Bioactive Saponins and Glycosides. VIII.[1] Notoginseng (1): New Dammarane-Type Triterpene Oligoglycosides, Notoginsenosides-A, -B, -C, and -D, from the Dried Root of *Panax notoginseng* (BURK.) F. H. CHEN

Masayuki YOSHIKAWA,*[,a] Toshiyuki MURAKAMI,[a] Takahiro UENO,[a] Kenichi YASHIRO,[a] Nobuko HIROKAWA,[a] Nobutoshi MURAKAMI,[a] Johji YAMAHARA,[a] Hisashi MATSUDA,[a] Reiko SAIJOH,[b] and Osamu TANAKA[c]

Kyoto Pharmaceutical University,[a] 5, Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan, New Product Development Division, Teikoku Seiyaku Co., Ltd.,[b] 9–19, Nihonbashi, Tomizawa-cho, Chuo-ku, Tokyo 103, Japan and Suzugamine Women's College,[c] 6–18, Inokuchi 4-chome, Nishi-ku, Hiroshima 733, Japan.
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The glycosidic fraction from the dried roots of *Panax notoginseng* (BURK.) F. H. CHEN was found to show protective effect on liver injury induced by D-galactosamine and lipopolysaccharide. From the glycosidic fraction with hepatoprotective effect, nine new dammarane-type triterpene oligoglycosides, notoginsenosides-A, -B, -C, -D, -E, -G, -H, -I, and -J and an acetylenic fatty acid glycoside, notoginsenic acid β-sophoroside, were isolated together with fourteen known dammarane-type triterpene oligoglycosides. The structures of notoginsenosides-A, -B, -C, and -D were determined on the basis of chemical and physicochemical evidence, which included the chemical correlation with ginsenoside-Rb_1 using photosensitized oxygenation, as follows: notoginsenoside A; 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*),25-tetrahydroxydammar-23-ene, B; 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*)-trihydroxydammar-25-en-24-one, C; 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*)-trihydroxy-24ξ-hydroperoxydammar-25-ene, and D; 3-*O*-[β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 20(*S*)-protopanaxadiol, respectively.

Key words notoginsenoside; *Panax notoginseng*; liver injury protective; bioactive saponin; dammarane-type triterpene oligoglycoside; notoginseng

Notoginseng, which is also called sanqi ginseng (or sanchi ginseng, 三七人参) and tienqi ginseng (or tienchi ginseng, 田七人参) in Chinese, is prepared by various processing methods from the main root with a small rhizome of *Panax notoginseng* (BURK.) F. H. CHEN.[2] Notoginseng has been prescribed in several Chinese formulas including "Yunnan Bai Yao (雲南白薬)," which is used for treatment of trauma and bleeding due to internal and external injury, and "Pien Tze Huang (片仔廣)," which is known as a specific medicine against hepatitis. Extensive chemical study was made on the constituents of notoginseng to determine its bioactive principles, and saponins, flavonoids, polysaccharides, and amino acids were isolated.[3,4] The saponin constituents, which are the principal ingredients of notoginseng, have been the subjects of many investigations and various dammarane-type triterpene oligoglycosides have been characterized from the roots, leaves, and buds of *P. notoginseng*.[4]

In the course of our studies to find bioactive saponins from natural medicines[1,5] and medicinal foodstuffs,[6] we have found a number of oleanane-type and dammarane-type triterpene oligoglycosides having an inhibitory effect on alcohol absorption, antiinflammatory, hypoglycemic, and antiallergic activities. In a continuing study, we have found that the glycosidic fraction obtained from the dried roots of *P. notoginseng* showed remarkable protective effect on liver injury induced by D-galactosamine and lipopolysaccharide. From the glycosidic fraction with the protective effect, nine new dammarane-type triterpene oligoglycosides, notoginsenosides-A (**1**), -B (**2**), -C (**3**), -D (**4**), -E, -G, -H, -I, and -J, and an acetylenic fatty acid glycoside, notoginsenic acid β-sophoroside, were isolated together with fourteen known dammarane-type triterpene oligoglycosides. This paper describes the hepatoprotective effect of the glycoside fraction from the dried roots of *P. notoginseng* and the structure elucidation of notoginsenosides-A (**1**), -B (**2**), -C (**3**), and -D (**4**).[7]

The glycosidic constituents of the dried roots of *P. notoginseng* cultivated in Yunnan Province, China, were separated through the procedure shown in Chart 1. Thus, the methanolic extract from the dried root was partitioned into an ethyl acetate–water mixture and the water phase was further extracted with 1-butanol. As shown in Table 1, the 1-butanol-soluble portion (so-called glycosidic fraction) was found to significantly inhibit the increase of serum GOT and GPT level induced by D-galactosamine and lipopolysaccharide injection, while this fraction showed only weak protective effect on CCl_4-induced liver injury in mice (Table 2). The 1-butanol-soluble portion was subjected to ordinary and reversed-phase silica-gel column chromatography and finally HPLC to afford notoginsenosides-A (**1**, 0.0065%), -B (**2**, 0.0042%), -C (**3**, 0.0056%), -D (**4**, 0.0038%), -E (0.0022%), -G (0.0016%), -H (0.0015%), -I (0.0047%), and -J (0.0009%) and notoginsenic acid β-sophoroside (0.007%). Furthermore, notoginsenoside-K[8] (**5**, 0.0042%), quinquenoside-R1[9] (**13**, 0.0023%), ginsenosides-Ra_3[10] (**6**, 0.026%) and -F1[11] (**14**, 0.0043%) were first isolated from notoginseng to-

* To whom correspondence should be addressed.

