HETEROCYCLES, Vol. 102, No. 5, 2021, pp. 955 - 960. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 9th February, 2021, Accepted, 5th March, 2021, Published online, 11th March, 2021
DOI: 10.3987/COM-21-14427

A NEW CHROMONE DERIVATIVE FROM ENDOPHYTIC FUNGUS *XYLARIA* sp. ECN212

Ken-ichi Nakashima,[a]* Junko Tomida,[b] Yoshiaki Kawamura,[b] and Makoto Inoue[a]

[a]Laboratory of Medicinal Resources, School of Pharmacy, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan. [b]Laboratory of Microbiology, School of Pharmacy, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan. E-mail: nakasima@dpc.agu.ac.jp

Abstract – A new chromone derivative as well as four known polyketides were isolated from cultures of the endophytic fungus *Xylaria* sp. ECN212. The structure of the new compound was determined by extensive spectroscopic analyses. Furthermore, the absolute configuration of the new compound was established by comparison of experimental and calculated electronic circular dichroism data. The new compound is a related compound of xylariacetal, isolated from *Xylaria* sp. ECN008 in our previous study.

Endophytic fungi are a rich source of natural products, which exhibit unique chemical structures and various pharmacological activities, rendering them potential medicinal compounds.[1,2] Our previous studies on endophytic fungi isolated in Japan have revealed that these organisms produce a diverse range of compounds.[3–5] Xylariacetal, a polyketide previously isolated from the fungus *Xylaria* sp. ECN008 possesses a rare carbon skeleton,[6] although agistatin D[7] and diplosporin[8] have been isolated from the culture broth of fungi as related compounds. In this study, a new compound, with the same carbon framework as that of xylariacetal, was isolated from the endophytic fungus *Xylaria* sp. ECN212, a strain different to that of the xylariacetal-producing fungus. Herein, we describe the isolation and structural analysis of the new compound, as well as the genetic comparison between ECN008 and ECN212.

Xylaria sp. ECN212 was isolated from the leaves of *Camptotheca acuminata* and identified by sequencing the D1/D2 26S rRNA gene and internal transcribed spacers (ITS) of rDNA.[9] The mycelia were extracted twice with MeOH, and the solution was evaporated to give an extract (27.6 g), which was partitioned between ethyl acetate and water. Compound **1** was isolated as a colorless gum from the ethyl

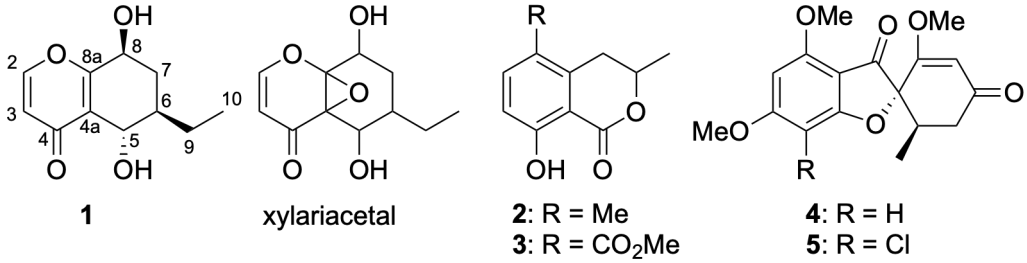

Figure 1. Structures of **1**–**5** and xylariacetal

acetate layer together with four known compounds, namely, 5-methylmellein (**2**),[10,11] 5-methoxycarbonylmellein (**3**),[10] 7-dechlorogriseofulvin (**4**),[12] and griseofulvin (**5**).[12]

The molecular formula of **1** was determined as $C_{11}H_{14}O_4$, based on the sodium adduct ion peak in the HRESIMS data. The IR spectrum contained absorption peaks correspinding to hydroxy (3383 cm^{-1}) and conjugated carbonyl groups (1645 cm^{-1}). The ^1H NMR spectrum exhibited signals indicative of a *cis*-disubstituted double bond [δ_H 7.81 (1H, d, J = 5.5 Hz, H-2), 6.33 (1H, d, J = 5.5 Hz, H-3)], a primary methyl [δ_H 0.97 (3H, t, J = 7.6 Hz, H_3-10)], two oxymethines [δ_H 4.58 (1H, dd, J = 1.8, 8.7 Hz, H-5), 4.72 (1H, ddd, J = 1.8, 5.9, 10.4 Hz, H-8)], two methylenes, a methine, and two hydroxy groups. The ^{13}C NMR and DEPT135 spectra indicated the presence of a tetrasubstituted double bond [δ_C 125.1 (C-4a), 164.0 (C-8a)] in addition to the *cis*-disubstituted double bond [δ_C 116.6 (C-3), 155.5 (C-2)]. The remaining carbon signals were assignable to a methyl [δ_C 10.9 (C-10)], two methylenes [δ_C 24.3 (C-9), 33.4 (C-7)], three sp^3 methines [δ_C 40.1 (C-6), 66.4 (C-8), 69.5 (C-5)], and a carbonyl carbon [δ_C 180.5 (C-4)].

