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Characterization of Ginseng Extracts*

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Abstract—In order to establish the chemical standards for the quality control of ginsentg extract, an approach for the assay of sapogenin contents in the part of main roots and fibrous side roots was performed by combination of preparative thin layer chromatographic procedure and vanillin-H_2SO_4 color reaction. The contents of dammarane aglycones as funcction of dammarane glycosides in 80%-EtOH extracts were analyzed by the method from the main roots and fibrous side roots of Korean ginseng grown for 4—6 years. The differences by their grown ages in the contents of dammarane glycosides, in the ratio of panaxadiol to panaxatriol contents, and in the amounts of 80% EtOH extract were not significant in the parts of main roots and fibrous side roots of Korean gingeng. Differences due to the part for medicinal uses were highly significant in all parameters mentioned, showing following results; in the main roots; 80% EtOH extract, 12.7—15.7 %: the ratio of aglycone composition, 0.955—1.012: dammarane glycoside (as diglucoside bases), 1.537—1.863; in the fibrous side roots; 80% EtOH extract, 26.0--26.02%: dammarane glycoside, 4.767—5.641: the ratio, 1.465—1.50.

To the dammarane glycosides of Korean ginseng has recently been paid much attention, due to their pharmacological activities,[1-3] although the significances of sinergetic and/or potentiating activities of other ginseng components are by no means overlooked. Furthermore, the glycosides are distributed especially in the plants of Panax genus, hence the chemical standards for the quality control of Korean ginseng and of its preparations in combination with or without other crude drugs should be established on the bases of dammarane glycoside content.

Since the specific color reaction for the dammarane series has not been found, a nonspecific color reaction with vanillin-H_2SO_4, which is commonly available for the assay of steroids,[5] is adopted for the assay of dammarane glycoside content in the main roots and the fibrous side roots of Korean ginseng. Although the colorimetric method using vanillin-H_2SO_4

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reaction is not selective to the dammarane glycosides, the reaction will be applicable in combination with the preparative-TLC for the selective assay of the components in the Korean ginseng or in the ginseng preparations, avoiding the interference due to β-sitosterol, oleanolic acid, and their glycosides.

In the present study, a detailed procedure for the specific assay of dammarane glycosides in Korean ginseng is established. By employing the assay prodceure, total glycoside content, the ratio of panaxadiol to panaxatriol content were determined in the main roots and in the fibrous side roots grown 4—6 years, and the amounts of 80% EtOH extract were also measured.

EXPERIMENTAL

Apparatus—Recording Spectrophotometer, Shimadzu Model QV-50, were used for the measurements of UV-absorption spectra and of color density.

Materials—Fresh Korean ginseng grown 4—6 years (Kangwha products) were dried at 50° untill the loss of weight could not be checked and pulverized to medium particle size. Fibrous side roots were separated from main roots and treated by same process.

Reagents—8% vanillin solution: Vanillin (Eastman Co.) was rechrystallized twice from aqueous-acetone and dissolved in EtOH to give 8% solution. The solution was kept in air-tight brown bottle.

Standard Solutions—The authentic samples of panaxadiol, panaxatriol, and Panax Saponin A were dissolved in EtOH to give 0.2% solution.

Extraction of Ginseng—The well dried powder (10g) of Korean ginseng were extracted four times on a boiling water bath with 50ml of 80% EtOH for 5 hours. After filtration through a glass filter, the residues were washed with 30ml of 80% EtOH four times. The volume of combined extract was adjusted to 200ml. One half of the combined extract was used for the assay of sapogenin content and the other half was concentrated to dryness in vacuo. The sapogenin content and the amounts of 80% EtOH extract are illustrated in table V.

Hydrolysis of Saponins—In order to estimate the optimum condition for hydrolysis, ten ml samples of 1.5%-solution of Ginseng Neutral Saponins [6] (in EtOH) were hydrolyzed by refluxing on a water bath with the addition of ten ml of H_2SO_4 (50% alcoholic). The final concentration of H_2SO_4 and the time of hydrolysis are given in table I. At the end of given time of hydrolysis, the hydrolysate was diluted with 25ml—H_2O an d extracted with 50ml-portion of ether three times. The combined ether extracts were washed with 5% $NaHCO_3$ and H_2O, dried with Na_2SO_4 and evaporated to dryness. The resulting residues were dissolved quantitatively in 5 ml of EtOH and the solutions were subjected to the preparative-TLC for the assay of panaxadiol and panaxatriol. The results are summarized in table III.

