



Two New Caffeoyl Threonate Esters from the Leaves of *Toxicodendron vernicifluum*

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Abstract – *Toxicodendron vernicifluum*, also called as *Rhus verniciflua* is a deciduous tree belonging to Anacardiaceae family. Two new caffeoyl threonate esters, rhuseols A (**1**) and B (**2**), together with 5-*O*-(*E*)-caffeoylquinic acid methyl ester (**3**) were isolated from the leaves of *T. vernicifluum*. The structures of isolated compounds were established by using 1D and 2D NMR in combination with HR-ESI-MS. Compounds **1** - **3** showed DPPH radical scavenging effects with IC₅₀ values of 47.9, 107.8 and 15.4 μM, respectively. Taken together, these compounds might contribute to the antioxidant properties of the leaves of *T. vernicifluum*, which will be useful for various oxidative stress mediated diseases.

Keywords – *Toxicodendron vernicifluum*, caffeoyl threonate esters, antioxidant effect

Introduction

Toxicodendron vernicifluum (Stokes) F.A. Barkley, also called as *Rhus verniciflua* is a deciduous tree belonging to Anacardiaceae family. It is widely distributed in Asia including Korea and also known as a lacquer tree. All parts of this plant are also used in traditional medicines and exerted diverse biological activities including antioxidant, anticancer, anti-inflammatory and antimicrobial effects have been reported.¹⁻⁴

Plants contain various bioactive substances. These substances are synthesized through biosynthesis, and each plant synthesizes different materials with specific biosynthetic enzymes. This represents the component diversity of each plant, which leads to the diversity of diversity. Differential composition and biological activity depending on the part have been reported by comparative analysis of different parts of plants.⁵⁻⁸ For example, tanshinones and phenolic acids are abundant in roots of *Salvia miltiorrhiza*, whereas flavonoids and triterpenes are abundant in stems and leaves. Our previous study showed the difference of chemical constituents of each part, such as bark, lignum, leaf and fruit using LC-MS-MS and PCA analysis, and this eventually led to differences in

biological efficacy.⁹ However, most of the studies on *T. vernicifluum* have been done on barks and there is little study on other parts.¹⁰⁻¹² Leaves are consumed as food, but little has been studied about its ingredients and benefits. This encourage us to investigate about the leaf of *T. vernicifluum* for wide application. In this study, three phenolic compounds, including two new compounds, were isolated from the leaves of *T. vernicifluum* and their structure were identified using spectroscopic analyses. Antioxidant efficacy of isolated compounds were also evaluated.

Experimental

Plant materials – The leaves of *T. vernicifluum* were collected from the farm in Buyeo, Korea in June 2016. After identification by the herbarium of the College of Pharmacy, Chungbuk National University, voucher specimen (CBNU2016-RVF) was deposited in a specimen room of the herbarium. The dried leaves of *T. vernicifluum* (1.5 kg) were extracted twice with 90% MeOH. The extract was evaporated under reduced pressure, which yielded the methanol extract (310 g). The methanol extract was suspended in water and partitioned successively with *n*-hexane, CH₂Cl₂ and EtOAc.

Extraction and isolation – The EtOAc fraction (RVE, 4.5 g) was chromatographed on a silica gel column chromatography and eluted with the mixture of CH₂Cl₂-

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MeOH with increasing polarity to give fifteen subfractions (RVE1-RVE15). RVE13 was chromatographed on MPLC (RP-18) and eluted with MeOH-H₂O (10:90 to 100:0 gradient) to obtain 13 fractions (RVE13A - RVE13M). Compounds **1** (4.6 mg), **2** (4.7 mg) and **3** (1.9 mg) were purified from RVE13A by Sephadex LH-20 column chromatography eluted with CH₂Cl₂-MeOH (1:1) and followed by semi-preparative HPLC (MeOH-H₂O, 20:80).

Rhuseoyl A (1): brown syrup; $[\alpha]_D^{25} +10.4$ (*c* 0.1, MeOH); UV (MeOH) λ_{\max} 216, 326 nm; IR_{max} 3324, 1654 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (CD₃OD, 100 MHz), see Table 1; ESI-MS (positive mode) *m/z* 335 [M+Na]⁺; HR-ESI-MS (positive mode) *m/z* 335.0737 (calcd for C₁₄H₁₆O₈Na 335.0743).

Rhuseoyl B (2): brown syrup; $[\alpha]_D^{25} +11.9^\circ$ (*c* 0.06, MeOH); UV (MeOH) λ_{\max} 217, 325 nm; IR_{max} 3323, 1654 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) and ¹³C NMR (CD₃OD, 100 MHz), see Table 1; ESI-MS (positive mode) *m/z* 335 [M+Na]⁺; HR-ESI-MS (positive mode) *m/z* 335.0733 (calcd for C₁₄H₁₆O₈Na 335.0743).

