Chem. Pharm. Bull. **46**(11) 1752—1757 (1998) Vol. 46, No. 11

Steroidal Glycosides from the Root of *Metaplexis japonica* M. Part II

Tsutomu Warashina* and Tadataka Noro

Institute for Environmental Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan.

Received June 17, 1998; accepted August 16, 1998

Fifteen new steroidal glycosides were obtained from the roots of *Metaplexis japonica* M. (Asclepiadaceae). These glycosides were confirmed to have ramanone, 12-*O*-acetylramamone, pergularin, 12-*O*-acetylpergularin and deacylmetaplexigenin as the aglycone and 2,6-dideoxyhexopyranoses as the sugar sequence by spectroscopic methods and chemical evidence.

Key words *Metaplexis japonica*; Asclepiadaceae; pregnane glycoside; ramanone; pergularin; deacylmetaplexigenin

In connection with a study on steroidal glycosides in the Asclepiadaceous family, we previously reported on pregnane glycosides which were obtained from the roots of *Metaplexis japonica* Makino.[1] In this paper, the structural elucidation of 15 new pregnane glycosides is described as part of our ongoing reseach.

Extraction and isolation of the roots of *M. japonica* M. were described in a previous report[1] and a pregnane glycoside fraction has now afforded 15 new compounds **1**—**15**.

To determine the component pregnanes and sugars, a mixture of pregnane glycosides was subjected to acid hydrolysis. The obtained aglycones were identified as ramanone,[2] pergularin,[3] 12-*O*-acetylramanone and 12-*O*-acetylpergularin in view of the ^{1}H- and ^{13}C-NMR spectra, $[\alpha]_D$ and melting point. The component sugars were fractionated by silica gel column chromatography and digitoxose, cymarose, oleandrose and canarose were obtained.

Compound **1** was suggested to have the molecular formula, $C_{44}H_{70}O_{14}$, based on FAB-MS [positive FAB-MS ion at *m/z* 845 $[M+Na]^+$]. In the ^{13}C-NMR spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those of 12-*O*-acetylramanone within the range of glycosylation shifts at C-3 (+6.2 ppm), C-2 (−2.2 ppm) and C-4 (−4.0 ppm). In the ^{1}H- and ^{13}C-NMR spectra, three anomeric proton and carbon signals were observed at δ 5.26, 5.13, 4.77 and δ 96.4, 100.5, 102.2. Thus, **1** was believed to be 12-*O*-acetylramanone 3-*O*-trioside. On acid hydrolysis of **1** with 0.2 N H_2SO_4, 12-*O*-acetylramanone, cymarose and oleandrose were obtained as the component aglycone and sugars. These sugars were identified as β-cymaropyranose and β-oleandropyranose based on the ^{13}C-NMR spectral data and the coupling constant of each anomeric proton signal (J=9.5, 2.0 Hz).[4—6] Moreover, the presence of the characteristic H-3 signals of cymaropyranose [δ 4.07 (1H, q, J=3.0 Hz) and 4.10 (1H, q, J=3.0 Hz)] in the ^{1}H-NMR spectrum suggested that the sugar moiety included two cymaropyranose and one oleandropyranose. Since the carbon signals due to the sugar moiety were consistent with those of cynanchoside C_2,[4,7] the sugar linkage was deduced to be 3-*O*-β-oleandropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside, and this linkage was confirmed based on the rotating frameshift difference nuclear Overhauser effect (ROE) spectra irradiating at each anomeric proton signal. Accordingly, the structure of **1** was determined to be 12-*O*-acetylramanone 3-*O*-β-oleandropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside.

Compound **2** showed the molecular formula $C_{44}H_{70}O_{15}$ based on FAB-MS [positive FAB-MS ion at *m/z* 861 $[M+Na]^+$]. As in the ^{1}H- and ^{13}C-NMR spectra, three anomeric proton and carbon signals were observed at δ 5.08, 5.12, 5.25 and δ 96.4, 100.5×2 along with the signals due to 12-*O*-acetylpergularin,[1] **2** was believed to be 12-*O*-acetylpergularin 3-*O*-trioside. On acid hydrolysis, **2** afforded cymarose as the component sugars, so the sugar moiety consisted of three cymarose. The carbon signals due to the sugar moiety and the coupling constant of each anomeric proton signal (J=9.5, 2.0 Hz) suggested that these cymarose were β-hexopyranosides.[4,5] Based on the consistence of the carbon signals of the sugar moiety with those of cynanchoside C_1,[4,8] the sugar linkage of **2** was deduced to be 3-*O*-β-cymaropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside, and this linkage was confirmed by difference ROE spectra irradiating at each anomeric proton signal. Hence, **2** was determined to be 12-*O*-acetylpergularin 3-*O*-β-cymaropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-cymaropyranoside.

Compounds **3**—**6** had molecular formulae, $C_{51}H_{82}O_{18}$, $C_{50}H_{80}O_{18}$, $C_{51}H_{82}O_{17}$ and $C_{51}H_{82}O_{18}$, respectively, based on FAB-MS. On acid hydrolysis, compounds **3**, **4** and **6** afforded 12-*O*-acetylpergularin, and **5** afforded 12-*O*-acetylramanone as the aglycone moiety. In the ^{1}H- and ^{13}C-NMR spectra of each compound, four anomeric proton and carbon signals were observed, so these compounds were thought to be tetraglycosides. The sugar moiety of each compound was determined by acid hydrolysis. As acid hydrolysis of **3** afforded cymarose as the component sugars, the sugar moiety of **3** was composed of cymarose alone. The coupling constant of each anomeric proton signal indicated the presence of two equatorial (J=4.5, 1.5 Hz) and two axial anomeric protons (J=9.5, 2.0 Hz). Thus, two cymarose had α-glycosidic linkage and the other two had β-glycosidic linkage.[9] The sugar sequence was decided by the consequences of the difference ROE experiments. The irradiation of the anomeric proton signal at δ 5.22 showed an ROE to the H-3 signal (δ 3.77) of the aglycone. The β-cymaropranose was attached to the C-3 position of the aglycone. Similarly, ROEs were observed between δ 4.97 (H-1 of the first α-cymaropyranose)/3.47 (H-4 of the first β-cymaropyranose); 5.24 (H-1 of the second β-cymaropyranose)/3.86 (H-4 of the first α-cymaropyranose); 4.97 (H-1 of the second α-cymaropyranose)/3.46 (H-4 of the second β-cymaropyranose). Based on the evidence, the structure of **3** was elucidated to be 12-*O*-acetylpergularin 3-*O*-α-cymaropyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-α-cymaropyranosyl-(1→4)-β-cymaropyranoside.

---

* To whom correspondence should be addressed.

That the sugar moiety of **4** consisted of two β-cymaropyranose, one β-oleandropyranose and one β-canaropyranose[10] as indicated from the results of acid hydrolysis and the ^{1}H- and ^{13}C-NMR spectra. The sugar sequence was determined by the difference ROE experiments irradiating at each anomeric proton. Therefore, the structure of **4** was concluded as shown in Chart 1.

