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Chiral Phase High-Performance Liquid Chromatographic Separation of Enantiomeric 1,2- and 2,3-*O*-Isopropylidene-*sn*-glycerols as 3,5-Dinitrophenylurethanes

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Abstract: High-performance liquid chromatographic separation of enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols was performed on two different stationary phases of opposite configurations: *N*-(*S*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-*tert*-leucine and *N*-(*R*)-1-(α-naphthyl)ethylaminocarbonyl-(*R*)-*tert*-leucine. This caused a reversal in the order of enantiomer elution. The isopropylideneglycerols were chromatographed as 3,5-dinitrophenylurethane derivatives. Complete enantiomer resolution, which permitted an accurate estimation of the optical purity of each enantiomer, was easily achieved on both columns (each 25 cm × 4.6 mm i.d.) within 15 min after an injection by an isocratic elution with hexane/dichloromethane/ethanol (40:12:3, by vol) as the mobile phase. The formations of diastereomeric π-π donor-accepter interaction, hydrogen bonding association, and dipole-dipole stacking between the solutes and the chiral stationary phases were considered as contributing factors to enantiomer resolution. Under optimal conditions, the optical purities of several commercially available 1,2-*O*-isopropylideneglycerol enantiomers were successfully determined in this study. The new method used in this study demonstrates that chiral phase high-performance liquid chromatography provides effective resolution, identification, and quantitation of synthetic 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol enantiomers.

Key words: isopropylideneglycerol, enantiomer, optical purity, chiral phase HPLC, 3,5-dinitrophenylurethane

1 Introduction

Enantiomeric 1,2-*O*-isopropylidene-*sn*-glycerol [(*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol] and 2,3-*O*-isopropylidene-*sn*-glycerol [(*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol] are the general intermediates required for the chemical synthesis of optically active glycerolipids, such as triacylglycerols and phospholipids (1-3). Therefore, accurately determining the optical purities of enantiomeric isopropylideneglycerols is essential for the synthesis of chiral glycerolipids in a high optical purity.

There were only a few reports on the chromatographic resolution and purity determination of enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols. Kodali reported a clear separation of enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols as benzoate derivatives by chiral phase high-performance liquid chromatography (HPLC) on a cellulose tribenzoate column (Chiralcel OB) using hexane/2-propanol (80:20, v/v) as the mobile phase (4). Terao *et al*. determined the optical yields in enzymic resolutions of racemic 1,2-*O*-iso-

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propylideneglycerol by HPLC using a Chiralcel OB or cellulose carbamate (Chiralcel OD) column after benzoylation of the hydroxy group (5). Maeda and Foglia also reported an effective separation of racemic 1,2-*O*-isopropylideneglycerol into enantiomers without any derivatization on Chiralcel OB (6). These methods using Chiralcel columns are useful in determining the optical purity and preparative separation of each enantiomer.

In this study, we developed a simple, sensitive, and accurate method to determine the optical purities of enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols using chiral-phase HPLC. This method is based on the simple derivatization of enantiomeric isopropylideneglycerols into 3,5-dinitrophenylurethanes (DNPU), which can be easily prepared at room temperature and are much more sensitive than benzoates. The subsequent separation on two chiral stationary phases of low molecular weight having opposite configurations (Sumichiral OA-4600 and 4600R) caused a reversal in the order of enantiomer elution. The OA-4600 column was used for the separation of enantiomeric 1- and 3-*O*-alkyl-*sn*-glycerols as bis-DNPU (7).

2 Experimental

2·1 Samples

Enantiomeric and racemic 1,2-*O*-isopropylideneglycerols having purities labeled 97% or more were obtained from Aldrich (Milwaukee, WI, U.S.A.), Sigma (St. Louis, MO, U.S.A.), Merck (Darmstadt, Germany), Tokyo Kasei Kogyo (Tokyo, Japan), and Nacarai Tesque (Kyoto, Japan). They were used in this study without further purification.

