g (0.01 mol) of 7 in 180 mL of ethyl acetate at −55 °C for 15 min. The reaction was stopped before the characteristic blue color of ozone was obvious, to minimize decomposition of the isocoumarin product. The system was flushed with nitrogen for 15 min and then 1 mL of dimethyl sulfide was added; the stirred solution was then allowed to warm to room temperature overnight. The solution was then washed with sodium bicarbonate (2×), 2 N HCl (2×), and saturated NaCl. After drying (Na_2SO_4), the solution was evaporated and the product was recrystallized from methanol: yield, 1.33 g (61%); mp 154–156 °C (lit.[16] mp 157–160 °C); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H), 3.88 (s, 3 H), 3.95 (s, 3 H), 6.08 (s, 1 H), 6.29 (d, 1 H, J = 2.2 Hz), 6.40 (d, 1 H, J = 2.2 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 19.40 (Me), 55.51 (OMe), 56.17 (OMe), 98.03 (C-7), 99.31 (C-5), 102.65 (C-8a), 103.61 (C-4), 142.36 (C-4a), 155.37 (C-3), 159.51 (C-1), 163.14 (C-8), 165.28 (C-6) ppm; H,C-COSY 2D NMR correlated the H's at 2.20, 3.88, 3.95, 6.08, 6.29, and 6.40 ppm with the C's at 19.40, 55.51, 56.17, 103.61, 102.65, and 99.31 ppm, respectively; UV λ_{max} (ε) 320 br (540), 288 sh (580), 276 sh (810), 242 (6400), 235 sh (4100) nm; MS m/e 220 (M^+, 100), 219 (31), 191 (65), 177 (20), 175 (21), 149 (71).

6,8-Dihydroxy-3-methylisocoumarin (1a). The demethylation of isocoumarin **1d** was done by scaling up the procedure of Hill.[18] The solid product obtained was sublimed at 0.1 Torr with the product coming over between 150 and 180 °C; a minor impurity that sublimes at less than 150 °C was removed. The yield was 87%: 1.37 g of **1a**, mp 250–252 °C (lit.[18] mp 250–252 °C); ^{1}H NMR (acetone-d_6) δ 2.22 (s, 3 H), 6.37 (s, 3 H), 9.6 (br s, 1 H), 11.2 (s, 1 H) ppm; ^{13}C NMR (acetone-d_6) δ 19.25 (Me), 99.56 (C-8a), 102.15 (C-7), 103.11 (C-5), 105.03 (C-4), 140.98 (C-4a), 155.30 (C-3), 164.56 (C-8), 166.31 (C-6), 166.99 (C-1) ppm; UV λ_{max} (ε) 329 sh (1900), 304 (3600), 252 (12 000), 241 (9200) nm; MS (solid probe) m/e 192 (M^+, 100), 177 (52), 149 (23), 121 (49), 69 (28), 60 (27), 57 (21), 55 (24).

3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin (2a). Using the procedure of Hill[18], isocoumarin **1d** was hydrogenated with a 10% Pd/C catalyst to give 3,4-dihydro-6,8-dimethoxyisocoumarin, which was then demethylated as above with BBr_3 in methylene chloride to give **2a**: mp 209–211 °C (lit.[18] mp 214–215 °C); ^{1}H NMR (acetone-d_6) δ 1.45 (d, 3 H, J = 6.3 Hz), 2.85 (AB q, 1 H, J = 16 Hz, J' = 11 Hz), 2.96 (AB q, 1 H, J = 16 Hz, J' = 3.6 Hz), 4.70 (m, 1 H), 6.27 (s, 1 H), 6.29 (s, 1 H), 9.44 (s, 1 H), 11.30 (s, 1 H) ppm; ^{13}C NMR (acetone-d_6) δ 20.82 (Me), 35.03 (C-4), 76.33 (C-3), 101.71 (C-4a), 101.92 (C-7), 107.41 (C-5), 143.21 (C-8a), 165.09 (C-8), 165.23 (C-6), 170.69 (C-1) ppm; H,C-COSY showed that the H's at 1.45, 2.85/2.96, 4.70, 6.27, and 6.29 ppm were connected to the C's at 20.82, 35.03, 76.33, 101.92, and 107.41 ppm, respectively; UV λ_{max} (ε) 308 (10 000), 243 (3800) nm.

