PLANT BIOCHEMISTRY OF GINSENG SAPONINS (I)

Saponins and Sapogenins from American Ginseng Plants.*

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Abstract

The sapogenins of two-and four-year-old American ginseng plants (*Panax quinquefolium* L.) (Araliaceae) collected in July and September were studied. American ginseng saponins (panaquilins) differ from Korean ginseng (*Panax ginseng* C. A. Meyer) saponins (ginsenosides). The American ginseng saponins separated and named were panaquilins A, B, C, D, E-1, E-2, E-3, G-1, G-2, (c) and (d). One-dimensional thin-layer chromatography did not completely separate panaquilin mixture and were sub ject to misinterpretation. The panaquilins were more accurately separated and identified by the two-dimensional thin-layer method established. Some differences in American ginseng saponins were dependent upon the plant age, time of collection, and part extracted. The American ginseng sapogenin components are panxadiol (panaquilins B and C), oleanolic acid (panaquilin D) and panaxatriol (panaquilin G-1). The panaquilins E-1, E-2 and E-3 mixture contains both panaxadiol and panaxatriol. The genins of panaquilins A, (c), (d) and G-2 were not identified. In addition, β-sitosterol and stigmasterol were identified from the root ether extracts.

Introduction

Since P. Lafitau, a missionary among the Iroquois Indians discovered the American ginseng plants near Montreal in 1716, both wild and cultivated ginseng roots have been exported to the Orient from the United States and Canada (1). Besides the term American ginseng the terms yang-shen, Cantonese renshen or whagi renshen have been used. Among the American Indians, the term *Garantoquen signitys* a resemblance to a man's thighs and legs.

American and Korean ginseng roots are products of *Panax quinquefolium* L. and *Panax ginseng* C. A. Meyer, respectively. They are economically important members of the family Araliaceae, and are cultivated for commercial purposes. Korean and American ginseng plants are similar in appearance and growth habits (2, 3, 4). Ginseng is a perennial herb normally collected after 5–7 years of cultivation. Wild ginseng plant roots from 30 to 75 years old have been reported (5). Both American and Korean ginseng roots are fleshy, light yellow brown, spindle-shaped and often divided so that it may resemble a human form. They have a unique weak odor, and a bitter then sweetish taste. The stem is single, straight and approximately 60 cm high with 3–5 long-stalked leaflets. American ginseng plants normally flower in June and Korean ginseng plants in April. The flower is small, yellowish green and on a single terminal umbel. The fruit is bright red, with

normally two seeds laterally compressed. The market of the cultivated root is based on color, maturity, size and form. The best quality root is of proper size and breaks with a somewhat waxy fracture. Young or undersized roots are dry, hard and glassy.

For at least two thousand years ginseng is reported to have contributed considerably to the health of the body and mind of the Oriental. Its therapeutic virtue is quite general (6–9). This is apparant even in its genus name, Panax, which is derived from the Greek *panakos* meaning a cure-all.

Little is known about the chemical nature of American ginseng plant saponins and sapogenins (10, 11). The chemical constituents of Korean ginseng roots have been extensively studied (12–17), and its saponin fraction is believed by many to be the therapeutic fraction (18–21). There are very few studies of the saponins present in the Korean and American ginseng above-ground parts (22, 23). These studies do not adequately explain the site of ginseng saponin biosynthesis as well as how to find a cheap saponin resource from the above-ground parts of ginseng plants.

In this study American ginseng root saponins were isolated and compared with those of Korean ginseng roots. Two-and four-year-old American ginseng plant saponins from various plant parts were studied at the beginning and the end of a growing season, and their age and season variation noted. The saponins present were determined qualitatively and semi-quantitatively by thin-layer chromatography. The genin component of American ginseng saponins was compared with those of Korean ginseng roots, and the distribution patterns of the genins in the plant noted.

Results and Discussions

The following abbreviations are used often in Tables throughout this study: JI-July collection: Sp-September collection; L-leaf; S-stem; F-fruit; R-root.

a. Extraction

On a dry weight basis, the average ether extract of American ginseng plants is 4.0%; the chlorform extract, 1.4%; the methanol extract-1, 33.1%; the methanol extract-2, 26.1%; and the residue containing principally saccharides from methanol extract-1, 7.0% (Table 1). The extract percentages of plants collected in July were generally lower than those plants collected in September.

Ether extracts generally contain fatty acids, hydrocarbon alcohols, highly unsaturated hydrocarbons including a unique odorous component, and phytosterols; chlorform extracts contain some pigments and unidentified constituents; methanol extract-1 residues contain ginseng saponins, carbohydrates, flavonoids and some nitrogen containing component; methanol extract-1 residues contain principally saccharides and some saponin impurities; and methanol extract-2 contain principally saponins.

Ether-souble extracts have been reported for American ginseng roots (0.60%), the above-ground parts (1.42%), callus tissues (0.92%) and Korean roots (0.82%) (24). The solvent extract percentages

Table 1. American Ginseng Plant Extracts.

Plant Material* Two-year-old	Dry Wt. (g)	Extracts (%) Ether	Chloroform	Methanol-1	Residue**	Methanol-2***
JIL	13.1	4.4	1.2	48.0	7.2	40.8
JIS	3.8	9.2	0.9	24.8	11.4	13.4
JIR	33.7	1.7	0.6	21.5	1.6	19.9
Plant (average)		5.1	0.8	31.4	6.9	24.7
SpL	4.3	5.4	2.0	45.3	13.6	31.7
SpS	1.2	5.6	2.9	36.6	4.6	32.0
SpR	43.0	1.2	0.8	28.6	10.8	17.8
Plant (average)		4.1	1.9	36.8	9.7	27.2
Four-year-old						
JIL	7.7	3.8	2.2	29.8	6.2	23.4
JIS	7.7	1.9	1.4	25.0	14.1	10.9
JIF	1.7	1.6	2.2	34.3	3.4	30.9
JIR	19.9	1.3	0.5	25.8	3.7	22.1
Plant (average)		2.2	1.6	28.7	6.9	21.8
SpL	20.0	6.5	2.0	50.0	0.2	49.8
SpS	30.7	7.0	0.3	25.8	2.1	23.7
SpR	50.0	1.0	NA	30.4	12.0	18.2
Plant (average)		4.8	1.2	35.4	4.8	30.6

*Jl-July collection; Sp-September collection; L-leaf; S-stem; F-fruit; R-root; NA-not available.

