References

(1) J.A. Osborn, F.H. Jardine, J.F. Yound and G. Wilkinson, *J. Chem. Soc.*, **A**, 1711 (1966).
(2) P.Z. Meakin, J.P. Jesson and C.A. Tolman, *J. Amer. Chem. Soc.*, **94**, 3240 (1972).
(3) J. Halpern and C.S. Wong, *Chem. Commun.*, 629 (1973).
(4) C.A. Tolman, P.Z. Meakin, D.L. Lindner and J.P. Jesson, *J. Amer. Chem. Soc.*, **96**, 2762 (1974).
(5) J. Halpern, in: Y. Ishi and M. Tsutsui, Eds., "Organotransition-Metal Chemistry", Plenum (1975).
(6) J. Halpern, T. Okamoto and A. Zakhariew, *J. Mol. Catalysis*, **2**, 65 (1977).
(7) Y. Ohtani, A. Yamagishi and M. Fujimoto, *Bull. Chem. Soc. Japan*, **52**, 69 (1979).
(8) W. Stroheimer and Endes, *Z. Naturforsch.*, **27b**, 1415 (1972).
(9) M.A. Bennett and D. L. Milner, *Chem. Commun.*, 581 (1967).
(10) W. Stroheimer and W. Rehder-Stirnweiss, *Z. Naturforsch.*, **24b**, 1219 (1969); **26b**, 61 (1971).
(11) W. Rehder-Stirnweiss and R. Fleischmann, *Z. Naturforsch.*, **25b**, 1481 (1970).
(12) J.C. Woo and C.S. Chin, submitted for publication.
(13) G.W. Parshall, "Homogeneous Catalysis", Wiley-Interscience, 1980, p. 92.
(14) W. Stroheimer, *Topics in Current Chemistry*, **25**, 71 (1972).
(15) B.R. James and N.A. Memon, *Can. J. Chem.*, **46**, 217 (1968).
(16) T. Onoda, *Z. Naturforsch.*, **24b**, 1493 (1963).
(17) R. Fleischmann, *J. Organomet. Chem.*, 29, C39 (1971).
(18) C.J. Moon and C.S. Chin, *J. Korean Chem. Soc.*, **26**, 253 (1982).
(19) L. Vaska, *Acc. Chem. Res.*, **1**, 355 (1968).
(20) It is well know that complex **1** reacts with H_2 and AN to give **2** and **3**, respectively.[19] Therefore, the possible olefin route for the hydrogenation of AN would be similar to eq. (1–a) where the dissociation of L occurs after the formation of the olefin adduct, $IrCl(olefin)(CO)(Ph_3p)_2$.
(21) J.P. Collman, C.T. Sears, Jr. and M. Kubota, *Inorg. Sytheses*, **11**, 101 (1968).
(22) L. Vaska and R.E. Rhodes, *J. Amer. Chem. Soc.*, **87**, 4970 (1965).

Polyacetylene Compounds from Panax Ginseng C. A. Meyer

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Two major and two minor polyacetylenes were isolated from fresh white Korean ginseng roots. The petroleum ether–ethyl ether fractions containing the polyacetylene compounds were collected through solvent fractionation, partition and silica gel column chromatography. Further separation of polyacetylenic fractions was proceeded by bonded normal phase HPLC utilizing a moderately nonpolar microparticulate column. The low pressure liquid chromatography was used for the semi-preparative separation. The chemical structures of the two major polyacetylenes separated were determined by UV, IR, ^1H NMR, ^{13}C NMR, mass spectra and elemental analysis. One of them is identified to be heptadeca-1-en-4, 6-diyne-3, 9, 10-triol, a new structure, and the other is heptadeca-1, 9-dien-4, 6-diyn-3-ol, known as panaxynol.

Introduction

Panax ginseng C. A. Meyer (Araliaceae) has been known for many years as the most valued among all herbal medicines and plants having mysterious effects in Korea, China and Japan. Since the saponin components from American ginseng (Panax quinquefolium L.) were isolated for the first time by Garriques in 1854,[1] much interest has been generated for the chemical, biochemical and pharmacological studies on ginsengs.

