blocked several steps earlier in the pathway and by the absence of a cell-free activity capable of reducing versicolorin A to 6-deoxyversicolorin A. Therefore, unlike the seemingly closely related case of emodin reduction to chrysophanol,[3] the deoxygenation that occurs between versicolorin A (**5**, R = OH) and sterigmatocystin (**6**) must proceed by means other than direct reduction of an anthraquinone. The metabolic events whereby the anthraquinone nucleus is cleaved, deoxygenated, and rearranged to a xanthone provide entry into the final stages of the aflatoxin biosynthetic pathway and are the object of current investigation.

Acknowledgment. Drs. P. R. O. Whittamore and S. G. Davis are thanked for many preliminary studies that made possible development of the bisfuran synthesis described above. We are grateful to the National Institutes of Health for financial support of this research (ES 01670). Major analytical instrumentation used was acquired with funds from the NIH and NSF (NMR: RR01934 and PCM 83-03176; MS: RR02318).

Registry No. 5 (R = OH), 6807-96-1; (±)-**5** (R = OH), 122741-51-9; **6**, 10048-13-2; **7**, 1162-65-8; **8**, 79834-12-1; **9**, 122623-60-3; **10**, 122623-61-4; **11**, 122623-62-5; **12**, 122623-63-6; **13**, 122623-64-7; **14** (R = H), 122623-65-8; **14** (R = OSEM), 122647-58-9; **15** (R = H), 122647-57-8; **15** (R = OSEM), 122623-70-5; **16** (R = H), 122623-66-9; **16** (R = OSEM), 122623-71-6; **17** (R = H), 122623-67-0; **17** (R = OSEM), 122647-59-0; **18** (R = H), 122623-68-1; **18** (R = OSEM), 122623-72-7; **19** (R = H), 122623-69-2; **19** (R = OSEM), 122623-73-8; [9-^{13}C]versicolorin A, 122623-74-9; 6-deoxyversicolorin A, 30517-65-8.

Partitioning of Tetrahydro- and Dihydrobisfuran Formation in Aflatoxin Biosynthesis Defined by Cell-Free and Direct Incorporation Experiments

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The fused dihydrobisfuran of the mycotoxin aflatoxin B_1 (**9**) contains an electron-rich double bond that undergoes metabolic activation in vertebrates to an epoxide which is capable of generating covalent lesions upon intercalation with doubly stranded DNA.[1] This structural element is formed from the six-carbon side chain of averufin (**1**) in three efficient oxidative steps leading to versicolorin A (**5**, Scheme I).[2] In addition to the dihydrobisfuran series of metabolites a family of tetrahydrobisfurans co-occurs in generally lesser amounts typified by aflatoxin B_2 (**7**). There is disagreement as to whether these two series are interconvertible in vivo or in fact arise from separate pathways.[3] We demonstrate here that partitioning between these two bisfuran groups takes place at the point of their formation in versicolorin B (**4**) and versicolorin A (**5**).

In 1980 Hsieh[4] and Dutton[5] independently reported the preparation of cell-free systems from *Aspergillus parasiticus* capable of carrying out the conversion of versiconal acetate (**3**) to versicolorin A (**5**). In the former a time course study revealed, surprisingly, at least four apparent intermediates in this process. With the intention of determining their structures, several protocols for cell breakage and spheroplast generation were surveyed in an effort to duplicate this conversion. Cell-free systems prepared by several methods, notably rapid disruption with glass powder in phosphate buffer,[6] gave clean, time-dependent reactions of versiconal acetate (**3**) to form not versicolorin A (**5**) but versicolorin B (**4**)[7,8] by way of the diol corresponding to hydrolysis of the acetate in **3**.[9] Complete conversion of **3** to **4** was readily achieved indicating that racemization of the former is rapid relative to the rate of selective enzymic processing of the (*S*)-antipode to bisfuran. Versicolorin A hemiacetal (**10**) was prepared from versicolorin A by acid-catalyzed hydration of the terminal double bond.[11] To test its potential intermediacy in dihydrobisfuran formation, incubation of this substrate with the cell-free extract above and in the presence of NAD^+, NADH, $NADP^+$, or NADPH gave only *reduction* to versicolorin B and no detectable dehydration to versicolorin A.

