ELSEVIER

Journal of Chromatography A, 829 (1998) 81–89

JOURNAL OF CHROMATOGRAPHY A

Highly sensitive method for the separation of enantiomeric and regioisomeric diacylglycerols as 2-anthrylurethanes by chiral-phase high-performance liquid chromatography with fluorescence detection

Hiroaki Okabe[a], Yutaka Itabashi[a,*], Toru Ota[a], Arnis Kuksis[b]

[a]*Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Japan*

[b]*Banting and Best Department of Medical Research, C.H. Best Institute, University of Toronto, 112 College Street, Toronto, Ontario M5G 1L6, Canada*

Received 15 April 1998; received in revised form 31 August 1998; accepted 6 October 1998

Abstract

A highly sensitive high-performance liquid chromatographic method was developed for the separation of enantiomeric 1,2- (*S* configuration) and 2,3-diacyl-*sn*-glycerols (*R* configuration) and regioisomeric 1,3-diacyl-*sn*-glycerols. For this purpose the diacylglycerols were converted to their 2-anthrylurethanes and subjected to chiral-phase HPLC with fluorescence detection. Satisfactory resolution of the enantiomers and regioisomers was achieved on a (*R*)-1-(1-naphthyl)ethylamine polymeric phase, using a mixture of *n*-hexane–dichloromethane–ethanol (150:10:1, v/v) as the mobile phase. The formation of various hydrogen bonding, dipole–dipole stacking and charge transfer complexes between the urethane derivatives and the stationary phase was thought to contribute to the enantiomer separation. The detection limit of 2-anthrylurethanes was 1 fmol when the signal-to-noise ratio was 3:1.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Diacylglycerols; 2-Anthrylurethanes

1. Introduction[1]

Chiral-phase high-performance liquid chromatographic separation of enantiomeric 1,2- (*S* configuration) and 2,3-diacyl-*sn*-glycerols (*R* configuration) on chiral stationary phases (CSPs) has been carried out exclusively as their ultraviolet (UV) sensitive derivatives, such as 3,5-dinitrophenylurethanes [1–3] and 1,2(2,3)-di-*O*-benzoyl-3-*tert.*-butyldimethylsilyl-glycerols [4]. On the other hand, normal-phase high-performance liquid chromatographic separation of enantiomeric diacylglycerols on achiral silica gel columns has been carried out for their diastereomers prepared with either chiral UV or fluorescent labeling reagents, such as (*S*)-(+)- or (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate [5,6], (*R*)-(+)-1-phenylethyl isocyanate [7], (*S*)-(+)-2-*tert.*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid [8] and fluoro-(1-naphthyl)acetic acid [9].

*Corresponding author. Fax: +81-138-40-8802, E-mail: yutaka@pop.fish.hokudai.ac.jp

[1]In order to designate the configuration of glycerol derivatives, the carbon atoms of glycerol are stereospecifically numbered (*sn*). When the secondary hydroxyl group is shown to the left of C-2 in a Fischer projection, the carbon atoms are numbered 1, 2 and 3 from top to bottom; the prefix ''*sn*'' is placed before the stem name of the compound [IUPAC–IUB Commission on Biochemical Nomenclature, Eur. J. Biochem., 2 (1967) 127–131; J. Lipid Res., 19 (1978) 114–128]. Thus, 1,2-diacyl-*sn*-glycerols and their anthrylurethane derivatives, which have the same configuration, are specified as *S* and *R*, respectively.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00870-X

Although, in general, fluorescence detection gives higher sensitivity than UV detection, the separation of enantiomeric diacylglycerols by highly sensitive chiral-phase high-performance liquid chromatography (HPLC) with fluorescence detection has not been achieved. Diacylglycerols play important roles as intermediates in the biosynthesis of triacyl-*sn*-glycerols and glycerophospholipids and as second messenger molecules that stimulate protein kinase C [10–12]. In the biochemical field of glycerolipids, a simple, accurate and highly sensitive method would be useful for the separation of the enantiomeric and regioisomeric diacylglycerols. This paper describes the separation of enantiomeric 1,2- and 2,3-diacyl-*sn*-glycerols and regioisomeric 1,3-diacyl-*sn*-glycerols as their 2-anthrylurethanes at the fmol level by chiral-phase HPLC with fluorescence detection.

2. Experimental

2.1. Materials

2-Aminoanthracene (96% purity) and triphosgene [bis(trichloromethyl)carbonate] (97% purity) were purchased from Aldrich (Milwaukee, WI, USA) and Junsei (Tokyo, Japan), respectively. Dry acetonitrile, toluene, chloroform, triethylamine and HPLC-grade *n*-hexane, dichloromethane and ethanol were obtained from Kanto (Tokyo, Japan). Monoacid 1,2-diacyl-*rac*- and 1,3-diacyl-*sn*-glycerols of lauric, myristic, palmitic, stearic and oleic acids, and enantiomeric 1,2- and 2,3-dipalmitoyl-*sn*-glycerols were those used previously [2].

