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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2008

Impact Factor: 3.8 · DOI: 10.1021/np070602x · Source: PubMed

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Benzofuran Derivatives from the Mangrove Endophytic Fungus *Xylaria* sp. (#2508)

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Received October 26, 2007

Three metabolites, named xyloketal J (**1**), xyloester A (**2**), and xyloallenolide B (**3**), together with the known substituted dihydrobenzofuran (**4**) were isolated from the mangrove endophytic fungus *Xylaria* sp. (#2508). Structures were determined by spectroscopic methods, mainly 1D and 2D NMR.

Our group has been searching for new bioactive compounds from marine microorganisms in the South China Sea. The ascomycete *Xylaria* sp. (#2508) collected on seeds in Mai Po mangrove, Hong Kong, produced a number of unique metabolites. In a previous study, the fungus was shown to produce xyloketals and xyloallenolides, two chemical families with novel frameworks, and exhibited strong activity against L-calcium channels and inhibition of acetylcholine esterase.[1–4,6–8] However, other metabolites produced were present in low quantities and their structures could not be elucidated. Subsequently, fermentation of the fungus in large scale yielded three metabolites, named xyloketal J (**1**), xyloester A (**2**), and xyloallenolide B (**3**) (which belong to a new series of compounds), and a known substituted dihydrobenzofuran (**4**).[9] It is biogenetically interesting that compounds **1**, **2**, and **3** possess a substituted dihydrobenzofuran unit (**4**). In this paper, we describe the isolation and structural elucidation of these three new metabolites.

The ethyl acetate extract of a fermentation broth of the fungus was subjected to silica gel column chromatography, which led to the isolation of **1**, **2**, **3**, and **4**. Their structures were elucidated by spectroscopic data, mainly 1D and 2D NMR spectra.

Xyloketal J (**1**) was obtained as colorless, blocky crystals. The HREIMS spectrum displayed a $[M]^+$ ion at *m/z* 518.2646 (calcd for $C_{32}H_{38}O_6$, 518.2663). In the MS spectrum, there was a characteristic $[M - 98]^+$ peak for xyloketals.[1,2] The ^1H, ^{13}C, and 2D NMR showed similar signals to xyloketal B (**5**) that we had previously reported.[1] The COSY spectrum revealed a contiguous sequence from H-4′ to H-7′. The correlation between H-11′ and H-5′ located CH_3-11′ at C-5′. In the HMBC experiment, the multiple correlations of C-2′ with H-4′, H-7′, H-10′, C-8′ with H-7′, H-6′, and between C-9′ and H-7′ were observed. All the data indicated the presence of the xyloketal B (**5**) subunit. In the ^{1}HNMR, the coupling constants for this position of **1** were very similar to that of xyloketal B (**5**).

Comparing the NMR spectrum of **1** with **5**, and eliminating signals similar to **5**,[1] there remained signals for a trisubstituted benzene ring (C-4, 6, 7, 9, CH-5, 8), one terminal double bond (CH_2-10 and C-11), an isolated proton (H-2), and a methyl group (CH_3-12). The structural fragment composed of these groups was elucidated by 2D NMR. In the HMBC spectra, the correlations between the methyl proton (H-12) and the terminal double bond (C-10 and C-11) indicated the presence of an isopropenyl group. The correlation between H-12 and C-2 and the correlations between H-3 and C-11 determined the contiguous sequence from C-12 to C-3. The substituted positions of the benzene ring were determined by the correlations between H-3 and C-4 and from H-13 to C-6, C-7, and C-5. These results unambiguously assembled a substituted dihydrobenzofuran unit that was similar to **4** and were consistent with 2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl)methanol reported in the literature.[9] The connection of units **4** and **5** could be established by the multiple correlations from H-13 to C-13′, C-12′, and C-9′.

