

Article

Acetylcholinesterase Inhibitory Meroterpenoid from a Mangrove Endophytic Fungus *Aspergillus* sp. 16-5c

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Abstract: One new meroterpenoid, named 2-hydroxyacetoxyldehydroaustin (1), together with nine known meroterpenoids, 11-acetoxyisoaustinone (2), isoaustanol (3), austin (4), austinol (5), acetoxydehydroaustin (6), dehydroaustin (7), dehydroaustanol (8), preaustinoide A2 (9), and 1,2-dihydro-acetoxydehydroaustin B (10), were isolated from the mangrove endophytic fungus, *Aspergillus* sp. 16-5c. These structures were characterized by spectroscopic analysis, further the absolute configurations of stereogenic carbons for Compounds 1, 3, 4, 6, 7, 8, 9, and 10 were determined by single crystal X-ray diffraction analysis using Cu K α radiation. Moreover, the absolute configurations of stereogenic carbons for Known Compounds 3, 7, 8, and 9 are identified here for the first time. Compounds 3, 7, and 8 showed acetylcholinesterase (AChE) inhibitory activity with IC₅₀ values of 2.50, 0.40, and 3.00 μ M, respectively.

Keywords: meroterpenoids; *Aspergillus* sp.; acetylcholinesterase (AChE) inhibitory

1. Introduction

Meroterpenoids are natural products derived from terpenoids mixed biosynthetic origin [1]. Meroterpenoids display structural diversity with widespread biological activities including antimicrobial, antiviral, and antitumoral activities and enzyme inhibition. Despite the structural diversity, austin and austin analogues are mixed polyketide–terpenoid meroterpenoids derived from 3,5-dimethylorsellinic acid, which were most often isolated from fungi [2]. Austin was first isolated as a novel polyisoprenoid mycotoxin from *Aspergillus ustus* in 1976 [3]. Subsequently, some austin analogues from *Aspergillus* sp. and *Penicillium* sp. have been reported [2–16]. As part of our ongoing investigations on novel bioactive compounds from mangrove endophytic-derived fungi [17–20], ten meroterpenoids, including one new meroterpenoids named 2-hydroxy-acetoxydehydroaustin (1), together with nine known meroterpenoids, 11-acetoxyisoaustinone (2), isoaustanol (3), austin (4), austinol (5), acetoxydehydroaustin (6), dehydroaustin (7), dehydroaustanol (8), preaustinoide A2 (9), and 1,2-dihydro-acetoxydehydroaustin B (10), were isolated from the fungus *Aspergillus* 16-5c, which was isolated from the leaves of the mangrove plant *Sonneratia apetala* collected on the coastal saltmarsh of the South China Sea (Figure 1). Compound 10 was reported as a mixture previously [16], but it is reported as a single compound here. The absolute configurations of stereogenic carbons for Compounds 3, 7, 8, and 9 are determined here for the first time (Figure 2). Herein, we report the isolation, structural elucidation, acetylcholinesterase (AChE) inhibitory activity, and cytotoxicity of these meroterpenoids.

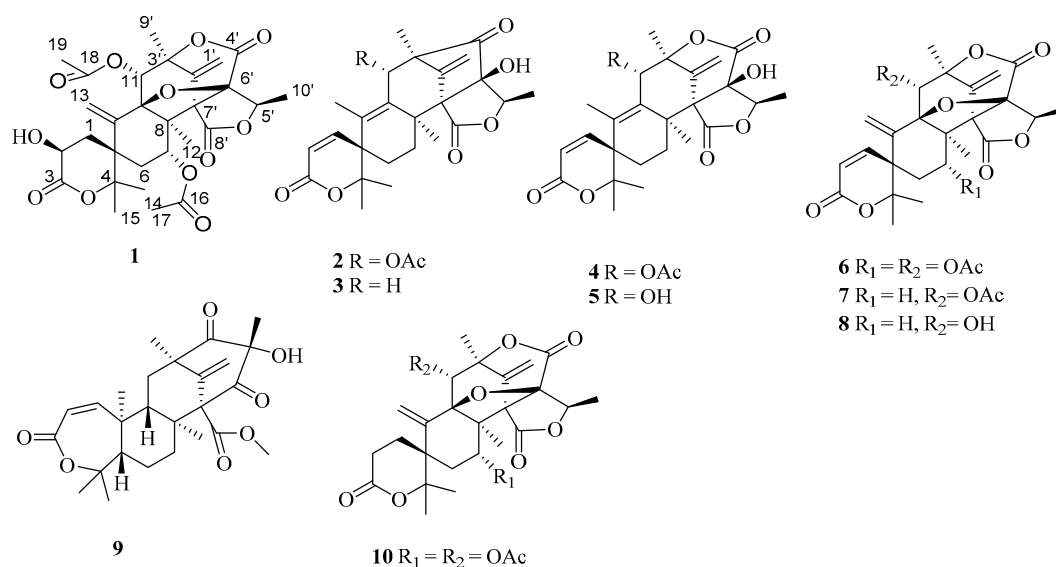


Figure 1. Structures of Compounds 1–10.

