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STEROIDAL SAPONINS FROM THE RHIZOMES OF *SMILAX SIEBOLDII*

SATOSHI KUBO, YOSHIHIRO MIMAKI, YUTAKA SASHIDA,* TAMOTSU NIKAIDO† and TAICHI OHMOTO†

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan; †Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan

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Key Word Index—*Smilax sieboldii*; Liliaceae; steroidal saponins; spirostanol glycosides; furostanol glycosides; laxogenin glycosides; tigogenin glycoside; cAMP phosphodiesterase; inhibitory activity.

Abstract—Six new steroidal saponins were isolated from the rhizomes of *Smilax sieboldii*. Their structures were determined by spectroscopic analysis and hydrolysis to be 3β-hydroxy-(25*R*)-5α-spirostan-6-one (laxogenin) 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L- arabinopyranosyl-(1→6)]-β-D-glucopyranoside, laxogenin 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside, 3β,27-dihydroxy-(25*S*)-5α-spirostan-6-one 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside, 26-*O*-β-D-glucopyranosyl-3β,22ξ,26-trihydroxy-(25*R*)-5α-furostan-6-one 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside, 26-*O*-β-D-glucopyranosyl-3β,22ξ,26-trihydroxy-(25*R*)-5α-furostan-6-one 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside and (25*R*)-5α-spirostan-3β-ol (tigogenin) 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside. The inhibition of cAMP phosphodiesterase by the saponins was evaluated.

INTRODUCTION

A number of *Smilax* plants have long been used in traditional medicine throughout the world [1, 2]. The occurrence of steroidal saponins in the plants is well documented [3]. In our previous paper [4], we reported on the isolation and structural determination of new steroidal saponins from *Smilax riparia* and *S. china*. We also investigated the constituents of the rhizomes of *S. sieboldii*, a climbing shrub native to Japan, and isolated six new steroidal saponins. In this paper we report the structural elucidation of the new saponins and the inhibitory effects of the saponins on cAMP phosphodiesterase.

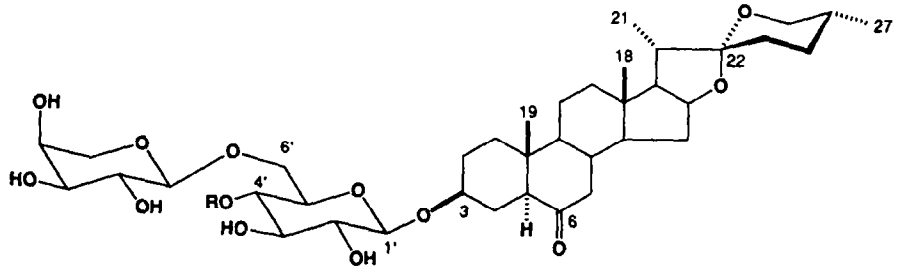
RESULTS AND DISCUSSION

The methanolic extract of the rhizomes of *S. sieboldii* was partitioned between chloroform and H_2O, and then between *n*-butanol and H_2O. A series of chromatographic separations of the chloroform-soluble phase, *n*-butanol-soluble phase and H_2O-residual phase furnished compounds **1**, **2** and **6**, **1** and **3**–**5**, and **1**, respectively.

Compound **1** was assigned the molecular formula $C_{44}H_{70}O_{18}$ {secondary ion (SI) mass spectrum *m/z* 909 $[M+Na]^+$ and 885 $[M-H]^+$ and elemental analysis}. The IR spectrum of **1** was consistent with the presence of hydroxyl group(s) (3400 cm^{-1}), a carbonyl group (1705 cm^{-1}) and a (25*R*)-spiroacetal moiety (985, 915, 895 and 860 cm^{-1}, intensity 915 < 895) [5–7]. The existence of a carbonyl group was also indicated by the UV (λ_{max} 284 nm, ε 109) and ^{13}C NMR (δ209.6) spectra. The ^1H NMR spectrum exhibited signals for two tertiary methyl protons at δ0.79 and 0.64, two secondary methyl protons at δ1.15 (*d*, *J* = 6.9 Hz) and 0.70 (*d*, *J* = 5.8 Hz), and three anomeric protons at δ5.51 (*d*, *J* = 7.9 Hz), 5.09 (*d*, *J* = 7.4 Hz) and 4.93 (*d*, *J* = 7.8 Hz). The above data suggested **1** to be a (25*R*)-spirostanol trisaccharide. On acid hydrolysis of **1** with 1 M hydrochloric acid in dioxane–H_2O (1:1), **1** was hydrolysed to give D-glucose and L-arabinose as the carbohydrate compounds, and an aglycone (**1a**), the structure of which was identified as 3β-hydroxy-(25*R*)-5α-spirostan-6-one (laxogenin) by its physical and spectral data [8]. The structure of the trisaccharide was determined by the following data. The measurement of the ^1H NMR spectrum in pyridine-d_5 minimized signal overlap and the proton spin system for each individual sugar was clarified by the ^1H–^1H COSY spectrum. Once the protons of each sugar were assigned, identification of the carbon signals was carried out by tracing out the one-bond ^1H–^{13}C connectivites through the use of the ^1H–^{13}C COSY spectrum. Comparison of the carbon assignments with those of reference methyl glycosides [9] indicated the presence of a terminal β-D-glucopyranosyl unit, a terminal α-L-arabinopyranosyl unit and an inner β-D-glucopyranosyl unit branched at the C-4 and C-6 positions. In the long-range ^1H–^{13}C COSY (COLOC) spectrum, the anomeric proton signal at δ5.51 (terminal glucose), 5.09 (terminal arabinose) and 4.93 (inner glucose) showed correlations with the carbon signals at δ81.1 (inner glucose C-4), 68.4 (inner glucose C-6) and 77.0 (aglycone C-3), respectively. Thus, the structure of the trisaccharide was determined to be β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranose and the full structure of **1** to be laxogenin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **2**, $C_{38}H_{60}O_{13}$ (SI mass spectrum: *m/z* 763 $[M+K]^+$ and 747 $[M+Na]^+$), yielded, like compound **1**, D-glucose, L-arabinose and laxogenin (**1a**) on acid hydrolysis. The ^{13}C NMR spectrum of **2** indicated the

