Acetylenic Compounds Isolated from Cultured Cells of *Asparagus officinalis*

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Three new acetylenic compounds, compounds I, II and III were isolated from the cultured cells of *Asparagus officinalis* L. (Liliaceae) and their structures identified as 1-methoxy-4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]-benzene, 4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]phenol and 4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl]phenol, respectively, from chemical and spectral analysis.

Key words *Asparagus officinalis*; acetylenic compound; cultured plant cell; structural elucidation; phenolics; Liliaceae

Asparagus officinalis L. is a liliaceous perennial plant whose young shoots are well known as the vegetables, "green asparagus" and "white asparagus." It is also known that the underground portion of this plant excretes plant-growth inhibitors,[1] which induce the phenomenon "allelopathy."[2] A lot of research has been published on its components, estimation of nutrient factors, such as vitamins, amino acids and carbohydrates, and on biotechnological aspects[3] involving tissue culture. This report is concerned with the isolation of three new compounds from the cultured cells of *A. officinalis* and the elucidation of their structures.

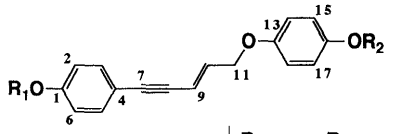
Three crystalline compounds, compounds I (**1**), II (**2**) and III (**3**) were isolated from a suspension of cultured cells of *A. officinalis* as described in Experimental. The empirical formulae of these compounds were assigned as $C_{19}H_{18}O_3$, $C_{18}H_{16}O_3$ and $C_{17}H_{14}O_3$, respectively, from high resolution (HR) mass spectral measurements. As far as their IR spectra were concerned, a sharp absorption band was observed at 2200 cm^{-1}, showing the presence of a triple bond.[4] From the UV spectral data [λ_{max}: 280—282 nm ($\log\varepsilon = 4.50$—4.46)], a conjugated system was also identified.

In the ^{1}H-NMR spectrum of **1**, two sets of AA'BB' spectra were found in the aromatic proton region at 6.8—7.4 ppm. Two sharp singlets at 3.77 ppm (3H) and 3.81 ppm (3H) were observed (Table 1), indicating the presence of two 1,4-disubstituted benzene rings, each of which has a methoxy group[4] as one of the substituents, in **1**. Residual signals in this spectrum were of the AMX_2 type appearing at 4.58 ppm (2H, dd, J=1.7, 5.1 Hz), 6.04 ppm (1H, dt, J=1.7, 15.9 Hz) and 6.33 ppm (1H, dt, J=5.1, 15.9 Hz). Consideration of their chemical shifts and J-values[4] led us to conclude that **1** has a *trans*-propenylene group attached to an oxygen atom by its methylene group. Since no acetylenic proton signals were observed in the spectrum, the acetylenic group was identified as a disubstituted one. Catalytic reduction of **1** on platinum(II) oxide in methanol gave its hexahydroderivative (**4**), supporting the presence of an acetylenic group and an olefinic group.

In the ^{1}H-NMR spectrum of **4**, signals due to five methylene groups appeared at 1.48 ppm (2H, collapsed quintet, J=approximately 7.8 Hz), 1.65 ppm (2H, quintet, J=7.7 Hz), 1.78 ppm (2H, collapsed quintet, J=approximately 7.1 Hz), 2.58 ppm (2H, t, J=7.7 Hz), and 3.89 ppm (2H, t, J=6.6 Hz). The multiplicities and chemical shifts of these signals supported a propenylene group in **1**, and also provided evidence of an acetylenic group and methylene group linked to the phenylic group and phenoxylic group, respectively.

Confirmative evidence that the position of the methoxy groups on the phenyl and phenoxy groups of **1** was in the *p*-position was obtained from nuclear Overhauser effect (NOE) measurements by the ^{1}H-NMR spectral subtruction method with respect to **4**. Irradiating the methoxy methyl signal on the aromatic rings at 3.76 and 3.78 ppm caused selective enhancement of their neighboring aromatic proton signals at 6.81 (2H, 10.1%) and 6.819 ppm (2H, 10.9%), respectively, and irradiating the methylene signals linked to the phenyl and phenoxy groups at 2.58 and 3.89 ppm caused enhancement of the aromatic proton signals at 7.09 (2H, 8.0%) and 6.816 ppm (2H, 13.7%). According to the spectral and chemical evidence above, the structure of **1** was assigned as 1-methoxy-4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]benzene (**1**) (Fig. 1). ^{13}C-NMR spectral data agreed with this conclusion (Table 2).

