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STEROIDAL SAPONINS FROM THE UNDERGROUND PARTS OF *CHLOROPHYTUM COMOSUM* AND THEIR INHIBITORY ACTIVITY ON TUMOUR PROMOTER-INDUCED PHOSPHOLIPIDS METABOLISM OF HELA CELLS

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Key Word Index—*Chlorophytum comosum*; Asphodeloideae; Liliaceae; steroidal saponins; spirostanol saponins; phospholipid metabolism inhibition; HeLa cells.

Abstract—Three new spirostanol pentaglycosides embracing β-D-apiofuranose were isolated from the fresh underground parts of *Chlorophytum comosum* together with four known saponins. The structures of new compounds were determined by spectroscopic data, including two-dimensional NMR, and partial acid-catalysed hydrolysis to be (25*R*)-5α-spirostane-2α, 3β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}, (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2) -*O*-[*O*-β-D-apiofuranosyl-(1 → 4) -β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} and hecogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}, respectively. The isolated saponins were examined for inhibitory activity using 12-*O*-tetradecanoylphorbor-13-acetate-stimulated ^{32}P-incorporation into phospholipids of HeLa cells as the primary screening test to identify new antitumour-promoter compounds.

INTRODUCTION

We have previously examined the underground parts of *Hosta longipes*, which is a member of the subfamily Asphodeloideae in Liliaceae, and isolated six new steroidal saponins [1]. As a part of our contribution to the study of plants belonging to this subfamily, we have now undertaken a phytochemical examination of the underground parts of *Chlorophytum comosum*, which is native to South Africa and used for treatment of bronchitis, fracture and burn as a folk medicine in China [2]. As a result of this study, three new spirostanol pentaglycosides embracing β-D-apiofuranose were isolated along with four known steroidal saponins. This paper reports the structural assignments of the new saponins and their inhibitory activity on 12-*O*-tetradecanoylphorbor-13-acetate (TPA)-stimulated ^{32}P-incorporation into phospholipids of HeLa cells; this is known as an excellent primary screening test for identifying new antitumour-promoter compounds [3–6].

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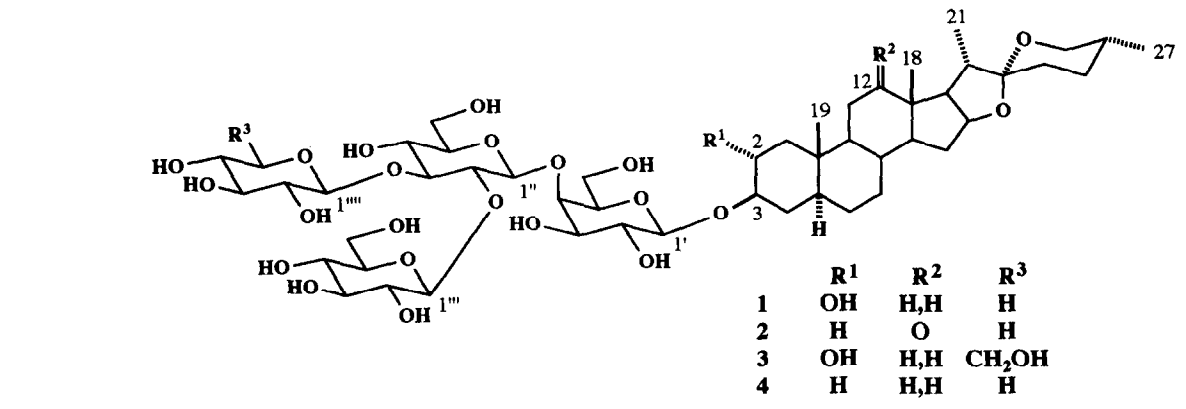
RESULTS AND DISCUSSION