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Manipulation of Enzymatic Regioselectivity by Structural Modification of Substrates

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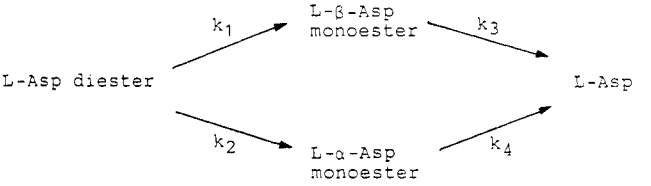
In recent years, hydrolytic enzymes such as proteases and lipases have been extensively used as catalysts for the preparation of optically active compounds.[1] This application has attracted attention of the organic chemists because of their usefulness as chiral catalysts.[2] For organic reactions, selectivity such as enantioselectivity, diastereoselectivity, and regioselectivity is one of the challenges[3] and may often be overcome by biocatalytic reactions.[4] In this article, we describe novel observations which demonstrate that either the α- or β-ester of L-aspartic acid diester may be preferentially hydrolyzed by chymotrypsin by changing the alcohol moiety of esters.

Scheme I



As shown in Scheme I, L-aspartic acid diester is enzymatically converted into two monoesters, which in turn may be further hydrolyzed to free L-aspartic acid. This two-step regioselective ester hydrolysis reaction is similar to that of the tandem asymmetric induction–kinetic resolution described by Sih and his co-workers.[5] Therefore, quantitative prediction of the amount of the diester, the monoesters, and the free amino acid at any extent of conversion can be calculated by using the kinetic parameters E_1, E_2, and α. These three kinetic parameters are defined by the ratios of the four kinetic constants (k_1, k_2, k_3, and k_4).[5] For the dimethyl, diisopropyl, and dibenzyl L-aspartate, the enzymes, subtilisin Carlsberg (Sigma, type VIII) and α-chymotrypsin (from bovine pancreas, 3× crystallized, Sigma, type II), cleave the α-ester group much faster than the β-ester group in both steps ($k_1 \gg k_2$; $k_4 \gg k_3$). Therefore, β-monoesters could accummulate in high yield, but the amount of α-monoesters is too low to be measured at any extent of conversion in the reaction

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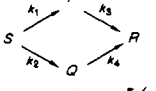
(5) Wang, Y. F.; Chen, C. S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1984**, *106*, 3695. The two-step reactions which have divergent reaction in the first step and convergent reaction in the second step can be expressed quantitatively as follows:



$$P = \frac{\alpha S_0}{(\alpha+1)(1-E_1)}\left[\left(\frac{S}{S_0}\right)^{E_1} - \left(\frac{S}{S_0}\right)\right] \quad (1)$$

$$Q = \frac{S_0}{(\alpha+1)(1-E_2)}\left[\left(\frac{S}{S_0}\right)^{E_2} - \left(\frac{S}{S_0}\right)\right] \quad (2)$$

$$R = S_0 - S - P - Q$$

k_1, k_2, k_3, and k_4 are apparent first-order rate constants. S_0 is the initial concentration of the substrate, where $\alpha = k_1/k_2$, $E_1 = k_3/(k_1 + k_2)$, and $E_2 = k_4/(k_1 + k_2)$.

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Table I. Kinetic Parameters for Hydrolysis of L-Aspartic Acid Diesters by Subtilisin and Chymotrypsin[a]

L-Asp diesters	α	E_1	E_2
subtilisin			
dicyclohexyl ester	18	0.03	0.47
dicyclopentyl ester	11	0.05	0.67
dicycloheptyl ester	10	0.04	0.56
chymotrypsin			
dicyclopentyl ester	0.63	0.08	0.04
dicyclohexyl ester	0.10	1.36	0.07
dicycloheptyl ester	–	–	–

[a] The definition and calculation of kinetic parameters are described in ref 5, and the standard deviations of kinetic parameters are ±30%.

mixtures. Hence, the kinetic parameters, E_1, E_2, and α, could not be calculated in these cases. For the cases of dicyclopentyl and dicyclohexyl L-aspartate, subtilisin Carlsberg still preferentially cleaves the α-esters; conversely, chymotrypsin attacks the β-esters preferentially. In order to calculate the kinetic parameters in these cases, experiments were carried out as follows: 1 mmol of L-aspartic acid diester was dissolved in 15 mL of 0.2 M phosphate buffer, pH 7.0, and then subtilisin (5 mg) or chymotrypsin (20 mg) was added. The reaction mixture was stirred at room temperature, and an aliquot (0.5 mL) was taken at various intervals for amino acid analyses. The amount of free aspartic acid and two monoesters can be measured very accurately, and the kinetic parameters are calculated from these data and are listed in Table I. The percentage of the diesters and the monoesters as a function of the percentage of aspartic acid are plotted by computer (Figure 1). Dicycloheptyl L-aspartate is a good substrate for subtilisin, which prefers to cleave the α-ester. On the other hand, this compound is hardly attacked by chymotrypsin. It is interesting to note that the alcohol moiety of esters in L-aspartic acid diester has a strong influence on the regioselective hydrolysis catalyzed by chymotrypsin.

