New Monoterpenoids and Polyketides from the Deep-Sea Sediment-Derived Fungus Aspergillus sydowii MCCC 3A00324

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Received: 16 October 2020; Accepted: 14 November 2020; Published: 17 November 2020

Abstract: Chemical study of the secondary metabolites of a deep-sea-derived fungus Aspergillus sydowii MCCC 3A00324 led to the isolation of eleven compounds (1–11), including one novel (1) and one new (2) osmane-related monoterpenoids and two undescribed polyketides (3 and 4). The structures of the metabolites were determined by comprehensive analyses of the NMR and HRESIMS spectra, in association with quantum chemical calculations of the 13C NMR, ECD, and specific rotation data for the configurational assignment. Compound 1 possessed a novel monoterpenoid skeleton, biogenetically probably derived from the osmane-type monoperpenoid after the cyclopentane ring cleavage and oxidation reactions. Additionally, compound 3 was the first example of the α-pyrone derivatives bearing two phenyl units at C-3 and C-5, respectively. The anti-inflammatory activities of 1–11 were tested. As a result, compound 6 showed potent inhibitory nitric oxide production in lipopolysaccharide (LPS)-activated BV-2 microglia cells with an inhibition rate of 94.4% at the concentration of 10 µM. In addition, a plausible biosynthetic pathway for 1 and 2 was also proposed.

Keywords: polyketides; monoterpenoids; deep-sea fungus; Aspergillus sydowii; ECD calculation

1. Introduction

Deep-sea-derived fungi inhabiting extreme sea environments have proven to be a new source for a wide array of structurally intriguing and biologically active secondary metabolites [1–3]. As of December 2019, about 700 new secondary metabolites were reported from deep-sea-derived fungi, such as terpenoids, polyketides, alkaloids, and steroids. Some of the metabolites featured a broad range of biological activities, for example, cytotoxicity [4,5] as well as antiviral [6,7], antibacterial [8,9], anti-inflammatory [10], and antiallergic effects [11]. In our continuing efforts to discover new or bioactive secondary metabolites from deep-sea-derived fungi [12–16], the fungus Aspergillus sydowii MCCC 3A00324, isolated from the South Atlantic Ocean deep-sea sediment (2246 m), attracted our attention due to the abundantly metabolic profile obtained by HPLC and TLC analysis (Figure S1, Supplementary Materials). A literature retrieval discovered that 52 out of 59 new compounds were isolated from the marine-derived Aspergillus sydowii fungus. The bisabolane-type sesquiterpenoids [17–24], xanthones [18,23,25], diphenyl ethers [20,26–28], and diketopiperazine alkaloids [29–31] were common secondary metabolites of the A. sydowii,
some of which exhibited a wide range of pharmacological activities. To date, only three undescribed polyketides, namely asperentin B [32], 2,3,5-trimethyl-6-(3-oxobutan-2-yl)-4H-pyran-4-one, and (2R)-2,3-dihydro-7-hydroxy-6,8-dimethyl-2-[(E)-propyl-enyl] chromen-4-one [33], have been reported from the deep-sea-derived A. sydowii. On the basis of the seldomly carried out chemical investigation and the abundantly metabolic profile of the A. sydowii MCCC 3A00324 fungus was carried out. In our previous research, 17 undescribed and 10 known sesquiterpenoids were obtained from the fermented cultures of the fungus [34]. In our continuous research on this fungus, eleven compounds (1–11) (Figure 1), including one novel (1) and one new (2) monoterpenoids as well as two new polyketides (3 and 4) were discovered. Compounds 1 and 2 were the first representatives of the osmane-related monoterpenoids discovered from the fungi, while 3 was the first example of the α-pyrone derivatives with two phenyl units at C-3 and C-5, respectively. As terpenoids usually possess anti-inflammatory activities [35–37], all compounds were subsequently evaluated for their anti-inflammatory effects. As a result, compound 6 exhibited strong inhibitory nitric oxide production in lipopolysaccharide (LPS)-activated BV-2 microglia cells with a 94.4% inhibition rate at 10 µM. Herein, the isolation, structure elucidation, and anti-inflammatory activities of these metabolites as well as the biosynthetic pathway of 1 and 2 are reported.

