Mokchae Konghak 31(3) : 30~34, 2003

Flavonol Glycosides from the Wood of *Platycarya strobilacea*[*1]

Hak-Ju Lee[*2,†], Youngki Park[*2], Yeong-Han Kwon[*3], Sung-Suk Lee[*2], and Don-Ha Choi[*2]

ABSTRACT

This study was carried out to investigate the constituents of *Platycarya strobilacea* (Juglandaceae) wood. To isolate compounds, wood was extracted with ethanol (EtOH) and then partitioned with petroleum ether, diethyl ether (Et_2O) and ethyl acetate (EtOAc) successively. After partitioned, diethyl ether fraction was subjected to column chromatography with various solvent system in silica gel and/or Sephadex LH-20. Structures were elucidated by spectroscopic methods including MS, ^1H, ^{13}C and 2D-NMR experiments.

Three compounds were isolated from the wood and identified as kaempferol 3-O-α-L-rhamnopyranoside (afzelin, Ⅰ), quercetin 3-O-α-L-rhamnopyranoside (quercitrin, Ⅱ), myricetin 3-O-α-L-rhamnopyranoside (myricitrin, Ⅲ).

Keywords: *Platycarya strobilacea,* wood, afzelin, quercitrin, myricitrin

1. INTRODUCTION

Platycarya strobilacea (Juglandaceae) is a small tree with pinnate leaves composed of seven to nineteen sessile, lanceolated toothed leaflets (Kim, 1995). It is distributed throughout China, Korea, Japan and Taiwan. This tree has been used in the traditional medicine. The roots of this plant are used for an antiphlogistic agent and diarrhea medicine. The constituents of the bark and leaves have been studies, but less is known of those in the wood (Kim *et al.*, 1998; Kim *et al.*, 1996). From the leaves of *P. strobilacea,* ursolic acid, gallic acid and eriodictyol were isolated. Tanaka *et al.* (1998) studied the distribution of ellagic acids and their derivatives in wood of *P. strobilacea.* This study was carried out to isolate and to identify the extractives from the wood of *P. strobilacea.*

2. MATERIALS and METHODS

2.1. Plant Materials

The wood of *P. strobilacea* (A 30 years old, DBH : 28 cm) was collected from Kumsan, Kyungnam, Korea during 1996 and dried at room temperature. After drying, these samples

*1 Received on December 21, 2002; accepted on January 20, 2003
*2 Div. Wood Chemistry & Microbiology, Korea Forest Research Institute, Seoul 130-712, Korea
*3 National Arboretum, Soheul-eup Pocheon-gun Gyeongi-do 487-821, Korea
† Corresponding author : Hak-Ju Lee (leehj99@foa.go.kr)

were ground by Wiley mill.

2.2. Extraction and Fractionation

Dried and ground wood of *P. strobilacea* was extracted twice with ethanol (EtOH) and then concentrated to give the crude extracts. The crude extracts were successively partitioned with organic solvents, such as petroleum ether, diethyl ether (Et_2O) and ethyl acetate (EtOAc). For the isolation of compounds, silica gel and Sephadex LH-20 were used for column chromatography with various solvents. To verify the purity of compounds, silica gel 60 F_{254} (Merck) TLC plate was used with solvent A (toluene-ethyl formate-formic acid = 5:4:1, v/v/v) and solvent B (acetone-EtOAc-H_2O = 10:10:1, v/v/v). After developing, UV (254 nm) detector was used for monitoring.

From Et_2O soluble fraction (40.01 g), isolation was performed. This part of extracts was chromatographed on silica gel column (3.5×42 cm) eluted with $CHCl_3$-MeOH (9:1~2:1, v/v) to give 6 sets of fractions (PS-1~PS-6).

2.3. Instrumental Analysis

For the determination of molecular weights of the isolated compounds, EI-MS was performed at 70 eV ionization energy by direct inlet probe method, using JEOL JMS-600W mass spectrometer. NMR spectra were obtained using a Varian UI 500 NMR spectrometer at the operating frequency of 500 MHz (^1H) and 125 MHz (^{13}C) at the Korea Basic Science Institute in Seoul. About 10 mg of each sample was dissolved in 0.75 mL of methanol-d_4 (CD_3OD) with TMS (tetramethylsilane) as an internal standard.

