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STEROIDAL GLYCOSIDES FROM *CYNANCHUM CAUDATUM*

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Key Word Index—*Cynanchum caudatum*; Asclepiadaceae; pregnane glycoside; 2,6-dideoxy-3-*O*-methylhexopyranose.

Abstract—The aerial part of *Cynanchum caudatum* afforded 10 new pregnane glycosides which had sarcostin or deacylmetaplexigenin as the aglycone moiety. The structures of these compounds were elucidated by spectroscopic methods and from chemical evidence.

INTRODUCTION

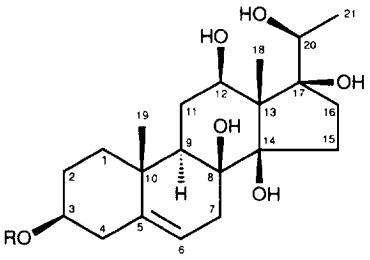
In connection with a study on the constituents of some plants in the Asclepiadaceae, we have investigated *Cynanchum caudatum* M. The isolation and structures of pregnane glycosides from the roots of this plant were previously reported [1, 2]. In the present paper, we describe the isolation and structural elucidation of 10 pregnane glycosides from the aerial part of this plant.

RESULTS AND DISCUSSION

The methanol extract of the aerial part of *C. caudatum* afforded new compounds **2**–**10** and **12**. An aglycone moiety obtained from **2**–**10** by acid hydrolysis was sarcostin (**1**), which was identified by comparison of the ^{13}C NMR spectral data, $[\alpha]_D$ and mp with previously reported values [3, 4]. The aglycone moiety of **12** was determined by comparing it with the reported data of the glycoside, wilfoside M1N, which had deacylmetaplexigenin (**11**) as the aglycone [5].

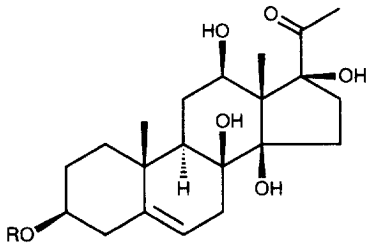
Compound **2** showed an anomeric proton and carbon signal at δ4.79 (1H, *dd*, J = 9.5, 2.0 Hz) and δ95.6 in the ^{1}H and ^{13}C NMR spectra, respectively. The signal from the C-3 of the aglycone at δ78.0 shifted downfield on comparison with that of sarcostin (**1**). This glycosilation shift indicated that the sugar moiety was attached to the C-3 position. The FAB-mass spectrum of **2** revealed a $[M + H]^+$ ion peak at m/z 527, which was larger by 144 mass units than that of sarcostin. Because this difference was derived from the sugar moiety, the molecular formula of this sugar was deduced to be $C_7H_{12}O_3$, and identified as cymarose by acid hydrolysis an comparison with an authentic sample (see Experimental). Thus, the structure of **2** was determined to be sarcostin 3-*O*-β-cymaropyranoside.

In the ^{1}H NMR spectrum of **3**, four anomeric proton signals were observed at δ4.84 (1H, *dd*, J = 9.5, 1.5 Hz), 4.76 (1H, *dd*, J = 9.5, 2.0 Hz), 4.46 (1H, *dd*, J = 9.5, 2.0 Hz) and 4.87 (1H, *dd*, J = 4.5, 1.5 Hz), and in the ^{13}C NMR spectrum, four anomeric carbon signals were also observed at δ96.1, 99.7, 101.4 and 96.9. Acid hydrolysis gave cymarose and oleandrose as the sugar moieties, and the relative ratio of these monosaccharides was

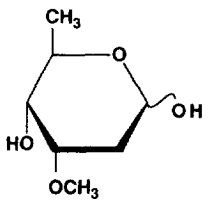


R

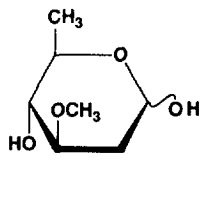
1: H
2: β-cym.
3: β-cym.-(4)-β-cym.-(4)-β-ole.-(4)-α-cym.
4: β-cym.-(4)-β-cym.-(4)-β-ole.-(4)-β-ole.
5: β-cym.-(4)-β-cym.-(4)-α-cym.
6: β-cym.-(4)-β-ole.-(4)-α-cym.
7: β-cym.-(4)-β-ole.-(4)-β-ole.
8: β-cym.-(4)-β-cym.-(4)-β-ole.
9: β-cym.-(4)-β-cym.
10: β-cym.-(4)-β-ole.
11: H
12: β-cym.-(4)-β-cym.-(4)-β-ole.-(4)-β-ole.



