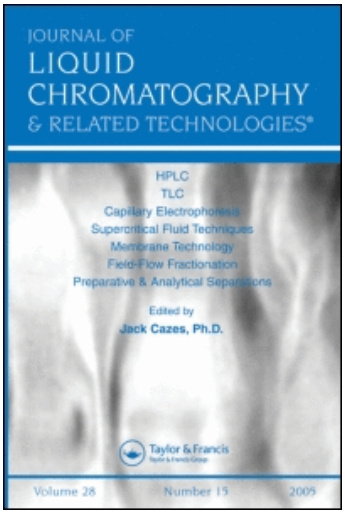

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HPLC SEPARATION OF THE DIASTEREOMERIC GLUTATHIONE ADDUCTS OF STYRENE OXIDE

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ABSTRACT

The four diastereomeric thioether adducts resulting from the addition of glutathione to racemic styrene oxide were separated on a Radial Pak C_{18} column using pH 7 Tris-phosphate buffer solution containing methanol as eluent. The benzylic thioether (1) eluted earlier than the benzylic alcohol (2) regioisomers. A complete stereochemical profile was established with the first eluting stereoisomer assigned as (S,R)-1, followed by (R,R)-1, (S,R)-2, and (R,R)-2,. The diastereomers with S configuration at the benzylic carbon emerged first for each set of regioisomers. The use of glutathione as a chiral probe for the analysis of enantiomerically enriched epoxides was illustrated with β-methylstyrene oxide formed from (1R,2S)-N,N-dimethylephedrium bromide during the course of a chiral phase-transfer synthesis of oxiranes.

INTRODUCTION

The reaction of glutathione (GSH) with electrophilic compounds is an important component in the defense mechanisms available to many organisms. This reaction is catalyzed by a group of enzymes known as the glutathione transferases (1). Among the substrates for these enzymes, epoxides constitute a

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major group of environmental interest. Epoxides are products of the oxidative metabolism of unsaturated hydrocarbons catalyzed by the cytochrome P450 dependent monoxygenase system (2). Styrene oxide has been found a useful substrate for the in vitro determination of glutathione transferase activities (3). In addition, this epoxide has provided a model for examining the stereochemical aspects of the reaction of GSH in the presence and absence of enzyme (4). The product profile for GSH and styrene oxide indicated various degrees of stereoselectivity for the chemical and enzymatic reactions. Further work was hampered by the lack of a suitable analytical procedure. Optimal analytical conditions provided only partial separation of the stereomeric GSH conjugates of styrene oxide (5).

In the present work we describe a modified reversed-phase HPLC (RP-HPLC) procedure for the separation of all the diastereomeric glutathione adducts of this epoxide. The practical value of this procedure is illustrated by examining the stereoisomer distribution resulting from reaction of GSH with a styrene oxide sample obtained by a phase- transfer procedure utilizing a chiral catalyst reported to produce epoxide with high enantiomeric enrichment (6).

MATERIALS AND METHODS

Enantiomerically pure styrene oxides were prepared from the corresponding mandelic acids. Reduction of (+)-(S)-mandelic acid with borane-dimethyl sulfide gave (+)-(S)-phenylethane diol

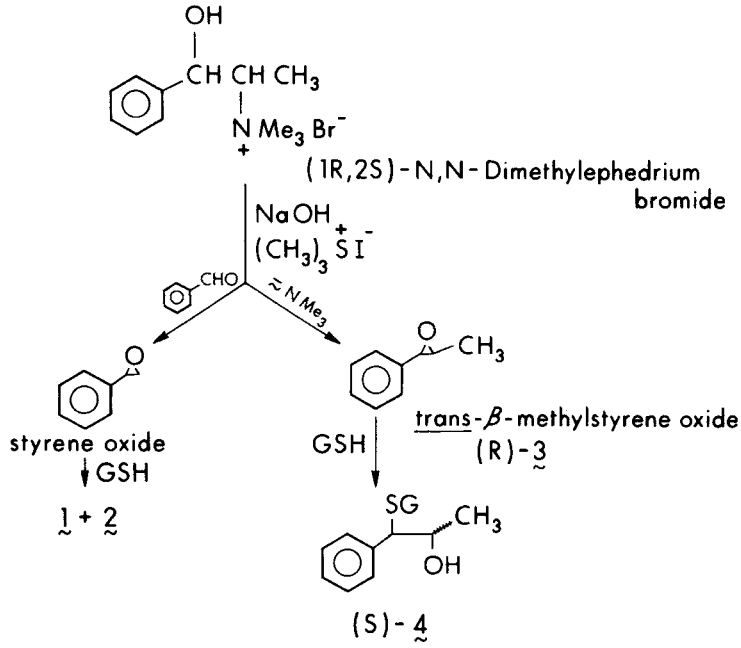
which by the orthoester procedure (7) gave (-)-(S)-styrene oxide ($[\alpha]_D^{25}$ -42.5° (c2.0, benzene), lit.(8)-41.5° (c5.0). Similarly, (-)-(R)-mandelic acid afforded (+)-(R)-styrene oxide $[\alpha]^{25}$ D+35.5°(c2.27, benzene), lit.(9) + 42.2° (c3.09).

