Luffariolides F and G, New Manoalide Derivatives from the Okinawan Marine Sponge Luffariella Sp.

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LUFFARIOLIDES F AND G, NEW MANOALIDE DERIVATIVES FROM THE OKINAWAN MARINE SPONGE *LUFFARIELLA* SP.

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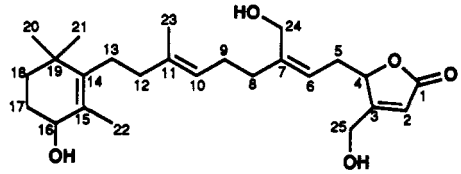
ABSTRACT.—Luffariolides F [**1**] and G [**2**], two new manoalide-related sesterterpenes with cytotoxic activities, have been isolated from the Okinawan marine sponge *Luffariella* sp. and their structures elucidated on the basis of spectroscopic data.

Manoalide (1) and related sesterterpenes (2–5) have been extensively investigated because of their potent anti-inflammatory activity and irreversible inhibition of phospholipase A_2 (6–10). We previously studied a sponge of the genus *Luffariella* (family Thorectidae; order Dictyoceratida) and isolated five new cytotoxic sesterterpenes, luffariolides A–E (11). Here we describe the isolation and structure elucidation of two other structurally related sesterterpenes, luffariolides F [**1**] and G [**2**], both possessing cytotoxic activity, from a different collection of *Luffariella* sp.

The sponge *Luffariella* sp. was collected off Kerama Islands, Okinawa and kept frozen until used. The MeOH extract was partitioned between EtOAc and a 1 M NaCl aqueous solution. The EtOAc-soluble fraction was subjected to Si gel flash cc eluted with $CHCl_3$/MeOH, followed by purification with reversed-phase hplc or gel filtration on a Sephadex LH-20 column, to give luffariolides F [**1**] (0.0005% yield, wet wt) and G [**2**] (0.0004%), together with the known sesterterpenes, 6*Z*-neomanoalide [**3a**] and 6*E*-neomanoalide [**3b**] (1.8:1 mixture, 0.001%) (2), manoalide [**4**] (0.001%) (1), 6*Z*-24-acetoxyneomanoalide [**5**] (0.001%)(4), 6*E*-neomanoalide-24-al [**6**] (0.0004%) (4), and (4*E*,6*E*)-dehydromanoalide [**7**] (0.0007%) (5).



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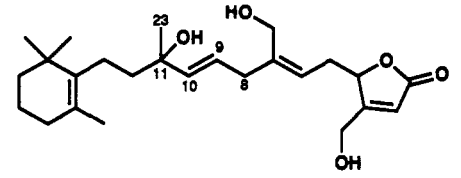

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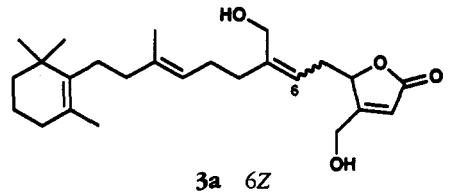
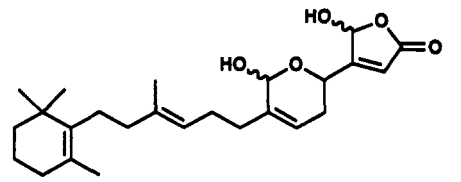


3a 6*Z*
3b 6*E*



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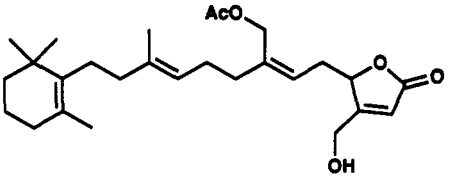
Luffariolide F [**1**] was obtained as a colorless oil, and its ir and uv spectra indicated the presence of an α,β-unsaturated ester (ν max 1740 cm^{-1}; λ max 210 nm) and hydroxyl (ν max 3420 cm^{-1}) groups. The molecular formula of **1** was suggested as $C_{25}H_{38}O_5$ by the ^1H- and ^{13}C-nmr spectra together with the hrfabms data (*m/z* 401.2669 $[M-H_2O+H]^+$, Δ −2.3 mmu). The ^{13}C-nmr spectrum of **1**



