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STEROIDAL SAPONINS FROM THE BULBS OF *TRITELEIA LACTEA* AND THEIR INHIBITORY ACTIVITY ON CYCLIC AMP PHOSPHODIESTERASE

YOSHIHIRO MIMAKI,* OSAMU NAKAMURA, YUTAKA SASHIDA,* TAMOTSU NIKAIDO† and TAICHI OHMOTO†

(School of Pharmacy, Tokyo University of Pharmacy and Life Science), 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan; †School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan

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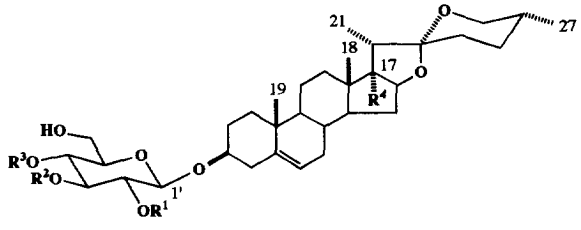
Key Word Index—*Triteleia lactea*; Allioideae; Liliaceae; bulbs; steroidal saponins; spirostanol saponins; cyclic AMP phosphodiesterase inhibition.

Abstract—The chemical compounds in the bulbs of *Triteleia lactea* have been analysed as a part of our systematic study of plants of the subfamily Allioideae in Liliaceae. Thirteen steroidal saponins, seven of which appeared to be new compounds, were isolated. The structures of the new saponins were elucidated on the basis of the spectroscopic analysis, including two-dimensional NMR techniques, and hydrolysis. Inhibitory activity of the isolated saponins on cyclic AMP phosphodiesterase was evaluated to identify new compounds with medicinal potential.

INTRODUCTION

In the course of our phytochemical screening of plants of the subfamily Allioideae in Liliaceae, we have examined the underground parts of *Allium giganteum* [1–3], *A. aflatunense* [2], *A. schubertii* [4, 5], *A. albopilosum* [6], *A. ostrowskianum* [6], *Agapanthus inapertus* [7] and *Ipheion uniflorum* [8], and isolated a considerable number of new steroidal glycosides. The results indicate that the Allioideae plants are a rich source of steroidal glycosides. In a continuation of our studies we investigated the constituents of the bulbs of *Triteleia lactea*, which is also classified in the subfamily Allioideae. This resulted in the isolation of 13 steroidal saponins, including seven new ones. We now describe the structural elucidation of the new saponins. The inhibitory activity of the isolated saponins on cyclic AMP phosphodiesterase was evaluated. This test provides a useful tool for screening of biologically active compounds present in a natural source [9–12].



	R¹	R²	R³	R⁴
1	α-L-Rhap	H	H	H
2	α-L-Rhap	H	H	OH
3	α-L-Rhap	H	α-L-Rhap	H
4	α-L-Rhap	H	α-L-Rhap	OH
5	α-L-Rhap	β-D-Glcp	H	H
6	α-L-Rhap	β-D-Glcp	H	OH
7	α-L-Rhap	β-D-Galp	H	H
7a	H	β-D-Galp	H	H
7b	H	H	H	H
8	α-L-Rhap	β-D-Galp	H	OH

RESULTS AND DISCUSSION

Standard extraction and fractionation of the fresh bulbs of *Triteleia lactea* eventually gave **1**–**13** from the 1-butanol-soluble fraction.

Compounds **1**–**6** were known constituents and identified as (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside} [13, 14], (25*R*)-spirost-5-ene-3β,17α-diol (pennogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside} [13], diosgenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside} (dioscin) [13–15], pennogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside} [15], diosgenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside} [16] and pennogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside} [17], respectively, by comparison of their spectral data and physical properties with literature values.

Compound **7** was obtained as an amorphous powder, $[\alpha]_D$ − 82.0° (methanol). The molecular formula, $C_{45}H_{72}O_{17}$, was estimated by the negative-ion FAB-mass spectrum, which showed a $[M]^-$ ion at *m/z* 884, and

*Authors to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data for **7**, **7a**, **7b**, **8**, **9**, nuatigenin and **10**–**13**