It is known that the stereospecificity of enzymes could sometimes be altered by modifying the structure of substrates.[6] For chymotrypsin, inversion of enantiospecificity could be achieved in some cases using artificial amino acid derivatives.[7] From the data listed in Table I, chymotrypsin prefers to hydrolyze the β-ester more than the α-ester for the dicyclopentyl and dicyclohexyl esters. However, the α-monoester and the β-monoester coexist in most extent of conversion for the case of dicyclopentyl aspartate and difficult to prepare α-cyclopentyl aspartate by the hydrolysis of chymotrypsin as shown in Figure 1a. As for dicyclohexyl aspartate, only the α-monoester accummulated in high yield (Figure 1b). We believe that the conformation of the cycloalkyl groups of substrates plays an important role in this change of enzymatic regioselectivity. This study provides a facile way to prepare L-aspartate derivatives for peptide synthesis.[8]

Experimental Section

L-Aspartic acid was purchased from Kyowa Fermentation Co., Tokyo, Japan. Solvents for synthesis were from ALPS Chemicals, Inc. Taipei, Taiwan. Thin-layer chromatography was performed on silica gel G. 60 (E. Merck, FRG) precoated on a glass plate. ^{1}H NMR spectra were recorded at 300 MHz. Subtilisin Carlsberg (type VIII) and chymotrypsin (from bovine pancreas, 3× crystallized, type II) were purchased from Sigma. The preparations of L-aspartate diesters were carried out by refluxing the mixture of L-aspartic acid, *p*-toluenesulfonic acid, and the desired alcohol in the benzene solution with a Dean–Stark receiver, and the yields were usually about 90%. The authentic samples of α-cycloalkyl aspartate and β-cycloalkyl aspartate were prepared according to the known chemical procedures.[8] The elution times of aspartic acid and its derivatives in amino acid analyzer (4150 LKB, Sweden) are 15.50 min for aspartic acid, 38.75 min for β-cyclopentylaspartate, 45.80 min for α-cyclopentylaspartate, 44.36 min for β-cyclohexylaspartate, 49.50 min for α-cyclohexylaspartate, 50.80 and 58.10 min for β-cycloheptylaspartate and its α-monoester.

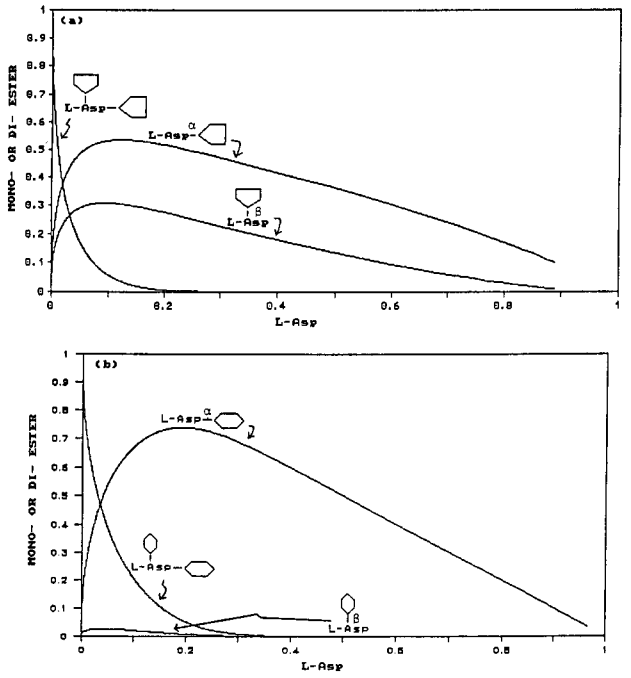

Figure 1. Profile of percent L-aspartic acid diesters and two monoesters as a function of percent free aspartic acid. The curves were generated on the basis of eq 1 and 2 in ref 5. (a) Dicyclopentyl L-aspartate (α = 0.63, E_1 = 0.08, E_2 = 0.04) and (b) dicyclohexyl L-aspartate (α = 0.10, E_1 = 1.36, E_2 = 0.07) hydrolyzed by chymotrypsin.

