Furanone Derivatives from *Aspergillus* sp. XW-12, an Endophytic Fungus in *Huperzia serrata*

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Two new 5-methoxyfuran-3(2*H*)-one derivatives, huaspenone A (**1**) and B (**2**), together with four known ones, *i.e.*, aspertetronin A (**3**), aspertetronin B (**4**) , gregatin E (**5**), and penicilliol A (**6**), were isolated from the cultures of an endophytic fungus *Aspergillus* sp. XW-12, derived from the stems of *Huperzia serrata*. The structures of the new isolates were established by detailed interpretation of the 1D- and 2D-NMR and HR-ESI-MS data.

Introduction. – The term endophyte refers to a bacterial or a fungal microorganism that colonizes interior organs of plants but does not have pathogenic effects on its host(s) [1]. As living in the special eco-environment, the endophytic fungi seem to be especially prolific producers of biologically active small molecules with which they probably regulate their interactions with hosts and competitors [2–9]. For the past few years, we have been engaged in the chemical investigation of endophytic fungi of *Huperzia serrata* [10], a well-known traditional Chinese herb medicine rich in lycopodium alkaloids. As a part of our ongoing program, we report the isolation and structural elucidation of the 5-methoxyfuran-3(2*H*)-one derivatives **1**–**6**[1]), of which **1** and **2** are new, from the cultures of an endophytic fungus *Aspergillus* sp. XW-12 (*Fig. 1*), which was isolated from the stems of *Huperzia serrata*. Here, we describe the isolation and structural elucidation of these compounds.

Results and Discussion. – The molecular formula of **1** was established as $C_{17}H_{24}O_6$ by HR-ESI-MS (*m*/*z* 325.1648 ($[M+H]^+$)), implying the presence of six degrees of unsaturation, in accordance with the ^{1}H- and ^{13}C-NMR data (*Table*). The IR absorption bands revealed the presence of one OH and two ketone functionalities. The latter were confirmed by ^{13}C-NMR signals at δ(C) 197.9 and 196.3. The ^{1}H-NMR spectrum of **1** showed the signals of three Me groups at δ(H) 1.27 (*d*, $J = 6.4$ Hz, Me(13), Me(17)) and 1.54 (*s*, Me(6)), and of two MeO groups at δ(H) 3.34 and 3.83. The presence of a pair of conjugated C=C bonds was easily established by the corresponding chemical shifts and coupling constants (δ(H) 5.67 (*d*, $J = 15.4$ Hz), 6.30 (*dd*, $J = 15.4$, 10.6 Hz), 6.16 (*dd*, $J = 15.2$, 10.6 Hz), and 5.79 (*d*, $J = 15.1$, 6.3 Hz)). In addition, two O-bearing CH groups at δ(H) 3.84–3.86, and 4.32–4.38 were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR and DEPT spectra revealed signals of 17 C-

