Mokchae Konghak 31(6) : 8~14, 2003

Extractives from Fruits of *Amorpha Fruticosa* (I)*1

Hyun-Jung Lee*2, Hak-Ju Lee*3†, Yeong-Han Kwon*4, Don-Ha Choi*3, and Ki-Hyon Paik*2

ABSTRACT

This study was carried out identify extractives of *Amorpha fruticosa*. In this study, one flavonoid glycoside, one ester and two rotenoids were isolated from fruits of *A. fruticosa*. The structures were determined as: kaempferol 7-O-α-L-rhamnopyranoside (I), methyl 3, 4, 5-trihydroxybenzoate (methyl gallate, II), tephrosin (III) and dalbinol (IV), respectively, on the basis of spectroscopic data.

Keywords : *Amorpha fruticosa, fruit, kaempferol 7-O-α-L-rhamnopyranoside, methyl 3, 4, 5-trihydroxybenzoate, tephrosin, dalbinol.*

1. INTRODUCTION

Amorpha fruticosa (Leguminosae) is a shrub originated from North America. This plant was introduced to Korea through China in about 1930. This plant has about three-meter height, and is flowering in May to June. Its seed is ripened in September, and usually has one seed per fruit. The studies on the chemical composition of its leaves and stems showed to include isoflavonoids such as amorphispironone, tephrosin, 12α-hydroxyamorphigenin, 12α-hydroxydalpanol, 6α,12α-dehydro-α-toxicorol,6α,12α-dehydro-deguelin, 6'-O-D-glucopyranosyl-dalpanol, glucopyranosyldalpanol (Takao *et al.*, 1993; Leping *et al.*, 1993; Hiroki *et al.*, 1993; Qiuyun *et al.*, 1998). The chemical composition of its root part consisted of prenylated flavoniods such as amorin, isoamorin, amoradicin, amoradinin, amorphaquinone, demethylmedicalpin, formononetin, calycosin (Masayoshi *et al.*, 1998; Rozsa *et al*, 1984, 1982).

This study was carried out identify the constituents of *A. fruticosa*. Instrumental analysis methods such as EI-MS (electric impact-mass spectroscopy) and NMR (nuclear magnetic resonance) spectroscopy were performed to identify compounds obtained from this study.

2. MATERIALS AND METHODS

2.1. Materials

The fruit of *A. fruticosa* was collected from

*1 Received on February 11, 2003; accepted on April 25, 2003.
*2 Department of Forest Resources and Environmental Science, Korea University, Seoul 136-701, Korea
*3 Div. Wood Chemistry & Microbiology Korea Forest Research Institute, Seoul 130-712, Korea
*4 National Arboretum, Soheul-eup Pocheon-gun Gyeongi-do, 487-821, Korea
† Corresponding author : Hak-Ju Lee (hakju99@hanmail.net)

Jinkyo-myun, Hadong-kun, Kyungnam, Korea in November, 2001.

2.2. Extraction and Fractionation

Dried and ground fruit of *A. fruticosa* was extracted with methanol (MeOH) and then concentrated to give the crude extracts. The crude extracts was successively fractioned with organic solvents, such as *n*-hexane, dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc).

2.3. Instrumental Analysis

For the determination of molecular weights of the isolated compounds, EI-MS was performed at 70eV ionization energy by direct inlet probe method, using JEOL JMS-600W mass spectrometer. Both ^{1}H- and ^{13}C-NMR spectra were obtained using a Varian UI 500 spectrometer at the Korea Basic Science Institute in Seoul.

2.4. Isolation of Compounds

2.4.1. Compound I

The EtOAc solubles (15.96 g) were chromatographed on a Sephadex LH-20 column (4.5×72 cm) using a MeOH solvent system. Each fraction was collected by 20 ml portions, and 90 fractions were obtained. These fractions were divided into 6 fractions (AFEA-1～AFEA-6) monitoring by thin layer chromatography (TLC; silica gel 60 F_{254}) with toluene-ethyl formate-formic acid (5:4:1, v/v/v). AFEA-4 (530 mg) was chromatographed on a Sephadex LH-20 column (3.3×55 cm) using a MeOH-EtOH (1:4, v/v) solvent system. Each fraction was collected by 10 ml portions, and 100 fractions were obtained. These fractions were divided into 3 fractions (AFEA-4-1～AFEA-4-3) monitoring by UV lamp. AFEA-4-2 (160 mg) was purified to give compound I of yellow crystal form.

