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Note

Gas chromatographic analyses of diastereomeric α-hydroxy acid amides using a chiral stationary phase

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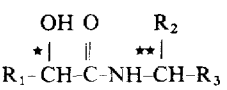
Several gas chromatographic (GC) resolutions of α-hydroxy acid derivatives have been performed with achiral[1–3] and chiral[4–8] stationary phases. Frank *et al.*[4,5] investigated the resolutions of enantiomeric α-hydroxy acid amides using Chirasil-Val as the chiral stationary phase. They proposed[5] a model for the chiral recognition mechanism of O-pentafluoropropionyl (O-PFP) lactic acid cyclohexyl amide on Chirasil-Val. The difference of the diastereomeric interaction with Chirasil-Val was explained by means of hydrogen bonding between NH and CO of carbamoyl groups[5].

However, the chiral recognition mechanism of the chiral stationary phase has not been fully investigated yet. In order to clarify the mechanism, it would be necessary to accumulate the separation data of many different kinds of compounds and to examine the relationship between the structure of the chiral stationary phase and the elution order of the isomers.

In this paper, the separation of diastereomeric O-trifluoroacetyl (O-TFA) α-hydroxy acid amides (I–IX) shown in Table I by using Chirasil-Val is described in terms of the structure of the amine moiety of the α-hydroxy acid amides and the

TABLE I

DIASTEREOMERS OF NINE KINDS OF α-HYDROXY ACID AMIDES



	R_1	R_2	R_3
$I_{R^*S^{**},SR,RR,SS}$	CH_3	CH_3	$COOCH_2CH(CH_3)_2$
$II_{RS,SR,RR,SS}$	CH_3	$CH(CH_3)_2$	$COOCH_2CH(CH_3)_2$
$III_{RS,SR,RR,SS}$	CH_3	$CH_2CH(CH_3)_2$	$COCH_2CH(CH_3)_2$
$IV_{RS,SR,RR,SS}$	CH_3	CH_3	Phenyl
$V_{RS,SR,RR,SS}$	CH_3	CH_2CH_3	Phenyl
$VI_{SR,RR,SS}$	CH_3	CH_3	Naphthyl
$VII_{RS,SR,RR,SS}$	Phenyl	CH_3	Phenyl
$VIII_{RS,SR,RR,SS}$	Phenyl	CH_2CH_3	Phenyl
$IX_{RS,SR,RR,SS}$	CH_3	Phenyl	$COOCH_2CH(CH_3)_2$

* Configuration of α-hydroxy moiety.
** Configuration of amine moiety.

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elution order of the diastereomers. It was found that the separation factors of (*S,S*)- to (*R,S*)-isomers were larger than those of (*R,R*)- to (*S,R*)-isomers. This tendency could be useful in clarifing the chiral recognition mechanism of the chiral stationary phase.

EXPERIMENTAL

Gas chromatography

A Hitachi 163 gas chromatograph equipped with a glass capillary column (25 m × 0.3 mm I.D.), which was coated with a chiral stationary phase, Chirasil-Val[4], was used. It was purchased from Applied Science Division, Milton Roy. The detection of the peaks was performed by a flame ionization detector. The temperature of the injection port was 250°C. The column temperature was programmed from 80°C to 170°C at a rate of 4°C/min. The pressure of the carrier gas (nitrogen) was 0.5 kg/cm^2, and the split ratio was 100:1. The retention times of the chromatographic peaks were measured by a Hitachi 834-30 chromatoprocessor.

Materials

(S,R)-Isomers and (S,S)-isomers of I–VI: $I_{SR,SS}$–$VI_{SR,SS}$. The pure (*S,R*)-isomers and (*S,S*)-isomers of I–VI were prepared[3] by the coupling of optically pure (*S*)- or (*R*)-amines and optically pure (*S*)-lactic acid with dicyclohexylcarbodiimide (DCC) in the presence of N-hydroxysuccinimide (HOSu).

(S,R)-Isomer of IX: IX_{SR}. The (*S,R*)-isomer of IX was obtained by the coupling of (*S*)-lactic acid and (*R*)-phenylglycine isobotyl ester in a reaction similar to the preparation of I_{SR}–III_{SR}. Yield, 88%. M.p. 59–60°C. $[\alpha]_D^{24}$ − 128.1 (*c* = 1.06, $CHCl_3$). Analysis: calculated for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.57; N, 5.01%; found: C, 64.23; H, 7.57; N, 5.03%.

