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Aestivalosides A–L, twelve pregnane glycosides from the seeds of *Adonis aestivalis*

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ABSTRACT

Eight adonilide (14,20α-epoxy-3β,20-dihydroxy-14β-pregn-5-en-18-oic acid γ-lactone) glycosides, named aestivalosides A–H, and four glycosides of the adonilide derivatives, named aestivalosides I–L, were isolated from the MeOH extract of seeds of *Adonis aestivalis*. Aestivalosides A–L were previously undescribed compounds, and were structurally characterized using spectroscopic techniques, including two-dimensional NMR, and chemical methods.

1. Introduction

Plants of the family Ranunculaceae have been reported to produce a huge variety of secondary metabolites such as alkaloids, saponins, and steroids, including bufadienolides and cardenolides, most of which showed significant biological activity (Hao et al., 2017). Our systematic phytochemical examinations of the Ranunculaceae plants were carried out with *Adonis aestivalis* Linné (Ranunculaceae) (Kubo et al., 2012), *A. amurensis* Regel et Radde (Kuroda et al., 2010; Kubo et al., 2015), *Anemone coronaria* Linné (Mimaki et al., 2009), *A. hupehensis* Lemoine var. *japonica* (Yokosuka et al., 2009), *Cimicifuga racemosa* Linné (Watanabe et al., 2002a; Mimaki et al., 2006), *Eranthis cilicica* Schott and Kotschy (Watanabe et al., 2003b; Kuroda et al., 2009), *Helleborus orientalis* Lamarck (Watanabe et al., 2002b, 2003a, 2005; Mimaki et al., 2003), and *Pulsatilla chinensis* Regel (Mimaki et al., 2001), and previously undescribed chromone derivatives, steroidal glycosides with the aglycone structures of cholestane, spirostan, furostan, cardenolide and bufadienolide, and triterpene glycosides were isolated and identified. The diversity in structure of the natural products in the Ranunculaceae plants prompted us to make a further analysis of the methanolic extract of *A. aestivalis* seeds, which resulted in the isolation of eight previously undescribed adonilide glycosides, named aestivalosides A–H (**1**–**8**), and four previously undescribed glycosides of the adonilide derivatives, named aestivalosides I–L (**9**–**12**) (Fig. 1). This paper mainly reports the structural determination of the previously undescribed glycosides.

2. Results and discussion

2.1. Structural elucidation

Aestivaloside A (**1**) was isolated from the seeds of *A. aestivalis* as an amorphous solid and shown by high resolution (HR) ESI-TOF MS to have a molecular formula of $C_{28}H_{40}O_7$ (*m/z*: 511.2654 $[M + Na]^+$). The IR spectrum of **1** displayed absorption bands at 3372 cm^{-1} and 1782 cm^{-1}, which were attributed to hydroxy groups and a carbonyl group, respectively. The ^1H NMR spectrum of **1** in C_5D_5N contained two three-proton singlets at δ_H 1.57 and 0.96, one three-proton doublet at δ_H 1.61 (d, $J = 6.2$ Hz), one olefinic proton signal at δ_H 5.38 (t-like, $J = 2.6$ Hz), and one methoxy singlet at δ_H 3.47, as well as one anomeric proton signal at δ_H 4.89 (dd, $J = 9.6$, 1.6 Hz). Acid hydrolysis of **1** with 0.025 M H_2SO_4 yielded an aglycone (**1a**) and D-oleandrose, which was the only sugar component. Compound **1a**

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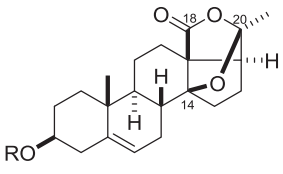
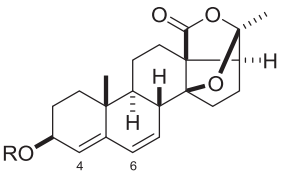
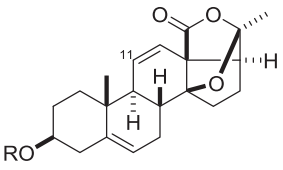


