A New Flavanone from the Wood of *Amorpha fruticosa* L.

Hyun-Jung Lee, Oh-Kyu Lee, Yeong-Han Kwon,† Don-Ha Choi, Ha-Young Kang, Hyeon-Yong Lee,‡ Ki-Hyon Paik,§ and Hak-Ju Lee*

*Div. Wood Chemistry & Microbiology, Korea Forest Research Institute, Seoul 130-712, Korea. *E-mail: leehj99@foa.go.kr*
†Korea National Arboretum, Pocheon 487-821, Korea
‡School of Biotechnology & Bioengineering, Kangwon National University, Chuncheon 200-701, Korea
§Dept. of Forest Resources and Environmental Science, Korea University, Seoul 136-701, Korea
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In the course of studies on phenolic compounds in leguminous plants, we have selected *Amorpha fruticosa*. *A. fruticosa* is a shrub originated from North America. This plant was introduced to Korea through China in 1930s.[1] This plant grows up to about three meters high and flowers in May to June. Its seed is ripened in September and usually has one seed per fruit.[1]

Numerous isoflavones,[2] flavanones,[3,4] and rotenoids[2,4-7] have been reported from the fruit, leaf, and root of this plant. However, phenolic compounds in the wood of *A. fruticosa* have less studied. In this study, the methanol (MeOH) extract of the wood of *A. fruticosa* was separated by column chromatography to give a new flavanone (Fig. 1), 3',5',7-trihydroxyflavanone (**1**). Its chemical structure was identified by instrumental analysis using ultraviolet (UV), infrared (IR), mass (MS), and nuclear magnetic resonance (NMR) spectrometer.

Compound **1** was isolated as a yellow amorphous solid. The UV λ_{max} (log ε) of compound **1** appeared at 424 (3.13) nm and 288 (3.36) nm. The IR spectrum disclosed a characteristic absorption for the conjugated carbonyl group (1670 cm^{-1}) and OH region (3422 cm^{-1}). In the EIMS of compound **1**, the molecular ion peak was observed at *m/z* 272 ($[M]^+$) (base ion) and the major ion peaks were *m/z* 255, 163, 150, and 137. The HREIMS of compound **1** gave a molecular ion peak at *m/z* 272.0679, corresponding to the molecular formula of $C_{15}H_{12}O_5$.

The structure of compound **1** was deduced from the analysis of ^1H and ^{13}C-NMR data (Table 1) aided with 2D NMR measurements (^1H-^1H COSY, NOESY, HMQC, and HMBC). The ^{13}C-NMR spectrum showed fifteen resonances, sorted by DEPT experiments into seven primary carbons, one secondary carbon, and seven quaternary carbons.

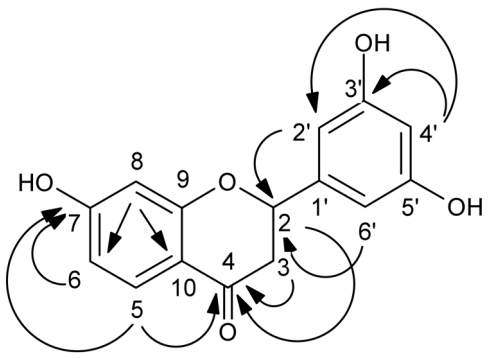

Figure 1. Key HMBC correlation of compound **1**.

The signal at δ 193.49 (*s*, C-4) was attributed to the carbonyl carbon. The long-range heteronuclear interactions of compound **1** were established by the HMBC spectrum which showed the *ortho* coupled proton signal δ 7.72 is connected to C-4. This proton signal was assigned to H-5 (δ 7.72, 1H, *d*, *J* = 9.0 Hz). The ^1H-^1H COSY spectrum revealed the connectivity of H-5 to H-6 (δ 6.50, 1H, *dd*, *J* = 1.5, 9.0 Hz). The proton signal at δ 6.35 (1H, *d*, *J* = 1.5 Hz) was assigned to the *meta* coupled aromatic H-8. ^1H-^{13}C connectivities of compound **1** were established by the HMQC spectrum which showed that H-5 and H-6 are connected to C-5 (δ 129.82, *d*) and C-6 (δ 111.78, *d*), respectively. Similarly, H-8 is connected to C-8 (δ 103.85, *d*). In the HMBC spectrum, the correlations between H-5 and C-4/C-9/C-7, H-6 and C-7/C-10/C-8, and H-8 and C-10/C-6 were observed. The correlation between H-6 and an oxygenated aromatic carbon (δ 165.53, *s*) was observed in the HMBC spectrum. From the above data, it was considered that a hydroxyl group is connected to C-7.

