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Original article

Two new β-hydroxy amino acid-coupled secoiridoids from the flower buds of *Lonicera japonica*: Isolation, structure elucidation, semisynthesis, and biological activities

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ABSTRACT

Two new β-hydroxy amino acid-coupled secoiridoids, named serinosecologanin (**1**) and threoninosecologanin (**2**), were isolated from an aqueous extract of the flower buds of *Lonicera japonic*a. Their uncommon structures including absolute configurations were determined by spectroscopic data analysis, and confirmed by semisynthesis from the co-occurring secologanin (**3**) and secologanic acid (**4**). Compounds **1** and **2** exhibited resistant activity against β-glucosidase from almonds and hesperidinase from *Aspergillus niger*, they also showed activity against the release of glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor with inhibition rates of 34.9 ± 3.1% and 53.6 ± 2.6%, respectively.

1. Introduction

Jin Yin Hua, the flower buds of *Lonicera japonica* Thunb. (Caprifoliaceae), also known as honeysuckle, is one of the most common ingredients of formulations used in traditional Chinese medicine for treating influenza, colds, fevers, and infections [1]. Chemical and pharmacological studies have resulted in characterization of constituents with different structural features and biological activities from extracts of this medicine, including caffeoyl quinic acids, secoiridoids, flavonoids, saponins, cerebrosides, polyphenols, and nitrogen containing iridoids [2–13]. As part of a program to assess the chemical and biological diversity of traditional Chinese medicines, we conducted detailed chemical analysis of an aqueous extract of the flower buds of *L. japonica*, since the flower bud decoction is practically used in the formulations. Our previous studies on the aqueous extract led to the isolation of 27 homosecoiridoids having structural characters of the secoiridoid nucleus coupled with *N*-substituted nicotinic acid or pyridine units (lonijaposides A–W), and the secoiridoid nucleus coupled with phenylpyruvic acid derived moieties (loniphenyruviridosides A–D) [14–16]. In addition, after the flower buds were extracted by water, the residue was further extracted with EtOH (95%), from which six new aromatic glycosides and 48 known compounds were characterized [17,18]. Some of these compounds showed antiviral activity against the influenza virus A/Hanfang/359/95 (H3N2) and Coxsackie virus B3 replication, as well as anti-inflammatory activity against the release of glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor and STAT-3 (signal transducers and activators of transcription 3) inhibitory activity. In continuing investigations on the aqueous extract, two uncommon β-hydroxy amino acid-coupled secoiridoids **1** and **2** have been characterized. We report herein the isolation, structure elucidation, semisynthesis, and biological activities of these two isolates.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a PE Model 343. UV spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). 1D- and 2D-NMR spectra were obtained at 500 MHz or 600 MHz for ^{1}H, and 125 MHz or 150 MHz

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for ^{13}C, respectively, on INOVA 500 MHz or SYS 600 MHz spectrometers with solvent peaks as references (unless otherwise noted). FABMS and HR-FABMS data were measured on a Micromass Auto spec-Ultima ETOF spectrometer. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HR-ESIMS data were, in turn, measured on an AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Pharmadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an instrument with a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector on a Prevail (250 × 10 mm i.d.) semi-preparative column packed with C_{18} (5 μm). Glass precoated silica gel GF254 plates were used for TLC. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

2.2. Plant material

See Ref. [14].

