

## Antiproliferative Constituents in Umbelliferae Plants. III.<sup>1)</sup> Constituents in the Root and the Ground Part of *Anthriscus sylvestris* HOFFM.

Ryuji IKEDA,<sup>a</sup> Tsuneatsu NAGAO,<sup>a</sup> Hikaru OKABE,<sup>\*a</sup> Yukitaka NAKANO,<sup>b</sup>  
Hisashi MATSUNAGA,<sup>b</sup> Mitsuo KATANO,<sup>c</sup> and Masato MORI<sup>b</sup>

Faculty of Pharmaceutical Sciences, Fukuoka University,<sup>a</sup> 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan  
and Faculty of Hospital Pharmacy<sup>b</sup> and Department of Surgery,<sup>c</sup> Saga Medical School, 5–1–1 Nabeshima,  
Saga 849–8501, Japan. Received October 7, 1997; accepted January 6, 1998

The MeOH extract of the root and the ground part of *Anthriscus sylvestris* HOFFM. showed a high inhibitory activity against MK-1, HeLa, and B16F10 cell growth *in vitro*. The activity was found only in the CHCl<sub>3</sub>-soluble fractions. From the CHCl<sub>3</sub>-soluble fraction of the root, faltarindiol, 1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1ξ-methoxy-2-propene, elemicin, and nemerosin were newly isolated in addition to deoxypodophyllotoxin (anthricin), anthriscusin, (–)-deoxypodorhizone, and anthriscinol methyl ether which were reported earlier as constituents of the root of this plant.

From the CHCl<sub>3</sub>-soluble fraction of the ground part, deoxypodophyllotoxin, (–)-deoxypodorhizone, nemerosin, and faltarindiol were isolated.

*In vitro* antiproliferative activities of the isolates against MK-1, HeLa, and B16F10 cells are reported.

**Key words** *Anthriscus sylvestris*; Umbelliferae; antiproliferative constituent; lignan; phenylpropanoid; acetylenic compound

In the preceding paper<sup>1)</sup> of this series, we described the application of the combination of MTT assay and ELISA for panaxytriol to the screening of the antiproliferative polyacetylenic compounds in the 21 Umbelliferae plants. We also reported in the same paper the isolation of panaxynol and faltarindiol from the root of *Heracleum moellendorffii* as the constituents which inhibit the growth of nude mouse-transplantable human gastric adenocarcinoma (MK-1), human uterus carcinoma (HeLa), and murine melanoma (B16F10) cells.

The MeOH extract of the root and the ground part of *Anthriscus sylvestris* HOFFM. showed a relatively low immunoreactivity in ELISA for panaxytriol, while the antiproliferative activities against three tumor cells were much higher than those of *Heracleum moellendorffii* root, indicating the presence of highly antiproliferative constituents in addition to the polyacetylenic compounds.

The constituents of this plant have already been investigated by other researchers,<sup>2)</sup> and the isolation of deoxypodophyllotoxin (**1**, anthricin), iso-anthricin, (–)-deoxypodorhizone (**2**), crocatone [1-(3'-methoxy-4',5'-methylenedioxyphenyl)propan-1-one], (Z)-2-angeloyloxymethyl-2-butenoic acid, anthriscusin (**3**), 1-(3'-methoxy-4',5'-methylenedioxyphenyl)-2ξ-angeloyloxypropan-1-one, and anthriscinol [1-(3'-methoxy-4',5'-methylenedioxyphenyl)-2-propen-3-ol] was reported.

Concerning the biological activities of these constituents, Kozawa *et al.*<sup>3)</sup> reported the insecticidal activities of **1**, (Z)-2-angeloyloxymethyl-2-butenoic acid, and anthriscinol methyl ether (**4**) on the adults of *Blattella germanica*, and on the larvae of *Culex pipiens molestus*, *Plutella xylostella*, and *Epilachna sparsa orientalis*. Compounds **1** and **2** are known to have an antitumor activity.<sup>4)</sup> Considering that **1** and **2** are the main lignans in this plant as claimed by Kozawa *et al.*, the antiproliferative activity of the extracts is very likely due to them.

The constituents of the root and the ground part were

thoroughly investigated again to check the presence of the antiproliferative polyacetylenes and other constituents using a combination of MTT assay and ELISA for panaxytriol.

