Structures of New Dammarane-Type Triterpene Saponins from the Flower Buds of *Panax notoginseng* and Hepatoprotective Effects of Principal Ginseng Saponins[1]

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The saponin fraction from the flower buds of *Panax notoginseng* exhibited protective effect on liver injury induced by D-galactosamine and lipopolysaccharide. From the saponin fraction with hepatoprotective effect, five new dammarane-type triterpene saponins, notoginsenosides-O (**1**), -P (**2**), -Q (**3**), -S (**4**), and -T (**5**), were isolated together with nine known protopanaxadiol oligoglycosides. The structures of the new saponins were elucidated on the basis of chemical and physicochemical evidence. The principal dammarane-type triterpene saponins from the roots and flower buds of *Panax notoginseng* were found to show potent hepatoprotective effects.

The Araliaceae plant *Panax notoginseng* (Burk.) F. H. Chen is cultivated on a large scale in Yunnan and Guang-Xi provinces of China. The main root of this plant, named notoginseng, is used for treatment of trauma and bleeding due to internal and external injury. As the principal constituents of this medicinal herb, various dammarane-type triterpene saponins were isolated from the roots, leaves, and seeds.[2] We also reported the isolation and structure elucidation of 13 dammarane-type triterpene saponins termed notoginsenosides-A−E and -G−N and an acetylenic fatty acid glycoside called notoginsenic acid β-sophoroside from notoginseng[3−5] and five dammarane-type triterpene saponins called quinquenosides I−V from American ginseng (the roots of *P. quinquefolium*).[6] Furthermore, we characterized immunological adjuvant activities of the principal dammarane-type triterpene saponins from notoginseng and American ginseng.[5]

In a continuing study, we have found that the saponin fraction from the flower buds of *P. notoginseng* showed hepatoprotective effect on liver injury induced by D-galactosamine (D-GalN) and lipopolysaccharide (LPS) in mice (Table 1). The flower buds of *P. notoginseng* have been used for treatment of hypertension, vertigo, tinnitus, and laryngopharyngitis, and several known dammarane-type triterpene saponins were hitherto isolated from the flower buds.[7] We isolated five new protopanaxadiol oligoglycosides called notoginsenosides-O (**1**), -P (**2**), -Q (**3**), -S (**4**), and -T (**5**) from the saponin fraction together with nine known protopanaxadiol oligoglycosides.

In this paper, we describe the isolation and structure elucidation of new saponins (**1**−**5**) from the flower buds of *P. notoginseng* as well as the hepatoprotective effects of the principal dammarane-type triterpene saponins from the flower buds and roots.

Results and Discussion

The methanolic extract from the flower buds of *P. notoginseng* cultivated in Yunnan province of China was partitioned into an ethyl acetate (EtOAc)−water mixture to furnish an EtOAc-soluble fraction and an aqueous phase. The aqueous phase was further extracted with *n*-butanol (*n*-BuOH) to give an *n*-BuOH-soluble fraction and an H_2O-soluble fraction. As shown in Table 1, the *n*-BuOH-soluble fraction (so-called saponin fraction) was found to significantly inhibit the increase of serum ALT and AST levels induced by D-GalN and LPS injection. The saponin fraction was subjected to normal-phase and reversed-phase silica gel column chromatography and finally HPLC to afford notoginsenosides-O (**1**, 0.010% from dried flower buds), -P (**2**, 0.011%), -Q (**3**, 0.014%,), -S (**4**, 0.029%), and -T (**5**, 0.008%) together with ginsenosides-Rb_1 (**6**,[8,9] 0.24%), -Rb_2 (**7**,[9,10] 0.29%), -Rb_3 (**8**,[11] 3.15%), -Rc (**9**,[9,12] 1.79%), and -Rd (**10**,[9,13] 0.010%), gypenosides IX (**11**,[14] 0.11%) and XVII (**12**,[15] 0.18%), and notoginsenosides-D (**13**,[3] 0.009%) and -Fa (**14**,[2e] 0.41%). The EtOAc-soluble fraction was also subjected to normal-phase and reversed-phase silica gel column chromatography and finally HPLC to furnish panaxynol[16] (0.011%), stigmast-7-en-3β-ol 3-*O*-β-D-glucopyranoside[17] (0.0006%), sitosterol 3-*O*-β-D-glucopyranoside[17] (0.0035%), and stigmasterol 3-*O*-β-D-glucopyranoside[17] (0.0047%).

Notoginsenoside-O (**1**) was isolated as colorless fine crystals of mp 196−198 °C from $CHCl_3$−MeOH. The IR spectrum of **1** showed strong absorption bands at 3410 and 1078 cm^{-1}, suggestive of an oligoglycosidic structure, and a weak band at 1655 cm^{-1} ascribable to an olefin function. In the negative-ion FABMS of **1**, a quasimolecular ion peak was observed at *m*/*z* 1047 $[M - H]^-$, while the positive-ion FABMS of **1** showed a quasimolecular ion peak at *m*/*z* 1071 $[M + Na]^+$. The molecular formula $C_{52}H_{88}O_{21}$ was determined by HRFABMS measurement of the quasimolecular ion peak $[M + Na]^+$. Acid hydrolysis of **1** with 0.1 M hydrochloric acid (HCl) liberated D-glucose and D-xylose, which were identified by HPLC analysis using an optical rotation detector.[18] The ^1H NMR (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[19] of **1** showed signals assignable to two β-D-glucopyranosyl moieties [δ 4.89 (1H, d, J = 7.7 Hz, H-1′), 5.09 (1H, d, J = 7.6 Hz, H-1″)] and two β-D-xylopyranosyl moieties [δ 4.97 (1H, d, J = 7.6 Hz, H-1‴), 5.19 (1H, d, J = 7.4 Hz, H-1⁗)]. The carbon signals of the sapogenin part in the ^{13}C NMR of **1** were found to be superimposable on those of 20(*S*)-protopanaxadiol 3,20-bisdesmosides,[2e,3,8−15] while the carbon signals of the glycoside moieties were similar to those of gypenoside IX (**11**),[14] except for the additional signals due to the terminal β-D-xylopyranosyl moiety of **1**. Comparison of the ^{13}C NMR data for **1** with those for **11** indicated the presence of a

