Steroidal Saponins from the Bulbs of *Allium karataviense*

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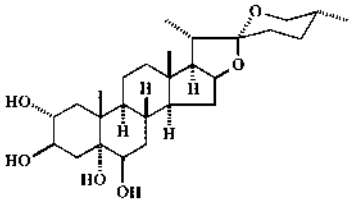
Chemical examination of the bulbs of *Allium karataviense* led to the isolation of five new spirostanol saponins (7—11) and a new furostanol saponin (12), together with a known steroidal sapogenin (1) and five known saponins (2—6). The structures of the new saponins were determined by detailed analysis of their spectral data, including two-dimensional NMR spectroscopy. The steroidal saponins produced by *A. karataviense*, except for 5 and 6, were found to be based upon (25*R*)-5α-spirostane-2α,3β,5,6β-tetrol (alliogenin) and contain a β-D-glucopyranosyl moiety with the formation of an *O*-glycoside linkage to C-2 of the polyhydroxylated steroidal skeleton as the common structural feature. The isolated compounds were evaluated for cytostatic activity against human promyelocytic leukemia HL-60 cells.

Key words *Allium karataviense*; Liliaceae; steroidal saponin; spirostanol saponin; furostanol saponin; leukemia HL-60 cell

Plants of the genus *Allium*, with more than 400 different species, have a wide distribution in the northern hemisphere and have been used both as food and as medicinal materials. The occurrence of steroidal saponins in various species of the genus *Allium* is well documented.[1] We have previously performed a systematic phytochemical screening study of ten *Allium* plants, *Allium giganteum*,[2] *A. aflatunense*,[2b] *A. schubertii*,[3] *A. albopilosum*,[4] *A. ostrowskianum*,[4] *A. chinense*,[5] *A. macleanii*,[6] *A. senescens*,[6] *A. sphaerosephalon*,[7] and *A. jesdianum*,[8] and isolated a variety of new steroidal saponins and cholestane glycosides, some of which appeared to possess unique chemical structures and exhibited significant biological activities. In a continuation of our studies on steroidal glycosides from the genus *Allium*, we have now examined the fresh bulbs of *Allium karataviense* REGEL, which is native to central Asia, especially, Turkestan, and is one of the best-known and grown dwarf species *Allium* for rock gardens. This study resulted in the isolation of five new spirostanol saponins (**7**—**11**) and a new furostanol saponin (**12**), together with a known steroidal sapogenin (**1**) and five known saponins (**2**—**6**). In this paper, we report the structural determination of the new saponins by detailed analysis of their spectral data, including two-dimensional NMR spectroscopy. The activity of the isolated compounds against human promyelocytic leukemia HL-60 cells was also investigated.

The methanolic extract prepared from the fresh bulbs of *A. karataviense* (7.0 kg) was repeatedly subjected to column chromatography on porous-polymer resin (Diaion HP-20), silica-gel, and octadecylsilanized (ODS) silica-gel to afford compounds **1**—**12** in the following yields; **1** (286 mg), **2** (3.13 g), **3** (52.0 mg), **4** (2.88 g), **5** (1.88 g), **6** (363 mg), **7** (23.0 mg), **8** (53.6 mg), **9** (61.7 mg), **10** (68.0 mg), **11** (315 mg), and **12** (118 mg). Compounds **1**—**6** were known steroidal constituents and identified as (25*R*)-5α-spirostane-2α,3β,5,6β-tetrol (alliogenin) (**1**),[2a,9] alliogenin 2-*O*-β-D-glucopyranoside (**2**),[2a] (25*R*)-3-*O*-acetyl-5α-spirostane-2α,3β,5,6β-tetrol 2-*O*-β-D-glucopyranoside (**3**),[2a] (25*R*)-3-*O*-benzoyl-5α-spirostane-2α,3β,5,6β-tetrol 2-*O*-β-D-glucopyranoside (**4**),[2a] (25*R*)-spirost-5-ene-2α,3β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**5**),[10] and alliogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**6**),[11] respectively. The ^{1}H- and ^{13}C-NMR spectral data of **6**, which were not described in the previous paper, are



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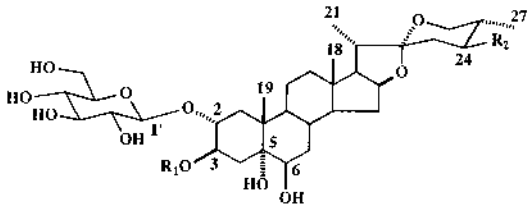
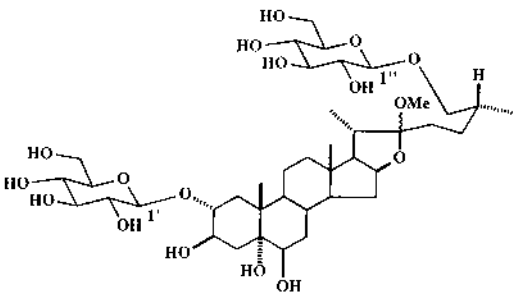


	R_1	R_2
2	**H**	H
3	Acetyl	**H**
4	Benzoyl	H
7	2-Hydroxybutyryl	H
8	Benzoyl	OH
9	H	O-Glc$^{1''}$
10	Benzoyl	O-Glc
11	**H**	O-Glc$^{1''}$-(2←1)-Glc$^{1'''}$



12

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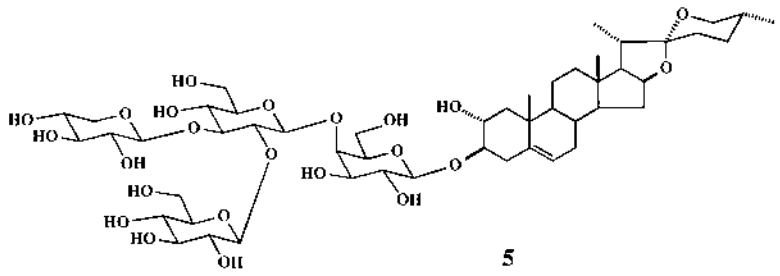

