Synthesis of the Optical Isomers of 4-[1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxybenzoic Acid (S-2) and Their Biological Evaluation as Antilipidemic Agent[1)]

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The enantiomers of (±)-4-[1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxybenzoic acid (S-2), a new antilipidemic agent having dual action on the plasma triglyceride (TG) and cholesterol (Cho) lowering effects, were prepared *via* separation by Chiralcel OJ column chromatography of their methyl ester and also by the same method as the described racemate's synthesis from optically active 1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-carboxylic acid respectively. These optically active carboxylic acids were prepared by the resolution of diastereomeric *N*-[(*S*)-(−)-[4-methyl-(α-methyl)benzyl]]-1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-carboxyamide using silica gel column chromatography, followed by deamination with N_2O_4. The absolute configurations for the enantiomers of S-2 were indirectly determined using X-ray analysis of the 4-bromo-2-fluorobenzamide of the (+)-4-[1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]-methyloxybenzoic acid. S-2 and its enantiomers showed an essentially equipotent activity on the fatty acid- and sterol-biosynthesis inhibition *in vitro*. On the other hand, in the *in vivo* activity, (*S*)-(+)-4-[1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxybenzoic acid (S-2E) was superior in the lowering abilities of the plasma TG and phospholipid(PL) and was chosen as a candidate for a novel antilipidemic agent. The difference in the *in vivo* activity among S-2 and its enantiomers was explained from the pharmacokinetics after administration *p.o.*

Key words optical isomer; antilipidemic agent; S-2E; X-ray crystallographic; pharmacokinetics

Pravastatin[3)] and Simvastatin,[4)] 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are representative antihyperlipemic agents, widely used in the clinic for drug treatment of hypercholesterolemia. These drugs decrease the serum cholesterol (Cho) level by the inhibition of sterol-biosynthesis in the liver, but their reducing effect on serum triglyceride (TG) is not remarkable. Recently, with the view for prevention of coronary heart disease involving atherosclerosis, the search for novel antihyperlipemic agents which decrease both serum TG and Cho levels has been in the limelight.[5)]

In our previous paper,[1)] we reported the synthesis of racemic 4-[1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]-methyloxybenzoic acid [S-2], which showed potent antihypercholesteromic and antilipidemic activities based on the inhibition of fatty acid- and sterol-biosynthesis. S-2 possesses one chiral center at the 4-position on the pyrroridinone ring resulting in the existence of two optical isomers. It is well known that in most cases, there are differences in the potency of the activity, toxicity and the biotransformation between the enantiomers. Therefore, it was of interest to us to find out the candidate drug with a good balance in the biological comparison (efficacies in the *in vivo* and safety test) of the enantiomers of S-2 (racemate). The present paper describes the preparation of the each enantiomer [(*S*)-(+)-**1** and (*R*)-(−)-**1**] through an optical resolution by a chiral column separation, as well as an optical resolution of 1-(4-*tert*-butyl)phenyl-2-pyrrolidone-4-carboxylic acid (**3**) and the determination of the absolute configuration for each enantiomer by X-ray analysis. The biological comparison of S-2 with its enantiomers is also reported.

Optical Resolution of S-2 [(±)-1] *via* Separation of Methyl Ester (±)-2 by HPLC Initial attempts at the optical resolution of racemate, S-2 [(±)-**1**] using the method of recrystallization of the diastereomeric salts with optically active amines were unsuccessful. Then with further work into the optical resolution, we planned optical separation by HPLC on the methyl ester (±)-**2**. Separation of the individual enantiomers of **2** was achieved using a Chiralcel OJ column[6)] by HPLC. The chromatographic profile of (±)-**2** and conditions are shown in Fig. 1. It was possible to separate the enantiomers of **2** on a preparative scale to ≧99% purity by HPLC. The ^{1}H-NMR and MS of the individual samples were identical with those of (±)-**2**. Moreover, samples collected from the two peaks of **2** gave opposite optical rotations; the 1st peak showed (+) optical rotation and the 2nd peak, (−) optical rotation. The optically active (+)-**2** and (−)-**2** obtained were subjected to hydrolysis to afford the optically active (+)-**1** and (−)-**1**, respectively. The preparative procedures for (+)-**1** and (−)-**1** are shown in Chart 2.

Determination of the Absolute Configuration for the Enantiomers 1 The absolute configuration of the enantiomers **1** was determined unequivocally by X-ray analysis. The optically pure enantiomer (+)-**1** was converted to the 4-bromo-2-fluorobenzamide of (+)-**1** for X-ray analysis. The absolute configuration of the 4-bromo-2-fluorobenzamide of (+)-**1** was determined by the anomalous dispersion effect of the bromine atoms. The structure was solved by the direct method with MULTAN78.[7)] On the basis of X-ray study on the 4-bromo-2-fluorobenzamide of (+)-**1**, the absolute stereochemistry of (+)-**1** and (+)-**1** were indirectly confirmed to be *S*-form and *R*-form, respectively.

