Asymmetric Hydrolysis of Prochiral Diesters with Pig Liver Esterase. Preparation of Optically Active Intermediates for the Synthesis of (+)-Biotin and (+)-α-Methyl-3,4-dihydroxyphenylalanine

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With pig liver esterase, 1,3-dibenzyl-4,5-cis-bis(alkyloxycarbonyl)-2-oxoimidazolidine (1) was asymmetrically hydrolyzed to (4S,5R)-1,3-dibenzyl-5-alkyloxycarbonyl-2-oxoimidazolidine-4-carboxylic acid (2). This acid 2 was reduced with lithium borohydride to (4S,5R)-1,3-dibenzyl-5-hydroxymethyl-2-oxoimidazolidine-4-carboxylic acid lactone (3), which is known to be converted to (+)-biotin (4). With the same esterase, diethyl 3,4-dimethoxyphenylmethyl(methyl)malonate (5) was asymmetrically hydrolyzed to (R)-ethyl hydrogen 3,4-dimethoxyphenylmethyl(methyl)malonate (6), which can be converted to (S)-α-methyl-3,4-dihydroxyphenylalanine (L-α-methyldopa) (9).

Asymmetric hydrolysis of the esters with enzymes or microorganisms is widely used as a convenient method to obtain optically active esters, acids or alcohols: The esters having an asymmetric center(s) at the alcohol part—(±)-cyclopentenyl acetate derivative for the prostaglandin synthesis, (±)-terpenyl acetates, (±)-acetates of alkynyl alcohols and α-hydroxy esters, (±)-1,2-diacetoxy-3-halo-propanes and (±)-1-acetoxy-2,3-dichloropropane—were asymmetrically hydrolyzed with lipases or microorganisms. The esters having an asymmetric center at the acid part—(±)-α-amino acid esters and (±)-α-substituted carboxylic acid esters—were asymmetrically hydrolyzed with chymotrypsin or microorganisms. Additionally, the prochiral diesters, such as dimethyl β-hydroxy-β-methylglutarate and the β-aminoglutarate ester derivatives, were asymmetrically hydrolyzed with pig liver esterase or chymotrypsin to produce the corresponding optically active acids.

We report here the asymmetric hydrolysis of three prochiral diesters—1,3-dibenzyl-4,5-cis-bis(alkyloxycarbonyl)-2-oxoimidazolidine (1) for the synthesis of (+)-biotin, diethyl 3,4-dimethoxyphenylmethyl(methyl)malonate (1) for the synthesis of antihypertensive (S)-α-methyl-3,4-dihydroxyphenylalanine (L-α-methyldopa) (9) and diethyl acetylamino(methyl) malonate (10)—with pig liver esterase.

(4S,5R)-1,3-Dibenzyl-5-hydroxymethyl-2-oxoimidazolidine-4-carboxylic acid lactone (3), a chiral key intermediate for the synthesis of (+)-biotin by the Hoffmann-La Roche process, has been synthesized by the reduction of either (4S,5R)-2 (R=cyclohexyl), obtained by the optical resolution of (+)-2 (R=cyclohexyl) with (-)-ephedrine, or (4S,5R)-2 (R=cholesteryl), obtained by the reaction of the anhydride of dicarboxylic acid 1 (R=H) with cholesterol. As an alternative method we attempted to prepare (4S,5R)-2 by asymmetrically hydrolyzing the prochiral meso-diester 1 with enzymes. The diester 1 has a special...
structure in which both the natural (S)-amino acid part (right-hand side) and the unnatural (R)-amino acid part (left-hand side) are combined in the same molecule as shown in Fig. 1. Enzymes are expected to preferentially hydrolyze the (S)-ester rather than the (R)-ester in 1. We investigated the hydrolysis of 1 with enzymes available and found that pig liver esterase hydrolyzed 1 with an appreciable asymmetric selectivity.

Hydrolysis of the diester 1 (R=Me) with pig liver esterase in the phosphate buffer (pH 7, 0.1 M) gave (+)-1,3-dibenzyl-5-methoxy-2-oxoimidazolidine-4-carboxylic acid (2; R=Me) in 71% (99% conversion) yield. Reduction of (+)-2 (R=Me) with lithium borohydride12) gave lactone 3 of [α]D +22.0° in 85% yield. Comparison of this optical rotation with the reported value12) showed that the product was (4S,5R)-3 of 38% enantiomeric excess (e.e.). This result indicated that the esterase hydrolyzed preferentially the (S)-amino acid ester in 1 (R=Me) to produce (4S,5R)-2 (R=Me) as expected.

