Steroidal Glycosides from the Root of *Cynanchum caudatum* M.

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Novel steroidal glycosides 2—4, 6—11, 13—19 and 20 were isolated from the roots of *Cynanchum caudatum* M. (Asclepiadaceae). The structures of these steroidal glycosides were determined on the basis of spectral and chemical evidence. All of these glycosides contain 2,6-dideoxyhexopyranoses as component sugars, and their structures were elucidated as esterified pregnane-type.

Key words *Cynanchum caudatum*; Asclepiadaceae; esterified pregnane glycoside; 2,6-dideoxyhexopyranose

Cynanchum caudatum M. (Asclepiadaceae) is said to be a toxic plant dating from ancient times, and is reported by Mitsuhashi and his colleagues[1,2] to contain significant quantities of esterified pregnane glycosides in its root. We decided to investigate this and other esterified pregnane glycosides from the root of this plant as part of our reseach on steroidal glycosides of the Asclepiadaceous plants.

The methanol extract of the root of *C. caudatum* M. was suspended in water. The suspension was then extracted with Et_2O and partitioned into an ether soluble fraction, a water soluble fraction and an ether–water insoluble deposit. The ether soluble fraction and ether–water insoluble deposit were combined and chromatographed on a silica gel column to give a steroidal fraction from which seventeen new pregnane glycosides (compounds **2—4**, **6—11**, **13—19** and **20**) were obtained in addition to cynanchoside C_2 (**1**),[2] calotroposides G (**5**) and E (**12**).[3]

Compound **2** was suggested to have the molecular formula $C_{49}H_{78}O_{16}$ based on FAB-MS. In the ^{13}C- and ^1H-NMR spectra, **2** showed three anomeric carbon and proton signals at δ 96.1, 99.7, 101.4 and δ 4.85 (dd, $J=9.5$, 2.0 Hz), 4.76 (dd, $J=9.5$, 2.0 Hz), 4.50 (dd, $J=9.5$, 2.0 Hz), and the carbon signals assignable to the aglycone moiety were similar to those of caudatin[1] within the range of glycosilation shifts at C-3 (+5.9 ppm), C-2 (−2.0 ppm) and C-4 (−3.2 ppm). Therefore, **2** was considered to be caudatin 3-*O*-trioside. On acid hydrolysis of **2**, caudatin, cymarose and oleandrose were obtained as the component aglycone and sugars, and analysis with GLC showed that the relative ratio of these monosaccharides was two cymaroses to one oleandrose (see Experimental). Thus, **2** consisted of caudatin, two cymaroses and one oleandrose. Since the signals to the sugar moiety in the ^1H- and ^{13}C-NMR spectra were consistent with those of cynanchoside C_2 (**1**) and calotroposide G (**5**), the sugar linkage was deduced to be 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside, and this sequence was confirmed based on the difference nuclear Overhauser effect (NOE) spectra irradiating from each anomeric proton signal. Accordingly, the structure of **2** was determined as shown in Chart 1.

Compounds **3**, **4**, **6** and **7** were also pregnane 3-*O*-triosides which had the same sugar linkage as that of **1**, **2** and **5**, because of the consistence of signals. Each aglycone moiety of **3**, **4**, **6** and **7** was determined to be ikemagenin,[4] penupogenin,[1] 12-*O*-benzoyl-deacylmetaplexigenin[5] and 12-*O*-benzoyl-sarcostin,[6] respectively, on methanolysis, acid and alkaline hydrolysis (see Experimental). Consequently, the structures of these compounds were determined as shown in Chart 1.

Compound **8** showed the $[M+H]^+$ ion peak at m/z 907, which suggested the molecular formula, $C_{49}H_{78}O_{15}$. On acid hydrolysis of **8**, cynanchogenin, D-cymarose and D-oleandrose were given, and analysis with GLC revealed that the component sugars were one cymarose and two oleandroses. To determine the sugar sequence, the difference NOE spectra were examined. The irradiation at the anomeric proton signal of D-cymaropyranose at δ 4.84 (dd, $J=9.5$, 2.0 Hz) caused enhancement of the signal intensity at δ 3.55 (m, H-3 of aglycone). Accordingly, the D-cymaropyranose was attached to the C-3 of the aglycone moiety. Similarly, NOEs were observed between δ 4.45 (dd, $J=9.5$, 2.0 Hz, H-1 of the first oleandropyranose)/3.22 (dd, $J=9.5$, 3.0 Hz, H-4 of cymaropyranose) and δ 4.72 (dd, $J=9.5$, 2.0 Hz, H-1 of the second oleandropyranose)/3.17 (t, $J=9.0$ Hz, H-4 of the first oleandropyranose). Based on the above evidence, **8** was identified as cynanchogenin 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **9** was determined to be caudatin 3-*O*-trioside having the same sugar sequence as **8**, since the signals assignable to the sugar moiety in the ^1H- and ^{13}C-NMR spectra were in good agreement with those of **8**.

Compounds **10** and **11** were hydrolyzed to cynanchogenin and caudatin, respectively, and cymarose and oleandrose whose relative ratio was determined to be one to one by GLC analysis. The ^1H- and ^{13}C-NMR signals to the sugar moieties corresponded to those of calotroposide E (**12**),[3] thus, the sugar sequences were the same as that of **12**, and the structures of **10** and **11** were elucidated as shown in Chart 1.

