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NOTE

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A Novel Indole-diterpenoid, JBIR-03 with Anti-MRSA Activity from *Dichotomomyces cejpii* var. *cejpii* NBRC 103559

Masahiro Ogata, Jun-ya Ueda, Midori Hoshi, Junko Hashimoto, Takuji Nakashima, Kozue Anzai, Motoki Takagi, Kazuo Shin-ya

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Abstract A new indole-diterpene, JBIR-03 (**1**), was isolated from the fungus *Dichotomomyces cejpii* var. *cejpii* NBRC 103559 and its structure was determined based on the spectroscopic data. **1** exhibited anti-MRSA (methicillin-resistant *Staphylococcus aureus*) activity and antifungal activity against apple Valsa canker-causing fungus, *Valsa ceratosperma*, while it exhibited no toxicity towards human cancer cells.

Keywords JBIR-03, indole-diterpene, *Dichotomomyces cejpii*, MRSA, *Valsa ceratosperma*

Introduction

During the past decade, nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals have become a serious clinical problem [1]. Vancomycin has been used for the treatment of infections due to MRSA. However, vancomycin-resistant *S. aureus* has recently been isolated [2]. The emergence of vancomycin-resistant bacterial strains is a very serious public health problem. Therefore, a new anti-MRSA antibiotic is clinically of interest. On the other hand, Valsa canker, caused by the fungus *Valsa ceratosperma* is a significant disease of apple in the Pacific Rim countries, including Japan, China, and Korea [3]. It is also found occasionally on pear and quince. In northern Japan, the disease is especially severe with more than 35% of orchards affected to some degree. However, information available to help with breeding against Valsa canker in apples is limited [4]. In our course of screening for anti-MRSA activity, we isolated a new indole-diterpenoid designated as JBIR-03 (**1**) from mycelium of *Dichotomomyces cejpii* var. *cejpii* NBRC 103559.

D. cejpii var. *cejpii* NBRC 103559 was cultured at 27°C for 14 days in 500-ml Erlenmeyer flasks each containing a solid medium consisting of 15 g oatmeal and 50 ml V8 Mix Juice (Campbell Soup Company). The mycelium

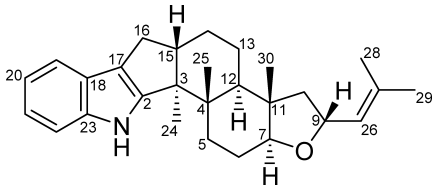

Fig. 1 Structure of JBIR-03 (**1**).

K. Shin-ya (Corresponding author)**:** Biological Information Research Center (BIRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan, E-mail: k-shinya@aist.go.jp
M. Takagi (Corresponding author)**, J. Ueda, M. Hoshi, J. Hashimoto:** Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan,
E-mail: motoki-takagi@aist.go.jp
M. Ogata: Department of Microbiology, Faculty of Pharmaceutical Sciences, Aomori University; 2-3-1 Koubata, Aomori-shi, Aomori, 030-0943, Japan
T. Nakashima, K. Anzai: NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan
M. Ogata, J. Ueda: These authors contributed equally to this work.

Table 1 Physico-chemical properties of JBIR-03 (**1**)

Appearance	Colorless needle
MP	142.5～148.0°C
$[\alpha]_D^{24.5}$	+46.2° (*c* 0.05, MeOH)
HR-ESI-MS (*m/z*)	
found	404.2953 $(M+H)^+$
calcd	404.2929
UV λ_{max} (MeOH) nm (log ε)	229 (4.42), 280 (3.76)
IR ν_{max} ($CHCl_3$) cm^{-1}	3477

(500 ml×8) was extracted with 80% Me_2CO. After concentration *in vacuo*, the residual concentrate was extracted with EtOAc (200 ml×3). After drying over Na_2SO_4, the organic layer was evaporated to dryness. The dried residue (0.48 g) was applied to normal-phase MPLC (Purif-Pack SI-60, Moritex) and developed with a *n*-hexane - EtOAc linear gradient system to yield an active fraction (40～55% EtOAc eluate). The active eluate was subjected to preparative reversed-phase HPLC (90% MeOH - H_2O, Senshu Pak PEGASIL ODS 20 i.d.× 150 mm) to yield **1** (1.0 mg; Rt, 14.5 minutes).

