Antiproliferative Constituents in Umbelliferae Plants. IV.[1] Constituents in the Fruits of *Anthriscus sylvestris* HOFFM.

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The constituents in the fruit of *Anthriscus sylvestris* HOFFM. were investigated, and four lignans [deoxypodophyllotoxin, morelensin, (−)-deoxypodorhizone, and (−)-hinokinin], one phenylpropanoid [1-(3′,4′-dimethoxyphenyl)-1ξ-hydroxy-2-propene], two phenylpropanoid esters [3′,4′-dimethoxycinnamyl (*Z*)-2-angeloyloxymethyl-2-butenoate and 3′,4′-dimethoxycinnamyl (*Z*)-2-tigloyloxymethyl-2-butenoate], and one polyacetylenic compound (falcarindiol) were isolated. Their antiproliferative activity against MK-1, HeLa and B16F10 cell lines is reported.

Key words *Anthriscus sylvestris*; Umbelliferae; antiproliferative activity; lignan; phenylpropanoid; polyacetylene

In the preceding paper[1] of this series, we reported the isolation of falcarindiol, some phenyl propanoids and lignans from the root and ground part of *Anthriscus sylvestris* HOFFM., and their antiproliferative activity against three tumor cell lines, human gastric adenocarcinoma (MK-1), human uterus carcinoma (HeLa), and murine melanoma (B16F10), was also reported.

As a continuation of our investigation into the antiproliferative constituents in this plant, we investigated the constituents of the fruit, and the antiproliferative activity of the isolates was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[2]

The dried fruits were percolated with $CHCl_3$, and then with MeOH. The $CHCl_3$ extract showed strong antiproliferative activity (ED_{50}: *ca.* 0.3 μg/ml) against MK-1 cells , while the MeOH extract had a very low activity (ED_{50}: 50 μg/ml).

The $CHCl_3$ extract was fractionated in almost the same manner as described in the preceding paper, and two lignans (**1**, **2**), two phenylpropanoid esters (**3**, **4**), and one phenyl propanoid (**5**) were isolated in addition to deoxypodophyllotoxin (**6**), (−)-deoxypodorhizone (**7**), and falcarindiol (**8**).

Compound **1** was obtained as a pale yellow oil, and the positive-ion high-resolution (HR) FAB-MS gave the molecular formula $C_{20}H_{18}O_6$. The ^{1}H-NMR and

Table 1. ^{1}H-NMR and ^{13}C-NMR Chemical Shifts of the Lignans

	6		**2**		**7**		**1**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1		128.3		128.3		131.5		131.6[a]
2	6.66 s	108.5	6.66 s	108.4	6.46 s	108.8	6.46 d (2)	108.8
3		147.1		146.8		147.9		147.9[b]
4		146.8		147.0		146.4		146.4[b]
5	6.52 s	110.5	6.50 s	110.4	6.69 dd (7, 1)	108.3	6.69 d (8)	108.3
6		130.7		133.1	6.47 dd (7, 2)	121.5	6.46 br dd (8, 2)	121.5
7	*ca.* 2.73	33.1	2.77 m	33.1	*ca.* 2.53	38.3	2.46 dd (17, 8)	38.4
8	2.72 m	32.8	2.73 m	32.6	*ca.* 2.49	41.0	2.46 m	41.3
9	3.91 m	72.0	3.90 m	72.1	3.87 dd (9, 8)	71.1	3.85 dd (9, 7)	71.1
	4.44 m		4.42 m		4.17 dd (9, 7)		4.12 dd (9, 7)	
1′		136.2		133.4		133.3		131.4[a]
2′	6.35 s	108.5	6.95 d (2)	114.6	6.36 s	106.4	6.63 d (2)	109.5
3′		152.5		148.1[a]		153.3		147.9[b]
4′		137.3		148.4[a]		137.1		146.5[b]
5′		152.5	6.68 d (8)	110.5		153.3	6.72 d (8)	108.3
6′	6.35 s	108.5	6.38 dd (8, 2)	122.7	6.36 s	106.4	6.59 dd (8, 2)	122.2
7′	4.60 d (3)	43.8	4.60 d (4)	43.3	2.88 dd (14, 7)	35.3	2.83 dd (14, 7)	34.9
					2.93 dd (14, 5)		2.98 dd (14, 5)	
8′	2.72 m	47.5	2.73 m	47.5	*ca.* 2.57	46.4	2.52 m	46.5
9′		174.8		174.9		178.1		178.3
$-OCH_2O-$	5.92 d (1)	101.2	5.91 d (1)	101.1	5.92 d (2)	101.0	5.93 d (2)	101.0 × 2
	5.94 d (1)		5.92 d (1)		5.93 d (2)		5.92 d (2) × 3	
C_3-OCH_3	3.75 s	56.3	3.84 s	56.0	3.83 s	56.1		
C_4-OCH_3	3.80 s	60.8	3.81 s	55.8	3.83 s	60.8		
C_5-OCH_3	3.75 s	56.3			3.83 s	56.1		

