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

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A new biflavanone (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 flavonoids, 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; biflavanone; 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.[1)] Previous studies[2—6)] 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 H_2O and Et_2O and then the H_2O layer was further extracted with EtOAc to afford Et_2O, EtOAc, and H_2O layers. Each Et_2O and H_2O layer was fractionated and purified by various column chromatographies as described in the Experimental section. Compounds **1** and **2**, along with isochamaejasmin,[6)] neochamaejasmin B,[3a)] chamaechromone,[4)] daphnodorin B,[7)] daphnodorin I,[8)] (+)-pinoresinol,[5)] (+)-lirioresinol B,[5)] matairesinol,[5)] (+)-wikstromol,[9)] (+)-lariciresinol,[10)] wikstroelide M,[11)] simplexin,[2)] umbelliferone,[12)] and daphnoretin,[13)] were obtained from the Et_2O layer. Compound **3** together with (+)-pinoresinol di-*O*-β-D-glucopyranoside,[14)] syringin,[15)] syringinoside,[15)] and skimmin[16)] were isolated from the H_2O 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 $C_{31}H_{24}O_{10}$, which was confirmed by elemental analysis. The ^{1}H- and ^{13}C-NMR spectra of **1** displayed signals due to a methoxyl group [δ_H 3.80 (3H, s), δ_C 56.1] and two sets of closely related signals, which were very similar to those of neochamaejasmin A,[3)] a symmetric biflavanone 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-methoxylneochaejasmin A.

Compound **2** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{35}H_{52}O_9$ on the basis of its high-resolution mass (HR-MS) spectrum. Both the ^{1}H- and ^{13}C-NMR spectra of **2** are very similar to those of wikstroelide M isolated from *Wikstroemia retusa* (Thymelaeaceae),[11)] 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 ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single-quantum correlation (HSQC), and heteronuclear maltiple bond connectivity (HMBC) (Fig. 2) techniques, the assignments of ^{1}H- and ^{13}C-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 ^{1}H–^{1}H 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).[11)]

Compound **3**, a white amorphous powder, displayed an

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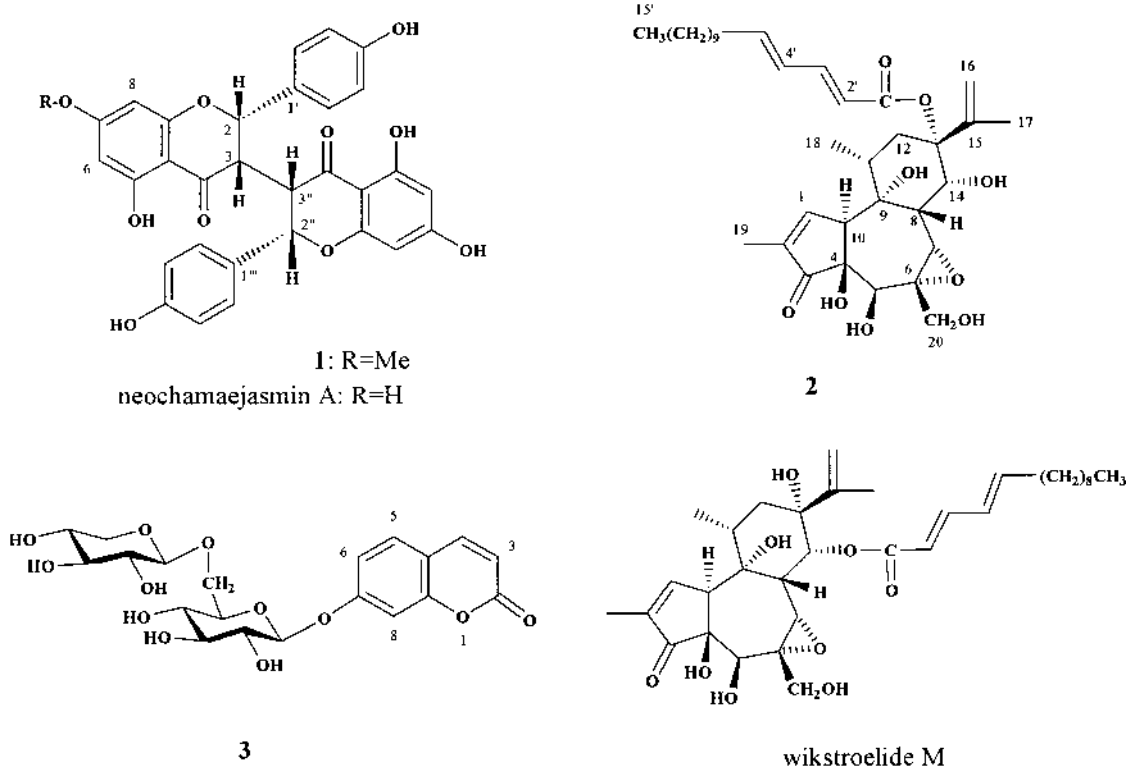

