PERGAMON

Phytochemistry 52 (1999) 1133–1139

PHYTOCHEMISTRY

Saponins from the roots of *Panax notoginseng*

Wei Guang Ma[a, b], Masanori Mizutani[a, b], Karl Egil Malterud[c], Shao Long Lu[d], Bertrand Ducrey[e], Satoshi Tahara[a, b,*]

[a]*Department of Applied Bioscience, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan*
[b]*CREST, Japan Science and Technology Corporation, 4-1-8 Honmachi, Kawaguchi 332-0012, Saitama, Japan*
[c]*Institute of Pharmacy, Department of Pharmacognosy, University of Oslo, PO BOX 1068 Blindern, N-0316 Oslo, Norway*
[d]*State-Owned Kunming Chinese Herbs Factory, Nanba, Kunming, Yunnan 650228, People's Republic of China*
[e]*Debio Recherche Pharmaceutique SA, Case postale 368 Route du Levant 146, CH-1920 Martigny, Switzerland*

Received 27 October 1998; received in revised form 11 May 1999

Abstract

The glycosidic fraction from the dried roots of *Panax notoginseng* (Burk.) F.H.Chen exhibited an inhibitory effect on zoospore motility of *Aphanomyces cochlioides*. Further study on this fraction afforded fourteen dammarane-type saponins. Their structures were determined on the basis of spectroscopic and chemical methods. Eleven were known compounds, and the three new ones were 3-*O*-[β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl 3β, 12β, 20 (*S*)-trihydroxydammar-24-ene, 3-*O*-β-D-glucopyranosyl 20-*O*-[α-L-arabinopyranosyl (1 → 2)-β-D-glucopyranosyl] 3β, 12β, 20 (*S*)-trihydroxydammar-24-ene and 6-*O*-β-D-glucopyranosyl 20-*O*-β-D-glucopyranosyl 3β, 6α, 12β, 20 (*S*), 25-pentahydroxydammar-23-ene, respectively. Each saponin displayed good inhibitory effects on zoospore motility. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Panax notoginseng*; Ariliaceae; Ethnic medicinal plant; Ginsenoside; Notoginsenoside; Bioactive saponin

1. Introduction

Panax notoginseng (Burk.) F.H.Chen (Araliaceae), a well-known medicinal plant indigenous to the mountains of Yunnan province, is used by ethnic peoples in Southwestern China for treatment of cardiovascular diseases, inflammation, different body pains, trauma, and internal and external bleeding due to injury. It has also been used as a tonic and haemostatic agent. Due to the demand for its important medicinal use in China, it was largely cultivated in Yunnan and Guang Xi Provinces. Extensive chemical studies on this plant proved the dammarane-type saponins to be the main bioactive principles (Yang, Kasai, Zhou & Tanaka, 1983; Yoshikawa et al., 1997a, 1997b; Zhao, Liu & Yang, 1996; Zhou et al., 1981;).

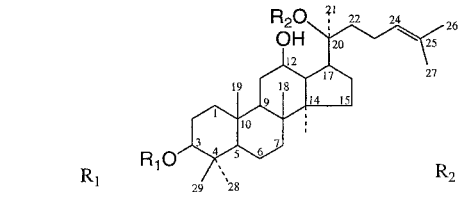
During the course of our studies in search of bioactive constituents from wild plants effective against zoospores of *Aphanomyces cochlioides* (a causative fungus of spinach root rot) (Horio et al., 1992; Horio, Yoshida, Kikuchi, Kawabata & Mizutani, 1993), the crude saponins from the roots of *P. notoginseng* showed an inhibitory effect on the zoospore motility. Guided by a zoospore bioassay, three new and eleven known dammarane-type saponins were isolated and identified from methanolic extracts of the roots. We wish to report here the isolation and structural elucidation of three novel saponins (**1**–**3**), as well as the inhibitory activity of these isolated saponins on the movement of zoospores of *A. cochlioides*.

2. Results and discussion

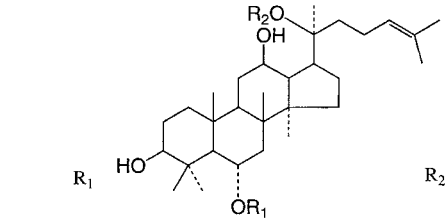
A crude glycosidic fraction from the methanol extracts of the dried roots of *P. notoginseng* was subjected repeatedly to Diaion HP-20, ordinary and reversed-phase silica-gel, as well as Sephadex LH-20

* Corresponding author. Tel.: +81-11-706-3840; fax: +81-11-747-5453.