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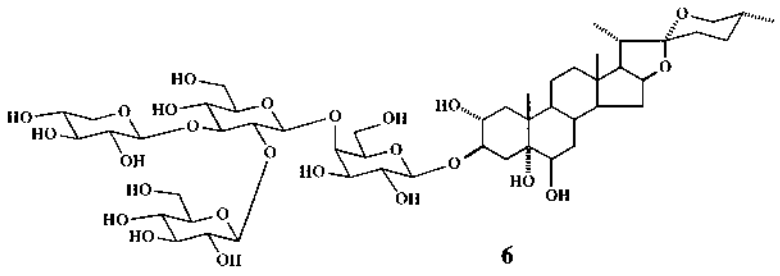

6

reported here in the Experimental section. Copies of the original spectra are obtainable from the authors.

Compound **7**, isolated as an amorphous solid, $[\alpha]_D$ −92.0° (methanol), showed in the high-resolution (HR) FAB-MS (positive mode), an $[M+Na]^+$ peak at *m/z* 735.3959 in accordance with the empirical molecular formula $C_{37}H_{60}O_{13}$, also deduced on the basis of ^{13}C-NMR data. The IR spectrum showed a characteristic absorption due to an ester carbonyl group at 1720 cm^{-1}, as well as a broad absorption attributable to hydroxyl groups near 3430 cm^{-1}. The ^{1}H-NMR spectrum of **7** in pyridine-d_5 showed two three-proton singlet signals at δ 1.54 and 0.88, and two three-proton doublet signals at δ 1.12 (J=7.0 Hz) and 0.67 (J=5.9 Hz), which were characteristic of a spirostanol derivative, and an anomeric proton signal at δ 5.20 (J=7.7 Hz). The ^{13}C-NMR spectrum exhibited a total of 27 carbon signals (C×4, CH×10, CH_2×9, and Me×4) arising from the aglycon moiety, and 6 signals due to a hexopyranosyl moiety, whose shift values [δ 102.5 (CH), 75.2 (CH), 78.3 (CH), 71.2 (CH), 78.5 (CH), 62.5 (CH_2)] corresponded to a β-D-glucopyranosyl group. In addition, the ^{13}C-NMR spectrum showed the presence of a 4 carbon atom substituent, the signals of these carbons being a methyl (δ 23.2), a methylene (δ 45.5), a hydroxymethine (δ 64.4), and an ester carbonyl (δ 171.6). The ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) experiment clarified that the proton signals that could be ascribed to the substituent included the spin system of a Me–CH(OH)–CH_2– group [δ 1.33 (3H, d, J=6.3 Hz), 4.62 (1H, m), 2.80 (1H, dd, J=15.3, 5.2 Hz), 2.76 (1H, dd, J=15.3, 7.4 Hz)]. The ^{1}H/^{13}C long-range correlations from the proton resonances at δ 2.80 and 2.76 to the carbonyl carbon observed in the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum finally allowed the identification of the substituent as a 2-hydroxybutyryl group. Cleavage of the acyl group by the treatment of **7** with 3% sodium methoxide in methanol gave **2**. In the ^{1}H-NMR spectrum of **7**, the H-3 proton of the aglycon was observed at δ 6.18 (ddd, J=11.2, 9.9, 6.1 Hz), suggesting the 2-hydroxybutyryl ester linkage formation at C-3 of the aglycon. This was confirmed by a long-range correlation between the signals for the H-3 proton and the carbonyl carbon of the 2-hydroxybutyryl ester group in the HMBC spectrum. Accordingly, the structure of **7** was determined to be (25*R*)-3-*O*-(2-hydroxybutyryl)-5α-spirostane-2α,3β,5,6β-tetrol 2-*O*-β-D-glucopyranoside. The absolute configuration of the 2-hydroxybutyryl ester moiety remained to be investigated.

Compound **8** was obtained as an amorphous solid, which was shown to have the molecular formula $C_{40}H_{58}O_{13}$ by the HR FAB-MS (*m/z* 747.3400 $[M+H]^+$) and ^{13}C-NMR data. The IR spectrum suggested the presence of an aromatic ester group (1700 cm^{-1}), as well as hydroxy groups (3425 cm^{-1}). The ^{1}H-NMR spectrum of **8** showed signals for four methyl groups of a spirostanol skeleton at δ 1.58 (3H, s), 1.15 (3H, d, J=7.0 Hz), 1.08 (3H, d, J=6.5 Hz), and 0.89 (3H, s), an anomeric proton of a hexopyranose at δ 5.23 (1H, d, J=7.7 Hz), and five aromatic protons assignable to a benzoyl group at δ 8.44 (2H, dd, J=7.8, 2.1 Hz) and 7.45 (3H). The ^{13}C-NMR spectrum of **8**, which included signals from a β-D-glucopyranosyl group and a benzoyl group along with 27 carbon signals from the aglycon moiety, was very similar to that of **4** with the exception of the signals due to the aglycon F-ring part (C-22—C-27). The molecular formula of **8** was higher by one oxygen atom than that of **4**. These facts suggested that the structure of **8** was closely related to that of **4** with a hydroxyl group in the F-ring part. Tracing out the spin-coupling system of the F-ring part through the ^{1}H–^{1}H COSY spectrum, with the Me-27 doublet at δ 1.08 (J=6.5 Hz) being used as the starting point of the analysis, led to assembly of the structure of the F-ring as –CH_2–CH(OH)–CH(Me)–CH_2–O– and confirmation of the presence of a C-24 hydroxyl group. The configurations at C-24 and C-25 were revealed to be 24*S* and 25*S*, respectively, by the multiplicity of the H-24 proton ($^3J_{H\text{-}24,H\text{-}23ax}$=10.5 Hz, $^3J_{H\text{-}24,H\text{-}23eq}$=4.8 Hz, $^3J_{H\text{-}24,H\text{-}25}$=10.5 Hz). Alkaline methanolysis of **8** with 3% sodium methoxide in methanol gave deacyl saponin (**8a**) and methyl benzoate. Enzymatic hydrolysis of **8a** with hesperidinase afforded D-glucose and an aglycon (**8b**). The ^{1}H- and ^{13}C-NMR spectra of **8b** also indicated the presence of an equatorial orientated hydroxyl group at C-24 [δ_H 3.99 (1H, ddd, J=10.3, 10.3, 4.0 Hz): δ_C 70.6 (CH)], as well as the presence of the C-2α, C-3β, C-5, and C-6β hydroxyl groups. In the HMBC spectrum, correlation peaks

