Isolation and Structural Determination of DTX-6, a New Okadaic Acid Derivative

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Diarrhetic shellfish poisoning is an illness caused by toxins accumulated in shellfish and produced by dinoflagellates, mainly of the *Dinophysis* and *Prorocentrum* genera. This paper reports the isolation and spectroscopic structural elucidation of a new compound, DTX-6 (**2**), an ester derivative of okadaic acid (OA) (**1**), isolated from an artificial culture of strain PLV2 of *Prorocentrum lima*.

The toxins responsible for diarrhetic shellfish poisoning (DSP) are okadaic acid (**1**) (OA) and its derivatives, produced by dinoflagellates of the *Dinophysis* and *Prorocentrum* genera.[1] These polyether toxins have attracted widespread attention both for their chemical structures and their pharmacological properties. For instance, it has been shown that OA is a potent and highly selective inhibitor of protein phosphatases as well as actively promoting tumors.[2]

Ester derivatives of DSP toxins, mainly diol esters, have also been found in *Prorocentrum lima* and *Prorocentrum concavum* cultures.[3,4] These compounds cannot be detected directly by HPLC with fluorescence, namely, Lee's procedure, which is still the one most widely used to detect DSP toxins,[5] due to the presence of an ester group which must be hydrolyzed prior to derivatization.[6]

We here report the isolation of a new toxin, DTX-6 (**2**), from the strain PL2V of *P. lima*, cultured in our laboratory. Compound **2** has been identified as an ester derivative of OA on the basis of its spectroscopical properties.

The organic extract obtained from 360 L of cultures was fractionated by gel filtration (LH-20, $CHCl_3$−MeOH−*n*-Hex, 1:1:2) and medium-pressure reversed-phase chromatography (Lobar LiChroprep RP-18, MeOH−H_2O, 85:15). The final purification was achieved by HPLC on a μ-Bondapak C18 column using first a gradient solvent system (A = CH_3CN−H_2O−AcOH, 50:50:0.1, B = CH_3CN−AcOH, 100:0.1, 1 h) and, second, an isocratic mode (MeOH−H_2O, 85:15), yielding 1.1 mg of pure compound **2**.

DTX-6, $[\alpha]^{25}_D$ +3.3 (*c* 0.10, $CHCl_3$), showed a pseudo molecular ion at *m*/*z* 913 $[M + H]^+$ in the mass spectrum, and the molecular formula $C_{51}H_{76}O_{14}$ was established by HRMS. Ester fragmentation at *m*/*z* 805 in the FAB mass spectrum, as well as the IR bands at 1738 and 1672 cm^{-1}, suggested the presence of ester and α,β-unsaturated carbonyl groups. The ^1H NMR spectrum of compound **2** clearly resembled that of OA,[7] but in addition exhibited new signals for an aldehyde proton centered at δ 9.67, two methylene groups showing A−B systems at δ 4.66, 4.55 (*J* = 13.4 Hz) and 3.07, 3.01 (*J* = 12.5 Hz), and two olefinic methylene groups at δ 6.16, 5.88 and 5.12, 4.95. On the basis of the correlations observed in the HSQC experiment, the related carbon chemical shift assignments were established as follows: δ 66.5 (C-1′), 33.8 (C-3′), 200.1 (C-5′), 114.9 (C-6′), and 126.5 (C-7′). The HMBC experiment showed correlations between the ester carbon signal C-1 (δ 176.2) and the H-1′ protons at δ 4.66 and 4.55, which were also correlated with the quaternary olefinic carbon C-2′ (δ 141.6) and the olefinic methylene group C-6′ (114.9), establishing the position of this moiety in the molecule. Other significant HMBC findings for the ester side chain are shown in Figure 1 and provided further confirmation of the proton and carbon assignments of compound **2** (Table 1). The relative stereochemistry at the chiral centers proved identical to that of OA (**1**) according to the ROESY experiment.

Table 1. ^1H and ^{13}C NMR Chemical Shift Data ($CDCl_3$) for DTX-6 (**2**)