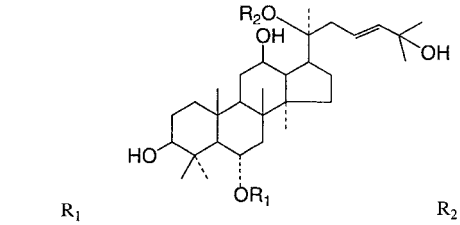
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00364-7



	R_1	R_2
1	Glc-(6-1)-Glc	Glc
2	Glc	Glc-(2-1)-Ara(pyr)
4	Glc-(2-1)-Glc	Glc
5	Glc-(2-1)-Glc	Glc-(6-1)-Glc
6	Glc-(2-1)-Glc	Glc-(6-1)-Ara(pyr)
7	Glc-(2-1)-Glc	Glc-(6-1)-Glc-(6-1)-Xyl



	R_1	R_2
8	Glc	H
9	Glc-(2-1)-Rha	H
10	Glc-(2-1)-Xyl	H
11	Glc	Glc-(6-1)-Glc
12	Glc	Glc
13	Glc-(2-1)-Xyl	Glc
14	Glc-(2-1)-Rha	Glc



	R_1	R_2
3	Glc	Glc

Glc: β-D-glucopyranosyl; Xyl: β-D-xylopyranosyl; Rha: α-L-rhamnopyranosyl; Ara(pyr): α-L-arabinopyranosyl

chromatography to afford fourteen saponins (**1**–**14**). Of these saponins, **4**–**14** were identified as ginsenosides-Rd (**4**) (Tanaka & Yahara, 1978), Rb_1 (**5**) (Yang et al., 1983), Rb_2 (**6**) (Sanada, Kondo, Shoji, Tanaka & Shibata, 1974), Rh_1 (**8**) (Zhou et al., 1981), Rg_2 (**9**) (Sanada et al., 1974), Rg_1 (**12**) (Mutsuura et al., 1983), Re (**14**) (Zhou et al., 1981) and notoginsenosides-R_4 (**7**) (Matsuura et al., 1983), R_2 (**10**) (Zhou et al., 1981), R_3 (**11**) (Matsuura et al., 1983), R_1 (**13**) (Matsuura et al., 1983), all of which were isolated previously from *Panax ginseng* and *P. notoginseng*. The new saponins, **1**–**3**, were named notoginsenosides K–M.

Saponin (**1**) was obtained as a colourless amorphous powder after freeze drying. Its molecular formula was deduced as $C_{48}H_{82}O_{18}$ from the HRFD mass {*m*/*z* 969.5384 (Int% 87.0); $[M]^+$ calcd for $C_{48}H_{82}O_{18}Na$:

Table 1
^{13}C NMR spectral data of saponins **1**–**3** (in pyridine-d_5 at 125 MHz)[a]

	1	**2**	Rd[b]	**3**	Rg_1[c]
Aglycone					
1	39.6	39.6	39.1	39.6	39.5
2	26.6[d]	26.7	26.7	27.9	27.6
3	88.8	88.9	88.9	78.8	78.6
4	39.1	40.0	39.6	40.3	40.1
5	56.3	56.3	56.4	61.4	61.3
6	18.4	18.3	18.5	79.6	77.8
7	35.1	35.1	35.2	45.0	44.9
8	40.0	39.1	40.0	41.1	41.0
9	50.2	50.1	50.2	49.9	49.9
10	36.9	36.8	36.9	39.4	39.5
11	30.7	29.9	30.8	31.0	30.8[d]
12	70.1	70.2	70.2	70.5	70.3
13	49.4	49.4	49.4	49.1	48.9
14	51.3	51.4	51.4	51.4	51.3
15	30.7	30.8	30.8	30.5	30.6[d]
16	26.7[d]	26.7	26.7	26.4	26.4
17	51.6	51.5	51.7	52.2	51.6
18	16.0	15.9	15.9[d]	17.6[d]	17.4[d]
19	16.2	16.2	16.3[d]	17.6[d]	17.4[d]
20	83.4	83.2	83.3	83.1	83.3
21	22.3	22.3	22.4	23.2	22.3
22	36.1	36.0	36.0	39.6	35.9
23	23.2	23.0	23.2	126.5	23.2
24	125.9	125.8	125.9	138.1	125.8
25	131.0	131.0	130.9	81.3	130.9
26	25.6	25.7	25.8	25.4[d]	25.7
27	17.9	17.7	16.6[d]	25.1[d]	17.7[d]
28	28.1	28.0	28.0	31.7	31.6
29	16.7	16.5	17.3[d]	16.3	16.2[d]
30	17.4	17.3	17.8[d]	16.9	17.0[d]
Sugar moieties					
3-Glc					
1	106.9	105.0	105.0		105.7
2	74.7	75.1	83.3		75.3
3	78.2[d]	78.1[d]	78.1[d]		80.0[d]
4	71.4[d]	71.6	71.6		71.6[d]
5	77.0	78.2[d]	78.1[d]		79.3[d]
6	70.2	62.8[d]	62.7		62.9
1	105.3		105.9		
2	75.7		77.0		
3	78.3[d]		79.1[d]		
4	71.6[d]		71.6		
5	78.6[d]		78.1[d]		
6	62.7[d]		62.7		
20-Glc					
1	98.0	98.4	98.2	98.3	98.1
2	75.1	83.4	75.0	75.4[d]	74.9
3	78.3[d]	77.1	78.1[d]	78.1[d]	78.8[d]
4	71.8[d]	71.6	71.6	71.5[d]	71.3[d]
5	79.1	78.3[d]	78.1[d]	78.6[d]	77.8[d]
6	63.0[d]	62.6[d]	62.7	63.0[d]	62.6
Ara					
1		106.0			
2		72.5			
3		74.8			
4		69.6			
5		65.8			
6-Glc					
1				105.9	
2				75.3[d]	
3				78.3[d]	

