Synthesis and Antibacterial Activity of the Metabolites of 9-Fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid (OPC-7251)

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The metabolites of 9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (OPC-7251) (1), which has a potent antibacterial activity against gram-positive bacteria, characteristically *Propionibacterium acnes*, were synthesized to confirm their structures and to examine their antibacterial activity. The structures of three major metabolites (2, 3a and 4) were identified by means of comparison with the synthetic compounds. The antibacterial activity of the metabolites (2, 3a and 4) was found to be lower than that of 1.

Keywords metabolite; 9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid; antibacterial activity; antibacterial agent; quinolone carboxylic acid

The quinolone carboxylic acid derivative (±)-9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (OPC-7251) (**1**), a new antibacterial agent, was synthesized by Ishikawa *et al.*[1] and is now under clinical trial. In studies on the metabolism of **1**, three metabolites, OPC-7251 analogues (**2** and **3a**) oxidized and hydroxylated on the piperidine ring and the sulfate derivative (**4**) of OPC-7251, were isolated from biological fluids of rats, rabbits and dogs (Chart 1).[2] In order to confirm the structures, the three metabolites (**2**, **3a** and **4**) were synthesized as described below. Stereoisomer (**3b**) of **3a** was also synthesized, and these compounds were tested for antibacterial activity against gram-positive and gram-negative bacteria.

Synthesis 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (**2**) was synthesized as shown in Chart 2. Reaction of the carboxylic acid (**5**)[3] with boric acid and acetic anhydride in acetic acid[4] gave the chelate

