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Prorocentin-4, a novel linear polyketide from a marine dinoflagellate *Prorocentrum* sp.

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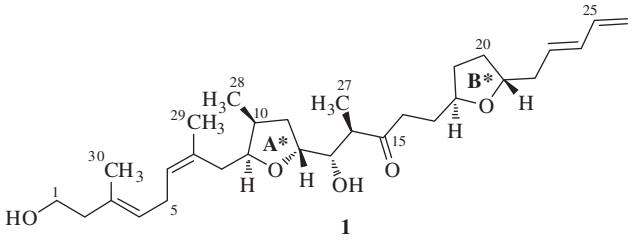
ABSTRACT

Prorocentin-4, isolated from a cultured marine dinoflagellate *Prorocentrum* sp. was possessed of two tetrahydrofuran rings, conjugated dienes, four branched methyl groups, and a ketone moiety. The gross structure was elucidated by spectroscopic studies and the relative stereostructure was determined on the basis of spectral data.

Marine dinoflagellates have been proved to be a rich source of the biologically significant and chemically complex natural products.[1] In particular, the dinoflagellates of the genus *Prorocentrum* are distributed in temperate and tropical oceans worldwide,[2] and many *Prorocentrum* species are known to produce unique bioactive secondary metabolites with a variety of chemical skeletons. For example, macrocyclic lactones, such as prorocentrolides,[3] hoffmanniolide,[4] and belizeanolide[5] were isolated from different OA-producing *Prorocentrum* species of *P. lima*, *P. maculosum*, *P. hoffmannianum,* and *P. belizeanum*. Spiro-prorocentrimine, a macrocyclic imine, was obtained from a non-OA-producing *Prorocentrum* species.[6] In addition, the linear polyether, prorocentin, was produced by Taiwanese strain of *P. lima*.[7] We have continued investigations on natural products from benthic *Prorocentrum* spp. living in coral reefs. Previously, two 17-membered macrolides from *Prorocentrum* sp. strain PL040104002 were reported.[8] Herein, we describe the isolation and structure elucidation of a novel linear polyketide, prorocentin-4 (**1**), which was possessed of two tetrahydrofuran rings and tetraenes moieties from the same *Prorocentrum* strain (PL040104002).

The dinoflagellate was isolated from the wash-off epiphytes of *Sargassum* spp., collected off South Bay, southern Taiwan. *Prorocentrum* sp. PL040104002 was cultivated at 25 ± 2 °C for 4 weeks in a natural seawater medium enriched with K nutrients.[9]



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*The relative stereochemistry between A and B rings is not correlated.

The harvested cells (1.24 kg wet weight, from 600 L culture) were extracted with methanol. After partial solvent evaporation, the aqueous methanol was fractionated into *n*-hexane, dichloromethane, and *n*-butanol solutions. The dichloromethane-soluble substance was subjected to a silica gel flash column (CH_2C1_2/MeOH, stepwise) and then was followed by LH-20 (MeOH) gel filtration chromatography. Final purification was achieved by reversed-phase HPLC (CH_3CN/H_2O = 55:45) to afford prorocentin-4 (**1**, 2.2 mg).

Prorocentin-4, $[\alpha]_D^{24}$ −8.9 (*c* 0.0005 g cm^{-3} in MeOH), was obtained as a colorless amorphous solid. UV absorption at 225 nm (log ε 3.2) was indicative of conjugated diene(s) and IR absorptions at 3442, 2929, and 1571 cm^{-1} were attributed to hydroxyl and alkyl groups. High resolution mass spectroscopy (HR-ESIMS) and the total number of carbons determined by ^{13}C NMR spectra suggested

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a molecular formula of $C_{30}H_{48}O_5$ (observed $[M+Na]^+$, m/z 551.3392; calcd for $C_{30}H_{48}O_5Na$, m/z 551.3394, Δ −0.2 mmu). Accordingly, seven sites of unsaturation were derived from the molecular formula. The resonances of five sp^2 methines (δ_C 121.0, 122.7, 131.0, 133.1, 137.1), one sp^2 methylene (δ_C 115.4), two sp^2 quaternary carbons (δ_C 134.6, 136.2), and a ketone (δ_C 215.2) in the ^{13}C NMR spectrum accounted for five of the seven sites of unsaturation. The remaining two sites of unsaturation had to be accounted for ring structures. Since HSQC and DEPTs data showed that 46 of the 48 hydrogen atoms were attached to carbons (4 methyl groups, 11 methylenes, and 12 methines), there should be 2 hydroxyl groups in **1**.

