

## Determination of novel nitrogen-containing metabolites after oral administration of swertiamarin to rats

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We investigated the metabolic fate of swertiamarin (1) in Wistar rats. Swertiamarin (1) is a principal component of *Swertia* herbs used in traditional medicine. Liquid chromatography/ion trap mass spectrometry detected new metabolites (*R*)-gentianol (4a) and (*S*)-gentianol (4b) in rat plasma, together with the known metabolite gentianine (2), all of which contained nitrogen. The structures of the metabolites were identified by comparing the retention times, as well as MS and MS/MS spectra with those of authentic compounds, which were synthesized from swertiamarin (1). We prepared (*S*)-gentianol (4b) by stereoselective reduction from gentianone (3) which is a new oxidation product of gentianine (2), and the absolute configuration was unequivocally determined using an improved Mosher's method.

**Key words** swertiamarin, metabolism, gentianine, gentianone, gentianol.

**Abbreviations** APCI, atmospheric pressure chemical ionization; DAD, diode array detector; ESI, electrospray ionization; HPLC, high performance liquid chromatography; LC/MS, high performance liquid chromatography/mass spectrometry; LC/MS/MS, high performance liquid chromatography/mass/mass spectrometry; SIM, selected ion monitoring chromatography; TIC, total ion current chromatogram.

### Introduction

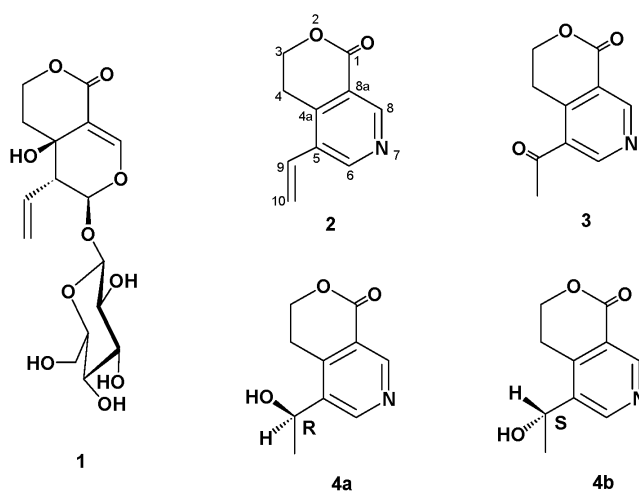
*Swertia* herbs belonging to Gentianaceae have been used to treat digestive dysfunction, acute and chronic dysentery, canker, and hepatitis in China, Japan and Korea.<sup>1)</sup> They also promote liver regeneration, repair liver fibrillation, clear fatty deposits within the liver and protect the liver from experimental damage by CCl<sub>4</sub>.<sup>2)</sup>

Swertiamarin (1, Fig. 1) is a bitter secoiridoid glycoside that is found in many *Swertia* species, including *Swertia japonica* Makino,<sup>3)</sup> *Swertia pseudochinensis* Hara,<sup>3)</sup> and *Swertia mussotii* Franch.<sup>4)</sup> It is the most abundant active constituent of *Swertia japonica*, which has been mainly used as a folk medicine in Japan to treat digestive disorders. Quality control and other evaluations of *S. japonica* have generally targeted swertiamarin (1), due to the high content of this compound (about 2–10% of the whole plant) according to the Japanese Pharmacopoeia. Swertiamarin (1) exerts a wide range of therapeutic effects. Studies of rats *in vivo* have shown that swertiamarin (1) is responsible for the anticholinergic action of *S. japonica* extract.<sup>5)</sup> Bhattacharya *et al.* found that swertiamarin (1) significantly reverses not only mangiferin-induced hyperactivity in albino mice and rats but also the potentiating effect of mangiferin on amphetamine toxicity in aggregated mice.<sup>6)</sup> Swertiamarin (1) also has antibacterial activity against *Bacillus dereus*, *Bacillus subtilis*, *Citrobacter freundii* and *Escherichia coli*,

*Proteus mirabilis* and *Serratia marcescens*.<sup>7)</sup>

Kubota *et al.*<sup>8)</sup> determined the structure of swertiamarin (1) in the 1960s. Swertiamarin (1) is chemically unstable, and transformed to gentianine (2) in the presence of NH<sub>4</sub><sup>+</sup>. El-Sedawy *et al.*<sup>9)</sup> enzymatically hydrolyzed swertiamarin (1) using β-glucosidase and identified gentiopicral and erythrocentaurin as the hydrolysates.

