Natural Product Sciences
14(3) : 177-181 (2008)

Polyacetylenes from the Tissue Cultured Adventitious Roots of *Panax ginseng* C.A. Meyer

Guang-Hua Xu, Soo-Jin Choo, In-Ja Ryoo, Young-Hee Kim, Kee-Yoeup Paek[1], and Ick-Dong Yoo*

Functional Metabolite Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea
[1]Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, 361-763, Korea

Abstract – Five polyacetylenes, ginsenoyne K (**1**), (*Z*)-1-methoxyheptadeca-9-en-4,6-diyne-3-one (**2**), panaxydol (**3**), panaxydiol (**4**), and (*E*)-heptadeca-8-en-4,6-diyne-3,10-diol (**5**) were isolated from the adventitious roots of *Panax ginseng* and their chemical structures were elucidated by interpretation of spectroscopic data. Among the isolated compounds, compounds **2** and **5** were reported for the first time as a natural product. Ginsenoyne K (**1**) showed dose-dependent inhibitory effect on dopa oxidase activity of tyrosinase.
Keywords – *Panax ginseng*, polyacetylenes, tyrosinase

Introduction

Ginseng, the roots of *Panax ginseng* C.A. Meyer (Araliaceae), has been known as a mystic medicinal herb in the East Asian countries, such as Korea, China and Japan since ancient times. Thousands of papers have reported its chemical constituents, biological activities and cultivation. The most well-known chemical constituent of ginseng is ginseng saponin, more than 30 ginsenosides have been reported from ginseng so far, which were main effective components responsible for the pharmacological and biological activities, such as antiaging effects, antidiabetic effects, anticancer effects, protection against physical and chemical stress, analgesic and antipyretic effects and so on (Park *et al.*, 1996). In addition, more than 20 polyacetylene derivatives have been isolated from ginseng, cytotoxicity (Ahn *et al.*, 1988; Matsunaga *et al.*, 1990) and ACAT inhibitory effects (Rho *et al.*, 2005) of them have been reported. Recently, tissue cultured methods of wild *Panax ginseng* were developed, which has made its mass production possible (Hahn *et al.*, 2003). The adventitious roots of *Panax ginseng* C.A. Meyer are regarded as an efficient alternative to cell culture or hairy root culture for biomass production due to its fast growth and stable metabolite production, and it was widely used as health functional foods and over-the-counter drugs. However, there are relatively few pharmacognostical reports of the adventitious roots of wild *Panax ginseng*. In this paper, we will report isolation of some polyacetylenes from the adventitious roots of *Panax ginseng* and their tyrosinase inhibitory activities.

*Author for correspondence
Fax: +82-42-860-4595; E-mail: idyoo@kribb.re.kr

Experimental

General procedure – Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. The NMR spectra were recorded on Varian Unity 400 spectrometer, chemical shifts were reported in ppm downfield from TMS. The EI-MS spectra were recorded on JMS-700 mass spectrometer (JEOL, Japan). Column chromatography was carried out on Merk silica gel (70 - 230 mesh) and Merck Lichroprep RP-18 gel (40 - 63 μm). TLC was performed on aluminum plates precoated with Kieselgel 60 F_{254} (Merck). For preparative HPLC, Waters 600 pump (Waters, USA), UV-8010 detector (Tosoh) and YMC-Pack Prep-ODS (20 × 250 mm) column were used.

