Chem. Pharm. Bull. **43**(10) 1734—1737 (1995) Vol. 43, No. 10

Steroidal Glycosides from Roots of *Cynanchum caudatum* M. II

Tsutomu WARASHINA* and Tadataka NORO

Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422, Japan.

Received April 12, 1995; accepted June 11, 1995

The roots of *Cynanchum caudatum* M. (Asclepiadaceae) afforded eleven new pregnane glycosides which had cynanchogenin, caudatin, 12-*O*-benzoyllineolon and 12-*O*-benzoyldeacylmetaplexigenin as the aglycone moiety and 2,6-dideoxy-3-*O*-methylhexopyranoses as component sugars. The structures of these compounds were elucidated by spectroscopic methods and from chemical evidence.

Key words *Cynanchum caudatum* M.; Asclepiadaceae; esterified pregnane glycoside; 2,6-dideoxy-3-*O*-methylhexopyranose

Cynanchum caudatum M. (Asclepiadaceae) has been reported by Mitsuhashi and his colleagues[1,2] to contain significant quantities of esterified pregnane glycosides in its roots. We also reported 17 esterified pregnane glycosides in a previous paper,[3] and in this paper the structural elucidation of eleven novel pregnane glycosides from its roots was mentioned as part of our research on steroidal glycosides of the Asclepiadaceous plants.

The extraction and isolation of the roots of *C. caudatum* M. were described in our previous report.[3] A crude pregnane glycoside fraction afforded eleven new pregnane glycosides (compounds **1**—**10**, **11**).

Compound **1** was suggested to have the molecular formula $C_{42}H_{66}O_{12}$ based on FAB-MS. In the ^{13}C- and ^{1}H-NMR spectra, **1** showed two anomeric carbon and proton signals at δ 96.1, 99.4 and δ 4.85 (dd, J=9.5, 2.0 Hz), 4.67 (dd, J=9.5, 2.0 Hz), and the carbon signals assignable to the aglycone moiety were similar to those of cynanchogenin[4] within the range of glycosylation shifts at C-3 (+6.1 ppm), C-2 (−1.9 ppm) and C-4 (−3.1 ppm). From the above evidence, **1** was considered to be cynanchogenin 3-*O*-diglycoside. On acid hydrolysis of **1**, cynanchogenin and cymarose were obtained as the component aglycone and sugar moieties (see Experimental), and these sugars were identified as β-D-cymaropyranose based on the ^{13}C-NMR spectral data and the (H, H) coupling constants of each anomeric proton signal (J=9.5, 2.0 Hz) in the ^{1}H-NMR spectrum.[5] In regard to the sugar sequence, the first β-D-cymaropyranose was attached to C-3 of cynanchogenin, because glycosylation shifts were observed around the C-3 position in the ^{13}C-NMR spectrum. In the difference nuclear Overhauser effect (NOE) spectrum, irradiation at the anomeric proton signal of the second β-D-cymaropyranose brought about an NOE to the H-4 signal of the first β-D-cymaropyranose. Therefore, **1** was identified as cynanchogenin 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **2** was suggested to have caudatin[4] as the aglycone and the same sugar linkage as that of **1** based on the ^{1}H- and ^{13}C-NMR spectra. Thus, the structure of **2** was determined to be caudatin 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **3** showed three anomeric carbon and proton signals at δ 96.1, 101.4, 96.9 and δ 4.85 (dd, J=9.5, 2.0 Hz), 4.47 (dd, J=9.5, 2.0 Hz), 4.87 (dd, J=9.5, 2.0 Hz), in addition to the signals assignable to cynanchogenin in the ^{13}C- and ^{1}H-NMR spectra. From the above evidence, **3** was considered to be cynanchogenin 3-*O*-triglycoside. On acid hydrolysis of **3**, cynanchogenin, cymarose and oleandrose were obtained as the component aglycone and sugars, and analysis with GLC of the sugar part showed that the relative ratio of these monosaccharides was two cymaroses to one oleandrose. Thus, **3** consisted of cynanchogenin, two cymaroses and one oleandrose. The magnitude of the (H, H) coupling constants indicated the presence of one equatorial (J=4.5, 1.5 Hz) and two axial anomeric protons (J=9.5, 2.0 Hz). The correlation spectroscopy (COSY) experiment established that the equatorial anomeric proton (δ 4.87) formed part of the connectivity network of cymaropyranose which, on the basis of the (H, H) coupling constants of the anomeric proton (J=4.5, 1.5 Hz), was involved in an α-glycosidic link, and the axial anomeric ones (δ 4.85, 4.47) formed part of cymaropyranose and oleandropyranose, respectively, which were involved in β-glycosidic linkages. Moreover, the configurations of two cymaropyranoses were determinded based on the ^{13}C-NMR spectral data. The chemical shift values for C-2 of two cymaropyranoses in **3** showed that α-linked cymaropyranose (δ 31.0) had the L-configuration and the β-linked cymaropyranose (δ 36.1) had the D-configuration.[5] The β-linked oleandropyranose was determined to have the D-configuration, because acid hydrolysis of a mixture of pregnane glycoside gave only D-oleandrose in addition to a D,L-cymarose mixture (see Experimental). Subsequently, to determine the sugar sequence, the difference NOE spectra were examined. Irradiation at the anomeric proton signal of β-D-cymaropyranose at δ 4.85 caused enhancement of the signal intensity at δ 3.55 (H-3 of aglycone). Accordingly, β-D-cymaropyranose was attached to C-3 of the aglycone moiety. Similarly, NOEs were observed between δ 4.47 (H-1 of β-D-oleandropyranose)/3.23 (H-4 of β-D-cymaropyranose) and δ 4.87 (H-1 of α-L-cymaropyranose)/3.12 (H-4 of β-D-oleandropyranose). Based on the above evidence, **3** was identified as cynanchogenin 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **4** was determined to be caudatin 3-*O*-triglycoside having the same sugar sequence as **3**, since the signals assignable to the sugar moiety in the ^{1}H- and ^{13}C-NMR spectra were in good agreement with those of **3**.

