# Malbrancheamide B, a novel compound from the fungus *Malbranchea aurantiaca*[1]

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## Abstract:

A new indole alkaloid, namely malbrancheamide B (**2**), was isolated from the culture medium and mycelia of the ascomycete *Malbranchea aurantiaca* along with malbrancheamide (**1**). Structural elucidation of **2** was carried out by a combination of mass spectrometry (MS) and $^{1}H$ and $^{13}C$ NMR spectroscopy analyses, as well as by comparison of the NMR data with those of **1**. According to the conformational studies using molecular mechanics analyses, compound **2** exists in one preferred conformation, which was optimised by density functional theory (DFT) calculations. Compound **2** is the second chlorinated indole alkaloid possessing a bicyclo [2.2.2] ring with an unusual relative configuration at C12a in the bicyclo [2.2.2] diazaoctane ring system. So far, these structural features seem to be unique for the alkaloids biosynthesised by the fungus *M. aurantiaca*.

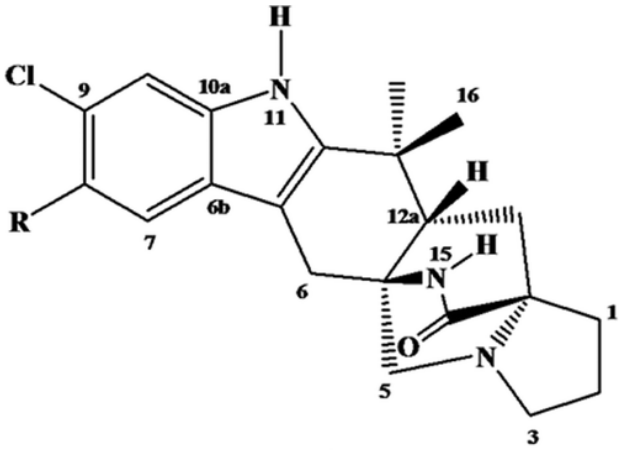
**Keywords:** *Malbranchea aurantiaca* | Malbrancheamide | Malbrancheamide B

## Article:

### 1. Introduction

Recently we reported the isolation and structural elucidation of malbrancheamide (**1**) (figure 1), an indole alkaloid possessing an unusual bicyclo [2.2.2] diazaoctane ring system[1]. This compound is related to the brevianamides, aspergamides, macfortines, paraherquamides, sclerotamides, and stephacidins[2,3,4]. These natural products have been isolated periodically from different strains of fungi of the genera *Aspergillus* and *Penicillium* since their discovery in 1969[3–7]. However, compound **1** was isolated from the culture of the ascomycete *Malbranchea aurantiaca* Singler and Carmich (Myxotrichaceae)[1]. Compound **1** is the first chlorinated indole alkaloid possessing the bicyclo [2.2.2] diazaoctane ring system. On the other hand, the relative configuration at C12a in the bicyclo [2.2.2] diazaoctane ring system is different to that of previous reported analogs. Altogether, these features make malbrancheamide (**1**) unique among

these of complex indole alkaloid derivatives. Compound **1** caused moderate inhibition of radicle growth of seedlings of *Amaranthus hypochondriacus* and inhibited the activation of the calmodulin (CaM)-dependent enzyme PDE1[1]. The latter effect was comparable to that of chlorpromazine, a well-characterised CaM antagonist. The inhibition mechanism of **1** was competitive with respect to CaM according to a kinetic analysis. 1-Hydroxy-2-oxoeremophil-1(10), 7(11), 8(9)-trien-12(18)-olide and penicillic acid were also isolated from this species[8]. The present work was undertaken to isolate additional phytotoxic agents from *M. aurantiaca* resulting in the isolation of malbrancheamide B (**2**) (figure 1), a new chlorinated compound related to **1**.