(S,S)-Isomer of IX: IX_{SS}. The (*S,S*)-isomer of IX was obtained from a reaction similar to the preparation of IX_{SR}. Yield 92%. M.p. 100–102°C. $[\alpha]_D^{24}$ + 117.3 (*c* = 1.03, $CHCl_3$). Analysis: calculated for $C_{15}H_{21}NO_4$: C, 64.49; H, 5.75; N, 5.01%; found: C, 64.51; H, 7.68; N, 5.32%.

Mixtures of (S,S)-isomers and (R,S)-isomers of I–III, IX. Diastereomeric mixtures of (*S,S*)-isomers and (*R,S*)-isomers of I–III were prepared by the coupling of racemic lactic acid and optically pure (*S*)-amino acid esters with DCC–HOSu. The crude products were purified by silica gel column chromatography [eluent; benzene–ethyl acetate (1:1)]. Chromatographically pure oily products were obtained.

Mixtures of (R,R)-isomers and (S,R)-isomers of I–III, IX. Diastereomeric mixtures of (*R,R*)-isomers and (*S,R*)-isomers of I–III were prepared by the coupling of racemic lactic acid and optically pure (*R*)-amino acid esters with DCC–HOSu. The crude products were purified by silica gel column chromatography [eluent; benzene–ethyl acetate (1:1)]. Oily products were obtained.

Mixtures of (S,S)-isomers and (R,S)-isomers of IV–VI. Diastereomeric mixtures of (*S,S*)-isomers and (*R,S*)-isomers of IV–VI were obtained by the catalytic hydrogenations[9,10] of pyruvamides whose amine moieties had an (*S*)-configuration.

Mixtures of (R,R)-isomers and (S,R)-isomers of IV–VI. Diastereomeric mixtures of (*R,R*)-isomers and (*S,R*)-isomers of IV–VI were obtained by the catalytic hydrogenations[9,10] of pyruvamides whose amine moieties had an (*R*)-configuration.

(S,R)-, (R,S)-, (R,R)-, and (S,S)-isomers of VII–VIII. The (*S,R*)-, (*R,S*)-, (*R,R*)- and (*S,S*)-isomers of VII–VIII were prepared by the coupling of the corresponding optically pure (S)- or (*R*)-amines and (*S*)- and (*R*)-mandelic acid by the DCC–HOSu method. The crude products were purified by silica gel column chromatography [eluent; benzene–ethyl acetate (1:1)]. Each diastereomer gave a single peak on the gas chromatograms.

Derivatization

O-TFA derivatives of I–III and VII–IX. To an α-hydroxy acid amide (5–10 mg), trifluoroacetic anhydride (1 ml) and dichloromethane (2 ml) were added. The mixture was refluxed for 15 min and was then evaporated to dryness. The residue was redissolved in chloroform and the resulting solution was injected onto the chromatograph.

O-TFA derivatives[10] *of IV–VI.* To an α-hydroxy acid amide (5–10 mg), trifluoroacetic acid (3 ml) saturated with hydrogen chloride was added, and the resulting solution was refluxed for 15 min. The residue obtained by evaporation was redis-

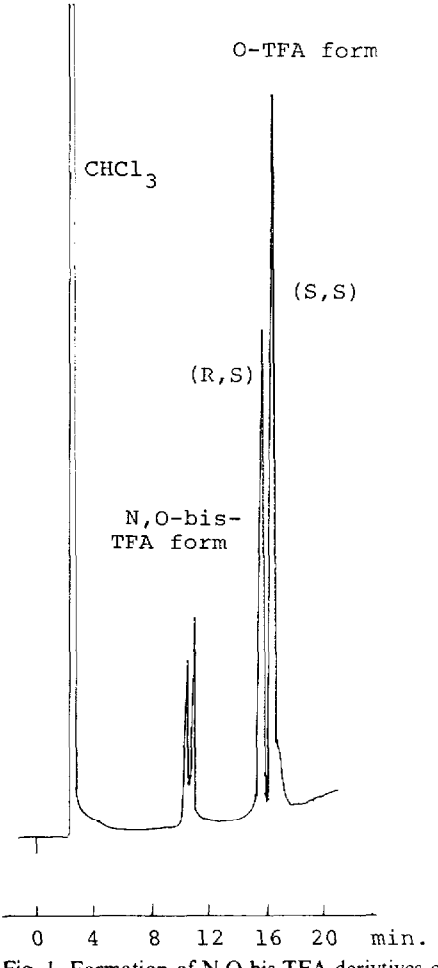

Fig. 1. Formation of N,O-bis-TFA derivtives of IV after reflux for 5 min.