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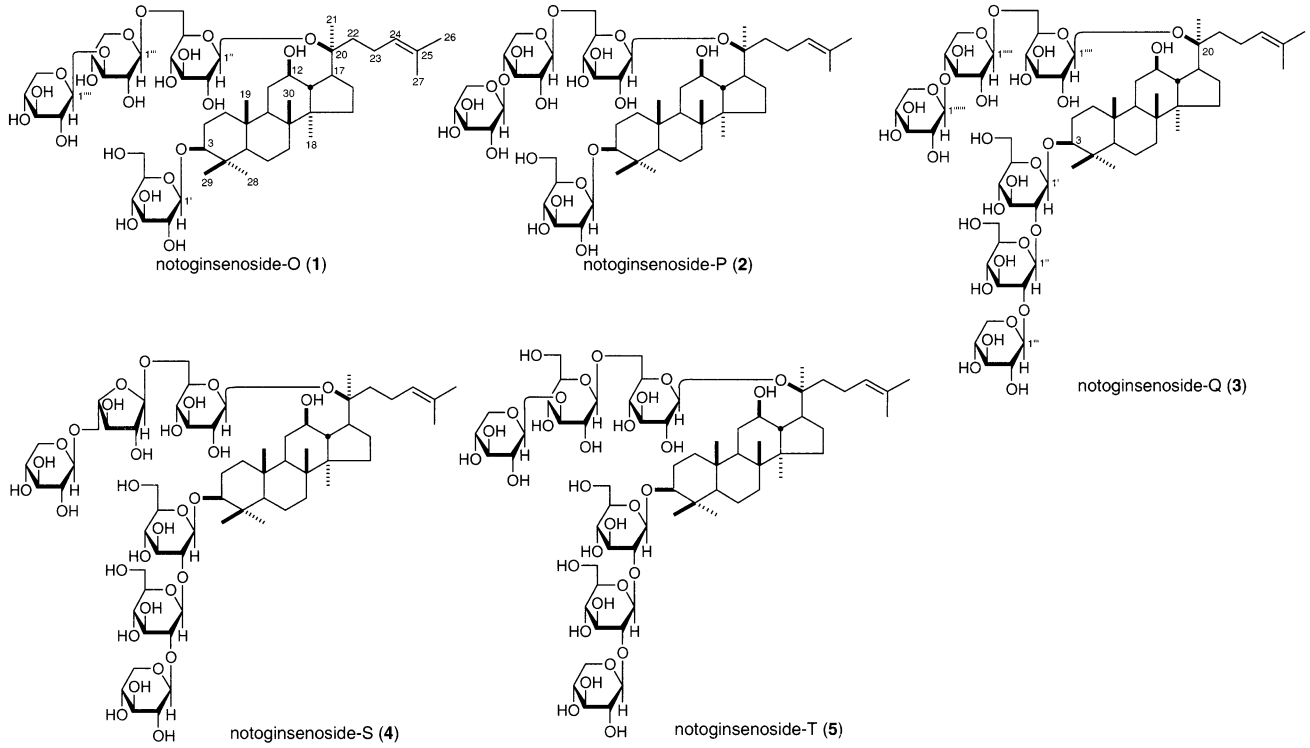
Table 1. Inhibitory Effects of the Saponin Fraction of the Methanolic Extract from the Flower Buds and Roots of *P. notoginseng* on D-GalN/LPS-Induced Liver Injury in Mice[a]

	dose (mg/kg, i.p.)	*N*	sALT		sAST	
			Karmen unit	inhibition (%)	Karmen unit	inhibition (%)
normal		10	16 ± 1**		51 ± 4**	
control		11	8193 ± 1339		6583 ± 1042	
saponin fraction from the flower buds	100	10	1552 ± 322**	81	1760 ± 329**	74
	200	10	867 ± 68**	90	1536 ± 71**	77
saponin fraction from the roots	100	9	2355 ± 833**	71	3018 ± 1371**	55
	200	10	557 ± 153**	93	671 ± 93**	91

[a] Each value represents the mean ± SEM. Significantly different from the control, $^{**}p < 0.01$.

Chart 1. Structures of New Notoginsenosides (**1**−**5**) from the Flower Buds of *P. notoginseng*

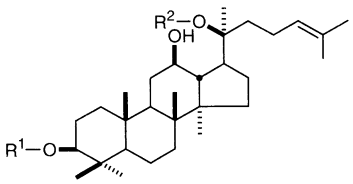


glycosilation shift at the 3‴-position of **1**. The glycoside structures bonding to the 3- and 20-hydroxyl groups in the 20(*S*)-protopanaxadiol part were determined by a heteronuclear multiple bond connectivity (HMBC) experiment on **1**. Namely, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 1⁗-proton and the 3‴-carbon, between the 1‴-proton and the 6″-carbon, and between the 1″-proton and the 20-carbon. On the basis of this evidence, the structure of notoginsenoside-O was characterized as 3-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxadiol 20-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranoside (**1**).