2.3. Extraction and isolation

For extraction and preliminary fractionation of the extract, see Refs. [14,15]. Fraction B2 (8.4 g) was separated by flash chromatography over RP silica gel, eluting with a gradient of EtOH (0–100%) in H_2O to give subfractions (B2-1–B2-20). B2-11 (1.2 g) was subjected to RP-HPLC, using CH_3CN–H_2O (12:88) containing 0.1% HOAc as the mobile phase, to afford fractions B2-11-1–B2-11-8. B2-11-6 (68 mg) was purified by RP-HPLC, using CH_3CN–H_2O (16:84) containing 0.1% HOAc as the mobile phase, to yield **2** (8.9 mg, 0.00007%). Fraction B4 (86 g) was chromatographed over a RP silica gel column, eluting with a gradient of EtOH (0–100%) in H_2O, to yield subfractions B4-1–B4-7, of which subfraction B4-7 (1.4 g) was further separated by flash chromatography over RP silica gel, eluting with a gradient of MeOH (0–50%) in H_2O to give subfractions B4-7-1–B4-7-4. B4-7-4 (108 mg) was subjected to RP-HPLC, using CH_3OH–H_2O (6:4) containing 0.1% HOAc as the mobile phase, to afford **1** (59.3 mg, 0.00049%).

Serinosecologanin (**1**): white amorphous powder, soluble in H_2O, MeOH, and EtOH; $[\alpha]_D^{20}$ −183.4 (*c* 0.40, H_2O); UV (H_2O) λ_{max} (log ε) 241.0 (4.06) nm; IR ν_{max} 3326, 2883, 1720 (sh), 1657, 1584, 1465, 1400, 1358, 1314, 1255, 1210, 1168, 1056, 1014, 913, 887, 837, 751, 713, 693, 633 cm^{-1}; ^1H NMR (D_2O, 600 MHz), see Table 1; ^{13}C NMR (D_2O, 150 MHz), see Table 1; (+)-FABMS *m/z* 444 $[M+H]^+$, 466 $[M+Na]^+$, 482 $[M+K]^+$; HR-ESIMS *m/z* 444.1512 $[M+H]^+$ (calcd. for $C_{19}H_{26}NO_{11}$ 444.1506).

Threninosecologanin (**2**): white amorphous powder, soluble in H_2O, MeOH, and EtOH; $[\alpha]_D^{20}$ −156.8 (*c* 0.37, H_2O); UV (H_2O) λ_{max} (log ε) 240 (4.36) nm; IR ν_{max} 3386, 2935, 1722, 1656, 1589, 1560, 1399, 1341, 1310, 1207, 1170, 1121, 1064, 1014, 955, 899, 840, 771, 751, 694 cm^{-1}; ^1H NMR (D_2O, 500 MHz), see Table 1; ^{13}C NMR (D_2O, 125 MHz), see Table 1; (+)-ESIMS *m/z* 458 $[M+H]^+$, 480 $[M+Na]^+$, 496 $[M+K]^+$; (−)-ESIMS *m/z* 456 $[M-H]^-$, 491 $[M+Cl]^-$; HR-ESIMS *m/z* 480.1494 $[M+Na]^+$ (calcd. for $C_{20}H_{27}NO_{11}Na$ 480.1482).

2.4. Enzymatic hydrolysis of **1** and **2**

Each compound (~1 mg) in H_2O (~1 mL) was treated with β-glucosidase from almonds (10 mg, 8.92 U/mg, Mw 135000, Sigma–Aldrich Corporation, USA), hesperidinase from *Aspergillus niger* (10 mg, 3 U/g, Sigma–Aldrich), or snailase (5 mg, Shanghai Sangon Biotech Co. Ltd., China) at 37 °C for 20–96 h. Thin layer chromatography (TLC, $CHCl_3$–MeOH–HOAc 3:1:0.3) detection indicated that **1** and **2** were not hydrolyzed by β-glucosidase and hesperidinase, but disappeared on hydrolysis with snailase. Then, an aqueous solution (1 mL) of each compound (5 mg) was treated with snailase (20 mg) at 37 °C for 12 h. The reaction mixtures were extracted with EtOAc (2 × 3 mL). The H_2O phases were separately concentrated to dryness, and the residues were chromatographed over silica gel, eluting with CH_3CN–H_2O (6:1), to yield glucose with $[\alpha]_D^{20}$ values of +47.3 (*c* 0.19, H_2O) and +43.1 (*c* 0.07, H_2O) from the hydrolysates of **1** and **2**. The solvent system

