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ISOFLAVONE GLYCOSIDES FROM THE BARK OF *Amorpha fruticosa*

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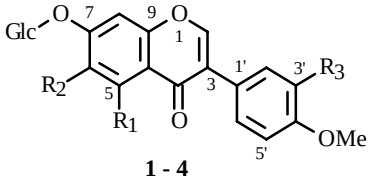
*Four known isoflavone glucosides have been isolated from the bark of Amorpha fruticosa, which is a traditional remedy plant, for the first time. They were elucidated as 3′-hydroxy-4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**1**), 4′,6-dimethoxyisoflavone-7-*O*-β-D-glucopyranoside (**2**), 4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**3**), and 3′,5-dihydroxy-4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**4**), based on the UV, FT-IR, EIMS, FABMS, HREIMS, and NMR (^{1}H and ^{13}C, DEPT, COSY, NOESY, HMQC, and HMBC) data.*

Key words: *Amorpha fruticosa*, isoflavone glucosides, 3′-hydroxy-4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside, 4′,6-dimethoxyisoflavone-7-*O*-β-D-glucopyranoside, 4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside, 3′,5-dihydroxy-4′-methoxyisofalvone-7-*O*-β-D-glucopyranoside.

Amorpha fruticosa L. (Leguminosae) is a shrub that flowers in May to June and originates from North America. This plant has used as a chinese folk medicine for hypertension, hematomas, and contutions [1]. *A. fruticosa* was introduced to Korea through China in 1930s. Although numerous isoflavones [2], flavanones [3, 4], and rotenoids [5–7] along with their biological activities have been reported from the fruit, leaf, and root of this plant, the chemical constituents in the bark of *A. fruticosa* have been less investigated.

In this study, the methanol extract from the bark of *A. fruticosa* was separated by column chromatography and the four known isoflavone glucosides, 3′-hydroxy-4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**1**), 4′,6-dimethoxyisoflavone-7-*O*-β-D-glucopyranoside (**2**), 4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**3**), and 3′,5-dihydroxy-4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (**4**) were isolated for the first time. Their chemical structures were identified by instrumental analysis using UV, IR, MS, and NMR spectrometer.



1 - 4

1: $R_1 = R_2 = H$, $R_3 = OH$
2: $R_1 = R_3 = H$, $R_2 = OMe$
3: $R_1 = R_2 = R_3 = H$
4: $R_1 = R_3 = OH$, $R_2 = H$

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TABLE 1. ^{1}H NMR (500 MHz, δ, ppm, J/Hz) Data of the Compounds

Position	Compound			
	1 (DMSO-d_6)	**2** (DMSO-d_6)	**3** (DMSO-d_6)	**4** (Methanol-d_4)
2	8.36 (1H, s)	8.41 (1H, s)	8.45 (1H, s)	8.14 (1H, s)
5	8.04 (1H, d, 8.5)	7.48 (1H, s)	8.06 (1H, d, 8.5)	
6	7.13 (1H, dd, 2.5, 8.5)		7.15 (1H, dd, 2.5, 8.5)	6.53 (1H, d, 2.0)
8	7.22 (1H, d, 2.5)	7.32 (1H, s)	7.25 (1H, d, 2.5)	6.70 (1H, d, 2.0)
2′	7.06 (1H, d, 1.0)	7.53 (1H, dd, 2.0, 7.0)	7.54 (1H, d, 9.0)	7.05 (1H, d, 1.5)
3′		7.00 (1H, dd, 2.0, 7.0)	7.00 (1H, d, 9.0)	
5′	6.96 (2H, d, 1.5)	7.00 (1H, dd, 2.0, 7.0)	7.00 (1H, d, 9.0)	6.99 (1H, d, 8.5)
6′	6.96 (2H, d, 1.5)	7.53 (1H, dd, 2.0, 7.0)	7.54 (1H, d, 9.0)	6.97 (1H, dd, 1.5, 8.5)
1″	5.09 (1H, d, 7.0)	5.16 (1H, d, 7.5)	5.12 (1H, d, 7.5)	5.04 (1H, d, 7.5)
2″	3.30 (1H, m)	3.31 (1H, m)	3.31 (1H, m)	3.48 (1H, m)
3″	3.44 (1H, m)	3.46 (1H, m)	3.47 (1H, m)	3.50 (1H, m)
4″	3.18 (1H, m)	3.17 (1H, m)	3.18 (1H, m)	3.44 (1H, m)
5″	3.32 (1H, m)	3.29 (1H, m)	3.32 (1H, m)	3.49 (1H, m)
6″	3.48 (1H, m), 3.72 (1H, m)	3.47 (1H, m), 3.70 (1H, m)	3.48 (1H, m), 3.73 (1H, m)	3.72 (1H, m), 3.92 (1H, m)
3′-OH	8.98 (1H, s)			
4′-OMe	3.79 (3H, s)	3.78 (3H, s)	3.79 (3H, s)	3.88 (3H, s)
6-OMe		3.88 (1H, s)		

