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# THE STRUCTURE OF DITRYPTOPHENALINE - A NEW METABOLITE OF ASPERGILLUS FLAVUS

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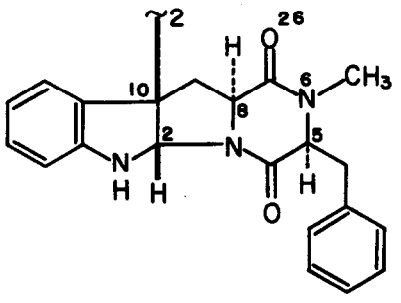
In the course of our search for mycotoxins present in contaminated food we investigated the secondary metabolites of several strains of Aspergillus flavus. The new metabolite 1 which we named ditryptophenaline was isolated from the mycelium of three of these fungal cultures (strains MIT-M25, 26,

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and 27). Methylene chloride extracts of the mycelium grown on either white corn or glutenous rice and harvested after ten days growth were precipitated with petroleum ether. After both column and preparative thin layer chromatography 1 was isolated as clear octahedral crystals from methylene chloride/methanol mixtures (mp 204-205° C); uv max (ethanol) 244 nm (ε 15,250) and 303 nm (ε 6,200); $[\alpha]_D^{23.5}$ -330°

(c. 52, $CH_2Cl_2$); ir ($CHCl_3$) 3415, 2995, 2930, 1662 br, 1611, 1498, 1480, 1470, 1455, 1401, 1355, 1323, 1270, 1162, 1151, 1135, 1078, 1052, 1027, 1020, 994, 974, 948, 906, 870, and 700 $cm^{-1}$. The high resolution electron impact mass spectrum[1] gave a parent ion at 692.3095 (calcd for $C_{42}H_{40}N_6O_4$ 692.3111) while the 90 MHz proton magnetic resonance spectrum measured in chloroform indicated that the molecule was a dimer: δ 1.59 (t, 1H, J = 12 Hz), 2.05 (d of d, 1H, J = 12 and 5 Hz), 3.03 (s, 3H), 3.21 (d of d, 1H, J = 14 and 5 Hz), 3.55 (d of d, 1H, J = 14 and 4 Hz), 3.65 (d of d, 1H, J = 12 and 5 Hz), 4.25 (br mult, 1H), 4.79 (s, 1H, exchanges), 4.89 (s, 1H), 6.5 - 6.9 (m, 2H), 6.95 - 7.3 (m, 4H), 7.4 - 7.7 (m, 3H). The $R_f$ of ditryptophenaline (1) measured on .25 silica gel developed with $CHCl_3$/MeOH 20:1 was .80 and a dark green color was produced with van Urk's reagent.[2]

In order to determine the structure of ditryptophenaline (1) and to define its stereochemistry a single crystal X-ray experiment was undertaken. Crystals grown as described above proved to be suitable, however since the crystals rapidly become opaque when dried a crystal was mounted in a Lindemann capillary with mother liquor. Preliminary experiments indicated that the space group was $P2_12_12_1$ with $a$ = 12.791 (6) A, $b$ = 14.731 (6) and $c$ = 19.91 (1) for a calculated density of 1.30 g/ml with Z = 4 (vide infra). Since at room temperature the X-ray scattering rapidly decreased with increasing θ angles, a cold stream of nitrogen (less than $-100^0$ C) was blown over the crystal to increase its scattering efficiency. Of the total of 2884 reflections measured using Cu radiation (λ = 1.5418 A) and ω-scan technique 2442 (85%) were considered observed ($1 \geq 3\sigma(1)$) and corrected for background, Lorentz and polarization effects. A multi-solution weighted tangent formula approach[3] was used to obtain an initial set of phases. Recycling of a suitable fragment in the tangent formula[4] generated a majority of the atoms. Subsequent electron density syntheses revealed the remainder of the non-hydrogen atoms. Two molecules of methanol were found to co-crystallize with each molecule of 1. Least squares refinements of positional and thermal parameters reduced the unweighted crystallographic residual to its current minimum of .085.[5] Tables 1, 2, 3, and 4 contain the fractional coordinates, bond distances, bond angles, and observed and calculated structure factors respectively.[6] All distances and angles are in agreement with commonly accepted values. Figure 1 contains a computer generated perspective drawing of ditryptophenaline (1).[7] The molecule contains two monomers of identical structure and stereochemistry related to each other in the solid phase by an approximate, non-crystallographic two-fold rotation. Each half possesses an indoline functionality which is cis fused to a pyrrolidine ring with the nitrogen in the a position. This ring is in turn fused to a fully substituted 2,5-dioxopiperazine ring containing a methyl substituent at N(6) and a benzyl group at C(5). Hydrogens at C(5) and C(8) are *syn* to each other but *re* to the hydrogen at C(2). The view of the molecule as seen perpendicular to the C(10)-C(10') bond has a doughnut shaped perspective. This arrangement in the solid phase allows for channels in the crystal structure which are filled by the molecules of methanol. One molecule of methanol is hydrogen bonded to O(26) with an O-O distance of 2.74 A, while the second molecule of methanol forms a hydrogen bond with the first with an O-O distance of 2.68 A. The absolute stereochemistry was not determined. Other fungal metabolites containing the same array of four fused rings include the sporidesmins,[8] the verticillins,[9] breviamide E,[10] chaetocin,[11]