**1** R = Cl
**2** R = H

**Figure 1.** Structures of alkaloids **1** and **2**.

## 2. Results and discussion

Compound **2** (figure 1) gave a positive colour with Dragendorff's and Ehrlich's reagents and exhibited UV absorptions at 231 and 292 nm. Structure elucidation of **2** was carried out by a combination of mass spectrometry (MS) and $^{1}H$ and $^{13}C$ NMR spectroscopy analyses, as well as by comparison of the NMR data with those of **1**. Thus, HREIMS gave a molecular ion $[M]^{+}$ at *m/z* 369.1589 (Calcd for $C_{21}H_{24}ON_3Cl$ 369.1608), 35 uma less than compound **1**. The $^{1}$HNMR spectrum of **2** (table 1) was almost identical to that of **1** except that the aromatic signals were observed as an ABX system [$\delta_H$ 7.31 (d, $J$ = 8.7 Hz), 6.94 (dd, $J$ = 1.8, 8.4 Hz) and 7.26 (d, $J$ = 1.7 Hz)] rather than the two singlets at $\delta_H$ 7.47 and 7.39 observed in the spectrum of **1**[1]. The $^{13}$C NMR spectrum (table 1) combined with the HSQC experiment revealed 21 carbon resonances and supported the presence of a lactam functionality and a mono-halogenated indole moiety. Altogether, this information suggested that **2** was a mono-chlorinated indole alkaloid similar to malbrancheamide (**1**). Detailed 2D-NMR spectral analyses (COSY, HETCOR, HMBC, and NOESY) led to the establishment of the connectivity of functional groups and, in turn, of the molecular structure. The position of the functional groups along the hexacyclic core was corroborated by an HMBC experiment (table 1). Thus, the correlations C-10a/H-7, C-6b/H-10, C-6a/H-6, H-7, C-5a/H-12a, C-12a/H-6, H-16, H-17, and C-11a/H-6, H-16, H-17 supported the position of the chlorine atom and the fusion of the indole nucleus to the dimethyl cyclohexane ring throughout C-6a and C-11a. On the other hand, the cross peaks C-5a/H-6, H-12a, H-13, H-5; C-12a/H-5 and C-13a/H-13, H-1, H-3, H-5 indicated that the bicyclo [2.2.2] diazaoctane ring

system has the same arrangement that of **1**. The location of the chorine atom at C-9 was corroborated by a NOESY experiment which showed the correlations H-10/H-11 and H-7/H-6A, H-6B. The relative stereochemistry at the chiral centers was elucidated as depicted on the basis of some key correlations observed in the NOESY spectrum which are summarised in figure 2. Biogenetic considerations as well as the Cotton effects observed in the CD spectrum of **2**, almost indistinguishable to that of **1**, tend to support that both compounds possess the same absolute configuration at the stereogenic centers.

