한국농화학회지 Vol. 23, No.4, Dec. 1980
J. Korean Agricultural Chemical Society

Saponin pattern of *Panax ginseng* root in relation to stem color

Hoon Park, Qwihee Park-Lee and Chong Hwa Lee
Korea Ginseng Research Institute, Seoul, Korea
(Received Dec. 15, 1980)

莖色度別 高麗人蔘根의 사포닌 樣相

朴　薰·朴 貴 姬·李 鍾 華
高麗人蔘硏究所

要　約

高麗人蔘根(紫莖種)의 中心部(形成層內部)와 外皮十皮層에 있는 ginsenoside를 고속액체크로마토그라피로 分析하고 莖의 紫色程度와의 關係를 檢討하였다. Ginsenoside의 單純相關에 依한 saponin 樣相의 類似度를 莖色度群間 같은 뿌리 또는 다른 뿌리間에 두部位에서 比較한 結果 莖色度는 saponin 樣相과 關聯되지 않는것으로 보였다. Saponin 樣相은 部位의 出處에 關係없이 서로 다른 部位間에 약간 달랐다. 各 ginsenoside 含量順位는 表皮十皮層에서 Rg_1>Re>Rb_1>Rb_2>Rc>Rg_2≥Rd>Rf이고 中心部에서는 Rg_1>Re≥Rg_2≥Rb_1≫Rb_2>Rc≥Rd>Rf였다.

Introduction

Ginseng saponins of Korea ginseng are present in the aerial part and root[1]. But only ginseng root has been used even though saponin content is much higher in leaves. This old practice may place less value on saponins than what the most of us generally thought with the fact that ginseng studies were mainly related to saponins. No one, however, can disclaim saponins as one of effective components even though other components such as peptides and nucleic acids in Korea ginseng were reported as effective recently[2,3].

Saponin study in relation to light environment[4], age[5] and seasonal change[6], however, is very limited. There is no information on varietal difference of saponin even though three varieties were reported[7] and purple stem variety seems to have many strains[4]. Investigation on saponin pattern is necessary for biosynthesis study of saponin, breeding program and quality improvement of root. In present study three groups different in percentage of purple colored region on stem were compared in saponin pattern(a series of each ginsenoside content).

Materials and Method

Ginseng root: Two healthy roots in each group different in percentage (10, 50 and 100%) of purple color region of stem but grown nearby were chosen without considering weight at harvest from field of *Jeug Pyeong* Experimental Station on October 5. Roots were washed and all lateral, fine and hair roots were eliminated. Remained main body was separated into central part (inside of cambium) and ot-

hers (epidermis plus cortex), dried at 70°C, oven, ground with mortor.

Saponin extraction: According to previous method[4] based on others[8,9] two grams of dry root powder were extracted 3 times for 3 hours each with 20m*l* of HPLC(High presure liquid chromatography) grade methanol at 60° C water bath. The extracts were pooled and evaporated *in vacuo* at 40°C to dryness. The residue was dissolved with 5m*l* HPLC grade water 5 times, transfered to separation funnel. Pooled water fraction was washed once with 50m*l* ethylether and 3 times with 50m*l* HPLC grade chloroform to eliminate nonpolar components.

Saponin in water layer was extracted 3 times with 50m*l* of HPLC grade n-buthanol. n-Buthanol layer was pooled and evaporated *in vacuo* to dryness until no n-buthanol smell is detectable. The residue was dissolved with 0.5 to 1.0m*l* of HPLC grade methanol for injection.

Saponin analysis[4,8,9]: Methanol solution(20 μl) was injected to HPLC(Waters Associate Model 244) and analytical conditions were as follow. μ-Bondapak Carbohydrate Analysis Column, RI detector, $CH_3CN/H_2O/BuOH=80:20:15$ solvent system, 1.5m*l*/min. flow rate, 1.0cm/min. chart speed, attenuator 8x.