Compound **13** showed the same molecular formula, $C_{56}H_{90}O_{18}$ as that of **10**, and afforded cynanchogenin as the aglycone, and three D-cymaroses and one D-oleandrose as the sugar moiety on acid hydrolysis. In the difference NOE spectra, irradiation at the anomeric proton of the first cymaropyranose [δ 4.84 (dd, $J=9.5$, 1.5 Hz)] brought about an NOE to H-3 of aglycone [δ 3.55 (m)]. That suggested D-cymaropyranose was located at the 3-hydroxy group of cynanchogenin. Similarly, correlation between δ 4.75 (dd, $J=9.5$, 1.5 Hz, H-1 of the

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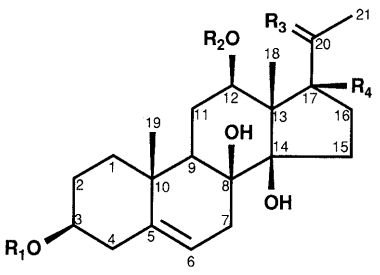
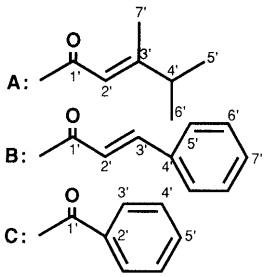




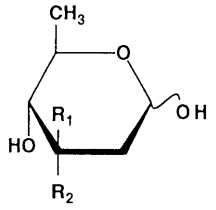
	R_2	R_3	R_4
I:	A	O	H
II:	A	O	OH
III:	B	O	H
IV:	B	β-OH, α-H	OH
V:	C	O	H
VI:	C	O	OH
VII:	C	β-OH, α-H	OH

R_1

a: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.
b: β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.
c: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.
d: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-cym.
e: β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.
f: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.
g: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-cym.
h: β-D-cym.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.$\overset{4}{-}$ β-D-cym.$\overset{4}{-}$ β-D-ole.

1:	I	-a	11:	II	-c
2:	II	-a	12:	VI	-c
3:	III	-a	13:	I	-d
4:	IV	-a	14:	II	-e
5:	V	-a	15:	I	-f
6:	VI	-a	16:	II	-f
7:	VII	-a	17:	I	-g
8:	I	-b	18:	II	-g
9:	II	-b	19:	I	-h
10:	I	-c	20:	II	-h

Chart 1



	R_1	R_2
D-oleandropyranose :	OCH_3	H
D-cymaropyranose :	H	OCH_3

Chart 2

second cymaropyranose)/3.21 (dd, J=9.5, 2.5 Hz, H-4 of the first cymaropyranose); δ 4.45 (dd, J=9.5, 1.5 Hz, H-1 of oleandropyranose)/3.21 (dd, J=9.5, 2.5 Hz, H-4 of the second cynmaropyranose) and δ 4.88 (dd, J=9.5, 2.0 Hz, H-1 of the third cymaropyranose)/3.16 (t, J=9.0 Hz, H-4 of oleandropyranose) were observed in the difference NOE spectra. Consequently, the sequence of sugars was established as presented in Table 4, and the structure of **13** was determined as shown in Chart 1.

Compound **14** was also considered to be caudatin 3-*O*-tetraside, which was composed of one D-cymaropyranose and three D-oleandropyranoses based on acid hydrolysis followed by analysis with GLC, the ^{1}H- and ^{13}C-NMR spectra. The sugar sequence was determined to be 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranoside by the difference NOE spectra irradiating at each anomeric proton signal. Therefore, the structure of **14** was concluded as shown in Chart 1.

Since the molecular formula of compounds **15**, **17**, **19** and **16**, **18**, **20** was suggested to be $C_{63}H_{102}O_{21}$ and $C_{63}H_{102}O_{22}$, respectively, by FAB-MS and the aglycones were identified as cynanchogenin and caudatin, respectively, the sugar chains of all compounds seemed to be composed of five 2,6-dideoxy-3-*O*-methylhexopyranoses. On acid hydrolysis followed by analysis with GLC, the relative ratio of the component sugars in **15**, **16** and **17**—**20** was determined to be two cymaroses to three oleandroses and three cymaroses to two oleandroses, respectively. Moreover, the ^{1}H- and ^{13}C-NMR signals for the sugar moieties in **15**, **16** and **17**, **18** were in good agreement with those of calotroposides C, D[3] and A, B.[7] Therefore, the structures of **15**—**18** were elucidated as in Chart 1. The sugar sequences of **19** and **20** were the same, because the ^{1}H- and ^{13}C-NMR signals of the sugar moiety were very

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

	I	II	III	IV	VI	VII
Aglycone						
1	38.8	38.8	38.8	38.9[a]	38.8	38.9[a]
2	28.9	28.9	28.9	29.1	29.0	29.0
3	77.9	77.8	77.9	77.9	77.9	77.9
4	38.8	38.8	38.8	38.8[a]	38.8	38.8[a]
5	141.3	140.8	141.4	139.8	140.7	139.8
6	117.3	117.5	117.3	118.3	117.6	118.3
7	34.5	34.2	34.5	34.6	34.3	34.5
8	74.8	74.3	74.9	73.9	74.3	74.0
9	44.1	43.8	44.1	43.5	43.7	43.5
10	37.3	37.2	37.3	37.1	37.2	37.1
11	24.4	24.3	24.3	24.7	24.2	24.7
12	71.0	71.6	72.1	74.4	73.2	74.7
13	55.1	57.9	55.2	56.1	58.4	56.3
14	86.7	88.0	86.7	87.9	88.0	87.9
15	33.3	33.1	33.3	33.4	33.3	33.3
16	21.3	31.9	21.4	31.7	32.0	31.8
17	60.0	91.5	59.9	87.9	91.5	87.9
18	15.0	9.4	14.9	11.2	9.5	11.1
19	18.8	18.6	18.9	18.2	18.6	18.2
20	209.2	208.7	209.4	71.3	209.3	71.0
21	31.8	27.1	32.0	17.6	27.3	18.2
Ester						
1′	166.0	166.6	165.6	166.2	165.3	165.4
2′	113.1	113.0	118.0	117.5	130.1	130.3
3′	165.7	165.9	145.0	146.2	128.4	128.7
4′	38.1	38.1	134.5	134.1	129.5	129.6
5′	20.9[a]	20.9[a]	128.1	128.3	133.1	133.4
6′	20.8[a]	20.8[a]	128.8	128.9	—	—
7′	16.5	16.5	130.2	130.7	—	—

Measured at 100.40 MHz in $CDCl_3$ solution at 35 °C. *a*) Assignment may be interchanged in each column.

