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NOTE

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New dammarane-type saponin from roots of *Panax notoginseng*

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Abstract A new ginsenoside and 22 known compounds were isolated from the roots of *Panax notoginseng* (Araliaceae). The structure of the new compound was elucidated from 2D-NMR and other spectral evidence.

Keywords *Panax notoginseng* · Araliaceae · Saponin · Notopanaxoside A

Introduction

Notoginseng is prepared by a curious processing method from the main root with a small rhizome of *Panax notoginseng* (Burk.) F.H. Chen. Notoginseng has been employed in Asia for many centuries in treatment of trauma and bleeding due to internal and external injury [1, 2]. An extensive chemical study was made of the constituents of notoginseng to determine its bioactive principles and saponins, flavonoids, polysaccharides, and acetylenic compounds [3]. In the present study, we report the isolation and structural elucidation of one new and 22 known compounds from the methanol extract of the roots of *P. notoginseng*.

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Materials and methods

General experimental procedures

Infrared (IR) spectra were recorded on a 1720 infrared Fourier transform spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). Optical rotations were measured with a DIP-370 digital polarimeter (JASCO, Tokyo, Japan). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, referenced to TMS) spectra were measured on an AVANCE 400 Fourier transform spectrometer (Bruker, Germany) and MS spectra were measured on a JMSD-300 mass spectrometer (JEOL). Column chromatographic supports were silica gel 60 N (63–210 mm; Kanto Kagaku, Tokyo, Japan); TLC: silica gel $60F_{254}$ (Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA), TOYOPEARL (TOSOH, Tokyo, Japan); and HPLC: silica gel (YMC-Pack SIL-06, 5 μm; YMC, Kyoto, Japan), gel-permeation column (H2001 and H2002; Shodex), and R-ODS-5 (YMC).

Plant material

The roots of *P. notoginseng* (cultivated in Yunnan Province, China) were donated by Yamada Yakken, Osaka, Japan. A voucher specimen (TU03005) was deposited at the herbarium of the Faculty of Pharmaceutical Sciences, University of Tokushima.

Extraction and isolation

The dried and cut roots of *P. notoginseng* (4.5 kg) were extracted three times with MeOH at 60°C. The MeOH extracts were concentrated in vacuo to give a residue (825 g), which was extracted and partitioned between *n*-hexane, AcOEt, *n*-BuOH, and H_2O to yield *n*-hexane extract (29.3 g), AcOEt extract (15.8 g), and *n*-BuOH extract (522 g). The *n*-hexane extract and AcOEt extract

were charged on an SiO_2 column chromatography eluted with *n*-hexane–AcOEt (6:1) to obtain ten fractions: 1 (5.7 g), 2 (5.6 g), 3 (1.9 g), 4 (2.1 g), 5 (2.1 g), 6 (2.2 g), 7 (1.5 g), 8 (0.7 g), 9 (4.1 g), and 10 (2.3 g). Each fraction was repeatedly purified by silica gel column chromatography, HPLC, and preparative TLC. Compounds were isolated from the fractions as follows:

- Fraction 1: compounds **10** (59 mg), **14** (12 mg)
- Fraction 2: compound **9** (2 mg)
- Fraction 3: compound **4** (5 mg)
- Fraction 4: compounds **6** (3 mg), **7** (9 mg), **8** (1 mg), **15** (5 mg), **17** (8 mg), **19** (6 mg)
- Fraction 5: compounds **16** (9 mg), **18** (11 mg), **20** (3 mg), **22** (8 mg)
- Fraction 6: compound **5** (36 mg)
- Fraction 7: compounds **11** (17 mg), **23** (14 mg)
- Fraction 8: compounds **12** (4 mg), **13** (6 mg)
- Fraction 9: compounds **1** (13 mg), **2** (916 mg)
- Fraction 10: compound **3** (704 mg)

The *n*-BuOH extract was charged on an SiO_2 column chromatography eluted with $CHCl_3$–MeOH (100:1) to obtain **21** (13 mg).

