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A Basic Study of the Amino Acid Residue in Protein. The Role of Hydrocarbon Groups in Enantiomer-differentiating Acylation

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The enantiomer-differentiating acylation (kinetic resolution) of 1-phenylalkylamines and their derivatives was carried out with (*S*)-2-phenylbutyric anhydride and its derivatives in aqueous and nonaqueous media. On the basis of the distributions of the two diastereomeric products, the molecular interactions between hydrocarbon residues responsible for the structural recognition of the reacting molecules were studied. In nonpolar media, the (*R*,*S*)-isomer was predominantly formed over the (*S*,*S*)-isomer. Moreover, the differentiation was mainly controlled by the size of the alkyl substituents of the substrates. In aqueous media, though, the (*S*,*S*)-isomer was predominantly formed. By a comparative study of the cyclohexyl analogs of the substrates and the reagents, it was shown that alkyl-phenyl and/or phenyl-phenyl interactions were important in the recognition process. A partitioning process of the substrate into the reagent phase was not effective in the differentiation of the enantiomer by the reaction in suspensions of optically active reagents in highly aqueous media.

Noncovalent molecular interactions as well as covalent interactions[1] play an important role in molecular recognition processes. However, only limited information is available for understanding the contributions of noncovalent interactions between small hydrocarbon residues of proteinous amino acids to a molecular recognition process.[2]

In previous reports[3,4] we have described the characterization of the function of small hydrocarbon residues in molecular recognition by analyzing the product distributions of the competitive acylation of two primary amines. Here, we wish to report the results of the enantiomer-differentiating acylation (kinetic resolution; Eq.1) of racemic primary amines with optically active acid anhydride in aqueous and nonaqueous media; we will also discuss the role of the hydrocarbon residues constituting a chiral center of substrates in the differentiation process. From the logarithmic molar ratio of the two diastereomeric products (ln r value: Eq.2), the differentiation efficiency of the reaction was evaluated. Since the nucleophilicities of amino groups in (*R*)-and (*S*)-substrates are identical with each other, the ln r value can be effected to reflect the interactions between hydrocarbon residues.

$$\left.\begin{matrix}(R)\text{-R-NH}_2\\(S)\text{-R-NH}_2\end{matrix}\right\} + (S)\text{-}(R^0\text{-CO})_2\text{O} \longrightarrow \begin{cases}(R,S)\text{-R-NHCO-R}^0\\(S,S)\text{-R-NHCO-R}^0,\end{cases} \quad (1)$$

$$\ln r = \ln\left([(S,S)\text{-R-NHCO-R}^0]/[(R,S)\text{-R-NHCO-R}^0]\right). \quad (2)$$

Results and Discussion

The Reaction System. The enantiomer-differentiating acylation of primary amine with acid anhydride (Eq. 1) is one of the simplest systems for the study of a differentiation process, for it readily proceeds without any side reactions. Acid anhydrides react quantitatively with amines not only under homogeneously-dissolved conditions in the solvent (Phase I), but also in a suspension of acid anhydride in an aqueous solvent (Phase II); however, the modes of differentiation in these solvents are completely different from each other. By the estimation of the solubility of acid anhydride under the present reaction conditions (see Experimental), it was shown that (*S*)-2-phenylbutyric anhydride was homogeneously dissolved in a water–dioxane mixture with $\chi_{H_2O}<0.77$ (Phase I). In a water–dioxane mixture with $\chi_{H_2O}>0.77$, a large fraction of the reagent was insoluble, resulting in a heterogeneous solution containing oily droplets of the reagent (Phase II).[3]

For the evaluation of the differentiation efficiency by means of the ln r value, the reaction should be carried out in the presence of so large an excess of the substrates to the reagent as to eliminate the effects of the concentration changes in the substrates during the reaction. To establish the appropriate reaction conditions, the ln r values were determined in water–dioxane ($\chi_{H_2O}=0.76$) or dioxane under a variety of relative molar ratios of the substrates to a reagent ([(*R*)-R-NH_2+(*S*)-R-NH_2]/[((*S*)-R^0-$CO)_2O$]). The ln r value became constant when the relative molar ratio of the substrates to a reagent was over four. The four molar equivalents of the substrates were treated with one molar equivalent of a reagent in the following experiments. Using this ratio of substrates to a reagent, it was also confirmed that the same ln r value was obtained in the presence and in the absence of triethylamine, an acid quencher. The ln r value was not affected by the (*S*)-2-phenylbutyric acid liberated during the reaction.