2.2. Preparation of 2-anthrylurethane derivatives

2-Anthryl isocyanate was synthesized as described elsewhere [13]. Briefly, to 2-aminoanthracene (20 mg, 0.1 mmol) dissolved in dry acetonitrile (2 ml), triphosgene (20 mg, 0.07 mmol) dissolved in the same solvent (1 ml) was added and kept at 100°C for 30 min. After the solvent was evaporated, the isocyanate was extracted with dry toluene (1 ml). 2-Anthrylurethane derivatives were prepared by the reaction of diacylglycerols (0.1 mg) with the isocyanate (5 mg) in dry toluene (1 ml) in a screw-capped test tube. After a 1 min preincubation at 60°C, dry triethylamine (50 μl) was added to initiate the reaction. After standing for 3 h at 25°C, the solvent was evaporated within 20 min under a stream of nitrogen at the same temperature and the resulting 2-anthrylurethanes were extracted with *n*-hexane. The crude 2-anthrylurethanes were purified by thin-layer chromatography (TLC) on Silica Gel 60 GF plates (20×20 cm, 0.5 mm thick layer, Merck, Darmstadt, Germany) using a mixture of *n*-hexane–dichloromethane–ethanol (40:10:3, v/v) as the developing solvent. Prior to use, the plates were activated at 110–120°C for 1 h. Bands were visualized under UV irradiation and the 2-anthrylurethanes were recovered from the adsorbent with diethyl ether.

2.3. Spectroscopy and spectrometry

Infrared (IR) spectra were taken on 3% carbon tetrachloride solutions with a Shimadzu IR-435 spectrophotometer (Shimadzu, Kyoto, Japan). UV and fluorescence spectra were taken on 2 μ*M* and 0.2 μ*M* *n*-hexane solutions with Hitachi U-2000 and F-2000 spectrophotometers (Hitachi, Tokyo, Japan), respectively. Flow injection electrospray ionization mass spectrometry (ESI-MS) was carried out in the negative ion mode with a Jeol JMS-SX102A magnetic instrument (Jeol, Tokyo, Japan). A sample of ca. 10 pmol/μl acetonitrile–methanol (1:2, v/v) was introduced into the ESI source at a flow-rate of 1 μl/min. The capillary skimmer voltage was set at 0 V and the chamber heater at 107°C. The mass spectrum was taken in the mass range 100–1000.

2.4. Chiral-phase HPLC

Chiral-phase HPLC was performed with a Hitachi L-6000 pump equipped with a Shimadzu RF-530 fluorescence detector and a column (250×4.6 mm I.D.) containing (*R*)-1-(1-naphthyl)ethylamine polymeric phase covalently bonded to 300 Å wide pore spherical silica (5-μm particles, YMC-Pack A-K03, YMC, Kyoto, Japan). A Guard-Pac pre-column module with a silica insert (Millipore, Milford, MA, USA) was attached to the inlet of the column. The analysis was done isocratically at ambient temperature (25–27°C) using *n*-hexane–dichloromethane–ethanol (150:10:1, v/v) as the mobile phase at a

constant flow-rate of 0.5 ml/min. Prior to use, the mobile phase was filtered with a 0.45-μm PTFE membrane filter (Fuji Film, Tokyo, Japan). The excitation (ex) and emission (em) wavelengths were 261 (ex) and 432 (em) nm, respectively. Peaks were also monitored at 254 nm using a Hitachi L-4000 UV detector. Usually, 50–100 fmol of a derivative per single diacylglycerol isomer dissolved in dichloromethane was injected into the column using a Rheodyne Model 7725i loop (20 μl) injector. Peak areas were measured using a Shimadzu integrator, Chromatopac C-R6A.