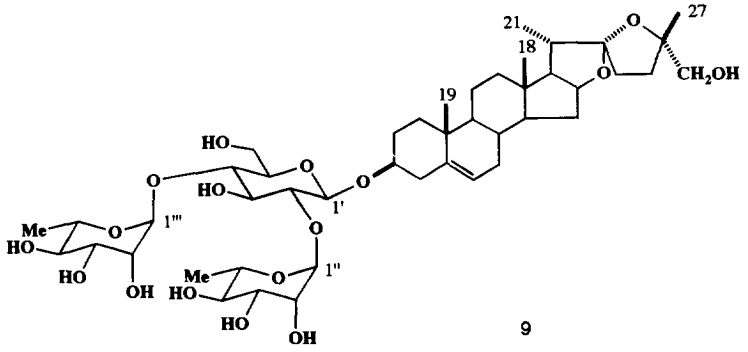
C	**7**	**7a**	**7b**	**8**	**9**	nuati-genin	**10**	**10***	**11**	**12**	**13**
1	37.5	37.4	37.5	37.6	37.5	37.8	45.6	45.5	37.3	45.6	45.6
2	30.1	30.2	30.3	30.1	30.2	31.7	70.5	70.5	29.9	70.4	70.4
3	77.9	78.3	78.2	77.9	78.0	71.3	84.4	84.2	77.6	84.4	84.4
4	38.8	39.3	39.4	38.8	39.0	43.5	34.1	34.0	34.9	34.0	34.0
5	140.8	140.9	141.0	140.8	140.9	142.0	44.7	44.7	44.8	44.6	44.7
6	121.9	121.8	121.7	121.9	121.8	120.3	28.2	28.2	29.0	28.1	28.1
7	32.4[a]	32.3	32.3[a]	32.1	32.3[a]	32.6[a]	32.2	32.2	32.5	32.1	32.1
8	31.8	31.7	31.7	32.4	31.8	32.2	34.7	34.7	35.3	34.6	34.6
9	50.3	50.3	50.3	50.3	50.4	50.5	54.5	54.5	54.5	54.4	54.4
10	37.2	37.1	37.1	37.2	37.2	37.0	36.9	37.0	35.9	36.9	36.9
11	21.1	21.1	21.1	21.0	21.1	21.2	21.5	21.5	21.3	21.5	21.5
12	39.9	39.9	39.9	32.5	39.8	40.0	40.1	40.2	40.2	40.1	40.0
13	40.5	40.5	40.5	45.2	40.6	40.6	40.8	40.9	40.8	40.8	40.8
14	56.7	56.7	56.7	53.1	56.6	56.6	56.4	56.4	56.5	56.4	56.4
15	32.3[a]	32.3	32.2[a]	31.8	32.2[a]	32.3[a]	32.3	32.3	32.2	32.3	32.3
16	81.1	81.1	81.1	90.1	81.1	81.1	81.2	81.2	81.2	81.1	81.5
17	63.0	62.9	62.9	90.2	62.7	62.6	63.1	63.1	63.1	63.0	63.0
18	16.4	16.4	16.4	17.2	16.1	16.2	16.6	16.7	16.6	16.6	16.6
19	19.4	19.4	19.4	19.5	19.4	19.6	13.5	13.5	12.4	13.4	13.4
20	42.0	42.0	42.0	44.8	38.5	38.5	42.0	42.1	42.0	42.0	41.9
21	15.0	15.0	15.0	9.7	15.2	15.2	15.0	15.0	15.0	15.0	15.0
22	109.3	109.3	109.3	109.9	120.3	120.9	109.2	109.4	109.2	109.2	109.4
23	31.9	31.9	31.9	32.1	33.8	33.8	31.9	31.9	31.9	31.9	33.2
24	29.3	29.3	29.3	28.8	32.6	32.6	29.3	29.3	29.3	29.3	29.0
25	30.6	30.6	30.6	30.5	85.7	85.6	30.6	30.7	30.6	30.6	144.5
26	66.9	66.9	66.9	66.7	70.1	70.1	66.9	67.0	66.9	66.9	65.0
27	17.3	17.3	17.3	17.3	24.1	24.1	17.3	17.3	17.3	17.3	108.6
1′	100.0	102.2	102.7	100.0	100.4		103.3	103.2	102.5	103.3	103.3
2′	77.2	74.1	75.4	77.2	78.9		72.6	72.4	73.1	72.6	72.6
3′	89.4	88.8	78.7[b]	89.4	76.9		75.6	75.4	75.6	75.5	75.5[a]
4′	69.7[b]	69.8	71.8	69.7[a]	77.9		78.9	79.0	79.6	79.2	79.2
5′	77.6	78.1	78.5[b]	77.7	78.2		75.8	75.7	75.4	75.7	75.7
6′	62.5	62.5	62.9	62.5	61.4		60.7	60.7	60.8	60.7	60.7
1″	102.3			102.3	102.0		104.3	104.2	104.7	104.0	104.0
2″	72.4			72.4	72.5		80.7	80.8	80.9	80.6	80.6
3″	72.8			72.8	72.8[b]		87.3	87.3	87.3	87.1	87.1
4″	74.2			74.2	74.2		70.4	70.2	70.4	70.4	70.4
5″	69.6[b]			69.6[a]	69.5		77.6	77.5	77.6	77.6	77.6
6″	18.7			18.7	18.5[c]		62.9	62.8	62.9	62.9	62.9
1‴	105.3	106.6		105.3	103.0		104.3	104.2	104.3	104.4	104.4
2‴	72.5	73.1		72.5	72.5		76.3	76.2	76.3	74.7	74.7
3‴	75.3	75.1		75.3	72.9[b]		83.9	83.6	83.6	87.8	87.8
4‴	70.1	70.2		70.1	73.9		69.7	69.6	69.4	69.7	69.7
5‴	77.5	77.4		77.5	70.5		78.0	78.0	78.4	77.8	77.8
6‴	62.1	62.2		62.1	18.6[c]		62.5	62.4	62.4	62.4[a]	62.4[b]
1⁗							102.8	102.8	102.8	105.4	105.4
2⁗							72.3	72.2	72.3	75.5	75.6[a]
3⁗							72.6	72.4	72.6	78.4[b]	78.4[c]
4⁗							74.1	73.9	74.2	71.6	71.6
5⁗							69.8	69.8	69.7	78.0[b]	78.0[c]
1⁗′							18.5	18.5	18.5	62.5[a]	62.5[b]
2⁗′							104.9	104.9	104.9	104.9	104.9
3⁗′							75.3	75.2	75.2	75.2	75.2
4⁗′							78.5	78.3	78.5	78.4	78.4
5⁗′							70.6	70.5	70.6	70.7	70.7
6⁗′							67.2	67.2	67.2	67.2	67.3

