Photooxidation of a Polyacetylene Compound from Panax Ginseng C.A. Meyer

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A major polyacetylene compound from Panax ginseng roots, heptadeca-1-en-4,6-diyn-3,9,10-triol, was irradiated with 300 nm UV light to obtain a photooxidized acetylenic compound having the conjugated en-on-diyne chromophore, heptadeca-1-en-4,6-diyn-9,10-diol-3-one. The same oxidation product was obtained exclusively by 4-(dimethylamino) pyridinium chlorochromate at room temperature.

Introduction

Panax ginseng C.A. Meyer has been known for many years as the most valued medicine among the 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], the chemical, biochemical and pharmacological studies for ginseng have been stimulated and carried out for a long time.

It was recently 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 leukemic lympocyte *in vitro*.[2] The petroleum ether-ethyl ether extracts from ginseng roots contain some polyacetylenic compounds which are responsible for the cytotoxicity described above.

A polyacetylene compound from ginseng roots was isolated by Takahashi et al. in 1964.[3,4] The chemical structure of the compound was turned out to be identical with falcarinol isolated from *Falcaria vulgaris* B.[5] and carotatoxin isolated from *Daucus carota* L.[6]. Wrobel et al. in 1973 also isolated another type of C_{17}-polyacetylene compounds from ginseng.[7]

Two major and two minor polyacetylenes were isolated from fresh Korean ginseng roots in our laboratory. The chemical structures of these two major polyacetylenes separated were determined to be heptadeca-1-en-4,6-diyn-3,9,10-triol and heptadeca-1,9-dien-4,6-diyn-3-ol, falcarinol.[8]

These polyacetylenes from ginseng are not thermally stable and very photolabile. We, therefore, investigated the photolysis and thermal oxidation of heptadeca-1-en-4,6-diyn-3,9,10-triol, a new polyacetylene compound from Korean ginseng roots.

Experimental

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 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.

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. Carbon-13 NMR spectrum was measured on an AM-200-SY Bruker spectrometer operating at 50.3 MHz. GC/MS spectrum was recorded on a VG 12-250 instrument with an ionization voltage of 70 eV. The GC was equipped with SE 30 capillary column.

Irradiation of Heptadeca-1-en-4,6-diyn-3,9,10-triol. About 10 mg of heptadeca-1-en-4,6-diyn-3,9,10-triol dissolved in nondegassed 15 m*l* of n-hexane:ethyl ether(20:1 v/v) were irradiated in a Rayonet Photochemical Reactor Model RPR-208 equipped with 254 nm mercury arc lamps or 300 nm fluorescent lamps (The Southern New England Ultraviolet Company). The samples were irradiated for 20 minutes with 254 nm UV light and for 10 hours with 300 nm UV light. After the irradiation, the resulting photoreaction mixtures were concentrated by bubbling nitrogen gas and analyzed by HPLC. The photoreaction product was isolated by semi-preparative HPLC.

Oxidation of Heptadeca-1-en-4,6-diyn-3,9,10-triol by 4-(Dimethylamimo) pyridinium chlorochromate. The heptadeca-1-en-4,6-diyn-3,9,10-triol (3×10^{-4} M) was added with stirring to a four fold excess of 4-(dimethylamino) pyridinium chlorochromate or manganese dioxide in methylene chloride solution (10 m*l*). After stirring for 15 hours, the mixtures were diluted with petroleum ether. The resulting solutions were passed through a Sep-pak cartridge (silica) and the product was analyzed by UV, HPLC, ^{1}H-NMR and ^{13}C NMR spectra.