3. Results and discussion

3.1. Preparation and purification of 2-anthrylurethanes

The diacylglycerols react readily with 2-anthryl isocyanate in toluene solution in the presence of triethylamine as a catalyst at ambient temperature to give the corresponding 2-anthrylurethanes. Fig. 1 shows the time course of the reaction of 1,2-dipalmitoyl-*rac*-glycerol with the isocyanate. The yields, which were calculated by comparison with the peak area of the standard sample (2-anthrylurethane of 1,2-dipalmitoyl-*rac*-glycerol), increased gradually with increasing reaction time up to 3 h (94%). No acyl migration of *sn*-1,2(2,3)-enantiomers to *sn*-1,3-regioisomers was observed during the reaction procedures. In order to obtain good HPLC chromatograms, isolation of the anthrylurethanes from the reaction products was necessary. The anthrylurethanes were clearly separated from the reaction products on silicic acid TLC plates. The R_F values of 2-anthrylurethanes of 1,3-dipalmitoyl-*sn*- and 1,2-dipalmitoyl-*rac*-glycerols were 0.64 and 0.59, respectively, while those of the underivatized dipalmitoylglycerols were 0.51 and 0.43, respectively. IR spectra of the 2-anthrylurethanes showed N–H stretch at 3440 cm^{-1} (w), C=O stretch at 1740 cm^{-1} (s) and C–H stretches at 2940 (s) and 2860 cm^{-1} (m). The negative ESI-MS spectrum of the 2-anthrylurethane of 1,2-dipalmitoyl-*rac*-glycerol is given in Fig. 2. The derivative gives a prominent quasimolecular ion $[M-H]^-$ at *m*/*z* 787 and a fragment ion $[RCOO]^-$ at *m*/*z* 255 for the acyl moiety. UV and fluorescence spectra of the derivative are given in Fig. 3. The excitation spectrum showed one major peak at 261 nm, while the emission spectrum showed three broad peaks. The maximum emission wavelength was 432 nm, indicating a large Stokes shift which gives strong fluorescence. The emission near 520 nm was due to Raman absorption of the solvent (*n*-hexane). The derivative also had an absorbance spectrum with two peaks at 258 and 267 nm, indicating that UV detection can be used but with lower sensitivity.

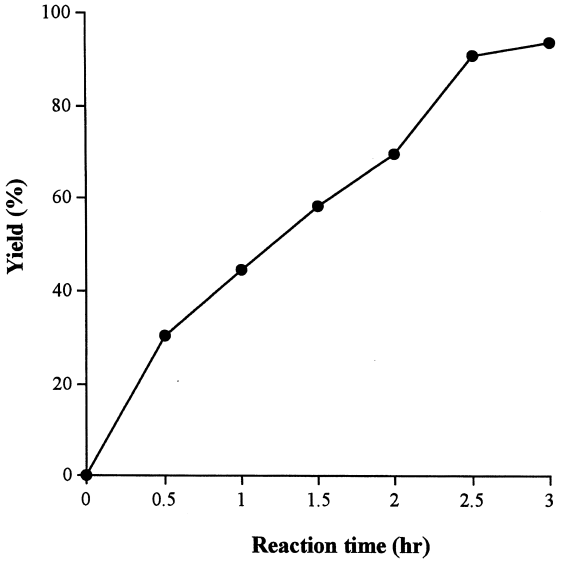

Fig. 1. Time-course of the reaction of 1,2-dipalmitoyl-*rac*-glycerol with 2-anthryl isocyanate.

3.2. Chiral-phase HPLC separation

Fig. 4 shows the chiral-phase HPLC resolution of the 2-anthrylurethanes of enantiomeric and regioisomeric dipalmitoylglycerols on a column containing (*R*)-1-(1-naphthyl)ethylamine polymeric phase (A-K03). Under fluorescence detection, three major peaks (approximately 50 fmol per each peak) eluted from the column within 30 min without baseline distortion and noise (Fig. 4A). Similar separation patterns were also observed for the other saturated and unsaturated diacylglycerols used. The chromatograms were characterized by almost complete resolution between the *sn*-1,2- and 2,3-enantio-

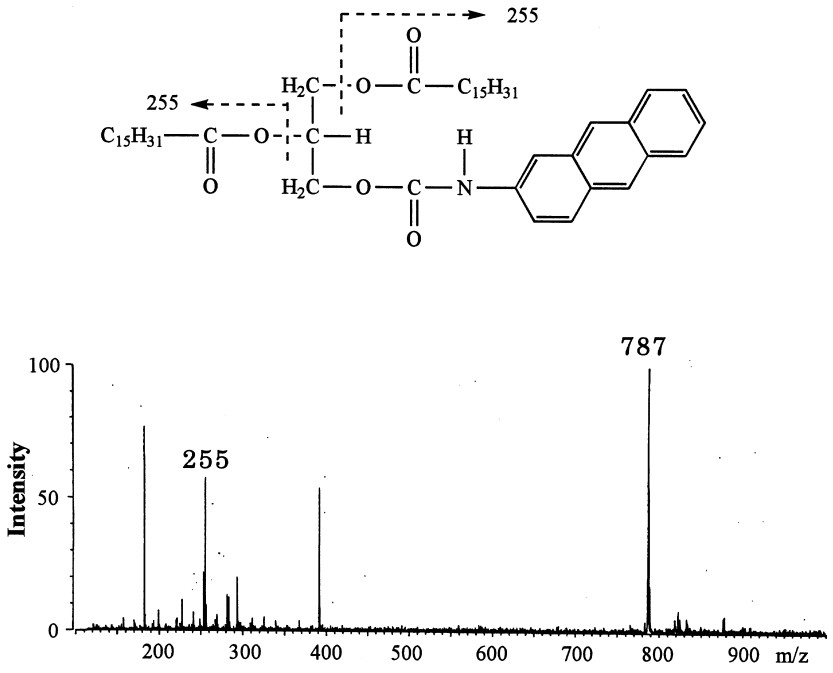

Fig. 2. Negative electrospray ionization mass spectra of the 2-anthrylurethane of 1,2-dipalmitoyl-*rac*-glycerol (M=788).