Syntheses of Optically Active Isomers [(+)- and (−)-1] Optical resolution of pyrrolidone-carboxylic acid [(±)-**3**]

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was conveniently achieved by converting it into a diastereomeric amide with an optically active amine. Acid chlorination of (±)-**3**, and subsequent coupling with (*S*)-(−)-**1**-(*p*-tolyl)ethylamine in the presence of triethylamine gave an approximately 1 : 1 mixture of the diastereomeric amides (**4A**, **4B**). By using column chromatography, optically active **4A** and **4B** were obtained from the first elution and continuous second elution, respectively. They were subjected to deamination according to the method of White[8] which afforded the optically active (*R*)-(−)-**3** from **4A** and (*S*)-(+)-**3** from **4B**, respectively as shown in Chart 3. Reduction of (*S*)-(+)-**3** and (*R*)-(−)-**3** with BH_3–tetrahydrofuran (THF) afforded the alcohols (*S*)-(−)-**5** and (*R*)-(+)-**5**, respectively. Subsequently mesylation of (*S*)-(−)-**5** and (*R*)-(+)-**5** with mesylchloride (MsCl) in the presence of triethylamine afforded the mesyl derivatives, (*S*)-(+)-**6** and (*R*)-(−)-**6**, respectively. The coupling of the mesyl compounds with methyl *p*-hydroxybenzoate at the presence of K_2CO_3 in *N*,*N*-dimethylformamide (DMF) gave (*S*)-(+)-**2** and (*R*)-(−)-**2**, respectively. Finally, they were hydrolyzed by heating with AcOH–HCl solution to give (*S*)-(+)-**1** from (*S*)-(+)-**2** and (*R*)-(−)-**1** from (*R*)-(−)-**2** as shown in Chart 4. Their structures were identified by comparison with the ^{1}H-NMR of the former optical compounds obtained by chiral column separation method. Moreover, their optical purities were determined by HPLC analysis using a chiral OD column[6] to be more than 99.2% ee and this data showed no isomerization took place during the reaction steps from the compound **3** to **1**.

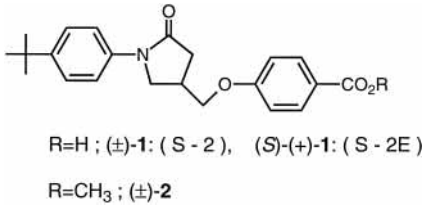

Chart 1

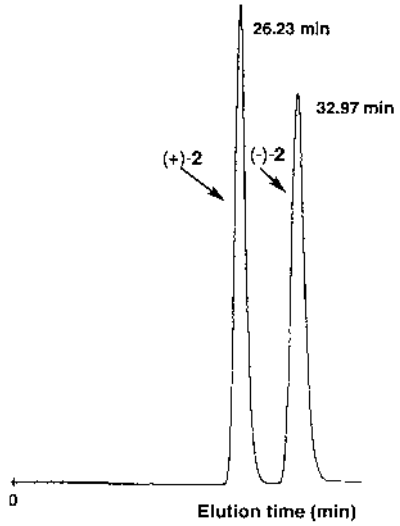

Fig. 1. HPLC Chromatogram of (±)-**2**

A 2 μl sample (10 mg in EtOH 2 ml) of (±)-**2** was injected onto a Chiralcel OJ column at 40 °C. The sample was eluted with *n*-hexane/ethanol(80 : 20) at a flow rate of 1 ml/min. Sample detection was monitored by UV absorption at 254 nm.

Biological Results and Discussion

The inhibitory activities against fatty acid- and Cho-biosynthesis of the racemate (±)-**1**, (S-2) and its enantiomers [(*S*)-(+)-**1** and (*R*)-(−)-**1**] were evaluated *in vitro* using rat liver slices according to the method of the previous paper.[1]

As shown in Table 1, the inhibitory activities *in vitro* of the two stereoisomeric compounds (enantiomers of S-2) were nearly equivalent with that of racemate (S-2).

Furthermore, in order to compare the three compounds in

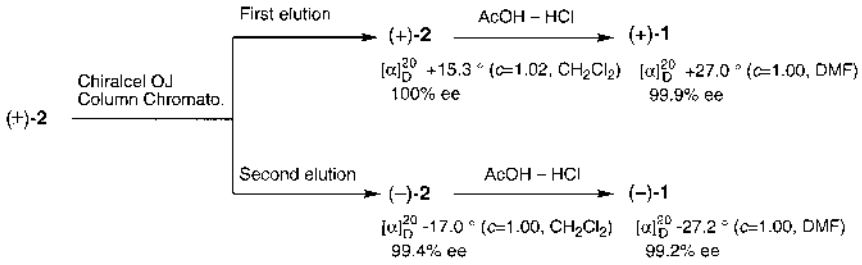

Chart 2

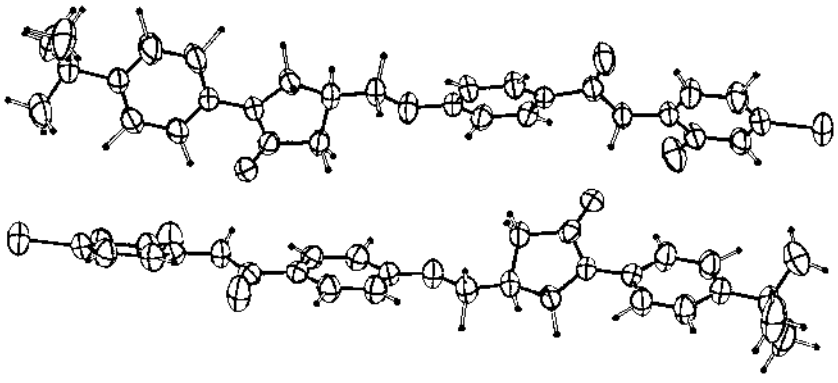

Fig. 2. Molecular Structures of the Two Independent Molecules in the 4-Bromo-2-fluorobenzamide of (+)-**1**

