MEDICINAL FOODSTUFFS. XXXIII. GASTROPROTECTIVE PRINCIPLES FROM BOESENBERGIA ROTUNDA (ZINGIBERACEAE)—ABSOLUTE STEREOSTRUCTURES OF DIELS–ALDER TYPE ADDITION PRENYLCHALCONES—

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Abstract —The methanolic extract from the rhizomes of Boesenbergia rotunda (Zingiberaceae) was found to exhibit potent inhibitory activities on ethanol- or indomethacin-induced gastric mucosal lesions in rats. Through the bioassay-guided separation, six new optically active Diels–Alder type addition prenylcalcones, (+)-panduratin A (1a), (–)-panduratin A (1b), (+)-4-hydroxypanduratin A (2a), (–)-4-hydroxypanduratin A (2b), (+)-isopanduratin A (3a), and (–)-isopanduratin A (3b) were isolated together with 12 known compounds [(4—14) and geraniol]. The absolute stereostructures of six new compounds were elucidated on the basis of physicochemical evidence including CD spectra. Among them, the enantiomeric mixtures of panduratin A (1a, 1b) and 4-hydroxypanduratin A (2a, 2b), and pinocembrin (4) showed gastroprotective effects on ethanol- or indomethacin-induced gastric mucosal in rats.

A Zingiberaceae plant, Boesenbergia rotunda (LINN.) MANSF. [syn. B. pandulata (ROXB.) SCHLTR., Thai Ginseng in English, Krachai in Thai] is distributed in Southeastern Asian countries such as Myanmar, Indonesia, Malaysia, and Thailand. 2,3 The rhizomes of this plant have been used for the treatment of oral diseases (dry mouth), stomach discomfort, stomach pain, leucorrhoea, diuretic, dysentery, and inflammation, etc, while the fresh rhizomes have been also used as a spice in Southern Asian countries. 2,3

Previously, flavonoids, 4–8 chalcones, 4,7 and prenylcalcones 4,6–11 were isolated from this herbal medicine. In the pharmacological studies, the extract and its constituents have been reported to show anti-inflammatory, 12 anti-tumor, 13 anti-mutagenic, 7 antioxidant, 14 anti-HIV-1 protease, 11 and anticancer 15 activities, and inhibitory activity on dengue-2 virus NS3 protease. 16 During the course of our
characterization studies on Thai medicinal foods such as *Albizia myriophylla*, *Salacia chinensis*, *Alpinia galanga*, *Piper chaba*, *Curcuma zedoaria* (Thai Zedoary), *Erycibe expansa*, and *Borassus flabellifer*, the methanolic extract from the dried rhizomes of *B. rotunda* was found to exhibit potent inhibitory activities on ethanol- or indomethacin-induced gastric mucosal lesions in rats. From the methanolic extract, six new optically active Diels–Alder type addition prenylchalcones, (+)-panduratin A (1a), (--)-panduratin A (1b), (+)-4-hydroxypanduratin A (2a), (--)-4-hydroxypanduratin A (2b), (+)-isopanduratin A (3a), and (--)-isopanduratin A (3b) were isolated together with 12 known compounds [(4–14) and geraniol]. This paper deals with the isolation and absolute stereostructure determination of six new prenylchalcones (1a, 1b, 2a, 2b, 3a, and 3b) as well as the gastroprotective effects of the principal constituents.