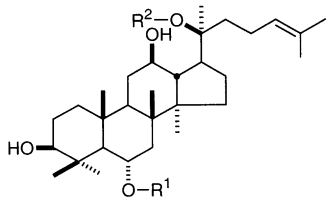
Notoginsenoside-P (**2**) was also isolated as colorless fine crystals of mp 194−196 °C (from $CHCl_3$−MeOH), and its IR spectrum showed absorption bands at 3431, 1684, and 1078 cm^{-1} ascribable to hydroxyl, olefin, and ether functions. In the negative-ion and positive-ion FABMS of **2**, quasimolecular ion peaks were observed at *m*/*z* 1047 $[M - H]^-$ and *m*/*z* 1071 $[M + Na]^+$, respectively. The molecular formula $C_{52}H_{88}O_{21}$, which was the same as that of **1**, was determined by HRFABMS measurement. The acid hydrolysis of **2** liberated D-glucose and D-xylose, which were identified by HPLC analysis using an optical rotation detector.[18] The ^1H NMR (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[19] of **2** showed signals due to two *β*-D-glucopyranosyl moieties [*δ* 4.91 (1H, d-like, H-1′), 5.09 (1H, d, *J* = 7.7 Hz, H-1″)] and two *β*-D-xylopyranosyl moieties [*δ* 4.89 (1H, d-like, H-1⁗), 4.90 (1H, d-like, H-1‴)] together with a 3,20-*O*-glycosyl-20(*S*)-protopanaxadiol part.[2e,3,8−15] The carbon signals in the ^{13}C NMR spectrum of **2** were similar to those of **1**, except for the signals due to the inner *β*-D-xylopyranosyl moiety (the 2‴, 3‴, 4‴, and 5‴-carbons). On comparison of the ^{13}C NMR data for **2** with those for gypenoside IX (**11**),[14] a glycosilation shift was observed around the 4‴-position of **2**. In the HMBC experiment on **2**, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 1⁗-proton and the 4‴-carbon, between the 1‴-proton and the 6″-carbon, and between the 1″-proton and the 20-carbon. Consequently, the structure of notoginsenoside-P was determined as 3-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxadiol 20-*O*-*β*-D-xylopyranosyl(1→4)-*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranoside (**2**).

Notoginsenoside-Q (**3**), which was isolated as colorless fine crystals of mp 194−196 °C (from $CHCl_3$−MeOH), showed absorption bands at 3410, 1655, and 1076 cm^{-1} ascribable to hydroxyl, olefin, and ether functions in the IR spectrum. In the negative-ion and positive-ion FABMS of **3**, quasimolecular ion peaks were observed at *m*/*z* 1341 $[M - H]^-$ and *m*/*z* 1365 $[M + Na]^+$, respectively, and HRFABMS analysis revealed the molecular formula of **3** to be $C_{63}H_{106}O_{30}$. By the acid hydrolysis of **3**, D-glucose and

Chart 2. Structures of Known Dammarane-Type Triterpene Saponins



	R^1	R^2
ginsenoside-Rb_1 (**6**):	Glc(1→2)Glc–	Glc(1→6)Glc–
ginsenoside-Rb_2 (**7**):	Glc(1→2)Glc–	Ara(p)(1→6)Glc–
ginsenoside-Rb_3 (**8**):	Glc(1→2)Glc–	Xyl(1→6)Glc–
ginsenoside-Rc (**9**):	Glc(1→2)Glc–	Ara(f)(1→6)Glc–
ginsenoside-Rd (**10**):	Glc(1→2)Glc–	Glc–
gypenoside IX (**11**):	Glc–	Xyl(1→6)Glc–
gypenoside XVII (**12**):	Glc–	Glc(1→6)Glc–
notoginsenoside-D (**13**):	Xyl(1→2)Glc(1→2)Glc–	Xyl(1→6)Glc(1→6)Glc–
notoginsenoside-Fa (**14**):	Xyl(1→2)Glc(1→2)Glc–	Glc(1→6)Glc–
notoginsenoside-Fc (**15**):	Xyl(1→2)Glc(1→2)Glc–	Xyl(1→6)Glc–



	R^1	R^2
ginsenoside-Re (**16**):	Rha(1→2)Glc–	Glc–
ginsenoside-Rg_1 (**17**):	Glc–	Glc–
ginsenoside-Rh_1 (**18**):	Glc–	H
20-*O*-glucoginsenoside-Rf (**19**):	Glc(1→2)Glc–	Glc–
notoginsenoside-R1 (**20**):	Xyl(1→2)Glc–	Glc–
notoginsenoside-R6 (**21**):	Glc–	*Glc(1→6)Glc–

Glc: β-D-glucopyranosyl, *Glc: α-D-glucopyranosyl, Xyl: β-D-xylopyranosyl, Ara(f): α-L-arabinofuranosyl, Ara(p): α-L-arabinopyranosyl, Rha: α-L-rhamnopyranosyl

