, 284, 210, 184, 159, 153, 144, 121 (base peak).

*(1R)-S-(1-phenyl-2-hydroxyethyl)glutathione* (*5*) *and (2S)-S-(2-phenyl-2-hydroxyethyl)glutathione* (*6*). Conjugates **5** and **6** were prepared from (+)-styrene oxide and glutathione. The reaction conditions and the purification of the product were identical to the above procedure (yield, 74.8%, based on styrene oxide). The positional isomers **5** and **6** could not be separated by hplc (system a or b) (Fig. 2C). The ratio of **5** to **6** was 70 : 30 as established by $^{13}C$ nmr (Table 2).

*Preparation of styrene oxide–glutathione conjugates 2,3,5 and 6.* The diastereoisomeric mixture of positional isomers was prepared from (±)-styrene oxide (240 mg) and glutathione (645 mg) as described above. The yield based on styrene oxide was 72.4%. The two peaks (Fig. 2A) were separated by hplc (system b) resulting in two fractions. The desalting of these fractions was performed on

Amberlite XAD-2 resin as described above, using water and methanol : water (1 : 1) as eluants. As shown by $^{13}C$ nmr analysis, the first peak contained the benzylic thioether diastereoisomer **2**, and the second peak was a mixture of the remaining isomers, **3**, **5**, and **6** (Table 2).

*Preparation of (1S)-S-(1-phenyl-2-hydroxyethyl)glutathione (2) and (1R)-S-(1-phenyl-2-hydroxyethyl)glutathione (5).* A solution of the protected bromohydrin **8** (500 mg) in dimethylformamide (10 ml) was added to a mixture of glutathione (700 mg) and potassium carbonate (500 mg) in 0.5 ml water. The suspension was stirred at room temperature for 24 hr. The solvent was evaporated under vacuum and the residue remaining was diluted with 30 ml water and extracted with ethyl acetate (5 × 15 ml). The aqueous solution was acidified to pH 3.1 with 5% $H_3PO_4$ and stirred at 60°C for 30 min. The solvent was removed *in vacuo*, and the remaining oil was extracted with methanol (5 × 25 ml). The oil, obtained upon methanol evaporation, was fractionated by column chromatography on a μBondapack $C_{18}$ (50 g) column. The elution was affected with water acidified to pH 4 with acetic acid, followed by a methanol–water mixture (1 : 4). A total of 35 fractions (15 ml) were collected and combined on the basis of tlc and hplc. Fractions 9–15 (water, pH 4) were combined and evaporated to dryness *in vacuo*. The remaining residue was redissolved in water (1 ml) and fractionated by hplc (system a). The fractions corresponding to the conjugates were collected and the solvents removed. The ammonium acetate was removed in high vacuum by heating the flask to 40°C. The remaining traces of the salt were removed by open column chromatography on a Porasil B $C_{18}$ column (10 g). Elution and collection of the product was as previously described.

First peak (hplc), diastereoisomer **2**: $[\alpha]_D^{23}$ + 50.2 (c 2.26, MeOH), uv $\lambda_{max}$ nm($\epsilon_{max}$) MeOH, 257 (301 and 263 (216). CI mass spectrum (methane as reagent gas) of methoxydimethyl ester of **2**. Found: $M^+ + 1$, 470.1958, $C_{21}H_{32}N_3O_7S$ requires 470.1959.

Second peak (hplc), diastereoisomer **5**: $[\alpha]_D^{23}$ − 68.4 (c 1.61 MeOH), uv $\lambda_{max}$ nm($\epsilon_{max}$) MeOH, 257 (242) and 263 (172). CI mass spectrum of the methoxy dimethyl ester of **5**. Found: $M^+ + 1$, 470.1970, $C_{21}H_{32}N_3O_7S$ requires 470.1959.

