Asian Journal of Chemistry; Vol. 26, No. 22 (2014), 7789-7791

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17847

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: ateeque97@gmail.com

Received: 18 April 2014; *Accepted*: 20 June 2014; *Published online*: 6 November 2014; AJC-16236

One new constituent lanost-24-en-3β-ol-3β-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*., ^{1}H and ^{13}C aided by FAB/MS and IR spectroscopy.

Keywords: *Panax ginseng*, Araliaceae, Heat processed roots, Lanost-24-en-3β-ol-3β-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 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] 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 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[6] 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*[6,18,19]. 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* roots[20-22].

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 ^{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

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. ^{1}H 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. FABMS data were recorded on a JMS-700 (JEOL, Japan) spectrometer instrument which was available at Korea Basic Science Institute, Seoul Korea. High-resolution ESIFT 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 C_2H_5OH 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 chromatograp-hed over Li chroprep 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-3β-ol-3β-D-xylopyranosyl-(2'→1'')-β-D-xylopyranoside (1): Yellow crystalline solid; m.p. 198-99 °C; $[\alpha]^{22}_D$ -34 (*c* 1, MeOH); IR (KBr, ν_{max}, cm^{-1}) 3357, 3260, 2923, 2854, 1457, 1377, 1257, 1075, 1046; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) (Table-1); FABMS (positive) *m/z* 693 [M + H]; ($C_{40}H_{69}O_9$) (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 $[M + H]^+$ was determined on the basis of FAB mass and ^{13}C NMR spectra and indicating nine degrees of unsaturation. The ^{1}H 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-3α 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].

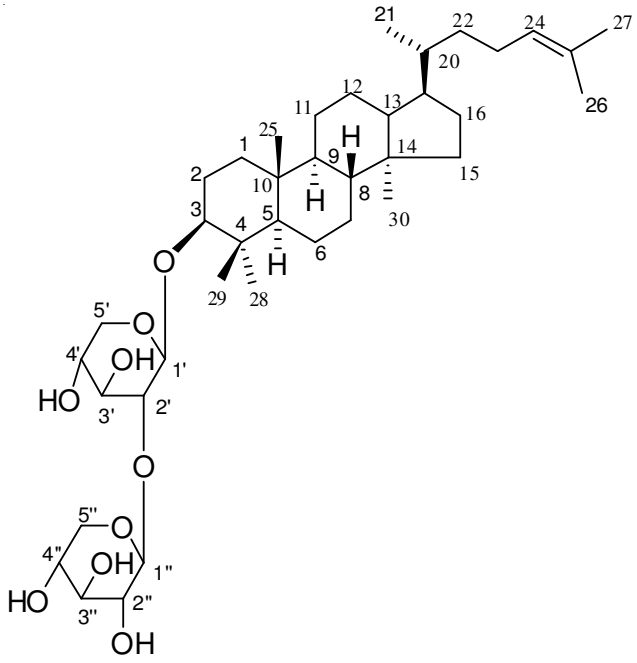

Fig. 1. Chemical structure of compound **1**

TABLE-1
^{1}H (600 MHz) AND ^{13}C NMR (150 MHz)
NMR DATA FOR **1** (CD_3OD)[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

[a]Coupling constants in parenthesis are given in hertz

^{1}H-^{1}H COSY spectrum of compound **1** showed correlations of H-3 with H_2-1, H_2-2, H_2-4 and H_2-1'; H-6 with H_2-4, H_2-7 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 H_2-2, H_2-4 and H-1'; C-24 with H_3-26, H_3-27, H_2-23 and H_2-22; 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 ^{13}C NMR data (Table-1) with the corresponding monosaccharides[27]. 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.

ACKNOWLEDGEMENTS

This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ 906938), Rural Development Administration, Republic of Korea. This work was also carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ 906938), Rural Development Administration, Republic of Korea.