a, b) Signal assignment in each vertical column may be interchangeable. Numbers in parentheses are coupling constants in Hz.

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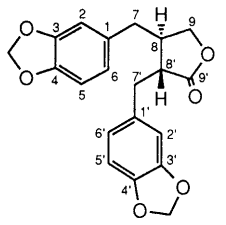
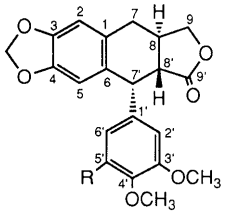
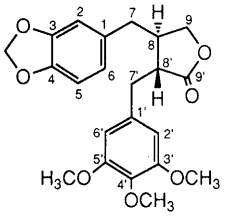


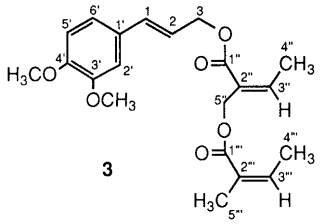
1 [(−)-hinokinin]



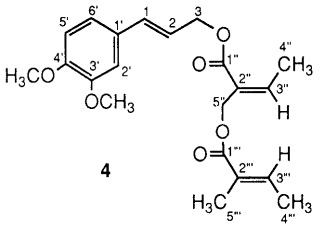
2 (morelensin): R=H
6 (deoxypodophyllotoxin): R=OCH_3



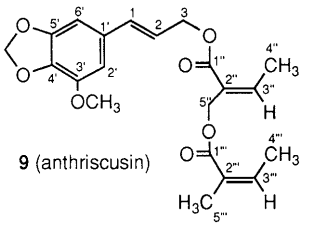
7 [(−)-deoxypodorhizone]



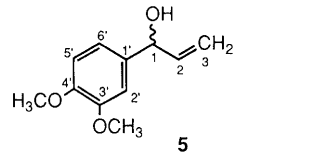
3



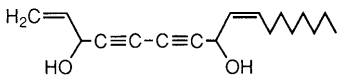
4



9 (anthriscusin)



5



8 (falcarindiol)

^{13}C-NMR spectra exhibited signals assignable to two 3,4-methylenedioxybenzyl groups and a γ-butyrolactone group (Table 1), and the spectra were similar to those of **7**. The CD spectrum showed a curve similar to that of **7**. From these spectral data, **1** was identified as (−)-hinokinin.[3]

Compound **2**, $C_{21}H_{20}O_6$, was obtained as a resin. The ^{1}H-NMR and ^{13}C-NMR spectra are similar to those of **6**, however, **2** has a methylenedioxy group and two methoxyl groups, one methoxyl group less than **6**. The heteronuclear multiple-bond correlation (HMBC) spectrum showed the correlation of one methoxyl proton signal (δ 3.81, s) with an oxygenated aromatic carbon signal (δ 148.1), and the other methoxyl proton signal (δ 3.84, s) correlated with another oxygenated aromatic carbon signal (δ 148.4). Two aromatic proton signals (δ 6.38, dd, J=8.0, 2.0 Hz; 6.95, d, J=2.0 Hz) correlated with the oxygenated carbon signal at δ 148.1 and a methine carbon signal at δ 43.3, and another aromatic proton signal (δ 6.68, d, J=8.0 Hz) showed a correlation with the oxygenated aromatic carbon signal at δ 148.4 and the C-substituted aromatic carbon signal (δ 133.4). These signal correlations clearly indicated the presence of a 3,4-dimethoxyphenyl group linked to the methine carbon, suggesting **2** to be 5′-desmethoxy-**6**. Compounds **2** and **6** showed almost the same CD spectra, indicating that the absolute configuration at C-8 and C-8′ is the same as in **6**. Therefore, **2** was identified as morelensin, a cytotoxic lignan isolated from the exudate of *Bursera morelensis* (Burseraceae).[4]

