ABSOLUTE CONFIGURATION OF OPTICAL ISOMERS OF 2-(4-CHLOROBENZOYLAMINO)-3-[2(1*H*)-QUINOLINON-4-YL]-PROPIONIC ACID (REBAMIPIDE)

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Abstract-The absolute configuration of enantiomers of 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propionic acid [(±)-**1**, rebamipide] was assigned. The key intermediate, (*S*)-quinoline derivative [(*S*)-**3**], was prepared by palladium-catalyzed coupling reaction of 4-iodoquinoline with organozinc compound. This quinolinylalanine derivative was also synthesized from α-amino acid derivative of 2(1*H*)-quinolinone [(+)-**4**], which was able to convert into optically active rebamipide. Therefore the absolute configuration of (+)- and (-)-rebamipide was found to be *R* and *S*, respectively.

Rebamipide# [2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propionic acid] [OPC-12759, (±)-1][1] is brought to clinical use as a novel antiulcer agent that enhances mucosal resistance. Rebamipide inhibited production of superoxide from polymorphonuclear leukocytes and scavenged hydroxyl radical *in vitro*.[2,3] Recently, it was found that rebamipide showed efficacy in restraining the interaction of neutrophil and

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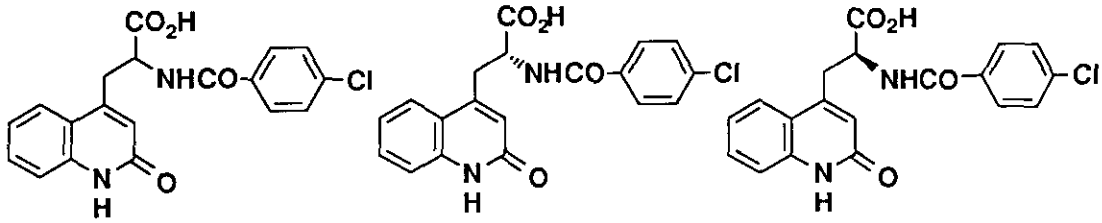
vascular endothelium, which was activated by *Helicobacter pylori* and nonsteroidal antiinflammatory drugs (NSAIDs).[4] In two earlier papers[5, 6] we have reported the synthesis of optically active rebamipide by using three efficient methods. It was suggested that the absolute configuration of α-amino acid derivative [(+)-**5**] synthesized from 2,5-dihydro-3,6-dimethoxy-2(*R*)-isopropylpyrazine[7] was (*S*)-configuration. However, compound (**5**) and its derivatives did not give a good crystal and the absolute configuration was unclear. We describe here the synthesis of (*S*)-quinolinylalanine and the determination of absolute configuration of optically active rebamipide..

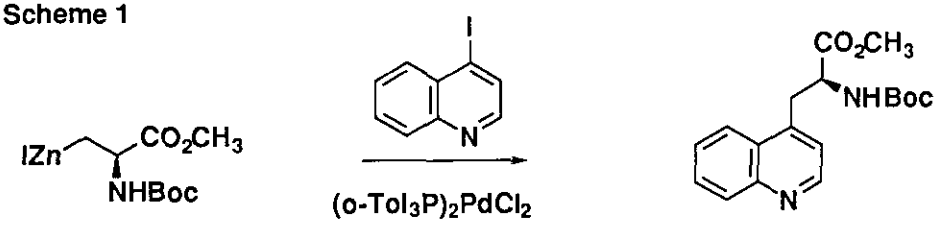
Figure 1



rebamipide (OPC-12759) **(*R*)-(+)-rebamipide** **(*S*)-(-)-rebamipide**

Palladium-catalyzed coupling reaction of freshly prepared organozinc compound (**2**)[8] with 4-iodoquinoline[9] at 60 ℃ for 6 hours provided 4-quinolinylalanine derivative [(*S*)-**3**] in 12 % yield. The optical purity of (*S*)-**3** appeared to be 96.7 % ee as determined by hplc using a chiral stationary phase column.

