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Rapid Hydrolysis of Ginseng Saponin by Microwave Oven Reaction: Application to Ginseng Drink Preparation

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INTRODUCTION

Various methods have been reported for the analysis of ginsenosides which include colorimetry (Woo *et al.*, 1973), HPLC (Besso *et al.*, 1979; Sakamoto *et al.*, 1975), ion chromatography (Park *et al.*, 1994), GC (Bombardelli *et al.*, 1980; Park *et al.*, 1991), and radioimmunoassay methods (Han and Han, 1981). Among these techniques, HPLC and GC methods have been widely accepted. In GC method all dammarane-type saponins are hydrolyzed to panaxadiol (PD) and panaxatriol (PT) followed by trimethylsilylation (TMS) and analyzed by GC using nonpolar stationary phase and flame ionization detector (FID). Since all dammarane-type saponins of ginseng appear in two peaks of PD-TMS and PT-TMS, the sensitivity is greatly improved and quantitation is less interfered by other compounds, which makes this method applicable to the analysis of complex mixture or low ginseng content preparations. The drawback of this method is that the hydrolysis of saponin takes a long time, typically 5.5 hours (Park *et al.*, 1991).

Recently we have reported microwave method for the hydrolysis of ginsenosides using home-made PTFE (polytetrafluoroethylene or Teflon) reaction vessel (Park *et al.*, 1993). The hydrolysis reaction was completed within 10 minutes with this method. However the reaction vessel used was too small, ca 200 μl, to apply it to the pharmaceutical whose ginseng contents is very low. In this communication, we report larger reaction vessel which enables hydrolysis of ginsenosides in ginseng drink preparation without sample pretreatment.

The reaction vessel used in this work was purchased from Parr Ins. Co. (USA) (Fig. 1). Other reagents and instruments including microwave oven and GC were the same as those of the pervious report (Park *et al.*, 1993). The typical analytical procedure is as follows.

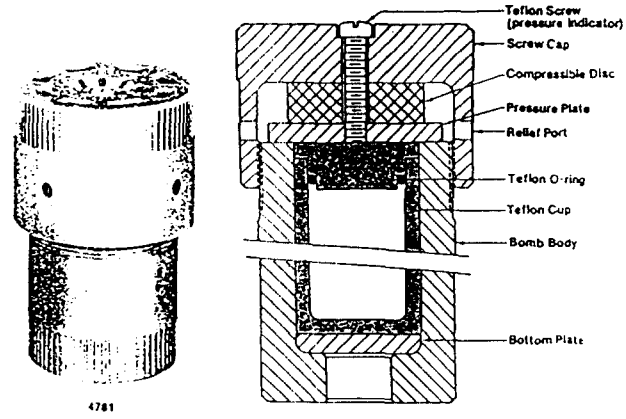

Fig. 1. PTFE reaction vesssel (23 ml, Parr).

The butanol fraction of white ginseng (50 mg) was dissolved in 10 ml of 5% H_2SO_4 in water and ethanol mixture ($H_2O : C_2H_5OH = 3 : 1$), and this solution was put in PTFE reaction vessel. The vessel, which was tightly closed, was placed in a microwave oven. The microwave reaction time was varied from 30 to 110 sec with 10 sec intervals. Immediately after the reaction, the vessel was cooled down in ice-water (Caution: The vessel should be completely cooled before opening it to prevent possible explosion). The reaction mixture was transferred to evaporating flask. 50 μl of the internal standard solution (50 mg of cholesterol in 25 ml of dichloromethane) was added and the solution was evaporated to dryness under reduced pressure. 4% NaOH (1 ml) was added to the residue and the solution was extracted with 10 ml of CH_2Cl_2 three times. Combined CH_2Cl_2 layer was backwashed with 4% NaOH (10 ml) and with distilled water (10 ml) twice to make aqueous layer neutral. CH_2Cl_2 layer was dehydrated with anhydrous sodium sulfate and transferred to reaction vessel for trimethylsilylation. The solvent was removed under the stream of nitrogen followed by the addition of 50 μl of pyridine and 50 μl of silylating reagent (HMDS : TMCS = 2 : 1). The solution was incubated for 40 minutes at 80℃, which was

