

Triterpene Saponins from Vietnamese Ginseng (*Panax vietnamensis*) and Their Hepatocytotoxic Activity

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The methanol extract of Vietnamese ginseng (*Panax vietnamensis*) was found to possess hepatocytotoxic effects on D-galactosamine (D-GalN)/tumor necrosis factor- α (TNF- α)-induced cell death in primary cultured mouse hepatocytes. Further chemical investigation of the extract afforded two new dammarane-type triterpene saponins, ginsenoside Rh₅ (**1**) and vina-ginsenoside R₂₅ (**2**), as well as eight known dammarane-type triterpene saponins, majonoside R₂ (**3**), pseudo-ginsenoside RT₄ (**4**), vina-ginsenosides R₁ (**5**), R₂ (**6**), and R₁₀ (**7**), ginsenosides Rg₁ (**8**), Rh₁ (**9**), and Rh₄ (**10**), and a known saponin protopanaxatriol oxide II (**11**). Their structures were elucidated on the basis of spectral analysis. In addition, by the using LC-electrospray ionization (ESI)-MS method, five known saponins, ginsenosides Rb₁, Rb₂, Rc, Rd, and Re (**12–16**), were also identified in the extract. Among the compounds isolated, majonoside R₂ (**3**), the main saponin in Vietnamese ginseng, showed strong protective activity against D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes. This demonstrates that the hepatocytotoxic effect of Vietnamese ginseng is due to dammarane-type triterpene saponins that have an ocotillol-type side chain, a characteristic constituent of Vietnamese ginseng.

Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv., Araliaceae) is a wild *Panax* species that was discovered in the mountains of Central Vietnam in 1973 and is used by ethnic minorities for treatment of many serious diseases and for enhancement of physical strength. Previous studies have demonstrated that Vietnamese ginseng is an important medicinal plant used in cultural traditional medicine. This ginseng contains a large quantity of ocotillol-type saponins, e.g., majonoside R₂, that are not present in *P. ginseng*.^{1–3} In addition, Vietnamese ginseng was reported to exhibit interesting pharmacological effects such as stimulatory and suppressive effects on the central nervous system and antitumor-promoting activity.⁴ However, the hepatocytotoxic activity of Vietnamese ginseng has not been reported, despite reports on the hepatocytotoxic activity of other ginsengs including *P. ginseng* and *P. notoginseng*.^{5,6} In our continuing study of biologically active hepatocytotoxic natural products in Vietnamese medicinal plants,⁷ we found that the MeOH extract of Vietnamese ginseng has a hepatocytotoxic activity against D-galactosamine (D-GalN)/tumor necrosis factor- α (TNF- α)-induced cell death in primary cultured mouse hepatocytes.⁸ The active constituents of Vietnamese ginseng were thus examined, and two new dammarane-type triterpene saponins were isolated and identified. In addition, eight known saponins and one known saponin were identified. Furthermore, using HPLC-electrospray ionization mass spectrometry (LCESIMS), five additional previously identified ginsenosides were found in the extract. This paper reports the isolation and structure elucidation of the two new dammarane-type triterpene saponins that possess hepatocytotoxic activity in vitro.

Results and Discussion

Air-dried roots and rhizomes of Vietnamese ginseng were extracted with refluxing MeOH, 50% aqueous MeOH, and

water to give MeOH, MeOH–H₂O, and H₂O extracts. These showed hepatocytotoxic activity against D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes, with cell survival rates of 91.8 \pm 3.6%, 59.7 \pm 6.9%, and 51.1 \pm 7.8%, respectively, at a concentration of 200 μ g/mL, compared to that of vehicle control (56.2 \pm 8.2%). The MeOH extract, which shows the strongest activity, was subjected to column chromatography with Diaion HP-20 followed by silica gel to give six fractions, of which fractions 1 and 2 exhibited strong activity. Further investigation of these two fractions led to the isolation of two new saponins, namely, ginsenoside Rh₅ (**1**, 0.015%) and vina-ginsenoside R₂₅ (**2**, 0.003%), as well as nine known compounds, majonoside R₂⁹ (**3**, 1.85%), pseudo-ginsenoside RT₄¹⁰ (**4**, 0.16%), vina-ginsenosides R₁¹ (**5**, 0.009%), R₂¹ (**6**, 0.0016%), and R₁₀³ (**7**, 0.007%), ginsenosides Rg₁¹¹ (**8**, 0.78%), Rh₁¹² (**9**, 0.021%), and Rh₄¹³ (**10**, 0.014%), and protopanaxatriol oxide II¹⁴ (**11**, 0.004%). (See Chart 1 for structures.)

