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NEW TYPE INDOLE DITERPENE, EUJINDOLES, FROM *EUPENICILLIUM JAVANICUM*

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Abstract – Two new indole diterpenes, 17-hydroxyeujindole (**1**) and 17-oxoeujindole (**2**), were isolated from the extract of *Eupenicillium javanicum* IFM 59075 (UC62). The structures containing the absolute configuration were determined by spectroscopic methods.

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 59075 showed a positive coloration with van Urk reagent.[2] Further fractionation of the extract led to the isolation of two new indole diterpenes, designated 17-hydroxyeujindole (**1**) and 17-oxoeujindole (**2**), along with 10,23-dihydro-24,25-dehydroaflavinine (**3**),[3] nominine (**4**)[4] and 2,3-anhydromevalonic acid δ-lactone.[5] In this paper, we report the isolation and structure determination of 17-hydroxyeujindole (**1**) and 17-oxoeujindole (**2**) containing their absolute configurations.

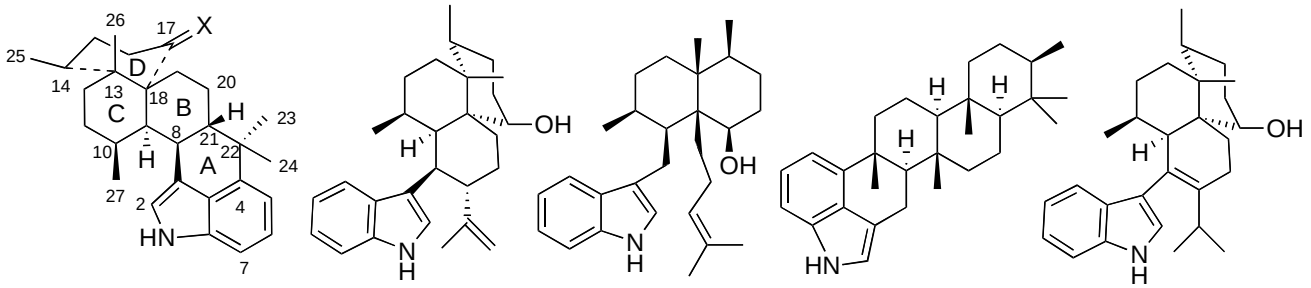
The molecular formula of **1** was determined as $C_{28}H_{39}NO$ by the HRFABMS, and that of **2** as $C_{28}H_{37}NO$ by HRFABMS. A positive coloration (blue) with van Urk reagent and the UV spectra suggested the presence of an indole moiety in **1** and **2**.

Proton and carbon NMR data for **1** and **2** are provided in Table 1. The ^{13}C NMR spectra of **1** and **2** revealed the presence of 8 and 9 sp^2 carbons, respectively. Considering the index of hydrogen deficiency (**1**; 10, **2**; 11), both **1** and **2** are hexacyclic containing indole ring, as **2** has one carbonyl group.

