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

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Abstract—A new polyyne, panaxyne, was isolated from the Korean red ginseng. The structure was determined as tetradeca-13-ene-1, 3-diyne-6, 7-diol by comparison of spectral data. The ED_{50} value of panaxyne as cytotoxicity against L1210 cell was 11.0 μg/ml. The lower cytotoxic activity of the substance relative to other ginseng polyynes is presumably due to lack of the essential structural part of hept-1-en-4, 6-diyne-3-ol.

Keywords—*Panax ginseng* • polyyne • panaxyne • cytotoxic against L1210

Korean red ginseng is the product prepared by steam heating the six years old fresh ginseng root. Therefore, it is expected that various chemical transformations would take place in the fresh ginseng components. In the course of searching for cytotoxic substances from Korean white ginseng root, six polyynes cytotoxic against L1210 cell have been isolated from it.[1~4] These chemically unstable polyynes could be sensitive to the steam heating. Therefore, the red ginseng could contain various transformed polyynes. In the previous report we found that the red ginseng contained panaxytriol as a major polyyne[5]. Panaxytriol was formed evidently by hydrolysis of panaxydol, the main polyyne of the fresh ginseng, during the heating. Thus it would be interesting to isolate the polyynes from the red ginseng and compare them with those isolated from the white ginseng. In this paper we report a new polyyne isolated from the red ginseng, its structure and cytotoxic activity against L1210 cell.

UV spectrum of the polyyne shows a typical absorption pattern for a conjugated diyne at 250, 239 and 227 nm. The presence of the diyne

Table I. ^{1}H-NMR spectral data for tetradeca-13-ene-1, 3-diyne-6, 7-diol (I) and its diacetate (III)

H	I	III
1	2.02s	2.00s
5	2.56d(5.6)	2.60dd(5.3, 17.1)
		2.57dd(5.9, 17.1)
6	3.65dt(4.5, 5.6)	5.11m
7	3.59dt(4.5, 5.9)	5.07m
8	1.51m	1.61m
9~11	1.2~1.4br, m	1.2~1.4br, m
12	2.06dt(6.7, 7.0)	2.03dt(6.7, 7.0)
13	5.81ddt(17.0, 10.2, 6.7)	5.81ddt(17.0, 10.2, 6.7)
14	5.00ddt(17.0, 1.8, 1.1)	5.00ddt(17.0, 1.8, 1.1)
	4.93ddt(10.2, 1.8, 1.1)	4.93ddt(10.2, 1.8, 1.1)
COCH$_3$		2.12s
		2.10s

(300 MHz, CDCl$_3$, δ, TMS as an internal standard)
The numbers in parentheses are J values in Hz.

H-C≡C-C≡C-CH₂-CH-CH-CH₂-CH₂-CH₂-CH₂-CH₂-CH=CH₂
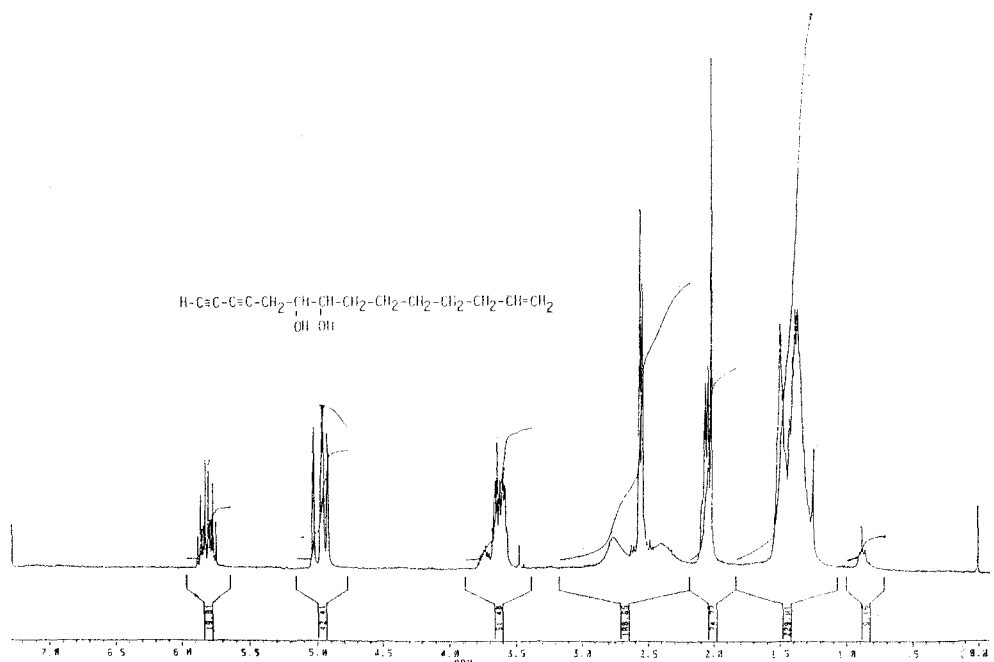 | |
                  OH OH

