The Regiochemistry of A-Ring-labelled Averufin Incorporation into Aflatoxin B_1

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A-Ring [^{13}C]-labelled averufin has been synthesized and found to be incorporated intact into aflatoxin B_1, where the labelling pattern in the fused cyclopentenone unequivocally establishes the origin of the carbon backbone as consistent with current biogenetic theories.

An ambiguity underlying investigation of the aflatoxin biosynthetic pathway is the possible misidentification of shunt metabolites as obligatory intermediates or *vice versa*.[1] Recent experiments[2] place on more solid footing the role of nor-

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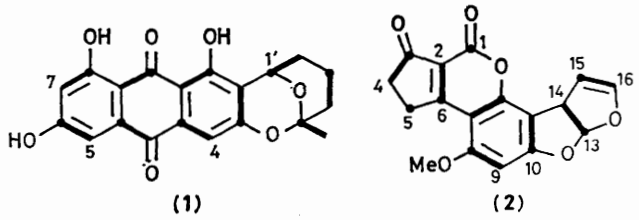

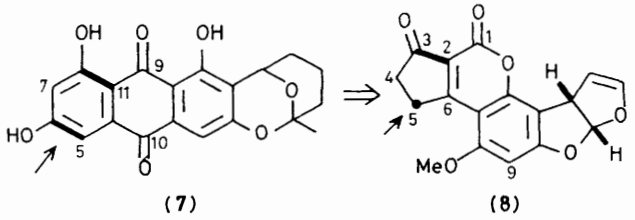

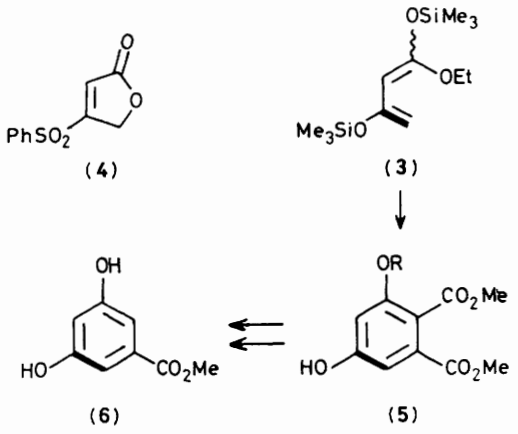

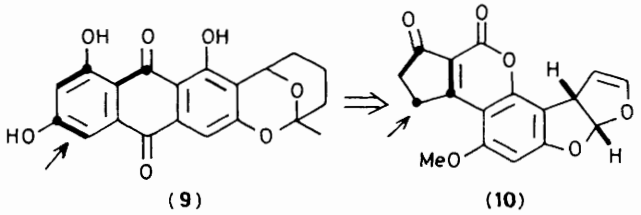

solorinic acid as the first-formed anthraquinone precursor followed linearly by averantin and averufin.[3] Labelled samples of averufin (**1**) bearing ^{13}C- and/or ^{2}H-isotope in the C_6-side chain have been shown to be specifically incorporated into the bisfuran of aflatoxin B_1 (**2**)[4] and the corresponding structural elements of versiconal acetate.[5] To complete this correlation, we establish herein that specimens of averufin (**7**) bearing A-ring [^{13}C]-labels are indeed specifically incorporated into the cyclopentenone carbons of aflatoxin B_1 (**2**) in the fashion suggested from earlier studies[6] {the pattern of [1,2-$^{13}C_2$]acetate labelling in (**1**) and (**2**) is indicated by heavy lines, the dot signifying C-1 }.

