Journal of Chromatography, 637 (1993) 29–34
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 882

Liquid chromatographic determination of chiral epoxides by derivatization with sodium sulphide, *o*-phthalaldehyde and an amino acid

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(First received October 27th, 1992; revised manuscript received January 12th, 1993)

ABSTRACT

A high-performance liquid chromatographic method for the enantiomeric determination of chiral epoxides is described. By derivatization with sodium sulphide, *o*-phthalaldehyde and an optically pure amino acid, chiral mono- and 2,2-disubstituted epoxides were converted into diastereomeric isoindole derivatives. Separation of the diastereomers was carried out by reversed-phase chromatography and the derivatives were detected fluorimetrically. For a series of nine monosubstituted epoxides, good enantioselectivity was obtained ($\alpha = 1.3$–1.7). To study the effect on the chromatographic behaviour of the isoindole adducts, derivatization was carried out using four different amino acids. The derivatization procedure was optimized using glycidyl butyrate as test compound. Both the precision and accuracy of the method were investigated. The method was applied to monitor the enantiomeric purity of glycidyl butyrate obtained by lipase-catalysed hydrolysis of the racemate.

INTRODUCTION

Optically pure epoxides (oxiranes) are important chiral building blocks for a wide variety of chiral pharmaceuticals [1]. In conjunction with the synthesis, analytical methods are required for the control of the enantiomeric purity of the epoxides. Both gas and liquid chromatographic methods have been described for determining epoxide enantiomers. Complexation gas chromatography has been used to determine the enantiomeric composition of epoxyalkanes [2–4]. More recently, cyclodextrin chiral stationary phases for capillary gas chromatography have been described for the determination of chiral epoxides [5,6].

By applying a two-reaction derivatization sequence, followed by high-performance liquid chromatographic (HPLC) analysis of the derivatives, the enantiomeric determination of several chiral epoxides could be achieved [7]. Recently, a direct HPLC method has been described for the resolution of glycidyl tosylate and glycidyl-3-nitrobenzenesulphonate enantiomers [8]. Because of the presence of salts and enzyme in reaction mixtures from bio-organic syntheses of chiral epoxides, HPLC will generally be preferred to gas chromatography for the determination of the enantiomers. The existing HPLC methods for chiral epoxides show some drawbacks, however. In the method of Gal [7], long

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0021-9673/93/$06.00

reaction times are required to perform the aminolysis step. Moreover, the complete derivatization sequence cannot be automated. On the other hand, the direct method of Shaw and Barton [8] is unsuitable for the analysis of aqueous mixtures of chiral epoxides.

In order to extend the scope of HPLC methods for enantiomeric epoxides, a new approach was evaluated. Epoxides are known to give the corresponding thioglycol derivatives when treated with hydrogen sulphide in the presence of sodium [9]. Based on this reaction, a spectrofluorimetric method has been developed for the assay of epoxides [10]. In this method, epoxides are converted into thioglycol derivatives and subsequently treated with *o*-phthalaldehyde (OPA) and taurine to form fluorescent isoindole adducts. The HPLC determination of isoindole derivatives of several thiols and amines has been extensively reported. By using an optically active thiol compound, *e.g.*, N-acetyl-L-cysteine in the OPA reaction, it was shown that this reaction was also applicable to the enantiomeric determination of amino compounds [11].

The aim of this work was to develop a sensitive and specific HPLC method for the enantiomeric determination of monosubstituted epoxides. To that end, the chiral epoxides were first converted into the corresponding chiral thioglycol derivatives by reaction with sodium sulphide, whereafter OPA and an optically pure amino acid were used as reagents to form diastereomeric isoindole compounds. The retention, enantioselectivity and resolution of the fluorescent diastereomers were examined by reversed-phase chromatography. The method was found to be suitable for monitoring the enantiomeric excess of glycidyl butyrate obtained by lipase-catalysed hydrolysis of the racemate.

