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Short communication

Antifouling activity against bryozoan and barnacle by cembrane diterpenes from the soft coral *Sinularia flexibilis*

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A B S T R A C T

In the present study, seven cembrane diterpenes were isolated from the soft coral *Sinularia flexibilis* by Toyopearl HW-40 column chromatography and High Performance Liquid Chromatography (HPLC). The diterpenes were identified as epoxycembrane A (**1**), sinularin (**2**), sinulariolide (**3**), (1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (**4**), 11-dehydrosinulariolide (**5**), (−)14-deoxycrassin (**6**) and dihydrosinularin (**7**). The antifouling activity of these compounds was examined by settlement assays, using the larvae of the bryozoan *Bugula neritina* and the barnacle *Balanus albicostatus*. With the exception of compound **2**, all compounds indicated significant antifouling activity and a variety of EC_{50} values. In particular, compound **6** exhibited remarkable anti-settlement activity against the two biofoulers (EC_{50} for *B. neritina* 3.90 μg ml^{-1}; EC_{50} for *B. albicostatus* 21.26 μg ml^{-1}) as well as low toxicity against *B. albicostatus* larvae ($LC_{50} > 100$ μg ml^{-1}), suggesting its potential as an environmentally friendly antifoulant. This is the first report on the antifouling activity of compounds **1** and **4**–**7**, further demonstrating the involvement of cembrane diterpenes in the chemical defense of soft corals against surface fouling.

1. Introduction

Toxic antifoulants such as tributyltin and copper have been widely used on the surfaces of artificial structures submerged in the sea in order to deter marine fouling organisms (Yebra et al., 2004; Muynck et al., 2009; Dafforn et al., 2011). However, a growing awareness of their adverse environmental impacts has led to strict regulations on their use as marine antifoulants (Thomas and Brooks, 2010; Dafforn et al., 2011). This has triggered the search for environmentally friendly natural antifouling products. A great deal of effort has been directed towards the screening of natural product antifoulants (NPAs) from marine organisms, especially sessile, soft-bodied marine species such as macroalgae, sponges and soft corals (Fusetani, 2011; LimnaMol et al., 2011; Patro et al., 2012; Qian et al., 2015). Despite being exposed to surface biofouling in the marine environment, some of these species are able to remain free of fouling. As they lack the necessary behavioral or physical defenses, it is believed that they resist epibiosis via the production of antifouling secondary metabolites (Schmitt et al., 1998; Dobretsov et al., 2004, 2015). Numerous NPAs have been isolated from sessile, soft-bodied marine organisms, suggesting that they are promising sources of NPAs.

Soft corals are one of the most prolific sources of bioactive marine natural products. A variety of secondary metabolites have been extracted from soft corals, indicating various cytotoxic, antibacterial, anti-inflammatory, antiviral and antifouling bioactivities (Wen et al., 2008; Lai et al., 2013;Cheng et al., 2015; Gomaa et al., 2015; Taira et al., 2015). Soft corals of the genus *Sinularia* are particularly well-known for containing bioactive substances with interesting chemical structures, including diterpenes, sesquiterpenes, polyhydroxylated steroids and polyamine compounds (Kamel and Slattery, 2005; Chen et al., 2012).

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Table 1
Compounds **1**–**7** isolated from the soft coral *Sinularia flexibilis*.

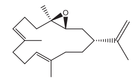
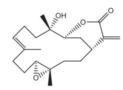
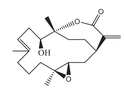
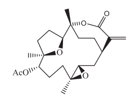
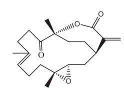
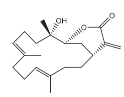
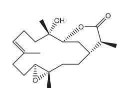
Compound	Name	Structure	Molecular weight	Property	Reference
1	Epoxycembrane A		288	Colorless oil	Bowden, (1981)
2	Sinularin		334	Colorless oil	Rymantas et al. (1978)
3	Sinulariolide		334	Colorless oil	Alfred and Weinheimer (1977)
4	(1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-Acetoxy-5,8:12,13-diepoxycembr-15 (17)-en-16,4-olide		334	Colorless oil	Su et al. (2000)
5	11-Dehydrosinulariolide		332	Clear crystal	Liu et al. (2011)
6	(−)14-Deoxycrassin		318	Colorless oil	Wen et al. (2008)
7	Dihydrosinularin		336	Colorless oil	Alfred and Weinheimer (1977)

Table 2
^{1}H NMR and ^{13}C NMR Data for Cembrane diterpenes **1**–**7** (δ in ppm, *J* in Hz).

