Seven Novel Linear Polyketides from *Xylaria* sp. NCY2

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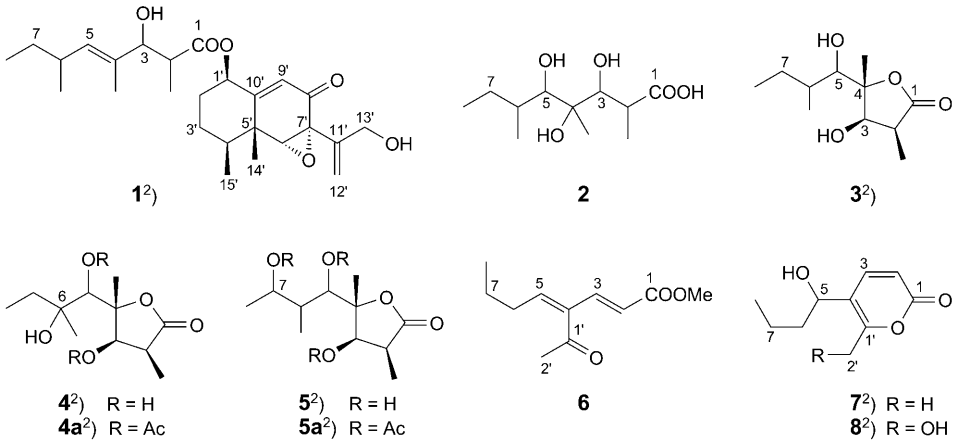
Seven novel polyketides, namely, 1-(xylarenone A)xylariate A (**1**), xylarioic acid B (**2**), xylariolide A (**3**), xylariolide B (**4**), xylariolide C (**5**), methyl xylariate C (**6**), and xylariolide D (**7**), together with the known one taiwapyrone (**8**), were isolated from the endophytic fungal strain *Xylaria* sp. NCY2 of *Torreya jackii* CHUN. Their structures were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments, and on the basis of HR-Q-TOF mass spectrometry. Antitumor and antibacterial assays of compounds **1**–**8** were carried out, which show moderate activities.

Introduction. – The genus *Xylaria* (family Xylariaceace) is rich in diverse natural products including terpenoids [1–5], cyclopeptides [6][7], polyketides [8][9], cytochalasins [10], xanthones [11][12], and unique unclassified xyloketals [13]. We isolated an endophytic fungal strain, named NCY2, from the medicinal plant *Torreya jackii* CHUN, collected from Jiangshi Nature Reserve Zone of Fujian Province, China, during November of 2004. This strain was determined to be *Xylaria* sp. based on its complete ITS1-5.8S-ITS2 gene sequences. Previously, three new sesquiterpenoids were isolated from the still suspension cultures of *Xylaria* sp. NCY2 in potato-dextrose (PD) medium [4]. Here, we report the isolation and structure elucidation of seven novel polyketides, *i.e.*, 1-(xylarenone A)xylariate A (**1**), xylarioic acid B (**2**), xylariolide A (**3**), xylariolide B (**4**), xylariolide C (**5**), methyl xylariate C (**6**), and xylariolide D (**7**), and the known polyketide taiwapyrone (**8**) [14] from this strain, together with the antitumor and antibacterial activities of compounds **1**–**8**[2]).

Results and Discussion. – 1. *Structure Elucidation.* The strain *Xylaria* sp. NCY2 was grown in still suspension PD medium at 28° and started to produce fruiting bodies after four weeks. However, at this culture stage, we were not able to isolate any natural products from the AcOEt extract of the fermentation broth. After two months of cultivation, the deer horn-shape fruiting bodies were full of the surface of fermentation medium, but the yield of natural products was still low. Therefore, we extended the fermentation period to six months according to the morphological change. The fermentation culture broths and mycelia of two different batches (two and six months,

[1]) Contributed equally to this work.

[2]) Arbitrary atom numbering. For systematic names, see *Exper. Part.*



resp.; see *Exper. Part*) were extracted successively with AcOEt and acetone, respectively. The crude extracts were purified by repeated column chromatography (*RP-18*, *Sephadex LH-20*, and SiO_2) to afford seven novel linear polyketides.

Compound **1** was obtained as a colorless oil. The molecular formula of **1** was determined to be $C_{26}H_{38}O_6$ according to the HR-Q-TOF-MS and NMR data. The IR absorption at 3447 cm^{-1} indicated the presence of OH groups. The ^{13}C-NMR and DEPT spectra of **1** (*Table 1*) exhibited 26 signals corresponding to six Me, five CH_2 (one bearing O-atom), eight CH (three bearing O-atom) groups, as well as seven

Table 1. *^1H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of* **1**. δ in ppm, J in Hz.

