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Short communication

Liquid chromatographic determination of less polar ginsenosides in processed ginseng

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Abstract

Reversed-phase LC with an evaporative light scattering detector (ELSD) is used for the determination of less polar ginsenosides in processed ginseng. These ginsenosides include ginsenosides F_4, Rg_3, Rg_5, Rg_6, Rk_1, Rk_3, Rs_3, Rs_4, and Rs_5. The method used a C_{18}-bonded silica column with a $CH_3CN/H_2O/CH_3COOH$ gradient elution. (20*R*) and (20*S*) epimers and geometric isomers at the C-20 position of ginsenosides, which are not generally separated by amino columns, were now clearly separated. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Panax ginseng*; Ginsenosides

1. Introduction

Ginseng, the radix of *Panax ginseng* C. A. Meyer, known as Korean ginseng, is one of the most widely used herbal medicines in the Orient. Recently, we reported that steaming the ginseng at high temperature greatly enhances its biological activity [1,2]. We also isolated several new ginsenosides from the steamed ginseng that are not present in white ginseng [3]. These were ginsenosides F_4, Rg_3, Rg_5, Rg_6, Rk_1, Rk_2, Rk_3, Rs_3, Rs_4, and Rs_5 (Fig. 1). Ginsenoside Rg_3 showed strong vasorelaxation activity [4] and anti-platelet aggregation activity [5]. Ginsenoside Rg_5, Rs_4 and Rs_3 also showed anti-cancer activity through the induction of apoptosis [6–8]. Therefore there is a need to analyze these ginsenosides which demonstrate important pharmacological activities.

However, the reported methods are not appropriate for the determination of new ginsenosides in processed ginseng since they show less polarity than the previously known ginsenosides. This paper describes the successful determination of these less polar compounds by RPLC–ELSD.

2. Experimental

2.1. Preparation of samples

Four-year-old fresh ginseng was purchased from the local ginseng market in Seoul. Ginsenosides Rb_1,

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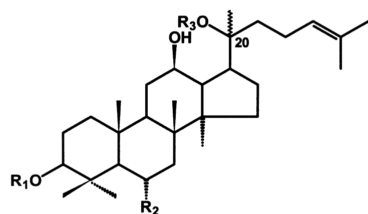
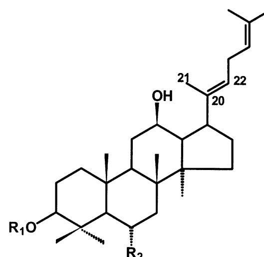


Ginsenoside	R_1	R_2	R_3
(20S)Rb_1	Glc-Glc-	H	Glc-Glc-
(20S)Rb_2	Glc-Glc-	H	Ara(p)-Glc-
(20S)Rc	Glc-Glc-	H	Ara(f)-Glc-
(20S)Rd	Glc-Glc-	H	Glc-
(20S), (20R)Rg_3	Glc-Glc-	H	H
(20S), (20R)Rs_3	Ac-Glc-Glc-	H	H
(20S)Re	H	Rha-Glc-O-	Glc-
(20S)Rg_1	H	Glc-O-	Glc-
(20S), (20R)Rg_2	H	Rha-Glc-O-	H
(20S)Rf	H	Glc-Glc-O-	Glc-
(20S), (20R)Rh_1	H	Glc-O-	H

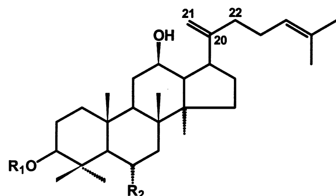


Ginsenoside	R_1	R_2
F_4	H	Rha-Glc-O-
Rg_5	Glc-Glc-	H
Rh_3[a)]	Glc-	H
Rh_4	H	Glc-O-
Rs_4	Ac-Glc-Glc-	H
Rs_6[a)]	H	Ac-Glc-O-

Glc: -D-glucopyranosyl, Ara(p): -L-arabinopyranosyl, Ac:6'-O-acetyl,

Ara(f): -L-arabinofuranosyl, Rha: -L-rhamnopyranosyl

a) : not determined



Ginsenoside	R_1	R_2
Rg_6	H	Rha-Glc-O-
Rk_1	Glc-Glc-	H
Rk_2[a)]	Glc-	H
Rk_3	H	Glc-O-
Rs_5	Ac-Glc-Glc-	H
Rs_7[a)]	H	Ac-Glc-O-

Fig. 1. Structure of ginseng saponins.