Compound **3**, $C_{21}H_{26}O_6$, exhibited in its ^{1}H-NMR and ^{13}C-NMR spectra signals assignable to a 3,4-dimethoxycinnamyl group, and the ester-linked angelic acid and sarracinic acid [(*Z*)-2-hydroxymethyl-2-butenoic acid] (Table 2). This spectral feature is very similar to that of anthriscusin (**9**) obtained from the root. The difference is the lack of a methylenedioxy group and the addition of one methoxyl group. Thus, **3** was identified as 3′,4′-dimethoxycinnamyl (*Z*)-2-angeloyloxymethyl-2-butenoate isolated from the leaves of *Bupleurum fruticosum* L.[5]

Compound **4**, $C_{21}H_{26}O_6$, exhibited ^{1}H-NMR and ^{13}C-NMR spectra very similar to those of **3**. There was a marked difference in the chemical shifts of two methyl groups (Table 2). Treatment of **3** with sodium methoxide gave methyl angelate, while **4** gave methyl tiglate. Therefore, the structure of **4** was determined as 3′,4′-dimethoxycinnamyl (*Z*)-2-tigloyloxymethyl-2-butenoate.

Compound **5**, $C_{11}H_{14}O_3$, was obtained as an oil. The ^{1}H-NMR spectrum exhibited signals of a 3,4-dimethoxyphenyl group, a hydroxymethylene group, and a terminal olefin group. Combination of these groups led to the structure of **5** being assigned as 1-(3′,4′-dimethoxyphenyl)-1ξ-hydroxy-2-propene. The ^{13}C-NMR signals were consistent with this structure.

The antiproliferative activity of the isolates from the fruits against three tumor cell lines, MK-1, HeLa, and B16F10 was evaluated by MTT assay. The 50% growth inhibition (ED_{50}, μg/ml) is listed in Table 3. Considering the content and activity, the antiproliferative effect of the extract from the fruit is mainly due to deoxypodophyllotoxin (**6**) as is the case with the root and ground part.

Experimental[6]

Plant Material The fruits were collected in August, 1996 in the suburbs of Fukuoka City and dried at room temperature.

Measurement of Antiproliferative Activity against Tumor Cell Lines Nude mouse-transplantable human gastric adenocarcinoma cells (MK-1), human uterus carcinoma cells (HeLa) and murine melanoma cells (B16F10) were used as tumor cell lines. Cellular growth was determined using the MTT-microculture tetrazolium assay described by Mosmann.[2] The detailed procedure was described in the previous paper.[7]

Extraction and Isolation The powdered fruits (100 g) were percolated with $CHCl_3$ (1.5 l), and then with MeOH (500 ml). The MeOH extract was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ percolate and the $CHCl_3$ extract were combined, concentrated and dried *in vacuo* to give a dark green resin ($CHCl_3$ ext. 17 g). The aqueous layer after $CHCl_3$ extraction was passed through a styrene polymer, Diaion HP-20 (150 ml) column and the column was washed with H_2O, and then with MeOH. The H_2O eluate was concentrated and dried *in vacuo* to give a brown residue (H_2O ext.). The MeOH eluate was also concentrated and dried *in vacuo* to give a brown powder (MeOH ext.). The antiproliferative activity (ED_{50}, μg/ml) was: $CHCl_3$ ext., *ca.* 0.3; MeOH ext., 50. The H_2O ext. exhibited no activity.