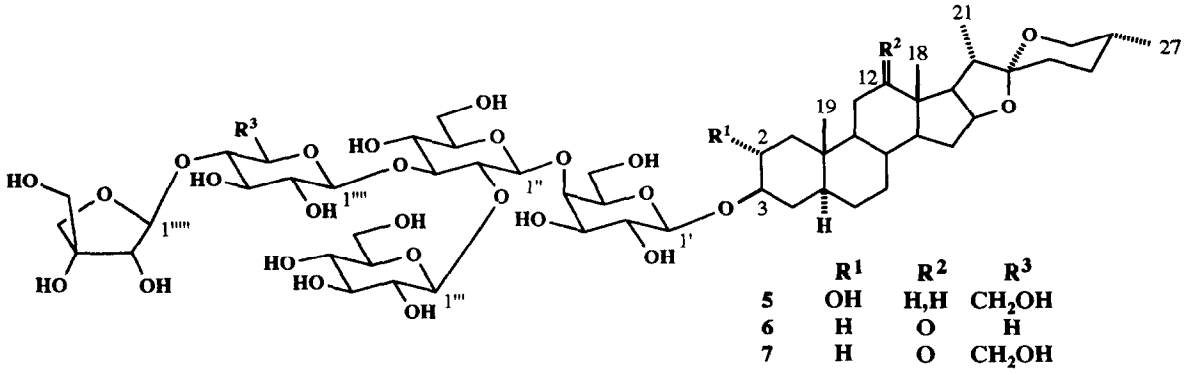
Fresh underground material of *C. comosum* was extracted with hot methanol and the extract was partitioned between 1-butanol and water. The 1-butanol-soluble phase was chromatographed on silica-gel, octadecylsilanized (ODS) silica gel and Diaion HP-20 to yield compounds **1**–**7** (see Experimental).

Compounds **1**–**4** were known spirostanol saponins and identified as (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} (F-gitonin) [7–9], (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} [1], gitogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} [1] and (25*R*)-5α-spirostan-3β-ol (tigogenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} (desgalactotigonin) [7, 10], respectively.

Compound **5** was obtained as an amorphous solid, $[\alpha]_D$ − 43.0° in chloroform–methanol (1 : 1). The molecu-



	R^1	R^2	R^3
1	OH	H,H	H
2	H	O	H
3	OH	H,H	CH_2OH
4	H	H,H	H



	R^1	R^2	R^3
5	OH	H,H	CH_2OH
6	H	O	H
7	H	O	CH_2OH

lar formula $C_{56}H_{92}O_{28}$ was deduced from the ^{13}C NMR spectrum with 56 signals, the negative-ion FAB-mass spectrum showing an $[M-H]^-$ at m/z 1211 and elemental analysis. The ^1H NMR spectrum of **5** displayed signals for typical steroid methyls; two appeared as singlets at δ0.81 and δ0.71 and the other two as doublets at δ1.13 ($J = 6.8$ Hz) and δ0.70 ($J = 6.3$ Hz), and five anomeric protons at δ5.93 (d, $J = 3.1$ Hz), δ5.57 (d, $J = 7.8$ Hz), δ5.26 (d, $J = 7.9$ Hz), δ5.13 (d, $J = 7.8$ Hz) and δ4.91 (d, $J = 7.7$ Hz). On comparison of the whole ^{13}C NMR spectrum of **5** with that of **3**, a set of five additional signals appeared at δ110.9 (CH), 77.5 (CH), 80.1 (C), 75.2 (CH_2) and 64.9 (CH_2), which could be assigned to a terminal apiofuranosyl unit [11,12]. Attempted hydrolysis of **5** with 0.2 M hydrochloric acid in dioxane–H_2O (1:1) at 100° for 2 hr gave **3** and D-apiose; the latter was identified by its specific rotation and direct HPLC comparison with an authentic sample obtained through partial hydrolysis of furcatin, a *p*-allylphenol glycoside embracing D-apiofuranosyl unit [13]. The linkage position of D-apiose was established by detailed interpretation of various two-dimensional NMR spectra, which were recorded in a mixed solvent of pyridine-d_5 and methanol-d_4 in a ratio of 11:1 to minimize signal overlap and remove exchangeable protons. All proton signals of the carbohydrate groups could be assigned by tracing out the spin system for each individual sugar through the ^1H–^1H COSY and HOHAHA spectra, starting from the anomeric proton signals. Assignments of the carbon signals were implemented by detecting one-bond ^1H–^{13}C couplings in the HMQC spectrum (Table 2). In the HMBC spectrum, the anomeric proton of the apiose at δ5.83 (d, $J = 3.4$ Hz) showed a $^3J_{C,H}$ correlation traversing the glycosidic linkage with C-4 (δ79.1) of the glucose attached to C-3 of the 2,3-disubstituted glucose (Fig. 1). The ^{13}C NMR chemical shift of the anomeric carbon of the apiose at δ110.9 indicated a β-orientation of the anomeric centre [14]. The above data unambigously identified the pentasaccharide structure as *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranose. The FAB-mass spectral fragments were assigned as shown in Fig. 1. Accordingly, the full structure of **5** was formulated as gitogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

