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STEROIDAL SAPONINS FROM *HOSTA LONGIPES* AND THEIR INHIBITORY ACTIVITY ON TUMOUR PROMOTER-INDUCED PHOSPHOLIPID METABOLISM OF HeLa CELLS

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Key Word Index—*Hosta longipes*; Liliaceae; steroidal saponins; spirostanol saponins; furostanol saponins; phospholipid metabolism inhibition; HeLa cells; antitumour-promoter activity.

Abstract—Three new spirostanol saponins and two new furostanol saponins were isolated from the underground parts of *Hosta longipes*. Their structures were determined to be (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside}, gitogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}, (25*R*)-5α-spirostan-3β-ol (tigogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}, 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-5α-furostane-2α,3β,22ξ,26-tetrol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside} and 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-5α-furostane-2α,3β,22ξ,26-tetrol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}, respectively. The isolated saponins and their derivatives were examined for inhibitory activity on 12-*O*-tetradecanoylphorbor-13-acetate-stimulated ^{32}P-incorporation into phospholipids of HeLa cells as the primary screening test to find new antitumour-promoter compounds.

INTRODUCTION

The genus *Hosta*, with about 20 species, belongs to the subfamily Asphodeloideae in Liliaceae and is distributed in east Asia. Takeda and co-workers [1–3] made a series of phytochemical examinations on the domestic *Hosta* plants in Japan such as *Hosta plantaginea*, *H. sieboldiana*, *H. longipes*, *H. montana* var. *liliflora* and *H. kiyosumiensis*. They surveyed the neutral fractions of the saponified methanolic extracts of the plant materials, which consequently led to the isolation of steroidal sapogenins. Although the steroids are expected to be present as glycosides in the plants, there has been no exploration of the steroidal saponins in the genus *Hosta*, except for our recent study on the constituents of *H. longipes*, in which we mainly reported new tetra- and penta-glycosides of spirostanols based on (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) [4]. Our additional attention to the steroidal saponins in *H. longipes* has resulted in finding a further three new spirostanol saponins and two new furostanol saponins. This paper reports the structural assignments of the new saponins, based on spectroscopic data and hydrolysis, and their inhibitory activity on 12-*O*-tetradecanoylphorbor-13-acetate (TPA)-stimulated ^{32}P-incorporation into phospholipids of HeLa cells, which is known as an excellent primary screening test for identifying new antitumour-promoter compounds [5–8].

RESULTS AND DISCUSSION

The 1-butanol-soluble phase of the methanolic extract of *H. longipes* was chromatographed on Diaion HP-20, on silica gel, and on octadecylsilanized (ODS) silica gel to give compounds **1**–**5**.

Compound **1** was obtained as an amorphous solid with the molecular formula $C_{39}H_{64}O_{13}$, which was deduced from the negative-ion FAB mass spectrum showing an $[M-H]^-$ ion at *m/z* 739 and the ^{13}C NMR spectrum (Table 1) with 39 signals, which were divided into C × 3, CH × 20, CH_2 × 11 and Me × 5 by the use of the various DEPT spectra. The glycosidic nature of **1** was inferred from the strong absorption bands at 3410 and 1045 cm^{-1}. The ^{1}H NMR spectrum showed signals for two tertiary methyl groups at δ 0.90 and 0.81 (each *s*), three secondary methyl groups at δ 1.61 (*d*, *J* = 6.2 Hz), 1.13 (*d*, *J* = 7.0 Hz) and 0.70 (*d*,

