

High-Performance Liquid Chromatographic Separation of Enantiomeric Benzyl Glycerides

The enantiomeric resolution of a series of 1,2-diacyl- and 1,2-mixed acid-diacyl-3-benzyl-*rac*-glycerols was investigated by high-performance liquid chromatography (HPLC). Of the racemic benzylglycerols studied, the 1-*O*-benzyl-2,3-*O*-isopropylidene-*rac*-glycerol, 1-acyl-3-benzyl-*rac*-glycerol and 2-acyl-3-benzyl-*rac*-glycerol structures could be resolved into their enantiomers. The latter were resolved on a silica (10 μm) column coated with cellulose tribenzoate by isocratic elution with hexane/isopropanol mixtures as mobile phase. The effects of para substitution of the benzyl moiety on the resolution of the acylbenzylglycerols by this HPLC method were evaluated. *Lipids* 27, 396-399 (1992).

1,2-Diacyl-*sn*-glycerols are activators of protein kinase C (PKC), an enzyme which plays an important role in signal transduction (1-5). To investigate the structural requirement of diacylglycerols as protein kinase C activators, enantiomeric 1,2-diacyl-*sn*-glycerols have been synthesized (4,5). Typical synthetic methods for the synthesis of enantiomeric 1,2-diacyl-*sn*-glycerols require optically active precursors, such as D-mannitol (6,7). The procedures typically involve a number of synthetic steps, produce relatively low yields, and cause racemization during synthesis.

A variety of chiral stationary phase (CSP) high-performance liquid chromatography (HPLC) columns have been developed recently and have been used to separate chiral compounds into their optical isomers (8). CSP HPLC columns also have been used on a preparative scale to isolate pure enantiomers (9). Thus, it may be easier to separate by HPLC selected racemic intermediates into their enantiomers, rather than to synthesize enantiomers from optically active compounds as starting materials. Using this approach, we recently reported the separation of enantiomeric alkyl glycerol ethers on a cellulose tribenzoate CSP HPLC column (10).

In the present study, we investigated the enantiomeric separation of several synthetic intermediates that are encountered in the synthesis of 1,2-diacylglycerols. We investigated the separation of 1,2-monoacid-diacylglycerols as well as of 1,2-mixed acid-diacylglycerols because different synthetic routes are required for their preparation.

MATERIALS AND METHODS

Materials. Oleic acid (99%, Extra Oleic 99) was obtained from Nippon Oil & Fats Co. (Tokyo, Japan). All other reagents used for the syntheses were obtained from Aldrich Chemical Co. (Milwaukee, WI). *n*-Hexane and isopropanol used for HPLC were obtained from American Burdick & Jackson (Muskegon, MI).

Analytical system. The HPLC system used in this study was a Beckman Model 110A instrument (Beckman Instruments, San Ramon, CA) which was connected to an ultraviolet detector (Spectroflow 773, Kratos Instruments, Ramsay, NJ) and an HP 3396A integrator (Hewlett-Packard, Avondale, PA); a Waters Differential Refractometer Model R 401 detector (Waters Associates, Milford, MA) was used as required. The analytical HPLC column used was a Microsorb Si, 4.6 mm i.d. \times 25 cm, 5 μm silica column (Rainin Instrument Co., Woburn, MA). The preparative silica column was a Dynamax Macro Si, 10 mm i.d. \times 25 cm, 8 μm (Rainin Instrument Co.). The CSP HPLC column was a Bakerbond Chiralcel OB, 4.6 mm i.d. \times 25 cm stainless steel column prepacked with 10 μm silica coated with cellulose tribenzoate (J.T. Baker, Phillipsburg, NJ). Columns were eluted isocratically with *n*-hexane/isopropanol (varying in composition from 96:4 to 99:1, v/v). In the case of the analytical column and the CSP column, sample concentrations were 1% (w/v) in mobile phase as solvent; injection volumes were 20 μL , and the flow rate was 0.5 or 1 mL/min. For preparative separations, sample concentrations were 50% (w/v), injection volumes were 100 μL , and the flow rate was 5 mL/min.

