Phenolic Glycosides from Roots of *Adenophora tetraphylla* Collected in Heilongjiang, China

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***Adenophora tetraphylla* (= *A. triphylla*, Campanulaceae) is a source of the traditional Chinese medicine "Shashen". From the roots of this plant, three new phenolic glycosides, called shashenosides I, II and III (2—4) were isolated together with siringinoside (1), β-sitosteryl-β-D-glucoside, linoleic acid and methyl stearate. The common aglycone of 2, 3 and 4 was identified as 3-methoxy-5-(2′-propenyl)-1,2-benzenediol [= 1-*O*-methyl-5-(2′-propenyl)-pyrogallol = 6-hydroxyeugenol, 6], and the structures of 2 and 3 were elucidated as 2,3-di-*O*-β-glucopyranoside and 3-*O*-α-arabinopyranosyl-(1→6)-β-glucopyranoside of 6, respectively. Shashenoside III (4) was formulated as 2-*O*-β-glucopyranoside of 3. No saponin was found in the roots.**

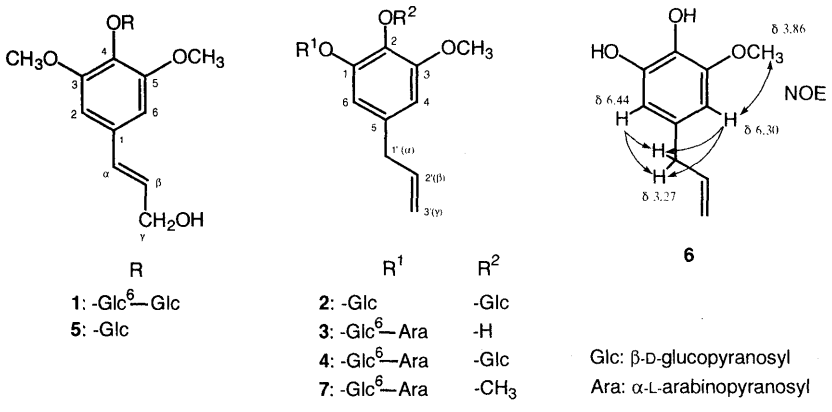
Keywords *Adenophora tetraphylla*; Campanulaceae; Shashen; shashenoside; Chinese traditional medicine; synapyl-glycoside; 3-methoxy-5-(2′-propenyl)-1,2-benzenediol; glycoside

The traditional Chinese medicine, Shashen (沙参), roots of *Adenophora* spp. (Campanulaceae) is known to be an antiinflammatory and antitussive drug used in the treatment of lung disease. From *Adenophora* species, Konno *et al.* reported the isolation of sterols and triterpenoids[1] and Du *et al.* isolated triterpenoids and coumarins.[2] *A. tetraphylla* FISCH. *ex* JACKSON (= *A. triphylla* (THUNB.) A.DC.,[3] Chinese name: 輪葉沙参, 南参; Japanese name: saiyoushajin) is one of source plants of this crude drug. We have conducted studies on water soluble constituents of dangshen (党参), roots of *Codonopsis* spp. (Campanulaceae).[4–6] Continuing our chemical studies on campanulaceous traditional Chinese medicines, the present paper reports the isolation and structural elucidation of four phenolic glycosides from roots of *A. tetraphylla* collected in Heilongjian province, China.

As in the case of Dangshen, a methanolic extract of the roots contained a large amount of saccharides, which was removed by chromatography on highly porous synthetic polymer (Diaion HP-20). The resulting crude glycoside fraction was separated by combination of a variety of chromatography as described in the Experimental section to give four glycosides, **1**—**4** together with β-sitosteryl-β-D-glucoside, linoleic acid and methyl stearate.

