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Two New C-3/C-3′′-Biflavanones from *Wikstroemia sikokiana*

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Two C-3/C-3′′-biflavanones named sikokianin A and sikokianin B were isolated from *Wikstroemia sikokiana* FRANCH. *et* SAV. and their structures were elucidated on the basis of spectral data.

Keywords——Thymelaeaceae; *Wikstroemia sikokiana*; ganpi; sikokianin A; sikokianin B; biflavanone; umbelliferone; coumarin

Introduction

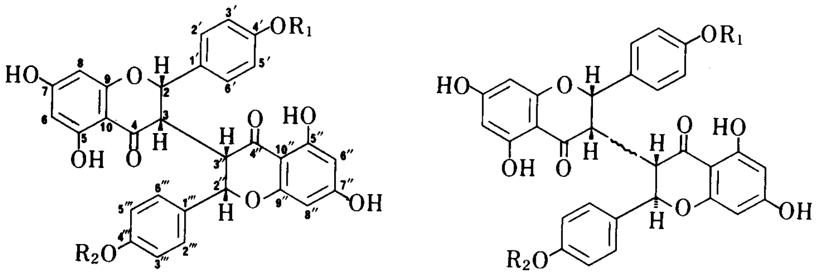
In previous papers,[2-5] we have reported novel C-3/C-3′′-biflavanones from the roots of *Stellera chamaejasme* L. (Thymelaeaceae) collected in China. In connection with these interesting biflavanones, we examined the chemical constituents of Thymelaeaceae plants growing in Japan and isolated two new C-3/C-3′′-biflavanones from *Wikstroemia sikokiana* FRANCH. *et* SAV. (Japanese name: Ganpi), which is used as a source material for Japanese paper. In this paper, we wish to describe the isolation and structure determination of these C-3/C-3′′-biflavanones, named sikokianin A (**1**) and sikokianin B (**2**), in addition to a known coumarin, umbelliferone (**3**).

Results and Discussion

Fresh roots of the above plant, which were collected in Ishikawa Prefecture in July, were disintegrated in large amounts of methanol and then the methanol extract was partitioned successively between water and hexane, benzene, ethyl acetate and butanol. The ethyl acetate extract was roughly separated by column chromatography on silica gel using a gradient solution of chloroform, ethyl acetate and methanol to give seven fractions. Three of them (fractions I, II and III) were further separated by a combination of repeated column chromatography on silica gel using a mixed solvent of chloroform and ethyl acetate and on reversed-phase silica gel using a mixed solvent of methanol and water and preparative thin layer chromatography (TLC) on silica gel using a mixed solvent of chloroform and ethyl acetate, to afford sikokianin A (**1**) and sikokianin B (**2**) in 0.004 and 0.025% yields, respectively, in addition to umbelliferone (7-hydroxycoumarin) (**3**).

Sikokianin A (**1**) has the molecular formula $C_{31}H_{24}O_{10}$ [*m/z* 579 $(M+Na)^+$ and 557 $(M+H)^+$ in the secondary ion mass spectrum (SIMS)] and contains a methoxyl group [δ_H 3.82 (3H, s) and δ_C 55.8 (q)]. Except for these points, there is a close resemblance between sikokianin A (**1**) and neochamaejasmin A (**4**)[3] in terms of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, as shown in Tables I and II. In the electron impact mass spectrum (EIMS) of sikokianin A (**1**), furthermore, fragment ion peaks at *m/z* 271 and 285 were observed. These data show that **1** has the structure dimerized at the C-3 positions of naringenin and methylnaringenin.

The position of the methoxyl group was determined by comparison of the chemical shifts of the C-3′ (C-3‴), C-4′ (C-4‴) and C-5′ (C-5‴) carbons (**1**: δ_C 114.9 (116.2), 160.6 (158.0) and 114.9 (116.2); **4**: δ_C 116.2, 157.7 and 116.2). Namely, the methoxyl group is located at the C-4′ position and the stereochemistry at the C-2/C-3 and C-2″/C-3″ positions has the same *cis–cis* geometry as that of neochamaejasmin A (**4**),[3] as judged from the J-values of the corresponding ^{1}H-NMR signals, all of which are 2 Hz. As mentioned above, the structure of sikokianin A should be represented as **1** or its antipode.



