

Chemical Constituents from Seeds of *Panax ginseng*: Structure of New Dammarane-Type Triterpene Ketone, Panaxadione, and HPLC Comparisons of Seeds and Flesh

Sachiko SUGIMOTO,^a Seikou NAKAMURA,^a Hisashi MATSUDA,^a Niichiro KITAGAWA,^b and Masayuki YOSHIKAWA^{*a}

^aKyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan; and ^bKoshiro Co., Ltd.; Chuou-ku, Osaka 541–0045, Japan. Received October 6, 2008; accepted December 3, 2008; published online December 5, 2008

A new dammarane-type triterpene ketone, panaxadione, was isolated from the seeds of *Panax ginseng* C. A. MEYER (Araliaceae) together with two dammarane-type and lupane-type triterpenes, an aromatic oligoglycoside, three sterol glycosides, and three dammarane-type triterpene oligoglycosides (ginsenosides Rd, Re, and Rg₂). The structure of a new compound was elucidated on the basis of physicochemical evidence. The relative contents of major ginsenosides in the seeds were compared with those of the flesh parts of young and mature fruits.

Key words panaxadione; *Panax ginseng*; dammarane-type triterpene; ginseng seed; Araliaceae

In the course of our characterization studies on the bioactive saponin constituents from natural medicines^{1–7} and medicinal flowers,^{8–12} we have reported the isolation and structure elucidation of malonylginsenosides Rb₁, Rb₂, Rc, and Rd,^{13,14} notoginsenosides A–E, G–N, and notoginsenic acid,^{15–17} and quinquenosides I–V¹⁸ from the roots of *Panax ginseng* C. A. MEYER (ginseng), *P. notoginseng* (BURK) F. H. CHEN (notoginseng), and *P. quinquefolium* L. (American ginseng). From the flower-buds of *P. ginseng*, *P. notoginseng*, and *P. quinquefolium*, we have also reported the isolation and structure elucidation of floralginsenosides A–K, La, Lb–P,^{19–21} notoginsenosides O–Q, S, T,²² and floralquinquenosides A–E,²³ respectively. Furthermore, we characterized immunological adjuvant,¹⁷ hepatoprotective,²² and gastro-protective²¹ activities of the principal dammarane-type triterpene saponins from the roots and flowers of those *Panax* species.

In a continuing study, we examined the chemical constituents from the seeds of *P. ginseng*. The fruits of *P. ginseng* were used as a protective for skin disease, and several dammarane-type triterpene saponins, ginsenosides Rb₂, Rc, Rd, Re, and Rg₁, have been identified as chemical constituents of the fruits.²⁴

This paper deals with the isolation and structure elucidation of a new dammarane-type triterpene ketone named panaxadione (**1**) from the seeds of *P. ginseng* as well as the comparison of major ginsenosides using HPLC analysis in the seeds with those in the flesh part of the fruits.

Results and Discussion

Isolation from Seeds of *P. ginseng* The seeds of *P. ginseng*, which was cultivated in Jilin province of China, were extracted with methanol. The methanolic extract (11.3% from the dried seeds) was partitioned into an EtOAc–H₂O [1:1 (v/v)] mixture to furnish an EtOAc-soluble fraction (6.2%) and aqueous layer. The aqueous layer was further extracted with 1-butanol (1-BuOH) to give 1-BuOH- (1.7%) and H₂O- (3.4%) soluble fractions. The EtOAc-soluble fraction was subjected to normal-phase and reversed-phase column chromatographies, and finally HPLC to give a new dammarane-type triterpene, panaxadione (**1**, 0.00043%) to-

gether with eight known compounds, 20(*S*)-protopanaxatriol²⁵ (**2**, 0.00052%), 3-keto-20(*S*)-protopanaxatriol²⁶ (**3**, 0.0010%), 3β-*trans*-feruloyloxy-16β-hydroxylup-20(29)-ene²⁷ (**4**, 0.017%), daucosterol²⁸ (**5**, 0.00097%), 5α,6β-dihydroxydaucoesterol²⁹ (**6**, 0.0010%), saringosteryl glucoside³⁰ (**7**, 0.0024%), and ginsenoside Rg₂³¹ (**8**, 0.0025%). The 1-BuOH-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatographies and repeated HPLC to give ginsenoside Rg₂³¹ (**8**, 0.0012%), ginsenoside Re³² (**9**, 0.0028%), ginsenoside Rd³³ (**10**, 0.0011%), and phenethyl alcohol xylopyranosyl(1→6)glucopyranoside³⁴ (**11**, 0.00072%).

