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ALKALOIDS FROM *ASPERGILLUS CAESPITOSUS*

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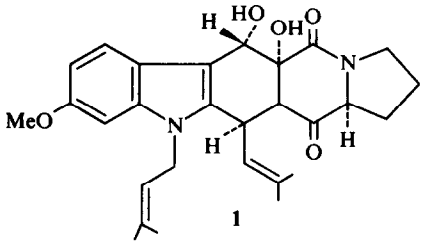
Key Word Index—*Aspergillus caespitosus*; alkaloids; dioxopiperazines; oxindole; myotoxins; tremorgens.

In our continuing studies on toxigenic food-borne fungi, isolates of *Aspergillus caespitosus* Raper and Thom were investigated. The isolate used was obtained from the Centraalbureaux voor Schimmelcultures, Baarn (C.B.S. 246.73) and is deposited in the culture collection of the S.A. Medical Research Council as MRC 271. The isolate, when cultivated on whole yellow maize, caused a neurotoxicity syndrome in rats characterized by sustained tremors and other neurological signs.

Schroeder *et al.* [1] isolated two known tremorgenic mycotoxins, viz. fumitremorgen B(**1**) [2] and verruculogen ≡TR_1 (**2**) [3] from *A. caespitosus* NRRL 1929 grown on autoclaved cracked maize. These neurotropic dioxipiperazines constitute an important group of mycotoxins [4, 5] which are elaborated by several microorganisms, e.g. *Aspergillus fumigatus* Fres [2, 6] (fumitremorgens A and B), *Penicillium paraherquei* Abe ex Smith (verruculogen) [7], *Penicillium verruculosum* Peyronel (verruculogen) [3], *Penicillium piscarium* Westling (verruculogen and fumitremorgen B) [8] and *Penicillium janthinellum* Biourge (verruculogen and fumitremorgen B) [9].

This paper relates the isolation of three alkaloids from *A. caespitosus* (MRC 271), viz. fumitremorgen B(**1**), TR_2 (**3**) [10] and cyclopiamine B (**4**) [11] and constitutes the first report on the natural occurrence of TR_2 and the production of cyclopiamine B by an *Aspergillus* species. Cyclopiamine B is known to be produced by *Penicillium cyclopium* Westling and *Penicillium urticae* Bainier only [11]. TR_2 was previously derived from the hydrogenation of the secondary–tertiary dialkyl peroxide, verruculogen in the presence of palladium on carbon.

The toxic principles were removed from the mouldered material by prolonged extraction with $CHCl_3$–MeOH (1:1). This crude extract was purified by solvent partition between $CHCl_3$–H_2O and a subsequent partition of the organic layer between hexane and 90% MeOH. The latter layer (1% by wt of the mouldered material) contained all the tremorgenic activity and was separated by partition chromatography on formamide-impregnated cellulose powder. Elution with hexane–C_6H_6 (1:1) gave fumitremorgen B (mp 212–213°) (0.02%). Subsequent elution with C_6H_6 gave a residue which was purified by crystallization to give TR_2 (mp 169–171°) (0.015%). The mother liquor was separated by chromatography on Si gel TLC under pressure; elution with $CHCl_3$–MeOH (19:1) gave a further amount of TR_2 and small quantities of a compound identified as the novel oxindole, cyclopiamine



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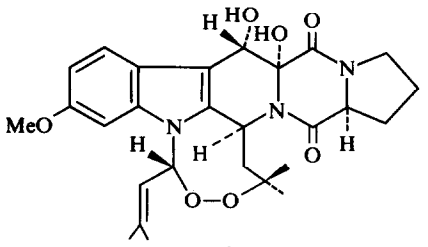

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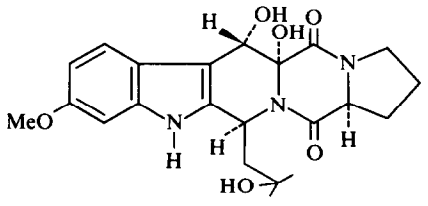

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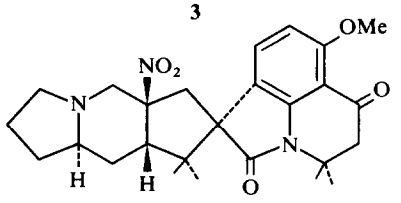

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B, mp 243–245° (0.00025%). The foregoing alkaloids **1**, **3** and **4** were fully characterized by direct comparison (spectroscopic and chromatographic properties) with reference samples.

The co-occurrence of fumitremorgen B (**1**) and TR_2 (**3**) in cultures of *A. caespitosus* established their biosynthetic relationship. Fumitremorgen B, TR_2 and cyclopiamine B seem to be biogenetically derived from tryptophan, proline and two units of dimethylallyl-pyrophosphate. The main structural differences originate from the different linkages of the two C_5-units which lead to the two novel structures.

EXPERIMENTAL

Isolation and purification. A. caespitosus (MRC 271) was grown on sterilized yellow maize (16 kg) for 21 days at 25° in 2 l. wide mouth glass jars (400 g/jar). The maize cultures were dried at *ca* 50° for 24 hr in a forced draught oven and milled to a fine meal. The dried mouldered meal (12 kg) was ground and extracted with $CHCl_3$–MeOH (1:1) for 2 days. The solvent was evapd and the residue (1.4 kg) dissolved in $CHCl_3$ (7 l.), washed with H_2O (3 × 3 l.) and dried (Na_2SO_4). Evapn of the $CHCl_3$ fraction furnished material which was partitioned between hexane (6 l.) and 90% MeOH (4 l.). The MeOH phase was evapd to dryness and the residue (125 g) separated by chromatography on formamide-impregnated cellulose powder (2.5 kg). Appropriate fractions were combined. Elution with hexane–C_6H_6 (1:1) gave fumitremorgen B (**1**) (2.4 g), mp 212–213° (from Me_2CO) (lit. [12] 211–212°), ν_{max} 1664 (amide CO) cm^{-1} and λ_{max}^{MeOH} 225, 276 and 294 nm (log ε 4.54, 3.80, and 3.85 respectively) and $[\alpha]_D^{21}$ + 6.4° (*c* = 1.0 in $CHCl_3$). Elution of the column with C_6H_6 gave after crystallization TR_2 (**3**) (2 g) and mother liquor. TR_2 had mp 169–171° (from Me_2CO); $\nu_{max}^{CHCl_3}$ 1668 (amide CO) cm^{-1} and λ_{max}^{MeOH} 222, 265 and 293 nm (log ε 4.51, 3.76 and 3.81 respectively) and $[\alpha]_D^{21}$ − 71.5° (*c* = 1.0 in $CHCl_3$). The mother liquor (1.3 g) was purified by column chromatography on Merck Si gel, Type H (200 g) using $CHCl_3$–MeOH (19:1) as eluant. The column was developed under 1 kg/cm² pres. Appropriate fractions were combined to yield TR_2 (0.1 g) and the bright blue-green fluorescent cyclopiamine B(**4**) (20 mg). It had mp 243–245° (from MeOH) (lit. [11] 245–246°) and ν_{max} 1720 (lactam CO), 1685 (ketone CO), 1550 and 1370 (aliphatic NO_2) cm^{-1}.

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