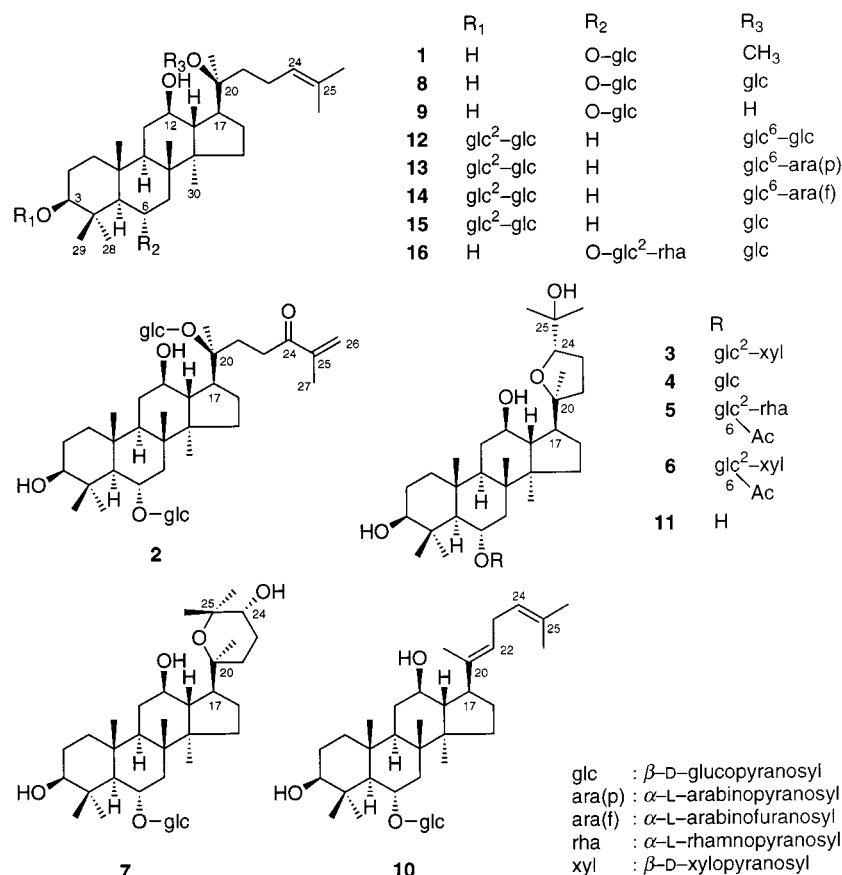
Ginsenoside Rh₅ (**1**) was obtained as a colorless amorphous solid with a $[\alpha]_D^{25}$ of +32.4°. Negative-ion HR-FABMS of **1** showed a quasi-molecular ion at m/z 651.4471 for the molecular formula C₃₇H₆₄O₉. The ¹H NMR spectrum of **1** displays signals due to eight tertiary methyls, an olefin, and an anomeric proton (Table 1). The ¹H and ¹³C NMR data of **1** are very similar to those of ginsenoside Rh₁ (**9**), except for signals indicating the presence of an extra methoxyl group (δ_H 3.22; δ_C 48.8), suggesting that **1** is a methyl ether of **9**. The methoxyl group could be located at C-20 based on the downfield shift of C-20 (**1**, δ_C 79.8; **9**, δ_C 73.0) and the highfield shifts of C-17 (**1**, δ_C 46.9; **9**, δ_C 54.7), C-21 (**1**, δ_C 21.1; **9**, δ_C 26.8), and C-22 (**1**, δ_C 35.1; **9**, δ_C 35.8). This was further supported by the long-range correlation between the methoxyl protons and C-20 in the HMBC spectrum. The stereochemistry of **1** was deduced based on the ROESY experiment, where the correlation between H₃-21 and H-17 indicated the configuration at C-20 to be *S*.¹⁵ The sugar moiety of **1** was determined to be β -D-glucopyranose by GC analysis of a chiral derivative of an acid hydrolysate.¹⁶ From these data, the structure of

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Chart 1



ginsenoside Rh₅ was determined to be 6-*O*- β -D-glucopyranosyl-20-*O*-methyl-dammar-24-en-3 β ,6 α ,12 β ,20(*S*)-tetrrol (**1**). Because the tertiary hydroxyl group at C-20 of dammarane-type triterpene saponins such as ginsenoside Rh₁ (**9**) has been reported to be very labile under acidic condition,^{15,17} we could not exclude the possibility that **1** is an artifact, even though acidic conditions in the extraction and isolation were not employed.