For the assay of sapogenin contents in Korean ginseng, the other one half of the com-

bined alcohol extract (100ml) mentioned was concentrated in vacuo to dryness and the resulting syrupy residue was dissolved in 25ml of EtOH. The EtOH solution was hydrolyzed by refluxing with 25ml of 10% H_2SO_4 for four hours. After the completion of hydrolysis, the hydrolysate was treated by the method mentioned above.

Preparative Thin Layer Chromatography of Sapogenins—Silica-gel G plates (20×5cm) of 0.3mm thickness were used. Each TLC samples of standard solutions of aglycones were chromatographed on a separate TLC plates. One hundred ul of TLC samples or standard solutions were applied quantitatively to form homogenous band by 100 ul-lambda pipet (Denmark) and developed 15cm by benzene-acetone (4 : 1) solvent. After drying, the chromatograms were exposed to the fine spray of H_2O and subsequently to the iodine-vapor. The resulted yellow bands of panaxadiol (R*f*: 0.45) and of panaxatriol (R*f*: 0.23) were collected quantitatively in 40ml-test tube, allowed to stand in the dark until the yellow color disappeared, and dried for one hour at 100°. The dried powder was used directly as the sample for color reaction.

Color Reaction[7]—The test tube with the powders of panaxadiol or panaxatriol bands were immersed into ice water bath. To the tube, one ml of EtOH and 0.4ml of 8% vanillin solution were added in the order and mixed well enough to make homogenous suspension. Ten ml of ice-cooled 72% H_2SO_4 was layered carefully with avoiding the agitation and mixed well all at once by shaking in an ice-water bath. The reaction mixture was heated ten min in a 60° water bath. After the completion of color development, the reaction tube was cooled in the ice water bath and the silica-gel powders were eliminated by centrifugation for ten min at 3000 rpm. The absorbance of supernatant was read at 550nm using absorption blank, which was prepared by same procedure with the equal amount of silica-gel powder.

RESULTS AND DISCUSSION

Colorimetric Procedure—Commercial product of vanillin should be rechrystallized before use, otherwise the absorption of blank was extra-ordinarily high, deviating the results from Lambert-Beer's rule. Every conditions of color reaction in experimentals should be kept strictly, since the final concentration of H_2SO_4, the heating temperature and the heating time affect the results critically. The color density at 550nm was stable within three hours at room temperature and was strictly dependent on the concentration of saponins or of aglycones. On account of slight deviation of experimental conditions, the slope of linear calibration curve was not fixed constant, hence the color reaction of standard should be performed simultaneously with that of samples.

Absorption Spectra—The color reaction of vanillin-H_2SO_4 reagent with triterpenoids did not show structure specificity in the given reaction condition. The absorption maximum of color showed at 550nm for both panaxadiol and panaxatriol, at 539nm for β-sitosterol and

at 538nm for oleanolic acid, hence the color density at 550nm would include a significant portion of absorption by oleanolic acid and β-sitosterol.

In order to assay the dammarane aglycones quantitatively, the aglycones should be purified by preparative thin layer chromatography. As shown in figure 1, panaxatriol produced the color of second absorption maximum at 470nm. However, a slight increase in the concentration of H_2SO_4 produced the disappearance of the second absorption maximum of panaxatriol, hence the peak will not be proposed as the diagnostic tool for the characterization of panaxatriol at present.

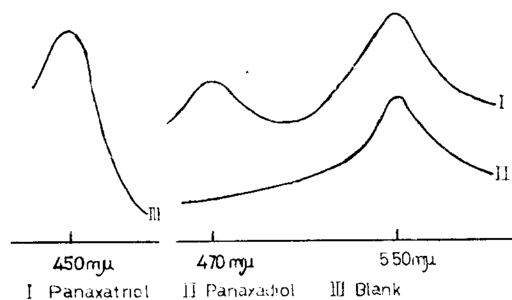

Fig. 1—Absorption spectra.

Quantitative Procedure of Preparative Thin Layer Chromatography—Thin layer chromatograms, which were developed by various solvent systems, were compared on their resolution of spots and on their R*f*-reproducibility, using the authentic sample mixture of panaxadiol, panaxatriol, oleanolic acid and β-sitosterol and the results are summarized in table 1.

Table I—TLC solvent and R*f*-value.