Xylarioyl A moiety			Xylarenone A moiety		
	δ(C)	δ(H)		δ(C)	δ(H)
C(1)	174.7 (*s*)		H–C(1′)	74.8 (*d*)	5.49 (br. *s*)
H–C(2)	43.6 (*d*)	2.63 (*dq*, J = 9.0, 7.2)	CH_2(2′)	31.0 (*t*)	2.06 (*dq*, J = 16.4, 3.0, H_β), 1.72 (*dq*, J = 16.4, 3.0, H_α)
H–C(3)	80.1 (*d*)	4.07 (*d*, J = 9.0)	CH_2(3′)	24.9 (*t*)	1.85 (*dq*, J = 16.4, 3.0, H_β), 1.56 (*dq*, J = 16.4, 3.0, H_α)
C(4)	132.5 (*s*)				
H–C(5)	136.4 (*d*)	5.21 (*d*, J = 9.5)	H–C(4′)	37.3 (*d*)	1.97 (*ddq*, J = 8.4, 6.6, 3.0)
H–C(6)	33.8 (*d*)	2.23–2.34 (*m*)	C(5′)	40.9 (*s*)	
CH_2(7)	30.0 (*t*)	1.20–1.26 (*m*), 1.29–1.35 (*m*)	H–C(6′)	69.2 (*d*)	3.40 (*s*)
			C(7′)	63.1 (*s*)	
Me(8)	11.0 (*q*)	0.86 (*t*, J = 7.4)	C(8′)	194.7 (*s*)	
Me–C(2)	14.2 (*q*)	1.00 (*d*, J = 7.2)	H–C(9′)	125.9 (*d*)	6.05 (*s*)
Me–C(4)	11.9 (*q*)	1.61 (*s*)	C(10′)	157.6 (*s*)	
Me–C(6)	20.5 (*q*)	0.92 (*d*, J = 6.7)	C(11′)	142.5 (*s*)	
			CH_2(12′)	116.4 (*t*)	5.32 (br. *s*, H_a), 5.40 (br. *s*, H_b)
			CH_2(13′)	63.8 (*t*)	4.35 (*d*, J = 13.5, H_a), 4.22 (*d*, J = 13.5, H_b)
			Me(14′)	18.6 (*q*)	1.32 (*s*)
			Me(15′)	15.8 (*q*)	1.15 (*d*, J = 6.7)

quaternary C-atoms, including two C=O groups. The presence of a xylarenone A [4] moiety in **1** was readily recognized by NMR-spectral comparison. The other C_{11} unit (*Fig. 1*) was determined to be 2,4,6-trimethyloct-4-enoyl moiety (named as xylarioyl A) by unambiguous NMR assignments with the aid of HSQC and HMBC experiments, particularly, the HMBCs from Me–C(2) to C(1), C(2) and C(3), from Me–C(4) to C(3), C(4), and C(5), from Me–C(6) to C(5), C(6), and C(7), and from Me(8) to C(6) and C(7). The configuration of C=C between C(4) and C(5) was determined to be (*E*) by the NOE correlations between H–C(3) and H–C(5). Thus, the structure of compound **1** was established to be 1-(xylarenone A)xylariate A.

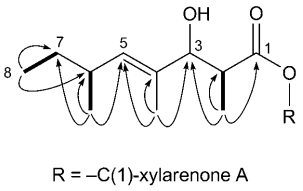


Fig. 1. *Selected HMBCs* (H → C) *and* $^1H,^1H$*-COSY correlations* (—) *of compound* **1**

The 2,4,6-trimethyloct-4-enoyl residue (named as xylarioyl A) of compound **1** is structurally interesting. This structure could be classified to be a tetraketide. Aromatic tetraketides, exemplified by 6-methylsalicylic acid [15][16], are not rare in fungi. However, linear tetraketides such as xylarioyl A are unusual. Hemibourgeanic acid, a structural unit of bourgeanic acid isolated from lichens, is a rare example [17]. Indeed, xylarioyl A is 4,5-didehydrohemibourgeanoyl. This led us to investigate whether this strain produced more structurally related polyketides. During the follow-up isolation process, we used an approach based on column chromatography over *Sephadex LH-20* with acetone. This method allowed us to trace relatively smaller molecules specifically, resulting in the isolation of compounds **2**–**8**.

Compound **2** was obtained as a colorless oil. The molecular formula was determined to be $C_{11}H_{22}O_5$ on the basis of the HR-Q-TOF-MS and NMR data. The IR spectrum exhibited absorptions at 3408 cm^{-1} for OH groups. The NMR data of **2** were similar to those of xylarioyl A (*Tables 2* and *3*), except that **2** contained two more O-bearing C-atoms (δ(C) 91.8 (C(4)) and 79.0 (C(5))) and no C=C group, indicating the adjacent trihydroxy substitution. Thus, compound **2** was determined to be 3,4,5-trihydroxy-2,4,6-trimethyloctanoic acid, named as xylarioic acid B.

