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New Aliphatic Alcoholic Acid Constituent from the Roots of *Panax ginseng*

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One new constituent 14α,15α,16α,17α,18α,19α,20α,21α-octahydroxy-16,17,18-trimethyl-*n*-triacontan-*trans*,*trans*-22,24-dien-1-oic acid was isolated and identified from the methanol extract of heat processed roots of *Panax ginseng*. The structure of compound was elucidated with the help of 600 MHz by 1D and 2D NMR (COSY, HSQC, HMBC) spectroscopic techniques aided by FAB MS and IR spectra.

Key Words: *Panax ginseng*, Araliaceae, Heat processed roots.

INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is one of the best known Chinese crude drugs and it has been investigated extensively in search bioactive principles[1]. Especially the polyglycosidic constituents, which are principal ingredient of ginseng, have been the subjects of many investigations and various ginsenosides have been characterized[1-3]. The roots of *P. ginseng* have been used in traditional medicine in Japan, China and Korea and are known for several bioactivity[4-8]. The biologically active constituents of these ginsengs have been pursued extensively[9]. Various ginsenosides, the dammarane-type triterpene oligoglycosides have been characterized as the principal ingredient of white ginseng[10,11].

Of the two kinds of ginseng, white ginseng is air dried and red ginseng is produced by steaming raw ginseng at 98-100 ºC for 2-3 h[12]. It has been reported that red ginseng is more effective in pharmacological activities than white ginseng[5,13-15]. The differences in biological activities and chemical constituents of red and white ginsengs have been reported. Ginseng saponins are known as ginsenosides and series of triterpenoids saponins have been reported and an important role in pharmacological activities[16].

Anticarcinogenic and antidiabetic effects of *P. ginseng* have been reported[4,17]. Polyacetylenes compounds have been isolated previously from the ginseng roots of *P. ginseng*[6,8-19]. The most well known chemical constituent of ginseng are ginsenosides, which are dammarane glycosides. Dammarane glycosides were reported from many parts of ginseng and heat processed *P. ginseng* roots[20-22].

In continuation of our previous work[23,24] on *P. ginseng* roots one more new compound 14α,15α,16α,17α,18α,19α, 20α,21α-octahydroxy-16,17,18-trimethyl-*n*-triacontan-*trans*, *trans*-22,24-dien-1-oic acid (**1**) was isolated as a natural product. This paper deals with the isolation and structure elucidation of compound (**1**) on the basis of ^{1}H and ^{13}C NMR, spectroscopic studies, including 2D NMR (COSY, HMBC, HSQC), FAB-MS and IR spectroscopy from the heat processed roots of *P. ginseng*.

EXPERIMENTAL

All chemicals were of analytical grade: *n*-hexane, EtOAc, MeOH, EtOH, H_2SO_4 and vanillin were purchased from Daejung Chemicals and Metals (Seoul, Korea). Thin layer chromatography was performed on precoated silica gel 60 F_{254} plates (Merck). Visulization of the TLC plates was performed using a 5 % H_2SO_4 in C_2H_5OH spray reagent. Column chromatography was performed using silica gel (70-230 mesh) and LiChroprep RP-18 (40-63 μm; ODS silica gel) from Merck. Optical rotation was measured with an instrument Ltd. (Seoul, Korea) model AA-10 polarimeter. IR spectra were recorded on a Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology (KIST), Seoul, Korea. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 600 (Germany) high resolution spectrometer operating at 600 and 150 MHz, respectively. This NMR machine was available at National Instrument center for Environment and Management (NICEM), Seoul National University (SNU), Seoul, South Korea). NMR experiments included COSY, HSQC and HMBC. NMR spectra were obtained in deuterated $CDCl_3$ using (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. FAB/MS data were recorded on

a JMS-700 (Jeol, Japan) spectrometer instrument which was available at Korea Basic Science Institute, Seoul 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 ºC 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.

