New Haliangicin Isomers, Potent Antifungal Metabolites Produced by

a Marine Myxobacterium[†]

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Haliangicin is a β-methoxyacrylate-type polyene antibiotic isolated from the unique marine myxobacterium *Haliangium ochraceum*. A further investigation of the extract of this microorganism has resulted in the isolation of haliangicin as well as its geometrical isomers. The configuration of the epoxide in haliangicin, which was unknown previously, was determined in this study. The planar structures with partial stereochemistry of these isomers were elucidated by spectroscopic analyses. The major isomer, *cis*-haliangicin, is a *cis* isomer of the epoxide portion of haliangicin. Haliangicins B~D are geometrical isomers of the polyene moiety, and present as an inseparable mixture of *cis* and *trans* epoxide isomers. Their antifungal activities were also evaluated in comparison with haliangicin.

Myxobacteria, common but unusual Gram-negative bacteria characterized by gliding behavior and fruiting body formation to form multicellular bodies, have recently been recognized as producers of novel antibiotics, and to date about 80 novel basic structures and more than 400 variants of these compounds have been isolated from myxobacteria.[1~3] Myxobacteria have lately drawn greater interest due to the discovery of the epothilones,[4,5] which show potent antitumor activity and mimic the biological effects of taxol.[6] The myxobacteria had been regarded as terrestrial microorganisms until recently, when some myxobacterial strains were successfully isolated from marine environments.[7] From a marine myxobacterial species of the genus *Haliangium*, a novel polyene antifungal antibiotic containing a β-methoxyacrylate moiety, haliangicin (1), was isolated, and its bioactivities and planar structure were reported.[8,9] In our search for additional antifungal agents from the marine isolate, we have found several new isomers of 1. *cis*-Haliangicin (2)

is a *cis* isomer about the epoxide moiety of 1, and haliangicins B~D (3~5) are geometrical isomers of the polyene moiety, as well as appear to be mixtures of *cis-trans* isomers of the epoxide moiety (Fig. 1). This paper reports the isolation and structural elucidation of these haliangicin isomers as well as their antifungal activity.

Isolation

The marine myxobacterium *Haliangium ochraceum* AJ 13395 was used as a haliangicin-producer throughout this study. Although it had been tentatively named *H. luteum* in the previous papers,[8,9] the new species name, *H. ochraceum*, was recently proposed for the strain with its detailed taxonomic description.[10] The marine myxobacterium was cultured at 27°C for 17 days in a culture medium containing 2% NaCl. The culture broths (total 9 liters) were centrifuged to collect the bacterial cells and an adsorbent resin (productivity enhancer), which were

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Fig. 1.   Structures of haliangicin (1) and its isomers 2~5.
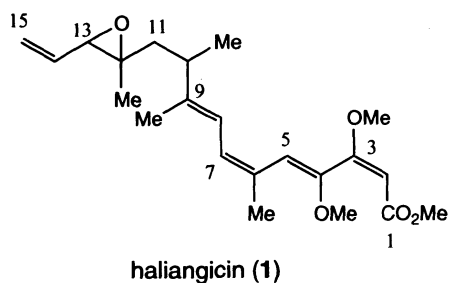
haliangicin (1)

Compound	Geometry			
	C2	C4	C6	Epoxide[a]
haliangicin (1)	E	Z	Z	trans
cis-halinagicin (2)	E	Z	Z	cis
haliangicin B (3) (+ cis isomer)	Z[b]	Z	Z	trans (+ cis)
haliangicin C (4) (+ cis isomer)	E	E[b]	Z	trans (+ cis)
haliangicin D (5) (+ cis isomer)	E	E[b]	E[b]	trans (+ cis)

[a] cis Isomers were minor components in all cases in the ratio of 2:1.
[b] Double bond geometries different from those of haliangicin (1).