Measurement of antioxidant activity – The antioxidant activity was evaluated by measuring the DPPH radical scavenging activity. Briefly, samples were mixed with freshly prepared DPPH solution. After shaking, the reaction mixtures were stand for 30 min at room temperature in dark places. The radical scavenging activity was determined by measuring the absorbance at 517 nm. The relative radical scavenging activity (%) was calculated as $[1 - \text{absorbance of solution with sample and DPPH} / \text{absorbance of solution with DPPH}] \times 100$.

Results and Discussion

Investigation on the constituents of *T. vernicifluum* leaves yielded three compounds including two new compounds (Fig. 1).

Compound **1** was purified as brown syrup and its molecular formula was determined as C₁₄H₁₆O₈ from the HRESI-MS (*m/z* 335.0737 [M+Na]⁺, calcd for C₁₄H₁₆NaO₈, 335.0743) and ¹³C NMR data. The IR spectrum of **1** suggested the presence of hydroxyl (3324 cm⁻¹) and carbonyl (1654 cm⁻¹) functionalities. In the aromatic region of ¹H NMR spectrum, signals attributed to 1,3,4-trisubstituted aromatic ring and a double bond in *trans*-configuration were observed at [δ_H 7.06 (1H, d, *J*=2.4 Hz, H-2'), 6.79 (1H, d, *J*=8.0 Hz, H-5'), 6.97 (1H, dd, *J*=8.0, 2.4 Hz, H-6')] and [δ_H 7.61 (1H, d, *J*=16.0 Hz, H-7'), 6.27 (1H, d, *J*=16.0 Hz, H-8')], respectively. The corresponding carbon signals at [δ_C 126.3 (C-1'), 113.7 (C-2'), 145.4 (C-3'), 148.3 (C-4'), 115.1 (C-5'), 121.7 (C-6')] and [δ_C 146.2 (C-7'), 113.2 (C-8')] in HSQC spectrum together with carbonyl signal at δ_C 166.8 (C-9') clearly showed the presence of *trans*-caffeoyl moiety. Besides aforementioned signals, the ¹H NMR spectrum revealed the presence of a methylene at δ_H 3.75 (1H, dd, *J*=11.2, 6.8 Hz, H-1a), 3.83 (1H, dd, *J*=11.2, 6.4 Hz, H-1b), two oxymethines at δ_H 5.30 (1H, ddd, *J*=6.8, 6.4, 2.4 Hz, H-2) and δ_H 4.52 (1H, d, *J*=2.4 Hz, H-3) and a methoxy group at δ_H 3.74 (3H, s, OCH₃). The correlations between H-1 and H-2, H-2 and H-3 in ¹H-¹H COSY spectrum suggested the presence of -CH₂-C(OH)-C(OH)- in **1**. The corresponding carbon signals at δ_C 59.5 (C-1), 74.3 (C-2),

Table 1. NMR spectroscopic data for compounds **1** and **2** (CD₃OD).

	1		2	
	δ_H	δ_C	δ_H	δ_C
1	3.75 (dd, 11.2, 6.8) 3.83 (dd, 11.2, 6.4)	59.5	4.27 brs 4.29 (d, 6.4)	64.3
2	5.30 (ddd, 6.8, 6.4, 2.4)	74.3	4.23 m	70.0
3	4.52 (d, 2.4)	69.2	4.32 (d, 2.4)	71.2
4		172.8		173.1
1'		126.3		126.3
2'	7.06 (d, 2.4)	113.7	7.06 (d, 2.4)	113.7
3'		145.4		145.4
4'		148.3		148.3
5'	6.79 (d, 8.0)	115.1	6.79 (d, 8.0)	115.1
6'	6.97 (dd, 8.0, 2.4)	121.7	6.97 (dd, 8.0, 2.4)	121.6
7'	7.61 (d, 16.0)	146.2	7.61 (d, 16.0)	145.9
8'	6.27 (d, 16.0)	113.2	6.27 (d, 16.0)	113.7
9'		166.8		167.5
OCH ₃	3.74 s	51.3	3.74	51.2

