Biosynthesis of Aflatoxins. Incorporation of [2-$^2H_3$]Acetate into Aflatoxin B_1 by *Aspergillus flavus*

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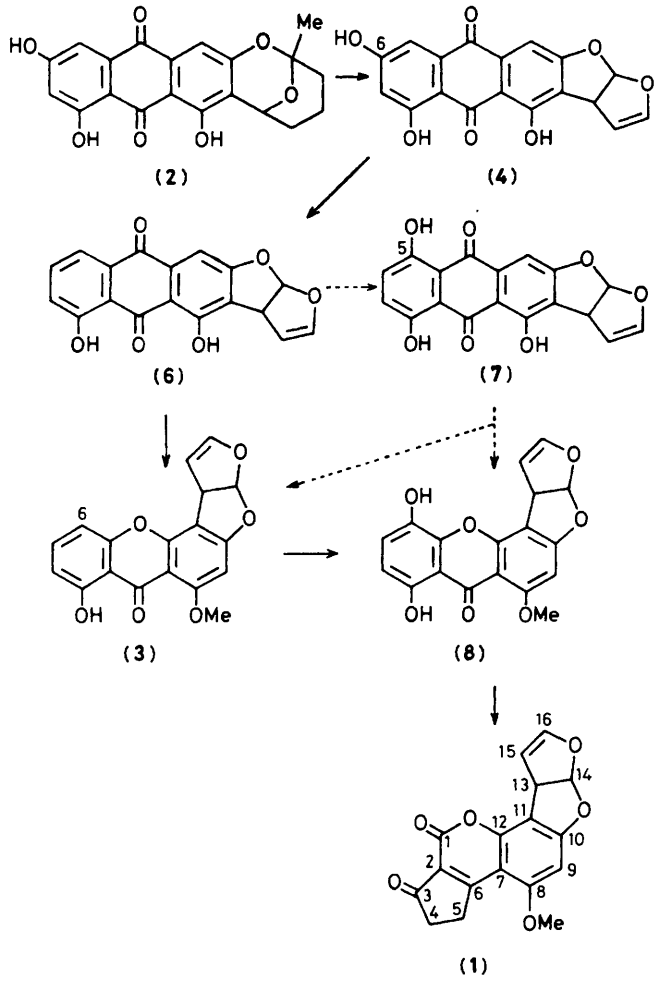
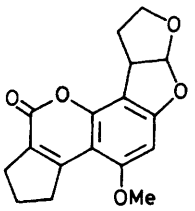
The observation of an NIH shift on incorporation of [$^2H_3$]acetate into aflatoxin B_1 in cultures of *Aspergillus flavus* provides evidence for the timing of deoxygenation and hydroxylation steps which are an essential part of the biosynthetic pathway.

Despite extensive studies the exact sequence of intermediates on the biosynthetic pathway leading to aflatoxin B_1 (**1**), the carcinogenic mycotoxin produced by the moulds *Aspergillus flavus* and *Aspergillus parasiticus*, is uncertain.[1] Even less is



Scheme 1



known of the detailed mechanisms responsible for the interconversion of the known intermediates. Accordingly we set out to elucidate the fate of acetate-derived hydrogen on incorporation into aflatoxin B_1 and its precursors, with a view to obtaining mechanistic information. We have recently reported ^2H labelling studies on averufin (**2**)[2] and sterigmatocystin (**3**)[3] which along with versicolorin A (**4**) are believed to be key intermediates on the biosynthetic pathway.[1] We now