R



D-cymaropyranose



D-oleandropyranose

determined to be three cymaroses to one oleandrose by GC analysis. The hetero multiple quantum coherence (HMQC) spectrum of **3** revealed cross-peaks between the anomeric carbon signals at δ96.1, 99.7, 101.4, 96.9 and the anomeric proton signals at 4.84, 4.76, 4.46, 4.87, respectively. Moreover, in the ^{1}H NMR spectrum, the characteristic H-3 signals of cymaropyranoses were observed at δ3.81 (1H, *q*, J = 3.0 Hz), 3.78 (1H, *q*, J = 3.0 Hz) and 3.58 (1H, *q*, J = 3.5 Hz). In the hetero nuclear multiple bond connectivity (HMBC) spectrum, $^3J_{CCCH}$s were confirmed between these characteristic H-3 signals and the anomeric carbon signals as follows, δ3.81 and 96.1, δ3.78 and 99.7, δ3.58 and 96.9. From the above results, the anomeric signals at δ4.84 (1H, *dd*, J = 9.5, 1.5), 96.1 and 4.76 (1H, *dd*, J = 9.5, 2.0), 99.7 were assigned to the H-1s and C-1s of two β-cymaropyranoses, and the anomeric signals at δ4.87 (1H, *dd*, J = 4.5, 1.5 Hz) and δ96.9 were assigned to the H-1 and C-1 of α-cymaropyranose. The remaining anomeric signals at δ101.4 and δ4.46 (1H, *dd*, J = 9.5, 2.0 Hz) belonged to the C-1 and H-1 of β-oleandropyranose. For the sugar linkage, $^3J_{COCH}$s were observed as follows in the HMBC spectrum, δ96.1 [the C-1 of the first β-cymaropyranose] and δ3.54 (1H, *m*) [the H-3 of the aglycone], δ99.7 [the C-1 of another β-cymaropyranose] and δ3.21 (1H, *dd*, J = 9.5, 3.0 Hz) [the H-4 of the first β-cymaropyranose], δ101.4 [the C-1 of β-oleandropyranose] and δ3.21 (1H, *dd*, J = 9.5, 3.0 Hz) [H-4 of the second β-cymaropyranose], δ96.9 [the C-1 of α-cymaropyranose] and δ3.12 (1H, *t*, J = 9.0 Hz) [the H-4 of β-oleandropyranose]. In the difference nuclear Overhauser effect (NOE) spectra, irradiation at the anomeric proton signal at δ4.84 of the first β-cymaropyranose revealed a NOE to the H-3 signal of the aglycone at δ3.54. Similarly, NOEs were observed between the anomeric proton signal of the second β-cymaropyranose at δ4.76 and the H-4 signal of the first β-cymaropyranose at δ3.21, the H-1 signal of β-oleandropyranose at δ4.46 and the H-4 signal of the second β-cymaropyranose at δ3.21, and between the H-1 signal of α-cymaropyranose at δ4.87 and the H-4 one of β-oleandropyranose at δ3.12. Based on the above information, the structure of **3** was determined to be sarcostin 3-*O*-α-cymaropyranosyl-(1 → 4)-β-oleandropyranosyl-(1 → 4)-β-cymaropyranosyl-(1 → 4)-β-cymaropyranoside.

Compound **4** had the same molecular formula as **3**. On hydrolysis, sarcostin, two cymaroses and two oleandroses were obtained. The sugar linkage was determined in the same manner as that of **3**. The structure of **4** was determined to be as shown.

Compounds **5**–**8** had the molecular formula $C_{42}H_{70}O_{15}$. Acid hydrolysis of these compounds suggested that they were composed of sarcostin and three 2,6-dideoxy-3-*O*-methylhexoses. Based on the ^{1}H, ^{13}C NMR and H–H COSY spectra, three 2,6-dideoxy-3-*O*-methylhexoses of **5**–**8** consisted of two β- and one α-cymaropyranoses; one β-, one α-cymaropyranose and one β-oleandropyranose; one β-cymaropyranose and two β-oleandropyranoses; and two β-cymaropyranoses and one β-oleandropyranose, respectively. The sugar sequences of these compounds were determined in the same manner as that of **3**. The structures of **5**–**8** were determined to be as shown.

