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Note

High-performance liquid chromatographic separation of monoalkylglycerol enantiomers on a chiral stationary phase

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Monoalkylglycerols (glyceryl monoethers) are widely distributed as minor components in the unsaponifiables of lipids from many animal tissues and bacteria, and occasionally as major components in those of marine animals such as sharks and ratfish. The naturally occurring glyceryl ethers possess the D-configuration, and they are an important source for the preparation of platelet activating factor[1].

Chromatographic separation of monoalkylglycerol enantiomers has not yet been achieved[2,3], although it would be useful for stereochemical studies on natural ether lipids and for determination of the optical purity of the synthesized glyceryl ethers. Recently, we reported the complete separation of monoacylglycerol enantiomers by high-performance liquid chromatography (HPLC) on (*S*)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine bonded to silica gel[4,5], which has been used for the separation of several aromatic and aliphatic chiral alcohols[6]. These results prompted us to study the HPLC separation of monoalkylglycerol enantiomers.

This paper presents a simple and accurate method for the analysis of enantiomeric 1- and 3-O-monoalkyl-*sn*-glycerols (corresponding to the D- and L-configurations, respectively) as their di-3,5-dinitrophenylurethane derivatives by HPLC on a chiral stationary phase.

EXPERIMENTAL

Samples

Optically active 1- and 3-O-hexadecyl-*sn*-glycerols were obtained from Bachem AG (Switzerland); racemic 1-O-hexadecyl- and 1-O-octadecylglycerols were obtained from Sigma (St. Louis, MO, U.S.A.) and Wako (Osaka, Japan), respectively. These samples were used without further purification, because gas chromatography on Silar 5CP of each sample as the acetyl derivatives[2] showed a single peak without that of the corresponding *sn*-2 isomer. Thin-layer chromatography (TLC) on silica gel G impregnated with boric acid[2] also showed the absence of the *sn*-2 isomers.

Preparation of derivatives

Di-3,5-dinitrophenylurethane derivatives of monoalkylglycerols were prepared on the basis of the procedures described for the derivation of several aromatic and aliphatic chiral alcohols[6]. About 0.5 mg of a monoalkylglycerol were dissolved in

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400 μl of dry toluene, and 1 mg of 3,5-dinitrophenyl isocyanate (Sumitomo, Osaka, Japan) and 40 μl of dry pyridine were added. The mixture was kept at 70°C for 1 h in a 0.5-ml glass vial with a PTFE-linked screw-cap with occasional shaking. After cooling, the solvent was removed at 40°C under reduced pressure. The resulting urethanes were purified by TLC on a silica gel GF plate (20 cm × 20 cm, thickness 0.25 mm) containing a fluorescence indicator (Analtech, Newark, DE, U.S.A.). Prior to use, the plate was developed with chloroform and then activated at 110–120°C for 3 h. The reaction mixture dissolved in 1 ml of chloroform was spotted and developed twice using *n*-hexane–1,2-dichloroethane–ethanol (40:10:2). The band of the urethanes (R_F 0.4–0.5) was marked under UV irradiation, and extracted from the adsorbent with diethyl ether.

HPLC

HPLC separation was carried out with a Shimadzu LC-6A instrument (Shimadzu Co., Kyoto, Japan) equipped with a chiral column (stainless steel, 25 cm × 4 mm I.D.) packed with 5-μm particles of N-(*S*)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine chemically bonded to γ-aminopropyl silanized silica, Sumipax OA-2100 (Sumitomo). A Guard-Pac precolumn module with a silica insert (Millipore, Bedford, MA, U.S.A.) was attached to the inlet of the chiral column. The analysis was done isocratically using *n*-hexane–1,2-dichloroethane–ethanol (all HPLC grade) (40:12:3) as the mobile phase at a constant flow-rate of 1 ml/min at 30°C. A 1-μl volume of the urethanes dissolved in 2 ml of chloroform was injected through a Rheodyne Model 7125 loop (20 μl) injector. Peaks were monitored at 0.08 a.u.f.s. with a Shimadzu SPD-6A variable-wavelength (195–350 nm) UV detector having an 8-μl flow cell. Peak-area percentages and retention times were measured with a Shimadzu integrator, Chromatopac C-R2AX.

