Antiproliferative Constituents from Umbelliferae Plants. V.[1)] A New Furanocoumarin and Falcarindiol Furanocoumarin Ethers from the Root of *Angelica japonica*

Toshihiro FUJIOKA,[a] Kazuhiro FURUMI,[a] Hiroko FUJII,[a] Hikaru OKABE,[a] Kunihide MIHASHI,*,[a]
Yukitaka NAKANO,[b] Hisashi MATSUNAGA,[b] Mitsuo KATANO,[c] and Masato MORI[b]

Faculty of Pharmaceutical Sciences, Fukuoka University,[a] 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan, Faculty of Hospital Pharmacy,[b] and Department of Surgery,[c] Saga Medical School, 5–1–1 Nabeshima, Saga 849–8501, Japan.

Received September 3, 1998; accepted October 14, 1998

The $CHCl_3$ extract of the root of *Angelica japonica* showed high inhibitory activity against human gastric adenocarcinoma (MK-1) cell growth. From this extract, a new furanocoumarin named japoangelone and four furanocoumarin ethers of falcarindiol, named japoangelols A—D, were isolated together with caffeic acid methyl ester, four polyacetylenic compounds (panaxynol, falcarindiol, 8-*O*-acetylfalcarindiol, and (9*Z*)-1,9-heptadecadiene-4,6-diyne-3,8,11-triol), eight coumarins (osthol, isoimperatorin, scopoletin, byakangelicin, xanthotoxin, bergapten, oxypeucedanin methanolate, and oxypeucedanin hydrate), and two chromones (3′-*O*-acetylhamaudol, and hamaudol). The structures of the new isolates were determined based on spectral evidence. The ED_{50} of isolates against MK-1, HeLa, and B16F10 cell lines are reported.

Key words *Angelica japonica*; Umbelliferae; antiproliferative constituent; furanocoumarin; japoangelol; falcarindiol furanocoumarin ethers

We recently reported the results of screening for antiproliferative polyacetylenic constituents in Umbelliferae plants[2)] using a combination of ELISA for panaxytriol and the microculture tetrazolium (MTT) assay, and also the isolation of falcarindiol and panaxynol from the root of *Heracleum moellendorfii* 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. In the course of our continuing search for antiproliferative compounds from Umbelliferae plants, we found that a MeOH extract of the root of *Angelica japonica* showed antiproliferative activity (ED_{50} 25 μg/ml) against MK-1 cells.

Several reports[3,4)] have dealt with the constituents of *Angelica japonica*, and nine chromones and fifteen coumarins were reported, however, there is no report on their cytotoxicity.

We have thoroughly investigated the constituents of the root for antiproliferative components using the MTT assay.[5)] This paper deals with the characterization of the isolates and their antiproliferative activities.

The air-dried and powdered root was extracted with $CHCl_3$ and MeOH. The $CHCl_3$ extract showed a higher growth inhibitory activity against MK-1 cells (ED_{50} 3.13 μg/ml) than that of the MeOH extract, and was fractionated by column chromatography on silica gel and a reversed-phase material, and each fraction was subjected to reversed-phase preparative HPLC to give a new furanocoumarin named japoangelone (**9**), and four new furanocoumarin ethers of falcarindiol named japoangelols A—D (**12**—**15**), in addition to caffeic acid methyl ester (**19**), four polyacetylenic compounds (panaxynol (**1**),[6)] 8-*O*-acetylfalcarindiol (**3**),[7)] falcarindiol (**17**),[8)] and (9*Z*)-1,9-heptadecadiene-4,6-diyne-3,8,11-triol (**20**)[8)]), eight furanocoumarins (osthol (**2**),[4)] isoimperatorin (**4**),[9)] scopoletin (**5**),[4)] byakangelicin (**6**),[10)] xanthotoxin (**7**),[11)] bergapten (**8**),[11)] oxypeucedanin methanolate (**10**),[12)] and oxypeucedanin hydrate (**11**)[13)]), and two chromones (3′-*O*-acetylhamaudol (**16**),[14)] and hamaudol (**18**)[14)]). The known compounds were identified by MS and NMR spectral comparison with reported data.

Japoangelone (**9**) was obtained as light yellow needles and the positive-ion high-resolution (HR) FAB-MS gave the molecular formula $C_{18}H_{16}O_8$. The ^{1}H-NMR spectrum indicated the presence of two *cis* disubstituted olefin groups, two methyl groups on an oxygenated quaternary carbon, a methoxyl group on an aromatic carbon, an oxymethyl group, and an oxymethylene group. The ^{1}H-NMR spectrum was very similar to that of byakangelicin (**6**), however, the signals of C1′-H, C2′-H, and two methyl groups appeared at lower magnetic field than those of **6**. The ^{13}C-NMR spectrum of **9** resembles to that of **6**, however, it shows one more carbonyl carbon signal (δ 153.6) and the chemical shifts of the side chain carbon signals are different from those of **6**, suggesting that **9** is the carbonic acid ester of **6**. The C–H and C–C connectivities were traced by checking the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) spectrum (Fig. 1), and the structure of **9** was determined as shown in Fig. 2, except for the stereochemistry at C2′.

Japoangelols A—D (**12**—**15**) were obtained as colorless oils, however, they gradually became yellow on standing at room temperature. NMR spectra suggested that they were conjugates of a polyacetylenic compound and furanocou-

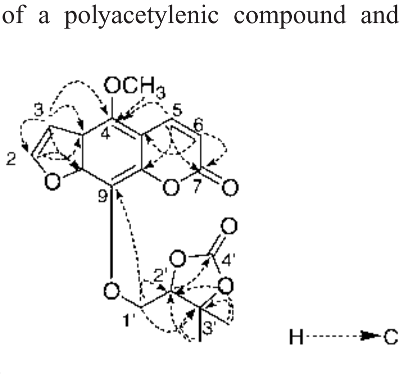

Fig. 1. ^{1}H–^{13}C Long-Range Correlations in **9**

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* To whom correspondence should be addressed.