Compound **3** was obtained as a colorless oil. The IR spectrum exhibited absorptions at 3354 cm^{-1} for OH groups. The ^1H- and ^{13}C-NMR spectra (*Tables 2* and *3*) along with the DEPT and HMQC experiments revealed the signals of four Me, one CH_2, four CH groups, and two quaternary C-atoms, including an ester C=O group (δ(C) 178.6 (C(1))), revealing a similar structure as compound **2**. The molecular formula of **3** was determined to be $C_{11}H_{20}O_4$ on the basis of the positive-ion-mode HR-Q-TOF-MS and NMR data. This corresponded to a structure with one molecular H_2O less than compound **2**, indicating that **3** was a lactone. Moreover, the ^1H-NMR spectrum of **3** was similar to that of compound **2** (*Table 2*), excluding the possibility of a β- or δ-lactone which would induce downfield shift of the H-atoms (H–C(3) or H–C(5)) at acyloxy-

Table 2. *^{1}H-NMR Data* (500 MHz, $CDCl_3$) *of* **2**–**4**, **4a**, *and* **5a**. δ in ppm, J in Hz.

H-Atom	**2**	**3**	**4**	**4a**	**5a**
H–C(2)	3.02 (*quint.*, $J=7.1$)	3.03 (*quint.*, $J=7.4$)	3.00 (*quint.*, $J=7.5$)	3.13 (*dq*, $J=7.2, 7.3$)	3.15 (*quint.*, $J=7.0$)
H–C(3)	4.44 (*d*, $J=7.1$)	4.57 (*d*, $J=7.4$)	4.74 (*d*, $J=7.5$)	5.72 (*d*, $J=6.4$)	5.31 (*d*, $J=6.5$)
H–C(5)	3.37 (*d*, $J=5.2$)	3.46 (*d*, $J=5.6$)	3.45 (*s*)	4.94 (*s*)	4.82 (*d*, $J=9.2$)
H–C(6)	1.56–1.62 (*m*)	1.56–1.62 (*m*)	–	–	2.01–2.05 (*m*)
CH_2(7) or CH(7)	1.19–1.21 (*m*), 1.56–1.62 (*m*)	1.19–1.21 (*m*), 1.56–1.62 (*m*)	1.63 (*dq*, $J=15.0, 7.5$), 1.78 (*dq*, $J=15.0, 7.5$)	1.50 (*q*, $J=7.5$)	5.05 (*dq*, $J=6.5, 1.8$)
Me(8)	0.88 (*t*, $J=7.4$)	0.94 (*t*, $J=7.4$)	0.93 (*t*, $J=7.5$)	0.91 (*t*, $J=7.5$)	1.21 (*d*, $J=6.5$)
Me–C(2)	1.18 (*d*, $J=7.1$)	1.27 (*d*, $J=7.4$)	1.30 (*d*, $J=7.5$)	1.17 (*d*, $J=6.4$)	1.16 (*d*, $J=6.5$)
Me–C(4)	1.33 (*s*)	1.39 (*s*)	1.52 (*s*)	1.51 (*s*)	1.34 (*s*)
Me–C(6)	0.96 (*d*, $J=6.8$)	1.02 (*d*, $J=6.8$)	1.31 (*s*)	1.31 (*s*)	1.13 (*d*, $J=7.0$)
AcO–C(3)	–	–	–	2.13 (*s*)	2.12 (*s*)
AcO–C(5)	–	–	–	2.17 (*s*)	2.10 (*s*)
AcO–C(7)	–	–	–	–	1.97 (*s*)

Table 3. *^{13}C-NMR Data* (125 MHz, $CDCl_3$) *of* **2**–**4**, **4a**, *and* **5a**. δ in ppm, J in Hz.

