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# A New Polyketide Derivative from the *Nicotiana tabacum* Symbiotic Fungus *Aspergillus japonicus* TE-739D

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**Abstract:** Filamentous fungi are very well known for producing a wide variety of secondary metabolites with important biological effects. In our study chemical exploration of the *Nicotiana tabacum* symbiotic fungus *Aspergillus japonicus* TE-739D led to the discovery of a new polyketide derivative, namely (3*S*,4*E*,6*E*,9*S*)-9-hydroxy-3,7-dimethyldeca-4,6-dienoic acid (**1**), along with three previously reported compounds **2**−**4**. The structures of these compounds were elucidated by using HRESIMS, NMR spectroscopic analyses, and quantum chemical calculations. Compounds **3** and **4** showed strong antibacterial activity against four representative bacterial strains including two Gram-negative species (*Agrobacterium tumefaciens* and *Xanthomonas oryzae*) and two Gram-positive *Bacillus* species (*B. cereus* and *B. subtilis*), with MIC values range from 1 to 16 μg/mL, respectively.

**Keywords:** Secondary metabolites; *Aspergillus japonicus*; polyketides; antibacterial activity. 

## 1. Fungal Source

The fungal strain *Aspergillus japonicus* TE-739D was isolated from the healthy leaves of *Nicotiana tabacum* grown in Enshi, Hubei Province (People's Republic of China). Based on the phylogenetic analyses of the 28S and internal transcribed spacer (ITS) rDNA regions, this fungus was identified as *A. japonicus*, which has been deposited in GenBank (NCBI) with the number of PP126510. The fungal strain was deposited in the China General Microbiological Culture Collection Center (CGMCC No. 40901).

## 2. Previous Studies

In recent years, plants-sourced endophytic/symbiotic fungi have gained attention for their ability to produce a wide range of bioactive secondary metabolites [1,2]. As a common group of filamentous fungi, *Aspergillus* species found in many environements are particularly noteworthy for their

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remarkable ability to create chemically diverse secondary metabolites [3]. These compounds exhibit broad bioactivities, including antimicrobial, antitumor, and herbicidal properties [4,5]. Our prior research on the *Nicotiana tabacum*-derived fungus *A. japonicus* identified two new polyketide derivatives and two cyclohexadepsipeptides [6,7], which demonstrated selective biological activities.

## 3. Present Study

In our ongoing investigations of natural products with intriguing structures from *Aspergillus* genus, chemical investigations of *A. japonicus* TE-739D yielded a new polyketide derivative (**1**) and three known analogs (**2**−**4**). The structural elucidation of **1**, which was identified as (3*S*,4*E*,6*E*,9*S*)-9-hydroxy-3,7-dimethyldeca-4,6-dienoic acid, was achieved through HRESIMS, NMR analyses, and quantum chemical calculations. Antimicrobial evaluation against Gram-positive (*Bacillus cereus* ATCC14579, *B. subtilis* ATCC 11562) and Gram-negative (*Agrobacterium tumefaciens* ATCC 11158, *Xanthomonas oryzae*) strains revealed potent activity against both two Gram-negative and two Gram-positive bacteria for **3** and **4** (MIC: 1−16 μg/mL), with exceptional efficacy against Gram-negative pathogens (MIC = 1 μg/mL).

The fungal strain *A. japonicus* TE-739D was cultured in PDA medium at 28 °C for five days. The agar was cut into small pieces (0.5 × 0.5 cm), which were subsequently transferred to 290 flasks each containing 0.4 L of Potato Dextrose Water medium at 28 °C for 30 days. After cultivation, the fermentation product was extracted with ethyl acetate (EtOAc) and the crude extract (68.8 g) was obtained with evaporated under vacuum. The extract was eluted with a stepwise petroleum ether (PE)−EtOAc mixtures (100:0, 90:10, 80:20, 70:30, 50:50, 30:70, and 0:100, v/v) to yield Fr.1−Fr.6. Fr.4 (eluted with PE−EtOAc 0:100) was further separated using reverse silica gel column chromatography (CC) with a gradient elution of MeOH−$H_2O$ (30:70−100:0, v/v) to yield six subfractions (Fr.4.1−Fr.4.6). Fr.4.2 was then separated by semi-preparative HPLC using MeCN−$H_2O$ (35:65, v/v) as an eluent to afford compound **1** (1.7 mg, $t_R$ 6.120 min).

