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STEROIDAL GLYCOSIDES FROM THE ROOTS OF *METAPLEXIS JAPONICA*

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Key Word Index—*Metaplexis japonica*; Asclepiadaceae; pregnane glycoside; 2,6-dideoxyhexopyranose.

Abstract—The roots of *Metaplexis japonica* afforded eleven new pregnane glycosides which had 12-*O*-acetylpergularin, metaplexigenin and deacylmetaplexigenin as the aglycone moiety and 2,6-dideoxy and 2,6-dideoxy-3-*O*-methylhexopyranoses as component sugars. The structures of these compounds were elucidated by spectroscopic methods and chemical evidence. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In connection with a study on the constituents of some plants in the Asclepiadaceae family, we have investigated the roots of *Metaplexis japonica* M. There have been previous reports of some pregnane-type genins from this plant [1–5], but their glycosides have not been researched yet. Thus, we decided to investigate the root of this plant, and eleven new pregnane glycosides were obtained. This paper describes the isolation and structural determination of these new pregnane glycosides.

RESULTS AND DISCUSSION

The methanol extract of the roots of *M. japonica* was suspended in water. The suspension was then extracted with diethyl ether and partitioned into an ether soluble fraction and a water soluble one. The ether soluble fraction and a part of the water soluble one were chromatographed on silica gel columns, to give a steroidal fraction from which eleven new pregnane glycosides (**1**–**10** and **11**) were obtained.

Compound **1** was suggested to have molecular formula $C_{30}H_{46}O_9$ based on the FAB-mass spectrum. In the ^{13}CNMR and ^{1}HNMR spectra, **1** showed one anomeric carbon and proton signals at δ 98.3 and 4.87 (*dd*, J = 9.5, 2.0 Hz), and the carbon signals assignable to the aglycone moiety were similar to those of 12-*O*-acetylpergularin within the range of glycosylation shifts at C-3 (+6.3 ppm), C-2 (−2.2 or −2.4 ppm) and C-4 (−3.9 or −4.0 ppm). From the above results, **1** was considered to be 12-*O*-acetylpergularin 3-*O*-monoglycoside. On acid hydrolysis of **1** with 0.2 N H_2SO_4, 12-*O*-acetylpergularin and oleandrose were obtained as the component aglycone and sugar moieties (see Section 3). The sugar was identified as β-oleandropyranose on the basis of the ^{13}CNMR spectral data and the (H, H) coupling constant of the anomeric proton signal (J = 9.5, 2.0 Hz) in the ^{1}HNMR spectrum. Thus, **1** was determined to be 12-*O*-acetylpergularin 3-*O*-β-oleandropyranoside.

Compound **2** showed two anomeric carbon and proton signals at δ 98.2. 100.4 (in pyridine-d_5) and 4.53 (*dd*, J = 9.5, 2.0 Hz), 4.72 (*dd*, J = 9.5, 2.0 Hz) (in $CDCl_3$), in addition to the signals of 12-*O*-acetylpergularin in the ^{13}C- and ^{1}HNMR spectra. Therefore **2** was considered to be 12-*O*-acetylpergularin diglycoside. On acid hydrolysis of **2**, 12-*O*-acetylpergularin and oleandrose were afforded as the aglycone and sugar moieties. This result and the ^{13}CNMR and ^{1}HNMR spectral data indicated that the component two monosaccarides were β-oleandropyranoses. In regard to the sugar sequence, the first β-oleandropyranose was attached to C-3 of 12-*O*-acetylpergularin, because glycosylation shifts were observed around the C-3 position in the ^{13}CNMR spectrum, and in the rotating frameshift difference nuclear Overhauser effect (ROE) spectrum, irradiation at the anomeric proton signal of the first β-oleandropyranose at δ 4.53 caused enhancement of the signal intensity at δ 3.53 (H-3

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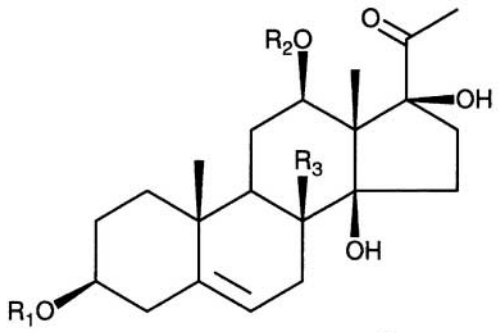


	R_2	R_3	
I:	Ac	H	(12-*O*-acetylpergularin)
II:	Ac	OH	(metaplexigenin)
III:	H	OH	(deacylmetaplexigenin)

R_1

a: (agly.^{3}) β-ole.
b: (agly.^{3}) β-ole. ^{4} β-ole.
c: (agly.^{3}) β-ole. ^{4} β-can. ^{4} β-ole.
d: (agly.^{3}) β-cym. ^{4} β-cym. ^{4} β-ole.
e: (agly.^{3}) β-ole. ^{4} β-can. ^{4} β-can. ^{4} β-ole.
f: (agly.^{3}) β-ole. ^{4} β-ole. ^{4} β-ole. ^{4} β-ole.
g: (agly.^{3}) β-dig. ^{4} β-cym. ^{4} α-cym. ^{4} β-cym. ^{4} α-cym.
h: (agly.^{3}) β-can. ^{4} β-cym. ^{4} α-cym. ^{4} β-cym. ^{4} α-cym.

