Spectral Assignments and Reference Data

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Structural and spectral assignments of six anthraquinone derivatives from the mangrove fungus (ZSUH-36)

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A new natural product named 6,8,1′-tri-*O*-methyl averantin(1) has been isolated together with five known anthraquinones 1′-*O*-methyl averantin(2), 6,8-di-*O*-methyl averufin (3) averufin (4), versicolorin C (5) and 6,8-di-*O*-methyl averufanin (6) from a mangrove endophytic fungus ZSUH-36 collected from the South China Sea. NMR techniques including COSY, HMQC, and HMBC were used to elucidate the structures of these compounds. We report the unambiguous assignments of the ^{1}H and ^{13}C NMR spectra of the new compound 6,8,1′-tri-*O*-methyl averantin(1).

Keywords: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; anthraquinone; secondary metabolites; structure elucidation

Introduction

Anthraquinone derivatives as a class of natural products are widespread in nature. They showed strong cytotoxic activity against cancer cell lines such as L1210,[1] LNCap,[2] and A431.[3] Previously, we investigated the chemistry of the fungus (ZSUH-36) and reported the isolation and structure elucidation of two anthraquinones and two xanthones from this endophytic fungus.[4] In a further study on the secondary metabolites of this mangrove endophytic fungus ZSUH-36, we isolated six anthraquinones which were the new natural product 6,8,1′-tri-*O*-methyl averantin (**1**) and five known anthraquinones, namely, 1′-*O*-methyl averantin (**2**),[5] 6,8-di-*O*-methyl averufin (**3**),[6] averufin (**4**),[7,8] versicolorin C (**5**),[8,9] and 6,8-di-*O*-methyl averufanin (**6**).[10] The structures of the compounds were elucidated on the basis of the 1D- and 2D-NMR experiment results, particularly those of COSY, HMQC, and HMBC. Among the known compounds reported previously in the literature, NMR data of compound **3** were incomplete with only proton results available. Therefore, we report the NMR spectral data of compound **1** and the 2D-NMR data of compound **3**. Compounds **2, 3**, and **6** were isolated for the first time as marine fungus metabolites.

Results and Discussion

The new compound 6,8,1′-tri-*O*-methyl averantin (**1**) was obtained as a red amorphous solid. It (Fig. 1) showed a molecular ion peak at *m/z* 414 in EIMS. Its molecular formula $C_{23}H_{26}O_7$ was assigned on the basis of HR-EIMS (*m/z* 414.1676, calcd 414.1673) and the NMR data, which indicated that the compound has 11° of unsaturation. The ^{13}C NMR and DEPT data implied that compound **1** has 1 methyl group, 3 methoxyl groups, 4 methylene carbons, 4 methine carbons, and 11 quaternary carbons including 2 carbonyl carbons at δ_C 187.3 and 182.7. The ^{1}H NMR spectrum and HMQC data (Table 1) revealed the presence of a hydrogen-bonded hydroxyl group [δ_H 13.99 (1H, s)], two signals of *meta*-coupled aromatic protons at δ_H 6.85 (d, 2.5) and 7.21 (d, 2.5), one singlet due to an aromatic proton at δ_H 6.94, three methoxyl groups at δ_H 3.89, 3.87, 3.35, and four methylene groups at δ_H 1.20–1.21(4H, overlapped, H-4′ and H-5′); 1.31 (1H, m, H-3′), 1.40 (1H, m, H-3′), 1.74 (1H, m, H-2′), and 1.77 (1H, m, H-2′)]. The ^{1}H and ^{13}C NMR (Table 1) spectra indicated that compound **1** was similar to averantin, except that the three methoxyl groups in compound **1** were replaced by three hydroxyl groups. Detailed assignments of the carbon and proton signals were unambiguously accomplished by analysis of 2D-NMR spectral data (Table 1). The structure of the six-carbon chain moiety was confirmed by sequential correlations of H–H COSY from H-2′ to H-1′, H-3′ and from H-6′ to H-4′, H-5′. In the HMBC spectrum, key correlations were observed between H-1′ and C-2, indicating that the six-carbon chain moiety was connected to C-2. Furthermore, the two aromatic protons at δ_H 6.94 (H-4) and 7.21 (H-5) gave cross-peaks with the carbonyl carbon at δ_C 182.7 (C-10) and C-2 (δ_C 119.8), C-3 (δ_C 162.9), C-9a (δ_C 111.0) and C-7 (δ_C 105.1), C-8a (δ_C 115.3), respectively, which were consistent with their locations on carbons C-4 and C-5, and the carbonyl group at C-10 (δ_C 182.7). The rest of the correlations have been summarized and listed in