Haliangicins B~D (3~5) are a mixture of *trans* and *cis* isomers about the epoxide moiety in a ratio of 2 : 1.

extracted with acetone. The acetone extract was chromatographed on silica gel. The haliangicin-containing fraction was applied to a second silica gel chromatography followed by preparative HPLC to obtain haliangicin (1), haliangicin B (3), haliangicin C (4), and haliangicin D (5) as mixtures of *trans*- and *cis*-epoxy isomers in the ratio of 2:1 (Fig. 2). Since the samples were quite susceptible to decomposition through oxidation in air and light at room temperature, they were handled in dim light and stored in benzene at −20°C. The fraction containing haliangicin (1) was further subjected to recycle HPLC to obtain pure haliangicin (1) and *cis*-haliangicin (2). Although haliangicins B (3), C (4) and D (5) contained their *cis*-epoxy isomers as the minor component (2 : 1 ratio), we did not succeed in the purification of these isomers.

The antifungal activity of the haliangicins against the phytopathogenic fungus *Phytophthora capsici* was evaluated by using a paper disc assay method. The minimum doses applied on a paper disc to inhibit the fungus growth in this assay were as follows: **1** (8 ng/disc), **2** (8 ng/disc), **3** (40 ng/disc), **4** (40 ng/disc), and **5** (200 ng/disc). A β-methoxyacrylate-type compound, cystothiazole A,[11] was used as a positive control (200 ng/disc).

Structural Elucidation

All the spectroscopic data for haliangicin (1) agreed well

Fig. 2.   A Preparative HPLC trace showing the separation of haliangicin (1) and its isomers (2~5).
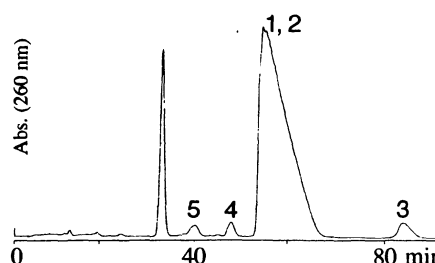
Each peak is a mixture of *trans* and *cis* isomers in a ratio of 2 : 1.

with the previous data reported by FUDOU *et al.*,[9] although the specific rotation was not in good agreement. The reported rotation was found to have been determined for a mixture of **1** and its *cis*-isomer **2** by comparison of NMR data. NOESY examination of **1** showed correlations of H11/H13 and H14/12-Me, suggesting a *trans* stereochemistry of the epoxy moiety (Fig. 3 and Table 4). The relative stereochemistry of the epoxide in **1**, though not stated clearly in the previous report,[9] has been determined for the first time in the present study.

Table 1.   NMR spectral data for *cis*-haliangicin (**2**) in CDCl$_3$.

Position	δ_H (mult, J in Hz)[a]	δ_C (mult)[b]	HMBC (to C)
1	-	167.0 (s)	-
2	5.26 (s)	95.8 (d)	3, 4
3	-	165.9 (s)	-
4	-	148.0 (s)	-
5	5.80 (s)	112.4 (d)	3, 4, 7, 6-Me
6	-	130.6 (s)	-
7	6.05 (d, 11.4)	126.3 (d)	5, 9, 6-Me
8	6.13 (d, 11.4)	120.7 (d)	6, 9, 10, 9-Me
9	-	141.8 (s)	-
10	2.39 (m)	40.6 (d)	8, 9, 11, 12, 9-Me, 10-Me
11a	1.46 (dd, 8.4, 14.0)	37.5 (t)	9, 10, 12, 10-Me, 12-Me
11b	1.80 (dd, 6.7, 14.0)		9, 10, 12, 13, 10-Me, 12-Me
12	-	62.3 (s)	-
13	3.18 (d, 7.1)	64.5 (d)	12, 14, 15, 12-Me
14	5.77 (ddd, 7.1, 10.5, 17.3)	133.9 (d)	13
15a	5.33 (d, 10.5)	119.6 (t)	13
15b	5.43 (d, 17.3)		13, 14
1-OMe	3.68 (s)	51.2 (q)	1
3-OMe	3.73 (s)	56.0 (q)	3
4-OMe	3.55 (s)	57.2 (q)	4
6-Me	2.09 (s)	23.6 (q)	5, 6, 7
9-Me	1.70 (s)	13.3 (q)	8, 9, 10
10-Me	0.97 (d, 6.9)	19.4 (q)	9, 10, 11
12-Me	1.33 (s)	22.5 (q)	11, 12, 13

[a] Recorded at 600 MHz.