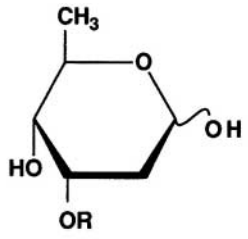
(agly.=aglycone,
ole.=oleandropyranose, can.=canaropyranose
cym.=cymaropyranose, dig.=digitoxopyranose)

1: I-a
2: I-b
3: I-c
4: I-d
5: II-d
6: I-e
7: I-f
8: I-g
9: II-g
10: III-g
11: I-h

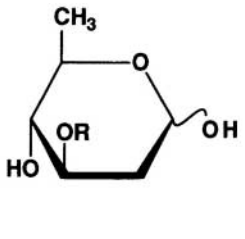
of aglycone). Accordingly, β-oleandropyranose was attached to C-3 of the aglycone moiety. Similarly, an NOE was observed between δ 4.72 (H-1 of another β-oleandropyranose) and δ 3.17 (H-4 of the first β-oleandropyranose). Therefore, **2** was identified as 12-*O*-acetylperguralin 3-*O*-β-oleandropyranosyl-(1 → 4)-β-oleandropyranoside.

Compound **3** was considered to be 12-*O*-acetylpergularin triglycoside, according to the consequences of the ^{1}HNMR and ^{13}CNMR spectra. On acid hydrolysis of **3**, oleandrose and canarose were obtained as the component sugars, and because two methoxyl proton signals were observed at δ 3.40 (*s*) and 3.41 (*s*) in the ^{1}H-NMR spectrum, these monosaccharides were two oleandroses and one canarose, which were decided to be β-hexopyranoses by the ^{13}C-NMR spectral data and the *J* values of the anomeric proton signals in the ^{1}HNMR spectrum. The sugar linkage was determined on the basis of the results of the ROE spectra. NOEs were observed between δ 4.53 (H-1 of the first β-oleandropyranose)/δ 3.53 (H-3 of the aglycone), δ 4.72 (H-1 of β-canaropyranose)/δ 3.16 (H-4 of the first β-oleandropyranose) and δ 4.49 (H-1 of the second β-oleandropyranose)/δ 2.98 (H-4 of β-canaropyranose) by irradiation of each anomeric proton signal. Thus, compound **3** was elucidated as 12-*O*-acetylpergularin 3-*O*-β-oleandropyranosyl-(1 → 4)-β-canaropyranosyl-(1 → 4)-β-oleandropyranoside.

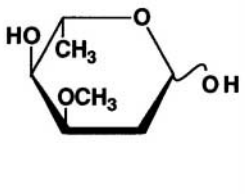
Compounds **4** and **5** were also considered to be 12-*O*-acetylated pregnane 3-*O*-triglycosides. On acid hydrolysis, the aglycone moieties of **4** and **5** confirmed 12-*O*-acetylpergularin and metaplexigenin [1], respectively. The sugar sequences of these com-



R=H: D-digitoxopyranose
R=CH_3: D-cymaropyranose



R=H: D-canaropyranose (D-olivopyranose)
R=CH_3: D-oleandropyranose



L-cymaropyranose

pounds were deduced to be β-oleandropyranosyl-(1 → 4)-β-cymaropyranosyl-(1 → 4)-β-cymaropyranoside, because of the consistency of signals assignable to the sugar moiety in the ^{1}HNMR and ^{13}CNMR spectra of cynanchoside C_2 [6] from *Cynanchum caudatum* M. From the above results, the structures of these compounds were concluded to be as shown.

Compounds **6** and **7** were considered to be 12-*O*-acetylpergularin 3-*O*-tetraglycosides. Acid hydrolysis followed by GC analysis and examination of their NMR spectra showed that the sugar moieties were composed of two β-canaropyranoses and two β-oleandropyranoses in **6**, and four β-oleandropyranoses in **7**. In **6**, the location of the sugar linkage was decided by interpretation the ROE spectra. Namely, NOEs were revealed between δ 4.53 (H-1 of the first β-oleandropyranose)/δ 3.53 (H-3 of the aglycone), δ 4.73 (H-1 of the first β-canaropyranose)/δ 3.16 (H-4 of the first β-oleandropyranose), δ 4.50 (H-1 of the second β-canaropyranose)/δ 2.97 (H-4 of the first β-canaropyranose) and δ 4.50 (H-1 of the second β-oleandropyanose)/δ 3.02 (H-4 of the second β-canaropyranose). In **7**, as the sugar chain constituted only four β-oleandropyranoses, the sugar linkage was considered to be β-oleandropyranosyl-(1 → 4)-β-oleandropyranosyl-(1 → 4)-β-oleandropyranosyl-(1 → 4)-β-oleandropyranoside. Hence, the structures of **6** and **7** were determined to be as shown.

