# Synthesis of Averufin and its Role in Aflatoxin $B_1$ Biosynthesis

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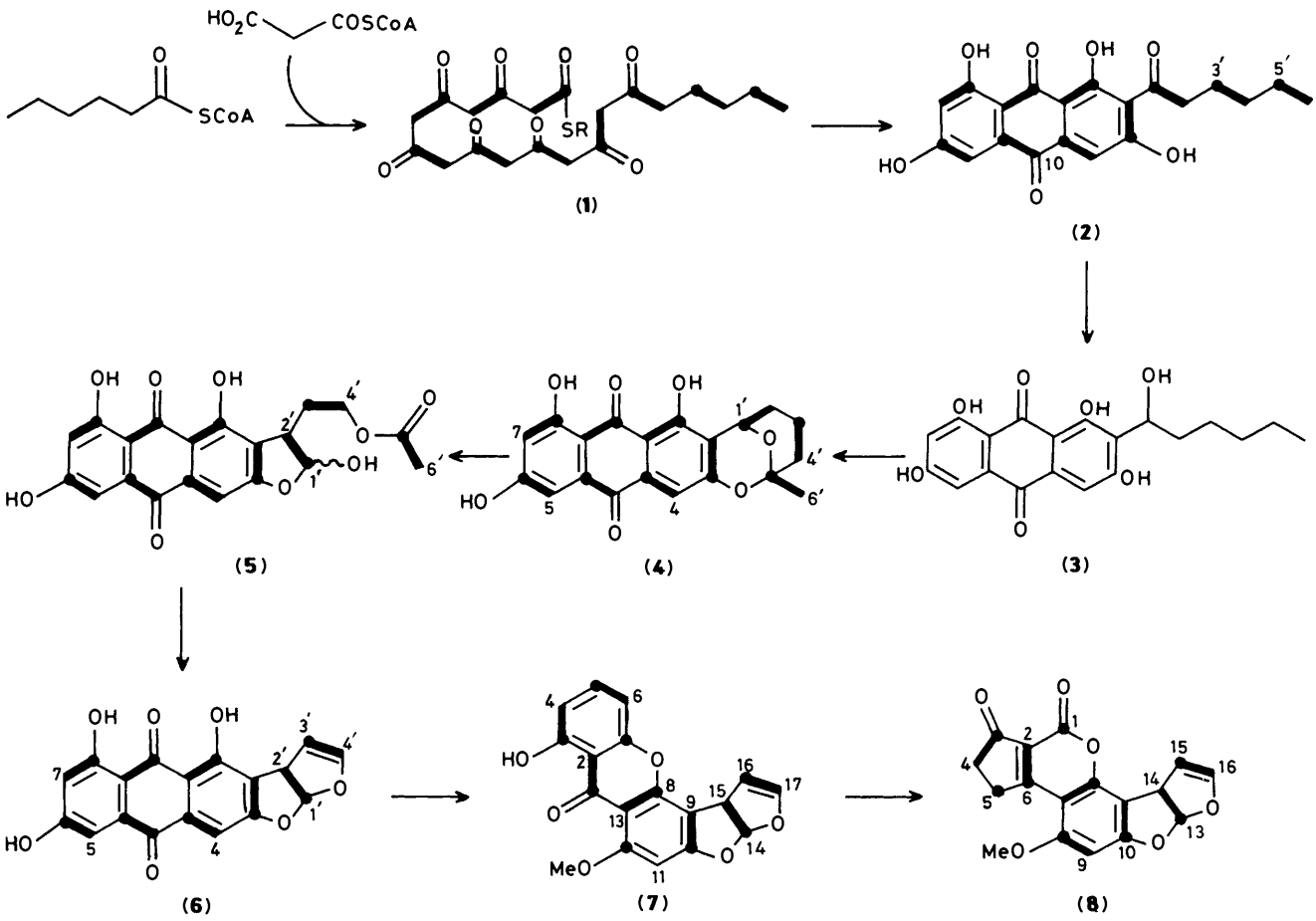
Described are two total syntheses of (±)-averufin (**4**) proceeding through a common intermediate and predicated on the efficient introduction of isotopic label(s) at side-chain and nuclear sites for the purpose of biosynthetic investigations of aflatoxin $B_1$ (**8**). Using these methods, (±)-[4′-$^{13}$C]- and -[1′-$^{13}$C,$^{2}$H]-averufin, (**65**) and (**68**) respectively, and a 1:1 mixture of (±)-[5,6-$^{13}C_2$]- and -[8,11-$^{13}C_2$]-averufin (**71**) were prepared and incorporated into aflatoxin $B_1$ using mycelial suspensions of *Aspergillus parasiticus* (SU-1). In each instance efficient and specific utilization of label was observed in the product by $^{13}$C-{$^{1}$H} n.m.r. spectroscopy, demonstrating the intact incorporation of averufin. When compared with earlier observations of [1,2-$^{13}C_2$]acetate incorporation, a complete correlation of the carbon skeleton from the intermediate anthraquinone stage of the pathway to the substituted coumarin of compound (**8**) was made possible, and the four carbons lost in this overall process were unambiguously identified. In the formation of the dihydrobisfuran, the anthraquinone nucleus migrates to C-2′ to branch the linear side-chain of averufin. Deuterium bound at C-1′ in averufin is carried to C-13 of aflatoxin. Preparation from (±)-[1′-$^{13}$C,$^{2}$H]averufin (**68**) of (±)-[1′-$^{13}$C,1′,4′,4′,6′,6′,6′-$^{2}H_6$]-averufin (**73**) and incorporation of the latter into versiconal acetate (**5**) demonstrated loss of the terminal two carbons of the averufin side-chain, presumably as acetate, by way of a Baeyer–Villiger-like oxidation.