TABLE 2. ^{13}C NMR (125 MHz, δ, ppm) Data of the Compounds

Position	Compound			
	1 (DMSO-d_6)	**2** (DMSO-d_6)	**3** (DMSO-d_6)	**4** (Methanol-d_4)
2	154.23 (d)	153.26 (d)	153.75 (d)	155.54 (d)
3	125.18 (s)	122.77 (s)	123.41 (s)	125.03 (s)
4	175.33 (s)	174.25 (s)	174.74 (s)	182.43 (s)
5	127.67 (d)	104.78 (d)	127.03 (d)	164.84 (s)
6	116.31 (d)	147.49 (s)	115.70 (d)	101.20 (d)
7	162.14 (s)	151.57 (s)	161.48 (s)	163.61 (s)
8	104.14 (d)	103.44 (d)	103.43 (d)	99.95 (d)
9	157.69 (s)	151.20 (s)	157.10 (s)	159.26 (s)
10	119.22 (s)	117.80 (s)	118.48 (s)	108.08 (s)
1′	124.30 (s)	124.25 (s)	124.04 (s)	124.94 (s)
2′	117.13 (d)	130.01 (d)	130.15 (d)	117.33 (d)
3′	146.78 (s)	113.60 (d)	113.68 (d)	147.55 (s)
4′	148.30 (s)	158.94 (s)	159.06 (s)	149.41 (s)
5′	112.73 (d)	113.60 (d)	113.68 (d)	112.74 (d)
6′	120.44 (d)	130.01 (d)	130.15 (d)	121.62 (d)
1″	100.77 (d)	99.64 (d)	99.97 (d)	101.70 (d)
2″	73.86 (d)	73.00 (d)	73.16 (d)	74.75 (d)
3″	77.93 (d)	77.18 (d)	77.24 (d)	78.42 (d)
4″	70.39 (d)	69.58 (d)	69.63 (d)	71.26 (d)
5″	77.20 (d)	76.72 (d)	76.50 (d)	77.89 (d)
6″	61.38 (t)	60.51 (t)	60.66 (t)	62.47 (t)
4′-OMe	56.41 (q)	55.11 (q)	55.20 (q)	56.48 (q)
6-OMe		55.83 (q)		

EXPERIMENTAL

General. The UV spectrum was recorded on a Hewlett Packard 8452A Diode Array spectrophotometer. The IR spectrum was recorded with a NEXUS FT-IR spectrophotometer. The FABMS spectrum was determined on a JEOL 700. The EIMS and HREIMS were obtained with a JEOL JMS-SX102A. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, COSY, NOESY, HMQC, and HMBC spectra were obtained with a Varian Unity-Inova 500 MHz. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} (0.2 mm, Merck) plates. 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 TLC was 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 bark 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). A voucher specimen (KNAb104-0019) was deposited at the Korea Forest Research Institute, Seoul, Korea.

Extraction and Isolation. The air-dried and powdered bark of *A. fruticosa* was extracted three times with MeOH at room temperature for 3 days each. The combined MeOH extracts were concentrated in vacuum at 40°C. The concentrated extract was successively partitioned with light petroleum ether (LPE), diethyl ether (Et_2O), and ethyl acetate (EtOAc).

