Bioactive Saponins and Glycosides. XI.[1] Structures of New Dammarane-Type Triterpene Oligoglycosides, Quinquenosides I, II, III, IV, and V, from American Ginseng, the Roots of *Panax quinquefolium* L.

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The methanolic extract and 1-butanol-soluble fraction of American ginseng, the roots of *Panax quinquefolium* L., were found to exhibit a protective effect on liver injury induced by D-galactosamine and lipopolysaccharide. Five new dammarane-type triterpene oligoglycosides called quinquenosides I, II, III, IV, and V were isolated together with fourteen known dammarane-type triterpene oligoglycosides such as chikusetsusaponin IVa, pseudo-ginsenoside-RC$_1$, malonyl-ginsenoside-Rb$_1$, and notoginsenosides-A, -C, and -K from the 1-butanol-soluble fraction. From the ethyl acetate-soluble fraction, four known acetylenic compounds and 6′-*O*-acetyl ginsenoside-Rg$_1$ were isolated. The structures of quinquenosides I, II, III, IV, and V were determined on the basis of chemical and physicochemical evidence as 3-*O*-[6-*O*-(*E*)-2-butenoyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-(β-D-glucopyranosyl) 20(*S*)-protopanaxadiol (quinquenoside I), 3-*O*-[6-*O*-(*E*)-2-octenoyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl] 20(*S*)-protopanaxadiol (quinquenoside II), 3-*O*-[β-D-glucopyranosyl (1→2)-6-*O*-acetyl-β-D-glucopyranosyl]-20-*O*-(β-D-glucopyranosyl) 20(*S*)-protopanaxadiol (quinquenoside III), 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]-3β,7β,20(*S*)-trihydroxydammar-5,24-diene (quinquenoside IV), and 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[α-D-glucopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl] 20(*S*)-protopanaxadiol (quinquenoside V).

Key words American ginseng; quinquenoside; *Panax quinquefolium*; hepatoprotective effect; dammarane-type triterpene oligoglycoside; bioactive saponin

American ginseng, the roots of *Panax* (*P.*) *quinquefolium* L. (Chinese names: 西洋参, 広東人参, or 花旗参), which is cultivated on a large scale in the U.S.A., Canada, and China and exported to China and other Asian countries, has been used for the same medicinal purpose as Ginseng. In regard to the chemical constituents of *P. quinquefolium* L., many dammarane-type triterpene oligoglycosides have been characterized from the roots, stems, leaves, and cultured cells[2] and also, cytotoxic acetylenic compounds have been reported from the roots of this plant.[3]

During the course of our screening to identify biologically active principles from natural medicines and medicinal foodstuffs,[4] we have found a number of oleanane-type and dammarane-type triterpene oligoglycosides with inhibitory effects on alcohol and glucose absorption,[5] as well as antiinflammatory,[6] antiallergic,[7] and hepatoprotective activities.[8] In a continuing study, we have found that the methanolic extract and 1-butanol-soluble fraction obtained from American ginseng, the roots of *P. quinquefolium*, showed a remarkable protective effect on liver injury induced by D-galactosamine and lipopolysaccharide. From the 1-butanol-soluble fraction with the hepatoprotective effect, five new dammarane-type triterpene oligoglycosides called quinquenosides I (**1**), II (**2**), III (**3**), IV (**4**), and V (**5**) were isolated together with fourteen known dammarane-type triterpene oligoglycosides. Four known acetylenic compounds and 6′-*O*-acetyl ginsenoside-Rg$_1$ (**19**) were isolated from the ethyl acetate-soluble fraction. In this paper, we describe the hepatoprotective effect of the methanolic extract and several fractions from American ginseng as well as the structure elucidation of quinquenosides I—V (**1**—**5**).[9]

The chemical constituents of American ginseng, the dried roots of *P. quinquefolium* cultivated in Wausau, the state of Wisconsin, U.S.A., were separated by the procedure shown in Chart 1. Thus, the methanolic extract from the dried roots was partitioned into an ethyl acetate–water mixture to furnish the ethyl acetate-soluble fraction and the water phase. The water phase was further extracted with 1-butanol to give the 1-butanol-soluble and the water-soluble fractions. As is apparent from Table 1, the methanolic extract and the 1-butanol-soluble fraction (so-called glycosidic fraction) were found to significantly inhibit the increase in serum GOT and GPT levels induced by D-galactosamine and lipopolysaccharide injection after a single oral administration at a dose of 500 and 200 mg/kg, respectively. On the other hand, although the ethyl acetate-soluble fraction showed a tendency to inhibit the increase in serum GOT and GPT following a single oral administration of 100 mg/kg, it exhibited potent toxicity at a dose of 200 mg/kg. The water-soluble fraction was found to lack this activity. The 1-butanol-soluble fraction was subjected to ordinary- and reversed-phase silica gel column chromatography and finally HPLC to afford quinquenosides I (**1**, 0.0006% from the natural medicine), II (**2**, 0.0008%), III (**3**, 0.0005%), IV (**4**, 0.0005%), and V (**5**, 0.0012%) together with notoginsenosides-A[8a] (**6**, 0.0006%), -C[8a] (**7**, 0.0004%), and -K[8b] (**8**, 0.0009%), malonyl-ginsenoside-Rb$_1$[10] (**9**, 0.0063%), pseudo-ginsenoside-RC$_1$[11] (**10**, 0.0018%), ginsenosides-Rb$_1$ (**11**,

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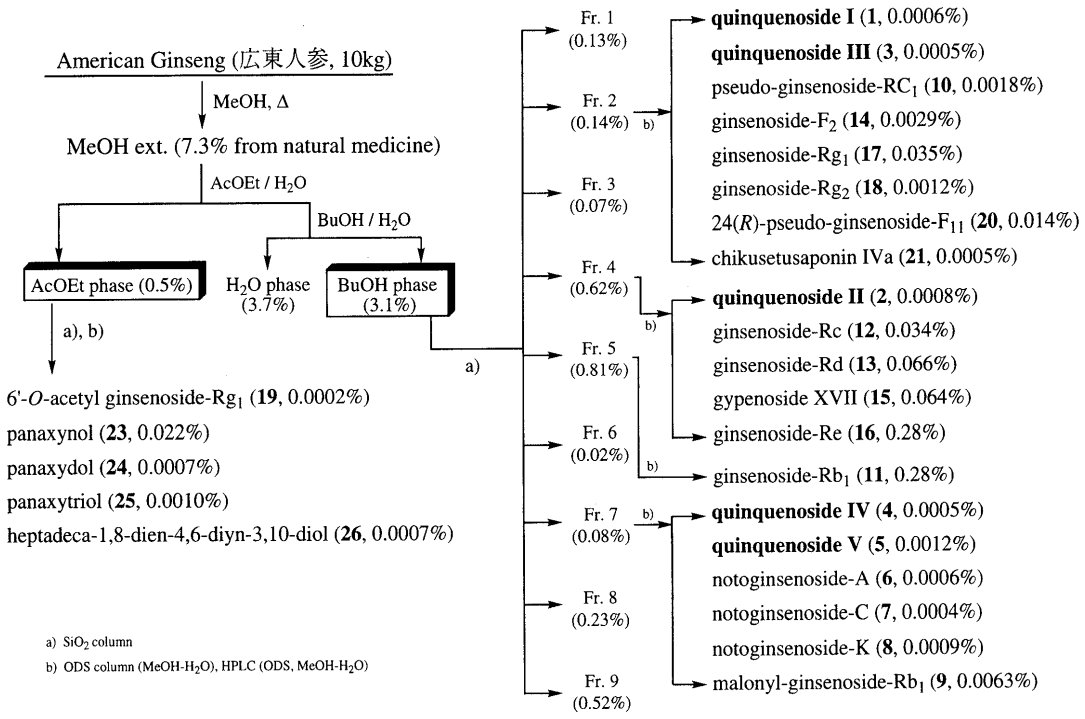


