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Lingshuiol, a novel polyhydroxyl compound with strongly cytotoxic activity from the marine dinoflagellate *Amphidinium* sp.☆

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Abstract—A novel polyhydroxy compound with a linear carbon-chain, lingshuiol (**1**), had been isolated from the cultured marine dinoflagellate *Amphidinium* sp. Its structure was elucidated by extensive analysis of 2D NMR spectral data. Lingshuiol possessed a powerful cytotoxic activity against A-549 and HL-60 cells in vitro with the IC_{50} of 0.21 and 0.23 μM, respectively.

Marine dinoflagellates are a rich source of structurally and biologically intriguing natural products; for example, okadaic acid,[1] brevetoxins,[2] and maitotoxin.[3] Among those polyether-cyclic compounds, amphidinols, and luteophanols are unique dinoflagellate metabolites since they are primarily made up of linear polyhydroxy structures. The first member of this group was isolated from the dinoflagellate *Amphidinium klebsii* as a potent antifungal substance by Yasumoto and co-workers.[4] A series of homologues has since been found in the genus.[5–10] As part of our ongoing research on the biologically active substances of Chinese marine organisms,[11,12] we made a collection of the dinoflagellate off the Lingshui Bay (the locality suggested the name assigned to the novel compound), Hainan Province, China. On separation of the toluene soluble fraction of a toluene/methanol (1:4) extract of the cultured dinoflagellate, we isolated a novel polyhydroxyl compound, lingshuiol (**1** Fig. 1). This paper describes the isolation and structure elucidation of this novel compound.

From the surface wash of seaweeds collected at Lingshui Bay, Hainan Province, China, we isolated a strain of *Amphidinium* sp., which was deposited to the Herbarium of South China Sea Institute of Oceanology, CAS for inspection (code-named as *Amphidinium* 2001-1). The

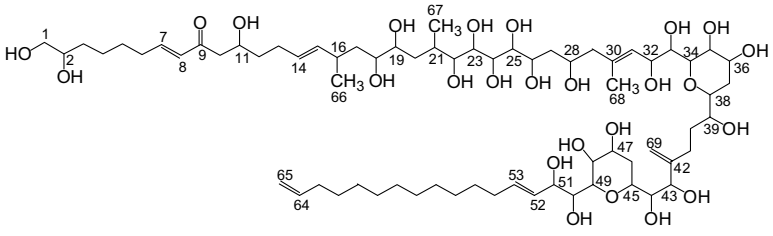

Figure 1. Chemical structure of **1**.

Keywords: Dinoflagellate; *Amphidinium* sp.; Lingshuiol; Antitumor.
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strain was grown unialgally in sterilized seawater enriched with an ES-1 supplement at 24 °C for 3–4 weeks under illumination of a 12 h light and 12 h dark cycle. When the cell density reached 500,000–600,000 cells/mL, the algae were harvested by filtration and extracted with toluene/methanol (1:4, 500 mL × 4). The combined extract was partitioned between toluene and 1 N NaCl solvent, the organic layer (9.0 g) was subjected to separation by normal phase and reversed-phase silica gel column chromatography, followed by purification with C_{18} HPLC (45% MeCN) to afford lingshuiol (**1**, 16.0 mg, 0.0008% wet weight).

Lingshuiol (**1**) was obtained as a pale yellow amorphous solid: $[\alpha]_D^{25}$ −8.0 (*c*, 0.28, MeOH); IR (KBr) ν_{max} 3411, 1705, 1624, 1070 cm^{-1}; UV λ_{max} (MeOH) 226 nm (ε 13,800); Electrospray ionization (ESI) MS of **1** prominently showed a quasimolecular ion peak at *m/z* 1373.8209 {$[M+Na]^+$, $\Delta + 2.6$ mmu}. The ^1H and ^{13}C NMR data[13] suggested that **1** contains one ketone carbon, two sp^2 quaternary carbons, 8 sp^2 methines, 2 sp^2 methylenes, 25 oxymethines, 1 oxymethylene, 2 sp^3 methines, 25 sp^3 methylenes, and 3 methyl groups. The molecular formula $C_{69}H_{122}O_{25}$ led to nine unsaturation equivalents, six of which were due to carbon–carbon double bonds, one due to the ketone carbon, the rest two were ascribable to two rings.

