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Anti-oxidative and anti-inflammatory activities of caffeoyl hemiterpene glycosides from *Spiraea prunifolia*

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ABSTRACT

Activity guided isolation of a *Spiraea prunifolia* extract yielded five caffeoyl hemiterpene glycosides: 4′-(6-*O*-caffeoyl-*β*-D-glucopyranosyl)-2′-methyl butyric acid, 1-O-caffeoyl-6-O-(4′-hydroxy-2′-methylene-butyroyl)-*β*-D-glucopyranoside, 1,2-O-dicaffeoyl-6-O-(4′-hydroxy-2′-methylene-butyroyl)-*β*-D-glucopyranoside, 1-O-caffeoyl-6-O-(4′-caffeoyl-2′-methylene-butyroyl)-*β*-D-glucopyranoside, and 1-O-caffeoyl-6-O-(4′-caffeoyl-3′-hydroxy-2′-methylene-butyroyl)-*β*-D-glucopyranoside, and nine known compounds. Structures were elucidated by analysis of 1D and 2D NMR spectra and FAB-MS. To evaluate the anti-oxidative and anti-inflammatory properties of all fourteen compounds, DPPH radical scavenging, NBT superoxide scavenging, and inhibition of nitric oxide production in LPS-stimulated RAW264.7 cells were examined. Three of the caffeoyl hemiterpene glycosides exhibited potent anti-oxidative and anti-inflammatory activities compared with Vitamin C and L-NMMA, which were used as positive controls.

1. Introduction

Spiraea prunifolia (Rosaceae), commonly called ‘Bridal wreath’, is widespread in northeast Asia. The young leaves, fruits, and roots of *S. prunifolia* have been used for the treatment of malaria, fever, and emetic conditions in traditional medicine (Choudhary et al., 2009; Oh et al., 2001; So et al., 1999a,b). *Spiraea* species have also been reported to contain various diterpenes, diterpene alkaloids, terpenoid glycosides, and flavonoids (Hou et al., 2009). In the present study, phytochemical isolation yielded five new caffeoyl hemiterpene glycosides (**1**–**5**), along with nine known phenolic compounds. Structures were elucidated by analysis of 1D and 2D NMR spectroscopic data. All of the isolated compounds were evaluated for anti-oxidative and anti-inflammatory properties by measuring their DPPH radical scavenging activity, NBT superoxide scavenging activity, and ability to inhibit nitric oxide production in LPS-stimulated RAW264.7 cells.

2. Results and discussion

2.1. Structure elucidation

Compound **1** had a molecular formula of $C_{20}H_{26}O_{11}$ as indicated by HR-FAB-MS data (*m/z* 441.1396 $[M-H]^-$, Calcd. $C_{20}H_{25}O_{11}$, 441.1397). Its ^1H NMR spectrum (Table 1) was consistent with a caffeoyl moiety in the aromatic region as determined by the presence of an aromatic ABX-spin system with a *meta*-coupled aromatic signal [δ 7.05 (1H, d, J = 2.1 Hz, H-5)], *ortho*-coupled aromatic resonance [δ 6.77 (1H, d, J = 8.4 Hz, H-8)] and *ortho*–*meta*-coupled aromatic signal [δ 6.95 (1H, dd, J = 2.1, 8.4 Hz, H-9)] for a 3,4-dihydroxyphenyl unit, and two olefinic protons [δ 6.29 and 7.57 (1H, d, J = 15.9 Hz, H-2,3)] for a *trans*-configured double bond. In addition, the ^1H and ^{13}C NMR spectra of **1** showed the presence of a glucopyranosyl moiety with one methylene [δ 4.32 (1H, dd, J = 6.0, 12.0 Hz, glc-6a) and 4.48 (1H, dd, J = 2.1, 12.0 Hz, glc-6b)], five methines [δ 4.29 (1H, d, J = 7.8 Hz, glc-1), 3.51 (1H, ddd, J = 9.0, 6.0, 2.4 Hz, glc-5), 3.41 (1H, t, J = 9.0 Hz, glc-3), 3.37 (1H, t, J = 9.0 Hz, glc-4), and 3.21 (1H, dd, J = 9.0, 7.8 Hz, glc-2)], and six carbons (δ 63.3, 70.3, 73.6, 74.0, 76.4 and 103.0). The large coupling constant of the anomeric proton at δ 4.28 (J = 7.8 Hz, glc-1) indicated that the glucopyranoside was in a β-configuration. The ^1H and ^{13}C NMR spectra of **1** also showed the presence of a hemiterpene moiety with a single methyl group [δ 1.12 (3H, d, J = 6.6 Hz, H-5′)], two methylenes [δ 1.65, 1.97 (each 1H, m, H-3′) and 3.60, 3.89 (each 1H, m, H-4′)], one methine [δ 2.49 (1H, m, H-2′)], and five carbons (δ 16.9, 33.7, 37.9, 67.8, 181.7). The ^1H–^1H COSY spectrum of **1** (Fig. 2) showed a correlation of the methyl group proton signal (H-5′) with the methine proton (H-2′). Further, the methine proton was also correlated with a methylene at δ 1.65/1.97 (H-3′). In addition, the two methylenes were correlated with each other. These data led to the elucidation of the hemiterpene structure as 4′-hydroxy-2′-methyl-butyrate. The connectivities of the caffeoyl,

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Table 1
^{1}H[a] and ^{13}C[b] NMR spectroscopic data for compounds **1**–**5**.