The rhizomes of *B. rotunda*, which were cultivated in Nakhonsithammarat province, Thailand, were extracted with methanol to give the methanolic extract (10.2% from the dried rhizomes). As shown in Table 1, the methanolic extract significantly inhibited ethanol- and indomethacin-induced gastric mucosal lesions in rats (*ED*$_{50}$ = 12.5, ca. 35 mg/kg, p.o., respectively). The methanolic extract was subjected to normal- and reversed-phase silica gel column chromatographies, and finally HPLC to give enantiomeric mixtures [panduratin A$^6$ (1a, 1b, 0.77%), 4-hydroxypanduratin A$^7$ (2a, 2b, 0.25%), and isopanduratin A$^{35}$ (3a, 3b, 0.36%)], and pinocembrin$^{4,36}$ (4, 1.82%), pinostrobin$^{4,36}$ (5, 1.96%), alpinetin$^{4,36}$ (6, 0.99%), 7,4'-dihydroxy-5-methoxyflavanone$^{37}$ (7, 0.0064%), 5,7-dihydroxy-8-geranylflavanone$^{38}$ (8, 0.012%), 7-methoxy-5-hydroxy-8-geranylflavanone$^{39}$ (9, 0.010%), cardamonin$^{4,36}$ (10, 0.014%), 2,6-dihydroxy-4-methoxydihydroalchalone$^{39}$ (11, 0.0034%), 2,4-dihydroxy-6-phenethylbenzoic acid methyl ester$^{40}$ (12, 0.0067%), geranyl-2,4-dihydroxy-6-phenethylbenzoate$^{36}$ (13, 0.018%), 5,6-dehydrokawain$^{41}$ (14, 0.028%), and geraniol$^{42}$ (0.080%).

**Table 1.** Effects of MeOH ext. on Gastric Lesions Induced by EtOH or Indomethacin in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>N</th>
<th>EtOH-induced gastric lesions</th>
<th>Indomethacin-induced gastric lesions</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lesion index (mm)</td>
<td>Inhibition (%)</td>
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<td>Control</td>
<td>—</td>
<td>8</td>
<td>116.8 ± 11.6</td>
<td>—</td>
</tr>
<tr>
<td>MeOH ext.</td>
<td>6.25</td>
<td>6</td>
<td>81.2 ± 10.0</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>6</td>
<td>53.0 ± 15.3**</td>
<td>54.6</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>36.8 ± 15.0**</td>
<td>68.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5</td>
<td>26.0 ± 3.0**</td>
<td>77.8</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>7</td>
<td>146.8 ± 9.7</td>
<td>—</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>2.5</td>
<td>4</td>
<td>80.9 ± 12.4</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>80.9 ± 12.4</td>
<td>54.6</td>
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<td></td>
<td>10</td>
<td>7</td>
<td>78.3 ± 15.9**</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>7</td>
<td>31.1 ± 11.6**</td>
<td>78.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>15.9 ± 3.1**</td>
<td>89.2</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>6</td>
<td>136.0 ± 10.6</td>
<td>—</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>12.5</td>
<td>5</td>
<td>123.4 ± 9.4</td>
<td>9.3</td>
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<td>25</td>
<td>7</td>
<td>87.2 ± 7.9**</td>
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<td></td>
<td>50</td>
<td>7</td>
<td>72.9 ± 10.1**</td>
<td>46.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7</td>
<td>64.4 ± 4.9**</td>
<td>52.6</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. Significantly different from the control group, *p*<0.05, **p**<0.01.
Although panduratin A, 4-hydroxypanduratin A, and isopanduratin A, which were Diels-Alder type addition prenylchalcones, were isolated as the enantiomeric mixtures. Although panduratin A, 4-hydroxypanduratin A, and isopanduratin...
A showed optical rotations, we found that their compounds are able to separate by HPLC using chiral column (Ceramospher Chiral RU-1 or RU-2). In order to separate their optically active compounds, those mixtures were purified by HPLC using chiral column to furnish (+)-panduratin A (1a, 0.41%), (–)-panduratin A8 (1b, 0.22%), (+)-4-hydroxypanduratin A (2a, 0.098%), (–)-4-hydroxypanduratin A8,36 (2b, 0.065%), (+)-isopanduratin A (3a, 0.14%), and (–)-isopanduratin A (3b, 0.12%), respectively.