No.	Developing solvent		R*f*-values			
			P.D.	P.T.	Olean.	β-sito.
1.	$CHCl_3$-Et_2O	(1 : 1)	0.39	0.22	0.48	0.59
2.	$CHCl_3$-Et_2O	(4 : 1)	0.41	0.26	0.50	0.59
3.	$CHCl_3$-Et_2O	(1 : 4)	0.16	0.07	0.27	0.46
4.	Benzene-EtOAc	(7 : 3)	0.32	0.18	0.40	0.51
5.	Et_2O (saturated with H_2O)		0.45	0.28	0.69	0.71
6.	Benzene-MeOH	(95 : 5)	0.35	0.10	0.35	0.70
7.	$CHCl_3$-MeOH	(20 : 1)	0.48	0.24	0.42	0.63
8.	Benzene-Acetone	(4 : 1)	0.45	0.23	0.65	0.83

Samples: Panaxadiol(P.D.), Panaxatriol(P.T.), Oleanolic acid(Olean.), and β-sitosterol (β-sito.) in $CHCl_3$.

Solvent No. 1, 5, and 8 showed good separation of the four components on the thin layer chromatogram. In case of preparative separation of the components from the hydrolysate of the extract, however, benzene-acetone (4 : 1) solvent was most suitable. As described in experimentals, the bands of panaxadiol or panaxatriol on the thin layer chromatogram were collected and subjected directly to the color reaction, eliminating the solvent extraction process of the compounds from Silica-gel powder. By using the procedure, the linearity of the calibration curve and the maximum recovery of the components were secured strictly in the preparative TLC process. Alternate procedure, in which the components for assay were extracted by solvents from the silica-gel powder before color reaction, was also examined on its linearity of calibration curve and on the recovery of substances.

Table II—The effect of alcohol concentration on the extraction of ginseng components.

Ethanol concn. %(V/V)		The amount of extract and colour density at 550mμ (No. of extraction)				
		1st.	2nd.	3rd.	4th.	Total
95	a	3.548	0.772	0.276	0.171	4.767
	b	0.857	0.2292	0.1498	0.081	1.317
80	a	4.511	0.709	0.171	0.073	5.464
	b	1.022	0.310	0.129	0.072	1.535
60	a	4.321	0.780	0.169	0.073	5.343
	b	1.400	0.426	0.189	0.075	2.090
40	a	4.163	0.880	0.230	0.089	5.362
	b	1.415	0.449	0.210	0.075	2.149

a : Colour density of extract when it was treated with vanillin-H_2SO_4 reagent (absorbance/1ml. alcohol extract)

b : Amount of alcohol extract desiccated/10gm ginseng.

Later process made the overall process unnecessarily complicate and increased rather the experimental error than the former process. As shown in table IV, the recovery of standard samples was practically 100% by the former process.

Extraction of Ginseng Root—The effect of alcohol concentration was examined on the efficiency of extraction of ginseng components. Ginseng was extracted four times by the procedure in experimentals using EtOH of various concentrations ranging from 40 to 95% for four times. An aliquot of each extract was subjected directly to the color reaction of vanillin-H_2SO_4 method and the remainders were concentrated to dryness in vacuo. The absorbance at 550nm and the weight of each extract are summarized in table II.

As shown in table II, the sums of absorbances or the sums of extract-weights were progressively increased by decreasing the alcohol concentration. However, dilution of alcohol concentration under 40% caused a significant swelling of ginseng power to impede the filtration procedure. From the results, it may be said that 60—80% is most adequate in

Table III—Recovery of aglycones on various hydrolysis condition.

Concentn. of H_2SO_4 %(w/w)	Time of hydrolysis hours	Absorbance at 550mμ** Panaxadiol	Panaxatriol
2.5	3	0.400	0.186
2.5	6	0.492	0.260
5.0	3	0.546	0.254
5.0	4	0.680	0.396
10.0	3	0.47	0.182
10.0	2	0.54	0.255
20.0	3	0.126	0.126
20.0	1	0.48	0.250

* Sulfuric acid was diluted with 50% aqueous alcohol.
** The aglycones were purified by preparative thin layer chromatography.

the extraction of ginseng for the analitical purpose.

Hydrolysis of Saponins—In order to estimate the optimum condition for hydrolysis, Ginseng Neutral Saponin mixture was hydrolyzed for 1—6 hours with H_2SO_4 of various concentration. After purification of the resulting aglycones by preparative thin layer

Table IV—Calibration data of overall assay procedure.