Structure of Panaxadione Panaxadione (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{23} +177.5^\circ$ in MeOH). The IR spectrum of **1** showed absorption bands at 3470 and 1712 cm⁻¹ ascribable to hydroxyl and carboxyl functions. The EI-MS of **1** showed a dehydrated molecular ion peak (M–H₂O)⁺ at *m/z* 470 together with a fragment ion peak (i) at *m/z* 143 (base peak), which was known to be characteristic for ocotillone-type triterpenes.³⁵ In the positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 511 (M+Na)⁺ and *m/z* 489 (M+H)⁺ and high-resolution FAB-MS analysis of the quasimolecular ion (M+Na)⁺ revealed the molecular formula of **1** to be C₃₀H₄₈O₅. The ¹H-NMR (methanol-*d*₃) and ¹³C-NMR (Table 1) spectra³⁶ of **1** showed signals assignable to eight tertiary methyls [δ 0.80, 0.82, 1.07, 1.09, 1.10, 1.27, 1.28, 1.31 (3H each, all s, H₃-30, 19, 21, 26, 27, 18, 28, 29)], two methines bearing an oxygen function [δ 3.70 (t-like, H-24), 3.99 (ddd, *J*=4.1, 11.0, 11.0 Hz, H-6)], and four methines [δ 1.72, (m, H-5), 1.90 (dd, *J*=4.1, 13.1 Hz, H-9), 2.52 (ddd, *J*=4.8, 9.6, 10.3 Hz, H-17), 3.04 (d, *J*=9.6 Hz, H-13)] together with eight methylenes, four quarternary carbons, two quarternary carbons bearing an oxygen function, and two carbonyl carbons.

The plane structure of **1** was constructed on the basis of H–H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) experiments. Thus, the H–H COSY experiment on **1** indicated the presence of partial structures written in bold lines as shown in Fig. 1. The carbon skeleton and the positions of functional groups were characterized by a HMBC experiment, which showed long-