Scheme 1



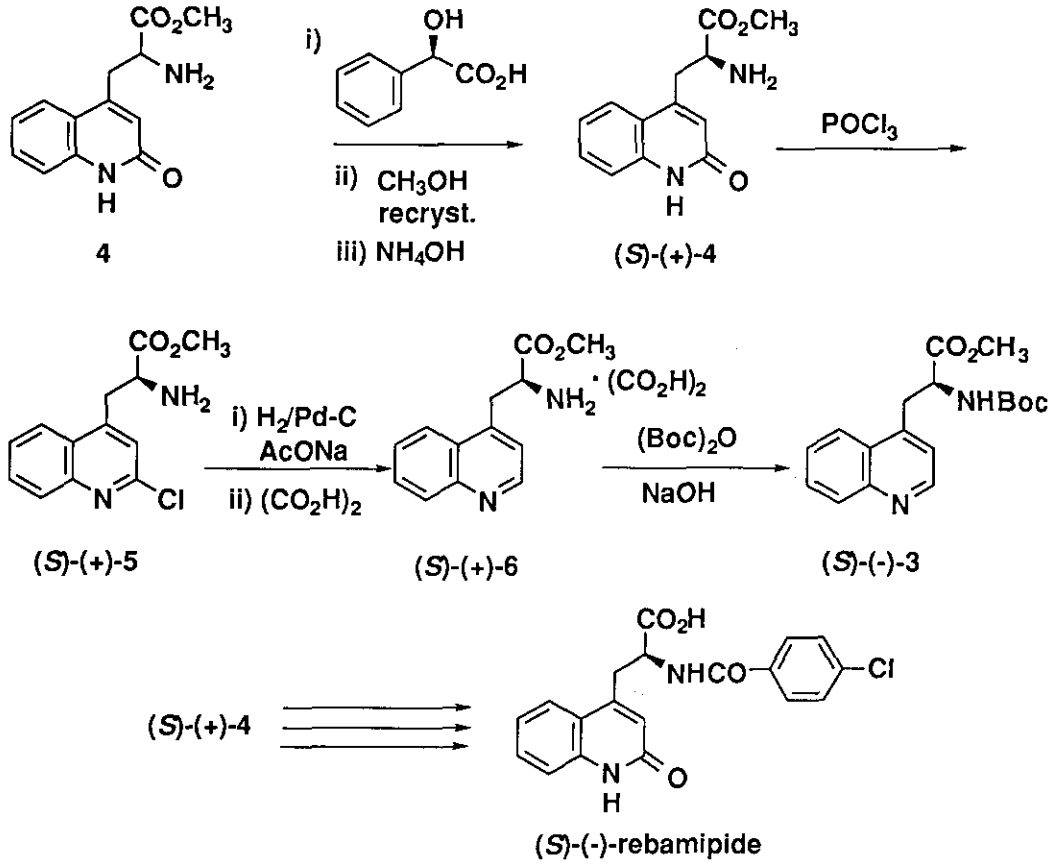
2 (*S*)-**3**

Next, we attempted to convert the 2(1*H*)-quinolinone derivative [(+)-**4**] to the 4-quinolinylalanine derivative [(*S*)-**3**)]. The α-amino acid derivative [(+)-**4**] of 2(1*H*)-quinolinone was synthesized by optical resolution of (rac)-**4** with D-(-)-mandelic acid.[6] The treatment of (+)-**4** with phosphoryl chloride ($POCl_3$) gave the 2-chloroquinoline derivative [(+)-**5**], followed by hydrogenation with palladium-charcoal (Pd-C) in the

presence of sodium acetate to give the quinoline compound [(+)-**6**]. Finally, treatment of (+)-**6** with di-*tert*-butyl dicarbonate in aqueous sodium hydroxide (NaOH)-1,4-dioxane afforded (-)-**3**. This compound was in good agreement with the retention time of hplc for the above synthesized (*S*)-**3**. In order to determine the optical purity of (*S*)-(-)-**3**, racemic compound (rac)-**3** was prepared from (rac)-**4** in the same manner as described for the synthesis of (*S*)-(-)-**3**.

Accordingly, the absolute configuration was for the isomers of (+)-**4**, (+)-**5** and (+)-**6** assigned as a *S* configuration. (-)-Rebamipide which was prepared from (*S*)-(+)-**4** was then established to the *S*.