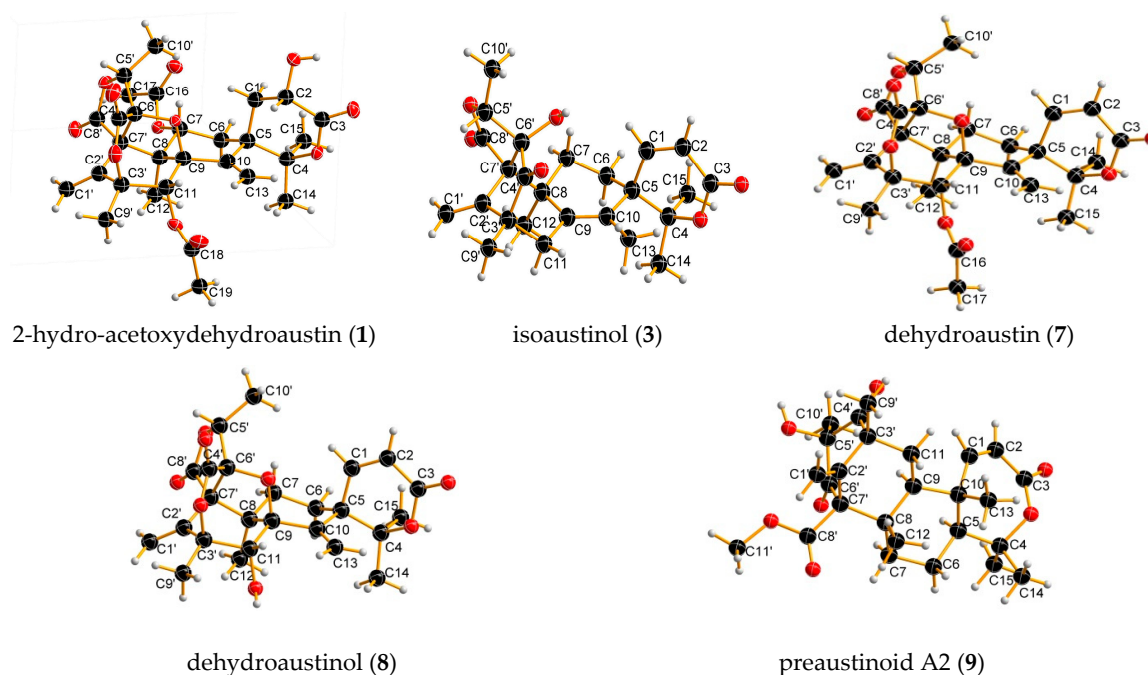


Figure 2. Perspective ORTEP drawings for 1, 3, 7, 8, and 9.

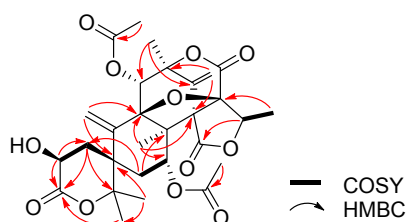
2. Results and Discussion

Compound **1** was obtained as colorless crystal, m.p. >300 °C. The HRESIMS result (m/z 575.2124 [M + H]⁺) suggested the molecular formula of Compound **1** as C₂₉H₃₄O₁₂ with 13 degrees of unsaturation. The data of ¹D-NMR (Table 1) combined with HSQC spectrum showed that **1** has seven methyl groups [δ_{H} 1.37, 1.43, 1.47, 1.58, 1.70, 2.05, 2.07 (each 3H, s)], two methylene groups, four oxygenated methines (δ_{H} 4.28/ δ_{C} 64.8, δ_{H} 5.35/ δ_{C} 67.8, δ_{H} 5.70/ δ_{C} 74.3, and δ_{H} 5.26/ δ_{C} 76.6), one terminal olefin moiety [δ_{H} 5.81, 5.85 (each 1H, $J = 1.5$ Hz) and δ_{H} 5.73, 6.14 (each 1H, s)], two acetyl groups (δ_{H} 2.05/ δ_{C} 20.8, 170.6 and δ_{H} 2.07/ δ_{C} 21.1, 168.9), and three others.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** and **2** (CDCl_3 , in ppm).