Syntheses. As shown in Figure 1, 1-*O*-benzyl-2,3-*O*-isopropylidene-*rac*-glycerol 2a was synthesized by benzylation of racemic glycerol acetone 1 with benzyl chloride in the presence of aq. NaOH and tetrabutylammonium bromide (11). 1-*O*-(4-Substituted-benzyl)-2,3-*O*-isopropylidene-*rac*-glycerols 2b-d were obtained in a similar manner.

As illustrated in Figure 2, 1-benzyloxy- and 1-(4-substituted-benzyloxy)-2,3-epoxy-propanes 5a-c were obtained by benzylation of epichlorohydrin 4 with benzyl or 4-substituted benzyl alcohols in the presence of aq. NaOH and tetrabutylammonium bromide. 1-*O*-Benzyl- and 1-*O*-(4-substituted-benzyl)-3-acyl-*rac*-glycerols 6a-c were obtained by acylation of 5a-c with fatty acid in the presence of tetraethylammonium bromide (12). Their 2-acyl-isomers 7a-c, formed as co-products in the latter reaction, were separated and purified by preparative HPLC on the silica column.

All compounds were identified by infrared (IR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and purity confirmed by analytical HPLC (Table 1).

RESULTS AND DISCUSSION

Enantiomeric HPLC separation of synthetic intermediates for enantiomeric 1,2-monoacid-diacyl-glycerol. We previously reported that racemic glycerol acetone 1 (Fig.

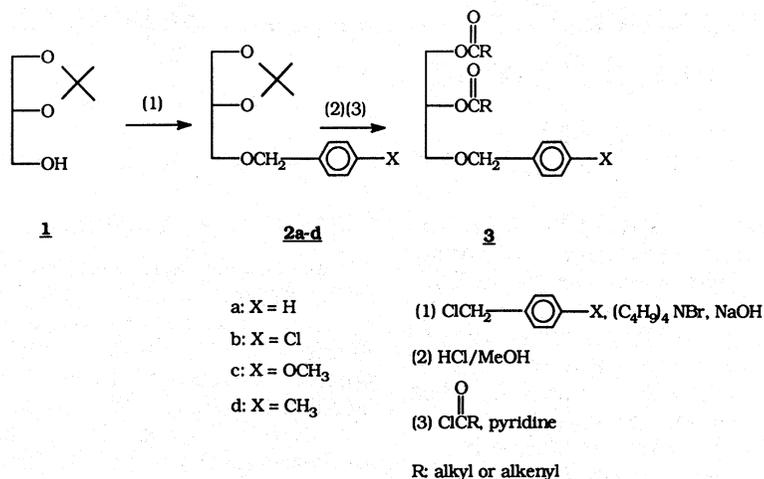


FIG. 1. Synthesis of racemic 1,2-diacylglycerols.