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

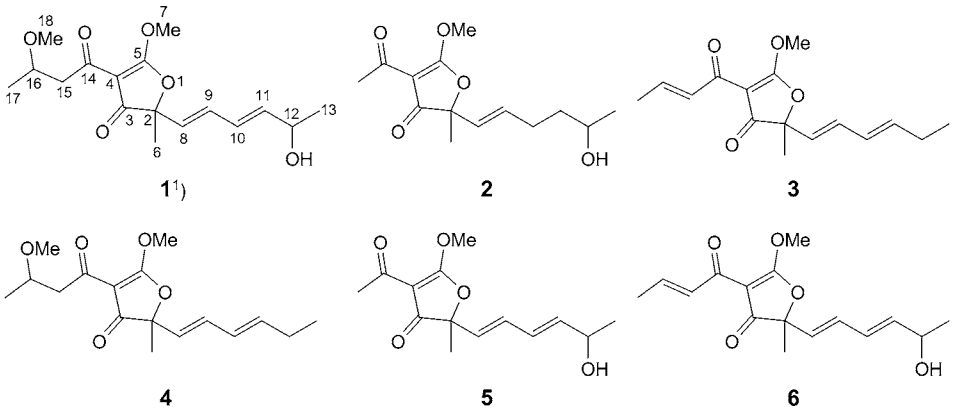

Fig. 1. *Compounds* **1**–**6**[1]) *isolated from* Aspergillus *sp. XW-12*

atoms, including five Me, one CH_2, and six CH groups, and five quaternary C-atoms. The data mentioned above showed that compound **1** was a 5-methoxyfuran-3(2*H*)-one derivative [11]. Analysis of the ^{1}H- and ^{13}C-NMR and HMQC spectra of **1** enabled us to assign all the H-atoms to the corresponding C-atoms. Two partial structures **a** (C(8) to C(13)) and **b** (C(15) to C(17)) were established by analysis of its 2D-NMR spectra, especially the ^{1}H,^{1}H-COSY data (*Fig. 2*). The assemblage of all C-atoms, including quaternary C-atoms and hetero atoms was mainly achieved by an HMBC experiment

Table. *^{1}H- and ^{13}C-*NMR *Data* (**1** in $CDCl_3$, **2** in (D_6)acetone, 500 and 125 MHz, resp.) *of* **1**–**2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)	–	91.2	–	91.4
C(3)	–	197.9	–	198.2
C(4)	–	107.0	–	106.7
C(5)	–	163.3	–	163.4
Me(6)	1.54 (*s*)	19.6	1.51 (*s*)	22.2
Me(7)	3.83 (*s*)	51.6	3.83 (*s*)	51.6
H–C(8)	5.67 (*d*, *J* = 15.4)	127.7	5.50 (*d*, *J* = 17.0)	126.0
H–C(9)	6.30 (*dd*, *J* = 15.4, 10.6)	130.7	5.83 (*dt*, *J* = 15.8, 6.8)	132.9
H–C(10) or CH_2(10)	6.16 (*dd*, *J* = 15.2, 10.6)	128.2	2.12–2.19 (*m*)	28.6
H–C(11) or CH_2(11)	5.79 (*dd*, *J* = 15.1, 6.3)	139.8	1.51–1.56 (*m*)	38.0
H–C(12)	4.32–4.38 (*m*)	68.2	3.76–3.80 (*m*)	67.4
Me(13)	1.27 (*d*, *J* = 6.4)	22.4	1.19 (*d*, *J* = 6.5)	23.6
C(14)	–	196.3	–	195.5
CH_2(15) or Me(15)	3.27 (*dd*, *J* = 13.6, 7.8), 3.11 (*dd*, *J* = 13.6, 5.2)	38.2	2.64 (*s*)	17.9
H–C(16)	3.84–3.86 (*m*)	74.6	–	–
Me(17)	1.27 (*d*, *J* = 6.4)	19.5	–	–
Me(18)	3.34 (*s*)	56.4	–	–

(*Fig. 2*). The connectivity of C(15) and C(4) *via* C(14) was deduced from the HMBCs CH_2(15)/C(4) and CH_2(15)/C(14). The attachment of the moiety **a** to C(2) was deduced from the correlations H–C(8)/C(2) and H–C(9)/C(2). The HMBC Me(18)/C(16) was indicative of the presence of an MeO group at C(16). An OH group was located at C(12) based on the molecular formula and the chemical shift of C(12) (δ(C) 68.2). This was additionally supported by the HMBCs H–C(12)/C(11) and H–C(12)/C(10). The large vicinal coupling constants clearly indicated that the C(8)=C(9) and C(10)=C(11) bonds had (*E*)-configuration. Thus, the structure of huaspenone A[1]) (**1**) was established, with the configuration at C(2), C(12), and C(16) not determined.

