Biosynthesis of Aflatoxins. Incorporation of [1,2-$^{13}C_2$]Acetate, [$^2H_3$]Acetate, and [1-^{13}C, $^2H_3$]Acetate into Sterigmatocystin in *Aspergillus versicolor*

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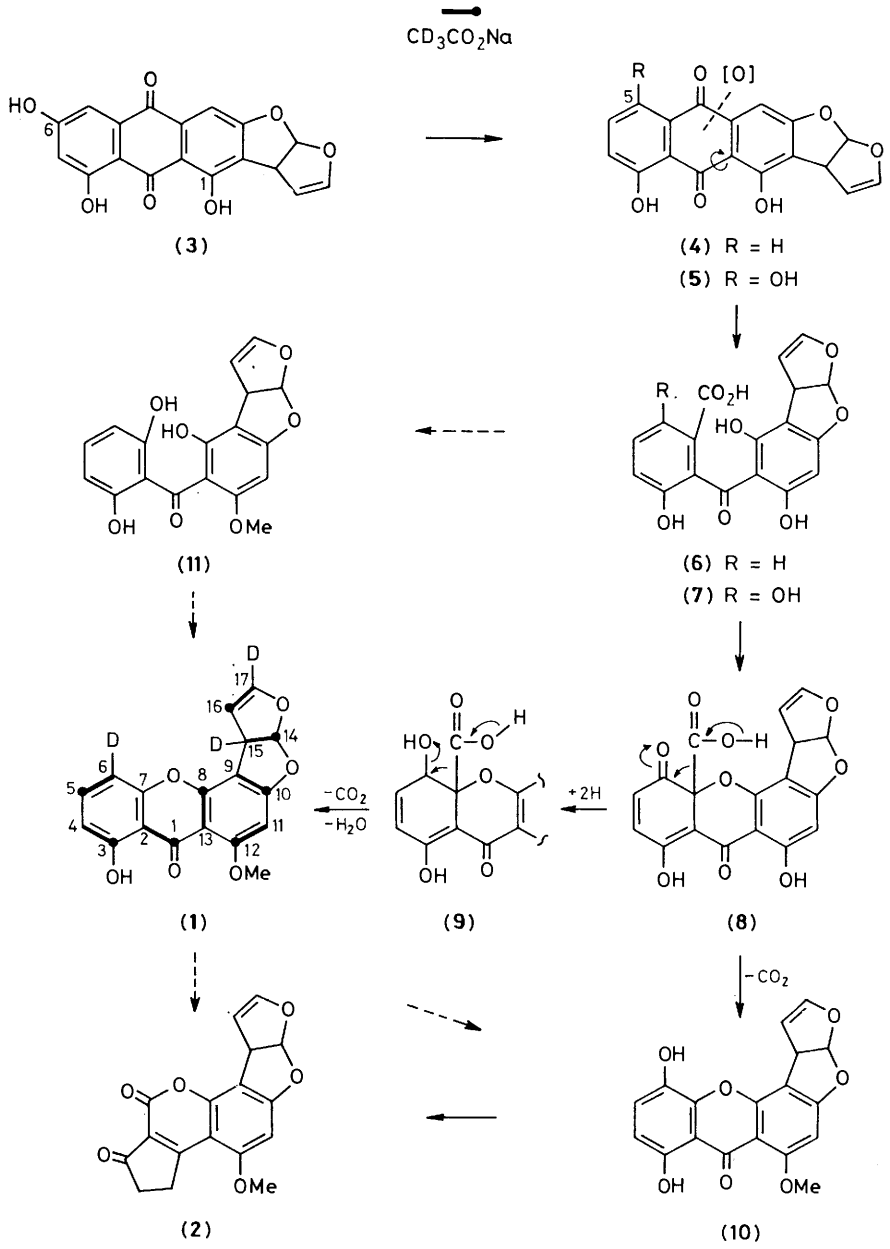
The complete ^{13}C–^{13}C coupling pattern in sterigmatocystin enriched from [$^{13}C_2$]acetate has been determined and confirms that no randomisation of labelling in ring-A occurs; ^2H-labelling studies indicate that ^2H from [$^2H_3$]acetate is retained at C-6 of sterigmatocystin to rule out mechanisms for xanthone ring formation requiring introduction of the phenolic hydroxy-group on this carbon.