Compounds **9** and **10** both had the molecular formula $C_{35}H_{58}O_{12}$. Compound **9** was hydrolysed to sarcostin and two cymaroses, and **10** to sarcostin, one cymarose and one oleandrose. Using the same procedures described previously, the structures were elucidated to be as shown.

The ^{1}H and ^{13}C NMR spectra of **12** suggested that it consisted of four monosaccharides and an aglycone moiety which was determined to be deacylmetaplexigenin by comparison with the NMR spectral data in the literature [5]. Since the NMR spectra of its sugar moiety were consistent with those of **4**, the sugar linkage was determined to be the same as that of **4**. Thus, the structure of **12** was determined to be as shown.

Table 1. ^{13}C NMR spectral data of the aglycone moiety of compounds **1**–**10** and **12**

C	1*	2–10†	12†
1	38.2	39.0[c]	38.9
2	30.9	29.1	29.0
3	70.3	78.0	77.9
4	42.1	38.9[c]	38.9
5	139.0	139.8	140.7
6	118.0	118.4	117.7
7	34.1	34.6	34.3
8	72.9	73.8	74.3
9	43.1	43.8	44.2
10	36.1	37.0	37.1
11	27.8	28.6	28.0
12	69.3	70.9[A]	69.5
13	57.1	57.8	60.9
14	87.5[a]	87.8[d]	87.8
15	33.4[b]	33.5	33.3
16	33.2[b]	32.5	32.5
17	87.9[a]	88.0[d]	91.9
18	10.2	10.1	7.7
19	17.7	18.4[B]	18.7
20	71.5	72.4[C]	213.8
21	17.1	17.0	28.2

Run at 100.40 and 125.65 MHz.

[a–d]Assignments may be interchanged in each column.

[A–C]Assignments may be interchanged between Tables 1 and 2.

*Measured in $(Me)_2SO$-d_6 at 35°.

†Measured in $CDCl_3$ at 35°.

The absolute configuration of each monosaccharide was not determined in any of the compounds.

EXPERIMENTAL

^1H and ^{13}C NMR were recorded at 500, 400 and 125.65, 100.40 MHz, respectively. TMS was used as int. standard.

Plant material. Cynanchum caudatum M. was collected in Shizuoka pref., Japan in August, 1993 and identified by by Prof. T. Noro (University of Shizuoka).

Extraction and isolation. Dried aerial parts of *C. caudatum* M. (2.4 kg) was extracted ×2 with MeOH under reflux. The extract was concd under red. pres. and the residue 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 the absorbed material was eluted with 50, 60 and 70% MeOH in H_2O and MeOH. The Et_2O layer was also concd and the residue dissolved in hexane–C_6H_6 (1:1). This soln was extracted with 80% MeOH in H_2O, and 80% MeOH in the H_2O layer was concd under red. pres. The residue of 80% MeOH in the H_2O layer (14.6 g) and the MeOH eluate of the Diaion HP-20 column (7.1 g) were combined, and rechromatographed on a silica gel column with the $CHCl_3$–MeOH system and a semi prep. HPLC (Develosil-ODS and YMC-ODS: MeCN–H_2O and MeOH–H_2O system) to give **2** (7 mg), **3** (10 mg), **4** (15 mg), **5** (13 mg), **6** (3 mg), **7** (3 mg), **8** (47 mg), **9** (9 mg), **10** (23 mg) and **12** (7 mg).

Compound **2**. Amorphous powder $[\alpha]_D^{26}$ +64.4° (MeOH; *c* 0.66). Calcd for $C_{28}H_{46}O_9 \cdot H_2O$: C, 61.74; H, 8.88. Found: C, 61.45; H, 9.04. FAB-MS *m/z*: 527 $[M + H]^+$, 549 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **3**. Amorphous powder $[\alpha]_D^{26}$ −2.9° (MeOH; *c* 0.40). Calcd for $C_{49}H_{82}O_{18} \cdot 2H_2O$: C, 59.14; H, 8.71. Found: C, 59.29; H, 8.78. FAB-MS *m/z*: 959 $[M + H]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **4**. Amorphous powder $[\alpha]_D^{26}$ +40.1° (MeOH; *c* 0.32). Calcd for $C_{49}H_{82}O_{18} \cdot 3H_2O$: C, 58.09; H, 8.75. Found: C, 58.33; H, 8.75. FAB-MS *m/z*: 959 $[M + H]^+$, 981 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **5** Amorphous powder $[\alpha]_D^{26}$ +4.4° (MeOH; *c* 1.26). Calcd for $C_{42}H_{70}O_{15} \cdot 2H_2O$: C, 59.28; H,

