6H-CH_3), ir: $\nu_{ar\text{-}H}$ = 3050 cm^{-1} (m), $\nu_{al\text{-}H}$ = 2990cm^{-1} (m), $\nu_{Si\text{-}CH_3}$ = 1260 cm^{-1} (vs).

1,1-Bis(trimethylsilyl)-2,3,4,5-tetraphenyl-1-silacyclopenta-2,4-diene The reaction of 2.10g of red-brownish 1,1-disodio-2,3,4,5-tetraphenyl-1-silacyclopenta-2,4-diene(silyl-dianion) with vapour of Me_3SiCl was proceeded in vacuum line for two hours in an identical manner as discribed above. The red-brownish color of the silyl-dianion changed to yellow. After crystallization from methanol a greenisch-yellow crystal of 1,1-bis(trimethylsilyl)-2,3,4,5-tetraphenyl-1-silacyclopenta-2,4-diene was obtained quantitatively. Yield: 2.41g (95%), m.p. = 99-100°C, Anal. calcd. for $C_{34}H_{38}Si_3$, C = 76.92%, H = 7.23%, found C = 76.87%, H = 7.21%, mass, m/e = 531, ^{1}H-nmr($CDCl_3$), 6.7-7.2 ppm (brd.m, 20H-C_6H_5), 0.1 ppm(s, 18H-CH_3), ir, $\nu_{ar\text{-}H}$ = 3050cm^{-1} (m), $\nu_{al\text{-}H}$ = 2950 cm^{-1} (m), ν_{SiCH_3} = 1245 cm^{-1} (vs).

Quenching 1,1,-disodio-2,3,4,5-tetraphenyl-1-silacyclopenta-2,4-diene with H_2O 1.22g of the red-brownish silyl-dianion was contacted with vapour of H_2O in vacuum line for 30 minutes. The red-brownish color changed to pale yellow immediately. After adding 30 m*l* of ether to this pale-yellow solid, the ether solution thus obtained was filtered and kept at -20°C for one day. A colorles crystal of 1,2,3,4-tetraphenylbutene-2 was obtained. Yield: 0.67g (71%), m.p. = 62°C, Anal. calcd. for $C_{28}H_{24}$, C = 93.29%, H = 6.66%, found C = 93.26%, H = 6.42%, mass, m/e = 360, ^{1}H-nmr($CDCl_3$), 6.95-7.21 ppm(brd.m, 20H-6_6H_5), 4.02 ppm(s, 4H-CH_2) ir, $\nu_{ar\text{-}H}$ = 3030 cm^{-1} and $\nu_{ar\text{-}H}$ = 2960 cm^{-1} (m). The residue was colorless and did not melt above 360°C. In ir only Si-O vibration was observed at 1020 cm^{-1} (vs).

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References

1. E. H. Braye, W. Hübel and I. Caplier, *J. Am. Chem. Soc.*, **83**, 4406 (1961).
2. H. Gilman, Steve G. Cottis and W.H. Atwell, *J. Am. Chem. Soc.*, **86**, 1956 (1964).
3. W. H. Atwell and D. R. Weyenberg, *J. Org. Chem.*, **32**, 885 (1967).
4. R. A. Benkeser, R. F. Grossman and G. M. Stanton, *J. Am. Chem. Soc.*, **84**, 4723 (1962).
5. R. A. Benkeser, R. F. Grossman and G.M. Stanton, *ibid* 84, 4727 (1962).
6. M. David Curtis, *J. Am. Chem. Soc., Comm.*, **89**, 4241 (1967).
7. H. Appler, L.W. Gloss, B. Mayer and W.P. Neumann, *J. Organomet. Chem.*, **291**, 9 (1985).
8. L. E. Gussel'Nikov and N.S. Nametkin, *Chem. Rev.*, **79**, 529 (1979).
9. M. Ishikawa, H. Sugisawa, O. Harata and M. Kumada, *J. Organomet. Chem.* **217**, 43 (1981).
10. W. -Ch. Joo, H. S. Hwang and J. H. Hong, *Bull. Kor. Chem. Soc.*, **6**, 348(1985) and refs. cited there.
11. M. David Curtis, *J. Am. Chem. Soc.*, **91**, 6011 (1969).
12. P. Jutzi and A. Karl, *J. Organomet. Chem.*, **214**, 289 (1981).
13. C. Eaborn, Organosilicon Compounds, Acad. Press N.Y. p. 357-360 (1960).
14. H. Gilman, D. J. Peterson and D. Wittenberg, *Chem. & Ind.* (rev.) 1479 (1958).

