

# A Novel Antimicrobial Indolizinium Alkaloid from *Aniba panurensis*

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## Abstract

Activity-guided fractionation of an *Aniba panurensis* organic solvent extract has led to the isolation of the novel alkaloid 6,8-didec-(1Z)-enyl-5,7-dimethyl-2,3-dihydro-1H-indolizinium, trifluoroacetic acid salt (**1**). Its structure was determined by NMR and mass spectrometry. Bioassays performed in vitro demonstrated toxicity of compound **1** to a drug resistant strain of *Candida albicans*.

## Introduction

High throughput screening of ~140,000 crude plant, marine, and fungal extracts from the DTP Natural Products Library has been carried out in an antimicrobial assay designed to detect novel chemotherapeutics for the treatment of drug resistant microorganisms responsible for opportunistic infections in immunocompromised patients. Several extracts from tropical trees of the genus *Aniba*, family Lauraceae, were determined to be toxic to an azole-resistant strain of *Candida albicans*.

A review of the literature revealed that the steam distillate from wood of several *Aniba* spp. which grow in Brazilian Amazonia, is a commercial product known as rosewood oil. Fungal and bacterial toxicities have been ascribed to essential oils.<sup>1,2</sup> Other compounds known from the genus *Aniba* include alkaloids,<sup>3,4,5</sup> styrylpyrones and neolignans,<sup>6</sup> and flavonoids,<sup>7</sup> none of which have reported antimicrobial activity. To determine the identity of the substance responsible for toxicity to azole-resistant *Candida albicans*, activity-guided fractionation of an organic solvent extract of *Aniba panurensis* (Meissn) Mez was undertaken. We report the isolation and structural elucidation of a novel alkaloid, 6,8-didec-(1Z)-enyl-5,7-dimethyl-2,3-dihydro-1H-indolizinium, as the trifluoroacetic acid salt (**1**).<sup>8</sup>

## Figure 1. Collection Data

**Plant Material.** *Aniba panurensis* (Meissn) Mez, family Lauraceae, and other *Aniba* spp. were collected near Bartica, Guyana, by Dr. S. Tawari of the New York Botanical Garden on September 11, 1992. Identification was done by the New York Botanical Garden, which maintains herbarium vouchers. Additional vouchers of each plant part extracted (wood, bark, and leaf) are maintained at the DTP Repository in Frederick, Maryland.

