Saponins of Plants of Panax Species Collected in Central Nepal, and Their Chemotaxonomical Significance. III.[1)]

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***Panax pseudo-ginseng* subsp. *pseudo-ginseng* has a carrot like root with a small rhizome. It was shown that the saponin composition of roots and rhizomes of this subspecies collected in Tibet and China was extremely poor. From the roots and rhizomes collected in Central Nepal, (specimen-PNct), only a small amount of an oleanolic acid saponin, β-D-glucopyranosyl-oleanolate (2) was isolated together with a polyacetylene-alcohol, panaxynol (3). In another specimen (specimen-PNs), also collected in Central Nepal, two oleanolic acid saponins, stipleanoside R2 (4) and chikusetsusaponin IV (5) were detected. No dammarane saponin was identified in either specimen. *P. pseudo-ginseng* subsp. *himalaicus* (Subsp-H) has a big rhizome with a small round root. From rhizomes and roots of this subsp. collected in Central Nepal (specimen-HNct), a fairly large amount of dammarane saponins, ginsenosides-Rb_1 (6), -Rd (7), -Re (9) and -Rg1 (10), gypenoside XVII (8), notoginsenoside-R_1 (11), majonoside-R2 (12) and pseudo-ginsenoside-F_{11} (13) were isolated, while no oleanane saponin (oleanolic acid saponin) was identified in this subsp. Based on the present and previous studies, medicinal evaluation and chemogeographical correlation of Himalayan *Panax* spp. are discussed.**

Key words saponin; *Panax pseudo-ginseng*; Araliaceae; Himalayan *Panax*; ginsenoside; chemotaxonomy

In the Central and Eastern Himalayas, South-Western China and Japan, several wild *Panax* species which have a close taxonomical relationship with each other are found. In 1970, Hara designated these Asian wild plants as subspecies or varieties of *P. pseudo-ginseng*; *Panax pseudo-ginseng* WALL. subsp. *pseudo-ginseng* HARA (tentatively named Subsp-P), *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA (tentatively named Subsp-H), *P. pseudo-ginseng* WALL. var. *bipinatifidus* HARA, *P. pseudo-ginseng* WALL. var. *angustifolius* HARA and *P. pseudo-ginseng* WALL. subsp. *japonicus* HARA (=*P. japonicus* C. A. MEYER).[2)] Of these species, Subsp-P has a carrot-like fleshy root with a small rhizome, growing rarely in the Himalayas, while Subsp-H and most of the other Asian wild *Panax* spp. have a long developing rhizome with a small round root.

From ginseng (*P. ginseng* C. A. MEYER, North-Eastern Asia), the well-known oriental traditional medicine, a number of biologically active dammarane saponins named ginsenosides were isolated together with an oleanane saponin, ginsenoside-Ro (=chikusetsusaponin V, a glucuronide-saponin of oleanolic acid, **1**).[3)] Comparative studies on saponins of *Panax* spp. have been extensively conducted.[4—6)] It has been revealed that the saponin content of a specimen of Subsp-P collected at Nielamu, Tibet was very low.[7)] For further confirmation of the saponin composition of this subsp., the present authors have investigated the specimens of Subsp-P growing near Kathmandu, Central Nepal where Hara and coworkers collected the specimens for their taxonomical studies.

For Subsp-H, which abundantly grows in the Eastern and Central Himalayas, a close relationship between the saponin-composition and locality of plants has been observed.[1,8—18)] In order to obtain further evidence on this chemogeographical observation, the present authors have conducted isolation, identification and analysis of saponins of a specimen of Subsp-H collected near Kathmandu, Central Nepal.

Results and Discussion

Because only small amounts of materials were available, the identification was carried out only for the major saponins (content or yield: >0.01%), as in the case of previous studies on Himalayan *Panax* spp.[1,7—10)]

TLC and HPLC of extracts of Subsp-P roots with small rhizomes, collected at Chautara, near Kathmandu, Central Nepal (specimen-PNct, 34.3 g) suggested a much lower saponin content than other *Panax* spp. Repeated chromatography of this extract afforded an oleanolic acid saponin, β-D-glucopyranosyl-oleanolate (**2**, yield: 0.052%, Fig. 1) which was previously isolated from Chinese *P. japonicus* C. A. MEYER var. *angustifolius* (Burk.) CHENG et CHU[19)] *etc*., while no ginseng major dammarane saponins, ginsenosides-Rb_1, -Rb_2, -Rc, -Rd (and their malonylated saponins), -Re and -Rg_1 were detected (Fig. 2). Together with **2**, a polyacetylene-alcohol, panaxynol[20,21)] (**3**, Fig. 1) already obtained from ginseng and several other *Panax* spp., was isolated and identified in a yield of 0.028%.

