Feruloyl sucrose derivatives from *Bistorta manshuriensis*

Ki Hyun Kim, Sang Wook Chang, and Kang Ro Lee

Abstract: *Bistorta manshuriensis* is a well-known Korean medicinal plant traditionally used to treat diarrhea. Phytochemical investigation of the aerial parts of *B. manshuriensis* led to the isolation of two new feruloyl sucrose derivatives, bistoroside A (**1**) and bistoroside B (**2**), together with three known compounds, helonioside A (**3**), helonioside B (**4**), and smilaside L (**5**). The structures of the new compounds were elucidated as (3,6-di-*O*-*Z*-feruloyl)-β-D-fructofuranosyl-(1→2)-α-D-glucopyranoside (**1**) and (3,6-di-*O*-*Z*-feruloyl)-β-D-fructofuranosyl-(1→2)-(6′-*O*-acetyl)-α-D-glucopyranoside (**2**) on the basis of extensive analysis of 1D and 2D NMR (HMQC and HMBC), HR-FAB-MS, and chemical evidence.

Key words: Bistorta manshuriensis, Polygonaceae, feruloyl sucrose derivatives, bistoroside A, bistoroside B.

Résumé : La *Bistorta manshuriensis* est une plante médicinale coréenne bien connue pour le traitement traditionnel de la diarrhée. Une étude phytochimique des parties aériennes du *B. manshuriensis* a permis d'isoler deux nouveaux dérivés féruloyles du sucrose, le bistoroside A (**1**) et le bistoroside B (**2**) aux côtés de trois composés connus, l'hélonioside A (**3**), l'hélonioside B (**4**) et le smilaside L (**5**). En se basant sur des études extensives de RMN 1D et 2D, de spectrométrie de masse à haute résolution et bombardement avec des atomes rapides (SM-HR-BAR) et de données chimiques, on a établi que le composé **1** est le (3,6-di-*O*-*Z*-féruloyl)-β-D-fructofuranosyl-(1→2)-α-D-glucopyranoside alors que le composé **2** est le (3,6-di-*O*-*Z*-féruloyl)-β-D-fructofuranosyl-(1→2)-(6′-*O*-acétyl)-α-D-glucopyranoside.

Mots-clés : Bistorta manshuriensis, polygonacée, dérivés féruloyles du sucrose, bistoroside A, bistoroside B.

[Traduit par la Rédaction]

Introduction

Bistorta manshuriensis KOM (Polygonaceae) (Korean name: Bum-ko-ri) is an erect perennial herb that is widely distributed throughout Korea.[1] The scientific name of this plant has also been used in China in combination with *Polygonum bistorta*. The rhizomes of *B. manshuriensis* have been used in traditional Korean medicine for the treatment of diarrhea.[1] In Chinese folk medicine, its rhizomes have been used to treat dysentery with bloody stools in acute gastroenteritis, acute respiratory infection, and venomous snake bite.[2] It has been reported that the crude extract of *P. bistorta* exhibits several potent pharmacological activities, including antibacterial, anti-inflammatory activities, and antimutation effect of 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1).[3–6] Previous phytochemical investigation of *P. bistorta* revealed the presence of triterpenoids, flavones, coumarins, and phenolic acids.[5–10] Despite several studies on *P. bistorta*, little is known about the phytochemical constituents of *B. manshuriensis*. Therefore, as a part of our continuing search for novel secondary metabolites from Korean medicinal plants, we investigated the constituents of the aerial parts of *B. manshuriensis* and isolated cerebrosides, flavonoids, and phenolic constituents from this source.[11] Herein, we report the further isolation and structure elucidation of two new feruloyl sucrose derivatives, bistoroside A (**1**) and bistoroside B (**2**), as well as three previously reported compounds, helonioside A (**3**), helonioside B (**4**), and smilaside L (**5**).

Results and discussion

The EtOAc-soluble portion of the 80% MeOH extract of the aerial parts of *B. manshuriensis* was subjected to repeated column chromatography on silica gel to afford two new feruloyl sucrose derivatives (**1**–**2**), together with three known compounds (**3**–**5**) (Fig. 1). Their structures were elucidated by 1D and 2D NMR, HR-FAB-MS, and comparison with published data.