Determination of glucose in **1**

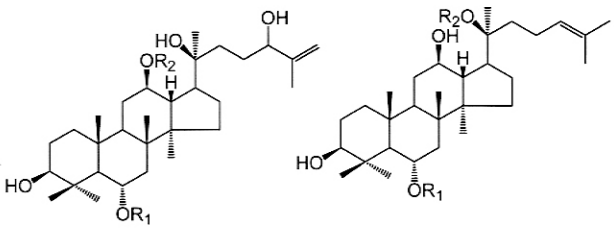
Compound **1** (3 mg) was refluxed with 5% hydrochloric acid for 2 h. Then the product was identified by comparing it with TLC of authentic glucose.

Results and discussion

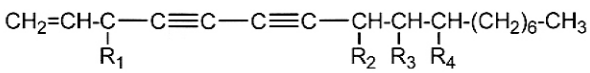
The methanol extract of roots of *P. notoginseng* was partitioned between *n*-hexane, AcOEt, *n*-BuOH, and H_2O. The *n*-hexane extract and AcOEt extract were purified by applying silica gel column chromatography, HPLC, and preparative TLC, repeatedly, to yield a new compound **1** (13 mg) and 22 known compounds (**2**–**23**).

Compounds **2**–**23** were identified as ginsenoside Rh_1 (**2**) [4], ginsenoside Rg_1 (**3**) [5], PQ-2 (**4**) [6], panaxytriol (**5**) [7], panaxydol chlorohydrine (**6**) [7], panaxydol (**7**) [7], (8*E*)-1,8-hepatadecadiene-4,6-diyene-3,10-diol (**8**) [8], ginsenoyne E (**9**) [7], panaxynol (**10**) [8], aromadendrane-7α,11α-diol (**11**) [9], aromadendrane-7β,11α-diol (**12**) [9], alloaromadendrane-7α,11α-diol (**13**) [9], spathulenol (**14**), 1β,6α-dihydroxyeudesm-4(15)ene (**15**) [10], 3-hydroxy-4-methoxybenzoic acid (**16**), cinnamic acid (**17**), *p*-coumaric acid 4-hydroxyphenyl ester (**18**), 2-methoxy-1H-pyrrole (**19**), succinic acid methyl ester (**20**), succinic acid monobuthyl ester (**21**), 5-hydroxy-3-methoxy dec-2-enoic acid (**22**), β-sitosterol-β-D-glucoside (**23**) [11]. Compounds **4**, **16**, and **19** were isolated for the first time from *P. notoginseng*.

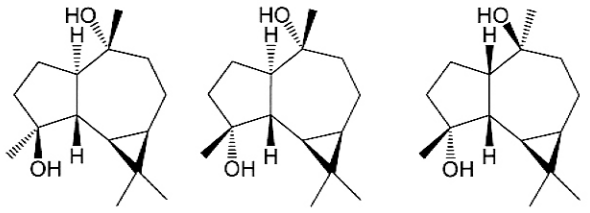
Compound **1** indicated the molecular formula $C_{36}H_{62}O_{10}$ by HRFAB-MS. The IR spectrum indicated the presence of hydroxyl (3,388 cm^{-1}). The ^1H NMR spectrum of **1** showed an exo-methylene [δ 5.26, 4.92 (both 1H, s, H-26)], a vinyl methyl group [δ 1.89 (3H, s)], and a glucose [δ 5.03 (1H, d, J=7.6 Hz, H-1′), 4.52 (1H, dd, J=2.8, 11.5 Hz, H-6′), 4.37 (1H, dd, J=5.4,



	R_1	R_2		R_1	R_2
1	glc	H	**2**	glc	H
1a	H	glc	**3**	glc	glc



	R_1	R_2	R_3	R_4
4	-H, -OH	-OH	-O-	
5	-H, -OH	-H	-OH	-OH
6	-H, -OH	-H	-OH	-Cl
7	-H, -OH	-H	-O-	
8	-H, -OH			-OH
9	=O	-H	-O-	
10	-H, -OH	-H		



11 **12** **13**

16 **19**

Fig. 1 The structure of isolated compounds (**1**–**13**, **16**, and **19**) from *P. notoginseng* and chikusetsusaponin-L_{9bc} (**1a**)

Table 1 ^{13}C NMR data for **1** (notopanaxoside A), **2** (ginsenoside Rh_1), and **1a** (chikusetsusaponin-L_{9bc}) (in C_5D_5N)