(+)-Panduratin A (1a) and (–)-panduratin A43 (1b), whose compositions were found to be ca. 2:1 ratio by HPLC analysis using chiral column (Figure 1), were isolated as white powders with positive and negative optical rotation (1a: \([\alpha]_D^{25} +102^\circ\), 1b: \([\alpha]_D^{25} –108^\circ\) both in MeOH), respectively. The electron ionization (EI)-MS of 1a and 1b showed the same molecular ion peak at m/z 406 (M+), and the molecular formula C26H30O4 was determined by high-resolution EI-MS measurement. The 1H- (CDCl3) and 13C-NMR (Table 2) spectra of 1a and 1b, which were assigned by various NMR experiments,44 showed the same signals assignable to three methyls \([\delta 1.52 (6H, s, 10', 11'-H_3), 1.78 (3H, s, 12'-H_3)]\), two methylenes \([\delta 2.03, 2.40 (1H each, both m, 5'_ax, 5'_eq-H)]\), three methines \([\delta 2.63 (1H, m, 2'-H), 3.43 (1H, m, 6'-H), 4.66 (1H, dd, J = 4.6, 11.3 Hz, 1'-H)]\), two trisubstituted oleins \([\delta 4.87 (1H, t-like, 8'-H), 5.43 (1H, br s, 4'-H)]\), and seven aromatic protons \([\delta 5.87 (2H, s, 3, 5-H), 7.10 (1H, m, 4''-H), 7.21 (4H, m, 2'',6'', 3'',5''-H)]\) together with a methoxyl group \([\delta 3.74 (3H, s, -OCH_3)]\). By comparison of the NMR data of 1a and 1b with those of panduratin A,6 the relative structures of 1a and 1b were confirmed. As shown in Figure 2, the circular dichroic (CD) spectrum of 1a was observed at 226 nm (\(\Delta\varepsilon +6.02\)), 263 nm (\(\Delta\varepsilon +2.39\)), and 297 nm (\(\Delta\varepsilon -1.29\)), which showed negative Cotton effect. On the other hand, the CD spectrum of 1b showed positive Cotton effect [226 nm (\(\Delta\varepsilon -6.58\)), 262 nm (\(\Delta\varepsilon -2.30\)), and 294 nm (\(\Delta\varepsilon +2.31\)]. On the basis of above-mentioned evidence, the absolute stereostructures of 1a and 1b were elucidated to be 1'R,2'S,6'R and 1'S,2'R,6'S orientations, respectively.

![Figure 1. Chiral HPLC Chromatogram of Panduratin A (1a, 1b)](image1)

HPLC condition: column: Ceramospher Chiral RU-1 (250 x 4.6 mm i.d.), detection: UV (254 nm), mobile phase: MeOH, flow rate: 1.0 ml/min.

![Figure 2. CD Spectra and Conformations of 1a and 1b](image2)
(+)-4-Hydroxyponduratin A (2a) and (−)-4-hydroxyponduratin A (2b) were isolated ca. 3:2 ratio and observed positive and negative optical rotations (2a: [α]D^25 +62°, 2b: [α]D^25 −62° both in MeOH), respectively. The same molecular formula C_{25}H_{28}O_{4} was determined from the molecular ion peak at m/z 392 (M⁺) by EI-MS and high-resolution EI-MS measurements of 2a and 2b. The 1H- (DMSO-d₆) and 13C-NMR (Table 2) spectra of 2a and 2b showed signals the same signals assignable to three methyls [δ 1.53, 1.55, 1.80 (3H each, all s, 11', 10', 12'-H₃)], two methylenes [δ 2.01, 2.37 (1H each, both m, 7'-H₂)], three methines [δ 2.56 (1H, m, 2'-H), 3.40 (1H, m, 6'-H), 4.76 (1H, dd, J = 4.5, 11.6 Hz, 1'-H)], two trisubstituted olefins [δ 4.94 (1H, br s, 8'-H), 5.45 (1H, t-like, 4'-H)], and seven aromatic protons [δ 8.00 (2H, s, 3', 5'-H), 7.10 (3H, m, 2''',6''', 4'''-H), 7.25 (2H, m, 3''',5'''-H)], which were very similar to those of 1a and 1b, except for the signals due to the lacking of a methoxyl group. Comparison of the NMR data of 2a and 2b with those of 4-hydroxyponduratin A led us to confirm the relative structures of 2a and 2b.

