Structure of Malyngamide C

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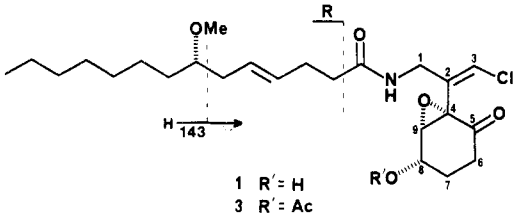
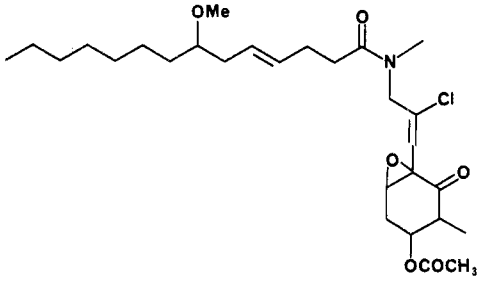
Malyngamide C (1), a chlorine-containing amide of 7(*S*)-methoxytetradec-4(*E*)-enoic acid, was found to be a major constituent of the lipophilic extract of *Lyngbya majuscula* from Fanning Island. The complete structure, including absolute stereochemistry, was determined by spectral and chemical studies of malyngamide C and several derivatives. Three structurally related compounds, viz., malyngamide C acetate (3), deoxymalyngamide C (4), and dideoxymalyngamide C (5), were shown to be minor constituents of the alga.

Malyngamides A and B are chlorine-containing amides of 7(*S*)-methoxytetradec-4(*E*)-enoic acid that are found in shallow water varieties of the marine blue-green alga *Lyngbya majuscula* in Hawaii.[2-4] Malyngamides D and E, on the other hand, are amides of 7-methoxy-9-methylhexadec-4(*E*)-enoic acid which are present in a deep water variety of *L. majuscula* from Enewetak.[5] We describe here the structure determination of malyngamide C (1), a chlorine-containing amide of 7(*S*)-methoxytetradec-4(*E*)-enoic acid isolated from a shallow-water variety of *L. majuscula* found on the reefs of Fanning Island in the Line Islands.[2] Malyngamide C shows many structural similarities to stylocheilamide (2) found in the sea hare *Stylocheilus longicauda* from Hawaii.[6,7] Stylocheilamide is presumably a dietary constituent of this gastropod mollusk and may arise from ingestion of *L. majuscula*, one of its favorite foods. Also described here are the structures of three related compounds from *L. majuscula* collected at Fanning Island, viz., malyngamide C acetate (3), deoxymalyngamide C (4), and dideoxymalyngamide C (5).



1 R′ = H
3 R′ = Ac



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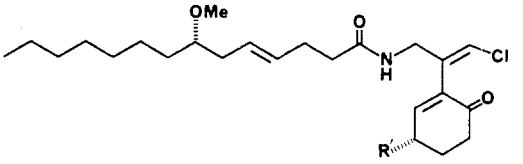
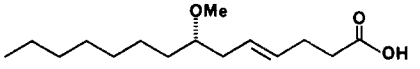


4 R′ = OH
5 R′ = H



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Malyngamide C (1), the major metabolite, was obtained as an optically active oil, $[\alpha]_D$ −27.4° in ethanol, by solvent partitioning, gel filtration, and HPLC of the lipophilic extract of the dried alga. The high-resolution electron-impact mass spectrum and ^{13}C NMR spectrum indicated that 1 had the molecular formula $C_{24}H_{38}ClNO_5$.[2] Proton NMR data as well as an intense mass spectral fragment ion peak at m/z 143 indicated that 1 was an amide of 7-methoxytetradec-4(*E*)-enoic acid. This partial structure was confirmed by acid hydrolysis of 1 to the known 7(*S*) acid 6.[2] Proton NMR data also showed that malyngamide C was a secondary alcohol. Acetylation of 1 with acetic anhydride and pyridine led to a monoacetate which was identical in all respects with the naturally occurring 3. A broad 1H triplet at 6.13 ppm which disappeared in the presence of D_2O, a carbon-13 signal at 172.6 ppm, and an IR carbonyl absorption at 1649 cm^{-1} indicated that 1 was a secondary amide. The ^1H NMR spectrum showed that the amide proton was coupled to an allylic methylene which in turn was coupled long range to a single olefinic proton. The data were consistent with a 2,3-disubstituted allylamide.

