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Luteophanol D, New Polyhydroxyl Metabolite from Marine Dinoflagellate *Amphidinium* sp.

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Abstract: Luteophanol D (**1**), a new polyhydroxyl linear carbon-chain metabolite, has been isolated from the cultured marine dinoflagellate *Amphidinium* sp., which was isolated from Okinawan marine acoel flatworm *Pseudaphanostoma luteocoloris*. The structure of **1** was elucidated by detailed analyses of 2D NMR spectra. Luteophanol D (**1**) possesses two tetrahydropyran rings and twenty-three hydroxyl groups on C_{63}-linear aliphatic chain with one exo-methylene and two methyl branches.

Keywords: dinoflagellate; *Amphidinium* sp.; luteophanol D; polyhydroxyl metabolite

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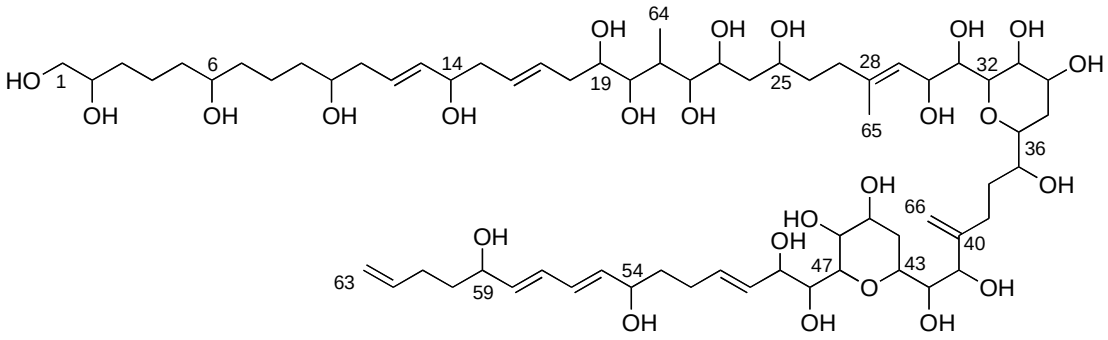
Introduction

During our continuing search for structurally unique secondary metabolites from marine dinoflagellates, we have isolated a series of cytotoxic macrolides, amphidinolides, as well as long chain polyhydroxyl metabolites from dinoflagellates *Amphidinium* sp. [1]. We previously investigated a strain of *Amphidinium* sp. (strain number Y-52), which was isolated from the inside cells of the Okinawan marine acoel flatworm *Pseudaphanostoma luteocoloris*, and isolated polyhydroxyl metabolites, luteophanols A ~ C [2-3]. Further investigation of extracts of the cultured dinoflagellate (Y-52) led to the isolation of a new polyhydroxyl metabolite, luteophanol D (**1**), possessing two tetrahydropyran rings and twenty three hydroxy groups on C_{63}-linear aliphatic chain. In this paper we describe the isolation and structure elucidation of **1**.



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Results and Discussion

The dinoflagellate was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement. The cultured algal cells were harvested by centrifugation and extracted with MeOH. The extract was partitioned between hexane and 1M NaCl aq, and the aqueous phase was successively extracted with $CHCl_3$ and then *n*-BuOH. The *n*-BuOH soluble materials were subjected to gel filtration on Sephadex LH-20 (MeOH and then MeOH/H_2O, 1:1) followed by purification with reversed-phase HPLC (MeOH/H_2O) to afford luteophanol D (**1**, 0.00023 %, wet weight).

ESIMS of luteophanol D { **1**, $[\alpha]_D^{23}$ +4.7° (*c* 0.25, MeOH)} showed the pseudomolecular ion peak at m/z 1329 $(M+Na)^+$, and its molecular formula, $C_{66}H_{114}O_{25}$, was established by HRESIMS [*m/z* 1329.7522 $(M+Na)^+$, Δ -2.5 mmu]. The UV and IR spectra indicated the presence of conjugated diene chromophore (λ_{max} 232 nm) and hydroxy group (υ_{max} 3430 cm^{-1}), respectively. The ^1H and ^{13}C NMR data revealed that **1** contained two sp^2 quaternary carbons, twelve sp^2 methines, two sp^2 methylenes, twenty-seven sp^3 methines, of which twenty-six were oxymethines, twenty-one sp^3 methylenes including one oxymethylene, and two methyl groups. Since eight out of ten elements of unsaturation implied by the molecular formula were accounted for, **1** was inferred to possess two rings. This suggested that luteophanol D (**1**) was a congener of luteophanol B or C.