The ^1H and ^{13}C NMR spectra of **6** ($C_{55}H_{88}O_{27}$) showed a close similarity to those of **2** and the presence of an additional terminal apiofuranosyl group. Partial hydrolysis was made on **6** with 0.2 M hydrochloric acid as for **5** to yield **2** and D-apiose. On comparison of the ^{13}C NMR of **6** with that of **2**, C-4 of the xylose was shifted to downfield by 9.1 ppm, and C-3 and C-5 were

Table 1. ^{13}C NMR spectral data for compounds 1–7*

C	1	2	3	4	5	6	7
1	45.6	36.7	45.6	37.2	45.6	36.6	36.6
2	70.4	29.6	70.5	29.9	70.5	29.7	29.7
3	84.3	77.1	83.2	77.4	84.2	77.1	77.1[a]
4	34.1	34.7	34.1	34.8	34.1	34.7	34.7
5	44.6	44.5	44.6	44.7	44.6	44.5	44.5
6	28.1	28.6	28.1	28.9	28.1	28.6	28.6
7	32.1	31.5[a]	32.1	32.4	32.1	31.5[a]	31.4[b]
8	34.6	34.4	34.6	35.5	34.6	34.4	34.3
9	54.4	55.5	54.4	54.5	54.4	55.5	55.5
10	36.9	36.3	36.9	35.8	36.9	36.3	36.3
11	21.4	38.0	21.4	21.3	21.4	38.0	37.9
12	40.1	212.7	40.1	40.2	40.1	212.7	212.7
13	40.8	55.4	40.8	40.8	40.7	55.4	55.3
14	56.3	55.9	56.3	56.4	56.3	55.9	55.9
15	32.2	31.8[a]	32.2	32.1	32.2	31.7[a]	31.7[b]
16	81.3	79.8	81.1	81.1	81.3	79.7	79.7
17	63.0	54.3	63.0	63.0	63.0	54.3	54.3
18	16.6	16.1	16.6	16.6	16.6	16.1	16.1
19	13.4	11.7	13.4	12.3	13.4	11.7	11.7
20	42.0	42.6	42.0	42.0	42.0	42.7	42.6
21	15.0	13.9	15.0	15.0	15.0	13.9	13.9
22	109.2	109.3	109.2	109.2	109.2	109.3	109.3
23	31.8	31.7[a]	31.8	31.8	31.8	31.8[a]	31.8[b]
24	29.3	29.2	29.3	29.3	29.3	29.3	29.2
25	30.6	30.6	30.6	30.6	30.6	30.6	30.6
26	66.9	67.0	66.9	66.9	66.9	67.0	67.0
27	17.3	17.3	17.3	17.3	17.3	17.3	17.3
Gal 1′	103.3	102.4	103.3	102.4	103.3	102.4	102.4
2′	72.5	73.2	72.6	73.2	72.5	73.2	73.2
3′	75.5	75.6	75.5	75.6	75.5	75.6	75.6
4′	79.4	79.9	79.7	79.9	79.7	79.9	80.2
5′	75.7	75.4	75.7	75.4	75.7	75.4	75.3
6′	60.6	60.6	60.5	60.6	60.6	60.6	60.6
Glc 1″	104.8[a]	105.0[b]	104.8[a]	105.0[a]	104.7	104.6	104.9[c]
2″	81.1	81.3	81.3	81.4	81.1	81.4	81.5
3″	87.0	86.9	88.7	86.8	88.4	86.4	88.2
4″	70.4	70.5	70.7	70.5	70.7	70.5	70.7
5″	77.6	77.6	77.5	77.6	77.5	77.6	77.5
6″	62.7	62.5	62.6	62.5	63.0	63.0	63.0
Glc 1‴	104.7[a]	104.8[b]	104.7[a]	104.9[a]	104.9	104.9	105.1[c]
2‴	76.1	76.2	76.0	76.2	76.1	76.3	76.2
3‴	78.1	78.7	78.2	78.7	78.2	78.8	78.7
4‴	71.4	71.1	71.3	71.0	71.3	71.1	71.0
5‴	78.5	77.8	78.6[b]	77.8	78.5	77.7	77.9
6‴	63.0	63.0	63.0	63.0	62.7	62.6	62.4
Xyl 1⁗	105.0	105.1	104.5	105.1	104.1	105.2	104.2
(Glc) 2⁗	75.1	75.1	75.3	75.1	75.0	75.0	75.0
3⁗	78.7	78.7	78.7[b]	78.7	76.6	76.3	76.6
4⁗	70.8	70.7	71.6	70.7	79.0	79.8	78.9
5⁗	67.3	67.3	78.4	67.3	76.9	64.4	77.0[a]
6⁗			62.3		61.1		61.1
Api 1⁗′					110.9	109.1	111.0
2⁗′					77.5	77.6	77.5
3⁗′					80.1	80.3	80.2
4⁗′					75.2	75.3	75.2
5⁗′					64.9	65.2	64.9

