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Luteophanols B and C, New Polyhydroxyl Compounds from Marine Dinoflagellate *Amphidinium* sp.

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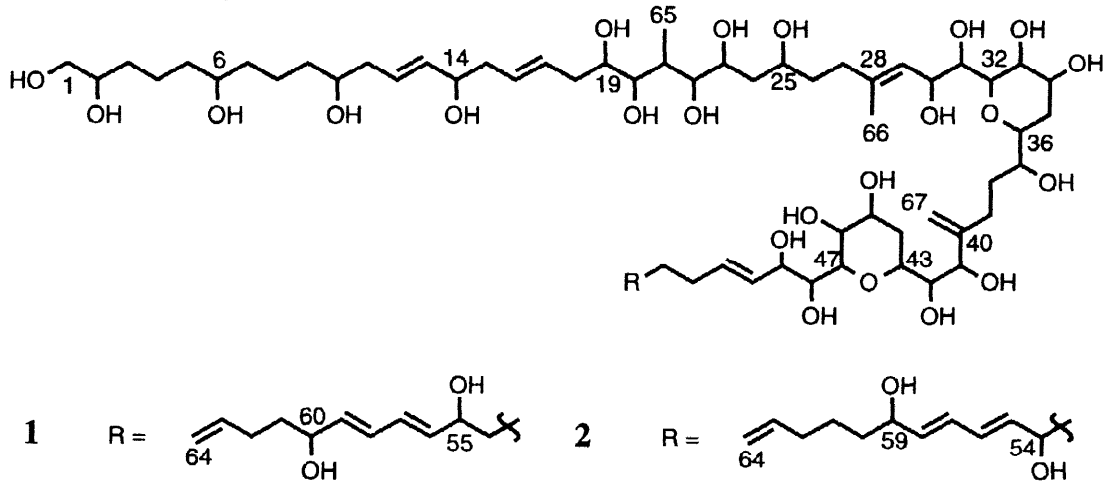
Abstract; Luteophanols B (**1**) and C (**2**), new polyhydroxyl linear carbon-chain compounds, have been isolated from the cultured marine dinoflagellate *Amphidinium* sp. The structures of **1** and **2** were elucidated by detailed analyses of two-dimensional NMR data containing HMBC, HMQC-RELAY, CH_2-selected E-HSQC, and CH_2-selected E-HSQC-TOCSY.

During our continuing search for structurally unique secondary metabolites from marine dinoflagellates,[1] we previously isolated a series of cytotoxic macrolides, amphidinolides, from dinoflagellates *Amphidinium* sp.[2] Recently we investigated another 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 a new polyhydroxyl compound, luteophanol A (**3**), consisting of a C_{57}-linear aliphatic chain possessing one sulfate ester.[3] Further investigation of extracts of the cultured dinoflagellate (Y-52) led to the isolation of two new polyhydroxyl compounds, luteophanols B (**1**) and C (**2**), both consisting of a C_{64}-linear aliphatic chain. In this paper we describe the isolation and structure elucidation of **1** and **2** on the basis of chemical degradation and newly developed 2D NMR experiments.



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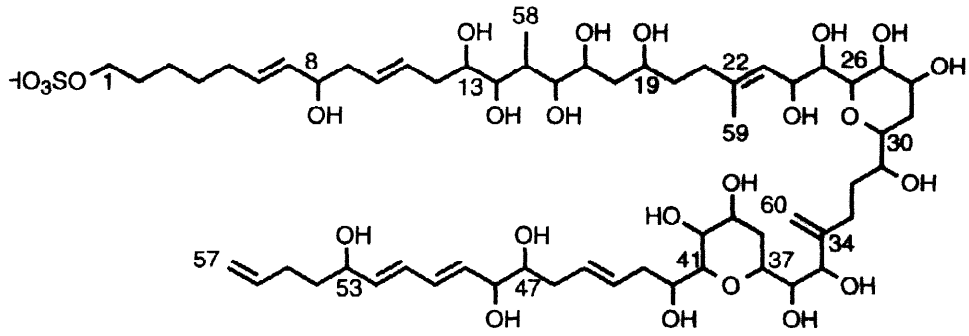

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The dinoflagellate was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement. The cultured algal cells were harvested by centrifugation and extracted with MeOH. The extract was partitioned between hexane and 1M 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 (ODS, 55 % MeOH) to afford luteophanols B (**1**, 0.00033 %, wet weight) and C (**2**, 0.00028%) together with luteophanol A (**3**).[3]