Compounds **8**–**10** were pregnane 3-*O*-pentaglycosides which had the same sugar sequences according to consistency of ^{1}HNMR and ^{13}CNMR spectral data, and their aglycones were determined to be 12-*O*-acetylpergularin, metaplexigenin and deacylmetaplexigenin [1, 7], respectively. On acid hydrolysis of each glycoside, digitoxose and cymarose were obtained as the component sugars, and four methoxyl signals seen at δ 3.37 (*s*), 3.38 (*s*), 3.53 (*s*) and 3.55 (*s*) in each ^{1}HNMR spectrum suggested that monosaccharides consisted of one digitoxose and four cymaroses. The *J* values indicated the presence of two equatorial (*J* = 4.5, 1.5 Hz) and three axial (*J* = 9.5, 2.0 Hz) anomeric protons. The COSY experiment of **10** established that equatorial anomeric protons (δ 4.94 and 4.97) formed part of the connectivity network of the cymaropyranoses which were involved in α-glycosidic linkages. The axial anomeric protons (δ 5.47, 5.12 and 5.24) of one digitoxopyranose and two cymaropyranoses, respectively, were involved in β-glycosidic limits. The sugar linkages were determined to be as shown on the basis of the examination of the ROE spectra after irradiating at each anomeric proton signal.

Compound **11** was also a 12-*O*-acetylpergularin 3-*O*-pentaglycoside, but β-digitoxopyranose was exchanged for β-canaropyranose in the component sugars, because of the appearance of the characteristic H-4 signal of the canaropyranose (δ 3.27 (*t*, *J* = 9.0 Hz)) and the disappearance of the H-4 one of digitoxopyranose (δ 3.46 (*dd*, *J* = 9.5, 3.0 Hz)) in the ^{1}HNMR spectrum. This was confirmed by acid hydrolysis followed by GC analysis of the sugar part. The sugar sequence was deduced by comparison of ^{1}HNMR and ^{13}CNMR spectral data with those of **8**, and decided by irradiating at the anomeric proton signals in the ROE spectra.

The absolute configuration of the monosaccarides in each compound was not determined with certainty. However, an acid hydrolysis of a mixture of pregnane glycosides afforded pure digitoxose, cymarose, oleandrose and canarose, whose $[\alpha]_D$ values suggested that oleandrose and canarose were the D-form [8, 9]. In regard to α-cymaropyranose, as the C-2 signal appeared approximately at δ 32 in the ^{13}C NMR spectrum, the α-cymaropyranose was deduced to be the L-form [10].

EXPERIMENTAL

^{1}HNMR and ^{13}CNMR spectra were recorded at 400 and 100.40 MHz, respectively. Chemical shifts were given on the δ (ppm) scale with tetramethylsilane as an internal standard.

Plant material

Metaplexis japonica M. was cultivated in the botanical garden and harvested on September 1995 in Shizuoka, Japan and identified by Professor Noro (University of Shizuoka).

Extraction and isolation

The air dried roots of *M. japonica* M. (300 g) were extracted 2× with MeOH under reflux. The extract was concentrated under red. pres. and the residue was suspended in H_2O. This suspension was extracted with Et_2O. The H_2O layer was passed through a Mitsubishi Diaion HP-20 column and absorbed material was eluted with 50% MeOH in water, 70% MeOH in water and MeOH. The Et_2O layer and the MeOH eluate of the HP-20 column were concentrated, and these residues were rechromatographed on a silica gel column with $CHCl_3$–MeOH system and semi-preparative HPLC (Develosil-ODS, PhA, C-8 and YMC-ODS: MeCN–H_2O and MeOH–H_2O systems) to give compounds **1** (4 mg), **2** (7 mg), **3** (2 mg), **4** (25 mg), **5** (10 mg), **6** (27 mg), **7** (2 mg), **8** (5 mg), **9** (4 mg), **10** (5 mg), **11** (2 mg).

Compound **1**. Amorphous powder. $[\alpha]_D^{27} - 78^\circ$ (MeOH; *c* 0.41). FAB-MS *m*/*z*: 573 $[M + Na]^+$. ^{13}C NMR and ^{1}H NMR: Tables 1 and 2.