The physico-chemical properties of **1** are summarized in Table 1. **1** was obtained as colorless needles (MP 142.5～148.0°C) and its molecular formula was determined to be $C_{28}H_{37}NO$ by HR-ESI-MS. The IR (ν_{max} 3477 cm^{-1}) and UV (λ_{max} 229, 280 nm) spectra of **1** suggested the presence of an indole moiety [5, 6]. The ^1H- and ^{13}C-NMR spectra (Table 2) revealed the signals of ten sp^2 carbons (C-2, δ_C 151.9; C-17, δ_C 117.8; C-18, δ_C 126.0; C-19 δ_H 7.28, δ_C 118.6; C-20, δ_H 6.92, δ_C 119.6; C-21, δ_H 6.95, δ_C 120.7; C-22, δ_H 7.28, δ_C 112.6; C-23, δ_C 141.8; C-26, δ_H 5.32, δ_C 127.7; C-27, δ_C 135.7), two oxygenated carbons (C-7, δ_H 3.30, δ_C 87.4; C-9, δ_H 4.90, δ_C 75.1), two vinyl methyl groups (C-28, δ_H 1.68, δ_C 18.1; C-29, δ_H 1.73, δ_H 26.0), and three methyl groups (C-24, δ_H 1.03, δ_C15.0; C-25, δ_H 1.11, δ_C 21.0; C-30, δ_H 0.91, δ_C15.4). In the ^1H-^1H COSY spectrum of **1**, spin couplings among aromatic protons 19-H (δ_H 7.28), 20-H (δ_H 6.92), 21-H (δ_H 6.95) and 22-H (δ_H 7.28) revealed the presence of a 1,2-disubstituted benzene ring. The sequence from 10-H (δ_H 1.97, 1.25) through 9-H (δ_H 4.90) to 26-H (δ_H 5.32) which in turn allylic coupled to methyl protons 28-H (δ_H 1.68) and 29-H (δ_H 1.73) established a 4-methylpent-3-en-2-ol moiety. In the same manner, a pentane and a propan-1-ol moiety were recognized. In the HMBC spectrum of **1**, the aromatic proton 19-H was *peri* coupled to aromatic quaternary carbon C-17 (δ_C 117.8), which was further long-range coupled to methylene protons 16-H (δ_H 2.61, 2.29). In addition, 16-H was long-range coupled to an aromatic carbon C-2 (δ_C 151.9). Taking into consideration these correlations and UV absorption *vide supra*, a 2,3-disubstituted indole residue was established. ^1H-^{13}C long-range couplings between 16-H and C-3 (δ_C 53.9), between a methyl proton 24-H (δ_H 1.03) and C-2, C-3, C-4 (δ_C 41.4) and C-15 (δ_C 50.1), and between a methyl proton 25-H (δ_H 1.11) and C-3, C-4, C-5 (δ_C 34.4) and C-12 (δ_C 47.6) elucidated the connectivity among these substructures as shown in Fig. 2A. Likewise, a methyl proton 30-H (δ_H 0.91) to C-7 (δ_C 87.4), C-10 (δ_C 49.6), C-11 (δ_C 46.1) and C-12 proved the tri-ring system. Finally, long-range couplings between the methylene proton 10-H and C-7, C-11 and C-12, and between an oxymethine proton 9-H (δ_H 4.90) and C-7 established the tetrahydrofuran moiety. Thus, the planar structure of **1** was determined as shown in Fig. 2A.

The relative configuration was assigned on the basis of coupling constants and the analysis of a ROESY experiment measured in pyridine-d_5. The large coupling constants for $J_{6H,7H}$ (11.7 Hz) and $J_{14H,15H}$ (10.5 Hz) suggested that 7-H and 15-H are in *axial* location. The ROESY correlations (Fig. 2B) between 7-H, 5-αH, 10-αH and 12-H, and between 24-H, 5-αH, 14-αH and 16-αH indicated these protons located the same direction. The ROESY correlations between 25-H, 6-βH, 13-βH, 15-H and 30-H, between 10-βH, 9-H and 30-H, and between 9-H and 30-H also revealed that 6-βH, 9-H, 10-βH, 13-βH, 15-H, 4-Me (25-H) and 11-Me (30-H) are in β-orientation. Thus, the structure of **1** was established as shown in Fig. 1. The structure of **1** resembled those of emindole SB [5] and paspaline [6], which were reported as indoloditerpenes except for the tetrahydrofuran moiety. Although lots of indolediterpenes such as emindole SB [5], paspaline [6], petromindole [7] and paxilline [8] were reported, the terminal ring system of these compounds commonly consisted of a 6-membered ring such as pyran. In contrast, the structure of **1** possessing the terminal ring system that consists of a 5-membered ring such as furan in this series of nodulisporic acids [9] is very rare.