*Author to whom correspondence should be addresed.



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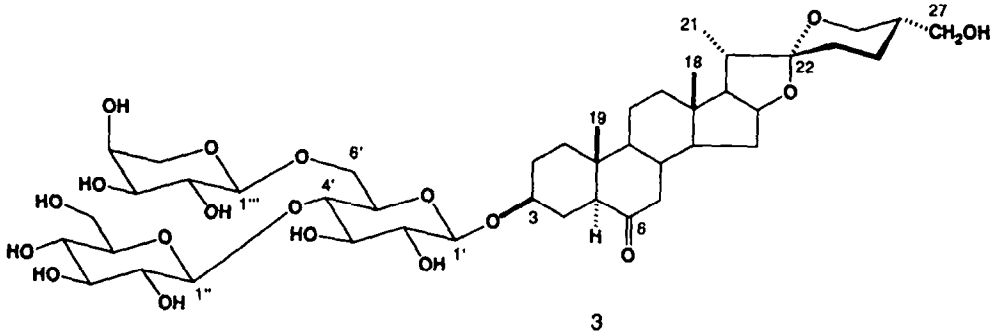
2 H



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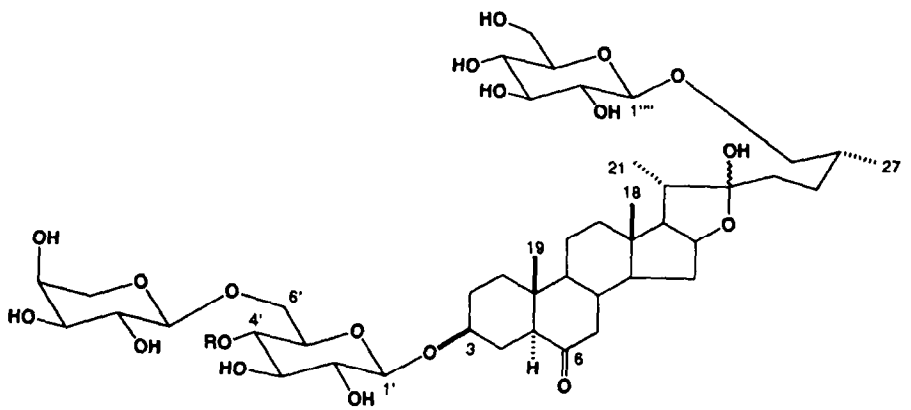

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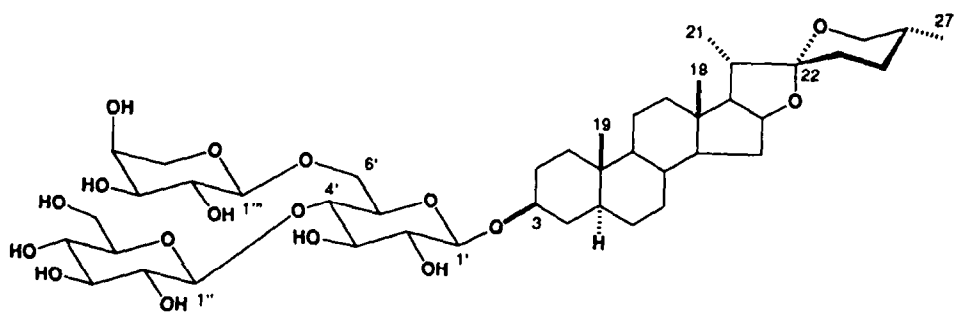
5 Glc