Compound **2**. Amorphous powder. $[\alpha]_D^{19} - 80^\circ$ (MeOH; *c* 0.30). FAB-MS *m*/*z*: 717 $[M + Na]^+$. ^{13}C NMR and ^{1}H NMR: Tables 1 and 2.

Table 1. ^{13}CNMR spectral data of compounds **1–11**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Aglycone moiety											
C-1	37.2[a]	37.2[a]	37.2[a]	37.3[a]	39.0[a]	37.3[a]	37.2[a]	37.2[a]	39.0[a]	39.0[a]	37.2[a]
2	30.2	30.2	30.2	30.2	29.9	30.2	30.2	30.2	29.9	30.0	30.1
3	77.4	77.6	77.6	77.2	77.7	77.6	77.6	77.2	77.7	77.7	77.6[b]
4	39.3	39.2	39.2	39.2	39.3[a]	39.3	39.2	39.2	39.3[a]	39.4[a]	39.2
5	139.9	139.9	139.9	139.9	139.4	139.9	139.9	139.9	139.4	139.5	139.9
6	121.9	121.9	121.9	121.9	119.1	122.0	121.9	121.8	119.1	119.4	121.9
7	26.7	26.6	26.7	26.6	33.7	26.7	26.7	26.7	33.7	34.2	26.6
8	37.3[a]	37.2[a]	37.1[a]	37.1[a]	74.3	37.2[a]	37.2[a]	37.2[a]	74.3	74.3	37.2[a]
9	43.2	43.2	43.2	43.2	44.6	43.2	43.2	43.2	44.5	45.0	43.2
10	37.5[a]	37.1[a]	37.2[a]	37.2[a]	37.4[b]	37.3[a]	37.1[a]	37.1[a]	37.4	37.4	37.1[a]
11	26.9	26.9	26.9	27.0	24.8	27.0	26.9	26.9	24.8	29.4	26.9
12	73.1[b]	73.1[b]	73.1[b]	73.1[b]	73.5	73.1[b]	73.1[b]	73.1[b]	73.6	69.0	73.1[c]
13	56.7	56.7	56.7	56.7	57.9	56.7	56.7	56.7[c]	57.9	60.5	56.7[d]
14	88.9	88.8	88.8	88.9	89.4	88.9	88.8	88.9	89.4	89.3	88.8
15	31.5	31.5	31.5	31.5	34.8	31.5	31.5	31.4	34.7	35.1	31.5
16	32.6	32.6	32.6	32.6	32.8	32.6	32.6	32.6	32.8	32.8	32.6
17	92.1	92.1	92.1	92.1	92.4	92.1	92.1	92.1	92.4	92.6	92.1
18	8.6	8.6	8.6	8.6	10.4	8.6	8.6	8.6	10.4	9.3	8.6
19	19.5	19.4	19.4	19.4	18.2	19.5	19.4	19.4	18.2	18.7[b]	19.4
20	209.6	209.6	209.6	209.6	210.1	209.6	209.6	209.6	210.1	209.5	209.6
21	27.4	27.4	27.4	27.4	27.1	27.4	27.4	27.4	27.5	27.8	27.4
Ac	169.7	169.7	169.7	169.8	169.8	169.8	169.7	169.7	169.8	–	169.7
	20.7	20.7	20.7	20.7	20.8	20.7	20.7	20.7	20.7	–	20.7
Sugar moiety											
	β-ole.	*β*-ole.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-ole.	*β*-ole.	*β*-dig.	*β*-dig.	*β*-dig.	*β*-can.
C-1	98.3	98.2	98.2	96.4	96.5	98.2	98.2	96.4	96.4	96.4	98.2
2	37.3[a]	37.9	38.0	37.3[a]	37.3[b]	37.9	37.8[c]	39.0	39.0	39.0	40.2
