Determination of molecular species of enantiomeric diacylglycerols by chiral phase high performance liquid chromatography and polar capillary gas-liquid chromatography

Y. Itabashi,[1] **A. Kuksis, and J.J. Myher**

Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6

Abstract A simple method is described for the determination of molecular species of enantiomeric *sn*-1,2- and sn-2,3-diacylglycerols derived from natural triacylglycerols by Grignard degradation. The method is based on a preparative separation of the enantiomeric diacylglycerols as 3,5-dinitrophenylurethane (DNPU) derivatives by high performance liquid chromatography (HPLC) on a chiral column (25 cm × 4.6 mm ID) containing R-(+)-1-(1-naphthyl)ethylamine as a stationary phase. This is followed by polar capillary gas–liquid chromatography (GLC) of the trimethylsilyl (TMS) ether derivatives of the enantiomeric diacylglycerols derived from the DNPU derivatives using trichlorosilane, which does not cause acyl migration and racemization during the reaction. The cleavage is better than 94% complete. The method was standardized with synthetic *sn*-1,2- and *sn*-2,3-dipalmitoyl- and *rac*-1,2-dioleoylglycerols and was applied to the identification and quantitation of individual molecular species of enantiomeric diacylglycerols generated by Grignard degradation of the triacylglycerols from corn oil, cocoa butter, and lard. —**Itabashi, Y., A. Kuksis, and J.J. Myher.** Determination of molecular species of enantiomeric diacylglycerols by chiral phase high performance liquid chromatography and polar capillary gas-liquid chromatography. *J. Lipid Res.* 1990. **31:** 2119–2126.

Supplementary key words 3,5-dinitrophenylurethane • trichlorosilane cleavage • trimethylsilyl ethers • cocoa butter • lard • corn oil

The chiral phase HPLC described by Takagi and Itabashi (1) provides a rapid and complete resolution of enantiomeric *sn*-1,2- and *sn*-2,3-diacylglycerols as 3,5-dinitrophenylurethane (DNPU) derivatives containing identical fatty acids, but resolution of enantiomers containing different fatty acids results in overlap or interdigitation of many molecular species, and identification of individual *sn*-1,2 and *sn*-2,3-enantiomers becomes difficult or impossible. Myher and Kuksis (2) obtained a physical resolution of the enantiomeric diacylglycerols via X-phosphatidylcholines and phospholipase C, and identified the individual molecular species of each enantiomer by polar capillary GLC or GLC-MS. Although polar capillary GLC of the TMS ether derivatives provides a detailed analysis of molecular species of the diacylglycerols, the preparation of the enatiomeric diacylglycerols by the enzymatic method is laborious, time-consuming, and potentially inaccurate in view of the multiple transformations.

Since the DNPU derivatives of diacylglycerols are not eluted effectively from polar or nonpolar GLC columns and yield only limited separations on reversed phase HPLC, for complete analysis of molecular species it is necessary to remove the DNPU groups by a chemical reaction. Urethanes are generally hydrolyzed in the presence of strong acids and bases, which cannot be used with diacylglycerol derivatives. A mild method of cleaving urethanes with chlorosilanes has been reported (3–5) and we have adopted it for the regeneration of the diacylglycerol moieties from the DNPU derivatives. The present study reports the conversion of DNPU derivatives into TMS ethers of diacylglycerols in better than 94% yield by reaction with trichlorosilane and trimethylchlorosilane at room temperature without affecting the acyl groups. A combination of the chiral phase HPLC of diacylglycerol DNPU derivatives with polar capillary GLC of diacylglycerol TMS ethers provides a simple method of determination of molecular species of enantiomeric diacylglycerols of natural origin.

Abbreviations: HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography; TLC thin-layer chromatography; DNPU, 3,5-dinitrophenylurethane; TMS, trimethylsilyl; ECN, equivalent carbon number.

[1]Present address: Department of Chemistry, Faculty of Fisheries, Hokkaido University, Hakodate, Japan.

MATERIALS AND METHODS

Samples

Trioleoylglycerol was obtained from Sigma Chemical Co. (St. Louis, Mo). *Sn*-1,2- and *sn*-2,3-dipalmitoylglycerols were available in the laboratory from a previous study (6). Corn oil, cocoa butter, and lard triacylglycerols were isolated from commercial products by silicic acid TLC using hexane–diethyl ether 7:3 as developing solvent. The *sn*-1,2(2,3)-diacylglycerols were prepared from the triacylglycerols by partial Grignard degradation and were purified by boric acid-TLC (7). The DNPU derivatives were prepared by reacting free diacylglycerols with 3,5-dinitrophenyl isocyanate in the presence of dry pyridine at room temperature; they were purifed by silicic acid TLC (6).