*Reaction of glutathione with 2-bromo-2-phenylethanol (7).* (a) Reaction in buffer, DMSO: A solution of the bromohydrin **7** (850 mg) in DMSO (1 ml) was added to GSH (1.5 g) in 60 ml of 0.1 *M* potassium phosphate buffer, pH 7.2, the mixture sonicated for 2 min, and stirred at room temperature for 24 hr. The workup of the reaction and separation of the products were done as above. A total of 240 mg of conjugates was obtained (14.4% yield). After separation of the peaks by hplc (system a), the first peak contained isomer **2** and the second peak contained 74% of isomer **5** and 26% of the two primary thioether diastereoisomers (**3** + **6**) as established by integration of their $^{13}C$ nmr spectra. (b) Reaction in buffer, MeOH: A mixture of GSH (1.5 g) in 50 ml 0.1 *M* phosphate buffer, pH 7.2, was added to the bromohydrin **7** (700 mg) in (50 ml) methanol and stirred for 24 hr at room temperature. The workup and separation of the peaks was accomplished as described above. A total of 850 mg (57% yield) of conjugates was obtained. According to $^{13}C$ nmr (Table 2), a mixture of the two positional isomers was formed.

## RESULTS AND DISCUSSION

The $^{13}C$ nmr spectrum of the GSH conjugates derived from (±)-styrene oxide is given in Fig. 1. The chemical shifts (Table 1) were assigned using model compounds (*13*) (*t*-butylthiol derivatives, mercapturic acid conjugates, and glutathione), the sford spectra, comparison of the spectra obtained from the conjugates derived from the optically pure enantiomers of styrene oxide, and from spectra of the peaks separated by hplc.

On the basis of the nmr spectrum (Fig. 1A) it is clear that more than one compound is present. There are three peaks in the region between 140 and 150 ppm, characteristic of substituted aromatic carbons, and three peaks in the region between 60 and 80 ppm, characteristic of carbons bearing one hydroxyl group. The sford spectrum shows that the peak at ~75 ppm is due to methine carbons. These results confirm the presence of both positional isomers of the GSH

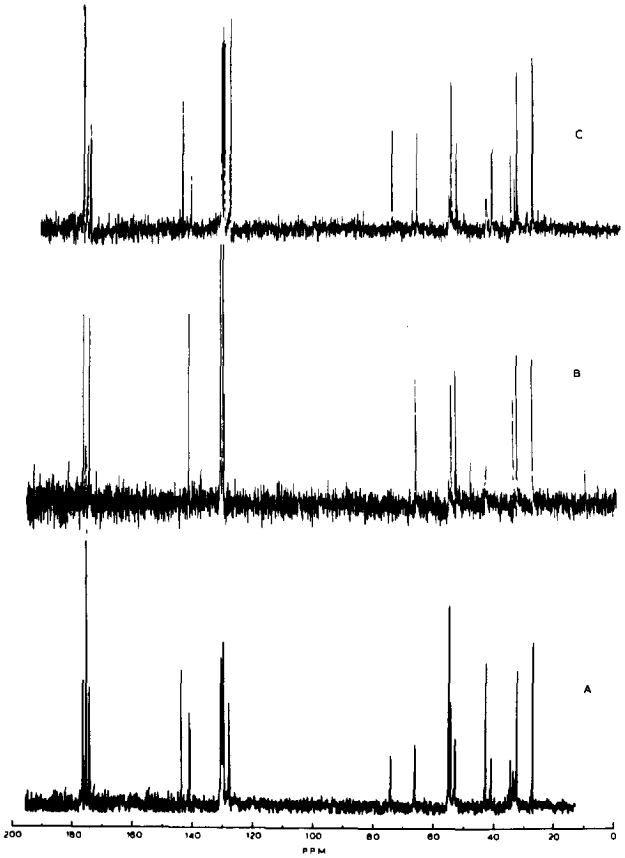
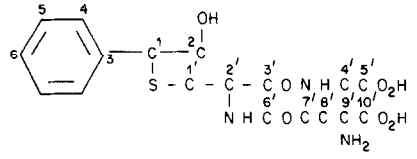



FIG. 1. $^{13}C$ nmr spectra: (A) of the glutathione conjugates formed from (±)-styrene oxide; (B) of the first eluting peak in the hplc separation (Fig. 2); and (C) of the latter eluting peak in the hplc separation (Fig. 2).