Each ginsenoside peak of chromatogram was identified by standard sample saponin. Rb_2 and Rg_1 were identified by cochromatography of each standard ginsenoside. Each ginsenoside content was calculated as Rg_1 equivalent from the peak area using Rg_1 calibration curve and then multiplied by appropriate factor to each ginsenoside as shown in Table 1. Factors were ratio of slope read out from each calibration curve.[9]

Saponin pattern similarity[10]: Saponin pattern was compared each other by simple correlation coefficient and when coefficient was positively significant two patterns were considered having similarity.

Results and Discussion

Calibration curve of standard ginsenoside Rg_1 was shown in Fig. 1. Slope is 26cm²/mg while that from the other's[9] was 28cm²/mg indicating little difference between two experiments. From this fact it is thought that the order of slopes among ginsenosides would be farely constant. Furtheremore analytical condsitions were similar with same model instrument. Thus slope ratio shown in Table 1 was used for the better precision to estimate each ginsenoside content. When the column was

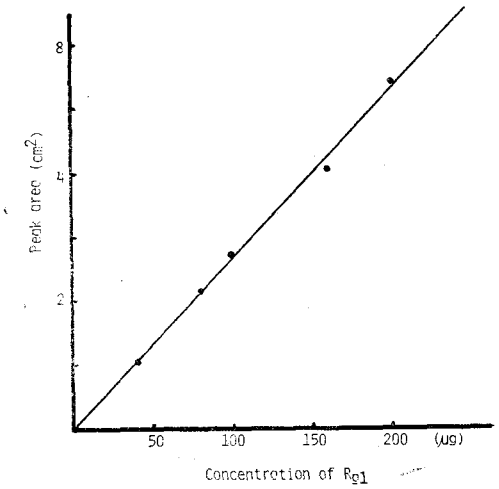

Fig. 1. Calibration curve of Rg_1 by HPLC.

Table 1. Ratio of response peak area of ginsenosides as of Rg_1†.

	Rg_2	Rg_1	Rf	Re	Rd	Rc	Rb_2	Rb_1
Rx/Rg_1	1.06	1.00	0.75	1.26	1.34	0.97	1.21	0.86

† calculated from Hong et al[9]

different the order of slope was different.[11]

Saponin pattern of epidermis-cotex different in stem color intensity are shown in Table 2. The order of each ginsenoside content seems to be similar each other. Saponin pattern of central part is shown in Table 3. The content of all

Table 2. Saponin pattern of epidermis–cortex of *P. ginseng* root in relation to stem color

Dark purple* (%)	Root No.	Ginsenoside(mg/100g dw)							
		Rg_2	Rg_1	Rf	Re	Rd	Rc	Rb_2	Rb_1
10	1	68.7	533	14.4	491	T	121	131	191
	2	91.6	557	26.0	436	81.1	121	209	390
	mean	80.1	545	20.2	464	40.6	121	169	290
50	1	67.6	555	T	543	87.5	133	197	330
	2	91.6	527	T	411	81.1	149	154	218
	mean	79.6	541	T	478	84.3	142	175	274
100	1	76.3	504	37.4	334	54.0	121	149	149
	2	81.4	485	36.0	363	45.0	83.8	102	186
	mean	78.9	495	36.7	349	49.4	103	126	168
Grand	mean	85.8	527	19.0	430	58.2	122	157	244

* Percentage of dark purple color on stem. T: trace.