Chiral phase-transfer procedure

By following the published procedure (6), benzaldehyde (10.7 mmol), (-)-(1R,2S)-N N-dimethylephedrium bromide (2.13 mmol), trimethylsulfonium iodide (11 mmol), and sodium hydroxide (20 mmol) were refluxed for 60 hr under argon in a mixture of water (18 ml) and dichloromethane (8 ml). The crude product was purified by filtration through deactivated neutral alumina, followed by elution with dichloromethane, to provide 0.63 g of an oil, $[\alpha]_D^{25}$+4.2° (c1.7, acetone), lit. +4.4° (c5.5, acetone). No significant product formation ocurred at room temperature. A control experiment was conducted as above except that benzaldehyde was omitted from the reaction mixture. The product, 0.17 g (57% yield), $[\alpha]_D^{25}$ + 79.75° (c1.6, acetone), was identified as trans-β-methylstyrene oxide by proton nmr and its reaction products with GSH (Scheme 1).

Glutathione Conjugates

The GSH conjugates were prepared by reaction of GSH (5 eq) with the corresponding epoxide (1 eq) in 0.5 M potassium bicarbonate for 12 hr at room temperature under argon. Isolation and purification methods for these compounds have been described (4,5).



SCHEME 1

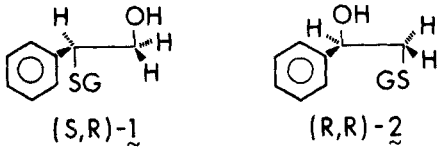
HPLC

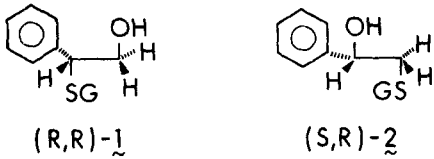
The equipment used consisted of a M6000A pump, model 440 UV absorbance detector (254nm), and a model U6K injector all from Waters Associates. The column used was a 8 mmID 5 micron C18 Radial-PAK (Waters Associates). A precolumn (5 micron C18-Spherisorb, Rainin Instruments Co.) and an in-line 2 micron filter were also used. A stock solution of Tris-phosphate buffer (pH 7) was prepared as follows: 5 ml of 85% phosphoric acid was added to 1000 ml of water and neutralized to pH 7 (Corning 125 pH meter with combination glass electrode) with tris-(hydroxylmethyl)aminomethane (Tris-base). Buffer A

consisted of 500 ml of stock solution plus 500 ml of 50 mM sodium sulfate solution. Buffer B contained 475 ml of stock, 25 ml methanol, and 500 ml 50 mM sodium sulfate. The column was conditioned by pumping 10 column volumes of B followed by 15 column volumes of A. For the separations, a step gradient procedure at a flow rate of either 3 ml/min or 2 ml/min was used. After sample injection, buffer A was pumped for 10 min followed by a step switch to buffer B until elution was complete. For reequilibration, 15 column volumes of buffer A were pumped through the column.

RESULTS AND DISCUSSION

The reaction of styrene oxide with glutathione produces two positional isomers, 1 and 2 (Fig. 1). Because of the chiral benzylic center in styrene oxide each positional isomer may consist of one or two diastereomers depending on the optical purity of the starting epoxide. As shown in Fig. 1 the benzylic thioether isomers are designated as (R,R) - 1 and (S,R) - 1, and the benzylic alcohols as (R,R) - 2 and (S,R) - 2. The first letter designates the absolute configuration at the benzylic carbon and the second letter the configuration of the asymmetric carbon of the cysteine residue in GSH. For simplicity, the configuration of the γ-glutamyl residue is not included in this notation. This nomenclature is preferred to rotation signs since, for styrene oxide, the magnitude and sign of rotation are solvent and





