Medicinal Flowers. XVI.[1] New Dammarane-Type Triterpene Tetraglycosides and Gastroprotective Principles from Flower Buds of *Panax ginseng*

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The oligoglycoside fraction from the flower buds of *Panax ginseng* C. A. MEYER (Araliaceae) was found to show protective effects on ethanol-induced gastric mucosal lesions in rats. From the oligoglycoside fraction, new dammarane-type triterpene tetraglycosides, floralginsenosides M, N, O, and P, were isolated together with the major oligoglycosides ginsenoside Rd and Re. The structures of the new floralginsenosides were elucidated on the basis of chemical and physicochemical evidence. Ginsenoside Rd (protopanaxadiol 3,20-*O*-bisdesmoside) exhibited inhibitory effects on ethanol- and indomethacin-induced gastric mucosal lesions in rats.

Key words floralginsenoside; ginseng flower; *Panax ginseng*; gastroprotective effect; dammarane-type triterpene tetraglycoside; medicinal flower

The flower buds of *Panax* (*P.*) *ginseng* C. A. MEYER (Araliaceae) have been used as an exhilarant and tonic in the form of health tea in traditional Chinese medicine. As the chemical constituents of ginseng flower, several dammarane-type triterpene glycosides were previously isolated,[2,3] but chemical and pharmacological studies on the bioactive constituents from the flower buds of *P. ginseng* were uncharacterized. Recently, we have reported the isolation and structural elucidation of dammarane-type triterpene diglycosides and triglycosides named floralginsenosides A–Lb together with 17 known dammarane-type triterpene glycosides including ginsenoside Rd (**5**) and ginsenoside Re (**6**).[4,5] As a continuing study on the bioactive constituents of medicinal flowers,[1,4—10] the saponin fraction (*n*-BuOH-soluble fraction) and ethyl acetate fraction from the flower buds of *P. ginseng* was found to show protective effects on ethanol-induced gastric mucosal lesions in rats [inhibition (%) at 250 mg/kg, *p.o.*= 63.0, 86.8, respectively]. From the oligoglycoside fraction, new dammarane-type triterpene tetraglycosides termed floralginsenosides M (**1**), N (**2**), O (**3**), and P (**4**) were isolated. This paper deals with the isolation and structural determination of four new floralginsenosides (**1**—**4**) as well as the gastroprotective effects of the principal dammarane-type triterpene glycosides, ginsenoside Rd (**5**, protopanaxadiol 3,20-*O*-bisdesmoside) and ginsenoside Re (**6**, protopanaxatriol 6,20-*O*-bisdesmoside).[11]

Isolation of Floralginsenosides The saponin (*n*-BuOH-soluble) and EtOAc fractions[4] from the methanolic extract of the flower buds of Chinese *P. ginseng* inhibited ethanol-induced gastric mucosal lesions in rats, as shown in Table 1. The saponin fraction was subjected to normal-phase and reverse-phase silica gel column chromatography to give nine fractions. Major fractions 6 and 7 were separated by reverse-phase silica gel column chromatography followed by HPLC to afford floralginsenosides M (**1**, 0.0023% from the dried flower buds), N (**2**, 0.0016%), O (**3**, 0.0055%), and P (**4**, 0.0090%) together with ginsenoside Rd (**5**, 1.3%)[12] and ginsenoside Re (**6**, 2.6%).[13]

Structures of Floralginsenosides Floralginsenoside M (**1**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −2.3° in MeOH). The IR spectrum of **1** showed strong absorption bands at 3470 and 1076 cm^{-1}, suggestive of the oligoglycosidic structure, and a weak band at 1655 cm^{-1} due to an olefin group. In the positive-ion fast atom bombardment (positive-ion FAB)-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1101 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 1077 $(M-H)^-$ together with fragment ion peaks at *m/z* 945 $(M-C_5H_9O_4)^-$ and *m/z* 765 $(M-C_{11}H_{20}O_{10})^-$ which were derived by cleavage of the glycoside linkages of the terminal pentose and pentose-hexose parts. The molecular formula $C_{53}H_{90}O_{22}$ was determined based on high-resolution MS measurement of the quasimolecular ion peak $(M+Na)^+$. Acid hydrolysis of **1** with aqueous hydrochloric acid (HCl,