*Spectra were measured in pyridine-d_5.

[a–c]Assignments may be interchanged.

Table 2. ^{1}H and ^{13}C NMR chemical shifts for oligosaccharide moiety of **5***

	^{1}H NMR	^{13}C NMR
Gal 1′	4.87 *d* (7.8)	103.2
2	4.45 *dd* (9.5, 7.8)	72.5
3	4.07 *dd* (9.5, 3.5)	75.4
4	4.51 *br d* (3.5)	79.8
5	4.01	75.7
6	4.52	60.6
	4.16 *dd* (10.9, 5.6)	
Glc 1″	5.09 *d* (7.9)	104.6
2	4.24 *dd* (8.9, 7.9)	81.2
3	4.11	88.1
4	3.71 *dd* (8.9, 8.9)	70.5
5	3.75 *ddd* (8.9, 7.9, 2.0)	77.4
6	4.38 *dd* (11.4, 2.0)	62.9
	3.95 *dd* (11.4, 7.9)	
Glc 1‴	5.52 *d* (7.9)	104.8
2	3.95	76.0
3	4.11	78.1
4	4.04	71.3
5	3.89	78.4
6	4.49 *br d* (10.2)	62.6
	4.36 *dd* (10.2, 5.7)	
Glc 1⁗	5.22 *d* (7.9)	104.1
2	3.90 *dd* (8.7, 7.9)	75.0
3	4.08	76.5
4	4.10	79.1
5	3.87	76.9
6	4.30 *br d* (11.6)	61.1
	4.12	
Api 1⁗′	5.83 *d* (3.4)	110.9
2	4.67 *d* (3.4)	77.4
3	—	80.1
4	4.69 *d* (9.5)	75.1
	4.28 *d* (9.5)	
5	4.09 (2H)	64.6

*Spectra were measured in pyridine-d_5–methanol-d_4 (11:1). *J* values in parentheses are expressed in Hz.

displaced upfield 2.4 and 2.9 ppm, respectively, accounting for the apiose linkage to the C-4 hydroxyl group of the xylose. This was well supported by the FAB-mass spectral fragment ion peak at *m/z* 1018 produced by the result of the elimination of the terminal glucose. The structure of **6** was determined to be hecogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

The ^{1}H and ^{13}C NMR spectra of **7** ($C_{56}H_{90}O_{28}$) indicated that the structure of the saccharide moiety of **7** was identical to that of **5** and that the aglycone corresponded to that of **2** and **6**. The structure of **7** was shown to be hecogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-apiofuranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

Compounds **5**–**7** are new spirostanol saponins. Previously, Li *et al.* [15] is reported the isolation of four new steroidal saponins, named chloromalosides A–D, from *C. malayense*, which is closely related to *C. comosum*. On comparison of the aglycone structures of the saponins from *C. comosum* investigated by us with those from *C. malayense*, the former is based on the (25*R*)-spirostan series, such as tigogenin, gitogenin and hecogenin, while the latter is based on the (25*S*)-spirostan series as in neotigogenin and neohecogenin. Chloromalosides B (**8**) and C (**9**) from *C. malayense*, and **5**–**7** from *C. comosum* are spirostanol pentaglycosides, and the saccharide sequences of the pentaglycoside of **5**–**7** are different from those of **8** and **9**. Compounds **5**–**7** embrace a terminal β-D-apiofuranosyl group and the galactose attached to the aglycone is substituted at the C-4 position by another saccharide. Conversely, the galactose attached to the aglycone of **8** and **9** is branched at C-2 and C-4. The above structural differences are recognized between the saponins of the two taxonomically related plants.