Table 2. ^{13}C NMR spectral data of the sugar moieties of Compounds **2**–**10** and **12**

C	2	3	4, 12	5	6	7	8	9	10
	Cym.	Cym.	Cym.	Cym.	Cym.	Cym.	Cym.	Cym.	Cym.
1	95.6	96.1	96.1	96.1	96.2	96.1	96.0	96.1	96.1
2	34.1	35.8[a]	35.7	35.8[e]	36.1[h]	35.8	35.6	35.7	35.8
3	77.5	77.1	77.1	77.2[f]	77.2	77.1	77.1	77.1	77.1
4	72.5[C]	82.5	82.5[c]	82.5	82.7	82.7	82.5	82.5	82.8
5	70.8[A]	68.4[b]	68.3[d]	68.6[g]	68.4	68.4	68.5	68.5	68.4
6	18.3[B]	18.2[B]	18.2[B]	18.3[B]	18.2[B]	18.2[B]	18.2[B]	18.3[B]	18.2[B]
		Cym.	Cym.	Cym.	Ole.	Ole.	Cym.	Cym.	Ole.
1	—	99.7	99.7	99.6	101.4	101.4	99.6	99.4	101.5
2	—	35.6[a]	35.7	35.9[e]	36.0[h]	36.4	35.6	33.8	35.4
3	—	77.1	77.1	77.3[f]	78.9	79.2	77.1	77.5	80.7
4	—	82.5	82.6[c]	81.6	81.5	82.3	82.6	72.5[C]	75.5
5	—	68.6[b]	68.6[d]	68.7[g]	71.7	71.0[A]	68.3	70.7[A]	71.6
6	—	18.2[B]	18.2[B]	18.2[B]	18.4[B]	18.4[B]	18.2[B]	18.2[B]	18.0[B]
		Ole.	Ole.	Cym.	Cym.	Ole.	Ole.		
1	—	101.4	101.4	98.3	96.9	100.2	101.4	—	—
2	—	36.1	36.4	31.0	31.0	35.5	35.4	—	—
3	—	78.8	79.2	74.8	75.1	80.8	80.6	—	—
4	—	81.4	82.3	72.2	72.2	75.5	75.4	—	—
5	—	71.7	71.0[A]	65.7	65.3	71.7	71.5	—	—
6	—	18.4[B]	18.2[B]	18.0[B]	17.8	18.0[B]	17.9	—	—
		Cym.	Ole.						
1	—	96.9	100.2	—	—	—	—	—	—
2	—	31.0	35.5	—	—	—	—	—	—
3	—	75.1	80.8	—	—	—	—	—	—
4	—	72.2	75.5	—	—	—	—	—	—
5	—	65.3	71.7	—	—	—	—	—	—
6	—	17.8	17.9	—	—	—	—	—	—
OMes	57.2	56.2	56.3	56.2	56.2	56.3	56.2	57.2	56.3
		56.3	56.7	58.1 × 2	56.3	56.7	58.0	58.0	58.2
		58.0	58.0		58.2	58.2	58.2		
		58.4	58.3						

Measured at 100.40 and 125.65 MHz in $CDCl_3$ solution at 35°.

[a–h]Assignments may be interchanged in each column.

[A–C]Assignments may be interchanged between Tables 1 and 2.

Table 3. ^{1}H NMR spectral data of the sugar moieties of compounds **2**–**10** and **12**

