Note

A Tricyclic Aromatic Polyketide Isolated from the Marine-Derived Fungus *Curvularia aeria*

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A novel tricyclic polyketide, curvulanone (1), was isolated from the marine-derived fungus *Curvularia aeria*. The structure of 1 was determined by NMR and single-crystal X-ray crystallography. 1 had a cyclopentabenzopyranone with 3-acetic acid structure that is rarely found in natural compounds. Monoamine oxidase and sirtuin 1 inhibitory test was exhibited and 1 showed their inhibitory activity.

Key words aromatic polyketide, fungal metabolite, *Curvularia* species, cyclopentabenzopyran-4-one, acetate-malonate pathway

Introduction

Fungi of the genus *Curvularia* produce a variety of structurally unusual secondary metabolites. The major secondary metabolite isolated from *Curvularia* spp. is curvularin (**2**), which consists of a 12-membered ring ketolactone with 1,3-dihydroxybenzene.[1)] Curvularin analogs, including *α*,*β*-dehydrocurvularin,[2)] sulfur-containing curvularin derivative,[3)] chlorine-substituted derivatives,[4)] and polyhydroxylated derivatives,[5)] have also been isolated. In addition, the aromatic polyketide curvulin (**3**) and curvulinic acid (**4**) have been isolated from *Curvularia* sp.[6)] They were biosynthesized from 1× acetyl CoA and 4× malonyl CoA, and both had a 2,4-dihydroxy-acetophenone-6-acetic acid moiety. Curvularin and curvulin derivatives are thought to be reaction intermediates that contribute to structural diversification. Two phenolic hydroxy groups have been reported to contribute to the generation of their esters.[7,8)] The coexistence of an alpha-proton at H-10 and carbonyl group at C-1 in the molecule may lead to cyclization under alkaline conditions. This reaction occurs in curvulinic acid methyl esters to generate scytalone.[9,10)] Scytalone has a 3,4-dihydro-1(2*H*)-naphthalenone structure and is an intermediate in fungal melanin pigment synthesis.[11)] Reduction and dehydration of scytalone convert the 3,4-dihydro-1(2*H*)-naphthalenone structure to 1,3,8-trihydroxynaphthalene.[10)]

The discovery of aromatic polyketides, which are biosynthetic intermediates of various secondary metabolites, may provide clues for better understanding the structural diversification of naturally occurring compounds. In the present study, we describe the isolation and structural determination of a novel aromatic polyketide with an unusual cyclopentabenzopyran-4-one skeleton and its plausible biosynthesis.

Results and Discussion

The marine-derived fungus *C. aeria* was isolated from barnacles. The isolate fermented barley media, and the fermentate was extracted with $CHCl_3$ and EtOAc. In our previous study, curvularin and dehydrocurvularin were isolated from the $CHCl_3$ extract, and we examined their antifungal activity.[12)] In the present study, the EtOAc extract was subjected to octadecylsilyl silica gel column chromatography, which led to the isolation of the new metabolite curvulanone (**1**) (Fig. 1).

Curvulanone (**1**) was obtained as colorless needles. Its molecular formula was determined by high resolution electron ionization-mass spectrometry (HR-EI-MS) to be $C_{14}H_{14}O_5$. The IR spectrum of **1** suggested the presence of a hydroxy group (3232 cm^{-1}) and two carbonyl groups (1714 and 1634 cm^{-1}). The ^{1}H-NMR spectrum showed signals attributed to three methylenes at δ_H 1.98, 2.09 (H-12), 1.80, 1.96 (H-13) and 1.82, 2.02 (H-14), a downshifted methine at δ_H 2.62 (H-10), an oxymethine at δ_H 4.83 (H-11), a pair of active methylenes at δ_H 3.85 and 3.90 (H-2), and two phenolic protons at δ_H 6.30 (H-4) and 6.24 (H-6). Furthermore, the ^{13}C-NMR spectrum showed signals for two methines at δ_C 51.4 (C-10) and 82.6 (C-11), four methylenes at δ_C 40.9 (C-2), 32.6 (C-12), 22.0 (C-13) and 27.6 (C-14), six aromatic carbons at δ_C 139.6 (C-3), 114.3 (C-4), 163.8 (C-5), 102.0 (C-6), 164.3 (C-7) and 110.5 (C-8), and a carbonyl carbon at δ_C 195.4 (C-9; Table 1). ^{1}H–^{1}H correlation spectroscopy (COSY) revealed correlations between H-10 with H-11 and H-14, and H-11 with H-12. Heteronuclear multiple bond connectivity (HMBC) correlations were observed from H-2 to C-3 and C-8, H-4 to C-2, C-5, C-6 and C-8, H-6 to C-7 and C-8, H-10 to C-8 and C-9, H-13 to C-10 and C-11,