The isolated saponins were evaluated for *in vitro* anti-tumour-promoter activity—measurement of inhibitory activity on TPA-stimulated ^{32}P-incorporation into phospholipides of HeLa cells. This is known to correlate well with antitumour-promoter effects *in vivo* [3–6]. Percentage inhibition at sample concentrations of 50 μg ml^{-1} or 5 μg ml^{-1} are listed in Table 3. Compounds **2**, **3** and **5**–**7** were cytotoxic to HeLa cells at 50 μg ml^{-1} and at the lower concentration (5 μg ml^{-1}), **2** and **6** exhibited 24.2% and 17.2% inhibition while **3** (8.9%) and **5** (1.5%) and **7** (2.5%) were even less potent. Compounds **1** and **4** exhibited 23.1% and 57.8% inhibition at 50 μg ml^{-1} without cytotoxicity towards HeLa cells.

EXPERIMENTAL

General. NMR (ppm, *J* Hz): Bruker AM-400 (400 MHz for ^{1}H NMR) or Bruker AM-500 (500 MHz for ^{1}H 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, RI-8010); TPA: Pharmacia PL Biochemicals; ^{32}P (carrier-free): Japan Radioisotope Associations. Furcatin, a *p*-allylphenol glycoside embracing D-apiofuranosyl group, was provided courtesy of Prof. M. Kikuchi, Tohoku College of Pharmacy.

Plant material. The plant material, *C. comosum* was purchased from Green Heart Co Ltd., Japan, and a voucher specimen is on file in our laboratory.

Extraction and isolation. Fresh underground parts of the plant material were extracted with hot MeOH. The resulting extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was divided into four frs (I–IV) by CC on silica-gel eluting with a step-wise gradient of CH_2Cl_2–MeOH (9:1, 6:1, 4:1, 2:1), and finally with MeOH. Fr. II was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (30:10:1, 20:10:1) to give compound **4** (56.7 mg). Fr. III was subjected to

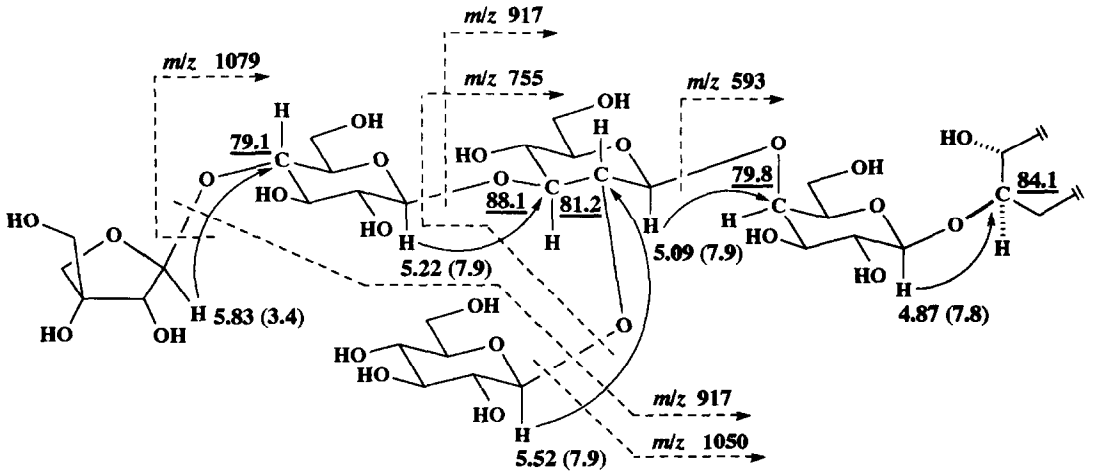
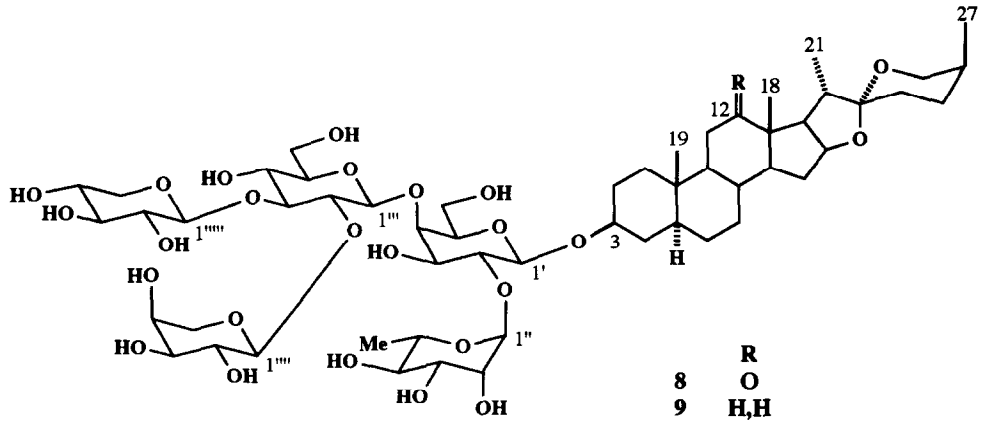


