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A NEW POLYETHER ACID FROM A COLD WATER MARINE SPONGE, A *PHAKELLIA* SPECIES

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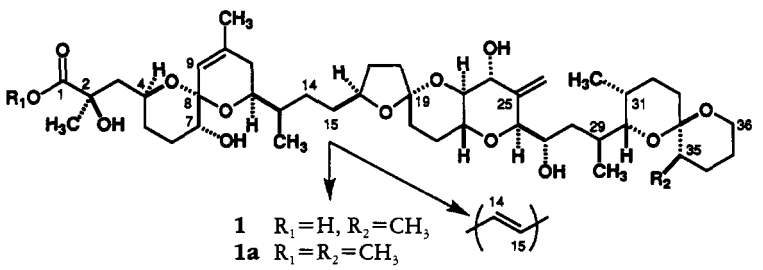
ABSTRACT.—A new polyether acid, 14,15-dihydrodinophysistoxin-1 [**1**], was isolated along with dinophysistoxin-1 [**2**] and okadaic acid [**3**], from an unidentified species of the sponge genus *Phakellia*. The structure of **1** was assigned by comparison of its ^{1}H- and ^{13}C-nmr and fabms spectral data to those of **2** and **3**, as well as interpretation of homonuclear 2D nmr data. Compounds **1–3** showed cytotoxicity against L-1210 leukemia cells with IC_{50} values of 3 ng/ml. The isolation, structure determination, and biological activities of these compounds are described (1).

Dinophysistoxin-1 [**2**] is a compound of the okadaic acid family first isolated by Yasumoto and co-workers from the mussel *Mytilus edulis* and identified by them as a causative toxin for diarrhetic shellfish poisoning in Japan and Europe (2,3). Later, compound **2** and okadaic acid [**3**] were shown to be produced primarily by the dinoflagellates, *Prorocentrum lima* and *Dinophysis fortii*, and accumulated by commercially important filter feeders such as shellfish and mussels (4,5). Okadaic acid [**3**] itself was initially isolated from the Pacific and Caribbean sponges, *Halichondria okadai* and *H. melanodocia*, respectively (6). Pacific *H. okadai* also contains **2** (7). Several okadaic acid- or dinophysistoxin-related compounds have been reported, including acanthifolicin, a 9,10-episulfide of **3**, isolated from the Caribbean sponge *Pandaros acanthifolium* (8), glycookadaic acid, a glycinamide of **3**, from *H. okadai* (7); okadaic acid esters and 2- and 7-deoxy okadaic acids from *P. lima* (9); and 7-acyl derivatives of **2** from the scallop *Patinopecten vessoensis* (9,10).

Okadaic acid [**3**] and dinophysistoxin-1 [**2**] induce illness in humans at very low doses. Oral intake of 32 μg of **2** is estimated to cause poisoning (11,12). Recently, both **2** and **3** were shown to promote tumors in a two-stage carcinogenesis study in mice (13,14), and also to inhibit protein phosphatases I and IIA through a mechanism similar to those of the microcystin hepatotoxic peptides and the polyfunctionalized fatty acid-derived calyculin (15,16). Structural requirements on protein phosphates were recently investigated of the above okadaic acid derivatives inclusive of 14,15-dihydro-okadaic acid, isolated from *H. okadai*.

In the course of our continuing search for biologically active compounds from marine organisms (18), a new compound, 14,15-dihydrodinophysistoxin-1 [**1**], along with **2** and **3**, was isolated as an active principle from a sponge of the



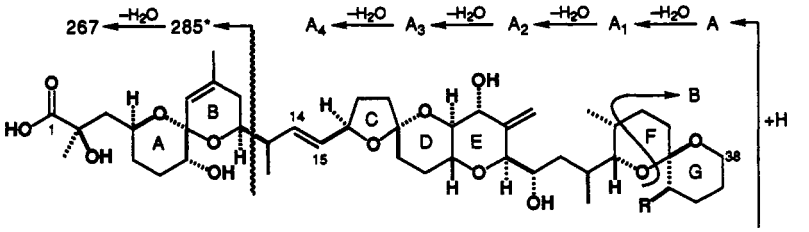
1 R_1=H, R_2=CH_3
1a R_1=R_2=CH_3

2 R_1=H, R_2=CH_3
2a R_1=R_2=CH_3
3 R_1=R_2=H

genus *Phakellia* by a bioassay-guided isolation scheme. Isolation, structure assignments, and biological activities of the above compounds are described here.