Chart 1

0.28%), -Rc (**12**, 0.034%), -Rd (**13**, 0.066%), -Re (**16**, 0.28%), -Rg_1 (**17**, 0.035%), -Rg_2 (**18**, 0.0012%), and -F_2 (**14**, 0.0029%), gypenoside XVII (**15**, 0.064%), 24(*R*)-pseudo-ginsenoside-F_{11}[12] (**20**, 0.014%), and chikusetsusaponin IVa (**21**, 0.0005%). Among those known dammarane-type triterpene oligoglycosides, **6**, **7**, **8**, **9**, **10**, and **21** were isolated for the first time from American ginseng.

The ethyl acetate-soluble fraction was purified by ordinary- and reversed-phase silica gel column chromatography and HPLC to provide 6'-*O*-acetyl ginsenoside-Rg_1 (**19**, 0.0002%), panaxynol (**23**, 0.022%), panaxydol (**24**, 0.0007%), panaxytriol (**25**, 0.0010%), and heptadeca-1,8-dien-4,6-diyn-3,10-diol (**26**, 0.0007%). Although **19** has been synthesized by the enzymatic treatment of ginsenoside-Rg_1,[13] this is the first report of its isolation in nature.

Structures of Quinquenosides I (1), II (2), III (3), IV (4), and V (5) Quinquenoside I (**1**) was obtained as colorless fine crystals of mp 172—175 °C. The IR spectrum of **1** showed absorption bands at 1716 and 1655 cm^{-1} ascribable to an α,β-unsaturated ester and olefin, and strong absorption bands at 3410 and 1076 cm^{-1} suggestive of an oligoglycosidic structure. The UV spectrum of **1** showed an absorption maximum at 211 nm (log ε, 4.3), which also suggested the presence of an α,β-unsaturated ester. In the negative-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1013 $(M-H)^-$, while the positive-ion FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 1037 $(M+Na)^+$ and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{52}H_{86}O_{19}$. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1) liberated ginsenoside-Rd (**13**)[12,14] together with crotonic acid (*trans*-2-butenoic acid). The organic acid was converted to the *p*-nitrobenzyl ester,[15] which was identified by HPLC.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **1** showed signals assignable to the ginsenoside-Rd part [δ 4.87 (d, J=7.6 Hz, 1'-H), 4.89 (dd-like), 4.97 (dd, J=1.5, 11.4 Hz) (6''-H_2), 5.16 (d, J=7.7 Hz, 1'''-H), 5.29 (d, J=7.6 Hz, 1''-H)] and the crotonic acid part [δ 1.67 (dd, J=1.6, 7.0 Hz, 4''''-H_3), 5.98 (br d, 2''''-H), 7.05 (dq, J=7.0, 15.6 Hz, 3''''-H)]. The position of the crotonyl group was characterized by a heteronuclear multiple bond correlation (HMBC) experiment (J_{CH}, 8 Hz) on **1**, which showed a long-range correlation between the 6''-methylene protons and the 1''''-carbonyl carbon (Fig. 1). Furthermore, comparison of the ^{13}C-NMR data for **1** with those for ginsenoside-Rd (**13**) revealed an acylation shift around the 6''-position. Consequently, the structure of quinquenoside I was determined as 3-*O*-[6-*O*-(*E*)-2-butenoyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-(β-D-glucopyranosyl) 20(*S*)-protopanaxadiol (**1**).

Quinquenoside II (**2**) was also isolated as colorless fine crystals of mp 168—170 °C and its IR spectrum showed absorption bands at 3400, 1717, 1655, and 1078 cm^{-1} due to a hydroxyl, olefin, and α,β-unsaturated ester. The negative- and positive-ion FAB-MS showed quasimolecular ion peaks at *m/z* 1231 $(M-H)^-$ and 1255 $(M+Na)^+$, respectively, and the molecular formula $C_{62}H_{104}O_{24}$ was determined by high-resolution MS. Alkaline hydrolysis of **2** liberated ginsenoside-Rb_1 (**11**)[14,17] together with *trans*-2-octenoic acid, which was identified by HPLC analysis of the *p*-nitrobenzyl ester. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **2** showed signals due to the ginsenoside-Rb_1 part [δ 4.86, 4.90 (both dd-like, 6''-H_2), 4.87 (d, J=6.6 Hz, 1'-H), 5.04 (d, J=8.0 Hz, 1''''-H), 5.09 (d, J=7.6 Hz, 1'''-H), 5.30 (d, J=7.2 Hz, 1''-H)] and the *trans*-2-octenoic acid part [δ 0.84 (t, J=7.4 Hz, 8''''-H_3), 6.02 (d, J=15.5 Hz, 2''''-H), 7.15 (dt, J=8.2, 15.5 Hz, 3''''-H)]. In the HMBC ex-