3	81.7	79.4	79.4	78.1[c]	78.1[c]	79.4	79.3	67.5	67.5	67.5	70.1
4	76.5	83.1	83.4	83.2[d]	83.2[d]	83.4	82.9[d]	83.4	83.4	83.4	88.5
5	72.9[b]	71.7	71.6	69.0[e]	69.0[e]	71.6	71.6[e]	68.6	68.6	68.6	70.9
		β-ole.	*β*-can.	*β*-cym.	*β*-cym.	*β*-can.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-cym.	*β*-cym.
C-1	–	100.4	100.4	100.5	100.5	100.4	100.2[f]	99.7	99.7	99.7	99.7
2	–	37.4[a]	40.0	37.3[a]	37.3[b]	40.0	37.8	36.8[a]	36.7[b]	36.7[c]	36.7
3	–	81.7	70.1	77.9[c]	77.9[c]	69.8[c]	79.3	77.7	77.7	77.7	77.7[b]
4	–	76.3	88.4	83.4[d]	83.4[d]	88.4	83.0[d]	82.3[d]	82.3[c]	82.3[d]	81.8
5	–	73.0[b]	71.1	69.1[e]	69.1[e]	71.0[d]	71.7[e]	69.4[e]	69.4[d]	69.4[e]	69.4[e]
			β-ole.	*β*-ole.	*β*-ole.	*β*-can.	*β*-ole.	α-cym.	α-cym.	α-cym.	α-cym.
C-1	–	–	101.4	102.2	102.2	101.4	100.2[f]	99.0	99.0	99.0	99.0
2	–	–	36.9[a]	37.3[a]	37.0[b]	39.5	37.9[c]	32.3[f]	32.2	32.3[f]	32.2
3	–	–	81.2	81.4	81.4	70.1[c]	79.3	73.1[b]	73.3	73.2	73.3
4	–	–	75.9	76.3	76.2	87.8	83.2[d]	77.7	77.7	77.7	77.7[b]
5	–	–	73.2[b]	73.0[b]	73.0	71.3[d]	71.8[e]	65.4	65.4	65.4	65.4
						β-ole.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-cym.	*β*-cym.
C-1	–	–	–	–	–	101.4	100.3[f]	95.6	95.6	95.6	95.6
2	–	–	–	–	–	36.9[a]	37.4[a]	36.7[a]	36.8[b]	36.8[c]	36.7
3	–	–	–	–	–	81.2	81.7	77.7	77.7	77.7	77.7[b]
4	–	–	–	–	–	75.9	76.4	82.2[d]	82.2[c]	82.2[d]	82.3
5	–	–	–	–	–	73.3[b]	73.0[b]	69.3[e]	69.3[d]	69.3[e]	69.7[e]
								α-cym.	α-cym.	α-cym.	α-cym.
C-1	–	–	–	–	–	–	–	99.0	99.0	99.0	99.0
2	–	–	–	–	–	–	–	32.2[f]	32.2	32.2[f]	32.2
3	–	–	–	–	–	–	–	76.4	76.4	76.4	76.4
4	–	–	–	–	–	–	–	73.3[b]	73.3	73.2	73.3[d]
5	–	–	–	–	–	–	–	66.5	66.5	66.5	66.5
6-Me	18.7	18.7	18.2 × 2	18.6 × 2	18.6 × 3	17.9	18.7	18.4	18.4	18.4[b]×2	18.1 × 2
		18.9	18.8	18.7		18.2 × 2	18.8 × 3	18.5 × 2	18.5 × 2	18.5[b]	18.5 × 2
						18.8		18.6 × 2	18.6 × 2	18.6[b]×2	18.6
3-OMe	57.0	57.0	57.2	57.1	57.0	57.2	57.0	56.6[c]	56.6	56.6	56.6[d]
		57.3	57.4	58.9 × 2	58.9 × 2	57.4	57.3 × 2	56.9	56.9	56.9	56.9
							57.4	58.3	58.3	58.3	58.3
								58.5	58.5	58.5	58.6