Vina-ginsenoside R₂₅ (**2**) was obtained as a colorless amorphous solid with $[\alpha]_{25}^D +30.8^\circ$. Negative-ion HR-FABMS of **2** showed a quasi-molecular ion at m/z 813.4633 for the molecular formula C₄₂H₇₀O₁₅. The ¹H NMR spectrum of **2** shows signals due to seven tertiary methyls, a terminal olefin, and two anomeric protons (Table 1). The ¹³C NMR spectrum, together with a DEPT experiment, indicated the presence of a terminal double bond and a ketone carbonyl group, consistent with the IR absorptions at 1660, 1635, and 890 cm⁻¹ for an α,β -unsaturated ketone group. Detailed analyses of the COSY, HMQC, and HMBC spectra allowed the assignment of all the ¹H and ¹³C NMR signals (Table 2) and indicated **2** to be a dammarane-type triterpene saponin with two β -glucopyranosyl units. The sugars were determined to be D-glucose by GC analysis of an acid hydrolysate as described above. The carbon signals assignable to the aglycone part of **2** were almost the same as those of ginsenoside Rg₁ (**8**), except for those of the side chain. The structure of the side chain moiety was determined by the long-range correlations between H₃-27 and C-24, C-25, and C-26 and between H₂-26 and C-24, C-25, and C-27 in the HMBC experiment (Figure 1a). Similarly, the long-range correlations between the anomeric protons of the two β -D-glucopyranosyl units and C-6 and C-20 in the HMBC spectrum indicated the two glucose units to be located at C-6 and C-20. The stereochemistry was deter-

mined from the ROESY correlations, including that between H₃-21 and H-17, as shown in Figure 1b. From these data, the structure of vina-ginsenoside R₂₅ was determined as 6-*O*- β -D-glucopyranosyl-20-*O*- β -D-glucopyranosyl-dammar-25-en-24-one-3 β ,6 α ,12 β ,20(*S*)-tetrrol (**2**).

In this paper, we report the identification of two new and nine known compounds from the MeOH extract of Vietnamese ginseng. However, 49 compounds have been previously reported as constituents of this plant.^{1-3,18} As such, we examined the MeOH extract by using the LCESIMS technique with the isolated compounds and another five ginsenosides, ginsenosides Rb₁ (**12**), Rb₂ (**13**), Rc (**14**), Rd (**15**), and Re (**16**), as authentic samples. These ginsenosides are major saponins of *P. ginseng*¹⁵ and used as a criteria for characterization of ginseng.¹⁹ Using negative-ion LCESIMS, the standard samples of **1-16** gave an [M + AcO]⁻ ion, but not an [M - H]⁻ ion. By comparing retention times of the [M + AcO]⁻ ions with those of authentic samples in the mass chromatogram, the peaks corresponding to **1-16** were identified (Figure 2). The total ion chromatogram (TIC) revealed that the saponins were eluted within 90 min, in order of decreasing polarity, and could be grouped into two major groups. The first group, which eluted before 40 min, consisted of protopanaxatriol saponins such as **2-8** and **16**, while the second group consisted of protopanaxadiol counterparts such as **12-15** and the sapogenin **11**. The chromatogram also demonstrated the presence in high content of majonoside R₂ (**3**), followed by ginsenoside Rg₁ (**8**), while the two newly isolated saponins were found to be minor constituents.

We examined the hepatocytotoxic activity of the 16 compounds against D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes (Table 2). Among the 16, compounds **3-6**, **10**, and **11** showed strong hepatocy-

