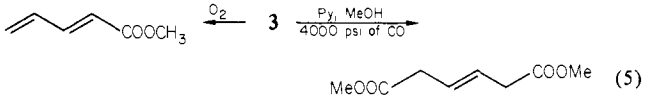
added ligands follows as trend $PPh_3 < P(OEt)_3 \ll PF_3 < (n\text{-octyl})_3PO < CO$).

Thus, a plausible comprehensive mechanism for reaction 1 is presented in Scheme II. Based on this mechanism, scavenging of $HCo(CO)_4$ from the reaction mixture should stop the catalytic cycle and result mostly in dicarbomethoxylation to dimethyl 3-hexenedioate, since we have observed that complex **3** undergoes carbomethoxylation to yield this diester (eq 5). Indeed, when



(5)

sodium carbonate is added to reaction 1, a stoichiometric reaction takes place, yielding mainly dimethyl hexenedioate esters. When reaction 1 is carried out in the presence of traces of oxygen, methyl 2,4-pentadienoate is also formed. This can also be explained by the intermediacy of **3**, which readily undergoes this oxidation process (eq 5). Studies aimed at direct observation of the methanolysis of the ion-pair **6** are now in progress.

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Registry No. 1, 83269-56-1; **2**, 83269-57-2; **3**, 83269-58-3; **4**, 83269-59-4; **5**, 64537-27-5; $Co_2(CO)_8$, 10210-68-1; py, 110-86-1; butadiene, 106-99-0; methyl 3-pentenoate, 818-58-6.

Supplementary Material Available: Tables of positional and thermal parameters for compound **2** (7 pages). Ordering information is given on any current masthead page.

Bisfuran Formation in Aflatoxin Biosynthesis: The Fate of the Averufin Side Chain

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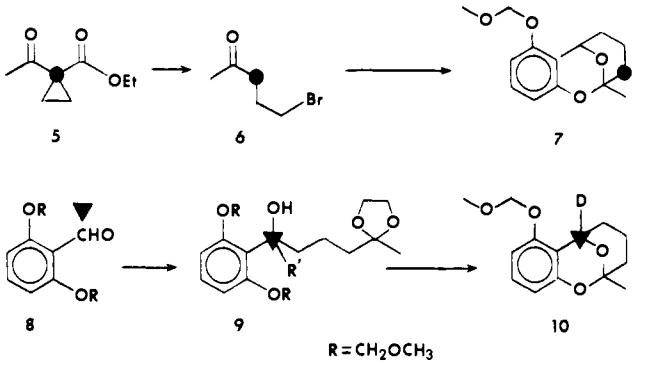
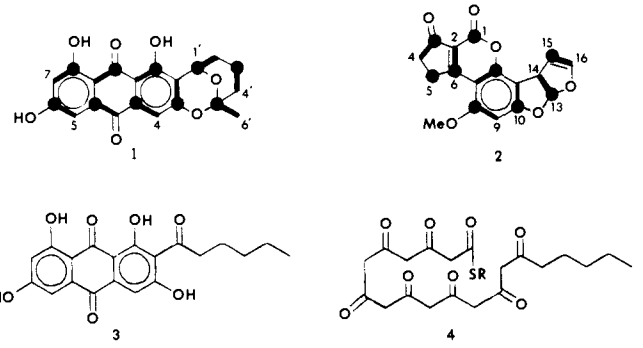
Biollaz, Büchi, and Milne in classic studies of aflatoxin B_1 (**2**) biosynthesis determined the origin of 13 of its 17 carbon atoms from acetate and methionine, results that indicated derivation of this toxin from a single, albeit highly rearranged, polyketide chain.[1] In the intervening years a chemically plausible series of intermediates has been advanced from extensive experiments using blocked mutants of *Aspergillus parasiticus* and metabolic inhibitors of the wild-type strain.[2-5] A second line of evidence has established[6] a common polyketide folding pattern through these intermediates as revealed in [1,2-$^{13}C_2$]acetate incorporation experiments (so indicated in **1** and **2** by heavy lines, the dot signifying C-1). However, the mechanistic details of the remarkable transformations required to connect the proposed intermediates of this pathway have been the object only of speculation for lack of discriminating experimental evidence. Described in this communication and its companion are experiments that specifically address the issue of bisfuran formation, the unique structural feature of this family of mycotoxins, and the seat of their lethal biological activity.[7]

Scheme I





Averufin (**1**) has emerged as the pivotal anthraquinone intermediate in the pathway to aflatoxin.[3,6] It is apparently derived by way of norsolorinic acid (**3**),[3,4] which may in turn be formulated in conventional fashion from a C_{20} polyketide, e.g., **4**.[8] As revealed in earlier work,[9,10] the linear C_6 ketal side chain of averufin (**1**), containing three intact acetate units, becomes branched in the C_4 bisfuran of aflatoxin B_1 (**2**), and one of the three acetate units is lost. Outlined in Scheme I are total syntheses of specifically labeled specimens of racemic averufin, (**11**) and (**13**), and below the results of studies that demonstrate their intact incorporation into aflatoxin B_1.