Compound **6** showed a $[M+Na]^+$ ion peak at m/z 1089, which suggested the molecular formula $C_{56}H_{90}O_{19}$.

* To whom correspondence should be addressed.

Acid hydrolysis of **6**, caudatin, cymarose and oleandrose were obtained, and analysis with GLC of the sugar part suggested that the relative ratio of these monosaccharides was three cymaroses to one oleandrose. Thus, **6** consisted of caudatin, three cymaroses and one oleandrose. Moreover, the ^{1}H- and ^{13}C-NMR spectral data and acid hydrolysis showed that these sugars were two β-D-cymaropyranoses, one α-L-cymaropyranose and one β-D-oleandropyranose. The sugar sequence was determined based on the consequences of the difference NOE spectra irradiating from each anomeric proton signal. Therefore, the structure of **6** was concluded to be as shown.

Compounds **5**, **7** and **8** were esterified pregnane 3-*O*-tetraglycosides which had the same sugar linkage as that of **6**, because of the consistency of signals assignable to the sugar moiety in the ^{1}H- and ^{13}C-NMR spectra. The aglycone moieties of **5**, **7** and **8** were identified as cynanchogenin, 12-*O*-benzoyllineolon[6] and 12-*O*-benzoyldeacymetaplexigenin,[6] respectively, on acid hydrolysis. Thus, the structures of these compounds were determined as shown.

Compound **9** was also deduced to be caudatin 3-*O*-tetraglycoside based on acid hydrolysis and the ^{1}H- and ^{13}C-NMR spectra. Moreover, the ^{1}H and ^{13}C signals assignable to the sugar moiety were very similar to those of cynanchogenin 3-*O*-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.[3] Therefore, the structure of **9** was elucidated as shown.

Compounds **10** and **11** were suggested to be cynanchogenin 3-*O*-pentaglycoside and caudatin 3-*O*-pentaglycoside, which have the same sugar linkage based on the ^{1}H- and ^{13}C-NMR spectra. Acid hydrolysis, followed by analysis with GLC of **10**, showed that the sugar sequence was composed of three cymaroses and two oleandroses. Moreover, three cymaroses were identified as two β-D- and one α-L-cymaropyranoses by the ^{1}H- and ^{13}C-NMR spectral data, and two oleandroses were two β-D-oleandropyranoses by acid hydrolysis. The sugar sequence was determined as shown based on the consequences of the difference NOE spectra irradiating from each anomeric proton signal.