C-Atom	**2**	**3**	**4**	**4a**	**5a**
C(1)	179.8 (*s*)	178.6 (*s*)	179.9 (*s*)	176.7 (*s*)	176.3 (*s*)
C(2)	40.7 (*d*)	40.2 (*d*)	40.1 (*d*)	38.3 (*d*)	38.1 (*d*)
C(3)	71.7 (*d*)	72.7 (*d*)	71.5 (*d*)	74.0 (*d*)	73.7 (*d*)
C(4)	91.8 (*s*)	90.3 (*s*)	90.9 (*s*)	87.1 (*s*)	86.9 (*s*)
C(5)	79.0 (*d*)	79.8 (*d*)	76.7 (*d*)	76.6 (*d*)	73.6 (*d*)
C(6)	36.5 (*d*)	36.6 (*d*)	74.5 (*s*)	74.7 (*s*)	37.8 (*t*)
C(7)	23.8 (*t*)	24.1 (*t*)	34.0 (*t*)	33.9 (*t*)	68.7 (*s*)
C(8)	11.3 (*q*)	11.2 (*q*)	8.0 (*q*)	7.8 (*q*)	17.4 (*q*)
Me–C(2)	8.9 (*q*)	9.3 (*q*)	9.1 (*q*)	8.7 (*q*)	8.7 (*q*)
Me–C(4)	16.7 (*q*)	16.6 (*q*)	18.1 (*q*)	18.5 (*q*)	15.2 (*q*)
Me–C(6)	16.9 (*q*)	16.9 (*q*)	22.3 (*q*)	22.5 (*q*)	10.8 (*q*)
*Ac*O–C(3)	–	–	–	20.4 (*q*), 169.9 (*s*)	20.4 (*q*), 169.7 (*s*)
*Ac*O–C(5)	–	–	–	20.7 (*q*), 170.1 (*s*)	20.7 (*q*), 170.5 (*s*)
*Ac*O–C(7)	–	–	–	–	21.2 (*q*), 170.7 (*s*)

substituted C-atoms. The relative configuration of **3** were established from the NOE spectra. The presence of NOE correlations H–C(2) ↔ H–C(3) ↔ H–C(5) ↔ H–C(2) indicated that Me–C(2) and Me–C(4) were in β-orientation, while H–C(3) was α-oriented (*Fig. 2*). Therefore, compound **3** was determined to be (3*R**,4*S**,5*R**)-4,5-dihydro-4-hydroxy-5-(1-hydroxy-2-methylbutyl)-3,5-dimethylfuran-2(3*H*)-one, named xylariolide A.

Compound **4** was obtained as a colorless oil. The molecular formula was determined to be $C_{11}H_{20}O_5$ on the basis of the positive-ion-mode HR-Q-TOF-MS and NMR data. The IR spectrum exhibited an absorption at 3361 cm^{-1} for OH groups. The ^{1}H- and ^{13}C-NMR spectra (*Tables 2* and *3*) along with the DEPT and HMQC experiments

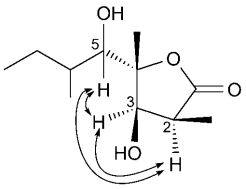


Fig. 2. *Selected NOE correlations of compound* **3** (H ↔ H)

revealed the signals of four Me, one CH_2, three CH groups, and three quaternary C-atoms, including an ester C=O group (δ(C) 179.9 (C(1))), similar to those of compound **3** except that **4** contained one more O-bearing quaternary C-atom (δ(C) 74.5 (C(6))), indicating a OH substitution at C(6). The relative configuration of **4** was determined on the basis of the same NOE correlations as those of **3**. Therefore, compound **4** was elucidated as (3*R**,4*S**,5*S**)-5-(1,2-dihydroxy-2-methylbutyl)-4,5-dihydro-4-hydroxy-3,5-dimethylfuran-2(3*H*)-one, named xylariolide B.

Compound **5** was obtained as a colorless oil. Inspection of the ^{1}H-NMR spectrum of **5** indicated that it was not a single compound. Before further purification, the mixture was acetylated with Ac_2O and pyridine at room temperature to afford two acetylated compounds, **4a** and **5a**, which were separated by repeated column chromatography over *Sephadex LH-20* with AcOEt. The molecular formula of compound **4a** was determined to be $C_{15}H_{24}O_7$ on the basis of the positive-ion-mode HR-Q-TOF-MS and NMR data (*Tables 2* and *3*), indicating the presence of two more Ac groups than in **4**. The ^{1}H-NMR spectrum of **4a** was similar to that of **4**, except that the chemical shifts of H–C(3) and H–C(5) were downfield-shifted from δ(H) 4.74 to 5.72, and from 3.45 to 4.94, respectively, revealing the locations of the two AcO groups at C(3) and C(5). Therefore, compound **4a** was determined as 1-[(2*R**,3*R**,4*S**)-3-(acetyloxy)-2,4-dimethyl-5-oxotetrahydrofuran-2-yl]-2-hydroxy-2-methylbutyl acetate, named 3,5-di-acetyl xylariolide B.

The molecular formula of compound **5a** was determined to be $C_{17}H_{26}O_8$ on the basis of the positive-ion-mode HR-Q-TOF-MS and NMR data (*Tables 2* and *3*), indicating the presence of one more Ac group than in **4a**. The ^{1}H-NMR spectrum of **5a** was similar to that of **4a**, except that H–C(7) appeared at δ(H) 5.05, indicating an AcO substitution at C(7). The relative configurations of **4a** and **5a** were determined on the basis of the same NOE correlations as those for **3** (*Fig. 2*). Accordingly, compound **5** was determined as (3*S**,4*R**,5*S**)-5-(1,3-dihydroxy-2-methylbutyl)-4,5-dihydro-4-hydroxy-3,5-dimethylfuran-2(3*H*)-one, named xylariolide C.