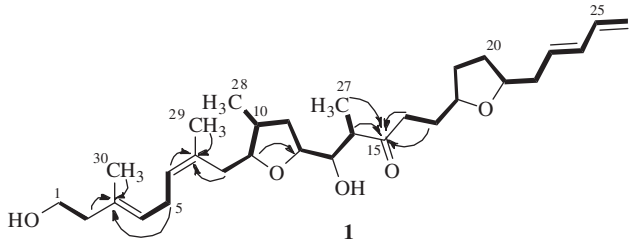

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Figure 1. Connectivities established by ^1H–^1H COSY, HSQC and HMBC. Heavy lines indicate the connectivities assigned on the basis of ^1H–^1H COSY and HSQC. Arrows denote the correlations between protons (tail) and carbons (head) around the quaternary carbons observed in the HMBC.

Detailed analyzes of NMR spectra, including ^1H–^1H COSY, selective TOCSY, and HSQC spectra of **1** led to assignments of proton connectivities for four partial structures of (**a**) from C1 to C2, (**b**) from C4 to C6, (**c**) from C8 to C14, including C10 and C14 methyl groups, and (**d**) from C16 and C26. These partial structures are designated by bold lines in Figure 1. Connectivities of four partial structures (**a**–**d**) were assigned on the basis of HMBC data. HMBC cross-peaks of H2/C3, H5/C3, H30/C2, H30/C3, and H30/C4 revealed connectivities of **a** to **b** through an olefinic quaternary carbon C3, to which a methyl group H30 was attached. Similarly, HMBC cross-peaks were observed for H5/C7, H6/C7, H8/C7, H29/C6, H29/C7, and H29/C8, thereby, revealing that the other olefinic quaternary carbon C7 connected **b** and **c**. The HMBC spectrum showed the correlations from sp^3 methine H14 (δ_H 2.78), methylene H16 (δ_H 2.60, 2.68), and a methyl group H27 (δ_H 1.12) to C15 (δ 215.2), indicating that partial structures **c** and **d** were connected through a ketone carbonyl (C15). Thus, the whole carbon backbone was able to be assembled, leaving the position of two hydroxyl groups and two ether rings to be determined. Of the six signals observed for oxygenated carbons, two hydroxyl groups were

Table 1
^1H and ^{13}C NMR data of **1** ($CDCl_3$ and CD_3OD)

