The Anti-Helicobacter pylori Flavonoids in a Brazilian Plant, Hyptis fasciculata, and the Activity of Methoxyflavones

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Two known flavonoids were isolated from a tropical medicinal plant, Hyptis fasciculata (Labiatae), found in Brazil. Flavonoids were identified as cirsilineol (1) and cirsimaritin (2) by spectroscopic means and were exhibited potent antibacterial activity against Helicobacter pylori, and cirsilineol (1) had weak antibacterial activity against Escherichia coli and Salmonella enteritidis. Following up on the relationship between anti-H. pylori activity and flavonoids with methoxy groups, several methoxy flavonoids were evaluated for proliferation of H. pylori.

Key words Helicobacter pylori; flavone; Hyptis fasciculata; methoxy; Labiatae

We have studied the anti-H. pylori activity of several methoxy flavonoids, as previous studies have indicated that the methoxy groups increase the anti-H. pylori activity of isoflavonoids.1) We have been studying Brazilian medicinal plants with antibacterial activity against Helicobacter pylori.2) Hyptis fasciculata Bentham (Labiatae) is native to Brazil, Argentina, and Uruguay, and is used as an expectorant, sudorific, and spasm sputum.3) Isolation of the constituents from this plant have not been studied previously. This study resulted in the isolation of five known compounds, cirsilineol (1), cirsimaritin (2), aurantiamide acetate, aurantiamide benzotate,4) and methoxynepetaeofolin, and two new diterpenoids.5) NMR and mass spectra were mainly used for the structural determinations. The obtained flavonoids exhibited positive activities against Helicobacter pylori.

MATERIALS AND METHODS

Experimental Instruments IR spectra were obtained using KBr discs in a Horiba FT-720 spectrophotometer. NMR spectra were recorded using a Bruker AVANCE 600 spectrophotometer at 600 MHz (1H) and 150 MHz (13C). All chemical shifts were given in ppm, with tetramethylsilane as an internal standard. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded using a Shimadzu UV2200 spectrophotometer. Mass spectra were obtained using a JASCO HX-100 spectrometer. Centrifugal liquid–liquid partition chromatography (CPC) was performed by using a Senshu LLB-M CPC system. HPLC was performed by using a JASCO 2000 HPLC system.

Plant Material The medicinal plant, Hyoptis fasciculata (2 kg), purchased in Brazil on March, 2001 (Code B2-154), was identified by Dr. G. Hashimoto (Centro de Pesquisas de Hístria, São Paulo).2) B2-154 was deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Isolation The aerial parts (1.45 kg) of the plant were mashed, and compounds were extracted with chloroform, and then extracted with methanol. Each extract solution was evaporated. The dry mass from the chloroform extract was 49.8 g and from the methanol extract it was 118.2 g. The chloroform extract was separated using an MPLC system. The materials were eluted with hexane, benzene, chloroform, ethyl acetate, acetone, and methanol in this order by using an MPLC column filled with silica gel. The methanol extract was partitioned with water and ethyl acetate. A water-soluble fraction (22.2 g) and an ethyl acetate-soluble fraction (30.0 g) were obtained. The ethyl acetate-soluble materials were distributed to the upper and lower layers using CPC in solvent system (CHCl3–CH3OH–H2O; 4: 4: 3). The lower layer was also separated by the similar MPLC method. The fractions obtained were purified by HPLC (ODS column, solvent; CH3OH: H2O; 50–80%) to obtain compound 1 (3.5 mg, 0.00024%) and compound 2 (19 mg, 0.0013%).

Compounds 1: Pale yellow fine needles; mp 193–195 °C. IR (KBr) cm−1: 1691, 1597, 1352, 1205, 1117, 1027. UV λmax (CH3OH) nm (ε): 280 (sh, 13000), 328 (27000), (+AlCl3) nm (ε): 375. EI-MS m/z: 344 (M+), 329, 298, 181, 153. HR-El-MS m/z: 344.0861 (C18H16O7; 344.0896). 1H-NMR (CD3OD) δ: 3.81 (3H, s, 7-OCH3), 3.83 (3H, s, 7- OCH3), 6.87 (1H, d, 8-H). 7.14 (1H, d, J = 8.9 Hz, 5'-H). 7.48 (1H, d, J = 1.9 Hz, 2'-H), 7.67 (1H, d, J = 8.9 Hz, 6'-H). 13C-NMR (CD3OD): 55.89 (s, C-3), 56.41 (q, 6-OCH3), 60.41 (q, 6-OCH3), 91.59 (d, C-5), 113.18 (d, C-6), 121.42 (s, C-1), 122.49 (s, C-3), 122.29 (d, C-4), 124.29 (s, C-7), 133.18 (s, C-6), 149.14 (s, C-3), 150.47 (s, C-7), 156.34 (s, C-2), 157.93 (s, C-2), 164.97 (s, C-2), 183.32 (s, C-4). This compound was identified as cirsimaritin from the spectral data.6)