![Figure 1. Chemical structures of 1–11 from Aspergillus sydowii MCCC 3A00324.](image)

2. Results and Discussion

Aspermonoterpenoid A (1), colorless oil, has the molecular formula of C_{10}H_{14}O_{4} as determined by the $^{13}$C NMR data and the HRESIMS spectrum ($m/z$ 221.0784, [M + Na]$^+$), indicating four degrees of unsaturation. The $^1$H NMR spectrum showed two olefinic protons (δ$_H$ 5.77, 6.66), a methine (δ$_H$ 3.36), and three methyls (δ$_H$ 1.24, 1.88, 2.14) (Table 1), while the $^{13}$C NMR and HSQC spectra exhibited four sp$^2$ carbons (δ$_C$ 117.0, 130.2, 144.5, 162.0) for two double bonds, two ester carbonyl carbons (δ$_C$ 170.4, 171.5), one methine (δ$_C$ 43.8), and three methyls (δ$_C$ 12.8, 17.0, 19.0) (Table 1). As all degrees of unsaturation were accounted for by two carbonyl carbons and two double bonds, an acyclic structure was required in 1. The COSY data of H-4 (δ$_H$ 3.36)/H$_3$-9 (δ$_H$ 1.24) and the HMBC cross-peaks from H$_3$-8 (δ$_H$ 1.88) to C-5 (δ$_C$ 144.5)/C-6 (δ$_C$ 130.2)/C-7 (δ$_C$ 171.5), H$_3$-9 to C-3 (δ$_C$ 162.0)/C-4 (δ$_C$ 43.8)/C-5, H$_3$-10 (δ$_H$ 2.14) to C-2 (δ$_C$ 117.0)/C-3/C-4, and from H-2 (δ$_H$ 5.77) to C-1 (δ$_C$ 170.4)/C-4/C-10 (δ$_C$ 17.0) established the planar structure of 1 as 2,4,5-trimethylhexa-2,5-dienedioic acid (Figure 2). The E geometries at Λ$^2$ and Λ$^5$ were resolved on the basis of the NOESY cross-peaks from H-2 to...
H-4 and from H3-8 to H-4 (Figure 2), which were further corroborated by the chemical shifts of Me-8 ($\delta_C 12.8$) and Me-10 ($\delta_C 17.0$). The sole chiral center of C-4 in 1 was resolved by the calculation of the specific rotation data using Gaussian 09 [38]. The specific rotation data of (4'S)-1 was calculated at the B3LYP/6-311+G(2d,p) level with the CPCM model in methanol, which has the same sign ([a]$_D^{25}$ +138) as that of the experimental data ([a]$_D^{25}$ +54), revealing the 4S configuration. Therefore, the structure of 1 was determined to be (2E,5E,4S)-2,4,5-trimethylhepta-2,5-dienedioic acid, which was named aspermonoterpenoid A. Noteworthily, 1 possessed a novel chained monoperpenoid skeleton, biogenetically probably derived from the osmane-type monoperpenoid after the cyclopentane ring cleavage and oxidation reactions [39,40].

Table 1. $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR spectroscopic data of 1 and 2 in CD$_3$OD.

| No. | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
|-----|------------|------------|------------|------------|
| 1   | 170.4, C   | 211.6, C   | 130.1, C   | 5.89, brs  |
| 2   | 170.4, C   | 211.6, C   | 130.1, C   | 5.89, brs  |
| 3   | 171.5, C   | 178.5, C   | 153, CH$_3$ | 1.29, d (7.2) |
| 4   | 12.8, CH$_3$ | 1.88, d (0.6) | 18.9, CH$_3$ | 1.28, d (7.1) |
| 5   | 19.0, CH$_3$ | 1.24, d (6.8) | 17.1, CH$_3$ | 2.13, s |

Figure 2. Key COSY, HMBC, and NOESY correlations of 1 and 2.