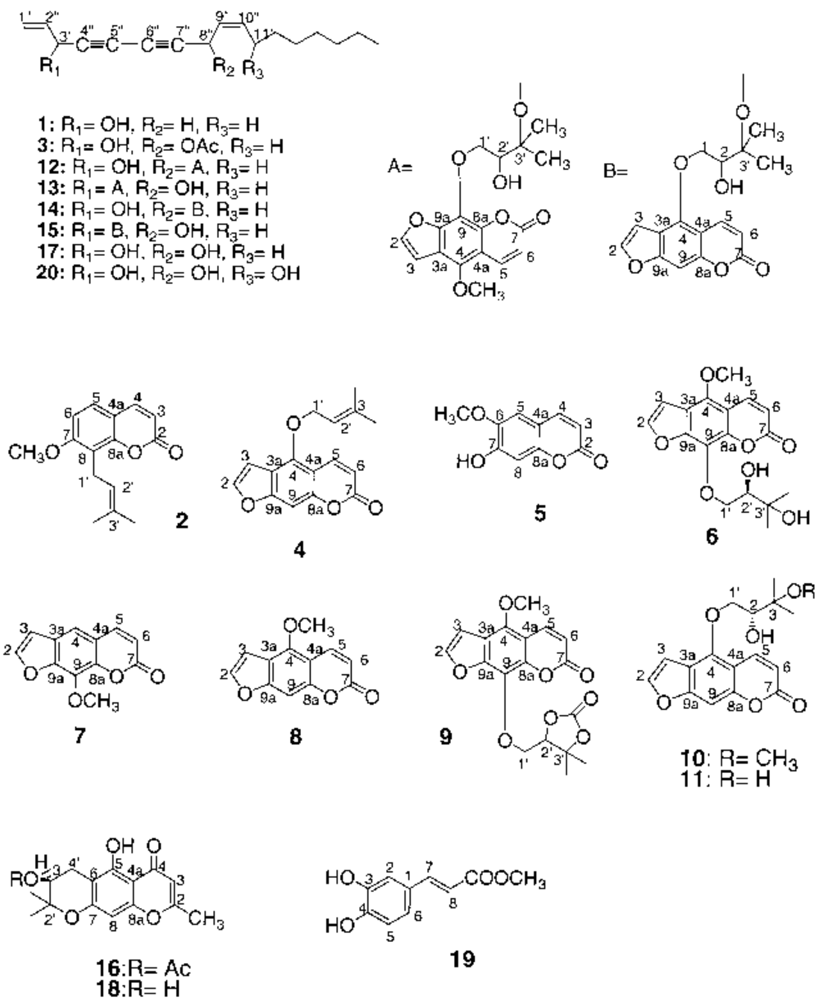

Fig. 2. Structures

marins.

The positive-ion HR FAB-MS of japoangelols A (**12**) and B (**13**) gave the molecular formula $C_{34}H_{40}O_8$, and both showed ^{1}H- and ^{13}C-NMR signals almost assignable to those of falcarindiol (**17**) and byakangelicin (**6**). In the ^{1}H-NMR spectrum of **12**, the proton signals of the two hydroxyl groups, C8″-O$\underline{H}$ of **17** and C3′-O$\underline{H}$ of **6**, were not observed even though the signals of the corresponding C3″-O$\underline{H}$ (δ 2.23) and C2′-O$\underline{H}$ (δ 3.08) were observed. The ^{13}C-NMR signal of C3′ appeared at δ 78.5, 7.0 ppm lower than that (δ 71.5) of **6**, suggesting that **17** links to C3′-O$\underline{H}$ of **6**. The down-field shift of the signal of the counterpart carbon (C8″) in **17** was not observed, however, the C8″-H signal showed a clear cross peak with a carbon signal (C3′) of **6** (see Fig. 3) in the HMBC spectrum. From this spectroscopic evidence, the location of the linkage was concluded to be C8″-*O*-C3′.

In the ^{13}C-NMR spectrum of **13**, the signal for C3′ appeared at δ 78.7, 7.2 ppm lower than that of **6**, and C3″-H and C3′ showed a cross peak in the HMBC spectrum, indicating that the location of the linkage is C3″-*O*-C3′.

The positive-ion HR FAB-MS of japoangelols C (**14**) and D (**15**) gave the molecular formula $C_{33}H_{38}O_7$, and both showed ^{1}H- and ^{13}C-NMR signals nearly assignable to those of falcarindiol (**17**) and oxypeucedanin hydrate (**11**). In the ^{1}H-NMR spectrum of **14**, the signals of C3″-O$\underline{H}$ (δ 2.18) of 17 and C2′-O$\underline{H}$ (δ 2.72) of **11** moiety were observed, however, the signals of C8″-O$\underline{H}$ and C3′-O$\underline{H}$ were not observed. The ^{13}C-NMR signal of C3′ appeared at δ 78.5, 6.8 ppm lower than that (δ 71.7) of **11**, suggesting that **17** links to C3′-O$\underline{H}$ of **11**. The location of the linkage was concluded to be C8″-*O*-C3′ by the HMBC experiment.

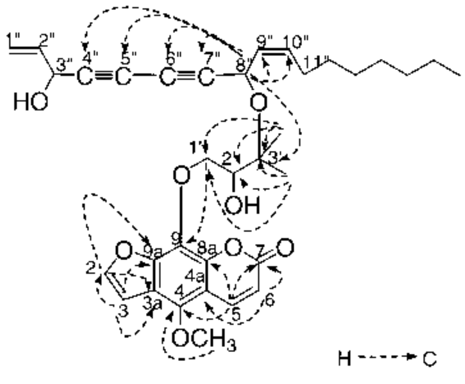

Fig. 3. ^{1}H–^{13}C Long-Range Correlations in **12**

In the ^{1}H-NMR spectrum of **15**, the signals for C8″-O$\underline{H}$ (δ 2.18) of **17** and C2′-O$\underline{H}$ (δ 2.72) of **11** were observed, and the ^{13}C-NMR signal of C3′ appeared at δ 78.7, 7.0 ppm lower than that of **11**. In the HMBC spectrum, C3″-H and C3′ showed a clear cross peak. Thus, the location of the linkage was concluded to be C3″-*O*-C3′.

