Mokchae Konghak 37(5) : 480~485, 2009

Ellagitannins from Bark of *Juglans mandshurica*[*1]

Dong-Joo Kwon[*2] and Young-Soo Bae[*2†]

ABSTRACT

Juglans mandshurica bark was collected and extracted with 70% aqueous acetone. The extracts were concentrated and then sequentially fractionated using *n*-hexane, CH_2Cl_2, EtOAc, and H_2O to be freeze dried. A portion of H_2O fraction was chromatographed on a Sephadex LH-20 column using aqueous methanol to isolate (*S*)-2,3-HHDP-D-glucopyranoside **(1)**. Some of EtOAc fraction was also treated on a Sephadex LH-20 column using aqueous methanol and EtOH-hexane mixture to purify pedunculagin **(2)**. The structures of these two epimeric ellagitannins were elucidated by spectroscopic tools, NMR and MS.

Keywords : *Juglans mandshurica*, bark, EtOAc fraction, H_2O fraction, epimeric ellagitannins

1. INTRODUCTION

Juglans mandshurica Maxim. (Juglandaceae), a fast growing deciduous tree, is widely distributed in China, Russia, and Korea. The tree has been used as a folk medicine for treatment of esophageal, gastric, cardiac and lung cancer (Kim, 1994). Other researchers reported that the volatile compounds of the species inhibit the growth of the neighbor plants and can be developed for chemurgy. Several naphthoquinones, naphthalenyl glycosides, α-tertalonyl glucopyranosides, flavonoids and diarylheptanoyl glucopyranosides have been isolated from this plant (Li *et al*., 2003; Min *et al*., 2003). In the previous study, we have already reported the isolation of several flavonoids and their glycosides, including two ellagitannins, from the bark of *J. mandshurica* (Kim *et al*., 2006; Si and Bae, 2007). In the present study, we report the isolation and structure elucidation of 2 ellagitannins from H_2O and EtOAc fractions of *J. mandshurica*. The compounds were separated by column chromatography using Sephadex LH-20 and their structures were identified by spectroscopic tools using ^{1}H NMR and ^{13}C NMR, including HMBC, and MALDI-TOF-MS.

2. MATERIALS and METHODS

2.1. Plant Material

A tree of *Juglans mandshurica* was collected in the experimental forest of Kangwon National University, Bong-Myung Ri, Gangwon Province, Korea in April 2007. The bark was strip-

*1 Received on July 24, 2009; accepted on September 1, 2009
*2 Dept. of Wood Science & Engineering, College of Forest and Environmental Sciences, Kangwon National University, Chuncheon 200-701, Korea.
† Corresponding author : Young-Soo Bae (e-mail: bae@kangwon.ac.kr)

ped and then air-dried at room temperature for 2 weeks before grinding.

2.2. General Experimental

^{1}H (600 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker Avance DPX 600 MHz spectrometers using tetramethylsilane (TMS) as an internal standard and chemical shifts are given in δ (ppm). MALDI-TOF-MS was performed with a Voyager-DE STR spectrometer. Column chromatography was done on a lipophilic Sephadex LH-20 (25～100 μm, Sigma) column. Eluents were collected using a fraction collector (Gilson FC 204). Thin layer chromatography (TLC) was performed on DC-Plastikfolien Cellulose F (Merck) plates and developed with TBAW (*t*-BuOH-HOAc-H_2O (3 : 1 : 1, v/v/v)) and 6% aqueous HOAc. Visualization was done by UV light (254 and 365 nm) or by spray with 1% ethanolic $FeCl_3$ solution followed by heating.

2.3. Extraction and Isolation

The air-dried, ground bark of *J. mandshurica* (3.2 kg) was immersed in 70% aqueous acetone at room temperature for 3 days. After filtration (Advantec No. 2), the bark sample was extracted two more times. The filtrates were combined and evaporated on a rotary evaporator under the reduced pressure at 40°C. The aqueous residue (500 g) was successively fractionated on a separatory funnel and freeze dried to give *n*-hexane (15.6 g), CH_2Cl_2 (20.6 g), EtOAc (130.0 g), and H_2O (237.6 g) soluble fractions.