2.4. Isolation of Compounds

Fraction PS-3 (5.07 g) was further subjected to repeated column (6.4×29 cm) chromatography on Sephadex LH-20 eluted with methanol to give 5 sets of fractions (PS-3-1~PS-3-5). The third fraction (PS-3-3, 276 mg) of PS-3 was rechromatographed on silica gel column (3.5×46 cm) chromatography with EtOAc-MeOH-H_2O (30:0.5:5, v/v/v) to give 5 sets of fractions (PS-3-3-1~PS-3-3-5). Compound I (18 mg) was obtained from the second fraction (GW-3-3-2) of GW-3-3 by further purification on preparative TLC (prep. TLC, Kiselgel 60, F_{254}, 2.0 mm layer thickness) of the upper part in EtOAc-MeOH-H_2O (4:1:5, v/v/v, upper layer) and from the lower part of this prep. TLC purification, Compound II (18 mg) was isolated. Compound III (9 mg) was obtained from PS-3-3-3 by further purification on prep. TLC in EtOAc-MeOH-H_2O (4:1:5, v/v/v, upper layer) solvent system.

2.5. Spectral Data of Compounds

Compound I. Yellow crystal. EI-MS *m/z* : 286 (M^+-146), 258, 229, 213, 184, 143, 121, 73. ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2. HMBC correlations : H-8→C-6/C-7/C-9/C-10, H-6→C-5/C-7/C-8/C-10, H-2'/H-6'→C-2/C-2'/C-6'/C-4', H-3'/ H-5'→C-1'/C-3'/C-4'/C-5', H-1"→C-3/C-5", H-2"→C-3"/C-4", H-3"→C-4", H-4"→C-3"/C-5", H-5"→C-3"/C-4", H-6"→C-4"/C-5". NOESY correlations : H-2'/6'↔H-5".

Compound II. Yellow crystal. EI-MS *m/z* : 302 (M^+-146, base ion), 285, 274, 245, 228, 199, 153, 137, 128. 113. ^1H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2. HMBC correlations : H-8→C-6/C-7/C-9/C-10, H-6→C-5/C-7/C-8/C-10, H-2'→C-1'/C-2/C-3'/C-4', H-5'→C-1'/C-3'/C-6', H-6'→C-2/C-5', H-1"→C-3/C-5", H-2"→C-3"/C-4", H-3"→C-4", H-4"→C-3"/C-5", H-5"→C-3"/C-4", H-6"→C-4"/C-5".

Compound III. Yellow crystal. EI-MS *m/z* :

Table 1. ^{1}H NMR spectral data of compounds I, II and III (500 MHz, CD_3OD).

H	I	II	III
Aglycone			
6	6.17 (*d*, 1.8)[a]	6.12 (*s*)	6.16 (*d*, 2.1)
8	6.34 (*d*, 1.8)	6.29 (*s*)	6.32 (*d*, 2.1)
2'	7.75 (*d*, 9.1)	7.28 (*s*)	6.90 (*s*)
3'	6.92 (*d*, 9.1)	-	-
5'	6.92 (*d*, 9.1)	6.85 (*d*, 7.9)	-
6'	7.75 (*d*, 9.1)	7.25 (*d*, 7.9)	6.90 (*s*)
rhamnose			
1"	5.36 (*d*, 1.8)	5.29 (*s*)	5.27 (*d*, 1.8)
2"	4.21 (*t*, 1.8)	4.16 (*s*)	4.18 (*dd*, 1.8, 3.4)
3"	3.70 (*dd*, 3.7, 9.2)	3.70 (*d*, 6.7)	3.74 (*dd*, 3.4, 9.5)
4"	3.30 (*dd*, 1.2, 4.8)	3.34 (*d*, 9.7)	3.26 (*dd*, 1.2, 3.1)
5"	3.32 (*m*)	3.36 (*m*)	3.47 (*m*)
6"	0.90 (*d*, 5.4)	0.88 (*d*, 6.1)	0.92 (*d*, 6.1)

[a] Values in parentheses are coupling constants, *J* values in Hz.

318 (M^+-146, base ion), 289, 261, 244, 219, 187, 153, 136, 108. 69. ^{1}H NMR (CD_3OD): Table 1; ^{13}C NMR (CD_3OD): Table 2. HMBC correlations : H-8→C-6/C-7/C-9/C-10, H-6→C-5/C-7/C-8/C-10, H-2'/6'→C-1'/C-2/C-2'/C-3'/C-4'/C-5'/C-6', H-1"→C-3/C-5", H-2"→C-3"/C-4", H-3"→C-2"/C-4", H-4"→C-3"/C-5", H-5"→C-3"/C-4", H-6"→C-4"/C-5". NOESY correlations : H-2'/6'↔H-5".

