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A Chemical Study of the Saponins and Flavonoids of Dwarf Ginseng (*Panax trifolius* L.) and Its Comparison to Related Species in the Araliaceae

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Abstract

Dwarf ginseng (*Panax trifolius* L.) is a member of the ginseng family (Araliaceae), which is indigenous to North America and is distributed from Southern Canada to the Northern United States. In total, nine compounds were isolated from the leaves of Dwarf gineng. Of these, four were identified as flavonoids and five were found to be ginsenosides. Two of the flavonoids were identified to be kaempferol-3, 7-dirhamnoside and kaempferol-3-gluco-7-rhamnoside. Four of the ginsenosides were identified as notoginsenoside-Fe, ginsenoside-Rd, ginsenoside-Rc and ginsenoside-Rb_3. The common aglycone of these ginsenosides was shown to be (20S)-protopanaxadiol. The identification of flavonoids and ginsenosides from the root, stem, leaf, flower and fruit of Dwarf ginseng was detected by Two-Dimensional Thin-Layer Chromatography (2D-TLC) and High Performance Liquid Chromatography (HPLC). The quantitation of flavonoids and ginsenosides from the root, stem, leaf, flower and fruit of Dwarf ginseng and related species such as Korean gineng (*Panax ginseng* C.A. Meyer) and American ginseng (*Panax quinquefolium* L.) was analyzed by HPLC only. Three flavonoids (Kaempferol derivatives) labelled compound 1 (10.8%), compound 3 (2.8%), and compound 4 (8.4%) were found in the root of Dwarf ginseng but not found in the roots of Korean ginseng and American ginseng. This is the first time that flavonoids have been found and identified in *roots* of the ginseng family (Araliaceae).

Introduction

Dwarf ginseng (*Panax trifolius* L.) is taxonomically related to *P. ginseng* C.A. Meyer, root of which (ginseng) is one of the most famous oriental drugs. However, Dwarf ginseg is indigenous to North America and is not presently used as either a food or drug.

The plant of Dwarf ginseng is small (5-15 cm) and delicate and has a whorl of three stalked leaves, each of which is divided into three stakless leaflets. The plant has a small round umbel of white flowers, which develop into greenish-yellow fruits, and a round tuberous root, which grows deep in the ground.

The chemistry of this plant has never been studied in any detail. Neverthless, a small quantity of root material of this plant has been extracted and some of the saponins have been identified by 2D-TLC [1,2]. These saponins were ginsenoside -Ro, -Rb_1, -Rb_2, -Rc, -Re, -Rf, -Rg_2, but none of the ginsenosides was confirmed chemically. Due to the low concentration of saponins present in the root [1] and the significance of the aerial parts as a new source of saponins, the leaves of this plant were used for this study. The present paper reports the isolation, identifiaction, structural determination and quantitation of flavonoids and saponins for Dwarf ginseg and related species of *Panax* in the ginseng family.

Experimental Methods

Plant Materials : Fresh plant materials of wild growing Dwarf ginseng were collected form Tyler Arboretum, Delaware County, Pennsylvania in the first week of May, 1983. The plant materials were freshly separated into five parts : root, stem, leaf, flower and fruit. Each part of the plant materials was then freeze-dried for 24 hours.

Extraction Procedures : Ground, freeze-dried materials of root (65.2 g), stem (148.3 g), leaf (314.6 g), flower (14.5 g), and fruit (76.1 g) of Dwarf ginseng were separately extracted with methanol at 70°C under Soxhlet Apparatus for 24 hours. After evaporation off the solvent under a reduced presure, crude residues were obtained.

These residues were suspended in water and extracted with ether to remove chlorophylls, fats, and impurities. The water layers were then extracted with water saturated n-butanol. The n-butanol layers were concentrated separately *in vacuo* to afford crude saponins. The extraction procedures for each individual part of the plant materials are shown in Figure 1.

Isolation Procedures: The yield of crude saponins from root, stem, leaf, flower, and fruit of Dwarf ginseng is shown in Table 1. The individual extraction yielded crude saponins from the root (0.7 g, yield from the dried material 1.07%), stem (13.6 g, 9.17%), leaf (27.8 g, 8.84%), flower (2.3 g, 15.86%), and fruit (7.5 g, 9.86 %). Due to the low yield of the saponins obtained from the root, stem, flower and fruit, the crude saponins of leaf were chosen to be the major source material for this research study.