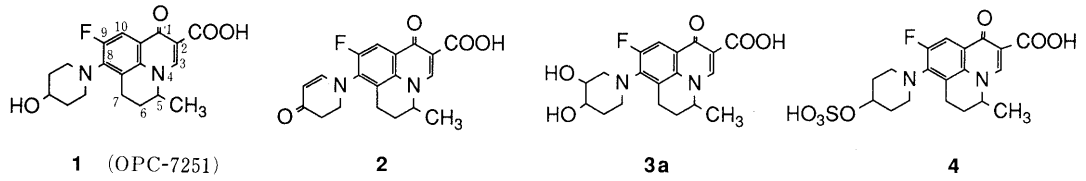

Chart 1

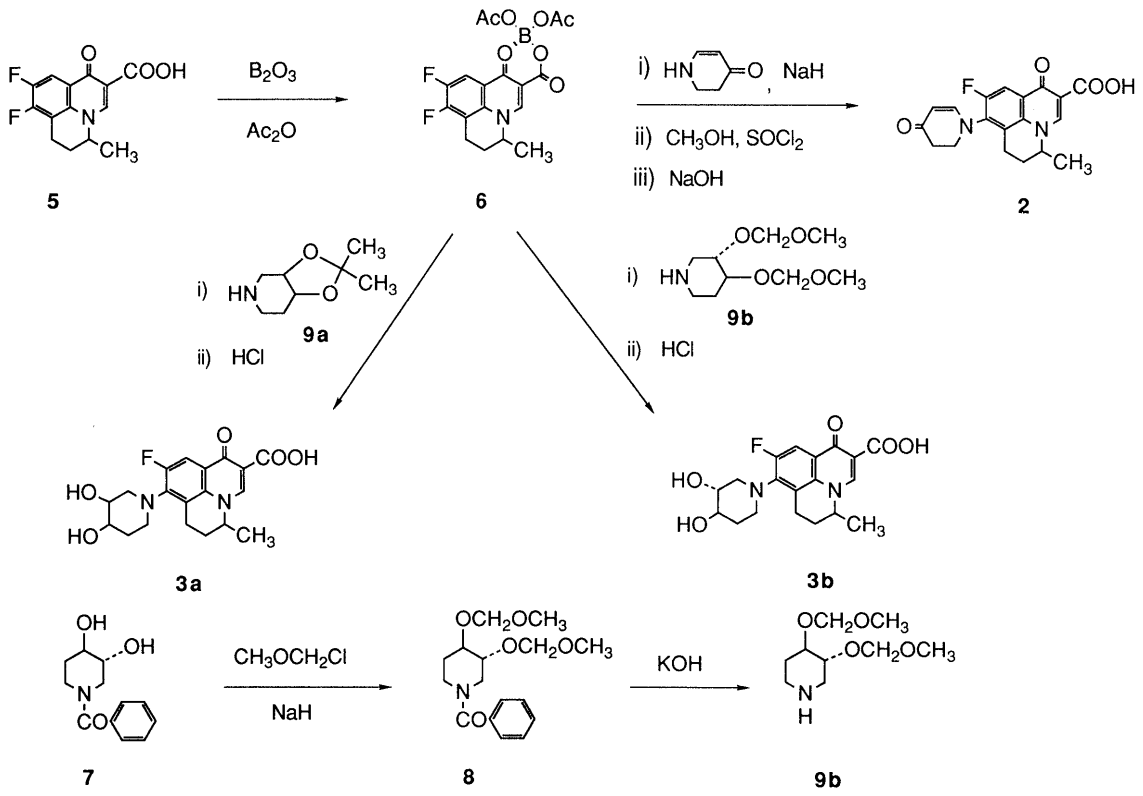

Chart 2

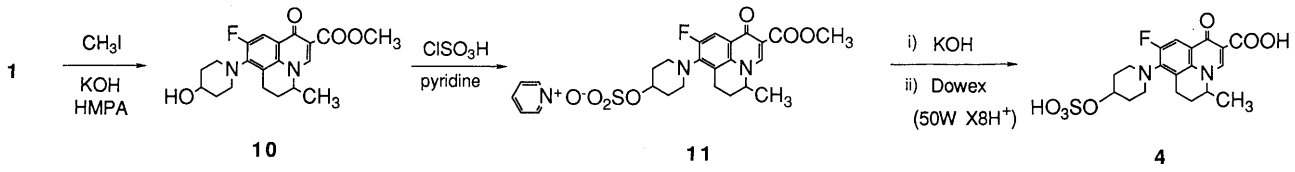

Chart 3

TABLE I. *In Vitro* Antibacterial Activity (Minimum Inhibitory Concentration, μg/ml)

Compd. No.	*S. aureus* 209 p	*E. coli* NIHJ JC-2	*Ps. aeruginosa* ATCC 10145	*P. acnes* ATCC 6919
1	0.024	0.39	3.13	0.10
2	0.20	0.78	6.25	0.10
3a	0.20	1.56	25	0.20
3b	0.20	3.13	25	0.20
4	>100	>100	>100	>100

compound (**6**), which was allowed to react with 4-oxo-1,2,3,4-tetrahydropyridine[5] in the presence of sodium hydride in *N*,*N*-dimethylformamide (DMF) to give the crude tetrahydropyridine derivative, which was esterified with methanol–thionyl chloride to give methyl 9-fluoro-6,7-dihydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate. Finally, the desired compound (**2**) was prepared by hydrolysis of the ester with sodium hydroxide.

For the confirmation of the structure of the metabolite (**3a**) two possible stereoisomers (**3a, b**) were synthesized by treatment of the chelate compound (**6**) with the protected piperidines (**9a, b**)[6] in the usual manner (Chart 2). The starting *trans*-dihydroxy piperidine (**9b**) was prepared by hydrolysis of the benzoyl derivative (**8**) with potassium hydroxide, and **8** was synthesized by alkylation of *trans*-1-benzoyl-3,4-dihydroxypiperidine (**7**) with methoxymethyl chloride in the presence of sodium hydride.

The sulfate of OPC-7251 was next synthesized by the pathway shown in Chart 3. OPC-7251 was esterified with methyl iodide in the presence of potassium hydroxide in hexamethylphosphoric triamide (HMPA)–ethanol[7] to give the ester (**10**), which was reacted with chlorosulfonic acid in pyridine to afford the pyridinium sulfate (**11**). Hydrolysis of **11** with potassium hydroxide gave the desired sulfate (**4**) (Chart 3).

The structures of the metabolites (**2**, **3a** and **4**) were identical with those of the corresponding synthetic compounds on the basis of nuclear magnetic resonance (NMR), mass spectrum (MS) and high performance liquid chromatographic comparisons.

