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Note

High-performance liquid chromatographic separation of alkane-1,2-diol enantiomers on a chiral slurry-packed capillary column

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There is a trend to reduce the column diameter in high-performance liquid chromatography (HPLC), because capillary HPLC has some advantages over conventional HPLC using 4–5 mm I.D. columns, such as a potentially high column efficiency, low consumption of stationary and mobile phases, a small amount of sample and easy coupling with mass spectrometry[1–3]. Various organic and inorganic compounds have been analysed by reversed-phase, normal-phase and size-exclusion HPLC using columns of 1 mm or less I.D.[1–3]. Chiral-phase HPLC has also been carried out using 1 mm I.D. microbore columns[4–6]. However, capillary HPLC, in which the column I.D. is less than 1 mm, using chiral stationary phases has rarely been applied[7].

We have previously studied separations of lipid enantiomers and reported HPLC separations of enantiomeric monoacyl-[8], monoalkyl-[9] and diacylglycerols[10,11] as their 3,5-dinitrophenylurethane (3,5-DNPU) derivatives on chiral stationary phases. These separations were obtained using conventional stainless-steel columns of 4 mm I.D.

This paper describes the separation of enantiomers of alkane-1,2-diols as their bis(3,5-DNPU) derivatives by HPLC using a chiral slurry-packed capillary column. The separation of the enantiomers of lower homologues of 1,2-diols (C_2–C_8) using the conventional columns with a chiral stationary phase has been described recently by Pirkle *et al.*[12].

The higher alkane-1,2-diols are widely distributed as their diesters with long-chain fatty acids in mammalian skin lipids and bird waxes[13]. In this study, higher 1,2-diols were analysed using a chiral slurry-packed capillary column of 0.32 mm I.D. packed with particles containing the chiral stationary phase.

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EXPERIMENTAL

Samples

Alkane-1,2-diols were obtained from 2-hydroxy acid methyl esters by reduction with lithium aluminium hydride. (*RS*)-2-Hydroxyhexadecanoic acid, (*RS*)-2-hydroxyoctadecanoic acid and (*S*)- and (*RS*)-2-hydroxy-4-methylpentanoic acids (leucic acids) of purity >98% were purchased from Tokyo Kasei (Tokyo, Japan), Larodan Fine Chemicals (Malmö, Sweden) and Sigma (St. Louis, MO, U.S.A.), respectively, and converted to the methyl esters with diazomethane.

The bis(3,5-DNPU) derivatives were prepared from 1 mg of alkane-1,2-diols and about 2 mg of 3,5-dinitrophenyl isocyanate in toluene in the presence of pyridine[9]. The pure urethane derivatives were isolated from the reaction mixture by thin-layer chromatography (TLC) on silicic acid[9], using chloroform–acetone (24:1) as the developing solvent.

UV spectra of the bis(3,5-DNPU) derivatives were taken in ethanol on a Hitachi (Tokyo, Japan) U-2000 spectrophotometer.

HPLC

A Model LC-6A single-plunger pump (Shimadzu, Kyoto, Japan) was used for HPLC analysis and column packing. The chiral packing material, N-(*S*)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine chemically bonded to 5-μm particles of γ-aminopropylsilanized silica (Sumipax OA-2100; Sumitomo Chemical, Osaka, Japan), was slurry-packed into a fused-silica tube (40 cm × 0.32 mm I.D.) (QuadRex, New Haven, CT, U.S.A.) using carbon tetrachloride–liquid paraffin (1:1) as the slurry solvent[14]. The slurry reservoir (stainless-steel tubing, 2.2 mm I.D.) was connected directly to the fused-silica column through a reducing union. The slurry was added to the reservoir and brought into the capillary tube by the pump. The particles, tightly packed under high pressure, were retained in the column by a PTFE frit (Kusano Kagaku, Tokyo, Japan), which was supported by narrower fused-silica tubing (10 cm × 75 μm I.D.) connected to the detector. A Shimadzu SPD-6A variable-wavelength UV detector equipped with a laboratory-made flow cell (*ca.* 0.05 μl)[15] was set at 226 nm and 0.08 a.u.f.s. The detector cell was a 0.32 mm I.D. fused-silica tube, and the polyimide coating where the UV light beam crosses was removed by burning. The end of the slurry-packed capillary column was protected by a stainless-steel tube (2 cm × 1/16 in. O.D.), and was connected directly to a Rheodyne Model 7520 injector with a 0.2-μl sample chamber.