Effects of Reaction Media on the Enantiomer-differentiation of 1-Phenylalkylamines. Four 1-phenylalkylamines, *i.e.* 1-phenylethylamine, 1-phenylpropylamine, 1-phenylbutylamine, and 1-phenyl-2-methylpropylamine, were treated with (*S*)-2-phenylbutyric anhydride in dioxane, benzene, or a water–dioxane mixture ($\chi_{H_2O}=0.76$). The resulting ln r values are listed in Table 1, together with the ν_x values of the alkyl group of the substrates.[5] In dioxane the ln r values were almost zero in the reaction of 1-phenylethylamine, which meant that no appreciable recognition of the substrate structure took place. The reaction of the other three amines resulted in negative ln r values, which meant that the (*S*)-reagent favored the reaction with (*R*)-substrates. The ln r value became negative with the increase in the size of the alkyl group (ν_x): The differentia-

TABLE 1. THE ln r VALUES OBTAINED IN THE ENANTIOMER-DIFFERENTIATING ACYLATION OF 1-PHENYLALKYLAMINES WITH (*S*)-2-PHENYLBUTYRIC ANHYDRIDE IN SEVERAL SOLVENTS

Substrate (R'-CH(C_6H_5)-NH_2)	Size of R'	ln r values		
	ν_x[5] for R'	In dioxane	In benzene	In water-dioxane[a]
R'=CH_3-	0.52	0.027	0.009	0.332
R'=CH_3CH_2-	0.56	−0.066	−0.222	0.289
R'=$CH_3CH_2CH_2$-	0.68	−0.104	−0.286	0.229
R'=$(CH_3)_2CH$-	0.76	−0.356	−0.560	0.200

a) The mole fraction of water in the water-dioxane mixture (χ_{H_2O}) used in the experiments was 0.76.

tion efficiency was highest in the reaction of the substrate carrying the largest alkyl group. A similar effect of alkyl goups on the ln r value was also found in the reaction in benzene. These results suggest that enantiomer-differentiation in these solvents is mainly controlled by the size of the substituents. Since nonpolar media reduce polar interactions between reactants, the contribution of polar interactions to a differentiation process will be less in benzene than that in dioxane. Therefore, the low polarity of benzene explains why the effect of the size of the alkyl group on the ln r value was more pronounced in benzene than in dioxane.

In Phase I of a water-dioxane mixture (χ_{H_2O}=0.76), the ln r value was positive in every case. The ln r decreased in the order of the increase in the size of the alkyl group (R'-). However, the effect of the size on ln r was not so remarkable as that in benzene or dioxane. These results suggest that the effect of the size on the differentiation was overlaid with some intermolecular interaction between reagents to make the reagent select the other enantiomer in aqueous media. Thus, the modes of enantiomer-differentiation are quite different in the reactions in aqueous media and in nonaqueous media.

In order to obtain further information about the effects of the water in reaction media on enantiomer-differentiation, the enantiomer-differentiating acylation of racemic 1-phenylpropylamine with (*S*)-phenylbutyric anhydride was carried out in a water-dioxane mixture with various compositions of water. The ln r values are plotted against χ_{H_2O} in the plot (○) in Fig. 1. In Phase I, where the reaction proceeds in a homogeneous solution, ln r linearly increased with the increase in χ_{H_2O} in changing its sign from negative to positive and it reached its maximum at χ_{H_2O}=0.76. In Phase II, ln r sharply decreased with increase in χ_{H_2O}. Thus, no efficient enantiomer-differentiation is attainable by a phase-transfer process of substrates from the aqueous phase to the optically active organic phase. The reaction in Phase I will be discussed here, because we are chiefly concerned with differentiation and molecular interactions between a reagent and a substrate under homogeneous conditions.

