Vigulariol, a New Metabolite from the Sea Pen *Vigularia juncea*

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A new eunicellin-type diterpenoid, vigulariol (**1**), has been isolated from the sea pen *Vigularia juncea*. Metabolite **1** has been obtained previously as a side product from a chemical reaction; however, it was isolated for the first time from natural resources. Also, this study established the full structure of **1** and afforded the detailed assignments of ^{1}H and ^{13}C NMR spectral data.

Sea pen octocorals of the genus *Vigularia* have been studied by marine natural products research groups and have yielded numbers of various novel secondary metabolites, including steroids and fatty acid derivatives.[1,2] Previous chemical studies on *Vigularia juncea* (Pallas) (phylum Cnidaria, class Octocorallia, order Pennatulacea, family Virgulariidae) by our research group has revealed that this marine organism produces various metabolites, such as a sesquiterpenoid junceol A and two diterpenoids sclerophytin A and cladiellisin.[3] In the course of our continuing study on the chemical constituents of *V. juncea*, one new diterpenoid, vigulariol (**1**) was isolated. We described herein the structure and biological activity of compound **1**.

Compound **1** was obtained as a colorless oil. The HREIMS of **1** established a molecular formula of $C_{20}H_{32}O_3$ (M^+ at m/z 320.2348), implying five degrees of unsaturation. The EIMS of **1** showed peaks at m/z 320 $[M]^+$ and 302 $[M - H_2O]^+$, revealing the presence of a hydroxy group. The ^{13}C NMR spectral data of **1** (Table 1) measured in $CDCl_3$ showed the presence of twenty carbon signals, which were identified by the assistance of DEPT spectrum as four methyls, five sp^3 methylenes, one sp^2 methylene, seven sp^3 methines (including three oxymethines), two sp^3 quaternary carbons and one sp^2 quaternary carbon. By the assistance of HMQC and HMBC correlations, the ^{1}H NMR spectrum of **1** (Table 1) showed the presence of four methyl groups, including two methyls (δ 0.76,

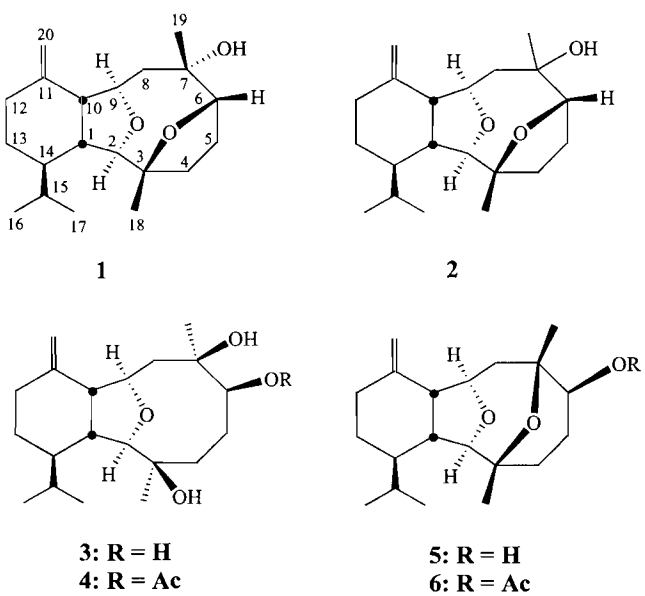

Chart 1.

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

C/H	^{1}H	^{13}C
1	2.18 m	45.9 (d)
2	3.65 s	90.1 (d)
3		85.8 (s)
4	1.78 (ddd, J = 12.5, 9.5, 9.5 Hz)	38.5 (t)
	2.18 m	
5	1.99 m	28.0 (t)
	2.45 m	
6	4.09 (t, J = 7.5 Hz)	87.1 (d)
7		74.5 (s)
8	1.85 (dd, J = 15.0, 2.5 Hz)	42.2 (t)
	2.27 (dd, J = 15.0, 4.5 Hz)	
9	4.06 (ddd, J = 8.0, 4.5, 3.0 Hz)	80.7 (d)
10	3.68 (t, J = 8.0 Hz)	47.6 (d)
11		148.0 (s)
12	2.05 (tt, J = 12.5, 2.0 Hz)	31.3 (t)
	2.24 (dt, J = 12.5, 3.0 Hz)	
13	1.00 (ddd, J = 13.0, 13.0, 3.0 Hz)	25.0 (t)
	1.71 m	
14	1.26 m	43.0 (d)
15	1.69 m	29.1 (d)
16	0.76 (d, J = 7.0 Hz)	15.5 (q)
17	0.96 (d, J = 7.0 Hz)	22.0 (q)
18	1.18 s	24.0 (q)
19	1.53 s	31.9 (q)
20	4.70 s, 4.72 s	110.0 (t)