RESULTS AND DISCUSSION

As with monoacylglycerols[5], the two hydroxy groups of monoalkylglycerols reacted readily with 3,5-dinitrophenyl isocyanate in the presence of pyridine upon heating for 30–60 min, and the urethanes purified by TLC gave good HPLC chromatograms. The urethane derivatives (R_F 0.47) were separated from impurities (R_F 0.36 and 0.52), formed from the reagent isocyanate, by double development TLC using *n*-hexane–1,2-dichloroethane–ethanol (40:10:2) as solvent.

Fig. 1 shows typical chromatograms of racemic and enantiomeric monoalkylglycerols as their di-3,5-dinitrophenylurethane derivatives on a chiral column, OA-2100. The retention times of the two separated peaks of racemic 1-O-hexadecylglycerol (Fig. 1A) were in agreement with those of the enantiomeric 1- and 3-O-hexadecyl-*sn*-glycerols (Fig. 1B and C), respectively. The identification of the peak components was also carried out by co-injection of the racemate with each enantiomer. A similar separation pattern to that in Fig. 1A was also obtained for racemic 1-O-octadecylglycerol. These racemic monoalkylglycerols were not separated by HPLC on a Zorbax CN column (5-μm particles, 25 cm × 4.6 mm I.D.) or on a Cosmosil 5SL silica column (5-μm particles, 20 cm × 4.6 mm I.D.; Nakarai, Kyoto, Japan) using the same mobile phase as for the OA-2100 column. Thus, the chromatograms are characterized by complete separation of the racemate into the enantiomers, sharp

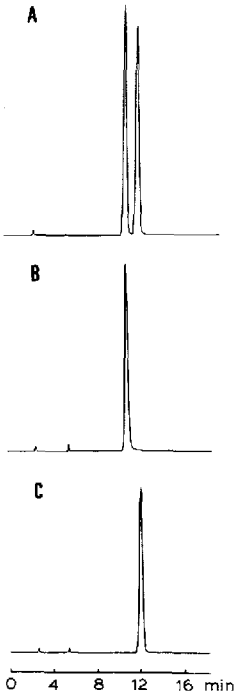

Fig. 1. Enantiomer separation of monoalkylglycerols as their di-3,5-dinitrophenylurethane derivatives on a chiral column, OA-2100. (A) Racemic 1-O-hexadecylglycerol; (B) enantiomeric 1-O-hexadecyl-*sn*-glycerol; (C) enantiomeric 3-O-hexadecyl-*sn*-glycerol. Peaks were monitored at 254 nm. Other HPLC conditions as in the text.

and symmetrical peaks within moderate retention times and faster elutions of *sn*-1 enantiomers than the corresponding *sn*-3 enantiomers as well as monoacylglycerols[4,5].

Table I gives chromatographic data for racemic 1-O-hexadecyl- and 1-O-octadecylglycerols on OA-2100. Favourable values for the separation factors and peak resolution were obtained for both racemic alkylglycerols. The separation factor, 1.13, and peak resolution, 2.0, were somewhat small in comparison with those reported[5] for the corresponding monoacylglycerols 1.19 and 2.5, respectively. The chiral column used in this study showed 7800 theoretical plates for the 3-O-hexadecyl-*sn*-glycerol peak. The separation factor of the homologues differing by two carbons was 1.02 for each *sn*-1 and *sn*-3 enantiomer, as calculated from the data in Table I. The shifts to longer retention times for the higher enantiomer homologues in normal-phase HPLC using OA-2100 can be explained by the lower polarity of the monoalkylglycerols having longer alkyl chains.