Sterigmatocystin (**1**), a carcinogenic mycotoxin produced by a number of *Aspergillus* species is believed to be an intermediate on the biosynthetic pathway to the even more potent aflatoxin B_1 (**2**) in *Aspergillus flavus* and *Aspergillus parasiticus*.[1] Versicolorin A (**3**) has in turn been proposed as an immediate precursor to sterigmatocystin and incorporation of label from versicolorin A into sterigmatocystin[2] and aflatoxin B_1[3] has been reported. However little is known of the biochemical steps responsible for the conversion of versicolorin A into sterigmatocystin, points of particular interest being the timing and mechanism of the required loss of the phenolic hydroxy-group on C-6 of versicolorin A, a process for which there is little biosynthetic precedent;[4] and the mechanisms of conversion of the anthraquinone into the xanthone ring system. Holker has proposed[5] a pathway similar to that shown in Scheme 1 which assumes loss of a hydroxy-group to give 6-deoxyversicolorin A (**4**) followed by *para*-hydroxylation either of (**4**)† or the product (**6**) of ring cleavage to permit an oxidative coupling mechanism for xanthone ring formation. The intermediate (**8**) formed in this way could function as a common precursor to both sterigmatocystin (**1**) and 6-hydroxysterigmatocystin (**10**). This implies that (**10**) and not sterigmatocystin would lie on the direct aflatoxin pathway, and there is indeed some indirect evidence for this.[6] Alternatively, a metabolic grid may be operating. A variation of this scheme in which the hydroxy-group is lost *after* formation of the xanthone ring system could account for the co-occurrence of sterigmatocystin, 6-methoxysterigmatocystin, and 5,6-dimethoxysterigmatocystin.[7]

Vederas has recently reported[8] incorporation studies with [1-^{13}C, $^{18}O_2$]acetate which indicate that the 1-hydroxy-group of versicolorin A becomes the xanthone ring oxygen of sterigmatocystin, and he proposed that xanthone formation occurs by an addition–elimination mechanism[9] on an enzyme-bound benzophenone intermediate (**11**). However all precedent suggests that a symmetrical intermediate of this type invariably results in randomisation of labelling,[10] whereas Tanabe has reported that [1,2-$^{13}C_2$]acetate is incorporated into sterigmatocystin without randomisation.[11] However poor spectral resolution and severe overlapping of signals allowed only a partial analysis of the coupling pattern. We now report labelling studies with ^{13}C- and ^2H-labelled acetates which bear upon the above proposals.

Labelled acetates were incorporated into sterigmatocystin by cultures of *Aspergillus versicolor* (NRRL 5219) essentially as previously described.[12] To minimise multiple labelling of individual molecules, sodium [1,2-$^{13}C_2$]acetate was diluted three-fold with unlabelled acetate prior to addition to cultures. Similarly [1-^{13}C, $^2H_3$]acetate was diluted with [$^2H_3$]-acetate prior to addition. The resultant labelled sterigmatocystin was analysed by ^{13}C and ^2H n.m.r. spectroscopy as appropriate. In the proton noise-decoupled 90.6 MHz ^{13}C n.m.r. spectrum of [$^{13}C_2$]acetate-enriched sterigmatocystin all the anticipated couplings were observed (Table 1). All the carbons in ring-A show only one coupling apart from C-7 which appears as a single resonance with no evidence whatsoever of coupling satellites, and so there is clearly no randomisation of labelling. Thus a symmetrical intermediate, *e.g.*, (**11**) would appear to be unlikely.

† 6-Deoxyversicolorin A (**4**) is a known metabolite of *A. versicolor* (ref. 5b). Its hydroxy-analogue (**5**) has been isolated as bisdeoxydehydrodothistromin from the phytotoxic fungus *Dothistroma pini* (ref. 18).