A methylene carbon signal at δ 45.03 (*t*) was assigned to C-3. The HMQC spectrum showed that C-3 is connected to the two H-3s (δ 2.70, 1H, *dd*, *J* = 3.0, 17.0 Hz and δ 3.00, 1H, *d*, *J* = 13.0, 17.0 Hz). The ^1H-^1H COSY spectrum presented the connectivity of H-3 to H-2. H-2 and H-3 of compound **1** showed signals characteristic of the flavanone moieties.

Three proton signals at δ 6.79 (1H, *m*), 6.80 (1H, *m*), and 6.93 (1H, *d*, *J* = 1.0 Hz) were assigned to H-6', H-4', and H-2', respectively. In the ^{13}C-NMR spectrum of compound **1**, two oxygenated aromatic carbon signals were observed at δ 146.51 (*s*, C-3') and 146.83 (*s*, C-5'). Therefore, it was postulated that two hydroxyl groups were connected to C-3' and C-5'.

According to the instrumental analysis performed, as a result, compound **1** was characterized as 3',5',7-trihydroxyflavanone.

Table 1. NMR data for compound **1** in methanol-d_4

Position	δ_H (ppm)	δ_C (ppm)	COSY	NOESY	HMBC
2	5.32 (1H, *dd*, *J* = 3.0, 12.5 Hz)	81.06 *d*	H-3	H-2'/H-6'	C-4
3	2.70 (1H, *dd*, *J* = 3.0, 17.0 Hz), 3.00 (1H, *dd*, *J* = 12.5, 17.0 Hz)	45.03 *t*	H-3/H-2		C-4/C-1'/C-2
4		193.49 *s*			
5	7.72 (1H, *d*, *J* = 9.0 Hz)	129.82 *d*	H-6		C-4/C-9/C-7
6	6.50 (1H, *dd*, *J* = 1.5, 9.0 Hz)	111.78 *d*	H-5		C-7/C-10/C-8
7		165.53 *s*			
8	6.35 (1H, *d*, *J* = 1.5 Hz)	103.85 *d*			C-10/C-6
9		166.92 *s*			
10		114.97 *s*			
1'		132.07 *s*			
2'	6.93 (1H, *d*, *J* = 1.0 Hz)	114.71 *d*		H-2	C-1'/C-2'/C-3'/C-4'/C-2
3'		146.51 *s*			
4'	6.80 (1H, *m*)	119.22 *d*			C-3'/C-2'
5'		146.83 *s*			
6'	6.79 (1H, *m*)	116.27 *d*		H-2	C-1'/C-2'/C-2

Experimental Section

General Methods. The UV spectrum was recorded on a Hewlett Packard 8452A Diode Array Spectrometer. The IR spectrum was recorded with a JASCO FT/IR-5300 spectrophotometer. The EIMS and HREIMS were obtained with a JEOL JMS-SX102A. The NMR spectra (^{1}H, ^{13}C, DEPT, COSY, NOESY, HMQC, HMBC) were recorded in methanol-d_4 using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in δ and coupling constants (J) in Hz. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity-Inova 500 MHz, operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). The thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} (0.2 mm, Merck) plates. The TLC plates were developed with solvent system A (toluene : ethyl formate : formic acid = 5 : 4 : 1, v/v/v) and B (acetone : ethyl acetate : H_2O = 10 : 10 : 1, v/v/v). The preparative TLC was performed on silica gel 60 F_{254} (2.0 mm, Merck) plates. The developed TLC plates were visualized under UV light at 254 nm and 365 nm. Silica gel 60 (40-100 μm, Kanto Chemical Co.) and Sephadex LH-20 (Amersham Pharmacia Biotech AB) were used for the column chromatography.