Bistoroside A (**1**) was isolated as a yellowish gum with a molecular formula of $C_{32}H_{38}O_{17}$ on the basis of the $[M + H]^+$ peak at *m/z* 695.2180 (calcd. for $C_{32}H_{39}O_{17}$: 695.2187) in the HR-FAB-MS. Its IR and UV spectra displayed absorption bands for the hydroxyl and α,β-unsaturated aromatic ester groups. The ^{1}H NMR spectrum (Table 1) of **1** showed two pairs of olefinic protons at δ 6.92 and 5.90 (each 1H, d, J = 13.0 Hz, H-7″, 8″), 6.89 and 5.83 (each 1H, d, J = 13.0 Hz, H-7‴, 8‴), and two aromatic moieties with ABX coupling patterns at δ 6.82 (d, J = 8.5 Hz, H-5″), 7.16 (dd, J = 8.5, 2.0 Hz, H-6″), and 7.83 (d, J = 2.0 Hz, H-2″) and at δ 6.78 (d, J = 8.5 Hz, H-5‴), 7.13 (dd, J = 8.5,

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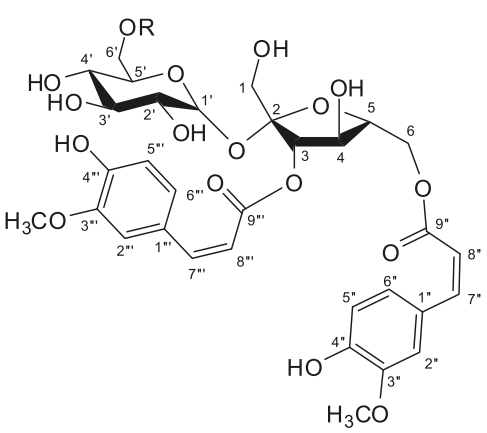
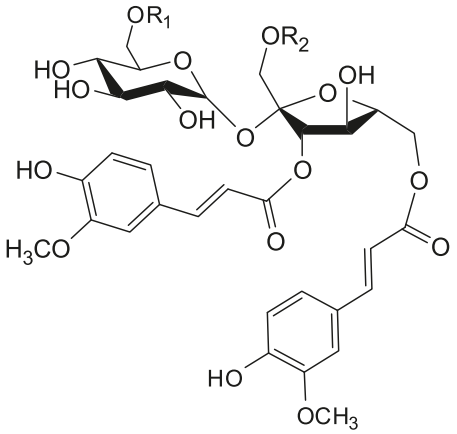
Fig. 1. Chemical structures of compounds **1**–**5**.



1 R = H
2 R = $COCH_3$



3 R_1 = H R_2 = H
4 R_1 = $COCH_3$ R_2 = H
5 R_1 = H R_2 = (*E*)-feruloyl

2.0 Hz, H-6‴), and 7.83 (d, *J* = 2.0 Hz, H-2‴). The coupling constant (13.0 Hz) of the proton signals at δ 6.92/5.90 and 6.89/5.83 suggested the presence of two pairs of *cis*-olefinic protons.[12] The above ^{1}H NMR data, along with two methoxy groups at the aromatic moieties as determined from the HMBC spectrum, indicated the presence of two *cis*-feruloyl units in **1**.[13] In addition, signals for eight oxygenated methines at δ 5.47 (d, *J* = 8.0 Hz, H-3), 4.42 (t, *J* = 7.0 Hz, H-4), 4.16 (m, H-5), 5.42 (d, *J* = 4.0 Hz, H-1′), 3.42 (dd, *J* = 10.0, 4.0 Hz, H-2′), 3.67 (t, *J* = 10.0 Hz, H-3′), 3.40 (t, *J* = 10.0 Hz, H-4′), and 3.95 (m, H-5′), three oxygenated methylenes at δ 3.60 and 3.65 (each 1H, d, *J* = 12.0 Hz, H-1), 4.48 (dd, *J* = 12.0, 4.0 Hz, H-6a), 4.54 (dd, *J* = 12.0, 7.0 Hz, H-6b), 3.78 (dd, *J* = 12.0, 6.0 Hz, H-6′a), and 3.91 (dd, *J* = 12.0, 2.5 Hz, H-6′b) were observed in the ^{1}H NMR spectrum. A characteristic anomeric signal at δ 5.42 with a small coupling constant (d, *J* = 4.0 Hz, H-1′), together with 12 oxygenated carbon signals containing two anomeric carbons (δ 105.1 and 93.3) in the ^{13}C NMR spectrum, suggested that **1** possessed a disaccharide moiety. Alkaline hydrolysis of **1** gave methyl (*Z*)-ferulate,[13] together with sucrose [α-D-Glc-(1→2)-β-D-Fru], which was confirmed by HMBC analysis indicating linkage of the two sugars and by comparing HPLC and optical rotation data with those of an authentic sample.[14] On further inspection of the HMBC spectrum, each (*Z*)-feruloyl moiety at C-3 and at C-6 in fructose (Fru) could be assigned unambiguously. The correlations between H-3 (δ 5.47) and H-6 (δ 4.48 and 4.54) of fructose and the corresponding carbonyl carbons (δ 168.3 and 168.4) of (*Z*)-ferulate were observed in the HMBC spectrum, respectively (Fig. 2). Thus, the structure of **1** was determined as (3,6-di-*O*-*Z*-feruloyl)-β-D-fructofuranosyl-(1→2)-α-D-glucopyranoside, and it was named bistoroside A (Fig. 1).

