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Antioxidative Constituents from the Woods of *Liriodendron tulipifera*[*1]

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ABSTRACT

Three flavonoids, quercetin, taxifolin, and kaempferol were isolated from the woods of *Liriodendron tulipifera*. Their structures were determined by spectral analysis. Based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method, the antioxidative activities of three isolated compounds and their acetates were measured in order to search for natural antioxidants. The IC_{50} of quercetin (1), taxifolin (2), and kaempferol (3) were 3.6, 3.9, and 4.1 $\mu g/m\ell$, respectively.

Keywords : *Liriodendron tulipifera*, Antioxidant activity, Radical scavenging effect, kaempferol, quercetin, taxifolin.

1. INTRODUCTION

Oxidation is known to be a major cause of material degradation. Free radicals, which have been recognized to be involved in several disease including cancer, are chemical fragments that cause oxidation and 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 as natural antioxidants. Because of the carcinogenic properties of some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), recently natural antioxidants from plants have received much attention (Cheung, *et al.*, 2003). In search for antioxidants from several Korean plants, the ethanolic extract from the woods of *L. tulipifera* was found to exhibit significant antioxidative activity, according to DPPH methods. *L. tulipifera* (yellow poplar) is one of the tallest trees, growing up to 120 feet tall. It has a long and straight trunk and large flowers. Yellow poplar leaves are shaped like a tulip blossom. It is also called tulip-tree, because of above reason. Fruits are cone-shaped, about three inches long.

From the *L. tulipifera*, lirionol, syringic acid methyl ester, dehydroglatheucine, glaucine, asimilobine, (+)-pinoresinol, (+)-syringaresinol, (+)-syringaresinol dimethyl ether, syringaldehyde,

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norushinsunine, *N*-acethylnornuciferine, (+)-liriofrin, (+)-liriotulipiferin, *O*-methyl-*N*-norliriinine, *N*-(2-hydroxy-phenylethyl)benzamide were isolated and identified (Chen *et al.*, 1978; Chen *et al.*, 1976). Liriodendronine, (-)-*N*-acetylnornuciferine, (-)-*N*-acetylasimilobine and (-)-tulifeoline have been also isolated and elucidated their structure from *L. tulipifera* (Chen *et al.*, 1976; Peter *et al.*, 1977).

In present paper, we report the isolation and structure elucidation of compounds from the woods of *L. tulipifera* and antioxidative activities of three compounds and their acetate of these compounds which were isolated from the woods of *L. tulipifera*.

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

The woods of *L. tulipifera* were collected at Experimental forest of Korea Forest Research Institute in Hoasung, Kyungki Province, during June, 2001 and dried at room temperature.

2.3. Extraction, Fractionation, and Isolation

Air dried woods of *L. tulipifera* were powdered and extracted twice with 95% EtOH and then evaporated to give the crude extracts. The crude extracts was successively partitioned with organic solvents, such as petroleum ether and ethyl acetate (EtOAc).

The EtOAc soluble fraction (63.49 g) was subjected to column chromatography on Sephadex LH-20 eluted with MeOH to yield 7 sets of fraction (MEA-1～MEA-7). Fraction MEA-7 (4.48 g) was rechromatographed on silica gel column chromatography with $CHCl_3$-MeOH (24:1, v/v) to give 5 subfractions (MEA-7-1～MEA-7-5). Among 5 fractions of MEA-7, second fraction (MEA-7-2) was compound 1 (16 mg). Fraction MEA-5 (5.55 g) was further subjected to repeated column chromatography on silica gel eluted with CH_2Cl_2-MeOH (24:1, v/v) to give 4 sets of fractions (MEA-5-1～MEA-5-4). Among 4 fractions, MEA-5-2 (170 mg) was purified by column chromatography on silica gel and eluted with a solvent system of CH_2Cl_2-MeOH (28:1, v/v) to give compound 2 (MEA-5-2-2, 30 mg). The MEA-6 (1.85 g) fraction was also chromatographed with a silica gel column ($CHCl_3$-MeOH, 20:1, v/v) to give 5 subfractions (MEA-6-1～MEA-6-5). The third fraction (MEA-6-3) was compound 3 (30 mg).