D-xylose were identified by HPLC analysis.[18] The ^{1}H NMR (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[19] of **3** showed the presence of three β-D-glucopyranosyl moieties [δ 4.89 (1H, d, J = 7.0 Hz, H-1′), 5.08 (1H, d, J = 7.3 Hz, H-1‴), 5.47 (1H, d, J = 9.1 Hz, H-1″)] and three β-D-xylopyranosyl moieties [δ 4.89 (1H, d, J = 7.0 Hz, H-1⁗), 4.89 (1H, d, J = 7.0 Hz, H-1⁗′), 5.35 (1H, d, J = 6.7 Hz, H-1‴)] together with a 3,20-*O*-glycosyl-20(*S*)-protopanaxadiol part.[2e,3,8−15] The carbon signals of the 20-*O*-triglycosidic moiety in the ^{13}C NMR spectrum of **3** were very similar to those of **2**, while the carbon signals of the 3-*O*-triglycosidic moiety were superimposable on those of notoginsenosids-D (**13**), -Fa (**14**), and -Fc (**15**).[2e] Comparison of the ^{13}C NMR data for **3** with those for **15** indicated a glycosilation shift at the 4⁗′-position of **3**. The oligoglycoside structures at the 3- and 20-positions of **3** were determined by a HMBC experiment, which showed long-range correlations between the following protons and carbons: H-1‴ and C-2″, H-1″ and C-2′, H-1′ and C-3; H-1⁗′ and C-4⁗′, H-1⁗′ and C-6⁗′, H-1⁗′ and C-20. This evidence led us to elucidate the structure of notoginsenoside-Q as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-20(*S*)-protopanaxadiol 20-*O*-β-D-xylopyranosyl(1→4)-β-D-xylopyranosyl(1→6)-β-D-glucopyranoside (**3**).

Notoginsenoside-S (**4**), obtained as colorless fine crystals of mp 186−188 °C (from $CHCl_3$−MeOH), showed absorption bands due to hydroxyl, olefin, and ether functions (3410, 1655, and 1076 cm^{-1}) in the IR spectrum. The molecular formula $C_{63}H_{106}O_{30}$ of **4** was also determined from the quasimolecular ion peaks {m/z 1341 [M − H]$^-$ and m/z 1365 [M + Na]$^+$} in the negative-ion and positive-ion FABMS and by HRFABMS measurement. The acid hydrolysis of **4** liberated L-arabinose, D-glucose, and D-xylose, which were identified by HPLC analysis.[18] The ^{1}H NMR (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[19] of **4** showed the presence of an α-L-arabinofuranosyl moiety [δ 5.59 (1H, br s, H-1⁗′)], three β-D-glucopyranosyl moieties [δ 4.89 (1H, d, J = 7.3 Hz, H-1′), 5.09 (1H, d, J = 6.7 Hz, H-1‴), 5.46 (1H, d, J = 7.6 Hz, H-1″)], and two β-D-xylopyranosyl moieties [δ 4.85 (1H, d, J = 7.6 Hz, H-1⁗″), 5.35 (1H, d, J = 6.4 Hz, H-1⁗)] together with a 3,20-*O*-glycosyl-20(*S*)-protopanaxadiol part.[2e,3,8−15] The carbon signals of the 3-*O*-triglycoside moiety in the ^{13}C NMR spectrum of **4** were superimposable on those of **3**, whereas the carbon signals of the 20-*O*-glycoside structure resembled those of ginsenoside-Rc (**9**),[9,12] except for the additional signals due to the terminal β-D-xylopyranosyl moiety. Comparison of the ^{13}C NMR data for **4** with those for **9** indicated two glycosilation shifts at the 2″- and 5⁗′-positions of **4**. The HMBC experiment of **4** exhibited long-range correlations between the following protons and carbons: H-1⁗ and C-2″, H-1″ and C-2′, H-1′ and C-3; H-1⁗′ and C-5⁗′, H-1⁗′ and C-6⁗′, H-1⁗′ and C-20. Consequently, the structure of notoginsenoside-S was characterized as 3-*O*-β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-20(*S*)-protopanaxadiol 20-*O*-β-D-xylopyranosyl(1→5)-α-L-arabinofuranosyl(1→6)-β-D-glucopyranoside (**4**).

Notoginsenoside-T (**5**), which was obtained as colorless fine crystals of mp 196−198 °C (from $CHCl_3$−MeOH), exhibited absorption bands due to hydroxyl, olefin, and ether functions (3410, 1655, and 1076 cm^{-1}) in the IR spectrum. The molecular formula $C_{64}H_{108}O_{31}$ of **5** was also determined from the quasimolecular ion peaks at m/z 1371 [M − H]$^-$ and m/z 1395 [M + Na]$^+$ in the negative-ion and positive-ion FABMS and by HRFABMS measurement. The acid hydrolysis of **5** liberated D-glucose and D-xylose, which were identified by HPLC analysis.[18] The ^{1}H NMR (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[19] of **5** showed signals assignable to four β-D-glucopyranosyl moieties [δ 4.90 (1H, d, J = 7.3 Hz, H-1′), 5.08 (1H, d, J = 7.0 Hz, H-1⁗′), 5.09 (1H, d, J = 7.3 Hz, H-1⁗″), 5.48 (1H, d, J = 8.9 Hz, H-1‴)] and two β-D-xylopyranosyl moieties [δ 5.22 (1H, d, J = 7.3 Hz, H-1⁗⁗′), 5.36 (1H, d, J = 6.7 Hz, H-1⁗)] together with a 3,20-*O*-glycosyl-20(*S*)-protopanaxadiol part.[2e,3,8−15] The carbon signals in the ^{13}C NMR spectrum of **5** resembled those of notoginsenoside-Fa (**14**),[2e] except for the additional signals due to the terminal β-D-xylopyranosyl moiety. Comparison of the ^{13}C NMR data for **5** with those for **14** indicated a glycosilation shift at the 3⁗′-position of **5**. In the HMBC experiment on **5**, long-range correlations between the following protons and carbons: H-1⁗ and C-2″, H-1″ and C-2′, H-1′ and C-3; H-1⁗′ and C-3⁗′, H-1⁗′ and C-6⁗′, H-1⁗′ and C-20. Consequently, the structure of notoginsenoside-T was elucidated as 3-*O*-β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-20-

