N-METHYL-1H-INDOLE-2-CARBOXAMIDE FROM THE MARINE FUNGUS *CLADOSPORIUM CLADOSPORIOIDES*

V. MANRÍQUEZ[A], A. GALDÁMEZ[A], B. VELIZ[A], J. ROVIROSA[A], A. R. DÍAZ-MARRERO[B], M. CUETO[B] J. DARIAS[B], C. MARTÍNEZ[C] AND A. SAN-MARTÍN[A].

[a]*Departamento de Química, Facultad de Ciencias, Universidad de Chile. Casilla 653, Santiago, Chile.*
[b]*Instituto de Productos Naturales y Agrobiología del CSIC, ZIP Code 38206, La Laguna, Tenerife, España*
[c]*Universidad de Santiago de Chile. Santiago. Chile.*
(Received: December 11, 2008 - Accepted: May 14, 209)

ABSTRACT

The crystal structure of N-methyl-1H-indole-2-carboxamide $C_{10}H_{10}N_2O$ was determined by single crystal X-ray diffraction. The structure can be described as consisting of an indole group and as substituent, linked at C2, the N-methylcarboxamide group. The molecular structure is essentially planar. The crystal packing results in N-H-------O hydrogen bonds which join the molecules into centrosymmetric dimeric rings. The knowledge of the crystal structure allows a complete assignment of the ^{1}H and ^{13}C-NMR spectra. The N-methyl-1H-indole-2-carboxamide is the first indole derivative isolated from marine fungus.

Keyword: Crystal structure, indole derivative, marine fungus

INTRODUCTION

Cladosporium (Hyphomycetes; mitosporic fungi) is the most frequently found genus of fungi in outdoor air in temperate climates. It has been isolated from many different types of soil and is a major colonizer of plant litter. The ability to sporulate heavily, ease of dispersal, and buoyant spores makes this fungus an important fungal allergen. Due to its ability to rapidly invade many different ecological niches (including marine habitat), *Cladosporium* is ubiquitous and therefore sometimes problematic. In this genus several secondary metabolites have been described, thus, from *C. cladosporioides* [1] and *C. tenuissimum* [2] it has been isolated lactones such as cladospolide A, B and C. cladospolide A and B were shown to be phytotoxic [2].

The compounds derived from indole are very common in nature and some have great biochemical importance, one of the most important and simple derivate of the indole is the amino acid tryptophan. Also, indole has been isolated of certain essences of flowers, like the oil of jasmine. Other substituted indoles compounds include alkaloids and the phytohormone auxin (indole-3-acetic acid). Furthermore, indole derivatives (alkaloids) had been found in several *Penicillum* strain [3]. The evaluation of topsentin analogues for biological activity showed that the introduction of a hydroxyl group enhances the cytotoxicity while bromination diminishes activity. Synthetic Methyl-indole-carboxamide was prepared by the imidazolide methods [4].

In our search for new interesting secondary metabolites, we collect, isolate and determine species of marine-derived fungi improve the cultivation for a productive fermentation in a 10 or 20-liter scale and characterize the obtained metabolites. In a previous culture of this fungus we isolated peroxyergosterol and p- methyl benzoic acid [5]. In a chemical analysis of *C. cladosporioides*, we now report the isolation of N-methyl-1H-indole-2-carboxamide. That is the first time that this indole derivative is isolated form marine fungus. This crystal structure analysis was intended for complete identification of an unknown compound, which was impossible to do with NMR spectra alone. Also, we relate here the X-ray structure to the ^{1}H, ^{13}C-NMR spectra of the N-methyl-1H-Indole-2-carboxamide, since the evidence can be useful for the future identification of substituted indole, which are not readily crystallized. To our knowledge no crystal structure on the present substituted indole has been reported.

EXPERIMENTAL

Source of material

Animal Material. - Samples of the marine sponge *Cliona sp.* were collected by scuba diving (-20 m) in IV Region (Los Molles), Chile, in April of 2004. A voucher fragment is kept under the registration, Universidad de Chile. Under sterile conditions, a piece of tissue from the inner part of the freshly collected sponge was cut and inoculated on malt agar slants consisting of 2% agar, 0.5% glucose, 0.5% yeast extract in filtered sea water. The inoculated agar slants were incubated at 20 °C, and from these a pure fungal culture was isolated after repeated inoculation on fresh malt agar plates. The Prof. E. Piontelli, Universidad de Valparaiso, Valparaiso, Chile, identified the isolated fungus as *Cladosporium cladosporioides*. The spores of fungi maintained on Czapeck agar slants at 8º C, were inoculated into the autoclaved Czapeck liquid medium. This medium was comprised of KNO_3 (2g/l), KCl (0.5g/l), $FeSO_4$ (0.01g/l), $MgSO_4$ (0.5g/L), KH_2PO_4 (1g/L), yeast extract (5g/L) and saccharose (30g/L) and was adjusted to pH 5.5. The strains were cultivated at 28º C in Erlenmeyer flasks that contained Czapeck liquid medium. After 20 days incubation at 20°C with shaking, the fermentation broth was filtered under suction, obtaining a mycelium and the broth (filtrate).