Results and Discussion

Analysis of Photoreaction Mixture. After the irradiation of aerated n-hexane/diethyl ether solution of heptadeca-1-en-4,6-diyn-3,9,10-triol with 254 nm or 300 nm UV light, the solution was chromatographed and monitored by HPLC using Radial Pak column. Figure 1 and 2 show the liquid chromatograms of reaction mixtures, respectively. When the sample is irradiated with 254 nm UV light, the polyacetylene was fully decomposed into unknown components after 20

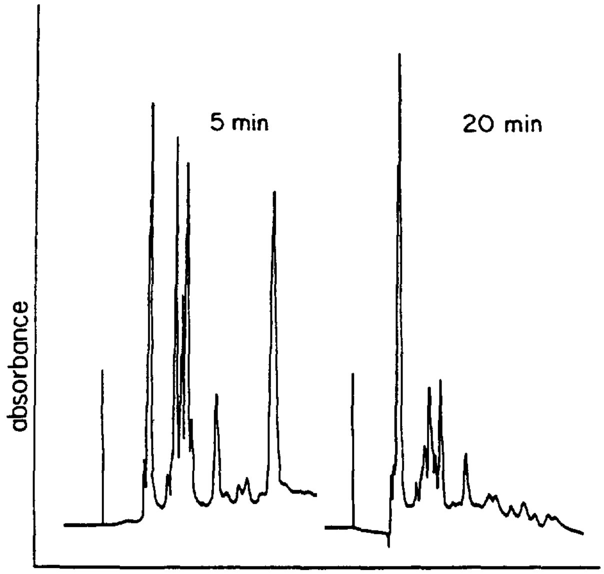


Figure 1. Liquid chromatogram of reaction mixture (254 nm).

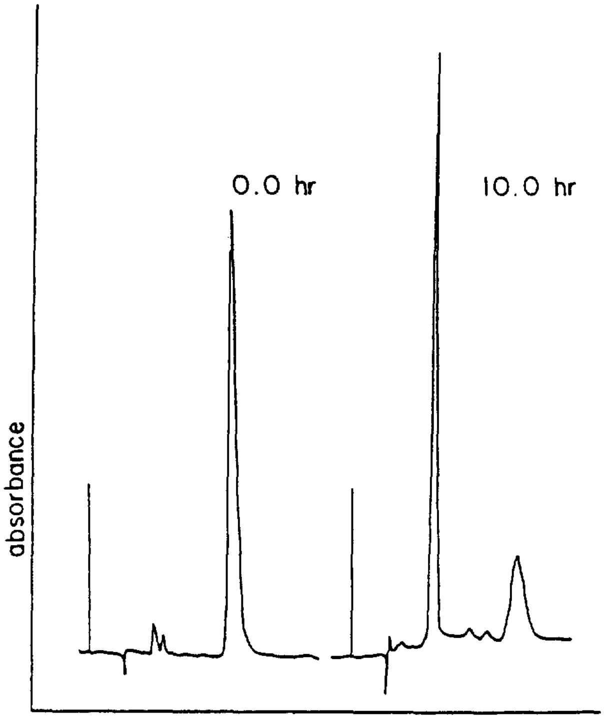


Figure 2. Liquid chromatogram of reaction mixture (300 nm).

minutes. It indicates that the polyacetylene compound is very unstable under 254 nm UV light. However, one major component having the shorter retention time in liquid chromatogram was obtained from the irradiation with 300 nm UV light. If the irradiation time was prolonged, the photoproduct disappeared because of the secondary photoreaction. The photoproduct under 300 nm was isolated by semi-preparative liquid chromatography and characterized by UV, IR, ^{1}H NMR and ^{13}C-NMR spectral analyses.

The UV spectrum of the isolated photoproduct (Figure 3) shows λ_{max} at 289, 272, 257 and 243 nm with band spacings of about 2000 cm^{-1} indicating the presence of polyacetylenic moiety. The bathochromic shift from the reactant suggests extended conjugation, probably conjugated en-on-diyne chromophore with one double bond, one ketone group and two triple bonds. As shown in Figure 4, UV spectrum of falcarinone, a compound with the en-on-diyne chromophore,

Figure 3. UV spectrum of photoproduct.

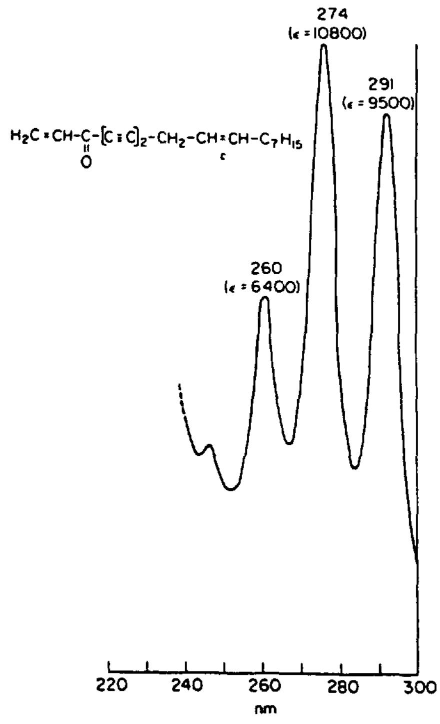


Figure 4. UV spectrum of falcarinone.

