New Chemical Constituent from *Panax ginseng* Roots

ILL-MIN CHUNG, SHENGXIANG YANG, SEUNG-HYUN KIM and ATEEQUE AHMAD*

Department of Applied Bioscience, College of Life and Environmental Sciences, Konkuk University, Seoul 143-701, Republic of Korea

*Corresponding author: Fax: +82 2 4467856; Tel: +82 2 4503730; E-mail: ateeqe97@gmail.com

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One new constituent lanost-24-en-3 β -ol-3 β -D-xylopyranosyl-(2'→1'')- β -D-xylopyranoside has been 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 NMR using 1D and 2D spectral methods viz., ^1H and ^{13}C aided by FAB/MS and IR spectroscopy.

Keywords: *Panax ginseng*, Araliaceae, Heat processed roots, Lanost-24-en-3 β -ol-3 β -D-xylopyranosyl-(2'→1'')- β -D-xylopyranoside.

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 principles¹. Especially the polyglycosidic constituents, which are principal ingredient of ginseng, have been the subjects of many investigations and various ginsenosides have been characterized¹⁻³. The roots of *P. ginseng* have been used in traditional medicine in Japan, China and Korea and are known for several bioactivity⁴⁻⁸. The biologically active constituents of these ginsengs have been pursued extensively⁹ and in recent years, 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 °C for 2-3 h¹². 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 reported⁶ and an important role in pharmacological activities¹⁶. 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*^{6,18,19}. 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* roots²⁰⁻²².

In continuation of our previous work^{23,24} on *P. ginseng* roots one more new compound **1** was isolated as a natural

product. This paper deals with the isolation and structure elucidation of compound **1** on the basis of ^1H 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

Melting points were determined using Electrochemical Engineering (Electrothermal, Seoul, South Korea) model IA9100 melting point apparatus. Optical rotations was measured with a model AA-10 polarimeter (Instrument Ltd. Seoul, Korea). IR spectra were recorded on a Thermo Mattson Scientific FT-IR model Nicolet 6700 spectrophotometer at the Korea Institute of Science and Technology (KIST) Seoul, South Korea. ^1H and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker Avance-600 spectrometer, available at the National Instrumentation Centre for Environmental Management (NICEM), College of Agriculture and Life Science, Seoul National University (SNU), Seoul, South Korea. NMR spectra was obtained in deuterated pyridine- d_5 and methanol- d_4 using tetramethylsilane (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. High-resolution ES/FT mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific) equipped with Dionex U 3000 HPLC system (NICEM, Seoul National University). All chemicals were of analytical grade. *n*-Hexane, ethyl acetate, methanol, ethanol, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals

(Seoul, South Korea). Thin layer chromatography was performed on precoated silica gel 60 F254 plates (Merck). Visualization of the TLC plates was performed using a 5 % H_2SO_4 in $\text{C}_2\text{H}_5\text{OH}$ spray reagent. Column chromatography (CC) was performed using silica gel (70-230 mesh) and LiChroprep RP-18 (40-63 μm ; ODS silica gel) from Merck. Authentic standards of sugars were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Fresh ginseng (*P. ginseng*) consisted of ground dried roots ginseng (6 years old) was collected from Ganghwado, South Korea. A voucher specimen (No. PG-R-11) has been deposited at the Department of Applied Bioscience, 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. After 70 % ethanol extraction, the extracts obtained from these red ginseng, the residues were called the by-products of red ginseng marc. These by-products were dried and powdered (297.8 g) was used for extraction in this study.

