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NEW DIOL ESTERS ISOLATED FROM CULTURES OF THE DINOFLAGELLATES *PROROCENTRUM LIMA* AND *PROROCENTRUM CONCAVUM*

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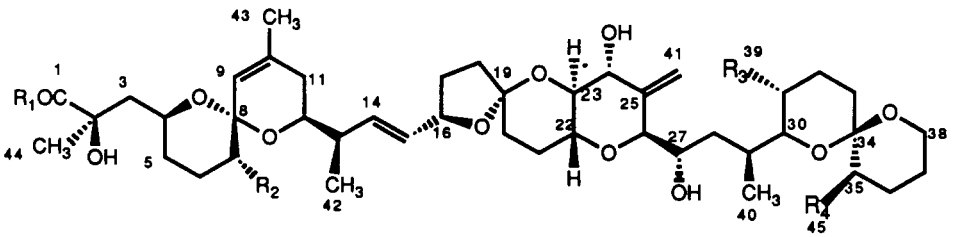
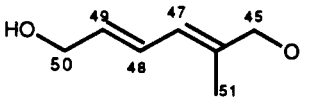
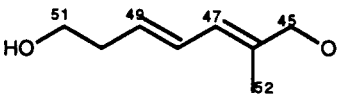
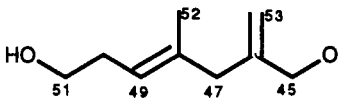
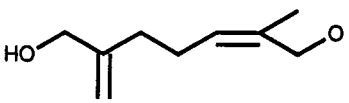
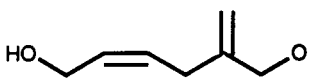
ABSTRACT.—During the course of obtaining okadaic acid [**1**] and DTX-1 [**2**] from large-scale cultures of the dinoflagellates *Prorocentrum concavum* and *Prorocentrum lima*, three new diol esters of okadaic acid have been isolated and their structures assigned as **5**, **6**, and **7** by spectroscopic methods.

The diarrhetic shellfish poisoning (DSP) toxins consist of a group of polyether metabolites **1–4**. Three of these (**1**, **2**, and **3**) are known to be produced by marine dinoflagellates belonging to the genus *Prorocentrum* or to be associated with species of the genus *Dinophysis* (1–5). The acyl derivative **4** has been found only in shellfish tissue and may not be a phytoplankton metabolite. Because of their toxicity and world-wide distribution, DSP toxins have had a disastrous effect upon the shellfish industry in many parts of the world (6). Interest in these toxins has been further heightened by the recent discovery that these compounds are phosphatase inhibitors and, as such, have a profound effect upon many cellular functions (7). As a prerequisite for a comprehensive study of the chemistry of the DSP toxins, it was necessary to obtain significant amounts of the parent toxin okadaic acid [**1**]. Several sources were examined, including the black sponge *Halichondria melanodociae* de Laubenfels from which **1** was originally isolated (8), extracts of toxic mussels known to contain DSP toxins, and non-axenic laboratory cultures of two dinoflagellates belonging to the genus *Prorocentrum* known to produce the toxins (5). It was eventually found that dinoflagellate cultures were the best source of the polyether metabolites, and large-scale cultivation of *Prorocentrum lima* (Ehr.) Dodge and *Prorocentrum concavum* Fukuyo provided an opportunity to investigate the minor DSP metabolites produced by these microalgae. In extracts of *P. concavum*, **1** and the esters **5** and **6** were found, whereas extracts of *P. lima* contained both **1** and DTX-1 [**2**], as well as the esters **6** and **7** and trace amounts of the newly identified DTX-2 [**3**] (9). Structural studies showed that the new compounds **5**, **6**, and **7** were esters of okadaic acid with C_7, C_8, and C_9 diols, respectively.