Position	1		2	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	2.23, dd (11.5, 13.4) 2.98, dd (7.4, 13.4)	37.1, CH_2	6.52, d (9.9)	146.7, CH
2	4.28, dd (7.4, 11.5)	64.8, CH	6.03, d (9.9)	120.0, CH
3		174.9, C		163.7, C
4		90.0, C		85.6, C
5		46.1, C		46.3, C
6	1.76, dd (11.9, 13.0) 1.90, dd (3.9, 13.0)	36.1, CH_2	2.66, td (13.5, 3.6) 1.79, dt (13.5, 3.6)	27.1, CH_2
7	5.35, dd (3.9, 11.9)	67.8, CH	1.67, dd (12.2, 3.6) 1.61, dd (12.2, 3.6)	27.0, CH_2
8		56.6, C		41.3, C
9		93.2, C		134.8, C
10		138.1, C		138.1, C
11	5.70, s	74.3, CH	5.74, s	74.1, CH
12	1.37, s	12.0, CH_3	1.57, s	23.0, CH_3
13	5.81, d (1.5) 5.85, d (1.5)	124.4, CH_2	1.75, s	15.2, CH_3
14	1.43, s	23.5, CH_3	1.37, s	25.9, CH_3
15	1.47, s	27.4, CH_3	1.20, s	22.8, CH_3
16		170.6, C	2.04, s	20.7, CH_3
17	2.07, s	20.8, CH_3		171.5, C
18		168.9, C		
19	2.05, s	21.1, CH_3		
1'	5.73, s 6.14, s	116.9, CH_2	5.41, d (0.9) 5.33, d (0.9)	111.7, CH
2'		136.9, C		142.6, C
3'		82.5, C		59.2, C
4'		168.9, C		209.5, C
5'	5.26, q (6.9)	76.6 d	4.35, q (6.4)	76.2, CH
6'		85.5, C		91.2, C
7'		61.6, C		66.4, C
8'		167.3, C		169.1, C
9'	1.58, s	19.6, CH_3	1.25, s	12.9, CH_3
10'	1.70, d (6.9)	13.8, CH_3	1.30, d (6.4)	12.7, CH_3

The above spectroscopic features suggested that **1** belonged to the austin analogue [16], whose planar structure is similar to the 1,2-dihydro-acetoxydehydroaustin B. However, the carbon chemical shifts values of C-1 (δ_{C} 37.1) and C-2 (δ_{C} 64.8) were different from those of 1,2-dihydro-acetoxydehydroaustin B. The detailed comparison of the NMR data of **1** with those of 1,2-dihydro-acetoxydehydroaustin B clearly confirmed that **1** was a new structure (Figure 3). The absolute configurations of stereogenic carbons for **1** were determined by single-crystal X-ray diffraction using Cu K α radiation [21]. Therefore, Compound **1** was named 2-hydroacetoxydehydroaustin. Its absolute configurations were 1S, 7R, 8S, 9R, 11S, 3'R, 5'R, 7'R.

**Figure 3.** Selected ^1H - ^1H COSY (bold line) and HMBC (arrow) correlations of Compound **1**.

Compound **2** was obtained as a colorless powder. Its molecular formula was assigned to be $C_{27}H_{32}O_8$ from the HRESIMS molecular ion peak at m/z 507.1988 $[M + Na]^+$. Comparison of the NMR data revealed that the structure of **2** resembled that of **3** except for the presence of an acetyl group. A carbon signal at δ_C 74.1 (C-11) was observed in **2** compared to that of **3** [7]. It was deduced that the acetyl group was connected to C-11 via the hydroxyl group in **2** supported by the HMBC correlation from H-11 (δ_H 5.74, 1H, s) to 11- CH_3CO (δ_C 171.5). The relative configuration was confirmed by the NOE correlations, and the correlation signals between 9- CH_3 (δ_H 1.25), 11- $OCOCH_3$ (δ_H 2.04), and 12- CH_3 (δ_H 1.57) showed that the 11-acetoxy, 12 and 9'- CH_3 were oriented at the same side. Furthermore, the NOE correlations between 10'- CH_3 (δ_H 1.30) and 6'-OH (δ_H 2.88) supported that the methyl and hydroxyl groups were at the same side. The NOE correlations of **2** were the same as **3**, in addition to their positive optical rotation values. Finally, the absolute configurations of stereogenic carbons for **2** were assigned as 5R, 8S, 11S, 3'R, 5'R, 6'R, 7'R. These results confirmed that **2** was consistent with 11-acetoxyisoaustinone [22].