Fig. 1. ^{1}H–^{13}C long-range correlations and FAB-mass spectral fragments of the oligosaccharide moiety of **5**. *J* values (Hz) in the ^{1}H NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C NMR chemical shifts.



CC on silica-gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1, 7:4:1) and ODS silica gel with MeCN–H_2O (1:1) to give **1** (66.9 mg), **2** (69.9 mg) and **3** (49.7 mg). Fr. IV was shown by TLC analysis to contain abundant saccharides along with steroidal saponins. The removal of the saccharides from the fr. IV was implemented by CC on Diaion HP-20 with increasing MeOH in H_2O. Chromatography of the 80% MeOH and MeOH eluate frs on silica gel eluting with $CHCl_3$–MeOH–H_2O (7:4:1) and $CHCl_3$–Et_2O–MeOH–H_2O (7:7:8:2), and on ODS silica-gel with MeOH–H_2O (3:2) and MeCN–H_2O (1:1) furnished **5** (64.6 mg), **6** (27.4 mg) and **7** (13.0 mg).

Compound **5**. Amorphous solid, $[\alpha]_D^{25}$ −43.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Found: C, 54.44; H, 7.72. Calcd for $C_{56}H_{92}O_{28}\cdot H_2O$: C, 54.62; H, 7.69). Negative-ion FAB-MS *m/z* 1211 $[M-H]^-$, 1079 $[M-$ apiosyl$]^-$, 1050 $[M-$ glucosyl$]^-$, 917 $[M-$ apiosyl − glucosyl$]^-$, 755 $[M-$ apiosyl − glucosyl × 2$]^-$, 593 $[M-$ apiosyl − glucosyl × 3$]^-$; IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2940 (CH), 1455, 1375, 1155, 1075, 920, 895; ^{1}H NMR (pyridine-d_5): δ5.93 (1H, *d*, *J* = 3.1 Hz, 1''''-H), 5.57 (1H, *d*, *J* = 7.8 Hz, 1'''-H), 5.26 (1H, *d*, *J* = 7.9 Hz, 1''''-H), 5.15 (1H, *d*, *J* = 7.8 Hz, 1''-H), 4.91 (1H, *d*, *J* = 7.7 Hz, 1'-H), 1.13 (3H, *d*, *J* = 6.8 Hz, 21-Me), 0.81 (3H, *s*, 18-Me), 0.71 (3H, *s*, 19-Me), 0.70 (1H, *d*, *J* = 6.3 Hz, 27-Me).