2.4. Acetylation

Compound (10 mg) was dissolved in pyridine (0.5 mℓ) and acetic acid anhydride (Ac_2O, 0.5 mℓ) was added. The reaction mixture was kept at 65°C for 24 hr. and then poured over crushed ice (30 mℓ) with stirring and left for 1 hr. Isolation of the insolubles was conducted by filtration using a small Büchner funnel. If filtration is unsuitable, extract the aqueous mixture with ether to remove the acetate derivatives. Purification of the acetate was performed by recrystallization or by TLC on silica gel.

2.5. Spectral Data of Compounds

Compound 1. Yellow powder. EI-MS *m/z* (rel. int., %) : 302 (M^+, 100), 275 (26.4), 165

Table 1. ^{1}H-NMR spectral data of compounds 1-3 (500 MHz, acetone-d_6)

Position	1	2	3
2	-	5.01 *d* (11.4)	-
3	-	4.59 *d* (11.4)	-
6	6.26 *d* (2.0)	5.98 *d* (2.1)	6.29 *d* (1.5)
8	6.51 *d* (2.0)	5.94 *d* (2.1)	6.66 *d* (1.5)
2'	7.80 *d* (2.0)	7.06 *d* (2.0)	8.15 *dd* (2.5, 9.5)
3'	-	-	7.03 *dd* (2.5, 9.5)
5'	6.99 *d* (8.5)	6.86 *d* (8.1)	7.03 *dd* (2.5, 9.5)
6'	7.69 *dd* (2.0, 8.5)	6.90 *dd* (2.0, 8.1)	8.15 *dd* (2.5, 9.5)

* *J* values in parentheses are recorded in Hz.

Table 2. ^{1}H-NMR spectral data of compounds 1a-3a (500 MHz, $CDCl_3$)

Position	1a	2a	3a
2	-	5.66 *d* (12.0)	-
3	-	5.43 *d* (12.0)	-
6	6.88 *d* (2.0)	6.79 *d* (2.0)	6.88 *d* (2.0)
8	7.34 *d* (2.0)	6.62 *d* (2.0)	7.34 *d* (2.0)
2'	7.70 *d* (2.0)	7.30 *d* (2.0)	7.87 *dd* (2.0, 7.0)
3'	-	-	7.27 *dd* (2.0, 7.0)
5'	7.36 *d* (8.5)	7.27 *d* (8.0)	7.27 *dd* (2.0, 7.0)
6'	7.73 *dd* (2.0, 8.5)	7.39 *dd* (2.0, 8.0)	7.87 *dd* (2.0, 7.0)
$COCH_3$	2.34 *s*	2.03 *s*	2.33 *s*
〃	2.34 *s*	2.30 *s*	2.35 *s*
〃	2.35 *s*	2.30 *s*	2.35 *s*
〃	2.35 *s*	2.30 *s*	2.44 *s*
〃	2.44 *s*	2.38 *s*	-

* *J* values in parentheses are recorded in Hz.

(11.1), 153 (45.0), 137 (9.5), 123 (12.5). ^{1}H-NMR, ^{13}C-NMR : Table 1, 3. ^{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'. pentaacetate 1a: Yellow powder. EI-MS *m/z* : 512 (M^+). ^{1}H-NMR, ^{13}C-NMR : Tables 2 and 3.

Compound 2. White crystal. EI-MS *m/z* (rel. int., %) : 304 (M^+, 88.8), 286 (25.1), 275 (99.8), 182 (11.8), 153 (100, base ion), 150 (41.1), 137 (9.8), 123 (93), 69 (13.2). ^{1}H-NMR, ^{13}C-NMR : Table 1, 3. ^{1}H-^{1}H COSY correlations : H-6↔H-8, H-5'↔H-6'/H-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-2/C-4/C-6', H-2→C-4/C-1', H-3→C-1'/C-2/C-4. pentaacetate 2a: Yellow powder. EI-MS *m/z* : 514 (M^+). ^{1}H-NMR, ^{13}C-NMR : Tables 2 and 3.

