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Panaxyne epoxide, A New Cytotoxic Polyyne from *Panax ginseng* Root against L1210 Cells

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Abstract □ A new polyacetylene compound with cytotoxic activity against L1210 cells having diyne-ene and epoxy moiety, named panaxyne epoxide, was isolated from *Panax ginseng* C.A. Meyer. The chemical structure of the polyacetylene was determined to be tetradeca-13-ene-1,3-diyne-6,7-epoxide by UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra.

Keywords □ polyacetylene, panaxyne-epoxide, *Panax ginseng*, tetradeca-13-ene-1,3-diyne-6,7-epoxide.

Since a polyacetylene, panaxynol was first isolated by Takahashi *et al.*[1] from *Panax ginseng* and later panaxydol and heptadeca-1-ene-4,6-diyne-3,9-diol by Wrobel *et al.*[2,3], their biological and pharmacological studies have been interested in. Polyacetylenes of Panax ginseng with antitumor activities have been studied by several laboratories. We reported seven polyynes showing the cytotoxic activities against L_{1210} cells *in vitro*[4-8].

Kanato and coworker[9] reported that panaxytriol which was isolated from Korea red ginseng powder inhibited the growth of several kinds of human and murine malignant cells *in vitro*. Fujimoto and coworker[10] purified panaxacol and dihydropanaxacol from dried callus of Panax ginseng and reported that these two polyacetylenes exhibited growth inhibition against Yoshida sarcoma cells in tissue culture.

In the course of searching for chemical characterization, Kitagawa *et al.*[1] found that panaxytriol were elucidated as characteristic constituent of red ginseng. We reassured the existance of panaxytriol in red ginseng, not in fresh or white ginseng.[12]

In previous report[8], We isolated panaxyne (tetradeca-13-ene-1,3-diyne-6,7-diol) from white and red ginseng which is similar to the chemical structure of panaxytriol having vicinal diol moiety. So, it was expected that panaxyne might be produced by hydrolysis of its epoxide form in fresh ginseng during steam heating of fresh ginseng which is one of manufacturing processes of red ginseng.

In this paper, we have isolated epoxide form of panaxyne from the petroleum ether extract of fresh ginseng and determined its structure.

EXPERIMENTAL METHODS

UV spectra were measured with Pye Unicam PU 8000. IR spectra were taken with Perkin-Elmer Model 599B. ^{1}H-NMR and ^{13}C-NMR spectra were measured with Bruker AM300. TMS was used as the internal standard and chemical shifts were expressed in ppm. The EI/MS spectra were determined on Varian Mat 212 MS combined with Varian 3700 GC. Kiesel gel 60 GF254 for thin layer chromatography and Kiesel gel 60(70-230 mesh ASTM) for silica gel column chromatography were also used. Further purification of each sample separated by silica gel column was performed by preparative HPLC using Allteck NH_2 column (Waters, 10 × 250mm).

Extraction and isolation

The six year old fresh ginseng roots (5 kg) were collected in Zeung Pyoung Farm of Korea Ginseng and Tobacco Research Institute, Chungbook province. It was freeze-dried, powdered and extracted with 15 *l* petroleum ether at room temperature for 24 hrs. The extraction was repeated three times under the same conditions. Petroleum ether extract (30g) was chromatographed on a si gel column with benzene to afford 5 fractions. The fraction 5 mostly contained acetyl panaxydol and panaxynol known by Ahn and Takahashi respectively.[1,7] The fraction 3 was rechromatographed on silica gel column with *n*-hexane/ethyl ether (97:3) to afford panaxyne-

epoxide and small impurities. Panaxyne-epoxide (45mg), visualized with anisaldehyde/sulfuric acid as brown color, was further purified by preparative HPLC under the following conditions;

column; Allteck NH_2 (10 × 250mm)
solvent; *n*-hexane/isopropyl alcohol (5:1)
flow rate; 3.0 ml/min.
detector; UV (254 nm) and RI

Panaxyne-epoxide

colorless oil, UV λ_{max} (*n*-Hexane): 225, 238, 251 nm. IR (ν_{max} cm^{-1}): 2230(C ≡ C), 1640(C = C), 3280 (≡C-H). ^{1}H-NMR δ ($CDCl_3$): 2.02(s, C-1), 2.68(dd, 5.5, 17.8Hz C-5a), 2.36(dd, 7.1, 17.8Hz, C-5b), 3.15(ddd, 4.2, 5.5, 7.1Hz, C-6), 2.97(dt, 4.2, 5.7Hz, C-7), 1.55(br, C-8), 1.30(br, C-9, 10, 11), 2.06(dt, 6.7, 7.0Hz, C-12), 5.81(ddt, 17.0, 10.2, 6.7 Hz, C-13), 5.00(ddt, 17.0, 1.8, 1.1Hz, C-14a), 4.93 (ddt, 10.2, 1.8, 1.1Hz, C-14b). MS *m/z*(%): 202 (M^+, 1), 91(100), 55(94), 67(88), 76(75), 105(46), 117(43), 131(30), 145(12), 159(7), 173(3), 183(2).