Table 2. ^{1}H-NMR Spectral Data of Aglycone and Ester Moieties

	I	II	III
Aglycone moiety			
3	3.55 (1H, m)	3.56 (1H, m)	3.56 (1H, m)
6	5.34 (1H, br s)	5.36 (1H, br s)	5.36 (1H, br s)
12	4.63 (1H, dd, J=10.0, 5.5 Hz)	4.56 (1H, dd, J=8.0, 6.5 Hz)	4.79 (1H, dd, J=11.5, 5.0 Hz)
18	1.53 (3H, s)	1.41 (3H, s)	1.59 (3H, s)
19	1.12 (3H, s)	1.13 (3H, s)	1.13 (3H, s)
21	2.14 (3H, s)	2.16 (3H, s)	2.17 (3H, s)
Ester moiety			
2′	5.51 (1H, t, J=1.5 Hz)	5.52 (1H, t, J=1.5 Hz)	6.29 (1H, d, J=16.0 Hz)
3′	—	—	7.63 (1H, d, J=16.0 Hz)
4′	2.34 (*)	2.35 (*)	—
5′	1.06 (3H, d, J=6.5 Hz)	1.06 (3H, d, J=6.5 Hz)	
6′	1.06 (3H, d, J=6.5 Hz)	1.06 (3H, d, J=6.5 Hz)	
7′	2.12 (3H, d, J=1.5 Hz)	2.12 (3H, d, J=1.5 Hz)	

	IV	VI	VII
Aglycone moiety			
3	3.55 (1H, m)	3.57 (1H, m)	3.56 (1H, m)
6	5.37 (1H, br s)	5.38 (1H, br s)	5.38 (1H, br s)
12	4.76 (*)	4.85 (*)	4.88 (1H, dd, J=10.5, 4.5 Hz)
18	1.56 (3H, s)	1.54 (3H, s)	1.64 (3H, s)
19	1.17 (3H, s)	1.13 (3H, s)	1.16 (3H, s)
21	1.10 (3H, d, J=6.5 Hz)	2.06 (3H, s)	1.06 (3H, d, J=6.5 Hz)
Ester moiety			
2′	6.64 (1H, d, J=16.0 Hz)	—	—
3′	7.76 (1H, d, J=16.0 Hz)	7.94 (2H, br d, J=8.0 Hz)	8.09 (2H, br d, J=8.0 Hz)
4′	—	7.43 (2H, br t, J=8.0 Hz)	7.47 (2H, br t, J=8.0 Hz)
5′		7.56 (1H, br t, J=8.0 Hz)	7.59 (1H, br t, J=8.0 Hz)
6′		—	—
7′		—	—

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

similar. The difference NOE experiments irradiating at each anomeric proton signal of **19** showed correlation between δ 4.84 (dd, J=9.5, 2.0 Hz, H-1 of the first cymaropyranose)/3.55 (m, H-3 of aglycone); δ 4.75 (dd, J=9.5, 2.0 Hz, H-1 of the second cymaropyranose)/3.21 (dd, J=9.5, 3.0 Hz, H-4 of the first cymaropyranose); δ 4.44 (dd, J=9.5, 2.0 Hz, H-1 of the first oleandropyranose)/3.20 (dd, J=9.5, 3.0 Hz, H-4 of the second cymaropyranose); δ 4.95 (dd, J=9.5, 2.0 Hz, H-1 of the third cymaropyrnose)/3.18 (t, J=9.0 Hz, H-4 of the first oleandropyranose) and δ 4.49 (dd, J=9.5, 2.0 Hz, H-1 of the second oleandropyranose)/3.24 (dd, J=9.5, 3.0 Hz, H-4 of the third cymaropyanose). Therefore, the structures of **19** and **20** were concluded as shown in Chart 1.

Each monosaccharide was determined to be β-D-2,6-dideoxyhexopyranose based on the report of Vleggaar *et al.*[8]

Experimental

Optical rotations were determined with a JASCO-360 digital polarimeter. FAB-MS spectra were taken on a JEOL JMS-SX120 spectrometer. ^{1}H- and ^{13}C-NMR were recorded at a JEOL JNM A-400 (400 and 100.40 MHz, respectively) spectrometer. Chemical shifts were given on the δ (ppm) scale with tetramethysilane as an internal standard. GLC was run on a Hitachi G-3000 gas chromatograph. HPLC was run on a JASCO 800 system instrument.

Isolation The air dried root of *C. caudatum* M. (1.9 kg) was extracted twice with MeOH under reflux. The extract was concentrated under reduced pressure and the residue was suspended in H_2O. This suspension was extracted with Et_2O. The Et_2O layer was also concentrated, and this residue and the Et_2O–water insoluble deposit were combined, and rechromatographed on a silica gel column with $CHCl_3$–MeOH system and semi-preparative HPLC [Develosil-ODS, PhA and YMC-ODS, C-8: MeCN–H_2O and MeOH–H_2O systems] to give compounds **1** (142 mg), **2** (60 mg), **3** (14 mg), **4** (12 mg), **5** (14 mg), **6** (7 mg), **7** (8 mg), **8** (10 mg), **9** (7 mg), **10** (160 mg), **11** (67 mg), **12** (4 mg), **13** (11 mg), **14** (6 mg), **15** (34 mg), **16** (18 mg), **17** (9 mg), **18** (12 mg), **19** (9 mg) and **20** (7 mg).

