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Isolation and Characterization of Antibacterial Carotane Sesquiterpenes from *Artemisia argyi* Associated Endophytic *Trichoderma virens* QA-8

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Abstract: Carotane sesquiterpenes are commonly found in plants but are infrequently reported in the fungal kingdom. Chemical investigation of *Trichoderma virens* QA-8, an endophytic fungus associated with the inner root tissue of the grown medicinal herb *Artemisia argyi* H. Lév. and Vaniot, resulted in the isolation and characterization of five new carotane sesquiterpenes trichocarotins I–M (**1–5**), which have diverse substitution patterns, and seven known related analogues (**6–12**). The structures of these compounds were established on the basis of a detailed interpretation of their NMR and mass spectroscopic data, and the structures including the relative and absolute configurations of compounds **1–3**, **5**, **9**, and **10** were confirmed by X-ray crystallographic analysis. In the antibacterial assays, all isolates exhibited potent activity against *Escherichia coli* EMBLC-1, with MIC values ranging from 0.5 to 32 μg/mL, while 7β-hydroxy CAF-603 (**7**) strongly inhibited *Micrococcus luteus* QDIO-3 (MIC = 0.5 μg/mL). Structure-activity relationships of these compounds were discussed. The results from this study demonstrate that the endophytic fungus *T. virens* QA-8 from the planted medicinal herb *A. argyi* is a rich source of antibacterial carotane sesquiterpenes, and some of them might be interesting for further study to be developed as novel antibacterial agents.

Keywords: *Artemisia argyi*; endophytic fungus; *Trichoderma virens*; carotane sesquiterpenes; antibacterial activity

1. Introduction

Artemisia argyi H. Lév. and Vaniot, a traditional medicinal and edible plant in China, is reported to have pleiotropic bioactivities and has been used for the treatment of a variety of diseases, such as amenorrhea, bruising, dysmenorrhea, inflammation, jaundice, malaria, and metrorrhagia [1–3]. This plant has been widely cultivated as a medicinal herb in Qichun county, Hubei province, China. *Artemisia argyi* and its endophytic fungi (*Trichoderma koningiopsis* and *T. virens*) have been the source of a wide range of biologically active natural products [4–9]. A number of bioactive constituents such as flavonoids [4], polysaccharides [5], terpenes [6,7], and polyketides [8,9], have been reported from the plant *A. argyi* and its associated endophytic fungi. Several of these components have been investigated to show anticoagulation, antioxidant, antimicrobial, and anticancer activities [4–10].

Endophytic fungi have recently become an important source of new chemical substances with interesting biological activities. A wide range of compound classes such as alkaloids, quinones, phenols, polyketides, and terpenoids have been reported from various sourced endophytic fungi, and some of these compounds have been evidenced to possess significant biological properties such as antibacterial, anticancer, antifungal, antiinfections, antioxidant, antivirus, and enzymatic inhibitory activities [6,8,9,11–13]. Paclitaxel and penicillin, two famous chemicals that are well developed and marketed, could also be produced by endophytic fungi [12]. Based on the above results, we undertook a chemical investigation into the endophytic fungus from *A. argyi* which resulted in the identification of six cadinane-type sesquiterpenes trichocadinins B–G from *Trichoderma virens* QA-8, an endophytic fungus associated with the inner root tissue of *A. argyi* [6]. Further work on the additional portions of the culture extract led to the identification of 12 carotane sesquiterpenes (**1**–**12**) (Figure 1), with five new (trichocarotins I–M, **1**–**5**) and seven known (**6**–**12**) [14–17] related analogues. Previous studies have shown that carotane sesquiterpenes have pleiotropic bioactivities, such as antifungal activity against *Candida albicans* strains, antifertility, anti-HIV activity, and inhibitory activity on the growth of etiolated wheat coleoptiles [14,17–21]. This paper describes the isolation, structure determination, stereochemical assignment, and antibacterial activities of the isolated compounds, and the results indicates that *T. virens* QA-8, an endophytic fungus from the planted medicinal herb *A. argyi*, has abundant antibacterial carotene sesquiterpenoids.
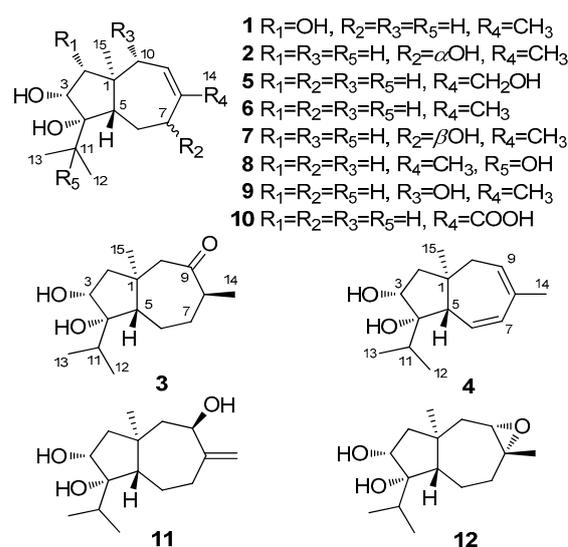
Figure 1. Structures of compounds **1**−**12**.