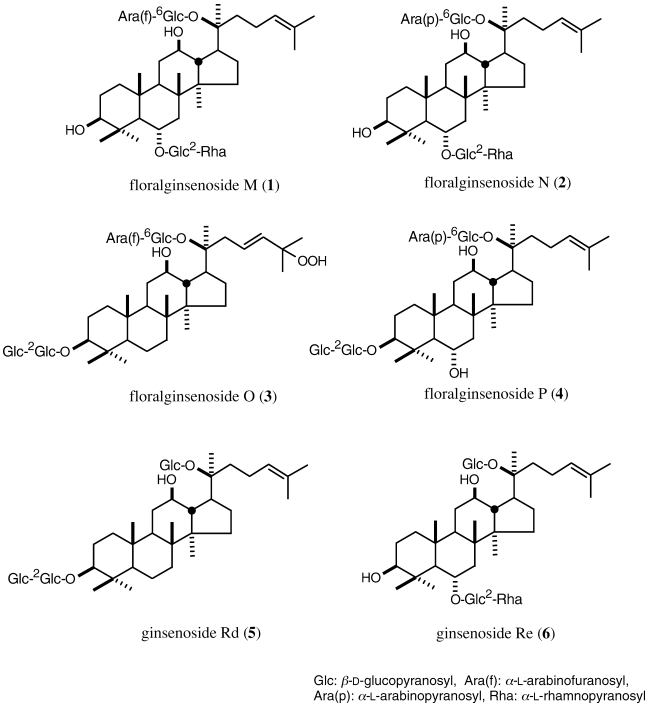

Chart 1. Structures of Floralginsenosides (**1**—**4**) and Ginsenosides Rd (**5**, **6**) from Flower-Buds of *P. ginseng*

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Table 1. Inhibitory Effects of the Saponin Fraction from the Flower of *P. ginseng* on Ethanol-Induced Gastric Mucosal Lesions in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	8	94.5±14.5	—
MeOH ext.	125	5	65.7±12.0	30.5
	250	5	61.6±19.2	34.8
EtOAc fraction	125	6	40.2±7.0*	57.5
	250	7	12.4±4.9**	86.8
Control	—	6	100.2±23.4	—
Saponin fraction	125	6	62.0±16.7	38.2
	250	6	37.0±11.1*	63.0
Control	—	8	94.5±14.5	—
H_2O fraction	500	6	68.5±20.8	27.5

Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

1.0 M) liberated L-arabinose, D-glucose, and L-rhamnose, which were identified in HPLC analysis using an optical rotation detector.[14,15] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned in various NMR experiments,[16] showed signals assignable to an aglycon part [δ 0.95, 0.98, 1.19, 1.35, 1.53, 1.64, 1.68, 2.06 (3H each, all s, H_3-30, 19, 18, 29, 21, 26, 27, 28), 3.44 (1H, br dd, J=3.8, 11.2 Hz, H-3), 4.10 (1H, m, H-12), 4.68 (1H, m, H-6), 5.32 (1H, m, H-24)] together with an α-L-arabinofuranosyl [δ 5.63 (1H, d, J=1.5 Hz, H-1‴)],[17—19] two β-D-glucopyranosyl [δ 5.13 (1H, d, J=7.8 Hz, H-1‴), 5.22 (1H, d, J=6.6 Hz, H-1′)], and an α-L-rhamnopyranosyl [δ 6.42 (1H, s, H-1″)] moieties. The carbon signals of the aglycon part in the ^{13}C-NMR spectra of **1** were found to be superimposable on those of 20(*S*)-protopanaxatriol 6,20-bisdesmosides,[13,20] while the carbon signals due to the 6- and 20-*O*-glycoside moieties were similar to those of ginsenoside Rg_2[21] and -F_5,[22] respectively. The structure of **1** was characterized using ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple-bond correlations (HMBC) experiments. The ^{1}H–^{1}H COSY experiment on **1** indicated the presence of partial structures (bold lines), and the HMBC experiment showed long-range correlations, as shown in Fig. 1. Consequently, the structure of floralginsenoside M (**1**) was determined as shown.

Floralginsenoside N (**2**), obtained as an amorphous powder with positive optical rotation, showed absorption bands at 3450, 1655, and 1076 cm^{-1} assignable to hydroxyl, olefin, and ether functions, respectively, in the IR spectrum. The positive-ion and negative-ion FAB-MS of **2** exhibited quasimolecular ion peaks at *m/z* 1101 $(M+Na)^+$ and *m/z* 1077 $(M-H)^-$, respectively, and fragment ion peaks were observed at *m/z* 945 $(M-C_5H_9O_4)^-$ and *m/z* 765 $(M-C_{11}H_{20}O_{10})^-$ in negative-ion FAB-MS. The molecular formula $C_{53}H_{90}O_{22}$ of **2** was determined from the quasimolecular ion peak and based on high-resolution MS measurement. The acid hydrolysis of **2** liberated L-arabinose, D-glucose, and L-rhamnose, which were identified in HPLC analysis using an optical rotation detector.[14,15] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **2** showed signals assignable to a protopanaxatriol part [δ 0.95, 0.98, 1.19, 1.35, 1.60, 1.64, 1.67, 2.06 (3H each, all s, H_3-30, 19, 18, 29, 21, 26, 27, 28), 3.43 (1H, dd, J=4.8, 11.5 Hz, H-3), 4.11 (1H, m, H-12), 4.63 (1H, m, H-6), 5.34 (1H, m, H-24)], an α-L-arabinopyranosyl [δ 4.97 (1H, d, J=5.7 Hz, H-1‴)], two β-D-glucopyranosyl [δ 5.08 (1H, d, J=7.7 Hz, H-1‴), 5.23 (1H, d-like, J=6.7 Hz, H-1′)], and an α-L-rhamnopyranosyl [δ 6.43 (1H, br s, H-1″)] moieties. The carbon signals in the ^{13}C-NMR spectrum of **2** resembled those of **1**, except for the terminal α-L-arabinopyranosyl moiety of **2**. Furthermore, the ^{1}H–^{1}H COSY and HMBC experiments on **2** showed correlations, as shown in Fig. 1. This evidence led us to formulate the structure of floralginsenoside N (**2**) as shown.

