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ANTITUMOR AGENTS, 115.[1] SESELIDIOL, A NEW CYTOTOXIC POLYACETYLENE FROM *SESELI MAIREI*

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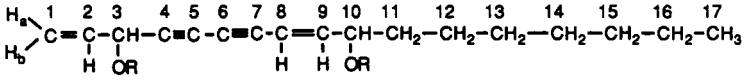
ABSTRACT.—Seselidiol [**1**], a new polyacetylene, has been isolated from the roots of *Seseli mairei*. On the basis of chemical and spectroscopic evidence, its structure has been established as heptadeca-1,8(*Z*)-diene-4,6-diyne-3,10-diol. Seselidiol and its acetate have been demonstrated to show moderate cytotoxicity against KB, P-388, and L-1210 tumor cells.

The roots of *Seseli mairei* Wolff (Umbelliferae), a plant occurring in the Yun-Nan and Si-Chuan areas of China, are known as "Zhu Ye Fang Feng" in Chinese folklore and used as herbal remedies for human inflammation, swelling, rheumatism, pain, and common cold (1). In the course of our continuing search for novel potent antitumor agents, the EtOH extract of the roots of this hitherto uninvestigated plant was found to show significant ($ED_{50} < 20$ μg/ml) cytotoxicity in KB, P-388, and L-1210 tumor cells. Bioassay-directed fractionation of the active extract has led to the isolation and characterization of a new cytotoxic principle, seselidiol [**1**]. We report herein the isolation and structural elucidation of **1**.

RESULTS AND DISCUSSION

The roots of *S. mairei* were extracted at room temperature with 95% EtOH. Si gel chromatography of the cytotoxic fractions followed by medium pressure liquid chromatography as described in the Experimental section afforded the oily compound



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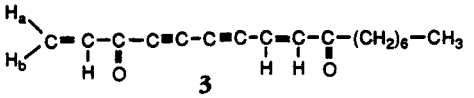

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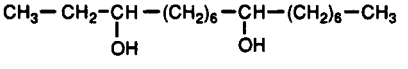

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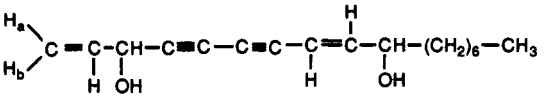

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[1]For part 114, see H. Tatematsu, M. Mori, T.H. Yang, J.J. Chang, T.T. Lee, and K.H. Lee, *J. Pharm. Sci.*, in press.

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seselidiol [**1**]. In pure form, **1** was unstable to light, air, and temperature; its color changed from yellowish to red-brown. The uv spectrum of **1** showed an intense absorbance, characteristic of an ene-diyne chromophore (λ max at 246, 256, 270, and 286 nm). The ir spectrum of **1** showed the presence of the acetylenic (2230 and 2140 cm^{-1}), vinyl (1640 cm^{-1}), and hydroxyl (3350 cm^{-1}) groups. The eims of **1** did not show the $[M]^+$ at *m/z* 260, due to its easy fragmentation. However, its diacetate **2** revealed the presence of a molecular ion at *m/z* 344, $C_{21}H_{28}O_4$, and prominent fragment at *m/z* 259, which were due to the loss of two acetyl groups and a proton. This result indicated that **1** is a C_{17} polyacetylene. The ^{1}H-nmr spectrum of **1** showed a complex spin system. This includes one terminal vinyl group, three olefinic, and two allylic protons at δ 4.49–5.95; twelve methylene protons of a straight hydrocarbon chain at δ 1.24–2.11; and one terminal methyl group at δ 0.88. The assignment of the ^{1}H-nmr spectrum of **1** was based on a homonuclear COSY experiment. The chemical shifts and coupling constants for H_a, H_b, and H-2 of **1** are identical to the three 1-ene protons of falcarinol (2,3), which is common in the Umbelliferae. The coupling constant for H-8 and H-9 in **1** is 8.3 Hz instead of 15.9 Hz as seen in heptadeca-1,8-diene-4,6-diyne-3,10-diol [**5**], which has recently been isolated from the Korean ginseng (*Panax ginseng*) root (4). Thus, compound **1** was assigned as a new C_{17} acetylenic compound bearing a C-8 and C-9 cis double bond instead of a trans one as found in **5**. This assignment was further substantiated by a ^{13}C-nmr DEPT (distortionless enhancement by polarization transfer) experiment as well as by comparison with published chemical shift values for closely related hydroxylated and carboxylated polyacetylenes (5,6). The chemical shifts for C-8 and C-9 in **1** were at δ 127.6 and 135.7 instead of δ 108.8 and 150.6, respectively as seen in **5**.