The racemic monoalkylglycerols separated on OA-2100 showed nearly the same peak-area ratios at different wavelengths (Table I). This is in support of the complete separation of the racemic monoalkylglycerols into the enantiomers, with no isomerization during the derivation procedures and the HPLC analysis. Thus, the optical purities of both of 1- and 3-O-hexadecyl-*sn*-glycerols used in this study were determined as more than 99% by the method described. Two very small peaks having

TABLE I

CHROMATOGRAPHIC DATA FOR RACEMIC MONOALKYLGLYCEROLS ON A CHIRAL COLUMN, OA-2100

Alkyl group	*Position*	V_R*	k'**	α***	R_s§	*UV(nm)*§§		
						230	*254*	*340*
Hexadecyl	*sn*-1	8.35	3.26	1.13	1.96	49.8	49.8	49.9
	sn-3	9.45	3.69			50.2	50.2	50.1
Octadecyl	*sn*-1	8.17	3.19	1.13	1.99	49.9	49.8	49.8
	sn-3	9.26	3.62			50.1	50.2	50.2

* V_R = Retention volume (ml) corrected by subtracting the column void volume (2.56 ml), determined by measuring the retention time of heptane with a refractive index detector.

** k' = Capacity ratio.

*** α = Separation factor.

§ R_s = Peak resolution = $2(t_2 - t_1)/(w_1 + w_2)$, where t = retention time and w = peak width.

§§ Peak-area ratio (%).

retention times of 2.6 and 5.4 min (Fig. 1) were due to the solvent chloroform and the reagent isocyanate, respectively.

Some racemic arylalkylcarbinols as their 3,5-dinitrobenzoates were separated by HPLC on a chiral stationary phase, 2,2,2-trifluoro-1-(9-anthryl)ethanol bonded to silanized silica[7]. The OA-2100 column, however, has no enantioselectivity for 3,5-dinitrobenzoates of various chiral alcohols except for some arylalkylcarbinols, but gives effective separations for their 3,5-dinitrophenylurethane derivatives. This may be attributed to the fact that the 3,5-dinitrobenzoates have no hydrogen atoms for hydrogen-bonding interaction with the stationary phase[6]. The two NH groups in the urethane derivatives of the monoalkylglycerols may contribute to hydrogen bonding with the OA-2100 stationary phase, which has two carboxyl groups per molecule (Fig. 2). Therefore, the stronger retentions of the *sn*-3 enantiomers suggest the formation of more stable hydrogen bonding. The steric hindrance between the

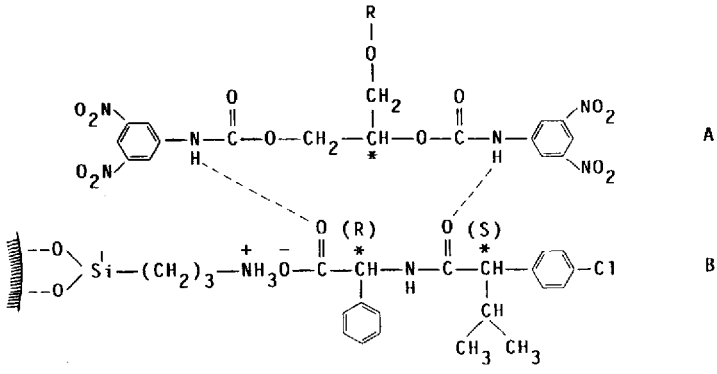

Fig. 2. Hydrogen bonds between di-3,5-dinitrophenylurethane derivatives of monoalkylglycerol (A) and the OA-2100 stationary phase (B).

phenyl group attached to the chiral carbon of the stationary phase and the alkoxy group of the solute will prevent the formation of the hydrogen bond with the *sn*-1 enantiomers. The carboxyl group of the urethanes would form a hydrogen bond with the hydrogen atom of the chiral amide group of the stationary phase[6]. Additionally, the 3,5-dinitrophenyl groups of the urethanes might contribute to the charge-transfer interaction with functional groups of the stationary phase having π electrons[7]. However, the main contribution to the enantiomer separation in this study arises from hydrogen-bond formation of the NH groups of the di-3,5-dinitrophenylurethane derivatives of the monoalkylglycerols.

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