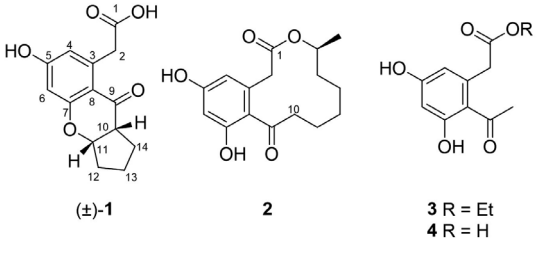

Fig. 1. Structures of **1** to **4**

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Table 1. NMR Spectroscopic Data for **1**

Curvulanone (**1**)		
Pos.	δ_C	δ_H (J in Hz)
1	174.9	
2	40.9	3.85 (d, 16.6)
		3.90 (d, 16.6)
3	139.6	
4	114.3	6.30 (d, 2.4)
5	163.8	
6	102.0	6.24 (d, 2.4)
7	164.3	
8	110.5	
9	195.4	
10	51.4	2.62 (dt, 9.7, 4.2)
11	82.6	4.83 (m)
12	32.6	1.98 (overlapped)
		2.09 (overlapped)
13	22.0	1.80 (overlapped)
		1.96 (overlapped)
14	27.6	1.82 (overlapped)
		2.02 (overlapped)

Measured in CD_3OD.

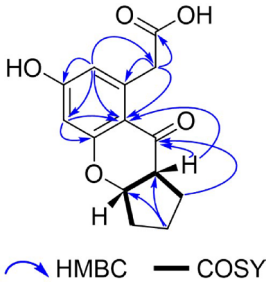

Fig. 2. 2D-NMR Correlations of **1**

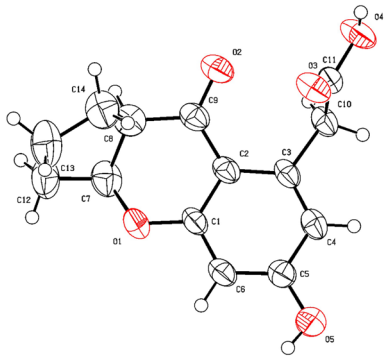

Fig. 3. X-Ray Crystal Structure of **1**

and H-14 to C-9 (Fig. 2). Moreover, HMBC correlations from H-2 to C-1 (δ_C 174.9) suggested the presence of carboxyl acid. These spectroscopic data for **1** suggested the presence of a cyclopentabenzopyran-4-one skeleton. Single crystal X-ray crystallography results showed the *cis*-orientation in regard to the ring juncture protons (H-10 and H-11 in Fig. 3). Moreover, no Cotton effects were observed in their electronic circular dichroism (ECD) spectrum, suggesting that **1** was racemic mixture.

