# Studies on Panax Acetylenes: Absolute Structure of a New Panax Acetylene, and Inhibitory Effects of Related Acetylenes on the Growth of L-1210 Cells

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**A new *Panax* acetylene, 3-oxo-PQ-1 (1), was isolated from *Panax quinquefolium*. The absolute configurations of 3-oxo-PQ-1 (1) and PQ-1 (2) were determined to be (9*R*,10*R*) and (3*R*,9*R*,10*R*), respectively, by synthesizing 1 and 2 starting from D-(−)-diethyl tartrate, and by synthesizing their stereoisomers from L-(+)-diethyl tartrate. The growth inhibitory effects of *Panax* acetylenes (1—8) and their stereoisomers against leukemia cells were tested. Unnatural acetylenes having the (3*S*)-configuration (2, 5, 6, 7, 8; $IC_{50}$=0.01—0.1 $\mu$g/ml) were found to be approximately ten times more potent than natural acetylenes ($IC_{50}$=0.1—1.0 $\mu$g/ml) with the (3*R*)-configuration. Potency differences due to the configuration at C-9 and C-10 were unrelated to this stereochemistry. The $C_{14}$-polyacetylenes, PQ-8 (4) and its isomer ($IC_{50}$=1.0—10.0 $\mu$g/ml), were found to exhibit weaker cytotoxicity than the $C_{17}$-polyacetylenes.**

**Key words** PQ-1; 3-oxo-PQ-1; *Panax* polyacetylene; absolute configuration; growth inhibition; L-1210

In previous papers[1—3] we reported the isolation and structural elucidation of eight new polyacetylenes (PQ-1—PQ-8) and six previously-identified polyacetylenes[4] (panaxytriol, panaxydol, acetylpanaxydol, panaxydiol, falcalinol, ginsenoyne G) from *Panax quinquefolium*. In addition, we reported the absolute configurations of PQ-3 (**3**), PQ-8 (**4**), panaxytriol (**5**), panaxydol (**6**), acetylpanaxydol (**7**) and panaxydiol (**8**), synthesizing them from D-(−)- or L-(+)-diethyl tartrate (Chart 1).[5,6] In this paper, we describe the isolation and structural determination of a new polyacetylene (**1**), and the growth inhibitory effects of *Panax* polyacetylenes (**1**—**8**) and their optical isomers against mouse leukemia cells (L-1210).

Dried roots of *P. quinquefolium* were extracted with ethyl acetate (EtOAc). The crude extract was chromatographed on a Diaion HP-20 resin column, then on a silica gel column, followed by high performance liquid chromatography (HPLC) to provide **1** and **2**. FAB-MS of **1** showed a $[M+H]^+$ ion peak at *m*/*z* 291 (291.1974), corresponding to the molecular formula $C_{18}H_{26}O_3$. The $^1$H- and $^{13}$C-NMR spectra of **1** indicated the presence of a carbonyl carbon ($\delta$ 177.77), four