[b] Recorded at 150 MHz. Multiplicity inferred using the DEPT pulse sequence.

cis-Haliangicin (**2**) and the other haliangicins (**3**~**5** and their *cis*-isomers) were thought to be isomers of **1** because they possess the same molecular formula, C$_{22}$H$_{32}$O$_5$, determined by positive ion ESI TOF-MS in high-resolution mode and by their similar NMR data to **1** (Tables 1~3). The resonances at δ 167.0, 165.8, 166.1, and 166.1 in the ^{13}C NMR spectra of **2**~**5**, respectively, indicated the presence of one carbonyl carbon in each compound, most likely an α,β-unsaturated ester judging from the IR absorption bands at 1717, 1720, 1726, and 1727 cm^{-1} for **2**~**5**, respectively. Analysis of 2D NMR data (DQF-COSY, HMQC, and HMBC) proved that the compounds **2**~**5** (and the *cis*-isomers of **3**~**5**) had the same gross structure as that of **1**. NOESY experiments finally enabled us to distinguish

each isomer, as discussed individually below (Fig. 3 and Table 4).

The NMR data for *cis*-haliangicin (**2**) are summarized in Table 1. Significant differences in the NMR chemical shifts between **1** and **2** are observed at the positions 11 and 12-Me, δ_H 1.46, 1.80 (H11), 1.33 (12-Me), δ_C 37.5 (C11), and 22.5 (12-Me) for **2**, and δ_H 1.28, 1.93 (H11), 1.22 (12-Me), δ_C 43.9 (C11), and 16.7 (12-Me) for **1**, suggesting that **2** is a stereoisomer of the epoxide in **1**. The NOE correlations of **2** (Table 4) revealed that the configuration of the tetraene moiety was the same as that of **1**. However, different NOE correlations were observed at epoxy portion, that is, the correlations of H13/12-Me and H11/H14 (Fig. 3), support a *cis* configuration of the epoxide in **2**. Thus, compound **2** is

Table 2. ^{1}H NMR data for haliangicins B (3)~D (5) and their cis isomers in CDCl$_3$.

| Position | δ_H (mult, J in Hz)^a | | |
	3 [cis-3]^b	4 [cis-4]^b	5 [cis-5]^b
2	5.56 [5.57] (s)	5.18 (s)	5.22 (s)
5	6.36 [6.37] (s)	5.80 [5.83] (s)	5.57 (s)
7	6.16 [6.17] (s)^c	5.98 (d, 10.9)	6.19 (d, 11.4)
8	6.16 [6.17] (s)^c	6.16 [6.17](d, 11.0)	6.08 [6.09] (d, 11.4)
10	2.43 [2.45] (m)	2.36 [2.38] (m)	2.37 [2.39] (m)
11a	1.37 [1.49] (dd, 8.7, 13.7)	1.32 [1.47] (dd, 9.2, 14.0)	1.32 [1.46] (dd, 9.1, 14.0)
11b	1.93 [1.78] (dd, 6.5, 13.7)	1.94 [1.79] (dd, 6.8, 14.00)	1.97 [1.80] (dd, 6.8, 14.0)
13	3.20 [3.18] (d, 7.1)	3.20 [3.18] (d, 7.1)	3.21 [3.18] (d, 7.1)
14	5.72 [5.77] (ddd, 7.1, 10.3, 17.3)	5.73 [5.77] (ddd, 7.1, 10.2, 17.3)	5.72 [5.77] (ddd, 7.1, 10.2, 17.4)
15a	5.33 [5.34] (d, 10.3)	5.33 (d, 10.2)	5.33 (d, 10.2)
15b	5.44 (d, 17.3)	5.44 [5.43] (d, 17.3)	5.44 (d, 17.4)
1-OMe	3.73 (s)	3.66 (s)	3.66 (s)
3-OMe	3.88 (s)	3.66 (s)	3.69 (s)
4-OMe	3.58 (s)	3.72 (s)	3.68 (s)
6-Me	2.09 (s)	1.83 (s)	1.78 (s)
9-Me	1.74 (s)	1.68 (s)	1.70 (s)
10-Me	1.04 [1.00] (d, 6.9)	1.02 [0.98] (d, 6.8)	1.02 [0.98] (d, 6.9)
12-Me	1.27 [1.34] (s)	1.26 [1.33] (s)	1.25 [1.33] (s)

^{a} Recorded at 600 MHz.