Rb_2, Rc and Rd were generous gifts from Korea Ginseng and Tobacco Research Institute (Daejon, Korea). Ginsenosides Rg_3, Rg_5, Rg_6, Rk_1, Rk_2, Rk_3, Rs_3, Rs_4 and Rs_5 were isolated and identified in our laboratory [3]. Two hundred g each of fresh ginseng, ginseng steamed at 100°C for 3 h, and ginseng steamed at 120°C for 3 h were refluxed with methanol for 6 h. The organic solvent was removed and the residue was dissolved in 1000 ml of water and extracted with 300 ml of dichloromethane. The aqueous layer was further extracted three times with 300 ml of water-saturated *n*-butanol. The *n*-butanol fraction was evaporated and the residue was dissolved in 40 ml of methanol, which was subjected to LC determination. The samples were filtered before injection.

2.2. Chromatographic conditions

The LC system consisted of two Hitachi (Tokyo, Japan) model L-7100 pumps coupled with a Rheodyne (Cotati, CA, USA) model 7125 injector, Sedex model 55 ELSD (SEDERE, Alfortville, France) and

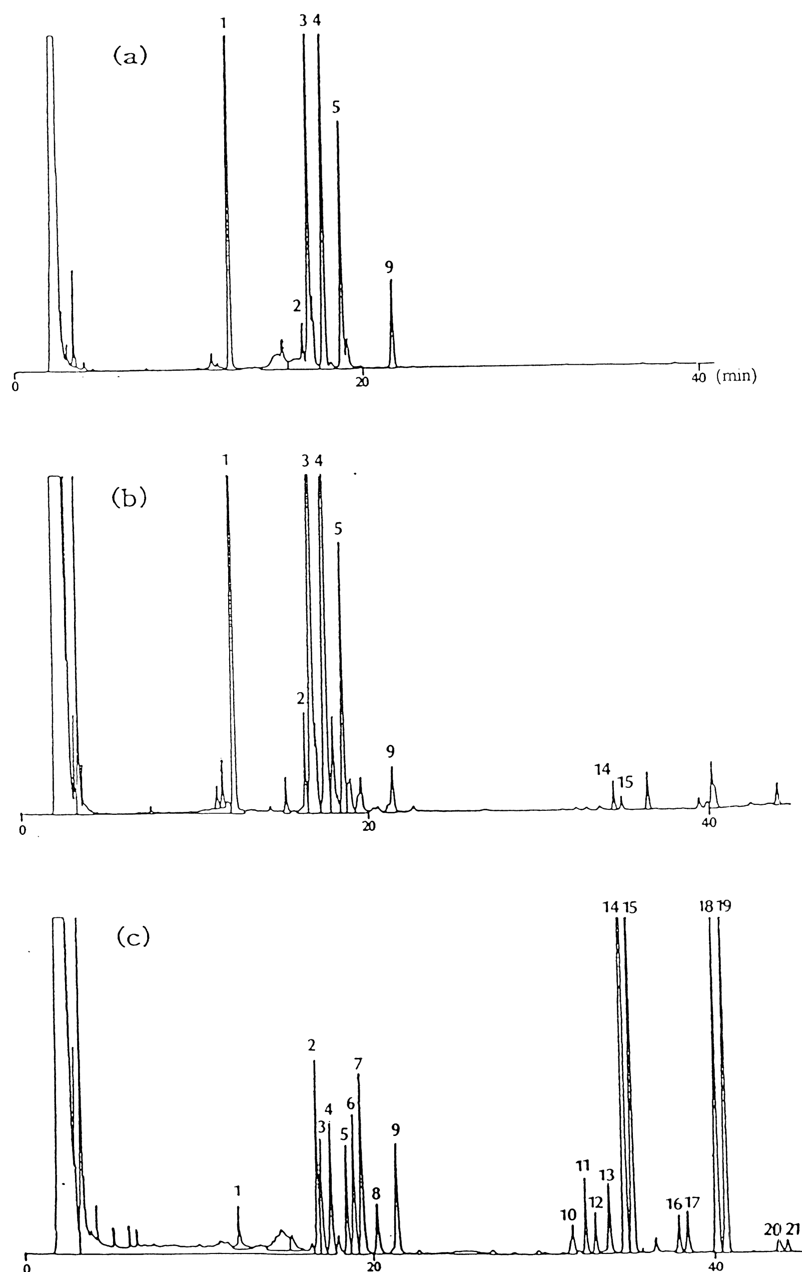


Fig. 2. Chromatogram of fresh ginseng (a), steamed ginseng at 100°C/3 h (b) and steamed ginseng at 120°C/3 h (c) (Peak identity: see Table 1).