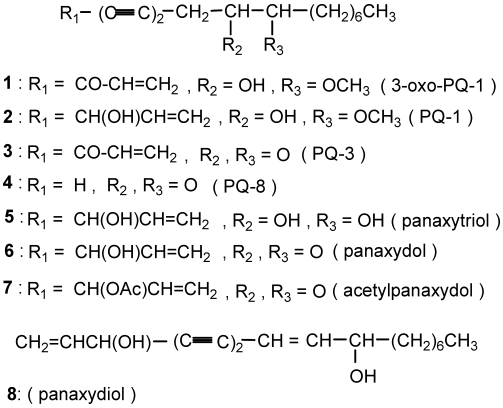


Chart 1

*sp* carbons ($\delta$ 65.38, 70.63, 76.85, 86.40), two oxymethine protons ($\delta$ 3.23, 3.76), a hydroxyl ($\delta$ 2.47) and a methoxyl group ($\delta$ 3.44, 8.24). The $^1$H- and $^{13}$C-NMR spectra were very similar to those of PQ-1 (**2**) except for the presence of a carbonyl group instead of a hydroxyl group in **2**. The HMBC spectrum of **1** exhibited the cross peaks due to the long-range correlations between the carbonyl carbon signal and the H-1 as well as H-2 signals, suggesting the carbonyl group should be located at C-3. Thus the structure of **1** was elucidated to be 9-hydroxy-10-methoxy-3-oxo-heptadeca-4,6-diyne. The absolute structures of **1** and **2** were determined by the syntheses of both enantiomers starting from (2*R*,3*R*)- and (2*S*,3*S*)-epoxy alcohols (**9a**, **b**) prepared from D-(−)- and L-(+)-diethyl tartrate as described previously.[7] Methylation of **9a** and **9b** with $CH_3I$–NaH gave 3-*O*-methyl ethers (**10a**, **b**), respectively, which were reacted with diacetylene in the presence of *n*-BuLi to provide 7-methoxy diacetylene alcohols (**11a**, **b**). Reaction of **11a** and **11b** with acrolein in the presence of *n*-BuLi yielded a diastereomeric mixture at C-3 of (9*R*,10*R*)-PQ-1 (**2a**) and its (9*S*,10*S*)-isomer (**2b**), respectively. Separation of **2a** and **2b** was achieved using CHIRAZYME catalyzed acetylation and hydrolysis procedures.[8] Treatment of **2a** and **2b** with vinyl acetate in the presence of CHIRAZYME L-2, C3 gave mixtures of acetates (**2a′-Ac**, **2b′-Ac**) and unreacted alcohols (**2a″**, **2b″**), respectively, which were separated by HPLC. The acetates (**2a′-Ac**, **2b′-Ac**) were hydrolyzed with CHIRAZYME L-2, C2 in phosphate buffer (pH 7.4) to provide alcohols **2a′** and **2b′**, respectively.[9] Oxidation of the **2a** and **2b** diastereomeric mixture with manganese dioxide gave (9*R*,10*R*)-3-oxo-PQ-1 (**1a**) and its (9*S*,10*S*)-isomer (**1b**), respectively (Chart 2).[10]

The stereochemistries at C-3 of **2a′**, **2a″**, **2b′** and **2b″** were determined using the modified Mosher method.[11] Treating alcohols **2a′**, **2a″**, **2b′** and **2b″** with (*R*)- and (*S*)-$\alpha$-methoxy-$\alpha$-(trifluoromethyl)phenylacetyl (MTPA) provided 3,9-di-*O*-MTPA esters. As shown in Table 1, the $H_2$-1 and H-2 signals of the **2a′**-(*R*)- and **2b′**-(*R*)-MTPA esters were shifted upfield

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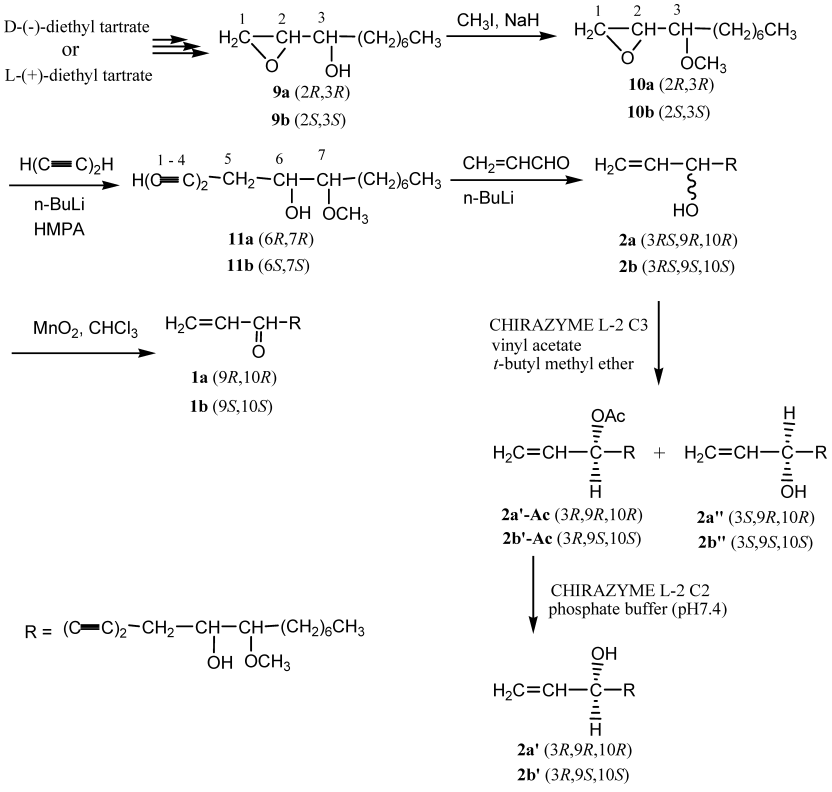