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

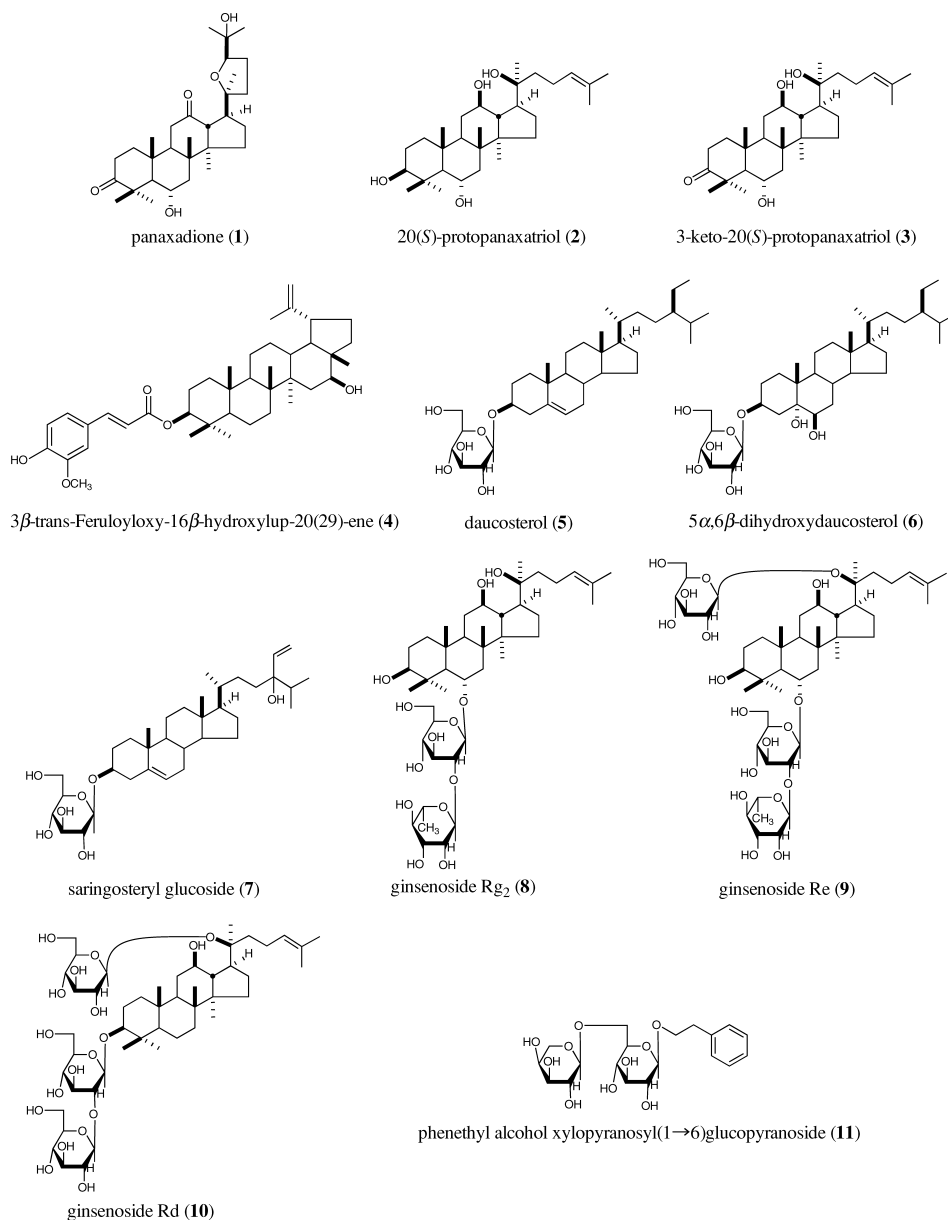


Chart 1. Isolated Constituents from the Seeds of *Panax ginseng*

range correlations between the following protons and carbons: H-2 and C-3; H-5 and C-4, 6, 10; H-7 and C-6, 8, 9; H-11 and C-9, 12; H-13 and C-12, 14, 17, 20; H-19 and C-5, 9, 10; H-18 and C-7, 8; H-21 and C-17, 20, 22; H-26, 27 and C-24, 25; H-28, 29 and C-3, 4, 5; H-30 and C-13, 14, 15. The stereostructure of **1** was determined by a nuclear Overhauser effect spectroscopy (NOESY) experiment, in which correlations were observed between the following proton pairs: H-6 and H-7β, 18, 19; H-18 and H-19, 13; H-21 and H-17, 23α, 24; H-24 and H-23α; H-30 and H-17. Furthermore, the proton and carbon signals of the hydrofuran part in the ¹H- and ¹³C-NMR data of **1** were superimposable on those of (20*S*,24*R*)-ocotillone-type triterpenes.³⁵ On the basis of this evidence, the structure of panaxadione was determined as 6α-hydroxy-3,12-dion-(20*S*),(24*R*)-epoxy dammarane (**1**).

Previously, it was reported that ginsenoside Re (**10**) was obtained from the fruits in a significantly high yield (6.0% from the dried fruits) together with ginsenosides Rb₁, Rc, Rd