2. Results and Discussion

2.1. Structural Elucidation of the New Compounds

Compound **1** was purified as colorless crystals and its molecular formula was determined as $C_{15}H_{26}O_3$ on the basis of HRESIMS data, implying three degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **1** indicated the presence of four methyls, three methylenes, five methines (including one olefinic and two oxygenated), and three non-protonated carbons (including one olefinic and one oxygenated) (Tables 1 and 2 and Figures S1 and S2 in the Supplementary Material). Comprehensive analysis of its ^{1}H and ^{13}C NMR data suggested that compound **1** belonged to carotane sesquiterpenes with structural similarity to that of CAF-603 (**6**, Figure 1) and differed from **6** mainly at the five-membered ring [14,15]. Compared to CAF-603 (**6**), resonances for the methylene group at δ_H/δ_C 1.39/50.3 (CH$_2$-2) of **6** disappeared in that of **1**. Instead, signals corresponding to an oxymethine group at δ_H/δ_C 3.09/78.4 (CH-2) were observed in the NMR spectra of **1**. Additionally, a signal for an additional hydroxy group was observed at δ_H 4.16 (s, OH-2) in the ^{1}H NMR spectrum of **1**. These data suggested that the methylene group CH$_2$-2 in **6** was replaced by an oxyme-

thine group in **1**. COSY and HMBC data (Figure 2) supported the above deduction. The planar structure of **1** was thus determined.

Table 1. ^{1}H NMR Data for Compounds **1–5** in DMSO-d_6 (500 MHz).

No.	1	2	3	4	5
2	3.09, d (7.0)	1.41, m (overlap)	1.50, m	1.64, m (overlap)	1.49, m (overlap)
3	3.63, d (7.0)	3.88, m	3.88, dd (7.2, 1.6)	3.87, m	3.88, t (4.4)
5	1.26, dd (11.4, 1.7)	1.41, m (overlap)	1.66, m	1.64, m (overlap)	1.36, m
6α	1.49, m	1.56, m	1.78, m	5.80, d (11.2)	1.30, m
6β	1.37, m	1.49, m	1.78, m		1.49, m (overlap)
7α	2.00, m (overlap)	4.03, m	1.43, m	5.39, d (11.2)	1.81, m (overlap)
7β	2.00, m (overlap)		1.43, m		2.11, dd (14.9, 3.2)
8			2.38, m		
9	5.32, br d (7.0)	5.26, m		5.57, m	5.50, br d (8.2)
10α	1.59, dd (14.4, 1.2)	1.87, dd (14.0, 9.1)	2.43, d (14.4)	2.18, m	1.81, m (overlap)
10β	2.07, dd (14.4, 8.9)	1.68, m	2.30, d (14.4)	2.18, m	1.99, dd (14.4, 8.9)
11	1.66, m	1.74, m	1.70, m	1.64, m (overlap)	1.69, m
12	0.79, d (6.9)	0.81, d (6.9)	0.79, d (6.9)	0.85, d (6.9)	0.81, d (6.9)
13	0.85, d (6.7)	0.88, d (6.9)	0.86, d (6.7)	0.78, d (6.9)	0.87, d (6.7)
14	1.70, s	1.71, s	0.96, d (6.9)	1.74, s	3.76, s
15	0.71, s	0.88, s	1.06, s	0.98, s	0.92, s
2-OH	4.16, s				
3-OH	5.39, s	5.21, d (4.2)	5.35, s		
4-OH	3.53, s	3.69, s	3.76, s		3.60, br s
7-OH		4.66, d (5.1)			

Table 2. ^{13}C NMR Data for Compounds **1–5** in DMSO-d_6 (125 MHz).

No.	1	2	3	4	5
1	44.5, C	40.7, C	40.2, C	44.6, C	41.6, C
2	78.4, CH	49.6, CH$_2$	50.3, CH$_2$	47.9, CH$_2$	50.0, CH$_2$
3	70.6, CH	70.1, CH	70.4, CH	71.8, CH	70.4, CH
4	79.0, C	82.5, C	82.1, C	82.1, C	82.6, C
5	54.3, CH	53.5, CH	55.4, CH	47.2, CH	57.7, CH
6	20.2, CH$_2$	31.7, CH$_2$	18.4, CH$_2$	143.8, CH	21.1, CH$_2$
7	34.1, CH$_2$	72.6, CH	33.0, CH$_2$	125.3, CH	30.0, CH$_2$
8	138.4, C	143.6, C	46.3, CH	129.0, C	142.6, C
9	122.3, CH	119.6, CH	214.3, C	127.4, CH	121.4, CH
10	40.0, CH$_2$	41.5, CH$_2$	57.3, CH$_2$	27.0, CH$_2$	42.1, CH$_2$
11	34.8, CH	34.4, CH	34.7, CH	35.0, CH	34.7, CH
12	17.6, CH$_3$	17.7, CH$_3$	17.6, CH$_3$	17.7, CH$_3$	17.7, CH$_3$
13	17.1, CH$_3$	17.2, CH$_3$	17.1, CH$_3$	17.3, CH$_3$	17.1, CH$_3$
14	27.1, CH$_3$	21.1, CH$_3$	22.8, CH$_3$	26.9, CH$_3$	66.9, CH$_2$
15	13.9, CH$_3$	20.9, CH$_3$	23.2, CH$_3$	21.4, CH$_3$	20.9, CH$_3$
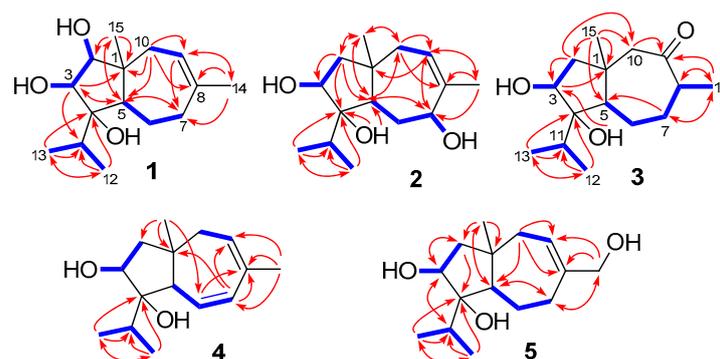
Figure 2. Key HMBC (arrows) and COSY (bold lines) correlations of compounds **1**−**5**.