RESULTS AND DISCUSSION

Several samples of the sponge *H. melanodociae*, collected from the Florida Keys, were examined for their DSP toxin content. Although the sponge did provide **1**, the yield of toxin was low (0.36 μg/g wet sponge) and purification was impaired by the presence of many additional interfering compounds in the sponge extract. A second approach was to use shellfish known to be contaminated with DSP toxins as indicated by the rat bioassay (2). Analysis of a lipid extract of the blended shellfish tissue indicated that the concentration of DSP toxins was only 5.0 μg/g wet tissue, and efficient large-scale purification was compromised by the considerable amount of fat in the sample. Finally, it was



	R_1	R_2	R_3	R_4
1	OH	OH	Me	H
2	OH	OH	Me	Me
3	OH	OH	H	Me
4	OH	O-acyl	Me	Me
5		OH	Me	H
6		OH	Me	H
7		OH	Me	H
8	OMe	OH	Me	H
9	OEt	OH	Me	H
10		OH	Me	H
11		OH	Me	H

found that the best method of obtaining sufficiently large quantities of okadaic acid and related DSP toxins was through large-scale cultivation of *P. concavum* and *P. lima*, in which, under the conditions of culture, the level of toxin production was 10–15 pg of okadaic acid [**1**] per cell.

During the course of isolation and purification of okadaic acid from dinoflagellate cells, tlc of partially purified fractions showed several compounds less polar than **1** and apparently related to the DSP toxins. Further purification of extract fractions from *P. concavum* yielded the diol ester derivatives **5** and **6** as colorless solids. Both compounds contained a conjugated diene system [uv λ max (MeCN) 238 nm], and **5** displayed a strong carbonyl stretch at 1742 cm^{-1} with complete absence of carboxyl OH stretch at 3300–2500 cm^{-1} in the ir spectra, suggesting that **5** was an ester of **1**. Flow injection analysis of **5** and **6** by ion-spray ms (10) showed $[MH]^+$ ions at *m/z* 915 and 929, respectively, and both showed a common intense collision-induced fragment ion at *m/z* 805 in ms-ms experiments. Accurate mass measurement of the $[MH]^+$ ions by liquid-assisted secondary ion mass spectrometry (lsims) was consistent with the molecular formulae $C_{51}H_{78}O_{14}$ and $C_{52}H_{80}O_{14}$ for **5** and **6**, respectively, which when compared with okadaic acid corresponds to an additional $C_7H_{10}O$ fragment in **5** and an additional $C_8H_{12}O$ fragment in **6**.

Because so many details of the ^{1}H- and ^{13}C-nmr spectra of **5** were similar to those of **1**, it was possible to identify the additional resonances associated with the $C_7H_{11}O$ fragment in this new metabolite. In the ^{13}C-nmr spectra of **5**, the seven additional resonances were identified as a single quaternary olefinic carbon (δ 131.9, C-46), three protonated olefinic carbons (δ 126.1, C-48; 127.6, C-47; and 133.5, C-49) two methylene carbons (δ 63.2, C-50, and 70.2, C-45) and a single methyl carbon (δ 14.4, C-51). The resonances of C-1, C-2, and C-4 were shifted upfield and that of C-3 downfield, with respect to **1**, while the remainder of the resonances were very similar to those for **1**, indicating that the carbon skeleton of the acyl moiety was identical with that of the parent acid.

The ^{1}H assignments of the alcohol moiety of the ester **5** were made on the basis of chemical shift arguments and the COSY data. The resonances of the low-field methylene group (H-45) at δ 4.73 and 4.56 were deshielded by the adjacent acyl group and appeared as a clean AB quartet (J = 12.8 Hz). The olefinic methyl resonance at δ 1.81 (H-51) was long-range-coupled (J = 1.1 Hz) to an olefinic proton at δ 6.11 (H-47). This proton was in turn coupled to a second olefinic proton at δ 6.48 (H-48, J = 11.0 Hz) that was also coupled to a third olefinic proton at δ 5.88 (H-49, J = 15.2 Hz). Finally, the latter proton was coupled to the protons of a second methylene group bonded to oxygen resonating at δ 4.23 (H-50, J = 5.7 Hz). This resonance showed further splitting presumably due to coupling (J = ca. 5 Hz) with a hydroxyl proton (δ 1.55) and to a long range coupling (J = 1.5 Hz) to H-48. This evidence indicates structure **5** for the ester. The nOe difference experiments established the geometry of the diene system as follows. An nOe (ca. 4% each way) between each of the H-45 protons and the olefinic proton at 6.11 ppm (H-47) indicated an *E* geometry for the first double bond, and this was supported by the absence of nOe between the same olefinic proton and the protons (H-51) of the methyl group. In the case of the second double bond (C-48, C-49), an nOe between the olefinic methines H-47 and H-49 (ca. 11% each way) indicated an *E* configuration, which was supported by the H-48, H-49 coupling value (J = 15.2 Hz) and the absence of an nOe between H-48 and H-49 as well as between H-47 and H-50.

