A Basic Study of Functions of Amino Acid Residues in Protein; A Study of Molecular Interactions Responsible for Differentiation of Hydrocarbon Residues.

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Chapter 1. INTRODUCTION

The author engaged in the elucidation of mechanism of enantioface-differentiating hydrogenation of methyl acetoacetate with asymmetrically modified Raney nickel catalyst (MRNi) for a few years in the past.[1,2] By this study, it was clearly shown that a pair of hydrogen-bonds were formed between a modifying reagent (tartaric acid) and a substrate (methyl acetoacetate) on the catalyst surface. And the formation of these hydrogen-bonds played a decisive role in controlling adsorbtion mode of substrate to the catalyst surface to result in an excellent differentiation of enantioface of substrate.

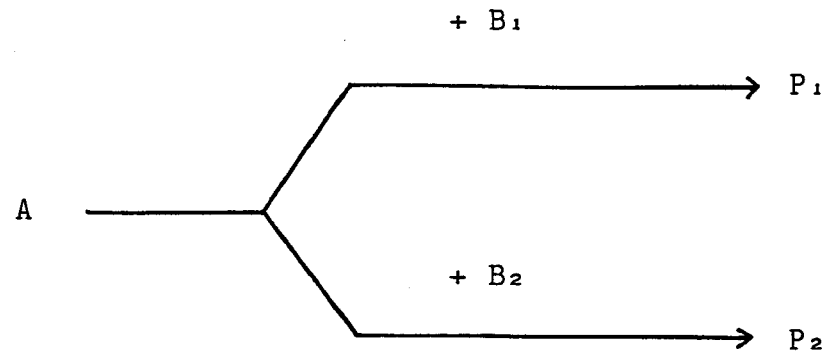
Occurrence of molecular recognition based on noncovalent interactions is a usual event in biological systems, e.g. in enzymatic reactions, in immuno reactions, in hormone actions, etc.[3] Importance of hydrogen-bonds in biological systems has already been well documented.[4]

Here the author intended to study the other important noncovalent interaction functioning in molecular recognition: the interaction between hydrocarbon groups, which must be essential in recognition of various organic compounds not only in biochemical reaction but also in organic reaction. However the interaction between hydrocarbon groups is expected to be so weak that it is very

difficult to monitor its function in a differentiation process under reaction conditions by means of a direct analysis of reaction rate or other physicochemical methods. Therefore only limited informations are available with respect to its contribution to a differentiation process.[5)]

In this study, the author attempted to acquire a suitable reaction system for monitoring interactions between hydrocarbon groups responsible for molecular recognition under reaction conditions.

One of the simplest differentiating reaction is a competitive parallel reaction as shown in Eq. 1. When a competitive reaction is carried out by employing a reagent (A) and two substrates (B_1 and B_2) carrying hydrocarbon groups, the product distribution ($r=P_1/P_2$) is controlled by the reactivities of functional groups in B_1 and B_2 and the interactions of hydrocarbon groups between A and B_1 and between A and B_2. If B_1 and B_2 have the same functional group with similar reactivity and different hyrocarbon groups with each other, r will reflect the result of differentiation of hydrocarbon groups in B_1 and B_2 by A on the basis of interactions between hydrocarbon groups. Therefore the product distribution (r) will be relevant parameter in evaluating interactions between hydrocarbon groups functioning in a differentiation process.



(Eq. 1)

Since noncovalent interactions are sensitively affected by the character of reaction media, the reaction which proceeds in a wide variety of reaction media will be adequate for the present study.

For investigation, the author has chosen the competitive acylation of primary amines with acid anhydride as shown in Eq. 2, since 1) the reaction readily proceeds in a quantitative manner in kinds of reaction media including an aqueous one, and gives kinetically controlled products; 2) the reaction products are stable and can readily be analyzed by quantitative gas chromatography; 3) the substrates and reagents carrying various hydrocarbon groups are available. A differentiation efficiency was evaluated in this study by a logarithmic molar ratio of two products: $\ln r = \ln ([R^1\text{-NHCO-}R^o]/[R^2\text{-NHCO-}R^o])$.

$$\left.\begin{matrix} R^1\text{-}NH_2 \\ \\ R^2\text{-}NH_2 \end{matrix}\right\} + (R^\circ\text{-}CO)_2O \longrightarrow \left\{\begin{matrix} R^1\text{-}NHCO\text{-}R^\circ \\ \\ R^2\text{-}NHCO\text{-}R^\circ \end{matrix}\right. \qquad \text{(Eq. 2)}$$

, where R°, R^1, and R^2 are hydrocarbon residues.

In biological systems, chiral recognition is also important as well as the direct differentiation of hydocarbon groups. Chiral recognition can be also investigated by the use of enantiomer-differentiating acylation (kinetic resolution) of racemic amines with optically active acid anhydride (Eq. 3). In this case, a differentiation efficiency is evaluated by a logarithmic molar ratio of two diastereomeric products: ln r' = ln ([(S,S)-R-NHCO-R°]/[(R,S)-R-NHCO-R°]).

$$\left.\begin{matrix} (S)\text{-}R\text{-}NH_2 \\ \\ (R)\text{-}R\text{-}NH_2 \end{matrix}\right\} + ((S)\text{-}R^\circ\text{-}CO)_2O \longrightarrow \left\{\begin{matrix} (S,S)\text{-}R\text{-}NHCO\text{-}R^\circ \\ \\ (R,S)\text{-}R\text{-}NHCO\text{-}R^\circ \end{matrix}\right. \qquad \text{(Eq. 3)}$$

, where R and R° are hydrocarbon residues.

Chapter 2. MATERIALS AND METHODS

2-1. 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-R1A using a 3 m- 5 mm or 1 m-5 mm o.d. glass column packed with 2% Silicone OV-17 on Chromosorb W (OV-17) at the stated temperature. For the analyses of reaction products in the enantiomer-differentiating acylation, a Silicone OV-101 capillary column, 30 m- 0.25 mm or 50 m- 0.25 mm, was employed. The preparative GLC was carried out with a Shimadzu 3 A instrument using a 3 m- 6 mm o.d. stainless column packed with OV-17.

2-2. Materials.

All chemicals except those noticed below were obtained from the commercial sources, and used without a further purification. Purity proofed acetanilide used as an internal standard for GLC analysis was obtained from Kishida Chemical Inc., Osaka. Methyl palmitate and methyl stearate of GLC analysis grade were obtained from Applied

Science Laboratories Inc., USA. Phenylacetic anhydride was prepared from the corresponding acid chloride and sodium salt of the acid by the conventional method. Needle crystals were obtained by the recrystallization from ether-hexane (mp. 71°C, lit. mp. 72°C). 2-Phenylbutyric anhydride was prepared from the corresponding acid by refluxing with the large excess of acetic anhydride followed by removal of acetic acid released by distillation. (S)-2-Phenylbutyric acid was obtained by the preferential recrystallization of (R)-1-phenylethylamine salt from water, $[\alpha]_D^{20}$ + 96.3° (c10, benzene).[6] (S)-2-Phenylbutyric anhydride was prepared by the published method,[6] $[\alpha]_D^{20}$ + 145° (c5, benzene). (S)-2-Cyclohexylbutyric acid was prepared from optically pure (S)-2-phenylbutyric acid by hydrogenation with platinum oxide at 8.2 kg/cm^2 hydrogen pressure at 65°C, $[\alpha]_D^{20}$ - 1.32° (c10, MeOH).[7] (S)-2-Cyclohexylbutyric anhydride was prepared by the published method.[7] (S)-2-Ethylhexanoic acid was obtained by the recrystallization of (R)-1-phenylethylamine salt from acetonitrile, $[\alpha]_D^{20}$ + 8.20° (neat).[8] (S)-2-Ethylhexanoic anhydride was prepared by the published method.[6] N-Acetyl-L-isoleucine N-hydroxysuccinimide ester was prepared from N-acetyl-L-isoleucine and N-hydroxysuccinimide by the treatment of dicyclohexylcarbodiimide with a conventional manner, and was purified by recrystallization from 2-propanol. 1-

Phenylpropylamine, 1-phenylbutylamine, and 1-phenyl-2-methylpropylamine were prepared from appropriate oximes, which were prepared from propiophenone, butyrophenone, and isobutyrophenone respectively, by hydrogenation with Raney nickel catalyst in acetic anhydride at 90 kg/cm^2 hydrogen pressure at 60°C and succesive hydrolysis with 6 N HCl. The NMR and IR spectra were consistent with the desired structure, and their boiling points were 98°C/20 mm Hg (lit. 99-100°C/16 mm Hg), 101-102°C/10 mm Hg (lit. 107-109°C/16 mm Hg), and 103-107°C/21 mm Hg (lit. 214°C/760 mm Hg) respectively. 1-Cyclohexylethylamine were prepared from N-acetyl-1-phenylethylamine by hydrogenation with platinum oxide in acetic acid at 8.2 kg/cm^2 hydrogen pressure at 60°C and succesive hydrolysis with 6 N HCl. (R)-1-Cyclohexylethylamine was prepared from commercially available (R)-1-phenylethylamine, $[\alpha]_D^{20}$ + 2.88° (c5, MeOH).[9] (R)-1-Methylbutylamine was obtained by the preferential recrystallization of (+)-camphor-10-sulfonic acid salt from water, $[\alpha]_D^{20}$ - 7.95° (c5, MeOH).[10] The NMR spectra are consistent with the desired structures. The authentic samples of various amides for quantitative GLC analysis of the competitive reaction were prepared from an appropriate acid chloride and amine by a conventional method, and they were purified by the preparative GLC. The NMR spectra, IR spectra, and elemental analysis of each

samples were consistent with the desired structure.

2-3. Reaction Procedure of Competitive Acylation of a Pair of Primary Amines.

When the concentration of an acylating reagent was 50 mM, the competitive acylation was carried out by the following procedure. In a flask, 50 μl portion of each amine (2 M dioxane solution) were dissolved in 0.8 ml of the solvent. A 100 μl portion of acid anhydride (0.5 M dioxane solution) was then added. all at once, with vigorous stirring at room temperature, after which the mixture was allowed to stand for 1 h. After a 100 μl portion of a stock solution of an internal standard (0.25 M dioxane solution) listed in Table 1, had been added, the volume of the reaction mixture was made up to 5 ml with dioxane. The amount of reaction products were determined by GLC with following an internal standard method. When the concentration of the acylating reagent was 6.25 mM, 25 μl of each amine solution, 50 μl of acid anhydride solution, 3.9 ml of solvent, and 50 μl of the internal standard solution were employed instead.

2-4. Reaction Procedure of Enantiomer-Differentiating Acylation.

In a flask, 100 μl portion of 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, 100 μl portion of optically active acid anhydride solution (0.5 M dioxane solution) was added all at once with a vigorous stirring at room temperature, after which the mixture was allowed to stand for 1 h. After a 100 μl portion of a stock solution of an internal standard (0.25 M dioxane solution), which will be described in detail 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 applied for the quantitative analysis of reaction products.

The analytical conditions of GLC and retention times of reaction products were summerized in Table 1.

(Table 1.)

2-5. Assignment of Diastereomeric Reaction Products in GLC Analysis.

The reaction shown in Eq. 4 affords two diastereomeric amides, i.e. (S,S)-isomer and (R,S)-isomer. These reaction products can be detected as two distinct peaks on gas chromatogram (Table 1). As far as the substrates and reagents employed in this study were concerned, the GLC peak with shorter retention time was assigned to (S,S)-isomer and the peak with longer retention time was assigned to (R,S)-isomer as a following manner.

Table 1. The retention time and analytical conditions in GLC of reaction products.

Compounds(R_A-CONH-R_B)[a]		Retention	GLC
R_A-	R_B-	time(min)	conditions[b]
$(CH_3)_2CHCH_2$-	CH_3CH_2-	5.68	A
	$CH_3(CH_2)_2$-	6.87	A
	$CH_3(CH_2)_3$-	8.18	A
	$CH_3(CH_2)_4$-	9.51	A
	$CH_3(CH_2)_5$-	10.82	A
	$(CH_3)_2CH$-	6.23	A
	$(CH_3)_2CHCH_2$-	7.60	A
	$(CH_3)_2CH(CH_2)_2$-	9.14	A
	$CH_3CH_2CH(CH_3)$-	7.12	A
	$C_6H_5CH_2$-	14.36	A
	$C_6H_5(CH_2)_2$-	15.28	A
	$C_6H_5(CH_2)_3$-	17.0	A
	$CH_3OCH_2CH_2$-	8.25	A
	$cC_6H_{11}CH_2$-	13.71	A
	$HOCH_2CH_2$-	13.0	B
$(CH_3)_2CH$-	$CH_3(CH_2)_2$-	5.39	A
	$CH_3(CH_2)_3$-	7.00	A
	$CH_3(CH_2)_4$-	9.43	A
$CH_3(CH_2)_2$-	$CH_3(CH_2)_3$-	7.96	A
	$C_6H_5(CH_2)_2$-	14.70	A
$(CH_3)_2CH(CH_2)_2$-	$CH_3(CH_2)_2$-	8.60	C
	$CH_3(CH_2)_3$-	9.90	C
	$CH_3(CH_2)_5$-	12.82	C
$C_6H_5CH_2$-	$CH_3(CH_2)_3$-	9.53	D
	$C_6H_5(CH_2)_2$-	20.49	D

Table 1. (continued)

Compounds(R_A-CONH-R_B)[a] R_A-	R_B-	Retention time(min)	GLC conditions[b]
$CH_3CH_2CH(C_6H_5)$-	$CH_3(CH_2)_3$-	10.1	D
	$C_6H_5(CH_2)_2$-	23.3	D
	$cC_6H_{11}CH_2$-	20.28	D
$CH_3CH_2CH(CH_3)CH(CH_3CONH)$-	$CH_3(CH_2)_2$-	13.5	A
	$CH_3(CH_2)_3$-	14.5	A
	$CH_3(CH_2)_4$-	15.6	A
	$CH_3(CH_2)_5$-	17.0	A
(S)-$CH_3CH_2CH(C_6H_5)$-	(S)-$CH_3CH(C_6H_5)$-	16.90	D
	(R)-$CH_3CH(C_6H_5)$-	17.70	D
	(S)-$CH_3CH_2CH(C_6H_5)$-	18.23	D
	(R)-$CH_3CH_2CH(C_6H_5)$-	19.26	D
	(S)-$CH_3(CH_2)_2CH(C_6H_5)$-	20.15	D
	(R)-$CH_3(CH_2)_2CH(C_6H_5)$-	21.09	D
	(S)-$(CH_3)_2CH(C_6H_5)$-	18.60	D
	(R)-$(CH_3)_2CH(C_6H_5)$-	19.90	D
	(S)-$cC_6H_{11}CH(CH_3)$-	19.5	E
	(R)-$cC_6H_{11}CH(CH_3)$-	20.4	E
	(S)-$CH_3(CH_2)_2CH(CH_3)$-	17.1	F
	(R)-$CH_3(CH_2)_2CH(CH_3)$-	17.9	F
(S)-$CH_3CH_2CH(cC_6H_{11})$-	(S)-$CH_3CH(C_6H_5)$-	16.6	G
	(R)-$CH_3CH(C_6H_5)$-	17.5	G
	(S)-$cC_6H_{11}CH(CH_3)$-	19.3	E
	(R)-$cC_6H_{11}CH(CH_3)$-	20.5	E
	(S)-$CH_3(CH_2)_2CH(CH_3)$-	22.1	F
	(R)-$CH_3(CH_2)_2CH(CH_3)$-	22.8	F
(S)-$CH_3(CH_2)_3CH(C_2H_5)$-	(S)-$CH_3CH(C_6H_5)$-	24.9	H
	(R)-$CH_3CH(C_6H_5)$-	25.9	H
	(S)-$cC_6H_{11}CH(CH_3)$-	31.7	H
	(R)-$cC_6H_{11}CH(CH_3)$-	32.3	H

Table 1. (continued)

Compounds(R_A-CONH-R_B)[a] R_A-	R_B-	Retention time(min)	GLC conditions[b]
(S)-$CH_3(CH_2)_3CH(C_2H_5)$-	(S)-$CH_3(CH_2)_2CH(CH_3)$-	23.5	I
	(R)-$CH_3(CH_2)_2CH(CH_3)$-	24.0	I

a) Cyclohexyl, phenyl and acetylamino groups are denoted by cC_6H_{11}, C_6H_5 and CH_3CONH, respectively.

b) GLC analytical conditions are indicated by A, B, C, etc.