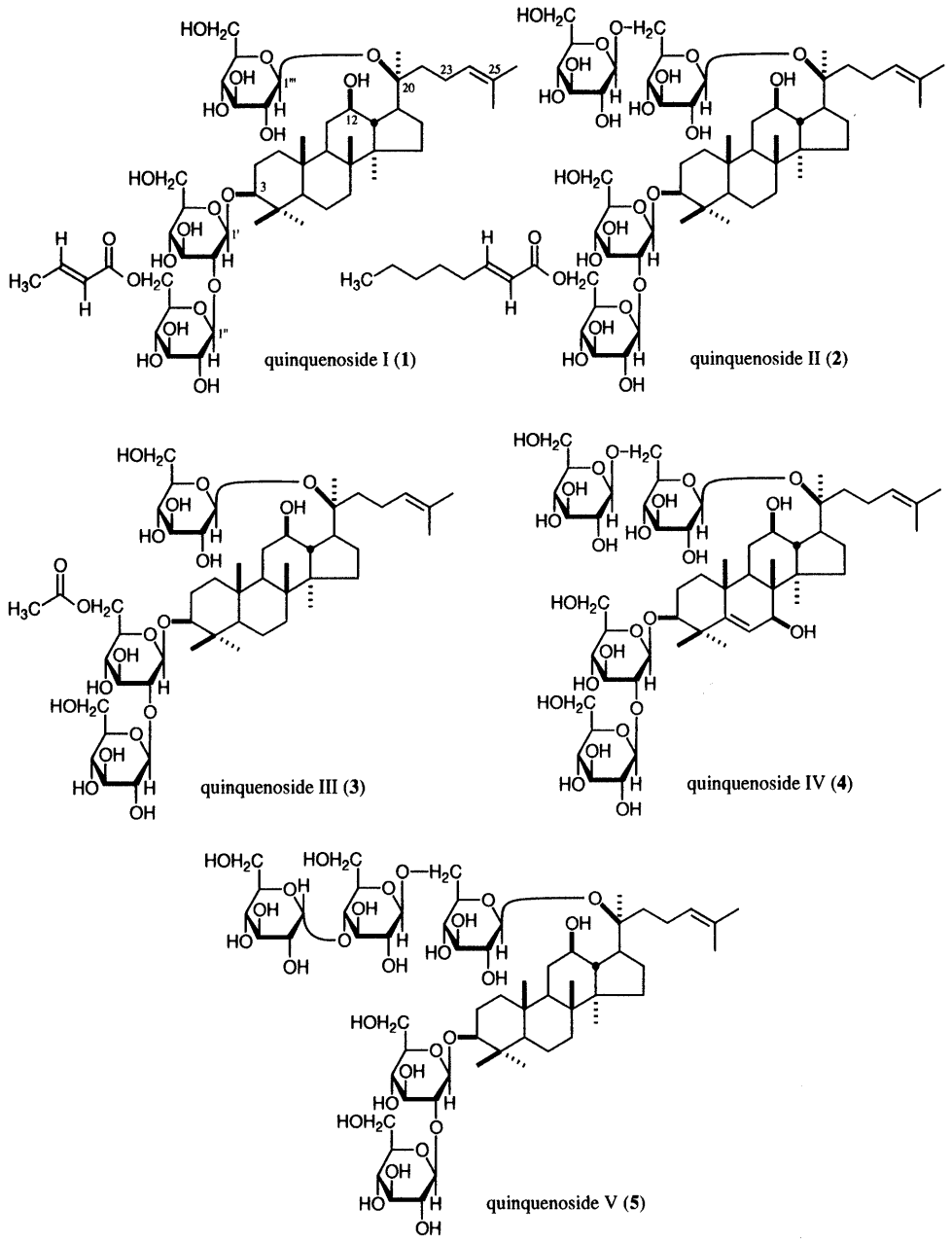
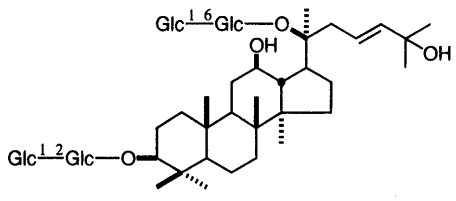


Chart 2

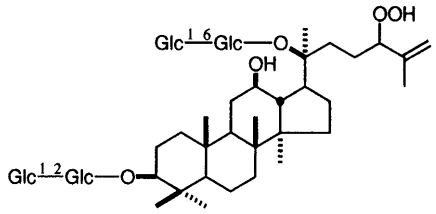
periment on **2**, a long-range correlation was observed between the 6″-methylene protons and the 1‴″-carbonyl carbon (Fig. 1). Finally, comparison of the ^{13}C-NMR data for **2** with those for **11** led us to formulate the structure of quinquenoside II as 3-*O*-[6-*O*-(*E*)-2-octenoyl-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-20-*O*-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] 20(*S*)-protopanaxadiol (**2**).

Quinquenoside III (**3**), obtained as colorless fine crystals of mp 167—169 °C, showed absorption bands at 3420, 1736, 1655, and 1078 cm^{-1} due to hydroxyl, ester, and olefin functions in its IR spectrum. The molecular formula $C_{50}H_{84}O_{19}$ was determined from its negative- and positive-ion FAB-MS [*m/z* 987 $(M-H)^-$ and 1011 $(M+Na)^+$] and by high-resolution MS measurement. Alkaline hydrolysis of **3** yielded ginsenoside-Rd (**13**) and acetic acid, which was identified by HPLC analysis of the *p*-nitrobenzyl ester. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[16] of **3** showed signals assignable to the ginsenoside-Rd part [δ 4.71 (dd, J=5.0, 11.3 Hz), 4.91 (br d) (6′-H$_2$), 4.84 (d, J=7.3 Hz, 1′-H), 5.16 (d, J=7.6 Hz, 1‴-H), 5.34 (d, J=7.6 Hz, 1″-H)] and an acetyl group [δ 2.02 (s)]. The HMBC experiment on **3** showed a long-range correlation between the 6′-methylene protons and the acetyl carbonyl carbon. In addition, an acetylation shift was observed around the 6′-carbon by comparison of the ^{13}C-NMR data for **3** with those for **13**. On the basis of above evidence, the structure of quinquenoside III was characterized as 3-*O*-[*β*-D-glucopyranosyl (1→2)-6-*O*-acetyl-*β*-D-glucopyranosyl]-20-*O*-(*β*-D-glucopyranosyl) 20(*S*)-protopanaxadiol (**3**).