Compound 3. Yellow powder. EI-MS *m/z*

Table 3. ^{13}C-NMR spectral data of compounds 1(a)-3(a) (125 MHz, $CDCl_3$)

Position	1*	1a	2*	2a	3*	3a
2	146.94	154.03 *s*	84.50	80.60 *d*	146.19	150.66 *s*
3	136.68	134.34 *s*	73.11	73.39 *d*	135.91	134.14 *s*
4	176.51	170.30 *s*	198.12	185.11 *s*	175.84	170.33 *s*
5	162.09	154.02 *s*	164.76	156.67 *s*	161.31	154.98 *s*
6	99.12	114.15 *d*	96.98	111.70 *d*	98.77	114.06 *d*
7	165.10	154.52 *s*	167.88	162.49 *s*	165.42	157.17 *s*
8	94.47	109.26 *d*	96.02	109.27 *d*	94.01	109.21 *d*
9	157.80	157.11 *s*	164.16	151.68 *s*	157.25	153.15 *s*
10	104.04	115.04 *s*	101.46	110.84 *s*	103.08	115.03 *s*
1'	123.72	126.70 *s*	129.77	13379 *s*	122.63	127.22 *s*
2'	115.68	124.11 *d*	115.72	123.07 *d*	129.72	129.84 *d*
3'	145.82	142.48 *s*	145.71	142.35 *s*	115.66	122.31 *d*
4'	148.33	144.63 *s*	146.52	143.15 *s*	159.60	154.46 *s*
5'	116.16	124.20 *d*	115.82	124.06 *d*	115.66	122.31 *d*
6'	121.45	128.02 *s*	120.80	125.59 *d*	129.72	129.84 *d*
$CO\underline{C}H_3$		20.71 *q*		20.51 *q*		20.81 *q*
〃		20.78 *q*		20.83 *q*		21.27 *q*
〃		20.86 *q*		20.86 *q*		21.38 *q*
〃		20.93 *q*		21.15 *q*		21.40 *q*
〃		21.37 *q*		21.36 *q*		-
$\underline{C}OCH_3$		167.92 *s*		168.04 *s*		168.01 *s*
〃		168.01 *s*		168.16 *s*		168.08 *s*
〃		168.10 *s*		169.21 *s*		169.10 *s*
〃		168.15 *s*		169.28 *s*		169.49 *s*
〃		169.54 *s*		169.33 *s*		-

* In acetone-d_6.

(rel. int., %) : 286 (M^+, 88.8), 259 (13), 230 (6), 154 (4), 121 (25), 93 (12). ^{1}H-NMR, ^{13}C-NMR : Table 1, 3. ^{1}H-^{1}H COSY correlations : H-2', 6'↔3', 5'. HMBC correlations : H-2'/H-6'→C-2/C-4', H-3'/H-5'→C-1'/C-4', H-6→C-5/C-7/C-8/C-10, H-8→C-6/C-7/C-9. pentaacetate 3a: EI-MS *m/z* : 454 (M^+). ^{1}H-NMR, ^{13}C-NMR : Tables 2 and 3.

2.6. Antioxidative Activity Test

MeOH solutions (4 mℓ) of samples at various concentrations were added to a solution of DPPH in MeOH (4.0×10^4 M, 1 mℓ) and the reaction mixtures were shaken vigorously. After storing mixtures for 30 min at room temperature, the remaining amounts of DPPH were determined by colorimetry (8452A Diode Array

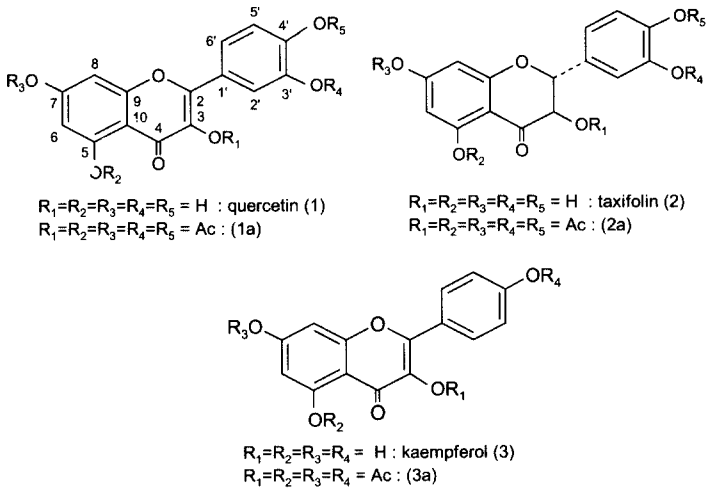

Fig. 1. Compounds isolated from the woods of *L. tulipifera*.