Table 2. ^{13}C NMR Data for Notoginsenosides-O (**1**), -P (**2**), -Q (**3**), -S (**4**), and -T (**5**)[a]

	1	2	3	4	5		1	2	3	4	5
C-1	39.3	39.3	39.3	39.3	39.3	C-1′	106.7	106.9	104.7	104.7	104.7
C-2	26.7	26.7	26.7	26.8	26.7	C-2′	75.7	75.8	83.0	83.0	83.0
C-3	88.7	88.9	89.1	89.1	89.1	C-3′	78.6	78.8	77.7	77.7	77.6
C-4	39.6	39.7	39.8	39.8	39.8	C-4′	71.9	72.1	71.7	72.1	71.5
C-5	56.4	56.5	56.5	56.5	56.5	C-5′	78.1	78.3	77.8	77.7	77.9
C-6	18.4	18.5	18.5	18.5	18.5	C-6′	63.1	63.2	63.0	63.0	63.0
C-7	35.1	35.2	35.2	35.2	35.2	C-1″	97.9	98.1	103.2	103.2	103.2
C-8	40.1	40.1	40.1	40.1	40.1	C-2″	75.3	75.0	84.6	84.4	84.6
C-9	50.2	50.3	50.3	50.2	50.3	C-3″	79.2	79.2	78.2	78.2	78.2
C-10	37.0	37.1	37.0	37.0	37.0	C-4″	71.4	71.7	71.2	71.1	70.7
C-11	30.7	30.8	30.9	30.9	29.9	C-5″	76.8	76.6	77.6	77.7	77.7
C-12	70.1	70.2	70.2	69.9	70.2	C-6″	70.1	70.2	63.1	63.1	63.1
C-13	49.5	49.7	49.7	49.5	49.6	C-1‴	105.2	105.5	106.4	106.4	106.4
C-14	51.7	51.5	51.5	51.5	51.4	C-2‴	73.4	73.7	75.6	75.9	75.9
C-15	30.7	31.0	30.8	30.8	30.7	C-3‴	86.2	75.6	79.2	79.2	79.2
C-16	26.8	26.6	26.8	26.9	26.8	C-4‴	70.8	76.8	70.7	70.7	69.9
C-17	51.7	51.7	51.7	51.5	51.7	C-5‴	66.1	64.5	67.4	67.4	67.4
C-18	16.1	16.1	16.1	16.1	16.1	C-1⁗	105.9	103.8	98.1	98.1	98.1
C-19	16.3	16.3	16.3	16.3	16.3	C-2⁗	74.7	74.6	74.9	74.9	74.9
C-20	83.4	83.5	83.5	83.7	83.6	C-3⁗	77.9	77.9	78.7	77.9	78.0
C-21	22.3	22.4	22.3	22.4	22.4	C-4⁗	71.0	71.0	72.0	72.0	72.0
C-22	36.2	36.2	36.2	36.2	36.2	C-5⁗	67.2	67.4	76.6	76.3	77.7
C-23	23.2	23.2	23.2	23.3	23.2	C-6⁗			70.1	68.3	69.7
C-24	125.9	126.1	126.1	126.0	126.1	C-1⁗′			105.5	110.0	104.9
C-25	130.8	131.0	131.0	131.1	131.1	C-2⁗′			73.7	83.4	74.2
C-26	25.8	25.8	25.8	25.8	25.8	C-3⁗′			75.9	78.2	87.6
C-27	18.0	17.9	17.9	17.9	17.5	C-4⁗′			76.8	84.6	71.2
C-28	28.1	28.2	28.1	28.1	28.1	C-5⁗′			64.5	70.3	78.1
C-29	16.8	16.8	16.7	16.7	16.7	C-6⁗′					62.5
C-30	17.5	17.5	17.5	17.4	18.0	C-1⁗″			103.8	105.7	106.3
						C-2⁗″			74.6	75.1	75.4
						C-3⁗″			77.9	77.6	77.1
						C-4⁗″			71.0	71.2	70.9
						C-5⁗″			67.4	67.1	67.3

[a] Measured in pyridine-d_5 at 125 MHz.

(*S*)-protopanaxadiol 20-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**5**).