Treatment of 1 with silver nitrate in hot ethanol failed to produce a precipitate, suggesting that the chlorine was vinylic as it was in malyngamide A[2] and stylocheilamide.[5] Since the ^{13}C NMR spectrum showed only four olefinic carbon signals which corresponded to the two carbon–carbon double bonds in the fatty acid and allylamide units, the chlorine had to be attached to either C-2 or C-3 of the allylamide. After an inventory of the elements in the fatty acid and allylamide units, the structure of a $C_6H_7O_3$ unit remained to be determined and this included the location of the secondary alcohol group.

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(2) Cardellina J. H., II; Dalietos, D.; Marner, F.-J.; Mynderse, J. S.; Moore, R. E. *Phytochemistry* **1978**, *17*, 2091.

(3) Cardellina J. H., II; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240.

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(6) Rose, A. F.; Scheuer, P. J.; Springer, J. P.; Clardy, J. *J. Am. Chem. Soc.* **1978**, *100*, 7665.

(7) The origin of 2 in *S. longicauda* appears to be dietary. *L. majuscula* is one of the favorite foods of this gastropod mollusk and is one of the seaweeds that the larvae (veligers) of *S. longicauda* settle on for metamorphosis. Switzer-Dunlap, M.; Hadfield, M. *J. Exp. Mar. Biol. Ecol.* **1977**, *29*, 245.

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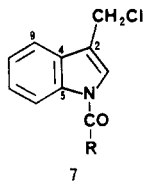
The ^{13}C NMR signal at 202 ppm and the IR absorption at 1724 cm^{-1} established the presence of a ketone group. A doublet of triplets at 2.57 ppm and a doublet of doublets at 2.24 ppm indicated that an ethylene group was adjacent to the ketone carbonyl. The β methylene of this group (1.93–2.0 ppm) was coupled to an oxygen-bearing methine (4.39 ppm) which in turn was coupled to another methine attached to oxygen (3.61 ppm). Since the methine signal at 4.39 ppm sharpened when the broad OH peak at 2.75 ppm was irradiated, this methine had to be bonded to the OH group. The methine resonating at 3.61 ppm, which was a 1:2:1 triplet (J = 1.1 Hz), displayed long-range coupling (W type) to one of the protons on the β methylene (1.93 ppm). This was verified by a decoupling experiment. The sizes of the various coupling constants in the $C_6H_7O_3$ unit strongly suggested that it was a substituted cyclohexanone. The remaining quaternary carbon had to connect the ketone carbon, the methine at 3.61 ppm and the oxygen on it, and the allylamide unit. The chemical shift of the β proton on the resulting α,β-epoxycyclohexanone agreed well with a value reported in the literature.[8]

The attachment of the cyclohexanone ring to C-2 and the chlorine to C-3 was established by the following chemical transformations. Malyngamide C underwent facile reduction with chromous chloride in acetic acid[9] to the enone **4**, a compound that was found to be identical with another minor constituent in the alga. As expected the epoxide methine signal at 3.61 ppm had shifted to 6.70 ppm for the β proton of a cyclohex-2-enone. Fortuitously compound **4** cyclized slowly to the indole **7** when a chloroform solution of **4** was allowed to stand for several weeks at −20 °C, clearly showing that the chlorine and the vinylic proton were bonded to the same carbon and that the cyclohexanone ring was fused to C-2 of **1**. The same structure was implied when it was found that malyngamide C reacted readily with hydrazine to give tetrahydrocinnoline **8**. The aromatic proton signal at 8.97 ppm had a chemical



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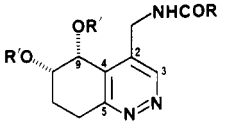
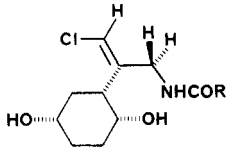