Plant material, induction, proliferation of callus and roots – Four-year old fresh mountain ginseng (*Panax. ginseng* C.A. Meyer) roots were collected, sterilized, and cultured as described by Paek et al (Ali *et al.*, 2006). Briefly, Cultures were maintained at 25 ± 2°C for 4 weeks for callus induction. For callus proliferation, the callus was subcultured on fresh medium every 4 weeks at 25 ± 2°C in darkness. Callus was transferred to solid Murashige and Skoog (MS) medium (1965) containing 3.0 mg/L

indole butyric acid (IBA) and 3% sucrose to induce adventitious roots at 25 ± 2°C in darkness. The induced adventitious roots were subcultured every 5 weeks in 400 mL conical flasks containing 100 mL liquid MS medium supplemented with 3.0 mg/ L IBA and 3% sucrose on a rotary shaker (100 rpm) at 25 ± 2°C in darkness. Adventitious roots were selected and proliferated further in 5 L airlift balloon type bubble bioreactors containing 4 L 3/4th strength MS liquid medium supplemented with 5.0 mg/L IBA, 0.1 mg/L kinetin, and 5% sucrose for 40 - 45 days and was named as Chungbuk National University Line 1 (CBN-1). These proliferated roots were used as explants for further experiments.

Extraction and isolation – The dried adventitious roots of *Panax ginseng* C.A. Meyer (1 kg) was extracted three times with MeOH at room temperature for seven days. The MeOH solution was concentrated under reduced pressure to give a residue (295 g) and it was partitioned between H_2O and *n*-hexane. The hexane extract (44 g) was loaded on a silica gel column and eluted with *n*-hexane/EtOAc in a gradient mode to give 10 fractions (PGH1-10). The fraction PGH4 was rechromatographed over a reverse phase column eluting with MeOH/H_2O in a step gradient to give 4 fractions (PGH41-4), the fraction PGH42 and PGH43 were purified by prep-HPLC to afford **1** (10 mg) and **2** (2 mg), respectively. The fraction PGH6 was subjected to Sephadex LH-20 column chromatography and eluted with CH_2Cl_2 : MeOH (1 : 1) to give 3 fractions (PGH61-3). The fraction PGH62 was rechromatographed on a silica gel column and eluted with *n*-hexane/CH_2Cl_2 in a gradient mode to give 4 fractions (PGH621-624). The subfraction PGH621 was purified by prep-TLC to afford **3** (35 mg). The fraction PGH8 was chromatographed on a Sephadex LH-20 column and eluted with CH_2Cl_2 : MeOH (1 : 1) to give 4 fractions (PGH81-4). The fraction PGH82 was further separated by prep-HPLC to afford **4** (90 mg) and **5** (7 mg).

Ginsenoyne K (1) – Pale yellow oil; $[\alpha]^{20}_D$ –25.2° (*c* 0.1, $CHCl_3$); UV λ_{max} (MeOH): 215, 241, 254, 269, 284 nm; EI-MS *m/z*: 276 $[M]^+$; ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.98 (1H, br s, OH), 6.25 (1H, dd, *J* = 16.0, 7.2 Hz, H-9), 5.97 (1H, ddd, *J* = 16.8, 10.4, 5.6 Hz, H-2), 5.79 (1H, d, *J* = 16.0 Hz, H-8), 5.49 (1H, dt, *J* = 17.2, 1.2 Hz, H-1b), 5.28 (1H, dt, *J* = 10.4, 1.2 Hz, H-1a), 4.99 (1H, br d, *J* = 5.2 Hz, H-3), 4.38 (1H, brq, *J* = 6.8 Hz, H-10), 1.63 (1H, m, H-11a), 1.47 (1H, m, H-11b), 1.22~1.37 (10H, m, –$(CH_2)_5$–), 0.89 (3H, t, *J* = 6.8 Hz, H-17); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.2 (C-9), 136.1 (C-2), 117.5 (C-1), 112.1 (C-8), 85.98 (C-10), 81.05 (C-4), 77.15 (C-7), 74.37 (C-6), 70.97 (C-5), 63.88 (C-3), 32.39 (C-11), 31.94 (C-15), 29.62 (C-13), 29.28 (C-14), 25.33 (C-12), 22.83 (C-16), 14.28 (C-17).