Separation and isolation: The ethyl acetate extract was separated on a silica gel column (70-230 mesh, 500 g, 4.5 × 95 cm) and was eluted with a gradient of *n*-hexane-$CHCl_3$ to yield 30 fractions (each of 250 mL): fractions 1-4 with *n*-hexane, fractions 5-6 with *n*-hexane-chloroform (7.5:2.5), fractions 7-8 with *n*-hexane-chloroform (1:1), fractions 9-10 with *n*-hexane-chloroform (2.5:7.5), fractions 11-12 with chloroform, fractions 13-14 with chloroform-methanol (99:1), fractions 15-18 with chloroform-methanol (98:2), fractions 19-22 with chloroform-methanol (97:3), fractions 23-26 with chloroform-methanol (96:4), fractions 27-30 with chloroform-methanol (95:0.5). Fraction 13-14 (0.6 g) was re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40-63 μm: 50 g; each fraction 50 mL). The elution was sequentially performed with methanol and water to yield five fractions. Fractions 1 with water-methanol (1:1), fractions 2 with water-methanol (2:8), fractions 3 with water-methanol (1:9), fractions 4 and 5 with methanol. Fraction 5 (0.2 g,) after rechromatography over silica gel with chloroform and methanol to yield one new compound **1** (54 mg).

14α,15α,16α,17α,18α,19α,20α,21α-octahydroxy-16,17,18-trimethyl-*n*-triacontan-*trans*,*trans*-22,24-dien-1-oic acid: Dark yellow semi-solid; R_f 0.38 ($CHCl_3$:MeOH; 9:1); $[\alpha]_D^{22}$-22.1º (c, 0.2, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3260, 2925, 2845, 1698, 1635, 1460, 1265, 1081, 915, 720; ^{1}H NMR ($CDCl_3$; 600 MHz): δ 5.92 (1H, m, $w_{1/2}$ = 14.2, H-22), 5.58 (1H, dd, *J* = 16.8, 9.6 Hz, H-23), 5.25 (1H, dd, *J* = 16.8, 9.6 Hz, H-24), 4.93 (1H, m, $w_{1/2}$ = 15.2 Hz, H-25), 4.36 (1H, br m, $w_{1/2}$ = 14.1 Hz, H-21β), 4.16 (1H, br m, $w_{1/2}$ =15.2 Hz, H-20β), 3.67 (1H, d, *J* = 17.8 Hz, H-19β), 3.64 (1H, d, *J* = 16.8 Hz, H-15β), 3.60 (1H, br m, $w_{1/2}$ = 16.5 Hz, H-14 β), 2.58 (2H, br s, H_2-2), 2.34 (2H, m, H_2-26), 2.04 (2H, m, H_2-13), 1.63 (2H, m, CH_2), 1.49 (4H, br s, 2 x CH_2), 1.29 (24 H, br s, 12 × CH_2), 1.25 (3H, br s, Me-35), 1.23 (3H, br s, Me-34), 1.21 (3H, br s, Me-33), 0.88 (3H, t, *J* = 6.5 Hz, Me-32); ^{13}C NMR ($CDCl_3$; 150 MHz); 177.20 (C-1), 138.94 (C-23), 136.10 (C-24), 116.94 (C-22), 114.28 (C-25), 78.25 (C-18), 74.88 (C-17), 73.07 (C-21), 72.15 (C-20), 70.76 (C-19), 68.87 (C-15), 63.20 (C-14), 34.01 (C-2), 33.77 (C-24), 33.65 (CH_2), 33.43 (CH_2), 31.77 (CH_2), 30.61 (CH_2), 30.01 (CH_2), 29.64 (CH_2), 29.53 (2 x CH_2), 29.39 (CH_2), 29.19 (2 x CH_2) 29.01 (CH_2), 28.87 (CH_2), 28.76 (CH_2), 28.54 (CH_2), 28.25 (CH_2), 25.57 (C-33), 24.83 (C-34), 22.62 (CH_2), 22.60 (CH_2), 14.05 (C-32); FAB MS *m/z* (rel. int.): 647 $[M + H]^+$ ($C_{35}H_{67}O_{10}$) (7.8), 405 (9.2), 373 (12.8), 329 (14.1), 273 (33.6), 241 (35.7), 211 (33.2).

