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Rapid Hydrolysis of Ginseng Saponin by Microwave Oven Reaction

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Abstract□A new and rapid method for the hydrolysis of ginsenosides to panaxadiol or panaxatriol was developed. It is based on the microwave oven reaction, which is high temperature and high pressure reaction. The optimal hydrolysis time using 5% H_2SO_4 solution was found at 10 min with PTFE reaction vessel in microwave oven, which is more than 30 times faster than the conventional hydrolysis method.

Key words□*Panax ginseng*, analysis of ginsenoside, microwave oven reaction, hydrolysis of ginsenoside, PTFE reaction vessel, GC analysis

Introduction

Various methods have been reported for the analysis of ginsenosides which include colorimetric,[1] HPLC,[2-5] GC,[6] and radioimmunoassay methods.[7] Among these techniques, colorimetric method has low selectivity, and diol- and triol-type ginsenosides cannot be quantitated individually by this method. Although some saponins can be quantitated by HPLC method, it is still difficult to analyse all saponins simultaneously, and the sensitivity of detection restricts its applicability to the microanalysis.

On the other hand, in GC method all ginsenosides are hydrolyzed to panaxadiol(PD) and panaxatriol(PT) as shown in Fig. 1 followed by trimethylsilylation(TMS) and analysis by GC using nonpolar stationary phase and flame ionization detector(FID). Because 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 to sapogenin takes a long time, typically 5.5 hours.[8]

In this study, we reduced the hydrolysis time by using polytetrafluoroethylene (PTFE or Teflon) vessel and microwave oven reaction.

Experiment

1. Materials and Reagents

The ginseng extract was provided by ILYANG Pharm. Co. Trimethylchlorosilane(TMCS) and hexamethyldisilazane(HMDS) were the products of Nakarai Chemical Co. All reagents used in this experiment were extra pure grade.

2. Apparatus and Instrument

The commercial microwave oven for household (Gold Star model ER-646JFK, 650W, GoldStar Co. Korea) and homemade PTFE reaction vessel (Fig. 2) were used for the hydrolysis reaction. GC was performed on Hewlett-Packard 5890 series II. Hewlett Packard 3394 A integrator or IBM-PC compatible computer with homemade software was used to record or process the chromatogram.

3. Optimization of the Hydrolysis Time

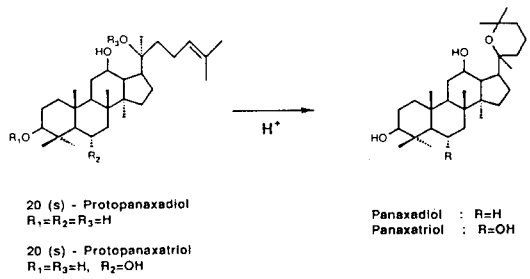

Fig. 1. Hydrolysis of ginsenosides.

The ginseng extract(100 mg) was dissolved in 1 m*l* of 5% H_2SO_4 in water and ethanol mixture (H_2O : C_2H_5OH=3 : 1), and 150 μ*l* of 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 1 to 12 mins with 1 min 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. 70 μ*l* of the internal standard solution(100 mg of chloesterol in 50 m*l* of chloroform) was added and the solution was evaporated to dryness under reduced pressure. 4% NaOH(10 m*l*) was added to the residue and extracted with 15 m*l* of CH_2Cl_2 three times. Combined CH_2Cl_2 layer was backwashed with 4% NaOH(15 m*l*) and with distilled water(15 m*l*) twice to make aqueous layer neutral. CH_2Cl_2 layer was dehydrated with sodium sulfate anhydrous and transfered to reaction vessel for trimethylsilylation. The solvent was removed under the stream of nitrogen followed by the addition of 50 μ*l* of pyridine and 50 μ*l* of silylating reagent (HMDS : TMCS=2 : 1). The solution was incubated for 40 minutes at 80℃, which was subjected to GC analysis. The conditions of GC analysis were as follows: Ultra 1 fused silica capillary column(0.32 mm i.d., 25 m, Hewlett-Packard), column temperature: 280℃ isothermal, injector: 290℃, FID: 300℃, sample size: 1 μ*l*, carrier gas: N_2(μ=2 cm/sec), split injection.