The molecular formula of aspermonoterpenoid B (2) was established to be C$_{10}$H$_{14}$O$_3$ on the basis of the HRESIMS spectrum at the sodium adduct ion peak at $m/z$ 205.0845 (calcd for C$_{10}$H$_{14}$O$_3$Na, 205.0841), requiring four degrees of hydrogen deficiency. The $^1$H NMR spectrum showed one olefinic proton ($\delta_H$ 5.89), three methines ($\delta_H$ 2.21, 2.76, 2.94), and three methyls ($\delta_H$ 1.28, 1.29, 2.13). Analyses of the $^{13}$C NMR spectrum, with the aid of the HSQC spectrum, revealed ten carbon signals attributable to two olefinic carbons ($\delta_C$ 130.1, 184.4) for a double bond, two carbonyl carbons for a keto ($\delta_C$ 211.6) and a carboxylic ($\delta_C$ 178.5) functionalities, three methines ($\delta_C$ 41.2, 44.8, 58.4), and three methyls ($\delta_C$ 15.3, 17.1, 18.9), which accounted for three degrees of unsaturation. The remaining one double bond equivalent indicated that a monocyclic skeleton was required in 2. The COSY cross-peaks of H-4 ($\delta_H$
2.76)/H-5 (δH 2.21)/H-6 (δH 2.94) and the HMBC correlations from H3-8 (δH 1.29) to C-5 (δC 58.4)/C-6 (δC 41.2)/C-7 (δC 178.5), H3-9 (δH 1.28) to C-3 (δC 184.4)/C-4 (δC 44.8)/C-5, H3-10 (δH 2.13) to C-2 (δC 130.1)/C-3/C-4, and from H-2 (δH 5.89) to C-1 (δC 211.6)/C-4/C-5 deduced the structure of 2 to be 3,4-dimethyl-5-isopropanic acidated cyclopentenone (Figure 2). The relative configuration of C-4 and C-5 in cyclopentenone ring moiety was determined as 4S* and 5R* on the basis of the NOESY correlation from H3-9 to H-5, in addition to the very weak interaction between H-4 and H-5 (Figure 2). Additionally, the relative configuration of C-6 was unreliable to be deduced by the NOESY data because of its location at the soft side chain. In order to assign the relative configuration, the two possible epimers (4S*,5R*,6S*)-2 (2a) and (4S*,5R*,6R*)-2 (2b) were subjected to the 13C NMR chemical shift theoretical calculation at the mPW1PW91/6-311+G(2d,p) level in methanol using Gaussian 09. As shown in Figure 3, the calculated 13C NMR data of 2a were nearly identical to those of the experimental NMR data, as corroborated by the correlation coefficient (R2) and root mean square error (RMSE) of 2a (R² = 0.9992, RMSE = 2.4436) and 2b (R² = 0.9988, RMSE = 2.7912), suggesting the 4S*, 5R*, and 6S* configurations. The absolute configuration of 2 was resolved on the basis of the ECD calculation, which was carried out at the B3LYP/6-311+G(2d,p) level in MeOH using the optimized geometries at the B3LYP/6-31+G(d,p) level in gas phase after conformational searches via the OPLS3 force field using Maestro 10.2. The ECD spectrum of 2a was calculated by the TDDFT method, which was in good agreement with the experimental ECD curve, indicating the 4S*, 5R*, and 6S* configurations (Figure 4). Interestingly, 2 was the first representative of the osmane-type monoterpenoids discovered from the fungi.

Figure 3. Linear regression analysis of the experimental 13C NMR data of 2 and calculated 13C NMR chemical shift data of 2a.