Samples[a] (μg.)	Absorbance (550mμ)	Found (μg)	Molecular yield of sapogenins
Panax Saponin A			
400	0.251	64.7	27.2%
820	0.504	131.4	26.93
1232	0.793	206.8	28.2
Panaxatriol			Average; 27.44±0.54
200	0.767	200.0	
Prosapogenin			
400	0.20	68.1	29.0
830	0.41	139.7	28.74
1250	0.61	207.8	28.33
Panaxadiol			Average; 28.69±0.28
200	0.587	200.0	

a : Fifty-fold amounts of samples were hydrolized by the method given in experiment. After the completion of hydrolysis, 100μl of aglycone solutions corresponding to the given amount of glycosides were analyzed through the preparative TLC-process in combination with the vanillin-H_2SO_4 color reaction.

b : Standard samples of panaxadiol and panaxatriol were treated simultaneously, however, the hydrolizing process was omitted.

c : Molecular yield of sapogenin was calculated based on their molecular formula: One mol of Panax Saponin A(M; 800)contains one mol of panaxatriol(M; 476) and one mol of prosapogenin (M; 784) contains one mol of panaxadiol(M; 460).

chromatography, the yield of panaxadiol and panaxatriol were compared by the absorbances of the vanillin-H_2SO_4 color reaction.

As shown in table III, the maximum yield of aglycones was attained in the test with 5%H_2SO_4-4 hour-hydrolysis. Under the conditions, the spots of glycosides unhydrolyzed were practically absent. Adopting the condition as standard process, the molecular recovery of aglycones in the process of hydrolysis were examined for both series of glycosides, of panaxadiol and panaxatriol series.

As shown in table IV, the molecular yield of aglycones under the given condition of hydrolysis are; 27.44% for panaxatriol from Panax Saponin A (Protopanaxatriol-diglucoside), and 28.69% for panaxadiol from Prosapogenin (Protopanaxadiol-diglucoside).

Table V—The amount of 80% alcohol extract and the sapogenin contents as function of panax saponins in Korean ginseng.

Ginseng: Part for use and ages	Amount of 80% alcohol extract (%).	Sapogenin content[a] found(%)		Glycoside content[b] (%)			Ratios	
		P.T.	P.D.	P.P.T.-diglucoside.	P.P.D.-diglucoside.	Total	Total glycoside /Amount of extract	P.D./P.T.
Main Root:								
6-years	12.71	0.130	0.124	0.803	0.734	1.537	0.121	0.955
4-years	15.74	0.153	0.155	0.942	0.921	1.863	0.118	1.012
Side Root:								
6-years	26.0	0.380	0.556	2.341	3.30	5.641	0.217	1.465
4-years	26.02	0.316	0.474	1.947	2.82	4.767	0.183	1.500

a : Sapogenin contents in table do not denote the actual sapogenin contents of Korean ginseng, since the degradation of sapogenins during acid hydrolysis of glycosides was not considered in the calculation of the sapogenin contents.

b : Glycoside content in table was calculated from the sapogenin content(found) by following equation.

$$\text{Glycoside content} = \frac{\text{Sapogenin content} \times 100}{\text{Recovery of aglycones}} \times \frac{\text{M.W. of P.P.T. or P.P.D.-diglucoside}}{\text{M.W. of P.T. or P.D.}}$$

Abbreviations; P.T.; Panaxatriol (M.W. 476), P.D.; Panaxadiol (M.W. 460), P.P.T.-diglucoside; Protopanaxatriol diglucoside (M.W. 800) P.P.D.-diglucoside; Protopanaxadiol diglucoside (M.W. 784)

In order to estimate the real content of aglycones or glycosides from the experimental values a working hypothesis is necessary in which the molecular recovery of aglycones may be fairly constant for all glycosides in ginseng irrespective of diversity on their glycoside structures.

Dammarane Glycoside Contents in Korean Ginseng—In the present study, the contents of dammarane glycosides in Korean ginseng were analyzed on the samples of 4—6 year grown ages and on the samples of fibrous side roots and the results are summarized in table V.

As shown in table, the differences in the dammarane glycoside contents and in the ratio of panaxadiol to panaxatriol content by the grown age of ginseng were not significant within 4-6 year grown samples. Compareing the same parameters with those of fibrous

side roots, both the dammarane glycoside content and the ratio of panaxadiol to panaxatriol content are higher in fibrous side roots than in the main roots. These facts will be utilized as the diagnostic tools for the characterization of main roots and fibrous side roots in their extract level.

CONCLUSION

1. A fairly accurate procedure for the specific assay of dammarane glycoside content in ginseng is formulated by combination of preparative thin layer chromatographic purification and vanillin-H_2SO_4 color reaction.

2. The ratio of panaxadiol to panaxtriol content was significantly different by the part for medicinal uses of Korean ginseng. This fact will be utilized as the diagnostic tool for the characterization of side root and of main root at their extract level.

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