**Residue: Insoluble material of methanol-1 extracted with cold methanol (5°C).

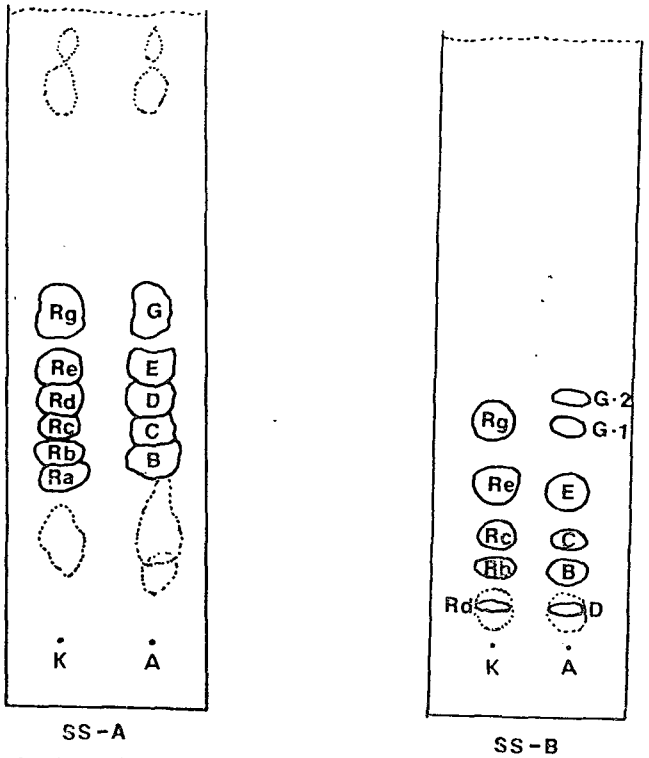
***Methanol-2: Soluble extract of methanol-1 extracted with cold methanol (5°C).

from American ginseng roots and above-ground parts (Table 1) are high as the materials were often exhaustively extracted in a Soxhlet apparatus.

b. One-dimensional Thin-layer Chromatographic Patterns

Thin-layer chromatographic (TLC) patterns of American ginseng saponins differed slightly from those of Korean ginseng root saponins (Fig. 1; Plate 1).

Fig. 1. One-dimensional Thin-layer Chromatograms of American and Korean Ginseng Root Saponins.



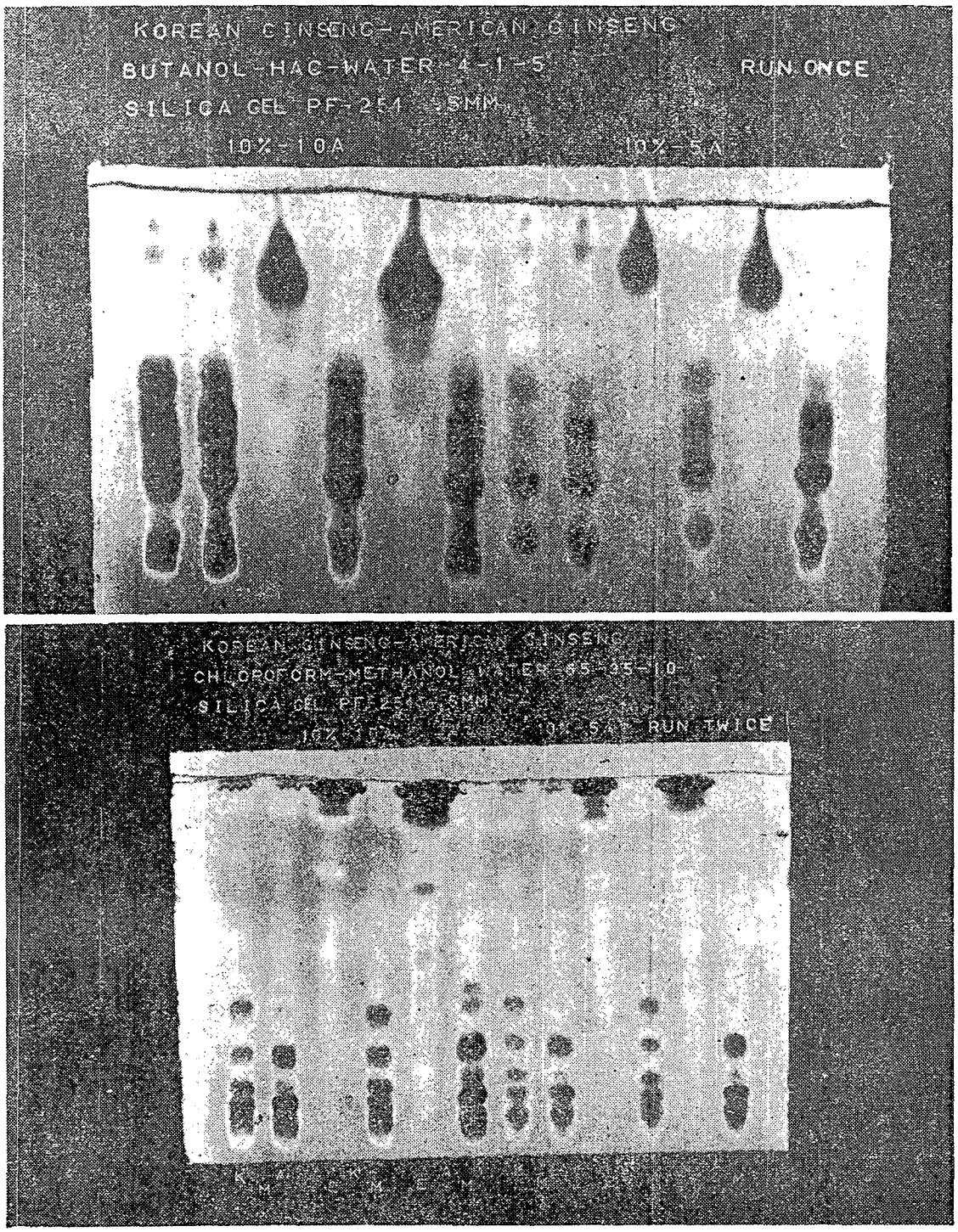
SS-A: Solvent system-A (n-butanol: acetic acid: water; 4:1:5, upper layer).
SS-B: Solvent system-B (methanol: chloroform: water; 65:35:10, lower layer).
K: Korean ginseng root methanol extract.
SS-A: Ginsenosides Ra, Rb, Rc, Rd, Re and Rg.
SS-B: Ginsenosides Rd, Rb, Rc, Re and Rg.
A: American ginseng root methanol extract.
SS-A: Panaquilins B, C, D, E and G.
SS-B: Panaquilins D, B, C, E, G-1 and G-2.

The Russian Elyakov *et al.* (25) first separated Korean ginseng root saponins (panaxosides) by column chromatography, and then proceeded to name them from thin-layer chromatograms. However, the Japanese Shibata *et al.* (26) examined ginseng saponin extracts (ginsenosides) directly by tlc after dividing the crude saponin extract into two groups. The first group contained ether or acetone precipitates (panaxadiol containing saponins ginsenosides Ro, Ra to $R_{(f)}$), and the second group was the ether supernatant (panaxatriol containing saponins-ginsenosides Rg_1, Rg_2 and Rg_3). The Russian and Japanese one-dimensional thin-layer chromatograms were not directly comparable probably due to the different isolation procedures used, or due to the habitat variation of the plants studied.

The solvent systems, and the tlc saponins rf-values reported by Shibata *et al.* (26) were used in this study. The term panaquilin is similar to the term panaquilon introduced by Garriques (27) for American ginseng saponins and it is used to identify the American ginseng saponins. In solvent system-A (SS-A) Korean ginseng root saponins contained ginsenoside Ra, but American ginseng saponin did not (Fig. 1; Plate 1). In solvent system-B (SS-B) American ginseng root saponins contained panaquilin G-2 but Korean ginseng did not. According to Shibata *et al.* (23) their one-dimensional thin-layer patterns of semipurified, ether-supernatant, Korean ginseng saponins contained large amounts of ginsenoside Rg_1 and relatively small amounts of ginsenosides Rg_3 as compared to that present in American ginseng saponins. Fig. 1 and plate 1 do not contain ginsenosides Rg_2 and Rg_3 as ginseng saponins.