Chart 2

Table 1

MTPA ester of **1** MTPA ester of **2**

| | H-1a | H-1b | H-2 | H-8a | H-8b | H-10 | $OCH_3$ | $[\alpha]_D$ |
|---|---|---|---|---|---|---|---|---|
| **1a** (9*R*,10*R*) | | | | +0.04 | +0.04 | −0.07 | −0.07 | +2.9° (*c*=0.59, $CHCl_3$) |
| **1b** (9*S*,10*S*) | | | | −0.04 | −0.04 | +0.07 | +0.07 | −3.5° (*c*=0.47, $CHCl_3$) |
| nat. 3-oxo-PQ-1 (**1**) | | | | +0.04 | +0.04 | −0.07 | −0.07 | +3.5° (*c*=0.69, $CHCl_3$) |
| **2a**′ (3*R*,9*R*,10*R*) | +0.07 | +0.09 | +0.11 | +0.03 | +0.03 | −0.07 | −0.07 | −19.0° (*c*=0.59, $CHCl_3$) |
| **2a**″ (3*S*,9*R*,10*R*) | −0.05 | −0.07 | −0.09 | +0.06 | +0.05 | −0.07 | −0.07 | +23.0° (*c*=2.09, $CHCl_3$) |
| **2b**′ (3*R*,9*S*,10*S*) | +0.06 | +0.08 | +0.10 | −0.05 | −0.04 | +0.07 | +0.07 | −24.9° (*c*=1.17, $CHCl_3$) |
| **2b**″ (3*S*,9*S*,10*S*) | −0.06 | −0.09 | −0.11 | −0.03 | −0.03 | +0.07 | +0.07 | +22.4° (*c*=0.78, $CHCl_3$) |
| nat. PQ-1 (**2**) | +0.06 | +0.09 | +0.11 | +0.03 | +0.03 | −0.07 | −0.07 | −21.7° (*c*=0.51, $CHCl_3$) |

$\Delta\delta$ ($=\delta S-\delta R$) values are expressed in ppm. $\Delta\delta$ values obtained from the MTPA esters of isomers (3-oxo-PQ-1 and PQ-1).

compared to those of **2a**′-(*S*)- and **2b**′-(*S*)-MTPA esters. In contrast, the signals due to $H_2$-1 and H-2 of **2a**″-(*R*)- and **2b**″-(*R*)-MTPA esters appeared downfield compared to those of **2a**″-(*S*)- and **2b**″-(*S*)-MTPA esters, respectively. This suggests that the stereochemistries of **2a**′ and **2b**′ at C-3 are in the *R*-configuration, and those of **2a**″ and **2b**″ are in the *S*-configuration. The optical rotation values and $^1$H-NMR chemical shifts of 3-oxo-PQ-1 (**1**) and PQ-1 (**2**) showed good accordance with **1a** and **2a**′, respectively (Table 1). Thus, the absolute configurations of **1** and **2** were confirmed to be (9*R*,10*R*) and (3*R*,9*R*,10*R*).