A: Silicone OV-17 3 m column was used. Column temperature was elevated from 100°C to 250°C by 10°C/min. Acetanilide was used as an internal standard.

B:Silicone OV-17 1 m column was used. Column temperature was elevated from 90°C to 250°C by 4°C/min. Acetanilide was used as an internal standard.

C: Silicone OV-17 3 m column was used. Column temperature was elevated from 100°C to 260°C by 10°C/min. Methyl palmitate was used as an internal standard.

D: Silicone OV-17 3 m column was used. Column temperature was elevated from 150°C to 250°C by 10°C/min. Methyl stearate was used as an internal standard.

E: Silicone OV-101 capillary column, 30 m - 0.25 mm, was used. Column temperature was 240°C. Acetanilide was used as an internal standard.

Table 1. (continued)

F: Silicone OV-101 capillary column, 30 m - 0.25 mm, was used. Column temperature was 170°C. Acetanilide was used as an internal standard.

G: Silicone OV-101 capillary column, 30 - 0.25 mm, was used. Column temperature was 220°C. Acetanilide was used as an internal standard.

H: Silicone OV-101 capillary column, 50 - 0.25 mm, was used. Column temperature was 175°C. Acetanilide was used as an internal standard.

I: Silicone OV-101 capillary column, 50 m - 0.25 mm, was used. Column temperature was 170°C. Acetanilide was used as an internal standard.

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

In the acylation of racemic 1-phenylpropylamine with (S)-2-phenylbutyric anhydride, the product with longer retention time was identified with (R,S)-isomer by a kinetic resolution of amine: When excess of racemic 1-phenylpropylamine was treated with (S)-2-phenylbutyric anhydride in benzene, the reaction product with longer retention time was produced in excess. And unreacted amine recovered showed a levorotatory power. Since (S)-1-phenylpropylamine is known to be levorotatory,[10] the reaction product with longer retention time was identified with (R,S)-isomer.

The configuration of 1-phenylbutylamine and 1-phenyl-2-methylpropylamine have not been determined yet. Therefore it is not possible to assign the configuration of reaction products on GLC. However the results of kinetic resolution of both amine were similar to the resuslt of 1-phenylpropylamine. That is, an isomeric product with longer

retention time was obtained in excess, while levorotatory unreacted amine was recovered in either case. Since the levorotatory amine has S configuration in other homologs, i.e. 1-phenylethylamine and 1-phenylpropylamine, the configuration of (-)-1-phenylbutylamine and (-)-1-phenyl-2-methylpropylamine was assumed to be S. Thus the isomeric product with shorter retention time in GLC was expected to be (S,S)-isomer and the other was (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 time were identified with the authentic (R,S)-isomer.

2-6. Solubility of Acid Anhydride in Aqueous Media.

The dissolved fraction of acylating reagent into water-dioxane mixture was determined by quenching it with a large excess of butylamine as following: A 100 μl portion of acylating reagent (0.5 M dioxane solution) was added to the solvent (0.9 ml or 4.9 ml) and mixed vigorously. The undissolved portion of the anhydride in the resulting mixture was separated from the solvent phase by centrifugation at 2000 rpm for 20 min. A 50 μl portion of butylamine (2 M dioxane solution) was added to a 100 μl portion of aqueous layer with 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 acid anhydride in aqueous media was estimated.

Chapter 3 RESULTS

3-1. The Competitive Reaction Conditions.

Acid anhydrides react quantitatively with amines not only under a homogeneously dissolved condition in the solvent (a reaction in Phase I) but also in a suspension of acid anhydride in aqueous solvent (a reaction in Phase II), however the modes of differentiation in these solvents are completely different from each other.

In this regard, it is necessary to determine the solubility of acid anhydride under reaction conditions beforehand. The fraction (%) of dissolved reagent is plotted against the mole fraction of water (χ_{H_2O}) in water-dioxane mixture, as shown in Fig. 1(a) and (b). To take an example, isovaleric anhydride was homogeneously dissolved in water-dioxane with $\chi_{H_2O} < 0.83$. In water-dioxane mixture with $\chi_{H_2O} > 0.83$, a large fraction of reagent was insoluble to result in a heterogeneous solution containing oily droplets of reagent. In the case of 2-phenylbutyric anhydride (Fig. 1(b)), it was completely soluble in water-dioxane with $\chi_{H_2O} < 0.77$, but not in water-dioxane with $\chi_{H_2O} > 0.77$.

(Fig. 1.)

For the evaluation of differentiation efficiency by ln r value or ln r' value, the reaction should be carried

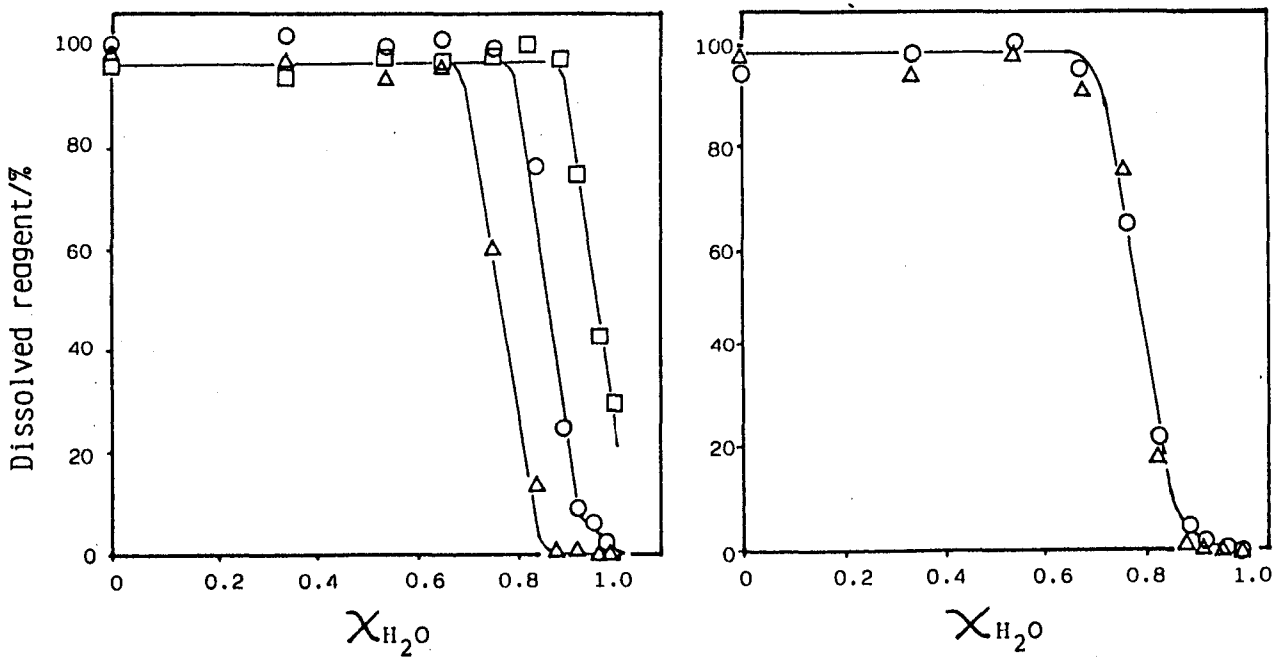


Fig. 1. The dependence of the amount of dissolved reagent (% of applied reagent) on the mole fraction of water (χ_{H_2O}) in water-dioxane mixture. In (a), 4-methylpentanoic anhydride (△), isovaleric anhydride (○), and isobutyric anhydride (□) were employed. Their concentration was 50 mM if all dissolved. In (b), 2-phenylbutyric anhydride (○) and phenylacetic anhydride (△) were employed. Their concentration was 12.5 mM if all dissolved.

out in the presence of so large excess of substrates to reagent as to eliminate the effects of the concentration changes of substrates during reaction. To establish the appropriate reaction conditions, the ln r values and the ln r' values were determined under a variety of molar ratios of substrates relative to reagent ($[R^1\text{-}NH_2+R^2\text{-}NH_2]/[(R^o\text{-}CO)_2O]$ and $[(R)\text{-}R\text{-}NH_2+(S)\text{-}R\text{-}NH_2]/[((S)\text{-}R^o\text{-}CO)_2O]$), respectively as shown in Fig. 2 and Fig. 3. In either case, the values became constant, when the relative molar ratio of substrates to reagent was over four. The four molar equivalents of substrates were treated with one molar equivalent of a reagent in the followling experiments. Under this ratio of substrates to a reagent, it was also confirmed that the same ln r value was obtained in the presence and absence of triethylamine, an acid quencher. It was also the case in the ln r' value. Thus the ln r and the ln r' values were not affected by carboxylic acid liberated during reaction. (Fig. 2 and Fig. 3.)

3-2. <u>The Competition of Alkylamines with Different Chain Lengths.</u>

The competitive acylation (Eq. 2) was carried out in order to monitor the molecular interactions between hydrocarbon residues in substrates and a reagent responsible for a differentiation. A pair of alkylamine were acylated

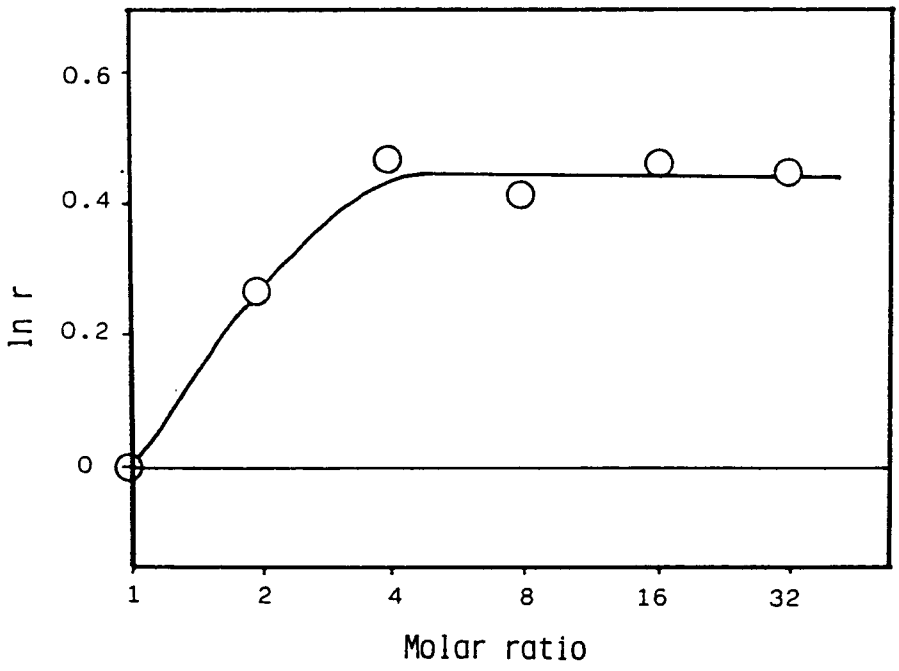


Fig. 2. The dependence of the ln r value on the molar ratio of substrates to a reagent ($[R^1\text{-}NH_2+R^2\text{-}NH_2]/[(R^0\text{-}CO)_2O]$). Isovaleric anhydride, hexylamine, and propylamine were employed as $(R^0\text{-}CO)_2O$, $R^1\text{-}NH_2$, and $R^2\text{-}NH_2$, respectively. The reaction was carried out in water-dioxane mixture of $\chi_{H_2O}=0.88$. The concentration of isovaleric anhydride was 6.25 mM.

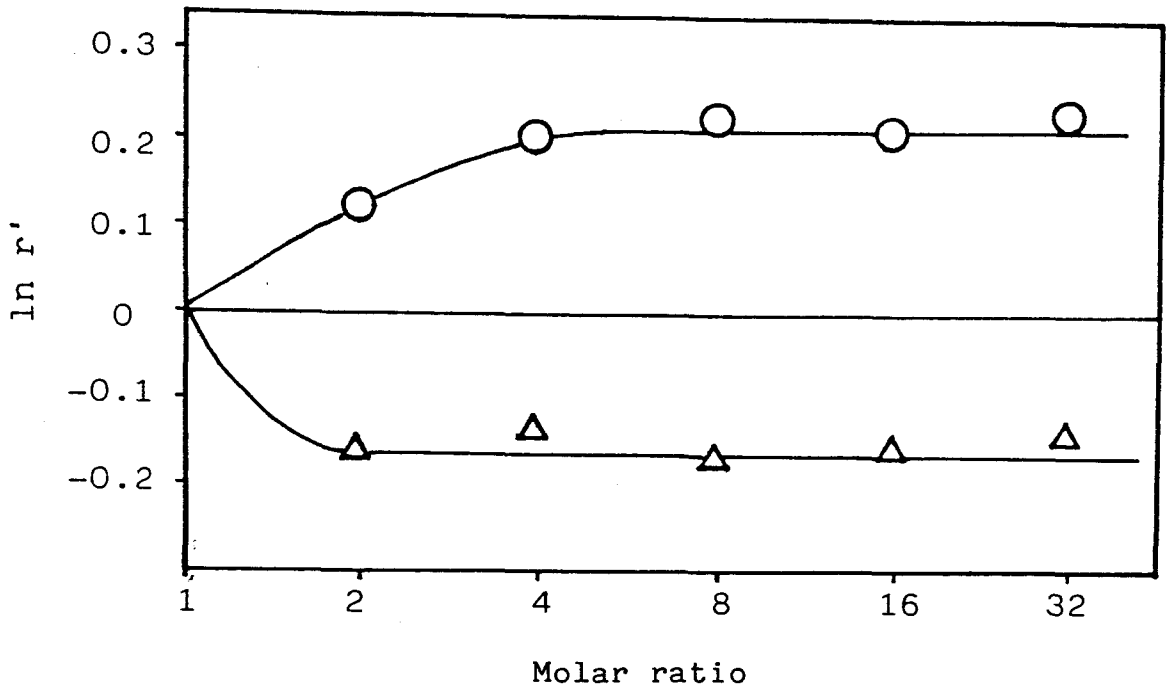


Fig. 3. The dependence of the ln r' value on the molar ratio of substrates to a reagent ([(R)-R-NH_2+(S)-R-NH_2]/[((S)-R^o-CO)$_2$O]). (S)-2-Phenylbutyric anhydride and racemic 1-phenylpropylamine were used as ((S)-R^o-CO)$_2$O and R-NH_2, respectively. The reaction was carried out in water-dioxane mixture (χ_{H_2O}=0.76) (○) or dioxane (△).

with isovaleric anhydride (R°=-$CH_2CH(CH_3)_2$) in benzene, dioxane, or water-dioxane mixture (χ_{H_2O}=0.88). The results are shown in Fig. 4(a) and (b).

(Figs. 4(a) and 4(b))

In dioxane or benzene, the ln r values were found to be substantially zero in every competition between unbranched alkylamines (Fig. 4(a)), and in the competition of a β- of γ-branched amine with an unbranched one (Fig. 4(b)). In the competition of an α-branched alkylamine, such as isopropylamine and s-butylamine, with the unbranched amines, large negative ln r values were obtained in every solvent: the formation of amides from α-branched amines was significantly suppressed, as is shown in Fig. 4(b).

In aqueous media (a water-dioxane mixture), the positive ln r values were obtained except in the competition of an α-branched amine with an unbranched one (Fig. 4(a) and (b)). The ln r values obtained in the competitions of unbranched amines in water-dioxane mixtures are shown in Table 2. Thus the reagent distinguished the longer alkylamine from the shorter one and preferentially gave the amide with the longer alkylamine.