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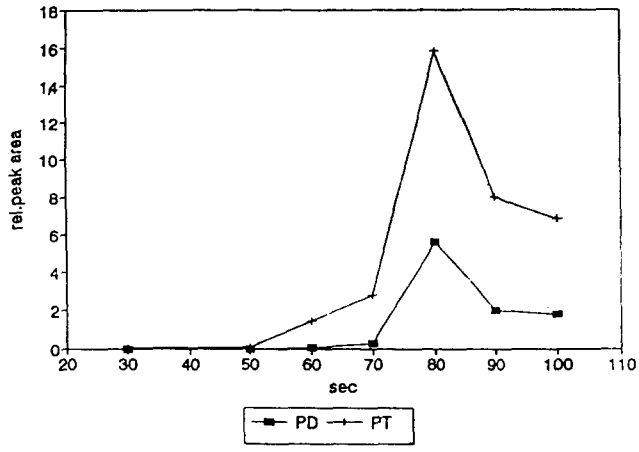

Fig. 2. Effect of hydrolysis time of white ginseng extract.

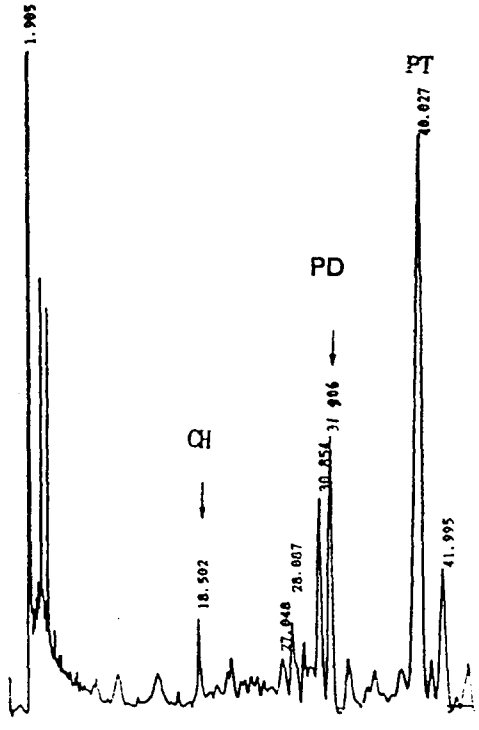

Fig. 3. Gas chromatogram of white ginseng butanol fraction; reaction for 80 sec; CH (cholesterol, internal standard), PD (panaxadiol), PT (panaxatriol); Ultra-1 fused-silica capillary column (0.32 mm i.d.×12 m, Hewlett-Packard), 280℃ (isothermal), injector temperature: 290℃, detector temperature : 290 ℃, injection volume: 1 μl, carrier gas: N_2 (μ=10.2 cm/sec) and split ratio(50:1).

subjected to GC analysis. The conditions of GC analysis were as follows: Ultra-1 fused-silica capillary column (0.32 mm i.d.×12 m, Hewlett-Packard), column temperature: 280℃ (isothermal), injector temperature: 290° C, detector temperature: 290℃, injection volume: 1 μl, carrier gas N_2 (μ=10.2 cm/sec) and split ratio of 50 : 1.

