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TWO NEW EUJINDOLES FROM *EUPENICILLIUM JAVANICUM*

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Abstract – Two new indole diterpenes, 8,21-dehydro-17-hydroxyeujindole (**3**) and 8,21-dehydro-17,20-epoxyeujindole (**4**) were isolated from the extract of *Eupenicillium javanicum* IFM 59075. The structures were determined by spectroscopic methods.

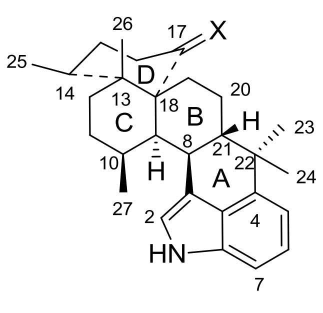
In view of structures, the indole diterpenes are one of large and diverse groups in natural products from fungi. Wicklow *et al.* found that sclerotia of *Asperigillus* and *Penicillium* spp. showed to be a rich source of secondary metabolites with insecticidal/antifeedant activity,[1] such as the aflavinins[2] and nominine,[3] which were the indole diterpenes. Recently we reported the isolation and the structural elucidation of 17-hydroxyeujindole (**1**) and 17-ketoeujindole (**2**), new type indole diterpenes, which showed positive coloration (blue) with van Urk reagent,[4] from *Eupenicillium javanicum*.[5] During our research, we found yellow spots on TLC with van Urk reagent in the organic extract of *E. javanicum* IFM 59075. As the result of purification, the substance showing three yellow spots by spraying van Urk reagent were ergosta-4,6,8(14),22-tetraen-3-one[6] and two new indole diterpenes, designated 8,21-dehydro-17-hydroxyeujindole (**3**) and 8,21-dehydro-17,21-epoxyeujindole (**4**). In this paper, we report the isolation and structure determination of **3** and **4**.

The molecular formula of **3** was determined as $C_{28}H_{37}NO$ by the HRFABMS, and that of **4** as $C_{28}H_{35}NO$ by HRFABMS. The ^{13}C NMR spectral data of **3** and **4** were similar to that of **1**, except for the appearance of two sp^2 carbons instead of that of two sp^3 ones in **1**, and that of a sp^3 methyne carbon attached to an oxygen atom in **4** instead of that of one methylene carbon in **1**.

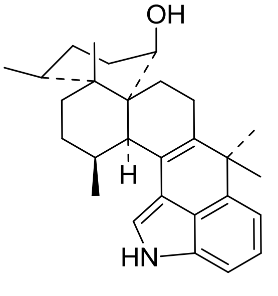
Table 1. ^{1}H and ^{13}C NMR spectral data for **3** and **4** in CDCl$_3$