4. Caution in Microwave Oven Reaction[9]

Before starting microwave oven reaction, one must fully understand the potential hazards of the microwave oven reaction. 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. The most important hazrd is explosion of the reaction vessel by overheating. When overheated the vapor pressure of the solvent or solution in the vessel increases dramatically with the pressure, and the strength of the PTFE material is reduced. Since PTFE is an excellent thermal insulator, the internal heat will be tranlated into higher internal temperature and pressure. Occasionally this leads leak or explosion of the PFTE reaction vessel, usually the cap is released from the body with explosion, however microwave oven was safe in our experience. Usually explosion was observed when the long-used reaction vessel is overheated. The heating time for this reaction vessel should never exceed 20 minutes.

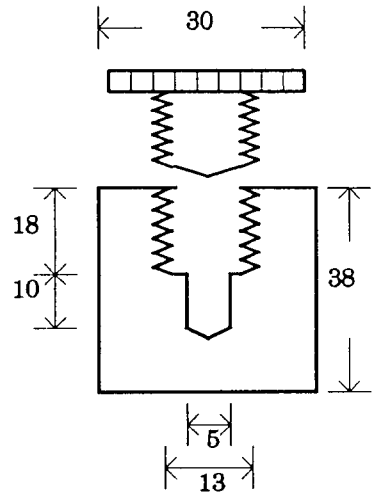

Fig. 2. PTFE reaction vessel(mm).

Always be sure that all parts, especially screw part, are clean and dry before assembly to prevent localized heating. The vessel must be completely cool to the touch before attempting to open. This is a slow process due to the insulating nature of the PTFE.

Results and Discussion

1. The Analysis of Standards by GC

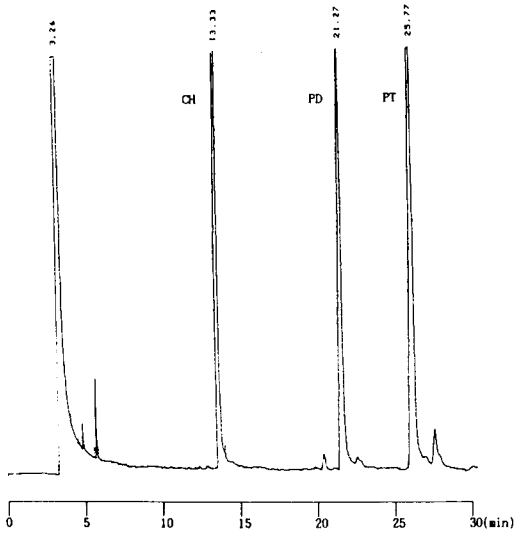

Fig. 3. Chromatogram of standard PD-TMS, PT-TMS and cholesterol-TMS.

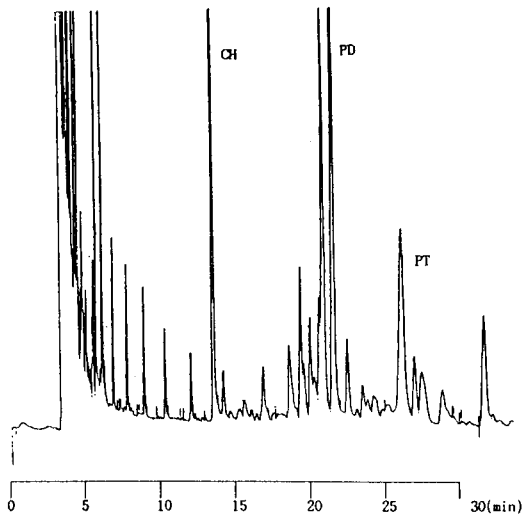

Fig. 5. Chromatogram of trimethylsilylated hydrolysates of ginseng extract.

