NOTE

JBIR-54, a new 4-pyridinone derivative isolated from *Penicillium daleae* Zaleski fE50

Akira Mukai¹, Aya Nagai¹, Shigeki Inaba², Motoki Takagi¹ and Kazuo Shin-ya³*The Journal of Antibiotics* (2009) **62**, 705–706; doi:10.1038/ja.2009.101; published online 23 October 2009**Keywords:** JBIR-54; *Penicillium daleae*; pyridinone

Penicillium is a versatile ascomyceteous fungi. It causes opportunistic infections and putrefaction of foods. *Penicillium* species are known to produce more than 900 documented bioactive compounds.¹ Furthermore, important pharmaceutical agents, namely, penicillins and compactin, have been isolated from *Penicillium* spp. We have reported that this fungus also produces valuable secondary metabolites, such as JBIR-12,² JBIR-27³ and JBIR-28.³ Therefore, we further screened for novel secondary metabolites from culture broths of *Penicillium* species. In this paper, we report the fermentation, isolation and structural determination of a new secondary metabolite, JBIR-54 (**1**), in addition to the taxonomy of the producing microorganism.

The *Penicillium daleae* Zaleski fE50 strain that produced **1** was isolated by the sodium dodecyl sulfate-yeast extract method⁴ from a soil sample collected in Fukui Prefecture, Japan. The soil was treated with a solution containing 6% bacto-yeast extract (BD Biosciences, San Jose, CA, USA) and 0.05% sodium dodecyl sulfate at 40 °C for 20 min. The solution was diluted with water, plated on potato dextrose agar plates, and incubated at 27 °C for a few weeks. Individual fungal colonies that appeared on the plate were transferred to potato dextrose agar slants and individual strains were maintained. The fE50 strain was identified by sequence analysis of the ribosomal DNA ITS region, and its microscopic features were observed by using a Zeiss Axioplan 2 imaging system (Carl Zeiss, Oberkochen, Germany). Sequence and morphological analysis revealed that the fE50 strain was 99.8% similar to the *P. daleae* strain NRRL 922. Thus, the strain was identified as *P. daleae* Zaleski.

The seed medium was potato dextrose broth (24 g l⁻¹ potato dextrose; BD Biosciences). The production medium containing 3 g brown rice (Hitomebore, Miyagi, Japan), 6 mg bacto-yeast extract (BD Biosciences), 3 mg sodium tartrate, 3 mg KH₂PO₄ and 9 ml H₂O was dispensed in 100-ml Erlenmeyer flasks. The fE50 strain was cultivated

in 50-ml test tubes containing 15 ml of seed medium. The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 3 days. Aliquots (1 ml) of the culture were transferred to 100-ml Erlenmeyer flasks containing the production medium and incubated in static culture at 27 °C for 14 days.

The culture of fE50 strain (100-ml Erlenmeyer flasks × 20) was extracted with 80% aqueous Me₂CO. The extract was evaporated *in vacuo*, and the residual aqueous concentrate was extracted with EtOAc (100 ml × 3). After drying over Na₂SO₄, the organic layer was evaporated to dryness. The dried residue (20.4 mg) was applied to a normal-phase medium-pressure liquid chromatography column (Purif-Pack SI-60; Moritex, Tokyo, Japan) and eluted with a chloroform–MeOH gradient (0–50% MeOH). The main fraction (0.98 mg) was finally separated by preparative HPLC using an L-column2 ODS (20 i.d. × 150 mm; Chemical Evaluation Research Institute, Tokyo, Japan) with 55% aqueous MeOH (flow rate, 10 ml min⁻¹, UV detection: 254 nm) to obtain the new compound **1** (0.48 mg; retention time, 14 min).

Compound **1** was obtained as a white powder ([α]_D²⁵ = –22.2; *c* = 0.1 in MeOH; UV (MeOH) $\lambda_{\max}$ (ϵ): 223 (15 000), 316 nm (9800), and its IR spectrum showed the characteristic absorptions of the carbonyl group ($\nu_{\max}$, 1669 cm⁻¹). The HR electron spray ionization-MS of **1** produced the (M+H)⁺ ion at *m/z* 236.1669 consistent with the molecular formula of C₁₄H₂₂NO₂ (calculated value for C₁₄H₂₂NO₂, 236.1651).