Compound 3 was isolated as a colorless oil, and its molecular formula was assigned to be C18H14O5 on the basis of the HRESIMS spectrum at the sodium adduct ion peak at m/z 333.0741, implying 12 indices of hydrogen deficiency. The 1H NMR spectrum exhibited four aromatic protons with dual intensity at δH 6.85 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), and 7.33 (d, J = 8.6 Hz, 2H), indicating the presence of two para-substituted benzene rings. Moreover, a methoxy (δH 3.85) and one olefinic (δH 7.59) protons were also observed (Table 2). The 13C NMR spectrum showed a total of 18 carbon resonance signals, including twelve aromatic carbons (δC 115.1 × 2, 116.3 × 2, 123.5, 124.6, 131.9 × 2, 133.2 × 2, 158.9, and 160.9) for two benzene units, four olefinic carbons (δC 107.1, 120.4, 149.5, and 167.1) for two double bonds, a carbonyl carbon (δC 166.2), and one methoxy group (δC 55.7) (Table 2). The COSY data of H-1′ (δH 7.33) and H-2′ (δH 7.01) and the HMBC cross-peaks from H-1′/H-5′ to C-3′ (δC 160.9) and C-3 (δC 107.1), H-2′/H-4′ to C-3′ and C-6′
possible epimers (4S*,5R*,6S*)-2 (2a) and (4S*,5R*,6R*)-2 (2b) were subjected to the 13C NMR chemical shift data, as given in Table 2. The COSY correlation between H-1' (δH 7.29)/H-2' (δH 6.85) and the HMBC correlations from H-1'/H-5' to C-3' (δC 158.9) and C-5 (δC 120.4) and from H-2'/H-4' to C-3' and C-6' (δC 123.5) in association with the chemical shift of C-3' established the p-hydroxyphenyl unit (ring A) to be resided at C-5. The remaining five olefinic carbons (δC 107.1, 120.4, 149.5, 166.2, and 167.1) constructed an α-pyrone moiety with a hydroxy at C-4 position, according to the HMBC cross-peaks from H-6 (δH 7.59) to C-2 (δC 166.2), C-4 (δC 167.2), C-5 (δC 120.4), and C-6', as well as their chemical shifts and the molecular formula. Therefore, the structure of 3 was elucidated to be 4-hydroxy-5-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-2H-pyran-2-one, which was named asperphenylpyrone. It is of note that 3 was the first example of the α-pyrone derivatives bearing two phenyl units at C-3 and C-5, respectively.

\[
\delta = 1.0144x - 0.5566
\]

\[
R^2 = 0.9992
\]

![Graph](image-url)

**Figure 4.** Experimental (Exp.) and calculated (Cal.) ECD spectra of 2 in methanol at the B3LYP/6-311+G(2d,p) level.

![Diagram](image-url)

**Figure 5.** Selected COSY and HMBC correlations of compounds 3 and 4.
Table 2. $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR spectroscopic data of 3 and 4.

| No. | $\delta$C | $\delta$H | No. | $\delta$C | $\delta$H |
|-----|----------|----------|-----|----------|----------|
| 2   | 166.2 C  |          | 2   | 156.6 C  |          |
| 3   | 107.1 CH |          | 3   | 144.8 C  |          |
| 4   | 167.1 C  |          | 4   | 113.4 CH | 7.49 s   |
| 5   | 120.4 C  | 4a       | 5   | 120.4 C  |          |
| 6   | 149.5 CH | 7.59 s   | 5a  | 128.9 CH | 8.23 d (1.8) |
| 1'  | 131.9 CH | 7.29 d (8.5) | 6   | 127.8 C  |          |
| 2'  | 116.3 CH | 6.85 d (8.5) | 7   | 129.5 CH | 7.95 dd (8.6, 1.8) |
| 3'  | 158.9 C  | 8        | 8   | 116.5 CH | 7.45 d (8.6) |
| 4'  | 116.3 CH | 6.85 d (8.5) | 8a  | 152.2 C  |          |
| 5'  | 131.9 CH | 7.29 d (8.5) | 9   | 166.9 C  |          |
| 6'  | 123.5 C  |          | 9a  | 133.2 CH | 7.33 d (8.6) |
| 1'' | 133.2 CH | 7.33 d (8.6) | 10  | 166.5 C  |          |
| 2'' | 115.1 CH | 7.01 d (8.6) | 3   | 120.4 C  |          |
| 3'' | 160.9 C  |          | 4   | 115.1 CH | 7.01 d (8.6) |
| 4'' | 133.2 CH | 7.33 d (8.6) | 5   | 124.6 C  |          |
| 5'' | 124.6 C  |          | 6   | 155.7 CH3 | 3.85 s   |
| OMe | 55.7 CH3 |          |     | 56.8 CH3 | 3.86 s   |

* Measured in CD$_3$OD.  
* Measured in DMSO-<i>d</i>_6.  
* Assignments in column could be interchanged.