Table 1. ^1H and ^{13}C NMR Data of Saponins **1** and **2** in Pyridine- d_5

no.	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	39.5	1.71 (1H, m); 1.08 (1H, m)	39.4	1.73 (1H, m); 1.01 (1H, m)
2	27.8	1.97 (1H, m); 1.84 (1H, m)	27.9	1.94 (1H, m); 1.88 (1H, m)
3	78.6	3.49 (1H, dd, 11.3, 4.7)	78.6	3.48 (1H, dd, 11.5, 4.4)
4	40.3		40.3	
5	61.4	1.41 (1H, d, 10.8)	61.4	1.37 (1H, d, 10.2)
6	80.0	4.38 (1H, td, 10.8, 3.0)	80.1	4.37 (1H, td, 10.2, 2.9)
7	45.1	2.45 (1H, dd, 12.7, 3.0); 1.92 (1H, m)	45.1	2.43 (1H, dd, 12.9, 2.9); 1.88 (1H, m)
8	41.0		41.1	
9	49.8	1.50 (1H, m)	50.0	1.50 (1H, m)
10	39.7		39.7	
11	31.0	2.07 (1H, m); 1.41 (1H, m)	30.9	2.03 (1H, m); 1.50 (1H, m)
12	70.3	3.68 (1H, td, 10.0, 5.4)	70.2	4.10 (1H, m)
13	48.6	1.8 (1H, dd, 11.2, 5.4)	49.1	2.03 (1H, m)
14	51.3		51.4	
15	30.7	1.56 (1H, m); 1.04 (1H, m)	30.6	1.64 (1H, m); 1.06 (1H, m)
16	26.2	1.61 (1H, m); 1.18 (1H, m)	26.7	1.73 (1H, m); 1.31 (1H, m)
17	46.9	2.30 (1H, m)	52.0	2.48 (1H, m)
18	17.5	1.02 (3H, s)	17.5	1.03 (3H, s)
19	17.4	1.12 (3H, s)	17.4	1.13 (3H, s)
20	79.8		83.1	
21	21.1	1.13 (3H, s)	21.9	1.53 (3H, s)
22	35.1	1.92 (1H, m); 1.18 (1H, m)	29.8	2.65 (1H, ddd, 14.4, 9.5, 4.7); 2.08 (1H, m)
23	22.7	2.25 (1H, m); 2.06 (1H, m)	32.8	3.30 (1H, ddd, 16.6, 10, 4.7); 3.03 (1H, ddd, 16.6, 9.5, 6.0)
24	125.5	5.23 (1H, t, 6.9)	202.4	
25	130.2		144.4	
26	25.8	1.66 (3H, s)	124.9	6.20 (1H, br. s); 5.66 (1H, br. s)
27	17.7	1.62 (3H, s)	17.8	1.85 (3H, s)
28	31.6	2.01 (3H, s)	31.7	2.05 (3H, s)
29	16.3	1.56 (3H, s)	16.3	1.60 (3H, s)
30	16.8	0.79 (3H, s)	17.1	0.81 (3H, s)
1'	105.9	4.96 (1H, d, 7.8)	105.9	4.98 (1H, d, 7.8)
2'	75.3	4.02 (1H, t, 7.8)	75.4	4.08 (1H, t, 7.8)
3'	79.5	4.17 (1H, m)	79.3	4.22 (1H, m)
4'	71.8	4.14 (1H, m)	71.8	4.20 (1H, m)
5'	78.0	3.88 (1H, m)	78.1	3.89 (1H, m)
6'	63.1	4.46 (1H, dd, 11.4, 2.4); 4.29 (1H, dd, 11.4, 5.4)	62.9	4.46 (1H, dd, 11.2, 2.0); 4.26 (1H, dd, 11.2, 5.8)
1''			98.0	5.10 (1H, d, 7.6)
2''			75.0	3.95 (1H, t, 7.6)
3''			79.6	4.17 (1H, m)
4''			71.6	4.10 (1H, m)
5''			78.2	3.90 (1H, m)
6''			63.0	4.49 (1H, dd, 11.5, 2.4); 4.33 (1H, dd, 11.5, 5.3)
MeO	48.8	3.22 (3H, s)		

toprotective activities, with IC_{50} values of 82.4, 74.8, 47.0, 63.2, 97.0, and 74.0 μM , respectively. Compounds **8**, **9**, **13**, and **16** showed moderate activity, with IC_{50} values of 111, 105, 105, and 119 μM , respectively. These compounds, with the exception of **10**, are all dammarane-type saponins possessing an ocotillol-type side chain. Compounds **2**, **12**, and **15** exhibited very weak or almost no hepatocytotoxic effects at a high concentration of 200 μM (Table 2). Previously, hepatoprotective effects of dammarane-type triterpene saponins such as **8** and **16** on D-GalN/lipopolysaccharide-induced liver injury model in mice⁵ and hepatocytotoxic activities of **8**, **9**, **12**, and **14–16** on D-GalN- and CCl_4 -induced cell death in primary cultured rat hepatocytes⁶ have been reported. However, this report describes the first example of hepatocytotoxic activity of the ocotillol-type saponins. The furan ring in the ocotillol-type side chain seems to be important for the hepatocytotoxic activity, while a pyran ring in the side chain does not (e.g., compound **7** is inactive). Silibinin, a positive control, showed strong activity, with an IC_{50} of 14.0 μM . However, glycyrrhizin, which has also been reported to exhibit hepatocytotoxic effects through membrane-stabilization via inhibition of lipid peroxidation,²⁰ exhibited only weak activity ($\text{IC}_{50} > 200 \mu\text{M}$) in this assay. The ocotillol-type saponins, such as majonoside R_2 (**3**), showed