The tan leafy sponge of the genus *Phakellia*, found at depths of 20 to 30 m off the coast of Maine, was collected by scuba in July 1985 and 1986. The biological activities of the extracts were measured qualitatively on site in Maine using CV-1 cytotoxicity, antiviral (HSV-1), and antimicrobial assays. Samples were stored frozen until used. The toluene/MeOH extracts of the sponge, both 1985 and 1986 specimens, showed cytotoxicity and some antiviral activity, but no antibacterial activity. From 2.7 kg of sample, separated by following CV-1, L-1210 cytotoxicity, and brine shrimp mortality, **2** (3.22 mg, 1.2×10^{-4}% yield from the wet organism), **1** (3.60 mg, 1.3×10^{-4}%, and **3** (1.45 mg, 5.3×10^{-5}%) were isolated using high-speed countercurrent chromatography (19) as a key separation tool.

Compounds **2** (dinophysistoxin) and **3** (okadaic acid) were identified by hrfabms ($C_{45}H_{71}O_{13}$, M+H, Δ 0.1 mDa and $C_{44}H_{68}NaO_{13}$, M+Na, Δ 0.3 mDa, respectively) and nmr data. The fabms spectrum of **1** showed a molecular ion at *m/z* 843 [M+Na] or at *m/z* 821 [M+H] with added oxalic acid. The molecular formula of **1**, $C_{45}H_{72}NaO_{13}$ (M+Na), was secured by hrfabms data (*m/z* 843.4830). Fabms/ms on the protonated molecular ion at *m/z* 821 showed fragmentations similar to those of compounds **2** and **3**: four sequential losses of 18 daltons for four hydroxyl groups, fragment ions at *m/z* 285 and 267 for the left half of the molecule, and fragment ion B at *m/z* 125 for the G ring (Scheme 1). The ^{1}H- and ^{13}C-nmr (APT and DEPT) spectra of **1** showed spectral patterns very similar to those of **2** except for the lack of proton and carbon signals for the 14,15-*trans*-disubstituted olefin observed in **2**, suggesting **1** is a 14,15-dihydro derivative of **2**. Treatment of **1** and **2** with CH_2N_2 gave corresponding methyl esters **1a** and **2a**, respectively, which yielded more highly resolved nmr spectra than the free acids. The ^{13}C-nmr data for **1a** showed all 46 carbons. Detailed examination of the ^{1}H-nmr data and the ^{1}H-^{1}H COSY spectrum of **1a** assigned most of the protons, allowing the structure of **1** to be determined as 14,15-dihydrodinophysistoxin-1. The absolute stereochemistries of **1** and **2** were concluded to be the same as those of **3**, since their optical rotations were of the same sign and of similar magnitude to those of **3**.



*$C_{14}H_{21}O_6$, Δ 4.5 mmu

Compound	Fragment Ion (*m/z*)					
	A (M+H)	A_1	A_2	A_3	A_4	B
1 (R=CH_3)*	821	803	785	767	749	125
2 (R=CH_3)	819	801	783	765	747	125
3 (R=H)	805	787	769	751	733	111

*14,15-dihydro

SCHEME 1

Compounds **1–3** showed strong cytotoxicity against L-1210 murine leukemia and CV-1 monkey kidney cells. Compound **2** also showed antiviral activity against HSV-1, with toxicity to the host cells. Compound **1** showed weaker antiviral activity against HSV-1 than that of **2**, although their cytotoxicities against L-1210 cells were essentially the same. Both **1** and **2** were slightly active against VSV on BHK cells (Table 1).

Compounds **1** and **2** showed slightly stronger cytotoxicity against L-1210 cells than **3**. Compound **1**, however, was less cytotoxic against CV-1 and BHK cells than **2** and **3**. Compounds **1** and **2** also showed moderate antiviral activity against HSV-1 and VSV and both compounds were more active against VSV than against HSV at the end point concentration (0.01 μg/ml) (Table 1).