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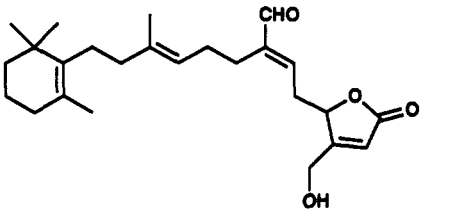

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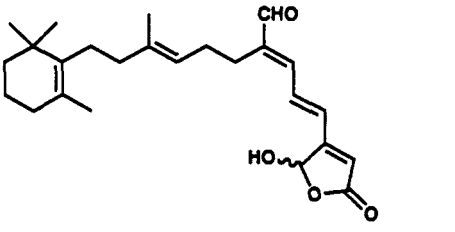

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showed signals due to an ester carbonyl, eight olefinic carbons, two sp^3 oxymethines, two sp^3 oxymethylenes, seven sp^3 methylenes, an sp^3 quaternary carbon, and four methyls. These data corresponded well to those of 6*Z*-neomanoalide [**3a**] (2) except for the presence of an additional oxymethine group (δ_C 70.3, d; δ_H 3.89, 1H, t, J=4.4 Hz) in **1** and the absence of an sp^3 methylene group. These differences were clearly due to the presence of a hydroxyl group at C-16. This deduction was supported by the ^1H-^1H COSY (cross-peaks: H-16/H_a-17, H-16/H_b-17, H_a-17/H_2-18, and H_b-17/H_2-18) and the HMBC (12) (cross-peaks: H-16/C-14, H-16/C-15, H-16/C-17, and H-16/C-22) spectra of **1**. The H-16 signal appeared as a triplet, being coupled with the methylene protons on C-17 by 4.4 Hz each. This result implied that the H-16 was equatorially oriented, and the hydroxyl group on C-16 was therefore axial. An attempt to determine the absolute stereochemistry of **1** by the modified Mosher method failed due to the failure of **1** to form the Mosher ester and the small amount of material available. The geometries of the $\Delta^{6,7}$ and $\Delta^{10,11}$ double bonds were deduced to be 6*Z* and 10*E* on the basis of the ^{13}C chemical shifts of C-24 (δ_C 60.3, t) and C-23 (δ_C 16.1 q) compared with those of 6*E*- and 6*Z*-neomanoalides (11). The structure of luffariolide F was therefore concluded to be 16-hydroxy-6*Z*-neomanoalide [**1**].

The ir (ν max 3400 and 1740 cm^{-1}) and uv (λ max 210 nm) absorptions of luffariolide G [**2**] indicated that it also possessed butenolide and hydroxyl groups. The ^1H- and ^{13}C-nmr data in combination with the hrfabms results (*m/z* 401.2693 $[M-H_2O+H]^+$, Δ+0.1 mmu) suggested the molecular formula of **2** to be $C_{25}H_{38}O_5$, being the same as that of luffariolide F [**1**]. The eims of **2** showed an intense peak at *m/z* 137, implying the presence of the alkylated cyclohexenyl end group commonly generated by manoalide-related sesterterpenes (8). The ^1H- and ^{13}C-nmr data of **2** were mostly parallel to those of 6*Z*-neomanoalide [**3a**] (2). The structural differences between **2** and **3a** were found in the C-9–C-11 part of the molecule. The DEPT experiment of **2** revealed the presence of an oxygenated sp^3 quaternary carbon (δ_C 73.7, s) which was assigned to C-11, bearing a tertiary methyl and a tertiary hydroxyl group as evidenced by the HMBC correlation between C-11 and methyl protons on C-23. The HMBC spectrum of **2** also showed a cross-peak from an olefinic proton at δ_H 5.59 (H-10) to C-11. In the ^1H-^1H COSY spectrum of **2**, coupling between H-10 and H-9 (δ_H 5.65; $J_{9,10}$=16.0 Hz, *E* configuration) was evident. H-9 in turn coupled to the methylene protons on C-8 (δ_H 2.84 and 2.76, each 1H, dt, J=15.0 and 6.5 Hz). From these observations an *E* double bond was shown to be present at the C-9

TABLE 1. Cytotoxic Activities of Compounds **1–7** (IC_{50} values, μg/ml).