Chiral phase HPLC

The analytical and preparative HPLC separations of enantiomeric *sn*-1,2- and *sn*-2,3-diacylglycerols as DNPU derivatives were performed on a Hewlett-Packard Model 1084 Liquid Chromatograph equipped with a chiral column (25 cm × 4.6 mm ID) containing R-(+)-1-(1-naphthyl)ethylamine polymeric phase chemically bonded to 300 A wide pore spherical silica (YMC-Pack A-KO3, YMC Inc., Kyoto, Japan), using an isocratic solvent system of hexane–dichloromethane–ethanol 40:10:1 at the flow rate of 0.8 ml/min at an oven temperature of 28 °C (8). For preparative HPLC, 40 μl of the injection solvent containing 80–90 μg DNPU were admitted to the column using an automatic sample injector, and enantiomerically pure *sn*-1,2- and *sn*-2,3-diacylglycerol DNPU derivatives were collected. The fractions from five injections were pooled to yield about 200 μg of each enantiomer. For analytical HPLC, a 10 μl volume containing 10–15 μg DNPU was injected using the same automatic sample injector.

Cleavage of diacylglycerol DNPU

To 200–300 μg of DNPU derivative dissolved in 400 μl of dry toluene, 40 μl triethylamine–dry toluene 1:20 (v/v) and 40 μl trichlorosilane–dry toluene 1:20 (v/v) were added in this order and gently shaken. After standing in a closed container for 16 h at 21–22 °C, two drops of water were added and the tubes were shaken vigorously for 30 sec using a Vortex mixer. Five ml of ether was added to the mixture, and the mixture was dried over anhydrous Na_2SO_4. The ether solution was filtered off through two Pasteur pipets containing Na_2SO_4, and the filtrate was then evaporated under nitrogen. The enantiomeric diacylglycerols were immediately converted to TMS ethers by reaction with trimethylchlorosilane–hexamethyldisilazane–pyridine (9).

Polar capillary GLC

GLC analysis of the TMS ethers was performed with a Hewlett-Packard Model 5880 Gas Chromatograph equipped with a flame ionization detector. A polar capillary column (RTx 2330, 15 m × 0.32 mm ID, Restek Corp., Bellefonte, PA) was operated isothermally at 260 °C with hydrogen as the carrier gas (2 psi head pressure). The diacylglycerol peaks separated according to carbon number and number of double bonds were identified by reference to standards and by the relative retention times tabulated previously (7).

RESULTS AND DISCUSSION

Cleavage of DNPU of model diacylglycerols

The regeneration of diacylglycerols from the DNPU derivatives is based on the method of Pirkle and Hauske (4), who demonstrated that trichlorosilane-induced cleavage was adequate for retrieving optically active carbinol moieties from the diastereomeric carbamates. Since epimerization, racemization, and rearrangement did not occur, it was inferred that carbon–oxygen bonds were not broken. The present study confirms these conclusions. **Fig. 1** shows the time course of cleavage of *sn*-2,3-dipalmitoylglycerol from its DNPU derivative using trichlorosilane. Unreacted DNPU diacylglycerol was recovered from a silicic acid TLC plate and the peak area in the HPLC chromatogram was compared with that obtained for the original DNPU at reaction time 0 min. Free diacylglycerols (R_f 0.41) and their DNPU derivatives (R_f 0.55) were clearly separated by TLC using petroleum ether (bp 60–80 °C)–dichloromethane–ethanol 40:10:3 as developing solvent. To avoid isomerization of *sn*-1,2(2,3)-diacylglycerols into X-1,3-diacylglycerols, the cleavage reaction was performed at room temperature, although this necessitated more than 10 h for a good yield (over 90%). Heating at 80 °C for 4 h, which also gave a good yield, isomerized an appreciable amount of the released diacylglycerols to the X-1,3-isomers. The yield increased gradually with increasing reaction time up to about 16 h (94%) and remained

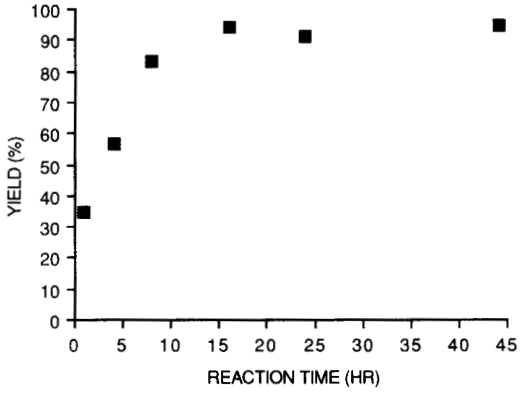

Fig. 1. Time-course of cleavage of the DNPU derivative of *sn*-2,3-dipalmitoylglycerol with trichlorosilane.

at this level at longer times (Fig. 1). There was no evidence that any of the released diacylglycerols would recombine with the 3,5-dinitrophenyl isocyanate liberated during the course of the reaction, but we have observed that the DNPU derivative can be produced from a diacylglycerol and an equimolar amount of dinitrophenyl isocyanate in the presence of triethylamine and trichlorosilane under the conditions of the cleavage reaction. The carbinol and isocyanate cleavage products described by Pirkle and Hauske (4) also did not appear to recombine under their reaction conditions.

