Studies on the Constituents of Dwarf Ginseng

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Phytochemical study of the leaves of *Panax trifolius* L. resulted in the isolation of two flavonoids and four ginsenosides. The two flavonoids were found to be kaempferol-3,7-dirhamnoside and kaempferol-3-gluco-7-rhamnoside. The four ginsenosides were identified as ginsenoside —Rd, —Rc, —Rb_3 and notoginsenoside-Fe. The common aglycones of these flavonoids and ginsenosides were shown to be kaempferol and (20S)-protopanaxadiol, respectively.

***Keywords*: *Panax trifolius*; dwarf ginseng: kaempferol-3,7-dirhamnoside; kaempferol-3-gluco-7-rhamnoside; ginsenoside —Rd, —Rc, —Rb_3; notoginsenoside-Fe.**

INTRODUCTION

Dwarf ginseng (*Panax trifolius* L.) is taxonomically related to *P. ginseng* C. A. Meyer, the root (ginseng) of which is one of the most famous oriental drugs. However, dwarf ginseng is indigenous to North America and is not presently used as either a food or drug. The chemistry of this plant has never been studied in any detail. Nevertheless, a small quantity of root material of this plant has been extracted and some of the saponins have been identified by two-dimensional TLC (Lee and Der Marderosian, 1981; Lui and Staba, 1980). These saponins were ginsenoside —R_0, —Rb_1, —Rb_2, —Rc, —Re, —Rf, —Rg_2, but none of the ginsenosides was confirmed chemically. Owing to the low concentration of saponins present in the root (Lee and Der Marderosian, 1981) and the significance of the aerial parts as a new source of saponins, the leaves of this plant were used for this study.

MATERIALS AND METHODS

General procedures. Melting points were determined with a Fisher–Johns melting point apparatus. Optical rotations were taken at room temperature 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 8450 A Diode Array Spectrophotometer. ^1H NMR spectra were measured at 300 MHz and ^{13}C NMR at 75 MHz with a Nicolet NT-300 Spectrometer, using tetramethylsilane as an internal standard. FAB-MS spectra were taken on a Kratos MS-50 High Resolution Mass Spectrometer with a FAB source and gun supplied by M-Scan, Ltd.

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Identification of the known flavonoids and saponins. All of the isolated flavonoids and saponins were identified by comparison of UV, IR, ^1H NMR, ^{13}C NMR, and MS spectra with known compounds, reported in the literature, and were also identified with authentic saponins by TLC: plate, silica gel 60 F254 precoated, Merck; solvent A, $CHCl_3$ + MeOH + EtOAc + BuOH + H_2O (4:4:8:1:2, lower phase); solvent B, $CHCl_3$ + MeOH + H_2O (65:35:10, lower phase), and solvent C, BuOH + EtOAc + H_2O (4:1:5, upper phase); detection, H_2SO_4.

Hydrolysis of flavonoids and saponins. Two milligrams each of the isolated flavonoids and saponins were heated with 3 mL of 1 N HCl in a sealed tube at 90 °C for 2 h on a Pierce Reacti-Therm Heating Module. The reaction mixtures were concentrated to dryness. The residues were then analyzed by TLC.

Thin-layer chromatography after hydrolysis. The residues after hydrolysis were redissolved in 50 μL of MeOH, and 10 μL of samples were spotted on TLC plates for developing. The products after hydrolysis were compared with authentic sugars in solvent B, $CHCl_3$ + MeOH + H_2O (65:35:10, lower phase); solvent D, BuOH + CH_3COOH + Et_2O + H_2O (9:6:3:1, homogeneous); and solvent E, 2-EtCOMe + CH_3COOH + MeOH (3:1:1, homogeneous). The TLC plates were sprayed with a mixture of 1.5% vanillin in EtOH + H_2SO_4 (9:1) followed by heating for 10 min. The aglycone of saponins was revealed as blue while flavonoids were seen as orange. The rhamnose, glucose, and arabinose showed yellow, black, and red respectively.

Extraction, separation, and identification of flavonoids and saponins of the leaves. The dried leaves (314.6 g), collected in Tyler Arboretum (Pennsylvania, USA) in May 1983, were extracted with hot MeOH which was then evaporated to dryness. A suspension of the

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resulting MeOH extract in H_2O was washed with Et_2O several times and then extracted repeatedly with BuOH saturated with H_2O. The combined BuOH layers were concentrated to dryness, affording a crude glycoside fraction (27.8 g, yield; 8.84%).