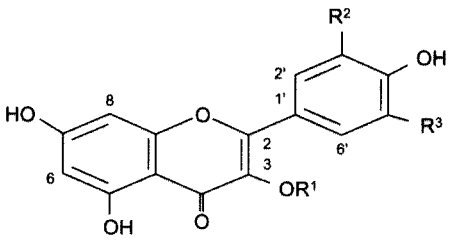
3. RESULTS and DISCUSSION

Flavonol glycosides were isolated from the diethyl ether soluble fraction of ethanol extracts of *P. strobilacea* by silica gel and Sephadex LH-20 column chromatography. Compound I was isolated as yellow crystal. The EI-Mass afforded an [M^+-146] at *m/z* 286. The ^{1}H NMR spectrum exhibited one set of *meta*-coupled aromatic protons (δ 6.17, H-6 and δ 6.34, H-8) and two sets of *ortho*-coupled aromatic

Table 2. ^{13}C NMR spectral data of compounds I, II and III (125 MHz, CD_3OD)

C	I	II	III
Aglycone			
2	159.14(*s*)	149.86(*s*)	159.43(*s*)
3	136.14(*s*)	136.14(*s*)	136.30(*s*)
4	179.50(*s*)	179.52(*s*)	179.67(*s*)
5	163.17(*s*)	163.13(*s*)	163.20(*s*)
6	100.27(*d*)	100.21(*d*)	99.78(*d*)
7	167.21(*s*)	167.07(*s*)	165.83(*s*)
8	95.06(*d*)	94.99(*d*)	94.66(*d*)
9	158.68(*s*)	158.61(*s*)	158.49(*s*)
10	105.57(*s*)	105.55(*s*)	105.87(*s*)
1'	122.66(*s*)	122.98(*s*)	121.91(*s*)
2'	131.88(*d*)	116.91(*d*)	109.57(*d*)
3'	116.54(*d*)	146.45(*s*)	146.83(*s*)
4'	161.62(*s*)	159.17(*s*)	137.88(*s*)
5'	116.54(*d*)	116.36(*d*)	146.83(*s*)
6'	131.88(*d*)	122.83(*d*)	109.57(*d*)
rhamnose			
1"	103.51(*d*)	103.51(*d*)	103.61(*d*)
2"	71.93(*d*)	71.90(*d*)	71.86(*d*)
3"	72.13(*d*)	72.11(*d*)	72.11(*d*)
4"	73.21(*d*)	73.26(*d*)	73.33(*d*)
5"	72.03(*d*)	72.01(*d*)	72.03(*d*)
6"	17.66(*q*)	17.64(*q*)	17.66(*q*)

protons (δ 7.75, H-2', H-6' and δ 6.92, H-3', H-5'). These signals were assigned to H-6, H-8, H-2', H-6' and H-3', H-5' of a 4', 5, 7-trihydroxyflavone skeleton, respectively. The corresponding carbons were identified by HMQC (^{1}H Detected Multiple Quantum Coherence) as, six methine carbon atoms at δ 100.27 (C-6), δ 95.06 (C-8), δ 131.88 (C-2', C-6') and δ 116.54 (C-3', C-5'), respectively. ^{13}C NMR spectrum suggested that the sugar moiety was attached to 3-hydroxyl group in compound I. From the *J* coupling constant (1.8 Hz) of the anomeric proton signals, the sugar which was linked to compond I was determined to be α-L-rhamnopyranose (Harborne, 1994). The peaks at δ 0.90 in ^{1}H NMR spectrum suggested the presence of rhamnose (Markham, K. R. 1982). These carbon chemical shifts were assigned by



afzelin (I) : R^1 = Rhamnose, R^2 = R^3 = H
quercitrin (II) : R^1 = Rhamnose, R^2= OH, R^3 = H
myricitrin (III) : R^1 = Rhamnose, R^2 = R^3 = OH

Fig. 1. Compounds isolated from the wood of *P. strobilacea*.

comparison with literature values (Dulce *et al.*, 1997; Agrawal, 1989). Based on all these data, compound I was characterized as kaempferol 3-*O*-α-L-rhamnopyranoside, afzelin (Fig. 1).

