

# Separation of secondary alcohol enantiomers using supercritical fluid chromatography

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## ABSTRACT

Secondary alcohol enantiomers were separated using pre-column derivatization with (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate and supercritical fluid chromatography. The chromatographic properties of the derivatized alcohols on three kinds of achiral stationary phases were investigated in this study. The selectivity of the two enantiomers depended on the properties of the stationary phase more than those of the mobile phase. The resolution properties depended on the species of secondary alcohols. The enantiomers of secondary alcohols with a longer carbon chain were better resolved, and the resolution became poor as the hydroxyl group was located towards the center of the carbon chain.

## INTRODUCTION

Optical resolution is an important subject in organic chemistry, biochemistry and pharmacology, and various separation methods such as crystallization and chromatography have been used for the purpose. The authors and other researchers have studied the optical resolution of secondary alcohol racemates with the use of enzymatic reactions [1–3]. It was required in those studies to determine the enantiomers quantitatively, and a chromatographic method using supercritical fluids as a mobile phase was used for the analysis.

Supercritical fluid chromatography (SFC) has recently become popular and has been applied to the analysis of various substances such as fatty acids and natural oils [4–6]. The advantages of SFC over liquid chromatography (LC) are lower fluid viscosity and higher efficiencies per unit time [7–9]. Further, SFC is more convenient than LC in operation, because solvent power, an important operational factor, is easily controlled by pressure and temperature of the supercritical fluids.

In this study, secondary alcohol enantiomers were derivatized into diastereomers before they were analyzed by SFC. This paper describes the influence

of operation pressure on the resolution behaviour as well as on the way the secondary alcohol derivatives resolved according to their molecular structures.

## EXPERIMENTAL

### *Apparatus*

For SFC, a modular supercritical fluid chromatograph (Jasco, Tokyo, Japan) was used. The operating conditions were as follows: temperature, 313 K; back-pressure, 10–25 MPa; mobile phase, carbon dioxide; flow-rate, 0.15 kg/h. A UV detector (Jasco) with a pressure-resistant cell was used at a wavelength of 275 nm. Three columns were used for SFC: Finepak OH (10  $\mu\text{m}$ , 250  $\times$  4.6 mm I.D., Jasco), Cosmosil NH<sub>2</sub> (10  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D., Nacalai Tesque, Kyoto, Japan) and Inertsil ODS (5  $\mu\text{m}$ , 150  $\times$  4.6 mm I.D., GL Science, Tokyo, Japan). A liquid chromatograph was also used to demonstrate the difference in chromatographic mode. The operating conditions for LC were as follows: mobile phase, cyclohexane containing 0.2% (v/v) ethanol; flow-rate, 30 ml/h; column, Finepak SIL (5  $\mu\text{m}$ , 250  $\times$  4.6 mm I.D., Jasco).

### Chemicals

The secondary alcohols used were 2-pentanol, 2-hexanol, 3-hexanol, 2-heptanol, 3-heptanol, 2-octanol, 3-octanol, 4-octanol, 2-nonanol, 3-nonanol, 4-nonanol, 2-decanol, 3-decanol, 4-decanol, 5-decanol, 2-undecanol, 3-undecanol, 4-undecanol, 5-undecanol, 2-dodecanol, 4-dodecanol and 5-dodecanol (Tokyo Kasei Kogyo, Tokyo, Japan). All of them were racemates. For a reagent to derivatize secondary alcohol enantiomers into diastereomers, (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate (Aldrich, Milwaukee, WI, USA) was used.

### Derivatization procedure

The methods for chromatographic separation of optical isomers may be divided into three groups: direct separation on chiral stationary phases, separation with chiral selecting reagents in the mobile phase, and separation of diastereomers formed by pre-column derivatization with chiral reagents [10]. Secondary alcohols have a simple molecular structure, so it is difficult to resolve their enantiomers directly on chiral stationary phases. Therefore, pre-column derivatization methods are often used for their optical resolution [11]. The derivatization procedure was as follows: 30  $\mu$ l of derivatizing reagent [10% (w/v) in toluene] were added to a secondary alcohol sample (1  $\mu$ l) and the mixture was heated at 363 K for 2–3 h. The reaction scheme is shown in Fig. 1. In this report, this derivatizing reagent and the resulting carbamates are briefly represented as (*R*)-1 and (*S,R*)-2 [or (*R,R*)-2]. The resulting derivatives were injected into the super-

