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Novel dammarane-type sapogenins from *Panax ginseng* berry and their biological activities

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ABSTRACT

Three new dammarane-type sapogenins (**1**, **3**, and **5**) together with two known ones (**2** and **4**) were isolated from the total hydrolyzed saponins extracted from *Panax ginseng* berry. Their structures were elucidated using a combination of 1D and 2D ^{1}H and ^{13}C NMR spectra and mass spectroscopy as 20(*R*)-25-methoxyl-dammarane-3β,12β,20-triol (**1**), 20(*R*)-25-methoxyl-dammarane-3β,6α,12β,20-tetrol (**2**), 20(*R*)-20-methoxyl-dammarane-3β,12β,25-triol (**3**), 20(*R*)-20,25-dimethoxyl-dammarane-3β,12β-diol (**4**), and (12*R*,20*S*,24*S*)-20,24-; 12,24-diepoxy-dammarane-3β-ol (**5**). Their antitumor activities were evaluated in six human cancer cell lines. The novel compounds **1** and **3** showed significant cytotoxic activity against the six cell lines. The IC_{50} values of **3** against HepG2, Colon205, and HL-60 were the lowest (8.78, 8.64, and 3.98 μM, respectively). Compounds **1** and 20(*S*)-25-OCH_3-PPD, which are a pair of configuration isomers, showed a 10- to 100-fold greater growth inhibition than ginsenoside-Rg_3 (an anti-cancer clinical agent in China). The data presented here may be useful for the development of novel anti-cancer agents.

The Araliaceae plant *Panax ginseng*, an ancient herbal medicine in traditional Chinese medicine, has been used for thousands of years.[1,2] In recent years, there has been an increasing interest in the use of ginseng and its preparation in the Western world.[3,4] Our interest in *P. ginseng* has focused on the isolation and characterization of its effective components, widely considered to be the ginsenosides, which include the ginseng saponins, mainly consisting of dammarane-type saponin derivatives.[5,6] Thus far, more than 100 naturally occurring dammarane-type saponins and the products of enzymatic conversion have been isolated from roots, stems, leaves, flowers, berries, and seeds of various *Panax* species. Many of these isolated compounds are regarded as the principal components responsible for the pharmaceutical and biological effects of ginseng.[7,8] Antitumor activity has been observed with several ginsenosides, and many studies indicate that the activities of these compounds are dependent upon their structures and that the aglycones are more effective than the glycosides.[9,10]

Since most studies have focused on the root of ginseng, there is limited data available with regard to the chemical components and cancer preventive potential of ginseng berry. In our previous work, various naturally occurring dammarane-type saponins (hydrolysates) were isolated from *P. ginseng* berry and some of them had obvious antitumor effects.[11–15] Some examples are the derivatives of protopanaxadiol ginsenoside-Rh_2[16–21] and ginsenoside-Rg_3,[22–25] which was approved for adjuvant chemotherapy in China.[26] We also obtained eleven compounds from the *P. ginseng* berry and evaluated their anti-cancer activity in vitro in several human cancer cell lines with diverse genetic backgrounds. Of the 11, 25-OH-PPD was identified as a new natural product whose IC_{50} values (10–60 μM) were 5- to 15-fold lower than those for ginsenoside-Rg_3.[27]

In this paper, we describe the isolation and structure elucidation of three new dammarane-type sapogenins from *P. ginseng* berry. Based on physicochemical characteristics and NMR data, the novel compounds were identified as 20(*R*)-25-methoxyl-dammarane-3β,12β,20-triol (**1**), 20(*R*)-20-methoxyl-dammarane-3β,12β,25-triol (**3**), and (12*R*,20*S*,24*S*)-20,24-; 12,24-diepoxy-dammarane-3β-ol (**5**). Biological activities of the three new aglycones and their known analogs, PPD, 25-OH-PPD, and 20(*S*)-25-OCH_3-PPD, were evaluated in six human cancer cell lines. The IC_{50} values of compounds **1** and **3** for most cell lines were close to 20(*S*)-25-OCH_3-PPD, which had a 5- to 15-fold greater cytotoxicity relative to PPD and a 10- to 100-fold increase over Rg_3.[28]

The total saponins were hydrolyzed with 18% HCl. The hydrolyzed preparation was then extracted with EtOAc and subjected