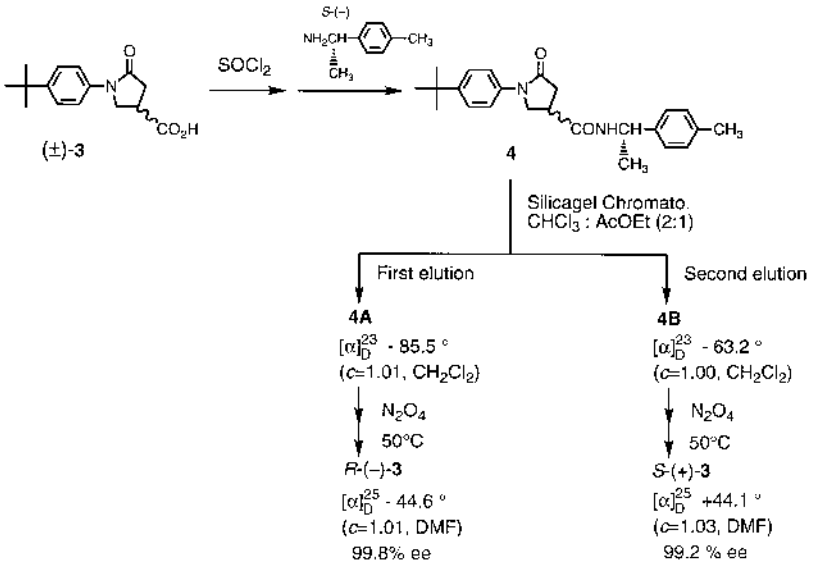

Chart 3

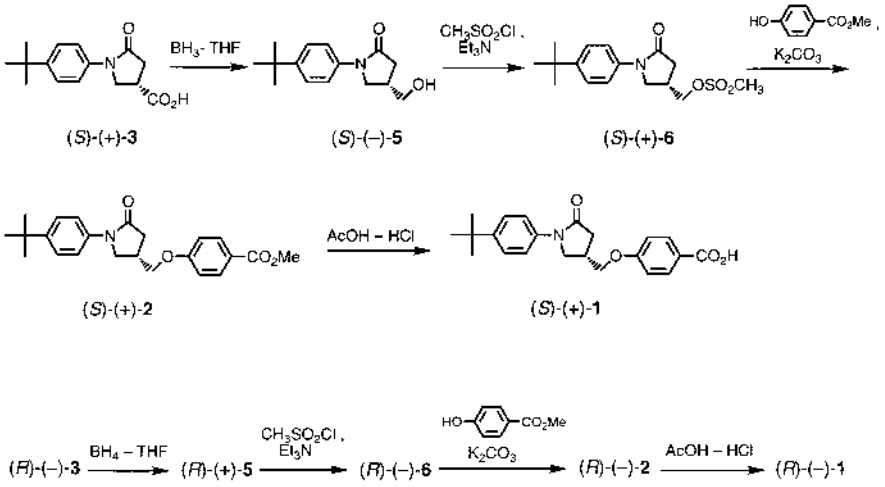

Chart 4

Table 1. Inhibitory Activities against Fatty Acid- and Sterol-Biosynthesis of Racemate (±)-**1**, *S*-(+)-**1** and *R*-(−)-**1** using Rats Liver Slices

Compd.	IC_{50} (μM)	
	Fatty acid	Sterol
Racemate (±)-**1**	4.9	11.4
S-(+)-**1**	4.8	11.7
R-(−)-**1**	4.5	12.0

in vivo, the plasma Cho, TG and phospholipid (PL) lowering effects on these three compounds [racemate {(S-2), (±)-**1**}, (*S*)-(+)-**1** and (*R*)-(−)-**1**] were assessed according to Kusama *et al.*[9] using Zucker fatty rats as the hypolipidemic model animal. When racemate, (±)-**1** and it's enantiomers [(*S*)-(+)-**1**, (*R*)-(−)-**1**] were orally administered at 30 mg/kg/d for 7 d, the levels of plasma TG and PL decreased remarkably on the third day after administration and the effects continued until the 7th day. Particularly, in comparison of the three compounds, the *S*-enantiomer [(*S*)-(+)-**1**] showed the most potent reducing effects on the 7th day (TG; 82.5%, PL; 44.7%). The evaluation of the plasma Cho lowering efficacy in the rodent *in vivo* based on the HMG-CoA reductase inhibitory activity is not general because it is well known[10] to be a disadvantage to use a rodent model. Therefore, as shown in Table 2, a significant difference in activity was not seen among the three compounds. On the other hand, as shown in Tables 1 and 2, though the *in vitro* inhibitory activities among three compounds [racemate (±)-**1**, (*S*)-(+)-**1**, (*R*)-(−)-**1**] were nearly similar, the reducing effects on plasma TG and PL *in vivo* were extremely different. To investigate the reason why, the pharmacokinetics of the three compounds were studied using SD rats at a dosage of 25 mg/kg, *p.o.* Data are shown in Table 3; both the *C*-max and area under the curve (*AUC*)-values of (*S*)-(+)-**1** in plasma were higher than that of racemate (±)-**1** and (*R*)-(−)-**1**. Moreover, the values of (*S*)-(+)-**1** on liver were also similar to the tendency of plasma and the plasma concentration of these three compounds was also measured concurrently the *in vivo* testing on Zucker fatty rats as shown in Table 2.

The plasma concentration of (*S*)-(+)-**1** was about 5 fold higher than that of the other two compounds, racemate (±)-**1**

Table 2. Hypolipidemic Effects and Concentration of Racemate (±)-**1** and Its Enantiomers (*S*-(+)-**1**, *R*-(−)-**1**) on the Plasma in Zucker Fatty Rats (n=4)

Compound	Hypolipidemic effects (% of reduction)						Concentration (μg/ml)	
	TC		TG		PL			
	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d
Racemate (±)-**1**	19.1	17.6	53.5	49.4	19.9	23.8	0.45	0.28
S-(+)-**1**	21.8	32.8	69.6*	82.5**	24.9	44.7*	1.16	1.48
R-(−)-**1**	37.3	38.6	50.8	59.5	30.3	38.7	0.38	0.29

*, **; Statistical significance from the control at $p<0.05$ and $p<0.01$ (Dunnett's test), respectively.