While investigating the metabolism of swertiamarin



**Fig. 1** Chemical structures of swertiamarin (1), gentianine (2), gentianone (3), (*R*)-gentianol (4a), and (*S*)-gentianol (4b).

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(1) by human intestinal bacteria,<sup>9)</sup> we identified erythrocentaurin and 5-hydroxymethylisochroman-1-one as the main metabolites of swertiamarin (1). We also discovered that one of the metabolic intermediates of swertiamarin (1) was further transformed by human intestinal bacteria to the biologically active, nitrogen-containing compound, gentianine (2). The concentration of nitrogen-containing metabolite is far lower than that of erythrocentaurin and 5-hydroxymethylisochroman-1-one, and such low concentrations cause difficulties in detecting them *in vivo*. To determine whether swertiamarin (1) can be transformed into the nitrogen-containing compound gentianine (2) *in vivo*, we used liquid chromatography/ion trap mass spectrometry to identify the metabolites, and developed a selective and sensitive LC/MS/MS method to measure the levels of nitrogen-containing metabolites in rat plasma samples.

## Materials and Methods

**Instruments.** Chromatograms and UV spectra were obtained using an Agilent 1100 series HPLC-DAD system (Agilent Technologies, Waldbronn, Germany) comprising a binary pump, thermostated column compartment and diode array detector (DAD). Characteristic peaks were identified using an Esquire 3000 mass spectrometer system with an electrospray ionization (ESI) source (Bruker Daltonik GmbH, Bremen, Germany). Samples were separated by HPLC as follows. **System A** was applied to a TSK gel ODS-80 Ts column (2.0 × 150 mm; i.d., 5 m) with a flow rate of 1.0 ml/min at 30°C in a stepwise gradient increasing from 10% (v/v) to 35% acetonitrile for 15 min, then to 100% acetonitrile for 5 min. Thereafter, the column was washed with 100% acetonitrile for 10 min and equilibrated with the starting eluant for 10 min. **System B** was applied to a SHISEIDO chiral CD-Ph column (4.6 × 250 mm; i.d., 5 m) with 5% acetonitrile at a flow rate of 1.2 ml/min at 30°C. The ESI-MS conditions were as follows: drying gas N<sub>2</sub>, 10.0 l/min; temperature, 300°C; nebulizer pressure, 50 psi; isolation width, 4; fragment amplification, 1.0; scan range, 50 - 1000 mass units.

**Solvents and chemicals.** Acetonitrile and all other organic solvents of analytical HPLC grade were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). [5,10,15,20-tetrakis(4-methoxyphenyl)-21*H*,23*H*-porphinato]cobalt(II) and triethylsilane were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Triethylamine, glucose and NaBH<sub>4</sub> were purchased from Nacalai Tesque Co. Ltd. (Kyoto, Japan). Dichloromethane, 2-propanol, NH<sub>4</sub>Cl, (±)-α-methoxy-(α-trifluoromethyl)phenylacetyl chloride, β-NADH, β-NADP, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemical (St. Louis, MO, USA). Baker's yeast was purchased from Oriental Yeast Co. Ltd. (Kyoto, Japan). Male Wistar rat microsomes were purchased from Charles River Laboratories (Yokohama, Japan).

The isolated and synthetic compounds were analyzed by IR, UV, MS, NMR and HPLC to confirm their structures and the purity was > 98%.

**Collection of urine, feces and plasma samples.** Male Wistar rats (8 weeks old) purchased from SLC Co. (Hamamatsu, Japan) were fed with standard laboratory chow for one week, fasted overnight and given free access to water before drug administration. Urine and feces samples were collected while the rats remained in metabolic cages. At intervals of 1, 2, 4, 6, 8, 12, 24 and 36 h after oral administration, the abdomen was exposed by a midline abdominal incision and blood samples were collected from the inferior vena cava using a heparinized injector. The blood samples were centrifuged at 8000 × g for 15 min to separate the plasma, and then all samples were stored at -20°C for later analysis.

**Sample preparation for LC/MS/MS.** Thawed urine and feces samples were dissolved in 3 volumes of MeOH, and then centrifuged at 8000 × g for 15 min. The supernatant was passed through a 0.45 μm Millipore syringe filter (Nihon Millipore, Tokyo, Japan) for LC/MS/MS analysis. Plasma samples were passed through solid phase extraction cartridges (Waters Co., Milford, MA, USA.) that had been washed with 3 ml of MeOH and equilibrated with 6 ml of water. The constituents were eluted with 2 - 3 ml of MeOH from the cartridge, and then the eluate was evaporated under a stream of nitrogen at 35°C. The residue was dissolved in 200 μl of MeOH and analyzed by LC/MS/MS.