Table 1 (*continued*)

	1	2	Rd[b]	3	Rg_1[c]
4				71.8[d]	
5				79.6	
6				62.9[d]	

[a] Assignments are based on HMQC, HMBC, ^1H–^1H COSY and ROESY spectra.
[b] Tanaka and Yahara (1978).
[c] Matsuura et al. (1983).
[d] Data in the vertical column are interchangeable.

969.5400}, FD mass and ^{13}C NMR spectral data. The FD mass spectrum of **1** exhibited quasimolecular ions at *m/z* 985 $[M+K]^+$ and 969 $[M+Na]^+$. The positive mass fragments appeared at *m/z* 807 $[M-hexose+Na]^+$, 748 $[M-hexose-2H_2O]^+$, 586 [748–hexose]$^+$ and 424 [586–hexose]$^+$, indicating the molecule contained three hexoses. Acid hydrolysis of **1** with HCl on a HPTLC plate (Ma, Wang, Zeng & Yang, 1991) proved the hexose was glucose. The ^1H NMR spectrum of **1** showed clearly the anomeric proton signals of glucoses at δ 4.91 (J = 8.0), 5.09 (J = 8.0) and 5.12 (J = 8.2), respectively. The large coupling constants (≥8.0 Hz) of the anomeric protons suggested the β-configuration of the glucose moieties. The carbon signals in the ^{13}C NMR spectrum of **1** were found to be nearly superimposable on those of ginsenoside-Rd (**4**), except for a few signals due to one of the glucose moieties (Table 1). Similar to compound **4**, compound **1** had only one glucose linked with the oxygen at the C-20 of the aglycone moiety, as shown by its FD mass spectrum. It is known that the glycosyl linkages of the C-20 hydroxyl group of dammarane saponins are rather unstable and no fragment peaks having a hydroxyl group or a glycosyl linkage at C-20 can be observed in the mass spectrum (Komori, Tanaka & Nagai, 1974; Kasai, Matsuura, Tanaka, Sanada & Shoji, 1977). The FD mass spectrum of **1**, as well as all the other saponins we isolated and determined from the target plant, confirmed that cleaving the glycosyl linkage at C-20 and losing two molecules of water to form a stable conjugated system at $\Delta^{12,13}$ and $\Delta^{17,20}$ occurred prior to the cleavage of glycosyl linkages at other positions. As described previously, the mass fragments at *m/z* 807 $[M-glucose+Na]^+$ and 748 $[M-glucose-2H_2O]^+$ of **1** demonstrated that there was only one glucose at the C-20 position. The disaccharide structures bonded to the C-3 position of the aglycone unit were characterized by its carbon data and confirmed by 2D NMR spectroscopic techniques. Upon comparing the ^{13}C NMR spectrum of **1**, to that of Rd (**4**) (Table 1), one of the C-6 signals of β-glucopyranosyl moiety was deshielded while that of C-5 was shielded, while other carbon signals remained almost unchanged. This glycosylation shift suggested that the terminal glucose unit was linked with the C-6 position of the inner glucose unit (Kasai, Suzuo, Asakawa & Tanaka, 1977; Itano, Yamasaki, Kihara & Tanaka, 1980). As illustrated in Fig. 1, the long range correlation between the H-1 (G-2) and the C-3, H-1 (G-3) and the C-6 (G-2) observed from the HMBC (heteronuclear multiple bond correlation) spectrum, as well as through-space interactions across glycosidic bonds observed in the ROESY (rotating frame nuclear Overhauser effect spectroscopy) spectrum, namely, between the H-1 (G-3) and the H-4, 5, 6 (G-2), H-1 (G-2) and the H-2, 3, 28, 29, confirmed not only the 1 → 6 linkage of the disaccharide but also the rest of the molecular structure. Consequently, the structure of saponin **1** was established as 3-*O*-[β-D-glucopyranosyl