Table 3. Pharmacokinetics of Racemate (±)-**1**, *S*-(+)-**1** and *R*-(−)-**1** after Administration with a Dosage of 25 mg/kg, *p.o.* in Rats

Compd.	Plasma		Liver	
	C max[a]	*AUC*[b]	*C* max[c]	*AUC*[d]
Racemate (±)-**1**	9.3	87.4	38.6	673.5
S-(+)-**1**	16.9	166.6	49.4	854.9
R-(−)-**1**	10.0	75.8	34.2	585.7

a) μg/ml, *b*) 0—48 h; μg·h/ml, *c*) μg/g, *d*) 0—48 h; μg·h/g.

Table 4. Observed and Calculated Bijvoet Pair Ratio on the Determination of the Absolute Configuration

h	*k*	*l*	*F*c (+)	*F*c (−)	*F*o (+)	*F*o (−)
0	2	1	116.17	113.68	120.14	117.60
0	3	1	79.68	75.16	81.74	76.76
1	2	1	62.91	64.91	63.74	65.75
−3	1	2	89.72	87.36	96.64	93.81
0	3	2	83.67	87.17	89.88	93.03
1	1	2	68.46	71.17	63.74	67.88
−2	2	3	81.47	87.86	93.57	99.83
−3	1	4	111.04	116.21	122.81	128.00
0	1	4	84.83	82.66	80.78	79.10
−1	3	5	99.44	101.88	105.21	107.40
3	1	5	63.65	59.74	68.84	64.15
3	1	7	73.92	76.49	82.96	85.38
−2	3	8	71.29	68.62	75.54	71.87
2	1	9	56.06	58.84	56.95	60.53
2	2	10	68.11	65.00	75.21	71.23
1	1	11	73.05	71.02	81.85	79.44

and (*R*)-(−)-**1** on the 7th day after administration. These pharmacokinetic data are reflected in the differences of *in vivo* data as follows in the order of TG and PL on the reducing effects: (*S*)-(+)-**1**>racemate, (±)-**1**=(*R*)-(−)-**1**.

In conclusion, the enantiomers of S-2 [(±)-**1**], (*S*)-(+)-**1** and (*R*)-(−)-**1** were prepared in fairly good yield with high optical purity *via* the optical resolution of 2-oxo-pyrrolidine-3-carboxylic acid, (±)-**3**, involving convenient silica gel column chromatographic separation. The absolute stereochemistry of the compound (+)-**1** was indirectly confirmed to be *S*-form by X-ray crystallography. In a comparison of racemate [(±)-**1**] with its enantiomers [(*S*)-(+)-**1**, (*R*)-(−)-**1**], biological activities *in vitro* were nearly similar and did not differ significantly. On the other hand, the hypotriglycemic and phospholipidemic activities of one enantiomer [(*S*)-(+)-**1**] were the most potent among the three compounds, and 1.4—1.6 and 1.3—2.0 fold stronger on day 7 of the test than the other two compounds [racemate (±)-**1**, (S-2), (*R*)-(−)-**1**]. It became clear that the plasma concentration and duration of the compounds affected the *in vivo* potency. Meanwhile, the hypocholesterolemic activity could not be sufficiently evaluated in the model system. The results of the *in vivo* studies has led us to select the enantiomer [(*S*)-(+)-**1**, S-2E] as a candidate for further development.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM EX-270 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ values (ppm). Elemental analysis were carried out with a Yanagimoto C. H. N Corder MT-2. MS were measured using JEOL JMS-SX 102 mass spectrometer. Optical rotations were measured on a Horiba SEPA-200 High Sensitive Polarimater using a cell with a 5 cm light path. Analytical HPLC was performed on a HPLC Chiralcel OJ or OD column[6] (0.46×25 cm; Daicel Chemical Co.), using a Shimadzu LC-4A Liquid Chromatograph. *n*-Hexane–ethanol (80 : 20) was used as the eluent.

Materials (±)-Methyl 4-[1-(*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]-methoxybenzoate [(±)-**2**] and (±)-1-(*tert*-butylphenyl)-2-oxo-pyrrolidine-4-carboxylic acid [(±)-**3**] were prepared according to the method of the previous paper.[1] (*S*)-(−)-4-methyl(α-methyl)-benzylamine was obtained from Yamakawa Chemical Ind. Co., Ltd. All reagents were purchased from commercial suppliers.

Separation[11] of Methyl 4-[1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxybenzoate, (±)-2 to (+)-2 and (−)-2 A 0.1% solution of (±)-**2** (1.6 g, 4.2 mmol) in a mixture of hexane and ethanol (ratio of 4 : 1, v/v, 1600 ml) was charged on the Chiralcel OJ column (20 cm, ϕ×50 cm, L) and systematically chromatographed with hexane–ethanol (ratio of 4 : 1) as an eluating solvent at 40 °C under vehicle speed of 760 ml/min. The first eluate was evaporated *in vacuo* to give (+)-**2** (0.7 g, 43.8%) as a white solid; mp: 137—138 °C. $[\alpha]_D^{20}$ +15.3° (c=1.02, CH_2Cl_2). *Anal*. Calcd for $C_{23}H_{27}NO_4$; C, 72.42; H, 7.13; N, 3.67. Found: C, 72.38; H, 7.27; N, 3.69. ^{1}H-NMR ($CDCl_3$) δ; 1.31 (9H, s), 2.52, 2.58 (1H, dd, J=6.2 Hz), 2.80—3.08 (2H, m), 3.78—3.88 (1H, m), 3.89 (3H, s), 4.00—4.15 (2H, m), 6.91 (2H, d, J=8.9 Hz), 7.39 (2H, d, J=8.9 Hz), 7.53 (2H, d, J=8.9 Hz), 8.00 (2H, d, J=8.9 Hz). The enantiomeric excess (ee) of (+)-**2** was determined to be 100% by Chiralcel OJ column[6] using the eluent of *n*-hexane–ethanol (80 : 20) at 40 °C. The second eluate was evaporated *in vacuo* to give (−)-**2** (0.6 g, 37.5%) as a white solid; mp: 137—138 °C. $[\alpha]_D^{20}$ −17.0° (c=1.00, CH_2Cl_2). *Anal*. Calcd for $C_{23}H_{27}NO_4$; C, 72.42; H, 7.13; N, 3.67. Found: C, 72.22; H, 7.08; N, 3.71. ^{1}H-NMR spectra was the same as that of (+)-**2**. The ee of (−)-**2** was determined to be 99.4% by the same manner as (+)-**2**.