Assembly of the carbon skeleton necessary for aflatoxin $B_1$ (**8**) biosynthesis is completed early in the pathway with the formation of norsolorinic acid (**2**). Apart from the notable utilization of a linear six-carbon primer, hexanoyl-CoA,[1] its presumed homologation, cyclization to an anthrone, and subsequent elevation[2] to the anthraquinone oxidation state [in structure (**2**)] are familiar processes in aromatic polyketide biosynthesis, although in fact poorly understood in their intimate details. Redox changes along the side-chain and oxidative rearrangements and cleavages mark the subsequent modifications of this template to generate ultimately the coumarin nucleus of aflatoxin $B_1$ bearing the fused dihydrobisfuran characteristic of this family of mycotoxins. The susceptibility of the isolated, electron-rich double bond of this latter structural feature to oxidation in mammalian tissue, especially liver and kidney,[3] combined with the molecular dimensions of this planar array, confer a lethal propensity to intercalate in GC-rich regions of doubly stranded DNA[4] and to deliver a reactive electrophilic site capable of forming covalent lesions at N-7 of guanine residues.[5] Identification and radiochemical experiments ordering[1,6–8] the proposed intermediates shown in Scheme 1 were made possible by the availability of *Aspergillus parasiticus* mutants which accumulate norsolorinic acid (**2**),[9] averantin (**3**),[6] averufin (**4**),[10] and versicolorin A (**6**).[11] Versiconal acetate (**5**)[12] appears in fermentations of the wild-type *A. parasiticus* grown in the presence of 10 p.p.m. of Dichlorovos®, an organophosphate insecticide, while sterigmatocystin (**7**), a known metabolite of *A. versicolor*,[13] has been detected in aflatoxigenic strains of *A. parasiticus* and *A. flavus*.[14] Incorporations of [1,2-$^{13}C_2$]acetate, principally by Steyn's group in Pretoria,[15] have provided an independent line of evidence supporting the sequence of intermediates shown in Scheme 1 by establishing a common polyketide folding pattern throughout the pathway as exemplified by structure (**1**).

The pivotal intermediate in Scheme 1 is averufin (**4**) whose ketal side-chain is oxidatively rearranged and cleaved, with loss of two carbons, to the bisfuran of versicolorin A (**6**), whereupon oxidative modification of the anthraquinone nucleus ensues with the sequential loss of two additional carbons to yield the xanthone of sterigmatocystin (**7**) and finally the substituted coumarin of aflatoxin (**8**). A number of transformations of biosynthetic significance are evident. The initial phase of work at Johns Hopkins was directed toward demonstrating the intact incorporation of averufin into aflatoxin. Specificity of labelling is an essential condition of intermediacy which, while probable, was not strictly proved in the radiochemical and stable isotope experiments noted above. Through the preparation and incorporation of selectively labelled specimens of averufin, specificity of incorporation could be unambiguously demonstrated; and, through the careful choice and location of marker, particular mechanistic questions could be addressed at the level of an advanced intermediate. This first phase of investigation focussed on the mode of bisfuran formation and on an overall mapping of carbons from the anthraquinone skeleton of (**4**) into the nuclear sites of (**8**). Some of these initial studies have been described in preliminary form.[16–18] We record here the full details of these experiments and the synthetic methods developed to support this and later phases of the investigation.

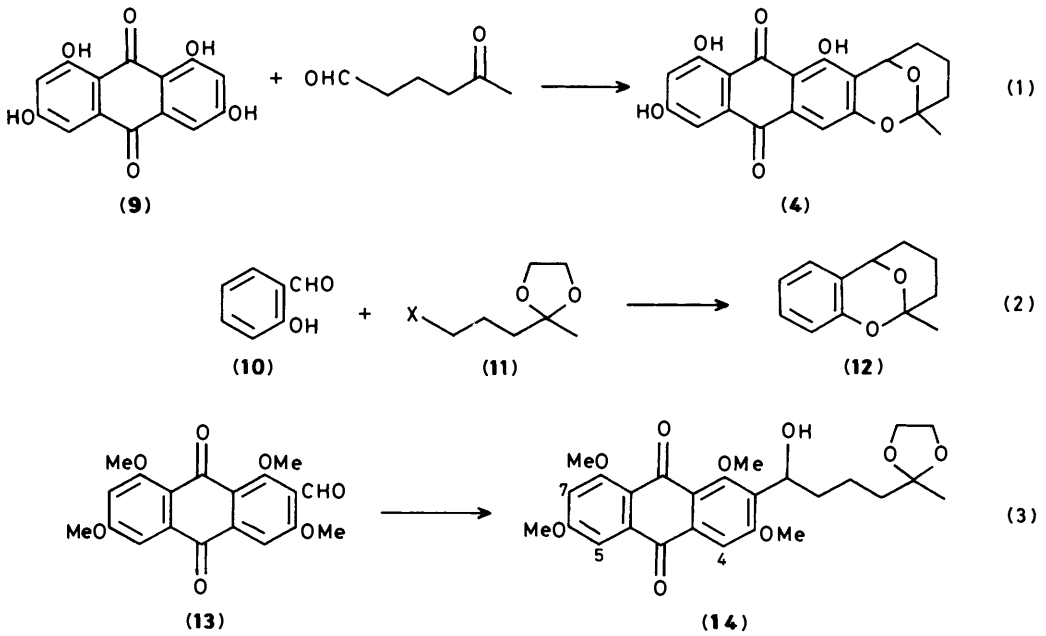
*Synthesis of Averufin* (**4**).—Perhaps the most obvious synthetic approach to averufin (**4**) would recognize the symmetry and synthetic accessibility of 1,3,6,8-tetrahydroxyanthraquinone (**9**). Castonguay and Brassard[19] investigated hydroxyalkylation of this substrate with 5-oxohexanal (2 equiv.), but were only able to achieve conversions of 6.5% into (**4**) after considerable experimentation [equation (1)]. In contemplating biosynthetic investigations of bisfuran formation, such a yield would not be satisfactory. Nonetheless, the virtues of introducing label into the $C_6$ side-chain of averufin late in a synthetic sequence by reaction with a preformed anthraquinone were readily apparent. It was known from the structure proof of averufin that salicylaldehyde (**10**) reacted with the Grignard reagent (**11**; X = MgCl) to afford compound (**12**) in good yield on acidic work-up[20] [equation (2)]. It seemed reasonable, therefore, that similar reaction of the tetramethoxyanthraquinone aldehyde (**13**) would give compound (**14**) from which averufin could be obtained after a deprotection step [equation (3)]. Concerns about steric or electronic retardation of such a reaction (the aldehyde is a multiply vinylogous ester) were assuaged by the fact that the analogous anthraquinone lacking methoxy substi-