Cyclopentabenzopyran-4-one derivatives rarely occur in nature. Some examples of the rare natural cyclopentabenzopyran-4-one derivatives include coniochaetones,[13–18] remisporine A,[19] diaportheones A and B,[20] preussochromones D–F,[21] and applanatumols X and Y[22]; however, **1** differs from the above-mentioned compounds as it has different substitution patterns. In particular, the substitution of acetic acid at C-3 is seen only in **1**. This suggests that the biosynthetic pathway of the cyclization pattern for the cyclopentabenzopyran-4-one scaffold in **1** differs from those of the previous compounds. Plausible biosynthetic pathways for **1**, curvulinic acid, and curvularin are shown in Fig. 4. Interestingly, *Curvularia* spp. appear to produce compounds with different carbon skeletons depending on the number of malonyl CoA units involved. Phenol intermediates are biosynthesized from acetyl CoA and malonyl CoA. Monocyclic aromatic polyketides culvulin (**3**) and curvulinic acid (**4**) are biosynthesized from 1× acetyl CoA and 4× malonyl CoA. The 2,4-dihydroxy-acetophenone-6-acetic acid moiety is a common structure for both **1** and curvularin (**2**). **2** is biosynthesized *via* esterification between C-1 and C-15, which generates a macrocyclic structure consisting of 12 elements. The biosynthetic pathway of **1** shows different cyclization forms. The hydroxy group at C-7 etherizes with C-11 to form a benzopyran-4-one scaffold. Furthermore, the carbon–carbon bond at C-10 to C-14 forms a cyclopentan moiety.

To investigate the bioactivities of **1** against monoamine oxidase (MAO) and sirtuin 1 (SIRT1), inhibitory activity tests were performed. MAO is one of the most important enzymes in Parkinson's disease, and MAO inhibitors are clinically used to treat the disease.[23] SIRT1 is involved in various pathologies. A correlation between MAO-A and SIRT1 was first reported by Jiang and colleagues.[24] In the present study, we found that **1** exhibited inhibitory activity against MAO-B ($IC_{50} = 55.8\,\mu M$), milder inhibitory activity against MAO-A ($IC_{50} = 117.9\,\mu M$), and weak inhibitory activity against SIRT1 ($IC_{50} = 107.9\,\mu M$).

Experimental

General Experimental Procedures All reagents and solvents were purchased from commercial suppliers and used without further purification. Melting points were determined on a MP apparatus (Yanaco Technical Science Corp., Tokyo, Japan). Optical rotation was measured with a P-2000 polarimeter (Jasco Corp., Tokyo, Japan). IR spectra were recorded with a IR Affinity-1S spectrophotometer (ATR, Shimazu Corp., Kyoto, Japan). UV spectra were recorded with a UV-1280 spectrophotometer (Shimazu Corp.). ECD spectra were acquired on a J-1500-150DS spectrophotometer (Jasco Corp.). One dimensional (1D) and 2D-NMR spectra were measured at 298 K with a Varian 400-MR (400 MHz) spectrometer (Agilent Technologies Japan, Ltd., Tokyo, Japan) and a Bruker Avance NEO 400 MHz spectrometer (Bruker Japan K.K., Kanagawa, Japan) using tetramethylsilane as the internal standard. Low- and high-resolution EI and FABMS spectra were measured with a JMS-700 spectrometer (JEOL, Tokyo, Japan). Column chromatography was performed using silica gel 60N (63–210 μm, Kanto Chemical, Tokyo, Japan). X-Ray diffraction measurements were performed at 273 K on a Bruker D8 Venture diffractometer equipped with a PHOTON II detector with Mo Kα radiation ($\lambda = 0.71073$ Å, Bruker Japan K.K., Kanagawa, Japan).

