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Haliangicin, a Novel Antifungal Metabolite Produced by a Marine Myxobacterium

2. Isolation and Structural Elucidation

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A novel antifungal antibiotic, haliangicin, was isolated from a culture broth of marine myxobacterium, *Haliangium luteum*. The planar structure of haliangicin was elucidated by spectroscopic analyses and was shown to be a new polyunsaturated compound containing β-methoxyacrylate moiety.

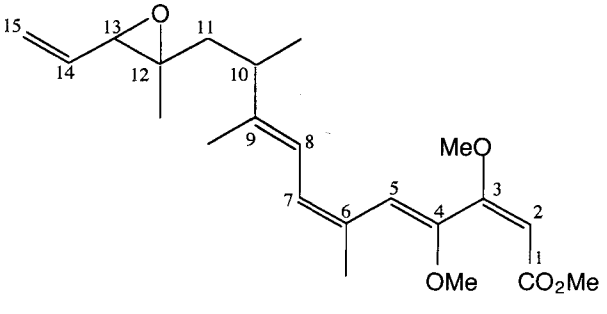
Myxobacteria are well-known as producers of novel antibiotics and more than eighty novel compounds have been reported from these microorganisms[1,2]. In the course of screening for bioactive compounds from myxobacteria of marine origin[3], a novel polyunsaturated antibiotic containing a β-methoxyacrylate moiety, haliangicin (**1**), was discovered. The fermentation and biological properties of haliangicin are the subject of the preceding paper[4]. In this paper, we describe the isolation and structural elucidation of haliangicin.

Results and Discussion

Isolation

From 20 liters of whole culture broth, bacterial cell mass and adsorber resin were collected by centrifugation and were extracted twice with five liters of acetone for 16 hours. The extracts were combined and filtered through a paper filter and concentrated with an evaporator until 800 milliliters of aqueous residue remained. This solution was further extracted twice with an equal volume of ethyl acetate. After concentration, the ethyl acetate extract (2.2 g) was subjected to solid phase extraction (Bond Elute C18, 10 g), washed with 40% methanol and eluted with 100% methanol. The active fraction (720 mg) was chromatographed on a semipreparative HPLC (CAPCELL PAK C18, UG120, 15×360 mm, Shiseido Co.; 70% CH_3CN isocratic elution (7 ml/minute)). The active fraction eluted at 21 minutes and was further subjected to another HPLC separation (Senshu Pak C6H5-4152N, 10×150 mm, Senshu Science Co.; 45% CH_3CN isocratic elution (4 ml/minute)) to give purified haliangicin (35 mg) as a light yellow oil.

Fig. 1. Structure of haliangicin (**1**).



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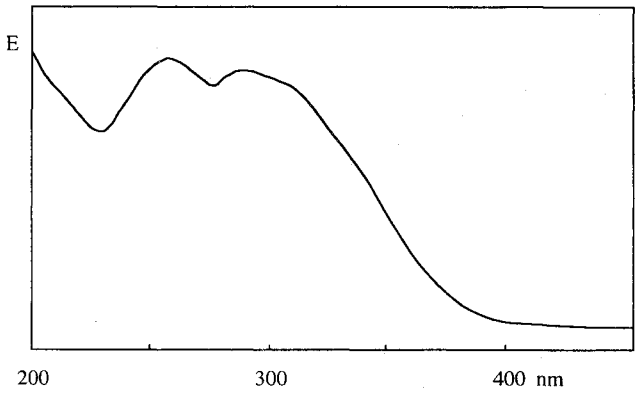
Structure Elucidation

Haliangicin (**1**) was shown to have the molecular formula of $C_{22}H_{32}O_5$ by the HR-FABMS and NMR data, indicative of six degrees of unsaturation. A resonance at 166.9 ppm in the ^{13}C NMR spectrum of **1** indicated the presence of one carbonyl carbon, likely an α,β-unsaturated ester carbonyl on the basis of the IR absorption data (1706, 1624 and 1150 cm^{-1}). The UV absorption spectrum in methanol solution is shown in Fig. 2. Haliangicin was quite susceptible to air oxidation and decomposed rapidly at room temperature when evaporated to dryness. For storage, the compound must be kept in a solvent, such as methanol, at −20°C. The physico-chemical properties of **1** are summarized in Table 1.

