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PIPERINE FROM AN *ULOCLADIUM* SP.

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Key Word Index—*Ulocladium* sp.; fungus; piperine; identification.

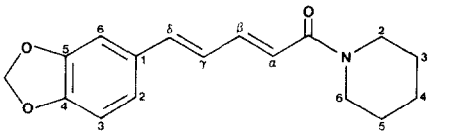
Abstract—Piperine was isolated from the mycelium of a *Ulocladium* species and identified by spectroscopic means. The compound showed antifungal activity in the *Cladosporium* TLC bioassay.

INTRODUCTION

Ulocladium is a common dematiaceous fungus, generally saprophytic on dead plant materials but it has also been isolated from soil. In the present paper we report the isolation of piperine, a major pungent factor of black pepper, from a species of *Ulocladium*.

RESULTS AND DISCUSSION

The isolated compound was a yellow crystalline substance, antifungal in nature when bioassayed against *Cladosporium cucumerinum* [1]. On mass spectral analysis it was found to have a $[M]^+$ at *m/z* 285 (95.7% rel. intensity) with fragment ions at *m/z* 201 (100), 173 (38), 135 (41), 115 (45), 97 (16) and 84 (31). The ion at *m/z* 84 is indicative of the presence of a piperidine ($C_5H_{10}N$) moiety in the structure. The absorbance at λ 341 nm ($\log \varepsilon$ 4.32) indicates the presence of an unsaturated system in the molecule. The IR spectrum exhibited bands at 1650 (α,β-unsaturated amide carbonyl), 1260, 1040 and 930 cm^{-1} (methylene grouping). However, the IR showed no peaks corresponding to an —NH group indicating that nitrogen could be in the form of a tertiary amide. 300 MHz ^1H NMR spectral analysis of the purified compound in $CDCl_3$ gave proton singlets at δ 6.92, 6.72 and a triplet at 6.82 indicating the presence of aromatic protons at positions C-6, C-2 and C-3. Unsaturation proton positions at C-α, C-δ, C-γ and C-β were confirmed by the doublet at δ6.38 (J = 15.3 Hz), 6.8 (J = 11.4 Hz), 6.73 (J = 15.8 Hz) and a multiplet at δ7.38. The presence of the methylene dioxy group in the molecule was confirmed by a singlet at δ5.96 while the presence of piperidine protons at positions C-2, C-6, C-3, C-4 and C-5 was supported by broad singlets at δ3.59, 3.56, 1.70 and 1.56.



All the foregoing data are in accordance with piperine reported from *Piper nigrum* [2]. The final structure was assigned when compared with the UV, NMR and mass spectral data of an authentic sample of piperine.

EXPERIMENTAL

Fungus. The *Ulocladium* sp. used in the present investigation was isolated from rapeseed samples collected from fields at Agriculture Canada Research Station, Beaverlodge, Alberta.

Isolation, extraction and purification of piperine. Fungus was grown on potato-dextrose agar plates for 20 days at 25°. Mycelial mat along with agar was extracted with Me_2CO, filtered and the filtrate dried *in vacuo*. The residue was dissolved in H_2O and extd with EtOAc. EtOAc exts were pooled and dried *in vacuo*. Antifungal activity of the EtOAc fraction was demonstrated using *Cladosporium*–silica gel TLC bioassay [1]. The antifungal compound was purified by prep. silica gel TLC (1 mm thickness, Terrochem Ltd, Edmonton) using EtOAc–hexane (1:4) as developing solvent. The active zone (R_f 1.0) was removed, eluted with MeOH, dried and subjected to UV, IR, NMR and MS analysis. Authentic piperine was purchased from Aldrich, USA.

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