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Implication of the Stereoisomers of Ginsenoside Derivatives in the Antiproliferative Effect of HSC-T6 Cells

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Supporting Information

ABSTRACT: Two ginsenoside derivatives (**9**, **10**) along with 10 known ginsenosides (**1**−**8**, **11**, and **12**) were isolated from BST204, which is a crude ginseng extract fermented by enzyme and acid hydrolysis. The two ginsenosides were determined as 12*β*,20(*S*),25-trihydroxydammara-3-*O*-*β*-D-glucopyranoside (**9**) and 12*β*,20(*R*),25-trihydroxydammara-3-*O*-*β*-D-glucopyranoside (**10**). Compounds **1**−**12** were categorized into stereoisomeric pairs differentiated by *R*- or *S*-configuration at C-20, the number or position of sugar residues at C-3 or C-6, and the type of derivative at C-21. Their structure−activity relationship was evaluated by the cell viability assay using HSC-T6 cells. Results showed that 20(*S*) (**3** > **4**, **7** > **8**, and **9** > **10**), a 2-hydroxy-2-methylbutyl moiety at C-21 (**3**, **7** > **9**), and the number of sugar residues at C-3 (**3** > **7**) significantly affected the antiproliferative activity on HSC-T6 cells. The inhibition of the cell proliferation of compound **3** was assessed by annexin-V/PI staining analysis using flow cytometry.

KEYWORDS: *ginsenosides, antiproliferative, HSC-T6 cells, Panax ginseng*

INTRODUCTION

Hepatic stellate cells (HSCs) in normal liver are the major storage sites of vitamin A presented as being quiescent.[1] As a result of liver injury induced by outside stimuli such as oxidative stress, apoptotic bodies, and the paracrine stimuli from adjacent cells, they are activated, led into myofibroblast-like cells, and produce the excessive collagen type I and extracellular matrix proteins that distort liver architecture by forming fibrous scars.[2,3] In response to liver damage, activated HSCs simultaneously become proliferative and eventually accumulate fibrogenic tissue, leading to liver fibrosis. Therefore, the suppression of activated HSC proliferation has been proposed as one of the therapeutic targets for the treatment of liver fibrosis.[4,5]

Ginsenosides are triterpene saponins isolated from the *Panax* genus.[6] A number of investigations showed various pharmacological properties including antidiabetic, anti-inflammatory, and antitumor activities of ginsenosides.[7] Most ginsenosides have a dammarane triterpene skeleton and are subdivided into protopanaxdiol (PPD) and protopanaxtriol (PPT) by the presence of a hydroxyl moiety at C-6. They are also classified by the type, number, or linkage position of sugars and the presence of a hydroxyl moiety at C-3, C-6, and C-20. It is known that the stereochemistry at C-20 and the type of derivatives at C-21 also contribute to the structural diversity of ginsenosides and their pharmacologic activities.[8−10]

In this study, we isolated 2 ginsenoside derivatives along with 10 known ginsenosides from the fermented ginseng extract BST204.[11,12] BST204 was transformed from the crude ginseng with ginsenoside-*β*-glucosidase and acid hydrolysis to enrich ginsenosides Rh2 and Rg3, which are the pharmacologically active chemicals in the different kinds of molecular mechanisms.[13] We assessed their structure−activity relationship depending on the type of aglycone, the presence, number, and type of sugar residue at C-3, C-6, and C-20, and the type of derivative at C-21 by the inhibitory activity on HSC-T6 cell proliferation.

MATERIALS AND METHODS

General Experiment Procedures. Column chromatography (CC) was carried out on Kiesgel 60 silica gel (40−60 *μ*m, 230−400 mesh, Merck, Whitehouse, NJ, USA), YMC-GEL ODS-A (5−150 *μ*m, YMC, Allentown, PA, USA), and Sephadex LH-20 (25−100 *μ*M, Pharmacia, Piscataway, NJ, USA), and TLC was carried out on Kiesgel 60 F_{254} coated normal silica gel and RP-18 F_{254} coated reverse-phase silica gel (Merck). The 1D and 2D NMR spectra were recorded on Bruker AMX 400, 500, and 600 spectrometers in pyridine-d_5. High-resolution and low-resolution ESIMS were obtained on a Tempo nano HPLC/QSTAR Elite (Applied Biosystems, Vernon Hills, IL, USA). The HPLC system consisted of a G-321 pump (Gilson, Middleton, WI, USA), a G-151 UV detector (Gilson), and a Kromacil C_{18} column (250 mm × 21.2 mm i.d.; 10 *μ*m, Kromacil, Sweden), and all chromatograms were monitored at 205 nm. HPLC grade solvents (Fisher Scientific, USA) were used in the AcCN−H_2O system. Detection of DNA cycle and apoptosis on HSC-T6 cells was conducted by flow cytometry (BD Biosciences, FACS Calibur, Franklin Lakes, NJ, USA).