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

C		**1**	**1a**	**2**	**2a**	**3**	**4**	**5**
1		45.8	46.5	45.8	45.7	37.3	45.8	45.8
2		70.7†	73.1	70.5	70.5	30.0	70.7	70.6
3		85.6	76.7	85.2	84.8	77.1†	85.7	85.3
4		33.7	37.2	33.5	34.1	34.4	33.8	33.5
5		44.7	45.3	44.7	44.7	44.7	44.8	44.8
6		28.2	28.4	28.2	28.1	29.0	28.2	28.2
7		32.2	32.2	32.1	32.1	32.4	32.1	32.1
8		34.7	34.7	34.6	34.6	35.3	34.7	34.6
9		54.5	54.7	54.4	54.4	54.5	54.5	54.5
10		36.9	37.6	36.9	36.9	36.0	36.9	36.9
11		21.5	21.6	21.5	21.5	21.3	21.4	21.4
12		40.1	40.2	40.1	40.1	40.2	40.0	40.0
13		40.8	40.9	40.8	40.8	40.8	41.2	41.1
14		56.4	56.5	56.3	56.4	56.5	56.3	56.3
15		32.3	32.4	32.3	32.3	32.2	32.3	32.3
16		81.2	81.2	81.1	81.1	81.1	81.4	81.4
17		63.1	63.1	63.0	63.0	63.1	64.3	64.3
18		16.6	16.7	16.6	16.6	16.6	16.3	16.3
19		13.5	13.8	13.5	13.4	12.4	13.5	13.5
20		42.0	42.0	42.0	42.0	42.0	40.5	40.5
21		15.0	15.0	15.0	15.0	15.0	16.5	16.5
22		109.2	109.2	109.2	109.2	109.2	112.7	112.7
23		31.9	31.9	31.8	31.8	31.8	30.8	30.8
24		29.3	29.3	29.3	29.3	29.3	28.2	28.2
25		30.6	30.6	30.6	30.6	30.6	34.2	34.2
26		66.9	66.9	66.9	66.9	66.9	75.2	75.2
27		17.3	17.3	17.3	17.3	17.3	17.2	17.2
OMe							47.3	47.3
Gal	1′	101.8		101.1	103.5	100.0	101.9	101.2
	2′	77.0		76.8	73.1	77.0†	76.9	76.9
	3′	76.5		76.4	75.8	76.4	76.4	76.4
	4′	70.8†		81.3	80.2	81.3	70.7	81.3
	5′	76.2		75.4	76.0	75.2	76.3	75.5
	6′	62.2		60.9	60.9	61.0	62.2	60.9
Rha	1″	102.2		102.3		102.3	102.1	102.3
	2″	72.5		72.3		72.4	72.4	72.3
	3″	72.8		72.7		72.8	72.8	72.7
	4″	74.2		74.1		74.1	74.2	74.1
	5″	69.4		69.4		69.4	69.4	69.4
	6″	18.5		18.5		18.6	18.5	18.5
Glc	1‴			107.2	107.2	107.2		107.2
	2‴			75.6	75.2	75.6		75.6
	3‴			78.9†	78.8†	78.9‡		78.9†
	4‴			72.2	72.3	72.2		72.2
	5‴			78.6†	78.5†	78.5‡		78.6†
	6‴			63.0	63.1	63.1		63.0
Glc	1⁗						105.0	105.0
	2⁗						75.2	75.2
	3⁗						78.6†	78.6‡
	4⁗						71.8	71.8
	5⁗						78.4†	78.5‡
	6⁗						63.0	63.1

*Spectra were measured in pyridine-d_5.

†,‡Assignments may be interchanged in each column.

J = 5.4 Hz), and two anomeric protons at δ 6.29 (*br s*) and 5.01 (*d*, J = 7.7 Hz). The signal at δ 1.61 was due to the methyl group of 6-deoxyhexopyranose. Acid hydrolysis of **1** with 1 M HCl in dioxane–H_2O (1:1) gave a steroidal sapogenin (**1a**), identified as (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) [9], together with D-galactose and L-rhamnose in a ratio of 1:1. Thus, compound **1** turned out to be a gitogenin diglycoside. The presence of a terminal α-L-rhamnopyranosyl moiety was indicated by the fragment ion peak at *m/z* 595 $[M-145]^-$ in the negative-ion FAB mass spectrum and characteristic six signals at δ 102.2, (CH), 72.5 (CH), 72.8 (CH), 74.2 (CH), 69.4 (CH) and 18.5 (Me) in the ^{13}C NMR spectrum [10, 11]. The remaining six signals due to another monosaccharide were assigned to a 2-*O*-glycosylated β-D-galactopyranoside [δ 101.8 (CH), 77.0 (CH), 76.5 (CH), 70.8 (CH), 76.2 (CH) and 62.2 (CH_2)] by comparing them with those of an authentic methyl β-D-galactopyranoside [10, 11], leading to the disaccharide structure as *O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside. This was in agreement with the ^{13}C assignments of alliospiroside B, the *O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside of (25*S*)-ruscogenin [12]. The dissaccharide was concluded to be linked to the C-3 hydroxyl position of the aglycone because, in the ^{13}C NMR spectrum of **1**, the signal due to C-3 shifted to a lower field by 8.9 ppm, whereas the signals due to C-2 and C-4 moved to upper fields by 2.4 and 3.5 ppm, as compared with those of **1a** (Table 1). From the data presented above, the full structure of **1** was established as gitogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside}.