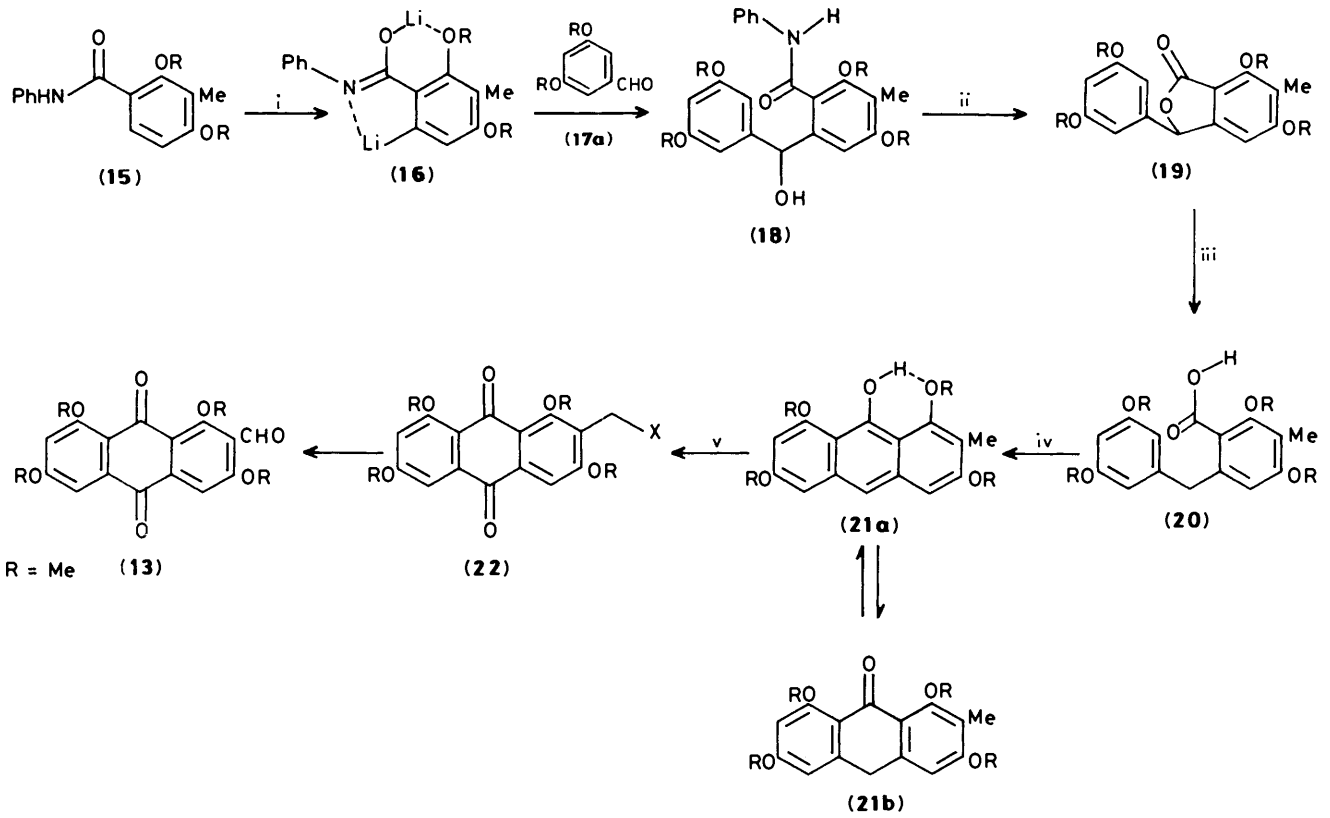


Scheme 1.

tuents in the A-ring readily underwent Wittig reaction with n-pentyltriphenylphosphorane.[19] Moreover, the three rings of the anthraquinone (**13**) could be readily differentiated in anticipation of later experiments where labels would reside in the A/B-rings of averufin (**4**) [*cf.* equations (1) and (3)].

The anthraquinone aldehyde (**13**) was initially obtained from a published multistep route utilizing classical Friedel–Crafts chemistry.[21] This labour-intensive sequence was soon abandoned in favour of that outlined in Scheme 2. Anilide (**15**) was prepared by carboxylation of 2-methylresorcinol,[22] tri-*O*-






**Scheme 2.** *Reagents and conditions:* i, Bu$^s$Li (2 equiv.), TMEDA, THF, −78 °C; ii, aq. HCl; iii, 5% Pd–C, $H_2$, HOAc, 85 °C; iv, TFAA, TFA; v, $CrO_3$, HOAc

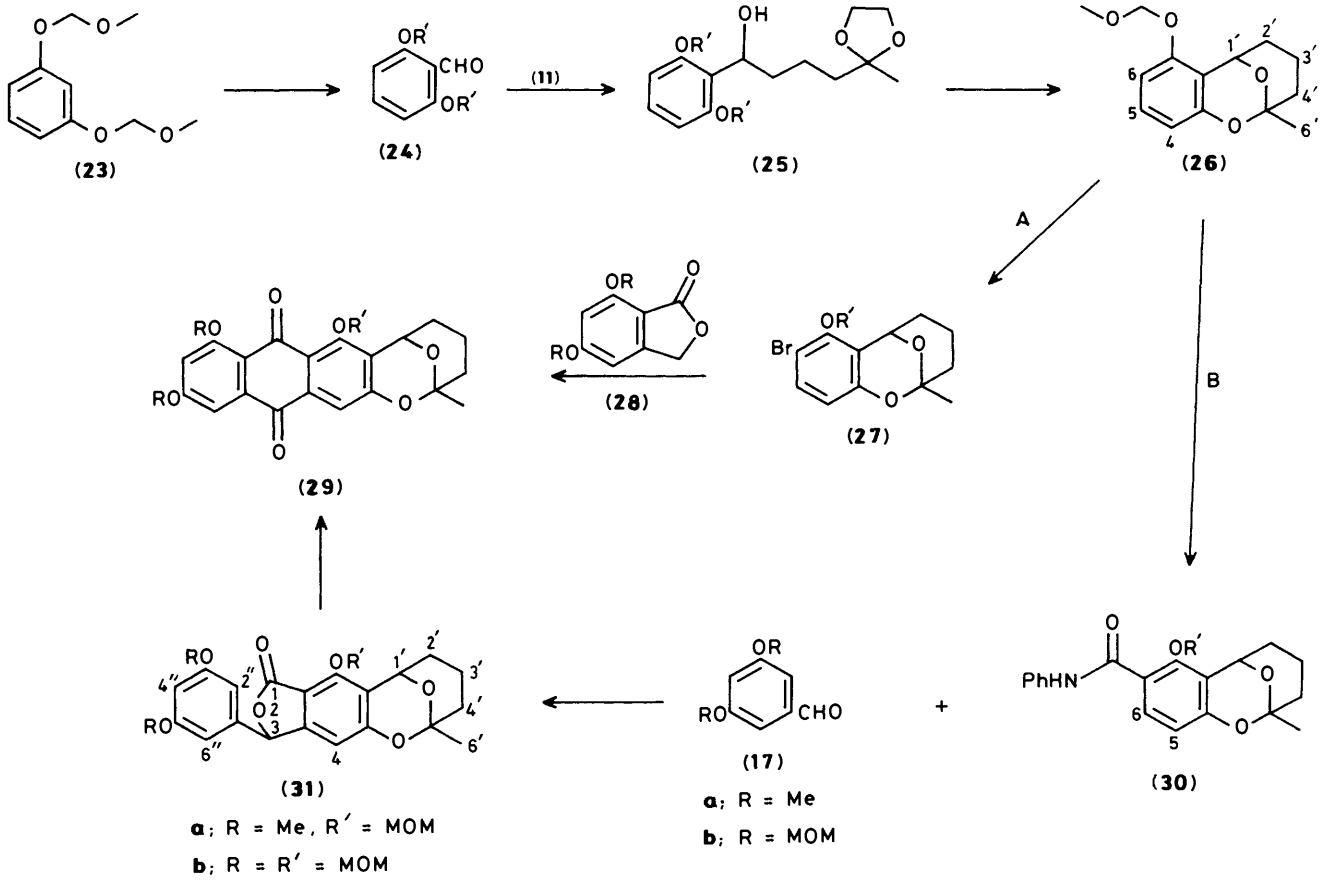
methylation with dimethyl sulphate, and reaction with aniline and sodium methoxide in refluxing benzene.[23] The dianion (**16**) was easily generated from compound (**15**) within 7 min at −78 °C* and reacted with 3,5-dimethoxybenzaldehyde (**17a**) to afford the labile hydroxy amide (**18**), which could not be isolated in pure form owing to its ready cyclization to the phthalide (**19**). Acidic work-up, therefore, gave compound (**19**) as a crystalline solid, m.p. 121—122 °C, in 86% yield.

