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PASPALININE, A TREMORGENIC METABOLITE FROM CLAVICEPS PASPALI STEVENS ET HALL

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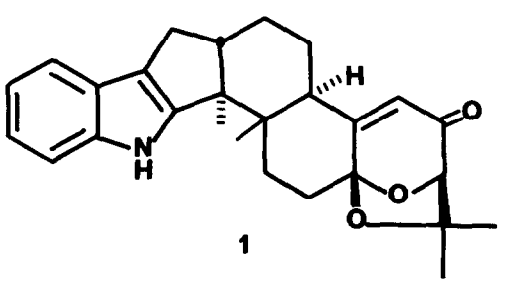
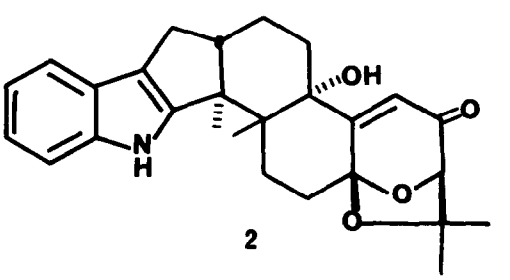
Summary· The structure of paspalinine, a tremorgenic fungal metabolite, is presented.

Previous studies on the mycelial components of the ergot fungus Claviceps paspali Stevens et Hall have led to the isolation[1] and structural elucidation of two indole-diterpenes, paspaline[2,3,5] and paspalicine (1).[4,5] Careful fractionation of the mycelial extracts has now provided a related minor metabolite which we name paspalinine. Particular interest in this compound arose from the fact that it causes tremors upon oral administration in cockerels, mice[6] and in domestic animals.[7] In this note we report experiments which establish structure 2 for the new tremorgenic agent



1



2

Crystals of paspalinine were obtained from methanol after repeated chromatography of the butyl acetate extract of the dried mycelium on silica gel using mixtures of benzene, toluene or hexane with 5-15% of ethyl acetate as solvents. The compound was found to possess the composition

$C_{27}H_{31}NO_4$ (MS: M^+ = 433) accounting for one oxygen atom more than in paspalicine (1). In contrast to the latter and in spite of the fact that it resists acetylation even under forcing conditions (Ac_2O/pyridine/4-dimethylaminopyridine) paspalinine contains a hydroxy group, as revealed by the IR-band at 3590 cm^{-1} next to the NH-absorption at 3480 cm^{-1} ($CHCl_3$). Since, in addition, it could be shown in biosynthetic experiments[8] that paspaline is a precursor in the formation of both paspalicine (1) and paspalinine (2), it was suspected that the latter represents the outcome of a simple hydroxylation of 1.

The close relationship between 1 and 2 is backed by comparison of their spectral properties. The UV spectrum of 2 with maxima at 232 (25,000), 250 (15,800 infl.) and 274 (8,000 infl.) nm matches very closely the ones reported for paspalicine[4] and paxilline[9] and thus betrays the presence of a 2,3-disubstituted indole chromophore and an α,β-unsaturated carbonyl group. As in 1, the latter is responsible for the appearance of characteristic IR-bands at 1669 and 1620 cm^{-1} (KBr). A study of the ^{13}C nmr spectrum of paspalinine (2) proved most revealing. In d_6-DMSO separate signals for all 27 carbon atoms are observed. The signals (in ppm from TMS) can be assigned as follows:[10] 196.6 (C-10); 169.5 (C-12), 152.7 (C-2),[12] 140.1 (C-24); 124.7 (C-19), 119.1, 118 4, 117.6 (C-20, C-21, C-22); 116.8 (C-11); 114.9 (C-18); 111.8 (C-23); 104.3 (C-7); 87.1 (C-9); 77.8 (C-27), 76.0 (C-13); 51.0 (C-3), 48.5 (C-16); 39.2 (C-4), 32.0 (C-14); 28.6 (C-28); 28.0 (C-6); 27.3 (C-17), 26.1 (C-5); 22.8 (C-29); 22.5 (C-26); 21.0 (C-15); 16.3 (C-25). Out of these 27 signals 26 correspond in multiplicity and approximate chemical shift to signals observed in the ^{13}C nmr of 1, their δ-values differing by less than ± 3 ppm, with the exception of the signal of C-14 which is shifted 8.7 ppm upfield in the spectrum of 1. The most striking feature of the spectrum is the absence of the doublet at 39.2 ppm which is characteristic for the 13-C-H group in 1 and its replacement by a new singlet at 76.0 ppm which is attributed to the 13-C-OH carbon in paspalinine.