^{b} The cis isomers are minor in all cases in the ratio of 2:1 and the chemical shifts due to the cis isomers are shown in brackets if different from those due to the trans isomers (3~5).

^{c} The protons H7 and H8 were distinguished when measured in C$_6$D$_6$ (400 MHz) to determine the geometry of C6 and C8: δ 6.26 (d, J = 11.3 Hz, H7) and 6.40 (d, J = 11.3 Hz, H8) for 3 and δ 6.26 (d, J = 11.3 Hz, H7) and 6.44 (d, J = 11.6 Hz, H8) for cis-3.

the epoxy cis-isomer of 1, that is, cis-haliangicin, though the relative stereochemistry between the epoxide (C12–C13) and C10 is still unknown.

The structural analysis of haliangicin B (3) was performed as a mixture of two isomers. A thorough analysis of the 2D NMR data for this mixture enabled us to assign all the NMR signals to each of the isomers (Tables 2 and 3). The same NOE correlations were observed for the epoxide moiety between 1 and 3 and between 2 and the isomer of 3 (Fig. 3). On the other hand, a strong NOE correlation between H2 and H5 in the tetraene moiety (Table 4) suggested 2Z configuration for both 3 and its isomer, unlike the 2E configuration for 1 and 2. The other olefins in the tetraene moiety had the same configuration as 1 and 2. Thus, haliangicin B (3) is the 2Z isomer of 1 and

the coexistent isomer is the 2Z isomer of 2, that is, cis-haliangicin B (cis-3).

Haliangicin C (4) showed the same NOE correlations as those of 1 except for the strong NOE correlations of H5/4-OMe (Table 4), suggesting 4E geometry. The concomitant minor isomer of 4 showed the same NOE correlations as those of 4 at the tetraene portion (Table 4), while different NOE correlations were observed for the epoxide moiety, as in the case of 3 and cis-3 (Fig. 3). Thus, haliangicin (4) is the 4E isomer of 1 and the concomitant isomer is the 4E isomer of 2, namely, cis-haliangicin C (cis-4).

In a similar way, the examination of the NOE correlations (Table 4, Fig. 3) revealed that haliangicin D (5) was the 4E,6E isomer of 1 and that the concomitant minor isomer was cis-haliangicin D (cis-5).

Table 3. ^{13}C NMR data for haliangicins B (3)~D (5) and their *cis* isomers in $CDCl_3$.

| Position | δ_C (mult)[a] | | |
	3 [*cis*-3][b]	4 [*cis*-4][b]	5 [*cis*-5][b]
1	165.8 (s)	166.1 (s)	166.1 (s)
2	100.3 (d)	95.4 (d)	95.5 (d)
3	164.8 (s)	166.6 (s)	166.6 (s)
4	149.9 (s)	150.7 (s)	149.3 (s)
5	115.5 (d)	103.4 (d)	110.4 (d)
6	129.9 (s)	129.2 (s)	130.0 (s)
7	128.5 [128.6] (d)	124.6 (d)	125.2 (d)
8	120.1 [120.3] (d)	120.4 [120.6] (d)	120.5 [120.7] (d)
9	143.8 (s)	141.0 (s)	141.9 (s)
10	40.5 [40.7] (d)	40.2 [40.5] (d)	40.4 [40.7] (d)
11	44.0 (t)	44.0 (t)	44.1 (t)
12	61.9 [62.1] (s)	62.0 (s)	62.0 [62.1] (s)
13	63.8 [64.4] (d)	63.9 [64.5] (d)	63.9 [64.5] (d)
14	133.5 [133.7] (d)	133.6 [133.8] (d)	133.6 [133.8] (d)
15	120.0 [119.7] (t)	120.0 [119.7] (t)	120.0 [119.7] (t)
1-OMe	51.1 (q)	51.1 (q)	51.1 (q)
3-OMe	60.1 (q)	56.0 (q)	55.9 (q)
4-OMe	58.8 (q)	55.9 (q)	55.9 (q)
6-Me	23.2 (q)	22.9 (q)	14.8 (q)
9-Me	13.5 [13.3] (q)	13.3 (q)	13.6 (q)
10-Me	19.4 [19.5] (q)	19.3 [19.4] (q)	19.3 (q)
12-Me	16.7 [22.4] (q)	16.8 [22.5] (q)	16.8 [22.5] (q)

[a] Recorded at 150 MHz, multiplicity inferred using the DEPT pulse sequence.

