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# AFLAVININE, A NOVEL INDOLE-MEVALONATE METABOLITE FROM TREMORGEN-PRODUCING ASPERGILLUS FLAVUS SPECIES

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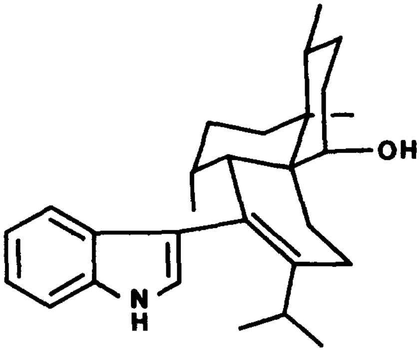
Summary. The structure of aflavanine, a new diterpene indole from A flavus, is presented.

Strains of Aspergillus flavus which produce the tremorgenic toxic indole aflatrem,[1,2] co-produce another major indole metabolite of very unusual structure. We now report the novel structure 1 for this indole, which we name aflavinine.



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Aflavinine, obtained as colorless needles, m.p. 102-4 °C from EtOAc, after column chromatography on silica gel with $CHCl_3$ cyclohexane (1:9) of a crude $CHCl_3$ extract of A. flavus cultures used to produce aflatrem,[1,3] was found by high-resolution MS to have a molecular ion of elemental composition $C_{28}H_{39}NO$, MW 405.

Crystals of composition, $C_{28}H_{39}NO\cdot CH_3\overset{O}{C}OCH_2CH_3$, were grown from an ethyl acetate solution. These crystals are triclinic and belong to the chiral space group P1 Accurate cell constants determined from a least-squares fit of fifteen high angle reflections were: $a$ = 8 288(2), $b$ = 8.853(3), $c$ = 10.477(3) Å, $\alpha$ = 98.29(2), $\beta$ = 102 91(2), and $\gamma$ = 79.49(2)° All unique diffraction maxima with

$2\theta \leq 114.1°$ were recorded in the $\omega$-scan mode using a computer controlled four-circle diffractometer and graphite monochromated $CuK\alpha$ (1.54178) x-rays. Of the 1967 reflections surveyed, 1577 (80%) were judged observed ($I > 3\delta(I)$) after correction for Lorentz, polarization and background effects

The structure was solved by a combination of direct phasing and Patterson methods.[4] All hydrogen atoms were located on a difference electron density synthesis and included in subsequent calculations. Full matrix least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for hydrogen converged to conventional crystallographic discrepancy index of 0.043.[4] The absolute configuration was not determined by the x-ray experiment. Additional crystallographic details such as the positional and thermal parameters, bond distances and angles and observed and calculated structure factors can be found in reference 5.[5]

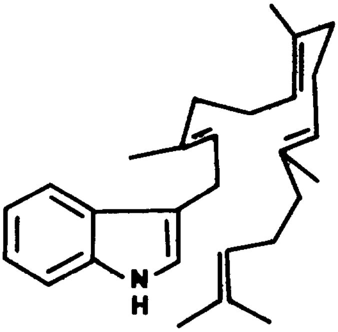
Figure 1 is a computer generated perspective drawing of aflavinine without hydrogens. The configuration of the substituents is 15(S*), 18(R*), 19(S*), 22(R*), and 23(S*) The cyclohexene ring is in a distorted half chair form since C(10), C(11), C(12), and C(23) are coplanar within 0.02 Å, and C(13) and C(14) deviate by 0.15 Å and 0.55 Å on different sides of this plane. All bond distances and angles agree well with accepted values for given bond types. There are two intermolecular hydrogen bonds, N(1)H--O(27) of 2.865 Å and O(27)H--O=C of 2.787 Å. All other intermolecular distances correspond to van der Waals contacts.

The UV of aflavinine 1, $\lambda$max 226 (27,000), 284(5300), 292(4500)nm, was characteristic of a 3-alkyl indole chromophore, rather than a 3-vinyl indole chromophore which has a strong absorption peak in the range 255-265 nm[6] due to conjugation of the extra-annular C=C with the indole aromatic ring system. The solution UV of aflavinine 1 is thus consistent with the extra-annular C=C being essentially orthogonal to the indole ring, as found in the solid state by the x-ray determination (78°).