(+)-Isopanduratin A (3a) and (−)-isopanduratin A (3b), the same molecular formula C_{26}H_{30}O_{4}, were also isolated ca. 7:6 ratio and observed with positive and negative optical rotation (3a: [α]D^19 +70°, 3b: [α]D^22 −67° both in MeOH), respectively. The proton and carbon signals in 1H- (CDCl₃) and 13C-NMR (Table 2) spectra of 3a and 3b were confirmed on those of isopanduratin A [three methyls {δ 1.51 (6H, s, 10', 11'-H₃), 1.79 (3H, s, 12'-H₃)}, two methylenes {δ 2.02, 2.40 (1H each, both m, 5''',6''', 4'''-H)}, 2.10, 2.23 (1H each, both m, 7'-H₂)], three methines [δ 2.49 (1H, m, 2'-H), 3.42 (1H, m, 6'-H), 4.49 (1H, dd, J = 4.6, 11.3 Hz, 1'-H)], two trisubstituted olefins [δ 4.86 (1H, t-like, 8'-H), 5.42 (1H, br s, 4'-H)], and seven aromatic protons [δ 5.90 (1H, s, 3'-H), 5.92 (1H, s, 5'-H), 7.09 (1H, m, 4'''-H), 7.19 (2H, m, 2''',6'''-H), 7.21 (2H, m, 3''',5'''-H)] together with a methoxyl group [δ 3.90 (3H, s, -OCH₃)]. The CD spectra of 2a and 3a showed negative Cotton effects (2a: 224 nm (+3.80), 263 nm (+1.44), and 302 nm (−0.54), 3a: 225 nm (+0.65), 267 nm (+0.38), and 306 nm (−0.07)], whereas 2b and 3b showed positive Cotton effects (2b: 224 nm (−4.35), 262 nm (−1.52), and 299 nm (+1.40), 3b: 224 nm (−0.50), 260 nm (−0.51), and 314 nm (+0.15)]. On the basis of above-mentioned evidence, the absolute stereostructures of 2a−3b were determined.

### Effects of Constituents on Gastric Lesions by EtOH or Indomethacin in Rats
Effects of the principal constituents, panduratin A (1a, 1b), 4-hydroxyponduratin A (2a, 2b), pinocembrin (4), pinostrobin (5), and alpinetin (6), on EtOH- or indomethacin-induced gastric lesions were examined.
Previously, we reported that several triterpene saponins,46–49 steroid saponins,50 sesquiterpenes,51,52 phanylpropanoids,21 and amide constituents26 showed protective effects on EtOH- and/or indomethacin-induced gastric lesions in rats. As shown in Table 3, (+)- and (–)-panduratin A (1a, 1b) and 4-hydroxypanduratin A (2a, 2b) showed protective effects on EtOH-induced gastric lesions, and also (+)- and (–)-panduratin A (1a, 1b) and 4 showed indomethacin-induced gastric lesions at a dose of each 10 mg/kg, p.o. Their gastroprotective effects were equivalent or stronger than those of reference compound, cimetidine (EtOH-induced: ED50 = 69 mg/kg, p.o.; indomethacin-induced: ED50 = 21 mg/kg, p.o., as shown in Table 1).

Table 3. Effects of Principal Constituents on Gastric Lesions Induced by EtOH or Indomethacin in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>EtOH-induced gastric lesions</th>
<th>Indomethacin-induced gastric lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Lesion index (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>121.9 ± 6.0</td>
<td>—</td>
</tr>
<tr>
<td>(+)- and (–)-Panduratin A (1a, 1b)</td>
<td>5.0</td>
<td>78.0 ± 10.6</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>39.5 ± 12.3**</td>
<td>67.6</td>
</tr>
<tr>
<td>(+)- and (–)-4-Hydroxypanduratin A (2a, 2b)</td>
<td>5.0</td>
<td>85.5 ± 12.9</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>53.9 ± 10.1**</td>
<td>55.8</td>
</tr>
<tr>
<td>Pinocembrin (4)</td>
<td>5.0</td>
<td>102.2 ± 13.2</td>
<td>16.2</td>
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<tr>
<td></td>
<td>10</td>
<td>91.6 ± 10.5</td>
<td>24.9</td>
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<tr>
<td>Pinostrobin (5)</td>
<td>5.0</td>
<td>98.0 ± 15.1</td>
<td>19.6</td>
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<tr>
<td></td>
<td>10</td>
<td>85.4 ± 14.3</td>
<td>29.9</td>
</tr>
<tr>
<td>Alpinetin (6)</td>
<td>5.0</td>
<td>110.4 ± 4.5</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>87.4 ± 13.1</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. Significantly different from the control group, *p<0.05, **p<0.01.