The second ester **6** was obtained from cultures of both *P. lima* and *P. concavum*. The ^{13}C-nmr data for this metabolite displayed a strong similarity with those of **5**. For example, all the resonances for the okadaic acid skeleton including the diol ester moiety could be observed, but in addition there was a resonance at δ 36.3 identified as a methylene carbon (C-50), which the HETCOR data showed was linked to two protons resonating as a broad doublet of triplets at δ 2.37 in the ^{1}H-nmr spectrum. The COSY data established that this additional methylene group was juxtaposed between an olefinic proton at δ 5.70 (H-49, J = 7.2 Hz) and the H-51 methylene group carrying oxygen (δ 3.69, J = 6.2 Hz) as depicted in structure **6**. The *E,E* geometry of the diene system was established by an nOe (ca. 5%) between the methylene group H-45 and the olefinic methine H-47, which in turn showed an nOe (average 9%) with the second olefinic methine H-49. As further evidence, the remaining methine H-48 also showed an nOe with the methylene group H-50.

Compound **7** was obtained in trace amounts as a colorless solid from large scale cultures of *P. lima* only, and like **5** and **6**, this third metabolite was also less polar than okadaic acid by tlc. Ion spray ms showed an $[MH]^+$ ion at *m/z* 943, indicating a mol wt of 942, which corresponds to an increase of 138 daltons over the mass wt of okadaic acid and is accommodated by an additional $C_9H_{14}O$ moiety. The HMQC data established that the alcohol moiety of **7** contained one protonated olefinic carbon (δ 123.7, C-49), one olefinic methylene (δ 114.2, C-53), four methylenes (δ 66.6, C-45; 44.1, C-47; 30.5, C-50; and 62.3, C-51), and a methyl carbon (δ 15.9, C-52).

Because details of the ^{1}H-nmr spectrum of **7** were very similar to those of **5** and **6**, it appeared that **7** was also an ester of okadaic acid, and once again it was possible to identify the signals of the extra fragment. The TOCSY data indicated two spin systems in this alcohol moiety. The first system comprised an AB quartet at δ 4.53 and 4.67, consistent with deshielding by an adjacent acyl group as in H-45 of **5** and **6**, and weakly coupled ($J<0.5$ Hz) to olefinic methylene protons resonating at 5.01 and 5.12 (H-53). The second spin system contained all the remaining resonances belonging to the alcohol moiety, and the COSY data revealed a consecutive sequence of coupled nuclei from the methylene protons H-51 (δ 3.65) bonded to oxygen, to another methylene H-50 (δ 2.29), to the olefinic proton (H-49) at δ 5.27, and finally to the methyl protons H-52 (δ 1.61, $J=0.9$ Hz). Further coupling from H-52 to the methylene group H-47 (δ 2.77, 2.80) was apparent from the TOCSY spectrum. Importantly, TOCSY cross peaks from the H-47 resonances to all resonances from the alcohol moiety showed that the two spin systems were linked at this point via weak coupling from H-53 to H-47. These data established the structure of the third ester as **7**. The sequence of protons deduced from the coupling data was confirmed by the NOESY spectra of **7**, which contained cross peaks between H-47 and H-49, and between H-50 and H-52, establishing the *E* geometry of the tri-substituted double bond.

