

## Inhibition of *Helicobacter pylori* Motility by (+)-Syringaresinol from Unripe Japanese Apricot

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**A methanol extract from unripe Japanese apricot showed inhibitory activity of *Helicobacter pylori* motility. Inhibitory compound 1 was isolated and identified as (+)-syringaresinol (1) by spectroscopic means. (+)-Syringaresinol (1) inhibited >90% of the *H. pylori* motility at a concentration of 500 µg/ml and the IC<sub>50</sub> value was 50 µg/ml.**

**Key words** *Helicobacter pylori*; (+)-syringaresinol; Japanese apricot

*Helicobacter pylori* was isolated from the gastric antrum of chronic gastritis patients. *H. pylori* is closely associated with gastritis and peptic ulcers and is even a bacterial risk factor for gastric cancer.<sup>1–4</sup> Therefore, eradication of the bacteria and inhibition of the urease are important for the treatment of patients with gastroduodenal diseases.

The standard treatment for *H. pylori* related disease is a combination of antimicrobial agents and anti-acid agent.<sup>5</sup> However, the drug resistant *H. pylori* against most effective antimicrobials, metronidazole and clarithromycin, is commonplace in many societies and is of particular concern as the major reason for treatment failure.<sup>6–8</sup> *H. pylori* is a spiral-shapes, strongly motile bacterium, and the motility is generally held to be a requirement for colonization of the stomach.<sup>9,10</sup> Thus, one possible approach for prevention of *H. pylori* infection would be to inhibit the *H. pylori* motility.

The Japanese apricot (*Prunus mume* SIBE. *et.* ZUCC.; Ume), a deciduous tree of the family Rosaceae, originated in the central and southern regions of China, and has now 400–500 varieties worldwide. The fruits of Japanese apricot are taken in foods as umeboshi, Bainiku-ekisu, pickled Japanese apricot, ume liquor and ume-based soft drinks. The fruit has been known to have various biological activities, and the fruit has been prescribed medicine for disorder of the stomach and intestines, quick recovery from fatigue, cough and diarrhea in Chinese traditional prescriptions.<sup>11,12</sup> However, very few reports are available that proofs of components from Japanese apricot are effective against diseases.

During the screening program to discover such compounds from natural products, Japanese apricot was found to show inhibitory activity against *H. pylori* motility. In this paper, we report the isolation and identification of inhibitor of *H. pylori* motility from unripe Japanese apricot.

### MATERIALS AND METHODS

**General** Melting point was measured on a Micro Melting Point Meter MP-500D. Optical rotation was measured on a Japan Spectroscopic Co. Ltd. DIP-1000 in CHCl<sub>3</sub>. The EIMS were obtained on a JEOL the Tandem MStation JMS-700. The IR spectra were determined with a JASCO FT/IR-

470 plus Fourier transform infrared spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained with a JEOL FX-500 (500.00 MHz, <sup>1</sup>H; 125.65 MHz, <sup>13</sup>C) spectrometer.

**Materials** Fresh Japanese apricot was obtained from Minabegawa Plum Research Center (Wakayama, Japan).

**Media and Bacterial Growth** *H. pylori* (*H. pylori* ATCC43504, American type, culture collected Rokville MD, U.S.A.) was grown on blood agar plates (Trypticase soy agar supplemented with 5% sheep blood; Becton Dickinson, Tokyo, Japan) for 4 d at 37 °C in a microaerophilic atmosphere (10% O<sub>2</sub> and 10% CO<sub>2</sub>). The colonies developed were then suspended in brain heart infusion (BHI) broth (Difco) containing 10% fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, U.S.A.), followed by incubation for 18 to 20 h at 37 °C in a microaerophilic atmosphere. The bacterial cultures were subjected to motion analysis.

**Motion Analysis** Bacterial motility was examined under an inverted, phasecontrast microscope with a Analysis Chamber (Neuroscience Co., Osaka, Japan) of specimens. The motility speed (in micrometers per second) was measured by using a motion analysis system with the program C-Imaging C-MEN (Complx Inc., Cramberry, PA, U.S.A.). Bacterial swimming in a liquid layer of BHI broth containing 10% FBS between a glass slide and a glass cover (106 to 107 CFU/ml) was continuously recorded 15 times with a 0.05-s analysis time each (a total of 0.75 s), and the swimming speed (in micrometers per second) of each bacterial cell in a specimen was obtained. This was performed in at least five different fields of a specimen, the swimming speeds of *ca.* 1000 bacterial cells were collected for each specimen, and the percent of motile bacteria was determined. Brownian motion of bacteria was estimated to be 0.4±0.3 µm/s using heated or formalin-treated, nonmotile bacteria, and the mean speed of ≥4.0 µm/s (speed 10 times higher than that of Brownian motion) was judged as positive motility; bacterial motility was also judged with the naked eye under a phasecontrast microscope. The swimming speed given in the text represents the mean speed of motile bacteria.

