DETERMINATION OF THE ENANTIOMERIC COMPOSITION OF CHIRAL EPOXIDES USING CHIRAL DERIVATIZATION AND LIQUID CHROMATOGRAPHY

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SUMMARY

There appears to be a need for convenient chromatographic methods for the analysis of the enantiomeric composition of chiral oxiranes. In this report a new procedure for such purposes is described. Racemic mono-, 2,2-disubstituted and *trans*-2,3-disubstituted oxiranes were reacted with simple volatile alkylamines. The aminoalcohol products were derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, and the resulting diastereomeric thioureas were resolved on C_{18} liquid chromatographic columns using methanol–water mobile phases and detection at 254 nm. In several cases, the order of elution of the derivatives could be related to the configuration of the starting epoxide. The procedure is simple and convenient to carry out despite the two-reaction derivatization sequence, and may have broad utility in the enantiomeric analysis of chiral epoxides.

INTRODUCTION

The chemistry, biochemistry, metabolism, toxicity, etc., of chiral epoxides (oxiranes) are of widespread and intense interest[1–15]. Relatively little has been published, however, on the chromatographic separation of enantiomeric epoxides, despite a vast and rapidly growing literature on the chromatographic resolution of the racemates of a variety of other compounds[16–24]. Schurig and Wistuba[25] described the resolution of the enantiomers of several volatile epoxides using optically active nickel complexes as stationary phase in capillary gas–liquid chromatographic columns. *trans*-Stilbene oxide[26] and 7,12-dimethylbenz[*a*]anthracene-5,6-oxide[27] have been resolved using (different) chiral liquid chromatography (LC) columns. The enantiomeric composition of styrene oxide (2-phenyloxirane) formed in liver microsomal incubations of styrene was determined by allowing the microsomal epoxide hydrolase to hydrolyze the epoxide to 1-phenylethane-1,2-diol, followed by chiral derivatization of the diol and LC separation of the resulting diastereomers[15]. Falk *et al.*[28] determined the enantiomeric composition of epoxyeicosatrienoic acids formed during cytochrome-P450-catalyzed oxygenation of arachidonic acid in a heroic procedure involving con-

version of the epoxides to alcohols, derivatization with a chiral isocyanate, and LC separation of the diastereomers. Much of the current interest in chiral epoxides is centered on epoxide metabolites of polycyclic aromatic hydrocarbons (PAHs) and several reports describing the determination of the enantiomeric composition of such metabolites have appeared. For example, the enantiomeric composition of enzymatically formed benz[a]anthracene-5,6- and 8,9-oxide were determined by reacting the epoxides with glutathione, followed by LC separation of the resulting diastereomers[29]. Each enantiomer of the 5,6-oxide gave two adducts with glutathione due to regioisomerism, while only one of the two possible regioisomeric adducts was obtained from each of the enantiomeric 8,9-oxides. The procedure was also complicated by the incomplete chromatographic separation of some of the isomeric adducts. Nevertheless, the analytical method was successfully used in a study of the stereochemistry of the cytochrome-P450 mediated epoxidation of benz[a]anthracene[29]. Extensive studies[13,30-35] in several laboratories on the nature and stereochemistry of the covalent binding of PAHs to biological macromolecules led to the use of polyguanylic acid as a chiral trapping agent for epoxide metabolites of benz[a]pyrene[33-35]. In the trapping reaction, the exocyclic amino group of guanine reacts at the benzylic epoxide carbon of the benzpyrene-derived epoxides. Both *syn* and *anti* ring-opening occurs, and therefore each enantiomer gives two adducts. In a complex procedure, the initial adducts were hydrolyzed with potassium hydroxide and then digested to guanosine derivatives with alkaline phosphatase. The guanosine derivatives were separated on reversed-phase LC columns[33-35].

