[Chem. Pharm. Bull.
34(8)3249—3251(1986)]

Stereostructures of Two Biflavanones from *Stellera chamaejasme* L.

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(Received February 24, 1986)

Two C-3/C-3''-biflavanones, chamaejasmine (**1**) and isochamaejasmin (**2**), were isolated from the roots of *Stellera chamaejasme* L. (Thymelaeaceae) and their stereostructures were elucidated on the basis of spectral and chemical evidence. Chamaejasmine was found to be a mixture of (−) and (+) forms (68 : 32) by high-performance liquid chromatographic analysis with a column for optical resolution.

Keywords——*Stellera chamaejasme*; Thymelaeaceae; chamaejasmine; (−)-chamaejasmine; (+)-chamaejasmine; isochamaejasmin; biflavanone; HPLC; optical resolution

Introduction

From the aqueous sodium hydroxide extract of *Stellera chamaejasme* L. (Thymelaeaceae), chamaejasmine was isolated as an optically inactive form by Hwang and Chang in 1979.[3] Chamaejasmine was the first C-3/C-3''-biflavonoid to be isolated and the structure was elucidated as **1**, having the *trans–trans* geometry at the C-2/C-3 and C-2''/C-3'' positions. However, the chirality at the C-3/C-3'' positions of **1** remained unsettled.

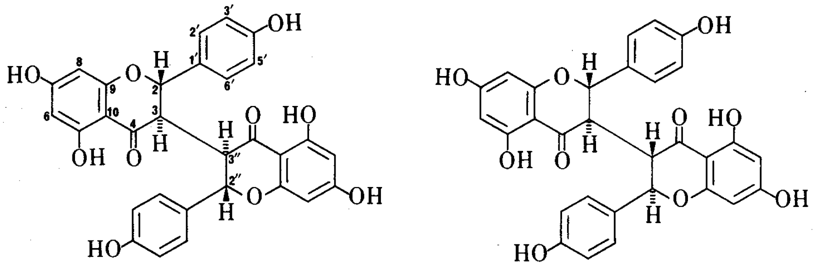
Recently we reported on the piscicidal constituents, diterpenes and lignans, of *Stellera chamaejasme* L.[4,5] We further examined the chemical components of the above plant and isolated a new C-3/C-3''-biflavanone named isochamaejasmin (**2**)[6] in addition to chamaejasmine (**1**).[7] Further, our chamaejasmine was optically active. In this paper, we wish to report the isolation and structures of chamaejasmine (**1**) and isochamaejasmin (**2**), including the chirality at the C-3/C-3'' positions.

Results and Discussion

The water soluble fraction described in the previous paper[5] was further partitioned between water and ethyl acetate. The ethyl acetate extract was roughly separated by column chromatography on silica gel using mixtures of chloroform–ethyl acetate to give several fractions. The second fraction was further separated by medium-pressure and high-performance liquid chromatography (MPLC and HPLC, respectively) on reversed-phase silica gel using a mixed solvent of methanol and water (70 : 30) to afford isochamaejasmin (**2**) ($[\alpha]_D$ 0°) as an amorphous powder.[6] The tailing part corresponding to isochamaejasmin in preparative HPLC was repeatedly collected. The combined fraction was repeatedly separated by preparative thin layer chromatography (TLC) to afford optically active chamaejasmine (**1**) ($[\alpha]_D$ −61.2°)[7] as an amorphous powder in a quite small yield compared to that of isochamaejasmin (**2**) (**1** : **2** = 1 : 44).

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of chamaejasmine (**1**), isochamaejasmin (**2**) and naringenin (**3**) are quite similar to one another except for remarkable differences of δ-values and multiplicity of the signals of the C-3 and C-3'' carbons (**1**; δ 51.2