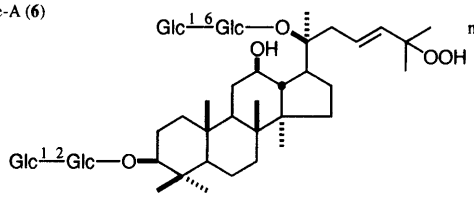
Quinquenoside IV (**4**) was obtained as colorless fine crystals of mp 190—192 °C. The IR spectrum of **4** showed absorption bands at 3413, 1650, and 1078 cm^{-1} ascribable to hydroxyl and olefin functions. Here again, the molecular formula $C_{54}H_{90}O_{24}$ of **4** was clarified from its nega-



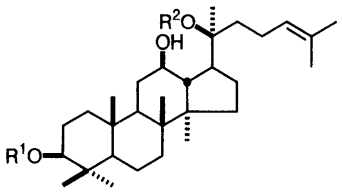
notoginsenoside-A (**6**)



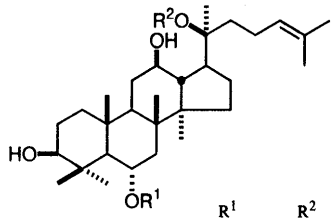
notoginsenoside-C (**7**)



notoginsenoside-K (**8**)

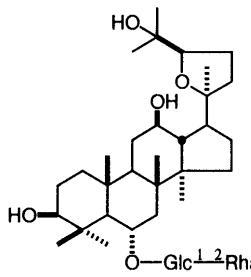


	R^1	R^2
malonyl-ginsenoside-Rb_1 (**9**) :	-Glc 2—1 Glc6—Mal	-Glc 6—1 Glc
pseudo-ginsenoside-RC_1 (**10**) :	-Glc 2—1 Glc 6—Ac	-Glc
ginsenoside-Rb_1 (**11**) :	-Glc 2—1 Glc	-Glc 6—1 Glc
ginsenoside-Rc (**12**) :	-Glc 2—1 Glc	-Glc 6—1 Ara
ginsenoside-Rd (**13**) :	-Glc 2—1 Glc	-Glc
ginsenoside-F_2 (**14**) :	-Glc	-Glc
gypenoside XVII (**15**) :	-Glc	-Glc 6—1 Glc

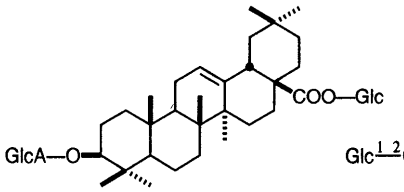


	R^1	R^2
ginsenoside-Re (**16**) :	-Glc 2—1 Rha	-Glc
ginsenoside-Rg_1 (**17**) :	-Glc	-Glc
ginsenoside-Rg_2 (**18**) :	-Glc 2—1 Rha	-H
6'-*O*-acetyl ginsenoside-Rg_1 (**19**) :	-Glc 6—Ac	-Glc

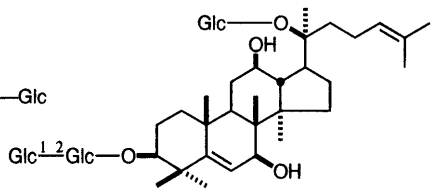
Glc : β-D-glucopyranosyl, GlcA : β-D-glucopyranosiduronic acid
Rha : α-L-rhamnopyranosyl, Ara : α-L-arabinofuranosyl
Mal : malonyl, Ac : acetyl



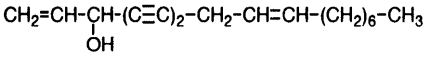
24(*R*)-pseudo-ginsenoside F_{11} (**20**)



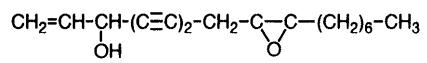
chikusetsusaponin IVa (**21**)



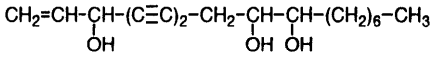
notoginsenoside-G (**22**)



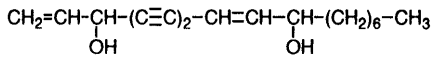
panaxynol (**23**)



panaxydol (**24**)



panaxytriol (**25**)



heptadeca-1,8-dien-4,6-diyn-3,10-diol (**26**)

Chart 3

tive- and positive-ion FAB-MS and by high-resolution MS. Thus, in the negative-ion FAB-MS of **4**, the quasi-molecular ion peak was observed at *m/z* 1121 $(M-H)^-$, while its positive-ion FAB-MS showed the quasi-molecular ion peak at *m/z* 1145 $(M+Na)^+$. Acid hydrolysis of **4** with 5% aqueous sulfuric acid–dioxane (1 : 1) liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolide derivative.[18] The ^{1}H-NMR (pyridine-d_5) spectrum[16] of **4** showed signals due to the β-sophorosyl moiety [δ 4.85 (d, J=7.4 Hz, 1'-H), 5.30 (d, J=7.6 Hz, 1''-H)], the β-gentiobiosyl moiety [δ 5.06 (d, J=7.6 Hz, 1''''-H), 5.12 (d, J=7.6 Hz, 1'''-H)], and the sapogenol moiety [δ 1.13, 1.16, 1.27, 1.41, 1.49, 1.61 (all s, 30, 19, 18, 29, 28, 26-H_3), 1.67 (s, 21, 27-H_3), 2.08 (t-like, 13-H), 3.33 (dd, J=4.2, 11.3 Hz, 3-H), 4.11 (m, 12-H), 4.68 (d-like, 7-H), 5.33 (t-like, 24-H), 5.82 (d, J=1.9 Hz, 6-H)]. The carbon signals due to the sugar moiety in the ^{13}C-NMR (Table 2) spectrum[16] of **4** were superimposable on those of ginsenoside-Rb_1 (**11**), whereas the carbon signals assignable to the sapogenol moiety were shown to be very similar to those of notoginsenoside-G (**22**).[8b] The HMBC experiment showed long-range cor-

Table 1. Inhibitory Effect of the Methanolic Extract, Ethyl Acetate-Soluble, 1-Butanol-Soluble, and Water-Soluble Fractions from American Ginseng on D-Galactosamine/Lipopolysaccharide-induced Liver Injury in Mice

	Dose (mg/kg, *i.p.*)	N	s-GPT (Karmen unit)	s-GOT (Karmen unit)
Normal	—	10	16±1[a]	51±4[a]
Control	—	10	3518±483	4458±564
MeOH ext.	500	10	246±10[a]	458±13[a]
AcOEt-soluble fraction	100	10	2992±567	3527±1013
n-BuOH-soluble fraction	100	9	3652±1039	3298±864
	200	9	954±259[b]	845±156[a]
H_2O-soluble fraction	200	10	4661±670	5085±860
Hydrocortisone	20	10	246±86[a]	258±76[a]

a) $p<0.01$, *b*) $p<0.05$.

relations between the following protons and carbons: 7-H and 6, 8-C; 6-H and 4, 7, 10-C; 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 20-C; 1⁗-H and 6‴-C (Fig. 1). In the difference rotating frame nuclear Overhauser effect spectroscopy (difference ROESY) analysis of **4**, ROE correlations were observed between the 7-proton and the 9-proton and between the 7-proton and the 30-H_3. These findings led us to confirm that the sapogenol of **4** was identical with that of **22**. Consequently, the structure of quinquenoside IV was identified as 3-*O*-[*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranosyl]-20-*O*-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl] 3*β*,7*β*,20(*S*)-trihydroxydammar-5,24-diene (**4**).