Fig. 2A shows the chiral phase HPLC separation of the DNPU derivatives of *rac*-1,2-dioleoylglycerol generated from trioleoylglycerol by partial Grignard degradation. A rapid and complete enantiomer resolution was obtained using the R-(+)-1-(-naphthyl)ethylamine polymer as a chiral stationary phase (8). It shows higher enantioselectivity for *sn*-1,2- and *sn*-2,3-diacylglycerols than the chiral phase column containing N-(R)-1-(1-naphthyl)ethylaminocarbonyl- (S)-valine used previously (1). The separation factor and peak resolution for *rac*-1,2-dioleoylglycerol peaks were 1.42 and 4.32, respectively [1.15 and 2.02 in the previous study (1)]. The increased peak resolution obtained on the present chiral column was essential for the preparative isolation of pure enantiomers.

Fig. 2B shows the polar capillary gas chromatogram of the TMS ethers of the *rac*-1,2-dioleoylglycerols retrieved from the DNPU derivative using trichlorosilane. There is a single major peak, which represents both enantiomers. The small peak (less than 1%) seen on the descending limb represents the 1,3-dioleoylglycerol isomer, which was present in the original DNPU (see Fig. 2A). Fig. 2B clearly shows that isomerization does not occur during the cleavage of the DNPU derivative. A small amount of the unreacted DNPU and the urea, which is produced from 3,5-dinitrophenyl isocyanate by addition of water to the reaction mixture, did not interfere with the analysis of the TMS ethers on the polar column. We have observed that the DNPU derivatives decompose on high-temperature GLC, probably yielding free diacylglycerols and their dehydration products (Y. Itabashi, A. Kuksis, and J. J. Myher, unpublished results). We have also observed that polar capillary GLC of the urea fraction (R_f 0.15) isolated from the reaction mixture by TLC on silica gel shows a major peak at 21.7 min (not shown in chromatograms). Thus, polar capillary GLC provides a good assessment of the quality of the diacylglycerols regenerated from the DNPU derivatives by trichlorosilane. Prior to conversion of the regenerated diacylglycerols' into the TMS ethers, however, the sample should be purified by borate-TLC to prevent elution of the decomposition products from the capillary column. Alternatively, the TMS ethers could be purified by normal phase HPLC. The absence of racemization of the cleavage products was assessed by recovering

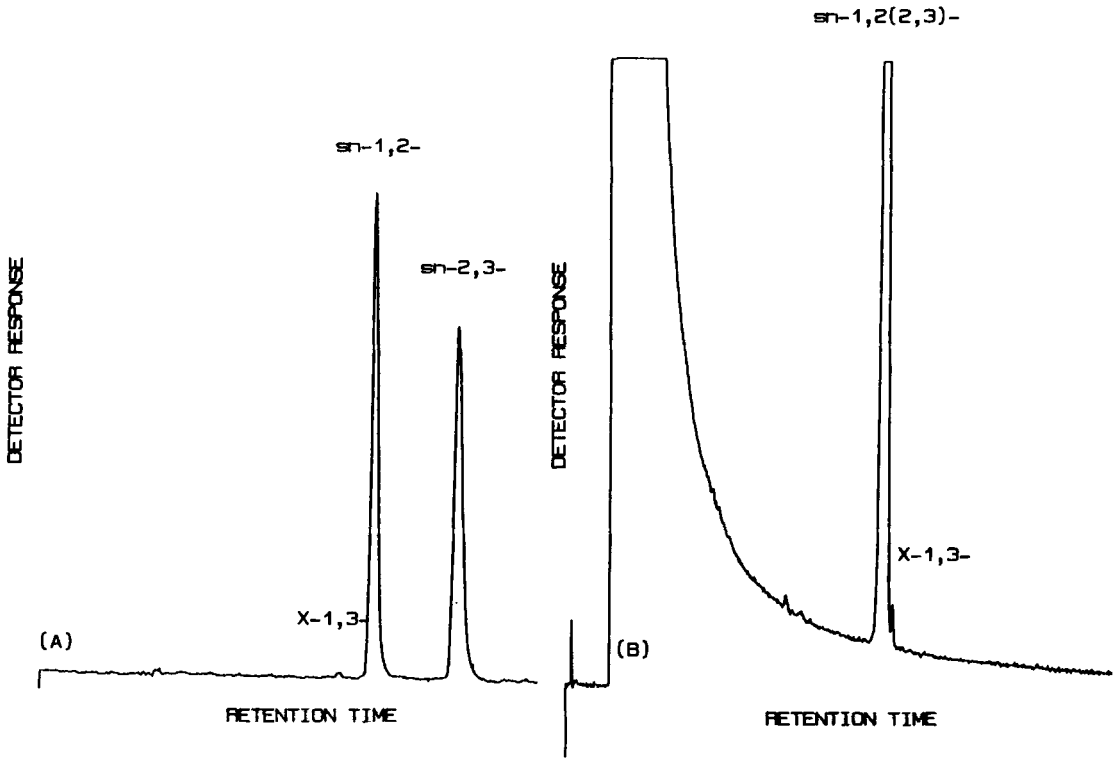

Fig. 2. Chiral-phase HPLC of the DNPU derivatives of *rac*-1,2-dioleoylglycerol (A) and polar capillary GLC of TMS ethers of the regenerated *rac*-1,2-dioleoylglycerols (B). The *sn*-2,3-dioleoylglycerol was eluted from the chiral HPLC column at 18.92 min. Capillary GLC was performed with hydrogen as carrier gas at 3 psi head pressure. All other HPLC and GLC conditions as given in text.

pure *sn*-2,3-dipalmitoylglycerol from the DNPU derivative, reconverting it to the DNPU, and reanalyzing it on the chiral column. A chiral phase HPLC of the DNPU derivative purified by TLC gave only a single peak of the *sn*-2,3-dipalmitoylglycerol, which indicates that a racemization also does not occur during the course of the reaction.