no inhibition against lipid peroxidation.²¹ Although the mechanism of hepatocytotoxic protection by Vietnamese ginseng is not clear, these data suggest that the protection mechanism of the Vietnamese ginseng, which contains ocotillol-type saponins as the major constituents, is different from that of glycyrrhizin.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-140 digital polarimeter at 25 $^{\circ}\text{C}$. NMR spectra were recorded on JEOL JNM GX-400 spectrometer in pyridine- d_5 , using TMS as an internal reference. IR spectra were taken on KBr disks on a Shimadzu IR-408 spectrophotometer. FABMS and LCESIMS were performed with a JEOL JMS-700T mass spectrometer, and glycerol was used as a matrix for FABMS.

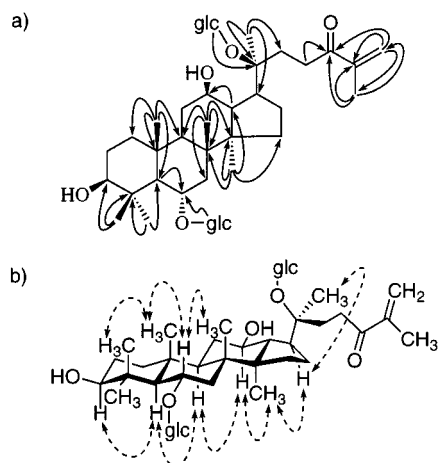
Plant Material. Vietnamese ginseng was collected at Quangnam Province, Vietnam, in November 1998, and the voucher sample (TMPU 010776) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. Air-dried roots and rhizomes of Vietnamese ginseng (700 g) were sequentially extracted with hot MeOH, hot 50% MeOH– H_2O , and water to give MeOH (198 g), MeOH– H_2O (163 g), and H_2O extracts (22 g). A portion of the MeOH extract (100 g) was subjected to column chromatography on Diaion HP-20, eluted with H_2O , MeOH, and

Table 2. Hepatocytotoxic Effect of Compounds Identified in Vietnamese Ginseng on D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes^a