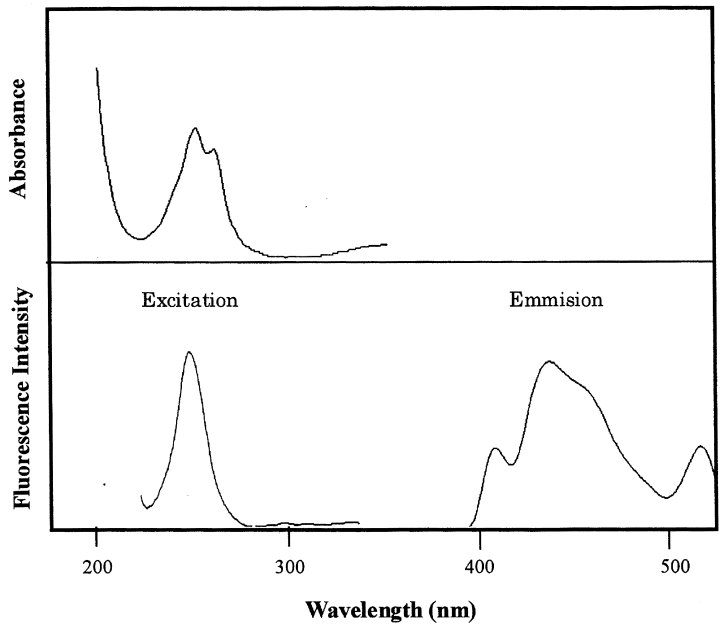

Fig. 3. UV and fluorescence spectra of the 2-anthrylurethane of 1,2-dipalmitoyl-*rac*-glycerol. The emission spectrum was recorded when excitation was set at 261 nm.

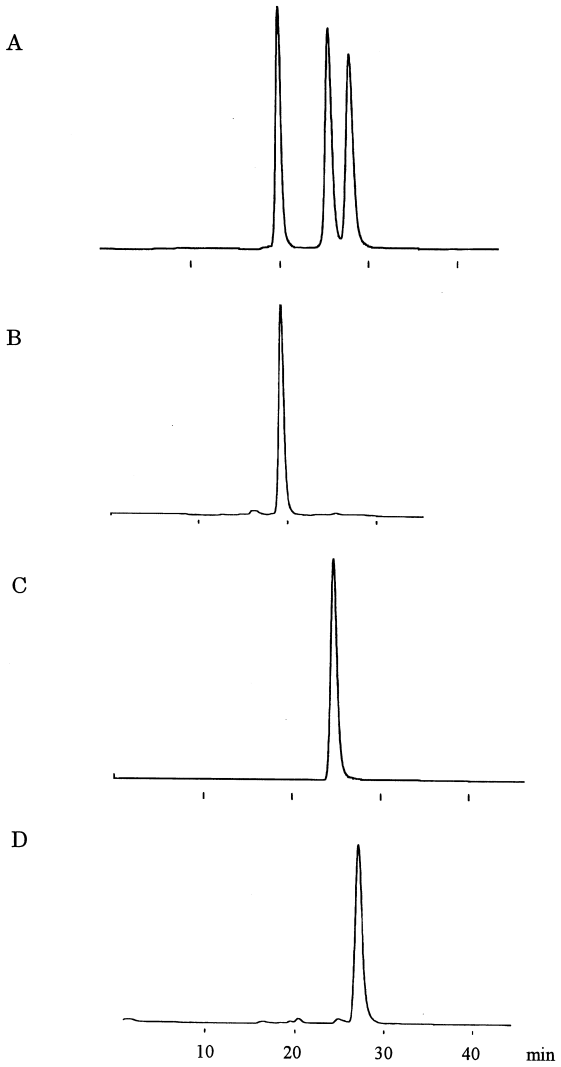

Fig. 4. Chiral-phase HPLC separation of the 2-anthrylurethanes of enantiomeric and regioisomeric diacylglycerols. (A) A mixture of 1,2-dipalmitoyl-*rac*-glycerol and 1,3-dipalmitoyl-*sn*-glycerol; (B) 1,3-dipalmitoyl-*sn*-glycerol; (C) 1,2-dipalmitoyl-*sn*-glycerol; (D) 2,3-dipalmitoyl-*sn*-glycerol. HPLC conditions as in Section 2.4.