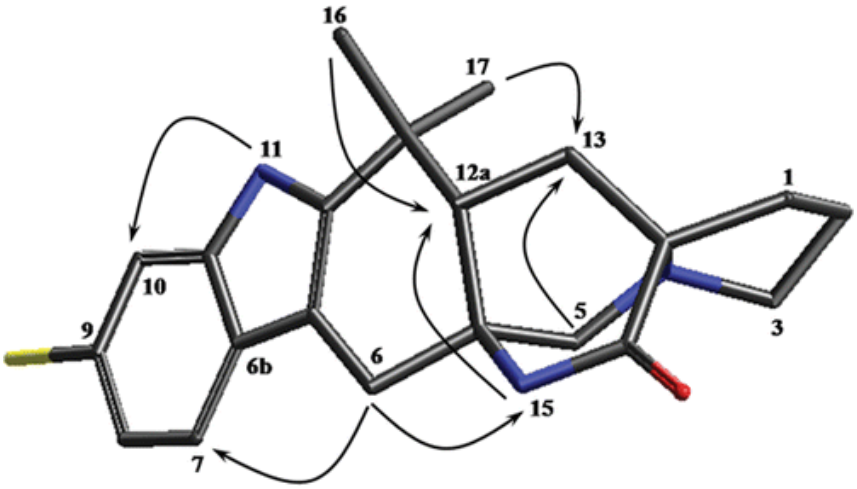



**Figure 2.** Optimized structure of compound **2** obtained by DFT analysis showing key NOESY correlations.

**Table 1.** $^{13}C$ and $^{1}H$ NMR data of malbrancheamide B **(2)**

| No. | $\delta^{13}C$ | $\delta^{1}H$ (*J* Hz) | HMBC |
|---|---|---|---|
| 1 | 28.5 | A 2.42 m | $H_2$, $H_3$, $H_{13}$ |
| | | B 1.34 m | |
| 2 | 22.4 | 1.72 m | $H_1$, $H_3$ |
| 3 | 55.2 | A 2.93 ddd (9.6; 6.0, 2.1) | $H_1$, $H_2$, $H_5$ |
| | | B 2.41 ddd (9.6; 6.0, 2.1) | |
| 5 | 59.5 | A 2.13 d (9.9) | $H_3$, $H_6$, $H_{12a}$ |
| | | B 3.25 d (9.9) | |
| 5a | 58.5 | – | $H_5$, $H_6$, $H_{12a}$, $H_{13}$, $H_{16}$ |
| 6 | 29.9 | A 2.79 d (15.9) | $H_5$, $H_{12a}$ |
| | | B 2.72 d (15.9) | |
| 6a | 103.7 | – | $H_6$, $H_7$ |
| 6b | 122.7 | – | $H_7$, $H_8$, $H_{10}$ |
| 7 | 120.7 | 7.31 d (8.7) | $H_8$, $H_{10}$ |
| 8 | 125.6 | 6.94 dd (1.8, 8.4) | $H_7$, $H_{10}$ |
| 9 | 126.6 | – | $H_7$, $H_8$, $H_{10}$ |
| 10 | 112.1 | 7.26 d (1.7) | $H_7$, $H_8$ |
| 10a | 135.4 | – | $H_7$, $H_{10}$ |
| 11 | – | 11.04 s | |
| 11a | 144.4 | – | $H_6$, $H_{16}$, $H_{17}$ |
| 12 | 34.1 | – | $H_{12a}$, $H_{13}$, $H_{16}$, $H_{17}$ |

| No. | $\delta^{13}C$ | $\delta^{1}H$ (*J* Hz) | HMBC |
|---|---|---|---|
| 12a | 46.9 | 2.07 m | $H_5$, $H_6$, $H_{13}$, $H_{16}$, $H_{17}$ |
| 13 | 33.9 | A 1.98 m | $H_1$, $H_2$ |
| | | B 1.91 m | |
| 13a | 64.1 | – | $H_1$, $H_3$, $H_5$, $H_{12a}$, $H_{13}$ |
| 14 | 173.1 | – | $H_1$, $H_{13}$ |
| 15 | – | 8.37 s | |
| 16 | 31.1 | 1.26 s | $H_{12a}$, $H_{17}$ |
| 17 | 26.6 | 1.31 s | $H_{12a}$, $H_{16}$ |

As part of the structural characterisation, a conformational analysis of the new compound **2** using molecular modelling techniques was carried out. The initial structure was built from standard fragments and minimised using molecular mechanics analysis as implemented in the HyperChem® 7 program. A preliminary searching protocol using the Monte Carlo method[9] was performed revealing a minimum energy conformer ($E = 51.86$ kcal mol$^{-1}$); this conformer was fully optimized by density functional theory (DFT) at the B3LYP/G31G* level theory[10]. In the DFT optimised structure ($E = -5200.84$ kcal mol$^{-1}$) ring *F* displays the same envelope conformation previously described for **1** with the atoms N4-C13a-C1-C2 located in the plane while C3 is out of the ring plane (figure 2). Due to the scarcity of the sample, the phytotoxic potential of **2** could not be established in the present investigation.

In conclusion, malbrancheamide B (**2**) is the second chlorinated indole alkaloid possessing a bicyclo [2.2.2] ring with an unusual relative configuration at C12a in the bicyclo [2.2.2] diazaoctane ring system. So far, these structural features seem to be unique for the alkaloids biosynthesised by the fungus *M. aurantiaca*.