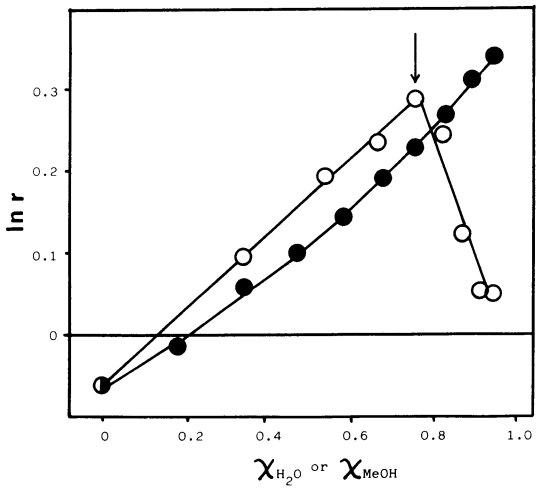

Fig. 1. The relationship between ln r value of the enantiomer-differentiating acylation with (*S*)-2-phenylbutyric anhydride and the mole fraction of water (χ_{H_2O}) or methanol (χ_{MeOH}). The ln r values were determined in the acylation of 1-phenylpropylamine in water-dioxane (○) or methanol-dioxane (●) mixed solvent.

As is shown by the (●) plot in Fig. 1, methanol had a similar effect on ln r to that of water in Phase I: ln r increased with the increase in χ_{MeOH} in changing its sign from negative to positive. Thus, the reaction in protic solvents, such as methanol and water, resulted in large positive ln r values.

The ln r values determined in various organic media are listed in Table 2, together with the solvent polarity parameters. The ln r value was very positive in polar media and almost zero or negative in nonpolar media. The dielectric constant or its related solvent parameters did not explain the increasing order of ln r values: the dielectric constants of DMF and acetonitrile are larger than that of methanol. However, it was in accordance with the increasing order of empirical solvent parameters, $E_T(30)$,[5] including the ln r values in water-dioxane (χ_{H_2O}=0.76, $E_T(30)$=52.4[7]) listed in Table 1. Thus, the remarkable changes in ln r must be a consequence of the elevation in polarity expressed by $E_T(30)$. These results suggest that the quite different molecular interactions from those caused by the bulkiness of hydrocarbon groups became important in the differentiaing process in highly polar reaction media, because bulkiness will not be affected so much by solvent polarity. In addition, water and methanol act as hydrogen-bond donors in the differentiation, since rather higher $E_T(30)$ values are assigned to hydrogen-bonded solvents than to those with high dielectric constants.[6]

Figure 2 shows the ln r *vs.* χ_{H_2O} plots in the enantiomer-differentiating acylation of 1-phenylethylamine in such aqueous media as water-dioxane, water-acetone, water-DMF and water-acetonitrile mixtures. In all cases, the ln r value increased linearly with an increase in χ_{H_2O} in Phase I, while it decreased in Phase II. The

TABLE 2. THE ln r VALUES IN ENANTIOMER-DIFFERENTIATING ACYLATION WITH (*S*)-2-PHENYLBUTYRIC ANHYDRIDE IN VARIOUS ORGANIC SOLVENTS

Solvent	Solvent Polarity[a]	ln r values	
	$E_T(30)$	Substrate structure	
	kcal mol^{-1} [c]	$CH_3CH(C_6H_5)$-NH_2	$CH_3CH_2CH(C_6H_5)$-NH_2
Methanol	55.5	0.37	0.38
Acetonitrile	46.0	0.29	0.24
DMF[b]	43.8	0.16	—
Acetone	42.2	0.15	—
Ethyl acetate	38.1	—	−0.07
Dioxane	36.0	0.03	−0.07
Benzene	34.5	0.01	−0.22

a) The empirical solvent polarity parameter $E_T(30)$-values are cited from "Solvent Effects in Organic Chemistry," by Christian Reichardt, Verlarg Chemie, New York (1979), pp. 270—272. b) *N*, *N*-Dimethylformamide is abbreviated as DMF. c) 1 cal=4.184 J.

