[Chem. Pharm. Bull. 36(10)4206—4208(1988)]

A New Cytotoxic Chlorine-Containing Polyacetylene from the Callus of *Panax ginseng*

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(Received May 2, 1988)

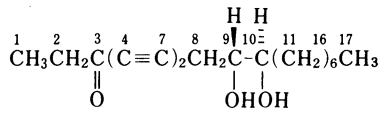
A new chlorine-containing polyacetylene (**3**) and panaxydol (**4**) have been isolated from the callus of *Panax ginseng*. The structure of **3** was confirmed by its ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR and mass spectral data. The new acetylene exhibited growth inhibition against leukemia cells (L-1210) in tissue culture.

Keywords——*Panax ginseng*; Araliaceae; callus; chlorine-containing C_{17}-polyacetylene; 1-chloro-9,10-epoxy-4,6-heptadecadiyne-2,3-diol; anticancer activity

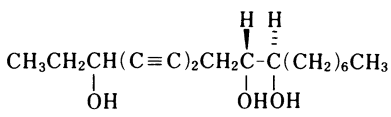
Introduction

From ancient times *Panax ginseng* C. A. MEYER has been considered as one of the most valuable drugs to be used in Korea, China and Japan. Studies on the constituents of *P. ginseng* have been mainly focused on the ginseng saponins. Since the anticancer activity of petrol extracts of the roots of *P. ginseng* was found,[1] the lipophilic portion of this plant has been extensively investigated. Several groups have isolated polyacetylene compounds, but, it has not been proved that the polyacetylenes in the plant are responsible for the growth inhibition of cancer cells.[2]

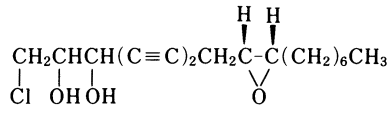
In the previous paper,[3] we reported the isolation and structural elucidation of two new polyacetylenes, panaxacol (**1**) and its dihydro derivative (**2**), from the callus of *P. ginseng*. Further studies on the constituents of the callus have led to the isolation of a new chlorine-containing polyacetylene, chloropanaxydiol (**3**), along with panaxydol (**4**),[4] which has been isolated from the roots of *P. ginseng* C. A. MEYER.



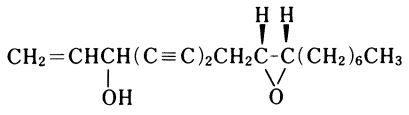
panaxacol (**1**)



dihydropanaxacol (**2**)



chloropanaxydiol (**3**)



panaxydol (**4**)

Chart 1

Results and Discussion

The compound (**3**) was obtained as an oil. Its mass spectrum (MS) exhibited the molecular ion (M^+) at *m/z* 312 and the (M+2) ion at *m/z* 314 in a peak ratio of 3:1, thereby suggesting the presence of a chlorine atom in the molecule. The infrared (IR) spectrum of **3** showed hydroxyl group (3560—3400 cm^{-1}) and triple bond (2260 cm^{-1}) absorptions. The proton magnetic resonance (^{1}H-NMR) spectrum of **3** showed the presence of a methyl group (δ 0.89), polymethylenes (δ 1.2—1.4), two nonequivalent methylene protons (δ 2.41 and 2.69), four methine protons (δ 2.98, 3.15, 3.92 and 4.54) and two nonequivalent methylene protons (δ 3.68 and 3.78) attached to the chlorine-bearing carbon. Acetylation of **3** with Ac_2O–pyridine gave a diacetate [*m/z* 396 (M^+): *m/z* 398 (M+2)=3:1] and caused the low field shift (δ 3.92→5.24 and δ 4.54→5.67) of two methine protons attached to the hydroxy-bearing carbons. In the carbon-13 magnetic resonance (^{13}C-NMR) spectrum of **3**, signals with large coupling constants were observed at δ 54.3 (J_{C-H}=178 Hz) and at δ 57.1 (J_{C-H}=171 Hz)