New Polyacetylene Compounds from Panax Ginseng C.A. Meyer†

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Two polyacetylene compounds having diyn-ene chromophore were isolated from fresh Korean ginseng roots through solvent fractionation, partition and silica gel column chromatography. The low pressure semi-preparative liquid chromatography and high performance preparative liquid chromatography were used for final separation of polyacetylenic fractions. The chemical structures of these polyacetylenes were determined to be heptadeca-1,8-dien-4,6-diyn-3,10-diol and heptadeca-1,4-dien-6,8-diyn-3,10-diol by UV, FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis.

Introduction

Panax ginseng C. A. Meyer belongs to the Araliaceae family and has been known for many years as the most valued medicine having mysterious effects among all the herbal medicines and plants in Korea, China and Japan.

The polyacetylene compound from ginseng roots was first obtained by Takahashi *et al.* as a yellowish viscous liquid through distillation and silicic acid column chromatography of the ether soluble neutral portion.[1,2] The structure was turned out to be identical with falcarinol isolated from Falcaria Vulgaris B.[3] and carotatoxin isolated from Daucus carota L..[4] Wrobel *et al.* also isolated other C_{17} polyacetylene compounds from ginseng.[5-7]

† Dedicated to Professor Sae-Hee Chang occasion of his 60th birthday.

In 1984, it was reported that the petroleum ether fraction extracted from Korean ginseng roots inhibits the growth of Sarcoma 180 or Walker carcinosarcoma 256 *in vivo* and L1210 leukematic lympocyte *in vitro*.[8]

Recently, Shim *et al.*[9] isolated another new C_{17} polyacetylene compound from fresh ginseng, heptadeca-1-en-4,6-diyn-3,9,10-triol. The compound showed anticancer activities against HRT-18 and HT-29 cells *in vitro*.

A few more new polyacetylene compounds were detected in the HPLC chromatogram of etheral extracts of ginseng. Isolation and characterization of these new polyacetylene compounds are carried out.

Experimental

Materials. The fresh ginseng roots obtained for these experiments were six years old. Extra pure methanol, petroleum ether, ethyl ether were used for solvent extraction as received or after distillation. Solvents for HPLC were HPLC grade n-hexane, ethyl ether and methylene chloride distilled in glass and filtered through membrane filter (0.45 μm) prior to use. Other solvents were used without further purification. Kiesel Gel 60 (70-230 mesh, ASTM) was used for silica gel column chromatography.

HPLC System. Each sample separated through a silica gel column was chromatographed with an Waters Associates Model 244 liquid chromatograph equipped with Model 6000A solvent delivery system, Model 440 UV absorbance detector fixed at 254 nm and U6K septumless universal injector. The bonded normal phase chromatographic column (μ-Bondapak CN) and preparative LiChrosorb CN column were used as received for the analytical and preparative purposes.