Table 1. ^{13}C-NMR Spectral Data of Aglycone and Ester Moieties

	I	II	III	IV
Aglycone				
1	38.8	38.8	38.9	38.8
2	28.9	29.0	28.9	29.0
3	77.9	77.9	77.9	77.8
4	38.8	38.8	38.9	38.8
5	141.3	140.8	141.4	140.7
6	117.4	117.6	117.3	117.6
7	34.5	34.3	34.5	34.3
8	74.8	74.4	74.8	74.3
9	44.1	43.8	44.1	43.7
10	37.3	37.2	37.3	37.2
11	24.4	24.3	24.3	24.2
12	71.0	71.6	72.4	73.2
13	55.1	58.0[a]	55.5	58.4
14	86.7	88.0	86.6	88.0
15	33.4	33.1	33.4	33.3
16	21.3	31.9	21.6	32.0
17	60.0	91.5	59.7	91.5
18	15.0	9.4	15.0	9.5
19	18.9	18.6	18.9	18.6
20	209.2	208.7	209.5	209.3
21	31.8	27.1	31.9	27.3
Ester				
1′	166.0	166.7	165.2	165.3
2′	113.1	113.1	130.3	130.1
3′	165.7	165.9	128.3	128.4
4′	38.1	38.2	129.5	129.5
5′	20.9[b]	20.9[b]	132.9	133.1
6′	20.8[b]	20.8[b]	—	—
7′	16.5	16.5	—	—

Measured at 100.40 MHz in $CDCl_3$ solution at 35 °C. *a*) Assignments may be interchanged between Tables 1 and 2. *b*) Assignments may be interchanged in each column.

Experimental

General Procedure Instrumental analysis was carried out as described previously.[3]

Isolation The procedure of extraction and isolation of pregnane glycosides was described in our previous report.[3] Isolated pregnane glycoside fraction afforded compounds **1** (11 mg), **2** (6 mg), **3** (8 mg), **4** (6 mg), **5** (5 mg), **6** (36 mg), **7** (6 mg), **8** (4 mg), **9** (30 mg), **10** (14 mg), **11** (9 mg).

Compound **1**: Amorphous powder. $[\alpha]_D^{20}$ −4.5° (c = 1.1, MeOH). *Anal.*

Table 2. ^{13}C-NMR Spectral Data of Sugar Moiety

	a	b	c	d	e
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
C-1	96.1	96.1	96.1	96.1	96.1
2	35.7	36.1	35.7[b]	35.6	35.6[b]
3	77.1	77.2	77.1	77.1	77.1
4	82.5	82.6	82.5	82.5	82.5[c]
5	68.5	68.4	68.4[c]	68.3[b]	68.3[d]
	D-cym.	D-ole.	D-cym.	D-cym.	D-cym.
1	99.4	101.4	99.7	99.7	99.7
2	33.8	36.1	35.6[b]	35.6	35.7[b]
3	77.4	78.8	77.1	77.1	77.1
4	72.5	81.5	82.5	82.5	82.6[c]
5	70.7	71.7	68.6[c]	68.5[b]	68.5[d]
		L-cym.	D-ole.	D-ole.	D-ole.
1	—	96.9	101.4	101.4	101.3
2	—	31.0	36.1[b]	36.4	36.1[b]
3	—	75.0	78.8	78.9	79.3
4	—	72.2	81.4	82.2	82.3[c]
5	—	65.2	71.7	71.1	71.0
			L-cym.	D-cym.	D-cym.
1	—	—	96.9	98.2	100.2
2	—	—	31.0	33.9	36.4[b]
3	—	—	75.1	77.6	78.9
4	—	—	72.2	72.5	81.5
5	—	—	65.3	71.1	71.8
					L-cym.
1	—	—	—	—	96.9
2	—	—	—	—	31.0
3	—	—	—	—	75.0
4	—	—	—	—	72.4
5	—	—	—	—	65.2
6s	18.2	17.7	17.7	18.2×2	17.7
	18.3	18.2	18.2×2	18.3	18.2×2
		18.4	18.4	18.4	18.4×2
OMes	57.2	56.2×2	56.2	56.5	56.2×2
	58.1[a]	58.4	56.3	57.1	56.8
			58.0	58.1[a]	58.0
			58.4	58.3[a]	58.2[a]

Measured at 100.40 MHz in $CDCl_3$ solution at 35°C. *a*) Assignments may be interchanged between Tables 1 and 2. *b*—*d*) Assignments may be interchanged in each column.