solved in chloroform. The chloroform solution was injected onto the gas chromatograph. The solutions of trifluoroacetic anhydride and dichloromethane in which α-hydroxy acid amides IV–VI were dissolved were refluxed, producing some N,O-bis-TFA α-hydroxy acid amides as shown in Fig. 1 (N,O-bis-TFA form of IV, M^+ = 383). However, the reflux of the sample solution in which trifluoroacetic acid saturated with hydrogen chloride was involved, did not give N,O-bis-TFA α-hydroxy acid amide.

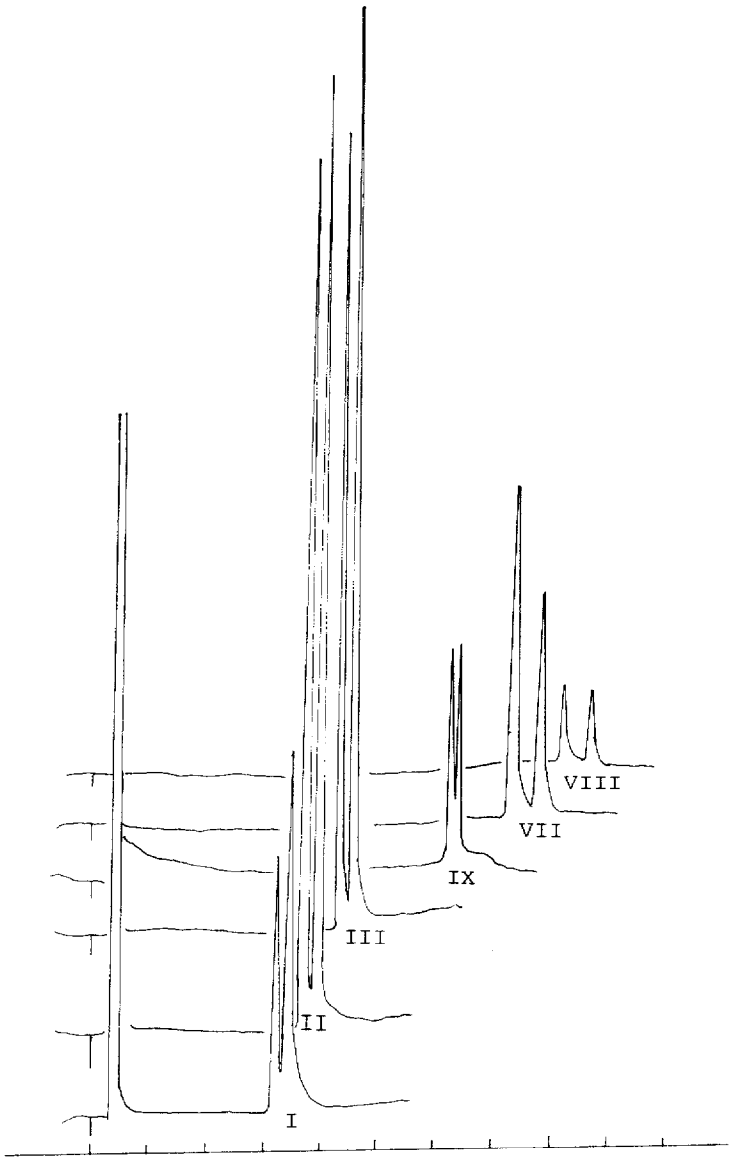

Fig. 2. Separation of (*R*,*S*)-isomers (first peak) and (*S*,*S*)-isomers (second peak) of I–III, VI–IX.

RESULTS AND DISCUSSION

The gas chromatograms of the mixtures of O-TFA derivatives of the (*S*,*S*)- and (*R*,*S*)-isomers are shown in Fig. 2. The gas chromatograms of the mixtures of O-TFA derivatives of the (*S*,*R*)- and (*R*,*R*)-isomers are shown in Fig. 3. Identifica-

