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ASPERSITIN-A NEW METABOLITE OF ASPERGILLUS PARASITICUS

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Summary: The structure of aspersitin, a new metabolite of Aspergillus parasiticus was determined by X-ray analysis, and by spectroscopic measurements.

In the course of a search for aflatoxins and biogenetically related fungal metabolites we had an opportunity to investigate Aspergillus parasiticus NRRL 3260.

The fungus was grown in a medium containing (per liter) glucose (autoclaved separately) (50 g), L-asparagine (10 g), $(NH_4)_2SO_4$ (3.5 g), KH_2PO_4 (10 g), $MgSO_4 \cdot 7H_2O$ (2 g), $CaCl_2 \cdot 2H_2O$ (75 mg), $ZnSO_4 \cdot 7H_2O$ (10 mg), $Na_2B_4O_7$ (5 mg) and $FeSO_4 \cdot 7H_2O$ (2 mg) at pH 4.5. Portions of the medium (500 ml each), in Fernbach flasks, were incubated without agitation at 28°C for 14 days. After killing cells and spores by boiling for 10 min, the suspensions were homogenized in a Waring blender, and extracted with chloroform. Preparative thin layer chromatography, (R_f 0.13 (15% acetone, 85% pentane)), gave a new metabolite $C_{14}H_{21}NO_4$, mp 58-60°, $[\alpha]D$ + 285° (CH_3OH) which we have named aspersitin (1).[2]

Esterification of 1 with p-bromobenzoyl chloride in pyridine gave the p-bromobenzoate (2), mp 215-217°. The IR spectrum demonstrated the presence of an ester, and the ^1H NMR spectrum[3] clearly excluded a rearrangement in the conversion of 1 to 2.

Crystals of the p-bromobenzoate were orthohombic, space group P2 2 2, with a = 9.565(2), b = 14.561(3), c = 15.688(3) A, and d_{calcd} = 1.369 g cm^{-3} for Z=4 ($C_{21}H_{24}BrNO_5$, M = 450.33). The intensity data were measured on a Hilger-Watts diffractometer (Ni filtered Cu K α radiation, θ - 2θ scans, pulse height discrimination). A crystal measuring approximately 0.10 x 0.10 x 0.75 mm was used for data collection; the data were corrected for absorption (μ = 30.9 cm^{-1}). A total of 1669 reflections were measured for θ <57°, of which 1475 were considered to be observed [I> 2.5σ (I)].

The structure was solved by the heavy-atom method and was refined by full-matrix least squares. Following preliminary anisotropic refinement of all nonhydrogen atoms, atoms C(10), C(11), and C(12) of the isobutyl group were found to be highly anisotropic. The anisotropy was interpreted as indicating that the isobutyl group was disordered. The three carbon atoms C(10), C(11), and C(12) were each split into two atoms of half weight, yielding two isobutyl groups oriented about 35° apart about the C(9)-C(8) bond. In the final refinement all nonhydrogen atoms were refined anisotropically except for the six half-weight carbon atoms which were refined isotropically. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R=0.049 and wR=0.055 for the 1475 observed reflections. The final difference map has no peaks greater than ± 0.5 e A^{-3}.

The absolute configuration is based on the anomalous scattering of the bromine atom and was established by refining both enantiomers. The final weighted R values were 0.0549 in the structure shown and 0.0631 for its antipode. Thus the former corresponds to the correct absolute configuration.

Aspersitin (1) is structurally related to humulone (3) and other 2-acylcyclohexane-1,3-diones[4] of plant origin.

Aspersitin (1) exhibits antibacterial activity against Staphylococcus aureus ATCC25923, S. epidermidis MIT-B-58, Bacillus subtilis ATCC6051, B. megaterium UNH, Proteus vulgaris ATCC6380, and Pseudomonas aeruginosa ATCC9721.