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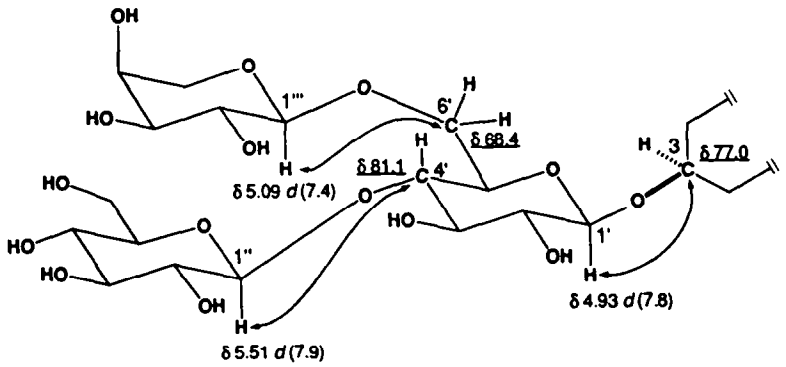

Fig. 1. ^1H–^{13}C long-range correlations of **1** in pyridine-d_5. J values (Hz) in the ^1H NMR spectrum are given in parentheses. Underlined figures indicate the ^{13}C NMR chemical shifts.

presence of α-L-arabinopyranosyl unit as a terminal monosaccharide and also established that this sugar was attached at the C-6 position of the inner β-D-glucopyranosyl unit. The structure of **2** was thus determined to be laxogenin 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **3**, $C_{44}H_{70}O_{19}$ (SI mass spectrum, *m/z* 925 $[M+Na]^+$ and 903 $[M+H]^+$), was shown to contain the same oligoside constituent as **1** by ^1H and ^{13}C NMR. Acid hydrolysis of **3** yielded D-glucose, L-arabinose and an aglycone (**3a**), $C_{27}H_{42}O_5$ (EI mass spectrum, *m/z* 446 $[M]^+$). The IR spectrum of **3a** displayed absorption bands of hydroxyl group(s) (3406 cm^{-1}) and a carbonyl group (1702 cm^{-1}). The ^1H NMR spectrum of **3a** resembled that of laxogenin (**1a**) with resonances for the H-18, H-19 and H-21 methyl groups at δ0.83 (*s*), 0.78 (*s*) and δ1.18 (*d*, $J=6.9$ Hz), respectively. However, the H-27 methyl doublet signal observed in the ^1H NMR spectrum of laxogenin (**1a**) at δ0.70 (*d*, $J=5.8$ Hz) was absent from that of **3a** and was replaced by the oxymethylene signals at δ3.73 (*dd*, $J=11.6$, 4.9 Hz) and 3.66 (*dd*, $J=11.6$, 7.8 Hz). The above data indicated that C-27 which is present as a methyl group in **1a** was modified to a hydroxymethyl group in **3a**. This was also supported by the fragment ion peaks at *m/z* 155 (base peak) and 131 in the EI mass spectrum [10]. The (25*S*)-configuration of the molecule was confirmed by the ^1H NMR parameters of the H-26 methylene protons at δ4.16 (*dd*, $J_{26eq,26ax}=11.1$ Hz, $J_{26eq,25ax}=3.8$ Hz) and 3.89 (*dd*, $J_{26ax,26eq}=11.1$ Hz, $J_{26ax,25ax}=11.1$ Hz). The structure of **3a** was elucidated as 3β,27-dihydroxy-(25*S*)-5α-spirostan-6-one. In the ^{13}C NMR spectrum of **3**, the single due to the C-3 was shifted downfield by 7.0 ppm, whereas the signals due to the C-2 and C-4 were shifted upfield by 1.8 and 4.8 ppm, respectively, as compared with those of **3a**. Thus, the sugar moiety was concluded to be attached at the C-3 hydroxyl group of the aglycone and the full structure of **3** was determined to be 3β,27-dihydroxy-(25*S*)-5α-spirostan-6-one 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **4**, $C_{44}H_{72}O_{19}$ (SI mass spectrum, *m/z* 927 $[M+Na]^+$) and **5**, $C_{50}H_{82}O_{24}$ (SI mass spectrum, *m/z* 1089 $[M+Na]^+$), were deduced to be 22-hydroxyfurostanol glycosides based on the IR, ^1H and ^{13}C NMR spectra. On enzymatic hydrolysis with β-glucosidase, **4** yielded **2** and D-glucose, and **5** yielded **1** and D-glucose.