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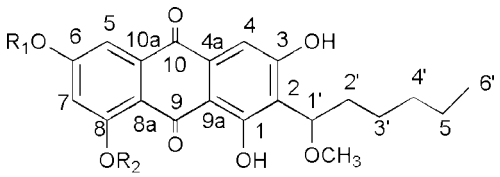
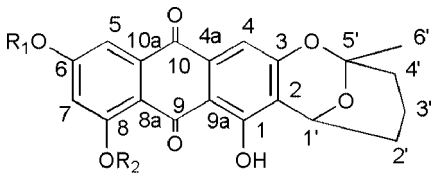


1 $R_1=R_2=CH_3$
2 $R_1=R_2=H$

Figure 1. The structures of compounds **1** and **2**.



3 $R_1=R_2=CH_3$
4 $R_1=R_2=H$

Figure 2. The structures of compounds **3** and **4**.

Table 1. NMR data of compound **1** (δ in ppm, J in Hz)

C no.	^{13}C NMR (DEPT)	^{1}H NMR	H–H COSY	HMBC
1	163.1			
2	119.8			
3	162.9			
4	108.1 CH	6.94(s)		C-2, C-3, C-10, C-9a
5	105.4 CH	7.21(d, 2.5)	H-7	C-7, C-10, C-8a
6	166.2			
7	105.1 CH	6.85(d, 2.5)	H-5	C-5, C-6, C-8, C-8a
8	164.4			
9	187.3			
10	182.7			
4a	134.0			
8a	115.3			
9a	111.0			
10a	138.0			
8-OCH_3	56.9	3.89(s)		C-8
9-OCH_3	56.5	3.87(s)		C-6
1′	79.8 CH	4.85(dd, 5.0, 8.0)	H-2′	C-2, C-3, C-7′
2′	35.2 CH_2	1.77(m)	H-1′, H-3′	C-1′
		1.74(m)	H-1′, H-3′	C-1′
3′	25.7 CH_2	1.31(m)	H-2′	C-5′
		1.40(m)	H-2′	C-4′
4′	32.3 CH_2	1.21 (overlapped)	H-6′	C-5′
5′	23.2 CH_2	1.20 (overlapped)	H-6′	C-4′
6′	14.2 CH_3	0.75(m)	H-4′, H-5′	C-4′, C-5′
1′-OCH_3	58.2	3.35(s)		C-1′
	1-OH	13.99(s)		C-1, C-2, C-9a

Table 2. NMR data of compound **3** (δ in ppm, J in Hz)

C no.	^{13}C NMR (DEPT)	^{1}H NMR	H–H COSY	HMBC
1	159.6			
2	116.8			
3	159.6			
4	107.0 CH	7.20(s)		C-2, C-3, C-10, C-9a
5	104.0 CH	7.45(d, 2.5)	H-7	C-7, C-10, C-8a
6	164.9			
7	104.8 CH	6.77(d, 2.5)	H-5	C-5, C-6, C-8, C-8a
8	162.8			
9	186.8			
10	182.6			
4a	132.5			
8a	115.3			
9a	104.0			
10a	137.6			
8-OCH_3	56.6	4.01(s)		C-8
8-OCH_3	56.0	3.97(s)		C-6
1′	67.1 CH	5.38(d, 3.0)	H-2′	C-1, C-2, C-3, C-3′, C-5′
2′	27.4 CH_2	1.88(m)	H-1′, H-3′	C-2, C-4′
		2.10(m)		C-1
3′	16.0 CH_2	1.53(m)	H-2′, H-4′	C-5′
		1.65(m)	H-2′, H-4′	C-1′, C-4′, C-5′
4′	35.9 CH_2	1.84(m)	H-3′	C-3′
		2.04(m)	H-3′	C-3′, C-5′, C-6′
5′	100.9			
6′	27.8 CH_3	1.58(s)		C-3, C-1′, C-4′
	1-OH	13.55(s)		C-1, C-2

Table 1. On the basis of these spectroscopy data the compound was identified as 6,8,1′-tri-*O*-methyl averantin (**1**).

Compound 1′-tri-*O*-methyl averantin (**2**) was isolated as a red amorphous powder with a molecular formula of $C_{21}H_{22}O_7$ assigned by using its EIMS and NMR data. Compared with the ^{1}H NMR data of **1**, compound **2** had two extra hydroxyl groups at δ_H 12.51, 10.15 and lacked two methoxyl groups at δ_H 3.89, 3.87. According to the above data this compound can be deduced as 1′-*O*-methyl averantin. This was also supported by the comparison of its ^{1}H and ^{13}CNMR spectra with those reported in the literature.[5] This is the first report that compound **2** was obtained from a marine fungus resource.