Yellow crystal. EI-MS *m/z*: 432 (M^+-146), 410, 286 (base ion), 258, 257, 208, 207, 153, 121. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.12 (3H, *d*, *J* = 6.14 Hz, CH3-6"), 3.35 (1H, *m*, H-4"), 3.43 (1H, *m*, H-5"), 3.64 (1H, *m*, H-3"), 3.85 (1H, *m*, H-2"), 4.81 (1H, *d*, *J* = 5.7 Hz, OH-3"), 4.93 (1H, *d*, *J* = 5.7 Hz, OH-4"), 5.15 (1H, *d*, *J* = 4.5 Hz, OH-2"), 5.54 (1H, *d*, *J* = 1.6 Hz, H-1"), 6.41 (1H, *d*, *J* = 2.0 Hz, H-6), 6.82 (1H, *d*, *J* = 2.0 Hz, H-8), 6.92 (2H, *dd*, *J* = 1.8, 7.2 Hz, H-3', 5'), 8.08 (2H, *dd*, *J* = 1.8, 7.2 Hz, H-2', 6'), 9.53 (1H, *s*, OH-3), 10.14 (1H, *s*, OH-4'), 12.47 (1H, *s*, OH-5). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 17.88 (*q*, CH3-6"), 69.80 (*d*, C-2"), 70.02 (*d*, C-5"), 70.20 (*d*, C-3"), 71.55 (*d*, C-4"), 94.29 (*d*, C-8), 98.34 (*d*, C-1"), 98.78 (*d*, C-6), 104.62 (*s*, C-10), 115.41 (*d*, C-3', 5'), 121.47 (*s*, C-1'), 129.61 (*d*, C-2', 6'), 135.98 (*s*, C-3), 147.43 (*s*, C-2), 155.69 (*s*, C-9), 159.32 (*s*, C-4'), 160.32 (*s*, C-5), 161.36 (*s*, C-7), 176.00 (*s*, C-4). HMBC correlations: OH-5→C-6/C-10/C-5, OH-4'→C-3'/C-5', OH-3→C-2, H-2'/6'→C-4'/C-2/C-6', H-3'/5'→C-3'/C-5'/C-1'/C-4', H-8→C-6/C-10/C-9/C-7, H-6→C-8/C-10/C-5/C-7, H-1"→C-5"/C-7, CH_3-6"→C-5"/C-4". NOESY correlations: H-1"↔H-6/H-8, OH-5↔OH-4'/OH-3.

2.4.2. Compound II

The AFEA-2 fraction (4.78 g) of EtOAc solubles was chromatographed on a silica gel column (5.5×45 cm) using a $CHCl_3$-MeOH (30:1, v/v) solvent system. Each fraction was collected by 20 ml portions, and 300 fractions were obtained. These fractions were divided into 5 fractions (AFEA-2-1～AFEA-2-5) monitoring by UV lamp. Compound II (59 mg) of white powder was isolated from AFEA-2-5 fraction.

White powder. EI-MS *m/z*: 184 (M^+), 153 (base ion), 125, 107, 79. ^{1}H-NMR (500 MHz, CD_3OD) : δ 3.79 (3H, *s*, OCH_3), 7.03 (2H, *s*,

H-2, 6). ^{13}C-NMR (125 MHz, CD_3OD): δ 52.26 (OCH3), 110.03 (C-2, 6), 121.44 (C-1), 139.75 (C-4), 146.49 (C-3, 5), 169.02 (C-7).

2.4.3. Compound III

The CH_2Cl_2 solubles were separated on a Sephadex LH-20 column (6.5×65 cm) using a MeOH-EtOH (1:1, v/v) solvent system. Each fraction was collected by 50 ml portions, and 85 fractions were obtained. These fractions were divided into 6 fractions (AFD-1～AFD-6) monitoring by UV (254 nm) lamp. AFD-2 (5.81 g) was chromatographed on a silica gel column (4.5×45 cm) using a benzene-EtOAc (5:1, v/v) solvent system. Each fraction was collected by 14 ml portions, and 450 fractions were obtained. These fractions were divided into 7 fractions (AFD-2-1～AFD-2-7). AFD-2-4 (100 mg) was rechromatographed on a silica gel column (3.5×29 cm) using a hexane-acetone (4:1, v/v) solvent system. Each fraction was collected by 3 ml portions, and 250 fractions were obtained. These fractions were divided into 4 fractions (AFD-2-4-1～AFD-2-4-4). AFD-2-4-3 (70 mg) was purified by preparative TLC with benzene-MeOH (50:1, v/v) which gave a compound III (18 mg).

