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NOTE

# A new pregnane hexaglycoside from *Adonis multiflora*

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**Abstract** A new pregnane hexaglycoside, named amurensioside L (**1**), was isolated from the whole plant of *Adonis multiflora*. Amurensioside L (**1**) emerged to be new pregnane hexaglycoside which we fully spectroscopically characterized. The chemical structure of compound **1** was established as 14*β*,20*R*-epoxy-3*β*-hydroxypregna-4,6-dien-18-oic acid-20-lactone 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-diginopyranosyl-(1 → 4)-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*O*-*β*-D-oleandropyranosyl-(1 → 4)-*O*-*β*-D-oleandropyranoside on the basis of spectroscopic analyses including nuclear magnetic resonance spectrometry, high-resolution fast atom bombardment mass spectrometry, and infrared spectroscopy.

**Keywords** *Adonis multiflora* · Amurensioside F · Amurensioside L · Pregnane hexaglycoside

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## Introduction

*Adonis* is a genus of perennial flowering herbs of the crowfoot family, Ranunculaceae, native to Europe and Asia. Korean *Adonis* comprised three species, *A. multiflora* Nishikawa & Koki Ito, *A. amurensis* var. *uniflora* Makino, and *A. pseudoamurensis* Wang (Suh et al. 2002).

*A. multiflora* is native to Korea, Japan, and Manchuria, and grows only on Jeju Island in Korea. *A. multiflora* has been mainly cultivated for an ornamental purpose, so far. It is a spring ephemeral herb growing in temperate deciduous forests, and can reach 20–25 cm tall at flowering with up to four yellow flowers per stem. The leaves are smooth, and sepals are pale yellow, oval, oblong, cuneate, or elliptic (Lee et al. 2003). However, with exception of a report on its inhibitory effect on elastase and tyrosinase activity (Moon et al. 2010), there have been no phytochemical and biological report on *A. multiflora*. We previously used thin-layer chromatography (TLC) to confirm the presence of pregnane glycosides in ethanolic whole plant extracts of *A. multiflora* based on UV absorption patterns and characteristic dark blue colors produced by spraying plates with a 10 % $H_2SO_4$ solution followed by heating. In this study, we isolated and identified new pregnane glycosides present in whole plants of *A. multiflora*.

## Materials and methods

### Isolation of pregnane glycoside

Whole plants of *A. multiflora* were collected on the mountains of Jeju Island Korea. Dried whole plants of *A. multiflora* (1.5 kg) were extracted with 70 % aqueous EtOH at room temperature for 24 h. The concentrated

**Table 1** $^{1}$H- (400 MHz) and $^{13}$C-NMR (100 MHz) data of amurensioside L (**1**) and its acetate (**1a**) ($CDCl_3$)