Table 1
Analytical characteristics of ginsenosides

Peak no. in Fig. 2	Ginsenoside	LODs(ng)[a]	Contents[b] % (w/w)	RSD(%) of Intra-day ($n=3$)	RSD(%) of Inter-day ($n=3$)
1	Re+Rg_1	30(Re), 45(Rg_1)	c	3.2	2.3
2	Rf	c	c	1.6	2.5
3	Rb_1	50	0.32	3.7	2.8
4	Rc	50	0.19	2.7	3.6
5	Rb_2	120	0.16	5.2	6.2
6	(20*S*)Rg_2	c	c	4.2	1.2
7	(20*R*)Rg_2+(20*S*)Rh_1	75(Rh_1)	c	0.59	0.65
8	(20*R*)Rh_1	c	c	5.8	2.7
9	Rd	100	0.30	1.1	4.1
10	Rg_6	75	0.16	0.53	5.1
11	F_4	170	0.20	0.54	4.9
12	Rk_3	c	c	2.2	2.2
13	Rh_4	c	c	0.71	3.6
14	(20*S*)Rg_3	70	3.6	0.11	2.6
15	(20*R*)Rg_3	35	2.5	4.6	8.5
16	(20*S*)Rs_3	65	0.05	0.32	2.5
17	(20*R*)Rs_3	100	0.05	1.8	2.6
18	Rk_1	75	2.9	0.04	2.8
19	Rg_5	110	3.3	4.0	1.0
20	Rs_5	65	0.02	4.2	6.5
21	Rs_4	110	0.03	1.5	2.7

[a] Limit of detection ($S/N=3$).
[b] w/w% in the extract of steamed ginseng at 120°C/3 h.
[c] Not determined in this authors wish to acknowledge the financial support of the Korea Research Foundation granted in 1997.

Chromatopac model C-R7Ae integrator (Shimadzu, Kyoto, Japan). ELSD conditions were optimized in order to achieve maximum sensitivity: temperature of the nebulizer was set for 40°C, and N_2 was used as the nubulizing gas at a pressure of 1.8 bar. Separation was performed on a Mightysil RP-18 (5 μm, 250×4.6 mm I.D., Kanto Chemical, Tokyo, Japan) column. A gradient elution system of A (CH_3CN:H_2O:5%CH_3COOH=15:80:5) and B (CH_3CN:H_2O=80:20) was used [0% B (0 min); 30% B (10 min); 50% B (25 min); 100% B (40 min); 100% B (50 min)]. The solvent flow-rate was 1.0 ml/min and the injection volume was 10 μl. A Finnigan LCQ (San Jose, CA, USA) ion-trap mass spectrometer with electrospary ionization (ESI) mode was used in the LC–MS method. ESI condition was as follows: source voltage 4.5 kV, sheath gas flow-rate 6 bar, auxiliary gas flow 2 bar, capillary voltage −9.3 V, capillary temperature 250°C, collision energy 10 V.

3. Results and discussion

We previously reported an LC determination of ginsenosides in processed ginseng using an amino column [2]. However, we discovered that epimers and geometric isomers do not separate on an amino column. (20*S*) ginsenoside Rg_3 did not separate from its (20*R*) epimer, (20*S*)Rg_2 from (20*R*)Rg_2, (20*S*)Rh_1 from (20*R*)Rh_1, and (20*S*)Rs_3 from (20*R*)Rs_3; geometric isomers of ginsenosides Rk_1 and Rg_5, Rg_6 and F_4, Rs_5 and Rs_4, and Rk_3 and Rh_4 were not separated either. Our efforts to separate these isomers by modifying the solvent system, column temperature and flow-rate were unsuccessful. As an alternative, the use of a reversed-phase separation was attempted.

An experiment with a C_{18}-bonded silica column with a CH_3CN: H_2O: CH_3COOH solvent system was successful in separating (20*S*) and (20*R*) epimers of ginsenosides Rg_2, Rh_1, Rg_3 and Rs_3.

Geometric isomers at the C-20 position i.e. ginsenosides Rg_6 and F_4, Rk_3 and Rh_4, Rk_1 and Rg_5, and Rs_5 and Rs_4 were also clearly separated through this system. It was found that the (20*S*) epimer of ginsenoside Rg_2, Rh_1, Rg_3 and Rs_3 were eluted earlier than its relevant (20*R*) epimer, and △20(21)-geometric isomers were eluted earlier than its relevant △20(22)-isomers.

RPLC traces of three kinds of ginseng extract are presented in Fig. 2. Peaks were identified by comparing retention times and LC–MS spectra with those of reference compounds.

Fig. 2 demonstrates that steaming the ginseng at 120°C increases the contents of less polar ginsenosides (peaks 10–21) and decreases polar ginsenosides (peaks 1–9). Through this method less polar ginsenosides which have been difficult to determine by usual methods were clearly detected. The calibration curves, plotted by double logarithmic coordinates [9], showed good linearity ($R = 0.995$–0.999). The slope was in the range of 1.36–1.90 [10]. Precision of the assay, represented by the relative standard deviation (RSD), was the range of 0.04–5.8% for intra-day variation and 0.65–8.5% for inter-day variation (Table 1).

In conclusion, the RPLC–ELSD method is a successful technique to determine less polar ginsenosides in processed ginseng.

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