The crude glycoside fraction was chromatographed on a Prep. LC/System-500 (Waters Asso. Inc.) by eluting with BuOH + EtOAc + H_2O (4:1:5, upper phase). A total of 24.0 g of the crude glycoside was injected six times (4.0 g each time) into the Prep. HPLC. Three silica cartridges (Waters Prep. Pak-500, 57 mm × 30 cm) were used. The chromatographic separation took a total of 9 h for the materials used. The crude glycoside was fractionated into 15 fractions with 300 mL per fraction. Corresponding fractions from Prep. HPLC runs were pooled and concentrated to dryness.

This report focused on fractions 2, 3, and 4 only. The rest of the fractions (5 to 15) were saved for isolation and identification in the future, and fraction 1 was found to contain mobile phase only.

From fraction 2, **1** and **2** were isolated by Semi-prep. HPLC using CH_3CN—H_2O (85:15) on a carbohydrate column (7.8 mm × 30 cm, Waters Assoc. Inc.) at a flow rate of 2.0 mL/min. Compounds **1** and **2** were identified to be kaempferol-3,7-dirhamnoside and kaempferol-3-gluco-7-rhamnoside, respectively. On acid hydrolysis, **1** afforded rhamnose only while **2** yielded glucose and rhamnose. Compound **1** was a yellow solid which precipitated out of the mobile phase by itself, mp 204–206 °C, $[\alpha]_D^{26} - 224 \pm 2°$ (c 0.615, MeOH). IR (KBr): 3400, 1650, 1600, 1490, 1350, 1170, 970, 835, 805 cm^{-1}. UV λ_{max}^{MeOH}: 205, 265, 345 nm. Found: C, 51.44%; H, 5.00%. $C_{27}H_{30}O_{14}$. $3H_2O$ requires: C, 51.27%, H, 5.68%. Compound **1** was identified by comparison of IR, UV, ^1H NMR, ^{13}C NMR and other physical constants with those data reported for kaempferol-3,7-dirhamnoside (Perkin, 1907; Hattori, 1952; Nakaoki and Morita, 1957; Markham *et al.*, 1978; Tanaka *et al.*, 1981; Zapesochnaya, 1982; Nakano *et al.*, 1983). Compound **2** was also a yellow solid which precipitated out of the mobile phase by itself, mp 232–234 °C, $[\alpha]_D^{26}$ $-101 \pm 3°$ (c 0.365, DMSO). IR (KBr): 3400, 1650, 1600, 1490, 1340, 1200, 1170, 970, 835, 805^{-1} cm. UV λ_{max}^{MeOH}: 205, 265, 350 nm. Found: C, 49.86%; H, 5.7%. $C_{27}H_{30}O_{15}$. $3H_2O$ requires: C, 50.00%; H, 5.55%. Compound **2** was identified by comparison of UV, ^1H NMR, ^{13}C NMR, and other physical constants with those data reported for kaempferol-3-gluco-7-rhamnoside (Aly *et al.*, 1975; Markham *et al.*, 1978; Rzadkowka-Bodalska and Olechnowicz-Stepien, 1975; Torck *et al.*, 1972).

From fraction 3, **1**, **2** and **3** were isolated by semi-prep. HPLC using CH_3CN—H_2O (86:14) on a carbohydrate column at a flow rate of 2.0 mL/min. Compound **3** was identified as notoginsenoside-Fe, which was a colorless amorphous solid, mp 178–182 °C. IR (KBr): 3400, 2960, 2940, 2880, 2070, 1040^{-1} cm. UV λ_{max}^{MeOH}: 204 nm. Found: C, 54.75%; H, 7.32%. $C_{47}H_{80}O_{17}$ requires C, 54.75%; H, 7.76%. On acid hydrolysis, **3** yielded glucose and arabinose. Bompound **3** was confirmed by comparison of ^{13}C NMR and other physical constants reported for notoginsenoside-Fe (Yang *et al.*, 1983).