Acid hydrolysis of **5**. A soln of **5** (20 mg) in 0.2 M HCl (dioxane–H_2O, 1:1) was heated at 100° for 2 hr under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column. A Sep-Pak C_{18} cartridge (Waters) was applied to fractionate the reaction mixture into sugar fr. using H_2O (20 ml) and sapogenin fr. using MeOH (20 ml). Each fr was chromatographed on silica gel eluting with $CHCl_3$–Et_2O–MeOH–H_2O (5:5:4:1) to yield D-apiose (1.1 mg) and **3** (11.4 mg), respectively. D-Apiose was identified by its specific rotation and HPLC analysis (column: Kaseisorb LC NH_2-60-5, Tokyo-Kasei-Kogyo, 4.6 mm i.d. × 250 mm, 5 μm; solvent: MeCN–H_2O (7:3); flow rate: 0.8 ml min^{-1}; detection RI). D-Apiose: $[\alpha]_D^{25}$ + 8.5° (H_2O; *c* 0.11); R_t 7.47 min. Authentic D-apiose (1.5 mg) was obtained from furcatin

Table 3. Inhibitory effect of the isolated saponins on TPA-enhanced ^{32}P-incorporation into phospholipids of HeLa cells*

Compound	Inhibition (%) 50 μg ml^{-1}	Inhibition (%) 5 μg ml^{-1}
1	23.1	—‡
2	—†	24.2
3	—†	8.9
4	57.8	—‡
5	—†	1.5
6	—†	17.2
7	—†	2.5

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

†The samples exhibited cytotoxicity towards HeLa cells.

‡Not measured.

(11.5 mg) through the same procedure as described above.

Compound **6**. Amorphous solid, $[\alpha]_D^{25}$ − 20.0° ($CHCl_3$–MeOH, 1:1 *c* 0.10). Negative-ion FAB-MS *m/z* 1179 $[M - H]^-$, 1048 $[M -$ apiosyl$]^-$, 1018 $[M -$ glucosyl$]^-$, 915 $[M -$ apiosyl − xylosyl$]^-$, 885 $[M -$ apiosyl − glucosyl$]^-$, 753 $[M -$ apiosyl − xylosyl − glucosyl$]^-$, 591 $[M -$ apiosyl − xylosyl − glucosyl × 2$]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1710 (C=O), 1455, 1425, 1375, 1260, 1160, 1070, 1040, 985, 920, 900, 865, 800; ^{1}H NMR (pyridine-d_5): δ5.72 (1H, *d*, *J* = 2.7 Hz, 1''''-H), 5.57 (1H, *d*, *J* = 7.4 Hz, 1'''-H), 5.20 (1H, *d*, *J* = 7.8 Hz, 1''''-H), 5.16 (1H, *d*,*J* = 7.9 Hz, 1''-H), 4.86 (1H, *d*, *J* = 7.7 Hz, 1'-H), 1.36 (3H, *d*, *J* = 6.9 Hz, 21-Me), 1.08 (3H, *s*, 18-Me), 0.70 (1H, *d*, *J* = 5.8 Hz, 27-Me), 0.66 (3H, *s*, 19-Me).

Acid hydrolysis of **6**. Compound **6** (8.5 mg) was subjected to acid hydrolysis as in the case of **5**, which gave **2** (4.3 mg) and D-apiose.

Compound **7**. Amorphous solid, $[\alpha]_D^{25}$ − 14.0° ($CHCl_3$–MeOH, 1:1; *c* 0.10). Negative-ion FAB-MS *m/z* 1209 $[M - H]^-$, 1077 $[M -$ apiosyl$]^-$, 1048 $[M -$ glucosyl$]^-$, 915 $[M -$ apiosyl − glucosyl$]^-$, 753 $[M -$ apiosyl − glucosyl × 2$]^-$, 591 $[M -$ apiosyl − glucosyl × 3$]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1705 (C=O), 1450, 1415, 1375, 1260, 1100, 1065, 915, 895; ^{1}H NMR (pyridine-d_5): δ5.95 (1H, *d*, *J* = 2.9 Hz, 1''''-H), 5.57 (1H, *d*, *J* = 7.6 Hz, 1'''-H), 5.26 (1H, *d*, *J* = 7.8 Hz, 1''''-H), 5.14 (1H, *d*, *J* = 7.9 Hz, 1''-H), 4.86 (1H, *d*, *J* = 7.3 Hz, 1'-H), 1.36 (3H, *d*, *J* = 6.7 Hz, 21-Me), 1.09 (3H, *s*, 18-Me), 0.70 (1H, *d*, *J* = 5.3 Hz, 27-Me), 0.66 (3H, *s*, 19-Me).

Cell culture and assay of ^{32}P-incorporation into phospholipids of the cultured cells. HeLa cells were cultured as a 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 μg ml^{-1} and/or 5 μg 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 phospholipid fraction was measured [3].

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