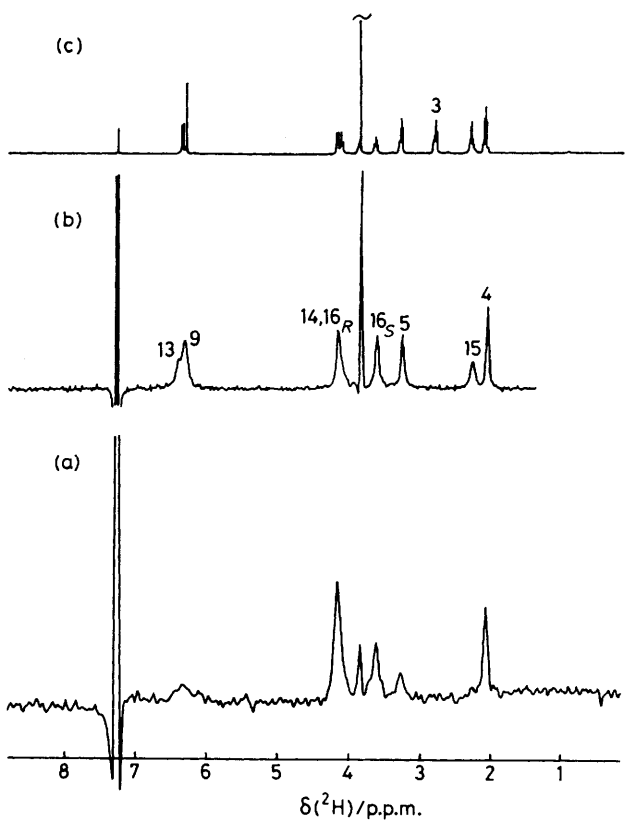


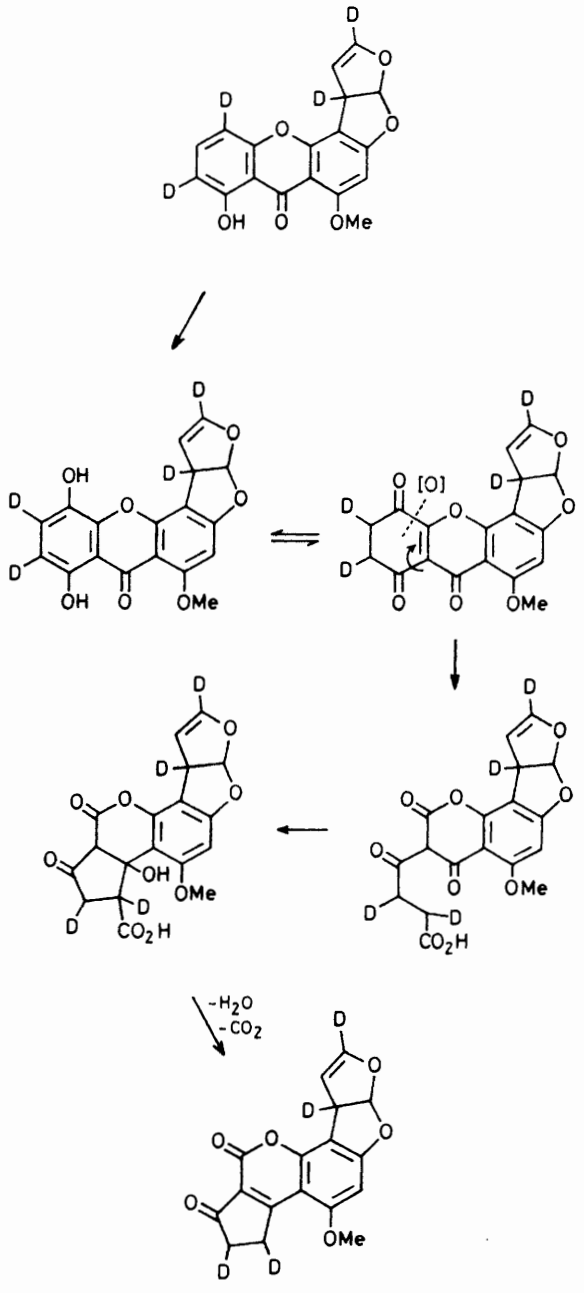
Figure 1. 55.28 MHz ^2H n.m.r. spectra of 3-deoxy-aflatoxin B_2 (**5**) derived from (a) [2-$^2H_3$]acetate-enriched aflatoxin B_1 and (b) from universally ^2H-enriched aflatoxin B_1. (c) shows the 360.13 MHz ^1H n.m.r. spectrum of (**5**).

report the results of extension of these studies to aflatoxin B_1 itself.

[2-$^2H_3$]Acetate was added to shaken cultures of *A. flavus* on low-salts medium and the aflatoxin B_1 (**1**) produced examined by ^2H n.m.r. spectroscopy. The resulting spectrum showed signals at δ_H 6.45, 4.72, 3.35, and 2.58 p.p.m. indicating that acetate-derived hydrogen is incorporated on carbons C-9 and/or C-16, C-14, C-5, and C-4. As the signals due to H-16 and H-9 are not resolved in aflatoxin B_1 itself, the labelled sample was converted by hydrogenation[4] into 3-deoxy-aflatoxin B_2 (**5**). The ^2H n.m.r. spectrum of (**5**) [Figure 1(a)] confirmed the above observations and indicated that most of the label was on C-16 with possibly a trace of ^2H incorporation at C-9. The 360.13 MHz ^1H n.m.r. spectrum of (**5**) [Figure 1(c)] and the 55.28 MHz ^2H n.m.r. spectrum of (**5**) derived from universally labelled[5] aflatoxin B_1 [Figure 1(b)] are shown for comparison.†

The crucial observation is the presence of acetate-derived hydrogen on C-5. The position is derived from the carboxy-carbon of acetate[6] and so it must be assumed that the ^2H label has migrated to C-5 from the adjacent carbon during the course of biosynthesis. The most likely mechanism would be

† Note that all the signals of interest are resolved. There is no signal for the C-3 methylene hydrogens in Figure 1(b) as these are introduced in the hydrogenation step.



Scheme 2

as a result of an NIH shift‡ indicating that hydroxylation of the carbon adjacent to that which becomes C-5 is an essential step in the cleavage and conversion of the aromatic ring which becomes the cyclopentenone moiety in aflatoxin B_1.

One of the outstanding problems concerned with the aflatoxin biosynthetic pathway is the timing of the required loss of the 6-hydroxy-function from versicolorin A (**4**). One of the more plausible proposals was that versicolorin A was converted first into 6-deoxyversicolorin A (**6**) which was then hydroxylated to give (**7**), both of which are known metabolites (Scheme 1). However we have recently shown that (**7**) cannot be an intermediate in the formation of sterigmatocystin (**3**) as ^2H is retained at C-6.[3] The present results indicate that an intermediate which has already lost the 6-hydroxy-function present in versicolorin A (**4**) is hydroxylated, otherwise no NIH shift could be observed. Thus the most probable sequence of events is that versicolorin A (**4**) is converted *via* (**6**), (**3**), and (**8**) into aflatoxin B_1. A less likely sequence, (**4**) → (**6**) → (**7**) → (**8**) → (**1**), would mean that sterigmatocystin is *not* an obligatory intermediate and cannot yet be entirely ruled out. Cleavage of the aromatic ring and formation of the cyclopentanone ring can occur by precedented procedures as outlined in Scheme 2. Further studies to delineate the exact sequence of events between versicolorin A and aflatoxin B_1 are in progress.

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‡ We have observed a similar NIH shift occurring during polyketide biosynthesis in studies on *O*-methyl-asparvenone,[7] a metabolite of *Aspergillus parvulus*.