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High Performance Liquid Chromatographic Isolation of Ginsenoside $-Rf$, $-Rg_2$ and $-Rh_1$

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高速液體 Chromatography에 依한 Ginsenoside—Rf, Rg_2 및 Rh_1의 分離

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요 약

人蔘사포닌중 소미량성분인 ginsenoside-Rf, $-Rg_2$ 및 $-Rh_1$을 造製用, 準造製用 및 分析用 HPLC를 使用하여 分離하였다. 本 方法은 신속하며 이들 소미량 ginsenoside의 分離 및 同定에 매우 有效하였으며 再순환 방식을 사용하여 달성되었다.

Introduction

The improvement of isolation techniques of saponin components in ginseng and its products is indispensable and of great importance for biochemical and pharmacological studies of *Panax ginseng* saponin. A number of works have been reported in literature for the isolation and identification of ginsenosides using chromatographic methods such as thin-layer chromatography(TLC),[1~6] droplet counter-current chromatography(DCC)[7] and gas-liquid phase chromatography (GLC)[8,9]. However, those isolation techniques are not only tedious and laborious but also inapplicable to obtain certain minor substances. Recently, a new technique using high performance liquid chromatography(HPLC)[11~12] has been accentuated as a useful tool for identification of natural-products. In the previous paper,[13] we found that application of HPLC technique to be also very efficient for the rapid isolation of major components of ginseng saponin—ginsenoside $-Rb_1$, $-Rb_2$, $-Rc$, $-Rd$, $-Re$ and $-Rg_1$.

The objective of this work was to develope a method using HPLC for isolation of minor components of ginseng saponin. Ginsenoside $-Rf$, $-Rg_2$ and $-Rh_1$ were isolated by preparative, semi-preparative and analytical HPLC.

Materials and Methods

Apparatus

Liquid chromatographs used in the investigation was a preparative HPLC (prep LC/System-500, Waters Associates), analytical and semi-preparative HPLC(ALC-201, Waters Associates, Inc., Milford, Mass., U.S.A.) equipped with Refractometer R-401(RI detector).

A prep PAK-500/Silica cartridge (57mm ID× 30cm), μ Bandapak C_{18} column(7.8mm ID×30 cm) and carbohydrate analysis column(3.9mm ID×30cm) were used for prep LC/System-500, semi-preparative and analytical identification, respectively. The solvent mixtures used for mobile phase of which ratio described in Figure I was filtered through a 0.45μ filter and degassed under vaccum while stirring before use.

Sample preparation

The four-year-old white ginseng(*Panax ginseng* C.A. Meyer), cultivated at Kum-San, Korea, were used for extraction of saponin and for isolation of ginsenoside-Rf, $-Rg_2$ and $-Rh_1$. As shown in Figure 1, 18g of crude saponin was extracted from 500g of white ginseng according to the same procedure decribed in the previous paper[13], This crude saponin obtained was fractionated into ten fractions using with the prep LC/System-500 equipped with two prep PAK-500/Silica cartridges. The mobile phase used was a mixed solventof n-butanol-ethyl acetate-water(4 : 1 : 5, upper phase) at a flow rate of 50ml/min. After each fraction was identified for its contents of authentic ginsenosides by using analytical HPLC, those fractions, Fr. 1(2.56g) and mixture (4.25g) of Fr. 2 and Fr. 3, which contained ginsenoside $-Rh_1$, $-Rg_2$ and -Rf were further sub-fractionated using with semi-preparative HPLC. As a result, seven sub-fractions of Fr. 1-1, Fr. 1-2... and Fr. 1-7 were obtained from Fr. 1 using acetonitrile-water(89 : 11) as a mobile phase. The 5 sub-fractions(Fr. 23-1, Fr. 23-2, ...Fr. 23-5) from the mixture of Fr. 2 and 3 (Fr. 23) was collected with using a mobile phase of acetonitrile-water(84 : 16). The both were carried out on μ Bondapak C_{18} column and at a flow rate of 8ml/min. The attenuation of RI detector was adjusted to a range of 8x to 16x. The authentic samples used for identification of ginsenoside -Rf, $-Rg_2$ and $-Rh_1$ were kindly donated from Dr. J. Shoji, School of Pharmaceutical Sciences, Showa University, Tokyo, Japan.

