Palytoxin Analogs from the Dinoflagellate *Ostreopsis siamensis*

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Palytoxin (**1**, $C_{129}H_{223}N_3O_{54}$, Figure 1) is one of the most potent and complex marine toxins.[1] Though first isolated from the zoanthid *Palythoa toxica*, its biogenetic origin was questioned because of marked seasonal and regional variations,[2] but the question was never pursued. Sporadic occurrence of palytoxin in an alga,[3] crabs,[4] and a herbivorous fish[5] also suggested that the toxin might be produced by a microorganism and transmitted to the marine food chain, as is the case with ciguatera, where the epiphytic dinoflagellate *Gambierdiscus toxicus* produces ciguatoxins and maitotoxin that are isolated from fish.[6] *Ostreopsis siamensis* drew our attention because of its close taxonomical relationship to *G. toxicus* and the potency of its toxins, named ostreocins.[7] In this Communication, we report the major ostreocin as a palytoxin analog.

O. siamensis (SOA1 strain) was collected at Aka Island, Okinawa, Japan, and grown for 30 days under the same culture conditions as *G. toxicus*.[7b] Cells harvested by filtration were extracted three times with MeOH/H_2O (1:1) at room temperature and then with MeOH/H_2O/HOAc (50:50:0.2) under reflux. Purification of ostreocins from the combined extracts was carried out by solvent partition and column chromatography.[8] Ostreocins were resolved into five constituents. Purification was monitored by mouse assay following characteristic UV maxima. The major constituent, ostreocin D, accounted for 70% of total toxicity. From 936 L of culture, 3.8 mg of ostreocin D was recovered.

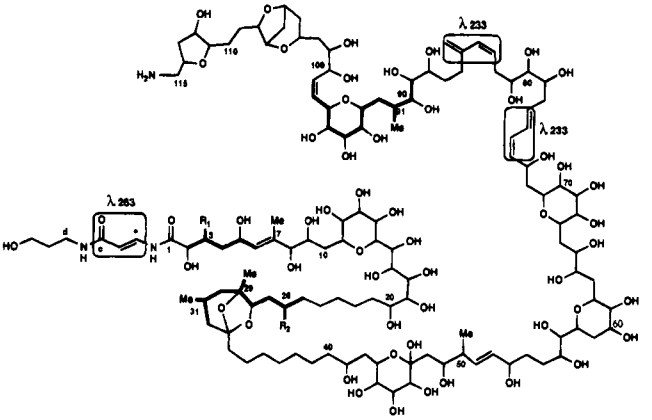

Figure 1. Planar structure of palytoxin (**1**) and ostreocin D (3,26-bisdesmethyldeoxypalytoxin). Bold lines denote connectivities elucidated by DQF-COSY, TOCSY, HSQC, and HMBC data. UV chromophores are bracketed.[12] Palytoxin, $R_1 = R_2 = Me$. Ostreocin D, $R_1 = R_2 = H$; OH → H (position not located).

Ostreocin D (OST-D) was a colorless amorphous solid: positive to ninhydrin; LD_{50} in mice, 0.75 μg/kg ip; $[\alpha]^{23}_D$ + 16.6 (*c* 0.121, H_2O); UV λ_{max} (H_2O) 234 (ε 35 000), 263 (ε 22 000) nm. Like palytoxin (**1**), OST-D gave two spots (R_f 0.61, 0.44) on TLC (silica gel, BuOH/H_2O/HOAc (4:2:1)).[4] The R_f values were comparable with those of **1** (R_f 0.63, 0.46). The UV spectrum indicates that OST-D shares the same chromophores with **1** (Figure 1). In the high mass range of the FABMS,[9] OST-D displayed cluster ions having a centroid at *m/z* 2636.51. The ion distribution pointed to a composition of $C_{127}H_{220}N_3O_{53}$ (MH^+, calcd 2636.47). Three nitrogen atoms in the molecule were further supported by triply charged ions in the electrospray ionization mass spectrum (ESIMS)[9]: *m/z* 2636.7 $(M + H)^+$, 1330.0 $(M + Na)^{2+}$, 894.3 $(M + 2Na)^{3+}$, 887.3 $(M + Na)^{3+}$. The difference in composition (C_2H_4O) between OST-D and palytoxin (**1**) suggested that two methyls and one hydroxyl of **1** were substituted by protons in OST-D. The ^1H NMR spectrum of OST-D[10] was virtually identical with that of **1**, except that OST-D displayed only two methyl doublets at about 0.95 ppm rather than four for **1**, and signals of H5 (4.59 ppm) and H6 (5.52 ppm) of **1** were shifted upfield to 4.48 and 5.46 ppm in OST-D. Extensive analysis of 2D NMR spectra (DQF-COSY, TOCSY, HSQC, and HMBC)[10] of **1** allowed us to follow proton connectivities in partial structures in the vicinity of the following methyl doublets at 0.95 ppm: H2−(3-Me)−H6, H25−(26-Me)−H28−(29-Me)−H30−(31-Me)−H32, H89−(91-Me)−H99[11] (Figure 1). As the signal assignments of H5 and H6 of **1** were already in the literature,[12] spin connectivities of H2−(3-Me)−H6 could be easily followed by TOCSY. In the spectra of OST-D, the signal corresponding to H3 (2.16 ppm) in **1** was absent, and signals due to H2−H6