EXPERIMENTAL

Materials

(±)-Epichlorohydrin, (±)- and (*R*)-styrene oxide, (±)- and (*S*)-propylene oxide, (*R*)- and (*S*)-glycidyl tosylate, (*R*)- and (*S*)-2-methylglycidyl 4-nitrobenzoate, (±)- and (*S*)-glycidol, (±)-glycidyl phenyl ether, L-valine, L-leucine, L-alanine and L-phenylalanine were obtained from Aldrich (Milwaukee, WI, USA). The optical purity of the L-amino acids, as determined by HPLC on a Crownpak CR(+) column, was 99.9% for all four compounds. (±)- And (*R*)-glycidyl butyrate and (±)-benzyl-3,4-epoxybutyrate were supplied by Andeno (Venlo, Netherlands). OPA, sodium sulphide hydrate and HPLC-grade methanol were obtained from Merck (Darmstadt, Germany). Water was purified with a Milli-Q system (Millipore). All other chemicals were of analytical-reagent grade.

Instrumentation

The chromatographic system consisted of a Hewlett-Packard (Palo Alto, CA, USA) Model 1081 B liquid chromatograph and a Gilson Model 231-401 autosampling injector for derivatization and injection. The injection loop had a 20-μl capacity. The columns used were Nucleosil-120-C_{18} (250 × 4.0 mm I.D., 5 μm) from Macherey–Nagel (Düren, Germany) and LiChrosorb RP-18 (250 × 4.0 mm I.D., 7 μm) from Merck. The flow-rate was 1 ml/min and the column temperature was kept at 40°C.

The derivatives were monitored with a Hitachi (Tokyo, Japan) Model F-1000 fluorescence detector using an excitation wavelength of 330 nm and an emission wavelength of 440 nm. Quantification was performed with a Hewlett-Packard Model 3350 laboratory automation system.

Eluent, reagent and derivatization procedure

The eluent consisted of 50 m*M* sodium acetate buffer (titrated to pH 6.0 with acetic acid)–methanol (45:55, v/v).

For sodium sulphide, a 100 m*M* solution was prepared and titrated to pH 10.0 with acetic acid. The buffer solution was prepared by titrating 12.5 m*M* sodium tetraborate solution with potassium dihydrogenphosphate to pH 8.3. OPA reagent was prepared by dissolving OPA in water–methanol (1:1, v/v) to a concentration of 150 m*M*. The final reagent concentration (30 m*M*) was obtained by dilution with buffer (pH 8.3). A 30 m*M* amino acid solution (L-Val, L-Phe, L-Leu or L-Ala) was prepared by dissolving the compound in buffer (pH 8.3). The

epoxide standards and samples were dissolved in methanol.

Derivatization and injection were performed automatically with a Gilson Model 231-401 system. Into a vial the following were successively dispensed: 100 μl of sample solution and 100 μl of sodium sulphide solution. The contents of the vial were mixed and allowed to stand for 15 minutes at room temperature, then 100 μl of OPA reagent and 100 μl of the amino acid solution were added. The 400-μl volume was mixed and allowed to stand for 15 min at room temperature. Finally, an aliquot of the reaction mixture was injected into the chromatographic system.

RESULTS AND DISCUSSION

Investigation of the enantioselectivity of the derivatives

A series of monosubstituted chiral epoxides, together with a 2,2-disubstituted epoxide, were converted into the corresponding thioglycol derivatives using the sodium sulphide procedure described by Sano and Takitani [10]. The reaction of OPA and L-Val with the thioglycol compounds yielded highly fluorescent derivatives, which are assumed to be isoindole fluorophores. Maximum fluorescence response for the derivatives was obtained at an excitation wavelength of 330 nm and an emission wavelength of 440 nm. The enantioselectivity of the isoindole derivatives was examined using reversed-phase chromatography.

In Table I, the capacity factor (k'), selectivity (α) and resolution (R_s) of the diastereomers are shown. It can be seen that for a broad range of monosubstituted epoxides good enantioselectivity was obtained ($\alpha = 1.3$–1.7). For 2-methylglycidyl 4-nitrobenzoate only partial separation of the enantiomers could be obtained. As this compound is the only 2,2-disubstituted epoxide in the series, the low α-value obtained may possibly be associated with the occurrence of an extra substituent at the chiral carbon. In the case of racemic glycidyl butyrate, two minor peaks (R′ and S′) occurred in the chromatogram which were partly separated from the main peaks (Fig. 1a). By changing the pH of the mobile phase from 6.0 to 4.6, the four peaks could be baseline separated (Fig. 1b). Analysis of non-racemic glycidyl butyrate showed that each of the two pairs of peaks gave the same enantiomeric excess (Fig. 1c). The occurrence of the two side-products may be explained by the fact that ring opening by treatment with Na_2S occurs at both carbons of the epoxide ring of glycidyl butyrate. Consequently, four diastereomeric reaction products will be formed after reaction with OPA–L-Val.