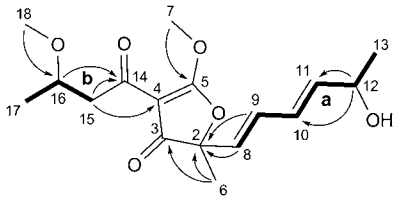

Fig. 2. *Key HMBC* (H → C) *and COSY* (—) *features of* **1**[1])

Compound **2** was isolated as yellow oil. It had the molecular formula $C_{14}H_{20}O_5$ as suggested, by the HR-ESI-MS (*m*/*z* 269.1386 ($[M+H]^+$)). The NMR data of **2** were very similar to those of **1**, indicating that they shared the same molecular skeleton. A major difference was that the downfield NMR signals of CH(10) and CH(11) of **1** were absent in the spectra of **2** (*Table*). Instead of those, two upfield CH_2 signals were observed in the NMR spectra of **2**, which suggested that the C(10)=C(11) bond of **1** was saturated in **2**. The correlations H–C(9)/CH_2(10) and CH_2(10)/CH_2(11) in the ^{1}H,^{1}H-COSY plot confirmed the above conclusion. Besides, the correlations CH_2(11)/H–C(12) and H–C(12)/Me(13) were also found in the ^{1}H,^{1}H-COSY plot; thus the structure of the side chain at C(2) was established. The Me group at δ(H) 2.64 (Me(15)) was placed at the terminus of the other side chain, next to the C(14)=O group, which was confirmed by the HMBC Me(15)/C(14). Therefore, huaspenone B was deduced to have structure **2** with the configuration at C(2) and C(12) not determined.

The four known compounds were identified as aspertetronin A (**3**) [12–14], aspertetronin B (**4**) [12][13], gregatin E (**5**) [13][15], and penicilliol A (**6**) [11] by comparison of their spectroscopic data with literature values.

This series of closely related 5-methoxyfuran-3(2*H*)-one derivatives belongs to the polyketide type metabolites. Aspertetronin A (**3**) and aspertetronin B (**4**) were firstly reported in 1969 [12][13]. Gregatin A (the enantiomer of **3**) and gregatins C and D, which were also 5-methoxyfuran-3(2*H*)-one derivatives, can cause wilting, death of leaves, and vascular browning of adzuki-bean and mung-bean cuttings, and had also an inhibitory action on a wide range of fungi and bacteria in antibiotic tests [15][16]. Besides, recent research revealed that a class of 5-methoxyfuran-3(2*H*)-one derivatives including penicilliol A (**6**) exhibited selective inhibitory activity on eukaryotic Y-family DNA polymerases, a target for antitumor and anti-HIV [11].

Experimental Part

General. All solvents used were of anal. grade (*Hangzhou Gaojing Fine Chemical Plant*). TLC: precoated silica gel GF_{254} plates (SiO_2; *Qingdao Haiyang Chemical Plant*). Column chromatography (CC): SiO_2 (230–400 mesh), SiO_2 *H-60*, *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries Ltd.*), *RP-18* (50 μm; *YMC ODS-A*), and *Toyopearl-HW-40C* gel (*Tosoh Corporation*). Optical rotations: *Rudolph-Autopol-IV* polarimeter. UV Spectra: *Shimadzu-UV-2450* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Thermo-Nicolet-6700* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-500* apparatus; δ in ppm rel. to Me_4Si, *J* in Hz. ESI- and HR-ESI-MS: *Agilent-6210* TOF LC/MS system; in *m*/*z*.