(Table 2)

In order to ascertain the mode of the differentiation in aqueous media, the competition of hexylamine with propylamine was carried out in water-dioxane

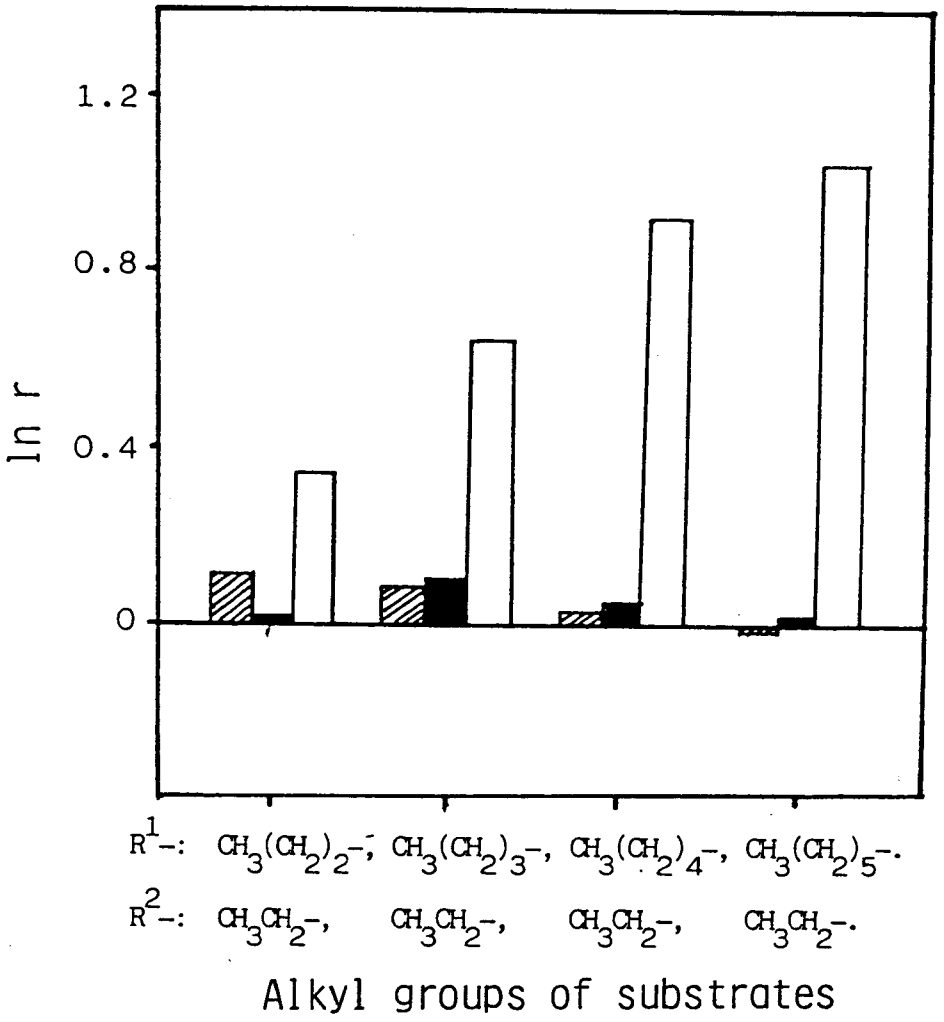


Fig. 4(a). The competitive acylation of a pair of alkylamine with isovaleric anhydride.

The reaction was carried out in benzene (▨), dioxane (■), or water-dioxane mixture at χ_{H_2O}=0.88 (□). The concentration of a reagent was 50 mM.

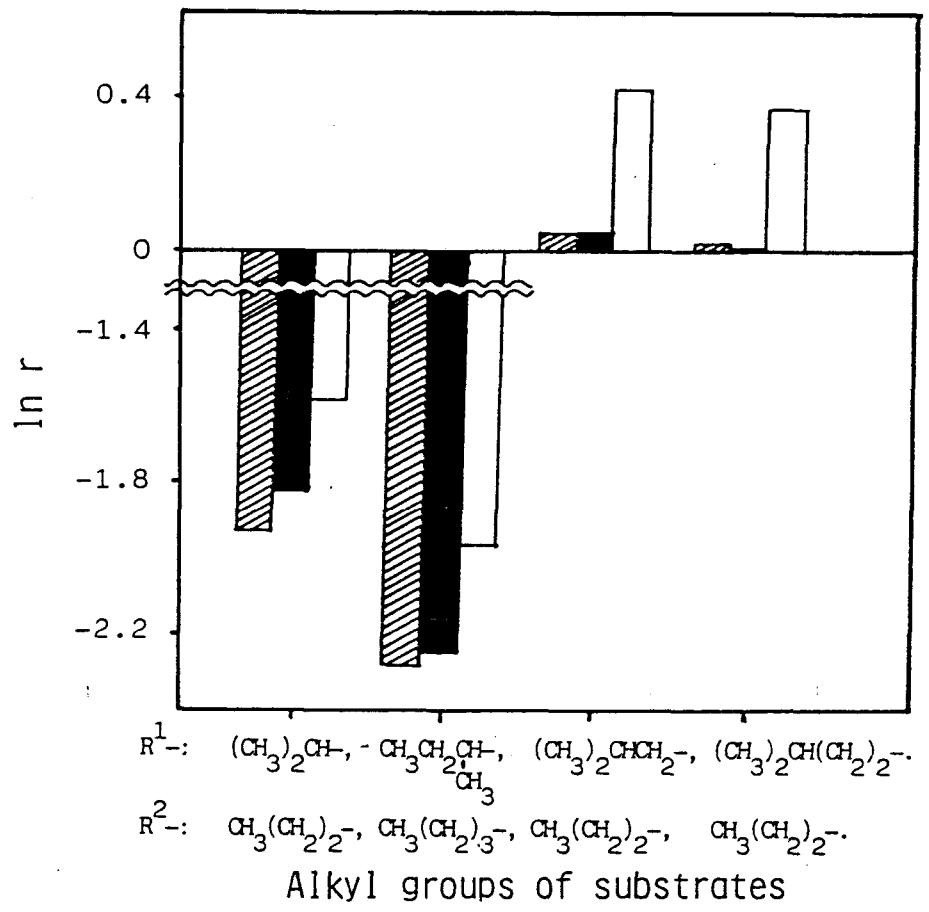


Fig. 4(b). The competitive acylation of a pair of alkylamine with isovaleric anhydride.

The reaction was carried out in benzene (▨), dioxane (■), or water-dioxane mixture at χ_{H_2O}=0.88 (□). The concentration of a reagent was 50 mM.

Table 2. The ln r values obtained in the competitive acylation of unbranched alkylamine with isovaleric anhydride in water-dioxane mixture (χ_{H_2O}=0.88)[a)]

R^1-NH_2 \ R^2-NH_2	CH_3CH_2-NH_2	$CH_3(CH_2)_2$-NH_2	$CH_3(CH_2)_3$-NH_2	$CH_3(CH_2)_4$-NH_2
$CH_3(CH_2)_2$-NH_2	0.330			
$CH_3(CH_2)_3$-NH_2	0.536	0.167(0.13)		
$CH_3(CH_2)_4$-NH_2	0.863	0.418(-0.10)	0.205	
$CH_3(CH_2)_5$-NH_2	1.023	0.621(0.04)	0.357	0.164

a) The concentration of a reagent was 50 mM. The results of the competitions, where the N-acetyl-L-isoleucine N-hydroxysuccinimide ester was used as a reagent, are given in parentheses.

mixtures with a variety of χ_{H_2O} values. The profile of the plots was apparently composed of two regions: Phase I and Phase II. For example, the plot (○) in Fig. 5(a) was composed of Phase I ($\chi_{H_2O} < 0.83$) and Phase II ($\chi_{H_2O} > 0.83$). The χ_{H_2O} values corresponding to the boundary between Phase I and Phase II are indicated by downward arrows hereafter. In Phase I, no effective differentiation of the alkyl-chain length was observed, while considerable differentiation was observed in Phase II, and the ln r value remarkable increased with the increase of χ_{H_2O}.

(Fig. 5(a))

In acylation carried out in solvents with $\chi_{H_2O} > 0.9$, it was observed that the reaction mixture became slightly turbid just after the addition of a reagent and that the turbidity disappeared instantaneously. The same ln r values were obtained in the competitive acylation carried out in two different manners: one was the reaction carried out by the addition of an acid anhydride to amine mixtures homogeneously dissolved in solvents (the standard procedure is described in Chap. 2, §2-3), while the other is the reaction carried out by the addition of amine mixtures to suspensions of reagents previously prepared in solvents with large χ_{H_2O} values. These facts strongly suggested that the occurrence of two regions in the plots of ln r vs. χ_{H_2O} is related to the solubility of acid anhydrides in water-

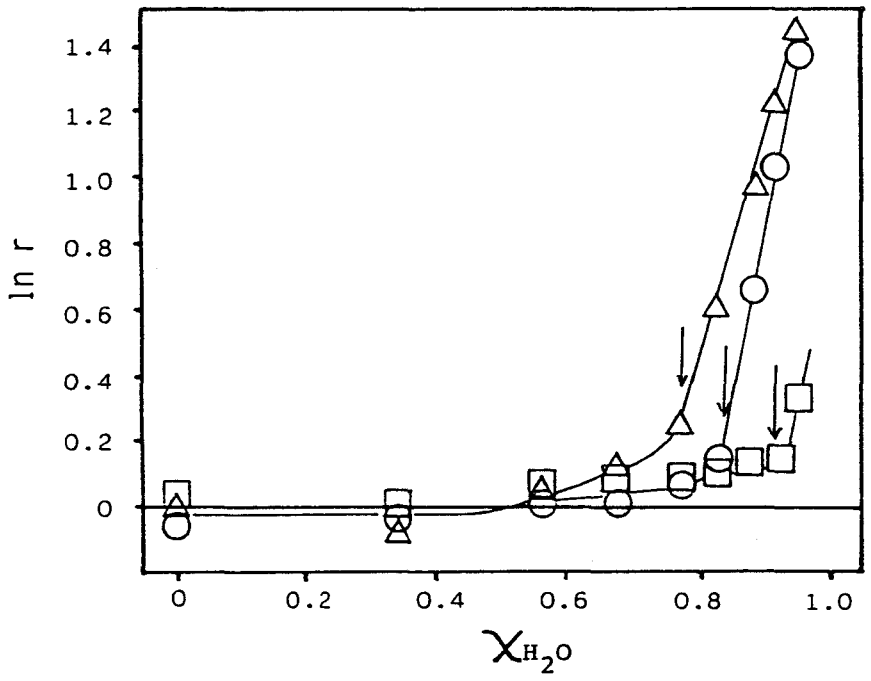


Fig. 5(a). The relationship between the mole fraction of water-dioxane (X_{H_2O}) and the ln r value of the competitive acylation of hexylamine (R^1-NH_2) and propylamine (R^2-NH_2). 4-Methylpentanoic anhydride (△), isovaleric anhydride (○), and isobutyric anhydride (□) were employed. The concentration of reagent was 50 mM.

dioxane mixed solvents. A comparison of Fig. 5(a) and Fig. 1(a) clearly shows that the reaction took place under homogeneous conditions in Phase I, while in Phase II it took place under heterogeneous conditions. Accordingly, it can well be understood that the boundary between Phase I and Phase II shifted to a larger χ_{H_2O} value, from 0.82 to 0.9, by the eight-fold dilution of the reagent (Fig. 5(b)).

(Fig. 5(b))

When cetyl trimethylammonium bromide (CTAB, 0.1 M aqueous solution) was added to the reaction system, no apparent phase separation of the reagent occurred. However, Phase II appeared even in the presence of CTAB, as is shown in Fig. 6. The boundary between Phase I and II shifted to a smaller χ_{H_2O} value. Thus the reagent incorporated into micelles provided by CTAB functioned much like the organic phases produced by the aggregation of reagents.

(Fig. 6)

When a water-soluble reagent such as the N-acetyl-L-isoleucine N-hydroxysuccinimide ester was used, Phase II did not appear. In this case, no effective differentiation of alkyl-chain length was observed, not even in solvents with a high χ_{H_2O} value as is shown in Table 2.

3-3. The Competition of an Alkylamine and a Phenylalkylamine.

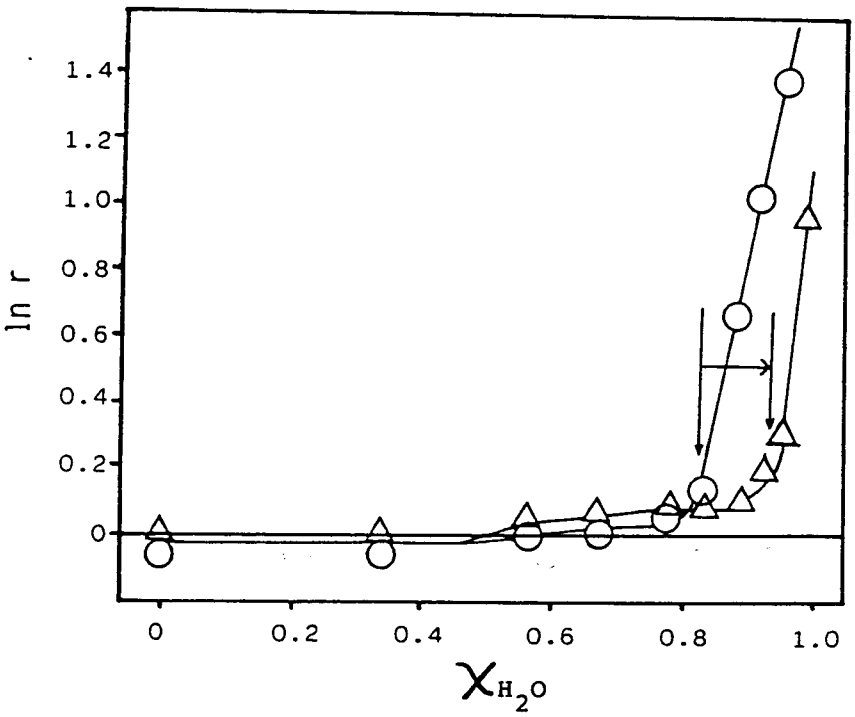


Fig. 5(b). The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation of hexylamine (R^1-NH_2) and propylamine (R^2-NH_2). Isovaleric anhydride was employed. Its concentration was 50 mM (○) or 6.25 mM (△).

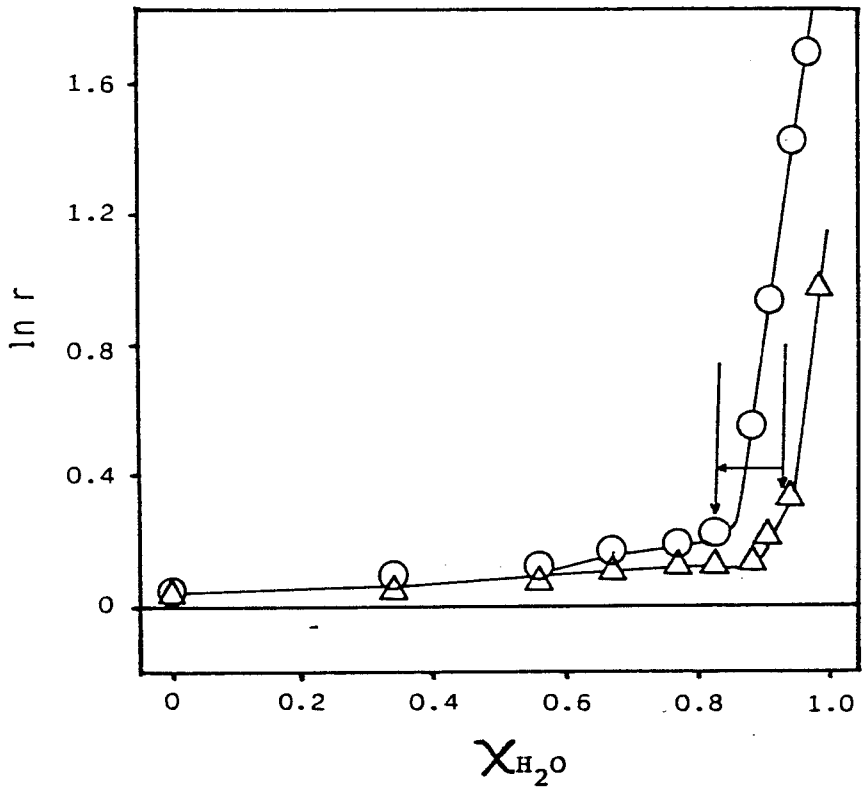


Fig. 6. The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation in the presence (○) or the absence (△) of CTAB.