Bistoroside B (**2**) was isolated as a yellowish gum. Its molecular formula was determined as $C_{34}H_{40}O_{18}$ from the $[M + H]^+$ peak at *m*/*z* 737.2298 (calcd. for $C_{34}H_{41}O_{18}$: 737.2293) in the HR-FAB-MS spectrum. The ^{1}H and ^{13}C NMR spectra of **2** were almost identical to those of **1** (Table 1), except for the presence of an acetyl group signal. This indicated that **2** possessed a structure similar to **1**, containing glucose (Glc) and fructose (Fru) units, two (*Z*)-feruloyl moieties, and one additional acetyl group. Acetylation of **2** yielded an octaacetate (**2a**) identical to that obtained from acetylation of **1**, indicating that the two (*Z*)-feruloyl moieties were located at C-3 and C-6 of the fructose unit in **2**, as in the structure of **1**. In the ^{1}H and ^{13}C NMR spectra of **2**, the signals of H-6′ and C-6′ in **2** were shifted to lower field (δ 4.13, δ 4.48, and δ 65.5 for **2**; δ 3.78, δ 3.91, and δ 62.6 for **1**), while that of C-5′ was shifted to higher field (δ 71.7 for **2**; δ 74.5 for **1**) when comparing the NMR spectra of **1** and **2**. This supported the assignment of the acetyl group at C-6′ of glucose. The HMBC experiment of **2** displayed correlations between the protons at δ 4.13 and 4.48 (H-6′ of Glc) and the carbonyl carbon at δ 172.5, confirming that the acetyl group was located at C-6′ of Glc. On the basis of this evidence, the structure of **2** was determined as (3,6-di-*O*-*Z*-feruloyl)-β-D-fructofuranosyl-(1→2)-(6′-*O*-acetyl)-α-D-glucopyranoside, and it was named bistoroside B (Fig. 1).

Three other known compounds obtained in this investigation were identified as helonioside A (**3**),[14] helonioside B (**4**),[14] and smilaside L (**5**)[15] by comparison of their spectroscopic data with those published in the literature (Fig. 1). Although several sucrose phenylpropanoid esters have previously been isolated from *Polygonum* sp.,[16] this is the first report of compounds from this class being isolated from a medicinal plant from the genus *Bistorta*.

Experimental

General experimental procedures

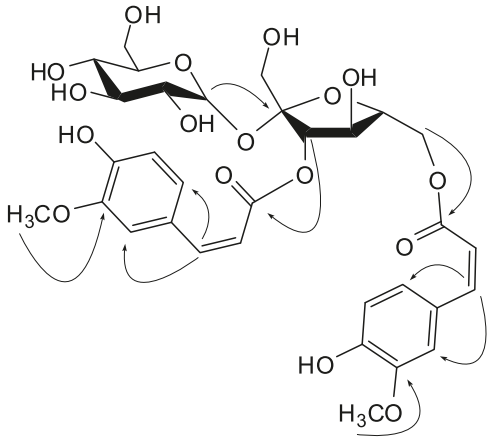
Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FTIR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV–vis spectrophotometer. FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^{1}H-^{1}H COSY, HMQC, and HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** in CD_3OD.