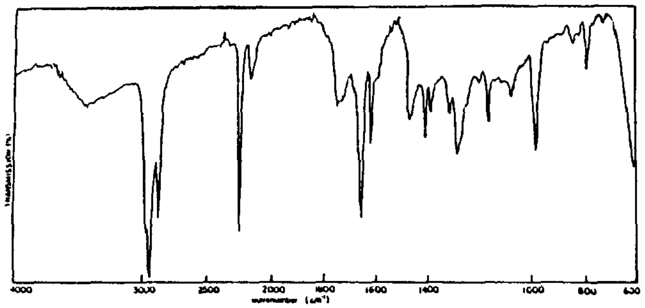


Figure 5. IR Spectrum of photoproduct.

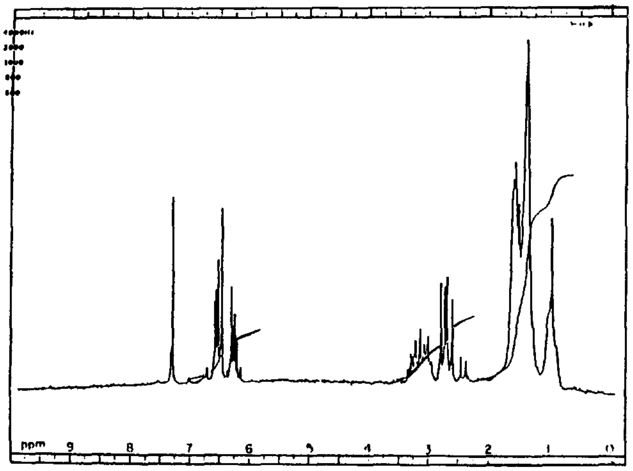


Figure 6. ^1H NMR spectrum of photoproduct.

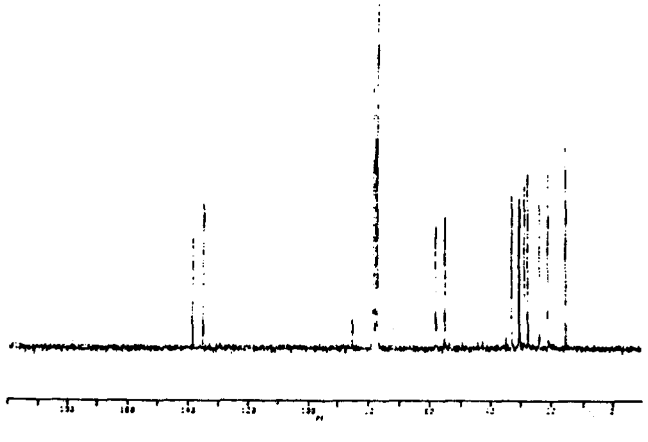
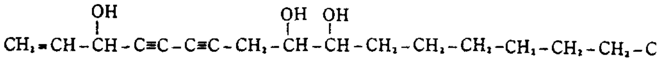


Figure 7. C-13 NMR spectrum of photoproduct.

Table 1. ^{13}C-NMR Spectral Data of Heptadeca-1-en-4,6-diyn-3,9,10-triol and its Oxidation Product



$$CH_2=CH-\overset{OH}{\overset{|}{C}H}-C\equiv C-C\equiv C-CH_2-\overset{OH}{\overset{|}{C}H}-\overset{OH}{\overset{|}{C}H}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$$

Carbon number	Chemical Shifts(ppm)	
	Reactant	Product
1	117.3	135.3
2	136.3	138.8
3	63.3	178.0
4	77.6	76.9
5	76.7	70.9
6	[a] 75.2	62.6
7	....	84.4
8	18.9	19.8
9	54.0	53.8
10	56.5	56.8
11	27.1	26.4
12	26.0	26.2
13	29.0	29.3
14	28.8	29.1
15	31.3	31.6
16	22.2	22.5
17	13.5	13.9

[a] A strong $CDCl_3$ solvent peak appears at about the same frequency (77 ppm) as that of C_6 and C_7 and these peaks are very hard to differentiate from each other.

