Dammarane-type Triterpene Saponins from *Panax japonicus*

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Six new dammarane-type saponins (**1**−**6**), together with 11 known saponins (**7**−**17**), were isolated from Ye-Sanchi, the underground part of *Panax japonicus* collected in the South of Yunnan Province, China. Their structures were elucidated by chemical and spectroscopic means.

Most of the therapeutic effects of *Panax* species (Araliaceae) are explained, at least in part, by the presence of a complex mixture of saponins often referred to as ginsenosides. As many of these compounds are notable for their effect on various biological processes,[1−6] the isolation of these compounds permits the study of other possible applications of this class of compounds as well as the evaluation of *Panax* drugs. To date, more than 30 ginsenosides have been isolated from *Panax* species,[4,5,7] including *Panax japonicus* C. A. Meyer, which grows wild throughout Japan and the region from Central to Southwest China. In Japan, its rhizome is used to promote the functional activity of the stomach and as an expectorant and antitussive agent,[8] while in China it is used as a tonic, antiinflammatory, and haemostatic agent.[9] A methanol extract of the underground part of *P. japonicus* was found to demonstrate a significant enhancement of the outgrowth activity on cultured human neuroblastoma cells.[10] Additionally, in the course of our study on the quality evaluation of *Panax* drugs in the world, we examined the phylogenetic relationship among 11 *Panax* taxa by gene sequence analysis of the 18S ribosomal RNA gene and *trn*K gene and found that *Panax* species bearing similar morphological and anatomical appearances contain very different DNA sequences.[11,12] To clarify the relationship between gene sequence and the chemical constituents in *Panax* species, we report herein the isolation and structural elucidation of ginsenosides from Ye-Sanchi (local name in Chinese), the underground part of *P. japonicus* collected from the South of Yunnan Province, China.

Results and Discussion

Repeated column chromatography of the *n*-BuOH-soluble fraction of the methanol extract of Ye-Sanchi, the underground part of *P. japonicus* C. A. Meyer, afforded six new dammarane-type saponins, yesanchinosides A−F (**1**−**6**), and 11 known compounds (**7**−**17**). The structures of **7**−**17** were identified as vina-ginsenoside R_1 (**7**),[13] (24*S*)-pseudoginsenoside F_{11} (**8**),[14] (24*S*)-pseudoginsenoside RT_4 (**9**),[15] vina-ginsenoside R_2 (**10**),[13] ginsenoside Rg_1 (**11**),[7] majonoside R_2 (**12**),[16] notoginsenoside R_6 (**13**),[7] vina-ginsenoside R_6 (**14**),[17] 20-*O*-Glu-ginsenoside Rf (**15**),[7] notoginsenoside R_1 (**16**),[7] and ginsenoside Re (**17**)[7] by comparison of their spectral data with those reported in the literatures. Of the 17 compounds isolated here, majonoside-R_2 (**12**) was found to be the major component. The structures of **1**−**6** were determined as follows.

Yesanchinoside A (**1**) was obtained as a white amorphous powder. The API-mass spectrum (positive and negative modes) of **1** showed quasimolecular ion peaks at *m*/*z* 881 $[M + Na]^+$ and 859 $[M + H]^+$, and 857 $[M - H]^-$, respectively, consistent with the molecular formula $C_{44}H_{74}O_{16}$, which was further confirmed by elemental analysis. The positive mass fragment appeared at *m*/*z* 655 [M − Ac − hexose + H]$^+$ and 457 [655 − hexose − $2H_2O$]$^+$, indicating the molecule contained two hexose units and an acetyl group. Acid hydrolysis of **1** with 10% HCl afforded D-glucose as the only sugar component, which was identified by GC−MS. The IR spectrum of **1** showed absorption bands at 3392 and 1737 cm^{-1} for hydroxyl and ester groups, respectively. The ^{1}H NMR spectrum of **1** (Table 1) analyzed by ^{1}H−^{1}H COSY and HMQC showed signals for a triterpene aglycone, an acetyl methyl (δ_H 2.11, 3H, s), and two glucose units (anomeric protons at δ_H 5.01 and 5.85), both with β-configuration ($J_{1,2}$ = 7.5 Hz). A comparison of ^{13}C NMR chemical shifts of the aglycone moiety in **1** (Table 2) with those reported for majonoside R_2 (**12**)[16] and 20(*S*),24(*S*)-epoxydammarane-3β,6α,12β,25-tetrol[16] indicated the presence of an ocotillol-type triterpene having C-20(*S*), C-24(*S*) configuration (C-20 appeared at δ_C 87.0, C-24 at δ_C 88.4, and C-26 at δ_C 26.6) [for derivatives with C-24(*R*) configuration C-20, C-24, and C-26 appeared at δ_C 86.5, 85.6, and 27.1, respectively].[16] In addition, signals for two glucose units (two anomeric carbons at δ_C 103.6 and 104.0) (Table 3) and an acetyl group (δ_C 170.8 and 21.0) were also observed. The downfield shift of C-6 and the long-range correlation observed in the HMBC spectrum of **1** between C-6 (δ_C 79.3) and H-1′ (δ_H 5.01) confirmed glycosylation at C-6 of the aglycone. Similarly, correlations between the carbon signal at δ_C 80.0 (C-2′) and both H-1′ and H-1″ (δ_H 5.85) suggested a 1 → 2 linkage mode for the two sugar units. Alkaline hydrolysis of **1** with 25% aqueous NH_4OH afforded **1a** (Chart 1). On going from **1** to **1a**, the carbon signals due to C-5′ and C-6′ of the glucosyl moiety were displaced downfield by 4.8 ppm (**1**, δ_C 75.0; **1a**, δ_C 79.8) and upfield by 2.1 ppm (**1**, δ_C 65.0; **1a**, δ_C 62.9), respectively, while other carbon signals were almost identical. Also, the chemical shift of H_2-6′ (δ_{H2} 4.80 br) was downfield from that of **1a** (δ_H 4.25 and 4.46). These observations suggested acetylation at C-6′ (δ_C 65.0) of a glucose unit. A fragment ion peak at *m*/*z* 695 $[M - glc - H]^-$ in the negative API-mass spectrum of **1** indicated that the acetylated glucose was not terminal.[4] Therefore, the structure of **1** was