**Synthesis of gentianine (2).** Gentianine was synthesized according to the method of Kubota *et al.*<sup>10)</sup>

**Synthesis of 5-acetyl-3,4-dihydropyrano[3,4-*c*]pyridin-1-one (3) and (±)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-*c*]pyridin-1-one (4).** A mixture of gentianine (2) (618 mg, 3.51 mmol), [5,10,15,20-tetrakis(4-methoxyphenyl)-21*H*,23*H*-porphinato]cobalt(II) (500 mg, 0.63 mmol), and triethylsilane (418 mg, 3.60 mmol) in 200 ml of 2-propanol-dichloromethane (1:1) was stirred at 28°C under oxygen at atmospheric pressure for 24 h. The solvent was removed under reduced pressure, and then the crude product was subjected to a silica gel column with CHCl<sub>3</sub>-acetone (20:1) to yield gentianone (3, 5-acetyl-3,4-dihydropyrano[3,4-*c*]pyridin-1-one) (606 mg, 3.17 mmol, 90% yield), followed by CHCl<sub>3</sub>-acetone (5:1) to yield (±)-gentianol (4, (±)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-*c*]pyridin-1-one) (68 mg, 0.35 mmol, 10% yield). **Gentianone (3):** Colorless needles. mp 166°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 9.38 (1H, s, H-8), 9.22 (1H, s, H-6), 4.53 (2H, t, *J* = 6.6 Hz, H-3), 3.47 (2H, t, *J* = 6.6 Hz, H-4), 2.71 (3H, s, H-10). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 197.7 (C-9), 162.8 (C-1), 154.3 (C-8), 154.0 (C-6), 148.9 (C-4a), 129.7 (C-5), 122.4 (C-8a), 66.3 (C-3), 29.1 (C-10), 25.7 (C-4). HR-EI-MS *m/z* 191.0564 [M]<sup>+</sup> (Calcd for C<sub>10</sub>H<sub>9</sub>NO<sub>3</sub> 191.0582). MS (ESI): *m/z*: 192 [M+H]<sup>+</sup>, 162 [M+H-HCHO]<sup>+</sup>. IR (KBr) cm<sup>-1</sup>: 1729, 1691. UV λ<sub>max</sub> nm: 226. (±)-**Gentianol (4):** Colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 9.08 (1H, s, H-8), 8.77 (1H, s, H-6), 5.10 (1H, q, *J* = 6.6 Hz, H-9), 4.53 (2H, t, *J* = 6.6 Hz, H-3), 3.16 (2H, t, *J* = 6.6 Hz, H-4), 1.55 (3H, d, *J* = 6.6 Hz, H-10). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 163.4 (C-1), 150.7 (C-8), 150.1 (C-6), 145.4 (C-4a), 136.7 (C-5), 120.9 (C-8a), 66.2 (C-3), 65.6 (C-9), 23.8 (C-4), 23.9 (C-10). HR-EI-MS *m/z* 193.0732 [M]<sup>+</sup> (Calcd for C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub> 193.0739). MS (ESI): *m/z*: 194 [M+H]<sup>+</sup>, 176 [M+H-H<sub>2</sub>O]<sup>+</sup>,

146 [M+H-H<sub>2</sub>O-HCHO], 118 [M+H-H<sub>2</sub>O-HCHO-CH<sub>2</sub>=CH<sub>2</sub>]<sup>+</sup>. IR (KBr) cm<sup>-1</sup>: 3312, 1726. UV λ<sub>max</sub> nm: 215.

**Synthesis of (S)-(-)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-c]pyridin-1-one (4b).** A mixture of commercial baker's yeast (52.4 g), glucose (25 g), MgSO<sub>4</sub> (50 mg), NH<sub>4</sub>Cl (58.8 mg), Na<sub>2</sub>HPO<sub>4</sub> (80.6 mg) and tap water in a round bottom flask was incubated in a shaking water bath for 2 h at 30 °C. A solution of gentianone (**3**, 400 mg, 2.09 mmol) in ethanol (3 ml) was then added. After 24 h of fermentation, the mixture was extracted with ethyl acetate. The organic layer was dried and concentrated *in vacuo*. The residue was subjected to silica gel column chromatography and eluted with CHCl<sub>3</sub>-acetone (5:2) to yield (S)-(-)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-c]pyridin-1-one [(S)-gentianol, **4b**] (26 mg, 0.14 mmol, 6.5% yield), ee > 98%, [α]<sub>D</sub><sup>20</sup> = -64° (c = 1.0, CHCl<sub>3</sub>).