The molecular formula of xyloester A (**2**) was determined as $C_{23}H_{30}O_5$ by HREIMS. The NMR spectra (^1H, ^{13}C, and 2D NMR) revealed the characteristic signals of compound **4** as a subunit of **2** (Table 2).[9] Similar to the treatment for **1**, and eliminating the signals of subunit **4** from the NMR spectra, there remained two carbonyl groups (δ_C 173.9, 170.4), one trisubstituted double-bond signals [δ_C 146.7, δ_C 128.6, δ_H 6.94 (t, $J = 8$ Hz)], one methine [δ_H 3.62 (q, 7.5 Hz, 1H), δ_C 37.7], two methyls [δ_H 1.37 (d, 1 Hz, 3H), δ_C 15.8 and δ_H 0.87 (t, 3H), δ_C 13.9], and four methylenes (Table 2). By HMBC, multiple correlations from H-16 to C-15, C-17, and C-18 located the positions of two carboxyl groups. According to the correlations from H-19 to C-15 and C-16, the CH_3-19 was located at C-16. The alkane chain from C-21 to C-25 could be assembled by analysis of COSY and HMBC spectra. The above analysis revealed the presence of a butanedioic acid structural unit. The linkage of subunit **4** and the acid unit was accomplished by the correlation between H-13 and C-15. Thus, the total structure of **2** was completely established.

Xyloallenolid B (**3**) was a colorless oil, with a molecular formula of $C_{23}H_{24}O_3$ on the basis of the NMR and HREIMS data (obsd *m/z* 348.1724 $[M]^+$, calcd for $C_{23}H_{24}O_3$, 348.1720). The NMR spectra for **3** also displayed all signals corresponding to subunit **4** as for compounds **1** and **2** (Table 3). The IR spectrum of **3** showed the characteristic absorptions for an allene functionality (1957 cm^{-1}).[6–8] The ^1H NMR spectrum showed an A_2MX_2 spin system due to H-23, 24, and 26, corresponding to the ^{13}C NMR signals of the allenic aromatic ether at δ_C 65.9, 87.1, and 76.5, respectively, and C-25 at δ_C 209.5. In addition, one AA′XX′ spin system at δ 6.90 (d, 2H, $J = 8.5$ Hz) and 7.28 (d, 2H, $J = 8$ Hz) revealed one *para*-disubstituted benzene ring. Combining this HMBC and COSY analysis (see Figure 2), a fragment containing an allenic aromatic ether was established. This new fragment and subunit **4** were connected by the correlation observed between H-13 and C-15.

Xyloallenolid B is one of many metabolites with an allene group from *Xylaria* sp. (#2508). Some are rare, such as xyloallenolid A, the structure of which is composed of an allene with cyclotripeptide. We have synthesized several allenic compounds and studied their

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10.1021/np070602x CCC: $40.75
Published on Web 05/24/2008

Table 1. NMR Data ($CDCl_3$) of **1**[a]