While attempted reduction of the phthalide (**19**) with zinc in aqueous acid[24] or base[25] failed to proceed, hydrogenolysis[26] provided an 84% yield of the *o*-benzylbenzoic acid (**20**). Mild Friedel–Crafts acylation with trifluoroacetic anhydride[27] (TFAA) in trifluoroacetic acid (TFA) generated the anthracenol (**21a**) [the tautomeric anthrone (**21b**) was not detected in the 80 MHz $^1$H n.m.r. or i.r. spectra of this product], which partly air-oxidized to quinone (**22**) during isolation. Oxidation of compound (**21a**) was best accomplished with excess of chromium(VI) trioxide[28] in aqueous acetic acid, and gave quinone (**22**; R = Me, X = H) in 48—51% yield for the two steps (Scheme 2) as canary yellow needles, m.p. 191—192 °C (lit.,[21] 192 °C). The transformation of (**22**; R = Me, X = H) to (**13**) was carried out with some slight modification of the literature procedure.[21] The use of high intensity illumination rather than the reported benzoyl peroxide catalysis provided the bromide (**22**; R = Me, X = Br) in quantitative yield. Acetolysis (**22**; X = OAc), saponification (**22**; X = OH), and pyridinium chlorochromate (PCC) oxidation[29] gave compound (**13**; R = Me) in 85% yield over four steps.

The reactions of aldehyde (**13**) were singularly disappointing. Organometallic reagents (**11**; X = MgCl, MgBr, or Li) under a variety of conditions of temperature and concentration gave red polymer and mixtures of products of ill-defined structure whose $^1$H n.m.r. spectra showed disappearance of the aldehyde resonance, reaction of 2 or 3 equiv. of compound (**11**) with (**13**) and in some cases loss of the 1-methoxy group (readily discerned by the upfield chemical shift of the anthraquinone 4-H). Similarly, attempted Wittig reaction of aldehyde (**13**) with (**11**; X = =PPh$_3$)[30] failed to give isolable products whose $^1$H n.m.r. spectra showed resonances corresponding to the expected olefin. Once again, multiple reactions with the anthraquinone nucleus appeared to have occurred along with some loss of the *O*-methyl protecting group at the C-1 hydroxy. The origin of this useless reactivity is unclear but presumably must stem from the added presence of the A-ring methoxy substituents (*vide supra*). Nonetheless, the i.r. spectrum of compound (**13**) shows a strong but unexceptional absorbance at 1 695 cm$^{-1}$ for the aldehyde carbonyl. Brief attempts to salvage the approach embodied in equation (3) by way of the benzylic bromide (**22**; X = Br) using Ramberg–Bäcklund chemistry or a malonic ester synthesis[31] (straightforward introduction of carbon label) were soon given up for a fundamentally different approach to the preparation of labelled averufin.

Early introduction of the ketal side-chain of averufin at the stage of a simple benzenoid intermediate would seem contrary to the first tenet of labelled synthesis that isotope should be introduced as late as possible. However, in the present instance such a plan would simplify the late phenol deprotection steps to reveal ultimately the bioactive natural product and perhaps simultaneously minimize competing chemical fates of the side-

* Complete deprotonation of C-6 was demonstrated on quenching with deuterium oxide and $^1$H n.m.r. analysis of the product. Heteroatom-directed metallation reactions have been thoroughly reviewed: D. W. Slocum and C. A. Jennings, *J. Org. Chem.*, 1976, **41**, 3653; H. W. Gschwend and H. R. Rodriquez, *Org. React.*, 1979, **26**, 1; P. Beak and V. Snieckus, *Acc. Chem. Res.*, 1982, **15**, 306.



