Preliminary Communication

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Synthesis of colletotrichumine A

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Abstract: A short synthesis of colletotrichumine A, an oxindole-pyrazine alkaloid isolated from the pathogenic fungus *Colletotrichum capsici*, is described.

Keywords: alkaloid; colletotrichumine A; indole; natural product; pyrazine.

The fungus *Colletotrichum capsici* is an economically damaging plant pathogen with a range of hosts including vegetables, legumes, cereals and various tree fruits [1]. The fungus causes the often devastating anthracnose in chili, one of the most important crops in the tropics [2].

The ethyl acetate extract of *C. capsici* was recently shown to contain colletotrichumine A (**1**), a structurally unique alkaloid comprising an oxindole fused to trimethylpyrazine moiety (Figure 1) [3]. Glume blotch in wheat is caused by *Septoria nodorum*, a pathogenic fungus that produces septorine (**2**), which is a pyrazine that causes a decoupling action on wheat mitochondria and likely contributes to the pathogenicity of the fungus [4]. Thus, we set out to determine if colletotrichumine A (**1**) plays a role in the pathogenicity of *C. capsici* and as such, set out to synthesize this natural product to provide a sufficient quantity for biological evaluation.

A classic Knoevenagel approach [5] was used to construct colletotrichumine A (**1** in Figure 1 and Scheme 1). Oxidation of the known alcohol **3** [6] with manganese dioxide gave 3,5,6-trimethylpyrazine-2-carbaldehyde (**4**) using the literature protocol [7]. The Knoevenagel condensation of **4** with 2-oxindole gave exclusively the desired regioisomer **1**, as determined by NOE studies (Scheme 1). The spectroscopic data of synthetic **1** were identical in all aspects to the natural product (Table 1).

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In summary, a straightforward synthesis of the oxindole-pyrazine alkaloid colletotrichumine A is reported. Biological evaluation of colletotrichumine A is in progress, the results of which will hopefully determine if this heteroaromatic compound plays a role in the pathogenicity of *C. capsici*.

Experimental

Commercially available reagents were used throughout without purification. Anhydrous solvents were used as supplied. All reactions were routinely carried out in oven-dried glassware under a nitrogen atmosphere. Analytical thin layer chromatography was performed using silica plates and compounds were visualized at 254 nm and/or 360 nm ultraviolet irradiation followed by staining with either alkaline permanganate or ethanolic vanillin solution. Melting points were recorded on an electrothermal melting point apparatus and are uncorrected. NMR spectra were recorded at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. Assignments were made with the aid of NOESY and HMBC experiments. High resolution mass spectra were obtained by electrospray ionization in positive ion mode at a nominal accelerating voltage of 70 eV on a microTOF mass spectrometer.

3,5,6-Trimethylpyrazine-2-carbaldehyde (4)

To a solution of 2-hydroxymethyl-3,5,6-trimethylpyrazine (**3**) [6] (40 mg 0.26 mmol) in ethanol (3 mL) was added manganese dioxide (1 g, 11.5 mmol) and the mixture was stirred for 1 h at room temperature, filtered and the solid washed with ethanol. The filtrate was concentrated *in vacuo* to yield compound **4** (39 mg, 0.26 mmol, 98%) as a colorless solid; mp 75–77°C; ^{1}H NMR: ($CDCl_3$): δ_H 10.16 (1 H, s, CHO), 2.80 (3 H, s, Me), 2.60 (3 H, s, Me), 2.46 (3 H, s, Me); ^{13}C NMR ($CDCl_3$): δ_C 194.6 (C=O), 155.5 (C), 151.8 (C), 150.2 (C), 141.9 (C), 22.4 (Me), 21.5 (Me), 18.5 (Me); ^{1}H NMR data consistent with the literature [6].

Colletotrichumine A (1)

A solution of 3,5,6-trimethylpyrazine-2-carbaldehyde (**4**) (40 mg, 0.26 mmol), 2-oxindole (35 mg, 0.26 mmol) and piperidine (0.02 mL) in EtOH (3 mL) was heated to reflux for 3 h. Upon cooling to room temperature, the mixture was concentrated *in vacuo*, diluted in ethyl acetate (30 mL), washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude solid was purified by flash chromatography on silica gel (30:70 EtOAc/CH_2Cl_2) to yield compound **1**

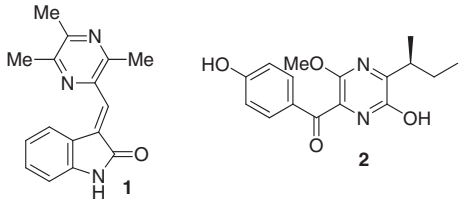
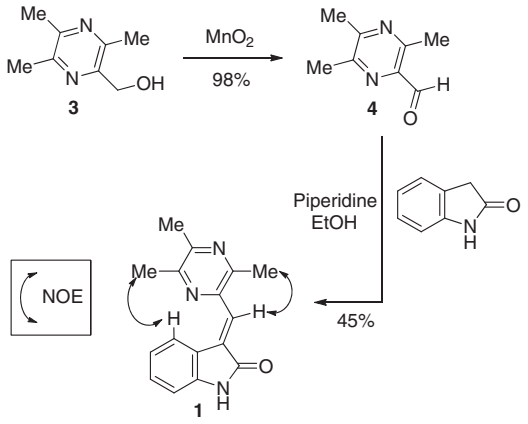
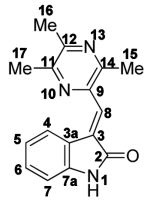


Figure 1 The fungal pyrazines colletotrichumine A (**1**) and septorine (**2**)



Scheme 1 Synthesis of colletotrichumine A (**1**)

Table 1 NMR data for synthetic and authentic colletotrichumine A (**1**).



Atom No.	Synthetic 1 (DMSO-d_6)		Colletotrichumine A (DMSO-d_6) [3]	
	δ_H (400 MHz)	δ_C (100 MHz)	δ_H (400 MHz)	δ_C (100 MHz)
1	10.65, s		10.64, s	
2	–	169.0	–	169.0
3	–	130.0	–	130.1
3a	–	121.3	–	121.4
4	8.60, br d (*J* 7.7)	127.2	8.58, br d (*J* 7.7)	127.3
5	6.98, br t (*J* 7.6)	121.1	6.96, br t (*J* 7.7)	121.2
6	7.28, br t (*J* 7.7)	130.9	7.27, br t (*J* 7.2)	130.9
7	6.88, br d (*J* 7.7)	109.7	6.87, br d (*J* 7.7)	109.8
7a	–	143.6	–	143.6
8	7.66, s	129.2	7.64, s	129.3
9	–	143.3	–	143.3
11	–	151.9	–	151.9
12	–	149.1	–	149.1
14	–	150.8	–	150.8
15	2.63, s	20.9	2.61, s	21.0
16	2.54, s	21.6	2.53, s	21.7
17	2.60, s	21.2	2.59, s	21.2

(18 mg, 0.07 mmol, 45%) as an orange solid; mp 255–257°C (lit [3] mp not stated); IR (neat): ν_{max} 3300, 1701, 1605, 1460, 1405, 1362, 1321, 747 cm^{-1}; For NMR data, see Table 1. ESI-HRMS. Calcd for $[C_{16}H_{15}N_3O + Na]^+$: *m/z* 288.1113. Found: *m/z* 288.1114.

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