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC[b]
2	85.8, CH	5.15, t (9)	C-12
3a	34.8, CH_2	3.27, dd (16, 9.5)	C-4
3b		2.96, dd (16, 8)	C-4, 10
4	127.1, qC		
5	124.9, CH	7.08, s	C-7, 9
6	132.4, qC		
7	127.9, CH	7.02,d (8)	C-5, 9
8	109.0, CH	6.69, d (8)	C-4, 6
9	158.3, qC		
10a	111.9, CH_2	4.90, s	C-2, 12
10b		5.07, s	C-2, 12
11	144.1, qC		
12	17.2, CH_3	1.76, s	C-2, 10, 11
13	28.1, CH_2	3.88, dd (28.5, 15.5)	C-5, 6, 7, 9″, 13′
2″	107.4, qC		
2′	107.6, qC		
4″a	74.0, CH_2	3.52, dd (17, 8.5)	C-2″, 5″
4″b		4.17, dd (17, 8.5)	C-6″
4′a	73.8, CH_2	3.55, dd (17, 8.5)	C-2′, 5′
4′b		4.18, dd (17, 8.5)	C-6′
5″	35.5, CH_2	2.13, m	C-4″, 7″
5′	35.3, CH_2	2.13, m	C-4′, 7′
6″	47.8, CH	1.75, ddd (7, 4.5, 1)	C-2″
6′	47.6, CH	1.91, ddd (6, 3.5, 1.5)	C-2′
7″a	19.1, CH_2	2.87, d (17.5)	C-2″, 6″, 8″, 9″
7″b		2.68, dd (16, 6)	C-5″, 6″, 8″, 9″
7′a	18.9, CH_2	2.76, d (16)	C-2′, 6′, 8′, 9′
7′b		2.68, dd (16.5, 5.5)	C-5′, 6′, 8′, 9′
8″	99.8, qC		
8′	98.4, qC		
9″	150.0, qC		
9′	149.9, qC		
10″	22.8, CH_3	1.49, s	C-2″, 6″
10′	23.1, CH_3	1.51, s	C-2′, 6′
11″	15.9, CH_3	1.06, d (6.5)	C-4″, 5″, 6″
11′	16.1, CH_3	1.07, d (6.5)	C-4′, 5′, 6′
12′	151.1, qC		
13′	127.1, qC		

[a] Measured at 500 MHz (^1H) and 125 MHz (^{13}C). [b] For the COSY correlations, see the Supporting Information.

Table 2. NMR Data ($CDCl_3$) of Xyloester A (**2**)[a]

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC[b]
2	86.0, CH	5.15, t (9)	C-4
3a	34.5, CH_2	3.01, dd (16, 8.5)	C-2, 4, 9, 11
3b		3.30, dd (16, 9.5)	C-4, 9, 11
4	126.9, qC		
5	125.5, CH	7.12, s	C-6, 9
6	131.3, qC		
7	128.1, CH	7.07, d (8)	C-9, 4
8	108.9, CH	6.73, d (8)	C-7
9	159.8, qC		
10a	112.1, CH_2	4.90, s	C-2, 12
10b		5.07, s	C-2, 12
11	143.9, qC		
12	17.2, CH_3	1.76, s	C-2, 11, 10
13	65.9, CH_2	5.02, s	C-5
15	173.5, qC		
16	37.7, CH	3.62, q (7.5)	C-15, 17, 18
17	146.7, qC		
18	170.4, qC		
19	15.8, CH_3	1.37, d (1)	C-15, 16
20	128.9, CH	6.94, t (8)	C-16, 18
21	31.5, CH_2	2.12, m	C-17, 23
22	28.7, CH_2	1.44, quintet	C-24
23	28.1, CH_2	1.29, m	C-21, 24
24	22.4, CH_2	1.29, m	C-25
25	13.9, CH_3	0.87, t (9)	C-21, 24

[a] Measured at 500 MHz (^1H) and 125 MHz (^{13}C). [b] For the COSY correlations, see the Supporting Information.

structure−activity relationships. Our results show that the allene group probably increases the antitumor activities of these compounds.[6–8]

Table 3. NMR Data ($CDCl_3$) of Xyloallenoid B (**3**)[a,b]

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC
2	85.9, CH	5.17, t (8.5)	C-3, 10, 12
3a	34.6, CH_2	3.03, dd (16, 9.5)	C-2, 4, 9, 11
3b		3.32, dd (16, 8.5)	C-4, 9, 11
4	126.9, qC		
5	124.9, CH	7.16, s	C-7, 9
6	130.4, qC		
7	128.3, CH	7.08, d (8)	C-5, 9, 13
8	108.8, CH	6.76, d (8)	C-6, 9
9	159.5, qC		
10a	112.1, CH_2	5.08, s	C-2, 12
10b		4.90, s	
11	144.0, qC		
12	17.2, CH_3	1.77, s	C-10
13	71.9, CH_2	4.43, s	C-5
15	71.5, CH_2	4.47, s	C-13, 17
16	130.8, qC		
17	129.4, CH	7.27, d (8)	C-15, 16, 21
18	114.8, CH	6.90, d (8.5)	C-19
19	157.9, qC		
20	114.8, CH	6.90, d (8.5)	C-18
21	129.4, CH	7.27, d (8)	C-17, 23
23	65.9, CH_2	4.57, dt (3, 7)	C-19, 24, 26
24	87.1, CH	5.39, tt (7)	C-23, 26
25	209.5, qC		
26	76.5, CH_2	4.86, dt (3, 7)	C-24

