Tetrahedron Letters 51 (2010) 5764–5766

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A novel symmetric sulfur-containing biscembranoid from the Formosan soft coral *Sinularia flexibilis*

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ARTICLE INFO

Article history:
Received 3 July 2010
Revised 2 August 2010
Accepted 10 August 2010
Available online 14 August 2010

Keywords:
Soft coral
Sinularia flexibilis
Thioflexibilolide A
Anti-inflammatory activity
Neuroprotective activity

ABSTRACT

A structurally unique symmetric sulfur-containing biscembranoid, namely, thioflexibilolide A (**1**), was isolated from the soft coral *Sinularia flexibilis*. The structure was determined by extensive spectroscopic analyses. Compound **1** has been found to possess significant anti-inflammatory and neuroprotective activities.

1. Introduction

Soft corals have been well recognized to be a rich source of secondary metabolites possessing variety of skeletons and biological activities.[1] Previously, we have discovered series of structurally novel and bioactive sesquiterpenoids,[2,3] norcembranoids,[4,5] cembranoids,[6–8] and steroids[9–11] from Taiwanese soft corals of the genus *Sinularia*. In our continuing search for bioactive metabolites from soft corals of Taiwanese waters, we investigated the chemical constituents of *Sinularia flexibilis*. This study has led to the discovery of a novel sulfur-containing biscembranolide, thioflexibilolide A (**1**), from the CH_2Cl_2 extract of this marine organism. The structure of **1** was determined on the basis of extensive spectroscopic analysis, including 2D NMR (^1H–^1H COSY, HMQC, HMBC, and NOESY) spectroscopy. Compound **1** demonstrated anti-inflammatory activity to inhibit the accumulation of the pro-inflammatory protein, inducible nitric oxide synthase (iNOS), in lipopolysaccharide (LPS)-stimulated microglial cells. Furthermore, the interesting neuroprotective activity of **1** against the damage of 6-OHDA (6-hydroxydopamine)-induced cytotoxic effect on SH-SY5Y cells was also observed.

2. Results and discussion

The soft coral *S. flexibilis* (2.1 kg) was collected by hand using scuba off the coast of Taitung County, located in the southeastern part of Taiwan. The acetone extract of the organism was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble portion (15.3 g) was subjected to column chromatography on silica gel using *n*-hexane/EtOAc mixture of increasing polarity as eluent. A fraction eluted with *n*-hexane/EtOAc (1:1) was further purified by reverse-phase HPLC (MeOH/H_2O, 2.3:1) to afford **1** (15.2 mg).

Thioflexibilolide A (**1**)[12] was obtained as colorless oil. The HRESIMS of **1** exhibited a $[M+Na]^+$ peak at *m*/*z* 725.4059 and established a molecular formula $C_{40}H_{62}O_8S$, implying ten degrees of unsaturation. The IR spectrum of **1** revealed the presence of hydroxy and ester functionalities from absorptions of 3460 and 1722 cm^{-1}. The ^{13}C NMR data of **1** showed the presence of 20 carbon signals in total (Table 1), which were assigned by the assistance of the DEPT spectrum to three methyls, eight sp^3 methylenes, five methines (including two oxymethines and one vinylic CH), one sp^2 carbonyl, and one sp^2 olefinic and two sp^3 oxygenated quaternary carbons. The NMR signals appearing at δ_C 172.2 (qC), 83.4 (CH), 48.2 (CH), 32.7 (CH), and 27.3 (CH_2) and the IR absorption at 1722 cm^{-1} were assigned to an δ-lactone functionality by comparison with the relevant data of similar functional group.[8] Furthermore, carbon signals of one trisubstituted double bond (δ_C 133.9 and 125.6), one trisubstituted epoxide (δ_C 62.9 and 58.9), one oxygen-bearing

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doi:10.1016/j.tetlet.2010.08.027

Table 1
^{1}H and ^{13}C NMR Data for compound **1**

Position	^{1}H[a]	^{13}C[b]
1	1.75 m	32.7, CH[d]
2	1.38 m	27.3, CH_2
	2.04 m	
3	4.06 d (10.7)[c]	83.4, CH
4		74.4, qC
5	1.81 m	38.2, CH_2
	1.64 m	
6	2.31 m	22.9, CH_2
	2.00 m	
7	5.26 t (7.3)	125.6, CH
8		133.9, qC
9	2.27 m	35.7, CH_2
	2.12 m	
10	1.95 m	25.4, CH_2
	1.61 m	
11	2.87 t (5.7)	62.9, CH
12		58.9, qC
13	2.07 m	34.6, CH_2
	1.24 m	
14	1.79 m	31.2, CH_2
	1.25 m	
15	2.47 m	48.2, CH
16		172.2, qC
17	3.25 dd (13.2, 4.8)	35.3, CH_2
	3.09 dd (13.2, 3.5)	
18	1.45 s	25.1, CH_3
19	1.67 s	16.4, CH_3
20	1.27 s	15.5, CH_3

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in Hz) in parentheses.
[d] Multiplicities deduced by DEPT.