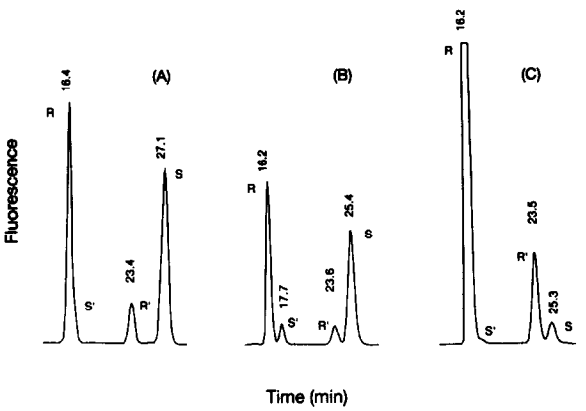


Fig. 1. Separation of the diastereomeric OPA–L-Val derivatives of (A, B) racemic and (C) non-racemic glycidyl butyrate. Conditions: column, LiChrosorb RP-18; mobile phase, 50 mM sodium acetate solution [(A) pH 6.0, (B, C) pH 4.6]–methanol (45:55, v/v); flow-rate, (A) 1 ml/min and (B, C) 1.5 ml/min.

The proposed structures for these isoindole derivatives are shown in Fig. 2. As in neutral and basic solution nucleophilic attack occurs predominantly at the sterically less hindered site of the epoxide [12], the main products formed will be those involving C-3 ring opening, whereas the minor products result from C-2 ring opening.

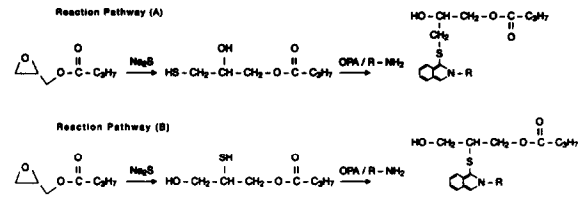
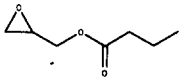
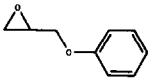
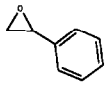
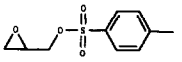
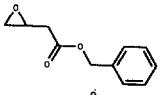
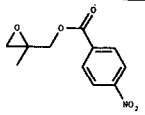


Fig. 2. Expected reaction pathway of glycidyl butyrate. (A) Pathway involving C-3 ring opening; (B) pathway involving C-2 ring opening.

TABLE I

CAPACITY FACTORS (k'), SELECTIVITIES (α) AND RESOLUTION (R_s) OF OPA–L-VAL DERIVATIVES OF RACEMIC EPOXIDES

Column: Nucleosil-120-C_{18}. For other conditions, see Experimental.

Structure	Compound	k'^a	α	R_s	MeOH(%)[b]
	Glycidol	0.81	1.28	1.99	55
	Glycidyl butyrate	3.25	1.60	9.24	55
	Epichlorohydrin	1.90	1.66	7.57	55
	Glycidyl phenyl ether	6.10	1.62	11.70	55
	Styrene oxide	3.28	1.60	8.95	55
	Propylene oxide	1.39	1.50	2.91	55
	Glycidyl tosylate	4.24	1.53	7.59	55
	Benzyl 3,4-epoxybutyrate	1.50	1.57	3.25	65
	2-Methylglycidyl 4-nitrobenzoate	9.71	1.02	<0.5	35

[a] Capacity factor of first-eluted diastereomer.
[b] Percentage of methanol in the mobile phase.

With respect to the other epoxides studied, the formation of side-products was always less than 1% of the main products. In instances where an optically pure form of the epoxides studied was available, the elution order was determined. Using OPA–L-Val reagent, an elution order of *S*, *R* was found for glycidol, styrene oxide and propylene oxide, whereas for glycidyl butyrate and glycidyl tosylate the *R*-form eluted before the *S*-form.