[b] The *cis* isomers are minor in all cases in the ratio of 2:1 and the chemical shifts due to the *cis* isomers are shown in brackets if different from those due to the *trans* isomers.

Discussion

Haliangicin (1) is the first antibiotic isolated from a marine myxobacterium.[8,9] Until this discovery, all the myxobacterial antibiotics had originated from terrestrial myxobacteria. Haliangicin (1) inhibits the growth of a variety of fungi but is inactive against bacteria. It interfered with the electron flow within the cytochrome b–c_1 segment in the mitochondrial respiratory chains.[8] While exploring further antifungal substances from the haliangicin-producing myxobacterium *H. ochraceum*, we have been able to isolate several isomers of haliangicin (1) and have determined their structures. Haliangicin (1) and its isomers belong to the class of compounds that contain a β-methoxyacrylate moiety, which exhibit antifungal activity.[8] To the best of our knowledge, all the antibiotics possessing a β-methoxyacrylate moiety have E configuration, such as cystothiazoles,[11,12] myxothiazols,[13] and melithiazols.[14,15] It is interesting to note that one of our isolates, haliangicin B (3), is the first example of a (Z)-β-methoxyacrylate of this class of antibiotics. Haliangicin (1) and its *cis*-isomer (2) show an almost identical activity against the fungus *P. capsici*. This observation indicates that the relative stereochemistry of the substituents on the epoxide ring, located far from the active center (β-methoxyacrylate), plays no crucial role in the antifungal activity of the haliangicins. The antifungal activities of the *cis*-isomers of 3~5, though pending because of the difficulty of isolation, are expected to be the same as those of the respective *trans*-

Table 4. NOESY correlations for haliangicins 1~5 in CDCl$_3$.

Position 1[a]		2	3 [cis-3][b]	4 [cis-4][b]	5 [cis-5][b]
2	3-OMe	3-OMe	5, 4-OMe		
5	8	8	2, 8[c], 3-OMe	8, 4-OMe, 6-Me	7, 6-Me
7	6-Me, 9-Me	6-Me, 9-Me	6-Me[c], 9-Me[c]	6-Me, 9-Me	5, 9-Me
8	5, 10, 10-Me	5, 10, 10-Me	5[c], 10[c]	5, 10, 10-Me	10, 6-Me, 10-Me
10	8, 11b, 10-Me, 12-Me	8, 11, 10-Me, 12-Me	8[c], 11, 9-Me, 10-Me, 12-Me	8, 11b, 9-Me, 10-Me, 12-Me	8, 11b, 10-Me, 12-Me
11a	10, 11b, 13, 10-Me	10, 11b, 14, 10-Me	11b, 13	10, 11b, 13, [14]	10, 11b, 13, [14]
11b	10, 11a, 12-Me	10, 11a, 12-Me	10, 11a	10, 11a	10, 11a
13	11a, 15b, 10-Me	14, 15b, 12-Me	11a, 15b, 10-Me, [12-Me]	14, 15b, 10-Me,	11, 14, 15b, 10-Me, [12-Me]
14	15a, 12-Me	11a, 15a	15a, 12-Me, [11a]	15a, 12-Me, [11a]	13, 15a, 12-Me, [11a]
15a	14, 15b	14, 15b	14, 15b	14, 15b	14, 15b
15b	13, 15a	13, 15a	13, 15a	13, 15a	13, 15a
3-OMe	2	2	5		2, 6-Me
4-OMe	6-Me	6-Me	2, 6-Me	5	5
6-Me	7	7, 4-OMe	7[c], 4-OMe	5, 7	5, 8
9-Me	7, 10, 10-Me	7, 10, 10-Me	7[c], 10, 10-Me	7, 10, 10-Me	7, 10-Me
10-Me	8, 10, 11a, 13, 9-Me	8, 10, 11a, 9-Me	8[c], 10, 13, 9-Me	8, 10, 13	8, 10, 13, 9-Me
12-Me	10, 11b, 14	10, 11b, 13	10, 11b, 14, [13]	10, 11b, 14, [13]	10, 11b, 14, [13]

[a] The NOESY data around the epoxide moiety of 1 were not reported in ref. 9.