Electrospray ionization (ESI) MS of luteophanol B {**1**, $[\alpha]_D^{20}$ +3.4° (*c* 0.21, MeOH)} showed the pseudomolecular ion peak at *m/z* 1343 $(M+Na)^+$, and its molecular formula, $C_{67}H_{116}O_{25}$, was established by HRESIMS [*m/z* 1343.7673 $(M+Na)^+$, Δ -3.1 mmu]. The UV and IR spectra indicated the presence of conjugated diene chromophore (λ_{max} 232 nm) and hydroxy group (ν_{max} 3430 cm^{-1}), respectively. The ^1H and ^{13}C NMR data (Table 1) 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-two 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. The carbon chemical shift deuterium-induced shift experiments using CD_3OH/C_5D_5N (2:1) and CD_3OD/C_5D_5N (2:1) revealed the presence of twenty-three hydroxyl groups. Four (C-32, C-36, C-43, and C-47) of twenty-seven oxygenated carbon signals (δ 67 ~ 85) did not show the deuterium-induced upfield shifts, thus suggesting that these four oxymethine carbons constituted two ether rings.

The C-10–C-52 part of luteophanol B (**1**) was elucidated by extensive 2D NMR experiments including ^1H-^1H COSY, TOCSY, ROESY, HSQC, HMBC, HMQC-RELAY,[4] and HMQC-TOCSY[4] (Figure 1). Detailed analyses of ^1H-^1H COSY, TOCSY, and HSQC spectra revealed the proton-connectivities from H_2-1 to H_2-3, from H-10 to H_2-18, from H-19 to H_2-27, from H_3-66 to H_2-39, from H-41 to H_2-52, from H_2-54 to H_2-61, and from H_2-62 to H_2-64. PFG-HMBC spectrum of **1** showed the cross-peaks for H-29/C-27, H-67/C-39, H-67/C-41, and H-39/C-40, indicating the 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

Table 1. ^1H and ^{13}C NMR Data of Luteophanols B (**1**) and C (**2**) in CD_3OD/C_5D_6N (2:1).