Quinquenoside V (**5**), isolated as colorless fine crystals of mp 192—194 °C, showed absorption bands due to hydroxyl and olefin groups (3399, 1655, 1075 cm^{-1}) in the IR spectrum. The molecular formula $C_{60}H_{102}O_{28}$ was determined from the quasimolecular ion peaks [*m/z* 1269

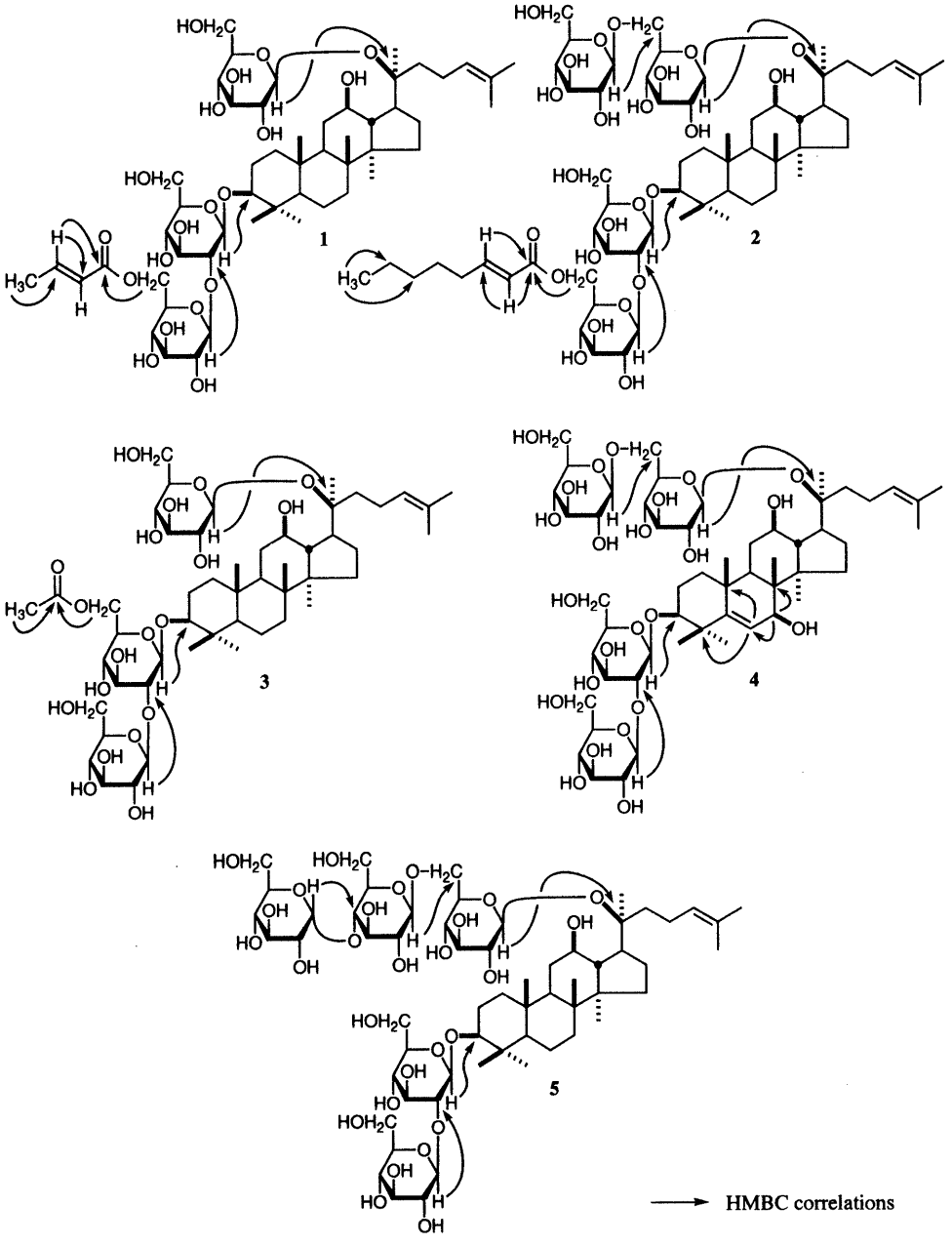


Fig. 1. Long-range Correlation in the HMBC Spectra of **1**, **2**, **3**, **4**, and **5**

Table 2. ^{13}C-NMR Data of Quinquenosides-I (**1**), -II (**2**), -III (**3**), -IV (**4**), and -V (**5**)

	1	2	3	4	5		1	2	3	4	5
C-1	39.3	39.2	39.3	39.7	39.3	Glc-1″	106.2	106.3	106.0	106.1	106.0
2	26.8	26.8	26.8	27.2	26.8	2″	76.8	76.8	77.0	77.0	77.0
3	89.3	89.3	89.5	88.1	89.1	3″	78.0	77.9	78.1	78.0	78.0
4	39.8	39.8	39.7	42.8	39.7	4″	71.1	70.9	71.9	71.9	71.8
5	56.5	56.4	56.6	147.2	56.5	5″	75.6	75.5	78.2	78.2	78.0
6	18.7	18.5	18.5	127.5	18.5	6″	64.4	64.4	63.0	62.9	62.8
7	35.3	35.1	35.2	71.3	35.1	Glc-1‴	98.3	98.1	98.3	98.2	98.1
8	40.1	40.0	40.1	42.4	40.0	2‴	75.2	74.8	75.2	74.9	74.9
9	50.3	50.2	50.3	47.5	50.2	3‴	79.2	79.2	79.3	79.1	79.1
10	37.0	36.9	37.1	38.1	37.0	4‴	71.7	71.5	71.8	71.7	71.6
11	30.8	30.8	30.8	33.2	30.8	5‴	78.2	77.0	78.2	77.0	77.0
12	70.3	70.1	70.2	69.8	70.2	6‴	62.9	70.2	62.9	70.3	70.2
13	49.5	49.5	49.6	50.5	49.5	Glc-1⁗		105.4		105.3	105.0
14	51.5	51.4	51.5	51.0	51.4	2⁗		75.2		75.3	74.8
15	31.0	30.7	31.0	34.4	30.9	3⁗		78.4		78.4	77.6
16	26.7	26.6	26.7	27.1	26.7	4⁗		71.7		71.8	81.4
17	51.8	51.6	51.8	51.2	51.6	5⁗		78.3		78.3	76.8
18	16.0	16.0	16.1	10.8	16.1	6⁗		62.8		62.9	61.9
19	16.3	16.3	16.3	20.4	16.3	Glc*-1⁗′					103.1
20	83.4	83.4	83.4	83.8	83.5	2⁗′					74.4
21	22.5	22.4	22.4	22.6	22.3	3⁗′					75.4
22	36.2	36.1	36.2	36.9	36.2	4⁗′					71.9
23	23.3	23.2	23.3	23.3	23.2	5⁗′					75.1
24	126.0	125.9	126.0	126.0	125.9	6⁗′					62.8
25	130.9	131.0	130.9	131.1	131.2						
26	25.7	25.8	25.7	25.8	25.8	Acyl group					
27	17.8	17.9	17.8	18.0	18.0	1	166.6	166.9	170.7		
28	28.1	28.0	28.2	28.4	28.1	2	123.8	121.7	20.8		
29	16.5	16.5	16.6	23.9	16.6	3	144.6	149.5			
30	17.4	17.4	17.4	18.2	17.5	4	17.7	32.2			
Glc-1′	104.9	105.0	105.0	104.9	105.0	5		27.8			
2′	84.3	84.4	83.3	83.6	83.6	6		31.5			
3′	78.5	78.5	78.0	78.3	78.3	7		22.6			
4′	71.6	71.4	71.5	71.7	71.7	8		14.1			
5′	78.0	78.1	74.7	78.0	78.1						
6′	62.9	62.8	64.4	62.8	62.9						