[b] The specific correlations for the cis isomers are in brackets.

[c] Determined by measurement in C$_6$D$_6$.

Fig. 3. Important NOE correlations and the partial stereochemistry of haliangicin (1) and its isomers.

R = tetraene moiety

haliangicin (1)
and
haliangicin B-D (3-5)

cis-haliangicin (2)
and
cis isomers of 3 - 5
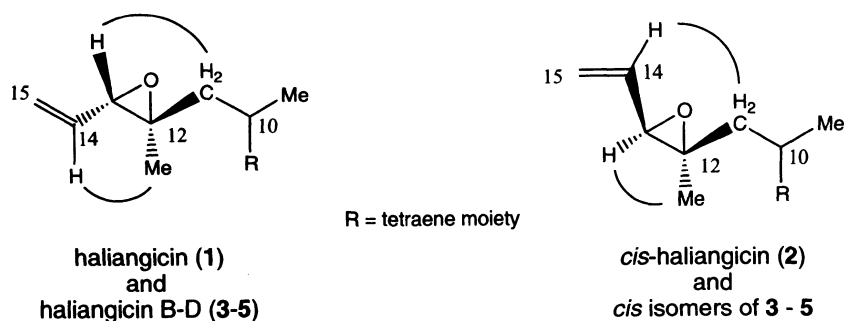
isomers **3~5** from analogy to the relationship between **1** and **2**. The geometrical isomers, haliangicins B (**3**)~D (**5**), show lower activities than haliangicin (**1**), suggesting that the configurations of the conjugated tetraene moieties (C2, C4, and C6) are important, to some extent for the antifungal activities. Determination of the absolute configurations at the three stereogenic centers of haliangicin (**1**) is now in progress.

Experimental

General

Evaporation of solvents was carried out using a rotary evaporator under reduced pressure at temperatures around 30~38°C. The residual samples were then vacuum dried. All the haliangicin samples were handled in dim light, or in the dark, with aluminum foil protection, and always stored as solutions in benzene at −20°C. Fuji Silysia silica gel BW-300 was employed for open column chromatography. Pre-coated silica gel 60 F_{254} plates (Merck) were used for thin-layer chromatography (TLC). HPLC was performed on a JASCO high-pressure gradient system equipped with PU-980 pumps. Recycle HPLC was performed on a Shimadzu LC-8A high-pressure isocratic system. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-7000S spectrometer. UV spectra were recorded on a JASCO Ubest-50 UV/VIS spectrophotometer. NMR spectra were recorded on a Bruker ARX 400 (400 MHz) or a Bruker ARX 600 (600 MHz) spectrometer. NMR chemical shifts were referenced to the solvent peak of δ_H 7.26 (residual CHCl$_3$) or 7.15 (residual C$_6$HD$_5$) for protons and δ_C 77.0 (CDCl$_3$) for ^{13}C. Mass spectra were recorded on an Applied Biosystems Mariner Biospectrometry Workstation in the positive ESI mode. Polypropylene glycol (PPG) was used as an internal standard for high-resolution analysis.

