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Amurensiosides A–K, 11 new pregnane glycosides from the roots of *Adonis amurensis*

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ABSTRACT

Five new pregnane tetraglycosides, amurensiosides A–E (**1**–**5**), two new pregnane hexaglycosides, amurensiosides F (**6**) and I (**9**), two new 18-norpregnane hexaglycosides, amurensiosides G (**7**) and H (**8**), and two new pregnane octaglycosides, amurensiosides J (**10**) and K (**11**), were isolated from the MeOH extract of the roots of *Adonis amurensis*. The structures of the new compounds were determined on the basis of extensive spectroscopic analysis, including two-dimensional (2D) NMR data, and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activity against HSC-2 human oral squamous cell carcinoma cells.

1. Introduction

Plants of the family Ranunculaceae can be found worldwide, but is common in the temperate and cold areas of the northern hemisphere, and consists of 58 genera totaling about 2500 species [1]. We have already made phytochemical screenings of three Ranunculaceae plants, *Helleborus orientalis* [2–5], *Cimicifuga racemosa* [6,7] and *Eranthis cilicica* [8,9], and isolated a variety of new steroidal, bufadienolide and tritrepene glycosides, and chromone derivatives. *Adonis amurensis* Regel et Radde is mainly found in Japan, Russia, and China. The chemical constituents of the roots of *A. amurensis* have been investigated, and more than 20 pregnanes and cardenolides [10–15] have been isolated and identified. As part of our continuous study on biologically active secondary metabolites from Ranunculaceae plants, we have now examined the fresh roots of *A. amurensis*, resulting in the isolation of 11 new pregnane glycosides, named amurensiosides A–K (**1**–**11**). This paper deals with the structure elucidation of the new glycosides on the basis of extensive spectroscopic analysis, including two-dimensional (2D) NMR data, and the results of hydrolytic cleavage. The cytotoxic activity of the glycosides against HSC-2 human oral squamous cell carcinoma cells is also described.

2. Experimental

2.1. General methods

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer and UV spectra on a JASCO V-520 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-value with reference to tetramethylsilane as an internal standard. ESI-TOF-MS data were obtained on a Waters-Micromass LCT (Manchester, U.K.) mass spectrometer. Porous-polymer polystyrene resin (Diaion HP-20, Mitsubishi-Chemical, Tokyo, Japan), silica gel (300 mesh, Fuji-Silysia Chemical, Aichi, Japan), and octadecylsilanized (ODS) silica gel (75 μm, Nacalai-Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 refractive index detector (Tosoh) or a Shodex OR-2 optical rotation detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C18 UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: Dulbecco's modi-

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fied Eagle medium (DMEM) (Gibco, Grand Island, NY, U.S.A.); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, U.S.A.); penicillin G and streptomycin sulfate (Meiji-Seika, Tokyo, Japan); and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were of biochemical reagent grade.

2.2. Plant material

A. amurensis was purchased from a nursery in Heiwaen, Japan, in April 2002 and was identified by Dr. Yutaka Sashida, emeritus professor of Medicinal Pharmacognosy at Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (Voucher No. 02-4-20-AM, Laboratory of Medicinal Pharmacognosy).

2.3. Extraction and isolation

The plant material (fresh weight, 16.2 kg) was extracted with hot MeOH (28 L). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (670 g) was passed through a Diaion HP-20 column eluted with 20% MeOH in H_2O, EtOH, and EtOAc. The EtOH eluate (fraction II, 110 g) was chromatographed on silica gel eluted with $CHCl_3$–MeOH gradients (19:1; 9:1; 4:1; 2:1), and finally with MeOH alone, to give six fractions (II-I to II-vi). Fraction II-iii was subjected to column chromatography on ODS silica gel eluted with MeOH–H_2O (2:3; 1:1; 3:2; 7:4; 7:3), silica gel with $CHCl_3$–MeOH (9:1), and to preparative HPLC using MeCN–H_2O (2:1; 1:1; 1:3) and MeOH–H_2O (9:2; 7:3) to yield **1** (58.9 mg), **2** (26.8 mg), **4** (41.8 mg), **5** (19.2 mg), **6** (9.5 mg), **9** (45.7 mg), and **11** (16.3 mg). Column chromatography of the EtOAc eluate (fraction III, 20 g) on silica gel eluted with $CHCl_3$–MeOH (19:1) followed by MeOH alone to gave fractions (III-i and III-ii). Fraction III-i was chromatographed on ODS silica gel eluted with MeOH–H_2O (3:2) to yield **10** (68.7 mg). Fraction III-ii was subjected to ODS silica gel column chromatography eluted with MeOH–H_2O (6:5) followed by MeCN–H_2O (1:1; 3:2), and preparative HPLC using MeCN–H_2O (1:1) to give **3** (23.9 mg), **7** (75.6 mg), and **8** (18.0 mg).

2.4. Preparation of 2,6-dideoxy sugars for comparison

A solution of the crude glycoside fraction (1.3 g) in 0.025 M HCl (dioxane–H_2O, 1:1, 200 mL) was heated at 95 °C for 5 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column and then chromatographed on ODS silica gel eluted with MeCN–H_2O (1:2) to give five fractions (I–V). Fraction II was subjected to column chromatography on ODS silica gel eluted with MeCN–H_2O (1:3), and silica gel eluted with hexane–EtOAc (1:9), EtOAc and $CHCl_3$–MeOH (19:1; 14:1) to yield D-cymarose (21.9 mg), D-diginose (2.9 mg), and D-oleandrose (23.0 mg). D-Cymarose: $[\alpha]_D^{27}$ +46.5° (*c* 0.10, H_2O); D-diginose: $[\alpha]_D^{27}$ +16.4° (*c* 0.05, H_2O); D-oleandrose: $[\alpha]_D^{27}$ −15.3° (*c* 0.10, H_2O) [16,17].

2.5. Amurensioside A (**1**)

An amorphous solid; $[\alpha]_D^{25}$ +10.2° (*c* 0.10, MeOH); UV λ_{max} nm (log ε): 231 (3.70); IR ν_{max} (film) cm^{-1}: 3409 (OH), 2933 (CH), 1712 (C=O), 1610 and 1450 (aromatic ring); HRESITOFMS *m*/*z*: 1085.5225 $[M+Na]^+$ (calcd for $C_{55}H_{82}O_{20}Na$, 1085.5230). ^1H and ^{13}C NMR (C_5D_5N): see Tables 1, 3 and 4.

2.6. Acid hydrolysis of **1**

A solution of **1** (20.0 mg) in 0.025 M HCl (dioxane–H_2O, 1:1, 5 mL) was heated at 95 °C for 90 min under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB column and then chromatographed on ODS silica gel eluted with MeCN–H_2O (1:1) to give an aglycone (12-*O*-benzoylisolineolon, 5.8 mg) and a sugar fraction (3.5 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex Sugar SC1011 (8.0 mm i.d. × 300 mm, 5 μm, Showa-Denko); solvent, H_2O; flow rate, 1.0 mL/min; column temperature, 80 °C; detection, RI and OR. Identification of D-glucose, D-cymarose, and D-diginose was carried out by comparison of their retention times and optical rotations with those of authentic samples; t_R (min) D-glucose (7.61, positive optical rotation), D-cymarose (9.14, positive optical rotation), and D-diginose (9.55, positive optical rotation).

2.7. Amurensioside B (**2**)

An amorphous solid; $[\alpha]_D^{25}$ −13.4° (*c* 0.10, MeOH); UV λ_{max} nm (log ε): 230 (4.11); IR ν_{max} (film) cm^{-1}: 3426 (OH), 2931 (CH), 1712 (C=O), 1608 and 1450 (aromatic ring); HRESITOFMS *m*/*z*: 1085.5277 $[M+Na]^+$ (calcd for $C_{55}H_{82}O_{20}Na$, 1085.5230); ^1H and ^{13}C NMR (C_5D_5N): see Tables 1, 3 and 4.

2.8. Acid hydrolysis of **2**

Compound **2** (12.5 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (12-*O*-benzoyllineolon, 3.8 mg) and a sugar fraction (3.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, and D-diginose; t_R (min) D-glucose (7.66, positive optical rotation), D-cymarose (9.17, positive optical rotation), and D-diginose (9.69, positive optical rotation).

2.9. Amurensioside C (**3**)

An amorphous solid; $[\alpha]_D^{25}$ +14.0° (*c* 0.10, MeOH); UV λ_{max} nm (log ε): 261 (3.46), 221 (3.89); IR ν_{max} (film) cm^{-1}: 3399 (OH), 2933 (CH), 1720 (C=O), 1592 and 1457 (aromatic ring); HRESITOFMS *m*/*z*: 1064.5435 $[M+H]^+$ (calcd for $C_{54}H_{82}NO_{20}$, 1064.5429); ^1H and ^{13}C NMR (C_5D_5N): see Tables 1, 3 and 4.

2.10. Acid hydrolysis of **3**

Compound **3** (10.3 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the aglycone (2.2 mg) and a sugar fraction (2.4 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.11. Amurensioside D (**4**)

An amorphous solid; $[\alpha]_D^{25}$ +27.6° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3411 (OH), 1685 (C=O); HRESITOFMS *m*/*z*: 981.5075 $[M+Na]^+$ (calcd for $C_{48}H_{78}O_{19}Na$, 981.5034); ^1H and ^{13}C NMR (C_5D_5N): see Tables 1, 3 and 4.

2.12. Acid hydrolysis of **4**

Compound **4** (20.0 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the aglycone (3.1 mg) and a sugar fraction (3.3 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, and D-diginose; t_R (min) D-glucose

Table 1
^{1}H NMR data (500 MHz, C_5D_5N) for compounds **1**–**5**.