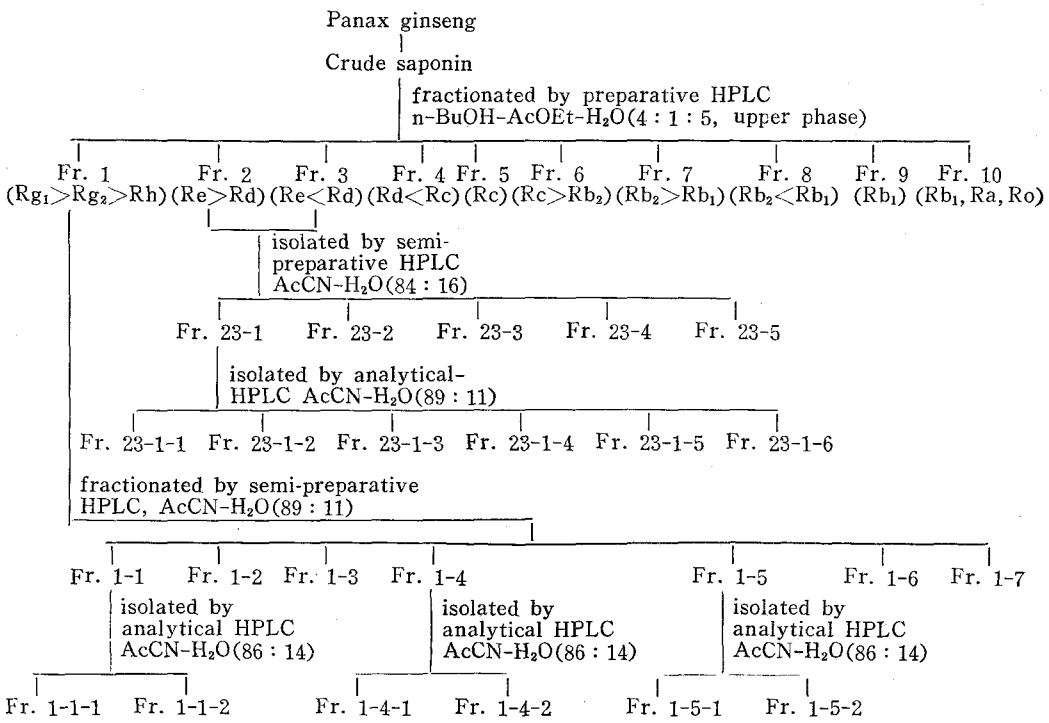

Fig. 1. Extraction and isolation of ginseng saponin

Results and Discussion

Sub-fractionation for isolation

Each of ten fractions(Fr. 1~Fr. 10) from crude saponin was identified by analytical HP LC for its ginsenosides. It was found that fraction 1 was the major fraction which contained ginsenoside -Rg_1, -Rg_2 and -Rh_1. The both fractions of II and III(Fraction 23) were found to have small amount of ginsenoside -Rf along with ginsenoside -Re and -Rd.

In order to separate ginsenoside -Rg_2 and -Rh_1, fraction I was sub-fractionated into 7 fractions by semi-preparative HPLC using a mixed solvent of acetonitrile-water(89 : 11) as amobile phase as shown in Figure 2. And then each sub-fraction was identified for its ginsenosides by analytical HPLC. It was found that ginsenoside-Rh_1, -Rg_2 and -Rg_1 were presented in sub-fractions 1-1, 1-4 and 1-5, respectively.

Also in order to separate ginsenoside -Rf, the mixture of fraction 2 and 3 was fractionated by semi-preparative HPLC using a mixed solvent of acetonitrile-water(84 : 16) as a mobile phase, and ginsenoside-Rf was found in sub-fraction 23-1.

The amount of the sub-fractions from 2.560 g of Fr. 1 and 0.984g of Fr. 23-1 are shown in Table 1. The dry weights of sub-fractions such as Fr. 1-1, Fr. 1-4 and Fr. 23-1-3 which were used for isolation of ginsenoside -Rh_1, -Rg_2 and -Rf, were obtained 97.5mg, 209.5mg and 175.0mg, respectively from 18g of crude saponin.

Isolation of ginsenoside -Rg_2 and -Rh_1

Ginsenoside -Rg_2 in sub-fraction 1-4 was isolated by analytical HPLC which was performed on a carbohydrate analysis column using a mixed solvent of acetonitrile-water(86 : 14) as a mobile phase at a flow rate of 2m*l*/min and the attenuation of 8 x for RI detector. As shown in Figure 3, sub-fraction 1-4 consisted 2 components. Therefore it was further separated into sub-fractions of 1-4-1 and 1-4-2. Ginsenoside-Rg_2 was identified from sub-fraction 1-4-1