Next, we examined the growth inhibitory effects of the polyacetylenes **1**—**8** and their optical isomers against L1210. As shown in Fig 1, the (3*S*)-isomers of PQ-1 (**2**, 3*R*,9*R*,10*R*), panaxytriol (**5**, 3*R*,9*R*,10*R*), panaxydol (**6**, 3*R*,9*R*,10*S*), acetylpanaxydol (**7**, 3*R*,9*R*,10*S*) and panaxydiol (**8**, 3*R*,10*S*) were found to be approximately ten times ($IC_{50}$=0.01—0.1 $\mu$g/ml) more potent than the natural polyacetylenes ($IC_{50}$=0.1—1.0 $\mu$g/ml) with (3*R*)-configuration. The epoxide ring at C-9 and C-10 are believed to have some effect on the activity of the compound, but no difference in activity between (9*R*,10*R*)- and (9*S*,10*S*)-isomers was observed. The corresponding 3-oxo-acetylenes (**1**, **3** and their stereoisomers) showed almost the same activities as the (3*R*)-isomers of PQ-1 (**2**), panaxytriol (**5**), panaxydol (**6**), acetylpanaxydol (**7**) and panaxydiol (**8**) ($IC_{50}$=0.1—1.0 $\mu$g/ml). The $C_{14}$-polyacetylene, PQ-8 (**4**) and its isomer were found to have weaker activities ($IC_{50}$=1.0—10.0 $\mu$g/ml) than the $C_{17}$-polyacetylenes.

## Experimental

The $^1$H- and $^{13}$C-NMR spectra were measured on a JEOL JNM-EX90 and

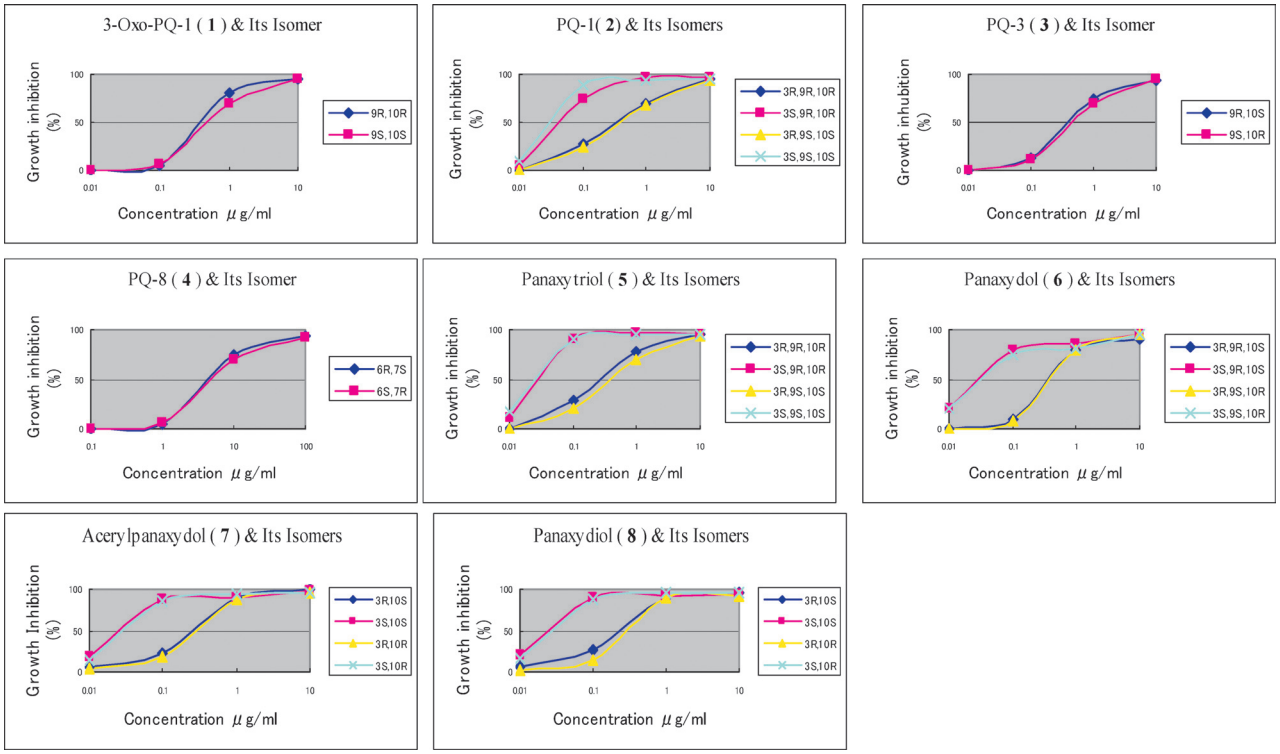


Fig. 1. Growth Inhibition Effects (%) of **1**—**8** and Their Stereoisomers against L-1210

Data are shown as the means from five independent experiments ($p<0.01$). The scale of $x$-axis are shown in the logarithm.

a JEOL JNM-$\alpha$500 spectrometer in $CDCl_3$ containing tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on a JEOL JMS-D 300 instrument. Waco-gel (C-300) was used for column chromatography. Optical rotations were measured on a JASCO DIP-370 polarimeter. A Senshu pack (PEGASIL Silica 60-5, 10$\phi$×250 mm) column was used for HPLC separations. The plant was purchased from UTIDA WAKANYAKU Co. Ltd.