Figure 7(a) shows the results of the competitive acylation between a phenylalkylamine and an unbranched alkylamine with isovaleric acid anhydride. Benzylamine, phenethylamine, or 3-phenylpropylamine was employed as R^1-NH_2, and butylamine was employed as R^2-NH_2. The profile of the plots is composed of two phases: Phase I ($\chi_{H_2O} < 0.83$) and Phase II ($\chi_{H_2O} > 0.83$). In Phase II, the ln r values increased remarkably with the increase of χ_{H_2O}. In Phase I, the ln r value increased from the negative to the positive with the increase in χ_{H_2O}. This increase of ln r was more pronounced when the phenyl group was substituted near the amino group.

(Fig. 7(a))

Figure 7(b) shows the ln r vs. χ_{H_2O} plots obtained by the competition of phenethylamine with propylamine, butylamine, or hexylamine. In Phase I, all the plots overlapped, even though the chain lengths of the alkylamines were different from one another. In Phase II, the slope of each plot was different. In the case of hexylamine, the ln r value decreased with the increase in χ_{H_2O} (the (□) plot).

(Fig. 7(b))

Figure 8 shows the results of the competitive acylation between phenethylamine and butylamine with several different types of reagent. In this case, the slopes of the plots in Phase I changed significantly with the change in

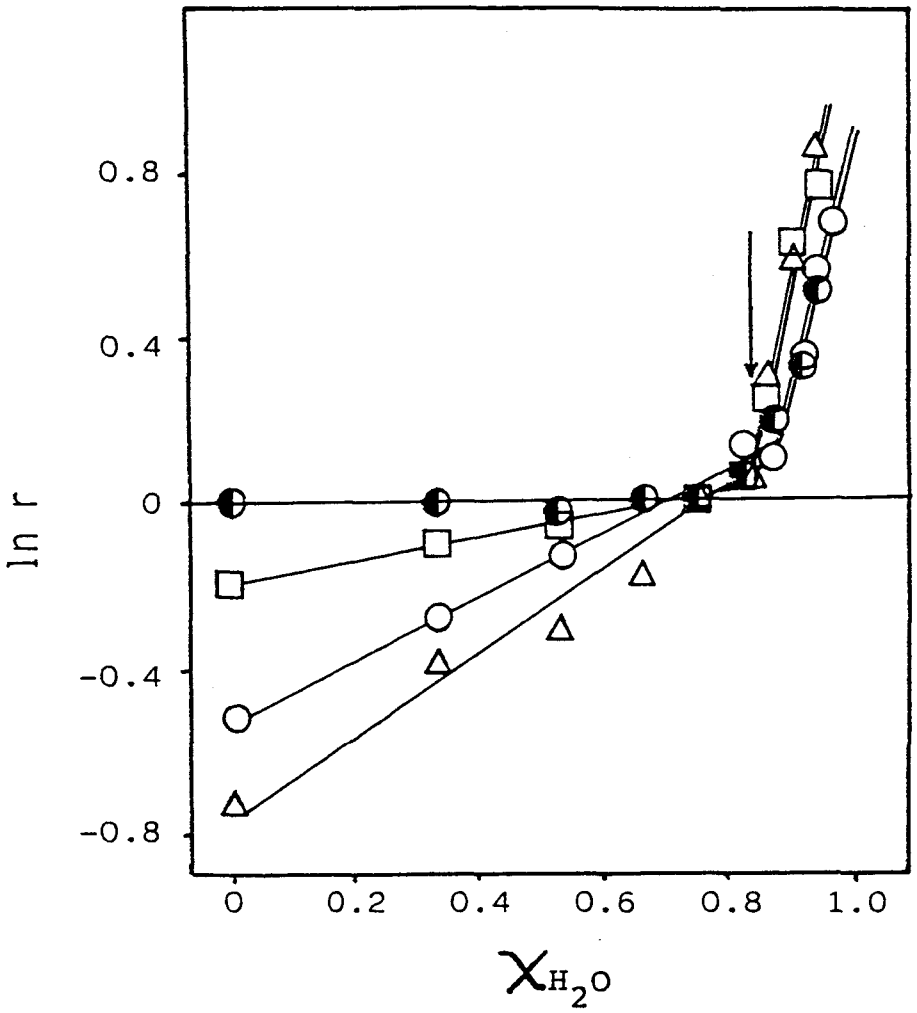


Fig. 7(a). The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation with isovaleric anhydride. Benzylamine (△), phenethylamine (○), 3-phenylpropylamine (□), and pentylamine (◐) were employed as R^1-NH_2. Butylamine was employed as R^2-NH_2 in every case. The concentration of a reagent was 50 mM.

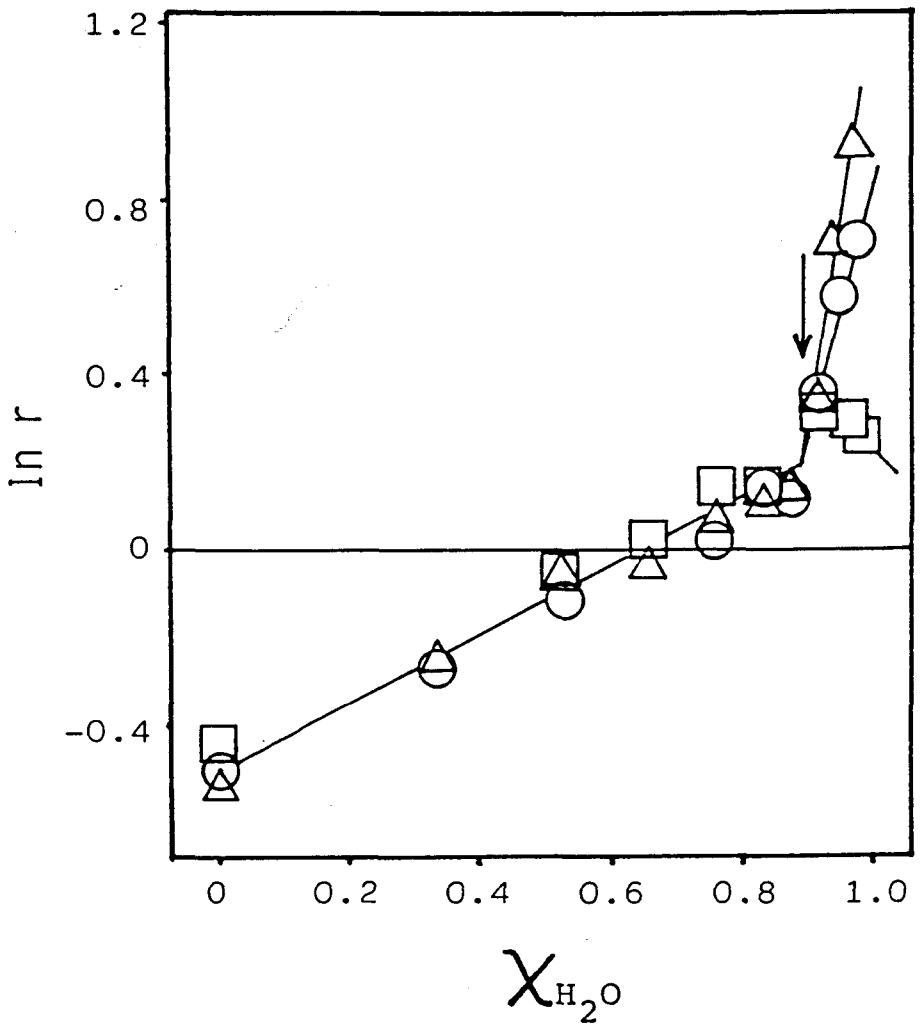


Fig. 7(b). The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation with isovaleric anhydride. Phenethylamine was employed as R^1-NH_2. As R^2-NH_2, propylamine (△), butylamine (○), and hexylamine (□) were employed. The concentration of a reagent was 6.25 mM.

the hydrocarbon residues of the reagents, whereas those of Phase II were similar to one another.

(Fig. 8)

Figure 9 shows the results of the competitive acylation between amines with different pKa's. The ln r value changed with χ_{H_2O} in Phase I only when benzylamine competed with others (the (○) and (△) plots). No change in the ln r value with a change in χ_{H_2O} was observed in any other combinations of amines (the (□) and (◧) plots). Thus, the changes in the ln r value with χ_{H_2O} in Phase I in the competition of phenylalkylamines with alkylamines are caused not by the difference in pKa's of the amines, but by the phenyl groups in the reactants.

(Fig. 9)

Figure 10 shows the results of the competitive acylation in methanol-dioxane or acetonitrile-dioxane mixtures. At any composition of these solvents, the reaction proceeded under homogeneous conditions which corresponded to Phase I in water-dioxane mixtures. As is shown by the (○) and (△) plots, the changes of the ln r value in Phase I were induced by either water or methanol, but no appreciable change in the ln r value was observed in acetonitrile-dioxane mixtures.

(Fig. 10)

Figure 11 shows the results of the competitive

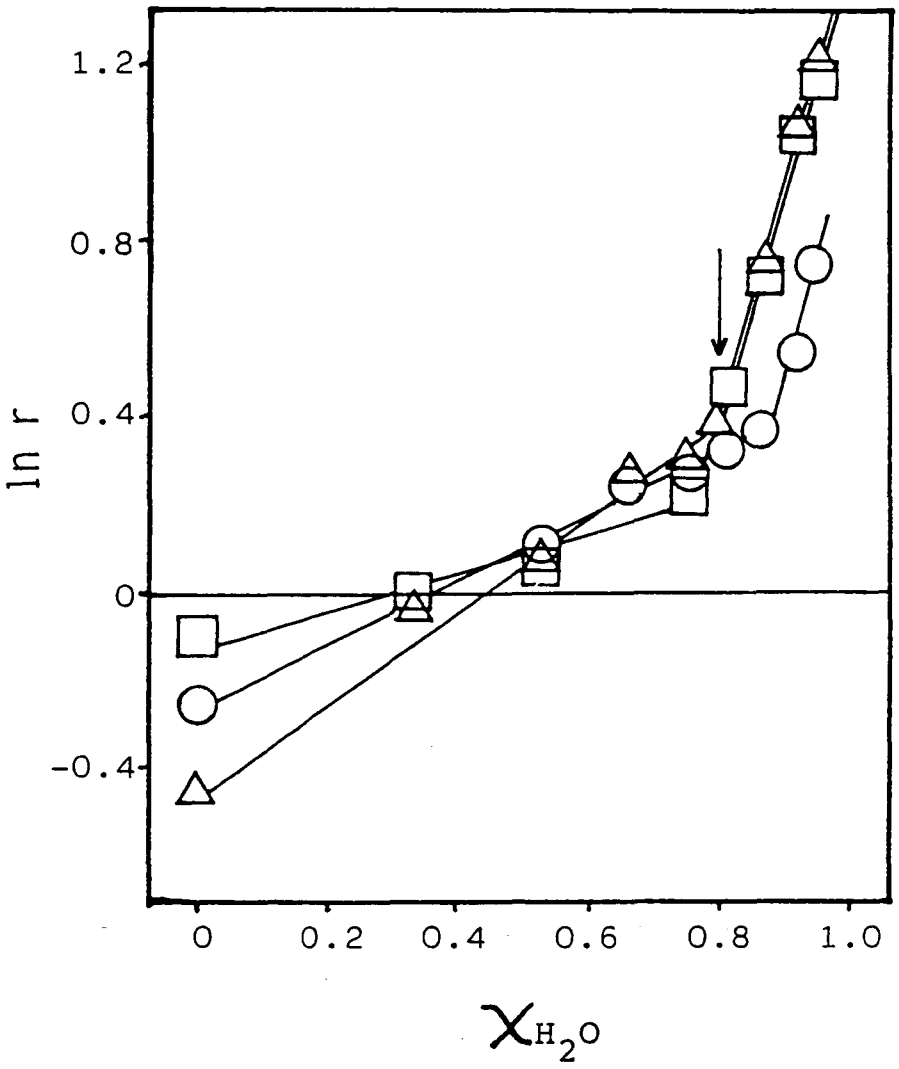


Fig. 8. The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation. Phenethylamine and butylamine were employed as R^1-NH_2 and R^2-NH_2, respectively. As a reagent, butyric anhydride (○), 2-phenylbutyric anhydride (△), and phenylacetic anhydride (□) were employed. The concentration of a reagent was 50 mM.

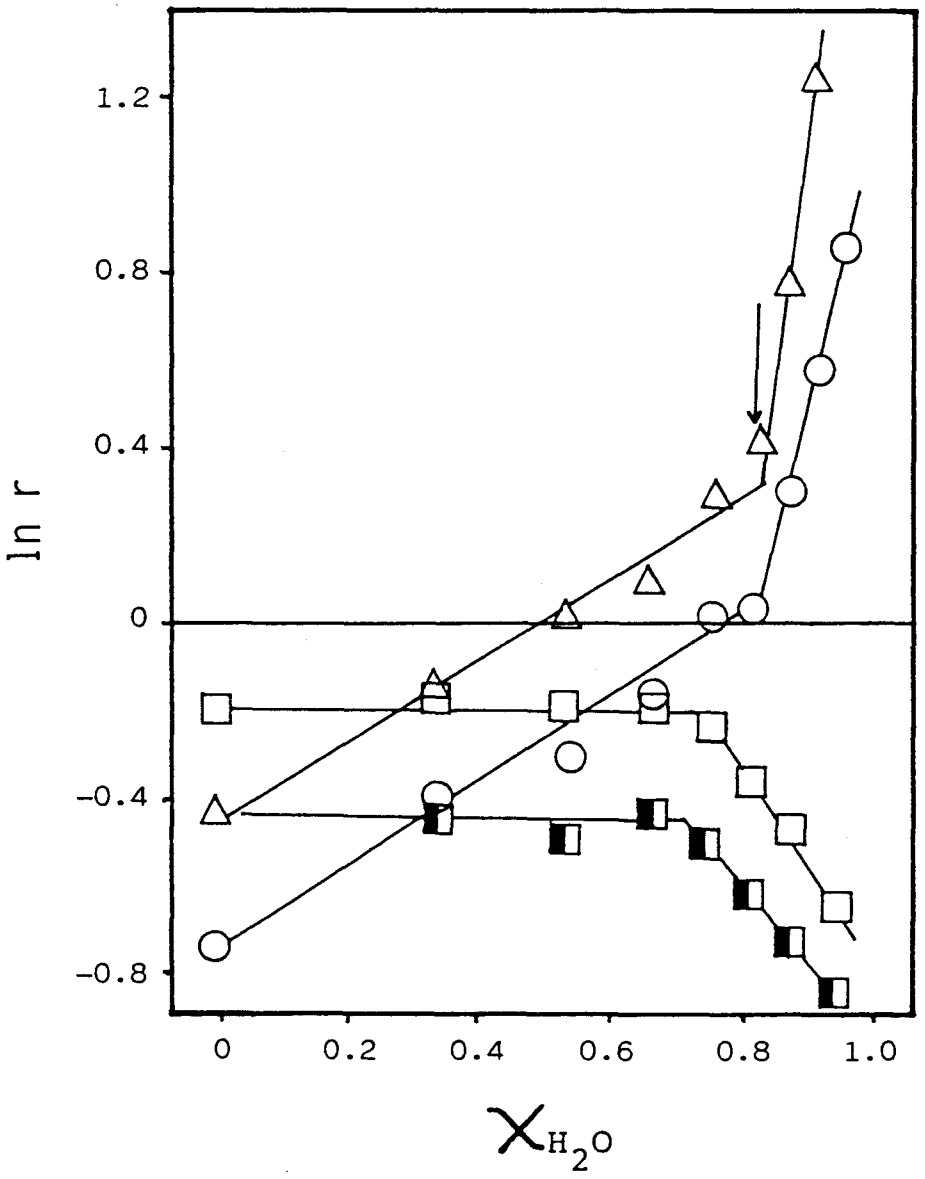


Fig. 9. The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation with isovaleric anhydride. The following combination of R^1-NH_2 and R^2-NH_2 were examined: benzylamine and butylamine (○), benzylamine and 2-methoxyethylamine (△), 2-methoxyethylamine and butylamine (□), and 2-aminoethanol and butylamine (◧). The pKa values of butylamine, benzylamine, 2-methoxyethylamine, and 2-aminoethanol were 10.64, 9.35, 9.28, and 9.5, respectively. The concentration of a reagent was 50 mM.

