Mokchae Konghak 33(5) : 86~91, 2005

Studies on Biological Activity of Wood Extractives (XVI)*1
- Antioxidant Components from the Bark of *Rhus chinensis* -

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ABSTRACT

Six compounds were isolated from the EtOAc and Et_2O fractions of the bark of *Rhus chinensis* by repeated column chromatography with SiO_2 and Sephadex LH-20. The structures were determined by instrumental analysis using MS and NMR spectrophotometer as: gallic acid (1), methyl gallate (2), 6, 7-dimethoxycoumarin (3), orcinol-β-D-glucoside (4), scopoletin (5), semialactone (6). Among these compounds, 6,7-dimethoxycoumarin (3) was isolated from this plant for the first time. To measure the antioxidant activity, the DPPH radical scavenging activity test was performed. Gallic acid (1) showed the strongest activity, while orcinol-β-D-glucoside (4), semialactone (5) and scopoletin (6) had the low activities.

Keywords : *Rhus chinensis*, bark, gallic acid, methyl gallate, coumarin, orcinol-β-D-glucoside, scopoletin, semialactone

1. INTRODUCTION

Free radicals, which have been recognized to be involved in several diseases including cancer, are chemical species that cause oxidation. Antioxidants act as free radical scavenger. Ageing may also be the result of the deleterious free-radical reactions which occur throughout cells and tissues (Maxwell, 1995). For this reason, great concern is focused on natural products including wood extractives.

In search for antioxidants using DPPH method from several Korean plants, the ethanolic extract from the bark of *Rhus chinensis* was found to exhibit significant antioxidant activity. *R. chinensis* (Anacardiacea) is a small tree, reaching up to 25 feet in height and has yellowish-white flowers. This plant is used in the traditional medicine to treat dysentery and diarrhea. From the leaf of *R. chinensis*, ellagic acid, gallic acid, shikimic acid were isolated and identified (Matsuda, 1966). Scopoletin, scopolin, orcinol, orcinol-β-D-glucoside, and methyl gallate were also found from the bark (Chung *et al.*, 1999).

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Biological activities such as antineoplastic effect and prophylactic activity were evaluated from the extracts of *R. chinensis* (Park *et al.*, 1993).

In present paper, we report the isolation and structure elucidation of compounds from the bark of *R. chinensis* and antioxidant activities of compounds isolated in this study.

2. MATERIALS and METHODS

2.1. General Procedure

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 spectrometer at the operating frequency of 500 MHz (^{1}H) and 125 MHz (^{13}C) at the Korea Basic Science Institute in Seoul.

2.2. Plant Materials

R. chinensis (25 yr.) was collected at Experimental forest of Korea Forest Research Institute in Jinju, Kyungnam Province March 2002 and dried at room temperature. Voucher specimens were deposited at the Korea Forest Research Institute, Seoul, Korea.

2.3. Extraction, Fractionation and Isolation

Air dried bark of *R. chinensis* was powdered and extracted twice with 95% ethanol (EtOH) and then evaporated to give the crude extracts. The crude extracts were successively partitioned with organic solvents, such as *n*-hexane, diethyl ether (Et_2O), and ethyl acetate (EtOAc).

The EtOAc soluble fraction was subjected to column chromatography on Sephadex LH-20 eluted with MeOH-EtOH (1:1, v/v) to yield 3 sets of fraction (REA-1～REA-3).

Fraction REA-2 was re-chromatographed on silica gel column chromatography with hexane-acetone (2:1, v/v) to give 9 subfractions (REA-2-1～REA-2-9). Among these fractions, 6th fraction (REA-2-6) was compound 1 (1.22 g) and 4th fraction (REA-2-4) was compound 2 (1.14 g). Fraction REA-1 was further subjected to repeated column chromatography on silica gel eluted with EtOAc-MeOH (9:1, v/v) to give 9 sets of fractions (REA-1-1～REA-1-9) and REA-1-1 was purified by column chromatography on silica gel and eluted with a solvent system of CH_2Cl_2-MeOH (150:1, v/v) to give compound 3 (REA-1-1-1, 20 mg). Compound 4 (50 mg) was fraction REA-1-3.

The Et_2O soluble fraction was subjected to column chromatography on Sephadex LH-20 eluted with MeOH-EtOH (1:1, v/v) to yield 8 sets of fraction (RE-1～RE-8). Fraction RE-2 was re-chromatographed on silica gel column chromatography with $CHCl_3$-MeOH (100:1, v/v) to give 3 subfractions (RE-2-1～RE-2-3). Fraction RE2-2 was further chromatographed on silica gel column chromatography with hexane-acetone (3:1, v/v) to give 5 subfractions (RE-2-2-1～RE-2-2-5). RE-2-2-2 was compound 5 (900 mg) and RE-2-2-4 was compound 6 (1.0 g).

