

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.^{1,2} Recently we obtained hairy roots of *O. pumila* and investigated their constituents, resulting in the isolation of camptothecin (**1**).^{3,4} 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.^{3,5} 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.

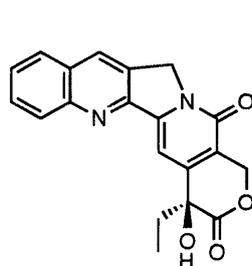
The hairy roots of *O. pumila* were subcultured every 4 weeks in liquid medium containing Gamborg's B5 salt and 2% sucrose at 25 °C on a rotary shaker (60 rpm) in the dark.⁵ The cultured medium (3.2 L) was extracted with CHCl₃ and then *n*-BuOH to give the CHCl₃ extract (223 mg) and the *n*-BuOH extract (714 mg), respectively. The CHCl₃ extract was subjected to medium pressure liquid chromatography (MPLC) using silica gel (CHCl₃-MeOH) and octadecylsilyl silica gel (ODS), and then HPLC (SiO₂, 10% MeOH-CHCl₃) to afford camptothecin (**1**, 0.9 mg). The *n*-BuOH extract was separated by SiO₂ open column chromatography eluted

with MeOH-CHCl₃ gradient. The 50-80% MeOH-CHCl₃ eluent was purified by the combination of silica gel flash column chromatography, MPLC (SiO₂, ODS), preparative TLC and HPLC (SiO₂) to give (3*S*)-pumiloside (**2**, 0.3 mg) and (3*R*)-deoxypumiloside (**3**, 0.1 mg).

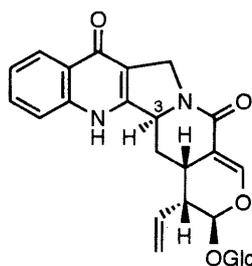
These compounds were identified by comparison of their spectroscopic data (¹H-NMR, UV) and HPLC behavior with those of authentic samples.

REFERENCES

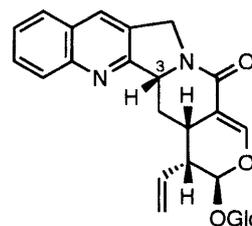
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Camptothecin (**1**)



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



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