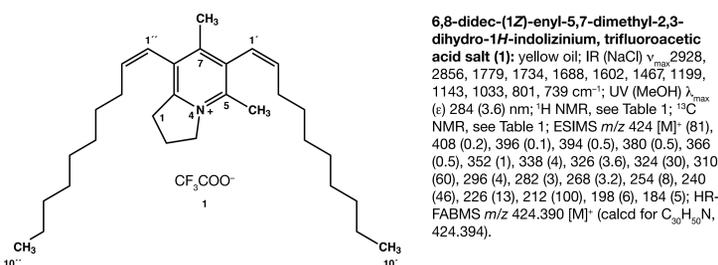
## Virtual Tree Guide of the Guianas



## Acknowledgment

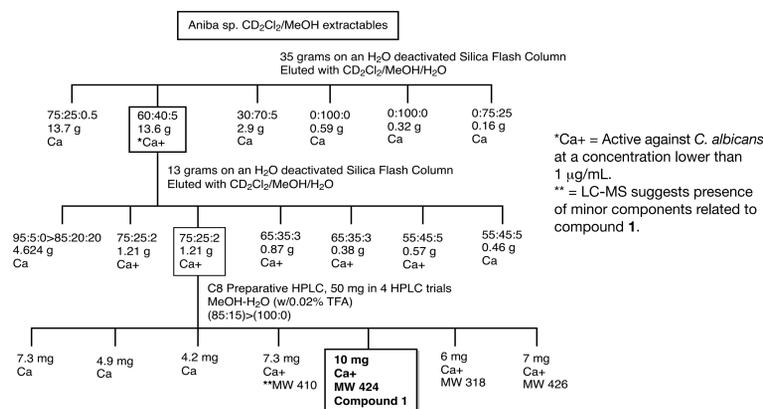
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**6,8-didec-(1Z)-enyl-5,7-dimethyl-2,3-dihydro-1H-indolizinium, trifluoroacetic acid salt (**1**):** yellow oil; IR (NaCl)  $\nu_{\text{max}}$  2928, 2856, 1779, 1734, 1688, 1602, 1467, 1199, 1143, 1033, 801, 739  $\text{cm}^{-1}$ ; UV (MeOH)  $\lambda_{\text{max}}$  (ε) 284 (3.6) nm; <sup>1</sup>H NMR, see Table 1; <sup>13</sup>C NMR, see Table 1; ESIMS  $m/z$  424 [M]<sup>+</sup> (81), 408 (0.2), 396 (0.1), 394 (0.5), 380 (0.5), 366 (0.5), 352 (1), 338 (4), 326 (3.6), 324 (30), 310 (60), 296 (4), 282 (3), 268 (3.2), 254 (8), 240 (46), 226 (13), 212 (100), 198 (6), 184 (5); HR-FABMS  $m/z$  424.390 [M]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>50</sub>N, 424.394).

**Figure 2.** Isolation of Compound **1** from *Aniba panurensis*.

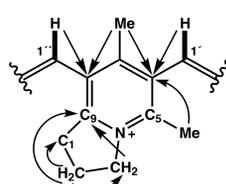


**Table 1.** 1D and 2D NMR Spectral Data and Assignments for Compound **1**<sup>a</sup>

C/H No.	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	COSY	HMBC (8 Hz)	1,1-ADEQUATE
1	3.31 t	32.6	2	2, 3	2, 9
2	2.49 p (7.7)	20.9	1, 3	1, 3	1, 3
3	4.73 t (7.7)	58.2	2	1, 2	2
5		148.1		3, 5-CH <sub>2</sub> , 7-CH <sub>3</sub> , 1"	5-CH <sub>3</sub>
5-CH <sub>3</sub>	2.63 s	18.1	1'		5
6		136.0		5-CH <sub>2</sub> , 7-CH <sub>3</sub> , 1"	1'
7		156.0		3, 5-CH <sub>2</sub> , 7-CH <sub>3</sub> , 1"	7-CH <sub>3</sub>
7-CH <sub>3</sub>	2.31 s	19.0	1', 1"		7
8		132.2		3, 5-CH <sub>2</sub> , 7-CH <sub>3</sub> , 1"	1"
9		154.3		1, 3, 5-CH <sub>2</sub> , 7-CH <sub>3</sub> , 1"	1
1"	6.23 br ab (11.3)	121.8	2'		6, 2'
1'	6.23 br ab (11.4)	120.9	2"	3"	8, 2"
2'	6.10 ab (11.3, 7.4)	139.4	1', 3'	1', 3', 4'	1', 3'
2"	6.07 ab (11.4, 7.4)	139.6	1'', 3''	1'', 3'', 4''	1'', 3''
3'	1.74 br q (7.4)	29.4	1', 2', 4'	1', 2'	2'
3''	1.80 dq (1.6, 7.4)	29.5	1'', 2'', 4''	1'', 2''	2''
4'	1.36 m	29.0	3'	3'	
4''	1.36 m	28.9	3''	3''	
5'	1.24-1.18 m	29.7	4'	4'	
5''	1.24-1.18 m	29.7	4''	4''	
6'	1.24-1.18 m	29.6	9'	9'	
6''	1.24-1.18 m	29.6	9''	9''	
7'	1.24-1.18 m	29.6	9'	9'	
7''	1.24-1.18 m	29.5			
8'	1.24-1.18 m	32.2	10'	9'	
8''	1.24-1.18 m	32.2	10''	9''	
9'	1.27 sextplt (7.0)	23.0	10'	8', 10'	
9''	1.27 sextplt (7.0)	23.0	10''	8'', 10''	
10'	0.86 t (7.0)	14.2		9'	
10''	0.86 t (7.0)	14.2		9''	

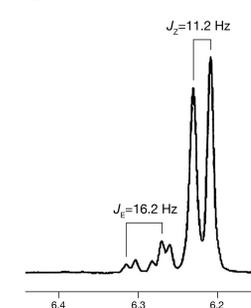
<sup>a</sup>Recorded at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C in CD<sub>2</sub>Cl<sub>2</sub> at 27°C.

