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Saponins of Zu-Tziseng, Rhizomes of *Panax japonicus* C.A. Meyer var. *major* (Burk.) C.Y. Wu *et* K.M. Feng, collected in Yunnan, China

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From rhizomes of *Panax japonicus* var. *major* (Chinese name: Zu-Tziseng) collected in Yunnan, China, two known oleanolic acid-saponins, chikusetsusaponin-IVa (**6**) and -V (**1**), two dammarane-saponins, ginsenoside-Rd (**3**) and notoginsenoside-R2 (**2**), and two new dammarane-saponins named majonoside-R1 (**7**) and -R2 (**13**) were isolated.

By means of ^{13}C-nuclear magnetic resonance (NMR) and mass spectrometry, the common aglycone of both new saponins was proved to be $3\beta,6\alpha,12\beta,25$-tetrahydroxy-(20*S*,24*S*)-epoxy-dammarane (**11**) (ocotillol-type triterpene) and the structures of **13** and **7** were elucidated to be 6-*O*-β-xylopyranosyl(1→2)-β-glucopyranoside and 6-*O*-β-sophoroside of **11**, respectively.

Keywords——*Panax japonicus* var. *major*; Araliaceae; Zu-Tziseng; oleanane-saponins; dammarane-saponins; ginsenosides; ocotillol-type triterpene; majonoside-R1 and -R2; ^{13}C-NMR

From the well-known oriental plant drug, Ginseng (roots of *Panax ginseng* C.A. Meyer (Araliaceae)), a number of dammarane-saponins have been isolated along with a relatively small amount of the oleanolic acid-saponin, ginsenoside-Ro (=chikusetsusaponin-V) (**1**).[1] It has also been reported that American Ginseng (roots of *P. quinquefolium* L.) and Sanchi-Ginseng (roots of *P. notoginseng* (Burk.) F.H. Chen, cultivated in Yunnan, China) are similar to Ginseng as regards the saponin composition as well as in the shape of their carrotlike roots.[2-4] In contrast to these *Panax* spp., Chikusetsu-ninjin (rhizomes of *P. japonicus* C.A. Meyer, which grows wild in Japan) and Himalayan Panax (rhizomes of *P. pseudo-ginseng* Wall. subsp. *himalaicus* Hara,[2] which grows wild in Eastern Himalaya) generally have long horizontally creeping rhizomes, and the saponin compositions of their rhizomes were found to be similar to each other in that both contain a large amount of oleanolic acid saponins along with the characteristic dammarane-saponins.[5,6] In South China, especially in Yunnan and Szechwan, several wild *Panax* spp. grow. Although the close botanical relationship of these Chinese wild *Panax* spp. to Chikusetsu-ninjin and Himalayan Panax has been pointed out, no chemical investigation of these Chinese *Panax* spp. has been reported as yet.