The bis(3,5-DNPU) derivatives dissolved in chloroform (HPLC grade) were injected with a 10-μl Hamilton syringe and analysed isocratically at ambient temperature (19–21°C) using *n*-hexane–1,2-dichloroethane–ethanol (all of HPLC grade) (40:12:3 or 20:5:1) as the mobile phase at a constant pressure of 10 kg/cm^2 (*ca.* 4 μl/min). Peak-area percentages and retention times were measured with a Shimadzu Chromatopac C-R6A integrator.

RESULTS AND DISCUSSION

Derivatives

As with monoacyl- and momoalkylglycerols[8,9], the two hydroxy groups of

alkane-1,2-diols reacted readily with 3,5-dinitrophenyl isocyanate in toluene solution in the presence of pyridine. The resulting bis(3,5-DNPU) derivatives were purified by preparative TLC on silicic acid.

In the TLC of the bis(3,5-DNPU) derivatives of monoalkylglycerols[6], *n*-hexane–1,2-dichloroethane–ethanol (20:5:1) was used as the developing solvent. This solvent system gave compact spots only when a small amount of sample (less than 1 mg) was applied on a TLC plate (20 cm × 20 cm, 0.25 mm thick layer). A similar observation was made for the bis(3,5-DNPU) derivatives of the alkane-1,2-diols. The solvent system employed in this study (chloroform–acetone, 24:1) gave clear chromatograms without tailing for the bis(3,5-DNPU) derivatives of both monoalkylglycerols and alkane-1,2-diols (R_F = 0.4 for hexadecane-1,2-diol), even when several milligrams of the samples were applied to the TLC plate. This is probably due to the greater solubility of the bis(3,5-DNPU) derivatives in this solvent.

Elemental analysis for the bis(3,5-DNPU) derivatives of octadecane-1,2-diol was as follows: found, C 55.10, H 6.57, N 11.58; calculated for $C_{32}H_{44}O_{12}N_6$, C 54.54, H 6.29, N 11.93%. The UV spectrum of the bis(3,5-DNPU) derivatives was essentially the same as that obtained for the monoacylglycerol bis(3,5-DNPU) derivatives (λ_{max} 226 nm)[8]. The 3,5-DNPU derivatives have sufficient sensitivity for HPLC detection over a wide range of UV wavelengths[8]. In our previous work[8–11], therefore, the 3,5-DNPU derivatives were detected at 254 nm, the wavelength generally used in UV detectors. To obtain a stable baseline at higher sensitivity, the bis(3,5-DNPU) derivatives in this study were monitored at 226 nm, which approximately doubles the sensitivity at 254 nm[8].

Separation

Fig. 1 shows the enantiomer separation of racemic hexadecane-1,2-diol as its bis(3,5-DNPU) derivatives on a chiral packed capillary column. Within 30 min, the racemate was clearly resolved into two components with nearly the same peak-area ratio, which demonstrates an effective separation into the enantiomers. The large and small peaks near 7 and 12 min were due to the solvent chloroform and the reagent isocyanate, respectively. A similar enantiomer separation to that in Fig. 1 was also obtained for racemic octadecane-1,2-diol. The chromatogram obtained for a mixture of the racemic and *S* enantiomeric 4-methylpentane-1,2-diol under the conditions used, showed clearly that the first and second peaks are the *R* and *S* enantiomers, respectively. Therefore, the enantiomer peaks obtained for racemic hexadecane- and octadecane-1,2-diols were also identified as shown in Fig. 1.

Table I gives chromatographic data for three racemic samples used. The enantiomer separations were examined using two mobile phases of different polarity. Although the lengthening of the retention times showed a slightly high separation factor and peak resolution, it also caused significant peak tailing. Such tailing is common for bands of high capacity factor (k'), and has been attributed to non-homogeneous adsorption sites[16]. The shifts to shorter retention times for the higher enantiomer homologues can be explained by the lower polarity of the alkane-1,2-diols with longer alkyl chains, which may interact weakly with the silica gel support. Similar shifts in the retention times of the enantiomer homologues were observed in the chiral-phase HPLC of the 3,5-DNPU derivatives of acyl and alkylglycerols with 4 mm I.D. columns containing the same packing material,

TABLE I

CHROMATOGRAPHIC DATA FOR ENANTIOMERIC ALKANE-1,2-DIOLS AS THEIR BIS(3,5-DNPU) DERIVATIVES ON A CHIRAL SLURRY-PACKED CAPILLARY COLUMN

RT = Retention time (min); k' = capacity factor; α = separation factor; R_s = peak resolution = $2(t_1 - t_2)/(w_1 - w_2)$, where t = retention time (min) and w = peak width (min); *TF* = tailing factor (%) = $100a/b$, where a and b are the baseline half-widths on the short- and long-time sides from the perpendicular drawn through the peak maximum, respectively.

