Pergamon

0031-9422(95)00626-S

Phytochemistry, Vol. 40, No. 6, pp. 1697–1698, 1995

0031-9422/95 $9.50 + 0.00

MACROPHOMINOL, A DIKETOPIPERAZINE FROM CULTURES OF *MACROPHOMINA PHASEOLINA*

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(*Received in revised form* 17 *July* 1995)

IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Macrophomina phaseolina*; Deuteromycetes; diketopiperazine; macrophominol.

Abstract—A diketopiperazine named macrophominol was isolated from the phytopathogenic fungus *Macrophomina phaseolina*.

INTRODUCTION

Macrophomina belongs to a group of deuteromycete fungi that has been identified as pathogenic to several highly commercially valued crops such as black bean, watermelon, sorghum, melon and sesame [1–4]. It is known that phytopathological fungi produce a number of metabolites with biological activities in plants and animals [5–7]. In particular, positive phytotoxicity (i.e. the ability to induce root rot) was displayed by *Macrophomina phaseolina* growing on *Phaseolus vulgaris*. Therefore, we set out to isolate the metabolites from this fungus.

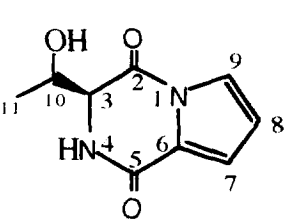
RESULTS AND DISCUSSION

Chloroform extraction of a phytotoxic liquid culture of *M. phaseolina*, followed by exhaustive chromatography of the organic residue obtained, allowed the isolation of a nitrogen-containing compound, which was present in a small amount. The ^{1}H and ^{13}C NMR spectra established the presence of 10 protons and nine carbons. The GC-mass spectrum indicated a molecular ion peak at *m/z* 194 $[M]^+$, suggesting the molecular formula to be $C_9H_{10}N_2O_3$. A fragmentation ion at *m/z* 150 $[M - 44]^+$ indicated the presence of the side chain at C-3. The ^{1}H NMR spectrum (Table 1) showed two signals at δ10.97 and 2.20 (exchangeable with D_2O) corresponding to one hydrogen on oxygen and one hydrogen on nitrogen, and a one-proton multiplet at δ4.56 corresponding to hydrogen geminal to the hydroxyl group. This proton coupled with the H-3 and H-11, according to the ^{1}H COSY experiment. Signals at downfield (δ7.51, 7.01 and 6.91) corresponded to the three pyrrolic aromatic hydrogens present in the bicyclic structure, and their connectivities were also established by the ^{1}H COSY experiment (Table 1). The ^{13}C NMR data confirmed the presence of two amide carbonyls (δ169.7 and 162.5), four unsaturated carbons (δ140.9, 137.3, 118.9 and 118.8), a saturated carbon directly bonded to oxygen (δ78.6), a saturated carbon directly bonded to nitrogen (δ67.7), and a saturated carbon (δ16.5). The above information led to the conclusion that the isolated compound, named macrophominol, has structure **1**.

Table 1. ^{13}C NMR (δ, $CDCl_3$, 50 MHz) data for the isolated diketopiperazine

H	δ (ppm)	^{1}H/^{1}H* connectivity	^{1}H/^{13}C* connectivity
3	4.56	H-10 (δ4.68)	C-3 (δ67.7)
4	2.20		
7	6.91	H-8 (δ7.51)	C-7 (δ118.8)
8	7.51	H-9 (δ7.01)	C-8 (δ137.3)
		H-7 (δ6.91)	
9	7.01	H-8 (δ7.51)	C-9 (δ118.9)
10	4.68	H-11 (δ1.49)	C-10 (δ78.6)
		H-3 (δ4.58)	
11	1.49	H-10 (δ4.68)	C-11 (δ16.5)

*Assignments were confirmed by ^{1}H COSY, DEPT and HETCOR experiments.



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EXPERIMENTAL

For the extraction process, a continuous flow liquid–liquid extractor was used for solvents denser than H_2O. TLC silica gel plates (Merck 60 GF_{254}, 0.2 mm thickness) were used. Mps uncorr.

A liquid–liquid extraction with $CHCl_3$ was performed on the liquid culture for 20 days. The solvent was then eliminated under red. pres., obtaining *ca* 8.3 g extract. This residue was purified by CC, using silica gel (Merck, 0.040–0.063 mm), with a hexane–EtOAc gradient and EtOAc–EtOH.

Fungal culture. The strain of *M. phaseolina* (Tassi) Goid (UDLAP 22-94) used for this study was donated by the Escuela Nacional de Ciencias Biológicas, I.P.N., México. The mycelium of the strain was originally inoculated in 40 Petri dishes, each containing 30 ml culture medium PDA (250 g potato, 10 g dry glucose, 10 g meat peptone, 15 g agar and 1000 ml H_2O, pH 5). They were incubated for 7 days at 36°, presenting homogeneous growth over the entire surface. Subsequently, 5 circles were taken (each 1.2 cm in diameter) from one of the fungal cultures, transferring them aseptically to a 500 ml flask, containing 250 ml potato dextrose broth (250 g potato, 10 g dry glucose, 10 g meat peptone and 1000 ml H_2O). 117 flasks were inoculated (29.25 l) in the same way and maintained in continuous agitation protected from direct light. After 7 days of growth, an homogeneous sample of the fungus was prepd for facilitating extraction of its components [8, 9].

Pathogenic test. This assay was carried out inoculating 2 black bean plants with 1 ml of a 5-day fungal culture grown at room temp. The 2 plants were inoculated by depositing the mycelial suspension in 3 cuts made to the stem of each plant. Root rotting was observed.

Isolation. From the frs eluted with hexane–EtOAc (4:1), 12 mg of **1** was obtained, mp 116–118°, $[\alpha]_D^{25}$ − 25.24° (EtOH; *c* 1.03 mg ml^{-1}). ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 10.97 (1H, *s*, HO-10); 7.51 (1H, *dd*, J = 7.5, 8.2 Hz, H-8); 7.01 (1H, *d*, J = 8.2 Hz, H-9); 6.91 (1H, *d*, J = 7.5 Hz, H-7); 4.68 (1H, *dq*, J = 6.6, 1.9 Hz, H-10); 4.56 (1H, *d*, J = 1.9 Hz, H-3); 2.20 (1H, *br s*, H-4); 1.49 (3H, *d*, J = 6.6 Hz, H-11). GC–MS 70 eV, (rel. int.): 194 $[M]^+$ (43), 177 $[M - OH]^+$ (5), 150 $[M - 44]^+$ (69), 121 $[M - 73]^+$ (100). ^{13}C NMR (50.0 MHz, $CDCl_3$); δ169.7 (C-5), 162.5, (C-2), 140.9 (C-6), 137.3 (C-8), 118.9 (C-9), 118.8 (C-7), 78.7 (C-10), 67.9 (C-3), 16.5 (C-11).

Acknowledgements—The authors thank the Escuela Nacional de Ciencias Biológicas, I.P.N., México, for the donation of the strain studied, and Dr Guillermo Delgado (Instituto de Química, UNAM) for interesting discussions and comments. Financial support from CONACYT 581300-5-3798N (Mexico) is acknowledged.

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