| Aglycone | | | | | | Sugar | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | $\delta_C$ | | HMBC* | $\delta_H$, coupling pattern, *J* in Hz | | C | $\delta_C$ | | HMBC* | $\delta_H$, coupling pattern, *J* in Hz | |
| | **1** | **1a** | | **1** | **1a** | | **1** | **1a** | | **1** | **1a** |
| 1 | 33.6 | 33.8 | – | – | – | 1′ | 98.2 | 98.4 | H-3 | 4.52, br.d, 9.6 | 4.55, dd, 9.6, 1.6 |
| 2 | 26.5 | 26.6 | – | – | – | 2′ | 36.9 | 36.9 | – | – | – |
| 3 | 74.5 | 74.6 | H-1, H-4, H-1′ | 4.22, br.dd, 7.1, 2.1 | 4.25, br.dd, 7.0, 2.0 | 3′ | 78.8 | 79.0 | – | – | – |
| 4 | 124.6 | 124.7 | H-3, H-6 | 5.37, br.s | 5.40, br.s | 4′ | 82.6 | 82.7 | – | – | – |
| 5 | 143.8 | 144.0 | H-3, H-6, H-7 | – | – | 5′ | 70.1 | 71.0 | – | – | – |
| 6 | 126.0 | 129.5 | H-4, H-8 | 5.91, dd, 10.0, 2.4 | 5.94, dd, 10.0, 2.8 | 6′ | 18.3 | 18.4 | – | 1.25, d, 6.4**** | 1.27, d, 6.0 |
| 7 | 129.4 | 126.1 | H-4, H-8 | 5.62, br.d, 10.8 | 5.67, br.d. 10.0 | OMe-3′ | 56.2 | 56.5 | H-3′ | 3.33, s***** | 3.35, s |
| 8 | 40.4 | 40.4 | – | 2.35, br.d, 10.4 | 2.40, br.d, 8.0 | 1′’ | 98.5 | 100.2 | H-4′ | 4.61, br.d, 9.6 | 4.38, dd, 9.6, 1.6 |
| 9 | 45.9 | 46.0 | – | – | – | 2′’ | 36.9 | 36.5 | – | – | – |
| 10 | 35.0 | 35.0 | – | – | – | 3′’ | 78.6 | 79.0 | | – | – |
| 11 | 20.0 | 20.1 | – | – | – | 4′’ | 82.0 | 81.6 | – | – | – |
| 12 | 22.2 | 22.3 | – | – | – | 5′’ | 70.9 | 71.3 | – | – | – |
| 13 | 59.3 | 59.4 | H-11, H-15, H-16 | – | – | 6′’ | 18.2 | 18.4 | – | 1.24, d, 6.4**** | 1.26, d, 6.0 |
| 14 | 89.9 | 90.0 | H-8, H-9, H-17 | – | – | OMe-3′’ | 56.4 | 56.6 | H-3′’ | 3.34, s,***** | 3.38, s |
| 15 | 28.6 | 28.8 | – | – | – | 1′’’ | 98.3 | 98.6 | H-4′’ | 4.92, br.d, 10.0 | 4.64, dd, 9.6, 1.6 |
| 16 | 18.8 | 18.9 | – | – | – | 2′’’ | 36.4 | 36.5 | – | – | – |
| 17 | 58.4 | 58.5 | H-15, H-12, H-21 | 2.68, br.d, 2.8 | 2.64, br.d, 2.4 | 3′’’ | 77.0 | 77.2 | – | – | – |
| 18 | 175.6 | 175.9 | H-12, H-17 | – | – | 4′’’ | 82.3 | 82.4 | – | – | – |
| 19 | 17.4 | 17.5 | – | 0.90, s | 0.94, s | 5′’’ | 68.5 | 68.7 | – | – | – |
| 20 | 113.3 | 113.4 | H-16, H-17, H-21 | – | – | 6′’’ | 18.1 | 18.1 | – | 1.18, d, 6.4**** | 1.20, d, 6.0 |
| 21 | 15.5 | 15.6 | – | 1.55, s | 1.59, s | OMe-3′’’ | 57.9 | 59.1 | H-3′’’ | 3.37, s***** | 3.46, s |
| | | | | | | 1′’’’ | 100.0 | 100.4 | H-4′’’ | 4.74, br.d, 8.8 | 4.79, br.d, 9.6 |
| Ac-2′’’’’’ | – | 169.1 | H-2′’’’’’, H-1′’’’’’ | – | 2.04, s*** | 2′’’’ | 36.4 | 36.2 | – | – | – |
| | – | 20.7** | – | – | | 3′’’’ | 76.4 | 76.8 | – | – | – |
| Ac-3′’’’’’ | – | 170.1 | – | – | 1.99, s*** | 4′’’’ | 82.2 | 82.2 | – | – | – |
| | – | 20.62** | – | – | | 5′’’’ | 68.1 | 68.3 | – | – | – |
| Ac-4′’’’’’ | | 169.2 | – | | 1.969, s*** | 6′’’’ | 18.1 | 18.2 | – | 1.14, d, 6.4**** | 1.17, d, 6.0 |
| | | 20.60** | – | | | OMe-3′’’’ | 56.5 | 59.1 | H-3′’’’ | 3.35, s***** | 3.45, s |
| Ac-6′’’’’’ | | 170.2 | H-5′’’’’’, H-6′’’’’’ | | 1.965, s*** | 1′’’’’ | 101.6 | 101.6 | H-4′’’’ | 4.36, br.d, 9.5 | 4.95, br.d, 9.5 |
| | | 20.60** | – | | | 2′’’’’ | 32.7 | 31.5 | – | – | – |
| | | | | | | 3′’’’’ | 79.2 | 79.4 | – | – | – |
| | | | | | | 4′’’’’ | 74.3 | 73.3 | – | – | – |
| | | | | | | 5′’’’’ | 70.1 | 70.2 | – | – | – |