  It appears from all of the above that there is a need for simpler and more widely applicable procedures for the enantiomeric analysis of epoxides. In the present report a new method for the chromatographic determination of the enantiomeric composition of several different types of chiral epoxides is described. The procedure involves ring-opening aminolysis of the epoxide with a simple primary amine, followed by derivatization of the resulting enantiomeric aminoalcohols with a chiral reagent and LC separation of the diastereomeric derivatives formed. The procedure is simple and practical to carry out despite the two-reaction derivatization sequence, requires only commercially available reagents and chromatographic columns, and may have wide applicability.

EXPERIMENTAL

Chemicals

  The following compounds were obtained from Aldrich (Milwaukee, WI, U.S.A.): styrene oxide, *trans*-2,3-epoxybutane, (1R,2R)-(+)-1-phenylpropylene oxide, propylene oxide, 3,3-dimethyl-1,2-epoxybutane, 1,2-epoxyoctane, 1,2-epoxy-3-phenoxypropane, 4-*tert.*-butylphenyl 2,3-epoxypropyl ether, *trans*-stilbene oxide, *n*-butylamine, isopropylamine, cyclopentylamine, isobutylamine, and cyclohexylamine. Other compounds obtained from commercial sources were: (S)-(−)-propylene oxide, Sigma (St. Louis, MO, U.S.A.); (R)-styrene oxide, Fluka (Hauppage, NY, U.S.A.); (±)- and (R)-α-methylbenzyl isothiocyanate, Trans World Chemicals (Kensington, MD, U.S.A.); 3-(α-naphthoxy)-1,2-epoxypropane, Chemical Dynamics (South Plainfield, NJ, U.S.A.); α-methylstyrene oxide, Chemtech Research (Hayward, CA, U.S.A.); acetonitrile and methanol, Burdick and Jackson Labs. (Muskegon, MI,

U.S.A.); ammonium phosphate monobasic, J. T. Baker (Phillipsburg, NJ, U.S.A.); 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT), Polysciences (Warrington, PA, U.S.A.). The above compounds were used as received, without further purification. Double-distilled water was used.

Derivatization procedures

Procedure A. A 50-μl aliquot of the epoxide (50 mg if the epoxide is a solid) was placed in a 3-ml Reactivial (Pierce, Rockford, IL, U.S.A.) and 200 μl of an amine (Table I) was added. The vial was tightly capped with a PTFE-lined cap and heated at 100°C in a heating block (Pierce) for a period depending on the epoxide (Table I). The vial was then removed from the heating block and allowed to cool to room temperature. The excess amine was evaporated in a stream of nitrogen, and a 0.5-mg or 0.5-μl portion of the product was placed in a conical test tube. A 100-μl aliquot of acetonitrile containing 2 mg TAGIT was added, the tube was vortexed, capped, and allowed to stand at room temperature for 30 min. Acetonitrile, 100 μl, and 50 μl of 0.02 M ammonium phosphate, pH 3, was added. The tube was vortexed and 2–5 μl aliquots were injected into the LC system.

Procedure B. The epoxide, *ca.* 0.5 mg, was placed in the Reactivial and 100 μl of the amine was added. The remainder of the procedure was identical to procedure A above, with the exception that after evaporation of the excess primary amine the TAGIT solution was added directly to the Reactivial.

Procedure C. Propylene oxide, 50 μl, was placed in the vial, and cyclohexyl-amine, 100 μl, and methanol, 100 μl, were added. The vial was vortexed and capped, and allowed to stand at room temperature for 68 h. A 1-μl aliquot was then derivatized with TAGIT as described above.

Procedure D. (1R,2R)-(+)-1-phenylpropylene oxide was reacted with *n*-butyl-amine according to Procedure A above for 72 h. Portions (1 mg) of the product were derivatized with racemic or (R)-α-methylbenzyl isothiocyanate as described[20].

Chromatography

A Waters Assoc. LC system consisting of a Model M-6000 solvent delivery system, a Model U6K injector, and a Model 440 asbsorbance detector was used. With one exception (see below), separations were carried out on a Beckmann (Berkeley, CA, U.S.A.) 150 × 4.6 mm I.D. column packed with Ultrasphere ODS of 5-μm particle size. A Waters Assoc. Nova-pak C_{18} column, 3.9 mm × 15 cm, was used in the analysis of the derivatives of epoxide 11. The mobile phases used (Table I) were prepared by first vacuum-filtering the individual components and then mixing in the appropriate ratio. The mobile phase was delivered at 1.0 ml/min, and the column effluent was monitored at 254 nm. The detector output was recorded using a Hewlett-Packard (Avondale, PA, U.S.A.) Model 3390 integrator.