Floralginsenoside O (**3**) was also obtained as an amor-

Table 2. ^{13}C-NMR Data for Floralginsenosides M (**1**), N (**2**), O (**3**), P (**4**)

	1	**2**	**3**	**4**
C-1	39.5	39.5	39.2	39.3
C-2	27.8	27.8	26.8	26.7
C-3	78.8	78.8	89.1	89.5
C-4	40.0	40.0	39.7	40.6
C-5	60.9	61.0	56.5	61.8
C-6	79.4	74.8	18.5	67.6
C-7	45.9	46.0	35.1	47.6
C-8	41.3	41.3	40.1	41.2
C-9	50.0	49.7	50.2	49.9
C-10	39.8	39.8	37.0	38.9
C-11	30.9	30.9	30.9	30.8
C-12	70.2	70.3	70.6	70.3
C-13	49.1	49.2	49.7	49.2
C-14	51.5	51.5	51.5	51.4
C-15	30.8	30.8	30.6	30.8
C-16	26.7	26.7	26.4	26.7
C-17	51.8	51.8	52.1	51.7
C-18	17.5	17.5	16.1	17.6
C-19	17.4	17.3	16.3	17.4
C-20	83.4	83.5	83.2	83.5
C-21	22.4	22.4	23.3	22.4
C-22	36.2	36.2	40.0	36.2
C-23	23.1	23.3	126.7	23.3
C-24	126.1	126.0	138.0	123.8
C-25	131.0	131.1	81.3	131.1
C-26	25.8	25.8	25.1	25.8
C-27	17.8	17.8	25.4	17.9
C-28	32.2	32.2	28.2	31.4
C-29	17.6	17.6	16.6	16.8
C-30	17.3	17.4	17.2	17.5
C-1′	101.8	101.8	105.1	105.3
C-2′	79.0	79.4	83.5	83.5
C-3′	78.2	78.5	78.0	78.0
C-4′	72.7	72.1	72.0	71.9
C-5′	78.2	78.2	78.0	78.1
C-6′	63.3	63.3	62.8	63.0
C-1″	101.9	101.9	106.0	106.0
C-2″	72.3	72.4	77.1	77.0
C-3″	72.4	72.4	78.8	78.5
C-4″	74.2	74.3	71.7	71.8
C-5″	69.5	69.5	78.1	78.1
C-6″	18.7	18.7	62.9	63.0
C-1‴	98.1	98.2	98.2	98.2
C-2‴	75.1	75.0	75.2	75.0
C-3‴	78.2	79.1	78.1	79.2
C-4‴	71.8	71.8	71.8	71.9
C-5‴	76.5	76.7	76.4	76.7
C-6‴	68.5	69.2	68.4	69.2
C-1⁗	110.6	104.5	110.2	104.5
C-2⁗	83.2	72.0	83.2	72.1
C-3⁗	78.5	74.1	78.4	74.1
C-4⁗	86.2	68.4	86.1	68.4
C-5⁗	63.0	65.4	62.8	65.4

Measured in pyridine-d_5 at 125 MHz, 150 MHz.