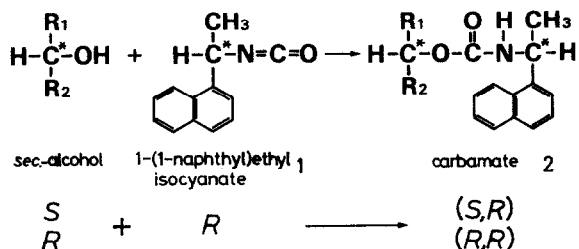


Fig. 1. Derivatization of racemic secondary alcohol with (*R*)-1-(1-naphthyl)ethyl isocyanate. The left-hand and right-hand symbols in parentheses indicate the configurations of the original alcohol and the derivatization reagent, respectively.

critical fluid chromatograph after being diluted with toluene.

### RESULTS AND DISCUSSION

#### Influence of operation pressure

The chromatograms of 2-octanol racemates derivatized with (*R*)-1 are shown in Fig. 2. Three columns were used for the separation, and the other operating conditions are described in the legend to Fig. 2. The strength of the interaction between an alcohol derivative and the stationary phases was of the order Inertsil ODS < Finepak OH < Cosmosil NH<sub>2</sub>. On each stationary phase, the (*S*)-2-octanol derivative [(*S,R*)-2] always eluted faster than the (*R*)-2-octanol derivative [(*R,R*)-2]. For other secondary alcohols, the elution order for the two isomers was also identical. Interestingly, the elution order was reversed when (*S*)-(+)-1-(1-naphthyl)ethyl iso-

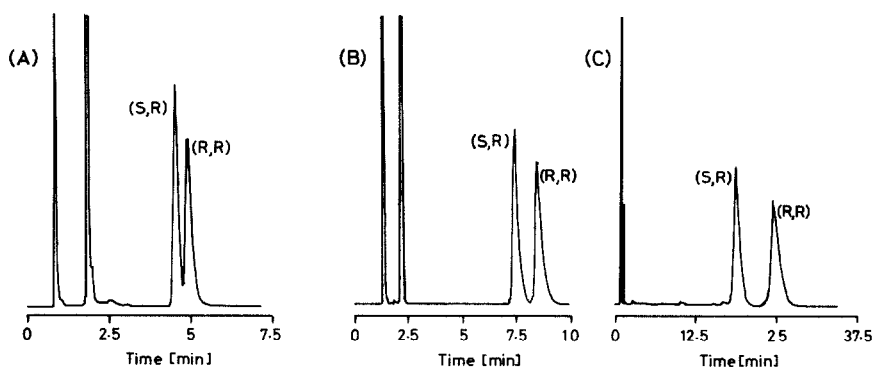


Fig. 2. Chromatographic separation of diastereomeric carbamates derived from racemic 2-octanol on (A) Inertsil ODS, (B) Finepak OH and (C) Cosmosil NH<sub>2</sub>. Back-pressure: (A) 10 MPa, (B) 20 MPa and (C) 25 MPa. See Experimental section for other conditions.

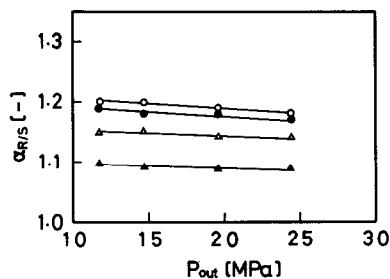


Fig. 3. Dependence of selectivity  $\alpha_{R/S}$  of four pairs of diastereomers on back-pressure. A Finepak OH column was used.  $\circ$  = 2-Dodecanol;  $\bullet$  = 2-decanol;  $\triangle$  = 2-heptanol;  $\blacktriangle$  = 2-pentanol. See Experimental section for other conditions.

cyanate was used as a derivatizing reagent, although the data were omitted in this report. This is because (*S,S*)-2 and (*R,R*)-2 are enantiomers of each other and show identical retention behavior on an achiral stationary phase.