2·2 Preparation of Derivatives

The isopropylideneglycerol DNPU derivatives were prepared following the procedures described for chiral diacylglycerol derivatization (8,9) (**Fig. 1**). Four to six mg of 3,5-dinitrophenyl isocyanate (Sumika Chemical Analysis Service, Osaka, Japan) dissolved in 0.5 mL of dry toluene and 20 μL of dry pyridine were added into 2-3 mg of isopropylideneglycerol dissolved in 0.5 mL of dry toluene. This mixture was kept at 30℃ for 30 min in a 10 mL PTFE-linked screw-cap-covered glass vial with occasional shaking, and the excess amount of the isocyanate was then decomposed with methanol. The solvent was removed at room temperature under a nitrogen stream. The residue containing the DNPU derivatives was dissolved with dichloromethane and then centrifuged at 1,000 × g for 5 min. An aliquot of the supernatant was subjected to HPLC analysis.

Pure DNPU derivatives of isopropylideneglycerols were obtained from the reaction mixture using preparative thin-layer chromatography (TLC) on a silica gel GF plate (20 cm × 20 cm, thickness 0.5 mm, Merck). Prior to use, the plate was activated at 110-120℃ for 1 h. The reaction mixture was dissolved in a small amount of dichloromethane, spotted, and then developed using hexane/dichloromethane/ethanol (40:20:3, by vol) as the developing solvent. Two bands containing the DNPU derivatives (Rf, 0.23) and impurities (Rf, 0.14) were detected under UV irradiation and extracted from the adsorbent with diethyl ether.

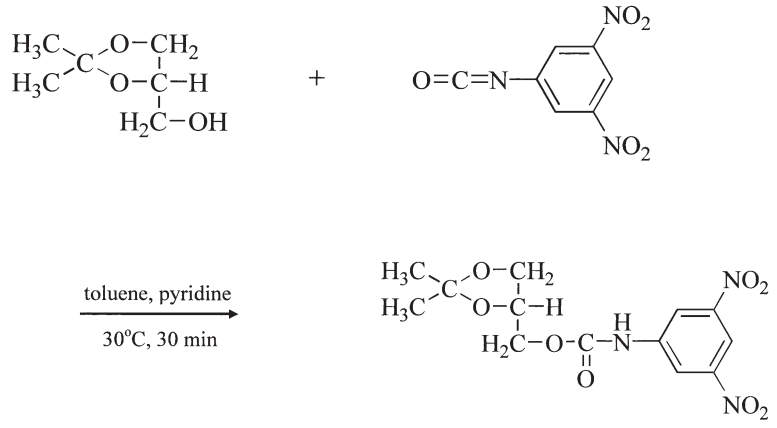

Fig. 1 Preparation of the 3,5-Dinitrophenylurethane (DNPU) Derivative of 1,2-*O*-Isopropylideneglycerol.

2·3 Spectroscopic Analysis

Flow injection electrospray ionization mass spectrometry (ESI-MS) was performed in the negative ion mode with an LCQ ion-trap mass spectrometer (Thermo Separation Products, San Jose, CA, U.S.A). The DNPU derivatives dissolved in chloroform/methanol (2:1, v/v, *ca*. 0.01 mg/mL) were directly introduced into the ESI prove using a syringe pump at a constant flow-rate of 3 μL/min. The heated capillary temperature was 200℃. The tube lens offset, capillary voltage, and spray voltage were -60 V, -19 V, and 4.2 kV, respectively. Computer software set the nitrogen sheath gas at 30 arbitrary units (arb). The mass spectra were taken in a mass range of *m*/*z* 150 - *m*/*z* 500. UV spectra of the 3,5-DNPU derivatives were taken in ethanol with a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

2·4 Chiral Phase HPLC

HPLC separation was carried out using a Hitachi L-6000 pump (Hitachi) equipped with 5 μm particle-packed columns of *N*-(*S*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-*tert*-leucine (Sumichiral OA-4600) and *N*-(*R*)-1-(α-naphthyl)ethylaminocarbonyl-(*R*)-*tert*-leucine (Sumichiral OA-4600R) chemically bonded to γ-aminopropyl silanaized silica (each 25 cm × 4.6 mm i.d., Sumika Chemical Analysis Service). A Sumipax PG-ODS filter (Sumika Chemical Analysis Service) was attached to the column inlet. The analysis was conducted isocratically using hexane/dichloromethane/ethanol (40:12:3, by vol) (all HPLC grade, Wako Pure Chemical, Osaka, Japan) as the mobile phase at a constant flow-rate of 0.5 mL/min. The column temperature was kept at 20℃ by a column oven (Model 557, GL Science, Tokyo, Japan). A 5 μL volume dichloromethane containing 1-2 μg DNPU derivatives was injected through a Rheodyne Model 7725i loop (20 μL) injector. Peaks were monitored at 254 nm using a Hitachi L-4000 UV detector with an 8 μL flow cell. Retention times, peak widths at half heights, and peak-area percentages were all measured with a Shimadzu integrator, Chromatopac C-R6A (Shimadzu, Kyoto, Japan).