The in vitro cytotoxicity assay against KB, HCT-8 (human colon carcinoma cells), P-388, and L-1210 was carried out according to a National Cancer Institute protocol (7) as described by Lee *et al.* (8). A comparison of the cytotoxicity of compounds **1–4** (Table 1) clearly indicated that the unsaturated bonds of **1** contribute to enhanced cytotoxicity in all tested cell lines, as hydrogenation of the double bonds of **1** led to the inactive saturated **4**. Oxidation of **1** yielded the diketo compound **3**, which was not cytotoxic in KB, P-388, and L-1210, but had an increased cytotoxicity in HCT-8.

TABLE 1. Cytotoxicity of Compounds **1–4** Against KB, HCT-8, P-388, and L-1210 Tumor Cells.

Compound	ED_{50} (μg/ml)			
	Cell line			
	KB	HCT-8	P-388	L-1210
1	1.0	10.0	4.9	3.3
2	4.0	7.8	5.2	5.5
3	>10.0	3.7	>10.0	>10.0
4	>10.0	>10.0	>10.0	>10.0
Etoposide	0.1		2.6	1.6
Adriamycin HCl		0.3		

Another cytotoxic polyacetylenic compound, which was isolated as the cytotoxic [ED_{50} (KB) = 2.0 μg/ml] and antileukemic [T/C = 165% (P-388 lymphocytic leukemia in mice) at 1 mg/kg, 3-day dosing] principle from *Cicuta maculata*, is cicutoxin (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were taken on a Kofler hotstage apparatus and are uncorrected. Uv spectra were recorded in MeOH on a Varian Cary 2200 spectrophotometer. Ir spectra were determined on a Perkin-Elmer 1320 spectrophotometer. Mass spectra were measured on a V.G. Micromass 70-70 instrument at 70 eV with a direct inlet system. ^{1}H; ^{1}H-^{1}H COSY, and ^{13}C-nmr spectra were obtained with a Varian EM-400 spectrometer. Si gel (Universal Absorbents Inc. 32–63 μm) was used for cc, and pre-coated Si gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for analytical tlc. The polyacetylene was detected by spraying with 10% H_2SO_4 solution containing 1% $Ce(SO_4)_2$, followed by heating. Hplc was carried out on a Shimadzu LC-6A system with an SPD-6AV uv detector. The column used in this system was TSK gel ODS-80Tm. MeOH-H_2O-HOAc (70:30:0.5) was used as the mobile phase, and the flow rate was 3 ml/min.

PLANT MATERIAL.—The roots of *S. mairei* were collected in Wan-Xian (Si-Chuan, China) in July 1987. The plant material was identified by Professor Ke-Min Dai of the Department of Pharmacognosy, School of Pharmacy, Shanghai Medical University, Shanghai, China. A voucher specimen is available for inspection at Professor Dai's laboratory.

EXTRACTION AND ISOLATION OF SESELIDIOL.—The air-dried roots of *S. mairei* (2 kg) were crushed and percolated with 95% EtOH at room temperature. The EtOH extract (271 g) was extracted with hexane. The hexane-soluble fraction (80 g) was partitioned between hexane and 90% MeOH (1:1). The cytotoxic MeOH extract (39 g) was chromatographed in $CHCl_3$ over Si gel with increasing polarity of Me_2CO. The active fraction [$CHCl_3$-Me_2CO (5:1)] was further purified by a medium pressure Si gel column to yield a brown oil after elution with hexane-Et_2O (3:2). Further repeated chromatography of this brown oil resulted in the isolation of compound **1** as a light yellowish oil (210 mg). The purification of this oil was achieved by analytical hplc [column TSK gel ODS-80Tm; solvent 70% MeOH/0.5% HOAc; flow rate 2.5 ml/min; detector uv 254 nm).

SESELIDIOL [**1**].—Seselidiol [**1**] was isolated in 0.0085% yield as a yellowish oil: $[\alpha]^{23}D$ +192° (c = 0.53, Et_2O); ir (KBr) 3350, 2920, 2850, 2230, 2140, 1640, 1450, 1380, 1300, 1110, 1010, 980, 930, 870, 780, 720 cm^{-1}; uv λ max (MeOH) 232, 246, 256, 270, 286 nm; ^{1}H nmr ($CDCl_3$) δ 5.95 (1H, ddd, $J_{1,2}$ = 10.2 and 17.0 Hz, $J_{2,3}$ = 5.2 Hz, H-2), 5.62 (1H, dt, $J_{9,10}$ = 1.4 Hz, $J_{10,11}$ = 7.3 Hz, H-10), 5.53 (1H, dd, $J_{8,9}$ = 8.3 Hz, $J_{9,10}$ = 1.4 Hz, H-9), 5.48 (1H, dd, $J_{1,2}$ = 17.0 Hz, H_a-1), 5.26 (1H, d, $J_{1,2}$ = 10.2 Hz, H_b-1), 5.21 (1H, d, $J_{8,9}$ = 8.3 Hz, H-8), 4.49 (1H, d, $J_{2,3}$ = 5.2 Hz, H-3), 2.11 (2H, ddd, J = 7.3, 5.8, and 1.5 Hz, H-11), 1.24–1.42 (10H, H-12, -13, -14, -15, -16), 0.88 (3H, t, $J_{16,17}$ = 6.9 Hz, 16-Me); ^{13}C nmr ($CDCl_3$) δ 14.2 (q, C-17), 22.6, 29.4, 29.3, 29.2, 27.9 (t, C-12, -13, -14, -15, -16), 31.9 (t, C-11), 58.6 (d, C-3), 63.5 (d, C-10), 68.7 (s, C-6), 68.9 (s, C-5), 78.3 (s, C-7), 79.9 (s, C-4), 117.3 (t, C-1), 127.6 (d, C-8), 134.5 (d, C-2), 135.7 (d, C-9); eims *m/z* 203, 175, 134, 105.