Fractionatin of crude ginseng saponins were performed by column chromatography on silica gel, but this method was very time-consuming. Therefore, the crude saponins obtained from leaf extracts were chromatographed on a Prep. LC/System-500 (Water Asso. Inc.) by eluting with n-butanol:ethyl acetate:water=4 : 1 : 5, v/v, upper phase). A total of 24.0 g of the crude saponins was injected 6 times (4.0 g/20 ml each) into the Prep. HPLC. Three silica cartridges (Water Asso. Inc., cat. no. 50041) were used. The chromatographic separation took a total of 9 hours for the material used. No fractionation profile was recorded. However, the crude saponins were fractionated into 15 fractions with 300 ml per fraction. Corresponding fractions from Prep. HPLC runs were pooled and concentrated to dryness. The fractionated saponins were then identified by TLC as shown in Figure 2, resulting with mixtures of 4 or 5 compounds for each fraction.

The color reaction with sulfuric acid after TLC se-

Fig. 1. Extraction Procedures of Crude Saponins of Dwarf Ginseng (*Panax trifolius* L.)

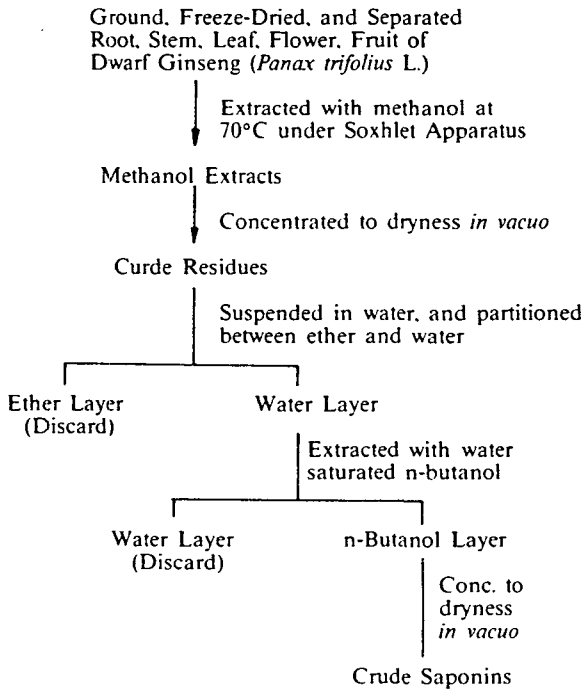


Table 1. The Actual Yield and Percentages of Crude Saponins (Obtained by Butanol Extraction) from Root, Stem, Leaf, Flower, and Fruit of Dwarf Ginseng (*Panax trifolius* L.)

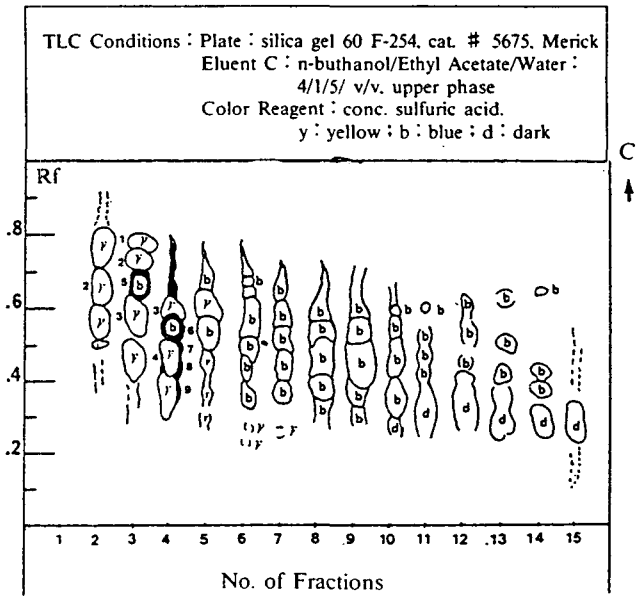
Plant Parts	Dried weight (g)	Crude Saponins (g)	% of Crude Saponins
Root	65.2	0.7	1.07
Stem	148.3	13.6	9.17
Leaf	314.6	27.8	8.84
Flower	14.5	2.3	15.86
Fruit	76.1	7.5	9.86
Total	618.7	51.9	8.39

paration showed three different colors among these 15 fractions. It was noted that there was no spot detected in fraction 1 which indicated that fraction 1 contained the mobile phase only. The yellow spots were found mainly in fraction 2 to 4, which were treated as new and unknown compounds other than ginsenosides, since blue is the characteristic color of ginsenosides. The blue spots were detected in fractions 2 to 14, which contained most of the ginsenosides. The dark spots were (located in fractions 10 to 15) unknown compounds at this stage.