The relative configuration of **1** was determined by the observed NOEs from H_3-15 to the protons of OH-2 and OH-4, from H-10α to the proton of OH-2, and from H-5 to H-2, H-3, and H-10β. The Cu Kα radiation single-crystal X-ray diffraction experiment resulted in Flack parameter 0.1(4) of **1**, which allowed the establishment of its absolute configuration as 1*S*, 2*R*, 3*R*, 4*S*, and 5*S* (Figure 4). Thus, the structure of **1** was identified and named as trichocarotin I.

Trichocarotin J (**2**) was originally isolated as an amorphous powder. Its molecular formula was also determined as $C_{15}H_{26}O_3$ based on the HRESIMS data. The ^{1}H NMR spectrum of **2** indicated the presence of an olefinic methyl moiety, an aliphatic singlet methyl, and an isopropyl unit, which are typical structural features of the known carotane sesquiternene members. The ^{1}H, ^{13}C, and DEPT NMR data of **2** (Tables 1 and 2 and Figures S8 and S9 in the Supplementary Material) showed almost identical spectral patterns to those of 7β-hydroxy CAF-603 (**7**) [15], with some minor variations for the chemical shifts of C-5 through C-9, and C-14 as well. Detailed inspection of the NMR data suggested that **2** is a diastereomer of **7**, epimeric at C-7. This was supported by the observed NOEs from H-5 to H-3, H-6β, H-7, and H-10β, and from H_3-15 to the protons of OH-3 and OH-4 (Figure 3). Upon slow evaporation of the solvent (MeOH:H_2O = 8:1) by storage in a refrigerator, quality single crystals of compound **2** were obtained. The structure and absolute configuration of **2** were further confirmed by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Figure 4). The Flack parameter 0.06(4) allowed for the establishment of the absolute configuration of **2** as 1*R*, 3*R*, 4*S*, 5*S*, and 7*R*.
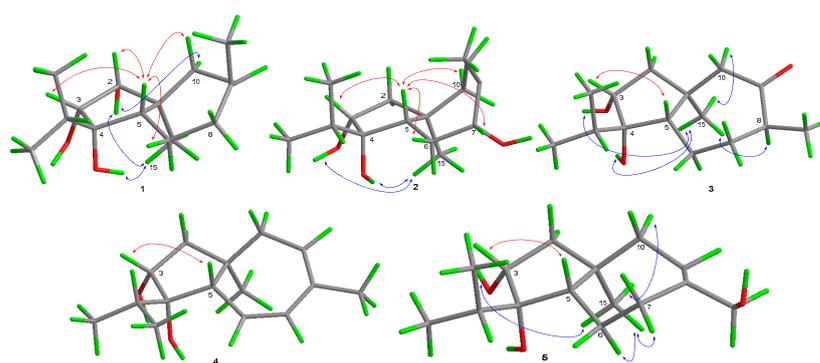
Figure 3. Key NOE correlations of compounds **1**–**5**.
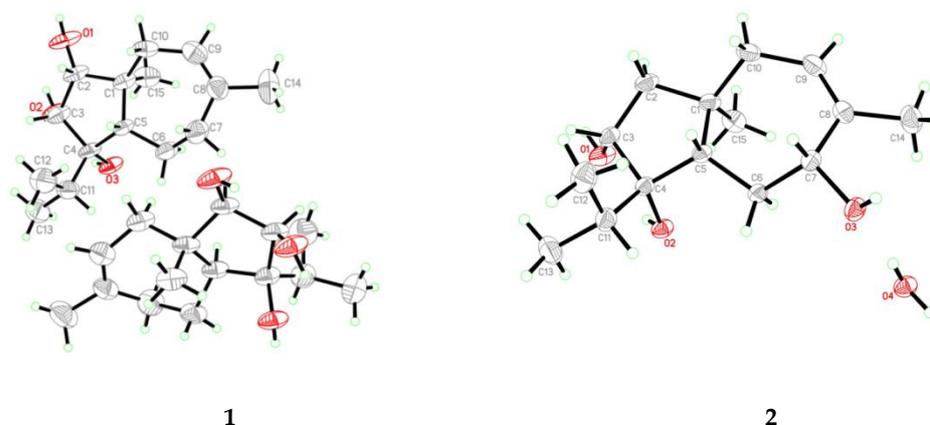
Figure 4. X-ray crystallographic structures of compounds **1** and **2**.

The molecular formula of trichocarotin K (**3**) was determined to be $C_{15}H_{26}O_3$ by HRESIMS. Its ^{1}H and ^{13}C NMR data revealed the presence of four methyls, four methylenes, four methines (one oxygenated), and three non-protonated (one oxygenated and one ketone) carbons (Tables 1 and 2 and Figures S15 and S16 in the Supplementary

Material). A detailed comparison of NMR data revealed that compound **3** differed from CAF-603 (**6**) [14,15] mainly at the seven-membered ring, and that resonances corresponding to the double bond (C-8 and CH-9) in **6** disappeared in that of **3**, while signals for an aliphatic methine (CH-8) and for a keto group (C-9) were observed in the NMR spectra of **3** (Tables 1 and 2). HMBC correlations from H-14 to C-7, C-8, and C-9 confirmed this deduction. Other COSY and HMBC correlations (Figure 2) further confirmed the planar structure of **3**.

The key NOE correlations from H$_3$-15 to H-8, H-10α, and to the protons of OH-3 and OH-4, and from H-3 to H-5 determined the relative configuration of **1** (Figure 3). Upon slow evaporation of the solvent (MeOH) by storing the sample in a refrigerator, quality single crystals of **3** were obtained, and the absolute configuration of **3** was thus determined as 1*S*, 3*R*, 4*S*, 5*S*, and 8*S* by X-ray diffraction analysis (Figure 5).