In this study, it has been demonstrated that a cold water sponge, a species of the genus *Phakellia*, is another filter feeder which accumulates okadaic acid-type compounds in concentrations comparable to other natural sources. The isolation of compounds **1–3** suggests that other filter feeders in Maine waters may contain these toxins as well, as is the case in Japanese or European waters, which could cause serious problems for public health or the fisheries industry if they were accumulated in mussels or shellfish. Cytotoxicity shown in different years could indicate a stable residue in the cold waters from a one-time algal bloom or a regular occurrence of such a bloom.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were obtained on an Ft-ir spectrophotometer using a NaCl plate. Optical rotations were measured employing a Na lamp (589 nm) and a 5-cm (1-ml) cell. Nmr spectra were obtained with a General Electric GN 500 FT nmr spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C). Chemical shifts are reported in ppm referenced to the $CHCl_3$ peak at 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C. Fabms data were obtained with VG Analytical ZAB or 70-SE 4F instruments operated in the fab mode. Fabms/cid/ms data were obtained with a VG70-SE 4F instrument using He as collision gas. Gravity columns were prepared with commercial grade Si gel (70–230 mesh), Kieselgel 60 (EM reagent, 70–230 mesh), tlc grade Si gel (Bio-Rad 2–10 μm, treated with NH_3 prior to use), reversed-phase Si gel (Amicon RP-18), or Sephadex LH-20 gel. Hplc was performed using an Ultrasphere reversed-phase C_{18} (250×4.6 mm, 5-μm particles, Altex) column. An Ito multi-layer coil separator-extractor (PC, Inc.) was used with a No. 10 column (2.6 mm i.d., 400-ml capacity) at

TABLE 1. Biological Activities of Compounds **1–3**.[a]

Sample	L-1210[b]	CV-1[c]	HSV[d]	VSV[d]	BHK[c]
1	3	0/0.1	±/0.1	ND[e]/0.1	T[f]
		0/0.01	−/0.01	+/0.01	6PT[g]/0.01
2	2	6L[h]/1	++/1	ND/1	
		L/0.1	+/0.1	ND/0.1	T
		0/0.01	−/0.01	±/0.01	8PT/0.01
3	ca. 5[i]	22/0.1[j]	NT[k]	NT	NT
		0/0.01			

[a]Performed by Dr. R.G. Hughes, except as noted.
[b]IC_{50} (ng/ml).
[c]Zone diameter (mm)/concentration (μg/ml).
[d]+++=very active, ++=active, +=moderately active, ±=marginally active, −=inactive/concentration (μg/ml).
[e]Not detected because of cytotoxicity.
[f]Toxic.
[g]Partially toxic.
[h]Light stain (toxic).
[i]Performed by Dr. G.R. Wilson.
[j]Performed by T.G. Perun.
[k]Not tested.

600 rpm for high-speed countercurrent chromatography (hsccc) (19).

ANIMAL MATERIAL.—The tan leafy sponge (29-VII-86-1-3 and 31-VII-86-1-1, a total of 6.6 kg wet wt) was collected off the west coast of Mark Island, Maine at depths of 20 to 30 m in July 1986. A voucher specimen prepared in aqueous EtOH was identified as a species of the genus *Phakellia* by Dr. S. Pomponi, Harbor Branch Oceanographic Institute, Inc., Fort Pierce, FL.

BIOASSAYS.—Cytotoxicity was measured on CV-1 monkey kidney, BHK baby hamster kidney, and L-1210 murine lymphoma cell lines. The brine shrimp assay was also used to monitor toxicity of crude fractions (20). Antiviral activity was measured by the plaque reduction method (21) using *Herpes simplex* type-1 and *Vesicular stomatitis* viruses grown on CV-1 and BHK cells, respectively.