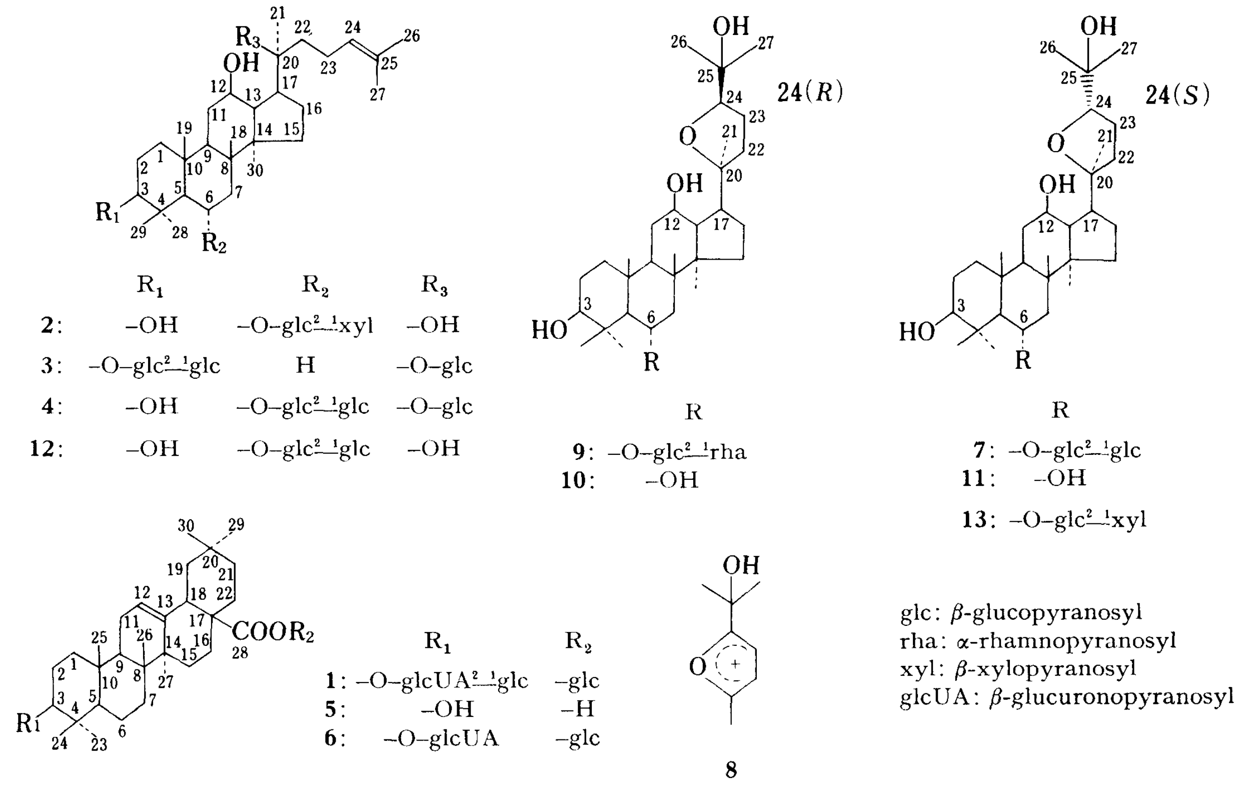
One of the Chinese *Panax* spp., *P. japonicus* C.A. Meyer var. *major* (Burk.) C.Y. Wu *et* K.M. Feng (=*P. pseudo-ginseng* Wall. var. *major* (Burk.) J.L. Li) grows from Tibet to Yunnan at altitudes of 2500—4500 m, and the internodes of its long creeping rhizomes are elongated and slender, being distinguished from those of Chikusetsu-ninjin, which has short and thick internodes. The rhizomes of this plant are the Chinese plant drug named Zu-Tziseng and have been used as an expectorant, antitussive, analgesic and hemostatic. The present paper deals with the isolation and structure determination of saponins of Zu-Tziseng.

A methanolic extract of the rhizomes collected in Likiang, North-West Yunnan at an altitude of 3500 m was suspended in water and extracted with ether and then with 1-butanol saturated with water. The resulting butanolic layer was concentrated to dryness to give a crude saponin-fraction which was chromatographed repeatedly to give seven saponins I—VII.

Saponins II and IV (yields: 0.03 and 0.67%, respectively) were proved to possess the dammarane-type aglycone by analysis of their ^{13}C-nuclear magnetic resonance (NMR) spectra, and they were identified respectively as the known saponins, notoginsenoside-R2 (**2**) (from Sanchi-Ginseng)4b) and ginsenoside-Rd (**3**) (from Ginseng).1a) Saponin V, which could not be obtained in a pure state owing to its low yield (0.01%), seemed to be identical with 20-gluco-ginsenoside-Rf (**4**) (from Ginseng)1b) on the basis of its ^{13}C-NMR spectrum.

Saponins VI and VII (yields: 0.19 and 0.95%, respectively), the aglycone of which was found to be oleanolic acid (**5**) by analysis of their ^{13}C-NMR spectra, were identified as chikusetsusaponin-IVa (**6**) and -V (=ginsenoside-Ro (**1**)), respectively, both of which have already been isolated from Chikusetsu-ninjin5) and Himalayan Panax6) as the major saponins.

Saponin III (**7**) (yield: 0.07%), a new saponin named majonoside-Rl, afforded glucose on acid hydrolysis, and its ^{1}H-NMR spectrum in pyridine-d_5 exhibited two anomeric proton signals at δ 4.93 (1H d, $J=7$ Hz) and 5.83 (1H d, $J=6$ Hz) due to β-glucosyl linkages. The mass spectrum (MS) of an acetate of **7** showed fragment ions at m/z 331 (glucose $(Ac)_4^+$), 619 (glucose $(Ac)_4$-glucose $(Ac)_3^+$) and a strong ion at m/z 143 (**8**) which is characteristic of the hydroxylisopropyltetrahydrofuran ring of ocotillol-type triterpenes such as pseudo-ginsenoside-F_{11} (**9**) (the leaf-saponin of Himalayan Panax).7) In our previous study on **9**, it was found that the C-24 epimers of ocotillol-type triterpenes could be distinguished from each other by the carbon signals due to the 24C and one of the methyl carbons (26C or 27C), *i.e.*, on going from the aglycone (**10**) (24-*R*) of **9** to its C-24 epimer (**11**) (24-*S*), signals attributable to 24C and 26C (or 27C) were displaced downfield, as shown in Table I.7) A comparison of the ^{13}C-NMR spectrum of **7** with those of **10** and **11** showed that signals due to the aglycone carbons of **7** appeared at almost the same positions as those of **11** except for the signals due to 6C and 7C. This indicated that **7** is a glycoside of **11** whose glycoside linkage must be located at its 6α-hydroxyl group.8) Further, carbon resonances of both sugar and aglycone moieties of **7** (Tables I and II) except for signals assignable to the side chain were observed at almost the same positions as those of ginsenoside-Rf (**12**), which was isolated from Ginseng and



	R_1	R_2	R_3
2:	-OH	-O-glc^{2}—^{1}xyl	-OH
3:	-O-glc^{2}—^{1}glc	H	-O-glc
4:	-OH	-O-glc^{2}—^{1}glc	-O-glc
12:	-OH	-O-glc^{2}—^{1}glc	-OH

R
9: -O-glc^{2}—^{1}rha
10: -OH

R
7: -O-glc^{2}—^{1}glc
11: -OH
13: -O-glc^{2}—^{1}xyl

	R_1	R_2
1:	-O-glcUA^{2}—^{1}glc	-glc
5:	-OH	-H
6:	-O-glcUA	-glc

8

glc: β-glucopyranosyl
rha: α-rhamnopyranosyl
xyl: β-xylopyranosyl
glcUA: β-glucuronopyranosyl

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts: Aglycone Moiety

	10	**11**	**9**	**7**	**13**
C- 1	39.4	39.5	39.4	39.7	39.6
C- 2	28.0	28.1	27.5	27.8	27.9
C- 3	78.3	78.4	78.1	78.0	78.1
C- 4	40.3	40.3	39.8	40.3	40.3
C- 5	61.8	61.9	60.7	61.5	61.4
C- 6	67.6	67.7	73.9	79.9	79.5
C- 7	47.4	47.5	45.8	45.0	44.9
C- 8	41.0	41.2	40.9	41.2	41.1
C- 9	50.4	50.2	49.9	50.4	50.3
C-10	39.4	39.3	39.4	39.7	39.6
C-11	32.3	32.2	31.8	32.4	32.6
C-12	71.1	70.8	71.0	71.0	70.9
C-13	48.3	49.1	48.1	49.2	49.2
C-14	52.0	52.2	52.0	52.3	52.3
C-15	31.7	32.6	31.6	32.4	32.6
C-16	25.4	25.8	25.3	25.8	25.8
C-17	49.3	49.5	49.2	49.5	49.5
C-18	17.7a)	17.8a)	17.7a)	17.9a)	17.9a)
C-19	17.4a)	17.2a)	17.4a)	17.3a)	17.2a)
C-20	86.6	87.0	86.5	87.1	87.1
C-21	26.9b)	26.9b)	26.8b)	27.1b)	27.0b)
C-22	32.8	32.6	32.6	32.4	32.6
C-23	28.6	28.7	28.7	29.0	28.7
C-24	85.6	88.4	85.7	88.4	88.4
C-25	70.2	70.0	70.2	70.1	70.0
C-26	27.1b)	26.6b)	27.0b)	26.7b)	26.6b)
C-27	27.6b)	29.0b)	27.5b)	29.0b)	29.0b)
C-28	31.8	31.9	31.9	32.4	31.7
C-29	16.4a)	16.5a)	16.8a)	16.8a)	16.7a)
C-30	18.2a)	18.1a)	18.0a)	17.9a)	17.9a)

δ ppm from internal TMS in C_5D_5N.
a, b) Values in any column may be reversed, though those given here are preferred.

TABLE II. ^{13}C-NMR Chemical Shifts: Sugar Moiety

		12	**7**	**2**	**13**
6-glc (inner)	1	103.2	103.6	103.6	103.5
	2	79.5	79.9	79.9	79.9
	3	78.7a)	78.6	78.8	78.8
	4	71.7	71.7	71.3a)	71.3a)
	5	79.8	79.9	80.3	80.4
	6	62.9	63.0	62.9	63.0
6-glc (terminal)	1′	103.2	103.9		
	2′	75.9	76.0		
	3′	78.4a)	78.6		
	4′	72.3	72.4		
	5′	79.8	79.9		
	6′	63.3	63.5		
6-xyl	1			104.9	104.9
	2			75.9	75.9
	3			78.8	78.8
	4			71.7a)	71.7a)
	5			67.3	67.3

glc: β-glucopyranosyl.
xyl: β-xylopyranosyl.
δ ppm from internal TMS in C_5D_5N.
a) Values in any column may be reversed, though those given here are preferred.

formulated as 6-*O*-β-sophoroside of 20(*S*)-protopanaxatriol.[1c] Recently, the assignment of carbon signals of the sugar moiety of **12** was substantiated by means of selective deuteration.[4b] These findings led to the formulation of **7** as 6-*O*-β-sophoroside of **11**, as shown in Chart 1.

The second new saponin I, named majonoside-R2 (**13**) (yield: 0.11%), afforded glucose and xylose on acid hydrolysis and its ^{1}H-NMR spectrum in pyridine-d_5 exhibited two anomeric proton signals at δ 4.92 (1H d, $J=6$ Hz) and 5.64 (1H d, $J=6$ Hz), indicating the presence of β-glycosyl linkages in **13**. The presence of an *O*-glucose-xylose moiety in **13** was demonstrated by the fragment ions at m/z 259 (terminal xylose $(Ac)_3{}^+$) and 547 (glucose $(Ac)_3$-xylose $(Ac)_3{}^+$) in the mass spectrum of its acetate. The mass spectrum of its acetate also showed a strong ion at m/z 143 (**8**) which was observed in the spectrum of the acetate of **7** (*vide supra*). In the ^{13}C-NMR spectrum of **13** (Table I), carbon signals due to its aglycone moiety were almost superimposable on those of **7**, indicating that **13** can also be formulated as a 6-*O*-glycoside of **11**. The carbon resonances due to the sugar moiety of **13** (Table II) were observed at almost the same positions as those of **2**.[4b] It follows that the structure of **13** can be formulated as 6-*O*-β-xylopyranosyl (1→2)-β-glucopyranoside of **11**, as shown in Chart 1.

It is noteworthy that, like Chikusetsu-ninjin and Himalayan Panax and unlike Ginseng, Sanchi-Ginseng and American Ginseng, Zu-Tziseng contains a large amount of the oleanolic acid-saponins, **1** and **6**, while its dammarane-saponin composition, **2**, **3**, **4**, **7**, and **13** is characteristic of this plant.

Experimental

General Procedure——NMR spectra were taken on a JEOL JNM PFT-100 spectrometer in C_5D_5N using tetramethylsilane (TMS) as an internal standard (^{1}H-NMR at 100 MHz and ^{13}C-NMR at 25.15 MHz).

MS were taken at 75 eV on a JEOL 01-SG-2 spectrometer by the direct inlet method; ionization current, 200 μA; accelerating voltage, 6—8 kV. Trimethylsilylation for MS: A sample of saponin (1—2 mg) was heated with *N*-trimethylsilyl imidazole (10 drops) in a sealed micro-tube at 80°C for 2—3 h. The reaction mixture was diluted with H_2O and then extracted with n-C_6H_{14}. The C_6H_{14} layer was washed with H_2O and concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was subjected to MS. Acetylation for MS: A sample of saponin (1—2 mg) was heated with $(CH_3CO)_2O$ (2—3 drops) and C_5H_5N (5—6 drops) in a sealed micro-tube at 80°C for 2—3 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature and then the residue was subjected to MS.

Identification of the Known Saponins——Each known saponin was identified by thin layer chromatography (TLC) on Kieselgel 60F254 (Merck) with $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer) and with EtOAc–EtOH–H_2O (8: 2: 1), and by reverse phase TLC on silica gel plates (RP-8 and RP-18 F254 (Merck)) with 60—75% MeOH (detection: H_2SO_4), as well as by ^{1}H-NMR and ^{13}C-NMR spectroscopy, optical rotation measurement and MS (as the acetate or trimethylsilyl ether) in comparison with an authentic sample.

Hydrolysis of a Saponin and Identification of the Resulting Monosaccharides——A saponin (a few mg) was heated with 10% HCl in H_2O–dioxane (1: 1) in a sealed micro-tube at 90°C for 2 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature. For gas liquid chromatography (GLC) analysis, the residue was trimethylsilylated by the same procedure as that used for MS. GLC: On a Shimadzu GC-4A gas chromatograph; glass column of 1.5% SE-30 on chromosorb W, 4 mm × 2 m; detector, FID; injection temperature, 200°C; column temperature, 180°C; carrier gas, N_2, 1.0 kg/cm^2.

Separation of Saponins——The dried and powdered rhizomes (1 kg), collected in Likiang, Yunnan, China were extracted with hot MeOH. The MeOH solution was concentrated to dryness to give an MeOH extract. A suspension of the extract in H_2O was washed with Et_2O and then extracted with 1-BuOH saturated with H_2O. The BuOH layer was concentrated to dryness to give a crude saponin fraction (70 g). This fraction was separated into six fractions, Fr-1—Fr-6 by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (30: 10: 1—30: 22: 6, homogeneous)).

Fr-1 was further chromatographed on silylated silica gel (Lobar-RP-8 (Merck)) (solvent: 70% MeOH) to give **2** (yield: 0.03%), colorless needles (from MeOH–H_2O), mp 184—186°C, $[\alpha]^{24}_{D}$ $+10.1°$ ($c=0.67$, MeOH). Another fraction from this column was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer)) and finally on silica gel (solvent: EtOAc–EtOH–H_2O (8: 2: 1, homogeneous)), affording **13** (yield: 0.11%), white powder (reprecipitated from MeOH–Et_2O), $[\alpha]^{15}_{D}$ $-2.4°$ ($c=1.13$, MeOH). *Anal.* Calcd for $C_{41}H_{70}O_{14}\cdot 2H_2O$: C, 59.83; H, 9.06. Found: C, 59.84; H, 8.76.

Fr-2 was chromatographed on silylated silica gel (*vide supra*) (solvent: 65% MeOH) and then on silica gel (solvent: EtOAc–EtOH–H_2O (8: 2: 1, homogeneous)), affording **7** (yield: 0.07%), white powder (reprecipitated from MeOH–Et_2O), $[\alpha]_D^{15}$ +1.0° (c=1.13, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{15}\cdot3.5H_2O$: C, 57.32; H, 9.05. Found: C, 57.54; H, 8.75.

Fr-3 was chromatographed on silylated silica gel (*vide supra*) (solvent: 75% MeOH), yielding **3** (yield: 0.67%), white powder, $[\alpha]_D^{24}$ +17.8° (c=0.67, MeOH).

Fr-4, after repeated column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (75: 35: 10, lower layer)) and on silylated silica gel (*vide supra*) (solvent: 60% MeOH), afforded a saponin (yield: 0.01%) which was not completely pure, though its ^{13}C-NMR spectrum was almost superimposable on that of 20-glucoginsenoside-Rf (**4**).

Fr-5 was recrystallized from MeOH–H_2O to give **6** (yield: 0.19%), colorless needles, mp 211—213°C, $[\alpha]_D^{22}$ +7.5° (c=1.11, MeOH).

Fr-6 was further chromatographed on silylated silica gel (*vide supra*) (solvent: 65% MeOH) to give **1** (yield: 0.95%), white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{16}$ +9.6° (c=0.69, MeOH).

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