H	**2**	**3**	**4, 12**
	Cym.	Cym.	Cym.
1	4.79 1H, *dd* (9.5, 2.0)	4.84 1H, *dd* (9.5, 1.5)	4.85 1H, *dd* (9.5, 2.0)
3	3.63 1H, *q* (3.0)	3.81 1H, *q* (3.0)	3.80 1H, *q* (3.0)
4	3.58*	3.21 1H, *dd* (9.5, 3.0)	3.21 1H, *dd* (9.5, 3.0)
5	3.58*	3.84 1H, *dq* (9.5, 6.5)	3.84 1H, *dq* (9.5, 6.5)
6	1.28 3H, *d* (6.5)	1.21 3H, *d* (6.5)	1.20 3H, *d* (6.5)
		Cym.	Cym.
1	—	4.76 1H, *dd* (9.5, 2.0)	4.75 1H, *dd* (9.5, 2.0)
3	—	3.78 1H, *q* (3.0)	3.78 1H, *q* (3.0)
4	—	3.21 1H, *dd* (9.5, 3.0)	3.21 1H, *dq* (9.5, 3.0)
5	—	3.86 1H, *dq* (9.5, 6.5)	3.86 1H, *dq* (9.5, 6.5)
6	—	1.22 3H, *d* (6.5)	1.21 3H, *d* (6.5)
		Ole.	Ole.
1	—	4.46 1H, *dd* (9.5, 2.0)	4.45 1H, *dd* (9.5, 2.0)
3	—	3.26*	3.37*
4	—	3.12 1H, *t* (9.0)	3.17 1H, *t* (9.0)
5	—	3.27 1H, *dq* (9.0, 6.5)	3.30 1H, *dq* (9.0, 6.5)
6	—	1.27 3H, *d* (6.5)	1.30[a] 3H, *d* (6.5)
		Cym.	Ole
1	—	4.87 1H, *dd* (4.5, 1.5)	4.72 1H, *dd* (9.5, 2.0)
3	—	3.58 1H, *q* (3.5)	3.15*
4	—	3.25*	3.15*
5	—	4.08 1H, *dq* (9.5, 6.5)	3.30 1H, *dq* (9.0, 6.5)
6	—	1.25 3H, *d* (6.5)	1.35[a] 3H, *d* (6.5)
OMes	3.44 3H, *s*	3.35 3H, *s*	3.40 3H, *s* × 2
		3.38 3H, *s*	3.44 3H, *s* × 2
		3.44 3H, *s*	
		3.45 3H, *s*	

Table 3. *Continued*

H	**5**	**6**	**7**
	Cym.	Cym.	Cym.
1	4.85 1H, *dd* (9.5, 1.5)	4.85 1H, *dd* (9.5, 2.0)	4.85 1H, *dd* (9.5, 1.5)
3	3.80 1H, *q* (3.0)	3.79 1H, *q* (3.0)	3.79 1H, *q* (3.0)
4	3.22 1H, *dd* (9.5, 3.0)	3.23 1H, *dd* (9.5, 3.0)	3.22 1H, *dd* (9.5, 3.0)
5	3.84 1H, *dq* (9.5, 6.5)	3.87 1H, *dq* (9.5, 6.5)	3.86 1H, *dq* (9.5, 6.5)
6	1.21 3H, *d* (6.5)	1.22 3H, *d* (6.5)	1.22 3H, *d* (6.5)
	Cym.	Ole.	Ole.
1	4.77 1H, *dd* (9.5, 1.5)	4.46 1H, *dd* (9.5, 2.0)	4.45 1H, *dd* (9.5, 1.5)
3	3.69 1H, *q* (3.0)	3.27*	3.37*
4	3.23 1H, *dd* (9.5, 3.0)	3.12 1H, *t* (9.0)	3.17 1H, *t* (8.5)
5	3.88 1H, *dq* (9.5, 6.5)	3.27 1H, *dq* (9.0, 6.5)	3.31*
6	1.21 3H, *d* (6.5)	1.27 3H, *d* (6.5)	1.30[b] 3H, *d* (6.5)
	Cym.	Cym.	Ole.
1	4.79 1H, *dd* (4.5, 1.5)	4.87 1H, *dd* (4.0, 1.5)	4.72 1H, *dd* (9.5, 2.0)
3	3.58 1H, *q* (3.5)	3.58 1H, *q* (3.5)	3.15*
4	3.28 1H, *dd* (9.0, 3.5)	3.27*	3.15*
5	4.04 1H, *dq* (9.0, 6.5)	4.08 1H, *dq* (9.5, 6.5)	3.31*
6	1.26 3H, *d* (6.5)	1.25 3H, *d* (6.5)	1.34[b], 3H, *d* (6.5)
1	—	—	—
2a	—	—	—
2b	—	—	—
3	—	—	—
4	—	—	—
5	—	—	—
6	—	—	—
OMes	3.39 3H, *s*	3.35 3H, *s*	3.40 3H, *s* × 2
	3.45 3H, *s*	3.38 3H, *s*	3.45 3H, *s*
	3.49 3H, *s*	3.45 3H, *s*	