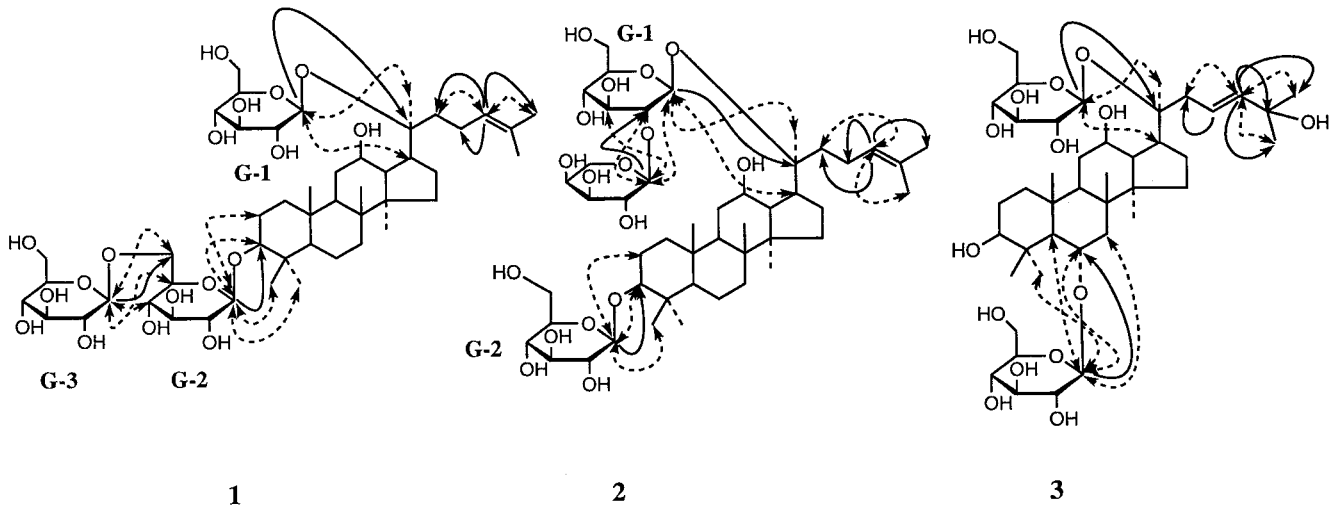


Fig. 1. Key HMBC and ROESY correlations for establishing sugar sequence, their locations on the aglycone and the double bond position in the side chain.

(1 → 6)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl 3β, 12β, 20 (*S*)-trihydroxydammar-24-ene.

Saponin **2** was also obtained as a colourless amorphous powder. The molecular formula of $C_{47}H_{80}O_{17}$ was determined from its HRFD mass {*m*/*z* 939.5272 (Int% 100); $[M]^+$ calcd for $C_{47}H_{80}O_{17}Na$: 939.5294}, FD mass and ^{13}C NMR spectral data. The quasimolecular ions in the FD mass spectrum appeared at *m*/*z* 955 $[M+K]^+$ and 939 $[M+Na]^+$, with prominent fragments at *m*/*z* 807 $[M–pentose+Na]^+$, 586 [807–hexose–$2H_2O$–Na]$^+$ and 424 [586–hexose]$^+$. Acid hydrolysis of **2** (same treatment as **1**) afforded arabinose and glucose as sugar components. According to its HMQC and ^1H–^1H COSY spectra, the signals due to the sugar moieties were assigned to two moieties of glucose and one arabinose (Tables 1 and 2). The configurations of the glucose and arabinose were identified as β- and α-type by comparing their proton coupling constants ($J_{1,2}=8.4$ Hz for β-glucose, $J_{1,2}=5.6$ Hz for α-arabinose) with reported data (Ma et al., 1991; Renault et al., 1997). The ^{13}C NMR data due to the aglycone moiety of **2** was in good agreement with those of **1**, suggesting that **2** and **1** possessed the same aglycone moiety and the same glycosylation positions on the aglycone. It was known that arabinose was the terminal sugar moiety of the disaccharide unit from its FD mass data (*m*/*z* 807 $[M–pentose+Na]^+$), however, it could be either linked with the glucose at the C-3 or C-20 positions. A downfield-shifted ^{13}C NMR resonance due to the glycosylation of one glucose appeared at δ 83.4, together with a small highfield shifted signal resonating at δ 77.1, indicating the glycosylation linkage between the disaccharide units was due to a 1 → 2 linkage (Yang et al., 1983). To confirm the structure of the disaccharide and its bonding position with the aglycone, HMBC and ROESY experiments were performed. Observation of a strong correlation in the HMBC spectrum between H-1 of arabinose and C-2 of inner glucose (G-1), as well as the Nuclear Overhauser effects in the ROESY spectrum between H-1 of arabinose and H-1, H-3 of inner glucose (G-1) (Fig. 1) confirmed the structure of the disaccharide. Moreover, the HMBC spectrum also exhibited intense long-range correlation between H-1 (G-1) and C-20 of aglycone unit. In addition, ROESY correlations were observed between H-1 (G-1) and H-17, H-21 of the aglycone unit contributing further evidence for attachment of the disaccharide moiety to C-20 of the aglycone. Thus the structure of compound **2** was elucidated as 3-*O*-β-D-glucopyranosyl 20-*O*-[α-L-arabinopyranosyl (1 → 2)-β-D-glucopyranosyl] 3β, 12β, 20 (*S*)-trihydroxydammar-24-ene.

