Biosynthesis of Aflatoxin B_1 from [2-^{13}C]- and [1,2-^{13}C]-Acetate

By PIETER S. STEYN,* ROBERT VLEGGAAR, and PHILIPPUS L. WESSELS

(*National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box* 395, *Pretoria*, 0001, *Republic of South Africa*)

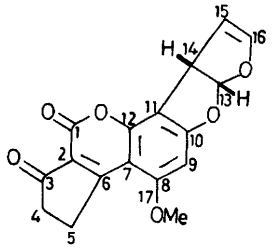
and DE BUYS SCOTT

(*Faculty of Agriculture, University of Pretoria, Pretoria*, 0002)

Summary The biosynthesis of aflatoxin B_1 from acetate, *via* two oxidative fissions of a preformed aromatic precursor, is established by the ^{13}C n.m.r. spectra of derivatives, enriched with [2-^{13}C]- and [1,2-^{13}C]-acetate.

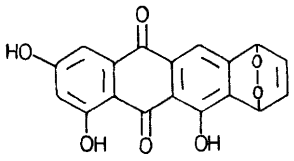
WE report results obtained on the biosynthesis of aflatoxin B_1 (**1**) using singly and doubly labelled ^{13}C-sodium acetate. The potent hepatocarcinogen aflatoxin B_1 is produced by cultures of *Aspergillus flavus* and *Aspergillus parasiticus*. An important structural and biosynthetic feature is the bisdihydrofuran unit which is also present in several related nonaketide derived fungal metabolites.[1] Extensive ^{14}C-labelling and degradation studies indicated that the aflatoxin molecule was totally derived from acetate units and that methionine contributed the methoxyl methyl group.[2] Biollaz *et al.*[2] postulated the currently accepted biogenesis by which a single polyacetate chain gave rise to the C_{18}-polyhydroxynaphthacene-endoperoxide (**2**) which

rearranged through a pyran intermediate to the bisdihydrofuran unit as in versicolorin A (**3**). The oxidative loss of two separate carboxyl acetate derived carbon atoms could then lead to aflatoxin B_1. Heathcote *et al.*[3] preferred an alternative route where a C_4 unit was linked to a preformed anthraquinone molecule; this hypothesis was supported by similar findings of Holker and Mulheirn[4] on the related sterigmatocystin which was converted into aflatoxin B_1 by cultures of *A.parasiticus*.[5]

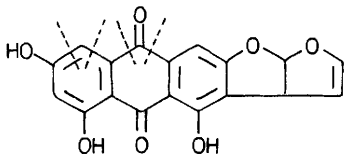


(1)

^{13}C n.m.r. spectroscopy was used to study this ambiguity. A culture of *A. flavus*, strain NRRL 3251 was grown by the replacement technique[6] and supplemented with either



(2)



(3)

sodium [2-^{13}C]-acetate (90%) or sodium [1,2-^{13}C]-acetate (90%). Aflatoxin B_1 was enriched approximately eightfold above the natural ^{13}C abundance, as established by mass spectroscopy.

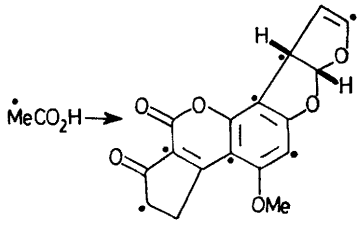
TABLE

^{13}C-Chemical shifts of aflatoxin B_1 and coupling constants ($^1J_{^{13}C-^{13}C}$/Hz) of [1,2-^{13}C]-acetate enriched aflatoxin B_1.

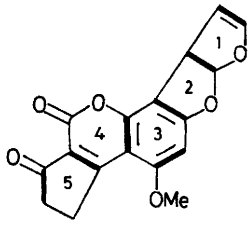
Carbon	δ p.p.m.[a]	$^1J_{^{13}C-^{13}C}$
1	155·2	—
2	117·4	60
3	201·3	40
4	35·1	40
5	29·0	—
6	177·1	60
7	104·0	64
8	161·6	71
9	90·9	71
10	165·8	61
11	107·9	61
12	153·0	64
13	113·6	33
14	47·9	33
15	102·7	75
16	145·4	75
17	56·6	—

[a] Relative to internal Me_4Si.