**Preparation of (R)-(+)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-c]pyridin-1-one (4a).** Sodium borohydride was added to a solution of gentianone (**3**, 100 mg, 0.52 mmol) in MeOH and toluene and the mixture was stirred for 10 min. The pH was adjusted 7.0 using 1N HCl and the mixture was concentrated *in vacuo*. The residue was applied to a silica gel column eluted with CHCl<sub>3</sub>-acetone (5:2) to yield (±)-gentianol (**4**, 91 mg, 0.47 mmol, 90% yield), which was then further subjected to preparative SHISEIDO chiral CD-Ph column chromatography (10.0 × 250 mm; i.d., 5 μm) at a flow rate of 3 ml/min at 30 °C to afford (R)-(+)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-c]pyridin-1-one [(R)-gentianol, **4a**] (2 mg), ee = 95%, [α]<sub>D</sub><sup>20</sup> = +59° (c = 1.0, CHCl<sub>3</sub>).

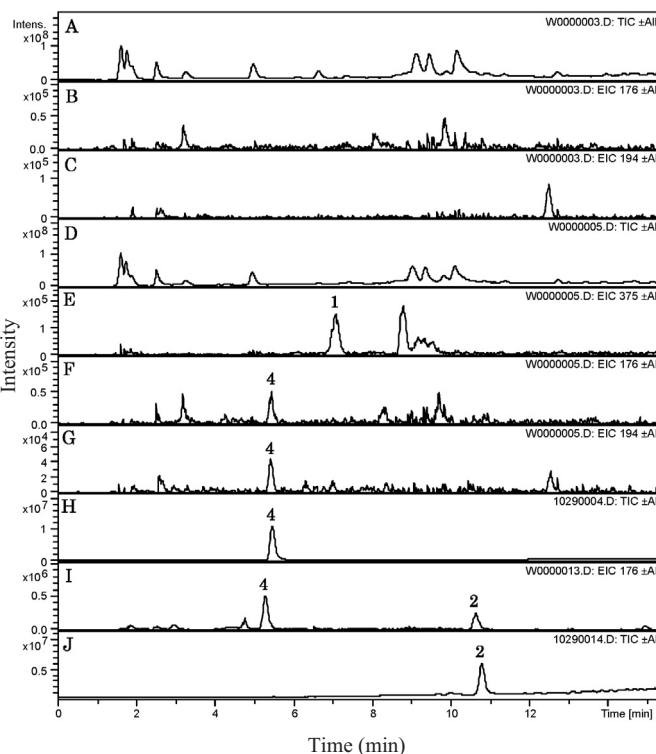
**α-Methoxy-α-(trifluoromethyl)phenylesters of (S)-(-)-5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-c]pyridin-1-one (A and B).** To a solution of (R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (100 mg, 0.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 ml), (S)-gentianol (**4b**, 9 mg, 0.05 mmol) and triethylamine (27.4 μl, 0.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 ml) was added dropwise at 0 °C. The reaction mixture was warmed to room temperature and stirred for 5 h, and then poured into ice-cold water (10 g). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> and then the extract was washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The organic layer was concentrated *in vacuo* and subjected to a silica gel column chromatography eluted with CHCl<sub>3</sub>-acetone (10:1) to yield colorless oil **A** (1 mg, 2.4 μmol, 5.2% yield). MS (ESI): 410 [M+H]<sup>+</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 9.24 (1H, s, H-8), 8.75 (1H, s, H-6), 6.20 (1H, q, J = 6.6 Hz, H-9), 4.49 (2H, m, H-3), 3.18 (1H, m, H-4), 3.05 (1H, m, H-4), 1.64 (3H, d, J = 6.6 Hz, H-10). **B** (1 mg, 2.4 μmol, 5.2% yield) was prepared in the same manner as described above using (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. MS (ESI): 410 [M+H]<sup>+</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 9.22 (1H, s, H-8), 8.61 (1H, s, H-6), 6.20 (1H, q, J = 6.6 Hz, H-9), 4.44 (2H, m, H-3), 3.12 (1H, m, H-4), 2.90 (1H, m, H-4), 1.70 (3H, d, J = 6.6 Hz, H-10).

