



## Fatty acid Constituent from the Heat Processed Roots of *Panax ginseng*

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One compound *n*-tetratriacont-20,23-dienoic acid (**1**) along with  $\beta$ -sitosterol- $\beta$ -D-glucopyranoside were isolated and identified from the heat processed roots of *Panax ginseng*. The structure of compound was elucidated with the help of 600 MHz NMR using 1D and 2D NMR (COSY, HSQC and HMBC) spectroscopic techniques aided by FABMS and IR spectra. The *n*-tetratriacont-20,23-dienoic acid (**1**) is reported for the first time from the roots of *Panax ginseng*.

**Key Words:** *Panax ginseng*, Araliaceae, Heat processed roots, *n*-Tetratriacont-20, 23-dienoic acid.

### INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most important oriental medicinal plants in Japan, Korea and China<sup>1,2</sup>. Of the two kinds of ginseng, white ginseng is air dried and red ginseng is produced by steaming raw ginseng at 98-100 °C for 2-4 h<sup>3</sup>. It has been reported that red ginseng is more effective in pharmacological activities than white ginseng<sup>4,7</sup>. The differences in biological activities and chemical constituents of red and white ginsengs have been reported. Ginseng saponins<sup>8</sup> are known as ginsenosides and have an important role in pharmacological activities<sup>9</sup>. Anticarcinogenic and antidiabetic effects of *Panax ginseng* have been reported<sup>10,11</sup>. Acetylenic compounds from the ginseng roots of *P. ginseng* have also been reported<sup>12,13</sup>. The most well known chemical constituent of ginseng is ginsenosides, which is a dammarane glycosides. Dammarane glycosides were reported from heat processed ginseng (Korean red Ginseng)<sup>14-17</sup>.

This paper deals with the isolation and structure elucidation of one compound as *n*-tetratriacont-20,23-dienoic acid (Fig. 1) on the basis of <sup>1</sup>H and <sup>13</sup>C NMR, spectroscopic studies, including 2D-NMR (COSY, HMBC, HSQC) FABMS and IR spectroscopy from the heat processed roots of *P. ginseng*. This is the first report of the isolated compounds (**1**) from the heat processed roots of *P. ginseng*. Due to significance of ginseng roots of this plant as a medicinal, the work in this area has already been done. The aim of the present investigation is to report some of the findings in the form of natural product from Korean red Ginseng.

### EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, methanol, ethanol, sulfuric acid and vanillin were purchased from Daejung Chemicals and Metals Co., Ltd, Korea. Precoated TLC plates (layer thickness 0.25 mm) and silica gel used for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63  $\mu$ m) were from Merck (Darmstadt, Germany). Authentic samples of standards were purchased from Sigma-Aldrich (USA). Optical rotation was measured on an AA-10 model polarimeter (Instruments Ltd, Seoul, South Korea). Both <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on a Bruker Avance 600 high resolution spectrometer operating at 600 and 150 MHz, respectively. This NMR machine was available at National Instrumentation Center for Environmental Management (NICEM) Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm ( $\delta$ ) and coupling constants (*J*) in hertz (Hz). FAB MS data were recorded on a JMS-700 (Jeol, Japan) spectrometer instrument which was available at Korea Basic Science Institute (KBSI) Daegu, South Korea. IR spectra were recorded on a Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea.

Fresh ginseng (*P. ginseng*) was cultivated of ground dried roots ginseng (6 years old) in Ganghwado, South Korea. A voucher specimen (No. PG-R-11) has been deposited at the Department of Applied Life Science, Konkuk University.

Korean Red ginseng was prepared by using non-peeled fresh ginseng, which was steamed at 98 °C for 2 h using an autoclave. The steamed ginseng after drying and powdered (297.8 g) was prepared for extraction.

**Extraction of Korean red Ginseng powder:** The Korean red ginseng powder (297.8 g) were immersed in methanol (3 × 1 L) for 3 days at room temperature and then the supernatant was concentrated under vacuum to yield (30.1 g) of the extract, which was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively to produce 5 g, 8.9 g and 14.2 g extract, respectively.