This research study focused on fractions 2, 3 and 4, because there were at least 4 yellow spots and 5 blue spots which were detected among these fractions. The yellow spots at this stage were still unknown, while at least two out of five blue spots which did not match with any of the known ginsenosides on TLC analysis.

Nevertheless, these two blue spots were also the major spots of fractions 5 to 10. Therefore, an investigation was made on isolation and identification of these 4 yellow spots and 5 blue spots of fractions 2, 3 and 4 as shown

Fig. 2. TLC Chromatogram of 15 Fractions Collected from the Crude Leaf Saponins of Dwarf Ginseng (*Panax trifolius* L.) by Preparative HPLC



in Figure 2. The rest of fractions 5 to 15 were saved for isolation and indentification work in the future.

From fraction 2, compound 1, 2 and 3 were isolated by semi-prep. HPLC using acetonitrile : water=85 : 15 on a carbohydrate column (30 cm×7.8 mm, Water Asso. Inc.) with Reflex Index (RI) detector at a flow rate of 2.0 ml/minute.

From fraction 3, compound 1, 2, 3, 4, and 5 were isolated by semi-prep. HPLC using acetonitrile : water= 86:14 on a carbohydrate column with RI detector at a flow rate of 2.0 ml/minute.

From fraction 4, compound 3, 4, 6, 7, 8 and 9 were isolated by semi-prep. HPLC using acetonitrile : water= 80:20 on a carbohydrate column with RI detector at a flow rate of 2.0 ml/minute.

In total, 9 compounds were isolated from fractions 2, 3 and 4 by semi-prep. HPCL procedure. Of these, compounds 1, 2, 3 and 4 were unknown compounds with yellow color, while compounds 5, 6, 7, 8 and 9 were ginsenosides because of their characteristic blue color showed after sulfuric acid spray and had no UV absorbance beyond 210 nm. Therefore, the corresponding compounds were pooled from fractions 2, 3 and 4 and weighed in total. These were compound 1 (45 mg), compound 2 (20 mg), compound 3 (35 mg), compound 4 (60 mg), compound 5 (20 mg), compound 6 (80 mg), compound 7 (5 mg), compound, 8 (2 mg), and compound 9 (1 mg). These compounds were then subjected to structural elucidation by IR, UV, NMR (Proton and C-13), FABMS, elemental analysis, hydroysis, optical rotation, and melting point.

Identification Procedures : The HPLC and 2D-TLC were used to identify the ginsenosides and flavonoids from the crude extracts of root, stem, leaf, flower, and fruit of Dwarf ginseng by using those nine compounds isolated from the leaf and those authentic ginsenosides obtained from Dr. Shoji and Dr. Tanaka in Japan. The analytical HPCL was performed on a carbohydrate column

(30cm×3.9 mm, P/N 84038, Waters Asso. Inc.) using acetonitrile : water=80 : 20 or 84 : 16 at a flow rate of 2.0 ml/minute with RI detector. The 2D-TLC was performed on the pre-coated TLC plates (Silica gel 60 F-254, cat. no 5765, E. Merck), developed separately in one direction in Eluent A (chloroform : methanol : ethyl acetate : n-butanol : water=4 : 4 : 8 : 1 : 2, v/v, lower phase) or Eluent B (chloroform : methanol : water=65 : 35 : 10, v/v, lower phase) for 10 cm. After air drying for 10 minutes, the plates were run in the second direction using Eluent C (n-butanol : ethyl acetate : water=4 : 1 : 5, v/v, upper phase) perpendicular to the first direction for a distance of 10 cm.

General Procedures : Melting points were determined with a Fisher-Johns melting point apparatus at room temperature. Optical rotations were taken with a Perkin-Elmer 141 Polarimeter. IR spectra were performed in KBr on a Nicolet FT-IR 7000 Series. UV spectra were recorded on a Hewlett Packard 8450A Diode Array Spectrophotometer. Proton NMR spectra were measured at 300 MHz and C-13 NMR at 75 MHz with a Nicolet NT-300 Spectrometer, using tetramethylsilane as an internal standard. FABMS spectra were taken on a Kratos MS-50 High Resolution Mass Spectrometer with a FAB source and gun supplied by M-Scan, Ltd.