Measured at 100.40 MHz in pyridine-d_5 solution at 35°C.
[a–f]Assignments may be interchangeable in each column.

Compound **3**. Amorphous powder. $[\alpha]_D^{27} - 82°$ (MeOH; *c* 0.18). FAB-MS *m/z*: 847 $[M + Na]^+$. ^{13}C NMR and ^{1}H NMR: Tables 1 and 2.

Compound **4**. Amorphous powder. $[\alpha]_D^{19} - 34°$ (MeOH; *c* 0.48). FAB-MS *m/z*: 861 $[M + Na]^+$. ^{13}C NMR and ^{1}H NMR: Tables 1 and 2.

Table 2. ^{1}HNMR spectral data of sugar moiety in compounds **1–11**

	1	**2**[#]	**3**[#]	**4**	**5**	**6**[#]	**7**[#]	**8**	**9**	**10**	**11**
	β-ole.	*β*-ole.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-ole.	*β*-ole.	*β*-dig.	*β*-dig.	*β*-dig.	*β*-can.
H-1	4.87 (*dd*, 9.5, 2.0)	4.53 (*dd*, 9.5, 2.0)	4.53 (*dd*, 9.5, 2.0)	5.26 (*dd*, 9.5, 2.0)	5.28 (*dd*, 9.5, 2.0)	4.53 (*dd*, 9.5, 2.0)	4.52 (*dd*, 9.5,2.0)	5.43 (*dd*, 9.5,2.0)	5.45 (*dd*, 9.5,2.0)	5.47 (*dd*, 9.5,2.0)	4.86 (*dd*, 9.5,2.0)
3	3.50 (*)	3.38 (*)	3.37 (*)	4.10 (*q*, 3.0)	4.10 (*q*, 3.0)	3.36 (*)		4.60 (*)	4.60 (*)	4.60 (*)	3.94 (*m*)
4	3.50 (*)	3.17 (*t*, 9.0)	3.16 (*t*, 9.0)	3.52 (*dd*, 9.5, 3.0)	3.52 (*dd*, 9.5, 3.0)	3.16 (*t*, 9.0)	3.15[a] (*t*, 9.0)	3.46 (*dd*, 9.5, 3.0)	3.46 (*brd*, 9.0)	3.46 (*dd*, 9.5, 3.0)	3.27 (*t*, 9.0)
5	3.61 (*dq*, 9.0, 6.5)	3.31 (*dq*, 9.0, 6.5)	3.30 (*dq*, 9.0, 6.0)	4.22 (*dq*, 9.5, 6.5)	4.22 (*dq*, 9.5, 6.5)	3.30 (*dq*, 9.0, 6.0)	3.30 (*dq*, 9.5, 6.5)	4.26 (*dq*, 9.5, 6.5)	4.26 (*dq*, 9.0, 6.0)	4.26 (*dq*, 9.5, 6.5)	3.53 (*)
6	1.61 (*d*, 6.5)	1.30[a] (*d*, 6.5)	1.29 (*d*, 6.0)	1.39 (*d*, 6.5)	1.39 (*d*, 6.5)	1.29 (*d*, 6.0)	1.29 (*d*, 6.5)	1.42 (*d*, 6.5)	1.42 (*d*, 6.0)	1.40 (*d*, 6.5)	1.39 (*d*, 6.0)
		β-ole	*β*-can.	*β*-cym.	*β*-cym.	*β*-can.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-cym.	*β*-cym.
H-1	–	4.72 (*dd*, 9.5, 2.0)	4.72 (*dd*, 9.5, 2.0)	5.13 (*dd*, 9.5, 2.0)	5.13 (*dd*, 9.5, 2.0)	4.73 (*dd*, 9.5, 2.0)	4.67 (*dd*, 9.5, 2.0)	5.12 (*dd*, 9.5, 2.0)	5.12 (*dd*, 9.5, 2.0)	5.12 (*dd*, 9.5, 2.0)	5.07 (*dd*, 9.5, 2.0)
3	–	3.17 (*)	3.59 (*ddd*, 11.5, 9.0, 5.5)	4.07 (*q*, 3.0)	4.07 (*q*, 3.0)	3.59 (*)		3.85 (*)	3.85 (*)	3.85 (*)	3.88 (*q*, 3.0)
4	–	3.17 (*)	2.98 (*t*, 9.0)	3.50 (*dd*, 9.5, 3.0)	3.50 (*dd*, 9.5, 3.0)	2.97 (*t*, 9.0)	3.16[a] (*t*, 9.0)	3.37 (*)	3.37 (*)	3.36 (*)	3.43 (*dd*, 9.5, 3.0)
5	–	3.31 (*dq*, 9.0, 6.5)	3.33 (*dq*, 9.0, 6.0)	4.19 (*dq*, 9.5, 6.5)	4.20 (*dq*, 9.5, 6.5)	3.35 (*dq*, 9.0, 6.0)		4.15 (*dq*, 9.5, 6.5)	4.15 (*dq*, 9.0, 6.0)	4.15 (*dq*, 9.5, 6.5)	4.20 (*)