The sugar sequences of **5** and **6** were determined to be β-oleandropyranosyl-(1 →4)-β-oleandropyranosyl-(1 →4)-β-cymaropyranosyl-(1 →4)-β-cymaropyranoside, based on the good agreement of the ^{1}H- and ^{13}C-NMR spectral data of **5** and **6** with those of calotroposide E.[11] So, the structures of **5** and **6** were concluded as shown in Chart 1.

The molecular formulae of compounds **7**—**12** were suggested to be $C_{58}H_{94}O_{21}$, $C_{58}H_{94}O_{21}$, $C_{56}H_{92}O_{19}$, $C_{56}H_{92}O_{20}$, $C_{56}H_{92}O_{21}$ and $C_{58}H_{94}O_{21}$, respectively, by observation of $[M+Na]^+$ ion peaks in FAB-MS. From the results of acid hydrolysis and the ^{1}H- and ^{13}C-NMR spectral data, **7**—**12** were determined to be pregnane 3-*O*-pentaosides whose aglycones were 12-*O*-acetylpergularin on **7**, **8** and **12**, ramanone on **9**, pergularin on **10** and deacylmetaplexigenin[12] on **11**. The ^{1}H- and ^{13}C-NMR spectral data of the sugar moiety of **7** were consistent with those of cynanchogenin 3-*O*-α-L-cymaropyranosyl-(1 →4)-β-D-oleandropyranosyl-(1 →4)-β-D-oleandropyranosyl-(1 →4)-β-D-cymaropyranosyl-(1 →4)-β-D-cymaropyranoside.[13] Thus, the structure of **7** was determined as shown in Chart 1.

The sugar sequences of **8**—**11** were considered to be the same based on the consistence of the ^{1}H-and ^{13}C-NMR spectral data. On acid hydrolysis of each compound, only cymarose was afforded along with each aglycone, and thus the component sugars were five cymarose; it was indicated that they consisted of two α-cymaropyranose and three β-cymaropyranose based on observation of the coupling constant of each anomeric proton signal. The sugar sequence was determined by the difference ROE experiments of **8**. The ROEs were observed between δ 5.24 (H-1 of the first β-cymaropyranose)/3.77 (H-3 of the aglycone); 5.09 (H-1 of the second β-cymaropyranose)/3.48 (H-4 of the first β-cymaropyranose); 4.97 (H-1 of the first α-cymaropyranose)/3.43 (H-4 of the second β-cymaropyranose); 5.24 (H-1 of the third β-cymaropyranose)/3.85 (H-4 of the first α-cymaropyranose); 4.97 (H-1 of the second α-cymaropyranose)/3.46 (H-4 of the third β-cymaropyranose). Accordingly, the sugar sequence in **8** was decided as 3-*O*-α-cymaroropyranosyl-(1 →4)-β-cymaropyranosyl-(1 →4)-α-cymaropyranosyl-(1 →4)-β-cymaropyranosyl-(1 →4)-β-cymaropyranoside, and the structures of **8**—**11** were determined as shown in Chart 1.

The component sugars of **12** were five cymarose which were composed of two β-cymaropyranose and three α-cymaropyranose. The sequence of the sugar moiety was decided as shown in Chart 1 in the same manner as that of **8**.

The molecular formula of compound **13** was $C_{64}H_{104}O_{24}$ on the basis of FAB-MS, and the ^{1}H- and ^{13}C-NMR spectra suggested that **13** was 12-*O*-acetylpergularin 3-*O*-hexaoside. Because **13** was hydrolyzed to 12-*O*-acetylpergularin, cymarose and digitoxose, and five methoxyl proton signals were observed in the ^{1}H-NMR spectrum (δ 3.37×2, 3.39, 3.56, 3.58), the sugar chain seemed to be composed of five cymarose and one digitoxose. Each anomeric proton signal was assigned in Table 2, based on the H–H correlation spectroscopy (COSY) spectrum. The coupling constants of the anomeric proton signals indicated that five cymarose consisted of two β-cymaropyranose and three α-cymaropyranose, and the digitoxose was β-digitoxopyranose.[14] The sugar linkage was determined to be 3-*O*-α-cymaropyranosyl-(1 →4)-β-digitoxopyranosyl-(1 →4)-β-cymaropyranosyl-(1 →4)-α-cymaropyranosyl-(1 →4)-α-cymaropyranosyl-(1 →4)-β-cymaropyranoside by the ROE spectra irradiating at the anomeric proton and/or the H-4 signals of each monosaccharide. Moreover, this sugar sequence was confirmed on the basis of the ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum. $^3J_{\mathrm{COCH}}$s were observed between δ 96.4 (C-1 of the first β-cymaropyranose)/3.76 (H-3 of the aglycone); 99.1 (C-1 of the first α-cymaropyranose)/3.47 (H-4 of the first β-cymaropyranose); 92.2 (C-1 of the second α-cymaropyranose)/3.70 (H-4 of the first α-cymaropyranose); 95.3 (C-1 of the second β-cymaropyranose)/3.86 (H-4 of the second α-cymaropyranose); 100.4 (C-1 of β-digitoxopyranose)/3.49 (H-4 of the second β-cymaropyranose); 98.4 (C-1 of the third α-cymaropyranose)/3.43 (H-4 of β-digitoxopyranose). Hence, the structure of **13** was elucidated as presented in Chart 1.

As compounds **14** and **15** showed $[M+Na]^+$ ion peaks at *m/z* 1409 on FAB-MS, their molecular formulae were suggested to be $C_{70}H_{114}O_{27}$. **14** and **15** seemed to be 12-*O*-acetylpergularin 3-*O*-heptaosides, whose component sugars were two β-cymaropyranose, two α-cymaropyranose, two β-digitoxopyranose and one β-oleandropyranose in **14**, and one β-cymaropyranose, two α-cymaropyranose, two β-digitoxopyranose and two β-oleandropyranose in **15** from the results of the ^{1}H-, ^{13}C-NMR and two-dimensional (2D) NMR (^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum, HMBC, COSY) spectra and acid hydrolysis. Their sugar sequences were determined to be as shown in Chart 1, using the same procedure of the difference ROE spectrum and the HMBC spectrum as for **13**.

Finally, absolute configuration of monosaccharides in each compound was not definitely determined, but acid hydrolysis of a mixture of pregnane glycosides afforded pure digitoxose, cymarose, oleandrose and canarose. Their optical rotation values suggested that oleadrose and canarose had D-form.[15,16] In regard to the identification of α-cymaropyranose, a signal appearing at approximately δ 32 could be assignable to C-2 of α-L-cymaropyranose in the ^{13}C-NMR spectra, and α-cymaropyranose was deduced to be L-form.[9]

Experimental

Optical rotation was determined with a JASCO-DIP1000 digital polarimeter. The melting point was measured with a Yanako-MP-S3. FAB-MS spectra were taken on a JEOL JMS-SX120 spectrometer in *m*-nitrobenzyl alcohol. ^{1}H- and ^{13}C-NMR were recorded on a JEOL JNM A-400 (400 and 100.40 MHz, respectively) spectrometer. Chemical shifts were given on the δ (ppm) scale with tetramethysilane (TMS) as an internal standard. GC was run on a Hitachi G-3000 gas chromatograph, and HPLC was run on a JASCO 800 system instrument.

