Biflavonones, Diterpenes, and Coumarins from the Roots of *Stellera chamaejasme* L.

Zhi-Hong JIANG,* Takashi TANAKA,* Takako SAKAMOTO,* Isao KOUNO,* Jia-Ao DUAN,† and Rong-Han ZHOU,‡

School of Pharmaceutical Sciences, Nagasaki University,* 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan and Department of Chemistry of Natural Resources, China Pharmaceutical University,† Nanjing 21038, China.

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A new biflavonone (1) with a C-3/C-3′ linkage, a new daphnane-type diterpene (2) acylated by an unsaturated fatty acid, and a new coumarin glycoside (3), along with six lignans, two phenylpropanoids, five daphnoids, two diterpenes, and three coumarins were isolated from the roots of *Stellera chamaejasme* L. (Thymelaeaceae). Elucidation of these secondary metabolites of *S. chamaejasme* L. supplied strong chemical verification of the close taxonomic relationships among the genera *Stellera, Daphne*, and *Wikstroemia*, all of which belong to the family Thymelaeaceae.

Key words *Stellera chamaejasme; Thymelaeaceae; biflavonone; daphnane diterpene; coumarin glycoside; chemotaxonomy*

The roots of *Stellera chamaejasme* L. (Thymelaeaceae) are a traditional Chinese medicine called lan du (roudoku in Japanese) that is used as diuretic and remedy for cough and skin diseases.† Previous studies‡–§ on the chemical constituents of this plant have resulted in reports on groups of biflavonoids, lignans, diterpenes, etc. To achieve a deeper understanding of the chemistry of this crude drug, we undertook detailed chemical studies on the roots of *S. chamaejasme* L. collected in Ningxia, China, leading to the isolation of three new compounds (1—3) and 18 known ones from the MeOH extract. In this paper, we describe the structure elucidation and chemotaxonomic significance of these secondary metabolites.

MeOH extract of the roots was partitioned between H₂O and Et₂O and then the H₂O layer was further extracted with EtOAc to afford Et₂O, EtOAc, and H₂O layers. Each Et₂O and H₂O layer was fractionated and purified by various column chromatographies as described in the Experimental section. Compounds 1 and 2, along with isochamaejasmin,† neochamaejasmin B,† chamaechromone,‡ daphnodorin B,† daphnodorin 1,§ (+)-pinoresinol,§ (+)-lirioresinol B,§ matairesinol,§ (+)-wikstromol,§ (+)-lariciresinol,§ wikstroelide M,§ isochamaejasmin,§ simplexin,§ umbelliferone,§ and daphnoretin,§ were obtained from the Et₂O layer. Compound 3 together with (+)-pinoresinol di-O-β-D-glucopyranoside,ったり, syringin,† syringinoside,† and skimmin† were isolated from the H₂O layer. The known compounds were identified by comparing their spectral and physical data with reported values.

**Compound 1, a pale yellow amorphous powder, exhibited an [M–H]− ion peak at *m/z* 555 in the negative FAB-MS spectrum, corresponding to a molecular formula C₃₅H₅₂O₉, which was confirmed by elemental analysis. The ¹H- and ¹³C-NMR spectra of 1 displayed signals due to a methoxyl group [δ₉ 3.80 (3H, s), δ₁₃ 56.1] and two sets of closely related signals, which were very similar to those of neo-chamaejasmin A,† a symmetric biflavonone from *S. chamaejasme* with a C-3/C-3′ linkage. The position of the methoxyl group was determined to be at C-7 by observation of the nuclear Overhauser effect (NOE) correlations in the difference NOE experiment between H-6 and the methoxyl group, and between H-8 and the methoxyl group (Fig. 1). The stereochemistries at the C-2/C-3 and C-2′/C-3′ positions were established to be cis, cis geometry on the basis of the J-values of the corresponding protons [5.46, 5.47 (each 1H, br s, H-2, 2), 3.04 (2H, br s, H-3, 3′)]. This was further supported by a difference NOE experiment that revealed NOE correlations (Fig. 1) between H-2 and H-3, and between H-2′ and H-3′. On the basis of above evidence, compound 1 was concluded to be 7-methoxyneochejasmin A.