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Table 1. Selected ^{1}H NMR Data of Aglycone Moieties in Compounds **1**−**6**[a] (in C_5D_5N)

position	**1**	**2**	**3**	**4**	**5**	**6**
3	3.46 (dd,11.2, 5.1)	3.52 (dd, 11.6, 4.5)	3.47 (dd, 11.8, 4.4)	3.46 (dd, 11.2, 4.6)	3.45 (dd, 11.7, 4.5)	3.44 (dd, 11.3, 4.7)
5	1.38 (d, 10.0)	1.36 (d, 10.8)	1.32 (d, 9.8)	1.36 (d, 10.4)	1.33 (d, 10.3)	1.29 (overlapped)
6	4.30 (dd, 10.4, 3.5)	4.35 (dd, 10.8, 3.2)	4.22 (dd, 9.8, 3.3)	4.34 (dd, 10.4, 2.9)	4.35 (m)	4.33 (m)
9	1.49 (m)	1.47 (m)	1.50 (m)	1.53 (m)	1.52 (m)	1.50 (m)
12	3.82 (ddd, 11.1, 11.0, 4.3)	3.91 (m)	3.90 (m)	3.89 (m)	3.78 (m)	3.88 (m)
13	1.89 (dd, 11.1, 11.0)	1.91 (dd, 11.0, 10.3)	1.88 (dd, 11.0, 10.3)	1.91 (dd, 11.0, 10.5)	1.90 (dd, 10.2, 9.8)	1.88 (dd, 11.0, 10.3)
17	2.35 (m)	2.30 (m)	2.28 (m)	2.30 (dd, 10.9, 8.4)	2.33 (m)	2.32 (m)
18	1.11 (s)	1.23 (s)	1.09 (s)	1.29 (s)	1.17 (s)	1.23 (s)
19	0.98 (s)	1.02 (s)	0.84 (s)	1.02 (s)	0.95 (s)	1.02 (s)
21	1.25 (s)	1.32 (s)	1.38 (s)	1.44 (s)	1.34 (s)	1.30 (s)
24	4.10 (m)	4.07 (m)	4.03 (m)	5.23 (d, 8.4)	5.31 (d, 7.8)	5.28 (d, 8.5)
26	1.57 (s)	1.49 (s)	1.64 (s)	1.57 (s)	1.60 (s)	1.58 (s)
27	1.57 (s)	1.51 (s)	1.64 (s)	1.57 (s)	1.60 (s)	1.60 (s)
28	2.02 (s)	1.63 (s)	1.76 (s)	2.02 (s)	2.07 (s)	2.05 (s)
29	1.53 (s)	1.36 (s)	1.47 (s)	1.64 (s)	1.65 (s)	1.60 (s)
30	0.77 (s)	0.81 (s)	0.70 (s)	0.88 (s)	0.94 (s)	1.01 (s)
CH_3CO	2.11 (s)			2.10 (s)		2.05 (s)

[a] Chemical shifts in ppm and coupling constants (in Hz) in parentheses.

Table 2. ^{13}C NMR Data of Aglycone Moieties in Compounds **1**−**6** (in C_5D_5N)