Saponin **3** showed quasimolecular ions at *m*/*z* 855 $[M+K]^+$ and 839 $[M+Na]^+$ in the FD mass spectrum. Prominent mass fragments appeared at *m*/*z* 636 $[M–hexose–H_2O]^+$, 618 $[M–hexose–2H_2O]^+$ and 457 $[618–hexose+H]^+$. Acid hydrolysis of **3** as for **1** afforded glucose as the sugar component. This information, together with the data from the ^{13}C NMR spectrum, allowed its molecular formula to be assigned as $C_{42}H_{72}O_{15}$ which was confirmed by its HRFD mass data {*m*/*z* 839.4738 (Int% 55.5); $[M]^+$ calcd for $C_{42}H_{72}O_{15}Na$: 839.4770}. The ^{13}C NMR chemical shifts due to the aglycone moiety and the sugar moieties attached at the C-6 (δ_C 79.6) and C-20 (δ_C 83.1) of **3** were in good agreement with those of ginsenoside Rg_1 (**12**) (Table 1). However, the aglycone M_r of **3** was 16 mass units higher than that of **12**. In addition, differences between the two saponins were also observed in the side chain. Compound **12**, which has a side chain common to the ginsenosides [2] displayed resonances at δ_C 125.8 (*d*) and 130.9 (*s*) for C-24 and C-25, respectively, while compound **3** had double bond carbons resonating at 126.5 (*d*) and 138.1 (*d*). A comparison of the chemical shifts of the two olefinic carbons of **3** with those of reported ginsenosides, such as chikusetsusaponin L9a (Yahara, Kasai & Tanaka, 1977) and notogisenosides-A, E, H (Yoshikawa et al., 1997a, 1997b) suggested that the double bond in the side chain of **3** was located between C-23 and C-24. The chemical shift of δ 81.3 for C-25 of **3** confirmed the presence of a hydroxyl group at this position. The long range correlation observed from the HMBC (between H-23 and C-22, H-24 and C-25, C-26, C-27) and ROESY (between H-22 and H-24, H-24 and H-25, H-26, H-27) spectra confirmed the position of the double bond in the side chain, as well as the rest of the structure shown in Fig. 1. Following comprehensive analysis of all 2D NMR spectra, the carbon and proton signals of **3** were unequivocally assigned as shown in Tables 1 and 2. The structure of **3** was thus 6-*O*-β-D-glucopyranosyl 20-*O*-β-D-glucopyranosyl 3β, 6α, 12β, 20 (*S*), 25-pentahydroxydammar-23-ene.

Compound **3** seems to be derived from compound **12** (Rg_1) by photosensitized oxygenation like those previously reported from the same plant (Yoshikawa et al., 1997a, 1997b).

The inhibitory effect on zoospore motility was evaluated for all compounds against *A. cochlioides* as described elsewhere (Horio et al., 1992), except for the modified preparation of samples: the sample was prepared in water solution at a certain concentration (ppm) and this solution was drawn into a micropipette with ring mark (1–5 μl) at a certain volume followed by drawing the same volume of zoospore suspension of *A. cochlioides*; the behavior of the zoospores were observed under a microscope. The minimum concentration for compounds **2**, **5** and **9** for motility inhibition was found to be 200 ppm, for **4** and **10** was 100 ppm and for **1** and **8** was 50 ppm, while compounds **3**, **6**–**7** and **11**–**14** showed no activity at 200 ppm. The minimum amount of positive control (portulaca con-

Table 2
^{1}H NMR spectral data of saponins **1**–**3** (in pyridine-d_5 at 500 MHz)[a]