Recently, it was reported[2,3] that the petroleum ether fraction extracted from Korean ginseng roots inhibits the growth of murine leukemia L5178Y and murine Sarcoma 180 cells *in vitro*, and also inhibits DNA, RNA and protein synthesis in murine ascitic Sarcoma 180 cells *in vitro*. The petroleum ether-etheral extracts from ginseng roots contain fatty acids, hydrocarbons, steroids, polyacetylene compounds, and glycosides. However, since it has not been known which of the components described above shows the cytotoxicity for the carcinoma cells, Panax ginseng has been investigated to determine the chemical composition of the plant root.

A polyacetylene compound from ginseng roots was isolated by Takahashi *et al.* in 1964.[4,5] The chemical structure of the compound was turned out to be identical with falcarinol

isolated from Falcaria vulgris B. and carotatoxin isolated from Daucus carota L..[7] Wrobel et al.[8~10] very recently also isolated other C_{17} polyacetylene compounds from ginseng such as panaxydol and heptadeca-1-en-4, 6-diyn-3, 9-diol. However, the clear spectral data or definite chemical structure of polyacetylenes from ginsengs are not unequivocally established at present due to the thermal and photochemical instability of the naturally occurring polyacetylene compounds and difficulty to isolate large enough quantities necessary for characterization because of the minute concentration of the compounds in the ginseng roots.

We have isolated two major polyacetylenes from the fresh ginseng C. A. Meyer roots and determined their chemical structures.

Materials and Methods

Materials. The fresh ginseng roots obtained for these experiments were six years old. Solvents for HPLC were HPLC grade n-hexane, ethyl ether and methylene chloride distilled in glass (Burdick and Jackson Lab. Inc.) and filtered through membrane filter (0.45 μm) prior to use. Kiesel gel 60 GF_{254} for thin layer chromatography and Kiesel gel 60 for silica gel column chromatography (70-230 mesh ASTM) were also used.

HPLC system. Each sample separated by 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) was used as received for the analytical purposes.

Spectroscopic Measurements

Ultraviolet absorption spectra were recorded with a Cary 17 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 283B grating spectrophotometer as neat liquid samples using sodium chloride windows. Pulsed proton NMR spectra were run on a Varian FT-80A NMR spectrometer at 79.542 MHz utilizing chloroform-d solvent as internal lock signal of chloroform-d solvent. Mass spectra were determined with a JEOL DX-300 GC/MS (low resolution) system through electron impact method. Elemental analyses were performed at Chemical Analysis Division of Korea Research Institute of Chemical Technology.

Isolation Methods

Fresh Korean ginseng roots (8 kg) were finely crushed up and extracted with methanol. Methanolic extracts were partitioned in the mixed solvents of petroleum ether-methanol (1:1). Petroleum ether layer was washed with 5 % NaOH solution several times. The solvent of the petroleum ether layer of ginseng extracts was evaporated and the crude oily mixture (4.5 g) remained was dissoved in mixed solvents of petroleum ether and ethyl ether for silica gel column chromat graphy. Stepwise gradient elution with petroleum ether/ethyl ether varying the polarity (from 5/1 to 2/1) gave two main fractions, A(ca. 120 mg) and B(ca. 330 mg), containing the polyacetylene compounds.

To monitor the polyacetylenes from A and B fractions using HPLC system, an analytical liquid chromatography was performed under the following conditions;

column: μ-Bondapak CN (3.9 mm ID × 30 cm)