Austin was first isolated from *Aspergillus ustus* in 1976 before the Flack constant (the absolute configurations were determined) reported in 1983, and its absolute configuration was not definite. Herein, the absolute configurations of stereogenic carbons for Known Compounds **3**, **7**, **8**, and **9** are identified for the first time by single crystal X-ray diffraction analysis using Cu $K\alpha$ radiation. The absolute configurations were shown as follows: **3** is 5R, 8S, 3'R, 5'R, 6'R, 7'R, **7** is 5R, 8R, 11S, 3'S, 5'R, 6'R, 7'R, **8** is 5R, 8R, 11S, 3'S, 5'R, 6'R, 7'R, and **9** is 5S, 8S, 9S, 10S, 3'R, 5'S, 7'R (see the Supplementary Materials).

Although meroterpenoids have a wide range of biological activities [2–16], few biological activities have been reported for austin and austin analogues. In this work, Compounds **1–10** were evaluated for their acetylcholinesterase (AChE) inhibitory activity using Huperzine A as a control (Table 2). The results showed Compounds **3**, **7**, and **8** were potential AChE inhibitors with IC_{50} values of 2.50, 0.40, and 3.00 μ M, respectively.

Table 2. Acetylcholinesterase (AChE) inhibitory activity of Compounds **1–10** ^a.

Compound	1	2	3	4	5	6	7	8	9	10	HUP ^b
	>50	>50	2.50	>50	>50	>50	0.40	3.00	>50	>50	0.07

^a Data are expressed in IC_{50} values (μ mol/L). ^b HUP (Huperzine A) used as positive control.

In addition, four human cancer cell lines (human breast cancer cell lines MCF-7 and MDA-MB-435, human hepatoma cell line HepG2, and human prostatic cancer cell line PC-3) were used in the in vitro cytotoxicity bioassay. The results showed that Compounds **1–10** have no cytotoxicity (>50 μ M) against human cancer cell lines.

3. Experimental Section

3.1. General

Melting points were determined on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Polartronic HHW5 digital polarimeter. IR spectra were measured on a Bruker Vector 22 spectrophotometer (Bruker, Billerica, MA, USA) using KBr pellets. The NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz for 1H and 100 MHz for ^{13}C in $CDCl_3$. All chemical shifts (δ) are given in ppm with reference to the solvent signal ($CDCl_3$, δ_H 7.26 for 1H , δ_C 77.23 for ^{13}C ; DMSO, δ_H 2.50 for 1H , δ_C 39.52 for ^{13}C), and coupling constants (J) are given in Hz. HRESIMS spectra were recorded on a Finnigan LCQ-DECA mass spectrometer (Thermo Scientific, Shanghai, China). ESIMS spectra were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer (Shimadzu, Taiwan). Single-crystal data were measured on an Oxford Gemini S Ultra diffractometer (Oxford Instrument, Oxfordshire, UK). Column chromatography (CC) was performed

on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia, Piscataway, NJ, USA).

3.2. Fungal Material

The fungus used in this study was isolated from a mangrove, leaves of *S. apetala*, which were collected in Hainan Island, China. The fungus was identified as *Aspergillus* sp. by the ITS region (deposited in GenBank, accession number JX993829). A voucher strain was deposited in the China Center for Type Culture Collection under patent depository number CCTCC M 2012358.

3.3. Extraction and Isolation

The fungus *Aspergillus* sp. 16-5c was fermented on autoclaved rice solid-substrate medium for 28 days at room temperature. The mycelia and solid rice medium were extracted with MeOH. Then, the MeOH layer was dried in vacuo to yield 6.8 g of organic extract. The extract was separated by column chromatography (CC) over silica gel eluting with a gradient of CHCl₃/MeOH from 1:0 to 1:45 to yield five fractions (Fractions 1–5). Fraction 3 (120 mg) was applied to Sephadex LH-20 CC, eluting with CHCl₃/MeOH (1:1) to obtain Compound **1** (0.8 mg), **2** (1.8 mg), and **3** (2.0 mg). Fraction 4 (65 mg) was applied to Sephadex LH-20, eluting with CHCl₃/MeOH (1:1) to obtain Compound **4** (1.2 mg), **5** (1.6 mg), and **6** (2.6 mg). Fraction 5 (30 mg) was applied to Sephadex LH-20 CC, eluting with CHCl₃/MeOH (1:1) to obtain Compound **7** (1.4 mg), **8** (5.6 mg), **9** (2.4 mg), and **10** (4.6 mg).