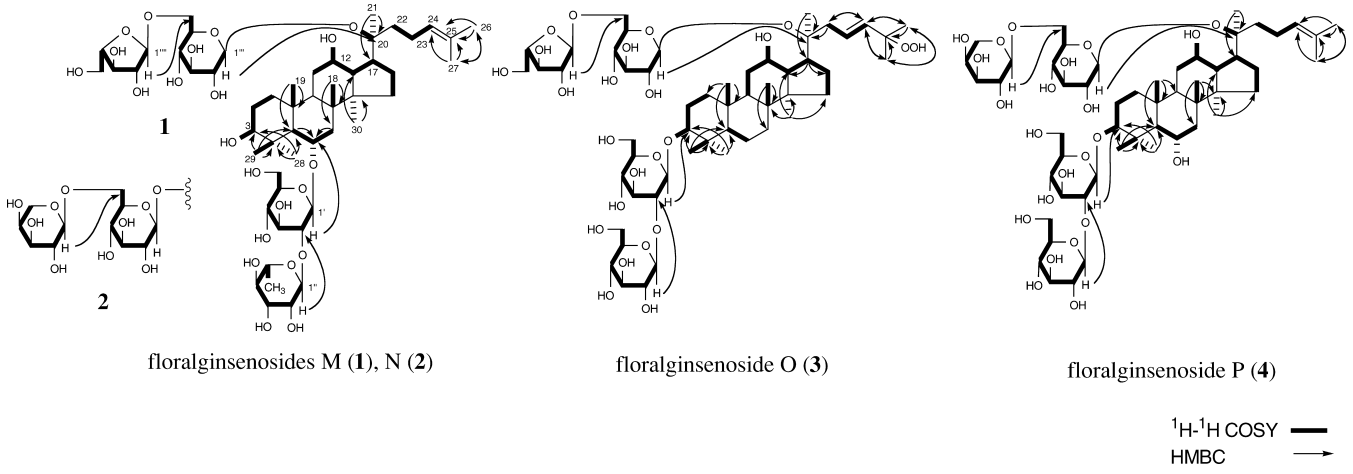

Fig. 1. Significant ^{1}H–^{1}H COSY and HMBC Correlations for Floralginsenosides (**1**—**4**)

phous powder with positive optical rotation and was shown to possess a hydroperoxide group based on its positive response to the *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent.[23] The IR spectrum of **3** showed absorption bands at 3415, 1686, and 1083 cm^{-1} assignable to hydroxyl, olefin, and ether groups, respectively. In positive-ion FAB-MS of **3**, a quasimolecular ion peak was observed at *m/z* 1133 $(M+Na)^+$, whereas negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 1109 $(M-H)^-$ together with fragment ion peaks at *m/z* 1093 $(M-OH)^-$, *m/z* 797 $(M-C_{11}H_{20}O_{10})^-$, and *m/z* 781 $(M-OH-C_{11}H_{20}O_{10})^-$. The molecular formula $C_{53}H_{90}O_{24}$ of **3** was determined based on high-resolution MS measurement. The acid hydrolysis of **3** provided D-glucose and L-arabinose, which were identified in HPLC analysis.[14,15] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **3** showed signals due to an aglycon part [δ 0.85, 0.89, 1.03, 1.10, 1.29, 1.59 (3H each, all s, H_3-19, 30, 18, 29, 28, 21), 1.60 (6H, H_3-26, 27), 3.27 (1H, dd, J=4.5, 11.7 Hz, H-3), 3.96 (1H, m, H-12), 6.10 (1H, d, J=15.8 Hz, H-24), 6.16 (1H, ddd, J=6.4, 7.9, 15.6 Hz, H-23)], together with an α-L-arabinofuranosyl [δ 5.62 (1H, d, J=1.4 Hz, H-1‴)],[17—19] and three β-D-glucopyranosyl moieties [δ 4.90 (1H, d, J=7.6 Hz, H-1′), 5.15 (1H, d, J=7.5 Hz, H-1‴), 5.34 (1H, d, J=7.7 Hz, H-1″)] moieties. The carbon signals in the ^{13}C-NMR spectrum of **3** were superimposable on those ginsenoside Rc,[24] except for the signals due to the side chain part (C-22—C-27), which were similar to those of notoginsenoside-E[25] and -K.[26] On the basis of this evidence and detailed examination of ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1), the structure of floralginsenoside O (**3**) was determined as shown.