Cell Line	Compound						
	1	**2**	**3**[a]	**4**	**5**	**6**	**7**
L1210	1.6	2.8	1.7	0.032	3.0	1.0	0.45
KB	33.4%[b]	30.8%[b]	36.9%[b]	0.31	44.8%[b]	5.6	2.1

[a]1.8:1 mixture of **3a** and **3b**.
[b]Inhibition (%) at 10 μg/ml.

and C-10 position. Thus the structure of luffariolide G was assigned as **2**.

Cytotoxic activities of luffariolides F [**1**] and G [**2**] together with those of known compounds **3–7** against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro were examined and are shown in Table 1.

EXPERIMENTAL

GENERAL METHODS.—The ir and uv spectra were recorded on JASCO A-102 and Shimadzu UV-220 spectrophotometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL GX-270 and EX-400 spectrometers. Fab mass spectra were obtained on a JEOL HX-110 spectrometer using 2-nitrobenzylalcohol as matrix. Ei mass spectra were recorded on a JEOL DX-303 spectrometer. Wako C-300 Si gel was used on glass cc, and tlc was carried out on Merck Si gel GF_{254}.

SPONGE MATERIAL.—The sponge *Luffariella* sp. (order Dictyoceratida; family Thorectidae), collected by scuba off Kerama Islands, Okinawa, was kept frozen until used. The specimen has dark yellow-brown conulose surface and light yellow-brown interior. The mesophyll is dense; the sponge is firm and slightly compressible. Primary and secondary skeletal fibers are the same size; the tertiary skeletal fibers are finer. The primary fibers are 55 μm wide and uncored. The fibers are stratified. The voucher specimen (SS-245) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University. This *Luffariella* sponge (SS-245) is apparently a different species from the *Luffariella* sponge (SS-15) of our previous study (11). The contents of the sesterterpenes in these two *Luffariella* sponges are also different; e.g., the latter did not contain manoalide [**4**].

ISOLATION.—The MeOH extract of the sponge (1.3 kg wet wt) was evaporated under reduced pressure, and the residue (40 g) was partitioned between EtOAc (400 ml×3) and 1 M NaCl (400 ml). The EtOAc-soluble material (4.6 g) was partially (3.8 g) subjected to Si gel flash cc with gradient elution of MeOH in $CHCl_3$ (0→100%). The fraction (216 mg) eluted with 10% MeOH in $CHCl_3$ was separated by the second Si gel column eluted with 0→50% MeOH in $CHCl_3$. The fraction (14 mg) eluted with 8% MeOH in $CHCl_3$ was further purified by a Sephadex LH-20 column (50% MeOH in $CHCl_3$) to give luffariolide F [**1**] (7.0 mg). The fraction (36.1 mg) of the second Si gel column eluted with 10% MeOH in $CHCl_3$ was subjected to a Sephadex LH-20 column (50% MeOH in $CHCl_3$), followed by reversed-phase hplc (YMC-Pack AM-323; 10×250 mm; 75% MeOH in H_2O; flow rate 2.0 ml/min) to give luffariolide G [**2**] (5.4 mg).