Table 1. ^{1}H-NMR Data (δ, J in Hz) for Compounds **6**, **9**, **11**—**15**, and **17** ($CDCl_3$, 500 MHz)

	12	**13**	**14**	**15**	**17**	**6**	**11**	**9**
H-2	7.63 (br d, 2.5)	7.64 (d, 2.5)	7.60 (br d, 2.5)	7.61 (br d, 2.5)		7.64 (d, 2.5)	7.60 (br d, 2.5)	7.64 (d, 2.0)
H-3	6.99 (br d, 2.5)	7.00 (d, 2.5)	7.01 (br dd, 1.0,2.5)	7.01 (br dd, 1.0,2.5)		7.01 (d, 2.5)	6.99 (dd, 1.0,2.5)	7.02 (d, 2.0)
4-OMe	4.17 (s)	4.18 (s)				4.19 (s)		4.20 (s)
H-5	8.11 (d, 10.0)	8.11 (d, 10.0)	8.23 (dd, 0.5,10.0)	8.23 (br d, 10.0)		8.12 (d, 10.0)	8.17 (d, 10.0)	8.12 (d, 10.0)
H-6	6.27 (d, 10.0)	6.28 (d, 10.0)	6.30 (d, 10.0)	6.30 (d, 10.0)		6.29 (d, 10.0)	6.26 (d, 10.0)	6.28 (d, 10.0)
H-9			7.14 (br s)	7.17 (br s)			7.15 (br s)	
H-1′	4.23 (dd, 8.0,10.0)	4.27 (dd, 8.0,10.0)	4.42 (dd, 7.5,10.0)	4.42 (dd, 8.0,10.0)		4.28 (dd, 8.0,10.0)	4.44 (dd, 8.0,10.0)	4.47 (dd, 7.5,10.5)
	4.63 (dd, 3.0,10.0)	4.60 (dd, 3.0,10.0)	4.59 (dd, 3.0,10.0)	4.59 (dd, 3.0,10.0)		4.61 (dd, 3.0,10.0)	4.55 (dd, 3.0,10.0)	4.58 (dd, 5.0,10.5)
H- 2′	3.99 (br d, 8.0)	3.99 (dd, 3.0,8.0)	3.96 (m)	3.96 (m)		3.84 (dd, 3.0,8.0)	3.90 (dd, 3.0,8.0)	4.72 (dd, 5.0,7.5)
3′-Me	1.33 (s)	1.33 (s)	1.30 (s)	1.30 (s)		1.29 (s)	1.31 (s)	1.67 (s)
	1.38 (s)	1.38 (s)	1.40 (s)	1.40 (s)		1.33 (s)	1.36 (s)	1.69 (s)
H-1″	5.22 (ddd, 1.0,1.0,10.0)	5.16 (ddd, 1.0,1.0,10.0)	5.22 (ddd, 1.0,1.5,10.0)	5.22 (br d, 10.0)	5.25 (ddd, 1.0,1.5,10.5)			
	5.44 (br d, 17.0)	5.39 (ddd, 1.0,1.5,17.0)	5.42 (ddd, 1.0,1.5,17.0)	5.42 (br d, 17.0)	5.46 (ddd, 1.0,1.5,17.0)			
H-2″	5.92 (ddd, 5.0,10.0,17.0)	5.81 (ddd, 5.0,10.0,17.0)	5.82 (ddd, 5.0,10.0,17.0)	5.82 (ddd, 5.0,10.0,17.0)	5.93 (ddd, 5.5,10.5,17.0)			
H-3″	4.91 (br s)	4.93 (br d, 5.0)	4.87 (br d, 5.0)	4.87 (br d, 5.0)	4.93 (br d, 5.5)			
H-8″	5.17 (br d, 8.0)	5.18 (br d, 8.0)	5.20 (br d, 9.0)	5.20 (br d, 8.0)	5.20 (br d, 8.0)			
H-9″	5.41 (br dd, 8.0,10.5)	5.50 (dddd, 1.0,1.5,8.0,10.5)	5.51 (dddd, 1.5,1.5,9.0,10.5)	5.51 (dddd, 1.5,1.5,8.0,10.5)	5.51 (dddd, 1.5,1.5,8.0,10.5)			
H-10″	5.46 (br dt, 10.5,7.5)	5.59 (ddt, 1.0,10.5,7.5)	5.61 (ddt, 1.0,10.5,7.5)	5.61 (ddt, 1.0,10.5, 7.5)	5.60 (ddt, 1.5,10.5,7.5)			
H-11″	2.07 (m)	2.10 (dq, 1.5,7.5)	2.11 (br q, 7.5)	2.11 (br q, 7.5)	2.11 (dq, 1.5,7.5)			
H-12″	1.36 (br t, 7.5)	1.36 (br t, 7.5)	1.36 (br t, 7.5)	1.38 (br t, 7.5)	1.38 (br t, 7.5)			
H-13″	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)			
H-14″	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)			
H-15″	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)			
H-16″	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)	1.28 (m)			
H-17″	0.88 (t, 7.0)	0.88 (t, 7.0)	0.87 (t, 7.0)	0.87 (t, 7.0)	0.88 (t, 7.0)			
C2′-OH	3.08 (br s)	N.O.[a]	2.72 (br s)	2.72 (br s)				
C3″-OH	2.23 (br s)		2.18 (br s)					
C8″-OH		N.O.[a]		2.18 (br s)				

a) Not observed.