	1					**2**	
positn.	$^{13}C^a$	$^1H^b$	HMBC	HMQC-RELAY	E-HSQC-TOCSY	$^{13}C^a$	$^1H^b$
1	68.29 t	3.62[c]	2,3	2	2,3	68.33 t	3.62[c]
2	74.00 d	3.77	1,3	1,3		73.94 d	3.77
3	35.48 t	1.49 1.63	1,2	2	2,4	35.51 t	1.49 1.63
4	23.76 t	1.48 1.78	2		3,5	23.78 t	1.51 1.76
5	39.39 t	1.47 1.52	6	6	4	39.43 t	1.47 1.52
6	72.79 d	3.63		5,7		72.75 d	3.63
7	39.34 t	1.51[c]	6	6	6,8	39.38 t	1.49[c]
8	23.75 t	1.46 1.75	6		7,9	23.75 t	1.46 1.74
9	38.74 t	1.46 1.56	10	10	8	38.79 t	1.48 1.55
10	72.75 d	3.68	12	9,11		72.71 d	3.68
11	42.32 t	2.25[c]	10,12	10,12	10,12	42.36 t	2.26[c]
12	129.41 d	5.80	10,13	11		129.36 d	5.81
13	137.29 d	5.65	12,14	12,14		137.35 d	5.67
14	73.94 d	4.16		13,15		73.94 d	4.17
15	43.00 t	2.31 2.34	14		14,16	43.03 t	2.30 2.33
16	130.71 d	5.66	17	15,17		130.71 d	5.69
17	131.69 d	5.75		16		131.73 d	5.79
18	39.08 t	2.27 2.68	17			39.14 t	2.27 2.68
19	73.41 d	3.93	20	20		73.51 d	3.77
20	80.40 d	3.78	19,65			80.47 d	3.82
21	35.85 d	2.67	65	65		35.85 d	2.71
22	81.07 d	3.78	23,65	21,23		81.12 d	3.82
23	73.19 d	3.94		22,24		73.05 d	3.96
24	42.08 t	1.70 2.12	23,25	23		42.13 t	1.70 2.13
25	72.42 d	4.02				72.47 d	4.02
26	37.71 t	1.68 1.72	25	25	25,27	37.79 t	1.68 1.70
27	37.47 t	2.14 2.28	29,66		26	37.49 t	2.12 2.26
28	139.32 s		29,66			139.29 s	
29	127.63 d	5.70	66	30,66		127.74 d	5.73
30	68.56 d	4.80	29	29,31		68.57 d	4.83
31	73.28 d	3.93	32			73.28 d	3.94
32	79.99 d	4.36	30,33	31		80.00 d	4.38
33	69.72 d	4.40				69.76 d	4.38
34	68.22 d	4.20	33			68.25 d	4.23
35	31.59 t	1.99 2.08	33,36	36		31.57 t	2.02 2.10
36	76.63 d	3.68	32,37			76.68 d	3.67
37	75.28 d	3.78				75.26 d	3.79
38	33.40 t	1.74 2.13		37	39	33.45 t	1.75 2.15
39	28.82 t	2.28 2.68	67		38	28.82 t	2.29 2.71
40	152.82 s		39,41,67			152.95 s	
41	77.49 d	4.45	42,67	42		77.44 d	4.49
42	76.06 d	3.57		41		76.11 d	3.57
43	71.42 d	4.29	44,47	42		71.49 d	4.31
44	32.68 t	1.69 2.39	43,46	43		32.77 t	1.69 2.41
45	68.09 d	4.24	43,44,47			68.13 d	4.26
46	69.52 d	4.40	45,47	45		69.59 d	4.44
47	81.42 d	4.08	45,46,49	46,48		81.44 d	4.11
48	72.97 d	4.27	47	47		73.05 d	4.29
49	74.89 d	4.69	48,50,51	48,50		74.90 d	4.73
50	130.43 d	5.86	49,51	49		130.64 d	5.86
51	135.21 d	5.85	49,52			135.15 d	5.85
52	30.43 t	2.16 2.19	51	51	53	30.04 t	2.07[c]
53	34.27 t	2.08[c]			52,54	38.78 t	1.61 1.67
54	38.46 t	1.61 1.68	55,56	55	53	83.08 d	3.58
55	83.19 d	3.60	56,57	56		134.64 d	5.44
56	135.35 d	5.49	55,57			134.30 d	6.31
57	134.36 d	6.25	55,56,58			130.83 d	6.33
58	130.87 d	6.33				139.25 d	5.33
59	139.32 d	5.83	60	60		72.60 d	4.21
60	72.97 d	4.21		59		37.79 t	1.65 1.69
61	35.08 t	2.08[c]	60	60	62	34.07 t	2.06[c]
62	31.68 t	2.11 2.16	63,64	63	63	31.40 t	2.15 2.18
63	140.34 d	5.81	64			140.37 d	5.81
64	115.97 t	4.93 5.02	62,63	63		116.18 t	4.92 5.00
65	7.79 q	1.16[d]	20			7.83 q	1.18[d]
66	18.20 q	1.76[d]	29			18.23 q	1.74[d]
67	113.48 t	5.02 5.20	39,41			113.39 t	5.03 5.19

[a]Recorded at 125 MHz. [b]Recorded at 600 MHz. [c]2H. [d]3H.

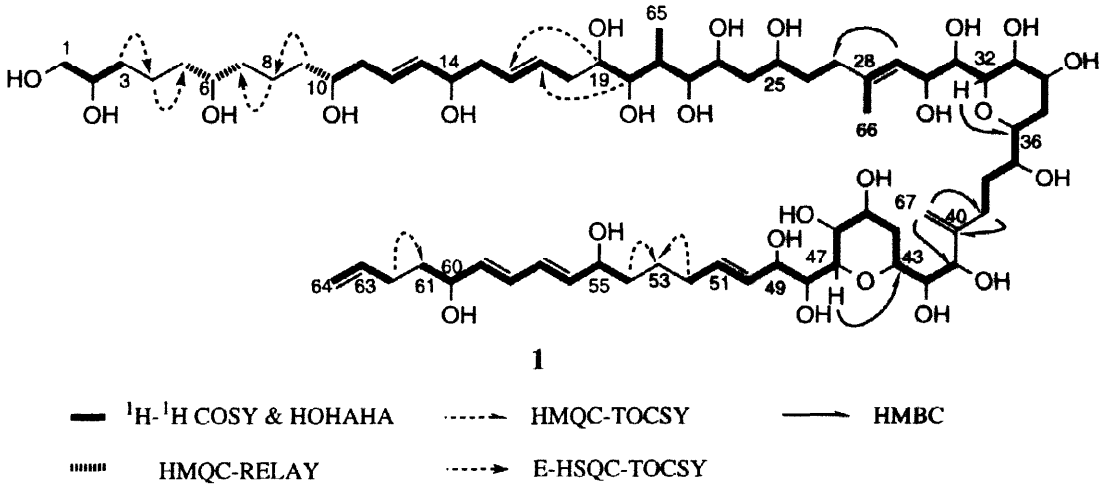
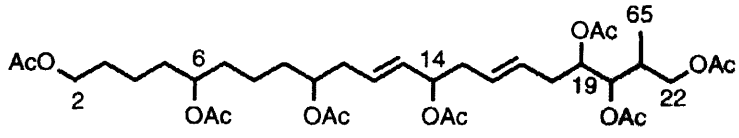