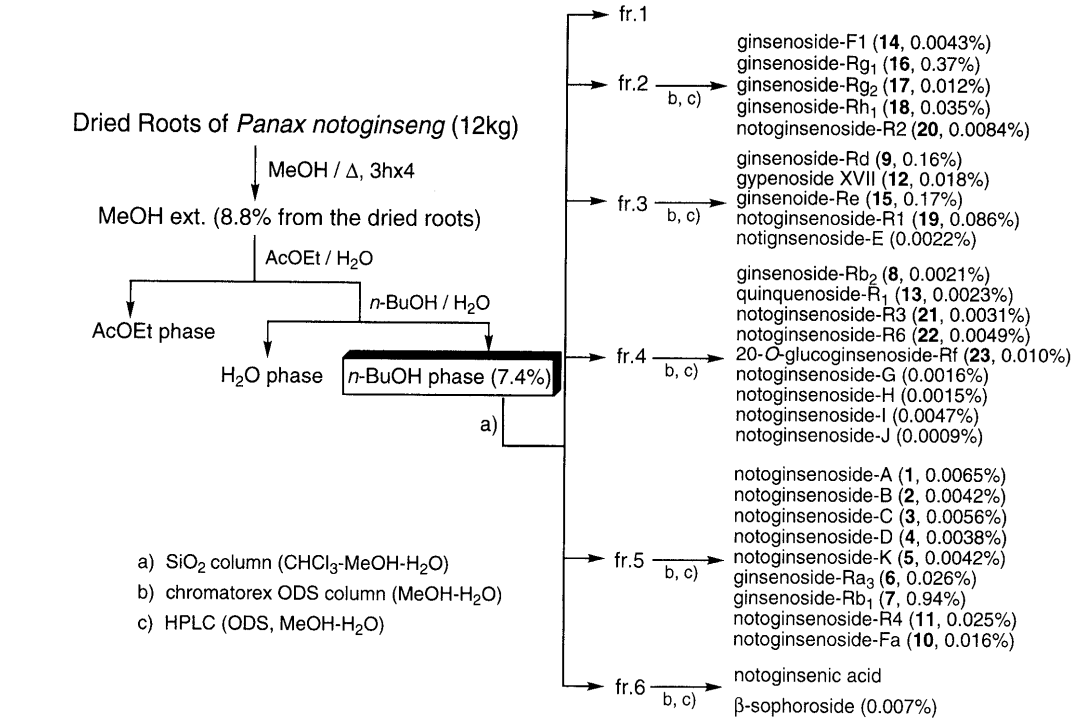
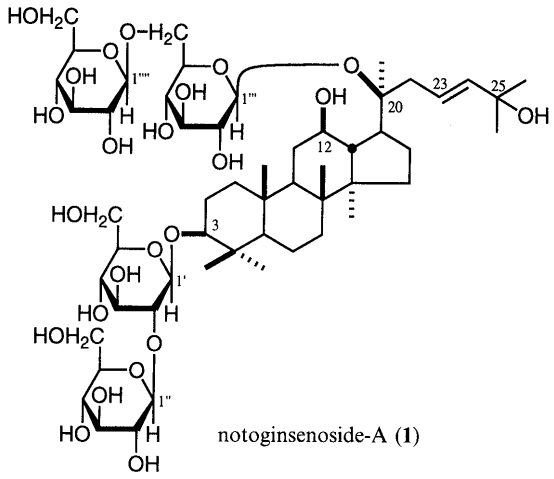
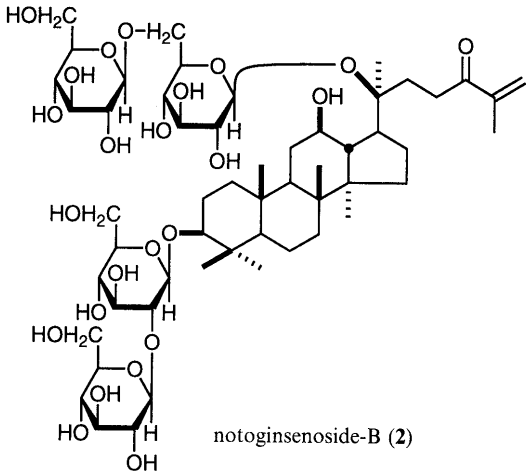
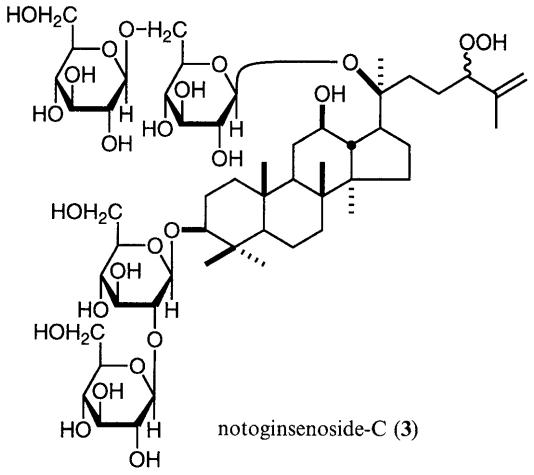


Chart 1


notoginsenoside-A (**1**)


notoginsenoside-B (**2**)


notoginsenoside-C (**3**)

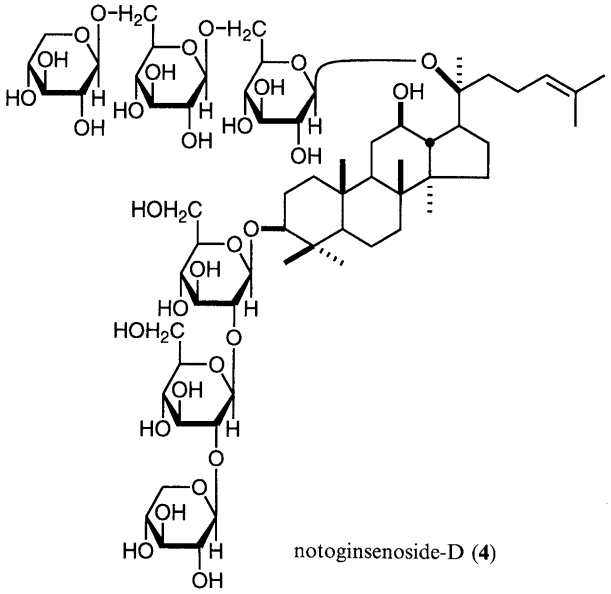
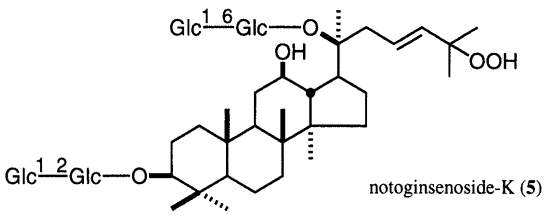
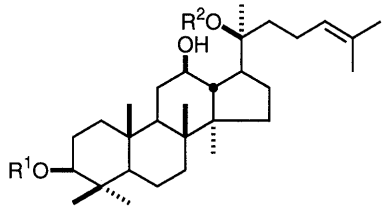

notoginsenoside-D (**4**)