Compound **1**, $C_{23}H_{34}O_{14}$ (from high resolution fast atom bombardment mass spectrometry (HR-FAB-MS)) afforded glucose on acid hydrolysis. Inspection of the negative FAB-MS as well as the ^1H- and ^{13}C-nuclear magnetic resonance (NMR) spectrum (in C_5D_5N) led to the identification of **1** as syringinoside (4-*O*-β-gentiobiosyl-3,5-dimethoxy-4-hydroxy-*trans*-cinnamylalcohol) which was recently isolated from *Wikstroemia sikokiana* FRANCH. *et* SAV. (Thymelaeaceae).[7]

Compound **2** called shashenoside I, $C_{22}O_{32}H_{13}$ (from HR-FAB-MS) afforded glucose and an aglycone (**6**), $C_{10}H_{12}O_3$ (from HR-FAB-MS) on acid hydrolysis. The ^1H-NMR spectrum of **6** (in CD_3OD) exhibited signals due to an allyl group at δ 3.27 (2H, dddd, J = 6.8, 1.6, 1.3, 0.6 Hz, $-C\underline{H}_2-CH=CH_2$), 5.92 (1H, ddt, J = 17.0, 10.1, 6.8 Hz, $-CH_2-C\underline{H}=CH_2$), 5.05 (1H, ddt, J = 10.1, 2.0, 1.3 Hz, $-CH_2-CH=C\underline{H}_2$ (*cis*)), 5.08 (1H, ddt, J = 17.0, 2.0, 1.6 Hz, $-CH_2-CH=C\underline{H}_2$ (*trans*)). Presence of an allyl group in **6** was also supported by the ^{13}C-NMR spectrum (in CD_3OD): signals at δ 40.1 ($-CH_2-$), 137.6 ($-CH=$), 115.7 ($=CH_2$). The ^{13}C-NMR (in CD_3OD) spectrum of **6** exhibited a methoxyl carbon signal at δ 56.2, four substituted aromatic carbon signals at δ 146.9, 143.9, 132.1, 130.7 and two unsubstituted aromatic carbon signals at δ 108.9, 103.5. The ^1H-NMR spectrum of **6** showed two hydroxy proton signals at δ 5.28 and 5.34 (each 1H, s), a methoxyl proton signal at δ 3.86



R

1: -Glc^6—Glc
5: -Glc

	R^1	R^2
2:	-Glc	-Glc
3:	-Glc^6—Ara	-H
4:	-Glc^6—Ara	-Glc
7:	-Glc^6—Ara	$-CH_3$

6

Glc: β-D-glucopyranosyl
Ara: α-L-arabinopyranosyl

Chart 1

(3H, s) and a couple of *meta*-located aromatic proton signals at δ 6.30 (1H, d, $J=1.8$ Hz) and 6.44 (1H, dt, $J=1.8$, 0.6 Hz, long range coupling with methylene protons). The nuclear Overhauser effect (NOE) difference spectrum of **6** revealed the presence of NOE between two aromatic proton signals and the allylic methylene signal as well as between one of the aromatic proton signals (at δ 6.30) and the methoxyl proton signal (see Chart 1). These results led to assignment of the structure of **6** as 3-methoxy-5-(2′-propenyl)-1,2-benzenediol [=1-*O*-methyl-5-(2′-propenyl)-pyrogallol=6-hydroxyeugenol]. Compound **6** was used as a starting material for syntheses of natural phenolic compounds such as (±)-eusiderin,[8] but this is the first example of the occurrence of this compound in nature as an aglycone of glycosides.

The ^{1}H-NMR spectrum of **2** in CD_3OD exhibited two β-glucosyl anomeric proton signals at δ 4.79 (1H, d, $J=7.9$ Hz) and 5.80 (1H, d, $J=7.9$ Hz). The ^{13}C-NMR spectrum of **2** in C_5D_5N exhibited signals assignable to two sets of terminal β-glucopyranosyl units together with signals due to the aglycone carbons. Accordingly, **2** can be formulated as 1,2-di-*O*-β-glucopyranoside of **6**.