Figure 1. Selected 2D NMR Data of Luteophanol B (**1**).

C-47) were assigned by the HMBC cross-peaks for H-32/C-36 and H-47/C-43. The connectivity between C-18 and C-19 was deduced from HMQC-TOCSY cross-peaks for H-19/C-16 and H-20/C-17. Thus the structure of C-9–C-52 of luteophanol B (**1**) was elucidated. The both termini for C-1–C-9 and C-52–C-64 were, however, unassignable from the HMQC and HSQC data, since the methylene carbon chemical shifts of C-4 (δ_C 23.76) and C-8 (δ_C 23.75), C-5 (δ_C 39.39) and C-7 (δ_C 39.34), and C-9 (δ_C 38.74) and C-54 (δ_C 38.46) were very close to one another, and the one-bond C–H correlations were severely overlapped.

To overcome this problem, we demonstrated the CH_2-selected editing HSQC (E-HSQC) experiment,[5,6] in which the narrow F_1 spectral width of δ_C 50 ~ 20 gave very high F_1 resolution, since the methylene carbon signals of **1** were resonated from δ_C 45 to 20 except for an oxymethylene (δ_C 68.29, C-1) and two exomethylenes (δ_C 115.97, C-64; δ_C 113.48, C-67), of which the proton chemical shifts were separated from those of other methylene protons. In the CH_2-selected E-HSQC spectrum of **1**, the cross-peaks for H_2-4 (δ_H 1.48 and 1.78)/C-4 (δ_C 23.76) and H_2-8 (δ_H 1.46 and 1.75)/C-8 (δ_C 23.75) and H_2-9 (δ_H 1.46 and 1.56)/C-9 (δ_C 38.74) and H_2-54 (δ_H 1.61 and 1.68)/C-54 (δ_C 38.46) were identified obviously. On the other hand, the cross-peaks for H_2-5/C-5 and H_2-7/C-7 were still overlapped, since both of proton and carbon chemical shifts were very close to each other. Further structural elucidation of the parts of C-1–C-9 and C-52–C-64 was performed by detailed analyses of the CH_2-selected E-HSQC-TOCSY[6] spectrum. The E-HSQC-TOCSY pulse sequence was constructed by the insertion of MLEV-17 spin locking before acquisition in the E-HSQC pulse sequence. In the E-HSQC-TOCSY spectrum of **1**, the correlations for H-3 (δ_H 1.63)/C-4 (δ_C 23.76), H-4 (δ_H 1.78)/C-5 (δ_C 39.39), H_2-9 (δ_H 1.56 and 1.46)/C-8 (δ_C 23.75), and H-8 (δ_H 1.75)/C-7 (δ_C 39.34) were observed, supporting the structure of the C-1–C-9 part. The structure of C-52–C-64 segment was assigned by the



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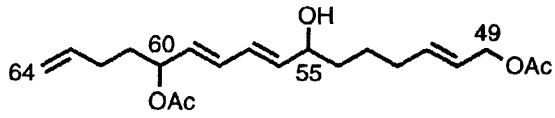

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cross-peaks for H_2-52 (δ_H 2.19 and 2.16)/C-53 (δ_C 34.27), H-54 (δ_H 1.68)/C-53, and and H_2-62 (δ_H 2.16 and 2.11)/C-61(δ_C 35.08).