^{1}H-NMR spectra of **2** and **3** resembled that of **1**, except



	R_1	R_2
1	CH_3	CH_3
2	H	CH_3
3	H	H
5	$COCH_3$	CH_3
6	$COCH_3$	$COCH_3$

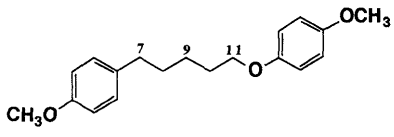


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Fig. 1. Structures of **1**—**6**

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Table 1. ^{1}H-NMR Data of **1**—**6**

Protons	1	2	3	4	5	6
2, 6	6.84 } (4H, AA′BB′)	6.77 } (4H, AA′BB′)	6.76 } (4H, AA′BB′)	6.82 } (4H, AA′BB′)	7.05 } (4H, AA′BB′)	7.05 } (4H, AA′BB′)
3, 5	7.37 }	7.33 }	7.33 }	7.09 }	7.44 }	7.44 }
7				2.58 (2H, t, 7.7)		
8				1.65 (2H, quin.)		
9	6.04 (1H, dt, 1.7, 15.9)	6.04 (1H, dt, 1.7, 15.9)	6.03 (1H, dt, 1.7, 15.9)	1.48 (2H, quin.)	6.04 (1H, dt, 1.7, 15.9)	6.05 (1H, dt, 1.7, 15.9)
10	6.33 (1H, dt, 5.1, 15.9)	6.33 (1H, dt, 5.1, 15.9)	6.32 (1H, dt, 5.1, 15.9)	1.78 (2H, quin.)	6.38 (1H, dt, 5.1, 15.9)	6.37 (1H, dt, 5.1, 15.9)
11	4.58 (2H, dd, 1.7, 5.1)	4.58 (2H, dd, 1.7, 5.1)	4.57 (2H, dd, 1.7, 5.1)	3.89 (2H, t, 6.6)	4.59 (2H, dd, 1.7, 5.1)	4.62 (2H, dd, 1.7, 5.1)
14, 18	6.85 } (4H, AA′BB′)	6.85 } (4H, AA′BB′)	6.80[a] } (4H, AA′BB′)	6.82 } (4H, AA′BB′)	6.85 } (4H, AA′BB′)	6.99[a] } (4H, AA′BB′)
15, 17	6.85 }	6.85 }	6.78[a] }	6.81 }	6.85 }	6.93[a] }
1-OMe	3.81 (3H, s)			3.78 (3H, s)		
16-OMe	3.77 (3H, s)	3.77 (3H, s)		3.76 (3H, s)	3.77 (3H, s)	
1-OCOMe					2.30 (3H, s)	2.30 (3H, s)
16-OCOMe						2.28 (3H, s)

Recorded at 200 MHz in $CDCl_3$ solution. Numbers in parentheses are *J*-values in Hz. *a*) In each vertical column chemical shifts may be interchanged. quin. = quintet.

for the lack of one or both of the two methoxy methyl signals observable in the spectrum of **1**. On acetylation they gave a monoacetate (**5**) and diacetate (**6**), respectively, indicating that **2** and **3** are monodesmethyl and bisdesmethyl derivatives of **1**. The structures of these compounds were assigned as 4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]phenol (**2**) and 4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl]phenol (**3**), respectively, by correlating them to **1** by a methylation reaction and comparing their MS. In the MS of **1**, **2** and **3**, base peaks assignable to the fragment ions containing *p*-substituted phenyl groups ($RO\text{–}C_6H_4\text{–}C{\equiv}CCH{=}CHCH_2^+$), which were caused by allylic fission,[4] appeared at *m/z*: 171 ($R=CH_3$), 157 ($R=H$) and 157 ($R=H$), respectively, and comparison of these values enabled us to assign the position of the methoxy group of **2** as shown in Fig. 1.