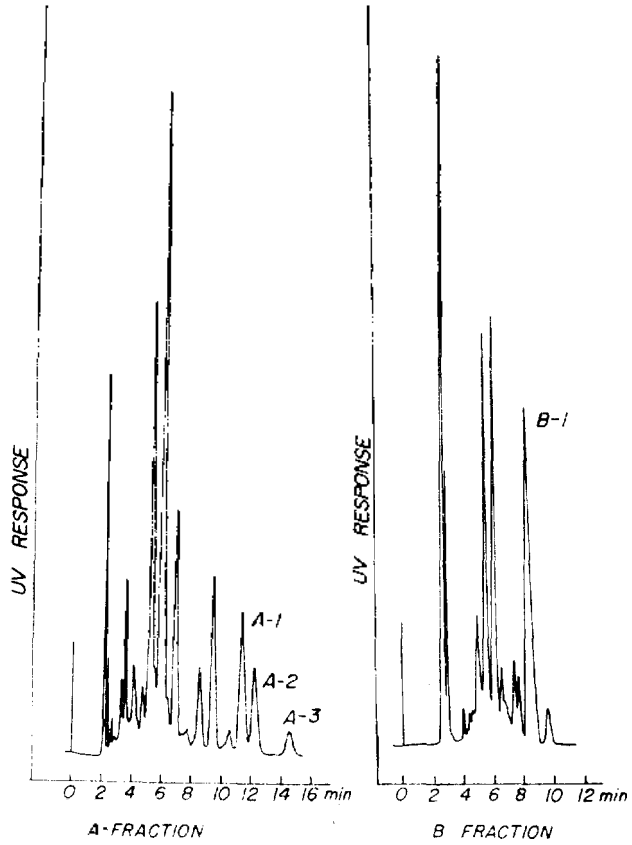


Figure 1. Liquid chromatogram of A and B fraction.

TABLE 1: The Typical UV λ_{max} of Diyne and Diyne-Ene System

Chromophore	λ_{max} (nm) (ε: molar extinction coefficient)
Diyne	255(200) 240(400) 230(300)
Diyne-Ene	280(15000) 265(18000) 251(13000) 238(6000) 210(42000)

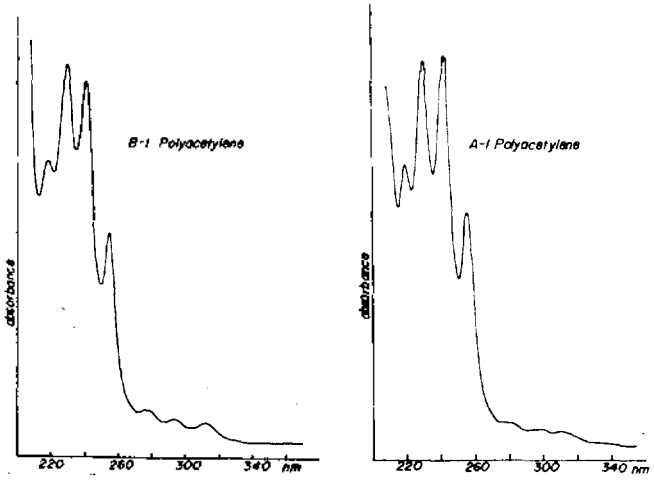


Figure 2a. UV spectra of A-1 and B-1 polyacetylene.

solvent: n-hexane/CH_2Cl_2 = 20/1 v/v for A fraction
n-hexane/Et_2O = 20/1 v/v for B fraction
flow rate: 1.0 ml/min.
detector: UV (254 nm)

To isolate polyacetylenes from each fraction, semipreparative liquid chromatography was carried out with the low pressure liquid chromatography under the following conditions;
column: Lichroprep Si 60 (40–63μm)
solvent: n-hexane/Et_2O = 3/1
flow rate: 5.0 ml/min.

In running semi-preparative liquid chromatography, the isolated polyacetylenes were collected into the bottles immersed in a dry ice-acetone bath and covered with aluminum foil to cut off the external light. The purity of each separated fraction was checked on a HPLC in analytical scale. For the spectroscopic measurements, collected fractions were concentrated by bubbling purified nitrogen gas to evaporate off the solvents. Residual solvent was removed by rotatory vacuum evaporator to obtain 7 mg of A–1 and 12 mg B–1 in pure form.

Anal. for B–1. Calcd. for $C_{17}H_{26}O_3$: C, 73.38; H, 9.35; O, 17.27. Found: C, 73.06; H, 9.40; O, 17.54.

