Carbon-13 Nuclear Magnetic Resonance Assignments and Biosynthesis of Aflatoxin B_1 and Sterigmatocystin

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The ^{13}C n.m.r. spectra of two secondary fungal metabolites of related structure, aflatoxin B_1 and sterigmatocystin, have been completely assigned. The distribution pattern of acetate units has been determined from ^{13}C n.m.r. spectra of aflatoxin B_1 derived from singly and doubly labelled [^{13}C]acetate. These results and other published data on ^{13}C-labelled sterigmatocystin agree with a biosynthetic scheme based on a C_{20} polyketide precursor.

THE biosynthesis of aflatoxin B_1 (1), a potent hepatocarcinogen produced by *Aspergillus flavus* and *A. parasiticus*, and the related metabolite sterigmatocystin (2), produced by *e.g. A. versicolor*, has been a topic of much conflicting speculation.[1-5] Extensive ^{14}C-labelling and degradation studies [3,5] indicated that the two metabolites are totally derived from acetate units, and that

[1] J. S. E. Holker and J. G. Underwood, *Chem. and Ind.*, 1964, 1865.
[2] R. Thomas, in ' Biogenesis of Antibiotic Substances,' ed. Z. Vaněk and Z. Hoštálek, Academic Press, London, 1965, pp. 155—167.
[3] M. Biollaz, G. Büchi, and G. Milne, *J. Amer. Chem. Soc.*, 1970, **92**, 1035.
[4] J. G. Heathcote, M. F. Dutton, and J. R. Hibbert, *Chem. and Ind.*, 1973, 1027.
[5] J. S. E. Holker and L. J. Mulheirn, *Chem. Comm.*, 1968, 1576.

methionine contributes the methoxy methyl group. Their acetate origin has subsequently been confirmed by feeding experiments with ^{13}C-labelled acetate.[6-8]

^{13}C N.m.r. spectroscopy provides an efficient tool for studying the ambiguities in the biosynthesis of aflatoxin B_1 and sterigmatocystin, as indicated in our preliminary shifts for the various protons facilitated the assignment of the C(4) and C(5) proton signals. The measured shifts, extrapolated to an equimolar ratio (Table 1), indicate that complexation occurs preferentially at the carbonyl groups and the signal at δ 2.62 is therefore attributed to the C(4) protons.

TABLE 1

Proton chemical shifts and proton–proton coupling constants of aflatoxin B_1 (1) and sterigmatocystin (2)

	Aflatoxin B_1				Sterigmatocystin	
Proton	δ_H [a]	$J_{H,H}$/Hz	LIS (p.p.m.) [b]	Proton	δ_H [a]	$J_{H,H}$/Hz
				4	6.74 (DD)	$J_{4,5}$ 8.2, $J_{4,6}$ 1.0
				5	7.84 (T)	$J_{5,6}$ 8.2
				6	6.80 (DD)	
4	2.62	AA'BB'	7.2			
5	3.39		3.0			
9	6.42 (S)		1.1	11	6.42 (S)	
13	6.80 (D)	$J_{13,14}$ 7.3	1.1	14	6.81 (D)	$J_{14,15}$ 7.0
14	4.75 (DT)	$J_{14,15} = J_{14,16} = 2.5$	2.5	15	4.78 (DT)	$J_{15,16} = J_{15,17} = 2.5$
15	5.46 (T)	$J_{15,16}$ 2.5	2.0	16	5.44 (T)	$J_{16,17}$ 2.5
16	6.46 (T)		0.7	17	6.50 (T)	
OMe	3.95 (S)		1.0	OMe	3.98 (S)	

[a] Relative to internal Me_4Si; S = singlet, D = doublet, T = triplet. [b] $Eu(fod)_3$-Induced shift extrapolated to an equimolar ratio.

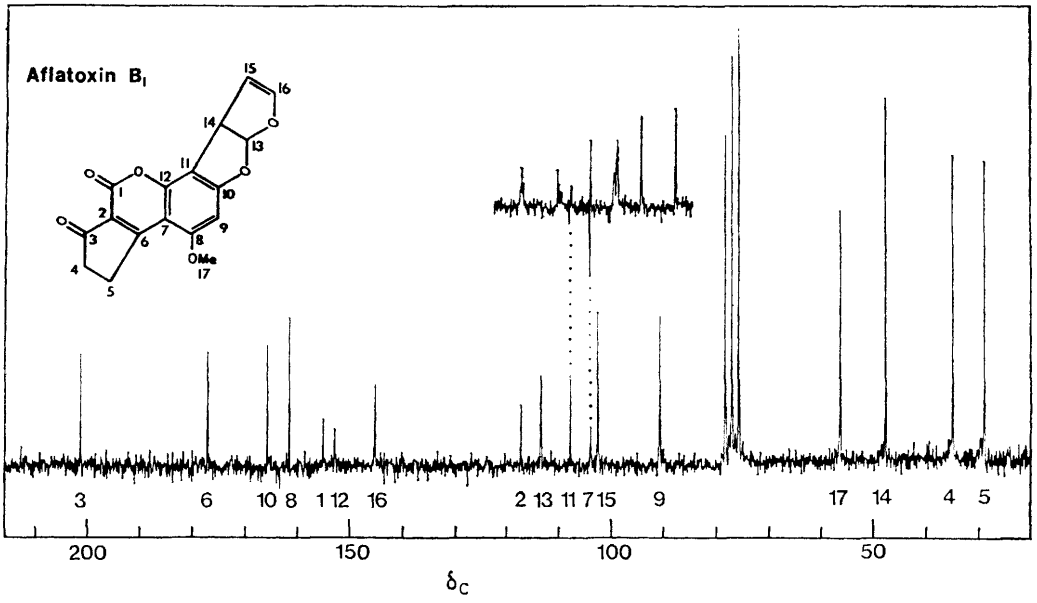


FIGURE 1 Natural-abundance proton-noise-decoupled 25.2 MHz ^{13}C n.m.r. spectrum of aflatoxin B_1 (1); spectral width 5 500 Hz; pulse delay 9 s; 90° r.f. pulse; transients 5 K. Insert: result of an SPI experiment (see text)

