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**Article**

# α-Glucosidase Inhibitors from the Roots of *Codonopsis lanceolata* Trautv.

**Suk Whan Jung, Ae Jin Han, Hae Jin Hong, Myoung Gun Choung[1], Kwan Su Kim and Si Hyung Park***

*Department of Medicinal Plant Resources, Division of Applied BioScience, Mokpo National University, Muan 534-729, Korea*
*[1]Department of Pharmacognosy Material Development, Kangwon National University, Samcheok 245-711, Korea*

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**The roots of *Codonopsis lanceolata* afforded tangshenoside I (1) and β-adenosine (2) as α-glucosidase inhibitors. Their structures were unambiguously determined by 1D and 2D NMR data including HMQC and HMBC experiments. Compounds 1 and 2 exhibited weak α-glucosidase inhibitory activities *in vitro* with $IC_{50}$ of 1.4 and 9.3 mM, respectively.**

**Key words:** *Codonopsis lanceolata, α-glucosidase inhibitor, tangshenoside I, β-adenosine*

*Codonopsis lanceolata* Trautv. is a plant of the Campanulaceae family, which is distributed throughout Korea, Japan and China. *C. lanceolata* has been cultivated and its roots have been used as food especially in Korea. Other Codonopsis species, *C. pilosula* and *C. tangshen* were used as medicine (Tang-Sam) for ulcers, memory improvement and immunostimulating; however, *C. lanceolata* was treated as an adulterant in Japan and China.[1,2] Several studies of the chemical constituents of *C. pilosula*, *C. tangshen* and *C. ussuriensis* have been reported in the literatures.[3-6] There are some reports on secondary metabolites of *C. lanceolata*; on the isolation of triterpenoid, saponin and alkaloid from the roots[7-10] and the isolation of flavonoids from the leaves.[11] The present report deals with the isolation, structure determination and α-glucosidase inhibition activity of tangshenoside I and adeonsine from the roots of *C. lanceolata*.

## Materials and Methods

**Plant material.** Roots of *C. lanceolata* were purchased from commercial markets and a voucher specimen is deposited in our laboratory.

**General experiments.** α-Glucosidase (from Baker's yeast) PNP-glucoside (*p*-nitrophenyl α-D-glucopyranoside), and XAD-16 resin were purchased from Sigma Co. (USA) and reverse phase (RP-18) silica gel (70-230 mesh, YMC GEL ODS-A) was purchased from YMC Co. (Japan). Compound **1** and **2** were isolated with open column chromatography and MPLC (Eyela VSP3050 system, Japan). The NMR spectra were recorded using Bruker Avanace 400 spectrometer. The mass spectra were obtained using Bruker HCT 3000 model equipped with ESI or APCI.

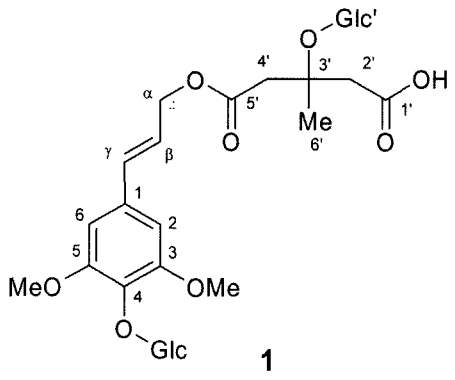
**Extraction and isolation.** Dried and chopped roots of *C. lanceolata* (5 kg) were extracted with hot water (20 *l*). The extract was filtered and passed through the XAD-16 resin column (10 × 20 cm) and the column was washed with 2 *l* of distilled water. The column was eluted with 50% MeOH and 100% MeOH, and the eluents were concentrated *in vacuo*. The 50% MeOH eluent was subjected to RP-18 column and eluted with water-MeOH (4 : 1) mixture to yield five subfractions. Subfraction II was purified by MPLC to obtain **1** and **2**.

**Tangshenoside I (1):** White powder; ESI-MS *m/z* 677 $[M-H]^-$, 701 $[M+Na]^+$; $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 6.77 (2H, s, H-2 and 6), 6.59 (1H,d, *J* = 15.8 Hz, H-γ), 6.33 (1H, dt, *J* = 6.0, 15.8 Hz, H-β), 4.93 (1H, d, *J* = 7.4 Hz, H-1-Glc), 4.66 (2H, d, *J* = 6.0 Hz, H-α), 4.44 (1H, d, *J* = 7.6 Hz, H-1-Glc'), 3.76 (6H, s, H-OMe), 3.58 (2H, m, H-6-Glc). 3.38 (2H, m, H-6-Glc'), 3.3-2.95 (m, Glc and Glc'), 2.89 (1H, d, *J* = 14.7 Hz, H-4'a), 2.65 (1H, d, *J* = 14.7 Hz, H-4'b), 2.51 (1H, d, *J* = 13.6 Hz, H-2'a), 2.33 (1H, d, *J* = 13.6 Hz, H-2'b), 1.31 (3H, s, H-6'); $^{13}$C-NMR (100 MHz) in Table 1.

