2-(Trifluoromethyl)-4(3*H*)-quinazalinone (47). Anthranilic amide (13.6 g, 0.1 mol) and trifluoroacetic anhydride (16.8 mL, 0.12 mol) in dioxane (50 mL) were stirred at 25 °C for 1 h whereupon the solvent was evaporated to yield *N*-(trifluoroacetyl)anthranilic amide (a known[66] compound), which was cyclized to the title compound by reflux (2 h) in acetic acid or (for small samples) by sublimation at 210 °C, yield 14.8 g (69%), mp 253–254 °C (lit.[67,68] mp 249–250 °C).

2,2-Bis(trifluoromethyl)-1,2-dihydro-4(3*H*)-quinazolinone (48). Anthranilic amide (13.6 g, 0.1 mol) and hexafluoroacetone hydrate (15 mL) in dioxane (50 mL) were refluxed for 2 h, whereupon the solvent was evaporated and the residue purified by sublimation (yielding centimeter-big crystals) or by recrystallization from aqueous ethanol, yield 19.4 g (71%), mp 110–112 °C: IR 3315, 3265, 1754, 1623, 1518, 1491, 1326, 1305, 1274, 1243, 1228, 1217, 1185, 1161, 1122, 1086, 976, 751, 718, 688 cm^{-1}; ^{13}C NMR, see the NMR chart.

Synthesis of Compound 29. 4(3*H*)-Quinazolinone (2.92 g, 0.02 mol) and 2-methylindole (2.62 g, 0.02 mol) were refluxed in acetic anhydride (35 mL) for 4 h. Excess of Ac_2O was then evaporated and the residue treated with methanol yielded crystals of **29**, 5.51 g (77%), mp 232–234 °C: IR 3260 (NH, br), 1711, 1695, 1649, 1608, 1491, 1468, 1372, 1309, 1242, 763, 748 cm^{-1}; MS, *m/z* (relative intensity) 361 (M^+, 24), 318 (8), 277 (11), 276 (100), 158 (35), 146 (38), 131 (40), 130 (100), only diagnostic peaks above *m/z* 100 are listed; ^{13}C NMR 171.1 (C=O), 168.9(C=O), 163.1 (C=O), 61.1 (d), 27.1 (q, $NCOCH_3$), 22.5 (q, $NCOCH_3$), 11.9 (q, indolic 2-CH_3) ppm, in addition 14 signals from the aromatic carbon atoms were observed between 139.4 and 105.3 ppm.

Acknowledgment. We thank Professors T. Kametani, T. Kamikado, and B. Danieli for provision of spectral information and samples. Generous gifts of isatoic anhydride from BASF, Ludwigshafen, Germany, and 3,4-dimethoxy- and 3,4-methylenedioxyisatoic anhydride from Dr. G. M. Coppola, Sandoz, Inc., East Hannover, NJ, are gratefully acknowledged.