communications.[8,9] A prerequisite for any biosynthetic study with ^{13}C-labelled precursors is the unambiguous assignment of the resonances in the natural-abundance ^{13}C n.m.r. spectrum of the compound under study.

The proton n.m.r. spectrum of aflatoxin B_1 (1), with the exception of the C(4) and C(5) protons, which give rise to an AA'BB' pattern at δ 2.62 and 3.39, has been previously assigned[10] (Table 1). The $Eu(fod)_3$-induced

The ^{13}C n.m.r. data of aflatoxin B_1 (1) derived from coupled nuclear Overhauser enhanced (n.O.e.), proton-noise-decoupled (p.n.d.), and proton off-resonance decoupled spectra are given in Table 2. The p.n.d. natural abundance ^{13}C n.m.r. spectrum is shown in Figure 1.

The proton-bearing carbon signals have been assigned by correlating the residual splittings in off-resonance decoupled spectra with the known proton chemical shifts.[11] The magnitudes of the observed directly

[6] M. Tanabe, T. Hamasaki, and H. Seto, *Chem. Comm.*, 1970, 1539.

[7] D. P. H. Hsieh, J. N. Seiber, C. A. Reece, D. L. Fitzell, S. L. Yang, J. I. Dalezios, G. N. la Mar, D. L. Budd, and E. Motell, *Tetrahedron*, 1975, **31**, 661.

[8] P. S. Steyn, R. Vleggaar, P. K. Wessels, and D. B. Scott, *J.C.S. Chem. Comm.*, 1975, 193.

[9] K. G. R. Pachler, P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J.C.S. Chem. Comm.*, 1975, 355.

[10] T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1965, **87**, 882.

[11] K. G. R. Pachler, *J. Magnetic Resonance*, 1972, **7**, 442.

bonded ^{13}C–H coupling constants (Table 2) support these assignments. Identical assignments for these proton-bearing carbon atoms, based on comparisons with model compounds, were recently reported by Hsieh *et al.*[7]

TABLE 2

^{13}C Chemical shifts, directly bonded ($^1J_{^{13}C,H}$) and long-range ($>^1J_{^{13}C,H}$) coupling constants of aflatoxin B_1 (1) and coupling constants ($^1J_{^{13}C,^{13}C}$) of [1,2-^{13}C]acetate-enriched aflatoxin B_1 (J in Hz)

Carbon atom	δ_C [a]	$^1J_{^{13}C,H}$	$>^1J_{^{13}C,H}$	$^1J_{^{13}C,^{13}C}$
1	155.2 (S)			S
2	117.4 (S)			60
3	201.3 (Stt)		6,3	40
4	35.1 (T)	131		40
5	29.0 (T)	134		S
6	177.1 (Stt)		7,3	60
7	104.0 (Sd)		6	64
8	161.6 (Sq)		4	71
9	90.9 (D)	165		71
10	165.8 (Sdd)		7,3	61
11	107.9 (Sdd)		9,3	61
12	153.0 (S)			64
13	113.6 (Dddd)	187	9,6,3	33
14	47.9 (Dq)	147	6	33
15	102.7 (Dddd)	182	13,5,2	75
16	145.4 (Ddt)	196	12,4	75
OCH_3	56.6 (Q)	146		S

[a] Relative to internal Me_4Si. Capital letters refer to the pattern resulting from directly bonded protons and small letters to long-range ^{13}C–H coupling. S = singlet, D or d = doublet, T or t = triplet, Q or q = quartet.

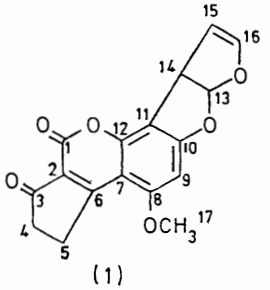
In the assignment of the quaternary carbon atom signals extensive use was made of techniques based on long-range (more than one bond) ^{13}C–H couplings. The result of an SPI (selective population inversion) experiment [12] in which a π-pulse of 0.085 s is applied at 3 Hz to higher field of the C(9) proton resonance is shown in an insert in Figure 1. The two affected resonances [δ 104.0 (Sd) and 107.9 (Sdd)] are assigned to C(7) and C(11), as three-bond ^{13}C–H couplings are usually larger than two-bond couplings in aromatic systems.[13] The smaller observed intensity changes of the resonance at δ 107.9 are due to the additional splitting of this resonance by the C(14) proton as well as the larger coupling (9 Hz) to the C(9) proton, which resulted in only a partial population transfer of the affected transition. The assignment of C(7) (δ 104.0) and C(11) (δ 107.9) signals is compatible with the observed long-range multiplicity of these signals and was confirmed by data obtained for [2-^{13}C]acetate-derived aflatoxin B_1 (see later).