position	**1**	**2**	**3**	**4**	**5**	**6**
1	39.5	39.6	39.6	39.4	39.4	39.7
2	27.8	27.7	27.7	27.9	27.7	27.6
3	78.6	78.2	78.0	78.6	78.3	78.3
4	40.1	40.0	40.2	40.2	40.0	39.9
5	61.4	61.4	61.4	61.4	60.8	60.7
6	79.3	79.5	79.7	77.9	74.1	73.7
7	45.3	44.9	45.2	45.4	45.8	46.1
8	41.2	41.2	41.2	41.2	41.2	41.3
9	50.3	50.2	50.2	50.0	49.6	49.6
10	39.7	39.6	39.3	39.7	39.6	39.7
11	32.2	32.2	32.0	30.9	30.8	30.8
12	70.8	70.8	70.8	70.2	70.2	70.2
13	49.2	49.2	48.9	49.3	49.1	49.2
14	52.3	52.3	52.2	51.4	51.4	51.4
15	32.6	32.6	32.2	30.6	30.8	30.8
16	25.8	25.7	25.8	26.6	26.6	26.6
17	49.6	49.6	49.4	51.6	51.6	51.6
18	17.9	17.8	17.7	17.6	17.5	17.4
19	17.1	17.2	17.0	17.4	17.6	17.6
20	87.0	87.1	87.0	83.3	83.4	83.5
21	26.9	26.9	26.9	22.3	22.3	22.3
22	32.6	32.5	32.4	36.6	36.1	36.1
23	28.6	28.7	28.9	23.2	23.2	23.2
24	88.4	88.4	88.4	125.9	125.9	125.9
25	70.0	70.0	70.6	130.9	131.0	131.0
26	26.6	26.5	26.5	25.7	25.8	25.8
27	28.9	29.0	30.0	17.7	17.9	17.9
28	31.7	32.2	31.7	32.0	32.1	32.1
29	16.8	17.5	16.7	16.5	17.3	17.3
30	17.8	16.8	17.7	17.4	17.3	17.4

established as 6-*O*-*β*-D-glucopyranosyl-(1→2)-6-*O*-acetyl-*β*-D-glucopyranosyl 20(*S*),24(*S*)-epoxydammarane-3*β*,6α,12*β*,-25-tetrol.

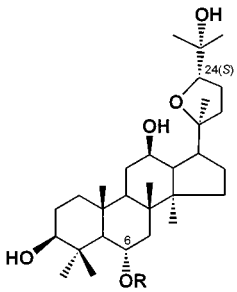
The spectral data of yesanchinoside B (**2**) was in part similar to that of **1**. However, the API-mass spectrum (positive mode) of **2** exhibited quasimolecular ion peaks at *m*/*z* 996 $[M + NH_4]^+$, 979 $[M + H]^+$ (*m*/*z* 977 $[M - H]^-$ in a negative mode), suggesting the presence of an extra hexose unit in **2**. Fragment ions observed at *m*/*z* 815 $[M -$ hexose $- H]^-$, 653 $[M -$ 2hexose $- H]^-$, and 491 $[M -$ 3hexose $- H]^-$ in the API-MS/MS spectrum (negative mode) indicated a stepwise elimination of three hexose units from the molecular ion peak at *m*/*z* 977 $[M - H]^-$. The molecular formula $C_{48}H_{82}O_{20}$ for **2** was finally established by elemental analysis. D-Glucose was the only sugar component detected by GC−MS of the acid hydrolysate. NMR spectra of **2** indicated the presence of a trisaccharide moiety (three anomeric carbon signals and three anomeric proton signals: δ_C 103.9 and δ_H 5.86, δ_C 103.8 and δ_H 4.93, δ_C 100.8 and δ_H 5.64). From the $J_{1,2}$ values of the glucose units, the *β*-configuration of the former two units (H-1′, J = 8.3 Hz and H-1″, J = 7.6 Hz) and the α-configuration of the latter one (H-1‴, J = 3.7 Hz) have been confirmed. The chemical shifts of NMR signals in the aglycone moiety of **2** were the same as the data in **1**. Similarly, the downfield shift of C-6 (δ_C 79.5) suggested that the trisaccharide moiety should be bound at C-6. Long-range correlation between the proton signal at δ_H 5.86 (H-1′) of the inner glucose unit and C-6 confirmed glycosylation at this carbon, and the downfield shift of C-2′ (δ_C 80.1) and C-6′ (δ_C 69.4) indicated substitutions at these carbons. The sequence of the trisaccharide moiety was suggested by its HMBC spectrum; correlation between C-2′ and proton signals at δ_H 5.86 (H-1′) and 4.93 (H-1″) indicated a 1→2 linkage between two *β*-D-glucose units, while the correlation between C-6′ and a proton signal at δ_H 5.64 (H-1‴) indicated a 1→6 linkage between the inner *β*-D-glucose and the α-D-glucose unit. From the foregoing findings, the structure of **2** was elucidated as 6-*O*-[α-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl 20(*S*),24(*S*)-epoxydammarane-3*β*,6α,12*β*,25-tetrol.

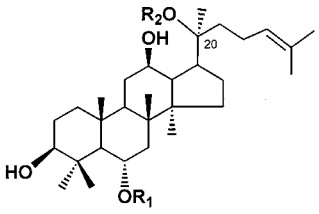
The molecular formula $C_{47}H_{80}O_{19}$ was assigned for yesanchinoside C (**3**) on the basis of API-mass spectra (molecular ion peaks at *m*/*z* 971 $[M + Na]^+$ and 949 $[M + H]^+$ in the positive mode) and elemental analysis. By acid hydrolysis and GC−MS analysis, D-glucose and D-xylose were identified. The ^{13}C chemical shifts in the aglycone part of **3**, **2**, and **1** are identical (Table 2), and the difference was only found for signals in the sugar part. Signals for three anomeric carbons were observed at δ_C 103.8 (C-1′), 104.9 (C-1″), and 105.3 (C-1‴) in the ^{13}C NMR spectrum (Table 3), and signals due to three anomeric protons with *β*-configuration were observed in the ^{1}H NMR spectrum at δ_H 4.99 (d, J = 7.1 Hz, H-1′), 5.21 (d, J = 7.8 Hz, H-1″), and 5.58 (d, J = 6.8 Hz, H-1‴). Their respective correlations were established from the HMQC spectrum. HMBC cor-