Fig. 1. 300 MHz ¹H-NMR spectrum of tetradeca-13-en-1,3-diyn-6,7-diol

was reassured by the IR absorption at 2230 cm⁻¹. The presence of four conjugated diyne carbons was confirmed by ¹³C-NMR spectrum (Table II); the resonances at 65.3 ppm with $J=105.6$ Hz in ¹³C-NMR, at 2.02 ppm in ¹H-NMR(singlet, Fig. 1 and Tab. I) of the substance and the peaks at 3,280 and 630 cm⁻¹ in the IR spectrum of the acetylated substance imply that a terminal alkynyl proton is present in the structure. The IR spectrum of the substance reveals further structural informations such as hydroxyl group(3,200∼3,400 cm⁻¹), many aliphatic C–H stretchings (2,980−2,860 cm⁻¹), a terminal allyl group (1,640 and 915 cm⁻¹) and a straight alkyl chain longer than N-butyl (725 cm⁻¹). A typical terminal allyl group is supported by the presence of the multiplet at 5.81 ppm(ddt, 17.0, 10.2, 6.7 Hz, H-13), three peaks at 4.93∼5.00 ppm(H-14) and sextet at 2.06 ppm(dt, 6.7 and 7.0 Hz, H-12) in the ¹H-NMR. The terminal allyl system could be also confirmed by coupled and decou-

Table II. ¹³C-NMR spectral data for tetradeca-13-ene-1,3-diyne-6,7-diol

Carbon number	Chemical shift (ppm)
1	65.3
2	68.0
3	66.8
4	74.4
5	24.6
6	72.0
7	72.9
8	33.6
9	25.4
10	29.0
11	28.7
12	33.4
13	138.9
14	114.3

(75.5 MHz, CDCl₃, TMS as an internal standard)