Registry No. **1**, 84-26-4; (±)-**2**, 518-18-3; **3**, 118-48-9; **4**, 16062-71-8; **5**, 77784-62-4; **6**, 95274-40-1; **10a**, 95274-41-2; **10b**, 95274-43-4; **11**, 95274-55-8; **12a**, 4119-10-2; **12b**, 95274-42-3; **16a**, 522-55-4; **16b**, 20999-50-2; **16c**, 51059-70-2; **16d**, 95274-64-9; **16e**, 95312-98-4; **17**, 38990-11-3; **18**, 33284-02-5; **19**, 60941-86-8; **20**, 95274-56-9; **21**, 59863-00-2; **22b**, 95312-97-3; **23**, 95274-44-5; **24a**, 95274-45-6; **24b**, 95274-46-7; **25a**, 95274-53-6; **25b**, 95274-54-7; **25c**, 95274-47-8; **26**, 95274-57-0; **29**, 95274-66-1; **30**, 55786-24-8; **31a**, 95274-59-2; **33a**, 95274-60-5; **33b**, 95274-61-6; **33c**, 72502-82-0; **40**, 68353-23-1; **44a**, 95274-63-8; **44b**, 91457-75-9; **44c**, 95274-62-7; **45**, 319-76-6; **46**, 22292-42-8; **47**, 26059-81-4; **48**, 95274-65-0; 2-$CH_3NHC_6H_4CO_2H$, 119-68-6; CH_3OCOCl, 79-22-1; 2-$CCl_3CONHC_6H_4CO_2H$, 4257-77-6; 3,5-Cl_2-2-$H_2NC_6H_2CO_2H$, 2789-92-6; 5-Cl-2-$H_2NC_6H_3CO_2H$, 635-21-2; 4,5-$(CH_3O)_2$-2-$H_2NC_6H_2CO_2H$, 5653-40-7; 2-$H_2NC_6H_4CONH_2$, 88-68-6; 2-$H_2NC_6H_4CONHCOCF_3$, 95274-68-3; $(CF_3)_2C(OH)_2$, 677-71-4; $HC(OC_2H_5)_3$, 122-51-0; *N*-methylisatoic anhydride, 10328-92-4; tryptamine, 61-54-1; 5-methoxytryptamine, 608-07-1; 10-methoxy-13b-(trifluoromethyl)-13b,14-dihydrorutaecarpine, 95274-67-2; 3,4-(methylenedioxy)isatoic anhydride, 57385-14-5; 3-[2-(3-indolyl)ethyl]-6,7-(methylenedioxy)-2-(trifluoromethyl)-4(3*H*)-quinazolinone, 95274-48-9; 3,4-(methylenedioxy)-13b-(trifluoromethyl)-13b,14-dihydrorutaecarpine, 95274-49-0; 3-[2-(3-indolyl]ethyl]-6,7-dimethoxy-2-(trifluoromethyl)-4(3*H*)-quinazolinone, 95274-50-3; 3,4-dimethoxyisaoic anhydride, 20197-92-6; 3-[2-(5-methoxy-3-indolyl)ethyl]-6,7-dimethoxy-2-(trifluoromethyl)-4(3*H*)-quinazoline, 95274-51-4; 2,3,10-trimethoxy-13b-(trifluoromethyl)-13b,14-dihydrorutaecarpine, 95274-52-5; 2-methyl-4*H*-3,1-benzoxazin-4-one, 525-76-8; 2-phenyl-4*H*-3,1-benzoxazin-4-one, 1022-46-4; 2-(trichloromethyl)-4*H*-3,1-benzoxazin-4-one, 41470-85-3; 2-methyl-4(3*H*)-quinazolinone, 1769-24-0; 3-[2-(3-indolyl)ethyl]-1-methyl-1,2-dihydro-4(3*H*)-quinazoline, 55786-32-8; 4(3*H*)-quinazolinone, 491-36-1; 2-methylindole, 95-20-5.

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Structures and Stereochemistries of Oscillatoxin B, 31-Noroscillatoxin B, Oscillatoxin D, and 30-Methyloscillatoxin D

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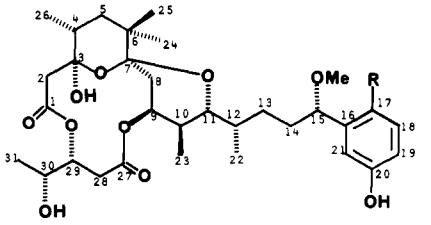
Four aplysiatoxin-related compounds, viz., oscillatoxin B (**3**), 31-noroscillatoxin B (**5**), oscillatoxin D (**6**), and 30-methyloscillatoxin D (**4**), have been isolated from marine blue-green algae belonging to Oscillatoriaceae. The structures and stereochemistries have been determined by spectral studies and chemical degradation. Oscillatoxin B was shown to be a mixture of the C-4 isomers B1 (**3a**) and B2 (**3b**).

The marine blue-green alga *Lyngbya majuscula* (Oscillatoriaceae) is the causative agent of a severe contact dermatitis in Hawaii[3] and Okinawa.[4] Two substances, aplysiatoxin (**1**) and debromoaplysiatoxin (**2**), which account for the highly inflammatory response associated with this dermatitis, have been isolated from *L. majuscula*,[5,6]



1 R = Br
2 R = H

as well as from the digestive gland of the seahare *Stylocheilus longicauda*,[7] a gastropod mollusk that feeds

(1) Work performed at the University of Hawaii while on sabbatical leave from the Department of Chemistry, University of Tasmania, Hobart, Tasmania, Australia, in 1981–1982.
(2) Lilly Research Laboratories, M-539, Eli Lilly & Co., Indianapolis, IN.
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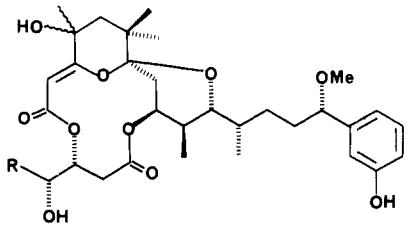
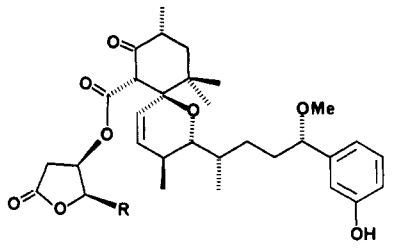
preferentially on the cyanophyte.