Figure 1. Selected 2D NMR data of luteophanol D (**1**).

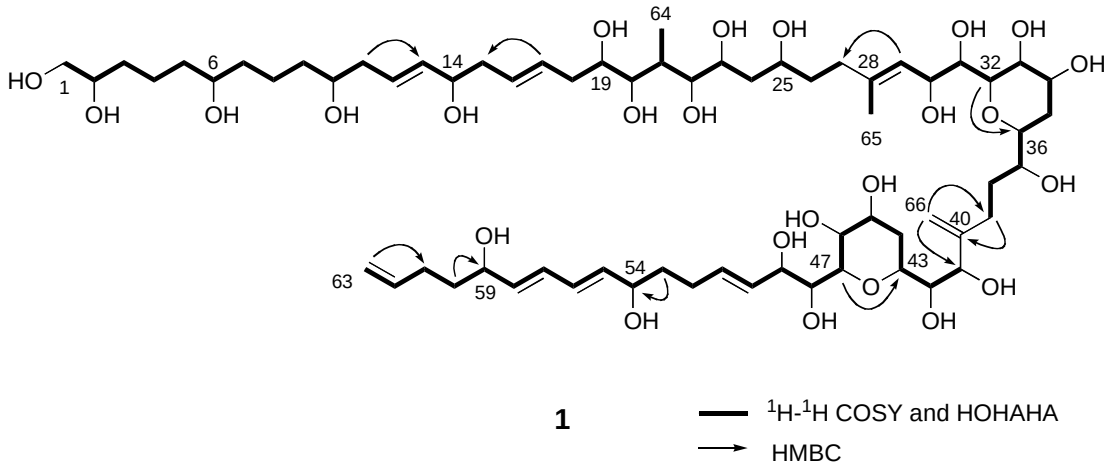

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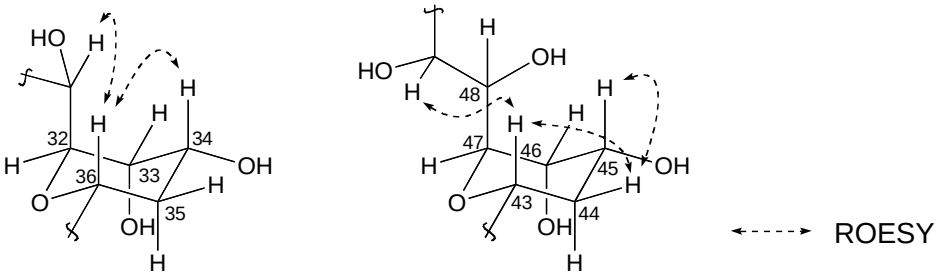
Figure 2. Selected ROESY data and relative stereochemistry of two tetrahydropyran rings of luteophanol D.



The structure of luteophanol D (**1**) was elucidated by extensive 2D NMR experiments including ^{1}H-^{1}H COSY, HOHAHA, ROESY, HSQC, and HMBC. Detailed analyses of ^{1}H-^{1}H COSY, HOHAHA, and HSQC spectra revealed the proton-proton connectivities from H_2-1 to H_2-27, from H_3-65 to H_2-39, and from H-41 to H_2-63. The HMBC spectrum of **1** showed cross-peaks for H-29/C-27, H_2-66/C-39, H_2-66/C-41, and H_2-39/C-40, indicating connecitivities of C-27 to C-28, C-39 to C-40, and C-40 to C-41. Two tetrahydropyran rings (C-32 to C-36 and C-43 to C-47) were assigned by HMBC cross-peaks for H-32/C-36 and H-47/C-43.