mers and excellent resolution of the *sn*-1,3-regioisomers from the enantiomers (Fig. 4A). The retention times of the peaks that eluted first, second and third were in agreement with those of 1,3-, 1,2- and 2,3-dipalmitoyl-*sn*-glycerols, respectively (Fig. 4B–D). Peak identification was confirmed by demonstration of complete overlapping upon co-injection of the diacylglycerol mixture (Fig. 4A) with appropriate synthetic isomer (Fig. 4B–D). The column used in this study had 5000 theoretical plates for the peak of 1,3-dipalmitoyl-*sn*-glycerol. The chromatograms shown in Fig. 4 were also obtained by UV detection, but fluorescence detection gave approximately 60-times higher sensitivity than UV detection at 254 nm. Detection of the 2-anthrylurethanes at 261 (ex)/432 (em) nm was approximately 530-times more sensitive than detection of the 3,5-dinitrophenylurethanes at 254 nm [1–3]. The detection limit for the 2-anthrylurethane of 1,2-dipalmitoyl-*rac*-glycerol with the fluorescence detector was 1 fmol when the signal-to-noise ratio was 3.

The elution order of the enantiomeric and regioisomeric diacylglycerols as 2-anthrylurethanes on (*R*)-1-(1-naphthyl)ethylamine (A-K03) was the same as that of the 3,5-dinitrophenylurethanes of diacylglycerols on the same CSP [3]. This suggests that the separation mechanism of the 2-anthrylurethanes on the CSP is essentially the same as that of the 3,5-dinitrophenylurethanes. The faster elution of the *sn*-1,3-regioisomers over the *sn*-1,2- and *sn*-2,3-enantiomers is probably due to their lower polarity, as observed on silicic acid TLC. In general, enantiomer resolution on such Pirkle-type CSPs is mainly due to attractive interactions between solutes and CSPs, i.e., hydrogen-bonding, π–π complexing and dipole stacking [14]. The CSP used in this study has the ability to serve either as a donor or an acceptor in hydrogen-bonding and to π–π complexing, and to participate in dipole stacking. Thus the diastereomeric hydrogen-bonding association, π–π donor-acceptor interaction and dipole–dipole interaction between the chiral (*R*)-1-(1-naphthyl)ethylamide group and the solutes were probably the major factors contributing to the separation of the enantiomeric 1,2- and 2,3-diacyl-*sn*-glycerols (Fig. 5). Partial enantiomer resolution of the 2-anthrylurethanes was obtained on *N*-(*R*)-1-(1-naphthyl)ethyl-aminocarbonyl-(*S*)-valine OA-4100) stationary phase which gave excellent enantiomer resolution for diacylglycerols as 3,5-dinitrophenylurethanes [1,2]. In order to obtain better enantiomer resolution of the 2-anthrylurethanes, we examined some commercially available CSPs containing 3,5-dinitrobenzoyl groups, which also could serve as π-acceptors. However, no resolution was obtained on (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine

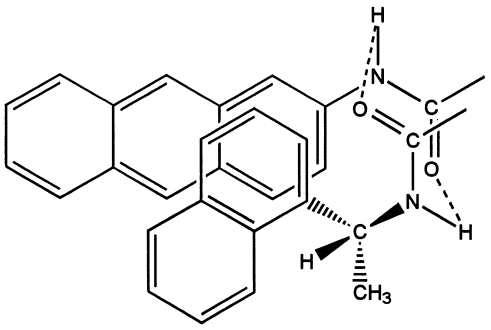

Fig. 5. Possible interactions between (*R*)-1-(1-naphthyl)ethylamine polymeric phase (A-K03) and 2-anthrylurethanes of diacylglycerols.

(Sumichiral OA-2000), (*S*)-*N*-(3,5-dinitrobenzoyl)-1-aminocarbonyl-(*S*)-valine (OA-3100), (*S*)-*N*-(3,5-dinitrobenzoyl)-1-aminocarbonyl-(*S*)-*tert.*-leucine (OA-3200) and (*S*)-*N*-(3,5-dinitrobenzoyl)-1-aminocarbonyl-(*R*)-phenylglycine (OA-3300), although a similar resolution to that on the A-K03 column used in this study was observed on (*R*)-*N*-(3,5-dinitrobenzoyl)-1-(*R*)-naphthylglycine (OA-2500), which also contains a naphthyl group. These observations suggest that the anthracene ring of the urethanes does not form effectively the diastereomeric π–π complex with the 3,5-dinitrobenzoyl group of the stationary phase as the two rings do not overlap upon the dipole–dipole stacking.