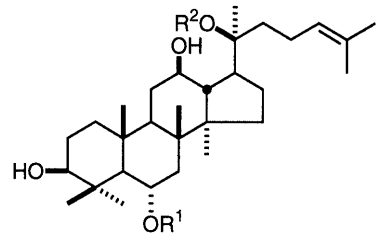
Chart 2



notoginsenoside-K (**5**)



	R^1	R^2
ginsenoside-Ra_3 (**6**)	-Glc 2—1 Glc	-Glc 6—1 Glc 3—1 Xyl
ginsenoside-Rb_1 (**7**)	-Glc 2—1 Glc	-Glc 6—1 Glc
ginsenoside-Rb_2 (**8**)	-Glc 2—1 Glc	-Glc 6—1 Ara
ginsenoside-Rd (**9**)	-Glc 2—1 Glc	-Glc
notoginsenoside-Fa (**10**)	-Glc 2—1 Glc 2—1 Xyl	-Glc 6—1 Glc
notoginsenoside-R4 (**11**)	-Glc 2—1 Glc	-Glc 6—1 Glc 6—1 Xyl
gypenoside XVII (**12**)	-Glc	-Glc 6—1 Glc
quinquenoside-R1 (**13**)	-Glc 2—1 Glc 6—Ac	-Glc 6—1 Glc



	R^1	R^2
ginsenoside-F1 (**14**)	-H	-Glc
ginsenoside-Re (**15**)	-Glc 2—1 Rha	-Glc
ginsenoside-Rg_1 (**16**)	-Glc	-Glc
ginsenoside-Rg_2 (**17**)	-Glc 2—1 Rha	-H
ginsenoside-Rh_1 (**18**)	-Glc	-H
notoginsenoside-R1 (**19**)	-Glc 2—1 Xyl	-Glc
notoginsenoside-R2 (**20**)	-Glc 2—1 Xyl	-H
notoginsenoside-R3 (**21**)	-Glc	-Glc 6—1 Glc
notoginsenoside-R6 (**22**)	-Glc	-Glc 6—1 Glc*
20-*O*-glucoginsenoside-Rf (**23**)	-Glc 2—1 Glc	-Glc

Glc : β-D-glucopyranosyl, Glc* : α-D-glucopyranosyl
Xyl : β-D-xylopyranosyl, Rha : α-L-rhamnopyranosyl
Ara : α-L-arabinopyranosyl

Chart 3

Table 1. Inhibitory Effect of the Glycosidic Fraction from the Dried Roots of *Panax notoginseng* on D-Galactosamine (D-GalN)/Lipopolysaccharide (LPS)

	Dose (mg/kg, i.p.)	*n*	Serum GPT (Karmen unit)	Serum GOT (Karmen unit)
Normal (saline)	—	5	14±1**	47±1**
Control (D-GalN/LPS)	—	11	8193±1339	6583±1042
Saponin fraction	100	9	2355±833**	3018±1371*
	200	10	557±153**	671±93**
Hydrocortisone	20	9	416±269**	391±188*

* $p<0.05$, ** $p<0.01$ *vs.* Control.

Table 2. Inhibitory Effect of the Glycosidic Fraction from the Dried Roots of *Panax notoginseng* on CCl_4-Induced Liver Injury in Mice

	Dose (mg/kg, i.p.)	*n*	Serum GPT (Karmen unit)	Serum GOT (Karmen unit)
Normal (saline)	—	5	21±3**	65±6**
Control (CCl_4)	—	12	5288±942	5394±811
Saponin fraction	100	11	3509±1038	3379±970
	200	11	3996±955	3814±922
Malotilate	100	9	73±10**	113±5**

** $p<0.01$ *vs.* Control.

gether with ginsenosides-Rb_1[10,12] (**7**, 0.94%), -Rb_2[12,13] (**8**, 0.0021%), -Rd[12,14] (**9**, 0.16%), -Re[14,15] (**15**, 0.17%), -Rg_1[16] (**16**, 0.37%), -Rg_2[11,15] (**17**, 0.012%), and -Rh_1[16b] (**18**, 0.035%), 20-*O*-glucoginsenoside-Rf[13,17] (**23**, 0.010%), notoginsenosides-Fa[8] (**10**, 0.016%), -R1[4d] (**19**, 0.086%), -R2[4d] (**20**, 0.0084%), -R3[4g] (**21**, 0.0031%), -R4[4g] (**11**, 0.025%), and -R6[4g] (**22**, 0.0049%), and gypenoside XVII[18] (**12**, 0.018%).