**Isolation of 3-Oxo-PQ-1 (1)** Dried roots of *P. quinquefolium* (1.0 kg) were powdered in a blender and extracted with EtOAc (1.0 l×3) by ultrasonication. The crude extract was chromatographed on a Diaion HP-20 resin (Nippon Rensui) column eluted successively with 1.5 l each of $H_2O$, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, then pure MeOH. The 80% MeOH fraction was chromatographed on silica gel (hexane : AcOEt=2 : 1) followed by HPLC (hexane : AcOEt=5 : 1) to provide **1** (10.4 mg, retention time: 11.0 min) and **2** (7.5 mg, retention time: 19.5 min).

3-Oxo-PQ-1 (**1**): $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=6.8 Hz, H-17), 1.20—1.40 (10H, br m, H-12—H-16), 1.56 (2H, m, H-11), 2.47 (1H, br s, –OH), 2.62 (1H, dd, $J$=6.1, 17.4 Hz, H-8), 2.70 (1H, dd, $J$=6.4, 17.4 Hz, H-8), 3.23 (1H, m), 3.44 (3H, s, –$OCH_3$), 3.76 (1H, m, H-9), 6.22 (1H, d, $J$=10.1 Hz, H-1), 6.41 (1H, dd, $J$=10.1, 17.3 Hz, H-2), 6.57 (1H, d, $J$=17.3 Hz, H-1); $^{13}$C-NMR ($CDCl_3$) $\delta$: 14.1 (C-17), 22.6 (C-16), 25.0 (C-15), 25.1 (C-8), 29.2 (C-14), 29.6 (C-13), 29.8 (C-11), 31.8 (C-12), 58.2 (–$OCH_3$), 65.4 (C-7), 70.6 (C-6), 70.6 (C-9), 76.9 (C-5), 82.0 (C-10), 86.4 (C-4), 134.4 (C-1), 137.7 (C-2), 177.8 (C-3), CI-MS: $m/z$: 291 $(M+1)^+$.

**(2*R*,3*R*)-Epoxy-3-methoxydecane (10a, b)** To a stirred suspension of NaH (434 mg, 60% in oil) in THF (15 ml), **9a** (622 mg) in THF (5 ml) and $CH_3I$ (3.1 g) were added successively at room temperature. After 1 h, 30 ml of water was added to the reaction mixture and then extracted with AcOEt (50 ml×2). The organic layer was washed with brine (50 ml×2), dried over $MgSO_4$ and concentrated under reduced pressure to leave an oil which was then chromatographed on a silica gel column (hexane : AcOEt=10 : 1) to provide **10a** (489 mg, 72.7%) as an oil. Methylation of **9b** was carried out in a similar manner as described above, to provide **10b** as an oil. **10a, b**: $^1$H-NMR ($CDCl_3$) $\delta$: 0.88 (3H, t, $J$=7.17 Hz), 1.20—1.40 (10H, br m), 1.57 (2H, m), 2.48 (1H, dd, $J$=2.8, 5.0 Hz), 2.76 (1H, dd, $J$=4.0, 5.0 Hz), 2.82 (1H, m), 2.94 (1H, ddd, $J$=2.8, 4.0, 7.0 Hz), 3.48 (3H, s); $^{13}$C-NMR ($CDCl_3$) $\delta$: 14.04, 22.60, 25.42, 29.15, 29.63, 31.75, 32.17, 42.95, 54.71, 57.74, 82.51. Low-MS: $m/z$: 186 $(M)^+$.