pled ¹³C-NMR at 114.3 (t, 96.8 Hz), 138.9(d, 100.7 Hz) and 33.4 (t, 34.9 Hz). Since two acetyl groups (2.12 and 2.10 ppm) were shown in the NMR of the acetylated product of the
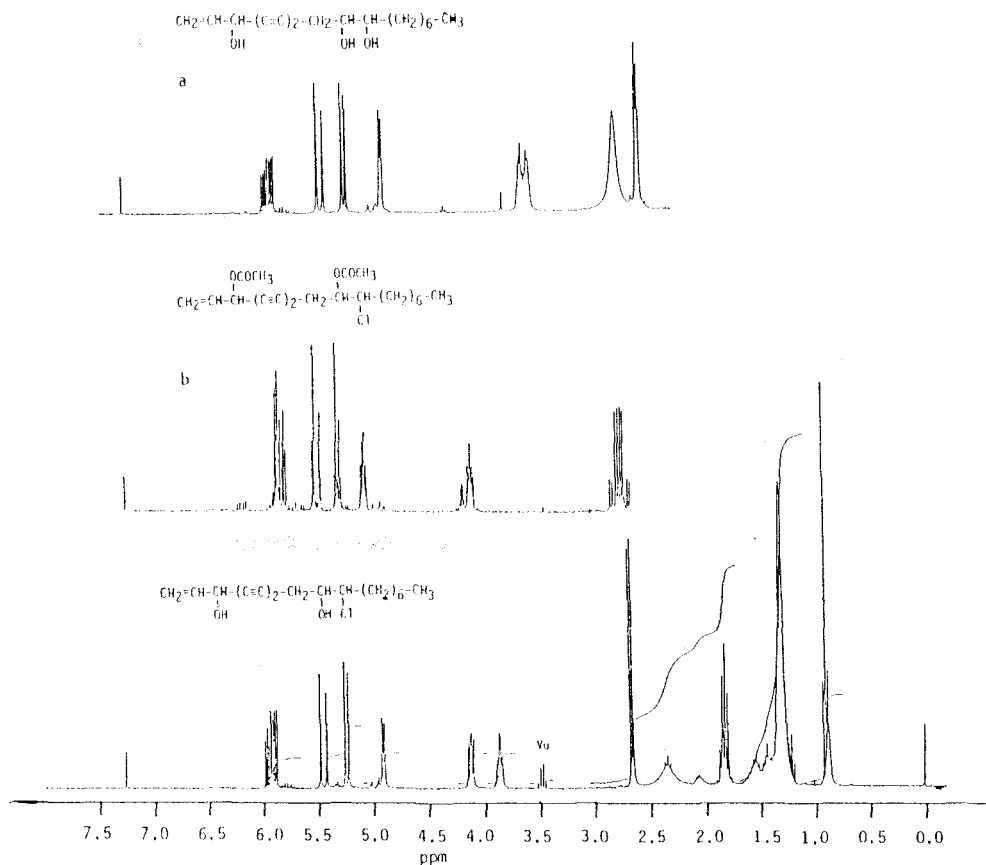
Fig. 2. 300 MHz ¹H-NMR spectrum of panaxytriol (a), acetylated panaxydol chlorhydrin (b) and panaxydol chlorhydrin (c).

substance, it is evident that the substance con- tains two hydroxyl groups The carbon atoms bound to the hydroxyl groups could be detected in ¹³C-NMR at 72.0 ppm(d, 71.4 Hz) and 72.9 ppm (d, 68.5 Hz). The chemical shifts of these carbon atoms correspond to those of a 1,2-diol. The N-butyl moiety appeared also in ¹H-NMR; a multiplet, theoretically a sextet, at 1.51 ppm corresponds to a methylene (C-8) group neigh- bored to a sec. alcoholic carbon(C-7) and the broad multiplet at 1.3 ppm to three methy- lene groups(C-9, C-10, C-11). The presence of only one methylene group (1.51 ppm) binding to an alcoholic carbon in ¹H-NMR proves that two hydroxyl groups must be vicinal to each other, that is a 6,7-diol, as the ¹³C-NMR data

suggests. If the hydroxyl groups stood more distant from each other, it is expected that more than two methylene groups bound to alcoholic carbons would appear in the range of 1.51 ppm. A doublet in ¹H-NMR at 2.56 ppm corresponds to a methylene group which is doublet with only one vicinal proton. These methylene protons have the same NMR-prope- rties as those of panaxydol chlorohydrine and panaxytriol (Fig. 2). Two protons of C-8 in the panaxydol chlorohydrine were shown up as a doublet at 2.66 ppm, which is transformed into an octet through acetylation of the neigh- bored hydroxyl group. This phenomenon means that the acetylation imposes a rigidity to the methylene group.[4] These results lead to the
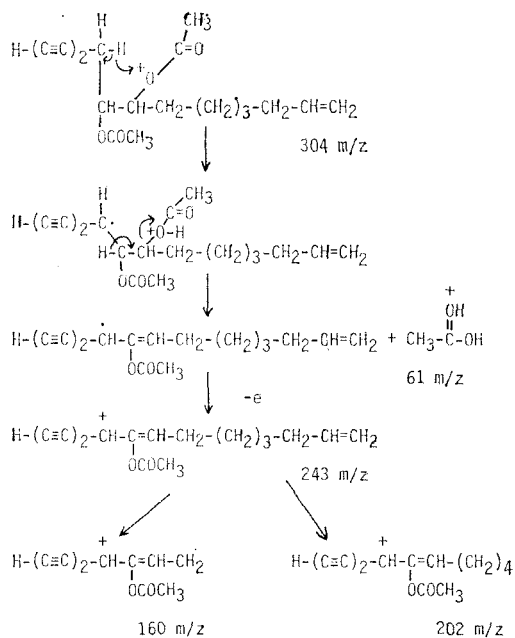
Scheme 1. Mass spectrometric fragmentation of tetradeca-13-en-1, 3-diyn-6, 7-diol-diacetate.