	1	**2**	**3**
Aglycone			
1	1.58[b]	1.56[b]	1.72[b]
	0.78[b]	0.88[b]	0.94[b]
2	2.21[b]	2.10[b]	1.92[b]
	1.84[b]	1.98[b]	1.83[b]
3	3.38 *dd* (10.2, 3.4)	3.25 *dd* (10.4, 2.5)	3.49 *dd* (10.9, 4.2)
5	0.69 *dd* (11.3, 2.5)	0.79 *d* (12.3)	1.42 *d* (12.4)
6	1.60[b]	1.50[b]	4.41 *dd* (12.4, 3.4)
	1.32[b]	1.34[b]	
7	1.45[b]	1.38[b]	2.51 *dd* (12.4, 3.2)
	1.17[b]	1.12[b]	1.86 *dd* (11.3, 3.4)
9	1.36[b]	1.33[b]	1.51[b]
11	1.98[b]	2.12[b]	2.05[b]
	1.54[b]	1.51[b]	1.52[b]
12	4.02[b]	4.08[b]	3.95[b]
13	1.94[b]	1.98[b]	2.02 *dd* (12.8, 10.4)
15	1.59[b]	1.61[b]	1.65[b]
	0.98[b]	0.92[b]	1.04[b]
16	1.82[b]	1.78[b]	1.69[b]
	1.36[b]	1.46[b]	1.34[b]
17	2.59 *dd* (12.1, 8.2)	2.23 *dd* (10.1, 7.3)	2.31 *dd* (12.1, 8.2)
18	0.96 *s*	0.95 *s*	1.18 *s*
19	0.92 *s*	0.79 *s*	1.06 *s*
21	1.62 *s*	1.54 *s*	1.54 *s*
22	3.14 *dd* (11.2, 7.8)	3.04 *dd* (10.4, 8.8)	3.14 *dd* (11.2, 7.8)
	2.36[b]	2.56[b]	2.71 *dd* (11.2, 7.6)
23	2.61[b]	2.41[b]	6.12[b]
	2.59[b]	2.23[b]	
24	5.29 *t* (9.8)	5.25 *t* (8.2)	6.14 *dd* (12.4, 0.5)
26	1.59 *s*	1.61 *s*	1.72 *s*
27	1.60 *s*	1.61 *s*	1.72 *s*
28	1.24 *s*	1.26 *s*	2.05 *s*
29	0.94 *s*	1.10 *s*	1.61 *s*
30	0.96 *s*	0.96 *s*	0.72 *s*
Sugar moieties			
3-Glc			
1	4.91 *d* (8.0)	4.89 *d* (8.4)	
2	4.02[b]	3.97[b]	
3	3.98[b]	3.92[b]	
4	3.98[b]	4.12[b]	
5	4.12[b]	4.22[b]	
6	4.74 *d* (10.3)	4.51 *d* (12.2)	
	4.31 *d* (10.3)	4.29[b]	
1	5.09 *d* (8.0)		
2	4.03[b]		
3	4.05[b]		
4	4.25[b]		
5	4.21[b]		
6	4.50 *d* (11.2)		
	4.28 *d* (11.2)		
20-Glc			
1	5.12 *d* (8.2)	5.08 *d* (8.4)	5.21 *d* (7.6)
2	3.91[b]	4.39[b]	3.98[b]
3	3.96[b]	4.13[b]	4.02[b]
4	4.13[b]	4.08[b]	4.12[b]
5	4.22[b]	4.12[b]	4.24[b]
6	4.50 *d* (11.2)	4.51 *d* (12.2)	4.49 *d* (11.2)
	4.28 *d* (11.2)	4.29[b]	4.31 *d* (11.2)
Ara			
1		5.38 *d* (5.6)	

(continued on next page)

Table 2 (*continued*)

	1	2	3
2		4.12[b]	
3		3.95[b]	
4		4.45[b]	
5		3.91[b]	
		4.28[b]	
6-Glc			
1			4.92 *d* (7.8)
2			4.09[b]
3			4.11[b]
4			4.18[b]
5			4.21[b]
6			4.49 *d* (11.2)
			4.31 *d* (11.2)

[a] Assignments are based on HMQC, HMBC, ^{1}H–^{1}H COSY and ROESY spectra. Coupling constants (*J* values in Hz) are shown in parentheses.

[b] Overlapped signals are reported without designating multiplicity.

stituents) was 30 ppm tested by particle method (Mizutani, Hashidoko & Tahara, 1998).

3. Experimental

3.1. General

Mps: uncorr. NMR: Brüker-AM500 spectrometer (C_5D_5N used as solvent and reference); Mass spectra: JEOL JMS-SX 102 A instrument; Optical rotation: JASCO DIP-370 polarimeter (in MeOH); IR: KBr; TLC: Merck HPTLC RP-18 WF254 plates; Detection: spraying with Godin reagent followed by 5% H_2SO_4 in MeOH; saccharide identification was carried out on Merck silica gel TLC plates (Ma et al., 1991).