**(6*R*,7*R*)-7-Methoxy-1,3-tetradecadiyn-6-ol (11a, b)** *n*-BuLi in hexane (1.1 ml, 1.53 mmol/ml) and HMPA (0.9 ml) were added dropwise to a stirred solution of diacetylene in THF (1.1 ml, 6.58 mmol/ml) at −60 °C. After 30 min, **10a** (489 mg) in THF was added and stirring was continued for 3 h at the same temperature. The reaction mixture was quenched with saturated $NH_4Cl$ solution (5.0 ml) and then extracted with AcOEt (50 ml×2). The organic layer was washed with brine (50 ml×2), dried over $MgSO_4$ and concentrated under reduced pressure to leave an oil which was then chromatographed on a silica gel column (hexane : AcOEt=7 : 1) to provide **11a** (467 mg, 75.3%) as an oil. The reaction of **10b** and diacetylene was carried out in a similar manner as described above, to provide **11b** as an oil. **11a, b**: $^1$H-NMR ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=7.0 Hz), 1.20—1.40 (10H, br), 1.57 (2H, m), 2.00 (1H, t, $J$=1.1 Hz), 2.50 (1H, ddd, $J$=1.1, 6.1, 17.3 Hz), 2.58 (1H, ddd, $J$=1.1, 6.4, 17.3 Hz), 3.24 (1H, m), 3.44 (3H, s), 3.72 (1H, m); $^{13}$C-NMR ($CDCl_3$) $\delta$: 14.08, 22.62, 24.39, 25.10, 29.21, 29.72, 29.85, 31.78, 58.30, 65.05, 66.38, 68.18, 70.71, 74.65, 81.89. CI-MS: $m/z$: 237$(M+1)^+$.

**Diastereomeric Mixture (2a, b) at C-3 of PQ-1** *n*-BuLi in hexane (321 $\mu$l, 1.60 mmol/ml) was added dropwise to a stirred solution of **10a** (48.8 mg) in THF (1 ml) at −78 °C. After 30 min, acrolein (67 $\mu$l) was added and stirring was continued for 3 h at the same temperature. The reaction mixture was quenched with saturated $NH_4Cl$ solution (2.0 ml) and then extracted with AcOEt (30 ml×2). The organic layer was washed with brine (30 ml×2), dried over $MgSO_4$ and concentrated under reduced pressure to provide an oil which was then chromatographed on a silica gel column (hexane : AcOEt=7 : 1) to provide **2a** (48.6 mg, 80.8%) as an oil. The reaction of **10b** and acrolein was carried out in a similar manner as described above, to provide **2b** as an oil. **2a, b**: $^1$H-NMR[11] ($CDCl_3$) $\delta$: 0.89 (3H, t, $J$=6.8 Hz), 1.20—1.40 (10H, br), 1.55 (2H, m), 2.53 (1H, dd, $J$=6.1, 17.3 Hz), 2.60 (1H, dd, $J$=6.4, 17.3 Hz), 3.24 (1H, m), 3.44 (3H, s), 3.71 (1H, m), 4.93 (1H, m), 5.26 (1H, d, $J$=10.1 Hz), 5.48 (1H, d, $J$=17.1 Hz), 5.95 (1H, ddd, $J$=5.3, 10.1, 16.4 Hz); $^{13}$C-NMR ($CDCl_3$) $\delta$: 14.11, 22.64, 24.64, 25.12, 29.22, 29.80, 31.57, 31.80, 58.32, 63.51, 66.02, 70.83, 71.09, 74.40, 78.43, 81.93, 117.12, 136.05. FAB-MS: $m/z$: 293$(M+1)^+$.

**Oxidation of 2a, b with $MnO_2$** $MnO_2$ (100 mg) was added to a stirred solution of **2a** (18.8 mg) in $CHCl_3$ (2.0 ml) and stirred for 3 h at room temperature. The reaction mixture was filtered and evaporated *in vacuo*. The residue was chromatographed on a silica gel column (hexane : AcOEt=5 : 1) to provide an oil, then the product was purified by HPLC (hexane : AcOEt=7 : 1) to give **1a** (13.6 mg, retention time=17.2 min) as an oil. Oxidation of **2b** was carried out in a similar manner as described above, to pro-

vide **1b** as an oil. **1a**, **b**: See the data for **1**.[12)]