3 Results and Discussion

3·1 Derivatives

As with 1,2-diacylglycerol (8,9), the hydroxy group of 1,2-*O*-isopropylideneglycerol readily reacted with 3,5-dinitrophenyl isocyanate in toluene with the presence of pyridine at room temperature (**Fig. 1**). Although the impurities in the reaction mixture did not interfere with subsequent HPLC analysis, the resulting DNPU derivatives were still purified by silicic acid TLC for spectroscopic analysis. These derivatives essentially gave the same UV spectra as those obtained from the bis-DNPU derivatives of enantiomeric 1- and 3-monoacyl-*sn*-glycerols (10). This indicated the sufficient absorption for HPLC detection over a wide range of UV wavelengths having ε values of 32,600 at 226 nm (λ_{max}) and 15,000 at 254 nm, which are much higher than benzoate (11).

Figure 2 illustrates the negative ESI-MS of the DNPU derivatives of the racemic and enantiomeric 1,2-*O*-isopropylideneglycerols. In the negative-ion mode, all of the mass spectra gave the same simple ionization pattern with a prominent deprotonated molecule $[M-H]^-$ at *m*/*z* 340 (base peak). The isotopic $[M+Cl]^-$ ions at *m*/*z* 376 and *m*/*z* 378 with the intensities of 3:1 were also observed in all of the spectra.

3·2 Resolution of Enantiomers

In order to obtain unambiguous conclusions on the enantiomer separation of 1,2-*O*-isopropylideneglycerols, two chiral stationary phases having opposite configurations were used in this study, and they caused a reversal in the elution order of enantiomeric 1,2-*O*- and 2,3-*O*-isopropylidene-*sn*-glycerols.

Figure 3 demonstrates chiral phase HPLC separation of the racemic and enantiomeric 1,2-*O*-isopropylideneglycerols as DNPU derivatives on *N*-(*S*)-1-(α-naphthyl)ethylaminocarbonyl-(*S*)-*tert*-leucine (Sumichiral OA-4600). Complete separation was achieved within 14 min after a sample injection. The retention times of the two separated racemate peaks (**Fig. 3**A) were consistent with those of the enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols (**Figs. 3**B and **3**C), respectively. Peak identification was performed by a co-injection of the racemate with each enantiomer (chromatograms not shown). The chromatograms obtained were characterized by the complete separation of the racemate into enantiomers, the sharp and symmetrical peaks within moderate retention times, and a faster elution of 1,2-*O*-isopropylidene-*sn*-glycerol than 2,3-*O*-isopropylidene-*sn*-glycerol. A complete enantiomer resolution was also obtained on an (*R*)-1-(α-naphthyl)ethylamine polymer column (YMC

A-K03), which gave an excellent resolution for the 3,5-DNPU derivatives of enantiomeric 1,2- and 2,3-diacyl-*sn*-glycerols (9,12). However, the elution times (30.5 min for the *sn*-2,3-enantiomer and 33.4 min for the *sn*-1,2-enantiomer) were much longer than those needed on the OA-4600 and 4600R.