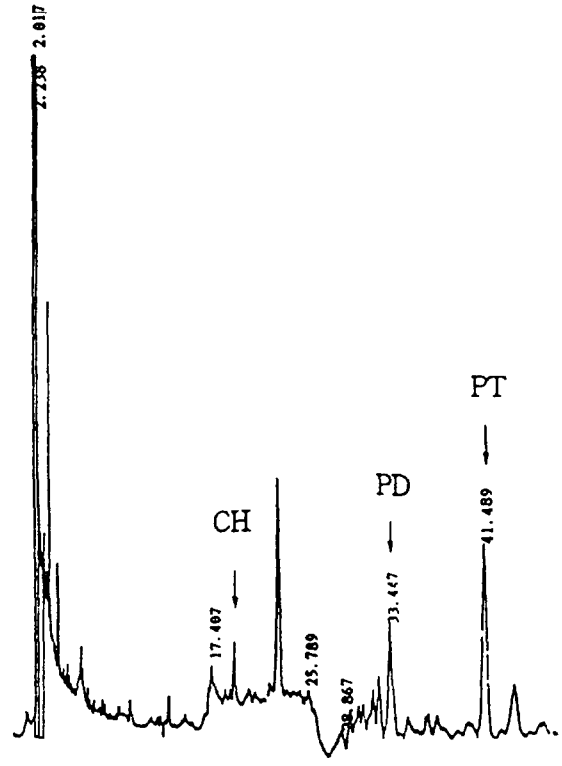

Fig. 4. Gas chromatogram of Ginseng drink preparation; reaction for 70 sec; CH (cholesterol, internal standard), PD (panaxadiol), PT (panaxatriol); Analytical conditions are the same as those in Fig. 3.

For the analysis of ginseng drink preparation, 5 ml of the preparation, equivalent to 30 mg of raw ginseng, was added to 5 ml of 10% H_2SO_4 in water and ethanol mixture to make $H_2O : C_2H_5OH = 3 : 1$ in solution. This solution was treated with the same method as white ginseng extract. Labelled constituents in 100 ml of ginseng drink preparation were as follows: 600 μl of ginseng fluid extract from 600 mg of white ginseng, 50 μl of epidemii fluid extract, 7 mg of ethyl parahydroxybenzoate, 3 mg of propyl parahydroxybenzoate, 60 mg of sodium benzoate, 5 mg of riboflavin sodium phosphate, 10 mg of tocopherol acetate, 20 mg of nicotinamide and 5 mg of pyridoxine hydrochloride. The conditions of GC analysis were the same as those of the white ginseng extract.

The relationship between hydrolysis time and intensities of PD-TMS and PT-TMS is given in figure 4 and 5. The peak intensity increased with the increase of hydrolysis time and showed maximum at 80 sec for white ginseng extract and at 70 sec for ginseng drink preparation. The rate of hydrolysis in this reaction vessel was much faster than that of previous report (Park *et al.*, 1993). This might be attributable to the structure of the reaction vessel. Since the vessel was larger than that used in the previous report, the microwave energy

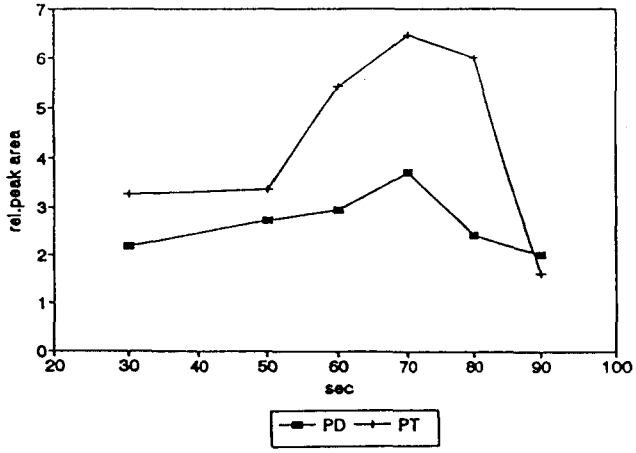

Fig. 5. Effect of hydrolysis time of ginseng drink preparation.

absorbed by the sample solution increased, which resulted in the rapid hydrolysis of ginsenosides. Figure 3 and 4 show the gas chromatograms of white ginseng extract and ginseng drink preparation, respectively. Correlation coefficient of calibration curve of ginseng extract was 0.977. However that of standard ginsenoside Rg_1 treated with the same method was 0.999. The linearity of the calibration curve of the ginseng extract was lower than that of the ginsenoside standard.

The hydrolysis rate in described method was over 250 times faster than the conventional method. Described method was applicable to the hydrolysis of ginsenosides in ginseng drink preparation.

The temperature and pressure generated within a microwave reaction vessel are dependent upon the solvent, the sample level, the length of exposure and power settings. Therefore, one must follow microwave digestion manual (Parr Ins. Co., USA; Kingston and Jessie, 1988; Park *et al.*, 1993).

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