Positions		**1**		**2**		**3**		**4**		**5**	
		δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)
		Aglycone		Aglycone		Aglycone		Aglycone		Aglycone	
1	ax	1.11		1.11		1.12		1.11 br dd	11.7, 3.5	3.30	
	eq	1.81		1.77		1.83		1.84		1.41	
2	ax	1.83		1.80		1.82		1.77		2.01	
	eq	2.07		2.07		2.10		2.05		2.16	
3		3.87 m	$W_{1/2}$ = 22.3	3.86 m	$W_{1/2}$ = 21.8	3.87 m	$W_{1/2}$ = 19.8	3.86 m	$W_{1/2}$ = 20.7	3.93 m	$W_{1/2}$ = 18.7
4	ax	2.46		2.43		2.59		2.47		2.63 (2H)	
	eq	2.57		2.56		2.47		2.56 dd	12.6, 3.4		
5		–		–		–		–		–	
6		5.35 br d	4.1	5.29 br s		5.35 br d	4.3	5.36 br s		5.39 br d	5.2
7	a	2.54		2.53		2.55		2.51		2.57	
	b	2.51		2.35		2.35		2.30		2.28	
8		–		–		–		–		–	
9		1.74 dd	12.9, 2.8	1.76		1.74 dd	12.9, 3.8	1.60 dd	12.9, 2.9	1.78 d	11.1
10		–		–		–		–		–	
11	ax	2.40		2.32		2.40		2.38 q-like	12.1	4.77 ddd	11.1, 11.1, 4.1
	eq	2.05		2.13		2.10		1.90		–	
12		5.25 dd	12.0, 3.8	5.41 dd	11.8, 4.0	5.20	12.0, 3.8	3.75 dd	11.9, 3.6	1.89	
		–		–		–		–		2.11	
13		–		–		–		–		–	
14		–		–		–		–		–	
15	a	2.00		2.10		2.12		2.30		2.26 (2H)	
	b	2.10		1.94		2.02		2.01		–	
16	a	1.95		2.54		2.31		2.17		1.90	
	b	2.26		1.84		1.94		1.95		2.02	
17		3.26 dd	9.3, 5.7	3.54		3.26 dd	9.2, 5.8	3.86 m		2.89 dd	9.5, 4.8
18		1.65 s		2.04 s		1.63 s		1.53 s		1.43 s	
19		1.37 s		1.33 s		1.38 s		1.37 s		1.80 s	
20		–		–		–		–		–	
21		2.20 s		2.11 s		2.23 s		2.28 s		2.20 s	
		Bz		Bz		Nic					
1		–		–		–					
2		–		–		–					
3		8.37 br d	7.8	8.27 br d	8.0	8.92 dd	4.8, 1.8				
4		7.53 br dd	7.8, 7.8	7.47 br dd	8.0, 8.0	–					
5		7.60 br dd	7.8, 7.8	7.53 br dd	8.0, 8.0	8.54 ddd	8.0, 1.8, 1.8				
6		7.53 br dd	7.8, 7.8	7.47 br dd	8.0, 8.0	7.43 dd	8.0, 4.8				
7		8.37 br d	7.8	8.27 br d	8.0	9.65 d	1.8				
		Cym′		Cym′		Cym′		Cym′		Cym′	
1′		5.29 dd	9.5, 1.6	5.28 dd	9.4, 1.3	5.29 br d	9.4	5.27 br d	9.5	5.31 dd	9.5, 1.4
2′	ax	1.92 ddd	12.0, 9.5, 2.9	1.92 ddd	12.0, 9.4, 2.9	1.91 ddd	12.0, 9.4, 2.9	1.87 ddd	12.0, 9.5, 3.0	1.92 ddd	12.4, 9.5, 2.9
	eq	2.34 br d	12.0	2.31 br d	12.0	2.35 br d	12.0	2.30 br d	12.0	2.31 br d	12.4
3′		4.09 q-like	2.7	4.09 q-like	2.7	4.09 q-like	2.6	4.06 q-like	2.7	4.07 q-like	2.5
4′		3.52 dd	9.5, 2.7	3.51 dd	9.4, 2.7	3.48 dd	9.2, 2.6	3.49 dd	9.7, 2.5	3.48 dd	9.7, 2.5
5′		4.23 m		4.23 m		4.23 m		4.21 m		4.20 m	
6′		1.40 d	6.3	1.40 d	6.2	1.37 d	6.4	1.36 d	6.3	1.36 d	6.2
OMe		3.61 s		3.61 s		3.41 s		3.59 s		3.59 s	
		Cym″		Cym″		Cym″		Cym″		Cym″	
1″		5.11 dd	9.5, 1.3	5.10 br dd	9.4, 1.1	5.11 br d	9.4	5.08 br d	9.5	5.09 dd	9.6, 1.4
2″	ax	1.78 ddd	12.4, 9.5, 2.7	1.78 ddd	12.4, 9.4, 2.9	1.79 ddd	12.4, 9.4, 2.9	1.76 ddd	12.4, 9.5, 2.9	1.78 ddd	12.4, 9.6, 2.9
	eq	2.33 br d	12.4	2.31 br d	12.4	2.31 br d	12.4	2.28 br d	12.4	2.30 br d	12.4
3″		4.00 q-like	2.7	4.00 q	2.7	4.00 q-like	2.6	3.98 q-like	2.6	3.98 q-like	2.4
4″		3.48 dd	9.6, 2.7	3.47 dd	9.3, 2.7	3.52 dd	9.2, 2.6	3.46 dd	9.5, 2.6	3.46 dd	9.6, 2.4
5″		4.17 m		4.16 m		4.20 m		4.13 m		4.15 m	
6″		1.37 d	6.1	1.38 d	6.2	1.40 d	6.4	1.36 d	6.3	1.36 d	6.2
OMe		3.41 s		3.41 s		3.61 s		3.39 s		3.39 s	
		Dgn‴		Dgn‴		Dgn‴		Dgn‴		Dgn‴	
1‴		4.65 dd	9.7, 1.7	4.65 dd	9.6, 1.7	4.65 br d	9.4	4.65 dd	9.6, 1.2	4.65 dd	9.6, 1.8
2‴	ax	2.30 q-like	11.0	2.30 q-like	11.2	2.34 q-like	11.0	2.27 q-like	10.5	2.28 q-like	11.0
	eq	2.18 br dd	12.2, 5.0	2.18 br dd	12.8, 5.1	2.19 br dd	12.5, 5.0	2.17 br dd	13.1, 5.0	2.16 br dd	13.1, 5.0
3‴		3.46 ddd	12.2, 4.3, 2.9	3.46 ddd	12.0, 4.0, 2.9	3.45 ddd	12.5, 4.5, 2.9	3.47 ddd	12.9, 4.5, 3.0	3.46 ddd	12.3, 4.7, 2.6
4‴		4.17 br s		4.17 br s		4.18 br s		4.18 br s		4.17 br s	
5‴		3.56 m		3.55 m		3.57 m		3.56 m		3.56	
6‴		1.55 d	6.4	1.55 d	6.3	1.64 d	6.2	1.54 d	6.0	1.54 d	6.3
OMe		3.39 s		3.39 s		3.39 s		3.39 s		3.39 s	
		Glc⁗		Glc⁗		Glc⁗		Glc⁗		Glc⁗	
1⁗		5.13 d	7.7	5.12 d	7.6	5.13 d	7.6	5.09 d	7.7	5.11 d	7.8
2⁗		3.95 dd	8.9, 7.7	3.96 dd	8.9, 7.6	3.96 dd	8.9, 7.6	3.92 dd	8.8, 7.7	3.94 dd	9.0, 7.8
3⁗		4.22 dd	8.9, 8.9	4.22 dd	8.9, 8.9	4.23 dd	8.9, 8.9	4.21 dd	8.8, 8.8	4.22 dd	9.0, 9.0
4⁗		4.16 dd	8.9, 8.9	4.17 dd	8.9, 8.9	4.18 dd	8.9, 8.9	4.15 dd	8.8, 8.8	4.15 dd	9.0, 9.0
5⁗		3.94 m		3.94 m		3.95 m		3.92 m		3.94 m	
6⁗	a	4.57 dd	11.7, 2.5	4.58 dd	11.6, 2.4	4.58 dd	11.6, 1.5	4.53 dd	11.7, 2.2	4.55 dd	11.6, 2.3
	b	4.36 dd	11.7, 2.5	4.36 dd	11.6, 5.8	4.36 dd	11.6, 5.8	4.34 dd	11.7, 5.7	4.35 dd	11.6, 5.7

Bz: benzoyl; Nic: nicotinoyl; Cym: β-D-cymaropyranosyl; Dgn: β-D-diginopyranosyl; Glc: β-D-glucopyranosyl.

Table 2
^{1}H NMR data (500 MHz, C_5D_5N) for compounds **6**, **6a**, and **7**–**11**.