Table 1
NMR spectroscopic data for compounds **1** and **2**.[a]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	5.58 d (1.8)	99.8	5.58 s	99.9
3	7.47 brs	151.2	7.46 d (1.5)	151.1
4		109.6		109.6
5	3.10 ddd (13.2, 6.0, 3.6)	26.5	3.08 ddd (14.0, 6.0, 3.5)	26.5
6α	1.43 ddd (13.2, 12.0, 9.6)	32.2	1.44 ddd (14.0, 12.0, 9.5)	32.3
6β	2.31 ddd (12.0, 3.6, 3.6)		2.30 ddd (12.0, 3.5, 3.5)	
7	5.13 dd (9.6, 3.6)	90.2	5.21 dd (9.5, 3.5)	90.1
8	5.50 ddd (16.8, 10.2, 10.2)	134.2	5.56 ddd (16.5, 10.5, 10.5)	134.2
9	2.85 ddd (10.2, 6.0, 1.8)	45.2	2.85 dd (10.5, 6.0)	45.2
10a	5.39 dd (16.8, 1.2)	123.6	5.39 d (16.5)	123.6
10b	5.31 dd (10.2, 1.2)		5.32 d (10.5)	
11		167.2		167.1
1′	4.86 d (8.4)	101.0	4.86 d (8.0))	101.0
2′	3.31 dd (9.6, 8.4)	75.4	3.31 dd (9.0, 8.0)	75.4
3′	3.52 t (9.6)	78.2	3.52 dd (9.0, 9.0)	78.2
4′	3.42 t (9.6)	72.3	3.42 dd (9.0, 9.0)	72.3
5′	3.54 m	79.0	3.54 m	79.0
6′a	3.94 dd (12.6, 1.8)	63.5	3.94 dd (12.5, 1.5)	63.5
6′b	3.75 dd (12.6, 6.0)		3.75 dd (12.5, 6.0)	
1″		176.6		177.2
2″	4.71 t (8.4)	60.8	4.12 d (8.0)	66.9
3″α	4.58 dd (9.0, 8.4)	70.8		
3″β	3.95 dd (9.0, 8.4)		4.27 dq (8.0, 6.0)	80.3
4″			1.53 d (6.0)	20.7

[a] NMR data (δ) were measured in D_2O for **1** at 600 MHz for ^1H and at 150 MHz for ^{13}C, respectively, and for **2** at 500 MHz for ^1H and at 125 MHz for ^{13}C. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ^1H–^1H gCOSY, gHSQC, and gHMBC experiments. The ^{13}C NMR data were presented as calculated using C-6′ (δ 63.5 ppm) as the reference.

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CH_3CN–H_2O (4:1) was used for TLC identification of glucose (R_f = 0.39).

2.5. Determination of the absolute configurations of amino acid units in 1 and 2

Compounds **1** (1.0 mg) and **2** (0.7 mg) were separately hydrolyzed with 6 N HCl (200 μL) in a sealed glass bomb at 110 °C for 16 h. The solutions were evaporated *in vacuo*. To the residues, FDAA [(1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide] solution in acetone (1%, 300 μL) and 6% aqueous triethylamine (150 μL, 1 M) were added. The mixture was stirred at 40 °C for 1 h, then diluted with H_2O (500 μL), and filtered. The standard FDAA-amino acids were prepared in the same way, using L-serine, D-serine, L-threonine, and D-threonine (1.1–1.3 mg), respectively. The FDAA-amino acid derivatives from the hydrolysate were compared with the standard FDAA-amino acids by HPLC analysis: Alltech Alltima C_{18} column (250 × 4.6, 5 μm), flow rate 1 mL/min, UV detection at 340 nm, mobile phase CH_3CN–H_2O (18:82) containing 1% AcOH. The retention times t_R, are as follows: FDAA derivative of the hydrolysate from **1**, 24.2 min; FDAA derivative of the hydrolysate from **2**, 32.4 min; FDAA-L-serine, 24.1 min; FDAA-D-serine, 26.5 min; FDAA-L-threonine, 32.4 min; and FDAA-D-threonine, 43.6 min.