Table 2. ^{13}C-NMR Data (δ) for Compounds **6**, **9**, **11**—**15**, and **17** ($CDCl_3$, 125 MHz)

	12	**13**	**14**	**15**	**17**	**6**	**11**	**9**
2	145.2	145.3	145.1	145.2		145.2	145.2	145.4
3	105.1	105.1	104.9	104.8		105.3	104.7	105.3
3a	114.8	114.7	114.1	114.4		114.6	114.3	114.6
4	144.6	144.7	148.7	148.7		144.9	148.6	145.3
4-OMe	60.8	60.8				60.8		60.7
4a	107.6	107.6	107.3	107.6		107.6	107.3	107.5
5	139.4	139.5	139.4	139.4		139.4	139.0	139.4
6	112.9	112.9	112.8	113.0		112.9	113.0	112.9
7	160.3	160.3	161.3	161.3		160.1	161.0	160.0
8a	144.0	144.0	152.6	152.6		144.0	152.6	143.9
9	127.2	127.2	94.5	94.7		126.9	94.8	126.1
9a	150.3	150.3	158.1	158.1		150.2	158.1	150.0
1′	75.7	75.6	74.2	74.2		76.1	74.5	70.4
2′	76.0	76.0	76.3	76.2		76.1	76.6	81.8
3′	78.5	78.7	78.5	78.7		71.5	71.7	83.9
3′-Me	22.1	22.1	22.2	21.9		25.1	26.6	21.2
	23.5	22.8	23.0	22.6		26.7	26.6	27.2
4′								153.6
1″	117.1	116.6	117.2	117.0	117.2			
2″	136.0	135.7	135.9	135.4	135.9			
3″	63.4	63.3	63.4	63.4	63.4			
4″	77.9	79.0	78.4	78.3	78.3			
5″	70.5	70.0	70.2	70.5	70.2			
6″	68.5	69.0	68.9	68.6	68.7			
7″	80.6	79.4	79.9	80.0	79.9			
8″	59.0	58.6	59.0	58.6	58.5			
9″	127.7	127.9	127.4	127.7	127.7			
10″	132.3	134.4	132.8	134.6	134.5			
11″	27.9	27.6	27.9	27.7	27.6			
12″	29.2	29.3	29.2	29.2	29.2			
13″	29.2	29.1	29.1	29.1	29.1			
14″	29.1	29.1	29.1	29.1	29.1			
15″	31.8	31.8	31.7	31.8	31.7			
16″	22.6	22.6	22.6	22.6	22.6			
17″	14.1	14.0	14.0	14.0	14.0			

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

Compounds	MK-1	HeLa	B16F10
1	0.3	54.7	19.5
2	82.7	53.0	61.3
3	3.2	35.5	36.3
4	>100	51.3	36.3
5	>100	>100	14.2
6	>100	>100	>100
7	61.0	17.6	32.0
8	41.7	9.4	>100
9	59.3	56.3	14.3
10	27.5	69.3	11.5
11	47.2	80.3	42.0
12	8.6	13.9	18.2
13	5.0	14.8	24.2
14	10.7	17.2	15.1
15	16.2	22.6	28.7
16	69.0	56.0	32.0
17	3.9	38.7	23.2
18	>100	>100	>100
19	2.5	3.1	1.8
20	2.2	29.3	17.0

Based on the spectral evidence described above, the structures of japoangelols A—D (**12**—**15**) are presented as shown in Fig. 2, though the stereochemistries of chiral centres are not determined.

The antiproliferative activities of the isolates against MK-1, HeLa and B16F10 cell lines were examined by MTT assay. The 50% growth inhibitions (ED_{50}) are summarized in Table 3. All furanocoumarins showed only weak antiproliferative activity, though some compounds did have specificity. Compounds **5**, **9**, and **10** showed higher activity to B16F10 cells than to MK-1 and HeLa cells, and **7** and **8** were more active to HeLa cells than to MK-1 and B16F10 cells. All polyacetylenes, **1**, **3**, **17**, and **20**, showed much greater antiproliferative activity against MK-1 cells than against HeLa and B16F10 cells. Among them, **1** showed the most potent antiproliferative activity against MK-1 cells growth. All falcarindiol furanocoumarin ethers, **12**—**15**, showed antiproliferative activity equally against the three tumor cell lines, though less active to MK-1 cells and more active to HeLa and B16F10 cells than falcarindiol. Compound **19** showed significant antiproliferative activity equally against all three cells. It is notable that compound **19**, a methyl ester of a common phenylpropanoid, showed rather high antiproliferative activity.

Experimental[15]

Plant Material The plant material was collected in October, 1995 at Genkai Machi, Fukuoka Prefecture.

Measurement of the 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 T. Mosmann.[5] The detailed procedure was described in the preceding paper.[2]

Extraction and Preliminary Fractionation The air-dried and powdered root (11 kg) was extracted first with CHCl$_3$ (72 l) and then with MeOH (72 l). The CHCl$_3$ solution was concentrated *in vacuo* to give fr. I (213.9 g). The MeOH solution was concentrated *in vacuo*. The residue was suspended in water and passed through a column of Diaion HP-20. The H$_2$O eluate was concentrated *in vacuo* to give fr. II (242.1 g). The column was then washed with MeOH and the eluate was concentrated *in vacuo* to give fr. III (77.5 g). The 50% growth inhibitions (ED$_{50}$, μg/ml) of frs. I, II, and III against MK-1 cells were 3.13, >100, and 12.5, respectively.