Table 3. Saponin pattern of central part of *P. ginseng* root in relation to stem color

Dark purple* (%)	Root No.	Ginsenoside(mg/100g dw)							
		Rg_2	Rg_1	Rf	Re	Rd	Rc	Rb_2	Rb_1
10	1	42.7	295	T	81.6	14.2	14.6	17.4	41.3
	2	66.1	163	17.0	120	17.7	17.5	34.8	70.6
	mean	54.5	229	8.5	101	15.9	16.1	26.1	56.0
50	1	77.3	264	T	103	17.7	30.3	40.7	88.6
	2	112	265	T	72.6	T	T	T	51.2
	mean	94.7	265	T	87.8	8.8	15.1	20.3	69.8
100	1	57.2	148	T	57.2	14.5	1.4	11.4	43.3
	2	85.4	178	T	62.9	T	T	T	31.0
	mean	71.3	163	T	60.1	7.2	0.7	5.7	37.2
Grand	mean	73.5	219	2.9	82.9	10.7	10.7	17.4	54.4

* Percentage of dark purple color on stem. T: trace.

ginsenosides is lower than that of epidermis–cortex. It is considerable amount though Tani *et. al.*[12] could not found any ginsenoside in xylem-pith by thin layer chromatography. Central part is the inside portion of cortex, that is, xylem-pith. The order of each ginsenosides content seems to be similar each other as it is in epidermis–cortex. To clarify the similarity between saponin pattern simple correlation analysis is used. The correlation coefficients between saponin patterns of two parts of different roots are shown in Table 4. Saponin pattern is very close (significant at $p=0.001\%$) in most cases between the same portions regardless of stem color (upper half of left side and bottom half of right side in Table 4) while it is less close (significant at $p=0.05$ or below) in many cases between the different parts regardless of stem color (bottom half of left and upper half of right side in Table 4).

Even in the same parts similarity is rather less between roots with the same color intensity

Table 4. Simple correlation coefficient between saponin patterns of two parts of *P. ginseng* root in relation to stem color

		Stem color (%)	Root 1					
			Central part			Epidermis−Cortex		
			10	50	100	10	50	100
Root 2	Central part	10	0.910	0.958	0.959	0.927	0.895	0.926
		50	0.955	0.964	0.989	0.780*	0.703*	0.856
		100	0.940	0.939	0.983	0.783*	0.695*	0.847
	Epidermis −Cortex	10	0.784*	0.871	0.808*	0.910	0.976	0.885
		50	0.889	0.922	0.867	0.983	0.970	0.978
		100	0.918	0.939	0.902	0.983	0.952	0.983

* Significant at p=0.05 and below, r=0.923 at p=0.001.
Root 1 and 2 indicate root number in Table 2 and 3.

Table 5. Simple correlation coefficient between saponin patterns of two parts of *P. ginseng* root in relation to stem color

		Stem color (%)	Root 1(2)					
			Central part			Epidermis−cortex		
			10	50	100	10	50	100
Root 1 (2)	Central part	10	—	0.971**	0.959**	0.856	0.780	0.944**
		50	0.914	—	0.984**	0.881	0.847	0.938*
		100	0.926**	0.997**	—	0.832*	0.786*	0.889
	Epidermis −Cortex	10	0.895	0.726*	0.705*	—	0.962**	0.972**
		50	0.926**	0.813*	0.806*	0.938**	—	0.915*
		100	0.964**	0.855	0.848	0.930**	0.982**	—

** Significant at p=0.001, * Significant at p=0.05 or below.
Root 1(2): indicates root number in Table 2 and 3.

(underlined in Table 1) than that between roots with different color intensity.

The same trend is shown in Table 5 in which pattern similarity is compared among the same replicate. Similarity between two parts of the same root (underlined in Table 5) is less than that between the same parts of different roots (upper half of left and lower half of right in Table 5). Pattern similarity of saponin between two parts of ginseng roots in relation to stem color may be clearly elucidated by Fig. 2.

From the above results it could be concluded that in main body of ginseng root the difference of saponin pattern is greater between different parts than between the same parts regardless of stem color. Thus an experienced analyzer can distinguish only the part but the origin of part according to of HPLC chromatogram saponin pattern as shown in Fig. 3.