Figure 4 shows chiral phase HPLC separation of racemic and enantiomeric 1,2-*O*-isopropylideneglycerols as DNPU derivatives on a column containing *N*-(*R*)-1-(α-naphthyl)ethylaminocarbonyl-(*R*)-*tert*-leucine (OA-4600R), which has a configuration opposite to that of OA-4600. Similar to the OA-4600 column, the OA-4600R column can also give complete racemate resolutions into enantiomers in a short retention time, but with a reversal in the elution order (**Figs. 4**B and **4**C). Peaks were identified in the same way as that employed for the OA-4600. No resolution was obtained for underivatized isopropylideneglycerol enantiomers and their ben-

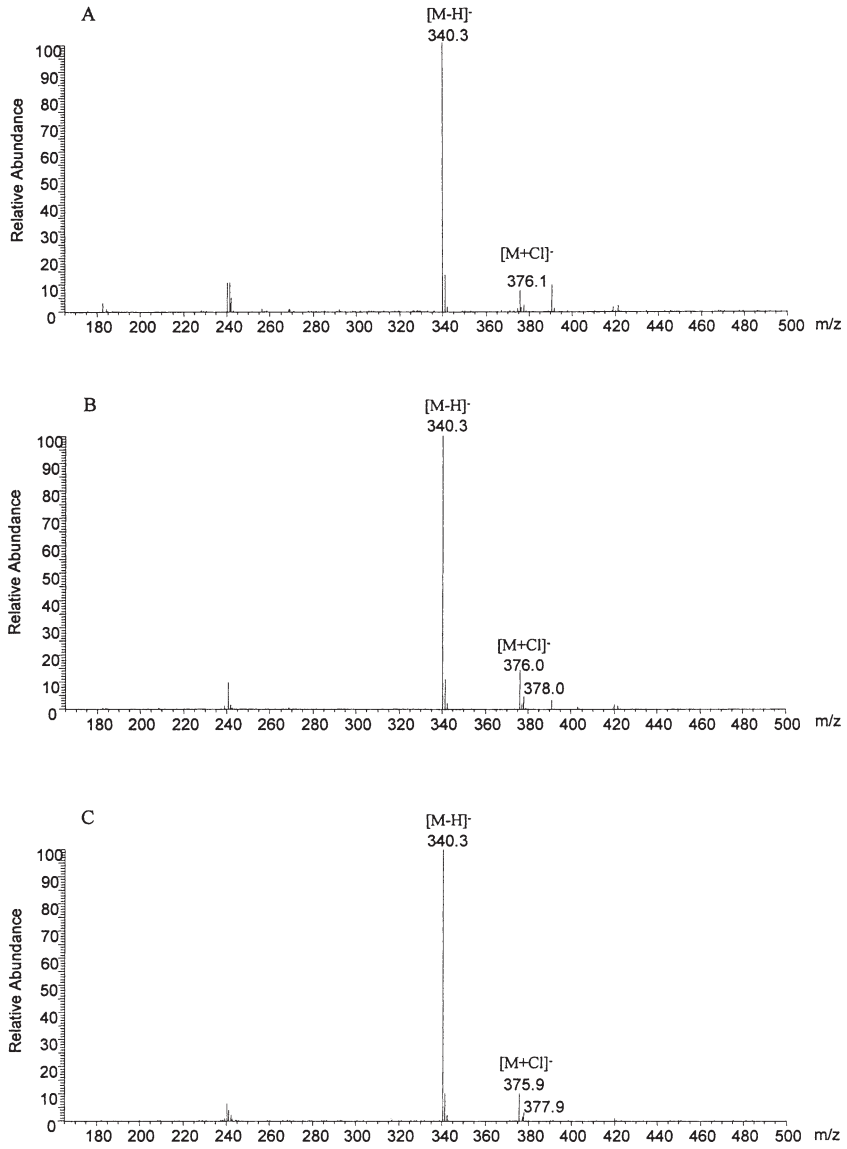

Fig. 2 Negative ESI Mass Spectra of the 3,5-Dinitrophenylurethane (DNPU) Derivatives of the Racemic and Enantiomeric 1,2-*O*-Isopropylideneglycerols. (A) 1,2-*O*-Isopropylidene-*rac*-glycerol. (B) 1,2-*O*-Isopropyridene-*sn*-glycerol. (C) 2,3-*O*-Isopropyridene-*sn*-glycerol. ESI-MS conditions as given in text.

zoate derivatives on both the OA-4600 and OA-4600R columns (chromatograms not shown).