The diol esters reported here are similar to esters **10** and **11** isolated previously from another culture of *P. lima* (11). In this work, the methyl ester **8** of okadaic acid was also found in extracts of both *P. concavum* and *P. lima*, but the ethyl ester **9** was found in *P. lima* only. Although methyl esters are not uncommon in nature, it is more unusual to find ethyl esters. The methyl ester, which was also observed by lc-ms analysis following Me_2CO extraction, was identified by comparison with synthetic material prepared from okadaic acid. The ethyl ester, found only in *P. lima* extracts using the same workup procedures as for *P. concavum*, was identified by ms and nmr data. In the case of *P. concavum*, it was also observed that the cellular concentration of okadaic acid decreases with time, while the concentration of the esters increases. This suggests that the esterified products may be shunt metabolites, produced at a certain stage in the growth cycle, or when the concentration of okadaic acid in the cell reaches a critical level.

The ability of okadaic acid to inhibit phosphatases 1 and 2A prompted an examination of other DSP derivatives as potential inhibitors (12,13), and it was found that the methyl ester **8** did not inhibit phosphatase 1 or 2A, suggesting that a free carboxyl group is essential for activity. The diol esters **5** and **6** isolated in this work have been examined for their phosphatase inhibition properties and were also found to be inactive (C.F.B. Holmes, personal communication), further supporting the idea that a free carboxyl group is essential for activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr, ms, ir, and uv spectra and optical data were obtained with the same instruments as previously described (14). Uv spectra were recorded in MeCN, the nmr data were obtained in $CDCl_3$, and chemical shifts are given in ppm with TMS as internal standard. Si gel was used for vlc and flash chromatography, Sephadex LH-20 for gel permeation chromatography, and C18 for reversed-phase chromatography. Tlc plates were eluted with toluene/Me_2CO/MeOH and visualized by acid and heat. Hplc was performed using a Vydac 201TP1010 (25 × 1 cm), and elution with MeCN/H_2O mixtures. All solvents used were hplc grade purchased from Anachemia. H_2O was purified using a Waters Millipore system.

SPONGE MATERIAL.—*H. melanodociae* was collected from the Keys in southern Florida in January 1989 and identified on site by WestWind SeaLab Supplies, Victoria, B.C., Canada. A voucher specimen is stored at Biological Chemistry, 1MB, Halifax, N.S.

SHELLFISH MATERIAL.—Frozen meat from blue mussels *Mytilus edulis* L. contaminated with DSP toxins and held in quarantine was obtained through the Netherlands Institute for Fishery Investigations,

Ijmuiden, and shipped frozen to IMB, Halifax. A voucher specimen is stored at Biological Chemistry, IMB, Halifax, N.S.

CULTURE CONDITIONS FOR *PROROCENTRUM* SPP.—The marine dinoflagellate *P. concavum* was obtained from the collection of Dr. R.W. Dickey, US-FDA, Fisheries Research Branch, Dauphin Island, AL, and brought into culture as already described (5). The *P. lima* strain, culture catalogue # 712, was obtained from the North East Pacific Culture Collection, University of British Columbia, Vancouver, B.C. Culture conditions for both *Prorocentrum* species were essentially as described (10) with culture temperature at 26° for *P. concavum* and 20° for *P. lima*. Cultures (50 liters) were grown in mechanically agitated fibreglass tanks suitably equipped with light and temperature control, and pH was maintained at 7.9 by CO_2 sparging. Cells were harvested by initial sedimentation in a conical container, followed by centrifugation.

EXTRACTION PROCEDURES.—The same extraction procedure was followed for sponge and shellfish materials. The wet tissue was blended and soaked in MeOH for 24 h and filtered, and the process repeated once more. After the second MeOH extraction, the tissue was extracted with Me_2CO. The organic extracts were combined and taken to dryness in vacuo. For phytoplankton material, wet biomass was soaked in MeOH (2 h) in centrifuge tubes. Cells were disrupted ultrasonically (3 × 15 sec), the total extract centrifuged, and the supernatant collected. The process was repeated three times. The supernatants were combined and taken to dryness in vacuo.