The fresh root was extracted and fractionated as described in the Experimental section, and from the CHCl<sub>3</sub> extract with a highest growth inhibitory activity against MK-1 cell growth, an acetylenic compound (**5**), two phenylpropanoids (**6** and **7**), and one lignan (**8**) were isolated in addition to deoxypodophyllotoxin (**1**), anthriscinol methyl ether (**4**), (–)-deoxypodorhizone (**2**), and anthriscusin (**3**).

Compound **5**, C<sub>17</sub>H<sub>24</sub>O<sub>2</sub>, was obtained as a colorless oil, however, it gradually colored yellow on being left standing at room temperature. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra showed the presence of a normal alkyl group, two acetylenic groups, two hydroxymethylene groups, *cis* olefin group and a terminal olefin group, and the molecular formula and these functional groups suggested that **5** was faltarindiol. The identity was confirmed by comparison of the NMR data with those reported.<sup>1)</sup>

Compound **6**, C<sub>12</sub>H<sub>14</sub>O<sub>4</sub>, was obtained as a pale yellow oil. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra (Table 1) showed signals assignable to a 3-methoxy-4,5-methylenedioxyphenyl group, a terminal olefin group and a methoxyl group on a methine carbon. Combination of these functional groups led to the structure of **6** being 1-(3'-methoxy-4',5'-methylenedioxyphenyl)-1ξ-methoxy-2-propene.

Compound **7**, C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>, was also obtained as a pale yellow oil. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra (Table 1) showed the presence of a 3,4,5-trimethoxyphenyl group and a terminal olefin group, indicating **7** to be 1-(3',4',5'-trimethoxyphenyl)-2-propene, *viz.* elemicin.

Compound **8**, C<sub>22</sub>H<sub>22</sub>O<sub>7</sub>, was obtained as a pale yellow resin. The <sup>1</sup>H-NMR spectrum (Table 2) showed signals of three methoxyl groups, a methylenedioxy group, five aromatic protons, a methylene group, an oxymethyl group,

\* To whom correspondence should be addressed.

Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Phenylpropanoids **4**, **6**, and **7**

	<b>4</b>		<b>6</b>		<b>7</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	6.49 br d (15.5)	132.2	4.50 d (6.5)	84.5	3.33 br d (6.5)	40.8
2	6.12 ddd (15.5, 6.0, 6.0)	124.8	5.89 ddd (17.0, 10.0, 6.5)	138.6	5.95 m	137.2
3	4.05 dd (6.0, 1.5)	73.0	5.19 ddd (10.0, 1.5, 1.5)	116.2	5.08 br d (10.0)	115.9
			5.26 ddd (17.0, 1.5, 1.5)		5.10 br d (17.0)	
1'		131.8		135.7		135.7
2'	6.54 d (1.5)	106.9	6.517 d (1.5) <sup>a)</sup>	101.1 <sup>b)</sup>	6.41 s	105.6
3'		143.6		149.0		153.2
4'		135.1		134.7		136.5
5'		149.2		143.7		153.2
6'	6.61 d (1.5)	100.1	6.523 d (1.5) <sup>a)</sup>	106.3 <sup>b)</sup>	6.41 s	105.6
OCH <sub>3</sub>	3.38 s (C <sub>3</sub> -)	57.9	3.32 s (C <sub>1</sub> -)	56.3	3.84 s (C <sub>3</sub> -)	56.1
	3.90 s (C <sub>3</sub> -)	56.6	3.90 s (C <sub>3</sub> -)	56.6	3.82 s (C <sub>4</sub> -)	60.8
OCH <sub>2</sub> O	5.95 s	101.4	5.95 s	101.4	3.84 s (C <sub>5</sub> -)	56.1

*a, b*) Assignment is interchangeable. Numbers in parentheses are coupling constants in Hz.