(*Z*)-1-Methoxyheptadeca-9-en-4,6-diyne-3-one (2) – Pale yellow oil; UV λ_{max} (MeOH): 210, 241, 254, 268, 285 nm; EI-MS *m/z*: 274 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.56 (1H, m, H-10), 5.36 (1H, m, H-9), 3.71 (2H, t, *J* = 6.6 Hz, H-1), 3.34 (3H, s, OCH_3), 3.11 (2H, d, *J* = 6.6 Hz, H-8), 2.80 (2H, t, *J* = 6.0 Hz, H-2), 2.02 (2H, t, *J* = 6.6 Hz, H-11), 1.2~1.4 (10H, m, –$(CH_2)_5$–), 0.85 (3H, t, *J* = 6.6 Hz, H-17); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 184.7 (C-3), 133.9 (C-10), 120.7 (C-9), 88.78 (C-4), 76.58 (C-7), 72.23 (C-5), 66.95 (C-1), 63.45 (C-6), 58.85 (OCH_3), 45.45 (C-2), 31.79 (C-15), 29.15 (C-12), 29.13 (C-13), 29.11 (C-14), 27.25 (C-11), 22.62 (C-16), 18.04 (C-8), 14.07 (C-17).

Panaxydol (3) – Brown oil; $[\alpha]^{20}_D$ –8.0° (*c* 0.01, $CHCl_3$); UV λ_{max} (MeOH): 208, 229, 240, 253, 267, 284 nm; EI-MS *m/z*: 274 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.94 (1H, ddd, *J* = 17.2, 10.0, 5.6 Hz, H-2), 5.47 (1H, dd, *J* = 16.8, 1.2 Hz, H-1b), 5.25 (1H, dd, *J* = 9.6, 1.0 Hz, H-1a), 4.92 (1H, br d, *J* = 5.2 Hz, H-3), 3.15 (1H, ddd, *J* = 7.2, 5.4, 3.9 Hz, H-9), 2.97 (1H, br td, *J* = 6.4, 4.0 Hz, H-10), 2.70 (1H, ddd, *J* = 17.6, 5.6, 1.0 Hz, H-8a), 2.39 (1H, ddd, *J* = 17.6, 6.8, 1.0 Hz, H-8b), 1.52 (2H, m, H-11), 1.24~1.40 (10H, m, –$(CH_2)_5$–), 0.89 (3H, t, *J* = 6.8 Hz, H-17); ^{13}C-NMR (100MHz, $CDCl_3$): δ 136.2 (C-2), 117.4 (C-1), 76.92 (C-7), 75.12 (C-4), 71.05 (C-5), 66.49 (C-6), 63.67 (C-3), 57.22 (C-10), 54.54 (C-9), 31.93 (C-15), 29.61 (C-13), 29.37 (C-14), 27.70 (C-11), 26.65 (C-12), 22.82 (C-16), 19.63 (C-8), 14.29 (C-17).

Panaxydiol (4) – Yellowish oil; $[\alpha]^{20}_D$ –32.5° (*c* 0.05, $CHCl_3$); UV λ_{max} (MeOH): 216, 241, 269, 284 nm; EI-MS *m/z*: 260 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.33 (1H, dd, *J* = 15.6, 5.1 Hz, H-9), 5.95 (1H, ddd, *J* = 17.1, 10.5, 6.0 Hz, H-2), 5.76 (1H, d, *J* = 15.9 Hz, H-8), 5.47 (1H, dd, *J* = 16.8, 1.1 Hz, H-1b), 5.26 (1H, dd, *J* = 10.5, 1.0 Hz, H-1a), 4.97 (1H, d, *J* = 5.1 Hz, H-3), 4.19 (1H, brq, *J* = 6.6 Hz, H-10), 1.52 (2H, brq, *J* = 7.6 Hz, H-11), 1.20~1.40 (10H, m, –$(CH_2)_5$–), 0.88 (3H, t, *J* = 7.5 Hz, H-17); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 150.2 (C-9), 136.2 (C-2), 117.5 (C-1), 108.3 (C-8), 80.63 (C-4), 77.75 (C-7), 73.74 (C-6), 72.28 (C-10), 71.12 (C-5), 63.88 (C-3), 37.09 (C-11), 31.98 (C-15), 29.63 (C-13), 29.39 (C-14), 25.41 (C-12), 22.84 (C-16), 14.29 (C-17).