In this report, selectivity  $\alpha_{R/S}$  defined according to eqn. 1 was used as an index for describing the chromatographic resolution.

$$\alpha_{R/S} = k'_R/k'_S \quad (1)$$

where  $k'_R$  and  $k'_S$  are the capacity factors of (*S,R*)-2 and (*R,R*)-2, respectively. The pressure dependence of  $\alpha_{R/S}$  is shown in Fig. 3. The chromatographic conditions are described in the legend to Fig. 3. This figure shows that the selectivity slightly decreased

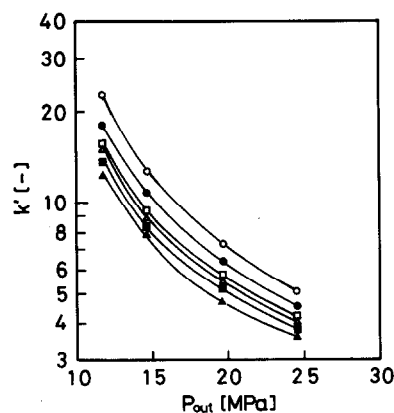


Fig. 4. Dependence of capacity factor of the carbamates derived from (*S*)-secondary alcohols on back-pressure. A Finepak OH column was used.  $\circ$  = 2-Dodecanol;  $\bullet$  = 2-decanol;  $\square$  = 2-octanol;  $\triangle$  = 2-heptanol;  $\blacksquare$  = 2-hexanol;  $\blacktriangle$  = 2-pentanol.

with increasing fluid pressure. This suggests that the stationary phase properties have a greater effect on the selectivity of the two diastereomers than mobile phase conditions. Fig. 4 shows the relation between capacity factor of (*S*)-secondary alcohol derivatives and the operation pressure. The capacity factor decreased with increasing pressure, because the solvent power of the mobile phase increased with the pressure. Figs. 3 and 4 show that the analysis time is easily controlled by regulating the fluid pressure, while the selectivity is hardly affected by the operating pressure.

In addition to selectivity, column efficiency was examined for evaluating the chromatographic separation. The separation ability of a column is often given in terms of the resolution factor  $R_s$ , defined by eqn. 2.

$$R_s = 2(t_R - t_S)/(W_R + W_S) \quad (2)$$

where  $t_R$  and  $t_S$  are retention times of (*R,R*)-2 and (*S,R*)-2, respectively, and  $W_R$  and  $W_S$  are band widths. If the bands show Gaussian distribution in shape, eqn. 2 can be rewritten as follows:

$$R_s = 1/4 \cdot N^{1/2}[(\alpha_{R/S} - 1)/\alpha_{R/S}][k'_R/(k'_R + 1)] \quad (3)$$

where  $N$  is the theoretical plate number of the column used. The resolution factor between (*R,R*)-2 and (*S,R*)-2 decreased with increasing carbon dioxide pressure, as shown in Fig. 5. The theoretical plate number showed a constant value, about 4500 for a Finepak OH column, in the range 12–25 MPa.

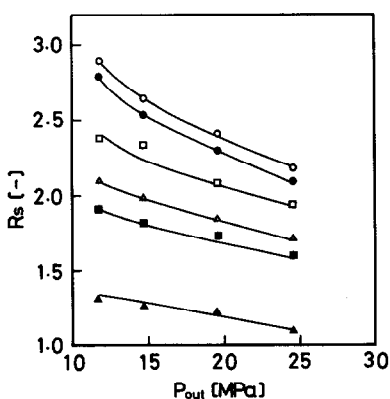


Fig. 5. Dependence of resolution factor  $R_s$  of six pairs of diastereomers on back-pressure. A Finepak OH column was used. For symbols see Fig. 4.

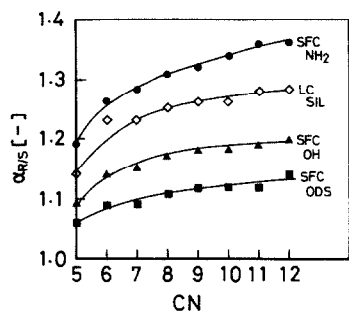


Fig. 6. Relation between selectivity  $\alpha_{R/S}$  and carbon number of 2-alkanols. Back-pressures for SFC were 15 MPa for Cosmosil  $\text{NH}_2$  and Finepak OH, and 10 MPa for Inertsil ODS. Operating conditions for SFC and LC are shown in the Experimental section.

This suggests that the decrease of  $R_s$  is due to decreases of both retention time and selectivity.

#### Selectivity and resolution factor of secondary alcohols enantiomers

2-Alkanol diastereomers derivatized with (*R*)-1 were separated by both SFC and LC. The relation between the selectivity  $\alpha_{R/S}$  and the carbon number of the alcohols is shown in Fig. 6. On every column, the selectivity increased with the carbon number,

with the curves gradually levelling off. A similar tendency was observed for LC.