Detailed analysis of DQF-COSY and TOCSY spectra of **1** led to the following four partial structures: from C-1 to C-9 (unit **a**), from C-10 to C-29 (unit **b**), from C-31 to C-41 (unit **c**), and from C-43 to C-69 (unit **d**). The proton connectivities for the two subunits from H_2-1 to H_2-3 ($\mathbf{a_1}$) and from H_2-6 to H-8 ($\mathbf{a_2}$) were evident from the DQF-COSY spectrum (Fig. 2a). The presence of conjugated carbonyl was supported by IR (ν_{max} 1705 cm^{-1}) and UV (λ_{max} 226 nm (ε 13,800). The connectivities from C-3 to C-4 were assigned by HMBC spectrum of **1**, which showed cross-peaks due to H-2/C-4 and H_2-3/C-4. The connectivities from C-5 to C-6 were revealed by HMBC correlations for H-7/C-5 and H_2-6/C-5. The assignment of C-4 connecting to C-5 was supported by the HMBC correlation for H_2-6/C-4. The geometry of the carbon–carbon double bond at Δ^7 was determined to be *E* since the ^1H–^1H coupling constant between H-7 and H-8 was found to be 16.2 Hz.

For partial structure **b**, connectivities from H_2-10 to H_2-17 ($\mathbf{b_1}$), from H-18 to H-24 ($\mathbf{b_2}$), and from H-25 to H_2-29 ($\mathbf{b_3}$) were evident from DQF-COSY cross-peaks (Fig. 2b). These two subunits $\mathbf{b_1}$ and $\mathbf{b_2}$ were connected by HMBC spectrum, which showed cross-peaks due to H-18/C-16 and H_2-17/C-18. The TOCSY spectra data revealed H-18 to be correlated with H-16/H_2-17 and H-19/H_2-20, also supporting the connection of $\mathbf{b_1}$ to $\mathbf{b_2}$ through C-17/C-18. A secondary methyl group (C-66, δ 21.4; H_3-66 δ 0.84, d, $J = 7.2$ Hz) was located on C-16, which was shown by the DQF-COSY cross-peak for H_3-66 (δ 0.84)/H-16 (δ 2.34). The HMBC correlations for H_3-66/C-15, H_3-66/C-16, H_3-66/C-17, and H-15/C-66 further confirmed this assignment. The *E* geometry of Δ^{14} double bond was assigned by the ^1H–^1H coupling constant ($J_{14,15} = 15.3$ Hz). Observation of an evident NOE between H-14 and the allylic methine proton H-16 also supported this conclusion.[5] Although there were no evident DQF-COSY cross-peaks observed from H-25 (δ 3.74) to any other protons, HMBC correlations for H-25/C-23 and H-25/C-24 undoubtedly determined and connection of $\mathbf{b_2}$ to $\mathbf{b_3}$. The TOCSY spectrum confirmed this determination, which revealed H-24 to be correlated to H-22, H-23, H-26, and H-27, respectively. A doublet (δ 0.93, 3H, d, $J = 6.7$ Hz, H_3-67) in the ^1H NMR spectrum of **1** suggested that there is another secondary methyl group locating in **1**. It was assigned on C-21 for that DQF-COSY cross-peak for H_3-67 (δ 0.93)/H-21 (δ 2.32) was evidently observed. This was further supported by HMBC spectrum (H_3-67/C-20, H_3-67/C-21, and H_3-67/C-22).

The ^1H and ^{13}C NMR data for C-31–C-54 of **1**,[13] including the NOEs and ^1H–^1H coupling patterns derived from the NOESY and other 2D NMR experiments, agreed quite well with those for C-31–C-54 of amphidinol 2[14] (**2** Fig. 4), indicating that they shared the same structure for that part (Fig. 2c and d). Analogously to luteophanol B,[10] the relative stereochemistry of the tetrahydropyran ring (from C-34 to C-38) in partial structure **c** was established to be the same as that of luteophanol B on the basis of NOESY spectrum (see Fig. 3).

Major structural alterations in partial structure **d** from amphidinol 2 resided in the terminus C-56–C-61, where

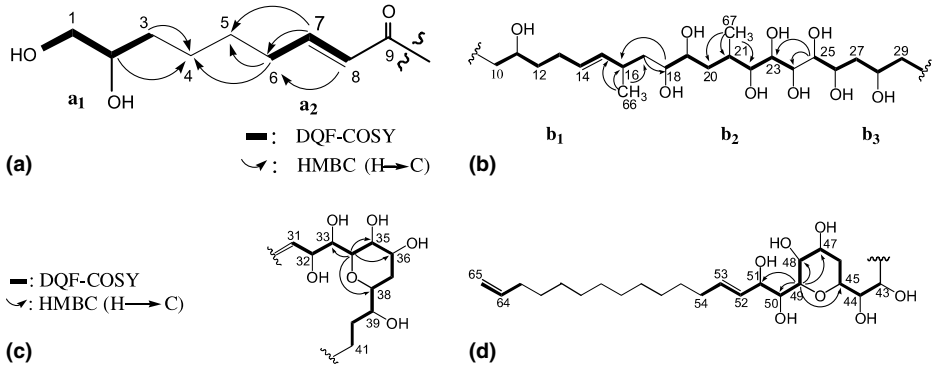

Figure 2. (**a**) Partial structure **a**; (**b**) partial structure **b**; (**c**) partial structure **c**; (**d**) partial structure **d**.