**Incubation of gentianine (2) with microsomes.** Incubation mixtures (1 ml) consisted of an NADPH-generating system, liver microsomes (1 mg/ml protein), and 1 mM gentianine (**2**) in potassium phosphate buffer (0.1 M,

pH 7.4). The NADPH-generating system contained 5 mM MgCl<sub>2</sub>, 1 mM β-NADH, 1 mM β-NADP<sup>+</sup>, 10 mM glucose 6-phosphate, and 5.0 units/ml of glucose-6-phosphate dehydrogenase. The reaction was initiated by adding the NADPH-generating system at 37 °C and stopped by adding an equal volume of ice-cold methanol. The residue obtained by centrifugation at 10,000 × g at 4 °C for 30 min was immediately passed through a 0.45 μm Millipore syringe filter, and the filtrates were analyzed by LC/MS.

## Results and Discussion

To profile potential metabolites of swertiamarin (**1**), rat plasma samples were collected after the oral administration of **1** at a dose of 200 mg/kg and initially analyzed by LC/MS in full-scan mode with a mass range from *m/z* 50 to 1000. No metabolite peaks were obvious in the total ion current chromatogram (TIC) (Fig. 2, D). Therefore, possible metabolites of **1** were screened from the TIC in either extract ion monitoring chromatography (EIC) or selected ion monitoring chromatography (SIM) mode, including erythrocentaurin (*m/z* 176, APCI), erythrocentauric acid (*m/z* 192, APCI), a glycine adduct of erythrocentauric acid



**Fig. 2** Total ion current chromatogram (TIC) and extract ion chromatograms (EIC).

**A,** TIC of blank plasma; **B,** EIC at *m/z* 176 of blank plasma; **C,** EIC at *m/z* 194 of blank plasma; **D,** TIC of plasma 6 h after oral administration of 200 mg/kg of swertiamarin (**1**); **E,** EIC at *m/z* 375 of the above plasma; **F,** EIC at *m/z* 176 of the above plasma; **G,** EIC at *m/z* 194 of the above plasma; **H,** TIC of (±)-gentianol (**4**); **I,** EIC at *m/z* 176 of plasma after 4 oral administration of 1000 mg/kg of **1** every 12 h; **J,** TIC of gentianine (**2**). All data were collected using system A as described in the experimental section.

( $m/z$  249, APCI and ESI) and 5-hydroxymethylisochroman-1-one ( $m/z$  178, APCI) as well as conjugates of 5-hydroxymethylisochroman-1-one with sulfuric acid ( $m/z$  258, APCI and ESI), with glucuronic acid ( $m/z$  354, APCI and ESI), with glucose ( $m/z$  340, APCI and ESI) or with acetic acid ( $m/z$  220, APCI and ESI) and other compounds. However, none of the possible metabolites described above was detected in the plasma. The EIC of gentianine (**2**) monitored at  $m/z$  176  $[M+H]^+$  did not contain an obvious ion at the corresponding retention time (10.8 min) of the authentic compound, but an ion was obvious at the retention time of 5.4 min with a protonated ion at  $m/z$  194 and an intense fragment ion at  $m/z$  176 (Fig. 2. F). This ion was recognized as a metabolite of **1** by comparing the TIC and EIC chromatograms and MS spectrum with those of a blank and gentianine (**2**). To further investigate the structure of the metabolite, full-scan MS/MS was performed to obtain characteristic fragment ions. The mass spectrum of the metabolite was almost identical to that of gentianine (**2**) except for an additional fragment ion at  $m/z$  194 (Fig. 3), suggesting that the compound was an intermediate in the metabolic course of swertiamarin (**1**) to gentianine (**2**), or maybe a hydroxylated product of gentianine (**2**). However, the metabolite was stable under acidic conditions during extraction from urine, indicating that it was not a metabolic intermediate, but rather a further metabolite of gentianine (**2**). When subjected to a chiral column chromatography, the metabolite

