SHORT REPORT

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Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Trichocarotin N: a New Carotane Sesquiterpene from the Marine-Derived Fungus *Trichoderma virens* QD-11

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(Received June 15, 2022; Revised July 21, 2022; Accepted August 03, 2021)

Abstract: A new carotane sesquiterpene, trichocarotin N (**1**), was isolated from a culture of the marine-derived fungus *Trichoderma virens* QD-11. The structure of this compound was established by detailed analysis of 1D/2D NMR and HRESIMS data. Trichocarotin N (**1**) exhibited moderate cytotoxicity against HeLa and MCF-7 cancer cell lines, with IC_{50} values of 32.4 and 41.6 μM, respectively.

Keywords: *Trichoderma virens;* carotane sesquiterpene; secondary metabolites; cytotoxic activity.

1. Fungal Source

An endophytic fungus, *Trichoderma virens* QD-11, was previously isolated from the marine red alga *Chondrus ocellatus* collected from the coastal zone of Qingdao, China, in June 2021. This strain was identified based on morphological characteristics and ITS regions of its rDNA, whose sequence data have been submitted to GenBank with the number ON565423. The fungus was deposited in Qingdao Hospital of Traditional Chinese Medicine, Qingdao, China, and assigned its registration number QD-11.

2. Previous Studies

Marine algicolous *Trichoderma* can produce abundant secondary metabolites with high structural diversity and intriguing bioactivities [1,2]. Previous studies have reported three novel polyketide-like compounds [1], two new sulfurated diketopiperazines [3], a new cadinane sesquiterpene and eight new carotane sesquiterpenes [4], as well as six new cadinane derivatives and two new carotane derivatives [5] from the marine algicolous fungus *T. virens*.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.349.2206.2492
Available online: August 14, 2022

3. Present Study

In our ongoing research on new and bioactive secondary metabolites from marine algicolous fungi, a new carotane sesquiterpene, trichocarotin N (**1**), three known carotane sesquiterpenes, trichocarotins A and B (**2** and **3**), CAF-603 (**4**), and two known cyclonerane sesquiterpenes, cyclonerodiol (**5**) and 9-cycloneren-3,7,11-triol (**6**), were isolated from *T. virens* QD-11 through chromatographic separation and identified by spectroscopic methods. Herein, the isolation, structural elucidation, and cytotoxic activity of compound **1** are described in detail.

The fungal strain *T. virens* QD-11 was cultured in PDA medium at 28 °C for three days. The PDA medium was cut into pieces (approximately 0.5 × 0.5 cm), which were inoculated into 50 × 1 L Erlenmeyer flasks preloaded with sterilized rice medium, each containing 50 g of rice and 100 mL of natural seawater. After fermentation at 28 °C for 30 days, the cultures were dried, smashed, and exhaustively extracted with EtOAc. The organic solvent was evaporated to dryness under reduced pressure to obtain 110 g of crude residue. The residue was subjected to silica gel column chromatography (CC) with step-gradient solvent systems of petroleum ether (PE)/EtOAc (from 10:1 to 1:1, v/v) and then CH_2Cl_2/MeOH (from 20:1 to 1:1) to give nine fractions. Fraction 4 (3.5 g), eluted with PE/EtOAc 1:1, was further purified by CC on RP-18 (MeOH/H_2O, 1:1) and then a Sephadex LH-20 column (MeOH) to give trichocarotin N (**1**) (1.8 mg).

Trichocarotin N (1): Colorless oil; $[\alpha]_D^{20}$ = +8.4° (*c* = 0.1, MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 277.1771 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

The cytotoxicity against four human tumour cell lines (HeLa, HuH-7, MCF-7, and NCI-H460) of the tested compound was evaluated according to previously reported methods [6,7]. The cells were exposed to the tested compounds at gradient concentrations (3.125, 6.25, 12.5, 25, 50, and 100 μM) for 72 h, and the positive control was epirubicin.

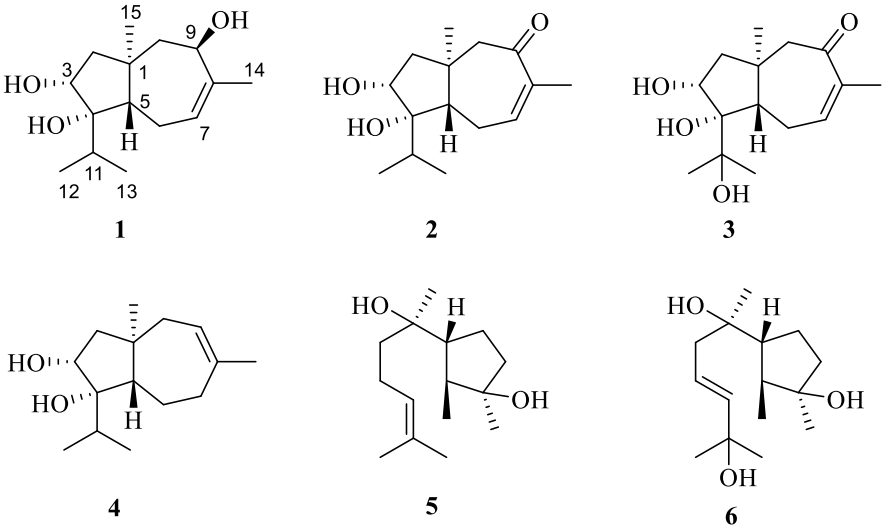

Figure 1. Chemical structures of isolated compounds **1-6**

Compound **1** was isolated as colourless oil. Its molecular formula was deduced to be $C_{15}H_{26}O_3$ by interpretation of HRESIMS (*m/z* 277.1771 $[M + Na]^+$). The ^{1}H NMR spectral data of **1** (Table 1) displayed notable proton signals attributed to one olefinic proton at δ = 5.57 (m, H-7), two oxygenated methine protons at δ = 4.28 (m, H-9) and 3.99 (dd, *J* = 7.9, 1.9 Hz, H-3), four methyl groups, including two methyl singlets at δ = 1.74 (s, H_3-14) and 1.14 (s, H_3-15), and two methyl doublets at δ = 0.95 (d, *J* = 6.8 Hz, H_3-12) and 0.87 (d, *J* = 6.9 Hz, H_3-13). Aided by the DEPT experiment, 15 resonances in the ^{13}C NMR spectrum (Table 1) were classified into four methyls, three methylenes,