For saponins from the roots and small rhizomes of Subsp-P collected at Mt. Shioupuri near Kathmandu, Central Nepal (specimen-PNs), identification was conducted only by comparison of TLC and HPLC due to shortage of material (roots: about 12 g, rhizomes: about 0.2 g). Two oleanolic acid saponins, stipleanoside R2 (**4**) and chikusetsusaponin IV (**5**) (Fig. 1) were detected together with a trace amount of unidentified saponins. Saponin (**4**) has been isolated from the rhizomes of *P. stipleanatus* growing in South China,[22)] and compound (**5**) has been isolated from the rhizomes of Japanese *P. japonicus*[23)] and several Himalayan and Chinese *Panax* spp. The amounts of these saponins in specimen-PNs were determined by HPLC[24)]; **4**: 0.10% in roots and 0.13% in rhizomes and **5**: 0.16% in both roots and rhizomes. Ginseng

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Table 1. Yields (%) of Major Saponins from Rhizomes of *Panax Pseudo-ginseng* subsp. *himalaicus* Collected in Central and Eastern Himalaya

	West ←						→ East
	HNg	HNcm	HNct	HNd	HBt	HBp	HBk
Dammarane saponins							
20(*S*)-Protopanaxadiol-type							
Ginsenoside-Rb_1 (**6**)	5.00*	1.70*	0.62	0.7	0.3	0.05	1.1
Ginsenoside-Rb_3 (**14**)	0.09	0.16	—	—	—	—	—
Ginsenoside-Rd (**7**)	0.94	2.20	0.20	0.1	0.2	0.07	—
Gypenoside-XVII (**8**)	0.39	0.11	0.095	0.08	—	0.03	—
Others	0.17	0.10	—	—	—	0.05	—
20(*S*)-Protopanaxatriol-type							
Ginsenoside-Re (**9**)	0.12	0.18	0.10	0.3	—	0.1	—
Ginsenoside-Rg_1 (**10**)	0.46	0.19	0.51	0.2	0.4	1.2	—
Ginsenoside-Rg_2 (**15**)	—	—	—	0.03	—	—	—
Notoginsenoside-R1 (**11**)	0.06	—	0.16	0.2	—	—	—
Notoginsenoside-R2 (**16**)	—	—	—	0.09	—	—	—
Others	—	—	—	0.02	0.1	0.02	—
Ocotillol-type							
Pseudo-ginsenoside-F_{11} (**13**)	—	—	0.036	0.01	0.07	—	—
24(*S*)-Pseudo-ginsenoside-F_{11} (**17**)	0.10	0.06	—	0.06	—	—	—
Majonoside-R2 (**12**)	0.10	—	0.19	0.1	—	—	—
Others	—	—	—	0.02	0.24	0.02	—
Dammarane saponin total	7.43	4.7	1.91	1.91	1.31	1.54	1.1
Oleanane saponins							
Chikusetsusaponin-IVa (**18**)	—	—	—	0.04	1.7	1.8	0.6
Chikusetsusaponin-IV (**5**)	—	—	—	—	—	—	0.3
Chikusetsusaponin-V (**1**)	—	—	—	0.5	0.4	0.1	7.3
Pseudo-ginsenoside-RT1 (**19**)	—	—	—	0.4	—	5.12	—
Others	—	—	—	—	—	0.12	—
Oleanane saponin total	0	0	0	0.94	2.1	7.13	8.2

Specimen-HNb[11]: collected at Ghorapani, the western foot of Mt. Annapurna, Central Nepal, altitude 2743 m. Specimen-HNcm[11]: collected at Chame, the eastern foot of Mt. Annapurna, Central Nepal, altitude 2700 m. * The quantitative HPLC analysis[24] indicated that 29% of **6** occurred as the malonyl ester, malonyl-ginsenoside-Rb_1, which was also isolated from HNg.[11] Specimen-HNct: collected at Chautara near Kathmandu, Central Nepal, altitude 3050 m. Specimen-HNd[1]: collected between Dhunche and Singkunba near Kathmandu, Central Nepal, altitude 2500 m. Specimen-HBt[10]: collected at Tzatogang, Western Bhutan, altitude 3100 m. Specimen-HBp[10] collected between Pari-la and Gasa, Western Bhutan, altitude 3550—2600 m. Specimen-HBk[8,9]: collected at Khosa, Western Bhutan, altitude 1800 m.

major dammarane saponins were not detected in roots or rhizomes.