Luffariolide F [**1**].—A colorless oil: $[\alpha]^{19}D$ −5.9° (*c*=0.67, MeOH); uv (MeOH) λ max 210 nm (ε 15000); ir (KBr) ν max 3420, 2950, 1740, 1440 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.02 (1H, br s, H-2), 5.24 (1H, dd, *J*=7.6 and 7.0 Hz, H-6) 5.08–5.11 (2H, m, H-10 and H-4), 4.50–4.53 (2H, m, H_2-25), 4.12 (2H, d, *J*=2.9 Hz, H_2-24), 3.89 (1H, t, *J*=4.4 Hz, H-16), 2.79 (1H, m, H_a-5), 2.55 (1H, m, H_b-5), 2.12-2.17 (4H, m, H_2-8 and H_2-9), 2.03 (4H, br s, H_2-12 and H_2-13), 1.74 (3H, s, H_3-22), 1.63 (3H, s, H_3-23), 1.80 (1H, m, H_a-17), 1.65 (1H, m, H_b-17), 1.43 (2H, m, H_2-18), 1.04 (3H, s, H_3-20), 0.97 (3H, s, H_3-21); ^{13}C nmr ($CDCl_3$) δ 172.4 (s, C-1), 116.1 (d, C-2), 171.1 (s, C-3), 81.7 (d, C-4), 30.3 (t, C-5), 120.0 (d, C-6), 143.3 (s, C-7), 35.6 (t, C-8), 28.0 (t, C-9), 123.3 (d, C-10), 136.4, (s, C-11), 39.7 (t, C-12), 26.7 (t, C-13), 142.2 (s, C-14), 128.7 (s, C-15), 70.3 (d, C-16), 32.7 (t, C-17), 34.6 (t, C-18), 35.4 (s, C-19), 28.6 (s, C-20), 27.0 (s, C-21), 16.9 (q, C-22), 16.1 (q, C-23), 60.3 (t, C-24), 58.7 (t, C-25) ppm; eims *m/z* (rel. int.) $[M-H_2O]^+$ 400 (4), 203 (9), 147 (18), 135 (100); hrfabms *m/z* 401.2669 (calcd for $C_{25}H_{37}O_4$ $[M-OH]^+$ 401.2692).

Luffariolide G [**2**].—A colorless oil: $[\alpha]D^{24}$ −9.5° (*c*=0.2, MeOH); uv (MeOH) λ max 210 nm (ε 8300); ir (KBr) ν max 3400, 2950, 1740 cm^{-1}; ^{1}H nmr (C_6D_6) δ 5.79 (1H, d, *J*=1.5 Hz, H-

2), 5.65 (1H, dt, J=16.0 and 6.5 Hz, H-9), 5.59 (1H, d, J=16.0 Hz, H-10), 5.07 (1H, m, H-6), 4.46 (1H, m, H-4), 3.97 (2H, s, H_2-24), 3.82 (1H, d, J=16.6 Hz, H_a-25), 3.72 (1H, d, J=16.6 Hz, H_b-25), 2.84 (1H, dd, J=15.0 and 6.5 Hz, H_a-8), 2.76 (1H, dd, J=15.0 and 6.5 Hz, H_b-8), 2.42 (1H, m, H_a-5), 2.26 (2H, m, H_2-13), 2.04 (1H, m, H_b-5), 1.96 (2H, m, H_2-13), 1.72 (3H, s, H_3-22), 1.71 (2H, m, H_2-12), 1.63 (2H, m, H_2-17), 1.51 (2H, m, H_2-18), 1.31 (3H, s, H_3-23), 1.17 (6H, s, H_3-20 and H_3-21); ^{13}C nmr ($CDCl_3$) δ 172.5 (s, C-1), 116.2 (d, C-2), 171.6 (s, C-3), 81.5 (d, C-4), 30.2 (t, C-5), 120.5 (d, C-6), 142.3 (s, C-7), 38.2 (t, C-8), 125.5 (d, C-9), 139.0 (d, C-10), 73.7 (s, C-11), 42.7 (t, C-12), 22.9 (t, C-13), 136.6 (s, C-14), 127.1 (s, C-15), 32.7 (t, C-16), 19.5 (t, C-17), 38.9 (t, C-18), 34.9 (s, C-19), 28.6 (q, C-20), 28.6 (q, C-21), 19.8 (q, C-22), 27.4 (q, C-23), 60.1 (t, C-24), 58.5 (t, C-25) ppm; eims *m/z* (rel. int.) $[M-H_2O]^+$ 400 (4), 385 (3), 367 (3), 287 (6), 137 (100); hrfabms *m/z* 401.2693 (calcd for $C_{25}H_{37}O_4$ $[M-OH]^+$ 401.2692).

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