The remaining resonance in the 90—120 p.p.m. region (δ 117.4) of the spectrum of aflatoxin B_1 arises from either C(2) or C(6). The corresponding carbon atoms in coumarin, C(3) and C(4), resonate at δ 116.3 and 142.8, respectively [14] whereas in styrene the α-carbon atom resonates at δ 135.8 and the β-carbon at δ 112.3.[15] Similarly, in cyclopent-2-enone the corresponding carbon atoms resonate at δ 132.9 and 164.2, respectively.[16]

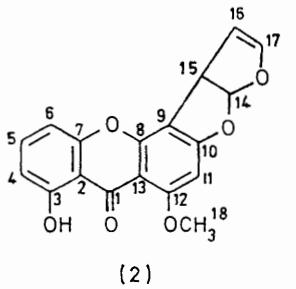
[12] K. G. R. Pachler and P. L. Wessels, *J. Magnetic Resonance*, 1973, **12**, 337.

[13] G. C. Levy and G. L. Nelson, ' Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley–Interscience, New York, 1972, p. 100, and references cited therein.

On the basis of these chemical shifts the peak at δ 117.4 should be assigned to C(2) in aflatoxin B_1. Additional evidence for this assignment was provided by data obtained for aflatoxin B_1 derived from [^{13}C]acetate (see later).



(1)



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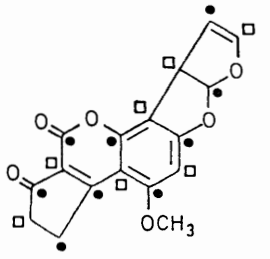
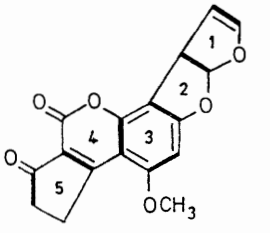


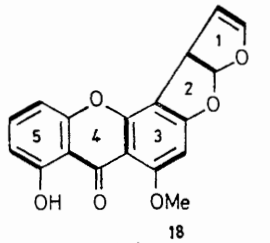
(3)

• C(1) of acetate

□ C(2) of acetate



(4)



(5)

Selective decoupling of the C(4) protons results in the collapse of the multiplets at δ 201.3 and 177.1 to triplets with couplings of 3 and 7 Hz, respectively. Conversely, decoupling the C(5) protons simplified the same multiplets to triplets with 6 and 3 Hz couplings, respectively. The effect of these selective proton decouplings on the appearance of the C(2) resonance could not be observed as a result of the overlap of the C(2) resonance and the high frequency leg of the C(13) resonance. The resonance at δ 201.3, characteristic of a ketone carbonyl carbon atom, is assigned to C(3). The consequential assignment of the signal at δ 177.1 to C(6) is corroborated by the data obtained for [1-^{13}C]acetate-derived aflatoxin B_1 (see later).

Selective decoupling of the C(13) proton removes a 6 Hz, a 2 Hz, and a 4 Hz splitting from the resonances due to C(14), C(15), and C(16), respectively. The resonance at δ 165.8 (Sdd) changes to a doublet (J 3 Hz) and is therefore attributed to C(10).

[14] R. D. Lapper, *Tetrahedron Letters*, 1974, 4293.

[15] J. B. Stothers, ' Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, p. 204.

[16] Reference 15, p. 193.

The resonances at δ 153.0 and 155.2 exhibit no long-range coupling and appear as singlets in the coupled n.O.e. spectrum, and the resonance at δ 161.6 is a multi-

has only one proton three bonds away, it follows that the resonance at δ 161.6(Sq) is due to C(8). Additional support for this assignment is obtained from the study of