(*E*)-Heptadeca-8-en-4,6-diyne-3,10-diol (5) – Yellow oil; $[\alpha]^{20}_D$ –13.8° (*c* 0.02, $CHCl_3$); UV λ_{max} (MeOH): 214, 240, 254, 267, 284 nm; EI-MS *m/z*: 262 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.32 (1H, dd, *J* = 16.0, 6.0 Hz, H-9), 5.77 (1H, d, *J* = 16.0 Hz, H-8), 4.43 (1H, t, *J* = 6.4 Hz, H-3), 4.20 (1H, q, *J* = 6.0 Hz, H-10), 1.77 (2H, br q, *J* =

7.6 Hz, H-2),1.54 (2H, br q, J = 7.6 Hz, H-11), 1.21~1.38 (10H, m, –$(CH_2)_5$–), 1.03 (3H, t, J = 7.2 Hz, H-1), 0.89 (3H, t, J = 6.4 Hz, H-17); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.9 (C-9), 108.4 (C-8), 83.11 (C-4), 77.10 (C-7), 73.91 (C-6), 72.29 (C-10), 69.79 (C-5), 64.46 (C-3), 37.11 (C-11), 31.98 (C-15), 39.91 (C-2), 29.64 (C-13), 29.39 (C-14), 25.41 (C-12), 22.84 (C-16), 14.29 (C-17), 9.54 (C-1).

Mushroom tyrosinase assay – Mushroom tyrosinase, L-tyrosine, and L-DOPA were purchased from Sigma Chemical (St. Louis, MO, USA). Tyrosinase activity was determined by spectrophotometry as described by Pomerantz with minor modifications (Pomerantz *et al.*, 1963]. Briefly, 20 μL of 10 mM L-DOPA, 168 μL of 100 mM phosphate buffer (pH 6.5), and 2 μL of test sample solution were mixed. Then 10 μL of mushroom tyrosinase (2000 U/mL) was added. The amount of dopachrome produced in the reaction mixture was determined against a blank (solution without enzyme) at 475 nm (OD475) using a spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The percent inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ Inhibition} = [(A - B) / A] \times 100$$

where A = absorbance at 475 nm without test sample and B = absorbance at 475 nm with test sample.

Results and Discussion

Repeated chromatography of hexane-soluble fraction of MeOH extract of the dried adventitious roots of *Panax ginseng* C.A. Meyer led to the isolation of five compounds (Fig. 1), and they were identified as Ginsenoyne K (**1**), (Z)-1-methoxyheptadeca-9-en-4,6-diyne-3-one (**2**), panaxydol (**3**), panaxydiol (**4**), and (E)-heptadeca-8-en-4,6-diyne-3,10-diol (**5**), respectively.

Compound **1** was isolated as pale yellow oil and had the molecular formula $C_{17}H_{24}O_3$ as determined by EI-MS, ^{1}H–NMR, ^{13}C-NMR and DEPT spectral data. The UV spectrum of **1** showed a typical absorption bands for a diyne chromophore (Bohlmann *et al.*, 1973). The ^{1}H NMR spectrum of **1** showed typical terminal vinyl proton signals at δ 5.97 (1H, ddd, J = 16.8, 10.4, 5.6 Hz, H-2), 5.49 (1H, dt, J = 17.2, 1.2 Hz, H-1b) and 5.28 (1H, dt, J = 10.4, 1.2 Hz, H-1a), and another vinyl signals at δ 6.25 (1H, dd, J = 16.0, 7.2 Hz, H-9), 5.79 (1H, d, J = 16.0 Hz, H-8) due to *trans*-double bond of Δ8,9, it also exhibited oxygenated methine signals at δ 4.99 (1H, br d, J = 5.2 Hz, H-3), 4.38 (1H, brq, J = 6.8 Hz, H-10), and a terminal methyl signal at δ 0.89 (3H, t, J = 6.8 Hz, H-17). The ^{13}C NMR spectrum of **1** showed 17 carbon peaks including