Isolation

The production culture of the myxobacterium *H. ochraceum* AJ 13395 was performed under the conditions reported previously.[8] The bacterial cells and the adsorption resin SP 207 (Nippon Rensui) was separated from a culture broth (6 liters) by centrifugation and extracted with acetone (600 ml) at room temperature for 3 days (one day should be sufficient). After filtration the filtrate was concentrated to obtain an oily crude sample (590 mg). TLC analysis (hexane/EtOAc 4 : 1) of the crude sample showed the major spot of haliangicins at Rf=0.42. The crude oil was chromatographed on silica gel (35 g) with 40 ml of

benzene, 40 ml each of 1%, 2%, 4%, 10%, 20%, 50% of ethyl acetate in benzene, 40 ml ethyl acetate, and then 40 ml of methanol as successive eluants. The haliangicin-containing fraction (138.7 mg) was combined with a second haliangicin-containing fraction (77.8 mg) obtained similarly from another culture broth (3 liters) and was applied to silica gel (10 g) column chromatography with hexane/ EtOAc 4 : 1 as an eluant to obtain a yellowish orange oil (184.7 mg). The oily material was then applied to preparative HPLC [Develosil ODS-5 (20×250 mm), Nomura Chemical Ltd.; UV at 260 nm, 60% CH$_3$CN, 9 ml/minute] to obtain haliangicin D (**5**) (0.4 mg, Rt=40.2 minutes), haliangicin C (**4**) (1.6 mg, Rt=47.9 minutes), haliangicin (**1**) (65.7 mg, Rt=54.2 minutes), and haliangicin B (**3**) (2.3 mg, Rt=84.9 minutes) as mixtures containing their corresponding *cis*-isomers, all as light yellow oils (Fig. 2). The mixture of **1** and the *cis*-isomer **2** was further subjected to recycle HPLC (Fig. 2) [Develosil ODS-5 (20×250 mm), UV at 360 nm, 75% MeOH, 9 ml/minute, 5- or 7-recycles for two injections] to obtain pure haliangicin (**1**) (36.5 mg) and *cis*-haliangicin (**2**) (19.6 mg) as light yellow oils.

Haliangicin (**1**): Light yellow oil, $[\alpha]_D^{22}$+34.6 (*c* 0.305, MeOH).

cis-Haliangicin (**2**): Light yellow oil, $[\alpha]_D^{22}$+29.3 (*c* 0.212, MeOH); UV (MeOH) λ_{max} 245 (ε 11000, sh), 295 (7600, sh) nm; IR (film) v_{max} 1717, 1617, 1457, 1143, 1050 and 927 cm^{-1}; ESI-MS *m/z* 377.2328 (M+H)$^+$, calcd for C$_{22}$H$_{33}$O$_5$ 377.2323. For ^{1}H and ^{13}C NMR and HMBC data refer to Table 1.

Mixture of Haliangicin B (**3**) and *cis*-**3** (2 : 1 Ratio): Light yellow oil, $[\alpha]_D^{22}$+38 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 254 (ε 9500), 341 (11000) nm; IR (film) v_{max} 1720, 1609, 1456, 1252, 1159, 1092 and 1056 cm^{-1}; ESI-MS *m/z* 377.2337 (M+H)$^+$, calcd for C$_{22}$H$_{33}$O$_5$ 377.2323; HMBC correlations H2/C3, H2/C4, H5/C3, H5/C4, H5/C7, H5/6-Me, H7/C5, H7/6-Me, H8/C10, H8/9-Me, H10/C8, H10/C9, H10/C11, H10/C12, H10/9-Me, H10/10-Me, H11/C9, H11/C10, H11/C12, H11/C13, H11/10-Me, H11/12-Me, H13/C11, H13/C12, H13/C14, H13/C15, H14/C13, H15/C13, H15/C14, 1-OMe/C1, 3-OMe/C3, 4-OMe/C4, 6-Me/C5, 6-Me/C6, 6-Me/C7, 9-Me/C8, 9-Me/C9, 9-Me/C10, 10-Me/C9, 10-Me/C10, 10-Me/C11, 12-Me/C11, 12-Me/C12, 12-Me/C13. For ^{1}H and ^{13}C NMR data and NOESY correlations refer to Tables 2, 3, and 4, respectively.

