Hydroxyversicolorone: Synthesis and Incorporation of a New Intermediate in Aflatoxin Biosynthesis

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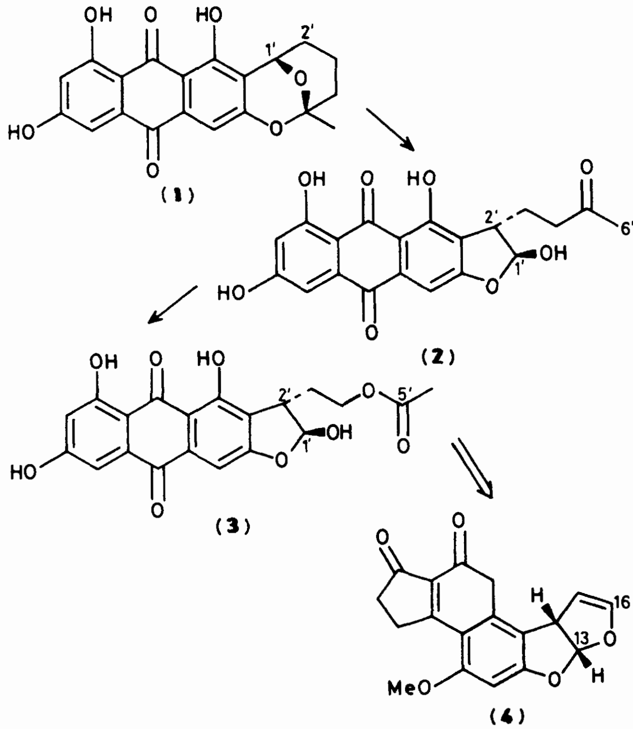
Hydroxyversicolorone, a natural product newly isolated from a blocked mutant of *Aspergillus parasiticus*, has been synthesized in labelled form and incorporated intact into aflatoxin B_1 by mycelial suspensions of wild-type *A. parasiticus.*

The conversion of the averufin (**1**) side chain into the dihydrobisfuran present in aflatoxin B_1 (**4**) is accomplished *in vivo* in three efficient oxidative stages with complete identification of the principal intermediates at each ascending oxidation step. Of these three net transformations, two are involved in the intermediate formation of versiconal acetate (**3**).[1] The proposed[2] order of these events is an oxidative anthraquinone migration from C-1′ to C-2′ to form (**2**), followed by a Baeyer–Villiger-like reaction at C-5′ to afford (**3**). To test this hypothesis, we prepared a specifically labelled sample of (**2**), a previously unknown natural product which we have dubbed 1′-hydroxyversicolorone[3] (*vide infra*), and here demonstrate its intact incorporation into aflatoxin B_1.