RESULTS

Epoxides 1–10 (Fig. 1) were reacted with a primary amine to obtain an ami-noalcohol (Fig. 2). The aminolysis of the epoxides was carried out either on a 50-mg (procedures A or C) or 0.5-mg (procedure B) scale. The reactivity of the epoxides toward the amines differed considerably, and therefore the reaction times had to be

TABLE I

SEPARATION OF ENANTIOMERIC EPOXIDES BY LC AFTER AMINOLYSIS AND REACTION WITH TAGIT

Epoxide*	Amine**	Procedure***	Mobile phase§	Separation factor[36] α	Peak resolution[37] R	t_R of TAGIT derivatives** (min)		Configuration of epoxide yielding faster-eluting derivative
1	Cyclohexyl	C	62:38	1.23	2.24	10.17	12.15	R-(+)
2	n-Butyl	A (24)	67:33	1.19	2.29	12.81	14.93	
3	Isobutyl	A (2)	75:25	1.15	2.14	8.04	9.07	
4	Isobutyl	A (4)	65:35	1.37	4.00	10.09	13.35	R-(+)
5	Isobutyl	B (2)	63:35	1.32	3.63	11.83	15.15	
6	Isobutyl	A (2)	75:25	1.31	3.88	11.72	14.88	
7	Isopropyl	B (2)	70:30	1.45	4.74	7.20	9.85	S
8	n-Butyl	A (24)	65:35	1.09	1.11	19.16	20.76	
9	n-Butyl	A (48)	60:40	1.18	1.72	7.38	8.43	
10	n-Butyl	A (70)	70:30	1.21	1.80	7.17	8.36	

* See Fig. 1.

** See derivatization scheme in Fig. 2.

*** See Experimental. The aminolysis reaction time (in h) for procedures A and B is in parentheses.

§ Volumes of methanol and water, respectively, were mixed in the proportions indicated. Other chromatographic conditions are given in the Experimental.

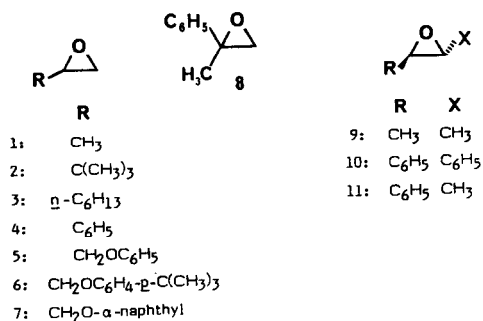|	R			R	X
1:	CH₃		9:	CH₃	CH₃
2:	C(CH₃)₃		10:	C₆H₅	C₆H₅
3:	n-C₆H₁₃		11:	C₆H₅	CH₃
4:	C₆H₅				
5:	CH₂OC₆H₅				
6:	CH₂OC₆H₄-p-C(CH₃)₃				
7:	CH₂O-α-naphthyl				

Fig. 1. Chemical structures of the epoxides studied. No absolute configuration is implied.
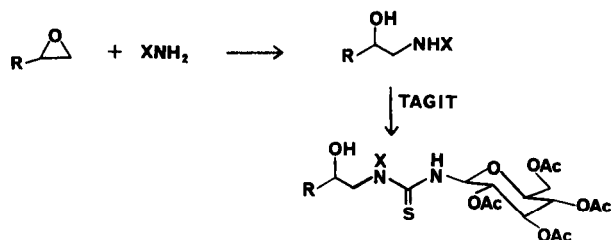

Fig. 2. The derivatization sequence.

adjusted accordingly to maximize the conversion of the epoxides to aminoalcohols (Table I). The extent of conversion was determined by monitoring the chromatographic peak areas due to the final derivatives *versus* time (data not shown).