Results and Discussion

Freshly prepared etheral extracts of the Panax ginseng C. A. Meyer were subjected to silica gel column chromatography. Two major polyacetylenic fractions, A and B, were obtained in the same order of the R_f values of silica gel thin layer chromatography. Since the isolation schemes following the classical chromatography and separation techniques such as solvent partition have many problems due to the high instability and due to the minute quantities of samples, complete separation of polyacetylene compounds from A and B fractions was accomplished by HPLC system utilizing a bonded normal phase

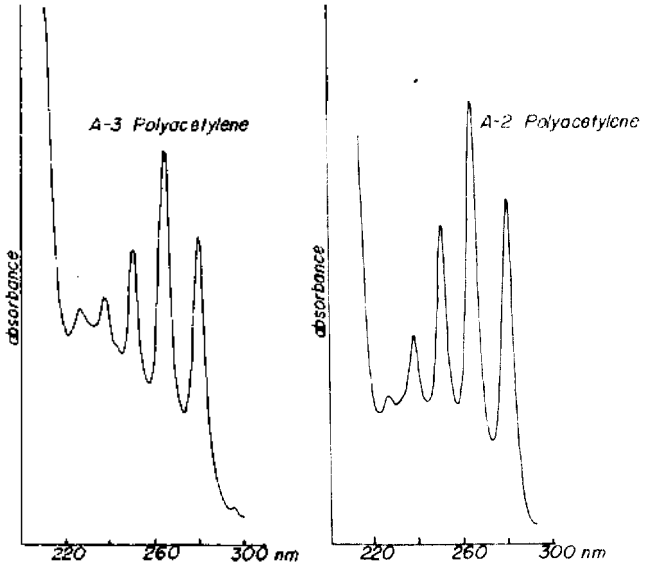


Figure 2b. UV spectra of A–2 and and A–3 polyacetylene.

TABLE 2: UV λ_{max} of Isolated Polyacetylenes

Component	λ_{max} (nm)	band spacing (cm^{-1})
A–1	257	2411
	242	1968
	231	2165
	220	
B–1	254	2297
	240	2193
	228	2223
	217	
A–2	279	2181
&	263	1977
A–3	250	2194
	237	2054
	226	3371
	210	