Positions		**6**		**6a**		**7**		**8**		**9**		**10**		**11**	
		δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)
		Aglycone		Aglycone		Aglycone		Aglycone		Aglycone		Aglycone		Aglycone	
1	ax	1.07		1.15		1.63		1.06		1.14		1.27		1.15	
	eq	1.77		1.84		2.30		1.74		1.82		1.87		1.87	
2	ax	1.67		1.75		1.71		1.71		1.71		1.37		1.85	
	eq	2.07		2.07		2.05		2.10		2.05		2.10		2.12	
3		3.80	$W_{1/2}$ = 22.6	3.83 m	$W_{1/2}$ = 21.5	3.76 m	$W_{1/2}$ = 23.9	3.78 m	$W_{1/2}$ = 24.1	3.80 m	$W_{1/2}$ = 22.5	3.87 m	$W_{1/2}$ = 23.2	3.91 m	$W_{1/2}$ = 24.1
4	ax	2.52		2.55		2.34		2.40		2.46		2.62		2.63	
	eq	2.34		2.56		2.57		2.62		2.62		2.53		2.53	
5		–		–		–		–		–		–		–	
6		5.36 t-like	2.5	5.32 t-like	2.6	5.41 dd	5.0	5.49 t-like	2.6	5.57 t-like	2.4	5.44 br d	4.4	5.45 br d	4.1
7	a	2.20		2.20		2.17		1.99		2.74		2.53		2.53	
	b	1.89		1.89		1.71		2.64		1.94		2.33		2.31	
8		1.95		1.99		2.48		2.82		1.99		–		–	
9		1.27		1.29		1.71		1.80		2.05		1.79		1.49	
10		–		–		–		–		–		–		–	
11	ax	2.35		2.26		2.32		2.65		1.91 (2H)		2.41		1.99	
	eq	1.60		1.65		2.42		2.51		–		2.07		1.44	
12	ax	2.07		1.30		–		–		4.88 br d	2.6	5.26		1.37	
	eq	1.31		2.11						–		–		1.49	
13		–		–		–		–		–		–		–	
14		–		–		–		–		–		–		–	
15	a	1.84		1.86		1.47 (2H)		6.40 d	4.0	1.78		2.08		2.12	
	b	1.77		1.77						1.50		2.04		1.87	
16	a	1.59 (2H)		1.58 (2H)		2.05		7.41 d	4.0	1.29		2.30		1.99	
	b					1.98				2.07		1.96		1.90	
17		2.06 brs		2.61 t-like	2.4	4.05	9.5, 4.9	–		3.21		3.27 dd	9.3, 5.7	2.82 dd	9.5, 4.9
18		–		–		–		–		1.04 s		1.64 s		1.33 s	
19		0.95 s		1.01 s		0.90 s		1.00 s		1.05 s		1.37 s		1.35 s	
20		–		–		–		–		3.97		–		–	
21		1.57 s		1.57 s		2.28 s		2.48 s		1.35 d	5.9	2.19 s		2.22 s	
												Bz			
1												–			
2												–			
3, 7												8.38 br d	7.8		
4, 6												7.53 br dd	7.8		
5												7.59 br dd	7.8		
		Ole′				Ole′		Ole′		Ole′		Ole′		Ole′	
1′		4.80 br d	9.6			4.80 dd	9.6, 1.6	4.81 dd	9.7, 1.6	4.81 dd	9.6, 1.6	4.83 dd	9.6, 1.3	4.85 dd	9.7, 1.7
2′	ax	1.83 q-like	10.8			1.78 ddd	11.8, 11.8, 9.6	1.81 ddd	11.8, 11.8, 9.7	1.81 ddd	11.3, 11.3, 9.6	1.77 ddd	11.8, 11.8, 9.6	1.80 ddd	11.8, 11.8, 9.7
	eq	2.43 br d	10.4			2.44 br d	11.8	2.45 br d	11.8	2.45 br d	9.9	2.49 br d	11.8	2.51 br d	11.8
3′		3.53				3.56		3.56		3.56		3.55		3.56	
4′		3.53 dd	9.5, 9.5			3.53 dd	9.5, 9.5	3.51 dd	9.5, 9.5	3.51 dd	9.5, 9.5	3.52		3.52	
5′		3.53 m				3.52 m		3.55 m		3.53 m		3.53 m		3.53 m	
6′		1.46 d	6.0			1.41 d	6.3	1.45 d	6.2	1.45 d	6.3	1.46 d	6.1	1.46 d	6.1
OMe		3.51 s				3.51 s		3.52 s		3.52 s		3.51 s		3.51 s	
		Ole″				Ole″		Ole″		Ole″		Ole″		Ole″	
1″		4.91 br d	9.7			4.90 dd	9.7, 1.7	4.91 dd	9.7, 1.7	4.91 dd	9.8, 1.5	4.91 br d	9.7	4.91 dd	9.7, 1.7
2″	ax	1.73 q-like	10.9			1.74 ddd	11.1, 11.1, 9.7	1.77 ddd	11.1, 11.1, 9.7	1.75 ddd	11.1, 11.1, 9.8	1.73 ddd	11.1, 11.1, 9.7	1.73 ddd	11.1, 11.1, 9.7
	eq	2.45 br d	10.6			2.49 br d	11.1	2.50 br d	11.1	2.47 br d	9.9	2.45 br d	11.1	2.51 br d	11.1
3″		3.55				3.56		3.54		3.56		3.55		3.56	
4″		3.52 dd	9.6, 9.6			3.53 dd	9.6, 9.6	3.53 dd	9.6, 9.6	3.51 dd	9.6, 9.6	3.52		3.52	

5″		3.53 m		3.52 m		3.51 m		3.53 m		3.53 m		3.53 m			
6″		1.42 d	6.1	1.44 d	6.2	1.45 d	6.4	1.44 d	6.4	1.42 d	6.1	1.42 d	6.2		
OMe		3.54 s		3.56 s		3.56 s		3.56 s		3.54 s		3.53 s			
		Cym‴		Cym‴		Cym‴		Cym‴		Ole‴		Ole‴			
1‴		5.28 br d	9.7	5.28 dd	9.6, 1.6	5.29 dd	9.7, 1.6	5.29 dd	9.7, 1.5	4.89 br d	9.7	4.89 dd	9.7, 1.7		
2‴	ax	1.84 ddd	12.2, 9.7, 2.9	1.83 ddd	12.1, 9.6, 3.1	1.83 ddd	12.1, 9.7, 3.1	1.84 ddd	12.0, 9.7, 2.9	1.73 ddd	11.1, 11.1, 9.7	1.73 ddd	11.1, 11.1, 9.7		
	eq	2.34 br d	13.5	2.34 br d	12.1	2.35 br d	12.1	2.35 br d	12.0	2.49 br d	11.1	2.51 br d	11.1		
3‴		4.07 q-like	2.7	4.07 q-like	2.8	4.08 q-like	2.9	4.07 q-like	2.8	3.55		3.56			
4‴		3.47 br d	10.7	3.46 dd	9.4, 2.8	3.46 dd	9.4, 2.9	3.46 dd	9.5, 2.8	3.52		3.52			
5‴		4.19 m		4.20 m		4.19 m		4.21 m		3.53 m		3.53 m			
6‴		1.36 d	6.3	1.36 d	6.2	1.36 d	6.2	1.35 d	6.3	1.42 d	6.0	1.42 d	6.0		
OMe		3.60 s		3.60 s		3.60 s		3.60 s		3.50 s		3.56 s			
		Cym⁗		Cym⁗		Cym⁗		Cym⁗		Cym⁗		Cym⁗			
1⁗		5.09 br d	9.4	5.08 dd	9.6, 1.5	5.09 dd	9.6, 1.5	5.09 dd	9.6, 1.4	5.29 br d	9.5	5.29 dd	9.7, 1.5		
2⁗	ax	1.79 ddd	12.3, 9.4, 2.9	1.76 ddd	12.3, 9.6, 3.1	1.77 ddd	12.3, 9.6, 3.1	1.77 ddd	12.4, 9.6, 2.7	1.85 ddd	12.0, 9.5, 2.9	1.84 ddd	12.0, 9.7, 2.9		
	eq	2.30 br d	13.1	2.32 br d	12.3	2.29 br d	12.3	2.29 br d	12.4	2.36 br d	12.0	2.35 br d	12.0		
3⁗		4.00 q-like	2.4	3.99 q-like	2.8	4.00 q-like	2.7	4.00 q-like	2.8	4.07 q-like	2.6	4.07 q-like	2.8		
4⁗		3.48 br d	10.9	3.48 dd	9.6, 2.8	3.48 dd	9.6, 2.7	3.48 dd	9.5, 2.8	3.47 dd	9.5, 2.6	3.46 dd	9.5, 2.8		
5⁗		4.19 m		4.19 m		4.17 m		4.16 m		4.17 m		4.21 m			
6⁗		1.39 d	6.2	1.38 d	6.2	1.38 d	6.2	1.37 d	6.5	1.33 d	6.8	1.35 d	6.3		
OMe		3.41 s		3.41 s		3.41 s		3.41 s		3.60 s		3.60 s			
		Dgn⁗′		Dgn⁗′		Dgn⁗′		Dgn⁗′		Cym⁗′		Cym⁗′			
1⁗′		4.65 br d	9.5	4.65 br d	9.6	4.65 dd	9.7, 1.7	4.65 dd	9.7, 1.8	5.10 br d	9.5	5.09 dd	9.6, 1.4		
2⁗′	ax	2.32 q-like	10.5	2.30 q-like	11.0	2.31 q-like	11.0	2.31 q-like	11.0	1.79 ddd	12.4, 9.5, 2.7	1.77 ddd	12.4, 9.6, 2.7		
	eq	2.20 br dd	12.6, 4.9	2.18 br dd	13.0, 5.1	2.18 br dd	12.4, 4.1	2.20 br dd	12.2, 5.0	2.29 br d	12.4	2.29 br d	12.4		
3⁗′		3.47 ddd	12.6, 4.4, 3.2	3.47 ddd	13.0, 4.4, 2.3	3.46 ddd	12.4, 4.3, 2.8	3.46 ddd	12.2, 4.3, 2.9	4.00 q-like	2.6	4.00 q-like	2.8		
4⁗′		4.17 br s		4.17 br s		4.17 br s		4.17 br s		3.48 dd	9.5, 2.6	3.48 dd	9.5, 2.8		
5⁗′		3.56 m		3.56 m		3.55 m		3.56 m		4.14 m		4.16 m			
6⁗′		1.55 d	6.5	1.55 d	6.3	1.56 d	6.4	1.55 d	6.3	1.35 d	6.5	1.37 d	6.5		
OMe		3.39 s		3.39 s		3.39 s		3.39 s		3.41 s		3.41 s			
		Glc⁗″		Glc⁗″		Glc⁗″		Glc⁗″		Dgn⁗″		Dgn⁗″			
1⁗″		5.12 d	7.8	5.12 d	7.8	5.13 d	7.8	5.13 d	7.8	4.66 br d	8.9	4.65 dd	9.7, 1.8		
2⁗″		3.95 dd	9.0, 7.8	3.95 dd	8.8, 7.8	3.96 dd	8.8, 7.8	3.96 dd	8.8, 7.8	2.32 q-like	11.0	2.31 q-like	11.0		
3⁗″		4.22 dd	9.0, 9.0	4.22 dd	8.8, 8.8	4.22 dd	8.8, 8.8	4.21 dd	8.8, 8.8	2.21 br dd	12.2, 5.0	2.20 br dd	12.2, 5.0		
4⁗″		4.15 dd	9.0, 9.0	4.15 dd	8.8, 8.8	4.15 dd	8.8, 8.8	4.15 dd	8.8, 8.8	3.45 ddd	12.2, 4.3, 2.9	3.46 ddd	12.2, 4.3, 2.9		
5⁗″		3.95 m		3.95 m		3.95 m		3.95 m		4.17 br s		4.17 br s			
6⁗″	a	4.57 dd	11.6, 2.1	4.56 br d	11.5, 2.2	4.57 dd	11.7, 2.4	4.57 br d	10.8	3.54 m		3.56 m			
	b	4.36 dd	11.6, 5.7	4.36 dd	11.5, 5.7	4.35 dd	11.7, 5.7	4.35 dd	10.8, 4.9	1.53 d	6.3	1.55 d	6.3		
										3.39 s		3.39 s			
										Glc⁗‴′		Glc⁗‴′			
										5.13 d	7.8	5.13 d	7.8		
										3.95 dd	8.8, 7.8	3.96 dd	8.8, 7.8		
										4.21 dd	8.8, 8.8	4.21 dd	8.8, 8.8		
										4.15 dd	8.8, 8.8	4.15 dd	8.8, 8.8		
										3.94 m		3.95 m			
										4.57 dd	11.6, 2.3	4.57 br d	10.8		
										4.35 dd	11.6, 5.8	4.35 dd	10.8, 4.9		