In two-year-old plants, the stems collected in July contained more panaquilin B than those collected in September (Table 2). Panaquilin B was usually present in high concentrations in the root. In four-year-old plants, panaquilin B was present in high concentrations in the leaf and root as compared to the stem. Panaquilin C was present more in the above-ground parts than in the roots. Panaquilin (d) was not present in the roots. The leaves of four-year-old plants contained more panaquilin (d) than that of the stems. Panaquilin D was consistantly present in the roots, and only occasionally in the above-ground portions. Panaquilin E was found in abundant concentrations at both collection periods and in most plant parts. Panaquilin G-1 was not present in two-year-old above-ground parts collected in July. Panaquilin G-2 was present

Plate 1. One-dimensional Thin-layer Chromatograms of American and Korean Ginseng Saponins.



A: American ginseng root (four-year-old).
K: Korean ginseng root (four-year-old).
M: Methanol extract. E: Ether extract.
10%-10A: 10% extract-10 lambda application.
10%-5A: 10% extract-5 lambda application.

Table 2. One-dimensional Thin-layer Chromatography of American Ginseng Plant Saponins*.

Plant Material**	Panaquilins*** B	C	(d)	D	E	G-1	G-2
Two-year-old							
JIL	+	+++	+	−	+++	+	++
JIS	+ +	+++	+++	−	+	−	+++
JIR	+ +	+	−	+	+ +	+	+
SpL	+	+ +	+	+	+++	+	++
SpS	+	+ +	+	−	+++	−	++
SpR	+++	+	−	+	+ +	+	+
Four-year-old							
JIL	+++	+++	+ +	−	+	−	+
JIS	+	+ +	+	+	+ +	−	+++
JIF	+	+++	−	−	+++	−	+++
JIR	+ +	+	−	+	+ +	+	+
SpL	+ +	+ +	+ +	−	+ +	+	+ +
SpS	+	+ +	+	+	+ +	+	+ +
SpR	+ +	+	−	+	+ +	+	+

*Silica gel plates developed in solvent system-B (chloroform: methanol: distilled water; 65:35:10, lower layer), and detected with ceric sulfate solution spray. The symbols (+++, + +, +++, + +, +) approximate the surface area observed after spraying.

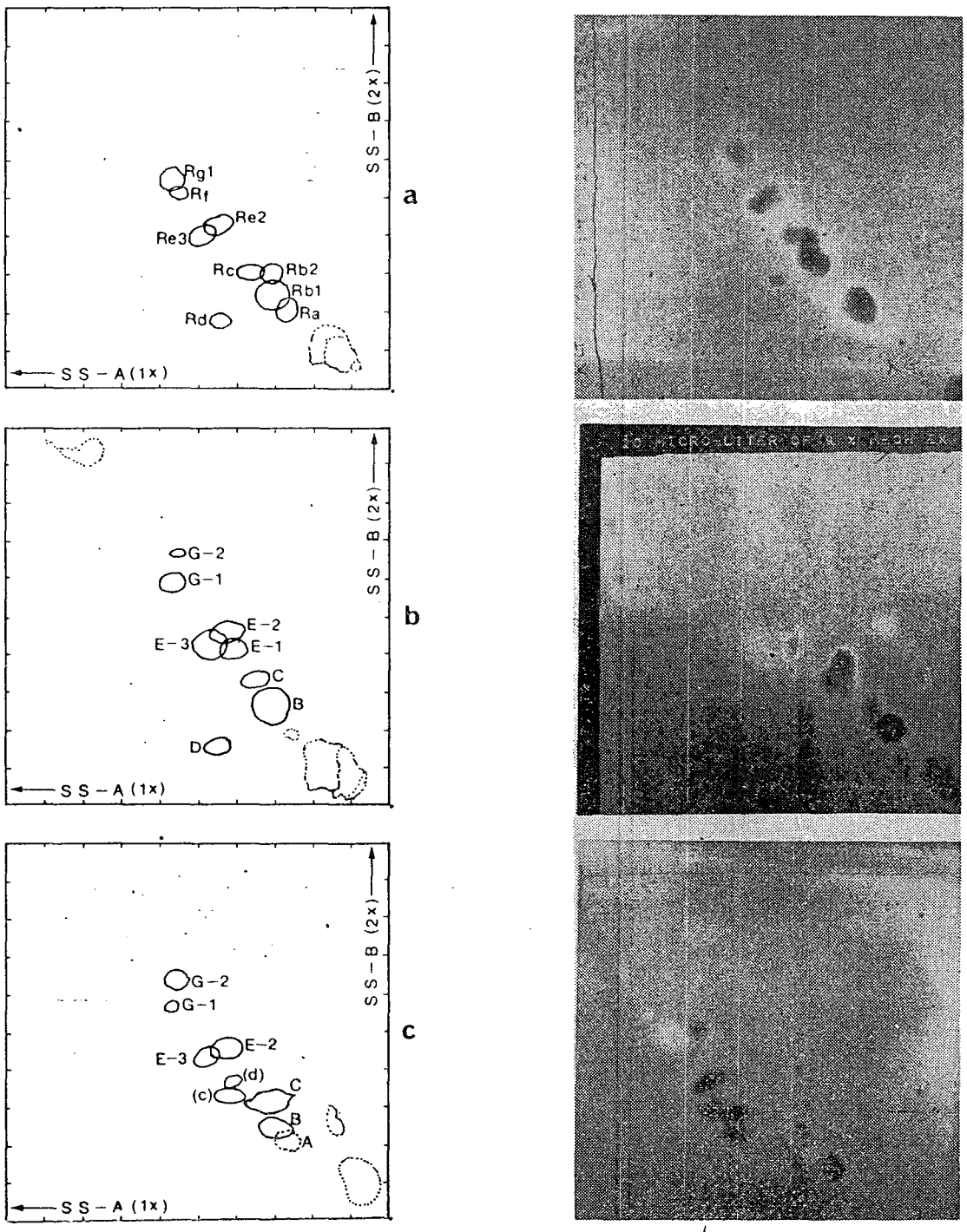
**Jl-July collection; Sp-September collection; L-leaf; S-stem; F-fruit; R-root.

***The panaquilin genins are: B and C-panaxadiol; D-oleanolic acid; E-both panaxadiol and panaxatriol: G-1-panaxatriol; (d) and G-2-unknown.

in all plant parts, and in considerably higher concentrations in the above-ground portions than in the roots.

Shibata *et al.* (23) reported ginsenosides Ra through $R_{(f)}$ to contain panaxadiol genin, ginsenosides Rg_1, Rg_2 and Rg_3 to contain panaxatriol genin, and ginsenoside Ro to contain oleanolic acid genin. In SS-A (Fig. 1) ginsenoside Ro is absent. It may be that ginsenosides Ro, $R_{(f)}$, Rg_2 and Rg_3 are artifacts. Elyakov *et al.* (28, 29) reported panaxosides A ~ C to contain panaxatriol genin, and panaxosides D ~ F to contain panaxadiol genin.

Figure 2 and Plate 2. Two-dimensional Thin-layer Chromatograms of American Ginseng Saponins (Panaquilins) and Korean Ginseng Saponins (Ginsenosides).



