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NEW DIOXOMORPHOLINE DERIVATIVES, JAVANICUNINE A AND B, FROM *EUPENICILLIUM JAVANICUM*

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Abstract – Two new dioxomorpholine derivatives, javanicunine A (**1**) and B (**2**) were isolated from the extract of *Eupenicillium javanicum* IFM 54704. These structures were determined by chemical and spectroscopic methods.

INTRODUCTION

We have been searching fungal metabolites which showed antifungal activity against pathogenic filamentous fungi, *Aspergillus fumigatus* and *A. niger*, and/or pathogenic yeasts, *Candida albicans* and *Cryptococcus neoformans*. During our research[1], we found that the organic extract of *Eupenicillium javanicum* IFM 54704 showed characteristic and strong antifungal activity against *A. fumigatus*. Further fractionation of the extract led to the isolation of two new dioxomorpholine derivatives, designated javanicunines A (**1**) and B (**2**), along with *p*-hydroxybenzoic acid and *p*-hydroxyphenylacetic acid. In this paper, we report the isolation and structure determination of javanicunines A (**1**) and B (**2**).

RESULTS AND DISCUSSION

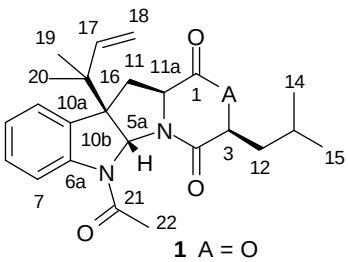
The molecular formula of **1** was determined as $C_{24}H_{30}N_2O_4$ by HREI-MS, and that of **2** as $C_{24}H_{30}N_2O_5$ by HRFAB-MS. An examination of the NMR spectral data (^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC) for **1** and **2** suggested a close structural similarity to fructigenine (**3**)[2]. The ^{13}C NMR spectrum of **1** (Table

1) was similar to that of **3**, except for the downfield-shifted carbon at C-3 (δ_C 77.2). From the molecular formula and the IR absorption at 1760 cm^{-1} of **1**, the plane structure of javanicunine A was assigned as **1**.

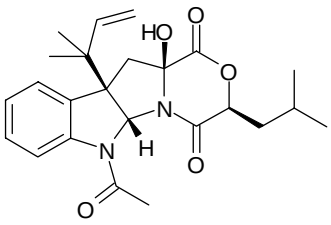
Table 1. ^1H and ^{13}C NMR spectral data for **1** and **2** in $CDCl_3$

No	**3**	**1**		**2**		HMBC correlations for **1**
	^{13}C	^{13}C	^1H	^{13}C	^1H	
1	165.9	168.5		167.3		
3	53.2	77.2	4.66 dd, 9.6, 2.8	77.0	5.00 dd, 10.4, 3.1	4, 12, 13
4	169.0	165.1		165.3		
5 a	79.4	79.4	5.92 br s	80.5	5.92 br s	6a, 10b, 16
6 a	143.2	142.8		142.1		
7	124.4 *	119.6	7.99 br d	120.7	8.06 br d	
8	128.9	129.3	7.34 td, 7.2, 1.5	130.0	7.42 td, 7.7, 1.2	6a, 7, 10
9	119.1 *	124.6	7.16 td, 7.5, 1.0	125.0	7.22 td, 7.4, 1.1	7, 10a
10	128.9	124.3	7.28 br d, 7.7	124.4	7.37 br d, 7.5	6a, 8, 10b
10 a	132.2	132.0		130		
10 b	60.8	60.9		59.2		
11	38.9	35.2	2.53 dd, 13.0, 10.8	42.0	2.94 d, 14.5	1, 10a, 10b, 11a, 16
			2.70 dd, 13.0, 6.4		2.69 d, 14.5	5a, 10a, 10b
11 a	59.0	57.7	4.00 dd, 10.8, 6.4	87.4		1, 11
12	35.8	37.7	1.82 m	37.6	1.76 m	3, 4, 13, 14
			1.89 m		1.87	4, 13, 14
13	24.4	23.8	1.90 m	23.8	1.91	
14	21.1	21.1	0.89 d, 6.1	21.2	0.89 d, 6.0	12
15	21.2	23.2	0.97 d, 4.4	23.3	0.97 d, 6.0	12
16	40.3	40.4		40.4		
17	143.0	142.8	5.79 dd, 17.4, 10.8	142.5	5.75 dd, 17.2, 10.8	10b, 16, 19, 20
18	114.5	114.8	5.12 d, 17.4	115.3	5.12 d, 17.2	16, 17
			5.15 d, 10.8		5.16 d, 10.8	16, 17
19	22.3	22.4	1.16 s	22.3	1.13 s	16, 17
20	22.5	23.0	0.97 s	22.9	0.96 s	16, 17
21	170.0	169.8		169.8		
22	23.2	23.5	2.63 br s	23.5	2.61 br s	21

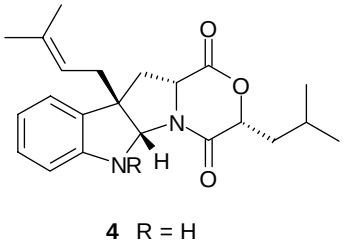
* Assignment should be reversed.