(**9**) and Rg₁ at 0.2, 0.1, 0.1, and 0.04% yields, respectively.²⁴ In this time, we merely isolated ginsenosides Rg₂ (**8**), Rd (**9**), and Re (**10**) in 0.0032%, 0.0011%, and 0.0030%, respectively, from the seeds. In order to clarify the distinction between the fruits and seeds of *P. ginseng*, we examined the principal ginsenoside compositions and relative contents of the young and mature flesh of Japanese *P. ginseng* using HPLC analysis.³⁷ From the immature green and mature red fruits of *P. ginseng* cultivated in Shimane prefecture of Japan, the young and full growth seeds and flesh parts were separated and extracted with MeOH, respectively. The MeOH extract were subjected to HPLC analysis.³⁷ As shown in Table 2, which showed relative contents to those of the roots, both flesh parts contained ginsenosides Rb₁, Rb₂, Rc, Re, and Rg₁ and their total contents were three to four times as amount as those of the roots. The ginsenosides composition and relative contents of the flesh part were similar to those of the flower buds.^{19–21} Whereas the seeds contained ginseno-

Table 1. ^{13}C -NMR (150 MHz) Data of **1**

Position	A	B
1	38.8	39.1
2	32.4	32.9
3	220.8	218.0
4	47.0	47.5
5	58.0	58.5
6	66.7	66.6
7	43.3	44.5
8	40.7	40.9
9	52.8	52.9
10	38.0	38.0
11	39.5	40.0
12	212.3	209.7
13	56.4	56.6
14	55.6	55.7
15	31.8	32.1
16	24.6	24.8
17	42.5	42.9
18	14.7	15.5
19	16.4	17.2
20	85.2	85.1
21	24.1	25.0
22	34.2	35.5
23	26.1	26.8
24	83.9	84.5
25	71.3	71.0
26	24.9	26.9
27	24.4	26.3
28	18.4	19.7
29	30.9	31.8
30	15.6	16.5

Measured in methanol- d_3 , (A) and pyridine- d_5 (B).

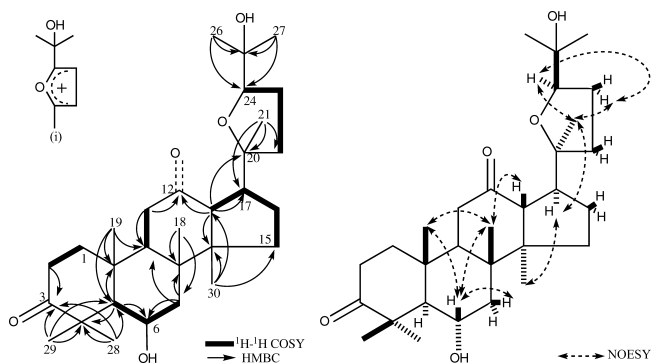


Fig. 1. Significant ^1H - ^1H COSY, HMBC, and NOESY Correlations and MS Base Peak (i) of Panaxadiolone (**1**)

sides Re and Rg₁ and their amounts were very small. This evidence indicated that the ginsenosides of the fruits were principally existent in the flesh part.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5\text{ cm}$); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^1H -NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C -NMR spectra, JEOL EX-270 (68 MHz) JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A_{VP} UV-VIS detectors. HPLC column, YMC-Pack ODS-A (250 \times 4.6 mm i.d.) and (250 \times 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: or-

dinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F₂₅₄ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO₄)₂-10% aqueous H₂SO₄ followed by heating.

Plant Material The seeds and flower buds of *P. ginseng* were cultivated in Jilin province, China, in November 2005 and identified by one of authors (M. Y.). The young and mature fruits (seeds and flesh) and roots were cultivated in Shimane prefecture of Japan in 2007.

Extraction and Isolation The dried seeds (1.0 kg) of *P. ginseng* cultivated in Jilin province of China were finely crushed and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (113.3 g, 11.3%). The methanolic extract (100 g) was partitioned into an EtOAc-H₂O [1:1 (v/v)] mixture to furnish an EtOAc-soluble fraction (55.0 g, 6.2%) and aqueous phase, which was extracted with 1-BuOH to give 1-BuOH- (15.4 g, 1.7%) and H₂O- (29.6 g, 3.4%) soluble fractions.

Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 2.0 kg), hexane:EtOAc (50:1 \rightarrow 20:1 \rightarrow 5:1 \rightarrow 1:1) \rightarrow CHCl₃:MeOH:H₂O (10:3:1 \rightarrow 7:3:1 lower phase \rightarrow 6:4:1 (v/v/v)) \rightarrow MeOH] of the EtOAc-soluble fraction (50.0 g) gave six fractions [fr. 1 (0.3 g), 2 (36.0 g), 3 (6.4 g), 4 (0.7 g), 5 (3.1 g), 6 (0.06 g)]. Fraction 4 (0.7 g) was subjected to reversed-phase silica gel column chromatography [21.0 g, H₂O-MeOH (40:60 \rightarrow 30:70 \rightarrow 20:80 \rightarrow 10:90, v/v) \rightarrow MeOH \rightarrow acetone] followed by HPLC [H₂O:MeOH (5:95) and H₂O:isopropanol (20:80)] to furnish 3 β -*trans*-feruloyloxy-16 β -hydroxylup-20(29)-ene (**4**, 134.6 mg). Fraction 5 (3.1 g) was subjected to reversed-phase silica gel column chromatography [100 g, H₂O:MeOH (40:60 \rightarrow 30:70 \rightarrow 20:80 \rightarrow 10:90) \rightarrow MeOH \rightarrow acetone] to provide twelve fractions [fr. 5-1 (494.3 mg), 5-2 (368.8 mg), 5-3 (231.8 mg), 5-4 (364.1 mg), 5-5 (160.3 mg), 5-6 (253.9 mg), 5-7 (70.8 mg), 5-8 (65.3 mg), 5-9 (91.5 mg), 5-10 (69.0 mg), 5-11 (247.6 mg), 5-12 (199.9 mg)]. Fraction 5-5 (160.3 mg) was purified by HPLC [H₂O:MeOH (35:65)] to give panaxadiolone (**1**, 3.5 mg). Fraction 5-6 (253.9 mg) was purified by HPLC [H₂O:MeOH (30:70)] to yield ginsenoside Rg₂ (**8**, 19.8 mg). Fraction 5-7 (70.8 mg) was separated by HPLC [H₂O:MeOH (25:75)] to give 20(*S*)-protopanaxatriol (**2**, 4.2 mg) and 3-keto-20(*S*)-protopanaxatriol (**3**, 8.4 mg). Fraction 5-9 (91.5 mg) was purified by HPLC [H₂O:MeOH (10:90)] to give saringosteryl glucoside (**7**, 19.5 mg). Fraction 5-10 (69.0 mg) was separated by HPLC [H₂O:MeOH (5:95)] to furnish hexadecanoic acid (6.5 mg) and 5 α ,6 β -dihydroxydaucosterol (**6**, 7.8 mg). Fraction 5-11 (247.6 mg) was purified by HPLC [H₂O:MeOH (3:97)] to give daucosterol (**5**, 5.2 mg).

The 1-BuOH-soluble phase (13.0 g) was subjected to normal-phase silica gel column chromatography [400 g, CHCl₃ \rightarrow CHCl₃:MeOH:H₂O (70:10:1 \rightarrow 50:10:1 \rightarrow 35:10:1 \rightarrow 20:3:1 \rightarrow 7:3:1, lower phase) \rightarrow MeOH] to give five fractions [fr. 1 (2.6 g), 2 (2.2 g), 3 (2.0 g), 4 (4.3 g), 5 (1.0 g)]. Fraction 2 (2.2 g) was further separated by reversed-phase silica gel column chromatography [60.0 g, H₂O:MeOH (80:20 \rightarrow 70:30 \rightarrow 60:40 \rightarrow 50:50 \rightarrow 40:60 \rightarrow 30:70) \rightarrow MeOH] followed by HPLC [H₂O:MeOH (60:40), (30:70)] to give phenethyl alcohol xylopyranosyl(1 \rightarrow 6)glucopyranoside (**11**, 5.5 mg) and ginsenoside Rg₂ (**8**, 8.8 mg). Fraction 3 (2.0 g) was subjected to reversed-phase silica gel column chromatography [60.0 g, H₂O:MeOH (40:60 \rightarrow 30:70 \rightarrow 20:80 \rightarrow 10:90) \rightarrow MeOH] followed by HPLC [H₂O:MeOH (50:50), (20:80)] to give ginsenoside Re (**9**, 21.3 mg) and Rd (**10**, 8.5 mg). The known compounds were identified by comparison of their physical data ([α]_D, IR, MS, and ^1H - and ^{13}C -NMR) with reported values.