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3H, d, $J = 7.0$ Hz and 0.96, 3H, d, $J = 7.0$ Hz) attached to a methine carbon and two methyls attached to oxygen-bearing carbons (δ 1.18, 3H, s and 1.53, 3H, s). The olefinic methylene protons were observed as singlets at δ 4.70 and 4.72. Furthermore, three oxygenated (δ 3.65, s, 4.06, ddd, $J = 8.0$, 4.5, 3.0 Hz, and 4.09, t, $J = 7.5$ Hz) methine protons and four nonoxygenated methine protons (δ 1.26, m, 1.69, m, 2.18, m, 3.68, t, $J = 8.0$ Hz) were identified.

The planar structure and all of the assignments of ^{1}H and ^{13}C NMR data of **1** were determined by the assistance of 2D NMR studies, including ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. ^{1}H–^{1}H COSY spectrum revealed proton sequences from H-1 to H-2, H-1 to H-10 and H-1 to H-14; H_2-4 to H-6; H_2-8 to H-10; H_2-12 to H_3-15 and H_3-16 and the long-range correlation between H-12 and H-20, as shown by the bold lines in Fig. 1. The HMBC correlations from H-20 to C-10 and C-12 indicated the connectivity between C-10 and C-12. The HMBC cross-peak from H-6 to C-3 suggested that C-3 and C-6 were linked through an oxygen to form a tetrahydrofuran ring. Furthermore, the methyl groups attached at C-3 and C-7 were confirmed by the HMBC correlations between H_3-18/C-2, C-3, C-4; and H_3-19/C-6, C-7, C-8. These data, together with the HMBC correlations between H-2 to C-4; H_2-5 to C-7; H_2-8 to C-6; H_3-16 and H_3-17 to C-14, C-15, unambiguously established the molecular framework of **1**.

Finally, the relative stereochemistry was well resolved by careful interpretation of the NOE correlations (Fig. 2). Key NOE correlations for **1** showed interactions between H-1 and H-10, and H-1 and H_3-18. Also, H_3-19 showed NOE responses with H-6 and H-10. Thus, H-1, H-6, H-10, H_3-18, and H_3-19 should be located on the β face. NOE correlations were also detected between H-14 and H-2, H-2 and H_3-18, revealing the α-orientations of H-2 and H-14, as suggested by a molecular model of **1**. On the basis of the above findings (Fig. 2), the structure of compound **1**, including the relative stereochemistry (1R^*, 2R^*, 3R^*, 6S^*, 7S^*, 9R^*, 10R^*, and 14R^*) was unambiguously established.

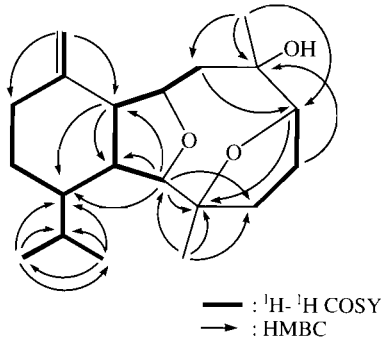

Fig. 1. ^{1}H–^{1}H COSY and HMBC correlations for **1**.

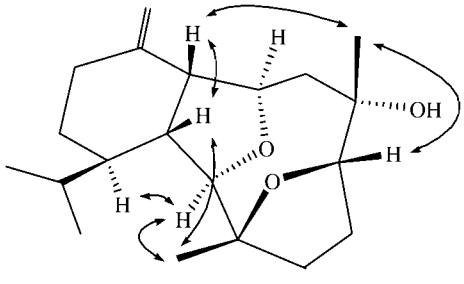

Fig. 2. Selective NOESY correlations of **1**.