Fractionation of the $CHCl_3$ Ext. The $CHCl_3$ ext. (152 g from 1 kg dried fruit) was roughly separated into two fractions by silica-gel column chromatography using benzene and benzene–acetone (9 : 1) as eluting

Table 2. ^{1}H-NMR and ^{13}C-NMR Signal Assignments of Phenylpropanoid Di-esters

	9		3		4	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	6.55 d (15)	134.0	6.61 br d (16)	134.3	6.61 br d (16)	134.2
2	6.14 ddd (16, 7, 7)	121.8	6.17 ddd (16, 7, 7)	121.1	6.17 ddd (16, 7, 7)	121.2
3	4.80 dd (7, 1)	64.8	4.82 dd (7, 1)	65.1	4.82 dd (7, 1)	65.3
1′		131.2		129.4		129.4
2′	6.60 d (2)	100.2	6.93 d (2)	109.1	6.94 d (2)	109.2
3′		149.2		149.3[a]		149.3
4′		135.3		149.1[a]		149.2
5′		143.6	6.82 d (8)	111.2	6.82 d (8)	111.2
6′	6.53 d (2)	107.2	6.92 dd (8, 2)	120.2	6.92 dd (8, 2)	120.0
1″		165.7		165.8		165.8
2″		127.9		128.0		128.0
3″	6.45 q (8)	143.4	6.45 q (8)	143.3	6.44 q (7)	143.0
4″	2.12 br d (8, 1)	15.7	2.12 br d (8)	15.8	2.12 br d (7)	15.8
5″	4.84 d (1)	65.1	4.85 br s	65.1	4.83 d (1)	65.0
1‴		167.6		167.6		167.7
2‴		127.7		127.8		128.5
3‴	6.03 br q (7)	138.0	6.02 qq (7, 2)	138.0	6.82 m	137.5
4‴	1.94 dd (7, 2)	15.7	1.94 dq (7, 2)	15.7	1.72 dd (7, 1)	14.3
5‴	1.85 br s	20.5	1.85 dq (2, 2)	20.5	1.79 t-like (1)	12.0
$-OCH_2O-$	5.96 s	101.5				
$C_{3'}$-OCH_3			3.90 s[a]	55.9	3.88 s	55.9
$C_{4'}$-OCH_3			3.88 s[a]	55.9	3.90 s	56.0
$C_{5'}$-OCH_3	3.90 s	56.6				

a) The signal assignment in each vertical column may be interchangeable. Numbers in parentheses are coupling constants in Hz.

Table 3. The Antiproliferative Activity (ED_{50}, μg/ml) against MK-1, HeLa, and B16F10 Cell Lines *in Vitro* ($n=3$)

Compds.	B16F10	HeLa	MK-1
1	2.72±0.53	2.58±0.33	1.67±0.53
2	0.084±0.006	0.053±0.020	0.087±0.012
3	37.1±1.0	59.0±1.7	38.4±1.9
4	24.5±4.3	23.2±7.7	19.2±0.7
5	10.6±1.1	15.3±3.7	5.9±0.9
6	0.085±0.023	0.033±0.002	0.022±0.015
7	1.61±0.27	1.26±0.10	0.74±0.17
8	18.3±1.1	55.3±5.1	2.8±0.5

solvents. The benzene eluate (120 g) consisted of non-polar oily substances (triglycerides, fatty acids) and showed no antiproliferative activity against MK-1 cells. The benzene–acetone (9 : 1) eluate (28 g) had an $ED_{50}<3$ μg/ml. This fraction was further fractionated by silica-gel open column chromatography and finally purified by preparative HPLC, almost in the same manner as described in the preceding paper,[1] and **1** (42 mg), **2** (10 mg), **3** (119 mg), **4** (13 mg), **5** (15 mg), deoxypodophyllotoxin (**6**) (1.1 g), (−)-deoxypodorhizone (**7**) (1.23 g), and falcarindiol (**8**) (90 mg) were isolated. The data for **6**, **7**, and **8** are shown in the preceding paper.[1]