Extraction and Isolation The air dried root of *M. japonica* M. (1.6 kg) was extracted twice with MeOH under reflux. The procedure after the extraction was described in our previous report.[1] Isolated pregnane glycoside fraction afforded compounds **1** (4 mg), **2** (2 mg), **3** (3 mg), **4** (3 mg), **5** (2 mg), **6** (5 mg), **7** (3 mg), **8** (4 mg), **9** (3 mg), **10** (5 mg), **11** (3 mg), **12** (2 mg), **13** (3 mg), **14** (3 mg) and **15** (2 mg).

Compound **1**: Amorphous powder. $[\alpha]_D^{25}$ $-53°$ (c=0.44, MeOH). FAB-MS *m/z*: 845 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **2**: Amorphous powder. $[\alpha]_D^{25}$ $-12°$ (c=0.23, MeOH). FAB-

Table 1. ^{13}C-NMR Spectral Data (Measured at 100.40 MHz in Py-d_5 Solution at 35 °C)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aglycone moiety															
C-1	$37.0^{a)}$	$37.1^{a)}$	$37.2^{a)}$	$37.3^{a)}$	$37.0^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.4^{a)}$	$37.3^{a)}$	39.0	$37.2^{a)}$	$37.2^{a)}$	$37.2^{a)}$	$37.2^{a)}$
2	30.3	30.2	30.2	30.2	30.3	30.2	30.2	30.2	30.2	30.3	30.0	30.2	30.2	30.2	30.2
3	77.3	77.3	77.2	77.3	77.3	77.3	77.3	77.2	77.4	77.3	$77.7^{a)}$	77.2	77.2	77.3	$77.6^{b)}$
4	39.3	39.4	39.2	39.2	39.2	39.2	39.2	39.2	39.3	39.3	39.4	39.2	39.2	39.2	39.2
5	139.9	139.9	139.8	139.9	139.9	139.9	139.9	139.9	140.0	139.9	139.5	139.8	139.9	139.8	139.9
6	122.1	121.8	121.8	121.8	122.1	121.8	121.8	121.8	122.3	122.2	119.4	121.8	121.8	121.8	121.9
7	27.2	26.6	26.6	26.6	27.2	26.6	26.6	26.6	27.4	26.8	34.3	26.6	26.6	26.6	26.7
8	$37.4^{a)}$	$37.2^{a)}$	$37.2^{a)}$	$37.3^{a)}$	$37.4^{a)}$	$37.3^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.0^{a)}$	74.3	$37.2^{a)}$	$37.2^{a)}$	$37.2^{a)}$	$37.2^{a)}$
9	43.5	43.2	43.2	43.2	43.5	43.2	43.2	43.2	43.9	43.8	45.0	43.2	43.2	43.2	43.2
10	$37.1^{a)}$	$37.2^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.3^{a)}$	$37.2^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$37.1^{b)}$	$37.2^{a)}$	$37.2^{a)}$	$37.1^{a)}$	$37.1^{a)}$
11	26.8	26.9	26.9	26.9	26.8	26.9	26.9	26.9	31.9	31.7	29.4	26.9	27.0	26.9	26.9
12	$72.9^{b)}$	73.1	$73.1^{b)}$	$73.1^{b)}$	$73.0^{b)}$	$73.1^{b)}$	$73.1^{b)}$	$73.1^{b)}$	68.4	68.2	$69.2^{c)}$	$73.1^{b)}$	$73.1^{b)}$	$73.0^{b)}$	$73.1^{c)}$
13	54.6	56.6	56.6	56.6	54.6	56.6	56.7	56.6	56.9	59.0	60.5	56.6	56.6	56.6	56.7
14	86.6	88.8	88.8	88.8	86.6	88.9	88.9	88.9	86.8	88.9	89.3	88.9	88.9	88.9	88.9
15	31.7	31.5	31.4	31.5	31.7	31.5	31.5	31.4	31.9	31.6	35.1	31.5	31.5	31.4	31.5
16	21.1	32.6	32.6	32.6	21.3	32.6	32.5	32.6	21.6	32.6	32.8	32.6	32.6	32.6	32.6
17	60.4	92.1	92.1	92.1	60.4	92.1	92.1	92.1	61.3	92.4	92.6	92.1	92.1	92.1	92.1
18	14.3	8.5	8.5	8.5	14.3	8.6	8.6	8.6	13.5	7.6	9.4	8.5	8.5	8.5	8.6
19	19.5	19.4	19.4	19.4	19.5	19.4	19.5	19.4	19.6	19.6	$18.4^{d)}$	19.4	19.4	19.4	19.4
20	209.6	209.6	209.6	209.6	209.5	209.6	209.6	209.6	210.3	209.0	209.5	209.6	209.6	209.6	209.6
21	31.9	27.4	27.4	27.4	31.9	27.4	27.4	27.4	31.4	27.7	27.8	27.4	27.4	27.4	27.4
Ac	169.8	169.7	169.7	169.7	169.8	169.7	169.7	169.7	—	—	—	169.7	169.7	169.7	169.7
	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7	—	—	—	20.7	20.7	20.7	20.7
Sugar moiety															
	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-ole.
C-1	96.4	96.4	96.3	96.4	96.4	96.4	96.4	96.4	96.4	96.4	96.4	96.4	96.4	96.4	98.1
2	$37.3^{a)}$	$37.3^{a)}$	$37.2^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.0^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{b)}$	$37.1^{a)}$	$37.1^{a)}$	$37.1^{a)}$	$36.8^{a)}$
3	77.9	$78.0^{b)}$	$77.7^{c)}$	$77.8^{c)}$	$78.1^{c)}$	$78.1^{c)}$	$78.1^{a)}$	$78.0^{c)}$	$78.0^{b)}$	$78.0^{b)}$	$78.0^{a)}$	77.7	$77.8^{c)}$	$77.8^{c)}$	79.3
4	$83.2^{c)}$	$83.4^{c)}$	$82.3^{d)}$	$83.4^{d)}$	$83.4^{d)}$	$83.4^{d)}$	83.2	83.4	83.4	83.4	83.4	$82.6^{c)}$	82.5	82.5	$82.1^{d)}$
5	$69.0^{d)}$	69.0	$69.2^{e)}$	$69.1^{e)}$	$69.1^{e)}$	$69.1^{e)}$	$68.9^{d)}$	$69.1^{d)}$	$69.0^{c)}$	$69.0^{c)}$	$69.0^{c)}$	$69.3^{d)}$	$68.9^{d)}$	$69.2^{d)}$	72.1