8 R′ = H
12 R′ = p-BrC_6H_4CO
13 R′ = p-$Me_2NC_6H_4CO$



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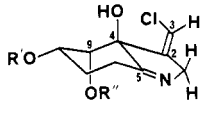

10 R′ = H ; R″ = RCO
11 R′ = RCO ; R″ = H

shift that was characteristic of a proton on C-3 of a pyridazine ring.[10]

Proton-difference NOE studies[11] were carried out next to define the geometry of the allylamide double bond. Unfortunately compounds **1**, **3**, and **4** failed to show any meaningful NOEs when the olefinic proton or the amide H was irradiated. Borohydride reduction of **4**, however, led to a diol **9** which showed appreciable enhancement of the signals for the C-1 methylene upon irradiation of the C-3 olefenic proton at 6.14 ppm. This indicated that the C-1 methylene and the proton on C-3 were cis, the same geometry shown by malyngamide A. Additional proof was obtained by NOE studies of **10** and **11**, two cyclic imines produced by a titanium tetraisopropoxide catalyzed rearrangement of **1**. Irradiation of the methylene signals at 4.3–4.5 ppm caused a positive NOE of the signal for the C-3 proton (at 6.232 ppm for **10** and 6.275 ppm for **11**), thereby confirming the cis relationship between the C-1 methylene and the olefinic proton.

Imine **10** had presumably been formed by intramolecular addition of the amide NH to the titanium-chelated carbonyl of **1**, followed by concerted migrations of the OH on C-5 to C-4 and the *N*-acyl group to the oxygen on C-9 with concomitant epoxide ring opening and formation of the imine double bond. Subsequent acyl transfer from the oxygen on C-9 to the oxygen on C-8 then produced imine **11**. All attempts to form **10** and **11** by other routes, for example, treatment of **1** with methanol/HCl, boron trifluoride etherate, or *p*-toluenesulfonic acid in benzene, failed, resulting mostly in polymeric material or anomalous products. The structures of **10** and **11** were deduced by detailed ^1H NMR analysis. Most helpful in concluding that **10** and **11** had the proposed structures were the homoallylic-type couplings observed between the C-1 protons and the β-proton on C-6.[12] The coupling constants indicated that the six-membered ring in **10** and **11** was in a chair conformation. This meant that the hydroxy group on C-4 had to be axial.

A circular dichroism study[13] was finally carried out on the bis-para-substituted benzoate esters **12** and **13** to assign the absolute stereochemistry of the three unknown chiral centers in malyngamide C. Conversion of **8** to **12** or **13** was achieved by reacting **8** with 2.3 equiv of the para-substituted benzoyl chloride in methylene chloride at 0 °C in the presence of 2.3 equiv of triethylamine and a catalytic amount of 4-(dimethylamino)pyridine.[14] The air-sensitive dibenzoates **12** and **13** showed negative first Cotton effects in the CD spectra at 257 and 326 nm, respectively, indicating that the absolute configurations at C-5 and C-6 in **8**, **12**, and **13** were *R* and *S*, respectively. This meant that the absolute stereochemistry of the cyclohexanone ring in **1** was 4*S*,8*S*,9*S*.

Experimental Section[11,15]

Isolation. *Lyngbya majuscula* was collected in shallow water near Cartwright Point, Fanning Island in April, 1977, and extracted with methanol and CH_2Cl_2. The extract was evaporated and the residual oil was distributed between ethyl acetate and water. Evaporation of the ethyl acetate layer gave an oil (7.2 g) which was partitioned between hexane and methanol/water (9:1). The concentration of the methanol/water layer was adjusted to