Compounds 2: Pale yellow fine needles; mp 267–269 °C. IR (KBr) cm−1: 1650, 1599, 1488, 1456, 1532, 1254, 1120, 829, 650. UV λmax (CH3OH) nm (ε): 276 (18000), 332 (25000), (+AlCl3) nm (ε): 360. EI-MS m/z: 314 (M+), 299, 271, 153. HR-El-MS m/z: 314.0763 (C17H14O6; 314.0791). 1H-NMR (CD3OD) δ: 3.87 (3H, s, 7-OCH3), 3.99 (3H, s, 6-OCH3), 6.79 (1H, d, 8-H), 6.94 (1H, d, 3-H), 7.31 (each 1H, d, J = 8.3 Hz, 3', 5'-H), 7.95 (each 1H, d, J = 8.3 Hz, 2', 6'-H). 13C-NMR (CD3OD): 56.40 (q, 7-OCH3), 60.58 (q, 6-OCH3), 91.55 (d, C-8), 103.77 (d, C-3), 106.39 © 2006 Pharmaceutical Society of Japan

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(s, C-10), 116.95 (d, C-3', 5'), 122.23 (s, C-1'), 128.99 (d, C-2', 6'), 133.13 (s, C-6), 153.50 (s, C-9), 153.69 (s, C-5), 159.35 (s, C-7), 162.89 (s, C-4'). This compound was identified as cirsilimarit in the spectral data.7,8

**Other Flavones** To test anti-

**Helicobacter pylori** activity, we used flavones that had been isolated from other plants previously (wogonin,21 apigenin,9,10 luteolin,9,10 crysoeriol,10 neogletin,11 pedalitin,11,12 3',4'-O-methylpedalitin,12 eupatorin,12) and subsequent made into commercially available agents purchased from Extrasynthese (genkwanin, pilon, sinensetin, 3',4',5',6,7-hexamethoxyflavone).

**Biological Assays** The bioassay was carried out as described in previous literature,13 by dissolving the sample in dimethyl sulfoxide (DMSO) and then adding water. IC90 (inhibition concentration of 90%) were defined as the inhibitor concentrations that produced 90% growth inhibition, compared with a solvent as control.

**RESULTS** Compounds from the aerial parts of the plant were extracted with chloroform, and then with methanol. The chloroform extract was separated using an MPLC system. The fractions obtained were purified using CPC (centrifugal liquid–liquid partition chromatography) and MPLC system. The fractions obtained were purified by HPLC (ODS column) to obtain compounds 1 and 2.

Compound 1, a pale yellow crystal with molecular formula 

\[\text{C}_{17}\text{H}_{14}\text{O}_6\] had spectral data resembling compound 1. It is particularly interesting that compounds 1 and 2 have a potent inhibitory activity (IC90) at a concentration of each 3.2 and 6.3 µg/ml against anti-

**Helicobacter pylori** activity, having selectivity for other microorganisms tested. These antibacterial tests were carried out by the method described in a previous paper.13 To examine the relationship between the anti-

**Helicobacter pylori** activity and methoxy flavonoid,14 the anti-

**Helicobacter pylori** activity of several related flavones was tested as shown in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
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<td>H</td>
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hibitory activity (IC<sub>90</sub>) at a concentration of 12.5 μg/ml. Flavones with an inhibitory activity at 25 μg/ml were pedalitin, sinensetin, luteolin, ngeletin, and 3',4',5,5',6,7-hexamethoxyflavone.

DISCUSSION

It was implied that number and their positions of methoxy-groups in the flavone molecules might affect for the value of anti-Helicobacter pylori activities as shown in Table 2. Flavones with potent activities had mainly adjacent dimethoxy-groups, especially positions of 6 and 7, and simultaneously some hydroxy groups in molecule as cirsilineol (1), cirsimaritin (2), and eupatorin. Though the correlation between chemical structure and level of activity intensity was not conclusively found, it is possible to understand the features to some extent. The relationship of membrane permeability and the requirement for methoxy groups may be significant.

Flavonoids and methoxyflavonoids are widespread and abundant in nature, especially in many types of food. Even if the anti-H. pylori activity is weak, the prevention of bacterial infection of the stomach can be expected due to the intake of food.

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REFERENCES