Hydrolysis Procedures : Two milligrams each of the isolated flavonoids and ginsenosides were heated with 3 ml of 1 N HCl in a sealed tube at 90°C for 2 hours on a Pierce Reacti-Therm Heating Module. The reaction mixtures were concentrated to dryness. The residues were then subjected to TLC analysis. The residues after hydrolysis were redissolved in 50 ul of methanol, and 10 ul of samples were spotted on TLC plates for developing. The products after hydrolysis were compaired with authentic sugars in Eluent B (chloroform : methanol : water=65 : 35 : 10, v/v, lower phase) and Eluent D (n-butanol : acetic acid : ether : water=9 : 6 : 3 : 1, v/v, homogenous). The TLC plates were sprayed with a mixture of 1.5% vanillin in ethanol : sulfuric acid=9 : 1 and followed by heating for 10 minutes. The aglycone of ginsenosides was revealed as blue in color while flavonoids was seen as orange color. The rhamnose, glucose, and arabinose showed yellow, black, and red color, respectively.

Quantitation Procedures : Two hundred and fifty milligrams of samples were weighed and transferred into a sealed tube with 10ml of water. The solution was shaken for 1 hour. The extracts were filtered through a 70-mm Whatman No 1 filter paper.

Five milliliters of aliquot were transferred to a sealed tube to which a 5 ml aliquot of water-saturated n-butanol was added. The solutions were then shaken for another 1 hour.

The n-butanol layer was separated and evaporated to dryness *in vacuo*. The residue was then redissolved in 50 μl of methanol of which a 20 μl of aliquot was injected into HPLC.

The quantitation of ginsenosides and flavonoids was performed on Gilson Gradient HPLC Kit (cat. no. 81-300, Gilson Medical Electronics, Inc.) using acetonitrile : water=gradient 85 : 15 to 5 : 95 on a Econo NH2 column (25 cm×4.6 mm, cat. no. 600 NH, Alltech Asso. Inc.) at a flow rate of 1.5 ml/minute with UV detection at 202 nm. For quantitaion, the external standard of ginsenosides and flavonoids were used. As parameter the peak areas were calculated by the intergrator and the retention times were recorded.

Results and Discussion

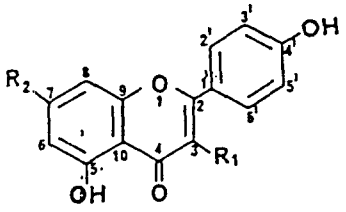
All the isolated ginsenosides and flavonoids were identified by comparison of IR, UV, NMR (Proton and C-13), and MS spectra with known compounds reported in literature (3-22), and were also identified with authentic compounds by TLC in Eluent A, B and C. In total, nine compounds were isolated from the leaves of Dwarf ginseng. Of these, four were identified as flavonoids and five were found to be ginsenosides.

Two of the flavonoids were identified to be kaempferol-3,7-dirhamnoside (compound 1) and kaempferol-3-gluco-7-rhamnoside (compound 3), while the other two (compounds 2 and 4) were not identified. Four of the ginsenosides were not identified to be notoginsenoside-Fe (compound 5), ginsenoside-Rd (compound 7), ginsenoside-Rc (compound 8) and ginsenoside-Rb_3 (compound 9), while the other ginsenoside (compound 6) was only partially identified because of its unusual sugar. The common aglycone of these ginsenosides was shown to be (20S)-protopanaxadiol. The structures and numbering systems of these flavonoids and ginsenosides isolated from the leaves of Dwarf ginseng are shown in Chart 1.