five methines [including one olefinic at δ = 124.8 (C-7), two oxygenated sp^3 at δ = 72.1 (C-3) and 70.4 (C-9)], and three quaternary carbons [including one olefinic at δ = 142.1 (C-8) and one oxygenated at δ = 84.4 (C-4)]. The above 1D NMR data of **1** showed high similarities to those of trichocarotin A, which was also isolated from *T. virens* [4]. The main difference was one oxygenated methine (C-9) in **1** replacing the carbonyl group in trichocarotin A, which was confirmed by the COSY correlation of H-9 with H-10 and the HMBC correlations from H_3-14 to C-7, C-8, and C-9. Other COSY and HMBC correlations (Figure 2) further confirmed the planar structure of **1**. The relative configuration of **1** was determined from its NOESY spectrum. The NOE correlations of H-11 with H-3, H-5, and H-6b indicated that C-11 was *syn* to H-3, H-5, and H-6b, while H-9 and C-15 were opposite to C-11 based on NOE correlations of H_3-15 with H-6a and H-9. However, the absolute configuration of this compound was unsolved. Compound **1** was named trichocarotin N.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (δ in ppm) in CD_3OD

No	δ_H (*J* in Hz)	δ_C, type
1	-	42.6, C
2	1.63, dd (13.9, 1.9); 1.57, dd (13.9, 7.9)	51.8, CH_2
3	3.99, dd (7.9, 1.9)	72.1, CH
4	-	84.4, C
5	1.50, dd (11.7, 2.6)	53.6, CH
6	2.15, m; 2.02, ddd (17.0, 8.3, 2.4)	25.3, CH_2
7	5.57, m	124.8, CH
8	-	142.1, C
9	4.28, m	70.4, CH
10	1.87, dd (12.8, 4.1); 1.36, dd (12.8, 11.0)	52.4, CH_2
11	1.75, heptet (6.9)	36.3, CH
12	0.95, d (6.8)	17.5, CH_3
13	0.87, d (6.9)	18.1, CH_3
14	1.74, s	23.3, CH_3
15	1.14, s	20.8, CH_3

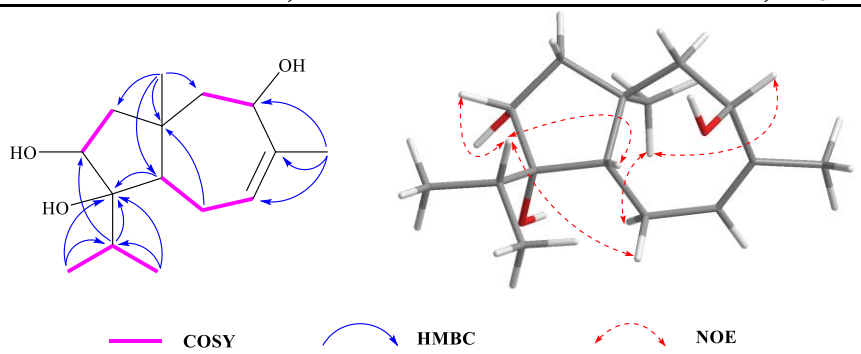

Figure 2. Key ^{1}H-^{1}H-COSY, HMBC, and NOE correlations for **1**

Trichocarotin N (**1**) was evaluated for its cytotoxic effect against HeLa, HuH-7, MCF-7, and NCI-H460 cell lines. As shown in Table 2, **1** exhibited moderate cytotoxicity against HeLa and MCF-7 cells with IC_{50} values of 32.4 and 41.6 μM, respectively. However, **1** was inactive towards HuH-7 and NCI-H460 cells (IC_{50} > 100 μM).

Table 2. Cytotoxic activity of trichocarotin N (**1**) (IC_{50}, μM)

Compounds	HeLa	HuH-7	MCF-7	NCI-H460
1	32.4 ± 0.7[a]	–[b]	41.6 ± 0.9	–
Epirubicin[c]	4.8 ± 0.3	3.1 ± 0.2	5.2 ± 0.4	6.5 ± 0.3

[a] mean ± SD, n = 3; [b] IC_{50} > 100 μM; [c] positive control.

Chemical investigation of the marine algicolous *T. virens* QD-11 resulted in the isolated of one new carotane sesquiterpene, trichocarotin N (**1**), three known carotane sesquiterpenes, trichocarotins A and B (**2** and **3**), CAF-603 (**4**), and two known cyclonerane sesquiterpenes, cyclonerodiol (**5**) and 9-cycloneren-3,7,11-triol (**6**). These compounds contribute to the chemical diversity of carotane and cyclonerane sesquiterpenes. The new compound, trichocarotin N (**1**), exhibited moderate cytotoxicity against HeLa and MCF-7 cells. Considering its insignificant cytotoxicity against cancer cells, chemical modification of trichocarotin N may improve its inhibitory activity against cancer cells.

Acknowledgments

This work was funded by Qingdao Science and Technology Project of TCM (NO.2021-zyyq05).

Supporting Information

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

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