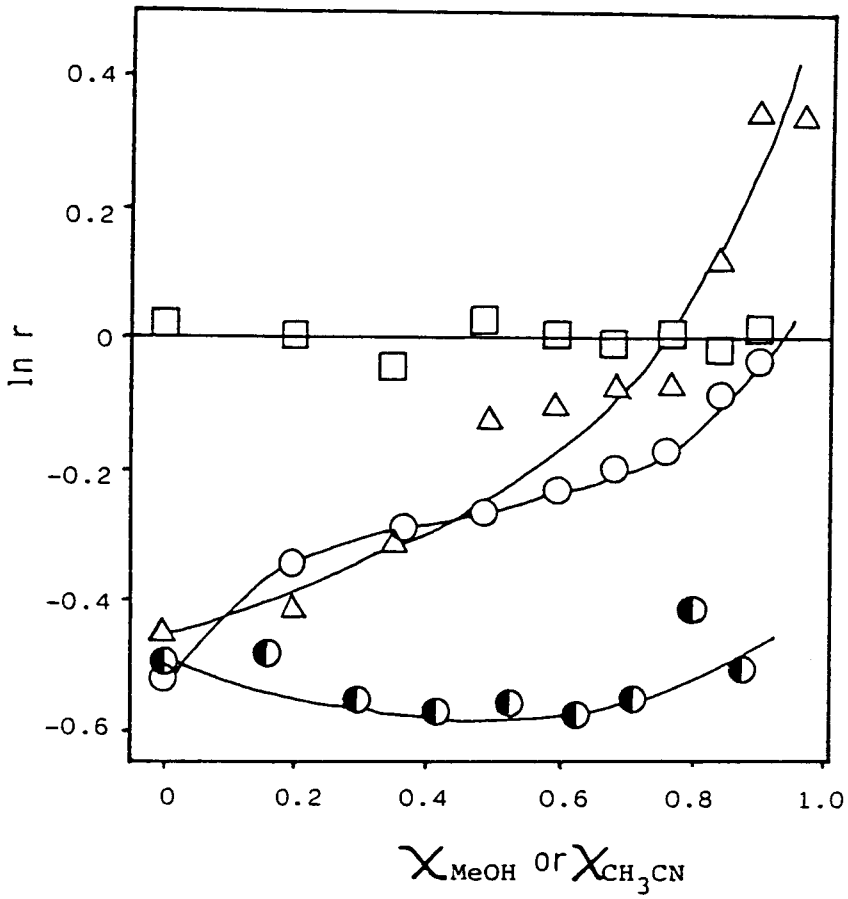


Fig. 10. The relationship between the mole fraction of methanol or acetonitrile in methanol-dioxane or acetonitrile-dioxane mixture (χ_{H_2O} or χ_{CH_3CN}) and the ln r value. The following combinations of R^1-NH_2, R^2-NH_2, and $(R^0\text{-}CO)_2O$ were examined: phenethylamine, butylamine, and isovaleric anhydride in methanol-dioxane (○) and in acetonitrile-dioxane (◐), phenethylamine, butylamine, and 2-phenylbutyric anhydride in methanol-dioxane (△), or hexylamine, butylamine, and isovaleric anhydride in methanol-dioxane (□). The concentration of a reagent was 50 mM.

acylation between cyclohexylmethylamine and butylamine. In the reaction of isovaleric anhydride, the (○) plot gave a very slight gradient in Phase I in comparison with the (△) plot, which shows the results of the competitive acylation between unbranched alkylamines, with the increase of χ_{H_2O}. In the reaction with 2-phenylbutyric anhydride (the (□) plot), an apparent gradient was observed, but it was smaller than that of the (△) plot in Fig. 7(a).

(Fig. 11)

3-4. The Competition of Enantiomeric Amines; Effects of Reaction Media.

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 water-dioxane mixture (χ_{H_2O} =0.76). The resulting ln r' values are shown in Fig. 12. In dioxane, the ln r' value was affected by the substituted alkyl groups: the ln r' values were almost zero in the reaction of 1-phenylethylamine which suggested no appreciable recognition of substrate structure took place. The reaction of another three amines resulted in the negative ln r' values, which suggested (S)-reagent favored the reaction with (R)-substrates. The similar effects of alkyl groups were observed in the reaction in benzene, where

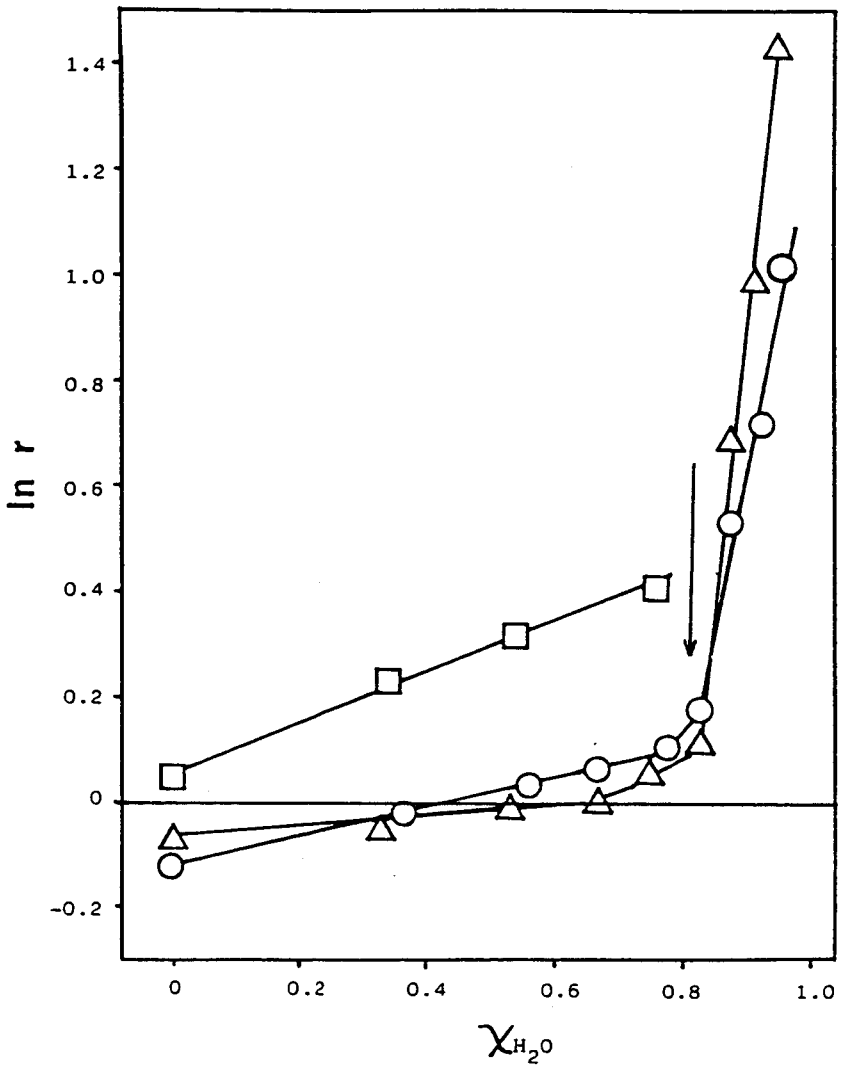


Fig. 11. The relationship between the mole fraction of water in water-dioxane mixture (χ_{H_2O}) and the ln r value of the competitive acylation. The following combinations of R^1-NH_2, R^2-NH_2 and $(R^\circ\text{-}CO)_2O$ were examined: cyclohexylmethylamine, butylamine and isovaleric anhydride (○), hexylamine, propylamine and isovaleric anhydride (△), and cyclohexylmethylamine, butylamine and 2-phenylbutyric anhydride (□).

the differentiation effeciency was higher than that in dioxane.

(Fig. 12)

In water-dioxane mixture (χ_{H_2O}=0.76), where the reaction took place in Phase I by giving positive ln r' value. Thus (S)-reagent favored the reaction with (S)-substrates. This indicated that the modes of enantiomer-differentiation were quite different in the reaction in an aqueous media and in nonaqueous media.

In order to obtain further information about the effects of water in reaction media on enantiomer-differentiation, the enantiomer-differentiating acylation of racemic 1-phenylpropylamine with (S)-phenylbutyric anhydride was carried out in water-dioxane mixture with various composition of water, as is shown in Fig. 13. In Phase II, where the reaction was expected to occur in the oily droplets of reagent with substrate incorporated from aqueous phase by phase transfer process, the ln r' value sharply decreased with increase of χ_{H_2O} to become substantially zero. In Phase I where reaction proceeds in homogeneous solution, the ln r' value linearly increased with increase of χ_{H_2O} in changing its sign from negative to positive. And it reached maximum at χ_{H_2O}=0.76.

(Fig. 13)

As shown in the (●) plot in Fig. 13, methanol had

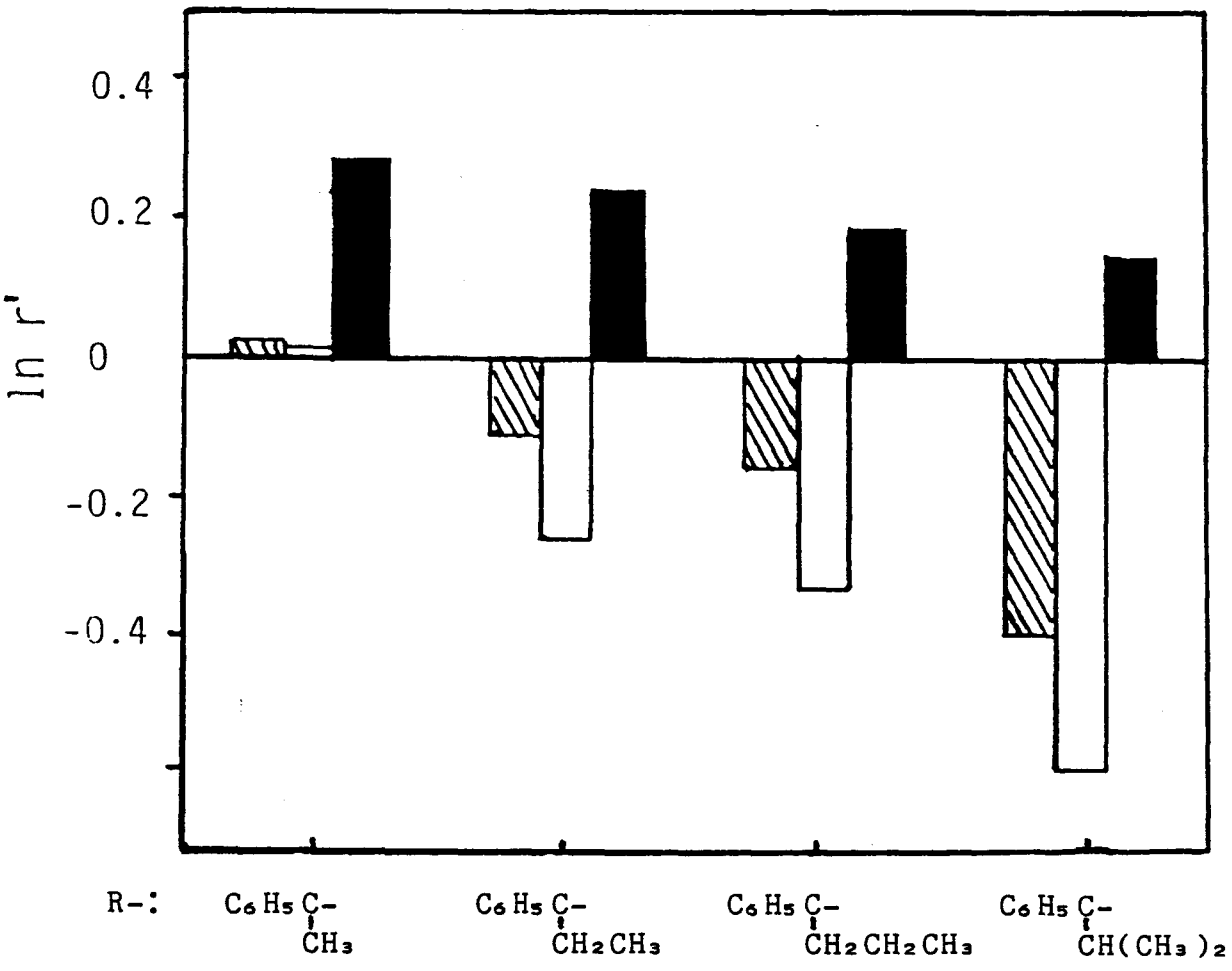


Fig. 12. Enantiomer-differentiating acylation of 1-phenylalkylamine with (S)-2-phenylbutyric anhydride in dioxane (▨), benzene (□), or water-dioxane mixture (X_{H_2O} =0.76) (■).

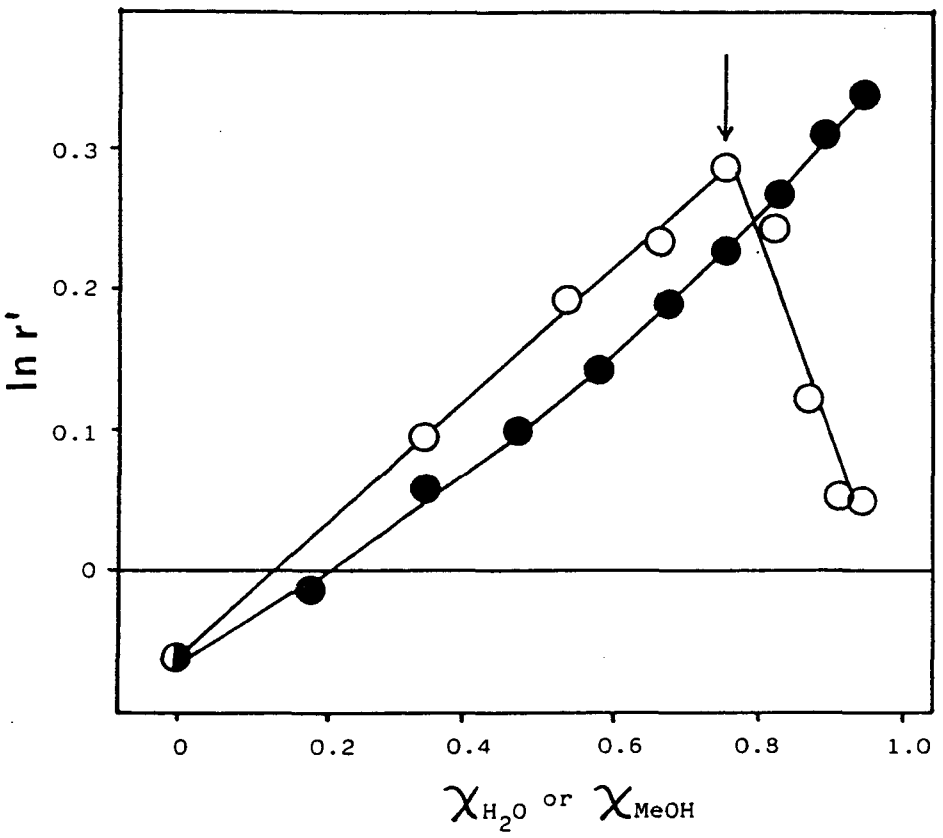


Fig. 13. The relationship between the ln r' value of the enantiomer-differentiating acylation with (S)-2-phenylbutyric anhydride and the mole fraction of water (χ_{H_2O}) or methanol (χ_{MeOH}). The ln r' values were determined in the acylation of 1-phenylpropylamine in water-dioxane (○) or methanol-dioxane (●) mixture.

a similar effect on the ln r' value to that of water in Phase I: the ln r' value increased with increase of χ_{MeOH} in changing its sign from negative to positive. In various organic media, the ln r' values were determined. The results are listed in Table 3 together with their solvent polarity parameters. The increasing order of the ln r' values was in accordance with the increasing order of empirical solvent polarity parameter $E_T(30)$.[11]

(Table 3)

Figure 14 shows the ln r' vs. χ_{H_2O} plots in enantiomer-differentiating acylation of 1-phenylethylamine in aqueous media such as water-dioxane, water-acetone, water-DMF and water-acetonitrile mixture. In all cases, the ln r' value linearly increased with increase of χ_{H_2O} in Phase I, while ln r' decreased in Phase II. The slopes in Phase I were similar with one another. In 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 are found in the figure. These results indicated that the effects of water on the ln r' value could not be accounted for only by its effects on polarity of mixed solvents.

