

## Camptothecin-related Alkaloids from Culture Medium of Hairy Roots of *Ophiorrhiza pumila*

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*Ophiorrhiza pumila* Champ. (Rubiaceae) which distributes in the Amami and Ryukyu Islands, Japan, Taiwan, Vietnam, Philippines, and southern China, produces an anti-tumor alkaloid, camptothecin (**1**), and its related alkaloids.<sup>1,2)</sup> Recently we obtained hairy roots of *O. pumila* and investigated their constituents, resulting in the isolation of camptothecin (**1**).<sup>3,4)</sup> In the course of our study on the camptothecin production by hairy roots of *O. pumila*, it was found by HPLC analysis that camptothecin was excreted into the culture medium.<sup>3,5)</sup> In the present paper, we report the isolation and identification of camptothecin (**1**), (3*S*)-pumiloside (**2**) and (3*R*)-deoxypumiloside (**3**) from the culture medium of *O. pumila* hairy roots.

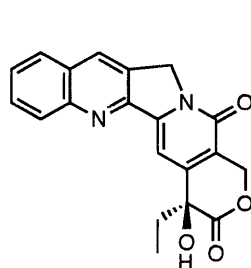
The hairy roots of *O. pumila* were subcultured every 4 weeks in liquid medium containing Gamborg's B5 salt and 2% sucrose at 25 °C on a rotary shaker (60 rpm) in the dark.<sup>5)</sup> The cultured medium (3.2 L) was extracted with CHCl<sub>3</sub> and then *n*-BuOH to give the CHCl<sub>3</sub> extract (223 mg) and the *n*-BuOH extract (714 mg), respectively. The CHCl<sub>3</sub> extract was subjected to medium pressure liquid chromatography (MPLC) using silica gel (CHCl<sub>3</sub>-MeOH) and octadecylsilyl silica gel (ODS), and then HPLC (SiO<sub>2</sub>, 10% MeOH-CHCl<sub>3</sub>) to afford camptothecin (**1**, 0.9 mg). The *n*-BuOH extract was separated by SiO<sub>2</sub> open column chromatography eluted

with MeOH-CHCl<sub>3</sub> gradient. The 50-80% MeOH-CHCl<sub>3</sub> eluent was purified by the combination of silica gel flash column chromatography, MPLC (SiO<sub>2</sub>, ODS), preparative TLC and HPLC (SiO<sub>2</sub>) to give (3*S*)-pumiloside (**2**, 0.3 mg) and (3*R*)-deoxypumiloside (**3**, 0.1 mg).

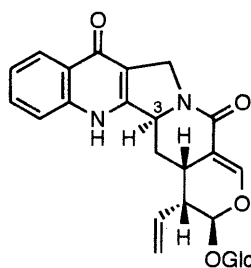
These compounds were identified by comparison of their spectroscopic data (<sup>1</sup>H-NMR, UV) and HPLC behavior with those of authentic samples.

### REFERENCES

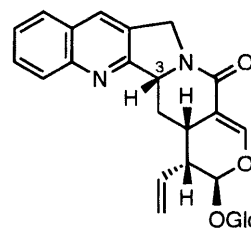
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Camptothecin (**1**)



(3*S*)-Pumiloside (**2**)



(3*R*)-Deoxypumiloside (**3**)