(d), **2**; δ 49.0 (d), **3**; δ 43.7 (t)) as shown in Table I. This shows that chamaejasmine and isochamaejasmin both have a structure dimerized at the C-3 position of the two naringenin molecules, and these two biflavanones must be stereoisomers. The stereochemistry at the C-2/C-3 and C-2″/C-3″ positions of chamaejasmine (**1**) and isochamaejasmin (**2**) was determined by comparison of the *J*-values of the corresponding protons (**1**, 12 and 12 Hz; **2**, 12 and 12 Hz). Namely, chamaejasmine and isochamaejasmin both have the *trans–trans* geometry. A structure having the *trans–trans* geometry can be represented as **1** or **2** except for the absolute configuration. The structure **1** has no symmetrical plane as seen in the case of *d*- or *l*-tartaric acid, while the structure **2** has a symmetrical plane as seen in the case of *meso*-tartaric acid. Therefore, optically active chamaejasmine and inactive isochamaejasmin can be represented as the *d*- or *l*-form (**1**) and the *meso*-form (**2**), respectively. This conclusion was supported by the following chemical evidence. On treatment with 5% aqueous sodium hydroxide (room temperature, 5 h), optically active chamaejasmine was easily isomerized to a mixture of optically inactive chamaejasmine and isochamaejasmin. This mixture was also obtained by the isomerization of isochamaejasmin under the same conditions. Consequently, optically inactive chamaejasmine obtained by alkali treatment should be a racemate. In fact, optically inactive chamaejasmine was found to be a 50 : 50 mixture of two enantiomers by HPLC analysis on an optical resolution column. Moreover, natural chamaejasmine isolated by us was unexpectedly found to be not optically pure by similar HPLC analysis carried out under the same conditions. Natural chamaejasmine was a mixture of (−) and (+) forms in the ratio of 68 : 32.[8] A similar phenomenon was seen in the case of germacrene-D, a ten-membered sesquiterpene from *Solidago altissima* (Compositae).[9]



chamaejasmine (**1**) isochamaejasmin (**2**)

Fig. 1

Table I. ^{13}C-NMR Spectral Data for Biflavanones and Naringenin

Position	Chamaejasmine (1)	Isochamaejasmin (2)	Naringenin (3)
2 2″	84.5 (d)	82.4 (d)	80.1 (d)
3 3″	51.2 (d)	49.0 (d)	43.7 (t)
4 4″	197.9 (s)	196.8 (s)	197.3 (s)
5 5″	165.4 (s)	165.3 (s)	165.0 (s)
6 6″	97.3 (d)	97.3 (d)	97.0 (d)
7 7″	168.4 (s)	168.1 (s)	168.0 (s)
8 8″	96.2 (d)	96.1 (d)	96.1 (d)
9 9″	164.3 (s)	164.3 (s)	164.5 (s)
10 10″	103.4 (s)	102.8 (s)	103.1 (s)
1′ 1‴	129.1 (s)	129.0 (s)	130.7 (s)
2′ 2‴	130.3 (d)	130.8 (d)	128.8 (d)
3′ 3‴	116.5 (d)	116.5 (d)	116.2 (d)
4′ 4‴	159.5 (s)	159.5 (s)	158.5 (s)
5′ 5‴	116.5 (d)	116.5 (d)	116.2 (d)
6′ 6‴	130.3 (d)	130.8 (d)	128.8 (d)

Finally, it seems that the isolation procedure using aqueous sodium hydroxide adopted by Hwang and Chang[3] resulted in racemization of optically active chamaejasmine and/or in isomerization of isochamaejasmin.

Experimental

Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Ultraviolet (UV) spectra were obtained on a JASCO UVIDEC-610 spectrometer. Infrared (IR) spectra were taken on a JASCO IR A-2 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra were taken on a JEOL FX-100 spectrometer; chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). Mass spectra (MS) were obtained on Hitachi M-52 and M-80 spectrometers. MPLC and HPLC were carried out with a JASCO TRIROTAR-V (detector: UVIDEC-100-V, 296 nm).

Isolation——The aqueous layer after partition between benzene and water, reported in the previous paper,[5] was subsequently extracted 3 times with AcOEt (each 500 ml) to afford an extract (140 g). A part of the extract (19 g) was chromatographed on silica gel (Merck 7734; 400 g) with mixtures of $CHCl_3$ and AcOEt (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6) (each 1000 ml). The second fraction (4.4 g) eluted with $CHCl_3$–AcOEt (8 : 2) was separated by MPLC (Develosil ODS 30/50, MeOH–H_2O (70 : 30) to afford crude isochamaejasmin (**2**). The crude product was purified by preparative HPLC (Develosil ODS-5, ϕ10 × 250, MeOH–H_2O (70 : 30) to give pure isochamaejasmin (**2**) (1775 mg). The tailing part corresponding to **2** in preparative HPLC was collected and repeatedly separated by preparative TLC (Merck 5744, 5715, 5642, $CHCl_3$–MeOH (10 : 1)) to afford optically active chamaejasmine (**1**) (40 mg).