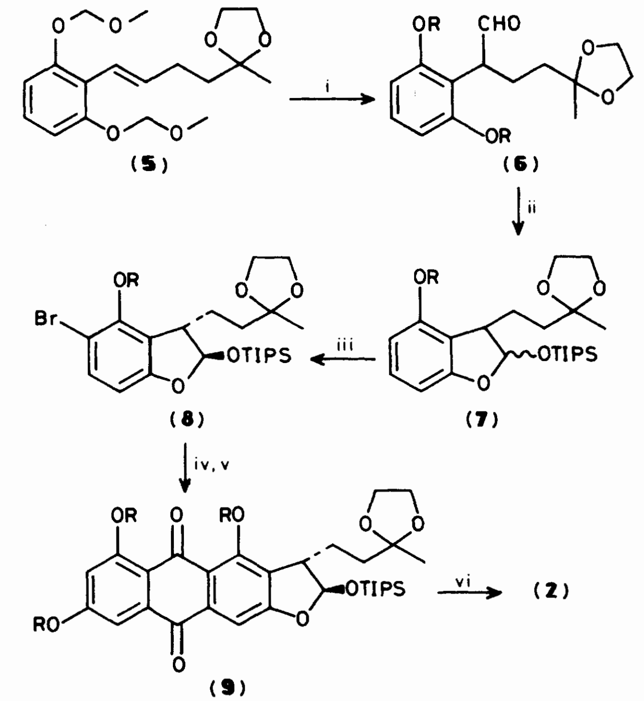
Hydroxyversicolorone (**2**) was synthesized as shown in Scheme 2. The alkene (**5**)[4] underwent oxidative rearrangement[5] to the aldehyde (**6**) in 75% yield. Attempted silyl enol ether formation using tri-isopropylsilyl triflate (TIPS-Tfl)[6] gave the expected product in only very low yield (5%) together with the differentially protected dihydrobenzofuran (**7**) (89% yield) as a chromatographically inseparable 6:1 *trans*:*cis* mixture. This latter reaction occurs rapidly, presumably owing to participation by one of the symmetry-equivalent methoxymethyl groups with the intermediate formed on initial reaction of the aldehyde with the silyl triflate. *ortho*-Metallation followed by treatment with cyanogen bromide[7,8] failed to give bromide (**8**) in more than trace amounts. Resort to electrophilic bromination, therefore, provided a 3:1 mixture of regioisomeric aryl bromides from which the major *trans*-aryl bromide (**8**) could be isolated in 52% yield (of a maximum *ca.* 64%) after flash chromatography. The anion of 5,7-bis(*O*-methoxymethyl)phthalide was reacted[7] with the benzyne generated *in situ*[7] by the dehydrobromination of (**8**) to give, after aerial oxidation, the anthraquinone (**9**) in 38% yield. Deprotection afforded racemic hydroxyversicolorone (**2**), m.p. 247—249 °C (decomp.), in 75% yield [10% overall from alkene (**5**)]; like versiconal acetate (**3**), (**2**) exists as a 1:1 mixture of isomeric hemiacetals formed to the anthraquinone 3-OH as shown and to the 1-OH.[9]†

Earlier studies have demonstrated that averufin (**1**) bearing a deuterium label at C-1′ gave rise to versiconal acetate (**3**)[1] and aflatoxin B_1 (**4**)[10] specifically labelled at C-1′ and C-13,

† The existence of hydroxyversicolorone as a 1:1 mixture of hemiacetal regioisomers made it possible subsequently to carry forward the mixture of all four isomeric bromides obtained in the reaction of (**7**) to (**8**) directly, without separation, to give (**2**) in an overall yield of 18% from (**5**).



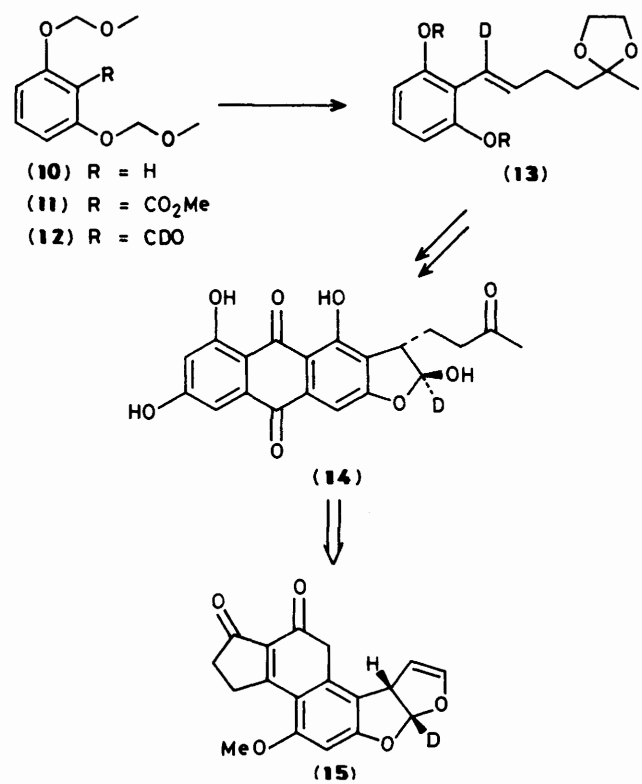
Scheme 1



Scheme 2. R = $-CH_2OMe$. *Reagents and conditions:* i, I_2, Ag_2O, dioxane–H_2O, 0 °C; ii, TIPS-Tfl, NEt_3, tetrahydrofuran (THF), 0 °C; iii, *N*-bromosuccinimide (NBS), 4 Å mol. sieves, $CHCl_3$, 0 °C → room temp., iv, lithium tetramethylpiperidide (5 equiv.), THF, −78 °C → −30 °C; HOAc and O_2, −30 °C → room temp.; vi, 1.5 M H_2SO_4, THF–H_2O, reflux.