Table 2.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of Lignans **1**, **2**, and **8**

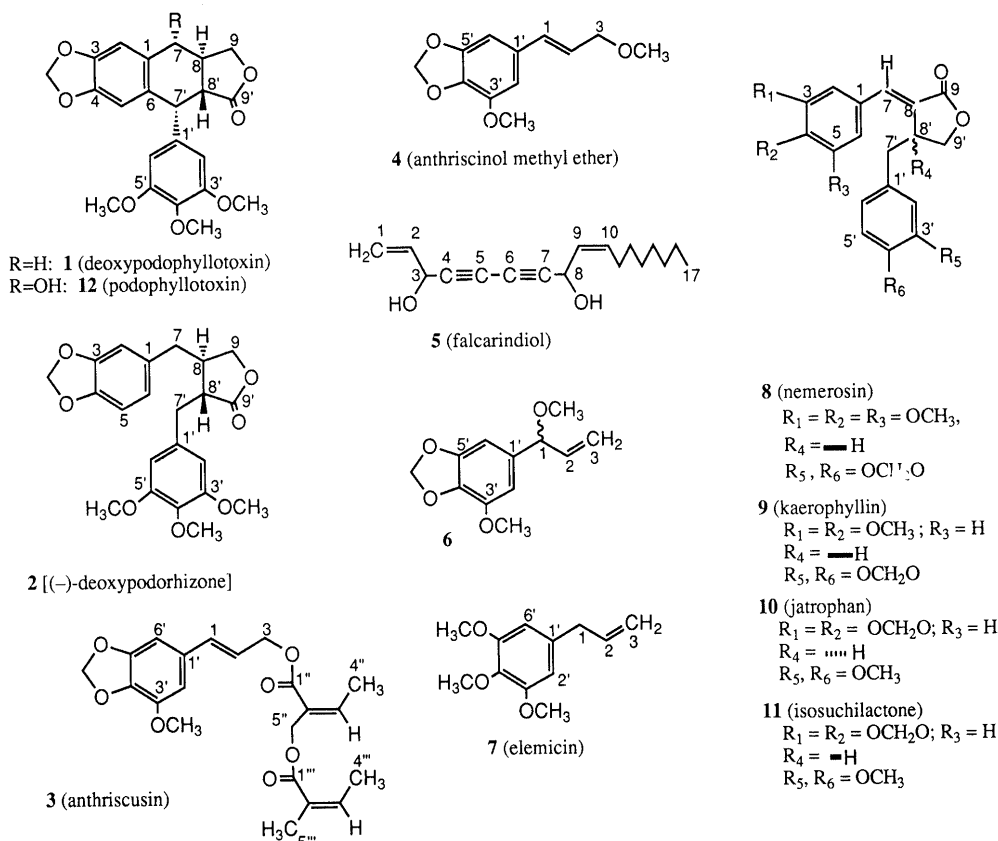
	<b>1</b>		<b>2</b>		<b>8</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1		128.3		131.5		129.4
2	6.65 s	108.5	6.46 br s	108.8	6.77 s	107.6 <sup>a)</sup>
3		147.1		147.9		153.4
4		146.8		146.4		140.0
5	6.52 s	110.5	6.69 dd (7.5, 1.0)	108.3		153.4
6		130.7	6.47 dd (7.5, 1.5)	121.5	6.77 s	107.5 <sup>a)</sup>
7	<i>ca.</i> 2.73	33.1	<i>ca.</i> 2.53	38.3	7.51 d (2.0)	137.6
	3.07 dd (17.0, 10.0)		<i>ca.</i> 2.62			
8	2.72 m	32.8	<i>ca.</i> 2.49	41.0		127.1
9	3.91 m	72.0	3.87 dd (9.0, 7.5)	71.1		172.2
	4.44 m		4.17 dd (9.0, 7.5)			
1'		136.2		133.3		131.3
2'	6.35 s	108.5	6.36 s	106.4	6.61 d (1.5)	109.0
3'		152.5		153.3		146.6 <sup>b)</sup>
4'		137.3		137.1		148.0 <sup>b)</sup>
5'		152.5		153.3	6.70 d (8.0)	108.4
6'	6.35 s	108.5	6.36 s	106.4	6.59 dd (8.0, 1.5)	121.8
7'	4.59 d (3.5)	43.8	2.88 dd (14.0, 7.0)	35.3	2.66 dd (15.0, 10.0)	37.8
			2.93 dd (14.0, 5.0)		3.02 dd (15.0, 5.0)	
8'	2.72 m	47.5	<i>ca.</i> 2.57	46.4	3.84 m	39.5
9'		174.8		178.4	4.25 dd (9.0, 2.0)	69.7
					4.30 dd (9.0, 7.0)	
OCH <sub>3</sub>	3.75 s (C <sub>3</sub> -)	56.3	3.83 s (C <sub>3</sub> -)	56.1	3.88 s (C <sub>3</sub> -)	56.3
	3.80 s (C <sub>4</sub> -)	60.8	3.83 s (C <sub>4</sub> -)	60.8	3.90 s (C <sub>4</sub> -)	60.9
	3.75 s (C <sub>5</sub> -)	56.3	3.83 s (C <sub>5</sub> -)	56.1	3.88 s (C <sub>5</sub> -)	56.3
OCH <sub>2</sub> O	5.92 d (1.0)	101.2	5.92 d (1.5)	101.0	5.91 d (1.5)	101.1
	5.94 d (1.0)		5.93 d (1.5)		5.92 d (1.5)	