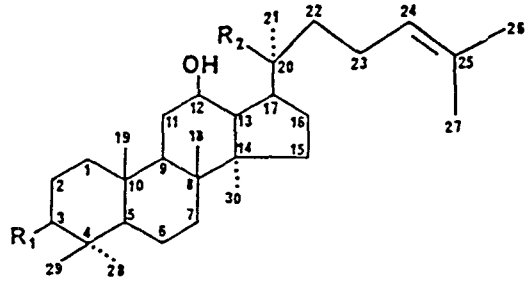
As shown in Table 2, compound 1 (kaempferol-3,7-dirhamnoside) was found in roots (10.8%), stems (7.0%), leaves (12.6%), flowers (13.9%), and fruits (11.0%). Compound 2 was not found in roots but was found in stems (3.8%), leaves (5.4%), flowers (3.7%), and fruits (1.0%). Compound 3 (kaempferol-3-gluco-7-rhamnoside) was found in roots (2.8%), stems (9.1%), leaves (15.1%), flowers (13.0%) and fruits (19.3%). Compound 4 was found in roots (8.4%), stems (17.3%), leaves (22.4%), flowers (21.5%) and fruits (17.6%). The quantitation as shown above indicated that a high yield of flavonoids was found in all parts of Dwarf ginseg. However, there were serveral other unisolated and unidentified flavonoids not included in the quantitation above, otherwise the quantitation of flavonoids would have been much higher than as shown above.

The presence of ginsenoside-Ro indicated that the higher yield was in the roots (4.3%) and fruits (4.5%) and fruits (4.5%), and the lower yield was in stem (1.0%), leaves (0.7%) and flowers (1.4%). Ginsenoside-Rb_1 was found in low yield throughout all parts of this plant : roots (0.7%), stems (0.4%), leaves (0.4%), flowers (0.8%) and fruits (0.5%). Ginsenoside-Rb_2 was also found in low yield throughout all parts of this plant : roots (0.4%), stems (0.2%), leaves (0.2%), flowers (0.3%) and fruits (0.3%). Ginsenosides-Rb_3 was not found in roots but was found in stems and flowers (1% each), leaves (0.4%), fruits (0.5%). Ginsenoside-Rc was not found in stems and fruits but was found in a high yield in leaves (4.3%) and in an averaged yield in roots (1.8%), and flowers (1.2%). Ginsenoside-Rd was not found in roots and fruits but was found in a high yield in stems (3.9%), leaves (3.5%), and in an averaged yield in flowers (1.5%). Notoginsenoside-Fe was found in a very high yield in stems (13.4%) and leaves (9.2%) but was not found in

Chart 1. The Structures and Numbering Systems of Flavonoids and Ginsenosides Isolated from the Leaf of Dwarf Ginesng (*Panax trifolius* L.)

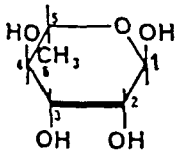


	R_1	R_2
1:	—O—Rha	—O—Rha
3:	—O—Glc	—O—Rha

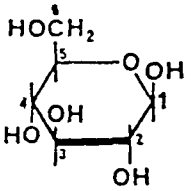


	R_1	R_2
5:	—O—Glc	—O—Glc^{6}—^{1}Ara(fur)
6:	—O—Glc	—O—Glc— ???
7:	—O—Glc^{2}—^{1}Glc	—O—Glc
8:	—O—Glc^{2}—^{1}Glc	—O—Glc^{6}—^{1}Ara (fur)
9:	—O—Glc^{2}—^{1}Glc	—O—Glc^{6}—^{1}Xyl

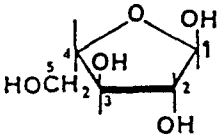
1: Kaempferol-3,7-dirhamnoside
3: Kaempferol-3-glucoside-7-rhamnoside
5: Notoginsenoside-Fe
6: Unidentified
7: Ginsenoside-Rd
8: Ginsenoside-Rc
9: Ginsenoside-Rb$_1$



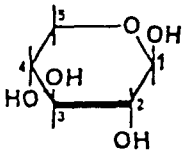
α-L-rhamnopyranose
Rha



β-D-glucopyranose
Glc



α-L-arabinofuranose
Ara(fur)



β-D-xylopyranose
Xyl

roots, flowers, and fruits. Compound 6 was not found in roots and flowers but was found in an anveraged yield in leaves (2.5%), in a low yield in stem (0.5%), and fruits (0.3%).

As shown in Table 3, the comparison of flavonoids in aerial parts of Dwarf ginseng, Korean ginseg, and American ginseng indicated that the Dwarf ginseng contained a significantly higher quantity of flavonoids than either of two species. For example: compound 1 (kaempferol-3, 7-dirhamnoside) was found in stems (7.0%), leaves (12.6%), flowers (13.9%), fruits (11.0%) of Dwarf ginseg; in stems (1.2%) but not in leaves of Korean ginseng; in leaves (8.1%), stems (0.9%), fruits (1.8%) of American ginseng. However, it was noted that a number of flavonoids found in the leaves and flowers was relatively higher than in stems and fruits among all three species.