SS-A: Solvent System-A (n-butanol: acetic acid: distilled water; 4:1:5; upper layer).
SS-B: Solvent System-B (methanol: chloroform: distilled water; 65:35:10; lower layer).
Silica gel PF-254, and ceric sulfate spray.
a: Korean ginseng roots (four-year-old, September collection): Ginsenosides Ra, Rb_1, Rb_2, Rc, Rd, Re_2, Re_3, $R_{(f)}$ and Rg_1.
b: American ginseng roots (four-year-old, September collection); Panaquilins B, C, D, E-1, E-2, E-3, G-1 and G-2.
c: American ginseng leaves (four-year-old, September collection): Panaquilins A, B, C, (c), (d), E-2, E-3, G-1 and G-2. (Panaquilin A appeared only in two-year-old September stem saponins).

Panaxadiol and panaxatriol were derived from protopanaxadiol and protopanaxatriol, respectively.

Panaquilins B and C were found to contain panaxadiol genin; panaquilin D to contain oleanolic acid genin; panaquilin E to contain both panaxadiol and panaxatriol genins; panaquilin G-1 to contain panaxatriol genin.

The data suggests that panaxadiol genin (protopanaxadiol) is present in all plants. Panaxatriol genin (protopanaxatriol) is present in two- and four-year-old plants with the exception of stems and fruits. Oleanolic acid is present in two-year-old roots and September leaves, and four-year-old roots and stems.

c. Two-dimensional Thin-layer Patterns

Two-dimensional thin-layer chromatograms were prepared by developing twice with SS-B and then once with SS-A (Fig. 2; Plate 2). Silica gel PF-254 was preferred to silica gel G as the resulting spots were better defined. The saponins were detected by spraying with ceric sulfate solution (3% in 3N sulfuric acid), rater than by a 10–50% sulfuric acid solution as was used by the Japanese and Russian workers.

The panaquilins can be more easily compared to the ginsenosides by two-dimensional tlc (Fig. 2; Plate 2). The panaquilins were named from the lowest rf-value to the highest rf-value in SS-A so that the results could be compared to those of Shibata *et al.* (11, 23, 30). Panaquilins G-1 and G-2 appeared after development with SS-B (Fig. 1), and panaquilins E-1, E-2 and E-3 only after development with both SS-A and SS-B (Fig. 2b and c.). In the SS-B panaquilins E-1, E-2 and E-3 had the same rf-value and panaquilin E-2 had a slightly higher rf-value. Panaquilins E-1 and G-2 are absent in Korean ginseng roots, and ginsenosides Ra and $R_{(f)}$ absent in American ginseng roots. Panaquilin B and ginsenoside Rb appear to differ chemically. Panaquilin B is a single compound, however, ginsenoside Rb will separate into ginsenosides Rb_1 and Rb_2 (Fig. 2a and b).

The above-ground saponins panaquilins A, (c) and (d) were not present in the root (Fig. 2b and c, and Table 3). Panaquilin (c) has the same rf-value as panaquilin C in SS-B and panaquilin E-2 in SS-A; and panaquilins (d), E-1 and E-2 the same rf-value as panaquilin D in SS-A. In SS-A, panaquilins (c) and (d) have similar rf-values. Panaquilin A was observed in two-year -old September stem saponins, and may be similar to ginsenoside Ra.

Some differences exist in American ginseng saponins that are related to the age, time of collection, and/or the plant part extracted (Table 3). Panaquilins B, C, E-2, E-3 and G-2 are present throughout the ginseng plant. Panaquilin A is present only in two-year-old stems collected in September, and panaquilin (c) is present only in July collected four-year-old leaves or two-year-old stems. Panaquilin G-1 was present in the roots and four-year-old September collected leaves. Panaquilins D, E-1 and G-1 are principally localized in the root, and panaquilins (c) and (d) in the above-ground parts. Panaquilins C and G-2 are in higher concentrations in the above-ground portions as compared to the roots.

Panaquilins E-1, E-2 and E-3; (c) and (d); ginsenosides Re_2 and Re_3; and Rb_1 and Rb_2 are not resolved by one-dimensional tlc, and panquilins D and G-1, and ginsenoside Rd may often be misinterpreted.

Panaxadiol and panaxatriol distribution was as noted from one-dimensional tlc data. However, oleanolic acid may be present only in the roots, and not the above-ground parts as suggested by the one-dimensional tlc data.

d. Isolation of American Ginseng Root Saponins

American ginseng saponins were isolated in order to obtain reference compound for tlc, to determine their ir, and to determine the tlc, ir and glc characteristic of their genins.

American ginseng saponins were isolated from methanol extract-2 (5 g). From this crude extract, panaquilin mixtures (fractions 1–6) were prepared by one-dimensional preparative tlc using SS-B (Table 4). The percentage of purified saponins (panaquilins) isolated from 5 g of methanol extract-2 was 17.4% (771 mg purified saponins and 98 mg saponin mixture), or 3.13% from dried ginseng roots. Total crude saponin concentrations have been reported for

Table 3. Two-dimensional Thin-layer Chromatography of American Ginseng Plant Saponins*.

Plant Material**	Panaquilins***									
Two-year-old	B	C	(c)	(d)	D	E-1	E-2	E-3	G-1	G-2
JIL	+	+++	+	+	−	+	+++	+++	+	+ +
JIS	+ +	+	+ +	+	−	−	+	+	−	+ +
JIR	+ +	+ +	−	−	+	+	+ +	+ +	+	+ +
SpL	+ +	+++	−	+	−	+	+++	+ +	−	+ +
SpS	+++	+ +	−	+	−	−	+++	+ +	−	+++
SpR	+ +	+++	−	−	+	+	+++	+++	+	+
Four-year-old										
JIL	+++	+ +	++	++	−	−	+	+	−	+ +
JIS	+++	+ +	−	+ +	−	−	+ +	+++	−	+ +
JIF	+++	+++	−	+	−	−	+ +	+++	−	+++
JIR	+ +	+ +	−	−	+	+	+−+	+++	+	+
SpL	+ +	+++	+	+	−	−	+ +	+++	+	+ +
SpS	+++	+ +	−	−	−	−	+ +	+++	−	+ +
SpR	+++	+ +	−	−	+	+	+ +	+++	+	+

*Silica gel plates developed in solvent system-B (2x) and then solvent system-A (1x), and detected with ceric sulfate spray.
The symbols (+++, + +, +++, ++, +) approximate the surface area observed by spraying.

**Jl-July collection; Sp-September collection; L-leaf; S-stem; F-fruit; R-root.

***The panaquilin genins are: B and C-panaxadiol; D-oleanolic acid; E-1, E-2 and E-3-either panaxadiol or panaxatriol; G-1-panaxatriol; (c), (d) and G-2-unknown.

Korean ginseng roots (2–4%), fiber-roots (8–13), above-ground parts(8–10%), flower parts(6–7%), and American ginseng roots (6–7)% (11, 22). All isolated saponins reacted positively for the Lieberman-Burchard test (31).

When examined by two-dimensional tlc, panaquilin B remained as a single spot. Ginsenoside Rb will separate into ginsenosides Rb_1 and Rb_2 (32) (Fig. 2a; Plate 2a). Panaquilins C, D, G-1 and G-2 remained as a single spot. Panaquilin E separated into panaquilins E-1, E-2 and E-3, and ginsenoside Re into ginsenosides Re_2 and Re_3.

The isolated panaquilin D contained saccharide impurities and was further purified by preparative

Table 4. Isolation of Panaquilins by Preparative Thin-layer Chromatography*.