Low Pressure Preparative Liquid Chromatography. Low pressure preparative liquid chromatography was performed by the following assembled liquid chromatograph system;

pumping system : Waters Associate Model 6000A
monitor : Bio-rad Model 1300 at 254 nm
column : LiChroprep Si 60 (40-63 μm) Lobar prepacked Column
flow rate : 5.0 m*l*/min

Spectroscopic Measurements. Ultraviolet absorption spectra were recorded with a Cary 17 spectrophotometer. Infrared spectra were recorded on a Analect Instruments FX-6160 FT-IR spectrophotometer as neat liquid samples using sodium chloride windows. Pulsed ^1H NMR spectra were run on a Brucker AM 200 NMR spectrometer at 200 MHz utilizing chloroform-d solvent. ^{13}C NMR spectra were run on a Brucker AM 200 NMR spectrometer (50.32 MHz) with wide band decoupler at spectral width of 200 ppm at ambient temperature and at 8K data points under internal lock signal of chloroform-d solvent. Mass spectra were determined with a JEOL DX-300 GC/MS system through electron impact method. Elemental analysis were run on a Perkin Elmer 240 C elemental analyzer.

Isolation of Polyacetylene Compounds. Fresh Korean ginseng roots were finely crushed up and extracted with methanol, and partitioned with the mixed solvents of petroleum ether: ethyl ether (4:1). Petroleum ether-ethyl ether layer was separated and concentrated under low vacuum.

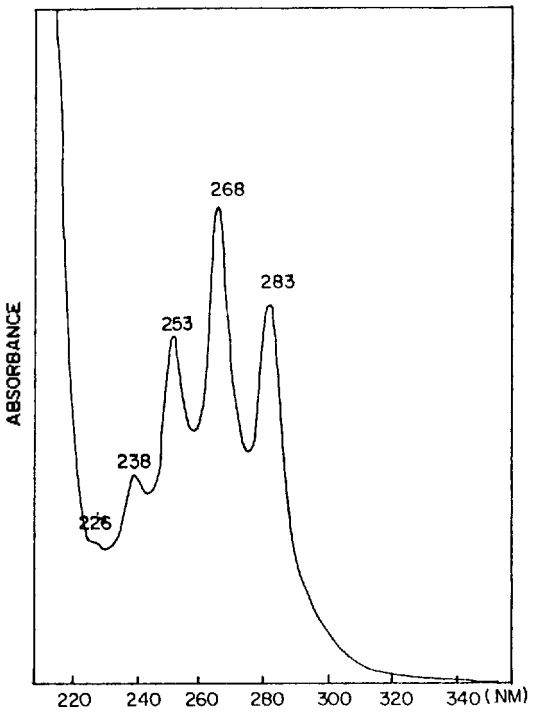


Figure 1. UV Spectrum of C-1 Polyacetylene (in MeOH).

The polyacetylenes were monitored by HPLC and isolated by semipreparative low pressure liquid chromatography. Stop flow injection method was used in the semipreparative liquid chromatography and finally the high performance liquid chromatography was carried out to isolate pure polyacetylenes under the following conditions:

column : preparative LiChrosorb CN (250 × 10 mm)
solvent : n-hexane/ethyl ether = 2/1
flow rate : 4.0 m*l*/min
detector : UV 254 nm

The isolated polyacetylenes were collected into the bottles covered with aluminum foil to cut off the external light and the purity of each fraction was checked by HPLC. Three polyacetylene compounds, A-1, B-1 and C-1, were isolated and characterized by various spectral data and comparing with previously published spectral data. Two of the three separated polyacetylenes, A-1 and B-1 were determined to be heptadeca-1,9-dien-4,6-diyn-3-ol and heptadeca-1-en-4,6-diyn-3,9,10-triol which were already reported and the remaining C-1 was found to be a new polyacetylene compound.

Thermal Stability of A Fraction. To test the thermal stability of A fraction from semipreparative liquid chromatography[10], A fraction was concentrated by bubbling purified nitrogen gas to evaporate off the solvent. Residue was heated in a water bath of 55°C for 12 hours, dissolved in n-hexane and ethyl ether (2:1) and tested the change by HPLC.

Results and Discussion

Characterization of C-1 Polyacetylene. The UV spectrum of C-1 (Figure 1) shows λ_{max} at 283, 268, 253, 238 and 226 nm with band spacing of about 2000cm^{-1} indicating the presence of diyn-ene chromophore with conjugated two triple bonds and one double bond.