Floralginsenoside P (**4**), obtained as an amorphous powder with positive optical rotation, exhibited absorption bands assignable to hydroxyl, olefin, and ether functions, respectively, in the IR spectrum. The molecular formula $C_{53}H_{90}O_{23}$ was determined from the positive-ion and negative-ion FAB-MS [*m/z* 1117 $(M+Na)^+$ and *m/z* 1093 $(M-H)^-$] and in high-resolution MS measurement. The acid hydrolysis of **4** gave D-glucose and L-arabinose.[14,15] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **4** showed signals due to a protopanaxatriol part [δ 0.93, 0.98, 1.07, 1.50, 1.62, 1.63, 1.67, 1.98 (3H each, all s, H_3-19, 30, 18, 29, 21, 26, 27, 28), 3.36 (1H, dd, J=4.6, 11.8 Hz, H-3), 4.13 (1H, m, H-12), 4.32 (1H, m, H-6), 5.32 (1H, m, H-24)] together with an α-L-arabinopyranosyl [δ 4.97 (1H, d, J=5.8 Hz, H-1‴)], and three β-D-glucopyranosyl moieties [δ 4.94 (1H, d, J=7.6 Hz, H-1′), 5.10 (1H, d, J=7.7 Hz, H-1‴), 5.38 (1H, d, J=7.7 Hz, H-1″)] moieties. The carbon signals due to the aglycon part in the ^{13}C-NMR spectra of **4** resembled those of protopanaxatriol glycosides,[27,28] while the signals due to the oligoglycoside moieties were similar to those of protopanaxadiol 3,20-bisdesmosides [ex. ginsenoside Rb_2 (**9**)].[24] The position of the glycoside linkages were determined in an HMBC experiment, which showed long-range correlations between the following protons and carbons: H-1′ and C-3; H-1″ and C-2′; H-1‴ and C-20; H-1⁗ and C-6‴ (Fig. 1). Finally, based on detailed examination of the ^{1}H–^{1}H COSY and HMBC experiments, floralginsenoside P (**4**) was clarified to be the rare 3,20-bisdesmoside of protopanaxatriol.

Protective Effects of Principal Glycoside Constituents (Ginsenoside Rd, Re) on Ethanol- and Indomethacin-Induced Gastric Mucosal Lesions in Rats Previously, we reported that several oleanane-type triterpene saponins,[29—32] steroid saponins,[33] sesquiterpenes,[34,35] phenylpropanoids,[36] amides,[37] and isothiocyanates[38] showed gastroprotective effects in several experimental models. On the other hand, Zhang *et al.* reported that the saponin fraction and a principal constituent, ginsenoside Re (**6**), from the flower of *P. ginseng* showed antiulcer effects on reserpine-induced gastric lesions in rats.[39] However, the antiulcer effects of ginsenoside Rd (**5**) remained uncharacterized. To compare the protective effects of ginsenosides Rd (**5**) and Re (**6**), both which are principal constituents from this flower, the protective effects of **5** and **6** on ethanol- and indomethacin-induced gastric mucosal lesions in rats were also examined. Ginsenoside Rd (**5**, protopanaxadiol 3,20-*O*-bisdesmoside) showed significant protective effects [inhibition (%) at 100 mg/kg, *p.o.*=57.6, 52.1, respectively] on ethanol- and indomethacin-induced gastric lesions in rats (Table 3). The effect of **5** on ethanol-induced gastric lesions was equipotent to that of a reference compound, cetraxate hydrochloride.[33] On the other hand, ginsenoside Re (**6**, protopanaxatriol 6,20-*O*-bisdesmoside) tended to inhibit ethanol-induced gastric lesions in rats [inhibition (%) at 100 mg/kg, *p.o.*=33.0], but not significantly. Furthermore, compound **6** did not inhibit indomethacin-induced gastric lesions. These findings suggest that the princi-

Table 3. Inhibitory Effects of Ginsenosides Rd (**5**) and Re (**6**) from the Flower of *P. ginseng* on Ethanol- or Indomethacin-induced Gastric Mucosal Lesions in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Ethanol-induced				
Control	—	8	137.5±15.8	—
Ginsenoside Rd (**5**)	50	8	86.1±12.6*	37.4
	100	8	58.3±14.9**	57.6
Ginsenoside Re (**6**)	50	8	116.9±12.3	15.0
	100	8	91.7±14.4	33.0
Control	—	6	148.4±9.8	—
Cetraxate hydrochloride[33]	75	6	87.2±7.4**	41.2
	150	6	51.0±4.0**	65.6
Indomethacin-induced				
Control	—	16	108.1±17.3	—
Ginsenoside Rd (**5**)	50	14	76.9±17.9	28.9
	100	14	51.8±8.9*	52.1
Ginsenoside Re (**6**)	50	14	96.8±12.0	10.5
	100	14	91.0±20.1	15.8
Control	—	6	81.3±6.7	—
Cetraxate hydrochloride[33]	75	6	58.7±7.5**	27.8
	150	6	13.4±3.2**	83.5

Values represent the means±S.E.M. Significantly different from the control group, $*p<0.05$, $**p<0.01$.

pal gastroprotective constituent of this flower is ginsenoside Rd (**5**) in our animal models, although the gastroprotective effects of minor constituents of this flower should be studied further.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270-MHz), JNM-LA500 (500-MHz), and JEOL ECA-600K (600-MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68-MHz) JNM-LA500 (125-MHz), and JEOL ECA-600K (150-MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, COSMOSIL-5C$_{18}$-MS-II (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F$_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO$_4$)$_2$–10% aqueous H$_2$SO$_4$ followed by heating.

Plant Material The flower buds of *P. ginseng* were cultivated in Jilin province, China, in November 2005 and identified by one of authors (M. Y.).