The species that grows in deep water on the pinnacles in the lagoon of Enewetak Atoll in the Marshall Islands contains, in addition to debromoaplysiatoxin and anhydrodebromoaplysiatoxin,[6] two minor constituents, viz., oscillatoxin B (3) and 30-methyloscillatoxin D (4). Com-



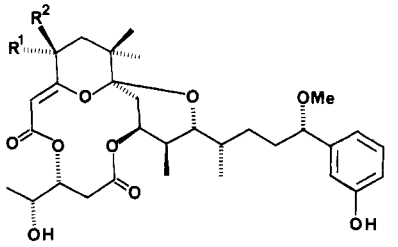
3 R = Me
5 R = H

pounds 3 and 4 are also present in other toxic Oscillatoriaceae, for example, in a mixture of two blue-green algae, *Schizothrix calcicola* and *Oscillatoria nigroviridis*, which grows on the seaward side of Enewetak Island. In this algal mixture 31-nortoxins are also present, the major one being oscillatoxin A.[8] Two related 31-nor compounds, viz., 31-noroscillatoxin B (5) and oscillatoxin D (6), are minor constituents in this algal mixture.



4 R = Me
6 R = H

In this paper we present results of spectral studies and chemical degradation which show that the oscillatoxins 3–6 have the structures and stereochemistries depicted in the structural formulas. Oscillatoxin B was found to be a 5:1 mixture of two C-4 isomers B1 (3a) and B2 (3b), the major one being B1.



	R^1	R^2
3a	OH	Me
3b	Me	OH

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Table I. ^{1}H NMR Data for New Oscillatoxins in Acetone-d_6

	3a[a]	3b[a]	5[b]	4[c]	6[c,d]
H-2	5.412 (s)	5.341	2.86	4.022 (d)	3.972
H-4				2.793 (m)	2.790
H-5_{ax}	2.054 (d)	2.053	2.07	1.391 (t)	1.374
H-5_{eq}	1.568 (d)	1.418	1.56	1.738 (dd)	1.690
H-8_{ax}	2.375 (dd)	2.405	2.39	5.758 (dd)	5.739
H-8_{eq}	1.728 (dd)	1.736	1.74		
H-9	4.800 (q)	4.814	4.78	5.500 (dd)	5.477
H-10	1.76 (m)	1.76	1.7–1.8	2.13 (m)	2.12
H-11	3.691 (dd)	3.696	3.66	3.137 (br d)	3.152
H-12	1.60 (m)	1.60	1.5–1.6	1.70 (m)	1.71
2 H-13	1.46 (m)	1.46	1.3–1.4	1.7–1.4 (m)	1.7–1.4
2 H-14	1.72 (m)	1.72	1.7–1.8	1.7–1.4 (m)	1.7–1.4
H-15	4.015 (dd)	4.004	4.01	4.022 (dd)	4.017
H-17	6.800 (dt)	6.787	6.79	6.781 (dt)	6.790
H-18	7.158 (t)	7.145	7.14	7.155 (t)	7.162
H-19	6.722 (ddd)	6.714	6.71	6.726 (ddd)	6.739
H-21	6.831 (dd)	6.825	6.82	6.813 (dd)	6.834
3 H-22	0.809 (d)	0.805	0.80	0.884 (d)	0.850
3 H-23	0.844 (d)	0.842	0.84	0.862 (d)	0.850
3 H-24	1.063 (s)	1.170	1.06	0.906 (s)	0.883
3 H-25	0.907 (s)	0.913	0.90	1.298 (s)	1.252
3 H-26	1.367 (s)	1.339	1.36	0.980 (d)	0.974
H-28	2.811 (dd)	2.807	2.77	2.515 (dd)	2.510
H-28′	2.700 (dd)	2.686	2.71	3.028 (dd)	2.998
H-29	5.133 (ddd)	5.118	5.07 (tt)	5.358 (ddd)	5.448 (ddt)
H-30	4.10 (m)	4.10	3.69 (d)	4.791 (dq)	4.521 (dd)
H-30′					4.415 (dd)
3 H-31	1.079 (d)	1.084		1.398 (d)	
3 H OMe	3.150 (s)	3.143	3.14	3.132 (s)	3.130
OH	10.36		10.40		
OH on 20	8.32		8.34	8.232	

[a] J (Hz) for **3a**: 5,5′ = −14.8; 8,8′ = −15.0; 8,9 = 3.3; 8′,9 = 2.8; 9,10 = 2.8; 10,11 = 10.6; 10,23 = 6.9; 11,12 = 1.4; 12,22 = 6.4; 14,15 = 7.9; 14′,15 = 4.7; 17,18 = 7.5; 17,19 = 1.4; 17,21 = 1.5; 28,28′ = −18.0; 28,29 = 3.3, 28′29 = 11.0, 29,30 = 4.2; 30,31 = 6.4. The coupling constants and multiplicities for **3b** are essentially the same. [b] Coupling constants and multiplicities for **5** are essentially the same as indicated for **3a** except $J_{29,30} = J_{29,30'} = 4$ Hz. [c] J (Hz) for **4**: 2,4 = 1.0; 5,5′ = −13.8; $5_{eq,4}$ = 7.0; 8,9 = 10.4; 8,10 = 1.7; 9,10 = 2.9; 10,11 = 10.0; 10,23 = 6.8; 11,12 = 1.0; 12,22 = 7.2; 14,15 = 4.5; 14′,15 = 7.5; 17,18 = 7.6; 17,19 = 1.1; 18,19 = 7.7; 19,21 = 2.5; 28,28′ = −18.1; 28,29 = 5.9; 28′,29 = 0.9; 29,30 = 4.0; 30,31 = 6.6. [d] Coupling constants and multiplicites for **6** are as indicated for **4** except the following J (Hz): 29,30= 1.1; 29,30′ = 4.7; 30,30′ = −10.9.