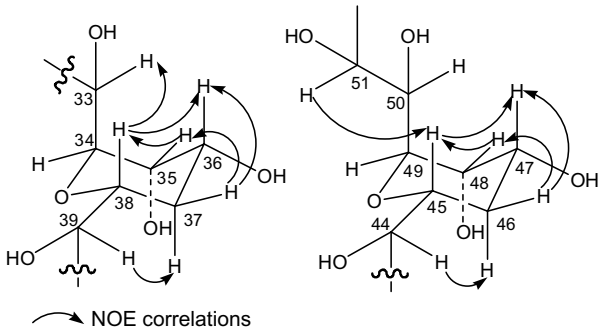

Figure 3. Relative stereochemistry of two tetrahydropyran rings of lingshuiol (**1**).

the conjugated triene moiety in amphidinol 2 was reduced to a saturated chain. Although the C_8-linear chain of C-55–C-62 could not be unambiguously assigned because of heavily overlapped proton signals (δ 1.05–1.60), the existence of eight methylene carbons for unit **d** was confirmed by ^{13}C NMR data (δ 28.7, 28.9, 29.0, 29.1, 29.2, 29.3, 29.3, 29.4). In a similar manner, the relative stereochemistry of the tetrahydropyran ring (from C-45 to C-49) in partial structure **d** was also established by analysis of NOESY spectrum (see Fig. 3).

Three partial structures **a**–**c** had to be connected through a ketone carbon (C-9) and a sp^2 quaternary carbon (C-30), respectively (Fig. 5). The HMBC correlations for H-7/C-9, H-8/C-9, H-8/C-10, H_2-10/C-9 suggested that the partial structures **a** and **b** were connected through a ketone carbon (C-9 δ 200.1). On the other hand, the HMBC spectrum showed cross-peaks due to H_3-68/C-29, H_3-68/C-30, H_3-68/C-31, H-31/C-29, H_2-29/C-30, and H_2-29/C-31, indicating that units **b** and **c** were connected through a sp^2 quaternary carbon (C-30 δ 135.0). The geometry of the carbon–carbon double bond at Δ^{30} was determined to be *E* by the NOESY spectrum, which showed significant correlations of H-31/H-29a and H-31/H-29b. The connectivity of partial structures **c** and **d** was revealed by HMBC spectrum (Fig. 5), which showed cross-peaks due to H_2-40/C-42, H_2-41/C-42, H-43/C-42, H-43/C-41, H-44/C-42, H_2-41/C-69, and H_2-69/C-41.

From all of these observations, we concluded the planar structure of lingshuiol as **1**. The central portion (C-30–C-54 moiety) of **1** was structurally common to those of amphidinols[4,5] and luteophanols.[9,10] lingshuiol, however, possessed different structural features from those of amphidinols and luteophanols in both ends of the molecule. Particularly, amphidinols comprised a hydrophobic polyene portion in one end of the molecule, and the corresponding portion of luteophanols contained two or three hydroxy groups with no conjugated triene, whereas lingshuiol possessed a saturated chain bearing a terminal carbon–carbon double bond, which may make this side of molecule less hydrophobic. The other end of **1** (C-1–C29) was quite different from those of amphidinols and luteophanols, and may come from different biosynthetic pathway. Lingshuiol (**1**) possessed powerful cytotoxic activity against A-549 and HL-60 cell lines in vitro with the IC_{50} of 0.21 and 0.23 μM, respectively.

Acknowledgements

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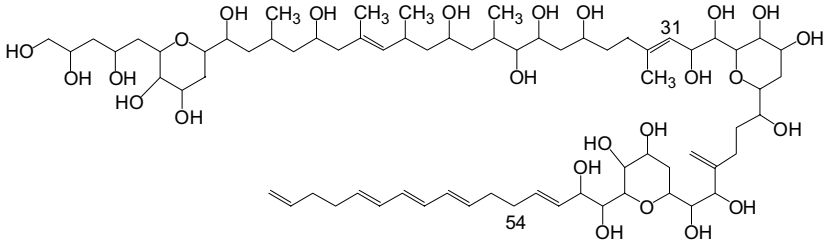

Figure 4. Chemical structure of amphidinol (**2**).