*a, b*) Assignment is interchangeable. Numbers in parentheses are coupling constants in Hz.

a trisubstituted olefin group, and a methine group which links to a methylene group, an oxymethyl group, and an olefin carbon. The  $^{13}\text{C}$ -NMR spectrum (Table 2) showed the signals of two *C*-substituted aromatic carbons, five *O*-substituted aromatic carbons, and an ester carbonyl carbon in addition to the carbon signals correlative to the above-mentioned proton signals.

The UV spectrum showed absorptions at 312, 296, and 233 nm. These data suggested that **8** is a  $\gamma$ -butyrolactone lignan which has a benzyl group and a benzylidene group. The  $^1\text{H}$ - $^1\text{H}$  chemical shift correlation spectro-

scopy (COSY) spectrum showed linkages of the benzylic methylene group ( $\delta$  2.66, H, dd,  $J=15.0, 10.0$  Hz; 3.02, H, dd,  $J=15.0, 5.0$  Hz) to a methine group ( $\delta$  3.84, H, m), which further connects to an oxymethyl group ( $\delta$  4.25, H, dd,  $J=9.0, 2.0$  Hz; 4.30, H, dd,  $J=9.0, 7.0$  Hz); the heteronuclear multiple-bond correlation (HMBC) spectrum showed a correlation of the aromatic protons ( $\delta$  6.77, 2H, s) with the oxygenated aromatic carbons ( $\delta$  140.0 and  $153.4 \times 2$ ) which are all correlated with the methoxyl protons. These correlations indicated the presence of a trimethoxyphenyl group. The aromatic protons ( $\delta$  6.77,



2H, s) are correlated with the olefin carbon ( $\delta$  137.6), and the proton ( $\delta$  7.51, H, d,  $J=2.0$  Hz) of this olefin group is correlated with the methine carbon ( $\delta$  39.5) and the ester carbonyl carbon ( $\delta$  172.2). The aromatic proton ( $\delta$  6.59, H, dd,  $J=8.0, 1.5$  Hz) in a methylenedioxyphenyl group showed correlation with the benzylic methylene carbon ( $\delta$  37.8). These data indicated that **8** is one of two geometric isomers (*7E* and *7Z*) of 2-(3,4,5-trimethoxybenzylidene)-3-(3',4'-methylenedioxybenzyl)- $\gamma$ -butyrolactone.

Gonzalez *et al.*<sup>5)</sup> reported that the <sup>1</sup>H-NMR spectrum of kaerophyllin (**9**) showed the olefin proton signal at  $\delta$  7.53 (d,  $J=1.8$  Hz), while that of isokaerophyllin (*7Z* isomer of **9**) appeared at  $\delta$  6.62 (d,  $J=1.5$  Hz). The chemical shift ( $\delta$  7.51) of the olefin proton of **8** indicated that it has the same geometry (*E*) as that of **9**. The absolute configuration at C-8' was suggested to be *R* by comparison of its rotation ( $-44.1^\circ$ ) with those of jatrophan (**10**, 8'*S*,  $+87^\circ$ )<sup>6)</sup> and isosuchilactone (**11**, 8'*R*,  $-83.3^\circ$ )<sup>7)</sup> The circular dichroism (CD) spectrum of **8** showed almost the same CD curve as that of **11**, supporting the 8'*R* configuration.<sup>7)</sup> Therefore, the structure of **8** was determined as shown. This compound was identified as anhydro-podorhizol<sup>8a)</sup> (=nemosin)<sup>8b)</sup> by comparison of the <sup>1</sup>H-NMR data reported.<sup>8)</sup>

The CHCl<sub>3</sub> extract of the ground part was fractionated essentially in the same manner as that from the root, and **1**, **2**, **5**, and **8** were isolated.