**Table 1** continued

| Aglycone | | | | | Sugar | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| C | $\delta_C$ | | HMBC* | $\delta_H$, coupling pattern, *J* in Hz | C | $\delta_C$ | | HMBC* | $\delta_H$, coupling pattern, *J* in Hz | |
| | **1** | **1a** | | **1** | | **1** | **1a** | | **1** | **1a** |
| | | | | | 6′′′′ | 17.1 | 17.1 | – | 1.23, d, 7.8**** | 1.21, d, 7.0 |
| | | | | | OMe-3′′′′ | 56.5 | 56.0 | H-3′′′′ | 3.32, s***** | 3.32, s |
| | | | | | 1′′′′′ | 104.8 | 98.4 | H-4′′′′ | 4.37, d, 7.8 | 4.77, d, 7.8 |
| | | | | | 2′′′′′ | 75.5 | 71.6 | – | – | 4.94, dd, 9.6, 7.8 |
| | | | | | 3′′′′′ | 77.2 | 72.8 | – | – | 5.20, dd, 9.6, 9.6 |
| | | | | | 4′′′′′ | 71.2 | 68.9 | – | – | 5.03, dd, 9.6, 10.0 |
| | | | | | 5′′′′′ | 77.3 | 71.3 | – | – | 3.62, m |
| | | | | | 6′′′′′ | 62.2 | 62.0 | – | – | 4.19, dd, 12.0, 2.4 |
| | | | | | | | | | | 4.11, dd, 12.0, 4.6 |

* Key correlations between the proton and the corresponding carbon signals in the HMBC spectrum of compound **1a**. **, ***, ****, *****, ****** The chemical shifts with same signals can be exchangeable

EtOH extracts (106 g) were poured into $H_2O$ (3 L) and successively extracted with $CH_2Cl_2$ (3 L × 3), AcOEt (3 L × 3), and *n*-BuOH (2.5 L × 3) to afford four fractions, namely, $CH_2Cl_2$ (AAC, 2.6 g), AcOEt (AAE, 0.7 g), *n*-BuOH (AAB, 12 g), and $H_2O$ (AAW, 89.2 g). The AAC (2.6 g) was applied to column chromatography (c.c.) [silica gel ($SiO_2$), ϕ 4 × 11 cm, $CH_2Cl_2$–MeOH = 18:1 → 15:1 → 7:1, 1.6 l of each] yielding 16 fractions, AAC-1–AAC-16. Fr. AAC-7 [194 mg, elution volume/total volume ($V_e/V_t$) 0.17–0.12] was applied to octadecyl silica gel (ODS) c.c. (3 × 5 cm) and eluted with MeOH–$H_2O$ (3:1, 1.2 l) to afford nine fractions along with a purified compound **1** [AAC-7-5, 78 mg, yield 0.0052 %, $V_e/V_t$ 0.78–0.82, TLC (ODS) Rf 0.47, MeOH–$H_2O$ = 7:1 ($SiO_2$) Rf 0.42, $CHCl_3$–MeOH = 10:1].