Table 3. ^{13}C NMR Data of Sugar Moieties in Compounds **1**−**6** (in C_5D_5N)

position	**1**	**2**	**3**	**4**	**5**	**6**
6-*O*-sugar	Glc	Glc	Glc	Glc	Glc	Glc
1′	103.6	103.9	103.8	105.9	101.8	101.4
2′	80.0	80.1	79.7	75.4	79.3	79.0
3′	78.6	78.2	78.7	79.3	78.3	78.3
4′	71.2	71.3	71.2	71.7	72.2	72.3
5′	75.0	76.4	79.9	75.1	78.3	75.4
6′	65.0	69.4	62.8	65.2	63.0	65.0
CO	170.8			170.8		170.8
CH_3	21.0			20.9		20.8
	Glc(1→2)	Glc(1→2)	Glc(1→2)		Rha(1→2)	Rha(1→2)
1″	104.0	103.8	104.9		101.8	102.0
2″	75.9	75.7	83.6		72.2	72.2
3″	78.5	78.6	77.9		72.2	72.2
4″	72.3	72.5	72.3		74.1	74.1
5″	79.8	79.8	78.5		69.5	69.4
6″	63.3	62.9	62.7		18.7	18.6
		Glc(1→6)	Xyl(1→2)			
1‴		100.8	105.3			
2‴		74.0	75.6			
3‴		75.3	78.6			
4‴		72.1	71.7			
5‴		74.2	67.3			
6‴		62.8				
20-*O*-sugar				Glc	Glc	Glc
1⁗				98.2	98.0	98.0
2⁗				75.0	74.9	74.9
3⁗				79.1	78.3	78.3
4⁗				71.4	71.5	71.5
5⁗				78.2	77.0	77.0
6⁗				62.9	71.7	71.7
					Glc(1→6)	Glc(1→6)
1⁗′					105.3	105.3
2⁗′					75.2	75.2
3⁗′					78.4	78.3
4⁗′					71.7	71.7
5⁗′					78.7	78.5
6⁗′					62.8	62.8

Chart 1





	R
1	Glc^2—Glc, ^{6}Ac
1a	Glc^2—Glc
2	Glc^2—Glc, ^{6}α-Glc
3	Glc^2—Glc^2—Xyl
7	Glc^2—Rha, ^{6}Ac
8	Glc^2—Rha
9	Glc
10	Glc^2—Xyl, ^{6}Ac
12	Glc^2—Xyl
14	Glc^2—Glc, ^{6}α-Glc

	R_1	R_2
4	Glc^6—Ac	Glc
5	Glc^2—Rha	Glc^6—Glc
6	Glc^2—Rha, ^{6}Ac	Glc^6—Glc
11	Glc	Glc
13	Glc	Glc^6—α-Glc
15	Glc^2—Glc	Glc
16	Glc^2—Xyl	Glc
17	Glc^2—Rha	Glc

Glc: β-D-glucopyranosyl
α-Glc: α-D-glucopyranosyl
Ac: acetyl
Xyl: β-D-xylopyranosyl
Rha: α-L-rhamnopyranosyl

relation between C-6 (δ_C 79.7) and H-1′ confirmed the glycosylation at C-6 of the aglycone, and correlations between the carbon signal at δ_C 79.7 (C-2′) and proton signals at δ_H 4.99 (H-1′) and 5.21 (H-1″) indicated a 1→2 linkage between the two glucose units. The second glucose unit had its C-2″ deshielded (+8.0 ppm) (Table 3) as a consequence of substitution by a xylose unit. Long-range correlation between C-2″ (δ_C 83.6) and proton signals at δ_H 5.21 (H-1″) and 5.58 (H-1‴) indicated that xylose is the terminal sugar unit with a 1→2 linkage to the second glucose unit. This sequence was confirmed by the fragment ion peaks at *m/z* 839 $[M - xyl + Na]^+$, 619 $[M - xyl - glc - 2H_2O + H]^+$ and 475 $[M - xyl - 2glc - H_2O + H]^+$ in the API-MS/MS spectrum (similar findings were also obtained from the negative mode). Accordingly, compound **3** was identified as 6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl 20(*S*),24(*S*)-epoxydammarane-3β,6α,12β,25-tetrol.