	1		**2**	
Position	^{1}H (J = Hz)	^{13}C	^{1}H (J = Hz)	^{13}C
β-D-Fru 1	3.60 d (12.0)	65.3	3.59 d (12.0)	65.2
	3.65 d (12.0)		3.64 d (12.0)	
2		105.1		104.4
3	5.47 d (8.0)	79.1	5.49 d (8.0)	78.4
4	4.42 t (7.0)	75.0	4.45 t (7.0)	73.8
5	4.16 m	81.3	4.15 m	80.8
6	4.48 dd (12.0, 4.0)	66.3	4.49 dd (12.0, 4.0)	65.3
	4.54 dd (12.0, 7.0)		4.52 dd (12.0, 7.0)	
α-D-Glc 1′	5.42 d (4.0)	93.3	5.43 d (4.0)	92.2
2′	3.42 dd (10.0, 4.0)	73.3	3.44 dd (10.0, 4.0)	72.6
3′	3.67 t (10.0)	74.9	3.64 t (10.0)	74.3
4′	3.40 t (10.0)	71.4	3.26 t (10.0)	71.5
5′	3.95 m	74.5	4.18 m	71.7
6′	3.78 dd (12.0, 6.0)	62.6	4.13 dd (12.0, 6.0)	65.5
	3.91 dd (12.0, 2.5)		4.48 dd (12.0, 2.5)	
Feruloyl 1″		128.2		127.6
2″	7.83 d (2.0)	115.17	7.82 d (2.0)	114.84
3″		147.93		147.93
4″		149.89		149.3
5″	6.82 d (8.5)	116.65	6.81 d (8.5)	115.26
6″	7.16 dd (8.5, 2.0)	127.1	7.16 dd (8.5, 2.0)	126.6
7″	6.92 d (13.0)	146.29	6.91 d (13.0)	145.5
8″	5.90 d (13.0)	115.9	5.89 d (13.0)	115.29
9″		168.4		167.7
OCH_3	3.89 s	56.68	3.88 s	56.1
Feruloyl 1‴		128.1		127.5
2‴	7.83 d (2.0)	115.16	7.81 d (2.0)	114.81
3‴		147.91		147.91
4‴		149.81		149.2
5‴	6.78 d (8.5)	116.63	6.78 d (8.5)	115.24
6‴	7.13 dd (8.5, 2.0)	127.0	7.11 dd (8.5, 2.0)	126.5
7‴	6.89 d (13.0)	146.26	6.86 d (13.0)	145.4
8‴	5.83 d (13.0)	115.8	5.82 d (13.0)	115.6
9‴		168.3		167.4
OCH_3	3.87 s	56.64	3.86 s	56.0
OAc-6′				172.5
			2.10 s	20.5

Note: NMR data were obtained at 500 MHz for ^{1}H and at 125 MHz for ^{13}C.

Fig. 2. Key HMBC (→) correlations of **1**.



Gilson 306 pump with Shodex refractive-index detector and Apollo Silica 5 μm column (250 × 10 mm). Silica gel 60 (Merck, 70–230 mesh and 230–400 mesh) and RP-C_{18} silica gel (Merck, 230–400 mesh) were used for column chromatography. The packing material for molecular-sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low pressure liquid chromatography was carried out over a LiChroprep Lobar®-A RP-18 (240 × 10 mm) column with a FMI QSY-0 pump (ISCO). Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (*v*/*v*).

Plant material

The aerial parts of *B. manshuriensis* (2.9 kg) were collected from Mount Daeduk, Gangwon Province, Korea, in June 2008. The plants were authenticated by one of the au-

thors (K.R.L.). A voucher specimen (SKKU-2008–6) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The dried aerial parts of *B. manshuriensis* (2.9 kg) were extracted three times at room temperature with 80% MeOH over a period of 3 days and evaporated under reduced pressure to give a crude extract (219 g). The crude extract was dissolved in distilled water (800 mL) and successively extracted with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH to provide *n*-hexane (10 g), CH_2Cl_2 (2 g), EtOAc (9 g), and *n*-BuOH extracts (32 g). The EtOAc-soluble extract (9 g) was subjected to normal-phase column chromatography (CC) over silica gel using $CHCl_3$/MeOH of increasing polarity (7:1 to 1:1) to give six fractions (E1–E6). Fraction E2 (1.1 g) was subjected to reversed-phase column chromatography over an RP-C_{18} silica gel using MeOH/H_2O (3:2) to provide five subfractions (E21–E25). Subfraction E21 (220 mg) was chromatographed on LiChroprep Lobar®-A RP-18 using MeOH/H_2O (1:1) to give four subfractions (E211–E214). Subfraction E213 (45 mg) was separated on Sephadex LH-20 column using 100% MeOH to yield bistoroside A (**1**, 8 mg) and helonioside A (**3**, 5 mg). Subfraction E22 (130 mg) was subjected to LiChroprep Lobar®-A RP-18 column using MeOH/H_2O (7:3) to afford three subfractions (E221–E223). Subfraction E223 (52 mg) was separated by preparative normal-phase HPLC using a solvent system of $CHCl_3$/MeOH (6:1) over 30 min at a flow rate of 2.0 mL/min to obtain bistoroside B (**2**, 26 mg) and helonioside B (**4**, 5 mg). Subfraction E23 (150 mg) was separated over LiChroprep Lobar®-A RP-18 column using MeOH/H_2O (1:1) to afford smilaside L (**5**, 8 mg).