Table 1 gives the chromatographic data for enantiomeric and regioisomeric diacylglycerols as their 2-anthrylurethanes on A-K03. Nearly the same values for the separation coefficients (α) were obtained for all enantiomeric diacylglycerols used. The α values between *sn*-1,2- and *sn*-2,3-enantiomers (1.13–1.14) were less than those of the 3,5-dinitrophenylurethanes on A-K03 (α=1.44) [3], but much higher (α=1.39–1.47) than those obtained on A-K03 (α=1.23) between *sn*-1,3-regioisomers and *sn*-1,2-enantiomers. Better resolution of *sn*-1,3-regioisomers from the corresponding *sn*-1,2- and *sn*-2,3-enantiomers on CSPs was observed on *N*-(*S*)-2-(4-chlorophenyl)isovaleroyl-(*R*)-phenylglycine (OA-2100) [1]. Thus R_s values between *sn*-1,2- and *sn*-2,3-enantiomers (1.51–1.61) were also less than those between *sn*-1,3-regioisomers and *sn*-1,2-enantiomers (4.05-4.52) (Table 1). The α and R_s values between the *sn*-1,3-regioisomers and *sn*-1,2-enantiomers increased with increasing retention volumes. These observations suggest that the resolution of *sn*-1,3-regioisomers from the corresponding *sn*-1,2-enantiomers is significantly affected by the mobile phase composition, although the enantiomer resolution was not improved by changing the composition. Each enantiomer of the 1,2-diacyl-*rac*-glycerols as 2-anthrylurethanes resolved on A-K03 showed nearly the same peak area ratio under UV and fluorescence detection (Table 1), indicating almost complete resolution of the racemates into enantiomers.

In addition to the enantiomeric and regioisomeric resolution, the CSP gives partial separation based on the carbon number and degree of unsaturation of the acyl groups, probably caused by the silica gel support. Fig. 6 shows the carbon number separation of the diacylglycerols as 2-anthrylurethanes on A-K03. Apparently, the resolution of the peaks of the homologues differing by four acyl carbons (R_s=0.8 and α=1.04–1.06) is low compared with the resolution of the enantiomer peaks (R_s=1.51–1.61 and α=1.13–1.14). Similar phenomena were observed for the 3,5-dinitrophenylurethanes on A-K03 [3] and other columns containing CSPs [1,2]. Fig. 7 shows plots of the logarithm of the retention volume (log V_r) versus the total acyl carbon numbers (CN) for 1,3-, 1,2- and 2,3-diacyl-*sn*-glycerols as 2-anthrylurethanes. Each line showed slightly positive deviations from straight lines and can be expressed by the following second-order equations: log V_r (*sn*-1,2)=$4\cdot10^{-4}CN^2-0.0320CN+1.56$, r^2=0.998; log V_r (*sn*-2,3)=$4\cdot10^{-4}CN^2-0.0294CN+1.57$, r^2=0.999; log V_r (*sn*-1,3)=$3\cdot10^{-4}CN^2-0.0300CN+1.41$, r^2=0.999, where V_r (*sn*-1,2), V_r (*sn*-2,3) and V_r (*sn*-1,3) indicate, respectively, the retention volumes of the 1,2-, 2,3- and 1,3-diacyl-*sn*-glycerols having the same acyl groups. These relationships would be useful for tentative identification of the enantiomeric and regioisomeric diacylglycerols. Such non-linear relationships between total acyl carbon numbers of diacylglycerols and their retention volumes on columns containing CSPs were also observed in the HPLC separation of the 3,5-dinitrophenylurethanes on *N*-(*S*)-2-(4-chlorophenyl)-isovaleroyl-D-phenylglycine chemically bonded to γ-aminopropyl silanized silica (OA-2100) [2]. Fig. 8 shows the separation of a mixture of saturated and unsaturated

Table 1
Chromatographic data for the diacylglycerols as 2-anthrylurethanes on (*R*)-1-(1-naphthyl)ethylamine polymeric phase, A-K03

Acyl group	Position	V_r (ml)[a]	k'[b]	α[c]	R_s[d]	Peak area ratio[e]	
						UV	FL
Dilauroyl	*sn*-1,3	7.80	2.23				
				1.39	4.05		
	sn-1,2	10.8	3.09			49.7	49.7
				1.13	1.60		
	sn-2,3	12.2	3.49			50.3	50.3
Dimyristoyl	*sn*-1,3	6.95	1.99				
				1.40	4.05		
	sn-1,2	9.77	2.79			48.5	47.8
				1.14	1.60		
	sn-2,3	11.1	3.17			51.5	52.2
Dipalmitoyl	*sn*-1,3	6.45	1.84				
				1.43	4.25		
	sn-1,2	9.25	2.64			50.1	50.5
				1.14	1.53		
	sn-2,3	10.5	3.00			49.9	49.5
Distearoyl	*sn*-1,3	6.05	1.73				
				1.47	4.52		
	sn-1,2	8.90	2.54			49.4	48.7
				1.14	1.51		
	sn-2,3	10.1	2.89			50.6	51.3
Dioleoyl	*sn*-1,3	7.50	2.14				
				1.42	4.28		
	sn-1,2	10.6	3.03			49.3	49.8
				1.13	1.61		
	sn-2,3	12.0	3.43			50.7	50.2