Bz: benzoyl; Ole: β-D-oleandropyranosyl; Cym: β-D-cymaropyranosyl; Dgn: β-D-diginopyranosyl; Glc: β-D-glucopyranosyl.

(7.63, positive optical rotation), D-cymarose (9.18, positive optical rotation), and D-diginose (9.65, positive optical rotation).

2.13. Amurensioside E (**5**)

An amorphous solid; $[\alpha]_D^{25}$ +15.7° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3401 (OH), 2933 (CH), 1689 (C=O); HRESITOFMS *m*/*z*: 981.4995 $[M+Na]^+$ (calcd for $C_{48}H_{78}O_{19}Na$, 981.5034); ^1H and ^{13}C NMR (C_5D_5N): see Tables 1, 3 and 4.

2.14. Acid hydrolysis of **5**

Compound **5** (10.0 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the aglycone (1.4 mg) and a sugar fraction (1.3 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.15. Amurensioside F (**6**)

An amorphous solid; $[\alpha]_D^{25}$ +0.49° (*c* 0.11, MeOH); IR ν_{max} (film) cm^{-1}: 3131 (OH), 1779 (C=O); HRESITOFMS *m*/*z*: 1249.6392 $[M+Na]^+$ (calcd for $C_{62}H_{98}O_{24}Na$, 1249.6345); ^1H and ^{13}C NMR: see Tables 2–4.

2.16. Acid hydrolysis of **6**

Compound **6** (8.3 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (**6a**) (adonilide, 1.5 mg) and a sugar fraction (4.1 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-oleandrose (9.20, negative optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.17. Adonilide (**6a**)

An amorphous solid; $[\alpha]_D^{25}$ +5.62° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3150 (OH), 2955 (CH), 1775 (C=O); HRESITOFMS *m*/*z*: 367.1887 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_4Na$, 367.1885); ^1H and ^{13}C NMR: see Tables 2 and 3.

2.18. Amurensioside G (**7**)

An amorphous solid; $[\alpha]_D^{25}$ −45.6° (*c* 0.10, MeOH); UV λ_{max} nm ($\log\varepsilon$): 250 (3.91); IR ν_{max} (film) cm^{-1}: 3093 (OH), 2965 (CH), 1710 (C=O); HRESITOFMS *m*/*z*: 1219.6228 $[M+Na]^+$ (calcd for $C_{61}H_{96}O_{23}Na$, 1219.6234); ^1H and ^{13}C NMR: see Tables 2–4.

2.19. Acid hydrolysis of **7**

Compound **7** (24.8 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the aglycone (5.1 mg) and a sugar fraction (9.9 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.41, positive optical rotation), D-oleandrose (9.16, negative optical rotation), D-cymarose (9.19, positive optical rotation), and D-diginose (9.60, positive optical rotation).

2.20. Amurensioside H (**8**)

An amorphous solid; $[\alpha]_D^{25}$ −22.6° (*c* 0.10, MeOH); UV λ_{max} nm ($\log\varepsilon$): 385 (3.81), 329 (3.90), 245 (3.99); IR ν_{max} (film) cm^{-1}: 3378 (OH), 2923 (CH), 1729 (C=O); HRESITOFMS *m*/*z*: 1233.6001 $[M+Na]^+$ (calcd for $C_{61}H_{94}O_{24}Na$, 1233.6032); ^1H and ^{13}C NMR: see Tables 2–4.

Table 3
^{13}C NMR data (125 MHz, C_5D_5N) for the aglycone moieties of compounds **1**–**6**, **6a**, and **7**–**11**.

Positions	**1**	**2**	**3**	**4**	**5**	**6**	**6a**	**7**	**8**	**9**	**10**	**11**
1	38.9	38.9	38.9	39.0	41.0	37.6	38.0	36.6	36.8	37.7	38.9	39.1
2	29.9	29.8	29.9	29.9	30.5	30.2	32.5	29.8	29.9	30.4	29.8	29.9
3	77.7	77.6	77.7	77.7	78.4	77.3	71.0	77.4	77.4	77.7	77.9	77.9
4	39.2	39.2	39.2	39.2	40.1	39.2	43.4	39.2	39.2	39.5	39.2	39.3
5	139.1	139.4	139.1	139.1	140.8	140.3	141.3	141.2	140.7	140.1	139.1	139.1
6	119.4	119.2	119.4	119.6	118.9	120.8	120.1	121.0	121.4	123.0	119.5	119.9
7	35.8	35.1	35.7	36.1	36.1	27.4	27.4	29.9	31.3	26.1	35.9	36.0
8	74.3	74.5	74.3	74.4	76.9	35.5	35.6	33.7	31.6	35.5	74.3	74.6
9	45.1	44.7	45.1	45.4	53.2	45.6	45.7	49.5	50.1	39.9	45.1	47.4
10	37.6	37.5	37.6	37.5	39.6	37.2	37.2	37.1	37.1	36.9	37.6	37.7
11	24.7	25.0	24.6	28.3	66.1	20.7	20.8	34.0	34.4	29.2	24.7	19.1
12	78.6	73.7	79.3	73.8	50.2	21.8	21.9	196.4	189.8	74.8	78.5	39.1
13	55.0	56.1	55.0	56.5	50.5	59.1	59.1	136.7	120.8	49.1	55.0	49.8
14	86.5	87.5	86.5	86.6	85.7	91.4	91.5	169.4	158.8	85.2	86.6	85.9
15	36.6	34.2	36.5	37.1	37.2	28.8	28.8	34.0	119.5	33.5	36.6	36.9
16	24.6	22.1	24.6	24.6	24.8	18.5	18.5	26.9	137.1	25.2	24.6	24.6
17	59.3	60.3	56.2	58.2	64.1	58.4	58.4	55.5	122.0	47.5	59.3	63.8
18	12.7	15.9	12.7	11.6	18.9	175.9	175.9	–	–	16.8	12.7	17.1
19	18.6	18.2	18.4	18.4	17.8	19.4	19.5	19.0	19.4	19.6	18.3	18.4
20	214.0	209.5	213.9	216.8	216.3	113.5	113.5	209.3	184.2	69.7	214.1	216.7
21	31.6	32.2	31.4	32.2	32.0	15.6	15.6	29.4	21.9	24.4	31.6	32.1
	Bz	Bz	Nic								Bz	
1	166.5	165.5	165.5								166.6	
2	131.2	131.3	–								131.2	
3	130.0	129.9	154.1								130.0	
4	129.1	128.9	126.9								129.1	
5	133.6	133.2	137.2								133.6	
6	129.1	128.9	124.0								129.1	
7	130.0	129.9	151.2								130.0	

Bz: benzoyl; Nic: nicotinoyl.

Table 4
^{13}C NMR data (C_5D_5N, 125 MHz) for the sugar moieties of compounds **1**–**11**.

