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 acel 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

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 acel 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.
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 CHCl₃ and then n-BuOH. The n-BuOH soluble materials were subjected to gel filtration on Sephadex LH-20 (MeOH and then MeOH/H₂O, 1:1) followed by purification with reversed-phase HPLC (MeOH/H₂O) to afford luteophanol D (1, 0.00023 %, wet weight).

ESIMS of luteophanol D {1, [α]D²⁺ +4.7° (c 0.25, MeOH)} showed the pseudomolecular ion peak at m/z 1329 (M+Na)⁺, and its molecular formula, C₆₆H₁₁₄O₂₅, 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 (λₓ max 232 nm) and hydroxy group (υₓ max 3430 cm⁻¹), respectively. The ¹H and ¹³C NMR data revealed that 1 contained two sp² quaternary carbons, twelve sp² methines, two sp² methylenes, twenty-seven sp³ methines, of which twenty-six were oxymethines, twenty-one sp³ 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).
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-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 connectivities 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 ($J_{32/33} = \sim 0$ Hz, $J_{33/34} = \sim 0$ 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, $J_{45/46} = \sim 0$ Hz, and $J_{46/47} = \sim 0$ 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$_{63}$-linear 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 dinofflagellate 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$_3$OD 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$_2$O, (1:1)] followed by reversed-phase HPLC (Develosil ODS-5, 10 x 250 mm; eluent: 55% MeOH/H$_2$O then 45% MeOH/H$_2$O; 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; [α]$_D^{23}$ +4.7° (c 0.25, MeOH); UV (MeOH) $\lambda_{\text{max}}$ 232 nm (ε 22800); IR $\nu_{\text{max}}$ 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$_3$OD/C$_5$D$_5$N 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-33); 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$_3$OD/C$_5$D$_5$N 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, 69.8; C-46, 46, 81.4; C-47, 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, 14.2; C-66, 113.5.

Reference and Notes


*Sample availability*: Not available.