The ^1H and ^{13}C NMR data (Table 2) and HMQC spectra showed four methyls (δ_H/δ_C: 1.00/19.2; 1.22/16.7; 1.67/13.5; 2.06/23.6), three methoxyls (δ_H/δ_C: 3.53/57.1; 3.65/51.2; 3.70/55.9), a methylene (δ_H/δ_C: 1.28, 1.93/43.9), two methines (δ_H/δ_C: 2.34/40.2; 3.17/63.9), a quarternary carbon (δ_C: 62.0) and eleven sp^2 carbons (δ_H/δ_C: 5.23/95.7; 5.29, 5.40/119.9; 5.73/133.5, δ_C: 112.2; 120.4; 126.2; 130.5; 141.2; 148.1; 165.8; 166.9).

Fig. 2. UV absorption spectrum of haliangicin in methanol.



HMBC experiments were carried out to ascertain the connectivity of the partial structures, and the results are shown in Fig. 3. The assignment of the observed correlation signals in the HMBC spectrum began with olefinic protons (δ 5.23, 5.75, 6.02 and 6.10 ppm). The signal of H-2 (δ 5.23) was correlated with C-3 (δ 165.8) and C-4 (δ 148.1). The signal of H-5 (δ 5.75) was correlated with C-3 (δ 165.8), C-4 (δ 148.1), C-7 (δ 126.2) and 6-Me (δ 23.6). The signal of H-7 (δ 6.02) was correlated with C-5 (δ 112.2), C-6 (δ 130.5), C-9 (δ 141.8) and 6-Me (δ 23.6). The signal of H-8 (δ 6.10) was correlated with C-6 (δ 130.5), C-9 (δ 141.8) and C-10 (δ 40.2). These correlations revealed the connectivity from C-2 to C-10. The correlations between C-12 and H-10, H-11 and H-13, and between C-13 and H-14 and H-15 indicated the connectivity from C-10 to C-15. The connectivity of 10-Me, C-10 and C-11, and C-13, C-14 and C-15 were supported by COSY results as shown in Fig. 3. The substitution pattern at 3-OMe, 4-OMe, 6-Me and 9-Me were deduced by HMBC correlation signals. The chemical shift values of C-12 (δ_C: 62.0) and C-13 (δ_H/δ_C: 3.17/63.9) together with the large $^1J_{CH}$ value of C-13 (166.6 Hz) suggest the presence of an epoxide ring. The correlation peak between a methoxyl group (δ_C: 3.65) and H-2 (δ_H: 5.23) in the NOESY spectrum denoted the connectivity of a methoxycarbonyl group to the conjugated tetraene group (Figure 3).

Table 1. Physico-chemical properties of haliangicin.

Appearance	light yellow oil
$[\alpha]_D^{25}$	+38° (c 0.5, MeOH)
Molecular formula	$C_{22}H_{32}O_5$
FAB-MS *m/z*	376 (M+), 273, 239, 219
HRFAB-MS	
calcd for $(M+H)^+$;	376.2248
found;	376.2250
UV λ_{max}^{MeOH} nm (ε)	257 (15900), 291 (15100)
IR ν_{max}^{CHCl3} cm^{-1}	3123, 1706, 1624, 1499, 1457, 1441, 1385, 1267, 1150, 1127, 1094
TLC (Rf value)[a]	0.42

[a] Silica gel plate; hexane-EtOAc (4:1).

Table 2. NMR data for haliangicin in $CDCl_3$.

Position	^1H [a]	^{13}C [b]
1	-	166.9(s)
2	5.23(s)	95.7(d)
3	-	165.8(s)
4	-	148.1(s)
5	5.75(s)	112.2(d)
6	-	130.5(s)
7	6.02(d,J =11.2)	126.2(d)
8	6.10(d,J =11.2)	120.4(d)
9	-	141.8(s)
10	2.34(m)	40.2(d)
11	1.28(dd, J =8.8, 13.6) 1.93(dd, J =8.8, 13.6)	43.9(t)
12	-	62.0(s)
13	3.17(d, J =7.2)	63.9(d)
14	5.73(ddd, J =7.2, 10.4, 17.2)	133.5(d)
15	5.29(d, J =10.4) 5.40(d, J =17.2)	119.9(t)
1-OMe	3.65(s)	51.2(q)
3-OMe	3.70(s)	55.9(q)
4-OMe	3.53(s)	57.1(q)
6-Me	2.06(s)	23.6(q)
9-Me	1.67(s)	13.5(q)
10-Me	1.00(d)	19.2(q)
12-Me	1.22(s)	16.7(q)

[a] Recorded at 400 MHz, [b] recorded at 100 MHz.