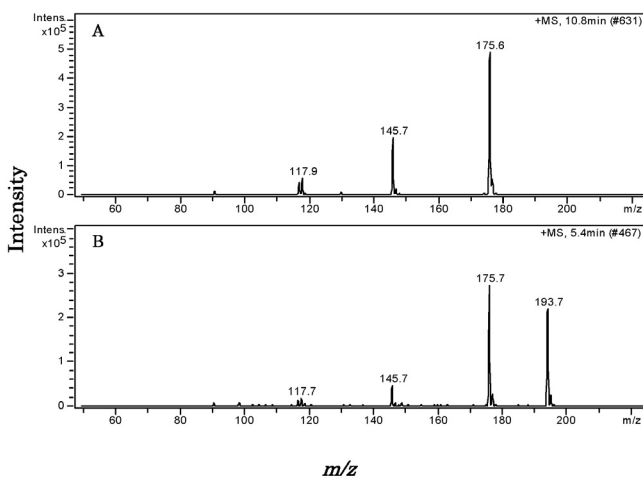


Fig. 3 Mass spectra of gentianine (A) and ( $\pm$ )-gentianol (B).

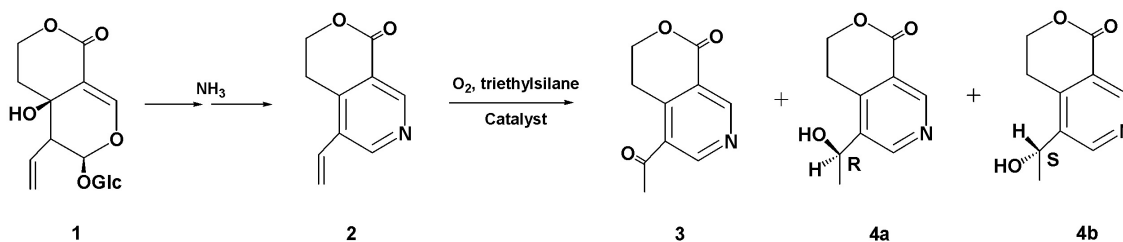


Fig. 5 Preparation of gentianine (**2**), gentianone (**3**), (*R*)-gentianol (**4a**), and (*S*)-gentianol (**4b**). Catalyst: [5,10,15,20-tetrakis(4-methoxyphenyl)-21*H*,23*H*-porphinato]cobalt (II).

was found to be a racemic mixture (Fig. 4), and also because the metabolite can be dehydrated to form gentianine (**2**) according to the mass spectrum, we speculated that hydration proceeded at the exo-methylene group of gentianine (**2**).

We synthesized the proposed alcohols using a modified method of Matsushita *et al.*<sup>11)</sup> with [5,10,15,20-tetrakis(2,6-dichlorophenyl)porphinato]cobalt (II) instead of [5,10,15,20-tetrakis(4-methoxyphenyl)-21*H*,23*H*-porphinato] cobalt (II), which changed the product ratio of ketone to alcohol (Fig. 5). We determined the structures of the synthetic ketone (5-acetyl-3,4-dihydropyrano[3,4-*c*]pyridin-1-one) and alcohols [ $(\pm)$ 5-(1-hydroxyethyl)-3,4-dihydropyrano[3,4-*c*]pyridin-1-one] by MS and NMR spectroscopy including  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and two-dimensional NMR, and named them gentianone (**3**) and ( $\pm$ )-gentianol (**4**). A comparison of retention times in the TIC, MS and MS/MS spectra showed that the synthesized alcohol was identical to the unknown metabolite (Fig. 2. H and Fig. 4. C).

Because of difficulties in separating the respective enantiomer using a preparative chiral column, we attempted optical resolution with lipase,<sup>12)</sup> and stereoselective reduction of gentianone (**3**) using the chiral Ru(II) complex,  $\text{RuCl}[(S,S)\text{-}N\text{-}(p\text{-toluenesulfonyl})\text{-}1,2\text{-diphenylethylenediamine}](p\text{-cymene})$ .<sup>13)</sup> However, neither method resulted in a satisfactory enantiomeric excess (about 70% and 50% ee for the

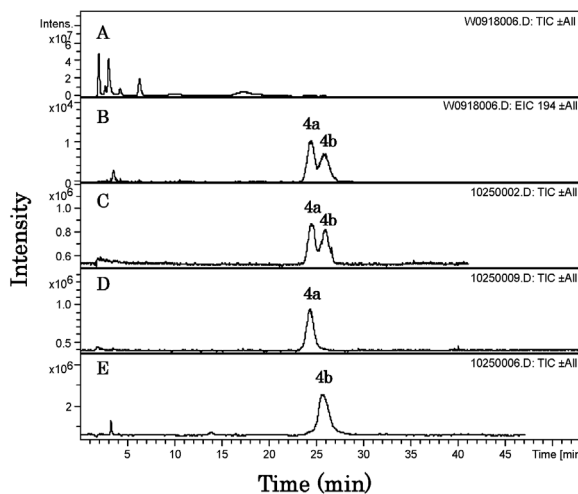


Fig. 4 TIC and EIC chromatograms monitored at  $m/z$  194.