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(9) Mills, J. S.; Bowers, A.; Djerassi, C.; Ringold, H. J. *J. Am. Chem. Soc.* **1960**, *82*, 3399.
(10) Tsujimoto, T.; Nomura, T.; Ifuru, M.; Sasaki, Y. *Chem. Pharm. Bull.* **1979**, *27*, 1169.
(11) Qualitative homonuclear ^1H NOEs were obtained by selective continuous irradiation (decoupler on, hetero mode) for 2 s, followed by data acquisition; off-resonance experiments were also performed in a similar manner, and the NOE enhancements were observed in difference spectra produced by subtracting off-resonance spectra from on-resonance spectra. The NOE enhancements were very roughly 5–10%.
(12) Jackman, L. M.; Sternhell, S.; "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon Press: Oxford, 1969, p 325.
(13) Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy. Exciton Coupling in Organic Stereochemistry"; University Science Books: Mill Valley, CA, 1983.
(14) Attempted benzoylation of **8** with excess benzoyl chloride in pyridine at 57 °C resulted in a mixture of products, none of which was the desired dibenzoate.
(15) Carbon-13 NMR spectra were determined at 75 MHz in $CDCl_3$ using the solvent signal as internal reference (76.90 ppm). ^1H–^{13}C connectivities were determined by using a phase-cycled 16-step heteronuclear chemical shift correlation map (CSCM) experiment. (Bax, A. "Two-Dimensional Nuclear Magnetic Resonance in Liquids"; Delft University Press: Delft, Holland, 1982.)

3:1 and extracted with CCl_4 and finally to 1:3 and extracted with chloroform. The $CHCl_3$ layer was evaporated to give 358 mg of oil which was chromatographed on a column of Sephadex LH-20 (2.5 × 190 cm) with chloroform/methanol (1:1) to give 205 mg of a green oil. Reverse-phase HPLC on a Whatman ODS-2 column with acetonitrile/water (3:1) yielded dideoxymalyngamide C (**5**, 6.2 mg), followed by malyngamide C (**1**, 162 mg), deoxymalyngamide C (**4**, 6.3 mg), and malyngamide C acetate (**3**, 10.7 mg). Proton NMR data for malyngamide C and related compounds are given in Table I.

Malyngamide C (**1**) had the following properties: oil, $[\alpha]_D$ −27.4° (*c* 5.8, EtOH); IR (neat) 3300, 1725, 1650, 990 cm^{-1}; FDMS, *m/z* 457 and 455 (M^+, 1:3); EIMS, *m/z* (relative intensity) 457 (M^+, 0.5), 455 (M^+, 1:5), 420 (17), 315 (40), 313 (63), 143 (65), 69 (100); high-resolution EIMS, *m/z* 313.110 (calcd for $C_{15}H_{20}NO_4{}^{35}Cl$, 313.108, MH − 143 ion resulting from cleavage of fatty acid C-6–C-7 bond), 143.144 (calcd for $C_9H_{19}O$, 143.144); ^{13}C NMR ($CDCl_3$) δ 202.0 (s, C-5), 172.6 (s), 133.3 (s, C-2), 130.5 (d), 127.5 (d), 122.3 (d, C-3), 80.7 (d), 66.2 (d, C-8), 65.3 (d, C-9), 61.8 (s, C-4), 56.4 (q), 40.2 (t, C-1), 35.6 (t), 35.4 (t, C-6), 33.3 (t), 31.8 (t), 29.7 (t), 29.2 (t), 28.4 (t), 25.3 (t), 24.8 (t, C-7), 22.6 (t), 14.1 (q).

Malyngamide C Acetate (3). A solution of malyngamide C (357 mg) in 1 mL of CH_2Cl_2, 2 mL of pyridine, and 1 mL of acetic anhydride under nitrogen was stirred overnight. Standard workup gave an oil which was subjected to HPLC (Partisil, 5% EtOH in 1:1 in hexane/EtOAc) to give **3** (209 mg) as an oil: $[\alpha]_D$ −32.4° (*c* 1.4 EtOH); IR (neat) 3300, 1720, 1650, 970 cm^{-1}; FDMS, *m/z* (relative intensity) 499 and 497 (1:3); high-resolution EIMS, *m/z* 497.255 (calcd for $C_{26}H_{40}NO_6Cl$, 497.254), 355.121 (calcd for $C_{17}H_{22}NO_5Cl$, 355.119), 143.143 (calcd for $C_9H_{19}O$, 143.144); ^{13}C NMR ($CDCl_3$) δ 200.7 (s, C-5), 172.2 (s), 170.2 (s, Ac), 133.0 (s, C-2), 130.6 (d), 127.5 (d), 122.6 (d, C-3), 80.6 (d), 68.3 (d, C-8), 62.4 (d, C-9), 61.4 (s, C-4), 56.3 (q), 40.2 (t, C-1), 36.4 (t), 36.2 (t), 34.9 (t, C-6), 33.2 (t), 31.7 (t) 29.6 (t), 29.2 (t), 28.4 (t), 25.2 (t), 22.6 (t), 21.4 (t, C-7), 20.9 (q, Ac), 14.0 (q).