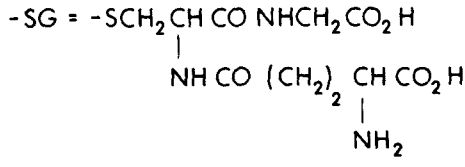


Figure 1. Relative stereochemistry of the GSH adducts of styrene oxide. The first letter designates the stereochemistry at the benzylic carbon center and the second letter the configuration of the cysteinyl residue in GSH.

concentration dependent (10). Reaction of GSH with optically pure (R)-styrene oxide would give rise to (S,R) - 1 and (R,R) - 2; (S)-styrene oxide would produce (R,R) - 1 and (S,R) - 2. Racemic styrene oxide would produce a mixture of all four diastereomers (Fig. 1).

RESULTS AND DISCUSSION

The separation of the glutathione conjugates of styrene oxide as originally developed (4) is illustrated in Fig. 2a.

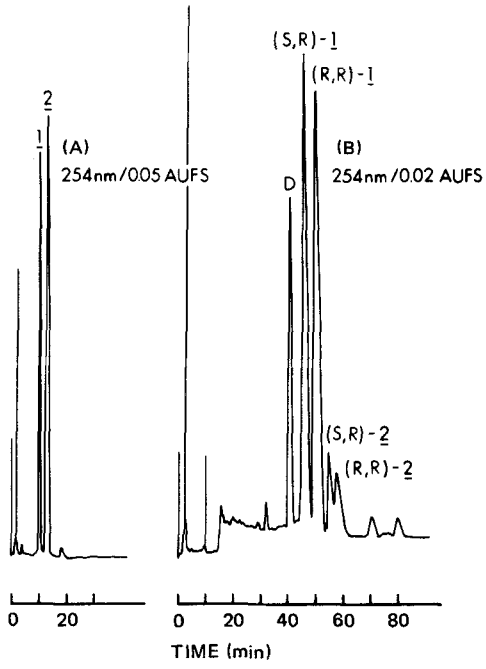


Figure 2. HPLC profiles of the diastereomeric GSH adducts of (±)-styrene oxide. Trace 2a, Tris-phosphate pH 3 buffer/15% MeOH; trace 2b, step gradient from buffer A (Tris-phosphate pH 7, 25mM sodium sulfate) to buffer B (Tris-phosphate pH 7, 2.5% MeOH, 25mM sodium sulfate). Flow rate was 2ml/min in both cases. For stereochemical N rotation see Fig. 1. The peak labeled D in Fig. 2b is 1-phenylethane 1,2-diol.

As described earlier, the first eluting peak contained a single benzylic thioether diastereomer, now identified as (S,R) - 1, the remaining three diastereomers coeluted in the second peak. These conditions were found useful in the analysis of thioether metabolites of styrene oxide but were not adequate for more detailed analysis of the stereochemical aspects of the alkylation reaction. In order to study the regio- and stereoselectivity of the enzymatic reaction we explored conditions that would allow separation of all four diastereomers (Fig. 1) and still be compatible with enzymatic samples. The separation of the four diastereomers from the reaction of racemic styrene oxide and GSH is shown in Fig. 2b. By operating at pH 7 with a minimal volume (2.5%) of methanol resolution is achieved in a reasonable time. The order of elution in this case is (S,R) - 1, (R,R) - 1,(S,R) - 2 and (R,R) - 2. Stereochemical assignments were based on the analysis of reaction products of GSH with optically pure epoxides the traces for which are shown in Fig. 3. The GSH adducts of (+)-(R)-styrene oxide (Fig. 3a) correspond to the first and last eluting peaks on the racemic sample (Fig. 2b). The (-)-(S)-sytrene oxide forms the GSH conjugates corresponding to the inside peaks (Fig. 3b) in the racemic sample (Fig. 2b). For both sets, the benzylic thioether (1) eluted ahead of the benzylic alcohol (2) regioisomer. These assignments were based on: 1) isolation and structural character-