Position	**1**		**2**		**3**		**4**		**5**	
	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)	δ C
Glucopyranosyl moiety										
1	4.29 d (7.8)	103.0	5.56 d (7.8)	94.3	5.78 d (8.4)	92.4	5.57 d (7.8)	94.2	5.56 d (7.8)	94.2
2	3.21 dd (9.0, 7.8)	73.6	3.45 dd (9.0, 7.8)	72.5	5.06 dd (9.6, 8.4)	72.6	3.45 dd (9.0, 7.8)	72.5	3.49 dd (9.0, 7.8)	72.6
3	3.41 t (9.0)	76.4	3.48 t (9.0)	76.4	3.75 t (9.6)	74.6	3.47 t (9.0)	76.5	3.58 t (9.0)	76.4
4	3.37 t (9.0)	70.3	3.41 t (9.0)	69.9	3.55 t (9.6)	70.1	3.42 t (9.0)	70.0	3.51 t (9.0)	70.0
5	3.51 ddd (9.0, 6.0, 2.4)	74.0	3.67 m	74.7	3.76 ddd (9.6, 5.4, 2.1)	74.9	3.69 ddd (9.0, 6.0, 1.8)	74.7	3.74 ddd (9.0, 6.0, 2.1)	74.6
6a	4.32 dd (12.0, 6.0)	63.3								
	4.28 dd (12.0, 5.4)	63.3	4.33 dd (12.0, 5.4)	63.1	4.32 dd (12.0, 6.0)	63.4	4.31 dd (12.0, 6.0)	63.2		
6b	4.48 dd (12.0, 2.4)		4.48 dd (12.0, 1.8)		4.52 dd (12.0, 2.1)		4.52 dd (12.0, 1.8)		4.56 dd (12.0, 2.1)	
Hemiterpene moiety										
1′		181.7		166.7		166.8		166.5		165.5
2′	2.49 m	37.9		137.1		137.1		136.8		140.2
3′	1.65/1.97 m	33.7	2.53 t (6.6)	34.9	2.56 t (6.6)	34.9	2.70 t (6.6)	31.4	4.81 t (4.8)	68.0
4′	3.60/3.89 m	67.8	3.66 t (6.6)	60.2	3.68 t (6.6)	60.2	4.33 m	62.4	4.28 d (4.8)	66.9
5′	1.12 d (6.6)	16.9	5.69/6.24 d (1.2)	126.7	5.71/6.27 d (1.2)	126.7	5.71/6.26 d (1.2)	127.1	6.06/6.41 br s	126.2
Caffeoyl moiety										
1″		167.7		166.2		166.9		167.7		167.7
2″	6.29 d (15.9)	113.5	6.30 d (15.9)	112.8	6.28 d (15.6)	113.1	6.28 d (15.9)	113.6	6.28 d (15.6)	113.5
3″	7.57 d (15.9)	145.8	7.66 d (15.9)	147.0	7.58 d (15.6)	147.5	7.63 d (15.9)	147.0	7.63 d (15.6)	147.0
4″		126.3		126.1		126.2		126.3		126.4
5″	7.05 d (2.1)	113.8	7.06 d (1.8)	113.8	7.02 d (1.8)	113.9	7.05 d (1.8)	113.9	7.05 d (1.8)	113.9
6″		145.4		145.4		145.4		145.4		145.4
7″		148.2		148.5		148.6		148.4		148.4
8″	6.77 d (8.4)	115.1	6.78 d (8.4)	115.1	6.76 d (8.4)	115.1	6.77 d (8.1)	115.1	6.75 d (7.8)	115.1
9″	6.95 dd (8.4, 2.1)	121.5	6.97 dd (8.4, 1.8)	121.9	6.96 dd (8.4, 1.8)	122.0	6.93 dd (8.1, 1.8)	121.9	6.91 dd (7.8, 1.8)	121.9
1‴						165.6		166.1		166.1
2‴					6.19 d (15.6)	112.2	6.21 d (15.9)	112.9	6.25 d (15.6)	112.9
3‴					7.58 d (15.6)	146.4	7.50 d (15.9)	145.6	7.55 d (15.6)	145.8
4‴						126.0		126.2		126.1
5‴					7.01 d (1.8)	113.7	7.02 d (1.8)	113.8	7.03 d (2.4)	113.8
6‴						145.3		145.3		145.3
7‴						148.3		148.1		148.1
8‴					6.75 d (8.1)	115.1	6.74 d (8.1)	115.1	6.77 d (8.4)	115.1
9‴					6.94 dd (8.1, 1.8)	121.7	6.91 dd (8.1, 1.8)	121.6	6.93 dd (8.4, 1.8)	121.6

[a] NMR data were measured in CD_3OD at 600 MHz.
[b] NMR data were measured in CD_3OD at 150 MHz.