Deoxymalyngamide C (4). To a stirred solution of malyngamide C (55 mg) in 2 mL of HOAc under CO_2 was added 0.3 mL of $CrCl_2$ solution (prepared[9] by zinc amalgam reduction of 500 mg of $CrCl_3$ in 0.2 mL of HCl and 2.0 mL of water). The mixture was stirred for 5 min, diluted with 50 mL water, and extracted with CH_2Cl_2. The extract was washed with 5% K_2CO_3, dried ($MgSO_4$), filtered, and evaporated to give an oil. Purification by HPLC (Partisil, 3% EtOH in 1:1 hexane/EtOAc) gave 19 mg (36%) of **4** as an oil: $[\alpha]_D$ −23° (*c* 6.6, EtOH); UV λ_{max} (EtOH) sh 235 nm (ε 6300); IR (neat) 3300, 1680, 1650, 1000 cm^{-1}; EIMS, *m/z* (relative intensity) 441 (M^+, 0.5), 439 (M^+, 2), 404 (31), 372 (16), 299 (32), 297 (44), 143 (56); high-resolution EIMS, *m/z* 404.283 (calcd for $C_{24}H_{38}NO_4$, 404.280); ^{13}C NMR ($CDCl_3$) δ 197.2 (s, C-5), 172.7 (s), 153.0 (d, C-9), 135.5 (s, C-4), 135.1 (s, C-2), 130.5 (d), 127.6 (d), 119.6 (d, C-3), 80.7 (d), 66.0 (d, C-8), 56.4 (q), 43.5 (t, C-1), 36.4 (t), 36.2 (t), 35.7 (t, C-6), 33.3 (t), 32.1 (t, C-7), 31.8 (t), 29.7 (t), 29.3 (t), 28.5 (t), 25.3 (t), 22.6 (t), 14.0 (q).

Dideoxymalyngamide C (5): oil; EIMS, *m/z* (relative intensity) 425 (M^+, 0.2), 423 (M^+, 0.5), 283 (16), 281 (29), 143 (35).

7. Deoxymalyngamide C (**4**) was allowed to stand in chloroform-*d* solution for several weeks in the freezer. Evaporation left an oil which was shown to be indole **7** by ^1H NMR and mass spectral analysis: UV λ_{max} (EtOH) 245 nm (ε 10 800), 253 (9600), 275 (6300), 317 (2700), 331 (2700); FDMS, *m/z* (relative intensity) 405 (52, M^+), 403. (100, M^+), 260(6), 143 (32); EIMS, *m/z* (relative intensity) 373 (0.2), 371 (0.5), 336 (1), 261 (1), 207 (3), 143 (26).

8. To a stirred solution of malyngamide C (113 mg) in 2 mL of MeOH under nitrogen was added 109 mg of hydrazine hydrate and 20 mg of HOAc. After 2 h at room temperature the mixture was placed in the freezer (−10 °C), whereupon after 2 days white crystals were formed. Recrystallization from EtOH gave 50 mg of **8**: mp 109 °C; $[\alpha]_D$ +16.7° (*c* 8.7, EtOH); UV (EtOH) λ_{max} 259 nm (ε 2400), 313 (430), (0.1 N HCl in EtOH) λ_{max} 250 nm (ε 5000), peak at 313 disappears; IR (neat) 3250, 1649, 1606 cm^{-1}; EIMS, *m/z* (relative intensity) 433 (1.0, M^+), 401 (1), 291 (4), 273 (4), 178 (34), 162 (20), 143 (17), 117 (50), 69 (100); ^{13}C NMR ($CDCl_3$) δ 173.6, 158.6, 150.0, 138.1, 134.5, 130.5, 127.7, 80.6, 68.3, 65.3, 56.3, 37.7, 36.1, 36.0, 33.3, 31.8, 29.7, 29.3, 28.4, 28.0, 25.3, 25.0, 22.6, 14.1.

