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Gymnodimine C, an Isomer of Gymnodimine B, from *Karenia selliformis*

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Gymnodimine C (**1**), an oxidized analogue of the spiro-imine algal toxin gymnodimine (**3**), was isolated from extracts of the cells of *Karenia selliformis*. The structure of gymnodimine C, determined by one- and two-dimensional ^{1}H NMR spectroscopy and mass spectrometry, was found to be isomeric with gymnodimine B (**2**) at C-18.

KEYWORDS: Gymnodimine; *Gymnodinium selliforme*; *Karenia selliformis*; shellfish toxin; spiro-imine

INTRODUCTION

Shellfish are sometimes contaminated by gymnodimine, a toxic spiro-imine, around New Zealand (*1*−*3*) and in the Mediterranean Sea (*4*). The presence of gymnodimine in shellfish gives rise to positive mouse bioassay screening tests for neurotoxic shellfish poisoning (NSP), despite the absence of known NSP toxins (e.g., brevetoxins) in the shellfish (*2*). Gymnodimine has been shown to be produced by *Karenia selliformis* (formerly *Gymnodinium selliforme*) (*2*, *5*) but may also be produced by other related species (*4*). To evaluate the potential for toxicity of gymnodimine in shellfish, we have commenced isolation of sufficient gymnodimine to permit oral toxicity studies on laboratory animals. During the course of these studies we have isolated large quantities of gymnodimine (**3**) (*6*) as well as small amounts of an oxidized analogue, gymnodimine B (**2**) (*5*) (**Figure 1**). Here we report the isolation and characterization of gymnodimine C (**1**), an oxidized analogue of gymnodimine that is isomeric with **2** at C-18.

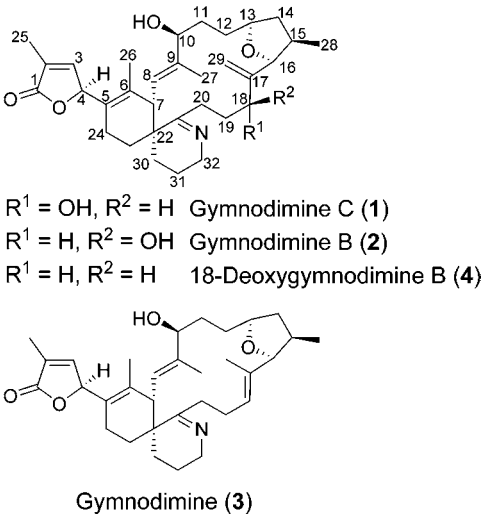

Figure 1. Structures of gymnodimine C (**1**), gymnodimine B (**2**), and gymnodimine (**3**). Also shown is 18-deoxygymnodimine B (**4**), a hypothetical isomer of **3** (*5*) from which **1** and **2** may have originated.

MATERIALS AND METHODS

General. Culturing of *K. selliformis* (CAWD79) and harvesting of its toxins and the analytical and preparative procedures used (HPLC, NMR, and MS) are as described previously (*5*) for the isolation of gymnodimine B (**2**).

Gymnodimine C (1). During fractionation of the cell extract from the culture by semipreparative HPLC during the purification of **2** (*5*), a peak was observed at 3.7 min that exhibited a UV spectrum similar to those of gymnodimine (**3**) (*5*) and gymnodimine B (**2**) (Figure 2 of ref *5*). Fractions containing this material were set aside for NMR and MS analyses, which identified the new component as an isomer of **2**, which we name gymnodimine C (**1**) (∼50 μg).

Gymnodimine C (**1**): EI-MS, *m*/*z* 523.3277 (M^+; $C_{32}H_{45}NO_5$ requires *m*/*z* 523.3298); for UV absorbance and HPLC retention data, see Figure 2 of ref *5*; for ^{1}H NMR assignments, see **Table 1**.

RESULTS AND DISCUSSION

During the isolation of gymnodimine B (**2**) from extracts of cells of *K. selliformis* grown in culture (*5*), a minor early-eluting peak was observed in the HPLC chromatogram that displayed a UV absorbance spectrum identical to those of gymnodimine (**3**) and gymnodimine B (**2**), consistent with the presence of a