Notoginsenosides-A (1), -B (2), and -C (3) Notoginsenoside-A (**1**) was obtained as colorless fine crystals of mp 197—200 °C from aqueous methanol. The IR spectrum of **1** showed strong absorption bands at 3432 and 1072 cm^{-1} suggestive of the oligoglycosidic structure. In the negative-mode FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1123 $(M-H)^-$, while two quasimolecular ion peaks were observed at *m/z* 1147 $(M+Na)^+$ and *m/z* 1169 $(M+2Na-H)^+$ in the positive-mode FAB-MS. The molecular formula $C_{54}H_{92}O_{24}$ was determined by high-resolution MS measurement of the quasimolecular ion peak $(M+Na)^+$. Methanolysis of **1** with 9% hydrogen chloride in dry methanol furnished a methyl glucoside. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[19] of **1** showed the presence of a β-sophorosyl part [δ 4.92 (d, $J=7.6$ Hz, 1′-H), 5.37 (d, $J=7.3$ Hz, 1″-H)] and a β-gentiobiosyl part [δ 5.18 (d, $J=7.6$ Hz, 1‴-H), 5.10 (d, $J=7.6$ Hz, 1⁗-H)]. The carbon signals in the ^{13}C-NMR spectra of **1** were found to be superimposable on those of ginsenoside-Rb_1 (**7**), except for the signals due to the side chain part (C-22—C-27) of the sapogenol moiety. The disaccharide structures bonding to the 3- and 20-positions of the sapogenol moiety were characterized by means of a heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-

range correlations were observed between the 1″-proton and the 2′-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. The side chain part of the sapogenol moiety was also determined by the HMBC experiment, which showed long-range correlations between the 23-proton [δ 6.19 (1H, ddd-like)] and the 22-carbon, between the 24-proton [δ 6.08 (d, J=15.6 Hz)] and the 25-carbon, and between the 26, 27-methyl protons [δ 1.55 (6H, s)] and 24, 25-carbons. Finally, the structure of notoginsenoside-A was determined by the chemical derivation from ginsenoside-Rb_1 (**7**) by photosensitized oxygenation in the presence of Rose Bengal (*vide infra*) as 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*),25-tetrahydroxydammar-23-ene (**1**).

Notoginsenoside-B (**2**) was also obtained as colorless fine crystals of mp 201—204 °C. The IR spectrum of **2** showed absorption bands due to hydroxyl and enone functions at 3410, 1655, 1638, and 1078 cm^{-1}, while the enone absorption was observed at 216 nm in its UV spectrum. The negative and positive-mode FAB-MS of **2** showed quasimolecular ion peaks at m/z 1121 $(M-H)^-$ and m/z 1145 $(M+Na)^+$, respectively, and the molecular formula $C_{54}H_{90}O_{24}$ was determined by high-resolution MS measurement. The methanolysis of **2** yielded methyl glucoside. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[19] of **2** showed a β-sophorosyl part [δ 4.93 (d, J=7.6 Hz, 1′-H), 5.38 (d, J=7.6 Hz, 1″-H)] and a β-gentiobiosyl part [δ 5.11 (d, J=7.3 Hz, 1‴-H), and 5.03 (d, J=7.2 Hz, 1⁗-H)], an exo-methylene [δ 5.77, 6.37 (both s, 26-H_2)], and a vinyl methyl group [δ 1.83 (s)]. The carbon signals in the ^{13}C-NMR (Table 3) of **2** were very similar to those of **1** and **7**, except for the signals due to the side chain part of the sapogenol moiety. In the HMBC experiment of **2**, long-range correlations were observed between the following protons and carbons [1″-H and 2′-C, 1′-H and 3-C, 1⁗-H and 6‴-C, 1‴-H and 20-C, 26-H_2 and 24, 25-C, 27-H_3 and 24, 25-C]. Notoginsenoside-B (**2**) was also synthesized by the photosensitized oxygenation of ginsenoside-Rb_1 (**7**) (*vide infra*). Consequently, the structure of notoginsenoside-B was characterized as 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*)-trihydroxydammar-25-en-24-one (**2**).

Notoginsenoside-C (**3**), isolated as colorless fine crystals of mp 199—202 °C, has a hydroperoxyl residue as shown by its positive response to the *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent and the ferrous thiocyanate reagent.[20] The IR spectrum of **3** showed hydroxyl and olefin absorption bands and the molecular formula $C_{54}H_{92}O_{25}$ was determined from its negative and positive-mode FAB-MS [m/z 1139 $(M-H)^-$, m/z 1163