Fraction	Rf-value	Crude Saponins (mg)	Panaquilin	Purified Saponin (mg)	M. P. **
1	0.14	1,320	D	56	162 ~ 165
2	0.23	800	B	315	193 ~ 195
3	0.30	270	C	83	152 ~ 154
4	0.41	460	E	276	184 ~ 188
5	0.55	70	G-1	26	152 ~ 155
6	0.63	60	G-2	15	150 ~ 152
Total	—	2,980	—	771	—

*Extraction from methanol extract-2 (5 g) of American ginseng roots (four-year-old, September collection). Solvent system-A was used to isolate panaquilin D, and solvent system-B for all other panaquilins.

**M. P.: Melting point, degrees centigrade, uncorrected.

Table 5. Suggested Similarity of Panaquilins, Ginsenosides and Panaxosides Isolated from American and Korean Ginseng Roots.

American Roots	Korean Roots		
Panaquilin*	Ginsenoside*	Ginsenoside**	Panaxoside***
—	—	Ro	—
—	Ra	Ra	F
B	Rb_1, Rb_2	Rb_1, Rb_2	E
C	Rc	Rc	D
D	Rd	Rd	—
E-1	—	—	—
E-2	Re_2	Rd	C
E-3	Re_3	Re	B
—	Rf	R(f)	—
G-1	Rg_1	Rg_1	A
G-2	—	Rg_2	—
—	—	Rg_3	—
—	—	Rh_1	—
—	—	Rh_2	—

*From this study. American and Korean ginseng root saponins examined by two-dimensional thin-layer chromatography (Fig. 2a, b).

**By Shibata *et al.* (26). Korean ginseng root saponins identified by one-dimensional thin-layer chromatography.

***By Elyakov *et al.* (25). Korean ginseng root saponins identified by one-dimensional thin-layer chromatography.

tlc using SS-A. The purified panaquilin D had a rf-value between panaquilins C and E in one-dimensional tlc using SS-A. To date, the Russians have not reported the isolation of a saponin containing oleanolic acid such as panaquilin D. The Japanese (12) reported an oleanolic acid containing saponin (ginsenoside Ro) present in one-dimensional tlc developed in the solvent system; n-butanol: ethyl acetate: distilled water (4:1:5, upper layer; modified SS-A). However, the two-dimensional tlc results obtained suggest that ginsenoside Ro may reside between ginsenosides Rc and Re, and that ginsenoside Ro might best be named ginsenoside Rd. The suggested similarity of panquilins, ginsenosides and panaxosides is shown in Table 5.

e. Isolation of American Ginseng Root Sapogenins

American ginseng root methanol extract-2 acid hydrolysates were separated by preparative tlc and contained panaxadiol (17.3%), panaxatriol (0.44%), and oleanolic acid (0.28%). The approximate ratio of panaxadiol to panaxatriol isolated from American ginseng roots by column chromatography is 6:5:1 or 40:1 by preparative tlc; whereas, that reported for Korean ginseng roots (four-year-old) is 1:1 (33). Oleanolic acid (panaquilin D genin) was present in American ginseng roots, but not in the above-ground plant parts when examined by two-dimensional tlc. The Japanese, but not the Russian, have reported oleanolic acid present in Korean ginseng roots and above-ground parts (24, 34). The minor ginseng species *Panax japonicum* and Himalayan *P. pseudoginseng* have been reported to contain large quantities of oleanolic acid and small quantities of panaxadiol (35–38).

f. Infra-red Spectra

Infra-red spectra of panaquilins and their genins are shown in Fig. 3 and 4, respectively. The ir of panaquilin B is similar to that of panaquilin E which is a mixture of panaquilins E-1, E-2 and E-3. A carbonyl group is present in panaxadiol containing panaquilin C (1720 cm^{-1}), and in oleanolic acid containing panaquilin D (1980 cm^{-1}). The carbonyl group of panaquilin C may reside in the carbohydrate part, whereas a carbonyl group resides within the genin of panaquilin D (oleanolic acid saponin). All panaquilins have a broad hydroxyl group band (3400 cm^{-1}).

The ir spectra shown for the American ginseng

Fig. 3. Infra-red Sepctra of Ginseng Saponins.

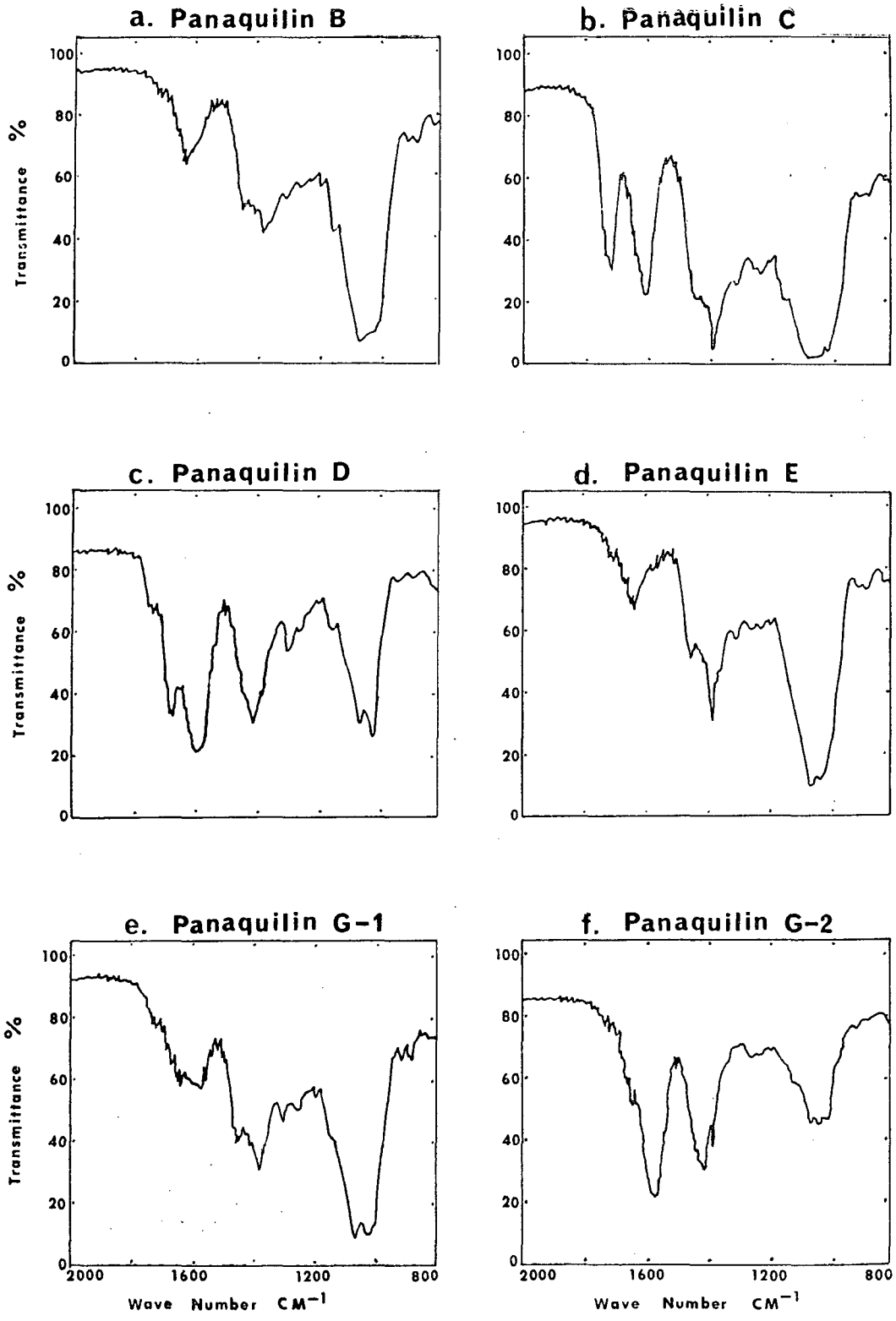


Fig. 4. Infra-red Spectra of Ginseng Sapogenins.