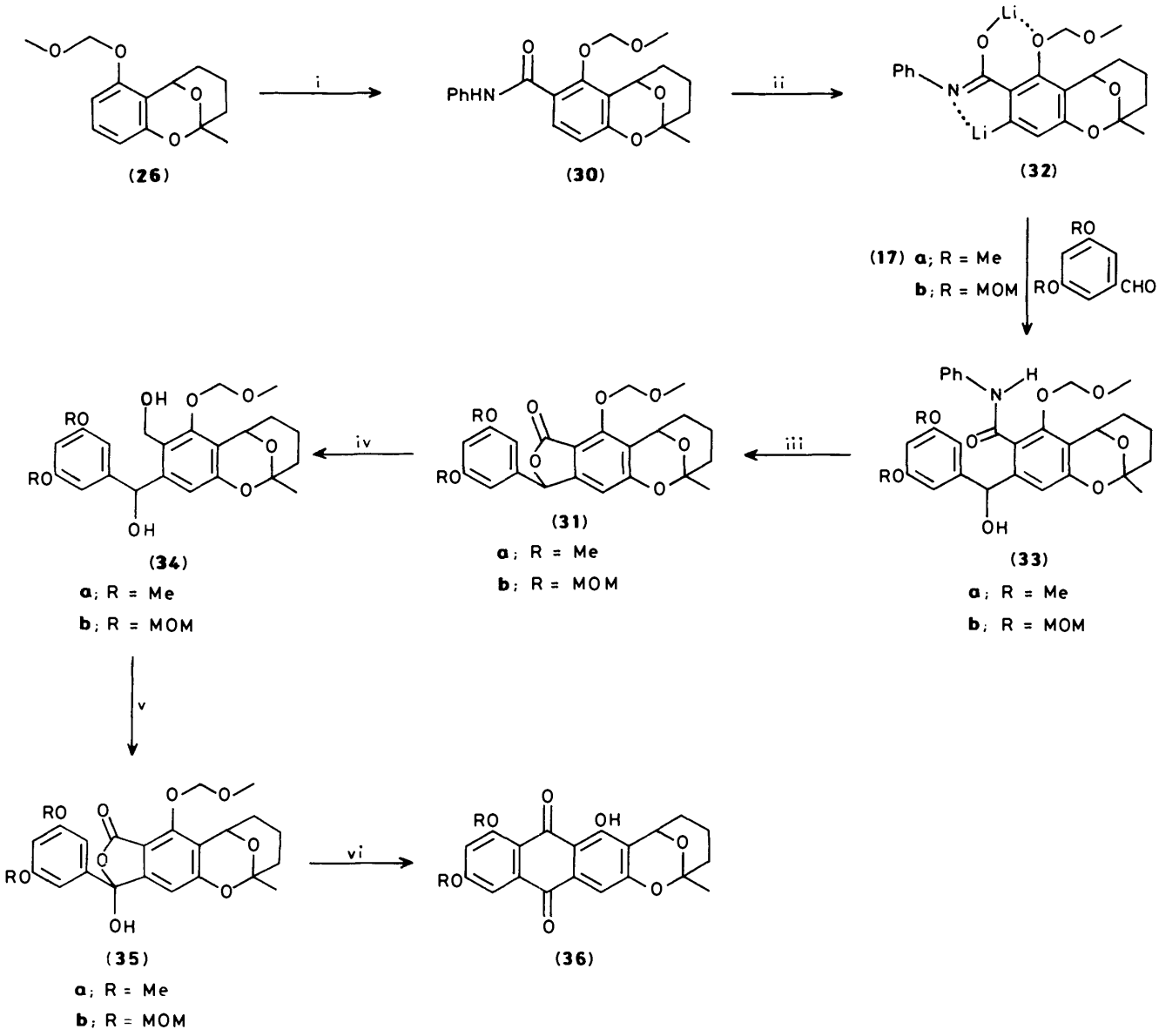

Scheme 3.

chain represented structure in (**14**) [equation (3)]. Moreover, an opportunity to realize a marked degree of convergence was identified in an intermediate such as (**26**) (Scheme 3) which would serve to differentiate the phenolic hydroxy groups and protect one of them in conjunction with the potentially troublesome benzylic alcohol as a base-stable internal ketal. Two conceptually distinct routes to convert (**26**) into protected forms of averufin (**29**) were designed and successfully reduced to practice as outlined in Scheme 3.

The choice of methoxymethyl (MOM) protecting groups was the key to both syntheses and was made for two reasons. First, final deprotection of compound (**29**; R = R′ = MOM) under mildly acidic conditions was expected[32] to give averufin (**4**) without undue destruction, and further, it was hoped that the inherently low electron density of anthraquinones would disfavour any subsequent reaction with the formaldehyde or formaldehyde equivalents liberated. Second, methoxymethyl is a directing group of intermediate strength which allows regiospecific aryl metallation and reaction with electrophiles in good yield.* As has been described previously,[33] bis(*O*-methoxymethyl)resorcinol (**23**) can be metallated in ether at 0 °C and on treatment with dimethylformamide (DMF) can give compound (**24**; R′ = MOM), which may be homologated with Grignard reagent (**11**; X = MgBr) to give the alcohol (**25**; R′ = MOM). Careful cyclization in good yield and without loss of the second MOM group to afford the tricycle (**26**) could be achieved presumably owing to neighbouring group participation by the benzylic hydroxy in the first MOM deprotection but not the second. Regiospecific metallation, now directed by a single MOM group, and reaction either with cyanogen bromide[34] (route A) or phenyl isocyanate[35] (route B) gave compounds (**27**) or (**30**), respectively, in high yield. The aryl bromide (**27**) may be visualized in the presence of a relatively non-nucleophilic base as lithium tetramethylpiperidide[36] to be a precursor of the 5,6-benzyne (route A). In the presence of the anion derived from 5,7-bis(methoxymethoxy)phthalide (**28**; R = MOM) this benzyne, generated *in situ*, was shown to undergo nucleophilic attack, whose regiochemistry was again governed by the methoxymethyl group in (**27**), to afford the anthraquinone (**29**) after air oxidation. The details of this synthetic approach to averufin have been reported previously.[33]

The second route, B, from (**26**) to (**29**), and hence to averufin (**4**), tied in closely with our favourable experience with the simpler synthesis illustrated in Scheme 2. This sequence was initially pursued with the series yielding methoxy groups in the A-ring of (**29**; R = Me, R′ = MOM) as shown in Scheme 4. Anilide (**30**; R′ = MOM) available in 95—98% yield from (**26**), was converted into its dianion (**32**) as described earlier for the case of (**15**) ⟶ (**16**) and upon reaction with aldehyde (**17a**) provided, after acidic work-up and silica gel chromatography, an 85—88% yield of the phthalide (**31a**) as a mixture of diastereoisomers. The presence of two diastereoisomers in roughly equal amounts was manifest in the 80 MHz $^1$H n.m.r. spectrum of compound (**31a**) by the existence of two signals for 3-H, the phthalide methine ($\delta_H$ 6.15 and 6.12) and the protons of C-6′ ($\delta_H$ 1.52 and 1.50). Attempted hydrogenolysis[26] of (**31a**) whether at high temperature, moderate pressure (40 lb in$^{-2}$),

* See footnote on page 3.



