Two New Saponins, Congmuyenosides A and B, from the Leaves of *Aralia elata* Collected in Heilongjiang, China

Hai-Xue KUANG,[a] Hui SUN,[a] Ning ZHANG,[a] Yoshihito OKADA,[b] and Toru OKUYAMA*,[b]

Heilongjiang University of Traditional Chinese Medicine,[a] Harbin 150040, China and Department of Pharmacognosy and Phytochemistry, Meiji College of Pharmacy,[b] 1-35-23 Nozawa, Setagaya-ku, Tokyo 154, Japan.
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Two new triterpenoidal saponins, congmuyenosides A and B, were isolated from the leaves of *Aralia elata* collected in Heilongjiang Province, China, and established as 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl hederagenin and 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl hederagenin, respectively, on the basis of chemical and spectral evidence.

Key words *Aralia elata*; congmuyenoside A; congmuyenoside B; triterpenoidal saponin

The bark of *Aralia elata* SEEM. (Chinese name: Ci Lao Ya, Japanese name: Taranoki) (Araliaceae) has been used for the treatment of neurathenia, rheumatic arthritis, hepatitis virus and diabetes, and has recently been used as a tonic in China.[1] It has been reported that triterpenoidal saponins were the main active components.[2,3] Eleven triterpenoidal saponins and two flavonoidal glycosides have been isolated from the materials in Japan by Saito *et al.*[4]

The *n*-butanol soluble part, after partition of the ethanol extract from the leaves of *A. elata*, was subjected to silica gel column chromatography to give six fractions (fr. I—fr. VI). The fr. V was repeatedly chromatographed on silica gel to give two saponins named congmuyenosides A (**1**) and B (**2**).

The molecular weight of congmuyenoside A (**1**), a white powder (MeOH), mp 262—264 °C, was determined to be 958 by the fast atom bombardment mass spectrum (FAB-MS) at *m/z* 981 $(M+Na)^+$ and at *m/z* 997 $(M+K)^+$. On acid hydrolysis, **1** gave hederagenin (**3**) (mp 331—333 °C) and only glucose as a sugar component.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in C_5D_5N) of **1** revealed signals due to six tertiary methyl groups (δ 0.86, 0.92, 0.98, 0.99, 1.05, 1.23), one trisubstituted olefinic proton (δ 5.45) and three anomeric protons (5.01, d, J=7.8 Hz; 5.30, d, J=7.8 Hz; 5.73, d, J=7.8 Hz). The ^{13}C-NMR spectrum of **1** showed signals of six quarternary carbons (δ 30.9, 36.9, 39.8, 42.0, 42.2, 46.5), one free carboxyl carbon (δ 180.2), a pair of olefinic carbons (δ 122.6, 144.9) and three anomeric carbons (δ 103.8, 103.9, 104.6).

By comparison of the ^{13}C-NMR spectral data of **1** (see Table 1) with hederagenin (**3**),[5] it was found that the signal due to C-3 shifted down field by approximately 10 ppm, signals due to C-2 and C-4 shifted to a higher field by 1.38 ppm and 0.6 ppm, respectively, and the signal due to C-23 also shifted to a higher field by approximately 3 ppm for a glycosylation shift, while the others were almost the same as those of **3**. This indicates that an aglycone of **1** was hederagenin with a sugar moiety composed of three glucose units at the C-3 position.

Comparison of ^{13}C-NMR spectral data of the sugar moiety of **1** with a known saponin (**4**),[6] which possesses a sugar moiety of [glc(1→2)][glc(1→4)]-glc-, isolated from *Phytolacca dodecandra* l'HERIT (Phytolaccaceae), and prosapogenin (**5**)[3] of Araloside G, which possesses a sugar moiety of [glc(1→3)][glc(1→4)]-glc-, isolated from the root and bark of *A. elata*, showed very different signals on the inner sugar moiety among the three saponins.

In addition, Mizutani *et al.*[7] reported the ^{13}C-NMR spectral data of methyl-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside (**6**). By comparison of the ^{13}C-NMR spectral data of **1** with **6**, both chemical shifts showed almost the same values.

The analysis of the heteronuclear single quantum coherence–total correlation spectroscopy (HSQC–TOCSY) revealed that the anomeric proton signals at δ 5.01, 5.30 and 5.73 showed correlations with the carbon signals at δ 70.0, 77.7, 79.4, 89.0, 103.9 ppm; 71.7, 75.5, 78.7×2, 104.6 ppm; and 72.3, 76.3, 77.8, 78.7, 103.8 ppm, respectively.

In the heteronuclear multiple bond connectivity (HMBC) spectrum of **1**, the anomeric proton signal at δ 5.01 (inner glucosyl) showed a correlation with the carbon signal at δ 83.4 due to the C-3 of hederagenin, and the anomeric proton signals at δ 5.30 and 5.73 showed correlations with the carbon signals at δ 89.0 (C-3 of inner glucosyl) and 79.4 (C-2 of inner glucosyl), respectively. All the carbon signals can be reasonably assigned as shown in Table 1.

