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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 br s	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.