A, TIC of plasma 6 h after oral administration of 200 mg/kg of swertiamarin (**1**); B, EIC at  $m/z$  194 of above plasma; C, TIC of ( $\pm$ )-gentianol (**4**); D, TIC of (*R*)-gentianol (**4a**); E, TIC of (*S*)-gentianol (**4b**). All data were collected using system B as described in the experimental section.

former and latter methods, respectively). We then attempted biotransformation using baker's yeast for stereoselective ketone reduction, since the analogous yeast-mediated reduction of 3-acetylpyridine<sup>14)</sup> and methyl 5-acetylnicotinate<sup>15)</sup> has been reported. Using this method, we synthesized one enantiomer (> 98% ee) from gentianone (3) despite a poor yield (6.5%).

Since baker's yeast stereoselectively reduced 3-acetylpyridine and methyl 5-acetylnicotinate to (*S*)-3-pyridinemethanol and methyl (*S*)-5-(1'-hydroxyethyl)nicotinate, respectively, we postulated that gentianone (3) would also be reduced to (*S*)-gentianol (4b). The product reduced by baker's yeast had the same optical rotation (negative) as (*S*)-3-pyridinemethanol and methyl (*S*)-5-(1'-hydroxyethyl)nicotinate, which supported our notion. To unequivocally determine the absolute configuration of the C-8 position of the synthesized enantiomers, we applied the improved Mosher's method<sup>16)</sup> to the alcohol. The compound was derivatized to the pair of isomers **A** and **B** with (*R*)-(-)-MTPA-Cl and (*S*)-(+)-MTPA-Cl, respectively (Fig. 6). Figure 7 shows that the proton signals at the pyridine and lactone rings in **A** were observed at lower fields than those of **B** ( $\Delta\delta$ : positive), while a signal due to a methyl group in

**A** was observed at higher fields than in **B** ( $\Delta\delta$ : negative). Based on this evidence, we determined the absolute configuration of the synthesized alcohol as *S* (Fig. 4. E).

Although ( $\pm$ )-gentianol (4) was detected in rat plasma after a single oral dose of 200 mg/kg, gentianine (2) was not clearly detectable. However, we detected a low concentration of gentianine (2) in the rat plasma when monitored by EIC after 4 repeated oral doses of 1,000 mg/kg swertiamarin (1) every 12 h (Fig. 2. I).

Gentianine (2) seemed to be further metabolized to ( $\pm$ )-gentianol (4) in the liver, and this was verified by incubating gentianine (2) *in vitro* with liver microsomes from male Wistar rats (data not shown). We therefore proposed that swertiamarin (1) was first hydrolyzed by  $\beta$ -glycosidase in the gut, where the unstable aglycone spontaneously reacted with ammonia to form gentianine (2). Gentianine (2) was further converted to ( $\pm$ )-gentianol (4) in the liver, and then excreted *via* the urine. Figure 8 shows the possible pathways from swertiamarin (1) to gentianine (2) through Schiff's bases (D and E) followed by intramolecular cyclization.

Gentianine (2) reportedly depresses the central nervous system, has anti-ulcerogenic action and inhibits gastric

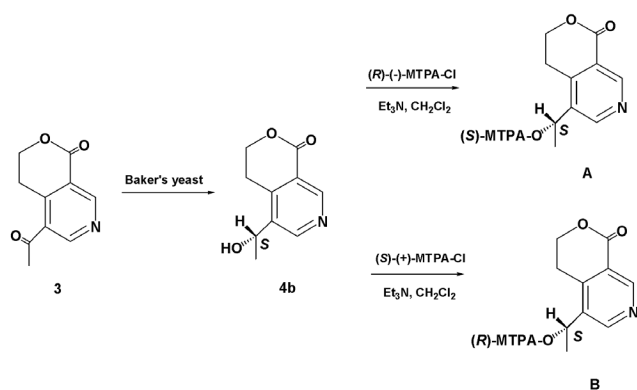


Fig. 6 Preparation of MTPA derivatives of (*S*)-gentianol (4b).