Five di- and one tri-substituted double bonds were indicated to have all *E* geometries by ROESY data (H-30/H_3-65, H-54/H-56, H-55/H-57, H-56/H-58, and H-57/H-59) and ^{1}H-^{1}H coupling constants ($J_{12,13}$ = 16 Hz, $J_{16,17}$ = 15 Hz, and $J_{51,52}$ = 15 Hz). Relative stereochemistry of the two tetrahydropyran rings (C-32 to C-36 and C-43 to C-47) was elucidated on the basis of ROESY data and ^{1}H-^{1}H coupling constants ($J_{32/33}$ = ~0 Hz, $J_{33/34}$ = ~0 Hz, $J_{34/35a}$ = 10 Hz, $J_{34/35b}$ = 2 Hz, $J_{35a/36}$ = 10 Hz, $J_{35b/36}$ = 2 Hz, $J_{43/44a}$ = 10 Hz, $J_{43/44b}$ = 2 Hz, $J_{44a/45}$ = 9 Hz, $J_{44b/45}$ = 2 Hz, $J_{45/46}$ = ~0 Hz, and $J_{46/47}$ = ~0 Hz) of **1** (Figure. 2). Thus, luteophanol D (**1**) was assigned as a luteophanol B congener lacking one methylene between C-52 and C-54 of luteophanol B.

Luteophanol D (**1**) possesses two tetrahydropyran rings and twenty-three hydroxyl groups on a C_{63}-1inear carbon chain with one exo-methylene and two methyl branches. Luteophanol D (**1**) contains a hydrophilic diene portion at C-54 ~ C-59, whereas known polyhydroxyl metabolites, amphidinols [4-8] and lingshuiols [9-10], previously isolated from dinoflagellates *Amphidinium* sp. comprise a hydrophobic moiety in this portion. The biosynthesis of amphidinols has been studied by acetate incorporation experiments [11]. Since the polyketide chain of **1** was shorter than those of luteophanols B and C by only one carbon, this truncation of the carbon chain might be occurred by unusual dinoflagellate polyketide biosynthesis. Luteophanol D (**1**) exhibited antibacterial activity against *Micrococcus luteus* (MIC, 33 μg/ml).

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Experimental

General

IR and UV spectra were recorded on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer. The 3.35 and 49.8 ppm resonances of residual CD_3OD were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were measured on JEOL JMX-SX102A spectrometer.

Cultivation and Isolation

The dinoflagellate *Amphidinium* sp. (strain number Y-52) was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement. The harvested cells of the cultured dinoflagellate (385 g wet weight, from 725 L of culture) were extracted with MeOH (1 L x 3). The MeOH extract (15.19 g) was partitioned between hexane (500 mL x 3) and 1M NaCl aq., and the aqueous phase was successively extracted with $CHCl_3$ (500 mL x 3) and then *n*-BuOH (500 mL x 3). The *n*-BuOH soluble fraction (4.47 g) was subjected to a Sephadex LH-20 column [MeOH and then MeOH/H_2O, (1:1)] followed by reversed-phase HPLC (Develosil ODS-5, 10 x 250 mm; eluent: 55% MeOH/H_2O then 45% MeOH/H_2O; flow rate: 2.5 mL/min; UV detection at 226 nm) to afford luteophanol D (**1**, 0.9 mg, 0.00023 % wet weight).