No	**1**		**3**					**4**				
	^{13}C	^{1}H	^{13}C	^{1}H^{a}		HMBC correltns^{b}	NOESY correltns^{bc}	^{13}C	^{1}H^{a}		HMBC correltns^{b}	NOESY correltns^{bc}
1		7.88		7.91	NH	3, 3a, 7a,	7		7.82	NH		7
2	116.5	6.81	114.5	6.83	d J = 1.8	3, 3a, 7a,	9, 10	116.4	6.98	d J = 1.7	3, 3a, 7a	9, 10
3	115.6		115.5					113.5				
3a	125.8		124.9					125.5				
4	142.3		140.7					140.2				
5	112.4	7.01	114.3	7.00	d J = 7.4	3a, 7, 22	23, 24	114.7	7.02	d J = 7.5	3a, 7, 22	23, 24
6	122.6	7.16	124.2	7.20	t J = 7.4	4, 5, 7a,		124.2	7.23	t J = 7.5	4, 7a	
7	107.8	7.16	107.3	7.08	d J = 7.4	3a, 5,	NH	107.3	7.11	d J = 7.5	3a, 5	NH
7a	134.0		134.0					133.7				
8	33.0	3.43	124.2					124.9				
9	36.7	2.79	39.3	2.93	dd J = 6.2, 1.5	3, 8, 10, 17, 18, 19, 21, 27	2, 11_{ax}, 14, 16_{ax}, 17	49.3	2.83	d J = 5.1	8, 10, 17, 18, 21, 27	2, 12_{d}, 14, 17
10	29.0	2.72	30.6	2.53	m	9, 11, 12, 18, 27	2	30.7	2.70	m		2, 12_{d}
11	28.6	1.99 $_{ax}$	26.0	1.98 $_{ax}$	m		9, 14	30.5	1.50	2H, m		26
		1.11 $_{eq}$		1.29 $_{eq}$	m		27					
12	27.8	1.57 $_{ax}$	27.5	1.61 $_{ax}$	m		19_{ax}, 26, 27	26.6	1.41 $_{u}$	m		
		1.22 $_{eq}$		1.26 $_{eq}$	m		25		1.79 $_{d}$	m	18	9, 10, 14
13	39.1		38.3					38.3				
14	31.3	2.31	31.3	2.30	m		9, 11_{ax}, 12_{eq}, 16_{ax}	27.9	2.05	m		9, 12_{d}, 17
15	25.3	1.75 $_{ax}$	25.4	1.69 $_{ax}$	m		25, 26	26.0	1.28 $_{u}$	qd J = 10.6, 3.9	17	25
		1.33 $_{eq}$		1.28 $_{eq}$	m				1.70 $_{d}$	m		25
16	29.5	2.00 $_{ax}$	30.4	1.83 $_{ax}$	tdd J = 13.8, 4.1, 3.1		9, 14	25.9	1.61 $_{u}$	m	17	26
		1.69 $_{eq}$		1.50 $_{eq}$	m				1.83 $_{d}$	m	17, 18	
17	69.1	4.61	70.9	4.03	brs	13, 15	9, 20_{u}	84.5	3.57	dd J = 12.6, 6.9	9	9, 14
18	43.5		41.3					48.1				
19	25.2	2.14 $_{eq}$	22.4	2.14 $_{eq}$	ddt J = 13.5, 6.6, 1.5	9, 18, 21	26	32.9	1.95 $_{u}$	dd J = 10.9, 4.6	9, 17	26
		1.84 $_{ax}$		1.92 $_{ax}$	ddd J = 13.5, 10.7, 7.5	17, 18, 20	12_{ax}, 27		1.99 $_{d}$	d J = 10.9	17	
20	20.9	1.91 $_{eq}$	22.8	2.49 $_{d}$	m	8, 19, 21	24	73.6	4.80	d J = 4.6	17, 18	23, 24
		1.67 $_{ax}$		2.43 $_{u}$	m	8, 19, 21	17, 23					
21	43.5	2.31	135.6					139.5				
22	37.8		40.6					39.8				
23	24.2	1.04	28.9	1.49	s	4, 21, 22, 24	5, 20_{u}	29.0	1.54	s	4, 21, 22, 24	5, 20
24	24.8	1.46	32.3	1.46	s	4, 21, 22, 23	5, 20_{d}	31.8	1.41	s	4, 21, 22, 23	5, 20
25	15.8	0.85	15.8	0.86	d J = 6.9	13, 14, 15,	12_{eq}, 15_{ax}	15.1	0.87	d J = 6.9	13, 14, 15	$15_{u,d}$
26	18.4	1.01	18.0	1.00	s	12, 13, 14, 18	12_{ax}, 15_{ax}, 19_{eq}	21.1	0.92	s	13, 14, 18	11, 16_{u}, 19_{u}
27	22.7	0.86	18.3	0.92	d J = 7.4	9, 11	11_{eq}, 12_{ax}, 19_{ax}	15.1	0.90	d J = 7.4	9	

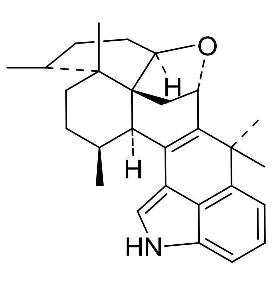
a Up-field proton of methylene is *u*, down-field is *d*. *b* Does not include a number of cross peaks observed for overlapping ^{1}H signals. *c* NOESY corretions between coupled protons are omitted.