As mentioned, Subsp-P has a carrot-like root, similar to the well-known traditional medicine, ginseng. For example, American ginseng (*P. quinquefolius* L.) and notoginseng (*P. notoginseng* (Burk.) Chen cultivated in Yunnan, China). Previously, Subsp-P was erroneously concluded to be a wild type of notoginseng, however, the present study coupled with previous studies[7,25] confirmed that the saponin content and composition of Subsp-P are very low and poor, being clearly different from ginseng and most other *Panax* spp. This evidence suggests that this subsp. may have no ginseng-like medicinal value. Recently, DNA analysis of Himalayan and Japanese *Panax* spp. indicated that on the phylogenic tree, Subsp-P forms a separate group with a large distance from other Himalayan types, as well as Japanese types.[25] Hara's proposal (*vide supra*)[2] that all Himalayan and Japanese races must be designated as subspecies or varieties of *P. pseudo-ginseng*, should be reinvestigated.

Regarding Subsp-H, from rhizomes with small roots (total 25.3 g) collected at Chautara, Sindhu Chouk District near Kathmandu, Central Nepal (specimen-HNct), the following dammarane-saponins were isolated and identified (Table 1, Fig. 1); saponins of 20(*S*)-protopanaxadiol; ginsenosides-Rb_1[26] (**6**, yield: 0.62%) and -Rd[26] (**7**, yield: 0.20%) and gypenoside-XVII[27] (**8**, yield: 0.095%), saponins of 20(*S*)-protopanaxatriol; ginsenosides-Re[28] (**9**, yield 0.10%) and -Rg_1[29] (**10**, yield 0.51%) and notoginsenoside-R_1[30] (**11**, yield: 0.16%) and ocotillol-type saponins (Table 1); majonoside-R2[31] (**12**, yield 0.19%) and pseudo-ginsenoside-F_{11}[32] (**13**, yield 0.036%). Saponins **6**, **7**, **9** and **10** are known as the major ginseng biologically active principles, and **11** has been isolated from notoginseng and several *Panax* spp. Furthermore, the content of these saponins in specimen-HNct were quantitatively determined by HPLC analysis[33]: **6**: 0.85%, **9**: 0.25%, and **10**: 0.77% (Fig. 2). Saponin **8** has been isolated from *Gynostemma pentaphyllum* and also from several *Panax* spp. Ocotillol-type saponins, **12** and **13** have been isolated from *Panax* spp. growing wild in South-Western China and the Himalayas. It was reported that **12** was also isolated from Vietnamese ginseng (*P. vietnamensis* Ha et Grushv.) in a yield of more than 5%,[34] and the biological activities of this saponin have been extensively investigated.[35] Oleanolic acid saponin was not isolated from this specimen.

As in the case of wild *Panax* spp. in South-Western China and Japan,[3—6] it has been disclosed that the content and composition of saponins in Subsp-H in the Himalayas are highly diverse, depending upon the locality (Table 1). It has been reported that specimens-HNg and -HNcm, collected around Mt. Annapurna, Nepal near the western limit of distribution of *Panax* spp., contain a significant amount of ginseng dammarane saponins without oleanolic acid saponin (oleanane saponin).[11] It appears that on going from the west to the east, the contents of dammarane saponins decrease, while those of oleanolic acid saponins increase. With regards a specimen collected between Dunche and Singkuba near