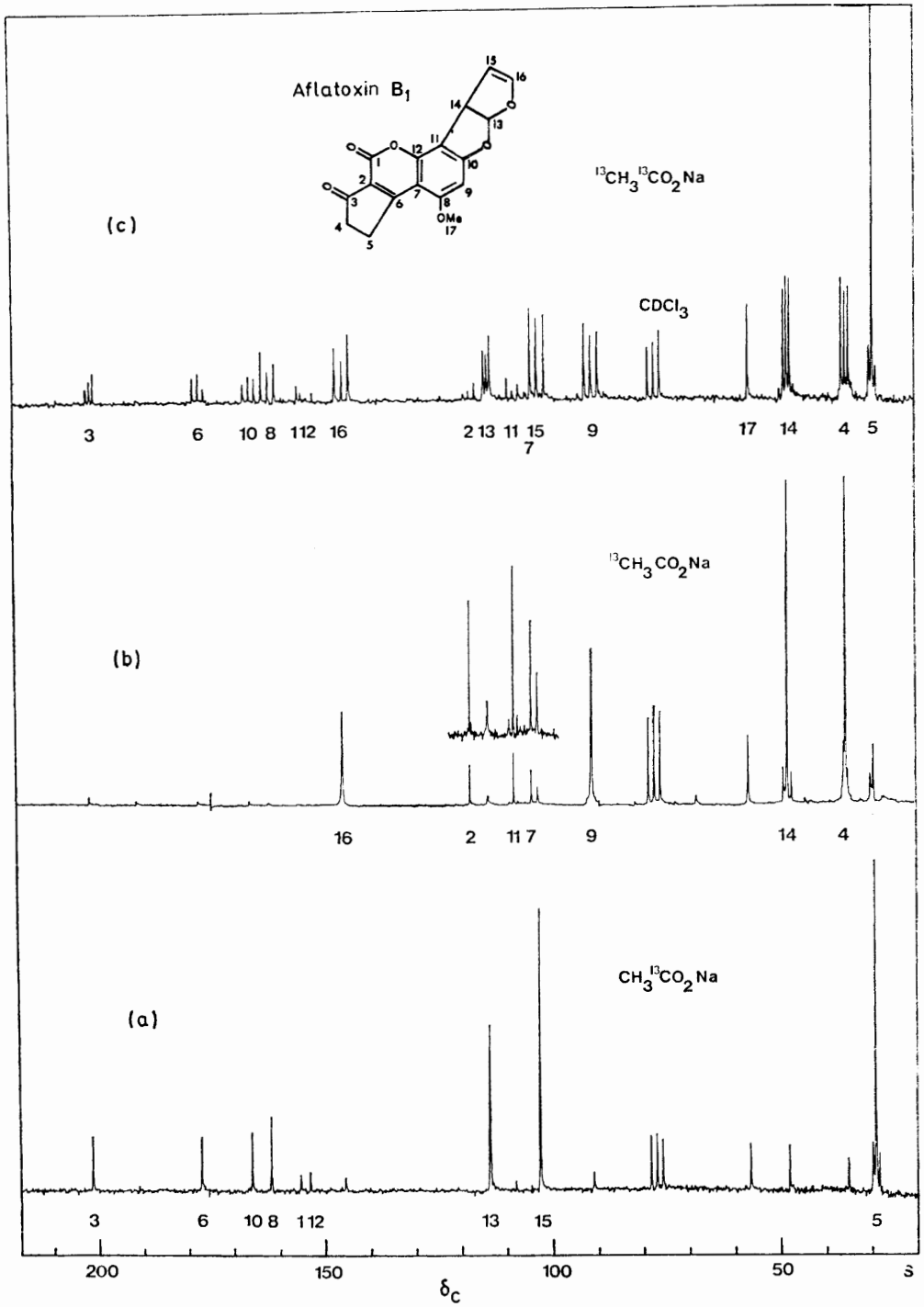


FIGURE 2 Proton-noise-decoupled 25.2 MHz ^{13}C n.m.r. spectra of aflatoxin B_1 derived from (a) [1-^{13}C]acetate; spectral width 5 500 Hz, pulse delay 5 s, 80° r.f. pulse, transients 40 K; (b) [2-^{13}C]acetate; spectral width 5 500 H; pulse delay 1 s 80° r.f. pulse, transients 32 K; (c) [1,2-^{13}C]acetate; spectral width 5 500 Hz, pulse delay 5 s, 80° r.f. pulse, transients 28 K

plet with at least quartet-like pattern. These resonances are ascribed to C(1), C(8), and C(12). As C(1) is four bonds removed from the nearest protons and as C(12)

sterigmatocystin (see later). The assignments of C(1) (δ 155.2) and C(12) (δ 153.0) are based on the results of a $Eu(fod)_3$-induced shift experiment. As complexation

occurs at the ketone carbonyl group [the signal due to C(4) which is a β-carbon atom relative to the complexation centre, shifts upfield by 5 p.p.m.[17]], addition of $Eu(fod)_3$ (*ca.* 1 : 4 molar ratio) resulted in a downfield shift of 4 p.p.m. for the signal at δ 155.2 [C(1)], whereas no shift was observed for the signal at δ 153.0 [C(12)].

The assignments of the quaternary carbon signals differ from those reported recently by Hsieh *et al.*[7] In their study, use of model compounds led to an interchange of the C(2) and C(7) assignments and a wrong assignment for C(1). Furthermore, C(8), C(10), and C(12) were not assigned individually.

assignment of the resonance at δ 107.9 to C(11) is confirmed by the ^{13}C–^{13}C coupling of 44 Hz between C(14) at δ 47.9 and C(11). The ^{13}C–^{13}C coupling of 21 Hz, seen clearly as satellites on the C(4) resonance and weakly on the resonance at δ 117.4 (Figure 2b, insert), lends further support to the assignment of the latter resonance to C(2). The geminal C–C coupling constant in acetone is 16.5 Hz.[19]

The labelling pattern of aflatoxin B_1 derived from the singly ^{13}C-labelled acetates is consonant with the reported ^{14}C-labelling patterns (3).[3]

