Metabolism of Ginseng Saponins and Its Significance

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Abstract

To follow the metabolic fate of aglycone of ginseng saponins, *in vitro* and *in vivo* experiments were performed. Incubation of 20(*S*)-prtopanaxatriol (**1**) with rat liver S9 fraction afforded unique ocotillol derivatives, 20, 24-epoxysides (**3** and **4**). Also 20(*S*)-prtopanaxadiol (**2**) gave the corresponding epoxides (**5**). Healthy volunteers were taken with Sanchi Ginseng, which contains protopanaxatriol and protopanaxadiol saponins and no ocotillol saponins. From the alkaline hydrolysate of the urine samples of these volunteers, **3** was detected as well as **1**, and the ratio of **3**/**1** increased up to 2.0 at the maximum at 50 hrs. Biochemical significance of the ocotillol derivatives is discussed, since the main bioactive saponin in *Panax vietnamensis* is an ocotillol-type saponin, majonoside R2 (**7**).

Introduction

Studies of the metabolism of Ginseng saponins have mainly focused on the deglycosylation of ginsenosides. These cumulative findings revealed that the uptake of ginsenosides occurs after complete or partial hydrolysis to yield sapogenins such as 20(*S*)-prtopanaxatriol (**1**) and 20(*S*)-prtopanaxadiol (**2**) or prosapogenins by the acidic conditions of stomach and intestinal flora (Han et al., 1982; Odani et al., 1983; Karikura et al., 1991; Hasegawa et al., 1996; Cui 1995). However, details on the metabolic pathway of the above primary metabolite after absorption from the digestive tract are not known. Very few report on the transformation of the aglycone moiety have been reported (Odani et al., 1983). The oxidation of **1** or **2** with organic peracids leads to its 24, 25-epoxide. However these epoxides are usually unstable and are immediately changed to 20, 24-epoxides (ocotillols) *i.e.* 20(*S*)-protopanaxatriol oxide-II (**3**, 24*S*-epimer) and -I

*Passed away on 30 August 2002, and this paper is dedicated to him.

(**4**, 24R) from **1**, and 20(*S*)-protopanaxadiol oxide-II (**5**, 24*S*-epimer) and -I (**6**, 24*R*) from **2**.

Since an ocotillol-type saponin, majonoside R2 (**7**, 6-xylosylglucoside of **3**) is a main constituent of Vietnamese ginseng, (*Panax vietnamensis*) and has strong biological activity, *i.e.* anti-fatigue and anti-tumor promoting activities, we tried to detect the ocotillol derivatives in human metabolite of ginseng saponins.

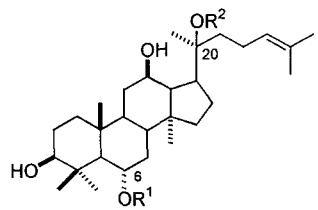
Materials and Methods

Standard samples

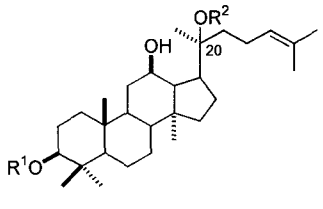
Compound **1** and **2** were obtained by alkaline hydrolysis of ginsenosides Re (**9**) and Rb_1 (**11**), respectively (Chen et al, 1987). Reference standard compounds **3** and **4** from **1**, and **5** and **6** from **2** were prepared by oxidation with *m*-chloroperbenzoic acid (Nagai et al, 1973).

Biotransformation

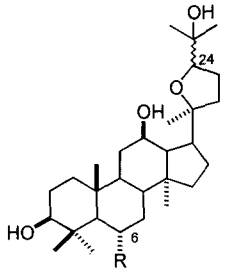
The sapogenins **1** or **2** (5 μmol in 50 μl of DMSO) was incubated with rat liver S9 fraction (0.25 g liver eq) of male Wister rat, pretreated with Phenobarbital as a cytochrome P450 inducer, at 37°C for 1 h in the presence of 0.5 mM $NADP^+$, 5 mM glucose-6-phosphate and 5 mM $MgCl_2$ in a final volume of 5.0 ml of 100 mM potassium phosphate buffer (pH 7.4). The metabolic fraction was quenched by addition of 15 ml of ice-cold MeOH. The metabolic fraction was separated by centrifugation and evaporated. The residue was dissolved into 1 ml of MeOH, and then subjected to HPLC and FAB-MS analysis.