Analysis of diacylglycerols derived from cocoa butter triacylglycerols

Fig. 3 shows the chiral phase HPLC profile of the DNPU derivatives of enantiomeric diacylglycerols derived from cocoa butter triacylglycerols by Grignard degradation, along with the polar capillary GLC of the molecular species of the regenerated diacylglycerols. Four major peaks are effectively resolved into two groups, which represent the *sn*-1,2- and *sn*-2,3-enantiomers (Fig. 3A). Enantiomerically pure *sn*-1,2 and *sn*-2,3-diacylglycerol DNPU derivatives were isolated on a micro-molar scale without cross-contamination by preparative chiral phase HPLC (Figs. 3B and 3C). In addition to complete enantiomer resolution, the chiral column also provides some segregation of the molecular species within each enantiomer type. This is due to complex interactions between the stationary and the mobile phase, which have not yet been exploited for effective resolution of molecular species. Such partial separations of molecular species were observed on other HPLC columns containing chiral stationary phases chemically bonded to a silica gel, which is probably responsible for the effect, as pointed out previously (1, 6). The diacylglycerol DNPU derivatives are eluted from the chiral column in order of decreasing ECN values (8). Thus, the two major peaks within each enantiomer type can be attributed to diacylglycerols having ECN values of 34 and 32, which contain 18:0-18:1 and 16:0-18:1 as main components, respectively. Figs. 3D and 3E show the polar capillary GLC profiles

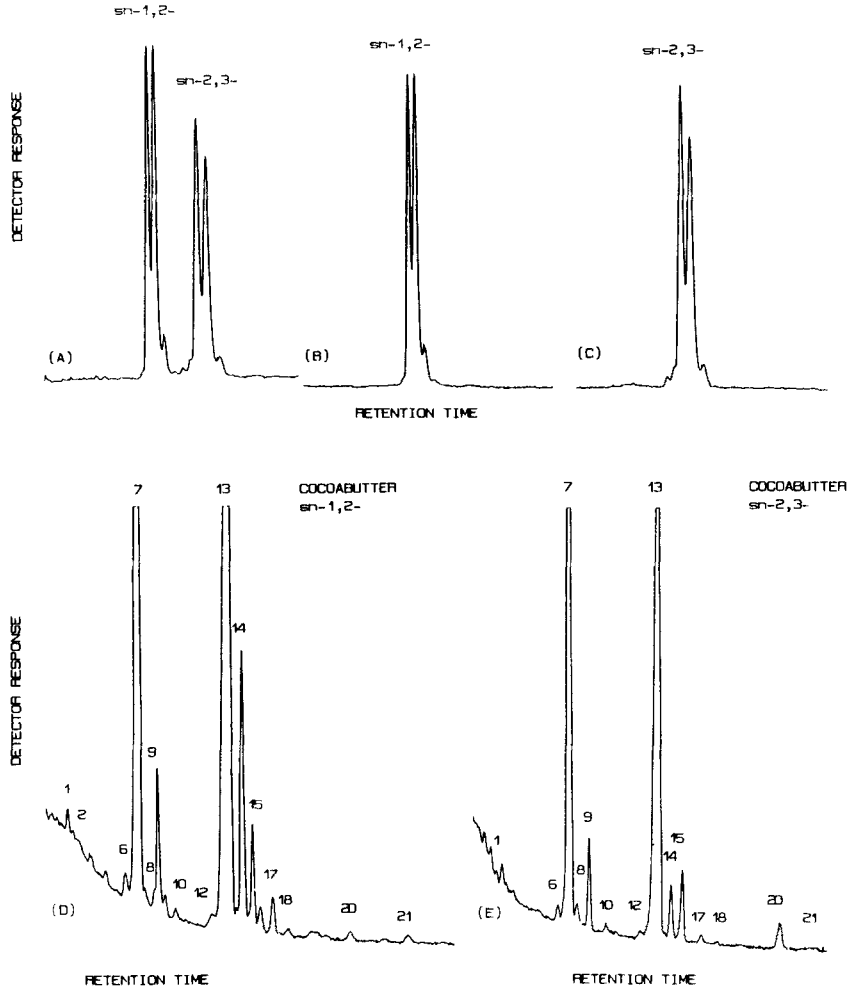

Fig. 3. Chiral phase HPLC of the DNPU derivatives of original cocoa butter *rac*-1,2-diacylglycerols (A) and of the *sn*-1,2- (B) and *sn*-2,3- (C) enantiomers after collection from the chiral column, along with polar capillary GLC of TMS ethers of the *sn* 1,2- (D) and *sn*-2,3- (E) diacylglycerols regenerated from DNPU derivatives of the enantiomers. Peak identification: 1, 16:0-16:0; 2, 16:0-16:1; 6, 16:0-18:0; 7, 16:0-18:1; 9, 16:0-18:2; 10, 17:0-18:1; 12, 18:0-18:0; 13, 18:0-18:1; 14, 18:1-18:1; 15, 18:0-18:2; 17, 18:1-18:2; 18, 18:0-18:3; 20, 20:0-18:1; 21, 20:0-18:2. HPLC and GLC conditions as given in text. The GLC retention time of dioleoylglycerol was 11.12 min (hydrogen head pressure 2 psi).