Microbial Strain. The fungal strain XW-12 was isolated from the healthy stems of *Huperzia serrata* collected from Xishuangbanna Tropical Plant Garden, Chinese Academy of Sciences, Yunnan Province, P. R. China, in September 2007. Plant samples were designed to undergo a process described by *Schulz* and co-workers as surface sterilization [17]. The surface-sterilized samples were cut into 0.5-cm fragments and placed onto the surface of potato-dextrose agar (PDA) as medium in *Petri* dishes with six fragments in each, which was then incubated at 28° for 4 d. During cultivation, the hyphal-tip method [18] was adopted for the purification of the endophytic fungi. The pure strains were then transferred to PDA slants and preserved at 4°. The fungal strain XW-12 was identified on the morphology level as *Aspergillus* sp. by Prof. *Wen-Hong Liu*, Zhejiang Chinese Medical University. The original culture (ZJUT HS-XW-12) was deposited at Zhejiang University of Technology, P. R. China. As the first step of cultivation, the fungal strain XW-12 was cultured in 500 ml *Erlenmeyer* flasks each containing 250 ml of liquid potato/dextrose medium (potato 200 g/l and glucose 20 g/l) to a total of 50 l (250 ml × 200) at 28°. The flasks were firstly inoculated on rotary shakers at 28° for 3 d at 180 r.p.m. and then cultivated for another 15 d at 28° without agitation.

Extraction and Purification. The culture was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated to *ca.* 2% of the original volume and then extracted with AcOEt (5 × 2 l). The AcOEt soln. was concentrated to afford a crude extract (23 g). This was subjected to CC (*MCI-CHP20P* gel, MeOH/H_2O 1:1 → 1:0): *Fractions 1–3. Fr. 1* (1.3 g) was separated by CC (*RP-18*, MeOH/H_2O 3:7 → 1:0): **2** (2 mg). *Fr. 2* (1.9 g) was applied to CC (SiO_2, petroleum ether/acetone/HCOOH 5:1:0.01 → 2:1:0.01) and then to CC (SiO_2, petroleum ether/acetone/HCOOH 25:1:0.01): **3** (2.8 mg) and **4** (5.6 mg). *Fr. 3* (2.5 g) was applied to CC (SiO_2, $CHCl_3$/MeOH 30:1 → 10:1) and then purified by CC (*Toyopearl HW-40C*, MeOH): **6** (12.7 mg), **1** (8 mg), and **5** (3 mg).

Huaspenone A (=*2-[(1*E*,3*E*)-5-Hydroxyhexa-1,3-dien-1-yl]-5-methoxy-4-(3-methoxy-1-oxobutyl)-2-methylfuran-3(2*H*)-one*; **1**): Yellow viscous oil. $[\alpha]_D^{20} = +30.8$ ($c = 0.36$, MeOH). IR (KBr): 3480, 2975, 2933, 1709, 1644, 1585. UV (MeOH): 265 (3.97), 232 (4.23). ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 325 ($[M+H]^+$). HR-ESI-MS: 325.1648 ($[M+H]^+$, $C_{17}H_{25}O_6^+$; calc. 325.1651).

Huaspenone B (=*4-Acetyl-2-[(1*E*)-5-Hydroxyhex-1-en-1-yl]-5-methoxy-2-methylfuran-3(2*H*)-one*; **2**): Yellow oil. $[\alpha]_D^{20} = +285.0$ ($c = 0.07$, MeOH). IR (KBr): 3364, 1594, 1512, 1425, 1148, 1102, 1018. UV (MeOH): 262 (3.71), 232 (3.91). ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 269 ($[M+H]^+$). HR-ESI-MS: 269.1386 ($[M+H]^+$, $C_{14}H_{21}O_5^+$; calc. 269.1389).

Aspertetronin A (=*2-[(1*E*,3*E*)-Hexa-1,3-dien-1-yl]-5-methoxy-2-methyl-4-[(2*E*)-1-oxobut-2-en-1-yl]furan-3-(2*H*)-one*; **3**): Colorless needles. $[\alpha]_D^{20} = +106.0$ ($c = 0.11$, MeOH; [12]: $[\alpha]_D = +133.0$ ($c = 0.3$, $CHCl_3$)). ^{1}H-NMR: in accordance with [12]. ^{13}C-NMR ((D_6)acetone): 13.4; 19.5; 22.1; 26.0; 51.2; 91.3; 107.3; 121.0; 127.3; 128.7; 132.0; 139.4; 145.4; 163.4; 196.3; 197.9. ESI-MS (pos.): 277 ($[M+H]^+$).

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