The 100 MHz $^{1}H$ nmr ($CDCl_3$, TMS int. ref.) showed a complex pattern of sharp peaks in the region $\delta$0.7-$\delta$1.2ppm for the 5 methyls of aflavinine, 4 of the methyls are split into doublets by methine protons. The -OH and -NH groups occurred at $\delta$4.5 and $\delta$8.04ppm, respectively. The indole $\alpha$-H appeared characteristically[7] as a sharp doublet at $\delta$6.76ppm, due to splitting by the indole NH, and was well separated from the benzene ring proton multiplet which appeared at $\delta$7.0-$\delta$7.5ppm.

The $^{13}C$ nmr (in $CDCl_3$) showed the 10 $sp^2$ carbons of aflavinine, at 141.1, 135.9, 127.6, 125.5, 121.8, 121.3, 119.6, 119.4, 118.6, and 111.0 ppm, the carbinol carbon at 71.15 ppm and 15 signals for the saturated carbons, at 43.7, 42.4, 38.5, 31.3, 31.0, 30.1, 29.1, 27.6, 25.7, 25.4, 21.9, 20.8, 20.5, 18.1 and 15.7 ppm.

The co-production of aflavinine 1 with aflatrem suggests that both of these metabolites may come from a common intermediate. Previous work has shown that the precursor 2 is involved in the production of paspalinine, paspaline and paspalicine.[8] Aflavinine has the same site of oxygenation as these metabolites but would require a different mode of cyclization and migration of a methyl group



**2**

Acknowledgements

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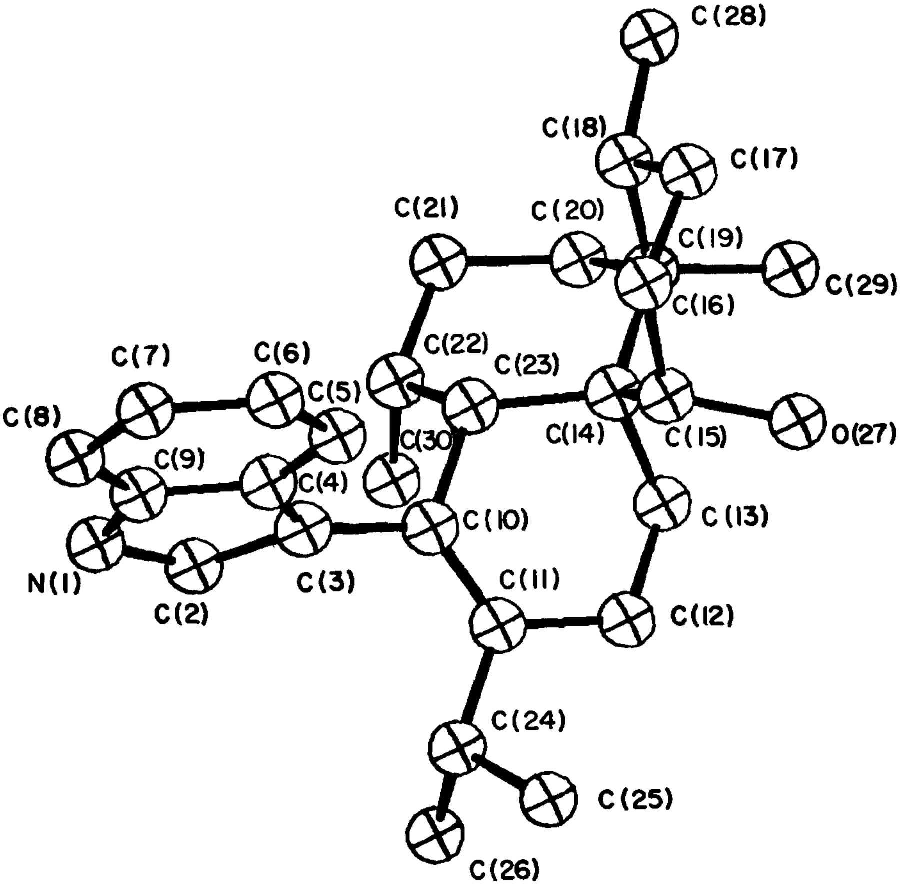

Fig. 1 A computer generated perspective drawing of the final x-ray model of aflavinine. Hydrogens are omitted for clarity and no absolute stereochemistry is implied.

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