Preparation of Fermented Ginseng Extract, BST204. BST204 was gifted from GCH&P Inc. (Sungnam, Korea). It was prepared with

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 9 and 10 in Pyridine-d_5[a]

	9[b]		10[b]	
position	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult
1	1.49, m, 0.75, m	39.1, CH_2	1.49, m, 0.75, m	39.1, CH_2
2	2.20, m, 1.38, m	26.7, CH_2	2.18, m, 1.36, m	26.7, CH_2
3	3.36, dd (4.4, 11.7)	88.8, CH	3.35, dd (4.4, 11.7)	88.7, CH
4		39.7, qC		39.6, qC
5	0.73, m	56.4, CH	0.71, m	56.3, CH
6	1.58−1.32, m	18.4, CH_2	1.52−1.36, m	18.4, CH_2
7	1.47, m, 1.22, m	35.2, CH_2	1.48, m, 1.22, m	35.1, CH_2
8		40.0, qC		40.0, qC
9	1.41 m	50.4, CH	1.41, m	50.3, CH
10		37.0, qC		36.9, qC
11	2.05, m, 1.54, m	32.1, CH_2	2.03, m, 1.52, m	32.1, CH_2
12	3.90, m	71.0, CH	3.90, m	70.8, CH
13	2.06, m	48.6, CH	2.00, m	49.2, CH
14		51.7, qC		51.7, qC
15	1.58, m, 1.02, m	31.4, CH_2	1.57, m, 1.02, m	31.4, CH_2
16	1.92, m, 1.81, m	27.2, CH_2	1.91, m, 1.80, m	26.6, CH_2
17	2.34, m	54.7, CH	2.40, m	50.7, CH
18	0.80, s	16.8, CH_3	0.81, s	16.7, CH_3
19	1.01, s	15.8, CH_3	1.00, s	15.8, CH_3
20		73.3, qC		73.3, qC
21	1.41, s	26.9, CH_3	1.38, s	22.8, CH_3
22	2.00, m, 1.63, m	36.5, CH_2	1.71, m	44.0, CH_2
23	2.16, m, 1.82, m	19.1, CH_2	2.12−1.97, m	18.7, CH_2
24	1.71, m	45.7, CH_2	1.71, m	45.5, CH_2
25		69.6, CH		69.7, qC
26	1.37, s	30.2, CH_3	1.40, s	30.1, CH_3
27	1.38, s	29.9, CH_3	1.40, s	29.9, CH_3
28	1.30, s	28.1, CH_3	1.30, s	28.1, CH_3
29	0.98, s	16.4, CH_3	0.98, s	16.3, CH_3
30	0.94, s	17.0, CH_3	0.94, s	17.3, CH_3
O-β-D-glucose				
1′	4.93, d (7.8)	106.9, CH	4.91. d (7.8)	106.9, CH
2′	4.02, m	75.8, CH	4.01, m	75.7, CH
3′	4.25, m	78.7, CH	4.22, t (8.8)	78.7, CH
4′	4.20, m	71.9, CH	4.18, t (8.8)	71.8, CH
5′	3.99, m	78.3, CH_3	3.98, m	78.3, CH_3
6′	4.57, dd (2.2, 11.7)	63.1, CH_2	4.56, dd (1.9, 11.7)	63.0, CH_2
	4.37 dd (5.4, 11.7)		4.37, dd (5.4, 11.7)	

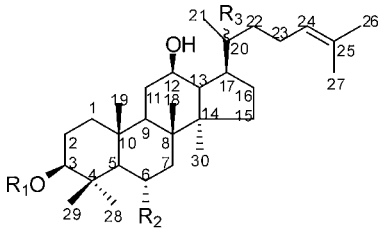
[a]Multiplicity of ^{13}C NMR data was determined by DEPT experiments. [b]^{1}H and ^{13}C NMR data were measured at 500 and 125 MHz, respectively.

a patented technology and the previously reported study.[11] Brifely, the harvested ginseng was extracted with ethanol repeatedly followed by reaction with an enzyme containing ginsenoside-β-glucosidase. After acid hydrolysis of the residue, the reactant was purified with HP-20 resin followed by washing out with distilled water and finally 95% ethanol. The 95% ethanol fraction was concentrated and designated BST204.