The flavonoids were found only in the roots of Dwarf ginseng (10.8% for compound 1, 2.8% for compound 3, and 8.4% for compound 4) but not in the roots of Korean ginseng nor in American ginseng. The presence of flavonoids in the roots of Dwarf ginseng was the first time ever reported in the ginseng family.

The aerial parts of all three species were found to be better source than the roots for obtaining ginsenosides. For example : ginsenoside-Rd was found in leaves (0.4%), in stems (19.4%) and in roots (0.5%) of Korean ginseng; in leaves (4.7%), in stems (6.9%), in fruits (3.1%), in roots (5.4%, 9 years old, sun-dried) and in roots (2.9%, 7 years old, fresh) of American ginseng; in stems (3.9%), in leaves (3.5%), in flowers (1.5%) but not in roots of Dwarf ginseng. However, it was also noted that the ginsenosides present in the aerial parts of Korean ginseng were relatively higher than the American ginseng and Dwarf ginseg. For example:ginsenoside-Rd was 19.4% in stems of Korean ginseng; 6.9% in stems of American ginseng; and 3.9% in stems of Dwarf ginseng.

It was also noted that compound 5 (notoginsenoside-Fe) and compound 6 were found only in Dwarf ginseng, but not in Korean ginseng nor in American ginseng. However, compound 5 and 6 were not found in the roots and flowers of Dwarf ginseng.

Ginsenosides of Re, Rf, Rg_1 and Rg_2 with (20S)-protopanaxatriol as aglycone were not found in any part of Dwarf ginseng but were found in all parts of Korean ginseng and American ginseng. These observations lead us to believe that the ginsenosides present in Dwarf ginseng are all derivatives of (20S)-protopanaxadiol.

No matter whether the roots are fresh or sun-dried,

Table 2. 2D-TLC Identification and HPLC Quantitation of Flavonoids and Ginsenosides from the Roots, Stems, Leaves, Flowers and Fruits of Dwarf Ginseng (*Panax trifolius* L.)

A. 2D-TLC Identification

Plant Parts	Compounds 1	2	3	4	5	6	7	8	9	11	12	13
Roots	+	−	+	+	−	−	−	+	−	+	+	+
Stems	+	+	+	+	+	+	+	−	+	+	+	+
Leaves	+	+	+	+	+	+	+	+	+	+	+	+
Flowers	+	+	+	+	−	−	+	+	+	+	+	+
Fruits	+	+	+	+	−	+	+	+	+	+	+	+

+:Present, −: Absent

B. HPLC Quantitation

Plant Parts	Compounds 1	2	3	4	5	6	7	8	9	11	12	13
Roots	10.8	−	2.8	8.4	−	−	−	1.8	−	0.4	0.7	4.3
Stems	7.0	3.8	9.1	17.3	13.4	0.5	3.9	−	1.0	0.2	0.4	1.0
Leaves	12.6	5.4	15.1	22.4	9.2	2.5	3.5	4.3	0.4	0.2	0.4	0.7
Flowers	13.9	3.7	13.0	21.5	−	−	1.5	1.2	1.0	0.3	0.8	1.4
Fruits	11.0	1.0	19.3	17.6	−	0.3	−	−	0.5	0.3	0.5	4.5

−: Absent or Unquantified

1 : Kaempferol-3,7-dirhamnoside
2 : Unidentified Flavonoid
3 : Kaempferol-3-gluco-7-rhamnoside
4 : Unidentifed Flavonoid
5 : Notoginsenoside-Fe
6 : Unidentified Ginsenoside
7 : Ginsenoside-Rd
8 : Ginsenoside-Rc
9 : Ginsenoside-Rb_3
11 : Ginsenoside-Rb_2
12 : Ginsenoside-Rb_1
13 : Ginsenoside-Ro

Table 3. The HPLC Quantitation of Flavonoids and Ginsenosides for Related Species in the Ginseng Family