Hydrolysis of (+)-2 and (−)-2 The compound, (+)-**2** (1.0 g, 2.6 mmol) was added to a solution of conc. HCl (3 ml) and acetic acid (9 ml). After stirring at 90 °C overnight, the reaction mixture was cooled to room temperature. The resulting precipitate was collected and washed with ethyl ether to give (+)-**1** (0.7 g, 77.9%) as a white solid; mp: 251—253 °C. $[\alpha]_D^{20}$ +27.0° (c=1.00, DMF). *Anal*. Calcd for $C_{22}H_{25}NO_4$; C, 71.91; H, 6.86; N, 3.81. Found: C, 71.79; H, 6.93; N, 3.80. ^{1}H-NMR δ; 1.27 (9H, s), 2.35—2.48(1H, m), 2.65—3.05 (2H, m), 3.62—3.78 (1H, m), 3.78—4.08 (1H, m), 4.08—4.20 (2H, m), 7.04 (2H, d, J=8.9 Hz), 7.38 (2H, d, J=8.9 Hz), 7.56 (2H, d, J=8.9 Hz), 7.89 (2H, d, J=8.9 Hz). The ee of (+)-**1** was determined to be 99.9% by Chiralcel OD column[6] using the eluent of *n*-hexane–ethanol–trifluoroacetic acid (80 : 20 : 0.2) at 30 °C. Compound (−)-**1** was prepared from (−)-**2** (2.6 g, 6.8 mmol) by the same manner as (+)-**1**. Yield 1.8 g (72.0%); mp: 252—253 °C. −27.2° (c=1.00, DMF). *Anal*. Calcd for $C_{22}H_{25}NO_4$; C,

71.91; H, 6.86; N, 3.81. Found: C, 71.84; H, 7.27; N, 3.71. ^{1}H-NMR spectra was the same as that of (+)-**1**. The ee of (−)-**1** was determined to be 99.2% by the same manner as (+)-**1**.

(+)-*N*-(4-Bromo-2-fluorophenyl)-4-[1-(4-*tert*-butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxy Benzamide A solution of (+)-**1** (1.36 g, 7.0 mmol), excess thionyl chloride (0.56 ml) and one drop of DMF in benzene (25 ml) was stirred under refluxing for 5 h. The reaction mixture was evaporated *in vacuo* to give the corresponding acid chloride. A solution of the acid chloride in CH_2Cl_2 (5 ml) was added dropwise to a solution of 4-bromo-2-fluoro aniline (0.74 g, 3.9 mmol) and triethylamine (0.46 ml, 4.5 mmol) in CH_2Cl_2 (20 ml) under ice-cooling. The reaction mixture was stirred for 2 h at room temperature. After cooling, the reaction mixture was diluted with ice water (10 ml), acidified with 0.1 N HCl. The organic layer was washed with water, dried ($MgSO_4$) and evaporated *in vacuo*. The resulting residue was recrystallized from ethyl acetate to give the amide (1.65 g, 83.0%) as colorless needles. mp: 201—203 °C. $[\alpha]_D^{25}$ +19.2° (c=0.5, $CHCl_3$). *Anal*. Calcd for $C_{28}H_{28}BrFN_{20}O_3$; C, 62.34; H, 5.23; N, 5.19. Found: C, 62.49; H, 5.28; N, 5.23. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (9H, S), 2.56 (1H, dd, J=17, 6 Hz), 2.86 (1H, dd, J=17, 9 Hz), 2.95—3.03 (1H, m), 3.33 (1H, dd, J=10, 5 Hz), 4.04—4.15 (3H, m), 6.99 (2H, d, J=9 Hz), 7.02—7.34 (2H, m), 7.40 (2H, d, J=9 Hz), 7.54 (2H, d, J=9 Hz), 7.85 (2H, d, J=9 Hz), 7.88—7.93 (1H, m), 8.36—8.42 (1H, m). MS m/z: 539 (M^+).