(125 MHz, pyridine-d_5).

$(M-H)^-$ and 1293 $(M+Na)^+$] in the negative- and positive-ion FAB-MS and by high-resolution MS. Acid hydrolysis of **5** liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolide derivative. The ^{1}H-NMR (pyridine-d_5) spectrum of **5** showed signals assignable to four β-D-glucopyranosyl moieties [δ 4.88 (d, J=7.6 Hz, 1′-H), 5.00 (d, J=7.9 Hz, 1⁗-H), 5.08 (d, J=7.6 Hz, 1‴-H), and 5.32 (d, J=7.9 Hz, 1″-H)], an α-D-glucopyranosyl moiety [δ 5.82 (d, J=4.0 Hz, 1⁗′-H)], and the protopanaxadiol part [δ 0.83, 0.96, 0.97, 1.09, 1.27, 1.68 (all s, 19, 30, 18, 29, 28, 27-H$_3$), 1.64 (s, 21, 26-H$_3$), 1.98 (t-like, 13-H), 3.26 (dd, J=4.2, 11.2 Hz, 3-H), 4.14 (m, 12-H), 5.31 (t-like, 24-H)]. The carbon signals in the ^{13}C-NMR (Table 2) spectrum on **5** closely resembled those of ginsenoside-Rb$_1$ (**11**), except for signals due to the terminal α-D-glucopyranosyl moiety in **5**. The HMBC experiment of **5** showed long-range correlations between the following protons and carbons: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 20-C; 1⁗-H and 6‴-C; 1⁗′-H and 4⁗-C. Comparison of the NMR data for **5** with those for several dammarane-type triterpene oligoglycosides having an α-D-glucopyranoside moiety[19] led us to characterize the structure of quinquenoside V as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[α-D-glucopyranosyl(1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] 20(*S*)-protopanaxadiol (**5**).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Quinquenosides I (1), II (2), III (3), IV (4), and V (5) and Known Compounds (6—21, 22—25) from the Roots of *Panax quinquefolium* L. The dried roots of *P. quinquefolium* L. (10 kg, cultivated in Wausau, the state of Wisconsin, U.S.A. and purchased through Teikoku Seiyaku Co., Ltd., Japan) were crushed and extracted four times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (732 g, 7.3% from natural medicine). The MeOH extract (360 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, *n*-BuOH-soluble, and H_2O-soluble portions under reduced pressure yielded the AcOEt-soluble (25 g, 0.5%), *n*-BuOH-soluble (152 g, 3.1%), and H_2O-soluble fractions (189 g, 3.7%).

The AcOEt-soluble fraction (23 g) was subjected to ordinary-phase silica gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., 1 kg), *n*-hexane–AcOEt (10 : 1→5 : 1→2 : 1, v/v)→$CHCl_3$–MeOH (5 : 1→2 : 1, v/v)→MeOH] followed by evaporation of the solvent under reduced pressure to furnish seven fractions [fr. 1 (3.1 g), fr. 2 (2.4 g), fr. 3 (2.7 g), fr. 4 (0.9 g), fr. 5 (6.2 g), fr. 6 (2.9 g), fr. 7 (3.4 g)]. Fraction 2 (1.9 g) was purified by reversed-phase silica gel column chromatography [Chromatorex ODS DM 1020T (Fuji Silysia Chemical Ltd., 76 g), MeOH–H_2O (80 : 20→90 : 10, v/v)→MeOH] to give panaxynol (**23**, 785 mg, 0.022%). Fraction 4 (0.9 g) was separated by reversed-phase silica gel column

chromatography [20 g, MeOH–H_2O (65:35→80:20→95:5, v/v)→MeOH] and ordinary-phase silica gel column chromatography [6 g, *n*-hexane–acetone (6:1, v/v)→MeOH] to give panaxydol (**24**, 31 mg, 0.0007%) and heptadeca-1,8-dien-4,6-diyn-3,10-diol (**26**, 33 mg, 0.0007%). Fraction 5 (5.4 g) was separated by reversed-phase silica gel column chromatography [162 g, MeOH–H_2O (50:50→60:40→80:20, v/v)→MeOH] and HPLC [YMC-Pack ODS (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH–H_2O (75:25, v/v)] to give panaxytriol (**25**, 41 mg, 0.0010%)]. Fraction 6 (1.2 g) was separated by reversed-phase silica gel column chromatography [36 g, MeOH–H_2O (50:50→70:30, v/v)→MeOH] and HPLC [MeOH–H_2O (65:35, v/v)] to give 6′-*O*-acetyl ginsenoside-Rg_1 (**19**, 9 mg, 0.0002%).