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Table 1. ^{1}H NMR Chemical Shifts (δ) for Gymnodimine C (**1**), Gymnodimine B (**2**),[a] and Gymnodimine (**3**)[a] in $CDCl_3$ Containing 0.1% v/v C_5D_5N

	gymnodimine C	gymnodimine B[b]	gymnodimine[b]
H-3	6.91 (br m)	6.91 (br m)	6.90 (br m)
H-4	5.85 (br s)	5.84 (br s)	5.82 (br s)
H-7	3.63 (br d)	3.63 (br d)	3.63 (br d)
H-8	5.31 (d, J = 10.5 Hz)	5.28 (br d, J = 10.7 Hz)	5.28 (d, J = 11.0 Hz)
H-10	3.94 (dd, J = 11.2, 2.5 Hz)	3.94 (dd, J = 11.6, 2.8 Hz)	4.00 (br s)
H-11	1.97, 1.57	2.08, 1.48	2.04, 1.67
H-12	1.36, 1.17	1.40,[c] 1.15	1.42, 1.16
H-13	4.00 (m)	4.09 (br t)	4.08 (br m)
H-14	1.71, 1.78	1.77–1.82	1.60, 1.49
H-15	2.65 (m)	2.71 (m)	2.18 (m)
H-16	3.89 (d, J = 8.7 Hz)	3.85 (d, J = 10.5 Hz)	3.97 (br s)
H-18	3.97 (m)	4.11 (d, J = 10.1 Hz)	5.06 (br s)
H-19	1.95, 2.06	1.49, 2.40[g]	2.03, 2.47
H-20	2.21, 2.62	2.23,[g] 2.64	2.48, 2.48
H-23	1.57, 1.77	1.54,[d] 1.77	1.49, 1.77
H-24	1.57, 2.06	1.54,[d] 2.06	1.55, 2.07
H-25	1.96 (br t, J = 1.8 Hz)	1.96 (br t, J = 1.5 Hz)	1.96 (br t, J = 1.7 Hz)
H-26	1.71 (br s)	1.71 (br s)	1.67 (br s)
H-27	1.78 (br d, J = 1.3 Hz)	1.91 (br s)	1.81 (br s)
H-28	0.98 (d, J = 6.6 Hz)	0.96 (d, J = 6.3 Hz)	1.09 (d, J = 7.1 Hz)
H-29	5.18 (br s)	5.32 (br s)	1.54 (br s)
	4.99 (br s)	5.19 (br d, J = 1.4 Hz)[f]	
H-30	1.57, 1.91	1.54,[e] 1.95	1.51, 1.94
H-31	1.57, 1.57	1.54,[e] 1.54[e]	1.51–1.57
H-32	3.45–3.52	3.57, 3.40	3.58, 3.39

[a] Assignments derived from a combination of ^{1}H, COSY, HMBC, and HSQC NMR data. [b] From ref *5*. Chemical shifts correspond closely to those determined by ref *7*. [c–e] Tentative assignment derived from analyses of partly overlapping COSY and/or TOCSY correlation peaks. [f] Determined with resolution enhancement (LB = –1.5, GB = 0.33). [g] Assignments are interchangeable.

butenolide moiety. Examination by mass spectrometry revealed an apparent molecular ion with the same atomic composition as that of **2**. Although NMR studies of the compound were severely constrained by the small amount of material, the structure and full ^{1}H NMR assignments of **1** were determined by application of one- and two-dimensional NMR spectroscopy.

The ^{1}H NMR spectrum of gymnodimine C (**1**), determined in $CDCl_3$ containing 0.1% C_5D_5N, included signals attributable to three olefinic methyl groups at 1.96 (H-25), 1.71 (H-26), and 1.78 (H-27) ppm, and a secondary aliphatic methyl group signal at 0.98 ppm (H-28). The chemical shifts and coupling constants for these signals are very close to those reported previously for the H-25−28 resonances of gymnodimine (**3**) and gymnodimine B (**2**) (*5*). The presence of two olefinic proton signals at 5.18 (H-29b) and 4.99 ppm (H-29a), attributable to the presence of an exocyclic 17(29)-olefinic methylene group, and low-field signals centered at 3.89 (H-16), 3.94 (H-10), 3.97 (H-18), and 4.00 ppm (H-13) (**Table 1**) was consistent with the formulation of **1** as 18-epi-**2**. Although the H-13 and H-18 multiplets were not resolved in the 400 MHz ^{1}H NMR spectrum of gymnodimine C, two-dimensional COSY and TOCSY data adequately differentiated the chemical shifts of the overlapping multiplets. Characteristic spectroscopic differences were observed in the H-10, H-13, H-16, and H-18 resonances of gymnodimines B and C (**Figure 2**). In particular, H-18 appears as a doublet at 4.11 ppm in **2**, whereas in **1** this resonance appears as a multiplet at 3.97 ppm and is partly concealed under the broad triplet-like H-13 signal.