Chart 1. Structures

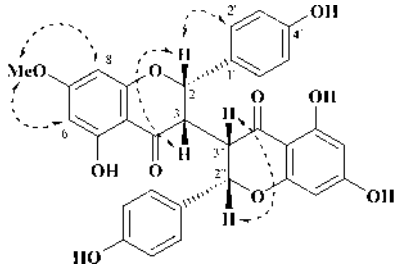

Fig. 1. NOE Correlations Observed for Compound **1**

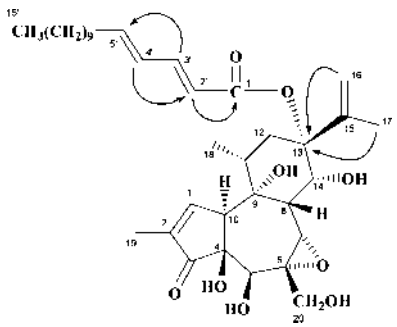

Fig. 2. Some Key HMBC Correlations (H to C) of Compound **2**

$[M+H]^+$ 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** (**3a**) 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 **3a** 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 β-linkage. Thus compound **3** was determined to be *O*-[β-D-xylopyranosyl(1→6) β-D-glucopyranosyl] 7-hydroxycoumarin.

In conclusion, we have isolated a new biflavanone (**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 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 mm, Mitsubishi Chemical Co., Ltd.) and Chromatorex ODS (100—200 mesh, Fuji Silysia Chemical). TLC was performed on precoated Kieselgel 60 F_{254} 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 H_2O and Et_2O. The Et_2O layer (155.7 g) was divided into fraction 1 (3.33 g), fraction 2 (6.79 g), fraction 3 (15.57 g), fraction 4 (58.7 g) and fraction 5 (52.9 g) by column chromatography over silica gel ($CHCl_3$–MeOH–H_2O, 10 : 0 : 0—9 : 1 : 0.1—7 : 3 : 0.5). Fractions 3, 4 and 5 were separately subjected to a combination of chromatographies over silica gel ($CHCl_3$–MeOH–H_2O, 10 : 0 : 0—9 : 1 : 0.1—7 : 3 : 0.5), MCI-gel CHP 20P (20—100% MeOH), Chromatorex ODS (40—100% MeOH) and Sephadex LH-20 (60—100% MeOH). Compounds **1** (33 mg), **2** (7 mg), (+)-pinoresinol (172 mg), (+)-lirioresinol B (34 mg), matairesinol (45 mg), (+)-wikstromol (210 mg), wikstroelide M (46 mg), simplexin (26 mg), umbelliferone (74 mg) and daphnoretin (59 mg) were obtained from fraction 3, isochamaejasmin (239 mg), neochamaejasmin B (380 mg), chamaechromone (657 mg) and (+)-lariciresinol (63 mg) from fraction 4, and daphnodorin B (62 mg) and daphnodorin I (799 mg) from fraction 5.

The H_2O layer was further extracted with EtOAc. The remaining H_2O 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 chromatographies over Chromatorex ODS (5—40% MeOH), MCI-gel CHP 20P (5—40% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 8 : 2 : 0.2—6 : 4 : 1) to furnish compounds **3** (574 mg), (+)-pinoresinol di-*O*-β-D-glucopyranoside (87 mg), syringin (654 mg), syringinoside (2.9 mg) and skimmin (44 mg).