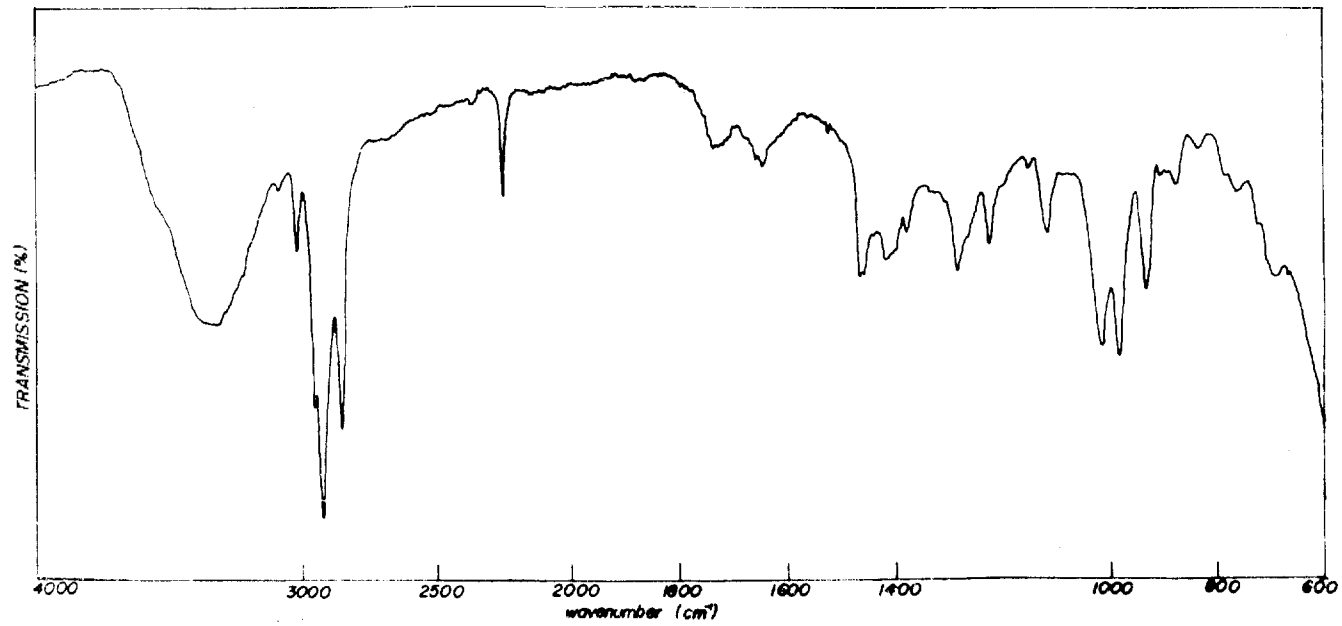


Figure 3a. IR spectra of A–1 polyacetylene.

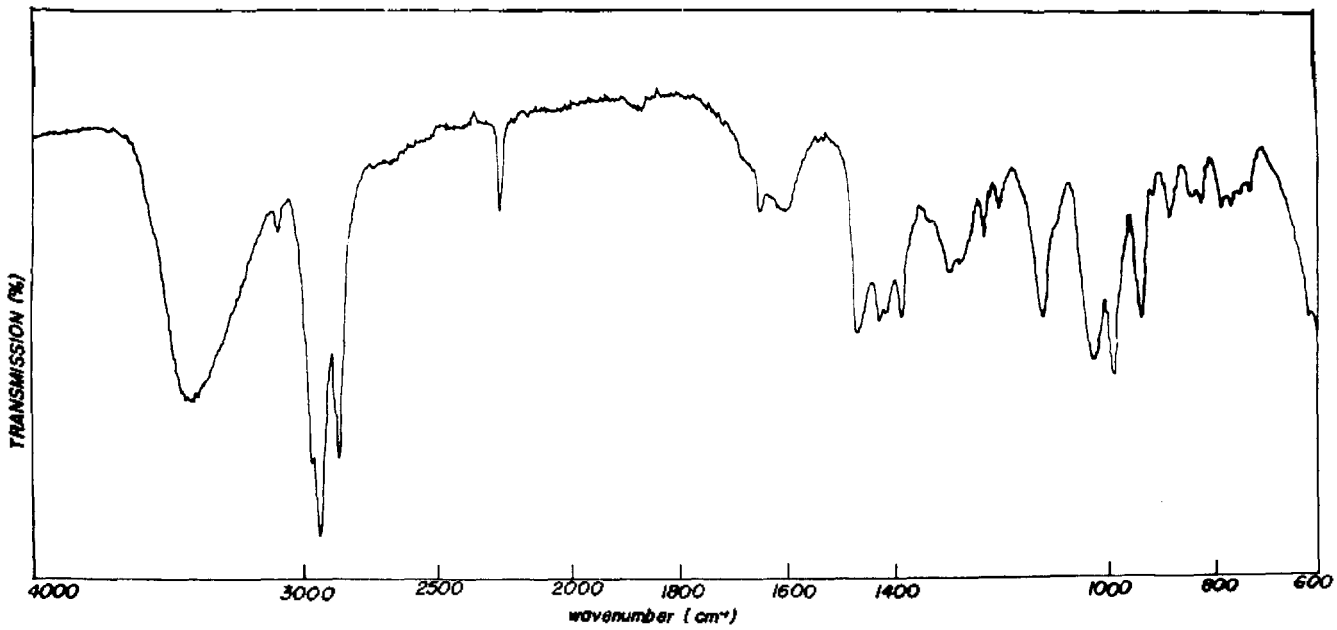


Figure 3b. IR spectra of B–1 polyacetylene.