Furthermore, the hydrolysis of the dipropyl ester 1 (R=n-Pr) with the same esterase gave (++)-2 (R=n-Pr) in 85% yield. Reduction of (++)-2 (R=n-Pr) with lithium borohydride12) gave (4S,5R)-3 of [α]D +43.8° (75% e.e.) in 64% yield. Recrystallization of this product gave (4S,5R)-3 of 87% e.e. This optical purity was confirmed by an NMR study with a chiral shift reagent Eu(tfc)₃. It is worth noting that the dipropyl ester 1 (R=n-Pr) was enzymatically hydrolyzed with a greater selectivity than the dimethyl ester 1 (R=Me). This result led us to examine the hydrolysis of a more sterically hindered diisopropyl ester 1 (R=i-Pr) with the same esterase, but 1 (R=i-Pr) was not hydrolyzed at all.

Meanwhile, (R)-alkyl hydrogen 3,4-dimethoxyphenylmethyl (methyl) malonate (6) is a chiral intermediate for the synthesis of (S)-α-methyl-3,4-dihydroxyphenylalanine (9) by the Hoechst process and was obtained by the optical resolution of (±)-6 (R=Me) with quinine.13) We planned to obtain (R)-6 by hydrolyzing asymmetrically the prochiral diester 5 with enzymes and found that pig liver esterase hydrolyzed 5 with an appreciable selectivity (Fig. 2).

Hydrolysis of 5 with the esterase gave (−)-6 in 86% yield. According to the reported method,13) (−)-6 was converted via a carbonylazide 7 to (−)-N-acetyl-α-methyl-3,4-dimethoxyphenylalanine ethyl ester (8) in 71% yield. The optical purity of (−)-8 thus obtained was estimated to be 59% e.e. by an NMR study with a chiral shift reagent Eu(tfc)₃ and its absolute configuration was deduced to be (S) on the basis of the fact that the (S)-methyl ester 8 (R=Me) had the (−)-rotation.14) This result showed that the esterase preferentially hydrolyzed the pro-(S)-ester...
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\begin{align*}
\text{NHAc} & \quad \text{esterase} \quad \text{NHAc} \\
\text{CH}_3\text{C}()\text{CO}_2\text{Et}_2 & \quad \text{CH}_3\text{C}()\text{CO}_2\text{Et} \\
\text{CO}_2\text{H} & \quad \text{IO}_2\text{H}
\end{align*}
\]

\[10 \quad (+)-11\]

Fig. 3. Enzymatic Preparation of (+)-Ethyl Hydrogen Acetylamino(methyl)malonate (11).

group of 5 to produce (R)-6. The (R)-ester 8 is known to be converted to L-α-methyldopa (9).

Similar hydrolysis of diethyl acetylamino (methyl) malonate (10) with pig liver esterase produced (+)-ethyl hydrogen acetylamino (methyl) malonate (11) (Fig. 3). The esterase from pig liver was found to be an interesting enzyme that has an ability to asymmetrically hydrolyze the prochiral diesters such as 1 and 5. Enzymes like this esterase need to be obtained from a microbial origin, because this esterase is expensive.

**EXPERIMENTAL**

Melting points were taken on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi EPI-G3 spectrometer and NMR spectra were measured on Varian HA-100 and T-60 spectrometers. Column chromatography was done with silica gel (Wakogel C-200). Pig liver esterase solution (30mg in 3 ml) (Boehringer Mannheim) and the enzymes described in the previous paper were used. Esterification was done by the method reported using thionyl chloride.

**Synthesis of 1,3-dibenzyl-4,5-cis-bis(methoxycarbonyl)-2-oxoimidazolidine (1; R=Me).** Thionyl chloride (0.41 g, 3.5 mmol) was added dropwise with stirring to a solution of 500mg (1.40 mmol) of 1,3-dibenzyl-2-oxoimidazolidine-4,5-dicarboxylic acid (1; R=H) in methanol (20 ml) cooled with water, and the mixture stirred at 40°C for 2hr. The mixture was then concentrated to give crystals (525mg) which were recrystallized from methanol to give 395mg (74%) of 1 (R=Me), mp 109- 111°C (from benzene-hexane); IR νmax cm⁻¹: 1700, 1735, 1750; NMR (10% in CDCl₃) δ: 1.20 (3H, d, J=6.5Hz), 3.93 (2H, s), 4.50 (4H, septet, J=6.5 Hz), 7.20 (10H, m).