The relative configurations of the acyclic part of compounds **1**–**5**, **4a**, and **5a** have not been established yet, and will demand further work, *e.g.*, by modified *Mosher*'s method as described for polyketides in [18][19].

Compound **6** was isolated as a colorless oil. The molecular formula was determined to be $C_{11}H_{16}O_3$ according to the positive-ion-mode HR-Q-TOF-MS and NMR data. The ^{13}C-NMR and DEPT spectra (*Table 4*) of **6** exhibited eleven signals, corresponding to three Me (one bearing O-atom), two CH_2, three olefinic CH groups, and three quaternary C-atoms, including two C=O and one olefinic one. The HMBCs from MeO to C(1), and from Me(2′) to C(1′) and C(4), from Me(8) to C(6) and C(7), from H–C(5) to C(3), C(4), C(1′), C(6) and C(7), and from H–C(3) to C(1) and C(2), led

to the establishment of the planar structure of **6**. The configuration of C=C between C(2) and C(3) was identified as (*E*) according to the coupling constant between H–C(2) and H–C(3), while the configuration of C=C between C(4) and C(5) was determined as (*Z*) according to NOE correlations between H–C(3) and H–C(5). Thus, compound **6** was determined to be methyl (2*E*,4*Z*)-4-acetylocta-2,4-dienoate, named methyl xylariate C.

Table 4. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **6**–**8**. δ in ppm, *J* in Hz.

Position	**6**[a])		**7**[a])		**8**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		167.5 (*s*)		162.8 (*s*)		163.6 (*s*)
H–C(2)	6.42 (*d*, *J* = 7.1)	123.7 (*d*)	6.22 (*d*, *J* = 7.1)	113.7 (*d*)	6.34 (*d*, *J* = 7.1)	115.7 (*d*)
H–C(3)	7.49 (*d*, *J* = 7.1)	136.3 (*d*)	7.52 (*d*, *J* = 7.1)	143.7 (*d*)	7.68 (*d*, *J* = 7.1)	145.2 (*d*)
C(4)		136.9 (*s*)		118.6 (*s*)		121.2 (*s*)
H–C(5)	6.82 (*t*, *J* = 5.2)	149.1 (*d*)	4.65 (*t*, *J* = 5.2)	68.1 (*d*)	4.47 (*t*)	67.2 (*d*)
CH_2(6)	1.57 (*q*, *J* = 9.5)	22.2 (*t*)	1.45–1.53 (*m*), 1.72–1.78 (*m*)	39.3 (*t*)	1.51–1.58 (*m*), 1.73–1.78 (*m*)	39.9 (*t*)
CH_2(7)	2.39–2.42 (*m*)	31.1 (*t*)	1.25–1.29 (*m*), 1.35–1.39 (*m*)	18.7 (*t*)	1.30–1.33 (*m*), 1.44–1.47 (*m*)	19.3 (*t*)
Me(8)	0.99 (*t*, *J* = 7.4)	13.8 (*q*)	0.95 (*t*, *J* = 7.4)	13.8 (*q*)	0.95 (*t*, *J* = 7.4)	13.6 (*q*)
C(1′)		198.6 (*s*)		158.4 (*s*)		159.3 (*s*)
C(2′)	2.33 (*s*)	27.2 (*q*)	2.26 (*s*)	17.0 (*q*)	4.42 (*s*)	58.4 (*t*)
MeO	3.76 (*s*)	51.7 (*q*)				

[a]) In $CDCl_3$. [b]) In CD_3OD.

Compound **7** was obtained as a colorless oil. The molecular formula was determined to be $C_{10}H_{14}O_3$ according to the positive-ion-mode HR-Q-TOF-MS and NMR data (*Table 4*). The NMR data of **7** were similar to those of **8**, identified as taiwapyrone (= 5-(1-hydroxybutyl)-6-(hydroxymethyl)-2*H*-pyran-2-one), by comparison with literature data [14]. However, a Me group (δ(C) 17.0) in **7** replaced the CH_2OH (δ(C) 58.4) in **8**. Accordingly, compound **7** was determined to be 5-(1-hydroxybutyl)-6-methyl-2*H*-pyran-2-one, named xylariolide D.

2. *Biological Studies.* Compounds **1**–**8** were tested in antitumor and antimicrobial assays *in vitro*. In the antitumor assay, compounds **1**–**8** exhibited weak activities against HepG2 and HeLa cells at a single concentration 10 μg/ml, the inhibitory rates were less than 30% relative to vehicle control. In the antibacterial assay, compounds **1**–**8** inhibited the growth of pathogenic bacteria *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 9372, and *Staphylococcus aureus* ATCC 25923 with *MIC* values above 10 μg/ml, but had no effects on the growth of yeasts (*Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* As 2.538) at a concentration of 10 μg/ml.