[a] Measured at 500 MHz (^1H) and 125 MHz (^{13}C). [b] For the COSY correlations, see the Supporting Information.

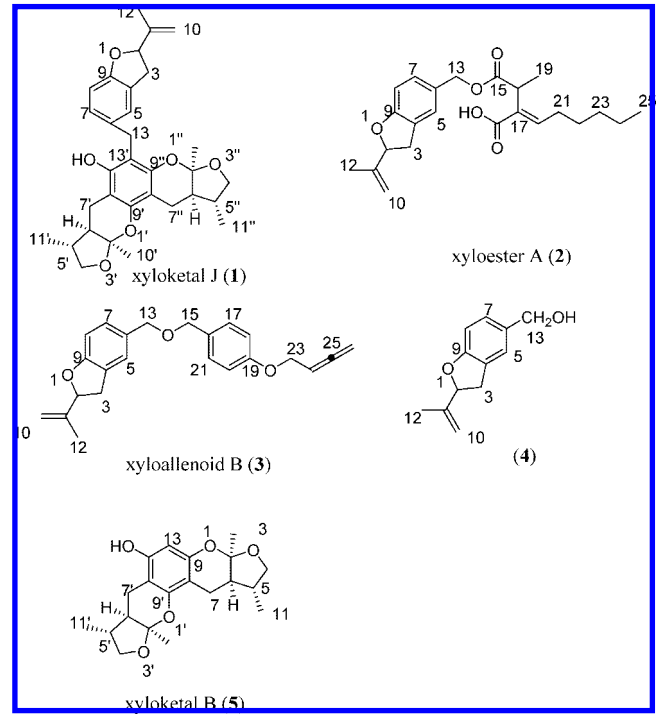

Figure 1. Metabolites **1**, **2**, **3**, **4**, and **5**.

Some 150 natural allenes have been found, many of which have intriguing biological activities.[5]

Xylaria sp. is a very prolific producer of novel metabolites, yielding three series of metabolites belonging to the xyloketal, allenoid, and dihydrobenzofuranoid classes. Further studies of the biosynthesis and ecological function of these diverse metabolites will be interesting.

The antibacterial activites of these three metabolites were tested, but they showed no effect against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Sarcina lutea* at 50 μg/mL. No further bioactivity studies could be performed due to the low yields of these compounds.

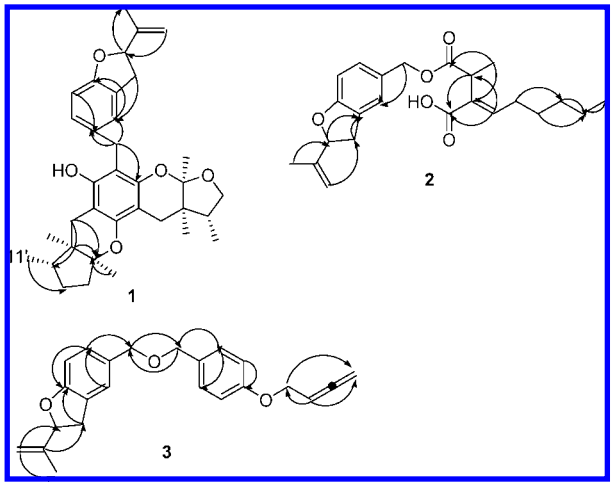

Figure 2. Key HMBC correlations of **1**, **2**, and **3**.