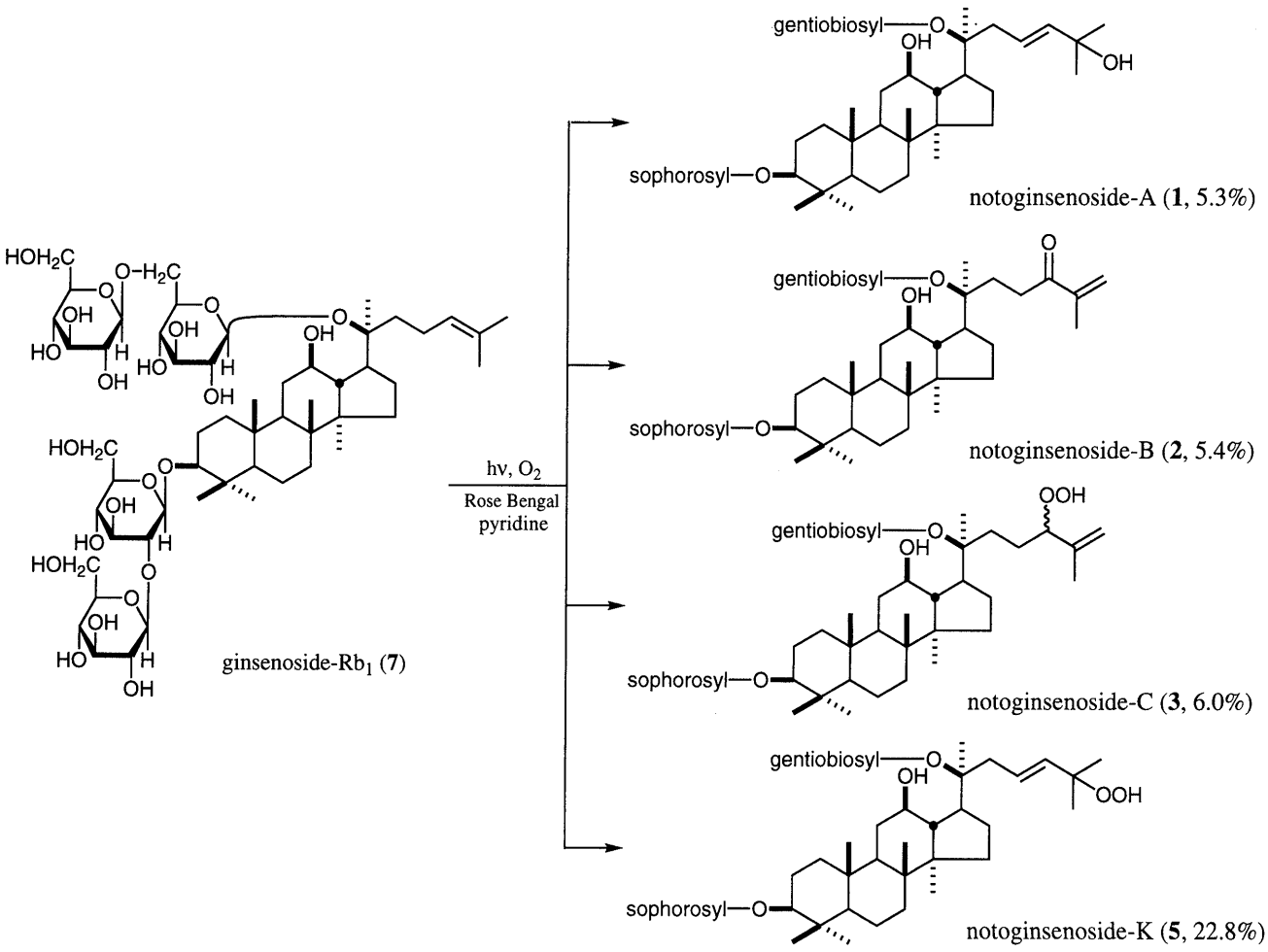


Chart 4. Photosensitized Oxygenation of Ginsenoside-Rb_1 (**7**)

Table 3. ^{13}C-NMR Data for Notoginsenosides-A (**1**), -B (**2**), -C (**3**), and -D (**4**) (125 MHz, Pyridine-d_5)

	1	2	3	4		1	2	3	4
C-1	39.2	39.2	39.2	39.2	Glc-1′	105.1	105.6	105.1	105.2
C-2	26.8	26.8	26.8	26.7	2′	83.3	83.5	83.3	83.1
C-3	89.1	89.0	89.0	89.0	3′	78.3	78.4	78.3	78.7
C-4	39.7	39.7	39.7	39.8	4′	71.7	71.7	71.6	71.6
C-5	56.4	56.4	56.4	56.5	5′	78.1	78.1	78.2	78.7
C-6	18.5	18.4	18.5	18.5	6′	62.8	62.8	62.8	63.3
C-7	35.1	35.1	35.1	35.2	Glc-1″	106.0	106.0	106.0	103.5
C-8	40.1	40.0	40.0	40.1	2″	77.1	77.1	77.1	85.0
C-9	50.1	50.2	50.2	50.3	3″	77.9	78.0	78.1	78.1
C-10	36.9	36.9	36.9	36.9	4″	71.8	71.7	71.7	72.3
C-11	30.8	30.8	30.9	30.8	5″	78.2	78.3	78.3	78.3
C-12	70.0	70.2	70.2	70.2	6″	62.7	62.7	62.7	63.3
C-13	49.5	49.5	49.4	49.5	Glc (Xyl)-1‴	98.1	98.0	98.1	106.8
C-14	51.5	51.5	51.4	51.4	2‴	75.1	74.8	74.8	76.3
C-15	30.6	30.7	30.8	30.8	3‴	78.7	79.4	79.1	79.6
C-16	26.4	26.7	26.7	26.7	4‴	71.6	71.3	71.5	72.0
C-17	52.1	52.1	51.7	51.6	5‴	77.1	76.7	77.0	67.8
C-18	16.0	16.0	16.0	16.0	6‴	70.5	70.3	70.0	
C-19	16.3	16.3	16.3	16.3	Glc-1⁗	104.8	105.1	105.2	98.5
C-20	83.4	83.2	83.4	83.5	2⁗	75.2	75.2	75.2	75.3
C-21	23.3	21.9	22.6	22.4	3⁗	78.3	78.5	78.4	78.3
C-22	39.7	32.8	32.6	36.3	4⁗	71.6	71.8	71.8	72.0
C-23	122.7	29.8	26.3	23.2	5⁗	78.3	78.3	78.3	77.4
C-24	142.3	202.2	90.0	126.0	6⁗	62.9	62.9	62.9	70.8
C-26	70.0	144.4	146.0	131.0	Glc-1′′′′′				105.8
C-26	30.6[a]	17.8	113.5	25.8	2′′′′′				75.5
C-27	30.7[a]	125.6	17.6	18.0	3′′′′′				78.7
C-28	28.1	28.1	28.1	28.1	4′′′′′				71.6
C-29	16.6	16.6	16.6	16.7	5′′′′′				77.4
C-30	17.2	17.4	17.4	17.5	6′′′′′				70.3
					Xyl-1′′′′′′				106.3
					2′′′′′′				75.3
					3′′′′′′				79.1
					4′′′′′′				71.2
					5′′′′′′				67.5

a) May be interchangeable.