	β-cym.	β-cym.	α-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	α-cym.	α-cym.	α-cym.	α-cym.
C-1	100.5	100.5	99.0	100.5	100.5	100.5	100.5	100.4	100.4	100.4	100.4	99.1	99.1	99.1	97.5
2	$37.3^{a)}$	$36.9^{a)}$	$32.2^{f)}$	$37.1^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.3^{a)}$	$37.4^{a)}$	$37.4^{a)}$	$37.4^{b)}$	$31.8^{e)}$	$31.9^{e)}$	31.9	31.9
3	78.1	$77.9^{b)}$	73.2	$78.0^{a)}$	$77.8^{c)}$	$77.8^{c)}$	$77.8^{c)}$	$77.7^{c)}$	$77.7^{b)}$	$77.7^{b)}$	$77.7^{a)}$	71.7	71.6	71.7	72.1
4	$83.4^{c)}$	$83.1^{c)}$	$77.7^{c)}$	$83.2^{d)}$	$83.2^{d)}$	$83.2^{d)}$	$82.4^{e)}$	82.3	82.2	$82.2^{d)}$	82.3	75.6	75.5	75.6	75.8
5	$69.1^{d)}$	69.0	65.4	$68.9^{e)}$	$68.9^{e)}$	$68.9^{e)}$	$69.1^{d)}$	$69.2^{d)}$	$69.2^{c)}$	$69.2^{c)}$	$69.0^{c)}$	$64.9^{f)}$	$64.9^{f)}$	$64.9^{e)}$	$64.4^{e)}$
	β-ole.	β-cym.	β-cym.	β-can.	β-ole.	β-ole.	β-ole.	α-cym.	α-cym.	α-cym.	α-cym.	α-cym.	α-cym.	α-cym.	α-cym.
C-1	102.2	100.5	95.6	102.1	102.0	102.0	102.0	99.0	99.0	99.0	99.0	92.3	92.2	92.2	92.3
2	$37.3^{a)}$	35.9	36.7	39.9	$37.4^{a)}$	$37.4^{a)}$	$37.3^{a)}$	$32.3^{f)}$	$32.3_{d)}$	$32.3^{e)}$	$32.2^{f)}$	$31.8^{e)}$	$31.9^{g)}$	31.9	31.9
3	81.4	78.8	$77.9^{c)}$	69.9	79.1	79.1	$79.1^{f)}$	$73.3^{b)}$	73.3	73.3	73.3	73.1	73.1	$73.0^{b)}$	$73.1^{c)}$
4	76.2	74.2	$82.5^{d)}$	88.1	82.7	82.7	$82.7^{e)}$	$77.7^{c)}$	$77.7^{b)}$	$77.7^{b)}$	$77.7^{a)}$	77.7	$77.8^{c)}$	$77.8^{c)}$	$77.9^{b)}$
5	$73.0^{a)}$	71.0	$69.4^{e)}$	71.0	71.7	71.7	71.7	65.4	65.4	65.4	65.4	$64.6^{f)}$	$64.6^{f)}$	$64.5^{e)}$	$64.5^{e)}$
			α-cym.	β-ole.	β-ole.	β-ole.	β-ole.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.
C-1	—	—	99.0	101.4	100.3	100.3	100.1	95.7	95.7	95.7	95.7	95.2	95.3	95.3	95.3
2	—	—	$32.3^{f)}$	$36.9^{a)}$	$37.6^{a)}$	$37.6^{a)}$	$37.6^{a)}$	36.7	36.7	36.7	36.7	36.7	36.8	$37.0^{a)}$	$37.0^{a)}$
3	—	—	76.4	81.2	81.7	81.7	$79.4^{f)}$	$77.7^{c)}$	$77.7^{b)}$	$77.7^{b)}$	$77.7^{a)}$	77.7	$78.0^{c)}$	$78.0^{c)}$	$78.0^{b)}$
4	—	—	$73.2^{b)}$	75.9	76.4	76.4	81.7	82.3	82.2	$82.3^{d)}$	82.3	$82.3^{c)}$	83.2	$83.3^{f)}$	$83.1^{f)}$
5	—	—	66.5	$73.3^{b)}$	$72.9^{b)}$	$73.0^{b)}$	72.3	$69.4^{d)}$	$69.4^{c)}$	$69.4^{c)}$	$69.4^{c)}$	$69.4^{d)}$	$69.1^{d)}$	$69.1^{d)}$	$69.2^{g)}$
							α-cym.	α-cym.	α-cym.	α-cym.	α-cym.	α-cym.	β-dig.	β-dig.	β-dig.
C-1	—	—	—	—	—	—	97.3	99.0	99.0	99.0	99.0	98.9	100.4	100.5	100.5
2	—	—	—	—	—	—	32.2	$32.2^{f)}$	$32.2^{d)}$	$32.1^{e)}$	$32.4^{f)}$	$32.2^{e)}$	38.5	38.8	$38.7^{h)}$
3	—	—	—	—	—	—	76.7	76.4	76.4	76.4	76.4	76.4	67.8	$67.5^{g)}$	$67.4^{i)}$
4	—	—	—	—	—	—	$73.4^{b)}$	$73.3^{b)}$	73.3	73.3	73.3	$73.2^{b)}$	80.8	$83.2^{f)}$	$83.2^{f)}$
5	—	—	—	—	—	—	66.0	66.5	66.5	66.5	66.5	66.5	$69.2^{d)}$	$68.7^{h)}$	$68.6^{g)}$
													α-cym.	β-dig.	β-dig.
C-1	—	—	—	—	—	—	—	—	—	—	—	—	98.4	99.9	99.9
2	—	—	—	—	—	—	—	—	—	—	—	—	$32.3^{g)}$	38.7	$38.8^{h)}$
3	—	—	—	—	—	—	—	—	—	—	—	—	76.6	$67.4^{g)}$	$67.5^{i)}$
4	—	—	—	—	—	—	—	—	—	—	—	—	72.7	$83.1^{f)}$	$83.3^{f)}$
5	—	—	—	—	—	—	—	—	—	—	—	—	67.3	$68.6^{h)}$	$68.7^{g)}$
														β-ole.	β-ole.
C-1	—	—	—	—	—	—	—	—	—	—	—	—	—	101.6	101.6
2	—	—	—	—	—	—	—	—	—	—	—	—	—	$36.8^{a)}$	$37.4^{a)}$
3	—	—	—	—	—	—	—	—	—	—	—	—	—	81.4	81.4
4	—	—	—	—	—	—	—	—	—	—	—	—	—	76.2	76.2
5	—	—	—	—	—	—	—	—	—	—	—	—	—	$72.9^{b)}$	$73.0^{c)}$
6s	18.5	18.4	18.6×3	18.2×2	18.5	18.5	18.5×2	18.5×2	18.5×4	18.5×4	$18.5^{d)}$×3	18.5×2	18.4×4	18.4×2	18.4×2
	18.6×2	18.6	18.7	18.5	18.6	18.6×2	18.6	18.6×3	18.6	18.6	$18.6^{d)}$×2	18.6×2	18.6	18.5	18.5×2
		19.0		18.6	18.7×2	18.7	18.7×2					18.7	18.7	18.6×3	18.6×2
														18.7	18.7
OMes	57.0	58.1	56.6	57.2	57.0	57.1	56.4	56.6	56.6	56.6	56.6	56.5	56.5	56.5	56.3
	58.8×2	58.8	56.9	58.8	57.3	57.3	56.8	57.0	57.0	57.0	57.0	56.6	56.8×2	56.9	56.7
		58.9	58.2	58.9	58.8	58.8	57.4	58.3	58.3	58.3	58.3	56.8	58.4	57.0	56.8
			58.4		58.9	58.9	58.8	58.6	58.5	58.6	58.6	58.3	58.8	58.4	57.0
							58.9	58.9	58.9	58.9	58.9	58.4		58.8	58.8

a—i): Assignments may be interchangeble in each column.