TABLE 1

$^{13}$C nmr CHEMICAL SHIFTS OF THE GSH CONJUGATES OF STYRENE OXIDE[a]



| Carbon/isomer | 2[b] | 3 | 5 | 6 |
|---|---|---|---|---|
| 1 | 54.20 | 74.79 | 53.34 | 74.95 |
| 2 | 67.25 | 42.10 | 66.42 | 41.96 |
| 3 | 141.95 | 144.41 | 140.92 | 144.38 |
| 4 | 131.44 | 128.48 | 130.75 | 128.43 |
| 5 | 130.61 | 131.14 | 129.92 | 130.90 |
| 6 | 130.61 | 130.61 | 129.92 | 129.92 |
| 1′ | 35.08 | 34.98 | 34.11 | 34.59 |
| 2′ | 55.67 | 55.67 | 54.99 | 54.99 |
| 3′ | 174.85 | 174.85 | 173.19 | 173.19 |
| 4′ | 44.24 | 44.24 | 45.29 | 45.29 |
| 5′[c] | 176.09 | 176.09 | 176.53 | 176.53 |
| 6′ | 176.84 | 176.84 | 177.74 | 177.74 |
| 7′ | 33.82 | 33.83 | 33.43 | 33.43 |
| 8′ | 28.68 | 28.68 | 28.68 | 28.68 |
| 9′ | 56.39 | 56.39 | 56.05 | 56.05 |
| 10′[c] | 175.92 | 175.92 | 176.53 | 176.53 |

[a] In ppm downfield from TMS.
[b] Structures refer to Fig. 3.
[c] Chemical shifts are pH dependent.

conjugates, and the doubling of certain other peaks in the spectrum indicates that each positional isomer is present as a mixture of diastereoisomers.

This sample was subjected to reverse phase, high-pressure liquid chromatography, which provided two peak fractions (Fig. 2A) which were collected and examined by means of $^{13}$C nmr spectroscopy. The first peak exhibits a $^{13}$C nmr spectrum (Fig. 1B) which, from the sford spectrum and chemical shift considerations, indicates that it is one of the diastereoisomers of the benzylic thioether conjugates. Similar considerations applied to the spectrum of the second peak (Fig. 1C) show that it contains the other benzylic thioether diastereoisomer plus the two diastereoisomers of the primary thioether conjugate.

At this point in the investigation it was possible to assign the $^{13}$C chemical shifts to the two diastereoisomers of the benzylic thioether conjugate; however, the relative stereochemistry of the diastereoisomers was still not clear. Furthermore, it was not possible to assign chemical shifts to the diastereoisomers of the primary thioether conjugate.

In order to resolve the questions regarding the assignments of the chemical shifts and the relative stereochemistry of the conjugates, the optically pure enantiomers of styrene oxide were prepared, the GSH conjugates prepared from

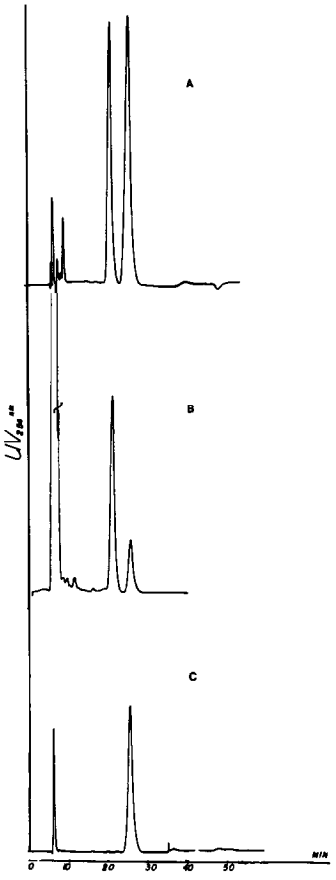



FIG. 2. Reverse phase hplc analysis of the glutathione conjugates from racemic (±)-styrene oxide (A), (−)-styrene oxide (B), and (+)-styrene oxide (C). The column used was a μBondapak $C_{18}$, 0.39 × 30 cm, with a flow rate of 1 ml/min. Eluent was monitored at 240 nm, solvent, 0.05 *N* $H_3PO_4$ buffered with triethylamine to pH 3.1/15% methanol.