(*S*)-*N*-[α-Methyl(4-methyl)benzyl]-(*R*)- and -(*S*)-[1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]carboxyamide (4A, 4B) A suspension of (±)-**3** (10.1 g, 38.3 mmol) and excess thionyl chloride (8 ml) in CH_2Cl_2 (10 ml) was stirred under refluxing for 1 h. The reaction mixture was evaporated *in vacuo* to give the corresponding acid chloride. A solution of the acid chloride in CH_2Cl_2 (20 ml) was added dropwise to a solution of (*S*)-(−)-4-methyl(α-methyl)benzyl amine (5.6 g, 41.5 mmol) and triethyl amine (5.03 g, 49.8 mmol) in CH_2Cl_2 (80 ml) under ice-cooling. The reaction mixture was stirred for 4 h at room temperature. The organic layer was washed with water, 0.1 N HCl and dried ($MgSO_4$), and then evaporated *in vacuo*. The resulting residue was chromatographed on a silica gel column. The chromatographic conditions are shown in Chart 3. The first elution gave **4A** (5.7 g, 39.4%) as a white solid, mp: 220—221 °C. $[\alpha]_D^{23}$ −85.5° (c=1.01, CH_2Cl_2). *Anal*. Calcd for $C_{24}H_{30}N_2O_2$; C, 76.16; H, 7.99; N, 7.40. Found: C, 75.93; H, 8.16; N, 7.35. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (9H, s), 1.48 (3H, d, J=6.6 Hz), 2.33 (3H, s), 2.62—2.91 (2H, m), 3.02—3.20 (1H, m), 3.85—3.95 (1H, m), 4.09—4.18 (1H, m), 5.02—5.18 (1H, m), 6.08 (1H, d, J=6.6 Hz), 7.10—7.50 (8H, m). The second elution gave **4B** (5.3 g, 36.6%) as a white solid, mp: 170—171 °C, $[\alpha]_D^{23}$ −63.2° (c=1.00, CH_2Cl_2). *Anal*. Calcd for $C_{24}H_{30}N_2O_2$; C, 76.16; H, 7.99; N, 7.40. Found: C, 76.16; H, 8.16; N, 7.39. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (9H, s), 1.48 (3H, d, J=7.0 Hz), 2.33 (3H, s), 2.68—3.00 (2H, m), 3.04—3.20 (1H, m), 3.80—3.90 (1H, m), 4.02—4.12 (1H, m), 5.00—5.15 (1H, m), 6.12 (1H, d, J=7.0 Hz), 7.10—7.45 (8H, m).

(*R*)-(−)- and (*S*)-(+)-1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-carboxylic Acid [(*R*)-(−)-3, (*S*)-(+)-3] A solution of **4A** (4.0 g, 10.6 mmol) in CH_2Cl_2 (100 ml) was added dropwise to a suspension of N_2O_4 (1.95 g, 21.2 mmol) and AcONa (3.0 g) in CCl_4 (15 ml) under 0 °C. The reaction mixture was stirred for 12 h at room temperature and then for 1 h at 50 °C. The organic layer was washed with water and extracted with saturated $NaHCO_3$ solution (100 ml×2). The aqueous layer was adjusted to pH=1 with 6 N HCl and stirred for 1 h under ice-cooling. The resulting precipitate was collected and washed with water to give (*R*)-(−)-**3** (1.17 g, 42.4%) as a white solid, mp: 234—235 °C. $[\alpha]_D^{25}$ −44.6° (c=1.01, DMF). *Anal*. Calcd for $C_{15}H_{19}NO_3$; C, 68.94; H, 7.33; N, 5.36. Found: C, 68.80; H, 7.61; N, 5.36. ^{1}H-NMR (dimethy sulfoxide(DMSO)-d_6) δ: 1.27 (9H, s), 2.60—2.90 (2H, m), 3.90—4.10 (3H, m), 7.38 (2H, d, J=8.5 Hz), 7.54 (2H, d, J=8.5 Hz). The ee of (*R*)-(−)-**3** was determined to be 99.8% by Chiralcel OJ column[6] using the eluent of *n*-hexane–isopropanol–trifluoroacetic acid (80 : 20 : 0.1). Compound (*S*)-(+)-**3** was prepared from **4B** (4.0 g, 10.6 mmol) by the same manner as (*R*)-(−)-**3**. Yield 1.25 g (45.3%), mp: 230—231 °C. $[\alpha]_D^{25}$ +44.1° (c=1.03, DMF). *Anal*. Calcd for $C_{15}H_{19}NO_3$; C, 68.94; H, 7.33; N, 5.36. Found: C, 68.93; H, 7.64; N, 5.39. ^{1}H-NMR spectra was the same as (*R*)-(−)-**3**. The ee of (*S*)-(+)-**3** was determined to be 99.2% by the same manner as (*R*)-(−)-**3**.

(*S*)-(−)- and (*R*)-(+)-1-(4-*tert*-Butylphenyl)-4-hydroxymethyl-2-oxo-pyrrolidine [(*S*)-(−)-5, (*R*)-(+)-5] 1 M BH_3–THF solution (12.6 ml) was added dropwise to a suspension of (*S*)-(+)-**3** (1.1 g, 4.2 mmol) in CH_2Cl_2 (20 ml) between 0 °C and −5 °C. The reaction mixture was stirred for 2 h at 0—3 °C and evaporated *in vacuo*. The resulting residue was chromatographed on a silica gel column using the eluent of chloroform–ethanol (1 : 1) to give (*S*)-(−)-**5** (0.3 g, 28.8%) as a white solid, mp: 88—89 °C. $[\alpha]_D^{23}$ −8.9° (c=1.03, CH_2Cl_2). *Anal*. Calcd for $C_{15}H_{21}NO_2$; C, 72.84; H, 8.56; N, 5.66. Found: C, 72.75; H, 8.97; N, 5.65. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (1H, s), 2.60—2.80 (2H, m), 3.60—3.80 (3H, m), 7.39 (2H, d, J=8.5 Hz), 7.50 (2H, d, J=8.5 Hz). The ee of (*S*)-(−)-**5** was determined to be 99.5% by Chiralcel OD column[6] using the eluent of *n*-hexane–ethanol (95 : 5) at 40 °C. Compound (*R*)-(+)-**5** was prepared from (*R*)-(−)-**3** (1.0 g, 3.8 mmol) by the same manner as (*S*)-(−)-**5**. Yield 0.56 g (59.2%), mp: 86—87 °C. $[\alpha]_D^{23}$ +10.1° (c=1.03, CH_2Cl_2). *Anal*. Calcd for $C_{15}H_{21}NO_2$; C, 72.84; H, 8.56; N, 5.66. Found: C, 72.71; H, 8.80; N, 5.72. ^{1}H-NMR spectra was the same as (*S*)-(−)-**5**. The ee of (*R*)-(+)-**5** was determined to be 100% by the same manner as (*S*)-(−)-**5**.