The NMR spectral data of yesanchinosides D−F (**4**−**6**) demonstrated common features characteristic for a protopanaxatriol-type triterpene glycosylated at C-6 and C-20, similar to those of **11**, **13**, and **15**−**17**.[7]

The molecular formula $C_{44}H_{74}O_{15}$ of **4** was established from the API-mass spectra (molecular ion peaks at *m/z* 881 $[M + K]^+$, 865 $[M + Na]^+$, and 843 $[M + H]^+$ in the positive mode) and elemental analysis. D-Glucose was only identified in the acid hydrolysate of **4**. The observation of carbon signals at δ_C 170.8 and 20.9, a proton signal at δ_H 2.10 (3H, s), and IR absorption band at 1745 cm^{-1} revealed the presence of an acetyl group. Alkaline hydrolysis of **4** gave **11** and indicated that the acetyl group was located at C-6 of one of the glucose units in **4**. The correlations of the anomeric carbon signals at δ_C 105.9 (C-1′) and 98.2 (C-1⁗) with anomeric proton signals at δ_H 4.99 (d, J = 7.1 Hz, H-1′) and 5.17 (d, J = 7.8 Hz, H-1⁗), respectively, were

established from the HMQC spectrum. The above coupling constants of the anomeric protons indicated β-configuration of the two glucose units. Long-range correlation between C-6 (δ_C 77.9) and H-1′, and between the carbon signal at δ_C 170.8 and the proton signal at δ_H 4.72 (H_b-6′), together with the downfield shift of C-6′ (δ_C 65.2) confirmed substitution at C-6 by a 6-acetyl-β-D-glucopyranosyl unit. The downfield shift of C-20 (δ_C 83.3) and the chemical shift of the anomeric carbon C-1′′′′ (δ_C 98.2) are characteristic of a protopanaxadiol (or triol)-triterpene acylated with a glucosyl unit at C-20.[7] On the basis of the above evidence, the structure of **4** was identified as 6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl]-20-*O*-(β-D-glucopyranosyl)-20(*S*)-protopanaxatriol.

From the API-mass spectra (*m/z* 1131 $[M + Na]^+$, 1107 $[M - H]^-$) and elemental analysis, yesanchinosides E (**5**) was assigned the molecular formula $C_{54}H_{92}O_{23}$. D-Glucose and L-rhamnose were identified as the sugar components in the acid hydrolysate of **5**, and their configurations were proved by GC−MS analysis. Fragment ions at *m/z* 961 $[M - rha - H]^-$, 945 $[M - glc - H]^-$, 799 $[M - glc - rha - H]^-$, 783 $[M - 2glc - H]^-$, 637 $[M - 2glc - rha - H]^-$, 619 $[M - 2glc - rha - H_2O - H]^-$, and 475 $[M - 3glc - rha - H]^-$ were observed in the negative mode API-MS/MS spectrum. The NMR spectral data of **5**, analyzed by the aid of HMQC, showed signals similar to that of ginsenoside Re (**17**) together with signals for an extra glucose unit. Since carbon signals of sugar residues of **5** (Table 3) resonate at almost the same positions as those of **17** and ginsenoside Rb_1,[7] the sugar residues at C-6 and C-20 of **5** were confirmed as shown in Chart 1. The correlation observed in the HMBC spectrum supported the above findings. Therefore, the structure of **5** was determined as 6-*O*-[α-L-rhammopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol.

The API-mass spectra (positive and negative modes) of yesanchinoside F (**6**) exhibited quasimolecular ion peaks at *m/z* 1173 $[M + Na]^+$ and 1149 $[M - H]^-$ respectively, consistent with the molecular formula $C_{56}H_{94}O_{24}$, which is 42 mass units more than that of **5**. The NMR data of **6** were almost undistinguishable from those of **5**. However, additional signals for an acetyl group [signals at δ_C 170.8 and 20.8 (δ_H 2.05)] indicated it to be the monoacetate of **5** (alkaline hydrolysis of **6** afforded **5**). The ^{13}C chemical shift values for the sugar residue attached to C-6 agreed well with those in **7** and pseudoginsenoside RS_1,[18] suggesting the presence of an α-L-rhamnopyranosyl-(1→2)-6-*O*-acetyl-β-D-glucopyranosyl moiety bound to C-6 through a glycosidic linkage. A comparison of the remaining carbon signals of the sugar chains with those of **5** and ginsenoside Rb_1[7] indicated the presence of glycosidation at C-20 of the aglycone in **6** with a β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl moiety. These findings established the structure of **6** as 6-*O*-[α-L-rhammopyranosyl-(1→2)-6-*O*-acetyl-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxatriol.

In the present study, nine ocotillol-type and eight protopanaxatriol-type saponins were isolated from Ye-Sanchi, the underground part of *P. japonicus* obtained in the south part of Yunnan Province, China. The ocotillol-type saponins were isolated in a remarkably high amount from this species. However, oleanolic acid saponins were not isolated at all. It is noteworthy that ocotillol-type derivatives have not been isolated so far from specimens of *P. japonicus* collected in Japan and oleanolic acid saponins were reported to be present in abundance in those specimens.[19] On the other hand, the 18S rRNA gene and *trn*K gene sequences of the specimen used in this study were found to be relatively close to those of *P. vietnamensis* Ha et Grushv. collected in central Vietnam and different from those of *P. japonicus* specimens collected in Japan.[20] Although in a previous study the main saponin of the underground part of *P. vietnamensis* was found to be majonoside-R2 (**12**), an ocotillol-type saponin, unlike the results of our present study, oleanolic acid saponins were also isolated.[13,17]