Table 1. ^{1}H-NMR Spectral Data for Compounds **7**—**11** in Pyridine-d_5

H	7	8	9	10	11
1ax	2.41 dd (12.3, 10.9)	2.48 dd (12.3, 11.1)	2.36 dd (12.3, 11.5)	2.47 dd (12.2, 11.6)	2.36 dd (12.2, 11.6)
eq	2.35 dd (12.3, 5.9)	2.38 dd (12.3, 5.6)	2.15 dd (12.3, 5.0)	2.38 dd (12.2, 5.6)	2.15 dd (12.2, 5.1)
2	4.73 ddd (10.9, 9.9, 5.9)	4.90 ddd (11.1, 9.8, 5.6)	4.39 ddd (11.5, 8.8, 5.0)	4.90 ddd (11.6, 10.1, 5.6)	4.40 ddd (11.6, 8.8, 5.1)
3	6.18 ddd (11.2, 9.9, 6.1)	6.30 ddd (11.0, 9.8, 6.1)	4.82 ddd (11.2, 8.8, 6.2)	6.31 ddd (10.8, 10.1, 6.1)	4.82 ddd (11.2, 8.8, 6.1)
4ax	2.86 dd (13.2, 11.2)	2.90 dd (13.2, 11.0)	2.97 dd (13.1, 11.2)	2.91 dd (13.2, 10.8)	2.97 dd (13.1, 11.2)
eq	2.40 dd (13.2, 6.1)	2.57 dd (13.2, 6.1)	2.38 dd (13.1, 6.2)	2.58 dd (13.2, 6.1)	2.38 dd (13.1, 6.1)
6	4.15 br s	4.19 br s	4.15 br s	4.18 br s	4.15 br s
16	4.57 q-like (7.3)	4.59 q-like (7.3)	4.56 q-like (6.9)	4.55 q-like (6.9)	4.50 q-like (7.3)
18	0.88 s	0.89 s	0.80 s	0.82 s	0.81 s
19	1.54 s	1.58 s	1.55 s	1.58 s	1.56 s
21	1.12 d (7.0)	1.15 d (7.0)	1.04 d (6.9)	1.06 d (6.9)	1.02 d (6.9)
23ax	—	2.00 dd (12.6, 10.5)	1.94 dd (12.9, 10.7)	1.96 dd (12.9, 10.5)	1.97 dd (12.9, 10.7)
eq	—	2.31 dd (12.6, 4.8)	2.63 dd (12.9, 4.8)	2.66 dd (12.9, 4.7)	2.62 dd (12.9, 4.8)
24	—	4.00 ddd (10.5, 10.5, 4.8)	3.99 ddd (10.7, 10.7, 4.8)	4.01 ddd (10.5, 10.5, 4.7)	3.95 ddd (10.7, 10.7, 4.8)
25	—	1.82 m	1.88 m	1.89 m	1.99 m
26ax	3.47 dd (10.7, 10.7)	3.58 dd (11.2, 11.2)	3.54 dd (11.4, 11.4)	3.56 dd (11.3, 11.3)	3.51 dd (11.4, 11.4)
eq	3.56 dd (10.7, 3.7)	3.69 dd (11.2, 4.8)	3.61 dd (11.4, 4.2)	3.63 dd (11.3, 5.0)	3.61 dd (11.4, 5.0)
27	0.67 d (5.9)	1.08 d (6.5)	1.12 d (6.4)	1.13 d (6.5)	1.24 d (6.4)
1′	5.20 d (7.7)	5.23 d (7.7)	5.16 d (7.8)	5.23 d (7.7)	5.17 d (7.8)
2′	4.01 dd (8.8, 7.7)	3.96 dd (8.9, 7.7)	4.11 dd (8.9, 7.8)	3.96 dd (9.0, 7.7)	4.12 dd (8.8, 7.8)
3′	4.30 dd (8.8, 8.8)	4.29 dd (8.9, 8.9)	4.30 dd (8.9, 8.9)	4.29 dd (9.0, 9.0)	4.31 dd (8.8, 8.8)
4′	4.31 dd (8.8, 8.8)	4.19 dd (8.9, 8.9)	4.23 dd (8.9, 8.9)	4.19 dd (9.0, 9.0)	4.24 dd (8.8, 8.8)
5′	4.00 ddd (8.8, 3.9, 1.9)	3.91 ddd (8.9, 4.6, 2.5)	4.09 ddd (8.9, 5.9, 2.0)	3.91 ddd (9.0, 4.9, 2.5)	4.10 ddd (8.8, 5.7, 2.2)
6′a	4.49 dd (11.8, 1.9)	4.41 dd (11.5, 2.5)	4.61 dd (11.5, 2.0)	4.41 dd (11.7, 2.5)	4.61 dd (11.6, 2.2)
b	4.44 dd (11.8, 3.9)	4.30 dd (11.5, 4.6)	4.33 dd (11.5, 5.9)	4.30 dd (11.7, 4.9)	4.34 dd (11.6, 5.7)
1″	—	—	4.89 d (7.7)	4.91 d (7.8)	4.91 d (7.3)
2″	—	—	4.04 dd (8.9, 7.7)	4.06 dd (9.0, 7.8)	4.22 dd (9.0, 7.3)
3″	—	—	4.20 dd (8.9, 8.9)	4.22 dd (9.0, 9.0)	4.24 dd (9.0, 9.0)
4″	—	—	4.27 dd (8.9, 8.9)	4.28 dd (9.0, 9.0)	4.29 dd (9.0, 9.0)
5″	—	—	3.85 ddd (8.9, 5.0, 2.2)	3.87 ddd (9.0, 5.1, 2.3)	3.92 ddd (9.0, 4.5, 2.8)
6″a	—	—	4.50 dd (11.5, 2.2)	4.51 dd (11.7, 2.3)	4.45 dd (11.3, 2.8)
b			4.38 dd (11.5, 5.0)	4.39 dd (11.7, 5.1)	4.38 dd (11.3, 4.5)
1‴	—	—	—	—	5.37 d (7.7)
2‴	—	—	—	—	4.11 dd (8.8, 7.7)
3‴	—	—	—	—	4.28 dd (8.8, 8.8)
4‴	—	—	—	—	4.23 dd (8.8, 8.8)
5‴	—	—	—	—	3.78 ddd (8.8, 4.9, 2.3)
6‴a	—	—	—	—	4.46 dd (11.7, 2.3)
b			—	—	4.33 dd (11.7, 4.9)
Acyl group					
2	2.80 dd (15.3, 5.2) 2.76 dd (15.3, 7.4)	8.44 dd (7.8, 2.1)	—	8.45 dd (7.7, 2.3)	—
3	4.62 m	7.45	—	7.45	—
4	1.33 d (6.3)	7.45	—	7.45	—
5	—	7.45	—	7.45	—
6	—	8.44 dd (7.8, 2.1)	—	8.45 dd (7.7, 2.3)	—