Compound **3** (Fig. 2) was isolated as a yellow solid. According to the LC-MS and NMR data, the molecular formula of **3** was established as $C_{22}H_{20}O_7$. The ^{13}C NMR and DEPT spectra showed 22 carbon signals, attributable to 1 methyl (δ_C 27.8), 2 methoxyl (δ_C 56.0, 56.6), 3 methylene (δ_C 16.0, 27.4, 35.9), 4 methine (δ_C 67.1, 104.0, 104.8, 107.0), 10 quaternary (δ_C 100.9, 104.0, 115.3, 116.8, 132.5, 137.6, 159.6, 159.6, 162.8, 164.9), and two ketone carbonyl (δ_C 182.6, 186.8) carbons. The ^{1}H NMR spectrum (Table 2) displayed a hydrogen-bonded hydroxyl group at δ_H 13.55 (1H, s), two *meta*-coupled aromatic proton signals at δ_H 6.77 and 7.45, one singlet due to an aromatic proton at δ_H 7.20, two methoxyl group at δ_H 4.01 and 3.97, one methyl group at δ_H 1.58, one methine at δ_H 5.38 (d, 3.0 Hz) attached to an oxygen atom, and three methylene groups at δ_H 1.88(1H, m), 2.10 (1H, m); 1.53 (1H, m), 1.65 (1H, m); and 1.84 (1H, m), 2.04 (1H, m). The assignments of the carbon and proton signals were unambiguously accomplished by a further analysis of the-2D NMR spectral data that are listed in Table 2. This was also the first report on 6,8-di-*O*-methyl averufin isolated from a marine fungus.

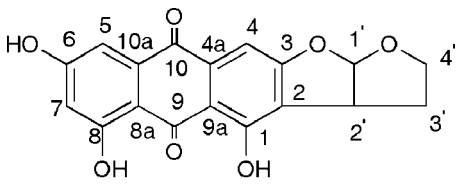


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Figure 3. The structure of compound **5**.

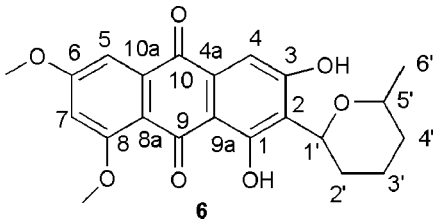

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Figure 4. The structure of compound **6**.

Compound **4** (Fig. 2) and compound **5** (Fig. 3) were also isolated from the fungus. Their structures were identified as averufin[7,8] and versicolorin C[8,9] by comparison of their spectroscopic data with those in the related literature.

Compound **6** (Fig. 4) was obtained as a red power. The molecular formula of this compound was $C_{22}H_{22}O_7$ confirmed by LC-MS which showed $[M - H]^-$ ion peak at *m/z* 397. This compound was isolated from a terrestrial fungus[10] but this is the first report from a marine fungus in the South China Sea.

Experimental

General experimental procedures

Mass spectra were recorded on a VG ZAB-HS double focusing mass spectrometer, IR spectra were measured on a Bruker EQUINOX 55 spectrophotometer, UV spectra were obtained on a Shimadzu UV-2501PC spectrophotometer. Column chromatography was carried out on silica gel (200–300 mesh; Qingdao Haiyang Chemicals).

Fungal strain

The fungus strain (ZSUH 36) was isolated from the Shenzhen mangrove *Acanthus ilicifolius Linn.* and the specimen was stored at the Department of Applied Chemistry, Sun Yat-sen University, Guangzhou, China. Starter cultures were maintained on cornmeal seawater agar.

Fermentation, extraction, and isolation

Plugs of agar-supporting mycelium growth were cut from a solid culture medium and transferred aseptically to a 500-ml Erlenmeyer flask containing 250 ml liquid medium (glucose 10 g/l, peptone 2 g/l, yeast extract 1 g/l, sea salt 2.5 g/l).[11] The fungus was cultured in 70 l and incubated at 28 °C, for 35 days. The broth was filtered through a cheesecloth. The mycelium (450 g) was air-dried and extracted with methanol. The filtrate was concentrated to 3 l below 60 °C and partitioned four times by shaking with an equal volume of ethyl acetate. The EtOAc extract (25.6 g) of the fungal mycelium was subjected to silica gel chromatography using a gradient from petroleum to ethyl acetate (fractions A–F) and then from ethyl acetate to methanol (fractions G–I). Fraction D (1.2 g) was further separated by preparative TLC (silica gel GF_{254}) developed by 2% methanol/chloroform (v/v) to afford fractions D_1–D_4. Fraction D_1 (226.0 mg) was subjected to a further column chromatography to afford compounds **1** (2.6 mg), **3** (56.2 mg), **4** (25.5 mg), **6** (2.5 mg). Fraction D_2 (86.4 mg) was again separated by PTLC to obtain **2** (12.6 mg) and **5** (18.7 mg).