**Figure 3.** Structurally Critical HMBC (8 Hz) Data



1,1-ADEQUATE experiments were critical in assigning connectivities of the quaternary carbons within the indolizinium ring system, C-7 ( $\delta_{\text{C}}$  156.0) connected to 7-methyl ( $\delta_{\text{C}}$  19.0); C-5 ( $\delta_{\text{C}}$  148.1) connected to 5-methyl ( $\delta_{\text{C}}$  18.1); C-6 ( $\delta_{\text{C}}$  136.0) and C-8 ( $\delta_{\text{C}}$  132.2) connected to  $\delta_{\text{C}}$  121.8 and  $\delta_{\text{C}}$  120.9, respectively. The last two carbons were assigned by three bond HMBCs with C-6 showing cross peaks to both 5-methyl and 7-methyl hydrogens, while C-8 shows only one HMBC to the 7-methyl hydrogens. The HMBC data supporting the assignments in the indolizinium ring are shown in Figure 3. The ring closure of C-1 to C-9 was confirmed by the HMBC connectivities from the hydrogens on C-1, C-2, and C-3 and the C-1' vinyl groups, in addition to the 1,1-ADEQUATE data.

**Figure 4.** <sup>1</sup>H NMR of Vinyl Protons



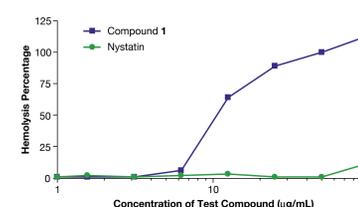
<sup>1</sup>H NMR of an impure sidecut reveals the presence of a minor species possessing the *E* configuration ( $J_z=16.2$  Hz), suggesting that the *Z* configuration ( $J_z=11.2$  Hz) is correct for compound **1**. This assignment was also supported by Advanced Chemistry Development software calculations which gave  $J_z=11.5$  Hz and  $J_z=16.1$  Hz.

**Table 2.** Spectrum of Antifungal Activity of **1**

Test Organism / Number of Strains Tested	MIC <sub>50</sub> Range (μg/ml)
<i>Candida albicans</i> / 8	0.5-1
<i>Candida lusitanae</i> / 2	1-4
<i>Candida glabrata</i> / 2	2
<i>Candida krusei</i> / 3	0.5-1
<i>Candida parapsilosis</i> / 1	0.5
<i>Cryptococcus neoformans</i> / 4	0.25-0.5

To further evaluate the potential of compound **1** as an antimicrobial chemotherapeutic, testing was conducted to determine the breadth of antifungal activity to clinically relevant strains. Compound **1** proved highly toxic, with minimal inhibitory concentrations in the low to sub-microgram range to 20 recent antibiotic resistant isolates (Table 2). In side-by-side testing against *Candida krusei*, the potency of **1** equaled that of amphotericin B and exceeded the potency of 5-fluorocytosine and fluconazole by factors of 16 and 32, respectively. In the DTP 60 human tumor cell panel, compound **1** had an LC<sub>50</sub> of ~10<sup>-6</sup> M with no selectivity toward any tumor type.

**Figure 5.** Hemolytic Activity of **1** and Nystatin



Compound **1** possessed potent hemolytic activity in the same molarity range as antifungal activity (Figure 5). The calculated log  $K_{ow}$  for **1** is 9.1, an indication of lipophilicity which exceeds the value set forth by "Lipinski's Rule of 5" for compounds that have favorable, drug-like properties.<sup>9</sup> Thus, lacking favorable chemical properties and possessing hemolytic activity, compound **1** would not be selected as a developmental candidate for antifungal or anticancer use.

## Conclusions

- 6,8-didec-(1Z)-enyl-5,7-dimethyl-2,3-dihydro-1H-indolizinium (**1**) shows a broad range of antimicrobial activity but is also toxic to human cells.
- Compounds closely related to **1** are present in *Aniba panurensis* organic extract.
- Synthetic modifications of **1** should be tried to improve selectivity.

## References and Notes

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