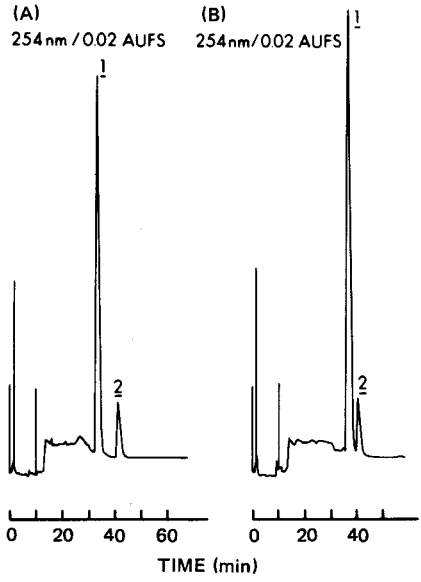


Figure 3. HPLC profiles of the diastereomeric GSH adducts of (+)-(R)-styrene oxide (trace 3a) and (-)-(S)-styrene oxide (trace 3b). Mobile phase and flow rate as on Fig. 2b. The numbers designate regioisomers (Fig. 1).

ization of the GSH conjugates of (+)-(R)-styrene oxide (14); 2) coinjection with authentic benzylic thioether samples (5). A step gradient was introduced as a cleaning step for samples from incubation mixtures. After pumping buffer A (0% methanol) for 10 min the solvent was manually switched at the pump head (M6000A pump) to buffer B (2.5% methanol). This procedure helps in the removal of large amounts of salts and other polar components and it provided for more reproducible separations. The early

eluting peak shown in Fig. 2b and identified as D corresponded to phenylethane 1,2-diol, demonstrated by coelution with authentic diol. This diol is formed by hydrolysis of styrene oxide and it is a potential contaminant in samples from enzymatic experiments. An organic amine in the buffer seemed to be critical for the separation of these compounds. We have used Tris-base because it is readily available in high purity although it is likely that other organic bases may produce similar results (11). As noted by Horvath (12), the role of organic bases in HPLC separations goes well beyond a simple buffering action and it is likely that ion pairing, and masking of silanols on the column bonded phase play an important role in the separation mechanism.

The ability to separate all four diastereomers from the reaction of styrene oxide with GSH provides an excellent tool to study the stereochemical aspects of the enzymatic and chemical reactions of GSH with styrene oxide. In addition to its obvious application to enzymatic studies, it was clear that we could use this approach to determine the optical purity of styrene oxide samples. The ring opening reaction of styrene oxide with GSH, under basic conditions, has been shown to occur without racemization (4,5) and this is verified by the traces shown in Fig. 3. As noted earlier the optical purity of styrene oxide is difficult to ascertain from the optical rotation of the sample since its value varies with

concentration and solvent. However, if a sample of styrene oxide of unknown optical purity is allowed to react with GSH as described here, the ratio of the first two eluting peaks will reflect the optical purity of the sample.

An attractive test for this approach was found in a recently published method (6) for the preparation of enantiomerically pure styrene oxides. Since these epoxides are now widely used in enzymatic reactions the operational simplicity and high optical yields obtained made this the synthetic method of choice. The reaction was conducted as described in the literature with (-)-(1R,2S)-N,N-dimethylephedrium bromide as the chiral phase-transfer catalyst. Under these conditions the (S)-styrene oxide was reported to be formed with enantiomeric purity up to 97% (6). The product obtained from this reaction was quenched with GSH (0.5 M $KHCO_3$ solution) and analyzed as described above. The trace for the product mixture (Fig. 4a) shows that all four diastereomers were formed. At the levels of enantiomeric enrichment reported (6) for this reaction a trace similar to Fig. 3b would have been anticipated. It follows that the level of optical induction by the chiral catalyst under the conditions reported is negligible. In a later note (13) the authors lamented contamination of their samples by β-methylstyrene oxide (3, Scheme 1) which could be formed from the ephedrium salt chiral catalyst. We have verified that this is indeed the case by:

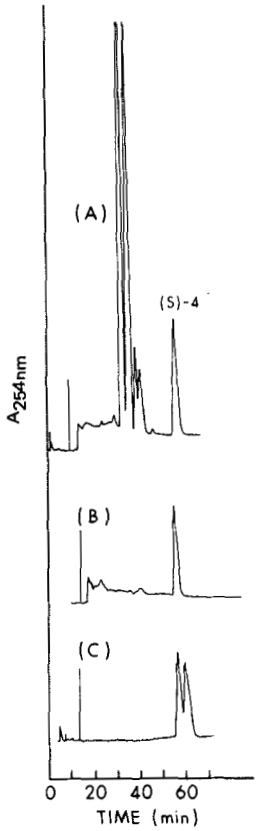


Figure 4. HPLC profiles of GSH adducts of (a) styrene oxide obtained in the presence of an ephedrium salt chiral phase-transfer catalyst; (b) optically pure β-methylstyrene oxide from fragmentation of the ephedrium salt catalyst; (c) racemic β-methylstyrene oxide. Flow rate was 3ml/min with mobile phase as on Fig. 2b.