Compound **2** was a more polar constituent than **1**. The negative-ion FAB mass spectrum showed an $[M-H]^-$ ion at *m/z* 901, shifted 162 mass units with respect to **1**. Acid hydrolysis of **2** gave **1a** and D-galactose, D-glucose and L-rhamnose in a ratio of 1:1:1. On comparison of the whole ^{13}C NMR spectrum of **2** with that of **1** (Table 1), a set of six additional signals, corresponding to a terminal β-D-glucopyranosyl unit, appeared at δ 107.2 (CH), 75.6 (CH), 78.9 (CH), 72.2 (CH), 78.6 (CH) and 63.0 (CH_2), and the signals due to the inner galactose moiety varied, while all other signals remained almost unaffected. It was observed that the signal of C-4 of the galactose was remarkably displaced downfield by 10.5 ppm to resonate at δ 81.3 as compared with that of **1**, suggesting that the C-4 position of the galactose was the glycosylated position to which the additional D-glucose was attached. Mild acid hydrolysis of **2** with 0.2 M HCl gave a partial hydrolysate (**2a**), the ^1H and ^{13}C NMR spectra (Table 1) of which gave evidence for the presence of a terminal β-D-glucopyranosyl unit and a 4-substituted β-D-galactopyranosyl unit [δ_H 4.93 (*d*, J = 7.8 Hz); δ_C 103.5 (CH), 73.1 (CH), 75.8 (CH), 80.2 (CH), 76.0 (CH) and 60.9 (CH_2)] [13]. The structure of **2a** was assigned as gitogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}. Accordingly, the full structure of **2** was characterized as gitogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}.

Comparison of the NMR data for **2** and **3** (Table 1) indicated that they possessed an identical triglycoside structure, but differed in the aglycone structure. Acid hydrolysis of **3** gave (25*R*)-5α-spirostan-3β-ol (tigogenin) (**3a**) and D-galactose, D-glucose and L-rhamnose in a ratio of 1:1:1. The structure of **3** was shown to correspond to that of **2** with the C-2 hydroxyl group missing, i.e. tigogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}.

Compound **4** was suggested to be a 22-methoxyfurostanol saponin by Ehrlich's test [14, 15], and the ^1H and ^{13}C NMR spectra (Table 1) [δ_H 3.26 (3H, *s*); δ_C 112.7 (C) and 47.3 (Me)] [10]. Enzymic hydrolysis of **3** with β-glucosidase gave D-glucose and **1**. The structure of **4** was formulated as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-5α-furostane-2α,3β,22ξ,26-tetrol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside}.

Compound **5** was also a 22-*O*-methoxyfurostanol saponin. The IR, ^1H NMR and ^{13}C NMR spectra (Table 1), and enzymic hydrolysis of **5**, which gave D-glucose and **2**, confirmed the structure of **5** to be 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-5α-furostane-2α,3β,22ξ,26-tetrol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-{β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}.