The unexpected results of these cell-free experiments led directly to the hypothesis shown in Scheme I that versicolorin B (**4**), first, was an intermediate in versicolorin A (**5**) formation, and, second, played a pivotal role in aflatoxin biosynthesis serving as the point of partition between tetrahydro- and dihydrobisfuran-containing products. In a direct test of this proposal averufin (**1**), an earlier

Scheme I

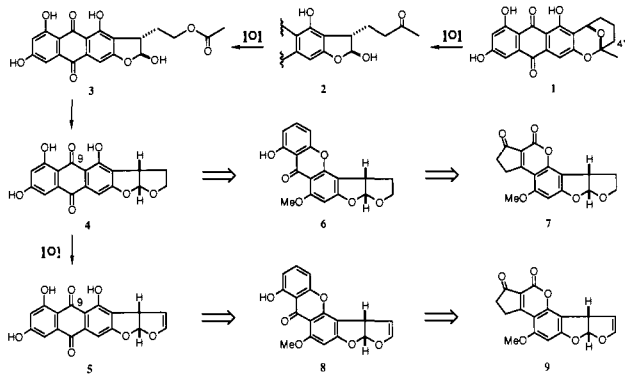

Table I. Incorporation of ^{13}C-Labeled Substrates into Aflatoxin B_1 and B_2

substrate	% incorp (SU-1) AFB_1	% incorp (SU-1) AFB_2
(±)-[4′-^{13}C]averufin	9	10
(±)-[9-^{13}C]versicolorin B(C)	9	25
(±)-[9-^{13}C]versicolorin A hemiacetal	8	15
(±)-[9-^{13}C]versicolorin A	20	0

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(6) Mycelial pellets of SU-1 were homogenized by using a "Bead-Beater" (Biospec, Bartlesville, OK) with 3.3 × their wet weight of glass powder (0.10-0.11 mm diamter) in 0.2 M potassium phosphate buffer (pH 7.5 at 22 °C) precooled in an ice/water bath. Duty cycles of 1 min were alternated with 1 min at rest for a total of 20 min. The homogenate was decanted and centrifuged (20 000 × g, 25 min at 0 °C); the supernatant was taken as the cell-free extract. See also ref 4.

(7) The identity of versicolorin B was established by HPLC comparisons to authentic materials (vide infra and accompanying paper) and by ^{1}H NMR and MS analyses.

(8) Versicolorin B and A are relatively difficult to separate chromatographically. Some of the conflicting claims in the literature may stem from this technical problem leading to product misidentification and/or radiochemical contamination.

(9) For comparison the diol (the name versiconal has been proposed for this compound by Gorst-Allman,[10] although it has not been isolated or synthesized and characterized prior to the present work) was prepared by saponification of versiconal acetate and independently correlated to other known structures (Brobst, S. W.; McGuire, S. M.; Townsend, C. A., unpublished).

(10) Gorst-Allman, C. P.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1987**, 163-168.

(11) Chen, P. N.; Kingston, D. G. I.; Vercellotti, J. R. *J. Org. Chem.* **1977**, *42*, 3599-3608.

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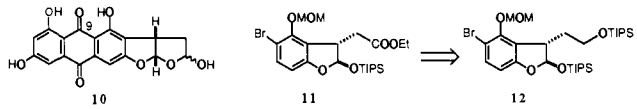
intermediate,[12,13] versicolorin B (**4**) and versicolorin A hemiacetal (**10**), which is reduced to **4** under cell-free conditions, should partition in ^{13}C-labeled form between aflatoxin B_2 (**7**) and B_1 (**9**). Versicolorin A (**5**), however, should incorporate label solely into aflatoxin B_1 (**9**).



10 11 12

Racemic [4'-^{13}C]averufin,[12] [9-^{13}C]versicolorin B (C),[14] [9-^{13}C]versicolorin A hemiacetal, and [9-^{13}C]versicolorin A were adminstered to mycelial suspensions of wild-type *A. parasiticus* (SU-1) under standard conditions.[2,13] After quantification by HPLC analysis of the aflatoxin B_1 and B_2 produced,[15] **7** and **9** were separated and further purified by preparative radial chromatography (silica gel, $CHCl_3$/acetone 20:1). The sites and extent of ^{13}C-incorporation were determined by $^{13}C\{^1H\}$ NMR spectroscopy and mass spectrometry. In each instance the locus of ^{13}C-enrichment in both aflatoxin B_1 and B_2 was that expected. The levels of ^{13}C-incorporation are summarized in Table I.

In *A. parasiticus* (SU-1) about 20 times more aflatoxin B_1 (**9**) is generated than B_2 (**7**). As might be predicted for an earlier pathway intermediate, i.e., averufin (**1**), steady-state flux through the proposed branch point metabolite versicolorin B (**4**) would be expected to lead to equal levels of isotopic labeling in **7** and **9**, as was observed. Addition of versicolorin B (**4**) itself, however, perturbs that steady state and a significantly enhanced enrichment of ^{13}C was observed in the minor metabolite aflatoxin B_2 (**7**). Entirely in keeping with the cell-free experiments above, label from the hemiacetal **10** partitioned similarly to versicolorin B, consistent with its reduction to this tetrahydrobisfuran prior to utilization in the pathway. In contrast, no label from versicolorin A (**5**) was detected in aflatoxin B_2 (**7**) indicating irreversible desaturation of versicolorin B to versicolorin A.