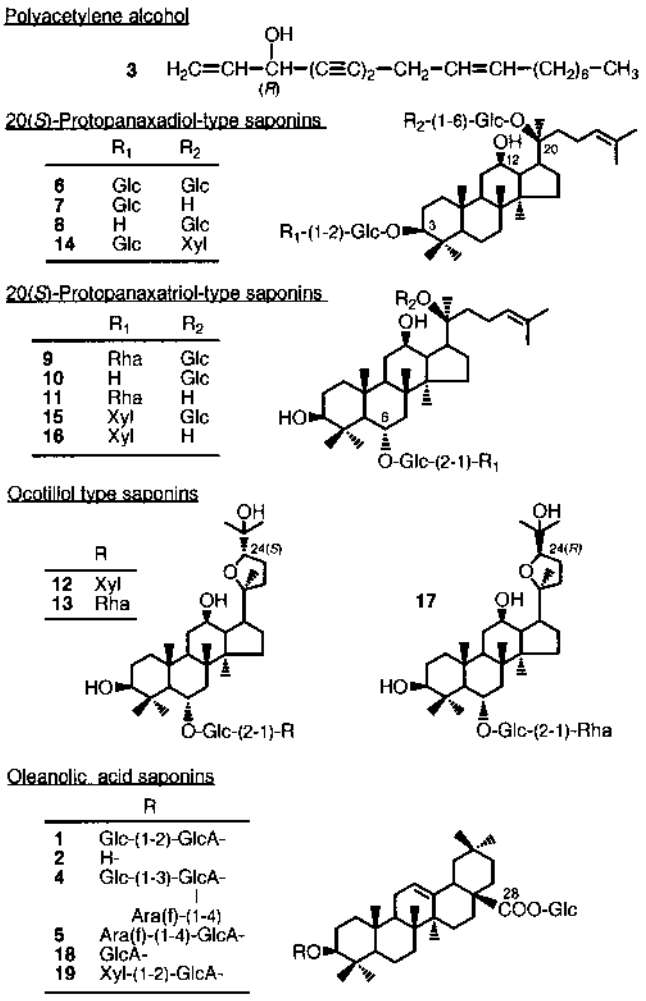

Fig. 1

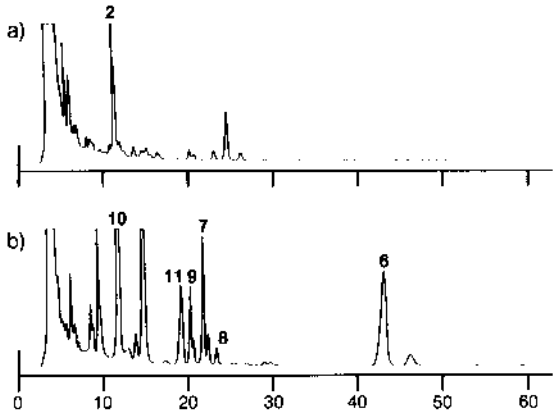

Fig. 2. HPLC Chromatogram

a) Specimen-PNct. b) Specimen-HNct. Conditions for HPLC Chromatograph: Shimadzu LC-10A; column: YMC-Pack A-612 (NH_2) (150×60 mm); mobile phase: 0.05 M phosphate buffer (pH 2.6)–CH_3CN (18 : 82); flow rate: 1.3 ml/min; detection: UV 202 nm, 0.08 AUFC.

Kathmandu (specimen-HNd in the previous study),[1] several oleanolic acid saponins such as chikusetsusaponins were isolated in a total yield of 0.94% together with a significant amount of dammarane saponins. In the present study, from specimen-HNct collected at another area near Kathmandu, dammarane saponins were identified without oleanolic acid saponin, as in the case of specimens-HNg and -Hncm. It is suggested that the areas near Kathmandu may be a chemo-geographical borderline of distribution of Subsp-H with and without oleanolic acid saponins. Comparison of the pharmacological activities of specimens-HNg and -Hncm, as well as specimens-HNct and Hnd, with those of ginseng, American ginseng, notoginseng and Vietnamese ginseng would be an attractive research subject. It should be emphasized that for utilization of these wild plants as a medicine, cultivation of the selected specimen must preserve natural resources, as well as supply sufficient amounts of medicinal materials with constant quality.

It has been demonstrated that rhizomes of Subsp-H collected in the Eastern Himalayas, the Singalila Range of Darjeeling, India[12—18] and Bhutan (specimens-HBt, -HBp and -HBk, Table 1)[8—10] contain large amounts of oleanolic acid saponins (yields: 2—8%) together with relatively small amounts of dammarane saponins, being similar to most *Panax* spp. growing wild in South-Western China and Japan.[3—6] The pharmacological activities of these Subsp-H from the Eastern Himalayas may be similar to those of Chinese and Japanese "Chikusetsu-ninjin" (*P. japonicus*=*P. pseudo-ginseng* subsp. *himalaicus*), being different from those of ginseng. It is also suggested that *Panax* spp., specimens-HBt, -HBp and -HBk from the Eastern Himalayas may be taxonomically distinct from specimens-HNg, -HNcm and -HNct from the Central Himalayas.