Alkane-1,2-diol	*Enantiomer*	*Mobile phase (n-hexane–1,2-dichloroethane–ethanol)*									
		40:12:3					*20:5:1*				
		RT	k'	α	R_s	*TF*	*RT*	k'	α	R_s	*TF*
4-Methylpentane-	*R*	31.23	4.00	1.08	0.73	–	63.45	9.17	1.09	0.87	–
	S	33.27	4.33			–	68.73	10.01			–
Hexadecane-	*R*	21.69	2.44	1.24	1.87	96.2	41.48	5.65	1.27	2.03	85.2
	S	25.38	3.02			88.9	50.92	7.16			75.0
Octadecane-	*R*	20.84	2.34	1.25	1.86	94.8	40.83	5.54	1.28	2.00	86.1
	S	24.46	2.92			90.2	50.62	7.11			76.0

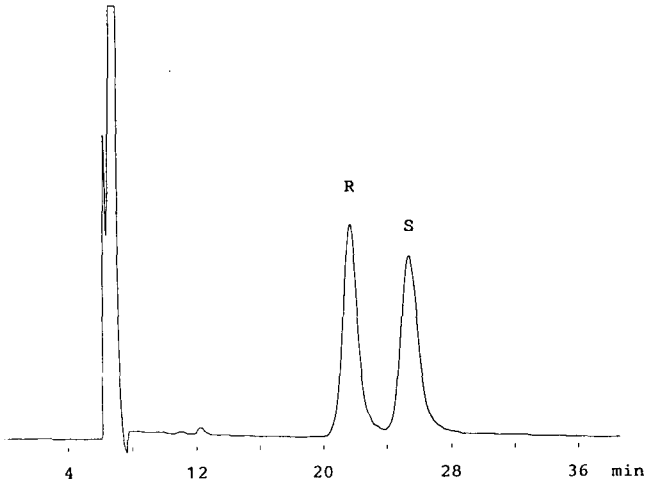

Fig. 1. Chiral-phase HPLC separation of hexadecane-1,2-diol enantiomers as their bis(3,5-DNPU) derivatives on a slurry-packed capillary column. Mobile phase: *n*-hexane–1,2-dichloroethane–ethanol (40:12:3). Other HPLC conditions as described in the text.

OA-2100[8–10]. Using the solvent system *n*-hexane–1,2-dichloroethane–ethanol (40:12:3), the separation factors of the *R* and *S* enantiomers of hexadecane- and octadecane-1,2-diols were 1.04 and 1.03, respectively, as calculated from the data in Table I. These values and the separation factors between *R* and *S* enantiomers hardly increased when using the lower polarity solvent system (20:5:1).

The chiral packed capillary column used in this study showed only 2800 theoretical plates for the *S* enantiomer peak of hexadecane-1,2-diol. The plate numbers were lower than that expected ($2 \cdot 10^4$–$3 \cdot 10^4$). This suggests that the column used in this study had not been well packed. Complete separations, however, were obtained for the long-chain alkane-1, 2-diol enantiomers as shown in Fig. 1 and Table I. The separation factor and peak resolution obtained for the *R* and *S* enantiomers of the long-chain alkane-1,2-diols are slightly higher than those of monoalkylglycerol enantiomers obtained using a 25 cm × 4 mm I.D. column packed with OA-2100[9], although the time of analysis was three times longer. The use of a column with more theoretical plates will give a higher resolution at a shorter retention time. The consumption of chiral material and mobile phase in this study were only *ca.* 1/100 and 1/250, respectively, of those in conventional HPLC (25 cm × 4 mm I.D. column, flow-rate 1 ml/min). The use of capillary columns therefore seems to be very economical in chiral-phase HPLC using expensive stationary phases.

The *S* enantiomers of monoacyl- and monoalkylglycerols as their bis(3,5-DNPU) derivatives were retained more strongly than the corresponding *R* enantiomers in conventional HPLC on the same chiral phase as that used in this study[8,9]. This supports the faster elution of the *R* enantiomers of alkane-1,2-diols as their bis(3,5-DNPU) derivatives on the chiral packed fused-silica column, and indicates that the mechanism of separation of alkane-1,2-diol enantiomers on the chiral stationary phase is essentially the same as that for monoacyl- and monoalkylglycerol

enantiomers. The formations of hydrogen bonds and charge-transfer complexes between the bis(3,5-DNPU) derivatives and the chiral stationary phase may contribute to the enantiomer separations, as discussed elsewhere[9,11].

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