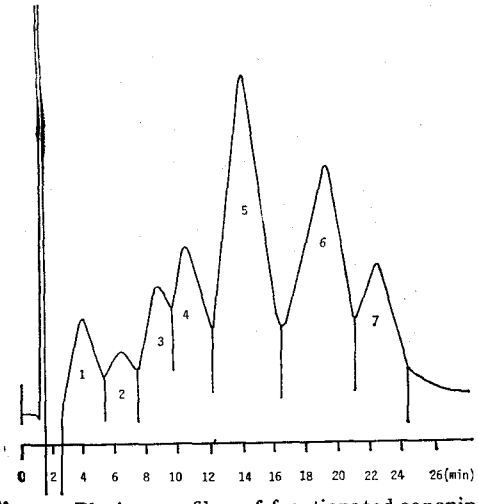

Fig. 2. Elution profiles of fractionated saponin (Fr. 1) by semi-preparative HPLC: 1, Fr. 1-1; 2, Fr. 1-2; 3, Fr. 1-3; 4, fr. 1-4; 5, Fr. 1-5; 6, Fr. 1-6; 7, Fr. 1-7.
Conditions; Packing : μ Bandapak C_{18}
Column : 7.8mm ID×30cm
Mobile phase : AcCN : H_2O=89 : 11(v/v)
Flow rate : 8m*l*/min
RI detector : Attenuation 16x
Sample load : 30mg/0.75m*l*/injection

Table 1. Amounts of sub-fractions obtained from fractionall and fraction 23-1.

Fraction No.	Dry weight (mg)	Fraction No.	Dry weight (mg)
Fraction 1	2,560	Fraction 23-1	984
Fr. 1-1	97.5	Fr. 23-1-1	43.2
Fr. 1-2	88.0	Fr. 23-1-2	262.4
Fr. 1-3	120.5	Fr. 23-1-3	175.0
Fr. 1-4	209.5	Fr. 23-1-4	75.2
Fr. 1-5	690.6	Fr. 23-1-5	60.2
Fr. 1-6	523.0	Fr. 23-1-6	131.5
Fr. 1-7	252.0		
Total	1,981.1	Total	747.5

using the same conditions and mixed solvent used for obtaining sub-fraction 1-4. This was followed by confirmation of its homogeneity using co-chromatographic method. The dry weight of ginsenoside-Rg_2 obtained was 165 mg which is correspond to 0.917% of crude saponin or 0.032% of white ginseng.

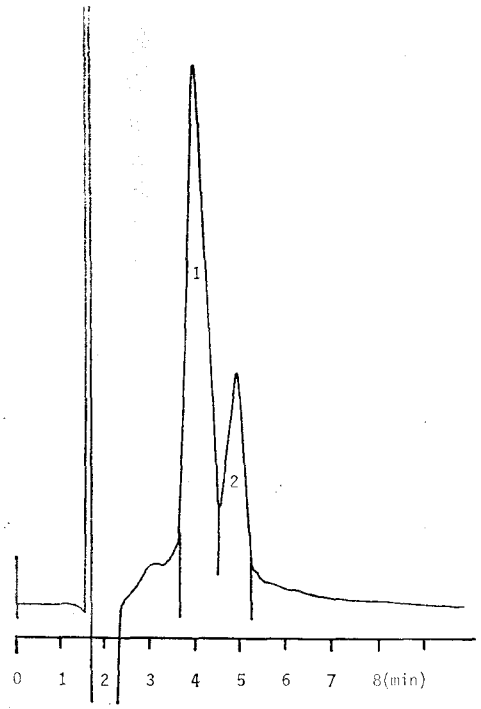

Fig. 3. Elution profile of fraction 1-4 by analytical HPLC: 1, Fr. 1-4-1; 2, Fr. 1-4-2.
Conditions; Packing : Carbohydrate analysis
Column : 3.9mm ID×30cm
Mobile phase : AcCN : H_2O=86 : 14(v/v)
Flow rate : 2ml/min
RI detector : Attenuation 8x

Ginsenoside-Rh_1 in sub-fraction 1-1 was isolated by analytical HPLC, which was performed by same conditions described for isolation for ginsenoside-Rg_2. The fraction 1-1 was further separated into sub-fractions of 1-1-1 and 1-1-2. The sub-fraction 1-1-2 had a peak which was overlapped with ginsenoside -Rh_1 perfectly(Figure 4). The Fr. 1-1-2 isolated was identified as authentic ginsenoside -Rh_1 by analytical HPLC using a mixed solvent of acetonitrile-water(90 : 10). The homogeneity was proved using co-chromatographic method. The yield of ginsenoside-Rh_1 was 71mg. The content of ginsenoside-Rh_1 in crude saponin and white ginseng was found to be 0.39% and 0.014%, respectively.

Isolation of ginsenoside-Rf

As shown in Figure 5, sub-fraction 23-1 was subjected to the isolation of ginsenoside-Rf.