No.	In $CDCl_3$[a]		No.	In CD_3OD[b]	
	δ_C	δ_H (mult. J in Hz)		δ_C	δ_H (mult. J in Hz)
1	62.5 (t)	3.60 (t, 6.4)	1	62.9 (t)	3.51 (t, 7.1)
2	31.5 (t)	2.27 (m)	2	32.5 (t)	2.25 (m)
3	136.2 (s)		3	136.0 (s)	
4	121.0 (d)	5.15 (tq, 7.4, 1.3)	4	122.7 (d)	5.20 (tq, 7.2, 1.2)
5	42.1 (t)	2.71 (m)	5	43.0 (t)	2.75
6	122.7 (d)	5.21 (t, 6.8)	6	124.0 (d)	5.29 (t, 7.0)
7	134.6 (s)		7	135.8 (s)	
8a	29.2 (t)	2.05 (m)	8a	30.2 (t)	2.11 (dd, 12.3, 6.6)
8b		2.22 (m)	8b		2.25 (dd, 12.3, 7.8)
9	82.5 (d)	3.82 (m)	9	83.9 (d)	3.93 (ddd, 5.1, 6.6, 7.8)
10	36.0 (d)	2.27 (m)	10	37.2 (d)	2.29 (m)
11a	36.0 (t)	1.60 (m)	11a	36.6 (t)	1.63 (ddd, 12.1, 7.7, 3.6)
11b		2.13 (ddd, 12.3, 7.6, 7.2)	11b		2.14 (ddd, 12.1, 7.7, 7.9)
12	77.4 (d)	4.03 (dt, 3.0, 7.6)	12	77.9 (d)	4.13 (dt, 2.1, 7.7)
13	76.3 (d)	3.48 (m)	13	76.6 (d)	3.48 (dd, 8.3, 2.1)
14	49.1 (d)	2.78 (dq, 7.7, 7.1)	14	50.5 (d)	2.91 (dq, 8.3, 7.1)
15	215.2 (s)		15	216.9 (s)	
16a	39.4 (t)	2.60 (ddd, 17.9, 8.6, 6.4);	16a	40.4 (t)	2.64 (m)
16b		2.68 (m)	16b		2.71 (m)
17a	29.4 (t)	1.71 (m)	17a	30.5 (t)	1.73 (m)
17b		1.80 (m)	17b		1.77 (m)
18	78.6 (d)	3.80 (m)	18	80.1 (d)	3.83 (m)
19a	30.9 (t)	1.48 (m)	19a	31.4 (t)	1.51 (m)
19b		1.51 (m)	19b		1.58 (m)
20a	30.5 (t)	1.88 (m)	20a	31.8 (t)	1.95 (m)
20b		1.93 (m)	20b		1.97 (m)
21	78.8 (d)	3.85 (m)	21	80.3 (d)	3.88 (m)
22a	39.2 (t)	2.22 (m)	22a	40.1 (t)	2.26 (m)
22b		2.34 (m)	22b		2.31 (m)
23	130.1 (d)	5.66 (dt, 15.2, 7.2)	23	131.9 (d)	5.70 (dt, 15.2, 7.2)
24	133.1 (d)	6.07 (dd, 15.2, 10.3)	24	134.5 (d)	6.11 (dd, 15.2, 10.3)
25	137.1 (d)	6.23 (dt, 16.8, 10.3)	25	138.5 (d)	6.31 (dt, 17.1, 10.3)
26a	115.4 (t)	4.96 (d, 10.3)	26a	115.7 (t)	4.95 (d, 10.3)
26b		5.09 (d, 16.8)	26b		5.09 (d, 17.1)
27	14.4 (q)	1.12 (d, 7.1)	27	14.3 (q)	1.06 (d, 7.0)
28	14.1 (q)	0.89 (d, 7.1)	28	14.4 (q)	0.93 (d, 7.0)
29	23.6 (q)	1.60 (d, 1.3)	29	25.7 (q)	1.61 (d, 1.1)
30	16.2 (q)	1.57 (s)	30	16.1 (q)	1.57 (s)

[a] Reference to residual solvent $CDCl_3$ signals at δ_H 7.24 and δ_C 77.0 and measured at 25 °C, 400 MHz for ^1H, and 100 MHz for ^{13}C. ^{13}C multiplicities were assigned from DEPT experiments.

[b] Reference to residual solvent CD_3OD signals at δ_H 3.3 and δ_C 49.0 and measured at 25 °C, 400 MHz for ^1H. and 100 MHz for ^{13}C. ^{13}C multiplicities were assigned from DEPT experiments.