Table 1 lists the chromatographic parameters for 1,2-*O*-isopropylidene-*rac*-glycerol DNPU derivatives obtained on the OA-4600 and OA-4600R columns. The values of separation factor (α) and peak resolution (*Rs*) obtained on the OA-4600R column were slightly bigger than those obtained on the OA-4600 column. This is probably because the OA-4600R has higher column efficiency than the OA-4600 does. The OA-4600 and OA-4600R columns used in this study showed 16,400 and 18,000 theoretical plates for the 2,3-*O*-isopropylidene-*sn*-glycerol peaks, respectively. Although the separation factors ($\alpha = 1.12$ and 1.14) were somewhat small in comparison to those obtained for the underivatized 1,2-*O*-isopropylideneglycerols ($\alpha = 1.21$) and their benzoate derivatives ($\alpha = 1.54$) on Chiralcel OB (4,6), much sharper peaks than those obtained on Chiralcel OB (*Rs* = 1.04 for underivatized isopropylideneglycerol) were observed on both the OA-4600 and OA-

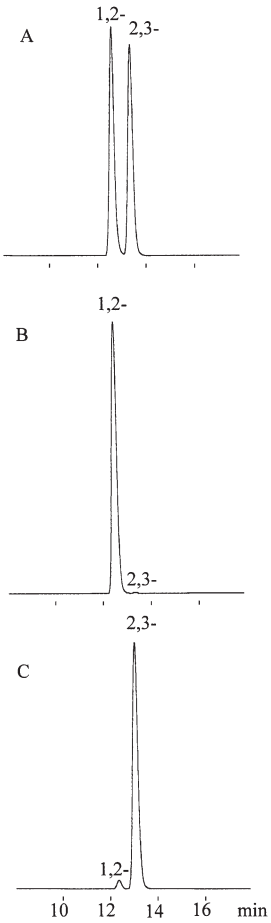

Fig. 3 Chiral Phase HPLC Separation of the 3,5-Dinitrophenylurethane (DNPU) Derivatives of Enantiomeric 1,2- and 2,3-*O*-Isopropylidene-*sn*-glycerols on *N*-(*S*)-1-(1-Naphthyl) ethylaminocarbonyl-(*S*)-*tert*-leucine (OA-4600).
(A) 1,2-*O*-Isopropylidene-*rac*-glycerol. (B) 1,2-*O*-Isopropylidene-*sn*-glycerol. (C) 2,3-*O*-Isopropylidene-*sn*-glycerol. HPLC conditions as given in text.

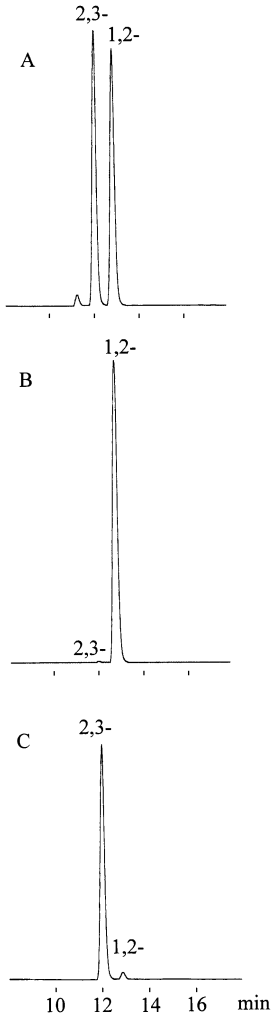

Fig. 4 Chiral Phase HPLC Separation of the 3,5-Dinitrophenylurethane (DNPU) Derivatives of Enantiomeric 1,2- and 2,3-*O*-Isopropylidene-*sn*-glycerols on *N*-(*R*)-1-(1-Naphthyl) ethylaminocarbonyl-(*R*)-*tert*-leucine (OA-4600R).
(A) 1,2-*O*-Isopropylidene-*rac*-glycerol. (B) 1,2-*O*-Isopropylidene-*sn*-glycerol. (C) 2,3-*O*-Isopropylidene-*sn*-glycerol. HPLC conditions as given in text.

Table 1 Chromatographic Data for Racemic 1,2-*O*-Isopropylideneglycerol as 3,5-Dinitrophenylurethane Derivative on Two Columns Containing Chiral Stationary Phases of Opposite Configurations, OA-4600 and OA-4600R.