2.6. Synthesis of 1 and 2

Secologanin (**3**) or secologanic acid (**4**) (30–50 mg) was refluxed with two molar equivalents of L-serine or L-threonine in acetonitrile or pyridine for 30–50 h, respectively. The reaction mixtures were evaporated to give corresponding residues which were separately isolated by preparative thin layer chromatography (PTLC) over silica gel, eluting with the mobile phase $CHCl_3$–MeOH–HOAc (3:1:0.3), to afford **1** and **2** (64–93% yields) from the reactions of **3** or **4** with L-serine and L-threonine, respectively. The ^{1}H NMR, ESIMS, and $[\alpha]_D^{20}$ data of **1** and **2** were consistent with those of the natural products.

2.7. Determination of β-glucosidase resistance of 1 and 2

Standard solutions (10 mmol/L) of **1**, **2**, and sweroside, and a solution (0.66 mg/mL) of β-glucosidase (from almonds) were prepared with a KH_2PO_4/K_2HPO_4 buffer (pH 6.8) [19]. A mixture of the standard solutions of sweroside (100 μL) and enzyme (100 μL), as well as a mixture of the standard solutions of sweroside (100 μL), enzyme (100 μL), and **1** (100 μL) or **2** (100 μL) were incubated at 37 °C. After incubation for 10, 30, and/or 60 min, the mixtures were analyzed by RP-HPLC using an Altech Brava C_{18} column (250 mm × 4.6 mm i.d., 5 μm) and mobile phase CH_3CN–H_2O (15:85) containing 0.1% HOAc. The chromatograms indicated that **1** and **2** were not hydrolyzed by β-glucosidase, whereas sweroside was hydrolyzed. In addition, the hydrolysis of sweroside with the enzyme was not disturbed by the presence of **1** and **2**.

2.8. Assays for pharmacological activities of 1 and 2

Details may be found in Refs. [14–16] and the references cited therein.

3. Results and discussion

Compound **1** was obtained as a white amorphous solid, $[\alpha]_D^{20}$ −183.4 (*c* 0.40, H_2O). Its IR spectrum showed absorption bands for hydroxy (3326 cm^{-1}) and carbonyl [1720 (sh), 1657, and 1584 cm^{-1}] functionalities. The positive FABMS of **1** exhibited pseudo molecular ion peaks at *m/z* 444 $[M+H]^+$, 466 $[M+Na]^+$, and 482 $[M+K]^+$. HRFABMS at *m/z* 444.1512 $[M+H]^+$ (calcd for $C_{19}H_{26}NO_{11}$, 444.1506) indicated the molecular formula $C_{19}H_{25}NO_{11}$, which was supported by the NMR data (Table 1). The ^{1}H NMR spectrum of **1** in D_2O showed signals attributed to a trisubstituted olefinic proton at δ_H 7.47 (brs, H-3), two acetal protons at δ_H 5.58 (d, *J* = 1.8 Hz, H-1) and 5.13 (dd, *J* = 9.6 and 3.6 Hz, H-7), and a monosubstituted vinyl group at δ_H 5.50 (ddd, *J* = 16.8, 10.2, and 10.2 Hz, H-8), 5.39 (dd, *J* = 16.8 and 1.2 Hz, H-10b), and 5.31 (d, *J* = 10.2 and 1.2 Hz, H-10b). In addition, the spectrum showed resonances due to two methines at δ_H 3.10 (*J* = 13.2, 6.0, and 3.6 Hz, H-5) and 2.85 (*J* = 10.2, 6.0, and 1.8 Hz, H-9) and a methylene at δ_H 1.43 (ddd, *J* = 13.2, 12.0, and 9.6 Hz, H-6α) and 2.31 (ddd, *J* = 12.0, 3.6, and 3.6 Hz, H-6β). Characteristic signals due to a β-glucopyranosyl unit were observed at δ_H 4.86 (d, *J* = 8.4 Hz, H-1′), 3.31 (dd, *J* = 9.6 and 8.4 Hz, H-2′), 3.52 (t, *J* = 9.6 Hz, H-3′), 3.42 (t, *J* = 9.6 Hz, H-4′), 3.54 (m, H-5′), 3.94 (dd, *J* = 12.6 and 1.8 Hz, H-6′a), and 3.75 (dd, *J* = 12.6 and 6.0 Hz, H-6′b). Also present was an ABX coupling system assignable to a serine unit at δ_H 4.58 (dd, *J* = 9.0 and 8.4 Hz, H-3″α), 3.95 (dd, *J* = 9.0 and 8.4 Hz, H-3″β), and 4.71 (t, *J* = 8.4 Hz, H-2″). Besides the resonances corresponding to the above protonated carbons (Table 1), the ^{13}C NMR and DEPT spectra of **1** showed resonances for two carbonyl carbons at δ_C 176.6 (C-1″) and 167.2 (C-11), and a quaternary olefinic carbon at δ_C 109.6 (C-4). These spectroscopic data suggest that **1** is an unusual secoiridoid glycoside [14–16] containing a serine unit. The suggestion was confirmed by comprehensive analysis of the 2D NMR data, which resulted in an unambiguous structure determination of **1**.