*(3S,4E,6E,9S)-9-hydroxy-3,7-dimethyldeca-4,6-dienoic acid (**1**):* colorless oil; UV (MeOH): $\lambda_{max}$ 239 nm; $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data, see Table 1; HRESIMS: *m/z* 213.1490 $[M + H]^+$ (calcd for $C_{12}H_{21}O_3^+$, 213.1485).

*Antibacterial Assay:* The antibacterial activities of the compounds **1**−**4** were evaluated against two Gram-positive bacteria (*Bacillus cereus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Agrobacterium tumefaciens* and *Xanthomonas oryzae*). Chloramphenicol was used as a positive control. The minimum inhibitory concentrations (MICs) of each compound were determined by broth micro-dilution method with certain modifications [6].

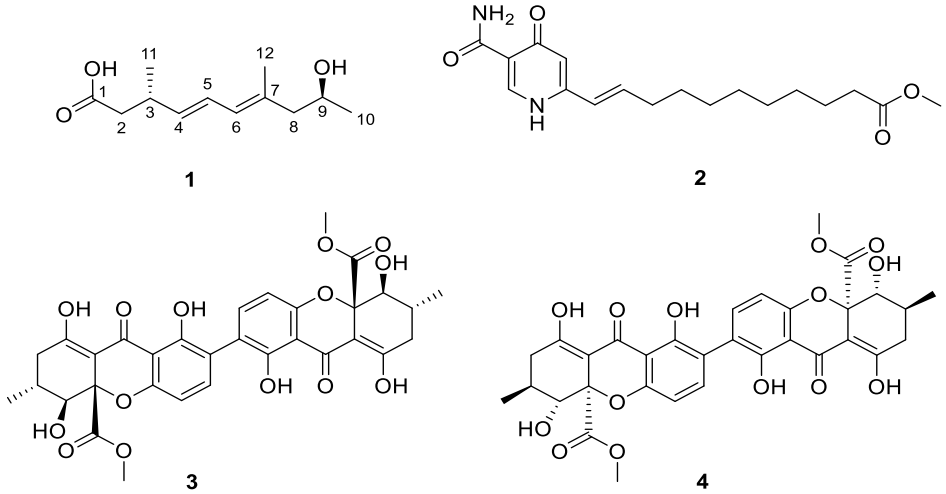


**Figure 1.** Structures of the isolated compounds **1**−**4**

Compound **1** was obtained as a colorless oil. Its molecular formula of $C_{12}H_{20}O_3$ was identified by the high-resolution electrospray ionization mass spectroscopy (HRESIMS) spectrum at *m*/*z* 213.1490 $[M + H]^+$ (calcd. for $C_{12}H_{21}O_3^+$, 213.1485) (Figure S1), implying three degrees of unsaturation. The $^1$H NMR data along with the HSQC spectrum (Table 1 and Figure S5) of **1** showed three olefinic protons resonating at $\delta_H$ 6.21 (1H, ddd, *J* = 15.2, 11.1, 1.2 Hz, H-5), 5.73 (1H, d, *J* = 11.1 Hz, H-6) and 5.50 (1H, dd, *J* = 15.2, 7.3 Hz, H-4), one oxygenated methine proton at $\delta_H$ 3.75 (1H, p, *J* = 6.3 Hz, H-9), one non-oxygenated methine proton at $\delta_H$ 2.60 (1H, m, H-3), two methylene groups at $\delta_H$ 2.21 (1H, dd, *J* = 15.0, 7.0 Hz, H-2a), 2.17 (1H, m, H-2b), and 2.11 (1H, dd, *J* = 13.3, 6.9 Hz, H-8a), 1.97 (1H, dd, *J* = 13.3, 6.2 Hz, H-8b), as well as three methyl groups at $\delta_H$ 1.69 (3H, s, $H_3$-12), 1.00 (3H, d, *J* = 6.8 Hz, $H_3$-10), 0.96 (3H, d, *J* = 6.1 Hz, $H_3$-11). The $^{13}$C NMR data (Table 1) combined with HSQC spectrum revealed 12 carbon resonances, including an ester carbonyl at $\delta_C$ 173.5 (C-1), four olefinic carbons at $\delta_C$ 136.3 (C-4), 134.6 (C-7), 126.3 (C-6) and 124.9 (C-5), one oxygenated methine at 64.7 (C-9), two aliphatic methylenes at $\delta_C$ 49.6 (C-8), 41.5 (C-2), one aliphatic methine at $\delta_C$ 33.2 (C-3), and three methyl groups at $\delta_C$ 23.5 (C-10), $\delta_C$ 20.1 (C-11) and $\delta_C$ 16.9 (C-12).