	OA-4600				OA-4600R			
Enantiomer	V_R[a]	k'[b]	α[c]	R_S[d]	V_R	k'	α	R_S
1,2-*O*-Isopropylidene-*sn*-glycerol	3.18	1.03			3.26	1.04		
			1.12	2.02			1.14	2.32
2,3-*O*-Isopropylidene-*sn*-glycerol	3.56	1.15			2.86	0.91		

[a] V_R = Retention volume (mL) corrected by subtracting the column void volume (3.10 mL for OA-4600; 3.14 mL for OA-4600R).

[b] k' = Capacity ratio.

[c] α = Separation factor.

[d] R_S = Peak resolution. $R_S = 1.18 \times (t_2 - t_1) / (w_1 + w_2)$, where t = retention time and w = peak width at half height.

Table 2 Optical Purity of Commercially Available 1,2-*O*-Isopropylideneglycerols Determined by Chiral-Phase HPLC on OA-4600R.

	Enantiomer (mol%)		
Commercially available isopropylideneglycerol	1,2-*O*-isopropylidene-*sn*-glycerol (*S* configuration)	2,3-*O*-isopropylidene-*sn*-glycerol (*R* configuration)	Enantiomer excess[a]
1,2-*O*-Isopropylidene-*rac*-glycerol (98%, Tokyo Kasei)	49.4	50.6	1.2
1,2-*O*-Isopropylidene-*rac*-glycerol (98%, Aldrich)	50.2	49.8	0.4
1,2-*O*-Isopropylidene-*sn*-glycerol (99%, Sigma)	99.3	0.7	98.6
2,3-*O*-Isopropylidene-*sn*-glycerol (98%, Sigma)	3.4	96.6	93.2
1,2-*O*-Isopropylidene-*sn*-glycerol (99%, Merck)	96.5	3.5	93.0
2,3-*O*-Isopropylidene-*sn*-glycerol (98%, Merck)	4.1	95.9	91.8
1,2-*O*-Isopropylidene-*sn*-glycerol (98%, Tokyo Kasei)	99.2	0.8	98.4
2,3-*O*-Isopropylidene-*sn*-glycerol (97%, Tokyo Kasei)	6.9	93.1	86.2
1,2-*O*-Isopropylidene-*sn*-glycerol (98%, Nacarai Tesque)	98.0	2.0	96.0
2,3-*O*-Isopropylidene-*sn*-glycerol (98%, Nacarai Tesque)	1.8	98.2	96.4

[a] Enantiomer excess (%) $= 100 \times (S - R)/(S + R)$ or $100 \times (R - S)/(R + S)$

4600R ($Rs = 2.02$ and 2.32, respectively).

Table 2 displays the optical purities of several commercially available 1,2-*O*-isopropylideneglycerol enantiomers determined by the chiral phase HPLC method used in this study. The racemic 1,2-*O*-isopropylideneglycerol resolved on both the OA-4600 and OA-4600R columns showed a 1:1 peak-area ratio at different wavelengths (only the data obtained at 254 nm are shown in the table). This is in support of the complete racemate separation into the enantiomers. The optical purities of the 1,2-*O*-isopropylidene-*sn*-glycerols obtained from Sigma, Merck, Tokyo Kasei Kogyo, and Nacarai Tesque were determined as 98.6%e.e., 93.0%e.e., 98.4%e.e., and 96.0%e.e., respectively, and those of the 2,3-*O*-isopropylidene-*sn*-glycerols were 93.2%e.e., 91.8%e.e., 86.2%e.e., and 96.4%e.e., respectively. The 1,2-*O*-isopropylidene-*sn*-glycerol can be obtained in a high ($>99\%$) optical purity from the 1,2,5,6-diacetonide of D-mannitol, as determined by measuring the optical rotation or by NMR. However, L-mannitol is not yet readily available, so alternative routes have been developed for enantiomeric 2,3-*O*-isopropylidene-*sn*-glycerol (1). Thus, the optical purity of 2,3-*O*-isopropylidene-*sn*-glycerol, obtained from L-serine after diazotation and reduction of the carboxyl group, was only 94.4% (1). The results we obtained using chiral phase HPLC reinforce the conclusion that the optical purity of 1,2-*O*-isopropylidene-*sn*-glycerol is generally higher than that of the corresponding 2,3-*O*-isopropylidene-*sn*-glycerol (**Table 2**).