The aminoalcohols were reacted with TAGIT, a chiral derivatizing agent[38], to give diastereomeric thioureas (Fig. 2). The derivatives produced from each racemic aminoalcohol were separated by reversed-phase LC (Table I). The resolution of styrene oxide is shown in Fig. 3.
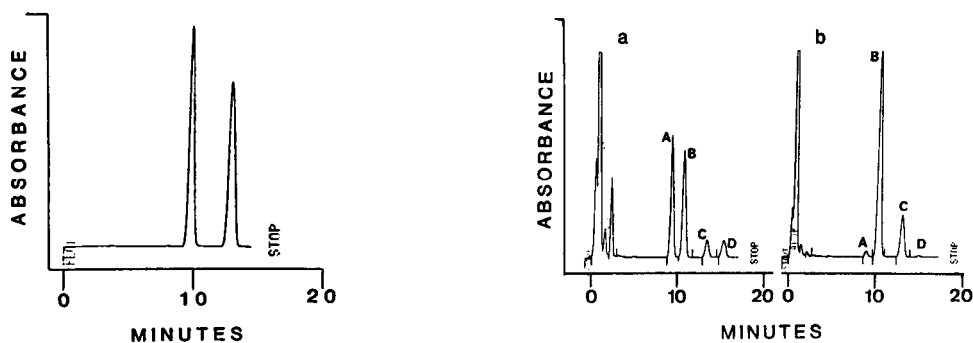

Fig. 3. The resolution of styrene oxide, 4.

Fig. 4. (a) Chromatogram obtained upon derivatization of (+)-11 with *n*-butylamine and (±)-AMBI. t_R of A, 9.69; B, 11.09; C, 13.80; D, 15.70 min. (b) Chromatogram obtained upon derivatizing (+)-11 with *n*-butylamine and R-AMBI. The small peaks at A and D are due to contamination of R-AMBI with S-AMBI[20].

The aminoalcohols produced by the reaction of (1R,2R)-(+)-1-phenylpropylene oxide [(+)-11] with n-butylamine were reacted with the chiral reagent (R)-α-methylbenzyl isothiocyanate (R-AMBI) and also with the racemic form of the reagent. The results are shown in Fig. 4. Two sets of diastereomeric peaks were obtained, in a ratio of 5.4:1, when the racemic derivatizing agent was used (Fig. 4a). As expected, when R-AMBI was used in the derivatization, only two peaks were obtained (Fig. 4b), in a ratio of 4.5:1. Thus, it appears that the ring opening occurred at both carbon atoms of the oxirane ring, but with considerable regioselectivity. The direction of regioselectivity, *i.e.*, the predominant site of attack by the amine, was not determined.

Individual enantiomers of propylene oxide and styrene oxide were available and were used to determine the order of elution of the corresponding derivatives (Table I).

DISCUSSION

The observation by us[38–40] and by others[41] that derivatization of racemic aminoalcohols with TAGIT provides diastereomeric derivatives which are generally readily resolvable by reversed-phase LC prompted our studies with chiral epoxides. It is well known that epoxides react with amines to give ring-opened aminoalcohol derivatives[42]. A key question in developing the method was the regioselectivity of the ring-opening reaction. It is known[43–45] that in the case of monosubstituted oxiranes the nucleophilic attack by the amine occurs at the less hindered (methylene) carbon (Fig. 2). Thus, the asymmetric center is not affected, and the resulting aminoalcohols retain the original configuration. Monosubstituted oxiranes 1–7 were reacted with amines, and the resulting aminoalcohols were derivatized with TAGIT. The two diastereomeric derivatives obtained from each of epoxides 1–7 were well separated by the chromatographic system used, as judged by the high values of peak resolution R obtained (Table I). The extent of resolution of the diastereomers obtained from 2-methyl-2-phenyloxirane (compound 8) was much smaller (Table I). This is not surprising, inasmuch as the presence of an additional substituent at the chiral center renders the steric differences between the diastereomeric derivatives smaller. The resolution of styrene oxide (4) is shown in Fig. 3.