Spectra were measured in pyridine-d_5 except for **10*** in pyridine-d_5–methanol-d_4 (10:1).
[a–c]Signals with same superscripts may be reversed in each column.

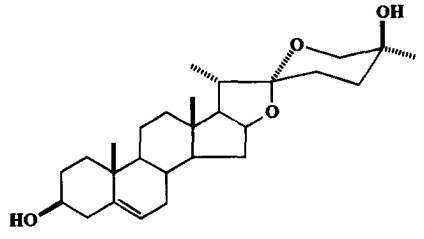
^{13}C NMR spectrum combined with the various DEPT spectra. The ^{1}H NMR spectrum of **7** showed signals for two tertiary methyl groups at δ1.07 and 0.84 (each *s*), three secondary methyl groups at δ1.74 (*d*, J = 6.2 Hz), 1.15 (*d*, J = 6.9 Hz) and 0.71 (*d*, J = 5.1 Hz), three anomeric protons at δ6.34 (*br s*), 5.01 (*d*, J = 7.8 Hz) and 4.94 (*d*, J = 7.2 Hz), and an olefinic proton at δ5.35 (*br d*, J = 4.5 Hz). The signal at δ1.74 was due to the methyl group of 6-deoxyhexopyranose. The above data were quite similar to those of **5**, suggesting **7** to be a diosgenin trisaccharide. Acid hydrolysis of **7** with 1 M hydrochloric acid in dioxane–H_2O (1:1) gave diosgenin, and D-glucose, D-galactose and L-rhamnose in a ratio of 1:1:1. The ^{13}C NMR assignments (Table 1) of the saccharide moiety of **7** were achieved by referring to those of authentic methyl glycosides and taking into account the known effects of *O*-glycosylation [18, 19], indicating the presence of a terminal α-L-rhamnopyranosyl unit (δ102.3, 72.4, 72.8, 74.2, 69.6 and 18.7), a terminal β-D-glalactopyranosyl unit (δ105.3, 72.5, 75.3, 70.1, 77.5 and 62.1) and a 2,3-disubstituted β-D-glucopyranosyl unit (δ100.0, 77.2, 89.4, 69.7, 77.6 and 62.5). The β-configurations of the anomeric centres of the glucose and galactose moieties were supported by the large $^{3}J_{H1-H2}$ values (7 Hz <). The anomeric centre of the rhamnose could not be deduced from the $^{3}J_{H1-H2}$ value. However, the ^{13}C shifts of C-3 and C-5 showed a remarkable difference between methyl α- and β-L-rhamnopyranosides [19]. The above data led to the structure of the saccharide moiety as rhamnosyl-(1 → 2)-[galactosyl-(1 → 3)]-glucose or galactosyl-(1 → 2)-[rhamnosyl-(1 → 3)]-glucose. Mild hydrolysis of **7** with 0.2 M hydrochloric acid at 100° for 1.5 hr gave L-rhamnose and partial hydrolysates (**7a** and **7b**). The ^{13}C NMR spectrum of **7a** showed the presence of a terminal β-D-galactopyranosyl unit (δ106.6, 73.1, 75.1, 70.2, 77.4 and 62.2) and a 3-substituted β-D-glucopyranosyl unit (δ102.2, 74.1, 88.8, 69.8, 78.1 and 62.5). Compound **7b** was identified as diosgenin 3-*O*-β-D-glucopyranoside (trillin) by the ^{1}H and ^{13}C NMR spectra [14]. Thus, the structure of **7** was determined to be diosgenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-galactopyranosyl-(1 → 3)]-β-D-glucopyranoside}.

The negative-ion FAB-mass spectrum of **8** showed a $[M - H]^-$ ion at *m/z* 899, suggesting the molecular formula, $C_{45}H_{72}O_{18}$. The ^{1}H and ^{13}C NMR spectra confirmed that **8** had the same sapogenol structure as **2**, **4** and **6**, and the same oligoside constituent as **7**. The structure of **8** was assigned as pennogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-galactopyranosyl-(1 → 3)-β-D-glucopyranoside}.