Extraction and isolation: The red ginseng marc 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 an extract, which was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol, successively, to produce 5, 8.9 and 14.2 g extracts, respectively. The BuOH (14.2 g) extract was subjected to column chromatography on silica gel (70-230 mesh, 500 g, 4.5×95 cm) and eluted with a gradient of *n*-hexane/ CHCl_3 /MeOH to yield 34 fractions (each of 250 mL): Frs. 1-4 with *n*-hexane, frs. 5-6 with *n*-hexane- CHCl_3 (7.5:2.5), frs. 7-8 with *n*-hexane- CHCl_3 (1:1), frs. 9-10 with *n*-hexane- CHCl_3 (2.5:7.5), frs. 11-12 with CHCl_3 , frs. 13-14 with CHCl_3 -MeOH (99:1), frs. 15-16 with CHCl_3 -MeOH (98:2), frs. 17-18 with CHCl_3 -MeOH (97:3), frs. 19-20 with CHCl_3 -MeOH (96:4), frs. 21-22 with CHCl_3 -MeOH (95:5), frs. 23-24 with CHCl_3 -MeOH (94:6), frs. 25-26 with CHCl_3 -MeOH (93:7), frs. 27-28 with CHCl_3 -MeOH (92:8), frs. 29-30 with CHCl_3 -MeOH (9:1), frs. 31-32 with CHCl_3 -MeOH (8.8:1.2), frs. 33-34 with CHCl_3 -MeOH (8.5:1.5). Fractions 29-30 (1.4 g) were chromatographed over LiChroprep RP-18 (ODS silica gel; 40-63 μm ; 100 g; 45×2 cm, each fraction 100 mL). Fractions 29-30 of the first column after additional rechromatography with elution sequentially performed with CH_3OH - H_2O , yielded 10 frs.: frs. 1-2 with H_2O -MeOH (1:1), frs. 3-4 with H_2O -MeOH (2:8), frs. 5-6 with H_2O -MeOH (1:9), frs. 7-10 with MeOH. Frs. 1-2 (0.8 g, 100 mL fraction) after rechromatography over silica gel with chloroform and methanol. The elution was sequentially performed with chloroform containing methanol 5, 10 and 12 % to yield one new compound **1** (29 mg).

Lanost-24-en-3 β -ol-3 β -D-xylopyranosyl-(2'→1'')- β -D-xylopyranoside (1**):** Yellow crystalline solid; m.p. 198-99 °C; $[\alpha]_D^{22}$ -34 (*c* 1, MeOH); IR (KBr, ν_{max} , cm^{-1}) 3357, 3260, 2923, 2854, 1457, 1377, 1257, 1075, 1046; ^1H NMR (methanol- d_4 , 600 MHz) and ^{13}C NMR (methanol- d_4 , 150 MHz) (Table-1); FABMS (positive) m/z 693 [$\text{M} + \text{H}$]; ($\text{C}_{40}\text{H}_{69}\text{O}_9$) (1.6).

Acid hydrolysis of compound 1: The solution of compound **1** (each 5 mg) was separately refluxed with 2 mL of 1 M hydrochloric acid-dioxane (1:1) in water bath at 70-80 °C for 4 h. After cooling, the reaction mixture was diluted with water and extracted with chloroform to yield aglycone and the

aqueous layer contained glycone was subjected to TLC (CHCl_3 : CH_3OH : H_2O :AcOH; 16:9:2:2) together with an authentic sample of xylose.

RESULTS AND DISCUSSION

Compound **1** (Fig. 1) was obtained as a yellow crystalline mass and its protonated molecular ion peak at m/z 693 [$\text{M} + \text{H}$] $^+$ was determined on the basis of FAB mass and ^{13}C NMR spectra and indicating nine degrees of unsaturation. The ^1H NMR spectrum of **1** showed signals a one-proton triplet at δ_{H} 5.07 (t, $J = 6.6$ Hz), respectively, which was assigned to the vinylic H-24 proton. A one-proton double doublet at δ 3.39 (dd, $J = 5.8, 9.3$ Hz), was ascribed to the oxymethine H-3 α proton. The three-proton singlets at δ 0.74, 1.10, 1.56, 1.60, 0.92, 0.98 and 0.79 and three-proton doublet at δ 0.2 (d, $J = 6.5$ Hz) were assigned to eight methyls (see Table-1). The sugar units in **1** were identified as β -xylopyranose by analysis of the coupling constants of the anomeric signals of sugar protons as one-proton doublets at δ 4.29 (d, $J = 7.8$, H-1') and 4.22 (d, $J = 7.2$, H-1''). The methylene protons in sugars appeared as double doublets at δ 3.78, 3.26 (dd, $J = 3.0, 3.0$) and 3.71, 3.69 (dd, $J = 4.2, 4.2$), were assigned for H-5' and H-5'' protons and other methylene protons appeared as multiplets resonated between δ 2.27-0.98 in lanostane skeleton, except C-12 protons appeared as triplet (t, $J = 7.8$ Hz). The ^{13}C NMR spectrum of **1** displayed 40 carbon signals, with 30 attributed to the aglycone part and 10 to disaccharide units (Table-1). Carbon signals were observed for vinylic carbons at δ 124.85 (C-24), 131.30 (C-25) and for an oxygenated methine carbon at δ 81.42 (C-3), with an anomeric carbons occurring at δ_{C} 104.93 (C-1') and 89.69 (C-1'') and other sugar carbons resonating in the range δ 76.26-61.81 and methyl carbons at δ 15.49 (C-18), 17.42 (C-19), 16.79 (C-21), 29.53 (C-26), 25.97 (C-27), 28.52 (C-28), 26.74 (C-29) and 16.05 (C-30). The compound was assigned after comparison of NMR values with the reported of such triterpenes^{25,26}.