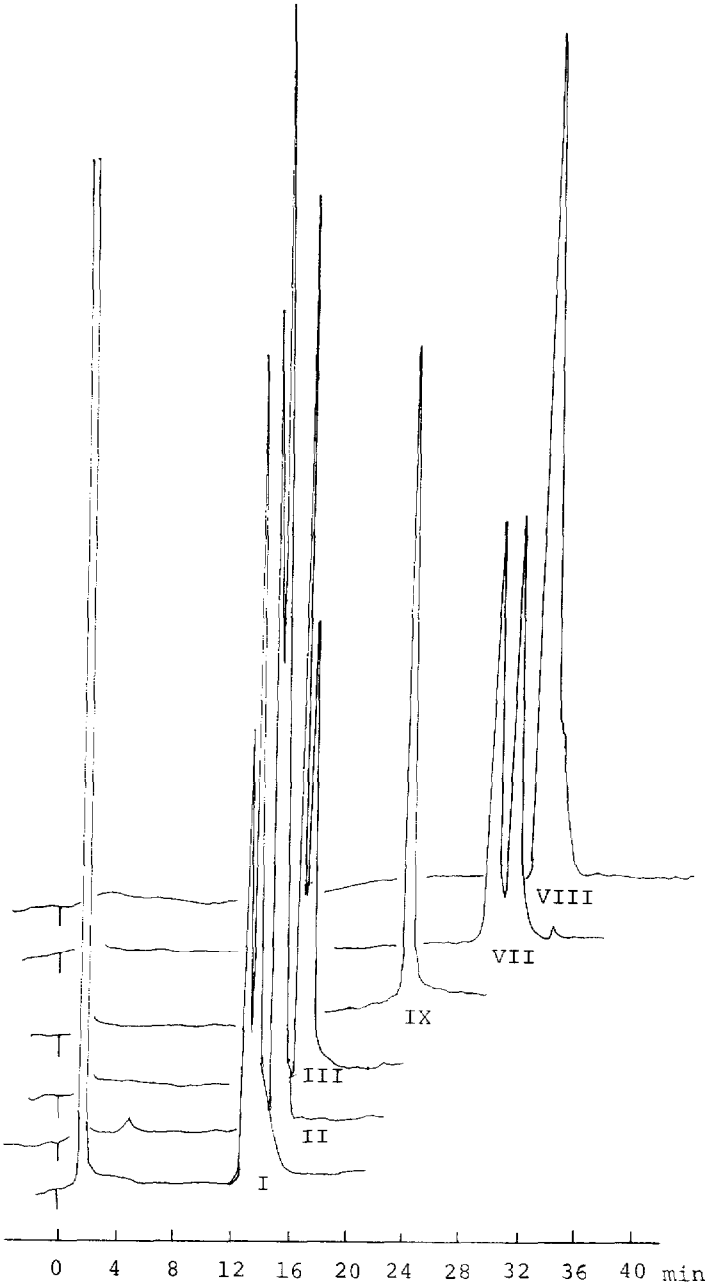

Fig. 3. Separation of (*S*,*R*)-isomers (first peak) and (*R*,*R*)-isomers (second peak) of I–III, VII–IX.

TABLE II

RETENTION TIMES AND SEPARATION FACTORS OF DIASTEREOMERS OF THE NINE α-HYDROXY ACID AMIDES

Conditions of gas chromatography: glass capillary column (25 m × 0.3 mm I.D.) coated with Chirasil-Val; crrier gas, nitrogen at the pressure of 0.5 kg/cm^2; oven temperature, programmed from 80 to 170°C at 4°C/min.

α-Hydroxy acid amides	*Retention times (min)*				*Separation factor*			
	*R*S***	*SR*	*RR*	*SS*	*SS/RS*	*RR/SR*	*SS/SR*	*RR/RS*
I	12.9	13.2	13.6	13.7	1.062	1.030	1.038	1.054
II	15.1	15.3	15.6	15.7	1.040	1.020	1.026	1.033
III	17.1	17.1	17.4	17.9	1.046	1.018	1.046	1.018
IV	13.1	13.2	13.2	13.8	1.053	1.000	1.045	1.008
V	14.8	15.0	15.3	15.7	1.061	1.020	1.047	1.034
VI	–	29.9	30.4	31.1	–	1.017	1.040	–
VII	29.6	30.1	31.6	31.4	1.061	1.050	1.043	1.068
VIII	32.9	34.5	34.5	34.8	1.058	1.000	1.009	1.049
IX	24.0	24.3	24.3	24.4	1.017	1.000	1.004	1.013

* Configuration of α-hydroxy moiety.
** Configuration of amine moiety.

tions of each of the diastereomeric peaks was carried out by comparison of their retention times with those of authentic diastereomers [(*S,S*)-isomer and (*S,R*)-isomer]. The retention times of all the diastereomers are listed in Table II along with the separation factors *SS/RS*, *RR/SR*, *SS/SR*, and *RR/RS*.