The infrared spectrum shows hydroxyl band at 3356

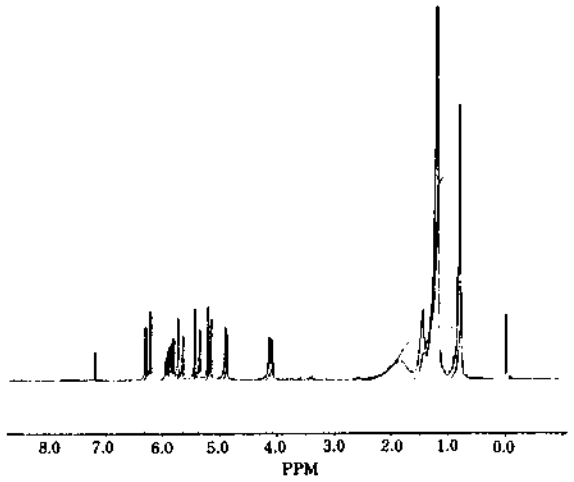


Figure 2. ^1H NMR Spectrum of C-1 Polyacetylene ($CDCl_3$).

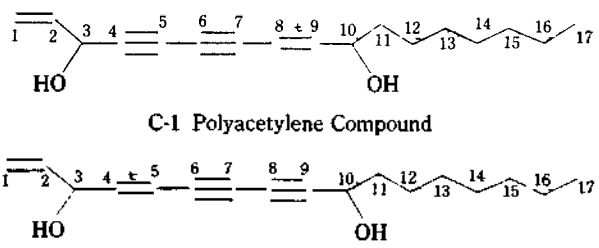
Table 1. ^{13}Chemical Shifts of C-1 and D-1 Polyacetylene ($CDCl_3$, TMS as an internal standard)

Carbon Number	Chemical Shift (ppm)	
	C-1	D-1
1	117.8	118.0
2	136.8	136.6
3	64.3	64.4
4	81.3	146.7
5	73.7	112.5
6	71.0	71.5
7	----	74.9
8	108.8	81.5
9	150.6	----
10	72.8	86.8
11	37.6	32.9
12	25.9	25.8
13	29.9	30.1
14	30.1	29.8
15	32.5	32.4
16	23.3	23.3
17	14.7	14.7

cm^{-1}, methylene group of aliphatic hydrocarbon at 2925 cm^{-1} and 2854 cm^{-1}, conjugated two triple bonds at 2234 cm^{-1}, C-O stretching of secondary hydroxyl group at 1129 cm^{-1}, terminal vinyl group at 1000-900 cm^{-1} and internal double bond of trans configuration at 955 cm^{-1}

The ^1H NMR spectrum (Figure 2) taken in chloroform-d shows a complex spin system of terminal vinyl group at 5.16-5.97 ppm, protons of internal trans double bond at 5.66-6.32 ppm with a coupling constant of 16 Hz, protons attached to secondary hydroxyl group at 4.90 and 4.11 ppm, methylene protons of straight hydrocarbon chains at 1.0-1.5 ppm and terminal methyl group protons at 0.81 ppm.

Table 1 shows the ^{13}C NMR chemical shifts according to the carbon number notated in Figure 3. The molecular skeleton of the polyacetylene compound can be easily recognized by proton decoupled ^{13}C NMR spectra. Typical aliphatic methylene carbons at 23.3, 32.5, 30.1, 29.9, 25.9 and 37.6 ppm, terminal methyl carbon of the straight aliphatic chain at



C-1 Polyacetylene Compound

D-1 Polyacetylene Compound

Figure 3. Chemical Structures of C-1 and D-1 Polyacetylene Compounds.