Experimental

Plant Material 1) Specimen-PNct: collected at Chautara, Sindhu Chouk District (north-eastern area of Kathmandu, Nepal, elevation 3050 m) in June, 1998 by Mr. Rajan Sakya, Himalayan Herbs Collection & Conservation, P. O. Box 10949, Kathmandu, Nepal. This specimen was supplied to us by Mr. Hideo Fujii, Hideo-Fuji Co. Ltd., 4–2–34 Takatanobaba, Shinjuku-ku, Tokyo 169–0075. A voucher specimen is deposited at Himalayan Herbs Collection & Conservation. 2) Specimen-PNs: collected near the top of Mt. Shioupuri (northern area of Kathmandu, Nepal, elevation 2500 m) in August, 1988 by Dr. Eyong-Chae Han, Korea Wild Ginseng Research Institute, 3RI Daegi, Wangsan, Gangnung City, 210–917 Korea. A voucher specimen is deposited at Korea Wild Ginseng Research Institute. 3) Specimen-HNct, collected at Chautara, Sindhu Chouk District (north-eastern area of Kathmandu, Nepal, elevation 3050 m) in June, 1998 by Mr. Rajan Sakya, Himalayan Herbs Collection & Conservation. This specimen was supplied by Mr. Hideo Fujii, Hideo-Fuji Co. Ltd. A voucher specimen is deposited at Himalayan Herbs Collection & Conservation.

Isolation of 2 and 3 from Specimen-PNct The rhizomes of specimen-PNct (34.3 g, collected in Central Nepal) were cut and extracted four times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (11.0 g, 32.1%). The MeOH extract (10.8 g) was partitioned into AcOEt–H_2O (1 : 1, v/v) solution. Removal of the solvent under reduced pressure from the AcOEt–soluble portion and aqueous phase yielded 1.3 g (3.8%) and 9.2 g (26.8%) of residue, respectively.

The AcOEt extract (1.2 g) was separated by normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 100 g), *n*-hexane–AcOEt (15 : 1→10 : 1→3 : 1, v/v)→MeOH], reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 15 g), MeOH–H_2O (80 : 20, v/v)→MeOH], and HPLC [MeOH–H_2O (80 : 20, v/v)] to give panaxynol (**3**, 13 mg, 0.028%).

The H_2O extract (9.2 g) was separated by reversed-phase silica gel column chromatography [1) 100 g, H_2O→MeOH→$CHCl_3$–MeOH–H_2O (6 : 4 : 1, v/v); 2) 15 g, MeOH–H_2O (60 : 40→70 : 30, v/v)→MeOH] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250×20 mm i.d.), MeOH–H_2O (60 : 40, v/v)] to give β-D-glucopyranosyl-oleanolate (**2**, 18 mg, 0.052%). The known compounds (**2**, **3**) were identified by comparison of authentic

samples.

Analysis of Neutral and Acidic Saponins of Rhizomes and Roots of Specimen-PNs The roots (11.906 g) was extracted 5 times with 60 ml of 70% MeOH at room temperature for 20 min and the combined extracts were concentrated to dryness below 40 °C. An aqueous suspension of the residue was chromatographed on a small column of highly porous polymer (MCI-gel CHP20P, 75—150 μm, 1×10 cm, Mitsubishi Chemical Industry) by eluting with H_2O, 40% MeOH and then MeOH (200 ml each). The MeOH eluate was concentrated to dryness and the residue (saponin fraction) was dissolved in 70% MeOH and subjected to quantitative analysis by HPLC according to the reported procedures[24]; on a ODS column, Ultron N-C18 (Chromato Packing Center, Kyoto, Japan) and on a column of Ultron NH_2 (Chromato Packing Center, Kyoto, Japan).

Qualitative analysis of saponins was carried out by TLC on Kiesel gel $60F_{254}$ (Merck) developing with H_2O saturated 1-BuOH, 1-BuOH–AcOH–H_2O (4 : 1 : 5, v/v, upper phase) or $CHCl_3$–MeOH–H_2O (65 : 35 : 10, v/v, lower phase), visualized with H_2SO_4.

Saponins in rhizomes (0.202 g) were also analyzed by the same procedures as above.