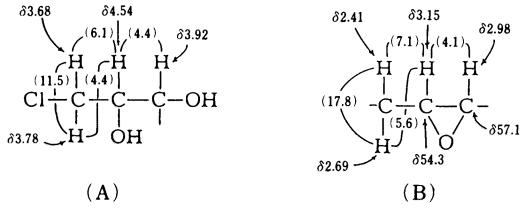


Chart 2. Partial Structures of **3**

The numbers in parentheses are *J* values in Hz.

which were correlated to the proton signals at δ 3.15 and at δ 2.98, respectively, by selective proton decoupling experiments. These large coupling constants and the chemical shifts of the protons (δ 2.98 and 3.15) indicated the presence of an epoxide ring including the carbons at δ 54.3 and 57.1. Detailed ^{1}H-NMR decoupling experiments on the diacetate revealed the partial structure of C-1—C-3 (A) and C-8—C-10 (B) (Chart 2). The configuration of the epoxide ring was assigned as *cis* from the coupling constant (J=4.1 Hz)[5] between H-9 and H-10. Furthermore, the presence of the methylene sequence from C-11 to C-17 could be presumed by comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **1** and **2**. Thus, the structure of **3** was confirmed as 1-chloro-9,10-epoxy-4,6-heptadecadiyne-2,3-diol.

Compound **4** was identified as panaxydol (**4**) from its spectral data described in the experimental section. Compounds **3** and **4** inhibited the growth of leukemia cells (L 1210) in tissue culture by 58% and 93%, respectively, at a concentration of 10 μg/ml. The details of the cytotoxicity of these polyacetylene compounds will be published elsewhere.

Experimental

Spectral data were obtained on the following instruments: IR spectra on a Shimadzu IR-430 in $CHCl_3$; ^{1}H-NMR and ^{13}C-NMR spectra on JEOL FX-400 and FX-100 instruments in $CDCl_3$ containing tetramethylsilane as an internal standard; MS on a Hitachi RMU-6M. The dried callus of *P. ginseng* C. A. MEYER was obtained from Nitto Electric Ind. Co., Ltd. (1-1 Shimohozumi, Ibaraki, Osaka 567, Japan).

Isolation of the Polyacetylene Compounds——The dried callus (4 kg) was powdered in a blender and extracted with EtOAc (4 l×3) under ultrasonication. After concentration of the EtOAc solution, the crude extract was chromatographed on Diaion HP-20 resin (Nippon Rensui) (eluted successively with 2 l each of 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, 90%, MeOH, MeOH and acetone). The growth inhibition of each fraction was tested against Yoshida sarcoma cells and only the 90% MeOH fraction was found to be active. The 90% MeOH fraction was chromatographed on silica gel (hexane:EtOAc=2:1) to give six fractions (fr. A—F). The active fraction (fr. C) was purified by high-performance liquid chromatography (HPLC) [Nucleosil 50-5 (Senshu),

8 × 300 mm; flow rate, 3 ml/min; hexane : EtOAc = 2 : 1] to give **1** [150 mg; retention time (t_R), 15 min], **2** (10 mg; t_R, 23 min) and **3** (15 mg; t_R, 12 min). The other active fraction (fr. B) was purified by HPLC [Nucleosil 50-5 (Senshu), 8 × 300 mm; flow rate, 3 ml/min; hexane : EtOAc = 3 : 1], followed by reverse-phase HPLC [ODS-2151 (Senshu), 6 × 150 mm, flow rate, 2 ml/min; MeOH : H_2O = 4 : 1] to give **4**[4] (35 mg; t_R, 5 min).

Chloropanaxydiol (3)——$[\alpha]_D$ −37.2 (MeOH, c = 0.2). MS m/z: 314 (M + 2), 312 (M^+), 294 (M − 18). IR: 3550, 3400 (OH), 2260 (acetylene) cm^{-1}. ^{1}H-NMR (400 MHz, δ): 0.89 (3H, t, J = 7.1 Hz, H-17), 1.25—1.4 (10H, br, H-12—H-16), 1.52 (2H, br m, H-11), 2.41 (1H, dd, J = 7.1, 17.8 Hz, H-8), 2.56 (1H, br s, OH), 2.69 (1H, dd, J = 5.6, 17.8 Hz, H-8), 2.76 (1H, br s, OH), 2.98 (1H, m, H-10), 3.15 (1H, ddd, J = 4.2, 5.6, 7.1 Hz, H-9), 3.68 (1H, dd, J = 6.1, 11.5 Hz, H-1), 3.78 (1H, dd, J = 4.4, 11.5 Hz, H-1), 3.92 (1H, d, J = 4.4 Hz, H-3), 4.54 (1H, m, H-2). ^{13}C-NMR (25 MHz, δ): 14.0 (C-17), 19.4 (C-8), 22.5 (C-16), 26.4, 27.5, 29.1, 29.3, 31.7 (C-11—C-15), 45.1 (C-1), 54.3 (C-9), 57.1 (C-10), 63.8 (C-3), 74.1 (C-2), 77.2, 78.5 (triple bond carbons).[6]