2.4. Spectral Data of Compounds

Compound 1. EI-MS *m/z* : 170 $[M]^{+}$, 153. ^{1}H-NMR (500 MHz, methanol-d_4) : δ 7.09 (2H, s, H-2 and H-6). ^{13}C-NMR (125 MHz, methanol-d_4) : δ 110.38 (C-2, 6), 121.97 (C-1), 139.57 (C-4), 146.35 (C-3, 5), 170.39 (C=O).

Compound 2. EI-MS *m/z* : 184 $[M]^{+}$, 153 (base ion). ^{1}H-NMR (500 MHz, methanol-d_4) : δ 7.09 (2H, s, H-2 and H-6), 3.76 (3H, s, $COCH_3$). ^{13}C-NMR (125 MHz, methanol-d_4) : δ 52.26 ($COCH_3$), 110.09 (C-2, 6), 121.50 (C-1), 139.72 (C-4), 146.45 (C-3, 5), 169.04 (C=O).

Compound 3. EI-MS *m/z* : 207$[M]^{+}$. ^{1}H-NMR (500 MHz, chloroform-*d*) : δ 3.93 (3H, s, OMe-

7), 3.96 (3H, s, OMe-6), 6.29 (1H, d, J = 9.5 Hz, H-3), 6.84 (1H, s, H-8), 6.87 (1H, s, H-5), 7.63 (1H, *d*, *J* = 9.5 Hz, H-4). ^{13}C-NMR (125 MHz, chloroform-*d*) : δ 56.60 (OMe-7), 56.62 (OMe-6), 100.28 (C-8), 108.34 (C-5), 111.69 (C-10), 113.77 (C-3), 143.50 (C-4), 146.63 (C-6), 150.29 (C-9), 153.14 (C-7), 161.60 (C-2). ^{1}H-^{1}H COSY correlations : H-4↔H-3. HMBC correlations: H-4→C-2/C-5/C-9. H-5→C-4/C-6/C-7/C-9/C-10. H-8→C-6/C-7/C-9/C-10. H-3→C-2/C-10. OMe-6→C-7. OMe-6→C-7.

Compound 4. EI-MS *m/z* : 286 $[M]^{+}$. ^{1}H-NMR (500 MHz, methanol-d_4) : δ 2.17 (3H, s, CH3), 3.13~3.29 (4H, overlapping, *m*, glc. H-2 to H-5), 3.49 (1H, *dd*, *J* = 2.2, 5.2 Hz, glc. H-6b), 3.69 (1H, *dd*, *J* = 1.3, 1.6 Hz, glc. H-6a), 4.74 (1H, *d*, *J* = 7.5 Hz, glc. H-1), 6.26 (3H, *m*, H-2, 4, 6). ^{13}C-NMR (125 MHz, methanol-d_4) : δ 21.63 (CH_3), 62.48 (glc. C-6), 71.32 (glc. C-4), 74.85 (glc. C-2), 77.94 (glc. C-3), 78.00 (glc. C-5), 102.15(glc. C-1), 102.22 (C-4), 109.77 (C-6), 111.21 (C-2), 141.24 (C-1), 159.15 (C-5), 160.02 (C-3).

Compound 5. EI-MS *m/z* : 468 $[M]^{+}$. ^{1}H-NMR and ^{13}C-NMR : see Table 1. ^{1}H-^{1}H COSY correlations : H-6↔H-8, H-5↔H-6′. 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-3′/C-6′, H-5′→C-1′/C-3′/C-4′. H-6′→C-2/C-4.

Compound 6. EI-MS *m/z* : 192 $[M]^{+}$. ^{1}H-NMR (500 MHz, DMSO-d_6) : δ 3.81 (3H, s, OMe-6), 6.21 (1H, *d*, *J* = 9.6 Hz, H-3), 6.77 (1H, s, H-8), 7.21 (1H, s, H-5), 7.90 (1H, *d*, *J* = 9.6 Hz, H-4). ^{13}C-NMR (125 MHz, DMSO-d_6) : δ 55.98 (OMe-6), 102.74 (C-8), 109.60 (C-5), 110.51 (C-10), 111.64 (C-4), 144.37 (C-3), 145.21 (C-6), 149.48 (C-7), 151.10 (C-9), 160.62 (C-2).

2.5. Antioxidant Activity Test

The antioxidant activity of compounds was assessed on the basis of the scavenging activity of the DPPH free radical method. Sample solution dissolved in MeOH (4 mℓ) was added to a solution of DPPH in MeOH (4.5×10^{-4} M, 1 mℓ) and the reaction mixture was shaken. After 30 min, the remaining amounts of DPPH were determined by colorimetry (8452A Diode Array Spectrophotometer, Hewlett Packard Co.) at 520 nm (Blois, 1958). The mixture of 4 mℓ MeOH with a solution of 1 mℓ DPPH was used as control. The mean values were obtained from triplicate experiments. Antioxidant activity of each compound was expressed by the ratio of the lowering of the DPPH solution in the absence of compound (control).