The p.n.d. ^{13}C n.m.r. spectrum of aflatoxin B_1 enriched

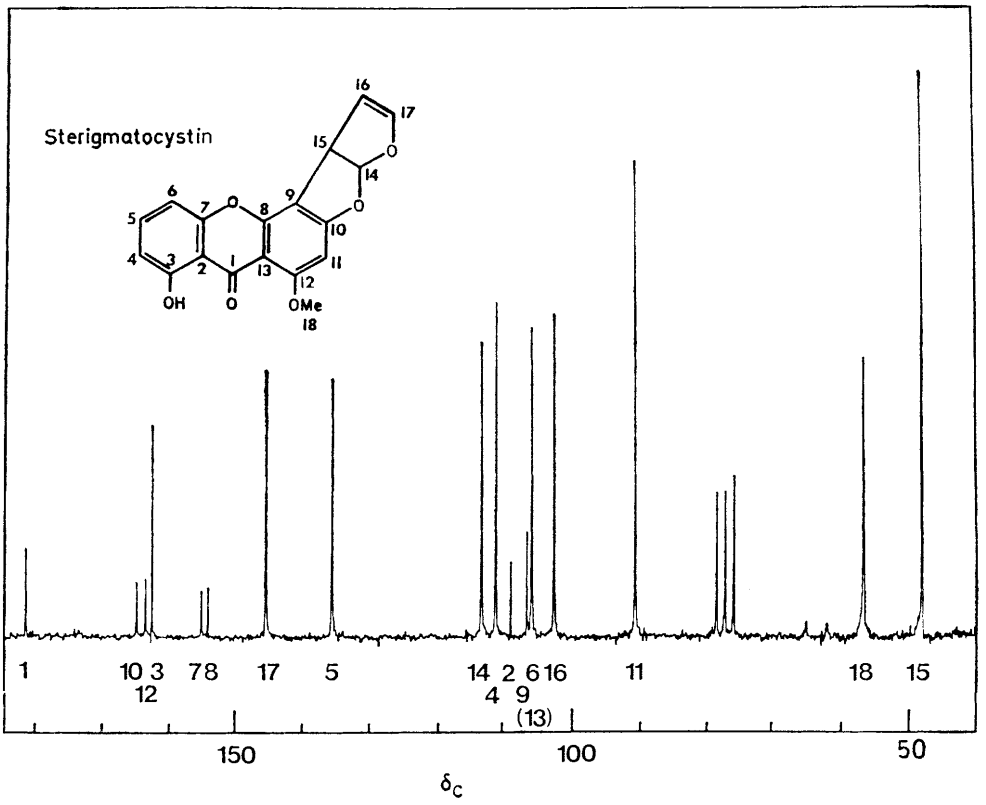


FIGURE 3 Natural-abundance proton-noise-decoupled 25.2 MHz ^{13}C n.m.r. spectrum of sterigmatocystin (2); spectral width 5 000 Hz; pulse delay 2 s; 75° r.f. pulse; transients 4 K

Additional evidence corroborating our assignments was provided by the spectra of [1-^{13}C]- and [2-^{13}C]-acetate-derived aflatoxin B_1.

Cultures of *A. flavus*, strain NRRL 3251, were grown by the replacement technique [18] and supplemented with sodium [1-^{13}C]-, [2-^{13}C]-, or [1,2-^{13}C]-acetate (all 90%). Aflatoxin B_1 was in each case enriched approximately eight-fold above the natural ^{13}C abundance, as determined by mass spectrometry.

The [1-^{13}C]acetate-derived aflatoxin B_1 spectrum (Figure 2a) shows nine enriched carbon signals. The coupling of 34 Hz between C(5) and C(6) provided additional evidence for the assignment of C(6) (δ 177.1).[7] Similarly the [2-^{13}C]acetate-derived aflatoxin B_1 spectrum (Figure 2b) shows seven enriched carbon signals. The with [1,2-^{13}C]acetate (Figure 2c) showed satellite resonances due to ^{13}C–^{13}C spin–spin couplings. The observed spin–spin coupling data (Table 2) indicated that C(2)–C(6), C(3)–C(4), C(7)–C(12), C(8)–C(9), C(10)–C(11), C(13)–C(14), and C(15)–C(16) originated from seven intact acetate units, arranged as shown in (4). Both the C(1) and C(5) resonances are enhanced in the ^{13}C spectrum of the sample derived from [1-^{13}C]acetate, but show no one-bond ^{13}C–^{13}C coupling in the doubly labelled derivative. These carbon atoms were therefore derived from two separate acetate units, each of which

[17] D. H. Williams, *Pure Appl. Chem.*, 1974, **40**, 25.
[18] D. P. H. Hsieh and R. I. Mateles, *Appl. Microbiol.*, 1971, **22**, 79.
[19] Reference 15, p. 374.

lost one acetate-derived *methyl* carbon atom in the biosynthesis of aflatoxin B_1.

The natural-abundance ^{13}C n.m.r. spectrum of sterigmatocystin (2) is shown in Figure 3. The ^{13}C n.m.r. data of (2) derived from coupled, p.n.d., and off-resonance decoupled spectra are given in Table 3. Methoxy and tertiary carbon signals have been assigned by correlating residual splittings in off-resonance proton-decoupled spectra with the known proton chemical shifts (Table 1). The proton chemical shifts are in agreement with reported values [20] with the exception of those of the C(4) and C(6) protons. The assignment of the C(4) (δ 6.74) and C(6) (δ 6.80) proton signals is based on the results of recent nuclear Overhauser effect experiments on sterigmatocystin and related compounds.[21] Directly bonded C–H couplings support the assignment coupled n.O.e. spectrum (Figure 4) the resonance centred around δ 111.0 shows two long-range couplings of 7 Hz, one of which is removed by exchanging the OH proton with deuterium. This clearly distinguishes C(4) from C(6), which is five bonds away from the hydroxy-proton.

Quaternary carbon signals have been assigned from long-range couplings, on the assumption that three-bond C–H couplings in aromatic systems are larger than two-bond couplings, and by comparison with chemical shifts in related compounds [dihydrosterigmatocystin, *O*-acetyldihydrosterigmatocystin, and aflatoxin B_1 (1)]. The following evidence also supports the assignments different from those of Seto *et al.*[22] [C(7), C(8), C(10), and C(12)].