Table 1. Inhibitory activity on cAMP phosphodiesterase of compounds **1–6**

	IC_{50} ($\times 10^{-5}$ M)
1	8.3
2	3.4
3	>500
4	>500
5	>500
6	3.2

The structures of **4** and **5** were characterized as 26-*O*-β-D-glucopyranosyl-3β,22ξ,26-trihydroxy-(25*R*)-5α-furostan-6-one 3-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside and 26-*O*-β-D-glucopyranosyl-3β,22ξ,26-trihydroxy-(25*R*)-5α-furostan-6-one 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **6**, $C_{44}H_{72}O_{17}$ (SI mass spectrum, *m/z* 895 $[M+Na]^+$), yielded D-glucose, L-arabinose and an aglycone (**6a**), identified as (25*R*)-5α-spirostan-3β-ol (tigogenin). The ^{13}C NMR spectrum of **6** confirmed that the structure of the sugar moiety was the same as that of **1**. The structure of **6** was characterized as tigogenin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside. Compounds **1–6** are new steroidal saponins.

The saponins were tested for cAMP phosphodiesterase inhibition [11, 12]. The IC_{50} inhibitory concentrations are indicated in Table 1. Compounds **1**, **2** and **6** showed considerable activity. Compounds **4** and **5**, which are the corresponding furostanol glycosides of **2** and **1**, respectively, and **3**, a 27-hydroxy derivative of **1**, showed no activity. The saponins isolated from other Liliaceae plants are now being assayed.

EXPERIMENTAL

General. NMR: 1D (Bruker AM-400) and 2D (Bruker AM-500); CC: silica gel (Fuji Davison), ODS (Nacalai Tesque), Sephadex LH-20 (Pharmacia) 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: Tosoh HPLC system (Tosoh: pump, Tosoh CCPM; detector, Tosoh RI-8010; controller, CCP controller PX-8010) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei, 10 i.d. × 250 mm, ODS 5 μm).

Plant material. The rhizomes of *S. sieboldii* were collected at Minamitsuru, Yamanashi prefecture, Japan. A voucher specimen is on file in our laboratory.

Extraction and isolation. The dried rhizomes of *S. sieboldii* (4.2 kg) were cut into pieces and extracted with MeOH under reflux. The extract was concd to almost dryness under red. pres., and the crude residue, after dilution with H_2O, was extracted with $CHCl_3$ and then with *n*-BuOH. The $CHCl_3$-sol. phase was fractionated on a silica gel column with a gradient mixt. of $CHCl_3$–MeOH (19:1, 9:1, 6:1, 4:1, 2:1) to give 6 fractions. Frs 4 and 5 were combined and chromatographed on Diaion HP-20 with an increasing amount of MeOH in H_2O. Further fractionation of the 80% MeOH and MeOH eluate frs on a Sephadex LH-20 column with MeOH, a silica gel column with a gradient mixt. of $CHCl_3$–MeOH (9:1, 6:1, 4:1, 2:1) and an ODS column with MeOH–H_2O (9:1) yielded compounds **2** (115 mg) and **6** (54.9 mg). Fr. 6 was separated by Diaion HP-20 CC with an H_2O–MeOH system, Sephadex LH-20 CC with MeOH and ODS CC with MeOH–H_2O (3:1) yielded **1** (153 mg). The *n*-BuOH-sol. phase was separated by silica gel CC with a gradient mixt. of $CHCl_3$–MeOH (19:1, 9:1, 6:1, 4:1, 2:1) into 8 fractions. Frs 6 and 7 were combined and chromatographed on Diaion HP-20 with a H_2O–MeOH system, on silica gel with a gradient mixt. of EtOAc–MeOH (9:1, 7:1, 5:1, 4:1) and with that of $CHCl_3$–MeOH–H_2O (175:25:2, 275:50:4, 100:25:2), on Sephadex LH-20 with MeOH, and on ODS with MeOH–H_2O (3:1, 2:1) to collect **1** (320 mg) and a mixt. of **3** and **4**. The mixt. was sepd by means of prep. HPLC with MeOH–H_2O (2:1) and H_2O–MeCN–2-methoxyethanol (11:9:2) to yield **3** (54.6 mg) and **4** (27.8 mg) as pure compounds. Fr. 8 was separated by Diaion HP-20 CC with an H_2O–MeOH system, silica gel CC with a gradient mixt. of EtOAc–MeOH (8:1, 6:1, 4:1, 3:1, 2:1) and with that of $CHCl_3$–MeOH (6:1, 4:1, 3:1, 2:1), and prep. HPLC with H_2O–MeCN (7:3) into **5** (103 mg). Compound **1** (35.7 mg) was also isolated from the H_2O-residual phase.