Position	1		2		3		4		5		6		7	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		40.4		33.7		35.1	3.21 m	35.7		34.9		33.4		36.9
2		33.6		22.6		31.4		33.2		32.6	1.42 m	26.3		27.7
3	2.83 dd (2.0,10.0)	63.2	3.97 d (11.0)	82.7		33.5		29.8		33.3	4.04 brd (11.0)	83.2	4.01 brd (8.0)	83.6
4		60.7		74.1		87		88.2		90.2		74.2		74.5
5		23.7		38.6	4.10 d (10.0)	68.7	4.38 dd (4.0,10.0)	83.4		209.3		39.5		38.1
6		38.3		27.8		35.8		26.7		30.1		22.7		23
7		123.9	5.23 t (11.0)	125.6		27		34.9		29.7	5.06 m	126.1	5.23 t (7.0)	125.9
8		135.1		134.5		134.8		85.4		135.2		134.6		133.8
9	5.10 t (6.4)	39.6		35.9	5.15 brd (9.0)	126.7	5.17 d (9.2)	77.9	5.09 t (6.9)	122.8		40.1		35.7
10		24.4		25.3		35.8		27.3		33.7		24.8		25.4
11	5.10 t (6.4)	124.3	2.79 brd (6.5)	62.9		38.1		34.3		37.6	5.07 m	126.6	2.87 t (6.0)	62.9
12		133.3		59		60.4		60		60.7		132.1		58.9
13		34.7		34.7	2.93 brd (10.7)	63.9	3.41 d (3.8,10.7)	60.8	3.18 dd (10.76,11.0)	62.3		36.1		34.8
14		29.8		32.9		31.7		33.5		31		31.8		31.2
15		148.6		140.1		144.4		145.4		143.7		140.2		42
16	1.63 s	110.7		167.2		169		169.5		167.7		167.3		174.7
17	4.68 brs 4.63 brs	18.5	6.47 brs 5.69 brs	127.9	6.29 brs 5.42 brs	124.1	6.27 brs 5.43 brs	123.6	6.33 brs 5.50 brs	125.8	6.47 d (2.4) 5.67 d (2.4)	126.4	1.37 d (7.0)	16.4
18	1.24 s	17	1.44 s	24.7	1.34 s	16	1.25 s	29.3	1.44 s	16.2	1.41 s	24.9	1.44 s	25.1
19	1.62 s	15.8	1.66 s	15.2	1.61 s	22.8	1.16 s	17.1	1.66 s	24.5	1.58 s	14.7	1.61 s	17.4
20	1.59 s	17.2	1.31 s	15.4	1.20 s	15.8	1.24 s	16.4	1.31 s	17.3	1.64 s	13.9	1.23 s	15.4
Acetoxyl							2.06 s	171.1 21.1						

Table 3
The known bioactivity of cembrane diterpenes from *Sinularia flexibilis*.

Activity	Compounds	References
Cytotoxic	Sinulaflexiolides D and E, flexilarin D, flexibilisolide C, 11-dehydrosinulariolide, sinularin,11-epi-sinulariolide acetate, sinulariolide, 11-acetylsinuflexolide, sinuflexolide, dihydrosinuflexolide, sinuflexibilin, 14-deoxycrassin, dehydrosinulariolide, dihydrosinularin, 3,4:8,11-bisepoxy-7-acetoxycembra-15 (17)-en-1,12-olide	Duh et al. (1998); Hsieh et al. (2003); Wen et al. (2008); Lin et al. (2009); Su et al. (2009); Shih et al. (2012); Su et al. (2013)
Anti-inflammatory	Flexibilisolides C and D, 11-dehydrosinulariolide, flexilarin D, (−)-sandensolide, 11-epi-sinulariolide acetate, isosinulaflexiolide K, sinulariolide, 11-epi-sinulariolide, sinulaflexiolide K, flexibilisquinone, 3,4:8,11-bisepoxy-7-acetoxycembra-15 (17)-en-1,12-olide	Shih et al. (2012); Y.F. Lin et al. (2013); Tsai et al. (2015)
Antimicrobial	Sinulariolide, flexibilide	Acerte et al. (1998)
Algacidal	Sinularin, 11-epi-sinulariolide	Michalek and Bowden (1997)