Resolution of *vic*-Dihydroxy Acid Diastereomers to Four Enantiomers by High-Performance Liquid Chromatography

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A chromatographic method is described for resolution of 9,10-*vic*-dihydroxyoctadecanoic acid. The *threo* and *erythro* isomers are both resolved into two enantiomers as di-3,5-dinitrophenylurethane derivatives by chiral-phase high-performance liquid chromatography on (*S*)-proline,(*R*)-1-(α-naphthyl)ethylamine stationary phase. Simultaneous separation of the *threo* and *erythro* dihydroxy acid mixtures to the four enantiomers is possible by this method.

KEY WORDS: Chiral-phase HPLC, diastereomer, dihydroxy fatty acid, enantiomer.

Diastereomers of *vic*-dihydroxy fatty acids can be easily resolved into the *threo* and *erythro* isomers by gas–liquid chromatography (1), thin-layer chromatography (TLC) (2) and high-performance liquid chromatography (HPLC) (3) on achiral stationary phases because diastereomers have different physical properties. However, the resolutions of *threo* and *erythro* isomers into their two enantiomers have not been reported. Recently, only *erythro* isomers of 5,6-, 8,9-, 11,12- and 14,15-*vic*-dihydroxyeicosatrienoic acids and of their corresponding saturated *vic*-dihydroxyeicosanoic acids, have been resolved by Capdevila and co-workers (4) to their enantiomers as the methyl or pentafluorobenzyl esters by chiral-phase HPLC with a Chiralcel OC or OD column (Daicel Chemical Industries, Tokyo, Japan). In our paper, complete resolutions of both *threo* and *erythro* isomers of 9,10-dihydroxy-*vic*-octadecanoic acid to their two enantiomers (Fig. 1) as their di-3,5-dinitrophenylurethane (3,5-DNPU) derivatives on a Sumichiral OA-4500 (OA-4500) chiral-phase column (Sumika Chemical Analysis Service, Osaka, Japan) are presented. Simultaneous separations of a mixture of the *threo* and *erythro* isomers to their four enantiomers can be carried out by this method.

EXPERIMENTAL PROCEDURES

Threo and *erythro*-9,10-dihydroxyoctadecanoic acids were obtained from Sigma Chemical Co. (St. Louis, MO). Preparation of di-3,5-DNPU derivatives from those acids was carried out by the procedures of Oi, Kitahara (5) and Takagi (6). The crude urethane products were purified by TLC on silica gel GF plates with *n*-hexane/dichloroethane/ethanol (40:15:5, vol/vol/vol) as the developing solvent.

HPLC separations were carried out with a Shimadzu LC-6A instrument (Shimadzu, Kyoto, Japan) with a chiral column (25 mm × 4.6 mm i.d.), packed with 5-μ particles of Sumichiral OA-4500 (*S*)-proline,(*R*)-1-(α-naphthyl)ethylamine chemically bonded to silanized silica. The analysis was done isocratically with a mixture of HPLC-grade *n*-hexane/chloroform/methanol/trifluoroacetic acid (65:20:15:0.1, by vol) as mobile phase at a constant flow rate of 1.0 mL/min at 25°C. The separations were monitored with a Shimadzu SPD-6A UV detector at 254 nm. Peak area percentages and retention times were measured with a Shimadzu integrator, Chromatopac CR6A. The injected amount was 2 μL of chloroform solution (2 mg/mL).

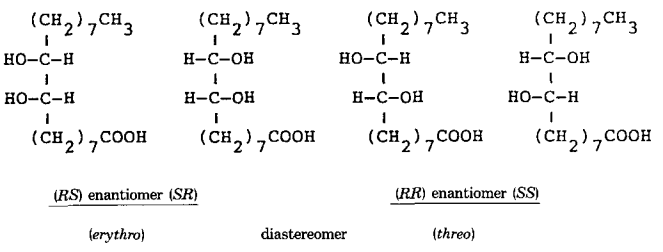

FIG. 1. Structure of 9,10-dihydroxyoctadecanoic acids by Fisher projection.