The separation factor *SS/RS* was larger than *RR/SR* under the conditions used in these analyses. This result could presumably be caused by the stronger affinity of the (*S*)-amide moiety in the α-hydroxy acid amide with the (*S*)-valine amide included in the chiral stationary phase. In the GC resolutions of N-TFA derivatives of racemic amino acid isopropyl esters on Chirasil-Val, N-TFA-(*R*)-amino acid isopropyl esters are eluted before N-TFA-(*S*)-amino acid isopropyl esters[4]. Therefore, the affinity of the (*S*)-amide moiety with the (*S*)-valine amide included in the chiral stationary phase [(*S*)–(*S*) interaction] would be stronger than that of the (*R*)-amide moiety with the (*S*)-valine amide moiety [(*R*)–(*S*) interaction]. In the GC resolutions of α-hydroxy acid amides, the distance between the α-hydroxyacyl moiety and the chiral stationary phase would become smaller for α-hydroxyacyl (*S*)-amine than for α-hydroxyacyl (*R*)-amine, because of the stronger (*S*)–(*S*) interaction. Therefore, the chilarity of the α-hydroxyacyl moiety would be recognized more clearly by the (*S*)–(*S*) interaction than by the (*R*)–(*S*) interaction.

However, there was no clear regularity between the separation factors *SS/SR* and *RR/RS*. The results seem to be due to the weak interaction between the O-TFA α-hydroxyacyl moiety and the chiral stationary phase.

The separation factor *SS/RS* of IX was the smallest of the nine α-hydroxy acid amides, and baseline separation was not obtained. With reference to the situation of the phenyl group, (*S*)-phenylglycine (amide moiety of IX) has the opposite configuration to those of (*S*)-α-methylbenzylamine, (*S*)-α-ethylbenzylamine, and (*S*)-α-(1-naphthyl)ethylamine which were the amide moieties of IV–VI. However, the sepa-

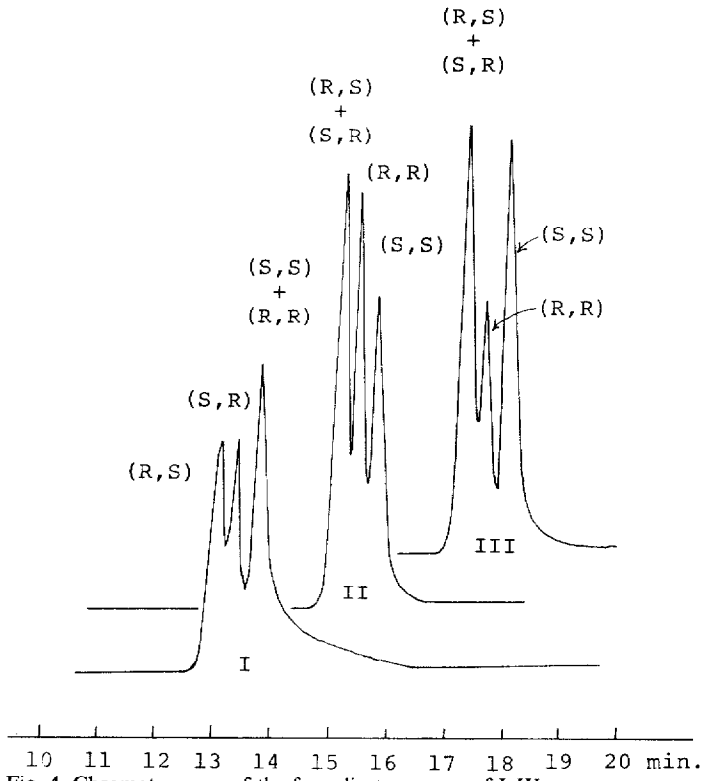

Fig. 4. Chromatograms of the four diastereomers of I–III.

ration factor *SS*/*RS* of IX was larger than *RR*/*SR*. This result may be interesting in connection with the relationship between the chiral recognition mechanism and the structure of α-hydroxy acid amides.

The elution order of the four diastereomers were $RS \geqslant SR \geqslant RR \geqslant SS$ in the analyses of I–VIII and IX. The elution order of the four diastereomers of VIII was $RS \geqslant SR \geqslant SS \geqslant RR$. The chromatograms of the four diastereomers of I–III are shown in Fig. 4.

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