The ^{13}C assignments for aflatoxin B_1 (Table) were based on proton-noise, off-resonance-decoupled (p.n.d.) as well as selective proton-decoupling and SPI experiments.[7] The enriched carbon atoms observed in the p.n.d. ^{13}C n.m.r. spectrum of aflatoxin B_1, obtained from feeding the organism [2-^{13}C]-acetate, are in complete agreement with the reported ^{14}C labelling pattern (**4**). The p.n.d. ^{13}C n.m.r. spectrum of aflatoxin B_1 enriched with [1,2-^{13}C]-acetate showed the presence of satellite resonances due to ^{13}C–^{13}C

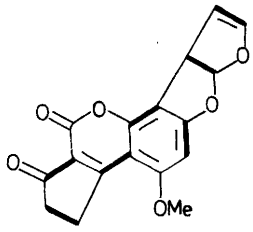


(4)

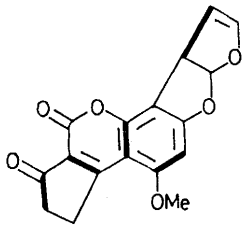


(5)

spin–spin couplings located symmetrically about the corresponding singlet peak arising from naturally occurring ^{13}C nuclei. The observed spin–spin coupling data (Table) indicated that C(2)–C(6), C(3)–C(4), C(12)–C(7), C(8)–C(9), C(10)–C(11), C(14)–C(13), and C(15)–C(16) originated from seven intact acetate units as shown in (**5**). The enhanced intensities of the uncoupled C(1) and C(5) signals relative to that of C(17) (OMe) proved that these carbon atoms were derived from two separate acetate units each of which lost one carboxyl acetate derived carbon atom in the biosynthesis of aflatoxin B_1.



(6)



(7)

The two possibilities for the expected arrangement of acetate units in aflatoxin B_1, if biosynthesised according to Biollaz[2] are shown in (**6**) and (**7**). From the data obtained it was clear that the observed arrangement of intact acetate units (**5**) differed from that postulated by Biollaz.[2] An important finding was that ring 3 in aflatoxin B_1 originated from the outer ring of an unknown C_{14} aromatic precursor and that the C_{18} naphthacene precursor[2] is no longer tenable. The results indicate the intermediacy of either a formal C_4 unit linked to a C_{14} precursor or an anthraquinone containing a linear C_6 chain, most likely derived from a single C_{20} polyketide. In both cases the mode of

head-to-tail linkage at C(11) and C(14) in aflatoxin B_1 can be explained by a mechanism proposed by Thomas.[8] Sterigmatocystin and the versicolorins are most probably derived biogenetically in a similar fashion.

We thank Dorothy Fennell for the culture of *Aspergillus flavus*.

(*Received, 28th November* 1974; *Com.* 1443.)

[1] W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 176.
[2] M. Biollaz, G. Büchi, and G. Milne, *J. Amer. Chem. Soc.*, 1970, **92**, 1035.
[3] J. G. Heathcote, M. F. Dutton, and J. R. Hibbert, *Chem. and Ind.*, 1973, 1027.
[4] J. S. E. Holker and L. J. Mulheirn, *Chem. Comm.*, 1968, 1576.
[5] D. P. H. Hsieh, M. T. Lin, and R. C. Yao, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 992.
[6] D. P. H. Hsieh and R. I. Mateles, *Appl. Microbiol.*, 1971, **22**, 79.
[7] K. G. R. Pachler and P. L. Wessels, *J. Magn. Resonance* ,1973, **12**, 337.
[8] R. Thomas, personal communication to M. O. Moss, Aflatoxin and related mycotoxins, 'Phytochemical Ecology,' ed. J. B. Harborne, Academic Press, London, 1972, p. 140.