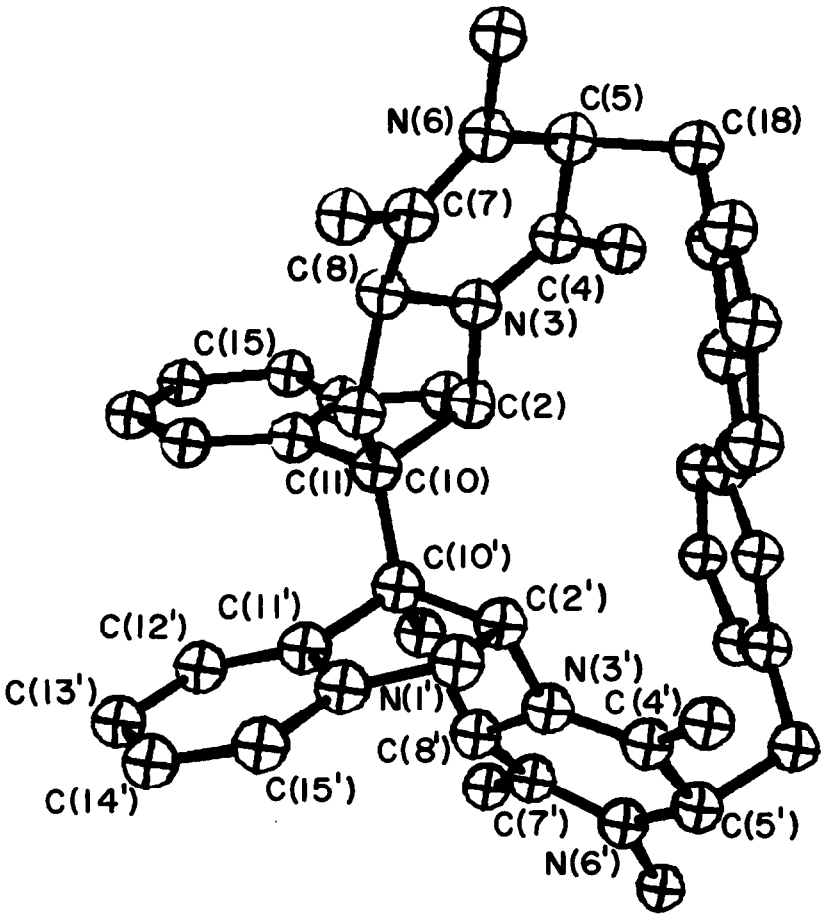


Figure 1. A computer generated perspective drawing from the crystal structure of ditryptophenaline (1). Hydrogens have been omitted for clarity.

and chetomin.[12] Verticillin A and chaetocin are of particular interest since they also contain a dimeric structure arranged analogously to 1. Ditryptophenaline (1), which possesses neither significant toxic ($LD_{50} > 200$ mg/kg) nor antibiotic properties, is formally derived from two molecules of tryptophan, two molecules of phenylalanine, and two methyl groups from the $C_1$ pool.

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