Biological Results

The antibacterial activity against gram-positive (*Staphylococcus* 209p and *Propionibacterium acnes* ATCC 6919) and gram-negative bacteria (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* ATCC 10145) was measured *in vitro* by the serial dilution method.[8] The results are summarized in Table I. The metabolites (**2**, **3a**) and compound (**3b**) exhibited potent antibacterial activity against *P. acnes*. The antibacterial activity of all metabolites and compound (**3b**) against the other bacteria tested was found to be of a lower potency than **1**.

Experimental

Melting points were determined on a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IRA-2 spectrometer. NMR spectra were recorded on a Bruker AC-200 spectrometer. MS were obtained on a Varian MAT-312 and JEOL JMS-SX102 instruments.

8,9-Difluoro-6,7-dihydro-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid B(OCOCH$_3$)$_2$-Chelate (6) A mixture of 8,9-difluoro-6,7-dihydro-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (303 g, 1.09 mol),[4] boric acid (41.6 g, 0.6 mol) and acetic anhydride (676 ml, 6.62 mol) in acetic acid (215 ml) was stirred at 120 °C for 1 h. The reaction mixture was allowed to cool (50 °C) and poured into isopropyl ether. The precipitated crystals were collected by filtration. Recrystallization from acetonitrile gave **6** (397 g, 90%) as colorless prisms, mp 203—206 °C (dec.). NMR (DMSO-d_6) δ: 1.51 (3H, d, J=6.76 Hz), 1.91 (6H, s), 2.20—2.40 (2H, m), 3.00—3.30 (2H, m), 5.20—5.40 (1H, m), 8.30 (1H, t, J=8.54 Hz), 9.62 (1H, s). IR (KBr): 1715, 1470, 1275, 1060 cm^{-1}. *Anal.* Calcd for $C_{18}H_{16}BF_2NO_7 \cdot 1/2H_2O$: C, 51.95; H, 4.12; N, 3.37. Found: C, 52.08; H, 4.08; N, 3.56.

9-Fluoro-6,7-dihydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid (2) Sodium hydride (60% dispersion in mineral oil, 0.6 g, 14.9 mmol) was added to a stirred and ice-cooled solution of 4-oxo-1,2,3,4-tetrahydropyridine (1.3 g, 13.6 mmol)[5] in DMF (18 ml) and the reaction mixture was stirred for 15 min. Then **6** (1.8 g, 4.5 mmol) was added and the reaction mixture was stirred at 60 °C for 4 h. The mixture was poured into water and acidified with dil. HCl. The resulting precipitate was collected by filtration, washed with water and dried over $MgSO_4$. This crude **2** was suspended in MeOH (10 ml) and thionyl chloride (0.58 ml) was added dropwise to the suspension. The mixture was refluxed for 3 h, then the solvent was evaporated off and the residue was dissolved in CH_2Cl_2. The solution was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography (silica gel; eluent, CH_2Cl_2 : MeOH = 30 : 1). Evaporation of the solvent gave methyl9-fluoro-6,7-dihydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (0.61 g, 64%). This ester derivative (0.61 g) was dissolved in a mixture of 30% NaOH solution (0.5 ml) and MeOH (10 ml) and refluxed for 2 h. After evaporation of the MeOH, the residue was dissolved in water and acidified with dil. HCl. The precipitate was collected by filtration and washed with water. Recrystallization from DMF gave **2** (0.46 g, 29%) as yellow powder, mp >300 °C. NMR (DMSO-d_6) δ: 1.43 (3H, d, J=6.6 Hz), 1.90—2.30 (2H, m), 2.30—2.85 (2H, m), 3.00—3.20 (2H, m), 3.70—4.10 (2H, m), 4.85—5.00 (1H, m), 5.00—5.15 (1H, m), 7.40—7.65 (1H, m), 8.05 (1H, d, J=10.6 Hz), 9.06 (1H, s), 14.90—15.10 (1H, br s). IR (KBr): 1715, 1660, 1590, 1460 cm^{-1}. MS *m/z* (%): 356 (M^+, 19), 313 (23), 312 (100), 269 (20), 255 (12). *Anal.* Calcd for $C_{19}H_{17}FN_2O_4$: C, 64.04; H, 4.81; N, 7.86. Found: C, 64.05; H, 4.84; N, 7.82.