Isolation. The lipophilic extract of *L. majuscula* collected from Reefer 8 and South Medren Pinnacles was subjected to gel filtration and reverse-phase chromatography[6] to give oscillatoxin B and 30-methyloscillatoxin D. Separation of oscillatoxin B into the B1 (**3a**) and B2 (**3b**) isomers was achieved by further chromatography on a cyano column. Attempts to crystallize **3a** and **3b** failed in our hands, but **4** could be obtained from methanol as colorless crystals. A similar procedure, i.e., gel filtration and reverse-phase chromatography, was used to isolate 31-noroscillatoxin B (**5**) and oscillatoxin D (**6**) from the lipophilic extract of the *S. calcicola*/*O. nigroviridis* mixture. Neither of the latter compounds was obtained crystalline.

Structure Determination. Oscillatoxin B1 (**3a**) was found to have an elemental composition $C_{32}H_{46}O_{10}$ on the basis of a field desorption mass spectral determination (molecular ion at m/z 590) and ^{1}H and ^{13}C NMR spectral data shown in Tables I and II. The ^{1}H NMR spectrum of oscillatoxin B1 bore a close resemblance to that of debromoaplysiatoxin (**2**),[6] strongly suggesting that oscillatoxin B1 possessed the same basic tetracyclic skeleton as debromoaplysiatoxin. Mass spectral data [intense fragment ion peaks at m/z 330, 298 (330 − MeOH), and 137] supported the presence of a moiety in **3a** represented by carbons 6–25 in debromoaplysiatoxin. There were two

Table II. ^{13}C NMR Data for New Oscillatoxins and Debromoaplysiatoxin

	2[a,b]	3a[a,b]	4[a,c]	6[d]
C-1	169.0 (s)	167.6	168.8	168.9
C-2	46.7 (t)	106.7 (d)	64.9	64.3
C-3	100.6 (s)	158.9	205.6	206.9
C-4	35.5 (d)	79.6	41.0 (d)	40.6
C-5	40.9 (t)	37.4	43.4	42.9
C-6	38.8 (s)	37.4	40.7	40.1
C-7	98.6 (s)	104.2	81.2	81.2
C-8	33.4 (t)	43.5	134.5 (d)	133.9
C-9	73.1 (d)	73.4	125.4	125.8
C-10	35.2 (d)	34.2	30.0	29.9
C-11	69.6 (d)	73.9	78.5	77.8
C-12	34.0 (d)	34.6	33.6	33.8
C-13	31.0 (t)	30.6	35.9	36.0
C-14	35.9 (t)	31.6	30.7	30.8
C-15	85.6 (d)	85.2	84.2	84.6
C-16	145.7 (s)	140.6	144.0	144.0
C-17	119.1 (d)	118.6	118.7	119.0
C-18	129.6 (d)	130.0	129.5	129.8
C-19	114.8 (d)	115.0	114.6	115.1
C-20	158.1 (s)	158.2	156.2	157.5
C-21	114.4 (d)	114.2	113.6	113.9
C-22	13.4 (q)	11.7	12.6	12.8
C-23	12.9 (q)	13.7	16.5	16.3
C-24	23.4 (q)	23.7	24.5	24.3
C-25	26.6 (q)	25.9	22.3	21.7
C-26	16.3 (q)	23.3	14.2	14.0
C-27	170.2 (s)	170.3	174.6	174.3
C-28	34.5 (t)	34.4	36.5	34.0
C-29	74.0 (d)	74.5	72.4	72.5
C-30	66.9 (d)	66.8	79.2 (d)	70.8 (t)
C-31	17.6 (q)	17.6	14.5	
OCH_3	56.4 (q)	56.6	56.6	56.1

[a] $^1H^{13}C$ connectivities were determined by using the phase-cycled 16-step heteronuclear chemical shift correlation map (CSCM) experiment. [b] 75 MHZ; in acetone-d_6 using solvent peak as internal standard (δ 29.8); multiplicities, when not indicated, are the same as in previous column. [c] 75 MHz; in $CDCl_3$ using solvent peak as internal standard δ 77.0); multiplicities, when not indicated, are the same as in previous column. [d] 75 MHz; in benzene-d_6 using solvent peak as internal standard (δ 128.0); multiplicities, when not indicated, are the same as in previous column.

basic differences, however, in the ^1H NMR spectrum. First of all the AB quartet for the C-2 methylene protons was missing and in its place was a one-proton olefinic signal at δ 5.412. Second, the 3 H doublet for the methyl group on C-4 was absent and instead there was another 3 H singlet in the spectrum.