EI-MS *m/z*: 410 (M^+), 208 (base ion), 207, 187, 165, 109. HMBC correlations: CH_3-2'→CH_3-3'/C-1'/C-13a, CH_3-3'→CH_3-2'/C-1'/C-13a, OMe-8→C-8, OMe-9→C-9, H-12→C-6a/C-10a, H-13a→C-1'/C-2, H-4→C-5a, H-10→C-7a/C-8/10a, H-13→C-1'/C-3, H-7→C-6a/C-8/C-10a, H-5→C-1a/C-3/C-6. NOESY correlations: H-13a ↔ CH_3-2'/CH_3-3', H-7↔OMe-8, H-10↔OMe-9, H-13↔H-13a, H-5↔H-4.

2.4.4. Compound IV

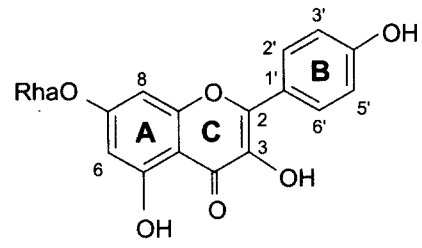
The AFD-3 fraction (6.0 g) of CH_2Cl_2 solubles was chromatographed on a silica gel column (5×35 cm) using a benzene-MeOH (5:1, v/v) solvent system. Each fraction was collected by 15 ml portions, and 120 fractions were obtained. These fractions were divided into 6 fractions (AFD-3-1～AFD-3-6) monitoring by UV lamp. AFD-3-2 (1.69 g) was chromatographed on a silica gel column (5.5×31 cm) using a $CHCl_3$-MeOH (4:1, v/v) solvent system. Each fraction was collected by 20 ml portions, and 300 fractions were obtained. These fractions were divided into 4 fractions (AFD-3-2-1～AFD-3-2-4). AFD-3-2-1 (1.30 g) was separated on a silica gel column (4.5×33 cm) using a hexane-acetone (2:1, v/v) solvent system. Each fraction was collected by 8 ml portions, and 300 fractions were obtained. These fractions were divided into 7 fractions (AFD-3-2-1-1～AFD-3-2-1-7). AFD-3-2-1-6 (151.3 mg) was separated on a silica gel column (2.5×25 cm) using a $CHCl_3$-MeOH (100:1, v/v) solvent system. Each fraction was collected by 10 ml portions, and 50 fractions were obtained. These fractions were divided into 2 fractions (AFD-3-2-1-6-1→AFD-3-2-1-6-2). AFD-3-2-1-6-1 (91 mg) was purified by prep. TLC with $CHCl_3$-MeOH (30:1, v/v) which gave a compound IV (26 mg).

EI-MS *m/z*: 426 (M^+), 208 (base ion), 207, 165, 149, 137, 107. HMBC correlations: H-13 →C-13a/C-1a/C-1'/C-3, OMe-8→C-8, OMe-9→C-9, H-2'→C-3'/C-1', H-12→C-6a/C-10a, H-12a →C-6a/C-7a, H-3'→C-2'/C-13a/C-1', H-13a→C-3', H-4→C-5a, H-10→C-7a/C-8/C-9, H-7→C-6a/C-8, H-5→C-1a/C-3/C-6. NOESY correlations: H-13a↔CH_2-13, H-7↔OMe-8, H-10↔OMe-9, CH_2-3'/CH_2-2', H-5↔H-4.