sample	conc (μ M)	cell survival rate (%)	inh. (%)	IC ₅₀ (μ M)	protection (%)	sample	conc (μ M)	cell survival rate (%)	inh. (%)	IC ₅₀ (μ M)	protection (%)
normal		100.0 \pm 15.0									
control		29.9 \pm 4.6									
silibinin	50	75.0 \pm 5.6**	149.7	14.0	64.3 \pm 8.1	8	200	48.3 \pm 3.3**	61.4	111	26.2 \pm 4.7
	25	60.0 \pm 3.1**	99.7		42.8 \pm 4.5		100	42.0 \pm 3.3**	40.6		17.3 \pm 4.8
	12.5	43.1 \pm 2.7**	43.4		17.3 \pm 3.6		50	41.6 \pm 1.0**	39.3		16.7 \pm 1.4
glycyrrhizin	200	36.2 \pm 2.4	20.3	>200	8.7 \pm 3.4	9	200	54.0 \pm 1.4**	80.5	105	34.3 \pm 2.0
	100	33.4 \pm 3.6	11.1		4.8 \pm 5.1		100	40.8 \pm 1.5**	36.6		15.6 \pm 2.2
	50	33.0 \pm 4.9	11.0		1.0 \pm 3		50	38.1 \pm 3.3**	27.4		11.7 \pm 4.7
1	200	22.7 \pm 0.9	-24.0			10	200	50.0 \pm 8.9**	67.1	97.0	35.1 \pm 6.9
	100	38.2 \pm 2.9*	27.9		11.9 \pm 4.1		100	45.0 \pm 4.6**	50.6		24.8 \pm 4.0
	50	36.9 \pm 3.8	23.5		13.1 \pm 1.2		50	34.0 \pm 2.8	13.7		8.0 \pm 1.5
2	200	44.3 \pm 5.3**	48.2		20.5 \pm 7.6	11	200	92.6 \pm 6.7**	209.9	74.0	92.2 \pm 9.6
	100	38.4 \pm 3.4*	28.3		12.0 \pm 4.9		100	45.3 \pm 2.3**	51.7		22.2 \pm 3.4
	50	35.6 \pm 1.1	19.2		8.2 \pm 1.6		50	40.2 \pm 4.5*	34.5		17.9 \pm 4.1
3	200	54.8 \pm 2.4**	83.3	82.4	35.5 \pm 3.4	12	200	39.6 \pm 1.6*	31.9		13.7 \pm 2.3
	100	46.4 \pm 4.4**	55.2		19.3 \pm 6.2		100	36.0 \pm 3.9	19.8		8.5 \pm 5.6
	50	41.1 \pm 2.4**	37.5		16.0 \pm 3.4		50	34.4 \pm 2.1	14.3		6.0 \pm 3.5
4	200	57.2 \pm 4.5**	91.5	74.8	39.0 \pm 6.4	13	200	60.3 \pm 2.2**	100.8	105	43.3 \pm 3.1
	100	48.7 \pm 3.3**	62.8		26.8 \pm 4.7		100	44.7 \pm 3.8**	48.8		21.0 \pm 5.4
	50	39.4 \pm 1.5*	31.8		13.6 \pm 2.2		50	36.7 \pm 2.6	22.1		7.6 \pm 2.1
5	100	57.0 \pm 10.5**	89.8	47.0	71.5 \pm 7.6	14	200	26.7 \pm 1.9	-11.3		
	50	45.6 \pm 3.2**	51.7		29.0 \pm 6.3		100	31.4 \pm 2.4	4.4		1.9 \pm 3.4
	25	33.1 \pm 5.3	10.1		22.0 \pm 7.2		50	32.1 \pm 1.8	6.7		3.2 \pm 2.9
6	200	72.9 \pm 9.1**	143.8	63.2	61.3 \pm 13.0	15	200	41.8 \pm 3.5**	39.1		16.8 \pm 5.0
	100	51.7 \pm 6.2**	72.9		31.1 \pm 8.8		100	35.9 \pm 1.6	19.5		8.4 \pm 2.3
	50	43.2 \pm 4.4**	44.7		19.0 \pm 6.3		50	35.3 \pm 3.9	17.5		7.4 \pm 6.5
7	200	35.2 \pm 1.5	17.8		7.6 \pm 2.2	16	200	52.4 \pm 1.5**	74.3	119	31.9 \pm 2.2
	100	38.3 \pm 4.0*	28.0		12.0 \pm 5.6		100	41.2 \pm 4.4*	37.0		15.9 \pm 6.2
	50	41.1 \pm 3.5*	37.6		16.0 \pm 5.1		50	36.4 \pm 0.9	21.1		8.9 \pm 1.4

^a Results are expressed as means \pm SD ($n = 4$; for normal and control, $n = 8$); * $p < 0.01$, ** $p < 0.001$ significantly different from control by Student's t -test.

**Figure 1.** Important HMBC (a) and ROESY (b) correlations of **2**.

CHCl₃, to give H₂O (51.6 g), MeOH (38.1 g), and CHCl₃ (3.8 g) fractions. The MeOH fraction was then chromatographed on silica gel with a CHCl₃-MeOH-H₂O (30:20:1 \rightarrow 30:20:5) gradient system to afford six fractions (fractions 1-6). Fraction 1 (3.5 g) was separated into three subfractions by silica gel column chromatography with CHCl₃-MeOH-H₂O (14:6:1). Subfractions 1 (80 mg) and 3 (140 mg) were subjected to preparative TLC with CHCl₃-MeOH-H₂O (14:6:0.5) to afford protopanaxatriol oxide II (**11**, 13 mg) and vina-ginsenoside R₁₀ (**7**, 33 mg), respectively. Subfraction 2 (1.9 g) was chromatographed on Cosmosil 75C₁₈-OPN with H₂O-MeOH-MeCN (2:1:1 \rightarrow 1:1:1) gradient system to give pseudo-ginsenoside RT₄ (**4**, 560 mg) and ginsenosides Rh₁ (**9**, 51 mg), Rh₄ (**10**, 49 mg), and Rh₅ (**1**, 16 mg). Fraction 2 (12.6 g) was subjected to column chromatography on silica gel with CHCl₃-MeOH-H₂O (14:6:1) to give three subfractions. Subfractions 1 (190 mg) and 3 (85 mg) were subjected to preparative TLC with CHCl₃-MeOH-H₂O (14:6:1) to give vina-ginsenosides R₁ (**5**, 30.5 mg) and R₂ (**6**, 5.6 mg) and vina-ginsenoside R₂₅ (**2**, 11 mg),

respectively. Subfraction 2 (11.0 g) was chromatographed on Cosmosil 75C₁₈-OPN with H₂O-MeOH-MeCN (2:1:1) to give majonoside R₂ (**3**, 6.1 g) and ginsenoside Rg₁ (**8**, 3.0 g).