***trans*-3,4-Dimethoxymethyloxypiperidine (9)** Sodium hydride (60%, 1.04 g, 26 mmol) was added to a stirred and ice-cooled suspension of *trans*-1-benzoyl-3,4-dihydroxypiperidine (**7**) (2.2 g, 10 mmol)[6] and molecular sieves 4A (1.5 g) in DMF (20 ml) and the mixture was stirred for 15 min. Then a solution of methoxymethyl chloride (2.7 ml, 36 mmol) in DMF (2 ml) was added and stirred at room temperature overnight. After removal of the molecular sieves by filtration, the filtrate was poured into water and extracted with toluene–AcOEt. The extract was dried over $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 2 : 1) to give **8** (2.18 g, 71%). This dimethoxymethyloxy derivative (2.18 g) was dissolved in 10% KOH solution (20 ml) and the mixture was refluxed for 2.5 h, and then cooled to room temperature. The reaction mixture was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and the solvent was evaporated off *in vacuo* to give **9** (1 g, 70%) as cololress oil. NMR ($CDCl_3$) δ: 1.40—1.60 (1H, m), 1.45—1.70 (1H, br s), 1.95—2.15 (1H, m), 2.50—2.70 (2H, m), 2.90—3.15 (1H, m), 3.20—3.35 (1H, m), 3.39 (6H, s),

3.40—3.65 (2H, m), 4.73 (4H, s). MS *m/z* (%): 206 ($M^+ + 1$, 1), 112 (15), 82 (18), 45 (100). This sample was used in the next step without purification.

9-Fluoro-6,7-dihydro-8-(*cis*-3,4-dihydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i,j*]quinolizine-2-carboxylic Acid (3a) A mixture of **6** (0.51 g, 1.25 mmol) and piperidine-*cis*-3,4-diol acetonide (0.79 g, 5 mmol)[6] in acetonitrile (4 ml) was refluxed with stirring for 4 h, then cooled to 60 °C. Dil. HCl (5%, 8 ml) was added to the above solution. The reaction mixture was stirred for 1 h and poured into ice-water. The resulting precipitates were collected by filtration. The filtrate was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated to dryness *in vacuo*. The residue and the above precipitates were combined and purified by preparative thin layer chromatography (silica gel; solvent, CH_2Cl_2: MeOH = 10 : 1) and recrystallized from EtOH–MeOH–hexane to give **3a** (0.1 g, 21%) as pale yellow powder, mp 251—252 °C. NMR ($CDCl_3$) δ: 1.23 (3H, d, J = 7.0 Hz), 1.85—2.10 (2H, m), 2.10—2.50 (4H, m), 2.80—3.80 (6H, m), 3.80—4.10 (2H, br s), 4.45—4.65 (1H, m), 8.05 (1H, d, J = 12.0 Hz), 8.70 (1H, s), 14.99 (1H, s). IR (KBr): 3400, 1715, 1625, 1450, 1070 cm^{-1}. MS *m/z* (%): 376 (M^+, 18), 332 (100), 245 (14), 244 (12), 229 (17), 44 (17). *Anal.* Calcd for $C_{19}H_{21}FN_2O_5 \cdot 1/2H_2O$: C, 59.92; H, 5.69; N, 7.35. Found: C, 59.90; H, 5.59; N, 7.26.

9-Fluoro-6,7-dihydro-8-(*trans*-3,4-dihydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i,j*]quinolizine-2-carboxylic Acid (3b) Compound **3b** (68 mg, 21%) was prepared from **6** (0.36 g, 0.88 mmol) and *trans*-3,4-(dimethoxymethyloxy)piperidine (0.72 g, 3.5 mmol) by a similar procedure to that used for **3a**. Yellow powder (from EtOH–hexane), mp 244—245 °C. NMR ($CDCl_3$) δ: 1.52 (3H, d, J = 6.75 Hz), 1.50—2.00 (4H, m), 2.80—3.90 (8H, m), 4.45—4.65 (1H, m), 8.05 (1H, d, J = 12.25 Hz), 8.70 (1H, s), 14.97 (1H, s). IR (KBr): 3450, 1720, 1625, 1450, 1070 cm^{-1}. MS *m/z* (%): 376 (M^+, 18), 333 (21), 332 (100), 245 (12), 244 (10), 229 (16), 44 (12). *Anal.* Calcd for $C_{19}H_{21}FN_2O_5 \cdot 3/4H_2O$: C, 58.53; H, 5.82; N, 7.18. Found: C, 58.32; H, 5.43; N, 7.17.