EXPERIMENTAL
The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrophotometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; 1H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; 13C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index and SPD-10Avp UV-VIS detectors; and HPLC column, YMC-Pack ODS-A (YMC Co., Ltd. 250 × 4.6 mm i.d. and 250 × 20 mm i.d.) and Ceramospher Chiral RU-1 and RU-2 (Shiseido Co., Ltd. 250 × 4.6 mm i.d. and 250 × 10 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase column chromatography; Silica gel BW-200 (Fuji Sylsia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Sylsia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with Silica gel 60F254 (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 F254S (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF254S (Merck, 0.25 mm) (reversed-phase) and detection was achieved by spraying with 1% Ce(SO4)2-10% aqueous H2SO4, followed by heating.
Plant Material
The rhizomes of *B. rotunda* were cultivated in Nakhonsithammarat province, Thailand in April 2002, and were identified by one of the authors, Y. Pongpiriyadacha (Lecturer of Faculty of Science and Technology, Rajamangala University of Technology Srivijaya). A voucher specimen (No. T-08) is on file in our laboratory.

Extraction and Isolation
The dried rhizomes of *B. rotunda* (990 g) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (100.6 g, 10.2% from the dried rhizomes). The methanolic extract (80.0 g) was subjected to normal-phase silica gel column chromatography [2.2 kg, hexane–EtOAc (25:1 → 10:1 → 1:1, v/v) → acetone → MeOH] to give nine fractions {Fr. 1 (0.5 g), Fr. 2 (2.7 g), Fr. 3 [= pinostrobin (5, 14.9 g, 1.90%)], Fr. 4 (11.1 g), Fr. 5 (13.1 g), Fr. 6 (19.0 g), Fr. 7 (5.4 g), Fr. 8 [= alpinetin (6, 7.8 g, 0.99%)], and Fr. 9 (5.5 g)}. Fraction 2 (2.7 g) was separated by reversed-phase silica gel column chromatography [80 g, MeOH–H$_2$O (80:20 → 95:5, v/v) → MeOH] to give 10 fractions {Fr. 2-1 (421.6 mg), Fr. 2-2 (52.1 mg), Fr. 2-3 (206.4 mg), Fr. 2-4 (49.4 mg), Fr. 2-5 (58.4 mg), Fr. 2-6 (36.3 mg), Fr. 2-7 (156.2 mg), Fr. 2-8 (330.0 mg), Fr. 2-9 (32.1 mg), and Fr. 2-10 (29.1 mg)}. Fraction 2-3 (206.4 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–H$_2$O (80:20, v/v)] to furnish pinostrobin (5, 17.1 mg, 0.0022%). Fraction 2-8 (330.0 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–H$_2$O (85:15, v/v)] to furnish 7-methoxy-5-hydroxy-8-geranylflavanone (9, 80.0 mg, 0.010%). Fraction 4 (11.1 g) was separated by reversed-phase silica gel column chromatography [333 g, MeOH-H$_2$O (50:50 → 60:40 → 70:30 → 80:20 → 90:10, v/v) → MeOH] to give 11 fractions {Fr. 4-1 (71.2 mg), Fr. 4-2 [= geraniol (630.5 mg, 0.080%)], Fr. 4-3 (87.9 mg), Fr. 4-4 [= pinostrobin (5, 400.0 mg, 0.051%)], Fr. 4-5 (126.5 mg), Fr. 4-6 [= panduratin A (1a, 1b, 4.70 g, 0.60%)], Fr. 4-7 (313.9 mg), Fr. 4-8 (454.1 mg), Fr. 4-9 (1.50 g), Fr. 4-10 (258.7 mg), and Fr. 4-11 (85.9 mg)}. Fraction 4-3 (87.9 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–H$_2$O (80:20, v/v)] to furnish pinostrobin (5, 13.8 mg, 0.0018%) and 2,4-dihydroxy-6-phenethylbenzoic acid methyl ester (12, 52.8 mg, 0.0067%). Fraction 4-7 (313.9 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeCN–H$_2$O (80:20, v/v)] to furnish 5,7-dihydroxy-8-geranylflavanone (8, 96.6 mg, 0.012%). Fraction 4-8 (454.1 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeCN–H$_2$O (80:20, v/v)] to furnish ganyl-2,4-dihydroxy-6-phenylbenzoate (13, 142.2 mg, 0.018%). Fraction 5 (13.1 g) was recrystallized with MeOH to furnish pinocembrin (4, 8.68 g, 1.10%) and mother liquid, which was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–H$_2$O (90:10, v/v)] to furnish five fractions {Fr. 5-2-1 [= pinocembrin (4, 696.1 mg, 0.089%)], Fr. 5-2-2 (87.1 mg), Fr. 5-2-3 (2.32 g), Fr. 5-2-4 [= isopanduratin A (3a, 3b, 206.8 mg, 0.026%)], and Fr. 5-2-5 (149.9 mg)}. Fraction 5-2-3 (2.32 g) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeCN–H$_2$O (75:25, v/v)] to furnish (2S)-panduratin A (1, 154.1 mg, 0.020%). Fraction 6 (19.0 g) was subjected by reversed-phase silica gel column chromatography [510 g, MeOH–H$_2$O (55:45, v/v) → MeOH] to give eight fractions {Fr. 6-1 (86.6 mg) Fr. 6-2 (89.7 mg), Fr. 6-3 [= pinocembrin (4, 4.90 g, 0.62%)], Fr. 6-4 (334.4 mg), Fr. 6-5 (327.8 mg), Fr. 6-6 (3.77 g), Fr. 6-7 (284.4 mg), and Fr. 6-8 (919.9 mg)}. Fraction 6-4 (334.4 mg) was purified by HPLC [RI
detector, YMC-Pack ODS-A, MeOH–1% aqueous AcOH (80:20, v/v)] to furnish cardamonin (10, 109.8 mg, 0.014%) and 2,6-dihydroxy-4-methoxydihydrochalcone (11, 27.0 mg, 0.0034%). Fraction 6-6 (1.04 g) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–1% aqueous AcOH (80:20, v/v)] to furnish panduratin A (1a, 1b, 314.4 mg, 0.15%) and isopandurtin A (3a, 3b, 721.3 mg, 0.33%). Fraction 7 (4.77 g) was separated by normal-phase silica gel column chromatography [143 g, hexane–EtOAc (1:1, v/v) → EtOAc] to give seven fractions {Fr. 7-1 (133.7 mg), Fr. 7-2 (92.3 mg), Fr. 7-3 (92.5 mg), Fr. 7-4 (753.5 mg), Fr. 7-5 (= 4-hydroxypanduratin A (2a, 2b, 754.6 mg, 0.11%), Fr. 7-6 (1.50 g), and Fr. 7-7 (809.0 mg)}. Fraction 7-4 (753.5 mg) was separated by reversed-phase silica gel column chromatography [24.4 g, MeOH–H₂O (60:40, v/v) → MeOH] to give 4-hydroxypanduratin A (2a, 2b, 124.8 mg, 0.018%). Fraction 7-5 (1.50 g) was separated by reversed-phase silica gel column chromatography [45.0 g, MeOH–H₂O (50:50, v/v) → MeOH] to give 4-hydroxypanduratin A (2a, 2b, 843.9 mg, 0.12%) and 5,6-dehydrokawain (14, 198.4 mg, 0.028%). Fraction 9 (5.5 g) was subjected by reversed-phase silica gel column chromatography [170 g, MeOH–H₂O (20:80 → 50:50 → 60:40, v/v) → MeOH] to give three fractions [Fr. 9-1 (926.0 mg), Fr. 9-2 (642.3 mg), and Fr. 9-3 (900.0 mg)]. Fraction 9-2 (642.3 mg) was purified by HPLC [RI detector, YMC-Pack ODS-A, MeOH–H₂O (50:50, v/v)] to furnish 4',7-dihydroxy-5-methoxyflavanone (7, 50.2 mg, 0.0064%).

