



Phenolic compounds from the leaves of *Cornus controversa*

Dongho Lee^a, Shin-Jung Kang^b, Seung-Ho Lee^c, Jaiseup Ro^b, Kyongsoon Lee^b,
A. Douglas Kinghorn^{a,*}

^aProgram for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

^bCollege of Pharmacy, Chungbuk National University, Cheongju 360-763, South Korea

^cCollege of Pharmacy, Yeungnam University, Kyongsan 712-749, South Korea

Received 11 May 1999; accepted 16 September 1999

Abstract

Two novel phenolic compounds from the leaves of *Cornus controversa* (Cornaceae) were characterized as (–)-2,3-digalloyl-4-(*E*)-caffeoyl-L-threonic acid and (–)-2-galloyl-4-(*E*)-caffeoyl-L-threonic acid, using spectroscopic methods. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Cornus controversa*; Cornaceae; Leaves; Phenolic compounds; Galloylcaffeoylthreonic acids; Kaempferol 3-*O*- α -L-rhamnoside

1. Introduction

Cornus controversa Hemsl. (Cornaceae) is a tree found in Korea and the People's Republic of China which has been used as an astringent and a tonic (Lee, 1993). Various flavonoids, phenolic compounds and terpenoids have been reported from this plant (Jang et al., 1998; Kurihara & Kikuchi, 1972; Lee, Lee, Chung, Ro, & Lee, 1995; Nakaoki & Morita, 1958; Nishino, Kobayashi, & Fukushima, 1988; Tanaka, 1971). In this investigation, four additional phenolic compounds, (–)-2,3-digalloyl-4-(*E*)-caffeoyl-L-threonic acid (**1**), (–)-2-galloyl-4-(*E*)-caffeoyl-L-threonic acid (**2**), (–)-4-(*E*)-caffeoyl-L-threonic acid (**3**) and kaempferol 3-*O*- α -L-rhamnoside (Matthes, Luu, & Ourisson, 1980), have been obtained from the leaves of *C. controversa*. The structure elucidation of the novel compounds **1** and **2** was performed by spectral data interpretation. The unambiguous assignments of the ¹H NMR signals of the known compound **3** (Han & Nahrstedt, 1993) are also presented.

2. Results and discussion

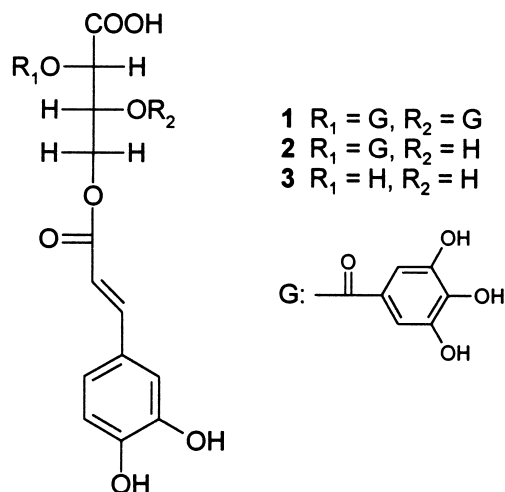
Compound **1**, $[\alpha]_D^{20} -38^\circ$, was obtained as an amorphous dark brown powder and gave a dark blue color in the FeCl₃ test. The negative-ion ESMS showed a quasimolecular ion at *m/z* 601. In the ¹H NMR spectrum of **1**, two proton singlets (δ_H 6.96 and 7.03) corresponded to two galloyl groups (Lee et al., 1995). Also, a broad singlet at δ_H 7.17, a doublet of doublets ($J = 8.5$ and 1.5 Hz) at δ_H 7.07, and a doublet ($J = 8$ Hz) at δ_H 6.85, which were observed as an AMX system, and two olefinic doublets ($J = 15.9$ Hz, *trans*), which were observed at δ_H 7.60 and 6.43, indicated the presence of a caffeoyl group (Han & Nahrstedt, 1993). The signals coupled to each other at δ_H 5.46 ($J = 3.2$ Hz), δ_H 5.86 (multiplet), δ_H 4.50 ($J = 5.1$ and 11.6 Hz) and δ_H 4.56 ($J = 7.3$ and 11.6 Hz) and the carbonyl carbon signal at δ_C 168.3, suggested the presence of threonic acid (Han & Nahrstedt, 1993). The positions of substitution of threonic acid were assigned using the HMBC technique as C-2 and C-3 (two galloyls) and C-4 (caffeoyl). Alkaline hydrolysis of **1** yielded gallic acid, caffeic acid, and L-threonic acid. The ¹H NMR spectral data of gallic acid and caffeic acid and the optical rotation of L-threonic acid

* Corresponding author.

were consistent with literature values (Han & Nahrstedt, 1993; Lee et al., 1995). Therefore, the structure of **1** was assigned as (–)-2,3-digalloyl-4-(*E*)-caffeoyl-L-threonic acid.