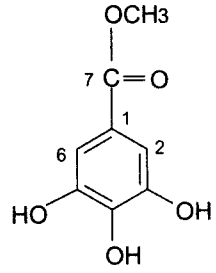
3. RESULTS AND DISCUSSION

3.1. Compound I

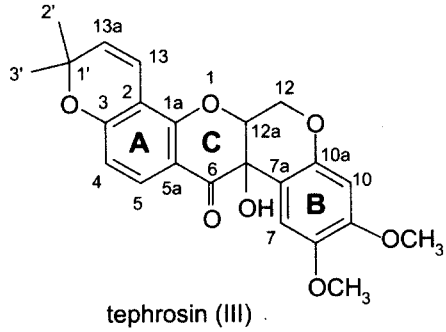
The compound I was obtained as yellow crystal. In the EI-MS of I, a molecular ion peak (M^+) was observed at *m/z* 432, and major ions



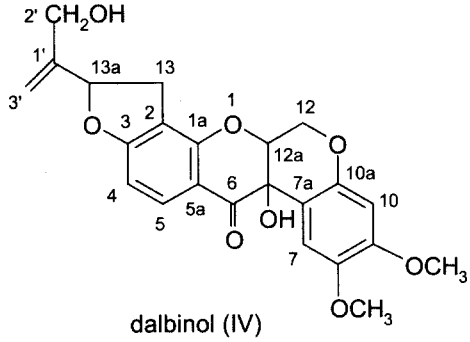
kaempferol-7-O-a-L-rhamnopyranoside (I)



methyl 3,4,5-trihydroxybenzoate (II)
(methyl gallate)



tephrosin (III)



dalbinol (IV)

Fig. 1. Compounds isolated from the fruit of *A. fruticosa.*

were observed at *m/z* 410, 286, 258, 208, 153 and 121. A base ion peak was observed at *m/z* 286. In the ^{1}H-NMR spectrum of I, two doublets at δ 6.41, 6.82 were caused by H-8, H-6 protons of the flavonoid A ring. Another two doublets at δ 6.92, 8.08 were the aromatic protons of H-3', H-5', H-2', H-6'-proton. Three singlets at δ 9.53 (1H, *s*, OH-3), 10.14 (1H, *s*, OH-4'), 12.47 (1H, *s*, OH-5) were attributed to hydroxyl group protons of 3, 4', 5 proton from aglycone moieties. In the NOESY spectrum of I, these hydroxyl group protons formed correlated peaks. It was suggested that these hydroxyl groups were near. A doublet signal of δ 5.54 (1H, *d*, J = 1.6 Hz, H-1") was assigned to H-1" anomer proton of a-L-rhamnose. A doublet signal at δ 1.12 (3H, *d*, J = 6.14 Hz, CH_3-6") was caused by rhamnose proton of 3H. In the DEPT (45°, 90°, 135°) and the ^{13}C-NMR spectrum, eleven primary carbons, one tertinary carbon and nine quaternary carbons were existed. The δ 176.00 (C-4) came from carbonyl group and δ 17.88 (*q*, CH_3-6") came from C-6" carbon of rhamnose (Agrawal, 1989; Harborne, 1994). In the HMBC spectrum of I, C-2 carbon was correlated with H-2, H-6' and C-7 carbon was correlated with H-1" anomer proton. So *a*-L-rhamnose was binded C-7 carbon. For the reasons stated above the compound I was determined as kaempferol-7-O-*a*-L-rhamnopyranoside (Fig. 1).

3.2. Compound II

The compound II was isolated as white powder. In the EI-MS of II, a molecular ion peak (M^+) was observed at *m/z* 184. In the ^{1}H-NMR spectrum, singlet signal of δ 3.79 (3H,

Table 1. ^{1}H- and ^{13}C-NMR data of compounds III and IV (CD_3OD)

	III		IV	
C	δH	δc	δH	δc
1a	-	156.44 *s*	-	157.60 *s*
2	-	109.13 *s*	-	113.17 *s*
3	-	160.38 *s*	-	167.58 *s*
4	6.46 (*d* 8.5)	111.37 *d*	6.49 (*d* 8.5)	104.78 *d*
5	7.70 (*d* 8.5)	128.14 *d*	7.78 (*d* 8.5)	129.61 *d*
5a	-	112.23 *s*	-	112.91 *s*
6	-	191.91 *s*	-	19168 *s*
6a	-	67.89 *s*	-	68.03 *s*
7	6.72 (*s*)	111.41 *d*	6.71 (*s*)	111.41 *d*
7a	-	108.44 *s*	-	108.64 *s*
8	-	143.80 *s*	-	143.80 *s*
9	-	151.69 *s*	-	151.67 *s*
10	6.54 (*s*)	101.19 *d*	6.50 (*s*)	101.14 *d*
10a	-	149.32 *s*	-	149.29 *s*
12	4.50 (*dd* 1.2, 12.5) 4.62 (*m*)	63.72 *t*	4.57 (*dd* 2.3, 12.0)	63.75 *t*
12a	4.62 (*m*)	76.75 *d*	4.62 (*d* 2.3)	76.50 *d*
13	6.60 (*d* 10.0)	115.00 *d*	3.02 (*dd* 8.0, 16.0) 3.32 (*dd* 8.0, 16.0)	31.34 *t*
13a	5.65 (*d* 10.0)	129.20 *d*	5.38 (*t* 8.0)	85.58 *d*
1'	-	77.81 *s*	-	147.90 *s*
2'	1.35 (*s*)	27.09 *q*	4.15 (*d* 3.0)	61.40 *d*
3'	1.43 (*s*)	27.49 *q*	5.18 (*s*) 5.24 (*s*)	110.36 *t*
8-OMe	3.68 (*s*)	55.97 *q*	3.67 (*s*)	55.98 *q*
9-OMe	3.78 (*s*)	55.18 *q*	3.76 (*s*)	55.18 *q*