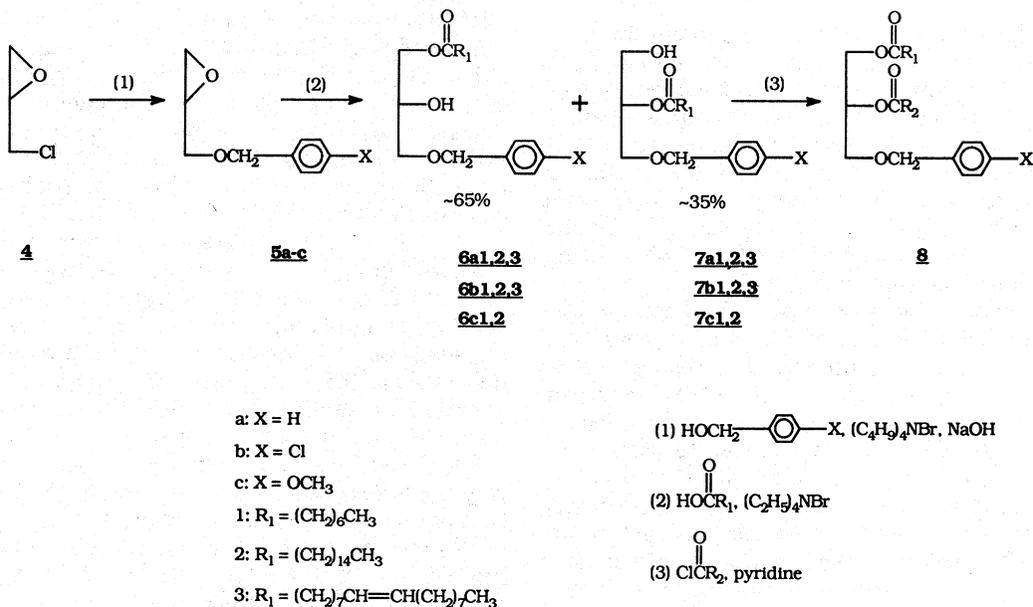


FIG. 2. Synthesis of racemic 1,2-mixed-acid-diacylglycerols.

1) and its 1-*O*-benzyl derivative **2a** (Fig. 1) can be separated into their enantiomers using a cellulose tribenzoate CSP HPLC column (abbreviated as "OB" below) (10). However, the enantiomers of glycerol acetonide may not be suitable synthetic intermediates for preparative isolation because they tend to racemize, and compound **2a** does not completely resolve into its enantiomer, which is required for preparative purposes (10). In an attempt to improve the enantiomeric resolution of **2a**, we prepared 4-substituted-benzyl analogs **2b-d** (Fig. 1) and examined their enantiomeric separation using the CSP HPLC column. Table 2 gives the chromatographic data obtained in the present study on the resolution of 1-*O*-(4-substituted-benzyl)-*rac*-glycerols. The separation factor (α) and resolution (R_s) values observed for **2a-d** were in the following order: compound **2c** > **2b** > **2d** > **2a**, which corresponds to a para substituent order of OCH₃ > Cl > CH₃ > H. Inter-

action between the phenyl moieties of the sample compound and the chiral stationary phase have been often cited in describing the mechanism of chiral recognition. It has been reported that electron-donating substituents on the phenyl moieties of the cellulose tribenzoate CSP (13) or the analyte compounds (14) improve the effective chiral recognition of the CSP. However, from our results for compound **2** (Fig. 1), it appears that the stronger the electron-donating substituent (e.g. OCH₃) or the electron-withdrawing (e.g. Cl) substituent effect on the phenyl moiety, the better an enantiomeric resolution is achieved.

We also examined the enantiomeric separation of several racemic 1,2-monoacid-diacyl-3-benzyl- or (4-substituted-benzyl)-glycerols **3** (Fig. 1) on the CSP column; however, none of these intermediates could be separated into enantiomers.

TABLE 1

HPLC Separation of Various Substituted Benzyl-*rac*-glycerols^a

Compound ^b	Retention time (min)	Chromatographic purity (%)	Mobile phase composition ^c
2a	4.5	94	98:2
2b	4.9	86	98:2
2c	5.4	87	98:2
2d	5.1	95	98:2
6a,1	11.1	88	98:2
6b,1	7.5	100	96:4
6c,1	8.3	100	96:4
6a,2	9.7	100	98:2
6b,2	11.9	95	98:2
6a,3	9.0	92	98:2
6b,3	8.4	97	97:3
7a,1	17.9	100	98:2
7b,1	10.0	100	96:4
7c,1	11.1	100	96:4
7a,2	14.4	100	98:2
7b,2	18.1	89	98:2
7a,3	13.6	81	98:2
7b,3	12.0	88	97:3

^aSeparations were obtained using a microsorb Si column (25 × 0.46 cm) at a flow rate of 1 mL/min.

^bStructure of compounds is given in Figures 1 and 2.

^cMobile phase, *n*-hexane/isopropanol (v/v).

TABLE 2

Enantiomeric Separation of 1-Benzylglycerols 2a-d

Compound	Capacity factor K'	Separation factor α	Resolution R _s
2a	3.31	1.19	0.70
2b	3.19	1.50	1.23
2c	11.08	1.54	1.46
2d	3.36	1.50	0.82

Because a desirable base-line separation ($R_s > 1$), a criterion that must be met to resolve enantiomers, was obtained with 2b and 2c, we considered these compounds useful as synthetic intermediates for preparing diacylglycerols in which both acyl residues are the same (Fig. 1).