Mixture of Haliangicin C (**4**) and *cis*-**4** (2 : 1 Ratio): Light yellow oil, $[\alpha]_D^{22}$−40 (*c* 0.04, MeOH); UV (MeOH) λ_{max} 247 (ε 9100), 277 (9200) nm; IR (film) v_{max} 1726, 1603, 1457, 1262, 1208, 1168, 1137, 1047 and 924 cm^{-1};

ESI-MS m/z 377.2343 $(M+H)^+$, calcd for $C_{22}H_{33}O_5$ 377.2323; HMBC correlations H2/C3, H2/C4, H5/C3, H5/C4, H5/C7, H5/6-Me, H7/C5, H7/C8, H7/C9, H7/6-Me, H8/C6, H8/C7, H8/C10, H8/9-Me, H10/C8, H10/C9, H10/C11, H10/9-Me, H10/10-Me, H11/C9, H11/C10, H11/C12, H11/C13, H11/10-Me, H11/12-Me, H13/C12, H13/C14, H13/C15, H14/C13, H15/C13, H15/C14, 1-OMe/C1, 3-OMe/C3, 4-OMe/C4, 6-Me/C5, 6-Me/C6, 6-Me/C7, 9-Me/C8, 9-Me/C9, 9-Me/C10, 10-Me/C9, 10-Me/C10, 10-Me/C11, 12-Me/C11, 12-Me/C12, 12-Me/C13. For ^1H and ^{13}C NMR data and NOESY correlations refer to Tables 2, 3, and 4, respectively.

Mixture of Haliangicin D (5) and cis-5 (2:1 Ratio): Light yellow oil, $[\alpha]_D^{22}-20$ (c 0.05, MeOH); UV (MeOH) λ_{max} 286 (ε 27000) nm; IR (film) v_{max} 1727, 1653, 1602, 1457, 1261, 1166, 1136, 1047, 923 and 805 cm^{-1}; ESI-MS m/z 377.2341 $(M+H)^+$, calcd for $C_{22}H_{33}O_5$ 377.2323; HMBC correlations H2/C3, H2/C4, H5/C3, H5/C4, H5/C7, H5/6-Me, H7/C5, H7/C9, H7/6-Me, H8-/C10, H8/9-Me, H10/C8, H10/C9, H10/C11, H10/9-Me, H10/10-Me, H11/C10, H11/C12, H11/C13, H11/10-Me, H11/12-Me, H13/C14, H13/C15, H14/C13, H15/C13, 1-OMe/C1, 3-OMe/C3, 4-OMe/C4, 6-Me/C5, 6-Me/C6, 6-Me/C7, 9-Me/C8, 9-Me/C9, 9-Me/C10, 10-Me/C9, 10-Me/C10, 10-Me/C11, 12-Me/C11, 12-Me/C12, 12-Me/C13. For ^1H and ^{13}C NMR data and NOESY correlations refer to Tables 2, 3 and 4, respectively.

Assay Method

The antifungal activity of the haliangicins was evaluated by a paper disc assay using the phytopathogenic fungus *Phytophthora capsici*. Since this filamentous fungus rarely produces spores, the activity was evaluated not by MIC but by the observation of aversion to a paper disc impregnated with a test sample. A piece of agar, 5 mm in diameter, containing mycelia of *P. capsici* previously grown on a synthetic agar medium was placed onto the center of a plate (9 cm in diameter) of the same agar medium (1 liter medium containing: sucrose 10 g, monosodium glutamate monohydrate 1.5 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $CaCl_2$ 0.1 g, agar 10 g, thiamine hydrochloride 1 mg, $Na_2B_4O_7 \cdot 10H_2O$ 0.088 mg, $CuSO_4 \cdot 5H_2O$ 0.393 mg, $Fe_2(SO_4)_3 \cdot 9H_2O$ 0.91 mg, $MnCl_2 \cdot 4H_2O$ 0.072 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.05 mg, $ZnSO_4 \cdot 7H_2O$ 4.403 mg, EDTA $\cdot$ 2Na 5 mg). After incubation at 25°C with 60% humidity for 2 days in the dark, the colony grew to a size of about 3~4 cm in diameter. Each paper disc (8 mm in diameter), which was impregnated with an EtOAc solution of a known amount of a sample and dried *in vacuo*, was placed one cm away from the edge of the colony. After incubation under the same conditions for an additional 29 hours when the edge of the colony reached the edge of the paper disc of the control, the distance between the edge of the colony and the paper disc was measured. The control disc was made by *in vacuo* drying of an EtOAc-containing paper disc.

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