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20(*R*)-Ginsenoside Rf: A new ginsenoside from red ginseng extract

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ABSTRACT

In spite of the general concept that herbal supplements are safe, there is a lack of appropriate quality control measures and regulations that often culminates in serious undesirable effects such as allergic reactions and renal and liver damage. Thus, there is a growing need to establish a suitable methodology that enables authentication and quality assurance of herbal products. The root of Panax ginseng C. A. Meyer (Araliaceae), commonly called ginseng, is traditionally recognized as a prominent herbal medicine in Far East Asia. There are two types of processed ginseng, white and red ginseng, based on processing methods, and these play a significant role in modifying ginsenosides, which are the major bioactive metabolites in these products. Herein we purify and characterize a new ginsenoside, 20(*R*)-ginsenoside Rf, utilizing NMR, UPLC-ESI-Q-TOF-MS and validate the metabolite is generated from its epimer, 20(*S*)-ginsenoside Rf during the steaming process to manufacture red ginseng. We further propose a relevant mechanism for the chemical conversion. This finding updates chemical profiling of ginseng products that can be employed in quality assurance and authentication.

1. Introduction

Despite the general conception that herbal supplements are safe, several products, including ginseng, have been reported to cause adverse effects such as allergic reactions and renal and liver damage (Ernst Md, 1998; Yap et al., 2007). These undesirable reactions are caused by many factors such as poor quality control methods (Yap et al., 2007). Quality control of herbal products is not well regulated because they are regarded as dietary supplements rather than medicines of which quality and safety are customarily administered by regulatory authorities (Hulse, 2004). Owing to the current formulations of ginseng productions such as powder, capsules and liquid extracts, the common authentication methods based on morphological and histological methods have been outdated (Um et al., 2001).

Ginseng, the root of *Panax ginseng* C. A. Meyer (Araliaceae), is traditionally employed as herb medicine in Far East Asia for the treatment of various diseases (Angelova et al., 2008). There are two common types of ginseng in the herbal supplement market according to the processing methods, white ginseng and red ginseng. White ginseng is produced by peeling and then air drying fresh ginseng and red ginseng is manufactured by steaming ginseng at 98–100 °C without peeling. The steaming process leads to significant variations in the chemical constituents such as the ginsenosides that are responsible for many of the biological activities of ginseng (Park et al., 2010; Zhang et al., 2012). Seventy ginsenosides have been identified from ginseng and its processed products (Christensen, 2008); Ginsenosides Rf, Rb_1, Rb_2, Rc, Rd, Re, Rg_1, and Rg_2 are major constituents of white and red ginseng, while ginsenosides Rh_1, Rg_3, Rg_5, Rg_6 Rs_{1-7}, and Rk_{1-3} are only found in red ginseng (Kim et al., 1996; Kitagawa et al., 1983). Comprehensive chemical profiling of red ginseng is still under way (Lee, 2010) and this is indispensable in the authentication and quality assurance of commercial ginseng products given that adulterations are constantly appearing that possess a chemical profile similar to that of genuine ginseng products (Kurtzweil, 1999). In our continued studies to update the chemical profile of red ginseng (Lee, 2010), we herein report the isolation and structural characterization of a new ginsenoside, 20(*R*)-ginsenoside Rf (**2**). This is an epimer of 20(*S*)-ginsenoside Rf (**1**), which was previously designated as "ginsenoside Rf". Furthermore, we validate that the new entity is generated from epimerization of **1** during the steaming process used in the production of red ginseng. The detailed NMR data of **1** are presented for the first time since it was identified (Sanada et al., 1974).

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2. Results and discussion

20(*S*)-ginsenoside Rf (**1**) was purified and identified utilizing NMR and ESI-Q-TOF-MS. The purified ginsenoside was acidified with 20 mM citric acid (pH 4.0) to simulate the conditions required for the production of red ginseng (Wu et al., 2012). The reaction mixture was purified to obtain 20(*R*)-ginsenoside Rf (**2**).