SESELIDIOL DIACETATE [**2**].—A solution of **1** (35 mg) in pyridine (2 ml) was acetylated with Ac_2O (3 ml) at room temperature overnight. After usual workup, the resulting product was chromatographed on Si gel in $CHCl_3$ to give seselidiol diacetate [**2**] (15 mg) as a yellowish oil: ir ($CHCl_3$) 3010, 2920, 2850, 2260, 2160, 1730, 1640, 1450, 1370, 1220, 1010, 940, 900 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.13 (1H, d, J = 8.8 Hz, H-9), 5.90 (1H, d, J = 5, 8 Hz, H-3), 5.86 (1H, ddd, J = 16, 10.0 and 5.8 Hz, H-2), 5.66 (1H, m, H-10), 5.54 (1H, d, J = 16 Hz, H_a-1), 5.47 (1H, d, J = 10.0 Hz, H_b-1), 5.35 (1H, d, $J_{8,9}$ = 8.8 Hz, H-8), 2.09 and 2.08 (6H, s each, Ac), 2.13 (2H, m, H-11), 1.4–1.2 (10 H, H-12, -13, -14, -15, -16), 0.88 (3H, t, $J_{16,17}$ = 6.9 Hz, 16-Me); eims *m/z* (rel. int.) [M − 1]$^+$ 343 (1.1), [M − 1 − Ac]$^+$ 285 (2.6), [M − 1 − Ac × 2]$^+$ 259 (1.2), 242 (17.8), [M − C_6H_{12}]$^+$ 175 (50.7), [M − C_7H_{14}]$^+$ 161 (94.5), 157 (100), 133 (33.5), 128 (64), 115 (76.8), 107 (25.2), 91 (49.6), 81 (58.1), 60 (68.4), 57 (65.0), 55 (57.6); hrms *m/z* [M − 1 − Ac × 2]$^+$ 259.16981 (calcd for $C_{17}H_{23}O_2$, 259.16979).

HEPTADECA-1,8(*Z*)-DIENE-4,6-DIYNE-3,10-DIONE [**3**].—A mixture of **1** (20 mg) and MnO_2 (160 mg) in CH_2Cl_2 (20 ml) was shaken for 4 h. The solution was filtered, concentrated, and purified by preparative tlc [$CHCl_3$-Me_2CO (5:1)] to furnish compound **3** as a very unstable oil: ir ($CHCl_3$) 3010, 2920, 2850, 2240, 1700, 1680, 1640, 1600, 1460, 1200 cm^{-1}; uv λ max (MeOH) 256, 271, 290 nm; ^{1}H nmr ($CDCl_3$) δ 6.32 (1H, dd, J = 17.5 and 10.5 Hz, H-2), 5.11 (1H, d, J = 8.7 Hz, H-8), 5.47 (1H, d, J = 8.7 Hz, H-9), 5.05 (1H, d, J = 17.5 Hz, H_a-1), 4.89 (1H, d, J = 10.5 Hz, H_b-1), 2.23 (1H, m, H-11), 1.4–1.2 (10 H, m, H-12, -13, -14, -15, -16), 0.89 (3H, t, 16-Me).

HEPTADECA-1,8-DIOL [**4**].—A solution of **1** (20 mg) in MeOH (2 ml) was hydrogenated with Pd-C (10 mg) for 36 h at room temperature in the dark. The reaction mixture was filtered, concentrated, and subjected to purification by use of preparative tlc [SiO_2, C_6H_6-Me_2CO (19:1)] to afford a completely hydrogenated product **4** as a colorless oil: ir ($CHCl_3$) 3430, 2960, 2840, 1450, 1370, 1210, 1020, 830, 720

cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.53 (2H, m), 1.6–1.3 (8H, m), 1.26 (20H, m), 0.91 (Me, t), 0.89 (Me, t); ^{13}C nmr ($CDCl_3$) δ 13.3, 14.9, 25.6, 27.5, 27.9, 28.4, 28.6, 29.6, 30.1, 31.3, 31.8, 35.3, 36.1, 36.9, 72.3, 74.3; eims *m/z* $[M-H_2O]^+$ 254, 236, 210, 208, 209, 152, 124, 123, 122, 97, 84, 83, 57.

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