TABLE 1. Composition of enantiomeric diacylglycerols generated by partial Grignard degradation from cocoa butter triacylglycerols

Molecular Species	Regenerated from DNPU			Original	Calculated[a]
	sn-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-
			mole %		
16:0-16:0	0.49	0.62	0.51	0.41	0.56
16:0-18:0	0.74	0.63	0.62	0.61	0.69
16:0-18:1	39.21	43.29	40.38	40.48	41.24
16:0-18:2	4.34	3.61	3.81	3.99	3.98
17:0-18:1	0.24	0.31	0.33	0.35	0.27
18:0-18:1	41.74	44.06	43.69	43.35	42.90
18:1-18:1	8.72	2.36	5.46	5.48	5.54
18:0-18:2	3.14	3.32	3.50	3.57	3.23
18:1-18:2	1.05	0.40	0.78	0.77	0.72
20:1-18:1	0.33	1.40	0.92	0.99	0.87

[a] (*sn*-1,2- + *sn*-2,3-Diacylglycerols)/2.

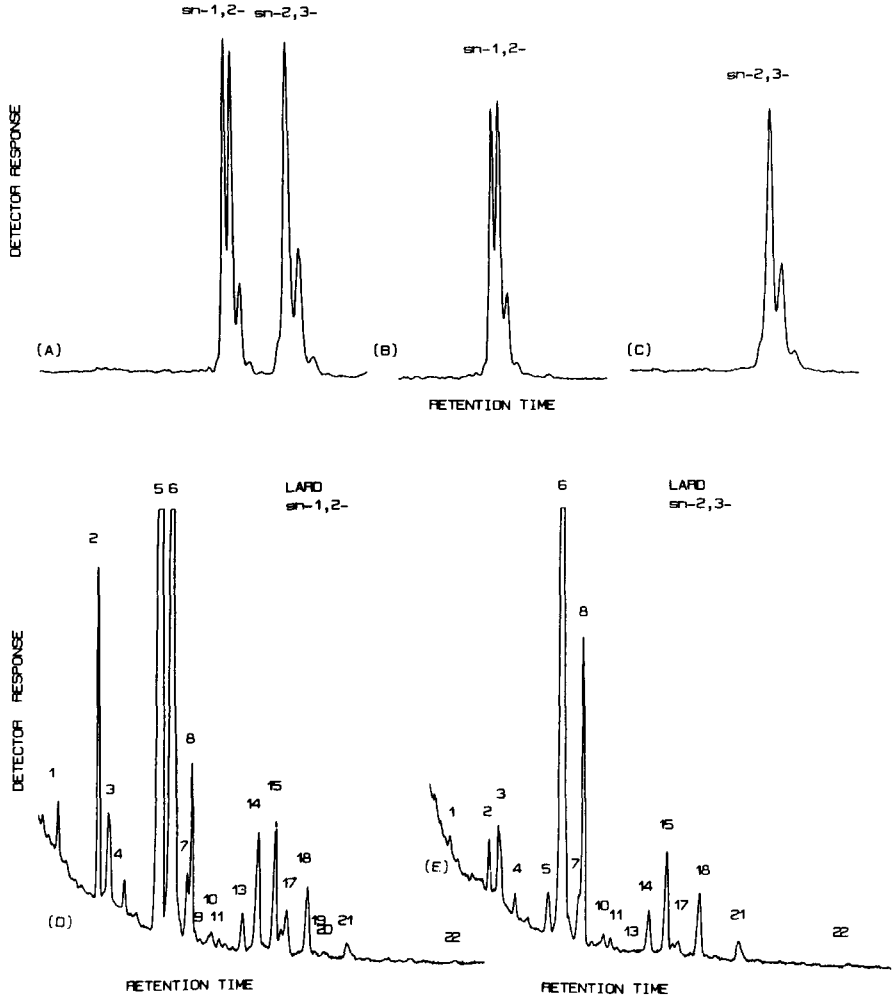

Fig. 4. Chiral phase HPLC of the DNPU derivatives of original lard *rac*-1,2-diacylglycerols (A) and of the *sn*-1,2- (B) and *sn*-2,3- (C) enantiomers after collection from the chiral HPLC column, along with polar capillary GLC of TMS ethers of the *sn*-1,2- (D) and *sn*-2,3- (E) diacylglycerols regenerated from DNPU derivatives of the enantiomers. Peak identification: 1, 14:0-16:0; 2, 14:0-18:0 + 16:0-16:0; 3, 14:0-18:1 + 16:0-16:1; 4, 14:0-18:2; 5, 16:0-18:0; 6, 16:0-18:1; 7, 16:1-18:1; 8, 16:0-18:2; 9, 17:0-18:1; 10, 16:1-18:2; 11, 16:0-18:3; 13, 18:0-18:0; 14, 18:0-18:1; 15, 18:1-18:1; 17, 18:0-18:2 + 16:0-20:2; 18, 18:1-18:2; 21, 18:2-18:2. HPLC and GLC conditions as given in text.