20(*S*)-Ginsenoside Rf (**1**) was obtained as a white powder from acetone. The specific rotation was established as $[\alpha]_D^{30}$ + 6.99 (*c* 1.0, methanol) and the melting point was observed as 197–198 °C, both of which are identical to the values reported by Sanada et al. (1974). The molecular formula was determined as $C_{42}H_{71}O_{14}$, deduced from ESI-Q-TOF-MS (obsd. $[M-H]^-$ at *m/z* 799.4940, calcd. $[M-H]^-$ = 799.4844). The characteristic ^1H and ^{13}C NMR signals for methyl groups (δ_H 0.81, 0.96, 1.16, 1.39, 1.48, 1.63, 1.66, 2.09 and δ_C 17,8, 18.0, 27.4, 26.2, 18.1, 32.4, 17.1, 17.2), and an olefinic signal (δ_H 5.33) implied that compound **1** was a protopanaxatriol derivative (Table 1) (Teng et al., 2002). A sophorose moiety was deduced from the NMR spectra displaying the typical signals of the two anomeric protons (δ_H 4.93, 5.93) and carbons (δ_C 104.2, 104.3), and other ^{13}C signals (δ_C 63.3, 63.7, 72.1, 72.7, 76.4, 78.2, 78.5, 78.8, 80.1, 80.3).

20(*R*)-Ginsenoside Rf (**2**) was acquired as white powder from methanol. The specific rotation was established as $[\alpha]_D^{20}$ −28.69 (*c* 0.1, methanol). The molecular formula was found to be identical to that of compound **1** (obsd. $[M-H]^-$ *m/z* 799.5131, calcd. $[M-H]^-$ = 799.4844). Further NMR analyses revealed that compound **2** shared structural similarities with 20(*S*)-ginsenoside Rf (**1**). 1D NMR spectra displayed the characteristic ^1H and ^{13}C NMR signals for a protopanaxatriol scaffold substituted with a sophorose moiety based on the two anomeric protons (δ_H 4.93, 5.93) and carbons (δ_C 104.2, 104.3), and relevant ^{13}C signals (δ_C 63.3, 63.7, 72.1, 72.7, 76.4, 78.2, 78.5, 78.8, 80.1, 80.3) (Table 1). The coupling constants of the anomeric protons (*J* = 8.1 Hz) confirmed β-configured glucosidic linkages. The stark difference of the NMR data between the two isomers was the chemical shift values of C-20, the stereogenic center in the side chain attached to the protopanaxatriol scaffold and its adjacent carbons, C-17, 21 and 22.

Previous NMR studies of 20-hydroxy-dammarane derivatives, C-17, C-21 and C-22 chemical shift values of 20(*S*)-dammarane derivatives are *ca.* 55, 27 and 35 ppm, respectively, which are distinctively different from those of the 20(*R*)-dammarane (*ca.* 50, 22 and 43 ppm, respectively) in cases in which 12-hydroxy group is β-oriented (Asakawa et al., 1977; Zhao et al., 1996). These diagnostic NMR deshielding properties in both epimers are attributed to non-bonded interactions associated with the conformation related to C-17–C-20 linkage which is restrained by the robust hydrogen bonding interaction between 12β and 20-hydroxy groups (Asakawa et al., 1977). The observed chemical shift values of each compound are within the above-mentioned ranges (Table 1), which permits the assignment of C-20 configuration of compound **1** and **2** as *S* and *R*, respectively. Therefore, the chemical structure of compound **2** was elucidated as 20(*R*)-protopanaxatriol 6-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and designated as 20(*R*)-ginsenoside Rf (Figs. 1–2).

The proposed mechanism by which ginsenoside 20(*S*)-ginsenoside Rf (**1**) was epimerized into 20(*R*)-ginsenoside Rf (**2**) in acidic conditions which simulated the thermal process employed in the production of red ginseng (Wu et al., 2012). The dehydration reaction produced a carbocation intermediate at C-20 of **1** and upon re-hydration an oxonium ion is generated, resulting in the conversion of the configuration at C-20 and the creation of **2** (Fig. 3).