The proton and protonated carbon signals in the NMR spectra of **1** were unequivocally assigned by interpreting the ^{1}H–^{1}H COSY and HMQC spectroscopic data. The ^{1}H–^{1}H COSY spectrum of **1** displayed homonuclear vicinal coupling correlations: H-1/H-9/H-5/H_2-6/H-7 and H-9/H-8/H_2-10, in addition to W-type correlations H-1/H-3/H-5 (Fig. 2, thick lines). These, combined with two- and three-bond correlations in the HMBC spectrum (Fig. 2, red arrows): H-1/C-3, C-5, C-8, and C-9; H-3/C-1, C-4, C-5, and C-11; H-5/C-1, C-3, C-4, C-6, C-7, C-8, C-9, and C-11; H_2-6/C-4, C-5, C-7, and C-9; H-7/C-5; H-8/C-1, C-5, C-9, and C-10; H-9/C-1, C-4, C-5, C-6, C-8, and C-10; and H_2-10/C-8 and C-9; as well as the chemical shifts of these proton and carbon resonances, revealed unambiguously the presence of a secoiridoid parent nucleus with 7-acetalic and 8(10)-olefinic functionalities in **1**. The COSY correlations H-1′/H-2′/H-3′/H-4′/H-5′/H_2-6′ and the HMBC correlations H-1/C-1′ and H-1′/C-1 verified the presence of a β-glucopyranosyl moiety at C-1 of the nucleus. Additionally, the COSY correlations H-2″/H_2-3″ and the HMBC correlations from H-2″ to C-1″, C-3″, C-7, and C-11 and from H_2-3″ to C-1″ and C-7, together with their chemical shifts, demonstrated the presence of the serine unit, of which C-2″ and C-3″ were connected through nitrogen and oxygen atoms to C-7 of the nucleus. The HMBC correlation from H-7 to C-11, combined with the molecular composition, indicated that the carbonyl carbon (C-11) was linked through the nitrogen atom to C-7 to form a lactam in **1**. Accordingly, the planar structure of compound **1** was elucidated as shown in Fig. 2.

In the NOE difference spectrum, irradiation of H-5 enhanced the H-6β, H-7, and H-9 resonances, while the H-5, H-6β, and H-3″β resonances were enhanced upon irradiation of H-7 (Fig. 3, red dashed lines). The enhancements revealed that H-5, H-6β, H-7, H-9, and H-3″β were cofacial on one side of the ring system. The enhancements of the H-1, H-8 and H_2-10 resonances upon irradiation of H-6α indicated that these protons were cofacial on the other side of the ring system. This suggested that the secoiridoid nucleus in **1**, with a β-oriented H-7, had the same configuration as that of the co-occurring secologanic acid, for which the absolute configuration was determined by a single-crystal X-ray crystallographic analysis using anomalous scattering