The resonance at δ 180.9 is assigned to C(1) on the basis of the chemical shift. Selective decoupling of the

TABLE 3

^{13}C Chemical shifts of sterigmatocystin and derivatives, and directly bonded ($^1J_{^{13}C,H}$) and long-range ($^{>1}J_{^{13}C,H}$) coupling constants (J in Hz) of sterigmatocystin

Carbon atom	δ_C [a]	$^1J_{^{13}C,H}$	$^{>1}J_{^{13}C,H}$	$^1J_{^{13}C,^{13}C}$ [b]	Dihydrosterigmatocystin δ_C	*O*-Acetyldihydrosterigmatocystin δ_C
1	180.9 (S)			58	181.0	173.8
2	108.8 (S)				108.8	115.9
3	162.1 (Sddd)		10,5,2		162.1	150.1
4	111.0 (Dt)	165	7	70	111.0	118.6
5	135.4 (D)	164		59	135.3	133.1
6	105.7 (Dd)	167	8	58	105.6	115.0
7	154.7 (Sdd)		12,3	S	154.7	155.7 †
8	153.7 (S)				154.3	153.6 †
9	106.4 (S)				105.3	104.8
10	164.3 (Sdd)		8,4		165.9	165.0
11	90.4 (D)	165		72	89.7	89.7
12	163.0 (Sqd)		7,3		163.2	163.1
13	105.7 ± 0.3 (S) *				(105.6)	107.7
14	113.1 (Dddd)	187	9,6,3	34	113.4	113.1
15	47.9 (Dq)	146	6	34	44.2	44.3
16	102.4 (Dddd)	181	13,5,2	76	31.4	31.5
17	145.1 (Ddt)	197	12,5	76	67.7	67.6
OCH_3	56.6 (Q)	146			56.6	56.7
CH_3CO						21.3
CH_3CO						168.7

[a] Chemical shifts relative to internal Me_4Si. Capital letters refer to the pattern resulting from directly bonded protons and small letters to long range ^{13}C–H coupling. S = singlet, D or d = doublet, T or t = triplet, Q or q = quartet. [b] From ref. 22.

* This resonance overlaps with that of C(6). The position was back-extrapolated from a $Eu(fod)_3$ experiment. † May be interchanged.

of aromatic, olefinic, and aliphatic carbon atoms. The absence of the resonances at δ 102.4 and 145.1 in dihydrosterigmatocystin (Table 3) further supports the assignment of these resonances to C(16) and C(17), respectively in sterigmatocystin and confirms that the previous assignment [6] of C(16) and C(14) should be reversed.[22]

The assignments of the proton-bearing carbon signals agree largely with those of Seto *et al.*[22] except for the crucial interchange of the C(4) and C(6) resonances. Neither data from the off-resonance proton-decoupled spectrum, because of the small difference in the proton chemical shifts, nor the acetylation shifts observed for *O*-acetyldihydrosterigmatocystin can be used to distinguish between these two carbon atoms. In the C(5) proton changes the resonances at δ 162.1 and 154.7 to a doublet of doublets (J 5 and 2 Hz) and a broad unresolved singlet, respectively. Deuterium exchange of the hydroxy-proton removes the 5 Hz coupling from the resonance at δ 162.1. This confirms the assignments of C(3) (δ 162.1) and C(7) (δ 154.7). The complex pattern at δ 163.0 is assigned to C(12) since decoupling of the methoxy-protons changes the signal to a simple doublet (J 2 Hz). This assignment is also supported by $Eu(fod)_3$-induced shift experiments. The methoxy carbon atom experiences the largest shift indicating that complexation occurs preferentially at the methoxy-group. The second biggest shift is shown by the C(12) resonance. The assignment of C(8) (δ 153.7) and C(10) (δ 164.3) follows

[20] E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1962, 4179.

[21] P. S. Steyn and R. Vleggaar, *J.C.S. Perkin I*, 1974, 2250.

[22] H. Seto, L. W. Cary, and M. Tanabe, *Tetrahedron Letters*, 1974, 4491.

from a comparison with the corresponding carbon signals of aflatoxin B_1 (1) at δ 153.0 and 165.8, respectively.

Assignments for the three quaternary carbon atoms [C(2), C(9), and C(13)], previously not assigned,[22] were made as follows. The resonance at δ 106.4 is shifted least by $Eu(fod)_3$ and is therefore assigned to C(9). Acetylation of the C(3) hydroxy-group in dihydrosterigmatocystin caused the resonance at δ 108.8 [C(2)] to shift to lower field (δ 115.9) with no significant effect on C(9) and C(13). This identifies the C(2) signal. The remaining resonance at δ 105.7 in the spectrum of sterigmatocystin is therefore assigned to C(13).

Great caution should be exercised when model compounds are used for the assignment of carbon signals in complex organic compounds. The effects of methoxy-substitution on aromatic carbon chemical shifts [23] have been used for the assignment of carbons 2—7 in sterigmatocystin.[22] The shift values as determined from our values for sterigmatocystin and those reported for 6-methoxysterigmatocystin, with the expected values in brackets, are (in p.p.m.): C(3) −9.0 (−6.8), C(4) −2.0 (+2.4), C(5) −14.8 (−15.0), C(6) +34.3 (+31.3), and C(7) −10.9 (−8.8). Although these values are in close agreement, with the possible exception of those for C(4), a completely wrong assignment was made.[22] Similarly, even if the observed chemical shift of the methoxy-bearing carbon atom in aflatoxin B_1 [C(8), δ 161.6] is used to assign the corresponding carbon signal in sterigmatocystin [C(12)], an interchange of the C(3) (δ 162.1) and C(12) (δ 163.0) assignments might result.