conclusion that the acetylation of the hydroxyl group on C-9 of panaxydol chlorohydrine makes the protons on C-8 magnetically unequivalent. Since this phenomenon was observed also in the case of the isolated substance, the methylene carbon atom must be bound to an alcoholic carbon atom, namely it is the C-5 atom in the structure. Molecular ion of the substance could not be observed in its mass spectrum. However, the acetylated substance showed its molecular ion peak at m/z 304 (Scheme 1), implying that the molecular weight of the substance is 220. The peaks at m/z 244 and 243 correspond to $(M-CH_3COOH)^+$ and $(M-CH_3COOH_2)^+$. The former ion is probably produced by a thermal decomposition. The peak at m/z 202 corresponding to $[(M-CH_3COOH_2)$ -allyl$]^+$ indicates the presence of a terminal allyl group. The ion peak at 160 proves the presence of 6, 7-diacetoxy groups.

All these findings lead to the conclusion that the substance is *tetradeca-13-ene-1, 3-diyne-6,*

7-diol, named panaxyne. Panaxyne showed an ED_{50} value of 11.0 $\mu g/ml$. The ED_{50} value, which is the concentration of the test substance to inhibit the growth of L1210 by 50% in reference of the untreated control, was determined by the procedure of Thayer and coworkers[6] with minor modification. The cytotoxic activity is lower than that of the panaxydol analogues $(ED_{50}=0.03\sim0.2\ \mu g/ml)$. 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 $(ED_{50}=2.1\ \mu g/ml)$. Panaxyne is not described in the literature. The polyyne was isolated also from white ginseng. Since panaxyne is contained in red ginseng greater amount than in white ginseng, it is evident that panaxyne was produced mainly during production of red ginseng as was the case of panaxytriol. This observation makes possible the assumption that 6, 7-epoxy form of panaxyne exists in the ginseng root.

Experimental

Instruments and reagents

UV spectra were measured with Pye Unicam PU 8000. IR spectra were taken with Perkin-Elmer Model 599B.

$^1H-$ and $^{13}C-$NMR spectra were measured with Bruker AM300. TMS was used as the internal standard and the chemical shifts were expressed in ppm. The mass spectra were determined on Varian Mat 212 MS combined with Varian 3700 GC. Kieselgel 60 (Merck) was used for CC and precoated Si plates (0.25 mm, Merck) for TLC. A reversed-phase column (Alltech NH2, 10×250 mm) was used for preparative HPLC.

Plant material

The ginseng root was collected in Zeung Pyoung Farm of Korea Ginseng and Tobacco Research Institute, Chungbook Province, Korea.

Isolation of the substance

Red ginseng (4 kg), obtained by saturated steam heating of fresh ginseng, was powdered and extracted with 15 *l* petroleum ether at room temp. for 24 hrs. The extraction was repeated three times under the same conditions. Evaporation of solvent (45 *l*) gave 37 g of residue. The dried extract was chromatographed repeatedly over a silica gel column (3.5×40 cm) with benzene/ether (9 : 1 to 1 : 1) gradiently, whereby 4 fractions were collected. The fractions 1, 2 and 4 mostly contained the known substances. The fraction 3 showed two main spots on Si TLC [petroleum ether/ether (1 : 1), Rf =0.18, 0.13], visualized with anisaldehyde/ sulfuric acid as brown and red colors, respectively. Rechromatography of the fraction 3 on the Si column with benzene/ether (9 : 1) gave compound I (Rf=0.18, 110 mg) and compound II (Rf=0.13, a negligible amount).

First compound I was further purified by HPLC under the following condition; column: solvent system: N-hexane/isopropyl alcohol (2 : 1). Flow rate: 3.0 ml/min. Detector: UV 254 nm and RI. The compound II must be collected in greater amount for a further management. Isolation of panaxyne from white ginseng was performed with the same manner.

Preparation of acetylpanaxydne from panaxyne was done in acetic anhydride/pyridine.

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