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to chromatography on a silica gel column. Elution was stepwise with a petroleum–EtOAc gradient (from petroleum–EtOAc, 50:1 to 2:1) to afford compounds **1**–**5**.[29]

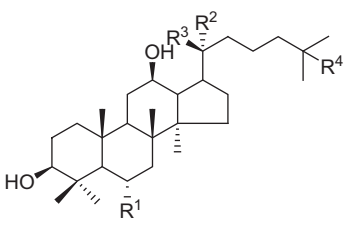
Compound **1** was obtained as colorless crystals. Its molecular formula was determined to be $C_{31}H_{56}O_4$ by ^{13}C NMR and ESI MS (*m/z* 493 $[M+H]^+$ and 515 $[M+Na]^+$), which was confirmed by HRE-IMS (*m/z* 515.4064 calcd for $C_{31}H_{56}O_4Na$, 515.4076). Positive Liebermann–Burchard and negative Molish reactions indicated that **1** is a sapogenin. The IR spectrum displayed absorption bands at 3283 and 1029 cm^{-1}, suggestive of the presence of hydroxyl groups. Its ^1H NMR spectrum (Table 1) showed signals for eight tertiary methyl groups at δ 0.91, 0.95, 1.04, 1.06, 1.14, 1.14, 1.24, 1.42 (each 3H, all s), a methoxyl proton at δ 3.14 (3H, s) and two protons of oxygen-bearing carbons at δ 3.45 (1H, m), 3.93 (1H, m). No signals from an olefinic or sugar moiety were evident. From the ^{13}C NMR spectrum,[30] the structure was determined to be a dammarane-type of aglycone with four oxygen-bearing carbons: δ 77.9 (C-3), 74.5 (C-25), 73.2 (C-20), 70.9 (C-12). The NMR data of **1** was similar to that of 20(*S*)-25-OCH_3-PPD (5) except for C-17 (50.8), C-21 (22.7), C-22 (43.9), which were used as the diagnostic signals for determination of the stereochemistry of C-20(*R*). Therefore, the structure was concluded to be 20(*R*)-25-OCH_3-PPD.

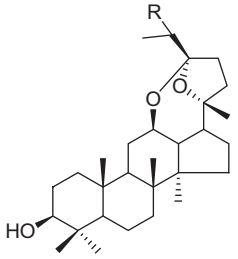
Table 1
^1H NMR spectral data for compounds **1**, **3**, and **5**

No.	1	3	5
1	0.91 (m), 1.56 (m)	0.91 (m), 1.65 (m)	0.80 (m), 1.55 (m)
2	1.24 (m), 1.80 (m)	1.28 (m), 1.81 (m)	1.14 (m), 1.88 (m)
3	3.54 (m)	3.43 (m)	3.42 (dd, *J* = 5.3, 11.0 Hz)
4			
5	0.81(m)	0.80 (m)	0.72 (m)
6	1.48 (m), 1.53 (m)	1.48 (m), 1.54 (m)	1.37 (m), 1.49 (m)
7	1.23 (m), 1.48 (m)	1.22 (m), 1.48 (m)	1.21 (m), 1.50 (m)
8			
9	1.50 (m)	1.51 (m)	1.28 (m)
10			
11	1.50 (m), 2.08 (m)	1.50 (m), 2.09 (m)	1.39 (m), 1.88 (m)
12	3.93 (m)	3.78 (m)	3.91 (m)
13	1.85 (m)	1.83 (m)	1.58 (m)
14			
15	1.10 (m), 1.48 (m)	1.04 (m), 1.48 (m)	1.00 (m), 1.60 (m)
16	1.28 (m), 1.84 (m)	1.23 (m), 1.86 (m)	1.00 (m), 1.50 (m)
17	2.41 (m)	2.35 (m)	2.09 (m)
18	1.06 (s)	1.04 (s)	0.89 (s)
19	0.95 (s)	0.89 (s)	0.86 (s)
20			
21	1.42 (s)	1.16 (s)	1.23 (s)
22	1.37 (m), 1.69 (m)	1.48 (m), 1.66 (m)	1.38 (m), 1.88 (m)
23	1.56 (m)	1.50 (m)	1.96 (m), 2.03 (m)
24	1.53 (m)	1.48 (m)	
25			
26	1.14 (s)	1.41 (s)	1.08 (d, *J* = 6.9 Hz)
27	1.14 (s)	1.41 (s)	1.14 (d, *J* = 7.3 Hz)
28	1.24 (s)	1.23 (s)	1.29 (s)
29	1.04 (s)	1.00 (s)	1.03 (s)
30	0.91 (s)	0.87 (s)	0.99 (s)
20-OCH3		3.16 (s)	
25-OCH3	3.14 (s)		

