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Note

Gas chromatographic separation of α-hydroxycarboxylic acid ester enantiomers using amino acid derivatives as chiral stationary phase

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It is well known that amino acid enantiomers in the form of their N-acyl esters[1,2] or N-acyl amides[3] can be resolved by gas chromatography (GC) with amino acid derivatives as optically active stationary phase. This separation has been attributed to diastereomeric interaction including hydrogen bonding between CONH groups.

In contrast, α-hydroxycarboxylic acid enantiomers have never been separated in the form of their O-acyl esters and it was necessary to use the corresponding O-acyl amides to resolve the enantiomers[4]. This has been considered to be due to the absence of a nitrogen-attached hydrogen in O-acyl α-hydroxycarboxylic acid esters.

In this paper we describe the direct separation of enantiomers of α-hydroxycarboxylic acid esters, which have no NH groups, using amino acid derivatives as chiral stationary phase.

EXPERIMENTAL

A Shimadzu GC-7A gas chromatograph equipped with a flame ionization detector was employed. Glass capillary columns (40 m × 0.25 mm I.D.) were coated with the optically active stationary phases N,N′-[2,4-(6-ethoxy-1,3,5-triazine)diyl]bis (L-valyl-L-valine isopropyl ester) (OA-200)[5], N,N′-[2,4-(6-ethoxy-1,3,5-triazine)diyl] bis(L-valyl-L-valyl-L-valine isopropyl ester) (OA-300)[6] and N,N′,N″-[2,4,6-(1,3,5-triazine)triyl]tris(N$^{\alpha}$-lauroyl-L-lysine *tert.*-butylamide) (OA-400)[7] which were prepared as described previously.

L- and DL-lactic acid and DL-α-hydroxybutyric acid were purchased from Wako (Osaka, Japan). The various esters or amines for GC were prepared from these acids by treatment with corresponding alcohols or amides. Some esters and amides were O-acylated with acetic anhydride or trifluoroacetic anhydride.

RESULTS AND DISCUSSION

The results are summarized in Tables I and II. A typical chromatogram is shown in Fig. 1.

Enantiomers of several α-hydroxycarboxylic acid esters were resolved into their antipodes when the α-hydroxy group was not acylated, as shown in Table I. The

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TABLE I

GC SEPARATION OF α-HYDROXYCARBOXYLIC ACID ESTER ENANTIOMERS

Chromatographed on 40 m × 0.25 mm I.D. glass capillary columns. Carrier gas: helium at 0.7 ml/min.

H R-C*-COOR' OH			*OA-400*				*OA-300 + OA-200 (1:1)*			
			Column temp. (°C)	*Retention time* (min)*		*Separation factor, α (2nd/1st)*	*Column temp. (°C)*	*Retention time* (min)*		*Separation factor, α (2nd/1st)*
X	*R*	*R'*		*1st peak*	*2nd peak*			*1st peak*	*2nd peak*	
H	CH_3	C_2H_5	90	3.48	3.60	1.034	130	1.4	1.4	1.000
H	CH_3	$CH(CH_3)_2$	90	3.84	3.95	1.029				
H	CH_3	$CH_2CH(CH_3)_2$	90	9.85	10.20	1.036	130	3.36	3.43	1.021
$COCH_3$	CH_3	$CH_2CH(CH_3)_2$	90	10.8	10.8	1.000	130	4.5	4.5	1.000
H	CH_3	cyclo-C_6H_{11}	100	32.0 (L)	33.1 (D)	1.034	130	16.9 (L)	17.4 (D)	1.030
H	CH_3	n-C_6H_{13}	100	24.5 (L)	25.4 (D)	1.037	130	12.5 (L)	12.8 (D)	1.024
$COCH_3$	CH_3	n-C_6H_{13}	100	24.4	24.4	1.000	130	16.1	16.1	1.000
H	C_2H_5	n-C_6H_{13}					130	18.1	18.6	1.028

* Measured from solvent peak.

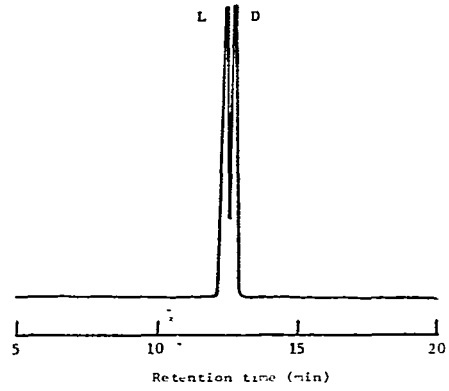


Fig. 1. Gas chromatogram of DL-*n*-hexyl lactate. Column: glass capillary (40 m × 0.25 mm I.D.) coated with OA-300 + OA-200 (1:1). Temperature: 130°C.

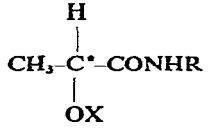
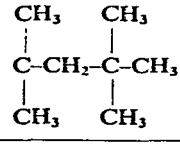
fact that enantiomers could not be resolved under the same chromatographic conditions when the α-hydroxy group was acylated indicates the free α-hydroxy group makes a large contribution to the separation of α-hydroxycarboxylic acid ester enantiomers.

The enantiomers of lactic acid amide were resolved even when the α-hydroxy group was acylated, as shown in Table II. This result suggests the CONH group exerts the main influence on the enantiomeric separation of these amides.

TABLE II

GC SEPARATION OF LACTIC ACID AMIDE ENANTIOMERS

Chromatographed on 40 m × 0.25 mm I.D. glass capillary columns coated with OA-300 + OA-200 (1:1). Carrier gas: helium at 0.6 ml/min. Column temperature: 150°C.

		*Retention time* (min)*		*Separation factor, α*
		1st peak	*2nd peak*	*(2nd/1st)*
X	*R*			
H	*n*-C_6H_{13}	161.1 (D)	163.8 (L)	1.017
$COCF_3$	*n*-C_6H_{13}	20.5 (D)	20.7 (L)	1.010
H	cyclo-C_6H_{11}	181.3 (D)	182.7 (L)	1.008
$COCF_3$	cyclo-C_6H_{11}	14.7 (D)	15.0 (L)	1.020
H		14.4	14.6	1.014
H		60.8	62.3	1.025

* Measured from solvent peak.

Peak identifications were made by chromatographing successively racemic and 1:3 mixtures of enantiomers of cyclohexyl-, *n*-hexyl esters and cyclohexyl-, *n*-hexyl amides of lactic acid. It is very interesting that L-isomers were eluted prior to D-isomers in the ester form, but D-isomers were eluted prior to L-isomers in the amide form. This reversal of the order of emergence on the same chiral stationary phase apparently indicates that the solute–solvent interaction in the ester form is different from that in the amide form. Hitherto it had been believed that the existence of a nitrogen-attached hydrogen was required for the formation of a strong diastereomeric association complex in the separation of enantiomers on amino acid derivatives.

It is noticeable that a free OH group linked to the asymmetric carbon atom is effective in the separation of enantiomers. We consider this finding throws new light on the mechanism of the separation of enantiomers.

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* *Editor's Note:* The paper by W. A. König and S. Sievers (*J. Chromatogr.*, 200 (1980) 189) was accepted by this journal on July 16th, 1980, and had not yet appeared when the present article was submitted.