REFERENCES

1. J. Shoji, in eds.: H. Oura, A. Kumagaki, S. Shibata, K. Takagi, Panax Ginseng, Kyoritsu Shuppan, Tokyo, pp. 10-41 (1981).
2. H. Besso, R. Kasai, Y. Saruwatari, T. Fuwa and O. Tanaka, *Chem. Pharm. Bull. (Tokyo)*, **30**, 2380 (1982).
3. I. Kitagawa, T. Taniyama, T. Hayashi and M. Yoshikawa, *Chem. Pharm. Bull. (Tokyo)*, **31**, 3353 (1983).
4. A.S. Attele, Y.-P. Zhou, J.-T. Xie, J.A. Wu, L. Zhang, L. Dey, W. Pugh, P.A. Rue, K.S. Polonsky and C.-S. Yuan, *Diabetes*, **51**, 1851 (2002).
5. W.M. Kim, J.M. Kim, S.B. Han, S.K. Lee, N.D. Kim, M.K. Park, C.K. Kim and J.H. Park, *J. Nat. Prod.*, **63**, 1702 (2000).
6. N. Fukuyama, M. Shibuya and Y. Orihara, *Chem. Pharm. Bull. (Tokyo)*, **60**, 377 (2012).
7. H. Matsunaga, M. Katano, H. Yamamoto, H. Fujito, M.K. Mori and T. Takata, *Chem. Pharm. Bull. (Tokyo)*, **38**, 3480 (1990).
8. M.C. Rho, H.S. Lee, S.W. Lee, J.S. Chang, O.E. Kwon, M.Y. Chung and Y.K. Kim, *J. Agric. Food Chem.*, **53**, 919 (2005).
9. B.H. Han, in ed.: H.W. Base, Korean Ginseng, Korean Ginseng Research Institute, Seoul, pp. 77-108 (1978).
10. O. Tanaka and R. Kasai, in eds.: W. Herz, H. Griesebach, G.W. Kirby and Ch. Tamm, Progress in the Chemistry of Organic Natural Products, Springer-Verlag Vienna, New York, pp. 1-76 (1984).
11. I. Kitagawa, T. Taniyama, M. Yoshikawa, Y. Ikenishi and Y. Nakagawa, *Chem. Pharm. Bull. (Tokyo)*, **37**, 2961 (1989).
12. D.Y. Kim, *J. Korean Agric. Chem. Soc.*, **16**, 60 (1973).
13. T.K. Takaku, K.Y. Kameda, K. Matsuura, H. Sekiya and H. Okuda, *Planta Med.*, **56**, 27 (1990).
14. S.D. Lee and H. Okuda, *Korean J. Ginseng Sci.*, **14**, 67 (1990).
15. J.H. Do, H.O. Lee, S.K. Lee, K.B. Noh, S.D. Lee and K.S. Lee, *Korean J. Ginseng Sci.*, **17**, 145 (1993).
16. K.Y. Nam, Contemporary Korean Ginseng: Chemical Constituents and Biological Activity; Korea Ginseng and Tobacco Research Institute: Daejeon, Korea, Chap. 3, pp. 56-134 (1996).
17. T.K. Yun, Y.S. Lee, Y.H. Lee, S.I. Kim and H.Yung, *J. Korean Med. Sci.*, **16(Suppl)**, S6 (2001).
18. K. Hirakura, K. Morita, M.K. Nakajima, Y. Ikeya and H. Mitsuhashi, *Phytochemistry*, **31**, 899 (1992).
19. S.C. Shim, S.K. Chang, C.W. Hur and C.K. Kim, *Phytochemistry*, **26**, 2849 (1987).
20. I.H. Park, N.Y. Kim, S.B. Han, J.M. Kim, S.W. Kwon, H.J. Kim, M.K. Park and J.H. Park, *Arch. Pharm. Res.*, **25**, 428 (2002).
21. J.H. Ryu, J.H. Park, J.H. Eun, D.H. Jung and D.H. Sohn, *Phytochemistry*, **44**, 931 (1997).
22. N.I. Baek, D.S. Kim, Y.H. Lee, J.D.C. Park, C.B. Lee and S.I. Kim, *Planta Med.*, **62**, 86 (1996).
23. I.M. Chung, M.Y. Kim, P. Nagella, Y.P. Hong and A. Ahmad, *Asian J. Chem.*, **25**, 1086 (2013).
24. I.M. Chung and M. Ali, *Asian J. Chem.*, **25**, 4667 (2013).
25. S.B. Mahato and A.P. Kundu, *Phytochemistry*, **37**, 1517 (1994).
26. M. Ali, Techniques in Terpenoid Identifications; Birla Publishers: New Delhi, pp 91-94 (2001).
27. D.Q. Yu and J.S. Yang, Handbook of Analytical Chemistry: Analysis of NMR Spectra, Chemical Industry Press, Beijing, edn. 2, Volume 7 (1999).