[a] Retention volume corrected by column void volume (3.5 ml).
[b] Capacity factor.
[c] Separation coefficients between *sn*-1,3- and *sn*-1,2-isomers, and *sn*-1,2- and *sn*-2,3-enantiomers.
[d] Peak resolution, $R_s = 1.18 \cdot (t_2 - t_1)/(W_{1(h/2)} + W_{2(h/2)})$, where t=retention time (min) and $W_{(h/2)}$=half-width (min).
[e] Peak area (%) of the 1,2-diacyl-*rac*-glycerols determined using UV and fluorescence (FL) detection.

diacylglycerols as their 2-anthrylurethanes on A-K03. Like the saturated diacylglycerols, 1,3-, 1,2- and 2,3-dioleoyl-*sn*-glycerols were also completely separated from each other within 30 min with almost the same retentions as dipalmitoylglycerols. The α value of 1.17 for the diacylglycerols differing by two double bonds, that is, distearoyl- and dioleoylglycerols, is almost equal to that of the diacylglycerols differing by eight acyl carbons, such as dimyristoyl- and distearoylglycerols. Thus, the chiral-phase HPLC of a mixture of saturated and unsaturated diacylglycerols gives complex chromatograms. Therefore, a preliminary separation by reversed-phase HPLC is necessary to obtain an accurate enantiomer composition of saturated and unsaturated molecular species. Chiral-phase HPLC coupled with mass spectrometry (MS) would also be useful for direct identification of the molecular species, as previously described in the chiral-phase LC–MS of the 3,5-dinitrophenylurethanes of the enantiomeric diacylglycerols [15].

The chiral-phase HPLC method with fluorescence detection allows for the separation of the enantiomeric and regioisomeric diacylglycerols as anthrylurethanes at the fmol level. The method should be applicable to trace analysis of chiral alcohols and

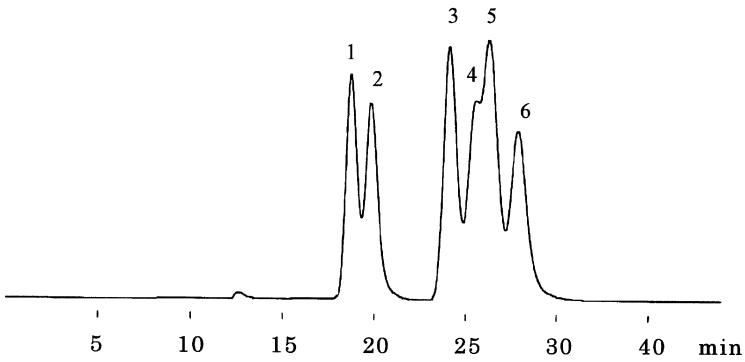

Fig. 6. Chiral-phase HPLC separation based on the acyl carbon number of diacylglycerols as 2-anthrylurethanes on a column containing (*R*)-1-(1-naphthyl)ethylamine polymeric phase, A-K03. Peaks: 1=1,3-Distearoyl-*sn*-glycerol; 2=1,3-dipalmitoyl-*sn*-glycerol; 3=1,2-distearoyl-*sn*-glycerol; 4=1,2-dipalmitoyl-*sn*-glycerol; 5=2,3-distearoyl-*sn*-glycerol; 6=2,3-dipalmitoyl-*sn*-glycerol. HPLC conditions as in Fig. 3.