each of the enantiomers, and the $^{13}C$ nmr spectra of the conjugates recorded. The $^{13}C$ nmr spectrum of the GSH conjugates derived from (−)-styrene oxide clearly shows the presence of both positional isomers as evidenced by two peaks each in the regions characteristic of the substituted aromatic carbons and the hydroxyl-bearing carbons and the sford spectrum. Furthermore, since only two peaks appear in each of the above regions it is clear that the two positional isomers are produced as single diastereoisomers, indicating that racemization of the styrene oxide did not occur during reaction with glutathione. The assignment of the relative stereochemistry of the individual diastereoisomers is based on the known absolute stereochemistry of the styrene oxide enantiomers (**1** and **4**) employed in the conjugation reactions. Thus, enantiomer **1**, (*R*)-(−)-styrene oxide, was synthesized from (*R*-(−)-mandelic acid and enantiomer **4**, (*S*)-(+)-styrene oxide, from (*S*)-(+)-mandelic acid, resulting in overall retention of configuration at the chiral center (*22–24*). It has been shown previously that the addition of sulfur

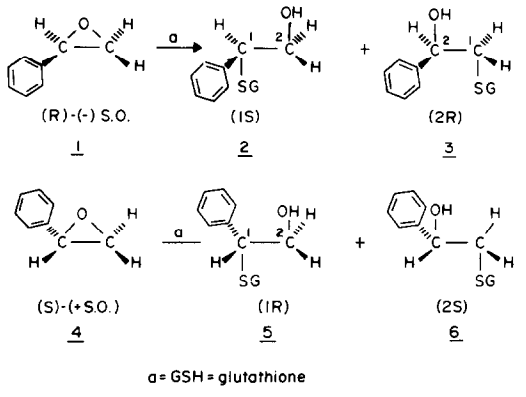



FIG. 3. Relative stereochemistry of the glutathione conjugates derived from the optically pure styrene oxides.

nucleophiles to an epoxide ring in slightly basic media occurs in a *trans* fashion with inversion of configuration at the carbon to which sulfur becomes attached (*25, 26*). Thus, the configuration of the benzylic carbon in conjugate **2** is 1(*S*) and 2(*R*) for conjugate **3** (Fig. 3).

The $^{13}C$ nmr spectra of the conjugates derived from (*S*)-(+)-styrene oxide also show the presence of both positional isomers as single diastereoisomers. The chemical shifts of certain carbons of **5** and **6** (Table 1) are slightly different from those of **2** and **3** confirming that different primary thioether conjugates are formed from each of the styrene oxide enantiomers—although the hplc trace (Fig. 2C) shows no separation of them under the conditions used in this investigation. Because of considerations similar to those above, the configuration of the benzylic carbon in the conjugates produced from (*S*)-(+)-styrene oxide is 1(*R*) for **5** and 2(*S*) for **6** (Fig. 3).

The above results demonstrate clearly that both possible positional isomers are produced as a mixture of diastereoisomers by the chemical reaction of (±)-styrene oxide with glutathione. $^{13}C$ nmr chemical shifts for each of the four isomers are given in Table 1.

With these results in hand, it became clear that $^{13}C$ nmr could be used to assay for glutathione transferase activity with styrene oxide as substrate. Either the substituted aromatic carbon peaks (~141 and 144 ppm) or the hydroxyl-bearing aliphatic carbons (~67 and 74 ppm) could be used to determine the presence of positional isomers. The hydroxyl-bearing carbon peaks at ~74 ppm could be used to determine the relative amount of the diastereoisomers of the primary thioether conjugates, and the substituted aromatic carbon peaks at 141 ppm could be used to determine the relative amount of the benzylic thioether diastereoisomers (Table 2, Fig. 1).

$^{13}C$ nmr has certain advantages over hplc in this investigation of the regioselectivity of the production of styrene oxide–glutathione conjugates, since the ratio of the positional isomers can be determined only for the conjugates derived from (−)-styrene oxide which are separated by hplc. Integration of the $^{13}C$ nmr

TABLE 2

STYRENE OXIDE GLUTATHIONE CONJUGATES DIASTEREOISOMERS FORMED FROM DIFFERENT SUBSTRATES[a]

| Substrate | Solvent | Yield (%) | 2 | 3 | 5 | 6 |
|---|---|---|---|---|---|---|
| ±SO + GSH | Buffer/DMSO | 72.4 | 33 | 17 | 33 | 17 |
| −SO + GSH | Buffer/DMSO | 78.4 | 66 | 34 | | |
| +SO + GSH | Buffer/DMSO | 74.8 | | | 70 | 30 |
| Bromohydrin 7 + GSH | Buffer/DMSO | 14.4 | 44 | 6 | 44 | 6 |
| Bromohydrin 7 + GSH | Buffer/MeOH | 57.6 | 32 | 18 | 32 | 18 |
| Protected bromohydrin 8 + GSH | DMF | 10.6 | 50 | | 50 | |