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

No	**1**					**2**				
	^{13}C	^{1}H		HMBC correltns[a]	NOESY correltns[a]	^{13}C	^{1}H		HMBC correltns[a]	NOESY correltns[a]
1		7.88	NH	2, 3, 3a, 7	2		7.91	NH	3, 3a	2
2	116.5	6.81	brt *J* = 1.7	3, 3a, 7a, 8	1, 9, 10	116.3	6.90	brt *J* = 1.7	3, 3a, 7	1, 9, 10
3	115.6					115.0				
3a	125.8					125.6				
4	142.3					142.7				
5	112.4	7.01	brt *J* = 4.0	3a, 4, 6, 7,22	24	112.5	6.99	dd *J* = 4.6, 3.4	4, 6, 7	24
6	122.6	7.16	m			122.8	7.16	m	4, 5, 7a	
7	107.8	7.16	m			107.7	7.15	m	3a, 5, 7a	
7a	134.0					134.0				
8	33.0	3.43	brdd *J* = 12.0, 4.0	3, 9, 10, 21	9, 17, 23	36.5	2.64	ddd *J* = 12.6, 5.1, 1.7	3, 10	9, 20_{ax}, 23
9	36.7	2.79	brt *J* = 5.7	8, 17, 19, 21, 27	2, 8, 11_{ax}, 14, 17	38.5	3.43	m	18, 19,	2, 8, 11_{ax}, 14, 16_{ax}
10	29.0	2.72	q *J* = 6.9	9, 12, 18, 27	2, 27	28.0	2.77	brq *J* =6.9	9, 18, 27	2, 9, 11_{eq}, 27
11	28.6	1.99 $_{ax}$	m	13, 27	9	28.8	2.18 $_{ax}$	m		9, 10, 12_{eq}, 14
		1.11 $_{eq}$	brd *J* = 13.8	9, 13	27		1.26 $_{eq}$	m		10, 27
12	27.8	1.57 $_{ax}$	ddd *J* = 14.3, 13.3, 2.7	13, 14	19_{ax}, 27	26.9	1.57 $_{ax}$	m	14	26, 27
		1.22 $_{eq}$	dt *J* = 13.3, 2.9	18	25, 26		1.35 $_{eq}$	dt *J* = 14.3, 3.4		11_{ax}, 14, 25, 26
13	39.1					44.8				
14	31.3	2.31 [c]	m		9, 16_{ax}	30.4	2.83	m		9, 11_{ax}, 16_{ax}, 25
15	25.3[b]	1.75 $_{ax}$	qd *J* = 13.2, 3.4	13, 14	25, 26	33.2	1.92 $_{eq}$	m		16_{ax}, 25
		1.33 $_{eq}$	brd *J* = 13.2	13, 14, 17	25		1.64 $_{ax}$	m	14	25, 26
16	29.5	2.00 $_{ax}$	m		14, 17	38.9	2.97 $_{ax}$	ddd *J* = 13.7, 11.5, 6.3	15, 17	9, 14, 15_{eq}
		1.69 $_{eq}$	m	14, 17	17		2.17 $_{eq}$	m	14	
17	69.1	4.61	brs	13	8, 9, $16_{ax,eq}$, 20_{ax}	217.0				
18	43.5[c]					58.0				
19	25.2[b]	2.14 $_{eq}$	brd *J* = 13.2	9, 20	26	24.0	2.14 $_{eq}$	m	17	20_{eq}, 26
		1.84 $_{ax}$	td *J* = 13.2, 4.0	17	12_{ax}, 21, 27		1.70 $_{ax}$	td *J* = 13.2, 5.2	17, 20	21, 27
20	20.9	1.91 $_{eq}$	m	21	24	21.5	2.01 $_{ax}$	qd *J* = 13.2, 5.1	21	8, 23
		1.67 $_{ax}$	m		23		1.80 $_{eq}$	m	8, 18	19_{eq}, 21, 24
21	43.5[c]	2.31 [c]	m		19_{ax}, 24, 27	42.6	2.31	td *J* = 12.6, 5.1	22, 23	19_{ax}, 20_{eq}, 24,
22	37.8					37.8				
23	24.2	1.04	s	4, 21, 22, 24	8, 20_{ax}	23.8	0.93	s	4, 21, 22, 24	8, 20_{ax}, 24
24	24.8	1.46	s	4, 21, 22, 23	5, 21	24.4	1.40	s	4, 21, 22, 23	5, 20_{eq}, 21, 23
25	15.8	0.85	d *J* = 8.6	13, 14, 15	12_{eq}, $15_{ax,eq}$	14.9	0.86	d *J* = 6.9	13, 14, 15	12_{eq}, 14, 15_{eq}, 26
26	18.4	1.01	s	12, 13, 14, 18	$12_{ax,eq}$, 15_{ax}, 19_{eq}	16.0	0.65	s	12, 13, 14, 18	$12_{ax,eq}$, 15_{ax}, 19_{eq}, 25
27	22.7	0.86	d *J* = 8.0	9, 10	10, 12_{ax}, 19_{ax}, 21	22.9	0.94	d *J* = 6.9	11	10, 11_{eq}, 12_{ax}, 19_{ax}

a Does not include a number of cross peaks observed for overlapping ^{1}H signals. *b* Assignments may be reversed. *c* The overlapping signals were resolved in acetone-d_6. The NOESY date at 14-H and 21-H were supported with the experiments in acetone-d_6. See in experimental.