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(8) The algal extracts were suspended in CH_2Cl_2 and extracted with MeOH/H_2O (1:1). The aqueous phase, after being concentrated, was extracted with 1-BuOH. The 1-BuOH extract was successively chromatographed on the following columns with solvents as indicated: Toyopearl HW60-fine, MeOH/H_2O (3:2), Inertsil C8, H_2O, H_2O/PrOH (4:1, *3:1*); CM Toyopearl 650M, 1% HOAC. The eluates were monitored by mouse assay. Resolution of ostreocins into five constituents and final purification of ostreocin D were achieved on Develosil TMS-5 with H_2O/CH_3CN (3:1) containing 0.1% HOAc. Eluates were monitored with a Hitachi C-6500 J3D Chromatosystem at 235 and 263 nm. All columns were washed with 10 mM aqueous EDTA-2Na before use.

(9) FABMS was measured with a JEOL JMS-SX 102A spectrometer and ESIMS with a Finnigan MAT TSQ-700 spectrometer.

(10) The NMR spectra were measured in 0.2% CD_3COOD/D_2O (1.4 μM) with the following three instruments: GSX-400 (JEOL 400 MHz), AMX500 (Brucker 500 MHz), and Unity 600 (Varian 600 MHz).

(11) ^1H and ^{13}C (in parentheses) assignments deduced from HSQC of **1** are as follows (0.2% CD_3COOD/D_2O): δ H2, 4.24, (75.8); H3, 2.16, (34.1); 3-Me, 0.92, (14.3); H4, 1.48, 1.81, (41.0); H5, 4.59, (66.7); H6, 5.52, (132.1); H25, 1.28, (39.1); H26, 1.63, (29.2); 26-Me, 0.93, (19.9); H27, 0.98, 1.49, (40.1); H28, 4.08, (80.2); C29, (83.4); 29-Me, 1.23, (21.2); H30, 1.20, 1.79, (45.2); H31, 2.06, (25.1); 31-Me, 0.93, (22.1); H32, 1.17, 1.75, (43.4); H89, 3.63, (74.2); H90, 3.49, (77.3); H91, 1.86, (33.1); 91-Me, 0.95, (15.8); H92, 1.40, 2.16, (27.0); H93, 4.18, (74.9); H94, 3.81, (71.8); H95, 3.71, (74.1); H96, 3.33, (75.6); H97, 4.32, (69.7); H98, 5.59, (134.1); H99, 5.65, (132.2).

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were shifted upfield, conforming to the absence of 3-Me. Because 29-Me resides on an oxygenated quaternary carbon, it gave a strong signal well separated from those of other methyls. The HMBC and HSQC spectra of palytoxin clarified the connectivities from H25 to H32 by showing cross-peaks from 29-Me to C28, C29, and C30 and from 31-Me to C30, C31, and C32 (see supplementary material). Assignment of C25, C27, C30, and C32 in **1** was done by combination of DQF-COSY and HSQC, as these methylenes were easily discernible from each other on the basis of their coupling patterns.[10] The presence of 31-Me and the absence of 26-Me in OST-D were confirmed by detecting $^3J_{HC}$ between 29-Me/C30 and 31-Me/C30 but no correlation between C27 and a doublet methyl in the HMBC spectra. The partial structure for C89−(91-Me)−C99 in **1** mainly consists of methines, and the protons on the tetrahydropyran ring (C93−C97) had large couplings. Thus, spin connections from the olefinic protons assigned to H98 and H99 were easily traced until C89 in TOCSY spectra of **1**. Chemical shifts of protons corresponding to H89−H99 were virtually unchanged in the spectra of the two toxins, indicating the presence of 91-Me in OST-D. The partial structures of OST-D thus elucidated were deduced to have the same stereochemistry as in **1**, on the basis of analysis of $^3J_{HH}$. Determining the position of the missing hydroxyl group in OST-D was hampered by heavily overlapping signals of methine protons and oxycarbons. Nevertheless, the spectral data obtained in this study support OST-D as the 3,26-bisdesmethyldeoxy analog of palytoxin, although, with the exception of the missing hydroxyl, the remainder of OST-D is believed to be identical regiochemically to palytoxin, this remains to be proven (Figure 1). ESIMS of the minor ostreocins indicated that none are identical with palytoxin. Not unexpectedly, the small structural changes barely affected the mouse lethality; interestingly, they reduced cytotoxicity and hemolytic potency: LD_{50} (ip), 0.50 and 0.75 μg/kg; IC_{50} against P388 cells, 0.2 and 2.5 pM; complete hemolysis of 0.5% mouse blood cell suspension, 1.5 and 39.5 nM for **1** and OST-D, respectively.

In the present study, palytoxin was not identified. Neither was it clear whether *O. siamensis* was a symbiont of *Palythoa* spp. Nonetheless, the present result is the first study to shed light on the etiology of palytoxin and paves the way for future biosynthetic studies of this fascinating molecule. The search for a palytoxin-producing strain and structural refinement of OST-D and its minor congeners are underway.

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Supplementary Material Available: 1D NMR, DQF-COSY, partial TOCSY, partial HSQC, and partial HMBC spectra of palytoxin and ostreocin D (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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