The *n*-BuOH extract (140 g) was subjected to ordinary-phase silica gel column chromatography [4.2 kg, $CHCl_3$–MeOH–H_2O (50:10:1→7:3:0.5→5:5:1, v/v)] to furnish nine fractions [fr. 1 (6.0 g, 0.13%), fr. 2 (6.5 g, 0.14%), fr. 3 (3.1 g, 0.07%), fr. 4 (28.0 g, 0.62%), fr. 5 (36.4 g, 0.81%), fr. 6 (0.83 g, 0.02%), fr. 7 (3.8 g, 0.08%), fr. 8 (10.4 g, 0.23%), fr. 9 (23.6 g, 0.52%)]. Fraction 2 (6.5 g) was separated by reversed-phase silica gel column chromatography [325 g, MeOH–H_2O (50:50→60:40→70:30→80:20, v/v)→MeOH] to give nine fractions [fr. 2-1 (1.7 g), fr. 2-2 (1.9 g), fr. 2-3 (38 mg), fr. 2-4 (911 mg), fr. 2-5 (321 mg), fr. 2-6 (226 mg), fr. 2-7 (381 mg), fr. 2-8 (348 mg), fr. 2-9 (213 mg)]. Fraction 2-2 (110 mg) was purified by HPLC [MeOH–H_2O (60:40, v/v)] to give ginsenoside-Rg_1 (**17**, 93 mg, 0.035%). Fraction 2-4 (100 mg) was purified by HPLC [MeOH–H_2O (65:35, v/v)] to give 24(*R*)-pseudo-ginsenoside-F_{11} (**20**, 56 mg, 0.014%). Fraction 2-5 (250 mg) was purified by HPLC [MeOH–H_2O (70:30, v/v)] to give ginsenoside-Rg_2 (**18**, 47 mg, 0.0012%) and chikusetsusaponin IVa (**21**, 21 mg, 0.0005%). Repeated HPLC [1) YMC-Pack ODS, MeOH–H_2O (80:20, v/v); 2) YMC-Pack Ph (YMC Co., Ltd., 250 × 20 mm, i.d.), MeOH–H_2O (80:20, v/v)] of fraction 2-7 (370 mg) yielded quinquenosides I (**1**, 25 mg, 0.0006%) and III (**3**, 20 mg, 0.0005%) and pseudo-ginsenoside-RC_1 (**10**, 77 mg, 0.0018%). Fraction 2-8 (270 mg) was purified by HPLC [MeOH–H_2O (85:15, v/v)] to give ginsenoside-F_2 (**14**, 105 mg, 0.0029%). Fraction 4 (20 g) was separated by reversed-phase silica gel column chromatography [500 g, MeOH–H_2O (60:40→80:20, v/v)→MeOH] to give seven fractions {fr. 4-1 (2.0 g), fr. 4-2 [ginsenoside-Re (**16**, 9.3 g, 0.28%)], fr. 4-3 (826 mg), fr. 4-4 (2.2 g), fr. 4-5 (5.2 g), fr. 4-6 (113 mg), fr. 4-7 (89 mg)}. Fraction 4-4 (100 mg) was purified by HPLC [MeOH–H_2O (75:25, v/v)] to give ginsenoside-Rc (**12**, 50 mg, 0.034%). Fraction 4-5 (750 mg) was purified by HPLC [MeOH–H_2O (80:20, v/v)] to give ginsenoside-Rd (**13**, 308 mg, 0.066%) and gypenoside XVII (**15**, 301 mg, 0.064%). Fraction 4-6 (112 mg) was purified by HPLC [MeOH–H_2O (85:25, v/v)] to give quinquenoside II (**2**, 27 mg, 0.0008%). Fraction 5 (15 g) was separated by reversed-phase silica gel column chromatography [500 g, MeOH–H_2O (60:40→90:10, v/v)→MeOH] and HPLC [MeOH–H_2O (75:25, v/v)] to give ginsenoside-Rb_1 (**11**, 5.2 g, 0.28%). Fraction 7 (3.8 g) was subjected by reversed-phase silica gel column chromatography [76 g, MeOH–H_2O (50:50→70:30, v/v)→MeOH] to give six fractions [fr. 7-1 (2.3 g), fr. 7-2 (141 mg), fr. 7-3 (107 mg), fr. 7-4 (322 mg), fr. 7-5 (986 mg), fr. 7-6 (132 mg)]. Fraction 7-2 (141 mg) was purified by HPLC [MeOH–H_2O (55:45, v/v)] to give quinquenoside IV (**4**, 23 mg, 0.0005%). Fraction 7-4 (322 mg) was purified by HPLC [MeOH–H_2O (60:40, v/v)] to give notoginsenosides-A (**6**, 28 mg, 0.0006%), -C (**7**, 21 mg, 0.0004%), and -K (**8**, 42 mg, 0.0009%). Repeated HPLC [1) MeOH–H_2O (70:30, v/v); 2) MeOH–1% aqueous AcOH (70:30, v/v)] of fraction 7-5 (986 mg) yielded malonyl-ginsenoside-Rb_1 (**9**, 281 mg, 0.0063%) and quinquenoside V (**5**, 52 mg, 0.0012%). The known compounds (**6**—**21**, **23**—**26**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[2,3,8,10-14]

Quinquenoside I (**1**): Colorless fine crystals from aqueous MeOH, mp 172—174 °C, $[\alpha]_D^{28}$ +34.6° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{86}NaO_{19}$ $(M+Na)^+$: 1037.5661. Found: 1037.5638. UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 211 (4.3). IR (KBr) cm^{-1}: 3410, 1716, 1655, 1076. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.84, 0.96, 0.97, 1.11, 1.31 (3H each, all s, 19, 30, 18, 29, 28-H_3), 1.61 (9H, s, 21, 26, 27-H_3), 1.67 (3H, dd, J=1.6, 7.0 Hz, 4′′′′-H_3), 1.98 (1H, t-like, 13-H), 3.27 (1H, dd, J=1.8, 11.0 Hz, 3-H), 4.10 (1H, m, 12-H), 4.87 (1H, d, J=7.6 Hz, 1′-H), 4.89 (1H, dd-like), 4.97 (1H, dd, J=1.5, 11.4 Hz) (6″-H_2), 5.16 (1H, d, J=7.7 Hz, 1‴-H), 5.25 (1H, t-like, 24-H), 5.29 (1H, d, J=7.6 Hz, 1″-H), 5.98 (1H, br d, 2′′′′-H), 7.05 (1H, dq, J=7.0, 15.6 Hz, 3′′′′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 1013 $(M-H)^-$, 945 $(M-C_4H_5O)^-$. Positive-ion FAB-MS m/z: 1037 $(M+Na)^+$.

Quinquenoside II (**2**): Colorless fine crystals from aqueous MeOH, mp 168—170 °C, $[\alpha]_D^{28}$ +22.5° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{62}H_{104}NaO_{24}$ $(M+Na)^+$: 1255.6815. Found: 1255.6792. UV λ_{max}^{MeOH} ($\log\varepsilon$): 205 (4.5). IR (KBr) cm^{-1}: 3400, 1717, 1655, 1078. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.84 (3H, t, J=7.4 Hz, 8′′′′′-H_3), 0.86, 0.98, 1.00, 1.12, 1.33, 1.62, 1.64, 1.67 (3H each, all s, 19, 30, 18, 29, 28, 26, 21, 27-H_3), 1.98 (1H, t-like, 13-H), 3.27 (1H, dd, J=4.3, 11.9 Hz, 3-H), 4.11 (1H, m, 12-H), 4.86, 4.90 (1H each, both dd-like, 6″-H_2), 4.87 (1H, d, J=6.6 Hz, 1′-H), 5.04 (1H, d, J=8.0 Hz, 1′′′′-H), 5.09 (1H, d, J=7.6 Hz, 1‴-H), 5.30 (1H, d, J=7.2 Hz, 1″-H), 5.32 (1H, t-like, 24-H), 6.02 (1H, d, J=15.5 Hz, 2′′′′-H), 7.15 (1H, dt, J=8.2, 15.5 Hz, 3′′′′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 1231 $(M-H)^-$, 1107 $(M-C_8H_{13}O)^-$. Positive-ion FAB-MS m/z: 1255 $(M+Na)^+$.