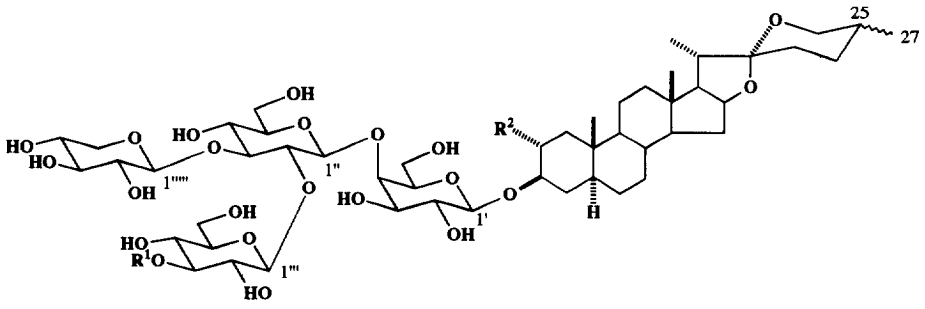
The ^{1}H NMR spectrum of **9** was similar to that of **3** except for the resonance owing to the H-27 methyl protons; δ0.71 (*d*, J = 5.5 Hz) in **3** and δ1.35 (*s*) in **9**. A quaternary carbon signal at δ120.3 in the ^{13}C NMR spectrum of **9** was typical of the 22,25-epoxyfurostanols (furospirostanols) [18]. Acid hydrolysis of **9** gave (25*S*)-spirost-5-ene-3β,25-diol (isonuatigenin) (**9a**), which must be produced as an artefact from (22*S*,25*S*)-22,25-epoxyfurost-5-ene-3β,26-diol (nuatigenin) under acidic conditions [20], and D-glucose and L-rhamnose in a ratio of 1:2. These data were indicative of **9** being a nuatigenin glycoside. The sequence of the monosaccharides was shown to be the same as that of **3** and **4** by the ^{13}C NMR spectrum. On comparison of the ^{13}C assignments of the aglycone part of **9** with those of authentic nuatigenin [20], signals owing to C-3 were shifted downfield by 6.7 ppm, and those due to C-2 and C-4 were upfield by 1.5 and 4.5 ppm, respectively, which accounted for the sac-



9



9a



		R^1	R^2
10	(25*R*)	α-L-Rha*p*	HO
11	(25*R*)	α-L-Rha*p*	H
12	(25*R*)	β-D-Glc*p*	HO
13	[25(27)-dehydro]	β-D-Glc*p*	HO

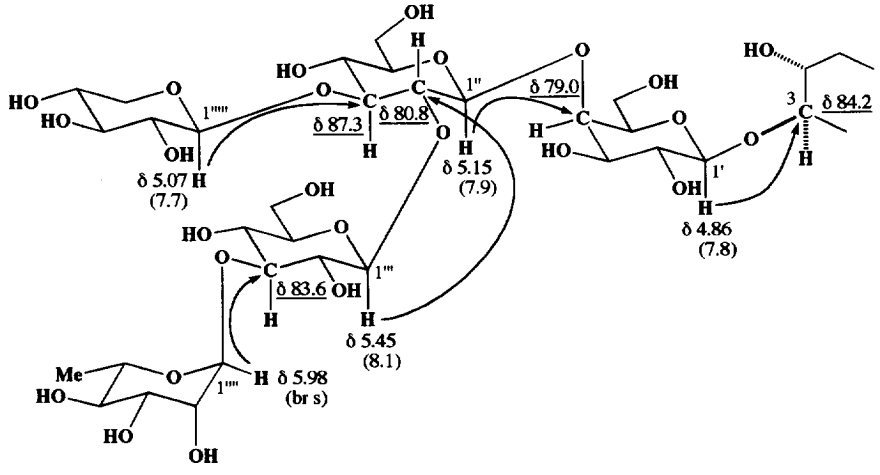


Fig. 1. ^1H–^{13}C long-range correlations of the saccharide moieties of **10** in pyridine-d_5–methanol-d_4 (10:1). *J* values (Hz) in the ^1H NMR spectrum are given in parentheses. Underlined values indicate ^{13}C NMR chemical shifts.

charide part linkage to the C-3β hydroxyl group of nuatigenin. The structure of **9** was formulated as nuatigenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside}.

Compounds **10**–**13** were much more polar constituents than **1**–**9**.