RESULTS AND DISCUSSION

TLC of the crude 3,5-DNPU products, prepared from the *threo* and *erythro* dihydroxyoctadecanoic acids, gave two bands (I: yellow band, R_f 0.3–0.4; II: detected under ultraviolet (UV) irradiation, R_f 0.6–0.7). The band II fractions from *threo* and *erythro* dihydroxyoctadecanoic acids showed only one spot on the TLC plates under the same conditions. The extracts from band II showed the typical UV spectra reported for the di-3,5-DNPU derivatives with a strong absorption at λ_{max} 226 nm, a weak absorption at λ_{max} 254 nm and a very weak absorption at 340 nm in ethanol solution (7). Band I in the TLC represents impurities formed from the excess of isocyanate.

Threo and *erythro* dihydroxyoctadecanoic acids have been separated as di-3,5-DNPUs by HPLC on Sumichiral OA-4000 and 4100 (Sumika Chemical Analysis Service) under the conditions used for the resolution of 1-monoacylglycerol enantiomers in a previous study (8). Each diastereomer was resolved to enantiomers. However, a mixture of *threo* and *erythro* isomers has shown only two peaks of enantiomers, and did not show resolution of the diastereomers. Separation of mixtures of *threo* and *erythro* dihydroxyoctadecanoic acids to their four enantiomers was achieved as 3,5-di-DNPU derivatives by HPLC on an OA-4500 chiral column under the conditions shown in Figure 2.

Assignments of the peaks to *threo* and *erythro* isomers were carried out with HPLC of each isomer under the same conditions. Complete enantiomer separations of *threo* and *erythro* isomers within 30 min, and sufficient space of the base line between enantiomer peaks in the figure show possibilities for more rapid separations. Both the separation factor and the peak resolution of enantiomers for the *threo* isomer are a little less than those for the *erythro* isomer. In reversed-phase HPLC, *erythro* isomers eluted earlier than *threo* isomers in resolutions of *erythro* dihydroxy fatty acid enantiomers as the trifluoroacetate or methyl ester (4). In normal-phase HPLC, used in this study, the *threo* isomer of dihydroxy acids reversely eluted earlier than the *erythro* isomer as di-3,5-DNPUs. The derivatives of the *erythro* dihydroxyoctadecanoic acids behave as more polar isomers in both

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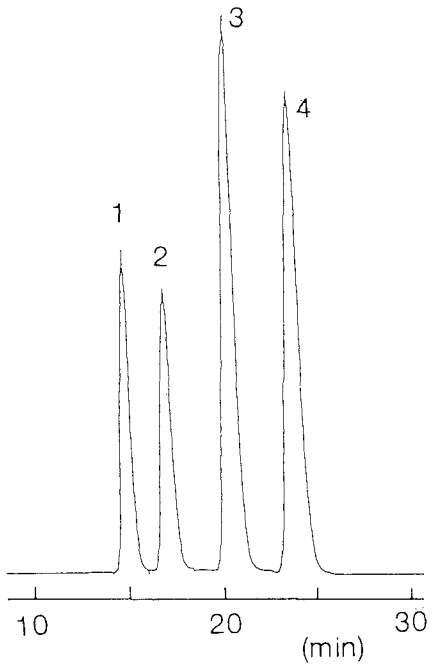

FIG. 2. Resolution of 9,10-dihydroxyoctadecanoic acids to four enantiomers as 3,5-dinitrophenylurethane derivatives on a column, Sumichiral 4500 (25 cm × 4.6 mm i.d.) (Sumika Chemical Analysis Service, Osaka, Japan). Hexane/chloroform/methanol/trifluoroacetic acid (65:20:15:0.1, by vol) is used as mobile phase. Flow rate, 1.0 mL/min; detection, 254 nm; peaks 1 and 2, *threo*; peaks 3 and 4, *erythro* diastereomers.

cases. The values of PA (area ratios of enantiomers in the racemates), shown in Table 1, are almost 1.0. It suggests the possibility of determining the enantiomer ratios by the method presented in this communication.

TABLE 1

Simultaneous Resolution of 9,10-Dihydroxyoctadecanoic Acid to Four Enantiomers

	Peak no.	Vr[a]	α_{enant}[b]	Rs[c]	PA[d]
	1	15.0			
Threo			1.15	2.09	1.002
	2	17.3			
				2.25	
	3	21.0			
Erythro			1.17	2.90	0.999
	4	24.6			

[a]Vr = retention volume (mL) corrected by substracting the column void volume (4.1 mL).
[b]α_{enant} = separation factor calculated from Vr of enantiomer pairs.
[c]Rs, peak resolution.
[d]PA, peak area ratio of enantiomers (peak 1/peak 2 and peak 3/peak 4).

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