**Isolation of the compounds from *n*-butanol extract:** The entire butanol extract was subjected to normal phase column chromatography over silica gel (600 g) to yield 26 fractions (each of 500 mL) with the following eluents: fractions 1-2 with chloroform, fractions 3-4 with chloroform:methanol (9.8:0.2), fractions 5-6 with chloroform:methanol (9.5:0.5), fractions 7-8 with chloroform:methanol (9:1), fractions 9-10 with chloroform:methanol (8:2), fractions 11-12 with chloroform:methanol (7:3), fractions 13-14 with chloroform:methanol (6:4), fractions 15-16 with chloroform:methanol (4:6), fractions 17-18 with chloroform:methanol (3:7), fractions 19-20 with chloroform:methanol (8:2), fractions 21-22 with chloroform:methanol (1:9) and fractions 23-26 with methanol. All fractions were examined by TLC. Fractions 1-4 were not further separated due to the low amount of the substance. Fractions 5-6 (0.9 g) were crystallized after the purification by column chromatography, yielding  $\beta$ -sitosterol- $\beta$ -D-glucoside (20 mg) whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fractions 23-26 (4.4 g) was re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40-63  $\mu$ m; 200 g; each fraction 100 mL). The elution was sequentially performed with methanol and water to yield 10 fractions. Fractions 1-2 with water:methanol (8:2), fractions 3-4 with water:methanol (6:4), fractions 5-6 with water:methanol (4:6), fractions 7-8 with methanol (2:8). Fractions 9-10 with methanol. Fraction 9-10 (1.1 g, 100 mL fraction) after rechromatography over silica gel with chloroform and methanol. The elution was sequentially performed with chloroform containing 0.5 and 1 % to yield one compound **1** (21 mg).

***n*-Tetratriacont-20,23-dienoic acid (1):** Colourless solid,  $R_f$  0.34 (CHCl<sub>3</sub>:MeOH; 9.8:0.2);  $[\alpha]_D^{25}$  -21.4 (c 0.1, CHCl<sub>3</sub>); IR

(KBr,  $\nu_{max}$ , cm<sup>-1</sup>): 3335, 2924, 2854, 1708, 1651, 1458, 1413, 1376, 1248, 1073, 1034, 991, 722; <sup>1</sup>H NMR (CDCl<sub>3</sub>);  $\delta$  5.38 (1H, m, H-23), 5.36 (1H, m, H-21), 5.34 (1H, m, H-20), 5.32 (1H, m, H-24), 2.78 (2H, t,  $J = 7.2$  Hz, H<sub>2</sub>-22), 2.34 (2H, dd,  $J = 7.2$  Hz, 7.8 Hz, H<sub>2</sub>-2), 2.06 (2H, m, H<sub>2</sub>-19), 2.03 (2H, m, H<sub>2</sub>-25), 1.64 (2H, m, CH<sub>2</sub>), 1.36 (16 H, br s, 8 × CH<sub>2</sub>), 1.32 (20 H, br s, 10 × CH<sub>2</sub>), 1.30 (8H, br s, 4 × CH<sub>2</sub>), 1.26 (4H, br s, 2 × CH<sub>2</sub>), 0.87 (3H, t,  $J = 7.1$  Hz, Me-34); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  180.37 (C-1), 130.13 (C-21), 129.94 (C-23), 127.85 (C-20), 127.70 (C-24), 34.06 (C-22), 31.89 (C-21), 31.49 (C-25), 29.63 (CH<sub>2</sub>), 29.54 (CH<sub>2</sub>), 29.40 (CH<sub>2</sub>), 29.31 (CH<sub>2</sub>), 29.20 (CH<sub>2</sub>), 29.10 (CH<sub>2</sub>), 29.03 (7 × CH<sub>2</sub>), 28.99 (6 × CH<sub>2</sub>), 27.16 (CH<sub>2</sub>), 27.14 (CH<sub>2</sub>), 25.58 (CH<sub>2</sub>), 24.61 (CH<sub>2</sub>), 22.65 (CH<sub>2</sub>), 22.53 (CH<sub>2</sub>), 14.01 (Me-34); FAB MS  $m/z$  (rel. int.) 505 [M+H]<sup>+</sup> (C<sub>34</sub>H<sub>64</sub>O<sub>2</sub>) (11.2), 459 (23.3), 363 (7.1), 337 (8.6), 323 (20.5), 207 (48.9).

## RESULTS AND DISCUSSION

Compound **1** was obtained as a colourless compound. Its IR spectrum exhibited characteristic absorption bands for carboxylic function (3335, 1708 cm<sup>-1</sup>), unsaturation (1651 cm<sup>-1</sup>) and long aliphatic chain (722 cm<sup>-1</sup>). The FAB mass and <sup>13</sup>C NMR spectral data led to established molecular formula (C<sub>34</sub>H<sub>64</sub>O<sub>2</sub>) ion peak  $m/z$  505. The mass fragmentation pattern of **1** is shown in Fig. 2.