Methyl 9-Fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i,j*]quinolizine-2-carboxylate (10) Potassium hydroxide (85%, 0.31 g, 4.6 mmol) was dissolved in HMPA (32 ml) and EtOH (18 ml) at 50 °C, and **1** (1.44 g, 4 mmol) and methyl iodide (1.25 ml, 20 mmol) were added to the above solution. The reaction mixture was stirred at 60 °C for 1.5 h. After evaporation of the solvent, the residue was poured into ice-water. The resulting precipitates were collected by filtration and recrystallized from DMF–H_2O to give **10** (1.36 g, 91%) as yellow powder, mp 178—182 °C. NMR ($CDCl_3$) δ: 1.48 (3H, d, J = 6.76 Hz), 1.60—1.90 (2H, m), 1.90—2.40 (6H, m), 2.70—3.45 (6H, m), 3.93 (3H, s), 4.35—4.55 (1H, m), 8.00 (1H, d, J = 12.74 Hz), 8.48 (1H, s). IR (KBr): 1690, 1610, 1450 cm^{-1}. *Anal.* Calcd for $C_{20}H_{23}FN_2O_4 \cdot 3/4H_2O$: C, 61.92; H, 6.37; N, 7.22. Found: C, 62.17; H, 6.13; N, 7.16.

Pyridinium 9-Fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-2-methoxycarbonyl-5-methyl-1-oxo-1*H*,5*H*-benzo[*i,j*]quinolizine Sulfate (11) Chlorosulfonic acid (0.67 ml, 10 mmol) was added dropwise to ice-cooled pyridine (20 ml) and the mixture was stirred at 50—55 °C for 30 min. Then **10** (0.75 g, 2 mmol) was added to the above solution and the reaction mixture was stirred at 50—55 °C for 6 h and then at room temperature overnight. After evaporation of the pyridine, the residue was poured into water. The resulting precipitates were collected by filtration and recrystallized from EtOH–hexane to give **11** (0.92 g, 86%) as colorless needles, mp 151—153 °C. NMR (DMSO-d_6) δ: 1.34 (3H, d, J = 6.70 Hz), 1.50—2.20 (6H, m), 2.70—3.00 (2H, m), 3.00—3.50 (4H, m), 3.73 (3H, s), 4.15—4.40 (1H, m), 4.60—4.75 (1H, m), 7.71 (1H, d, J = 12.95 Hz), 7.95—8.05 (2H, m), 8.45—8.60 (1H, m), 8.63 (1H, s), 8.89 (2H, d, J = 5.08 Hz). IR (KBr): 1700, 1450, 1240, 1225 cm^{-1}. *Anal.* Calcd for $C_{25}H_{27}FN_3O_7S \cdot 3/2H_2O$: C, 53.66; H, 5.40; N, 7.51. Found: C, 53.62; H, 5.41; N, 7.43.

9-Fluoro-6,7-dihydro-5-methyl-8-(4-sulfoxy-1-piperidyl)-1-oxo-1*H*,5*H*-benzo[*i,j*]quinolizine-2-carboxylic Acid (4) A solution of **11** (213 mg, 0.4 mmol) in 0.1 N KOH (15 ml) and MeOH (8 ml) was allowed to stand overnight. The reaction mixture was subjected to column chromatography (solvent, water) on Dowex 50 W-X8. After removal of the solvent, the residue was recrystallized from MeOH to give **4** (72 mg, 38%) as yellow needles, mp 193—197 °C (dec.). NMR (DMSO-d_6) δ: 1.40 (3H, d, J = 6.80 Hz), 1.50—2.20 (6H, m), 2.70—3.80 (6H, m), 4.10—4.40 (1H, m), 4.80—5.00 (1H, m), 7.86 (1H, d, J = 12.60 Hz), 9.00 (1H, s), 15.30 (1H, br s). IR (KBr): 1715, 1625, 1450, 1230 cm^{-1}. FAB-MS (neg.) *m/z*: 439 $[M-H]^-$. *Anal.* Calcd for $C_{19}H_{21}FN_2O_7S \cdot 2H_2O$: C, 47.90; H, 5.29; N, 5.88. Found: C, 48.06; H, 4.72; N, 5.76.

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