The structure of **1** was elucidated by a series of NMR techniques, such as constant time heteronuclear multibond correlation (CT-HMBC)⁵ and double-quantum filtered (DQF)-COSY. The direct C–H connectivity was established by heteronuclear single quantum coherence (see Table 1). In the DQF spectrum, the sequence from a methyl proton 1-H₃ (δ_{H} 1.01) to an olefinic proton 6-H (δ_{H} 5.66)

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Table 1 ^1H - and ^{13}C -NMR data of JBIR-54 (**1**)

Position	δ_{C}	δ_{H} (J in Hz)
1	13.6	1.01, t (7.5, 7.5)
2	26.0	2.12, m
3	139.2	5.81, m
4	128.1	6.01, dd (10, 15)
5	130.5	6.28, dd (10, 15)
6	133.3	5.66, d (15)
7	73.4	
7-OH		1.90
8	28.1	1.54, s
9	168.6	
10	95.4	4.97, s
11	193.2	
12	43.3	2.26, dd (6, 12) 2.35, dd (6, 12)
13	49.0	3.76, m
14	20.5	1.33, d (6.5)
14-NH		5.79

The ^{13}C (125 Hz) and ^1H (500 Hz) NMR spectra were obtained using a Varian NMR system 500 NB CL (Palo Alto, CA, USA) in CDCl_3 , and the solvent peak was used as an internal standard (δ_{C} 77.0 p.p.m.; δ_{H} 7.26 p.p.m.).

Figure 1 Structure of **1**, and ^1H - ^1H (bold lines), main ^1H - ^{13}C (solid arrows) and ^1H - ^{15}N (dotted arrow) correlations in 2D NMR.

through a methylene proton 2- H_2 (δ_{H} 2.12), an olefinic proton 3- H (δ_{H} 5.81), an olefinic proton 4- H (δ_{H} 6.01) and an olefinic proton 5- H (δ_{H} 6.28) was observed as shown in Figure 1. Another spin coupling system was detected from the methylene proton 12- H_2 (δ_{H} 2.26, 2.35) to the methyl proton 14- H_3 (δ_{H} 1.33) through the methine proton 13- H (δ_{H} 3.76). A 2,3-dihydropyridin-4-one moiety was identified by the following correlation. Long-range couplings from the olefinic proton 10- H (δ_{H} 4.97) to the carbonyl carbon C-11 (δ_{C} 193.2) and

methylene carbon C-12 (δ_{C} 43.3), as well as from the methine proton 13- H to C-11 were observed by CT-HMBC. Moreover, ^1H - ^{15}N long-range couplings from 10- H and 14- H_3 to an amine nitrogen atom 9- NH were observed. On the basis of the ^{13}C chemical shift at C-13 (δ_{C} 49.0), this nitrogen atom was confirmed to be substituted at the C-13 position. Thus, these members form a cyclic structure, which is 2-methyl-2,3-dihydropyridin-4-one (Figure 1). The long-range couplings from the methyl proton 8- H_3 (δ_{H} 1.54) to the olefinic carbon C-6, quaternary carbon C-7 (δ_{C} 73.4) and olefinic quaternary carbon C-9 (δ_{C} 168.6), which in turn is long-range coupled from the olefinic proton 10- H , as well as from the hydroxyl proton 7-OH (δ_{H} 1.90) to the olefinic carbon C-6 (δ_{C} 133.3), quaternary carbon C-7 and olefinic quaternary carbon C-9 revealed that the side chain moiety was substituted at the C-9 position. The coupling constants between 3- H and 4- H ($J=15\text{ Hz}$), and 5- H and 6- H ($J=15\text{ Hz}$) indicated that the configurations at C-3 and C-5 are *trans*. Thus, the structure of **1** was determined as shown in Figure 1. Streptazone D, a derivative of **1**, is a known secondary metabolite of *Streptomyces* sp.⁶ To the best of our knowledge, this is first report of streptazone D analogs from fungi.

The 2-pyridinone analogs are reported to be non-nucleoside reverse-transcriptase inhibitors that can be used as anti-HIV agents.⁷ The antimicrobial activities of 2- and 4-pyridinone analogs have also been studied.^{8,9} Therefore, we attempted to investigate the cytotoxic and antimicrobial activities of **1**. The results showed that **1** did not exhibit cytotoxic activity against several cancer cell lines or antibacterial activity against *Micrococcus luteus* and *Escherichia coli*.

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