The infrared spectrum of oscillatoxin B1 exhibited a strong band at 1720 cm^{-1}, which was characteristic of an α,β-unsaturated ester carbonyl. The best position for the olefinic double bond of this sytem was between C-2 and C-3, since all of the signals for the 3,4-dioxygenated pentanoate unit could be found. Since the UV spectrum of oscillatoxin B1 was essentially identical in acidic, neutral, and basic media, a hydroxy group could not be present on C-3.[9] The most logical gross structure for oscillatoxin B1 was therefore **3**.

The relative stereochemistry of the bis-lactone system and the side chain was shown to be identical with that in debromoaplysiatoxin by ^1H NMR studies, in particular NOE difference spectral studies. The ester oxygen on C-9 had to be axially oriented since H-9 (δ 4.800) showed small couplings to the proton on C-10 (δ 1.76) and both protons on C-8 (δ 2.735, 1.728). A large coupling was observed between the protons on C-10 and C-11 (δ 3.691), which indicated that the methyl group on C-10 and the side chain at C-11 had to be oriented equatorially.

Irradiation of the methyl group at C-10 (δ 0.844) resulted in significant NOEs for the methine protons on C-9 (δ 4.800), C-11 (δ 3.691), C-12 (δ 1.60), and C-29 (δ 5.133). Examination of a Dreiding model indicated that the proton on C-29 could only come close to the methyl group on C-10 if the relative stereochemistry was 7*S**,9*S**,10*R**,11*R**,29*R**.

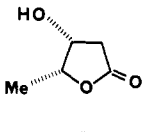
The positive NOE between the methyl group at C-10 and the proton on C-12 indicated that these groups were eclipsed in the preferred conformation of oscillatoxin B1 in solution. The large coupling $J_{10,11}$ = 11.6 Hz and the small coupling $J_{11,12}$ = 1.4 Hz supported this conclusion.

Irradiation of the (equatorial) methyl group on C-6 resonating at δ 1.063 resulted in positive NOEs for the axial proton on C-8 (δ 2.375) and the equatorial proton on C-5 (δ 1.568), whereas irradiation of the (axial) methyl group on C-6 absorbing at δ 0.907 induced a strong positive NOE for the methyl group at C-4 (δ 1.367). These experiments clearly established the relative stereochemistry of C-4 and C-7 and showed that the C-4 methyl group was axial. As the relative stereochemistry of C-7 had already been shown to be 7*S**, the one at C-4 had to be 4*R**.

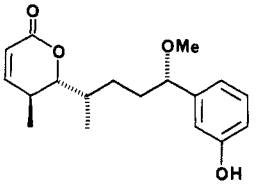
Optical and chemical degradative studies indicated that oscillatoxin B1 had the absolute stereochemistry depicted in **3a**.

Oscillatoxin B1 (**3a**) in methanol did not exhibit an optical rotation $[\alpha]_D$. Its circular dichroism (CD) spectrum in methanol, however, showed a negative Cotton effect with $[\theta]_{243}$(MeOH) −54 000, one presumably associated with the α,β-unsaturated ester system. A Cotton effect for the phenol chromophore adjacent to the asymmetric carbon at C-15 (265–290 nm)[6] could not be detected.

Acid hydrolysis of **3a** with 0.5 N HCl in aqueous ethanol produced **7** and **8**. The γ-lactone **7**, $[\alpha]_D$(MeOH) +76°,



7



8

exhibited a positive CD spectrum, $[\theta]_{220}$(MeOH) +980°, which was essentially identical with the one already described for (4*R*,5*R*)-4-hydroxy-5-methyl-4,5-dihydrofuran-2-one from acid hydrolysis of **2** and anhydrodebromoaplysiatoxin;[6] C-4 and C-5 in **7** and C-29 and C-30 in **3a** were therefore all *R*.

The δ-lactone **8** exhibited a negative CD curve, $[\theta]_{255}$(MeOH) −8600°, indicating that the absolute stereochemistry at C-5 in **8** and C-11 in **3a** was *R*. In (+)-parasarbic acid, which is known to have the opposite configuration at C-5, a positive CD curve, $[\theta]_{262}$ +7430, is observed.[10-12] The large axial–axial coupling, $J_{4,5}$ = 11 Hz, in **8** showed that the configuration at C-4 is *S*. The absolute stereochemistry at C-10 in **3** was therefore 10*R*.