Compound **2** was obtained as colorless crystals. Its molecular formula was determined to be $C_{31}H_{56}O_5$ by ^{13}C NMR and ESI MS (*m/z* 509 $[M+H]^+$ and 531 $[M+Na]^+$), which was confirmed by HRE-IMS (*m/z* 531.4036 calcd for $C_{31}H_{56}O_5Na$, 531.4025). Positive Liebermann–Burchard and negative Molish reactions suggested **2** to be a sapogenin. The IR spectrum displayed strong absorption bands at 3364 and 1060 cm^{-1}, suggestive of the presence of hydroxyl groups. Its ^1H NMR spectrum showed signals for eight tertiary methyl groups at δ 0.94, 1.03, 1.12, 1.14, 1.14, 1.40, 1.45, 1.99 (each 3H, all s), a methoxyl proton at δ 3.12 (3H, s) and three protons of oxygen-bearing carbons at δ 3.53 (1H, m), 3.91 (1H, m), and 4.40 (1H, m). From the ^{13}C NMR spectrum, the structure was determined to be a dammarane-type of aglycone with five oxygen-bearing carbons: δ 77.9 (C-3), 74.1 (C-25), 72.7 (C-20), 70.4 (C-12), 67.3 (C-6). The NMR data of **2** was similar to that of 20 (*S*)-PPT[28] except for the side chain, which was observed to be similar to that of **1** by comparison of their NMR spectral data. Therefore, the structure of **2** was determined to be 20(*R*)-25-methoxyl-dammarane-3β,6α,12β,20-tetrol (Fig. 2).

Compound **3** was obtained as colorless crystals. Its molecular formula was determined as $C_{31}H_{56}O_4$ by ^{13}C NMR and ESI MS (*m/z* 493 $[M+H]^+$ and 515 $[M+Na]^+$) and was confirmed by HREIMS (*m/z* 515.4066 calcd for $C_{31}H_{56}O_4Na$, 515.4076). Positive Liebermann–Burchard and negative Molish reactions inferred **3** to be a sapogenin. The IR spectrum displayed strong absorption bands at 3301 and 1037 cm^{-1}, suggestive of the presence of hydroxyl groups. Its ^1H NMR spectrum (Table 1) showed signals for eight tertiary methyl groups at δ 0.87, 0.89, 1.00, 1.04, 1.16, 1.23, 1.41, 1.41 (each 3H, all s), a methoxyl proton at δ 3.16 (3H, s) and two protons of oxygen-bearing carbons at δ 3.43 (1H, m) and 3.78 (1H, m). From the ^{13}C NMR, the structure was determined to be a dammarane-type of aglycone with four oxygen-bearing carbons: δ 80.3 (C-20), 78.0 (C-3), 70.5 (C-25), 69.5 (C-12). The NMR data





1	R^1 = H	R^2 = OH	R^3 = H	R^4 = OCH_3
2	R^1 = OH	R^2 = OH	R^3 = H	R^4 = OCH_3
3	R^1 = H	R^2 = OCH_3	R^3 = H	R^4 = OH
4	R^1 = H	R^2 = H	R^3 = OCH3	R^4 = OCH_3
20 (S)-25-OCH_3-PPD	R^1 = H	R^2 = H	R^3 = OH	R^4 = OCH_3

5	R = H
aglycone of gynoside E	R = OH

Figure 1. Structures for compounds **1**–**5**.