Scheme 2



EXPERIMENTAL

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Infrared (ir) spectra were recorded on a JASCO IRA-2 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were

recorded on a Bruker A-200 spectrometer. Mass spectra (ms) were obtained on a Varian MAT-312 instrument. Optical rotations were measured on a DIP-360 digital polarimeter (Japan Spectroscopic Co., Ltd.).

Reaction of Organozinc Reagent (2) with 4-Iodoquinoline

A suspension of methyl 2(*R*)-[[(1,1-dimethylethyl)oxy]carbonyl]amino-3-iodopropionate (2.9 g, 8.8 mmol) and zinc-copper couple (1.16 g, 17.8 mmol) in benzene (30 ml) and dimethylacetamide (3 ml) was sonicated for 1 h and then was stirred at 60 ℃ for 30 min under N_2. To this reaction mixture was added a solution of 4-iodoquinoline (2.25 g, 8.8 mmol) and bis-[tri(o-methoxy)phenylphosphine]palladium dichloride (0.69 g, 0.88 mmol) in benzene (30 ml) and dimethylacetamide (3 ml) and the mixture was stirred at 60 ℃ for 6 h under N_2. After cooling the insoluble material was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2:MeOH=30:1) to give (*S*)-(-)-**3** (0.36 g, 12 %) as pale yellow oil. $[\alpha]_D^{25}$-39.44° (c=1, MeOH), nmr ($CDCl_3$) δ : 1.40 (9 H, s), 3.65 (3 H, s), 3.40-3.70 (2 H, m), 4.70-4.80 (1 H, m), 5.05-5.15 (1 H, br s), 7.20 (1 H, d, *J*=4.4 Hz), 7.56-7.76 (2 H, m), 8.07-8.15 (2 H, m), 8.82 (1 H, *J*=4.4 Hz). Fab-ms (pos.) m/z : 331.1681 $[M-H]^+$.

Determination of the Optical Purity of (*S*)-3 Obtained from (*S*)-(+)-Serine

Compound (*S*)-**3** obtained from (*S*)-(+)-serine was subjected to hplc (column, ULTRON-ES OVM, 4.6 mm i.d. x 25 cm; solvent, acetonitrile:20 mM KH_2PO_4=1:9; detection, uv 254 nm). The optical purity was determined to be 96.7 % ee.

Methyl (+)-(2*S*)-Amino-3-[2(1*H*)-quinolinon-4-yl]propionate [(*S*)-(+)-4]

Compound [(*S*)-(+)-**4**] D-(-)-mandelic acid salt (6.9 g, 21 %) was prepared according to the reported method[5] with racemic **4** (20 g, 81.2 mmol) and D-(-)-mandelic acid (12.0 g, 78.9 mmol) in EtOH (200 ml). Then the salt was dissolved in water (30 ml), adjusted to pH 9 with a 25 % ammonia solution and the solution was extracted with $CHCl_3$. The extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The

residue was recrystallized from MeOH-AcOEt-hexane to give (*S*)-(+)-**4** (3.4 g, 17 %) as white powder, mp150-151 ℃, $[\alpha]_D^{24}$ +35.8° (c=0.2, MeOH), nmr ($CDCl_3$) δ : 1.51 (2 H, br s), 2.97 (1 H, dd, *J*=8.7, 13.8 Hz), 3.45 (1 H, dd, *J*=4.9, 13.4 Hz), 3.75 (3 H, s), 3.89 (1 H, dd, *J*=4.9, 8.7 Hz), 6.67 (1 H, s), 7.22-7.30 (1 H, m), 7.48-7.58 (2 H, m), 7.77 (1 H, d, *J*=7.9 Hz), 12.71 (1 H, br s). Ms m/z (%): 246 (M^+, 7), 187 (17), 170 (10), 160 (20), 159 (100). 142 (6), 141 (6), 130 (11), 88 (14). Ir (KBr): 2950, 2840, 1740, 1660, 1440, 1200, 770 cm^{-1}. *Anal.* Calcd for $C_{13}H_{13}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.34; H, 5.76; N, 11.36.