3·3 Separation Mechanism

Figure 5 demonstrates possible interactions between the DNPU derivatives of the enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols and the OA-4600 chiral stationary phase. This stationary phase contains a naphthyl group as the π-donor, two chiral centers at the amine, and an amino acid (*tert*-leucine) bonded with an

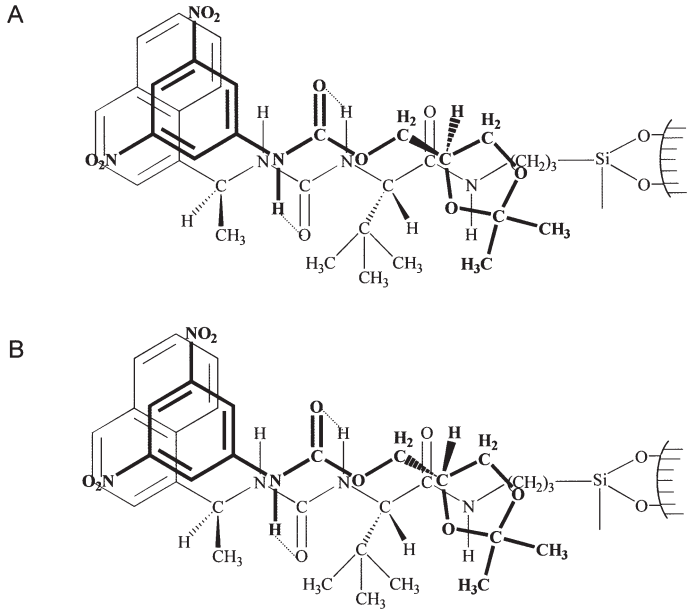

Fig. 5 Possible Interactions between the 3,5-Dinitrophenylurethane (DNPU) Derivatives of 1,2-*O*-Isopropylideneglycerol Enantiomers (boldface) and the OA-4600 stationary phase (lightface). The formation of the diastereomeric hydrogen bonding association, dipole-dipole interaction, and π-π donor-acceptor interaction between the solute and the stationary phase may contribute to the enantiomer separation.
(A) 1,2-*O*-Isopropylidene-*sn*-glycerol. (B) 2,3-*O*-Isopropylidene-*sn*-glycerol.

NHCONH group. The CONH and NHCONH groups in the molecule have the abilities to serve either as donors or acceptors in hydrogen bonding (13). The NH group in the DNPU derivatives of the isopropylideneglycerols may contribute to form hydrogen bonding with the stationary phase, which has two carboxyl groups in the molecule. The amide groups in both the solute and stationary phase may form dipole-dipole stacking. Pirkle and Pochapsky used H^1-NMR to discover the existence of these interactions, which are formed between enantiomer molecules and chiral stationary phases (14). Thus, the stronger retentions of the 2,3-*O*-isopropylidene-*sn*-glycerol on the OA-4600 column (**Fig. 3**) indicate more stable formations of hydrogen bonding, charge transfer complex, and dipole-dipole stacking than those of the 1,2-*O*-isopropylidene-*sn*-glycerol enantiomer. When these interactions are drawn for both enantiomeric 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerols **(Figs. 5**B and **5**C), we can see a steric hindrance between the stationary phase and the chiral center of the 1,2-*O*-isopropylidene-*sn*-glycerol. This steric hindrance prevents the formation of the diastereomeric interactions between the DNPU and the OA-4600 stationary phase, and causes the earlier elution of the 1,2-*O*-isopropylidene-*sn*-glycerol. With the OA-4600R, which has an opposite configuration to the OA-4600, the steric hindrance is also reversed for the two enantiomers and causes the earlier elution of the 2,3-*O*-isopropylidene-*sn*-glycerol DNPU derivative.

In conclusion, chiral phase HPLC of the enantiomeric isopropylideneglycerol DNPU derivatives on two chiral stationary phases having opposite configurations provides an exact and reliable method for the determination of their optical purities.

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