A*	B*	C*	D*	% of Content Flavonoids 1*	2*	3*	4*	5*	6*	Ginsenosides Rd	Rc	Rb_3	Rb_2	Rb_1	Ro	Rg_2	Rg_1	Rf	Re
CMF-208	K	L	SD	−	−	0.8	−	−	−	0.4	3.5	−	0.1	4.3	2.0	0.01	0.3	4.4	2.2
CMF-209	K	S	SD	1.2	−	−	−	−	−	19.4	−	8.3	−	0.9	0.7	0.1	1.2	1.8	11.6
CMF-210	K	R	SD(6)	−	−	−	−	−	−	0.5	1.4	−	−	−	0.6	0.2	1.0	0.2	1.4
CMF-211	A	L	SD	8.1	10.5	0.7	7.0	−	−	4.7	−	0.3	−	0.5	0.4	0.4	0.3	1.7	4.8
CMF-212	A	S	SD	0.9	7.2	−	−	−	−	6.9	−	1.0	−	0.3	2.7	0.5	0.2	0.3	3.1
CMF-213	A	FT	SD	1.8	13.4	2.2	−	−	−	3.1	9.3	1.0	0.2	0.5	3.1	0.01	2.9	2.8	7.0
CMF-214	A	R	SD(9)	−	−	−	−	−	−	5.4	12.4	0.3	−	−	3.0	0.3	2.7	0.4	4.3
CMF-215	A	R	SD(13)	−	−	−	−	−	−	5.2	12.9	1.1	−	−	6.3	1.0	3.2	0.8	5.7
CMF-220	A	R	F(7)	−	−	−	−	−	−	2.9	10.3	−	−	−	1.5	1.4	2.4	1.5	2.2
CMF-221	A	R	F(16)	−	−	−	−	−	−	5.3	12.5	−	−	−	2.4	2.1	2.6	1.9	4.2
CMF-222	A	R	F(45)	−	−	−	−	−	−	6.6	14.0	−	−	−	3.7	2.2	2.7	2.4	4.9
CMF-223	D	R	FD	10.8	−	2.8	8.4	−	−	−	1.8	−	0.4	0.7	4.3	−	−	−	−
CMF-224	D	S	FD	7.0	3.8	9.1	17.3	13.4	0.5	3.9	−	1.0	0.2	0.4	1.0	−	−	−	−
CMF-225	D	L	FD	12.6	5.4	15.1	22.4	9.2	2.5	3.5	4.3	0.4	0.2	0.4	0.7	−	−	−	−
CMF-226	D	FW	FD	13.9	3.7	13.0	21.5	−	−	1.5	1.2	1.0	0.3	0.8	1.4	−	−	−	−
CMF-227	D	FT	FD	11.0	1.0	19.3	17.6	−	0.3	−	−	0.5	0.3	0.5	4.5	−	−	−	−

A* CMF : Cold Mountain Farm, New York.
B* K : Korean Ginseng A: American Ginseng D: Dwarf Ginseng
C* L : Leaf S : Stem R : Root FT : Fruit FW : Flower
D* SD:Sun-Dired F : Fresh FD: Freeze-Dried (6) :the number in the ( ) was the ages (years)
1*: Kaempferol-3,7-dirhamnoside 2* : Unidentified Flavonoid 3* : Kaempferol-3-gluco-7-rhamnoside
4*: Unidentified Flavonoid 5* : Notoginsenoside-Fe 6* : Unidentified Ginsenoside

the greater the age of American ginseng, the higher the amounts of ginsenosides were found. For example: ginsenoside-Rc was 10.3% for 7 years, 12.5% for 16 years, and 14.0% for 45 years of fresh roots; and was 12.4% for 9 years, 12.9% for 13 years of sun-dried roots.

Further studies are in progress to further elucidate the chemistry of Dwarf ginseng and related species in the ginseng family.