(Fig. 14)

3-5. <u>The Competition of Enantiomeric Amines; A Role of Hydrocarbon Groups in Aqueous Media.</u>

Table 3. The ln r' values in enantiomer-differentiating acylation with (S)-2-phenylbutyric anhydride in various organic solvents.

Solvent	Solvent Polarity[a]	ln r' value	
	$E_T(30)$ (kcal/mol)	Substrate Structure $CH_3CH(C_6H_5)-NH_2$,	$CH_3CH_2CH(C_6H_5)-NH_2$
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, Verlag Chemie, New York (1979), pp. 270-272.

b) N,N-Dimethylformamide is abbreviated as DMF.

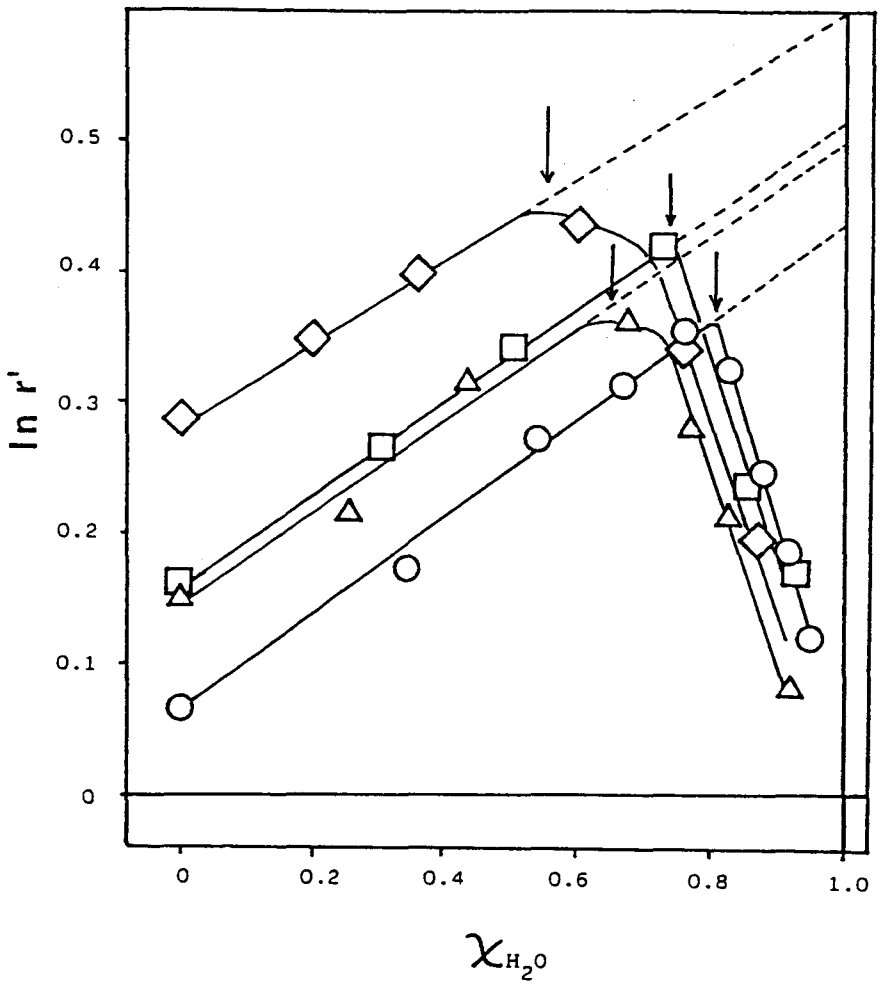


Fig. 14. The relationship between the ln r' value in enantiomer-differentiating acylation and the mole fraction of water (χ_{H_2O}) in several mixed solvents. (S)-2-Phenylbutyric anhydride and 1-phenylethylamine were employed as a reagent and a substrate, respectively. The reaction was carried out in water-dioxane (○), water-acetone (△), water-DMF (□) or water-acetonitrile (◇) mixture.

As shown in the previous section, it was common to acylation of 1-phenylalkylamine derivatives with (S)-2-phenylbutyric anhydride that the ln r' value exhibited a large change with increase of water content of reaction media in Phase I. To know a 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 15 shows the results of enantiomer-differentiation of 1-phenylethylamine with three kinds of optically active acid anhydride. In the reaction with (S)-2-phenylbutyric anhydride (the (○) plot), no significant differentiation took place in dioxane ($\chi_{H_2O}=0$), and the ln r' value linearly increased with increase of χ_{H_2O} in Phase I. In Phase II, the ln r' value decreased to become substantially zero at a very high water region. In the case of (S)-2-cyclohexylbutyric anhydride (the (△) plot), the profile of the plot was smaller than that of the (○) plot. In the case of (S)-2-ethylhexanoic anhydride (the (□) plot), no appreciable differentiation took place in either Phase I or Phase II, even if a slight increase of the ln r' value was detectable in Phase I.

(Fig. 15)

The results of enantiomer-differentiation of 1-cyclohexylethylamine with optically active anhydride are

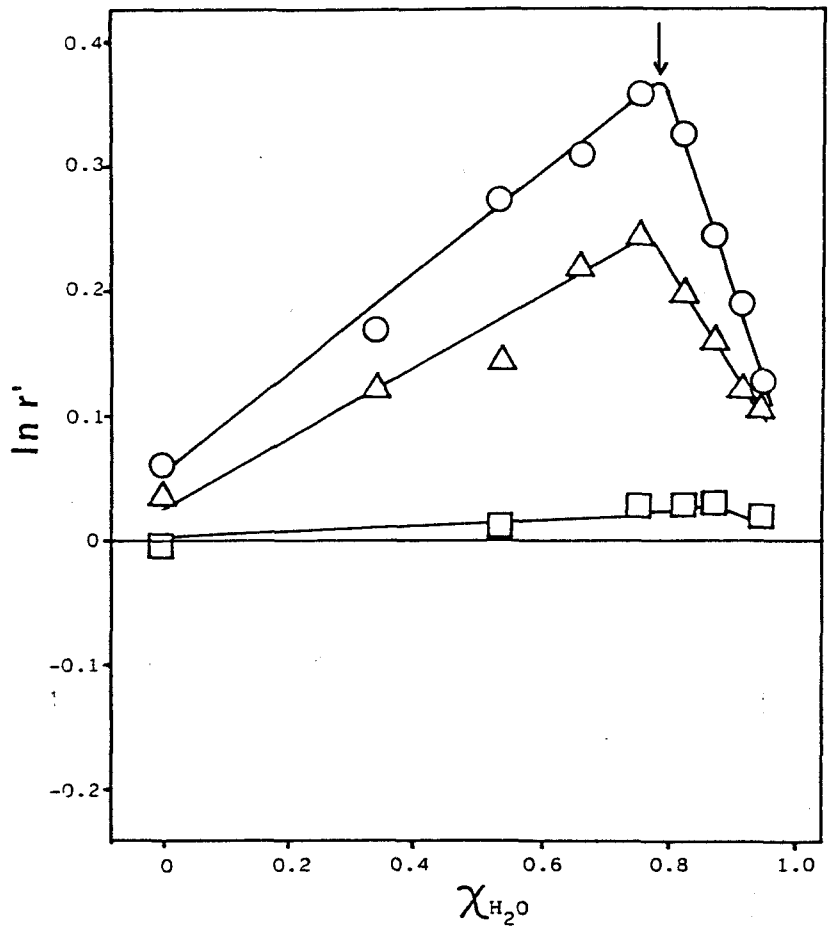


Fig. 15. The relationship between the ln r' value in the acylation of racemic 1-phenylethylamine and χ_{H_2O} in water-dioxane mixture. As a reagent, (S)-2-phenylbutyric anhydride (○), (S)-2-cyclohexylbutyric anhydride (△) and (S)-2-ethylhexanoic anhydride (□) were employed.

shown in Fig. 16. In differentiation with (S)-2-phenylbutyric anhydride (the (○) plot), a positive ln r' value was obtained in dioxane (χ_{H_2O} =0), and the ln r' value stayed constant in Phase I. In Phase II the ln r' value decreased with increase of χ_{H_2O}. In other cases (the (△) and (□) plots), no appreciable differentiation took place in the entire region of χ_{H_2O}.

(Fig. 16)

The results of enantiomer-differentiation of 1-methylbutylamine with optically acid anhydride are shown in Fig. 17. In dioxane (χ_{H_2O} =0), small positive, small negative and substantially zero ln r' values were obtained in the reaction with (S)-2- phenylbutyric anhydride, (S)-2-cyclohexylbutyric anhydride and (S)-2-ethylhexanoic anhydride, respectively. In every case, the ln r' value stayed constant in the entire region of χ_{H_2O}.

(Fig. 17)

Thus large increase of the ln r' value in Phase I was observed only when both reagent and substrates carried phenyl groups (Fig. 12, Fig. 13 and the (○) plot in Fig. 15). In the reaction between reagent and substrates carrying saturated hydrocarbon residues (the (△) and (□) plots in Fig. 16 and Fig. 17), ln r' did not change in either phase I or Phase II at all. These facts indicated that the presence of a phenyl group in a reagent and

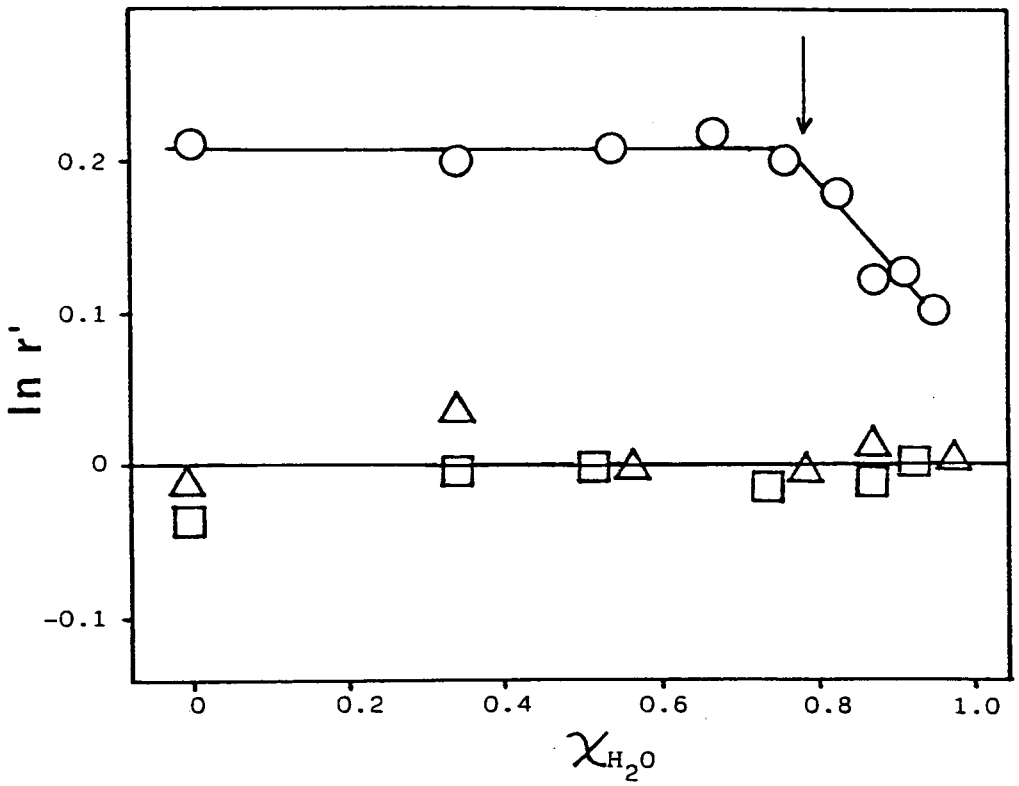


Fig. 16. The relationship between the ln r' value in the acylation of racemic 1-cyclohexylethylamine and χ_{H_2O} in water-dioxane mixture. As a reagent, (S)-2-phenylbutyric anhydride (○), (S)-2-cyclohexylbutyric anhydride (△) and (S)-2-ethylhexanoic anhydride (□) were employed.

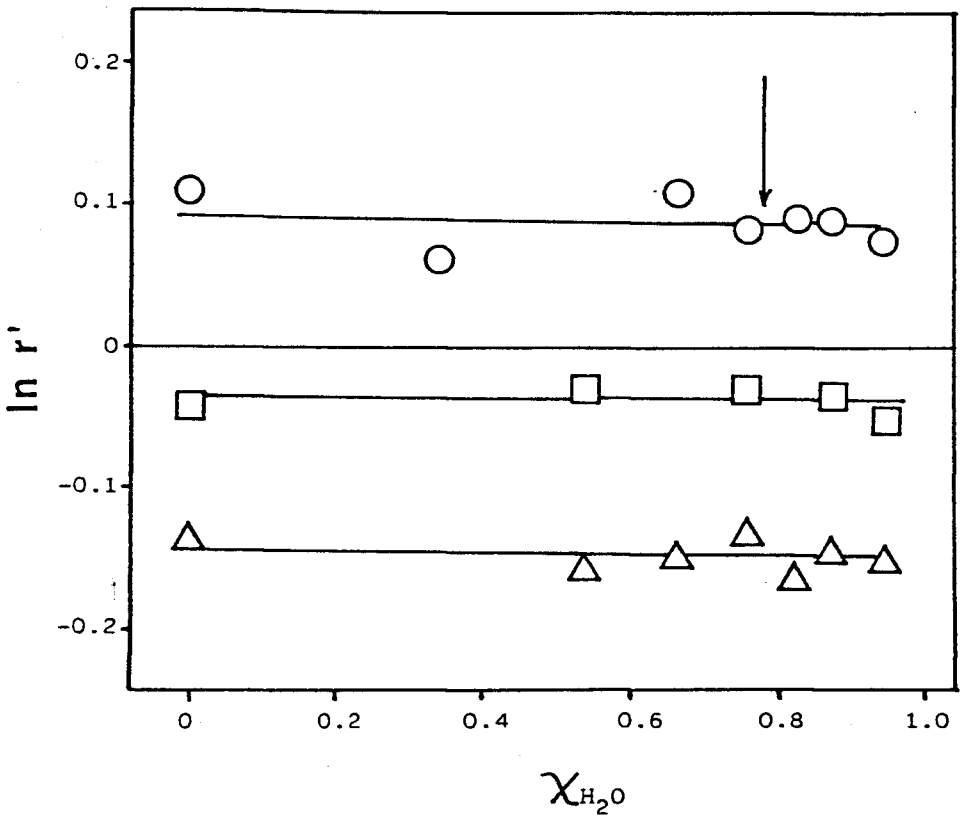


Fig. 17. The relationship between the ln r' value in the acylation of racemic 1-methylbutylamine and X_{H_2O} in water-dioxane mixture. As a reagent, (S)-2-phenylbutyric anhydride (○), (S)-2-cyclohexylbutyric anhydride (△) and (S)-2-ethylhexanoic anhydride (□) were employed.

substrates was essential for the characteristic differentiation which took place in polar aqueous media under homogeneous conditions (Phase I).