Compound **1**. Amorphous powder (509 mg), $[\alpha]_D^{25}$ −69.0° (MeOH; *c* 0.12). (Found: C, 56.46; H, 8.01. Calc. for $C_{44}H_{70}O_{18}\cdot 5/2H_2O$: C, 56.70; H, 8.11%.). SI MS *m/z* 909 $[M+Na]^+$, 885 $[M-H]^+$, 776 $[M-\text{pentose}+Na+OH]^+$, 755 $[M-\text{pentose}+H_2O+H]^+$, 748 $[M-\text{hexose}+Na+H_2O+H]^+$, 725 $[M-\text{hexose}+H_2O+H]^+$; UV λ_{max}^{MeOH} nm (ε): 284 (109); CD (MeOH; *c* 9.03 × 10^{-4}) nm (θ): 291 (−3212); IR ν_{max}^{KBr} cm $^{-1}$: 3400 (OH), 2950 and 2875 (CH), 1705 (C=O), 1445, 1420, 1375, 1265, 1235, 1225, 1170, 1150, 1040, 985, 950, 915, 895, 860, 775 [(25*R*)-spiroacetal, intensity 915 < 895]; ^{1}H NMR (pyridine-d_5): δ4.53 (1H, *q*-like, *J* = 7.0 Hz, H-16), 3.96 (1H, *m*, H-3), 3.59 (1H, *dd*, *J* = 10.6, 3.6 Hz, H-26a), 3.49 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-26b), 2.37 (1H, *dd*, *J* = 12.7, 4.1 Hz, H-7eq), 2.16 (1H, *dd*, *J* = 12.6, 2.4 Hz, H-5), 2.00 (1H, *dd*, *J* = 12.7, 12.7 Hz, H-7ax), 1.15 (3H, *d*, *J* = 6.9 Hz, H-21), 0.79 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.8 Hz, H-27), 0.64 (3H, *s*, H-19). Signals for the saccharide moiety are shown in Table 2.

Acid hydrolysis of compound **1**. Compound **1** (200 mg) was dissolved in 1 M HCl (dioxane–H_2O, 1:1) and the mixt. was refluxed for 1 hr under an N_2 atmosphere. The reaction mixt., after cooling, was neutralized with 1 M NaOH and chromatographed on silica gel with $CHCl_3$–Me_2CO (6:1) and $CHCl_3$–MeOH–H_2O (20:10:1) to D-glucose (41.5 mg), L-arabinose (6.5 mg) and an aglycone (**1a**) (79.7 mg), identified as laxogenin. D-Glucose: $[\alpha]_D^{25}$ +35.2° (H_2O; *c* 0.24); TLC, R_f 0.38 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). L-Arabinose: $[\alpha]_D^{25}$ +118.3° (H_2O; *c* 0.12); TLC, R_f 0.45 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). Laxogenin (**1a**): $[\alpha]_D^{25}$ −34.9° ($CHCl_3$; *c* 0.18). (Found: C, 73.07; H, 9.29. Calc. for $C_{27}H_{42}O_4\cdot 1/2H_2O$: C, 73.76; H, 9.86%.) EI MS *m/z* (rel. int.): 430 $[M]^+$ (4), 358 (12), 316 (20), 287 (18), 139 (100), 115 (30); UV λ_{max}^{MeOH} nm (ε): 281 (132); CD (MeOH; *c* 1.16 × 10^{-3}) nm (θ): 293 (−2621); IR ν_{max}^{KBr} cm $^{-1}$: 3450 and 3220 (OH), 2950 and 2875 (CH), 1705 (C=O), 1450, 1430, 1375, 1360, 1335, 1280, 1260, 1240, 1170, 1150, 1090, 1055, 1015, 1000, 985, 975, 955, 915, 895, 860, 790, 750 [(25*R*)-spiroacetal, intensity 915 < 895]; ^{1}H NMR (pyridine-d_5): δ4.54 (1H, *q*-like, *J* = 6.6 Hz, H-16), 3.83 (1H, *m*, H-3), 3.58 (1H, *dd*, *J* = 10.6, 3.8 Hz, H-26a), 3.49 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-26b), 2.39 (1H, *dd*, *J* = 12.5, 3.5 Hz, H-7eq), 2.28 (1H, *dd*, *J* = 12.1, 2.9 Hz, H-5), 2.02 (1H, *dd*, *J* = 12.5, 12.5 Hz, H-7ax), 1.15 (3H, *d*, *J* = 6.9 Hz, H-21), 0.81 (3H, *s*, H-18), 0.77 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 5.9 Hz, H-27).

Table 2. ^{1}H NMR chemical shifts for oligosaccharide units of **1**

	^{1}H NMR
Glc 1′	4.93 *d* (7.8)
2′	3.88 *dd* (9.3, 7.8)
3′	4.22 overlapping
4′	4.45 *dd* (9.3, 9.3)
5′	3.96 *m*
6′	4.83 *br d* (10.8)
	4.68 *dd* (10.8, 3.6)
Glc 1″	5.51 *d* (7.9)
2″	4.07 *dd* (8.8, 7.9)
3″	4.38 *dd* (8.8, 8.8)
4″	4.24 overlapping
5″	4.17 *ddd* (8.8, 5.6, 2.4)
6″	4.48 *dd* (11.6, 2.4)
	4.29 *dd* (11.6, 5.6)
Ara 1‴	5.09 *d* (7.4)
2‴	4.46 *dd* (8.7, 7.4)
3‴	4.06 *dd* (8.7, 2.2)
4‴	4.23 overlapping
5‴	4.25 overlapping
	3.72 *br d* (11.0)

Spectrum was measured in pyridine-d_5.