Compound 4 has the molecular formula of C$_{11}$H$_8$O$_5$ as determined by the positive HRESIMS spectrum (m/z 243.0268, [M + Na]$^+$) and the $^{13}$C NMR data, indicating eight degrees of unsaturation. The $^1$H NMR spectrum showed three aromatic protons ($\delta$H 7.45 (d, $J$ = 8.6 Hz, H-8), 7.95 (dd, $J$ = 8.6, 1.8 Hz, H-7), and 8.23 (d, $J$ = 1.8 Hz, H-5)) for a 1,2,4-trisubstituted benzene ring, a singlet olefinic proton ($\delta$H 7.49), and one methoxy ($\delta$H 3.86), whereas the $^{13}$C NMR spectrum exhibited 11 carbon signals, including six aromatic carbons ($\delta$C 116.5, 120.4, 127.8, 128.9, 129.5, and 152.2) for a benzene ring, two olefinic carbons ($\delta$C 113.4, 144.8) for a double bond, two ester carbonyl carbons (156.6, 166.9), and a methoxy group ($\delta$C 56.8). The aforementioned NMR data were very similar to those of the known coumarin derivative [41], with the exception of the presence of an additional aromatic methine ($\delta$H 7.95, $\delta$C 129.5) in 4 instead of an oxygenated nonprotonated sp$^2$ carbon. The additional olefinic proton was appointed at C-7 ($\delta$C 129.5) on the basis of the COSY cross-peaks from H-7 ($\delta$H 7.95) to H-8 ($\delta$H 7.45) and H-5 ($\delta$H 8.23) as well as the HMBC correlations from H-5 to C-4 (C$_{115.3}$), C-7/C-9 ($\delta$C 166.9)/C$_{8a}$ ($\delta$C 152.2) and from H-7 to C-5 (C$_{128.9}$), C-9, and C-8a (Figure 5). Thus, the structure of 4 was established as a 7-deoxylated derivative of the above known compound, which was given the trivial name of aspercoumarine acid.

In addition, seven known compounds were established to be pestalotiolactone A (5) [42,43], 3,7-dihydroxy-1,9-dimethyldibenzofuran (6) [44], diorcinol (7) [45], cordyol C (8) [46], 4-(3',4'-dihydroxyphenyl)-2-butanone (9) [47], 3,4,5-trimethoxybenzoic acid (10) [48], and 4-hydroxybenzoic acid (11) [49] on the basis of comparison of their NMR and specific rotation data with those reported in the literature.

Compound 1 was a novel chained monoterpoid. The plausible biosynthetic pathway for 1 and 2 was proposed (Scheme 1). Starting from the GPP, hydrosis, oxygenation, and cyclization reaction occurrence constructed the monocyclic ring monoterpoid osmane. The osmane might undergo carbon–carbon bond cleavage to form a key intermediate A, which was further oxygenated to yield 1. Additionally, the osmane might also undergo oxygenation to form 2.
with a model 201 variable wavelength UV were measured using a Chirascan spectrometer (Applied Photophysics inc., Leatherhead, Surrey, UK). Autopol IV automatic polarimeter (Rudolph Research Analytical, Newburgh, NY, USA). ECD spectra (TMS) as an internal standard. Semi-preparative HPLC was performed using an Alltech LS class pump column (YMC Co., Ltd. Kyoto, Japan) was used for the purification. Column chromatography (CC) HRESIMS data were measured using a Xevo G2 Q-TOF mass spectrometer (Waters, Milford, MA, USA). Optical rotation data were measured on the basis of the Rudolph Instruments Inc., Shanghai, China). Interestingly, compound 6 (10 µM) showed potent anti-inflammatory activities with the inhibition rate of 94.4%.

Table 2. 1H (400 MHz) and 13C (100 MHz) NMR spectroscopic data of 3. Interestingly, compound 6 (10 µM) showed potent anti-inflammatory activities with the inhibition rate of 94.4%.