The order of each ginsenoside content in the epidermis−cortex is $Rg_1 > Re > Rb_1 > Rb_2 \geq Rc > Rg_2 \geq Rd > Rf$ (grand mean of Table 1) while that in the central part is $Rg_1 > Re \geq Rg_2 \geq Rb_1 \gg Rb_2 > Rc \geq Rd > Rf$ (grand mean of Table 2). In the central part the order of ginsenoside content is more variable due to little difference in content among Re, Rg_2, Rb_1 and also between Rc and Rd. But in the epidermis-cortex the order is farely constant with gradual decrease as shown in Fig. 4.

Total saponin content of root was reported in relation to age[5] various parts,[1,5,9,12,13] and

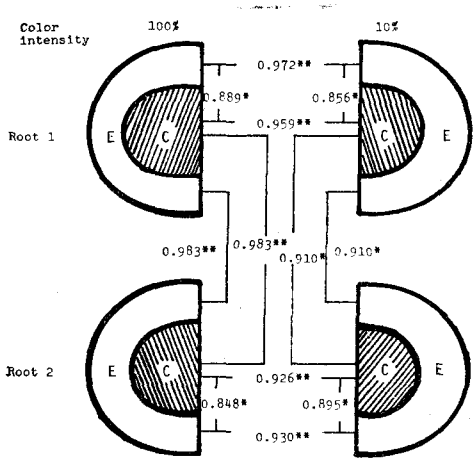

Fig. 2. Saponin pattern similarity between parts of *P. ginseng* roots with 10 or 100% of purple colored region on stem.
C : Central part(inside of cambium), E : Epidermis plus cortex.
**, * : Significant at p=0.001 and p=0.01 respectively.

seasonal change[6]. Quantitative analysis of saponin pattern is, however, very rare and hardly found in various parts of main body which has been used as the best quality portion.

Saponin pattern of whole root will depend on the ratio of two parts. Since stem color intensity is not associated with saponin pattern

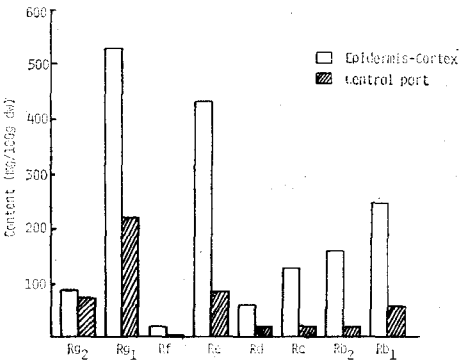

Fig. 4. Content of ginsenosides in two parts of *Panax ginseng* root.