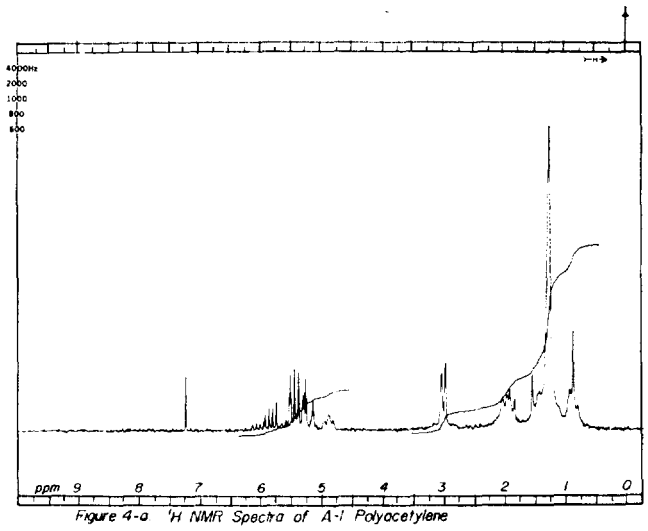


Figure 4a. ^{1}H NMR spectra of A–1 polyacetylene.

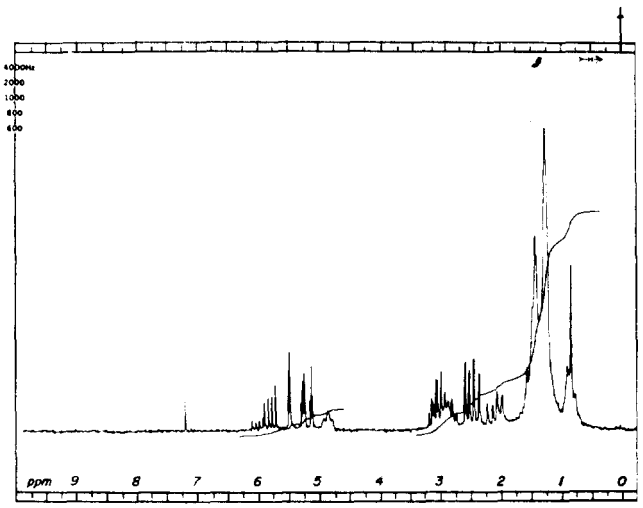


Figure 4b. ^{1}H NMR spectra of B–1 polyacetylene.

column having moderately low polarity.

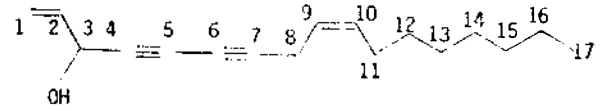
From the chromatograms shown in Figure 1, four polyacetylenes, A–1, A–2, A–3, and B–1, were obtained and each component was tested by the UV spectra and compared with the previously published UV data as shown in Table 1.[11] The UV spectra of separated components in Figure 2 show the typical polyacetylene vibrational bands with the spacings of ca. 2000 cm^{-1} indicating the presence of the conjugated acetylenes. Spectral data tabulated in Table 2 show λ_{max} of each peak of the polyacetylenes. They can be classified into two main polyacetylenic groups having two different characteristic UV chromophore, namely diyne system with two conjugated triple bonds for A–1 and B–1 component and diyne-ene system with conjugated two triple bonds and one double bond for A–2 and A–3 component. Since A–2 and A–3 components have higher molar absorption coefficients at 254 nm but show smaller peak intensity on the liquid chromatogram than A–1 and B–1 polyacetylenes, they must exist in much smaller quantities than A–1 and B–1 and preparative isolation of A–2 and A–3 for further physical characterizations is not carried out.