Isolation of Ginsenosides from BST204. The dried BST204 (90 g) was subjected to normal silica gel CC (20 × 5 cm) with a mixture of $CHCl_3$ and MeOH (10:1, 7:1, 5:1, 3:1, 4 l) and give 12 subfractions (BST204-1−12). Among them, the BST204-6 fraction (8.7 g) was chromatographed by RP C_{18} silica gel CC ($MeOH/H_2O$ = 8.5:1.5; 10 × 3 cm) and separated into *S*/*R*-Rh2-rich fraction (2.0 g). The stereoisomers of Rh2 were isolated into *S*-Rh2 (**3**, 300 mg) and *R*-Rh2 (**4**, 210 mg), respectively, with preparative RP C_{18} HPLC system ($AcCN/H_2O$ = 55:45; 13 min/mL). The mixture of *S*/*R*-Rh1 (**1** and **2**), **9** (280 mg), and **10** (100 mg) was prepared from BST204-8 fraction by RP C_{18} silica gel CC ($MeOH/H_2O$ = 7:3; 10 × 3 cm) and purified with a preparative RP C_{18} HPLC system ($AcCN/H_2O$ = 50:50, 70:30; 13 min/mL). The BST204-10 fraction was subjected to RP C_{18} silica gel CC eluting with a mixture of $MeOH/H_2O$ (7:3; 10 × 3 cm) to give the mixture of *S*/*R*-Rg2 (**5**, 150 mg; **6**, 100 mg) and *S*/*R*-Rg3 (**7**, 500 mg, **8**, 400 mg). They were isolated by preparative RP C_{18} HPLC using $AcCN/H_2O$ (35:65, 10 mL/min). The stereoisomers of 25-trihydroxydammara-3-*O*-β-D-glucopyranoside (**11** and **12**) were obtained from the BST204-11 fraction with the condition of RP C_{18} silica gel CC ($MeOH/H_2O$ = 3:1; 10 × 3 cm) followed by preparative RP C_{18} HPLC using $AcCN/H_2O$ (35:65, 13 mL/min).

12β,20(*S*),25-Trihydroxydammara-3-*O*-β-D-glucopyranoside (9). Obtained as a whitish amorphous powder; $[\alpha]^{25}_D$ +10.9 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3433.6, 2959.2, 2917.8, 1636.3, 1468.5, 1383.7 cm^{-1}; ESIMS (positive mode) *m*/*z* 641 $[M + H]^+$; HRESIMS (positive mode) *m*/*z* 641.4605 $[M + H]^+$ (calcd for 641.4623); ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1

12β,20(*R*),25-Trihydroxydammara-3-*O*-β-D-glucopyranoside (10). Obtained as a whitish amorphous powder; $[\alpha]^{25}_D$ +22.7 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3385.4, 2948.6, 2919.7, 1632.5, 1469.5, 1379.8 cm^{-1}; ESIMS (positive mode): *m*/*z* 641 $[M + H]^+$; HRFABMS (positive mode) *m*/*z* 641.4603 $[M + H]^+$ (calcd for 641.4623); ^{1}H



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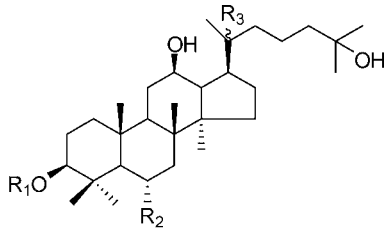

II

	R_1	R_2	R_3		R_1	R_2	R_3
		I		**7**	*β*-D-glu-*O*-*β*-D-glu	H	*β*-OH
1	H	*O*-*β*-D-glu	*β*-OH	**8**	*β*-D-glu-*O*-*β*-D-glu	H	*α*-OH
2	H	*O*-*β*-D-glu	*α*-OH			**II**	
3	*β*-D-glu	H	*β*-OH	**9**	*β*-D-glu	H	*β*-OH
4	*β*-D-glu	H	*α*-OH	**10**	*β*-D-glu	H	*α*-OH
5	H	*O*-*β*-D-glu-*α*-L-rha	*β*-OH	**11**	H	*O*-*β*-D-glu	*β*-OH
6	H	*O*-*β*-D-glu-*α*-L-rha	*α*-OH	**12**	H	*O*-*β*-D-glu	*α*-OH

Figure 1. Structures of compounds **1**−**12** isolated from BST204.

NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1

HSC-T6 Cell Culture and Cell Viability Test. HSC-T6 cells were maintained in DMEM (Sigma, St. Louis, MO, USA) to which were added 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma) at 37 °C in a humidified incubator containing 5% CO_2 gas. For the cell viability assay, the cells were seeded in a 48-well plate at a density of 1.5×10^5 cells/well and incubated for 24 h. The cell viability test on HSC-T6 cell proliferation was obtained by measuring the activity of enzymes that reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan dyes, giving a purple color.[14] All tested compounds were dissolved in DMSO (final concentration = 0.1%) and diluted with distilled water to reach a desired concentration. HSC-T6 cells were treated with vehicle (0.01%) or compounds **1**−**12** at concentrations (50 and 100 μM) for 24 or 48 h. The inhibitory activity of compounds **1**−**12** on cell proliferation was calculated as 100 × (absorption of well treated with compounds/absorption of control treated with vehicle). Data were expressed as the mean of three independent experiments. (−)-EGCG (purity > 95%, Sigma-Aldrich) was used as a positive control.

Flow Cytometry Analysis for Apoptosis. The DNA cycle and apoptosis after treatment of compounds from BST204 were measured by flow cytometry.[15] After 24 h of incubation with the density of 1×10^6 cells/well in a 6-well plate, the cells were treated with the selected compounds for 24 h. The cells were washed with cold PBS twice, trypsinized, and centrifuged at room temperature. The measurement of apoptosis was performed in flow cytometry using an FITC−Annexin V Apoptosis Detection Kit (BD Pharmigen, Franklin Lakes, NJ, USA) according to the manufacturer's manual.

Statistical Analysis. The evaluation of statistical significance was determined by the one-way ANOVA test, with $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) considered to be statistically significant.

RESULTS AND DISCUSSION

A fermented ginseng extract (90 g), BST204, was prepared with patented processes such as ginsenoside-*β*-glucosidase and acid hydrolysis. BST204 is subjected to normal phase and reverse phase column chromatographies in turn to give 2 ginsenoside derivatives, **9** and **10**, with 10 ginsenoside derivatives **1**−**8**, **11**, and **12**. The names of those compounds are 20(*S*)-ginsenoside Rh1 (**1**),[16] 20(*R*)-ginsenoside Rh1 (**2**),[17] 20(*S*)-ginsenoside Rh2 (**3**),[18] 20(*R*)-ginsenoside Rh2 (**4**),[19] 20(*S*)-ginsenoside Rg2 (**5**),[17] 20(*R*)-ginsenoside Rg2 (**6**),[20] 20(*S*)-ginsenoside Rg3 (**7**),[21] 20(*R*)-ginsenoside Rg3 (**8**),[22] 12*β*,20(*S*),25-trihydroxydammara-6-*O*-*β*-D-glucopyranoside (**11**),[17] and 12*β*,20(*R*),25-trihydroxydammara-6-*O*-*β*-D-glucopyranoside (**12**)[17] (Figure 1). The structures of compounds **1**−**12** were unequivocally determined by 1D and 2D NMR and MS experiments as well as comparison with the literature.

Compound **9** is a whitish, amorphous powder; the molecular formula is $C_{36}H_{56}O_{10}$, established by the positive HRESIMS (m/z 641.4605 $[M + H]^+$, calcd for 641.4623). In the ^{1}H NMR spectrum, the signals at $^H\delta$ 4.93 (1H, d, J = 7.8 Hz, H-1′), 3.36 (1H, dd, J = 4.4, 11.7 Hz, H-3), 1.41 (3H, s, H-21), 1.38 (3H, s, H-27), 1.37 (3H, s, H-26), 1.30 (3H, s, H-28), 1.01 (3H, s, H-19), 0.98 (3H, s, H-29), 0.94 (3H, s, H-30), and 0.80 (3H, s, H-18) displayed the presence of an anomeric proton of a sugar residue and a hydroxyl moiety and seven methyl groups,