3. RESULTS and DISCUSSION

3.1. Identification of Compounds

Repeated column chromatography with SiO_2 and Sephadex LH-20 of the EtOAc and Et_2O fractions from the bark of *R. chinensis* led to the isolation of six compounds.

The compound 1 was obtained as a white needle and the EI-MS presented a signal at *m/z* 170 $[M]^{+}$. The ^{1}H-NMR spectrum of compound 1 showed one aromatic singlet (δ 7.09) assigned to H-2 and H-6. The ^{13}C-NMR spectrum exhibited typical signals for a galloyl at δ 110.38 (C-2, 6), 121.97 (C-1), 139.57 (C-4), and 146.35 (C-3, 5). The assigned proton and carbon chemical signals were compared with the literature values (Kim *et al.*, 1997a). Consequently the structure of compound 1 was concluded to be the gallic acid (Fig. 1).

The compound 2 was obtained as a white amorphous powder. The EI-MS presented a signal at *m/z* 184 and the ^{1}H-NMR spectrum indicated the presence of a methoxyl group at 3.76 (3H, s, $COOCH_3$). A ^{13}C-NMR signal at 169.04 was assigned to carboxyl carbon (C=O, C-7), C-1 at δ 121.50, C-4 at δ 139.72, and C-2 and C-6 at δ 110.0, respectively. The assigned proton and carbon chemical signals

Table 1. ^{1}H- and ^{13}C-NMR data of semialactone (5) in CD_3OD

Position	$^{1}H^{a)}$	*J* (Hz)	$^{13}C^{a)}$	H coupled with $C^{b)}$
1 (a)	1.70 *m*		30.06 *t*	
1 (b)	1.97 *m*		-	
2 (a)	1.05 *m*		35.55 *t*	
2 (b)	2.18 *m*		-	H-2, H-4, H-5, H-8
3	0.87 *m*		98.13 *s*	H-3, H-20
4	2.04 *m*		35.47 *s*	H-20
5	1.13 *m*		49.33 *d*	H-5
6 (a)	1.49 *m*		19.83 *t*	
6 (b)	1.70 *m*		-	H-5, H-19
7 (a)	1.12 *m*		33.08 *t*	
7 (b)	1.39 *m*		-	H-3, H-9
8	2.45 *m*		39.67 *s*	H-10
9	1.49 *m*		45.36 *d*	H-9
10	2.50 *m*		40.44 *s*	H-9, H-10, H-13
11 (a)	1.70 *m*		23.11 *t*	H-10, Me-18
11 (b)	-		-	H-14
12 (a)	1.25 *m*		25.29 *t*	H-1, H-2, H-3
12 (b)	1.70 *m*		-	
13	2.18 *m*		44.99 *d*	H-10
14	-		49.91 *s*	H-15
15 (a)	1.28 *m*		33.11 *t*	H-15, Me-16
15 (b)	1.49 *m*		-	H-13
16 (a)	1.70 *m*		29.49 *t*	H-3, H-7, H-9
16 (b)	2.18 *m*		-	H-5
17	2.96 *d*	9.0, 19.0	40.03 *d*	
18	0.88 *s*		15.41 *q*	H-5
19 (a)	3.73 *dd*	1.5, 9.0	67.99 *t*	H-9
19 (b)	4.23 *dd*	2.5, 8.5	-	
20	-		149.22 *s*	H-10
21 (a)	5.23 *s*		113.41 *t*	H-13
21 (b)	5.28 *s*		-	
22	4.75 *dd*	3.5, 12.5	80.78 *d*	
23 (a)	2.34 *m*		29.01 *t*	
23 (b)	2.53 *m*		-	
24	6.60 *d*	6.0	139.13 *d*	
25	0.85 *m*		128.37 *s*	
26	-		165.99 *s*	
27	1.93 *s*		16.99 *q*	
28	1.03 *s*		26.76 *q*	
29	0.99 *s*		18.45 *q*	
30	0.90 *s*		16.54 *q*	

a) in ppm; b) in HMBC spectrum.

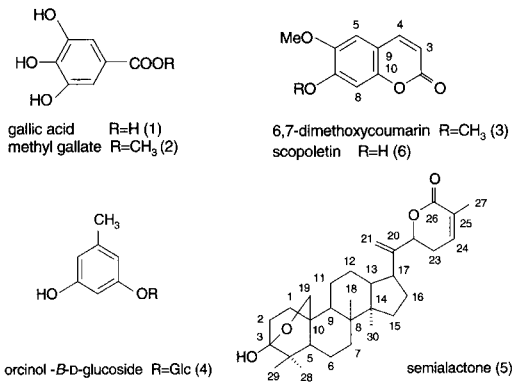

Fig. 1. Chemical structures of compounds isolated from the bark of *R. chinensis*.

were compared with the literature values (Park *et al.*, 2003). Consequently the structure of compound 2 was concluded to be the methyl gallate (Fig. 1).