9. To a stirred solution of deoxymalyngamide C (**4**, 20 mg) in 1 mL of EtOH at room temperature under nitrogen was added

Table I. Proton NMR Data for Malyngamide C and Related Compounds[a,b]

	1	3	4	5	7	8	9[c]	10	11	12[d]	13[e]
NH	6.131 (6.2, 4.7)	6.10	5.4 (6.2–5.8)	6.15 (5.4)		6.828 (6.0)	6.507 (5.9, 4.3)			6.271 (5.9)	6.293
1α	4.033 (−14.1, 4.7, 0.8)	3.85	3.82 (−14.9, 5.8, 1.1)	3.917 (5.4, 0.9)	6.480 (3.2)	4.525 (6.1)	3.882 (−14.9, 5.9, 1.3)	4.485 (−20.2, 2.9, 2.0)	4.498 (−19.8, 2.9, 2.0)	4.430 (−16.3, 5.9)	4.462
1β	3.812 (−14.1, 6.2, 1.3)	3.96	3.72 (−14.9, 6.2, 1.1)	3.917 (5.4, 0.9)			3.776 (−14.9, 4.3, 1.3)	4.310 (−20.2, 2.4, 2.0)	4.393 (−19.8, 2.4, 2.0)	4.327 (−16.3, 5.9)	4.372
3	6.380 (1.3, 0.8)	6.38	5.933 (1.1)	6.264 (0.9)	4.673 (3.2)	8.966	6.137 (1.3)	6.232 (2.0)	6.275 (2.0)	9.080	9.102
5							4.083 (2.6)				
6α	2.568 (−16.9, 4.4, 3.9)	2.6	2.66 (−18.0, 4.5)	2.49	8.323 (8.1, 0.7)	3.423 (−18.0, 4.9)	1.5–1.9	2.57 (−13.6, 5.0, 2.3)	2.568 (−13.2, 3.8)	3.658 (−18.5, 5.6, 3.3)	3.617
6β	2.242 (−16.9, 12.5, 6.1)	2.2	2.44 (−18.0)	2.49		3.072 (−18.0, 10.0, 6.0)	1.5–1.9	2.65 (−13.6, 12.5, 5.4, 2.9, 2.4)	2.66 (−13.2, 8.5, 2.9, 2.4)	3.347 (−18.5, 11.4, 6.9)	3.333
7α	1.928 (−13.1, 12.5, 9.5, 4.4)	2.0	2.0	2.05	7.286 (8.1, 7.2, 1.3)	2.10 (−14.1, 10.6, 10.0, 5.0)	1.5–1.9	1.767 (−13.0, 12.5, 12.0, 5.0)	1.988	2.48 (−12.0, 11.7, 11.4, 5.6)	2.505
7β	2.013 (−13.1, 6.1, 5.5, 3.9, 1.1)	2.0	2.0	2.05		2.20 (−14.1, 6.0, 5.0, 3.6)	1.5–1.9	2.0 (−13.0, 5.4, 4.7, 2.3, 1.4)	1.988	2.3	2.31
8	4.392 (9.5, 5.5, 1.1)	5.4	4.15	2.50	7.031 (7.8, 7.2, 0.7)	3.998 (10.6, 3.7, 3.6)	3.695 (10.7, 4.2)	4.492 (12.0, 4.7, 2.8)	5.466	5.495 (11.7, 3.5)	5.429 (10.8, 3.4)
9	3.611 (1.1)[f]	3.63	6.700	6.926 (4.2)	7.358 (7.8, 1.3)	4.996 (3.7)	1.5–1.9	5.902 (2.8, 1.4)	4.667 (2.4, 0.5)	6.801 (3.5)	6.710 (3.4)