Luteophanol D (**1**). Colorless amorphous solid; $[\alpha]_D^{23}$ +4.7° (*c* 0.25, MeOH); UV (MeOH) λ_{max} 232 nm (ε 22800); IR υ_{max} 3430, 2920, 1640, and 1050 cm^{-1}; ESIMS *m/z* 1329 $(M + Na)^+$; HRESIMS *m/z* 1329.7522 $(M + Na)^+$. Calcd. for $C_{66}H_{114}O_{25}Na$, 1329.7547; ^{1}H NMR (CD_3OD/C_5D_5N 2:1) δ 3.62 (2H, H_2-1); 3.75 (m, 1H, H-2); 1.50 (1H, H-3a); 1.65 (1H, H-3b); 1.47 (1H, H-4a); 1.75 (1H, H-4b); 1.50 (2H, H_2-5); 3.63 (1H,H-6); 1.50 (2H, H_2-7); 1.47 (1H, H-8a); 1.75 (1H, H-8b); 1.47 (1H, H-9a); 1.58 (1H, H-9b); 3.68 (1H, H-10); 2.25 (2H, H_2-11); 5.80 (1H, H-12); 5.63 (1H, H-13); 4.17 (1H,H-14); 2.35 (2H, H_2-15); 5.67 (1H, H-16); 5.76 (1H, H-17); 2.30 (1H, H-18a); 2.70 (1H, H-18b); 3.75 (1H, H-19); 3.77 (1H, H-20); 2.67 (1H, H-21); 3.77 (1H,H-22); 3.95 (1H, H-23); 1.70 (1H, H-24a); 2.13 (1H, H-24b); 4.02 (1H, H-25); 1.70 (2H, H_2-26); 2.10 (1H, H-27a); 2.32 (1H, H-27b); 5.70 (1H, H-29); 4.80 (1H, H-30); 3.90 (1H,H-31); 4.33 (1H, H-32); 4.40 (1H,H-33); 4.20 (1H, H-34); 2.02 (1H, H-35a); 2.11 (1H, H-35b); 3.68 (1H, H-36); 3.77 (1H, H-37); 1.75 (1H, H-38a); 2.19 (1H, H-38b); 2.32 (1H, H-39a); 2.69 (1H, H-39b); 4.45 (1H, H-41); 3.58 (1H, H-42); 4.30 (1H, H-43); 1.70 (1H,H-44a); 2.40 (1H, H-44b); 4.26 (1H, H-45); 4.39 (1H, H-46); 4.08 (1H, H-47); 4.28 (1H, H-48); 4.70 (1H, H-49); 5.90 (1H, H-50); 5.86 (1H, H-51); 2.18 (2H, H_2-52); 1.65 (1H, H-53a); 1.70 (1H, H-53b); 4.18 (1H, H-54), 5.73 (1H, H-55); 6.30 (1H, H-56), 6.30 (1H, H-57), 5.78 (1H, H-58), 4.18 (1H, H-59), 1.60 (1H, H-60a); 1.65 (1H, H-60b); 2.15 (2H, H_2-61); 5.83 (1H, H-62); 4.93 (1H, H-63a); 5.01 (1H, H-63b); 1.18 (d3H, H_3-64); 1.75 (3H, H_3-65); 5.03 (1H, H-66a); 5.18 (1H, H-66b); ^{13}C NMR (CD_3OD/C_5D_5N 2:1) δ C-1, 68.3; C-2, 74.0; C-3, 35.5; C-4, 23.8; C-5, 39.4; C-6, 72.8; C-7,

39.4; C-8, 23.8; C-9, 38.8; C-10, 72.8; C-11, 42.3; C-12, 129.4; C-13, 137.7; C-14, 74.0; C-15, 43.0; C-16, 131.2; C-17, 131.7; C-18, 39.2; C-19, 74.0; C-20, 80.4; C-21, 35.9; C-22, 81.1;C-23, 73.2; C-24, 42.3; C-25, 72.5; C-26, 37.7; C-27, 37.5;C-28, 139.4; C-29, 127.7; C-30, 68.6; C-31, 73.3; C-32, 80.2; C-33, 69.8; C-34, 68.2; C-35, 31.6; C-36, 76.6; C-37, 75.3; C-38, 33.7; C-39, 28.9; C-40, 152.9; C-41, 77.4; C-42, 76.2; C-43, 71.5; C-44, 32.7; C-45, 68.1; C-46, 69.8; C-47, 81.4; C-48, 73.0; C-49, 75.0; C-50, 130.5; C-51, 135.4; C-52, 30.4; C-53, 38.8; C-54, 73.0; C-55, 132.3; C-56, 131.5; C-57, 131.5; C-58, 138.5; C–59, 73.0; C-60, 38.5; C-61, 31.4; C-62, 140.4; C-63, 115.9; C-64, 7.8; C-65, 18.2;C-66, 113.5.

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Sample availability: Not available.