Hydrolysis of panaxyne-epoxide

30mg of panaxyne-epoxide were dissolved in 5 ml of 30% acetic acid and stirred at 60 C for 72 hrs. The reaction mixture was neutralized with 2% NaOH and extracted with ethyl ether. The ethyl ether layer was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by a preparative HPLC under above mentioned conditions using *n*-hexane/isopropyl alcohol (2:1), yielding 15 mg of the hydrolysed product (panaxyne).

Panaxyne

colorless oil, UV(*n*-Hexane) λ_{max}; 227, 239, 250 nm. IR(ν_{max} cm^{-1}); 3300(OH), 2230(C≡C), 1640 (C=C), 3280(≡C-H). ^{1}H-NMR δ($CDCl_3$); 2.02(s, C-1), 2.56(d, 5.6Hz, C-5), 3.65(dt, 4.5, 5.6Hz, C-6), 3.59(dt, 4.5, 5.9Hz, C-7), 1.51(m, C-8), 1.3(br, C-9, 10, 11), 2.06(dt, 6.7, 7.0Hz, C-12), 5.81(ddt, 17.0, 10.2, 6.7Hz, C-13), 5.00(ddt, 17.0, 1.8, 1.1Hz, C-14a), 4.93(ddt, 10.2, 1.8, 1.1Hz, C-14b).

RESULT AND DISCUSSION

The petroleum ether fraction was obtained by the method described in the experiment part subjected to silica gel column chromatography and followed by a preparative HPLC. A new polyacetylene was obtained at higher Rf value than of acetyl panaxydol. The UV spectrum of this compound showed λ_{max} at 225, 238, 251 nm indicating the presence of the conjugated diyne. Bands at 2230, 1640 and 3280 cm^{-1} in the IR spectrum indicated the presence of acetylenic, olefinic and terminal alkynyl proton, respectively. Four conjugated diyne carbons was confirmed by ^{13}C-NMR spectrum (Table I) at 72.9, 68.0, 66.6, and 65.5 ppm. The terminal allyl system could be confirmed at 138.9, 114.4 and 33.6 ppm. The carbon atom bounded to the epoxy groups could be detected at 56.9 and 54.2 ppm. The other carbon atoms showed nearly the same absorption peaks as panaxyne.

Table I. ^{13}C-NMR spectral data for panaxyne epoxide (1) and panaxyne (2)

C	1	2
1	65.5	65.3
2	68.0	68.0
3	66.6	66.8
4	72.9	74.4
5	19.2	24.6
6	54.2	72.0
7	56.9	72.9
8	27.5	33.6
9	26.3	25.4
10	28.9	29.0
11	28.7	28.7
12	33.6	33.4
13	138.9	138.9
14	114.4	114.3

^{1}H-NMR spectrum of this compound was also similar to that of panaxyne (Fig. 1-b). The presence of the multiplet at 5.81 ppm (C-13), three peak at 4.93-5.00 ppm (C-14) and sextet at 2.06 ppm (C-12) in the ^{1}H-NMR is typical for the terminal allyl groups, and singlet at 2.02 ppm for a terminal alkyne proton. But structural difference of the two polyynes occurs only in the range of C-5 to C-7. Two quartets at 2.68 and 2.36ppm in the structure (Fig. 1-a) correspond apparently to an ab-sub system of an ABX which is nearly the same as that of panaxydol. This means that two methylene protons on C-5 are magnetically non-equivalent.

In order to verify the presence of the epoxy group, this compound was hydrolysed with 30% acetic acid. On hydrolysing the epoxy group, the two quartets were transformed into a doublet proving that the structural rigidity of epoxy has disappeared. The corresponding peaks of panaxyne are located at 2.56ppm (Fig. 1-b). In these cases we can