The negative-ion FAB-mass spectrum of compound **10** gave a $[M - H]^-$ ion at *m/z* 1195 corresponding to the molecular formula, $C_{56}H_{92}O_{27}$. The ^1H NMR spectrum showed five anomeric proton signals at δ6.06 (*br s*), 5.49 (*d*, *J* = 8.0 Hz), 5.21 (*d*, *J* = 7.9 Hz), 5.12 (*d*, *J* = 7.5 Hz) and 4.90 (*d*, *J* = 7.2 Hz). On acid hydrolysis of **10**, it liberated an aglycone, identified as (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) [21], and D-glucose, D-galactose, D-xylose and L-rhamnose in a ratio of 2:1:1:1. Based on the above data, **10** was inferred to be gitogenin pentasaccharide. The combined use of the two-dimensional NMR techniques allowed the structural assignments of the pentasacchride moiety without such chemical degradation studies as permethylation followed by hydrolysis or partial hydrolysis, which often consume relatively large amounts of material. The sequential assignments of the ^1H resonances for each monosaccharide were established by the detailed inspection of the ^1H–^1H correlation spectroscopy (COSY) and homonuclear Hartmann–Hahn (HOHAHA) spectra, which were measured in pyridine-d_5–methanol-d_4 (10:1) to minimize signal overlap. Once the protons of each sugar were identified, assignments of the ^{13}C signals were routine using the ^1H-detected heteronuclear multiple quantum coherence (HMQC) spectrum (Table 2). Comparison of the ^{13}C shifts thus assigned with those of the reference methyl glycosides [18, 19] and taking into account the known effect of *O*-glycosylation, and the result of acid hydrolysis indicated that **10** was composed of a terminal α-L-rhamnopyranosyl unit (δ102.8, 72.2, 72.4, 73.9, 69.8 and

Table 2. ^1H and ^{13}C NMR spectral data for the saccharide moiety of **10**

		^1H	^{13}C
Gal	1′	4.86 *d* (7.8)	103.2
	2′	4.42 *dd* (9.5, 7.8)	72.4
	3′	4.08 *dd* (9.5, 3.1)	75.4
	4′	4.53	79.0
	5′	4.01	75.7
	6′	4.49	60.7
		4.14	
Glc	1″	5.15 *d* (7.9)	104.2
	2″	4.19 *dd* (8.4, 7.9)	80.8
	3″	4.02 *dd* (8.4, 8.4)	87.3
	4″	3.73 *dd* (8.4, 8.4)	70.2
	5″	3.74	77.5
	6″	4.41	62.8
		4.00	
Glc	1‴	5.45 *d* (8.1)	104.2
	2‴	3.89 *dd* (9.1, 8.1)	76.2
	3‴	4.14 *dd* (9.1, 9.1)	83.6
	4‴	3.86 *dd* (9.1, 9.1)	69.6
	5‴	3.74	78.0
	6‴	4.41	62.4
		4.32 *dd* (12.1, 5.3)	
Rha	1⁗	5.98 *br s*	102.8
	2⁗	4.58 *br d* (3.3)	72.2
	3⁗	4.39 *dd* (9.4, 3.3)	72.4
	4⁗	4.16 *dd* (9.4, 9.4)	73.9
	5⁗	4.77 *dq* (9.4, 6.2)	69.8
	6⁗	1.57 *d* (6.2)	18.5
Xyl	1⁗′	5.07 *d* (7.7)	104.9
	2⁗′	3.83 *dd* (8.7, 7.7)	75.2
	3⁗′	3.92 *dd* (8.8, 8.7)	78.3
	4⁗′	4.02	70.5
	5⁗′	4.16	67.2
		3.58 *dd* (11.1, 11.1)	

Spectra were measured in pyridine-d_5–methanol-d_4 (10:1). *J* values in parentheses are expressed in Hz.

Table 3. Inhibitory activity on cyclic AMP phosphodiesterase of **1**–**13**

Compound	IC_{50} ($\times 10^{-5}$ M)
1	13.1
2	12.7
3	11.3
4	18.0
5	6.1
6	17.2
7	16.2
8	38.9
9	10.4
10	14.2
11	10.9
12	8.4
13	9.1
Papaverine	3.0

18.5), a terminal β-D-xylopyranosyl unit (δ104.9, 75.2, 78.3, 70.5 and 67.2), 3-substituted β-D-glucopyranosyl unit (δ104.2, 76.2, 83.6, 69.6, 78.0 and 62.4), 2,3-disubstituted β-D-glucopyranosyl unit (δ104.2, 80.8, 87.3, 70.2, 77.5 and 62.8) and 4-substituted β-D-galactopyranosyl unit (δ103.2, 72.4, 75.4, 79.0, 75.7 and 60.7). The β-configurations of the anomeric centres of the glucose, galactose and xylose moieties were supported by the large $^3J_{H1-H2}$ values (7 Hz <). Finally, the $^1H-^{13}C$ long-range correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide or aglycone confirmed the sugar sequence. In the ^1H-detected heteronuclear multiple-bond correlation (HMBC) spectrum, the anomeric proton signals at δ5.98 (rhamnose), 5.45 (3-substituted glucose), 5.15 (2,3-disubstituted glucose), 5.07 (xylose) and 4.86 (4-substituted galactose) showed correlations with the carbon signals at δ83.6 (C-3 of 3-substituted glucose), 80.8 (C-2 of 2,3-disubstituted glucose), 79.0 (C-4 of 4-substituted galactose), 87.3 (C-3 of 2,3-disubstituted glucose) and 84.2 (C-3 of aglycone), respectively. Thus, the structure of the saccharide moiety was solved, and the full structure of **10** was formulated as gitogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

Acid hydrolysis of **11** gave an aglycone, identified as (25*R*)-5α-spirostan-3β-ol (tigogenin) [22], and D-glucose, D-galactose, D-xylose and L-rhamnose in a ratio of 2:1:1:1. The ^1H and ^{13}C NMR spectra proved **11** to have the same saccharide sequence as **10**. The structure of **11** was determined to be tigogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