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respectively (Table 1). The ^{13}C NMR spectrum showed the existence of a sugar residue at $^{C}\delta$ 106.9 (C-1′), 75.8 (C-2′), 78.7 (C-3′), 71.9 (C-4′), 78.3 (C-5′), and 63.1 (C-6′) and four hydroxyl moieties at $^{C}\delta$ 88.7 (C-3), 71.0 (C-12), 73.3 (C-20), and 69.6 (C-25). On the basis of the above spectral data, it was inferred that **9** is a ginsenoside derivative having a sugar residue and no olefinic moiety. In the HMBC experiment, the correlation peaks from $^{H}\delta$ 2.34 (H-17), 1.41 (H-21), and 2.00 and 1.63 (H-22) to $^{C}\delta$ 73.3 (C-20) and from $^{H}\delta$ 1.38 (H-27) and 1.37 (H-26) to $^{C}\delta$ 69.6 (C-25) indicated that each hydroxyl moiety was substituted at C-20 and C-25, respectively, and the derivative at C-21 was assigned as a 2-hydroxyl-2-methylbutyl moiety (Figure 2). The stereochemistry at C-20

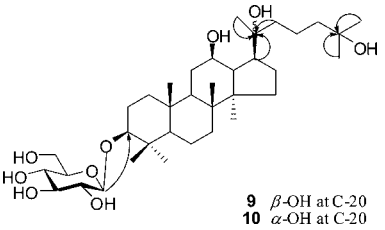

Figure 2. Key correlations of compounds **9** and **10** in 2D NMR spectral data.

was determined by the differences in the ^{13}C NMR chemical shifts at C-17, C-21, and C-22. The differences between **9** and **11** were not significant as all of the signals at C-17, C-21, and C-22 were below 0.1 ppm. The stereochemistry at C-20 was determined to be *S*-configured as that of **11**. It was determined that the position of a sugar residue is at C-3 by the cross-link between $^{H}\delta$ 4.93 (H-1′) and $^{C}\delta$ 88.7 (C-3). The magnitude of the coupling constant at H-1′ (J = 7.8 Hz) suggested that a sugar residue was oriented at the β-side. By comparison with the spectral data of **3**, which is an identical structure except for the derivative at C-21, a sugar residue at C-3 in **9** was assigned as D-glucose.[18] On the basis of these observations, the structure of **9** was identified as 12β,20(S),25-trihydroxydammara-3-*O*-β-D-glucopyranoside.

Compound **10** was a white, amorphous powder, and $C_{36}H_{56}O_{10}$ (m/z 641.4603 [M + H]$^+$, calcd for 641.4623) was deteremined as the molecular formula. The ^{1}H NMR spectral data of **10** were similar to those of **9**, except for the signal at $^{H}\delta$ 2.40 (H-17). In the ^{13}C NMR spectrum, the signals at $^{C}\delta$ 26.6 (C-16, −0.6 ppm), 50.7 (C-17, −4.0 ppm), and 22.8 (C-21, −4.1 ppm) were significantly up-shifted and that at $^{C}\delta$ 44.0 (C-22, +7.5 ppm) was down-shifted in comparison of those of **9**, respectively. The differences of the chemical shifts at C-17, C-21, and C-22 are used for determining the stereochemistry at C-20. By comparison with the literature and the ^{13}C NMR chemical shifts of **12**, these results indicated the structure of **10** was the 20(R)-epimeric isomer of **9**.[17] The stereochemistry of ginsenoside derivatives is mainly determined as the configuration of a hydroxyl moiety at C-20. The structure of **10** was assigned as 12β,20(R),25-trihydroxydammara-3-*O*-β-D-glucopyranoside.

Ginsenosides are called ginseng saponins, which are the major pharmacologically active compounds of ginseng species. With the development of the technology revolution, the applications of LC-MS/MS and NMR analyses facilitated the isolation and identification of more than 100 ginsenosides. Ginsenosides have a wide range of therapeutic and pharmacological applications, such as antioxidation, angiogenesis modulation, anti-inflammation, neuroprotection, antihyperglycemic activity, and antitumor activity in vitro and in vivo.[13] In addition, some of ginsenosides, such as Rg1 and Rb1, showed antifibrotic activity in vitro and in vivo models.[23,24]