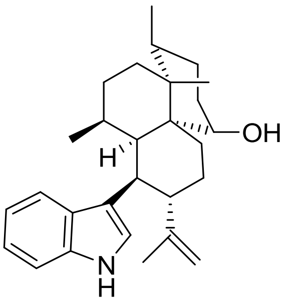
1 X = *ax*-OH, *eq*-H
2 X = O



3



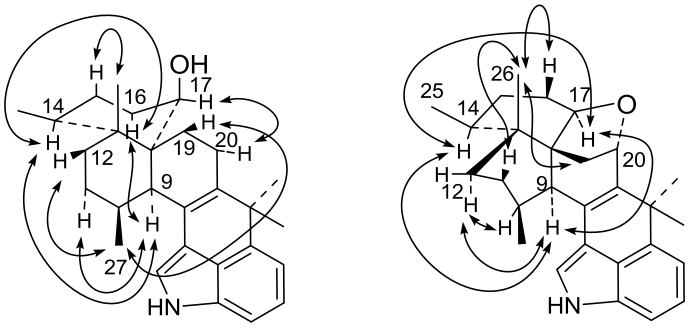
4



5

Proton and carbon NMR data for **3** and **4** are provided in Table 1. In the HMBC of **3**, the cross peaks from 9-H (δ_H 2.93) were observed to the two sp^2 carbons at δ_C 124.2 and δ_C 135.6, along with C-10 (δ_C 30.6) and C-18 (δ_C 41.3). The cross peaks from the two tertiary methyl protons (δ_H 1.46 and 1.49) were observed to the carbon at δ_C 135.6, along with C-4 (δ_C 140.7) of indole moiety. Considering the above result, structure of **3** was assigned as 8,21-dehydro-17-hydroxy-eujindole. This conclusion was supported by other HMBC correlation peaks indicated in Table 1.

The NOESY correlations of 27-H$_3$ (δ_H 0.92) with 12-H*ax* (δ_H 1.61) and 19-H*ax* (δ_H 1.92), and of 9-H with 11-H*ax* (δ_H 1.98), 14-H (δ_H 2.30) and 16-H*ax* (δ_H 1.83), of 14-H with 16-H*ax,* and even of 17-H (δ_H 4. 03) with 20-H*u* (δ_H 2.43)[7] suggested that both C and D rings were in a chair conformation and the B-C and C-D ring junctions were *cis*. The relative stereochemistry of **3** was thus assigned as depicted in structure **3**. This conclusion was supported by other NOESY correlation peaks showed in Table 1.



3 4

Figure 1. NOESY correlation of **3** and **4**

In the HMBC of **4**, the cross peaks from 9-H (δ_H 2.83) also were observed to the two sp^2 carbons (δ_C 124.9 and δ_C 139.5) and C-19 (δ_C 32.9), along with C-10 (δ_C 30.7), C-17 (δ_C 84.5) and C-18 (δ_C 48.1). The cross peaks from the two tertiary methyl protons (δ_H 1.41 and 1.54) were observed to the carbon at δ_C 139.5, along with C-4 (δ_C 140.2) of indole moiety. Further HMBC correlations of 17-H (δ_H 3.57) with C-20 (δ_C 73.6) suggested that **4** possessed an ether ring. In the ^{1}H-^{1}H COSY spectrum of **4**, the signal at δ_H 4.80 was connected with those of 19-H$_2$ (δ_H 1.95 and 1.99). Considering the above result, structure of **4** was assigned as 8,21-dehydro-17,20-epoxyeujindole. This conclusion was supported by other HMBC correlation peaks indicated in Table 1. The relative stereochemistry of **4** also was established by the NOESY experiments. NOESY correlations of 9-H with 14-H (δ_H 2.05) and 17-H (δ_H 3.57), of 26-H$_3$ (δ_H 0.92) with 11-H (δ_H 1.50), 16-H*u* (δ_H 1.61) and 19-H*u* (δ_H 1.95), and ever of 12-H*d* (δ_H 1.79) with 9-H suggested that both C and D rings were in boat conformation, and B-C and C-D ring junctions were both *cis*. This conclusion also was supported by other NOESY correlation peaks.