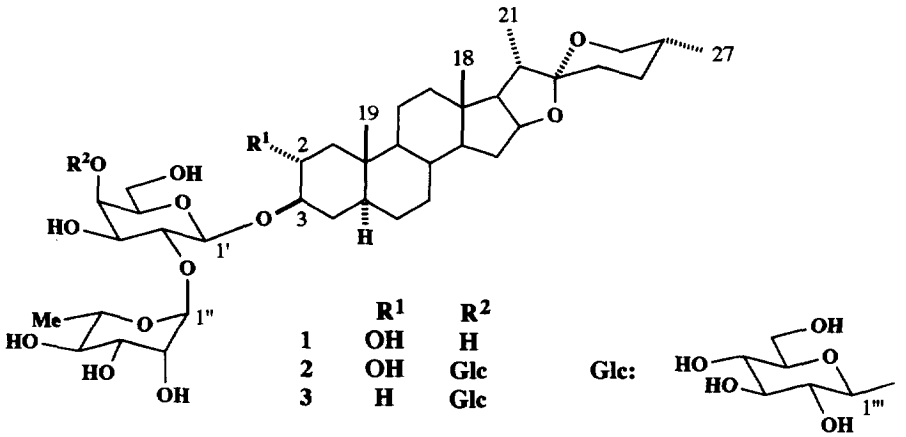
Compounds **1**–**5** are all new steroidal saponins. The isolated saponins and their derivatives were evaluated for an *in vitro* antitumour-promoter activity by measurement of their inhibitory activity on TPA-stimulated ^{32}P-incorporation into phospholipids of HeLa cells (Table 2). This is known to correlate well with antitumour-promoter effects *in vivo* [5–8]. Compounds **1** and **3** inhibited the phospholipid metabolism with percentage values of 77.8 and 45.6, respectively, at a sample concentration of 50 μg ml^{-1}, while the aglycones (**1a** and **3a**) and the furostanol saponins (**4** and **5**) did not act as inhibitors. The spirostanol saponins (**2** and **2a**) were cytotoxic towards HeLa cells at 50 μg ml^{-1} and at the lower concentration (5 μg ml^{-1}) they exhibited very weak or no activity.

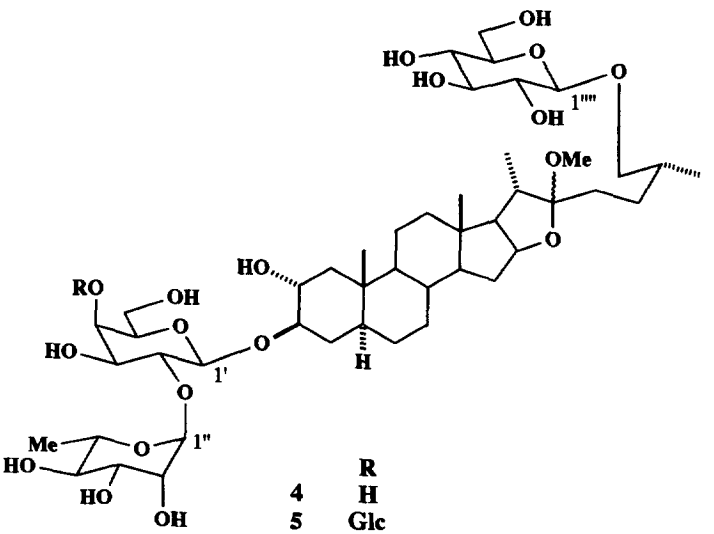
EXPERIMENTAL

General. NMR (ppm, J Hz): Bruker AM-400 (400 MHz for ^1H NMR); 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, UV-8000); TPA: Pharmacia PL Biochemicals; ^{32}P (carrier-free): Japan Radioisotope Assoc.

Plant material. Underground parts of *H. longipes* were collected in Gunma Prefecture, Japan, and a voucher specimen is on file in our laboratory.

Extraction and isolation. The fresh plant material





(10 kg) were extracted with hot MeOH (15 l × 2). The crude MeOH extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract was divided into three frs (I–III) by subjecting it to silica gel CC and elution with a gradient of CH_2Cl_2–MeOH (9:1; 4:1; 2:1; 1:1) and finally with MeOH. Fr. II was subjected to CC on silica gel, eluting with $CHCl_3$–MeOH–H_2O (20:10:1), and on ODS silica gel, eluting with MeOH–H_2O (4:1), to give **1** (1.38 g). After removal of saccharides from fr. III by CC on Diaion HP-20 with an increasing amount of MeOH in H_2O, it was chromatographed on silica gel, eluting with $CHCl_3$–Et_2O–MeOH–H_2O (7:7:8:2), to give a further four frs (IIIa–IIId). Fr. IIIc was subjected to CC on ODS silica gel, eluting with MeOH–H_2O (4:1), and on silica gel, eluting with $CHCl_3$–Et_2O–MeOH–H_2O (5:5:4:1), to yield **2** (1.35 g) and **3** (117 mg). Fr. IIId was subjected to CC on silica gel, using $CHCl_3$–Et_2O–MeOH–H_2O (5:5:4:1), and on ODS silica gel, using MeOH–H_2O (1:1), to furnish **4** (474 mg) and **5** (130 mg).