Compound: **1** white amorphous powder; $[\alpha]_D^{25}$ + 2.1° (*c* 0.82, MeOH); infrared spectroscopy (IR, KBr, *v*): 3420, 1730, and 1622 $cm^{-1}$; negative high-resolution fast atom bombardment mass spectrometry (HR/FAB/MS *m/z* 1223.6206 $[M–H]^-$ (calcd. for $C_{62}H_{95}O_{24}$ 1223.6213)); $^1H$ and $^{13}C$ nuclear magnetic resonance spectrometry (NMR) (see Table 1).

### Acetylation of amurensioside L.

Compound **1** (10 mg) was dissolved in pyridine (2 mL), added by acetic anhydride (2 mL) drop by drop in ice bath and stirred at room temperature for 8 h. The reaction mixture was poured in $H_2O$ (30 mL) and extracted with AcOEt (30 mL × 3). The organic phase was successively washed with 5 % HCl solution (60 mL), a saturated $NaHCO_3$ (60 mL), and a saline water (60 mL). And the solution was dehydrated by pouring anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The concentrates were purified by $SiO_2$ c.c. (Pasteur pipette, 1 × 7 cm, $CHCl_3$–MeOH = 18:1, 40 mL) to give an acetylated compound **1a** [9.8 mg, TLC ($SiO_2$) Rf 0.85, $CHCl_3$–MeOH = 10:1].

Compound **1a**: white amorphous powder; IR (KBr, *v*): 3010, 1735, and 1625 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1.

## Results and discussion

EtOH extracts of the whole plants of *A. multiflora* were partitioned into $CH_2Cl_2$, AcOEt, *n*-BuOH, and $H_2O$ fractions. The TLC analysis indicated that the pregnane glycosides were mainly included in $CH_2Cl_2$ fraction. The $SiO_2$ and ODS column chromatography for the $CH_2Cl_2$ resulted in the isolation of a new pregnane glycoside (**1**) with the isolation yield 0.0052 % from dry plant. The chemical structure was determined based on the spectroscopic data such as NMR, HR/FAB/MS, and IR spectra.