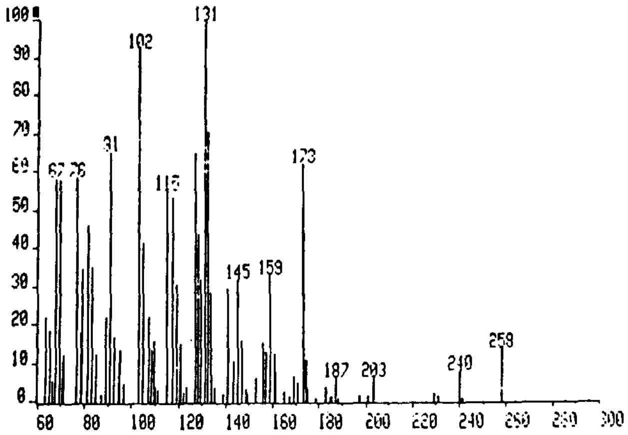


Figure 8. Mass spectrum of photoproduct.

is very similar to that of the photoproduct.[5]

The infrared spectrum of photoproduct (Figure 5) shows a strong band at 2240 cm^{-1} indicating the presence of triple bonds. Carbonyl stretching at 1655 cm^{-1} indicates that this carbonyl group is conjugated with other chromophores. Hydroxyl group intensity at 3400 cm^{-1} is decreased.

The ^1H NMR spectrum of photoproduct (Figure 6) shows the terminal vinyl group shifted to downfield at 6.20-6.58 ppm, secondary hydroxyl group at 3.61-3.20, methylene protons of aliphatic hydrocarbon chain at 1.15-1.55 and terminal methyl group at 0.99 ppm. The C_3-H proton peak at 4.79-4.92 ppm in starting polyacetylene compound was lost in the spectra of photoproduct due to the conversion of allylic alcohol to ketone.

The ^{13}C-NMR data of photoproduct (Figure 7) and the starting material are compared and summarized in Table 1. The peak of C_3 at 63.3 ppm in the reactant is shifted to 178 ppm which can be attributed to the conversion of alcohol to ketone. The peak of terminal vinyl carbon C_1 is shifted from 117.3 to 135.29 ppm indicating the conjugation of double bond with carbonyl group.[9] There are some difficulties in identifying the quaternary carbons of triple bonds because of the strong $CDCl_3$ solvent peak around the same frequency.

Mass spectrum (Figure 8) does not show molecular ion peak as expected but shows various reasonable fragment ions. The dehydration peaks from molecular ion of the product are

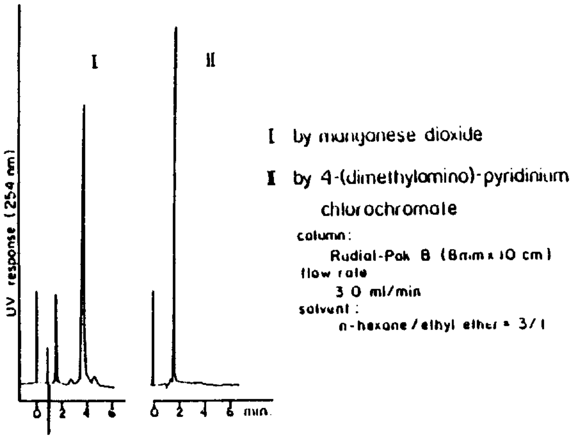


Figure 9. Liquid chromatogram of thermal reaction.