Assignments of the ^{1}H-NMR chemical shifts of **1** were carried out by NOE measurements (irradiating at the methoxy and methylene signals), H–H correlation spectroscopy (COSY) and two dimensional nuclear Overhauser effect spectroscopic experiments, as well as the spectral information described above. Its ^{13}C-NMR assignments were made based on C–H COSY spectral measurements and consideration of the chemical shift data. For the other compounds, **2**—**6**, their shift data were compared with those of **1**. In order to confirm the assignments for the acetylenic carbon nuclei signals of the acetylenic compounds, an incredible natural abundance double quantum transfer spectrum was recorded for **5**. The resulting assignments are presented in Tables 1, 2.

This report describes the occurence of natural compounds with a new type of skeleton and our interest lies in the biosynthetic pathway of these compounds. Although the occurence of numerous acetylenic compounds have been reported,[5] no reports of the isolation of any from *Asparagus* species have been presented. In our preliminary study, minute amounts of these compounds have also been detected in the *A. officinalis* plant and screening tests for these compounds in other plants of the *Asparagus* species have been undertaken in our laboratory out of chemotaxonomical interest.

Table 2. ^{13}C-NMR Data of **1**—**3**

Carbons	1[a]	2[a]	3[b]
1	159.72	155.72	157.41
2, 6	114.05	115.51	115.63
3, 5	133.03	133.24	133.24
4	115.40	115.69	114.37
7	90.66	90.46	91.07
8	85.93	85.90	85.52
9	112.59	112.56	112.80
10	137.15	137.24	137.09
11	68.68	68.68	69.03
13	152.65	152.65	152.13
14, 18	115.95	115.98	116.10
15, 17	114.78	114.81	116.30
16	154.23	154.23	151.13
1-OMe	55.30		
16-OMe	55.77	55.80	

Recorded at 50 MHz. *a*) In $CDCl_3$ solution. *b*) In $CDCl_3 + CD_3OD$ solution.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured with tetramethylsilane (TMS) as an internal reference. Chemical shifts are expressed in ppm downfield from TMS and the coupling constants (*J*) in Hz. The following instruments were used: mp, Yanagimoto micro melting-point apparatus; IR spectra, Shimadzu IR-435 IR spectrometer; UV spectra, Shimadzu UV-300 UV spectrometer; ^{1}H- and ^{13}C-NMR spectra, JEOL FX-200 and GSX-500 NMR spectrometers; HR-MS and MS, JEOL DX-300 GC-mass spectrometer. For preparative HPLC, a JASCO 880-PU pump, 875-UV detector, Megapak $SILC_{18}$ analytical column (i.d. 8 mm) and methanol mobile phase were used. For column chromatography, Merck Kiesel gel 60 (70—230 mesh) was used.

Material Suspensions of cultured cells of *A. officinalis* L., which had been induced from segments of aseptically germinated seedlings and subcultured for over five years under suspension conditions, were used. For this experiment, the material was cultured in Linsmaier–Skoog medium,[6] containing 3% glucose and 10^{-6} M 2,4-dichlorophenoxyacetic acid, on a reciprocal shaker (96—98 rpm) at 27 °C in the dark. The cultured cells were harvested at the stationary phase, washed with water and dried under a flow of hot air at 65 °C in a temperature-controlled oven (Yamato Scientific Co., Ltd.).

Extraction and Isolation Powdered cells were extracted with hot $CHCl_3$. After filtration, the filtrate was evaporated to dryness. The

benzene-soluble portion of the $CHCl_3$ extract was subjected to silica gel column chromatography and eluted successively with benzene and $CHCl_3$. After concentration, the first portion of the benzene fraction was treated with HPLC to separate crude **1**, which was recrystallized from methanol (MeOH) to give pure **1**. The remaining portion of the benzene fraction and $CHCl_3$ fraction were treated in the same way to give crude **2** and **3**, which were recrystallized from CCl_4 and benzene to give pure **2** and **3**, respectively.