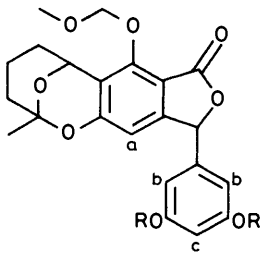

**Scheme 4.** *Reagents and conditions:* i, BuLi, $Et_2O$; then PhNCO; ii. Bu$^s$Li (2-equiv.), TMEDA, THF, −78 °C; iii, aq. $H_2SO_4$; iv, $LiAlH_4$, THF; v, $KMnO_4$, aq. $Me_2CO$, $NaHCO_3$; vi, TFAA, TFA

with sonication, with highly active catalyst (fresh palladium black [37]), or combinations of these resulted only in recovery of starting phthalide (**31a**). Likewise, attempted reduction with zinc in aqueous base[20] failed to proceed to any significant extent. Apparently, the presence of the bulky, basket-like side-chain in compound (**31a**) prevented access to the catalyst surface so that no reduction could occur.

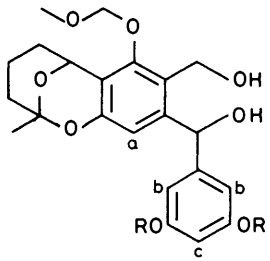
The phthalide (**31a**) could, however, be smoothly reduced with lithium aluminium hydride[38] to provide a mixture of diastereoisomeric diols (**34a**). The 80 MHz $^1$H n.m.r. spectrum of (**34a**) revealed *inter alia* two signals for 4-H ($H_a$, $\delta_H$ 6.58 and 6.37), the methine hydrogen of the ring-bridging carbon ($\delta_H$ 6.00 and 5.90, no change upon addition of deuterium oxide), the hydrogens of the hydroxymethylene group ($\delta_H$ 4.61 and 4.50, no change upon addition of deuterium oxide), and the protons of C-6′ (1.49 and 1.48 p.p.m.). Initially, oxidation of (**34a**) was conducted with PCC[29] in order to obtain the *o*-benzoylbenzaldehyde (**37a**) (see Table) for spectral and chromatographic comparison. Owing to the acidic nature of the oxidant, a 12% recovery of the phthalide (**31a**) was obtained (presumably resulting from oxidation of the hemiacetal). However, oxidation of diol (**34a**) with potassium permanganate[39] in aqueous acetone pH 8.5 provided pseudoacid (**35a**) in *ca.* 85% yield with no detectable competing phthalide formation. The 80 MHz $^1$H n.m.r. spectrum of compound (**35a**) revealed no signal multiplicity for the mixture of diastereoisomers at 80 MHz (*vide infra*). The pseudoacid structure (**35a**) was assigned primarily on the basis of the chemical shifts observed for the aromatic protons in the 80 MHz $^1$H n.m.r. spectrum (Table). The i.r. spectrum revealed a single carbonyl stretch (1 760 cm$^{-1}$) consistent with a γ-lactone structure.

Cyclization of compound (**35a**) with TFAA[27] led to 6,8-di-*O*-methylaverufin (**36**; R = Me) in 80% yield after silica gel chromatography. Averufin had been reported to be stable to

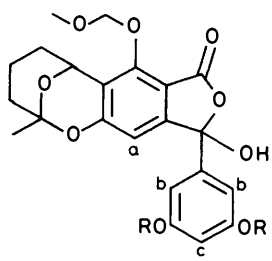
**Table.** $^1$H N.m.r. chemical shift comparisons for compounds (**31**), (**34**), (**35**), and (**37**)[a]



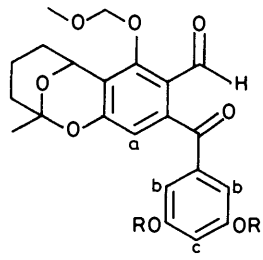
(**31**)



(**34**)



(**35**)



(**37**)

| | (**31a**) (R = Me) | (**31b**) (R = $CH_2OMe$) | (**34a**) (R = Me) | (**34b**) (R = $CH_2OMe$) | (**35a**) (R = Me) | (**35b**) (R = $CH_2OMe$) | (**37a**) (R = Me) | (**37b**) (R = $CH_2OMe$) |
|---|---|---|---|---|---|---|---|---|
| $H_a$, s | 6.43 | 6.46 | 6.58, 6.37 | 6.55, 6.39 | 6.64 | 6.64, 6.61 | 6.61 | 6.62 |
| $H_b$, d | 6.43 | 6.61 | 6.59 | 6.76 | 6.73 | 6.90 | 6.92 | 7.08 |
| $H_c$, t | 6.43 | 6.74 | 6.40 | 6.68 | 6.45 | 6.76 | 6.63 | 6.93 |

[a] Spectra recorded for $CDCl_3$ solutions.