The structures of C-1–C-10 and C-51–C-64 parts were confirmed by degradation experiments for **1** as follows. Luteophanol B (**1**) was treated with $NaIO_4$ followed by reduction with $NaBH_4$ and acetylation with acetic acid and pyridine. The reaction mixture was purified by silica gel HPLC to afford two segments **4** and **5**.[7] ESIMS data [*m/z* 735 $(M+Na)^+$] and ^1H NMR data including ^1H-^1H COSY and TOCSY spectra of **4** revealed that the segment **4** corresponded to the heptaacetate of C-2–C-22 fragment generated by fission of the 1,2-diol bonds of C-1–C-2 and C-22–C-23 of **1**. The segment **5** showed the pseudomolecular ion at *m/z* 373 $(M+Na)^+$ in the ESIMS. The structure of segment **5** was assigned to be the diacetate of the C-49-C-64 fragment by analyses of ^1H-^1H COSY and TOCSY data.[8] Therefore, the gross sturucture of luteophanol B was elucidated to be **1**.

Relative stereochemistry of the two tetrahydropyran rings (C-32–C-36 and C-43–C-47) were deduced from ROESY correlations and ^1H-^1H coupling constants as shown in Figure 2. The trisubstituted double bond (C-28–C-29) and the two disubstituted double bonds at the diene system (C-56–C-59) were assigned to have all *E*-geometries from the following ROESY data: H-30/H_3-66, H-55/H-57, H-56/H-58, H-57/H-59, and H-58/H-60. Geometries of the three remaining disubstituted double bonds (C-12–C-13, C-16–C-17, and C-50–C-51) were concluded to be all *E* by the ^1H-^1H coupling constants ($J_{12,13}$ = 16 Hz, $J_{16,17}$ = 15 Hz, and $J_{51,52}$ = 15 Hz, respectively).

Luteophanol C {**2**, $[\alpha]_D^{20}$ +12° (*c* 0.19, MeOH)} was revealed to have the same molecular formula, $C_{67}H_{116}O_{25}$, as that of luteophanol B (**1**) by HRESIMS data [*m/z* 1343.7704 $(M+Na)^+$, Δ ±0 mmu]. The ^1H and ^{13}C NMR data (Table 1) indicated that **2** contained two sp^2 quaternary carbons, twelve sp^2 methines, two sp^2 methylenes, twenty-seven sp^3 methines including twenty-six oxymethines, twenty-two sp^3 methylenes including one oxymethylene, and two methyl groups. Detailed analyses of 2D NMR data such as ^1H-^1H COSY, TOCSY, HSQC, CH_2-selected E-HSQC (Figure 3), HMBC, HMQC-RELAY, HMQC-TOCSY, and CH_2-selected E-HSQC-TOCSY spectra revealed that luteophanol

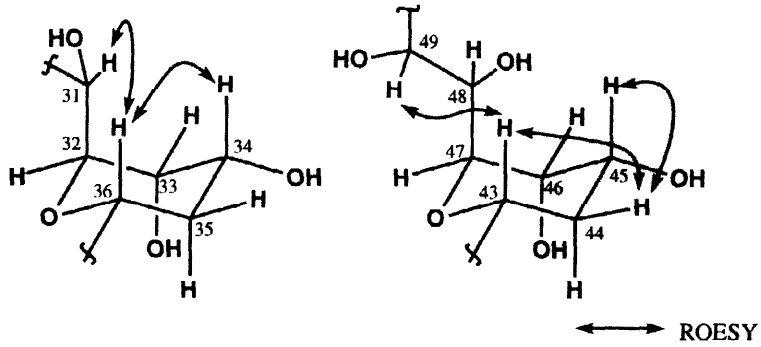

Figure 2. Relative Stereochemistry of Two Tetrahydropyran Rings of Luteophanol B (**1**).
The coupling constants for these moieties (H/H in Hz) are as follows: 32/33 = ~0 Hz, 33/34 = ~0 Hz, 34/35α = 10 Hz, 34/35β = 2 Hz, 35α/36 = 10 Hz, 35β/36 = 2 Hz, 43/44α = 10 Hz, 43/44β = 2 Hz, 44α/45 = 9 Hz, 44β/45 = 2 Hz, 45/46 = ~0 Hz, and 46/47 = ~0 Hz