Extraction and Isolation The dried flower buds of *P. ginseng* (1.0 kg) were finely cut and extracted four times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the methanolic extract (386 g, 38.6%). The methanolic extract (370 g) was partitioned in an EtOAc–H$_2$O (1 : 1, v/v) mixture, and the aqueous phase was further extracted with *n*-BuOH. Removal of the solvent from the EtOAc-soluble, *n*-BuOH-soluble, and H$_2$O-soluble fractions under reduced pressure yielded 43.3 g (4.3%), 216.7 g (21.7%), and 107.1 g (11.0%) of the residue, respectively.

Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 3.0 kg), CHCl$_3$–MeOH–H$_2$O (50 : 10 : 1—35 : 10 : 1—7 : 3 : 1 lower layer—6 : 4 : 1, v/v/v)–MeOH] of the *n*-BuOH-soluble fraction (134.0 g) gave nine fractions [fr. 1 (0.3 g), 2 (1.1 g), 3 (13.5 g), 4 (13.6 g), 5 (55.0 g), 6 (15.9 g), 7 (12.1 g), 8 (5.9 g), 9 (4.3 g)]. Fraction 4 (13.6 g) was separated by reverse-phase silica gel column chromatography [400 g, MeOH–H$_2$O (30 : 70—40 : 60—50 : 50—60 : 40—70 : 30—80 : 20—90 : 10, v/v)–MeOH] to furnish 10 fractions [fr. 4-1 (0.34 g), fr. 4-2 (0.42 g), fr. 4-3 (0.31 g), fr. 4-4 (0.16 g), fr. 4-5 (1.16 g), fr. 4-6 (0.08 g), fr. 4-7 (0.19 g), fr. 4-8 (1.63 g), fr. 4-9 (0.58 g), fr. 4-10 (6.61 g)]. Fraction 4-5 (0.23 g) was purified by HPLC [MeOH–H$_2$O (55 : 45, v/v)] to give ginsenoside Re (**6**, 56 mg, 0.041%). Fraction 4-10 (0.20 g) was purified by HPLC [MeOH–H$_2$O (75 : 25, v/v)] to give ginsenoside Rd (**5**, 115 mg, 0.57%). Fraction 5 (55.0 g) was separated by reverse-phase silica gel column chromatography [400 g, MeOH–H$_2$O (40 : 60—50 : 50—60 : 40—70 : 30—80 : 20—90 : 10, v/v)–MeOH] to furnish 10 fractions [fr. 5-1 (5.30 g), fr. 5-2 (3.31 g), fr. 5-3 (1.15 g), fr. 5-4 (32.3 g), fr. 5-5 (0.38 g), fr. 5-6 (0.68 g), fr. 5-7 (1.10 g), fr. 5-8 (0.17 g), fr. 5-9 (0.71 g), fr. 5-10 (10.7 g)]. Fraction 5-3 (0.13 g) was purified by HPLC [MeOH–H$_2$O (50 : 50, v/v)] to give ginsenoside Re (**6**, 84 mg, 0.10%). Fraction 5-4 (0.10 g) was purified by HPLC [MeOH–H$_2$O (50 : 50, v/v)] to give ginsenoside Re (**6**, 53 mg, 2.4%). Fraction 5-5 (0.17 g) was purified by HPLC [MeOH–H$_2$O (60 : 40, v/v)] to give ginsenoside Re (**6**, 13 mg, 0.0043%). Fraction 5-9 (0.15 g) was purified by HPLC [MeOH–H$_2$O (75 : 25, v/v)] to give ginsenoside Rd (**5**, 15 mg, 0.010%). Fraction 5-10 (0.16 g) was purified by HPLC [MeOH–H$_2$O (75 : 25, v/v)] to give ginsenoside Rd (**5**, 65 mg, 0.65%). Fraction 6 (15.9 g) was separated by reverse-phase silica gel column chromatography [500 g, MeOH–H$_2$O (40 : 60—50 : 50—60 : 40—70 : 30—80 : 20—90 : 10, v/v)–MeOH] to furnish 12 fractions [fr. 6-1 (0.08 g), fr. 6-2 (1.14 g), fr. 6-3 (0.14 g), fr. 6-4 (0.65 g), fr. 6-5 (0.39 g), fr. 6-6 (0.20 g), fr. 6-7 (0.67 g), fr. 6-8 (1.42 g), fr. 6-9 (0.49 g), fr. 6-10 (9.90 g), fr. 6-11 (1.63 g), fr. 6-12 (0.68 g)]. Fraction 6-6 (0.20 g) was purified by HPLC [MeOH–H$_2$O (50 : 50, v/v)] to give floralginsenoside M (**1**, 16 mg, 0.0023%), N (**2**, 11 mg, 0.0016%). Fraction 6-7 (0.17 g) was purified by HPLC [MeOH–H$_2$O (55 : 45, v/v)] to give ginsenoside Re (**6**, 58 mg, 0.0033%). Fraction 6-9 (0.49 g) was purified by HPLC [MeCN–H$_2$O (30 : 70, v/v)] to give floralginsenoside O (**3**, 11 mg, 0.0055%). Fraction 6-11 (0.32 g) was purified by HPLC [MeOH–H$_2$O (75 : 25, v/v)] to give ginsenoside Rd (**5**, 32 mg, 0.049%). Fraction 7 (12.1 g) was separated by reverse-phase silica gel column chromatography [400 g, MeOH–H$_2$O (40 : 60—50 : 50—60 : 40—70 : 30, v/v)–MeOH] to furnish 12 fractions [fr. 7-1 (0.70 g), fr. 7-2 (0.12 g), fr. 7-3 (0.17 g), fr. 7-4 (0.19 g), fr. 7-5 (0.19 g), fr. 7-6 (0.60 g), fr. 7-7 (1.14 g), fr. 7-8 (0.53 g), fr. 7-9 (1.43 g), fr. 7-10 (5.15 g), fr. 7-11 (0.19 g), fr. 7-12 (0.48 g)]. Fraction 7-8 (0.12 g) was separated by HPLC [MeCN–H$_2$O (27 : 73, v/v)] to give floralginsenoside P (**4**, 14 mg, 0.0090%). Fraction 7-11 (0.19 g) was purified by HPLC [MeOH–H$_2$O (80 : 20, v/v)] to give ginsenoside Rd (**5**, 33 mg, 0.0048%). Fraction 8 (5.9 g) was separated by reverse-phase silica gel column chromatography [400 g, MeOH–H$_2$O (20 : 80—30 : 70—40 : 60—50 : 50—60 : 40—70 : 30, v/v)–MeOH] to furnish 12 fractions [fr. 8-1 (0.99 g), fr. 8-2 (0.19 g), fr. 8-3 (0.04 g), fr. 8-4 (0.04 g), fr. 8-5 (0.11 g), fr. 8-6 (0.02 g), fr. 8-7 (0.03 g), fr. 8-8 (0.11 g), fr. 8-9 (0.92 g), fr. 8-10 (0.67 g), fr. 8-11 (0.64 g), fr. 8-12 (1.10 g)]. Fraction 8-10 (0.17 g) was purified by HPLC [MeOH–H$_2$O (50 : 50, v/v)] to give ginsenoside Re (**6**, 54 mg, 0.029%).