Isolation of Dammarane-Type Saponins from Specimen-HNct The rhizomes of specimen-HNct (25.3 g, collected in Central Nepal) were cut and extracted four times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (7.8 g, 30.8% from natural medicine). The MeOH extract (7.5 g) was partitioned into a 1-BuOH–H_2O (1 : 1, v/v) solution. Removal of the solvent under reduced pressure from the 1-BuOH-soluble portion yielded 3.6 g (14.2%) of residue. The 1-BuOH extract (3.4 g) was subjected to normal-phase silica gel column chromatography [100 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer→65 : 35 : 10, lower layer→6 : 4 : 1, v/v)→MeOH] to give seven fractions [fr. 1 (357 mg, 1.4%), fr. 2 (135 mg, 0.5%), fr. 3 (619 mg, 2.4%), fr. 4 (373 mg, 1.5%), fr. 5 (434 mg, 1.7%), fr. 6 (1088 mg, 4.3%), fr. 7 (387 mg, 1.5%)]. Fraction 3 (600 mg) was separated by reversed-phase silica gel column chromatography [20 g, MeOH–H_2O (1 : 1, v/v)→MeOH] and HPLC [MeOH–H_2O (60 : 40, v/v)] to give ginsenoside-Rg_1 (**10**, 128 mg, 0.51%), majonoside R2 (**12**, 47 mg, 0.19%), and pseudo-ginsenoside-F_{11} (**13**, 9 mg, 0.036%). Fraction 4 (373 mg) was separated by reversed-phase silica gel column chromatography [15 g, MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] and HPLC [1) MeOH–H_2O (60 : 40, v/v); 2) MeOH–H_2O (85 : 15, v/v)] to give notoginsenoside-R1 (**11**, 41 mg, 0.16%), ginsenosides-Re (**9**, 26 mg, 0.10%) and -Rd (**7**, 50 mg, 0.20%), and gypenoside-XVII (**8**, 24 mg, 0.095%). Fraction 6 (600 mg) was purified by HPLC [MeOH–H_2O (75 : 25, v/v)] to give ginsenoside-Rb_1 (**6**, 156 mg, 0.62%). Those known dammarane-type saponins were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values and authentic samples.

Quantitative Analysis of Specimens-PNct and -HNct The method of HPLC quantitative analysis was basically the same as described in our previous paper.[33] Thus, powdered ginseng (<100 mesh, PNct 0.7897 g, HNct 0.5396 g) was extracted with 60% aqueous MeOH (20 ml) three times for 30 min. After removal of the solvent from the aqueous MeOH extracts under reduced pressure, the residue was dissolved in H_2O (10 ml) and then the water solution was subjected to Sep-Pak C18 column, which was eluted with H_2O (20 ml), 40% aqueous MeOH (20 ml), and MeOH (20 ml). The MeOH–eluted fraction was concentrated under reduced pressure and the residue was dissolved in 0.05 M phosphate buffer (pH 2.6)–CH_3CN (18 : 32, 1 ml), which was subjected to HPLC analysis. HPLC conditions : chromatograph : Shimadzu LC-10A; column: YMC-Pack A-612 (NH_2) (150×6 mm i.d.); mobile phase: 0.05 M phosphate buffer (pH 2.6)–CH_3CN (18 : 32); flow rate: 1.3 ml/min; detection: UV 202 nm, 0.08 AUFS.