The Et_2O-soluble (97.1 g) was separated on a Sephadex LH-20 column (8.0 × 50.0 cm) using a MeOH–EtOH (1:1, v/v) solvent system to yield 36 fractions (250.0 mL each). These fractions were divided into 3 portions (ABO-1~ABO-3) on the basis of TLC profiles. ABO-2 (89.1 g) was chromatographed on a Sephadex LH-20 column (8.0 ×45.0 cm) using a MeOH–EtOH (1:4, v/v) solvent system to yield 70 fractions (250.0 mL each). These fractions were divided into 4 portions (ABO-2-1~ABO-2-4). ABO-2-4 (22.6 g) was chromatographed on silica gel column (6.5 ×45.0 cm) using a benzene–MeOH (5:1, v/v) solvent system to yield 80 fractions (100.0 mL each). These fractions were divided into 3 portions (ABO-2-4-1~ABO-2-4-3). ABO-2-4-2 (3.5 g) was subjected to the silica gel column (4.0 × 50.0 cm) with EtOAc–MeOH (5:1, v/v) to yield 250 fractions (15.0 g each). These fractions were divided into 4 portions (ABO-2-4-2-1~ABO-2-4-2-4). ABO-2-4-2-3 (350.5 mg) was purified to give compound **1**, a white powder. The MeOH solution of portion ABO-2-3 was divided into MeOH insoluble (ABO-2-3A) and soluble fractions (ABO-2-3B). ABO-2-3A (12.9 g) was subjected to the silica gel column (4.5 × 55.0 cm) with $CHCl_3$–MeOH (10:1, v/v) to yield 120 fractions (100 mL each). These fractions were divided into 5 portions (ABO-2-3A-1~ABO-2-3A-5). ABO-2-3A-2 (683.3 mg) was purified to give compound **2**, white powder. ABO-2-3A-4 (800.0 mg) was separated on the silica gel column (4.5 × 55.0 cm) using a $CHCl_3$–EtOH (10:1, v/v) solvent system to yield 500 fractions (20.0 g each). These fractions were divided into 6 portions (ABO-2-3A-4-1~ABO-2-3A-4-6). Compound **3** was isolated from fraction ABO-2-3A-4-5 (25.0 mg). ABO-2-3A-5 (230.0 mg) was subjected to the silica gel column (2.5 × 50.0 cm) with $CHCl_3$–MeOH (10:1, v/v) to yield 200 fractions (15.0 g each). These fractions were divided into 5 portions (ABO-2-3A-5-1~ABO-2-3A-5-5). ABO-2-3A-5-3 (170.0 mg) was chromatographed on the silica gel column (2.5×50.0 cm) using a benzene–MeOH (6:1, v/v) solvent system to yield 130 fractions (10.0 g each). These fractions were divided into 3 portions (ABO-2-3A-5-3-1~ABO-2-3A-5-3-3). Compound **4** was isolated from fraction ABO-2-3A-5-3-2 (10.1 mg).

3′-Hydroxy-4′-methoxyisoflavone-7-*O*-*β*-D-glucopyranoside (1), white powder. UV (DMSO, λ_{max}, nm) (log ε): 294 (3.72); + NaOH (0.1 M) (log ε): 306 (3.68). IR (KBr, ν_{max}, cm^{-1}): 3419 (OH), 1645 (C=O), 1570, 1510, 1078, 1026. FABMS *m/z*: 447 ($[M+H]^+$), 446 ($[M]^+$). EIMS *m/z*: 446 ($[M]^+$), 284 (base ion), 269, 241, 213, 137, 105, 73. HREIMS *m/z*: 446.1204 ($[M]^+$, calcd. for $C_{22}H_{22}O_{10}$, 446.1213). COSY: H-5↔H-6, H-1″↔H-2″, H-6″↔H-6″/H-5″. NOESY: H-8↔H-1″, H-5′↔OMe-4′. HMBC: OH-3′→C-4′/C-2′, H-2→ C-4/C-9/C-3/C-1′, H-5→C-4/C-7/C-9, H-8→C-7/C-9/C-10/C-6, H-6→C-10/C-8, H-2′→C-1′/C-6′/C-4′/C-3′, H-6′→C-5′, H-5′→C-3′, H-1″→C-7/C-5″/C-2″, OMe-4′→C-4′, H-3″→C-5″, H-5″→C-1″, H-2″→C-1″ .