## 3. Experimental section

### 3.1. General experimental procedures

The UV spectrum was recorded on a Shimadzu 160 UV spectrometer in MeOH solution. The CD spectrum was registered on a JASCO 720 spectropolarimeter at 25°C in MeOH solution. NMR spectra including COSY, NOESY, HMBC, and HSQC experiments were recorded in DMSO-$d_6$ on a Varian spectrometer at 300 MHz ($^1$H) or 75 MHz ($^{13}$C) NMR, using tetramethylsilane (TMS) as internal standard. HREIMS was obtained on a JEOL JMS-AX505HA mass spectrometer. Column chromatography: silica gel 60 (70–230 mesh, Merck). TLC was performed on precoated silica gel 60 F254 plates (Merck).

### 3.2. Fungal material

The isolate of *M. aurantiaca* was obtained from bat guano collected at the Juxtlahuaca cave located in Ramal del Infierno, State of Guerrero, Mexico, in 1998. A voucher specimen (24428) is deposited in the National Herbarium (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City. Three 2-L Erlenmeyer flasks, each containing 2 L of PDB (Difco), were individually inoculated with one 1-cm$^2$ agar plug taken from a stock culture of *M.*

*aurantiaca* maintained at 4°C on potato dextrose agar (PDA). Flask cultures were incubated at environmental temperature and aerated by agitation on an orbital shaker at 200 rpm for 15 days.

### 3.3. Extraction and isolation

After incubation, all flask contents were combined and filtered. The combined culture filtrate (6 L) was extracted exhaustively with EtOAc (3 × 6 L). The combined organic phase was filtered over anhydrous $Na_2SO_4$ and concentrated in vacuo to give a dark brown solid (1.5 g). The mycelium was macerated with EtOAc (3 × 2 L). After evaporating the solvent *in vacuo*, the combined mycelial and culture extract (2.0 g) was subjected to silica gel (200 g) open column chromatography eluting with a gradient of $CH_2Cl_2$–EtOAc (10 : 0/0 : 10) and EtOAc : MeOH (10 : 0/0 : 10) to yield nine major primary fractions (FI-FIX). Bioactivity in the bioautographic bioassay showed one active pool: FIV (100 mg), eluted with EtOAc–MeOH (9 : 1). Fraction IV (100 mg) was submitted to prepare TLC on Silica gel eluted with EtOAc–MeOH (9 : 1); after usual work up **1** (15 mg) and **2** (3 mg) were obtained.

### 3.4. Molecular modeling calculations

Minimum energy structures were generated using the MMF94 (Monte Carlo Protocol) as implemented in the HyperChem 7 Program (Hypercube Inc., Gainesville, Florida). The conformational search was carried out by exploring torsional internal degrees of freedom of dihedral angles selected by the automatic set up procedure. The torsional angles considered were: C12a-C13-C13a, C13a-C1-C2, C1-C2-C-3, C2-C3-N4, N4-C5-C5a. The rotation angle was 30°. The minimum energy conformer was fully optimised by DFT at the B3LYP/G31G* level.

Malbrancheamide B (**2**)

Glassy solid. $[\alpha]_D$ + 50° (c1, MeOH). IR (KBr) $\nu_{max}$: 3300, 1740, 1670, 1461, 1315, 1250 $cm^{-1}$. UV $\lambda_{max}$ (MeOH) (log ε): 231 (3.95), 292 (4.80) nm: CD (MeOH) $\lambda_{max}$ (Δε): 244 ($2 \times 10^6$), 217 ($1.3 \times 10^6$), 226 ($1.2 \times 10^6$), 296 ($1.5 \times 10^5$) nm; $^1H$ and $^{13}C$ NMR data, see table 1.
EIMS *m/z* (rel. int.) 369 (7.5), 371 (2.00), 365 (6.5), 325 (100), 311 (27.1), 228 (39.1), 206 (17.3), 163 (31.8), 164 (97.3), 163 (25), 135 (17.3), 120 (10), 96 (8.2); HREIMS: *m/z* 369.1589, calculated for $C_{21}H_{24}ON_3Cl$: 369.1608.

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