Quinquenoside III (**3**): Colorless fine crystals from aqueous MeOH, mp 167—169 °C, $[\alpha]_D^{28}$ +24.3° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{50}H_{84}NaO_{19}$ $(M+Na)^+$: 1011.5504. Found: 1011.5510. IR (KBr) cm^{-1}: 3420, 1736, 1655, 1078. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.86, 0.96, 0.97, 1.10, 1.29, 2.02 (3H each, all s, 19, 30, 18, 29, 28, 2′′′′-H_3), 1.61 (9H, s, 21, 26, 27-H_3), 1.98 (1H, t-like, 13-H), 3.26 (1H, dd, J=4.9, 11.6 Hz, 3-H), 4.11 (1H, m, 12-H), 4.71 (1H, dd, J=5.0, 11.3 Hz), 4.91 (1H, br d) (6′-H_2), 4.84 (1H, d, J=7.3 Hz, 1′-H), 5.16 (1H, d, J=7.6 Hz, 1‴-H), 5.25 (1H, t-like, 24-H), 5.34 (1H, d, J=7.6 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 987 $(M-H)^-$, 945 $(M-C_2H_3O)^-$. Positive-ion FAB-MS m/z: 1011 $(M+Na)^+$.

Quinquenoside IV (**4**): Colorless fine crystals from aqueous MeOH, mp 190—192 °C, $[\alpha]_D^{28}$ +39.1° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{90}NaO_{24}$ $(M+Na)^+$: 1145.5720. Found: 1145.5734. Calcd for $C_{54}H_{89}Na_2O_{24}$ $(M+2Na-H)^+$: 1167.5539. Found: 1167.5598. IR (KBr) cm^{-1}: 3413, 1650, 1078. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.13, 1.16, 1.27, 1.41, 1.49, 1.61 (3H each, all s, 30, 19, 18, 29, 28, 26-H_3), 1.67 (6H, s, 21, 27-H_3), 2.08 (1H, t-like, 13-H), 3.33 (1H, dd, J=4.2, 11.3 Hz, 3-H), 4.11 (1H, m, 12-H), 4.68 (1H, d-like, 7-H), 4.85 (1H, d, J=7.4 Hz, 1′-H), 5.06 (1H, d, J=7.6 Hz, 1′′′′-H), 5.12 (1H, d, J=7.6 Hz, 1‴-H), 5.30 (1H, d, J=7.6 Hz, 1″-H), 5.33 (1H, t-like, 24-H), 5.82 (1H, d, J=1.9 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 1121 $(M-H)^-$, 959 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS m/z: 1145 $(M+Na)^+$, 1167 $(M+2Na-H)^+$.

Quinquenoside V (**5**): Colorless fine crystals from aqueous MeOH, mp 192—194 °C, $[\alpha]_D^{28}$ +24.4° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{102}NaO_{28}$ $(M+Na)^+$: 1293.6456. Found: 1293.6481. Calcd for $C_{60}H_{101}Na_2O_{28}$ $(M+2Na-H)^+$: 1315.6275. Found: 1315.6245. IR (KBr) cm^{-1}: 3399, 1655, 1075. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.96, 0.97, 1.09, 1.27, 1.68 (3H each, all s, 19, 30, 18, 29, 28, 27-H_3), 1.64 (6H, s, 21, 26-H_3), 1.98 (1H, t-like, 13-H), 3.26 (1H, dd, J=4.2, 11.2 Hz, 3-H), 4.14 (1H, m, 12-H), 4.88 (1H, d, J=7.6 Hz, 1′-H), 5.00 (1H, d, J=7.9 Hz, 1′′′′-H), 5.08 (1H, d, J=7.6 Hz, 1‴-H), 5.31 (1H, t-like, 24-H), 5.32 (1H, d, J=7.9 Hz, 1″-H), 5.82 (1H, d, J=4.0 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 1269 $(M-H)^-$, 1107 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS m/z: 1293 $(M+Na)^+$, 1315 $(M+2Na-H)^+$.

Alkaline Hydrolysis of Quinquenosides I (1), II (2), and III (3) A solution of quinquenosides (**1**, **2**, **3**, 20 mg each) in 10% aqueous KOH–50% aqueous dioxane (1:1, v/v, 5 ml) was stirred at 37 °C for 15 min. After removal of the solvent from a part (0.1 ml) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1.5 h. The reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl esters of crotonic acid (**a**), *trans*-2-octenoic acid (**b**), and acetic acid (**c**). HPLC conditions: column, YMC-Pack ODS (250 × 4.6 mm i.d.); mobile phase, MeOH–H_2O (70:30, v/v); flow rate, 1.0 ml/min; t_R: **a**, 11.2 min; **b**, 15.2 min; **c**, 8.0 min.

The rest of the reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a crude product (20 mg), which was subjected to ordinary-phase silica-gel column chromatography [600 mg, $CHCl_3$–MeOH–H_2O (7:3:1, lower layer)] to give desacyl saponin [ginsenosides-Rd (**13**, 15.0 mg from **1**, 14.8 mg from **3**) and -Rb_1 (**11**, 15.1 mg from **2**)], which was identified from an authen-

tic sample by TLC, HPLC, $[\alpha]_D$, and ^{1}H- and ^{13}C-NMR spectra comparisons.

Acid Hydrolysis of Quinquenosides IV (4) and V (5) A solution of quinquenosides (**4**, **5**, 2 mg each) in 5% aqueous H_2SO_4–dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was passed through a Sep-Pak C18 cartridge eluting with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) at 60 °C for 1 h. After the reaction was complete, the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i) from **4** and **5**. GLC conditions: column, Supelco SPR™-1, 0.25 mm (i.d.) × 30 m; column temperature, 230 °C; t_R, i: 24.2 min.

D-Galactosamine/Lipopolysaccharide-Induced Liver Injury The method described by Tiegs *et al.*[20] 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-galactosamine hydrochloride (Wako Pure Chemical Industries, Ltd.) and lipopolysaccharide (from *Salmonella enteritidis*, Sigma Chemical Company) was injected intraperitoneally at a dose of 350 and 10 mg/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.

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References and Notes

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