THE GINSENOSIDES OF VARIOUS GINSENG PLANTS AND SELECTED PRODUCTS

JOSEPH HON-CHIU LUI and E. JOHN STABA

Department of Pharmacognosy, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT.—Three previously reported ginsenoside extraction methods were used to extract *Panax quinquefolius* (American ginseng), *Panax ginseng* (Oriental ginseng) and *Panax pseudoginseng* var. *notoginseng* (Sanchi ginseng). The ginsenoside concentration present in the extracts was determined by weight and by spectrophotometry. A two-dimensional thin-layer chromatographic method was used to show concentration trends of the ginsenosides present in cultivated American and Canadian ginseng, wild American ginseng, Korean cultivated white and processed red ginseng, Chinese processed red ginseng, Sanchi ginseng, Japanese ginseng (*Panax pseudoginseng* subsp. *japonicus*), ground-nut ginseng (*Panax trifolius*), Siberian ginseng (*Acanthopanax senticosus*) and selected products.

The (20S)-protopanaxadiol ginsenosides (Rb1, Rb2, Rc or Rd) and the (20S)-protopanaxatriol ginsenosides (Re, Rf, Rg1 or Rg2) were detected in both American Korean, Chinese and Sanchi ginseng. The highest total ginsenoside concentration was observed in Sanchi ginseng. Japanese ginseng and ground-nut ginseng leaves contained high concentrations of Ro ginsenosides and panaxadiol ginsenosides. Panax-type ginsenosides were not detected in Siberian ginseng, American wild red ginseng (*Rumex hymenosepalus*) and Korean ginseng cigarettes.

Panax ginseng C. A. Meyer (Oriental ginseng) or *Panax quinquefolius* L. (American ginseng), Araliaceae, roots are often used to prepare a beverage to correct human physiological imbalances (adaptogenic) (1) and the leaves are occasionally processed into cigarettes. The American species is cultivated or collected wild in North America and the Oriental species is cultivated in either Korea, People's Republic of China or Japan. The *Panax trifolius* L. (ground-nut or dwarf ginseng) species is indigenous to North America and is not presently used as either a food or drug. Other ginseng species sold for a diversity of medical uses in Hong Kong are *Panax pseudoginseng* var. *notoginseng* (Burkill) Hoo and Tseng (Sanchi ginseng), *Panax pseudoginseng* subsp. *japonicus* (Nees) Hara (Japanese ginseng) and *Acanthopanax senticosus* Harms. (Siberian ginseng).

The ginsenosides present in *Panax* and its commercial products have been separated and identified by one-dimensional (2–5) and two-dimensional (6) thin-layer chromatography. The ginsenosides have been quantified by gas chromatography (7), gas chromatography-mass spectrometry (8), high pressure liquid chromatography (9), droplet counter-current chromatography (DCC) (10) and spectrodensitometry (11). Ginsenosides are present in the above-ground and the below-ground portions of *Panax* (12, 13). The ginsenoside genin is either oleanolic acid (Ro), (20S)-protopanaxadiol (Rb1, Rb2, Rc & Rd) or (20S)-protopanaxatriol (Re, Rf, Rg1 & Rg2). The ginsenoside glycosides often separate chromatographically in groups containing the same genin.

The identity and/or the amount of ginsenosides present in American, Oriental and other ginseng plants and products is controversial. This report shows the concentration trends of the ginsenosides present in various ginseng plant and products as determined by a standard extraction procedure, two-dimensional thin-layer chromatography, and spectrophotometry. The ginsenosides detected in the leaf and root of *Panax trifolius*, wild American ginseng root, ginseng cigarettes, ginseng ampules, and in various geographical sources of *Acanthopanax senticosus* represents largely new and previously unpublished data.

MATERIALS AND METHODS

The sources of the botanical materials and products studied are reported in table 1.

EXTRACTION.—American, Korean and Sanchi ginsenosides were extracted by Methods I (11), II (12), and III (14).

METHOD I.—A sample (2 gm) was placed in a 150 ml Erlenmeyer flask and water (40 ml) was added. After shaking (30 min), additional water (80 ml) was added, and the shaking continued for an additional 30 min. The mixture was filtered, and the first 24–34 ml of filtrate was discarded. An aliquot (40 ml) of filtrate was collected and extracted with an equal volume of water-saturated *n*-butanol for 30 min with gentle shaking. The *n*-butanol extract was separated, evaporated to dryness, and weighed. The residue was redissolved to make a Sample Extract (10 ml methanol/gm sample) for two-dimensional tlc and spectrophotometric analysis.