Fungal Material The fungus *C. aeria* was isolated from

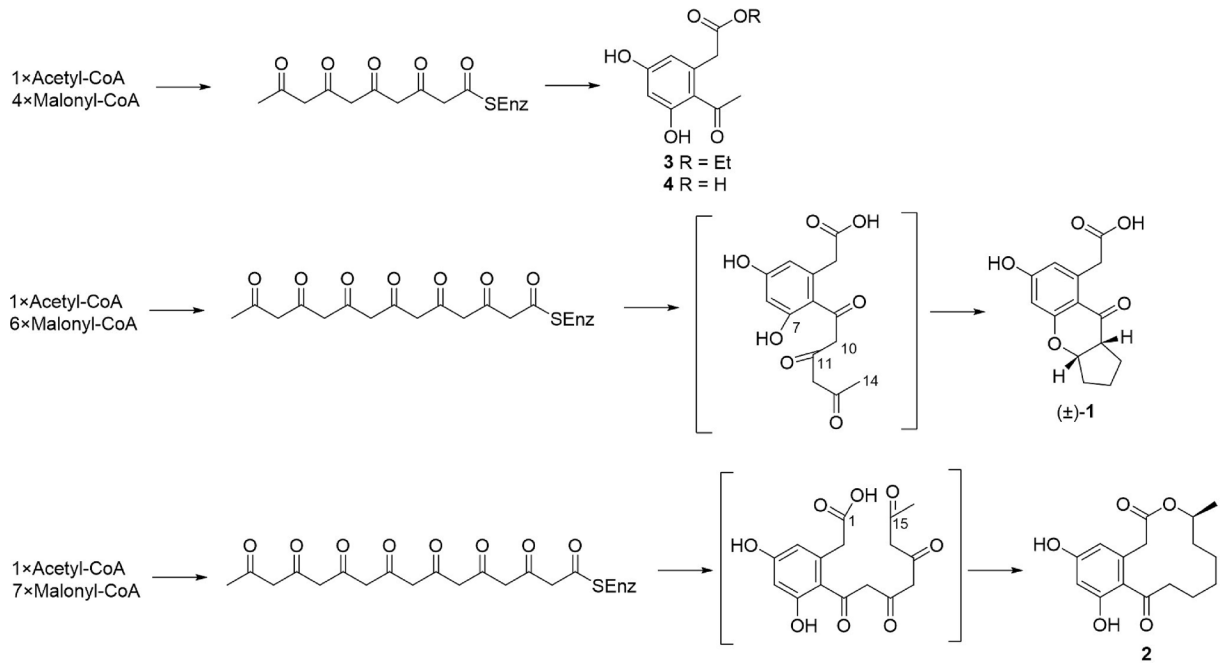

Fig. 4. Plausible Biosynthetic Pathways of Phenolic Polyketides Isolated from *Curvularia* spp.

barnacles obtained at Kashima-city, Ibaraki Prefecture, Japan, in September 2020. The isolate was speciated by ribosomal DNA (rDNA) sequence analysis. The internal transcribed spacer regions 1 and 2 and the 5.8S rDNA in the ribosomal RNA (rRNA) gene of the isolate were identical to those of an epitype strain of *C. aeria*. A voucher specimen (JU-M017) was deposited at the department of Bioorganic Chemistry, Faculty of Pharmacy Pharmaceutical and Sciences, Josai University.

Fermentation and Extraction *C. aeria* was pre-incubated on PGY agar medium (2% peptone: Kyokuto Pharmaceutical Industrial, Tokyo, Japan; 2% glucose: FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan; 1% yeast extract: Becton Dickinson and Company, Franklin Lakes, NJ, U.S.A., and 2% agar: Becton Dickinson and Company) at 27 °C. After pre-incubation, *C. aeria* was inoculated into 1000 mL Roux flasks (12 flasks) containing barley (200 g per flask, Hakubaku, Yamanashi, Japan). Flasks were statically incubated at 26 °C for 28 d. The fermented substrate was extracted with $CHCl_3$ and EtOAc.

Isolation and Purification The EtOAc extract (17.4 g) was fractionated by silica-gel column chromatography (Si C. C.) with $CHCl_3$/MeOH (100 : 1, 50 : 1, 25 : 1 and 10 : 1, followed by MeOH) to yield six fractions (a–f). Fraction d (1.4 g) was further subjected to Si C. C. ($CHCl_3$/MeOH) and octadecylsilyl (ODS) C. C. (MeOH/H_2O), yielding the seven fractions (da–dh). Compound **1** (15.9 mg) was isolated as fraction db. Fraction c (2.5 g) was subjected to Si C. C. ($CHCl_3$/MeOH) to yield six fractions (ca–cf). Curvularin (**2**, 601.4 mg) was obtained as fraction ce.

Curvulanone (**1**): Colorless needles, m.p. 225–227 °C (dec.); $[\alpha]_D^{23}$ −0.2 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.02), 236 (3.83), 313 (3.57) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) ±0; IR (ATR) 3232, 1714 1634, 1573, 1481, 1167 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-EI-MS *m/z* 262.0833 $[M]^+$ (Calculated for $C_{14}H_{14}O_5$, 262.0841).