2-Hydroacetoxydehydroaustin (**1**): colorless crystals (MeOH/CHCl₃); m.p. >300 °C; $[\alpha]_D^{20} = +60$ (*c* 0.01, MeOH); UV (MeOH): λ_{\max} 210, 236 nm; IR (KBr): ν_{\max} 3528, 2998, 1748, 1431, 1384, 1214 and 1040 cm⁻¹; ¹H-NMR(CDCl₃, 400 MHz), ¹³C-NMR(CDCl₃, 100 MHz), see Table 1; ESIMS 575[M + H]⁺; HRESIMS *m/z* = 575.2124, [M + H]⁺ (calcd for C₂₇H₃₂O₈, 574.2050).

11-Acetoxyisoaustinone (**2**): colorless crystals (MeOH/CHCl₃); m.p. >300 °C; $[\alpha]_D^{20} +150$ (*c* 0.1 MeOH); IR (KBr) ν_{\max} 3466, 3108, 2988, 2944, 2890, 1754, 1705, 1450, 1377, 1287, and 1226 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz) and ¹³C-NMR (CDCl₃, 100 MHz), see Table 1; ESIMS *m/z* 507 [M + Na]⁺; HRESIMS *m/z* 507.1988 [M + Na]⁺ (calcd for C₂₇H₃₂O₈Na, 507.1994).

3.4. X-Ray Crystallographic Analysis of **1**, **3**, **4**, **6**, **7**, **8**, **9** and **10**

All single crystal X-ray diffraction data were collected at 150(2) K on an Oxford Gemini S Ultra diffractometer with Cu K α radiation ($\lambda = 1.54178$ Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the “ride on” method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. Crystallographic data for **1**, **3**, **4**, **6–10** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC 1533328, 12 Union Road, Cambridge CB2 1EZ, UK (fax: t44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data of **1**: C₂₉H₃₄O₁₂, Mr = 574.56, Orthorhombic, *a* = 8.8330(2) Å, *b* = 12.2482(2) Å, *c* = 12.3432(2) Å, $\alpha = 90^\circ$, $\beta = 97.342(2)^\circ$, $\gamma = 90^\circ$, *V* = 1324.44(4) Å³, space group P21, *Z* = 2, *Dx* = 1.363 mg/m³, μ (Cu K α) = 0.850 mm⁻¹, and *F*(000) = 1064. Crystal dimensions: 0.40 × 0.33 × 0.29 mm. Independent reflections: 4336 (*R*_{int} = 0.0392). The final *R*1 values were 0.0258, *wR*2 = 0.0659 (*I* > 2 σ (*I*)). Flack parameter = 0.08 (8). CCDC number: 1533329.

Crystal data of **3**: C₂₅H₃₀O₆, Mr = 426.49, Monoclinic, *a* = 11.5899(2) Å, *b* = 7.4432(2) Å, *c* = 12.0804(2) Å, $\alpha = 90^\circ$, $\beta = 92.060(2)^\circ$, $\gamma = 90^\circ$, *V* = 1041.45(4) Å³, space group P21, *Z* = 2, *Dx* = 1.360 mg/m³, μ (Cu K α) = 0.786 mm⁻¹, and *F*(000) = 456. Crystal dimensions: 0.30 × 0.23 × 0.22 mm. Independent reflections: 3696 (*R*_{int} = 0.0549). The final *R*1 values were 0.0476, *wR*2 = 0.1215 (*I* > 2 σ (*I*)). Flack parameter = 0.01 (2). CCDC number: 921711.

Crystal data of **4**: $C_{27}H_{32}O_9$, Mr = 500.53, Orthorhombic, $a = 7.71560(10)$ Å, $b = 14.9523(2)$ Å, $c = 21.1376(3)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 2438.56(6)$ Å³, space group P212121, $Z = 4$, $D_x = 1.363$ mg/m³, μ (Cu K α) = 0.850 mm⁻¹, and $F(000) = 1064$. Crystal dimensions: 0.40 mm \times 0.33 mm \times 0.29 mm. Independent reflections: 4336 ($R_{int} = 0.0392$). The final R_1 values were 0.0258, $wR_2 = 0.0659$ ($I > 2\sigma(I)$). Flack parameter = 0.08 (12). CCDC number: 921707.