Hydrolysis of L-Aspartate Dicycloalkyl Esters by Subtilisin. Ten millimoles of the substrate was dissolved in 50 mL of 0.3 M phosphate buffer (pH 7.0), and then 200 mg of subtilisin was added. The reaction mixture was stirred at room temperature until the substrate could not be detected by the TLC method. The reaction mixture was extracted with ethyl acetate to remove the cycloalkyl alcohol, which was released from the substrates. The aqueous phase was collected, concentrated, adjusted to pH 6.2, and cooled in a refrigerator to complete the precipitation of the product, which was collected and dried in vacuo over P_2O_5. In order to confirm the structure of the products, the products were analyzed by amino acid analyses and ^{1}H NMR spectra and compared with those of authentic samples. ^{1}H NMR spectra (D_2O) of β-cycloalkyl aspartate: δ 0.95–1.63 (9 H for cyclopentyl ester or 11 H for cyclohexyl ester or 13 H for cycloheptyl ester, m), 2.80–3.03 (2 H, m), 4.16–4.20 (1 H, t). β-Cyclopentyl aspartate: yield 1.69 g (84%); $[\alpha]^{25}_D$ +1.20° (c = 1.0, 0.1% HOAc). β-Cyclohexyl aspartate: yield 1.7 g (79%); $[\alpha]^{25}_D$ +2.40° (c = 1.0, 0.1% HOAc). β-Cycloheptyl aspartate: yield 1.7 g (74%); $[\alpha]^{25}_D$ +3.60° (c = 1.0, 0.1% HOAc).

Hydrolysis of L-Aspartate Dicyclohexyl Ester by Chymotrypsin. Ten millimoles of the substrate was dissolved in 50 mL of 0.3 M phosphate buffer (pH 7.0), and then 100 mg of chymotrypsin was added. The procedures for the reactions and isolation of the product were the same as those described above.

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The product, α-cyclohexyl aspartate, was identified by amino acid analysis and ^{1}H NMR spectrum. ^{1}H NMR: δ 0.95–1.63 (11 H, m), 2.65–2.85 (2 H, m), 4.16–4.20 (1 H, t, J = 5.1 Hz); yield 1.3 g (60%); $[\alpha]^{25}_D$ +5.2° (c = 2.0, 1% HOAc).

Registry No. H-Asp-OH, 56-84-8; chymotrypsin, 9004-07-3; dicyclopentyl L-aspartate, 121329-69-9; dicyclohexyl L-aspartate, 121329-70-2; dicycloheptyl L-aspartate, 121329-71-3; β-cyclopentyl L-aspartate, 121329-72-4; β-cyclohexyl L-aspartate, 112259-66-2; β-cycloheptyl L-aspartate, 107164-80-7; α-cyclohexyl L-aspartate, 121329-73-5; subtilisin, 9014-01-1.

Camphoryl Sulfide as a Chiral Auxiliary and a Mediator for One-Step Synthesis of Optically Active 1,2-Diaryloxiranes

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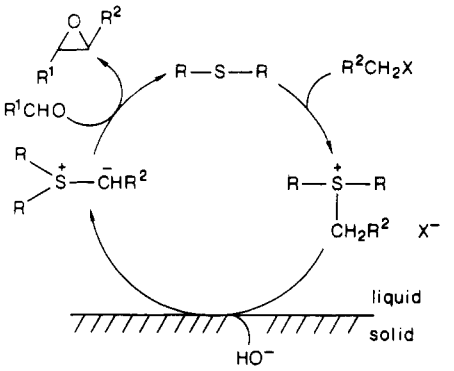
Sulfonium salts have been known to serve as either alkylating or alkylidene transfer agents after conversion to the corresponding ylides, which are widely employed in synthetic organic chemistry.[1] One advantage of sulfur ylides in organic synthesis is that the starting sulfides can be readily recovered after treatment of ylides with, e.g., carbonyl compounds and recycled to regenerate the starting ylides. Recently, we reported a convenient one-pot synthesis of oxiranes using a reaction system composed of alkyl sulfides, alkyl halides, and aldehydes in the presence of solid KOH under phase-transfer conditions.[2] In these reactions, the sulfides are converted initially to sulfonium salts and then subsequently to the corresponding ylides in situ under phase-transfer conditions. Thus, the sulfide works as a mediator that transfers an alkyl group to the aldehyde. The reaction cycle is shown in Scheme I. As a modification of this reaction, if one used an optically active sulfide as a mediator, one could obtain optically active oxiranes in one pot. We tried several optically active sulfides and found that those derived from camphorsulfonic acid work as both mediators and chiral auxiliaries for the formation of optically active oxiranes in moderate enantiomeric excess. Herein we report the results.