The infrared spectra of A–1 show hydroxyl group at 3400 cm^{-1}, methylene group at 2940 and 2863 cm^{-1}, conjugated triple bonds at 2260 cm^{-1}, C–O stretching of secondary hydroxyl group at 1120 cm^{-1}, terminal vinyl group at 1000–900 cm^{-1} and internal double bond of *cis* configuration at 690 cm^{-1}. Those of B–1 show also the same absorption bands at 3400 cm^{-1}, 2940 cm^{-1}, 2863 cm^{-1}, 2260 cm^{-1}, 1120 cm^{-1} and 1000–900 cm^{-1} as the A–1 spectra. Two differences between A–1 and B–1 spectra were observed, one being the difference of the relative peak intensity at 1120 cm^{-1} and the other the absence of peak at 690 cm^{-1} in B–1 spectra. The reason of the former difference is attributed to the presence of more secondary hydroxyl groups in B–1 than in A–1 polyacetylene. The

TABLE 3: Chemical Shifts of ^{13}C NMR Spectra

Carbon Number	Chemical Shifts (ppm)		
	A-1	B-1	
1	117.2	117.3	
2	136.4	136.3	
3	63.4	63.3	
4	—	77.6	
5	—	76.7	not assigned
6	—	75.2	
7	—		
8	19.3	18.9	
9	122.1	54.0	
10	126.6	56.5	
11	26.8	27.1	
12	26.0	26.0	
13	28.8	29.0	
14	28.7	28.8	
15	31.4	31.3	
16	22.2	22.2	
17	13.6	13.5	

A-1 heptadeca-1,9-dien-4,6-diyne-3-ol



B-1 heptadeca-1-en-4,6-diyne-3,9,10-triol

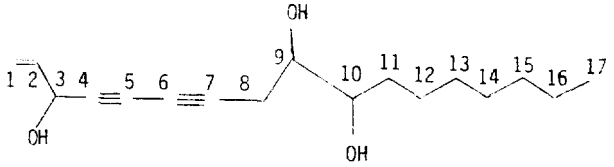


Figure 5. Chemical structure of isolated polyacetylenes.

absence of 690 cm^{-1} peak in the B-1 spectra indicates the absence of an internal double bond in B-1 in contrast to the A-1 polyacetylene. From the UV and IR spectral analysis, it is clear that both A-1 and B-1 polyacetylenes have the similar molecular skeletons and functional groups with minor differences.

The ^1H NMR spectra of A-1 polyacetylene taken in chloroform-d show complex spin system of terminal vinyl group at 5.14-6.13 ppm, protons of internal double bond at 5.37-5.48 ppm, allylic protons of terminal vinyl group at 4.80-4.96 ppm, two protons between conjugated triple bond and internal double bond in *cis*-form at 2.27-3.03 ppm, two allylic protons of internal double bond connected to a hydrocarbon chain at 1.83-2.06 ppm, methylene protons of straight hydrocarbon chains at 1.26 ppm, and terminal methyl group protons of aliphatic hydrocarbon chain at 0.86 ppm. The ^1H NMR spectra of B-1 polyacetylene show the same functional groups as A-1 polyacetylene and show the proton peaks at 5.13-6.10 ppm, 4.79-4.92 ppm, 1.28-1.44 ppm, and 0.85 ppm. However, the absence of peaks at 5.37-5.48 ppm range indicates the absence of protons bound to the internal double bond of aliphatic hydrocarbon chain. The presence of more secondary hydroxyl groups in B-1 than A-1 polyacetylene may have caused the very complex spin system in the range of 1.98-3.19 ppm in B-1.

Table 2 shows the chemical shifts of ^{13}C for A-1 and B-1 polyacetylenes according to the carbon number notated in Figure 5. The molecular skeleton of each polyacetylene can be easily recognized by the use of ^{13}C NMR spectral data. The proton wide band decoupled ^{13}C NMR spectra of A-1 polyacetylene show the typical aliphatic methylene carbons at 22.2, 31.4, 28.7, 28.8, 26.0 and 26.8 ppm, terminal methyl carbon of the straight aliphatic chain at 13.6 ppm, two carbons of terminal vinyl group at 117.2 and 136.4 ppm, carbon of allylic position of terminal vinyl group at 63.4 ppm, methylene carbon strongly shielded by conjugated triple bonds at 19.3 ppm, and two carbons of internal double bond at 122.1 ppm. Except for a few carbons of different functional groups, those of B-1 polyacetylene show the same absorption peaks at 22.2, 31.3, 28.8, 29.0 26.0, 27.1, 13.5, 117.3, 136.3, 63.3 and 18.9 ppm as expected. The resonance peaks of quarternary carbons of conjugated triple bonds shown at 77.6, 76.7 and 75.2 ppm in B-1 polyacetylene are not definitely assigned because only three are observed instead of four, one being superimposed with solvent peaks. Although many spectral transients were executed to obtain better signal/noise ratio, quarternary carbons of A-1 polyacetylene could not be found because of the large solvent peaks and back ground noise. The carbon-13 NMR spectra of A-1 show upfield shift of two carbons, from 126.6 and 122.1 ppm to 56.5 and 54.0 ppm compared to B-1 polyacetylene. This indicates the presence of more secondary hydroxyl groups in B-1 and substantiates the previous suggestion.