Fig. 7 shows the chromatograms of diastereomers derived from racemic 2-, 3-, 4- and 5-decanols. The bands of (*R,R*)-2 and (*S,R*)-2 came closer together as the hydroxyl group became located towards the center of the carbon chain in the original alcohol, and the bands of two diastereomers derivatized from racemic 5-decanol finally overlapped. Similar behavior was observed on other columns. Fig. 8 shows the effect of the position of the hydroxyl group on the selectivity,  $\alpha_{R/S}$ . On each column, the selectivity decreased as the hydroxyl group approached the center of the alcohol. This shows that the structural difference between two diastereomers becomes minor as the structures of original alcohols become symmetric. Fig. 6 also suggests that the selectivity depends on the deviation of the hydroxyl group from the center of a carbon chain.

Fig. 9 shows the relation between the capacity factor of the diastereomers and the position of hydroxyl group in the alcohols. While the capacity factor of (*S,R*)-2 scarcely varied with the position of hydroxyl group, that of (*R,R*)-2 decreased as the hydroxyl group moved toward the center of the alcohol. This means that the structural variation

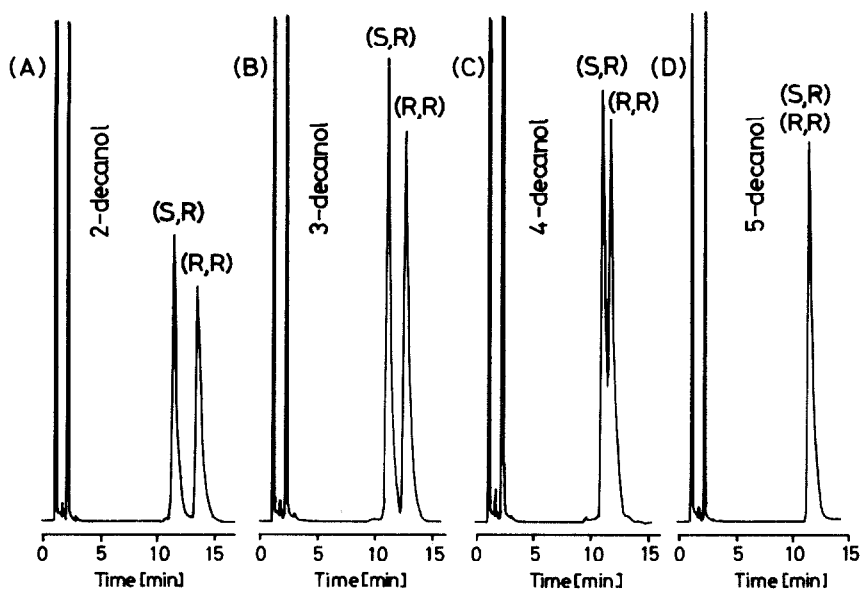


Fig. 7. Chromatographic separation of diastereomers derived from racemic (A) 2-decanol, (B) 3-decanol, (C) 4-decanol, and (D) 5-decanol. A Finepak OH column was used at 15 MPa of back-pressure.

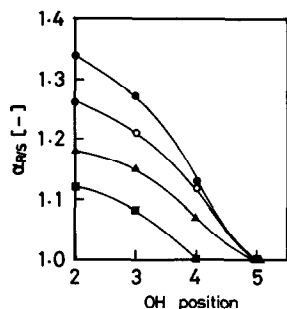


Fig. 8. Variation of selectivity,  $\alpha_{R/S}$ , versus the position of hydroxyl group of secondary decanols. ● = SFC  $\text{NH}_2$ ; ○ = LC SIL; ▲ = SFC OH; ■ = SFC ODS. See Fig. 6 for operating conditions.

accompanying hydroxyl group position is larger in (*R,R*)-2 than in (*S,R*)-2. For reference, a stereochemical formula of the secondary alcohol derivative is shown in Fig. 10. Figs. 9 and 10 lead to the following suggestion: so long as  $R_1$  is longer than  $R_2$  (where  $R_1 + R_2$  is constant), the interaction between a diastereomer and a stationary phase is affected by the difference in carbon chain length between  $R_1$  and  $R_2$ .