Fractionation of Fr. I, and Isolation of Constituents Fraction I (105.6 g) was chromatographed on silica gel using CHCl$_3$–MeOH–H$_2$O (1 : 0 : 0→7 : 3 : 0.5) as the eluting solvents and separated into 7 fractions [I-1 (13.1 g), I-2 (62.1 g), I-3 (10.2 g), I-4 (11.8 g), I-5 (3.3 g), I-6 (2.2 g), and I-7 (1.6 g)]. Fractions I-2, I-3, and I-4 showed significant growth inhibitory activity. Fraction I-2 (62.12 g) was further chromatographed on silica gel using hexane–isopropanol (50 : 1→5 : 1) to give 7 fractions [I-2-1 (3.4 g), I-2-2 (5.9 g), I-2-3 (0.9 g), I-2-4 (0.5 g), I-2-5 (21.1 g), I-2-6 (13.8 g), and I-2-7 (4.7 g)]. Fraction I-2-2 was chromatographed on YMC gel ODS-A using MeOH–H$_2$O (3 : 2→1 : 0) to give **1** (57 mg), **2** (654 mg), and **3** (100 mg). From frs. I-2-3 and I-2-4, stigmasterol (720 mg) and **4** (326 mg) were isolated, respectively. Fraction I-2-6 was chromatographed on YMC gel ODS-A using MeOH–H$_2$O (1 : 5→1 : 0) to give 7 fractions [I-2-6-1 (0.4 g), I-2-6-2 (2.3 g), I-2-6-3 (1.3 g), I-2-6-4 (1.7 g), I-2-6-5 (3.0 g), I-2-6-6 (0.7 g), and I-2-6-7 (3.6 g)]. From the first 2 fractions, **5** (30 mg), **6** (93 mg), **7** (187 mg), and **8** (54 mg) were isolated, respectively. Fraction. I-2-6-4 was chromatographed on silica gel using benzene–acetone (1 : 0→0 : 1) to give **9** (9 mg), **10** (15 mg), and **11** (23 mg). Fraction I-2-6-6 was chromatographed on silica gel using hexane–AcOEt (5 : 1→0 : 1) and then subjected to preparative HPLC on an ODS column using 60% MeOH to give **12** (28 mg), **13** (38 mg), **14** (43 mg), and **15** (49 mg). Fraction I-3 (10.2 g) was chromatographed on silica gel using hexane–isopropanol (30 : 1→3 : 1) to give 2 fractions. The first fraction was subjected to preparative HPLC on an ODS column using 80% MeOH to give **16** (116 mg). The second fraction was chromatographed on YMC gel ODS-A using MeOH–H$_2$O (1 : 1→1 : 0) and then subjected to preparative HPLC on an ODS column using 80% MeOH to give **17** (136 mg). Fraction I-4 (11.9 g) was chromatographed on silica gel using hexane–isopropanol (40 : 1→5 : 1) to give 4 fractions [I-4-1 (1.3 g), I-4-2 (4.4 g), I-4-3 (0.9 g), and I-4-4 (4.0 g)]. Fraction I-4-2 was chromatographed on YMC gel ODS-A using MeOH–H$_2$O (2 : 5→1 : 0) to give **17** (210 mg) and **18** (30 mg). Fraction I-4-3 was subjected to preparative HPLC on an ODS column using 60% MeOH to give **19** (212 mg). Fraction I-5 (3.3 g) was chromatographed on silica gel using hexane–isopropanol (30 : 1→0 : 1) and YMC gel ODS-A using MeOH–H$_2$O (1 : 1→1 : 0) to give **20** (25 mg).

Compound **1** (Panaxynol): A colorless oil. $[\alpha]_D^{29}$ −9.8° (c=0.48, Et$_2$O). [lit. $[\alpha]_D^{20}$ −22.5° (c=1.0, Et$_2$O)]. Positive-ion HR FAB-MS m/z: 267.1725 ([M+Na]$^+$) (Calcd for C$_{17}$H$_{24}$ONa: 267.1733). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 0.88 (t-like, J=7.0 Hz, 3H, H-17), 1.28 (m, 8H, H-13-16), 1.36 (t-like, J=7.0 Hz, 2H, H-12), 2.02 (br q, J=7.0 Hz, 2H, H-11), 3.03 (br d, J=7.0 Hz, 2H, H-8), 4.90 (br d, J=5.0 Hz, 1H, H-3), 5.23 (ddd, J=1.0, 1.0, 10.0 Hz, 1H, H-1), 5.37 (dtt, J=10.5, 2.0, 7.0 Hz, 1H, H-9), 5.45 (ddd, J=1.0, 1.0, 17.0 Hz, 1H, H-1), 5.51 (dtt, J=10.5, 2.0, 7.0 Hz, 1H, H-10), 5.94 (ddd, J=5.0, 10.0, 17.0 Hz, 1H, H-2). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 14.0 (C17), 17.7 (C8), 22.6 (C16), 27.2 (C11), 29.1 (C14), 29.2 (C12), 29.2 (C13), 31.8 (C15), 63.5 (C3), 64.1 (C6), 71.3 (C5), 74.3 (C4), 80.3 (C7), 117.0 (C1), 121.9 (C9), 133.1 (C10), 136.2 (C2).

Compound **2** (Osthol): Colorless needles (MeOH–H$_2$O). mp 79—81 °C. (lit. mp 78—80 °C). Positive-ion HR FAB-MS m/z: 267.0999 ([M+Na]$^+$) (Calcd for C$_{15}$H$_{16}$O$_3$Na: 267.0998). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 1.67, 1.84 (each br s, 3H, C3′-CH$_3$), 3.53 (d, J=7.5 Hz, 2H, H-1′), 3.91 (br s, 3H, C7-OCH$_3$), 5.22 (m, 1H, H-2′), 6.21 (d, J=9.5 Hz, 1H, H-3), 6.82 (d, J=9.0 Hz, 1H, H-6), 7.27 (d, J=9.0 Hz, 1H, H-5), 7.59 (d, J=9.5 Hz, 1H, H-4). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 17.9 (C3′-CH$_3$), 21.9 (C1′), 25.7 (C3′-CH$_3$), 56.0 (C7-OCH$_3$), 107.4 (C6), 113.0 (C3), 113.0 (C4a), 121.1 (C2′), 126.2 (C5), 132.6 (C3′), 143.7 (C4), 152.9 (C8a), 160.2 (C7), 161.3 (C2).

Compound **3** (8-*O*-Acetylfalcarindiol): A colorless oil. $[\alpha]_D^{29}$ +133.3° (c=0.39, CHCl$_3$). Positive-ion HR FAB-MS m/z: 325.1780 ([M+Na]$^+$) (Calcd for C$_{19}$H$_{26}$O$_3$Na: 325.1783). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 0.87 (t, J=7.0 Hz, 3H, H-17), 1.28 (m, 8H, H-13—16), 1.38 (br t, J=7.5 Hz, 2H, H-12), 2.07 (s, 3H, OAc), 2.13 (br q, J=7.5 Hz, 2H, H-11), 4.93 (br d, J=5.5 Hz, 1H, H-3), 5.25 (br d, J=10.0 Hz, 1H, H-1), 5.46 (br d, J=17.0 Hz, 1H, H-1), 5.48 (br dd, J=9.0, 10.5 Hz, 1H, H-9), 5.66 (br dt, J=10.5, 7.5 Hz, 1H, H-10), 5.93 (ddd, J=5.5, 10.0, 17.0 Hz, 1H, H-2), 6.14 (d, J=9.0 Hz, 1H, H-8). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 14.0 (C17), 20.8 (OAc), 22.6 (C16), 27.7 (C11), 29.1 (C12), 29.1 (C13), 29.1 (C14), 31.8 (C15), 60.1 (C8), 63.4 (C3), 69.2 (C6), 70.2 (C5), 76.5 (C7), 78.6 (C4), 117.3 (C1), 123.8 (C9), 135.8 (C2), 136.4 (C10), 169.4 (OAc).