[a] Results are given as percentage of each diastereoisomer formed as established by $^{13}C$ nmr integretation of the peaks error ±5%. SO, Styrene oxide; bromohydrin 7, 2-bromo-2-phenylethanol; protected bromohydrin 8, α-ethoxyethyl ether of compound 7; 2, (1*S*)-*S*-(1-phenyl-2-hydroxyethyl)glutathione; 3, (2*R*)-*S*-(2-phenyl-2-hydroxyethyl)glutathione; 5, (1*R*)-*S*-(1-phenyl-2-hydroxyethyl)glutathione; 6, (2*S*)-*S*-(2-phenyl-2-hydroxyethyl)glutathione; GSH, glutathione.

spectra of the GSH conjugates produced from (±)-styrene oxide (Table 2) shows a preference for the benzylic thioether conjugates (66:34). These results are in agreement with recent results obtained from an analysis of the 270 MHz 'H nmr spectrum of the conjugates (*15*). However, it is important to point out that even at 270 MHz, the chemical shift dispersion does not allow one to quantitate the mixture of isomers for the relative amounts of the diastereoisomers.

For further studies, we required the two major biologically formed isomers 2 and 5. Due to the formation of both positional isomers during the conjugation of styrene oxide with GSH, and failure to separate conjugate 5 from the primary thioether conjugates 3 and 6 by our best hplc system, we were forced to develop a synthesis specifically for the benzylic thioether styrene oxide–glutathione conjugates. Attempts to use the unprotected bromohydrin 7 under different conditions of coupling with GSH produced both positional isomers, due to the partial recyclization of the bromohydrin to styrene oxide (Table 2, Fig. 4). By using the protected bromohydrin 8, however, the recyclization to styrene oxide was avoided.

The $^{13}C$ nmr spectra of the GSH conjugates produced from the protected bromohydrin 8 show that an equal mixture of the diastereoisomers of the benzylic thioether conjugate is present. There is no evidence for the presence of the primary thioether conjugates. High-pressure liquid chromatographic separation of the above benzylic thioether conjugates results in two peaks which were subsequently shown by $^{13}C$ nmr to be identical to the diastereoisomer 2 obtained using (−)-styrene oxide (first eluting peak) and 5 obtained from (+)-styrene oxide (second eluting peak).

The synthetic and hplc procedures reported here permit the preparation and separation of the benzylic thioether diastereoisomers of the sytrene oxide–gluthathione conjugates in reasonable yield. Furthermore, this synthetic procedure can be easily modified to produce the radiolabeled diastereoisomers with high specific activity. Since the benzylic thioether conjugates of styrene oxide are

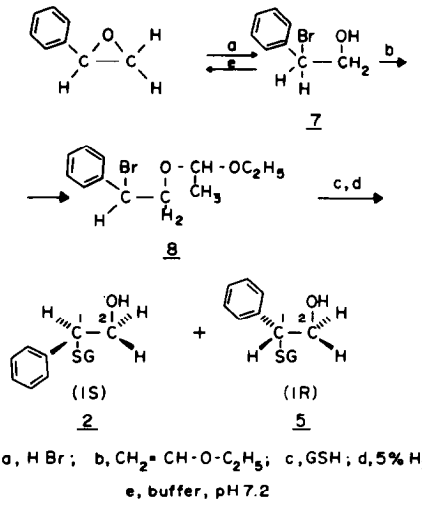



FIG. 4. Synthetic scheme for the preparation of the benzylic glutathoine conjugates derived from racemic styrene oxide.

the major isomers produced enzymatically (*14*), these conjugates should prove useful in future studies of the influence of stereochemistry on the biotransformation and excretion of gluthathione conjugates.