3   **5**
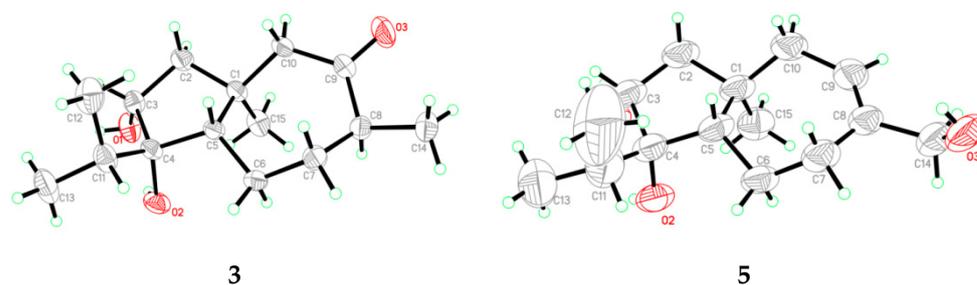
Figure 5. X-ray crystallographic structures of compounds **3** and **5**.

Compound **4** was isolated as a colorless oil and its molecular formula was determined as C$_{15}$H$_{24}$O$_2$ by HRESIMS. Its ^{1}H and ^{13}C NMR data revealed the presence of four methyls, two methylenes, six methines (one oxygenated and three olefinic), and three non-protonated (one oxygenated and one olefinic) carbons (Tables 1 and 2 and Figures S22 and S23 in the Supplementary Material). These data suggested that **4** had the same carbon skeleton as that of CAF-603 (**6**) [14,15]. Actually, compound **4** was a C-6 and C-7 deprotonated product of CAF-603. This was verified by chemical shifts and COSY correlations of H-6 with H-5 and H-7 (Figure 2). In the NOESY experiments, the NOE from H-3 to H-5 indicated the co-facial orientation of these groups (Figure 3). However, no other diagnostic NOEs were observed, and thus the relative configuration could not be characterized by NOESY experiments. According to the literature reports [14–16], the relative configuration of all the congeners of CAF-603 was deduced to be same at the five-membered ring. The absolute configuration of **4** was tentatively deduced as 1*R*, 3*R*, 4*S*, 5*S* on the basis of biogenic considerations. Thus, the structure of compound **4** was characterized and was named trichocarotin L.

Trichocarotin M (**5**), initially obtained as a colorless waxy solid, was determined to possess the molecular formula C$_{15}$H$_{26}$O$_3$, based on HRESIMS data. The ^{1}H and ^{13}C NMR data of **5** (Tables 1 and 2 and Figures S29 and S30 in the Supplementary Material) closely resembled those of 14-hydroxy CAF-603 (trichocarane B), whose relative configuration was determined by NOESY spectrum [17], but its absolute configuration was not clarified. The relative configuration of **5** was deduced to be the same as that of 14-hydroxy CAF-603 based on the NOESY data (Figure 3). However, the optical rotation of compound **5** ([α]25 D +16 (*c* 0.25, CHCl$_3$)) has an opposite sign to that of 14-hydroxy CAF-603 ([α]25 D –28.0 (*c* 0.20, CHCl$_3$)) [17]. Thus, trichocarotin J (**5**) might be the enantiomer of 14-hydroxy CAF-603. To further confirm this deduction we turned our efforts to a crystallographic study of this compound. Upon slow evaporation of the solvent (MeOH:H$_2$O = 15:1) by storage in a refrigerator, quality single crystals of compound **5** were obtained, and the relative and absolute configuration of **5** was therefore established by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Figure 5). The Flack parameter 0.3(2) of **5** allowed the unambiguous confirmation of the absolute configuration of **5** as 1*R*, 3*R*, 4*S*, and 5*S*, and named trichocarotin M, which is an enantiomer of 14-hydroxy CAF-603.

In addition to new compounds **1–5**, seven known analogues including CAF-603 (**6**) [14,15], 7β-hydroxy CAF-603 (**7**) [15], trichocarotins E–H (**8–11**) [16], and trichocarane A (**12**) [17] were also isolated and identified. Their structures were elucidated by comparing their NMR data with those reported in the literature. It deserve to mention that the absolute configurations of compounds **9** and **10** were previously assigned solely from a biogenetic perspective [16], but in the present study the single-crystal X-ray diffraction (Figure 6) was used to confirm their absolute configurations.

9 **10**
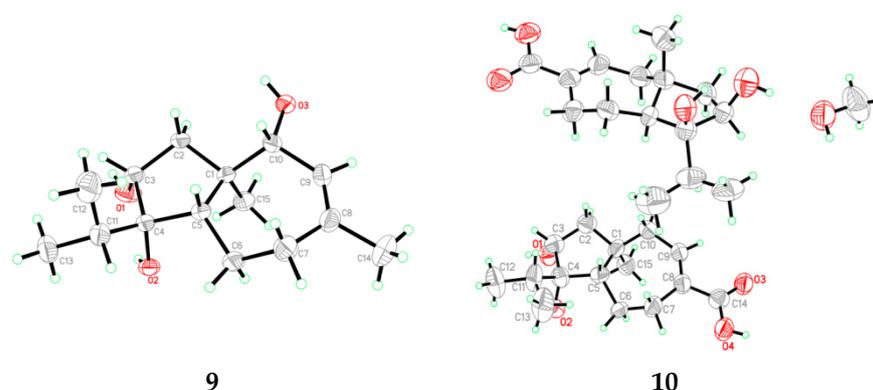
Figure 6. X-ray crystallographic structures of compounds **9** and **10**.

2.2. Antibacterial Activities of the Isolated Compounds

Compounds were assayed for their antibacterial activities against human pathogens *Escherichia coli* EMBLC-1 and *Micrococcus luteus* QDIO-3. Statistical analysis by ANOVA for various concentrations when assessed for *E. coli* and *M. luteus* showed potent inhibitory growth both at concentrations ≥ MICs when compared to that at concentrations < MICs, and the MICs were obtained. Significance of all the statistical tests was predetermined at $p < 0.05$. As a result, each of these compounds showed strong inhibitory activity against *E. coli*, with MIC values ranging from 0.5 to 16 μg/mL, and the activity of compounds **3–5**, **8**, and **11** are as active as that of the positive control (chloramphenicol, MIC = 0.5 μg/mL) (Table 3). In addition, compounds **6–8** showed potent activity against *M. luteus* with MIC values of 4, 0.5, and 2 μg/mL, respectively, and the activity of compound **7** was stronger than that of chloramphenicol (MIC = 1 μg/mL).