	R^1	R^2
1:	H	H
8:	-Glc	Glc
9:	-Glc^2-Rha	Glc
10:	-Glc^2-Xyl	Glc
14:	-Glc	H



	R^1	R^2
2:	H	H
11:	-Glc^2-Glc	-Glc^6-Glc
12:	-Glc^2-Glc	-Glc
15:	-Glc	-Glc
16:	H	-Glc



3: R=OH, 24*S*
4: R=OH, 24*R*
5: R=H, 24*S*
6: R=H, 24*R*
7: R=O-Glc^2-Xyl, 24*S*

Drug

The fine powder of Sanchi Ginseng (*Panax notoginseng*) was supplied by Yunnan Baiyao Co. Ltd, China, which contains ginsenoside Rg_1 (**8**, 3.35%), Re (**9**, 0.61%) and notoginsenoside-R1 (**10**, 0.89%) as protopanaxatriol saponins, and ginsenoside Rb_1 (**11**, 2.42%) and Rd (**12**, 0.59%) as protopanaxadiol saponins. The saponin contents were determined with HPLC method at detector wavelength: 202 nm, using TSK-GEL ODS-80Ts (4.6 mm id × 15 cm, Toso), mobile phase water/acetonitril (4:1) for protopanaxatriol-type saponins and (69:31) for protopanaxadiol-type saponins, flow rates: 1.0 ml/min.

Administration of Sanchi Ginseng

The finely powdered Sanchi Ginseng (2 g) was taken in 200 ml of water, following, by five healthy male volunteers (age: 26-48) who had not taken any ginseng of ginseng preparations for at least one week prior to the experiment. Urinary samples were collected separately 30 min before administration of the drug, and for eight successive period as follows: 0-3 hr, 3-9 hr, 9-15 hr, 15-24 hr, 24-33 hr, 33-48 hr, 48-57 hr, and 57-72 hr after administration of the drug. The volume of urine at each sampling period was measured, with 5 ml of each sample used for analysis. This study was carried out with informed consent.