J values in parentheses are expressed in Hz.

Compound **2**. Amorphous powder (115 mg), $[\alpha]_D^{30}$ −98.7° (MeOH; *c* 0.25). (Found: C, 61.52; H, 8.36. Calc. for $C_{38}H_{60}O_{13}\cdot H_2O$: C, 61.44; H, 8.41%.) SI MS *m/z* 769 $[M+2Na-H]^+$, 763 $[M+K]^+$, 747 $[M+Na]^+$; UV λ_{max}^{MeOH} nm (ε): 282 (108); CD (MeOH; *c* 1.63 × 10^{-3}) nm (θ): 293 (−2515); IR ν_{max}^{KBr} cm $^{-1}$: 3425 (OH), 2950 and 2890 (CH), 1710 (C=O), 1455, 1430, 1380, 1265, 1245, 1230, 1175, 1050, 1005, 980, 955, 920, 900, 865, 780 [(25*R*)-spiroacetal, intensity 920 < 900]; ^{1}H NMR (pyridine-d_5): δ4.98 (1H, *d*, *J* = 6.6 Hz, H-1″), 4.94 (overlapping with H_2O signal, H-1′), 4.53 (1H, *q*-like, *J* = 7.0 Hz, H-16), 3.59 (1H, *dd*, *J* = 10.5, 3.7 Hz, H-26a), 3.49 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 2.35 (1H, *dd*, *J* = 12.4, 4.2 Hz, H-7eq), 2.19 (1H, *dd*, *J* = 12.0, 2.9 Hz, H-5), 2.00 (1H, *dd*, *J* = 12.4, 12.4 Hz, H-7ax), 1.15 (3H, *d*, *J* = 6.9 Hz, H-21), 0.78 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.7 Hz, H-27), 0.65 (3H, *s*, H-19).

Acid hydrolysis of compound **2**. Compound **2** (5.0 mg) was hydrolysed with 1 M HCl (dioxane–H_2O, 1:1) as for **1**. D-Glucose, L-arabinose and laxogenin (**1a**) were detected in the

Table 3. ^{13}C NMR spectral data for compounds **1**, **1a**, **2**, **3**, **3a**, **4**, **5** and **6**

C	1	1a	2	3	3a	4	5	6
1	36.7	37.0	36.8	36.7	37.0	36.7	36.7	37.2
2	29.5	31.3	29.6	29.5	31.3	29.5	29.4	30.0
3	77.0	70.0	76.8	77.0	70.0	76.8	77.0	77.6
4	27.0	31.8[a]	27.0	27.0	31.8	27.0	27.0	34.8
5	56.5	57.0	56.4	56.5	56.9	56.4[a]	56.5[a]	44.6
6	209.6	209.9	209.1	209.6	209.9	209.7	209.6	28.9
7	46.7	46.8	46.7	46.7	46.8	46.8	46.8	32.4
8	37.4	37.5	37.3	37.4	37.4	37.3	37.4	35.2
9	53.7	53.9	53.6	53.7	53.8	53.7	53.7	54.4
10	40.8	40.9	40.8	40.9	40.9	40.8	40.9	35.8
11	21.5	21.7	21.5	21.5	21.6	21.5	21.5	21.3
12	39.6	39.8	39.6	39.6	39.7	39.6	39.7	40.1
13	41.1	41.2	41.1	41.1	41.1	41.4	41.4	40.8
14	56.5	56.6	56.4	56.5	56.6	56.3[a]	56.4[a]	56.4
15	31.8	31.9[a]	31.8	31.8	31.8	32.0	32.0	32.1
16	80.9	80.9	80.8	81.1	80.9	80.8	80.8	81.1
17	62.8	62.9	62.8	62.8	62.9	63.8	63.8	63.0
18	16.5	16.5	16.4	16.5	16.5	16.5[b]	16.5[b]	16.6
19	13.1	13.3	13.1	13.1	13.2	13.1	13.1	12.3
20	42.0	42.0	42.0	42.1	42.1	40.5	40.6	42.0
21	15.0	15.0	15.0	15.0	15.0	16.4[b]	16.4[b]	15.0
22	109.3	109.3	109.2	109.7	109.7	110.6	110.7	109.2
23	31.8	31.9[a]	31.8	31.5	31.5	37.1	37.1	31.8
24	29.2	29.2	29.2	24.0	24.0	28.3	28.4	29.2
25	30.6	30.6	30.6	39.2	39.2	34.2	34.2	30.6
26	66.9	66.9	66.9	64.1[a]	64.1[a]	75.2	75.2	66.9
27	17.3	17.3	17.3	64.4[a]	64.4[a]	17.4	17.4	17.3
1′	102.0		102.1	102.0		102.1	102.0	102.1
2′	74.8		75.2	74.8		75.2	74.7	74.7
3′	76.6		78.5	76.6		78.5[c]	76.5	76.5
4′	81.1		71.9	81.1		71.9	81.1	81.1
5′	74.9		77.0	74.9		77.0	74.9	74.8
6′	68.4		69.7	68.4		69.7	68.4	68.3
1″	104.9		105.5	104.9		105.4	104.9	104.8
2″	75.2		72.3	75.2		72.3	75.2	75.2
3″	78.5		74.5	78.5		74.4	78.5[c]	78.4
4″	71.9		69.1	71.9		69.1	71.8[d]	71.8
5″	78.2		66.5	78.2		66.5	78.2	78.1
6″	62.6			62.6			62.6	62.5
1‴	105.7			105.7			105.6	105.6
2‴	72.6			72.6			72.5	72.5
3‴	74.6			74.6			74.6	74.6
4‴	69.7			69.7			69.7	69.8
5‴	67.2			67.2			67.1	67.2
6‴								
1⁗						104.9	104.9	
2⁗						75.2	75.2	
3⁗						78.6[c]	78.6[c]	
4⁗						71.7	71.7[d]	
5⁗						78.4[c]	78.4[c]	
6⁗						62.8	62.8	