The absolute configuration of 17-oxoeujindole was determined as depicted in **2** in the previous paper.[5] The absolute configurations of **3** and **4** have not been determined, but those structures may be as shown in structures **3** and **4** containing the absolute configuration because of the co-occurrence of **2**.

It seems that yellow spot on TLC by spraying van Urk reagent were derived from 8,21-dehydroeujindole derivatives. 8,21-Dehydro-17,20-epoxyeujindole (**4**) possessed a pentacyclic diterpene core fused to carbons at C-3 and C-4 in an indole moiety. The indole diterpene had the pentacyclic diterpene core is quite rare, except for indole diterpenes containing acetal moiety, which had diterpene core connected to carbons at C-2 and C-3 in indole, such as aflatrem[8,9] and paspalitrem[10] of the tremorgenic mycotoxin.

18 Strains of *E. javanicum* were examined for the capacity producing eujindole derivatives. TLC analysis of the $CHCl_3$ extract of each strain grown on moist rice for 3 weeks revealed that two strains (strains IFM 60196 and 60197) possessed the producing capacity of eujindole derivatives. **1**, **2**, **4** and 10,23-Dihydro-24,25-dehydroaflavinine (**5**) were detected in the $CHCl_3$ extract of *E. javanicum* IFM 60196, and **1**, **2** and **5** in that of *E. javanicum* IFM 60197.

EXPERIMENTAL

General Experimental Procedures. For general experimental details see ref. 5.

Fungal Material. The studied strains were 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 numbers IFM 59075, 60196 and 60197. 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. The 18 strains of *E. javanicum* were cultured at 25 °C for 21 days in Erlenmeyer flask (100 mL) containing 30 g of soaked rice in each flask.

Extraction and Isolation. The fermented rice was extracted with $CHCl_3$-MeOH (6:4) 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 (14.4 g) was partitioned 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 (9:1) gave a fraction showing a positive coloration for van Urk reagent (blue). The positive fraction was purified by low-pressure liquid chromatography on a silica gel column using cyclohexane-acetone (12:1) to give ergosta-4,6,8(14),22-tetraen-3-one, which showed a positive coloration for van Urk reagent (yellow), and three fractions. The most polar fraction was purified by HPLC on silica gel column [cyclohexane-acetone (12:1)] to give 8,21-dehydro-17,21-epoxy-eujindole (**4**) (4 mg), 17-Hydroxy-eujindole (**1**) (35 mg) and 17-oxoeujindole (7 mg) in this elution order. The most polar fraction was also purified by HPLC on silica gel column [cyclohexane-acetone (12:1)] to give 17-oxoeujindole (7 mg) and 8,21-dehydro-17-hydroxyeujindole (**3**) (2 mg).

8,21-Dehydro-17-hydroxyeujindole (**3**): Colorless microcrystal (cyclohexane); mp 211.0 – 211.8 °C; $[\alpha]_D^{24}$ -81° (*c* 0.07, CH_2Cl_2); UV (MeOH) λ_{max} (log ε): 239 (4.19), 276 (3.86), 297 (3.81), 314 (sh 3.73) nm; IR νmax 3499 (NH), 3239 (OH), 1444, 749 cm^{-1}; HRFAB(+)MS *m/z* : 404.2918 $[M + H]^+$ (calcd for 404.2953 for $C_{28}H_{38}NO$).

8,21-Dehydro-17,20-epoxyeujindole (**4**): Colorless leaves (acetone); mp(dec.) 257.3 – 258.3 °C; $[\alpha]_D^{24}$ -332° (*c* 0.15, CH_2Cl_2); UV (MeOH) λ_{max} (log ε): 234 (4.32), 278 (3.95), 298 (4.02), 308 (3.99), 317 (sh 3.84) nm; IR νmax 3228 (NH) cm^{-1}; HRFAB(+)MS *m/z* : 402.2809 $[M + H]^+$ (calcd for 402.2797 for $C_{28}H_{36}NO$).

The assignments of ^1H and ^{13}C NMR signals for **3** and **4** in $CDCl_3$ are summarized in Table 1.

ACKNOWLEDGEMENTS

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