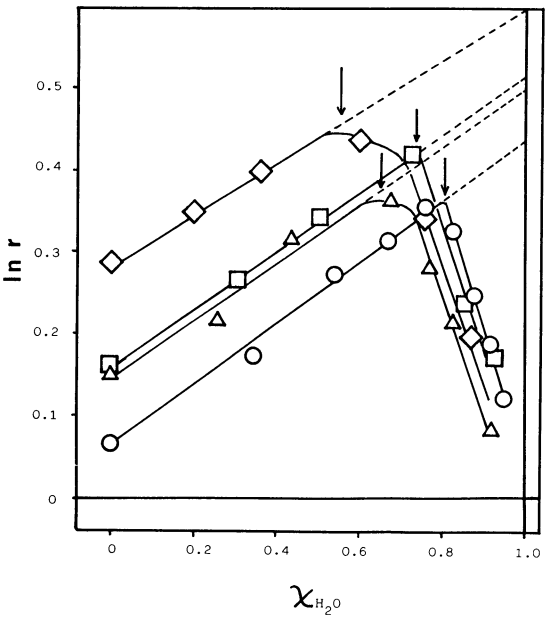

Fig. 2. The relationship between ln r value in enantiomer-differentiating acylation and the mole fraction of water (χ_{H_2O}) in several mixed solvents. (*S*)-2-Phenylbutyric anhydride and 1-phenylethylamine were employed as a reagent and a substrate respectively. The reaction was carried out in water–dioxane (○), water–acetone (△), water–DMF (□), or water–acetonitrile (◇) mixed solvent.

slopes in Phase I were similar with one another. In the water–acetonitrile mixture, the ln r value became as large as 0.44 at a maximum. The estimated ln r values in water obtained by extrapolation were not converged, as may be found in the figure. Without taking into account the solvophobic effect, the large ln r values in protic solvents cannot be explained.

The Role of Hydrocarbon Groups in Enantiomer-differentiating Acylation in Aqueous Media. As has been shown above (Table 1, Figs. 1 and 2), in the acylation of 1-phenylalkylamine derivatives with (*S*)-2-phenylbutyric anhydride the ln r value commonly exhibited a large change with an increase in the water content of the reaction media in Phase I. To ascertain the role of phenyl groups in the differentiation process, the reactions between reactants carrying no phenyl group were compared with those between reactants carrying a phenyl group.

Figure 3 shows the results of the enantiomer-differentiation of 1-phenylethylamine. In the reaction with (*S*)-2-phenylbutyric anhydride (plot (○)), no significant differentiation took place in dioxane (χ_{H_2O}=0), and the ln r value increased linearly with an increase in χ_{H_2O} in Phase I. In Phase II, the ln r value decreased to substantially zero in the very-high-water region. In the case of (*S*)-2-cyclohexylbutyric anhydride (plot (△)), the profile of the plot was the same as that of the (○) plot, though its gradient in Phase I was smaller than that of the (○) plot. In the case of (*S*)-2-ethylhexanoic anhydride (plot (□)), no appreciable differentiation took place in either Phase I or Phase II, even if a slight increase in the ln r value was detectable in Phase I.

Figure 4 shows the results of the enantiomer-differentiation of 1-cyclohexylethylamine and 1-methylbutyl-

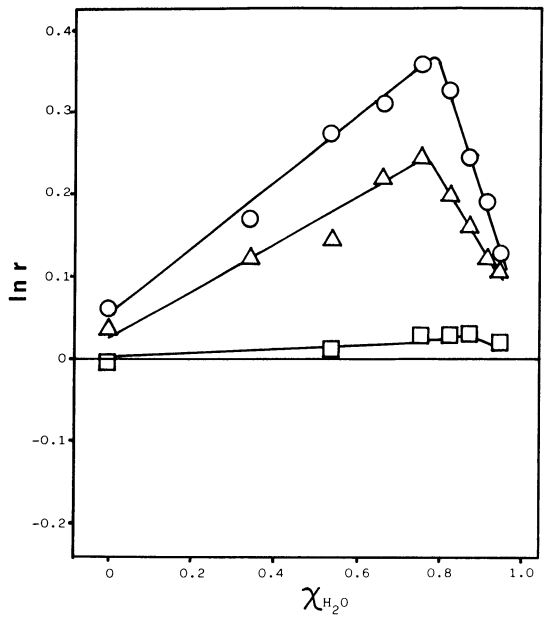

Fig. 3. The relationship between the ln r value in the acylation of racemic 1-phenylethylamine and χ_{H_2O} in water–dioxane mixed solvents. As a reagent, (*S*)-2-phenylbutyric anhydride (○), (*S*)-2-cyclohexylbutyric anhydride (△), and (*S*)-2-ethylhexanoic anhydride (□) were employed.