Panduratin A (1a, 1b, 0.77% from the dried rhizomes, 100.0 mg) was purified by HPLC [UV detector (254 nm), Ceramospher Chiral RU-1, MeOH] to furnish (+)-panduratin A (1a, 53.7 mg, 0.41%) and (−)-panduratin A (1b, 28.0 mg, 0.22%). 4-Hydroxypanduratin A (2a, 2b, 0.25% from the dried rhizomes, 82.0 mg) was purified by HPLC [UV detector (254 nm), Ceramospher Chiral RU-1, MeOH] to furnish (+)-4-hydroxypanduratin A (2a, 32.0 mg, 0.098%) and (−)-4-hydroxypanduratin A (2b, 21.4 mg, 0.065%). Isopandurtin A (3a, 3b, 0.36% from the dried rhizomes, 60.0 mg)] was purified by HPLC [UV detector (254 nm), Ceramospher Chiral RU-2, MeCN–H₂O (65:35, v/v)] to furnish (+)-isopanduratin A (3a, 22.5 mg, 0.14%) and (−)-isopanduratin A (3b, 19.2 mg, 0.12%).

(+)-Panduratin A (1a): a white powder, [α]D²⁵ +102° (c 0.29, MeOH). High-resolution EI-MS: Calcd for C₂₆H₃₀O₄ (M⁺): 406.2144. Found: 406.2146. CD [MeOH, nm, (Δε)]: 226 (+6.02), 263 (+2.39), 297 (−1.29). UV [MeOH, nm (log ε)]: 291 (4.14). IR (KBr): 3483, 1630, 1597, 1098, 1051, 1020 cm⁻¹. EI-MS m/z (%): 406 (M⁺, 5), 271 (100).


1a and 1b: ¹H-NMR (CDCl₃, 500 MHz) δ: 1.52 (6H, s, 10°, 11°-H₃), 1.78 (3H, s, 12°-H₃), 2.03, 2.40 (1H each, both m, J = 4.5, 11.3 Hz, 1°-H), 2.63 (1H, m, 7°-H), 3.74 (3H, s, -OCH₃), 4.66 (1H, dd, J = 4.6, 11.3 Hz, 1°-H), 4.87 (1H, t-like, 8°-H), 5.43 (1H, br s, 4°-H), 5.87 (2H, s, 3, 5°-H), 7.10 (1H, m, 4°-H), 7.21 (4H, m, 2°, 6°, 3°, 5°-H). ¹³C-NMR (CDCl₃, 125 MHz) δ: given in Table 2.


2a and 2b: 1H-NMR (DMSO-d6, 500 MHz) δ: 1.53, 1.55, 1.80 (3H each, all s, 11', 10', 12'-H3), 2.01, 2.37 (1H each, both m, 5'α, 5'eq-H), 2.07, 2.25 (1H each, both m, 7'-H2), 2.56 (1H, m, 2'-H), 3.40 (1H, m, 6'-H), 4.76 (1H, dd, J = 4.5, 11.6 Hz, 1'-H), 4.94 (1H, br s, 8'-H), 5.45 (1H, t-like, 4'-H), 5.83 (2H, s, 3, 5-H), 7.14 (3H, m, 2'',6'', 4''-H), 7.25 (2H, m, 3'',5''-H). 13C-NMR (DMSO-d6, 125 MHz) δc: given in Table 2.

(−)-Isopanduratin A (3a): a white powder, [α]D19 +70° (c 2.80, MeOH). High-resolution EI-MS: Calcd for C26H30O4 (M+): 406.2144. Found: 406.2143. CD [MeOH, nm (Δε)]: 225 (−0.65), 267 (−0.38), 306 (−0.07). UV [MeOH, nm (log ε)]: 292 (4.16). IR (KBr): 3480, 1628, 1215, 1109 cm−1. EI-MS m/z (%): 406 (M+, 1), 167 (100).