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco DIP-360 digital polarimeter. IR spectra were measured in KBr using a Jasco FT/IR-230 infrared spectrometer. ^1H and ^{13}C NMR spectra were obtained with a JEOL JNA-LA 400WB-FT spectrometer (^1H, 400 MHz; ^{13}C, 100 MHz), the chemical shifts being represented as ppm with TMS as an internal standard. The API-mass spectra (positive and negative modes) were taken on a Perkin-Elmer SCIEX API-*III* biomolecular mass analyzer. HPLC was performed on a Jasco PU-1587 intelligent preparative pump equipped with a Jasco UV-1575 intelligent UV/vis detector and a Jasco 807-IT integrator; column, YMC-Pack ODS-AQ (S-5μm, 12 nm, 250 × 20 mm i.d.); flow rate, 8.0 mL/min; detection, UV at 203 nm. GC analysis was carried out with a GC-17A gas chromatograph (Shimadzu, Japan) fitted with a DB-1 column (0.25 mm i.d. × 30 m) (J&W Scientific), coupled to an Automass System II benchtop quadrupole mass spectrometer (JEOL, Japan); column temp, 50−230 °C, 15 °C/min; Carrier gas, He at a flow rate of 50 mL/min.

Plant Material. The underground part of *P. japonicus* (Ye-Sanchi) was collected in August 1999 from Jinping County of Yunnan Province, China. A voucher specimen (TMPW No.19759) has been deposited in the Museum of Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University (TMPW), Toyama, Japan.

Extraction and Isolation. The dried powdered underground part of *P. japonicus* (500 g) was extracted four times with methanol (2.5 l) under reflux. After removal of the solvent, the methanol extract (150 g) was obtained. This extract was suspended in H_2O (1.5 l) and successively extracted with ethyl acetate (4 × 300 mL) and *n*-BuOH saturated with H_2O (4 × 300 mL). The *n*-BuOH-soluble fraction was evaporated to dryness to give 72 g of dry residue. This residue was subjected to column chromatography over Sephadex LH-20 eluted with methanol, to give 56 g of a crude saponin mixture. This mixture was applied on a column of Si gel, and elution was started with a gradient solvent system ($CHCl_3$−CH_3OH−H_2O, 10:1:0 → 6:4:1) to obtain 27 fractions. Fraction 6 (880 mg) was chromatographed on Sephadex LH-20 (eluted with 55% MeOH) and purified by HPLC elution with CH_3CN−H_2O, 25:75 v/v, giving vina-ginsenoside R_1 (**7**) (40 mg), while (24*S*)-pseudoginsenoside F_{11} (**8**) (19 mg) and (24*S*)-pseudoginsenoside RT_4 (**9**) (63 mg) were obtained by using 28% CH_3CN in H_2O as a mobile phase. Similarly, Fraction 7 afforded yesanchinosides A (**1**) (10 mg) and D (**4**) (8 mg) and vina-ginsenoside R_2 (**10**) (563 mg); fraction 9 gave ginsenoside Rg_1 (**11**) (56 mg) and majonoside R_2 (**12**) (1.275 g). Fraction 23 (3.5 g) was further chromatographed on a RP-18 column eluted with 40% → 90% CH_3OH; the 45% eluted subfraction was then purified by HPLC to afford yesanchinoside E (**5**) (11 mg), notoginsenoside R_6 (**13**) (13 mg), vina-ginsenoside R_6 (**14**) (13 mg), and yesanchinoside B (**2**) (10 mg); the 55% eluted subfraction gave 20-*O*-glu-ginsenoside Rf (**15**) (28 mg), notoginsenoside R_1 (**16**) (63 mg), and yesanchinoside C (**3**) (13 mg), while ginsenoside Re (**17**) (39 mg) and yesanchinoside F (**6**) (5 mg) were obtained from the 70% eluted subfraction.

Yesanchinoside A (**1**): white amorphous powder, $[\alpha]_D^{20}$ +7.1° (*c* 0.1, 40% CH_3CN); IR (KBr) ν_{max} 3392, 2931, 2875, 1737, 1637, 1566 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 1, 2, and 3); API-MS (positive mode, MeOH) *m/z* 881 $[M + Na]^+$,

859 [M + H]$^+$, 655 [M − Ac − glc + H]$^+$, and 457 [M − 2glc − Ac − 2H$_2$O + H]$^+$; (negative mode) *m/z* 857 [M − H]$^-$, 653 [M − glc − Ac − H]$^-$; APIMS/MS (negative mode, parent ion *m/z* 857) 857 [M − H]$^-$, 815 [M − Ac − H]$^-$, 737 [857−120]$^-$, 695 [M − glc − H]$^-$, 653 [M − glc − Ac − H]$^-$, 635 [695 − 60]$^-$, 695 [815 − 120]$^-$, 491 [M − 2glc − Ac − H]$^-$; *anal*. C 60.27%, H 8.68%, calcd for $C_{44}H_{74}O_{16}$·H$_2$O, C 60.01%, H 8.63%.

Yesanchinoside B (**2**): white amorphous powder, $[\alpha]_D^{20}$ +11.3°(*c* 0.1, 40% CH$_3$CN); IR (KBr) ν_{max} 3392, 2931, 2874, 1639, 1563 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Tables 1, 2, and 3); APIMS (positive mode, MeOH/NH$_4$OAc) *m/z* 996 [M + NH$_4$]$^+$, 979 [M + H]$^+$; APIMS/MS (positive, parent ion *m/z* 996) 961 [M − H$_2$O + H]$^+$, 799 [M − glc − H$_2$O + H]$^+$, 637 [M − 2glc − H$_2$O + H]$^+$, 475 [M − 3glc − H$_2$O + H]$^+$, 457 [M − 3glc − 2H$_2$O + H]$^+$, 439 [M − 3glc − 3H$_2$O + H]$^+$, 421 [M − 3glc − 4H$_2$O + H]$^+$; (negative, MeOH/NH$_4$OAc) *m/z* 1037 [M + OAc]$^-$, 977 [M − H]$^-$, 961 [M − OH]$^-$, 815 [M − glc − H]$^-$, 637 [M − 2glc − OH]$^-$; APIMS/MS (negative, parent ion *m/z* 977) 977 [M − H]$^-$, 815 [M − glc − H]$^-$, 797 [M − glc − H$_2$O−H]$^-$, 653 [M − 2glc − H]$^-$, 617 [M − 2glc − 2H$_2$O − H]$^-$, 491 [M − 3glc − H]$^-$. *anal*. C 56.80%, H 8.48%, calcd for $C_{48}H_{82}O_{20}$·2H$_2$O, C 56.56%, H 8.46%.

Yesanchinoside C (**3**): white amorphous powder, $[\alpha]_D^{20}$ +5.9° (*c* 0.1, 40% CH$_3$CN); IR (KBr) ν_{max} 3389, 2923, 2865, 1630, 1556 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Tables 1, 2, and 3); API-MS (positive mode, MeOH) *m/z* 971 [M + Na]$^+$, 949 [M + H]$^+$, 817 [M − xyl + H]$^+$, 655 [M − xyl − glc + H]$^+$, 493 [M − xyl − 2glc + H]$^+$, 457 [M − xyl − 2glc − H$_2$O + H]$^+$; APIMS/MS (positive, parent ion *m/z* 949) 839 [M − xyl + Na]$^+$, 619 [M − xyl − glc − 2H$_2$O + H]$^+$, 475 [M − xyl − 2glc − H$_2$O + H]$^+$, 457 [M − xyl − 2glc − 2H$_2$O + H]$^+$, 439 [M − xyl − 2glc − 3H$_2$O + H]$^+$, 421 [M − xyl − 2glc − 4H$_2$O + H]$^+$; (negative, MeOH/NH$_4$OAc) *m/z* 1007 [M + OAc]$^-$, 947 [M − H]$^-$, 875 [M + OAc − xyl]$^-$, 815 [M − xyl − H]$^-$, 653 [M − xyl − glc − H]$^-$; APIMS/MS (negative, parent ion *m/z* 947) 947 [M − H]$^-$, 815 [M − xyl − H]$^-$, 653 [M − xyl − glc − H]$^-$, 491 [M − xyl − 2glc − H]$^-$. *anal*. C 57.32%, H 8.54%, calcd for $C_{47}H_{80}O_{19}$·2H$_2$O, C 57.11%, H 8.51%.

Yesanchinoside D (**4**): white amorphous powder, $[\alpha]_D^{20}$ +13.6° (*c* 0.1, 40% CH$_3$CN); IR (KBr) ν_{max} 3396, 2931, 1745, 1647, 1556 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Tables 1, 2, and 3); API-MS (positive mode, MeOH) *m/z* 865 [M + Na]$^+$, 843 [M + H]$^+$, 823 [M − Ac + Na]$^+$, 685 [M − glc − H$_2$O + Na]$^+$; APIMS/MS (positive, parent ion *m/z* 865) 865 [M + Na]$^+$, 703 [M − glc + Na]$^+$, 685 [M − glc − H$_2$O + Na]$^+$, 481 [M − 2glc − Ac − H$_2$O + Na]$^+$, 463 [M − 2glc − Ac − 2H$_2$O + Na]$^+$; (negative, MeOH) *m/z* 841 [M − H]$^-$, 799 [M − Ac−H]$^-$. *anal*. C 60.14%, H 8.88%, calcd for $C_{44}H_{74}O_{15}$·2H$_2$O, C 59.95%, H 8.85%.