The <sup>1</sup>H NMR spectrum of **1** showed two one-proton multiplets at  $\delta$  5.38 and 5.36 was assigned to vinylic H-23 and H-21, respectively. Two one-proton multiplets at  $\delta$  5.34 and 5.32 were assigned to vinylic H-20 and H-24, respectively. Two-proton triplets at  $\delta$  2.78 ( $J = 7.2$  Hz) and two protons double doublet at  $\delta$  2.34 ( $J = 7.2, 7.8$  Hz) were associated to vinylic carbon H<sub>2</sub>-22 and H<sub>2</sub>-2, respectively. Two two-proton multiplets at  $\delta$  2.06 and 2.03 were attributed to H<sub>2</sub>-19 and H<sub>2</sub>-25. The remaining methylene protons were resonated between  $\delta$  1.64-1.26. Three protons triplets at  $\delta$  0.87 were associated correspondingly to C-34 primary methyl protons. The <sup>13</sup>C NMR spectrum of **1** displayed important signals for carboxylic carbons at  $\delta$  180.37 (C-1), vinylic carbons at  $\delta$  130.13 (C-21), 129.94 (C-23), 127.85 (C-20) and 127.70 (C-24) and methyl carbon at  $\delta$  14.01 (C-34). The remaining methylene carbons were resonated between at  $\delta$  34.06-22.53. The <sup>1</sup>H-<sup>1</sup>H COSY spectrum of **1** showed correlation of vinylic protons H-20 and H-21 with the adjacent methylene protons

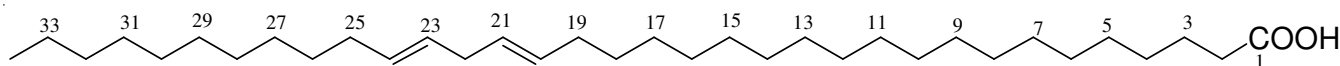


Fig. 1. Chemical structure of *n*-tetratriacont-20,23-dienoic acid (**1**)

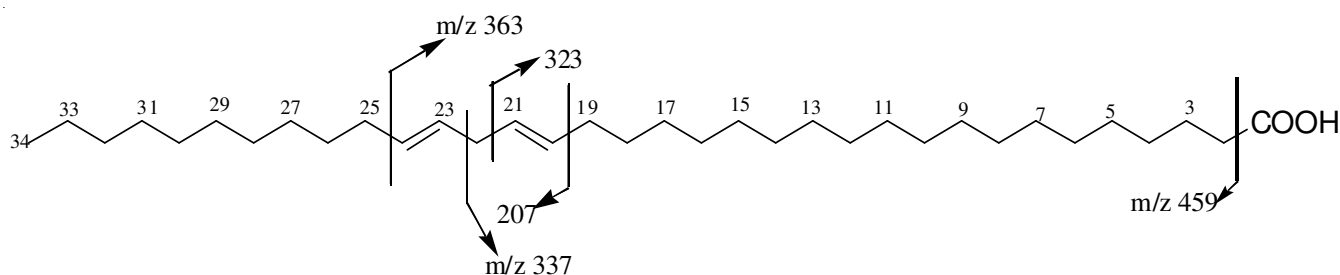


Fig. 2. Fragmentation pattern of *n*-tetratriacont-20,23-dienoic acid (**1**)

H<sub>2</sub>-19 and H<sub>2</sub>-22; H-23 and H-24 with H<sub>2</sub>-22 and H<sub>2</sub>-25; H<sub>2</sub>-2 with H<sub>2</sub>-3, H<sub>2</sub>-4 and H<sub>2</sub>-5; H<sub>3</sub>-34 with adjacent methylene protons. The HMBC spectrum correlations of C-1 with H<sub>2</sub>-2, H<sub>2</sub>-3 and H<sub>2</sub>-4; C-20 with H<sub>2</sub>-19 and H-12; C-21 with H<sub>2</sub>-22, H-20 and H<sub>2</sub>-19; C-30 with methylene protons. The HSQC spectrum experiment showed key-correlation between the vinylic proton at  $\delta$  5.38 and 5.36 with carbon signals at  $\delta$  129.94 and 130.13, respectively and at  $\delta$  5.34 and 5.32 and the carbon signals  $\delta$  127.85 and 127.70, respectively and between methyl protons at  $\delta$  0.87 and the corresponding carbon signals at  $\delta$  14.01. On the basis of this evidence the structure of **1** has been established as *n*-tetratriacont-20,23-dienoic acid.

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