The optical and proton NMR spectral data for oscillatoxin B1 (**3a**) and its degradation products show that the absolute configurations of eight of the nine asymmetric carbons are 4*R*,7*S*,9*S*,10*R*,11*R*,12*S*,29*R*,30*R*. Since all these centers have the same absolute configurations as debro-

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moaplysiaxotin (2),[6] the absolute stereochemistry at C-15 in **3a** is probably also 15*S*, the same as it is in **2**.

Similar studies of oscillatoxin B2 and 31-noroscillatoxin B showed that their gross structures and relative stereochemistries are as shown in **3b** and **5**, respectively.

The elemental compositions of oscillatoxin D (**6**) and 30-methyloscillatoxin D (**4**) were found to be $C_{31}H_{42}O_8$ and $C_{32}H_{44}O_8$, respectively, by high-resolution mass spectrometry. The EI mass spectra of **4** and **6** both showed an intense fragment ion peak at m/z 137, but the characteristic m/z 330 peak present in the mass spectra of debromoaplysiatoxin and oscillatoxin B was missing. ^1H NMR analysis indicated, however, that the C-10 to C-23 portion was present in both oscillatoxin Ds. Further analysis showed that C-7 to C-11 were located in a dihydropyran ring where the double bond was between C-8 and C-9 (J = 10.4 Hz). Unlike in **2** and **3**, where C-7 was a ketal carbon, C-7 was now a carbon that had only one oxygen attached to it (^{13}C signal at δ 81.2 for both **4** and **6**).

^1H NMR and IR spectral data (band at 1788 cm^{-1} for **4**) indicated that the 3,4-dioxygenated pentanoate unit was present in a γ-lactone ring. 30-Methyloscillatoxin D (**4**) possessed a methyl substituent (C-31) on the γ-lactone ring, but oscillatoxin D (**6**) differed from (**4**) in that this methyl group was missing.

^1H–^1H NOE experiments allowed us to assign the relative stereochemistries of **4** and **6**. Irradiation of one of the (axial) methyl groups on C-6 (δ 1.298) in **4** resulted in positive NOEs on the protons on C-2 (δ 4.022) and C-4 (δ 2.793). This meant that the C-2 and C-4 protons had to be in axial positions. Interestingly there was a small long-range coupling ($J_{2,4}$ = 1 Hz) between the C-2 and C-4 protons which could not be described as a W-type coupling.

Irradiation of the other (equatorial) methyl group on C-6 (δ 0.906) gave NOE enhancements of the signals for the proton at C-10 (δ 2.13) and the axial proton on C-5 (δ 1.391). The double bond therefore had to be attached axially to the cyclohexanone system resulting in the ether oxygen being in an equatorial position. Irradiation of the methyl group at C-10 (δ 0.862) produced positive NOEs on the signals for the methyl group at C-30 (δ 1.398) and the protons at C-11 (δ 3.137) and C-12 (δ 1.70), indicating that **4** had the same relative stereochemistry as established for δ-lactone **8** from oscillatoxin B1. The same stereochemistry was further supported by another NOE experiment whereby the signal for the proton at C-10 (δ 2.13) was enhanced when the methyl group at C-12 (δ 0.884) was irradiated.

The foregoing data lead us to the conclusion that the relative stereochemistry of 30-noroscillatoxin D (**4**) is 2*S**,4*R**,7*R**,10*S**,11*R**,12*S**,29*R**,30*R**. Oscillatoxin D (**6**) has the same relative stereochemistry based on similar data.

The absolute stereochemistry at C-15 was shown to be *S* for both **4** and **6**. The CD curves exhibited positive Cotton effects, viz., single peak, $[\theta]_{277}$ +2300° for **6** and a doubled peak $[\theta]_{270}$ +1950° and $[\theta]_{285}$ +4900° for **4**. Comparison of the CD curves with those for debromoaplysiatoxin and oscillatoxin A[6] indicated that the absolute configuration at C-15 was *S* for **4** and **6**. Compounds **4** and **6** were not degraded to lactone **7**, but the absolute configurations for the asymmetric centers in **4** and **6** are most likely the same as those in debromoaplysiatoxin.

Experimental Section

Spectral Analysis. ^1H NMR spectra were determined at 300 MHz and ^{13}C NMR spectra at 75 MHz. Proton chemical shifts are reported in δ units relative to acetone-d_5 (δ 2.04) and $CHCl_3$ (δ 7.24) as internal standard; ^{13}C chemical shifts are relative to acetone-d_6 (δ 29.80), $CDCl_3$ (δ 77.00), and benzene-d_6 (δ 128.00). ^1H homonuclear connectivities were determined by using the phase-cycled 16-step COSY experiment as described by Bax.[13] ^1H–^{13}C connectivities were determined using the phase-cycled 16-step heteronuclear shift correlation map (CSCM) experiment.[13] ^1H NOEs were obtained by selective continuous irradiation (decoupler on, hetero mode) for 3 s followed by data acquisition and recycling delay (decoupler off) for 3 s. Off-resonance experiments were also performed in a similar manner; NOE enhancement were observed in difference spectra produced by subtracting on-resonance from off-resonance spectra.