carbon	δ ^1H	δ ^{13}C	carbon	δ ^1H	δ ^{13}C
1		176.2	27	4.04	64.6
2		75.3	28	0.98; 1.32	35.2
3	1.68; 2.06	44.2	29	1.85	31.0
4	3.96	68.7	30	3.26	75.2
5	1.36; 1.91	31.7	31	1.76	27.2
6	1.75; 1.82	27.3	32	1.86; 1.99	26.2
7	3.35	71.3	33	1.36; 1.51	30.3
8		96.0	34		95.5
9	5.31	121.5	35	1.44; 1.64	35.8
10		138.2	36	1.40; 1.61	18.7
11	1.85; 1.88	32.9	37	1.50; 1.83	25.4
12	3.38	71.2	38	3.54; 3.64	60.2
13	2.22	42.1	39	0.90	10.7
14	5.56	135.8	40	1.03	15.6
15	5.47	130.1	41	5.02; 5.35	113.1
16	4.68	79.3	42	1.01	16.0
17	1.51; 2.15	30.6	43	1.72	22.7
18	1.86; 2.04	37.1	44	1.39	27.6
19		105.7	1′	4.55; 4.66	66.5
20	1.36; 1.49	32.7	2′		141.6
21	1.76; 1.82	26.4	3′	3.01; 3.07	33.8
22	3.63	70.9	4′		145.7
23	3.41	76.6	5′	9.67	200.1
24	4.09	70.6	6′	4.95; 5.12	114.9
25		143.9	7′	5.89; 6.16	126.5
26	3.92	85.0			

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer 241 polarimeter. The IR spectrum was measured on a Bruker IFS55 spectrometer. The NMR spectra were obtained with a Bruker AVANCE 500 MHz instrument. Chemical shifts are reported relative to TMS, and coupling constants are given in Hz. HRMS was performed on a VG AutoSpec FISON spectrometer. HPLC was carried out with an LKB 2248 system equipped with a differential diffractometer detector. Si gel CC and TLC were performed on Si gel Merck 60 G. TLC plates were visualized by spraying with H_2SO_4−H_2O−AcOH (1:4:20) and heating.

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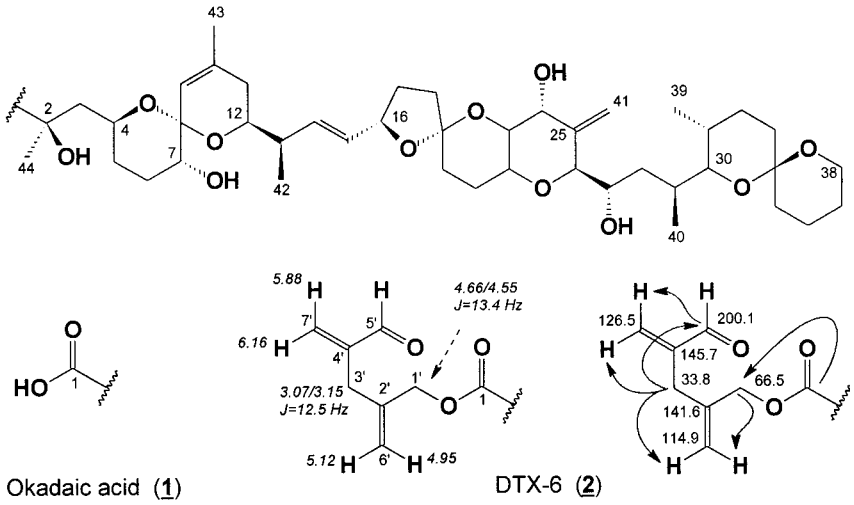

Figure 1. Okadaic acid (**1**) and DTX-6 (**2**). Chemical shift data and arrows indicate significant HMBC correlations for ester side chain in compound **2**.

Culture. Cultures of the PL2V strain of the dinoflagellate *Prorocentrum lima* were carried out by inoculating 8 × 80 L tanks, each containing 40 L of a Guillard K medium with 5 L of *P. lima* culture grown and incubated under constant white fluorescent illumination at 25 °C for 3 weeks.

Extraction and Isolation of DTX-6. *P. lima* cells were harvested by continuous centrifugation at 7000 rpm. The cells were sonicated and extracted with acetone. The solvent was evaporated, and the resultant extract was successively chromatographed by gel filtration on a Sephadex LH-20 column eluted with a mixture of $CHCl_3$−MeOH−*n*-Hex (1:1:2) and over a medium-pressure reversed-phase Lobar LiChroprep RP-18 column with MeOH−H_2O (85:15). Final purification of compound **2** was achieved on a μ-Bondapak C18 HPLC column using a gradient solvent system, A = CH_3CN−H_2O−AcOH, 50:50:1, B = CH_3CN−AcOH, 100:0.1, 1 h, followed by an isocratic elution [MeOH−H_2O, (85:15)], over the same column, yielding 1.1 mg of pure substance.

DTX-6 (2): white amorphous powder; $[\alpha]^{25}_D$ +3.3 (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} 242 and 270 nm; IR ($CHCl_3$) ν_{max} 3424, 2954, 2919, 2850, 1738, 1672, 1462, 1378, 1212, and 1078 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FAB MS *m*/*z* 913, 805, 798, 741, 680, 401, 368; HRMS 912.52368 (calcd for $C_{51}H_{76}O_{14}$, 912.523507).

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