# Conversion of Averufin derived from [1,2-$^{13}$C]Acetate into Aflatoxin $B_1$

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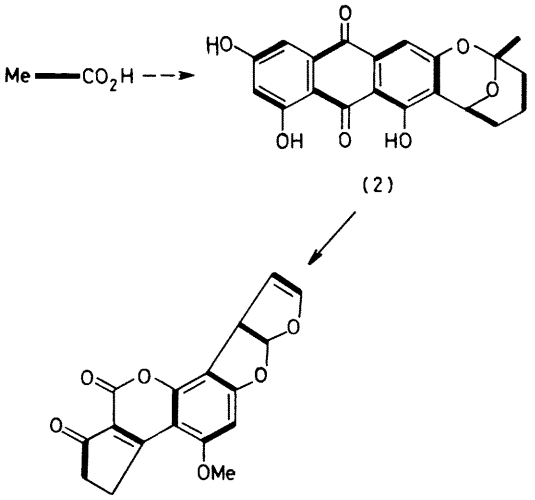
*Summary* The incorporation of label from averufin derived from [1,2-$^{13}$C]- and [1-$^{14}$C]-acetate supports the intermediacy of averufin in the biosynthesis of aflatoxin $B_1$.

BIOSYNTHETIC studies have shown that aflatoxin $B_1$ (**1**) is produced *via* the acetate–polymalonate biosynthetic pathway by *Aspergillus flavus* and *Aspergillus parasiticus*.[1,2]

The sequence of biochemical events leading to the aflatoxins was elucidated by using metabolic inhibitors and blocked mutants of *A. parasiticus*.[3] Averufin (**2**) is recognized as a pivotal intermediate in aflatoxin biosynthesis owing to its accumulation in the mutant, *A. parasiticus* ATCC 24551,[4] and its efficient conversion into aflatoxin $B_1$ by wild-type cultures *e.g.*, *A. parasiticus* ATCC 15517.[5] Its intermediary role in the biosynthetic pathway leading to aflatoxin $B_1$ was recently supported by its ready conversion into sterigmatocystin, a well-established precursor of aflatoxin $B_1$.[6] This study on the biosynthesis of the aflatoxins was undertaken to provide further evidence that averufin (**2**) is incorporated intact into aflatoxin $B_1$ (**1**). Previous studies have been questioned since enzymatic degradation of the side-chain of averufin could give rise to labelled acetate which, on incorporation, would give aflatoxin $B_1$ with the observed activity and labelling pattern.

Averufin (**1**), derived from [1,2-$^{13}$C]acetate,[7] (21·8 mg) was admixed with [$^{14}$C]averufin, derived from [1-$^{14}$C]acetate, (1·043 $\mu$Ci) to give averufin with a specific activity of 17·23 $\mu$Ci mmol$^{-1}$. This $^{13}$C/$^{14}$C-labelled averufin in methanol was introduced into aflatoxin-producing cells of *A. parasiticus* ATCC 15517, which had been resuspended in the resting cell medium of Hsieh and Mateles.[8] The incubation was performed at 30 °C and the culture was shaken at 200 r.p.m. for 20 h. The formed aflatoxin $B_1$ (0·193 $\mu$Ci, 17·7 mg), purified by thin layer and high pressure liquid chromatography, had a specific activity of 3·40 $\mu$Ci mmol$^{-1}$. The efficient conversion of averufin into aflatoxin $B_1$ (absolute incorporation 18·5%) and the low dilution value (5·1) observed for the process indicates that the precursor is incorporated intact. This dilution value was corroborated by the decrease (80%) in the ratio of the satellite peaks compared to the natural abundance peaks in the proton noise decoupled (p.n.d.) $^{13}$C n.m.r. spectra of the labelled averufin and the derived aflatoxin $B_1$. The p.n.d. $^{13}$C n.m.r. spectrum of the aflatoxin $B_1$ showed the same labelling pattern and directly bonded coupling constants, $^1J$(CC), as that of [1,2-$^{13}$C] acetate-derived aflatoxin $B_1$.[2]




(2)

(1)

In a subsequent experiment, [$^{14}$C]averufin (3·36 mg; 10·88 $\mu$Ci mmol$^{-1}$) in acetone was added to a replacement medium,[9] which contained no carbon source (500 ml in ten 250 ml flasks) over a period of 8 h. The incubation was performed at 25 °C and the culture was shaken at 160 r.p.m. for 22 h. The averufin (1·8 mg) and formed aflatoxin $B_1$ (0·33 mg; 6·04 $\mu$Ci mmol$^{-1}$) were isolated. A lower dilution value (1·8) was obtained while the absolute incorporation of averufin into aflatoxin $B_1$ was similar (14%) (corrected for recovered precursor).

These data, and the more efficient incorporation of averufin (18·5%) into aflatoxin $B_1$ than acetate (1·0%), support the central role of averufin in aflatoxin biosynthesis. The established labelling pattern in the following metabolites support the sequence: acetate-polymalonate → averufin[7] → versiconal acetate[10] → versicolorin A[11] → sterigmatocystin[2] → aflatoxin $B_1$.[2]

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