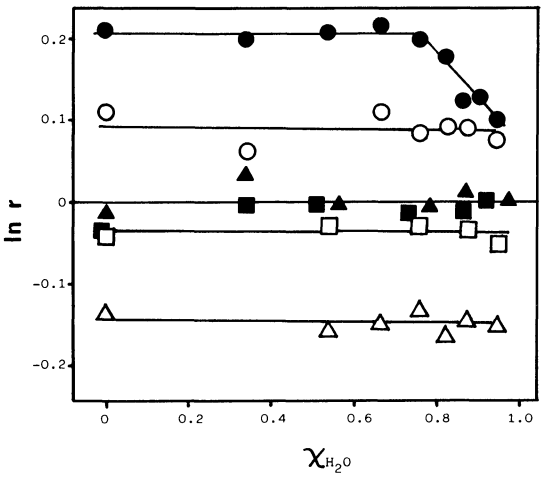

Fig. 4. The relationship between ln r value and χ_{H_2O} in water-dioxane mixed solvent. In the acylation of racemic 1-cyclohexylethylamine, (*S*)-2-phenylbutyric anhydride (●), (*S*)-2-cyclohexylbutyric anhydride (▲), and (*S*)-2-ethylhexanoic anhydride (■) were employes as a reagent. In the acylation of racemic 1-methylbutylamine, (*S*)-2-phenylbutyric anhydride (○), (*S*)-2-cyclohexylbutyric anhydride (△), and (*S*)-2-ethylhexanoic anhydride (□) were employed as a reagent.

amine. In no case, did any efficient differentiation take place, and the ln r value stayed constant in Phase I.

Thus, the large increase in the ln r value in Phase I was specific for the reaction where both reagent and substrate carry a phenyl group (Table 1, Figs. 1 and 3 plot (○)). In the reaction between a reagent and a substrate carrying only saturated hydrocarbon residues (plots (△), (□), (▲), and (■) in Fig. 4), the ln r value did not change at all in either Phase I or Phase II. These facts indicate that the existence of an alkyl-phenyl group in a reagent and the substrate is responsible for the interaction being evident in polar aqueous media. The plot (▲) in Fig. 4 suggests that the geometrical feature of a phenyl group are unimportant for the occurrence of this interaction.

The effect of a phenyl group in reactant molecules on enantiomer-differentiation in Phase I qualitatively parallels that found in the competitive acylation of two amines previously reported.[3] In that earlier report, we showed that the distinction of a phenyl group from alkyl groups easily took place due to the attractive alkyl-phenyl or phenyl-phenyl interaction, but that the distinction of alkyl groups with different chain lengths did not take place in polar aqueous media. The present results can be explained in terms of these attractive interactions. That is, enantiomer-differentiation results from a distinction between alkyl and phenyl groups in substrate molecules by means of alkyl and/or phenyl groups in a reagent molecule on the basis of alkyl-alkyl and alkyl-phenyl interactions and/or alkyl-phenyl and phenyl-phenyl interactions. On the other hand, in reaction between a reagent and a substrate carrying only saturated hydrocarbon groups, the enantiomer-differentiation does not take place, since neither alkyl-phenyl nor phenyl-phenyl interaction can be expected.

In the reaction where a phenyl group and a cyclohexyl group participated (the plot (△) in Fig. 3), the ln r value increased in Phase I, though the gradient of the plot was not large. This must be correlated with the fact that a phenyl group could slightly distinguish a branched alkyl group from an unbranched one.[8]

The results of the present study show that a phenyl group significantly influences the recognition of hydrocarbon groups by means of attractive alkyl-phenyl and/or phenyl-phenyl interactions[3] in aqueous media as well as by means of interaction based on its molecular size.

The attractive alkyl-phenyl and phenyl-phenyl interactions seem unlikely to be hydrophobic interactions between hydrocarbon groups, since no attractive interaction was found between alkyl groups (an alkyl-alkyl interaction). These attractive interactions might be a complex expression of noncovalent interactions induced in polar aqueous media. At this stage, weak solvophobic interaction and electron donor-acceptor interaction including CH···π[9] are probable candidates of the physicochemical entity of these attractive interactions.