Table 3. ^{1}H-NMR Spectral Data of the Suger Moiety

	a	b	c	d	e
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
H-1	4.85 (dd, 9.5, 2.0)	4.85 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 2.0)	4.84 (dd, 9.5, 1.5)	4.84 (dd, 9.5, 1.5)
3	3.81 (q, 3.0)	3.79 (q, 3.0)	3.81 (q, 3.0)	3.80 (q, 3.0)	3.80 (q, 3.0)
4	3.22 (dd, 9.5, 3.0)	3.23 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.86 (dq, 9.5, 6.5)	3.87 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)	3.84 (dq, 9.5, 6.5)
6	1.22 (d, 6.5)	1.23 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
	D-cym.	D-ole.	D-cym.	D-cym.	D-cym.
1	4.67 (dd, 9.5, 2.0)	4.47 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 2.0)	4.75 (dd, 9.5, 1.5)	4.75 (dd, 9.5, 1.5)
3	3.62 (q, 3.0)	3.27 [a]	3.78 (q, 3.0)	3.78 (q, 3.0)	3.78 (q, 3.0)
4	3.19 (dd, 9.5, 3.0)	3.12 (t, 9.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)	3.21 (dd, 9.5, 3.0)
5	3.56 (dq, 9.5, 6.5)	3.27 (dq, 9.0, 6.5)	3.87 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)	3.86 (dq, 9.5, 6.5)
6	1.27 (d, 6.5)	1.27 (d, 6.5)	1.22 (d, 6.5)	1.21 (d, 6.5)	1.21 (d, 6.5)
		L-cym.	D-ole.	D-ole.	D-ole.
1	—	4.87 (dd, 4.5, 1.5)	4.46 (dd, 9.5, 2.0)	4.45 (dd, 9.5, 1.5)	4.45 (dd, 9.5, 2.0)
3	—	3.58 (q, 3.5)	3.27 [a]	3.36 [a]	
4	—	3.27 [a]	3.12 (t, 9.0)	3.16 (t, 9.0)	3.15 (t, 9.0)
5	—	4.09 (dq, 9.5, 6.5)	3.32 (dq, 9.0, 6.5)	3.29 (dq, 9.5, 6.5)	3.30 (dq, 9.0, 6.5)
6	—	1.25 (d, 6.5)	1.27 (d, 6.5)	1.28 (d, 6.5)	1.28 (d, 6.5)
			L-cym.	D-cym.	D-ole.
1	—	—	4.87 (dd, 4.5, 1.5)	4.88 (dd, 9.5, 1.5)	4.68 (dd, 9.5, 2.0)
3	—	—	3.58 (q, 3.5)	3.62 (q, 3.0)	
4	—	—	3.27 [a]	3.21 (dd, 9.5, 3.0)	3.12 (t, 9.0)
5	—	—	4.08 (dq, 9.5, 6.5)	3.60 (dq, 9.5, 6.5)	3.30 (dq, 9.0, 6.5)
6	—	—	1.25 (d, 6.5)	1.29 (d, 6.5)	1.29 (d, 6.5)
					L-cym.
1	—	—	—	—	4.88 (dd, 4.5, 1.5)
3	—	—	—	—	3.58 (*q*, 3.5)
4	—	—	—	—	3.28 [a]
5	—	—	—	—	4.08 (dq, 9.5, 6.5)
6	—	—	—	—	1.24 (d, 6.5)
OMes	3.43 (s)	3.36 (s)	3.35 (s)	3.40 (s)	3.37 (s)
	3.45 (s)	3.38 (s)	3.38 (s)	3.43 (s)	3.38 (s)
		3.46 (s)	3.44 (s)	3.44 (s)	3.40 (s)
			3.45 (s)	3.45 (s)	3.44 (s)
					3.45 (s)

Measured at 400 MHz in $CDCl_3$ solution at 35 °C. *a*) Overlapping with other signals.