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## REFERENCES

1. W. J. STORCK, *Chem. Eng. News* **56,** 32 (1978).
2. *Chem. Eng. News* **56,** 11 (1978).
3. J. M. DALY, "Handbook of Experimental Pharmacology," Vol. XXVIII, Part 2 (B. B. Brodie and J. R. Gillette, Eds.), pp. 255–311. Springer-Verlag, New York, 1971.
4. K. C. LEIBMAN AND E. ORTIZ, *J. Pharmacol. Exp. Ther.* **173,** 242 (1970).
5. B. L. VAN DUUREN, S. NELSON, L. ORRIS, E. D. PALMES, AND F. L. SCHMITT, *J. Nat. Cancer Inst.* **31,** 41 (1963).
6. P. MILVY AND A. J. GARRO, *Mutat. Res.* **40,** 15 (1976).
7. C. DE MEESTER, F. PONCELET, M. ROBERFROID, J. RONDELET, AND M. MERCIER, *Mutat. Res.* **56,** 147 (1977).
8. H. VAINIO, R. PAAKKONEN, R. RONNHOLM, V. RAUNIO, AND O. PELKONEN, *Scand. J. Work Environ. Health* **3,** 177 (1976).
9. B. J. DOWTY, D. R. CARLISLE, AND J. L. LASETER, *Environ. Sci. Technol.* **9,** 762 (1975).
10. D. M. JERINA AND J. R. BEND, "Biological Reactive Intermediates" (D. J. Jollow, J. I. Kocsis, R. Snyder, and H. Vainio, Eds.), pp. 207–236. Plenum, New York, 1977.

11. R. MORGENSTERN, J. W. DEPIERRE, AND L. ERNSTER, *Biochem. Biophys. Res. Commun.* **87**, 657 (1979).
12. F. OESCH, *Xenobiotica* **3**, 305 (1973).
13. R. H. COX, O. HERNANDEZ, B. YAGEN, B. R. SMITH, J. D. MCKINNEY, AND J. R. BEND, "The Chemistry of Environmental Agents as Potential Human Hazards" (J. D. McKinney, Ed.). Ann Arbor Science, Ann Arbor, Mich., 1980, in press.
14. O. HERNANDEZ, B. YAGEN, R. H. COX, B. R. SMITH, G. L. FOUREMAN, J. R. BEND, AND J. D. MCKINNEY, "The Chemistry of Environmental Agents as Potential Human Hazards" (J. D. McKinney, Ed.). Ann Arbor Science, Ann Arbor, Mich., 1980, in press.
15. J. PACHECKA, P. GARIBOLDI, L. CANTONI, G. BELVEDERE, E. MUDDINI, AND M. SALMONA, *Chem.-Biol. Interact.* **27**, 313 (1979).
16. S. P. JAMES AND D. A. WHITE, *Biochem. J.* **104**, 914 (1967).
17. F. SEUTTER-BERLAGE, L. P. C. DELBRESSINE, F. L. M. SMEETS, AND H. C. J. KETELAARS, *Xenobiotica* **8**, 413 (1978).
18. P. JENNER AND B. TESTA, *Drug Metab. Rev.* **2**, 117 (1978).
19. A. V. DEAN, S. J. LAN, K. J. KRIPALANI, L. T. DIFAZIO, AND E. C. SCHREIBER, *Xenobiotica* **7**, 549 (1977).
20. J. B. STOTHERS, "Carbon-13 NMR Spectroscopy," p. 49, Academic Press, New York, 1972.
21. S. L. SHAPIRO, H. SOLOWAY, AND L. FREEDMAN, *J. Amer. Chem. Soc.* **80**, 6060 (1958).
22. C. DUPIN AND J. F. DUPIN, *Bull. Soc. Chim. Fr.* 249 (1970).
23. K. MISLOW, *J. Amer. Chem. Soc.* **73**, 3954 (1951).
24. E. L. ELIEL AND D. W. DELMONTE, *J. Org. Chem.* **21**, 596 (1956).
25. A. M. JEFFREY, H. J. C. YEH, D. M. JERINA, R. M. DEMARINIS, D. H. FOSTER, D. E. PICCOLA, AND G. A. BERCHTOLD, *J. Amer. Chem. Soc.* **96**, 6929 (1974).
26. F. A. BELAND AND R. G. HARVEY, *J. Amer. Chem. Soc.* **98**, 4963 (1976).