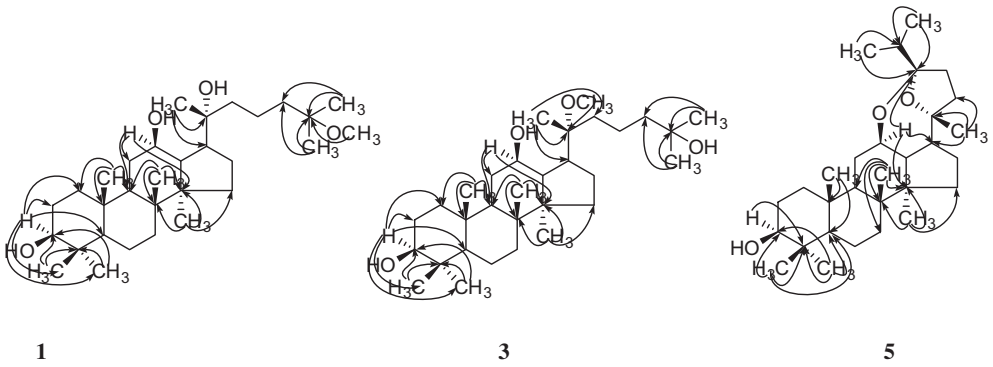


Figure 2. The important HMBC correlations of **1**, **3**, and **5**.

of **3** was similar to that of **1** except for the side chain, especially the C-20 downfield from δ 73.2 to 80.3 and the C-20 upfield from 74.5 to 70.5. In the HMBC spectrum, the long-range correlations were observed between δ 3.16 (OCH_3) and δ 80.3 (C-20); δ 1.16 (H-21) and δ 80.3 (C-20), 50.3 (C-17), 36.6 (C-22); δ 1.41 (H-26, H-27) and δ 70.5 (C-25), 45.0 (C-24), which suggested the methoxyl was connected to C-20 and C-25 was substituted by hydroxyl. In the NOESY spectrum of **3**, the signals at δ 3.16 (OCH_3) showed correlation with δ 3.78 (H-12) and δ 0.87 (H-30), indicating the configuration of C-20 is *R*. On the basis of above data[31], the structure of **3** was determined to be 20(*R*)-20-methoxyl-dammarane-3β,12β,25-triol (Fig. 2).

Compound **4** was afforded as colorless crystals. Its molecular formula was determined as $C_{32}H_{58}O_4$ by ^{13}C NMR and ESI MS (*m/z* 507 $[M+H]^+$ and 529 $[M+Na]^+$) and was confirmed by HREIMS (*m/z* 529.4224 calcd for $C_{32}H_{58}O_4Na$, 529.4233). Positive Liebermann–Burchard and negative Molish reactions suggested **4** to be a sapogenin. The IR spectrum displayed strong absorption bands at 3377 and 1062 cm^{-1}, showing the presence of hydroxyl groups. Its ^1H NMR spectrum showed signals for eight tertiary methyl groups at δ 0.89, 0.90, 1.00, 1.04, 1.14, 1.14, 1.16, and 1.23 (each 3H, all s), two methoxyl proton at δ 3.16 (6H, s) and two protons of oxygen-bearing carbons at δ 3.43 (1H, m) and 3.80 (1H, m). From the ^{13}C NMR spectrum, the structure was determined to be a dammarane-type of aglycone with four oxygen-bearing carbons: δ 78.0 (C-3), 74.4 (C-25), 80.3 (C-20), and 70.6 (C-12). The NMR data of **4** was similar to that of **3** except for the C-24, C-25, C-26, and C-27 of the side chain, which were observed to be similar to that of 20 (*S*)-25-OCH_3-PPD[28] by comparison of their NMR spectral data. Therefore, the structure of **4** was concluded to be 20(*R*)-20,25-dimethoxyl-dammarane-3β,12β-diol (Fig. 2). The conclusion was confirmed by HMQC, HMBC, and NOESY spectral data.