Enantiomeric HPLC separation of synthetic intermediates for the preparation of enantiomeric 1,2-mixed acid-diacyl glycerols. The synthetic scheme used for preparing racemic 1,2-mixed acid-diacylglycerols is shown in Figure 2 (11,12). Compounds 6a-6c are candidates for separation into their enantiomers because they contain the requisite functional groups, namely an ester, a hydroxy group and an aromatic ring. We have previously shown that these structural features enhance the enantiomeric separation on the CSP column (10). In the present study, we also prepared several 1-*O*-benzyl- and 1-*O*-(4-substituted-benzyl)-3-acyl-glycerols 6a-c,1-3 to examine their enantiomeric separation on the CSP column. The 2-acyl isomers 7a-c,1-3, which are formed as co-products, were purified and isolated by preparative HPLC and their enantiomeric separation was also investigated.

Table 3 gives the chromatographic data for the racemic 1-acyl and 2-acyl-3-benzyl-glycerols prepared in this study.

Compounds 6a-c,1-3 were separated only partially into their enantiomers or were not separated at all on the CSP column. On the other hand, the corresponding 2-acyl isomers 7a-c,1-3 were resolved into their enantiomers with a good separation factor (α) and resolution (R_s) and with most giving a base-line separation ($R_s > 1$). These results demonstrate that an ester group at the 2-position of the glycerol backbone contributes more substantially to the enantiomeric separation than does an ester group at the 3-position of the glycerol backbone. This is in agreement with our previous study where we reported that 1-*O*-hexadecyl-2-*O*-benzyl-*rac*-glycerol was successfully separated into its enantiomers by the CSP column, whereas the corresponding 3-benzyl isomer was not resolved at all (10). From our previous results and based on the present data, it is concluded that a selected functional group, *e.g.*, the ester or benzyl group, needs to be attached at the 2-position of the glycerol backbone to accomplish a successful enantiomeric separation on a CSP column.

The effect of the substituents on the phenyl moiety on enantiomeric separation was less clear because, in the case of the octanoyl and oleoyl glycerols 7a1 and 7a3, the non-substituted benzyl derivatives showed better enantiomeric separation than the substituted benzyls 7b1 and

TABLE 3

Enantiomeric Separation of 1(2)-Acyl-3-benzylglycerols 6 and 7

	<i>rac</i> -Glycerol position			Enantiomeric separation		
	1 ^a	2	3	K'	α	R _s
6a,1	phenyl		octanoyl	10.7	1.19	0.83
6b,1	4-chlorophenyl		octanoyl	12.7	1.22	0.81
6c,1	4-methoxyphenyl		octanoyl	28.3	1.10	0.4
6a,2	phenyl		palmitoyl	1.4	1.0	0.4
6b,2	4-chlorophenyl		palmitoyl	6.2	1.15	0.54
6a,3	phenyl		oleoyl	4.1	1.15	0.4
6b,3	4-chlorophenyl		oleoyl	5.0	1.0	0.4
7i,1	phenyl	octanoyl		10.3	1.53	1.26
7b,1	4-chlorophenyl	octanoyl		13.9	1.18	0.81
7c,1	4-methoxyphenyl	octanoyl		26.4	1.33	1.04
7a,2	phenyl	palmitoyl		2.8	1.0	0.4
7b,2	4-chlorophenyl	palmitoyl		7.2	1.34	1.13
7i,3	phenyl	oleoyl		4.8	1.60	1.40
7b,3	4-chlorophenyl	oleoyl		6.3	1.82	1.17

^aThe phenyl moiety referred to is that of the benzyl group located at position-1 of glycerol (see Fig. 2).

7b3. We also attempted to separate several racemic 1,2-mixed acid-diacyl-3-benzyl- or (4-substituted-benzyl)-glycerols **8** using the CSP column. However, enantiomeric separation was not achieved.

Based on the present data we conclude that 2-acyl-3-benzyl-glycerols, such as compounds **7a-c** (Fig. 2, Table 3), have the best potential as intermediates for the preparative enantiomeric separation of the racemic compounds. Because the 2-acyl-3-benzyl-glycerols are co-products of the synthetic pathway we have used (Fig. 2), alternate synthetic routes by which the 2-acyl isomers are obtained as major products are under investigation.

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