(*S*)-(+)- and (*R*)-(−)-[1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]-methyl Methanesulfonate [(*S*)-(+)-6, (*R*)-(−)-6] A solution of triethylamine (0.14 g, 1.3 mmol) in CH_2Cl_2 (0.5 ml) was added dropwise to a solution of (*S*)-(−)-**5** (0.2 g, 0.85 mmol), and methanesulfonyl chloride (0.12 g, 1.0 mmol) in CH_2Cl_2 (5 ml) under ice-cooling. The reaction mixture was stirred for 1 h at 0—5 °C and evaporated *in vacuo*. The resulting residue was chromatographed on a silica gel column using the eluent of chloroform–ethanol (10 : 1) to give (*S*)-(+)-**6** (0.26 g, 94.1%) as a white solid, mp: 114—115°C. $[\alpha]_D^{23}$ +1.98° (c=1.01, CH_2Cl_2). *Anal*. Calcd for $C_{16}H_{23}NO_4S$: C, 59.05; H, 7.12; N, 4.30. Found: C, 58.64; H, 7.38; N, 4.38. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (9H, s), 2.40—2.47 (1H, dd, J=6.0 Hz), 2.75—3.00 (2H, m), 3.05 (3H, s), 3.70—3.78 (1H, m), 3.95—4.05 (1H, m), 4.20—4.35 (2H, m), 7.39 (2H, dd, J=8.9 Hz), 7.49 (2H, dd, J=8.9 Hz).

Compound (*R*)-(−)-**6** was prepared from (*R*)-(+)-**5** (0.4 g, 1.6 mmol) by the same manner as (*S*)-(+)-**6**. Yield 0.48 g (91.3%), mp: 113—114 °C. $[\alpha]_D^{23}$ −2.94° (c=1.02, CH_2Cl_2). *Anal*. Calcd for $C_{16}H_{23}NO_4S$; C, 59.05; H, 7.12; N, 4.30. Found: C, 58.62; H, 7.34; N, 4.44. ^{1}H-NMR spectra was the same as (*S*)-(+)-**6**.

(*S*)-(+)- and (*R*)-(−)-Methyl 4-[1-(4-*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]methyloxy Benzoate [(*S*)-(+)-2, (*R*)-(−)-2] A suspension of (*S*)-(+)-**6** (0.21 g, 0.65 mmol), methyl 4-hydroxybenzoate (0.1 g, 0.66 mmol) and K_2CO_3 (0.1 g, 1.0 mmol) in DMF (5 ml) was stirred for 37 h at 60 °C. The reaction mixture was triturated with ice-water and adjustified to pH=1.0 with 0.2 N HCl. The resulting precipitate was collected and recrystallized from methanol–water to give (*S*)-(+)-**2** (0.22 g, 89.4%) as white needles, mp: 137—138 °C. *Anal*. Calcd for $C_{23}H_{27}NO_4$; C, 72.42; H, 7.13; N, 3.67. Found: C, 72.38; H, 7.27; N, 3.69. The ee of (*S*)-(+)-**2** was determined to be 99.6% by Chiralcel OJ column[6] using the eluent of *n*-hexane–ethanol (80 : 20). The optical rotation and ^{1}H-NMR were the same as that of (+)-**2** obtained by the Chiralcel column separation method. Compound (*R*)-(−)-**2** was prepared from (*R*)-(−)-**6** (0.3 g, 0.92 mmol) by the same manner as (*S*)-(+)-**2**. Yield 0.33 g (93.8%), mp: 137—138 °C. *Anal*. Calcd for $C_{23}H_{27}NO_4$; C, 72.42; H, 7.13; N, 3.67. Found: C, 72.22; H, 7.08; N, 3.71. The ee of (*R*)-(−)-**2** was determined to be 99.8% by the same manner as (*S*)-(+)-**2**. The optical rotation and ^{1}H-NMR were the same as that of (−)-**2** obtained by the Chiralcel column separation method.

(*S*)-(+)- and (*R*)-(−)-[1-(*tert*-Butylphenyl)-2-oxo-pyrrolidine-4-yl]-methyloxy-benzoic Acids [(*S*)-(+)-1, (*R*)-(−)-1] A suspension of (*S*)-(+)-**2** (0.11 g, 0.29 mmol) and 2 N NaOH (0.3 ml) in methanol (4 ml) was stirred at 60 °C for 17 h. The reaction mixture was acidified (pH=1.0) with 6 N HCl. The resulting precipitate was collected, washed with water and methanol to give (*S*)-(+)-**1** (0.08 g, 75.5%) as a white powder. The mp, ee and ^{1}H-NMR spectra of the (*S*)-(+)-**1** obtained above method were nearly the same as (+)-**1**. *Anal*. Calcd for $C_{22}H_{25}NO_4$; C, 71.91; H, 6.68; N, 3.81. Found: C, 71.79; H, 6.93; N, 3.80. Compound (*R*)-(−)-**1** was prepared from (*R*)-(−)-**2** (0.2 g, 0.52 mmol) by the same manner as (*S*)-(+)-**1**. The mp, ee and ^{1}H-NMR spectra of (*R*)-(−)-**1** were nearly the same as (−)-**1**. *Anal*. Calcd for $C_{22}H_{25}NO_4$; C, 71.91; H, 6.68; N, 3.81. Found: C, 71.82; H, 6.80; N, 3.94.