EXTRACTION AND ISOLATION.—The sponge (2.7 kg wet wt) was successively extracted with *i*-PrOH, Me_2CO, and MeOH. The combined extract was concentrated *in vacuo*, and the residual aqueous suspension was extracted with EtOAc. The organic layer was concentrated to dryness and the residue was partitioned (hexane-MeOH, 2:1, 750 ml). Each layer was concentrated to give a solid. Since both fractions showed cytotoxicity (brine shrimp mortality, 80–90% mortality at 20 μg/ml after 12 h), the upper layer was partitioned again between upper and lower layers of the same solvent system, after which the lower layer gave bioactive Fraction A (4.76 g). A portion of Fraction A (3.8 g) was partitioned between the lower and the upper layers of the same solvent system to give an upper layer (2.19 g, inactive) and a lower layer (Fraction B, 1.56 g, brine shrimp mortality 100% after 12 h at 10 μg/ml). Fraction B was separated by hsccc with EtOAc-heptane-MeOH-H_2O (7:4:4:3, upper layer as the mobile phase, 18–20 ml/fraction, flow rate 2 ml/min), and the bioactive Fraction C, which contained a mixture of **1** and **2** (19.2 mg), and Fraction D, which contained **3** (9.4 mg), were obtained. Fraction C (80 mg), was separated on a Sephadex LH-20 column with CH_2Cl_2-MeOH (1:1) then Si gel [tlc grade, treated with NH_3 (gas), 15 fractions] followed by hplc on a reversed-phase (C_{18}) column with MeOH-H_2O (7:2) to give **1** (3.60 mg, 1.3×10^{-4}% yield from wet sponge) and **2** (3.22 mg, 1.2×10^{-4}%).

Dinophysistoxin-1 [**2**].—Slightly yellowish solid: $[\alpha]^{25}D$ +19° (*c*=0.32, $CHCl_3$) [lit. (14) $[\alpha]^{20}D$ +28° (*c*=4.7, $CHCl_3$)]; fabms *m/z* 819 (M+H), 801, 783, 765, 285, 267; hrfabms *m/z* calcd for $C_{45}H_{71}O_{13}$ (M+H), M_r 819.4895, found M_r 819.4894 (hrfabms).

14,15-Dihydrodinophysistoxin-1 [**1**].—Colorless amorphous solid: $[\alpha]^{25}D$ +19° (*c*=0.36, $CHCl_3$); ir (film) ν max 3435, 2934, 2876, 1589, 1076, 1005, 985, 754 cm^{-1}; ^1H nmr δ 5.43 (1H, br s), 5.32 (1H, br s), 5.05 (1H, br s), 4.2–4.0 (4H, m), 3.95 (1H, d, *J*=9 Hz), 3.6–3.3 (m), 3.25 (1H, dd, *J*=10 and 1 Hz), 1.76 (3H, s), 1.32 (3H, s), 1.02 (3H, d, *J*=6.5 Hz), 0.93 (3H, d, *J*=6.5 Hz), 0.91 (3H, d, *J*=7.0 Hz), 0.88 (3H, *J*=7.0 Hz); ^{13}C nmr δ 175.3 s, 144.7 s, 129.4 s, 121.7 d, 113.0 t, 105.7 s, 98.0 s, 96.5 s, 84.9 d, 79.1 d, 76.5 d, 74.9 d, 72.8 d, 71.2 d, 71.1 d, 69.9 d, 69.2 d, 64.9 d, 60.1 d, 39.1 d, 38.4 d, 37.3 t, 35.4 t, 33.5 t, 33.4 t, 33.3 t, 31.5 t, 31.3 q, 30.4 t, 27.7 d, 27.6 t, 27.4 t, 26.7 t, 26.6 t, 26.5 t, 26.1 t, 23.3 q, 16.9 q, 16.2 q, 14.9 q, 10.8 q (41 carbons observed); fabms *m/z* 843 (M+Na), 270; hrfabms *m/z* calcd for $C_{45}H_{72}NaO_{13}$, M_r (M+Na) 843.4871, found M_r 843.4830.

Okadaic acid [**3**].—Fraction D (140 mg of above) was successively separated by hsccc with EtOAc-heptane-MeOH-H_2O (7:4:4:3, 18 ml/fraction, flow rate 1.8 ml/min using the upper layer as the mobile phase), a reversed-phase (C_{18}) Si gel column, and a Si gel column (treated with NH_3 prior to use) to give **3** as a light yellow glass (1.45 mg, 5×10^{-5}% yield from wet sponge): $[\alpha]^{24}D$ +18.7° (*c*=1.45, $CHCl_3$) [lit. (7) $[\alpha]^{20}D$ +21° (*c*=0.33, $CHCl_3$)]; fabms *m/z* 827 (M+Na), 805 (M+H), 788, 769, 751, 285, 267; hrfabms *m/z* calcd for $C_{44}H_{68}NaO_{13}$, M_r (M+Na) 827.4553; found M_r 827.4550.