Chamaejasmine (1)——$[\alpha]_D$ $-61.2°$ ($c=0.49$, MeOH). High-resolution MS Found: m/z 271.0614; $C_{15}H_{11}O_5$ requires 271.0606 (1/2 M^+). UV λ_{max}^{MeOH} nm (ε): 296 (34700). IR ν_{max}^{KBr} cm^{-1}: 3300, 1620. ^{1}H-NMR (CD_3OD) δ: 2.78 (2H, d, $J=12$ Hz), 5.78 (2H, d, $J=12$ Hz), 5.80 (2H, near s), 5.89 (2H, d, $J=1$ Hz), 6.76 (4H, d, $J=8$ Hz), 6.96 (4H, d, $J=8$ Hz); (acetone-d_6) δ: 2.92 (2H, d, $J=12$ Hz), 5.85 (2H, d, $J=12$ Hz), 5.87 (2H, d, $J=2$ Hz), 5.95 (2H, d, $J=2$ Hz), 6.84 (4H, d, $J=8$ Hz), 7.02 (4H, d, $J=8$ Hz), 11.92 (2H, s).

Isochamaejasmin (2)——$[\alpha]_D$ 0° ($c=0.4$, MeOH). High-resolution MS Found: m/z 271.0614; $C_{15}H_{11}O_5$ requires 271.0606 (1/2 M^+). UV λ_{max}^{MeOH} nm (ε): 296 (30200). IR ν_{max}^{KBr} cm^{-1}: 3350, 1620. ^{1}H-NMR (CD_3OD) δ: 3.72 (2H, d, $J=12$ Hz), 4.90 (2H, d, $J=12$ Hz), 5.74 (2H, s), 5.88 (2H, s), 6.75 (4H, d, $J=8$ Hz), 7.00 (4H, d, $J=8$ Hz); (acetone-d_6) δ: 3.84 (2H, d, $J=12$ Hz), 4.92 (2H, d, $J=12$ Hz), 5.80 (2H, s), 5.94 (2H, s), 6.86 (4H, d, $J=8$ Hz), 7.12 (4H, d, $J=8$ Hz), 11.92 (2H, s).

Isomerization of Optically Active Chamaejasmine (1)——A solution of optically active chamaejasmine (**1**) (5 mg) in 5% NaOH (0.5 ml) was stirred at room temperature for 5 h. The solution was made acid with 10% HCl and extracted twice with AcOEt (5 ml). The extract was washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated to afford a residue, which was separated by preparative TLC (Merck 5715, 5642, $CHCl_3$–MeOH (10 : 1)) to give optically inactive chamaejasmine (**1**) (1.5 mg) and isochamaejasmin (**2**) (2 mg).

Isomerization of Isochamaejasmin (2)——According to the same procedure as described above, the treatment of isochamaejasmin (**2**) (6 mg) with 5% NaOH (0.5 ml) gave optically inactive chamaejasmine (**1**) (2 mg) and isochamaejasmin (**2**) (2 mg).

Chromatographic Resolution of Chamaejasmine (1)——HPLC analysis of chamaejasmine (**1**) was carried out under the following conditions; column, Chiralpak OT (+) ϕ4.6 × 250, mobile phase, MeOH–H_2O (70 : 30), flow rate, 0.5 ml/min. The ratio of (−)- and (+)-chamaejasmine was determined on the basis of the area ratio of the two peaks: (−)-chamaejasmine, t_R 10.4 min; (+)-chamaejasmine, t_R 14.1 min.

References and Notes

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7) Although we could not directly compare our chamaejasmine with Prof. Hwang's sample because of his accidental death in 1982, the spectral data of our product were in good agreement with the reported data[3,6] except for the value of optical rotation.
8) No change of the ratio was observed after preparative HPLC on reversed phase silica gel and/or preparative TLC on silica gel.
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