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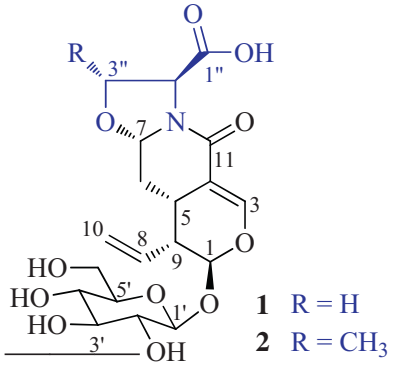


Fig. 1. Structures of compounds 1 and 2.

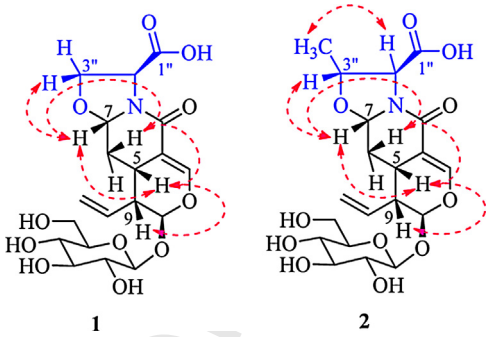
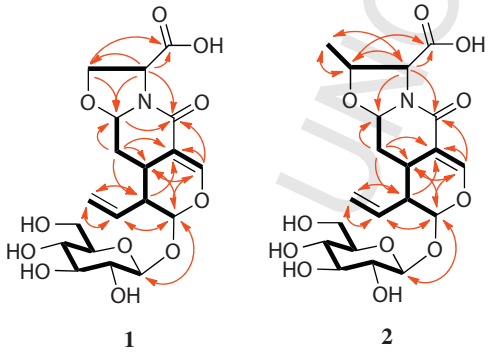


Fig. 3. NOE enhancements (red dash arrows) in the NOE difference spectra of **1** and **2**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

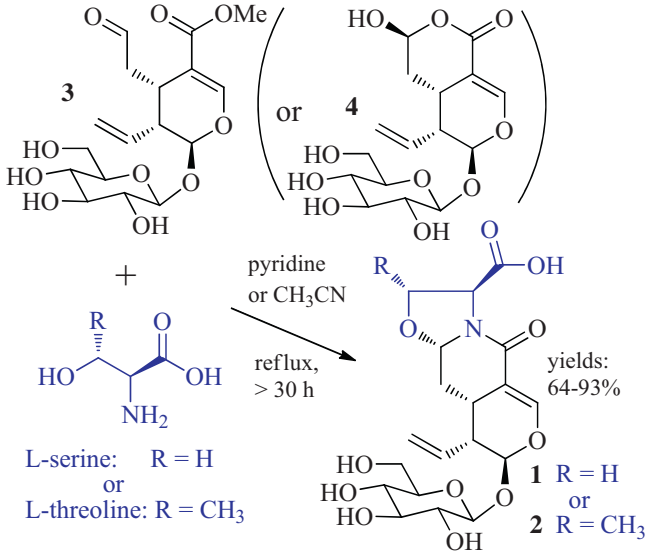
of Cu Kα radiation [15]. In addition, the H-2″ resonance was not enhanced by irradiation of H-7, and, in turn, irradiation of H-2″ did not enhanced the H-7 resonance. This suggests that H-7 is opposite H-2″ on the oxazolidine ring in **1**. Acid hydrolysis of **1**, followed by Marfey's analysis of the hydrolysate [20], revealed a production of L-serine on hydrolysis. This supports a *trans*-orientation of H-7 and H-2″. Enzymatic hydrolysis of **1** with snailase, produced glucose identified on the basis of TLC by comparison with an authentic sugar sample. The glucose isolated from the hydrolysate gave a positive optical rotation, $[\alpha]_D^{20}$ +47.3 (*c* 0.19, H_2O), indicating that it was D-glucose [14,15]. Based on the enzymatic and acidic hydrolyses and the aforementioned NOE enhancements, the absolute configuration of **1** was assigned as shown in Fig. 1. Therefore, the structure of **1** was determined and the compound was designated as serinosecologanin.