Compound **3**, called shashenoside II, $C_{21}H_{30}O_{12}$ (from HR-FAB-MS), yielded glucose, arabinose and the aglycone (**6**) on acid hydrolysis. The ^{1}H-NMR spectrum of **3** in CD_3OD showed two anomeric proton signals at δ 4.75 (1H, d, $J=7.3$ Hz) and 4.29 (1H, d, $J=6.8$ Hz). The ^{13}C-NMR spectrum of **3** in CD_3OD exhibited signals due to a terminal α-arabinopyranosyl unit and a 6-linked β-glucopyranosyl unit, indicating the presence of an α-arabinopyranosyl-(1→6)-β-glucopyranosyl moiety. In order to elucidate the location of the glycosyl moiety, a methyl ether (**7**) was prepared from **3** by treatment with diazomethane. As reported, in the ^{1}H-NMR spectra of **5**,[4] signals due to two aromatic and two methoxy protons appear at the same position, respectively, showing the symmetrical nature of the aromatic ring moiety. In contrast, the ^{1}H-NMR spectrum of **7** in CD_3OD exhibited signals due to the two aromatic and two methoxy proton signals at dfferent positions: at δ 6.66, 6.55 (each 1H, d, $J=1.8$ Hz, C-2,6), 3.81, 3.79 (each 3H, s, OCH_3), leading to the structure of **3** as 1-*O*-α-arabinopyranosyl-(1→6)-β-glucopyranoside of **6**. Presence of NOE between the signals at δ 3.81 and 6.55 as well as absence of NOE of another aromatic signal at δ 6.66 with both the methoxyl proton signals supported this formulation.

Compound **4**, called shashenoside III, $C_{27}H_{40}O_{17}$ (from HR-FAB-MS) gave glucose, arabinose and **6** on acid hydrolysis. The ^{1}H-NMR spectrum of **4** in CD_3OD exhibited three anomeric proton signals at δ 4.94 (1H, d, $J=7.3$ Hz), 4.89 (1H, d, $J=6.3$ Hz), 4.29 (1H, d, $J=6.8$ Hz). Comparison of the ^{13}C-NMR of **4** in CD_3OD with that of **3** revealed the presence of a terminal β-glucopyranosyl unit together with a α-arabinopyranosyl-(1→6)-β-glucopyranosyl moiety in **4**. On mild acid hydrolysis, **4** afforded **3**. It follows that **4** can be formulated as 1-*O*-β-glucopyranoside of **3**.

It has been mentioned that Shashen contains some saponins, and can be used as a substitute of Ginseng. However, in the present study, no saponin was isolated from the roots.

Experimental

General Procedures ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a JEOL JNM GX-400 NMR spectrometer. FAB-MS was taken on a JEOL JMS SX-102 mass spectrometer by the direct inlet method. Preparative high performance liquid chromatography (HPLC) was carried out on a column of TSK-gel ODS-120T (21.5 mm i.d. × 30 cm): detection, ultraviolet (UV) at 254 nm; flow rate, 6 ml/min. Acid hydrolysis of glycosides and identification of the resulting monosaccharides by gas-liquid chromatography (GLC) were conducted in the usual manner.

Extraction and Separation Dried roots (8 kg) collected at Xiaoxinganling, Heilongjiang Province of China, were extracted with hot MeOH to give MeOH-extract (700 g). A suspension of the MeOH-extract (350 g) in H_2O was chromatographed on a column of Diaion HP-20 (Mitsubishi Kasei Co., Ltd.) with H_2O and then with MeOH. The H_2O-eluate was composed of a large amount of saccharides and other highly water soluble substances. Twenty grams of the MeOH-eluate (total: 40 g) was subjected to chromatography on silica gel with $CHCl_3$–MeOH–H_2O (30 : 10 : 1 then 14 : 6 : 1, homogenous) to give six fractions tentatively designated as fr. I—VI in their order of elution. Further chromatography of fr. I on silica gel with $CHCl_3$–MeOH (20 : 1) afforded β-sitosteryl 3-*O*-β-glucoside (150 mg) and linoleic acid (10 mg). Fraction II was chromatograhed on silica gel with $CHCl_3$–MeOH (6 : 1), affording methyl stearate. All of these compounds were identified by comparison of thin layer chromatography (TLC) and the ^{1}H- and ^{13}C-NMR spectra with corresponding authentic specimens.

Fractions II and III were respectively separated by HPLC on an octadecyl silica (ODS) column with 30% MeOH to give **3** (90 mg) from fr. III and **2** (228 mg) from fr. IV. Fractions V and VI were respectively subjected to chromatography on LiChroprep RP-18 (40—63 mμ, Merck) with 25% MeOH followed by HPLC on an ODS column with 24% MeOH, affording **1** (30 mg) from fr. V and **4** (40 mg) from fr. VI.