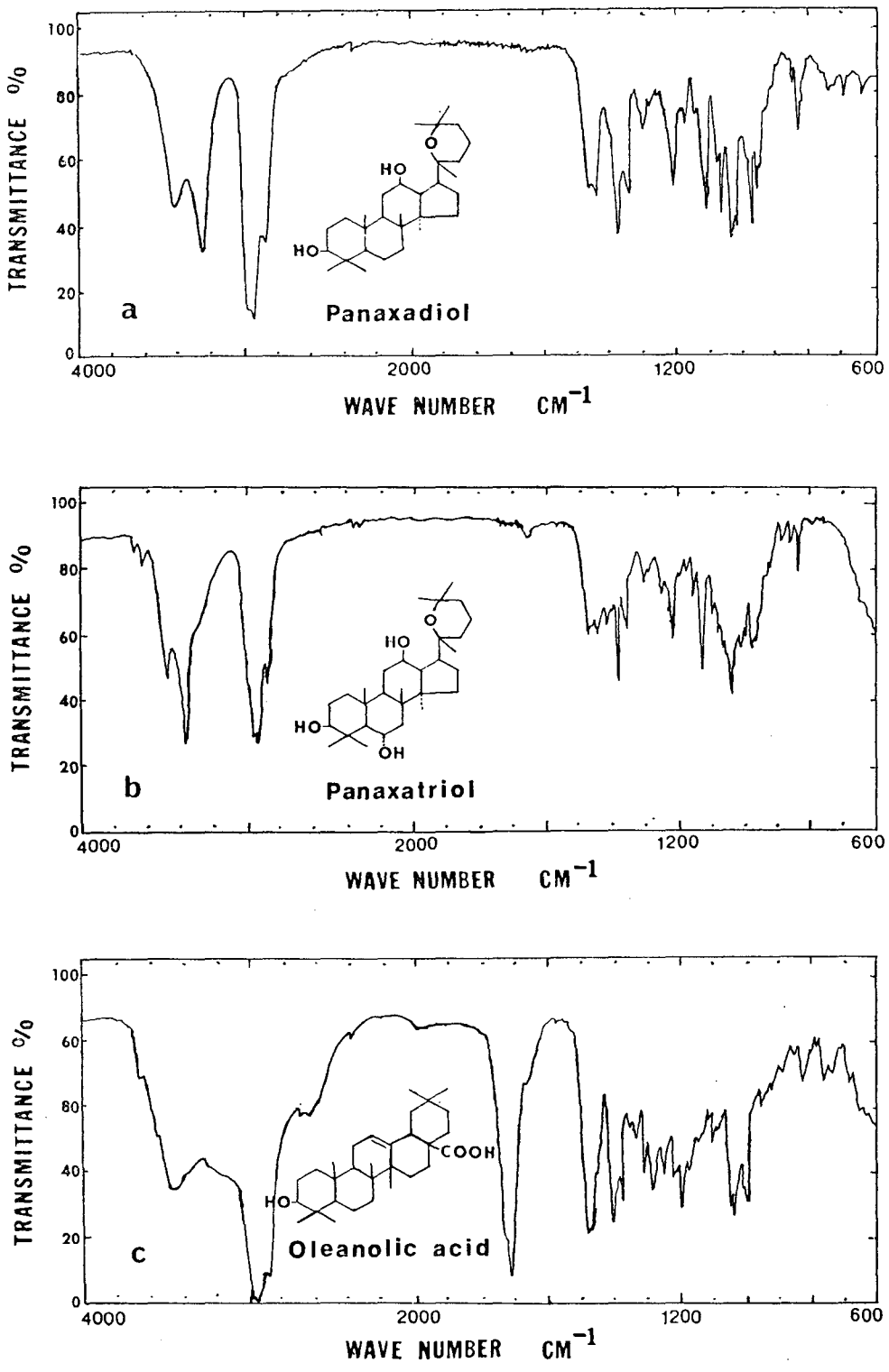
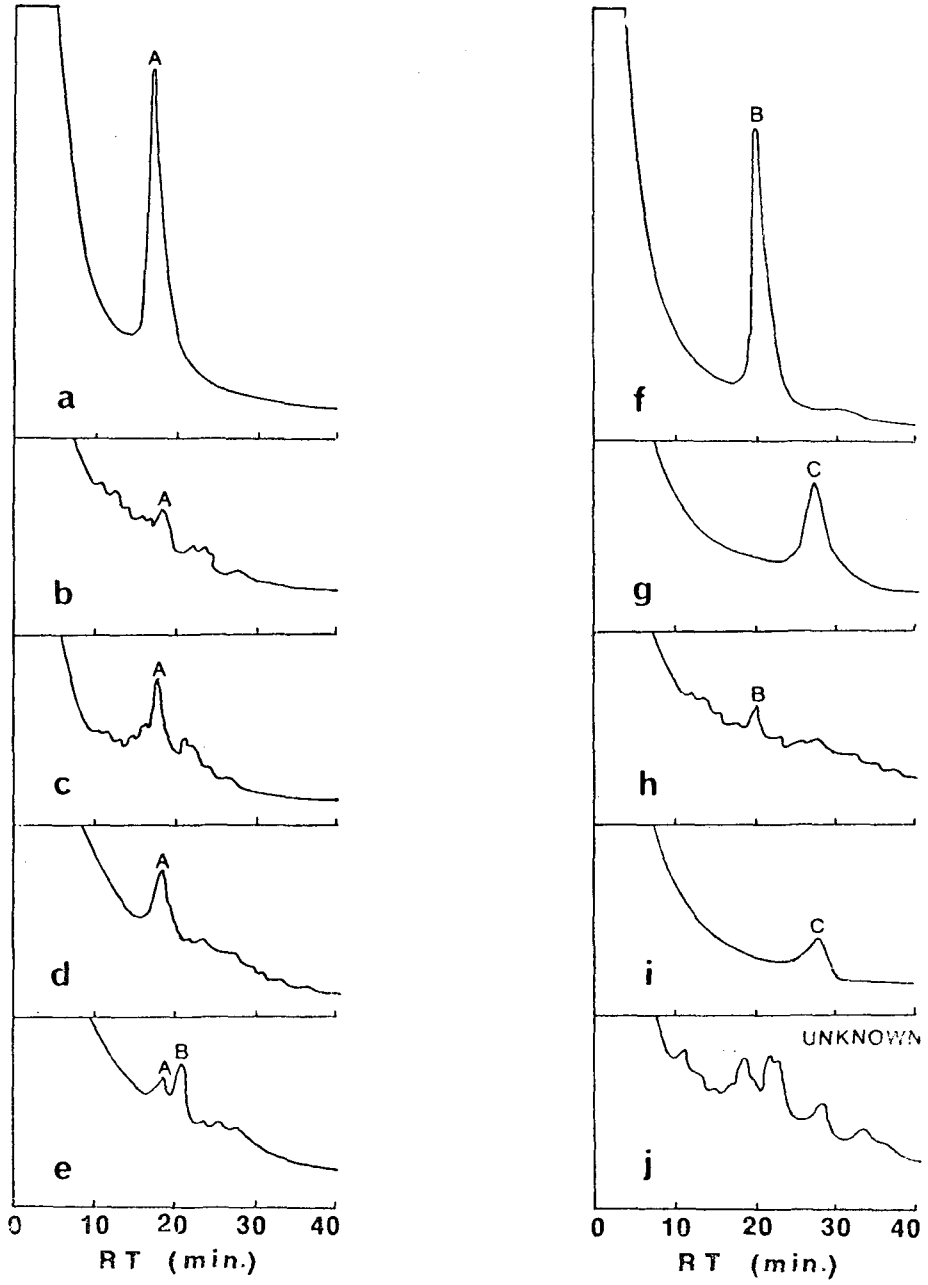