	1	2	1a		1	2	1a
1	39.5	39.5	39.0	6-glc			
2	28.0	28.0	27.3	1′	106.1	106.0	
3	78.6	78.7	78.4	2′	75.5	75.6	
4	40.3	40.4	40.3	3′	79.7	79.6	
5	61.5	61.5	61.7	4′	71.9	72.1	
6	80.1	80.1	67.6	5′	78.2	78.1	
7	45.5	45.3	47.2	6′	63.1	63.3	
8	41.2	41.2	41.0	12-glc			
9	50.3	50.3	49.9	1′			100.1
10	39.7	39.8	39.4	2′			75.1
11	32.2	32.1	28.0	3′			78.3
12	71.1	71.1	78.6	4′			71.1
13	48.3	48.4	46.4	5′			77.3
14	51.7	51.7	52.0	6′			62.4
15	31.3	31.3	31.3				
16	26.9	26.7	26.9				
17	54.9	54.8	54.2				
18	17.4	17.5	17.4				
19	17.7	17.7	17.3				
20	73.2	73.2	73.5				
21	27.4	27.1	27.0				
22	32.3	35.9	32.2				
23	30.7	23.1	30.0				
24	76.1	126.4	75.9				
25	150.0	130.8	150.1				
26	109.9	25.9	109.7				
27	18.5	17.7	18.6				
28	31.7	31.8	31.9				
29	16.4	16.4	16.4				
30	16.9	16.9	17.4				

11.5 Hz, H-6′), 4.24 (2H, m, H-3′, 4′), 4.09 (1H, dd, $J=7.6$, 8.4 Hz, H-2′), 3.95 (1H, m, H-5′)]. The ^{13}C NMR spectra of **1** were very similar to those of **2**, except for the signals due to the side chain part of the sapogenol moiety. They were also similar to the side chain moiety of **1a** (chikusetsusaponin-L_{9bc}) [12] (Fig. 1), except for the signals due to the sapogenol moiety. In the HMBC experiment of **1**, long-range correlations were observed between the following protons and carbons [H-1′ and C-6, H-27 and C-24, 25, 26]. From those results, the structure of **1** named notopanaxoside A was assigned as shown.

Notopanaxoside A (**1**)

Colorless amorphous. $[\alpha]_D^{25}+33.1°$ (c 0.45, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3,388. HRFAB-MS m/z: 655.4440 $[M+H]^+$ (calculated for $C_{36}H_{63}O_{10}$: 655.4421). ^1H NMR (C_5D_5N) δ_H: 5.26 (1H, s, H-26), 5.03 (1H, d, $J=7.6$ Hz, H-1′), 4.92 (1H, s, H-26), 4.52 (1H, dd, $J=2.8$, 11.5 Hz, H-6′), 4.42 (1H, m, H-6), 4.41 (1H, m, H-24), 4.37 (1H, dd, $J=5.4$, 11.5 Hz, H-6′), 4.24 (2H, m, H-3′, 4′), 4.09 (1H, dd, $J=7.6$, 8.4 Hz, H-2′), 3.95 (1H, m, H-5′), 3.89 (1H, m, H-12), 3.52 (1H, brd, $J=7.9$ Hz, H-3), 2.51 (1H, dd, $J=3.2$, 13.0 Hz, H-7), 2.30 (3H, m, H-17, 22, and 23), 2.13 (1H, m, H-11), 2.11 (1H, m, H-13), 2.07 (3H, s, H-28), 1.90 (1H, m, H-7), 1.89 (3H, s, H-27), 1.88 (1H, m, H-2), 1.84 (1H, m, H-2), 1.79 (1H, m, H-16), 1.75 (1H, m, H-22), 1.67 (1H, m, H-1), 1.61 (1H, m, H-15), 1.59 (3H, s, H-29), 1.55 (2H, m, H-9, 23), 1.54 (1H, m, H-11), 1.44 (1H, m, H-5), 1.41 (3H, s, H-21), 1.36 (1H, m, H-16), 1.17 (3H, s, H-18), 1.06 (1H, m, H-15), 1.03 (1H, m, H-1), 1.02 (3H, s, H-19), 0.81 (3H, s, H-30). ^{13}C NMR (C_5D_5N) (see Table 1).

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