J values in parentheses are expressed in Hz.

were observed between the anomeric proton of the glucosyl residue and the C-2 carbon of the aglycon at δ 77.7, and between the H-3 proton of the aglycon at δ 6.30 (ddd, J=11.0, 9.8, 6.1 Hz) and the carbonyl carbon of the benzoyl group. This confirmed that the glucosyl residue was bonded to the C-2 hydroxyl group of the aglycon and the benzoyl group to the C-3 hydroxyl, as in **4**. The structure of **8** was formulated as (24*S*,25*S*)-3-*O*-benzoyl-5α-spirostane-2α,3β,5,6β,24-pentol 2-*O*-β-D-glucopyranoside.

Compound **9** was obtained as an amorphous solid. The HR FAB-MS of **9** showed an $[M+Na]^+$ ion at *m/z* 827.4111, consistent with the molecular formula $C_{39}H_{64}O_{17}$. The ^{1}H-NMR spectrum contained two anomeric proton signals at δ 5.16 (1H, d, J=7.8 Hz) and 4.89 (1H, d, J=7.7 Hz), as well as four steroid methyl proton signals at δ 1.55 (3H, s), 1.12 (3H, d, J=6.4 Hz), 1.04 (3H, d, J=6.9 Hz), and 0.80 (3H, s). Acid hydrolysis of **9** with 1 M hydrochloric acid in dioxane–H_2O (1 : 1) gave D-glucose and several artifactual sapogenols. The ^{1}H- and ^{13}C-NMR assignments of **9**, which were carried out by analysis of the ^{1}H–^{1}H COSY spectrum followed by ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectral data, confirmed the identity of the aglycon of **9** with that of **8** and the presence of two terminal β-D-glucopyranosyl units [δ 104.6, 75.2, 78.5, 71.8, 78.5, 62.7; δ 106.4, 75.6, 78.6, 71.7, 78.0, 62.8] in **9**. In the HMBC spectrum, the anomeric proton signals at δ 5.16 and 4.89 showed long-range correlations with the respective carbon signals at δ 85.0 (C-2 of aglycon) and 81.4 (C-24 of aglycon). The structure of **9** was shown to be (24*S*,25*S*)-5α-spirostane-2α,3β,5,6β,24-pentol 2,24-di-*O*-β-D-glucopyranoside.

Compound **10** was isolated as an amorphous solid. An accurate $[M+Na]^+$ ion at *m/z* 931.4286 in the HR FAB-MS corresponded to the molecular formula $C_{46}H_{68}O_{18}$. The spectral properties of **10** were essentially analogous to those of

Table 2. ^{13}C-NMR Spectral Data for Compounds **7**, **8**, **8a**, **8b**, and **9**—**12** in Pyridine-d_5