[a] Chemical shifts in ppm (coupling constants in Hz) at 300 MHz; in $CDCl_3$ (about 10–15 mg/0.5 mL) using residual $CHCl_3$ signal as internal reference (7.25 ppm). Numbering systems for **2–13** based on one for **1**. [b] NMR data for 7(*S*)-methoxytetradec-4(*E*)-enoyl group of **1**: δ 5.457 (m, 2 H), 3.311 (s), 3.149 (*p*, 1 H), 2.3 (m, 4 H), 2.2 (m, 2 H), 1.42 (m), 1.254 (m), 0.864 (t, 3 H). These chemical shifts do not change appreciably in other compounds listed in this table. [c] Proton on C-4 (1.5–1.9 ppm) should be axial since coupling constants indicate that C-5 H is equatorial and C-8 H is axial. [d] Chemical shifts for *p*-bromobenzoate groups: δ 7.921, 7.892, 7.610, 7.581. [e] Chemical shifts for *p*-dimethylaminobenzoate groups: δ 7.840, 7.691, 6.610, 6.507. [f] Triplet; shows *W* coupling to 7β proton.

sodium borohydride in EtOH (30 μL, 0.5 M). After 30 min, 10 mL of water was added followed by 0.3 mL of HOAc. The product was extracted from the mixture with EtOAc and the extract was dried ($MgSO_4$), filtered, and evaporated to give a clear oil. Purification by HPLC (Partisil, 3% EtOH in EtOAc) afforded 6 mg of diol **9**: EIMS, m/z (relative intensity) 445 (0.5 M^+), 443 (1, M^+), 408 (35), 390 (23), 376 (20), 358 (15), 303 (22), 301 (33), 143 (55), 69 (100).

10 and 11. To a stirred solution of malyngamide C (**1**, 59 mg) in 1 mL of CH_2Cl_2 at 25 °C under nitrogen was added 0.2 mL of titanium tetraisopropoxide. The mixture was stirred overnight, then diluted with water, and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered, and evaporated to yeild an oil which was purified by HPLC (Partisil, 5% EtOH in 1:1 hexane/EtOAc) to yield compounds **10** (2.2 mg) and **11** (1.7 mg).

Compound 10 had the following properties: oil, $[\alpha]_D$ +125° (c 0.16, MeOH); IR (neat 3200, 1755, 1660, 815 cm^{-1}; EIMS, m/e (relative intensity) 457 (1, M^+), 455 (4, M^+), 425 (1), 423 (3), 315 (4), 313 (9), 217 (33), 199 (18), 143 (82), 69 (100).

Compound 11 had the following properties: oil, $[\alpha]_D$ +40° (c 0.3, MeOH); IR (neat) 3360, 3110, 1735, 1650, 815 cm^{-1}; EIMS, m/z (relative intensity) 457 (8, M^+), 455 (20, M^+), 425 (2), 423 (4), 315 (15), 313 (41), 219 (30), 217 (55), 199 (43), 143 (97), 69 (100).

12 and 13. To a stirred solution of compound **8** (20 mg, 0.05 mmol) in 1.5 mL of CH_2Cl_2 cooled to 0 °C under nitrogen was added 4-(dimethylamino)pyridine (0.005 mmol) followed by triethylamine (0.12 mmol) and p-bromobenzoyl chloride (0.12 mmol). The mixture was allowed to stir at 0 °C for 6 h and at room temperature for 1 h. Water was added and the organic layer was washed with 5% HCl followed by aqueous sodium bicarbonate and saturated brine, filtered through cotton, and evaporated to give an air-sensitive oil. Purification by HPLC (Partisil, 1% EtOH in 9:1 EtOAc/hexane) gave 7.9 mg (20%) of bis(p-bromobenzoate) **12**: EIMS, m/z (relative intensity) 599 (1), 597 (1, M^+ - BrC_6H_4COOH), 397 (25), 143 (71), 69 (100); CD (MeOH) $[\Theta]_{257}$ -80 000°, $[\Theta]_{249}$ 0°, $[\Theta]_{241}$ +12 8000°.