| Compounds | Anti-NO (%) | Cell Viability Inhibition (%) |
|-----------|-------------|-------------------------------|
|           | 20 µM | 10 µM | 20 µM | 10 µM |
| 1         | 33.5 ± 1.5 | 10.2 ± 2.0 | 0.7 ± 0.1 | 0.3 ± 1.2 |
| 2         | 34.0 ± 1.4 | 22.7 ± 1.4 | 4.6 ± 2.6 | -1.1 ± 0.4 |
| 3         | 22.7 ± 1.5 | 13.2 ± 1.3 | 4.1 ± 7.6 | 3.3 ± 3.1 |
| 4         | 28.3 ± 0.7 | 18.0 ± 2.0 | 3.5 ± 3.7 | 2.01 ± 1.4 |
| 5         | 39.1 ± 1.6 | 25.1 ± 0.8 | 10.0 ± 0.2 | 3.2 ± 1.7 |
| 6         | 101.4 ± 2.4 | 94.4 ± 0.0 | -1.6 ± 5.1 | -0.8 ± 3.6 |
| 7         | 55.0 ± 1.4 | 35.4 ± 2.4 | 4.1 ± 3.8 | -1.5 ± 3.5 |
| 8         | 30.7 ± 0.8 | 18.0 ± 0.8 | 1.8 ± 4.7 | -0.1 ± 8.2 |
| 9         | 39.3 ± 0.7 | 30.4 ± 1.9 | 2.3 ± 0.1 | -1.1 ± 3.7 |
| 10        | 44.8 ± 0.7 | 33.0 ± 0.7 | 0.4 ± 1.3 | 0.2 ± 1.8 |
| 11        | 42.7 ± 1.3 | 30.8 ± 2.6 | 1.9 ± 2.4 | -0.5 ± 3.1 |

3. Materials and Methods

3.1. General Experimental Procedures

UV spectra were recorded using a UV8000 UV/Vis spectrophotometer (Shanghai Metash Instruments Inc., Shanghai, China). Optical rotation data were measured on the basis of the Rudolph Autopol IV automatic polarimeter (Rudolph Research Analytical, Newburgh, NY, USA). ECD spectra were measured using a Chirascan spectrometer (Applied Photophysics inc., Leatherhead, Surrey, UK). HRESIMS data were measured using a Xevo G2 Q-TOF mass spectrometer (Waters, Milford, MA, USA). The 1H, 13C, HSQC, COSY, HMBC, and NOESY spectra were measured on the basis of the Bruker Avance 400 FT NMR spectrometer (Bruker Company, Fällanden, Switzerland) with tetramethylsilicone (TMS) as an internal standard. Semi-preparative HPLC was performed using an Alltech LS class pump with a model 201 variable wavelength UV/Vis detector, and a YMC packed ODS-A (250 × 10 mm, 5 µm) column (YMC Co., Ltd. Kyoto, Japan) was used for the purification. Column chromatography (CC)
was carried out using a Sephadex LH-20 (Amersham Biosciences, San Francisco, CA, USA), ODS-A-HG (YMC Co., Ltd. Kyoto, Japan), and silica gel (Qingdao Marine Chemistry Co., Ltd., Qingdao, China). The TLC analyses were carried out with the precoated silica gel plates by heating after spraying with vanillin sulfuric acid chromogenic reagent (Xilong Scientific Co., Ltd., Shantou, China).

3.2. Fungal Material and Identification

The fungus was isolated from the deep-sea sediment at the depth of 2246 m sampling from the South Atlantic Ocean (W13.6639°, S14.2592°), and it was identified to be Aspergillus sydowii on the basis of the morphology and the internal transcribed spacer (ITS) region of the rDNA sequence. The ITS gene sequence was deposited in GenBank and assigned the accession no. MN918102. The fungus was preserved at the Marine Culture Collection of China (MCCC), and assigned the accession no. MCCC 3A00324. Therefore, the producing fungus was named Aspergillus sydowii MCCC 3A00324.

3.3. Fermentation, Extraction, and Isolation

The fungus was cultivated in a potato dextrose agar (PDA) plate under 25 °C for four days, and then the fresh mycelia and spores were inoculated into 500 mL Erlenmeyer flasks (×2), each containing 100 mL potato dextrose broth (PDB) medium and followed by cultivation in a rotary shaker under 25 °C at 200 rpm for four days. The seed cultures were subsequently inoculated to 30 Erlenmeyer flasks (1 L) (each containing 80 g rice and 120 mL sea water) after autoclaving at 121 °C for 22 min. The fermentation was performed under static conditions at 25 °C for 26 days.