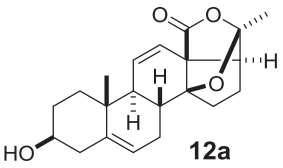
	R
aestivaloside A (**1**)	S_1
aestivaloside B (**2**)	S_2
aestivaloside C (**3**)	S_3
aestivaloside D (**4**)	S_4
aestivaloside E (**5**)	S_5
aestivaloside F (**6**)	S_6
aestivaloside G (**7**)	S_7
aestivaloside H (**8**)	S_8



	R
aestivaloside I (**9**)	S_1
aestivaloside J (**10**)	S_3
aestivaloside K (**11**)	S_6



	R
aestivaloside L (**12**)	S_3



12a

S_1=Ole-
S_2=Ole-(1→4)-Ole-
S_3=Glc-(1→4)-L-Cym-(1→4)-Ole-
S_4=Glc-(1→4)-Glc-(1→4)-Ole-(1→4)-Ole-
S_5=Glc-(1→4)-Glc-(1→4)-Ole-(1→4)-D-Cym-
S_6=Glc-(1→6)-Glc-(1→4)-L-Cym-(1→4)-Ole-
S_7=Glc-(1→4)-Glc-(1→4)-Ole-(1→4)-Ole-(1→4)-D-Cym-
S_8=Glc-(1→6)-Glc-(1→4)-Dgn-(1→4)-D-Cym-(1→4)-D-Cym-

Fig. 1. Structures of aestivalosides A-L (**1**–**12**).

was identified as adonilide (14,20α-epoxy-3β,20-dihydroxy-14β-pregn-5-en-18-oic acid γ-lactone) by comparison of its optical rotation ($[\alpha]_D^{25}$ 12.8) and spectral data with those in the literature ($[\alpha]_D^{25}$ 13.2) (Shimizu et al., 1978). Identification of this sugar, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate, using a combination of refractive index (RI) and optical rotation (OR) detectors. The relatively large $^3J_{H\text{-}1,\ H\text{-}2ax}$ value of the anomeric proton (9.6 Hz) of the D-oleandropyranosyl unit (Ole) suggested that it had the β-anomer. The HMBC spectrum of **1** displays a long-range correlation from the anomeric proton (H-1) of Ole to C-3 of the aglycone at δ_C 77.1 (Fig. 2). Thus, **1** was established as adonilide 3-*O*-β-D-oleandropyranoside.

Aestivalosides B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), and H (**8**) were isolated as amorphous solid and shown by HRESI-TOFMS to have molecular formulae of $C_{35}H_{52}O_{10}$, $C_{41}H_{62}O_{15}$, $C_{47}H_{72}O_{20}$, $C_{47}H_{72}O_{20}$, $C_{47}H_{72}O_{20}$, $C_{54}H_{84}O_{23}$, and $C_{54}H_{84}O_{23}$, respectively. On enzymatic hydrolysis or enzymatic hydrolysis followed by acid hydrolysis, these compounds afforded adonilide (**1a**) or the corresponding adonilide glycosides, suggesting **2**–**8** were adonilide glycosides.

Aestivaloside B (**2**) and **1** differed by a mass of 144.0772 corresponding to $C_7H_{12}O_3$, and the ^{1}H NMR spectrum of **2** contained signals for two anomeric protons, one at δ_H 5.00 (dd, J = 9.8, 1.9 Hz) and one at δ_H 4.82 (dd, J = 9.7, 1.8 Hz). Acid hydrolysis of **2** produced **1a** and D-oleandrose. In the ^{1}H–^{1}H COSY and HMQC spectra of **2**, all the proton and carbon signals arising from the sugar moieties were assigned to a terminal β-D-oleandropyranosyl unit [Ole″: δ_H 5.00 (dd, J = 9.8, 1.9 Hz); δ_C 100.3, 37.4, 81.6, 76.3, 73.0, 18.9 (C-1″-C-6″), 57.0 (OMe)] and an inner β-D-oleandropyranosyl residue [δ_H 4.82 (dd, J = 9.7, 1.8 Hz); δ_C 98.0, 37.8, 79.3, 83.1, 71.6, 18.7 (C-1′-C-6′), 57.2 (OMe)] glycosylated at C-4 (Ole′). The HMBC spectrum of **2** exhibited a long-range correlation between H-1 of Ole″ at δ_H 5.00 and C-4 of Ole′ at δ_C 83.1, whereas H-1 of Ole′ at δ_H 4.82 had a correlation with C-3 of the aglycone at δ_C 77.2 (Fig. 2). These correlations indicated that the oleandropyranosyl-(1→4)-oleandropyranosyl diglycoside unit was attached to C-3 of the aglycone. Therefore, the structure of **2** was elucidated as adonilide 3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside.