Spectra were measured in pyridine-d_5.

[a–d]Assignments with the same superscripts may be reversed in each vertical column.

reaction mixt. by TLC. Laxogenin: TLC, R_f 0.40 ($CHCl_3$–MeOH, 19:1).

Compound **3**. Amorphous powder (54.6 mg), $[\alpha]_D^{27}$ −55.5° (MeOH; *c* 0.26). (Found: C, 56.94; H, 7.70. Calc. for $C_{44}H_{70}O_{19}\cdot 3/2H_2O$: C, 56.82; H, 7.91%.).SI MS *m/z* 925 [M + Na]$^+$, 903 [M + H]$^+$; UV λ_{max}^{MeOH} nm (ε): 286 (99); CD (MeOH; *c* 9.13 × 10^{-4}) nm (θ): 292 (−3178); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1710 (C=O), 1450, 1375, 1255, 1255, 1170, 1160, 1065, 1040, 955, 905, 860, 775;^{1}H NMR (pyridine-d_5): δ5.50 (1H, *d*, *J* = 7.9 Hz, H-1″), 5.09 (1H, *d*, *J* = 7.4 Hz, H-1‴), 4.92 (1H, *d*, *J*

= 7.8 Hz, H-1'), 4.55 (1H, *q*-like, *J* = 6.9 Hz, H-16), 2.36 (1H, *dd*, *J* = 12.8, 4.0 Hz, H-7eq), 2.16 (1H, *dd*, *J* = 12.5, 2.2 Hz, H-5), 2.00 (1H, *dd*, *J* = 12.8, 12.8 Hz, H-7ax), 1.16 (3H, *d*, *J* = 6.9 Hz, H-21), 0.79 (3H, *s*, H-18), 0.64 (3H, *s*, H-19).

Acid hydrolysis of compound **3**. Compound **3** (29.0 mg) was hydrolysed with 1 M HCl (dioxane–H_2O, 1:1) as for **1**. The crude reaction mixt. was chromatographed on silica gel with $CHCl_3$–Me_2CO (5:1) and $CHCl_3$–MeOH–H_2O (20:10:1) to yield D-glucose (4.6 mg), L-arabinose (1.0 mg) and an aglycone (**3a**) (5.2 mg). Compound **3a**: $[\alpha]_D^{25}$ −66.8° ($CHCl_3$; *c* 0.12). EI MS *m/z* (rel. int.): 446 $[M]^+$ (2.8), 371 (2.6), 316 (14), 287 (21), 155 (100), 131 (42); UV λ_{max}^{MeCN} nm (ε): 282 (224); CD (MeOH; *c* 1.93 × 10^{-3}) nm (θ): 299 (−3167); IR ν_{max}^{Film} cm^{-1}: 3406 (OH), 2940 and 2872 (CH), 1702 (C=O), 1456, 1381, 1262, 1241, 1220, 1174, 1140, 1100, 1059, 1040, 1000, 985, 974, 960, 912, 867, 803, 754; ^1H NMR (pyridine-d_5): δ4.57 (1H, *q*-like, *J* = 6.7 Hz, H-16), 4.16 (1H, *dd*, *J* = 11.1, 3.8 Hz, H-26a), 3.89 (1H, *dd*, *J* = 11.1, 11.1 Hz, H-26b), 3.86 (1H, *m*, H-3), 3.73 (1H, *dd*, *J* = 11.6, 4.9 Hz, H-27a), 3.66 (1H, *dd*, *J* = 11.6, 7.8 Hz, H-27b), 2.41 (1H, *dd*, *J* = 12.2, 3.6 Hz, H-7eq), 2.29 (1H, *dd*, *J* = 13.3, 2.7 Hz, H-5), 2.02 (1H, *dd*, *J* = 12.2, 12.2 Hz, H-7ax), 1.18 (3H, *d*, *J* = 6.9 Hz, H-21), 0.83 (3H, *s*, H-18), 0.78 (3H, *s*, H-19)