The fermented material was fragmented using a stick and extracted successively with EtOAc three times, and then evaporated under reduced pressure to get an EtOAc extract (16.2 g). The extract was subjected to the vacuum liquid chromatography column on silica gel eluting with a gradient of CH$_2$Cl$_2$ and MeOH (1:0 to 0:1) to furnish fractions A and B. The fraction B (8.5 g) was subsequently separated by CC on ODS with MeOH/H$_2$O elution (30–100%) to obtain fourteen fractions (Fr.1-Fr.14). Fraction Fr.3 (147 mg) was separated by CC on silica gel eluting with petroleum ether (PE)/EtOAc (10:1) to yield two subfractions. The former was further purified by semi-preparative HPLC with the mobile phase of MeOH/H$_2$O (3:7) to yield 2 (1.8 mg) and 5 (21.0 mg), whereas the latter was separated by semi-preparative HPLC (CH$_3$CN/H$_2$O, 1:9) to get 11 (0.5 mg). Fraction Fr.5 (333 mg) was chromatographed by CC on silica gel eluting with CH$_2$Cl$_2$/MeOH (30:1) to furnish two subfractions, whereas the former was further separately purified by semi-preparative HPLC using CH$_3$CN/H$_2$O (19:81) elution to obtain 10 (4.0 mg), and the latter using MeOH/H$_2$O (7:18) elution to yield 4 (3.0 mg). Fraction Fr.7 (489 mg) was subjected to silica gel CC with CH$_2$Cl$_2$ and MeOH gradient elution (1:0→0:1) to yield two subfractions (Fr.7-1 and Fr.7-2). Subfraction Fr.7-1 was separated by semi-preparative HPLC purification (CH$_3$CN/H$_2$O, 23:77) to get 1 (1.4 mg) and 3 (3.8 mg). Subfraction Fr.7-2 was purified by semi-preparative HPLC (CH$_3$CN/H$_2$O, 37:63) to yield 9 (1.2 mg). Fraction Fr.11 (146 mg) was separated by silica gel CC eluting with CH$_2$Cl$_2$/acetone gradient from 1:0 to 0:1 to furnish two subfractions, whereas the former was further separated using CC on a Sephadex LH-20 (MeOH) and semi-preparative HPLC (CH$_3$CN/H$_2$O, 3:7) to obtain 7 (18.5 mg) and 8 (3.0 mg), respectively. Fraction Fr.13 (545 mg) was subjected to CC on silica gel eluting with PE/EtOAc (1:0→0:1) to get four subfractions (Fr.13-1→Fr.13-4). Subfraction Fr.13-1 was subjected to semi-preparative HPLC with the mobile phase of CH$_3$CN/H$_2$O (39:61) to yield 6 (1.0 mg).

Aspermonoterpenoid A (1): colorless oil; [α]$_D^{25}$ +54 (c 0.06, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 223 (1.59) nm; $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS m/z 221.0784 [M + Na]$^+$ (calcd. for C$_{10}$H$_{14}$O$_4$Na, 221.0790).

Aspermonoterpenoid B (2): colorless oil; [α]$_D^{25}$ −38 (c 0.07, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 229 (0.96) nm; ECD (MeOH) $\lambda_{max}$ (Δε) 199 (-21.88), 222 (-5.77), 231 (-13.46), 260 (-0.15) nm; $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS m/z 205.0845 [M + Na]$^+$ (calcd. for C$_{10}$H$_{14}$O$_3$Na, 205.0841).
Asperphenylpyrone (3): colorless oil; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 204 (1.67), 248 (0.85), 315 (0.28) nm; $^1$H and $^{13}$C NMR data, see Table 2; HRESIMS m/z 333.0741 [M + Na]$^+$ (calcd. for C$_{18}$H$_{14}$O$_5$Na, 333.0739).

Aspercoumarine acid (4): white powder; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 204 (0.38), 296 (0.15) nm; $^1$H and $^{13}$C NMR data, see Table 2; HRESIMS m/z 243.0268 [M + Na]$^+$ (calcd. for C$_{11}$H$_8$O$_5$Na, 243.0269).

3.4. BV-2 Cell Culture and Treatment

The BV-2 microglia cells were cultured in DMEM medium containing 10% fetal bovine serum and antibiotics (100 units/mL of penicillin and 100 g/mL of streptomycin) and maintained in a humidified 5% CO$_2$ incubator (Beijing Luxi Technology Co., Ltd., Beijing, China) at 37 °C. For the experiment, cells were seeded into 24-well plates (2 × 10$^5$ cells/well) overnight. Next day, cells were incubated with fresh culture medium containing indicated concentration of the tested compounds for half an hour and following LPS treatment (1 µg/mL). Cells were treated vehicle (DMSO, 0.1%) as control.