**Compound 2** was isolated as a white amorphous powder. Its molecular formula was determined to be C₃₅H₅₂O₉ on the basis of its high-resolution mass (HR-MS) spectrum. Both the ¹H- and ¹³C-NMR spectra of 2 are very similar to those of wikstroelide M isolated from *Wikstroemia retusa* (Thymelaeaceae),† suggesting the existence of a daphnane-type diterpene moiety and an unsaturated fatty acid with a diene system in the molecule. With the aid of ¹H–¹H correlation spectroscopy (COSY), heteronuclear single-quantum correlation (HSQC), and heteronuclear multiple bond connectivity (HMBC) (Fig. 2) techniques, the assignments of ¹H- and ¹³C-NMR signals of 2 were completed. It was found that the signals due to the diterpene moiety closely resembled those of wikstroelide M, except for an upfield shift by 1.25 ppm for the H-14 signal and downfield shift by 10.4 ppm for C-13. We concluded that the location of the ester linkage of the fatty acid unit to the diterpene moiety is at the 13-hydroxyl group.

As compound 2 is 14 mass unit (a methylene) larger than wikstroelide M, the fatty acid unit in 2 was suggested to be pentadecadienoic acid. Furthermore, based on the ¹H–¹H COSY and HMBC correlations (Fig. 2), two double bonds were determined to be conjugated and they are adjacent to the ester carbonyl carbon of the fatty acid. The stereochemistry of the diene system of the fatty acid unit was assigned to be 2′E, 4′E because large coupling constants (16 Hz) between H-2′ and H-3′ and between H-4′ and H-5′ were observed. Thus the structure of 2 was determined as shown in Fig. 2. Compound 2 contains an unsaturated fatty acid with odd carbon numbers, which is the same as that of wikstroelide N isolated from *W. retusa* (Thymelaeaceae).†

**Compound 3, a white amorphous powder, displayed an**
[M+H]^+ ion peak at m/z: 457 in the positive FAB-MS spectrum. Its formula was determined to be C_{20}H_{24}O_{12} on the basis of its molecular weight, results of elemental analysis, and \(^{13}\)C-NMR spectral data. The presence of an umbelliferone unit in addition to a pentose and a hexose in the molecule of \(3\) was deduced from its \(^1\)H- and \(^{13}\)C-NMR spectral data, suggesting \(3\) to be a glycoside of umbelliferone. By calculating the coupling constants of the sugar proton signals of the acetylated \(3\) (\(3\)a) which were assigned based on the \(^1\)H–\(^1\)H COSY spectrum, the sugars in \(3\) were clarified to be a xylopyranose and a glucopyranose. Moreover, since the chemical shift of C-6 of the glucose moiety is 66.2 ppm, and no downfield shift was observed for H-6 signals of the glucose moiety in \(3\)a compared with those in \(3\), the glycosidic linkage was determined to be located at C-6 of the glucose moiety. To clarify the absolute stereochemistry of the sugars, \(3\) was subjected to acid hydrolysis with HCl to liberated D-glucose and D-xylose together with aglycone umbelliferone. The anomeric protons of each sugar appeared as a doublet (\(J=7\) Hz), suggesting each has \(\beta\)-linkage. Thus compound \(3\) was determined to be \(O\)-[\(\beta\)-D-xylopyranosyl(1→6) \(\beta\)-D-glu-curonic acid] 7-hydroxycoumarin.