C	7	8	8a	8b	9	10	11	12
1	38.5	38.9	39.7	42.3	39.6	38.9	39.6	39.6
2	77.1	77.7	85.1	73.7	85.0	77.7	85.1	85.2
3	74.9	76.4	71.5	73.8	71.4	76.4	71.4	71.4
4	37.8	37.9	40.2	41.2	40.2	37.9	40.3	40.2
5	74.9	75.0	74.9	75.7	74.9	75.0	74.9	74.9
6	75.0	75.0	75.3	75.6	75.3	75.0	75.3	75.3
7	35.7	35.7	35.8	35.9	35.7	35.7	35.7	35.7
8	30.1	30.1	30.2	30.3	30.1	30.0	30.1	30.1
9	45.6	45.6	45.8	46.0	45.7	45.6	45.7	45.7
10	40.5	40.4	40.7	41.1	40.6	40.5	40.7	40.6
11	21.5	21.5	21.6	21.7	21.5	21.5	21.6	21.5
12	40.3	40.3	40.4	40.5	40.3	40.3	40.3	40.5
13	41.0	41.0	41.0	41.0	40.9	41.0	41.0	41.3
14	56.1	56.2	56.4	56.4	56.3	56.1	56.3	56.2
15	32.3	32.3	32.3	32.3	32.1	32.2	32.2	32.2
16	81.2	81.6	81.6	81.6	81.6	81.6	81.6	81.4
17	63.1	62.8	62.8	62.8	62.6	62.6	62.6	64.4
18	16.6	16.6	16.7	16.7	16.6	16.6	16.7	16.3
19	17.9	18.0	18.1	18.5	18.1	18.0	18.1	18.1
20	42.0	42.3	42.3	42.3	42.1	42.1	42.1	40.6
21	15.0	15.0	15.0	15.0	14.9	14.9	14.9	16.6
22	109.2	111.8	111.8	111.8	111.5	111.6	111.5	112.6
23	31.8	41.8	41.8	41.9	40.8	40.9	40.7	30.8
24	29.2	70.6	70.6	70.6	81.4	81.5	81.7	28.2
25	30.6	39.9	39.9	40.0	38.2	38.2	38.1	34.2
26	66.8	65.3	65.3	65.3	65.1	65.1	65.2	75.2
27	17.3	13.6	13.6	13.6	13.4	13.5	13.7	17.2
OMe	—	—	—	—	—	—	—	47.2
1′	102.5	103.4	104.7	—	104.6	103.4	104.7	104.7
2′	75.2	75.3	75.2	—	75.2	75.3	75.2	75.2
3′	78.3	78.4	78.5	—	78.5	78.4	78.5	78.5
4′	71.2	71.7	71.9	—	71.8	71.7	71.8	71.8
5′	78.5	78.3	78.5	—	78.5	78.4	78.5	78.5
6′	62.5	62.9	62.8	—	62.7	62.9	62.7	62.7
1″	—	—	—	—	106.4	106.4	104.2	105.0
2″	—	—	—	—	75.6	75.7	83.8	75.2
3″	—	—	—	—	78.6	78.6	78.0	78.6
4″	—	—	—	—	71.7	71.7	71.7	71.7
5″	—	—	—	—	78.0	78.0	78.4	78.5
6″	—	—	—	—	62.8	62.9	62.8	62.9
1‴	—	—	—	—	—	—	106.4	—
2‴	—	—	—	—	—	—	77.0	—
3‴	—	—	—	—	—	—	78.3	—
4‴	—	—	—	—	—	—	71.5	—
5‴	—	—	—	—	—	—	77.8	—
6‴	—	—	—	—	—	—	62.6	—
Acyl group								
1	171.6	131.9	—	—	—	131.9	—	—
2	45.5	130.3	—	—	—	130.3	—	—
3	64.4	128.7	—	—	—	128.7	—	—
4	23.2	132.9	—	—	—	132.9	—	—
5	—	128.7	—	—	—	128.7	—	—
6	—	130.3	—	—	—	130.3	—	—
7	—	166.8	—	—	—	166.8	—	—

9 and suggestive of **10** being a bisdesmosidic spirostanol saponin of the same type. The existence of a benzoyl group in **10** was indicated by the IR (1705 cm^{-1}), ^{1}H-NMR [δ 8.45 (2H, dd, J=7.7, 2.3 Hz), 7.45 (3H)], and ^{13}C-NMR [δ 166.8 (C=O), 132.9, 131.9, 130.3×2, 128.7×2]. Treatment of **10** with 3% sodium methoxide in methanol produced methyl benzoate and a steroidal saponin, which was identified as **9**. The ester linkage at the aglycon C-3 was formed from benzoic acid, as was evident from a long-range correlation between the resonanes of the H-3 proton at δ 6.31 (ddd, J= 10.8, 10.1, 6.1 Hz) and the carbonyl carbon of the benzoyl ester group. Thus, the structure of **10** was assigned as (24*S*,25*S*)-3-*O*-benzoyl-5α-spirostane-2α,3β,5,6β,24-pentol 2,24-di-*O*-β-D-glucopyranoside.

Compound **11** was obtained as an amorphous solid. Its molecular formula ($C_{45}H_{74}O_{22}$) was deduced from the FAB-MS (negative mode), which showed an $[M-H]^-$ ion at m/z 965, ^{13}C-NMR data, and elemental analysis. The ^{1}H-NMR spectrum showed signals for three anomeric protons at δ 5.37 (1H, d, J=7.7 Hz), 5.17 (1H, d, J=7.8 Hz), and 4.91 (1H, d, J=7.3 Hz) together with signals for four steroid methyls at δ 1.56 (3H, s), 1.24 (3H, d, J=6.4 Hz), 1.02 (3H, d, J=6.9 Hz), and 0.81 (3H, s). Acid hydrolysis of **11** with 1 M hydrochloric acid gave D-glucose and several degradation products from the aglycon. The ^{1}H- and ^{13}C-NMR assignments of **11** indicated that it was a 2,24-bisdesmosidic spirostanol saponin closely related to **9** with two terminal β-D-glucopyranosyl units (δ_H 5.17/δ_C 104.7, 75.2, 78.5, 71.8, 78.5, 62.7; δ_H 5.37/δ_C 106.4, 77.0, 78.3, 71.5, 77.8, 62.6) and a 2-substituted β-D-glucopyranosyl unit (δ_H 4.91/δ_C 104.2, 83.8, 78.0, 71.7, 78.4, 62.8). In the HMBC spectrum of **11**, the anomeric proton signals at δ 5.37 (terminal glucosyl), 5.17 (terminal glucosyl), and 4.91 (2-substituted glucosyl) were correlated to the three-bond coupled carbon signals at δ 83.8 (C-2 of 2-substituted glucosyl), 85.1 (C-2 of aglycon), and 81.7 (C-24 of aglycon), respectively. This was evidence for one β-D-glucopyranosyl moiety linkage to C-2 of the aglycon and the diglycoside, *O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl moiety to C-24. Thus, the structure of **11** was elucidated as (24*S*,25*S*)-5α-spirostane-2α,3β,5,6β,24-pentol 2-*O*-β-D-glucopyranosyl 24-*O*-{*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside}.