Ginsenoside Rh₅ (1): colorless amorphous solid; [α]_D²⁵ +32.4° (c 2.39, MeOH); IR (KBr) ν_{\max} 3400, 1650 cm⁻¹; ¹H and ¹³C NMR, see Table 2; HRFABMS m/z 651.4471 [calcd for C₃₇H₆₃O₉ (M - H)⁻, 651.4472].

Vina-ginsenoside R₂₅ (2): colorless amorphous solid; [α]_D²⁵ +30.8° (c 0.6, MeOH); IR (KBr) ν_{\max} 3400, 1660, 1635, 890 cm⁻¹; ¹H and ¹³C NMR, see Table 2; HRFABMS m/z 813.4633 [calcd for C₄₂H₆₉O₁₅ (M - H)⁻, 813.4637].

Sugar Analysis of 1 and 2.¹⁶ Each compound (1 mg) was hydrolyzed with 1 M HCl (H₂O-dioxane, 1:1; 0.5 mL) at 80 °C for 4 h. The reaction mixture was neutralized with Amberlite IRA67 (OH⁻ form), and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in 0.1 mL of pyridine, to which L-cysteine methyl ester hydrochloride in pyridine (0.1 M, 0.1 mL) was added. The mixture was heated at 60 °C for 2 h. Then, trimethylsilylimidazole (0.1 mL) was added, and the mixture was heated at 60 °C for 1.5 h. The reaction mixture was partitioned between hexane and water (0.1 mL each), and the hexane layer was analyzed on a Shimadzu GC14AH gas chromatograph; column, Shimadzu CBJ17-S30-025, 0.32 mm \times 30 m; column temperature, 230 °C; detector temperature, 270 °C; injection temperature, 270 °C. Standard D- and L-glucose gave one peak at t_R 5.89 and 6.25 min, respectively.

LC-ESI-MS Analysis. The MeOH extract of Vietnamese ginseng was applied to a Diaion-HP20 column and then eluted successively with water and MeOH, and the MeOH eluate was evaporated to dryness under vacuum. The residue was redissolved in HPLC grade MeOH at a concentration of 10 mg/mL and filtered with an Acrodisc LC13 filter (Gelman, Ann Arbor, MI) prior to LC-MS analysis. Ginsenosides Rb₁ (**12**), Rb₂ (**13**), and Rd (**15**) were purchased from Alps Pharmaceutical Industries Co., Ltd. (Gifu, Japan), and ginsenosides Rc (**14**) and Re (**16**) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). For use in LCMS analyses, the samples were dissolved in HPLC grade MeOH at a concentration of 1