The antiproliferative activities against MK-1, HeLa, and B16F10 were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.<sup>9)</sup> The antiproliferative activities of podophyllotoxin (**12**) were also measured for comparison. The 50% growth inhibi-

Table 3. Antiproliferative Activity (ED<sub>50</sub>,  $\mu$ g/ml) against MK-1, HeLa, and B16F10 Cell Lines *in Vitro*

Compds.	MK-1	HeLa	B16F10
<b>1</b>	0.0010	0.0005	0.0007
<b>2</b>	0.3	0.8	0.35
<b>3</b>	2.5	2.1	3.0
<b>4</b>	2.9	2.4	2.5
<b>5</b>	3.2	21.4	11.3
<b>6</b>	100	95	> 100
<b>7</b>	4.5	2.0	2.6
<b>8</b>	0.7	0.4	0.7
<b>12</b>	0.006	0.0025	0.001

tions (ED<sub>50</sub>) are summarized in Table 3.

Judging from the content and the antiproliferative activity of the respective compound, it can safely be said that the main antiproliferative component in the root and the ground part of *Anthriscus sylvestris* is deoxy podophyllotoxin.

#### Experimental

The instruments and materials used in this work were as follows: JASCO DIP-360 digital polarimeter (specific rotation), JEOL JNM GX-400 and JNM A-500 spectrometers (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra), JEOL JMS HX-110 mass spectrometer (MS spectra), Shimadzu UV-200S spectrometer (UV spectra), JASCO J-720 spectrometer (CD spectra), TOSOH CCPD liquid chromatograph equipped with a refractive index (RI) detector, RI-8010 (HPLC). For preparative scale HPLC, a Cosmosil 5SL column (Nacalai Tesque), 20 mm i.d.  $\times$  250 mm long, was used. The solvents are noted in the text, and the flow rate of the solvent was set at 6 ml/min. For column chromatography, Kieselgel 60, 70–230 mesh (E. Merck), and MCI Gel HP-20 (Mitsubishi Chemical Industries, Ltd.) were used.

The NMR spectra were measured in  $\text{CDCl}_3$ , and chemical shifts were shown on the  $\delta$ -scale using tetramethylsilane as an internal standard. The signal assignment was performed using the  $^1\text{H}$ - $^1\text{H}$  COSY,  $^1\text{H}$ - $^{13}\text{C}$  COSY, and heteronuclear multiple bond connectivity (HMBC) techniques together with the ordinary analyses of one-dimensional spectra. The instruments and materials used for the biological experiments were shown in the preceding paper<sup>1)</sup> of this series.

**Plant Materials** The plant materials were collected in May, 1995 at Genkai Machi, Fukuoka Prefecture.

**Detection of the Polyacetylenic Compounds** The ELISA for panaxytriol was used to detect the polyacetylenic compounds in the extract. The detailed procedure was described earlier.<sup>1,10)</sup>

**Measurement of Antiproliferative Activities against Tumor Cell Lines** MK-1, HeLa, and B16F10 were used as tumor cell lines. Cellular growth was evaluated using the MTT-microculture tetrazolium assay described by Mosmann.<sup>9)</sup> The detailed procedure was given in the preceding paper.<sup>1)</sup>

**Extraction and Preliminary Fractionation** The fresh root (12 kg) was chopped and homogenized in MeOH (30 l). The MeOH extract was suspended in  $\text{H}_2\text{O}$  (2 l) and extracted twice with  $\text{CHCl}_3$  (1 l  $\times$  2). The  $\text{CHCl}_3$  solution was concentrated *in vacuo* to give a reddish brown resinous residue (Fr. I, 53 g). The aqueous layer after extraction with  $\text{CHCl}_3$  was passed through a column of Diaion HP-20 (1 l) and the column was washed with  $\text{H}_2\text{O}$ . The  $\text{H}_2\text{O}$  eluate was concentrated *in vacuo* to give a brown residue (Fr. II). The column was then washed with MeOH (2 l) to give a brown eluate which was concentrated and dried *in vacuo* to give a brown powder (Fr. III, 14.7 g). The 50% growth inhibition ( $\text{ED}_{50}$ , mg/ml) of the Fr. I, II and III against MK-1 cells was, 0.024, 3.13 and 1.56, respectively, and the immunoreactivity in the ELISA ( $\text{B}/\text{B}_0$ , % at 200  $\mu\text{g}/\text{ml}$ ) was 63.1, 100.0 and 54.2, respectively.

