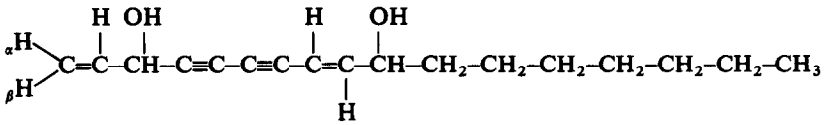

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Residual solvents were removed by a rotary vacuum evaporator.

Heptadeca-1,8-dien-4,6-diyn-3,10-diol (1). ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. st.): δ6.27 (1H, *dd*, J = 15.9, 5.6 Hz, C-9), 5.89 (1H, *ddd*, J = 17.2, 9.6, 5.4 Hz, C-2), 5.70 (1H, *d*, J = 15.9 Hz, C-8), 5.41 (1H, *dd*, J = 17.2, 2.1 Hz, C-1), 5.19 (1H, *dd*, J = 9.6, 2.1 Hz, C-1), 4.91 (1H, *d*, J = 5.4 Hz, C-3), 4.12 (1H, *dt*, J = 5.6, 2.0 Hz, C-10), 1.39 (12H, *m*, $-(CH_2)_6-$), 0.81 (3H, *t*, C-17); UV λ_{max}^{MeOH} nm: 283, 268, 253, 238, 226; IR ν_{max}^{NaCl} cm^{-1}: 3356 (–OH), 2234 (C≡C), 955 (CH=CH); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ150.6 (C-9), 136.8 (C-2), 117.8 (C-1), 108.8 (C-8), 81.3 (C-4), 73.7 (C-5), 72.8 (C-10), 71.0 (C-6), 64.3 (C-3), 37.6 (C-11), 32.5 (C-16), 30.1 (C-14), 29.9 (C-13), 25.9 (C-12), 23.3 (C-15), 14.7 (C-17). (Found: C, 78.18; H, 9.34. $C_{17}H_{24}O_2$ requires C, 78.47: H, 9.23%).

Acknowledgement—This investigation was supported by the Korea Advanced Institute of Science and Technology, and Pacific R & D center.

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Phytochemistry, Vol. 26, No. 10, pp. 2850–2852, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

ACETYLENES FROM THE CALLUS OF *PANAX GINSENG*

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(*Revised received* 27 *February* 1987)

Key Word Index—*Panax ginseng*; Araliaceae; C_{17}-polyacetylenes; callus; anticancer activity.

Abstract—Two new polyacetylenes were isolated from dried callus of *Panax ginseng*. The structures of the polyacetylenes were confirmed as heptadeca-3-oxo-4,6-diyne-9,10-diol and its dihydro derivative by their IR, ^{1}H NMR, ^{13}C NMR and mass spectra, and some chemical reactions. The new acetylenes exhibited growth inhibition against Yoshida sarcoma cells in tissue culture.

INTRODUCTION

From ancient times *Panax ginseng* C. A. Mayer has been considered as one of the most valuable drugs to be used in Korea, China and Japan. Studies on the constituents of *P. ginseng* have been mainly focused on the ginseng saponins. Since the anticancer activity of petrol extracts of the roots of *P. giseng* was found [1], the lipophilic portion of this plant has been extensively investigated. Several groups have isolated polyacetylene compounds, however, it has not been proved that the polyacetylenes in the plant are responsible for the growth inhibition of cancer cells [2].

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We now report on the isolation and structural elucidation of the new anticancer active polyacetylenes, panaxacol (1) and its dihydro derivative (2) from the callus of *P. ginseng*.

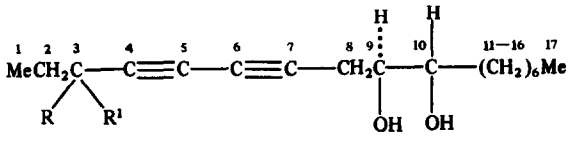
RESULTS AND DISCUSSION

Panaxacol (1) was obtained as a colourless solid. Its high resolution mass spectrum showed the molecular ion at *m/z* 278.1844, $C_{17}H_{26}O_3$. Its IR spectrum showed the presence of hydroxyl groups (3560–3300 cm^{-1}), acetylenic bonds [2240 (*s*) and 2150 (*m*) cm^{-1}] and a carbonyl group (1660 cm^{-1}). The ^1H NMR spectrum indicated the presence of two methyl groups (δ0.88 and 1.15), polymethylenes (δ1.2–1.4) and two methine protons attached to hydroxyl-bearing carbon atoms (δ3.60 and 3.70). Reduction of 1 with sodium borohydride gave dihydropanaxacol (2) which was identical with the minor acetylene from the callus (^1H NMR and MS: *m/z* 280 $[M]^+$, $C_{17}H_{28}O_3$). Detailed ^1H NMR decoupling experiments on 1 and 2 revealed the partial structures of C-1–C-3 and C-8–C-10. The structure of C-10–C-17 was determined from the results of the following experiments. Oxidative cleavage of 2 with sodium periodate, followed by reduction of the oxidation products with sodium borohydride gave an alcohol which was identified as *n*-octyl alcohol by comparison of its GC/MS spectrum with that of an authentic sample. From the above mentioned results and the ^{13}C NMR data of 1 and 2, panaxacol and dihydropanaxacol were confirmed as 4,6-heptadecadiyne-3-oxo-9,10-diol (1) and 4,6-heptadecadiyne-3,9,10-triol (2), respectively.