The 4-pyrone moiety was confirmed by HMBC correlations of H-2/C-3, C-4, C-8a, H-3/C-4, and C-4a and the characteristic deshielding of C-2 and C-8a. The DQF COSY spectroscopic data and HMBC correlations of H-5/C-4a, C-8a, H-7/C-5, C-8a, H-8/C-4a, C-8a, H_2-9/C-5, C-7, H_3-10/C-6, C-9, and 5-OH/C-5, C-6 revealed the carbon sequence from C-5 to C-8 with an ethyl group at C-6. Furthermore, the linkage between C-4a and C-5 was confirmed by an HMBC correlation from H-5 to a carbonyl group (C-4). Thus, **1** was identified as 6-eyhyl-5,8-dihydroxy-5,6,7,8-tetrahydrochromone.

The relative configuration of **1** was established based on a combination of NOEs and the coupling constants between key protons. The NOE between H-5/H-7β indicated that H-5 and H-7β were in axial positions on the β face. Similarly, H-6 and H-8 were axially positioned on the opposite face (α), as shown by the NOE between H-6/H-8. The coupling constants between H-5/H-6 (J = 8.7 Hz), H-6/H-7β (J = 12.8 Hz), and H-7β/H-8 (J = 10.4 Hz) indicated that the four hydrogen atoms are in axial positions. In addition, other coupling constants, including the homoallylic coupling between H-5/H-8 (J = 1.8 Hz), further corroborated the assumed relative configuration.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for **1**[a] (δ in ppm, *J* in Hz)

Positions	**1** ($CDCl_3$)	
	δ_C, type	δ_H
2	155.4, CH	7.81 (1H, d, *J* = 5.5)
3	116.5, CH	6.33 (1H, d, *J* = 5.5)
4	180.5, C	
4a	125.1, C	
5	69.4, CH	4.58 (1H, dd, *J* = 1.8, 8.7)
6	40.1, CH	1.63 (1H, m)
7α	33.3, CH_2	2.30 (1H, ddd, *J* = 2.7, 5.9, 12.8)
7β		1.45 (1H, td, *J* = 10.4, 12.8)
8	66.4, CH	4.72 (1H, ddd, *J* = 1.8, 5.9, 10.4)
8a	164.0, C	
9α	24.2, CH_2	1.37 (1H, m)
9β		1.92 (1H, m)
10	10.8, CH_3	0.97 (3H, t, *J* = 7.6)
5-OH		4.95 (1H, br s)
8-OH		2.91 (1H, br s)

[a] ^{1}H- and ^{13}C-NMR spectra were measured at 400 and 100 MHz, respectively.

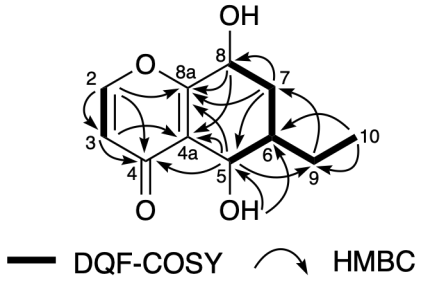

Figure 2. Key COSY (bold) and HMBC (arrows) correlations in **1**

The absolute structure of **1** was determined by comparison of calculated and experimental electronic circular dichroism (ECD) spectra. After conformational analysis, geometry optimization was performed using density functional theory (DFT) with the CAM-B3LYP/6–311+G(d,p) level of theory. ECD spectra of the DFT-optimized conformers were obtained by calculations using time-dependent DFT (TDDFT) with the CAM-B3LYP/6–311+G(d,p) level of theory. The calculated ECD spectrum is the Boltzmann weighted average of all the possible conformers. Because the experimental ECD spectrum was in good agreement with the calculated spectrum, the configuration of **1** was determined to be (5*S*, 6*R*, 8*S*).