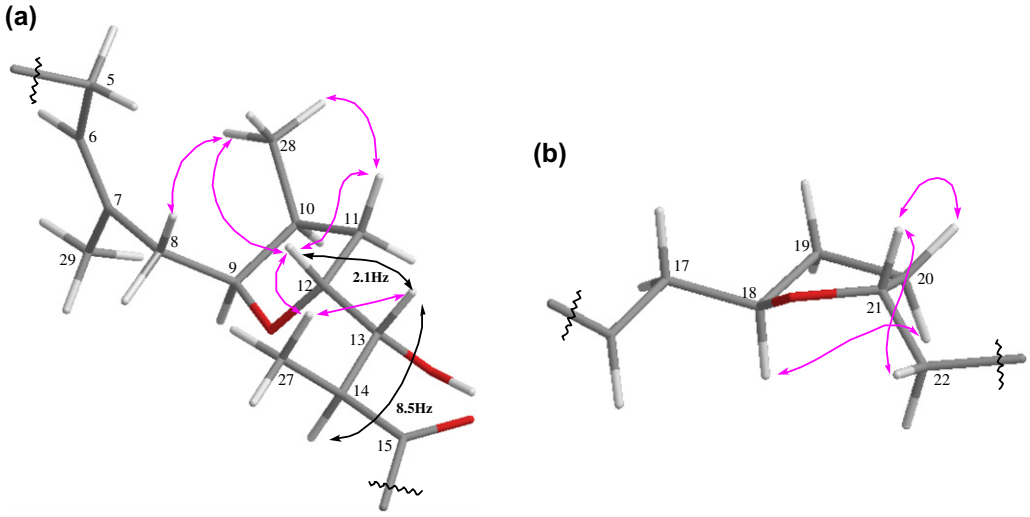

Figure 2. The relative stereochemistry for (a) C9–C15 and (b) C18–C21. The pink-arrows show NOESY correlations, and the black-arrows show the $^{3}J_{H,H}$ coupling constants.

assigned to be attached to terminal oxymethylene (C1, δ_C 62.5, δ_H 3.60) and oxymethine (C13, δ_C 76.3, δ_H 3.48) because of a cross peak between H13 and a broad-doublet hydroxyl proton in COSY spectrum. Two five-membered ether rings were constructed, based on the relative positions of four oxymethines (C9, δ_C 82.5; C12, δ_C 77.4; C18, δ_C 78.6; C21, and δ_C 78.8). The ether linkage between C9/C12 was further confirmed by HMBC correlations of H9 (δ_H 3.82) to C12 and H12 (δ_H 4.03) to C9. Therefore, a planar structure of prorocentin-4 (**1**) was elucidated. A summary of the assignments of all the protons and carbons mentioned above are shown in Table 1.

The geometry of $\Delta^{3,4}$, $\Delta^{23,24}$, and $\Delta^{25,26}$ double bonds was determined to be *E* by the ^{1}H–^{1}H coupling constant ($J_{23,24}$ = 15.2 Hz, and $J_{25,26b}$ = 16.8 Hz) as well as the ^{13}C chemical shifts. The carbon chemical shifts of the C30 vinyl methyl group (δ_C 12.6) suggested that the trisubstituted $\Delta^{3,4}$ double bond was *E* configuration. The carbon chemical shift of the C29 vinyl methyl group (δ_C 23.6) suggested that the trisubstituted $\Delta^{6,7}$ double bond was *Z* configuration.[10] These assignments were further supported by the NOESY data (H2/H4, H5/H8). The relative stereochemistry of five chiral centers around the 3-methyl *trans*-2,5-hydrofuran ring was elucidated by detailed analysis of NOESY correlations, $^{3}J_{H,H}$, and $^{2,3}J_{C,H}$,[11] as shown in Figures 2 and 3. The geminal and vicinal coupling constants were obtained from the selective TOCSY, 2D J-resolved, and DQF-COSY spectra,[12] measured in CD_3OD. The measurement of heteronuclear coupling constants ($^{2,3}J_{C,H}$) relied on the analysis of G-BIRD$_{R,X}$-HSQMBC.[13] The relative configurations of the chiral centers present in the ring at C9–C12 were initially established with NOESY analysis. The clear cross peaks were observed between the methyl group (H28) at C10 and H12, H28 and H11a, and H28 and H8a (Fig. 2). These data imply a *cis* relationship for the H9/H10 proton pair and a *trans* relationship for H10/H12. This assignment can be further supported by $^{3}J_{H,H}$, and $^{2,3}J_{C,H}$ values. The $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ coupling constants for the tetrahydrofuran ring were shown in Figure 3a. The medium coupling constants of $^{3}J_{H10,H11b}$ = 7.9 Hz, $^{3}J_{H12,H11a}$ = 7.7 Hz, and $^{3}J_{H12,H11b}$ = 7.7 Hz were elucidated by the near eclipsed conformation along C10–C11 and C11–C12 strains. The heteronuclear coupling constants of $^{3}J_{C28,H9}$ = 7.5 Hz, $^{3}J_{C28,H11a}$ = 3.8 Hz, $^{3}J_{C28,H11b}$ = 7.0 Hz, $^{3}J_{C9,H11a}$ = 6.0 Hz, $^{3}J_{C9,H11b}$ = 2.8 Hz, $^{3}J_{C13,H11b}$ = 7.1 Hz, and $^{2}J_{C12,H11b}$ = −6.2 Hz were measured (Fig. 3a). As shown in Figure 3b, $^{3}J_{H12,13}$ (2.1 Hz) revealed the typical value of gauche-orientation of H12/H13. The coupling constants of $^{2}J_{C13,H12}$ (−2.3 Hz) and $^{3}J_{C11,H13}$ (2.9 Hz) indicate that H12 is anti to C13–OH and H13 is gauche to C11. With respect to C13–C14 bond, the values of $^{3}J_{H13,14}$ (8.5 Hz) indicated that relative stereochemistry between H13 and H14 was anti-oriented. The $^{3}J_{C27,H13}$, $^{3}J_{C15,H13}$, and $^{2}J_{C13,H14}$ were measured to be 3.5, 3.2, and −5.0 Hz, respectively. These indicate that H13 is gauche to C27 and H14 is gauche to C13-OH. NOESY correlation was observed for H12/H27 which supported the relative assignment as showed in Figure 3c. These data unambiguously established the *syn*-H12/H13 and *anti*-H13/H14 configuration (Fig. 2). The anti-oriented configuration of the C18–C21 tetrahydrofuran ring was proposed by the lack of NOE between H18/H21.