Methyl (+)-(2*S*)-Amino-3-(2-chloroquinolin-4-yl)propionate [(*S*)-(+)-5]

A mixture of (*S*)-(+)-**4** (3.2 g, 13 mmol) and phosphorylchloride (25 ml, 268 mmol) was refluxed for 20 min. After removal of $POCl_3$, the residue was poured into ice-water, adjusted to pH 9 with a 25 % ammonia solution and extracted with $CHCl_3$. The extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2:MeOH=100:1) and recrystallized from Et_2O-hexane to give (*S*)-**5** (1.7 g, 49 %) as colorless prisms, mp 55-57 ℃, $[\alpha]_D^{24}$ +36.16° (c=0.5, MeOH), nmr ($CDCl_3$) δ : 1.59 (2 H, br s), 3.16 (1 H, dd, *J*=8.5, 13.8 Hz), 3.60 (1 H, dd, *J*=5.1, 13.8 Hz), 3.73 (3 H, s), 3.89 (1 H, dd, *J*=5.1, 8.5 Hz), 7.32 (1 H, s), 7.56-7.78 (2 H, m), 8.05 (2 H, d, *J*=7.1 Hz). Ms m/z (%): 265 (M^++1, 1), 264 (M^+, 0.4), 205 (7), 188 (9), 179 (32), 178 (13), 177 (100), 141 (6), 140 (5), 88 (25). Ir (KBr): 3370, 1730, 1585, 1560, 1280, 1190, 1170, 1150, 1100, 770 cm^{-1}. *Anal.* Calcd for $C_{13}H_{13}N_2O_2Cl$: C, 58.99; H, 4.95; N, 10.58. Found: C, 58.90; H, 4.88; N, 10.63.

Methyl 2-Amino-3-(2-chloroquinolin-4-yl)propionate [(rac)-5]

Compound (rac)-**5** (3.6 g, 67 %) was prepared by a procedure similar to that used for (*S*)-(+)-**5** with (rac)-**4** (5.0 g, 20 mmol) and $POCl_3$ (30 ml, 322 mmol) as colorless prisms, mp 77-78 ℃. Ir (KBr): 3370, 1730, 1580, 1280, 1190, 1170, 1150, 1100, 770 cm^{-1}. *Anal.* Calcd for $C_{13}H_{13}N_2O_2Cl$: C, 58.99; H,

4.95; N, 10.58. Found: C, 59.16; H, 4.95; N, 10.60.

Methyl (+)-(2*S*)-Amino-3-(4-quinolinyl)propionate Oxalate [(*S*)-(+)-6]

A mixture of (*S*)-(+)-**5** (1.2 g, 4.5 mmol), AcONa (0.37 g, 4.5 mmol) and 10 % Pd-C (0.6 g) in AcOH (50 ml) was stirred at 60-70 ℃ under atmospheric pressure of hydrogen until the theoretical amount of hydrogen had been absorbed. The mixture was cooled to room temperature, the catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was poured into ice-water and adjusted to pH 9 with a 25 % ammonia solution and extracted with $CHCl_3$. The extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2:MeOH=50:1) to give (*S*)-**6** free base (0.7 g, 67 %) as colorless oil. Nmr ($CDCl_3$) δ: 1.68 (2 H, br s), 3.19 (1 H, dd, *J*=8.5, 13.8 Hz), 3.63 (1 H, dd, *J*=5.1, 13.8 Hz), 3.70 (3 H, s), 3.91 (1 H, dd, *J*=5.1, 8.5 Hz), 7.28 (1 H, d, *J*=4.4 Hz), 7.58-7.76 (2 H, m), 8.06-8.15 (2 H, m), 8.83 (1 H, d, *J*=4.4 Hz). Ms m/z (%): 231 (M^++1, 6), 154 (10), 144 (14), 143 (100), 88 (10). The oxalic acid salt was recrystallized from EtOH as white powder, mp 170-175 ℃(decomp.). $[\alpha]_D^{24}$ +26.25° (c=0.2, MeOH), Ir (KBr): 3425, 3000, 2950, 2650, 1750, 1600, 1510, 1440, 1300, 770, 710 cm^{-1}. *Anal.* Calcd for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 56.69; H, 5.34; N, 9.28.