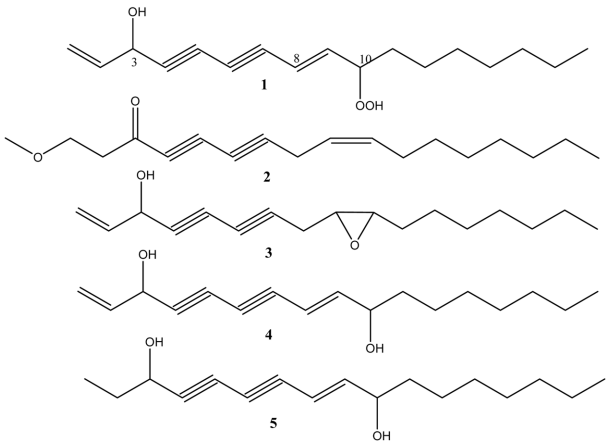

Fig. 1. Chemical structures of compounds isolated from *Panax ginseng*.

four olefinic carbons at δ 146.2 (C-9), 136.1 (C-2), 117.5 (C-1), 112.1 (C-8), four conjugated acetylene carbons at δ 81.05 (C-4), 77.15 (C-7) and 74.37 (C-6), and one oxygen-bearing methine carbon at δ 63.88 (C-3). Another oxygenated methine carbon downfield shifted +13.7 ppm to δ 85.98 (C-10) when compared with that of panaxydiol, indicating the presence of a hydroperoxy group at C-10. On the basis of above evidences, compound **1** was identified as Ginsenoyne K by direct comparison its spectral data with those of literature (Hirakura *et al.*, 1992).

Compound **2** was obtained as pale yellow oil. Its molecular formula, $C_{18}H_{26}O_2$, was determined by EI-MS, ^{1}H–NMR, ^{13}C-NMR and DEPT spectral data. The ^{1}H NMR spectrum of **2** was very similar to 1-methoxy-(9*R*,10*S*)-epoxyheptadecan-4,6-diyn-3-one (Lee *et al.*, 2004), showing an oxygen-bearing methylene proton at δ 3.71 (2H, t, J = 6.6 Hz, H-1), a methoxy signal at δ 3.34 (3H, s, OCH_3), eight methylene proton at δ 3.11 (2H, d, J = 6.6 Hz, H-8), 2.80 (2H, t, J = 6.0 Hz, H-2), 2.02 (2H, t, J = 6.6 Hz, H-11) and 1.2~1.4 (10H, m, –$(CH_2)_5$–), and a terminal methyl proton at δ 0.85 (3H, t, J = 6.6 Hz, H-17). However, the epoxy methine signals at δ 2.68/2.53 were substituted by two olefinic proton signals at δ 5.56 (1H, m, H-10) and 5.36 (1H, m, H-9). The ^{13}C NMR spectrum of **2** exhibited 18 carbons including a carbonyl signal at δ 184.7 (C-3), two vinyl carbons at δ 133.9 (C-10) and 120.7 (C-9), four conjugated acetylene carbons at δ 88.78, 76.58, 72.23 and 63.45, one methoxy group at δ 58.85, one oxygenated methylene carbon at δ 66.95, eight methylene carbons at upfield region and a terminal methyl carbon at δ 14.07. Thus, compound **2** was elucidated as (*Z*)-1-methoxyheptadeca-9-en-4,6-diyne-3-one by comparison of its spectral data with those of as analogy, panaxynol (Yang *et al.*, 2008). While this compound has been

synthesized previously (Magalhaes *et al.*, 1992), it has not been isolated as a natural product so far and the ^{13}C NMR data is presented here for the first time.