$(M+Na)^+$, and *m/z* 1185 $(M+2Na-H)^+$] and by high-resolution MS measurement. By the methanolysis of **3**, a methyl glucoside was obtained. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[19] of **3** showed signals assignable to a β-sophorosyl part [δ 4.91 (d, $J=7.3$ Hz, 1′-H), 5.36 (d, $J=7.6$ Hz, 1″-H)] and a β-gentiobiosyl part [δ 5.09 (d-like, 1‴-H), and 5.10 (d, $J=7.2$ Hz, 1⁗-H)], exo-methylene [δ 5.10, 5.30 (both m, 26-H_2)], vinyl methyl [δ 1.96 (s, 27-H_3)], and a methine proton bearing a hydroperoxyl group[12] [δ 4.79 (dd-like, 24-H)]. The carbon signals in the ^{13}C-NMR (Table 3) spectra of **3** closely resembled those of **1**, **2**, and **7**, except for a few signals due to the side chain part of the sapogenol moiety. In the HMBC experiment of **3**, long-range correlations were observed between the following protons and carbons [1″-H and 2′-C, 1′-H and 3-C, 1⁗-H and 6‴-C, 1‴-H and 20-C, 22-H_2 and 23, 24-C, 24-H and 25-C, 26-H_2 and 24, 25, 27-C, 27-H_3 and 24, 25, 26-C]. This evidence led us to presume that **3** was an oxygenated derivative of **7**. To verify this presumption, **7** was subjected to the photosensitized oxygenation in the presence of Rose Bengal in a Pyrex tube. The products were separated by reversed-phase silica-gel column chromatography and HPLC to give notoginsenosides-A (**1**, 5.3%), -B (**2**, 5.4%), -C (**3**, 6.0%), and -K (**5**, 22.4%). Thus, the structure of notoginsenoside-C is now known to correspond to **3** {3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 3β,12β,20(*S*)-trihydroxy-24ξ-hydroperoxydammar-25-ene}, except for its C-24 configuration.

Notoginsenoside-D (4) Notoginsenoside-D (**4**) was isolated as colorless fine crystals of mp 207—210 °C and the IR spectrum showed absorption bands assignable to hydroxyl group. Here again, the molecular formula $C_{64}H_{108}O_{31}$ of **4** was determined from the quasimolecular ion peaks observed in the negative and positive-mode FAB-MS and by high-resolution MS measurement. Namely, quasimolecular ion peaks were observed at *m/z* 1395 $(M+Na)^+$ and *m/z* 1417 $(M+2Na-H)^+$ in the positive-mode FAB-MS of **4**, while the negative-mode FAB-MS showed the quasimolecular ion peak at *m/z* 1371 $(M-H)^-$ in addition to fragment ion peaks at *m/z* 1237 $(M-C_5H_9O_4)^-$ and *m/z* 1106 $(M-C_{10}H_{18}O_8)^-$. The methanolysis of **4** liberated a methyl glucoside and a methyl xyloside in a 2:1 ratio. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[19] of **4** showed signals assignable to an 20(*S*)-protopanaxadiol moiety, four β-D-glucopyranosyl parts [δ 5.52 (d, $J=7.2$ Hz, 1′-H), 4.92 (d, $J=7.9$ Hz, 1″-H), 5.12 (d, $J=7.6$ Hz, 1⁗-H), and 5.02 (d, $J=7.6$ Hz, 1′′′′′-H)] and two β-D-xylopyranosyl parts [δ 5.38 (d, $J=7.0$ Hz, 1‴-H) and 4.94 (d, $J=8.5$ Hz, 1′′′′′′-H)]. The oligosaccharide structures bonding to the

3 and 20-positions of the 20(*S*)-protopanaxadiol moiety were characterized by HMBC experiment, and exhibited long-range correlations between the following protons and carbons [1‴-H and 2″-C, 1″-H and 2′-C, 1′-H and 3-C, 1⁗‴-H and 6⁗‴-C, 1⁗‴-H and 6⁗″-C, 1⁗″-H and 20-C]. Finally, comparison of the ^{13}C-NMR data for **4** with those for notoginsenosides-Fa (**10**)[8] and -R4 (**11**)[4g] led us to formulate the structure of notoginsenoside-D as 3-*O*-[*β*-D-xylopyranosyl (1→2)-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranosyl]-20-*O*-[*β*-D-xylopyranosyl (1→6)-*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl] 20(*S*)-protopanaxadiol (**4**).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described earlier.[5,6]

Isolation of Notoginsenosides-A (1), -B (2), -C (3), -D (4), -E, -G, -H, -I, and -J and Notoginsenic Acid *β*-Sophoroside from the Dried Roots of *Panax notoginseng* (Burk.) F. H. Chen The dried roots of *P. notoginseng* (12 kg, cultivated in Yunnan Province, China and purchased through Teikoku Seiyaku Co., Ltd., Japan) were crushed and then extracted with MeOH three times under reflux. Evaporation of the solvent from the extract under reduced pressure gave the MeOH extract (1050 g, 8.8% from natural medicine). The extract (510 g) was partitioned into an AcOEt–H_2O mixture and the H_2O-soluble portion was further extracted with *n*-BuOH. Removal of the solvent from the AcOEt-soluble, the *n*-BuOH-soluble, and the H_2O-soluble portions under reduced pressure yielded the AcOEt extract (44 g, 0.75%), the *n*-BuOH extract (430 g, 7.4%), and the H_2O extract (38 g, 0.65%).