A portion of EtOAc fraction (33 g) was chromatographed on a Sephadex LH-20 column using MeOH-H_2O (4:1, 4 ℓ) as an eluting solvent to give 5 fractions: EF-1 (1.06 g), EF-2 (17.1 g), EF-3 (7.8 g), EF-4 (3.2 g), and EF-5 (2.8 g). EF-3 (7.8 g) was rewashed with MeOH-H_2O (3:1, 3 ℓ) on a Sephadex LH-20 column to afford 3 fractions: EF-3-1 (20 mg), EF-3-2 (160 mg), and EF-3-3 (7.6 g). EF-3-3 (7.6 g) was treated again on a Sephadex LH-20 column with MeOH-H_2O (1:2, 2 ℓ) to get 4 subfractions: EF-3-3-1 (150 mg), EF-3-3-2 (860 mg), EF-3-3-3 (2.28 g), and EF-3-3-4 (3.7 g). EF-3-3-2 (860 mg) was rechromatographed on a Sephadex LH-20 column with MeOH-H_2O (1:2 and 1:5, 1.5 ℓ) and EtOH-Hexane (3:2, 1 ℓ) to isolate compound **2** (80 mg).

The H_2O fraction (32 g) was treated on a Sephadex LH-20 column with MeOH-H_2O (1:1, 4 ℓ) to afford 4 fractions: WF-1 (24.2 g), WF-2 (1.8 g), WF-3 (2.4 g), and WF-4 (3.0 g). WF-1 (15.0 g) was reapplied on a Sephadex LH-20 column with MeOH-H_2O (1:3, 2 ℓ) to get 3 subfractions: WF-1-1 (10.9 g), WF-1-2 (630 mg), and WF-1-3 (520 mg). WF-1-2 (600 mg) was chromatographed again with MeOH-H_2O (3:1 and 1:5, 1 ℓ) to give compound **1** (320 mg).

2.3.1. Compound 1

Yellowish amorphous powder. R_f : 0.22 (TBAW) and 0.53 (6% HOAc). MALDI-TOF-MS : Calculated for $C_{20}H_{18}O_{14}$ 482, Found *m/z* 505 $[M + Na]^+$, 521 $[M + K]^+$. ^{1}H and ^{13}C-NMR see Table 1.

2.3.2. Compound 2

Yellowish amorphous powder. R_f : 0.33 (TBAW) and 0.71 (6% HOAc). MALDI-TOF-MS; Calculated for $C_{34}H_{24}O_{22}$ 784, Found *m/z* 807 $[M + Na]^+$, 823 $[M + K]^+$. ^{1}H and ^{13}C-NMR see Table 2.

3. RESULTS and DISCUSSION

(*S*)-2,3-HHDP-D-glucopyranoside (**1**) and pedunculagin (**2**) (Fig. 1) were isolated from H_2O and EtOAc fractions of *J. mandshurica* bark, respectively, by Sephadex LH-20 column chro-

Table 1. ^{1}H (600 MHz) and ^{13}C-NMR (125 MHz) values of compound **1** (CD_3OD, *J* in Hz)

	^{1}H		^{13}C	
	α-Anomer	β-Anomer	α-Anomer	β-Anomer
Glucose				
1	5.32 *d* (*J* = 3.4)	4.90 *d* (*J* = 8.1)	91.86	95.19
2	4.96 *dd* (*J* = 3.4, 9.5)	4.73 *t* (*J* = 8.6)	76.11	78.41
3	5.36 *t* (*J* = 9.4)	5.04 *t* (*J* = 9.5)	78.86	81.17
4	3.71～3.79 *m*	3.71～3.79 *m*	68.71	68.49
5	3.93 *m*	3.46 *m*	73.24	78.37
6	3.73～3.92 *m*	3.73～3.92 *m*	62.14	62.28
Galloyl-A,B				
1′, 1″			126.84, 126.92, 127.11, 127.19	
2′, 2″			115.23, 115.35	
3′, 3″			144.56, 144.61	
4′, 4″			137.22, 137.31	
5′, 5″			145.77, 145.82	
6′, 6″	6.59 *s*, 6.67 *s*	6.60 *s*, 6.67 *s*	107.73, 107.96, 108.00, 108.06	
7′, 7″			170.68, 170.51, 171.28, 171.25	

