Journal of the Pharmaceutical Society of Korea
21, 163-166 (1977)

Studies on the Analysis of Dammarane Aglycones of Korean Ginseng

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(Received July 5, 1977)

Abstract—To establish a convenient quantitative method for dammarane glycosides in Korean ginseng, the ginseng roots harvested at the cultivation areas of Ga Pyeong, Geum San and Jeung Pyeong were dried, powdered, extracted with methanol and hydrolyzed. The ginseng root obtained at Gang Hwa was divided into three parts: main root, lateral root and cortex, and then these were treated in the same manner as the above. The various hydrolysates thus obtained were subjected to the analysis by an instrument coupled with flame ionization detector. The results showed that panaxadiol contents in the ginsengs of the three different cultivation sites were similar. However, the lateral root of Gang Hwa ginseng was found to contain the largest amount of panaxadiol among the three parts of ginseng. This method of the analysis for panaxadiol in ginseng was found to be one with relative rapidity and ease.

Ginseng has long been utilized as an excellent drug for health in Asia. Especially Korean ginseng has been known as the best of various ginsengs. The fact that ginseng has been extensively investigated recently by many scientists indicates a world-wide attention to ginseng. Although ginseng is one of the major drugs exported from Korea, there is no adequate and convenient method for quality control of various ginseng preparations. The authors made attempts to establish a quantitative method for dammarane glycosides which are considered to be the main effective constituents of ginseng.

Woo *et al.* reported a spectrophotometric method using preparative TLC as a means for separating dammarane aglycones and vanilin-sulfuric acid as coloring reagent[1]. Sakamoto *et al.* presented a gas chromatographic method for quantitative determination of trimethylsilylated panaxadiol and panaxatriol after hydrolysis of ginseng saponins with dilute mineral acid.[2] For analysis of the saponins, Namba *et al.* reported a quantitative TLC method

utilizing a new apparatus equipped with a flame ionization detector.[3~4] Analyses of saponins in ginseng tissue cultures were shown by Furuya *et al.* and Jhang *et al.*, respectively.[5~6] Hiai *et al.* recently published an estimation method of ginseng saponins using vanillin and sulfuric acid.[7~9]

The authors made methanol extracts of the three different parts of Gang Hwa ginseng and of the ginseng roots collected at three areas of cultivation in Korea. This paper reports the results of thinchrography of the hydrolysates of these methanol extracts.

EXPERIMENTAL

Materials—To compare the constituents of the ginseng roots of three different areas of cultivation, four-year-old ginseng roots were harvested at the farms of Geum San, Ga Pyeong and Jeung Pyeong. These roots were found to contain about 12% moisture.

Six-year-old ginseng roots of Gang Hwa area were harvested, washed, dried and divided into three parts: lateral root, cortex and main root. These were used for the experiments.

Instruments—Thinchrograph, model TFG-10 type(K.K. Iatron, Japan) and a recorder, model 056-4019 (Hitachi, Japan) were used in the analysis.

Extraction—Ginseng samples were powdered, placed into a flask with methanol (30 ml to two g of the sample), and refluxed on a water bath for five hours. This prodedure was repeated thrice and the extractives were combined and evaporated under reduced pressure.

Hydrolysis—The above extractive was dissolved in 30 ml of 50% ethanol and filtered. To the filtrate, were added 30 ml of 10% sulfuric acid and the mixture was refluxed for six hours. After ethanol was evaporated, distilled water was added and the mixture was extracted with ether for three times. After the ether extractive was washed with 30 ml of 5% sodium hydroxide solution, it was washed again with 30 ml of distilled water. The moisture in the ether extractive was removed by adding small quantity of anhydrous sodium sulfate. Then the extractive was evaporated and the residue was dissolved in three ml of absolute ethanol for use in the experiments.

TLC—The plates with silica gel G(Merck Co.) were activated by keeping them at 120°C for one hour and cooled in a desiccator. Five μl of the sample solution were spotted on the plate and it was developed in a system of solvents and sprayed with 50% alcoholic sulfuric acid.

Solvent system—The examination of different ratios of several solvents revealed that a mixture of benzene : acetone(4 : 1) gave the best result in separating sapogenins.

Detection by thinchrograph—After one μl of the sample solution was spotted on a synchrod with a microsyringe, it was dried and developed in a developing chamber containing a solvent mixture of benzene: acetone (4 : 1). When the solvent was removed by drying, it was subjected to the flame ionization detector to obtain the curves on the graph. The quantities of the sapogenins were calculated by using the calibration curve of the peak area.

Preparation of calibration curve—Standard panaxadiol (3. 167 mg) was dissolved in two ml of absolute ethanol and one ml of the solution was spotted on synchrods with a micro-syringe and after the development they were subjected to the flame ionization detector to obtain the peaks.