**Synthesis of dipropyl ester 1 (R=n-Pr).** A mixture of thionyl chloride (0.98 g, 8.2 mmol) and 1 (R=H) (110mg, 0.31 mmol) in isopropanol (5 ml) was refluxed for 20 hr and concentrated to dryness. As the residue still contained a monocarboxylic acid 2 (R=n-Pr), the esterification reaction was repeated. The reaction mixture was next concentrated and dissolved in dichloromethane. The organic solution was washed with dil. NaHCO₃, dried, concentrated and chromatographed with benzene. Elution with benzene-ethyl acetate (97 : 3) gave 112mg (82%) of 1 (R=n-Pr) as an oil, IR νmax cm⁻¹: 1720, 1750; NMR (10% in CDCl₃) δ: 1.20 (3H, d, J=6.5Hz), 3.93 (2H, s), 4.50 (4H, septet, J=6.5 Hz), 4.94 (2H, q, J=6.5 Hz), 7.20 (10H, m).

**Asymmetric hydrolysis of 1 (R=Me) to (4S,5R)-1,3-dibenzyl-5-methoxycarbonyl-2-oxoimidazolidine-4-carboxylic acid (2; R=Me) with pig liver esterase and the chemical conversion of (4S,5R)-2 (R=Me) to (4S,5R)-1,3-dibenzyl-5-hydroxymethyl-2-oxoimidazolidine-4-carboxylic acid lactone (3).** Dimethyl ester 1 (R=Me) (126 mg, 0.33 mmol) was dissolved in methanol (4 ml) with warming. To the solution were successively added with stirring the sodium phosphate buffer (pH 7, 0.1 m) (5 ml) and the pig liver esterase solution (3 mg in 0.3 ml). The mixture was stirred at 25°C for 24 hr, basified with dil. NaHCO₃ and extracted with ethyl acetate to remove the unreacted 1. From the extract was recovered 38mg (0.1 mmol) of 1. The aqueous layer was acidified with dil. H₂SO₄ and extracted with ethyl acetate. The extract was finally dried and concentrated to give (4S,5R)-2 (R=Me) (84 mg, 99%), 2lβ +2.75° (c=0.40, CHCl₃).

According to the method which was described for ester 2 (R=cyclohexyl), (4S,5R)-2 (R=Me) (82 mg, 0.22 mmol) was reduced with LiBH₄. The reduced product was chromatographed with benzene-hexane (7 : 3). Elution with benzene-ethyl acetate (96 : 4) gave (4S,5R)-3 (60 mg, 85%), 3β +22.0° (c=0.30, benzene). The optical purity of this product was estimated to be 38% e.e. on the basis of the reported value of [α]β +58.2° (c=1, benzene).

**Asymmetric hydrolysis of dipropyl ester 1 (R=n-Pr) to (4S,5R)-1,3-dibenzyl-5-hydroxymethyl-2-oxoimidazolidine-4-carboxylic acid lactone (3).** Dipropyl ester 1 (R=n-Pr) (183 mg, 0.42 mmol) was dissolved in acetone (3 ml). To the stirred solution were successively added the phosphate buffer (pH 7, 0.1 m) (15 ml) and the esterase solution (9 mg in 0.9 ml). The suspension was stirred at 25°C for 24 hr, basified with dil. NaHCO₃ and washed with ethyl acetate. The aqueous layer was acidified and extracted...
with ethyl acetate. The extract was finally dried and concentrated to give \( (4S,5R)-2 \) \((R=\text{-Pr}) \) (141 mg, 85%), \([\alpha]_D^20 + 43.8^\circ \) (c = 0.73, benzene) (75% e.e.).

According to the method,\(^1\)\(^2\) the product was reduced with LiBH\(_4\) to \( (4S,5R)-3 \) (73 mg, 64%), \([\alpha]_D^7 + 43.8^\circ \) (c = 0.73, benzene) (75% e.e.). Recrystallization from benzene-cyclohexane gave 3 (50 mg), mp 107 ~ 112°C; \([\alpha]_D^7 + 50.4^\circ \) (c = 0.50, benzene) (87% e.e.).

IR \( \nu \text{cm}^{-1} \): 700, 1210, 1420, 1440, 1705, 1780.

100 MHz NMR (10% in CDCl\(_3\)) \( \delta \): 3.78-4.12 (4H), 4.41 (2H, ABq, \( \delta = 15\text{Hz} \), CH\(_2\)Ph), 4.63 (2H, ABq, \( \delta = 15\text{Hz} \), CH\(_2\)Ph), 7.25 (10H, m).