C (**2**) possessed the same gross partial structure as C-1–C-52 part of luteophanol B (**1**). The structure of C-52–C-64 part was assigned by the following CH_2-selected E-HSQC-TOCSY correlations; H-49/C-52, H-49/C-53, H-55/C-52, H-55/C-53, H-60/C-61, H-60/C-62, H-60/C-63, H_2-64/C-61, and H_2-64/C-62. Five di- and one trisubstituted double bonds were indicated to have all *E*-geometries by ROESY data (H-30/H_3-66, 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–C-36 and C-43–C-47) was elucidated to be the same as that of **1** on the basis of ROESY data (H-31/H-36, H-34/H-36, H-43/H-44β, H-43/H-49, and H-44β/H-45) 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 **2**. Thus the structure of luteophanol C was concluded to be **2**.

Luteophanols B (**1**) and C (**2**) possess two tetrahydropyrans rings and twenty-three hydroxyl groups on a C_{64}-linear carbon chain with one exo-methylene and two methyl branches, while luteophanol A (**3**) is a polyhydroxyl compound consisting of C_{57}-linear carbon chain with one sulfate ester. The partial structures of C-11–C-48 and C-61–C-64 parts of luteophanols B (**1**) and C (**2**) corresponded to those of C-5–C-42 and C-54–C-57 parts of luteophanol A (**3**). Luteophanols B (**1**) and C (**2**) contain a hydrophilic diene portion at C-55 ~ C-60 and C-54 ~ C-59, respectively, whereas amphidinols previously isolated as potent antifugal metabolites from dinoflagellates *Amphidinium* sp.[9~12] comprise a hydrophobic polyene system in this portion.

In this paper it was demonstrated that E-HSQC[5] and E-HSQC-TOCSY[6] are very useful tools for structure elucidations of complex natural products possessing many aliphatic methine and methylene carbons such as luteophanols B (**1**) and C (**2**). Especially, the CH_2-selected E-HSQC-TOCSY[6] experiments seem to be suitable to assign very closely resonated carbon signals, since this method

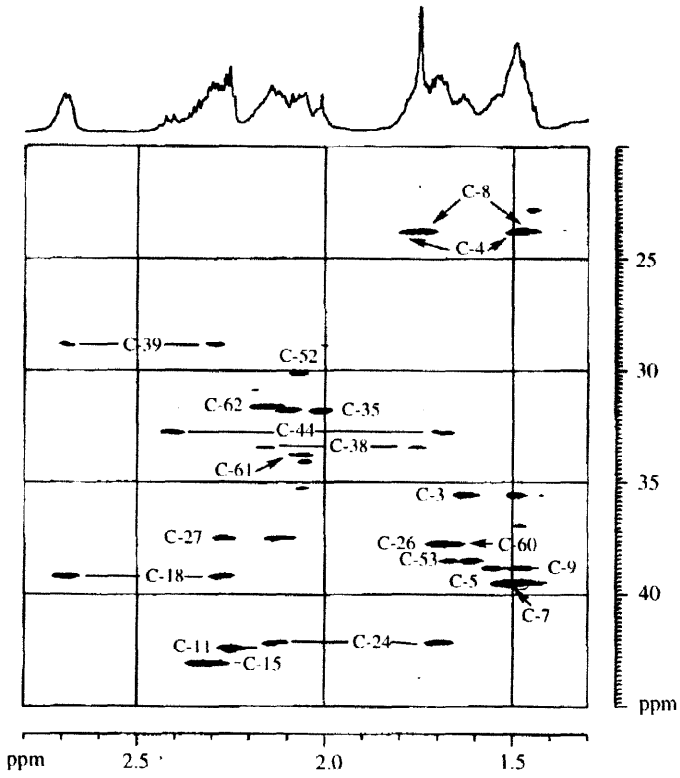

Figure 3. CH_2-Selected E-HSQC Spectrum (Part) of Luteophanol C (**2**).

affords the high resolution in F_1 axis by limiting the F_1 spectral width, in which sp^2 methylene carbons are resonated.[6]

EXPERIMENTAL SECTION

General Procedure. IR and UV spectra were recorded on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. Optical rotations were measured on JASCO DIP-360 polarimeter. ESI mass spectra were obtained using sample dissolved in MeOH with a flow rate 2 μL/min on a 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)] to give luteophanol A (**3**, 0.004 %, wet weight) and the mixture (13.3 mg) of luteophanols B (**1**) and C (**2**). The mixture was purified by reversed-phase HPLC (Develosil ODS-5, 10 x 250 mm; eluent: 55 % MeOH/ H_2O; flow rate: 2.5 mL/min; UV detection at 226 nm) to afford luteophanols B (**1**, 1.3 mg, 0.00033 % wet weight, t_R 18.4 min) and C (**2**, 1.1 mg, 0.00028 %, t_R 15.2 min).