Compound **5** was isolated as colorless needles. Its molecular formula was determined as $C_{30}H_{50}O_3$ by ^{13}C NMR and ESI MS (*m/z* 459 $[M+H]^+$ and 481 $[M+Na]^+$) data analysis, and confirmed by HREIMS (*m/z* 481.3650 calcd for $C_{30}H_{50}O_3Na$, 481.3658). Positive Liebermann–Burchard and negative Molish reactions suggested **5** to be a sapogenin. The IR spectrum displayed strong absorption bands at 3374 and 1062 cm^{-1}, suggestive of the presence of hydroxyl groups. Its ^1H NMR spectrum (Table 1) showed signals for six tertiary methyl groups at δ 0.80, 0.89, 0.91 1.04, 1.16, and 1.23 (each 3H, all s) and two secondary methyl groups at δ 1.00 (6H, d, J = 7.3 Hz), two protons of oxygen-bearing carbons at δ 3.21 (1H, m) and 3.70 (1H, m). From the ^{13}C NMR spectrum,[32] the structure was determined to be an aglycone with four oxygen-bearing carbons: δ 78.9 (C-3), 72.5 (C-12), 87.0 (C-20) including a hemiacetal carbon at δ 111.6 (C-24). The structure was further concluded to be a PPD-type aglycone with an epoxy on the side chain. The NMR spectral data of **5** were similar to those of the aglycone part of gynoside E except for the signals of C-25, C-26, and C-27 on the side chain. In the HMBC spectrum (Fig. 2), the following long-range correlations between the methyl protons δ 1.00 (H-26, H-27) and δ 111.6 (C-24), 37.0 (C-25), δ 1.23 (H-21) and δ 87.0 (C-20), 29.0 (C-22), as well as the proton at δ 3.70 (H-12) and δ 111.6 (C-24), 52.2 (C-17), 48.7 (C-14) were observed. These data further indicated the presence of 12,24; 20,24-diepoxy on part of the side chain and the absence of the hydroxyl substituted at C-25. Thus, the structure of compound **5** was established as (12*R*,20*S*,24*S*)-20,24-; 12,24-diepoxy-dammaran-3β-ol[32] (Fig. 2).

Compounds **1**–**5** were evaluated for cytotoxic activities in six cell lines including MCF-7, HepG2, Du145, Colon205, A549, and HL-60.[33] The cells were cultured with test materials for 72 h and results expressed as $IC_{50} \pm SE$ are summarized in Table 2 and Figure 3.

It was clear that compounds **1**–**3** showed significant cytotoxic activity against all six cell lines. Compounds **2** and **3** showed selective cytotoxic activities against Colon205 and HL-60 cells, respectively. The cytotoxic activity of compound **5** was much lower than compounds **1–3**. The cytotoxic activity of compound **4** was the lowest of all of the five compounds. The IC_{50} of compounds **1**–**3** were lower than 25-OH-PPD, which was a new natural product recently isolated from the fruits of *P. ginseng*, whose IC_{50} values (10–60 μM) were already 5- to 15-fold lower than those for ginsenoside-Rg_3[26] in MCF-7, HepG2, Du145, and Colon205. The IC_{50}s of

Table 2
Biological activities of the compounds **1**–**5**

Compounds	$IC_{50} \pm SE$ (μM)					
	MCF-7	HepG2	Du145	Colon205	A549	HL-60
1	13.65 ± 2.79	15.74 ± 1.79	16.11 ± 1.72	11.20 ± 4.15	33.57 ± 2.94	12.65 ± 3.83
2	35.63 ± 2.93	48.30 ± 5.09	23.29 ± 3.51	1.66 ± 4.99	37.42 ± 0.61	50.25 ± 4.20
3	15.2 ± 6.21	8.78 ± 0.37	12.43 ± 0.60	8.64 ± 3.67	18.20 ± 3.92	3.98 ± 0.28
4	–	–	–	–	–	–
5	48.96 ± 2.41	64.14 ± 1.22	–	70.65 ± 5.21	–	42.39 ± 3.44
PPD	6.62 ± 2.34	39.42 ± 1.15	60.41 ± 1.23	49.16 ± 3.48	99.89 ± 1.59	27.06 ± 1.25
25-OH-PPD	34.16 ± 4.21	45.83 ± 2.18	–	18.86 ± 0.97	29.87 ± 1.23	8.03 ± 0.35
20(*S*)-25-OCH_3-PPD	8.34 ± 1.57	8.32 ± 2.49	11.83 ± 3.52	14.2 ± 1.28	35.62 ± 2.27	8.48 ± 5.41