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. The radical scavenging 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

Repeated column chromatography with SiO_2 and Sephadex LH-20 of the EtOAc fraction from the woods of *L. tulipifera* led to the isolation of three compounds. The compound 1 was obtained as a yellow powder and the EI-MS presented a signal at *m/z* 302 $[M]^+$, corresponding to molecular formula $C_{15}H_{10}O_7$. The ^{1}H-NMR spectrum of compound 1 showed two *meta*-coupled doublets (δ 6.51 and 6.26) assigned to H-8 and H-6, respectively, of ring A in the flavonoid skeleton. The ^{1}H-NMR spectrum of 1 also exhibited, one *meta*-coupled aromatic proton (δ 7.80), one *ortho*, *meta*-coupled aromatic proton (δ 7.69) and one *ortho*-coupled aromatic proton (δ 6.99) attributable to H-2', H-6' and H-5' of B ring, respectively. It was supported by ^{13}C-NMR and DEPT spectra, which showed a total of 15 carbons consisting of five methines and ten quaternary carbons. Acetylation of compound 1 gave a pentaacetate, 1a, EI-mass spectrum *m/z*: 512 $[M]^+$. Consequently the structure of compound 1 was concluded to be 3, 3', 4', 5, 7-pentahydroxyflavone, quercetin (Shen *et al.*, 1993; Agrawal, 1989).

The ^{1}H-NMR spectrum of compound 2 showed two mechine (δ 4.59 and 5.01), *meta*-coupled aromatic (δ 5.94 and 5.98) and ABX-type aromatic (δ 6.86, 6.90, and 7.06) proton signals. In ^{13}C-NMR spectrum, 15 carbon signals were shown and suggested that tetrahydroxyflavanonol structure. The assigned carbon chemical shifts signals were compared with literature values (Ishimaru *et al.*, 1995). Consequently, the structure of compound 2 was concluded to be 3',4',5,7-tetrahydroxyflavanonol, taxifolin.

The ^{1}H-NMR spectrum of compound 3 exhibited two set of doublets at δ 7.03 (2H, *dd*, J = 2.5, 9.5 Hz) and 8.15 (2H, *dd*, J = 2.5, 9.5 Hz) that were assigned to H-3', H-5' and H-2', H-6', respectively. In ^{13}C-NMR spectrum, C-8 at δ 94.01, C-6 at δ 98.77, C-10 at δ 103.08, C-1'

Table 4. DPPH free radical scavenging activities of the compounds and their acetates isolated from the woods of *L. tulipifera*

Compounds	DPPH radical scavenging activity IC_{50} ($\mu g/m\ell$)[1)]
Quercetin	3.6
Taxifolin	3.9
Kaempferol	4.1
Pentaacetate Quercetin	62.3
Tetraacetate Kaempferol	> 100
BHT	9.8
α-Tocopherol	13.5

[1)] The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

at δ 122.63, C-3 at δ 135.91, C-9 at δ 157.25, C-4' at δ 159.60, C-5 at δ 161.31, C-7 at δ 165.42, C-4 at δ 175.84 and two sets of symmetrical carbons, C-2', C-6' and C-3', C-5' at δ 129.72 and δ 115.66, respectively, were assigned. The identity of compound 3 was thus confirmed to be 3,4',5,7-tetrahydroxyflavone, kaempferol (Park, 2002). These three compounds are the first report from *L. tulipifera.*

The free radical scavenging activities of three compounds obtained from the woods of *L. tulipifera* and their acetates were shown in Table 1. Among four isolated compounds, compound 1 (quercetin) exhibited highest scavenging activity value on DPPH with IC_{50} value 3.6 $\mu g/m\ell$. From the above results, the radical scavenging activity of quercetin which has hydroxyl group on C-3' was higher than that of kaempferol which has hydrogen on C-3'. Recently, natural antioxidants are receiving much attention and flavonoids are regarded as efficient antioxidants by scavenging oxygen radicals. Therefore, this study indicates that these isolated compounds may be useful for the treatment of oxidative damage and have potential possibility to be natural antioxidants.

4. CONCLUSION

From the EtOAc fraction of the woods of *L. tulipifera*, three compounds 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 as follows: quercetin, taxifolin, and kaempferol. The IC_{50}, which indicates 50% decrease of DPPH radical, of three compounds obtained from *L. tulipifera* woods were 3.6, 3.9, and 4.1 $\mu g/m\ell$, respectively.

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