Compound **2**, $[\alpha]_D^{20} -27^\circ$, was obtained as an amorphous dark brown powder and gave a dark blue color in the FeCl_3 test. The negative-ion ESMS showed a quasimolecular ion at m/z 449. The ^1H NMR spectrum of **2** was similar to that of **1** except for the absence of one galloyl signal and an observed upfield shift of the H-3 signal at δ_{H} 4.53 of the threonic acid moiety. Analysis of the ESMS, ^1H , and ^{13}C NMR data suggested that compound **2** is a derivative of **1** missing one galloyl group at the C-3 position of threonic acid. The positions of the galloyl and caffeoyl substituents were confirmed to be at C-2 and C-4 on threonic acid, respectively, using the HMBC technique. The products of alkaline hydrolysis of **2** were also the same as those of **1**. The structure of **2** was therefore identified as (–)-2-galloyl-4-(*E*)-caffeoyl-L-threonic acid (**2**).

The ^1H NMR spectrum of **3** was also similar to that of **1** except for the absence of two galloyl groups and the consequent upfield shift of two proton signals of the threonic acid moiety. The signals at δ_{H} 4.09 ($J = 2.4$ Hz), δ_{H} 4.05 ($J = 2.5$ and 6.3 Hz), and δ_{H} 4.12 ($J = 6.4$ Hz) were assigned unambiguously to the C-2, C-3, and C-4 positions of threonic acid, respectively, by 2D NMR techniques.



3. Experimental

MPs uncorr.; optical rotations: Perkin-Elmer model 241 polarimeter; UV: Beckman DU-7 spectrometer; IR: ATI Mattson Genesis series FT-IR spectrophotometer. ^1H , ^{13}C , COSY, HMQC and HMBC NMR experiments were conducted on a Bruker DPX-300

Table 1

^1H and ^{13}C NMR data for compounds **1** and **2** in $\text{DMSO}-d_6^a$

Carbon	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		168.3		172.7
2	5.46, d (3.2)	71.0	5.33, brs	73.0
3	5.86, <i>m</i>	69.5	4.53, <i>m</i>	70.0
	4.50, dd (5.1, 11.6)	62.0	4.31, dd (6.6, 11.0)	65.3
	4.56, dd (7.3, 11.6)		4.41, dd (6.3, 11.0)	
Caffeoyl				
1'		125.4		127.6
2'	7.17, brs	114.6	7.07, brs	115.3
3'		145.4		146.8
4'		148.5		149.7
5'	6.85, d (8)	115.8	6.80, d (8.5)	116.5
6'	7.07, dd (1.5, 8.5)	122.0	6.98, dd (1.6, 8.3)	123.3
7'	7.60, d (15.9)	146.5	7.71, d (15.9)	148.2
8'	6.43, d (15.9)	112.9	6.38, d (16)	114.2
9'		165.9		165.9
2-Galloyl				
1''		118.8		121.1
2'', 6''	6.96, s	108.7	7.10, s	110.2
3'', 5''		145.3		146.5
4''		138.7		140.0
7''		165.5		165.5
3-Galloyl				
1'''		118.6		
2''', 6'''	7.03, s	108.9		
3''', 5'''		145.3		
4'''		138.5		
7'''		164.9		

^a TMS was used as the internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses.

spectrometer and a GE Omega 500 MHz spectrometer. Mass spectra were obtained on a HPLC-ESMS system (Hewlett-Packard 5989B mass spectrometer, 59987A electrospray interface) and a Finnigan MAT 90 instrument (70 eV).

3.1. Plant material

Fresh leaves of *Cornus controversa* were collected in Cheongju, Korea, in May 1992. A voucher specimen has been deposited in the College of Pharmacy, Chungbuk National University, Cheongju, Korea.

3.2. Extraction and isolation

The fresh plant material (8 kg) was cut and extracted with 80% aqueous acetone (3×10 L). The extracts were combined and concentrated in vacuo at 40°C . The resultant precipitation was removed by filtration and the filtrate was concentrated again. Fractionation was initiated by column chromatography over Sephadex LH-20 as stationary phase using a H_2O –MeOH gradient as mobile phase to afford three fractions. Again using a H_2O –MeOH gradient as

mobile phase, fraction 2 was passed sequentially over MCI-gel CHP 20P and Sephadex LH-20, to afford compounds **1** (20 mg) and **2** (15 mg). Fraction 3 from the initial column was purified further using MCI-gel CHP 20P as stationary phase with a H₂O–MeOH gradient as mobile phase to afford compound **3** (450 mg) and five subfractions. Of these, subfraction 3 was chromatographed on TSK-gel Toyopearl HW 40F and Cosmosil 75 C₁₈-OPN with a H₂O–MeOH gradient and EtOH, respectively, to afford kaempferol 3-*O*- α -L-rhamnoside (30 mg). This compound was identified by comparison of its physical and spectroscopic data to published values (Matthes et al., 1980).