The *n*-BuOH extract (120 g) was subjected to normal-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 3 kg), $CHCl_3$–MeOH–H_2O (50 : 10 : 1→7 : 3 : 0.5→5 : 5 : 1, v/v)→MeOH] followed by evaporation of the solvent under reduced pressure to furnish six fractions [fr. 1 (18.2 g), fr. 2 (17.0 g), fr. 3 (14.8 g), fr. 4 (6.3 g), fr. 5 (51.7 g), fr. 6 (7.0 g)]. Fraction 2 (17.0 g) was separated by reversed-phase silica-gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical, Ltd., 500 g), MeOH–H_2O (60 : 40, v/v)→MeOH] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH–H_2O (70 : 30, v/v)] to give ginsenosides-F1[11] (**14**, 70 mg, 0.0043%), -Rg_1[16] (**16**, 6018 mg, 0.37%), -Rg_2[11,15] (**17**, 195 mg, 0.012%), and -Rh_1[16b] (**18**, 569 mg, 0.035%) and notoginsenoside -R2[4d] (**20**, 137 mg, 0.0084%). Fraction 3 (14.8 g) was separated by reversed-phase silica-gel column chromatography [400 g, MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] to give fr. 3-1 (8.6 g), fr. 3-2 (5.9 g), and fr. 3-3 (0.2 g). Fraction 3-1 (8.6 g) was purified by HPLC [MeOH–H_2O (65 : 35, v/v)] to give ginsenoside-Re[14,15] (**15**, 2765 mg, 0.17%) and notoginsenoside-R1[4d] (**19**, 1399 mg, 0.086%). Fraction 3-2 (5.9 g) was purified by HPLC [MeOH–H_2O (85 : 15, v/v)] to give ginsenoside-Rd[12,14] (**9**, 2603 mg, 0.16%), gypenoside XVII[18] (**12**, 293 mg, 0.018%), and notoginsenoside-E (33 mg, 0.002%). Fraction 4 (6.3 g) was separated by reversed-phase silica-gel column chromatography [800 g, MeOH–H_2O (50 : 50→70 : 30→90 : 10)→MeOH] to give fr. 4-1 (0.5 g), fr. 4-2 (2.3 g), and fr. 4-3 (3.5 g). Fraction 4-2 (2.3 g) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give 20-*O*-glucoginsenoside-Rf[13,17] (**23**, 163 mg, 0.010%), notoginsenosides-R3[4g] (**21**, 50 mg, 0.0031%), -R6[4g] (**22**, 80 mg, 0.0049%), -G (26 mg, 0.0016%), and -H (24 mg, 0.0015%). Fraction 4-3 (3.5 g) was purified by HPLC [1) YMC-Pack Ph (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH–H_2O (70 : 30, v/v), 2) YMC-Pack ODS-A, CH_3CN–H_2O (40 : 60, v/v)] to give quinquenoside-R1[9] (**13**, 37 mg, 0.0023%), ginsenoside-Rb_2[10a,12] (**8**, 34 mg, 0.0021%), and notoginsenosides-I (76 mg, 0.0047%) and -J (15 mg, 0.0009%). Fraction 5 (51.7 g) was separated by reversed-phase silica-gel column chromatography [1 kg, MeOH–H_2O (60 : 40→70 : 30, v/v)] to give fr. 5-1 (18.3 g), fr. 5-2 (29.7 g), and fr. 5-3 (3.1 g). Fraction 5-1 (18.3 g) was purified by HPLC [MeOH–H_2O (63 : 35, v/v)] to give notoginsenosides-A (**1**, 106 mg, 0.0065%), -B (**2**, 68 mg, 0.0042%), -C (**3**, 91 mg, 0.0056%), and -K[8] (**5**, 68 mg, 0.0042%). Fraction 5-2 (29.7 g) was purified by HPLC [MeOH–H_2O (75 : 25, v/v)] to give notoginsenosides-D (**4**, 62 mg, 0.0038%), -R4[4g] (**11**, 407 mg, 0.025%), and -Fa[8] (**10**, 260 mg, 0.016%) and ginsenosides-Rb_1[10,12] (**7**, 15290 mg, 0.94%) and -Ra_3[10] (**6**, 423 mg, 0.026%). Fraction 6 (7.0 g) was separated by reversed-phase silica-gel column chromatography (150 g, H_2O→MeOH) and HPLC [MeOH–H_2O (20 : 80, v/v)] to give notoginsenic acid *β*-sophoroside (114 mg, 0.007%). Known compounds (**5**—**23**) were identified by comparison of their physical data ($[\alpha]_D$, IR, and ^{1}H- and ^{13}C-NMR) with reported values.[4,8-18]

Notoginsenoside-A (**1**): Colorless fine crystals from aqueous MeOH, mp 197—200 °C, $[\alpha]_D^{24}$ +18.9° (*c* = 0.1, MeOH). High-resolution positive-mode FAB-MS (*m/z*): Calcd for $C_{54}H_{92}O_{24}Na$ $(M+Na)^+$: 1147.5876. Found: 1147.5895. IR (KBr): 3432, 1632, 1072 cm^{-1}. ^{1}H-NMR (pyridine-d_5, *δ*): 0.85, 0.89, 1.03, 1.11, 1.29, 1.61 (3H each, all s, 19, 30, 18, 29, 28, 21-H_3), 1.55 (6H, s, 26, 27-H_3), 3.28 (1H, dd, *J* = 3.4, 11.2 Hz, 3-H), 4.34 (1H, m, 12-H), 4.92 (1H, d, *J* = 7.6 Hz, 1′-H), 5.10 (1H, d, *J* = 7.6 Hz, 1⁗‴-H), 5.18 (1H, d, *J* = 7.6 Hz, 1‴-H), 5.37 (1H, d, *J* = 7.3 Hz, 1″-H), 6.08 (1H, d, *J* = 15.6 Hz, 24-H), 6.19 (1H, ddd-like, 23-H). ^{13}C-NMR: given in Table 3. Negative-mode FAB-MS (*m/z*): 1123 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1147 $(M+Na)^+$, 1169 $(M+2Na-H)^+$.

Notoginsenoside-B (**2**): Colorless fine crystals from aqueous MeOH, mp 201—204 °C, $[\alpha]_D^{23}$ +17.8° (*c* = 0.1, MeOH). High-resolution positive-mode FAB-MS (*m/z*): Calcd for $C_{54}H_{90}O_{24}Na$ $(M+Na)^+$: 1145.5720. Found: 1145.5717. UV (MeOH, nm): 216 (log *ε* 3.5). IR (KBr): 3410, 1655, 1638, 1078 cm^{-1}. ^{1}H-NMR (pyridine-d_5, *δ*): 0.82, 0.92, 0.97, 1.11, 1.28, 1.59, 1.83 (3H each, all s, 19, 18, 30, 29, 28, 21, 27-H_3), 3.26 (1H, dd-like, 3-H), 4.18 (1H, m, 12-H), 4.93 (1H, d, *J* = 7.6 Hz, 1′-H), 5.03 (1H, d, *J* = 7.2 Hz, 1⁗‴-H), 5.11 (1H, d, *J* = 7.3 Hz, 1‴-H), 5.38 (1H, d, *J* = 7.6 Hz, 1″-H), 5.77, 6.37 (1H each, both s, 26-H_2). ^{13}C-NMR: given in Table 3. Negative-mode FAB-MS (*m/z*): 1121 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1145 $(M+Na)^+$.