Each glucose was determined to be a β-configuration from the coupling constants of signals due to an anomeric proton in the ^{1}H-NMR spectrum of **1**.

By all of the above evidence, the structure of congmuyenoside A (**1**) was established as 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl hederagenin.

Congmuyenoside B (**2**) was obtained as colorless needles from MeOH, mp 283—284 °C. The FAB-MS revealed an $(M+K)^+$ ion at *m/z* 1159 and an $(M+Na)^+$ ion at *m/z* 1143, indicating that the molecular weight is 1120. On acid hydrolysis, compound **2** yielded hederagenin (**3**) as an aglycone and only glucose as a sugar component.

The ^{13}C-NMR signals of **2** were assigned as shown in Table 1 using the same methods. The ^{13}C-NMR signals of the aglycone moiety were quite similar to those of **1**. These spectral data indicated that **2** is a monodesmosidic tetraglucoside of hederagenin in which the sugar moieties

* To whom correspondence should be addressed.

Table 1. ^{13}C-NMR Spectral Data for Saponins **1** and **2**

	1	2		1	2
C-1	38.7	38.7	C-28	180.2	180.9
C-2	26.2	26.2	C-29	33.6	33.2
C-3	83.4	83.1	C-30	23.7	23.8
C-4	42.2	42.2	Glc-1	103.9	103.8
C-5	48.1	48.1	Glc-2	79.4	79.3
C-6	18.3	18.3	Glc-3	89.0	88.9
C-7	33.0	33.0	Glc-4	70.0	70.0[a]
C-8	39.8	39.8	Glc-5	77.7[a]	77.6
C-9	48.1	48.0	Glc-6	62.5[b]	62.5[b]
C-10	36.9	36.9	Glc′-1	103.8	103.6
C-11	23.9	23.8	Glc′-2	76.3	76.3
C-12	122.6	122.6	Glc′-3	78.7	78.6[c]
C-13	144.9	144.9	Glc′-4	72.3	72.2
C-14	42.0	42.0	Glc′-5	77.8[a]	77.6
C-15	28.4	28.4	Glc′-6	63.2[b]	62.1[b]
C-16	23.8	23.8	Glc″-1	104.6	104.1
C-17	46.5	46.5	Glc″-2	75.5	74.1
C-18	42.2	42.2	Glc″-3	78.7	87.7
C-19	46.7	46.7	Glc″-4	71.7	69.9[a]
C-20	30.9	31.0	Glc″-5	78.7	78.0
C-21	34.3	34.3	Glc″-6	62.4[b]	63.2[b]
C-22	33.3	33.2	Glc‴-1		105.2
C-23	64.9	65.0	Glc‴-2		75.0
C-24	13.4	13.4	Glc‴-3		78.5[c]
C-25	16.0	16.0	Glc‴-4		71.6
C-26	17.5	17.5	Glc‴-5		78.0
C-27	26.0	26.0	Glc‴-6		62.5[b]

a—*c*) Signals may be interchangeable in each column.

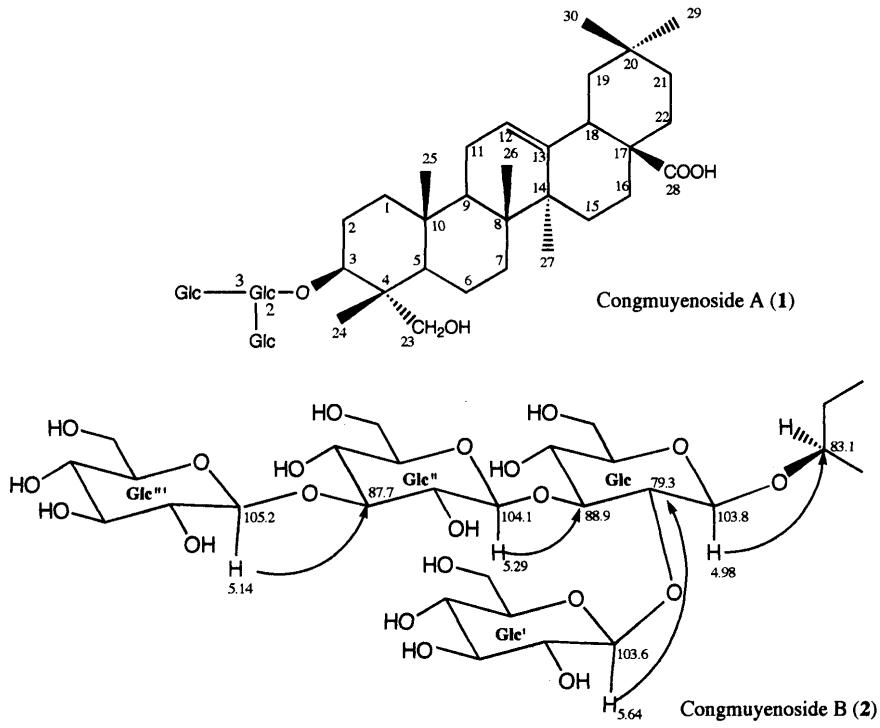

Chart 1

are linked to the C-3 hydroxyl group.