Luteophanol B (1). Colorless amorphous solid; $[\alpha]_D^{20}$ +3.4° (*c* 0.21, MeOH); UV (MeOH) λ_{max} 232 nm (ε 21300); IR (KBr) ν_{max} 3430, 2925, 1630, 1385, and 1070 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS *m/z* 1343.77 $(M + Na)^+$; HRESIMS *m/z* 1343.7673 $(M + Na)^+$. Calcd. for $C_{67}H_{116}O_{25}Na$, 1343.7704.

Luteophanol C (2). Colorless amorphous solid; $[\alpha]_D^{20}$ +12° (*c* 0.19, MeOH); UV (MeOH) λ_{max} 233 nm (ε 23500); IR (KBr) ν_{max} 3430, 2925, 1640, 1385, and 1065 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS *m/z* 1343.77 $(M + Na)^+$; HRESIMS *m/z* 1343.7704 $(M + Na)^+$. Calcd. for $C_{67}H_{116}O_{25}Na$, 1343.7704.

NMR Experiments. The NMR samples of luteophanols B (**1**) and C (**2**) were prepared by dissolving 2.5 mg each in 400 μL of CD_3OD-C_5D_5N (2:1). ^1H and 2D NMR spectra were recorded on a Bruker AMX-600 spectrometer, while ^{13}C NMR spectra were measured on a Bruker ARX-500 spectrometer. For HSQC, HMBC, HMQC-RELAY, and HMQC-TOCSY, a total of 512 increments of 2*K* data points were collected. The HMBC were recorded using standard pulse sequence with *Z*-axis pulsed field gradient. Sine-bell shaped gradient pulses were used with a 5:3:4 ratio and 1 ms duration, and maximum strength was 25.0 G cm^{-1}. For HMBC, 50 ms delay time was used for long range C–H coupling. For HMQC-TOCSY a mixing time for TOCSY was 60 ms. A BIRD pulse sequence in the HMQC-TOCSY and HMQC-RELAY experiments was used for suppressing proton signals bonded to ^{12}C, and suppression of one-bond C-H correlations was performed by an insertion of 3.48 ms delay time before broad-band carbon decoupling at the beginning of acquisition time.

The CH_2-selected E-HSQC experiments were carried out using the following pulse sequence proposed by Davis with slight modification;[5] RD–BIRD[$90°_x(^1H)$–Δ–$180°_y(^1H,^{13}C)$–Δ–$90°_{-x}(^1H)$–BD]–$90°_x(^1H)$–Δ/2–$180°_x(^1H,^{13}C)$–Δ/2–$90°_{\Phi1}(^1H)$–$90°_{\Phi2}(^{13}C)$–editing[τ/2–$\beta°_x(^1H)$–$180°_{\Phi3}(^{13}C)$–τ/2]–t_1/2–$180°_y(^1H)$–t_1/2–$90°_x(^1H,^{13}C)$–Δ/2–$180°_y(^1H,^{13}C)$–Δ/2–$AQ_{\Phi4}(^1H,^{13}C$-decoupling); Φ1 = 2(y), 2(-y); Φ2 = x, 2(-x), x; Φ3 = 4(x), 4(y), 4(-x), 4(-y); Φ4 = 2(x, -x), 2(-x, x). For CH_2-selection, the editing flip angle β and the delay τ were π and 3.7 ms ($^1J_{CH}$ = 135 Hz), respectively.[6] The delays RD (repetition delay), BD (BIRD delay), and Δ were 2.0 s, 0.3 s, and 3.7 ms, respectively. 512 experiments, each with 64 scans, were performed with 1*K* data points in F_2. The spectral width in F_1 and F_2 was 4545 (ca. 30 ppm) and 6666 Hz, respectively. Zero-filling was carried out in F_1 and F_2 to give data sets of 1*K* and 2*K* points, respectively. The data were processed using squared cosine-bell window function in both dimensions before 2D Fourier transformation.