1 X = *ax*-OH, *eq*-H
2 X = O

3 **4** **5** **6**

Three proton spin systems observed at 6-H and 7-H (δ 7.16, 2H, m) and 5-H (7.01, 1H, br t) in **1** and at 6-H (δ 7.16, 1H, m), 7-H (7.15, 1H, m) and 5-H (6.99, 1H, dd) in **2** may be assigned to the adjacent protons of the benzene ring of the indole moiety, whereas the protons at 2-H (δ 6.81 in **1** and 6.90 in **2**) correlated with NH protons (δ 7.88 and 7.91, respectively) in ^{1}H-^{1}H COSY spectra should be assigned to

the proton at C-2 in the indole moiety. The ^{13}C NMR spectrum of **1** was similar to that of aflavinine (**6**),[6] except for the appearance of one quaternary carbon, two methynes and two tertiary methyls instead of that of two olefinic carbons and isopropyl group in **6**. The cross peaks between two tertiary methyl protons at 23-H_3 (δ_H 1.04) and 24-H_3 (δ_H 1.46) and two carbons at C-22 (quaternary carbon at δ_C 37.8) and at C-4 (δ_C 142.3) of the indole moiety in the HMBC spectrum of **1** suggested that the C-22 was attached to C-4 in the indole moiety. Considering the above result, structures of 17-hydroxyeujindole was assigned as **1**. This conclusion was supported by other HMBC correlation peaks indicated in Table 1.

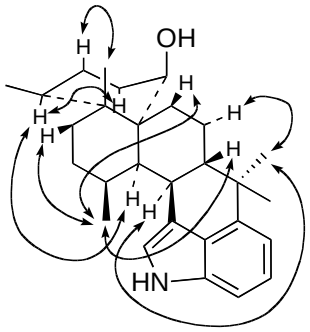


Figure 1. NOESY correlation of 17-hydroxyeujindole (**1**)

The relative stereochemistry of **1** was assigned by analogy to that of aflavinines and supported by the results of NOESY experiments showed in Table 1. The NOESY correlations of 23-H_3 (δ_H 1.04) with 8-H (δ_H 3.43) and 20-H_{ax} (δ_H 1.67), of 17-H (δ_H 4.61) with 8-H, of 27-H_3 (δ_H 0.86) with 12-H_{ax} (δ_H 1.57), 19-H_{ax} (δ_H 1.84) and 21-H (δ_H 2.31), and even of 9-H (δ_H 2.79) with 11-H_{ax} (δ_H 1.99) suggested that both B and C rings were in a chair conformation and A-B and B-C ring junctions were *tans* and *cis*, respectively. Further correlations of 14-H (δ_H 2.31) with 9-H and 16-H_{ax} (δ_H 2.00), and of 26-H_3 (δ_H 1.01) with 15-H_{ax} (δ_H 1.75) indicated that D ring also was in a chair conformation and C-D ring junction was *cis*. Above results revealed relative stereochemistry of **1** corresponding to that of aflavinines. The structure of 17-hydroxyeujindole was thus assigned as depicted in structure **1**. This conclusion was supported by other NOESY correlation peaks and the result of NOESY experiment in acetone-d_6.