Fig. 5. Gas-liquid Chromatograms of Reference Standards and Hydrolyzed Products of Panaquilins.



Retention time (RT) of saponin controls.

a. Panaxadiol-18' 55" (A).
b. Hydrolyzed panaxadiol containing methanol and 30% hydrochloric acid (4:1).

Gas-liquid chromatograms of hydrolyzed saponins.

c. Panaquilin B
d. Panaquilin C
e. Panaquilin E
f. Panaxadiol-21' 30" (B).
g. Oleanolic acid-28' 18" (C).
h. Panaquilin G-1
i. Panaquilin D
j. Panaquilin G-2

sapogenins (Fig. 4) are identical to those reported by the Japanese (30) and Russians (39) for Korean ginseng, and were used to assist in identifying the isolated American ginseng sapogenins.

h. Gas-liquid Chromatography.

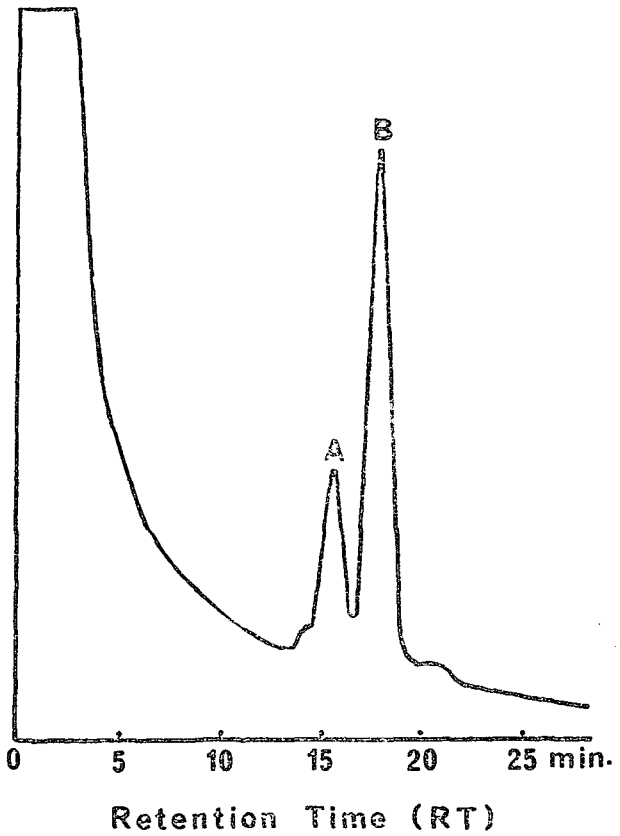
Isolated panaquilins, and their genins, were studied by glc in order to establish a procedure for rapid identification and/or assay.

Chromatograms of standard genins are shown in Fig. 5a, b and f. The Russian workers (40, 42) have suggested that panaxoside hydrolysates contained many artifacts. The chromatogram of a hydrolysates contained many artifacts. The chromatogram of a hydrolyzed panaquilin does contain a number of unidentified peaks. The hydrolysates of panaquilins B and C contained panxadiol (Fig. 5c and d), that of panaquilin D contained oleanolic acid (Fig. 5i), and that of panquilin G-1 contained panaxatriol (Fig. 5h). Panaquilins E-1, E-2 and E-3 hydrolysates contained both panaxadiol and panaxatriol (Fig. 5e). The genin content of panaquilin G-2 could not be identified (Fig. 5j) by the glc procedure used. Panaquilins A, (c) and (d) from the aboveground parts were not isolated nor the ir genins studied and identified. The panaxadiol/panaxatriol ratio in panaquilin E hydrolysates is approximately 1:2. The identity of panaxadiol, panaxatriol and oleanolic acid in the panaquilin hydrolysates was verified by tlc.

Ether extracts (10 g) of American ginseng roots contained a sterol mixture (82 mg) which was isolated by column chromatography. The m.p., ir and rf-value of the mixture were identical with standard β-sitosterol. However, glc (Fig. 6) indicated that the mixture contained a 1:3 ratio of stigmasterol and β-sitosterol, as well as minor constituents. Commercial β-sitosterol (58–63%) and campesterol (37–42%), or stigmasterol (34%) (43). Resolution of phytosterol mixtures, or their derivatives, has been done by glc (44) or tlc (45).

Lin (46) isolated stigmasterol from Manchurian *P. ginseng* fiber-roots, and Takahashi *et al.* (47–49) isolated both β-sitosterol and its glucoside (daucosterin) form Korean ginseng roots. Wong (50) isolated phytosterol (m.p. 132–134°C) identical with β-sitosterol from American ginseng roots. Stigmasterol and β-sitosterol are reported here for the first time as existing together in American ginseng roots. Perhaps these phytosterols exist in the plant as β-sitosteryl glucoside (daucosterin) and stigmasteryl glucoside. Stigmasteryl glucoside is known to be present in *Adenanthera pavonina* (*Mimosaceae*) seeds and leaves (51).

Tig. 6. Gas-liquid Chromatograms of β-Sitosterol and Stigmasterol.



Peak A: Stigmasterol (RT-15' 36").
Peak B: β-Sitosterol (RT-17' 57").
β-Sitosterol: Stigmasterol 3:1.
Minor peaks: Unknown (RT-14' 05" and RT-20' 50").

Materials and Methods

Plant Materials

Plant materials were obtained from the Fromm Brothers Farm, Hamburg, Wisconsin. The two-and four-year-old American ginseng plants (*Panax quinquefolium* L.) and four-year-old Korean ginseng roots (*Panax ginseng* C. A. Meyer) used in this

study were collected on July 20–21, and September 13–15, 1971 and dried at 50°C or at room temperature.

Chemical Standards

Panaxatriol was obtained from S. Shibata (Faculty of Pharmaceutical Sciences, University of Tokyo, Japan), panaxadiol from S. Shibata and Th. Wagner-Jauregg (Forschungsabteilung der Siegfried AG., Zofingen AG., Switzerland), and oleanolic acid from Z. Kaspryk (Department of Biochemistry, University of Warsaw, Poland). Stigmasterol was purchased from Sigma Chemical Co., St. Louis, Missouri, and β-sitosterol from Nutritional Biochemicals Corp., Cleveland, Ohio.