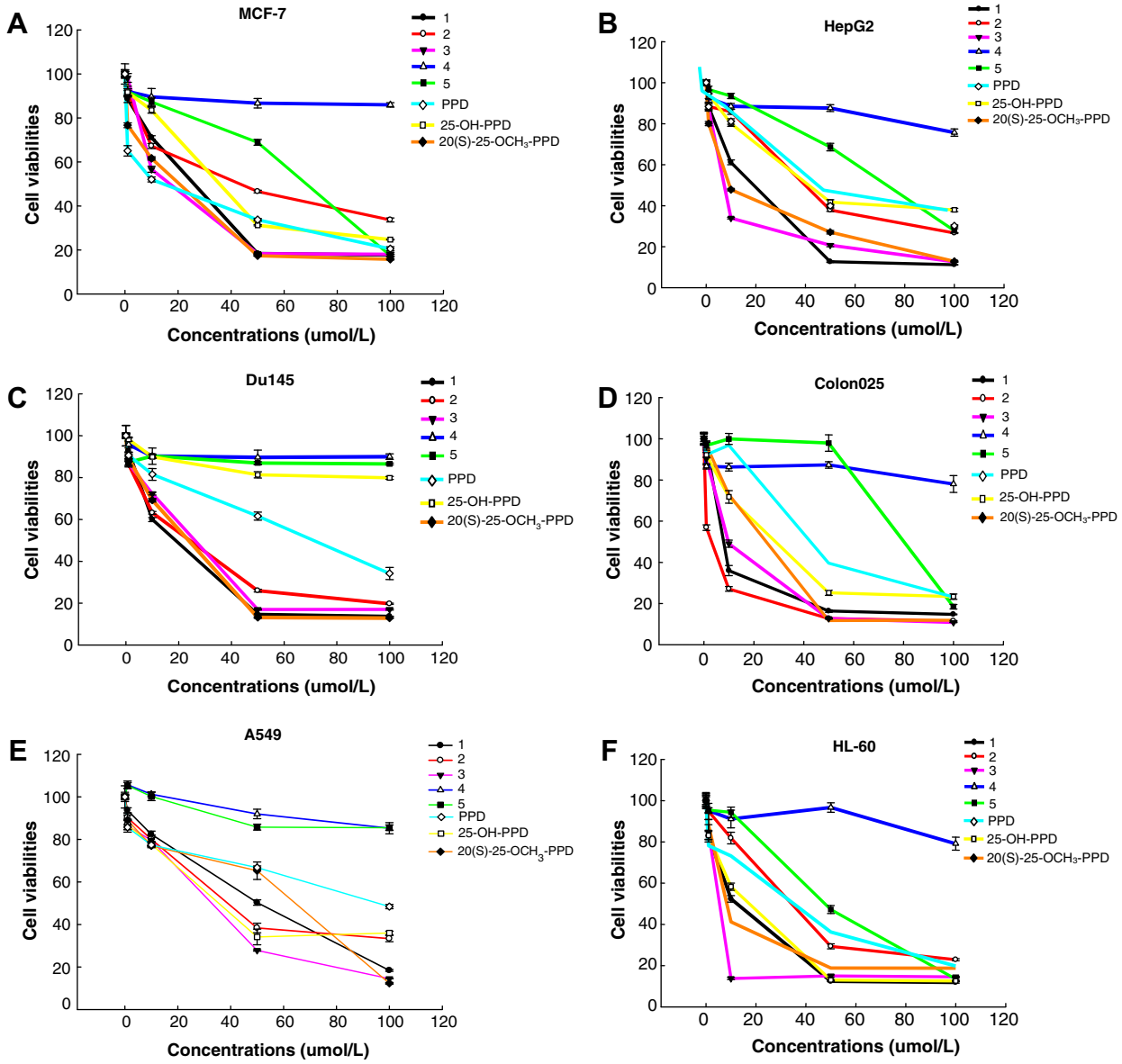


Figure 3. Cell viabilities of six cell lines (MCF-7, HepG2, Du145, Colon205, A549, and HL-60) after incubated with test compounds. The cell viabilities were significant lower than the negative control group. Cells were treated with 1, 10, 50, and 100 μmol/L for 72 h, respectively.

compounds **1**–**3** were lower than PPD in six cell lines except for MCF-7. Compared with 20(*S*)-25-OCH_3-PPD, a novel dammarane-type triterpene sapogenin which had reportedly exerted the strongest activity among any of the known ginsenosides tested for cytotoxic effects,[28] a similar efficacy was apparent for compounds **1**–**3** in all tumor cell lines. For compound **2**, its IC_{50} value against the Colon205 cells was much lower than that of 20(*S*)-25-OCH_3-PPD, which may suggest that it has selective toxicity on this type of tumor cell.

In summary, the present study demonstrated that the compounds **1** and **3** had significant activities towards a broad spectrum of human cancer cells. In addition, the discovery and structural identification of the active sapogenins may provide an opportunity to develop novel compounds for cancer prevention and therapy.