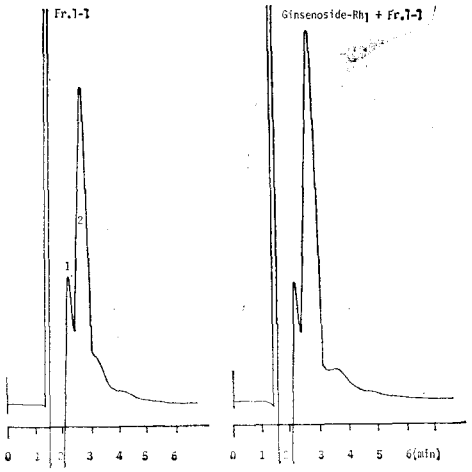

Fig. 4. Elution profiles of fraction 1-1 and co-chromatogram of ginsenoside-Rh_1 added to fraction 1-1 by analytical HPLC: 1, Fr. 1-1-1; 2, Fr. 1-1-2.
Conditions; Packing : Carbohydrate analysis
Other conditions are same as shown in Fig.3

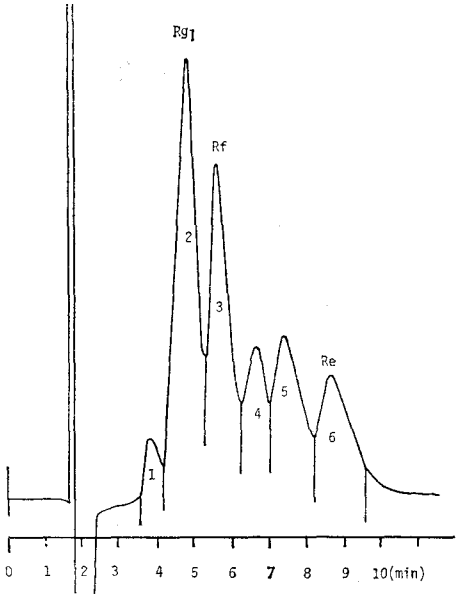

Fig. 5. Elution profile of fraction 23-1 by analytical HPLC: 1, Fr. 23-1-1; 2, Fr. 23-1-2; 3, Fr. 23-1-3; 4, Fr. 23-1-4; 5, Fr. 2 3-1-5; 6, Fr. 23-1-6.
Conditions; Packing : Carbohydrate analysis
Other conditions are same as shown in Fig.3

It was further separated into 6 sub-fractions using analytical HPLC. The mobile phase was a mixed solvent of acetonitrile-water(86 : 14) It was found that sub-fraction 23-1-3 was the main fraction which contained ginsenoside -Rf.

A further sub-frationation was carried out for fraction 23-1-3 to isolate ginsenoside-Rf using the same solvent of acetonitrile-water(Figure 6). Sub-fraction 23-1-3-2 isolated was identified as ginsenoside-Rf by analytical HPLC using a mixed solvent of acetonitrile-water(86 : 14) and the homogeneity examined with authentic ginsenoside-Rf by co-chromatographic method. The amount of ginsenoside-Rf obtained from 500g of white ginseng was 137mg which is correspond to 0.76% of crude saponin.

Acknowledgement

The authors are grateful to Dr. J. Shoji, School of pharmaceutical Sciences, Showa university, Tokyo, Japan, for his donation of authentic ginsenosides.

Abstract

The minor components of saponin-ginsenoside-Rf, -Rg_2 and -Rh_1 were isolated from *Panax ginseng* C.A. Meyer by preparative, semi-preparative and analtical high performance liquid chromatography. The rapid method developed in this work was proved to be very effective in separation and isolation of these minor ginsenosides. A further isolation was achieved by using the recycling technique.

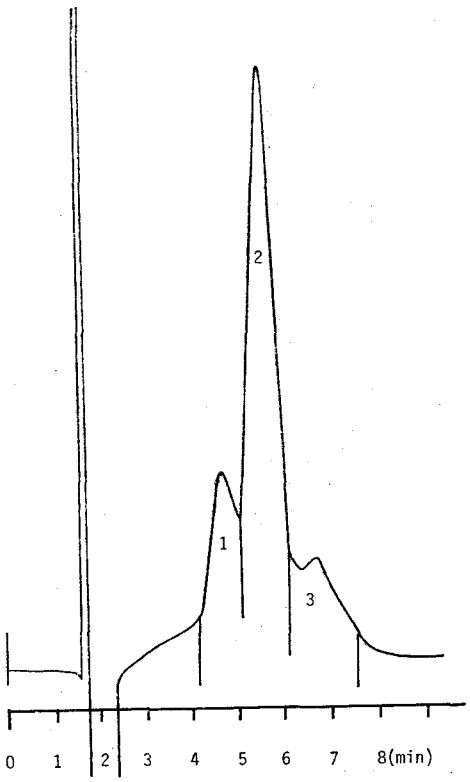

Fig. 6. Elution profile of fraction 23-1-3 by analytical HPLC: 1, Fr. 23-1-3-1; 2, Fr. 23-1-3-2; 3, Fr. 23-1-3-3.
Conditions; same as shown in Fig. 3.

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