**Extraction and Isolation** The methanol extract of unripe Japanese apricot was fractionated to search for inhibitor

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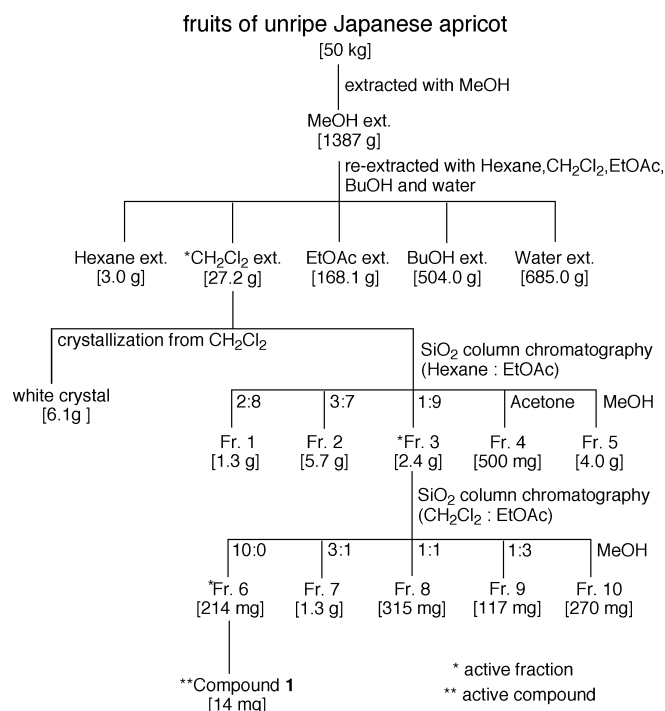


Fig. 1. Isolation Scheme of Inhibitor from Unripe-Japanese Apricot

of *H. pylori* motility (Fig. 1). Unripe Japanese apricot (50 kg) was refluxed with methanol for 12 h to give a methanol extract (1387 g). This extract was suspended in water and re-extracted with hexane, dichloromethane, ethyl acetate, butanol and water, respectively. Each fraction was concentrated under reduced pressure to give hexane (3 g), dichloromethane (27.2 g), ethyl acetate (168.1 g), butanol (504 g), and water (685 g) fractions. The dichloromethane fraction showed inhibitory effect of *H. pylori* motility. The dichloromethane fraction was fractionated to fractions 1—5 by silica gel column chromatography with hexane and ethyl acetate as eluents. Fr. 3 showed a potent inhibitory effect. Repeated column chromatography of Fr. 3 on silica gel using motion analysis as a guide afforded inhibitory compound **1** (14 mg).

(+)-Syringaresinol (**1**): Needles; mp 184—186 °C;  $[\alpha]_D^{21} +24.3^\circ$  ( $c=0.1$ , CHCl<sub>3</sub>); IR  $\nu_{\max}^{\text{KBr}} \text{ cm}^{-1}$ : 3419, 1614, 1517, 1461, 1213; EI-MS  $m/z$ : 418 (M<sup>+</sup>).

## RESULTS AND DISCUSSIONS

The methanol extract of unripe Japanese apricot was fractionated to search for suppressive compound of *H. pylori* motility. Test samples were evaluated at concentration of 500 μg/ml. The dichloromethane extract exhibited inhibitory effect on the *H. pylori* motility. To isolate the inhibitor, fractionation of the dichloromethane extract was carried out as described in Fig. 1. The inhibitor was isolated by SiO<sub>2</sub> column chromatography and identified as (+)-syringaresinol (Fig. 2) by comparing their spectral data to that previously reported.<sup>13,14)</sup>

Compound **1** was tested for inhibitory effect of *H. pylori* motility. Compound **1** inhibited >90% of *H. pylori* motility at a concentration of 500 μg/ml, and the IC<sub>50</sub> value was 50 μg/ml. *H. pylori* has multiple flagella, and exhibits strong motility.<sup>15)</sup> The motility contributed by the flagella is neces-

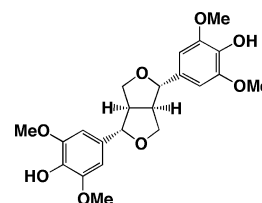


Fig. 2. Chemical Structure of (+)-Syringaresinol

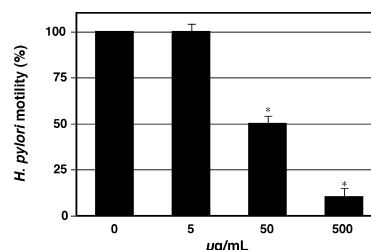


Fig. 3. Inhibitory Effect of Compound **1** on the *H. pylori* Motility

The data represent means ± standard deviations (error bars) of a thousand *H. pylori*. \* $p < 0.05$ .

sary for colonization of the gastric mucosa and development of gastritis by *H. pylori*.<sup>9,10)</sup> Therefore, the inhibition of the motility of the *H. pylori* is expected to be an efficient approach for prevention of the gastric mucosa by *H. pylori*. Tsutsui *et al.* reported the inhibitory effects of rabeprazole and its thioether derivative on the motility.<sup>16)</sup> (+)-Syringaresinol (**1**) showed potent inhibitory effect *in vitro* *H. pylori* motility assay. Thus, compound **1** may prevent the colonization by *H. pylori* of the gastric mucosa and gastritis. To clarify the mechanism of inhibition of *H. pylori* motility by compound **1**, more studies are necessary.

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