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THE STRUCTURE OF PAXILLINE, A TREMORGENIC METABOLITE OF *PENICILLIUM PAXILLI* BAINIER

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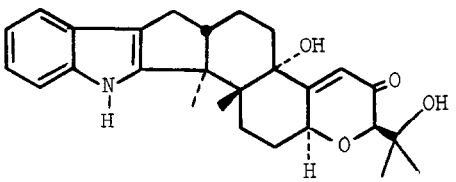
The fungus *Penicillium paxilli* Bainier produces a metabolite paxilline ($C_{27}H_{33}NO_4$) capable of inducing severe tremors in mice with an ED_{50} of 25 mg/kg. These tremors are sustained for several hours. In contrast to other reported tremorgens[2], paxilline is not especially toxic with an LD_{50} of 150 mg/kg in mice. Isolation procedures, IR, and UV data have been reported in a preliminary paper.[2] We wish to report the structure of paxilline (1).



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Paxilline (1) crystallizes as large clear cubes from acetone-heptane mixtures. Preliminary diffraction experiments showed that paxilline belongs to the common orthorhombic space group $P2_12_12_1$ with a=31.009(3), b=11.522(1), c=7.707 (1) Å. All unique data with $\theta \leq 57°$ was collected on a fully automated four-circle diffractometer using an ω-scan technique. A total of 2186 reflections were measured in this fashion. After correction for Lorentz, background, and polarization effects a total of 1840 reflections were judged observed.

Structure solution proceeded routinely by application of a multiple solution tangent formula approach.[3] When all thirty-two nonhydrogen atoms had been located

it became obvious that there was an acetone of crystallization tightly held to the indole NH. Inclusion of the solvent acetone and all thirty-three hydrogens of paxilline in full-matrix least-squares refinements lowered the conventional discrepancy index to 0.040 for the 1840 observed reflections.[4]

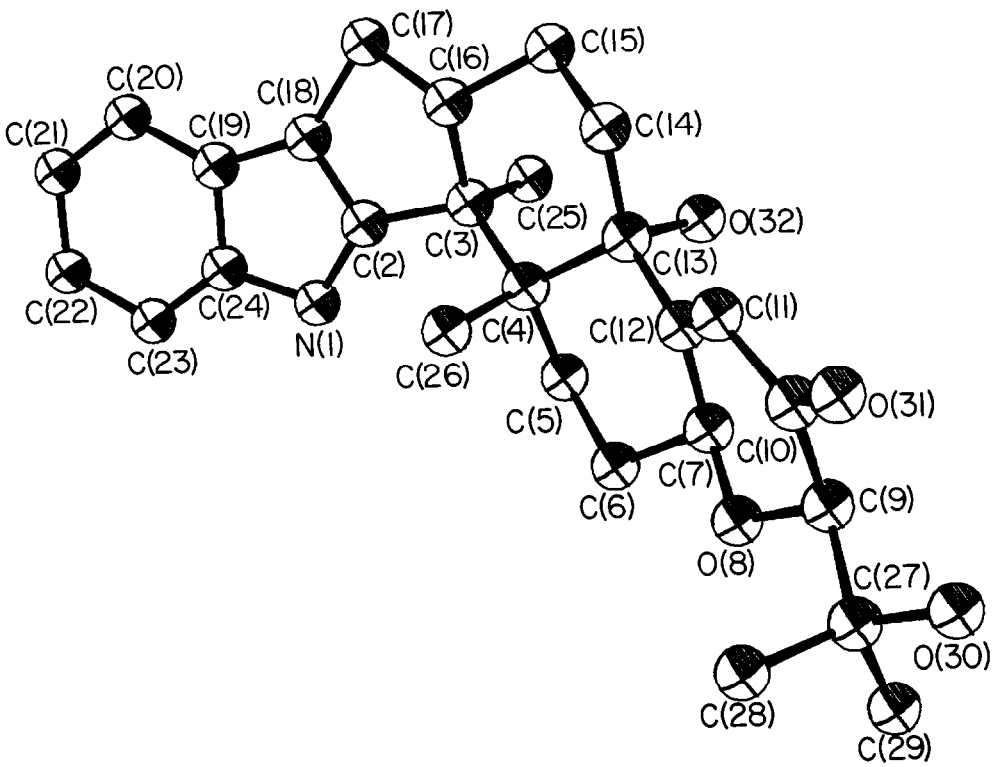
As can be seen paxilline (1) presents a non-linear array of six rings. An indole moiety is fused at the C(2) and C(18) positions to a cyclopentane ring which is itself fused in a trans fashion to a cyclohexane ring to form a linear tetracyclic-arrangement. An additional trans equatorial fused cyclohexane ring at C(4) and C(13) produces a bend in the molecule which is completed by a β-pyrone ring. Both cyclohexane rings are in the chair conformation. If there was a CH_3 at C(12) (vide infra) the structure could be formally derived from the union of a regular diterpene plus an indole. In general all bond distances agree well with generally accepted values and there are no abnormally short intermolecular contacts.[5] There is a linear hydrogen bond from the indole NH to the oxygen of the acetone of crystallization with a distance of 2.88 Å. The O(30)H forms an intramolecular hydrogen bond to O(30) with a 2.71 Å distance and an intermolecular hydrogen bond to O(32)H of 2.81 Å.