Crystal data of **6**: $C_{29}H_{32}O_{11}$, Mr = 556.55, Monoclinic, $a = 8.8841(3)$ Å, $b = 12.5166(4)$ Å, $c = 12.1254(5)$ Å, $\alpha = 90^\circ$, $\beta = 99.052(4)^\circ$, $\gamma = 90^\circ$, $V = 1331.54(8)$ Å³, space group P21, $Z = 2$, $D_x = 1.388$ mg/m³, μ (Cu K α) = 0.897 mm⁻¹, and $F(000) = 588$. Crystal dimensions: 0.44 mm \times 0.35 mm \times 0.21 mm. Independent reflections: 4474 ($R_{int} = 0.0620$). The final R_1 values were 0.0326, $wR_2 = 0.0740$ ($I > 2\sigma(I)$). Flack parameter = 0.01 (1). CCDC number: 921708.

Crystal data of **7**: $C_{29}H_{33}NO_9$, Mr = 539.56, Hexagonal, $a = 11.20730(10)$ Å, $b = 11.20730(10)$ Å, $c = 36.3299(5)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 120^\circ$, $V = 3951.82(7)$ Å³, space group P65, $Z = 6$, $D_x = 1.360$ mg/m³, μ (Cu K α) = 0.841 mm⁻¹, and $F(000) = 1716$. Crystal dimensions: 0.44 mm \times 0.35 mm \times 0.32 mm. Independent reflections: 4596 ($R_{int} = 0.0815$). The final R_1 values were 0.0597, $wR_2 = 0.1612$ ($I > 2\sigma(I)$). Flack parameter = 0.01 (1). CCDC number: 921709.

Crystal data of **8**: $C_{25}H_{28}O_8$, Mr = 456.47, Orthorhombic, $a = 7.8409(2)$ Å, $b = 11.6017(2)$ Å, $c = 23.9529(4)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 2178.94(8)$ Å³, space group P212121, $Z = 4$, $D_x = 1.391$ mg/m³, μ (Cu K α) = 0.862 mm⁻¹, and $F(000) = 968$. Crystal dimensions: 0.37 mm \times 0.31 mm \times 0.22 mm. Independent reflections: 3880 ($R_{int} = 0.0455$). The final R_1 values were 0.0277, $wR_2 = 0.0684$ ($I > 2\sigma(I)$). Flack parameter = 0.01 (2). CCDC number: 921710.

Crystal data of **9**: $C_{26}H_{34}O_7$, Mr = 458.53, Orthorhombic, $a = 8.29600(10)$ Å, $b = 13.9817(2)$ Å, $c = 19.5230(2)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 2264.52(5)$ Å³, space group P212121, $Z = 4$, $D_x = 1.345$ mg/m³, μ (Cu K α) = 0.793 mm⁻¹, and $F(000) = 984$. Crystal dimensions: 0.33 mm \times 0.30 mm \times 0.25 mm. Independent reflections: 4027 ($R_{int} = 0.0434$). The final R_1 values were 0.0280, $wR_2 = 0.0728$ ($I > 2\sigma(I)$). Flack parameter = 0.08 (12). CCDC number: 921712.

Crystal data of **10**: $C_{29}H_{34}O_{11}$, Mr = 558.56, Orthorhombic, $a = 8.29600(10)$ Å, $b = 13.9817(2)$ Å, $c = 19.5230(2)$ Å, $\alpha = 90^\circ$, $\beta = 98.7090(10)^\circ$, $\gamma = 90^\circ$, $V = 2264.52(5)$ Å³, space group P21, $Z = 2$, $D_x = 1.345$ mg/m³, μ (Cu K α) = 0.793 mm⁻¹, and $F(000) = 984$. Crystal dimensions: 0.33 mm \times 0.30 mm \times 0.25 mm. Independent reflections: 4027 ($R_{int} = 0.0434$). The final R_1 values were 0.0280, $wR_2 = 0.0728$ ($I > 2\sigma(I)$). Flack parameter = 0.04 (11). CCDC number: 1533328.

3.5. Assays for Enzyme Inhibiting Activities and Cytotoxic Activities

These two experiments were conducted according to reference procedures [14,23].

4. Conclusions

One new and nine known meroterpenoids (**1–10**) were isolated and identified from the culture of the endophytic fungus *Aspergillus* sp. 16-5c. The absolute configurations of Known Compounds **3**, **7**, **8**, and **9** were first identified. Compounds **3**, **7**, and **8** showed acetylcholinesterase (AChE) inhibitory activity with IC_{50} values of 2.50, 0.40, and 3.00 μ M, respectively.