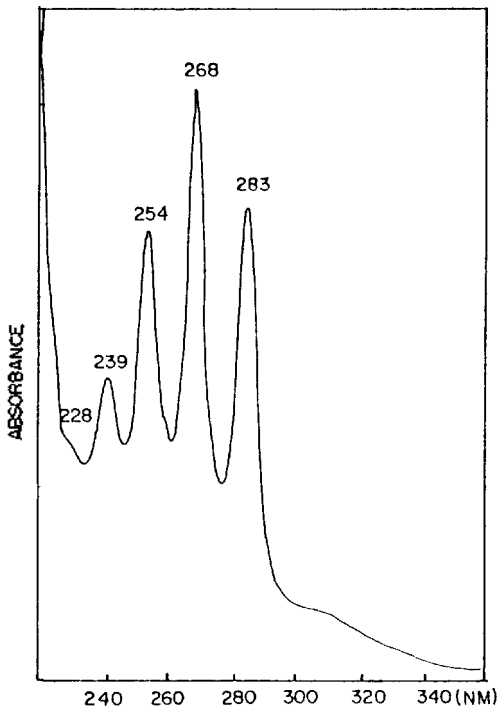
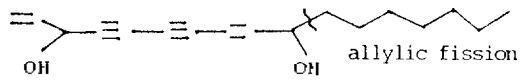


Figure 4. UV Spectrum of D-1 Polyacetylene (in MeOH).

14.7 ppm, two carbons of terminal vinyl group at 117.8 and 136.8 ppm, carbon of allylic position of terminal vinyl group at 64.3 ppm, two carbons of internal double bond at 108.8 and 150.6 ppm, and carbon of allylic position of internal vinyl group at 72.8 ppm are clearly shown in the spectra. The resonance peaks of quarternary carbons of conjugated triple bonds shown at 81.3, 73.7, and 71.0 ppm are not definitely assigned because only three are observed instead of four, one being superimposed with solvent peaks.

The mass spectrum determined by the electron impact method do not shows the molecular ion peak. but show the fragment peak at 161 probably due to allylic fission.



The elemental analysis data is consistent with the expected molecular formula $C_{17}H_{24}O_2$, as shown below

calculated for $C_{17}H_{24}O_2$

C, 78.47; H, 9.23; O, 12.30

found

C, 78.18; H, 9.34; O, 12.48

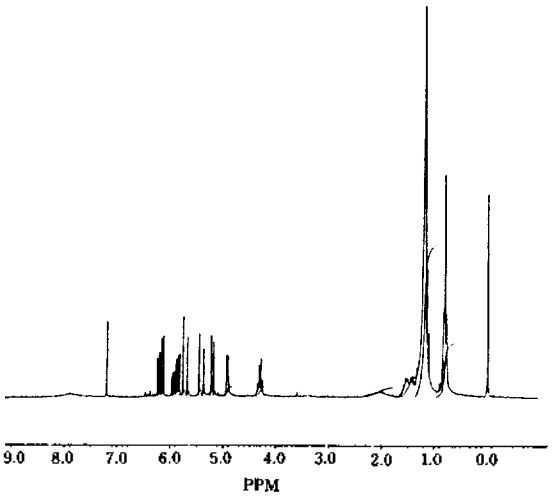


Figure 5. ^{1}H NMR Spectrum of D-1 Polyacetylene ($CDCl_3$).

It is; therefore, concluded that the C-1 polyacetylene is heptadeca-1,8-dien-4,6-diyn-3,10-diol.

Heat-transformed Polyacetylene Compound from A-fraction. When A-fraction was heated in a water bath of 55°C, a new component (D-1) was detected in the HPLC chromatogram. This component was isolated by preparative HPLC and characterized by various physical methods.

UV spectrum (Figure 4) shows λ_{max} at 283, 268, 254, 239, and 228 nm with band spacing of about 2000cm^{-1} indicating that the compound is also a polyacetylenic compound having diyn-ene chromophore.

The infrared spectrum shows hydroxyl group peak at 3417 cm^{-1}, methylene group of aliphatic hydrocarbon at 2928 and 2857 cm^{-1}, conjugated two triple bonds and one double bond at 2211 cm^{-1}, C-O stretching of secondary hydroxyl group at 1093 and 1019 cm^{-1}, terminal vinyl group at 1000-900 cm^{-1} and trans internal double bond at 954 cm^{-1}.