20(*R*)-Ginsenoside Rf (**2**) purified from the epimerization reaction was compared with red ginseng extract by UPLC-ESI-Q-TOF-MS (Fig. 4) to validate that the proposed epimerization is occurring during the thermal process for the production of red ginseng. The chromatography clearly demonstrated that 20(*R*)-ginsenoside Rf (**2**) purified from our epimerization experiment existed in the red ginseng extract based on the observed molecular ion mass and retention time (Fig. 4). This implied that 20(*R*)-ginsenoside Rf (**2**) was epimerized from 20(*S*)-ginsenoside Rf (**1**)

Table 1
^1H (900 MHz) and ^{13}C (226 MHz) NMR data of 20(*S*)-ginsenoside Rf (**1**) and 20(*R*)-ginsenoside Rf (**2**) in pyridine-d_5.

No. C	**1**		**2**		No. C	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.71–1.65 m 0.99–0.98 m	39.8	1.68–1.66 m 0.98–0.96 m	39.8	22	1.70 m	36.2	1.71 m	43.6
2	1.85–1.89 m 1.78–1.81 m	28.1	1.80–1.82 m 1.90–1.86 m	28.2	23	2.60 m	23.4	2.52 m	22.9
3	3.48 dd (11.7, 4.5)	79.0	3.49 m	79.0	24	5.33 t (6.3)	126.7	5.31 t (7.2)	126.4
4		40.0		40.0	25		131.1		131.2
5	1.39 ov	61.8	1.40 ov	61.8	26	1.66 s	26.2	1.70 s	26.2
6	4.30–4.32 ov	80.2	4.33–4.39 ov	80.2	27	1.63 s	18.1	1.64 s	18.1
7	2.42 dd (12.6, 2.7) 1.95 t (11.7)	45.4	2.44 dd (12.6, 2.7) 1.97 t (12.6)	45.4	28	2.09 s	32.4	2.10 s	32.5
8		41.5		41.5	29	1.48 s	17.1	1.50 s	17.1
9	1.52 m	50.5	1.55 m	50.5	30	0.81 s	17.2	0.86 s	17.5
10		40.6		40.6	1′	4.93 d (7.2)	104.2	4.95 d (8.1)	104.2
11	1.53–1.49 m 2.11–2.02 m	36.2	1.55 m 2.14–2.13 m	32.6	2′	4.49 m ov.	80.1	4.50 m ov.	80.1
12	3.90–3.88 m	71.4	3.93–3.91 m	71.3	3′	4.33 m ov.	80.3	4.39 m ov.	80.3
13	2.05–2.03 m	48.6	2.03 t(10.8)	49.3	4′	4.16 m	72.1	4.15 m	72.1
14		52.0		52.1	5′	3.86 m	78.5	3.87 m	78.5
15	1.17–1.15 m	31.6	1.24–1.19 m	31.7	6′	4.49 m ov. 4.33 m ov.	63.3	4.49 m ov. 4.33 m ov.	63.3
16	1.36–1.33 m 1.91–1.85 m	27.2	1.32–1.30 m 1.90–1.86 m	27.0	1″	5.93 d (7.2)	104.3	5.93 d (8.1)	104.3
17	2.31–2.27 m	55.1	2.37–2.34 m	51.0	2″	4.20 m	76.4	4.21 m	76.5
18	1.16 s	17.8	1.22 s	17.8	3″	4.27 m	78.8	4.26 m	78.8
19	0.96 s	18.0	0.98 s	18.0	4″	4.21 m	72.7	4.22 m	72.7
20		73.3		73.4	5″	3.96 m	78.2	3.97 m	78.2
21	1.39 s	27.4	1.40 s	23.1	6″	4.49 m ov. 4.38 m	63.7	4.49 m ov. 4.39 m	63.7