ISOLATION OF **1**, **5**, **6**, AND **8** FROM *P. CONCAVUM*.—The MeOH extracts of *P. concavum* cells (326 g wet wt) were combined and evaporated to dryness under vacuum. A portion of this residue was chromatographed on Si gel (100 g). Fractions eluted with EtOAc and EtOAc-MeOH (90:10) afforded residues A (35 mg) and B (0.72 g), respectively, both of which contained DSP-related compounds by tlc and nmr analysis. Residue A was fractionated by Sephadex LH-20 column chromatography using MeOH as eluent. The DSP-toxin-containing fractions were combined and further purified by preparative hplc [C18 column, MeCN-H_2O (55:45)]. Four major peaks were observed in the chromatogram (uv detection at 210 nm) which were collected following repeated chromatography to yield **1** (2.5 mg), **5** (1.6 mg), **6** (0.8 mg), and **8** (0.7 mg), respectively. Residue B was similarly purified by Sephadex LH-20 cc and preparative hplc, yielding **1** (12 mg).

ISOLATION OF **1**, **2**, **6**, **7**, **8**, AND **9** FROM *P. LIMA*.—After the MeOH extracts of cultivated *P. lima* cells (47 g wet wt) were combined and evaporated to dryness, the residue was chromatographed on Si gel (100 g). Fractions eluted with EtOAc, EtOAc-MeOH (95:5), and EtOAc-MeOH (90:10) were all found to contain DSP compounds by tlc analysis. Similar fraction were combined and subjected to Sephadex LH-20 cc, using MeOH as eluent, to afford two DSP-rich residues C (21.7 mg) and D (276.7 mg). Residue D was further subjected to C18 reversed-phase open cc to give **1** (22 mg) and a mixture (13.7 mg) of other DSP compounds. In the final hplc separation of this mixture, four less polar fractions were collected to give **2** (2.5 mg), **6** (0.3 mg), **8** (0.6 mg), and **9** (0.3 mg). Residue C was purified by a combination of Sephadex LH-20 and C18 reversed-phase cc and a final clean-up by C18 reversed-phase semi-preparative hplc, to yield **7** (0.5 mg).

Okadaic acid [**1**] *from* P. concavum *and* P. lima.—Mp 163–164°; $[\alpha]^{20}D$ +53.3; ir ν max 2937, 2874, 1736, 1452, 1382, 1078 cm^{-1}; hrlsims *m/z* $[MH]^+$ 805.4745 (calcd for $C_{44}H_{69}O_{13}$, 805.4738). All nmr data were identical with the corresponding data for an authentic sample of okadaic acid.

DTX-1 [**2**] *from* P. lima.—This material had the same retention time on C_{18} reversed-phase chromatography and displayed nmr and ms data identical to those of an authentic sample of DTX-1.

Compound **5** *(diol ester of okadaic acid) from* P. concavum.—Ir ($CHCl_3$) ν max 2950, 1742, 1455, 1382 cm^{-1}; uv λ max 238 nm; lsims ms *m/z* $[MH]^+$ 915, $[MH\text{-}110]^+$ 805, $[MH-110-H_2O]^+$ 787, $[MH-110-2H_2O]^+$ 769; hrlsims *m/z* $[MH]^+$ 915.5084 (calcd for $C_{51}H_{79}O_{14}$, 915.5470); ^1H nmr (300 MHz) δ 6.48 (1H, ddt, J = 15.2, 11.0, 1.5 Hz), 6.11 (1H, bd, J = 11.0 Hz), 5.88 (1H, dt, J = 15.2, 5.7 Hz), 5.58 (1H, dd, J = 15.2, 7.7 Hz), 5.49 (1H, dd, J = 15.2, 7.1 Hz), 5.38 (1H, t, J = 1.9 Hz), 5.33 (1H, q, J = 1.3 Hz), 5.05 (1H, t, J = 1.8 Hz), 4.83 (OH, s), 4.73, 4.56 (2H, AB, J = 12.8 Hz), 4.49 (1H, td, J = 8.0, 7.1 Hz), 4.23 (2H, bt, J = ca. 6 Hz), 4.15–3.92 (3H, m), 3.94 (1H, d, J = 9.6 Hz), 3.70–3.52 (4H, m), 3.44 (1H, t, J = 9.7 Hz), 3.37 (1H, dd, J = 10, 8 Hz), 3.28 (1H, dd, J = 10.2, 2.2 Hz), 2.90 (OH, d, J = 6.3 Hz), 2.54 (OH, s), 2.30 (1H, bq, J = 7.2 Hz), 1.81 (3H, d, J = 1.1 Hz), 1.73 (3H, d, J = 1.1 Hz), 1.39 (3H, s), 1.06 (3H, d, J = 6.4 Hz), 1.03 (3H, d, J = 6.8 Hz), 0.98 (1H, m), 0.93 (3H, d, J = 7.0 Hz), other overlapped multiplets from 2.24 to 1.24 (31H); ^{13}C nmr (75.5 MHz) δ 176.1 (s), 143.7 (s), 138.8 (s), 135.3 (d), 133.5 (d), 131.9 (s), 131.1 (d), 127.6 (d), 126.1 (d), 121.7 (d), 112.4 (t), 105.8 (s), 96.1 (s), 95.6 (s), 84.8 (d), 79.0 (d), 76.6 (d), 75.3 (s), 75.0 (d), 71.6 (d), 70.8 (d), 70.8 (d), 70.2 (t), 69.6 (d), 68.5 (d), 64.6 (d), 63.2 (t), 60.3 (t), 44.1 (t),