Extraction and Isolation.-The filtrate was partitioned with ethyl acetate. This extract (1.43 g) was fractionated by column chromatography with Sephadex LH-20 using methanol as solvent system. Some fractions were subjected to column chromatography on silica gel. The column chromatography was eluted with mixtures of *n*-hexane-ethyl acetate (0 up to 30%).Elution with *n*-hexane-ethyl acetate (30%v/v) afforded 74 mg of the product. The solid formed was recrystallized from a mixture of n-hexane-chloromethane. The title compound was obtained as colorless crystal.

X-ray Structure Determination

A needle-shaped single crystal of the title compound with dimensions 0.1 x 0.08 x 0.4 mm, was mounted on glass fiber with the long axis of the crystals oriented roughly parallel to the length of the fiber. Single-Crystal X-ray Diffraction data were obtained with the use of graphite-monochromatized MoKα radiation (λ = 0.71073 Å) at 293 K on a Bruker AXS SMART CCD area detector Diffractometer. Intensity data for 5055 total reflections were collected in the φ/ω scan mode. The collection of the intensity data was carried out with the program SMART [6]. Cell refinement and data reduction were done using SAINT [7]. Cell parameters were obtained from reductions data. The observed Laue symmetry and systematic extinctions clearly pointed to the space groups $P2_1/c$ (Nº14). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares. The H1 atom at N1 was located in difference Fourier map with N-H distance of 0.85 Å, all others H atoms were calculated geometrically with C-H distances of 0.93 Å (aromatic) or 0.96 Å (methyl). The H atoms were allowed to ride on their parent C or N atoms with fixed isotropic Uiso(H) = kUeq (carrier), where k=1.5 for methyl groups and k=1.2 for all other H atoms. The final cycle of refinement included all anisotropic displacement parameters converged to final R=0.0534 and wR_2= 0.1275, for 1092 observed reflections and 133 variable parameters. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.127 eÅ^{-3} and –0.116 eÅ^{-3}. To solve and refine the structure was used the SHELXL package of crystallographic programs [6]. Atomic scattering factors were taken from SHELXTL/PC. The Molecular graphics used to prepare material for publication were done using DIAMOND [8].

NMR spectroscopy

The NMR spectra ^{1}H NMR and ^{13}C NMR, HSQC, HMBC, NOESY and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. All chemical shifts are reported with respect to the acetone-d_6 solvent peak (δ_H 2.04 ppm; δ_C 29.80 ppm).

 e-mail: vmanriqu@uchile.cl

RESULTS AND DISCUSSION

The crystal data are summarized in Table 1. Selected bond angles with their estimated standard deviation (esd`s) are given in Table 2. The bond lengths and angles are within the expected ranges and similar to those observed previously in other indole derivatives [9,10]. A perspective view of the molecule with the atom labelled and displacement ellipsoids draw at the 50% probability level is given in Figure 1.

Table 1. Data collection and structure refinement parameter

Crystal Data	$C_{10}H_{10}ON_2$
Crystal	colorless block
Size	0.1 x 0.08 x 0.4 mm
Space group	Monoclinic, $P2_1/c$
Cell constants	
a	11.168(2) Å
b	5.2482(10) Å
c	15.874(3) Å
β	106.876(3) º
Z	4
F(000)	368
Wavelength, λ (MoKα)	0.71073 Å
Linear absorption coefficient, μ	0.087 mm^{-1}
Data Collection	
Diffractometer	Bruker AXS SMART CCD
Scan mode	φ/ω
T measurement	293 K
θ max	25.00 º
θ min	1.91º
Measurement reflections	5055
Independent reflections	1539
Reflection with $I > 2\sigma(I)$	1092
R_{int}	0.0599
Refinement	
Solution by	Direct methods
Refinement on F^2	Full-Matrix Least-Squares
Program	SHELXL-97
$R_{gt}(F)$	0.0534
$wR_{ref}(F^2)$	0.1278
S	1.064
Parameters	133
$\Delta\rho_{max}$	0.127 e $Å^{-3}$
$\Delta\rho_{min}$	-0.116 e $Å^{-3}$