Table 2. ^{1}H (600 MHz) and ^{13}C-NMR (125 MHz) values of compound **2** (CD_3OD, *J* in Hz)

	^{1}H		^{13}C	
	α-Anomer	β-Anomer	α-Anomer	β-Anomer
Glucose				
1	5.36 *d* (*J* = 3.8)	4.95 *d* (*J* = 8.1)	92.29	95.83
2	4.86[a]	4.86[a]	76.31	78.84
3	5.47 *t* (*J* = 9.7)	5.26 *t* (*J* = 9.5)	76.50	78.25
4	5.09 *m*	5.09 *m*	70.48	70.05
5	4.57 *m*	4.14 *m*	67.83	73.33
6	3.81 *dd* (*J* = 1.7, 12.8) 5.31 *m*	3.81 *d* (*J* = 12.6) 5.31 *m*	64.16	64.23
Galloyl-A,B,C,D				
1′, 1″, 1‴, 1⁗			125.94, 126.02, 126.19, 126.24 126.43, 126.51, 126.64, 126.72	
2′, 2″, 2‴, 2⁗			114.93, 115.02, 115.45, 115.59 115.66, 116.62	
3′, 3″, 3‴, 3⁗			144.66, 144.74, 144.82, 144.89 145.04	
4′, 4″, 4‴, 4⁗			137.16, 137.25, 137.56	
5′, 5″, 5‴, 5⁗			145.90, 145.85	
6′, 6″, 6‴, 6⁗	6.58 *s*, 6.35 *s*, 6.52 *s*, 6.61 *s*	6.60 *s*, 6.36 *s* 6.49 *s*, 6.61 *s*	107.70, 107.78, 107.87, 108.25 108.68	
7′, 7″, 7‴, 7⁗			169.10, 169.16, 169.63, 169.76 170.27, 170.42, 170.85, 170.89	

[a]Overlapped with solvent signal.

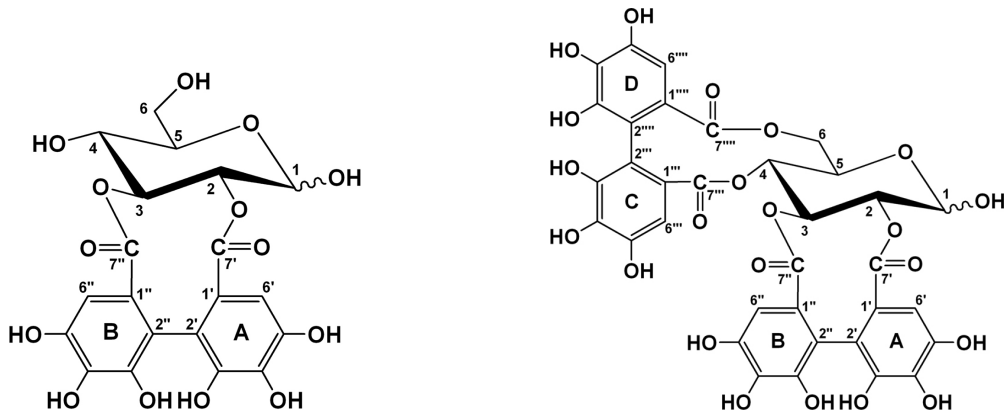

Fig. 1. Chemical structures of compounds **1** and **2**.

matography using aqueous methanol and EtOH-hexane mixture as eluting solvents. The structures were elucidated by NMR, including 2D-NMR (HMQC, HMBC), and MALDI-TOF MS.