Compound **4**. Amorphous powder (27.8 mg), $[\alpha]_D^{25}$ −44.8° (EtOH; *c* 0.30). (Found: C, 57.09; H, 8.10. Calc. for $C_{44}H_{72}O_{19}\cdot H_2O$: C, 57.25; H, 8.08%.). SI MS *m/z* 927 $[M + Na]^+$, 887 $[M - OH]^+$; UV λ_{max}^{EtOH} nm (ε): 285 (75); CD (EtOH; *c* 9.62 × 10^{-4}) nm (θ): 291 (−2911); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930 (CH), 1695 (C=O), 1445, 1375, 1255, 1170, 1155, 1070, 1040, 1005, 900, 775, 725, 695; ^1H NMR (pyridine-d_5): δ4.98 (overlapping with H_2O signal, H-1' and H-1''), 4.81 (1H, *d*, *J* = 7.6 Hz, H-1'''), 4.39 (1H, *q*-like, *J* = 5.9 Hz, H-16), 1.34 (3H, *d*, *J* = 6.8 Hz, H-21), 0.99 (3H, *d*, *J* = 6.7 Hz, H-27), 0.83 (3H, *s*, H-18), 0.65 (3H, *s*, H-19).

Compound **5**. Amorphous powder (103 mg), $[\alpha]_D^{28}$ −46.3° (EtOH; *c* 0.28). (Found: C, 55.14; H, 7.81. Calc. for $C_{50}H_{82}O_{24}\cdot H_2O$: C, 55.34; H, 7.80%.).SI MS *m/z* 1106 $[M + K + H]^+$, 1089 $[M + Na]^+$; UV λ_{max}^{MeOH} nm (ε): 280 (102); CD (MeOH; *c* 1.26 × 10^{-3}) nm (θ): 293 (−2698); IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2940 (CH), 1700 (C=O), 1445, 1380, 1260, 1225, 1160, 1065, 1035, 900, 860, 775, 715, 695; ^1H NMR (pyridine-d_5): δ5.49 (1H, *d*, *J* = 7.9 Hz, H-1''), 5.09 (1H, *d*, *J* = 7.3 Hz, H-1'''), 4.92 (1H, *d*, *J* = 7.7 Hz, H-1'), 4.81 (1H, *d*, *J* = 7.6 Hz, H-1''''), 1.34 (3H, *d*, *J* = 6.9 Hz, H-21), 0.99 (3H, *d*, *J* = 6.7 Hz, H-27), 0.84 (3H, *s*, H-18), 0.65 (3H, *s*, H-19).

Enzymatic hydrolysis of compounds **4** and **5**. A mixt. of **4** (25.0 mg) and β-glucosidase (12.5 mg) was dissolved in AcOH/AcONa buffer (pH 5) (5 ml) and incubated at room temp. overnight. The reaction mixt. was chromatographed on silica gel with $CHCl_3$–MeOH H_2O (20:10:1) to yield **2** and D-glucose (3.2 mg). Compound **5** (30.0 mg) on hydrolysis with β-glucosidase (15.0 mg) as for **4** yielded **3** (14.6 mg) and D-glucose (2.5 mg).

Compound **6**. Amorphous powder (54.9 mg), $[\alpha]_D^{30}$ −46.6° (MeOH; *c* 0.28). (Found: C, 57.75; H, 8.27. Calc. for $C_{44}H_{72}O_{17}\cdot 2H_2O$: C, 58.13; H, 8.43%.). SI MS *m/z* 895 $[M + Na]^+$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1450, 1380, 1260, 1155, 1045, 980, 955, 920, 895, 860, 800, 730, 700 [(25*R*)-spiroacetal, intensity 920 < 895]; ^1H NMR (pyridine-d_5): δ5.54 (1H, *d*, *J* = 7.9 Hz, H-1''), 5.11 (1H, *d*, *J* = 7.4 Hz, H-1'''), 4.93 (1H, *d*, *J* = 7.5 Hz, H-1'), 4.56 (1H, *q*-like, *J* = 7.1 Hz, H-16), 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), 0.83 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.6 Hz, H-27), 0.68 (3H, *s*, H-19).

Acid hydrolysis of compound **6**. Compound **6** (5.3 mg) was hydrolysed with 1 M HCl (dioxane–H_2O, 1:1) as for **1**. D-Glucose, L-arabinose and tigogenin (**6a**) were detected in the reaction mixt by TLC. Tigogenin: TLC, R_f 0.66 ($CHCl_3$–MeOH, 19:1).

Assay of phosphodiesterase. Phosphodiesterase activity was assayed by the method as described in the previous paper [11].

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