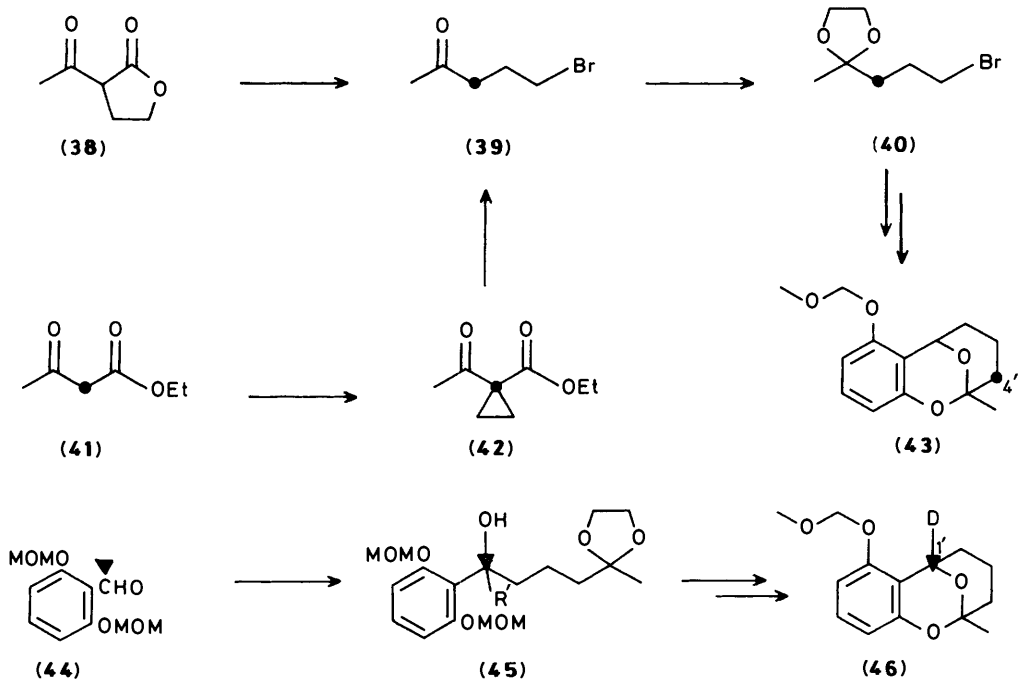
50% hydrobromic acid in acetic acid at reflux for 4 hours.[32] However, attempted demethylation of (**36**; R = Me) under these and a variety of other conditons gave only extremely low yields of the desired product accompanied by large amounts of highly coloured polar impurities. Clearly, more readily removable protecting groups were needed for the A-ring. Therefore, 3,5-bis(methoxymethoxy)benzaldehyde (**17b**) was prepared (not shown but fully described in the Experimental section). Reaction of compound (**17b**) with dianion (**32**) (Scheme 4) led, after acidic work-up and silica gel chromatography, to 83—89% yields of the phthalide (**31b**); again, the presence of two diastereoisomers was revealed in the 80 MHz $^1$H n.m.r. spectrum of compound (**31b**) by two signals for 3-H ($\delta_H$ 6.14 and 6.12) and the protons of C-6′ ($\delta_H$ 1.52 and 1.51). Reduction of (**31b**) with lithium aluminium hydride[48] provided the diastereoisomeric mixture of diols (**34b**) whose 80 MHz $^1$H n.m.r. spectrum revealed *inter alia* two signals for 3-H ($H_a$, $\delta_H$ 6.55 and 6.39), the methine hydrogen of the ring-bridging carbon ($\delta_H$ 5.99 and 5.90), and the protons of C-6′ ($\delta_H$ 1.49 and 1.48). Oxidation of (**34b**) with PCC provided the benzophenone (**37b**) (Table) and the phthalide (**31b**), while permanganate oxidation[39] as above provided pseudoacid (**35b**) [two 80 MHz $^1$H n.m.r. signals observed only for 4-H ($H_a$, $\delta_H$ 6.64 and 6.61)], whose structure was assigned on the basis of the chemical shifts observed for the aromatic protons in its 80 MHz $^1$H n.m.r. spectrum (Table) and the position of the carbonyl stretch (1 755 $cm^{-1}$) observed in its i.r. spectrum. Upon treatment of pseudoacid (**35b**) with TFAA[27] in TFA at room temperature, an orange precipitate formed within 10 min. After being stirred for an additional 2 h, the TFA and TFAA were removed under reduced pressure, and silica gel chromatography of the residue yielded (±)-averufin (63%) [53% from (**31b**)], identical in all respects with authentic material.[20,32,33]

The target molecule (**4**), therefore, was prepared in an overall yield of 40—45% through effectively five steps from the tricyclic ketal (**26**) by path B, Scheme 3. This net conversion compares favourably with the shorter benzyne route[33] (path A, Scheme 3) where averufin could be obtained in 25—30% yield from (**26**). It will be seen in the present discussion and more fully in later papers that each of these approaches has particular strengths that may be turned to advantage to prepare labelled materials flexibly and efficiently for investigation of the aflatoxin biosynthetic pathway. The first applications of these syntheses were the preparation of side-chain-labelled specimens of averufin to examine the formation of the bisfuran moiety in (**8**) and, second, the introduction of A-ring labels to map carbon utilization from the anthraquinone nucleus to averufin (**4**) to the coumarin moiety of aflatoxin $B_1$. These syntheses will be taken in order.

*Side-chain-labelled Averufin.*—Large-scale preparations of the bromide (**40**) (Scheme 5) were carried out from 2-acetylbutyrolactone (**38**).[40] Initial attempts to synthesize lactone (**38**) by reaction of ethyl sodioacetoacetate and ethylene oxide generated *in situ* from 2-bromoethanol[41] were unpromising. Attention was, therefore, turned to preparation of the cyclopropane (**42**), first described (as the unlabelled parent) by Perkin in 1885.[42] The reaction between ethyl acetoacetate (**41**) and 1,2-dibromoethane was conducted under a wide variety of conditions to determine the optimum conditions; these afforded a *ca.* 65% isolated yield of the cyclopropane (**42**).[32] Treatment of compound (**42**) (Scheme 5) with 48% hydrobromic acid under an inert atmosphere in the dark effected ring opening, saponification, and decarboxylation to provide 5-bromopentan-2-one (**39**) in 80% yield. Ketalization with ethylene glycol in benzene and azeotropic removal of water gave, after fractional distillation, the bromide (**40**) in *ca.* 40% overall yield from (**41**). However, when this sequence was conducted without purification of the ester (**42**), *i.e.* when a crude alkylation-product mixture containing the excess of 1,2-dibromoethane was used, isolated yields of the bromide (**40**) were 49—51% overall. Fractional distillation readily provided compound (**40**) uncontaminated by 1,2-dibromoethane. In turn, the ethyl acetoacetate (**41**) required for this sequence could be prepared in 80—85% yield by treatment of ethyl acetate with lithium hexamethyldisilazide (LiHMDS) (2 equiv.) at −78 °C in tetrahydrofuran (THF) and reaction with acetyl chloride.[43]

Therefore, ethyl [2-$^{13}$C]acetate (90% enriched) was homologated to give ethyl [2-$^{13}$C]acetoacetate, and this was converted as above into the labelled bromoketal (**40**). Generation of the organolithium (**11**; X = Li) from (**40**) was carried out with lithium dispersion containing 2% sodium[44] in ether followed by the addition of 2,6-bis(methoxymethoxy)benzaldehyde (**24**; R′ = MOM) to generate, finally (Scheme 5), tricyclic ketal (**43**) [*cf.* (**24**)→(**26**), Scheme 3]. Further elaboration of compound (**43**) (*cf.* Scheme 4) gave a sample of (±)-[4′-$^{13}$C]averufin (**65**) (Scheme 7).