As part of our studies to characterize the bioactive components of natural medicines,[20−31] we have reported several compounds with *in vivo* and *in vitro* hepatoprotective effects from the roots of *Bupleurum scorzonerifolium*,[20,21] the seeds and fruit of *Hovenia dulcis*,[22] the roots of *Angelica furcijuga*,[23] the bark of *Betula platyphylla* var. *japonica*,[24] the rhizomes of *Curcuma zedoaria*,[25−27] the young seedpods of *Pisum sativum*,[28] the roots and stems of *Salacia reticulata*,[29] the flowers of *Tilia argentea*,[30] and the whole plants of *Anastatica hierochuntica*.[31] Since notoginseng is prescribed as a principal herb in Chinese traditional preparations, as a specific medicine against hepatitis, we examined the effects of the saponin fraction and saponin constituents from the flower buds and roots of *P. notoginseng* on D-GalN/LPS-induced liver injury as a continuous study for hepatoprotective principles. D-GalN/LPS-induced liver injury is recognized to develop from immunological responses.[32] This type of liver injury occurs in two ways: (1) depletion of uridine triphosphate and increased sensitivity of hepatocytes to TNF-α induced by D-GalN; (2) release of pro-inflammatory mediators, such as TNF-α, from LPS-activated macrophages (Kupffer's cells).[33] Apoptosis of hepatocytes induced by TNF-α is reported to be important in D-GalN/LPS-induced liver injury.[33] As shown in Table 3, serum alanine transaminase (sALT) and aspartate transaminase (sAST) were markedly elevated in D-GalN/LPS-treated mice, and hydrocortisone, an inhibitor of activation of macrophages, strongly inhibited the increase in sALT and sAST levels. Principal dammarane-type saponins from the flower buds and roots showed hepatoprotective effects. Particularly, ginsenosides-Rb_3 (**8**), -Rc (**9**), -Rd (**10**), and -Re (**16**) and gypenoside XVII (**12**) were found to exhibit substantial hepatoprotective effects, and also it is interesting to note that the major saponins of the flower buds of *P. notoginseng*, ginsenosides-Rb_3 (**8**) and -Rc (**9**), showed stronger activity than those of the major saponins of the roots of *P. notoginseng* and *P. ginseng*, ginsenosides-Rb_1 (**6**) and -Rg_1 (**17**).

Experimental Section

General Experimental Procedures. 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; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; ^{1}H NMR spectra, JEOL LNM-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL LNM-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-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) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The flower buds of *Panax notoginseng* was purchased in Kunming, Yunnan Province, China, in September 2001, and identified by one of the authors, M.Y. A voucher of the plant is on file in our laboratory (2001.09. Yunnan-33).

Extraction and Isolation. The dried flower buds of *P. notoginseng* (500 g) were finely cut and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the methanolic extract (214

Table 3. Inhibitory Effects of the Principal Saponins from the Flower Buds and Roots of *P. notoginseng* on D-GalN/LPS-Induced Liver Injury in Mice[a]

	dose (mg/kg, i.p.)	*N*	sALT		sAST	
			Karmen unit	inhibition (%)	Karmen unit	inhibition (%)
normal		8	22 ± 1**		76 ± 5**	
control		12	7956 ± 1470		5855 ± 1123	
ginsenoside-Rb_3 (**8**)	50	10	1872 ± 492**	77	1702 ± 388**	72
	100	10	748 ± 106**	91	1144 ± 67**	80
ginsenoside-Rc (**9**)	50	10	1844 ± 794**	77	1244 ± 493	80
	100	10	896 ± 75**	89	813 ± 60**	87
control		20	6406 ± 886		5967 ± 858	
ginsenoside-Rb_1 (**6**)	100	8	4283 ± 1442	33	3591 ± 1076	40
ginsenoside-Rd (**10**)	100	10	227 ± 8**	97	464 ± 17**	93
gypenoside XVII (**12**)	100	9	358 ± 60**	95	617 ± 43**	91
ginsenoside-Re (**16**)	100	7	542 ± 198*	92	649 ± 177*	90
ginsenoside-Rg_1 (**17**)	100	5	4515 ± 1767	30	5272 ± 2169	12
ginsenoside-Rh_1 (**18**)	100	6	470 ± 130*	93	571 ± 101*	90
20-*O*-glucoginsenoside-Rf (**19**)	100	6	4252 ± 1409	34	3628 ± 1240	40
notoginsenoside-R1 (**20**)	100	8	851 ± 312*	87	703 ± 102*	89
notoginsenoside-R6 (**21**)	100	6	4383 ± 1577	32	3383 ± 1180	44
hydrocortisone	20	10	96 ± 16**	99	163 ± 15**	97

[a] Each value represents the mean ± SEM. Significantly different from the control, *$p < 0.05$, **$p < 0.01$.

g, 42.8%). The methanolic extract (213 g) was partitioned in an EtOAc−H_2O (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_2O-soluble fractions under reduced pressure yielded 22.6 g (4.5%), 146.3 g (29.4%), and 44.1 g (8.9%) of the residue, respectively.

Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 2.5 kg), $CHCl_3$−MeOH− H_2O (10:3:1 lower layer−6:4:1, v/v/v)−MeOH] of the *n*-BuOH-soluble fraction (73.6 g) gave six fractions [Fr. 1 (0.8 g), 2 (3.7 g), 3 (10.2 g), 4 (30.3 g), 5 (25.8 g), 6 (2.8 g)]. Fraction 4 (30.0 g) was separated by reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Co., Ltd., 900 g), MeOH−H_2O (70:30, v/v)−MeOH] to furnish five fractions [Fr. 4-1 (0.93 g), Fr. 4-2 (0.83 g), Fr. 4-3 (26.90 g), Fr. 4-4 (1.04 g), Fr. 4-5 (0.30 g)]. Fraction 4-3 (2.79 g) was separated by reversed-phase silica gel column chromatography [85 g, MeOH−H_2O (25:75−30:70, v/v)−MeOH] to give ginsenosides-Rb_1 (**6**, 62 mg, 0.24%), -Rb_3 (**8**, 810 mg, 3.15%), and -Rc (**9**, 460 mg, 1.79%). Fraction 4-4 (1.00 g) was further separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., Kyoto, Japan, 250 × 20 mm i.d.), MeOH−H_2O (80:20, v/v)] to give notoginsenosides-O (**1**, 24 mg, 0.010%) and -P (**2**, 26 mg, 0.011%), ginsenoside-Rd (**10**, 24 mg, 0.010%), and gypenosides IX (**11**, 262 mg, 0.11%) and XVII (**12**, 429 mg, 0.18%). Fraction 5 (25.0 g) was separated by reversed-phase silica gel column chromatography [750 g, MeOH−H_2O (60:40−70:30, v/v)−MeOH] to furnish six fractions [Fr. 5-1 (0.82 g), Fr. 5-2 (0.92 g), Fr. 5-3 (2.75 g), Fr. 5-4 (3.72 g), Fr. 5-5 (15.90 g), Fr. 5-6 (0.89 g)]. Fraction 5-4 (2.00 g) was separated by HPLC [YMC-Pack ODS-A, MeOH-1% aqueous acetic acid (AcOH) (65:35, v/v)] to give notoginsenosides-Q (**3**, 18 mg, 0.014%), -S (**4**, 38 mg, 0.029%), -T (**5**, 10 mg, 0.008%), -D (**13**, 12 mg, 0.009%), and -Fa (**14**, 536 mg, 0.41%). Fraction 5-5 (300 mg) was separated by HPLC [YMC-Pack ODS-A, CH_3CN-1% aqueous AcOH (35:65, v/v)] to give ginsenoside-Rb_2 (**7**, 13 mg, 0.29%).

Normal-phase silica gel column chromatography [600 g, hexane−EtOAc (50:1−25:1−10:1−5:1−1:1, v/v)−EtOAc] of the EtOAc-soluble fraction (15.4 g) gave four fractions [Fr. 1 (3.77 g), 2 (0.42 g), 3 (5.72 g), 4 (5.49 g)]. Fraction 2 (0.42 g) was separated by reversed-phase silica gel column chromatography [750 g, MeOH−H_2O (75:25, v/v)−MeOH] and then HPLC [YMC-Pack ODS-A, MeOH−H_2O (80:20, v/v)] to give panaxynol (39 mg, 0.011%). Fraction 4 (5.49 g) was separated by reversed-phase silica gel column chromatography [170 g, MeOH−H_2O (90:10, v/v)−MeOH] and then HPLC [YMC-Pack ODS-A, MeOH−H_2O (95:5, v/v)] to give stigmast-7-en-3β-ol 3-*O*-β-D-glucopyranoside (2 mg, 0.0006%), sitosterol 3-*O*-β-D-glucopyranoside (12 mg, 0.0035%), and stigmasterol 3-*O*-β-D-glucopyranoside (16 mg, 0.0047%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values.[2e,3,8−15]

Notoginsenoside-O (1): colorless fine crystals (mp 196−198 °C from $CHCl_3$−MeOH); $[\alpha]_D^{28}$ +0.3° (*c* 1.30, MeOH); IR (KBr) ν_{max} 3410, 1655, 1078 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.84, 0.98, 0.98, 0.99, 1.29, 1.63, 1.63, 1.68 (3H each, all s, H_3-19, 18, 30, 29, 28, 26, 21, 27), 4.89 (1H, d, J = 7.7 Hz, H-1′), 4.97 (1H, d, J = 7.6 Hz, H-1‴), 5.09 (1H, d, J = 7.6 Hz, H-1″), 5.19 (1H, d, J = 7.4 Hz, H-1⁗), 5.34 (1H, d-like, H-24); ^{13}C NMR data, see Table 2; positive-ion FABMS *m*/*z* 1071 [M + Na]$^+$; negative-ion FABMS *m*/*z* 1047 [M − H]$^-$; HR-FABMS: *m*/*z* 1071.5708 (calcd for $C_{52}H_{88}O_{21}Na$ [M + Na]$^+$, 1071.5716).

Notoginsenoside-P (2): colorless fine crystals (mp 194−196 °C from $CHCl_3$−MeOH); $[\alpha]_D^{28}$ +2.1° (*c* 1.00, MeOH); IR (KBr) ν_{max} 3431, 1684, 1078 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.98, 0.99, 1.00, 1.29, 1.63, 1.63, 1.67 (3H each, all s, H_3-19, 30, 18, 29, 28, 26, 21, 27), 4.89 (1H, d-like, H-1⁗), 4.90 (1H, d-like, H-1‴), 4.91 (1H, d-like, H-1′), 5.09 (1H, d, J = 7.7 Hz, H-1″), 5.31 (1H, d-like, H-24); ^{13}C NMR data, see Table 2; positive-ion FABMS *m*/*z* 1071 [M + Na]$^+$; negative-ion FABMS *m*/*z* 1047 [M − H]$^-$; HRFABMS: *m*/*z* 1071.5721 (calcd for $C_{52}H_{88}O_{21}Na$ [M + Na]$^+$, 1071.5716).