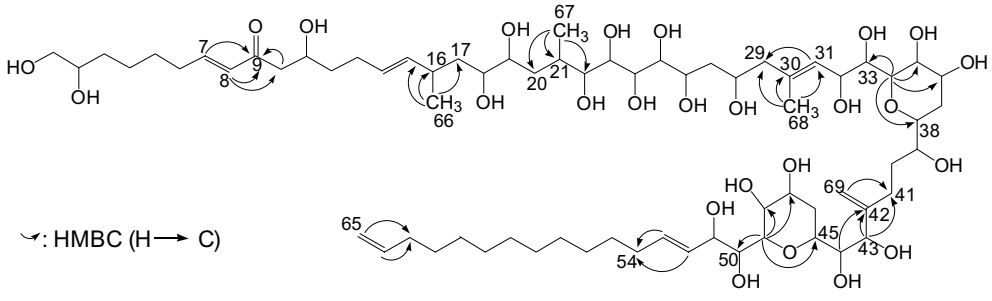

Figure 5. Key HMBC correlations of **1**.

References and notes

1. Tachibana, K.; Scheuer, J.; Kikuchi, H.; Engen, D. V.; Clardy, J.; Schmitz, F. *J. Am. Chem. Soc.* **1981**, *103*, 2469–2470.
2. Lin, Y.-Y.; Risk, S. M.; Engen, D. V.; Clardy, J.; Golik, J.; James, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775, 514–515.
3. Murata, M.; Naoki, H.; Matsunaga, S.; Satake, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1994**, *116*, 708–710.
4. Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861.
5. Paul, G. K.; Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 6279–6282.
6. Paul, G. K.; Matsumori, N.; Konoki, K.; Sasaki, M.; Murata, M.; Tachibana, K. *Harmful Toxic Algal Blooms* **1996**, 503–506.
7. Paul, G. K.; Matsumori, N.; Konoki, K.; Murata, M.; Tachibana, K. *J. Mar. Biotechnol.* **1997**, *5*, 124–128.
8. Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1994**, *121*, 870–871.
9. Doi, Y.; Ishibashi, M.; Nakamichi, H.; Kosaka, T.; Ishikawa, T.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3820–3823.
10. Kubota, T.; Tsuda, M.; Doi, Y.; Takabashi, A.; Nakanichi, H.; Ishibashi, M.; Fukushi, E.; Kawabata, J.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 14455–14464.
11. Shao, Z.-Y.; Zhu, D.-Y.; Guo, Y.-W. *J. Nat. Prod.* **2002**, *65*, 1675–1677.
12. Shao, Z.-Y.; Cai, J.-N.; Ye, Q.-Z.; Guo, Y.-W. *J. Asian Nat. Prod. Res.* **2002**, *4*, 205–209.
13. ^{13}C NMR chemical shifts of **1** (CD_3OD/C_5D_5N 2:1): 66.3 (C-1), 71.8 (C-2), 33.1 (C-3), 25.1 (C-4), 28.0 (C-5), 32.1 (C-6), 148.0 (C-7), 130.8 (C-8), 200.0 (C-9), 47.2 (C-10), 67.2 (C-11), 37.2 (C-12), 28.5 (C-13), 128.8 (C-14), 135.2 (C-15), 33.4 (C-16), 40.5 (C-17), 72.2 (C-18), 71.8 (C-19), 37.5 (C-20), 29.9 (C-21), 71.5 (C-22), 73.8 (C-23), 68.8 (C-24), 77.2 (C-25), 69.1 (C-26), 39.9 (C-27), 66.3 (C-28), 48.2 (C-29), 135.0 (C-30), 128.2 (C-31), 66.7 (C-32), 71.5 (C-33), 77.9 (C-34), 67.8 (C-35), 66.1 (C-36), 29.5 (C-37), 74.6 (C-38), 73.2 (C-39), 31.4 (C-40), 26.8 (C-41), 150.8 (C-42), 75.6 (C-43), 74.0 (C-44), 69.4 (C-45), 30.1 (C-46), 66.2 (C-47), 67.6 (C-48), 79.4 (C-49), 71.0 (C-50), 73.0 (C-51), 128.0 (C-52), 133.8 (C-53), 32.3 (C-54), 28.7, 28.9, 29.0, 29.1, 29.2, 29.3, 29.3, and 29.4 were all assigned to carbons of methylene (C-55–C-62) with protons resonating