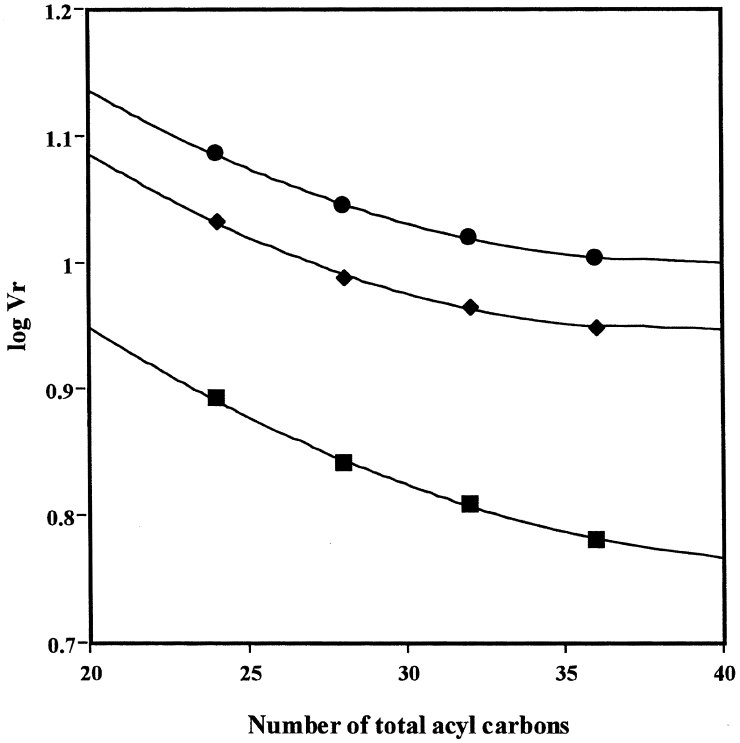

Fig. 7. Plots of log retention volume (V_r) vs. number of total acyl carbon atoms (CN) for the enantiomeric and regioisomeric diacylglycerols as 2-anthrylurethanes separated by HPLC on a column containing a (*R*)-1-(1-naphthyl)ethylamine polymeric phase, A-K03.

other chiral lipid components containing hydroxy groups, such as monoacylglycerols and hydroxy fatty acids.

Acknowledgements

We thank Kenji Watanabe (Faculty of Agriculture,

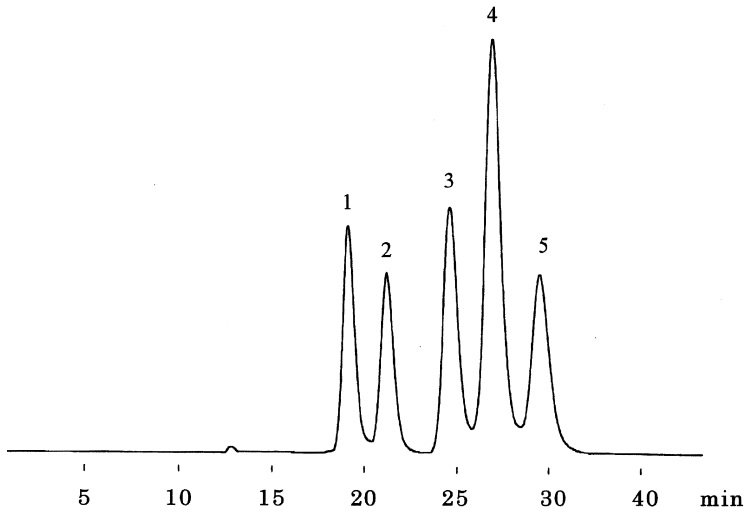

Fig. 8. Chiral-phase HPLC separation based on the degree of unsaturation of diacylglycerols as 2-anthrylurethanes on a column containing (*R*)-1-(1-naphthyl)ethylamine polymeric phase, A-K03. Peaks: 1=1,3-Distearoyl-*sn*-glycerol; 2=1,3-dioleoyl-*sn*-glycerol; 3=1,2-distearoyl-*sn*-glycerol; 4=2,3-distearoyl-*sn*-glycerol+1,2-dioleoyl-*sn*-glycerol; 5=2,3-dioleoyl-*sn*-glycerol. HPLC conditions as in Fig. 3.

Hokkaido University) for MS analyses and Fumiko Aoki (Sumika Chemical Analysis Service) for useful suggestions on CSPs.

References

[1] Y. Itabashi, T. Takagi, J. Chromatogr. 402 (1987) 257.
[2] T. Takagi, Y. Itabashi, Lipids 22 (1987) 596–600.
[3] Y. Itabashi, A. Kuksis, L. Marai, T. Takagi, J. Lipid Res. 31 (1990) 1711.
[4] H. Uzawa, H. Ohrui, H. Meguro, T. Mase, A. Ichida, Biochim. Biophys. Acta 1169 (1993) 165.
[5] P. Michelsen, E. Aronsson, G. Odham, B. Åkesson, J. Chromatogr. 350 (1985) 417.
[6] P. Laakso, W.W. Christie, Lipids 25 (1990) 349.
[7] E. Rogalska, S. Ransac, R. Verger, J. Biol. Chem. 265 (1990) 20271.
[8] J.H. Kim, Y. Nishida, H. Ohrui, H. Meguro, J. Chromatogr. A 693 (1995) 241.
[9] P.E. Sonnet, J.E. Oliver, R.M. Waters, G. King, S. Panicker, Chem. Phys. Lipids 78 (1995) 203.
[10] R. Lehner, A. Kuksis, Prog. Lipid Res. 35 (1996) 169.
[11] G.L. Scherphof, in: C. Gregor (Ed.), Phospholipids Handbook, Marcel Dekker, New York, 1993, Ch. 22, pp. 777–800.
[12] Y. Nishizuka, Science 258 (1992) 607.
[13] Y. Itabashi, A. Kuksis and H. Okabe, manuscript in preparation.
[14] J.M. Finn, in: M. Zief, L.J. Crane (Eds.), Chromatographic Chiral Separations, Marcel Dekker, New York, 1988, pp. 355–401.
[15] Y. Itabashi, L. Marai, A. Kuksis, Lipids 26 (1991) 951.