Compound I: Yield; 0.2%; mp 124.0—125.5 °C. HR-MS *Anal.* Calcd for $C_{19}H_{18}O_3$ (M^+, *m/z*): 294.1256. Found: 294.1256. MS *m/z*: 294 (M^+), 171 (base peak). IR ($CHCl_3$): 2200 cm^{-1}. UV λ_{max}^{MeOH} nm (log ε): 280 (4.50). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound II: Yield, 0.2%; mp 140.0—140.5 °C. HR-MS *Anal.* Calcd for $C_{18}H_{16}O_3$ (M^+, *m/z*): 280.1100. Found: 280.1099. MS *m/z*: 280 (M^+), 157 (base peak). IR ($CHCl_3$): 2200 cm^{-1}. UV λ_{max}^{MeOH} nm (log ε): 282 (4.46). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound III: Yield, 0.05%; mp 182 °C (dec.). HR-MS *Anal.* Calcd for $C_{17}H_{14}O_3$ (M^+, *m/z*): 266.0943. Found: 266.0943. MS *m/z*: 266 (M^+), 157 (base peak). IR ($CHCl_3$): 2200 cm^{-1}. UV λ_{max}^{MeOH} nm (log ε): 282 (4.47). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Catalytic Reduction of Compound I **1** (8.7 mg) was dissolved in MeOH (10 ml), and catalytically reduced with H_2 on PtO_2 under standard conditions. After stirring for 10 min at room temperature (r.t.), the mixture was filtered and the filtrate evaporated to dryness. The residue was treated with HPLC to give crude **4**, which was recrystallized from MeOH to give pure **4**.

Hexahydro-derivative of **1** (**4**): mp 79.0—80.0 °C; MS *m/z*: 300 (M^+), 121 (base peak). ^{1}H-NMR: see Table 1.

Acetylation of Compound II To a solution of **2** (2.0 mg) in pyridine (0.5 ml), acetic acid anhydride (0.5 ml) at r.t. was added. After 10 min, MeOH was added to the mixture, then the solution was evaporated to dryness under reduced pressure and the residue was recrystallized from MeOH to give the pure monoacetate of **2** (**5**).

Monoacetate of **2** (**5**): mp 96.5—97.0 °C; MS *m/z*: 322 (M^+), 157 (base peak). ^{1}H-NMR: see Table 1. ^{13}C-NMR ($CDCl_3$) δ: 21.08 (COMe), 55.77 (16-OMe), 68.56 (C11), 87.27 (C8), 89.73 (C7), 112.10 (C9), 114.81 (C15, 17), 115.94 (C14, 18), 120.91 (C4), 121.64 (C2, 6), 132.68 (C3, 5), 138.19 (C10), 150.58 (C1), 152.62 (C13), 154.29 (C16), 168.95 (C=O).

Acetylation of Compound III To a solution of **3** (1.8 mg) in pyridine (0.5 ml), acetic acid anhydride (0.5 ml) at r.t. was added. After 10 min, MeOH was added to the mixture, then the solution was evaporated to dryness under reduced pressure and the residue was recrystallized from MeOH to give the pure diacetate of **3** (**6**).

Diacetate of **3** (**6**): MS *m/z*: 350 (M^+), 157 (base peak). ^{1}H-NMR: see Table 1.

Methylation of Compound II To a solution of **2** (1.6 mg) in MeOH was added an ether solution (1 ml) of diazomethane. After 24 h, the solution were evaporated to dryness and recrystallized from MeOH to give the pure methyl ether of **2** (mp 124.0—125.5 °C). This compound was identified as **1** by comparison of the ^{1}H-NMR spectrum and MS with those of an authentic sample.

Methylation of Compound III To a solution of **3** (1.4 mg) in MeOH was added an ether solution (1 ml) of diazomethane. After 24 h, the solution were evaporated to dryness and recrystallized from MeOH to give the pure dimethyl ether of **3** (mp 124.0—125.5 °C). This compound was identified as **1** by comparison of the ^{1}H-NMR spectrum and MS with those of an authentic sample.

References and Notes

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