In a biochemical field, a special aromatic character was postulated by Némethy[10] to characterize amino acid side chains. It was shown by the study of the structure-activity relationship that the aromatic character and the charge-transfer interactions were remarkably important in the expression of the biological activity of oligopeptides.[11] By the analysis of evolutionary changes in proteins, the relative mutabilities of Tyr or Phe can be said to be rather lower than those of Leu, Ile, and Val.[12] The probability of intermutation between Tyr and Phe was rather high, but probability of the mutation of Tyr or Phe to Leu, Ile, or Val was low.[12] These findings must be explained by the unique character of the phenyl groups that participate in the molecular recognition process through alkyl-phenyl or phenyl-phenyl interaction.

Experimental

Instruments. The ^{1}H-NMR and IR spectra were taken with a JEOL FX-100 spectrometer and a Shimadzu IR 27 G spectrometer respectively. The optical rotation was measured with a Perkin Elmer 241 polarimeter. The analytical GLC was carried out with a Shimadzu GC 6A gas chromatograph equipped with a Shimadzu Chromatopac C-R 1A apparatus, using a 3 m×5 mm o. d. glass column packed with 2% Silicone OV-17 on a Chromosorb W (OV-17) or Silicone OV-101 capillary column, 30 m×0.25 mm or 50 m×0.25 mm, at the stated temperature. The preparative GLC was carried out with a Shimadzu 3A instrument using a 3 m×6 mm o.d. stainless column packed with OV-17.

Materials. All the chemicals except those noted below were obtained from commercial sources and were used without further purification. The pure acetanilide used as an internal standard for the GLC analysis was obtained from Kishida Chemical, Inc., Osaka. Methyl stearate of a GLC analysis grade was obtained from Applied Science Laboratories, Inc., USA. (*S*)-2-Phenylbutyric acid was obtained by the preferential recrystallization of (*R*)-1-phenylethylamine salt from water; $[\alpha]_D^{20}$ +96.3° (*c* 10, benzene).[13] (*S*)-2-Phenylbutyric anhydride was prepared by the published method,[13] $[\alpha]_D^{20}$ +145° (*c* 5, benzene). (*S*)-2-Cyclohexylbutyric acid was prepared from optically pure (*S*)-2-phenylbutyric acid by hydrogenation with platinum oxide at a hydrogen pressure of 8.2 kg/cm^2 at 65°C, $[\alpha]_D^{20}$ −1.32° (*c* 10, MeOH).[14] (*S*)-2-Cyclohexylbutyric anhydride was

TABLE 3. THE RETENTION TIMES AND ANALYTICAL CONDITIONS IN GLC ANALYSES OF THE REACTION PRODUCTS

Compound(R_A-CONH-R_B)[a] R_A-	R_B-	Retention time/min	GLC condition[b]
(*S*)-$CH_3CH_2CH(C_6H_5)$-	(*S*)-$CH_3CH(C_6H_5)$-	16.90	A
	(*R*)-$CH_3CH(C_6H_5)$-	17.70	A
	(*S*)-$CH_3CH_2CH(C_6H_5)$-	18.23	A
	(*R*)-$CH_3CH_2CH(C_6H_5)$-	19.26	A
	(*S*)-$CH_3(CH_2)_2CH(C_6H_5)$-	20.15	A
	(*R*)-$CH_3(CH_2)_2CH(C_6H_5)$-	21.09	A
	(*S*)-$(CH_3)_2CH(C_6H_5)$-	18.60	A
	(*R*)-$(CH_3)_2CH(C_6H_5)$-	19.90	A
	(*S*)-$cC_6H_{11}CH(CH_3)$-	19.5	B
	(*R*)-$cC_6H_{11}CH(CH_3)$-	20.4	B
	(*S*)-$CH_3(CH_2)_2CH(CH_3)$-	17.1	C
	(*R*)-$CH_3(CH_2)_2CH(CH_3)$-	17.9	C
(*S*)-$CH_3CH_2CH(cC_6H_{11})$-	(*S*)-$CH_3CH(C_6H_5)$-	16.6	D
	(*R*)-$CH_3CH(C_6H_5)$-	17.5	D
	(*S*)-$cC_6H_{11}CH(CH_3)$-	19.3	B
	(*R*)-$cC_6H_{11}CH(CH_3)$-	20.5	B
	(*S*)-$CH_3(CH_2)_2CH(CH_3)$-	22.1	C
	(*R*)-$CH_3(CH_2)_2CH(CH_3)$-	22.8	C
(*S*)-$CH_3(CH_2)_3CH(C_2H_5)$-	(*S*)-$CH_3CH(C_6H_5)$-	24.9	E
	(*R*)-$CH_3CH(C_6H_5)$-	25.9	E
	(*S*)-$cC_6H_{11}CH(CH_3)$-	31.7	E
	(*R*)-$cC_6H_{11}CH(CH_3)$-	32.3	E
	(*S*)-$CH_3(CH_2)_2CH(CH_3)$-	23.5	F
	(*R*)-$CH_3(CH_2)_2CH(CH_3)$-	24.0	F