41.7 (d), 37.1 (t), 35.9 (t), 35.2 (t), 32.8 (t), 32.7 (t), 31.8 (t), 31.0 (d), 30.6 (t), 30.3 (t), 27.4 (t), 27.4 (q), 27.3 (d), 26.4 (t), 26.3 (t), 25.4 (t), 23.0 (q), 18.7 (t), 16.2 (q), 15.8 (q), 14.4 (q), 10.6 (q).

Compound **6** *(diol ester of okadaic acid) from* P. concavum.—Uv λ max 238 nm; lsims *m/z* $[MH]^+$ 929, $[MH-124]^+$ 805, $[MH-124-H_2O]^+$ 787, $[MH-124-2H_2O]^+$ 769; hrlsims *m/z* $[MH]^+$ 929.5607 (calcd for $C_{52}H_{81}O_{14}$, 929.5626); ^{1}H nmr (300 MHz) δ 6.33 (1H, ddt, *J* = 15.0, 10.8, 1.3 Hz), 6.06 (1H, dq, *J* = 11.0, 1.2 Hz), 5.70 (1H, dt, *J* = 15.1, 7.2 Hz), 5.56 (1H, dd, *J* = 15.2, 7.9 Hz), 5.47 (1H, dd, *J* = 15.2, 7.1 Hz), 5.35 (1H, t, *J* = 1.9 Hz), 5.30 (1H, q, *J* = 1.4 Hz), 5.02 (1H, t, *J* = 1.7 Hz), 4.79 (OH, s), 4.67, 4.53 (2H, AB, *J* = 12.5 Hz), 4.47 (1H, td, *J* = 7.8, 6.9 Hz), 4.13–3.93 (3H, m), 3.91 (1H, d, *J* = 9.6 Hz), 3.69 (2H, t, *J* = 6.2 Hz), 3.62–3.47 (4H, m), 3.41 (1H, t, *J* = 9.7 Hz), 3.34 (1H, dd, *J* = 9.8, 6.9 Hz), 3.26 (1H, dd, *J* = 10.2, 2.2 Hz), 2.84 (OH, d, *J* = 6.1 Hz), 2.53 (OH, s), 2.37 (2H, dt, *J* = 6.9, 6.2 Hz), 2.26 (1H, q, *J* = 7.3 Hz), 1.76 (3H, d, *J* = 1.1 Hz), 1.71 (3H, d, *J* = 1.0 Hz), 1.36 (3H, s), 1.03 (3H, d, *J* = 6.5 Hz), 1.01 (3H, d, *J* = 6.9 Hz), 0.96 (1H, m), 0.90 (3H, d, *J* = 7.0 Hz), other overlapped multiplets from 2.2 to 1.2 (30H); ^{13}C nmr (75.5 MHz) δ 176.2 (s), 143.7 (s), 138.8 (s), 135.4 (d), 131.8 (d), 131.1 (d), 130.2 (s), 128.4 (d), 128.3 (d), 121.8 (d), 112.4 (t), 105.8 (s), 96.1 (s), 95.6 (s), 84.9 (d), 79.1 (d), 77.0 (d), 75.4 (s), 75.1 (d), 71.6 (d), 70.9 (d), 70.9 (d), 70.6 (t), 69.6 (d), 68.6 (d), 64.7 (d), 61.9 (t), 60.3 (t), 44.1 (t), 41.8 (t), 37.2 (t), 36.3 (t), 35.9 (t), 35.3 (t), 32.8 (t), 32.8 (t), 31.8 (t), 31.1 (d), 30.7 (t), 30.4 (t), 27.4 (d), 27.4 (q), 27.4 (t), 26.5 (t), 26.4 (t), 25.5 (t), 23.0 (q), 18.8 (t), 16.2 (q), 15.9 (q), 14.4 (q), 10.7 (q).