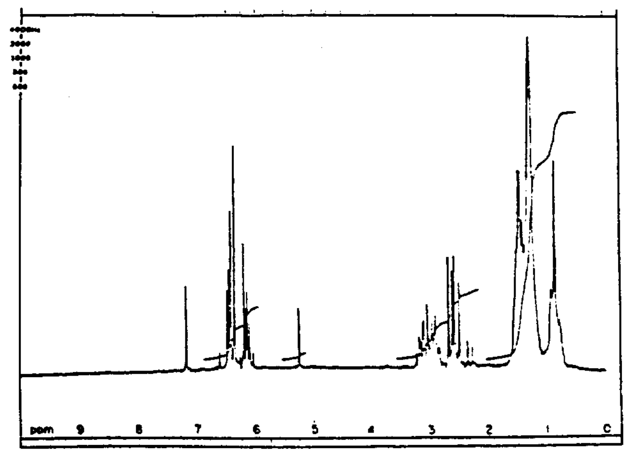


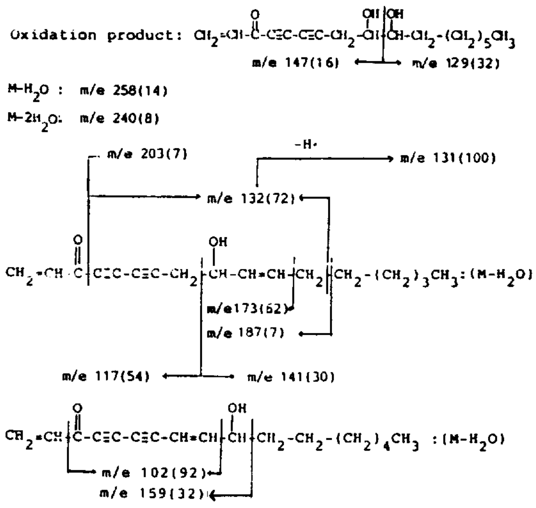
Figure 10. ¹H NMR spectra of thermal product.

shown at m/e 258 (M-H_2O, 14%) and m/e 240 (M-$2H_2O$, 8%). The other fragment ions are shown at m/e 203 ($C_{14}H_{19}O$, 7%), 187($C_{13}H_{17}O_2$, 7%), 159($C_{10}H_7O_2$, 32%), 131(C_9H_7O, 100%), and 173($C_{12}H_{11}O_2$, 62%) all of which are reasonable for the proposed structure of the product.

From the above spectral data, it is evident that heptadeca-1-en-4,6-en-diyn-3,9,10-triol is converted into heptadeca-1-4,6-diyn-9,10-diol-3-one on irradiation with 300 nm UV light.

Thermal Oxidation Reaction. In order to oxidize the allylic alcohol selectively by the mild oxidizing reagents, heptadeca-1-en-4,6-diyn-3,9,10-triol was oxidized by 4-(dimethylamino) pyridinium chlorochromate or manganese dioxide. After the reaction for 15 hours at room temperature, the reaction mixture was analyzed by HPLC (Figure 9). Most of the starting polyacetylene compound were recovered intact when manganese dioxide is used as the oxidizing agent, while most of the reactant were transformed into an oxidation product by 4-(dimethylamino) pyridinium chlorochromate. The product isolated by semi-preparative liquid chromatography has the same spectral data and retention time in liquid chromatogram as the phtooxidation product, heptadeca-1-en-4,6-diyn-9,10-diol-3-one. Figure 10 shows the same ¹H NMR spectrum of the thermal product as the photooxidation compound.

Analysis of GC/MS spectrum of the photooxidation product of heptadeca-1-en-4,6-diyn-3,9,10-triol.



* Numbers in parenthesis indicate the relative intensity.

Conclusion

A major polyacetylenic compound isolated from Panax ginseng roots, heptadeca-1-en-4,6-diyn-3,9,10-triol, was found to be very photolabile under 254 nm and 300 nm UV light. The aerated solution of the compound was irradiated with 300 nm UV light to obtain a photooxidized acetylenic compound, heptadeca-1-en-4,6-diyn-9,10-diol-3-one. The same oxidation product was obtained by oxidation of the reactant with 4-(dimethylamino) pyridinium chlorochromate at room temperature. The yellowish coloring of ginseng when stored for a long period of time may be attributed to this oxidation phenomenon. This is supported by the observation of this oxidized polyacetylene in the white dried ginseng, Baik Sam.

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