[2-^{13}C]Ethyl acetate (90% enriched) was homologated[11] to [2-^{13}C]ethyl acetoacetate, which was treated with 1,2-dibromoethane (acetone/K_2CO_3) to afford cyclopropane derivative **5**.[12] Hydrobromination 48% HBr) gave [3-^{13}C]-5-bromo-2-pentanone (**6**)[13] in 45% overall yield. Protection of **6**, formation of the corresponding lithium reagent (2% Na/Li) followed by reaction with 2,6-bis(methoxymethyl)benzaldehyde and partial deprotection as previously described,[14] gave the 4′-labeled tricyclic ketal **7** in

† Research Fellow of the Alfred P. Sloan Foundation 1982-1984.

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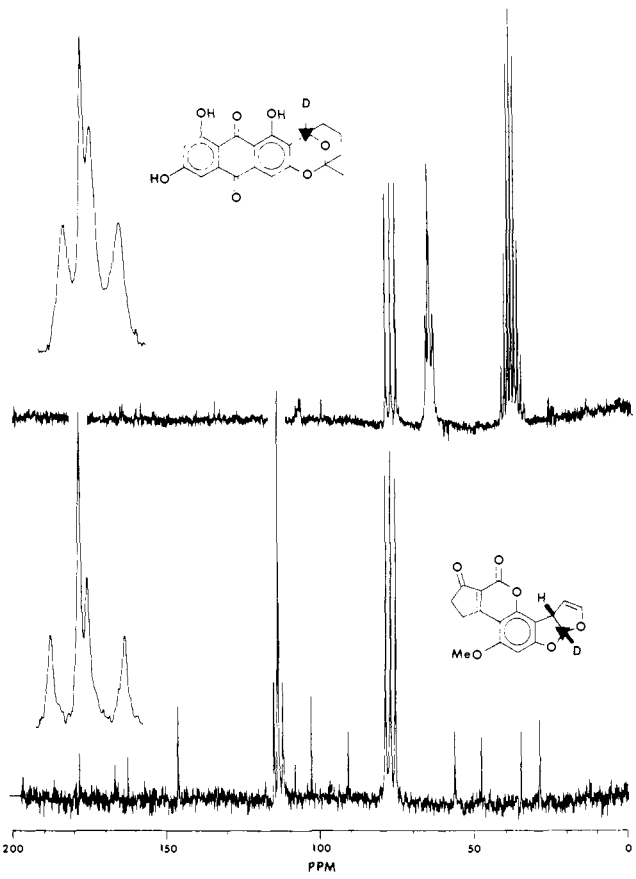
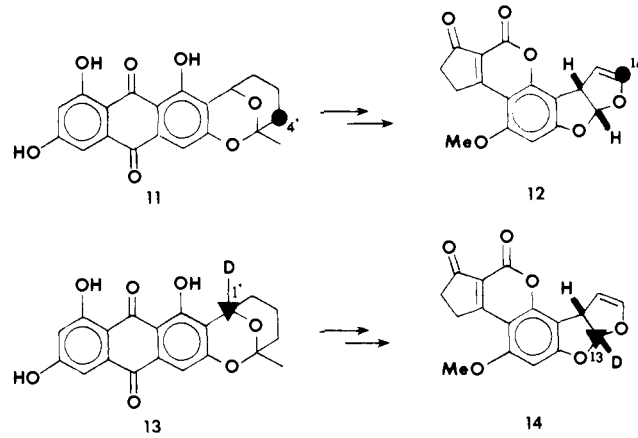


Figure 1. $^{13}C\{^1H\}$ NMR spectra of (top) [1′-^{2}H,^{13}C]averufin (**13**; 15 mg in 2.5 mL 1:1 $CDCl_3$:Me_2SO-d_6 25 000 transients) and (bottom) derived aflatoxin B_1 (**14**; 16 mg in 0.5 mL $CDCl_3$, 22 250 transients) obtained under the following conditions: Varian CFT-20, 20 MHz; spectral width 4000 Hz, 8K points, acquisition time 1.023 s, pulse delay 2.25 s.