Compound **12** was obtained as an amorphous solid and its molecular formula was $C_{40}H_{68}O_{17}$ from the HR FAB-MS (m/z 843.4301 $[M+Na]^+$) and ^{13}C-NMR data. The fundamental structure of **12** was shown to be a 22-methoxy-furostanol saponin by Ehrlich's test,[12] and by the ^{1}H-NMR [δ 3.27 (3H, s)] and ^{13}C-NMR [δ 112.6 (C), 47.2 (Me)] spectra.[13] The ^{1}H-NMR spectrum showed signals for four steroid methyl groups at δ 1.60 (3H, s), 1.21 (3H, d, J=6.9 Hz), 1.04 (3H, d, J=6.7 Hz), and 0.89 (3H, s), and two anomeric protons at δ 5.21 (1H, d, J=7.7 Hz) and 4.90 (1H, d, J=7.7 Hz). Enzymatic hydrolysis of **12** with β-D-glucosidase gave **2** and D-glucose. The structure of **12** was shown to be (25*R*)-26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furostane-2α,3β,5,6β,22ξ-pentol 2-*O*-β-D-glucopyranoside.

Compounds **7**—**12** are new steroidal saponins based upon (25*R*)-5α-spirostane-2α,3β,5,6β-tetrol (alliogenin) and contain a β-D-glucopyranosyl moiety with the formation of an *O*-glycoside linkage to C-2 of the polyhydroxylated steroidal skeleton as the common structural feature. Alliogenin 2-*O*-glucoside and its derivatives, including a glucoside of the C-6 isomer of alliogenin, have been found only in *A. giganteum*,[2a,c] *A. aflatunense*,[2b] *A. albopilosum*,[4] and *A. macleanii*,[6] and are thought to be characteristic of some limited species of the genus *Allium*.

Cytostatic activity of the isolated compounds against human promyelocytic leukemia HL-60 cells was evaluated. The cells were continuously treated with each sample for 72 h, and cell growth was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Compound **5** exhibited considerable cytostatic activity with an IC_{50} value of 2.4 μg/ml, compared with etoposide used as a positive control (IC_{50} 0.3 μg/ml), while the other compounds were inactive (IC_{50}>10 μg/ml).

Experimental

Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO A-100 spectrophotometer. NMR spectra were recorded on a Bruker DPX-400 spectrometer (400 MHz for ^{1}H-NMR) or on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H-NMR) using XWIN-NMR pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a VG AutoSpec E mass spectrometer. Elemental analysis was carried out using an Elementar Vario EL elemental analyzer. Diaion HP-20 (Mitsubishi-Kasei, Japan), silica gel (Fuji-Silysia Chemical, Japan), and ODS silica gel (Nacalai Tesque, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a Tosoh HPLC system comprised of a CCPM pump, a CCP controller PX-8010, a UV-8000, and Rheodyne injection port with a 20 μl sample loop. A Capcell Pak C_{18} column (4.6 mm i.d.×250 mm, ODS, 5 μm, Shiseido, Japan) was employed for HPLC analysis. The following materials and reagents were used for cell culture and assay of cytostatic activity: microplate reader, Inter Med Immuno-Mini NJ-2300 (Japan); 96-well flat-bottom plate, Iwaki Glass (Japan); HL-60 cells, ICN Biomedicals (U.S.A.); RPMI 1640 medium, GIBCO BRL (U.S.A.); MTT, Sigma (U.S.A.). All other chemicals used were of biochemical reagent grade.

Plant Material The bulbs of *A. karataviense* were purchased from a nursery in Heiwaen, Japan. The bulbs were cultivated and a voucher of the plant is on file in our laboratory.

Extraction and Isolation The plant material (fresh weight, 7.0 kg) was extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure and passed through a Diaion HP-20 column eluting with increasing amounts of MeOH in H_2O. Column chromatography of the MeOH eluate portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (9 : 1; 4 : 1; 2 : 1; 1 : 1), and finally with MeOH alone, gave seven fractions (I—VII). Fraction I was chromatographed on silica gel eluting with $CHCl_3$–MeOH (12 : 1) and ODS silica gel with MeOH–H_2O (8 : 3) to give **4** (2.88 g). Fraction II was subjected to a silica gel column eluting with $CHCl_3$–MeOH (6 : 1), and the sapogenin and saponin mixture thus obtained was dissolved in $CHCl_3$–MeOH–H_2O. After being set aside for 12 h, it precipitated an amorphous solid, which was filtered off to give **1** (286 mg). The filtrate was chromatographed on silica gel eluting with $CHCl_3$–MeOH (7 : 1) and ODS silica gel with MeOH–H_2O (8 : 5) and MeCN–H_2O (5 : 8; 1 : 2) to yield **3** (52.0 mg), **7** (23.0 mg), and **8** (53.6 mg). Fraction III was dissolved in $CHCl_3$–MeOH–H_2O and the deposited precipitate was filtered off to give **2** (3.13 g). The filtrate was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH (5 : 1) and ODS silica gel column chromatography with MeOH–H_2O (8 : 3; 1 : 1) and MeCN–H_2O (1 : 3) to afford **10** (68.0 mg). Fraction V was dissolved in $CHCl_3$–MeOH–H_2O and the precipitated compound was filtered off to give **5** (1.88 g). Fraction VII was subjected to an ODS column eluting with MeOH–H_2O (8 : 11) and divided into three additional fractions (VIIa—VIIc). Fraction VIIa was purified by column chromatography on silica gel eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) and ODS silica gel with MeOH–H_2O (2 : 3; 1 : 2) to furnish **9** (61.7 mg), **11** (315 mg), and **12** (118 mg). Fraction VIIb was dissolved in $CHCl_3$–MeOH–H_2O and the precipitate was filtered off to give **6** (363 mg).