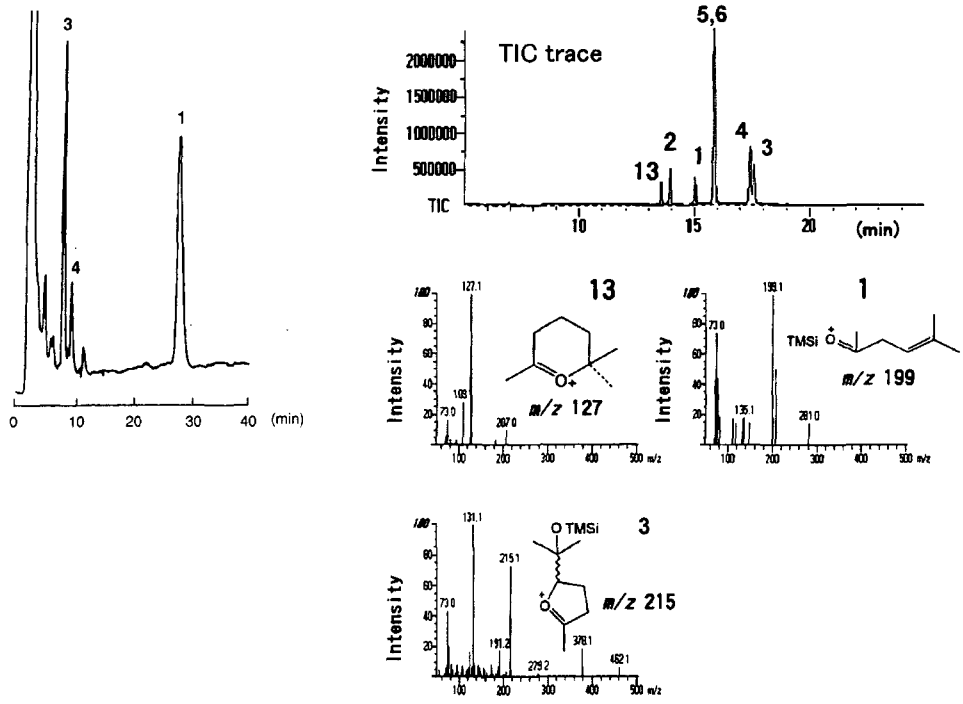
Analysis of urine samples

The human urine (5 ml) was centrifuged and applied to a Sep-Pak C18 cartridge. After washing the cartridge with 20 ml of water and 10 ml of 25% aqueous MeOH, the MeOH fraction was eluted with 3 ml of MeOH and then applied to the alkaline cleavage (Cui 1995). The TMS derivative of the resulted reaction mixture was applied to GC-MS analysis.

Results

In vitro

Incubation of **1** with rat liver S9 fraction at 37°C for 1 hr afforded unique 20,24-epoxysides (**3** and **4**). A typical HPLC chromatogram (column, YMC-Pack ODS-A 100 × 4.6 mm; solvent, 70% aq. MeOH, flow rate, 1 ml/min; detection, reflective index) is shown. The amount of total substance changed was estimated to be 40% of **1**, and the formation of 24*S* epimer (**3**, 40% of the total metabolites) was higher than that of 24*R* epimer (**4**, *ca.* 4 %). The similar reaction



proceeded with 20(*S*)-prtopanaxadiol (**2**) to give the corresponding epoxide (**5**, 50% of the total metabolites). The identity was confirmed by HR-MS and HPLC. In the latter case, the total yield is about 20%, and the 24*R* epimer (**6**) cannot be detected due to the overlapping of the peak. (Kasai et al., 2000), (Fig. 1).

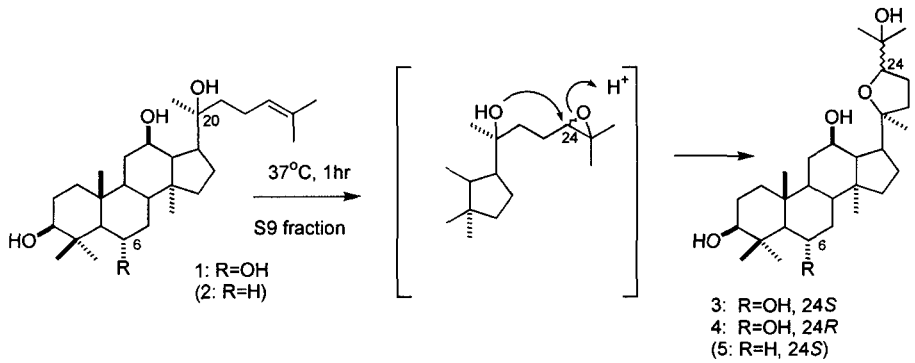


Fig. 1. Biotransformation of triterpene side chain.

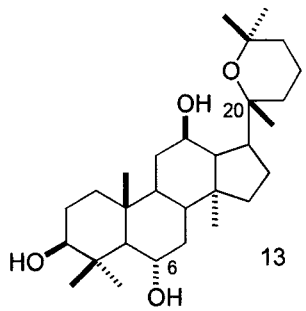


Fig. 2. GC-MS chromatogram in the total ion current (TIC) trace and EI mass spectra of the reference standards (**13**), **1** and **3**.

In vivo

Evaluation of analytical method

TMS derivatives of the reference standards and internal standard, panaxatriol (**13**) were well resolved on GC-MS except the overlapping of **5** and **6**, and exhibited the characteristic ions at *m/z* 199 for **1** and **2**, *m/z* 215 for **3-6**, and *m/z* 127 for **13**, due to the fragmentation of side chains of dammarane skeleton (Fig. 2).

Therefore, GC-MS analysis of the sapogenins in urine was carried out with the EI positive SIM method monitored at *m/z* 199, 215 and 127. No trace of sapogenins were detected in any of the urine samples collected before administration from volunteers, who have not taken any kind of ginseng products for 1 week.