Panaxadiolone (**1**): A white powder; [α]_D²³ +177.5° ($c=0.23$, MeOH); High-resolution positive-ion FAB-MS: Calcd for C₃₀H₄₈O₅Na (M+Na)⁺: 511.3399. Found: 511.3391; IR (NaCl): ν_{max} 3470, 2991, 1712, 1468 cm⁻¹; ^1H -NMR (methanol- d_3 , 600 MHz) δ_{H} : 0.80, 0.82, 1.07, 1.09, 1.10, 1.27, 1.28, 1.31 (3H each, all s, H₃-30, 19, 21, 26, 27, 18, 28, 29), 1.56 (1H, m, H-7 α), 1.65 (1H, m, H-7 β), 1.72 (1H, m, H-5), 1.76 (1H, m, H-23 β), 1.89 (1H, m, H-23 α), 1.90 (1H, dd, $J=4.1$, 13.1 Hz, H-9), 2.52 (1H, ddd, $J=4.8$, 9.6, 10.3 Hz, H-17), 3.04 (1H, d, $J=9.6$ Hz, H-13), 3.70 (1H, t-like, H-24), 3.99 (1H, ddd, $J=4.1$, 11.0, 11.0 Hz, H-6); ^1H -NMR (pyridine- d_5 , 600 MHz) δ_{H} : 0.81, 0.85, 1.22, 1.23, 1.36, 1.41, 1.65, 1.68 (3H each, all s, H₃-19, 30, 21, 18, 26, 27, 29, 28), 1.91 (1H, m, H-5), 3.16 (1H, d, $J=9.6$ Hz, H-13), 3.95 (1H, t, $J=7.6$ Hz, H-24); ^{13}C -NMR: given in Table 1; EI-MS (%): 470 (M-H₂O, 13), 143 (100); Positive-ion FAB-MS m/z : 511 (M+Na)⁺, 489 (M+H)⁺.

