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ASPERTOXIN, A HYDROXY DERIVATIVE OF O-METHYLSTERIGMATOCYSTIN FROM AFLATOXIN-PRODUCING CULTURES OF *ASPERGILLUS FLAVUS*

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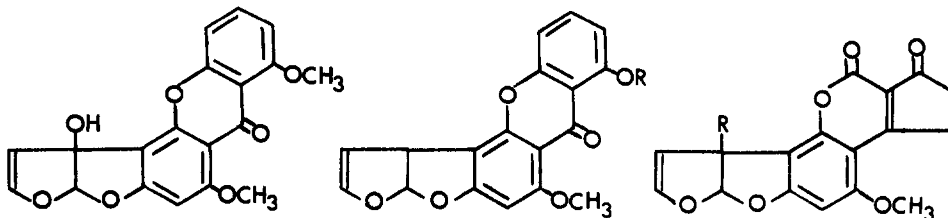
A preliminary report from our laboratories[1] described the isolation and biological activity of a new toxic metabolite from an aflatoxin-producing culture of *Aspergillus flavus*, and the name "aspertoxin" was tentatively assigned to the compound. On the basis of further investigations of this substance, we propose that aspertoxin is a hydroxy derivative of another metabolite of *A. flavus*, O-methylsterigmatocystin (II, R=CH_3),[2] and is represented by formula I. Aspertoxin also bears a close resemblance to aflatoxin M_1 (III, R=OH),[3,4] which is one of a



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number of aflatoxins produced by the mold *A. flavus*. The obvious structural resemblance of aflatoxin B_1 (III, R=H)[5] to a metabolite of the mold *Aspergillus versicolor*, sterigmatocystin (II, R=H),[6] and the fact that the aflatoxins and sterigmatocystin are derived from the same genus, led to the early postulation that sterigmatocystin, or a sterigmatocystin precursor, may be a biosynthetic precursor of the aflatoxins.[7] The very recent discovery that a simple sterigmatocystin derivative, O-methylsterigmatocystin (II, R=CH_3),[2] is elaborated by an

aflatoxin-producing strain of A. flavus, and the present report that aspertoxin is the second sterigmatocystin derivative to be isolated from A. flavus cultures, furnish support for the above hypothesis.

Aspertoxin is a colorless, crystalline material which undergoes decomposition, without melting, in the range 240-280° and affords a complex mixture (TLC) of highly colored products. The molecular formula of aspertoxin was established by high resolution mass spectrometry; the compound exhibited a molecular ion peak at m/e= 354.074200, and the mass calculated for $C_{19}H_{14}O_7$ is 354.073949. Elemental analysis indicates that aspertoxin crystallizes as the hydrate (Calcd. for $C_{19}H_{14}O_7 \cdot H_2O$: C, 61.29; H, 4.33; Found: C, 61.20; H, 4.45). Treatment of aspertoxin with acetic anhydride in pyridine at room temperature yielded aspertoxin acetate (IV), $C_{21}H_{16}O_8$, m.p. 119-121°. The mass spectrum of IV showed the molecular ion peak at m/e= 396.

The NMR spectrum of aspertoxin acetate (IV) is presented as Figure 1. The signal due to

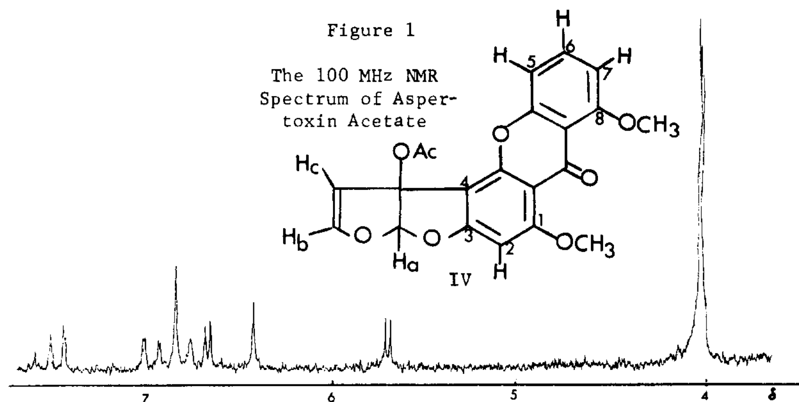

Figure 1

The 100 MHz NMR Spectrum of Aspertoxin Acetate

the acetyl protons is not shown in Figure 1, and appears as a singlet at δ2.17(3H). Signals due to the methoxyl hydrogens are prominent at δ4.07 and δ4.10(6H). The argument for the attachment of the four carbon side chain at C_4 of the xanthone ring rather than at C_2 is based on the chemical shift observed for the isolated aromatic proton at C_2 (see IV, above). This proton gives a singlet at δ6.41(1H), and the position of this signal agrees closely with the chemical shifts observed for the corresponding aromatic protons in O-methylsterigmatocystin (II, R=CH_3, δ6.38)[2] and sterigmatocystin (II, R=H, δ6.45).[6]