^{13}C N.m.r. data on [1,2-^{13}C]acetate-derived sterigmatocystin have been reported previously.[22] The p.n.d. spectrum showed only one singlet, which we assign to C(7). Our ^{13}C assignments (Table 3) and the reassigned one-bond ^{13}C–^{13}C couplings from reference 22 allow only one arrangement of intact acetate units in sterigmatocystin, as shown in (5). The ^{13}C assignments of sterigmatocystin by Seto *et al.*[22] lead to the incorrect arrangement of intact acetate units, *viz.* the location of only two intact acetate units in ring 3, and furthermore to a different folding of the original polyketide chain.

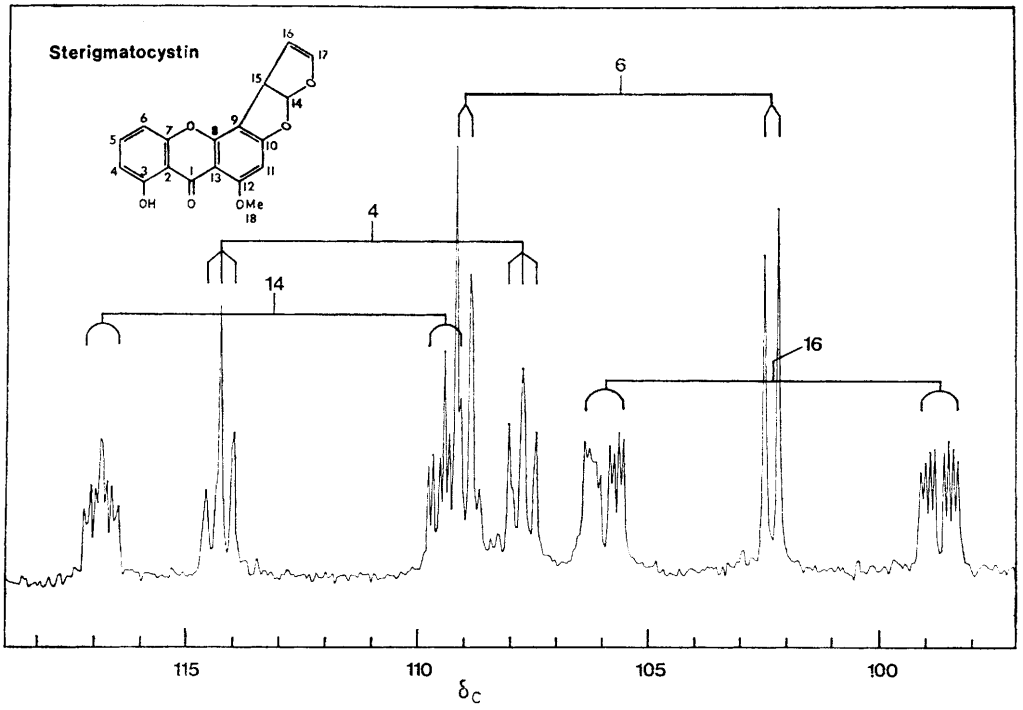


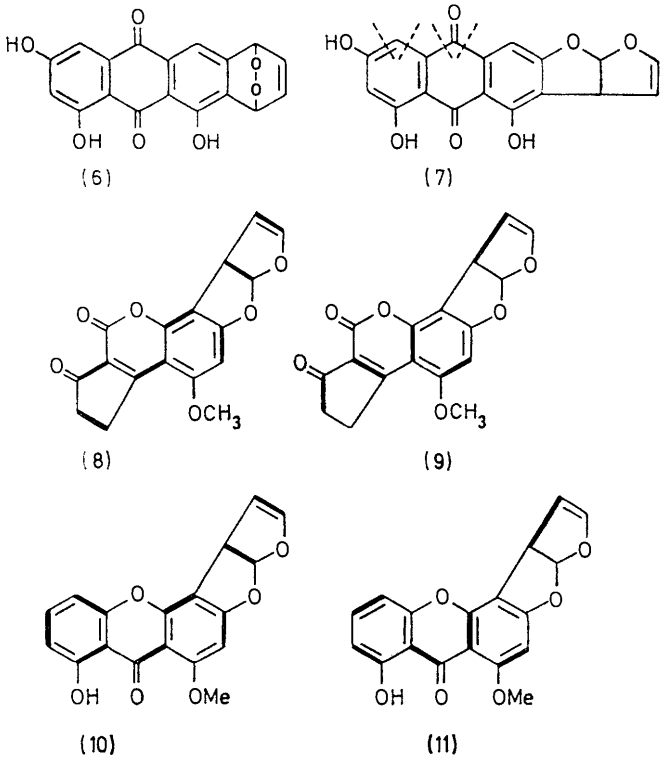
FIGURE 4 Part of the natural-abundance coupled n.O.e. 25.2 MHz ^{13}C spectrum of sterigmatocystin (2); total spectral width 5 000 Hz; 75° r.f. pulse; transients 17 K; decoupler on time 2 s

On the basis of their extensive ^{14}C-labelling studies on aflatoxin B_1 (1), Biollaz *et al.*[3] postulated the currently accepted biogenesis by which a single polyacetate chain gives rise to the C_{18} polyhydroxynapthacene *endo*-peroxide (6) which rearranges through a pyran intermediate to the bisdihydrofuran unit as in versicolorin A (7). The consecutive oxidative loss of two separate acetate-derived *methyl* carbon atoms can then lead first to sterigmatocystin and secondly to aflatoxin B_1. Heathcote *et al.*[4] preferred an alternative route where a C_4 unit is linked to a preformed anthraquinone molecule; however, feeding experiments showed that C_4 units were very poorly incorporated into aflatoxin B_1.[4]