methine (δ_C 83.4), and one oxygenated quaternary carbon (δ_C 74.4) were observed. The ^{1}H NMR spectrum of **1** also showed signals of one olefinic proton (δ 5.26, t, J = 7.3 Hz), one oxymethine proton (δ 4.06, d, J = 10.7 Hz), and one trisubstituted epoxy proton (δ 2.87, t, J = 5.7 Hz), which were all confirmed by HSQC, ^{1}H–^{1}H COSY, and HMBC correlations. The three 3H singlets appearing in the ^{1}H NMR spectrum at δ1.67, 1.45, and 1.27 were assigned to an olefinic methyl, one methyl attached to a quaternary oxygenated carbon, and one methyl of a trisubstituted epoxide in the molecule, respectively. From the above observations and molecular formula of **1**, it is clear that **1** is a symmetric dimer of a cembranoid ($C_{20}H_{31}O_4$). On the basis the NMR spectral data, and by extensive analyses of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 1), it was found that the molecular framework for the monomer of **1** should be nearly identical with that of sinularin (**2**),[13] which also was known as flexibilide.[14] The presence of the sp^3 sulfur-containing methylene (δ_H 3.25 and 3.09; δ_C 35.3) and the absence of the sp^2 α-methylene group of **2** in **1** indicated that two identical monomers were connected by a sulfur atom to form this biscembranoid, as further evidenced by the long-range HMBC correlations from H_2-17 to C-17′ (δ 35.3, CH_2). Therefore, the

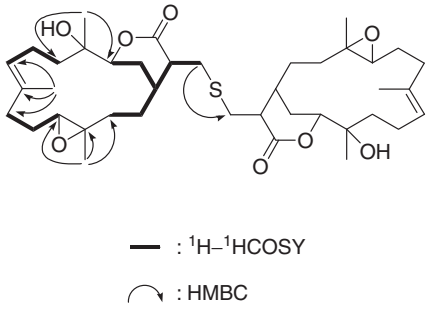


Figure 1. ^{1}H–^{1}H COSY and HMBC correlations for **1**.

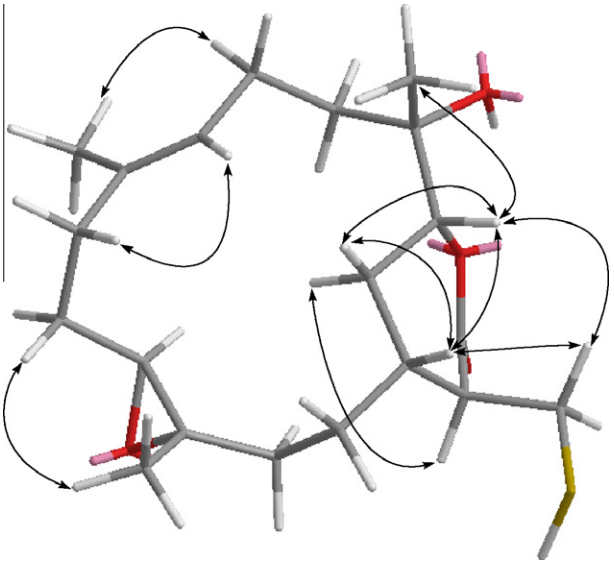
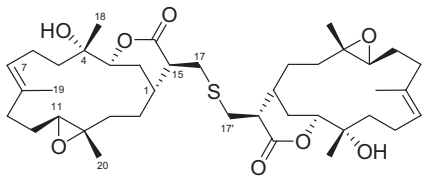
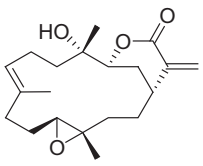


Figure 2. Key NOESY correlations of **1**.

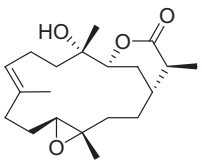
planar structure of **1** was established. The relative configurations of all chiral centers of **1** were mostly confirmed to be the same as these of **2** by comparison of the chemical shifts and coupling constants for protons of both compounds and was further proven by NOE correlations (Fig. 2). The structure of **1** was thus found to possess the (1R^*, 3R^*, 4S^*, 11S^*, 12S^*, and 15S^*)-relative configuration. Furthermore, the absolute structure of **1** was suggested to be the same as that of dihydrosinularin (**3**), as both compounds have the same sign of optical rotation and similar $[\alpha]_D$ values, and on the basis that **3** has been isolated from the same organism.[13,14] Therefore, the structure of thioflexibilolide A (**1**) was established and was found to be the first biscembranoid with a sulfide linkage.