Table 1. ^1H NMR spectral data for panaxacol (1) and dihydropanaxacol (2) (400 MHz, $CDCl_3$, TMS as internal standard)

H	1	2
1	1.15 *t* (7.3)	1.02 *t* (7.3)
2	2.60 *q* (7.3)	1.74 m
3	—	4.37 *t* (6.4)
8	2.65 *dd* (6.4, 16.9)	2.57 *dd* (6.4, 16.9)
8	2.68 *dd* (5.6, 16.9)	2.59 *dd* (5.6, 16.9)
9	3.70 *m*	3.64 *m*
10	3.60 *m*	3.59 *m*
11	1.51 *m*	1.50 *m*
12	1.2–1.4 *br, m*	1.2–1.4 *br, m*
16	$W_{1/2}$ = 25 Hz	$W_{1/2}$ = 25 Hz
17	0.88 *t* (7.1)	0.88 *t* (7.1)

The numbers in parenthesis are *J* values in Hz.



1 Panaxacol R, R^1 = O
2 Dihydropanaxacol R = H, R^1 = OH

Table 2. ^{13}C NMR spectral data for panaxacol (1) and dihydropanaxacol (2) (100 MHz, $CDCl_3$, δ)

C	1	2
1	8.0	9.4
2	38.8	30.7
3	187.8	63.9
4	75.9	77.3
5	72.4	69.6
6	65.5	66.5
7	86.9	77.6
8	25.3	24.9
9	72.0	72.2
10	73.1	73.1
11	31.9	31.9
12	25.7	25.6
13	29.6	29.6
14	29.3	29.3
15	33.6	33.5
16	22.7	22.7
17	14.1	14.1

Finally, the relative configuration at C-9 and C-10 was confirmed by measurement of the N.O.E. effect on dihydropanaxacol acetonide (3). Thus, irradiation of the C-8 protons of 3 resulted in enhancement of H-9 (9.4%) and H-10 (7.7 %), suggesting that the relative configuration of H-9 and H-10 was *anti* [3]. The new acetylenes 1 and 2 inhibited the growth of Yoshida sarcoma cells in tissue culture [4] by 95% and 80% respectively at a concentration of 10 μg/ml.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were measured on a JEOL GX-400 in $CDCl_3$ containing TMS as an internal standard. The dried callus of *Panax ginseng* C. A. Mayer was obtained from NITTO electric company Ltd (1-1 Shimohozumi, Ibaraki, Osaka 567, Japan).

Isolation of panaxacol and dihydropanaxacol. The dried callus (2 kg) was powdered in a blender and extracted with EtOAc (2l × 3). After concentration of the EtOAc soln, the crude extract was chromatographed on Diaion HP-20 resin (Nippon Rensui) (eluted successively with 2l each of H_2O, 20% MeOH, 40% MeOH, 60% MeOH 80% MeOH, 90% MeOH, MeOH and Me_2CO). The growth inhibition of each fraction was tested against Yoshida sarcoma cells and only the 90% MeOH fraction (2.7 g) was found to be active. The 90% MeOH fraction was chromatographed on silica gel (hexane–EtOAc, 2:1) to give five fractions (Fr. A–Fr. E). The active fraction (Fr. C) was purified by HPLC [Nucleosil 50–5 (Senshu), 8 × 300 mm, flow rate: 3 ml/min, hexane–EtOAc, 2:1] to give panaxacol (100 mg, R_t: 15 min) dihydropanaxacol (5 mg, R_t, 25 min)

Panaxacol (1) readily polymerized at room temp. to give an insoluble purple substance. $[\alpha]_D^{22}$ +19.5° (MeOH; *c* 1.0), IR ν_{max} cm^{-1}: 3500–3300, 2930 (*s*), 2850 (*m*), 2240 (*s*), 2150 (*m*), 1660 (*s*), 1455 (*m*), 1400 (*m*), 1375 (*m*); UV λ_{max} nm (log ε): 254 (3.60), 268 (3.71), 284 (3.62); EIMS 70 eV, *m/z* (rel. int): 278 $[M]^+$ (3.0), 260 $[M-H_2O]^+$ (3.0), 159 $[M-C_8H_7O]^+$ (28.0), 120 $[M-C_9H_{18}O_2]^+$ (100).

Dihydropanaxacol (2). $[\alpha]_D^{22}$ +13.5° (MeOH, *c* 1.0), IR ν_{max} cm^{-1}: 3500 ~ 3300, 2910 (*s*), 2850 (m), 2220 (w), 1485 (w), 1450 (m), 1370 (m); UV λ_{max} nm (log ε): 230 (2.73), 242 (2.69) 255 (2.52); EIMS 70 eV, *m/z* (rel. int): 280 $[M]^+$ (2.5), 262 $[M-H_2O]^+$ (11.3), 233 $[M-C_2H_7O]^+$ (21.3), 159 $[M-C_8H_9O]^+$ (25.0), 104 $[M-C_9H_{20}O_3]^+$ (100).