3.5. Nitrite Quantification

The concentration of nitrite in culture medium was determined using a Griess Reagent Kit (Thermo Fisher, Shanghai, China). Briefly, 75 µL of cell culture supernatants were reacted with an equal volume of Griess Reagent Kit for 30 min at room temperature, and absorbance of diazonium was obtained at a wavelength of 560 nm. Nitrite production by vehicle stimulation was designated as 100% inhibition compared to LPS stimulation for the experiment.

3.6. Computational Details

3.6.1. $^{13}$C NMR Calculation of 2

Conformational searches were carried out using the Maestro 10.2 program (Schrödinger Inc., NY, USA) at the OPLS3 molecular mechanics force field within an energy window of 3.0 kcal/mol. The results exhibited 8 conformers for (4$^S$*,5$^R$*,6$^S$*)-2 and (4$^S$*,5$^R$*,6$^R$*)-2, respectively. The conformers were further optimized at the B3LYP/6-31+G(d,p) level in gas phase using Gaussian 09 (Gaussian, Inc., Wallingford, CT, USA) [38]. The conformers with a Boltzmann population over 1% were selected for the NMR calculations. The NMR data were calculated by the GIAO method at the mPW1PW91/6-311+G(2d,p) level with the IEFPCM model in methanol. Finally, the calculated NMR data were averaged according to the Boltzmann distribution for each conformer and then fitted to the experimental values by linear regression.

3.6.2. ECD Calculation of 2

The eight conformers of (4$^S$*,5$^R$*,6$^S$*)-2 were optimized by density functional theory (DFT) calculations at the B3LYP/6-31+G(d,p) level in gas phase using Gaussian 09. The energies, oscillator strengths, and rotational strengths of the first 60 electronic excitations were calculated by the TDDFT method at the B3LYP/6-311+G(2d,p) level in methanol. The ECD spectrum was simulated using SpecDis (version1.70, Berlin, Germany) by applying the Gaussian band shapes with sigma = 0.3 eV. Finally, the calculated ECD data were weighted and then summed up each stable conformer on the basis of the Boltzmann distribution.

4. Conclusions

In summary, four new compounds, including one novel (1) and one new (2) monoterpenoids and two new polyketides (3 and 4), were obtained from the EtOAc extract of the deep-sea-derived fungus Aspergillus sydowii MCCC 3A00324, together with seven known compounds (5–11). The structures of metabolites were determined by comprehensive analyses of the NMR and HRESIMS spectra, in association with quantum chemical calculations of the ECD, $^{13}$C NMR, and specific rotation data for their configurational assignment. Compound 1 possessed a novel monoterpenoid skeleton,
biogenetically probably derived from the osmane-type monoterpenoid after the cyclopentane ring cleavage and oxidation reactions. Additionally, 2 was the first osmane-type monoterpenoid representative discovered from the fungi, while 3 was the first example of the α-pyrones derivatives bearing two phenyl units at C-3 and C-5, respectively, indicating that the deep-sea-derived fungi are a unique source of the structurally novel compounds. Compound 6 exhibited significant inhibitory effects against NO secretion in LPS-activated BV-2 microglia cells (94.4% inhibition rate, 10 µM), suggesting the potential application for the anti-inflammatory agents.

**Supplementary Materials:** The following are available online at [http://www.mdpi.com/1660-3397/18/11/561/s1](http://www.mdpi.com/1660-3397/18/11/561/s1), Figure S1: The HPLC and TLC metabolic profile of the study fungus, Figures S1-1–S4-7: HRESIMS, 1H, 13C, HSQC, COSY, and HMBC spectra of new compounds 1–4, and NOESY spectra of 1 and 2.

**Author Contributions:** S.N., Z.S., and S.P. isolated and identified the fungus. S.N. isolated and elucidated the structures. L.Y. and T.C. performed the anti-inflammatory assay. B.H. and G.Z. designed and coordinated the study. S.N., B.H., and G.Z. wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was financially supported by the Scientific Research Foundation of the Third Institute of Oceanography (2018018), the National Natural Science Foundation of China (81901133), and the COMRA program (DY135-B2-08).

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

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