Initial attempts to prepare A-ring labelled averufin centred on the reaction of the known[7] siloxydiene (**3**) with the butenolide (**4**)‡ which was prepared in four steps from α-bromo-γ-butyrolactone.§ Only poor yields, *ca.* 25%, of the desired 5,7-dihydroxyphthalide were obtained under a variety of conditions together with significant quantities of dark polymeric material and other unidentified products owing presumably to the adverse effects of the liberated benzenesulphinic acid coproduct. Reaction of (**3**), however, with dimethyl acetylenedicarboxylate[7a] gave (**5**) as a mixture of products (R = H and Et) in 55—60% yield. Therefore, [1,2-$^{13}C_2$]acetyl chloride was homologated[8] to give ethyl [3,4-$^{13}C_2$]acetoacetate and converted into (**3**). Reaction as above gave doubly labelled (**5**) as a mixture (R = H:R = Et, 1:2) which, after separation by silica chromatography, de-ethylation,[9] saponification–decarboxylation,[10] and esterification, afforded (**6**), labelled as shown, in 20% overall yield from ethyl [3,4-$^{13}C_2$]acetoacetate. Methyl [2,3-$^{13}C_2$]-3,5-dihydroxybenzoate (**6**) was converted as described earlier[4a,11] into a 1:1 mixture of (±)-[5,6-$^{13}C_2$]- and [8,11-$^{13}C_2$]-averufin (**7**) in 10% yield from (**6**).

The mixture of doubly-labelled averufins (**7**) (20 mg) was administered to suspensions of 48 h-old mycelial pellets of *Aspergillus parasiticus* (ATCC 15517).[4a,12] After 48 h the aflatoxin B_1 produced was isolated by chloroform extraction and silica gel chromatography. $^{13}C\{^1H\}$-N.m.r. analysis of the crystallized toxin (**8**) gave a strong singlet at δ 29.0 p.p.m. indicating approximately 10% specific incorporation of [^{13}C]-label at C-5.[13] In addition, enhanced doublets centred at δ 117.3 and 201.2 p.p.m. (J_{CC} 57 Hz) were observed that may be securely assigned[13] to C-2 and -3 in (**8**). Therefore, one of the two paired ^{13}C-labels 5/6 or 8/11 in averufin (**7**) may be correlated through intact incorporation to carbons 2/3 in aflatoxin B_1 (**8**). For comparison, the relevant patterns of labelling in averufin and aflatoxin B_1 [*cf.* (**1**) and (**2**)] from [1,2-$^{13}C_2$]acetate are presented in (**9**) and (**10**). Common to both experiments is the C-5/6 unit [arrow in (**7**) and (**9**)] which leads uniquely to the single label at C-5 in (**8**) and (**10**); one labelled carbon is lost. This position is further known[6] to be derived from C-1 of acetate. Therefore, C-6 of averufin transforms specifically to C-5 of aflatoxin B_1. It must be, therefore, that C-8/11 in averufin correlate specifically to C-2/3 in aflatoxin B_1 (**8**). It then follows that C-7/8 in (**9**) translate to C-3/4 in (**10**) and lastly that C-11/9 in (**9**) lead to the intact acetate unit C-2/6 in (**10**).

In conclusion, preparation and incorporation of averufin specifically [^{13}C]-labelled in the A-ring has further confirmed the intact incorporation of this key anthraquinone intermediate into aflatoxin B_1. While the apparent loss of the averufin 6-hydroxy group remains to be studied, the labelling pattern found in the fused cyclopentenone ring of aflatoxin, in reference to previously observed patterns of [1,2-$^{13}C_2$]-acetate incorporation in both (**1**) and (**2**), unequivocally establishes the folding of the progenitor polyketide backbone, readily identifiable in averufin, through the cleavage and rearrangement steps to aflatoxin B_1.

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‡ It has been noted that certain phenylsulphonyl substituted dienophiles undergo facile additions to even unreactive dienes; see for example: M. Shen and A. G. Schultz, *Tetrahedron Lett.*, 1981, 3347.

§ i, But-2-en-4-olide (C. C. Price and J. M. Judge, *Org. Synth., Coll. Vol. V*, 1973, 255); ii, β-phenylthio-γ-butyrolactone [PhSH–NaH (catalytic)–tetrahydrofuran, room temperature (r.t.), 80%]; iii, 3-phenylthiobut-2-en-4-olide (*N*-chlorosuccinimide–$CHCl_3$, r.t., 70%); iv, 3-phenylsulphonylbut-2-en-4-olide (2 equiv. *m*-chloroperbenzoic acid–$CHCl_3$, r.t., 70%).

the facilities to do so. The National Institutes of Health are gratefully acknowledged for financial support.

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