Positions	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	Cym′	Cym′	Cym′	Cym′	Cym′	Ole′	Ole′	Ole′	Ole′	Ole′	Ole′
1′	96.4	96.4	96.4	96.3	96.5	97.9	98.0	98.1	98.0	98.0	98.0
2′	37.2	37.2	37.1	37.0	37.3	37.9	37.9	37.9	37.9	37.8	37.8
3′	78.0	78.0	78.0	77.9	78.2	79.3	79.3	79.3	79.4	79.3	79.3
4′	83.4	83.4	83.4	83.3	83.6	83.3	83.1	83.2	83.1	83.3	83.3
5′	69.0	69.0	69.0	69.0	69.2	71.5	71.5	71.6	71.5	71.6	71.6
6′	18.6	18.6	18.6	18.5	18.8	18.8	18.7	18.8	18.8	18.8	18.8
OMe	58.8	58.8	58.8	58.7	58.9	57.3	57.3	57.5	57.3	57.5	57.2
	Cym″	Cym″	Cym″	Cym″	Cym″	Ole″	Ole″	Ole″	Ole″	Ole″	Ole″
1″	100.4	100.4	100.4	100.3	100.6	100.2	100.2	100.2	100.2	100.2	100.2
2″	36.5	36.5	36.5	36.3	36.6	37.9	37.9	37.9	37.9	37.9	37.9
3″	77.5	77.5	77.5	77.4	77.6	79.2	79.1	79.1	79.2	79.3	79.3
4″	83.1	83.1	83.1	83.0	83.3	83.0	83.0	83.0	83.0	83.0	83.1
5″	68.9	68.9	68.9	68.9	69.1	71.8	71.8	71.8	71.8	71.7	71.7
6″	18.6	18.6	18.6	18.5	18.7	18.7	18.7	18.7	18.7	18.7	18.7
OMe	58.3	58.3	59.4	58.2	58.5	57.5	57.5	57.3	57.5	57.4	57.4
	Dgn‴	Dgn‴	Dgn‴	Dgn‴	Dgn‴	Cym‴	Cym‴	Cym‴	Cym‴	Ole‴	Ole‴
1‴	102.7	102.6	102.7	102.5	102.8	98.5	98.5	98.5	98.5	100.2	100.2
2‴	32.8	32.8	32.8	32.8	33.0	36.9	36.9	36.9	36.9	37.9	37.9
3‴	79.8	79.8	80.0	79.7	80.0	78.0	78.0	78.0	78.1	79.1	79.1
4‴	73.8	73.7	73.7	73.7	73.9	83.2	83.2	83.2	83.2	82.9	82.9
5‴	71.0	71.0	71.0	70.9	71.2	69.2	69.2	69.2	69.2	71.8	71.8
6‴	17.9	17.9	17.9	17.8	18.0	18.4	18.4	18.4	18.4	18.7	18.7
OMe	56.2	56.2	58.3	56.1	56.4	58.7	58.7	58.7	58.8	57.3	57.3
	Glc⁗	Glc⁗	Glc⁗	Glc⁗	Glc⁗	Cym⁗	Cym⁗	Cym⁗	Cym⁗	Cym⁗	Cym⁗
1⁗	105.0	105.0	104.9	104.8	105.1	100.4	100.4	100.4	100.4	98.5	98.5
2⁗	75.9	75.9	75.9	75.8	76.1	36.4	36.4	36.4	36.5	36.9	37.0
3⁗	78.4	78.4	78.4	78.2	78.5	77.5	77.4	77.5	77.5	78.1	78.1
4⁗	71.9	71.9	71.9	71.8	72.0	83.1	83.1	83.1	83.2	83.2	83.3
5⁗	78.5	78.5	78.6	78.5	78.7	69.0	69.0	69.0	69.0	69.2	69.2
6⁗	63.1	63.1	63.1	62.9	63.3	18.6	18.6	18.6	18.6	18.4	18.4
OMe						58.3	58.3	58.3	58.3	58.8	58.8
						Dgn⁗′	Dgn⁗′	Dgn⁗′	Dgn⁗′	Cym⁗′	Cym⁗′
1⁗′						102.7	102.6	102.7	102.7	100.4	100.4
2⁗′						32.8	32.8	32.8	32.8	36.5	36.5
3⁗′						79.8	79.8	79.8	79.8	77.5	77.5
4⁗′						73.8	73.7	73.7	73.8	83.1	83.0
5⁗′						71.0	71.8	71.0	71.0	69.0	69.0
6⁗′						17.9	17.9	17.9	17.9	18.6	18.6
OMe						56.2	56.2	56.2	56.2	58.3	58.3
						Glc⁗″	Glc⁗″	Glc⁗″	Glc⁗″	Dgn⁗″	Dgn⁗″
1⁗″						105.0	104.4	104.9	105.0	102.7	102.7
2⁗″						75.9	75.9	75.9	75.9	32.8	32.8
3⁗″						78.4	78.4	78.4	78.4	79.8	79.8
4⁗″						71.9	71.9	71.9	71.9	73.8	73.8
5⁗″						78.5	78.5	78.5	78.5	71.0	71.0
6⁗″						63.1	63.1	63.2	63.2	17.9	17.9
OMe										56.2	56.2
										Glc⁗‴′	Glc⁗‴′
1⁗‴′										105.0	105.0
2⁗‴′										75.9	75.9
3⁗‴′										78.4	78.4
4⁗‴′										71.9	71.9
5⁗‴′										78.5	78.6
6⁗‴′										63.1	63.2

Cym: β-D-cymaropyranosyl; Dgn: β-D-diginopyranosyl; Ole: β-D-oleandropyranosyl; Glc: β-D-glucopyranosyl.

2.21. Acid hydrolysis of *8*

Compound **8** (9.6 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the aglycone (1.3 mg) and a sugar fraction (1.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-oleandrose (9.20, negative optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.22. Amurensioside I (*9*)

An amorphous solid; $[\alpha]_D^{25}$ −14.0° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3397 (OH), 2933 (CH), 1637 (C=O); HRESITOFMS *m*/*z*: 1233.7043 $[M+H]^+$ (calcd for $C_{62}H_{105}O_{24}$, 1233.6995); ^{1}H and ^{13}C NMR: see Tables 2–4.

2.23. Acid hydrolysis of *9*

Compound **9** (20.9 mg) was subjected to acid hydrolysis as described for **1** to give several decomposed compounds of the

aglycone (4.3 mg) and a sugar fraction (5.5 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-oleandrose (9.20, negative optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.24. Amurensioside J (*10*)

An amorphous solid; $[\alpha]_D^{25}$ +2.79° (*c* 0.10, MeOH); UV λ_{max} nm (log ε): 231 (4.01); IR ν_{max} (film) cm^{-1}: 3392 (OH), 2931 (CH), 1714 (C=O), 1601 and 1450 (aromatic ring); HRESITOFMS *m*/*z*: 1517.7706 $[M+Na]^+$ (calcd for $C_{76}H_{118}O_{29}Na$, 1517.7655); ^1H and ^{13}C NMR: see Tables 2–4.

2.25. Acid hydrolysis of *10*

Compound **10** (14.2 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (12-*O*-benzoylisolineolon, 3.1 mg) and a sugar fraction (5.3 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-oleandrose (9.20, negative optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.26. Amurensioside K (*11*)

An amorphous solid; $[\alpha]_D^{25}$ +4.30° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3399 (OH), 2931 (CH), 1687 (C=O); HRESITOFMS *m*/*z*: 1397.7471 $[M+Na]^+$ (calcd for $C_{69}H_{114}O_{27}Na$, 1397.7397); ^1H and ^{13}C NMR (C_5D_5N): see Tables 2–4.

2.27. Acid hydrolysis of *11*

Compound **11** (12.1 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (fukujusone, 2.1 mg) and a sugar fraction (2.6 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose, D-cymarose, D-oleandrose, and D-diginose; t_R (min) D-glucose (7.69, positive optical rotation), D-oleandrose (9.20, negative optical rotation), D-cymarose (9.22, positive optical rotation), and D-diginose (9.59, positive optical rotation).

2.28. HSC-2 cell culture assay

HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate in a humidified 5% CO_2 atmosphere. Cells were trypsinized and inoculated at 6×10^3 to 1.2×10^4 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA, U.S.A.) and incubated for 24 h. After washing once with PBS, they were

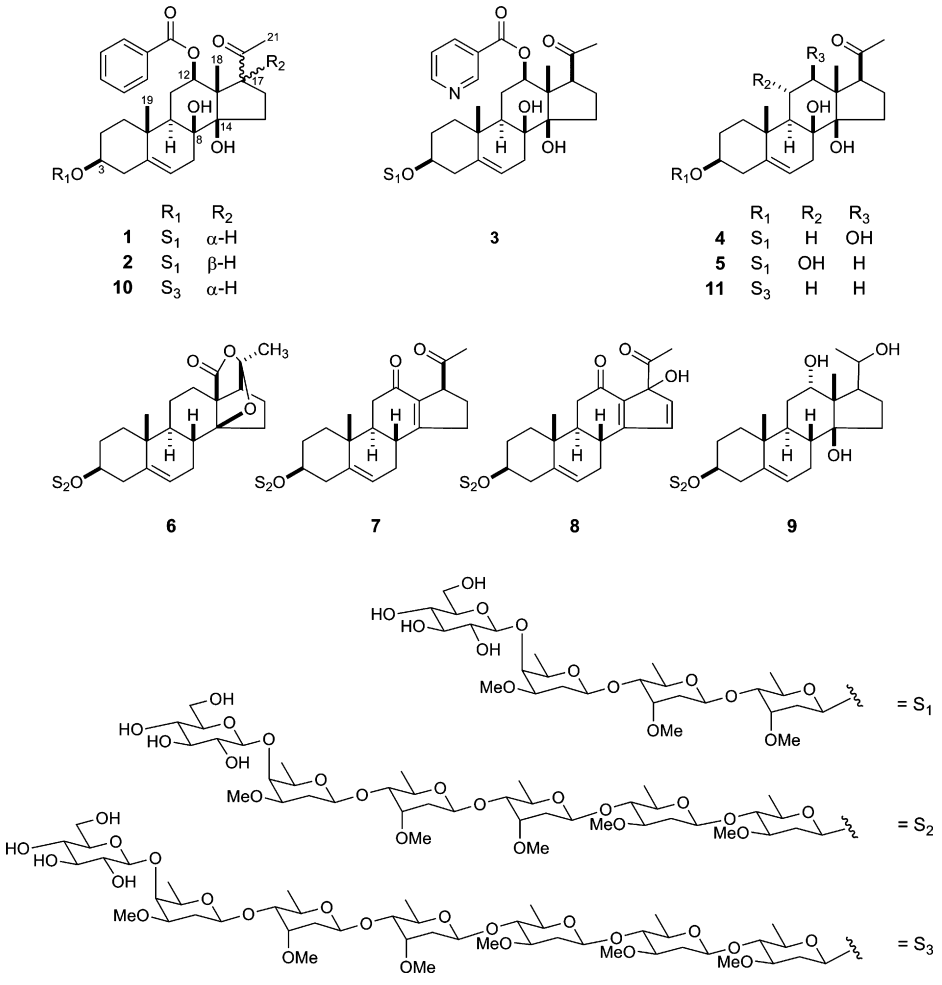

Fig. 1. Structures of compunds **1**–**11**.

treated for 24 h without or with increasing concentrations of test compounds. They were then washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10 [18,19]. The IC_{50} value, which reduces the viable cell number by 50%, was determined from dose–response curve.