Acknowledgments

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References and notes

1. Coon, J. T.; Ernst, E. *Drug Saf.* **2002**, *25*, 322.
2. Zhu, M.; Chan, K. W.; Ng, L. S.; Chang, Q.; Chang, S.; Li, R. C. *J. Pharm. Pharmacol.* **1999**, *5*, 175.
3. Helms, S. *Altern. Med. Rev.* **2004**, *9*, 259.
4. Sparreboom, A.; Cox, M. C.; Acharya, M. R.; Figg, W. D. *J. Clin. Oncol.* **2004**, *22*, 2489.
5. Attele, A. S.; Wu, J. A.; Yuan, C. S. *Biochem. Pharmacol.* **1999**, *58*, 1685.
6. Gillis, C. N. *Pharmacology* **1997**, *54*, 1.
7. Jia, L.; Zhao, Y. Q. *Curr. Med. Chem.* **2009**, *16*, 2475.
8. Jia, L.; Zhao, Y. Q.; Liang, X. J. *Curr. Med. Chem.* **2009**, *16*, 2924.
9. Popovich, D. G.; Kitts, D. D. *Arch. Biochem. Biophys.* **2002**, *406*, 1.
10. Shibata, S. J. *Korean Med. Sci.* **2001**, *16*, 28.
11. Zhao, Y. Q.; Yuan, C.; Lu, H. *Zhongguo Zhong Yao Za Zhi* **1991**, *16*, 678.
12. Zhao, Y. Q.; Yuan, C. L. *Zhongguo Zhong Yao Za Zhi* **1993**, *18*, 296.
13. Yu, M.; Zhao, Y. Q. *Chin. Tradit. Herb. Drugs* **2002**, *33*, 404.
14. Yu, M.; Zhao, Y. Q. *Chin. Tradit. Herb. Drugs* **2004**, *35*, 869.
15. Wang, D.; Chui, R. H.; Zhao, Y. Q. *Mod. Chin. Med.* **2008**, *10*, 12.