Table 2. Inhibitory effect of the isolated saponins and their derivatives on TPA-stimulated ^{32}P-incorporation into phospholipids of HeLa cells*

Compounds	Inhibition (%) (50 μg ml^{-1})	Inhibition (%) (5 μg ml^{-1})
1	77.8	—‡
1a	3.9	—
2	†	5.2
2a	†	0
3	45.6	—
3a	0	—
4	0	—
5	0	—

*Data, expressed as percentage of inhibition on TPA-stimulated ^{32}P-incorporation, the deviations of which are within 5%.

†The samples exhibited cytotoxicity towards HeLa cells.

‡Not measured.

Compound **1**. Amorphous solid, $[\alpha]_D^{25}$ −70.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 739 $[M-H]^-$, 595 $[M-$rhamnosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2935 (CH), 1450, 1375, 1240, 1175, 1125, 1045, 980, 950, 920, 895, 865, 815, 780, 700. ^1H NMR (pyridine-d_5): δ 6.29 (1H, *br s*, 1″-H), 5.01 (1H, *d*, J = 7.7 Hz, 1′-H), 4.86 (1H, *dq*, J = 9.3, 6.2 Hz, 5″-H), 4.81 (1H, *br d*, J = 3.3 Hz, 2″-H), 4.66 (1H, *dd*, J = 9.2, 7.7 Hz, 2′-H), 4.61 (1H, *dd*, J = 9.3, 3.3 Hz,

3″-H), 4.55 (1H, *q*-like, $J = 7.0$ Hz, 16-H), 4.48 (1H, *br d*, $J = 3.4$ Hz, 4′-H), 4.47 (1H, *dd*, $J = 10.8$, 5.9 Hz, 6′a-H), 4.38 (1H, *dd*, $J = 10.8$, 5.1 Hz, 6′b-H), 4.28 (1H, *dd*, $J = 9.3$, 9.3 Hz, 4″-H), 4.27 (1H, *dd*, $J = 9.2$, 3.4 Hz, 3′-H), 4.15–4.07 (2H, overlapping, 2-, 5′-H), 3.89 (1H, *ddd*, $J = 11.2$, 8.6, 5.3 Hz, 3-H), 3.59 (1H, *dd*, $J = 10.4$, 3.1 Hz, 26a-H), 3.50 (1H, *dd*, $J = 10.4$, 10.4 Hz, 26b-H), 1.61 (3H, *d*, $J = 6.2$ Hz, 6″-Me), 1.13 (3H, *d*, $J = 7.0$ Hz, 21-Me), 0.90 (3H, *s*, 19-Me), 0.81 (3H, *s*, 18-Me), 0.70 (3H, *d*, $J = 5.4$ Hz, 27-Me).

Acid hydrolysis of **1**. A soln of **1** (100 mg) in 1M HCl (dioxane–H_2O, 1:1, 8 ml) was heated at 100° for 2 hr under Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and subjected to ODS silica gel CC, eluting initially with H_2O and then with MeOH. The MeOH eluate fr. was chromatographed on silica gel using $CHCl_3$–MeOH (19:1) to give **1a** (45 mg), which was identified by its mass, IR, ^{1}H NMR and ^{13}C NMR spectra.