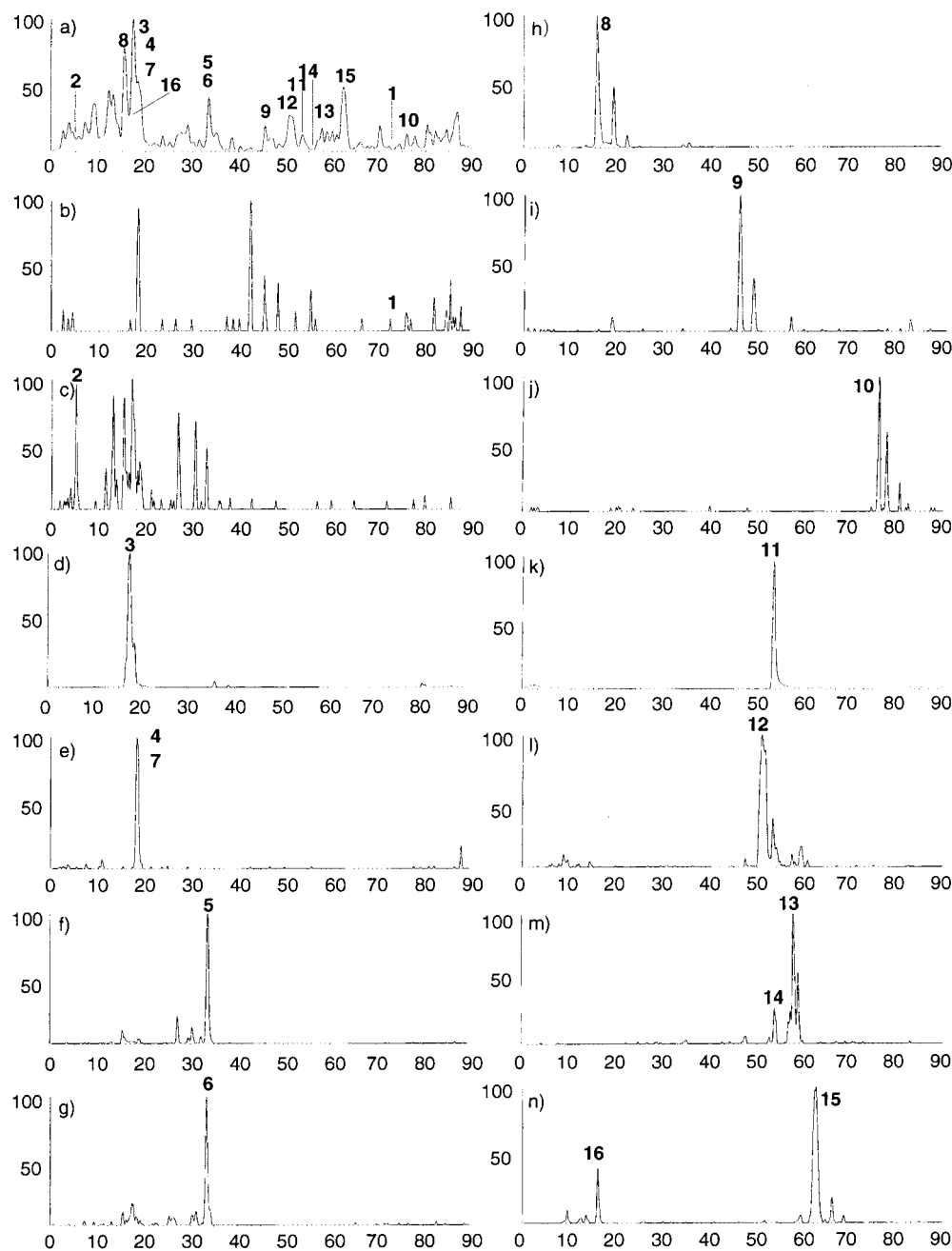


Figure 2. LCESIMS chromatograms of Vietnamese ginseng saponins. (a) Total-ion chromatogram (TIC). (b–n) Mass chromatograms monitored at the $[M + AcO]^-$ ion of **1** (m/z 711), **2** (m/z 873), **3** (m/z 845), **4** and **7** (m/z 713), **5** (m/z 901), **6** (m/z 887), **8** (m/z 859), **9** (m/z 697), **10** (m/z 679), **11** (m/z 551), **12** (m/z 1167), **13** and **14** (m/z 1137), and **15** and **16** (m/z 1005).

mg/mL. HPLC separation was accomplished using a Hewlett-Packard HP-1100 system with a Waters Symmetry C_{18} column ($5 \mu m$, 2.1×150 mm) at a column temperature of $40^\circ C$. The mobile phase was a gradient system of (A) 8 mM NH_4OAc (pH 7.0) and (B) CH_3CN : 0–20 min, 80–75% A, 20–25% B; 20–45 min, 75–70% A, 25–30% B; 45–70 min, 70–60% A, 30–40% B; 70–80 min, 60–40% A, 40–60% B; 80–90 min, 60% A, 40% B; flow rate, 0.2 mL/min. Negative-ion ESIMS were obtained by a full range scan from 500 to 1300 unit in 20 s.

d-GalN/TNF- α Induced Cell Death in Primary Cultured Mouse Hepatocytes. Mouse liver parenchymal cells were isolated according to the procedure described previously by Seglen.²² In brief, the liver was perfused Ca^{2+} -free Hanks' balanced salt solution (HBSS) containing 0.5% bovine serum albumin (BSA) and 5 mM ethyleneglycol-*O*,*O*-bis(2-aminoethyl)-*N,N,N,N*-tetraacetic acid (EGTA), then recirculated with collagenase solution composed of Ca^{2+} -free HBSS, 0.075% collagenase, 4 mM $CaCl_2$, and 0.005% trypsin inhibitor. The isolated hepatocytes were cultured in William's E medium

supplemented with 10% calf serum, 100 IU/mL penicillin G, 100 $\mu g/mL$ streptomycin, 100 μM dexamethasone, and 50 ng/mL insulin and incubated in 96-well plastic plates (1.5×10^4 cells/well). After 2 h preincubation, the medium was replaced with fresh medium containing d-GalN (0.5 mM) and test samples at various concentrations. Thirty minutes later, TNF- α (100 ng/mL) was added to each well, and the viability of the hepatocytes was assessed 18 h thereafter by MTT colorimetric reaction.²³

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