Table 3. Antibacterial activity of compounds **1–12** (MIC, μg/mL).

No.	1	2	3	4	5	6	7	8	9	10	11	12	Chloramphenicol
EC	16	32	0.5	0.5	0.5	16	16	0.5	16	16	0.5	8	0.5
ML	–	32	–	–	8	4	0.5	2	–	–	32	8	1

EC: *E. coli*. ML: *M. luteus*; –: no activity (MIC > 64 μg/mL).

Structure-activity relationship analysis revealed that the OH substitution at C-11 increased the activities against both *E. coli* and *M. luteus* (**8** vs. **6**), while the OH group at C-14 increased the activity against *E. coli* and decreased the activity against *M. luteus* (**5** vs. **6**). Similarly, the 8,9-epoxy group at the seven-membered ring also increased the activity against *E. coli* and decreased the activity against *M. luteus* (**12** vs. **6**).

3. Materials and Methods

3.1. General Experimental Procedures

The general experimental procedures and the apparatus used in the current work are the same as that described in our previous report [6]: an SGW X-4 micro-melting-point apparatus, an Optical Activity AA-55 polarimeter, a PuXi TU-1810 UV-visible spectrophotometer, a JASCO J-715 spectropolarimeter, a Bruker Avance 500 spectrometer, an API QS-

TAR Pulsar 1 mass spectrometer, analytical HPLC: a Dionex HPLC system, equipped with P680 pump (Dionex), silica gel GF254 precoated plates, 100–200 mesh and 200–300 mesh silica gel, 40–63 μm RP-18 reverse-phase Si gel, and Sephadex LH-20.

3.2. Plant and Fungal Materials

The fungus *T. virens* QA-8 was isolated from the fresh inner root tissue of the Compositae medical plants *A. argyi* collected at Qichun, Hubei Province, in central China in July 2014 and was identified by analysis of its ITS region of the rDNA. The primers used for PCR are ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), and the total length of sequenced ITS is 616bp. The BLAST search showed that the amplified ITS sequence (GenBank accession no. MK224593) has 100% homology with other members of the genus *T. virens* (compared with MT256290.1). The strain QA-8 is cryopreserved at −80 °C in 20% aqueous glycerol at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology of the Chinese Academy of Sciences (IOCAS).

3.3. Fermentation, Extraction and Isolation

The culture of *T. virens* QA-8 was grown on PDA medium at 28 °C for 7 days and were then inoculated in a 1L-Erlenmeyer flask containing solid rice medium consisting of 70 g rice, 0.1 g corn flour, 0.3 g peptone, and 100 mL distilled water. After 30 days, the whole fermented cultures (180 flasks) were extracted with EtOAc and the combined EtOAc solution was concentrated under reduced pressure to yield 97.4g extract. The extract was fractionated by Si gel vacuum liquid chromatography (VLC) to yield 10 fractions (Frs. 1–10). Purification of Fr. 3 (3.0 g), Fr. 4 (3.7 g), Fr. 5 (11.0 g), and Fr. 6 (22.7 g) by reversed-phase column chromatography (CC) over Lobar LiChroprep RP-18 with a MeOH-H_2O gradient (from 10:90 to 100:0) yielded six subfractions (Frs. 3.1–3.6), nine subfractions (Frs. 4.1–4.9), 10 subfractions (Frs. 5.1–5.10), and 15 subfractions (Frs. 6.1–6.15), respectively. Fr. 3.5 (341 mg) was further purified by CC on Si gel eluting with a PE–acetone gradient (from 50:1 to 10:1) to obtain compound **6** (185.9 mg). Fr. 4.9 (325 mg) was purified by CC on Si gel eluting with a CH_2Cl_2–acetone gradient (from 20:1 to 5:1) and then by preparative TLC (plate: 20 × 20 cm, developing solvents: PE–EtOAc, 1:1) to yield compounds **3** (7.9 mg) and **10** (18.3 mg). Fr. 5.5 (436 mg) was purified by CC on Si gel eluting with a CH_2Cl_2−acetone gradient (from 100:1 to 20:1) and then purified by CC on Sephadex LH-20 (MeOH) to give compounds **7** (3.6 mg), **8** (8.9 mg) and **12** (9.3 mg), respectively. Fr. 5.8 (275 mg) was purified by CC on Si gel eluting with a CH_2Cl_2−acetone gradient (from 100:1 to 10:1) and then purified by CC on Sephadex LH-20 (MeOH) and preparative TLC (plate: 20 × 20 cm, developing solvents: PE/EtOAc, 1:1) yielded compounds **2** (7.2 mg) and **9** (5.5 mg). Fr. 6.2 (136 mg) was further purified by preparative TLC (plate: 20 × 20 cm, developing solvents: CH_2Cl_2− EtOAc, 5:1) to afford compound **1** (6.5 mg). Fr. 6.7 (94 mg) was purified by CC on Sephadex LH-20 (MeOH) to give compound **11** (5.8 mg). Fr. 6.8 (582 mg) was purified by CC on Si gel eluting with a CH_2Cl_2−MeOH gradient (from 200:1 to 10:1) and then purified by preparative TLC (plate: 20 × 20 cm, developing solvents: PE/acetone/acetic acid, 3:1:0.04) to obtain compounds **4** (8.9 mg) and **5** (12.0 mg).