Compound **1**: A pale yellow amorphous powder, IR (neat) cm^{-1}: 3338, 1638, 1518, 1372, 1303. UV λ_{max} (MeOH) nm (ε): 298 (47674). $[\alpha]_D^{26}$ +153.2° (*c*=0.3, MeOH). *Anal.* Calcd for $C_{31}H_{24}O_{10}\cdot 2H_2O$: C, 62.84; H, 4.76. Found: C, 62.73; H, 4.86. Negative FAB-MS *m/z*: 555 $[M-H]^-$. ^{1}H-NMR (500 MHz, acetone-d_6): δ 11.6 (2H, s, 5-OH, 5″-OH), 7.08, 7.07 (each 2H, d, *J*=8 Hz, H-2′, 6′, 2‴, 6‴), 6.80, 6.79 (each 2H, d, *J*=8 Hz, H-3′, 5′, 3‴, 5‴), 6.01, 5.93 (each 1H, d, *J*=1 Hz, H-6, 8), 5.88, 5.80 (each 1H, *J*=2 Hz, H-6″, 8″), 5.46, 5.47 (each 1H, br s, H-2, 2″), 3.80 (3H, s, OMe), 3.04 (2H, br s, H-3, 3′). ^{13}C-NMR (75 MHz, acetone-d_6): δ 197.3, 196.7 (C-4, 4″), 168.5 (C-7), 167.5 (C-7″), 164.2, 163.9 (C-5, 5″), 163.6×2 (C-9, 9″), 157.6×2 (C-4′, 4‴), 127.6×4 (C-2′, 6′, 2‴, 6‴), 127.4, 127.3 (C-1′, 1‴), 115.8×4 (C-3′, 5′, 3‴, 5‴), 103.6, 102.9 (C-10, 10″), 96.3, 95.3, 94.9, 93.9 (C-6, 8, 6″, 8″), 81.2, 81.1 (C-2, 2″), 56.1 (OMe), 47.2×2 (C-3, 3″).

Compound **2**: A white amorphous powder, IR (neat) cm^{-1}: 3448, 2925, 2855, 1705. $[\alpha]_D^{26}$ +6.1° (*c*=0.6, $CHCl_3$). EI-MS *m/z*: (rel. int.%): 616 (M^+, 2), 598 (5), 584 (12). HR-electron impact (EI)-MS *m/z*: 616.3634 (M^+) (Calcd for $C_{35}H_{52}O_9$: 616.3612). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.65 (1H, br s, H-1), 7.26 (1H, dd, *J*=15, 11 Hz, H-3′), 6.17 (1H, dd, *J*=15, 11 Hz, H-4′), 6.13 (1H, m, H-5′), 5.76 (1H, d, *J*=15 Hz, H-2′), 5.26, 5.21 (each 1H, br s, H-16), 4.42 (1H, br s, H-14), 4.26 (1H, br s, H-5), 3.97 (1H, br s, H-10), 3.87 (1H, d, *J*=12 Hz, H-20a), 3.62 (1H, d, *J*=12 Hz, H-20b), 3.36 (2H, s, H-7, 8), 1.77 (3H, s, H-19), 1.81 (3H, s, H-17), 1.00 (3H, d, *J*=6 Hz, H-18), 0.88 (3H, t, *J*=7 Hz, H-15′). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 209.6 (C-3), 165.4 (C-1′), 162.8 (C-1), 146.1 (C-3′), 145.9 (C-5′), 142.4 (C-15), 134.4 (C-2), 128.1 (C-4′), 119.1 (C-16), 117.3 (C-2′), 84.5 (C-13), 78.6 (C-14), 77.1 (C-9), 72.5 (C-4), 71.2 (C-5), 64.7 (C-20), 64.6 (C-7), 61.8 (C-6), 49.9 (C-10), 38.3 (C-11), 37.5 (C-8), 35.1 (C-12), 33.1 (C-6′), 32.0, 29.8, 29.6, 29.5, 29.4, 29.3, 28.8, 22.8 (C-7′, 8′, 9′, 10′, 11′, 12′, 13′, 14′), 19.7 (C-17), 18.1 (C-18), 14.2 (C-15′), 9.9 (C-19).