Compound **4** (Isoimperatorin): Light yellow prisms (MeOH–H$_2$O). mp 109—110 °C. (lit. mp 108—110 °C). Positive-ion HR FAB-MS m/z: 293.0790 ([M+Na]$^+$) (Calcd for C$_{16}$H$_{14}$O$_4$Na: 293.0790). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 1.72, 1.80 (each br s, 3H, C3′-CH$_3$), 4.92 (m, 2H, H-1′), 5.54 (m, 1H, H-2′), 6.26 (d, J=10.0 Hz, 1H, H-6), 6.96 (d, J=2.5 Hz, 1H, H-3), 7.15 (br s, 1H, H-9), 7.60 (d, J=2.5 Hz, 1H, H-2), 8.16 (d, J=10.0 Hz, 1H, H-5). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 18.2 (C3′-CH$_3$), 25.8 (C3′-CH$_3$), 69.7 (C1′), 94.1 (C9), 105.0 (C3), 107.4 (C4a), 112.5 (C6), 114.1 (C3a), 119.0 (C2′), 139.6 (C5), 139.8 (C3′), 144.9 (C1), 148.9 (C4), 152.6 (C8a), 158.1 (C9a), 161.3 (C7).

Compound **5** (Scopoletin): Light yellow needles (MeOH–H$_2$O). mp 203—205 °C. (lit. mp 202—204 °C). Positive-ion HR FAB-MS m/z: 193.0494 ([M+H]$^+$) (Calcd for C$_{10}$H$_9$O$_4$: 193.0501). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 3.78 (s, 3H, C6-OCH$_3$), 6.30 (d, J=9.5 Hz, 1H, H-3), 7.05 (s, 1H, H-5), 7.11 (s, 1H, H-8), 7.69 (d, J=9.5 Hz, 1H, H-4′). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 56.2 (C6-OCH$_3$), 104.1 (C8), 109.6 (C5), 111.1 (C8a), 112.4 (C3), 116.5 (C4a), 144.0 (C4), 146.2 (C6), 153.0 (C7), 161.4 (C2).

Compound **6** (Byakangelicin): Yellow needles (hexane–acetone). mp 127—128 °C. (lit. mp 124.5—125.5 °C). $[\alpha]_D^{29}$ +19.0° (c=0.40, C$_5$H$_5$N). [lit. $[\alpha]_D^{23}$ +10.0° (c=0.05, C$_5$H$_5$N)]. Positive-ion HR FAB-MS m/z: 334.1055 ([M]$^+$) (Calcd for C$_{17}$H$_{18}$O$_7$: 334.1052). ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Compound **7** (Xanthotoxin): Colorless needles (hexane–isopropanol). mp 146—147 °C. (lit. mp 144—145 °C). Positive-ion HR FAB-MS m/z: 217.0500 ([M+H]$^+$) (Calcd for C$_{12}$H$_9$O$_4$: 217.0500). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 4.29 (s, 3H, C9-OCH$_3$), 6.35 (d, J=9.5 Hz, 1H, H-6), 6.82 (d, J=2.0 Hz, 1H, H-3), 7.34 (s, 1H, H-4), 7.69 (d, J=2.0 Hz, 1H, H-2), 8.10 (d, J=10.0 Hz, 1H, H-5). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 61.2 (C9-OCH$_3$), 107.7 (C3), 112.9 (C4), 114.7 (C6), 116.5 (C4a), 126.1 (C3a), 132.8 (C9), 143.0 (C8a), 144.3 (C5), 146.6 (C2), 147.7 (C9a), 160.4 (C7).

Compound **8** (Bergapten): Colorless needles (hexane–acetone). mp 188—189 °C. (lit. mp 190—191 °C). Positive-ion HR FAB-MS m/z: 217.0493 ([M+H]$^+$) (Calcd for C$_{12}$H$_9$O$_4$: 217.0501). ^{1}H-NMR (CDCl$_3$, 500 MHz) δ: 4.26 (s, 3H, C4-OCH$_3$), 6.26 (d, J=10.0 Hz, 1H, H-6), 7.01 (dd, J=1.0, 2.5 Hz, 1H, H-3), 7.12 (dd, J=0.5, 1.0 Hz, 1H, H-9), 7.59 (d, J=2.5 Hz, 1H, H-2), 8.14 (dd, J=0.5, 10.0 Hz, 1H, H-5). ^{13}C-NMR (CDCl$_3$, 125 MHz) δ: 21.9 (C9-CH$_3$), 60.1 (C4-OCH$_3$), 105.0 (C3), 106.4 (C4a), 112.5 (C6), 112.7 (C3a), 139.2 (C5), 144.8 (C2), 149.6 (C4), 152.7 (C8a), 158.4 (C9a), 161.1 (C7).