However, the actual configuration around the chiral centre for the first- (or second-) eluting stereoisomer was the same for all five compounds mentioned. In the determination of chiral amino compounds by means of OPA in combination with a chiral thiol, the enantioselectivity of the diastereomeric adducts is influenced by the chemical structure of the amino compound [13]. We investigated the effects of four amino acids, L-Ala, L-Val, L-Phe and L-Leu, on the retention characteristics of the corresponding diastereomeric isoindoles obtained from glycidyl butyrate. The results are given in Fig. 3. Under the chromatographic conditions employed, baseline resolution could be obtained for all four diastereomers of glycidyl butyrate with either

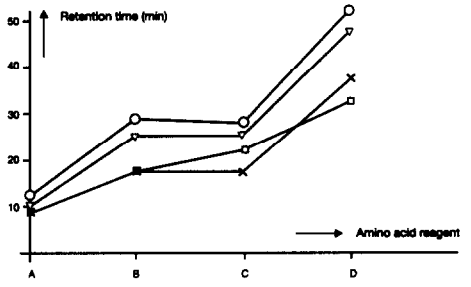


Fig. 3. Retention times of OPA–amino acid adducts of glycidyl butyrate using (A) L-Ala, (B) L-Val, (C) L-Phe and (D) L-Leu as chiral selectors. □ = (*R*)-glycidyl butyrate; ○ = (*S*)-glycidyl butyrate; ▽ = (*R*)-glycidyl butyrate (C-2 ring opening); × = (*S*)-glycidyl butyrate (C-2 ring opening). Column, LiChrosorb RP-18. For other conditions, see Experimental.

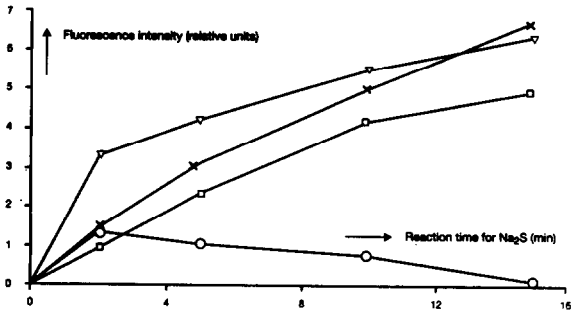


Fig. 4. Fluorescence response of the OPA–L-Phe adduct of (*R*)-glycidyl butyrate as a function of reaction time with sodium sulphide at different pH values: □ = pH 8; × = pH 10; ▽ = pH 12; ○ = pH 13.

L-Phe or L-Leu as chiral selectors. However, L-Phe offered the shortest analysis time.

The commercial availability of both D- and L-forms of amino acids is another important aspect in their use as chiral reagents for the determination of the enantiomeric excess of epoxides, because a proper selection between the D- and the L-forms of the amino acid gives the possibility of eluting an enantiomeric impurity before the corresponding enantiomer which is in excess, thus facilitating the accurate determination of the diastereomeric peak-area ratio.

Optimization of the derivatization procedure

Initially, the conversion of the epoxides into the corresponding thioglycol derivatives was carried out as described earlier [10]. Using this procedure, derivatives of glycidyl esters were formed in low yields.

In order to optimize the formation of thioglycol derivatives of glycidyl esters, derivative formation was studied using sodium sulphide solutions of different pH. The conversion of (*R*)-glycidyl butyrate with OPA–L-Phe reagent into the corresponding isoindole adduct at different pH as a function of reaction time is shown in Fig. 4. For sodium sulphide solutions with pH between 8 and 12, an increase in derivative formation with time was noted. At pH 13, derivative formation declined with time and an increase in the isoindole adduct of glycidol was measured (not shown), indicating hydrolysis of glycidyl butyrate. A sodium sulphide solution at pH 10 was chosen for further experiments. Using a 250-fold molar excess of OPA–L-Phe reagent, maximum fluorescence of glycidyl butyrate was obtained within 15 min. The detection limit for the first-eluting derivative of glycidyl butyrate, based on a signal-to-noise ratio of 3, was 2 pmol.

