Biosynthetic Studies on the Fusarins, Metabolites of *Fusarium moniliforme*

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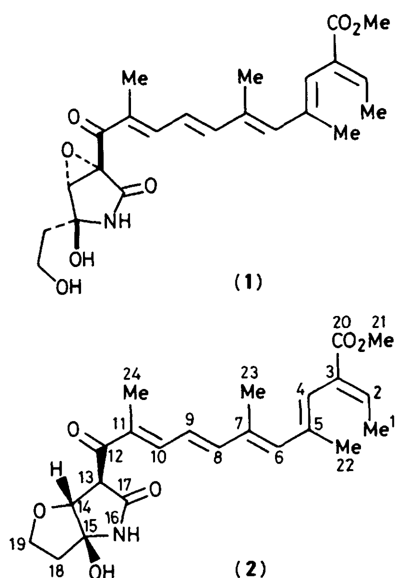
The incorporation of (2*S*)-[*methyl*-^{13}C]methionine and [1,2-$^{13}C_2$]acetate into fusarin A, a metabolite of *Fusarium moniliforme*, points to a biosynthetic pathway involving the condensation of a C_{14}-polyketide and a C_4 intermediate, *e.g.* oxaloacetate, of the Krebs tricarboxylic acid cycle.

Extracts of several strains of *Fusarium moniliforme*, one of the most prevalent fungi associated with maize throughout the world,[1] exhibit mutagenic activity.[2] We recently reported the structure elucidation of a potent mutagen, fusarin C (**1**), isolated from maize cultures of *F. moniliforme* Sheldon, strain MRC 826.[3] Structural analysis strongly suggests a polyketide derivation for the metabolite but the origin of the substituted 2-pyrrolidone moiety in (**1**) is obscure. Possible routes include: (a) the condensation of two preformed polyketide chains;[4,5] or (b) a 1,2-bond migration of a single polyketide chain;[6] or (c) the oxidative cleavage of a suitably oxygenated phenyl ring;[7] or (d) the cleavage of the terminal acetate unit of a C_{18}-polyketide chain. This diversity in possible biosynthetic routes prompted us to investigate the biosynthesis of fusarin C (**1**) in order to identify the pathway.



In our biosynthetic studies we used an isolate of *F. moniliforme*, strain MRC 826, which consistently produced mainly fusarin A (**2**). Cultures of *F. moniliforme* were grown in the dark at 23 °C on cakes of yellow maize meal containing 50% water. Studies on the course of fermentation indicated the initial appearance of fusarin A on day 7 which reached a level of 8 mg per 100 g of maize on day 14.

High resolution mass spectrometric analysis of the molecular ion, *m/z* 415.1993, of fusarin A (**2**) gave the molecular formula as $C_{23}H_{29}NO_6$; the metabolite had $\lambda_{max.}$ (MeOH) 352 nm (ε 21300) and $\nu_{max.}$ ($CHCl_3$) 3410, 1710, 1625, 1600, and

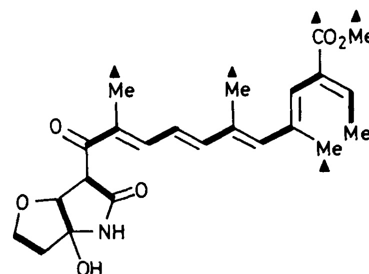


Figure 1. Arrangement of intact acetate units in fusarin A (**2**): ▲ derived from (2*S*)-[*methyl*-^{13}C]methionine.

Table 1. N.m.r. data for fusarin A (**2**).[a]

Carbon atom	δ[b]	^1J(CH)/Hz	^1J(CC)/Hz[c]	% Enrichment[d]	δ[e]	J(HH)/Hz
1	16.12 Q	126.6	42.9	0.26	1.779 dd	7.2, 1.5
2	140.27 D	157.3	43.1	0.30	6.960 qd	7.2, 1.2
3	130.86 S		57.5	0.22		
4	126.63 D	158.1	57.4	0.23	6.085 m	
5	137.79 S		55.3	0.23		
6	140.96 D	151.7	55.1	0.20	6.328 s br	
7	135.41 S		54.9	0.22		
8	149.16 D	152.9	54.9	0.21	6.880 d	15.0
9	124.01 D	152.6	57.2	0.22	6.684 dd	15.0, 11.0
10	146.13 D	152.7	57.2	0.22	7.512 dq	11.0, 1.1
11	134.44 S		56.1	0.21		
12	197.70 S		55.8	0.20		
13	57.19 D	141.0	43.1	0.26	4.351 s	
14	86.23 D	160.1	36.8	0.13	4.223 d[f]	1.2
15	94.95 S		36.7	0.12		
17	171.08 S		43.1	0.22		
18	37.89 T	133.2	34.3	0.12	2.365 ddd	12.8, 8.9, 8.6
					2.260 ddd	12.8, 6.5, 3.9
19	68.85 T	148.4	34.1	0.11	4.084 ddd	8.9, 8.6, 3.9
					3.992 ddd	8.9, 8.9, 6.5
20	167.73 S					
21	52.05 Q				3.717 s	
22	18.92 Q				1.740 d	1.4
23	14.31 Q				2.108 d	1.3
24	11.77 Q				1.970 d	1.2