The genus of *Xylaria* is known as a rich source of natural products including polyketides, terpenoids, cyclopeptides, and unique unclassified xyloketals. Among those compounds, one of the terpenoids, sordarin [20], is particularly interesting because of its significant antifungal activity *via* inhibiting elongation factor of fungal protein synthesis [21]. The strain *Xylaria* sp. NCY2 was shown to be rich in terpenoids by the isolation of three new sesquiterpenoids belonging to two structural types,

eremophiladiene and brasiladiene [4]. This report of seven new linear polyketides from *Xylaria* sp. NCY2 presents the first isolation of linear polyketides from this genus. Overall, our work, together with previous reports, indicates that further efforts are worth pursuing to exploit the potential of this genus for the discovery of more bioactive natural products.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 and 80–100 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), SiO_2 GF_{254} (*Merck*), *RP-18* (*Merck*), and *Sephadex LH-20* (*Amersham Biosciences*) were used. TLC: Precoated SiO_2 GF_{254} plates (0.20–0.25 mm, *Qingdao Marine Chemical Factory*). Optical rotations: *Perkin-Elmer 341* polarimeter with $CHCl_3$ as solvent. UV Spectra: *Varian Cary 50* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet FT-IR 360* in KBr; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DRX-500* spectrometer, at 500 (^1H), and 125 (^{13}C) MHz; in $CDCl_3$; δ in ppm rel. to Me_4Si, J in Hz. HR-Q-TOF-MS: *Bruker Daltonics BioTOF-Q* mass spectrometer; in *m*/*z* (rel. %).

Isolation and Fermentation of the Fungal Strain. The fungus strain NCY2 was isolated from *Torreya jackii* CHUN collected during November of 2004 from Jiangshi Nature Reserve Zone of Fujian Province, China, by the hyphal tip method. The strain NCY2 shows a white mycelial growth on the surface of PDA plates, and the main character of it is the emergence of black club stromas around the center of the colony 7 d after inoculation. This strain was identified as *Xylaria* sp. by partial sequence analysis of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rDNA gene. A nucleotide-to-nucleotide BLAST query of the NCBI database yielded *Xylaria* sp. TP5BS72 as the closest match to the ITS rDNA of NCY2 (99%). The mycelium of NCY2 grown on PDA plates was used to inoculate in 1-l *Erlenmeyer* flasks containing 200 ml of PD medium (potato 200 g/l, glucose 20 g/l, natural pH). The flasks were incubated on a rotary shaker for 5 d at 28° with shaking at 160 rpm to afford seed cultures. The seed cultures were transferred into flasks (20 l) containing 5 l of PD medium and cultivated in still at 28°. These fermentations were performed twice. During the first time, 83 l were cultivated for six months, and the second time, 36 l for two month.

Extraction and Isolation. All fermentation broths were processed by the same procedure and filtered with four layers of cheese cloth to produce two parts, *i.e.*, mycelium and filtrate; the filtrates, which were concentrated to 20 l and 3 l for each fermentation, resp. The filtrates were extracted exhaustively with equal volume of AcOEt while the mycelia were extracted three times with acetone at r.t. to afford *CY1* (34.7 g; filtrate of six months fermentation), *CY2* (17.2 g; mycelia of six months fermentation), *CY3* (7.1 g; two months filtrate), and *CY4* (13.3 g; two months mycelia), resp.

CY1 (34.7 g) was divided into two parts and subjected to MPLC (*RP-18* (170 g); gradient aq. acetone (30, 50, and 70%, resp., 2 l each)). All eluants were pooled on the basis of TLC analysis into three fractions *CY1A–CY1C*. *CY1A* (4.78 g) was subjected to MPLC (*RP-18* (170 g); acetone/H_2O 10:90, 20:80, and 30:70 (2 l each)), and pooled into five fractions *CY1A-1–CY1A-5* according to TLC analysis. *CY1A-1* (831 mg) was subjected to CC (*Sephadex LH-20* (140 g); MeOH) to produce fraction *CY1A-1-1* which was further purified by CC (SiO_2 (8 g); petroleum ether (PE)/AcOEt 10:1) to afford **2** (140 mg). *CY1A-2* (499 mg) was subjected to CC (*Sephadex LH-20* (140 g); MeOH), and further purified by CC (SiO_2 (10 g); PE/AcOEt 30:1, 20:1, and 10:1) to give **6** (7 mg). *CY1A-3* (672 mg) was subjected to CC (*Sephadex LH-20* (140 g); MeOH), and further purified by recrystallization in MeOH to produce **3** (23 mg). *CY1B* (4.1 g) was subjected to CC (*Sephadex LH-20* (140 g); MeOH) to afford four fractions: *CY1B-1* (1.129 g), *CY1B-2* (920 mg), *CY1B-3* (424 mg), and *CY1B-4* (185 mg). *CY1B-2* (920 mg) was subjected to MPLC (*RP-18* (80 g); acetone/H_2O 20:80 and 30:70 (1 l each)) to afford four fractions: *CY1B-2-1* (32 mg), *CY1B-2-2* (83 mg), *CY1B-2-3* (149 mg), and *CY1B-2-4* (197 mg). *CY1B-2-1* (32 mg)