Chapter 4. DISCUSSION

4-1. The Reaction Systems.

The competitive acylation of primary amine with acid anhydride (Eq. 2 and Eq. 3) is advantageous for analysis of differentiation process in terms of product distribution, since it gives the stable and kinetically controlled products. In the reaction system shown in Eq. 2, the molecular interactions between hydrocarbon residues will be directly reflected on the ln r value. The rate determining step of the reaction is the nucleophilic attack by an amino group on a carbonyl carbon of the reagent.[12] Therefore the difference in the nucleophilicity of amino groups of substrates must be carefully taken into account in interpreting the ln r values. However the rate of acylation is known to be insensitive to a small change in nucleophilicity of an amino group indexed by its pKa, when an excess amount of amine (pKa $>$ 9) is acylated by a reagent carrying a good leaving group such as an acid anhydride.[13]

In the enantiomer-differentiating acylation, the nucleophilicity of the amino group of competing substrates is assumed to be identical with each other, since they are enantiomers. In this respect, the reaction system of Eq. 3 is advantageous to that of Eq. 2 in evaluation of interaction between hydrocarbon residues. As shown in

Scheme I, the noncovalent interactions between hydrocarbon residues (A - E interaction, B - G interaction, C - F interaction, etc.) undoubtedly play a desicive role in a differentiation of chirality, in addition to the interaction between functional groups, X - Y . However the effect of a certain elementary interaction (A - E interaction, B - G interaction, etc.) cannot be unequivocally evaluated by the ln r' values, but the values will express only a topological difference of total interactions between three hydrocarbon residues and functional group around a chiral carbon atom. Therefore a molecular interaction between each hydrocarbon residue cannot be unequivocally evaluated from the ln r' value. Thus the comparative study of these two reaction systems (Eq. 2 and Eq. 3) is important.

(Scheme I)

4-2. Differentiation of Substrate Molecules in Aprotic Reaction Media.

In reaction shown in Eq. 2 in aprotic media, such as dioxane and benzene, the reagent molecule could distinguish only an α-branched substrate from an unbranched one, while it could distinguish neither the difference in the alkyl-chain length of unbranched substrates nor β- and γ-branched substrates from unbranched one (Fig. 4(a) and (b)).

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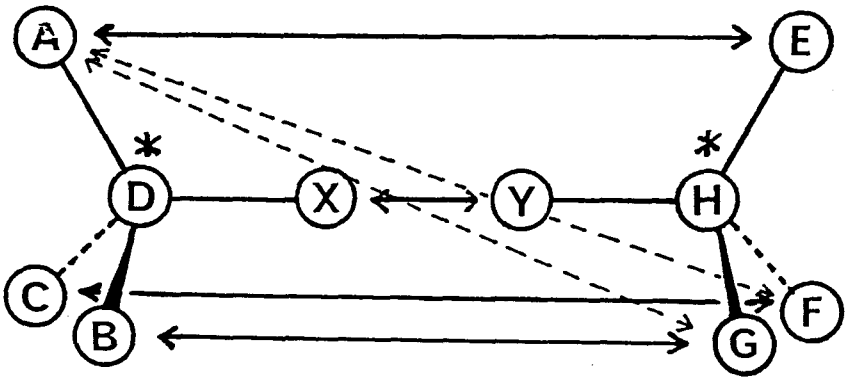

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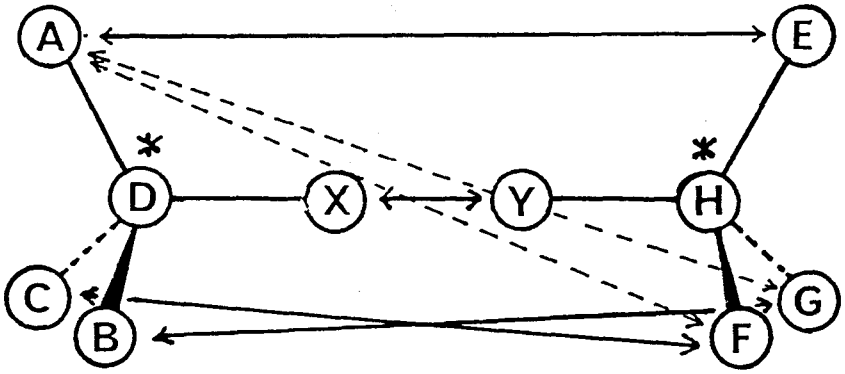

a reagent molecule enantiomeric substrate molecules

Scheme I. Schematic representation of enantiomer-differentiation.

In enantiomer-differentiating acylation (Eq. 3), the reagent distinguished the chirality of substrates carrying branched alkyl group more effectively than that of substrate carrying unbranched one (Fig. 12). When the ln r' values in dioxane (▨) or benzene (□) shown in Fig. 12 are plotted against ν_x value, a measure of bulkiness,[14] of alkyl substituent in substrates in Fig. 18, the ν_x values of alkyl skelton of substrates was linearly correlated to the ln r' values. The result indicates that the enantiomer-differentiation is mostly controlled by bulkiness of hydrocarbon residues in aprotic media.[15]

(Fig. 18)

4-3. Differentiation of Substrate Molecules in Protic Reaction Media.

In Phase I of water-dioxane mixtures, the reaction proceeded under homogeneous conditions, where the differentiation of substrates by a reagent must controlled by molecular interactions. As may be found in Fig. 5(a) and (b), no effective differentiation of the chain length of alkyl groups took place, even in solvents with high χ_{H_2O} value, as long as the reaction took place in Phase I. Thus it was shown that there is no characteristic interaction contributing to the differentiation of the chain length of alkyl groups in Phase I. The absence of interaction between

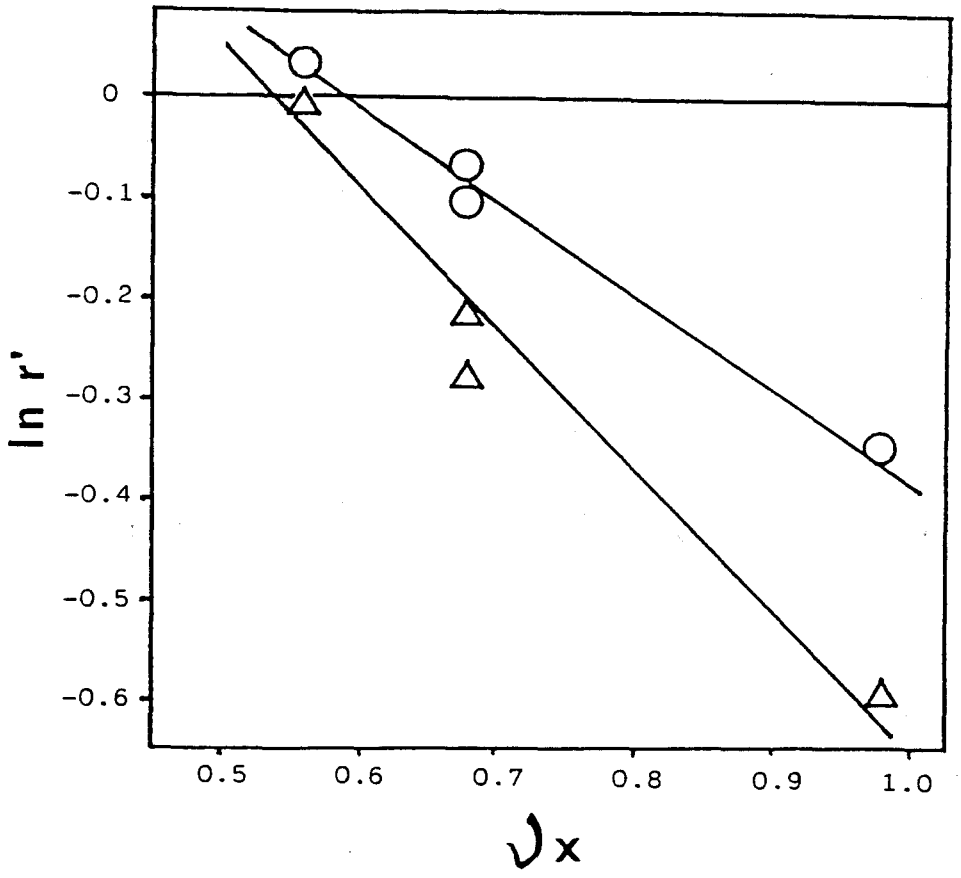

Fig. 18. The relationship between the ln r' value and ν_x value of carbon chains in the substrates. The reaction was carried out in dioxane (○) or benzene (△).

alkyl groups in the reagent and substrate is also supported by the fact that the slope of plots in Phase I in Fig. 7(b) were not affected by the alkyl-chain length of R^2-NH_2.

However the ln r value increased with the increase in χ_{H_2O} from a negative value to a positive one in the competitive acylation of a phenylalkylamine and alkylmaine by isovaleric anhydride in Phase I. As is found in Fig. 7(a), the slopes of the plots depended on the structural features of the phenylalkylamines. As is found in Fig. 7(b), the slope of the plot depended on phenylalkylamine and was not changed by the change in the chain length of alkylamines. The results shown in Fig. 9 exclude the possibility that the slope of a plot depends on the difference in the pKa's of the competing amines. These facts can be explained in terms of an attractive interaction between the phenyl group of a substrate and the alkyl group of a reagent (an alkyl-phenyl interaction) that was induced by the addition of water to the reaction media.

When phenylacetic anhydride is used in the competitive acylation between phenethylamine and butylamine, the slope of the ln r vs. χ_{H_2O} plot in Phase I will represent the difference between a phenyl-phenyl interaction (an interaction between phenyl groups of a substrate and a reagent) and an alkyl-phenyl interaction. The slope of the plot (□) in Fig. 8 showed a more gentle gradient than that

of the (○) plot in Fig. 8 or than those of the plots in Fig. 7(b). The results show that a phenyl-phenyl interaction was also induced by the addition of water to solvents. The former was a little stronger than, or comparable to the latter.

Since the alkyl-phenyl interaction occurred not only in water, but also in methanol which forms strong intermolecular hydrogen bonds, the interaction must be solvophobic in character. No alkyl-phenyl interaction occurred in dioxane or acetonitrile (ε=37.5)[16] which has comparable dielectric constant to that of methanol (ε=32.6),[15] but does not form hydrogen bonds (Fig. 10).

By the (△) plot in Fig. 11 with the (△) plot in Fig. 7(a), it is clearly shown that no attractive interaction occurs, when a benzene ring of a substrate is replaced by a saturated hydrocarbon ring. Therefore electron system of a phenyl group must be important for occurrence of the attractive alkyl-phenyl interaction.

In Phase II of water-dioxane mixture, the alkyl chains of substrates were remarkably differentiated by a reagent (Fig. 5 and Table 2). These results are in clear contrast to the results in Phase I. In Phase II, the added reagents separated from the solvent phase to make fine oily droplets prior to the reaction with amines. Therefore almost all of the reaction must proceed inside the

hydrophobic domain constituted by acid anhydride. Moreover, the ln r value must be determined by the relative concentration of substrates transferred from an aqueous phase. The results shown in Table 2, Fig. 5 and Fig. 6 suggest that the formation of a hydrophobic domain in the aqueous phase is indispensable for the effective distinction of alkyl-chain length.

In general, the hydrophobicity of a compound is evaluated from its partition constant between water and a certain organic solvent (i.e. π value).[17] Figure 19 shows the relationship between the ln r value and the difference in carbon number between alkyl groups in the competition with ethylamine. The ln r value has been linearly correlated with the difference in the carbon numbers of the hydrocarbon residues.[18] Thus the ln r values is Phase II can be well explained by the hydrophobicity of hydrocarbon groups.

Since the partition constant of enantiomeric substrates is identical with each other, no efficient enantiomer-differentiation was attainable in Phase II by a phase transfer process of substrates from an aqueous phase to an organic phase. Even if there is optically active organic phase, efficient enantiomer-differentiation does not take place.

(Fig. 19)

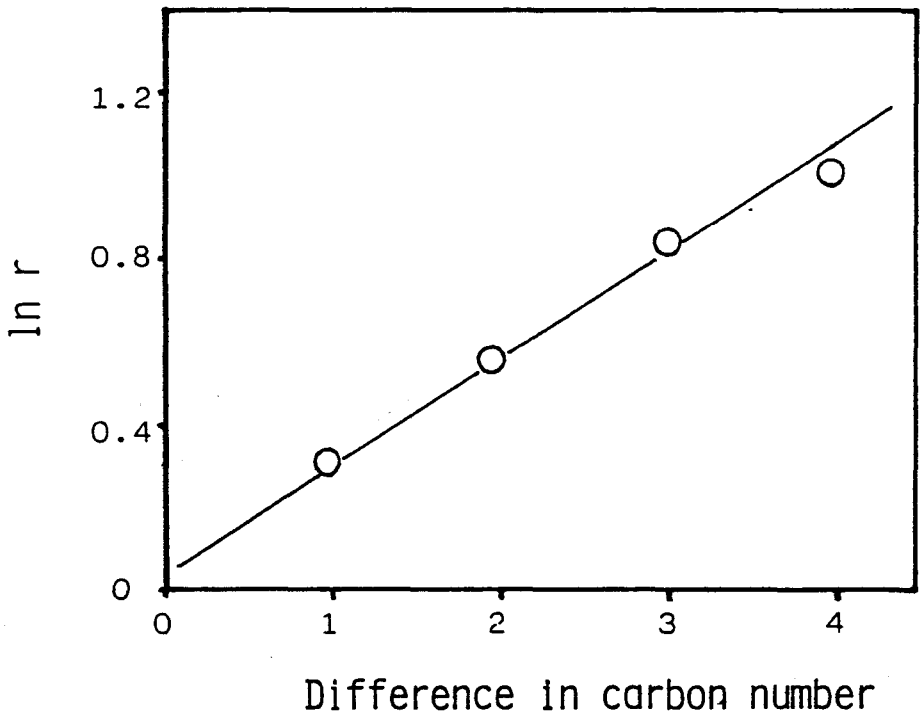

Fig. 19. The relationship between the ln r value and the difference in carbon number of alkyl chain of substrates in the competition of unbranched alkylamine and ethylamine.

In the competition of phenethylamine with hexylamine, the slope of the plot in Phase II showed a negative gradient, while in the competition with another shorter alkylamines the slopes of plots were positive (Fig. 7(b)). These facts can be explained by the published finding that the phenylethyl group is more hydrophobic than the butyl group and less hydrophobic than the hexyl group.[18] In the results shown in Fig. 7(b), it is also noteworthy that the slopes of the plots in Phase I are all positive, regardless of their variation from positive to negative in Phase II. These results indicate that the alkyl-phenyl interaction resulting in the differentiation in Phase I has a different character from a conventionally called hydrophobicity contributing to the differentiation in Phase II.[18,19]

Since an alkyl-phenyl interaction and a phenyl-phenyl interaction are molecular interactions functioning under homogeneous conditions, it is expected that they participate in enantiomer-differentiation as an elemental interaction. The results of enantiomer-differentiation in water-dioxane mixture in Phase I (Fig. 12) showed clear contrast to that in organic media. In all cases, the positive ln r' values were obtained. The interactions other than bulkiness are expected to take major part in chiral recognition.

Moreover, the large increase of ln r' in Phase I was specific for the reaction where both reagent and substrates carried phenyl groups (Fig. 12, Fig. 13 and the (○) plot in Fig. 15). In the reaction between reagent and substrates carrying only saturated hydocarbon residues (the (△) and (□) plots in Fig. 16 and Fig. 17), the ln r' value did not change in either Phase I or Phase II at all. These facts indicate that the presence of a phenyl group in a reagent and substrates is responsible for the characteristic differentiation in polar aqueous media.