Compound **1** is considered to be a biosynthetic precursor of xylariacetal, as it produces xylariacetal via epoxidation by monooxygenases, such as cytochrome P450. However, xylariacetal was not detected in the *Xylaria* sp. ECN212. The nucleotide sequences of the internal transcribed spacer (ITS) and the D1/D2

domain of the 26S rDNA gene were determined. Similarities in the sequences of the ITS and 26S rDNA genes among the strains of the genus *Xylaria* were 71.9 to 99.8% and 93.4 to 100.0%, respectively. ITS and 26S rDNA gene similarities between the ECN008 and ECN212 strains were 99.8% and 99.3%, respectively. These results indicate that the two strains were genetically closely related.

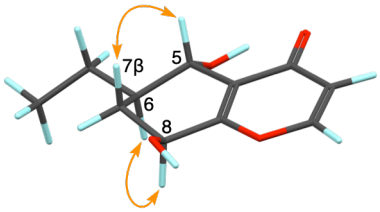

Figure 3. Key NOEs (orange arrows) observed for **1**

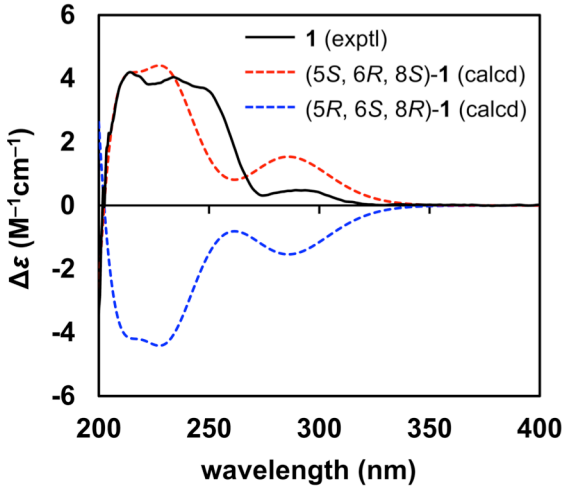

Figure 4. Experimental ECD spectrum of **1** compared with the calculated spectrum at the CAM-B3LYP/6-311+G(d, p)//CAM-B3LYP/6-311+G(d, p) level (Scaling factor = 0.8)

EXPERIMENTAL

General. Optical rotation values were recorded on a JASCO P-1020 polarimeter. UV spectra were obtained using a Hitachi U-2900 spectrometer. ECD spectra were acquired on a JASCO J-820 spectropolarimeter, and IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer. NMR spectra were acquired on a JEOL JNM-ECZ 400S spectrometer with tetramethylsilane as the internal standard. ESIMS data were obtained using an Agilent 6230 LC/TOF mass spectrometer. DNA sequencing was performed using an Applied Biosystems 3130 Genetic Analyzer. Silica gel AP-300 (Toyota Kako) and Sephadex LH-20 (GE Healthcare) were used for column chromatography (CC). Silica gel 60 F_{254} (Merck) was used for thin-layer chromatography.

Fungal Strain Isolation and Identification. *Camptotheca acuminata* leaves were collected in February 2016 at the Medicinal Plant Garden of Setsunan University (Osaka, Japan). The isolation and

identification of endophytic fungi were conducted according to a previously described method.[13] On the basis of DNA sequencing of the ITS of rDNA and the D1/D2 domain of the 26S rDNA, the isolate was found to belong to the genus *Xylaria*. The sequence data for *Xylaria* sp. ECNs 008 and 212 have been deposited at the DNA Data Bank of Japan (DDBJ) under access numbers LC603052 (ITS of ECN008), LC603053 (ITS of ECN212), LC603054 (26S rDNA of ECN008), and LC603055 (26S rDNA of ECN212). The strains were deposited at the Department of Microbiology, School of Pharmacy, Aichi Gakuin University.