Table 2. ^{1}H-NMR Spectral Data of Sugar Moiety

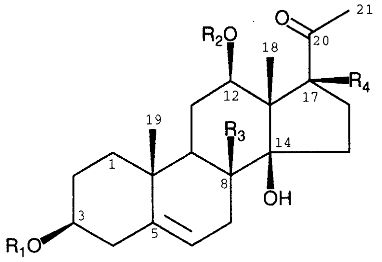
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym	β-cym.	β-cym.
H-1	5.26 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	5.22 (dd, 9.5, 2.0)	5.26 (dd, 9.5, 2.0)	5.26 (dd, 9.5, 2.0)	5.26 (dd, 9.5, 2.0)	5.26 (dd, 9.5, 2.0)	5.24 (dd, 9.5, 2.0)
3	4.10 (q, 3.0)	4.08 (*)	3.91 (q, 3.0)	4.09 (q, 3.0)	4.09 (q, 3.0)	4.09 (q, 3.0)	4.10 (q, 3.0)	4.06 (q, 3.0)
4	3.51 (dd, 9.5, 3.0)	3.50 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.51 (dd, 9.5, 3.0)	3.51 (*)	3.52 (*)	3.52 (*)	3.48 (dd, 9.5, 3.0)
5	4.22 (dq, 9.5, 6.5)	4.21 (dq, 9.5, 6.5)	4.20 (dq, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.22 (dq, 9.5, 6.5)	4.19 (*)
6	1.39 (d, 6.5)	1.39 (d, 6.5)	1.38 (d, 6.5)	1.39 (d, 6.5)	1.39 (d, 6.5)	1.39 (d, 6.5)	1.39 (d, 6.5)	1.38 (d, 6.5)
	β-cym.	β-cym.	α-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.
H-1	5.13 (dd, 9.5, 2.0)	5.12 (dd, 9.5, 2.0)	4.97 (dd, 4.5, 1.5)	5.12 (dd, 9.5, 2.0)	5.13 (dd, 9.5, 2.0)	5.13 (dd, 9.5, 2.0)	5.13 (dd, 9.5, 2.0)	5.09 (dd, 9.5, 2.0)
3	4.07 (q, 3.0)	4.06 (*)	3.76 (*)	4.05 (q, 3.0)	4.04 (q, 3.0)	4.04 (q, 3.0)	4.04 (q, 3.0)	3.88 (q, 3.0)[a]
4	3.50 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.86 (dd, 8.5, 3.5)	3.47 (*)	3.47 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.47 (*)	3.43 (dd, 9.5, 3.0)
5	4.19 (dq, 9.5, 6.5)	4.18 (dq, 9.5, 6.5)	4.66 (dq, 8.5, 6.5)	4.17 (dq, 9.5, 6.5)	4.18 (dq, 9.5, 6.5)	4.18 (dq, 9.5, 6.5)	4.18 (dq, 9.5, 6.5)	4.15 (dq, 9.5, 6.5)
6	1.42 (d, 6.5)	1.37 (d, 6.5)	1.52 (d, 6.5)	1.37 (d, 6.5)	1.40 (d, 6.5)	1.40 (d, 6.5)	1.39 (d, 6.5)	1.34 (d, 6.5)
	β-ole.	β-cym.	β-cym.	β-can.	β-ole.	β-ole.	β-ole.	α-cym.
H-1	4.77 (dd, 9.5,2.0)	5.08 (dd, 9.5, 2.0)	5.24 (dd, 9.5, 2.0)	4.77 (**)	4.71 (dd, 9.5, 2.0)	4.71 (dd, 9.5, 2.0)	4.70 (dd, 9.5, 2.0)	4.97 (dd, 4.5, 1.5)
3		3.75 (q, 3.0)	3.88 (q, 3.0)	3.94 (m)				3.75 (*)
4	3.48 (*)	3.52 (*)	3.46 (dd, 9.5, 3.0)	3.30 (t, 9.5)				3.85 (dd, 8.5, 3.0)
5	3.59 (*)	4.10 (dq, 9.5, 6.5)	4.19 (dq, 9.5, 6.5)	3.57 (dq, 9.5, 6.0)	3.55 (*)[a]	3.55 (*)[a]	3.53 (*)	4.64 (dq, 8.5, 6.5)
6	1.58 (d, 6.5)	1.53 (d, 6.5)	1.38 (d, 6.5)	1.45 (d, 6.0)	1.48 (d, 6.5)[b]	1.48 (d, 6.5)[b]	1.45 (d, 6.5)	1.52 (d, 6.5)[b]
			α-cym.	β-ole.	β-ole.	β-ole.	β-ole.	β-cym.
H-1	—	—	4.97 (dd, 4.5, 1.5)	4.82 (dd, 9.5,2.0)	4.99 (dd, 9.5, 2.0)	4.99 (dd, 9.5, 2.0)	4.87 (dd, 9.5, 2.0)	5.24 (dd 9.5, 2.0)
3	—	—	3.70 (q, 3.5)	3.51 (*)			3.43 (*)	3.89 (q, 3.0)[a]
4	—	—	3.59 (dd, 8.5, 3.5)	3.48 (*)			3.40 (t, 8.5)	3.46 (dd, 9.5, 3.0)
5	—	—	4.53 (dq, 8.5, 6.5)	3.66 (dq, 8.5, 6.0)	3.62 (*)[a]	3.62 (*)[a]	3.49 (*)	4.19 (*)
6	—	—	1.51 (d, 6.5)	1.52 (d, 6.0)	1.60 (d, 6.5)[b]	1.60 (d, 6.5)[b]	1.41 (d, 6.5)	1.38 (d, 6.5)
							α-cym.	α-cym.
H-1	—	—	—	—	—	—	5.10 (dd, 4.5, 1.5)	4.97 (dd, 4.5, 1.5)
3	—	—	—	—	—	—	3.73 (q, 3.5)	3.71 (q, 3.5)
4	—	—	—	—	—	—	3.60 (*)	3.59 (*)
5	—	—	—	—	—	—	4.64 (dq, 9.5, 6.5)	4.53 (dq, 8.5, 6.5)
6	—	—	—	—	—	—	1.55 (d, 6.5)	1.51 (d, 6.5)[b]
H-1	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
H-1	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
OMes	3.47 (s)	3.47 (s)	3.38 (s)×2	3.49 (s)	3.49 (s)	3.48 (s)	3.35 (s)	3.37 (s)
	3.58 (s)	3.59 (s)	3.53 (s)	3.60 (s)	3.53 (s)	3.52 (s)	3.44 (s)	3.38 (s)
	3.63 (s)	3.63 (s)	3.57 (s)	3.63 (s)	3.59 (s)	3.59 (s)	3.50 (s)	3.53 (s)
					3.63 (s)	3.63 (s)	3.58 (s)	3.56 (s)
							3.63 (s)	3.61 (s)

