Notes

A NEW INDOLE FROM *Penicillium daleae*

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Receptors for 5-hydroxytryptamine (5-HT)[1] are currently classified as 5-HT_1-like, 5-HT_2 and 5-HT_3. The 5-HT_1-like class has been further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes. Evaluation of the receptor binding profile of sumatriptan (GR 43175), an approved drug for the acute treatment of migraine, strongly suggests that its stimulation of the 5-HT_{1D} receptor subtype is relevant to the reported antimigraine effects[2]. In our screening for 5-HT_{1D} receptor agonists from microbial sources, we discovered a novel indole alkaloid and determined its structure to be 3-(dimethylaminomethyl)-1-(1,1-dimethyl-2-propenyl)indole (**1**) (Fig. 1). This note describes the fermentation, isolation, physico-chemical characterization and biochemical properties of **1**.

Penicillium daleae was obtained from the late Dr. CHARLES THOM in 1942 (strain No. 260-5034.116, Peoria 920), maintained at the Merck Culture Collection in lyophilized form as MF1134 and deposited as ATCC74115. RAPER and THOM[3] described this culture and assigned it as NRRL 2025. MF1134 was added under aseptic conditions to 54 ml of a seed medium in a 250-ml plain Erlenmeyer flask. The seed medium consisted of yeast extract (Difco) 0.4%, malt extract (Difco) 1% and glucose 0.4%, pH 7.0. The flask was maintained at 22°C on a rotary shaker for 3 days. A second stage seed culture was obtained by repeating the above procedure using 1 ml of the first seed culture as inoculum per flask and incubated for 2 days. A 24 ml inoculum from the second seed culture was added to 425 ml of the liquid phase of production medium in a 1-liter Erlenmeyer flask. The liquid phase of the production medium consisted of glucose 1%, fructose 1.5%, sucrose 4%, NZ Amine type E (Sheffield Products) 0.4%, urea 0.4%, K_2HPO_4 0.05%, KCl 0.025%, $MgSO_4 \cdot 7H_2O$ 0.025%, $ZnSO_4 \cdot 7H_2O$ 0.09% and $CaCO_3$ 0.8%. This liquid phase and seed mixture was mixed vigorously with 1,250 ml of vermiculite, as the solid phase, in a 4-liter roller jar. Incubation was then performed at 22°C on a roller machine for 19 days.

Fermentation in eight roller jars was extracted with methyl ethyl ketone (5,600 ml) for three hours at room temperature. Filtration over Whatman number 3 filter paper and flash evaporation afforded 6 g dry weight. Partition between water (20 ml) and methyl ethyl ketone (4 × 30 ml) yielded 2 g dry weight from the organic layers. Preparative chromatographic purification included three columns: a) Whatman partisil 10 ODS-3 (2.2 × 50 cm) using 20~25% acetonitrile in 0.1% TFA (aq) as mobile phase; b) E. Merck Silica gel 60 (20 g, 40~63 μm, 2.5 × 10 cm) using 0.5% triethylamine in acetone-hexane, 1:5~2:1, as mobile phase; and c) Whatman partisil 10 ODS-3 (0.94 × 50 cm) using 20~25% acetonitrile-0.1% TFA (aq) as mobile phase. The actual sequence consisted of two rounds on a), once on b), once on a) again, and then once on c). 35 mg of homogeneous **1** was obtained in this fashion with a Rf of 0.17 [E. Merck Silica gel 60F, 0.2 mm thickness, hexane-acetone-triethylamine, 50:50:0.5] and a k' of 4.72 [Whatman partisil 5 ODS-3, acetonitrile-0.1% TFA (aq), 3:7].

The physico-chemical properties of **1** are as follows: UV λ_{max}^{MeOH} nm (ε) 223 (12,100), 273 (4,890), 279 (5,090), 289 (4,120); FT-IR ν_{max} (ZnSe) cm^{-1} 2991, 1679, 1550, 1460, 1202, 1135; HREI-MS *m/z* 242.1766 (M, Calcd for $C_{16}H_{22}N_2$: 242.1783), 198 [M−$N(CH_3)_2$], 173 [M−$C(CH_3)_2(CH=CH_2)$], 130.0636 (base peak, for C_9H_8N: 130.0657). The *m/z* 198 ion results from the expected facile loss of

Fig. 1. The structure of **1**.

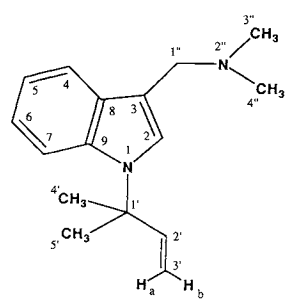

Table 1. ^1H and ^{13}C NMR chemical shifts assignment of **1**.

Atom No.	^1H NMR[a] Chemical shift	Multiplicity (J=Hz)	^{13}C NMR[b] Chemical shift	Multiplicity ($^1J_{CH}$=Hz)
2	7.70	s	130.0	d (183)
3	N.A.		103.3	s
4	7.71	m	119.4	d (160)
5	7.16	m	121.5	d (160)
6	7.16	m	122.8	d (159)
7	7.59	m	115.8	d (164)
8	N.A.		130.4	s
9	N.A.		137.1	s
1′	N.A.		61.0	s
2′	6.17	dd (10.5, 17.5)	144.8	d (157)
3′			114.5	t (157)
a	5.14	d (17.5)		
b	5.24	d (10.5)		
4′	1.79	s	28.3	q (128)
5′	1.79	s	28.3	q (128)
1″	4.49	s	53.5	t (143)
2″	N.A.		N.A.	
3″	2.86	s	42.5	q (143)
4″	2.86	s	42.5	q (143)

[a] 400 MHz and [b] 100 MHz spectra were recorded in CD_3OD at 20°C. Chemical shifts are reported in ppm from TMS.
N.A.: Not applicable.