6	–	1.35[a] (*d*, 6.5)	1.31 (*d*, 6.0)	1.42 (*d*, 6.5)	1.42 (*d*, 6.5)	1.30 (*d*, 6.0)	1.31[b] (*d*, 6.5)	1.28 (*d*, 6.5)	1.27 (*d*, 6.0)	1.27 (*d*, 6.5)	1.28 (*d*, 6.0)
			β-ole.	*β*-ole.	*β*-ole	*β*-can.	*β*-ole.	*α*-cym.	*α*-cym.	*α*-cym.	*α*-cym.
H-1	–	–	4.49 (*dd*, 9.5, 2.0)	4.77 (*dd*, 9.5, 2.0)	4.76 (**)	4.50 (*dd*, 9.5, 2.0)	4.67 (*dd*, 9.5, 2.0)	4.94 (*dd*, 4.5, 1.5)	4.94 (*)	4.94 (*dd*, 4.5, 1.5)	4.92 (*dd*, 4.5, 1.5)
3	–	–	3.20 (*)	3.48 (*)	3.48 (*)	3.62 (*)		3.75 (*)	3.75 (*q*, 3.0)	3.75 (*q*, 3.5)	3.74 (*)
4	–	–	3.16 (*t*, 9.0)	3.48 (*)	3.48 (*)	3.02 (*t*, 9.0)	3.17 (*t*, 9.0)	3.85 (*dd*, 8.5, 3.0)	3.85 (*)	3.85 (*dd*, 8.0, 3.5)	3.84 (*dd*, 8.5, 3.0)
5	–	–	3.41 (*)	3.60 (*)	3.59 (*)	3.43 (*)		4.63 (*dq*, 8.5, 6.5)	4.62 (*dq*, 8.5, 6.0)	4.63 (*)	4.62 (*dq*, 8.5, 6.5)
6	–	–	1.36 (*d*, 6.0)	1.58 (*d*, 6.5)	1.58 (*d*, 6.5)	1.33 (*d*, 6.0)	1.32[b] (*d*, 6.5)	1.51 (*d*, 6.5)	1.51 (*d*, 6.0)	1.51 (*d*, 6.5)	1.51 (*d*, 6.5)
						β-ole.	*β*-ole.	*β*-cym.	*β*-cym.	*β*-cym.	*β*-cym.
H-1	–	–	–	–	–	4.50 (*dd*, 9.5, 2.0)	4.72 (*dd*, 9.5, 2.0)	5.24 (*dd*, 9.5, 2.0)	5.24 (*dd*, 9.5, 2.0)	5.24 (*dd*, 9.5, 2.0)	5.23 (*dd*, 9.5, 2.0)
3	–	–	–	–	–	3.21 (*)	3.15 (*)	3.89 (*q*, 3.0)	3.88 (*q*, 3.0)	3.88 (*q*, 3.0)	3.88 (*q*, 3.0)
4	–	–	–	–	–	3.16 (*t*, 9.0)		3.46 (*dd*, 9.5, 3.0)	3.46 (*brd*, 9.5)	3.46 (*dq*, 9.5, 3.0)	3.46 (*dd*, 9.5, 3.0)
5	–	–	–	–	–	3.41 (*)	3.31 (*)	4.19 (*dq*, 9.5, 6.5)	4.19 (*dq*, 9.5, 6.0)	4.19 (*dq*, 9.5, 6.5)	4.19 (*)
6	–	–	–	–	–	1.36 (*d*, 6.0)	1.34 (*d*, 6.5)	1.38 (*d*, 6.5)	1.38 (*d*, 6.0)	1.38 (*d*, 6.5)	1.38 (*d*, 6.0)
								α-cym.	*α*-cym.	*α*-cym.	*α*-cym.
H-1	–	–	–	–	–	–	–	4.97 (*dd*, 4.5, 1.5)	4.97 (*dd*, 4.5, 1.5)	4.97 (*dd*, 4.5, 1.5)	4.97 (*dd*, 4.5, 1.5)
3	–	–	–	–	–	–	–	3.70 (*q*, 3.5)	3.70 (*q*, 3.0)	3.70 (*q*, 3.0)	3.71 (*q*, 3.0)
4	–	–	–	–	–	–	–	3.59 (*)	3.59 (*dd*, 9.0, 3.0)	3.60 (*)	3.60 (*)
5	–	–	–	–	–	–	–	4.53 (*dq*, 8.5, 6.5)	4.53 (*dq*, 9.0, 6.0)	4.53 (*dq*, 9.0, 6.5)	4.53 (*dq*, 8.5, 6.5)
6	–	–	–	–	–	–	–	1.51 (*d*, 6.5)	1.51 (*d*, 6.0)	1.51 (*d*, 6.5)	1.51 (*d*, 6.0)
OMes	3.47 (*s*)	3.40 (*s*) × 2	3.40 (*s*)	3.47 (*s*)	3.47 (*s*)	3.39 (*s*)	3.38 (*s*)	3.37 (*s*)	3.37 (*s*)	3.37 (*s*)	3.35 (*s*)
			3.41 (*s*)	3.58 (*s*)	3.58 (*s*)	3.41 (*s*)	3.40 (*s*) × 2	3.38 (*s*)	3.38 (*s*)	3.38 (*s*)	3.38 (*s*)
				3.64 (*s*)	3.63 (*s*)		3.41 (*s*)	3.53 (*s*)	3.53 (*s*)	3.53 (*s*)	3.53 (*s*)
								3.55 (*s*)	3.55 (*s*)	3.55 (*s*)	3.55 (*s*)