From fraction 4, **2**, **4**, **5**, and **6** were isolated by Semi-prep. HPLC using CH_3CN—H_2O (80:20) on a carbonydrate column at a flow rate of 2.0 mL/min. Compounds **4**, **5** and **6** were identified only by TLC comparison in solvents A, B, C with authentic saponins to be ginsenoside —Rd, —Rc and Rb_3, respectively.

RESULTS AND DISCUSSION

The crude glycoside obtained from the dried leaves of dwarf ginseng (*P. trifolius* L.) was subjected to Prep. and Semi-prep. HPLC separations (Choi *et al.*, 1980; Nagasawa *et al.*, 1980a,b) to give two flavonoids and four saponins. The flavonoids were found to be kaempferol-3,7-dirhamnoside **1** and kaempferol-3-gluco-7-rhamnoside **2**. The saponins were identified as notoginsenoside-Fe **3**, ginsenoside-Rd **4**, ginsenoside-Rc **5** and ginsenoside-Rb_3 **6** (Fig. 1).

The IR spectrum of **1** suggested the presence of a hydroxyl group (3400 cm^{-1}), a carbonyl group (1650 cm^{-1}), a double bond (1600 cm^{-1}), and an aromatic ring (835, 805 cm^{-1}). On acid hydrolysis with 1 N HCl, **1** yielded kaempferol **7** and rhamnose. The ^1H NMR spectrum (DMSO) of **1** showed two secondary methyl groups (each 3H, d, J = 5.4 Hz at 0.8 and 1.1 ppm), six multiple methine protons at 3.1–3.7 ppm, two singlet methine protons at 3.8 and 3.9 ppm, two anomeric protons at 5.3 and 5.5 ppm, six aromatic protons (each 1H, d, J = 2.1 Hz at 6.4 and 6.8 ppm, and each 2H, d, J = 8.7 Hz at 6.9 and 7.8 ppm), six hydroxyl groups (each 1OH, d, J = 5.7 Hz at 4.6 ppm, J = 3.9 Hz at 4.7 ppm, J = 5.7 Hz at 4.8 ppm, J = 5.4 Hz at 4.9 ppm, J = 4.2 Hz at 5.0 ppm, and J = 4.2 Hz at 5.1 ppm). These observations indicated that **1** was kaempferol bisrhamnoside. The two fragments from FAB-MS of *m/z* 432 (kaempferol + rhamnose − H_2O), *m/z* 286 (kaempferol), and molecular weight of *m/z* 578 (kaempferol + 2 rhamnoses—$2H_2O$) confirmed the observations of ^1H NMR and acid hydrolysis. The location of two rhamnosyl groups was shown to be at the C-3 and C-7 hydroxyl groups on **7** by comparing the ^{13}C NMR spectrum (DMSO) of **1** with those data of **7** (Markham *et al.*, 1978). That is, as shown in Table 1, the signals ascribable to C-2, C-3 and C-7 in **1** were shifted by +10.9, −1.7, and −2.8 ppm, respectively, compared with those of **7**. These observations indicated the presence of rhamnosyl linkages to the hydroxyl groups at both C-3 and C-7 on **7**. Accordingly, **1** was identified to be kaempferol-3,7-dirhamnoside, being assumed to be identical with kaempferitrin (Perkin, 1907).

On acid hydrolysis, **2** afforded rhamnose and glucose together with **7**. Similar patterns of IR and UV spectra were observed for **1** and **2**. However, the ^1H NMR spectrum (DMSO) of **2** showed one secondary methyl group (3H, d, J = 5.5 Hz at 1.1 ppm), seven multiple methine protons and one methylene proton at 3.1–3.7 ppm, one singlet methine proton at 3.9 ppm, one triplet hydroxyl proton at 4.3 ppm, two anomeric protons at 5.5 and 5.6 ppm, six aromatic protons (each 1H, d, J = 2.1 Hz at 6.4 and 6.8 ppm, each 2H, d, J = 8.7 Hz at 6.9 and 8.1 ppm),