Compound **3**: A white amorphous powder, IR (neat) cm^{-1}: 3400, 1708, 1620, 1234. $[\alpha]_D^{26}$ −83.4° (*c*=1.0, H_2O). *Anal.* Calcd for $C_{20}H_{24}O_{12}\cdot 7/4H_2O$: C, 49.23; H, 5.68. Found: C, 49.34; H, 5.41. Positive FAB-MS *m/z*: 457 $[M+H]^+$. ^{1}H-NMR (300 MHz, D_2O): δ 7.93 (1H, d, *J*=9 Hz, H-4), 7.58 (1H, d, *J*=9 Hz, H-5), 7.12 (1H, d, *J*=2 Hz, H-8), 7.10 (1H, dd, *J*=2, 9 Hz, H-6), 6.35 (1H, d, *J*=9 Hz, H-3), 5.22 (1H, d, *J*=7 Hz, glc-1), 4.43 (1H, d, *J*=7 Hz, xyl-1), 4.18 (1H, dd, *J*=5, 15 Hz, xyl-5a), 3.94 (1H, dd, *J*=5, 12 Hz, glc-6a). ^{13}C-NMR (75 MHz, D_2O): δ 164.8 (C-2), 160.6 (C-7), 155.5 (C-8a), 146.3 (C-4), 130.6 (C-5), 115.3 (C-8, 4a), 113.8 (C-6), 104.5, 104.7 (glc-1, xyl-1), 100.7 (C-3), 76.7, 76.5, 76.3, 74.1, 73.8, 70.5, 70.2 (glc-2, 3, 4, 5, xyl-2, 3, 4), 66.2 (glc-6), 69.6 (xyl-5).

Acetylation of 3 Compound **3** (30 mg) was acetylated with pyridine (1 ml) and Ac_2O (1 ml) at room temperature overnight. The reaction mixture was subjected to silica gel column chromatography eluted with hexane–EtOAc (3 : 1—2 : 1) to give **3a** (26 mg): white amorphous powder, $[\alpha]_D^{26}$ −55.5° (*c*=1.2, $CHCl_3$). Positive FAB-MS *m/z*: 731 $[M+Na]^+$, 709 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.69 (1H, d, *J*=10 Hz, H-4), 7.50 (1H, d, *J*=9 Hz, H-5), 6.94 (1H, d, *J*=2 Hz, H-8), 6.93 (1H, dd, *J*=2, 9 Hz, H-6), 6.32 (1H, d, *J*=10 Hz, H-3), 5.31 (1H, t, *J*=9 Hz, glc-3), 5.25 (1H, dd, *J*=8, 9 Hz, glc-2), 5.16 (1H, d, *J*=8 Hz, glc-1), 5.08 (1H, t, *J*=9 Hz, xyl-3), 5.06 (1H, dd, *J*=9, 10 Hz, glc-4), 4.94 (1H, m, xyl-4), 4.91 (1H, dd, *J*=7, 9 Hz, xyl-2), 4.52 (1H, d, *J*=7 Hz, xyl-1), 4.10 (1H, dd, *J*=5, 12 Hz, xyl-5a), 3.90 (1H, m, glc-5), 3.89 (1H, dd, *J*=2, 12 Hz, glc-6a), 3.68 (1H, dd, *J*=5, 12 Hz, glc-6b), 3.28 (1H, dd, *J*=9, 12 Hz, xyl-5b).

Acid Hydrolysis of 3 A solution of **3** (22 mg) in 2 N HCl/H_2O (10 ml) was heated at 90 °C for 3 h. After neutralizing with 1 N NaOH/H_2O, the solution was extracted with Et_2O (3×10 ml) and the combined organic phase was evaporated to dryness under reduced pressure to yield umbelliferone (8 mg). The water layer was evaporated to dryness under reduced pressure and subjected to silica gel chromatography [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5—6 : 4 : 1)] to afford D-glucose (4.2 mg): $[\alpha]_D^{26}$ +24.8° (*c*=0.3, H_2O, measured 24 h after dissolving in H_2O) and D-xylose (7.4 mg): $[\alpha]_D^{26}$ +14.5° (*c*=0.6, H_2O, measured 24 h after dissolving in H_2O).

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