16. Fei, X. F.; Wang, B. X.; Tashiro, S.; Li, T. J.; Ma, J. S.; Ikejima, T. *Acta Pharmacol. Sin.* **2002**, *23*, 315.
17. Tran, Q.; Tezuka, Y.; Banskota, A. H.; Tran, Q. K. *J. Nat. Prod.* **2001**, *64*, 1127.
18. Nakata, H.; Kikuchi, Y.; Tode, T. *Jpn. J. Cancer Res.* **1998**, *89*, 733.
19. Cheng, C. C.; Yang, S. M.; Huang, C. Y.; Chen, J. C.; Chang, W. M.; Hsu, S. L. *Cancer Chemother. Pharmacol.* **2005**, *55*, 531.
20. Jiang, H.; Fan, G. H. *Chin. J. Clin. Oncol. Rehabil.* **2004**, *11*, 289.
21. Oh, M.; Choi, Y. H.; Choi, S. *Int. J. Oncol.* **1999**, *14*, 869.
22. Liu, W. K.; Xu, S. X.; Che, C. T. *Life Sci.* **2000**, *67*, 1297.
23. Li, X.; Guan, Y. S.; Zhou, X. P. *Sichuan Da Xue Xue Bao Yi Xue Ban* **2005**, *36*, 217.
24. Xu, T. M.; Xin, Y.; Cui, M. H.; Jiang, X.; Gu, L. P. *Chin. Med. J.* **2007**, *120*, 584.
25. Chen, J. X.; Peng, H. M.; Pu, S. P.; Guo, Y. P. *Zhongguo Zhong Yao Za Zhi* **2007**, *32*, 1680.
26. Liu, L. W.; Ye, G. C. *Chin. J. Cancer Prev. Treat.* **2004**, *11*, 957.
27. Wang, W.; Zhao, Y. Q.; Rayburn, E.; Hill, D. L.; Wang, H.; Zhang, R. W. *Cancer Chemother. Pharmacol.* **2007**, *59*, 589.
28. Zhao, Y. Q.; Wang, W.; Han, L.; Rayburn, E. R.; Hill, D. L.; Wang, H.; Zhang, R. W. *Med. Chem.* **2007**, *3*, 51.
29. Hydrolysis and isolation: the total saponins (100 g) were hydrolyzed with 18% HCl (500 mL). The hydrolyzed preparation was then extracted with EtOAc (500 mL) three times and the extract was subjected to chromatography on a silica gel column (400 g). Elution was stepwise with a petroleum–EtOAc gradient (petroleum–EtOAc, 10:1, 5:1, 2:1, 1:1, 1:2, 1:3, 1:4) to afford compounds **1**, **2**, **3**, **4**, and **5**.
30. Spectral data for **1**: colorless crystals, $[\alpha]_D^{20}$ +8.0 (*c* 0.1, MeOH); IR ν_{max} 3283, 2944, 1466, 1385, 1082, 1029 cm^{-1}; ^{13}C NMR (75 MHz, pyridine-d_5): 39.4 (C-1), 28.3 (C-2), 77.9 (C-3), 40.1 (C-4), 56.4 (C-5), 18.8 (C-6), 35.2 (C-7), 39.6 (C-8), 50.5 (C-9), 37.4 (C-10), 31.5 (C-11), 70.9 (C-12), 49.3 (C-13), 51.8 (C-14), 32.3 (C-15), 26.7 (C-16), 50.8 (C-17), 16.4 (C-18), 16.5 (C-19), 73.2 (C-20), 22.7 (C-21), 43.9 (C-22), 18.1 (C-23), 41.2 (C-24), 74.5 (C-25), 25.1 (C-26), 25.1 (C-27), 28.6 (C-28), 15.9 (C-29), 17.4 (C-30), 49.0 (25-OCH_3). HRESIMS *m*/*z* 515.4064 $[M+Na]^+$ (calcd for $C_{31}H_{56}O_4Na$, 515.4076).
31. Spectral data for **3**: colorless crystals, $[\alpha]_D^{20}$ +7.0 (*c* 0.1, MeOH); IR ν_{max} 3301, 2961, 1467, 1385, 1262, 1168, 1084, 1037, 802 cm^{-1}; ^{13}C NMR (75 MHz, pyridine-d_5): 39.4 (C-1), 28.2 (C-2), 78.0 (C-3), 40.1 (C-4), 56.4 (C-5), 18.8 (C-6), 35.2 (C-7), 39.6 (C-8), 49.7 (C-9), 37.4 (C-10), 31.3 (C-11), 69.5 (C-12), 48.4 (C-13), 51.8 (C-14), 31.4 (C-15), 26.3 (C-16), 50.3 (C-17), 16.4 (C-18), 16.5 (C-19), 80.3 (C-20), 17.8 (C-21), 36.6 (C-22), 18.8 (C-23), 45.0 (C-24), 70.5 (C-25), 30.2 (C-26), 30.1 (C-27), 28.7 (C-28), 15.9 (C-29), 17.4 (C-30), 48.9 (20-OCH_3). HRESIMS *m*/*z* 515.4066 $[M+Na]^+$ (calcd for $C_{31}H_{56}O_4Na$, 515.4076).
32. Spectral data for **5**: ^{13}C NMR (75 MHz, pyridine-d_5): 39.4 (C-1), 28.3 (C-2), 78.0 (C-3), 39.6 (C-4), 56.3 (C-5), 18.7 (C-6), 35.1 (C-7), 39.9 (C-8), 50.1 (C-9), 37.5 (C-10), 29.8 (C-11), 73.1 (C-12), 49.3 (C-13), 49.1 (C-14), 32.0 (C-15), 23.5 (C-16), 52.9 (C-17), 16.4 (C-18), 16.2 (C-19), 87.3 (C-20), 27.8 (C-21), 29.4 (C-22), 38.2 (C-23), 117.7 (C-24), 38.0 (C-25), 17.7 (C-26), 17.7 (C-27), 28.7 (C-28), 17.7 (C-29), 18.2 (C-30). HRESIMS *m*/*z* 481.3650 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3Na$, 481.3658).
33. In vitro cytotoxicity bioassay: the carcinoma cell lines MCF-7, HepG2, Du145, Colon205, A549, and HL-60 were used as the target cells in the cytotoxicity assay. For drug exposure experiments, after exposuring the drug with cells for 72 h, 10 μL of MTT solution (2.5 mg/ml) was added to each well, and the tumor cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 air for 4 h. At the end of incubation, the growth medium was removed and replaced with 100 μL of DMSO (at room temperature). After agitating on a vortex for 10 min, the absorbance was determined at 492 nm as reference on a Bio-Rad (model 550) microplate reader to calculate 50% inhibition concentration (IC_{50}). DMSO and MTT were purchased from Sigma Chemical Co., Ltd, USA.