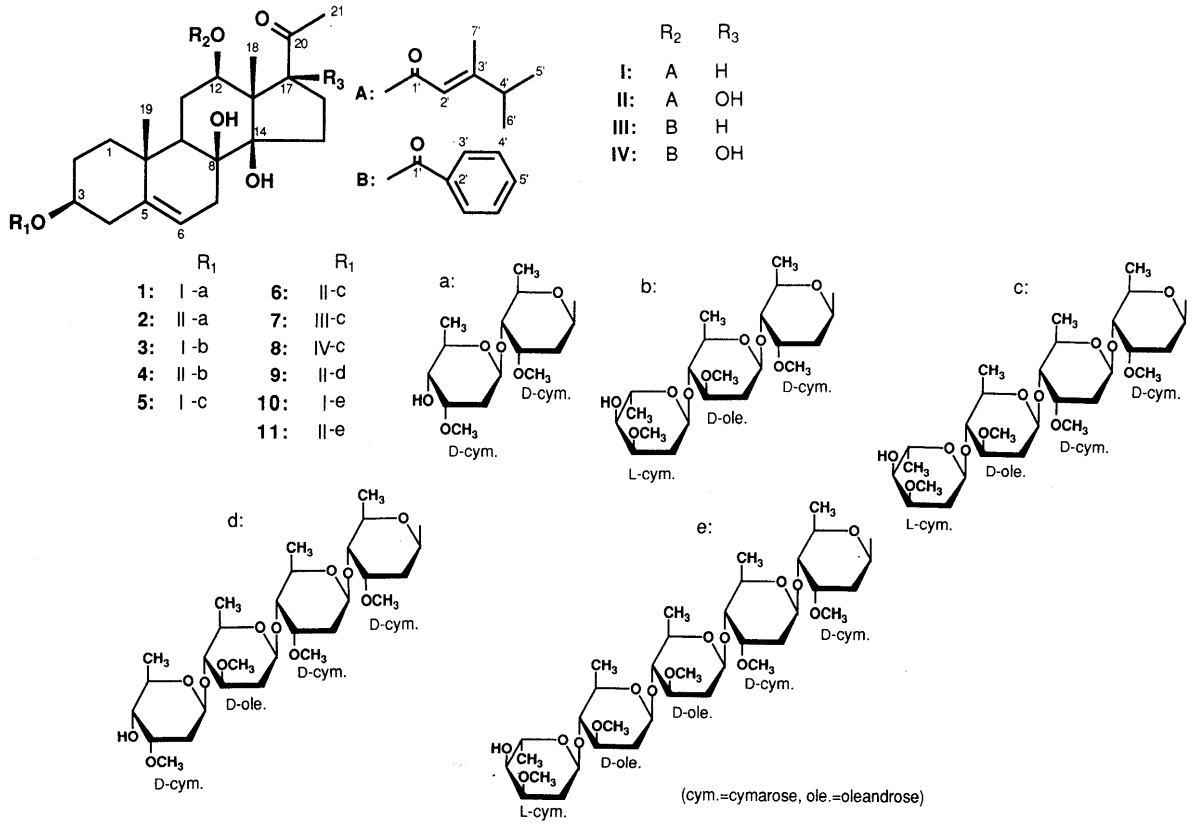

Chart 1

Calcd for $C_{42}H_{66}O_{12}\cdot 3/4H_2O$: C, 64.97; H, 8.76. Found: C, 65.04; H, 8.65. FAB-MS *m/z*: 763 $[M+H]^+$, 785 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **2**: Amorphous powder. $[\alpha]_D^{20}$ +11.9° (*c*=0.56, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{13}\cdot 3/4H_2O$:C, 63.66; H, 8.58. Found: C, 63.69; H, 8.69. FAB-MS *m/z*: 779 $[M+H]^+$, 801 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **3**: Amorphous powder. $[\alpha]_D^{20}$ −48.2° (*c*=0.77, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{15}\cdot 3/2H_2O$: C, 63.00; H, 8.74. Found: C, 62.88; H, 8.72. FAB-MS *m/z*: 907 $[M+H]^+$, 929 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **4**: Amorphous powder. $[\alpha]_D^{20}$ −47.4° (*c*=0.57, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{16}\cdot H_2O$: C, 62.53; H, 8.57. Found: C, 62.83; H, 8.69. FAB-MS *m/z*: 945 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **5**: Amorphous powder. $[\alpha]_D^{20}$ −26.2° (*c*=0.47, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{18}\cdot H_2O$: C, 62.90; H, 8.67. Found: C, 62.90; H, 8.67. FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **6**: Amorphous powder. $[\alpha]_D^{20}$ −28.1° (*c*=1.8, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{19}\cdot 3/2H_2O$: C, 61.46; H, 8.57. Found: C, 61.41; H, 8.47. FAB-MS *m/z*: 1089 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **7**: Amorphous powder. $[\alpha]_D^{20}$ −26.2° (*c*=0.54, MeOH). *Anal.* Calcd for $C_{56}H_{84}O_{18}\cdot H_2O$: C, 63.26; H, 8.15. Found: C, 63.02; H, 8.35. UV λ_{max}^{MeOH} nm (log ε): 230 (4.14), 275 (3.23), 280 (3.18). FAB-MS *m/z*: 1067 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **8**: Amorphous powder. $[\alpha]_D^{20}$ −32.2° (*c*=0.44, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 (4.15), 273 (3.14), 281 (3.07). FAB-MS *m/z*: 1083 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **9**: Amorphous powder. $[\alpha]_D^{20}$ +6.7° (*c*=3.0, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{19}\cdot H_2O$: C, 61.97; H, 8.54. Found: C, 61.89; H, 8.55. FAB-MS *m/z*: 1089 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **10**: Amorphous powder. $[\alpha]_D^{20}$ −46.9° (*c*=1.4, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{21}\cdot 1/2H_2O$: C, 62.82; H, 8.62. Found: C, 63.08; H, 8.69. FAB-MS *m/z*: 1217 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Compound **11**: Amorphous powder. $[\alpha]_D^{20}$ −19.1° (*c*=0.90, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{22}\cdot H_2O$: C, 61.55; H, 8.53. Found: C, 61.67; H, 8.67. FAB-MS *m/z*: 1233 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—3.