In conclusion, we have isolated a new biflavanone (\(1\)) with a C-3/C-3\(^0\) linkage, a new daphnane-type diterpene (\(2\)) acylated by an unsaturated fatty acid, and a new coumarin glycoside (\(3\)), along with 18 known compounds, namely, five flavonoids, six lignans, two phenylpropanoids, two diterpenes, and three coumarins, from the roots of \(S.\) chamaejasme \(L.\) (Thymelaeaceae). Although daphnodorin B, daphnodorin I, wikstromol, wikstroelide M, and skimmin were isolated from the genus Stellera for the first time, most of these known compounds have been reported to exist in \(Daphne\) and \(Wikstroemia\) plants belonging to the family Thymelaeaceae. These secondary metabolites supply a strong chemical confirmation of the close taxonomic relationships among the genera Stellera, Daphne, and Wikstroemia.

**Experimental**

**General** Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-400 spectrometer. \(^1\)H- and \(^{13}\)C-NMR spectra were recorded on Varian Unity plus 500 and
Varian Gemini 300 spectrometers. Coupling constants (J) are expressed in Hz, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. MS were recorded on a JEOL JMS DX-303 spectrometer. Column chromatographies were performed with Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 mm, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (75—150 mesh, Mitsubishi Chemical Co., Ltd.), and Chromatotron ODS (100—400 mesh, Fuji Silysia Chemical). TLC was performed on precoated Kieselgel F254 plates (0.2 mm thick, Merck), and spots were detected by ultraviolet (UV) illumination and by spraying with 10% sulfuric acid reagent.

Plant Material The root of *S. chamaejasme* (1.75 kg) was collected in Ningxia, China in August 1997, and was identified by one of the authors (J.-A. Duan). A voucher specimen has been deposited in the China Pharmaceutical University, Nanjing, China.

Extraction and Isolation MeOH extract of the roots (1.7 kg) was partitioned between H2O and Et2O. The Et2O layer (155.7 g) was divided into 1620, 1234. [MeOH], MCI-gel CHP 20P (5—40% MeOH) and silica gel (CHCl3—MeOH), MCI-gel CHP 20P (75—150 mm, Mitsubishi Chemical Co., Ltd.), MCI-gel CHP 20P (0—100% MeOH) and Sephadex LH-20 (60—100% MeOH). Compounds 1 (33 mg), 2 (7 mg), (+)-pinocembrin (172 mg), (+)-lirioresinol B (34 mg), matairesinol (45 mg), (+)-wikstroemin (210 mg), wikstroelide M (46 mg), simplexin (26 mg), umbellifere (74 mg) and daphnodorin (54 mg) were obtained from fraction 3, isochamaejasmin (239 mg), neochamaejasmin B (380 mg), chaemeachromin (657 mg) and (+)-lariciresinol (63 mg) from fraction 4, and daphnodorin B (65 mg) and daphnodorin I (79 mg) from fraction 5. The H2O layer was further extracted with EtOAc. The remaining H2O layer was then chromatographed over MCI-gel CHP 20P (0—100% MeOH) to afford fraction 1 (16.8 g), fraction 2 (30.1 g) and fraction 3 (30.4 g). Fraction 2 was subjected to chromatography over Chromatorex ODS (5—40% MeOH), MCI-gel CHP 20P (5—40% MeOH) and silica gel (CHCl3—MeOH—H2O, 8:2:0:2—6:4:1) to furnish compounds 3 (574 mg), (+)-pinocembrin β-D-glucoside (87 mg), syringin (654 mg), syringin-noside (2.9 mg) and skimmian (44 mg).

Compound 1 A pale yellow amorphous powder, IR (neat) cm−1: 3388, 1683, 1518, 1372, 1303. UV λmax (MeOH) nm (ε): 298 (47676). [α]D26 +153.2° (c=0.3, MeOH). Anal. Caled for C14H12O2: H 2.52; C 74.02; H 2.52 (Cu 20.3%); H 2.25; C 73.06; H 2.58 (Cu 20.3%); H 2.52 (Cu 20.3%).

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References