Compound **3** was isolated brown oil and had the molecular formula $C_{17}H_{24}O_2$ as determined by EI-MS, ^1H–NMR, ^{13}C-NMR and DEPT spectral data. The ^1H NMR spectrum of **3** exhibited an allylic alcohol moiety at δ 5.94 (1H, ddd, J= 17.2, 10.0, 5.6 Hz, H-2), 5.47 (1H, dd, J= 16.8, 1.2 Hz, H-1b), 5.25 (1H, dd, J= 9.6, 1.0 Hz, H-1a) and 4.92 (1H, br d, J= 5.2 Hz, H-3), two oxygen-bearing methine at δ 3.15 (1H, ddd, J= 7.2, 5.4, 3.9 Hz, H-9), 2.97 (1H, br td, J= 6.4, 4.0 Hz, H-10), and a triplet peak at δ 0.89 (3H, t, J= 6.8 Hz) due to a methyl group. The ^{13}C NMR spectrum of **3** showed two vinyl carbons at δ 136.2 (C-2) and 117.4 (C-1), four sp carbons at δ 76.92 (C-7), 75.12 (C-4), 71.05 (C-5), and 66.49 (C-6), three oxygen-bearing methines at δ 63.67 (C-3), 57.22 (C-10), and 54.54 (C-9), one methyl carbon at δ 14.29, and seven methylenes at δ 31.93, 29.61, 29.37, 27.70, 26.65, 22.82 and 19.63. In addition, the chemical shifts of two methine was about 1 and 15 ppm upfield in ^1H and ^{13}C NMR spectra, respectively, indicating an epoxide ring at C-9/C-10. Therefore, compound **3** was identified as panaxydol by comparison its NMR spectral data with those reported in the literature (Hirakura *et al.*, 1991; Beak *et al.*, 1996).

Compound **4** was obtained as yellowish oil and had the molecular formula $C_{17}H_{24}O_2$ as determined by EI-MS, ^1H-NMR, ^{13}C-NMR and DEPT spectral data. The ^1H NMR spectrum of **4** exhibited an allylic alcohol moiety at δ 5.95 (1H, ddd, J= 17.1, 10.5, 6.0 Hz, H-2), 5.47 (1H, dd, J= 16.8, 1.1 Hz, H-1b), 5.26 (1H, dd, J= 10.5, 1.0 Hz, H-1a) and 4.97 (1H, d, J= 5.1 Hz, H-3), two vinyl protons at δ 6.33 (1H, dd, J= 15.6, 5.1 Hz, H-9), 5.76 (1H, d, J= 15.9 Hz, H-8) corresponding to a *trans* double bonds, a hydroxylmethine proton at δ 4.19 (1H, brq, J= 6.6 Hz, H-10), a terminal methyl protons at δ 0.88 (3H, t, J= 7.5 Hz, H-17), and six methylene groups between δ 1.20-1.50. The ^{13}C NMR spectrum of **4** showed four vinyl carbons at δ 150.2 (C-9), 136.2 (C-2), 117.5 (C-1), 108.3 (C-8), four sp carbons at δ 80.63 (C-4), 77.75 (C-7), 73.74 (C-6), 71.12 (C-5), two oxygen-bearing methines at δ 72.28 (C-10), 63.88 (C-3), one methyl carbon at δ 14.29 (C-17), and six methylenes at δ 37.09 (C-11), 31.98 (C-15), 29.63 (C-13), 29.39 (C-14), 25.41 (C-12), 22.84 (C-16). The spectral data discussed herein was in good agreement with those reported in the literature (Shim *et al.*, 1987) and compound **4** was identified as panaxydiol.