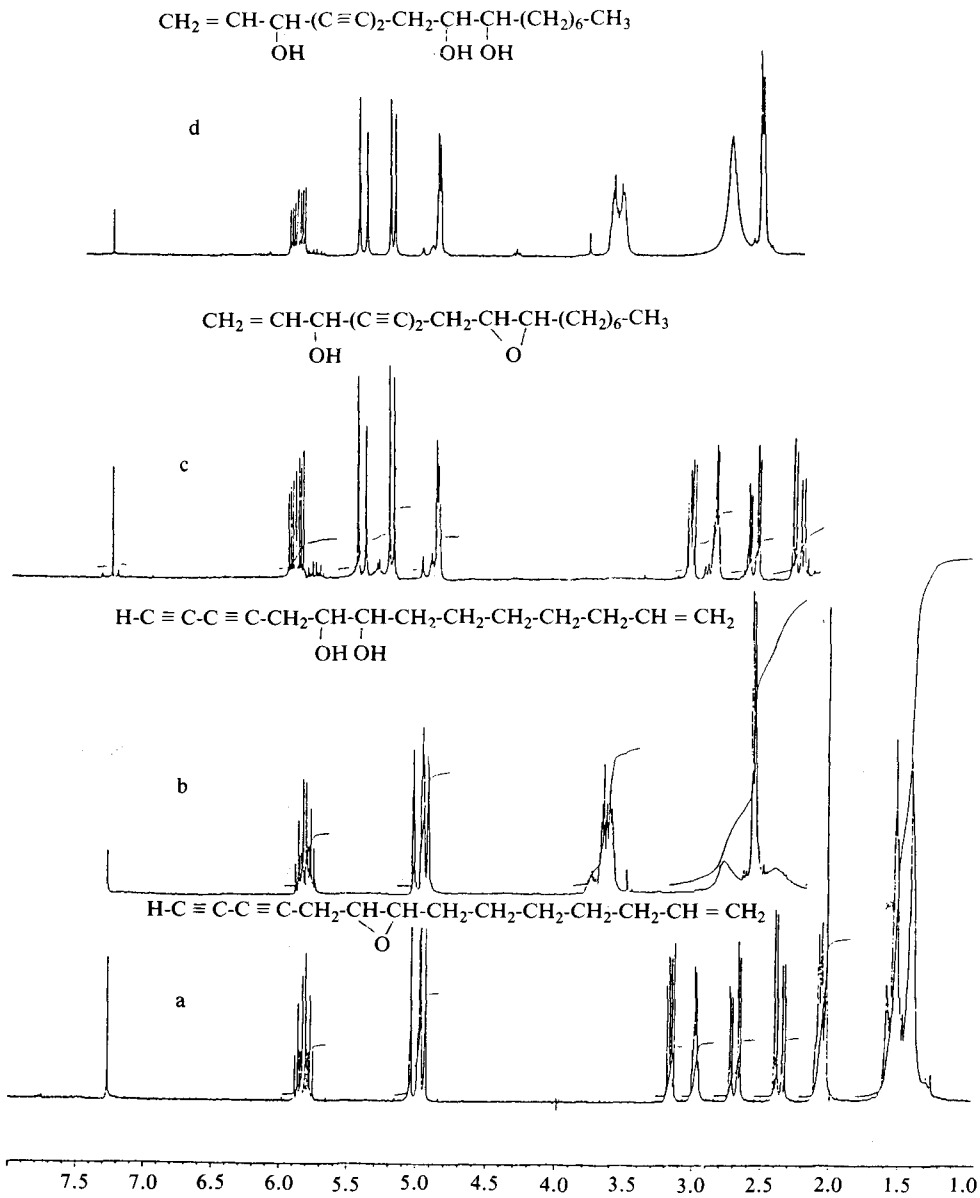

Fig. 1. 300MHz ^{1}H-NMR spectrum of panaxyne epoxide(a), panaxyne(b), panaxydol(c) and panaxytriol(d).

recognize that these two spectra in Fig. 1 are similar to those of panaxydol (Fig. 1-c) and panaxytriol (Fig. 1-d).[5] Molecular ion was observed at 202 *m/z* in its EI mass spectrum, and the peak at 105 *m/z* corresponds to H-(C = C)$_2$-CH$_2$-CH-$\mathbf{CH}^+$.

All these findings lead to the conclusion that the substance is tetradeca-13-ene-1,3-diyne-6,7-epoxide, named panaxyne epoxide showed an ED_{50} value of 6.0 μg/m*l*. The ED_{50} value, which is the concentration of the test substance to inhibit the growth of L_{1210} by 50% in reference of the untreated control, was determined by the procedure of Thayer and coworkers[13] with minor modification.

The cytotoxic activity of panaxyne epoxide is lower than those of the panaxydol (ED_{50} = 0.03-2 μg/m*l*) analogues.[7] This is due to the lack of the essential structural moiety of the cytotoxic ginseng polyynes, hept-1-ene-4,6-diyne-3-ol, for the cytotoxicity (ED_{50} = 2.1 μg/m*l*).

It was reported that the cytotoxicity of panax-

ydol was approximately ten times higher than other polyene in Panax ginseng and the presence of epoxy group in the structure was responsible for the enhancement of the activity.[7] But in case of panaxyne epoxide, its activity was slightly higher than panaxyne ($ED_{50} = 11.0\ \mu g/ml$) in spite of its epoxy group. This implies that the cytotoxicity-enhancing effect of the epoxide group in these series of the compounds is dependent on the position of the epoxide function in the structure.

All these results ascertain that the essential structure for a cytotoxicity of panaxydol analogue is hept-1-ene-4,6-diyne-3-ol.

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