Detailed analyses of COSY spectra optimized for the detection of short- and long-range couplings, and a series of TOCSY spectra acquired with mixing times of 8, 30, 100, and 300 ms, led to the complete assignment of the ^{1}H resonances of gymnodimine C (**1**) presented in **Table 1**. Notable features of

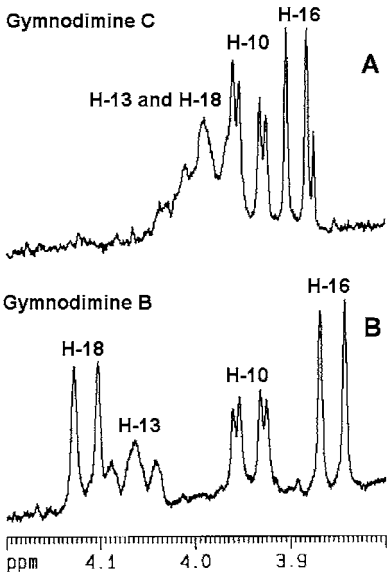

Figure 2. Selected region of the ^{1}H NMR spectrum of **A.** gymnodimine C (**1**) and **B.** gymnodimine B (**2**), showing characteristic spectral differences between the two compounds.

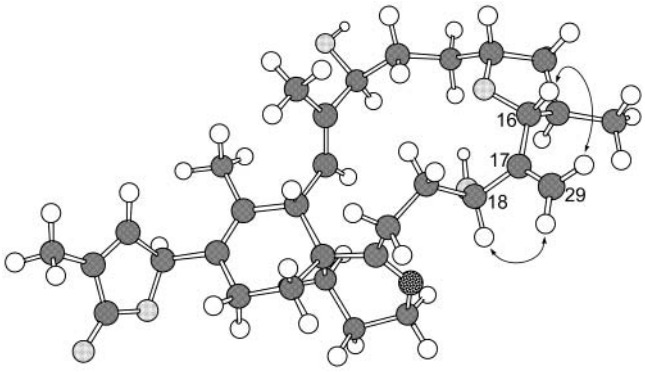

Figure 3. Preferred conformation of gymnodimine C (**1**) as predicted by molecular modeling (MM2*), with NOE enhancements and ROESY correlations observed for H-29$_E$, H-29$_Z$, H-16 and H-18 shown.

this analysis were the occurrence of the H-19 methylene proton signals at 1.95 and 2.06 ppm (revealed by COSY and TOCSY correlations with H-18) and the occurrence of the H-11 methylene protons at 1.57 and 1.97 ppm (revealed by COSY and TOCSY correlations with H-10). The equivalent resonances of gymnodimine B (**2**) occur at 1.49 and 2.40 ppm (H-19) and 1.48 and 2.08 ppm (H-11), respectively. These differences, along with smaller differences in the chemical shifts of H-10, H-13, and H-27 of **1** and **2** (**Table 1**), are consistent with the view that changing the stereochemistry at C-18 causes significant changes in the conformation of the macrocyclic rings of **1** and **2**.

Molecular modeling studies identified a low-energy macrocyclic ring conformation (**Figure 3**) in which there was a hydrogen bond between the 18-OH group and the 13(16)-ether oxygen atom. ROESY and NOE difference data identified NOEs between H-29$_Z$ (4.99 ppm) and H-16 (3.91 ppm, 10.8%) and between H-29$_E$ (5.18 ppm) and H-18 (3.97 ppm, 3.1%). These observations are consistent with the view that H-16 (3.91 ppm) and H-18 (3.97 ppm) are both β-oriented and that the18-OH group of gymnodimine C (**1**) is therefore α-oriented.

Gymnodimine C (**1**) contains an exocyclic methylene δ to the imine carbon, a feature it also shares with gymnodimine B

(**2**), the pinnatoxins and spirolides but not with gymnodimine (**3**) (*5*). It is unlikely that **1** and **2** are produced artifactually from **3** during extraction and isolation, because this would require both isomerization and oxidation. Furthermore, the observation that **1** and **2** were present in the cellular extract but were not detected in the culture filtrate, which contained ~90% of the gymnodimine (**3**) present in the total culture (*5*), is consistent with the proposition that **1** and **2** are not artifacts produced from the more abundant **3** during isolation. Possibly, **1** and **2** originate from a precursor such as 18-deoxygymnodimine B (**4**), which itself is an isomer of **3**, through oxidation (*5*). Such a precursor could also have a role in the biogenesis of **3**. From what little is known about the structure−activity relationships within the fast-acting spiro-imine toxins, it can be expected that **1** and **2** will be toxic, as the spiro-imine moiety appears to be crucial to the biological activity of these compounds.

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Supporting Information Available: COSY, TOCSY, and ROESY NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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