Accuracy of determination of enantiomeric excess

For several epoxides studied, the diastereomeric isoindole derivatives showed different specific fluorescence intensities. Differences of

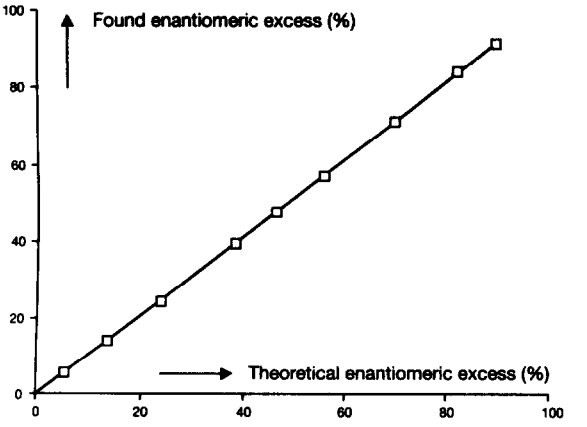


Fig. 5. Plot of theoretical enantiomeric excess *versus* found enantiomeric excess for glycidyl butyrate. Reagent: OPA–L-Phe.

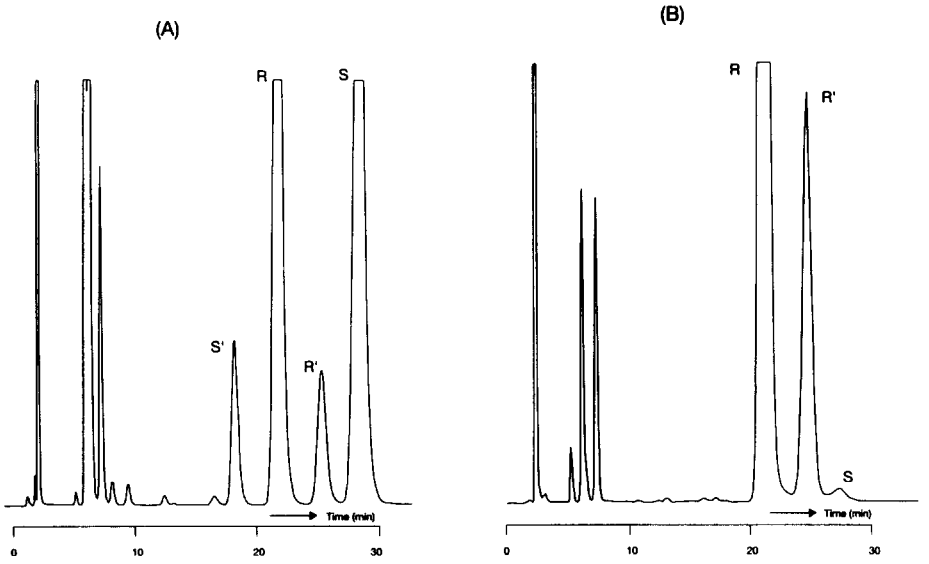


Fig. 6. Chromatograms of (A) racemic glycidyl butyrate and (B) (*R*)-glycidyl butyrate from enantioselective hydrolysis. S', R' = side-products from C-2 ring-opening. Column, LiChrosorb RP-18; reagent, OPA–L-Phe. For other conditions, see Experimental.

up to 20% were obtained for some epoxides. Therefore, quantitative measurements were made by comparing the peak areas of compounds of the same enantiomeric form.

The accuracy of the method, using the OPA–L-Phe reagent, was checked by determining the enantiomeric excess of enantiomeric mixtures of glycidyl butyrate, made up by weighing amounts of both the *R* and the racemic forms. The results are given in Fig. 5. Linear regression analysis indicated that the correlation coefficient was 0.99997. To illustrate the applicability of the method, representative chromatograms of glycidyl butyrate are shown in Fig. 6. That of the racemic substrate is given in Fig. 6a, and that of (*R*)-glycidyl butyrate obtained from an enantioselective hydrolysis experiment on the laboratory scale in Fig. 6b. The calculated enantiomeric excess of the product shown in Fig. 6b was 98.5%, with a relative standard deviation of 0.2% ($n = 4$).

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