Sample Preparation for HPLC The powdered sample (50 mg) in water

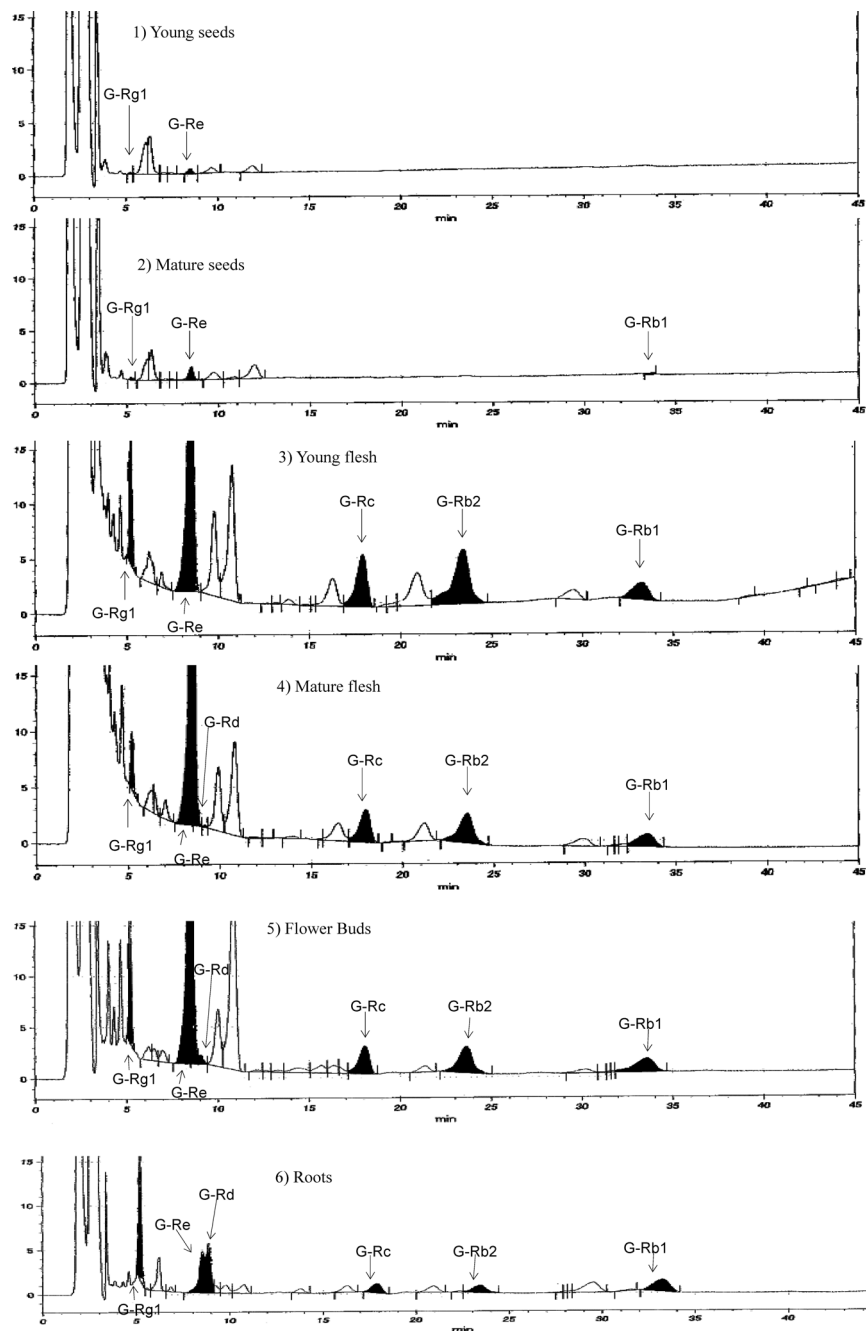


Fig. 2. HPLC Patterns of Ginsenoside Constituents in the Seeds, Flesh, Flower Buds, and Roots

Table 2. The Relative Contents of Ginsenosides in the Young and Mature Seeds and Flesh of Fruits, Flower Buds, and Roots of Japanese *P. ginseng*

	G-Rb ₁	G-Rb ₂	G-Rc	G-Rd	G-Re	G-Rg ₁	Total
1) Young seeds	N.D.	N.D.	N.D.	N.D.	0.021	0.003	0.024
2) Mature seeds	0.003	N.D.	N.D.	N.D.	0.054	0.005	0.062
3) Young flesh	0.221	0.755	0.461	N.D.	2.214	0.301	3.952
4) Mature flesh	0.172	0.335	0.284	0.006	2.175	0.111	3.084
5) Flower buds	0.234	0.324	0.255	0.033	2.254	0.303	3.403
6) Roots	0.193	0.099	0.094	0.030	0.224	0.361	1.000

Each value in the column means the ratio of the HPLC peak area of the ginsenoside which is calculated on the basis of the HPLC peak area (value: 1.000) due to the roots of ginsenosides.

(25 ml) was ultrasonicated for 30 min. The part (10 ml) of the extract solution (25 ml) was subjected to Sep-pak C₁₈ cartridge column [H₂O 20 ml, H₂O–MeOH (3→10) 20 ml, MeOH 5 ml]. The MeOH eluate was subjected to HPLC analysis.

HPLC System and Conditions Shimadzu LC-10A VP HPLC system equipped with CLASS-VP workstation (Shimadzu Co., Ltd., Japan) was used for quantitative analysis. HPLC analysis was performed on a YMC-Pack NH2 A-612 column (6.0 mm i.d.×150 mm L., 10 μl) at 40 °C. The mobile phase consisted of acetonitrile–phosphate buffer (82:18) with a flow rate of 1.4 ml/min. The detectable wavelength was set at 203 nm.

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