(−)-Isopanduratin A (3b): a white powder, [α]D22 −67° (c 2.10, MeOH). High-resolution EI-MS: Calcd for C26H30O4 (M+): 406.2144. Found: 406.2143. CD [MeOH, nm (Δε)]: 224 (−0.50), 260 (−0.51), 314 (+0.15). UV [MeOH, nm (log ε)]: 292 (4.16). IR (KBr): 3490, 1684, 1215, 1109 cm−1. EI-MS m/z (%): 406 (M+, 1), 167 (100).

3a and 3b: 1H-NMR (CDCl3, 500 MHz) δ: 1.51 (6H, s, 10', 11'-H3), 1.79 (3H, s, 12'-H3), 2.02, 2.40 (1H each, both m, 5'α, 5'eq-H), 2.10, 2.23 (1H each, both m, 7'-H2), 2.49 (1H, m, 2'-H), 3.42 (1H, m, 6'-H), 3.90 (3H, s, -OCH3), 4.49 (1H, dd, J = 4.6, 11.3 Hz, 1'-H), 4.86 (1H, t-like, 8'-H), 5.42 (1H, br s, 4'-H), 5.90 (1H, s, 3-H), 5.92 (1H, s, 5-H), 7.09 (1H, m, 4''-H), 7.19 (2H, m, 2'',6''-H), 7.21 (2H, m, 3'',5''-H). 13C-NMR (CDCl3, 125 MHz) δc: given in Table 2.

**Bioassay Method**

**Animals**

Male Sprague-Dawley rats weighing about 230–250 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 24–26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious rats unless otherwise noted. The experimental protocols were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

**Effect of EtOH- or Indomethacin-induced Gastric Mucosal Lesions in Rats**

The acute gastric lesions were induced by oral administration of EtOH and indomethacin according to the method described previously.21,26,46–52 Briefly, 99.5% EtOH (1.5 mL/rat) and indomethacin (20 mg/kg, dissolved in 5% aqueous sodium bicarbonate, and then diluted in water and neutralized with 0.2 m HCl
and adjusted to 1.5 mL/rat) were administered to 24–26 h fasted rats using a metal orogastric tube. One hour after administration of EtOH or 4 h after administration of indomethacin, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 10 mL 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature, the lengths of gastric lesions were measured and the total length (mm) was expressed as a lesion index. The test samples and cimetidine were suspended in 5% acacia solution. Omeprazole was suspended in 0.5% CMC-Na. Test samples in vehicle and vehicle only (control group) were administered orally at a dose of 5.0 mL/kg 1 h prior to the application of EtOH and indomethacin.

Statistics
Values were expressed as means±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett’s test was used. Probability (P) values less than 0.05 were considered significant. ED_{50} values were estimated based on linear regressions of probit-transformed values of inhibition (%).

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REFERENCES AND NOTES
33. We presented the isolation and structural elucidation of these compounds (1a, 1b, 2a, 2b, 3a, and 3b) from the rhizomes of *B. ratunda* at the 46th Symposium on the Chemistry of Natural Products on October, 2004.34

43. Previously, isolation studies of compounds 1b ([α]D²⁹ −24.62°) and 2b ([α]D²⁵ −10.44° both in EtOH) were reported except for a chiral HPLC purification and absolute stereostructure determination. Thus this paper is the first report for the separation and absolute stereosturuture determination of optically active compounds (1b and 2b).

44. The ¹H- and ¹³C-NMR spectra of 1a—3b were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), homocorrelation spectroscopy (¹H-¹H COSY), heteronuclear multiple quantum coherence (HMQC), and HMBC experiments.

45. Spartan (version '02, Wavefunction, Inc., Irvine, CA) was used to build and optimize the conformations of 1a and 1b (Figure 2) using MOPAC (AM1) program. Those conformations were also supported by the NOE correlations in the nuclear Overhauser enhancement spectroscopy (NOESY) experiments, respectively (data not shown).