Values are in ppm (δH and δC). ^{1}H- and ^{13}C-NMR spectra were measured at 500 MHz and 125 MHz, respectively. Figures in parentheses are coupling constants (*J*) in Hz.

s, OCH_3) was caused by methoxyl group proton and singlet of δ 7.03 (2H, *s*, H-2, 6) was caused by H-2, H-6 of aromatic ring. The ^{13}C-NMR spectrum showed six signals. The δ 110.03 (C-2, 6) was caused by primary carbon of C-2, C-6 and δ 121.44 (C-1), 139.75 (C-4), 146.49 (C-3, 5) were caused by quaternary carbon of C-1, C-4, C-3, C-5. The δ 169.02 (C-7) was assignable to carbonyl group. So the compound II was determined as methyl 3, 4, 5-tryhydroxybenzoate or methyl gallate (Fig. 1).

3.3. Compound III

In the EI-MS of III, a molecular ion peak (M^+) was observed at *m/z* 410, and major ions were observed at *m/z* 208, 207, 187, 165. A base ion peak was observed at *m/z* 208. In the ^{1}H-NMR spectrum of III, two singlet signals of δ 1.35 (3H, CH_3-2'), δ 1.43 (3H, CH_3-3') were caused by methyl group protons of H-2' and H-3' side chains, two singlet signal of δ 3.68 (3H, OMe-8) and δ 3.78 (3H, OMe-9) were caused by methoxyl group protons of H-8 and

H-9. Two doublet signals of δ 6.46 (1H, *d*, *J* = 8.5 Hz, H-4) and 7.70 (1H, *d*, *J* = 8.5 Hz, H-5) were assignable to H-4 and H-5 protons of isoflavonoid A ring. Two singlet signals of δ 6.54 (1H, *s*, H-10) and 6.72 (1H, *s*, H-7) were caused by CH-10 and CH-7 of methine proton. In the NOESY spectrum of III, methyl group proton of CH_3-2' and CH_3-3' side chain were correlated with H-13a. H-7 protons was correlated with OCH_3-8 of methoxyl group proton, and H-10 was correlated with OCH_3-9 methoxyl group proton. The ^{13}C-NMR spectrum of the compound III showed twenty-three signals. There were seven primary carbons, one secondary carbon, 4 tertinary carbons and 11 quaternary carbons. Both δ 27.09 (CH_3-2') and δ 27.49 (CH_3-3') were assignable to methyl carbon of C-2' and C-3' side chain. The δ 55.18 (OCH_3-9) and δ 55.97 (OCH_3-8) were assignable to methoxyl group carbon. The δ 191.91 was caused by carbonyl group (Harborne, 1975; 1982). The HMBC spectrum showed that the peak of CH_3-2' and CH_3-3' proton was correlated with CH_2-12 methylene proton while the peak of C-1' carbon and C-13a carbon was correlated with C-6a and C-10a carbon. For the evidence detected above, the compound III was determined as tephrosin or rotenoid compound of isoflavonoid skeleton (Fig. 1).