Compound **7** *(unconjugated diol ester of okadaic acid) from* P. lima.—Lc-ms-ms *m/z* $[MH]^+$ 943, $[MH-156-2H_2O]^+$ 751; ^{1}H nmr (500 MHz) δ 5.56 (1H, dd, *J* = 8.1, 15.2 Hz), 5.49 (1H, dd, *J* = 7.7, 15.2 Hz), 5.38 (1H, t, *J* = 2 Hz), 5.33 (1H, q, *J* = 1.4 Hz), 5.27 (1H, tq, *J* = 7.3, 0.9 Hz), 5.12 (1H, m), 5.05 (1H, t, *J* = 1.6 Hz), 5.01 (1H, m), 4.86 (OH, s), 4.67, 4.53 (2H, AB, *J* = 13.3 Hz), 4.47 (1H, dt, *J* = 7.8, 6.8 Hz), 4.12 (1H, m), 4.07 (1H, t, *J* = 9.8 Hz), 3.97 (1H, tt, *J* = 11.1, 1.5 Hz), 3.94 (1H, d, *J* = 9.5 Hz), 3.68–3.53 (6H, m), 3.42 (1H, t, *J* = 9.8 Hz), 3.37 (1H, dd, *J* = 9.6, 7.4 Hz), 3.28 (1H, dd, *J* = 10.2, 2.1 Hz), 3.13 (OH, d, *J* = 6.5 Hz), 2.80, 2.77 (2H, AB, *J* = 14.8 Hz), 2.53 (OH, s), 2.32–2.23 (3H, m), 2.17 (1H, m), 1.73 (3H, d, *J* = 1.2 Hz), 1.61 (3H, d, *J* = 1.0 Hz), 1.41 (3H, s), 1.05 (3H, d, *J* = 6.4 Hz), 1.02 (3H, d, *J* = 6.9 Hz), 0.97 (1H, t, *J* = 12.4 Hz), 0.92 (3H, d, *J* = 7.0 Hz), other overlapped multiplets from 2.6 to 1.3 (31H); ^{13}C nmr (500 MHz, 125.8 MHz, from HMQC data, carbons of the diol moiety bonded to hydrogen only) δ 123.7 (d), 114.4 (t), 66.6 (t), 62.3 (t), 44.1 (t), 30.5 (t), 15.9 (q).