Notoginsenoside-Q (3): colorless fine crystals (mp 194−196 °C from $CHCl_3$−MeOH); $[\alpha]_D^{28}$ −0.6° (*c* 0.70, MeOH); IR (KBr) ν_{max} 3410, 1655, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.81, 0.96, 0.96, 1.10, 1.27, 1.62, 1.62, 1.66 (3H each, all s, H_3-19, 18, 30, 29, 28, 26, 21, 27), 4.89 (1H, d, J = 7.0 Hz, H-1′), 4.89 (1H, d, J = 7.0 Hz, H-1⁗), 4.89 (1H, d, J = 7.0 Hz, H-1⁗⁗), 5.08 (1H, d, J = 7.3 Hz, H-1‴), 5.31 (1H, d-like, H-24), 5.35 (1H, d, J = 6.7 Hz, H-1‴), 5.47 (1H, d, J = 9.1 Hz, H-1″); ^{13}C NMR data, see Table 2; positive-ion FABMS *m*/*z* 1365 [M + Na]$^+$; negative-ion FABMS *m*/*z* 1341 [M − H]$^-$; HRFABMS *m*/*z* 1365.6681 (calcd for $C_{63}H_{106}O_{30}Na$ [M + Na]$^+$, 1365.6667).

Notoginsenoside-S (4): colorless fine crystals (mp 186−188 °C from $CHCl_3$−MeOH); $[\alpha]_D^{28}$ −8.7° (*c* 1.40, MeOH); IR (KBr) ν_{max} 3410, 1655, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.81, 0.94, 0.96, 1.11, 1.27, 1.61, 1.63, 1.67 (3H each, all s, H_3-19, 30, 18, 29, 28, 26, 21, 27), 4.85 (1H, d, J = 7.6 Hz, H-1⁗⁗), 4.89 (1H, d, J = 7.3 Hz, H-1′), 5.09 (1H, d, J = 6.7 Hz, H-1‴), 5.32 (1H, d-like, H-24), 5.35 (1H, d, J = 6.4 Hz, H-1‴), 5.46 (1H, d, J = 7.6 Hz, H-1″), 5.59 (1H, br s, H-1⁗⁗); ^{13}C NMR data, see Table 2; positive-ion FABMS *m*/*z* 1365 [M + Na]$^+$; negative-ion FABMS *m*/*z* 1341 [M − H]$^-$; HRFABMS *m*/*z* 1365.6653 (calcd for $C_{63}H_{106}O_{30}Na$ [M + Na]$^+$, 1365.6667).

Notoginsenoside-T (5): colorless fine crystals (mp 196−198 °C from $CHCl_3$−MeOH); $[\alpha]_D^{28}$ +6.8° (*c* 1.20, MeOH); IR (KBr) ν_{max} 3410, 1655, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.96, 0.98, 1.11, 1.28, 1.64, 1.64, 1.69 (3H each,

all s, H_3-19, 30, 18, 29, 28, 26, 21, 27), 4.90 (1H, d, $J = 7.3$ Hz, H-1′), 5.08 (1H, d, $J = 7.0$ Hz, H-1⁗), 5.09 (1H, d, $J = 7.3$ Hz, H-1‴), 5.22 (1H, d, $J = 7.3$ Hz, H-1⁗′), 5.34 (1H, d-like, H-24), 5.36 (1H, d, $J = 6.7$ Hz, H-1‴), 5.48 (1H, d, $J = 8.9$ Hz, H-1″); ^{13}C NMR data, see Table 2; positive-ion FABMS *m*/*z* 1395 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1371 $[M - H]^-$; HRFABMS *m*/*z* 1395.6759 (calcd for $C_{64}H_{108}O_{31}Na$ $[M + Na]^+$, 1395.6772).

Acid Hydrolysis of Notoginsenosides-O (1), -P (2), -Q (3), -S (4), and -T (5). A solution of **1**−**5** (3 mg each) in 0.1 M HCl−1,4-dioxane (1:1, v/v, 0.5 mL) was heated under reflux for 2 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 rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN-H_2O (75:25, v/v); flow rate 0.8 mL/min; column temperature, room temperature. Identification of D-xylose, L-arabinose, and D-glucose 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: 9.3 min (D-xylose, positive optical rotation), 10.5 min (L-arabinose, positive optical rotation), and 12.3 min (D-glucose, positive optical rotation), respectively.

Protective Effect on D-GalN/LPS-Induced Liver Injury in Mice. The method described by Tiegs et al.[34] was modified and used for this experiment. Briefly, male ddY mice weighing about 25−27 g were fasted for 20 h before the experiment. The D-GalN (350 mg/kg) and LPS (10 μg/kg, from *Salmonella enteritidis*, Sigma) dissolved in saline were injected intraperitoneally to produce liver injury. Test sample was given intraperitoneally 1 h before D-GalN/LPS injection. Blood samples were collected from the inflaorbital venous plexus 10 h after D-GalN/LPS injection. sALT and sAST were determined by the Reitman-Frankel method (commercial kit, S.TA-Test Wako, Wako Pure Chemical Industries Co. Ltd., Osaka).

Statistics. Values are expressed as means ± SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

References and Notes

(1) This paper is number 21 in our series Bioactive Saponins and Glycosides. For paper number 20, see: Yoshikawa, M.; Morikawa, T.; Nakano, K.; Pongpiriyadacha, Y.; Murakami, T.; Matsuda, H. *J. Nat. Prod.* **2002**, *65*, 1638−1642.

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