Yesanchinoside E (**5**): white amorphous powder, $[\alpha]_D^{20}$ +1.5° (*c* 0.1, 40% CH$_3$CN); IR (KBr) ν_{max} 3392, 2931, 2874, 1649, 1549 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Tables 1, 2, and 3); API-MS (positive mode, MeOH) *m/z* 1131 [M + Na]$^+$, 985 [M − glc + K]$^+$; APIMS/MS (positive, parent ion *m/z* 1131) 1131 [M + Na]$^+$, 951 [M − glc − H$_2$O + Na]$^+$, 807 [M − 2glc + Na]$^+$, 789 [M − 2glc − H$_2$O + Na]$^+$; (negative) *m/z* 1107 [M − H]$^-$; APIMS/MS (negative, parent ion *m/z* 1107) 1107 [M − H]$^-$, 961 [M − rha − H]$^-$, 945 [M − glc − H]$^-$, 799 [M − glc − rha − H]$^-$, 783 [M − 2glc − H]$^-$, 765 [M − 2glc − H$_2$O − H]$^-$, 637 [M − 2glc − rha − H]$^-$, 619 [M − 2glc − rha − H$_2$O − H]$^-$, 475 [M − 3glc − rha − H]$^-$. *anal*. C 55.76%, H 8.43%, calcd for $C_{54}H_{92}O_{23}$·3H$_2$O, C 55.60%, H 8.40%.

Yesanchinoside F (**6**): white amorphous powder, $[\alpha]_D^{20}$ +3.3° (*c* 0.1, 40% CH$_3$CN); IR (KBr) ν_{max} 3394, 2926, 1740, 1648, 1553 cm^{-1}; ^{1}H NMR and ^{13}C NMR (see Tables 1, 2, and 3); API-MS (positive mode, MeOH/NH$_4$OAc) *m/z* 1173 [M + Na]$^+$, 971 [M − glc − H$_2$O + H]$^+$; APIMS/MS (positive, parent ion *m/z* 1173) 1173 [M + Na]$^+$, 849 [M − 2glc + Na]$^+$, 831 [M − 2glc − H$_2$O + Na]$^+$; (negative, MeOH/NH$_4$OAc) *m/z* 1149 [M − H]$^-$, 1107 [M − Ac − H]$^-$, 1089 [M − HOAc − H]$^-$; APIMS/MS (negative, parent ion *m/z* 1149) 1107 [M − Ac − H]$^-$, 1089 [M − HOAc − H]$^-$, 961 [M − rha − Ac − H]$^-$, 945 [M − glc − Ac − H]$^-$, 783 [M − 2glc − Ac − H]$^-$, 781 [M − glc − rha − HOAc − H]$^-$, 765 [M − 2glc − HOAc − H]$^-$, 637 [M − 2glc − rha − Ac − H]$^-$, 619 [M − 2glc − rha − HOAc − H]$^-$, 601 [M − 2glc − rha − HOAc − H$_2$O − H]$^-$, 475 [M − 3glc − rha − H]$^-$. *anal*. C 56.66%, H 8.26%, calcd for $C_{56}H_{94}O_{24}$·2H$_2$O, C 56.57%, H 8.20%.

Alkaline Hydrolysis of Compounds 1, 4, and 6. To a solution of **1** (4.2 mg), **4** (2.9 mg), and **6** (2.2 mg) in H$_2$O (2.0 or 1.5 mL) was added a portion (2.0 or 1.5 mL) of 50% aqueous NH$_4$OH, respectively. After stirring at 35 °C for 16 h, the solution was neutralized by adding 2.0 M HCl solution and then desalted with Amberlite MB-3. The eluate was concentrated under reduced pressure and subjected to preparative HPLC (mobile phase, CH$_3$CN−H$_2$O, 22:78 v/v; flow rate, 8.0 mL/min) to afford **1a** (t_R = 16.3 min, 2.7 mg), **11** (t_R = 19.2 min, 1.8 mg), and **5** (t_R = 25.6 min, 1.4 mg) from **1**, **4**, and **6**, respectively. **1a**[16] was obtained as a white amorphous powder, mp 173−176 °C; $[\alpha]_D^{20}$ +1.3° (*c* 0.1, 40% CH$_3$CN). The HPLC, API-MS, IR, and ^{1}H NMR data of **5** and **11** were in good agreement with those of yesanchinoside E and ginsenoside Rg$_1$,[7] respectively.

Determination of Sugar Components.[21,22] Compounds **1**−**6** (each 0.8 mg) were hydrolyzed with 10% HCl in 40% CH$_3$-CN solution by reflux at 80 °C for 4 h. After neutralization with 2.0 M NaOH, the mixture was extracted with CHCl$_3$. The water layer was desalted with Amberlite MB-3 and evaporated to dryness under reduced pressure. The residue was dissolved in anhydrous pyridine (100 μL), and 200 μL of 0.1 M L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 1 h, then 150 μL of HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane−pyridine, 2:1:10) was added, and the mixture was stirred at 60 °C for another 30 min. After centrifugation, the supernatant was directly subjected to GC analysis. The sugar derivatives obtained from compounds **1**, **2**, and **4** showed a single peak (t_R at 21.55 min) comparable with that of a D-glucose derivative, while **3** showed two peaks (t_R at 21.55 and 16.33 min) for D-glucose and D-xylose derivatives, respectively. Compounds **5** and **6** showed similar peaks for their sugar derivatives at t_R 21.55 min (for a D-glucose derivative) and 18.04 min (for a L-rhamnose derivative). Derivatives obtained for standard L-glucose and L-xylose had their t_R at 22.38 and 19.05 min, respectively.

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