Compound 2 Amorphous powder. $[\alpha]_D^{20}$ +5.1° (c=2.2, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{16}\cdot H_2O$: C, 62.53; H, 8.57. Found: C, 62.45; H, 8.64. FAB-MS m/z: 923 $[M+H]^+$, 945 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 3 Amorphous powder. $[\alpha]_D^{20}$ +9.3° (c=0.23, MeOH). *Anal.* Calcd for $C_{51}H_{74}O_{15}\cdot 5/2H_2O$: C, 63.01; H, 8.19. Found: C, 63.21; H, 8.22. UV λ_{max}^{MeOH} nm (log ε): 217 (4.25), 222 (4.19), 279 (4.40). FAB-MS m/z: 949 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 4 Amorphous powder. $[\alpha]_D^{20}$ +27.9° (c=1.2, MeOH). *Anal.* Calcd for $C_{51}H_{76}O_{16}\cdot 2H_2O$: C, 62.43; H, 8.22. Found: C, 62.52; H, 8.29. UV λ_{max}^{MeOH} nm (log ε): 217 (4.19), 223 (4.16), 280 (4.20). FAB-MS m/z: 945 $[M+H]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 6 Amorphous powder. $[\alpha]_D^{20}$ $\simeq$0° (c=0.55, MeOH). *Anal.* Calcd for $C_{49}H_{72}O_{16}\cdot 3/2H_2O$: C, 62.34; H, 8.01. Found: C, 62.19; H, 8.03. UV λ_{max}^{MeOH} nm (log ε): 230 (4.19), 274 (3.35), 281 (3.32). FAB-MS m/z: 939 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 7 Amorphous powder. $[\alpha]_D^{20}$ +16.9° (c=0.77, MeOH). *Anal.* Calcd for $C_{49}H_{74}O_{16}\cdot 2H_2O$: C, 61.62; H, 8.23. Found: C, 61.41; H, 8.10. UV λ_{max}^{MeOH} nm (log ε): 230 (4.16), 274 (3.19), 281 (3.12). FAB-MS m/z: 919 $[M+H]^+$, 941 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 8 Amorphous powder. $[\alpha]_D^{20}$ −29.1° (c=0.98, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{15}\cdot 2H_2O$: C, 62.40; H, 8.76. Found:C, 62.57; H, 8.61. FAB-MS m/z: 907 $[M+H]^+$, 929 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 9 Amorphous powder. $[\alpha]_D^{20}$ −8.9° (c=0.71, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{16}\cdot 3/2H_2O$: C, 61.94; H, 8.59. Found: C, 61.96;

Table 3. ^{13}C-NMR Spectral Data of Sugar Moiety

	a	b	c	d	e	f	g	h
	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.	D-cym.
1	96.1	96.1	96.1	96.1	96.1	96.1	96.1	96.1
2	35.7[a]	35.9	35.4[a]	35.6	35.9[a]	35.6[a]	35.6[a]	35.7[a]
3	77.1[b]	77.1	77.1[b]	77.1	77.1	77.1	77.1	77.1[b]
4	82.6[c]	82.7	82.5[c]	82.5	82.7[b]	82.5[b]	82.5[b]	82.6[c]
5	68.3[d]	68.4	68.3[d]	68.3[a]	68.4	68.3[c]	68.3[c]	68.4[d]
	D-cym.	D-ole.	D-cym.	D-cym.	D-ole.	D-cym.	D-cym.	D-cym.
1	99.7	101.4	99.7	99.7	101.4	99.7	99.7	99.7
2	35.6[a]	36.4	35.5[a]	35.6	36.4[c]	35.7[a]	35.5[a]	35.7[a]
3	77.0[b]	79.2	77.1[b]	77.1	79.1[d]	77.1	77.1	77.1[b]
4	82.5[c]	82.3	82.6[c]	82.5	82.4[b]	82.6[b]	82.4[b]	82.7[c]
5	68.6[d]	71.0	68.5[d]	68.5[a]	71.1	68.5[c]	68.6[c]	68.7[d]
	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.	D-ole.
1	101.4	100.2	101.3	101.4	100.1[e]	101.4	101.4	101.5[e]
2	35.4[a]	35.5	36.3	36.4	36.5[c]	36.4[d]	36.4[d]	36.4
3	80.6	80.8	79.1	78.9	79.3[d]	79.3[e]	79.0[e]	78.9
4	75.4	75.5	82.4[c]	82.2	82.5[b]	82.3[f]	82.4[b]	82.1
5	71.6	71.7	71.0	71.1	71.1	71.0[g]	71.1[f]	71.3[f]
			D-ole.	D-cym.	D-ole.	D-ole.	D-ole.	D-cym.
1	—	—	100.1	98.2	100.2[e]	100.2[h]	100.1	98.5
2	—	—	35.6[a]	33.9	35.5[a]	36.5[d]	36.5[d]	35.6[a]
3	—	—	80.7	77.6	80.8	79.1[e]	79.2[e]	77.2[b]
4	—	—	75.4	72.5	75.5	82.5[f]	82.3[b]	82.5[c]
5	—	—	71.7	71.1	71.7	71.7[g]	71.7[f]	68.9[d]
						D-ole.	D-cym.	D-ole.
1	—	—	—	—	—	100.1[h]	98.2	101.6[e]
2	—	—	—	—	—	35.5[d]	33.9	35.4[a]
3	—	—	—	—	—	80.8	77.6	80.7
4	—	—	—	—	—	75.5	72.5	75.5
5	—	—	—	—	—	71.7	71.3[f]	71.6[f]
6s	17.9	18.0	17.9	18.2×2	18.0	18.0	18.2×2	18.0
	18.2×2	18.2	18.1	18.3	18.2	18.2×2	18.3	18.3×3
		18.4	18.2	18.4	18.4×2	18.4×2	18.4×2	18.3
			18.4					
OMes	56.2	56.3	56.3	56.5	56.3	56.3	56.7×2	56.3
	58.0	56.7	56.6	57.1	56.7	56.7	57.1	56.6
	58.2	58.2	58.0	58.1	56.8	56.8	58.1	58.0×2
			58.2	58.3	58.2	58.1	58.3	58.3
						58.2		