	9	**10**	**11**	**12**	**13**	**14**	**15**
	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-ole.
H-1	5.25 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	5.27 (dd, 9.5, 2.0)	5.22 (dd, 9.5, 2.0)	5.22 (dd, 9.5, 2.0)	5.22 (dd, 9.5, 2.0)	4.74 (dd. 9.5, 2.0)
3	4.06 (q, 3.0)	4.06 (q, 3.0)	4.06 (q, 3.0)	3.90 (*)[a]	3.90 (q, 3.0)	3.90 (q, 3.0)	3.42 (*)
4	3.47 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.47 (*)	3.37 (t, 8.5)
5	4.19 (dq, 9.5, 6.5)	4.19 (dq, 9.5, 6.5)	4.19 (dq, 9.6, 6.5)[a]	4.19 (*)	4.20 (dq, 9.5, 6.5)	4.19 (*)	3.45 (*)
6	1.38 (d, 6.5)[a]	1.37 (d, 6.5)	1.38 (d, 6.5)[b]	1.38 (d, 6.5)	1.38 (d, 6.5)	1.38 (d, 6.5)[a]	1.41 (d, 6.5)
	β-cym.	β-cym.	β-cym.	α-cym.	α-cym.	α-cym.	α-cym.
H-1	5.08 (dd, 9.5, 2.0)	5.08 (dd, 9.5, 2.0)	5.08 (dd, 9.5, 2.0)	4.96 (dd, 4.5, 1.5)	4.96 (dd, 4.5, 1.5)	4.96 (brd, 4.5)	5.07 (dd, 4.5, 1.5)
3	3.87 (*)	3.87 (q, 3.0)[a]	3.88 (*)	3.86 (*)	3.87 (*)	3.87 (*)	3.88 (q, 3.0)
4	3.43 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.43 (dd, 9.5, 3.0)	3.70 (dd, 8.5, 3.0)	3.70 (dd, 8.5, 3.0)	3.70 (dd, 8.5, 3.0)	3.72 (dd, 8.5, 3.0)
5	4.15 (dq, 9.5, 6.5)	4.14 (dq, 9.5, 6.5)	4.15 (dq, 9.5, 6.5)	4.65 (dq, 8.5, 6.5)	4.65 (dq, 8.5, 6.5)	4.65 (dq, 8.5, 6.5)	4.73 (dq, 8.5, 6.5)
6	1.34 (d, 6.5)	1.34 (d, 6.5)	1.34 (d, 6.5)	1.50 (d, 6.5)	1.50 (d, 6.5)	1.51 (d, 6.5)	1.53 (d, 6.5)
	α-cym.	α-cym.	α-cym.	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 (*)	5.20 (*)	5.21 (dd, 4.5, 1.5)	5.21 (*)	5.22 (dd, 4.5, 1.5)
3	3.75 (*)	3.75 (*)	3.75 (q, 3.5)	3.76 (q, 3.0)	3.77 (q, 3.0)	3.77 (q, 3.0)	3.79 (q, 3.0)
4	3.85 (dd, 8.5, 3.0)	3.84 (dd, 8.5, 3.0)	3.85 (dd, 8.5, 3.0)	3.85 (dd, 8.5, 3.0)	3.86 (dd, 9.5, 3.0)	3.86 (dd, 8.5, 3.0)	3.87 (dd, 8.5, 3.0)
5	4.63 (dq, 8.5, 6.5)	4.63 (dq, 8.5, 6.5)	4.64 (dq, 8.5, 6.5)	4.53 (*)[b]	4.55 (dq, 8.5, 6.5)	4.55 (dq, 8.5, 6.5)	4.57(dq, 8.5, 6.5)
6	1.51 (d, 6.5)	1.51 (d, 6.5)	1.51 (d, 6.5)	1.51 (d, 6.5)[c]	1.52 (d, 6.5)	1.53 (d, 6.5)	1.53 (d, 6.5)
	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.	β-cym.
H-1	5.23 (dd, 9.5, 2.0)	5.23 (dd, 9.5, 2.0)	5.24 (dd, 9.5, 2.0)	5.24 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	5.25 (dd, 9.5, 2.0)	5.26 (dd, 9.5, 2.0)
3	3.88 (*)	3.88 (q, 3.0)[a]	3.88 (*)	3.89 (*)[a]	4.05 (q, 3.0)	4.06 (q, 3.0)	4.06 (q, 3.0)
4	3.46 (dd, 9.5, 3.0)	3.45 (dd, 9.5, 3.0)	3.46 (dd, 9.5, 3.0)	3.47 (dd, 9.5, 3.0)	3.49 (dd, 9.5, 3.0)	3.50 (*)	3.50 (dd, 9.5, 3.0)
5	4.19 (dq, 9.5, 6.5)	4.19 (dq, 9.5, 6.5)	4.20 (dq, 9.5, 6.5)[a]	4.19 (*)	4.18 (dq, 9.5, 6.5)	4.20 (*)	4.19 (dq, 9.5, 6.5)
6	1.36 (d, 6.5)[a]	1.37 (d, 6.5)	1.36 (d, 6.5)[b]	1.38 (d, 6.5)	1.34 (d, 6.5)	1.36 (d, 6.5)[a]	1.36 (d, 6.5)
	α-cym.	α-cym.	α-cym.	α-cym.	β-dig.	β-dig.	β-dig.
H-1	4.97 (dd, 4.5, 1.5)	4.97 (dd, 4.5, 1.5)	4.97 (*)	4.97 (dd, 4.5, 1.5)	5.24 (dd, 9.5, 2.0)	5.31 (dd, 9.5, 2.0)	5.30 (dd, 9.5, 1.5)
3	3.70 (q, 3.5)	3.70 (q, 3.5)	3.70 (q, 3.5)	3.70 (*)	4.46 (*)	4.63 (*)	4.63 (brs)
4	3.60 (*)	3.60 (*)	3.60 (*)	3.60 (*)	3.43 (dd, 9.5, 3.0)	3.47 (*)	3.47 (*)
5	4.53 (dq, 8.5, 6.5)	4.52 (dq, 8.5, 6.5)	4.53 (dq, 8.5, 6.5)	4.54 (*)[b]	4.12 (dq, 9.5, 6.5)	4.25 (dq, 9.5, 6.5)	4.25 (dq, 9.5, 6.5)
6	1.51 (d, 6.5)	1.51 (d, 6.5)	1.51 (d, 6.5)	1.52 (d, 6.5)[c]	1.37 (d, 6.5)	1.38 (d, 6.5)	1.38 (d, 6.5)

Table 2. (continued)