Identification of the sapogenins in urine after consumption of Sanchi Ginseng

The aqueous extract of Sanchi Ginseng was applied to the alkaline cleavage according to the present method. The SIM chromatogram showed two peaks of **1** and **2** without the appearance of their 20,24-epoxide, showing that the drug was free from the ocotillol-type sapogenins and saponins.

After the oral consumption of a single dose (2 g) of Sanchi Ginseng by five volunteers, **1** and **3** were identified in the collected urine after alkaline cleavage by the comparison with authentic samples (Fig. 3). The peak assigned to be **4,** 24 R-epimer of **3**, was also observed as a small shoulder on the peak **3**. In addition, small peaks of **2** and **5** (or **6**) were detected in the urine.

Excretion profiles of 1 and 3 in alkaline treated human urine

The excretion profiles of **1** and **3** in urine are shown in Fig. 4. After oral intake of Sanchi

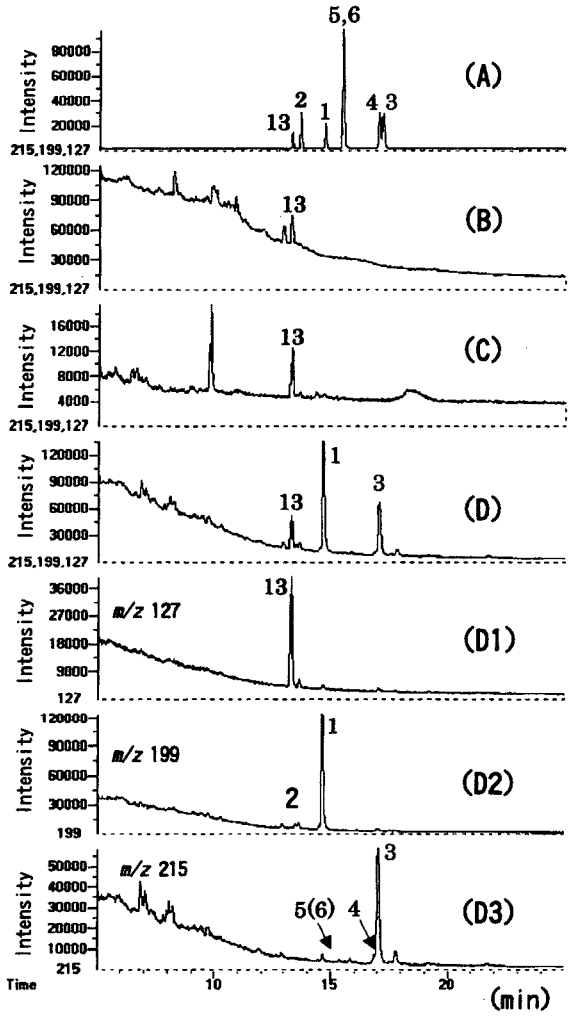


Fig. 3. Ion chromatograms of the standards (**A**), human urine blank treated with alkaline cleavage (**B**), metabolites excreted into human urine without alkaline cleavage (**C**) and with alkaline cleavage (**D**) after consumption of Sanchi Ginseng by EI positive SIM method. Ion chromatograms of **D** monitored by m/z 127 (**D1**), 199 (**D2**) and 215 (**D3**).

Ginseng as a single dose (2 g), **1** was recovered in urine within 3 hr. The urinary excretion of **1** was liable to variation and reached a peak at 15-33 hr among the volunteers. On the contrary, **3** could not be detected in the samples for at least 9 hr, with a maximum excretion observed at from 15-33 hr after administration. The ratio of **3** to **1** in urine of the same volunteer increased time dependently until 57 hr after consumption of Sanchi Ginseng (Fig. 5). The 2 g of the powdered Sanchi Ginseng contains 55 mg of **1**. The cumulative recoveries for the administered dose were