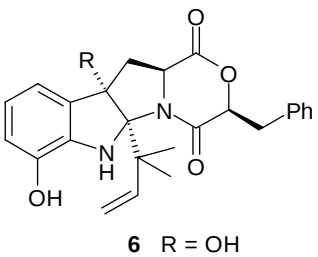
1 A = O
3 A = NH



2



4 R = H
5 R = CHO



6 R = OH
7 R = OAc

The stereochemistry of **1** including the absolute configuration was established by difference NOE experiments and acid hydrolysis as follows. Irradiation of the signal for the 11a-H (δ_H 4.00) brought about enhancement of the 3-H signal (δ_H 4.66) and one of hydrogens at C-11 (δ_H 2.70). Irradiation of the 19-methyl signal (δ_H 1.16) caused enhancement of the other upfield proton at C-11 (δ_H 2.53) and the 5a-H signal (δ_H 5.92). Above results and the coupling constant between the 11a-H and 11-H signals revealed **1**

having the 1,1-dimethylallyl group and the 5a-H on the opposite face of the molecule from 11a-H and 3-H. An amino acid derived from **1** by hydrolysis with 6M HCl was identified with L-tryptophan by TLC to resolve enantiomeric compounds. The structure of javanicunine A was thus assigned as depicted in structure **1** with the absolute stereochemistry shown.

The ^{13}C NMR spectrum of javanicunine B (**2**) was similar to that of **1**, except for the downfield-shifted carbon at C-11a (δ_C 87.4) having no proton. Irradiation of the 19-methyl signal (δ_H 1.13) in **2** brought about enhancements of the 5a-H signal (δ_H 5.92) and the downfield proton at C-11 (δ_H 2.94). Accordingly, the dimethylallyl group, the 5a-H, and the downfield proton at C-11 in **2** were on the same face of the molecule. Considering the molecular formula and the presence of the large downfield-shifted β-proton at C-11 (δ_H 2.94), javanicunine B (**2**) seems to have β-OH at C-11a[3]. The absolute configuration of **2** has not yet been determined, but it was assumed to be as shown in the structure (**2**) because of the co-occurrence of **1**.

Although dioxopiperazines are common as fungal metabolites, dioxomorpholines are quite rare. Mollenines A (**4**) and B (**5**)[4] from *Eupenicillium molle*, and PF1233 A (**6**) and B (**7**)[5] from *Aspergillus niveus* are only known as naturally occurring dioxomorpholines derived from tryptophan. Javanicunines A (**1**) and B (**2**) have same stereochemistry as common dioxopiperazines from fungus, though that of **4** – **7** differs from common dioxopiperazine, such as **3** and roquefortine C[6]. Unfortunately, both **1** and **2** showed no antifungal activity. Further studies are required.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 photopolarimeter. The UV and IR spectra were recorded on a JASCO V-560 and a JASCO FT/IR-4100 spectrophotometers, respectively. EI and FABMS spectra were taken with a JEOL-MS600W spectrometer. ^1H and ^{13}C NMR spectra were recorded on a JNM AL-300 (^1H, 300.40 MHz; ^{13}C, 75.45 MHz) spectrometer, using $CDCl_3$ solutions containing tetramethylsilane as an internal standard. The TLC to resolve enantiomeric compounds was carried out on MACHEREY-NAGEL chiralplate (solvent: MeOH : H_2O : MeCN = 1 : 1 : 4)[6,7].

Culture, Extraction and Isolation. *Eupenicillium javanicum* IFM 54704 was cultured at 25 °C for 21 days in 10 Roux flasks containing 250 g of moist rice in each flask. The fermented rice was extracted with CH_2Cl_2-MeOH (1:1) and the organic layer was evaporated *in vacuo*. The resultant extract was suspended in H_2O and extracted with EtOAc, and then the organic layer was evaporated *in vacuo*. The EtOAc extract (23g), which showed the strong antifungal activity against *A. fumigatus*, was separated by column chromatography on SiO_2 (400 g) into six fractions: CH_2Cl_2 (50:1) (10.5g), CH_2Cl_2-EtOH (20:1) (3.8g), CH_2Cl_2-EtOH (10:1) (6.2g), CH_2Cl_2-EtOH (5:1) (0.5 g), CH_2Cl_2-EtOH (1:1) (0.8 g), and

EtOH (0.5 g). The 3rd fraction [CH_2Cl_2-EtOH (10:1)] was purified by LPLC on a SiO_2 column using benzene-acetone (10:1) followed by the further purification of HPLC on SiO_2 column [cyclohexane-acetone (10:1)] to give javanicunines A (**1**) (35 mg), B (**2**) (3 mg), *p*-hydroxybenzoic acid (7mg), *p*-hydroxyphenylacetic acid (11 mg) in this elution order.

Javanicunine A (**1**) : Colorless amorphous; $[\alpha]_D^{22}$ -152.3° (*c* = 1.0, CH_2Cl_2); IR ν_{max}: 1760(CO_2), 1670 (CO) cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε): 247 (3.95), 278 (3.14), 285 (3.09); EI-MS *m/z* : 410 (M^+, 18), 368 (29), 299 (base peak), 157 (27), 130 (19); HREIMS *m/z* : 410.1094 (M^+, 410.1103 for $C_{24}H_{30}N_2O_4$). The assignments of ^1H and ^{13}C NMR signals are summarized in Table 1.

Javanicunine B (**2**) : Colorless amorphous; $[\alpha]_D^{22}$ -118.5° (*c* = 0.20, CH_2Cl_2); IR ν_{max}: 1760(COO), 1700 (CO) cm^{-1}; UV (MeOH) λ_{max} (log ε): 246 (3.87), 278 (3.13), 285 (3.09); HRFAB(+)MS *m/z* : 427.2260 (MH^+, 427.2233 for $C_{24}H_{31}N_2O_5$). The assignments of ^1H and ^{13}C NMR signals are summarized in Table 1.

Acid hydrolysis of Javanicunines A (1). The tube containing **1** (3mg) and 6M-HCl (0.5 mL) was sealed under vacuum after being flushed with argon, and kept at 115°C for 22 hrs. After evaporated to dryness and neutralized with 0.5M-Na_2CO_3, the reaction mixture compared directly with D- and L-tryptophan on the TLC to resolve enantiomeric compounds[6,7].

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