The ^{13}C NMR spectrum of **2** was similar to that of **1**, except for the presence of the carbonyl carbon (δ_C 217.0) instead of one of the carbon bearing a hydroxyl group in **1**. From ^{1}H-^{1}H COSY, HMQC and HMBC spectra, the plane structure of **2** was assigned as 17-oxoeujindole oxidized at C-17 of **1**. The relative stereochemistry of **2** also was established by the NOESY experiments. NOESY correlations of 23-H_3 (δ_H 0.93) with 8-H (δ_H 2.64) and 20-H_{ax} (δ_H 2.01), of 19-H_{ax} (δ_H 1.70) with 21-H (δ_H 2.31), of 27-H_3 (δ_H 0.94) with 12-H_{ax} (δ_H 1.57) and 19-H_{ax}, of 9-H (δ_H 3.43) with 11-H_{ax} (δ_H 2.18), 14-H (δ_H 2.83)

and 16H$_{ax}$ (δ_H 2.97), and even of 26-H$_3$ (δ_H 0.65) with 15-H$_{ax}$ (δ_H 1.64) suggested that all B, C and D rings were in a chair conformation and A-B, B-C and C-D ring junctions were *tans*, *cis*, and *cis*, respectively. Consequently, the relative configuration of **2** also has as same as that of **1**. This conclusion also was supported by other NOESY correlation peaks.

Because **2** possess substituted cyclohexanone moiety having a chair form, the CD spectrum was examined to propose the absolute stereochemistry. Kirk and Klyne proposed the empirical calculation of CD contribution of the *cis*-decalons from experimental data.[7] According to their conclusion, *cis*-decalone in **2** was in Class c2*ax*. The CD spectrum of **2** indicated a negative Cotton effect at 292 nm ($\Delta\varepsilon$ -1.4). The absolute configuration of 17-oxoeujindole (**2**) was thus determined as depicted in **2**. The absolute configuration of **1** has not been determined, but it was assumed to be as shown in structure (**1**) because of the co-occurrence of 17-oxoeujindole (**2**). 10,23-Dihydro-24,25-dehydroaflavinine (**3**) and nominine (**4**) were isolated from the extract of the strain used in this study and identified by comparison with published data.[4,5] Though the absolute configurations of **3** and **4** have not yet been determined, those structures also may be assigned as depicted in structures **3** and **4** with the absolute stereochemistry shown because of the co-occurrence of **2**.

In view of structures, the indole diterpenes are one of large and diverse groups in natural products from fungi. A number of indole diterpenes have the common tetracyclic diterpene core connected to carbons at C-2 and C-3 in an indole moiety, such as aflatrem,[6,8] penitrems[9] and paxilline,[10] which are the tremorgenic mycotoxin. However, indole diterpenes possessed the tetracyclic diterpene core fused to carbons at C-3 and C-4 in an indole moiety is quiet rare. Petromindole (**5**)[11] from *Petromyces muricatus* is only known as naturally occurring compound, though the ring formation of the diterpene moiety differs from that of **1** and **2**.

Aflavinines and petromindole were isolated from sclerotia and ascostromata, respectively. 17-Hydoxyeujindole (**1**) also was detected mainly in the $CHCl_3$ extract of the ascomata of the strain IMF 59075 grown on potato dextrose agar (PDA), along with **2** and **3**. We have now an interest that *E. javanicum* may have the capability producing indole diterpenes such as aflavinines and eujindoles.

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 spectrophotometer, respectively. CD curves were recorded on a JASCO J-820 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECA500 (^{1}H, 500.16 MHz; ^{13}C, 125.77 MHz) spectrometer, using $CDCl_3$ or acetone-d_6 solution containing TMS as an internal standard. FABMS was taken with a JEOL JMS-MS700V spectrometer.

Fungal Material. The studied strain was isolated from a cultivated soil in Chiba, Japan, identified as *Eupenicillium javanicum* based on morphology (by T. Y.), and deposited at the Medical Mycology Research Center, Chiba University, under the accession number IFM 59075 (UC 62). The strain IFM 59075 was cultured at 25 °C for 21 days in 10 Roux flasks containing 250 g of soaked rice in each flask.