3.4. Spectroscopic Data

Trichocarotin I (**1**): colorless crystals; mp 99–101 °C; [α]25 D −5.56 (*c* 0.18, MeOH); ECD (3.15 mM, MeOH) λ_{max} (Δε) 215 (−3.35), 249 (+1.75) nm; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 253.1812 [M − H]− (calcd for $C_{15}H_{25}O_3$, 253.1809).

Trichocarotin J (**2**): colorless crystals; mp 88–90 °C; [α]25 D −10.64 (*c* 0.47, MeOH); ECD (4.33 mM, MeOH) λ_{max} (Δε) 223 (−28.47), 262 (+20.03) nm; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 253.1810 [M − H]− (calcd for $C_{15}H_{25}O_3$, 253.1809).

Trichocarotin K (**3**): colorless crystals; mp 92–93 °C; [α]25 D +98.41 (*c* 0.63, MeOH); ECD (5.12 mM, MeOH) λ_{max} (Δε) 219 (+5.15), 234 (+5.39), 294 (+7.79) nm; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 253.1808 [M − H]− (calcd for $C_{15}H_{25}O_3$, 253.1809).

Trichocarotin L (**4**): colorless oil; [α]25 D +26.32 (*c* 0.19, MeOH); ECD (7.20 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 243 (–10.60) nm; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 235.1709 [M – H]$^-$ (calcd for $C_{15}H_{23}O_2$, 235.1704).

Trichocarotin M (**5**): colorless crystals; mp 131–133 °C; [α]25 D +85.92 (*c* 0.71, MeOH) or [α]25 D +16.00 (*c* 0.25, CHCl$_3$); ECD (5.51 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 209 (–4.84), 220 (+2.44), 240 (–1.72) nm; ^{1}H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m/z* 299.1869 [M + HCOO]$^-$ (calcd for $C_{16}H_{27}O_5$, 299.1864).

3.5. X-Ray Crystallographic Analysis of Compounds **1**–**3**, **5**, **9**, and **10**

The crystallographic data were collected on a Bruker Smart1000 or Bruker D8 Venture CCD diffractometer. The data were corrected for absorption by using the program SAD-ABS [22,23]. The structures were solved by direct methods with the SHELXTL software package [24]. The structures were refined by full-matrix least-squares techniques [25].

Crystal data for compound **1**: $C_{15}H_{26}O_3$, F.W. = 254.36, Monoclinic space group *P*2(1), unit cell dimensions *a* = 6.9632(6) Å, *b* = 12.8384(13) Å, *c* = 16.8664(18) Å, *V* = 1506.5(3) Å^3, $\alpha = \gamma = 90°$, $\beta = 92.3770(10)°$, *Z* = 4, d_{calcd} = 1.121 mg/m^3, crystal dimensions 0.36 × 0.08 × 0.05 mm^3, μ = 0.605 mm^{-1}, *F*(000) = 560. The 3967 measurements yielded 2231 independent reflections. The final refinement gave R_1 = 0.0649 and wR_2 = 0.1385 [$I > 2\sigma(I)$]. The Flack parameter was 0.1(4).

Crystal data for compound **2**: $C_{15}H_{26}O_3 \cdot H_2O$, F.W. = 272.37, Orthorhombic space group *P*2(1)2(1)2(1), unit cell dimensions *a* = 7.7040(9) Å, *b* = 13.802(4) Å, *c* = 14.5355(16) Å, *V* = 1545.6(5) Å^3, $\alpha = \beta = \gamma = 90°$, *Z* = 4, d_{calcd} = 1.171 mg/m^3, crystal dimensions 0.18 × 0.16 × 0.12 mm^3, μ = 0.668 mm^{-1}, *F*(000) = 600. The 2796 measurements yielded 2746 independent reflections. The final refinement gave R_1 = 0.0293 and wR_2 = 0.0804 [$I > 2\sigma(I)$]. The Flack parameter was 0.06(4).

Crystal data for compound **3**: $C_{15}H_{26}O_3$, F.W. = 254.36, Orthorhombic space group *P*2(1)2(1)2(1), unit cell dimensions *a* = 7.4144(10) Å, *b* = 7.5400(10) Å, *c* = 26.394(4) Å, *V* = 1475.5(4) Å^3, $\alpha = \beta = \gamma = 90°$, *Z* = 4, d_{calcd} = 1.145 mg/m^3, crystal size 0.22 × 0.20 × 0.18 mm, μ = 0.617 mm^{-1}, *F*(000) = 560. The 2675 measurements yielded 2588 independent reflections after. The final refinement gave R_1 = 0.0347 and wR_2 = 0.1193 [$I > 2\sigma(I)$]. The Flack parameter was −0.1(3).

Crystal data for compound **5**: $C_{15}H_{26}O_3$, F.W. = 254.36, monoclinic space group *P*2(1), unit cell dimensions *a* = 7.6721(12) Å, *b* = 7.4046(12) Å, *c* = 13.155(3) Å, *V* = 746.3(3) Å^3, $\alpha = \gamma = 90°$, $\beta = 92.961(14)°$, *Z* = 2, d_{calcd} = 1.132 mg/m^3, crystal dimensions 0.16 × 0.15 × 0.14 mm, μ = 0.610 mm^{-1}, *F*(000) = 280. The 2201 measurements yielded 1459 independent reflections. The final refinement gave R_1 = 0.0542 and wR_2 = 0.1530 [$I > 2\sigma(I)$]. The Flack parameter was 0.3(2) in the final refinement for all 2201 reflections with 1459 Friedel pairs.