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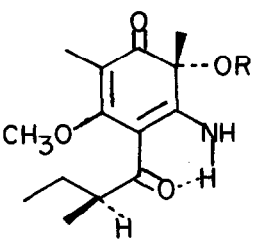
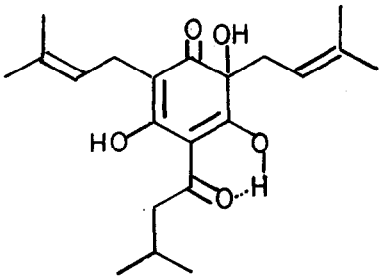
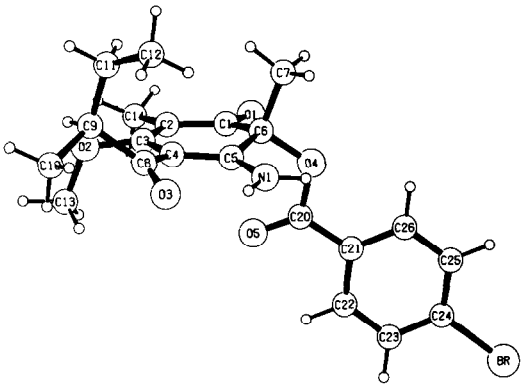


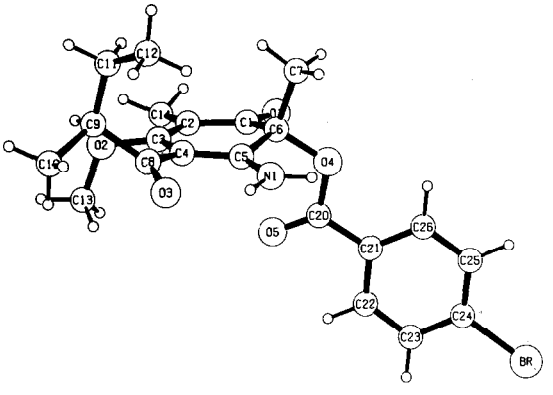


1 R = H.

2 R = OCO-p-BrC6H4.

3





A stereodrawing of aspersitin p-bromobenzoate (2).

References and Notes

1. Fellow of the Deutsche Forschungsgemeinschaft.
2. Aspersitin exhibited the following spectroscopic properties: UV max (C_2H_5OH) (ε) 399 (4700), 316 (11,700), 239 (8500), 214 (sh) (11,300) nm; IR (CCl_4) 3450, 1650, 1610, 1550 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 10.0 (br s, 1, D_2O exchangeable), 6.65 (br s, 1, D_2O exchangeable), 4.11 (s, 1, D_2O exchangeable), 3.79 (s, 3), 3.22 (sext, 1, J = 6.8 Hz), 1.90 (s, 3), 1.72 (d, quint, 1, J = 13.2 Hz, 6.6 Hz), 1.57 (s, 3), 1.38 (d, quint, 1, J = 13.2 Hz, 6.6 Hz), 1.06 (d, 3, J = 7.2 Hz), 0.89 (t, 3, J = 7.5 Hz); ^{13}C NMR (62.5 MHz, C_6D_6) δ 204.8 (s), 199.6 (s), 172.7 (s), 171.3 (s), 106.7 (s), 99.3 (s), 76.4 (s), 60.0 (q, J = 71 Hz), 45.4 (d, J = 56 Hz), 34.3 (q, J = 70 Hz), 27.3 (t, J = 67 Hz), 17.3 (q, J = 70 Hz), 11.9 (q, J = 70 Hz), 9.0 (q, J = 65 Hz); mass spectrum m/e (rel intensity) 267 (M^+, 16), 225 (12), 224 (81), 193 (10), 182 (14), 168 (11), 165 (13), 152 (16), 151 (11), 140 (36), 139 (11), 85 (12), 83 (21), 80 (14), 77 (14), 69 (11), 68 (17), 67 (12), 57 (74), 55 (24), 54 (10), 53 (23), 43 (100), 42 (21), 41 (72); high resolution: calc. for $C_{14}H_{21}NO_4$: 267.14705, found: 267.14764.
3. ^1H NMR absorptions (250 MHz, $CDCl_3$) at δ 0.88 (t, J = 7.4 Hz 3), 1.12 (d, J = 6.6 Hz. 3), 1.40 (m, J = 6.6 Hz, 1), 1.70 (m, J = 6.6 Hz, 1), 1.72 (s, 3), 1.90 (s, 3), 3.29 (m, J = 6.6 Hz, 1), 3.79 (s, 3), 7.60 (d, J = 8.8 Hz, 2), 7.94 (d, J = 8.8 Hz 2).
4. Stevens, R. Chem. Revs. 1967, 67, 19. Hassall, C. H. Progress in Org. Chem. 1958, 4, 115. Ashurst, P. R. Progress in the Chem. of Org. Nat. Prod. 1967, 25, 63.

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