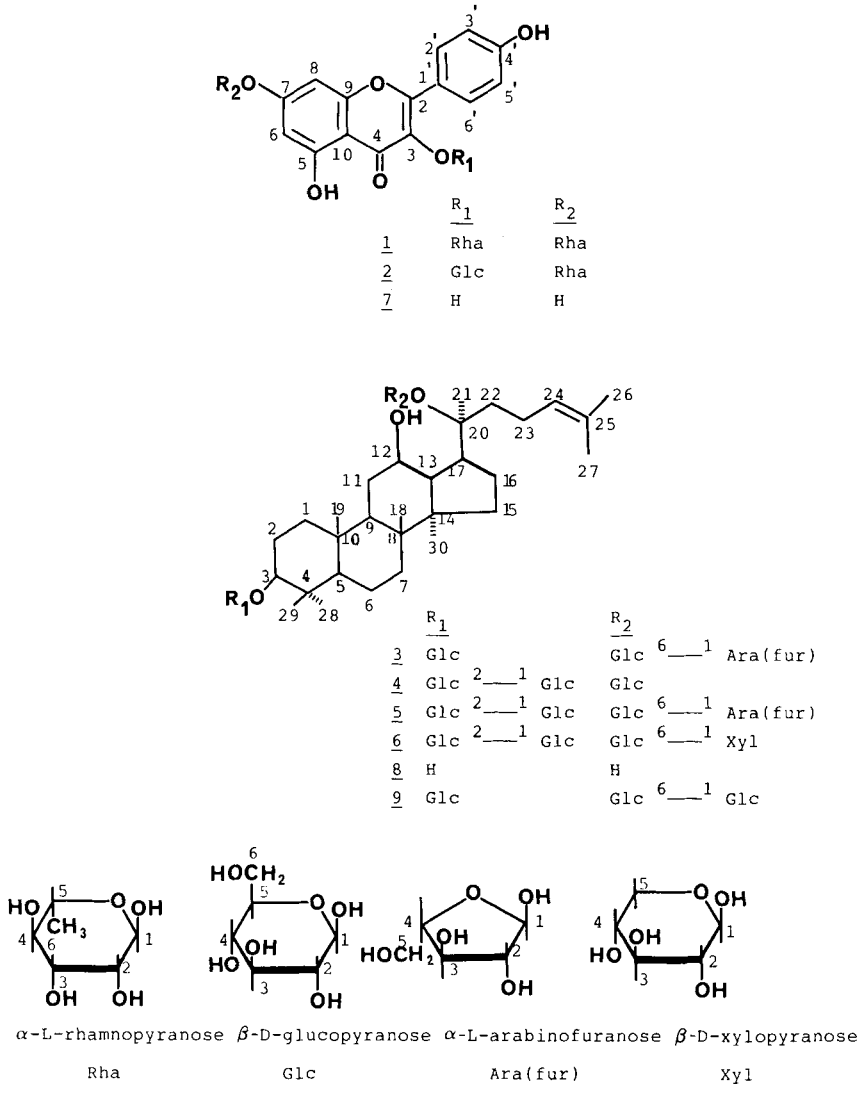

Figure 1. Structures of flavonoids and ginsenosides.

and six hydroxyl protons (each 1OH, d, J = 5.4 Hz at 4.8 ppm, J = 5.7 Hz at 4.9 ppm, J = 0.8 Hz at 5.0 ppm, J = 3.9 Hz at 5.1 ppm, J = 4.2 Hz at 5.2 ppm, J = 4.2 Hz at 5.3 ppm). These observations indicated that **2** was kaempferol glucorhamnoside. The two fragments from FAM-MS of *m/z* 448 (kaempferol + glucose − H_2O), *m/z* 432 (kaempferol + rhamnose − H_2O), and molecular weight of *m/z* 594 (kaempferol + glucose + rhamnose − $2H_2O$) confirmed the observations of ^1H NMR and acid hydrolysis. The location of glucosyl and rhamnosyl groups was shown to be at the C-3 and C-7 hydroxyl groups, respectively, on **7** by comparison of the ^{13}C NMR spectrum (DMSO) of **2** with those data of **7** (Markham *et al.*, 1978). That is, as shown in Table 1, the signals ascribable to C-2, C-3 and C-7 in **2** were shifted by +9.0, −2.2, and −2.4 ppm, respectively, compared with those data of **7**. These observations indicated the presence of glucosyl linkage to the hydroxyl group at C-3 and rhamnosyl linkage to the hydroxyl group at C-7 on **7**. Accordingly, **2** was identified to be kaempferol-3-gluco-7-rhamnoside.