Methyl 2-Amino-3-(4-quinolinyl)propionate Oxalate [(rac)-6]

Compound (rac)-**6** free base (1.1 g, 63 %) was prepared by the procedure similar to that used for (*S*)-(+)-**6** with (rac)-**5** (2.0 g, 7.6 mmol), sodium acetate (0.62 g, 7.6 mmol) and 10 % Pd-C (1.0 g) in AcOH (80 ml). The oxalic acid salt was recrystallized from EtOH as white powder, mp 212-214 ℃ (decomp.). Ir (KBr): 3450, 3275, 2850, 1740, 1510, 1230, 1190, 720 cm^{-1}. *Anal.* Calcd for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 56.20; H, 4.92; N, 8.67.

Methyl (-)-(2*S*)-(*tert*-Butoxycarbonylamino)-3-(4-quinolinyl)propionate [(*S*)-(-)-3]

Di-*tert*-butyl dicarbonate (1.04 g, 4.8 mmol) was added to a solution of (*S*)-**6** free base (0.5 g, 2.2 mmol)

in 1,4-dioxane (30 ml) and 1 N sodium hydroxide solution (2.2 ml, 2.2 mmol) cooled in ice and the mixture was stirred at the same temperature for 1 h. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2:MeOH=100:1) and recrystallized from Et_2O-hexane to give (*S*)-(-)-**3** (0.4 g, 56 %) as white powder, mp 93-94 ℃, $[\alpha]_D^{24}$ -43.36° (c=1, MeOH), ms m/z (%): 331 (M^++1, 5), 330 (M^+, 0.9), 274 (19), 257 (11), 213 (35), 154 (19), 144 (13), 143 (100), 115 (10). Ir (KBr): 3330, 1740, 1680, 1535, 1290, 1170, 770 cm^{-1}. *Anal.* Calcd for $C_{18}H_{22}N_2O_4$:C, 65.44; H, 6.71; N, 8.48. Found: C, 65.56; H, 6.73; N, 8.58.

Methyl 2-(*tert*-Butoxycarbonylamino)-3-(4-quinolinyl)propionate [(rac)-3]

Compound (rac)-**3** (0.7 g, 49 %) was prepared by the procedure similar to that used for (*S*)-(-)-**3** with (rac)-**6** free (1.0 g, 4.3 mmol), di-*tert*-butyl dicarbonate (2.1 g, 9.5 mmol), 1N NaOH (4.3 ml, 4.3 mmol) in dioxane (30 ml) as white granules, mp 89.5-91.5 ℃. Ir (KBr): 3225, 2970, 1750, 1710, 1240, 1170, 780 cm^{-1}. *Anal.* Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.46; H, 6.76; N, 8.50.

Determination of the Optical Purities of (*S*)-(-)-3 Derived from 2(1*H*)-Quinolinone Derivative [(*S*)-(+)-4]

Compound (*S*)-(-)-**3** derived from 2(1*H*)-quinolinone derivative [(*S*)-(+)-**4**] was subjected to hplc [column, ULTRON-ES OVM, 4.6 mm i.d. x 25 cm; solvent, acetonitrile:20 mM KH_2PO_4=1:9; detection, uv 254 nm). The optical purity was determined to be 99.0 % ee. The compound (rac)-**3** was analyzed under the same conditions with that of (*S*)-(-)-**3**. The chromatogram showed two peaks with almost the same area intensity, and the reten-tion times were 7.92 min (49.7 %) and 10.88 min (50.3 %). The former peak was identical with that of (*S*)-(-)-**3**.

Determination of the Absolute Configurations of (+)- and (-)-1

The absolute configuration of the optically active rebamipide were determined as follows. The absolute stereochemistry of the quinolinylalanine (**3**) which derived from commercially available (*S*)-(+)-serine was determined to be *S*. The quinolinylalanine synthesized from α-amino acid derivative [(+)-**4**] of 2(1*H*)-quinolinone had in good agreement with the retention time of hplc for the above quinolinylalanine [(*S*)-**3**], suggesting that the starting (+)-**4** has (*S*)-configuration. Thus, the absolute configuration of (-)-rebamipide prepared from (*S*)-(+)-**4** was determined to be *S* and that of (+)-rebamipide was found to be *R*.

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