Compound **1** was isolated as a white amorphous powder, which showed UV absorption, and appeared as a dark blue spot on the TLC plates upon spraying with 10 % sulfuric acid followed by heating. The IR spectrum showed absorbance bands corresponding to hydroxyl (3420 $cm^{-1}$), ester (1730 $cm^{-1}$), and olefin (1622 $cm^{-1}$) groups. The molecular weight was determined to be 1224 from the molecular ion peak *m/z* 1223 $[M–H]^-$ in the negative FAB-MS spectrum, and a molecular formula of $C_{62}H_{96}O_{24}$ was determined from the highly resolved molecular ion peak ($[M–H]^-$, *m/z* 1223.6206, calc. for $C_{62}H_{95}O_{24}$, 1223.6213) in the negative HR-FAB-MS. The $^1H$ and $^{13}C$ NMR spectra of compound **1** were very similar to those of a pregnane hexaglycoside previously isolated from *A. amurensis*, amurensioside F (Kuroda et al. 2010), with the exception of the presence of two double bonds instead of one. We also noted signals representative of one aldohexose and five 2,6-deoxy-3-methyl aldohexoses that appeared as six hemiacetals [$\delta_C$ 104.8, 101.6, 100.0, 98.5, 98.3, 98.2; $\delta_H$ 4.95 (br.d, $J = 9.5$ Hz), 4.79 (br.d, $J = 9.6$ Hz), 4.77 (d, $J = 7.8$ Hz), 4.64 (dd, $J = 9.6$, 1.6 Hz), 4.55 (dd, $J = 9.6$, 1.6 Hz), 4.38 (dd, $J = 9.6$, 1.6 Hz)], five methoxies [$\delta_C$ 57.9, 56.5, 56.5, 56.4, 56.2; $\delta_H$ 3.46 (s), 3.45 (s), 3.38 (s), 3.35 (s), 3.32 (s)], and five doublet methyls [$\delta_C$ 18.3, 18.2, 18.1, 18.1, 17.1; $\delta_H$ 1.27 (d, $J = 6.0$ Hz), 1.26 (d, $J = 6.0$ Hz), 1.21 (d, $J = 0.0$ Hz), 1.20 (d, $J = 6.0$ Hz), 1.17 (d, $J = 6.0$ Hz)]. An aglycone was observed, consisting of one ester carbon of a five-membered lactone ring ($\delta_C$ 175.6), one ketal carbon ($\delta_C$ 113.3), one olefin quaternary carbon ($\delta_C$ 143.8), three olefin methines [$\delta_C$ 129.4, 126.0, 124.6; $\delta_H$ 5.94 (dd, $J = 10.0$, 2.8 Hz), 5.67 (br.d. $J = 10.0$ Hz), 5.40 (br.s)], one oxygenated quaternary carbon ($\delta_C$ 89.9), one oxygenated methine ($\delta_C$ 74.5; $\delta_H$ 4.25, br.dd, $J = 7.0$, 2.0 Hz), and two singlet methyls [$\delta_C$ 17.4, 15.5; $\delta_H$ 1.59 (s), 0.94 (s)]. The complicated overlapping of NMR signals in the oxygenated and upfield regions prevented full identification of all signals, and thus acetylation of compound **1** was performed. The acetate **1a** showed one single spot with higher Rf value than compound **1** on the $SiO_2$ TLC. In addition, no hydroxyl group absorption bands were observed in the IR spectrum. The NMR data of **1a** such as six hemiacetals [$\delta_C$ 101.6, 100.4, 100.2, 98.6, 98.4, 98.4; $\delta_H$ 4.92 (br.d, $J = 10.0$ Hz), 4.74 (br.d, $J = 8.8$ Hz), 4.61 (br.d, $J = 9.6$ Hz), 4.52 (br.d, $J = 9.6$ Hz), 4.37 (d, $J = 7.8$ Hz), 4.36 (br.d, $J = 9.5$ Hz)], five methoxies [$\delta_C$ 59.1, 59.1, 56.6, 56.5, 56.0; $\delta_H$ 3.37 (s), 3.35 (s), 3.34 (s), 3.33 (s), 3.32 (s)], five methyls [$\delta_C$ 18.4, 18.4, 18.2, 18.1, 17.1; $\delta_H$ 1.25 (d, $J = 6.4$ Hz), 1.24 (d, $J = 6.4$ Hz), 1.23 (d, $J = 7.8$ Hz), 1.18 (d, $J = 6.4$ Hz), 1.14 (d, $J = 6.4$ Hz)], four acetyls [$\delta_C$ 169.1, 170.1, 169.2, 170.2, 20.7, 20.62, 20.60. 20.60; $\delta_H$ 2.04 (s), 1.99 (s), 1.969 (s), 1.965 (s)], and numerous methylenes and oxygenated methines due to the sugar moiety confirmed the sugar of **1a** as 2,3,4,6-tetraacetyl-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-diginopyranosyl-(1 → 4)-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*O*-*β*-D-oleandropyranosyl-(1 → 4)-*O*-*β*-D-oleandropyranose. The key signals of the aglycone moiety were identified as one ester carbon ($\delta_C$ 175.9), one ketal carbon ($\delta_C$ 113.4), one olefin quaternary carbon ($\delta_C$ 143.8), three olefin methines [$\delta_C$ 124.7, 129.5, 126.1; $\delta_H$ 5.91 (dd, $J = 10.0$, 2.4 Hz), 5.62 (br.d, $J = 10.8$ Hz), 5.37 (br.s)], one oxygenated quaternary carbon ($\delta_C$ 90.0), one oxygenated methine [$\delta_C$ 74.6; $\delta_H$ 4.22 (br.dd, $J = 7.1$, 2.1 Hz)], and two singlet methyls [$\delta_C$ 17.5, 15.6; $\delta_H$ 1.55 (s), 0.90 (s)], indicating that compound **1a** has one more double bond compared to the aglycone of amurensioside F, adonilide. The positions of the sugars and key functional groups were determined from the HMBC experiment. The correlations of H-1′/C-3; H-4/C-2, C-6, and C-10; H-6/C-8; H-7/C-5, C-8, and C-14; H-17/C-14; H-16 and H-21/C-20; and H-12b/C-18 in the HMBC spectrum of **1a** confirmed