Assignments of ^{13}C NMR signals of dammarane triterpenes including (20S)-protopanaxadiol **8** have been established (Asakawa *et al.*, 1977), and the glycosylation shifts have been investigated for a variety of glycosides. Therefore, ^{13}C NMR spectroscopy is a highly effective means of determining the structure of the aglycone without hydrolysis. By comparison of the ^{13}C NMR spectra of **3** with those of known dammarane saponins, **4**, **5**, **6**, and gypenoside-XVII **9**, it was found that all of the carbon signals due to the aglycone moiety of **3** appeared almost superimposable over those of **4**, **5**, **6**, and **9** as shown in Table 2 (Besso *et al.*, 1982a, b; Tanaka and Yahara, 1978; Yang *et al.*, 1983). This indicated that the genuine aglycone of **3** must be represented by **8** and glycosyl linkages should be located both at the C-3 and C-20 hydroxyl groups (Itano *et al.*, 1980; Kasai *et al.*, 1977). Inspection of the anomeric carbon signals disclosed the presence of three sugar units in **3** (98.2, 106.9, and 110.2 ppm). Upon comparison of the ^{13}C NMR spectrum of sugar moieties of **3** with that of **4**, **5**, **6**, and **9** as shown in Table 3, two glucoses and one arabinose were strongly recommended.

On acid hydrolysis, **3** afforded glucose and arabinose which confirmed the observations of ^{13}C NMR. The molecular weight of **3** was determined

Table 1. ^{13}C NMR chemical shifts of flavonoids in DMSO-d_6

Carbon No.		7	1	1–7	2	2–7
	2	146.8	157.7[a]	+10.9	155.8[a]	+9.0
	3	135.6	133.9	−1.7	133.4	−2.2
	4	175.9	177.8	+1.9	177.5	+1.6
	5	160.7	160.7	+0.0	160.7	+0.0
	6	98.2	98.9	+0.7	99.2	+1.0
	7	163.9	161.1	−2.8	161.5	−2.4
	8	93.5	94.0	+0.5	94.4	+0.9
	9	156.2	155.8[a]	−0.4	156.6[a]	+0.4
	10	103.1	105.7	+2.6	105.6	+2.5
	1′	121.7	120.0	−0.3	120.6	−1.1
	2′	129.5	130.5	+1.0	130.8	+1.3
	3′	115.4	115.5	+0.1	115.0	−0.4
	4′	159.2	160.3	+1.1	160.0	+0.8
	5′	115.4	115.5	+0.1	115.0	−0.4
	6′	129.5	130.5	+1.0	130.8	+1.3
7-Rha	1		98.8		98.4	
	2		70.2[b]		69.7[b]	
	3		70.5[b]		70.2[b]	
	4		71.8[c]		71.5	
	5		70.0		69.9[b]	
	6		17.7		17.7	
3-Rha	1		101.9			
	2		70.4[b]			
	3		70.6[b]			
	4		71.5[c]			
	5		70.1[b]			
	6		17.3			
3-Glc	1				100.8	
	2				74.1	
	3				76.4[c]	
	4				69.9[b]	
	5				77.3[c]	
	6				60.8	

[a,b,c] Assignment in the vertical column may be reversed, though those given here are preferred.

Table 3. ^{13}C NMR chemical shifts of the sugar moieties of saponins in C_5D_5N

Carbon No.		3	4	5	6	9
3-Clc	1	106.9	105.0	104.9	104.3	106.7
	2	75.9	83.3	83.1	83.4	75.6
	3	79.3[a]	78.1[a]	77.8[a]	77.7[a]	79.0[a]
	4	72.2	71.6	71.5	71.7	71.6
	5	78.4[a]	78.1[a]	77.8[a]	77.3[a]	78.6[a]
	6	63.2	62.7	62.6	62.9	62.9
Glc	1		105.9	105.6	105.6	
	2		77.0	76.8	76.7	
	3		79.1[a]	78.9[a]	78.1[a]	
	4	71.6	71.5	71.7		
	5	78.1[a]	78.0[a]	78.1[a]		
	6	62.7	62.9	62.8		
20-Glc	1	98.2	98.2	97.9	97.9	97.9
	2	75.1	75.0	74.9	74.8	75.1
	3	79.0[a]	78.1[a]	78.0[a]	78.8[a]	78.1[a]
	4	72.0	71.6	71.5	71.7	71.6
	5	76.6[a]	78.1[a]	76.3[a]	76.6[a]	76.8[a]
	6	68.6	62.7	68.3	69.8	71.6
Xyl	1				105.2	
	2				74.2	
	3				78.8[a]	
	4				70.8	
	5				66.4	
Ara (fur)	1	110.2		109.9		
	2	83.4		83.3		
	3	78.8		78.6[a]		
	4	86.1		85.8		
	5	62.1		62.6		
Glc	1					105.1
	2					74.6
	3					78.1[a]
	4					71.6
	5					78.1[a]
	6					62.7