Independent evidence for the location of the hydroxy group came from analysis of the 100 MHz ^1H nmr (d_5-pyridine, δ-values in ppm from TMS int. ref.). In addition to the expected features, (exchangeable protons at 4.98 (OH) and 11.35 (NH), 4 aromatic protons at 7.0-7.8, C-9-H at 4.65 (d, J=1 Hz), four methyls at 1.22, 1.40, 1.56 and 1.78 (each 3H, s) and 11 aliphatic protons at 1.9-3.5, the spectrum displays at δ 6.03 a doublet (J=1 Hz) for the C-11 proton. The corresponding signal in the spectrum of 1 has a similar position (δ 5.9) but a different multiplicity (dd; J_1=1, J_2=2). The smaller coupling constant has been shown[4] by double resonance experiments to arise from a long-range interaction with C-9-H and accordingly the larger coupling can be safely assigned to interaction with the allylic proton at C-13. Therefore, the absence of the second coupling in the spectrum of 2 defines the point of attachment of the hydroxy group in paspalinine as C-13. Thus, all of the available data are consistent with structural formula 2 for paspalinine

The validity of this formula has been checked in a single crystal x-ray diffraction experiment. Paspalinine is difficult to crystallize but slow evaporation of a dioxane solution resulted in small but usable crystals for x-ray diffraction purposes. The crystals were orthorhombic with a = 9 801(4),

$\underset{\sim}{b}$ = 10.555(3) and $\underset{\sim}{c}$ = 21.605(7) Å. Systematic extinctions and density measurements indicated one molecule of $C_{27}H_{31}NO_4$ per asymmetric unit in the orthorhombic space group $P2_12_12_1$. A set of intensity data with all unique reflections with $2\theta \leq 114.1°$ was collected using a 1°/min, ω-scan technique with graphite monochromated CuKα (1.54178Å) x-rays. Only 645 (43%) of the 1497 reflections surveyed were judged observed after correction for Lorentz, polarization and background effects Attempts to solve this structure using conventional direct methods procedures failed repeatedly. This is not surprising in view of the poor scattering ability of the crystals at higher 2θ values. The structure was finally solved by using structural information from paspalicine (1).[5] In view of the isostructural nature of paspalicine (1) and paspalinine (2) the phase angles of the 100 strongest normalized structure factors (E's) of paspalinine (2) were assumed to be those of paspalicine (1). These phase angles were expanded to 200 E's using the tangent formula and refined.[16] All nonhydrogen atoms of paspalinine were visible in the resulting E-synthesis.[16] Refinement by least-squares procedures was troublesome because of the limited data. The current model uses <u>all</u> x-ray intensities and has anisotropic nonhydrogen atoms and isotropic hydrogens. The current residual is 0.084 for this model (see reference 17 for additional crystallographic details). Figure 1 is a computer generated perspective drawing of paspalinine (2) less hydrogens.

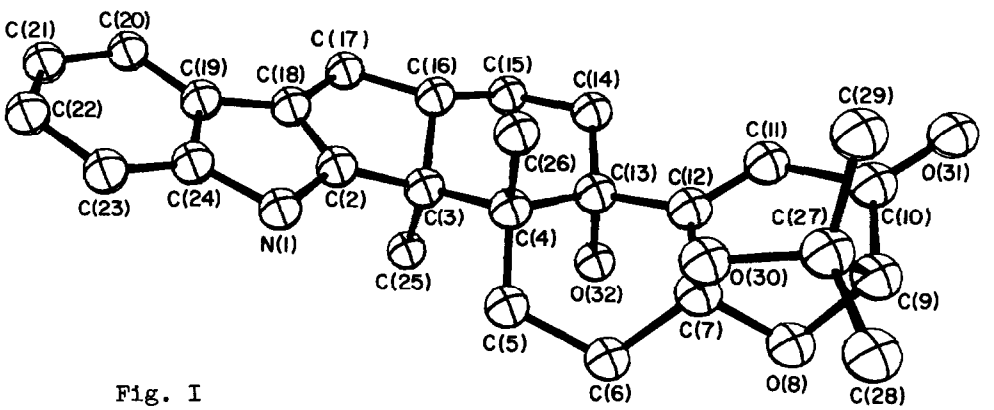


Fig. I

The absolute configuration of paspalinine indicated in 2 follows from the known[3] absolute configuration of paspaline and the observed[8] biosynthetic transformation paspaline → 2

Paspalinine bears a close relationship to both paspalicine[4,5] and paxilline.[9] Knowledge of its structure has proved of crucial importance in the elucidation of still more complex tremorgens.[18,19]

Acknowledgement

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17. Crystallographic coordinates have been deposited with the Cambridge Crystallographic Data Centre.
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19. Recently Dr. R. J. Cole, using the structure of paspalinine which we made available to him, reported the structure of other related indole-mevalonate metabolites. See ref. 6 above.

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