glucose, and hemiterpene groups were elucidated by HMBC correlation signals. The HMBC spectrum of **1** (Fig. 2) established a correlation between the H-1 of glucose and the C-4′ of hemiterpene. The HMBC spectrum of **1** also showed a correlation between H-6 of glucose and carbonyl C-1″ of the caffeoyl moiety. Based on these data, **1** was determined to be 4′-(6-*O*-caffeoyl-*β*-D-glucopyranosyl)-2′-methyl butyric acid (Fig. 1). The naturally occurring caffeoyl hemiterpene glycosides have been rarely reported – aohada-glycosides (A, B and C) from *Ilex macropoda* (Fuchino et al., 1997) and rotundarpenosides (A and B) from *Ilex rotunda* (Kim et al., 2012) and hymenosides (N, O, P and V) from *Hymenophyllum barbatum* (Toyota et al., 2002). Comparing with these, **1** possessed different type of hemiterpene or sugar moiety.

Compound **2** had a molecular formula of $C_{20}H_{24}O_{11}$ as indicated by HR-FAB-MS data (*m/z* 439.1240 $[M-H]^-$, Calcd. $C_{20}H_{23}O_{11}$, 439.1240). Its ^{1}H NMR spectrum (Table 1) was consistent with a caffeoyl moiety in the aromatic region as determined by the presence of an aromatic ABX-spin system, *meta*-coupled aromatic signal [δ 7.06 (1H, d, *J* = 1.8 Hz, H-5″)], *ortho*-coupled aromatic resonance [δ 6.78 (1H, d, *J* = 8.4 Hz, H-8″)] and *ortho*–*meta*-coupled aromatic signal [δ 6.97 (1H, dd, *J* = 1.8, 8.4 Hz, H-9″)] for a 3,4-dihydroxyphenyl unit, and two olefinic protons [δ 6.30 and 7.66 (1H, d, *J* = 15.9 Hz, H-2″,3″)] for a *trans*-configured double bond. In addition, the ^{1}H and ^{13}C NMR spectra of **2** indicated the presence of a glucopyranosyl moiety with one methylene [δ 4.28 (1H, dd, *J* = 5.4, 12.0 Hz, glc-6a) and 4.48 (1H, dd, *J* = 1.8, 12.0 Hz, glc-6b)], five methines [δ 5.56 (1H, d, *J* = 7.8 Hz, glc-1), 3.67 (1H, m, glc-5), 3.48 (1H, t, *J* = 9.0 Hz, glc-3), 3.45 (1H, dd, *J* = 9.0, 7.8 Hz, glc-2), and 3.41 (1H, t, *J* = 9.0 Hz, glc-4)], and six carbons (δ 63.3, 69.9, 72.5, 74.7, 76.4 and 94.3). The large coupling constant of the anomeric proton at δ 5.56 (*J* = 7.8 Hz, glc-1) indicated that the glucopyranoside as in a *β*-configuration. The ^{1}H and ^{13}C NMR spectra of **2** also established the presence of a hemiterpene moiety, exomethylene protons [δ 5.69 and 6.24 (each 1H, d, *J* = 1.2 Hz, H-5′)], two methylenes [δ 2.53 (2H, t, *J* = 6.6 Hz, H-3′), δ 3.66 (2H, t, *J* = 6.6 Hz, H-4′)], and five carbons (δ 34.9, 60.2, 126.7, 137.1, 166.7). The HMBC spectrum of **2** (Fig. 3) showed correlations between the exomethylene proton signals (H-5′) with the quaternary carbon at δ 166.7 (C-1′) and the methylene carbon at δ 34.9 (C-3′). These data led to the elucidation of the hemiterpene structure as 4′-hydroxy-2′-methylene-butyrate. The connectivities of the caffeoyl, glucose, and hemiterpene groups were elucidated by HMBC correlation signals. The HMBC spectrum of **2** showed a correlation between the H-1 of glucose and carbonyl C-1″ of the caffeoyl moiety. The HMBC spectrum of **2** also showed a correlation between H-6 of glucose and C-1′ of the hemiterpene. Therefore, **2** was deduced as 1-*O*-caffeoyl-6-*O*-(4′-hydroxy-2′-methylene-butyroyl)-*β*-D-glucopyranose (Fig. 1). The hemiterpene glycoside portion of compound **2**, excluding the caffeoyl moiety, was previously isolated from a *Tulipa* species and named 6-Tuliposide A (Christensen, 1999, 1995a,b; Christensen and Kristiansen, 1999, 1995; Tschesche et al., 1968). Thus, **2** was named 1-caffeoyl-6-tuliposide A.

Compound **3** was isolated as a pale yellow amorphous powder. Its HR-FAB-MS (*m/z* 601.1556 $[M-H]^-$ Calcd. for $C_{29}H_{29}O_{14}$, 601.1557) indicated that the molecular formula was $C_{29}H_{30}O_{14}$. The ^{1}H and ^{13}C NMR spectra of **3** (Table 2) were similar to those of **2** except for the presence of other signals for a caffeoyl moiety,