Crystal data for compound **9**: $C_{15}H_{26}O_3$, F.W. = 254.36, Orthorhombic space group *P*2(1)2(1)2(1), unit cell dimensions *a* = 6.9592(2) Å, *b* = 11.0588(4) Å, *c* = 19.4762(9) Å, *V* = 1498.90(10) Å^3, $\alpha = \beta = \gamma = 90°$, *Z* = 4, d_{calcd} = 1.127 mg/m^3, crystal dimensions 0.30 × 0.10 × 0.07 mm^3, μ = 0.608 mm^{-1}, *F*(000) = 560. The 2603 measurements yielded 2124 independent reflections. The final refinement gave R_1 = 0.0403 and wR_2 = 0.0908 [$I > 2\sigma(I)$]. The Flack parameter was 0.1(3).

Crystal data for compound **10**: $2C_{15}H_{24}O_4 \cdot CH_3OH$, F.W. = 568.73, Orthorhombic space group *P*2(1)2(1)2(1), unit cell dimensions *a* = 7.2324(7) Å, *b* = 15.2789(15) Å, *c* = 28.581(3) Å, *V* = 3158.3(5) Å^3, $\alpha = \beta = \gamma = 90°$, *Z* = 4, d_{calcd} = 1.196 mg/m^3, crystal size 0.21 × 0.08 × 0.03 mm, μ = 0.704 mm^{-1}, *F*(000) = 1240. The 5499 measurements yielded 1880 independent reflections. The final refinement gave R_1 = 0.1114 and wR_2 = 0.2422 [$I > 2\sigma(I)$]. The Flack parameter was 0.0(7) in the final refinement for all 5499 reflections with 1880 Friedel pairs.

3.6. Antibacterial Assays

Antibacterial evaluation against human pathogens *E. coli* EMBLC-1 and *M. luteus* QDIO-3 was carried out by the microplate assay with three repetitions [26]. *E. coli* and *M. luteus* (95 μL × 5 × 10^5 CFU/mL per well) were cultured at 37 °C in LB medium containing 1% peptone, 0.5% yeast extract, 1% NaCl and distilled water with 5 μL various

concentrations of compounds **1**–**12** in each well of 96-well plates for 24 h. The pathogens used in the assays were obtained from the Institute of Oceanology, Chinese Academy of Sciences. Chloramphenicol was used as positive control.

4. Conclusions

In summary, five new carotane sesquiterpenes trichocarotins I–M (**1**–**5**) and seven known analogues (**6**–**12**) were identified from the culture extract of endophytic fungus *Trichoderma virens* QA-8. Their structures were elucidated by a detailed interpretation of the spectroscopic data and the structures and absolute configurations of compounds **1**–**3**, **5**, **9**, and **10** were confirmed by X-ray crystallographic analysis. The crystal structures of the known compounds **9** and **10** are reported for the first time. The absolute configurations of this kind of sesquiterpene were barely presumed by biosynthesis in previously published reports [16], and some of them were not even determined [14,17], but in the present study single crystal X-ray diffraction was used to confirm their absolute configurations. Compounds **3**–**5**, **8**, and **11** showed inhibitory activity against *E. coli* (MIC = 0.5 μg/mL) and compound **7** showed the strongest activity against *M. luteus* (MIC = 0.5 μg/mL), which are similar to or stronger than that of the positive control. Our results suggested that some of these compounds could be interesting and could lead compounds into a further development of novel antibacterial agents.

Supplementary Materials: The following are available online at https://www.mdpi.com/2079 -6382/10/2/213/s1, Figures S1–S35: Selected 1D and 2D NMR and HRESIMS spectra of **1**–**5**; Figures S36 and S37: Crystal packing of **1**, **9** and **10**.

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References

1. Song, X.W.; Wen, X.; He, J.W.; Zhao, H.; Li, S.M.; Wang, M.Y. Phytochemical components and biological activities of *Artemisia argyi*. *J. Funct. Foods* **2019**, *52*, 648–662. [CrossRef]
2. Nakasugi, T.; Nakashima, M.; Komai, K. Antimutagens in gaiyou (*Artemisia argyi* levl. et vant.). *J. Agric. Food Chem.* **2000**, *48*, 3256–3266. [CrossRef] [PubMed]
3. Zhang, L.B.; Duan, J.A.; Lv, J.L. Phytochemistry and bioactivities of sesquiterpenoids from the *Artemisia* species. *J. Chin. Pharm. Sci.* **2017**, *26*, 317–334. [CrossRef]
4. Lv, J.L.; Li, Z.Z.; Zhang, L.B. Two new flavonoids from *Artemisia argyi* with their anticoagulation activities. *Nat. Prod. Res.* **2018**, *32*, 632–639. [CrossRef] [PubMed]
5. Zhang, P.; Shi, B.; Li, T.; Xu, Y.; Jin, X.; Guo, X.; Yan, S. Immunomodulatory effect of *Artemisia argyi* polysaccharide on peripheral blood leucocyte of broiler chickens. *J. Anim. Physiol. Anim. Nutr.* **2018**, *1*, 1–8. [CrossRef] [PubMed]
6. Shi, X.S.; Meng, L.H.; Li, X.M.; Li, X.; Wang, D.J.; Li, H.L.; Zhou, X.W.; Wang, B.G. Trichocadinins B−G: Antimicrobial cadinane sesquiterpenes from *Trichoderma virens* QA-8, an endophytic fungus obtained from the medicinal plant *Artemisia argyi*. *J. Nat. Prod.* **2019**, *82*, 2470–2476. [CrossRef] [PubMed]

7. Zhang, L.; Yang, Y.M.; Wang, S.X.; Ren, Z.; Cheng, Y.X. Three new sesquiterpenoids with cytotoxic activity from *Artemisia argyi*. *Nat. Prod. Res.* **2019**, *2*, 1–7. [CrossRef]

8. Shi, X.S.; Wang, D.J.; Li, X.M.; Li, H.L.; Meng, L.H.; Li, X.; Pi, Y.; Zhou, X.W.; Wang, B.G. Antimicrobial polyketides from *Trichoderma koningiopsis* QA-3, an endophytic fungus obtained from the medicinal plant *Artemisia argyi*. *RSC Adv.* **2017**, *7*, 51335–51342. [CrossRef]