METHOD II.—A sample (10 gm) was extracted in a Soxhlet apparatus for 24 hrs with chloroform. The residue was air-dried and extracted similarly for 24 hrs with methanol (100 ml). The methanol extract was diluted with water (500 ml) and extracted three times with water-water-saturated *n*-butanol (total vol. 350 ml). The *n*-butanol layers were combined and processed as in Method I.

METHOD III.—A sample (10 gm) was placed in a 150 ml Erlenmeyer flask and 75 ml of chloroform-methanol-water (1:2:0.8) was added. The mixture was shaken for two hrs and filtered. The residue was re-extracted with the chloroform-methanol-water mixture (20 ml) for one hour and then filtered. The combined filtrates were diluted with water (25 ml) and separated into two phases (chloroform and methanol-water). The methanol-water phase was diluted 5-fold with water and extracted three times with water-saturated *n*-butanol (total vol. 350 ml). The *n*-butanol layers were combined and processed as in Method I.

TWO-DIMENSIONAL TLC.—Sample Extracts (15 μl) and the standard mixture (10 μl) were applied to silica gel G plates. The standard ginsenosides mixture contained Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 ,Rg2, F11 (1 mg ginsenoside/ml methanol). Thin-layer plates were first developed with solvent system I (chloroform-methanol-water, 65:35:10, lower phase) and then solvent system II (*n*-butanol-ethylacetate-water, 4:1:5, upper phase). The plates were developed to a solvent height of 10.5 cm in both solvent system I and II. After development, the plates were air dried and sprayed with freshy prepared anisaldehyde reagent [p-anisaldehyde (0.5 ml), 70% perchloric acid (5.0 ml), acetone (10 ml) and water (40 ml)].

SPECTROPHOTOMETRY.—The spectrophotometric method was used to determine the total ginsenosides present in each sample analyzed (15). Each Sample Extract (40 μl) was diluted to 0.5 ml with ethanol and reacted at 60° for 10 min with 8% vanillin solution (0.5 ml) and 72% sulfuric acid (5 ml). The absorbance of the reaction mixture was read at 544 nm against a blank solution. The total amount of ginsenosides in each sample was determined by the molar ratios.

RESULTS AND DISCUSSION

The results of the three different ginsenoside extraction methods are summarized in table 2. The weight of the ginsenosides extracted (butanol extract) and the spectrophotometrically determined ginsenosides from extraction Method II was considerably higher than that obtained from extraction Methods I or III. Extraction Method II was, therefore, used for all materials analyzed in table 1.

Two-dimensional thin-layer chromatography enabled the materials to be evaluated for individual ginsenosides (Fig. 1). (20S)-Protopanaxadiol (Rb1, Rb2, Rc and Rd) and (20S)-protopanaxatriol (Re, Rf, Rg1 and Rg2) are separated chromatographically in groups. In general, the triol-type ginsenosides resolve in the one-dimensional systems between Rf 0.53–0.65 and the diol-type ginsenosides between Rf 0.25–0.47. In the same chromatographic system, oleanolic-acid-type ginsenosides (Ro type) resolve at approximately Rf 0.18 and the ocotillone type ginsenoside (F11) at approximately Rf 0.7.

The total ginsenoside concentration and the relative amount of each ginsenoside is stated in table 1. The two-dimensional tlc patterns of American ginseng, wild American ginseng and Canadian ginseng roots were similar to each other and contained all of the known ginsenosides in the standard mixture except Rf and

TABLE 1. Ginsenosides in various ginseng plants and products.