$N(CH_3)_2$ as directed by the indole moiety. Similarly, the m/z 173 ion results from loss of the prenyl group. The m/z 130 base peak is the diagostic indole ion which in this case results (nominally) from the m/z 198 ion *via* loss of the prenyl group with transfer of a proton. FAB-MS displays $(M+H)^+$ at m/z 243 and the m/z 198 fragment is the base peak. The plus matrix peaks are also observed.

Extensive 1D (^1H, decoupling, and ^{13}C: broad band decoupled, gated, and attached proton test) and 2D NMR [including ^1H-^1H COSY, ^{13}C-^1H COSY and NOESY] experiments (data not shown) allowed the assignment of **1** as a new indole alkaloid, 3-(dimethylaminomethyl)-1-(1,1-dimethyl-2-propenyl)indole. The chemical shift assignments are listed in Table 1. The exceptionally down field nature of 2-H in **1** (δ 7.70 ppm) as compared to related compounds[4], indole (δ 6.68 ppm), 1-methylindole (δ 6.82 ppm) and 3-methylindole (δ 6.80 ppm), is supported by correlation between 2-H and C-2 in a ^{13}C-^1H COSY experiment. The C-2 chemical shift in **1** (δ 130.0 ppm) is comparable to that reported[5] for 1-methylindole (δ 129.0 ppm) in perdeuterodioxane. In a NOESY experiment with a 500 millisecond mixing time, 2-H showed a positive NOE

Fig. 2. Postulated rotamers of **1**.

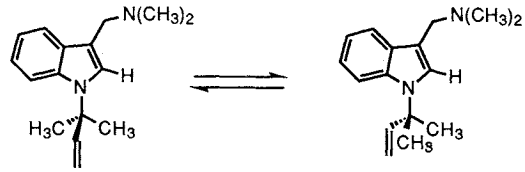

Table 2. Effect of **1** on binding to 5-HT receptor subtypes.

Assay	% Inhibition of **1** @ μM 4.1	41
$5\text{-}HT_{1D}$	44	81
$5\text{-}HT_{1A}$	20	62
$5\text{-}HT_2$	42	86

correlation with both 1″-H_2 (δ 4.49 ppm) and the two methyl singlets of the prenyl side chain at δ1.79 ppm (4′-H_3 and 5′-H_3). This NOE data can be accommodated by postulating the existence of two sterically favored rotamers where NOE interactions between 2-H and both 4′ and 5′-methyls are anticipated (see Fig. 2) and where, in each case, 2-H falls in the deshielding zone of C-2′~C-3′ double bond. In addition, in a ^{13}C-^1H long range COSY experiment, correlations between 1″-H_2 with both C-2 and C-8 (δ 130.4 ppm) were observed. These correlations strongly support the structure shown.

$5\text{-}HT_{1D}$ radioligand binding assay was carried out according to HEURING and PEROUTKA[6] with slight modifications as follows. Polypropylene tubes containing ^3H-5-HT (2 nM), cyanopindolol (100 nM), mesulergine (100 nM) and crude pig striatal membranes (10 mg wet weight per tube) in a final assay volume of 1 ml were used. All reagents and tissues were made up in 50 mM Tris-HCl containing 0.1% ascorbate, 10 μM pargyline and 4 mM $CaCl_2$ (pH 7.7 at room temperature). 5-HT (10 μM) was used to define non-specific binding. The incubation was initiated by the addition of membranes and carried out for 30 minutes at 37°C. Following incubation, membranes were rapidly filtered under vacuum through Whatman GF/B filters using a Brandel Cell Harvester, followed by 2×3 ml washes with 50 mM Tris-HCl (pH 7.7 at room temperature). Bound radioactivity was determined by liquid scintillation spectrometry. $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ radioligand binding assays were performed according to HALL *et al.*[7] and TITELER *et al.*[8] respectively. Results of these binding assays are presented as the mean of trip-

licates in Table 2. **1** has less affinity for 5-HT_{1A} than for 5-HT_{1D} and 5-HT_2 receptor subtypes, for which it has similar affinity and thus is not selective between the latter two. For comparison, sumatriptan shows a rank order of affinity for these subtypes: 5-HT_{1D} > 5-HT_{1A} > 5-HT_2 with pIC_{50}'s of 7.7, 6.3 and < 5.0, respectively.

Functionally, **1**, at up to a 10 μM concentration, lacks any 5-HT_{1D} agonist action in a guinea pig brain preparation using the method of WAEBER *et al.*[9].

1 does not seem to possess the potential of sumatriptan as a therapeutic agent. However, *N*-alkylated indoles are unusual natural products, especially as microbial metabolites. The present producing strain seems able to *N*-alkylate indoles. To really assess the effects of such a substitution in the search for antimigraine therapy, it may be worthwhile to incorporate such structural features into medicinal chemistry programs.

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