[a] Assignment in the vertical column may be reversed, though those given here are preferred.

by FAB-MS to be m/z 916. When **3** was run in thioglycerol, fragments of m/z 713, 587, 533 and 407 were recorded. The difference between 713 and 533 was 180, just like the difference between 587 and 407, both of which gave significant observations of having two glucoses for **3**. In addition, when **3** was run with NaCl in thioglycerol, fragments of m/z 1047 and 735 were recorded. While **3** was run with KCl in thioglycerol, fragments of m/z 1063 and 751 were also recorded. The difference between the two fragments above was m/z 312 which was interpreted as glucose + arabinose − H_2O (180 + 150 − 18). These

Table 2. ^{13}C NMR chemical shifts of the aglycone moieties of saponins in C_5D_5N

Carbon No.	8	3	4	5	6	9
1	39.6	39.3	39.1	39.0	39.4	39.2
2	28.2	26.7	26.7	26.6	26.7	26.6
3	77.9	88.9	88.9	89.0	89.1	88.7
4	39.5	39.7	39.6	39.6	39.7	39.6
5	56.3	56.5	56.4	56.3	56.5	56.3
6	18.7	18.5	18.5	18.3	18.4	18.4
7	35.2	35.2	35.2	35.1	35.1	35.1
8	40.0	40.1	40.0	39.9	40.1	40.0
9	50.4	50.3	50.2	50.1	50.2	50.1
10	37.3	37.0	36.9	36.8	37.0	36.9
11	32.0[b]	30.9[b]	30.8[b]	30.7[b]	30.8[b]	30.6[b]
12	70.9	70.2	70.2	70.2	70.2	70.1
13	48.5	49.5	49.4	49.5	49.5	49.4
14	51.6	51.5	51.4	51.4	51.5	51.3
15	31.8[b]	30.8[b]	30.8[b]	30.8[b]	30.8[b]	30.6[b]
16	26.8	26.8	26.7	26.6	26.7	26.6
17	54.7	51.7	51.7	51.6	51.7	51.5
18	16.2[a]	16.4[a]	16.3[a]	16.2[a]	16.2[a]	16.2[a]
19	15.8[a]	16.1[a]	15.9[a]	15.9[a]	16.2[a]	16.0[a]
20	72.9	83.4	83.3	83.1	83.5	83.3
21	26.9	22.5	22.4	22.2	22.3	22.3
22	35.8	36.2	36.0	36.0	36.2	36.1
23	22.9	23.3	23.2	23.1	23.1	23.1
24	126.2	126.1	125.9	125.9	125.9	125.8
25	130.6	131.0	130.9	130.9	130.9	130.8
26	25.8	25.9	25.8	26.7	25.7	25.7
27	17.6[a]	18.0[a]	17.8[a]	17.8[a]	17.9[a]	17.8[a]
28	28.6	28.2	28.0	28.0	28.1	28.0
29	16.4[a]	16.8[a]	16.6[a]	16.5[a]	16.5[a]	16.7[a]
30	17.0[a]	17.5[a]	17.3[a]	17.3[a]	17.5[a]	17.5[a]

[a,b] Assignment in the vertical column may be reversed, though those given here are preferred.

observations indicated the presence of two glucoses and one arabinose in **3**, and one of the glucoses was linked to arabinose as the disaccharide. Therefore, based on acid hydrolysis, FAB-MS and ^{13}C NMR, **3** was determined to be notoginsenoside-Fe (Yang *et al.*, 1983).

Compounds **4**, **5**, and **6** were identified only by TLC comparison with authentic saponins in three solvent systems to be ginsenoside —Rd, —Rc, and —Rb_3, respectively.

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