The effects of a phenyl group in reactant molecules on the enantiomer-differentiation in Phase I is qualitatively in accordance with those found in the competitive acylation (Eq. 2). The above mentioned results of enantiomer-differentiating acylation (Eq. 4) are explicable with an alkyl-phenyl interaction and a phenyl-phenyl interaction. That is, enantiomer-differentiation is resulted from a distinction between alkyl and phenyl group in substrate molecules by alkyl and/or phenyl groups in a reagent molecule on the basis of alkyl-alkyl and alkyl-phenyl interactions. On the other hand, in the reaction between a reagent and substrates carrying only saturated hydrocarbon groups, no enantiomer-differentiation takes place, since neither alkyl-phenyl nor phenyl-phenyl interactions is expected.

In the reaction in which a phenyl group and a cyclohexyl group participated (the (△) plot in Fig. 15), 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 unbranched one (Fig. 11). The result shown by the (○) plot in Fig. 16 cannot be simply explicable with the size of substituents.

The correspondence of an increasing order of the ln r' values in various organic media to $E_T(30)$ (Table 3) suggests that the responsible molecular interactions of the differentiation in "highly polar" media are quite different from those caused by bulkiness of hydrocarbon residues, because bulkiness will not be affected so much by solvent polarity. At least, the remarkable changes of ln r' from negative to positive as shown Fig. 13 must be in part a consequence of elevation in polarity. However the remarkable effect of methanol (the (●) plot in Fig. 13) and the effects of addition of water in several organic solvents (Fig. 14) indicate that the effect of water on ln r' cannot be accounted for only by its effects on polarity of mixed solvents. In addition to a polar effect, water and methanol must contribute to occurrence of an alkyl-phenyl interaction and a phenyl-phenyl interaction by acting as a hydrogen-bond donor, since $E_T(30)$ values which well reflects

hydrogen-bond donor character of solvents rather than the dielectric constants were in accordance with these results.[16)]

In this section, it has been shown that there are two different modes of differentiation of hydrocarbon groups in aqueous media. One is the differentiation based on the partition of substrates between an aqueous media and the hydrophobic aggregates of reagents in Phase II. Distinction of alkyl-chain length is possible by this mode of differentiation. The other is the differentiation based on characteristic molecular interactions, such as the alky-phenyl interaction and the phenyl-phenyl interaction in Phase I. The interaction between alkyl groups (an alkyl-alkyl interaction) is not so large as to contribute to this mode of differentiation.

In the former mode, the formation of a hydrophobic domain is vital for the differentiation of relatively short alkyl chains. This is an important part of understanding the role of the hydrophobic domain in macromolecules carrying small size-alkyl groups.

In the latter mode of differentiation, the unique function of phenyl group is concerned with a molecular recognition through an alkyl-phenyl interaction or phenyl-phenyl interaction. The attractive alkyl-phenyl and phenyl-phenyl interactions seem unlikely to be a hydrophobic interaction, sice no attractive interaction was found

between alkyl group (an alkyl-alkyl interaction). These attractive interactions might be the complex expression of noncovalent interactons occurring in protic media. At this stage, a combination of weak solvophobic interaction and electron donor-acceptor interaction including CH···π [20] are probable candidates of physicochemical entity of these attractive interactions.

4-4. General Considerations.

As a noncovalent interaction between hydrocarbon groups, a repulsive interaction due to their bulkiness and a solvophobic (hydrophobic) interaction occurring in hydroxylic solvents are generally accepted.

In nonpolar aprotic media, a reagent molecule can differentiate α-branched alkyl group from unbranched one on the basis of the difference in bulkiness of substrates, but cannot differentiate unbranched alkyl groups with different chain-lengths, as has been discussed in the section 4-2. Even in the reaction in Phase I in aqueous media, the differentiation of unbranched alkyl groups with different alkyl chain-lengths could not be achieved. Thus unbranched alkyl groups with different chain-lengths have no useful function in a differentiation process. Only a branching of alkyl group must be effectively functioning in differentiation of alkyl groups. It is quite interesting

that there is no unbranched alkyl residue in proteinous amino acid residues.

In the present study, it has not been possible to differentiate alkyl-chain length under homogeneous conditions in aqueous media (Phase I). The alkyl-alkyl interaction is rather small and negligible. Knowles and his coworkers reported that the rate of acylation was remarkably enhanced in an aqueous alkyl groups.[21] This phenomenon was interpreted in terms of the "proximity effect" caused by hydrophobic bonding between hydrocarbon groups in aqueous media. However, further critical reexaminations should be made to prove this effect, as has been pointed out Guthrie.[22] The hydrophobic interaction between such small- and medium-size hydrocarbon groups as proteinous amino acid residues and its function in a differentiation process have not been reported. At least, it is indicated that "proximity effect" is not generally applicable. Especially its contribution to the differentiation of small- and medium-size alkyl groups must be negligible.

The author has explicitly demonstrated that the attractive alkyl-phenyl interaction and phenyl-phenyl interaction contribute to a differentiation process by the analysis of product distributions obtained in aqueous media under homogeneous conditions (Phase I), though the alkyl-alkyl interaction is negligible. Thus aromatic hydrocarbon

groups behave very differently from saturated hydocarbon groups in a differentiation process.

This kind of peculiarity of aromatic hydrocarbon groups has been implicated by several stereo-differentiating reaction systems. For example, in efficient enantioface-differentiating hydrogenation of α,β-unsaturated acids or esters by Wilkinson catalyst, the presence of phenyl groups in substrate and chiral phosphine ligands was necessary for obtaining excellent results.[23] It was noticed that the use of water or alcohols as a reaction media often resulted in good results in this system. The peculiartity of a phenyl groups was also reported in enantioface-differentiating reduction of alkyl phenyl ketones.[24]

Furthermore, several findings implying the function of aromatic hydrocarbon residues in a differentiation process has been reported in a biological field. For example, the presence of aromatic amino acid residues in the interfacial recognition site of phospholipase A_2[25] and in the antigen binding site of the Bence Jones protein Mcg is well documented.[26] By the analysis of evolutionary changes in proteins, the relative mutabilities of Tyr or Phe is rather low than that of Leu, Ile or Val.[27] The probability of intermutation between Try and Phe was rather high, but the probability of mutation between Try or Phe to Leu, Ile or Val was low.[27] The

findings suggest that aromatic hydrocarbon residues in protein not only provide a hydrophobic domain, but also have some additional functions. The presence of alkyl-phenyl interaction and phenyl-phenyl interaction and their functions in a differentiation process as has been shown by the present study will present experimental grounds for the function of aromatic hydocarbon groups in protein.

It has been shown by the present study that, once aqueous phase (hydrophilic domain) and organic phase (hydrophobic domain) were formed by phase separation in the reaction system (Phase II), an efficient differentiation of small-size hydrocarbon groups takes place by partitioning of substrates. A property of hydrocarbon groups characterized by their partition coefficient between water and organic solvents is known as hydrophobicity, which is extensively determined for many compounds from simple hydrocarbons to proteinous amino acids.[28] Since partition constant sensitively varies in a manner depending on carbon number of hydrocarbon groups, it is possible to differentiate small-size hydrocarbon groups by a partitioning process.

Importance of the differentiation mode shown in Phase II is exemplified by organic synthetic processes utilizing liposomes or micelles.[29] The close relationship between partition constants and pharmacological activities indicates that this differentiation mode is also functioning

in biological systems.[30] The function of hydrophobic domain in protein must be understandable in this respect. It is also known to be possible to differentiate hydrocarbon groups on highly polar solid surface by making hydrophobic domain artificially: fatty acid modified nickel catalyst can hydrogenate alkenes in a manner of differentiating their hydrocarbon groups.[31]

It should be noticed in conclusion that analysis of the product distributions of a simple competitive reaction is effective in evaluating very weak interactions between hyrocarbon groups and that a competitive acylation of primary amines with acid anhydride is an adequate system for this purpose.

Chapter 5. SUMMARY

In this study, the author showed a new approach for characterization of noncovalent interactions between hydrocarbon groups such as proteinous amino acid residues. An analysis of the product distributions of a simple competitive reaction is proved to be effective in evaluating weak noncovalent interactions between hydrocarbon groups, and an acylation of primary amine with acid anhydride is an adequate system for this purpose: The two types of competitive acylation of a pair of monofunctional primary amines with acid anhydride were carried out in protic and aprotic media. One was competitive acylation of a pair of amines carrying different hydrocarbon residues with each other (Eq. 2). The other was enantiomer-differentiating acylation of racemic amine with optically active acid anhydride (Eq. 3). On the basis of distribution of products (the ln r and ln r' values), molecular interactions between hydrocarbon residues responsible for differentiation of reacting molecules were studied.

In aprotic media, the differentiation was mainly controlled by the size of hydrocarbon residues near the reaction center. In aqueous media, two different modes of differentiation were functioning. In solvents with a high water content, where the reaction proceeded under heterogeneous condititons, the differentiation was due to

partition of substrates between an aqueous media and hydrophobic aggregates of reagent. The differentiation was controlled by the difference in hydrophobicity of hydrocarbon residues. Effective distinction of alkyl-chain length was possible by this mode of differentiation, but a differentiation of enantiomer did not take place. In solvents with a low water content, where the reaction proceeded under homogeneous conditons, the differentiation of a phenyl group from an alkyl group occurred. From the dependency of the product distribution on water content in reaction media, an attractive interaction induced by water was postulated between alkyl and phenyl groups (an alkyl-phenyl interaction) or phenyl groups (a phenyl-phenyl interaction). It was shown that these interactions were also important in a enantiomer-differentiation.

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Chapter 7. REFERENCES AND NOTES

1) T. Harada, Y. Hiraki, Y. Izumi, J. Muraoka, H. Ozaki, and A. Tai, Proc. 6th Int. Congr. Catal., London (1976), 1024.

2) A. Tai, T. Harada, Y. Hiraki, and S. Murakami, Bull. Chem. Soc. Jpn., **56**, 1414 (1983); Y. Hiraki, K. Ito, T. Harada, and A. Tai, Chem. Lett., **1981**. 131.

3) Z. Simon, Angew. Chem., Int. Ed. Engl., 13 719 (1974).

4) G.E. Schulz and R.H. Schirmer, "Principles of Protein Sturcture,"Springer-Verlag, New York (1980), chap. 3; C. Chothia, Nature, **248**, 338 (1974).

5) J.B. Jones, "Application of Biochemical Systems in Organic Chemistry," ed by J.B. Jones, C.J. Sih, and D. Perlman, John Wiley Sons, New York (1976), part I, pp. 15-28; P.D. Ross and S. Subramanian, Biochemistry, **20**, 3096 (1981); P. Manavalan and P.K. Ponnuswamy, Nature, **275**, 673 (1978).

6) A. Horeau, "Stereochemistry Fundamentals and Methods," ed by H.B. Kagan, Georg Thieme Publishers, Vol. 3, Stuttgart (1977), pp. 52-93.

7) P.A. Levene, R.E. Marker, and A. Roten, J. Biol. Chem., **100**, 589 (1933).

8) P.A. Levene, J. Biol. Chem., **91**, 687 (1931).

9) H. Herlinger, H. Kleimann, and I. Ugi, Justus Liebigs

Ann. Chem., 706, 37 (1967).

10) P.A. Levene, J. Biol. Chem., 120, 759 (1937).

11) C. Reichardt, "Solvent Effects in Organic Chemistry," Verlag Chemie, Weinheim (1979), p. 237.

12) J.F. Kirsch and W.P. Jenks, J. Am. Chem. Soc., 86, 837 (1964); W.P. Jenks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York (1969), chap. 10.

13) W.P. Jenks and M. Gilchrist, J. Am. Chem. Soc., 90, 2622 (1968).

14) M. Charton, J. Am. Chem. Soc., 97, 1552 (1975); Since ν_x values directly corresponding to substrate structure (R-CH(C_6H_5)-) were not found in the literature, ν_x values for alkyl skelton (R-CH_2-) were plotted against ln r' in Fig. 18: ν_x=0.56 for 1-phenylethylamine, 0.68 for 1-phenylpropylamine, 0.68 for 1-phenylbutylamine and 0.98 for 1-phenyl-2-methylpropylamine.

15) O. Cervinka and L. Hub, J. Chem. Soc., Chem. Commun., 1966, 761; Collect. Czech. Chem. Commun., 32, 2295 (1967); A. Horeau, " Sterochemistry Fundamentals and Methods," ed by H.B. Kagan, Georg Thieme Publishers, Vol. 3, Stuttgart (1977), p. 70.

16) C. Reichardt, " Solvent Effects in Organic Chemistry," Verlag Cehmie, New York (1979), p. 237 and p. 270.

17) C. Hansch and T. Fujita, J. Am. Chem. Soc., 86, 1616 (1964).

18) C. Tanford, "The Hydrophobic Effect," John Wiley Sons, New York (1973), chap. 2.

19) W.P. Jenks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York (1969), chap. 8.

20) M. Hirata, K. Abe, H. Tashiro, and M. Nishio, Chem. Lett., **1982**, 777; J. Uzawa, S. Zushi, Y. Kodama, Y. Fukuda, K. Nishihata, K. Umemura, M. Nishio, and M. Hirota, Bull. Chem. Soc. Jpn., **53**, 3623 (1980).

21) J.R. Knowles and C.A. Parsons, Nature, 221, 53 (1969); C.A. Blyth and J.R. Knowles, J. Am. Chem. Soc., **93**, 3017 (1971); C.A. Blyth and J.R. Knowles, J. Am. Chem. Soc., **93**, 3021 (1971); D.G. Oakenfull, J. Chem. Soc., Perkin Trans. 2, 1006 (1973); D.G. Oakenfull, J. Chem. Soc., Chem. Commun ., **1970**, 1655.

22) J.P. Guthrie, J. Chem. Soc., Chem. Commun., **1972**, 897.

23) W.S. Knowles, M.J. Sabacky, and B.D. Vineyard, J. Chem. Soc., Chem. Commun., **1972**, 10; W.S. Knowles, M.J. Sabacky, and B.D. Vineyard, Ann. N.Y. Aced. Sci., **172**, 232 (1970).

24) R. Macleod, F.J. Welch, and H.S. Mosher, J. Am. Chem. Soc., **82**, 876 (1960); M.S. Biernbaum and H.S. Mosher, J. Org. Chem., **36**, 3168 (1971); R. Noyori, I. Tomino, Y. Tanimoto, J. Am. Chem. Soc., **101**, 3129 (1979).

25) "Lipid-Protein Interactions," ed by P.C. Jost and O.H. Griffith, John Wiley Sons, New York (1982), chap. 3.

26) A.B. Edmondson, K.R. Ely, R.L. Girling, E.E. Abola, M. Schiffer, F.A. Westholm, M.D. Fausch, and H.F. Deutsch, Biochemistry, **13**, 3816 (1974).

27) M.O. Dayhoff, R.M. Schwartz, and B.C. Orcutt, "Atlas of Protein Sequences and Structure 5," (Supplement 3), National Biochemistry Research Foundation, Silver Spring (1978), chap. 22.

28) M.J. Harris, T. Higuchi, and J.H. Rytting, J. Phys. Chem., **77**, 2694 (1973),J.A. Reynolds, D.B. Gilbert, and C. Tanford, Proc. Nat. Acad. Sci., USA, **71**, 2925 (1974); N. Tanaka and E.R. Thornton, J. Am. Chem. Soc., **99**, 7300 (1977).

29) J.H. Fendler and E.J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academec Press (1975) J.M. Brown, S.K. Baker, A. Colens, and J.R. Darwent, "Enzymic and non-enzymic catalysis," ed by P. Dunniel, A. Wiseman, and N. Blackebrough, Ellis Horwood Limited. (1980), chap. 5.

30) C. Hansch, Acc. Chem. Res., 2, 232 (1969); D. Henry, J.H. Block, J.L. Anderson, and G.R. Carlson, J. Med. Chem., **19**, 619 (1976).

31) H. Ozaki, A. Tai, S. Matsukiyo, T. Harada, and Y. Izumi, Shokubai (Catalyst), **25**, 314 (1983).