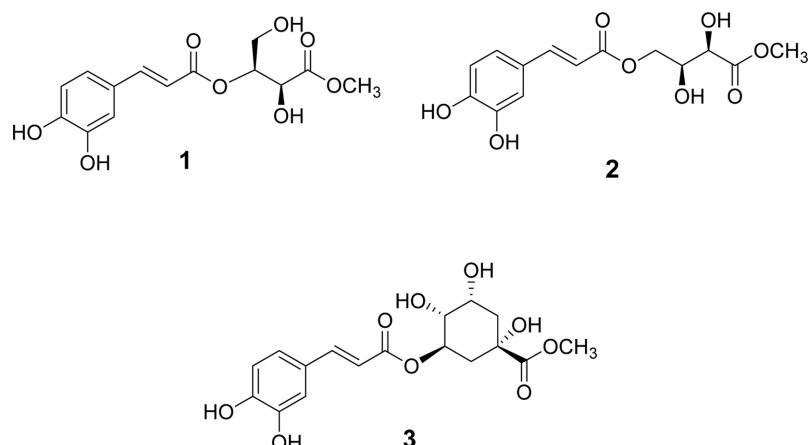


Fig. 1. Chemical structures of compounds 1 - 3.

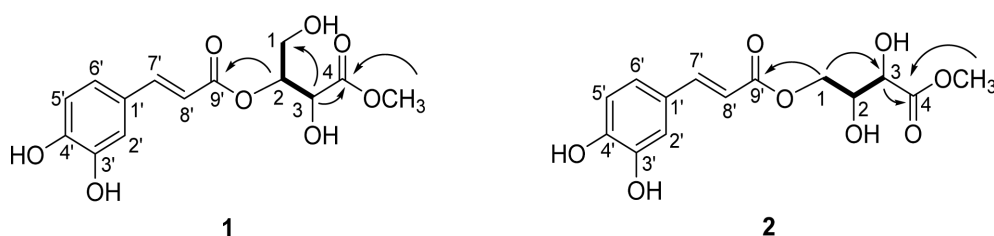


Fig. 2. Chemical structures and key ^1H - ^1H COSY (---) and HMBC (—) correlations of compounds 1 and 2.

69.2 (C-3), 51.3 (OCH₃), and carbonyl signal at δ_{C} 172.8 in the HSQC spectrum together with HMBC correlations of H-1 to C-2 and C-3, H-3 to C-4 and positive value of optical rotation suggested the presence of threonic acid.¹³ The HMBC correlations from δ_{H} 3.74 (3H, s, OCH₃) to δ_{C} 172.8 (C-4) determined the position of a methoxy group (Fig. 2). The connection between a threonyl group and a caffeoyl moiety was assigned by the HMBC correlation from H-2 to C-9', a carbonyl of caffeoyl moiety. Taken together, compound 1 was determined as shown in Fig. 1 and named ruseoyl A.

Compound 2 was purified as brown syrup and same molecular formula of C₁₄H₁₆O₈ was deduced from a pseudomolecular ion [M+Na]⁺ of m/z 335.0733 at the HRESI-MS analysis. Comparison of the ^1H and ^{13}C data of 2 with those of 1 disclosed that 2 also has *trans*-caffeoyl acid moiety. The presence of threonic acid was suggested by the signals at δ_{H} 4.27 (1H, brs, H-1a), 4.29 (1H, d, $J=6.4$ Hz, H-1b), 4.23 (1H, m, H-2) and 4.32 (1H, d, $J=2.4$ Hz, H-3), displaying ^1H - ^1H COSY correlations between H-1 and H-2, H-2 and H-3 as in 1. Further examination of NMR data, however, disclosed the differences in the chemical shifts of H-1, H-2 and H-3. The chemical shift of H-1 was downfield shifted from δ_{H} 3.75 and 3.83 to δ_{H} 4.27 and 4.29, whereas that of H-2

was upfield shifted from δ_{H} 5.30 to δ_{H} 4.27 and 4.23. In addition, HMBC correlation from H-1 to C-9' was observed in 2, instead of correlation from H-2 to C-9' in 1 (Fig. 2). Therefore, 2 was suggested to differ from 1 in the linkage between caffeoyl acid and threonic acid as carbonyl of caffeoyl moiety was connected to C-1 of threonic acid. Taken together, compound 2 was determined as shown and named ruseoyl B.

Compound 3 was identified as 5-*O*-(*E*)-caffeoylquinic acid methyl ester by the spectroscopic data analysis and comparison with those of published values.¹⁴

Evaluation of biological activity of isolated compounds suggested the DPPH radical scavenging effects of compounds 1 - 3 with IC₅₀ values of 47.9, 107.8 and 15.4 μM , respectively. Taken together, our present study suggested that these compounds might contribute to the antioxidant properties of the leaves of *T. vernicifluum*, which will be useful for various oxidative stress mediated diseases.

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