**Adenosine (2):** White powder; APCI-MS *m/z* 266 $[M-H]^-$, 268 $[M+H]^+$; $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 8.41 (1H, s, H-8), 8.19 (1H, s, H-2), 7.41 (2H, br. s, $NH_2$), 5.93 (1H, d, *J* = 6.2 Hz, H-1'), 4.67 (1H, dd, *J* = 4.7, 6.2 Hz, H-2'), 4.20 (1H, dd, *J* = 3.1, 4.7 Hz, H-3'), 4.02 (1H, ddd, *J* = 3.0, 3.1, 6.5 Hz, H-4'), 3.73 (1H, dd, *J* = 3.0, 12.1 Hz, H-5'a), 3.61 (1H, dd, *J* = 6.5, 12.1 Hz, H-5'b); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 156.20 (C-6), 152.73 (C-2), 149.41 (C-4), 140.27 (C-8), 119.71 (C-5), 88.24 (C-1'), 86.24 (C-4'), 73.78 (C-2'), 71.00 (C-3'), 62.03 (C-5').

**α-Glucosidase inhibition assay.** α-Glucosidase (final concentration, 0.05 unit/m*l*) in 150 μ*l* of 50 mM phosphate buffer (pH 6.7) was premixed with the samples at various concentrations. 2 mM *p*-nitrophenyl glucopyranoside (150 μ*l*) as a substrate in the same phosphate buffer was added to the mixture to start the reaction. The reaction was incubated at 37°C for 20 min and stopped by adding 1 m*l* of 1 M $Na_2CO_3$. The α-glucosidase inhibiting activity was determined by

*Corresponding author
Phone: 82-61-450-2662; Fax: 82-61-450-6443
E-mail: shp@mokpo.ac.kr



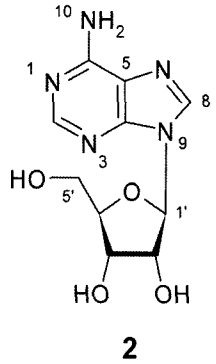




**Fig. 1. Structures of isolated compounds 1 and 2 from the roots of *C. lanceolata*.**

measuring the *p*-nitrophenol released from PNP-glucoside at 400 nm. The $IC_{50}$ value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.

## Results and Discussion

**Structure determination.** The structure of each compound was identified by MS, 1D and 2D NMR experiments and comparing the corresponding NMR spectral data with those in the literature (Fig. 1).

Compound **1** was obtained as a colorless residue with ions at *m/z* 677 $[M-H]^-$ and 701 $[M+Na]^+$ by ESI-MS spectrometry. Molecular ion peak was increased to 710 $[M\text{-}d_9+Na]^+$ when the compound was dissolved in $D_2O$. These results mean the molecular weight of compound **1** is 678 and this compound has nine exchangeable protons. The analysis of $^1H$ NMR, $^{13}C$ NMR, DEPT, HMQC and HMBC spectral data gave unambiguous $^1H$ and $^{13}C$ assignments for aglycone of compound **1**. NMR spectra were obtained from three different solvents DMSO-$d_6$, $CD_3OD$ and pyridine-$d_5$ and DMSO-$d_6$ showed the best results. Four aromatic carbon signals indicated the presence of a symmetric aromatic ring. HMBC experiment showed the three carbon signals corresponding to allyl alcohol were connected with the aromatic ring system. Coupling constant between H-β (δ 6.33) and H-γ (δ 6.59) was

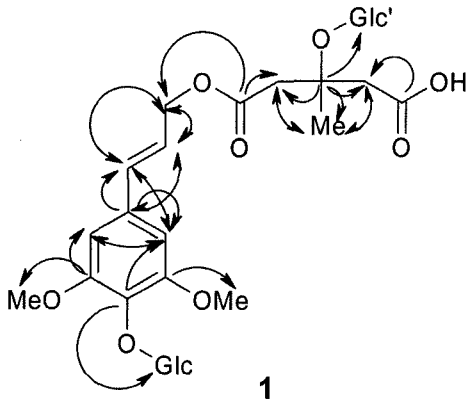



**Fig. 2. Important HMBC correlations in compound 1.**

15.8 Hz, meaning that these two protons have *trans* conformation. Overlapped two singlet *O*-Me group was proven to connect to C-3 and 5 carbon of aromatic ring by HMBC experiment (Fig. 2). One anomeric proton (δ 4.93) of glucose showed the cross peak with C-4 carbon of aromatic ring in HMBC spectrum. A partial structure of compound **1** was identified as syringin from the above results. Another part of compound **1** was 3'-glucosyl-3'-methylglutaric acid. Another anomeric proton (δ 4.44) of glucose showed the cross peak with C-3' quaternary carbon of 3'-methylglutaric acid in HMBC spectrum. Originally 3-methylglutaric acid has symmetric structure, however the carbon signals of compound **1** showed asymmetric patterns because one carboxyl group was esterified with hydroxyl group of syringin. $^{13}C$ NMR data of 3'-methylglutaryl moiety of tangshenoside I was not correctly assigned in the previous report (Table 1 right column). Carbonyl carbon (δ 174.78 not 170.51) was proven to connect with allylic carbon (δ 64.33) of syringin moiety in HMBC experiment. Compound **1** was identified as tangshenoside I (syringin 3'-glucosyl-3'-methylglutarate) by MS and NMR data. Tangshenoside I have been isolated from *Codonopsis tangshen*, *C. pilosula* and *C. ussuriensis*, however, it is the first report from *C. lanceolata*.