of the TMS ethers of the cocoa butter diacylglycerols retrieved from the DNPU derivatives. In addition to the major 16:0-18:1 and 18:0-18:1 species, both enantiomers contain 16:0-18:2, 18:1-18:1, and 18:0-18:2 as minor species along with trace amounts of other components of lower and higher carbon and unsaturation number. **Table 1** gives the quantitative composition of the enantiomeric diacylglycerols derived from cocoa butter. The *sn*-1,2-enantiomers contain significantly more 18:1-18:1 and less 16:0-18:1 than the *sn*-2,3-enantiomers. Table 1 also shows good agreement among the compositions of the original *sn*-1,2-(2,3)-diacylglycerols, the *sn*-1,2(2,3)-diacylglycerols retrieved from the DNPU derivatives, and the *sn*-1,2(2,3)-diacylglycerols reconstituted from the separate analyses of the *sn*-1,2- and *sn*-2,3-enantiomers. These findings agree closely with those derived by polar capillary GLC analysis of the *sn*-1,2- and *sn*-2,3-diacylglycerol moieties of cocoa butter triacylglycerols obtained by Grignard degradation and phospholipase C resolution of *rac*-phosphatidylcholines prepared as intermediates (S. Pind, A. Kuksis, J. J. Myher, L. Marai, and D. Kritchevsky, unpublished results).

Analyses of diacylglycerols derived from lard triacylglycerols

Fig. 4 shows the chiral phase HPLC profile of the DNPU derivatives of enantiomeric diacylglycerols derived from lard triacylglycerols by Grignard degradation, along with the polar capillary GLC profiles of the molecular species of the regenerated diacylglycerols. Three major peaks can be recognized in the *sn*-1,2-enantiomer region and two in the *sn*-2,3-enantiomer region (Figs. 4A-4C). On the basis of the ECN concept (8), the three peaks in the *sn*-1,2-enantiomer can be attributed to diacylglycerols with ECN values of 34, 32, and 30, respectively. Similarly, the two peaks in the *sn*-2,3-enantiomer can be attributed to diacylglyercols with ECN values 32 and 30, respectively. Enantiomerically pure *sn*-1,2- and *sn*-2,3-diacylglycerol DNPU derivatives were isolated on a micromolar scale without cross-contamination by preparative chiral phase HPLC. Figs. 4D and 4E show the polar capillary GLC profiles of the *sn*-1,2- and the *sn*-2,3-diacylglycerols of lard triacylglycerols after removal of the DNPU groups and trimethylsilylation. Again, the transformation into the TMS derivatives was effective and free of isomerization. The molecular species are completely resolved in each enantiomer. The main components are made up of 16:0 and 18:0 saturated and 16:1 and 18:1 unsaturated fatty acids, which account for the ECN values ranging from 30 to 34. **Table 2** gives the quantitative composition of the major and minor diacylglycerol moieties of the lard triacylglycerols. All species are present in both enantiomers, but not in the same proportions, as already known from stereospecific analyses (2). Table 2 also compares the composition of the original *sn*-1,2(2,3)-diacylglycerols and those reconstituted from the combined chiral phase HPLC and polar capillary GLC analyses. The good agreement indicates that the cleavage reaction proceeded equally well with all molecular species. The compositions of the *sn*-1,2- and *sn*-2,3-diacylglycerols correspond closely to those determined earlier via *rac*-phosphatidylcholines and phospholipase C (2).

Analysis of diacylglycerols derived from corn oil triacylglycerols

Fig. 5 shows the chiral phase HPLC profile of the DNPU derivatives of enantiomeric diacylglycerols generated from corn oil triacylglycerols by Grignard degradation, along with the polar capillary GLC profiles of the molecular species of the recovered diacylglycerols. Six major peaks are separated clearly into two groups, which represent the *sn*-1,2- and *sn*-2,3-enantiomers (Figs. 5A-5C). The major peaks

TABLE 2. Composition of enantiomeric diacylglycerols generated by partial Grignard degradation from lard triacylglycerols

Molecular Species	Regenerated from DNPU			Original	Calculated[a]
	sn-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-
			mole %		
14:0-16:0	1.04	0.36	0.61	0.54	0.70
16:0-16:0	6.93	2.17	4.53	4.15	4.55
16:0-16:1	3.75	5.12	4.29	3.87	4.43
14:0-18:2	0.85	0.99	0.94	0.83	0.92
16:0-18:0	36.29	2.19	19.34	18.93	19.24
16:0-18:1	30.52	56.41	43.23	43.25	43.46
16:0-18:2[b]	5.70	16.50	11.05	11.29	11.10
16:1-18:2	0.46	0.67	0.61	0.73	0.57
16:0-18:3	0.17	0.53	0.35	0.46	0.35
18:0-18:0	0.99	tr	0.54	0.64	0.50
18:0-18:1	4.06	2.52	3.45	3.67	3.29
18:1-18:1	4.17	6.04	5.28	5.52	5.11
18:1(n-7)-18:1	0.65	0.56	0.59	0.67	0.60
18:0-18:2	1.57	0.92	1.35	1.36	1.24
18:1-18:2	2.23	3.80	3.01	3.13	3.02
18:2-18:2	0.62	1.22	0.83	0.96	0.92

[a] (*sn*-1,2-Diacyl + *sn*-2,3-diacyl)/2.
[b] Contains 16:1-18:1.