Table 2: Selected bond angles and torsion angles (º)

	Angles	Atoms	Angles
O10-C10-C2	120.66(18)	N1-C2-C10	119.04(17)
N11-C10-C2	117.88(18)	C3-C2-C10	132.36(18)
O10-C10-C2-N1	-9.8	N11-C10-C2-N1	172.6
N11-C10-C2-C3	-11.7	O10-C10-C2-C3	165.9

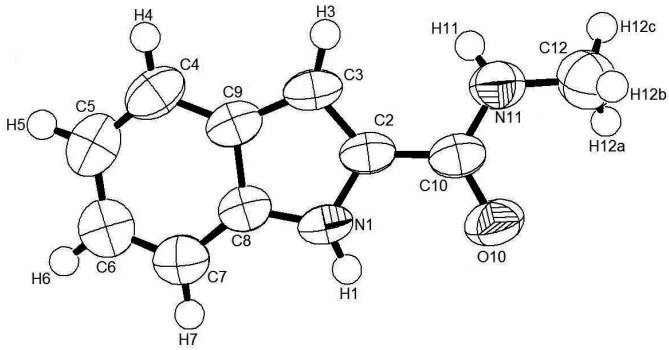

Fig 1: The molecular structure of the title compounds; showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 50% probability level.

The molecular structure of the title compound consists of an indole group linked by C2 to the N-methyl carboxamide substituent. The plane defined by the indole group (max. deviation 0.0105 Å) is the main plane of the molecule. The conjugation of the amide group with the indole system is a little less favoured in the title compounds , torsion angle N1-C2-C10-O10 = 9.8º, than in the related compounds N-3-methoxyphenyl-2-(5-methoxyindole) carboxamide [9], where the corresponding torsion angle is 7.9º. On the other hand, the conformation at the amide N atom is similar to the related compounds reported by Ianelli et al. [9], the methyl group is cis to the carbonyl O atom with respect to the carboxamide C-N bond, with torsion angle O10-C10-N11-C12=1.1º. The most import feature of this molecule is its planarity, with O10 at 0.2771 Å out of the mean plane defined by the indole group. Thus, the structure of the title compound is essentially planar and shows no unusual geometrical features. The crystal packing results in N-H-------O hydrogen bonds (Table 3). Pairs of intermolecular N-H-------O hydrogen bonds join the molecules into centrosymmetric dimeric rings, which are stacking parallel to **c** axes with alternate aromatic rings (Figure 2). Thus, aromatic π-π interactions are absent.

Table 3. Hydrogen-bonding geometry (Å, º).

D-H….A	d(D-H)	d(H….A)	d(D….A)	< DHA
N1-H1….$O10^{i}$	0.85(2)	2.06(2)	2.871(2)	161(2)

Symmetry code: (i) -x+1, -y+2, -z

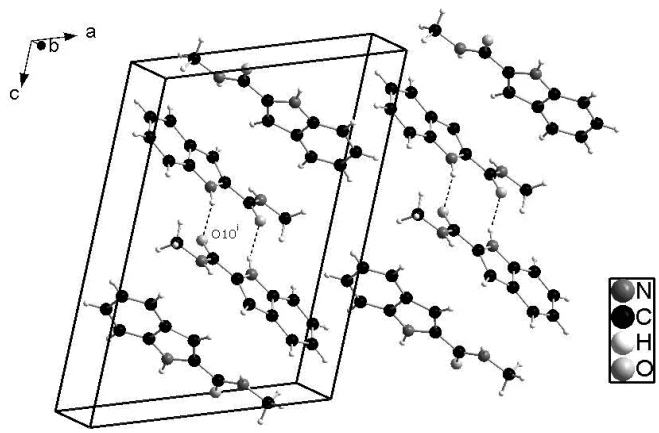

Fig. 2: A view crystal packing showing the N1-H1...$O10^{i}$ hydrogen bond. [Symmetry code: (i) -x+1, -y+2, -z].