Fermentation, Extraction, and Isolation. The fungus *Xylaria* sp. ECN212 was inoculated onto 50 plates composed of 2% malt extract agar. After incubation at 27 °C for 30 days, the fermented materials were extracted with MeOH (1 L, each 48 h) at room temperature (~ 28 °C), and the solution was evaporated in vacuo to obtain the MeOH extract (27.6 g). The MeOH extract was partitioned three times with EtOAc and water, and the EtOAc solution was concentrated under vacuum to yield the EtOAc soluble fraction (2.7 g). The fraction was chromatographed on a silica gel column eluting with $CHCl_3$/MeOH (step gradient 1:0, 30:1, 10:1, and 8:1, v/v) to give fractions (Frs.) 1–6. Fr. 2 was separated using a Sephadex LH-20 column and eluted with MeOH to obtain three combined subfractions. The second subfraction was further purified on a silica gel column eluting with *n*-hexane/acetone (2:1, v/v) to yield **2** (5.8 mg) and **3** (1.1 mg). Fr. 3 was likewise separated on a Sephadex LH-20 column and eluted with MeOH to obtain four combined subfractions. The third subfraction was further purified on a silica gel column with benzene/EtOAc (4:1, v/v) to yield **4** (13.2 mg) and **5** (11.3 mg). Fr. 5 was chromatographed on a Sephadex LH-20 column and eluted with MeOH to obtain **1** (12.5 mg).

(5*S*, 6*R*, 8*S*)-6-Ethyl-5,8-dihydroxy-5,6,7,8-tetrahydrochromone (1): colorless gum; $[\alpha]_D^{25}$ + 59.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 253 (3.88), 276 (2.62) nm; ECD (MeOH) λ (Δε) 214 (+ 4.20), 234 (+ 4.04), 249 (+ 3.65), 294 (+ 0.48); IR (KBr) 3383, 2963, 2932, 2876, 1645, 1597, 1456, 1437, 1314, 1244, 1142, 1125, 1059, 1009, 959, 849, 829 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HRESIMS *m/z* 233.0784 $[M + Na]^+$ (calcd. 233.0784 for $C_{11}H_{14}O_4$).

Computational Methods. Potential conformers of **1** were generated using CONFLEX 8 with the MMFF94s force field and a search limit of 5 kcal/mol. The suggested conformers were optimized by DFT calculations using Gaussian 16 at the CAM-B3LYP/ 6–311+G(d,p) level with the CPCM solvent model. Each optimized conformer was further calculated employing the TDDFT method and generated ECD spectra. The calculated ECD spectra were the Boltzmann-weighted average at 298 K of possible conformers (UV correction = + 15 nm, band width σ = 0.3 eV).

ACKNOWLEDGEMENTS

This study was supported by JSPS KAKENHI (Grant Number JP18K14939).

REFERENCES

1. S. Gouda, G. Das, S. K. Sen, H.-S. Shin, and J. K. Patra, *Front. Microbiol.*, 2016, **7**, 1538.
2. A. Schueffler and T. Anke, *Nat. Prod. Rep.*, 2014, **31**, 1425.
3. K. Nakashima, J. Tomida, T. Hirai, Y. Kawamura, and M. Inoue, *J. Nat. Prod.*, 2019, **82**, 3347.
4. K. Nakashima, J. Tomida, T. Kamiya, T. Hirai, Y. Morita, H. Hara, Y. Kawamura, T. Adachi, and M. Inoue, *Tetrahedron Lett.*, 2018, **59**, 1212.
5. K. Nakashima, J. Tomida, T. Tsuboi, Y. Kawamura, and M. Inoue, *Beilstein J. Org. Chem.*, 2020, **16**, 2100.
6. K. Nakashima, J. Tomida, M. Tabata, T. Hirai, Y. Morita, Y. Kawamura, and M. Inoue, *Heterocycles*, 2017, **94**, 308.
7. A. Gohrt, S. Grabley, R. Thiericke, and A. Zeeck, *Liebigs Ann. Chem.*, 1996, 627.
8. A. A. Chalmers, C. P. Gorst-Allman, N. P. J. Kriek, W. F. O. Marasas, P. S. Steyn, and R. Vleggaar, *S. Afr. J. Chem.*, 1978, **31**, 111.
9. H. A. Raja, A. N. Miller, C. J. Pearce, and N. H. Oberlies, *J. Nat. Prod.*, 2017, **80**, 756.
10. M. W. Sumarah, E. Puniani, B. A. Blackwell, and J. D. Miller, *J. Nat. Prod.*, 2008, **71**, 1393.
11. A. Ballio, S. Barcellona, and B. Santurbano, *Tetrahedron Lett.*, 1966, **7**, 3723.
12. J.-H. Park, G.-J. Choi, S.-W. Lee, K.-M. Kim, H.-S. Jung, K.-S. Jang, K.-Y. Cho, and J.-C. Kim, *J. Microbiol. Biotechnol.*, 2005, **15**, 112.
13. K. Nakashima, J. Tomida, T. Hirai, Y. Kawamura, and M. Inoue, *J. Nat. Med.*, 2019, **73**, 480.