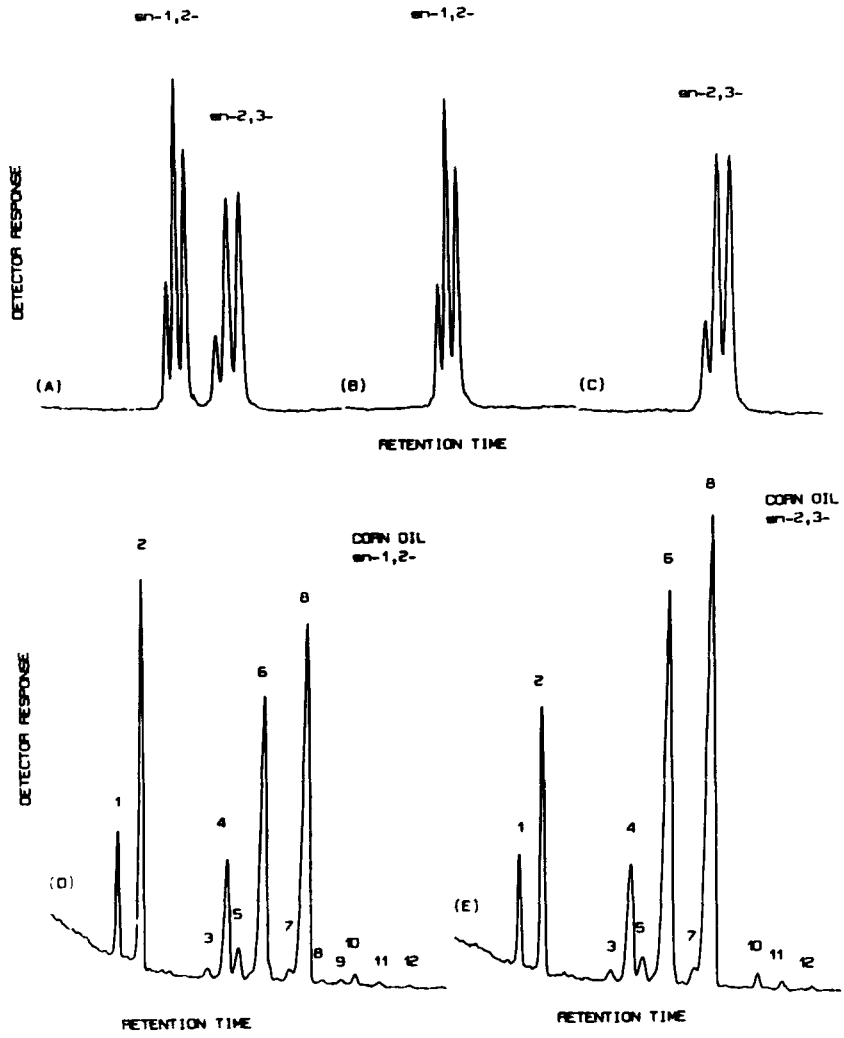

Fig. 5. Chiral phase HPLC of the DNPU derivatives of original corn oil *rac*1,2,-diacylglycerols (A) and of the *sn*-1,2- (B) and *sn*-2,3- (C) enantiomers after collection from the chiral HPLC column, along with polar capillary GLC of TMS ethers the *sn*-1,2- (D) and *sn*-2,3- (E) diacylglycerols regenerated from DNPU derivatives of the enantiomers. Peak identification: 1, 16:0-18:1; 2, 16:0-18:2; 3, 18:0-18:1; 4, 18:1-18:1; 5, 18:0-18:2; 6, 18:1-18:2; 8, 18:2-18:2; 10, 18:2-18:3; 11, 20:0-18:2. HPLC and GLC conditions as given in text.

in each enantiomer group correspond in retention times to diacylglycerols with ECN values of 28–32 (8). Enantiomerically pure *sn*-1,2- and *sn*-2,3-diacylglycerol DNPU derivatives were isolated on a micromolar scale without cross-contamination by preparative HPLC. Figs. 5D and 5E show the polar capillary GLC profiles of the TMS ethers of the corn oil diacylglycerols retrieved from the DNPU derivatives. In addition to 16:0-18:1, 16:0-18:2, 18:1-18:1, 18:1-18:2, and 18:2-18:2 as major components, 18:0-18:1 and 18:0-18:2 are found as minor components in each enantiomer. The species account for the observed ECN values of 28–32. **Table 3** gives the quantitative composition of the diacylglycerols from corn oil triacylglycerols. All species found in this study are common to each enantiomer, but minor differences are seen in proportions of the individual components, as would be anticipated from the known enantiomeric nature of the original corn oil triacylglycerols (10). Table 3 also compares the composition of the original *sn*-1,2(2,3)-diacylglycerols, those retrieved from DNPU derivatives, and those reconstituted from the knowledge of the enantiomer composition. The good agreement indicates that the cleavage proceeded well with all molecular species. The composition of the *sn*-1,2(2,3)-diacylglycerols corresponds rather closely to the peak area ratios determined earlier (11).