Notoginsenoside-C (**3**): Colorless fine crystals from aqueous MeOH, mp 199—202 °C, $[\alpha]_D^{22}$ +14.4° (*c* = 0.1, MeOH). High-resolution positive-mode FAB-MS (*m/z*): Calcd for $C_{54}H_{92}O_{25}Na$ $(M+Na)^+$: 1163.5826. Found: 1163.5873. IR (KBr) : 3410, 1655, 1078 cm^{-1}. ^{1}H-NMR (pyridine-d_5, *δ*): 0.83, 0.94, 0.99, 1.10, 1.28, 1.63, 1.96 (3H each, all s, 19, 30, 18, 29, 28, 21, 27-H_3), 1.97 (m), 2.55 (m), (22-H_2), 3.27 (1H, dd, *J* = 2.9, 11.0 Hz, 3-H), 4.14 (1H, m, 12-H), 4.79 (1H, dd-like, 24-H), 4.91 (1H, d, *J* = 7.3 Hz, 1′-H), 5.09 (1H, d-like, 1‴-H), 5.10, 5.30 (1H each, both m, 26-H_2), 5.10 (1H, d-like, 1⁗‴-H), 5.36 (1H, d, *J* = 7.6 Hz, 1″-H). ^{13}C-NMR: given in Table 3. Negative-mode FAB-MS (*m/z*): 1139 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1163 $(M+Na)^+$, 1185 $(M+2Na-H)^+$.

Notoginsenoside-D (**4**): Colorless fine crystals from aqueous MeOH, mp 207—210 °C, $[\alpha]_D$ +6.5° (*c* = 0.1, MeOH). High-resolution positive-mode FAB-MS (*m/z*): Calcd for $C_{64}H_{108}O_{31}Na$ $(M+Na)^+$: 1395.6772. Found: 1395.6761. IR (KBr): 3410, 1638, 1085 cm^{-1}. ^{1}H-NMR (pyridine-d_5, *δ*): 0.80, 0.95, 0.98, 1.11, 1.28, 1.62 (3H each, all s, 19, 18, 30, 29, 28, 26-H_3), 1.66 (6H, s, 21, 27-H_3), 3.29 (1H, dd, *J* = 3.3, 10.4 Hz, 3-H), 4.08 (1H, m, 12-H), 4.92 (1H, d, *J* = 7.9 Hz, 1″-H), 4.94 (1H, d, *J* = 8.5 Hz, 1⁗⁗‴-H), 5.02 (1H, d, *J* = 7.6 Hz, 1⁗″-H), 5.12 (1H, d, *J* = 7.6 Hz, 1⁗‴-H), 5.31 (1H, d-like, 24-H), 5.38 (1H, d, *J* = 7.0 Hz, 1‴-H), 5.52 (1H, d, *J* = 7.9 Hz, 1′-H). ^{13}C-NMR: given in Table 3. Negative-mode FAB-MS (*m/z*): 1371 $(M-H)^-$, 1239 $(M-C_5H_9O_4)^-$, 1106 $(M-C_{10}H_{18}O_8)^-$. Positive-mode FAB-MS (*m/z*): 1395 $(M+Na)^+$, 1417 $(M+2Na-H)^+$.

Methanolysis of Notoginsenosides-A (1), -B (2), -C (3), and -D (4) A solution of notoginsenosides (1 mg each of **1**, **2**, **3**, and **4**) in 9% HCl–dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i) from **1**, **2**, and **3**; i and methyl xyloside (ii) from **4**; GLC conditions: column CBR1-M25-025 [0.25 mm (i.d.) × 25 m], injector temperature: 140 °C, detector temperature: 280 °C, column temperature: 140—240 °C, 5 °C/min, initial time: 5 min, He flow rate: 15 ml/min, t_R: i: 17.8, 18.2, 19.2 min, ii: 15.8, 16.2 min.

Photosensitized Oxygenation of Ginsenoside-Rb_1 (7) A solution of **7** (200 mg) and Rose Bengal (20 mg) in dry pyridine (5 ml) was irradiated for 1.5 h with a 400 W Hg lamp at room temperature (25 °C) in a Pyrex tube under an O_2 atmosphere. Removal of the solvent from the filtrate under reduced pressure gave a crude product (198 mg), which was purified by reversed-phase silica-gel column chromatography [10 g, MeOH–H_2O (60 : 40, v/v)→MeOH] and HPLC [MeOH–H_2O (65 : 35, v/v)] to give notoginsenosides-A (**1**, 10.8 mg, 5.3%), -B (**2**, 11.0 mg, 5.4%), -C (**3**,

12.4 mg, 6.0%), and -K (**5**, 46.1 mg, 22.4%), which were identified with authentic notoginsenosides-A, -B, -C, and -K by TLC, IR, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR spectral comparisons.

D-GalN/LPS-Induced Liver Injury The method described by Tiegs *et al.*[21] was modified and used for the experiment. Male ddY mice weighing about 25—30 g were used. After 20 h of fasting, a mixture of D-GalN (D-galactosamine hydrochloride, Wako Pure Chemical Industries, Ltd.) and LPS (lipopolysaccharide, from *Salmonella enteritidis*, Sigma Chemical Company) was injected intraperitoneally at a dose of 350 mg/kg and 10 μg/kg) to produce liver injury. Each test sample was administered intraperitoneally 1 h before D-GalN/LPS injection. Blood samples were collected 10 h after D-GalN/LPS injection, and serum GPT and GOT levels were determined.

CCl_4-Induced Liver Injury Male ddY mice weighing about 25—30 g were used. After 20 h of fasting, a mixture of 10% (v/v) CCl_4 in olive oil was injected subcutaneously at a dose of 5 ml/kg to produce CCl_4-induced liver injury. Each test sample was administered intraperitoneally 1 h before CCl_4 injection. Blood samples were collected 20 h after injection, and serum GPT and GOT levels were determined by means of "S.TA-test Wako." Test compounds were administered as a suspension of 1% CMC-Na.

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