Compound **8** *(methyl ester of okadaic acid) from* P. concavum *and* P. lima.—Ir ν max 2946, 1744, 1564, 1438, 1383, 1070; lsims $[MH]^+$ 819, $[MH-H_2O]^+$ 801, $[MH-2H_2O]^+$ 783; ^{1}H nmr (300 MHz) δ 5.56 (1H, dd, *J* = 15.2, 7.6 Hz), 5.49 (1H, dd, *J* = 15.2, 7.0 Hz), 5.41 (1H, bd, *J* = 1.9 Hz), 5.33 (1H, q, *J* = 1.5 Hz), 5.07 (1H, t, *J* = 1.6 Hz), 4.47 (1H, td, *J* = 7.5, 6.8 Hz), 4.11 (1H, t, *J* = 8 Hz), 4.09 (1H, t, *J* = 10 Hz), 3.95 (1H, t, *J* = 11.3 Hz), 3.94 (1H, d, *J* = 8.0 Hz), 3.81 (3H, s), 3.66 (1H, td, *J* = 10.7, 3.5 Hz), 3.63–3.50 (2H, m), 3.42 (1H, t, *J* = 8.5 Hz), 3.38 (1H, dd, *J* = 9.1, 7.8 Hz), 3.29 (1H, dd, *J* = 8.3, 2.1 Hz), 2.28 (1H, q, *J* = 7.5 Hz), 1.72 (3H, d, *J* = 1.0 Hz), 1.36 (3H, s), 1.06 (3H, d, *J* = 6.5 Hz), 1.03 (3H, d, *J* = 6.9 Hz), 0.99 (1H, t, *J* = 13.2 Hz), 0.93 (3H, d, *J* = 7.0 Hz); ^{13}C nmr (125 MHz) δ 177.0 (s), 144.1 (s), 139.7 (s), 135.7 (d), 131.0 (d), 121.7 (d), 112.5 (t), 105.8 (s), 96.1 (s), 95.6 (s), 84.9 (d), 79.0 (d), 76.6 (d), 75.6 (s), 75.1 (d), 71.6 (d), 71.0 (d), 70.7 (d), 69.6 (d), 68.4 (d), 64.6 (d), 60.3 (t), 52.8 (q), 44.0 (t), 41.9 (d), 37.2 (t), 35.9 (t), 35.3 (t), 33.0 (t), 32.8 (t), 31.8 (t), 31.1 (d), 30.7 (t), 30.4 (t), 27.7 (q), 27.4 (d), 27.3 (t), 26.5 (t), 26.4 (t), 25.5 (t), 23.0 (q), 19.8 (t), 16.2 (q), 15.9 (q), 10.7 (q).

Compound **9** *(ethyl ester of okadaic acid) from* P. lima.—Lc-ms-ms $[MH]^+$ 833, $[MH-H_2O]^+$ 815; ^{1}H nmr (500 MHz) δ 5.53 (1H, dd, *J* = 15.2, 7.5 Hz), 5.49 (1H, dd, *J* = 15.2, 7.1 Hz), 5.39 (1H, t, *J* = 1.9 Hz), 5.33 (1H, q, *J* = 1.4 Hz), 5.06 (1H, t, *J* = 1.6 Hz), 4.48 (1H, dt, *J* = 8.1, 6.9 Hz), 4.35 (1H, dq, *J* = 10.6, 7.1 Hz), 4.18 (1H, dq, *J* = 10.5, 7.3 Hz), 4.10 (1H, tt, *J* = 8.1, 2.3 Hz), 4.08 (1H, bt, *J* = 10 Hz), 3.98 (1H, tt, *J* = 11.3, 2.0 Hz), 3.95 (1H, d, *J* = 9.7 Hz), 3.66 (1H, td, *J* = 12.3, 2.5 Hz), 3.60 (1H, dd, *J* = 8.4, 6.0 Hz), 3.55 (1H, bd, *J* = 12 Hz), 3.43 (1H, t, *J* = 9.8 Hz), 3.39 (1H, dd, *J* = 9.2, 7.3 Hz), 3.28 (1H, dd, *J* = 10.3, 2.1 Hz), 2.53 (OH, s), 2.28 (1H, q, *J* = 7.5 Hz), 1.73 (3H, d, *J* = 1.2 Hz), 1.36 (3H, s), 1.31 (3H, t, *J* = 7.2 Hz), 1.06 (3H, d, *J* = 6.5 Hz), 1.02 (3H, d, *J* = 6.7 Hz), 0.99 (1H, bt, *J* = 13.2 Hz), 0.92 (3H, d, *J* = 6.9 Hz), other overlapped multiplets from 2.2 to 1.24 (34H).

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