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Short communication

Intramolecular formation of cyclic ether by dehydration of 20(*S*)-ginsenoside Rg_3

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ABSTRACT

Two new conversion ginsenosides having cyclic ether together with ginsenoside Rg_5, Rk_1, and Rz_1 were isolated from dehydration products of 20(*S*)-ginsenoside Rg_3. On the basis of NMR spectroscopic analyses and comparison with spectral data of ginsenoside Rg_3 as a starting material, the chemical structures of two new ginsenosides were established as 12β-O-20(*S*)-ginsenoside Rg_3 and 12β-O-20(*R*)-ginsenoside Rg_3. The compounds were named as neoginsenoside L_1 and L_2 respectively. The conversion mechanism was expected to be accomplished by the formation of a tertiary carbocation or intramolecular nucleophilic displacement. The two new ginsenosides confirmed the existence from red ginseng extract by liquid chromatography.

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) has been traditionally used as a precious medicine in Far East Asia such as Korea, China and Japan (Tyler, Brady, & Robbers, 1988). The commercially available ginseng roots are classified into two forms, red ginseng (roots steamed at 98–100 °C without peeling) and white ginseng (roots air-dried after peeling). The most well known chemical constituents from ginseng are ginsenosides as a dammarane glycoside. By now, some 50 different kinds of ginsenosides have been identified from ginseng (Cheng, Na, Bang, Kim, & Yang, 2008). Ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 which are considered naturally occurring constituents are common constituents of white and red ginseng, whereas ginsenoside Rg_2, Rg_3, Rh_1, Rh_2, Rg_5, and Rk_1 are known to be unique constituents of red ginseng as artifacts from the common ginsenosides (Kim et al., 1996; Kitagawa, Yoshikawa, Yoshigara, Hayashi, & Taniyama, 1983; Ryu, Park, Eun, Jung, & Sohn, 1997). Ginsenosides are categorised into three types, protopanaxadiol, protopanaxatriol ginsenosides, and oleanane type ginsenosides. Protopanaxadiol ginsenosides such as ginsenoside Rb_1, Rb_2, Rc, and Rd can be readily converted into ginsenoside Rg_3 by either acid treatment or heating in red ginseng manufacturing process (Han et al., 1982; Park, 2004). Furthermore, ginsenoside Rg_3 can be readily converted into two isomers such as ginsenoside Rg_5 and Rk_1 (Park et al., 2002). Recently, we have reported about ginsenoside Rz_1 which was a geometric isomer of ginsenoside Rg_5 and Rk_1 as conversion products of ginsenoside Rg_3 (Lee et al., 2009). In these cases conversions probably occur by the E1 mechanism, by way of intermediate carbocations (Fig. 1). In our further search for denatured ginsenosides, two new ginsenosides which have cyclic ether and were converted from ginsenoside Rg_3, were found in red ginseng extract. In this paper, we will describe the isolation of new ginsenosides from dehydrolysate of ginsenoside Rg_3, structure elucidation, dehydration mechanism of Rg_3, and the existence of two new compounds from red ginseng extract.

2. Materials and methods

2.1. Materials

Red ginseng extract was purchased from a selling area managed by the Korea Ginseng Corporation (Daejon, Korea). The 20(*S*)-ginsenoside Rg_3 was isolated from the red ginseng extract as reported previously and identified (Cheng et al., 2008) by ^{1}H NMR, ^{13}C NMR and MS spectroscopy. Other reagents used in this work were of extra pure grade.