RESULTS AND DISCUSSION

Compound **1** (Fig. 1) was obtained as dark yellow semi-solid mass and its IR spectrum of **1** showed characteristic absorption bands for carboxylic group (3260, 1698 cm^{-1}), unsaturation (1635 cm^{-1}) and aliphatic chain (720 cm^{-1}). The FAB mass of **1** showed a molecular ion peak at *m/z* 647 $[M + H]^+$ consistent to the molecular formula of a resinous acid $C_{35}H_{66}O_{10}$. The ion fragments arising at *m/z* 211 [C_{19}-C_{20} fission]$^+$, 241 [C_{18}-C_{19} fission]$^+$, 405 $[M-241]^+$ indicated the presence of the hydroxyl group at C-19, C-20 and C-21 with unsaturated aliphatic chain. The ion peak generating at *m/z* 273 [C_{15}-C_{16} fission, CHOH- CHOH $(CH_2)_{12}$ COOH]$^+$ the location of the hydroxyl groups at C-14 and C-15 (Fig. 2).

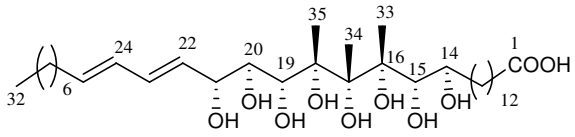

Fig. 1. Chemical structure of compound **1**

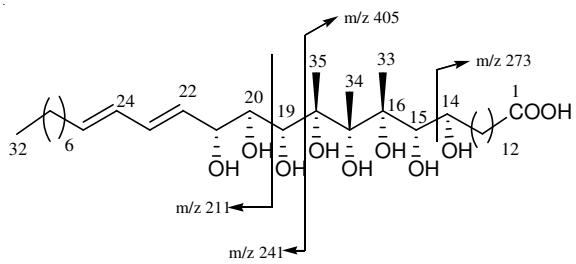

Fig. 2. Fragmentation patterns of compound **1**

The ^{1}H NMR spectrum of **1** exhibited three one-proton signals as multiplets at δ 5.92 ($W_{1/2}$ = 14.7 Hz) and as double doublets at δ 5.58 (*J* = 16.8, 9.6 Hz), 5.25 (*J* = 16.8, 9.6 Hz) and 4.93 ($W_{1/2}$ = 15.2 Hz) assigned to *trans*-oriented H-22, H-23, H-24 and H-25, respectively. Five one proton signals as broad multiplets at δ 4.36 ($W_{1/2}$ =14.1 Hz), 4.16 ($W_{1/2}$= 15.2 Hz) and 3.60 ($W_{1/2}$= 16.5 Hz) and as doublets as δ 3.67 (*J* =17.8 Hz) and 3.64 (*J* = 16.8 Hz), were attributed correspondingly to β-oriented carbinol H-21, H-20, H-14, H-19 and H-15 protons. The methylene protons resonated between δ 2.58-1.29. Three broad singlets at δ 1.25, 1.23 and 1.21 and triplets at δ 0.88 (*J* = 6.5 Hz), all integrating for three protons each were associated with tertiary C-35, C-34, C-33 and primary C-32 methyl protons, respectively, all attached to saturated carbons. The ^{13}C NMR spectrum of **1** displayed signals for carboxylic carbons at δ 177.20 (C-1), vinylic carbons between δ 138.94-114.28; carbinol carbons from δ 78.25 to 63.20, methylene carbons in the range of δ 34.01-28.25 and at δ 22.62 and methyl carbons at δ 22.57 (Me-33), 24.83 (Me-34), 22.62 (Me-35) and 14.05 (Me-32), The ^{1}H-^{1}H COSY spectrum of **1** showed correlations of H-25 with H_2-26, H-24 and H-23; H-21 with H-22, H-20 and H-19; H-19 with H-20 and Me-25; and H-15 with H-14 and Me-23. The HMBC spectrum exhibited

interactions of C-1 with H_2-2; C-14 with H_2-13 and H-15; C-21 with H-22, H-20 and H-19; C-18 with H-19 and Me-35; C-33 with H-15 and Me-34. The HSQC spectrum of **1** showed interactions of vinylic protons of H-22, H-23, H-24 and H-25 with the vinylic carbons C-22, C-23, C-24 and C-25, respectively; and carbinol protons H-14, H-15, H-19, H-20 and H-21 with the respective carbons C-14, C-15, C-19 C-20 and C-21. On the basis of these evidences the structures of **1** has been elucidated as 14α,15α,16α,17α,18α,19α,20α,21α-octahydroxy-16,17,18-trimethyl-*n*-triacontan-*trans*,*trans*-22,24-dien-1-oic acid. This is a new aliphatic alcoholic acid.

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