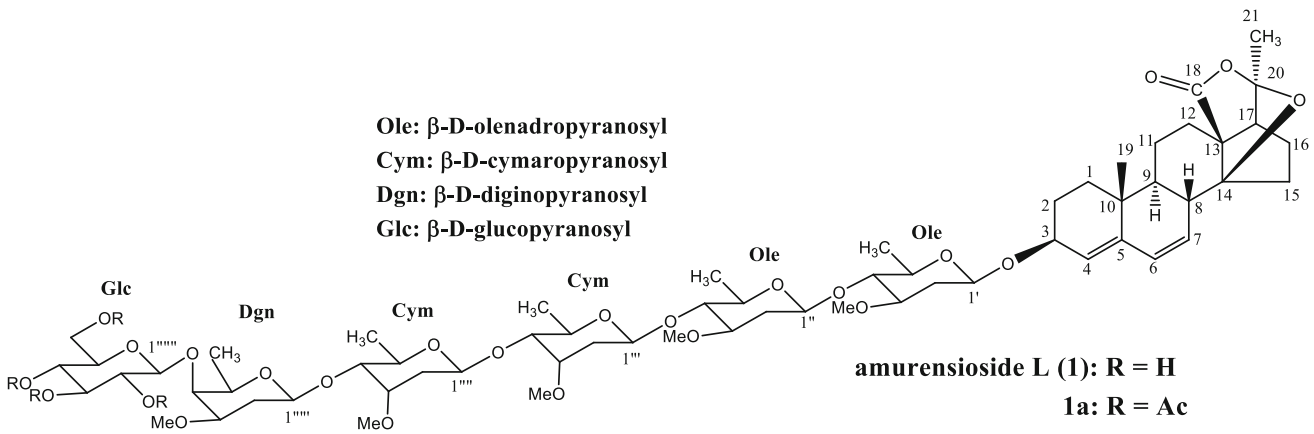


**Fig. 1** Chemical structure of amurensioside L (**1**) and its acetate (**1a**)

the positions of the sugar, β-D-oleandropyranose, the double bonds, the ketal, and the ester of the lactone ring, as shown in Fig. 1. Therefore, **1a** was identified as 2‴‴‴, 3‴‴‴, 4‴‴‴, 6‴‴‴-tetraacetyl-14β,20*R*-epoxy-3β-hydroxypregna-4,6-dien-18-oic acid-20-lactone 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-oleandropyranoside. All remaining carbon and major proton signals of compounds **1** and **1a** were identified by analysis of the DEPT, $^{1}$H–$^{1}$H COSY, NOESY, HSQC, and HMBC spectra (Table 1), as well as by comparison of the data with those of amurensioside F (Kuroda et al. 2010). Finally, the chemical structure of compound **1** was determined to be 14β,20*R*-epoxy-3β-hydroxypregna-4,6-dien-18-oic acid-20-lactone 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-oleandropyranoside, a new pregnane glycosides which we named amurensioside L.

Pregnane glycosides have been reported to show a variety of biological activities such as antimicrobial activity, antioxidant (Reddy et al. 2013), and cytotoxic activity against HSC-2 (Kuroda et al. 2010).

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