1: $R_1=CH_3$, $R_2=H$
4: $R_1=R_2=H$
6: $R_1=R_2=CH_3$

2a: $R_1=CH_3$, $R_2=H$
2b: $R_1=H$, $R_2=CH_3$
5: $R_1=R_2=H$

Fig. 1

Sikokianin B (**2**) has the same molecular formula [$C_{31}H_{24}O_{10}$; m/z 579 $(M+Na)^+$ and 557 $(M+H)^+$ in SIMS and m/z 285 and 271 in EIMS] as sikokianin A (**1**) and its NMR data are quite similar to those of **1**, as shown in Tables I and II, indicating that sikokianin B is a stereoisomer of sikokianin A. Namely, the stereochemistry at the C-2/C-3 and C-2″/C-3″ positions in **2** has the *cis–trans* geometry ($J_{2-H}=4$ Hz and $J_{2''-H}=9$ Hz)[6] which is observed in neochamaejasmin B (**5**).[3] The position of the methoxyl group [δ_H 3.72 (3H, s), δ_C 55.8 (q)] was determined as follows. The chemical shift values (δ_C 114.8 and 114.8) of the 3′ and 5′ or 3‴ and 5‴ carbons show that the methoxyl group is located at the C-4′ or C-4‴ position. However, the analyses of the spectral data could not clearly distinguish the position of the methoxyl group. The structure of sikokianin B can consequently be represented as **2a** or **2b** and the chirality at the C-3/C-3″ positions remains unsettled. Finally, from a biogenetic point

TABLE I. ^{1}H-NMR Data for C-3/C-3″-Biflavanones **1**, **2**, **4** and **5** in CD_3OD

Position	Sikokianin A (**1**)	Sikokianin B (**2**)	Neochamaejasmin A (**4**)	Neochamaejasmin B (**5**)
3 (3″)	2.91 (1H, d, J=2Hz)	3.3 (2H, superimposed on peaks of Me group of the solvent)	2.90 (2H, d, J=4Hz)	3.00 (1H, d, J=4Hz)
3″ (3)	2.98 (1H, d, J=2Hz)			3.28 (1H, d, J=12Hz)
4′ (4‴)	3.82 (3H, s)	3.72 (3H, s)		
2 (2″)	5.31 (1H, d, J=2Hz)	5.16 (1H, d, J=9Hz)	5.26 (2H, d, J=4Hz)	5.34 (1H, d, J=12Hz)
2″ (2)	5.36 (1H, d, J=2Hz)	5.50 (1H, d, J=4Hz)		5.42 (1H, d, J=4Hz)
6 (6″)	5.73 (1H, d, J=0.5Hz)	5.75 (2H, s)	5.72 (2H, d, J=2Hz)	5.81 (1H, s)
6″ (6)	5.75 (1H, d, J=0.5Hz)			5.86 (1H, s)
8 (8″)	5.86 (1H, d, J=0.5Hz)	5.83 (1H, s)	5.96 (2H, d, J=2Hz)	5.95 (1H, s)
8″ (8)	5.88 (1H, d, J=0.5Hz)	5.94 (1H, s)		6.02 (1H, s)
3′,5′ (3‴,5‴)	6.63 (2H, d, J=9Hz)	6.72 (2H, d, J=8Hz)	6.68 (4H, d, J=8Hz)	6.6—7.2 (8H, complex)
3‴,5‴ (3′,5′)	6.77 (2H, d, J=9Hz)	6.76 (2H, d, J=9Hz)		
2′,6′ (2‴,6‴)	6.93 (2H, d, J=9Hz)	7.02 (2H, d, J=8Hz)	6.94 (4H, d, J=8Hz)	
2‴,6‴ (2′,6′)	7.04 (2H, d, J=9Hz)	7.15 (2H, d, J=9Hz)		

TABLE II. ^{13}C-NMR Data for C-3/C-3″-Biflavanones **1**, **2**, **4** and **5** in CD_3OD