Experimental Section

General Experimental Procedures. Melting points were determined on an X-4 micromelting point apparatus and were uncorrected. Optical rotations were measured on a Schmidt + Haensch Polartronic HH W5 polarimeter and were uncorrected. UV spectra were measured on a Shimadzu UV-2501 PC spectrophotometer. IR spectra were measured on a Bruker Vector 22 spectrophotometer. ^{1}H and ^{13}C NMR data were recorded on a Varian Inova 500 MB NMR spectrometer operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively (TMS as internal standard). HREIMS were measured on a Thermo MAT95XP high-resolution mass spectrometer and EIMS on a Thermo DSQ EI-mass spectrometer. Si gel (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd.) was used for column chromatography (CC).

Fungal Strain. The ascomycete *Xylaria* sp. (#2508) was isolated from the seeds of an angiosperm tree in Mai Po mangrove, Hong Kong, and deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, China. Plugs of agar with mycelium were cut and transferred aseptically into a 250 mL Erlenmeyer flask containing 100 mL of liquid GYT medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L) and incubated on a rotary shaker at 25 °C for 5−7 days. The mycelium was harvested, resuspended in 300 L of liquid GYT medium, and incubated at 25 °C for 72 h.

Extraction and Separation of Metabolites. The cultures (300 L) were filtered through cotton yarn and concentrated in vacuo to 5 L below 50 °C and extracted with an equal volume of EtOAc. Dried extract (45 g) was subjected to Si gel CC (200−300 mesh, 500 g) and eluted with a gradient of petroleum ether and EtOAc, to obtain **1** (2.5 mg), **2** (1 mg), **3** (2.3 mg), and **4** (1 mg) from the EtOAc−petroleum ether (15:85, 8:92, 20:80, and 8:92) fractions, respectively.

Xyloketal J (1): colorless, blocky crystals; mp 58−59 °C; $[\alpha]^{25}_D$ +25 (*c* 0.012, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 284 (4.00), 218 (4.76) nm; IR (KBr) ν_{max} 3426 (br), 2956, 2928, 1615, 1459, 1382, 1204, 1114, 1007, 872 cm^{-1}; ^{1}HNMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 MHz), and 2D NMR data, see Table 1; HREIMS *m/z* 518.2646 (calcd for $C_{32}H_{38}O_6$, 518.2663).

Xyloester A (2): colorless, blocky crystals; mp 158−159 °C; $[\alpha]^{25}_D$ +15.9 (*c* 0.025, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 230 (3.48), 284 (3.32) nm; IR (KBr) ν_{max} 2929, 2858, 1689, 1492, 1459, 1377, 1299, 1253, 1199, 1092, 1061, 967, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz),^{13}C NMR ($CDCl_3$, 125 MHz), and 2D NMR data, see Table 2; HREIMS *m/z* 386.2086 (calcd for $C_{23}H_{30}O_5$, 386.2088).

Xyloallenolid B (3): colorless oil; $[\alpha]^{25}_D$ −13.2 (*c* 0.025, MeOH); IR (KBr) ν_{max} 3425 (br), 2921, 2854, 1957, 1703, 1610, 1511, 1490, 1442, 1377, 1244, 1171, 1110, 1076, 1015, 905, 821 cm^{-1}; ^{1}HNMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 MHz), and 2D NMR data, see Table 3; HREIMS *m/z* 348.1724 (calcd for $C_{23}H_{24}O_3$, 348.1720).

Acknowledgment. This research was supported by the National Natural Science Foundation of China (20772162, 20572136), the 863 Foundation of China (2007AA09Z448, 2006AA09Z422), the Science Foundation of Guangdong Province, China (2007A032600001), and the Star Lake Biotechnology Co., Inc., Zhaoqing, Guangdong, China.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP070602X