4′,6-Dimethoxyisoflavone-7-*O*-*β*-D-glucopyranoside (2), white powder. UV (DMSO, λ_{max}, nm) (log ε): 320 (3.90), 288 (3.92); + NaOH (0.1 M) (log ε): 354 (3.91), 290 (4.11). IR (KBr, ν_{max}, cm^{-1}): 1635 (C=O), 1514, 1087, 1022. FABMS *m/z*: 461 ($[M+H]^+$), 460 ($[M]^+$). EIMS *m/z*: 460 ($[M]^+$), 340, 312, 298 (base ion), 283, 255, 240, 227, 166, 132, 123, 89, 69, 60. HREIMS *m/z*: 460.1378 ($[M]^+$, calcd. for $C_{23}H_{24}O_{10}$, 460.1369). COSY: H-2′/H-6′↔H-3′/H-5′, H-1″↔H-2″, H-6″↔H-6″/H-5″. HMBC: H-2→C-4/C-9/C-1′/C-3, H-2′/H-6′→ C-4′/C-3, H-5→C-4/C-9/C-6/C-10, H-8→C-9/C-6/C-10, H-3′/H-5′→C-4′/C-1′, H-1″→C-7, OMe-6→C-6, OMe-4′→C-4′, H-6″→C-5″, H-3″→C-5″.

4′-Methoxyisoflavone-7-*O*-*β*-D-glucopyranoside (3), pale yellow powder. UV (DMSO, λ_{max}, nm) (log ε): 290 (3.99); + NaOH (0.1 M) (log ε): 298 (4.16). IR (KBr, ν_{max}, cm^{-1}): 1645(C=O), 1541, 1076. FABMS *m/z*: 430 ($[M]^+$). EI-MS *m/z*: 430 ($[M]^+$), 314, 298, 268 (base ion), 267, 253, 225, 197, 168, 168, 132, 117, 89, 73, 60. HREIMS *m/z*: 430.1263 ($[M]^+$, calcd. for

$C_{22}H_{22}O_9$, 430.1264). COSY: H-5↔H-6, H-2′/H-6′↔H-3′/H-5′, H-1″↔H-2″, H-6″↔H-6″, H-3″↔H-4″, H-4″↔H-5″. NOESY: H-2↔H-2′, H-8↔H-1″, H-3′/H-5′↔OMe-4′. HMBC: H-2→ C-4/C-9/C-1′/C-3, H-5→C-4/C-7/C-9/C-6, H-2′→C-4′/C-6′/C-3/C-3′/C-5′, H-6′→C-4′/C-2′/C-3/C-3′/C-5′, H-8→C-7/C-9/C-10/C-6, H-6→C-7/C-10/C-8, H-3′→C-4′/C-1′, H-5′→C-4′/C-1′, H-1″→C-7/C-3″, OMe-4′→C-4′, H-5″→C-4″, H-2″→C-1″/C-4″, H-3″→C-1″, H-4″→C-5″.

3′,5-Dihydroxy-4′-methoxyisoflavone-7-*O*-*β*-D-glucopyranoside (4), pale yellow powder. UV (DMSO, λ_{max}, nm) (log ε): 288 (3.73); + NaOH (0.1 M) (log ε): 288 (3.74). IR (KBr, ν_{max}, cm^{-1}): 3421 (OH), 1645 (C=O), 1504, 1078. FABMS *m/z*: 462 ($[M]^+$). EIMS *m/z*: 462 ($[M]^+$), 430, 301, 300 (base ion), 285, 257, 229, 153, 133, 105, 85, 60. HREIMS *m/z*: 462.1167 ($[M]^+$, calcd. for $C_{22}H_{22}O_{11}$, 460.1162). COSY: H-1″↔H-2″, H-6″↔H-6″/H-5″, H-5″↔H-4″. NOESY: H-2↔H-6′, H-1″↔H-8/H-6. HMBC: H-2→C-4/C-9/C-3/C-1′, H-2′→C-4′/C-3′/C-3, H-5′→C-3′, H-8→C-10, H-6→ C-5/C-7/C-10, H-1″→C-7, OMe-4′→C-4′, H-3″→C-2″,H-2″→C-3″, H-4″→C-5″.

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