Measured at 100.40 MHz in $CDCl_3$ solution at 35 °C. *a*—*h*) Assignments may be interchanged in each column.

H, 8.53. FAB-MS *m/z*: 923 $[M+H]^+$, 945 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 10 Amorphous powder. $[\alpha]_D^{20}$ −13.1° (*c*=1.6, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{18}\cdot 3/2H_2O$: C, 62.38; H, 8.69. Found: C, 62.22; H, 8.55. FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 11 Amorphous powder. $[\alpha]_D^{20}$ −0.45° (*c*=1.1, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{19}\cdot 3/2H_2O$: C, 61.46; H, 8.56. Found: C, 61.70; H, 8.55. FAB-MS *m/z*: 1089 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 13 Amorphous powder. $[\alpha]_D^{20}$ +7.0° (*c*=1.2, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{18}\cdot H_2O$: C, 62.90; H, 8.67. Found: C, 63.08; H, 8.69. FAB-MS *m/z*: 1073 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 14 Amorphous powder. $[\alpha]_D^{20}$ +0.74° (*c*=0.68, MeOH). *Anal.* Calcd for $C_{56}H_{90}O_{19}\cdot 3/2H_2O$: C, 61.46; H, 8.57. Found: C, 61.40; H, 8.61. FAB-MS *m/z*: 1089 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 15 Amorphous powder. $[\alpha]_D^{20}$ −18.9° (*c*=1.7, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{21}\cdot 3/2H_2O$: C, 61.90; H, 8.66. Found: C, 62.11; H, 8.71. FAB-MS *m/z*: 1217 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 16 Amorphous powder. $[\alpha]_D^{20}$ −5.9° (*c*=1.1, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{22}\cdot H_2O$: C, 61.54; H, 8.52. Found: C, 61.43; H, 8.61. FAB-MS *m/z*: 1233 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 17 Amorphous powder. $[\alpha]_D^{20}$ −4.5° (*c*=0.66, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{21}\cdot H_2O$: C, 62.36; H, 8.64. Found: C, 62.30; H, 8.65. FAB-MS *m/z*: 1217 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 18 Amorphous powder. $[\alpha]_D^{20}$ +15.8° (*c*=1.2, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{22}\cdot H_2O$: C, 61.54; H, 8.53. Found: C, 61.30; H, 8.55. FAB-MS *m/z*: 1233 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 19 Amorphous powder. $[\alpha]_D^{20}$ −8.1° (*c*=0.86, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{21}\cdot 1/2H_2O$: C, 62.82; H, 8.62. Found: C, 62.96; H, 8.86. FAB-MS *m/z*: 1217 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Compound 20 Amorphous powder. $[\alpha]_D^{20}$ +5.9° (*c*=0.76, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{22}\cdot 2H_2O$: C, 60.66; H, 8.56. Found: C, 60.57; H, 8.61. FAB-MS *m/z*: 1233 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: Tables 1—4.

Degradation of the Crude Pregnane Glycoside The ether layer and insoluble residue were chromatographed on a silica gel column, and the crude pregnane glycoside fraction was eluted with $CHCl_3$–MeOH (97 : 3). This fraction (*ca.* 70 mg) was dissolved in AcCl–MeOH (1 : 10) (2 ml) and heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the precipitate was removed by filtration. The solvent was evaporated off under reduced pressure from the filtrate to give a product. Purification of the product by HPLC (YMC-ODS, 65% MeOH) afforded cynanchogenin [I, (R_1=H) 12 mg] and caudatin [II, (R_1=H) 11 mg]. Other esterified aglycone moieties, ikamagenin [III (R_1=H)], penupogenin [IV (R_1=H)] and 12-*O*-benzoyl-deacylmetaplexigenin [VI (R_1=H)], were acquired by metha-