1′,6,8-Tri-*O*-methyl averantin (**1**): EIMS (*m/z*): 414 (M^+, 4), 399 (4), 382 (42), 353 (16), 343 (32), 339 (10), 325 (100), 313 (20), 310 (10).

1′-*O*-Methyl averantin (**2**): EIMS (*m/z*): 386 (M^+, 11), 368 (13), 354 (96), 325 (39), 315 (100), 311 (40), 297 (94), 285 (84), 272 (23), 255 (11), 244 (9), 213 (8), 155 (6). ^{1}H NMR (500 MHz, Acetone-d_6): δ 12.75 (OH), 12.51 (OH), 10.15 (OH), 9.75 (OH), 7.21 (1H, d, 2.4 Hz, H-5), 7.11 (s, H-4), 6.62 (1H, d, 2.4 Hz, H-7), 4.96 (1H, dd, 5.0, 8.0, H-1′), 3.49 (s, 1′-OCH_3), 1.83 (2H, m, H-2′), 1.41 (2H, m, H-3′), 1.33 (4H, overlapped, H-4′, 5′), 0.88 (t, 6.8, 6′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.4 (C-9), 182.4 (C-10), 166.8 (C-6), 166.7 (C-8), 165.0 (C-1), 163.7 (C-3), 137.2 (C-10a), 135.8 (C-4a), 120.4 (C-2), 111.1 (C-8a), 111.0 (C-4), 110.6 (C-9a), 110.5 (C-5), 109.9 (C-7), 80.7 (C-1′), 59.3 (1′-OCH_3), 36.1 (C-2′), 33.3 (C-4′), 26.7 (C-3′), 24.2 (C-5′), 15.3 (C-6′).

6,8-Di-*O*-methyl averufanin (**6**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.74 (OH), 9.75 (OH), 7.38 (d, 2.4 Hz, H-5), 7.16 (s, H-4), 6.71 (d, 2.4 Hz, H-7), 5.11 (dd, 1.5, 11.1 Hz, H-1′), 3.96 (s, 5-OCH_3), 3.92 (s, 7-OCH_3), 3.68 (ddd, 5.4, 6.3, 11.2 Hz, H-5′), 1.94 (m, H-2′), 1.76 (m, H-4′), 1.40 (m, H-3′), 1.29 (d, 7.0 Hz, H-6′). LC-MS (*m/z*): 397 (M^-, 100), 382 (16), 367 (42), 353 (48), 337 (36), 312 (66), 309 (47), 297 (41).

NMR data

NMR spectra (1H, COSY, HSQC and HMBC) were recorded at 25 °C using TMS as internal reference on Varian Inova 500NB NMR spectrometer (^{1}H, 499.77 MHz; ^{13}C, 125.7 MHz) equipped with a triple resonance inverse cold probe cooled to 25 K. The ^{1}H NMR and ^{13}C NMR spectra provided digital resolutions of 0.25 and 0.17 Hz, respectively. The ^{1}H and ^{13}C chemical shifts were referenced to the residual solvent peak of Acetone-d_6 at δ 2.05 and 30.8 ppm and $CDCl_3$ at δ 7.26 and 77.0 ppm, for proton and carbon respectively. All sample concentrations ranged from 5 to 15 mg ml^{-1} with a total volume of 0.5 ml for each sample. The ^{1}H sweep width was set at 7506 Hz for all experiments with a 45° pulse for ^{1}H and a ^{13}C 90° pulse. The gradient COSY was acquired with 200 F1 increments with 32 scans per increment. A sinebell weighting was applied to each dimension and zero filled to 2 K points. The gradient HMQC was acquired with ^{13}C sweep width of 32 000 Hz and 256 t1 increments. Each increment was acquired with 32 transients. A sinebell function was applied to the F2 dimension before zero filling to 2 K points, and a sinebell was applied to the F1 and zero filled to 2 K points before Fourier transformation. A one-bond coupling constant delay was set using 140 Hz, and WURST decoupling was applied during acquisition. The gradient HMBC was acquired using 64 transients per increment with 256 F1 increments. A sweep width of 32 000 Hz was used for the ^{13}C dimension. The one-bond coupling constant of 140 Hz and a long-range coupling constant of 8 Hz were used to set the delays in the pulse sequence. A sinebell weighting was applied to both ^{1}H and ^{13}C dimensions and zero filled to 4 and 1 K, respectively.

Acknowledgements

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