The Inhibitory Activities toward Sterols and Fatty Acids Biosynthesis Using Rats Liver Slices[1] (*in Vitro*) Male Wistar rats (weight; about 200 g) were sacrificed, their livers were taken out, perfused with cold Krebs–Ringer bicarbonate (KRB) solution and cut into small slices. Using the small liver slices, the test was carried out according to the methods of the literature.[10] Small liver slices (100 mg) were weighed and added into the KRB (1 ml) containing [^{14}C]acetic acid (2 mCi/2 mmol) and the prescribed amount of test compounds, and the mixture was reacted with shaking at 37 °C for 2 h under an atmosphere of 95% O_2/5% CO_2. Thereafter, to the reaction mixture was added 15% solution of potassium hydroxide in ethanol (1 ml), and further heated at 75 °C for 2 h. After cooling, petroleum ether (2 ml) was added to the mixture, and it was shaken and separated into layers. The organic layer (upper layer) was extracted and concentrated to dryness. Then thereto digitonin solution (1 ml) was added and sterols were collected

in the resulting precipitation fraction. This fraction was washed with diethyl ether and dissolved in acetic acid (1 ml), and the radioactivity of the sample was measured to determine the inhibitory activity toward sterols biosynthesis. On the basis of the value obtained in the control test, in which the above procedure was repeated except that no test compound was used, the concentration (mM) of the test compound inhibiting 50% inhibitory concentration (IC_{50}) was determined. On the other hand, hydrochloric acid was added into the lower layer obtained by extraction with petroleum ether in the above procedure, and the mixture was extracted with petroleum ether under acidic conditions, and the organic layer was concentrated and then the radioactivity was measured likewise to determine the inhibitory activity toward fatty acid biosynthesis. Likewise as above, on the basis of the inhibitory activity for fatty acid biosynthesis obtained in the control test, 50% inhibitory concentration (IC_{50}) toward fatty acids biosynthesis of the test compounds was determined. Thus the obtained results are shown in Table 1.

Hypolipidemic Effects on Plasma in Zucker Fatty Rats ($n=4$) (*in Vivo*)[9] Racemate(±)-**1**, and its enantiomers (*S*)-(+)-**1** and (*R*)-(−)-**1** were suspended with 0.5% (w/v) HPMC solution and prepared as 30 mg in 5 ml. Zucker fatty rat (male, 7 months old, Charles River Japan) were used for this study. Animals were equally divided into four groups(control, racemate(±)-**1**, *S*-(+)-**1**, *R*-(−)-**1**, $n=4$) by their plasma lipid levels and each compound (30 mg/kg BW) was orally administered for 7 d. On the 4th day before administration and 24 h after final administration, blood was withdrawn from the abdominal vein, and plasma was obtained by centrifugation. Plasma total cholesterol (TC), TG and PL were enzymatically determined with colorimetric assays, and serum lipid levels were compared with the control. Thus the obtained results are shown in Table 2.

Pharmacokinetics of Compounds [(±)-1, (*S*)-(+)-1, (*R*)-(−)-1] on SD Rats Racemate(±)-**1**, (*S*)-(+)-**1** and (*R*)-(−)-**1** suspended with 0.5% (w/v) HPMC solution were administrated orally at the dose of 25 mg/kg to SD rats. Blood samples after administration were withdrawn from inferior vena cava. Blood samples were centrifuged and processed to plasma. Plasma levels of three compounds [(±)-**1**, (*S*)-(+)-**1**, (*R*)-(−)-**1**] were determined using liquid/liquid extraction followed by reversed-phase HPLC with ultraviolet detection. Thus the obtained results are shown in Table 3.

Crystal Structure of 4-Bromo-2-fluorobenzamide Derivative of (+)-1 A colorless needle crystal was obtained from ethanol and a crystal size, 0.40×0.22×0.11 mm was used for X-ray study.

Intensity data was measured on a Enraf-Nonius CAD4R diffractometer using CuKα radiation. The unit cell parameters were $a=10.561$ (1), $b=9.668$ (1), $c=25.258$ (1) Å, $\beta=92.32$ (1)° in space group $P2_1$, ($Z=4$). Of the 4327 reflections measured with $2\phi \leqq 136°$ employing a ω scan, 4231 were obtained independently at the level of $F>5\sigma(F)$, absorption correction was applied. The structure was solved by the direct method with MULTAN78,[7] and refined by least-squares to give an *R* value of 0.067. At this stage 16 reflections with large Bijvoet differences were selected and the absolute configuration was determined from the measurement of these reflections. The structure with the correct chirality was refined by the block-diagonal least-squares. Unit weight was given to all reflections, and anisotropic temperature factors were used for all non-hydrogen atoms. Of the 56 H atoms, 41 were located from the difference Fourier syntheses, 15 were calculated assuming ideal geometry and were included in the refinement with isotropic temperature factors. The final *R* and *Rw* values were 0.043 and 0.043, respectively. The atomic scattering factors, including the anomalous dispersion terms, were taken from the International Tables for X-ray Crystallography.[12] Calculations were performed using the UNICS-III[13] system on a FACOM M-1800. All observed Bijvoet ratios were in agreement with those calculated for the chosen enantiomer shown in Fig. 2, and the observed and calculated Bijvoet ratios are shown in Table 4. The tables of atomic coordinates, temperature factor, bond lengths and angles, and structure factors have been deposited as supplementary materials.

References and Notes

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