Compound II was also obtained as yellow crystal. The EI-MS presented a signal at *m/z* 302, corresponding to molecular formula $C_{15}H_{10}O_7$ without sugar moiety. The ^{1}H NMR spectrum exhibited one set of singlet aromatic protons at δ 6.12 (1H) and 6.29 (1H) that were assigned to H-6 and H-8, respectively. The ^{1}H NMR spectrum of compound II showed one singlet and two *meta*-coupled doublets and assigned to H-2' (δ 7.28), H-6' (δ 7.25) and H-5' (δ 6.85) of ring B. These spectra meant that compound II was quercetin glycoside. In the HMBC (^{1}H Detected Multiple Bond Connectivity) spectrum, the anomeric proton signal at δ 5.29 (rhamnopyranosyl moiety, H-1") showed long-range correlations with the ^{13}C signals at δ 136.14 (C-3 of aglycone). The position of rhamnose was suggested to be at C-3 of ring C. In ^{13}C NMR spectrum, C-8 at δ 94.99, C-6 at δ 100.21, C-10 at δ 105.55, C-2' at δ 116.91, C-5' at δ 116.36, C-6' at δ 122.83, C-1' at δ 122.98 and C-7 at δ 167.07 were assigned shown in Table 2. The ^{13}C NMR signal at δ 179.52 was assigned to carboxyl carbon (C=O, C-4). These carbon chemical shifts were assigned by comparison with literature values (Ning *et al.*, 1997). Consequently, the structure of compound II was concluded to be quercetin 3-*O*-α-L-rhamnopyranoside, quercitrin (Fig. 1).

Compound III was isolated as yellow crystal. The EI-Mass afforded an [M^+-146] at *m/z* 318. The ^{1}H NMR spectrum of compound III showed signals for four aromatic protons, two *meta*-coupled proton (δ 6.16, J = 2.1 Hz, H-6 and δ 6.32, J = 2.1 Hz, H-8) and one sets of singlet protons (δ 6.90, H-2', H-6'). In ^{1}H NMR spectrum of compound III, signals at δ 6.32 exhibited the secondary methyl for rhamnosyl methyl and anomeric proton doublet at δ 5.27 (1H, *d*, J = 1.8 Hz) showed the presence of rhamnose of a α-configuration (Lee, M. W., 1994). These results meant that compound III was myricetin glycoside. These carbon chemical shifts were assigned by comparison with literature values (Harborne and Mabry, 1982). Based on all these data, the structure of compound III assigned as myricetin 3-O-α-L-rhamnopyranoside, named myricitrin (Fig. 1).

4. CONCLUSIONS

From diethyl ether fraction of the wood of *P. strobilacea*, three flavonol glycosides were isolated by column chromatography using Sephadex LH-20 and/or silica gel and identified using EI-MS and ^{1}H and ^{13}C NMR spectroscopy. The identified three compounds were as follows; afzelin (I), quercitrin (II) and myricitrin (III).

REFERENCES

1. Agrawal, P. K. 1989. Carbone-13 NMR of Flavonoids. Elsevier, New York. pp. 334~335.
2. David, N. S. Hon, and N. Shiraishi. 1990. Wood

and Cellulosic Chemistry. Marcel Dekker INC. New York. pp. 217~219.
3. Dulce, H. S., Y. Massayoshi, and J. K. Massuo. 1997. Flavonoids from *Iryanthera sagotiana*. *Phytochemistry*. 46: 579~582.
4. Harborne, J. B. 1994. The flavonoids: Advances in research since 1986. Champman & Hall. London. pp. 442~473.
5. Harborne, J. B. and T. J. Mabry. 1982. The flavonoids: Advances in research. Champman & Hall. London. pp. 52~134.
6. Kim, T. W. 1995. The Woody Plants of Korea in Color. Kyo-Hak Publishing Co. Seoul, Korea. pp. 60.
7. Kim, C. M., Y. S. Kwon, and J. H. Kim. 1998. Flavonoids from the stem bark of *Platycarya strobilacea*. *Kor. J. Pharmacogn.* 29(4): 353~356.
8. Kim, I. Y., S. H. Lee, and T. S. Cho. 1996. Isolation of anticancer agents from the leaves of *Platycarya strobilacea* S. et Z. *Kor. J. Pharmacogn.* 27(3): 238~245.
9. Lee, M. W. 1994. Flavonoids from the leaves of *Betula platyphylla* var. latifolia. *Kor. J. Pharmacogn.* 25(3): 199~203.
10. Markham, K. R. 1982. Techniques of Flavonoid Identification. Academic Press, London New York. pp. 75~78.
11. Ning, Z., O. Hideaki, I. Yoshinori, H. Eiji, T. Anki, and T. Yoshio. 1997. Three flavonol glycoside from leaves of *Myrsine seguinii*. *Phytochemistry*. 46: 943~946.
12. Tanaka, T., Z. H. Jiang, and I. Kouno. 1998. Distribution of ellagic acid derivatives and a diarylheptanoid in wood of *Platycarya strobilacea*. *Phytochemistry*. 47(5): 851~854.