Supplementary Materials: The following are available online, Figure S1: The NMR spectrum of compounds **1–10**, and the perspective ORTEP drawings for compounds **1**, **3**, **4**, **6**, **7**, **8**, **9** and **10**.

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Author Contributions: Conceived and designed the experiments: Xishan Huang, Zhigang She, and Yuhua Long. Performed the experiments: Ze'en Xiao, Yuhua Long, Hui Cui, Xinglie Liu, Shitong Wen Analyzed the data:

Zhigang She, Xishan Huang, Ze'en Xiao, and Yuhua Long. Contributed reagents/materials/analysis tools: Xishan Huang, Yuhua Long. Wrote the paper: Xishan Huang, Yuhua Long. Read and approved the final manuscript: Xishan Huang, Yuhua Long, Zhigang She.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cornforth, J.W. Terpenoid biosynthesis. *Chem. Br.* **1968**, *4*, 102–106. [[PubMed](#)]
2. Geris, R.; Simpson, T. Meroterpenoids produced by fungi. *Nat. Prod. Rep.* **2009**, *26*, 1063–1094. [[CrossRef](#)] [[PubMed](#)]
3. Chexal, K.; Springer, J.; Clardy, J.; Cole, R.; Kirksey, J.; Dorner, J.; Cutler, H.; Strawter, B. Austin, a novel polyisoprenoid mycotoxin from *Aspergillus ustus*. *J. Am. Chem. Soc.* **1976**, *98*, 6748–6750. [[CrossRef](#)] [[PubMed](#)]
4. Ahmed, S.A.; Scott, F.E.; Stenzel, D.J.; Simpson, T.J.; Moore, R.W.; Trimble, L.A.; Arai, K.; Vederas, J.C. Studies on the biosynthesis of the mycotoxin austin, a meroterpenoid metabolite of *Aspergillus ustus*. *J. Chem. Soc. Perkin Trans. I.* **1989**, 807–816.
5. Chen, J.-W.; Luo, Y.-L.; Hwang, M.-J.; Peng, F.-C.; Ling, K.-H. Territrem B, a tremorgenic mycotoxin that inhibits acetylcholinesterase with a noncovalent yet irreversible binding mechanism. *J. Biol. Chem.* **1999**, *274*, 34916–34923. [[CrossRef](#)] [[PubMed](#)]
6. Lo, H.; Entwistle, R.; Guo, C.; Ahuja, M.; Szewczyk, E.; Hung, J.; Chiang, Y.; Oakley, B.; Wang, C. Two separate gene clusters encode the biosynthetic pathway for the meroterpenoids austinol and dehydroaustinol in *Aspergillus nidulans*. *J. Am. Chem. Soc.* **2012**, *134*, 4709–4720. [[CrossRef](#)] [[PubMed](#)]
7. Fill, T.P.; Pereira, G.K.; Geris dos Santos, R.M.; Rodrigues-Fo, E. Four additional meroterpenes produced by *Penicillium* sp. found in association with *Melia azedarach*. Possible biosynthetic intermediates to austin. *Z. Naturforsch.* **2007**, *62b*, 1035–1044.
8. Geris dos Santos, R.M.; Rodrigues-Fo, E. Meroterpenes from *Penicillium* sp. found in association with *Melia azedarach*. *Phytochemistry* **2002**, *61*, 907–912. [[CrossRef](#)]
9. Dos Santos, R.M.G.; Rodrigues-Fo, E. Structures of meroterpenes produced by *Penicillium* sp., an endophytic fungus found associated with *Melia azedarach*. *J. Braz. Chem. Soc.* **2003**, *14*, 722–727. [[CrossRef](#)]
10. Geris dos Santos, R.M.; Rodrigues-Fo, E. Further meroterpenes produced by *Penicillium* sp., an endophyte obtained from *Melia azedarach*. *Z. Naturforsch.* **2003**, *58c*, 663–669.
11. Fukuyama, K.; Katsube, Y.; Ishido, H.; Yamazaki, M.; Maebayashi, Y. The absolute configuration of desacetylaustin isolated from *Emericella nidulans* var. dentate. *Chem. Pharm. Bull.* **1980**, *28*, 2270–2271. [[CrossRef](#)]
12. Hayashi, H.; Mukaiharu, M.; Murao, S.; Arai, M.; Lee, A.; Clardy, J. Acetoxydehydroaustin, a new bioactive compound, and related compound neo-austin from *Penicillium* sp. MG-11. *J. Biosci. Biotechnol. Biochem.* **1994**, *58*, 334–338. [[CrossRef](#)]
13. Scott, F.; Simpson, T.; Trimble, L.; Vederas, J. Biosynthesis of the meroterpenoid austin, by *Aspergillus ustus*: synthesis and incorporation of carbon-13, oxygen-18-labeled ethyl 3,5-dimethylorsellinate. *J. Chem. Soc. Chem. Commun.* **1986**, *3*, 214–215. [[CrossRef](#)]
14. Scudiero, D.; Shoemaker, R.; Paull, K.; Monks, A.; Tierney, S.; Nofziger, T.; Currens, M.; Seniff, D.; Boyd, M. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.* **1988**, *48*, 4827–4833. [[PubMed](#)]
15. Simpson, T.; Ahmed, S.; McIntyre, C.; Scott, F.; Sadler, I. Biosynthesis of polyketide-terpenoid (meroterpenoid) metabolites andibenin B and andilesin A in *Aspergillus varicolor*. *Tetrahedron* **1997**, *53*, 4013–4034. [[CrossRef](#)]
16. Song, Y.-X.; Qiao, L.-T.; Wang, J.-J.; Zeng, H.-M.; She, Z.-G.; Miao, C.-D.; Hong, K.; Gu, Y.-C.; Liu, L.; Lin, Y.-C. Two new meroterpenes from the mangrove endophytic fungus *Aspergillus* sp. 085241B. *Helv. Chim. Acta.* **2011**, *94*, 1875–1879. [[CrossRef](#)]
17. Huang, H.; Feng, X.; Xiao, Z.; Liu, L.; Li, H.; Ma, L.; Lu, Y.; Ju, J.; She, Z.; Lin, Y. Azaphilones and p-terphenyls from the mangrove endophytic fungus *Penicillium chermesinum* (ZH4-E2) isolated from the South China Sea. *J. Nat. Prod.* **2011**, *74*, 997–1002. [[CrossRef](#)] [[PubMed](#)]
18. Huang, X.; Huang, H.; Li, H.; Sun, X.; Huang, H.; Lu, Y.; Lin, Y.; Long, Y.; She, Z. Asperterpenoid A, a new sesterterpenoid as an inhibitor of mycobacterium tuberculosis protein tyrosine phosphatase B from the culture of *Aspergillus* sp. 16-5c. *Org. Lett.* **2013**, *15*, 721–723. [[CrossRef](#)]