Reduction of **1** *with* $NaBH_4$. An excess amount of $NaBH_4$ (4.0 equivalents) was added to a soln of 1 (74 mg) in MeOH (1.0 ml) with stirring at room temp. After 1 hr, a satd NaCl soln (10 ml) was added and the mixture extracted with EtOAc (20 ml × 3). The combined organic layer was washed with satd NaCl soln (10 ml), dried over Na_2SO_4 and concd under red. pressure to leave an oil which was purified by HPLC [Nucleosil 50–5 (Senshu), 8 × 300 mm, flow rate: 3.0 ml/min, hexane–EtOAc, 1:1] to give **2** (50 mg, R_t, 9 min). The ^1H NMR and GC/MS spectra of **2** were identical with those of the natural product.

Preparation of dihydropanaxacol acetonide (**3**). A mixture of **2** (3 mg) and a catalytic amount of camphor sulphonic acid in 2,2-dimethoxypropane (500 μl) was stirred for 30 min at room temp. After completion of the reaction, a satd $NaHCO_3$ soln (2 ml) was added and the mixture extracted with EtOAc (5 ml × 3). The combined organic layer was washed with satd NaCl soln (5 ml × 2), dried over Na_2SO_4 and concentrated *in vacuo* to give 3 mg of **3**. ^1H NMR (400 MHz, $CDCl_3$ + D_2O): δ0.89 (3H, *t*, *J* = 7.1 Hz, H-17), 1.01 (3H, *t*, *J* = 7.3 Hz, H-1), 1.20–1.40 (10 H, *br*, *m*, $W_{1/2}$ = 25 Hz, H-12–H-16), 1.41 (6H, *s*, *gem*-dimethyl), 1.56 (2H, *m*, H-11), 2.58 and 2.62 (2H, *ABq*, *J* = 5.4, 16.9 Hz, H-8), 1.74 (2H, *m*, H-2), 3.73 (1H, *ddd*, *J* = 5.4, 5.4, 7.8 Hz, H-9), 3.81 (1H, *ddd*, *J* = 4.2, 7.8, 7.8 Hz, H-10), 4.37 (1H, *t*, *J* = 6.1 Hz, H-3); EIMS 70 eV, *m/z* (rel. int): 305 $[M-Me]^+$ (15.3), 245 $[M-C_3H_7O_2]^+$ (16.3), 199 $[M-C_8H_9O]^+$ (100).

Detection of n-*octyl alcohol from* **2**. To a stirred mixture of **2** (10 mg), THF (300 μl), H_2O (300 μl) and $NaIO_4$ (74 mg) were added at room temp. After 20 min, a satd NaCl soln (10 ml) was added and the mixture extracted with EtOAc (20 ml × 3). The combined organic layer was dried over Na_2SO_4 and concd under red. pressure to give an oil. This oil was dissolved in MeOH (500 μl) and an excess $NaBH_4$ (5 mg) was added with stirring. After 20 min, a satd NaCl soln (10 ml) was added and the mixture extracted with EtOAc (20 ml × 3). The combined organic layer was dried over Na_2SO_4, and then concentrated under reduced pressure to leave an oil. *N*-Octyl alcohol was detected in the oil by GC/MS [column OV-1, 1.0 m × 3 mm, temp. programmed: 50–250° at 10°/min, injection temp. 200°: *m/z* (rel. int): 130 $[M]^+$ (3.0), 112 $[M-H_2O]^+$ (10.0), 84 $[M-C_2H_4O]^+$ (100).

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Phytochemistry, Vol. 26, No. 10, pp. 2852–2854, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

FOUR TERPENOIDS FROM *CEDRUS LIBANOTICA*

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(*Revised received* 3 *February* 1986)

Key Word Index—*Cedrus libanotica*; Pinaceae; sesquiterpenes.

nAbstract—Structures are proposed for four new terpenoids, α-torosol, β-torosol, andirolactone and *trans*-atlanton-6-ol, isolated from the neutral fraction of the petroleum ether extract of *Cedrus libanotica* wood.

INTRODUCTION

Cedar (*Cedrus libanotica*), which is a needle-leaf tree, grows in southern Turkey and Lebanon. The tar, which is obtained from its wood, is used to cure various diseases [1]. Though several studies have been made on the other two cedars (*C. deodora* and *C. atlantica*) [2–7], a detailed chemical study has not yet been performed on *C. libanotica*. We now wish to report the isolation and structures of four new terpenoids, named α-torosol, β-torosol, andirolactone and *trans*-atlanton-6-ol, from the neutral fraction of the petroleum ether extract of *C. libanotica*.

RESULTS AND DISCUSSION

Two sesquiterpene alcohols, α-torosol (**1**) and β-torosol (**2**), were isolated as a mixture from a silica gel column and were separated using preparative thin layer chromato-