Results and Discussion

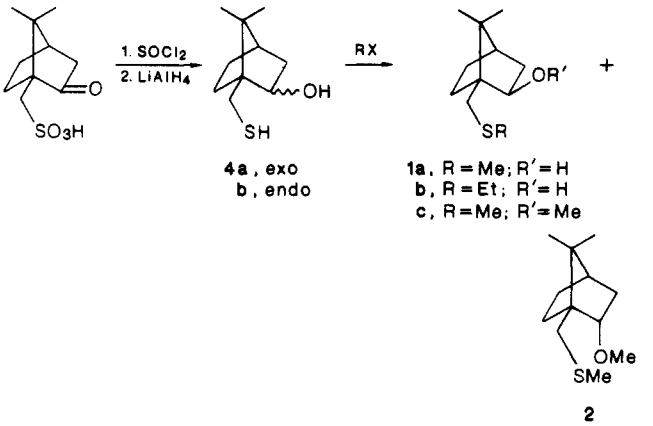
Optically active sulfides **1** and **2** used in the reactions were prepared starting from (+)-camphorsulfonic acid in three steps as shown in Scheme II.

Generally, the synthesis of optically active oxiranes was carried out by using an equimolar amount of aryl aldehyde (3 mmol) and benzyl bromide (3 mmol) in the presence of a half molar equivalent of the sulfide **1** (1.5 mmol) in THF or CH_3CN under liquid–solid two-phase conditions. Powdered KOH was used as a base. The products were separated and identified by NMR and IR spectra and elemental analysis. The yields and enantiomeric excess of the oxiranes thus obtained are presented in Table I. In order to elucidate the substituent effects on the chemical yields and enantiomeric excess for preparation of *trans*-1,2-diaryloxiranes, several para-substituted aryl aldehydes and benzyl bromides were reacted by using the sulfide **1a** as a mediator and the results are summarized in Table II. Recently, Sharpless and his co-workers reported a one-step synthesis of optically active oxiranes in high chemical and optical yields.[4] On the other hand, Wynberg et al. also prepared optically active oxiranes in moderate optical

Scheme I. Reaction Cycle for Formation of Oxiranes



Scheme II

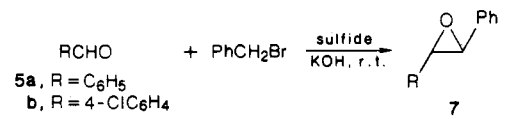


4a, exo
b, endo

1a, R = Me; R′ = H
b, R = Et; R′ = H
c, R = Me; R′ = Me

2

Table I. Preparation of Optically Active Oxiranes

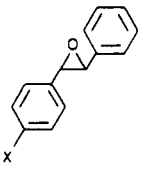


5a, R = C_6H_5
b, R = 4-ClC_6H_4

7

sulfide	RCHO	solvent	time, h	yield,[e] %	% ee[a]	confgn
1a	**5a**	CH_3CN	36	100[b]	47	(+)-*R,R*
1c	**5a**	THF	48	16[b]	14	(−)-*S,S*
1a	**5b**	THF	36	15[c]	34	(+)-*R,R*
1a	**5b**	CH_3CN	36	100[c]	43	(+)-*R,R*
1a[d]	**5b**	CH_3CN	36	230[c]	31	(+)-*R,R*
1b[d]	**5b**	CH_3CN	36	90[c]	31	(+)-*R,R*
1c	**5b**	THF	48	30	28	(−)-*S,S*
2	**5b**	THF	48	17	7	(+)-*R,R*

[a] Determined by HPLC analysis using Chiralpack OT(+) of Daicel Chemical Ind., Ltd. [b] Product is **7a**. [c] Product is **7b**. [d] Mole ratio of RCHO/$PhCH_2Br$/sulfide = 10:10:1. [e] The yields were calculated on the basis of the sulfide used in the reactions.



7a, X = H
b, X = Cl

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