The mass spectra of A-1 polyacetylene determined by the electron impact method show the molecular ion peak at 244, $M^+-C_6H_{13}$ peak by allylic fission[12,13] at 159 but those of B-1 polyacetylene neither show the molecular ion peak at 278 nor the typical fragment peak probably due to the highly unstable property of the B-1 polyacetylene. The same phenomena were observed from the panaxydol, 9, 10-epoxy-3-hydroxyheptadeca-1-en-4,6-diyne.[9]

Thus the spectral data of B-1 show some differences from those of previously reported other ginseng polyacetylenes such as panaxydol,[9] falcarinol,[4] and heptadeca-1-en-4, 6-diyn-3, 9-diol.[10] The major differences are intense secondary hydroxyl group peak at 1120 cm^{-1} in the IR spectra, complex peak pattern at 1.98-3.19 ppm in ^1H NMR spectra due to hydroxyl groups in 9, 10 position, and elemental analysis data of $C_{17}H_{26}O_3$.

From these results, it is concluded that the A-1 polyacetylene is heptadeca-1, 9-dien-4, 6-diyn-3-ol which is identical with the previously isolated panaxynol or falcarinol and the

B-1 polyacetylene is heptadeca-1-en-4, 6-diyn-3, 9, 10-triol. This is a newly found polyacetylene compound among the C_{17} naturally occurring polyacetylenes in Korean ginseng roots.

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References

(1) J.-H. Kim, "Korean Ginseng", Ed. H.-W. Bae, P.62, Korea Ginseng Research Institute, Seoul, 1978.

(2) W. I. Hwang and S. M. Cha, Proceedings of the Second International Ginseng Symposium, P. 43, Seoul, 1978.

(3) Y. S. Yun, Se Y. Lee, B. S. Kim and T. K. Yun, *J. Korean Biochem.*, **13**. 203 (1980).

(4) M. Takahashi and M. Yoshikura, *J. Pharmac. Soc. Japan*, **84**. 752 (1964).

(5) M. Takahashi and M. Yoshikura, *ibid.*, 757 (1964).

(6) F. Bohlmann, U. Niedballa and K.-M. Rode, *Chem. Ber.*, **99**. 3552 (1966).

(7) D. G. Crosby and N. Aharonson, *Tetrahedron*, **23**. 465 (1967).

(8) J. T. Wrobel, Z. Dabowski, H. K. Gielzynska, A. Iwanow, K. Kabzinska, J. Popławski and J. Ruszkowska, *Tluszcze Srodki Piorace Kosmet.*, **17**. 63 (1973).

(9) J. Poplawski, J. T. Wrobel and T. Glinka, *Phytochem.*, **19**. 1539 (1980).

(10) Z. Dabrowski, J. T. Wrobel and K. Wojtasiewicz, *Phytochem.*, **19**. 2464 (1980).

(11) F. Bohlmann, T. Burkhardt and C. Zero, in "Naturally Occuring Acetylenes" P. 4, Academic Press, London and New York, 1973.

(12) Sir Ewart R. H. Jones, S. Safe and V. Thaller, *J. Chem. Soc. (C)* 1220 (1966).

(13) R. K. Bentley, D. Bhattacharjee, Sir Ewart R. H. Jones, and V. Thaller, *J. Chem. Soc. (C)*. 685 (1969).