Compound **5** was obtained as yellow oil. Its molecular formula, $C_{17}H_{26}O_2$, was determined by EI-MS, ^1H-NMR, ^{13}C-NMR and DEPT spectral data. The ^1H NMR spectrum of **5** exhibited two vinyl protons at δ 6.32 (1H, dd, J= 16.0, 6.0 Hz, H-9), 5.77 (1H, d, J= 16.0 Hz, H-8) due to a *trans* double bonds at Δ8,9, two hydroxylmethine proton at δ 4.43 (1H, t, J= 6.4 Hz, H-3), 4.20 (1H, q, J= 6.0 Hz, H-10), two terminal methyl protons at δ 1.03 (3H, t, J= 7.2 Hz, H-1), 0.89 (3H, t, J= 6.4 Hz, H-17), and six methylene groups at δ 1.77 (2H, br q, J= 7.6 Hz, H-2),1.54 (2H, br q, J= 7.6 Hz, H-11), 1.21~1.38 [10H, m, –$(CH_2)_5$–]. These NMR data suggested compound **5** was a hydrate of panaxydiol (Hirakura *et al.*, 1991) at Δ1,2. The ^{13}C NMR spectrum of **5** confirmed the suggested structure of **5**, displaying two vinyl carbons at δ 149.9 (C-9) and 108.4 (C-8), four sp carbons at δ 83.11 (C-4), 77.10 (C-7), 73.91 (C-6), and 69.79 (C-5), two oxygen-bearing methines at δ 72.29 (C-10) and 64.46 (C-3), two methyl carbon at δ 14.29 (C-17) and 9.54 (C-1), and seven methylene carbons at δ 37.11 (C-11), 31.98 (C-15), 39.91 (C-2), 29.64 (C-13), 29.39 (C-14), 25.41 (C-12), and 22.84 (C-16). Based on above evidences, the chemical structure of compound **5** was elucidated as (*E*)-heptadeca-8-en-4,6-diyne-3,10-diol. This compound has been synthesized before (Schulte *et al.*, 1977) but not reported as a natural product so far, and here we present its NMR spectral data for the first time.

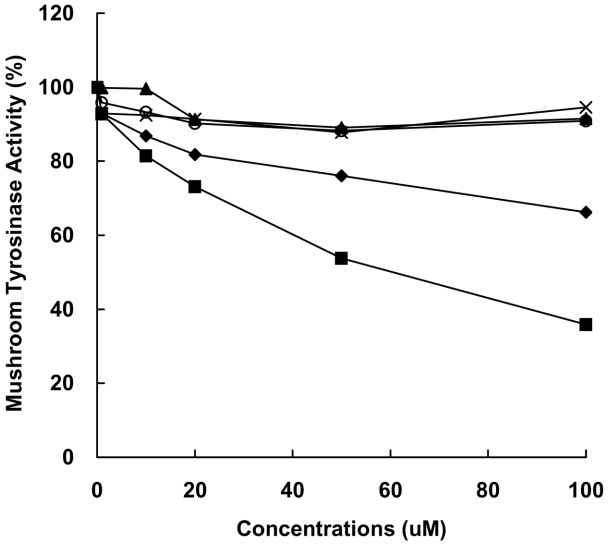

Fig. 2. Effects of polyacetylenes on the mushroom tyrosinase activity. Dose-dependent inhibitory effect of polyacetylenes on dopa oxidase activity of tyrosinase in represented as % of control. Ginsenoyne K (◆), Panaxydol (×), Panaxydiol (▲), (E)-heptadeca-8-en-4,6-diyne-3,10-diol (○), Kojic acid (■).

The isolated compounds were tested for their inhibitory effects against murine tyrosinase activity. As a result, compound **1** showed dose-dependent inhibitory effect on dopa oxidase activity of tyrosinase as shown in Fig. 2,

and it inhibited 30% of the tyrosinase activity at a concentration of 100 μM. However, other isolated compounds exhibited no tyrosinase inhibitory activities.

Acknowledgements

This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050432).