14,15-Dihydrodinophysistoxin-1 methyl ester [**1a**].—Compound **1** (3.0 mg) was treated with CH_2N_2 in Et_2O (3 ml) at room temperature for 20 min. The reaction product was passed through a short Si gel column with $CHCl_3$-MeOH (4:1) to give a colorless oil (3.08 mg), which was purified by reversed-phase hplc (C_{18}, MeOH-H_2O, 7:1) to give **1a** (1.97 mg, oil, 66%): ^1H nmr δ 5.41 (1H, br s, H-25a), 5.06 (1H, br s, H-25b), 5.02 (1H, m, H-9), 4.11 (1H, br d, *J*=10.0 Hz, H-24), 4.06 (1H, dd, *J*=11.5 and 4.5 Hz, H-7), 4.02 (1H, m, H-16), 3.94 (1H, d, *J*=9.5 Hz, H-26), 3.59 (1H, m, H-38a), 3.56 (1H, m, H-22), 3.55 (1H, m, H-12), 3.50 (1H, m, H-38b), 3.38 (1H, dd, *J*=10.0 and 10.0 Hz, H-23), 3.25 (1H, d, *J*=10.0 and 1.5 Hz, H-30), 2.09 (1H, dd, *J*=15.0 and 2.5 Hz, H-3a), 1.73 (3H, s, Me-10), 1.68 (1H, d, *J*=15.0 Hz, H-3b), 1.36 (3H, s, Me-2), 1.32 (1H, ddd, *J*=14.0, 10.5, and 3.5 Hz, H-28a), 1.15 (1H, ddd, *J*=12.5, 5.0, and 3.3 Hz, H-33b), 1.03 (3H, d, *J*=6.5 Hz, Me-29), 0.92 (3H, d, *J*=6.5 Hz, Me-35), 0.91 (3H, d, *J*=5.5 Hz, Me-13), 0.90 (3H, d, *J*=6.5 Hz, Me-31) (assignment by COSY); ^{13}C nmr δ 176.7 s, 143.3 s, 139.1 s, 121.4 d, 112.6 t, 105.6 s, 97.9 (?), 96.1 s, 84.9 d, 78.6 d, 75.4 s, 76.6 d, 74.8 d, 72.3 d, 71.5 d, 70.9 d, 69.6 d, 68.8 d, 64.5 d, 59.5 d, 52.6 d, 44.0 t, 39.0 d, 38.4 d, 37.0 t, 35.1 t, 33.4 t, 33.1 t, 32.9 t, 31.4 t, 31.1 q, 30.05, 29.9 t, 27.7 d, 27.5 t, 27.4 t, 27.3 d, 26.6 t, 26.4 t, 26.3 t, 26.0 t, 23.1 q, 16.8 q, 16.0

q, 14.8 q, 10.7 q; fabms *m/z* 835 (M+H), 817, 799, 783, 285, 267; hrfabms *m/z* calcd for $C_{46}H_{75}O_{13}$, M_r (M+H) 835.5208; found M_r 835.5166.

Dinophysistoxin-1-methyl ester [**2a**].—Compound **2** (3.3 mg) was treated with CH_2N_2 as above to give methyl ester **2a** (1.64 mg, 50%, colorless amorphous): ^{1}H nmr δ 5.55 (1H, dd, J=15 and 7.5 Hz), 5.48 (1H, dd, J=15 and 7.0 Hz), 5.40 (1H, dd, J=2.0 and 2.0 Hz), 4.47 (1H, dd, J=7.0 and 7.0 Hz), 4.08 (2H, m), 3.95 (1H, dd, J=9.0 Hz), 3.94 (1H, m), 3.81 (3H, s), 3.62–3.55 (3H, m), 3.51 (1H, br d, J=9.5 Hz), 3.41 (1H, t, J=10.0 Hz), 3.39 (1H, t, J=8.0 Hz), 3.25 (1H, dd, J=10.0 and 2.0 Hz), 2.28 (1H, dd, J=7.5 and 7.5 Hz), 1.73 (3H, s), 1.36 (3H, s), 1.03 (3H, d, J=6.5 Hz), 1.03 (3H, d, J=7.0 Hz), 0.91 (3H, d, J=6.5 Hz), 0.90 (3H, d, J=7.0 Hz); ^{13}C nmr δ 177.2 s, 144.1 s, 138.1 s, 135.9 d, 131.4 d, 121.8 d, 112.6 t, 105.9 s, 98.0 s, 96.2 s, 85.1 d, 79.1 d, 76.6 d, 75.6 s, 74.8 s, 71.6 d, 70.9 d, 69.6 d, 68.9 d, 64.5 d, 59.5 t, 52.9 q, 44.1 t, 41.9 d, 39.0 d, 37.2 t, 35.1 t, 33.0 t, 32.8 t, 31.7 t, 31.2 q, 30.7 t, 29.7 t, 27.8 d, 27.5 t, 27.4 d, 27.3 t, 26.5 t, 26.4 t, 26.0 t, 23.1 q, 16.8 q, 16.0 q, 15.8 q, 10.9 q; fabms *m/z* 833 (M+H, weak peak), 815 (M−H_2O), 299, 281; hrfabms *m/z* calcd for $C_{46}H_{73}O_{13}$, M_r (M+H) 833.5050, found M_r 833.4989 (hrfabms).