Bistoroside A (1)

Yellowish gum. $[\alpha]_D^{25}$ – 6.5 (*c* 0.25, MeOH). UV λ_{max} (MeOH) nm: 326, 299 (sh), 235, 217. IR (KBr) ν_{max}: 3354, 2945, 2833, 1699, 1453, 1597, 1278, 1030 cm^{-1}. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data: see Table 1. HR-FAB-MS *m*/*z*: 695.2180 $[M + H]^+$ (calcd. for $C_{32}H_{39}O_{17}$: 695.2187).

Bistoroside B (2)

Yellowish gum. $[\alpha]_D^{25}$ – 4.7 (*c* 0.65, MeOH). UV λ_{max} (MeOH) nm: 327, 299 (sh), 235, 218. IR (KBr) ν_{max}: 3357, 2945, 2833, 1701, 1598, 1518, 1453, 1278, 1121, 1029 cm^{-1}. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data: see Table 1. HR-FAB-MS *m*/*z*: 737.2298 $[M + H]^+$ (calcd. for $C_{34}H_{41}O_{18}$: 737.2293).

Alkaline hydrolysis of 1 and 2

Compounds (**1**: 2.5 mg and **2**: 6.0 mg) were hydrolyzed with 3% KOH/MeOH (3 mL) at room temperature for 15 min. The reaction mixture was neutralized with 2N HCl (monitored with indicator paper) and filtered. The filtrate was concentrated under reduced pressure to give a residue that was chromatographed over Sephadex LH-20 (100% MeOH) and preparative normal-phase HPLC ($CHCl_3$/MeOH/H_2O, 9:7:1.5) to afford methyl (*Z*)-ferulate[13] (0.3 mg from **1**; 1.2 mg from **2**) and sucrose (0.8 mg from **1**; 2.7 mg from **2**). The sucrose was identified by comparison of its optical rotation value, $[\alpha]_D^{25}$ + 26.6 (*c* 0.15, MeOH) with that of an authentic sample[14] and by co-TLC comparison with an authentic sample ($CHCl_3$/MeOH/H_2O, 9:7:1.5; R_f = 0.31). The sucrose was detected by co-injection of an authentic sample on preparative normal-phase HPLC using a solvent system of $CHCl_3$/MeOH/H_2O (9:7:1.5) at a flow rate of 2.0 mL/min in the same manner above, giving a single peak at 11.03 min.

Acetylation of 1 and 2

To each compound (**1**: 3.0 mg; **2**: 3.0 mg) mixed with pyridine (1 mL), acetic anhydride (1 mL) was added. Each solution was stirred at room temperature for 24 h. Each reaction solution was extracted with $CHCl_3$ three times to give each octaacetate, which was purified by a silica gel Waters Sep-Pak® Vac 6cc (hexane/EtOAc, 5:1). Octaacetate of **1** (**1a**): colorless gum. FAB-MS *m*/*z*: 1031 $[M + H]^+$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.93, 7.91 (1H each, d, *J* = 2.0 Hz, H-2″, H-2‴), 7.34–7.10 (4H, m, H-5″, H-5‴, H-6″, H-6‴), 7.02, 6.97 (1H each, d, *J* = 13.0 Hz, H-7″, H-7‴), 5.95, 5.88 (1H each, d, *J* = 13.0 Hz, H-8″, H-8‴), 5.71 (1H, d, *J* = 3.5 Hz, H-1′), 5.63 (1H, d, *J* = 6.0 Hz, H-3), 5.55 (1H, t, *J* = 6.0 Hz, H-4), 5.45 (1H, t, *J* = 9.5 Hz, H-3′), 5.01 (1H, t, *J* = 9.5 Hz, H-4′), 4.90 (1H, dd, *J* = 9.5, 3.5 Hz, H-2′), 4.40, 4.17 (1H each, d, *J* = 12.0 Hz, H_2-1), 4.55–4.15 (6H, m, H-5, H_2-6, H-5′, H_2-6′), 3.91, 3.88 (3H each, s, OCH_3), 2.30, 2.28 (3H each, s, OAc × 2), 2.13, 2.12, 2.10, 2.05, 1.97, 1.81 (3H each, s, OAc × 6). Octaacetate of **2** (**2a**) was identical to **1a** in terms of ^1H NMR and TLC.

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