We focused on finding therapeutic agents that have antifibrotic activity from natural products. HSC-T6 cells, immortalized hepatic stellate cell line, were used as the screening tool to determine the antifibrotic activity of compounds.[25,26] The activation of hepatic stellate cells is considered as the important cause of the aggravation of liver fibrosis, and thus the inhibition of the proliferation of these

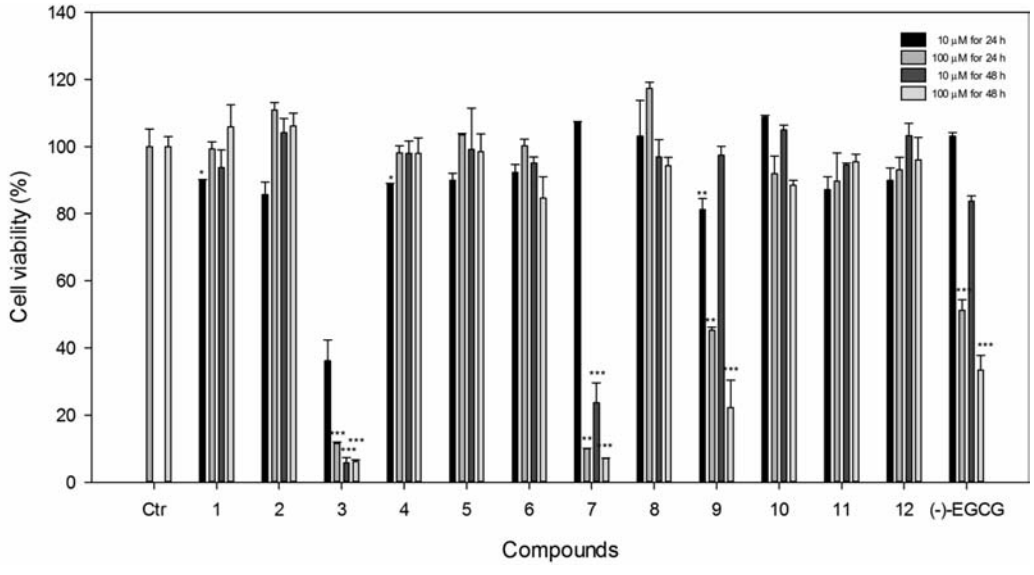

Figure 3. Inhibitory activity of compounds **1**−**12** on HSC-T6 cell proliferation. HSC-T6 cells were treated with compounds **1**−**12** at concentrations of 10 and 100 μM for 24 and 48 h. Cell viability (%) was determined using the MTT assay as described under Materials and Methods. The values shown are the mean ± SD of data (n = 3). 0.01% DMSO, which did not show any toxic effect on HSC-T6 cells, was used for the vehicle. Results significantly differed from vehicle-alone treatment: *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.001$.