Compound **13** was prepared by a similar procedure using p-(dimethylamino)benzoyl chloride.[16] After the reaction water was added and the organic layer was washed 2× with water followed by saturated $NaHCO_3$ and brine solutions, filtered, and evaporated to give a dark yellow residue. This material was passed through a Bond Elut silica column (Analytichem International) with EtOAc and then purified by HPLC (Partisil, 5% EtOH in EtOAc) to give a 30% yield of the bis[p-(dimethylamino)benzoate] **13**, CD (MeOH) $[\Theta]_{326}$ -177 000°, $[\Theta]_{314}$ 0°, $[\Theta]_{302}$ +114 000°.

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Registry No. **1**, 70622-52-5; **3**, 96845-19-1; **4**, 96845-20-4; **5**, 96845-21-5; **7**, 96845-23-7; **8**, 96845-22-6; **9**, 96845-24-8; **10**, 96845-25-9; **11**, 96845-26-0; **12**, 96845-27-1; **13**, 96845-28-2.

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Marine Natural Products: Spongiane Derivatives from the Sponge *Igernella notabilis*

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Three new diterpene lactones, 7α,17β-dihydroxy-15,17-oxidospongian-16-one 7-butyrate (**2**), 7α,17β-dihydroxy-15,17-oxidospongian-16-one 7-acetate (**3**), and 7α,17β-dihydroxy-15,17-oxidospongian-16-one (**4**), isolated from the Caribbean sponge *Igernella notabilis* are reported. The structure of **2** was determined by X-ray analysis and those of **3** and **4** were established by comparison of their spectral data with that of **2**. Lactone **2** crystallizes in the space group $P4_1$ with cell dimensions (138 K) $a = b = 10.782$ (6) Å and $c = 18.531$ (13) Å. The crystal structure was determined from 2302 data and the final R value was 0.032.

Kazlauskas et al. have reported[1] the isolation of a series of sponge diterpene metabolites designated spongianes, e.g. **1**, which are related to isoagatholactone[2], another sponge metabolite. In our continuing search for biologically active compounds, we have isolated from the Caribbean sponge *Igernella notabilis* (Duch & Mich.) three diterpene lactones **2**-**4** that have spongiane skeletons. The structure of one of these was determined by X-ray crystallographic analysis and the others by spectral comparisons.

For the major metabolite **2**, mp 197-198 °C, $[\alpha]$ -37.2°, the formula $C_{24}H_{36}O_6$ was suggested by the low-resolution FD mass spectrum, m/z 421 ($M^+ + 1$), and this was supported by ^{13}C NMR data indicating 24 carbons. The infrared spectrum contained bands at 3600, 1780, and 1730 cm^{-1}, compatible with hydroxyl, γ-lactone, and ester groups, and the latter were further evidenced by ^{13}C absorptions at δ 172.3 and 176.6. The acyl group of the ester was identified as n-butyryl by the occurrence of an ion in the FD mass spectrum corresponding to loss of $C_4H_7O_2$ (m/z 332) and by ^1H NMR data: δ 2.37 (2 H, t), 1.67 (2 H, sextet), 0.99 (3 H, t), see Table I. The ^1H NMR spectrum also showed three quaternary methyl signals (δ 0.75, 0.76, 0.93). The ^{13}C NMR spectrum revealed the presence of two acetal carbons [$-CH(O)_2$] and confirmed the absence of any double bonds. Hence, of the 7 degrees of unsaturation present in **2**, 5 were due to rings. Since the proton dispersion was inadequate to allow structure determination by NMR analysis, single-crystal X-ray diffraction was used to determine the complete structure which is shown in formula **2**.

A perspective view of **2** is shown in the ORTEP plot in Figure 1. An attempt to determine the absolute configuration of **2** by using the anomalous dispersion of Cu radiation by O atoms did not give conclusive results, but the majority of Bijvoet differences[3] (60%) out of 20 Friedel's

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