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References

1. Lee, T. M. and Der Marderosian, A. (1981) *J. Pharm. Sci.* 70, 89.
2. Lui, J. H.-C. and Staba, E. J. (1980) *J. Nat. Prod.* 43, 340.
3. Nagasawa, T., Yokozawa, T. and Nishimo, Y. (1980) *Chem. Pharm. Bull.* 28, 2059.
4. Nagasawa, T., Choi, J. H., Nishimo, Y. and Oura, H.(1980) *Chem. Pharm. Bull.* 28, 3701.
5. Choi, J. H., Kim, W. J., Hong, S. K., Oh, S. K. and Oura, H. (1980) *J. Korean Agr. Chem. Soc.* 23, 206.
6. Markham, K. R., Ternal, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* 34, 1389.
7. Perkin, A. G. (1907) *J. Chem. Soc.* 91, 435.
8. Asakawa, J., Kasai, R., Yamasaki, K. and Tanaka, O. (1977) *Tetrahedron* 33, 1935.
9. Tanaka, O. and Yahara, S. (1978) *Phytochem.* 17, 1353.
10. Besso, H., Kasai, R., Saruwatari, Y., Fuwa, T. and Tanaka, O. (1982) *Chem. Pharm. Bull.* 30, 2380.
11. Besso, H., Kasai, R., Wei, J., Wang, J.-F., Saruwatari, Y., Fuwa, T. and Tanaka, O. (1982) *Chem. Pharm. Bull.* 30, 4534.
12. Yang, T. -R., Kasai, R., Zhou, J. and Tanaka, O. (1983) *Phytochem.* 22, 1473.
13. Kasai, R., Suzuo, M., Asakawa, J. and Tanaka, O.(1977) *Tetrahedron Letters* 175.
14. Itano, K., Yamasaki, K., Kihara, C. and Tanaka, O. (1980) *Carbohydr. Res.* 87, 27.
15. Hattori, S. (1952) *Nature* 168, 788.
16. Nakaoki, T. and Morita, N. (1957) *J. Pharm. Soc. (Japan)* 77, 108.
17. Tanaka, N., Murakami, T., Saiki, Y., Chen, C.-M. and Gomez, L.D. (1981) *Chem. Pharm. Bull.* 29, 3455.
18. Zapesochnaya, G. G. (1982) *Khim. Prir. Soedin.* 6, 695.
19. Nakano, K., Takatani, M., Tominatsu, T. and Nohara, T. (1983) *Phytochem.* 22, 2831.
20. Rzadkowska-Bodalska, H. and Olechnowicz-Stepien, W. (1975) *Pol. J. Pharmacol. Pharm.* 27, 335.
21. Aly, H. F., Geiger, H., Schucker, U., Waldrum, H., Velde, G. V. and Mabry, T. J. (1975) *Phytochem.* 14, 1613.
22. Torch, M., Pinkas, M. and Bezanger-Beauquesne, L. (1972) *Phytochem.* 11, 3065.

O. Tanaka : What was the yield of saponins as compared to your last data and I would like to know about the procedures used for identification.

A. D. Maderosin : The data given today is accurate and shows a higher percentage of saponins because they were carried out by modern HPLC procedures. The data of 1981 were low because now we used the absolute spectrodensitometric procedure for quantification of saponins.

왜생삼 (*Panax trifolius* L.)의 사포닌과 프라보노이드의 화학적 연구 및 오가과에 속하는 유연종과의 성분 비교연구

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북미가 원산지인 왜생삼 (*Panax trifolius* L.)은 인삼속(오가과)에 속하며 카나다 남부에서 북미에 걸쳐 서식한다. 왜생삼 잎에서 4종류의 프라보노이드와 5종류의 진세노사이드로 확인된 총 9종류의 화합물을 분리하였다. 2종류의 프라보노이드는 kaempferol-3,7-dirhamnoside 와 kaempferol-3-gluco-7-rhamnoside로 각각 확인되었다. 4종류의 진세노사이드는 각각 notoginsenoside-Fe, ginsenoside-Rd, ginsenoside-Rc와 ginsenoside-Rb$_1$로 확인되었으며, 이들 왜생삼의 ginsenoside 공통된 골격구조는 (20S)-protopanaxadiol인 것으로 밝혀졌다. 프라보노이드와 진세노사드의 동정은 왜생삼의 뿌리, 줄기, 잎, 꽃과 열매에서 추출하여 2차원 박층 그로마토그라피 (2D-TLC)와 고압 액체크로마토그라피(HPLC)로 하였다. 왜생삼과 근연종인 고려인삼(*Panax ginseng* C.A. Meyer)및 미국삼(*Panax quinquefolium* L.)의 뿌리, 줄기, 잎, 꽃과 열매에서 추출한 프라보노이드와 진세노시드의 정량은 고압 액체크로마토그래피 만을 사용하여 분석하였다. 화합물 1,3과 4로 명명한 kaempferol 유도체인 프라보노이드 3가지는 왜생삼의 뿌리에 10.8%, 2.8%와 8.4%가 각기 함유되어 있었으나 고려인삼과 미국삼에는 함유되어 있지 않았다. 오가과 인삼속식물 뿌리에서 프라보노이드가 확인, 동정된 것은 이것이 처음이다.