Syringinoside (**1**): A white powder, $[\alpha]_D^{18}$ −22.6° ($c=0.31$, MeOH), HR-FAB-MS (positive) *m/z*: Calcd for $[C_{23}H_{34}O_{14}+Na]^+$ 557.1846. Found 557.1884. FAB-MS *m/z* (negative): 533 $(M-H)^-$, 371 $(M-Glc-H)^-$, 209 $(M-Glc_2-H)^-$. ^{1}H-NMR in C_5D_5N: δ 6.89 (2H, s), 6.64 (1H, dt, $J=16.1$, 5.2 Hz), 6.86 (1H, d, $J=16.1$ Hz), 5.71 (1H, d, $J=6.8$ Hz), 4.93 (1H, d, $J=7.8$ Hz), 4.57 (2H, d, $J=5.2$ Hz), 3.83 (6H, s). ^{13}C-NMR in CD_3OD: aglycone carbons, δ 57.0 (2C), 63.6, 105.5, 130.0 (2C), 131.0, 131.3, 135.3, 154.3 (2C); sugar carbons, δ 62.8, 69.3, 71.4, 71.7, 75.2, 75.7, 77.9 (4C), 104.5, 105.1.

Shashenoside I (**2**): A pale yellow powder, $[\alpha]_D^{18}$ −47.6° ($c=0.42$, H_2O). HR-FAB-MS (positive) *m/z*: Calcd for $[C_{22}H_{32}O_{13}+Na]^+$ 527.1741. Found: 527.1780. FAB-MS (negative) *m/z*: 503 $(M-H)^-$, 341 $(M-Glc-H)^-$, 179 $(M-Glc_2-H)^-$. ^{1}H-NMR in CD_3OD: δ 3.84 (3H, s, OCH_3), 6.81, 6.60 (each 1H, d, $J=1.8$ Hz, a pair of *meta*-located aromatic protons) and allyl group signals at δ 3.29 (2H, ddd, $J=6.4$, 1.6, 1.3 Hz), 5.95 (1H, ddt, $J=17.0$, 10.1, 6.8 Hz), 5.05 (1H, ddt, $J=10.1$, 2.0, 1.3 Hz), 5.06 (1H, ddt, $J=17.0$, 2.0, 1.6 Hz). ^{13}C-NMR in CD_3OD: aglycone carbons, δ 41.2 (C-α), 57.0 (OCH_3), 109.3, 112.0 (C-6,2), 116.3 (C-γ), 135.5 (C-4), 138.5 (C-β), 138.7 (C-1), 152.4, 154.3 (C-3,5); sugar carbons, δ 62.5, 62.6 (C-6), 71.3, 71.4 (C-4), 75.1, 75.7 (C-2), 77.7, 78.0, 78.4 (2C) (C-3,5), 103.8, 105.5 (C-1).

A solution of **2** (45 mg) in a mixture of 2% HCl and 50% dioxan for 2 h at 75 °C. The reaction mixture was extracted with C_6H_6 and the benzene extract was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give **6**: A yellow powder. HR-EI-MS *m/z*: Calcd for $C_{10}H_{12}O_3^+$ 180.0786. Found: 180.0766.

Shashenoside II (**3**): A yellow powder, $[\alpha]_D^{18}$ −51.4° ($c=0.68$, MeOH). HR-FAB-MS (positive) *m/z*: Calcd for $[C_{21}H_{30}O_{12}+Na]^+$ 497.1635. Found: 497.1651. ^{1}H-NMR in CD_3OD: δ 6.66, 6.54 (each 1H, d, $J=1.8$ Hz, C-2,4), 5.94 (1H, ddt, $J=16.8$, 10.1, 6.8 Hz, C-β), 5.06 (1H, dd, $J=16.8$, 1.8 Hz, C-γ*trans*), 5.03 (1H, ddt, $J=10.1$, 1.8, 1.0 Hz, C-γ*cis*), 4.75 (1H, d, $J=7.3$ Hz, anomeric H), 4.29 (1H, d, $J=6.6$ Hz, anomeric H), 3.82 (3H, s, OCH_3), 3.29 (2H, dd, $J=6.8$, 1.0 Hz, C-α). ^{13}C-NMR in CD_3OD: aglycone carbons, δ 40.9 (C-α), 56.8 (OCH_3), 108.9, 112.3 (C-6,2), 115.9 (C-γ), 132.5 (C-1), 136.0 (C-4), 139.1 (C-β), 146.7, 149.6 (C-5,3); anomeric carbons δ 104.3, 104.8; α-arabinoside carbons, δ 66.6 (C-5), 69.2 (C-4), 72.3 (C-2), 74.1 (C-3); β-glucoside carbons, δ 69.3 (C-6), 71.3 (C-4), 74.8 (C-2), 77.0 (C-5), 77.4 (C-3). On acid hydrolysis in the same way as **2**, **3** afforded glucose, arabinose and **6**. Shashenoside III (**3**) (15 mg) was treated with CH_2N_2 in Et_2O–MeOH and the product was purified by HPLC on an ODS column with 35% MeOH to give a methyl ether **7**: A yellow powder. HR-FAB-MS (positive) *m/z*: Calcd for $[C_{22}H_{32}O_{12}+Na]^+$ 511.1791. Found: 511.1820. ^{1}H-NMR in CD_3OD: δ 6.66 (1H, d, $J=1.8$