Compound **6**: Amorphous solid, $[\alpha]_D^{25}$ −86.0° ($CHCl_3$–MeOH, 1 : 1, c= 0.10). FAB-MS (negative mode) m/z: 1081 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2930 and 2875 (CH), 1450, 1420, 1375, 1235, 1200, 1150, 1060, 1035, 975, 955, 915, 890, 860. ^{1}H-NMR (pyridine-d_5) δ: 5.60 (1H, d, J=7.7 Hz, H-1‴), 5.29 (1H, d, J=7.8 Hz, H-1⁗), 5.24 (1H, d, J=7.9 Hz, H-1″), 4.82 (1H, d, J=7.8 Hz, H-1′), 1.56 (3H, s, Me-19), 1.15 (3H, d, J=6.9 Hz, Me-21), 0.92 (3H, s, Me-18), 0.70 (3H, d, J=5.6 Hz, Me-27). ^{13}C-NMR (pyridine-d_5) δ: 41.3, 70.8, 83.0, 38.1, 75.3, 75.3, 35.6, 30.2, 45.7, 40.3, 21.5, 40.4, 40.9, 56.2, 32.3, 81.2, 63.0, 16.7, 18.3, 41.9, 15.0, 109.2, 31.7, 29.2, 30.5, 66.8, 17.3 (C-1—C-27), 103.5, 72.6, 75.5, 79.0, 75.5, 60.4 (C-1′—C-6′), 104.6, 81.2, 86.7, 70.4, 77.5, 62.9 (C-1″—C-6″), 104.8, 76.1, 78.0, 71.3, 78.4, 62.7 (C-1‴—C-6‴), 104.9, 75.1, 78.6, 70.7, 67.3 (C-1⁗—C-5⁗).

Compound **7**: Amorphous solid, $[\alpha]_D^{27}$ −92.0° (MeOH, c=0.10). HR FAB-MS (positive mode) m/z: 735.3959 $[M+Na]^+$ ($C_{37}H_{60}O_{13}$·Na, Calcd for 735.3932). IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2950 and 2885 (CH), 1720 (C=O), 1455, 1375, 1255, 1240, 1170, 1150, 1060, 1040, 975, 955, 915, 895, 860.

Alkaline Methanolysis of 7 Compound **7** (5 mg) was treated with 3% NaOMe in MeOH (2 ml) at room temperature for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo, Japan) column and chromatographed on silica gel using $CHCl_3$–MeOH (4 : 1) to yield **2** (2.8 mg).

Compound **8**: Amorphous solid, $[\alpha]_D^{27}$ −106.0° (MeOH, c=0.10). HR FAB-MS (positive mode) m/z: 747.3400 $[M+H]^+$ ($C_{40}H_{59}O_{13}$, Calcd for 747.3956). IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 2935 and 2890 (CH), 1700 (C=O), 1605 and 1585 (aromatic ring), 1455, 1380, 1320, 1275, 1210, 1175, 1120, 1060, 1025, 990, 950, 890.

Alkaline Methanolysis of 8 Compound **8** (15 mg) was treated with 3% NaOMe in MeOH (4 ml) at room temperature for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and chromatographed on silica gel using $CHCl_3$–MeOH (19 : 1; 3 : 1) to yield **8a** (12.6 mg) and methyl benzoate (1.8 mg). Compound **8a**: amorphous solid, $[\alpha]_D^{24}$ −88.0° (MeOH, c=0.10). FAB-MS (negative mode) m/z: 641 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 3390 (OH), 2925 (CH), 1445, 1375, 1205, 1160, 1035, 985, 950, 885. ^{1}H-NMR (pyridine-d_5) δ: 5.16 (1H, d, J=7.8 Hz, H-1′), 4.81 (1H, ddd, J=11.3, 8.8, 6.1 Hz, H-3), 4.57 (1H, q-like, J=7.4 Hz, H-16), 4.38 (1H, ddd, J=11.5, 8.8, 5.3 Hz, H-2), 4.16 (1H, br s, H-6), 3.99 (1H, ddd, J=10.7, 10.7, 4.8 Hz, H-24), 3.68 (1H, dd, J=11.3, 4.8 Hz, H-26eq), 3.57 (1H, dd, J=11.3, 11.3 Hz, H-26ax), 1.57 (3H, s, Me-19), 1.15 (3H, d, J=6.9 Hz, Me-21), 1.07 (3H, d, J=6.5 Hz, Me-27), 0.88 (3H, s, Me-18). Methyl benzoate was identified by direct TLC comparison with an authentic sample. *Rf* 0.34 (hexane–$CHCl_3$, 2 : 1); 0.45 (hexane–EtOAc, 19 : 1).

Enzymatic Hydrolysis of 8a Compound **8a** (10 mg) was dissolved in an AcOH/AcOK buffer (pH 4.3, 5 ml) with hesperidinase (Sigma) (20 mg) and the mixture was incubated at room temperature for 72 h. The crude product was passed through a Diaion HP-20 column, firstly with H_2O–MeOH (4 : 1) as mobile phase and then with MeOH to give the sugar and sapogenin fractions. The sugar fraction was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to give D-glucose (0.9 mg). The sapogenin fraction was purified by a silica gel column eluting with $CHCl_3$–MeOH (4 : 1) to give an aglycon (**8b**) (3.1 mg). D-Glucose was identified by direct TLC comparison with an authentic sample. *Rf* 0.39 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1). Compound **8b**: amorphous solid, $[\alpha]_D^{30}$ −82.0° (MeOH, c=0.10). EI-MS m/z: 480.3114 $[M]^+$ ($C_{27}H_{44}O_7$, Calcd for 480.3087). IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 2930 (CH), 1450, 1375, 1275, 1205, 1170, 1030, 990, 950, 895. ^{1}H-NMR (pyridine-d_5) δ: 4.83 (1H, ddd, J=11.7, 9.1, 5.7 Hz, H-3), 4.58 (1H, q-like, J=7.3 Hz, H-16), 4.44 (1H, ddd, J=11.4, 9.1, 5.1 Hz, H-2), 4.21 (1H, br s, H-6), 3.99 (1H, ddd, J=10.3, 10.3, 4.0 Hz, H-24), 3.69 (1H, dd, J=11.3, 4.8 Hz, H-26eq), 3.58 (1H, dd, J=11.3, 11.3 Hz, H-26ax), 1.68 (3H, s, Me-19), 1.16 (3H, d, J=6.9 Hz, Me-21), 1.07 (3H, d, J=6.5 Hz, Me-27), 0.91 (3H, s, Me-18).