	9	10	11	12	13	14	15
					α-cym.	β-dig.	β-dig.
H-1	—	—	—	—	5.08 (dd, 4.5, 1.5)	5.38 (dd, 9.5, 2.0)	5.38 (dd, 9.5, 1.5)
3	—	—	—	—	3.72 (*)	4.63 (*)	4.63 (brs)
4	—	—	—	—	3.61 (*)	3.47 (*)	3.47 (*)
5	—	—	—	—	4.45 (*)	4.30 (dq, 9.5, 6.5)	4.30 (dq, 9.5, 6.5)
6	—	—	—	—	1.42 (d, 6.5)	1.40 (d, 6.5)	1.41 (d, 6.5)
						β-ole.	β-ole.
H-1	—	—	—	—	—	4.80 (dd, 9.5, 2.0)	4.80 (dd, 9.5, 2.0)
3	—	—	—	—	—		
4	—	—	—	—	—	3.43 (t, 8.5)	3.42 (*)
5	—	—	—	—	—	3.57 (*)	3.57 (dq, 9.5, 6.5)
6	—	—	—	—	—	1.49 (d, 6.5)	1.49 (d, 6.5)
OMes	3.37 (s)	3.37 (s)	3.37 (s)	3.37 (s)×3	3.37 (s)×2	3.37 (s)×2	3.32 (s)
	3.38 (s)	3.38 (s)	3.38 (s)	3.52 (s)	3.39 (s)	3.46 (s)	3.39 (s)
	3.53 (s)	3.53 (s)	3.53 (s)	3.55 (s)	3.56 (s)	3.55 (s)	3.42 (s)
	3.55 (s)	3.55 (s)	3.55 (s)		3.58 (s)	3.59 (s)	3.46 (s)
	3.61 (s)	3.61 (s)	3.60 (s)				3.58 (s)

Measured at 400 MHz in Py-d_5 solution at 35 °C. *a*—*c*): Assignments may be interchangeble in each column. *: Overlapping with other signals. **: Overlapping with H_2O signal.



	R_2	R_3	R_4	
I:	Ac	H	H	(12-*O*-acetylramanone)
II:	H	H	H	(ramamone)
III:	Ac	H	OH	(12-*O*-acetylpergularin)
IV:	H	H	OH	(pergularin)
V:	H	OH	OH	(deacylmetaplexigenin)

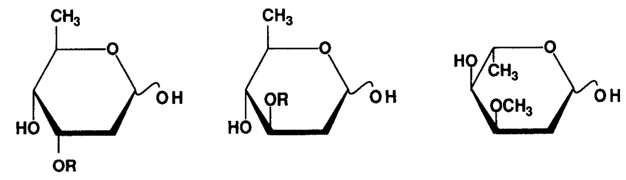
R_1

a: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-ole.
b: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-cym.
c: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ α-cym.$\xrightarrow{4\ 1}$ β-cym. $\xrightarrow{4\ 1}$ α-cym.
d: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-can. $\xrightarrow{4\ 1}$ β-ole.
e: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-ole. $\xrightarrow{4\ 1}$ β-ole.
f: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-ole. $\xrightarrow{4\ 1}$ β-ole.$\xrightarrow{4\ 1}$ α-cym.
g: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ α-cym. $\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ α-cym.
h: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ α-cym.$\xrightarrow{4\ 1}$ α-cym. $\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ α-cym.
i: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ α-cym.$\xrightarrow{4\ 1}$ α-cym. $\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-dig.$\xrightarrow{4\ 1}$ α-cym.
j: (Agly.$\xrightarrow{3\ 1}$) β-cym.$\xrightarrow{4\ 1}$ α-cym.$\xrightarrow{4\ 1}$ α-cym. $\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-dig.$\xrightarrow{4\ 1}$ β-dig.$\xrightarrow{4\ 1}$ β-ole.
k: (Agly.$\xrightarrow{3\ 1}$) β-ole.$\xrightarrow{4\ 1}$ α-cym.$\xrightarrow{4\ 1}$ α-cym. $\xrightarrow{4\ 1}$ β-cym.$\xrightarrow{4\ 1}$ β-dig.$\xrightarrow{4\ 1}$ β-dig.$\xrightarrow{4\ 1}$ β-ole.

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

1: I-a
2: III-b
3: III-c
4: III-d
5: I-e
6: III-e
7: III-f
8: III-g
9: II-g
10: IV-g
11: V-g
12: III-h
13: III-i
14: III-j
15: III-k

Chart 1



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

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

L-cymaropyranose

Chart 2

MS *m/z*: 861 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2

Compound **3**: Amorphous powder. $[\alpha]_D^{25}$ −105° (c=0.41, MeOH). FAB-MS *m/z*: 1005 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **4**: Amorphous powder. $[\alpha]_D^{25}$−33° (c=0.32, MeOH). FAB-MS *m/z*: 991 $[M+Na]^+$, 969 $[M+H]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **5**: Amorphous powder. $[\alpha]_D^{25}$ −57° (c=0.19, MeOH). FAB-MS *m/z*: 989 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **6**: Amorphous powder. $[\alpha]_D^{25}$ −35° (c=0.55, MeOH). FAB-MS *m/z*: 1005 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **7**: Amorphous powder. $[\alpha]_D^{25}$ −56° (c=0.37, MeOH). FAB-MS *m/z*: 1149 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **8**: Amorphous powder. $[\alpha]_D^{25}$ −89° (c=0.45, MeOH). FAB-MS *m/z*: 1149 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **9**: Amorphous powder. $[\alpha]_D^{25}$ −92° (c=0.35, MeOH). FAB-MS *m/z*: 1091 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **10**: Amorphous powder. $[\alpha]_D^{25}$ −77° (c=0.47, MeOH). FAB-MS *m/z*: 1107 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **11**: Amorphous powder. $[\alpha]_D^{25}$ −54° (c=0.35, MeOH). FAB-MS *m/z*: 1123 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **12**: Amorphous powder. $[\alpha]_D^{25}$ −161° (c=0.20, MeOH). FAB-MS *m/z*: 1149 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **13**: Amorphous powder. $[\alpha]_D^{25}$ −110° (c=0.31, MeOH). FAB-MS *m/z*: 1279 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **14**: Amorphous powder. $[\alpha]_D^{25}$ −83° (c=0.27, MeOH). FAB-MS *m/z*: 1409 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

Compound **15**: Amorphous powder. $[\alpha]_D^{25}$ −105° (c=0.19, MeOH). FAB-MS *m/z*: 1409 $[M+Na]^+$. ^{13}C- and ^{1}H-NMR: Tables 1 and 2.

As all compounds could be acquired only in a small amount, their elemental analyses were not performed.

Acid Hydrolysis of a Mixture of Pregnane Glycosides A mixture of pregnane glycosides (*ca.* 500 mg) was heated at 60 °C for 5.5 h with dioxane (8 ml) and 0.2 N H_2SO_4 (2 ml) to obtain the aglycones and sugars. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness. Purification of the residue by HPLC (YMC-ODS, 52.5% MeOH in water or 35% MeOH in water) afforded 12-*O*-acetylramanone [I (R_1=H) 6 mg], ramanone [II (R_1=H) 3 mg], 12-*O*-acetylpergularin [III (R_1=H) 29 mg] and pergularin [IV (R_1=H) 4 mg].

I: $[\alpha]_D^{26}$ −89° (c=0.41, MeOH). ^{13}C-NMR (Py-d_5 at 35 °C): δ 209.6 (C-20), 169.9 (CH$_3$C*O), 140.8 (C-5), 121.4 (C-6), 86.7 (C-14), 72.9 (C-12), 71.1 (C-3), 60.4 (C-17), 54.6 (C-13), 43.5 (C-9), 43.3 (C-4), 37.6, 37.3, 37.2 (C-1, 8, 10), 32.5 (C-2), 31.9, 31.7 (C-15, 21), 27.2, 26.9 (C-7, 11), 21.1 (C-16), 20.8 (C*H$_3$CO), 19.6 (C-19), 14.4 (C-18). ^{1}H-NMR (Py-d_5 at 35 °C): δ 5.43 (br s, H-6), 4.96 (dd, 11.5, 4.5, H-12), 3.82 (m, H-3), 3.57 (t, 8.5, H-17), 2.28 (s, H-21), 2.08 (td, 11.5, 4.5, H-8), 2.04 (s, CH*$_3$CO), 1.64 (s, H-18), 1.04 (s, H-19).