Extraction and Isolation. The fermented rice was extracted with $CHCl_3$-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 (13.4 g) was portioned between MeCN and hexane. The MeCN soluble portion (3.6 g) was separated by column chromatography on silica gel (80 g), eluting with $CHCl_3$ containing increasing amounts of acetone. Elution with $CHCl_3$-acetone (20:1) gave a fraction showing a positive coloration for van Urk reagent (blue). The positive fraction was purified by LPLC on a silica gel column using cyclohexane-EtOAc (8:1) followed by the further purification of HPLC on silica gel column [cyclohexane-EtOAc (6:1)] to give 17-Hydroxyeujindole (**1**) (35 mg), nominine (**4**) (2 mg), 17-oxoeujindole (**2**) (7 mg), 10,23-dihydro-24,25-dehydroaflavinine (**3**) (2 mg) and 2,3-anhydromevalonic acid δ-lactone (6 mg)[5] in this elution order.

17-Hydroxyeujindole (**1**): Colorless needles (hexane-acetone); mp 129.3-130.2 °C; $[\alpha]_D^{22}$ -43.7 ° (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε): 226 (4.51), 283 (3.73), 293 (3.66) nm; IR νmax 3480 (NH), 3287 (OH) cm^{-1}; HRFAB(-)MS *m/z* : 404.2925 $[M - H]^+$ (calcd for 404.2953 for $C_{28}H_{38}NO$); ^1H NMR (acetone-d_6) δ 9.75 (NH) 7.08 (7-H, d, *J*=8.0), 6.99 (2-H, m), 6.98 (6-H, m), 6.87 (5-H, d, *J*=6.9), 4.59 (17-H, brs), 3.42 (8-H, dd, *J*=12.0, 5.2), 2.85 (9-H, m), 2.78 (10-H, m), 2.29 (19-H_{eq}, m), 2.27 (14-H, m), 2.24 (21-H, m), 2.02 (11-H_{ax}, m), 1.94 (16-H_{ax}, m), 1.81 (15-H_{ax}, ddd, *J*=15.1, 12.6, 3.2), 1.8-1.7 (20-H_2, m), 1.67 (19-H_{ax}, m), 1.59 (16-H_{eq}, m), 1.55 (12-H_{ax}, td, *J*=14.3, 2.8), 1.39 (24-H_3, s), 1.20 (15-H_{eq}, m), 1.16 (12-H_{eq}, dt, *J*=14.3, 3.4), 1.04 (11-H_{eq}, brd, *J*=13.1), 1.00 (26-H_3, s), 0.95 (23-H_3, s), 0.81 (27-H_3, d, *J*=7.4), 0.79 (25-H_3, d, *J*=6.9); ^{13}C NMR (acetone-d_6) δ 142.5(C-4), 135.3(C-7a), 126.8 (C-3a), 122.7 (C-6), 118.1 (C-2), 115.3 (C-3), 112.3 (C-5), 108.8 (C-7), 68.3 (C-17), 44.6 (C-21), 44.3 (C-18), 39.9 (C-13), 38.3 (C-22), 37.2 (C-9), 33.8 (C-8), 32.0 (C-14), 30.6 (C-16), 30.0 (C-10), 29.4 (C-11), 28.7 (C-12), 26.4 (C-15), 26.1 (C-19), 25.2 (C-24), 24.5 (C-23), 23.1 (C-27), 21.6 (C-20), 18.8 (C-26), 16.2 (C-25); NOESY data (H↔H#) 14-H↔9-H, 16-H_{ax}; 21-H↔19-H_{ax}, 24-H_3, 27-H_3. The other NOESY data were the same as the results in $CDCl_3$ in Table 1. The assignments of ^1H and ^{13}C NMR signals for **1** in $CDCl_3$ are summarized in Table 1.

17-Oxoeujindole (**2**): Colorless amorphous; $[\alpha]_D^{22}$ -28.7 ° (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε): 225 (4.51), 282 (3.83), 292 (3.76) nm; IR νmax 3406 (NH), 1683 (C=O) cm^{-1}; CD (MeOH) $\Delta\varepsilon$ (nm) -1.4 (292); HRFAB(+)MS *m/z* : 404.2930 $[M + H]^+$ (calcd for 404.2953 for $C_{28}H_{38}NO$). The assignments of ^1H and ^{13}C NMR signals for **2** in $CDCl_3$ are summarized in Table 1.

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