The 100MHz pmr spectrum of paxilline (1) was obtained using a Varian HA-100 spectrometer with acetone-d_6 as solvent and TMS as internal standard at δ0.00.[6] Assignments of the absorptions listed below are made with reference to Fig. 1. The spectrum shows absorptions at δ1.04, s, 3H, CH_3 (26); 1.22, s, 6H, CH_3 (28, 29); 1.40, s, 3H, CH_3 (25); 1.7-2.95, bm, CH, CH_2 (5, 6, 14, 15, 16, 17); 3.70, d, J=2 Hz, 1H, CH (9); 4.05, s, 1H, OH (32); 4.08, s, 1H, OH(30); 4.94, m, 1H, CH (7); 5.85, d, J=2 Hz, 1H, CH (11); 6.95, m, 2H, CH (21, 22); 7.30, m, 2H, CH (20, 23); 9.82, s, 1H, NH (1). Irradiation of the multiplet at δ4.94 collapsed the doublets at δ3.70 and δ5.85 to singlets. Irradiation at δ1.85 sharpened the multiplet at δ4.94 to a broad singlet. Addition of D_2O to the sample caused the disappearance of absorptions at δ4.05, 4.08 and 9.82. We assign the δ1.40 resonance to CH_3 (25) on the basis of the expected deshielding by the neighboring hydroxyl and proximity to the aromatic ring. Addition of $Eu(fod)_3$ caused a shift of the δ4.08 peak to lower field at the same time the isopropyl moiety was affected to approximately the same extent while the protons on the NH and the other hydroxyl were relatively unaffected.

The CD spectrum[6] of paxilline (1) showed positive Cotton effects for the first two bands (θ_{335nm} + 5.0 x 10^3; θ_{300nm} + 1.09 x 10^4); a third band at shorter wavelength appeared to give a negative Cotton effect but not all of this band was observed.

Two closely related compounds, paspaline (2) and paspalicine (3) have recently been isolated from the fungus Claviceps paspali and their gross structures elucidated by chemical means.[7] Paspaline (2) is particularly interesting because of the angular methyl at C(12) indicates the regular mevalonate origin of this new family of compounds. Studies on the biosynthesis of paxilline (1) are currently in progress.

Figure 1. A computer generated perspective drawing of paxilline (1). Hydrogens are not shown and no absolute stereochemistry is implied.



REFERENCES

1. Camille and Henry Dreyfus Teacher-Scholar Grant Awardee 1972-77 and Fellow of the Alfred P. Sloan Foundation 1973-75.

2. R.J. Cole, J.W. Kirksey and J.M. Wells, Can. J. Microbiol., 20, 1159 (1974).

3. G. Germain, P. Main and M.M. Woolfson, Acta Cryst., B24, 274 (1970).

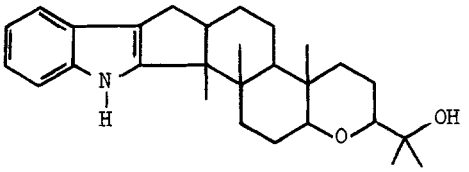
4. The following library of crystallographic programs was used: C.R. Hubbard, C.O. Quicksall, and R.A. Jacobson, "The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFT and FRIEDEL," USAEC Report IS-2625, Iowa State University-Institute for Atomic Research, Ames, Iowa, 1971; W.R. Busing, K.O. Martin, and H.A. Levy, "A Fortran Crystallographic Least Squares Program," USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965; C. Johnson, "ORTEP, A Fortran Thermal-Ellipsoid Plot Program," U.S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

5. International Tables for X-Ray Crystallography, Vol. III., Kynoch Press, Birmingham, England, 1952.

6. The authors wish to thank D. Lokensgard for obtaining the NMR data and C. Christianson for obtaining the CD spectrum.

7. Paspaline (2): G. Stamm, R. Gysi, A. Leutwiler, W. Acklin and D. Arigoni, manuscript submitted, cf. R. Gysi, Ph.D. Thesis Nr. 4990, ETH Zurich

Paspalicine (3): A. Leutwiler, W. Acklin and D. Arigoni, manuscript submitted, cf. A. Leutwiler, Ph.D. Thesis Nr. 5163, ETH Zurich.



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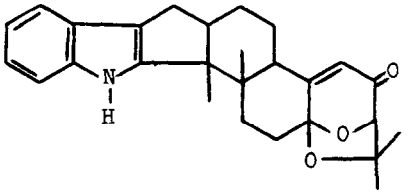

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The isolation and preliminary characterization of paspaline and paspalicine is described in a paper by Th. Fehr and W. Acklin, Heh. Chim. Acta, 49, 1907 (1966).