Acid Hydrolysis of a Mixture of Pregnane Glycoside A mixture (*ca.* 500 mg) was heated at 60 °C for 2 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 a D,L-cymarose mixture and D-oleandrose 13 mg, $[\alpha]_D^{24}$ −11.9° (*c*=1.34, 24 h after dissolution in H_2O).[7]

Acid hydrolysis of Compounds 1—11 Compounds **1**—**11** (*ca.* 0.5 mg) dissolved in dioxane (4 drops) and 0.2 N H_2SO_4 (1 drop) were heated at 60 °C for 90 min. After hydrolysis, the reaction mixtures were passed through an Amberlite IRA-60E column and the eluates were concentrated to dryness. The residue was analyzed by HPLC to identify the aglycone by comparison with the authentic samples [conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 75% MeOH in H_2O; t_R (min), cynanchogenin 8.8, caudatin 9.6, 65% MeOH in H_2O; t_R (min), 12-*O*-benzoyllineolon 9.0, 12-*O*-benzoyldeacylmetaplexigenin 10.4]. 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 (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From each glycoside, cymaritol acetate and oleandritol acetate were detected by GLC [condition: column, Supelco SP-2380 capillary column 0.25 mm × 30 m; column temperature 200 °C; carrier gas, N_2; t_R (min), cymaritol acetate 5.8, oleandritol acetate 6.5]. The relative ratio of each monosaccharide was determined based on the peak area.

Acknowledgement We thank the staff of the Central Analytical Laboratory of this university for elemental analysis and measurement of MS.

References and Notes

1) Wada K., Hayashi K., Mitsuhashi H., Bando H., *Chem. Pharm. Bull.*, **27**, 2252—2254 (1979).
2) Wada K., Hayashi K., Mitsuhashi H., Bando H., *Chem. Pharm. Bull.*, **30**, 3500—3504 (1982).
3) Warashina T., Noro T., *Chem. Pharm. Bull.*, **43**, 977—982 (1995).
4) Bando H., Amiya T., Sato E., Mitsuhashi H., *Chem. Pharm. Bull.*, **28**, 2258—2260 (1980).
5) Vleggaar R., van Heerden F. R., Anderson L. A. P., Erasmus G. L., *J. Chem. Soc., Perkin Trans. 1*, **1993**, 483—487.
6) Kitagawa I., Zhang R., Park J. D., Beak N. I., Takeda Y., Yashikawa M., Shibuya H., *Chem. Pharm. Bull.*, **40**, 2007—2013 (1992).
7) Nakagawa T., Hayashi K., Wada K., Mitsuhashi H., *Phytochemistry*, **39**, 607—612 (1983).