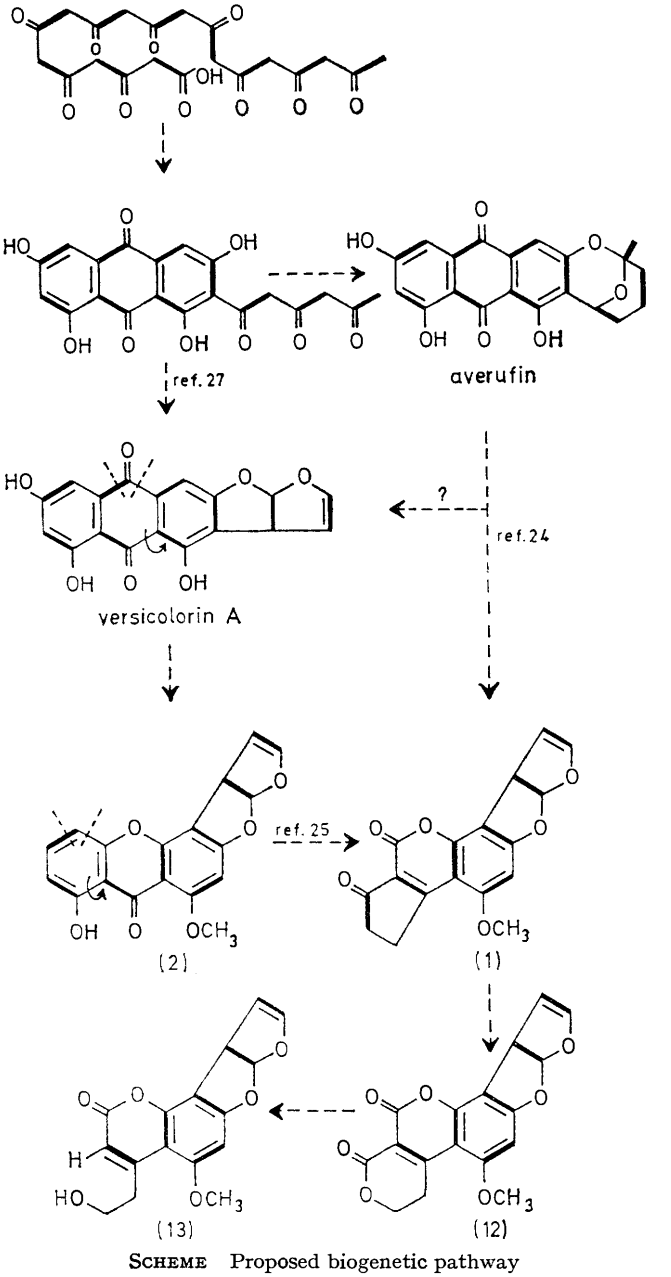
The two possibilities for the expected arrangement of acetate units in aflatoxin B_1 and sterigmatocystin, if biosynthesised according to Biollaz [3] are shown in (8) and (9) and in (10) and (11), respectively. From our data it was clear that the observed arrangements of acetate units in aflatoxin B_1 (4) and sterigmatocystin

[23] R. H. Levin, J. Y. Lallemand, and J. D. Roberts, *J. Org. Chem.*, 1973, **38**, 1983.

(5) differed from those postulated by Biollaz *et al.*[3] An important finding is that ring 3 contains three acetate units and must therefore be derived from the outer ring of an aromatic precursor. A C_{18} naphthacene precursor [3] is thus no longer tenable. Our results on aflatoxin B_1 and sterigmatocystin are in agreement with the biosynthesis from a single C_{20} polyketide precursor which is folded in only one mode leading to averufin and sterigmatocystin (Scheme). The proposed C_{20} pathway (Scheme) is supported by recent experiments which established that averufin [24] and sterigmatocystin [25] can be converted very efficiently by cultures of *A. parasiticus* into aflatoxin B_1. The precursor–product relationship between acetate and averufin has recently been demonstrated by using [^{13}C]acetate.[26] The unique head-to-head linkage of the two acetate units in (1) and (2) for the coupling of the dihydrofuran ring and the aromatic system can be adequately accommodated by a mechanism proposed by Thomas.[2,27]



Aflatoxin G_1 (12) and parasiticol (13) [28] are most probably derived biogenetically in a similar fashion, as shown in the Scheme.



SCHEME Proposed biogenetic pathway

EXPERIMENTAL

100 MHz ^{1}H N.m.r. spectra (solvent $CDCl_3$) were recorded with a Varian HA-100 spectrometer (Me_4Si as lock signal and internal reference). 25.2 MHz ^{13}C N.m.r. spectra were obtained from solutions in $CDCl_3$ with a Varian XL-100-15 FT spectrometer equipped with a 16 K Varian 620i computer and a gated gyrocode decoupler; 12 mm sample tubes were used, except for ^{13}C-enriched aflatoxin B_1 (5 mm tubes). Spectral width, pulse angle, pulse delay, and number of transients are specified on the Figures. Acquisition times were chosen to obtain the maximum number (8192) of data points. SPI experiments were performed as described previously.[12]

[5/1932 *Received*, 3*rd October*, 1975]

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