For the pulse sequence of CH_2-selected E-HSQC-TOCSY, the trim (2.5 ms) and MLEV-17 composite pulses (mixinig time; 60 ms) were inserted between the last $\Delta/2$ and AQ in the E-HSQC pulse sequence described above. The spectrum was measured with the F_2 spectral width of 6666 Hz in $1K$ data points using 512 transients for each of 256 t_1 increments of the F_1 spectral width of 4545 Hz.

$NaIO_4$ Oxidation of Luteophanol B (1). A THF/phosphate buffer (1 M, pH 7.0) solution (1:1, 500 μL) of luteophanol B (**1**, 2.8 mg) was treated with $NaIO_4$ (6 mg) at room temperature for 1 h. After evaporation of the solvent *in vacuo*, the residue was extracted with MeOH (150 μL x 3) and the solvent was evaporated. To MeOH solution (300 μL) of the residue was added $NaBH_4$ (7.5 mg) at 0 °C, and stirring was continued at room temperature for 1 h. After addition of phosphate buffer (1 M, pH 7.0, 25 μL) at 0 °C, the reaction mixture was stirred for 30 min. After the solvent was removed by N_2 stream, the residue was treated with pyridine (250 μL) and acetic anhydride (250 μL) at room temperature for 18 h. After evaporation of the solvent, the residue was partitioned between $CHCl_3$ (150 μL x 3) and H_2O (150 μL). The organic layer was evaporated, and the residue was purified by silica gel HPLC (YMC Pack silica-06, YMC Co., Ltd., 4.6 x 250 mm; eluent, hexane/*i*-propanol, 95:5; flow rate, 1 mL/min; RI detection) to afford compounds **4** (0.8 mg, t_R 48 min) and **5** (0.4 mg, t_R 5.6 min). **4:** ^{1}H NMR ($CDCl_3$) δ_H 0.98 (3H, d, J = 6.3 Hz, H_3-65), 1.37 (4H, m, H_2-4 and H_2-8), 1.55 ~ 1.64 (8H, H_2-3, H_2-5, H_2-7, and H_2-9), 2.05 (3H, s, AcO), 2.06, (3H, s, AcO), 2.08 (6H, s, AcO x 2), 2.10 (3H, s, AcO), 2.12 (3H, s, AcO), 2.13 (3H, s, AcO), 2.15 (1H, m, H-21), 2.25 ~ 2.35 (6H, m, H_2-11, H_2-15, H_2-18), 3.93 (2H, m, H_2-22), 4.07 (2H, t, J = 7.6 Hz, H_2-2), 4.87 (2H, m, H-6 and H-10), 5.06 (1H, m, H-19), 5.08 (1H, m, H-20), 5.28 (1H, m, H-14), 5.42 (2H, m, H-16 and H-17), 5.44 (1H, m, H-13), and 5.62 (1H, m, H-12); ESIMS *m/z* 735 $(M + Na)^+$; HRESIMS 735.3603 $(M + Na)^+$. Calcd. for $C_{36}H_{56}O_{14}Na$, 735.3568. **5:** ^{1}H NMR ($CDCl_3$) δ_H 1.46 (2H, m, H_2-53), 1.60 (1H, m, H-54), 1.65 (1H, m, H-54), 1.68 (1H, m, H-61), 1.76 (1H, m, H-61), 2.05 (6H, s, AcO x 2), 2.10 (2H, m, H_2-52), 2.18 (2H, m, H_2-62), 3.55 (1H, m, H-55), 4.52 (2H, m, H_2-49), 4.96 (1H, m, H-64), 5.03 (1H, m, H-64), 5.28 (1H, m, H-60), 5.52 (1H, m, H-50), 5.55 (1H, m, H-56), 5.62 (1H, m, H-59), 5.75 (1H, m, H-51), 5.80 (1H, m, H-63), 6.14 (1H, dd, J = 10.5 and 16.5 Hz, H-57), and 6.23 (1H, dd, J = 10.3 and 16.5 Hz, H-58); ESIMS *m/z* 373 $(M + Na)^+$; HRESIMS 373.2003 $(M + Na)^+$, Calcd. for $C_{20}H_{30}O_5Na$, 373.1991.

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