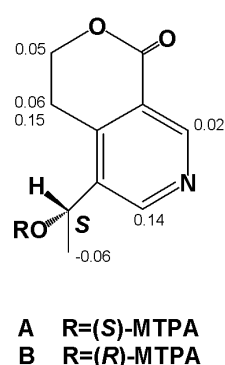


Fig. 7  $\Delta\delta$  Values (ppm) obtained by  $\delta A$ - $\delta B$ .

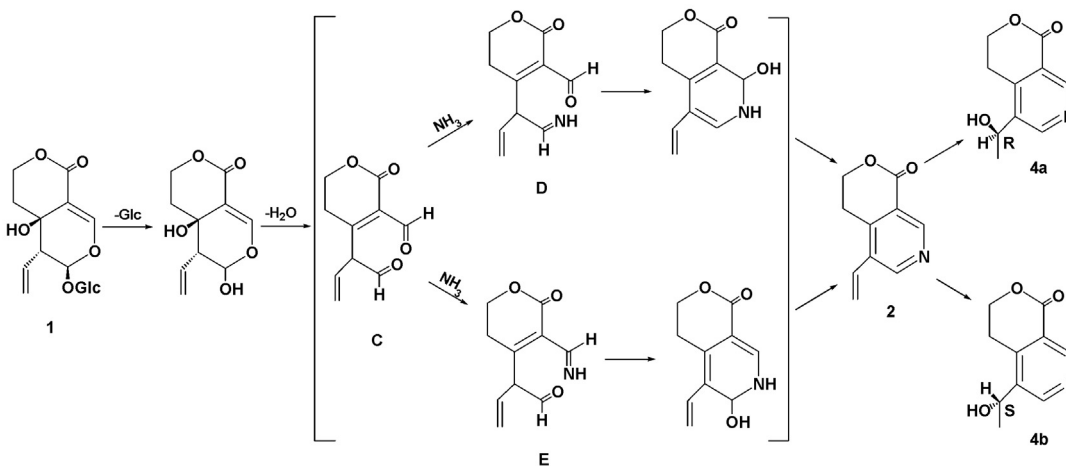


Fig. 8 Proposed metabolic pathways for conversion of swertiamarin (1) to nitrogen-containing compounds *in vivo*.

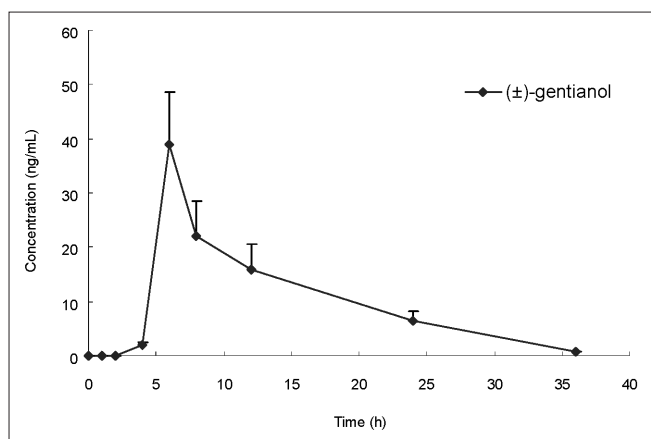


Fig. 9 Time course of (±)-gentianol (4) in rat plasma after oral administration of 200 mg/kg of swertiamarin (1) ( $n = 3$ ).

secretion.<sup>17)</sup> It is probable that other metabolites such as (±)-gentianol (4) also play an important role for the pharmacological effects of swertiamarin *in vivo*. The bioactivity of new metabolite (±)-gentianol (4) will be investigated in more detail.

Most natural iridoids and secoiridoids exist as glycosides with high polarity which causes difficulties with physiological absorption. Many pharmacokinetic studies<sup>18-20)</sup> have revealed that the bioavailability of iridoids and secoiridoids after oral administration is very low due to the first pass effect of gastric acid hydrolysis, followed by metabolic activities by intestinal bacteria and possibly, by the liver. We quantified (±)-gentianol (4) in rat plasma (Fig. 9). However, the plasma concentrations of the metabolites were low. We therefore speculated that most of the metabolites including nitrogen-containing compounds are converted to other compounds that are undetectable by LC/MS or form adducts with other physiological substances *in vivo*.

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