Compound **9** (Japoangelone): Light yellow needles (MeOH–H$_2$O). mp 162—164 °C. $[\alpha]_D^{31}$ +9.3° (c=0.12, CHCl$_3$). Positive-ion HR FAB-MS m/z: 361.0916 ([M+H]$^+$) (Calcd for C$_{18}$H$_{17}$O$_8$: 361.0924). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **10** (Oxypeucedanin Methanolate): Colorless needles (MeOH–H$_2$O). mp 126—127 °C. (lit. mp 123—125 °C). $[\alpha]_D^{31}$ +19.8° (c=0.25, CHCl$_3$). [lit. $[\alpha]_D^{20}$ +12.0° (CHCl$_3$)]. Positive-ion HR FAB-MS *m/z*: 319.1179 ([M+H]$^+$) (Calcd for $C_{17}H_{19}O_6$: 319.1182). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.24, 1.27 (each s, 3H, C3′-CH_3), 3.27 (s, 3H, C3′-OCH_3), 3.94 (dd, *J*=3.0, 7.5 Hz, 1H, H-2′), 4.38 (dd, *J*=7.5, 10.0 Hz, 1H, H-1′), 4.57 (dd, *J*=3.0, 10.0 Hz, 1H, H-1′), 6.28 (d, *J*=10.0 Hz, 1H, H-6), 7.00 (dd, *J*=1.0, 2.0 Hz, 1H, H-3), 7.16 (br t, *J*=1.0 Hz, 1H, H-9), 7.59 (d, *J*=2.0 Hz, 1H, H-2), 8.22 (dd, *J*=1.0, 10.0 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 20.7 (C3′-CH_3), 20.8 (C3′-CH_3), 49.3 (C3′-OCH_3), 74.4 (C1′), 76.0 (C3′), 76.2 (C2′), 94.6 (C9), 104.9 (C3), 107.4 (C4a), 112.9 (C6), 114.1 (C3a), 139.3 (C5), 145.0 (C2), 148.9 (C4), 152.7 (C8a), 158.2 (C9a), 161.1 (C7).

Compound **11** (Oxypeucedanin Hydrate): Light yellow needles (MeOH–H_2O). mp 132—134 °C. (lit. mp 130—131.5 °C). $[\alpha]_{\mathrm{D}}^{30}$ +15.4° (*c*=0.35, EtOH). [lit. $[\alpha]_{\mathrm{D}}^{21.5}$ +15.2° (*c*=1, $CHCl_3$)]. Positive-ion HR FAB-MS *m/z*: 305.1032 (M+H]$^+$) (Calcd for $C_{16}H_{17}O_6$: 305.1025). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **12** (Japoangelol A): A colorless oil. $[\alpha]_{\mathrm{D}}^{29}$ +111.8° (*c*=0.50, $CHCl_3$). Positive-ion HR FAB-MS *m/z*: 599.2646 ([M+Na]$^+$) (Calcd for $C_{34}H_{40}O_8$Na: 599.2621). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **13** (Japoangelol B): A colorless oil. $[\alpha]_{\mathrm{D}}^{24}$ +138.4° (*c*=0.17, $CHCl_3$). Positive-ion HR FAB-MS m/z: 599.2635 ([M+Na]$^+$) (Calcd for $C_{34}H_{40}O_8$Na: 599.2621). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **14** (Japoangelol C): A colorless oil. $[\alpha]_{\mathrm{D}}^{29}$ +117.5° (*c*=0.19, $CHCl_3$). Positive-ion HR FAB-MS *m/z*: 546.2633 ([M]$^+$) (Calcd for $C_{33}H_{38}O_7$: 546.2617). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **15** (Japoangelol D): A colorless oil. $[\alpha]_{\mathrm{D}}^{24}$ +219.4° (*c*=0.16, $CHCl_3$). Positive-ion HR FAB-MS *m/z*: 569.2502 ([M+Na]$^+$) (Calcd for $C_{33}H_{38}O_7$Na: 569.2515). ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Compound **16** (3′-*O*-Acetylhamudol): Colorless needles (MeOH–H_2O). mp 126—127 °C. (lit. mp 129.5—130 °C). $[\alpha]_{\mathrm{D}}^{29}$ −33.5° (*c*=0.34, $CHCl_3$). [lit. $[\alpha]_{\mathrm{D}}^{25}$ −28.4° (*c*=0.88, $CHCl_3$)]. Positive-ion HR FAB-MS *m/z*: 341.1001 ([M+Na]$^+$) (Calcd for $C_{17}H_{18}O_6$Na: 341.1001). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.34, 1.36 (each s, 3H, C2′-CH_3), 2.07 (s, 3H, C3′-$OCOCH_3$), 2.33 (s, 3H, C2-CH_3), 2.77 (dd, *J*=5.0, 17.5 Hz, 1H, H-4′), 2.99 (dd, *J*=5.0, 17.5 Hz, 1H, H-4′), 5.11 (t, *J*=5.0 Hz, 1H, H-3′), 5.99 (s, 1H, H-3), 6.33 (s, 1H, H-8). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 20.5 (C2-CH_3), 21.0 (C3′-$OCO\underline{C}H_3$), 22.6 (C4′), 23.0 (C2′-CH_3), 24.7 (C2′-CH_3), 69.8 (C3′), 76.7 (C2′), 94.8 (C8), 102.4 (C6), 104.4 (C4a), 108.4 (C3), 156.2 (C8a), 158.7 (C7), 159.5 (C5), 166.7 (C2), 170.2 (C3′-$O\underline{C}OCH_3$), 182.5 (C4).

Compound **17** (Falcarindiol): A colorless oil. $[\alpha]_{\mathrm{D}}^{24}$ +147.6° (*c*=0.16, $CHCl_3$). [lit. $[\alpha]_{\mathrm{D}}^{24}$ +219.4° (*c*= 4.6, $CHCl_3$)]. Positive-ion HR FAB-MS *m/z*: 283.1674 ([M+Na]$^+$) (Calcd for $C_{17}H_{24}O_2$Na: 283.1674). ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Compound **18** (Hamaudol): Light yellow needles (MeOH–H_2O). mp 201—203 °C. (lit. mp 202—202.5 °C). $[\alpha]_{\mathrm{D}}^{30}$ −6.5° (*c*=0.35, $CHCl_3$). [lit. $[\alpha]_{\mathrm{D}}^{25}$ −22.0° (*c*=0.46, $CHCl_3$)]. Positive-ion HR FAB-MS *m/z*: 277.1078 ([M+H]$^+$) (Calcd for $C_{15}H_{17}O_5$: 277.1076). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.35, 1.39 (each s, 3H, C2′-CH_3), 2.32 (s, 3H, C2-CH_3), 2.73 (dd, *J*=5.5, 17.0 Hz, 1H, H-4′), 2.95 (dd, *J*=5.5, 17.0 Hz, 1H, H-4′), 3.87 (t, *J*=5.5 Hz, 1H, H-3′), 5.98 (br s, 1H, H-3), 6.31 (s, 1H, H-8). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 20.5 (C2-CH_3), 22.0 (C2′-CH_3), 24.9 (C2′-CH_3), 25.4 (C4′), 68.8 (C3′), 78.4 (C2′), 94.8 (C8), 102.9 (C6), 104.4 (C4a), 108.3 (C3), 156.2 (C8a), 159.0 (C7), 159.7 (C5), 166.7 (C2), 182.5 (C4).