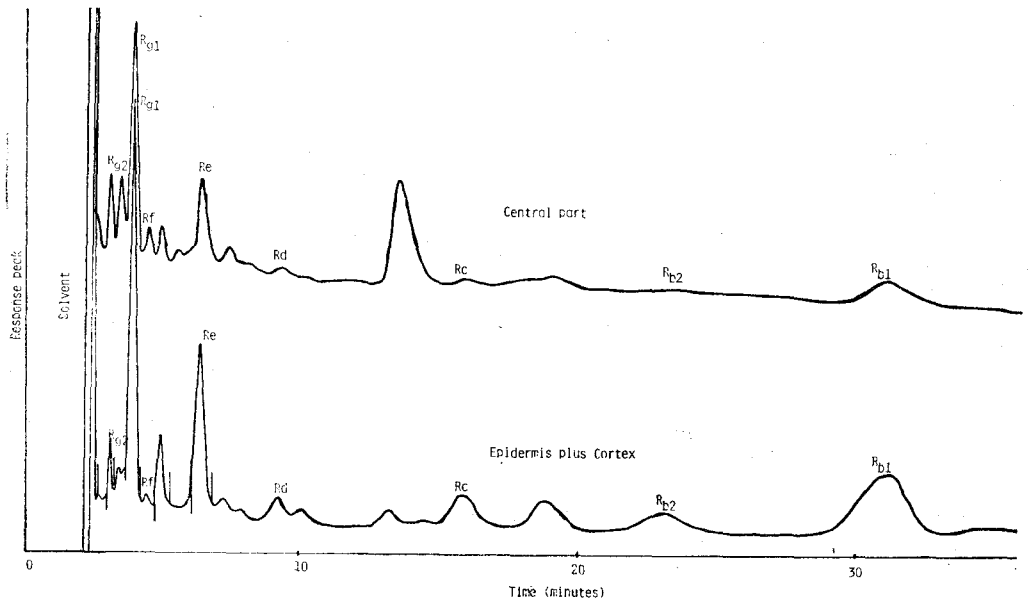

Fig. 3. Saponin patterns in central part and epidermis-cortex of *Panax ginseng* root(var. atropurpureacaulo) by HPLC.

in two parts if there is difference between saponin patterns of whole roots it will suggest that the ratio of two parts is associated with stem color intensity. The proportion of each part in study of saponin pattern for ginseng root seems very important.

Acknowledgement:

Authors thank Mr. J.H. Choi Korea Ginseng

Res. Inst. for generous supply of standard ginsenosides.

Abstract

Ginsenosides in two parts (central part and epidermis-cortex) of main body of Korea ginseng root (purple stem variety) were analyzed by high performance liquid chromatography in relation to purple color intensity on stem. Pattern similarity of saponin by simple correlation of ginsenosides between the same or different parts of root in the same or different group showed that stem color was not associated with saponin pattern in two parts.

Saponin pattern was slightly different between different parts regardless of stem color. The order of each ginsenoside content was $Rg_1 > Re > Rb_1 > Rb_2 > Rc > Rg_2 \geq Rd > Rf$ in epidermis-cortex while $Rg_1 > Re \geq Rg_2 \geq Rb_1 \gg Rb_2 > Rc \geq Rd > Rf$ in central part.

Literature cited

1. Bae, H.Y.: Korean Ginseng. Korea Ginseng Research Institute (1980)
2. Okuda, H.: Proceedings of the 2nd Internatl. Ginseng Symp. 75, Korean Ginseng Res. Inst. (1978)
3. Okuda, H. and Yoshida, R.: Proc. 3rd Internatl. Ginseng Symp. Korea Ginseng Res. Inst., in Press (1980)
4. Park, H.: Proc. of the 3rd Internatl. Ginseng Symp. Korea Ginseng Res. Inst., in Press (1980)
5. Lee, C.H. and Nam, K.Y.: J. Korean Food Sci., 10 : 263 (in Korean) (1978)
6. Kim, S.K., Sakamoto, I., Morimoto, K., Sakata, M., Yamasaki K., and Tanaka, O.: Proc. 3rd Internatl. Ginseng Symp. Korea Ginseng Res. Inst. in Press (1980)
7. Imamura, A.: Ginseng History Vols. 4 and 6. (in Japanese) (1939)
8. Namba, T., Yoshizaki, M., Tomimori, T., Mitsui, K. and Hase, J.: Yakugaku Zasshi, 94 : 252 (in Japanese) (1974)
9. Hong, S.K., Park, E.K., Lee, C.Y. and Kim, M.U.: Yakhak Hoeji, 23 : 181 (in Korean) (1979)
10. Park, H., Lee, J.S. and Lee, C.Y.: J. Korean Agric. Chem., 20 : 247(1977)
11. Soldati, F.: Proc. 3rd Internatl. Ginseng Symp. Korea Ginseng Res. Inst., in Press (1980)
12. Tani, T., Katsuki, T., Arichi, S., Kubo, M. and Okasaki, O.: Ginseng Seminar Abstract. 59. Japan (in Japanese). (1979)
13. Kim. M.U., Choi, K.J. and Cho, Y.H.: Ginseng Res. Rep. 375 Korea Ginseng Res. Inst. (in Korean). (1978)