**Fractionation of Fr. I, and Isolation of Constituents** Fraction I (53 g) was repeatedly chromatographed on silica gel using hexane, hexane-AcOEt (9:1  $\rightarrow$  1:1) and separated into 4 fractions (Fr. I-1  $\rightarrow$  Fr. I-4). Fraction I-1 (9 g) was further chromatographed on silica gel using benzene-acetone (99:1) to give 4 fractions (Fr. I-1-1  $\rightarrow$  Fr. I-1-4). From the first 3 fractions, a fatty acid mixture (848 mg), a triglyceride mixture (214 mg), and **6** (1.47 g) were isolated, respectively. The last fraction (Fr. I-1-4) was subjected to preparative HPLC on a silica gel column using benzene-acetone (19:1) to give **7** (44 mg), **3** (268 mg), and **4** (161 mg). Fraction I-2 (10 g) was chromatographed on silica gel using hexane-AcOEt (4:1  $\rightarrow$  1:1) to give a phytosterol mixture (1.20 g) and **5** (1.81 g). Fraction I-3 (15 g) was crystallized from hexane-AcOEt to give **1** (6.96 g). The mother liquor was subjected to preparative HPLC on a silica gel column using benzene-acetone (19:1) to give another crop of **1** (870 mg), **2** (1.89 g), and **8** (287 mg).

**Fractionation of the  $\text{CHCl}_3$  Extract from the Ground Part** The  $\text{CHCl}_3$  extract (26.8 g) from the dried ground part (1 kg) was repeatedly chromatographed on silica gel using benzene and benzene-acetone (19:1  $\rightarrow$  9:1), fractionating into four fractions, fr. 1 (5.01 g), fr. 2 (10.4 g), fr. 3 (2.2 g), and fr. 4 (8.07 g). Fraction 1 consisted mainly of fatty oil, and fr. 4 was a mixture of polar compounds. Fraction 2 was further chromatographed on silica gel using benzene-acetone (97:3  $\rightarrow$  9:1) to give 2 fractions, fr. 1-1 (7.55 g) and fr. 1-2 (2.90 g). Fraction 2-1 contained oily materials and coloring materials such as chlorophylls. Fraction 2-2 was repeatedly subjected to preparative HPLC on silica gel column using hexane-EtOAc (1:1) to give a mixture of phytosterols (952 mg), **8** (127 mg), **2** (464 mg) and **1** (502 mg). Fraction 3 was subjected to preparative HPLC on a silica gel column using hexane-iso-PrOH (9:1) to give **5** (16 mg).

**Compound 1:** Colorless prisms from hexane-AcOEt, mp 168–170  $^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{25} - 129.7^\circ$  ( $c=0.99$ , MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 292 (3.65). Positive-ion HR FAB-MS  $m/z$ : 398.1366 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{22}\text{H}_{24}\text{O}_7$ : 398.1366).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 2. Compound **1** was identified as deoxypodophyllotoxin by comparison of  $^1\text{H}$ -NMR data with those reported by Kozawa *et al.*<sup>2b)</sup> and  $^{13}\text{C}$ -NMR data by Van Uden *et al.*<sup>11)</sup>

**Compound 2:** A pale yellow resin,  $[\alpha]_{\text{D}}^{25} - 19.3^\circ$  ( $c=1.00$ , MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 285 (3.52), 227 sh (4.01). Positive-ion HR FAB-MS  $m/z$ : 400.1520 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{22}\text{H}_{24}\text{O}_7$ : 400.1522).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 2. Compound **2** was identified as (-)-deoxypodophyllotoxin by comparison of the  $^1\text{H}$ -NMR data with those reported.<sup>2b,12)</sup>