Floralginsenoside M (**1**): A white amorphous powder; $[\alpha]_D^{26}$ −2.3° ($c=0.87$, MeOH); IR (KBr) ν_{max} 3470, 2928, 1655, 1076 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.95, 0.98, 1.19, 1.35, 1.53, 1.64, 1.68, 2.06 (3H each, all s, H$_3$-30, 19, 18, 29, 21, 26, 27, 28), 3.44 (1H, br dd, $J=3.8$, 11.2 Hz, H-3), 4.10 (1H, m, H-12), 4.68 (1H, m, H-6), 5.13 (1H, d, $J=7.8$ Hz, H-1‴), 5.22 (1H, d, $J=6.6$ Hz, H-1′), 5.32 (1H, m, H-24), 5.63 (1H, d, $J=1.5$ Hz, H-1⁗), 6.42 (1H, s, H-1″); ^{13}C-NMR data, see Table 1; positive-ion FAB-MS *m/z* 1101 [M+Na]$^+$; negative-ion FAB-MS *m/z* 1077 [M−H]$^-$, *m/z* 945 [M−C$_5$H$_9$O$_4$]$^-$, *m/z* 765 [M−C$_{11}$H$_{20}$O$_{10}$]$^-$; HR-FAB-MS: *m/z* 1101.5826 (Calcd for C$_{53}$H$_{90}$O$_{22}$Na [M+Na]$^+$, 1101.5826).