Material[1]	Total ginsenosides (w/w%)[2]		Relative amount of ginsenosides[3]									
	Wt	Sp	Ro	Rb1	Rb2	Rc	Rd	Re	Rf	Rg1	Rg2	F11
Plant roots												
1. American ginseng (fibered)	28.8	4.7	+	++++	+	+++	+++	++++	–	++	++	–
2. American ginseng	14.1	5.9	+	++++	+	++	++++	++++	–	++	++	–
3. Wild American ginseng	12.2	8.0	+	++++	+	++	++	++++	–	++	+++	–
4. Canadian ginseng	17.8	6.1	+	++++	+	++	+++	++++	–	++	++	–
5. Korean white ginseng	11.0	3.7	+	+++	++	+++	++	+++	++	++++	tr	–
6. Korean red ginseng	5.2	2.3	+	+++	++	++	+	+++	+	++++	+	+
7. Chinese red ginseng	7.5	2.7	+	+++	++	++	+	+++	++	++++	+	+
8. *Panax trifolius*	3.0	0.8	+	+	–	–	–	–	–	–	–	–
9. Sanchi ginseng	11.3	8.7	tr	++++	tr	+	+	+++	–	++++	–	+
10. *Panax japonicus*	13.2	6.6	++++	+	++	++	+++	tr	+	+	–	–
11. Siberian ginseng	N	N	–	–	–	–	–	–	–	–	–	–
Plant leaves												
12. *Panax trifolius*	11.1	3.3	++++	++++	++++	++	–	–	–	–	–	–
13. American ginseng	13.8	6.6	tr	+++	–	+	+++	++	–	+	++	+
Commercial products												
14. Korean ginseng cigarettes	N	N	–	–	–	–	–	–	–	–	–	–
15. Korean tobacco cigarettes	N	N	–	–	–	–	–	–	–	–	–	–
16. Sliced Korean red ginseng root	5.5	1.7	+	++	+	+	+	++	+	+++	+	+
17. Chinese Panax ginseng extract	10.3	1.9	+	++	++	++	+++	++	+	+	+	–
18. Siberian ginseng extract	N	N	–	–	–	–	–	–	–	–	–	–
19. Siberian ginseng root tablets	N	N	–	–	–	–	–	–	–	–	–	–
20. Wild American red ginseng root (*Rumex hymenosepalus*)	N	N	–	–	–	–	–	–	–	–	–	–

[1]Material Sources:

Plant roots

1. *Panax quinquefolius* L., Araliaceae, dried, cultivated, fibered-white ginseng root, Fromm Brothers, Inc., Hamburg, Wi., 54438, 1978 Crop.
2. *P. quinquefolius* L., dried, cultivated, white ginseng root, Fromm Brothers, Inc., 1976 crop.
3. *P. quinquefolius* L., dried, wild (more than 6 yrs old), white ginseng root, S. Goodman & Sons, Louisville, KY. 40202, 1978 collection.
4. *P. quinquefolius* L., dried, cultivated (4 yr. old), white ginseng root, Race-Hellyer, Waterford, Ontario NOE IYO, Canada, 1978 crop.
5. *P. ginseng* C. A. Meyer, dried, cultivated (approx. 6 yrs. old), white ginseng root, collected by E. J. Staba in Republic of Korea, 1974 crop.

6. *P. ginseng* C. A. Meyer, dried, cultivated (probably 6 yrs. old), red ginseng root, Office of the Monopoly, Republic of Korea, 1974 crop or earlier, Cutty style 20 and 30–Earth classification.
7. *P. ginseng* C. A. Meyer, dried, cultivated, red ginseng root, People's Republic of China, from Po-chien Inc., Los Angeles, CA. 90029.
8. *P. trifolius* L., driuel, roots, collected in Minnesota, Dr. Gerald B. Ownbey, Department of Botany, University of Minnesota, St. Paul, Herbarium Specimen No. 345879.
9. *P. Pseudo-ginseng* Wall. var. *notoginseng* Hoo & Tseng, dried, roots, People's Republic of China, Sanchi (Tienchi), Po Chien Inc., Los Angeles, CA. 90006.
10. *P. pseudo-ginseng* Wall subsp. *japonicus* Harn, dried, roots obtained from Japan prior to 1973, Dr. Jung Yun Kim, Los Angeles, CA. 9006.
11. *Acanthopanax senticosus* Harms., Araliaceae, (Eleutherococcus senticosus, Siberian ginseng, Tsu Wu Chu), dried, roots, Heliung Kiang province, People's Republic of China, from Botanical Research Laboratory, Santa Cruz, CA. 95061, 1977 collection.

Plant leaves

12. *P. trifolius* L., dried, leaves from Washington, D.C. area, Dr. James A. Duke, Beltsville, MD. 20702, Voucher Duke No. 18279, 1978 collection.
13. *P. quinquefolius* L., dried, cultivated leaves from 4 yr old plants, Fromm Brothers, Inc., September, 1974 crop.