**Acetylation of a C-3 Diastereomeric Mixture (2a, b) of PQ-1 with Lipase (CHIRAZYME L-2, C3)** Lipase (CHIRAZYME L-2, C3, 250.0 mg) and vinyl acetate (40 $\mu$l) were added to a stirred solution of **2a** (37.5 mg, 0.35 mmol) in *t*-butyl methyl ether (10.0 ml) and the mixture was stirred overnight at room temperature. The reaction mixture was filtered with celite, concentrated *in vacuo*, then the residue was purified by HPLC (hexane : AcOEt=4 : 1) to provide **2a′-Ac** (35.2 mg, retention time=8.0 min) and **2a″** (28 mg, retention time=14.0 min) as oils. Acetylation of **1b** was carried out in a similar manner as described above, to provide **2b′-Ac** and **2b″** as oils. **2a′-Ac**, **2b′-Ac**: $^1$H-NMR $\delta$: 0.89 (3H, t, $J$=6.99 Hz), 1.20—1.40 (10H, br), 1.54 (2H, m), 2.11 (3H, s), 2.54 (1H, dd, $J$=6.25, 17.3 Hz), 2.60 (1H, dd, $J$=6.43, 17.3 Hz), 3.23 (1H, m), 3.43 (3H, s), 3.70 (1H, m), 5.34 (1H, d-like, $J$=10.1 Hz), 5.54 (1H, d-like, $J$=16.4 Hz), 5.87 (1H, m), 5.91 (1H, m).

**Hydrolysis of 2a′-Ac, 2b′-Ac with Lipase (CHIRAZYME L-2, C2)** Compound **2a′-Ac** (64.3 mg) was dissolved in 0.5 ml of acetone and 4.5 ml of pH 7.4 phosphate buffer, then lipase (CHIRAZYME L-2, C2, 180 mg) was added. The reaction mixture was stirred overnight at room temperature, then filtered with celite and extracted with AcOEt (30 ml). The organic layer was washed with brine (30 ml×2), dried over $MgSO_4$ and evaporated *in vacuo* to provide an oil. The residue was purified by HPLC (hexane : AcOEt=4 : 1) to give **2a′** (48 mg, retention time=14.0 min) as an oil. Hydrolysis of **2b′-Ac** was carried out in a similar manner as described above, to provide **2b′** as an oil.

**MTPA Ester of PQ-1 and Its Isomers** Five drops (large excess) of (*S*)-(+)- or (*R*)-(−)-MTPA-Cl was added to a stirred solution of PQ-1 or its isomers (5.0 mg, 0.05 mmol) in pyridine (1.0 ml) and stirred overnight at room temperature. The mixture was diluted with AcOEt (30 ml), washed successively with 10% HCl (20 ml) and saturated $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by HPLC to give (*R*)-(+)- or (*S*)-(−)-MTPA esters of PQ-1 or its isomers.

**Growth Inhibition Activity** The effects of *Panax* polyacetylenes (**1**—**8**) and their optical isomers on the growth of leukemia cells (L 1210) were investigated as follows. Cells were suspended in RPMI medium 1640 (GIBCO) containing 100 units/ml of penicillin G sodium, 100 $\mu$g/ml of streptomycin sulfate and 10% fetal bovine serum supplemented with L-glutamine. Cells were plated in a 96 well microplate (100 $\mu$l/well) at a density of $3\times10^3$ cells/well. After incubating the plate for 24 h at 37 °C in a 5% $CO_2$ humidified atmosphere, the test compounds (**1**—**8** and their isomers) in MeOH (2 $\mu$l/well) at various concentrations were added and the plate was incubated further for 48 h under the same conditions. Control wells ($n$=5) containing the same volume of MeOH (2 ml/well) were incubated in each assay. Ten microliters of Cell Counting Kit-8 (Dojindo)[13,14)] was added to each well, then the microplate was incubated for 3 h in a 5% $CO_2$ atmosphere at 37 °C. The absorbance ($A$) of each well was measured at 450 nm using a Microplate reader (MTP-450, Corona Electric). The percent inhibition was calculated by comparing $A_{control}$ and $A_{treated}$.

$$\text{percent inhibition (\%)} = 100 - A_{treated}/A_{control} \times 100$$

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