Table 4. ^{1}H-NMR Spectral Data of Sugar Moiety

	a	b	c	d
	D-cym.	D-cym.	D-cym.	D-cym.
1	4.85 (1H, dd, J=9.5, 2.0 Hz)	4.84 (1H, dd, J=9.5, 2.0 Hz)	4.84 (1H, dd, J=9.5, 2.0 Hz)	4.84 (1H, dd, J=9.5, 1.5 Hz)
3	3.80[a)]	3.79 (1H, q, J=3.0 Hz)	3.80 (1H, q, J=2.5 Hz)	3.80 (1H, q, J=2.5 Hz)
4	3.21 (1H, dd, J=9.5, 2.5 Hz)	3.22 (1H, dd, J=9.5, 3.0 Hz)	3.21 (1H, dd, J=9.5, 2.5 Hz)	3.21 (1H, dd, J=9.5, 2.5 Hz)
5	3.84 (1H, dq, J=9.5, 6.5 Hz)	3.86 (1H, dq, J=9.5, 6.5 Hz)	3.84 (1H, dq, J=9.5, 6.5 Hz)	3.84 (1H, dq, J=9.5, 6.5 Hz)
6	1.22 (3H, d, J=6.5 Hz)	1.22 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)
	D-cym.	D-ole.	D-cym.	D-cym.
1	4.76 (1H, dd, J=9.5, 2.0 Hz)	4.45 (1H, dd, J=9.5, 2.0 Hz)	4.75 (1H, dd, J=9.5, 2.0 Hz)	4.75 (1H, dd, J=9.5, 1.5 Hz)
3	3.80[a)]	3.37[a)]	3.78 (1H, q, J=2.5 Hz)	3.78 (1H, q, J=2.5 Hz)
4	3.23 (1H, dd, J=9.5, 2.5 Hz)	3.17 (1H, t, J=9.0 Hz)	3.21 (1H, dd, J=9.5, 2.5 Hz)	3.21 (1H, dd, J=9.5, 2.5 Hz)
5	3.88 (1H, dq, J=9.5, 6.5 Hz)	3.31 (1H, dq, J=9.0, 6.5 Hz)	3.86 (1H, dq, J=9.5, 6.5 Hz)	3.86 (1H, dq, J=9.5, 6.5 Hz)
6	1.21 (3H, d, J=6.5 Hz)	1.30 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)
	D-ole.	D-ole.	D-ole.	D-ole.
1	4.50 (1H, dd, J=9.5, 2.0 Hz)	4.72 (1H, dd, J=9.5, 2.0 Hz)	4.45 (1H, dd, J=9.5, 2.0 Hz)	4.45 (1H, dd, J=9.5, 1.5 Hz)
3	3.17[a)]	3.16[a)]	3.36[a)]	3.36[a)]
4	3.12 (1H, t, J=9.0 Hz)	3.14[a)]	3.17 (1H, t, J=9.0 Hz)	3.16 (1H, t, J=9.0 Hz)
5	3.28 (1H, dq, J=9.0, 6.5 Hz)	3.31 (1H, dq, J=9.5, 6.5 Hz)	3.31 (1H, dq, J=9.0, 6.5 Hz)	3.29 (1H, dq, J=9.0, 6.5 Hz)
6	1.32 (3H, d, J=6.5 Hz)	1.34 (3H, d, J=6.5 Hz)	1.29 (3H, d, J=6.5 Hz)	1.28 (3H, d, J=6.5 Hz)
			D-ole.	D-cym.
1	—	—	4.72 (1H, dd, J=9.5, 2.0 Hz)	4.88 (1H, dd, J=9.5, 2.0 Hz)
3	—	—	3.17[a)]	3.62 (1H, q, J=3.0 Hz)
4	—	—	3.13 (1H, t, J=9.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)
5	—	—	3.31 (1H, dq, J=9.0, 6.5 Hz)	3.60 (1H, dq, J=9.5, 6.5 Hz)
6	—	—	1.34 (3H, d, J=6.5 Hz)	1.29 (3H, d, J=6.5 Hz)
1	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	—	—
OMes	3.39 (3H, s)	3.40 (3H, s) × 2	3.40 (3H, s) × 2	3.40 (3H, s)
	3.45 (3H, s) × 2	3.45 (3H, s)	3.45 (3H, s) × 2	3.43 (3H, s)
				3.44 (3H, s)
				3.45 (3H, s)