19. Li, H.; Huang, H.; Shao, C.; Huang, H.; Jiang, J.; Zhu, X.; Liu, Y.; Liu, L.; Lu, Y.; Li, M.; et al. Cytotoxic norsesquiterpene peroxides from the endophytic fungus *Talaromyces flavus* isolated from the mangrove plant *Sonneratia apetala*. *J. Nat. Prod.* **2011**, *74*, 1230–1235. [[CrossRef](#)] [[PubMed](#)]
20. Wen, L.; Cai, X.; Xu, F.; She, Z.; Chan, W.; Vrijmoed, L.L.P.; Jones, E.B.G.; Lin, Y. Three metabolites from the mangrove endophytic fungus *Sporothrix* sp. (#4335) from the South China Sea. *J. Org. Chem.* **2009**, *74*, 1093–1098.
21. Flack, H.D. On enantiomorph-polarity estimation. *Acta Crystallogr. Sect. A.* **1983**, *39*, 876–881. [[CrossRef](#)]
22. Arunpanichlert, J.; Rukachaisirikul, V.; Phongpaichit, S.; Supaphon, O.; Sakayaroj, J. Meroterpenoid, isocoumarin, and phenol derivatives from these agrass-derived fungus *Pestalotiopsis* sp. PSU-ES194. *Tetrahedron* **2015**, *71*, 882–888. [[CrossRef](#)]
23. Tang, H.; Wei, Y.B.; Zhang, C.; Ning, F.X.; Qiao, W.; Huang, S.L.; Ma, L.; Huang, Z.S.; Gu, L.Q. Synthesis, biological evaluation and molecular modeling of oxoisoaporphine and oxoaporphine derivatives as new dual inhibitors of acetylcholinesterase/ butyrylcholinesterase. *Eur. J. Med. Chem.* **2009**, *44*, 2523–2532. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of Compounds **1, 2, 3, 4, 5, 6, 7, 8, 9** and **10** are available from the authors.



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