3. Results and discussion

The fresh roots of *A. amurensis* were extracted with MeOH, and the MeOH extract was passed through a Diaion HP-20 column eluted with 20% MeOH in H_2O, EtOH, and EtOAc. Through multiple chromatographic steps over silica gel and ODS silica gel, as well as preparative HPLC, amurensiosides A (**1**; 58.9 mg), B (**2**; 26.8 mg), D (**4**; 41.8 mg), E (**5**; 19.2 mg), F (**6**; 9.5 mg), I (**9**; 45.7 mg), and K (**11**; 16.3 mg) were isolated from the EtOH eluate, and amurensiosides C (**3**; 23.9 mg), G (**7**; 75.6 mg), H (**8**; 18.0 mg), and J (**10**; 68.7 mg) were isolated from the EtOAc eluate (Fig. 1).

Amurensioside A (**1**) was obtained as an amorphous solid and was shown to have a molecular formula of $C_{55}H_{82}O_{20}$ on the basis of the HRESITOFMS data, exhibiting an $[M+Na]^+$ peak at *m/z* 1085.5225. The IR spectrum of **1** showed absorption bands for hydroxy groups at 3409 cm^{-1} and a carbonyl group at 1712 cm^{-1}. The UV absorption at 231 nm ($\log\varepsilon$ 3.70) is consistent with the presence of an aromatic ring in the structure of **1**. The ^1H NMR spectrum of **1** in C_5D_5N contained three three-proton singlet signals at δ 2.20, 1.65, and 1.37, and one olefinic proton signal at δ 5.35 (br d, J = 4.1 Hz), which were characteristic of the pregn-5-en-20-one skeleton, as well as signals for four anomeric protons at δ 5.29 (dd, J = 9.5, 1.6 Hz), 5.13 (d, J = 7.7 Hz), 5.11 (dd, J = 9.5, 1.3 Hz), and 4.65 (dd, J = 9.7, 1.7 Hz). The methyl carbon signals at δ 18.6 (2× CH_3) and 17.9, and proton signals at δ 1.55 (d, J = 6.4 Hz), 1.40 (d, J = 6.3 Hz), and 1.37 (d, J = 6.1 Hz), respectively, were indicative of **1** possessing three deoxy sugars. In addition, the presence of a benzoyl group was shown by the following ^1H and ^{13}C NMR signals; δ_H 8.37 (2H, br d, J = 7.8 Hz), 7.60 (1H, br dd, J = 7.8, 7.8 Hz), 7.53 (2H, br dd, J = 7.8, 7.8 Hz)/δ_C 166.5 (C=O), 133.6 (C), 131.2 (C), 130.0 (CH ×2), and 129.1 (CH ×2). Acid hydrolysis of **1** with 0.025 M HCl gave a pregnane aglycone with a benzoyl group, identified as 12β-(benzoyloxy)-3β,8β,14β-trihydroxypreg-5-en-20-one (12-*O*-benzoylisolineolon, **1a**) by comparison with literature data [20], and D-cymarose, D-diginose, and D-glucose as the carbohydrate components. Identification of the monosaccharides, including their absolute configurations, was carried out by direct HPLC analysis of the hydrolysate, using a combination of refractive index (RI) and optical rotation (OR) detectors. The ^1H–^1H COSY and TOCSY experiments with **1** allowed for the sequential assignments from H-1 to H_2-6 or Me-6 of each monosaccharide. Their signal multiplet patterns and coupling constants (Table 1) indicated the presence of two β-D-cymaropyranosyl ($^4C_1$) units, a β-D-diginopyranosyl ($^4C_1$) unit, and a β-D-glucopyranosyl ($^4C_1$) unit. The proton resonances correlated with those of the one-bond coupled carbons using the HMQC spectrum. The existence of a terminal β-D-glucopyranosyl unit (Glc′′′′), a C-4 substituted β-D-diginopyranosyl unit (Dgn′′′), and two C-4 substituted β-D-cymaropyranosyl units (Cym′, Cym′′) was confirmed by comparison of their carbon chemical shifts with those of the sugar moieties of reported steroidal glycosides [16,17,21]. The β-orientations of the anomeric centers of the Cym′, Cym′′, Dgn′′′, and Glc′′′′ units were ascertained by the relatively large $^3J_{H\text{-}1,\,H\text{-}2(ax)}$ values of their anomeric protons (7.7–9.7 Hz). In the HMBC spectrum of **1** (Fig. 2), long-range correlations were observed between δ 5.13 (H-1 of Glc′′′′) and δ 73.8 (C-4 of Dgn′′′), δ 4.65 (H-1 of Dgn′′′) and δ 83.1 (C-4 of Cym′′), δ 5.11 (H-1 of Cym′′) and δ 83.4 (C-4 of Cym′), and between δ 5.29 (H-1 of Cym′) and δ 77.7 (C-3 of aglycone), establishing the sequence of the sugar moieties. From the above spectroscopic and chemical data, the structure of **1** was formulated as 12-*O*-benzoylisolineolon 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Amurensioside B (**2**) was deduced to be $C_{55}H_{82}O_{20}$ on the basis of the HRESITOFMS data (*m/z* 1085.5277 $[M+Na]^+$). The ^1H and ^{13}C NMR spectra of **2** were almost identical to those of **1**, except for the signals due to the H-17 and Me-18 protons and C-12 and C-20 carbons, suggesting that **2** is an epimer of **1** with regard to the configuration at C-17, that is, 17α. This was confirmed by the presence of a NOESY correlation between δ 3.54 (H-17) and δ 2.04 (Me-18). Furthermore, acid hydrolysis of **2** with 0.025 M HCl furnished 12-*O*-benzoyllineolon [22] as the aglycone, and D-cymarose, D-diginose, and D-glucose as the sugar residues. Thus, the structure of **2** was formulated as 12-*O*-benzoyllineolon 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Amurensioside C (**3**) was isolated as an amorphous solid with a molecular formula, $C_{54}H_{81}NO_{20}$, as determined by the HRESITOFMS data (*m/z* 1064.5435 $[M+H]^+$). The spectral properties of **3** were essentially analogous with those of **1**, except for the aromatic region signals for the benzoyl moiety. The aromatic acid linked to the aglycone moiety of **3** was suggested to be nicotinic acid by the ^1H NMR [δ 9.65 (d, J = 1.8 Hz), 8.92 (dd, J = 4.8, 1.8 Hz), 8.54 (ddd, J = 8.0, 1.8, 1.8 Hz), and 7.43 (dd, J = 8.0, 4.8 Hz)] and ^{13}C NMR [δ 165.5 (C=O), 154.1 (CH), 151.2 (CH), 137.2 (CH), 126.9 (C), and 124.0 (CH)] spectra. The linkage position of the nicotinoyl group to C-12 of the aglycone moiety was ascertained by a long-range correlation from the H-12 proton at δ 5.20 (dd, J = 12.0, 3.8 Hz) to the carbonyl carbon resonance of the nicotinoyl moiety at δ 165.5 in the HMBC spectrum of **3**. Thus, **3** was identified as 12-*O*-nicotinoylisolineolon 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Amurensioside D (**4**) was obtained as an amorphous solid. On the basis of the HRESITOFMS (*m/z* 981.5075 $[M+Na]^+$) data, its

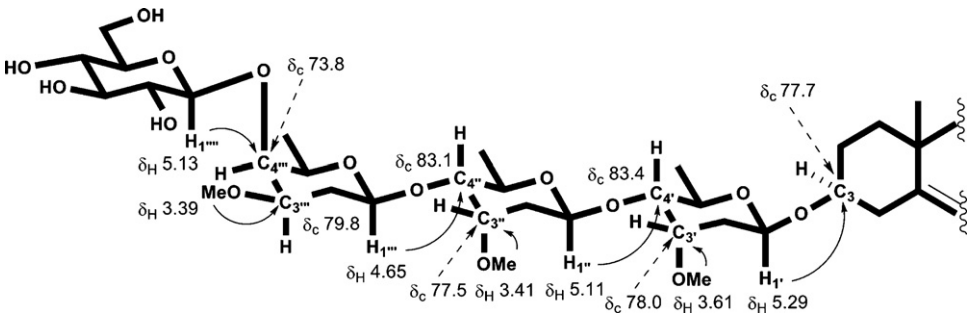

Fig. 2. HMBC correlations of the sugar moiety of **1**.

molecular formula was determined to be $C_{48}H_{78}O_{19}$, which was less than that of **1** by C_6H_4O, corresponding to a benzoyl unit. When the ^1H and ^{13}C NMR spectra of **4** were compared with those of **1**, the signals assigned to the benzoyl group could not be detected, and the carbon resonance of C-12 of the aglycone moiety was shifted upfield by 4.8 ppm and observed at δ 73.8 in **4**. All other ^1H and ^{13}C NMR signals, including those assignable to the tetraglycoside moiety, appeared at almost the same positions between the two compounds. Thus, the structure of **4** was established as isolineolon 3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Amurensioside E (**5**) was isolated as an amorphous solid with a molecular formula, $C_{48}H_{78}O_{19}$, which was determined on the basis of the HRESITOFMS data (*m*/*z* 981.4995 $[M+Na]^+$). Acid hydrolysis of **5** with 0.025 M HCl gave D-cymarose, D-diginose, and D-glucose, together with several unidentified degradation products; no aglycone could be obtained. The molecular formula of **5** was the same as that of **4**, and the ^1H and ^{13}C NMR spectra of **5** were essentially analogous to those of **4** with the exceptions of the signals assignable to the ring C part of the aglycone. In the HMBC spectrum of **5**, the Me-18 angular methyl protons at δ 1.43 exhibited correlations with not only two quaternary carbons at δ 50.5 (C-13) and 85.7 (C-14) but also a methylene carbon at δ 50.2 (C-12), which was associated with methylene protons at δ 2.11 and 1.89 (H_2-12) by analysis of the HMQC spectrum. In the ^1H–^1H COSY spectrum, the methylene proton signals at δ 2.11 and 1.89 exhibited correlations with an oxymethine proton at δ 4.77 (H-11). The oxymethine proton, in turn, displayed a correlation with a methine proton at δ 1.78 (d, J = 11.1 Hz, H-9). These data are consistent with the presence of a hydroxy group at C-11 of the aglycone moiety of **5**. NOE correlations between δ 4.77 (H-11) and δ 1.43 (Me-18)/δ 1.80 (Me-19) in the PHNOESY spectrum, and the proton spin-coupling constants between H-9 and H-11 ($^3J_{H\text{-}9,\,H\text{-}11}$ = 11.1 Hz) and between H-11 and H-12 ($^3J_{H\text{-}11,\,H\text{-}12ax}$ = 11.1 Hz, $^3J_{H\text{-}11,\,H\text{-}12eq}$ = 4.1 Hz) indicate that the configuration of the C-11 hydroxy group is α. Thus, **5** was defined as 3β-[(*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-diginopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl)oxy]-8β,11α,14β-trihydroxypregn-5-en-20-one.