between δ 1.05–1.60, 33.5 (C-63), 138.8 (C-64), 113.6 (C-65), 21.4 (C-66), 12.6 (C-67), 16.4 (C-68), 111.6 (C-69). The center peak of ^{13}C value of C_5D_5N was taken as standard at δ 136.2. ^{1}H NMR chemical shifts of **1** (CD_3OD/C_5D_5N 2:1): 3.46 (m, H_2-1); 3.56 (m, H-2); 1.32 (m, H-3a); 1.42 (m, H-3b); 1.28 (m, H-4a); 1.42 (m, H-4b); 1.28 (m, H-5a); 1.32 (m, H-5b); 2.03 (m, H_2-6); 6.78 (dt, $J = 16.2$, 6.6 Hz, H-7); 6.03 (d, $J = 16.2$ Hz, H-8); 2.56 (m, H-10a); 2.64 (m, H-10b); 4.06 (m, H-11); 1.44 (m, H-12a); 2.15 (m, H-12b); 1.98 (m, H-13a); 2.06 (m, H-13b); 5.35 (m, H-14); 5.17 (dd, $J = 15.3$, 8.1 Hz, H-15); 2.34 (m, H_2-16); 1.30 (m, H-17a); 1.42 (m, H-17b); 3.46 (m, H-18); 3.58 (m, H-19); 1.44 (m, H-20a); 1.76 (m, H-20b); 2.32 (m, H-21); 3.94 (br d, $J = 9.0$ Hz, H-22,); 3.72 (m, H-23); 4.30 (br d, $J = 6.4$ Hz, H-24); 3.74 (m, H-25); 4.16 (m, H-26); 1.68 (m, H-27a); 1.78 (m, H-27b); 4.13 (m, H-28); 2.10 (m, H-29a); 2.20 (m, H-29b); 5.58 (br d, $J = 8.4$ Hz, H-31); 4.65 (dd, $J = 8.4$, 1.8 Hz, H-32); 3.79 (dd, $J = 9.0$, 1.8 Hz, H-33); 4.16 (m, H-34); 4.22 (br s, H-35); 4.05 (m, H-36); 1.82 (m, H-37a); 1.92 (m, H-37b); 3.54 (m, H-38); 3.63 (m, H-39); 1.60 (m, H-40a); 1.98 (m, H-40b); 2.14 (m, H-41a); 2.52 (m, H-41b); 4.32 (br d, $J = 6.4$ Hz, H-43); 3.43 (m, H-44); 4.13 (m, H-45); 1.54 (m, H-46a); 2.24 (m, H-46b); 4.08 (m, H-47); 4.24 (br s, H-48); 3.92 (br d, $J = 9.0$ Hz, H-49); 4.10 (m, H-50); 4.53 (dd, $J = 7.2$, 3.0 Hz, H-51); 5.67 (m, H-52); 5.73 (m, H-53); 1.86 (m, H_2-54); 1.05–1.60 (m, H_2-55–H_2-62); 1.84 (m, H-63); 5.64 (m, H-64); 4.78 (br d, $J = 10.2$ Hz, H-65a); 4.85 (dd, $J = 17.1$, 1 Hz, H-65b); 0.84 (d, $J = 7.2$ Hz, H-66); 0.93 (d, $J = 7.2$ Hz, H-67); 1.64 (br s, H-68); 4.90 (br s, H-69a); 5.04 (br s, H-69b). The center peak of ^{1}H value of C_5D_5N was taken as standard at δ 7.59.
14. ^{13}C NMR chemical shifts of corresponding carbons of amphidinol 2 (**2**) ($CD_3OD/C_5D_5N/D_2O$ 2:1:0.1): 126.2 (C-31), 67.7 (C-32), 72.3 (C-33), 79.1 (C-34), 68.7 (C-35), 67.3 (C-36), 30.5 (C-37), 75.7 (C-38), 74.4 (C-39), 32.3 (C-40), 27.8 (C-41), 151.4 (C-42), 76.6 (C-43), 74.9 (C-44), 70.4 (C-45), 31.5 (C-46), 67.2 (C-47), 68.5 (C-48), 80.5 (C-49), 71.9 (C-50), 71.9 (C-51), 129.2 (C-52), 134.5 (C-53), 33.4 (C-54). The center peak of ^{13}C value of CD_3OD was taken as standard at δ 49.8.