Measured in Pyridine-d_5 at 35°C.
[#]:Measured in $CDCl_3$ at 35°C.
*:Overlapping with other signals.
**:Overlapping with H_2O signal.
[a–b]Assignments may be interchangeable in each column.

Compound **5**. Amorphous powder. $[\alpha]_D^{19} - 0°$ (MeOH; *c* 0.23). FAB-MS *m/z*: 855 $[M + H]^+$, 877 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **6**. Amorphous powder. $[\alpha]_D^{19} - 66°$ (MeOH; *c* 1.5). FAB-MS *m/z*: 955 $[M + H]^+$, 977 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **7**. Amorphous powder. $[\alpha]_D^{27} - 70°$ (MeOH; *c* 0.20). FAB-MS *m/z*: 1005 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **8**. Amorphous powder. $[\alpha]_D^{27} - 95°$ (MeOH; *c* 0.45). FAB-MS *m/z*: 1135 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **9**. Amorphous powder. $[\alpha]_D^{27} - 66°$ (MeOH; *c* 0.43). FAB-MS *m/z*: 1151 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **10**. Amorphous powder. $[\alpha]_D^{19} - 60°$ (MeOH; *c* 0.50). FAB-MS *m/z*: 1109 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Compound **11**. Amorphous powder. $[\alpha]_D^{27} - 99°$ (MeOH; *c* 0.45). FAB-MS *m/z*: 1135 $[M + Na]^+$. ^{13}C NMR and ^1H NMR: Tables 1 and 2.

Acid hydrolysis of a mixture of pregnane glycosides

A mixture of pregnane glycosides (320 mg) was heated at 60° for 4.5 h with dioxane (6 ml) and 0.2 N H_2SO_4 (1.5 ml). After hydrolysis, this reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The H_2O layer was deacidified with Amberlite IRA-60E and the eluate was concentrated to dryness. The residue was chromatographed on a silica gel column with a $CHCl_3$–MeOH–H_2O (7:1:1.2 bottom layer) system to obtain cymarose (21 mg), oleandrose (12 mg) ($[\alpha]_D^{24}$ −9.6° (*c* 1.2, 24 h after dissolution in H_2O)), digitoxose (7 mg) and canarose (7 mg) ($[\alpha]_D^{24}$ +22° (*c* 0.69, 24 h after dissolution in H_2O)). The $CHCl_3$ layer was also concentrated to dryness. Purification of the product by HPLC (YMC-ODS, 52.5, 50% MeOH in water) yielded 12-*O*-acetylpergularin [I (R_1 = H) 24 mg] and metaplexigenin [II (R_1 = H) 4 mg].

I (R_1 = H): $[\alpha]_D^{20}$ −63° (MeOH; *c* 1.9) ^{1}HNMR (pyridine-d_5 at 35°C): δ 5.42 (*m*, H-6), 4.87 (*dd*, 11.5, 4.5, H-12), 3.81 (*m*, H-3), 3.27 (*m*, H-16), 2.49 (*s*, H-21), 2.07 (*s*, H-Ac), 1.65 (*s*, H-18), 1.04 (*s*, H-19). ^{13}CNMR (pyridine-d_5 at 35°): δ 209.6 (C-20), 169.7 (CH_3C^*O), 140.8 (C-5), 121.2 (C-6), 92.1 (C-17), 88.9 (C-14), 73.1 (C-12), 71.1 (C-3), 56.7 (C-13), 43.3, 43.2 (C-4, 9), 37.5, 37.2 × 2 (C-1, 8, 10), 32.6, 32.4 (C-2, 16), 31.5 (C-15), 27.4 (C-21), 27.0 (C-11), 26.6 (C-7), 20.7 (C^*H_3COO), 19.6 (C-19), 8.6 (C-18).

II (R_1 = H): mp 268–271°C (acetone). [α]-1.3° (pyridine; *c* 0.35). ^{1}HNMR (pyridine-d_5 at 35°C): δ 5.33 (*brs*, H-6), 4.98 (*dd*, 11.5, 4.5, H-12), 3.84 (*m*, H-3), 2.47 (*s*, H-21), 2.07 (*s*, H-AC), 1.92 (*s*, H-18), 1.40 (*s*, H-19).

Acid hydrolysis of compounds **1–10** *and* **11**

A soln of each compound (*ca* 0.5 mg) in dioxane (4 drops) and 0.2 N H_2SO_4 (1 drop) was heated at 60° for 1 h. After hydrolysis, this soln was passed through an Amberlite IRA-60E column and concentrated to dryness. The residues from **1–10** and **11** were analyzed by HPLC to identify the aglycone with the authentic samples [conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 30% MeOH in water; R_t (min), deacylmetaplexigenin 14.2, 52.5% MeOH in water; R_t (min), 12-*O*-acetylpergularin 12.8, metaplexigenin 10.0].

Subsequently, for sugar analysis, the remaining residue of acid hydrolysis was reduced with $NaBH_4$ (*ca*. 1 mg) for 1 h at room temp. The reaction mixture was passed through an Amberlite IR-120B column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with MeOH, and the residue was acetylated with acetic anhydride and pyridine (3 drops each) at room temp. for one night. The reagents were evaporated off *in vacuo*. From each glycoside, cymaritol acetate, oleandritol acetate, digitoxitol acetate and canaritol acetate were detected by GC [condition: column, Supelco SP-2380™ capillary column 0.25 mm × 30 m, column temperature 200°, carrier gas, N_2, R_t (min); cymaritol acetate 7.4, oleandritol acetate 8.3 digitoxitol acetate 10.7, canaritol acetate 12.0].

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