The ^{1}H NMR spectrum of D-1 polyacetylene (Figure 6) taken in chloroform-d shows also complex spin system of terminal vinyl group at 5.16-5.17 ppm, protons of internal trans double bond at 5.68-6.24 ppm with a coupling constant of 16 Hz, protons attached to secondary hydroxyl group at 4.93 and 4.32 ppm methylene protons of hydrocarbon chain at 1.11-1.58 ppm and terminal methyl group protons at 0.88 ppm.

Table 1 shows the ^{13}C chemical shifts for D-1 polyacetylene according to the carbon number notated in Figure 3. The proton band decoupled ^{13}C spectrum shows the typical aliphatic methylene carbons at 23.3 32.4, 29.8, 30.1, 25.8, and 32.9ppm, terminal methyl carbon of the straigt aliphatic chain at 14.7 ppm, two carbons of terminal vinyl group at 118.0 and 136.6 ppm, carbon of allylic position of terminal vinyl group at 64.4 ppm, two carbons of internal double bond at 112.5 and 146.7 ppm, and carbon of secondary hydroxyl group at 86.8 ppm. The resonance peaks of quarternary carbons of conjugated triple bonds shown at 81.6, 74.9, and 71.5 ppm are not definitely assigned because only three are observed instead of four, one being superimposed with solvent peaks.

The mass spectra determined by electron impact method do not show the molecular ion peak.

The elemental analysis data is consistent with the expected molecular formula, $C_{17}H_{24}O_2$, as shown below.

Calculated for $C_{17}H_{24}O_2$

C, 78.47; H, 9.23; O, 12.30

Found

C, 78.76; H, 9.18; O, 12.06

The compound, D-1 polyacetylene, is thus determined to be heptadeca-1,4-dien-6,8-diyn-3,10-diol.

Conclusion

Two polyacetylenes were isolated through solvent fractionation, partition, semi-preparative liquid chromatography and preparative high performance liquid chromatography from Panax ginseng C. A. Meyer.

These two polyacetylenes were determined to be heptadeca-1,8-dien-4,6-diyn-3,10-diol and heptadeca-1,4-dien-6,8-diyn-3,10-diol by various physical methods.

One of the polyacetylenes is a naturally occurring polyacetylene compound, but the other is a heat-transformed polyacetylene compound from unidentified component of Panax ginseng C. A. Meyer.

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References

1. M. Takahashi and M. Yoshikura, *J. Pharmc. Soc. Japan*, **84**, 752 (1964).
2. M. Takahashi and M. Yoshikura, *J. Pharmc. Soc. Japan*, **84**, 757 (1964).
3. F. Bohlmann, U. Niedballa, and K. M. Rode, *Chem. Ber.*, **99**, 3552 (1966).
4. D. G. Crosby and N. Aharonson, *Tetrahedron*, **23**, 465 (1967).
5. J. T. Wrobel, Z. Dabowski, H. K. Gielzynska, A. Iwanow, K. Kabzinska, J. Poplawsik, and J. Ruszkowska, *Tluszcze Srodki Piorace Kosmet.*, **71**, 163 (1971).
6. J. Poplawski, J. T. Wrobel, and T. Glinka, *Phytochem.*, **19**, 1539 (1980).
7. *Z. Dabrowski, J. T. Wrobel, and K. Wojtasiewicz, Phytochem.*, **19**, 2464 (1980).
8. W. I. Hwang and S. K. Oh, *Korean J. Ginseng Science*, **8**, 153 (1984).
9. *S. C. Shim, H. Y. Koh, and B. H. Han, Phytochem.*, **22**, 1817 (1983).
10. S.-K. Chang, H. Y. Koh, and S. C. Shim, *Korean J. Ginseng Science*, **10**, 21 (1986).