Acid hydrolysis of **12** gave gitogenin, and D-glucose, D-galactose and D-xylose in a ratio of 3:1:1. On comparison of the ^{13}C NMR spectrum of **12** with that of **10**, a set of signals corresponding to a terminal β-D-glucopyranosyl unit appeared instead of the signals assignable to a terminal α-L-rhamnopyranosyl unit; all other signals remained unaffected. These data led to assurance of the saccharide structure as glucosyl-(1 → 3)-glucosyl-(1 → 2)-[xylosyl-(1 → 3)]-glucosyl-(1 → 4)-galactose, which was well supported by agreement of the ^1H and ^{13}C signals owing to the saccharide part of **12** with those of diurnoside 1 from *Cestrum diurnum* [23]. The structure of **12** was confirmed to be gitogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

All spectral data of **13** were almost superimposable with those of **12**. Lack of the C-27 methyl group and the presence of an exomethylene group, as compared with **12**, were the only differences recognizable in the ^1H and ^{13}C NMR spectra of **13** [^1H NMR: δ4.82 and 4.78 (each H, *br s*, $C=CH_2$); ^{13}C NMR: δ144.5 ($C=CH_2$) and 108.6 ($C=CH_2$)], indicating that **13** was a 25(27)-dehydro derivative of **12**. The structure of **13** was assigned as spirost-25(27)-ene-2α,3β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 3)-

O-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

Compounds **7**–**13** are new steroidal saponins. The isolated saponins were evaluated for inhibitory activity on cyclic AMP phosphodiesterase (Table 3) [9–12]. The diosgenin glycosides with a 2,3-branched or 2,4-branched trisaccharide (**3**, **5** and **7**) exhibited IC_{50} values of 11.3, 6.1 and 16.2×10^{-5} M, respectively, and introduction of a hydroxyl group at C-17α reduced their activities approximately by half [**4**: 18.0; **6**: 17.2; **8**: 38.9 ($\times 10^{-5}$ M)], while the diosgenin diglycoside (**1**) and its 17α-hydroxy derivative (**2**) had almost equal activity [**1**: 13.1; **2**: 12.7 ($\times 10^{-5}$ M)]. The saponins bearing a pentasaccharide also showed considerable activity [**10**: 14.2; **11**: 10.9; **12**: 8.4; **13**: 9.1 ($\times 10^{-5}$ M)]. However, their activities were not always more potent than those of the saponins with a disaccharide or a trisaccharide, suggesting that the number of sugar moieties is not essential to the activity.

EXPERIMENTAL

General. NMR (ppm, *J* Hz): 1D (Bruker AM-400) and 2D (Bruker AM-500); CC: silica gel (Fuji-Silysia Chemical), ODS silica gel (Nacalai Tesque) and Diaion HP-20 (Mitsubishi-Kasei); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck); HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-Kasei-Kogyo, 10 mm i.d. × 250 mm, ODS, 5 μm) for prep. HPLC or with a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for the analyt. HPLC.

Chemicals. Beef heart phosphodiesterase: Boehringer. Snake venom nucleotidase and cyclic AMP: Sigma. [^{3}H]Cyclic AMP: Radiochemical Center.

Extraction and isolation. Fresh bulbs of *T. lactea* (4.4 kg) purchased from Heiwaen, Japan, were cut into pieces and exhaustively extracted with hot MeOH. The MeOH extract was concd under red. pres., and the viscous concentrate was partitioned between H_2O and *n*-BuOH. CC of the *n*-BuOH extract on silica gel and elution with CH_2Cl_2–MeOH, with an increasing proportion of MeOH (6:1; 4:1; 2:1), and finally with MeOH, gave three frs (I–III).

Fr. II was passed through a Diaion HP-20 column with H_2O gradually enriched with MeOH. Chromatography of the MeOH eluate fr. on ODS silica gel CC with MeOH–H_2O (4:1) and silica gel with $CHCl_3$–MeOH–H_2O (50:10:1) to yield **2** (629 mg) and **4** (462 mg) as pure compounds, and **1**, **3** and **9** with a few impurities. Final purification of **1**, **3** and **9** was carried out by prep. HPLC with MeOH–H_2O (87:13; 83:17); **1** (45 mg), **3** (35 mg) and **9** (10 mg).

Fr. III was subjected to a Diaion HP-20 CC and the MeOH eluate fr. was chromatographed on ODS silica gel CC with MeOH–H_2O (4:1; 7:3) and silica gel with $CHCl_3$–MeOH–H_2O (40:10:1; 30:10:1) to give **5** (16 mg), **6** (39 mg), **7** (201 mg), **8** (579 mg), **11** (36 mg), **12** (128 mg) and **13** (32.9 mg) as pure compounds, and **10** as almost pure compound. Compound **10** was purified by prep. HPLC with MeOH–H_2O (83:17); **10** (76 mg).