RESULTS AND DISCUSSION

The results obtained by using the flame ionization detector in analyzing the hydrolysates of ginseng constituents showed that this method detected more spots than that of spraying sulfuric acid on TLC plates(Fig. 1), indicating that thinchrography seems more effective than the ordinary TLC method in analyzing ginseng constituents.

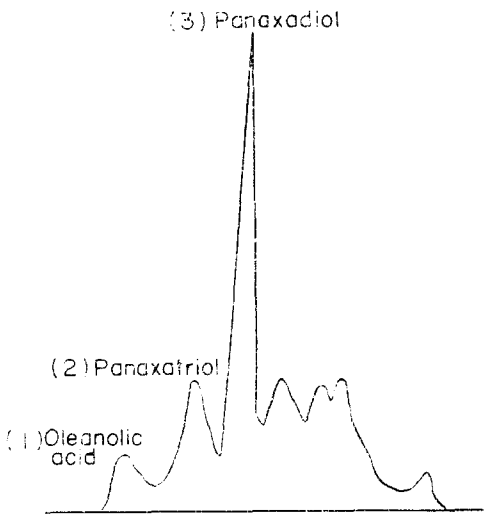

Fig 1—Quantitative thinchrogram of the hydrolysates of the methanol extract of Jeung Pyeong ginseng.

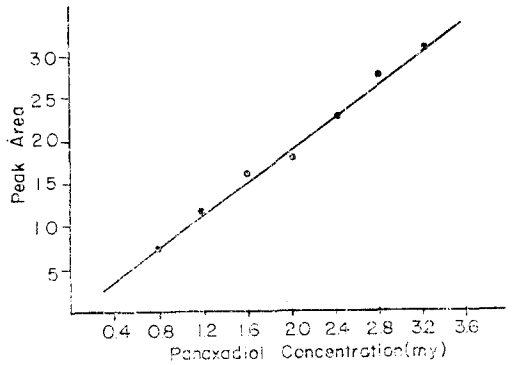

Fig. 2—Calibration curve of panaxadiol concentration and peak area by thinchrograph.

Table I—The amount of panaxadiol detected from MeOH extract hydrolysate of the ginsengs harvested at three cultivation areas

Cultivation Area	Ga Pyeong	Geum San	Jeung Pyeong
Panaxadiol(mg/2 g)	2.88	2.85	2.53

Table II—The amount of panaxadiol detected from MeOH extract hydrolysate of the three different parts of ginseng

Part used	Loteral root	Cortex	Main root
Panaxadiol(mg/2g)	294	0.75	0.36

Among the five systems of developing solvents, a mixture of benzene : acetone (4 : 1) was found to be effective one for separating the constituents.

As shown in Table I the contents of panaxadiol in the roots which were collected at the three different areas of cultivation showed no significant difference. However, panaxadiol was always found to be the major aglycone among the five constituents in the hydolysates of all the three ginsengs. The panaxadiol contents in the three parts of the Gang Hwa root varied noticeably as shown in Table II. That is, the lateral root contained the largest quantity, the cortex the next, and the main root the least among the three

parts.

Comparison of the peaks on the thinchrogram with those of the standard panaxadiol showed that the third peak is that of panaxadiol and that the second appears to be that of panaxatriol. Woo *et al.* determined the panaxadiol and panaxatriol contents in the main and side roots of ginseng.[1] Their results showed an identical tendency in the panaxadiol contents with that of ours. The results which the authors obtained are also in agreement with those of Namba *et al.*[3-4]

The calibration curve for standard panaxadiol was shown to be a straight line. Although these results can be applied for the quantitative analysis of ginseng constituents, additional data on panaxatriol would be necessary, since the panaxadiol contents differed depending upon the part used. However, the fact that panaxadiol was always the major aglycone in the ginsengs suggests that it may be useful as an indicator compound of dammarane glycosides in ginseng.

CONCLUSION

1. The panaxadiol contents in the ginseng roots which were collected at the three cultivation areas of Ga Pyeong, Geum San and Jeung Pyeong showed no significant difference.

2. The panaxadiol contents in the three parts of Gang Hwa ginseng root showed that its lateral root contained the largest quantity, that its cortex the second, and that its main root the least among the three parts.

These results indicate that the hydrolysate of the methanol extract of ginseng can be quantitatively determined by thinchrography with relative ease.

Acknowledgments—This study was supported in part by Tae Pyeong Yang Cultural Foundation in Seoul and we acknowledge with gratitude the grant. We wish also to express our thanks to Prof. Won Sick Woo, Director of the Natural Products Research Institute, Seoul National University, for supplying panaxadiol standard.

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