respectively (see Scheme 1). Therefore, we chose to prepare [1′-^{2}H]hydroxyversicolorone (**14**) as outlined in Scheme 3. Bis(*O*-methoxymethyl)resorcinol (**10**) was metallated[7] and treated with dimethylcarbonate to afford ester (**11**). Lithium aluminium deuteride reduction followed by pyridinium chlorochromate (PCC) oxidation gave aldehyde (**12**)[7] in 60% overall yield from (**11**). Elaboration of (**12**) by a Wittig–Schlösser reaction as previously described[4] provided[8] the [1′-^{2}H]alkene (**13**) (Scheme 3), from which a specimen of [1′-^{2}H]hydroxyversicolorone (**14**) was obtained as above.

[1′-^{2}H]Hydroxyversicolorone (**14**) (34 mg) was administered to 17 250 ml Erlenmeyer flasks each containing wet mycelial pellets (10 g; 48 h old)[8,11] of *Aspergillus parasiticus* (SU-1) suspended in a low-sugar replacement medium (100 ml).[8,12] After 48 h, the aflatoxin B_1 (**15**) produced was isolated[8,10] and gave a ^{2}H n.m.r. spectrum in chloroform having a single resonance whose chemical shift (δ 6.81 p.p.m.) and integrated intensity relative to that of natural abundance deuteriochloroform‡ indicated a 13% incorporation of label specifically at C-13. This incorporation rate was separately estimated to be 14% by mass spectrometry.



Scheme 3

‡ Using the natural abundance deuterium signal of a solvent as an integration reference requires knowing accurately the concentration of the solute, the actual abundance of deuterium in the solvent, and the T_1 of the deuteriated solvent. The quadrupole moment of deuterium leads to generally short T_1 values, such that these nuclei in most organic compounds are fully relaxed at the end of a normal spectral acquisition. However for small, symmetrical solvent molecules, they can become significantly long. For example, chloroform (5% $CDCl_3$ in $CHCl_3$) was observed to have a T_1 of 1.38 ± 0·045 s (22 °C). Therefore, to a 1 s acquisition time was added a 6 s delay to allow 5 × T_1 for relaxation of the solvent to occur. Moreover, after standardization of the reagent grade solvent (Aldrich Gold Label, filtered through alumina) against a known concentration of crystalline reference material of high deuterium content (mass spectrometric estimation), the abundance in the solvent was determined to be 0.019 ± 0.001%, or *ca.* 120% of 'natural abundance.'

Concurrent with completion of the total synthesis of (**2**) (Scheme 2), hydroxyversicolorone was isolated from a previously undescribed mutant of *A. parasiticus* and its structure was independently determined.[9] The observation of hydroxyversicolorone as a natural product, confirmation of its structure by total synthesis, and the present demonstration of its intact incorporation into aflatoxin B_1 at a level comparable to that of averufin,[10] strongly suggest that (**2**) lies between averufin (**1**) and versiconal acetate (**3**) in the pathway to the mycotoxin. The established (1′-*S*) configuration of averufin[13] and the observed retention of the 1′-oxygen–5′-carbon bond from averufin to the acetate carbonyl of versiconal acetate[14] provide stereochemical and mechanistic support beyond the present findings for the proposed sequence of oxidative events indicated in Scheme 1. Therefore, in summary, migration of the anthraquinone nucleus in averufin (**1**) from C-1′ to C-2′ precedes Baeyer–Villiger-like oxidation in the biosynthesis of versiconal acetate (**3**). A third overall oxidative transformation then yields the dihydrobisfuran substructure, which is preserved through the subsequent steps of the pathway to aflatoxin B_1.

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