was then purified by CC (*Sephadex LH-20* (68 g); acetone) and finally purified by CC (SiO_2 (800 mg); $CHCl_3$/MeOH 200 : 1 and 100 : 1) to afford **4** (4.5 mg).

CY2 (17.2 g) was partitioned between PE and MeOH, and the MeOH extract was subjected to MPLC (*RP-18* (170 g); gradient aq. acetone (30, 50, and 70%, resp., 2 l each)) to produce nine fractions *CY2A – CY2I*. *CY2D* (970 mg) was purified by CC (*Sephadex LH-20* (140 g); twice eluted with MeOH and acetone, resp.), subjected to MPLC (*RP-18* (30 g); acetone/H_2O 53 : 47), then further purified by chromatography (*Sephadex LH-20* (40 g); twice MeOH and AcOEt, resp.), and finally purified by CC (SiO_2 (600 mg); PE/AcOEt 20 : 1, 15 : 1, and 10 : 1) to yield **1** (7.5 mg).

CY3 (7.1 g) was subjected to MPLC (*RP-18* (170 g); gradient aq. acetone (30, 50, and 70%, resp., 2 l each)) to afford seven fractions *CY3A – CY3G*. *CY3A* (546 mg) was purified by several chromatographies (*Sephadex LH-20* (140 g); MeOH and acetone), then subjected to MPLC (*RP-18* (30 g); MeOH/H_2O 5 : 95), further purified by CC on SiO_2 (200 mg) eluted with $CHCl_3$/MeOH 15 : 1, and finally purified by chromatography (*Sephadex LH-20* (40 g); AcOEt) to yield a mixture **4**/**5** (22 mg), which was acetylated with Ac_2O in the presence of pyridine. The acetylated mixture of **4** and **5** was purified by several chromatographies (*Sephadex LH-20* (40 g); AcOEt) to give **4a** (7 mg) and **5a** (8 mg). *CY3B* (2.7 g) was subjected to CC (*Sephadex LH-20* (140 g); MeOH) to afford two fractions *CY3B-1* and *CY3B-2*. *CY3B-1* (1.1 g) was purified by repeated CC (*Sephadex LH-20* (200 g, 68 g); acetone) and MPLC (*RP-18*; (30 g); gradient aq. MeOH) and then further purified by CC (SiO_2 (2.5 g); PE/AcOEt 20 : 1, 15 : 1, and 10 : 1) to yield **7** (53 mg). *CY3B-2* (428 mg) was subjected to CC (*Sephadex LH-20* (200 g); acetone) and then subjected to MPLC (*RP-18* (30 g); MeOH/H_2O 15 : 85, 25 : 75, and 35 : 65), subjected CC (*Sephadex LH-20* (68 g); acetone), and finally purified by CC (SiO_2 (4 g); $CHCl_3$/AcOEt 10 : 1) to yield **8** (100 mg).

1-(Xylarenone A)xylariate A (= *(1a*R,*4*R,*7*S,*7a*R,*7b*R*)-1a,2,4,5,6,7,7a,7b-Octahydro-1a-[1-(hydroxymethyl)ethenyl]-7,7a-dimethyl-2-oxonaphth[1,2-b]oxiren-4-yl (4*E*)-3-Hydroxy-2,4,6-trimethyloct-4-enoate*; **1**). Colorless oil. $[\alpha]_D^{20} = +135.3$ ($c = 0.744$, $CHCl_3$). IR: 3447, 2961, 2932, 2875, 1732, 1678, 1458, 1379, 1166, 1019, 880, 756. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-Q-TOF-MS: 469.3442 ($[M+Na]^+$, $C_{26}H_{38}NaO_6^+$; calc. 469.2566).

Xylarioic Acid B (= *3,4,5-Trihydroxy-2,4,6-trimethyloctanoic Acid*; **2**). Colorless oil. $[\alpha]_D^{20} = -2.98$ ($c = 0.054$, $CHCl_3$). IR: 3408, 2965, 2939, 1747, 1223, 1037, 992, 948. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. HR-Q-TOF-MS: 257.1467 ($[M+Na]^+$, $C_{11}H_{22}NaO_5^+$; calc. 257.1365).