Compound 3 and 4 were elucidated as 6, 7-dimethoxycoumarin and orcinol-β-D-glucoside, respective, by interpretation of their spectral data, such as EI-MS, 1D-NMR, and 2D-NMR (COSY, DEPT, HMQC, and HMBC) and by comparison of already reported spectroscopic data (Chung *et al.*, 1999).

Compound 5 was isolated as a white powder and gave a EI-MS molecular ion peak at *m/z* 468. The ^{1}H-NMR spectrum showed signals for five tertinary methyl groups as sharp singlets at δ 0.88, 0.90, 0.99, 1.03, and 1.09 and these were assignable to H-18, H-30, H-29, H-28, and H-27, respectively. The ^{13}C-NMR spectrum and DEPT experiments showed the present of six quaternary carbons, eight methines, elevene methylene, and five methyl group. In the HMBC spectrum, H-21 was correlated with C-17 and C-22 and also showed cross peak between C-20 and H-13, H-16, H-17, and H-22 (Fig. 2). From the coupling constant of H-22 (H-22, *dd*, J = 3.5, 12.5) in ^{1}H-NMR spectrum, the configuration was deduced *S*. From the results above and the comparison of literature values (Lee *et al.*, 2001), the compound 5 was elucidated as semialactone (Fig. 1).

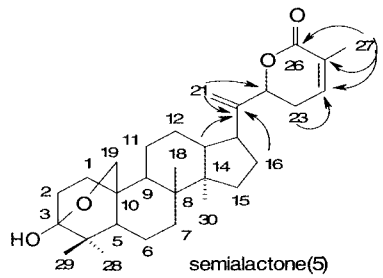

Fig. 2. Selected HMBC correlations of compound 5.

The compound 6 was obtained as a white needle powder. The ^{1}H-NMR spectrum indicated the presence of a methoxyl group (δ 3.81, 3H, s, OMe-6). In the ^{1}H-NMR spectrum, two doublets were observed at δ 6.21 and 7.90 and two singlets at δ 6.77 (1H, s) 7.21 (1H, s,) and these were assignable to H-3, H-4, H-8, and H-5, respectively. Based on these results and the values previously reported data in the literature (Kim *et al.*, 1997b), this compound was identified as 7-hydroxy-6-methoxy-coumarin, scolpoletin (Fig. 1).

3.2. Antioxidant Activity

The antioxidant activities of six compounds obtained from the bark of *R. chinensis* were shown in Fig. 3. Among six compounds, compound 1 and 2 exhibited higher scavenging activity on DPPH radical. However, compound 4, 5, and 6 have little antioxidant activity. The antioxidant activity of gallic acid which has hydroxyl group on C-7 was higher (90.28% at 10 μg/mℓ) than that of methyl gallate which has methoxyl group on C-7 (89.74% at 10 μg/mℓ). According to the reports of Cooper-Drive *et al.* (1998) and Park *et al.* (2004), the antioxidant activity of phenolic compounds is based on the number and position of hydroxyl groups.

Recently, natural antioxidants are receiving much attention, therefore, this study indicates that these isolated compounds may be useful for

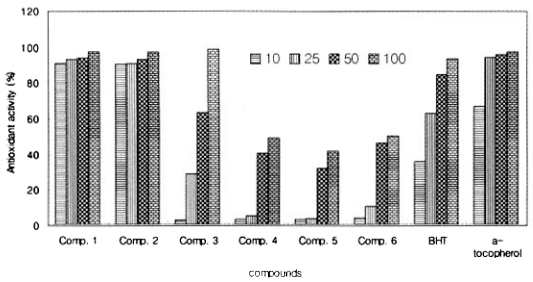

Fig. 3. Antioxidant activity of isolated compounds from the bark of *R. chinensis*.

the treatment of oxidative damage and have potential possibility to be natural antioxidants.

4. CONCLUSION

From the EtOAc and Et_2O fraction of the bark of *R. chinensis*, six compounds were isolated by column chromatography using Sephadex LH-20 and/or silica gel and identified as follows: gallic acid (1), methyl gallate (2), 6,7-dimethoxycoumarin (3), orcinol-β-D-glucoside (4), semialactone (5), and scopoletin (6). Antioxidant activity of six acompounds was measured by the DPPH free radical scavenging method. From the results, methyl gallate showed the strongest action, while orcinol-β-D-glucoside (4), semialactone (5), and scopoletin (6) had the low activities.

ACKNOWLEDGEMENT

The authors thank for the Korea Basic Science Institute in Seoul, Korea for the NMR experiments.

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