Tricholumin A, a Highly Transformed Ergosterol Derivative from the Alga-Endophytic Fungus *Trichoderma asperellum*

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**ABSTRACT:** Tricholumin A (1) with an unprecedented carbon skeleton was isolated from the fungus *Trichoderma asperellum* cf44-2, an endophyte from the marine brown alga *Sargassum* sp. Its structure and relative configuration were identified by extensive 1D/2D NMR and mass spectrometric data, and the absolute configuration was assigned by X-ray diffraction and ECD calculations. Compound 1 represents a highly transformed ergosterol derivative, and it exhibited inhibition of some pathogenic microbes and marine phytoplankton species.

Natural steroids with various physiological functions are almost ubiquitous in plants, animals, and microbes of terrestrial and marine origin. They commonly feature a cyclopentano perhydrophenanthrene nucleus, and oxidation, substitution, and cyclization of this core and its affiliated methyls and side chains greatly diversify the structures. Filamentous fungi have proven to be prolific sources of ergosterol and its congeners. Except for the regular ergosteroids, a few derivatives with cleaved, contracted, and expanded ring systems have been discovered from them. These derivatives often possess high novelty and have attracted much attention for natural product research. Marine-derived fungi have contributed a number of novel ergosterol analogues with great diversity and intriguing bioactivity, encouraging further investigation toward them. As a result, one novel ergosterol derivative (1) with a unique carbon scaffold was isolated and identified from the marine alga-endophytic fungus *Trichoderma asperellum* cf44-2. Herein, the isolation, structure elucidation, and bioactivity as well as possible biogenetic pathway of this compound are described in detail.

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Received: September 4, 2018
Published: September 26, 2018

DOI: 10.1021/acs.orglett.8b02821
Org. Lett. 2018, 20, 6306–6309
Thus, the planar structure of 1 was assigned as a highly transformed ergosterol derivative (Figure 1).

The relative configuration of 1 was established in part by analysis of coupling constants and NOE correlations (Figure 2). H-1a and H-3 were oriented to be axial according to their respective constants. The double bond between C-6 and C-7 was assigned a Z geometry by their small coupling constants, and it was syn to C-28 and H-1b by the NOE correlations of H-28 with H-1b and H-6. Furthermore, the cofacial property of C-7 and C-27 was verified by the obvious NOE correlation between H-7 and H-27. Additionally, H-15, H-18, and C-26 were allowed to be on the same face of ring D based on the NOE correlations of H-26 with H-15 and H-18. However, the relative configurations between rings B and D and at C-20 and C-21 remained unable to be resolved on the basis of the NMR data.

The flexible architecture of 1 prompted us to ascertain its absolute configuration through crystallography. Fortunately, a suitable single crystal was obtained in MeOH with a drop of water after repeated efforts and was subjected to the X-ray diffraction analysis using Cu Kα radiation. As a result, the absolute configuration of 1 was assigned to be 3S, 5S, 8R, 10R, 14S, 15R, 18S, 19S, 20R, and 21R (Figure 3). To further confirm the absolute configuration of 1, its electronic circular dichroism (ECD) spectrum was determined in MeOH, which exhibited a positive Cotton effect at 310 nm. Ring B with two carbonyl groups should be responsible for this peak, due to the lack of any chromophore in the other moieties. Regardless of rotations of the hydroxy and methyl groups as well as the side chain, two energy-minimized conformers (1A and 1B) (Figure 2) within a 3 kcal/mol energy threshold from the global minimum were obtained after conformational optimization at the B3LYP/6-31G(d) level in MeOH with the integral equation formalism variant of the polarizable continuum model via Gaussian 09 software, and then they were used to simulate the ECD spectrum at the same level through the time-dependent density function theory method. The Boltzmann-weighted ECD curve, depicted by SpecDis software with σ = 0.2, agreed well with the experimental one (Figure 4), which corroborated the absolute configuration of 1.