Single-Crystal X-Ray Crystallography Analyses **1** was crystallized from *n*-hexane-EtOAc to give colorless needles. The structures were refined with full matrix least-squares calculations on F2 using SHELXL-97.[25] Crystal data for **1**: $C_{14}H_{14}O_5$, space group *P*-1 (#2), $a = 7.0658$ (9) Å, $b = 8.4618$ (10) Å, $c = 10.9075$ (13) Å, $\alpha = 75.199$ (5)°, $\beta = 79.525$ (4)°, $\gamma = 81.275$ (4)°, $V = 616.19$ (13) Å^3, $Z = 2$, $D_{calc} = 1.413$ g/cm^3, $R = 0.1436$, $wR_2 = 0.4708$. Crystallographic data for **1** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under reference number CCDC 2298619. The data can be obtained free of charge at http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi, or from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44-1223-336-033; e-mail: data_request@ccdc.cam.ac.uk.

MAO-A and -B Inhibitory Assay MAO-A and MAO-B inhibitory activities were assayed using the method in the literature with slight modification.[26] Three microliters of human recombinant MAO-A solution (M7316, Sigma-Aldrich, St. Louis, MO, U.S.A.) or 7 μL of MAO-B solution (M7441, Sigma-Aldrich) was diluted with 1100 μL of potassium phosphate buffer (0.1 M, pH 7.4). One hundred and forty microliters of potassium phosphate buffer, 8 μL of kynuramine (final concentration is 30 μM, Sigma-Aldrich) in potassium phosphate buffer, and 2 μL of dimethyl sulfoxide (DMSO) containing tested compound or pargyline (final DMSO concentration of 1% (v/v), DMSO dissolved without tested compound was used as control), were mixed and pre-incubated at 37 °C for 10 min. Fifty microliters of diluted MAO-A or MAO-B solution was then added to each well. The reaction mixture was further incubated at 37 °C and the reaction was stopped after 20 min by the addition of 75 μL of 2 M NaOH. The product generated by MAO-A or MAO-B, 4-quinolinol, is fluorescent and was measured at Ex 310 nm/Em 400 nm using a microplate reader (SPECTRA MAX M2, Molecular Devices, Tokyo, Japan). DMSO without test compound was used as the negative control, and pargyline (Sigma-Aldrich) was used as a positive

control. The IC_{50} values were estimated using Prism software (version 5.02; GraphPad, San Diego, CA, U.S.A.). Pargyline was used as positive control (IC_{50} value was 4.0 μM for MAO-A, 1.5 μM for MAO-B).

SIRT1 Deacetylation Assay The SIRT1 enzyme reaction was performed on a half-volume 96 well microplate with SIRT1 direct fluorescent screening assay kit 10010401 (Cayman Chemical, Ann Arbor, MI, U.S.A.). Ten microliters of human recombinant SIRT1 was diluted by 900 μL of assay buffer (1 mg/mL bovine serum albumin (BSA), 1 mM $MgCl_2$, 2.7 mM KCl, 137 mM NaCl, and 50 mM Tris−HCl pH 8.0). SIRT1 direct peptide (10 μL) was diluted by 980 μL of assay buffer. SIRT1 solution (5 μL), assay buffer (25 μL) and test compounds DMSO solution (5 μL) were added to the well. Three point five microliters of NAD^+ assay buffer solution (50 mM) and SIRT1 direct peptide solution (140 μL) was mixed and 15 μL of this solution was added to each well and incubated at room temperature for 45 min. The 50 μL of the solution (the mixture of 7.5 μL nicotinamide solution (50 mM) and 400 μL of SIRT developer assay buffer solution (0.25 mg/400 μL)) was added to each well. After incubation for 30 min. at room temperature fluorescent of each well was measured at Ex 350 nm/Em 450 nm using a microplate reader (SPECTRA MAX M2). The IC_{50} values were estimated using Prism software. Sirtinol (IC_{50} value was 7.9 μM, FUJIFILM Wako Pure Chemical Corporation) was used as positive control.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

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