Amurensioside F (**6**) was obtained as an amorphous solid, and its molecular formula was determined to be $C_{62}H_{98}O_{24}$ by the HRESITOFMS data (*m*/*z* 1249.6392 $[M+Na]^+$). The ^1H and ^{13}C NMR spectral properties of **6** were suggestive of a pregnane hexaglycoside. Acid hydrolysis of **6** with 0.025 M HCl gave a six-membered pregnane derivative (**6a**; $C_{21}H_{28}O_4$) as the aglycone, and D-cymarose, D-diginose, D-oleandrose and D-glucose as the carbohydrate components. The pregnane (**6a**) appeared to have a structure identical to adonilide, which was isolated by Shimizu et al. in 1967 [10]. However, no spectroscopic data for adonilide are available. The structure of **6a**, 14β,20-epoxy-3β-hydroxypregn-5-ene-13-carboxylic acid-20-lactone, was identified by combined analysis of the ^1H–^1H COSY, HMQC, HMBC, and NOESY spectra (Figs. 3 and 4).

The severe overlapping of the proton signals for the sugar moieties of **6** excluded the possibility of a complete assignment in a straightforward way using conventional 2D NMR methods, such as the ^1H–^1H COSY, 2D TOCSY, and HMQC spectra. Analysis of the 1D TOCSY spectra followed by interpretation of the ^1H–^1H COSY, HSQC–TOCSY, and HMQC spectra allowed for the exact sugar sequences of the sugars to be determined. The ^1H NMR subspectra of the individual monosaccharide units were obtained by using selective irradiation of easily identifiable anomeric proton signals at δ 5.28, 5.12, 5.09, 4.91, 4.80, and 4.65, as well as irradiation of other non-overlapping proton signals at δ 1.55, 1.46, 1.42, 1.39 and 1.36 in a series of 1D TOCSY experiments [23,24]. Subsequent analysis of the ^1H–^1H COSY spectrum resulted in the sequential assignments of all the proton resonances due to the six monosaccharide units, including identification of their signal multiplet patterns and coupling constants. The proton resonances correlated with those of the corresponding one-bond coupled carbons using the HMQC and HSQC–TOCSY spectra, leading to unambiguous assignments of the carbon shifts. Comparison of the carbon chemical shifts thus assigned with those reported in the literature [16,17,21], taking into account the known effects of the *O*-glycosylation shift, indicate that **6** contains two 4-substituted β-D-oleandropyranosyl ($^4C_1$) units (Ole′ and Ole″), two 4-substituted β-D-cymaropyranosyl ($^4C_1$) units (Cym‴ and Cym⁗), a 4-substituted β-D-diginopyranosyl ($^4C_1$) unit (Dgn⁗′), and a terminal β-D-glucopyranosyl ($^4C_1$) unit (Glc⁗″). The β-orientations of the anomeric centers of these monosaccharides were supported by the relatively large $^3J_{H\text{-}1,\,H\text{-}2ax}$ values of their anomeric protons (7.8–9.7 Hz). In the HMBC spectrum of **6** (Fig. 5), correlation peaks were observed between δ 5.12 (H-1 of Glc⁗″) and δ 73.8 (C-4 of Dgn⁗′), δ 4.65 (H-1 of Dgn⁗′) and δ 83.1 (C-4 of Cym⁗), δ 5.09 (H-1 of Cym⁗) and δ 83.2 (C-4 of Cym‴), δ 5.28 (H-1 of Cym‴) and δ 83.0 (C-4 of Ole″), δ 4.91(H-1 of Ole″) and δ 83.3 (C-4 of Ole′), and between δ 4.80 (H-1 of Ole′) and δ 77.3 (C-3 of aglycone). All of these data for **6** are consistent with the structure adonilide 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)-β-D-oleandropyranoside.

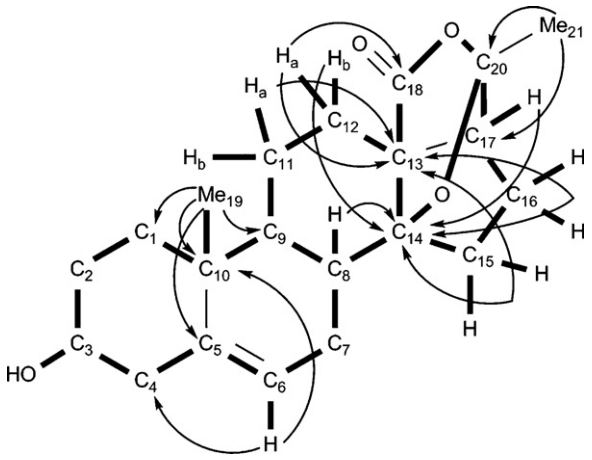

Fig. 3. Key HMBC correlations of **6a**.

Amurensioside G (**7**), isolated as an amorphous solid, showed an $[M+Na]^+$ ion at *m*/*z* 1219.6228 in HRESITOFMS, corresponding to the empirical molecular formula $C_{61}H_{96}O_{23}$. Comparison of the ^1H and ^{13}C NMR spectral data of **7** with those of **6** revealed that the structures of the ring A and B portions of the aglycone and the hexaglycoside moiety attached to C-3 of the aglycone are identical to that of **6**. Analysis of the ^1H–^1H COSY, 2D TOCSY,

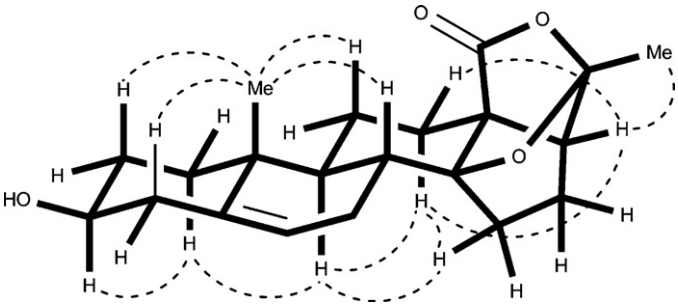

Fig. 4. Key NOE correlations of **6a**.

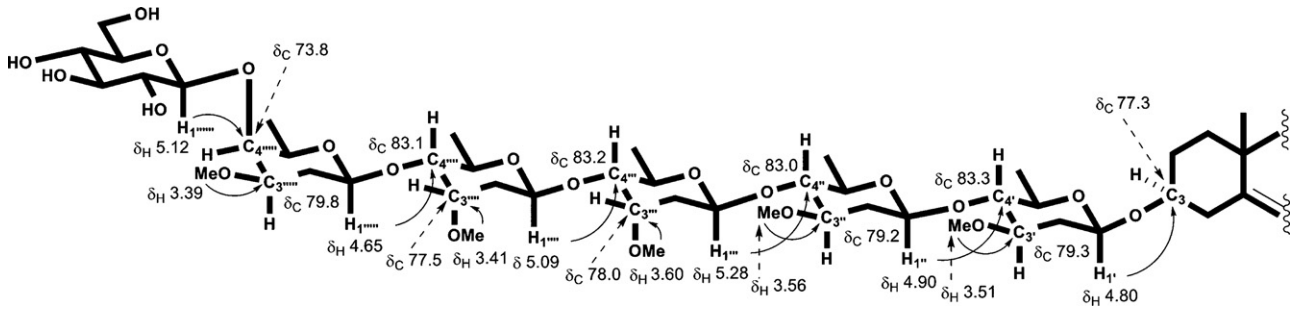

Fig. 5. HMBC correlations of the sugar moiety of **6**.

HMQC, HMBC, and NOESY spectra of **7** allowed for the structure of the aglycone moiety of **7** to be assigned as 3β-hydroxy-18-norpregna-5,13(14)-diene-12,20-dione (fukujusonorone), which was reported by Shimizu et al. in 1969 [12]. Although fukujusonorone could not be obtained by acid hydrolysis of **7**, the structure was unequivocally identified by analysis of the ^{1}H–^{1}H COSY, 2D TOCSY, HMQC, HMBC, and NOESY spectra. The structure of **7** was characterized as fukujusonorone 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)-β-D-oleandropyranoside.

Amurensioside H (**8**) had a molecular formula of $C_{61}H_{94}O_{24}$ on the basis of HRESITOFMS (*m*/*z* 1233.6001 [M+Na]$^+$). The ^{1}H and ^{13}C NMR spectral features of **8** showed a close similarity to those of **7**. On comparison of the ^{1}H and ^{13}C NMR spectra of **8** with those of **7**, the methine proton and carbon signals assigned to H-17/C-17 at δ_H 4.05 and δ_C 55.5, were missing from the spectrum of **8**. Instead, a quaternary carbon signal bearing a hydroxy group was observed at δ 122.0, accompanied by significant upfield shifts of C-13 (by 15.9 ppm) and C-20 (by 25.1 ppm). Furthermore, the coupled methylene proton signals at H_2-15 and H_2-16 at δ 2.05 and 1.98 were replaced by a pair of olefinic proton signals at δ 6.40 and 7.41, which were associated with the corresponding olefinic carbon signals at δ 119.5 and 137.1, respectively. Thus, **8** was assigned as the C-15/C-16 dehydro and C-17 hydroxy derivative of **7**. The presence of a double bond between C-15 and C-16, and a hydroxy group at C-17 was supported by HMBC correlations between δ 2.48 (Me-21) and δ 122.0 (C-17)/δ 184.2 (C-20), δ 6.40 (H-15) and δ 120.8 (C-13)/δ 158.8 (C-14)/C-17, and between δ 7.41 (H-16) and C-13/C-14/C-17. The hexaglycoside attached to C-3 of the aglycone was confirmed to be the same as that of **6** and **7** by analysis of the HMBC spectrum of **8**, and the results of acid hydrolysis. Thus, the structure of **8** was formulated as 3β-[(*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)-β-D-oleandropyranosyl)oxy]-17-hydroxy-18-norpregna-5,13,15-triene-12,20-dione. The configuration at C-17 remains to be determined.