Compound **2** was obtained as a white amorphous solid, $[\alpha]_D^{20}$ –156.8 (*c* 0.37, H_2O). The positive ESIMS exhibited pseudo molecular ion peaks at *m/z* 458 $[M+H]^+$, 480 $[M+Na]^+$, and 496 $[M+K]^+$. The molecular formula $C_{20}H_{27}NO_{11}$, with one CH_2 unit more than that of **1**, was indicated by HR-ESIMS at *m/z* 480.1494 $[M+Na]^+$ (calcd. for $C_{20}H_{27}NO_{11}Na$ 480.1482). The UV, IR and NMR spectroscopic features of **2** were similar to those of **1**. Comparing the NMR data between **2** and **1** (Table 1) indicated replacement of the serine unit in **1** by a threonine moiety in **2**. This was confirmed by 2D NMR data analysis of **2**. In particular, the ^1H–^1H COSY correlations H-2″/H-3″/H_3-4″ and the HMBC correlations H-2″/C-1″, C-3″, and C-4″; H-3″/C-1″; H_3-4″/C-2″ and C-3″ (Fig. 2), in combination with their shifts, proved the presence of the threonine unit in **2**. Additionally, the HMBC correlations H-2″/C-7 and C-11 verified the connection of C-7 and C-11 of the secoiridoid moiety to the nitrogen atom of the threonine unit. Although the correlations H-3″/C-7 and H-7/C-3″ were not observed in the HMBC spectrum of **2** due to the limited amount of sample, the chemical shift of C-7 (δ_C 90.1), similar to that of **1**, supports the connection between the threonine oxygen atom and C-7. In the NOE difference spectrum of **2**, irradiation of H-2″ enhanced the H_3-4″ resonance, and the H-7 resonance was enhanced by irradiation of H-3″ (Fig. 3). In turn, when H-7 was irradiated, the H-3″, H-5, and H-6β resonances were enhanced. These enhancements not only indicated that **2** possessed the configuration shown in Fig. 1, but also confirmed the oxygen-bridged connection between C-3″ and C-7. The absolute configuration of **2** was verified by the isolation of D-glucose {$[\alpha]_D^{20}$ +43.1 (*c* 0.07, H_2O)} from the enzymatic hydrolysate and by Marfey's analysis indicating release of L-threonine from the acid hydrolysate. Therefore, the structure of compound **2** was determined and named threoninosecologanin.

Secologanin (**3**) is proposed to be the biogenetic precursor of about 2000 alkaloids and other bioactive natural products, such as reserpine, vincristine, and camptothecin [21], and has been extensively used in synthetic and biosynthetic studies of monoterpene alkaloids and related natural products. This indicates that **1** and **2** could be biosynthesized from the co-occurring secologanin (**3**) and/or secologanic acid (**4**) [14,15]. To provide chemical support to this proposition, confirm the absolute configurations, and assay their biological activities, compounds **1** and **2** were synthesized by refluxing **3** or **4** with L-serine or L-threonine in acetonitrile or pyridine for 30–50 h, respectively (Scheme 1). Interestingly, the reactions did not take place in acetonitrile or pyridine at 20 °C even



Q3 **Fig. 2.** Main ^1H–^1H COSY (black thick lines) and HMBC correlations (red arrows, from ^1H to ^{13}C) of **1** and **2**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Scheme 1. Synthesis of compounds 1 and 2.