The geometrical configuration of the conjugated tetraene moiety was also predicted from the NOESY experiment as shown in Fig 3. To clarify this assertion, an HSQC experiment was conducted. In the long range ^1H-^{13}C correlation spectrum, strong cross peaks were observed for H2-C4, H7-C5 and H8-9Me in comparison with those for H5-C3, H7-6Me and H8-C10 (Table 3). Since the three-bond correlation signals are expected to be stronger in the *trans*- than in the *cis*-configuration, the H2-C4, H7-C5 and H8-9Me exist in the *trans*-configuration, and H5-C3, H7-6Me and H8-C10 in the *cis*-configuration.

From above results, the planar structure of haliangicin was determined to be a novel tetraene type compound containing both an epoxide ring and a β-methoxyacrylate moiety as shown in Fig. 1 (**1**). The determination of the absolute configurations at the three chiral carbons (10,12 and 13 positions) is now in progress.

Experimental

General

UV absorption spectra were measured with a Beckman model DU 640 spectrophotometer. High performance liquid chromatography was performed on Waters996 HPLC with

Fig. 3. ^1H-^1H COSY, HMBC and NOESY correlation of haliangicin.

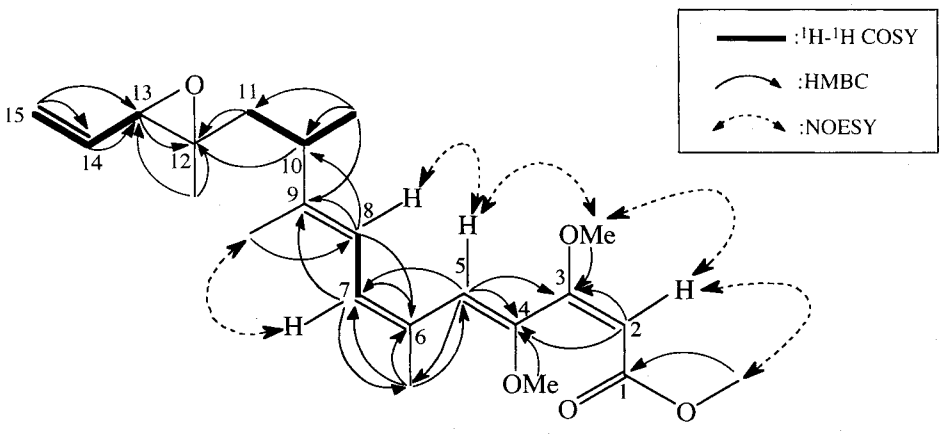


Table 3. Relative intensities of the three-bond ^1H-^{13}C correlation signals of haliangicin.

Cross peak	H2-C4	H5-C3	H7-C5	H7-6Me	H8-9Me	H8-C10
Relative intensities [a]	1.0	0.2	0.5	0.3	0.5	0.4

[a] The values are based on the signal intensity of H2-C4 cross peak.

a photodiode array detector (Waters 997). IR spectra were recorded with a Jasco IR-810 infrared spectrophotometer. Optical rotations were measured with a Jasco DIP-370 polarimeter in a 10 cm microcell. Mass spectra were obtained with a JEOL JMS-DX 300 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DMX 400 spectrometer.

Determination of *cis*-/*trans*-Conformations by HSQC Experiments

To detect the three-bond ^{1}H-^{13}C correlation peaks such as H2-C4, H5-C3, H7-C5, H7-6Me, H8-9Me and H8-C10, a long range ^{1}H-^{13}C correlation experiment was carried out with a 14 ms delay for the ^{1}H-^{13}C coherence transfer[5]. Observed peak intensities depend on their three-bond coupling constants, reflecting the *cis*- or *trans*-configurations.

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