3.4. Compound IV

In the EI-MS of IV , a molecular ion peak (M^+) was observed at *m/z* 426. In the ^{1}H-NMR spectrum, two signals of δ 3.67 (3H, *s*, OMe-8) and δ 3.76 (3H, *s*, OMe-9) were caused by the protons of OCH_3-8 and OCH_3-9 methoxyl group. Two singlet signals of δ 6.50 (1H, *s*, H-10) and δ 6.71 (1H, *s*, H-7) were caused by the CH-10, CH-7 methine proton of isoflavonoid B ring. The δ 4.15 (2H, *d*, *J* = 3.0 Hz, H-2') was assigned to the CH_2-2' methylene proton. Both δ 5.18 (1H, H-3') and δ 5.24 (1H, *s*, H-3') were assigned to the CH_2-3' methylene proton. Both δ 4.48 (1H, H-12) and δ 4.57 (1H, *dd*, *J* = 2.2, 12.0 Hz, H-12) were derived from the CH_2-12 methylene proton. The δ 4.62 (1H, *dd*, *J* = 1.0, 2.5 Hz, H-12a) was derived from CH-12a methine proton. The total number of signals of ^{13}C-NMR spectrum were 23. There were 6 primary carbons, 4 secondary carbons, 2 tertinary carbons, and 11 quaternary carbons. The δ 31.34 was assigned to C-13 carbon. The δ 85.58 was assigned to C-13a-carbon. Both δ 147.90 and δ 110.36, δ 61.40 were derived from C-1', C-3', C-2' carbon. The δ 55.18 (OCH_3-9) and δ 55.98 (OCH_3-8) were assignable to OCH_3-9 and OCH_3-8 methoxyl group carbon. Using of other peaks of the 2D-NMR spectrum compoud IV was determined as dalbinol or rotenoid compound of isoflavonoid skeleton (Fig. 1).

4. CONCLUSION

One flavonoid glycoside, one ester compound and two rotenoids were isolated from the fruit of *A. fruticosa*. The structures were determined as: kaempferol 7-O-*a*-L-rhamnopyranoside (I), methyl 3, 4, 5-trihydroxybenzoate (methyl gallate, II), tephrosin (III) and dalbinol (IV) respectively, on the basis of spectroscopic data.

REFERENCES

1. Agrawal, P. K., 1989. Carbon-13 NMR of Flavonoids. Elsevier. pp. 564.
2. Harborne, J. B., 1994. The Flavonoids. Advances in Research since 1986. Chapman & Hall. pp. 676.
3. Harborne, J. B., T. J. Mabry, and H. Mabry. 1975. The Flavonoids Part I. Academic press. pp. 630.
4. Harborne, J. B. and T. J. Mabry. 1982. The Flavonoids: Advances in Research. Chapman & Hall. pp. 744.

5. Hiroki T., M. Kokumai, T. Konoshima, M. Kozuka, M. Haruna, K. Ito, J. R. Estes, L. Li, H. K. Wang, and K. H. Lee. 1993. Structural Elucidation and Chemical Conversion of Amorphispironone, a Novel Spironone from *Amorpha fruticosa*, to Rotenoids. Chem. Pharm. Bull. 41(1): 187～190.
6. Leping L. and H. K. Wang. 1993. Rotenoids and Isoflavones as Cytotoxic Constituents from *Amorpha fruticosa*. Jounal of Natural Products. 56(5): 690～698.
7. Masayoshi O., T. Tanaka, and M. Linuma. 1998. A prenylated flavanone from roots of *Amorpha fruticosa*. Phytochemisry. 48(5): 907～909.
8. Qiuyun Q., B. Zhicheng, S. Deyu, W. Shengrui, and G. Zhonghua. 1998. Reserch on the insecticide components in the Leaves of *Amorpha fruticosa* L. Acta Bot. Boreal. Occident. sin. 18(2): 311～313.
9. Rozsa Z., J. Hohmann, K. Szendrei, I. Mester, and J. Reisch. 1984. Amoradin, amoradicin and amoradinin, Three prenylflavanones from *Amorpha fruticosa*. Phytochemisry. 23(8): 1818～1819.
10. Rozsa Z., J. Hohmann, J. Reisch, I. Mester, and K. Szendrei. 1982. Amorinin, A prenylated chromenoflavanone from *Amorpha fruticosa*. Phytochemisry. 21(7): 1827～1828.
11. Takao K., H. Terada, M. Kokumai, and M. Kozuka. 1993. Studies on inhibitors of tumor promotion, XII. Rotenoids from *Amorpha fruticosa*. Jounal of Natural Products. 56(6): 843～848.