Commercial products

14. *P. ginseng* C. A. Meyer, Korean ginseng cigarettes.
15. Korean tobacco cigarettes.
16. *P. ginseng* C. A. Meyer, Pure Sliced Korean red ginseng.
17. *P. ginseng* C. A. Meyer, People's Republic of China, ampules containing ginseng extract for oral use.
18. *A. senticosus* Harms., Unadulterated concentrated Siberian ginseng alcoholic extract.
19. *A. senticosus* Harms., U.S.S.R. Imported Siberian ginseng root tablets.
20. *Rumex hymenosepalus* Torr., Polygonaceae (Canaigre, Wild American Red Ginseng), probably root material used to prepare capsules.

[a]Total ginsenosides concentration (w/w%), determined by direct weighing (Wt) and spectrophotometrically (Sp). N = None-detected.

[b]tr = trace amount. The symbols (+/++, ++, +/++ & +) represent the surface area observed after spraying the chromatographed test sample (15 μl) and standard (10 μl; Conc: 1 mg ginsenoside/ml) with anisaldehyde detecting reagent. The standard ginsenosides reaction are estimated as (+).

TABLE 2. Comparison of ginsenoside extraction methods.[1]

Root samples[2]	Method I		Method II		Method III	
	Wt.	Sp.	Wt.	Sp.	Wt.	Sp.
American white ginseng (fibered)	4.0±0.2	2.2±0.1	28.8±1.1	4.7±0.2	4.9±0.2	4.0±0.1
Korean white ginseng	3.5±0.2	1.6±0.1	11.0±0.8	3.7±0.1	4.2±0.3	3.2±0.1
Sanchi ginseng	7.6±0.3	4.7±0.2	11.3±0.7	8.7±0.3	10.4±0.6	7.7±0.2

[1]The extraction procedures used were those reported by Der Marderosian (Method I; 11), Staba (Method II; 12) and Chung (Method III; 14). The total ginsenoside concentration (w/w%) was determined by direct weighing (Wt.) or spectrophotometrically (Sp.). Data obtained from three replicates.

[2]The root samples extracted were items 1, 5 and 9 (table 1, Material Sources).