1 was examined for antimicrobial activities against *S. aureus* N315, MRSA (*S. aureus* N315 ΔI-HR), *Bacillus subtilis* JCM2499, *Pseudomonas aeruginosa* JCM5962, *Escherichia coli* IID 5208 and *V. ceratosperma* which is resistant to known antifungal compound such as griseofulvin. **1** inhibited the growth of Gram-positive and Gram-negative bacteria at concentrations of 32 and 64 μg/ml, respectively. **1** also inhibited the growth of *V. ceratosperma* with an MIC value of 128 μg/ml. To the contrary, **1** did not show any cytotoxic activity against a human HT-1080 fibrosarcoma cell line at a concentration of 100 μM.

Table 2 ^{13}C (150 MHz)- and ^{1}H (600 MHz)-NMR data for **1**

	^{13}C[a]	^{1}H[a]	^{1}H[b]
2	151.9		
3	53.9		
4	41.4		
5	34.4	1.94 (m)	1.88 (m)
		1.87 (m)	1.79 (m)
6	23.5	1.88 (m)	1.82 (m)
		1.78 (m)	1.74 (m)
7	87.4	3.30 (m)	3.28 (dd, 11.7, 1.8)
9	75.1	4.90 (m)	4.94 (q, 8.2)
10	49.6	1.97 (dd, 11.5, 7.1)	1.88 (ddd, 11.2, 6.7, 2.1)
		1.25 (m)	1.17 (m)
11	46.1		
12	47.6	1.74 (m)	1.78 (m)
13	26.4	1.58 (m)	1.53 (br q, 12.5)
		1.53 (m)	1.41 (m)
14	26.3	1.75 (m)	1.75 (m)
		1.67 (m)	1.64 (m)
15	50.1	2.75 (br q, 10.5)	2.79 (m)
16	28.3	2.61 (dd, 12.9, 6.5)	2.76 (m)
		2.29 (dd, 12.9, 10.9)	2.45 (t, 10.6)
17	117.8		
18	126.0		
19	118.6	7.28 (br d, 7.9)	7.55 (m)
20	119.6	6.92 (br t, 7.5)	7.21 (td, 7.6, 1.5)
21	120.7	6.95 (br t, 7.5)	7.25 (td, 7.9, 1.5)
22	112.6	7.28 (br d, 7.9)	7.70 (br d, 7.6)
23	141.8		
24	15.0	1.03 (s)	1.11 (s)
25	21.0	1.11 (s)	1.38 (m)
26	127.7	5.32 (dd, 8.8, 1.0)	5.51 (dd, 8.5, 1.2)
27	135.7		
28	18.1	1.68 (s)	1.69 (s)
29	26.0	1.73 (s)	1.62 (s)
30	15.4	0.91 (s)	0.89 (s)

[a] Measured in methnol-d_4 including 4.0% $CDCl_3$. [b] Measured in pyridine-d_5.

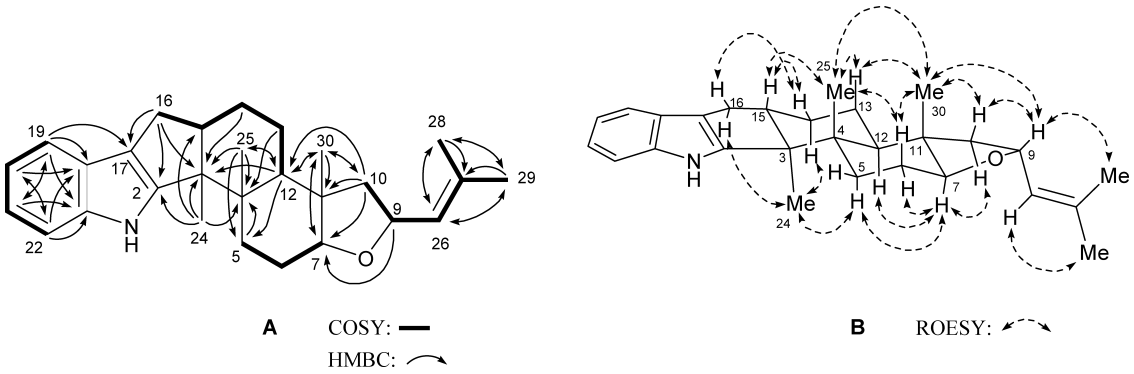

Fig. 2 Key correlations in ^{1}H-^{1}H COSY (solid line) and HMBC (solid arrow, A), and ROESY experiments (dashed arrow, B) of **1**.

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