Chloropanaxydiol Acetate——A mixture of chloropanaxydiol (10 mg), pyridine (1.5 ml) and acetic anhydride (150 μl) was stirred at room temperature overnight. Usual work-up gave panaxachlor acetate (10 mg). High-resolution MS m/z: 396.1711 (M^+), $C_{21}H_{29}ClO_5$. ^{1}H-NMR (400 MHz, δ): 0.89 (3H, t, J = 7.1 Hz, H-17), 1.2—1.4 (10H, m, H-12—H-16), 1.4—1.6 (2H, m, H-11), 2.11 (3H, s, CH_3CO), 2.14 (3H, s, CH_3CO), 2.40 (1H, dd, J = 6.8, 16.8 Hz, H-8), 2.68 (1H, dd, J = 5.6, 16.8 Hz, H-8), 2.97 (1H, m, H-10), 3.13 (1H, m, H-9), 3.78 (2H, d, J = 5.1 Hz, H-1), 5.24 (1H, dd, J = 5.1, 5.1 Hz, H-2), 5.67 (1H, d, J = 5.1 Hz, H-3). ^{13}C-NMR (25 MHz, δ): 14.0 (C-17), 19.5 (C-8), 20.5 (CH_3CO × 2), 22.5 (C-16), 26.4, 27.1, 29.1, 29.3, 31.6 (C-11—C-15), 41.9 (C-1), 54.0 (C-9), 56.8 (C-10), 63.1 (C-3), 69.1, 77.6 (triple bond carbons),[6] 168.8, 169.5 ($CH_3\underline{C}O$ × 2).

Panaxydol——$[\alpha]_D$ −19.5 (MeOH, c = 0.6). MS m/z: 260 (M^+), $C_{17}H_{24}O_2$. IR: 3600—3400 (OH), 2260 (triple bond) cm^{-1}. ^{1}H-NMR (400 MHz, δ): 0.89 (3H, t, J = 7.1 Hz, H-17), 1.2—1.4 (10H, m, H-12—H-16), 1.4—1.6 (2H, m, H-11), 2.15 (1H, br s; OH), 2.39 (1H, dd, J = 7.1, 17.6 Hz, H-8), 2.71 (1H, dd, J = 5.4, 17.6 Hz, H-8), 2.98 (1H, m, H-10), 3.15 (1H, ddd, J = 4.1, 5.4, 7.1 Hz, H-9), 4.93 (1H, br s; H-3), 5.26 (1H, ddd, J = 1.2, 1.2, 10.3 Hz, H-1), 5.47 (1H, ddd, J = 1.2, 1.2, 17.1 Hz, H-1), 5.95 (1H, ddd, J = 5.4, 10.3, 17.1 Hz, H-2). ^{13}C-NMR (25 MHz, δ): 14.0 (C-17), 19.5 (C-8), 22.6 (C-16), 26.4 (C-12), 27.5 (C-11), 29.1 (C-14), 29.4 (C-13), 31.8 (C-15), 54.3 (C-9), 56.9 (C-10), 63.4 (C-3), 60.2, 66.4, 75.1, 76.7 (acetylenic carbons), 117.0 (C-1), 136.2 (C-2).

References and Notes

1) S. C. Shim and H. Y. Koh, *Bull. Korean Chem. Soc.*, **4**, 183 (1983).
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6) The other two acetylenic carbon signals might be overlapped with the $CDCl_3$ signals.