	e	f	g	h
	D-cym.	D-cym.	D-cym.	D-cym.
1	4.84 (1H, dd, J=9.5, 2.0 Hz)	4.84 (1H, dd, J=9.5, 1.5 Hz)	4.84 (1H, dd, J=9.5, 2.0 Hz)	4.84 (1H, dd, J=9.5, 2.0 Hz)
3	3.79 (1H, q, J=3.0 Hz)	3.80 (1H, q, J=3.0 Hz)	3.80 (1H, q, J=3.0 Hz)	3.80 (1H, q, J=3.0 Hz)
4	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)
5	3.86 (1H, dq, J=9.5, 6.5 Hz)	3.84 (1H, dq, J=9.5, 6.5 Hz)	3.84 (1H, dq, J=9.5, 6.5 Hz)	3.84 (1H, dd, J=9.5, 6.5 Hz)
6	1.22 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)
	D-ole.	D-cym.	D-cym.	D-cym.
1	4.44 (1H, dd, J=9.5, 2.0 Hz)	4.75 (1H, dd, J=9.5, 1.5 Hz)	4.75 (1H, dd, J=9.5, 2.0 Hz)	4.75 (1H, dd, J=9.5, 2.0 Hz)
3	3.35[a)]	3.78 (1H, q, J=3.0 Hz)	3.78 (1H, q, J=3.0 Hz)	3.78 (1H, q, J=3.0 Hz)
4	3.16 (1H, t, J=9.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.20 (1H, dq, J=9.5, 3.0 Hz)
5	3.29[a)]	3.86 (1H, dq, J=9.5, 6.5 Hz)	3.86 (1H, dq, J=9.5, 6.5 Hz)	3.86 (1H, dq, J=9.5, 6.5 Hz)
6	1.28 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)	1.21 (3H, d, J=6.5 Hz)
	D-ole.	D-ole.	D-ole.	D-ole.
1	4.66 (1H, dd, J=9.5, 2.0 Hz)	4.44 (1H, dd, J=9.5, 1.5 Hz)	4.44 (1H, dd, J=9.5, 2.0 Hz)	4.44 (1H, dd, J=9.5, 2.0 Hz)
3	3.35[a)]	3.35[a)]		3.35[a)]
4	3.18 (1H, t, J=9.0 Hz)	3.16 (1H, t, J=9.0 Hz)	3.15 (1H, t, J=9.0 Hz)	3.18 (1H, t, J=9.0 Hz)
5	3.32[a)]	3.29 (1H, dq, J=9.0, 6.5 Hz)	3.29 (1H, dq, J=9.0, 6.5 Hz)	3.28[a)]
6	1.32 (3H, d, J=6.5 Hz)	1.28 (3H, d, J=6.5 Hz)	1.28 (3H, d, J=6.5 Hz)	1.28 (3H, d, J=6.5 Hz)
	D-ole.	D-ole.	D-ole.	D-cym.
1	4.72 (1H, dd, J=9.5, 2.0 Hz)	4.67 (1H, dd, J=9.5, 2.0 Hz)	4.66 (1H, dd, J=9.5, 2.0 Hz)	4.95 (1H, dd, J=9.5, 2.0 Hz)
3	3.16[a)]	3.35[a)]		3.80 (1H, q, J=3.0 Hz)
4	3.13 (1H, t, J=9.0 Hz)	3.17 (1H, t, J=9.0 Hz)	3.17 (1H, t, J=9.0 Hz)	3.24 (1H, dd, J=9.5, 3.0 Hz)
5	3.31[a)]	3.33 (1H, dq, J=9.0, 6.5 Hz)	3.31 (1H, dq, J=9.0, 6.5 Hz)	3.89 (1H, dq, J=9.5, 6.5 Hz)
6	1.34 (3H, d, J=6.5 Hz)	1.32 (3H, d, J=6.5 Hz)	1.31 (3H, d, J=6.5 Hz)	1.24 (3H, d, J=6.5 Hz)
		D-ole.	D-cym.	D-ole.
1	—	4.71 (1H, dd, J=9.5, 2.0 Hz)	4.87 (1H, dd, J=9.5, 2.0 Hz)	4.49 (1H, dd, J=9.5, 2.0 Hz)
3	—	3.17[a)]	3.62 (1H, q, J=3.0 Hz)	3.16[a)]
4	—	3.13 (1H, t, J=9.0 Hz)	3.21 (1H, dd, J=9.5, 3.0 Hz)	3.12 (1H, t, J=9.0 Hz)
5	—	3.31 (1H, dd, J=9.0, 6.5 Hz)	3.60 (1H, dq, J=9.5, 6.5 Hz)	3.28[a)]
6	—	1.34 (3H, d, J=6.5 Hz)	1.29 (3H, d, J=6.5 Hz)	1.32 (3H, d, J=6.5 Hz)
OMes	3.39 (3H, s)	3.39 (3H, s)	3.39 (3H, s)	3.39 (3H, s)
	3.40 (3H, s)	3.40 (3H, s)	3.42 (3H, s) × 2	3.40 (3H, s)
	3.41 (3H, s)	3.41 (3H, s)	3.44 (3H, s)	3.44 (3H, s) × 2
	3.45 (3H, s)	3.44 (3H, s) × 2	3.45 (3H, s)	3.45 (3H, s)

Measured at 400 MHz in $CDCl_3$ solution at 35 °C. Signal assignments were done based on the results of 2D-NMR (HMQC/C-H COSY, HMBC and COSY) and the decoupling experiments. *a)* Overlapping with other signals.

nolysis of their glycosides (**3**, **4**, **6** and **12**). But methanolysis to acquire 12-*O*-benzoyl-sarcostin [VII (R_1=H)] was not performed, because so little of its glycoside was isolated.

I (R_1=H): mp 170—172 °C (ether–hexane). $[\alpha]_D^{20}$ −48.6° (*c*=1.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$ at 35°C) δ: 5.51 (br s, H-2′), 5.35 (t-like, H-6), 4.64 (dd, 10.5, 5.5 Hz, H-12), 3.56 (m, H-3), 3.14 (t, 9.0 Hz, H-17), 2.24 (s, H-21), 2.13 (br s, H-7′), 1.54 (s, H-18), 1.14 (s, H-19), 1.06 (d, 6.5 Hz, H-5′, 6′). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 209.2 (C-20), 166.1 (C-1′), 165.8 (C-3′), 141.2 (C-5), 117.4 (C-6), 113.1 (C-2′), 86.7 (C-14), 78.4 (C-8), 71.8 (C-3), 71.0 (C-12), 60.0 (C-17), 55.0 (C-13), 44.1 (C-9), 41.9 (C-4), 38.7 (C-1), 38.1 (C-4′), 37.1 (C-10), 34.5 (C-7), 33.4 (C-15), 31.8 (C-21), 30.8 (C-2), 24.4 (C-11), 21.2 (C-16), 20.9, 20.8 (C-5′, 6′), 18.9 (C-19), 16.5 (C-7′), 15.0 (C-18).

II (R_1=H): $[\alpha]_D^{20}$ +16.2° (*c*=1.1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$ at 35 °C) δ: 5.53 (br s, H-2′), 5.36 (t-like, H-6), 4.57 (t-like, 8.0 Hz, H-12), 3.56 (m, H-3), 2.84 (m, H-16), 2.16 (s, H-21), 2.13 (br s, H-7′), 1.41 (s, H-18), 1.15 (s, H-19), 1.07 (d, 6.5 Hz, H-5′, 6′). ^{13}C-NMR ($CDCl_3$) δ: 208.7 (C-20), 166.7 (C-1′), 166.0 (C-3′), 140.5 (C-5), 117.7 (C-6), 113.1 (C-2′), 91.5 (C-17), 88.0 (C-14), 74.4 (C-8), 71.9 (C-3), 71.7 (C-12), 58.0 (C-13), 43.8 (C-9), 42.0 (C-4), 38.7 (C-1), 38.2 (C-4′), 37.0 (C-10), 34.3 (C-7), 33.2 (C-15), 31.9 (C-16), 30.9 (C-2), 27.1 (C-21), 24.3 (C-11), 20.9, 20.8 (C-5′, 6′), 18.7 (C-19), 16.7 (C-7′), 9.4 (C-18).