Compound **1** was obtained as yellowish amorphous powder. The molecular formula was deduced to be $C_{20}H_{18}O_{14}$ on the basis of the peak at *m/z* 505 $[M + Na]^+$ in the positive MALDI-TOF MS. In the ^{1}H-NMR spectrum, the two galloyl protons gave the signals at δ 6.59, 6.67 (Hα-6′,6″) and δ 6.60, 6.67 (Hβ-6′,6″). Two symmetric protons of galloyl group should show one signal at about δ 7.10 (Si and Bae, 2007). However, the signals of H-6′ and H-6″ in the spectrum were upfield shifted about 0.5～0.6 ppm due to the mutual steric effect from the adjacent substituent group, the galloyl moiety that is linked between C-2′ and C-2″. The anomeric protons showed two signals at δ 5.32 (J = 3.4 Hz) and 4.90 (J = 8.1 Hz) suggesting α-D- and β-D-glucose, respectively (Hobley *et al.*, 1996). H-2 and H-3 signals were observed at δ 4.96, 4.73 and δ 5.36, 5.04, respectively, that were downfield shifted about 1.5 ppm due to the bonding of the galloyl group to C-2 and C-3. H-4, H-5 and H-6 gave multiple signals at between at δ 3.71 and 3.93. According to the above ^{1}H-NMR data, compound **1** suggest an epimer of a digalloyl glucose. In the ^{13}C-NMR spectrum, two galloyl groups showed very complicate overlapped signals. Two carbonyl carbons appeared at δ 170.68, 170.51 (Cα-7′,7″) and δ 171.28, 171.25 (Cβ-7′,7″), methine carbons at δ 107.73, δ 107.96 (Cα-6′,6″) and δ 108.00, 108.06 (Cβ- 6′,6″), respectively. C-2′ and C-2″ signals appeared at δ 115.23, 115.35 for both α- and β- anomer. These chemical shift values were downfield shifted about 7～8 ppm compare to those of C-6′ and C-6″ and suggest that C-2′ and C-2″ are linked together (Moharram *et al*., 2003). The other six epimeric carbons (C-3′,4′, 5′,3″,4″,5″) showed overlapped six signals at δ 137.22～145.82. In the glucose moiety, anomeric carbon signals were observed at δ 91.86 (Cα-1) and δ 95.19 (Cβ-1), respectively. C-2 and C-3 signals appeared about 4 ppm downfield signals compare to those of the original glucose at δ 76.11, 78.41 and δ 78.86, 81.17. These facts mean that the galloyl groups are attached to C-2 and C-3 of the glucose. In the HMBC spectrum, correlations were observed between H-2 and C-7′, H-3 and C-7″, H-6′ and C-2′, and H-6″ and C-2″, which confirm the proper structure

linkages of compound **1** as shown in Fig. 1. According to the combination of spectroscopic and literature data (Yoshida *et al*., 1984; Moharram *et al*., 2003), compound **1** was elucidated as (*S*)-2,3-HHDP (hexahydroxydiphenoyl)-D-glucopyranoside.

Compound **2** was obtained as yellowish amorphous powder. The molecular formula was deduced to be $C_{34}H_{24}O_{22}$ on the basis of the peak at *m/z* 807 $[M+Na]^+$ in the positive MALDI-TOF MS. The ^{1}H- and ^{13}C-NMR spectrum is similar to those for compound **1**, except for the difference of the hydroxy group located on the hexahydroxydiphenoyl (HHDP) moiety at C-4 and C-6 position. In the ^{1}H-NMR spectrum, the galloyl protons signal were observed at δ 6.35～6.61 (H α/β-6′,6″,6‴,6⁗), and the anomeric protons at δ 5.36 (J = 3.8 Hz) and 4.95 (J = 8.1 Hz). H α/β-2,3,4,6 showed downfield shifted multiple signals at δ 4.86～5.47 increasing about 1.5 ppm compare to those of normal glucose unit. In the ^{13}C-NMR spectrum, the carbonyl carbon of galloyl groups apparently showed 8 signals at δ 169.10～170.89 indicating the presence of four galloyl of α- and β-glucose units. The other galloyl carbons indicated the chemical shift values that are similar to those of compound **1**. Anomeric carbon signals were observed at δ 92.29 (C α-1) and δ 95.83 (C β-1), C α/β-4,6 signals gave about 2 ppm downfield shifted signals at δ 64.16～70.48 indicating that these two carbons contain galloyl units. In the HMBC spectrum, correlations were observed between H-2 and C-7′, H-3 and C-7″, H-4 and C-7‴, H-6 and C-7⁗, H-6′ and C-2′, H-6″ and C-2″, H-6‴ and C-2‴, and H-6⁗ and C-2⁗ which confirmed the proper structure linkage as shown in Fig. 1. According to the combination of spectroscopic and literature data (Lee and Yang, 1994; Chang *et al*., 1995; Naoki *et al*., 2008), compound **2** was elucidated as pedunculagin.

