Biosynthesis of Aflatoxins. Incorporation of [4′-$^2H_2$]Averufin into Aflatoxin B_1 by *Aspergillus flavus*

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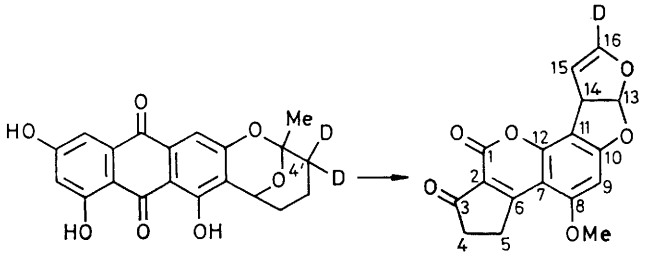
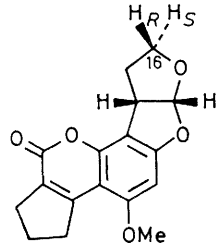
The regiospecific incorporation of ^2H from [4′-$^2H_2$]averufin into aflatoxin B_1 by cultures of *Aspergillus flavus*, as demonstrated by ^2H n.m.r. spectroscopy, confirms the intermediacy of averufin in the biosynthesis of aflatoxin B_1.

The biosynthesis of the aflatoxins, potent carcinogenic mycotoxins produced by the common fungi *Aspergillus flavus* and *A. parasiticus*, has been a subject of intensive and continuing study[1,2] since their isolation. Averufin (**1**), a metabolite of decaketide origin,[3] also produced by *A. parasiticus*, appears to be the first isolable intermediate on the aflatoxin biosynthetic pathway and several experiments have been described which indicate that averufin is incorporated into aflatoxin B_1 (**2**). However, all these experiments have used averufin labelled biosynthetically from ^{13}C or ^{14}C labelled acetate and so, despite the sometimes impressive incorporations reported, they are subject to the criticism that degradation of the side-chain could produce labelled acetate which on reincorporation would give identically labelled aflatoxin B_1. As the role of averufin as a key intermediate on the aflatoxin pathway is crucial to all current biosynthetic proposals we have prepared a *specifically* labelled averufin and examined its incorporation into aflatoxin B_1.

Averufin was subjected to an acid catalysed exchange reaction by stirring at room temperature for 6 days in a mixture prepared by addition of ^2H_2O to acetyl chloride in dimethyl sulphoxide.[4] This resulted in complete and specific exchange of the 4′-hydrogens only, as shown by *inter alia* the disappearance of the C-4′ resonance from the proton noise decoupled ^{13}C n.m.r. spectrum of (**1**) and ^2H n.m.r. spectroscopy. [4′-$^2H_2$]Averufin in acetone solution was fed to shaken cultures of *A. flavus* on low salts medium and the aflatoxin B_1 produced was analysed by ^2H n.m.r. spectroscopy. The resultant spectrum, Figure 1(a), showed a strong signal at δ_H 6.45 corresponding to the anticipated signal for H-16. The signal at



(**1**) (**2**)



(**3**)

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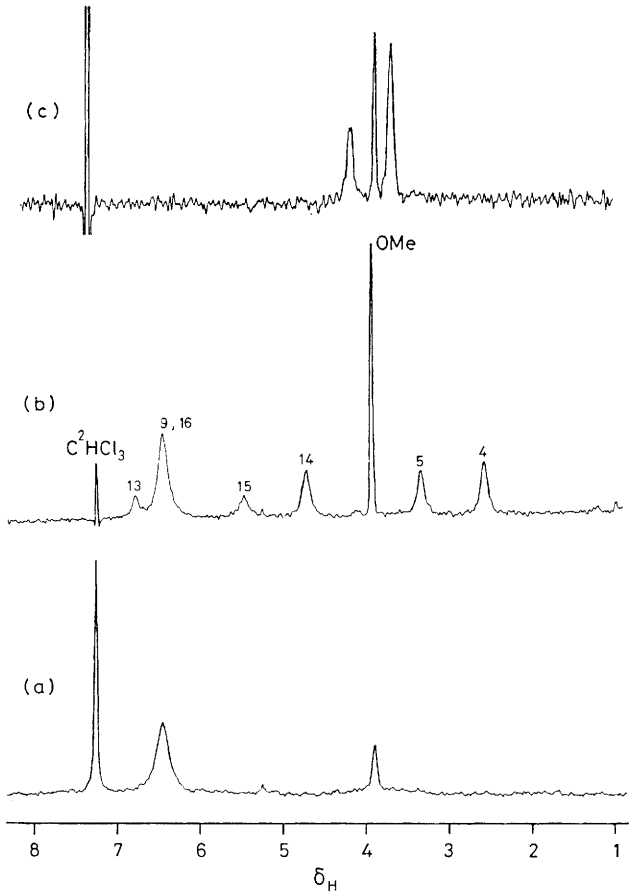

Figure 1. ^2H N.m.r. spectra of (a) [4′-$^2H_2$]averufin enriched aflatoxin B_1 (**2**), (b) [*U*-^2H]aflatoxin B_1, and (c) [4′-$^2H_2$]averufin enriched 3-deoxy-aflatoxin B_2 (**3**). All spectra were determined for $CHCl_3$ solutions at 55.28 MHz on a Bruker WH-360 spectrometer, with resolution enhancement.

δ_H 3.89 is due to natural abundance deuterium in the 8-methoxy-group.

For comparison the ^2H spectrum of uniformly labelled aflatoxin B_1 is shown in Figure 1(b). This sample is produced by the simple expedient of growing *A. flavus* on a medium supplemented with 10% ^2H_2O. As the spectrum shows, the signals due to H-16 and H-9 are not resolved and so to ensure that labelling from [4′-$^2H_2$]averufin has occurred at H-16 only the labelled aflatoxin B_1 was converted into 3-deoxy-aflatoxin B_2 (**3**) by catalytic hydrogenation[5] and its ^2H n.m.r. spectrum determined. This, Figure 1(c), shows that the signal at 6.45 p.p.m. has disappeared to be replaced by 2 signals at 3.59 and 4.07 p.p.m. due to the 16-*pro-S* and 16-*pro-R* hydrogens, respectively.[6] This indicates that reduction of aflatoxin B_1 is not completely stereospecific but the greater intensity of the

signal at 3.59 p.p.m. means that reduction from the less hindered β face *i.e.* the 15-*re*, 16-*re* face, predominates.

In conclusion these results prove that averufin is indeed incorporated intact into aflatoxin B_1 and so averufin is now firmly established as an obligate intermediate on the aflatoxin biosynthetic pathway.

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