35% yield from **6**. Aldehyde **8**, prepared from [^{13}C]dimethylformamide,[14] was converted to alcohol **9** (R′ = H).[14] Careful Sarett oxidation[15] followed immediately by reduction with lithium aluminum deuteride generated **9** (R′ = D), which was cyclized as before[14] to doubly labeled tricyclic ketal **10** (ca. 85% deuterium incorporation estimated by ^{1}H NMR). The labeled ketals **7** and **10** were separately converted to [4′-^{13}C]- and [1′-^{2}H,^{13}C]averufin (**11** and **13**, respectively) by extension of the synthetic route published earlier.[14]

[4′-^{13}C]averufin (**11**, 40 mg)[16] was administered to ten 250-mL Erlenmeyer flasks each containing 10 g of wet 48-h-old mycelial pellets[18] of *A. parasiticus* (ATCC 15517) suspended in 100 mL of low sugar replacement medium.[19] After 24 h the aflatoxin B_1 produced was isolated by chloroform extraction and silica gel chromatography. $^{13}C\{^1H\}$ NMR analysis of the purified toxin **12** gave a strong singlet at δ 145.1, indicating a >20% specific incorporation of averufin (**11**) and showing enrichment at C-16 exclusively[20,21] as shown in **12**. The site of label was verified in the NOE-enhanced coupled ^{13}C NMR spectrum where two-bond coupling to H-15 (11.6 Hz) and three-bond coupling to H-13 and H-14 (4.5 Hz) was observed. Therefore, the central of the three intact acetate units of the averufin (**1**) side chain becomes the outer unit of the two retained in the bisfuran of aflatoxin B_1 (**2**).



The $^{13}C\{^1H\}$ NMR spectrum of 1′ doubly labeled averufin **13** is displayed in Figure 1 (top). At δ 66.2 there appears a singlet corresponding to about 15% (vide supra) of $^{13}C/^1H$ material present. Superimposed on this signal is a three-line manifold centered at δ 65.9 ($^1J_{C-D}$ = 22.6 Hz) constituting the major, doubly labeled $^{13}C/^2H$ species. The relative intensity of these latter resonances appears disproportionately weak owing to the considerably reduced efficiency of ^{13}C relaxation in the absence of a bound proton in the $^{13}C\{^1H\}$ experiment.[22] Submission of averufin (**13**, 40 mg) as above to replacement cultures of *A. parasiticus* but for 40 h rather than 24 h afforded, after isolation and purification, aflatoxin B_1 (**14**). The $^{13}C\{^1H\}$ NMR spectrum (Figure 1, bottom) revealed again significant enrichment at a single site, C-13.[20,21] In the expansion a singlet is observed at δ 113.3 and superimposed is a 1:1:1 triplet at δ 113.0 ($^1J_{C-D}$ = 28.5 Hz) corresponding to the $^{13}C/^1H$- and $^{13}C/^2H$-labeled entities, respectively. Most important, the relative intensities of lines arising from carbon label bound to hydrogen and deuterium are substantially if not completely unchanged in the transformation of **13** to **14**, indicating no loss of deuterium in the process.

In sum, therefore, the inner and central two acetate units of the averufin (**1**) side chain are utilized in vivo to construct the bisfuran of aflatoxin B_1 (**2**) as shown in **12** and **14**; the terminal unit is lost. The process by which this loss takes place will be taken up in the accompanying paper. In the course of the branching transformation of the side chain, migration of the tetrahydroxyanthraquinone nucleus from C-1′ to C-2′ must occur in such a way that deuterium is retained at C-1′. A Favorski-like process is therefore excluded[23,24] but other suggested mechanisms involving rearrangement of an open-chain epoxide[10] or a pinacol-like rearrangement[25] remain consistent with existing data.

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Registry No. (±)-**1**, 79896-28-9; (±)-**2**, 10279-73-9; **5**, 83152-78-7; **6**, 83152-79-8; (±)-**7**, 83159-82-4; **8**, 83152-80-1; (±)-**9** (R′ = H), 83152-81-2; (±)-**9** (R′ = D), 83152-82-3; (±)-**10**, 83152-83-4; (±)-**11**, 83152-84-5; (±)-**12**, 83152-85-6; (±)-**13**, 83152-86-7; (±)-**14**, 83159-83-5.

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