4. CONCLUSIONS

Two epimeric ellagitannins, (*S*)-2,3-HHDP-D-glucopyranoside (**1**) and pedunculagin (**2**), were isolated from H_2O and EtOAc fractions, respectively, of *Juglans mandshurica* bark by Sephadex LH-20 column chromatography using aqueous methanol and EtOH-hexane mixture as eluting solvents. Their structures were characterized by NMR, including two dimensional HMBC, and MALDI-TOF MS. To our best knowledge, these epimeric ellagitannins are first isolated from the bark of *Juglans mandshurica*.

ACKNOWLEDGEMENT

This study was partially supported by the Basic Research Program for Forest Science of Korea Forest Service. We are also thank to Dr. Ji-Sook Ryu, Central Laboratory of Kangwon National University, for measuring the NMR spectra.

REFERENCES

1. Chang, J. H., J. H. Cho, H. H. Kim, K. P. Lee, M. W. Lee, S. S. Han, and D. I. Lee. 1995. Antitumor activity of pedunculagin, one of the ellagitannin. Archives of Pharmacal Research 18(6): 396～401.
2. Hobley, P., O. Howarth, and R. N. Ibbett. 1996. ^{1}H and ^{13}C NMR shifts for aldopyranose and aldofuranose monosaccharides: Conformational analysis and solvent dependence. Magnetic Resonance in Chemistry 34(10): 755～760.
3. Kim, J. K., C. L. Si, and Y. S. Bae. 2006. Phenolic compounds from bark of *Juglans mandshurica*. Journal of Korean Wood Science and Technology 34(6): 51～60.
4. Kim, T. W. 1994. The Woody Plants of Korea in Color. Koyhak Press, Seoul, pp. 58～59.
5. Lee, I. R. and M. Y. Yang. 1994. Phenolic compounds from *Duchesnea chrysantha* and their cy-

totoxic activities in human cancer cell. Archives of Pharmacal Research 17(6): 476～479.
6. Li, G., M. L. Xu, H. G. Choi, S. H. Lee, Y. D. Jahng, C. S. Lee, D. C. Moon, M. H. Woo, and J. K. Son. 2003. Four new diarylpeptanoids from the roots of *Juglans mandshurica*. Chemical & Pharmaceutical Bulletin 51(3): 262～264.
7. Min, B. S., S. Y. Lee, J. H. Kim, J. K. Lee, J. J. Kim, D. H. Kim, Y. H. Kim, H. Joung, H. K. Lee, N. Nakamura, H. Miyashiro, and M. Hattori. 2003. Anti-complement activity of constituents from the stem bark of *Juglans mandshruica*. Biological & Pharmaceutical Bulletin 26(7): 1042～1044.
8. Moharram, F. A., M. S. Marzouk, S. A. El-Toumy, A. A. Ahmed, and E. A. Aboutabl. 2003. Polyphenols of *Melaleuca quinquenervia* leaves-Pharmacological studies of grandinin. Phytotherapy Research 17(7): 767～773.
9. Naoki, K., H. Ito, T. Hatano, and T. Yoshida. 2008. Phloroglucinol diglycosides accompanying hydrolyzable tannins from *Kunzea ambigua*. Phytochemistry 59(18): 3080～3086.
10. Si, C. L. and Y. S. Bae. 2007. Antioxidant effect of *Juglans mandshurica* bark gallotannins. Journal of Forest Science 23(1): 1～4.
11. Yoshida, T., T. Hatano, T. Okuda, M. U. Memon, T. Shingu, and K. Inoue. 1984. Spectral and chromatographic analyses of tannins. I. ^{13}C nuclear magnetic resonance spectra of hydrolyzable tannins. Chemical & Pharmaceutical Bulletin 32 (5): 1790～1799.