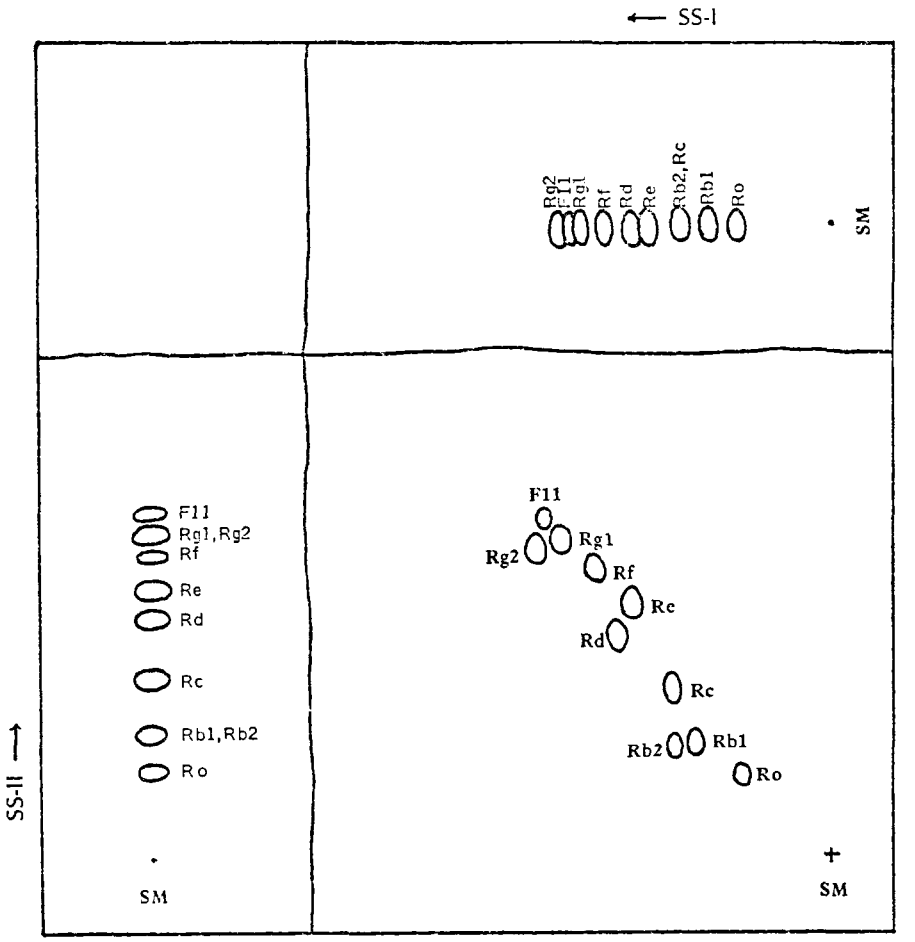
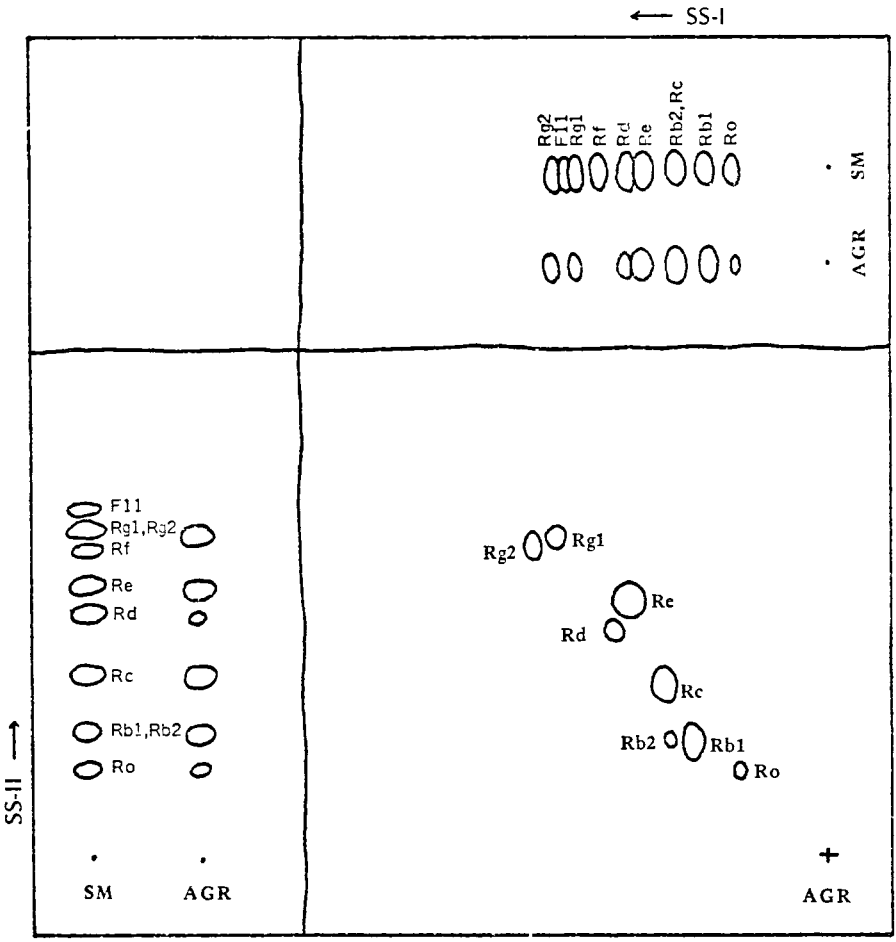


FIG. 1. Thin-layer chromatogram.
(a) One and two-dimensional resolution of standard ginsenoside mixture (SM): Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2 and F11.



1(b)

(b) One-dimensional resolution of external standard ginsenoside mixture and one and two-dimensional resolution of American ginseng root (AGR) extract.

F11. The presence (5, 9, 10, 13) or absence (10, 11) of Rg1 in American ginseng is controversial. The concentration of spectrophotometrically assayed total ginsenosides in wild American ginseng root was higher (8.0 w/w%) than that in cultivated American ginseng root (5.9 w/w%) and Canadian ginseng root (6.1 w/w%).

American ginseng leaf extracts had a similar two-dimensional tlc pattern as that of its root and contained F11 but less Ro and no Rb2. The results for Ro, Rb2 and F11 are similar to those previously reported (12, 13). Ginsenoside F11 was also isolated from the leaves of Himalyan ginseng (16). Interestingly, no ginsenosides were detected in Korean ginseng cigarettes.