References

Ahn, B.Z. and Kim, S.I., Relation between structure and cytotoxic activity of panaxydol analogs against L1210 cells. *Arch. Pharm.* **321**, 61-63 (1988).

Ali, M.B., Hahn, E.J. and Paek, K.Y., Copper-induced changes in the growth, oxidative metabolism, and saponin production in suspension culture roots of *Panax ginseng* in bioreactors. *Plant Cell Rep.* **25**, 1122-1132 (2006).

Beak, N.I., Kim, D.S., Park, J.D., Lee, Y.H., and Kim, S.I., Polyacetylenes from the rhizome of Korean red ginseng. *Korean J. Ginseng Sci.* **20**, 30-35 (1996).

Bohlmann, F., Burkhardt, T. and Zdero, C., Naturally occurring acetylenes. London: Academic Press, 1973.

Hahn, E.J., Kim, Y.S., Yu, K.W., Jeong, C.S., and Paek, K.Y., Adventitious root cultures of *Panax ginseng* C.A. Meyer and ginsenoside production through large-scale bioreactor system. *J. Plant Biotechnol.* **5**, 1-6 (2003).

Hirakura, K., Morita, M., Nakajima, K., Ikeya, Y., and Mitsuhashi, H., Three acetylenic compounds from roots of *Panax ginseng*. *Phytochemistry* **31**, 899-903 (1992).

Hirakura, K., Morita, M., Nakajima, K., Ikeya, Y., and Mitsuhashi, H., Polyacetylenes from the roots of *Panax ginseng*. *Phytochemistry* **30**, 3327-3333 (1991).

Hirakura, K., Morita, M., Nakajima, K., Ikeya, Y., and Mitsuhashi, H., Three acetylated polyacetylenes from the roots of *Panax ginseng*. *Phytochemistry* **30**, 4053-4055 (1991).

Lee, S.W., Kim, K.H., Rho, M.C., Chung, M.Y., Kim, Y.H., Lee, S.K., Lee, H.S., and Kim, Y.K., New polyacetylenes, DGAT inhibitors from the roots of *Panax ginseng*. *Planta Med.* **70**, 197-200 (2004).

Magalhaes, A.F., Magalhaes, E.G., Leitao Filho, H.F., and Junior, V.N., Polyacetylenes of brazilian species of *Didymopanax* (Araliaceae). *Biochem. Syst. Ecol.*, 20, 783-784 (1992).

Matsunaga, H., Katano, M., Yamamoto, H., Fujito, H., Mori, M., and Takata, K., Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C.A. Meyer. *Chem. Pharm. Bull.* **38**, 3480-3482 (1990).

Park, J.D., Recent studies on the chemical constituents of Korean Ginseng (*Panax ginseng* C.A. Meyer). *Korean J. Ginseng Sci.* **20**, 389-415 (1996).

Pomerantz, S.H., Separation, purification, and properties of two tyrosinases from hamster melanoma. *J. Biol. Chem.* **238**, 2351-2357 (1963).

Rho, M.C., Lee, H.S., Lee, S.W., Chang, J.S., Kwon, O.E., Chung, M.Y., and Kim, Y.K., Polyacetylenic compounds, ACAT inhibitors from the roots of *Panax ginseng*. *J. Agric. Food Chem.* **53**, 919-922 (2005).

Schulte, K.E. and Potter, B., Constituents of medicinal plants. XXX. Polyacetylenes from *Pituranthus tortuosus* (Desf.) Bnth. and Hook. *Arch. Pharm.* **310**, 945-963 (1977).

Shim, S.C., Chang, S.K., Hur, C.W., and Kim, C.K., A polyacetylenic compound from *Panax ginseng* roots. *Phytochemistry* **26**, 2849-2850 (1987).

Yang, R.L., Yan, Z.H., and Lu, Y., Cytotoxic phenylpropanoids from carrot. *J. Agric. Food Chem.* **56**, 3024-3027 (2008).

(Accept September 2, 2008)