The optical purity (87% e.e.) obtained by comparison with the reported value\(^2\) was confirmed as follows: The 100 MHz NMR spectrum of the product in the presence of \( \text{Eu}(\text{tfc})_3 \) (0.2 mol eq) showed its optical purity to be ca. 86% e.e., on the basis of a lower field doublet of an AB quartet at \( \delta = 4.63 \), the doublet signals of \( (4S,5R)-3 \) and \( (4R,5S)-3 \) being shifted downwards by 3.92 and 3.55 ppm, respectively.

Asymmetric hydrolysis of diethyl 3,4-dimethoxy-phenylmethyl (methyl) malonate (5) to \( (R)-\text{ethyl hydrogen 3,4-dimethoxyphenylmethyl (methyl) malonate (6) and the } \)

chemical conversion of \( (R)-6 \) to \( (S)-\text{N-acetyl-a-methyl-3,4-dimethoxyphenylalanine ethyl ester (8). To a stirred solution of 5 (200mg, 0.62 mmol) in ethanol (1 ml) was added the phosphate buffer (pH 7, 0.1 m) (4ml). To the suspension was added the esterase solution (2mg in 0.2ml), and the mixture stirred at 25°C for 21 hr. Water was added and the mixture extracted with dichloromethane. From the extract was recovered 5 (94mg, 0.29 mmol). The aqueous solution was then acidified and extracted with dichloromethane. The extract was finally dried and concentrated to give \( (R)-6 \) (84mg, 86%), \([\alpha]_D^2 \) + 2.38° (c = 1.68, CHCl\(_3\)).

NMR (10% in CDCl\(_3\)) \( \delta \): 1.26 (3H, t, \( \delta = 7\text{Hz} \)), 1.37 (3H, s), 3.18 (2H, s), 3.80 (6H, s), 4.20 (4H, q, \( \delta = 7\text{Hz} \)), 6.68 (3H, m), 10.07 (1H, s).

According to the method\(^3\) which has been reported for \( \text{L-methyl ester 6 (R = Me), (R)-ethyl ester 6 was converted to carbonylazide 7, NMR (10% in CDCl} \(_3\) \( \delta \): 1.27 (3H, t, \( J = 7.5\text{Hz} \)), 1.35 (3H, s), 3.18 (2H, s), 3.87 (6H, s), 4.20 (2H, q, \( J = 7.5\text{Hz} \)), 6.72 (3H, m) and then 7 was transformed to (S)-8. The product 8 was purified by chromatography with dichloromethane to give (S)-8 (62 mg, 71%), \([\alpha]_D^2 \) + 23.8° (c = 0.5, acetone); 100 MHz NMR (10% in CDCl\(_3\) \( \delta \): 1.30 (3H, t, \( J = 7.5\text{Hz} \)), 1.62 (3H, s), 1.92 (3H, s), 3.27 (2H, ABq, \( J = 13.5\text{Hz} \)), 3.76 (3H, s), 3.79 (3H, s), 4.18 (2H, q, \( J = 7.5\text{Hz} \)), 6.14 (1H, br s), 6.47 ~ 6.77 (3H). The 100 MHz NMR spectrum of (S)-8 in the presence of \( \text{Eu}(\text{tfc})_3 \) (0.22 mol eq) showed its optical purity to be 59% e.e. by a singlet signal due to \( \alpha-\text{CH}_3 \), the signals of the (S)-isomer and (R)-isomer being shifted downwards by 1.19 and 0.95 ppm, respectively.

Hydrolysis of diethyl acetylamino (methyl) malonate (10) with the esterase. To a stirred solution of 10 (161 mg, 0.70 mmol) in ethanol (1 ml) were added the phosphate buffer (pH 7, 0.1 m) (4ml) and the esterase solution (2 mg in 0.2 ml) and then the mixture stirred at 25°C for 24 hr. It was extracted with ethyl acetate to give 10 (45 mg, 0.195 mmol) recovered. The aqueous solution was acidified and extracted with ethyl acetate to produce ethyl hydrogen acetylamino(methyl)malonate (11) (83 mg, 81%). \([\alpha]_D^2 \) + 33.4° (c = 0.83, EtOH); NMR (10% in CDCl\(_3\) \( \delta \): 1.27 (3H, t, \( \delta = 7.5\text{Hz} \)), 1.35 (3H, s), 3.18 (2H, s), 3.87 (6H, s), 4.20 (2H, q, \( J = 7.5\text{Hz} \)).

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