[a] Recorded on a Bruker WM-500 spectrometer. [b] Relative to Me_4Si; solvent CD_2Cl_2. Capital letters refer to the pattern resulting from directly-bonded (C,H)-couplings. S = singlet, D = doublet, T = triplet, Q = quartet. [c] Values obtained from the broad-band proton-decoupled ^{13}C spectrum of fusarin A derived from [1,2-$^{13}C_2$]acetate. [d] Enrichments, due to incorporation of [1,2-$^{13}C_2$]acetate, were calculated by means of the formula reported in ref. 9. [e] Relative to Me_4Si; solvent CD_2Cl_2. s = singlet, d = doublet, q = quartet, and br = broad. The chemical shifts of the N-16 proton (δ 6.825 br) and the hydroxy proton (δ 4.93) are concentration dependent. [f] Doublet due to coupling to the N-16 proton.

1580 cm^{-1}. The assignment of the resonances in the ^1H and ^{13}C n.m.r. spectra of fusarin A, as collated in Table 1, will be reported in a full paper.

On feeding (2*S*)-[*methyl*-^{13}C]methionine (90 atom %, 500 mg per 375 g of maize), admixed with (2*S*)-[*methyl*-^{14}C]methionine (50 μCi) as a tracer, to cultures of *F. moniliforme*, fusarin A with a specific activity of 4.87 μCi $mmol^{-1}$ was obtained. This result corresponds to a dilution value of 15.4 (assuming the presence of 5 labelled positions and thus an enrichment factor[8] of 6.2). The broad-band proton-decoupled ^{13}C n.m.r. spectrum of the enriched metabolite showed enhancement of the signals attributed to C-20 (enrichment factor 5.3), C-21 (9.1), C-22 (6.9), C-23 (5.7), and C-24 (7.9).

The arrangement of intact acetate units in fusarin A was studied by addition of [1,2-$^{13}C_2$]acetate (91 atom % ^{13}C, 2.0 g per 375 g of maize) to cultures of *F. moniliforme*. All the resonances in the proton-decoupled ^{13}C n.m.r. spectrum of the enriched fusarin A, with the exception of those for C-20—C-24, exhibited low intensity one-bond (C,C) couplings. The measured ^1J(CC) values are given in Table 1 and prove the presence of nine intact acetate units arranged as shown in Figure 1: C-1–C-2, C-3–C-4, C-5–C-6, C-7–C-8, C-9–C-10, C-11–C-12, C-13–C-17, C-14–C-15, and C-18–C-19. The enrichment at each labelled site, deduced from the intensities of the satellite signals arising from one-bond (C,C)-coupling relative to that of the central signal,[9] provided additional information on the biosynthesis of fusarin A. Both C-1 and C-2 showed somewhat higher enrichment than the average (0.22%) observed for the twelve carbon atoms C-3—C-13 and C-17. In contrast the average enrichment for C-14, C-15, C-18, and C-19 (0.12%) is distinctly lower (see Table 1). This difference in the enrichment levels points to a biosynthetic pathway for fusarin A involving a C_{14}-polyketide derived from an acetyl-CoA starter unit (C-1 and C-2) and 6 malonyl-CoA units, and a C_4 unit derived most probably from a product of the Krebs tricarboxylic acid cycle, oxaloacetate. A similar biosynthetic pathway involving a polyketide chain and oxaloacetate or a related intermediate of the tricarboxylic acid cycle has been demonstrated for the tetronic acids, carolic and carlosic acid, metabolites of *Penicillium charlesii*,[10] and for the marticins, metabolites of *Fusarium martii*.[11]

The involvement of oxaloacetate in the biosynthesis of fusarin A implies that C-15 is derived from C-2 of oxaloacetate and furthermore that the two contiguous carbon atoms, C-15 and C-18 are derived from C-2 of acetate. Incorporation of either [1-^{13}C]- or [2-^{13}C]-acetate into fusarin A would test these conclusions. However, the low enrichment obtained on incorporation of [1,2-$^{13}C_2$]acetate precludes the detection of enrichment at these carbon atoms using singly ^{13}C-labelled acetate. This was indeed the case. The incorporation of [1-^{13}C]acetate (99 atom % ^{13}C, 2.5 g per 375 g of maize) was subject to a too high dilution and no reliable enhancement factors[8] were obtained from the proton-decoupled ^{13}C n.m.r. spectrum of the enriched fusarin A.

The nature of polyketide biosynthesis indicates that C-1–C-2 constitutes the chain-initiating acetate unit which is extended by six malonyl-CoA units to form the proposed C_{14}-polyketide

chain. Thus C-2, C-4, C-8, C-10, C-12, and C-17 are derived from C-1 of acetate as the subsequent methylations of the C_{14}-polyketide occur at positions derived from C-2 of acetate.

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