1



2



3

The anti-inflammatory activity of **1** against the accumulation of pro-inflammatory iNOS and COX-2 proteins in microglial cells stimulated with LPS was evaluated according to a previous study.[15] It was found that at 10 μM compound **1** did not show cytotoxicity against microglial cells but significantly reduced the iNOS expression to 26.2 ± 11.2%, relative to the control cells treated with LPS only. By measuring the ability to reduce the 6-OHDA (6-hydroxydopamine)-induced neurotoxicity in neuroblastoma SH-SY5Y, a human dopaminergic neuron often used for the study of Parkinson's disease,[16,17] the neuroprotective effect of **1** against the damage of 6-OHDA toward SH-SY5Y cells was assayed by a method reported previously.[18] It was observed that the cytotoxicity of

6-OHDA on SH-SY5Y cells was significantly reduced by pretreatment of **1** at various concentrations. The relative neuroprotective activities of **1** at 0.001, 0.01, 0.1, 1, and 10 μM were 37.2 ± 4.3%, 73.2 ± 4.0%, 30.8 ± 8.6%, 31.2 ± 6.2% and 29.9 ± 8.5%, respectively. Thus, **1** exhibited significant neuroprotective activity at 0.01 μM. From the above neurological activity results, it seems that further investigation of **1** for its therapeutic potential for neurodegenerative diseases is worthwhile.

Acknowledgments

The financial support of this work was provided by the National Science Council (NSC-98-2113-M-110-002-MY3) and Ministry of Education (98C031702) of Taiwan awarded to J.-H.S.

References and notes

1. Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165–237, and previous reviews in this series.
2. Chao, C.-H.; Hsieh, C.-H.; Chen, S.-P.; Lu, C.-K.; Dai, C.-F.; Wu, Y.-C.; Sheu, J.-H. *Tetrahedron Lett.* **2006**, *47*, 2175–2178.
3. Chao, C.-H.; Hsieh, C.-H.; Chen, S.-P.; Lu, C.-K.; Dai, C.-F.; Sheu, J.-H. *Tetrahedron Lett.* **2006**, *47*, 5889–5891.
4. Tseng, Y.-J.; Ahmed, A. F.; Dai, C.-F.; Chiang, M.-Y.; Sheu, J.-H. *Org. Lett.* **2005**, *7*, 3813–3816.
5. Ahmed, A. F.; Su, J.-H.; Shiue, R.-T.; Pan, X.-J.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. *J. Nat. Prod.* **2004**, *67*, 592–597.
6. Ahmed, A. F.; Tai, S.-H.; Wen, Z.-H.; Su, J.-H.; Wu, Y.-C.; Hu, W.-P.; Sheu, J.-H. *J. Nat. Prod.* **2008**, *71*, 946–951.
7. Lu, Y.; Huang, C.-Y.; Lin, Y.-F.; Wen, Z.-H.; Su, J.-H.; Kuo, Y.-H.; Chiang, M. Y.; Sheu, J.-H. *J. Nat. Prod.* **2008**, *71*, 1754–1759.
8. Su, J.-H.; Ahmed, A. F.; Sung, P.-J.; Chao, C.-H.; Kuo, Y.-H.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 1134–1139.
9. Su, J.-H.; Tseng, Y.-J.; Huang, H.-H.; Ahmed, A. F.; Lu, C.-K.; Wu, Y.-C.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 850–852.
10. Ahmed, A. F.; Hsieh, Y.-T.; Wen, Z.-H.; Wu, Y.-C.; Sheu, J.-H. *J. Nat. Prod.* **2006**, *69*, 1275–1279.
11. Chen, B.-W.; Su, J.-H.; Dai, C.-F.; Wu, Y.-C.; Sheu, J.-H. *Bull. Chem. Soc. Jpn.* **2008**, *81*, 1304–1307.
12. Thioflexibilolide A (**1**): colorless oil; $[\alpha]_D^{25}$ −51 (*c* 1.52, $CHCl_3$); IR (neat) ν_{max} 3460, 1722 cm^{-1}; ^{13}C and ^1H NMR data (300 MHz; $CHCl_3$), see Table 1; ESIMS *m*/*z* 725 $[M+Na]^+$; HRESIMS *m*/*z* 725.4059 $[M+Na]^+$ (calcd for $C_{40}H_{62}O_8SNa$, 725.4063).
13. Weinheimer, A. J.; Matson, J. A.; Hossain, M. B.; van der Helm, D. *Tetrahedron Lett.* **1977**, *18*, 2923–2926.
14. Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Schonholzer, P.; Coll, J. C. *Aust. J. Chem.* **1978**, *31*, 1817–1824.
15. Jean, Y.-H.; Chen, W.-F.; Sung, C.-S.; Duh, C.-Y.; Huang, S.-Y.; Lin, C.-S.; Tai, M.-H.; Tzeng, S.-F.; Wen, Z.-H. *Br. J. Pharmacol.* **2009**, *158*, 713–725.
16. Kitamura, Y.; Kosaka, T.; Kakimura, J. I.; Matsuoka, Y.; Kohno, I.; Nomura, Y.; Taniguchi, T. *Mol. Pharmacol.* **1998**, *54*, 1046–1054.
17. Blum, D.; Torch, S.; Lambeng, N.; Nissou, M.; Benabid, A. L.; Sadoul, R.; Verna, J. M. *Prog. Neurobiol.* **2001**, *65*, 135–172.
18. Lee, K. Y.; Sung, S. H.; Kim, Y. C. *J. Nat. Prod.* **2006**, *69*, 679–681.