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AFLATREM, A TREMORGENIC TOXIN FROM ASPERGILLUS FLAVUS

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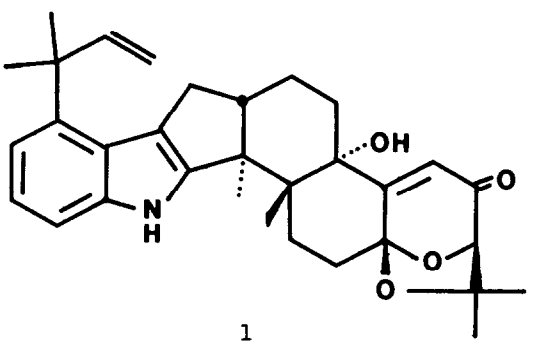
Summary The structure of aflatrem, a tremorgenic toxin from A. flavus is reported.

A substance produced by several strains of Aspergillus flavus grown on various foodstuffs[1,2] has the ability to produce in mice, trembling, convulsions, and, if the dose is high enough, death.[1,2] In this note we describe experiments which lead to expression 1 for this substance which we name aflatrem. We recently determined[3] structure 2 for the tremorgen paspalinine from Claviceps paspali



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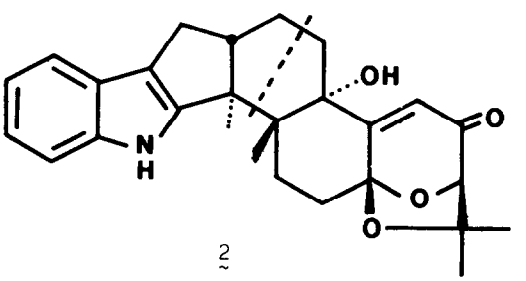

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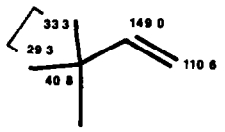
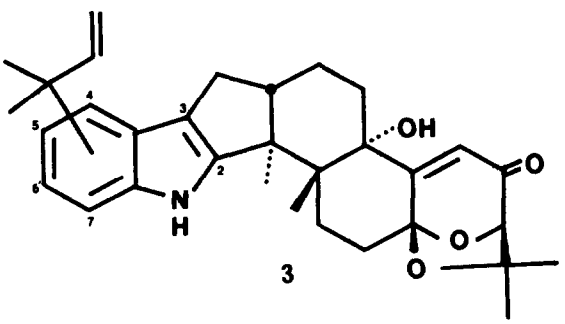
and were struck by the remarkable similarity between 1 and 2. Several lines of evidence suggested that aflatrem was paspalinine (2) extended by a reversed isopentyl group attached to the aromatic portion. The UV spectrum, λ_{max} (EtOH) 231(27750), 282(9050) and 292(sh) (7850)nm suggested a 2,3-disubstituted indole, and the indolic nature was further supported by characteristic TLC color reactions.[4] However, significant absorption (~10,000) in the UV at 250nm, showed that aflatrem, like paspalinine, also possessed an α,β-unsaturated carbonyl moiety.[3] The mass spectrum of aflatrem showed a parent ion at m/e 501 ($C_{32}H_{39}NO_4$) while paspalinine's parent ion occurred at m/e 433 ($C_{27}H_{31}NO_4$). A fragmentation of paspalinine (dotted line in 2) to yield a strong peak at m/e 182 and

which was also observed in several related compounds appeared to be of diagnostic value. Aflatrem showed an analogous fragmentation resulting in a strong m/e 250 peak, suggesting that $-C_5H_8$ was attached to the aromatic portion. D_2O exchange in the mass spectrometer established the presence of two exchangeable hydrogens in the molecule. This observation is satisfied by the presence of an indole NH proton, and a hydroxyl group in the molecule. The IR spectrum (KBr) showed -NH and -OH stretching bands at 3420 and 3390 cm^{-1}, and also an intense carbonyl absorption at 1680 cm^{-1} indicative of an α,β-unsaturated carbonyl group.

The ^{13}C nmr spectra of paspalinine (2)[3] and aflatrem (1) are virtually identical with the following changes. One aromatic carbon in aflatrem has undergone an ~20 ppm downfield shift appropriate for tert-alkyl group substitution.[5] Five new signals (29.3, 33.3, 40.8, 110.6 and 149.0 ppm) have appeared in the aflatrem spectrum. The new carbon signals are attributable to an α,α-dimethylallyl (reversed isopentenyl) substituent which must be attached to the aromatic ring. These signals are assigned in the following fashion, in good agreement with related molecules.[6] One carbon present in paspalinine at 27.0 ppm has moved to 29.1 ppm.