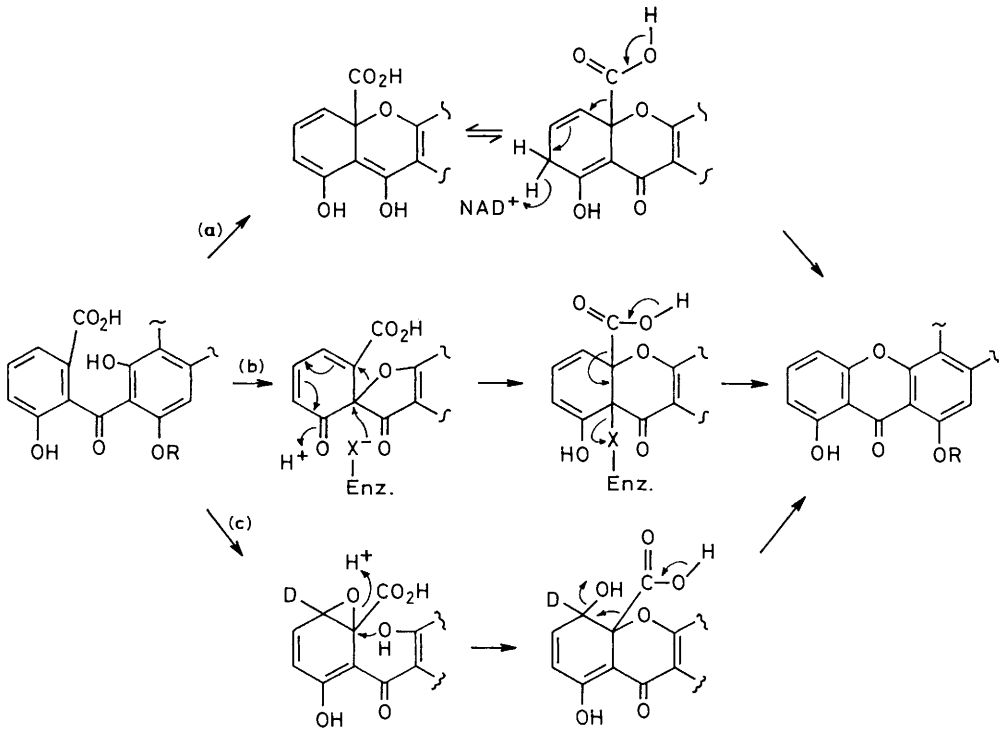
Scheme 1

In the p.n.d. ^{13}C n.m.r. spectrum of [1-^{13}C, $^{2}H_3$]acetate-enriched sterigmatocystin, the resonances due to carbons 5, 14, and 16 show β-^{2}H isotope shifts (Table 1) indicating the incorporation of ^{2}H at C-6, C-15, and C-17 only.[13] This was confirmed by the 55.3 MHz ^{2}H n.m.r. spectra in which sterigmatocystin itself showed signals at δ_H 6.83 p.p.m. (assigned[14] to 4-H and/or 6-H), 6.52 (11-H and/or 17-H), and 4.77 (15-H). On conversion into 3-*O*-methyldihydrosterigmatocystin the signals moved to δ_H 6.98, 4.18, and 3.69 p.p.m. allowing their unambiguous assignment to 6-H, 15-H and 17-H *pro-R*, and 17-H *pro-S*, respectively.[15] There is no evidence for incorporation of ^{2}H at C-4 or C-11,‡ reflecting the results obtained previously for incorporation of [$^{2}H_3$]acetate into averufin.[16]

The observed retention of acetate-derived hydrogen at C-6 is of crucial importance as it rules out all mechanisms for xanthone formation necessitating introduction of a phenolic hydroxy-group at this carbon during the biosynthesis of sterigmatocystin. On the basis of the existing information, three mechanisms appear to be feasible. These, outlined in Scheme 2, are Michael addition followed by oxidative decarboxylation, path (a), oxidative coupling to give a spiro-intermediate, *cf.* erdin,[17] followed by rearrangement and decarboxylation, path (b), or addition to an epoxide,§ path

‡ Incorporation of ^{2}H into C-4 and C-11 of sterigmatocystin has been reported, but no experimental details are available (U. Sankawa, Abstr. 28th Congress, I.U.P.A.C., Vancouver, Canada, 1981).

§ This or a very similar arene oxide would necessarily be involved in the formation of both (**7**) and (**11**) from (**6**).



Scheme 2. Possible mechanisms of xanthone ring formation.

Table 1. 90.6 MHz ^{13}C n.m.r. spectral data of sterigmatocystin (**1**) enriched from labelled acetates.

Carbon	δ_C[a](p.p.m.)	J_{C-C}[a]/Hz	$\Delta\delta_C$ (p.p.m.)[b]
1	181.2	56	
2	109.0	56	
3	162.3	69	
4	111.2	69	
5	135.5	58	−0.10
6	105.7	58	
7	154.9	—	
8	154.0	66	
9	106.5	62	
10	164.5	62	
11	90.5	71	
12	163.3	71	
13	106.0	66	
14	113.2	33	−0.04
15	48.0	33	
16	102.4	76	−0.08
17	145.3	76	
OMe	56.7	—	

[a] Sterigmatocystin enriched from [1,2-$^{13}C_2$]acetate. [b] β-^2H-isotope shift for sterigmatocystin enriched from [1-^{13}C, $^2H_3$]acetate.

(c). The latter mechanism is the one we prefer as it has the merit of leading to the previously proposed intermediate (**9**) which by concerted decarboxylation and elimination would give sterigmatocystin, or by oxidation to (**8**) followed by decarboxylation would give 6-hydroxysterigmatocystin.

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