3.3. (–)-2,3-Digalloyl-4-(*E*)-caffeoyl-L-threonic acid (**1**)

Amorphous dark brown powder; mp 215–217°; $[\alpha]_D^{20}$ –38° (*c* 0.05, MeOH); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 220 (2.79), 282 (2.45), 331 (2.22); IR ν_{\max} (KBr) cm^{–1}: 3420, 1700, 1624; ¹H and ¹³C NMR data of **1**, see Table 1; HMBC correlations: H-2/C-1, C-7''; H-3/C-7''; H-4/C-9; H-7'/C-1', C-6'; H-8'/C-1', C-9'; H-2'', 6''/C-7''; H-2''', 6'''/C-7'''; ESMS *m/z*: [M–H][–] 601; EIMS (70 eV) *m/z* (rel. int.): 170 (100), 163 (10), 153 (78), 136 (10), 126 (26).

3.4. (–)-2-Galloyl-4-(*E*)-caffeoyl-L-threonic acid (**2**)

Amorphous dark brown powder; mp 128–130°, $[\alpha]_D^{20}$ –27° (*c* 0.09, MeOH); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 255 (4.98), 294 (5.28), 330 (5.27); IR ν_{\max} (KBr) cm^{–1}: 3367, 1697, 1609; ¹H and ¹³C NMR data of **2**, see Table 1; HMBC correlations: H-2/C-1, C-7''; H-4/C-9'; H-7'/C-1, C-9'; H-8'/C-2', C-6', C-9'; H-2'', 6''/C-7''; ESMS *m/z*: [M–H][–] 449; EIMS (70 eV) *m/z* (rel. int.): 170 (87), 163 (100), 153 (79), 136 (12), 135 (40), 125 (27).

3.5. (–)-4-(*E*)-Caffeoyl-L-threonic acid (**3**)

Amorphous bright yellow powder; mp 195–198°; $[\alpha]_D^{20}$ –17° (*c* 0.05, MeOH) (lit. $[\alpha]_D^{20}$ –23° (*c* 1.27, H₂O)); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 219 (4.02), 235 sh (3.89), 243 (3.90), 305 sh (4.04), 327 (4.09); IR ν_{\max} (KBr) cm^{–1}: 3421, 1636; ¹H NMR (500 MHz, DMSO-*d*₆): δ 4.05 (1H, dt, *J* = 2.5, 6.3, H-3), 4.09 (1H, d, *J* = 2.4, H-2), 4.12 (2H, d, *J* = 6.4, H-4), 6.28 (1H, d, *J* = 15.9, H-8'), 6.78 (1H, d, *J* = 8.2, H-5'), 7.01 (1H, dd, *J* = 1.8, 8.2, H-6'), 7.07 (1H, d, *J* = 1.7, H-2'), 7.51

(1H, d, *J* = 15.9, H-7'); HMBC correlations: H-2/C-1; H-3/C-4; H-4/C-3, C-9'; H-7'/C-1', C-2', C-6', C-9'; H-8'/C-1', C-9', ¹³C NMR and MS, consistent with literature values (Han & Nahrstedt, 1993).

3.6. Hydrolysis of **1–3**

Samples of **1–3** (10 mg) were dissolved in MeOH, with 1% NaOH added, and then each mixture was stirred for 4 h at 20°C. The reactants were acidified with 1 N HCl and separated into organic-soluble and water-soluble fractions by partitioning with EtOAc. The EtOAc-soluble fraction was purified by passage over Sephadex LH-20 and then the resultant gallic acid and caffeic acid were subjected to spectroscopic evaluation (Han & Nahrstedt, 1993; Lee et al., 1995). The water-soluble fraction was dried, and the resultant L-threonic acid was subjected to an optical rotation measurement (Han & Nahrstedt, 1993).

Acknowledgements

The authors thank Dr. Darrick S.H.L. Kim, Dr. Dong Seon Kim, Mr. Soobong Park and Mr. Nam-Cheol Kim, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago (UIC), for helpful discussions. Also, we are grateful to Dr. Young Geun Shin and Mr. R. Dvorak, Department of Medicinal Chemistry and Pharmacognosy, UIC, for the mass spectral data and the Research Resources Center, UIC, for providing assistance and spectroscopic equipment.

References

- Han, R., & Nahrstedt, A. (1993). *Planta Medica*, 59, 71.
- Jang, H. M., Hwang, B. Y., Kim, M. S., Lee, D., Kang, S. J., Ro, J. S., & Lee, K. S. (1998). *Korean Journal of Pharmacognosy*, 29, 225.
- Kurihara, T., & Kikuchi, M. (1972). *Yakugaku Zasshi*, 98, 969.
- Lee, D., Lee, S. H., Chung, S. R., Ro, J., & Lee, K. (1995). *Korean Journal of Pharmacognosy*, 26, 327.
- Lee, T. B. (1993). In *Illustrated Flora of Korea* (p. 539). Seoul, Korea: Hyangmoonsa.
- Matthes, H. W. D., Luu, B., & Ourisson, G. (1980). *Phytochemistry*, 19, 2643.
- Nakaoki, T., & Morita, N. (1958). *Yakugaku Zasshi*, 78, 558.
- Nishino, C., Kobayashi, K., & Fukushima, M. (1988). *Journal of Natural Products*, 51, 1281.
- Tanaka, K. (1971). *Phytochemistry*, 10, 719.