Xylariolide A (= *(3*R*,4*S*,5*R*)-4,5-Dihydro-4-hydroxy-5-(1-hydroxy-2-methylbutyl)-3,5-dimethylfuran-2(3*H*)-one*; **3**). Colorless oil. $[\alpha]_D^{20} = +7.55$ ($c = 0.054$, $CHCl_3$). IR: 3354, 2970, 1750, 1061, 985. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. HR-Q-TOF-MS: 239.1367 ($[M+Na]^+$, $C_{11}H_{20}NaO_4^+$; calc. 239.1259).

Xylariolide B (= *(3*R*,4*S*,5*S*)-4,5-Dihydro-5-(1,2-dihydroxy-2-methylbutyl)-4-hydroxy-3,5-dimethylfuran-2(3*H*)-one*; **4**). Colorless oil. $[\alpha]_D^{20} = +8.3$ ($c = 0.025$, $CHCl_3$). IR: 3361, 2973, 1751, 1076, 988. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. HR-Q-TOF-MS: 255.2637 ($[M+Na]^+$, $C_{11}H_{20}NaO_5^+$; calc. 255.1208).

Acetyl Xylariolide B (= *1-[(2*R*,3*R*,4*S*)-3-(Acetyloxy)-2,4-dimethyl-5-oxotetrahydrofuran-2-yl]-2-hydroxy-2-methylbutyl Acetate*; **4a**). Colorless oil. $[\alpha]_D^{20} = +31.1$ ($c = 0.7$, $CHCl_3$). IR: 3497, 2981, 2940, 1781, 1749, 1374, 1227, 1034. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. HR-Q-TOF-MS: 339.1464 ($[M+Na]^+$, $C_{15}H_{24}NaO_7^+$; calc. 339.1420).

Acetyl Xylariolide C (= *1-[(2*R*,3*S*,4*R*)-3-(Acetyloxy)-2,4-dimethyl-5-oxotetrahydrofuran-2-yl]-2-methylbutane-1,3-diyl Diacetate*; **5a**). Colorless oil. $[\alpha]_D^{20} = +32.0$ ($c = 0.054$, $CHCl_3$). IR: 1788, 1764, 1373, 1234, 1024. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*, resp. HR-Q-TOF-MS: 381.1611 ($[M+Na]^+$, $C_{17}H_{26}NaO_8^+$; calc. 381.1525).

Methyl Xylariate C (= *Methyl (2*E*,4*Z*)-4-Acetylocta-2,4-dienoate*; **6**). Colorless oil. $[\alpha]_D^{20} = +0.43$ ($c = 0.054$, $CHCl_3$). UV (MeOH): 241 (3.03), 243 (3.04), 247 (2.99). IR: 1727, 1705, 1436, 1015, 950. ^{1}H- and ^{13}C-NMR: see *Table 4*. HR-Q-TOF-MS: 219.2307 ($[M+Na]^+$, $C_{11}H_{16}NaO_3^+$; calc. 219.0997).

Xylariolide D (= *5-(1-Hydroxybutyl)-6-methyl-2*H*-pyran-2-one*; **7**). Colorless oil. $[\alpha]_D^{20} = -27.5$ ($c = 0.035$, $CHCl_3$). UV (MeOH): 301 (4.18). IR: 3433, 2960, 2932, 2873, 1736, 1714, 1557, 1296, 829. ^{1}H- and ^{13}C-NMR: see *Table 4*. HR-Q-TOF-MS: 205.1797 ($[M+Na]^+$, $C_{10}H_{14}NaO_3^+$; calc. 205.0841).

Taiwapyrone (= *5-(1-Hydroxybutyl)-6-(hydroxymethyl)-2*H-*pyran-2-one*; **8**). Colorless oil. $[\alpha]_D^{20} = -1.5$ ($c = 0.015$, $CHCl_3$). IR: 3386, 2960, 2933, 2872, 1710, 1637, 1559, 1025, 833. ^{1}H- and ^{13}C-NMR: see *Table 4*. HR-Q-TOF-MS: 221.0778 ($[M+Na]^+$, $C_{10}H_{14}NaO_4^+$; calc. 221.0790).

Biological Studies. Cytotoxicities of compounds **1**–**8** were investigated using the human cancer cell lines HepG2 and HeLa, following the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) standards [22], and cisplatin (DDP) was used as a positive control in this experiment. The antibacterial activities of **1**–**8** were tested against bacteria (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 9372, and *Staphylococcus aureus* ATCC 25923) and yeasts (*Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* As 2.538), using a similar *MIC* method with 96-well microplates [23]. Three replicates were performed for each compound at a concentration of 10 μg/ml.

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