Plant Material. The wood of *A. fruticosa* was collected from Hadong-kun, Kyungnam, Korea in June, 2003 and identified by Dr. Y. H. Kwon (Korea National Arboretum, Pocheon, Korea). The voucher specimen (KNAb104-0019) has deposited at Korea Forest Research Institute, Seoul, Korea.

Extraction and Isolation. The air-dried and powdered wood of *A. fruticosa* was extracted three times with MeOH at room temperature for 3 days each. The combined MeOH extracts were concentrated under vacuum at 40 °C. The concentrated extract was partitioned with *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc). The $CHCl_3$-soluble (180.0 g) was separated on the Sephadex LH-20 column (6.5 × 55.0 cm) using MeOH-EtOH (1 : 1, v/v) solvent system to yield 28 fractions (250.0 mL each). These fractions were divided into 3 portions (AWC-1~AWC-3) on the basis of TLC profiles. AWC-3 (10.0 g) was subjected to the silica gel column (6.5 × 45.0 cm) with benzene-MeOH (5 : 1, v/v) to yield 50 fractions (100 mL each). These fractions were divided into 3 portions (AWC-3-1~AWC-3-3). AWC-3-1 (1.9 g) was chromatographed on the silica gel column (5.5 × 40.0 cm) with benzene-EtOAc (5 : 1, v/v) to yield 160 fractions (100 mL each). These fractions were divided into 5 portions (AWC-3-1-1~AWC-3-1-5). AWC-3-1-4 (290.0 mg) was subjected to the silica gel column (4.5 × 40.0 cm) with $CHCl_3$-MeOH (17 : 1, v/v) to yield 300 fractions (15.0 g each). These fractions were divided into 2 portions (AWC-3-1-4-1~AWC-3-1-4-2). AWC-3-1-4-2 (120.0 mg) was separated on the silica gel column (4.5 × 40.0 cm) with *n*-hexane-acetone (2 : 1, v/v) to yield 300 fractions (10.0 g each). These fractions were divided into 4 portions (AWC-3-1-4-2-1~AWC-3-1-4-2-4). AWC-3-1-4-2-2 (210.0 mg) was chromatographed on the silica gel column (2.5 × 50.0 cm) with CH_2Cl_2-MeOH (20 : 1, v/v) to yield 100 fractions (15.0 g each). These fractions were divided into 2 portions (AWC-3-1-4-2-2-1~AWC-3-1-4-2-2-2). AWC-3-1-4-2-2-2 (120.0 mg) was subjected to the silica gel column (3.0 × 60.0 cm) with CH_2Cl_2-MeOH (25 : 1, v/v) to yield 200 fractions (12.0 g each). These fractions were divided into 2 portions (AWC-3-1-4-2-2-2-1~AWC-3-1-4-2-2-2-2). AWC-3-1-4-2-2-2-2 (100.0 mg) was separated on the silica gel column (3.0 × 50.0 cm) CH_2Cl_2-MeOH (30 : 1, v/v) to yield 250 fractions (15.0 g each). These fractions were divided into 2 portions (AWC-3-1-4-2-2-2-2-1~AWC-3-1-4-2-2-2-2-2). AWC-3-1-4-2-2-2-2-2 (10.0 mg) was separated on the Sephadex LH-20 column (2.5 × 70.0 cm) using acetone to yield 100 fractions (10.0 g each). These fractions were divided into 2 portions (AWC-3-1-4-2-2-2-2-2-1~AWC-3-1-4-2-2-2-2-2-2). Compound **1** (3.7 mg) was

isolated from AWC-3-1-4-2-2-2-2-2-1.

3',5',7-Trihydroxyflavanone (1). Yellow amorphous solid. UV (MeOH) λ_{max} nm (log ε): 424 (3.13), 288 (3.36). UV (MeOH+0.1 M NaOH) λ_{max} nm (log ε): 434 (3.25), 336 (3.52). IR (KBr) ν_{max}: 3422 (OH), 2361, 1670 (C=O), 1282. EI-MS *m/z*: 272 ($[M]^+$) (base ion), 255, 163, 150, 137. HREIMS *m/z*: 272.0679 ($[M]^+$, calcd. for $C_{15}H_{12}O_5$, 272.0685). ^{1}H-NMR (methanol-d_4, 500 MHz), ^{13}C-NMR (methanol-d_4, 125 MHz), COSY, NOESY, and HMBC: Table 1.

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