Table 3. *Continued*

H	8	9	10
	Cym.	Cym.	Cym.
1	4.85 1H, *dd* (9.5, 1.5)	4.86 1H, *dd* (9.5, 2.0)	4.86 1H, *dd* (9.5, 2.0)
3	3.81 1H, *q* (3.0)	3.81 1H, *q* (3.0)	3.82 1H, *q* (3.0)
4	3.21 1H, *dd* (9.5, 3.0)	3.22 1H, *dd* (9.5, 3.0)	3.24 1H, *dd* (9.5, 3.0)
5	3.84 1H, *dq* (9.5, 6.5)	3.86 1H, *dq* (9.5, 6.5)	3.87 1H, *dq* (9.5, 6.5)
6	1.22 3H, *d* (6.5)	1.22 3H, *d* (6.5)	1.23 3H, *d* (6.5)
	Cym.	Cym.	Ole.
1	4.76 1H, *dd* (9.5, 1.5)	4.68 1H, *dd* (9.5, 2.0)	4.50 1H, *dd* (9.5, 2.0)
3	3.81 1H, *q* (3.0)	3.62 1H, *q* (3.0)	3.17*
4	3.23 1H, *dd* (9.5, 3.0)	3.18 1H, *dd* (9.5, 3.0)	3.13 1H, *t* (8.5)
5	3.87 1H, *dq* (9.5, 6.5)	3.56 1H, *dq* (9.5, 6.5)	3.29 1H, *dq* (8.5, 6.5)
6	1.21 3H, *d* (6.5)	1.28 3H, *d* (6.5)	1.32 3H, *d* (6.5)
	Ole.		
1	4.50 1H, *dd* (9.5, 1.5)	—	—
3	3.17 1H, *ddd* (12.5, 9.0, 4.5)	—	—
4	3.12 1H, *t* (9.0)	—	—
5	3.28 1H, *dq* (9.0, 6.5)	—	—
6	1.32 3H, *d* (6.5)	—	—
1	—	—	—
3	—	—	—
4	—	—	—
5	—	—	—
6	—	—	—
OMes	3.39 3H, *s*	3.43 3H, *s*	3.39 3H, *s*
	3.45 3H, *s* × 2	3.45 3H, *s*	3.46 3H, *s*

Run at 400 and 500 MHz in $CDCl_3$ solution at 35°.

Signal assignments were done based on the consequences of 2D-NMR (HMQC/C-H COSY, HMBC and COSY) and the decoupling experiments.

*Overlapping with other signals.

[a,b]Assignments may be interchanged in each column.

8.76. Found: C, 59.22; H, 8.79. FAB-MS *m/z*: 815 $[M + H]^+$, 837 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **6** Amorphous powder $[\alpha]_D^{26}$ + 4.5° (MeOH; *c* 0.33). FAB-MS *m/z*: 815 $[M + H]^+$, 837 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **7** Amorphous powder $[\alpha]_D^{26}$ + 32.0° (MeOH; *c* 0.38). FAB-MS *m/z*: 815 $[M + H]^+$, 837 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **8**. Amorphous powder $[\alpha]_D^{26}$ + 51.8° (MeOH; *c* 0.35). Calcd for $C_{42}H_{70}O_{15}\cdot 2H_2O$: C, 59.28; H, 8.76. Found: C, 59.42; H, 8.94. FAB-MS *m/z*: 815 $[M + H]^+$, 837 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **9**. Amorphous powder $[\alpha]_D^{26}$ + 40.5° ($CHCl_3$; *c* 0.55). Calcd for $C_{35}H_{58}O_{12}\cdot 2H_2O$: C, 59.47; H, 8.84. Found: C, 59.41; H, 8.88. FAB-MS *m/z*: 671 $[M + H]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **10**. Amorphous powder $[\alpha]_D^{26}$ + 41.4° (MeOH; *c* 0.35). Calcd for $C_{35}H_{58}O_{12}\cdot 2H_2O$: C, 59.47; H, 8.84. Found: C, 59.53; H, 8.94. FAB-MS *m/z*: 671 $[M + H]^+$. ^1H and ^{13}C NMR: Tables 1–3.