a) Cyclohexyl and phenyl groups are denoted by cC_6H_{11} and C_6H_5 respectively. b) GLC analytical conditions are indicated by A, B, C, *etc.* A: A 3-m Silicone OV-17 column was used. The column temperature was elevated from 150 °C to 250 °C by 10 °C/min. Methyl stearate was used as the internal standard. B: A Silicone OV-101 capillary column, 30 m×0.25 mm, was used. The column temperature was 240 °C. Acetanilide was used as the internal standard. C: A Silicone OV-101 capillary column, 30 m×0.25 mm, was used. The column temperature was 170 °C. Acetanilide was used as the internal standard. D: A Silicone OV-101 capillary column, 30 m×0.25 mm, was used. The column temperature was 220 °C. Acetanilide was used as the internal standard. E: A Silicone OV-101 capillary column, 50 m×0.25 mm, was used. The column temperature was 175 °C. Acetanilide was used as the internal standard. F: A Silicone OV-101 capillary column, 50 m×0.25 mm, was used. The column temperature was 170 °C. Acetanilide was used as the internal standard.

prepared by the published method.[13] (*S*)-2-Ethylhexanoic acid was obtained by the recrystallization of (*R*)-1-phenylethylamine salt from acetonitrile, $[\alpha]_D^{20}$ +8.20° (neat).[15] (*S*)-2-Ethylhexanoic anhydride was prepared by the published method.[13] 1-Phenylpropylamine, 1-phenylbutylamine, and 1-phenyl-2-methylpropylamine were prepared from the appropriate oximes, which had themselves been prepared from propiophenone, butyrophenone, and isobutyrophenone respectively by hydrogenation with a Raney nickel catalyst in acetic anhydride at a hydrogen pressure of 90 kg cm^{-2} at 60 °C and by succesive hydrolysis with 6 M HCl (1 M=1 mol dm^{-3}). The NMR and IR spectra were consistent with the desired structure; their boiling points were 98 °C/20 mmHg (1 mmHg≈133.322 pa) (lit, 99—100 °C/16 mmHg), 101—102 °C/10 mmHg (lit, 107—109 °C/16 mmHg), and 103—107 °C/21 mmHg (lit, 214 °C/760 mmHg) respectively. 1-Cyclohexylethylamine was prepared from *N*-acetyl-1-phenylethylamine by hydrogenation with platinum oxide in acetic acid at a hydrogen pressure of 8.2 kg cm^{-2} at 60 °C and by successive hydrolysis with 6 M HCl. (*R*)-1-Cyclohexylethylamine was prepared from commercially available (*R*)-1-phenylethylamine; $[\alpha]_D^{20}$ +2.88° (*c* 5, MeOH).[16] (*R*)-1-Methylbutylamine was obtained by the preferential recrystallization of (+)-camphor-10-sulfonic acid salt from water; $[\alpha]_D^{20}$ −7.95° (*c* 5, MeOH).[17] The NMR spectra are consistent with the desired structures. The authentic samples of various amides for GLC analysis were prepared from an appropriate acid chloride and amine by a conventional method; they were then purified by preparative GLC. The NMR, IR spectra, and results of elemental analysis of each sample were consistent with the desired structures.