Furthermore, compound **2** had a set of additional signals corresponding to an inner β-D-glucopyranosyl unit compared to **1** in the ^{13}C-NMR spectrum. The presence of a downfield carbon signal at δ 87.7 ppm due to a sugar moiety revealed that the terminal β-D-glucopyranosyl (glc‴) was branched to the C-3 of glc′ or glc″. In the H–C correlation spectroscopy (COSY) spectrum of **2**, the anomeric proton signals at δ 4.98 (H-1), 5.14 (H-1‴), 5.29 (H-1″) and 5.64 (H-1′) ppm showed correlations with the anomeric carbon signals at δ 103.8, 105.2, 104.1 and 103.6 ppm, respectively. HSQC–TOCSY revealed that the anomeric proton signals at δ 4.98, 5.14, 5.29 and 5.64 showed correlations with the carbon signals at δ 70.0, 77.6,

79.3, 88.9, 103.8 ppm; 71.6, 75.0, 78.0, 78.5, 105.2 ppm; 69.9, 74.1, 78.0, 87.7, 104.1 ppm; and 72.2, 76.3, 77.6, 78.6, 103.6 ppm.

On the other hand, in the HMBC spectrum of **2**, the signal of an anomeric proton at δ 4.98 ppm showed correlation with the signal of a carbon due to the C-3 of the aglycone moiety at 83.1 ppm, and the signal of an anomeric proton (H-1‴) at δ 5.14 ppm showed correlation with the signal of a carbon due to C-3″ of a middle glucosyl at δ 87.7 ppm, whereas the signal of anomeric protons (H-1′, H-1″) at δ 5.64 and 5.29 ppm showed correlation with the signal of carbon due to the C-2 and C-3 of the inner glucosyl at δ 79.3 and 88.9 ppm, respectively. These data indicate the presence of a [glucopyranosyl(1→2)][glucopyranosyl(1→3)-glucopyranosyl(1→3)]-glucopyranosyl moiety in the structure of **2**. Each glucose was determined to be a β-configuration from the coupling constants as well.

By all of the above evidence, the structure of **2** was concluded to be 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl hederagenin.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. FAB-MS spectra were obtained with a JEOL JMS DX-302 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL A-500 spectrometer using tetramethylsilane as an internal standard.

Isolation of Saponins 1 and 2 The air dried leaves (1 kg) of *Aralia elata* were extracted with hot EtOH (3 l), and the combined solution was concentrated *in vacuo* to a syrup, followed by suspension in water. The suspension was extracted with petroleum ether, chloroform and *n*-butanol, sequentially, to give the corresponding extract after removal of the solvent. The *n*-butanol soluble part (55 g) was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (5 : 1 : 0.1) to give six fractions (fr. I—VI). Fraction V was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (3 : 1 : 0.1) to give two saponins named congmuyenoside A (**1**) (59 mg) and B (**2**) (74 mg).

Congmuyenoside A (**1**): A white powder, mp 262—264 °C, $[\alpha]_D$ +25.5° (C_5H_5N), $C_{48}H_{78}O_{19}$, FAB-MS (pos.) *m/z*: 997 $(M+K)^+$, 981 $(M+Na)^+$. ^{1}H-NMR (500 MHz, ppm, in C_5D_5N): δ 0.86, 0.92, 0.98, 0.99, 1.05, 1.23 (3H, s each), 5.45 (1H, t-like), 5.01 (1H, d, J=7.8 Hz), 5.30 (1H, d, J=7.8 Hz), 5.73 (1H, d, J=7.8 Hz). ^{13}C-NMR (125 MHz, ppm, in C_5D_5N) as shown in Table 1.

Congmuyenoside B (**2**): A white powder, mp 283—284 °C, $[\alpha]_D$ +30.0° (C_5H_5N), $C_{54}H_{88}O_{24}$, FAB-MS (pos.) *m/z*: 1159 $(M+K)^+$, 1143 $(M+Na)^+$. ^{1}H-NMR (500 MHz, ppm, in C_5D_5N): δ 0.86, 0.91, 0.98, 0.99, 1.04, 1.22 (3H, s each), 5.45 (1H, t-like), 4.98 (1H, d, J=7.8 Hz), 5.14 (1H, d, J=7.8 Hz), 5.29 (1H, d, J=7.9 Hz), 5.64 (1H, d, J=7.8 Hz). ^{13}C-NMR (125 MHz, ppm, in C_5D_5N) as shown in Table 1.

Acid Hydrolysis of 1 and 2 Each saponin (**1**, **2**) was heated with 7% H_2SO_4 at 80 °C for 12 h. The reaction mixture was neutralized by $Ba(OH)_2$ and filtrated. The filtrate was extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness, followed by column chromatography on silica gel to give hederagenin (**3**) which was identified by direct comparison on silica gel TLC with the authentic sample. The aqueous layer was identified as D-glucose by direct comparison on silica gel TLC and PC with the authentic sample.

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