**Compound 3:** A pale yellow oil. Positive-ion HR FAB-MS  $m/z$ :

388.1520 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{22}\text{H}_{24}\text{O}_7$ : 388.1522).  $^1\text{H}$ -NMR  $\delta$ : 1.85 (3H, brs, 5''-H), 1.94 (3H, brd,  $J=7.3$ , 4''-H), 2.12 (3H, brd,  $J=7.5$ , 1.0 Hz, 4''-H), 3.90 (3H, s,  $\text{C}_3$ -OCH<sub>3</sub>), 4.80 (2H, brd,  $J=6.5$ , 3-H), 4.84 (2H, brs, 5''-H), 5.96 (2H, s, -OCH<sub>2</sub>O-), 6.03 (H, br q,  $J=7.3$  Hz, 3''-H), 6.14 (H, ddd,  $J=16.0$ , 6.5, 6.5 Hz, 2-H), 6.45 (H, q,  $J=7.5$  Hz, 3''-H), 6.60 (H, d,  $J=1.5$  Hz, 6'-H), 6.55 (H, d,  $J=16.0$  Hz, 1-H), and 6.53 (H, d,  $J=1.5$  Hz, 2'-H).  $^{13}\text{C}$ -NMR  $\delta$ : 15.7 (C-4''), 15.8 (C-4''), 20.5 (C-5''), 56.6 (C<sub>3</sub>-OCH<sub>3</sub>), 64.8 (C-3), 65.1 (C-5''), 107.2 (C-2'), 101.5 (-OCH<sub>2</sub>O-), 100.2 (C-6'), 121.8 (C-2), 127.7 (C-2''), 127.9 (C-2''), 131.2 (C-1'), 134.0 (C-1), 135.4 (C-4'), 138.0 (C-3''), 143.4 (C-3''), 149.2 (C-5'), 143.6 (C-3'), 165.7 (C-1''), and 167.6 (C-1''). Compound **3** was identified as anthriscusin by comparison of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data with those reported by Kozawa *et al.*<sup>2b)</sup>

**Compound 4:** A pale yellow oil. Positive-ion HR FAB-MS  $m/z$ : 222.0891 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{12}\text{H}_{14}\text{O}_4$ : 222.0892).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 3. The  $^1\text{H}$ -NMR data was compared with that of anthriscinol<sup>2b)</sup> and **4** was determined to be anthriscinol methyl ether.

**Compound 5:** A colorless oil which turns yellow on standing,  $[\alpha]_{\text{D}}^{25} + 144.8^\circ$  ( $c=1.01$ , MeOH). Positive-ion HR FAB-MS  $m/z$ : 283.1674 ( $[\text{M}+\text{Na}]^+$ ) (Calcd for  $\text{C}_{17}\text{H}_{24}\text{NaO}_2$ : 283.1674). Compound **5** was identified as falcariindiol by comparison of the NMR data with those reported.<sup>1)</sup> The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data were shown in the preceding paper.<sup>1)</sup>

**Compound 6:** A pale yellow oil.  $[\alpha]_{\text{D}}^{23} + 1.46^\circ$  ( $c=1.00$ , MeOH). Positive-ion HR FAB-MS  $m/z$ : 222.0884 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{12}\text{H}_{14}\text{O}_4$ : 222.0892).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 3.

**Compound 7:** A pale yellow oil. Positive-ion HR FAB-MS  $m/z$ : 208.1098 ( $[\text{M}]^+$ ) (Calcd for  $\text{C}_{12}\text{H}_{16}\text{O}_3$ : 208.1099).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 3.

**Compound 8:** A pale yellow resin,  $[\alpha]_{\text{D}}^{25} - 44.8^\circ$  ( $c=0.99$ , MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 312 (4.17), 296 (4.14), 233 (4.20). CD ( $c=0.62 \times 10^{-4}$ , MeOH)  $[\theta]^{25}$  (nm): -8000 (240), -4500 (300). Positive-ion HR FAB-MS  $m/z$ : 399.1434 ( $[\text{M}+\text{H}]^+$ ) (Calcd for  $\text{C}_{22}\text{H}_{23}\text{O}_7$ : 399.1366).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR: shown in Table 2.

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