Isolation. *Lyngbya majuscula* was collected in the lagoon of Enewetak Atoll in the Marshall Islands at Reefer 8 and South Medrin Pinnacles and the freeze-dried alga (10.5 kg) was processed as previously described.[6]

Separation of the toxic fraction from phenylporasil B chromatography was achieved by reverse-phase HPLC on a Whatman Partisil M9 10/50 ODS-3 column using water/methanol (1:4) as the eluant (80 mg per injection). Oscillatoxin B (**3**) and 30-methyloscillatoxin D (**4**) were eluted between anhydrodebromoaplysiatoxin and debromoaplysiatoxin (**2**).[6]

Oscillatoxin B (**3**) (0.62 g, (6×10^{-3})% based on dried alga) was obtained as a white solid which decomposed at 89 °C: IR (KBr) ν_{max} 3410, 1720 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 278 nm (1750), 272 (1980), 243 (3820), 235 (5030), 216 (9350). Separation of oscillatoxin B (12 mg) into the two isomers was achieved by HPLC on a 30-cm Waters Bondapak CN column (10% ethyl acetate/hexane) to give 3 mg of oscillatoxin B1 (**3a**) and 1 mg of oscillatoxin B2 (**3b**).

30-Methyloscillatoxin D (**4**) (100 mg, 10^{-3}%) was isolated and crystallized from methanol to give small colorless crystals, mp 166 °C; high-resolution EIMS, m/z 556.300 (calcd for $C_{32}H_{44}O_8$ 556.304); IR (KBr) ν_{max} 3295, 1788, 1765, 1705, 690 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 280 nm (1800), 274 (2030), 224 (5150), 211 (8200), 208 (24 000).

A mixture of *Schizothrix calcicola* and *Oscillatoria nigroviridis* (2.4-kg wet weight) was collected on the seaward side of Enewetak Island and extracted with dichloromethane to give 4.0 g of oil. The oil was partitioned between hexane and 10% H_2O/MeOH. The aqueous MeOH layer was separated, adjusted to 25% H_2O/MeOH by the addition of water, and extracted with CCl_4. The CCl_4 extract was evaporated and the residue (1.0 g) was subjected to gel filtration on Sephadex LH-20 with 1:1 $CHCl_3$/MeOH followed by reverse-phase HPLC on a μC-18 column with 1:1 CH_3CN/H_2O. Final purification by HPLC on μPorasil with $CH_3CN/CHCl_3$ gave pure oscillatoxin B (**3**), 30-methyloscillatoxin D (**4**), 31-noroscillatoxin B (**5**), and oscillatoxin D (**6**). Purification was monitored by ^1H NMR spectroscopy.

31-Noroscillatoxin B (**5**) (50 mg) was obtained as a colorless gum; FDMS, m/z 576.

Oscillatoxin D (**6**) (50 mg, (5×10^{-4})%) was obtained as colorless oil; high-resolution EIMS, m/z 542.289 (calcd for $C_{31}H_{42}O_8$, 542.287).

Acid Hydrolysis of Oscillatoxin B (3). Oscillatoxin B (**3**) (40 mg) was dissolved in 5 mL of 0.5 N HCl and 2 mL of EtOH and refluxed under nitrogen for 1 h. The EtOH was removed in a stream of nitrogen and the remaining solution was freeze dried. The residue was subjected to short-path distillation (140 °C, 10^{-3} mmHg). The distillate was chromatographed on a Whatman Partisil M9 10/50 column (EtOAc) to give 2.4 mg (29%) of the γ-lactone **7** and 0.42 mg (2%) of the δ-lactone **8**. Compound **7**: $[\alpha]_D$(MeOH) +76°; $[\theta]_{220}$(MeOH) +980°; ^1H NMR ($CDCl_3$) δ 1.430 (d, 6.5 Hz, Me on C-5), 1.92 (br, OH), 2.552 (dd, −17.8 and 1.2 Hz, H-3), 2.800 (dd, −17.8 and 5.6 Hz, H-3), 4.44 (br ddd, H-4), 4.556 (qd, 6.5 and 3.8 Hz, H-5). Compound **8**: $[\theta]_{255}$(MeOH) −8600°; ^1H NMR ($CDCl_3$) δ 0.930 (d, 6.8 Hz, Me on C-6), 1.038 (d, 7.3 Hz, Me on C-4), 1.45 (m, 2 H on C-7), 1.55 (m, 2H on C-8), 1.70 (m, H-6), 2.61 (m, H-4), 3.192 (s, OCH_3), 4.002 (dd, 11.0 and 2.4 Hz, H-5), 4.007 (dd, 5.8 and 7.0 Hz, H-9), 5.208 (s br, OH), 5.921 (dd, 9.7 and 2.6 Hz, H-3), 6.624 (dd, 9.7, 2.1 Hz, H-2), 6.749 (ddd, 8.0, 2.5, and 1.1 Hz, H-13), 6.791 (dd, 2.5 and 1.5 Hz, H-11), 6.810 (dt, 7.5 and 1.1 Hz, H-15), 7.190 (dt, 8.0 and 0.5 Hz, H-14);