Methanolysis of Compounds 3, 4, 6 and 12 Compounds **3** (10 mg), **4** (8 mg) and a mixture of **6** and **12** (total 8 mg) were dissolved in AcCl–MeOH (1 : 10) (1 ml), respectively. Subsequent reaction and purification was done with the above methods to give ikemagenin [III (R_1=H) 3 mg], penupogenin [IV (R_1=H) 1 mg] and 12-*O*-benzoyl-deacylmetaplexigenin [VI (R_1=H) 2 mg].

III (R_1=H): $[\alpha]_D^{20}$ −18.5° (*c*=0.28, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 217 (4.23), 222 (4.16), 279 (4.39). ^{1}H-NMR ($CDCl_3$ at 35°C) δ: 7.63 (d, 16.0 Hz, H-3′), 7.51 (m), 7.38 (m) (aromatic protons), 6.30 (d, 16.0 Hz, H-2′), 5.36 (t-like, H-6), 4.79 (dd, 11.5, 4.5 Hz, H-12), 3.57 (m, H-3), 3.19 (t, 9.5 Hz, H-17), 2.17 (s, H-21), 1.60 (s, H-18), 1.15 (s, H-19). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 209.3 (C-20), 165.6 (C-1′), 145.0 (C-3′), 141.2 (C-5), 134.5 (C-4′), 130.3 (C-7′), 128.8 (C-6′), 128.1 (C-5′), 117.9, 117.4 (C-6, 2′), 86.7 (C-14), 74.9 (C-8), 72.1, 71.9 (C-3, 12), 59.9 (C-17), 55.2 (C-13), 44.1 (C-9), 42.0 (C-4), 38.7 (C-1), 37.1 (C-10), 34.5 (C-7), 33.4 (C-15), 32.0 (C-21), 30.8 (C-2), 24.4 (C-11), 21.4 (C-16), 18.9 (C-19), 14.9 (C-18).

IV (R_1=H): $[\alpha]_D^{20}$ +70.8° (*c*=0.12, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 217 (4.26), 222 (4.20), 279 (4.41). ^{1}H-NMR ($CDCl_3$ at 35 °C) δ: 7.76 (d, 16.0 Hz, H-3′), 7.55 (m), 7.40 (m) (aromatic protons), 6.45 (d, 16.0 Hz, H-2′), 5.38 (t-like, H-6), 4.77 (dd, 11.5, 4.5 Hz, H-12), 3.66 (m, H-20), 3.55 (m, H-3), 1.53 (s, H-18), 1.19 (s, H-19), 1.10 (d, 6.5 Hz, H-21). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 166.2 (C-1′), 146.2 (C-3′), 139.6 (C-5), 134.2 (C-4′), 130.7 (C-2′), 129.0 (C-6′), 128.3 (C-5′), 118.4 (C-2′), 117.5 (C-6), 87.9 (C-14, 17), 74.4 (C-12), 74.0 (C-8), 72.0 (C-3), 71.3 (C-20), 56.1 (C-13), 43.5 (C-9), 42.1 (C-4), 38.8 (C-1), 36.9 (C-10), 34.6 (C-7), 33.4 (C-15), 31.7 (C-16), 31.0 (C-2), 24.8 (C-11), 18.3 (C-19), 17.7 (C-21), 11.2 (C-18).

VI (R_1=H): $[\alpha]_D^{20}$ ≃0° (*c*=0.19, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 230 (4.11), 273 (3.03), 281 (2.95). ^{1}H-NMR ($CDCl_3$ at 35 °C) δ: 7.94 (br d, 7.5 Hz), 7.54 (br t, 7.5 Hz) 7.44 (br t, 7.5 Hz) (aromatic protons), 5.38 (t-like, H-6), 4.86 (dd, 10.0, 6.5 Hz), 3.57 (m, H-3), 2.86 (m, H-16), 2.06 (s, H-21), 1.54 (s, H-18), 1.15 (s, H-19). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 209.3 (C-20), 165.4 (C-1′), 140.6 (C-5), 133.1 (C-5′), 130.0 (C-2′), 129.5 (C-4′), 128.5 (C-3′), 117.7 (C-6), 91.5 (C-17), 88.0 (C-14), 74.3 (C-8), 73.3 (C-12), 71.9 (C-3), 58.4 (C-13), 43.7 (C-9), 42.0 (C-4), 38.8 (C-1), 37.0 (C-10), 34.3 (C-7), 33.4 (C-15), 32.0 (C-2), 30.9 (C-16), 27.3 (C-21), 24.3 (C-11), 18.6 (C-19), 9.5 (C-18).

Hydrolysis of Compounds 2—4, 6—11, 13—19 and 20 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 90 min. After hydrolysis, this solution was passed through an Amberlite IRA-60E column and concentrated to dryness. The residues from **2**, **8**—**11**, **13**—**19** and **20** were analyzed by HPLC to identifiy the aglycone with authentic samples [Conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 75% MeOH in water; t_R (min), cynanchogenin 8.8, caudatin 9.6]. Moreover, the residue from **7** was divided into two parts, and one was dissolved in 5% NaOH–MeOH (*ca* 0.2 ml) and refluxed for 1 h. The resulting mixture was neutralized by passing through an Amberlite IR-120B column, and the eluate was concentrated. The residual aglycone and ester moieties were identified by HPLC with authentic samples [Conditions: column, YMC-ODS 4.6 mm × 25 cm; flow rate, 1.0 ml/min, 40% MeOH in water, t_R (min), sarcostin 10.8, 22.5% MeCN in water + 0.05% trifluoroacetic acid, benzoic acid 13.2]. 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.

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References and Notes

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