The planar structure of compound **1** was determined by 2D NMR analyses. According to the HMBC correlations from $H_2$-2 to C-1/C-3/C-4/C-11, from H-3 to C-1/C-2/C-4/C-5/C-11, from H-4 to C-2/C-3/C-6/C-11, from H-5 to C-3/C-6/C-7, from H-6 to C-4/C-5/C-8/C-12, from $H_2$-8 to C-6/C-9/C-10, from $H_3$-10 to C-8/C-9, from $H_3$-11 to C-2/C-3/C-4, and from $H_3$-12 to C-6/C-7/C-8, the planar structure of compound **1** was elucidated as a straight chain polyketide as shown in Figure 2. The large coupling constants (15.2 Hz) between H-4 and H-5 supported the *E*-configuration of the double bond C-4/C-5. Due to lack of the key NOE correlations, the configurations of C-6/C-5 could not be determined. In order to address the configuration, the GIAO $^{13}$C NMR calculations at the mpw1pw91/6-311+g(2d,p) (PCM = DMSO)//B3LYP/6-31+G(d,p) level of theory using the reported procedure and scaling parameters [8] revealed that (4*E*,6*E*)-3*S**9*S**-**1** fitted best to the experimental data, for having the smallest MAE/RMSD values (1.09/2.11) compared to the remaining candidate structures (Tables 2 and 3).

The absolute configuration of **1** (four candidate structures: (4*E*,6*E*)-3*S*9*S*-**1**; (4*E*,6*E*)-3*S*9*R*-**1**; (4*E*,6*E*)-3*R*9*S*-**1**; (4*E*,6*E*)-3*R*9*R*-**1**) was determined by ECD calculation at the B3LYP/6-31+g(d)//B3LYP/6-31+g(d,p) level by using the solvent model (PCM = MeOH). The calculated ECD spectrum of 3*S*,9*S*-**1** matched the experimental ECD well (Figure 3). Therefore, the absolute configuration of **1** was assigned, and **1** was named (3*S*,4*E*,6*E*,9*S*)-9-hydroxy-3,7-dimethyldeca-4,6-dienoic acid.

In addition, the known compounds were identified based on the literature data as himeic acid G (**2**) [9], secalonic acid A (**3**) [10], and secalonic acid D (**4**) [10].

**Table 1.** $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR data of compound **1** ($\delta$ in ppm) in DMSO-$d_6$

| No | $\delta_H$ (*J* in Hz) | $\delta_C$, type |
|---|---|---|
| 1 | | 173.5 C |
| 2 | 2.21 dd (15.0, 7.0) | 41.5 $CH_2$ |
| | 2.17 m | |
| 3 | 2.60 m | 33.2 CH |
| 4 | 5.50 dd (15.2, 7.3) | 136.3 CH |
| 5 | 6.21 ddd (15.2, 11.1, 1.2) | 124.9 CH |
| 6 | 5.73 d (11.1) | 126.3 CH |
| 7 | | 134.6 C |
| 8 | 2.11 dd (13.3, 6.9) | 49.6 $CH_2$ |
| | 1.97 dd (13.3, 6.2) | |
| 9 | 3.75 p (6.3) | 64.7 CH |
| 10 | 1.00 d (6.8) | 23.5 $CH_3$ |
| 11 | 0.96 d (6.1) | 20.1 $CH_3$ |
| 12 | 1.69 s | 16.9 $CH_3$ |