Compound **1**: A pale yellow oil, $[\alpha]_D^{24}$ −30.0° ($c=1.08$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 287 (3.83), 235 (3.83). CD ($c=0.56\times10^{-4}$, MeOH) $[\theta]^{25}$ (nm): −6300 (234), −1260 (280). Positive-ion HR FAB-MS *m/z*: 354.1102 ($[M]^+$) (Calcd for $C_{20}H_{18}O_6$: 354.1103). The ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Compound **2**: A pale yellow resin, $[\alpha]_D^{23}$ −83.9° ($c=0.46$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 292 sh (3.60), 283 (3.74), 227 sh (4.02). CD ($c=0.58\times10^{-4}$, MeOH) $[\theta]^{25}$ (nm): +7900 (238), −6650 (274), −950 (298). Positive-ion HR FAB-MS *m/z*: 368.1257 ($[M]^+$) (Calcd for $C_{21}H_{20}O_6$: 368.1260). The ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Compound **3**: A pale yellow oil. Positive-ion HR FAB-MS *m/z*: 374.1732 ($[M]^+$) (Calcd for $C_{21}H_{26}O_6$: 374.1729). The ^{1}H- and ^{13}C-NMR data are shown in Table 2.

Compound **4**: A pale yellow oil. Positive-ion HR FAB-MS *m/z*: 374.1726 ($[M]^+$) (Calcd for $C_{21}H_{26}O_6$: 374.1729). The ^{1}H- and ^{13}C-NMR data are shown in Table 2.

Compound **5**: A pale yellow oil, $[\alpha]_D^{25}$ +1.46° ($c=1.00$, MeOH). Positive-ion HR FAB-MS *m/z*: 194.0944 ($[M]^+$) (Calcd for $C_{11}H_{14}O_3$: 194.0943). ^{1}H-NMR δ: 3.87 (3H, s, $C_{4'}$-OCH_3), 3.88 (3H, s, $C_{3'}$-OCH_3), 5.15 (H, br d, $J=6.0$ Hz, 1-H), 5.19 (H, ddd, $J=10.4$, 1.5, 1.2 Hz, 3-Ha), 5.34 (H, ddd, $J=17.0$, 1.5, 1.2 Hz, 3-Hb), 6.05 (H, ddd, $J=17.0$, 10.5, 6.0 Hz, 2-H), 6.84 (H, d, $J=8.0$ Hz, 5′-H), 6.90 (H, dd, $J=8.0$, 2.0 Hz, 6′-H), 6.92 (H, d, $J=2.0$ Hz, 2′-H). ^{13}C-NMR δ: 55.9*[1] ($C_{3'}$-OCH_3), 56.0*[1] ($C_{4'}$-OCH_3), 75.1 (C-1), 109.7 (C-2′), 111.2 (C-5′), 114.9 (C-3), 118.7 (C-6′), 135.4 (C-1′), 140.3 (C-2), 148.7*[2] (C-3′) and 149.2*[2] (C-4′).*[1,2]: Signal assignment is interchangeable.

Identification of the Component Organic Acids of 3 and 4 2.0 mg each of **3**, **4**, and **9** was dissolved in 0.05 N MeONa in MeOH (0.2 ml) and the solution was stirred at room temperature for 22 h. After neutralization with 0.05 N aqueous acetic acid, hexane (0.2 ml) was added and the mixture was shaken. After centrifugation, the hexane layer was subjected to gas liquid chromatography (column, Carbowax-20M, 0.24 mm i.d. × 25 m long; carrier gas, He, 30 cm/s; column oven temperature, 50 °C; injection port temperature, 200 °C; detector temperature, 200 °C). Methyl angelate and methyl tiglate were used as standards. The reaction product of **3** showed 2 peaks with a t_R (min) of 4.30, 12.22, that of **4** gave 2 peaks (t_R: 6.46, 12.22) and that of **9** gave 2 peaks (t_R: 4.30, 12.22). The t_R values of methyl angelate and methyl tiglate were 4.30 and 6.46, respectively.

The peak with t_R 12.22 is methyl sarracinate, and the cinnamyl alcohol derivatives were not eluted at the oven temperature used.

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References and Notes

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(1995).
6) The instruments, materials and methods used in this work were the same as those shown in the Part III. Shimadzu gas chromatograph GC-17A was used for GC analysis of the organic acid methyl esters. The NMR spectra were measured in $CDCl_3$ (^{1}H-NMR, 500 MHz; ^{13}C-NMR, 125 MHz), and chemical shifts were shown on the δ-scale using tetramethylsilane as an internal standard. The signal assignment was performed using the ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY), ^{1}H–^{13}C COSY, and HMBC techniques together with the ordinary analyses of one-dimensional spectra.
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