Hz, C-2), 6.55 (1H, d, $J=1.8$ Hz, C-6), 5.96 (1H, m, C-β), 5.10 (1H, dd, $J=16.7$, 2.0 Hz, C-γ*trans*), 5.04 (1H, dd, $J=10.0$, 2.0 Hz, C-γ*cis*), 4.94 (1H, d, $J=7.0$ Hz, anomeric H), 4.26 (1H, d, $J=6.7$ Hz, anomeric H), 3.81 (3H, s, 5-OCH_3), 3.79 (3H, s, 4-OCH_3), 3.29 (2H, d, $J=7.3$ Hz, C-α).

Shashenoside III (**4**): A yellow powder, $[\alpha]_D^{18}$ $-42.2°$ ($c=0.45$, MeOH). HR-FAB-MS (positive) *m/z*: Calcd for $[C_{27}H_{40}O_{17}+Na]^+$ 659.2163. Found: 659.2184. Calcd for $[C_{27}H_{40}O_{17}+K]^+$ 675.1903. Found: 675.1940. FAB-MS (negative) *m/z*: 635 $(M-H)^-$, 473 $(M-Glc-H)^-$, 341 $(M-Glc-Ara-H)^-$, 179 $(M-Glc_2-Ara-H)^-$. ^{1}H-NMR in CD_3OD: δ 6.77, 6.66 (each 1H, d, $J=1.8$ Hz, C-2,6), 5.96 (1H, m, C-β), 5.07 (2H, m, C-γ), 4.94 (1H, d, $J=7.3$ Hz, anomeric H), 4.89 (1H, d, $J=6.3$ Hz, anomeric H), 4.29 (1H, d, $J=6.8$ Hz, anomeric H), 3.82 (3H, s, OCH_3), 3.31 (2H, d, $J=6.6$ Hz, C-α). ^{13}C-NMR in CD_3OD: aglycone carbons, δ 41.2 (C-α), 57.2 (OCH_3), 109.4, 112.1 (C-2,6), 116.4 (C-γ), 135.6 (C-4), 138.6 (C-β), 138.7 (C-1), 152.0, 154.4 (C-3,5); anomeric carbons, δ 103.7, 105.0, 105.4; α-arabinoside carbons, δ 66.6 (C-5), 69.3 (C-4), 72.4 (C-2), 74.2 (C-3); β-glucoside carbons, δ 62.5 (C-6), 69.4 (C-6), 71.2 (C-4), 71.3 (C-4), 75.0 (C-2), 75.8 (C-2), 77.1, 77.4, 77.9, 78.3 (C-3,5). On acid hydrolysis in the same way as **2**, **4** gave glucose, arabinose and **6**.

A solution of **4** (15 mg) in 1% H_2SO_4 (20 ml) was heated at 70 °C for 3 h. The reaction mixture was neutralized with diluted NaOH solution and then extracted with 1-BuOH saturated with H_2O. The BuOH extract was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (30 : 10 : 1, homogeneous) to give **3** which was identified by comparison of the ^{1}H- and ^{13}C-NMR spectra, optical rotation and TLC with those of an authentic sample.

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