A new cytotoxic drimane sesquiterpene

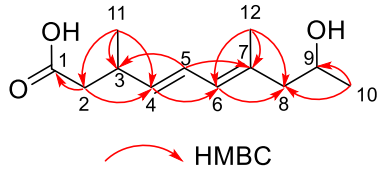


**Figure 2**. Key HMBC correlations for **1**

**Table 2.** GIAO $^{13}$C NMR calculation of four candidate configurations for 4*E*,6*E*-**1**

| Position | Exp. $\delta_C$ | Cal. $\delta_C$ [a] (3*R**9*R**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*R**9*S**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*S**9*R**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*S**9*S**-1) | Δ$\delta$ (Cal. − Exp.) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 173.5 | 172.0 | -1.5 | 172.2 | -1.3 | 172.2 | -1.3 | 172.0 | -1.5 |
| 2 | 41.5 | 42.9 | 1.4 | 46.9 | 5.4 | 44.5 | 3.0 | 43.1 | 1.6 |
| 3 | 33.2 | 36.4 | 3.2 | 41.5 | 8.3 | 38.6 | 5.4 | 36.8 | 3.6 |
| 4 | 136.3 | 135.3 | -1.0 | 137.2 | 0.9 | 136.2 | -0.1 | 135.7 | -0.6 |
| 5 | 124.9 | 129.0 | 4.1 | 131.6 | 6.7 | 130.8 | 5.9 | 128.9 | 4.0 |
| 6 | 126.3 | 126.2 | -0.1 | 127.7 | 1.4 | 126.8 | 0.5 | 126.5 | 0.2 |
| 7 | 134.6 | 142.1 | 7.5 | 143.8 | 9.2 | 143.3 | 8.7 | 142.1 | 7.5 |
| 8 | 49.6 | 49.5 | -0.1 | 52.7 | 3.1 | 50.6 | 1.0 | 50.0 | 0.4 |
| 9 | 64.7 | 64.8 | 0.1 | 67.7 | 3.0 | 65.7 | 1.0 | 65.2 | 0.5 |
| 10 | 23.5 | 21.8 | -1.7 | 25.1 | 1.6 | 22.4 | -1.1 | 22.3 | -1.2 |
| 11 | 20.1 | 17.0 | -3.1 | 25.2 | 5.1 | 21.3 | 1.2 | 17.7 | -2.4 |
| 12 | 16.9 | 15.8 | -1.1 | 20.4 | 3.5 | 17.5 | 0.6 | 16.4 | -0.5 |
| | | RMSD | 2.17 | RMSD | 3.63 | RMSD | 2.66 | RMSD | 2.11 |
| | | MAE | 1.19 | MAE | 1.50 | MAE | 1.17 | MAE | 1.09 |

[a] $^{13}$C NMR calculations were performed at the mpw1pw91/6-311 + G(2d,p) (PCM = MDSO)//B3LYP/6-31 + G(d,p) level.