In conclusion, formation of the critical dihydrobisfuran is a three-step process from versiconal acetate (**3**) involving an acetate hydrolysis/cyclization sequence to give versicolorin B (**4**) and an apparent oxidative desaturation step to irreversibly form versicolorin A (**5**). Both 1'-hydroxyversicolorone (**2**) and versiconal acetate (**3**) are isolated as racemates.[16] Cell-free experiments have demonstrated, however, that versiconal acetate (**3**) can be *completely* converted to (−)-versicolorin B (**4**) suggesting that either a single, bifunctional enzyme or two enzymes selectively process one enantiomer of **3** to establish the absolute configuration of the critical bisfuran ring system in **4**. The stereoelectronic arguments made earlier,[17] based on the absolute configuration of averufin (**1**),[18] would give **2** and **3** in the correct configuration for conversion to bisfurans but fail, however, to account for the competing facility of these intermediates to racemize relative to their rates of forward reaction in the biosynthetic pathway. Once formed, separate pathways diverge from versicolorin B (**4**) to give rise to the tetrahydro- and dihydrobisfuran-containing aflatoxins **7** and **9**. The hydroxylated species **10**, which might be imagined to be involved in the oxidation of **4** to **5**, does not undergo dehydration under either cell-free or whole-cell conditions, but is reduced to versicolorin B.[19]

Acknowledgment. We are grateful to the National Institutes of Health for financial support of this research (ES 01670). Major analytical instrumentation used was acquired with funds from the NIH and NSF (NMR: RR01934 and PCM 83-03176; MS: RR 02318).

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(14) Reduction of **11** with $LiAlH_4$ and protection of the resulting alcohol gave **12**, which was converted in 45% overall yield to versicolorin C (the racemate of versicolorin B) essentially as described for versicolorin A (see preceding paper).

(15) Modification of the method of Schmidt et al. (Schmidt, R.; Mondani, J.; Ziegenhagen, E.; Dose, K. *J. Chromatogr.* **1981**, *207* 435-438).

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(19) Hydration, enzymic or chemical, of dihydrobisfurans followed by reduction to the tetrahydro series may provide a minor route for conversion between these bisfuran groups.[3] The results in Table I indicate that this is not a significant process for versicolorin A or for later dihydrobisfuran-containing intermediates in the pathway under the present experimental conditions.

Regioselectivity and Diastereoselectivity in Free-Radical Macrocyclization

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The development of free-radical methods for the synthesis of complex organic molecules continues to attract much attention.[1] Construction of five-membered rings has been one of the cornerstones of recent synthetic developments, but the synthesis of large rings by free-radical cyclization has also recently been reported.[2] Macrocycle yields can be as high as 90% for secondary or tertiary radicals if the alkene is electron deficient. Steric effects have a profound effect on the reaction.[3,4] We report here on new substrates, **1a-d**, for macrocyclization that have X = CH_2 and Z = COOEt or $CONR_2$ (Scheme I). Cyclizations of these compounds are highly selective for the endocyclic product. Furthermore, substrates where Z is a chiral amide derived from alanine, **1c** and **1d**, give products that are highly enriched in one of the four possible diastereomeric cyclic products.

The synthesis of the substrates **1a-d** is straightforward, the key step being phosphonate coupling to form the electrophilic alkene.[5] For compounds **1b-d**, for example, the coupling reaction between $Br(CH_2)_mCOCH_2PO(OEt)_2$[6] and the α dicarbonyl $HCOCONR_2$ gave $Br(CH_2)_mCOCH{=}CHCONR_2$ (70%), which was converted to the iodide by reaction with NaI (quantitative). The substrate **1a** was prepared via the sequence $Br(CH_2)_{12}COCHO$ + $EtOOCCH_2PO(OEt)_2 \rightarrow Br(CH_2)_{12}COCH{=}CHCOEt$ (70%) $\rightarrow$ **1a** (quantitative).

Mixtures of cis and trans geometric isomers were sometimes formed in the coupling reaction, and, if formed, the cis isomers were converted to the trans by reaction with I_2. The dicarbonyl $HCOCONR_2$ was prepared by reaction of the nitrate $O_2NOCH_2CONR_2$ with NaOAc in DMSO;[7] $Br(CH_2)_{12}COCHO$ was prepared by Swern oxidation of $Br(CH_2)_{12}CHOHCH_2OH$.[8] The pyrrolidine used for the amide of **1c** and **1d** was prepared by the method of Schlessinger, and the starting material used in this synthesis was the unnatural (*R*)-alanine since cyclization

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(5) See, for example, Roush, W. R.; Coe, J. W. *Tetrahedron Lett.* **1987**, *28*, 921.

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