Reaction Procedure of Enantiomer-differentiating Acylation. In a flask, a 100-μl portion of a racemic mixture of a chiral amine (2 M dioxane solution) was dissolved in 3.8 ml of a reaction medium. To the resulting amine solution, a 100-μl portion of an optically active acid anhydride solution (0.5 M dioxane solution) was added all at once under vigorous stirring at room temperature; the mixture was then allowed to stand for 1 h. After a 100-μl portion of the internal standard (0.25 M dioxane solution), which will be specified in the following section, had been added for GLC analysis, the volume of the reaction mixture was made up to 5 ml with dioxane. GLC was used for the quantitative analysis of the products of the sample solution.

Assignment of Diastereomeric Reaction Products in GLC Analysis. The reaction shown in Eq. 1 affords two diastereomeric amides, *i.e.* the (*S,S*)-isomer and the (*R,S*)-isomer. These reaction products can be detected as two distinct peaks on a gas chromatogram. The amount of the products were determined by quantitative GLC using an internal-standard method. The analytical conditions and the retention times of the reaction products are listed in Table 3.

As far as the substrates and reagents employed in this study were concerned, the GLC peak with a shorter reten-

tion time was assigned to the (*S,S*)-isomer, while the peak with a longer retention time was assigned to the (*R,S*)-isomer, in the following manner.

In the acylation of racemic 1-phenylethylamine with (*S*)-2-phenylbutyric anhydride, the reaction product with a shorter retention time was identified with the authentic *N*-[(*S*)-1-phenylethyl]-(*S*)-2-phenylbutyramide, which had been prepared from (*S*)-2-phenylbutyric anhydride and (*S*)-1-phenylethylamine by GLC. Therefore, the reaction product with a longer retention time was identified as the (*R,S*)-isomer.

In the acylation of racemic 1-phenylpropylamine with (*S*)-2-phenylbutyric anhydride, the product with a longer retention time was identified as the (*R,S*)-isomer by a kinetic resolution of the amine. When an excess of racemic 1-phenylpropylamine was treated with (*S*)-2-phenylbutyric anhydride in benzene, the reaction product with a longer retention time was produced in excess. Moreover, the unreacted amine recovered showed a levorotatory power. Since (*S*)-1-phenylpropylamine is levorotatory,[17] the reaction product with a longer retention time was identified as the (*R,S*)-isomer.

The configurations of 1-phenylbutylamine and 1-phenyl-2-methylpropylamine have not yet been determined. Therefore, it is not possible to assign the configuration of the reaction products on the GLC. However, the results of the kinetic resolutions of both amines were the same as in the case of 1-phenylpropylamine. That is, the isomeric product with a longer retention time was obtained in excess, while levorotatory unreacted amine was recovered in either case. Since the levorotatory amine has the *S* configuration in other homologues, *i.e.*, 1-phenylethylamine and 1-phenylpropylamine, the configuration of (−)-1-phenylbutylamine and (−)-1-phenyl-2-methylpropoylamine was assumed to be also *S*. Thus, the product amide isomer with a shorter retention time in the GLC was identified as the (*S, S*)-isomer, and the other, is the (*R, S*)-isomer.

In all other cases, authentic (*R, S*)-isomers were prepared from the corresponding amine and acid anhydride. The diastereomeric products with longer retention times were identified with the authentic (*R, S*)-isomer.

Solubility of Acid Anhydride in Aqueous Media. The fraction of the acylating reagent dissolved in the water-dioxane mixture was determined by quenching it with a large excess of butylamine as follows: A 100-μl portion of (*S*)-2-phenylbutyric anhydride (0.5 M dioxane solution) was added to the solvent (0.9 ml), and the mixture was stirred vigorously. The resulting mixture was centrifuged at 2000 min^{-1} for 20 min to separate the undissolved portion of the anhydride from the solvent phase. A 50-μl portion of butylamine (2 M dioxane solution) was added to the 100-μl portion of the supernatant obtained under a vigorous stirring, after which the mixture was allowed to stand for 1 h to complete the reaction. By the quantitative GLC analysis of *N*-butylamide, the solubility of 2-phenylbutyric anhydride in aqueous media was estimated.

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