**Table 3.** GIAO $^{13}$C NMR calculation of four candidate configurations for 4*E*,6*Z*-**1**

| Position | Exp. $\delta_C$ | Cal. $\delta_C$ [a] (3*R**9*R**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*R**9*S**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*S**9*R**-1) | Δ$\delta$ (Cal. − Exp.) | Cal. $\delta_C$ [a] (3*S**9*S**-1) | Δ$\delta$ (Cal. − Exp.) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 173.5 | 173.5 | 0.0 | 174.7 | 1.2 | 174.7 | 1.2 | 173.5 | 0.0 |
| 2 | 41.5 | 40.2 | -1.3 | 56.0 | 14.5 | 56.0 | 14.5 | 40.2 | -1.3 |
| 3 | 33.2 | 37.7 | 4.5 | 46.3 | 13.1 | 46.2 | 13.0 | 37.7 | 4.5 |
| 4 | 136.3 | 137.7 | 1.4 | 140.9 | 4.6 | 140.9 | 4.6 | 137.7 | 1.4 |
| 5 | 124.9 | 125.7 | 0.8 | 133.6 | 8.7 | 133.6 | 8.7 | 125.7 | 0.8 |
| 6 | 126.3 | 128.8 | 2.5 | 130.7 | 4.4 | 130.7 | 4.4 | 128.8 | 2.5 |
| 7 | 134.6 | 138.5 | 3.9 | 143.5 | 8.9 | 143.5 | 8.9 | 138.5 | 3.9 |
| 8 | 49.6 | 41.9 | -7.7 | 50.9 | 1.3 | 50.9 | 1.3 | 41.9 | -7.7 |
| 9 | 64.7 | 66.0 | 1.3 | 78.0 | 13.3 | 77.9 | 13.2 | 66.0 | 1.3 |
| 10 | 23.5 | 21.0 | -2.5 | 32.1 | 8.6 | 32.0 | 8.5 | 21.0 | -2.5 |
| 11 | 20.1 | 19.0 | -1.1 | 32.9 | 12.8 | 32.9 | 12.8 | 19.0 | -1.1 |
| 12 | 16.9 | 24.0 | 7.1 | 36.1 | 19.2 | 36.0 | 19.1 | 24.0 | 7.1 |
| | | RMSD | 2.74 | RMSD | 7.86 | RMSD | 7.85 | RMSD | 2.74 |
| | | MAE | 1.52 | MAE | 3.06 | MAE | 3.06 | MAE | 1.52 |

[a] $^{13}$C NMR calculations were performed at the mpw1pw91/6-311 + G(2d,p) (PCM = MDSO)//B3LYP/6-31 + G(d,p) level.

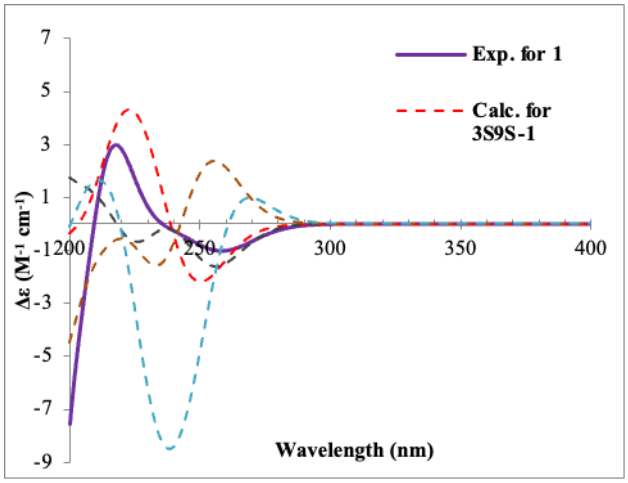


**Figure 3.** Calculated and experimental ECD spectra of **1**

The antibacterial activities of compounds **1**−**4** were assessed against four representative bacterial strains (Table 4). As shown in Table 4, compounds **3** and **4** exhibited potent antibacterial activity against all tested strains, with minimum inhibitory concentration (MIC) values ranging from 1 to 16 μg/mL. Notably, their antimicrobial potency surpassed that of the positive control chloramphenicol under identical experimental conditions. In contrast, compounds **1** and **2** demonstrated no detectable inhibitory effects against any of the tested bacterial (MIC > 128 μg/mL).

**Table 4.** Antibacterial activities of the isolated compounds **1**−**4** (MIC, μg/mL).

| Compound | *A. tumefaciens* | *X. oryzae* | *B. cereus* | *B. subtilis* |
|---|---|---|---|---|
| **1** | >128 | >128 | >128 | >128 |
| **2** | >128 | >128 | >128 | >128 |
| **3** | 1 | 1 | 8 | 2 |
| **4** | 1 | 1 | 16 | 4 |
| Chloramphenicol[a] | 64 | 4 | 16 | 16 |

[a] positive control.

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## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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A new cytotoxic drimane sesquiterpene

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