Compound **9**: Amorphous solid, $[\alpha]_D^{27}$ −78.0° (MeOH, c=0.10). HR FAB-MS (positive mode) m/z: 827.4111 $[M+Na]^+$ ($C_{39}H_{64}O_{17}$·Na, Calcd for 827.4041). IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2920 (CH), 1455, 1370, 1255, 1155, 1060, 1020, 885.

Acid Hydrolysis of 9 A solution of **9** (6.5 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column and fractionated by a Sep-Pak C_{18} cartridge (Waters, U.S.A.) successively eluting with H_2O, H_2O–MeOH (8 : 2), and MeOH to give a sugar fraction (2.1 mg) and an aglycon fraction (3.4 mg). TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction 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 °C for 4 h, followed by addition of AcOH (0.2 ml) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4 : 1; 1 : 1, each 5 ml) mixtures as eluting solvent. The H_2O–MeCN (1 : 1) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh, Japan) with EtOH (10 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of a monosaccharide,[14] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2 : 3); flow rate, 0.8 ml/min; detection, UV 230 nm. The derivative of D-glucose was detected; t_R (min): 17.19.

Compound **10**: Amorphous solid, $[\alpha]_D^{27}$ −60.0° (MeOH, c=0.10). HR FAB-MS (positive mode) m/z: 931.4286 $[M+Na]^+$ ($C_{46}H_{68}O_{18}$·Na, Calcd

for 931.4303). IR ν_{max} (KBr) cm^{-1}: 3425 (OH), 2935 and 2895 (CH), 1705 (C=O), 1605 and 1585 (aromatic ring), 1455, 1375, 1310, 1280, 1160, 1060, 1025, 955, 895.

Alkaline Methanolysis of 10 Compound **10** (30.2 mg) was treated with 3% NaOMe in MeOH (6 ml) at room temperature for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and chromatographed on silica gel using $CHCl_3$–MeOH (19 : 1; 2 : 1) to yield **9** (23.3 mg) and methyl benzoate (2.1 mg).

Compound **11**: Amorphous solid, $[\alpha]_D^{27}$ −67.0° (MeOH, c=0.10). FAB-MS (negative mode) m/z: 965 $[M-H]^-$, 803 $[M-glucosyl]^-$. *Anal*. Calcd for $C_{45}H_{74}O_{22}\cdot H_2O$: C, 54.87; H, 7.78. Found: C, 56.68; H, 8.08. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2920 (CH), 1450, 1370, 1250, 1165, 1065, 1020, 945, 885.

Acid Hydrolysis of 11 Compound **11** (5.8 mg) was subjected to acid hydrolysis as described for **9** to give a sugar fraction (1.3 mg). The monosaccharide constituent in the fraction was converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, which was then analyzed by HPLC. The derivative of D-glucose was detected; t_R (min): 17.34.

Compound **12**: Amorphous solid, $[\alpha]_D^{27}$ −70.0° (MeOH, c=0.10). HR FAB-MS (positive mode) m/z: 843.4301 $[M+Na]^+$ ($C_{40}H_{68}O_{17}\cdot Na$, Calcd for 843.4354). IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2925 and 2880 (CH), 1455, 1370, 1240, 1155, 1060, 1020, 955, 885, 830, 815. ^{1}H-NMR (pyridine-d_5) δ: 5.21 (1H, d, J=7.7 Hz, H-1′), 4.90 (1H, d, J=7.7 Hz, H-1″), 3.27 (3H, s, OMe), 1.60 (3H, s, Me-19), 1.21 (3H, d, J=6.9 Hz, Me-21), 1.04 (3H, d, J=6.7 Hz, Me-27), 0.89 (3H, s, Me-18).

Enzymatic Hydrolysis of 12 Compound **12** (6.7 mg) was dissolved in an AcOH/AcONa buffer (pH 5, 5 ml) with β-D-glucosidase (Sigma, EC 3. 2. 1. 21) (16.4 mg) and incubated at room temperature for 12 h. The crude mixture was chromatographed on ODS silica gel eluting with MeOH–H_2O (8 : 3) to yield **2** (4.8 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample. *Rf* 0.43 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Cell Culture Assay HL-60 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum supplemented with L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. The leukemia cells were washed and resuspended in the above medium to 3×10^4 cells/ml, and 196 μl of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μl of EtOH–H_2O (1 : 1) solution containing the sample was added to give the final concentrations of 0.01—10 μg/ml; 4 μl of EtOH–H_2O (1 : 1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure.[15] The MTT assay was carried out according to a modification of the method of Sargent and Tayler as follows. After termination of the cell culture, 10 μl of 5 mg/ml MTT in phosphate buffered saline was added to each well and the plate was reincubated in 5% CO_2/air for 4 h at 37 °C. The plate was then centrifuged at 1500 g for 5 min to precipitate cells and formazan. An aliquot of 150 μl of the supernatant was removed from every well, and 175 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min, and then read on a microplate reader at 550 nm. Compound **5** showed more than 50% of cell growth inhibition at a sample concentration of 10 μg/ml. A dose response curve was plotted for **5**, and the concentration giving 50% inhibition (IC_{50}) was calculated.

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