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

TABLE-1
¹H (600 MHz) AND ¹³C NMR (150 MHz)
 NMR DATA FOR **1** (CD₃OD)^a

Position	δ _H (J in Hz)	δ _C
1	2.77 m, 1.94 m	34.65
2	2.05 m, 1.98 m	27.65
3	3.29 dd (5.8, 9.3)	81.42
4	-	38.81
5	1.92 m	53.40
6	1.48 m, 1.38 m	17.95
7	1.08 m, 0.98 m	27.65
8	1.68 m	48.11
9	1.34 m	49.89
10	-	36.63
11	1.23 m, 1.25 m	22.05
12	2.22 t (7.8)	33.97
13	-	41.65
14	-	51.39
15	1.77 m, 1.46 m	30.91
16	1.52 m, 1.31 m	34.69
17	1.49 m	51.39
18	0.74 br s	1.49
19	1.10 br s	32.27
20	1.23 m	32.27
21	0.82 d (6.5)	16.79
22	1.66 m, 1.71 m	39.06
23	1.80 m, 1.77 m	25.03
24	5.07 t (6.6)	124.85
25	-	131.30
26	1.56 br s	29.53
27	1.60 br s	25.97
28	0.92 br s	26.52
29	0.98 br s	26.74
30	0.79 br s	16.05
1'	4.29 d (7.8)	104.93
2'	3.76 m	76.27
3'	3.36 m	73.96
4'	3.47 dd (5.4, 5.1)	70.68
1''	4.22 d (7.2)	89.69
2''	3.37 m	75.36
3''	3.25 m	73.20
4''	3.49 dd (5.4, 4.8)	69.98
5''	3.71 d (4.2), 3.69 d (4.2)	61.81

^aCoupling constants in parenthesis are given in hertz

¹H-¹H COSY spectrum of compound **1** showed correlations of H-3 with H₂-1, H₂-2, H₂-4 and H₂-1'; H-6 with H₂-4, H₂-7 and H-8; and H-2' with H-1' and H-3'; H-2'' with H-1'' and H-3''. The HMBC spectrum of **1** showed interactions of C-3 with H₂-2, H₂-4 and H-1'; C-24 with H₃-26, H₃-27, H₂-23 and H₂-22; C-6' with H-1''; C-6 with H₂-7 and H-8. The HSQC spectrum of **1** showed correlations of H-3 at δ 3.39 with C-3 at δ 81.42; H-1' at δ 4.29 with C-1' at δ 104.93 and H-1'' at δ 4.22 with C-2'' at δ 89.69. Acid hydrolysis of **1** yielded xylopyranose (co-TLC comparable). The identity xylopyranose units of **1** were determined by comparison of the ¹³C NMR data (Table-1) with the corresponding monosaccharides²⁷. On the basis of this evidence, the structure of **1** was established as lanost-24-en-3β-ol-3β-D-xylopyranosyl-(2'→1'')-β-D-xylopyranoside.

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