White and red Korean ginseng and Chinese red ginseng have similar two-dimensional ginsenoside patterns. All the ginsenosides in the standard mixture were present in these samples. Ginsenoside F11, which is present in *Panax* leaf

material, was also found in two Korean red ginseng (Material 6 & 16) and one Chinese red ginseng root (Material 7) samples. A trace amount of Rg2 was found in Korean white ginseng, and a large amount of Rg2 was found in Korean red or Chinese red ginseng. Ginsenoside Rg2 contains one less glucose residue than ginsenoside Re. The steaming and drying process used to manufacture red ginseng (17) may be responsible for the conversion of Re to Rg2. A larger total ginsenoside content was observed in Korean white ginseng (3.7 w/w%) than the processed red form (2.3 w/w%). The two-dimensional ginsenoside pattern of Chinese *Panax ginseng* extract was similar to that of Chinese red ginseng root, but the total ginsenoside content was less than that of the root.

Sanchi ginseng contained the largest total ginsenoside content (8.7 w/w%) and larger proportions of Rb1, Re, and Rg1 than Rc, Rd and F11. Small amounts of Ro and Rb1 were detected in *Panax trifolius* roots and large amounts of Ro, Rb1, Rb2 and Rc in its leaves.

Although very high extract concentrations of Siberian ginseng and its commercial products were applied to two-dimensional tlc, they did not contain ginsenosides. No ginsenosides were found in American wild red ginseng (*Rumex hymenosepalus*) although Rg1 is reported as present (18).

ACKNOWLEDGMENTS

The authors are very grateful to the individuals and companies cited in table 1 (Material Sources—Plant Roots and Leaves) for their generosity in supplying materials for analysis. We also thank Dr. S. Shibata, Meiji College of Pharmacy, Tokyo, Japan, for supplying ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg2 and Dr. O. Tanaka, Hiroshima University School of Medicine, Kasumi, Hiroshima, Japan, for supplying ginsenoside F11.

Received 25 July 1979.

LITERATURE CITED

1. H. Bae, "Korean Ginseng," 2nd Ed., Korea Ginseng Research Institute, Republic of Korea, 1978, p. 7.
2. S. Sanada, J. Shoji and S. Shibata, *Yakugaka Zasshi*, **98**(8), 1048 (1978).
3. S. Sanada and J. Shoji, *Shoyakugaku Zasshi*, **32**(2), 96 (1978).
4. T. Ando, O. Tanaka and S. Shibata, *Syoyakugaku Zaashi*, **25**(1), 28 (1971).
5. H. Wagner and A. Wurmbock, *Deutsche Apotheker-Zeitung*, **117**(20), 743 (1977).
6. J. J. Jhang, E. J. Staba and J. Y. Kim, *In Vitro*, (4), 253 (1974).
7. C. H. Brieskorn and A. Mosandl, In: "Proceedings of the 2nd International Ginseng Symposium," Korea Ginseng Research Institute, Seoul, Korea, 1978, pp. 99–102.
8. E. Bombardelli, A. Bonati, B. Gabetta and E. M. Martinelli, In: "Proceedings of the 2nd International Ginseng Symposium," Korea Ginseng Research Institute, Seoul, Korea, 1978, pp. 29–40.
9. O. Sticher and F. Soldati, *Planta Medica*, **36**, 30 (1979).
10. A. Otsuka, Y. Morita, Y. Ogihara and S. Shibata, *Planta Medica*, **32**, 9 (1977).
11. L. E. Liberti and A. Der Marderosian, *J. Pharm. Sci.*, **78**(10), 1487 (1978).
12. S. E. Chen and E. J. Staba, *Lloydia*, **41**(4), 361 (1978).
13. J. Y. Kim and E. J. Staba, In: "Proceedings of International Ginseng Symposium," Korea Ginseng Research Institute, Seoul, Republic of Korea, 1974, pp. 77–113.
14. N. J. Chung, In: "Proceedings of the 2nd International Ginseng Symposium," Korea Ginseng Institute, Seoul, Korea, 1978, pp. 115–134.
15. S. Hiai, H. Oura, Y. Odaka and T. Nakajima, *Planta Medica*, **28**: 363 (1975).
16. O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).
17. H. Bae, "Korean Ginseng," 2nd ed., Korea Ginseng Research Institute, Republic of Korea, 1978, pp. 221.
18. W. Ziglar, *Whole Foods* (*The Natural Foods Business Journal*), **2**(4), 48 (1978).