3.2. Plant material

The roots of *P. notoginseng* (cultivated in Yunnan province, China) was purchased in a drug company in Kunming city (Yunnan) and was confirmed by Dr Sun Hang (Kunming Botanical Institute). A voucher specimen (KUN No. 0560429) was deposited at the herbarium of the Kunming Botanical Institute (Yunnan, China).

3.3. Extraction and isolation

The air-dried roots of the plant (115 g) were powdered and then extracted in boiling MeOH. After filtration, excess solvent was removed under reduced pressure to give a residue (ca. 10 g). This MeOH extract was suspended in water and defatted with hexane followed by partitioning with *n*-BuOH. The combined BuOH layers were concd to dryness, affording a crude glycosidic fraction (ca. 8 g). The crude glycosidic fraction was chromatographed on a column of highly porous polymer resin (Diaion HP-20, Nippon Rensui CO.) eluting with 10% MeOH, 30% MeOH, 60% MeOH (aqueous/MeOH) and MeOH to provide four fractions (1–4). Fraction 4 (MeOH, ca. 3 g) was separated on silica gel eluting with $CHCl_3$–MeOH–H_2O (7:3:0.5 and then 6:4:1) to give pure compound **5** (50 mg, Rb_1) and crude **7**, together with twenty subfractions (A1–A20). Pure **7** (15 mg, R_4) was obtained from Lobar column (RP-18, 40–63 μm, 1.5 × 15 cm id MERCK) chromatography using MeOH–H_2O (7:3) as an eluant. Fractions A6 and A12 were purified by using Sephadex LH-20 eluted with MeOH to afford compounds **8** (20 mg, Rh_1) and **4** (40 mg, Rd), respectively. Fraction A9 provided two pure compounds, **9** (20 mg, Rg_2) and **10** (10 mg, R_2), after purification on a Lobar column (RP-18, 40–63 μm, 1.5 × 15 cm id MERCK, MeOH–H_2O; 6:4). Fraction A11 was subjected to Lobar column chromatography (RP-18, 40–63 μm, 1.5 × 15 cm id MERCK; MeOH–H_2O; 75:25) to give pure **2** (5 mg). Fraction 12 (combined with A13–15) was separated on silica gel eluting with $CHCl_3$–MeOH–H_2O (7:3:0.5) to give pure **1** (8 mg). Compounds **11** (8.3 mg, R_3) and **6** (10 mg, Rb_2) were obtained with the same separation condition (Lobar column RP-18, 40–63 μm, 1.5 × 15 cm id MERCK; MeOH–H_2O; 6:4) from the fractions A16 and A17 (A17 combined with A18–19), respectively. Fraction 3 (60% MeOH, ca. 4 g) was separated on silica gel eluting with $CHCl_3$–MeOH–H_2O (7:3:0.5) to afford pure compound **12** (1 g, Rg_1) and seven subfractions (B1–B7). Repeated purification of fraction B2 on Lobar column chromatography (RP-18, 40–63 μm, 1.5 × 15 cm id MERCK; MeOH–H_2O; 1:1) to provided compounds **3** (10 mg), **13** (35 mg, R_1) and **14** (12 mg, Re), respectively.

3.4. Saponin **1**

Colourless amorphous powder, mp 190–194°. HPTLC RP-18 (MeOH–H_2O, 6:4) R_f 0.14; Silica gel TLC ($CHCl_3$–MeOH–H_2O, 7:3:0.5) R_f 0.24; $[\alpha]_D^{21}$ +13.2° (*c* 0.45, MeOH); IR(KBr) ν_{max} cm^{-1} 3387, 2926, 1638, 1385, 1077, 652; HRFD-MS *m/z* 969.5384 (Int% 87.0) [$C_{48}H_{82}O_{18}Na$]; FD-MS *m/z* (rel int%): 985(18) $[M+K]^+$, 969(100) $[M+Na]^+$, 807(8) [M–glucose+Na]$^+$, 748(21) [M–glucose–$2H_2O$]$^+$, 586(53) [748–glucose]$^+$, 424(7) [586–glucose]$^+$; ^{13}C and ^1H NMR spectral data: Tables 1 and 2.

3.5. Saponin **2**

Colourless amorphous powder, mp 189–192°. HPTLC RP-18 (MeOH–H_2O, 8:2) R_f 0.27; Silica gel TLC ($CHCl_3$–MeOH–H_2O, 6:4:1) R_f 0.63; $[\alpha]_D^{21}$ +34.5 (*c* 0.08, MeOH); IR(KBr) ν_{max} cm^{-1} 3387, 2926, 1638, 1385, 1077, 652; HRFD-MS *m/z* 939.5272 (Int% 100); [$C_{47}H_{80}O_{17}Na$]; FD-MS *m/z* (rel int%): 955(34) $[M+K]^+$, 939(100) $[M+Na]^+$, 807(32) [M–arabinose+Na]$^+$, 586(43) [807–glucose–$2H_2O$–Na]$^+$, 424 (12) [586–glucose]$^+$; ^{13}C and ^1H NMR spectral data: Tables 1 and 2.