Signals for the three protons in aspertoxin acetate (IV) at δ5.66 (doublet, J ≈ 2 Hz), δ6.64 (doublet, J ≈ 2 Hz), and δ6.83 (singlet) may be attributed to protons H_c, H_b and H_a,

respectively. The coupling between protons H_b and H_c was demonstrated by a double irradiation experiment. The position and coupling of the two vinyl protons (H_b and H_c), and the position of the acetal proton (H_a) signal are similar to those observed for the corresponding protons in aflatoxin M_1 (III, R=OH).[4] The two vinyl protons in aflatoxin M_1 appear as doublets ($J \approx 2$ Hz) at $\approx \delta 5.8$ and $\approx \delta 6.9$. The single acetal proton in aflatoxin M_1 gives a singlet at $\approx \delta 6.6$.

The remaining three aromatic protons H_5, H_6 and H_7 give rise to an ABC spectrum which can be analyzed roughly by the ABX approximation. The X portion consists of a triplet* at $\delta 7.52$ with a spacing of about 8 Hz. The AB portion cannot be seen in its entirety, since the acetal proton (H_a) peak is superimposed on it. The discernible absorptions are three doublets with a spacing of about 1 Hz, whereas the fourth such doublet seems to be merged with the acetal proton peak. With this assumption, the analysis for the ABX pattern yields the following approximate parameters: $J_{AB} = 1$ Hz; $J_{AX} = J_{BX} = 8$ Hz; $\nu_A = 698$Hz and $\nu_B = 678$Hz (measured downfield from TMS). The appearance of the X portion as a triplet is typical for $J_{AX} = J_{BX}$. That $\nu_A - \nu_B \neq 0$ was borne out further by the results of strong irradiation at the frequency of the center of the $\delta 7.52$ triplet: the AB portion changed into two signals at $\delta 6.98$ and $\delta 6.78$, their position corresponding to ν_A and ν_B as found above. The assignment of A, B and X to H_5, H_6 and H_7 is then as follows: (A,B) = (H_5,H_7), and X = H_6. The values for $J_{AX} = J_{BX}$ (8 Hz) and J_{AB} (1 Hz) fall in the ranges for J_{ortho} and J_{meta} in benzenoid systems. It should be mentioned also that the present ABX pattern bears a striking resemblance to that of the corresponding protons in O-methylsterigmatocystin (II, R=CH_3)[2] which consists of a triplet at $\delta 7.48$ (1 H) with spacings of 8.1 Hz and a complex band centered at $\delta 6.82$ (2H).

The U.V. spectrum of aspertoxin has λ_{max}^{MeOH} at 310 mμ and 241 mμ (log ϵ 4.08 and 4.53, respectively). The U.V. spectrum of O-methylsterigmatocystin has λ_{max}^{MeOH} at 310 mμ and 236 mμ (log ϵ 4.224 and 4.614, respectively).[2] The U.V. spectrum of aspertoxin is unaffected by the addition of acid or base.

The I.R. spectrum of aspertoxin (KBr disc) shows strong absorptions at 3354 cm^{-1}(OH), 1656 (γ-pyrone carbonyl), 1630, 1587, 1575, (phenyl), 1460, 1269, 1133 (C-O-C), 894, and 811 cm^{-1} (isolated aromatic H). The I.R. spectrum of aspertoxin acetate (in $CHBr_3$) is compared with the reported I.R. spectrum of O-methylsterigmatocystin (in $CHBr_3$)[8] in Table I.

* The tall peak at $\delta 7.45$ next to the highest-field component of the triplet is due to $CHCl_3$.

The shapes and relative intensities of the major peaks in the spectrum of O-methylsterigmatocystin[2] are remarkably similar to those of aspertoxin and aspertoxin acetate.

TABLE I

COMPARISON OF I.R. SPECTRA OF O-METHYLSTERIGMATOCYSTIN[8] AND ASPERTOXIN ACETATE. ν_{MAX} IN CM^{-1} (S = STRONG, M = MEDIUM, W = WEAK).

ASPERTOXIN ACETATE (IV)			O-METHYLSTERIGMATOCYSTIN (II, R=CH_3)		
1739 s	1414 m	1083 m	---	1418 m	1075 m
1655 s	1380 w	---	1662 s	1382 w	1040 w
1640 s	1341 m	1011 m	1643 s	1347 m	1016 m
1617 (sh)	1262 s	965 m	*	1267 s	970 m
1592 s	---	890 w	1603 s	1250 m	890 w
1469 s	1230 s	850 w	1473 s	1229 m	840 w
1430 m	---	811 m	1438 m	1204 m	811 m

*Ref. 9 does not list an absorption near 1617 cm^{-1}; however, Ref. 2, which presents a photograph of the I. R. spectrum of O-methylsterigmatocystin, shows that the compound does indeed have a shoulder absorption near 1620 cm^{-1}.

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