Compound **12**. Amorphous powder $[\alpha]_D^{26}$ + 8.2° (MeOH; *c* 0.67). Calcd for $C_{49}H_{80}O_{18}\cdot 2H_2O$: C, 59.26; H, 8.52. Found: C, 59.43; H, 8.54. FAB-MS *m/z*: 957 $[M + H]^+$, 979 $[M + Na]^+$. ^1H and ^{13}C NMR: Tables 1–3. ^{13}C NMR data of aglycone moiety in pyridine-d_5 soln at 35°: δ9.4 (C-18), 18.4[a] (C-19), 27.9 (C-21), 29.5 (C-11), 30.0 (C-2), 32.8, 35.1 (C-15, 16), 34.3 (C-7), 37.4 (C-10), 39.0 (C-4), 39.4 (C-1), 45.0 (C-9), 60.5 (C-13), 69.0 (C-12), 74.3 (C-8), 77.8[b] (C-3), 89.3 (C-14), 92.6 (C-17), 119.5 (C-6), 139.5 (C-5), 209.6 (C-20). ([a,b]Assignments may be interchanged with a part of the signals in sugar moiety at δ18.5[a], 18.6[a], 18.7[a], 18.8[a] and 78.1[b].) The elemental analysis of **6** and **7** was not performed because of the small amounts available.

Acid hydrolysis of compound **8**. Compound **8** (*ca* 11 mg) dissolved in dioxane (1 ml) and 0.2 M H_2SO_4 (5 drops) was heated at 60° for 90 min. After hydrolysis, this reaction mixture was passed through a Mitsubishi Diaion HP-20 column and eluted with H_2O and MeOH. The MeOH eluate was concd to dryness and the residue recrystallized with MeOH–$CHCl_3$ to give sarcostin (**1**) (1 mg), mp 258–262°. $[\alpha]_D^{26}$ + 62.5° (MeOH; *c* 0.36) (lit. mp 262–264° $[\alpha]_D^{25}$ + 63.4°; MeOH; *c* 0.09) [4]. Subsequently, for sugar analysis, the H_2O layer was passed through an Amberlite IR-60E column and the eluate was concd to dryness, and the residue reduced with $NaBH_4$ (*ca* 1 mg) for 1 hr at room temp. The reaction mixture was passed through an Amberlite IR-120B column and the eluate concd to dryness. Boric acid was removed by co-distillation with MeOH, and the residue was acetylated

with Ac_2O and pyridine (1 drop each) at 100° for 1 hr. The reagents were evapd off *in vacuo*. From each glycoside, cymaritol acetate and oleandritol acetate were detected by GC [Conditions: column Supelco SP-2380 capillary column (0.25 mm × 30 m); column temp. 200°; carrier gas N_2; R_t (min); cymaritol acetate 6.4, oleandritol acetate 7.2]. The relative ratio of each monosaccharide was determined based on the peak area,

Acid hydrolysis of compounds **2–7**, **9–10** *and* **12**. Compounds **2–7**, **9–10** and **12** (*ca* 0.3 mg) dissolved in dioxane (4 drops) and 0.2 M H_2SO_4 (1 drop) were heated at 60° for 90 min, and the subsequent reactions were performed as before. Each residue of **2–7**, **9** and **10** was analysed by HPLC to identify the aglycone by comparison with an authentic sample. [Conditions: column YMC-ODS, flow rate 1.0 ml min^{-1}; 40% MeOH in H_2O; R_t (min); sarcostin, 10.8]. The remaining residue was reduced with $NaBH_4$ and then acetylated with pyridine and Ac_2O as before. From each glycoside, cymaritol acetate and oleandritol acetate were identified by GC with the same conditions as described previously.

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REFERENCES

1. Wada, K., Hayashi, K., Mitsuhashi, H. and Bando, H. (1979) *Chem. Pharm. Bull.* **27**, 2252.
2. Wada, K., Hayashi, K., Mitsuhashi, H. and Bando, H. (1982) *Chem. Pharm. Bull.* **30**, 3500.
3. Yamagishi, T., Hayashi, K., Mitsuhashi, H., Imanari, M. and Matsushita, K. (1973) *Tetrahedron Letters* 3527.
4. Kaur, K. J., Khare, A. and Khare, M. P. (1985) *Phytochemistry* **24**, 3007.
5. Tsukamoto, S., Hayashi, K. and Mitsuhashi, H. (1983) *Chem. Pharm. Bull.* **33**, 2294.