2.2. Methods

2.2.1. General procedures

Optical rotations were measured using DIP-370 digital polarimeter (JASCO, Tokyo, Japan). The ^{1}H NMR spectra (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker Avance 600 MHz NMR spectrometer (BRUKER, Karlsruhe, Germany) and the chemical shifts were reported in ppm using the solvent as reference. ESI-MS data was obtained on a Waters Acquity SQDetector on Acquity LC system (Waters, MA, USA). HRFABMS spectra were measured on

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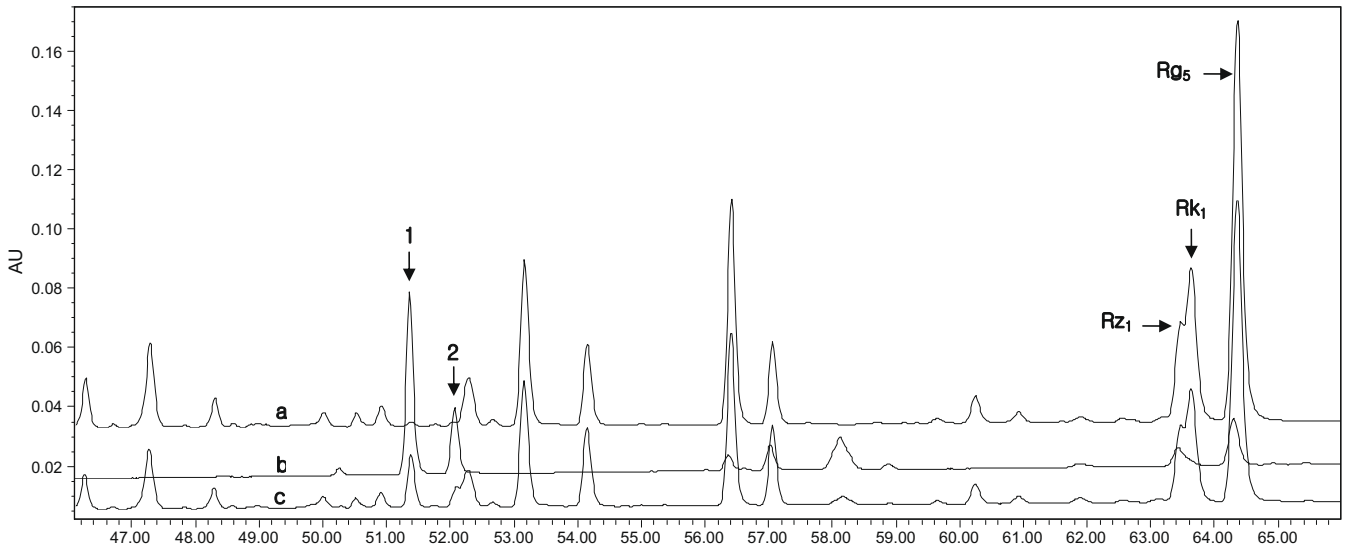

Fig. 1. Comparison of three chromatographies, which were red ginseng extract (a), the hydrolysate of ginsenoside Rg_3 (b), and the mixture (c). Mobile phase; aqueous acetonitrile (20 → 65%) for 70 min, flow rate, 1.6 mL/min, column temperature; 40 °C, detection wave length; 203 nm.

JMS 700 mass (JEOL, Tokyo, Japan). Preparative LC used Waters DeltaPrep preparative chromatography system with XTerra Prep MSC_{18} (5 μm, 30 × 100 mm) column (Waters, MA, USA).

2.2.2. Dehydration of ginsenoside Rg_3

A solution of 20(*S*)-ginsenoside Rg_3 (200 mg) in 20 mM citric acid (pH 3, 20 mL) was heated at 100 °C for 3 h. After cooling, the reaction mixture was neutralised with 0.01% NaOH, then subjected to an ODS column in order to remove of water soluble by-products, eluted with acetonitrile (70%) and finally, concentrated on reduced pressure to yield a neoginsenosides rich extract, consecutively.

2.2.3. Isolation of neoginsenosides

The neoginsenosides rich extract (200 mg) was chromatographed on ODS column, eluted with water–acetonitrile (9:11) to obtain a mixture of compounds 1 and **2**. Sequential preparative liquid chromatography (Waters DeltaPrep) on ODS column (XTerra Prep MSC_{18}) led to isolation of two new compounds **1** (40 mg) and **2** (10 mg).