Aestivaloside C (**3**) differed from **2** by a mass of 162.0580 corresponding to $C_6H_{10}O_5$, and the ^{1}H NMR spectrum of **3** contained signals for three anomeric protons at δ_H 5.07 (dd, J = 4.5, 1.8 Hz), 5.04 (d, J = 7.7 Hz), and 4.77 (dd, J = 9.6, 1.6 Hz). When **3** was enzymatically hydrolyzed by naringinase, D-glucose and **3a** were obtained. Upon acid hydrolysis, **3a** afforded **1a** as the aglycone, along with L-cymarose and D-oleandrose. In the ^{1}H–^{1}H COSY and HMQC spectra of **3**, all the proton and carbon signals arising from the sugar moieties were assigned to a terminal β-D-glucopyranosyl ($^4C_1$) unit (Glc‴), a 4-substituted α-L-cymaropyranosyl ($^1C_4$) unit (Cym″), and a 4-substituted β-D-oleandropyranosyl ($^4C_1$) unit (Ole′). The Glc, L-Cym, Ole units were assigned as β-, α-, and β-anomers, respectively, from the J values of their anomeric protons (7.7 Hz in Glc‴, 4.5, 1.8 Hz in L-Cym″, and 9.6, 1.6 Hz in Ole′). The HMBC spectrum of **3** exhibited correlations between H-1 of Glc‴ (δ_H 5.04) and C-4 of L-Cym″ (δ_C 79.1), between H-1 of L-Cym″ (δ_H 5.07) and C-4 of Ole′ (δ_C 82.0), and between H-1 of Ole′ (δ_H 4.77) and C-3 of the aglycone (δ_C 77.2) (Fig. 2). The above data are consistent with **3** having the following structure: adonilide 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-oleandropyranoside.

The molecular formula of aestivaloside D (**4**) was larger than that of **2** by a mass of 324.1100, which was equivalent to $C_{12}H_{20}O_{10}$, corresponding to two hexose units. Compound **4** had four anomeric protons signals in its ^{1}H NMR spectrum: δ_H 5.19 (d, J = 7.9 Hz), 5.08 (d, J = 7.9 Hz), 4.90 (dd, J = 9.7, 1.5 Hz), and 4.80 (dd, J = 9.6, 1.6 Hz). Enzymatic hydrolysis of **4** yielded **2** and D-glucose, suggesting that the structure of **4** was composed of **2** and two glucopyranosyl units. Using the same procedures as described for **3**, all the ^{1}H and ^{13}C NMR signals arising from the sugar moieties of **4** were assigned to two 4-substituted β-D-glucopyranosyl units (Glc‴ and Glc⁗) and two 4-substituted β-D-oleandropyranosyl units (Ole′ and Ole″). The HMBC spectrum of **4** displayed long-range correlations between H-1 of Glc⁗ (δ_H 5.19) and C-4 of Glc‴ (δ_C 81.7), between H-1 of Glc‴ (δ_H 5.08) and C-4 of Ole″ (δ_C 83.1), between H-1 of Ole″ (δ_H 4.90) and C-4 of Ole′ (δ_C 83.1), and between H-1 of Ole′ (δ_H 4.80) and C-3 of the aglycone (δ_C 77.2) (Fig. 2). Accordingly, the structure of **4** was identified as adonilide 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside.