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Chemical and Morphological Study on Chinese *Panax japonicus* C. A. MEYER (Zhujie-Shen)

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From rhizomes of *Panax japonicus* collected in Yunnan, China (Chinese name: Zhujie-Shen), various oleanolic acid saponins, chikusetsusaponins-IVa (**1**), -IV (**7**) and -V (**2**) were isolated in high yields, together with the methyl ester of **2**. All these compounds have already been isolated from rhizomes of the same species of Japanese (Japanese name: Chikusetsu-Ninjin) and Himalayan *Panax* (*P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA). On the other hand, dammarane saponins of Zhujie-Shen were found to consist of ginsenosides-Rd (**3**), -Re (**9**), -Rg_1 (**10**), -Rg_2 (**11**), notoginsenoside-R2 (**4**) and pseudo-ginsenoside-F_{11} (**13**), being significantly different from those of Chikusetsu-Ninjin and Himalayan *Panax*.

No characteristic morphological difference in external or internal structures was observed between the Chinese and Japanese specimens.

Keywords——Chinese wild *Panax*; *Panax japonicus*; Zhujie-Shen; Chikusetsu-Ninjin; Araliaceae; rhizome; chikusetsusaponin; oleanolic acid saponin; ginsenoside; dammarane saponin

In the south of China, various wild *Panax* spp. (Araliaceae) are distributed. In contrast to Ginseng, American Ginseng and Sanchi-Ginseng, which have a carrot-like root, most of the Chinese wild *Panax* spp. have long horizontal rhizomes like *Panax japonicus* C. A. MEYER (=*P. pseudo-ginseng* WALL. subsp. *japonicus* HARA, grows wild in Japan; Japanese name: Chikusetsu-Ninjin or Tochiba-Ninjin) and Himalayan *Panax* (*P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA).[1,2] Since the close botanical relationship between these Himalayan, Chinese and Japanese wild *Panax* spp. has been pointed out, chemical studies on these Chinese wild plants are of interest, not only from the viewpoint of pharmacognosy but also from a plant-geographical point of view. Recently, we have isolated from rhizomes of *P. japonicus* C. A. MEYER var. *major* (BURK.) C. Y. WU et K. M. FENG (Chinese name: Zu-Tziseng, collected in Likiang, Yunnan) two known oleanolic acid saponins, chikusetsusaponins-IVa (**1**) and -V (**2**) (=ginsenoside-Ro), which have already been isolated from rhizomes of Chikusetsu-Ninjin[3] and Himalayan *Panax*[4] as their major saponins by Shoji *et al.* Besides these oleanolic acid saponins, several dammarane saponins, ginsenoside-Rd (**3**) and notoginsenoside-R2 (**4**) and new ocotillol-type saponins named majonosides-R1 (**5**) and -R2 (**6**) were also isolated from rhizomes of Zu-Tziseng.[5]

In Yunnan, Guizhou, Hunan, Jiangxi, Zhejiang and Szechwan, at lower altitudes (1500—2500 m) than those at which Zu-Tziseng is found, there grows another Chinese wild *Panax* named "Zhujie-Shen, Zhujie-Sanchi or Zhao-Shen," which has a bamboo-like long horizontally creeping rhizome, and which has been considered to be botanically identical with Japanese *P. japonicus* (Chikusetsu-Ninjin).[2] The present paper reports a chemical and morphological comparison of rhizomes of this medicinal plant with Chikusetsu-Ninjin, both

of which have been used as an expectorant, analgesic and antitussive in China and Japan, respectively.

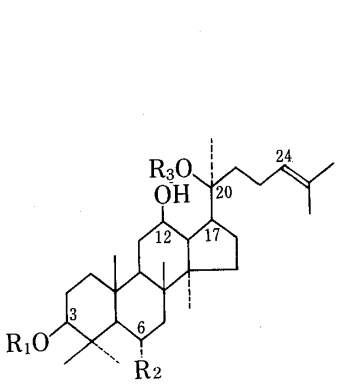
The rhizomes of Zhujie-Shen collected at Zhaotong, Yunnan were extracted with methanol. A crude saponin fraction (yield: about 20%) of the methanolic extract was separated by selective solvent precipitation followed by repeated chromatography to give ten saponins, tentatively named A-J in decreasing order of polarities on a thin layer chromatogram (TLC) on silica gel.

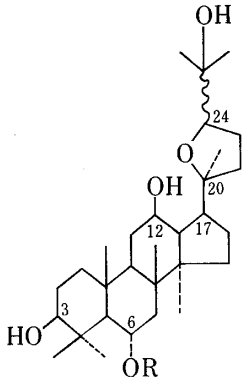
Three major saponins, A, B and C (yields: 3.1, 3.4 and 2.8%, respectively) were proved to be identical with oleanolic acid saponins, **2**, chikusetsusaponin-IV (**7**) and **1**, respectively, all of which have already been isolated from Chikusetsu-Ninjin and Himalayan *Panax* as their major saponins.[3,4]

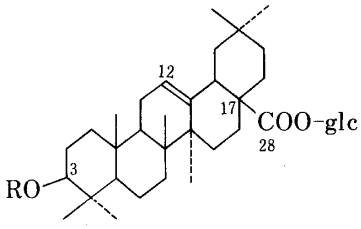
The aglycones of all of the other saponins are dammarane-type triterpenes. Saponin E (yield: 0.04%) was identified as **3**, a saponin of 20 (*S*)-protopanaxadiol (**8**) already isolated from Ginseng roots.[6] Saponins D, G and I (yields: 0.12, 0.15 and 0.05%, respectively) were identical with ginsenosides-Re (**9**), -Rg_1 (**10**) and -Rg_2 (**11**), all of which were also isolated from Ginseng roots as saponins of 20 (*S*)-protopanaxatriol (**12**).[7,8] Saponin H (yield: 0.02%) was identical with **4**, a saponin of **12** recently isolated from roots of Sanchi-Ginseng[9] and also from rhizomes of Zu-Tziseng.[5] Saponin-J (yield: 0.24%) was found to be identical with an ocotillol-type saponin, pseudo-ginsenoside-F_{11} (**13**) which was previously isolated from leaves of Himalayan *Panax*[10] and also from leaves of American Ginseng.[11]

The remaining saponin F (yield: 0.04%) was identified as the methyl ester of **2** (**14**). Since **14** could not be detected in a methanolic extract of rhizomes of Zu-Tziseng, which also contains a fairly large amount of **2**, this ester did not seem to be an artifact formed during the extraction of the rhizomes with hot methanol.

Although the present study revealed that the dammarane saponin composition of Zhujie-Shen (Chinese *P. japonicus*) is significantly different from that of Chikusetsu-Ninjin (Japanese *P. japonicus*), the external structures of both plants are very similar, being difficult to distinguish morphologically from each other.[2] The internal structure of the rhizome of







	R_1	R_2	R_3
3 :	-glc^2—glc	-H	-glc
4 :	-H	-O-glc^2—xyl	-H
8 :	-H	-H	-H
9 :	-H	-O-glc^2—rha	-glc
10 :	-H	-O-glc	-glc
11 :	-H	-O-glc^2—rha	-H
12 :	-H	-OH	-H

	R	
5 :	-glc^2—glc	24 (*S*)
6 :	-glc^2—xyl	24 (*S*)
13 :	-glc^2—rha	24 (*R*)

glc = β-D-glucopyranosyl
xyl = β-D-xylopyranosyl
rha = α-L-rhamnopyranosyl
glcUA = β-D-glucuronopyranosyl
ara(f) = α-L-arabinofuranosyl

	R
1 :	-glcUA
2 :	-glcUA^2—glc
7 :	-glcUA^4—ara(f)
14 :	^{6}COOCH$_3$ | -glcUA^2—glc

Chart 1

TABLE I. Anatomical Characters of Rhizomes of *Panax japonicus*

	Chikusetsu-Ninjin[12]	Chikusetsu-Ninjin[a]	Zhujie-Shen (Yunnan)
Cork layer	5—7 layers of thin-walled cells regularly elongated to tangential	5—7 layers of thin-walled cells regularly elongated to tangential	6—8 layers of thin-walled cells occasionally secondary growth
Cortex	Several layers of sclerenchymatous cells elongated to tangential	3—4 layers of sclerenchymatous cells elongated to tangential	2—4 layers of sclerenchymatous cells elongated to tangential
Cambium	Irregular cells continued obviously	Continued obviously	Continued obviously
Resin canal	7—8 neighboring cells 1 or 2 layers circularly in the upper parts of the phloem	6—7 neighboring cells 2—3 layers circularly in the upper parts of the phloem	6—8 neighboring cells 2—3 layers circularly in the upper parts of the phloem occasionally in xylem
Fiber bundle	Upper or central parts of the xylem	Occasionally in the xylem	Occasionally in the xylem
Starch grain	Almost all tissue	Almost all tissue	Almost all tissue
Clustered calcium oxalate	Scattered in cortex and xylem	Scattered in cortex not in xylem	Scattered in cortex not in xylem

a) Collected in Hiroshima.

TABLE II. Comparison of Saponins of *Panax* spp.

Aglycone	20(*S*)-Protopanaxadiol	20(*S*)-Protopanaxatriol	Oleanolic acid
P. ginseng (Ginseng) (root)	G-Ra_1,[a] -Ra_2,[a][13] -Rb_3,[a] -Rb_1 [0.47], -Rb_2 [0.21], -Rc [0.26], -Rd (**3**) [0.15]	G-Re(**9**) [0.15], -Rf [0.05], -Rg_1 (**10**) [0.21], -Rg_2 (**11**),[a] -Rh_1,[a] 20-glc-Rf[a]	G-Ro (**2**)[a]
P. japonicus (Chikusetsu-Ninjin) (rhizome) (Japan)	C-III [1.17], -Ia[a]	G-Rg_2 (**11**)[a]	C-V (**2**) [5.35], -IV (**7**) [0.43], -IVa,[a] -Ib[a]
P. pseudo-ginseng subsp. *himalaicus* (Himalayan *Panax*) (rhizome) (Bhutan)	G-Rb_1 [1.05]	—	C-V (**2**) [7.25], -IV (**7**) [0.3], -IVa (**1**) [0.6]
P. japonicus var. *major* (Zu-Tziseng) (rhizome) (Yunnan)	G-Rd (**3**) [0.67]	M-R1 (**5**) [0.07],[b] -R2 (**6**) [0.11],[b] 20-glc-Rf,[a] N-R2 (**4**)[a]	C-V (**2**) [0.95], -IVa (**1**) [0.19]
Chinese *P. japonicus* (Zhujie-Shen) (rhizome) (Yunnan)	G-Rd (**3**)[a]	G-Re (**9**) [0.12], -Rg_1 (**10**) [0.15], -Rg_2 (**11**) [0.05], N-R2 (**4**),[a] P-F_{11} (**13**) [0.24][b]	C-V (**2**) [3.1], -IV (**7**) [3.4], -IVa (**1**) [2.8], Me ester of V (**14**)[a]

a) Minor saponin. *b*) Ocotillol type.
G, ginsenoside; C, chikusetsusaponin; M, majonoside; N, notoginsenoside; P, pseudo-ginsenoside; (G-Ro=C-V); [ ]=yield %.

Chikusetsu-Ninjin was previously reported by Mitsuno *et al.*[12] The present authors compared the internal structure of the rhizome of Zhujie-Shen with that of Chikusetsu-Ninjin collected in Hiroshima, and the results are summarized in Table I. Although some discrepancies in the

structure of the canbium and in the distribution of crystals of calcium oxalate were found between Mitsuno's data and the present results, no significant difference in the internal structure was observed between Chikusetsu-Ninjin and Zhujie-Shen except for the amount of crystals of calcium oxalate in the cortex; the crystals were more abundant in the Chinese specimen than in Chikusetsu-Ninjin.

The saponin compositions of underground parts of *Panax* spp., including the result of the present study, are summarized in Table II, which indicates that unlike Ginseng, American Ginseng and Sanchi-Ginseng, rhizomes of Japanese wild *Panax* (Chikusetsu-Ninjin), Chinese wild *Panax* spp. (Zhujie-Shen and Zu-Tziseng) and Himalayan *Panax* contain large amounts of the common oleanolic acid saponins, while the dammarane saponins are characteristic of each species.

Further chemotaxonomical studies on *Panax* spp. of East Asia as well as the locality dependence of their chemical constituents are in progress as a part of our China–Japan co-operative research on medicinal plants.

Experimental

The identification of known saponins was performed by comparisons of physical constants, mass spectrum (MS) as the acetate or trimethylsilyl ether, ^{13}C nuclear magnetic resonance (NMR) in C_5D_5N or DMSO-d_6 and TLC behavior with those of corresponding authentic samples. The details are given in our previous paper.[5] For separation by reverse phase column chromatography, a Lobar column size-B LiChroprep RP-8 (Merck No. 11804) was used.

Extraction and Separation of Saponins——Dried and powdered rhizomes (250 g) of Zhujie-Shen collected in Zhaotong, Yunnan at an altitude of 2100 m were extracted with hot MeOH to give an MeOH extract (after evaporation) in a yield of 37%. An aqueous suspension of this MeOH extract was washed with ether and then extracted with 1-BuOH saturated with H_2O. On concentration *in vacuo*, the BuOH layer afforded a crude saponin fraction in a yield of 20%. A saturated solution of this fraction in MeOH (*ca.* 300 ml) was poured into an excess of CH_3COCH_3 (*ca.* 2 l) to give a precipitate, which was further subjected to this MeOH–CH_3COCH_3 precipitation. This process was repeated again to yield the final precipitate, tentatively named PPT, which consisted mainly of **1**, **2** and **7** (separation of PPT: see last part of this section). The combined supernatants were evaporated to dryness and the residue was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (30:10:1, 30:15:2, 30:20:5 and finally 30:23:7, homogeneous)) to provide seven fractions named Fr. 1—7 in order of elution.

Fr. 1 was subjected to chromatography on a reverse phase column (solvent: 70—75% MeOH), to give **13** (white powder, $[\alpha]_D^{11}$ −14.8° (c=0.67, MeOH), yield 0.24%), a mixture of **11** and **4** and finally **10** (white powder, $[\alpha]_D^{11}$ +19.1° (c=0.67, MeOH), yield 0.15%). The mixture of **11** and **4** was acetylated with Ac_2O and anhydrous C_5H_5N in the usual way and the resulting mixture of acetates was chromatographed on silica gel (solvent, n-C_6H_{14}–$CHCl_3$–EtOAc (1:1:1, homogeneous)), yielding the acetates of **11** and **4**. Both acetates were subjected to deacetylation with 2.5% KOH in EtOH at 80 °C for 1 h, followed by neutralization with ion exchange resin (Amberlite MB-3), affording **11**, colorless needles from EtOH, mp 185—188 °C, $[\alpha]_D^{17}$ −16.3° (c=0.64, DMSO) and **4**, colorless prisms from MeOH–H_2O, mp 184—187 °C, $[\alpha]_D^{16}$ +16.1° (c=0.67, MeOH) in yields of 0.05 and 0.02%, respectively.

Fr. 2 was chromatographed on a reverse phase column (solvent: 70% MeOH) then on silica gel (solvent: EtOAc–EtOH–H_2O (8:2:1, homogeneous)) to give **14**, white powder, $[\alpha]_D^{11}$ +4.5° (c=0.67, MeOH), yield: 0.04%.