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UV (MeOH) λ_{max} (ϵ) 280 nm (1740), 275 (1880), 225 (7230), 207 (16600); high-resolution EIMS, m/z 304.167 (calcd for $C_{18}H_{24}O_4$, 304.167).

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Registry No. **3a**, 95189-16-5; **3b**, 95189-17-6; **4**, 95069-54-8; **5**, 95098-07-0; **6**, 95069-53-7.

Synthesis of 3-Aryl-3,4-dihydroisocoumarins[1]

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The use of the *o*-tolyloxazoline as a common intermediate for the general synthesis of a series of 3-aryl-3,4-dihydroisocoumarins is described. The synthesis involves three single and convenient steps and provides the products in good yields. The products were characterized by analysis of their mass and high-resolution 500-MHz proton NMR spectral data.

Introduction

Dihydroisocoumarins, similar to isocoumarins and coumarins, are a class of naturally occurring lactones exhibiting various biological activities.[3] 3-Substituted-3,4-dihydroisocoumarins occur as mycotoxins,[4,5] fungal metabolites,[6] and are a principal sweetness component of Japanese sweet tea.[7,8] Several synthetic methods have been reported for the syntheses of 3-substituted-3,4-dihydroisocoumarins,[5-13] but most require multistep syntheses. In connection with our interest in their sweetness property and their possible toxicity, we have developed a simple and convenient method for the synthesis of 3-aryl-3,4-dihydroisocoumarins.

Results and Discussion

The method developed involves three steps (Scheme I). According to the method of Meyers et al.[14] for the synthesis of bromophenyloxazoline, reaction of *o*-toluyl chloride with 2-amino-2-methyl-1-propanol in methylene chloride at 0 °C under argon afforded *N*-(2-methyl-3-hydroxyprop-2-yl)-*o*-toluamide (1) in quantitative yield. Cyclization of 1 with thionyl chloride gave 2-(*o*-(tolyl)-4,4-dimethyl-2-oxazoline (**2**)[15] in 92% yield. Lithiation of **2** with *n*-butyllithium in ethyl ether at 0 °C proceeded rapidly to form the deep red anion **3** (Scheme I). Addition of aryl al-

Scheme I

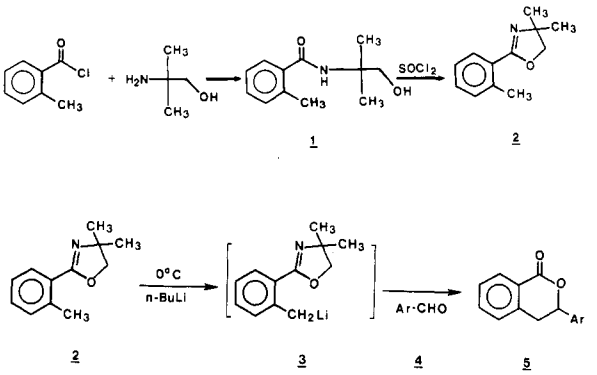

Table I. Formation of 3-Aryl-3,4-dihydroisocoumarins from *o*-Tolyloxazoline and Aryl Aldehydes or 1-Acetylnaphthalene

compd	3-aryl-3,4-dihydroisocoumarins	yield, %
benzaldehyde (**4a**)	3-phenyl-3,4-dihydroisocoumarin (**5a**)	72
p-tolualdehyde (**4b**)	3-*p*-tolyl-3,4-dihydroisocoumarin (**5b**)	80
p-methoxybenzaldehyde (**4c**)	3-*p*-methoxy-3,4-dihydroisocoumarin (**5c**)	65
o-fluorobenzaldehyde (**4d**)	3-*o*-fluoro-3,4-dihydroisocoumarin (**5d**)	78
1-naphthaldehyde (**4e**)	3-(1-naphthyl)-3,4-dihydroisocoumarin (**5e**)	87
2-naphthaldehyde (**4f**)	3-(2-naphthyl)-3,4-dihydroisocoumarin (**5f**)	68
1-acetylnaphthalene (**4g**)	3-methyl-3-(1-naphthyl)-3,4-dihydroisocoumarin (**5g**)	56

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