Prorocentin-4 (**1**) is a new type of linear polyether from a benthic marine dinoflagellate belonging to genus *Prorocentrum*. The compound is possessed of two tetrahydrofuran rings, tetraenes, four branched methyl groups, and a ketone moiety. The biosynthetic pathway for the functional group of 3-methyl *trans*-2,5-dihydrofuran seems common in marine dinoflagellates. The

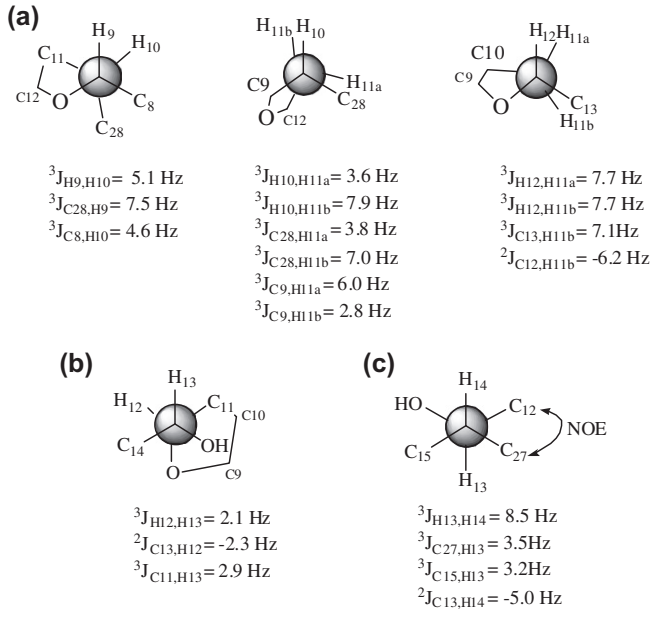

Figure 3. The relative stereochemistry determined for (a) the C9–C12 fragment, (b) C12–C13 fragment,and (c) C13–C14 fragment.

3-methyl *trans*-2,5-dihydrofuran moiety with the same stereostructure of *cis* H2/H3 and *trans* H3/H5 proton pairs was found in prorocentin-4 (**1**), amphidinolides (C, C2, F, and T1–T5)[14], amphidinin B[15], and gambieric acids (A–D).[16] These polyethers and macrolides were isolated from marine dinoflagellates belonging to genus of *Prorocentrum*, *Amphidinium,* and *Gambierdiscus*. Although, prorocentins, isolated form *P. lima* strain PL021117001 showed potent cytotoxic activities,[7] prorocentin-4 showed no evidence of cytotoxicity against CCRF-CEM human T-cell acute lymphoblastic leukemia cells and DLD-1 human colon adenocarcinoma cells in vitro (>40 μg/mL).

Acknowledgments

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