Compound **19** (Caffeic Acid Methyl Ester): A brown powder. Positive-ion HR FAB-MS *m/z*: 194.0588 ([M]$^+$) (Calcd for $C_{10}H_{10}O_4$: 194.0579). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 3.75 (s, 3H, $COOCH_3$), 6.25 (d, *J*=16.0 Hz, 1H, H-7), 6.78 (d, *J*=8.0 Hz, 1H, H-5), 6.94 (dd, *J*=2.0, 8.0 Hz, 1H, H-6), 7.04 (d, *J*=2.0 Hz, 1H, H-2), 7.54 (d, *J*=16.0 Hz, 1H, H-8). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 52.0 ($COO\underline{C}H_3$), 114.9 (C7), 115.2 (C2), 116.5 (C5), 122.9 (C6), 127.4 (C1), 146.8 (C3), 146.9 (C8), 149.5 (C4), 169.8 ($\underline{C}OOCH_3$).

Compound **20** ((9*Z*)-1,9-Heptadecadiene-4,6-diyne-3,8,11-triol): A colorless oil. $[\alpha]_{\mathrm{D}}^{24}$ +64.7° (*c*=0.08, $CHCl_3$). [lit. $[\alpha]_{\mathrm{D}}^{24}$ +83.7° (*c*=0.09, $CHCl_3$)]. Positive-ion FAB-MS *m/z*: 299 ([M+Na]$^+$). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.89 (t, *J*=7.0 Hz, 3H, H-17), 1.28 (m, 8H, H-13—16), 1.49, 1.62 (m, 2H, H-12), 4.43 (br q, *J*=8.0 Hz, 1H, H-11), 4.93 (d, *J*=5.0 Hz, 1H, H-3), 5.25 (br d, *J*=10.0 Hz, 1H, H-1), 5.28 (d, *J*=8.0 Hz, 1H, H-8), 5.46 (br d, *J*=17.0 Hz, 1H, H-1), 5.59 (dd, *J*=8.0, 11.0 Hz, 1H, H-10), 5.64 (dd, *J*=8.0, 11.0 Hz, 1H, H-9), 5.93 (ddd, *J*=5.0, 10.0, 17.0 Hz, 1H, H-2). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 14.1 (C17), 22.6 (C16), 25.2 (C15), 29.2 (C14), 31.8 (C13), 37.1 (C12), 58.6 (C11), 63.3 (C3), 67.9 (C8), 69.1 (C6), 70.1 (C5), 78.7 (C7), 79.4 (C4), 117.3 (C1), 129.6 (C9), 135.8 (C10), 136.0 (C2).

References and Notes

1) Part IV: Ikeda R., Nagao T., Okabe H., Nakano Y., Matsunaga H., Katano M., Mori M., *Chem. Pharm. Bull.*, **46**, 875—878 (1998).
2) Part II: Nakano Y., Matsunaga H., Mori M., Katano M., Okabe H., *Biol. Pharm. Bull.*, **21**, 257—261 (1998).
3) Hata K., Nitta A., *Yakugaku Zasshi*, **80**, 742—746 (1960); *idem*, *ibid.*, **85**, 173—175 (1965).
4) Baba K., Hata K., Kimura Y., Matsuyama Y., Kozawa M., *Chem. Pharm. Bull.*, **29**, 2565—2570 (1981).
5) Mosmann T., *J. Immunol. Methods*, **65**, 55—63 (1983).
6) Hirakura K., Morita M., Nakajima K., Ikeya Y., Mitsuhashi H., *Phytochemistry*, **31**, 899—903 (1992).
7) Bohlmann F., Zdero C., *Chem. Ber.*, **108**, 511—514 (1975).
8) Matsuura H., Saxena G., Farmer S. W., Hancock R. E. W., Towers G. H. N., *Planta Medica*, **62**, 256—259 (1996).
9) Woo W. S., Lee C. K., Shin K. H., *Planta Medica*, **45**, 234—236 (1982).
10) Thastrup O., Lemmich J., *Phytochemistry*, **22**, 2035—2037 (1983).
11) Elgamal M. H. A., Elewa N. H., Elkhrisy E. A. M., Duddeck H., *Phytochemistry*, **18**, 139—143 (1979).
12) Atkinson E., Boyd D. R., Grundon M. F., *Phytochemistry*, **13**, 853—855 (1983); Mendez J., Castro-Poceiro J., *ibid.*, **22**, 2599—2601 (1983); Alkinson E., Boyd D. R., Grundon M. F., *ibid.*, **13**, 853—855 (1974).
13) Nielsen E. B., Lemmich J., *Acta Chem. Scand.*, **23**, 962—966 (1969).
14) Sasaki H., Taguchi H., Endo T., Yoshioka I., *Chem. Pharm. Bull.*, **30**, 3555—3562 (1982).
15) The instruments and materials used in this work were the same as those in Part III.[16] ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR spectra were measured in $CDCl_3$. Chemical shifts are expressed on the δ scale with tetramethylsilane as an internal standard. The signal assignment was based on comparison with data reported for compounds having similar structures, and confirmed with the aid of NMR spectral techniques (^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMQC, phase-sensitive NOESY, long-range ^{1}H–^{13}C COSY, and HMBC spectra).
16) Part III: Ikeda R., Nagao T., Okabe H., Nakano Y., Matsunaga H., Katano M., Mori M., *Chem. Pharm. Bull.*, **46**, 871—874 (1998).