Amurensioside I (**9**) was shown to have a molecular formula of $C_{62}H_{104}O_{24}$ on the basis of HRESITOFMS (*m*/*z* 1233.7043 [M+H]$^+$). The ^{1}H and ^{13}C NMR signals of the aglycone part of **9** were similar to those of pregn-5-ene-3β,14β,20-triol (calogenin) 3-*O*-glycosides [25]. However, the signals for the C-12 methylene group at δ_H 1.17 and 1.74, and δ_C 37.6 in the calogenin glycosides were displaced by those assigned to a hydroxymethine group at δ_H 4.88 and δ_C 74.8 in **9**. An HMBC correlation between δ 1.84 (Me-18) and δ 74.8 (C-12) is consistent with the presence of a hydroxy group at C-12. The proton spin-coupling constant between H-12 and H_2-11 (2.6 Hz), along with an NOE correlation between H-12 and Me-18, indicate that the C-12 hydroxy group is an α-axial-orientated configuration. Unfortunately, no information concerning the configurations at C-17 and C-20 of the aglycone of **9** could be obtained from the NOE spectral data. The configurations at C-17 and C-20 remain to be determined. As for the sugar sequence of **9**, the hexaglycoside attached to C-3 of the aglycone was shown to be identical with that of **6**–**8** on the basis of the ^{1}H and ^{13}C NMR, and HMBC spectral data, and the results of acid hydrolysis. Thus, the structure of **9** was established as 12α,14β,20-trihydroxypregn-5-en-3β-yl *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)-β-D-oleandropyranoside.

Amurensioside J (**10**) was obtained as an amorphous solid, and its molecular formula was determined to be $C_{76}H_{118}O_{29}$ on the basis of HRESITOFMS (*m*/*z* 1517.7706 [M+Na]$^+$). The ^{1}H NMR spectrum of **10** showed signals for three tertiary methyl groups at δ 2.19, 1.64, and 1.37, one olefinic proton at δ 5.44 (br d, *J* = 4.4 Hz), and five aromatic protons of a benzene ring at δ 8.38 (2H, br d, *J* = 7.8 Hz), 7.59 (1H, br dd, *J* = 7.8 Hz) and 7.53 (2H, br dd, *J* = 7.8 Hz), as well as signals for seven anomeric protons at δ 5.29 (br d, *J* = 9.7 Hz), 5.13 (d, *J* = 7.8 Hz), 5.10 (br d, *J* = 9.5 Hz), 4.91 (br d, *J* = 9.7 Hz), 4.89 (br d, *J* = 9.7 Hz), 4.83 (dd, *J* = 9.6, 1.3 Hz) and 4.66 (br d, *J* = 8.9 Hz), six methoxy protons at δ 3.60, 3.54, 3.51, 3.50, 3.41 and 3.39 (each s), and six secondary methyl protons at δ 1.53 (d, *J* = 6.3 Hz), 1.46 (d, *J* = 6.1 Hz), 1.42 (d, *J* = 6.0 Hz), 1.42 (d, *J* = 6.1 Hz), 1.35 (d, *J* = 6.5 Hz) and 1.33 (d, *J* = 6.8 Hz). Acid hydrolysis of **10** with 0.025 M HCl gave 12-*O*-benzoylisolineolon (**1a**) as the aglycone,

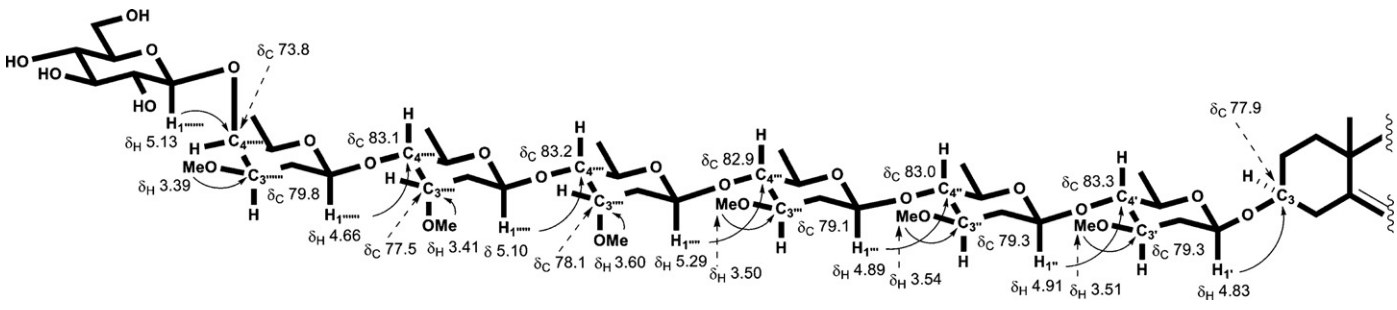

Fig. 6. HMBC correlations of the sugar moiety of **10**.

Table 5
Cytotoxic activity of compounds **1**–**11** against HSC-2 cells.

Compounds	IC_{50} (μg/mL)	Compounds	IC_{50} (μg/mL)
1	66	**7**	>120
2	26	**8**	>120
3	>120	**9**	>120
4	47	**10**	>120
5	58	**11**	>120
6	>120	Melpharan	13

HSC-2 (human oral squamous cell carcinoma).

and D-cymarose, D-diginose, D-oleandrose, and D-glucose as the carbohydrate moieties. These data suggest that **10** is a 12-*O*-benzoylisolineolon heptaglycoside. In the ^{13}C NMR spectrum of **10**, the signal due to C-3 of the aglycone residue was observed at δ 77.9, indicating that the sugar is linked to C-3, as in **1**. Using the same procedures as described for **6**, all the ^{1}H and ^{13}C NMR signals arising from the sugar moieties of **10** were assigned to three 4-substituted β-D-oleandropyranosyl ($^{4}C_1$) units (Ole′, Ole″, and Ole‴), two 4-substituted β-D-cymaropyranosyl ($^{4}C_1$) unit (Cym⁗ and Cym⁗′), a 4-substituted β-D-diginopyranosyl ($^{4}C_1$) unit (Dgn⁗′), and a terminal β-D-glucopyranosyl ($^{4}C_1$) unit (Glc⁗′). In the HMBC spectrum of **10** (Fig. 6), long-range correlations were observed between δ 5.13 (H-1 of Glc⁗′) and δ 73.8 (C-4 of Dgn⁗′), δ 4.66 (H-1 of Dgn⁗′) and δ 83.1 (C-4 of Cym⁗′), δ 5.10 (H-1 of Cym⁗′) and δ 83.2 (C-4 of Cym⁗), δ 5.29 (H-1 of Cym⁗) and δ 82.9 (C-4 of Ole‴), δ 4.89 (H-1 of Ole‴) and δ 83.0 (C-4 of Ole″), δ 4.91 (H-1 of Ole″) and δ 83.2 (C-4 of Ole′), and between δ 4.83 (H-1 of Ole′) and δ 77.9 (C-3 of aglycone). Thus, the structure of **10** was characterized as 12-*O*-benzoyllineolon 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-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside.

Amurensioside K (**11**) was determined to be $C_{69}H_{114}O_{27}$ on the basis of HRESITOFMS (*m*/*z* 1387.7471 $[M+Na]^+$). The deduced molecular formula of **11** was lower than that of **10** by $C_7H_5O_2$. When the ^{1}H and ^{13}C NMR spectra of **11** were compared with those of **10**, the signals assignable to the benzoyloxy moiety attached to C-12 of the aglycone were absent from the spectrum of **11**. Acid hydrolysis of **11** with 0.025 M HCl gave 3β,8β,14β-trihydroxypregnan-5-en-20-one (fukujusone) [14] as the aglycone, and D-cymarose, D-diginose, D-oleandrose, and D-glucose as the carbohydrate moieties. Analysis of the HMBC spectrum of **11** and the results of acid hydrolysis indicate that the heptaglycoside attached to C-3 of the aglycone is the same as that of **10**. Thus, the structure of **11** was established as fukujusone 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-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside.

Amurensiosides A–K (**1**–**11**) were evaluated for their cytotoxic activity against HSC-2 cells, using a modified MTT assay method (Table 5). Although **3** and **6**–**11** did not show any apparent cytotoxicity at a sample concentration of 120 μg/mL, **1**, **2**, **4**, and **5** were moderately cytotoxic to HSC-2 cells with IC_{50} values of 66, 26, 47, and 58 μg/mL, respectively, while that of melphalan used as a positive control was 13 μg/mL.

The present phytochemical study of the roots of *A. amurensis* resulted in the isolation and characterization of 11 new pregnane glycosides named amurensiosides A–K. The structures of these new compounds are notable for the following viewpoints of natural product chemistry. The aglycones of **5**, **8**, and **9** are new polyoxygenated pregnane derivatives. Although the aglycones of **6** and **7**, adonilide and fukujusonorone, respectively, were reported in the 1960s, their glycosides such as **6** and **7** have not been previously reported. The oligoglycosides attached to C-3 of the aglycone of **1**–**11** are novel tetra-, hexa-, and heptaglycosides containing deoxysugars characteristic of plant pregnane and cardiotonic glycosides.

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