Compound **7**. Amorphous powder, $[\alpha]_D^{26}$ −82.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 884 $[M]^-$, 722 $[M - \text{galactosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1445, 1375, 1240, 1115, 1040, 980, 960, 915, 895, 860 [intensity 915 < 895, (25*R*)-spiroacetal]. ^{1}H NMR (pyridine-d_5): δ6.34 (1H, *br s*, H-1″), 5.35 (1H, *br d*, *J* = 4.5 Hz, H-6), 5.01 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.94 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.59 (1H, *dd*, *J* = 10.4, 2.6 Hz, H-26a), 3.51 (1H, *dd*, *J* = 10.4, 10.4 Hz, H-26b), 1.74 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.15 (3H, *d*, *J* = 6.9 Hz, H-21), 1.07 (3H, *s*, H-19), 0.84 (3H, *s*, H-18), 0.71 (3H, *d*, *J* = 5.1 Hz, H-27).

Acid hydrolysis of compound **7**. A soln of **7** (10 mg) in 1 M HCl (dioxane–H_2O, 1:1) was heated at 100° for 3 hr under an Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and fractionated by Sep-Pak C_{18} cartridge (Waters) eluting with H_2O–MeOH (4:1) followed by MeOH to give the sugar fr. and the sapogenin fr. From the sapogenin fr., diosgenin (3.9 mg) was obtained as the genuine aglycone. The sugar fr. (3.5 mg) was diluted with H_2O (1 ml) and treated with (−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 ml) at 40° for 4 hr, followed by acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixt. was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4:1, 10 ml; 1:1, 10 ml; 1:9, 10 ml). The H_2O–MeCN (1:9) eluate fr. was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a mixture of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analysed by HPLC [24, 25]. Derivatives of D-glucose, D-galactose and L-rhamnose were detected in a ratio of 1:1:1.

Partial hydrolysis of compound **7**. A soln of **7** (40 mg) in 0.2 M HCl (dioxane–H_2O, 1:1) (6 ml) was heated at 100° for 1.5 hr under an Ar atmosphere. The reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and purified by silica gel CC with $CHCl_3$–MeOH–H_2O (45:10:1) to give partial hydrolysates, **7a** (6.3 mg) and **7b** (8.9 mg). Compound **7a**: amorphous powder, $[\alpha]_D^{27}$ −34.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FAB-MS *m/z*: 738 $[M]^-$, 575 $[M - \text{galactosyl}]^-$. IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2935 (CH), 1450, 1375, 1240, 1120, 1065, 1050, 980, 960, 920, 900, 865 [intensity 920 < 900, (25*R*)-spiroacetal]. ^{1}H NMR (pyridine-d_5): δ5.34 (1H, *br d*, *J* = 4.5 Hz, H-6), 5.27 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.99 (1H, *d*, *J* = 7.7 Hz, H-1′), 3.59 (1H, *dd*, *J* = 10.5, 3.2 Hz, H-26a), 3.51 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 1.15 (3H, *d*, *J* = 6.9 Hz, H-21), 0.91 (3H, *s*, H-19), 0.84 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.3 Hz, H-27).

Compound **8**. Amorphous powder, $[\alpha]_D^{26}$ −90.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 899 $[M - H]^-$, 754 $[M - \text{rhamnosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3445 (OH), 2945 (CH), 1455, 1375, 1245, 1150, 1050, 975, 955, 915, 900, 890. ^{1}H NMR (pyridine-d_5): δ6.34 (1H, *br s*, H-1″), 5.33 (1H, *br d*, *J* = 4.7 Hz, H-6), 5.01 (1H, *d*, *J*

= 7.8 Hz, H-1‴), 4.92 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.52 (2H, *br d*, *J* = 7.3 Hz, H-26), 1.74 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.23 (3H, *d*, *J* = 7.2 Hz, H-21), 1.01 (3H, *s*, H-19), 0.97 (3H, *s*, H-18), 0.69 (3H, *d*, *J* = 5.6 Hz, H-27).

Compound **9**. Amorphous powder, $[\alpha]_D^{26}$ −70.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 884 $[M]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930 (CH), 1455, 1375, 1115, 1035. ^1H NMR (pyridine-d_5): δ6.36 (1H, *br s*, H-1″), 5.82 (1H, *br s*, H-1‴), 5.31 (1H, *br d*, *J* = 5.2 Hz, H-6), 4.93 (1H, *d*, *J* = 7.9 Hz, H-1′), 1.76 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.62 (3H, *d*, *J* = 6.2 Hz, H-6‴), 1.35 (3H, *s*, H-27), 1.10 (3H, *d*, *J* = 6.9 Hz, H-21), 1.05 (3H, *s*, H-19), 0.81 (3H, *s*, H-18).