Floralginsenoside N (**2**): A white amorphous powder; $[\alpha]_D^{23}$ +0.3° ($c=0.42$, MeOH); IR (KBr) ν_{max} 3450, 2961, 1655, 1076 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.95, 0.98, 1.19, 1.35, 1.60, 1.64, 1.67, 2.06 (3H each, all s, H$_3$-30, 19, 18, 29, 21, 26, 27, 28), 3.43 (1H, dd, $J=4.8$, 11.5 Hz, H-3), 4.11 (1H, m, H-12), 4.63 (1H, m, H-6), 4.97 (1H, d, $J=5.7$ Hz, H-1⁗), 5.08 (1H, d, $J=7.7$ Hz, H-1‴), 5.23 (1H, d-like, $J=6.7$ Hz, H-1′), 5.34 (1H, m, H-24), 6.43 (1H, br s, H-1″); ^{13}C-NMR data, see Table 1; positive-ion FAB-MS *m/z* 1101 [M+Na]$^+$; negative-ion FAB-MS *m/z* 1077 [M−H]$^-$, *m/z* 945 [M−C$_5$H$_9$O$_4$]$^-$, *m/z* 765 [M−C$_{11}$H$_{20}$O$_{10}$]$^-$; HR-FAB-MS: *m/z* 1101.5824 (Calcd for C$_{53}$H$_{90}$O$_{22}$Na [M+Na]$^+$, 1101.5821).

Floralginsenoside O (**3**): A white amorphous powder; $[\alpha]_D^{30}$ +7.8° ($c=0.13$, MeOH); IR (KBr) ν_{max} 3415, 2943, 1686 1083 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.85, 0.89, 1.03, 1.10, 1.29, 1.59 (3H each, all s, H$_3$-19, 30, 18, 29, 28, 21), 1.60 (6H, H$_3$-26, 27), 3.27 (1H, dd, $J=4.5$, 11.7 Hz, H-3), 3.96 (1H, m, H-12), 4.90 (1H, d, $J=7.6$ Hz, H-1′), 5.15 (1H, d, $J=7.5$ Hz, H-1‴), 5.34 (1H, d, $J=7.7$ Hz, H-1″), 5.62 (1H, d, $J=1.4$ Hz, H-

1‴″), 6.03 (1H, d, J=16.0 Hz, H-24), 6.21 (1H, ddd, J=6.5, 8.1, 15.5 Hz, H-23); ^{13}C-NMR data, see Table 1; positive-ion FAB-MS m/z 1133 $[M+Na]^+$; negative-ion FAB-MS m/z 1109 $[M-H]^-$, m/z 1093 $[M-OH]^-$, m/z 797 $[M-C_{11}H_{20}O_{10}]^-$, m/z 781 $[M-OH-C_{11}H_{20}O_{10}]^-$; HR-FAB-MS: m/z 1133.5729 (Calcd for $C_{53}H_{90}O_{24}Na$ $[M+Na]^+$, 1133.5720).

Floralginsenoside P (**4**): A white amorphous powder; $[\alpha]_D^{29}$ +18.4° (c=0.50, MeOH); IR (KBr) ν_{max} 3451, 2943, 1655, 1078 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.93, 0.98, 1.07, 1.50, 1.62, 1.63, 1.67, 1.98 (3H each, all s, H_3-19, 30, 18, 29, 21, 26, 27, 28), 3.36 (1H, dd, J=4.6, 11.8 Hz, H-3), 4.13 (1H, m, H-12), 4.32 (1H, m, H-6), 4.94 (1H, d, J=7.6 Hz, H-1′), 4.97 (1H, d, J=5.8 Hz, H-1‴″), 5.10 (1H, d, J=7.7 Hz, H-1‴), 5.32 (1H, m, H-24), 5.38 (1H, d, J=7.7 Hz, H-1″); ^{13}C-NMR data, see Table 1; positive-ion FAB-MS m/z 1117 $[M+Na]^+$; negative-ion FAB-MS m/z 1093 $[M-H]^-$; HR-FAB-MS: m/z 1117.5775 (Calcd for $C_{53}H_{90}O_{23}Na$ $[M+Na]^+$, 1117.5771).

Acid Hydrolysis of Floralginsenosides M (1), N (2), O (3), and P (4) A solution of **1**—**4** (1 mg each) in HCl 1.0 M (1.0 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical mobile phase, MeCN–H_2O (75 : 25, v/v); flow rate 0.80 ml/min; column temperature, room temperature. Identification of D-glucose, L-arabinose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with that of an authentic sample. t_R: 6.2 min (L-rhamnose, negative optical rotation), 7.8 min (L-arabinose, positive optical rotation), 8.6 min (D-glucose, positive optical rotation), respectively.

Effect on Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats Acute gastric lesions were induced by oral administration of ethanol and indomethacin according to the method described previously.[31,35—38] Briefly, 99.5% ethanol and indomethacin (20 mg/kg, dissolved in 5% sodium bicarbonate, and then diluted in water and neutralized with HCl 0.2 M and adjusted to 1.5 ml/rat) were administered to 24—26-h fasted rats using a metal orogastric tube. One hour after administration of ethanol or 4 h after administration of indomethacin, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 1.5% formalin 10 ml to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature, the lengths of gastric lesions were measured as previously described, and the total length (mm) was expressed as a lesion index.

Statistics Values are expressed as mean±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (p) values less than 0.05 were considered to represent a statistically significant difference.

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