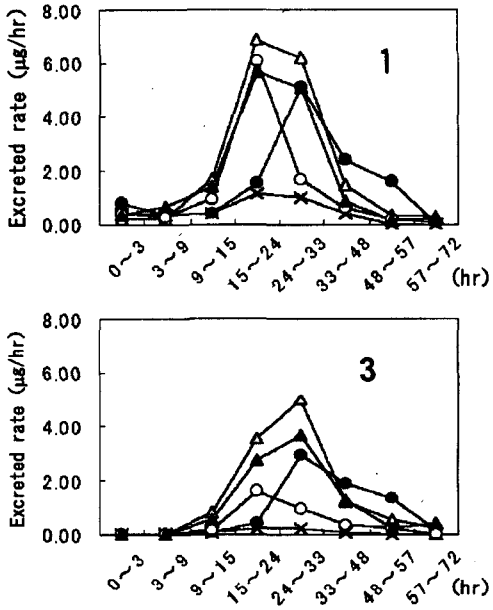


Fig. 4. Excretion profiles of 20(S)-protopanaxatriol (**1**) and 20(S)-protopanaxatriol 20*S*,24*S*-epoxide (**3**) excreted into human urine after consumption of Sanchi Ginseng. Five volunteers are respectively indicated by symbols.

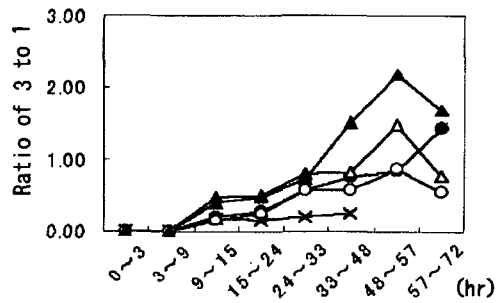


Fig. 5. Time course for the ratio by the excreted amounts of **3** to **1** into human urine in the same volunteer. Five volunteers are respectively indicated by symbols.

respectively in the range of 0.05-0.29% (average: 0.19%) for **1** and 0.009-0.19%(average: 0.11%) for **3**. Total recoveries of **1** and **3** excreted for each volunteer were found to be between 0.06 and 0.48% (average: 0.29%) of the dose. It is to be noted that without alkaline hydrolysis, no peaks in the SIM chromatogram were observed (Matsuura et al, 2002) (Fig. 3C).

Discussion

The partial or complete hydrolysis of ginsenosides under the acidic conditions of the stomach and intestinal flora is the important metabolic pathway leading to the uptake of ginsenosides. In *in vivo* experiments (Odani et al., 1983, Hasegawa et al., 1996, Akao et al., 1998) using intestinal bacteria of human and rat in the presence of ginsenoside Rg_1 (**8**) and Rb_1 (**11**), ginsenosides-Rh_1 (**14**) and compound K (**15**) were found to be the main intermediate to yield the aglycone **1** and **2**, respectively. However the details on the metabolic pathway of ginsenosides after absorption from the digestive tract are not yet clear, especially in human. Our first finding that **1** and **2** with

rat liver microsomes resulted in the identification of their 20,24-epoxides suggested that it might be possible to yield the ocotillol side-chain in human liver via the formation of **1** and **2** or their prosapogenins in the digestive tract after oral administration of 20(*S*) protopanaxatriol-type and 20(*S*) protopanaxadiol-type saponins. In the second experiment, compound **3** was first identified in the human urine along with **1** and trace amount of **4** with the alkaline cleavage after the ingestion of Sanchi Ginseng. No peaks of **1** and **3** in human urine after consumption of Sanchi Ginseng were observed without alkaline cleavage. These findings indicate that the major urinary metabolites of dammarane saponins of Ginseng are not sapogenins, but instead suggest that they exist primarily in the form of glycosides. Further experiments regarding the identification of these metabolites are in progress.

We have been studying chemical and pharmacological aspects of Vietnamese ginseng (*Panax vietnamensis*) for ten years (Yamasaki, 2000). During the course of our study, we found that the main saponin is an ocotillol type, majonoside R2 (**7**) (Duc et al., 1994). This saponin (**7**) showed very strong biological activity, such as attenuator of psychological stress-induced pathophysiological change (Huong et al., 1995) and remarkable anti-tumor-promoting activity (Konoshima et al., 1996, Konoshima et al., 1999). Accordingly we assume that the real biologically active compounds of ginseng might be ocotillol type compounds such as **3**.

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