1) a control sample without benzaldehyde and quenched with GHS; 2) coelution of authentic β-methylstyrene oxide glutathione adduct (4) with the product obtained above. The late eluting peak designated as (S)-4 on Fig. 4a corresponded to the glutathione conjugate of β-methylstyrene oxide (3) formed when benzaldehyde was ommitted. Fig. 4c shows the diastereomers derived from racemic β-methylstyrene oxide. It is clear that the extent of optical induction, if any, is very remote from the original claim. The optical activity detected in the product(s) is largely, if not exclusively, derived from optically pure β-methylstyrene oxide formed by the elimination of trimethylamine from the chiral phase-transfer catalyst. These conclusions are summarized in Scheme 1. The elimination of trimethylamine proceeds with retention of configuration at the benzylic carbon and the configuration at this site of the β-methylstyrene oxide (3) is R. Reaction of 3 with GSH has been shown to be regioselective with the sulfur nucleophile adding exclusively to the benzylic carbon (4). Thus, the configuration of the GSH adduct of 3 becomes S at this center, and in relation to the adducts from racemic 3, it corresponds to the first eluting peak (Fig. 4b and 4c). Similarly, by examining the traces of the adducts of (R)-and (S)-styrene oxide (Fig.2 and 3) one finds that for each set of regioisomers (i.e. 1 and 2) the diastereomer with S configuration at the benzylic center elutes

earlier than the corresponding R diastereomer ((S)-1 vs (R)-1, and (S)-2 vs (R)-2). This undoubtedly reflects a strong stereochemical influence in the separation mechanism involving these compounds. Further studies are necessary in order to ascertain whether this observation may be of use in elucidating the stereochemistry of GSH adducts of epoxides. The present analytical procedure significantly expands the range of experiments possible, with styrene oxide as substrate, in the study of the glutathione transferase enzymes. In addition, an interesting observation, the use of GSH as a chiral trap may develop into a practical approach to the determination of the optical purity of epoxides, particularly those from metabolic processes.

REFERENCES

1. Boyland, E., and Chassed, L.F., Adv. Enzymol. 32, 173 (1969).
2. Coon, M.J. and Persson, A.V., in Enzymatic Basis of Detoxication, Vol. 1, Jakoby, W.B., ed. Academic Press, New York, 1980, p. 117. 3. James, M.O., Fouts, J.R., and Bend, J.R., Biochem. Pharmacol., 15, 187 (1976).
4. Hernandez, O., Yagen, B., Cox, R.H., Smith, B.R., Foureman, G.L., Bend, J.R., and McKinney, J.D., in The Chemistry of Environmental Agents as Potential Human Hazard, McKinney, J.D., ed., Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1980, p. 425.

5. Yagen, B., Hernandez, O., Bend, J.R., and Cox, R.H., Biorg. Chem., 10, 299 (1981).

6. Hiyama, T., Mishima, T., Sawada, H., and Nozaki, H., J. Am. Chem. Soc., 97, 1626 (1975).

7. Newman, M.S., and Olson, D.R., J. Org. Chem., 38, 4203 (1973).

8. Pirkle, W.H., and Rinaldi, P.L., J. Org. Chem., 43, 3803 (1978).

9. Imuta, M., Kawai, K., and Ziffer, H., J. Org. Chem., 45, 3352 (1980).

10. Berti, G., Bottari, F., and Macchia, B., Ann. Chim. (Rome), 58, 1101 (1963).

11. Rivier, J.F., J. Liquid Chromatogr., 1, 343 (1978).

12. Melander, W.R., Stoveken, J., and Horvath, C., J. Chromatogr., 185, 111 (1979).

13. Himaya, T., Mishima, T., Sawada, H., and Nozaki, H., J. Am. Chem. Soc., 98, 641 (1976).

14. Cox, R.H., Hernandez, O., Yagen, B., Smith, B.R., McKinney, J.D., and Bend, J.R., in The Chemistry of Environmental Agents as Potential Human Hazards, McKinney, J.D., ed., Ann Arbor Science Publishers, Inc., Ann Arbor, MI 1980, p. 403.