Selectivity relates the free energy difference as represented by eqn. 4.

$$\ln \alpha_{R/S} = -(\Delta G_R^\circ - \Delta G_S^\circ)/RT \quad (4)$$

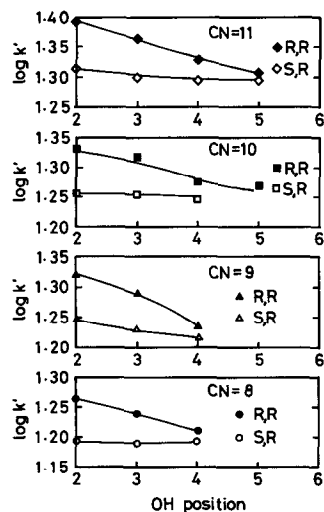


Fig. 9. Retention behavior of diastereomers, (*R,S*)-2 and (*R,R*)-2, with respect to the position of hydroxyl group in secondary alcohols. A Finepak OH column was used at 12 MPa of back-pressure. See Experimental section for operating conditions.

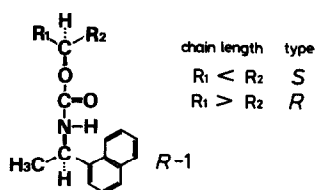


Fig. 10. Structure of the carbamate derived from secondary alcohol and (*R*)-1-(1-naphthyl)ethyl isocyanate.

where  $\Delta G_R^\circ$  and  $\Delta G_S^\circ$  are free energy changes and they are related to partition coefficients of (*R,R*)-2 and (*S,R*)-2, respectively. The free energy difference,  $-(\Delta G_R^\circ - \Delta G_S^\circ)$ , of each stationary phase is listed in Table I. The resolution factor between two dia-

TABLE I

$-(\Delta G_R^\circ - \Delta G_S^\circ)$  VALUES (kJ/mol) FOR 2-, 3-, 4- AND 5-DECANOL DERIVATIVES ON THREE KINDS OF COLUMNS

See Experimental section for operating conditions. Column 1 = Inertsil ODS, back-pressure 10 MPa; column 2 = Finepak OH, back-pressure 15 MPa; column 3 = Cosmosil  $\text{NH}_2$ , back-pressure 15 MPa.

Original alcohol	$-(\Delta G_R^\circ - \Delta G_S^\circ)$ (kJ/mol)		
	Column 1	Column 2	Column 3
2-Decanol	0.32	0.43	0.76
3-Decanol	0.20	0.36	0.62
4-Decanol	0	0.18	0.32
5-Decanol	0	0	0

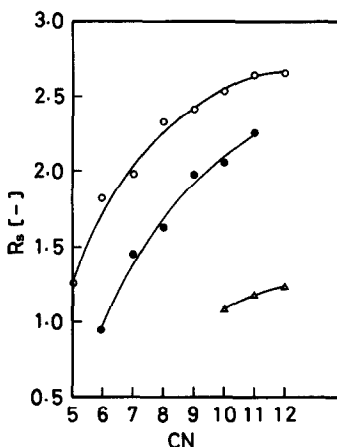


Fig. 11. Relation between resolution factor and carbon number of secondary alcohols. A Finepak OH column was used at 15 MPa of back-pressure. Position of hydroxyl group: ○ = C-2; ● = C-3; △ = C-4. See Experimental section for operating conditions.

stereomers was plotted as a function of the carbon number of the alcohols (Fig. 11), and the position of the hydroxyl group was used as another parameter. The resolution factor increased with the carbon number, because the selectivity increased with the carbon number, as already mentioned above. The resolution factor of 4-alkanol is smaller than those of 2- and 3-alkanols, since its selectivity is also smaller, as shown in Fig. 8. From the above findings, it is concluded that the separation behavior of racemic alcohols derivatized with (*R*)-1 depends on carbon number and the position of the hydroxyl group of the original alcohols.

#### CONCLUSION

Secondary alcohol enantiomers were derivatized with (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate and the resulting diastereomers were separated by SFC on achiral stationary phases. The selectivity of the two diastereomers was more affected by the properties of the stationary phases than by those of the supercritical carbon dioxide used as a mobile phase. Both selectivity and resolution factor increased with the carbon number of the secondary alcohol enantiomers, while both decreased as the hydroxyl group of the original alcohols became situated more to-

wards the center of the molecules. The results mean that the configuration of various atomic groups in the diastereomers is significant in the interaction between a solute and a stationary phase.

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