The main difficulty found to elucidate the structure was to determinate the position of carboxamide group. The most frequently indole derivative (i.e tryptophan) has a substituent mainly at position 3. The experimental data, mainly HMBC gave correlations that was not sufficient to assign unambiguously all the NMR signals. Thus, the knowledge of the crystal structure of the N-methyl-1H-indole-2-carboxamide allows the complete interpretation and assignment of the NMR spectra. Analysis of the ^{1}H- and ^{13}C NMR spectra (Table 4) plus

the H-H COSY and HMBC experiments allowed us to assign unequivocally all ^{1}H and ^{13}C signals of this compound. The molecular formula of the title compound was deduced as $C_{10}H_{10}N_2O$ (*m/z* = 174.1990) by HREIMS and ^{13}C NMR data. ^{1}H NMR spectrum showed the proton signal at δ 7.06 (1H, s), assigned to olefinic proton. Aromatic proton appeared at δ 7.60 (1H, d, *J* = 7.9 Hz), 7.05 (1H, ddd, *J* = 0.6, 6.9, 6.9 Hz), 7.20 (1H, ddd, *J* = 0.6, 6.9, 6.9 Hz) and 7.56 (1H, br s). Two broad singlets at δ 11.02 and 7.82 were assigned to NH protons. Finally, a methyl group at δ 2.96 (d, *J* = 4.7 Hz) completed the ^{1}H NMR spectrum. The ^{13}C NMR spectra (Table 4) shown one methyl group, five methine groups, one quaternary carbon and one carbonyl group at δ163.5. All data are consistent with the solved molecular structure of N-methyl-1H-indole-2-carboxamide.

Table 4. NMR data for N-methyl-1H-indole-2-carboxamide (500 MHz, δ ppm, J Hz, acetone-d_6)

#	^{1}H- RMN	^{13}C RMN	HMBC	COSY	NOESY
1-NH	11.02 br s	---			H-7
2	---	133.2			
3	7.06 (s)	103.1	C-2, C-8, C-9		NH-11
4	7.60 (d, 7.9)	122.9	C-3, C-6, C-8	H-5	
5	7.05 (ddd, 0.6, 6.9, 6.9)	121.2	C-7, C-9	H-4, H-6	
6	7.20 (ddd, 0.6, 6.9, 6.9)	124.8	C-4, C-5, C-8	H-5, H-7	
7	7.56 (d, 8.2)	113.6	C-5, C-9	H-6	NH-1
8	---	138.2			
9	---	129.2			
10	---	163.5			
11-NH	7.82 br s	---		H_3-12	H-3
12	2.96 (d, 4.7)	26.8	C-2, C-10	NH-11	

ACKNOWLEDGEMENTS

The authors thank M. T. Garland and Andres Ibañez for X-ray measurement and the FONDAP Nº 11980002 for Bruker SMART CCD single crystal diffractometer. This work was supported by Fondecyt 1040895 , Proyecto Anillo ACT-38 and Program of Cooperation between CSIC(Spain)-Universidad de Chile.

Supplementary Information: Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 706185. Copies of the data can be obtained, free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK ; fax: +44 1223 336033 ; or e-mail: deposit@ccdc.cam.ac.uk).

REFERENCES

[1] A. Hirota, H. Sakai, A Isogai, Y. Kitano, T. Ashida, H. Hirota, T. Takahashi , *Agric. Biol. Chem*. **49**, 903, (1985**).**
[2] Y. Fuji, A. Fukuda, T. Hamasaki, I. Ichimoto, H. Nakajima, *Phytochemistry*, **40**, 1443, (1995).
[3] N. F. Zelenkova, N. G. Vinokurova, M. U. Arinbasarov, *Appl Biochem Micro.***, 39***,* (1), 44, (2003).
[4] J. Pigulla; *Archiv der Pharmazie* (Weinheim, Germany), **312**, (1), 12, (1979).
[5] A. San-Martín, K. Painemal, Y. Diaz, C. Martinez, J. Rovirosa, *J. Argent. Chem. Soc.***, 93,** (4/6), 247, (2005).
[6] SMART, SAINTPLUS V6.02, SHELXTL V6.10 and SADABS; Bruker Analytical X-ray Instruments Inc., Madison, Wisconsin, USA.
[7] G. M. Sheldrick, SHELXL-97. Program for the Refinement of Crystal Structures. University of Göttingen, Germany, (1997).
[8] K. Brandenburg, DIAMOND. Visual Crystal Structure Information System, Version 2.1e Crystal Impact GbR, Bonn, Germany, (1999).
[9] S. Ianelli, M. Nardelli and D. Belletti, *Acta Cryst. ,* **C51**, 1341, (1995).
[10] S. Basu, S. Ghosh, S. Ghosh, M. Helliwell and A. K. Mukherjee, *Acta Cryst.,* **C64**, 595, (2008).