Individual enantiomers were available for compounds 1 and 4, allowing the determination of the order of elution of the derivatives. In each case, the derivative derived from the R epoxide elutes first (Table I). From previous studies[38] in our laboratory, the order of elution of the TAGIT derivatives of the amino alcohol formally derived from 7 and isopropylamine was known, and since the asymmetric center is not affected in the derivatization sequence, the order of elution of the diastereomeric derivatives could be related to the configuration of epoxide 7. As indicated in Table I, the derivative of (S)-7 elutes first. This apparent difference between compounds 1 and 4 on the one hand, and epoxide 7 on the other, is indeed only an apparent difference: the configurational designations (R *vs.* S) are opposite due to the nature of the substituents at the chiral center, but the actual configurations around the chiral center are the same. Furthermore, we have shown[38,40] that, when derivatized with TAGIT, the enantiomeric pairs of several other aminoalcohols display the same order of elution as those derived from 1, 4 and 7. Therefore, while the

number of compounds studied is small, it is tempting to suggest that the present method may be used to assign the absolute configuration of monosubstituted oxiranes. Clearly, more compounds will have to be studied in order to confirm this point.

The extent of resolution of the *trans*-2,3-disubstituted oxiranes (9 and 10) was smaller than that of the monosubstituted compounds, but baseline separation of the diastereomers was still readily achieved (Table I). The ring-opening reaction in compounds 9 and 10 takes place at a chiral center. Such ring-opening reactions of epoxides proceed via inversion[46], and the products in the case of 9 and 10 are the *erythro* aminoalcohols. No evidence was found for epimerization leading to mixtures of the *erythro* and *threo* aminoalcohols, inasmuch as such diastereomeric aminoalcohols are expected to be separable as their TAGIT derivatives[39].

In order to explore the applicability of the method to oxiranes having two different substituents in the 2- and 3-positions, respectively, the derivatization of 11 was examined. After the reaction of (+)-11 with *n*-butylamine, the aminoalcohol products were derivatized with R-AMBI. This reagent is similar in resolving ability to TAGIT and is a useful chiral reagent developed in our laboratory for the LC separation of enantiomers[30]. The aminoalcohols were also reacted with (±)-AMBI, which, under the chromatographic conditions used (*i.e.*, non-chiral column and mobile phase), is equivalent to derivatizing racemic 11 with R-AMBI. The results are shown in Fig. 4. Nucleophilic attack by the amine occurred at both carbon atoms of the oxirane ring, with a regioselectivity of *ca.* 5:1. The direction of regioselectivity, *i.e.*, the preferred site of attack, was not determined. It is also clear that if (±)-11 were to be derivatized using R-AMBI in the second step (Fig. 2), peaks B and C in Fig. 4a would be derived from (+)-11, and peaks A and D from (−)-11. These experiments demonstrated that (a) R-AMBI may be usable in place of TAGIT in the procedure; this is also suggested by our observation that the aminoalcohol derived from the reaction of epoxide 7 with isopropylamine is readily resolvable by LC via derivatization with R-AMBI[20]; the potential applicability of R-AMBI is significant inasmuch as it costs *ca.* 50 times less than TAGIT; (b) the method described may be applicable to 2,3-disubstituted oxiranes with non-identical substituents, since the regioisomers as well as the diastereomers may be separable, as demonstrated for epoxide 11 (Fig. 4).

The method developed has several advantages: the procedure is simple and convenient to carry out despite the two-reaction derivatization sequence, since the excess volatile alkylamine used is easily evaporated at the end of the aminolysis, and derivatization with TAGIT is simple and rapid; the retention times of the derivatives are relatively short; only commercially available reagents and chromatographic columns are used. A disadvantage of the technique may be the somewhat long aminolysis reaction times required for some of the epoxides. Also, some sensitive functional groups may not survive the vigorous aminolysis conditions required for less reactive epoxides.

In conclusion, a convenient and potentially widely applicable method for the enantiomeric analysis of chiral epoxides has been developed.

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