*2.2.4. Existence test of **1**, and **2** from red ginseng extract*

In order to confirm the existences of **1** and **2** from red ginseng extract, the chromatographic analysis is as follows. Analyses were performed on Waters HPLC system (Waters, USA), consisting of Alience 2695 Separations Module, 2996 Photodiode Array Detector, and Ampower software, Discovery C18 column (4.6 mm id × 25 cm, 5 μm) (Supelco, USA). The column temperature was kept at a constant temperature of 40 °C, and the mobile phase flow rate was 1.6 mL/min. The detection wave length was 203 nm. The mobile phases consisted water (A) and acetonitrile (B) using a gradient elution of 20–32% B at 0–40 min, 32–50% B at 40–55 min, 50–65% B at 55–70 min, and the re-equilibration time of mobile phase condition was 10 min.

*2.2.5. Neoginsenoside L_1 (**1**)*

$C_{42}H_{70}O_{12}$, amorphous powder, α_D^{25} − 23.2° (MeOH, *c* 0.03), ESI-MS *m*/*z* 784 $[(M + H_2O) - H]^+$, 766 $[M - H]^+$, HRFABMS *m*/*z* 768.49454 $[M + H]^+$, ^1H and ^{13}C NMR and ROESY data, see Table 1.

*2.2.6. Neoginsenoside L_2 (**2**)*

$C_{42}H_{70}O_{12}$, amorphous powder, α_D^{25} − 7.5° (MeOH, *c* 0.01), ESI-MS *m*/*z* 784 $[(M + H_2O) - H]^+$, 766 $[M - H]^+$, HRFABMS *m*/*z* 768.49453 $[M + H]^+$, ^1H and ^{13}C NMR and ROESY data, see Table 1.

3. Result and discussion

Five conversion products (**1** and **2**, ginsenoside Rz_1, Rk_1, and Rg_5) were shown on the chromatography from dehydrolysate of 20(*S*)-ginsenoside Rg_3 in citric acid water (Fig. 1 b). In the products, compounds 1 and **2** were isolated at 16–20 min by preparative chromatography system with 45% acetonitrile (15 mL/min) easily. In order to confirm the existence of **1** and **2** from red ginseng extract, chromatographic analyses were performed against red ginseng extract, hydrolysate of ginsenoside Rg_3, and the mixture (Fig. 1). On the chromatogram of the hydrolysate of ginsenoside Rg_3, compounds 1 and **2** appeared together with other conversion products such as ginsenoside Rz_1, Rk_1, and Rg_5 (Fig. 1 b). In these peaks of five conversions, compounds 1 and **2** appeared like traces at the chromatogram of red ginseng extract (Fig. 1 a). The chromatogram of the mixture, red ginseng extract and hydrolysate of ginsenoside Rg_3, gave proof of the existence of **1** and **2** in red ginseng extract (Fig. 1 c). The specificities of compounds **1** and **2** from red ginseng extract were given by *m*/*z* 784 and 766 at LC–ESIMS spectra of both.

A new compound, named neoginsenoside L_1 (**1**), together with another new compound, neoginsenoside L_2 (**2**) was isolated from the acid treated 20(*S*)-ginsenoside Rg_3. Compound **1**, an amorphous solid, had a molecular formula of $C_{42}H_{70}O_{12}$ determined by high resolution positive ion FABMS (at *m*/*z* 768.49454 $[M + H]^+$). The negative ESI-MS spectrometry showed a rehydrated ion peak at *m*/*z* 784 $[(M + H_2O) - H]^+$ and molecular peak at *m*/*z* 766 $[M - H]^+$, probably, of the hydrolysing processes at the ether bond in the ionisation process. The ^1H and ^{13}C NMR spectral data assignments of **1** were achieved via analysis of the HMQC, HMBC, ^1H–^1H COESY and DEPT spectra. The three-dimensional arrangements of **1** determinated by NOE from ROESY data. The NMR spectra of **1** showed the presence of two sugars from the two anomeric protons at δ 4.96 (d, 7.2 Hz) and 5.41 (d, 7.8 Hz) in the ^1H NMR spectrum, and the two anomeric carbon signals at δ 105.6 and 106.5 in the ^{13}C NMR spectrum. These NMR data explain that the