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References and Notes

1) Part II: Morita T., Kong Y. C., But P. P., Ng K. H., Yip T. T., Kasai R., Tanaka O., *Chem. Pharm. Bull.*, **34**, 4368—4372 (1986).
2) Hara H., *Journ. Jap. Bot.*, **45**, 197—212 (1970).
3) Tanaka O., Kasai R., "Progress in the Chemistry of Organic Natural Products," ed. by Herz W., Griesebach H., Kirby G. H., Tamm Ch., Springer-Verlag, Wien, New York, 1984, Vol. 46, pp. 1—76.
4) Tanaka O., *Pure & Appl. Chem.*, **62**, 1281—1284 (1990).
5) Tanaka O., *Gakujutsu Geppo*, **44**, 590—596 (1991).
6) Tanaka O., *Gakujutsu Geppo*, **47**, 607—614 (1994).
7) Morita T., Zhou J., Tanaka O., *Chem. Pharm. Bull.*, **34**, 4833—4835 (1986).
8) Kondo N., Shoji J., Tanaka O., *Chem. Pharm. Bull.*, **21**, 2702—2711 (1973).
9) Kondo N., Shoji J., *Chem. Pharm. Bull.*, **23**, 3282—3285 (1975).
10) Tanaka O., Morita T., Kasai R., Kinouchi J., Sanada S., Ida Y., Shoji J., *Chem. Pharm. Bull.*, **33**, 2323—2330 (1985).
11) Namba N., Matsushige K., Morita T., Tanaka O., *Chem. Pharm. Bull.*, **34**, 730—738 (1986).
12) Shukla Y. N., Thakur R. S., *Phytochemistry*, **24**, 1091—1092 (1985).
13) Shukla Y. N., Thakur R. S., *Phytochemistry*, **25**, 2201—2203 (1986).
14) Shukla Y. N., Thakur R. S., *Planta Med.*, **54**, 367—368 (1988).
15) Shukla Y. N., Thakur R. S., *Phytochemistry*, **27**, 3012—3014 (1988).
16) Shukla Y. N., *Planta Med.*, **55**, 396 (1989).
17) Shukla Y. N., Thakur R. S., *Phytochemistry*, **29**, 239—241 (1990).
18) Shukla Y. N., Thakur R. S., *Phytochemistry*, **31**, 1046—1048 (1992).
19) Wang Z., Jia Z.-J., Zhu Z.-Q., Yang C.-R., Zhou J., Kasai R., Tanaka O., *Acta Botanica Sinica*, **27**, 618—624 (1985).
20) Takahashi M., Yoshikura M., *Yakugaku Zasshi*, **86**, 1053—1056 (1966) and references cited therein.
21) Bernart M. W., Hallock Y. F., Cardellina J. H., Boyd M. R., *Tetrahedron Lett.*, **35**, 993—994 (1994).
22) Yang C.-R., Jiang Z. D., Zhou J., Kasai R., Tanaka O., *Acta Botanica Yunnanica*, **7**, 103—108 (1985).
23) Kondo N., Shoji J., Nagumo N., Komatsu N., *Yakugaku Zasshi*, **89**, 846—850 (1969).
24) Yamaguchi H., Kasai R., Matsuura H., Tanaka O., Fuwa T., *Chem. Pharm. Bull.*, **36**, 3468—3473 (1988).
25) Watanabe T., Kawaguchi K., Suzuki J., Yoshikawa T., Takano A., Isoda S., Kohda H., Malla K. J., *Natural Medicines*, **52**, 426—433 (1998).
26) Sanada S., Kondo N., Shoji J., Tanaka O., Shibata S., *Chem. Pharm. Bull.*, **22**, 421—428 (1974).
27) Takemoto T., Arihara S., Nakajima T., Okuhira M., *Yakugaku Zasshi*, **103**, 1015—1023 (1983).
28) Sanada S., Kondo N., Shoji J., Tanaka O., Shibata S., *Chem. Pharm. Bull.*, **22**, 2407—2412 (1974).
29) Nagai Y., Tanaka O., Shibata S., *Tetrahedron*, **27**, 881—892 (1971).
30) Zhou J., Wu M., Taniyasu S., Besso H., Tanaka O., Saruwatari T., Fuwa T., *Chem. Pharm. Bull.*, **29**, 2844—2850 (1981).
31) Morita T., Kasai R., Tanaka O., Zhou J., Yang T.-R., Shoji J., *Chem. Pharm. Bull.*, **30**, 4341—4346 (1982).
32) Tanaka O., Yahara S., *Phytochemistry*, **17**, 1353—1358 (1978).
33) Yoshikawa M., Fukuda Y., Hatakeyama S., Murakami N., Yamahara J., Taniyama T., Hayashi T., Kitagawa I., *Yakugaku Zasshi*, **113**, 460—467 (1993).
34) Duc N. M., Kasai R., Ohtani K., Ito A., Nham N. T., Yamasaki K., Tanaka O., *Chem. Pharm. Bull.*, **41**, 2010—2014 (1993).
35) *a*) Huong N. T. T., Matsumoto K., Yamasaki K., Duc N. M., Nham N. T., Watanabe H., *Pharmcol. Biochem. Behav.*, **57**, 285—291 (1997); *b*) Huong N. T. T., Matsumoto K., Yamasaki K., Watanabe H., *Life Sci.*, **61**, 395—402 (1997) and references cited therein.