Gitogenin (**1a**). Amorphous solid, $[\alpha]_D^{25}$ −52.0° ($CHCl_3$–MeOH, 1:1; *c* 0.05). Negative-ion FABMS *m/z*: 431 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2935 and 2875 (CH), 1445, 1375, 1235, 1175, 1050, 975, 950, 920, 895, 865, 700. ^{1}H NMR (pyridine-d_5): δ 6.12 and 6.00 (each 1H, *br s*, OH × 2), 4.56 (1H, *q*-like, $J = 6.8$ Hz, 16-H), 4.04 (1H, *m*, 2-H), 3.84 (1H, *m*, 3-H), 3.59 (1H, *dd*, $J = 10.5$, 3.7 Hz, 26a-H), 3.50 (1H, *dd*, $J = 10.5$, 10.5 Hz, 26b-H), 1.14 (3H, *d*, $J = 7.0$ Hz, 21-Me), 0.89 (3H, *s*, 19-Me), 0.84 (3H, *s*, 18-Me), 0.70 (3H, *d*, $J = 5.7$ Hz, 27-Me). A 2-mg portion of the H_2O eluate fr. (monosaccharide mixt.) was dissolved in H_2O (1 ml), to which (−)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (8 mg) in EtOH (1 ml) were added. After being set aside at 40° for 4 hr followed by addition of HOAc (0.2 ml) and evapn to dryness, the reaction mixt. was acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temp. for 12 hr. The crude mixt. was passed through a Sep-Pak C_{18} cartridge (Waters), eluting initially with H_2O–MeCN (4:1) (10 ml) and then with MeCN (10 ml). The MeCN eluate fr. was further passed through a TOYOPAK IC-SP M cartridge (Tosoh) with EtOH (20 ml) to give 1-[(*S*]-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were analysed by HPLC under the following conditions: column, TSK-gel ODS-Prep (Tosoh, 250 × 4.6 mm i.d., 5 μm); solvent: MeCN–H_2O (2:3); flow rate: 0.8 ml min^{-1}; detection: UV (230 nm). The derivatives of D-galactose and L-rhamnose were detected; R_t 18.99 min (D-galactose derivative), 25.46 min (L-rhamnose derivative).

Compound **2**. Amorphous solid, $[\alpha]_D^{25}$ −112.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 901 $[M-H]^-$, 755 $[M-$ rhamnosyl$]^-$, 739 $[M-$ glucosyl$]^-$, 593 $[M-$ rhamnosyl − glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2930 (CH), 1445, 1370, 1235, 1120, 1045, 980, 915, 895, 810, 750, 695. ^{1}H NMR (pyridine-d_5): δ 6.18 (1H, *br s*, 1″-H), 5.18 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 4.95 (1H, *d*, $J = 7.8$ Hz, 1′-H), 3.59 (1H, *dd*, $J = 10.5$, 3.0 Hz, 26a-H), 3.50 (1H, *dd*, $J = 10.5$, 10.5 Hz, 26b-H), 1.61 (3H, *d*, $J = 6.2$ Hz, 6″-Me), 1.13 (3H, *d*, $J = 6.9$ Hz, 21-Me), 0.92 (3H, *s*, 19-Me), 0.81 (3H, *s*, 18-Me), 0.70 (3H, *d*, $J = 5.4$ Hz, 27-Me).

Partial hydrolysis of **2**. A soln of **2** (150 mg) in 0.2 M HCl (dioxane–H_2O, 1:1, 8 ml) was heated at 100° for 30 min under Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU column and subjected to CC on silica gel, eluting initially with $CHCl_3$–Et_2O–MeOH–H_2O (14:8:7:1) and then with MeOH to give a partial hydrolysate (**2a**) (86.3 mg) and L-rhamnose.

Compound **2a**. Amorphous solid, $[\alpha]_D^{25}$ −50.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 756 $[M]^-$, 593 $[M-$ glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2940 (CH), 1445, 1375, 1240, 1175, 1075, 1045, 985, 955, 925, 895, 865, 700. ^{1}H NMR (pyridine-d_5): δ 5.28 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 4.93 (1H, *d*, $J = 7.8$ Hz, 1′-H), 3.59 (1H, *dd*, $J = 10.5$, 3.5 Hz, 26a-H), 3.50 (1H, *dd*, $J = 10.5$, 10.5 Hz, 26b-H), 1.13 (3H, *d*, $J = 7.0$ Hz, 21-Me), 0.81 (3H, *s*, 18-Me), 0.72 (3H, *s*, 19-Me), 0.70 (3H, *d*, $J = 5.5$ Hz, 27-Me).