On the basis of these studies it is concluded that trichlorosilane degradation provides a mild and effective method for retrieving natural diacylglycerols from their DNPU derivatives without isomerization and destruction. Furthermore, the present combination of chiral phase HPLC separation of

TABLE 3. Composition of enantiomeric diacylglycerols generated by partial Grignard degradation from corn oil triacylglycerols

Molecular Species	Regenerated from DNPU			Original	Calculated[a]
	sn-1,2-	*sn*-2,3-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-	*sn*-1,2(2,3)-
			mole %		
16:0-18:1	6.80	4.72	5.77	5.64	5.76
16:0-18:2	21.86	11.80	17.38	16.85	16.83
18:0-18:1	0.63	0.58	0.59	0.65	0.61
18:1-18:1	9.04	7.99	8.36	8.14	8.51
18:0-18:2	2.41	1.73	1.99	2.02	2.07
18:1-18:2	25.19	32.49	28.58	28.02	28.84
18:2-18:2	34.07	40.69	37.33	38.68	37.38

[a] (*sn*-1,2-Diacyl + *sn* -2,3-diacyl)/2.

the enantiomers with polar capillary GLC of the molecular species of the recovered diacylglycerols constitutes an effective practical method for determining the structure of enantiomeric diacylglycerols, except for reverse isomers, which still remain unresolved. Alternatively, the recovered diacylglycerols could be converted into UV-absorbing derivatives and the molecular species resolved by reversed phase HPLC (12). Either method should be applicable to stereospecific analyses of triacylglycerols as well as to establishing the chiral nature of molecular species of diradylglycerols occurring in the free form in tissues and cell cultures.

These studies were supported by funds from the Medical Research Council of Canada, Ottawa, and the Heart and Stroke Foundation of Ontario, Toronto, Canada.

Manuscript received 5 June 1990.

REFERENCES

1. Takagi, T., and Y. Itabashi. 1987. Rapid separations of diacyl- and dialkylglycerol enantiomers by high performance liquid chromatography on a chiral stationary phase. *Lipids.* **22:** 596-600.
2. Myher, J. J., and A. Kuksis. 1979. Stereospecific analysis of triacylglycerols via racemic phosphatidylcholines and phospholipase C. *Can. J. Biochem.* **57:** 117-124.
3. Greber, G., and H. R. Kricheldorf. 1968. A new synthesis of isocyanates and isothiocyanates. *Angew. Chem. Internat. Edit.* **7:** 941.
4. Pirkle, W. H., and J. R. Hauske. 1977. Trichlorosilane-induced cleavage. A mild method for retrieving carbinols from carbamates. *J. Org. Chem.* **42:** 2781-2782.
5. Corey, E. J., and S. Hashimoto. 1981. A practical process for large-scale synthesis of (S)-5-hydroxy-6-*trans*-8, 11, 14-*cis*-eicosatetraenoic acid (5-HETE). *Tetrahedron Lett.* **22:** 299-302.
6. Itabashi, Y., and T. Takagi. 1987. High performance liquid chromatographic separation of diacylglycerol enantiomers on a chiral stationary phase. *J. Chromatogr.* **402:** 257-264.
7. Myher, J. J., and A. Kuksis. 1982. Resolution of natural diacylglycerols by gas-liquid chromatography on polar capillary columns. *Can. J. Biochem. Cell Biol.* **60:** 638-650.
8. Itabashi, Y., A. Kuksis, L. Marai, and T. Takagi. 1990. HPLC resolution of diacylglycerol moieties of natural triacylglycerols on a chiral phase consisting of bonded (R)-(+)-1-(1-naphthyl) ethylamine. *J. Lipid Res.* **31:** 1711-1717.
9. Myher, J. J., A. Kuksis, L. Marai, and S. K. F. Yeung. 1978. Microdetermination of molecular species of oligo- and polyunsaturated diacylglycerols by gas chromatography-mass spectrometry of their *tert*-butyl dimethylsilyl ethers *Anal. Chem.* **50:** 557-561.
10. Brockerhoff, H., and M. Yurkowski. 1966. Stereospecific analyses of several vegetable fats. *J. Lipid Res.* **7:** 62-64.
11. Myher, J. J., and A. Kuksis. 1975. Improved resolution of natural diacylglycerols by gas-liquid chromatography on polar siloxanes. *J. Chromatogr. Sci.* **13:** 138-145.
12. Blank. M. L., M. Robinson, V. Fitzgerald, and F. Snyder. 1984. Novel quantitative method for determination of molecular species of phospholipids and diglycerides. *J. Chromatogr.* **298:** 473-482.