Fr. 3 was chromatographed on a reverse phase column (solvent: 85% MeOH), yielding **3**, white powder, $[\alpha]_D^{11}$ +19.0° (c=0.67, MeOH), yield 0.04%.

Chromatography of Fr. 4 on silica gel (solvent: EtOAc–EtOH–H_2O (8:2:1, homogeneous) afforded **9**, colorless needles from 50% MeOH, mp 203—205 °C, $[\alpha]_D^{11}$ −7.6° (c=0.67, MeOH), yield 0.12%.

Fr. 5 was subjected to chromatography on highly porous polymer (MCI Gel CHP-20P, Mitsubishi Chem. Co., Ltd.) (solvent: 70% MeOH) followed by deionization with ion exchange resin (Dowex 50W-X1), affording **1** and **7**. Fr. 6, a mixture of **1**, **2** and **7** was derived to methyl esters with CH_2N_2 in MeOH–Et_2O and the resulting mixture of esters was separated repeatedly by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (30:10:1, homogeneous)) to give the methyl esters of **1**, **2** and **7**, all in of very low yields (negligible in relation to the calculation of total yields). Fr. 7 was deionized with ion exchange resin (Dowex 50W-X1) and purified by reprecipitation by addition of EtOAc to its MeOH solution, affording **2**.

The aforementioned PPT was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous)) to give **2** and a mixture of **1** and **7**, the later of which was separated by chromatography on highly porous polymer (*vide supra*, solvent: 70% MeOH), yielding **1** and **7**. These saponins, **1**, **2** and **7** were further purified by treatment with ion exchange resin (Amberlite MB-3).

Total yields of **1**, **2** and **7** were 2.8, 3.1 and 3.4%, respectively; **1**, colorless prisms from 50% MeOH, mp 216—

218 °C (dec.), $[\alpha]_D^{23}$ +12.6° (c=1.09, MeOH); **2**, white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{20}$ +15.9° (c=0.98, MeOH); **7**, white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{22}$ −9.0° (c=1.01, C_5H_5N).

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References

1) H. Hara, *J. Jap. Botany*, **45**, 197 (1970).
2) J. Zhou, M.-Z. Wu, T.-R. Yang and C.-Y. Wu, *Acta Phytotaxonomica Sinica*, **13**, 29 (1975).
3) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968); N. Kondo, J. Shoji, N. Nagumo and N. Komatsu, *ibid.*, **89**, 846 (1969); N. Kondo, K. Aoki, H. Ogawa, R. Kasai and J. Shoji, *Chem. Pharm. Bull.*, **18**, 1558 (1970); N. Kondo, Y. Marumoto and J. Shoji, *ibid.*, **19**, 1103 (1971); T. D. Lin, N. Kondo and J. Shoji, *ibid.*, **24**, 253 (1976).
4) N. Kondo and J. Shoji, *Chem. Pharm. Bull.*, **21**, 2705 (1973); N. Kondo and J. Shoji, *ibid.*, **23**, 3282 (1975).
5) T. Morita, R. Kasai, O. Tanaka, J. Zhou, T.-R. Yang and J. Shoji, *Chem. Pharm. Bull.*, **30**, 4341 (1982).
6) S. Sanada, N. Kondo, J. Shoji, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.*, **22**, 421 (1974).
7) Y. Nagai, O. Tanaka and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
8) S. Sanada, N. Kondo, J. Shoji, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.*, **22**, 2407 (1974).
9) J. Zhou, M.-Z. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari and T. Fuwa, *Chem. Pharm. Bull.*, **29**, 2844 (1981).
10) O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).
11) S. E. Chen, E. J. Staba, S. Taniyasu, R. Kasai and O. Tanaka, *Planta Med.*, **42**, 406 (1981).
12) M. Mitsuno and K. Hukai, *Shoyakugaku Zasshi*, **7**, 10 (1953).
13) H. Besso, R. Kasai, Y. Saruwatari, T. Fuwa and O. Tanaka, *Chem. Pharm. Bull.*, **30**, 2380 (1982) and references cited therein.