Analytical Procedures

Infra-red spectra were prepared from potassium bromide pellets and with a Beckman IR-33 Spectrometer. Melting points were determined using a Fisher-Johns melting point apparatus (Fisher Scientific Co., Chicago, Illinois), and uncorrected.

a. Preparative Thin-layer Chromatography

Silica gel G and silica gel PF-254 (for preparative tlc) were used to prepare chromatograph plates with a DeSaga apparatus (Brinkma Instruments Inc., Great Neck, Long Island). The slurry was prepared by mixing the adsorbent (40g) with a 2:1 distilled water and methanol mixture (75 or 95 ml), and vigorously shaking for 50–60 sec. in a 500-ml Erlenmeyer flask covered with parafilm. The coated plates (thickness 0.25 mm, 0.50 mm or 1.00 mm) were air-dried for approximately 20 min., activated at 120°C for 50–60 min., and stored over calcium sulfate in a dessicator until used.

Samples (100 mg) for preparative tlc were dissolved in methanol (1 ml) and streaked by means of a pippette on the plate (80mg sample on 1-mm, 40mg on 0.5-mm and 20 mg on 0.25-mm thick plates). The plates were developed to a height of 15 cm from the application point in a glass jar containing either solvent system-A (SS-A) (n-butanol:acetic acid:distilled water; 4:1:5, upper layer), or solvent system-B (SS-B) (chlorform:methanol:distilled water; 65:35:10, lower layer). The saponin bands were detected by spraying a small side portion of the plate with ceric sufate solution (3% in 3N sulfuric acid). While observing under u. v. light sections of the saponin bands were scraped off and then eluted with methanol (2x) (approximately 10 ml to 1g of silica gel powder) using a glass sintered funnel. The methanol solutions were evaporated under a tungsten lamp, and the residue weighed.

Crude saponin hydrolysates were developed with either benzene-acetone (3:1) or ether.

b. One-dimensional Thin-layer Chromatography

The procedure is essentially as described in the above. The slurry was made by mixing silica gel G (30g) or silica gel PF254 (30g) with a mixture (60 or 75 ml) of distilled water and methanol (2:1). Samples (10 mg) were dissolved in methanol (0.1 ml), and aliquots (10–20 lambda) applied to plates.

c. Two-dimensional Thin-layer Chromatography

A sinlge sample was spotted at a point 2.5 cm from two adjacent sides of a tlc plate. The plate was developed in SS-B (2x), air-dried, and then developed in SS-A (1x) with the plate turned at right angle to the point of sample application. The plate was developed to a solvent front height of 10 cm from the sample spot.

d. Gas-liquid Chromatography

Gas-liquid chromatography (glc) (Varian Aerograph Model 1740, Varian/Analytical Instrument Division, Palo Alto, California) was used to separate ginseng sapogenins. The instrument contained 1/4 "x6' glass columns with 80–100 mesh Aeropak (stationary phase) coated with 3% OV-17 (liquid phase). The glc temperature conditions were: injector (300°C), detector (300°C) and column (275°C). The gas flow rates were: nitrogen (40 ml/min.), hydrogen (27 ml/min.) and air (300 ml/min).

Samples (1mg) for injection were silylated with Tri-Sil (0.5ml) (Pierce Chemical Co., Rockford, Illinois) at 60°C for 15 min., and then diluted up to 1 ml with pyridine.

Extraction

Ginseng plants were extracted by a modification of the procedure by Shibata *et al.* (30, 32). Coar-

sely ground ginseng roots (50g) were Soxhlet extracted with ether for 24 hrs. The ethereal extract was concentrated in a rotary evaporator, dried and weighed. The extraction thimble containing the root powder was allowed to air dry after which it was Soxhlet extracted with chlorform for 24 hrs., and then methanol (300ml) for 24 hrs. The methanol solution was concentrated in a rotary evaporator at 50°C, dried over calcium sulfate in a dessicator for 24 hrs., or under a tungsten lamp, and weighed. The dried methanol extract (1g) was treated with cold methanol (10ml, 5°C). The residue was discarded, and the filtrate was evaporated, dried, and weighed. Methanol extract-2 was prepared by dissolving the methanol extract (approximately 1g) in methanol (10ml).

Hydrolysis

Crude saponin (34g) was hydrolyzed with a mixture (500 ml) of methanol 30% hydrochloric acid (4:1) by refluxing on a steam bath for 5 hrs. (22). After hydrolysis, methanol was removed in a rotary evaporator at 50°C. Absolute ethanol (100ml) was added to the residue and evaporated. This procedure was repeated twice in order to remove residual hydrochloric acid. The residue was then extracted with absolute ethanol (340ml), filtered, and concentrated to 100 ml. The black precipitate formed upon addition of distilled water (300 ml) was removed by filtration. The filtrate was extracted with ether (4x, 400ml) in a separatory funnel. The ether layers and the black precipitate (hydrolyzed saponins) were combined, dried over anhydrous sodium sulfate over night, and filtered. The filtrate was then evaporated and the residue weighed (8g).

Isolation of Ginseng Sapogenins Panaxadiol, Panaxatriol and Oleanolic Acid (22)

The hydrolysate (1.48g) was dissolved in ether 3 ml) and applied to a silica gel column (60g, 1.5 × 60 cm). The column was eluted with a mixture (300 ml) of n-hexane and acetone (4:1) and 5-ml fractions were collected. When examined by tlc, fractions 18–20 contained principally panaxadiol. Panaxadiol was recrystallized from ethyl acetate twice to form colorless needles (189mg, mp. 247–250°C). Fractions 29–61 were combined, evaporated and dissolved in acetone (61mg; 1.5 ml). Panaxatriol was isolated from this fraction by preparative tlc, and recrystallized from benzene to form colorless needles (29 mg, 235–238°C).

Preparative tlc of crude saponin hydrolysates (2.5g) gave panaxadiol (432 mg) and panaxatriol (11mg). An oleanolic acid fraction (7mg) was obtained, but it was difficult to recrystallized. To obtain pure oleanolic acid, 50mg of oleanolic acid saponin (panaquilin D) was hydrolyzed with a mixture (10ml) of methanol and 30% hydrochloric acid (4:1) for 5 hrs. on a water bath. Upon evaporation of methanol, oleanolic acid crystals deposited. Oleanolic acid was recrystallized from methanol (10mg, m.p. 305–310°C).

Isolation of B-Sitosterol (48)

Ether extracts (10g) were dissolved in ether (100 ml) and treated twice with 5% sodium hydroxide solution (50ml) to principally remove fatty acids and lipids. Ether layers were combined and concentrated in a rotary evaporator. The residue was dissolved in petroleum ether (b.p. 30–60°C, 10ml) and applied to a column (1.5 × 60 cm) containing alumina (200 g, neutral, grade 1, M. Woelm, Eschwege, Germany). Small amount of petroleum ether (1ml, 2x)was used to wash completely the sample remaining inside the column. The column was then eluted with ether (1 l.) and 10-ml fractions collected. Fractions 20–29 were concentrated and contained principally β-sitosterol. The residue was dissolved in 95% ethanol (150ml), and distilled water was added drop by drop until the solution became turbid. The turbid solution was heated in a water bath until clear, filtered while hot through a glass sintered funnel, and allowed to stand overnight in a refrigerator. The β-sitosterol scale-like colorless crystals were recrystallized from 90% acetone to form fine needles (82 mg, m.p. 138–139°C) (rf-value 0.35 in benzene-ethylacetate (3:1), and 0.80 in benzene-acetone 3:1)).

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