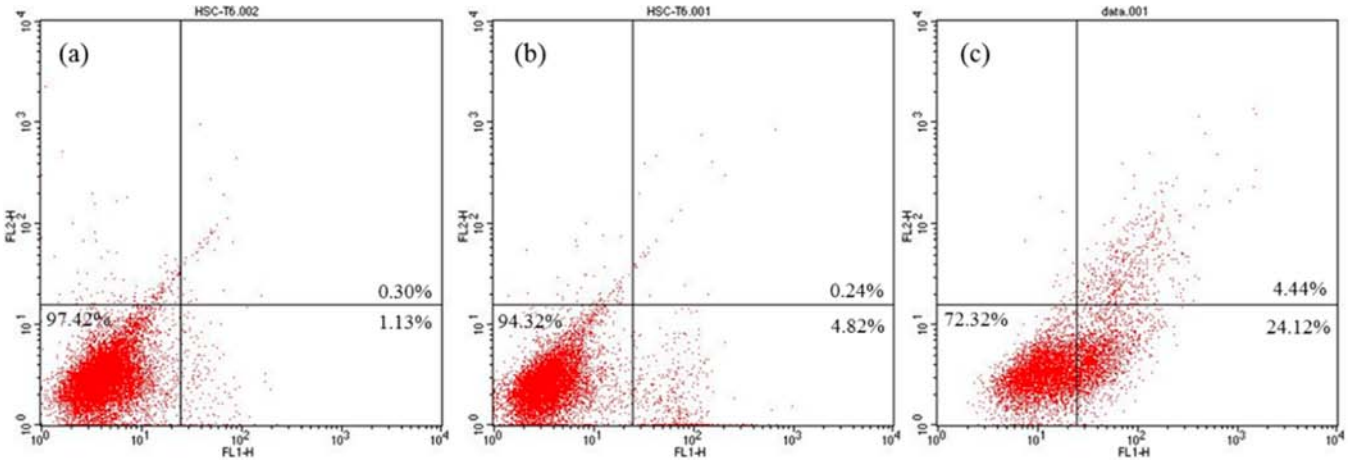

Figure 4. Effect of compound **3** on apoptosis in HSC-T6 cells. HSC-T6 cells were treated with compound **3** at the concentration of 20 μM for 6 h (b) and 24 h (c). Apoptosis was determined using flow cytometry as described under Materials and Methods. (a) Vehicle-treated. The values shown are represented as percentage and representative of three independent experiments.

cells has been the target for the treatment of hepatic fibrosis.[27] To the best of our knowledge, there has been no attempt to systematically evaluate the structure−activity relationship of the stereoisomers of ginsenosides on the inhibition of HSC-T6 cell proliferation. From BST204, we isolated 12 less polar ginsenoside derivatives (**1**−**12**) than those from crude ginseng. It was expected that **9** and **10** are the derivatives during the process of mild acid hydrolysis to yield BST204. At C-20, all of the derivatives had α- or β-hydroxyl moieties (**1** and **2**, **3** and **4**, **5** and **6**, **7** and **8**, **9** and **10**, and **11** and **12**) instead of the sugar residue. They are also categorized into several groups according to the type of the aglycone (**1**, **2**, **5**, **6**, **11**, and **12**, proptopanaxtriols (PPTs); and **3**, **4**, and **7**−**10**, protopanaxdiols (PPDs)), the number and location of the sugar residue at C-3 or C-6 (**1**−**12**), and the type of derivative at C-21 (**1**−**8**, dimethylallyl; and **9**−**12**, 2-hydroxyl-2-methylbutyl) (Figure 1). We examined the antiproliferative effects on HSC-T6 cells according to the structure of compounds **1**−**12** (Figure 3). First, PPT derivatives (**1**, **2**, **5**, **6**, **11**, and **12**) did not show any significant inhibitory activity on the proliferation of HSC-T6 cells (Figure 3). In PPD derivatives, the configuration of a hydroxyl moiety at C-20 significantly affected the antiproliferative activity in HSC-T6 cells. Compounds **3**, **7**, and **9** having β-OH at C-20 showed more potent inhibitory activity than compounds **4**, **8**, and **10** with α-OH. In compounds **3**, **7**, and **9**, the dimethylallyl moiety at C-21 instead of the 2-hydroxyl-2-methylbutyl moiety affected the inhibitory activity on HSC-T6 cell proliferation (**3** and **7** > **9**). In addition, the number of the sugar residue at C-3 was interestingly the important factor affected by the cell viability. Among compounds **3** and **7** with β-OH at C-20 and the dimethylallyl moiety at C-21, compound **3**, having a glucose residue at C-3, showed more significant inhibition activity than compound **7**, having two glucoses, in dose- and time-dependent manners. Taken together, it would be concluded that the type of PPD and the presence of β-OH at C-20 were significant inhibitory elements on HSC-T6 cell proliferation. Also, the number of the glucose moiety at C-3 and the dimethylallyl moiety at C-21 quietly affected the inhibitory activity on cell viability. Thus, we investigated whether the inhibitory activity of those compounds on HSC-T6 cell proliferation is induced by apoptosis. To evaluate the apoptotic effect of **3** at 20 μM, we additionally examined the stage of apoptosis using the annexin-V/PI double-staining assay (Figure 4). Over time, the proportion of annexin-V^+/PI^--stained cells implied early apoptosis was dramatically increased from 4.82% (6 h) to 24.12% (24 h), and late apoptotic cells (annexin-V^+/PI^+, 0.24%) were also increased to 4.44%. Overall, the structure of ginsenosides plays a role in the inhibitory activity on the proliferation of ginsenosides of HSC-T6 cells. Especially, the *S*-configuration of the hydroxyl moiety at C-20 and dimethylallyl at C-21 in aglycone and the presence of only a glucose residue at C-3 are the most important factors affecting cell viability via arrest of the DNA cycle and inducement of apoptosis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral data including 1D and 2D NMR, HR-ESIMS data of compounds **9** and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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