Compound **3**. Amorphous solid, $[\alpha]_D^{25}$ −57.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 885 $[M-H]^-$, 739 $[M-$ rhamnosyl$]^-$, 723 $[M-$ glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2910 (CH), 1440, 1360, 1165, 1110, 1045, 975, 915, 890, 690. ^{1}H NMR (pyridine-d_5): δ 6.22 (1H, *br s*, 1″-H), 5.18 (1H, *d*, $J = 7.9$ Hz, 1‴-H), 4.92 (1H, *d*, $J = 7.6$ Hz, 1′-H), 3.59 (1H, *dd*, $J = 10.5$, 2.9 Hz, 26a-H), 3.50 (1H, *dd*, $J = 10.5$, 10.5 Hz, 26b-H), 1.70 (3H, *d*, $J = 6.2$ Hz, 6″-Me), 1.14 (3H, *d*, $J = 6.8$ Hz, 21-Me), 0.87 (3H, *s*, 19-Me), 0.82 (3H, *s*, 18-Me), 0.70 (3H, *d*, $J = 5.4$ Hz, 27-Me).

Acid hydrolysis of **2** *and* **3**. Each soln of **2** (10 mg) and **3** (20 mg) in 1 M HCl (dioxane–H_2O, 1:1, 2 ml) was treated identically to that of **1** to give **1a** (3.5 mg) and saccharide fr., and **3a** (7.2 mg) and saccharide fr., respectively. Compound **3a** was identified by direct comparison with a commerically available authentic sample (Sigma). D-Galactose, D-glucose and L-rhamnose in a ratio of 1:1:1 in both the saccharide frs of **2** and **3** were identified by converting them into 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives followed by HPLC analysis; R_t 18.89 min (D-galactose derivative), 22.54 min (D-glucose derivative), 25.46 min (L-rhamnose derivative).

Compound **4**. Amorphous solid, $[\alpha]_D^{25}$ −63.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 934 $[M]^-$. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2930 (CH), 1445, 1375, 1265, 1040, 950, 905, 890, 815, 780, 700. ^{1}H NMR (pyridine-d_5): δ 6.25 (1H, *d*, $J = 1.0$ Hz, 1″-H), 4.98 (1H, *d*, $J = 7.8$ Hz, 1′-H), 4.83 (1H, *d*, $J = 7.8$ Hz, 1⁗-H), 3.26 (3H, *s*, OMe), 1.62 (3H, *d*, $J = 6.2$ Hz, 6″-Me), 1.18 (3H, *d*, $J = 6.9$ Hz, 21-Me), 1.01 (3H, *d*, $J = 6.6$ Hz, 27-Me), 0.88 (3H, *s*, 19-Me), 0.79 (3H, *s*, 18-Me).

Compound **5**. Amorphous solid, $[\alpha]_D^{25}$ −53.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FABMS *m/z*: 1095 $[M]^-$: IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2925 (CH), 1450, 1375, 1255, 1065, 1035, 900, 815, 695. ^{1}H NMR (pyridine-d_5): δ 6.23 (1H, *br d*, 1″-H), 5.17 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 4.94 (1H, *d*, $J = 7.8$ Hz, 1′-H),

4.85 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 3.26 (3H, *s*, OMe), 1.62 (3H, *d*, $J = 6.2$ Hz, 6″-Me), 1.18 (3H, *d*, $J = 6.9$ Hz, 21-Me), 1.01 (3H, *d*, $J = 6.6$ Hz, 27-Me), 0.91 (3H, *s*, 19-Me), 0.79 (3H, *s*, 18-Me).

Enzymic hydrolysis of **4** *and* **5**. Compound **4** (20 mg) was treated with β-glucosidase (20 mg) in HOAc–NaOAc buffer (pH 5, 2 ml) at room temp. for 5 hr. The reaction mixt. was chromatographed on silica gel, eluting initially with $CHCl_3$–MeOH–H_2O (30:10:1) and then with MeOH to give the corresponding spirostanol saponin (**1**) (10.5 mg) and D-glucose. Compound **5** (20 mg) was also converted by the same procedure into the corresponding spirostanol saponin (**2**) (9.4 mg).

Cell culture and assay of ^{32}P*-incorporation into phospholipids of the cultured cells.* HeLa cells were cultured as monolayer in Eagle's minimum essential medium supplemented with 10% calf serum under a humidified atmosphere of 5% CO_2 in air. HeLa cells were incubated with the test samples (50 $\mu g\ ml^{-1}$ and/or 5 $\mu g\ ml^{-1}$), and after 1 hr, ^{32}P (370 kBq culture^{-1}) was added with or without TPA (50 nM). Incubation was continued for 4 hr and then the radioactivity incorporated into the phospholipid fr. was measured [5].

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