3.6. Saponin **3**

Colourless amorphous powder, mp 208–212°. HPTLC RP-18 (MeOH–H_2O, 1:1) R_f 0.35; Silica gel TLC ($CHCl_3$–MeOH–H_2O, 7:3:0.5) R_f 0.28; $[\alpha]_D^{21}$ +31.2 (*c* 0.25, MeOH); IR(KBr) ν_{max} cm^{-1} 3387, 2933, 1655, 1385, 1041; HRFD-MS *m/z* 839.4738 (Int% 55.5) [$C_{42}H_{72}O_{15}Na$]; FD-MS *m/z* (rel int%): 855(25) $[M+K]^+$, 839(100) $[M+Na]^+$, 636(80) [M–glucose–H_2O]$^+$, 618(98) [M–glucose–$2H_2O$]$^+$, 457(17) [618–glucose+H]$^+$; ^{13}C and ^1H NMR spectral data: Tables 1 and 2.

Acknowledgements

The authors are grateful to Mr Kenji Watanabe and Dr Eri Fukushi (GC-MS & NMR laboratory, Faculty of Agriculture, Hokkaido University) for measuring 2D NMR and mass spectra. The first author wishes to express his sincere thanks to Prof. Hajime Iwamura (Department of Applied Life Science, Graduate School of Agriculture, Kyoto University) for his kindness in accepting him as a member of his research project of CREST for JST.

References

Horio, T., Kawabata, Y., Takayama, T., Tahara, S., Kawabata, J., Fukushi, Y., Nishimura, H., & Mizutani, J. (1992). *Experientia*, *48*, 411.

Horio, T., Yoshida, K., Kikuchi, H., Kawabata, J., & Mizutani, J. (1993). *Phytochemistry*, *33*, 807.

Itano, K., Yamasaki, K., Kihara, C., & Tanaka, O. (1980). *Carbohydrate Research*, *87*, 27.

Kasai, R., Matsuura, K., Tanaka, O., Sanada, S., & Shoji, J. (1977). *Chemical and Pharmaceutical Bulletin*, *25*, 3277.

Kasai, R., Suzuo, M., Asakawa, J., & Tanaka, O. (1977). *Tetrahedron Letters, 2*, 175.

Komori, T., Tanaka, O., & Nagai, Y. (1974). *Organic Mass Spectrometry*, *9*, 744.

Ma, W. G., Wang, D. Z., Zeng, Y. L., & Yang, C. R. (1991). *Phytochemistry*, *30*, 3401.

Matsuura, H., Kasai, R., Tanaka, O., Saruwatari, Y-I., Fuwa, T., & Zhou, J. (1983). *Chemical and Pharmaceutical Bulletin*, *31*, 2281.

Mizutani, M., Hashidoko, Y., & Tahara, S. (1998). *FEBS Letters*, *438*, 236.

Renault, J. H., Ghedira, K., Thepenier, P., Lavaud, C., Zeches-Hanrot, M., & Le Men-Olivier, L. (1997). *Phytochemistry*, *44*, 1321.

Sanada, S., Kondo, N., Shoji, J., Tanaka, O., & Shibata, S. (1974). *Chemical and Pharmaceutical Bulletin*, *22*, 2407.

Tanaka, O., & Yahara, S. (1978). *Phytochemistry*, *17*, 1353.

Yahara, S., Kasai, R., & Tanaka, O. (1977). *Chemical and Pharmaceutical Bulletin*, *25*, 2041.

Yang, C. R., Kasai, R., Zhou, J., & Tanaka, O. (1983). *Phytochemistry*, *22*, 1473.

Yoshikawa, M., Murakami, T., Ueno, T., Hirokawa, N., Yashiro, K., Murakami, N., Yamahara, J., Matsuda, H., Saijoh, R., & Tanaka, O. (1997a). *Chemical and Pharmaceutical Bulletin*, *45*, 1056.

Yoshikawa, M., Murakami, T., Ueno, T., Yashiro, K., Hirokawa, N., Murakami, N., Yamahara, J., Matsuda, H., Saijoh, R., & Tanaka, O. (1997b). *Chemical and Pharmaceutical Bulletin*, *45*, 1039.

Zhao, P., Liu, Y. Q., & Yang, C. R. (1996). *Phytochemistry*, *41*, 1419.

Zhou, J., Wu, M. Z., Taniyasu, S., Besso, H., Tanaka, O., Saruwatari, Y., & Fuwa, T. (1981). *Chemical and Pharmaceutical Bulletin*, *29*, 2844.