After the structure determination of **1**, we found that this compound has been obtained previously as a side product **2** during an enantioselective total synthesis of sclerophytins A (**3**) and B (**4**).[4] This synthetic route not only successfully prepared **3** and **4**, but also caused the structure revision for both compounds from the originally reported structures, **5** and **6**, respectively.[5,6] However, our study has led to the isolation of **1** for the first time from natural sources. In addition, we successfully elucidated the full structure of **1** (including the absolute structure, as shown in formula **1**, as sclerophytin A (**3**) also has been isolated from *V. juncea*[3]). Moreover, our work also provides the full assignment for the ^{1}H and ^{13}C NMR spectral data of **1**.

Vigulariol (**1**) has thus been found to be a diterpenoid related to eunicellin-type metabolites, which have been isolated previously from gorgonians and soft corals.[7–9] Also, **1** has been shown to exhibit cytotoxicity against A 549 (human lung adenocarcinoma) cell culture system with IC_{50} of 18.33 μg/mL.

Experimental

General Methods. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. The IR spectrum was recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Bruker AVANCE DPX300 FT-NMR at 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H, respectively, in $CDCl_3$. EIMS was obtained with a VG Quattro GC/MS spectrometer. HREIMS spectral data were recorded on a Finnigan MAT-95XL mass spectrometer. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Isolation and Purification. The sea pen *V. juncea* (0.8 kg fresh wt) was collected by hand at the Penghu Islands, located on the west coast of Taiwan, in August 2000, at depths of 0.3–0.5 m; it was stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Resources, National Sun Yat-Sen University (specimen no. KTSC-103). The freeze-dried material was minced and extracted exhaustively with EtOAc (3 L × 5). The organic layers were combined and concentrated under vacuum to afford a dark brown viscous residue (8.7 g), which was subjected to column chromatography on silica gel, using hexane, hexane and EtOAc mixture of increasing polarity, and finally pure EtOAc, to yield 12 fractions. Fraction 8 was eluted with hexane–EtOAc (5:1); it was further purified by normal phase HPLC (5 μm, 25 mm × 250 mm), run isocratically by applying hexane–EtOAc (5:1) with a flow rate of 5 mL/min, to afford 3.5 mg of compound **1** (t_R 50.5 min).

Vigulariol (1): colorless oil (3.5 mg); $[\alpha]_D^{27}$ 3.6° (*c* 0.24, $CHCl_3$); IR (neat): ν_{max} 3437, 2932, 1645, 1373, 1249; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel. int.) 320 [8, $(M)^+$], 302 [2, $(M - H_2O)^+$], 277 (2), 259 (3), 179 (25), 85 (100); HREIMS *m/z* 320.2348 (calcd for $C_{20}H_{32}O_3$, 320.2353).

Cytotoxicity testing. Cytotoxicity assays of the test compound **1** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[10,11]

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References

1 A. S. R. Anjaneyulu, C. V. S. Prakash, U. V. Mallavadhani, and K. V. S. Raju, *J. Indian Chem. Soc.*, **69**, 150 (1992).

2 A. S. R. Anjaneyulu and C. V. S. Prakash, *Indian J. Chem.*, **33B**, 55 (1994).

3 S.-P. Chen, P.-J. Sung, C.-Y. Duh, C.-F. Dai, and J.-H. Sheu, *J. Nat. Prod.*, **64**, 1241 (2001).

4 P. Bernardelli, O. M. Moradei, D. Friedrich, J. Yang, F. Gallou, B. P. Dyck, R. W. Doskotch, T. Lange, and L. A. Paquette, *J. Am. Chem. Soc.*, **123**, 9021 (2001).

5 P. Sharma and M. Alam, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 2537.

6 M. Alam, P. Sharma, A. S. Zektzer, G. E. Martin, X. Ji, and D. van der Helm, *J. Org. Chem.*, **54**, 1896 (1989).

7 O. Kennard, D. G. Watson, L. Riva di Sanseverino, B. Tursch, R. Bosmans, and C. Djerassi, *Tetrahedron Lett.*, **9**, 2879 (1968).

8 R. Kazlauskas, P. T. Murphy, R. J. Wells, and P. Schönholzer, *Tetrahedron Lett.*, **18**, 4643 (1977).

9 V. Roussis, W. Fenical, C. Vagias, J.-M. Kornprobst, and J. Miralles, *Tetrahedron*, **52**, 2735 (1996), and references cited therein.

10 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, and M. R. Boyd, *Cancer Res.*, **48**, 589 (1988).

11 R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, and M. R. Boyd, *Cancer Res.*, **48**, 4827 (1988).