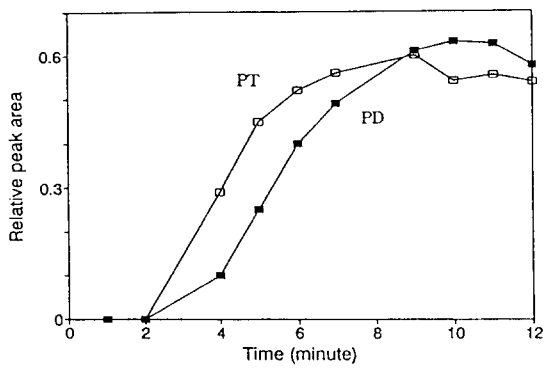

Fig. 4. Effect of hydrolysis time of ginseng extract using PTFE reaction vessel.

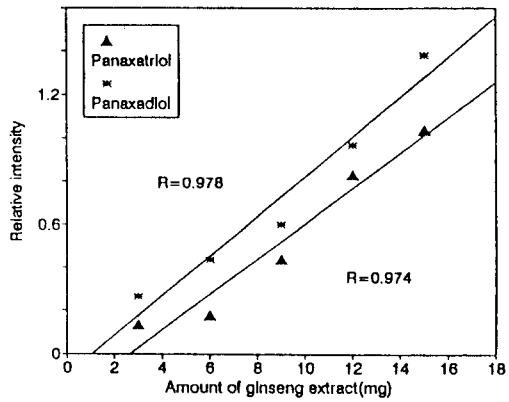

Fig. 6. Calibration curves of ginseng extract.

Fig. 3 is the gas chromatogram of TMS-ethers of cholesterol(CH, internal standard), panaxadiol (PD), and panaxatriol(PT). The relative retention times of PD and PT to cholesterol was 1.60, 1.93, respectively, which were very close to those reported using OV-101 column(1.69, 2.07, respectively).[8]

2. Optimum Hydrolysis Time

The hydrolysis time of ginseng extract solution using microwave energy and PTFE reaction vessel varied from 1 to 12 mins with the interval of 1 min, and the relative peak intensity of PD-TMS and PT-TMS to the internal standard is given in Fig. 4. As shown in Fig. 4, the peak intensity increased with an increase in hydrolysis time and showed maximum at 9 min for PT, and at 10 min for PD. PT reached maximum earlier than PD which coinsides with the results obtained by conventional reflux-hydrolysis method.[8]

3. The Calibration Curve of Ginsenosides

Ginseng extract(3~15 mg) was hydrolyzed by microwave oven reaction for 10 minutes followed by silyation and GC analysis. Fig. 5 is the chromatogram of the ginseng extract, and Fig. 6 shows the relationship of the amount of ginseng extract and

the relative peak intensities of PD and PT. As shown in Fig. 6, the correlation coefficients for PD and PT were 0.978 and 0.974, respectively. On the other hand, when the ginsenoside Rb_1 and Rg_1 standards were treated by the same method, the correlation coefficients of the calibration curve were 0.989 and 0.999, respectively. Therefore, the linearity of the calibration curve from ginseng extract was lower than that of ginsenoside standard. We think that coexisting compounds in ginseng extract influence the hydrolysis of saponin. Modification of the method to increase linearity is under progress.

Conclusion

1. Rapid hydrolysis method of ginsenosides under the high temperature and high pressure using PTFE reaction vessel and microwave oven was established.

2. The optimum hydrolysis time of ginseng extract in 5% H_2SO_4 solution was found at 10 min which is more than 30 times faster than the conventional hydrolysis method.

요 약

전자렌지의 고온 고압 반응을 이용하여 인삼 사포닌을 사포게닌으로 신속하게 가수분해하는 방법을 개발하였다. 인삼 추출물의 5% 황산 용액을 PTFE 반응 용기에 넣고 전자렌지에서 가수분해시킨 결과 10분에서 가장 양호한 결과를 나타내었다. 이는 일반적으로 사용되는 환류에 의한 가수분해 방법보다 30배 이상 빠른 것이다.

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