II: $[\alpha]_D^{23}$ −56° (c=0.33, MeOH). ^{13}C-NMR (Py-d_5 at 35 °C): δ 210.3 (C-20), 140.9 (C-5), 121.7 (C-6), 86.8 (C-14), 71.2 (C-3), 68.5 (C-12), 61.3 (C-17), 56.9 (C-13), 44.0 (C-9), 43.4 (C-4), 37.7 (C-1), 37.3, 37.2 (C-8, 10), 32.6 (C-2), 32.0, 31.9 (C-15, 21), 31.5 (C-11), 27.4 (C-7), 21.6 (C-16), 19.8 (C-19), 13.5 (C-18). ^{1}H-NMR (Py-d_5 at 35 °C): δ 5.48 (br s, H-6), 3.90 (dd, 11.5, 4.5, H-12), 3.84 (m, H-3), 3.56 (t, 9.0, H-17), 2.45 (s, H-21), 2.09 (td, 12.5, 4.5, H-8), 1.73 (s, H-18), 1.09 (s, H-19).

III: $[\alpha]_D^{20}$ −63° (c=1.9, MeOH). ^{13}C-NMR (Py-d_5 at 35 °C): δ 209.6 (C-20), 169.8 (CH$_3$C*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$_3$CO), 19.6 (C-19), 8.6 (C-18). ^{1}H-NMR (Py-d_5 at 35 °C): δ 5.42 (br s, H-6), 4.87 (dd, 11.5, 4.5, H-12), 3.81 (m, H-3), 2.49 (s, H-21), 2.07 (s, CH*$_3$CO), 1.65 (s, H-18), 1.04 (s, H-19).

IV: mp 226—240 °C (acetone). $[\alpha]_D^{26}$ −21° (c=0.68, MeOH). ^{13}C-NMR (Py-d_5 at 35 °C): δ 209.1 (C-20), 140.9 (C-5), 121.5 (C-6), 92.4 (C-17), 88.9 (C-14), 71.2 (C-3), 68.3 (C-12), 59.1 (C-13), 43.8 (C-9), 43.4 (C-4), 37.7 (C-1), 37.2, 37.1 (C-8, 10), 32.6, 32.5 (C-2, 16), 31.8, 31.7 (C-11, 15), 27.7 (C-21), 26.8 (C-7), 19.8 (C-19), 7.6 (C-18). ^{1}H-NMR (Py-d_5 at 35 °C): δ 5.47 (br s, H-6), 3.84 (m, H-3), 3.83 (overlapped, H-12), 2.65 (s, H-21), 1.76 (s, H-18), 1.09 (s, H-19).

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), digitoxose (7 mg) amd canarose (7 mg).

As to absolute configuration of each monosaccharide, oleandrose and canarose were believed to have D-form based on their optical rotation values.

D-Oleandrose: $[\alpha]_D^{24}$ −9.6° (c=1.2, 24 h after dissolution in H_2O).

D-Canarose: $[\alpha]_D^{24}$ +22° (c=0.69, 24 h after dissolution in H_2O).

Acid Hydrolysis of Compounds 1—15 A solution of each compound (*ca.* 0.5 mg) in dioxane (4 drops) and 0.2 N H_2SO_4 (1 drop) was heated at 60 °C for 1 h. After hydrolysis, this solution was passed through an Amberlite IRA-60E column and concentrated to dryness. The residues from **1**—**15** were analyzed by HPLC to identify the aglycone with authentic samples [conditions:column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min, 35% MeOH in water; t_R (min), deacylmetaplexigenin 13.2, ramanone 21.6, pergularin 25.0, 52.5% MeOH in water; t_R (min), 12-*O*-acetylramanone 12.0, 12-*O*-acetylpergularin 20.0].

Subsequently, for sugar analysis, the remaining residue of acid hydrolysis was reduced with $NaBH_4$ (*ca.*1 mg) for 1 h at room temperature. 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 temperature 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 [Conditions: column, Supelco SP-2380^{TM} capillary column 0.25 mm×30 m, column temperature 200 °C, carrier gas N_2, t_R (min); cymaritol acetate 7.3, oleandritol acetate 8.3, digitoxitol acetate 10.6, canaritol acetate 11.9].

Acknowlegments We thank the staff of the Central Analytical Laboratory of this university for measurements of MS.

References and Notes

1) Warashina T., Noro T., *Phytochemistry*, (accepted).
2) Mitsuhashi H., Nomura T., *Chem. Pharm. Bull.*, **13**, 1332—1340 (1965).
3) Mitsuhashi H., Nomura T., Hirano M., *Chem. Pharm. Bull.*, **14**, 717—726 (1966).
4) Mitsuhashi H., Hayashi K., *Shoyakugaku Zasshi*, **39**, 1—27 (1985).
5) Tsukamoto S., Hayashi K., Kaneko K., Mitsuhashi H., *Chem. Pharm. Bull.*, **34**, 3130—3134 (1986).
6) Tsukamoto S., Hayashi K., Kaneko K., Mitsuhashi H., *J. Chem. Soc., Perkin Trans. 1*, **1988**, 787—790.
7) Wada K., Hayashi K., Mitsuhashi H., Bando H., *Chem. Pharm. Bull.*, **27**, 2252—2254 (1979).
8) Wada K., Hayashi K., Mitsuhashi H., Bando H., *Chem. Pharm. Bull.*, **30**, 3500—3504 (1982).
9) Vleggaar R., van Heerden F. R., Anderson L. A. P., Erasmus G. L., *J. Chem. Soc., Perkin Trans. 1*, **1993**, 483—487.
10) Abe F., Yamauchi T., *Chem. Pharm. Bull.*, **36**, 621—626 (1988).
11) Shibuya H., Zhang R., Park J. D., Baek N. I., Takeda Y., Yoshikawa M., Kitagawa I., *Chem. Pharm. Bull.*, **40**, 2647—2653 (1992).
12) Tsukamoto S., Hayashi K., Mitsuhashi H., *Chem. Pharm. Bull.*, **33**, 2294—2304 (1985).
13) Warashina T., Noro T., *Chem. Pharm. Bull.*, **43**, 1734—1737 (1995).
14) Zielinski J., Jereczek E., Sowinski P., Falkowski L., Rudowski A., Borowski E., *J. Antibiotics.*, **32**, 565—568 (1979).
15) Nakagawa T., Hayashi K., Wada K., Mitsuhashi T., *Tetrahedron*, **39**, 607—612 (1983).
16) Miyamoto M., Kawamatsu Y., Shinohara M., Nakadaira Y., Nakanishi K., *Tetrahedron*, **22**, 2785—2799 (1966).