Compound **10**. Amorphous powder, $[\alpha]_D^{26}$ −74.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 1195 $[M - H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2940 (CH), 1445, 1375, 1240, 1065, 1035, 975, 920, 895, 860 (intensity 920 < 895, (25*R*)-spiroacetal). ^1H NMR (pyridine-d_5): δ6.06 (1H, *br s*, H-1⁗), 5.49 (1H, *d*, *J* = 8.0 Hz, H-1‴), 5.21 (1H, *d*, *J* = 7.9 Hz, H-1″), 5.12 (1H, *d*, *J* = 7.5 Hz, H-1⁗), 4.90 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.58 (1H, *dd*, *J* = 10.6, 3.3 Hz, H-26a), 3.50 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-26b), 1.61 (3H, *d*, *J* = 6.2 Hz, H-6⁗), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 0.82 (3H, *s*, H-18), 0.74 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 5.5 Hz, H-27). ^1H NMR (pyridine-d_5–methanol-d_4, 10:1): δ1.11 (3H, *d*, *J* = 6.9 Hz, H-21), 0.80 (3H, *s*, H-18), 0.74 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 5.9 Hz, H-27). Signals for the saccharide moiety are shown in Table 2.

Compound **11**. Amorphous powder, $[\alpha]_D^{26}$ −48.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 1179 $[M - H]^-$, 1046 $[M - xylosyl]^-$, 869 $[M - rhamnosyl - glucosyl]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1450, 1375, 1240, 1150, 1065, 1040, 980, 920, 895, 860 [intensity 920 < 895, (25*R*)-spiroacetal]. ^1H NMR (pyridine-d_5): δ6.07 (1H, *br s*, H-1⁗), 5.48 (1H, *d*, *J* = 8.0 Hz, H-1‴), 5.16 (1H, *d*, *J* = 7.9 Hz, H-1″), 5.12 (1H, *d*, *J* = 7.5 Hz, H-1⁗), 4.86 (1H, *d*, *J* = 7.3 Hz, H-1′), 3.59 (1H, *dd*, *J* = 10.5, 3.0 Hz, H-26a), 3.51 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 1.62 (3H, *d*, *J* = 6.2 Hz, H-6⁗), 1.14 (3H, *d*, *J* = 6.9 Hz, H-21), 0.83 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 5.5 Hz, H-27), 0.68 (3H, *s*, H-18).

Compound **12**. Amorphous powder, $[\alpha]_D^{26}$ −58.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 1211 $[M - H]^-$, 1080 $[M - xylosyl]^-$, 887 $[M - glucosyl \times 2]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2935 (CH), 1375, 1240, 1155, 1070, 1040, 980, 920, 895, 860 [intensity 920 < 895, (25*R*)-spiroacetal]. ^1H NMR (pyridine-d_5): δ5.58 (1H, *d*, *J* = 7.3 Hz, H-1‴), 5.21 (1H, *d*, *J* = 7.9 Hz, H-1⁗), 5.17 (1H, *d*, *J* = 7.7 Hz, H-1″), 5.10 (1H, *d*, *J* = 7.8 Hz, H-1⁗), 4.91 (1H, *d*, *J* = 8.2 Hz, H-1′), 3.59 (1H, *dd*, *J* = 10.5, 2.9 Hz, H-26a), 3.50 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 0.81 (3H, *s*, H-18), 0.71 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 5.2 Hz, H-27).

Compound **13**. Amorphous powder, $[\alpha]_D^{26}$ −44.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z*: 1209 $[M - H]^-$, 1079 $[M - xylosyl]^-$, 885 $[M - glucosyl \times 2]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2930 (CH), 1370, 1155, 1070, 1035, 920, 890. ^1H NMR (pyridine-d_5): δ5.58 (1H, *d*, *J* = 7.3 Hz, H-1‴), 5.21 (1H, *d*, *J* = 7.9 Hz, H-1⁗), 5.17 (1H, *d*, *J* = 7.7 Hz, H-1″), 5.10 (1H, *d*, *J* = 7.8 Hz, H-1⁗), 4.92 (overlapping with H_2O signal, H-1′), 4.82 and 4.78 (each 1H, *br s*, H-27), 1.09 (3H, *d*, *J* = 6.9 Hz, H-21), 0.81 (3H, *s*, H-18), 0.72 (3H, *s*, H-19).

Acid hydrolysis of compounds **9**, **10**, **11** *and* **12**. Compounds **9** (4 mg), **10** (6.4 mg), **11** (6.2 mg) and **12** (8 mg) were subjected to acid hydrolysis as in the case of **7**. Compound **9** gave isonuatigenin (1.7 mg), and D-glucose and L-rhamnose (1:2), **10** gave gitogenin (2.1 mg), and D-glucose, D-galactose, D-xylose and L-rhamnose (2:1:1:1), **11** gave tigogenin (2.2 mg), and D-glucose, D-galactose, D-xylose and L-rhamnose (2:1:1:1), and **12** gave gitogenin (2.2 mg), and D-glucose, D-galactose and D-xylose (3:1:1).

Assay of cAMP phosphodiesterase activity. The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously [10, 11]. The assay consisted of a two-step isotopic procedure. Tritium-labelled cAMP was hydrolysed to 5′-AMP by phosphodiesterase and the 5′-AMP was then further hydrolysed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; BIO-RAD) to adsorb all charged nucleotides and to leave [^3H]adenosine as the only labelled compound to be counted.

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