9. Shi, X.S.; Li, H.L.; Li, X.M.; Wang, D.J.; Li, X.; Meng, L.H.; Zhou, X.W.; Wang, B.G. Highly oxygenated polyketides produced by *Trichoderma koningiopsis* QA-3, an endophytic fungus obtained from the fresh roots of the medicinal plant *Artemisia argyi*. *Bioorg. Chem.* **2020**, *94*, 103448. [CrossRef]

10. Zhang, X.W.; Wang, S.; Tu, P.F.; Zeng, K.W. Sesquiterpene lactone from *Artemisia argyi* induces gastric carcinoma cell apoptosis via activating NADPH oxidase/reactive oxygen species/ mitochondrial pathway. *Eur. J. Pharmacol.* **2018**, *837*, 164–170. [CrossRef]

11. Anoumedem, E.G.M.; Mountessou, B.Y.G.; Kouam, S.F.; Narmani, A.; Surup, F. Simplicilones A and B isolated from the endophytic fungus *Simplicillium subtropicum* SPC3. *Antibiotics* **2020**, *9*, 753. [CrossRef] [PubMed]

12. Manganyi, M.C.; Ateba, C.N. Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. *Microorganisms* **2020**, *8*, 1934. [CrossRef]

13. Das, M.; Prakash, H.S.; Nalini, M.S. Antibacterial metabolites from *Bipolaris specifera*, an endophytic fungus from the endemic medicinal plant, *Zingiber nimmonii* (J. Graham) Dalzell. *Biotech* **2020**, *10*, 317–324. [CrossRef]

14. Watanabe, N.; Yamagishi, M.; Mizutani, T.; Kondoh, H.; Omura, S.; Hanada, K.; Kushida, K. CAF-603: A new antifungal carotane sesquiterpene isolation and structure elucidation. *J. Nat. Prod.* **1990**, *53*, 1176–1181. [CrossRef] [PubMed]

15. Yasumura, R.; Ashtekar, K.D.; Tonouchi, A.; Nehira, T.; Borhan, B.; Hashimoto, M. 7-*β*- and 10-*β*-Hydroxylated congeners of CAF-603; elucidation of absolute configuration of CAF-603 family, and their SAR studies in the anti-fungal activity. *Tetrahedron* **2013**, *69*, 9469–9474. [CrossRef]

16. Shi, Z.Z.; Fang, S.T.; Miao, F.P.; Yin, X.L.; Ji, N.Y. Trichocarotins A−H and trichocadinin A, nine sesquiterpenes from the marine-alga-epiphytic fungus *Trichoderma virens*. *Bioorg. Chem.* **2018**, *81*, 319–325. [CrossRef]

17. Macías, F.A.; Varela, R.M.; Simonet, A.M.; Cutler, H.G.; Cutler, S.J.; Eden, M.A.; Hill, R.A. Bioactive carotanes from *Trichoderma virens*. *J. Nat. Prod.* **2000**, *63*, 1197–1200. [CrossRef] [PubMed]

18. Ondeyka, J.G.; Ball, R.G.; Garcia, M.L.; Dombrowski, A.W.; Sabnis, G.; Kaczorowski, G.J.; Zink, D.L.; Bills, G.F.; Goetz, M.A.; Schmalhofer, W.A.; et al. A carotane sesquiterpene as a potent modulator of the Maxi-K channel from *Arthrinium phaesospermum*. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 733–734. [CrossRef]

19. Lee, S.H.; Hensens, O.D.; Helms, G.L.; Liesch, J.M.; Zink, D.L.; Giacobbe, R.A.; Bills, G.F.; Stevens-Miles, S.; Garcia, M.L.; Schmalhofer, W.A.; et al. L-735,334, a novel sesquiterpenoid potassium channel-agonist from *Trichoderma virens*. *J. Nat. Prod.* **2004**, *58*, 1822–1828. [CrossRef]

20. Singh, M.M.; Agnihotri, A.; Garg, S.N.; Agarwal, S.K.; Gupta, D.N.; Keshri, G.; Kamboj, V.P. Antifertility and hormonal properties of certain carotane sesquiterpenes of *Ferula jaeschkeana*. *Planta Med.* **1988**, *54*, 492–494. [CrossRef] [PubMed]

21. Zhao, Y.X.; Zhou, J.; Wang, Q.; Dai, H.F.; Zheng, Y.T.; Ma, Q.Y.; Zhang, X.; Huang, S.Z. Anti-HIV terpenoids from *Daphne aurantiaca* Diels. Stems. *RSC Adv.* **2015**, *5*, 80254–80263. [CrossRef]

22. Cambridge Crystallographic Data Centre. Crystallographic Data of Compounds 1-3, 5, 9, and 10 as CCDCs1890738, 2014253, 2014254, 1967785, 1890742, and 1890734. Available online: http://www.ccdc.cam.ac.uk/data_request/cif (accessed on 8 February 2021).

23. Sheldrick, G.M. *SADABS, Software for Empirical Absorption Correction*; University of Gottingen: Gottingen, Germany, 1996.

24. Sheldrick, G.M. *SHELXTL, Structure Determination Software Programs*; Bruker Analytical X-ray System Inc.: Madison, WI, USA, 1997.

25. Sheldrick, G.M. *SHELXL, Program for the Refinement of Crystal Structures*; University of Gottingen: Gottingen, Germany, 2014.

26. Abdulwahab, H.G.; Harras, M.F.; El Menofy, N.G.; Hegab, A.M.; Essa, B.M.; Selim, A.A.; Sakr, T.M.; El-Zahabi, H.S.A. Novel thiobarbiturates as potent urease inhibitors with potential antibacterial activity: Design, synthesis, radiolabeling and biodistribution study. *Bioorg. Med. Chem.* **2020**, *28*, 115759. [CrossRef] [PubMed]