Biosynthetically, compound 1 could be traced back to the ergosterol that was also obtained in the present study. A plausible biogenetic pathway was proposed, as shown in Scheme 1. Initiation by dehydrogenating ergosterol at C-14 and C-15 generates tetraenol 1a, followed by further deprotonation at C-9 to yield intermediate 1b. Its C-5 position is then attacked by OH- to form tetraendiol 1c, which undergoes oxidation by dioxygenases and monoxygenases to afford 1d. After the

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Table 1. 1H and 13C NMR Data for 1 (in CDCl3)

| pos | δH (J in Hz) | δC, type |
|-----|-------------|----------|
| 1a  | 2.49, td (13.6, 3.8) | 30.7, CH2 |
| 1b  | 1.66, m | |
| 2a  | 2.02, m | 29.9, CH2 |
| 2b  | 1.52, m | |
| 3   | 4.14, dddd (10.5, 10.5, 5.0, 5.0) | 67.3, CH |
| 4   | 1.90, m | 43.7, CH2 |
| 5   | 95.9, C | |
| 6   | 6.16, d (5.8) | 137.0, CH |
| 7   | 6.02, d (5.9) | 129.5, CH |
| 8   | 110.4, C | |
| 9   | 209.1, C | |
| 10  | 54.8, C | |
| 11a | 2.70, br t (13.0) | 33.5, CH2 |
| 11b | 1.82, m | |
| 12a | 3.03, br d (12.8) | 37.6, CH2 |
| 12b | 2.20, td (12.6, 4.3) | |
| 13  | | 213.6, C |
| 14  | | 54.6, C |
| 15  | 2.23, m | 54.7, CH |
| 16a | 1.73, m | 29.0, CH2 |
| 16b | 1.44, m | |
| 17a | 1.66, m | 30.9, CH2 |
| 17b | 1.39, m | |
| 18  | 1.81, m | 50.7, CH |
| 19  | 1.97, m | 36.8, CH |
| 20  | 3.39, dd (7.1, 5.5) | 79.3, CH |
| 21  | 1.50, m | 41.8, CH |
| 22  | 2.07, m | 26.6, CH |
| 23  | 0.82, d (6.3) | 16.5, CH3 |
| 24  | 0.92, d (6.9) | 22.3, CH3 |
| 25  | 0.80, d (6.6) | 10.7, CH3 |
| 26  | 1.21, d (6.6) | 24.9, CH3 |
| 27  | 1.06, s | 21.3, CH3 |
| 28  | 1.18, s | 19.2, CH3 |

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Figure 1. Structure and key COSY and HMBC correlations of 1.

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cleavage between C-8 and C-9 and intramolecular SN2 reaction accompanied by a series of electron and proton transfers, two carbonyl groups and one norbornyl group are created, as shown in 1e and 1f. Subsequent oxidation produces 1g, and further cyclization through an SN2 reaction and cleavage due to ring strain of the norbornyl moiety leads to the formation of 1 via 1h. The highlights of these transformations are the oxidative cleavage between C-8 and C-9 of ergosterol and construction of ring D in 1.

To develop new inhibitors against harmful microalgae and pathogenic bacteria that greatly threaten marine aquaculture, compound 1 was evaluated for inhibition of four phytoplankton species (Chattonella marina, Heterosigma akashiwo, Karlodinium veneficum, and Prorocentrum donghaiense) and five aquatic pathogens (Vibrio anguillarum, V. harveyi, V. splendidus, and Pseudoalteromonas citrea). The results show that 1 could inhibit the four phytoplankton species tested, with IC50 values of 0.56, 0.37, 0.59, and 0.27 μg/mL, respectively. It also exhibited weak antibacterial activity against V. harveyi, V. splendidus, and P. citrea with inhibitory zones of 10, 7.5, and 8.0 mm, respectively, at 50 μg/disk. On the other hand, its antifungal activity against Glomerella cingulata, a phytopathogen in agriculture, was also assayed, and the MIC value was determined to be 12 μg/mL.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02821. Experimental section, Cartesian coordinates, 1D/2D NMR, HREIMS, IR, and UV spectra (PDF)

Accession Codes

CCDC 1864431 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support from the National Natural Science Foundation of China (31670355), the Natural Science Foundation for Distinguished Young Scholars of Shandong Province (JQ201712), the Open Fund of Key Laboratory of Experimental Marine Biology, the CAS (KF2017NO4), the Youth Innovation Promotion Association of the CAS (2013138), and the Self-Fund from Yantai Institute of Coastal Zone Research, the CAS (Y75503012) is gratefully acknowledged.

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