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LITERATURE CITED

1. R. Sakai, "Biologically Active Compounds from Tunicates and a Sponge," Ph.D. Thesis, University of Illinois, Urbana, IL, 1991.
2. M. Murata, M. Shimatani, H. Sugitani, Y. Oshima, and T. Yasumoto, *Bull. Jpn. Soc. Sci. Fish.*, **48**, 549 (1982).
3. M. Kumagai, T. Yanagi, M. Murata, T. Yasumoto, M. Kat, P. Lassus, and J.A. Rodriguez-Vazquez, *Agric. Biol. Chem.*, **50**, 2853 (1986).
4. Y. Murakami, Y. Oshima, and T. Yasumoto, *Bull. Jpn. Soc. Sci. Fish.*, **48**, 69 (1982).
5. T. Yasumoto, *Kagaku Zokan*, **111**, 3 (1987).
6. K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Gopichand, and F.J. Schmitz, *J. Am. Chem. Soc.*, **103**, 2469 (1981).
7. D. Uemura, in: "Bioorganic Marine Chemistry," Ed. by P.J. Scheuer, Springer-Verlag, New York, 1991, Vol. 4, pp. 1–31.
8. F.J. Schmitz, R.S. Prasad, Y. Gopichand, M.B. Hossain, and D. van der Helm, *J. Am. Chem. Soc.*, **103**, 2467 (1981).
9. T. Yasumoto and M. Murata, *Chem. Rev.*, **93**, 1897 (1993).
10. T. Yasumoto, N. Seino, Y. Murakami, and M. Murata, *Biol. Bull.*, **172**, 128 (1987).
11. T. Yasumoto, M. Murata, Y. Oshima, M. Sano, G.K. Matsumoto, and J. Clardy, *Tetrahedron*, **41**, 1019 (1985).
12. T. Yanagi, M. Murata, K. Torigoe, and T. Yasumoto, *Agric. Biol. Chem.*, **53**, 525 (1989).
13. M. Suganuma, H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada, and T. Sugimura, *Proc. Natl. Acad. Sci., U.S.A.*, **85**, 1768 (1988).
14. H. Fujiki, M. Suganuma, H. Suguri, S. Yoshizawa, K. Takagi, N. Uda, K. Wakamatsu, K. Yamada, M. Murata, T. Yasumoto, and T. Sugimura, *Jpn. J. Cancer. Res.*, **79**, 1089 (1988).
15. P. Cohen, C.F.B. Holmes, and Y. Tsukitani, *Trends Biochem. Sci.*, **15**, 98 (1990).
16. C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, and G.A. Codd, *FEBS Lett.*, **264**, 187 (1990).
17. A. Takai, M. Murata, K. Torigoe, M. Isobe, G. Mieskes, and T. Yasumoto, *Biochem. J.*, **284**, 539 (1992).
18. E.A. Jares-Erijman, C.P. Bapat, A. Lithgow-Bertelloni, K.L. Rinehart, and R. Sakai, *J. Org. Chem.*, **58**, 5732 (1993).
19. D.G. Martin, in: "Countercurrent Chromatography." Ed. by N.B. Mandava and Y. Ito, Marcel Dekker, New York, 1988, pp. 565–582.
20. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
21. A.C. Schroeder, R.G. Hughes, Jr., and A. Bloch, *J. Med. Chem.*, **24**, 1078 (1981).

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