Position	Sikokianin A (**1**)		Sikokianin B (**2**)		Neochamaejasmin A (**4**)		Neochamaejasmin B (**5**)	
2 2″	81.7$^{a)}$	81.8$^{a)}$ (d)	81.4	82.9 (d)	81.6	81.6 (d)	81.3	83.1 (d)
3 3″	47.9	47.9 (d)	49.5	50.8 (d)	47.7	47.7 (d)	49.4	50.8 (d)
4 4″	197.4	197.4 (s)	196.0	198.5 (s)	197.3	197.3 (s)	196.1	198.4 (s)
5 5″	165.0	165.0 (s)	165.0	165.4 (s)	164.7	164.7 (s)	164.9	165.2 (s)
6 6″	96.8	96.8 (d)	97.2	97.3 (d)	96.7	96.7 (d)	97.1	97.2 (d)
7 7″	168.4	168.4 (s)	168.3	168.4 (s)	168.0	168.0 (s)	167.9	168.0 (s)
8 8″	95.9	95.9 (d)	96.1	96.5 (d)	95.8	95.8 (d)	96.0	96.4 (d)
9 9″	164.5	164.5 (s)	163.3$^{a)}$	165.0$^{a)}$ (s)	164.3	164.3 (s)	163.2$^{a)}$	164.9$^{a)}$ (s)
10 10″	103.6	103.6 (s)	103.7$^{a)}$	105.0$^{a)}$ (s)	103.5	103.5 (s)	103.7$^{a)}$	105.1$^{a)}$ (s)
1′ 1‴	129.3	128.2 (s)	130.1$^{a)}$	128.5$^{a)}$ (s)	128.1	128.1 (s)	128.4	128.6 (s)
2′ 2‴	128.2	128.2 (d)	128.5	130.2 (d)	128.1	128.1 (d)	128.9	130.2 (d)
3′ 3‴	114.9	116.2 (d)	114.8$^{a)}$	116.4$^{a)}$ (d)	116.2	116.2 (d)	116.1	116.4 (d)
4′ 4‴	160.6	158.0 (s)	161.3$^{a)}$	158.5$^{a)}$ (s)	157.7	157.7 (s)	158.3	158.7 (s)
5′ 5‴	114.9	116.2 (d)	114.8$^{a)}$	116.4$^{a)}$ (d)	116.2	116.2 (d)	116.1	116.4 (d)
6′ 6‴	128.2	128.2 (d)	128.5	130.2 (d)	128.1	128.1 (d)	128.9	130.2 (d)
MeO	55.8	— (q)	55.8$^{a)}$	—$^{a)}$ (q)	—	—	—	—

a) Values may be interchanged.

of view, sikokianin A seems to be an alkylated product of neochamaejasmin A (**4**) and a precursor of chamaejasmenin B (**6**).[2,3]

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-810 spectrometer. ^{1}H- 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.

Extraction——Fresh roots of the plant (1.5 kg) were extracted twice with MeOH (10 l) for 1 week at room temperature. Evaporation of MeOH under reduced pressure afforded a brownish residue (140 g), which was partitioned 3 times between hexane (400 ml) and 50% aqueous MeOH (1200 ml). The aqueous MeOH solution was concentrated to half the initial volume under reduced pressure, followed by dilution with water (600 ml). The aqueous solution was extracted 3 times successively with benzene (400 ml), AcOEt (400 ml), and BuOH (300 ml). The yields of the hexane-, benzene-, AcOEt-, BuOH-, and water-soluble fractions were 20, 7.5, 39, 15, and 78 g, respectively.

Isolation——A part of the above AcOEt extract (15 g) was chromatographed on silica gel (Merck 7734, 300 g) with a gradient of $CHCl_3$, AcOEt, and MeOH to afford seven fractions. The first fraction (0.9 g) eluted with $CHCl_3$–AcOEt (2 : 1) (600 ml) was repeatedly chromatographed on silica gel with $CHCl_3$–AcOEt (2 : 1) to yield umbelliferone (**3**) (21 mg), which was identified by comparison of the IR, MS, and ^{1}H-NMR data with those of an authentic sample. The second fraction eluted with $CHCl_3$–AcOEt (1 : 1) (600 ml) was further separated by a combination of column chromatography on silica gel using $CHCl_3$–AcOEt (2 : 1) and on reversed-phase silica gel (Develosil ODS-30/50) using MeOH–H_2O (75 : 25) to afford sikokianin B (**2**) (145 mg) as an amorphous powder. The third fraction (1.9 g) was separated by column chromatography on silica gel using $CHCl_3$–AcOEt (7 : 3) followed by preparative TLC on silica gel using $CHCl_3$–AcOEt (1 : 1) to afford sikokianin A (**1**) (23 mg) as colorless needles.

Sikokianin A (1)——Colorless needles from aqueous MeOH, mp 230—232 °C. $[\alpha]_D$ +150° ($c=0.74$, MeOH). High-resolution MS Found *m/z*: 285.0760; $C_{16}H_{13}O_5$ requires 285.0761, Found *m/z*: 271.0588; $C_{15}H_{11}O_5$ requires 271.0605. UV λ_{max}^{MeOH} nm (ε): 298 (32000). IR ν_{max}^{KBr}: 3450, 1640 cm^{-1}. ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

Sikokianin B (2)——Amorphous powder, $[\alpha]_D$ +242° ($c=1.0$, MeOH). High-resolution MS Found *m/z*: 285.0779; $C_{16}H_{13}O_5$ requires 285.0761, Found *m/z*: 271.0619; $C_{15}H_{11}O_5$ requires 271.0605. UV λ_{max}^{MeOH} nm (ε): 298 (31000). IR ν_{max}^{KBr}: 3350, 1640 cm^{-1}. ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

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References and Notes

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