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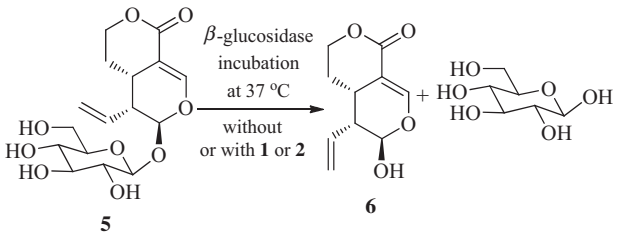

Scheme 2. Hydrolysis of sweroside (5).

after 24 h [21]. The physical–chemical properties of the synthetic **1** and **2** were identical with those of the natural products. Because a single diastereomer of **1** or **2** was generated in the reaction, with H-7 having the axial β orientation as shown by the splitting patterns and coupling constants ($J_{6\alpha,7\beta}$ = 9.6 Hz and $J_{6\beta,7\beta}$ = 3.6 Hz), the stereoselectivity in the formation of **1** or **2** was similar to that in the condensation of **3** with functionalized amines. This indicates a similar stereo-controlled mechanism for the reaction [21].

In the enzymatic hydrolyses of compounds **1** and **2**, neither compound could be hydrolyzed by common enzymes of plant origin (β-glucosidase from almonds and hesperidinase from *A. niger*), but could be hydrolyzed by snailase, of animal origin. In contrast, other analogs from the same materials [14–16], such as **3**, **4**, and sweroside (**5**) can readily be hydrolyzed by all the enzymes. Thus, resistance and/or inhibitory activities of **1** and **2** against β-glucosidase were preliminarily investigated. Compound **5**, without or with **1** or **2**, was hydrolyzed using β-glucosidase (Scheme 2). HPLC analysis revealed that time-dependent hydrolysis of **5** was not disturbed by the presence of **1** or **2** in the hydrolytic system, although **1** or **2** was not hydrolyzed (Figs. S27, S28, and S29 in Supporting information). This indicates that **1** and **2** are not inhibitors of the enzyme and they are resistant to the β-glucosidase. Therefore, these unusual structures are not substrates of the enzyme.

In preliminary *in vitro* assays, compounds **1** and **2**, at 10 μmol/L, showed inhibitory activity against the release of glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor, with inhibition rates of 34.9 ± 3.1% and 53.6 ± 2.6%, respectively, while the positive control (ginkgolide B) exhibited an inhibition rate of 73.0 ± 3.3% at the same concentration [14]. The protective activities of the compounds against neurotoxicity induced by serum deprivation in PC12 cells were investigated by the MTT method. The results showed that serum deprivation induced significant inhibition of MTT reduction, at a concentration of 10 μmol/L. Compounds **1** and **2** showed cell viability from 71.0 ± 2.4% (control) to 77.5 ± 3.0 and 78.9 ± 1.6, respectively, indicating that **2** may be effective in neurodegenerative disorders. Compounds **1** and **2** were also assessed for their activities against influenza virus A/Hanfang/359/95 (H3N2), Coxsackie virus B3, and HIV-1 replication, as well as against several human cancer cell lines [16], but all were inactive at a concentration of 10 μmol/L.

4. Conclusion

Two β-hydroxy amino acid-coupled secoiridoids, serinosecologanin (**1**) and threoninosecologanin (**2**) were isolated from an aqueous extract of the flower buds of *L. japonica*. Their unique structures, including their absolute configurations, were elucidated by spectroscopic data analysis and synthesis. Compounds **1** and **2** exhibited activity against the release of glucuronidase in rat polymorphonuclear leukocytes, induced by the platelet-activating factor, as well as resistance against β-glucosidase from almonds and hesperidinase from *A. niger*. This is the first example of natural iridoid glycosides resistant to the β-glucosidases of plant origin. These results, combined with our previous studies [14–18], suggest that the diverse constituents have pharmacological contributions to the traditional uses of the flower buds of *L. japonica*. Semisynthesis of **1** and **2** from the co-occurring abundant analogs secologanin (**3**) and/or secologanic acid (**4**), not only supports their biosynthetic relationship, but also provides an important approach to synthesize and modify the structures of the minor constituents for further investigation of their biological activities and structure–activity relationships.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.05.037.

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