The 100 MHz ^1H nmr spectrum (d_6-DMSO, δ values in ppm from TMS int. ref.) of paspalinine (2) and aflatrem (1) show a number of identical signals. Both spectra show exchangeable protons at δ 5.50 (-OH) and δ 10.5 (indole NH). Identical assigned resonances also occur for the lone vinyl proton at δ 4.38 (1H, s); the methine proton at δ 5.72 (1H, s), four methyls at δ 1.07 (3H, s), δ 1.10 (3H, s), δ 1.30 (3H, s) and δ 1.36 (3H, s). Aflatrem (1) shows new resonances appropriate for the α,α-dimethylallyl substituent δ 6.21 (1H, dd, J_{cis}=11 Hz, J_{trans}=17 Hz), δ 4.44 (1H, dd, J_{cis}=11 Hz, J_{gem}=1.5 Hz), δ 4.80 (1H, dd, J_{trans}=17 Hz, J_{gem}=1.5 Hz) and δ 1.41 (s, 6H). Also paspalinine shows four aromatic protons (δ 6.9, 2H, m, and δ 7.26, 2H, m) while aflatrem shows only three (δ 6.84, 2H, 'd'; δ 7.18, 1H, m). In view of these results and the fact that the relative stereochemistries of all compounds of this type, i.e. paxilline,[7] paspaline,[8] paspalicine,[8] and paspalinine[3] are the same, structure 3 may be written for aflatrem.



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The remaining problem is to define the point of attachment of the α,α-dimethylallyl substituent to the aromatic ring. In indole the ^{1}H nmr signals for H-4 (δ 7.55) and H-7 (δ 7.40) lie significantly further downfield than those from H-6 (δ 7.08) and H-5 (δ 7.00).[9,10] In a number of indoles related to aflatrem we found this generalization holds in d_6-DMSO solution: the H-4′ and H-7′ protons (i.e. the benzene ring α-protons) lie significantly downfield from the H-5′ and H-6′ protons (i.e the benzene ring β-protons). Thus, paspaline shows this pattern with the two α-protons at δ 7.26 and the two β-protons at δ 6.90. In aflatrem one of the downfield resonances disappears indicating that the substitution has taken place at C-4′ or C-7′. The presence in the ^{13}C nmr spectrum of aflatrem[12] of the diagnostic indole C-7′ signal[11] at 111.2 ppm shows that this carbon bears no substituent, and thus the alkyl substituent is placed at C-4′. A single frequency off-resonance decoupling experiment showed that the downfield proton of aflatrem (δ 7.18) is coupled to the C-7′ signal at 111.2 ppm. Further, the methylene carbon attached to the indole ring in aflatrem appears at 29.1 ppm in the ^{13}C nmr, c.f. 27.0 ppm[13] for the same carbon in paspalinine; we attribute this downfield shift to a through space effect of the reversed isopentenyl group located at C-4′.

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References and Notes

1. B.J. Wilson and C.H. Wilson, Science, 144, 177 (1964).
2. B.J. Wilson, in "Microbial Toxins, Vol VI", A. Ciegler, S. Kadis and S.J. Ajl (editors), Academic Press, New York, 1977, pp. 274-277.
3. R.T. Gallagher, J. Finer and J. Clardy, Tetrahedron Lett., accompanying paper.
4. E. Stahl (editor), "Thin-Layer Chromatography", Springer-Verlag, Academic Press Inc., New York, 1965.
5. J.B. Stothers, p. 97, in "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.
6. R. Cardillo, C. Fuganti, G. Gatti, D. Ghiringhelli and P. Grasselli, Tetrahedron Lett., 3163 (1974); see also heohexene, spectrum 187 in L.F. Johnson and W.C. Jankowski (editors), "Carbon-13 NMR Spectra", J. Wiley and Sons, Inc., New York, 1972.
7. J.P. Springer, J. Clardy, J.M. Wells, R.J. Cole and J.W. Kirksey, Tetrahedron Lett., 2531 (1975).
8. J.P. Springer and J. Clardy, Tetrahedron Lett., accompanying paper.

9. P. J. Black and M. L. Heffernan, Aust. J. Chem., 18, 353 (1965).

10. The δ values quoted from ref. 9 above are for solutions in acetone. We determined the spectrum of indole in d_6-DMSO, d_6-acetone and d_6-DMSO-d_6-acetone solvent combinations, and verified that the published assignment was also valid for indole in d_6-DMSO. We also measured the following δ values in d_6-DMSO 2,4-dimethylindole, 7.10 (H-7), 6.88 (H-6), 6.70 (H-5); 2,5-dimethylindole, 7.15 (H-4), 7.14 (H-7), 6.79 (H-6); 2,6-dimethylindole, 7.25 (H-4), 7.05 (H-7), 6.74 (H-5).

11. In the ^{13}C nmr spectra of indole, benzene-ring methylated indoles, and numerous indole alkaloids, the unsubstituted C-7′ carbon appears within 108-112 ppm from TMS, which is well upfield from the other benzene ring carbons and hence easily identified.

12. The following ^{13}C nmr signals (in d_6-DMSO, ppm from TMS) were observed for aflatrem 196 7, 169 4, 151.8, 149.0, 140.5, 138.5, 122.8, 118.6, 116.6, 115.3, 114 0, 111.2, 110.6, 103.9, 86.7, 77.7, 75.8, 50.0, 47.8, 40.8, 38.9, 33.3, 31 5, 29.3, 29.1, 28.3, 27.8, 25.7, 22.7, 22.3, 20.6, 16.0.

13. This ^{13}C assignment for paspalinine was determined by Prof. Arigoni, personal communication from W. Acklin.

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