Compound **2** was obtained as white powder with ions at *m/z* 266 $[M-H]^-$ and 268 $[M+H]^+$ by APCI-MS spectrometry. The product ions of *m/z* 266 (negative) and 268 (positive) were *m/z* 134 [M-H-pentose]$^-$ and [M + H-pentose]$^+$ by APCI-MS/MS, respectively. The product ions in tandem MS experiments mean compound **2** has pentose. Odd number of molecular weight 267 indicated the presence of odd number of nitrogen in this compound by nitrogen rule. Compound **2** was identified as β-adenosine by MS and NMR data. Five carbon signals corresponding to ribose and another five carbon signals corresponding to adenine in $^{13}C$ NMR were identical with those published for β-adenosine.[12] And the configuration of H-1' was assigned as β form by H-1' coupling constant (6.2 Hz).

**α-Glucosidase inhibition activity.** Compounds **1** and **2** exhibited weak α-glucosidase inhibitory activities under the assay conditions with $IC_{50}$ of 1.4 and 9.3 mM, respectively. 1-

**Table 1.** $^{13}$C-NMR data of tangshenoside I (1)

| No | DMSO-$d_6$[a] | $CD_3OD$[a] | pyridine-$d_5$[a] | $D_2O$[b] |
|---|---|---|---|---|
| 1 | 131.66 | 134.58 | 132.91 | 134.2 |
| 2,6 | 104.82 | 105.63 | 104.33 | 105.1 |
| 3,5 | 152.75 | 154.32 | 152.62 | 153.3 |
| 4 | 134.35 | 136.13 | 134.27 | 134.4 |
| $\alpha$ | 64.33 | 66.04 | 65.07 | 57.0 |
| $\beta$ | 123.18 | 124.42 | 123.20 | 66.3 |
| $\gamma$ | 133.18 | 134.88 | 133.39 | 124.3 |
| O-Me | 56.41 | 57.07 | 56.18 | 134.4 |
| 1' | 174.78 | 178.35 | 177.50 | 173.3 |
| 2' | 45.42 | 47.67 | 46.50 | 44.3 |
| 3' | 76.19 | 78.11 | 77.21 | 78.2 |
| 4' | 43.82 | 45.02 | 43.96 | 47.4 |
| 5' | 170.51 | 172.77 | 171.83 | 176.7 |
| 6' | 24.98 | 25.13 | 24.39 | 24.8 |
| G-1 | 102.47 | 105.26 | 103.63 | 103.8 |
| G-2 | 74.18[c] | 75.66[c] | 74.24[c] | 74.5[c] |
| G-3 | 76.62[d] | 78.29[d] | 76.86[d] | 77.0[d] |
| G-4 | 69.94 | 71.34 | 69.95 | 70.3 |
| G-5 | 77.29[d] | 78.08[d] | 76.50[d] | 76.6[d] |
| G-6 | 61.18 | 62.65 | 61.23 | 61.5 |
| G'-1 | 96.78 | 98.28 | 96.93 | 97.2 |
| G'-2 | 73.74[c] | 75.15[c] | 73.76[c] | 74.0[c] |
| G'-3 | 76.56[d] | 77.73[d] | 76.43[d] | 76.6[d] |
| G'-4 | 69.89 | 71.23 | 69.50 | 70.0 |
| G'-5 | 77.08[d] | 77.72[d] | 76.2[d] | 76.5[d] |
| G'-6 | 60.82 | 62.41 | 60.59 | 61.2 |

[a]Measured with 100 MHz NMR in this experiment.
[b]Data from Reference 3.
[c,d]May be exchanged in measurement.

Deoxynojirimycin, a well known α-glucosidase inhibitor, was used as a positive control. $IC_{50}$ of 1-deoxynojirimycin was 0.21 mM under the same conditions. Various α-glucosidase inhibitors have been reported, and the potencies of inhibitory activity were different with the sources of enzyme. Catechin, an inhibitor of yeast α-glucosidase, did not show any inhibitory activity on mammalian α-glucosidase. Some α-glucosidase inhibitors for mammalian showed a low effect on microbial α-glucosidase. Therefore, compound **1** and **2** should be tested with various α-glucosidase including other carbohydrate digestion enzymes.

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