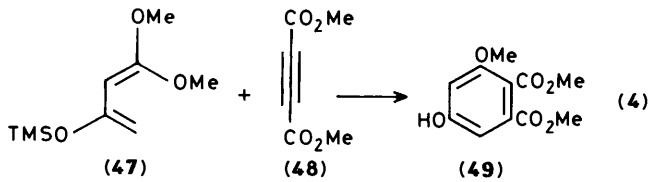
To prepare a specimen of averufin double labelled at C-1′, tricyclic ketal (**46**) was required. Bis(*O*-methoxymethyl)re-




Scheme 5.

sorcinol (**23**) (Scheme 3) was metallated as before[33] and then treated with [$^{13}$C] DMF to give the labelled benzaldehyde (**44**) (Scheme 5). Exchange of the formyl hydrogen in deuterium oxide in the presence of potassium cyanide[45] was slow and gave only about 70% of the $^{2}H_{1}$-species in eight days. Seeking to take advantage[46] of the relatively high kinetic isotope effects observed in the reactions of transition metal oxidants,[47] we reduced compound (**44**) with lithium aluminium deuteride and reoxidized (PCC) the product to give [$^{2}H_{1}$]-(**44**) containing 85—90% deuterium at the desired site, but unfortunately in only 60% overall yield. Therefore, compound (**44**) was directly treated with Grignard reagent (**11**; X = MgBr)[34] to give the alcohol (**45**; R′ = H), which was carefully oxidized with chromium(VI) trioxide–pyridine[48] and the product immediately reduced to give (**45**; R′ = D). Cyclization to (**46**) bearing 90% deuterium at C-1′ was carried out in 51% overall yield from compound (**44**). A sample of (±)-[1′-$^{13}$C,$^{2}$H]averufin (**68**) (Scheme 7) was prepared from the tricycle (**46**) by the route developed in Scheme 4.

A-*Ring-labelled Averufin*.—Of the three rings that comprise the anthraquinone nucleus of averufin (**4**), only the C-ring remains intact in aflatoxin $B_1$ (**8**). Comparatively few routes from simple acyclic precursors[49] are available to prepare A/B-ring synthons specifically labelled at nuclear sites to map the post-bisfuran rearrangement processes. As outlined earlier in Scheme 3, 3,5-bis(methoxymethoxy)benzaldehyde (**17b**) or 5,7-bis(methoxymethoxy)phthalide (**28**; R = MOM) would be appropriate targets for such a synthesis.

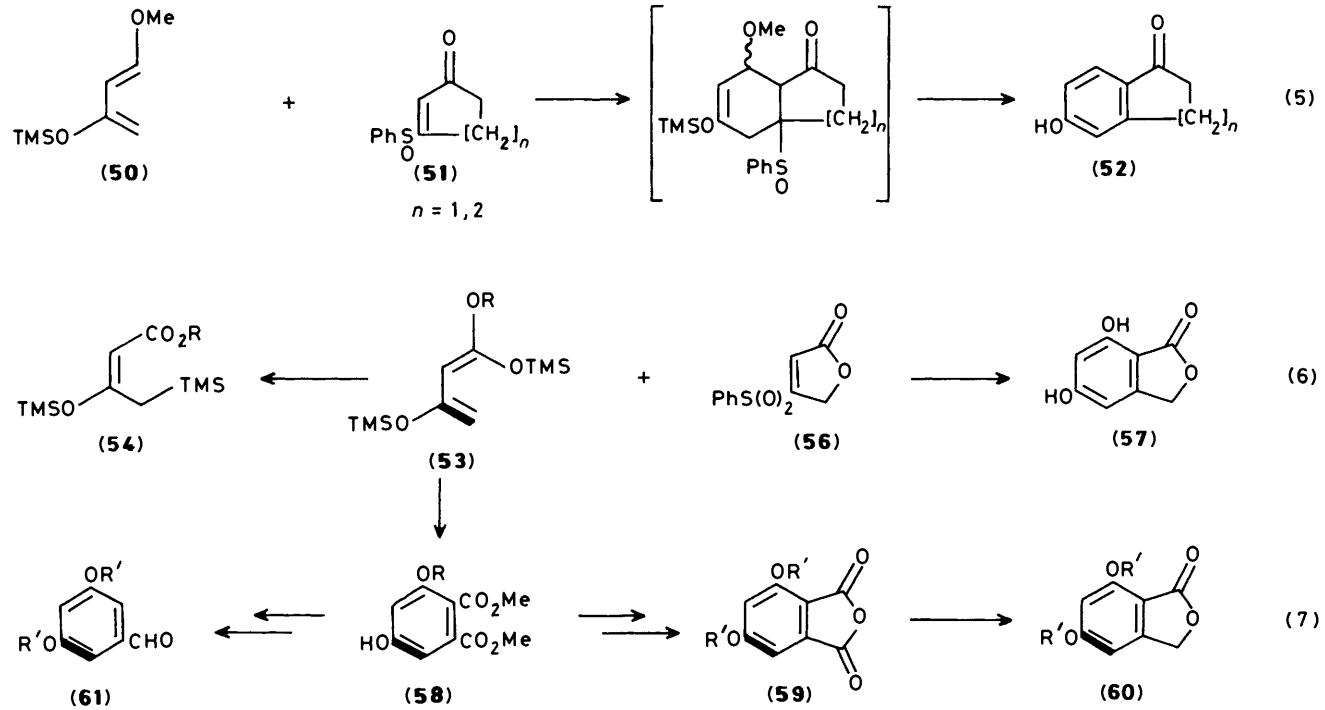


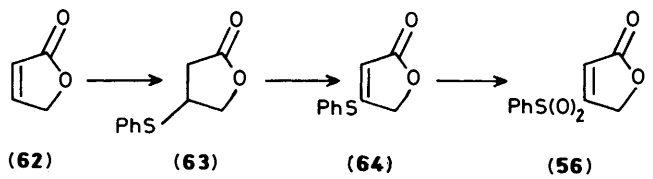

(4)

Our thinking about the preparation of these 4- or 4,5-substituted resorcinol derivatives was shaped by the concurrent studies of Danishefsky on the Diels–Alder reactions of siloxydienes.[50] Two examples in particular pointed the way. The Brassard–Danishefsky[51,52] diene (**47**) was known[52] to react with dimethyl acetylenedicarboxylate (DMAD) (**48**) to afford the dimethyl phthalate (**49**) [equation (4)]. Similarly, cycloaddition of the siloxy diene (**50**) with β-phenylsulphinyl enones (**51**), after intermediate loss of methanol and benzenesulphinic acid, afforded ketones (**52**) [equation (5)] at the correct aryl oxidation state.[53] The plan, therefore, centred on the preparation of 1-ethoxy-1,3-bis(trimethylsiloxy)buta-1,3-diene[54] (**53**; R = Et) in labelled form ultimately from acetate. Envisioned Diels–Alder reactions of compound (**53**), however, were likely to be thwarted by competitive[55] (and presumably unimolecular) silyl migration to form ester (**54**). Hope was renewed by the timely report of Schultz[57] that ethyl phenylsulphonylpropiolate (**55**) underwent Diels–Alder reaction with a series of dienes


(**55**)

at room temperature; and, like phenylsulphinyl,[53] phenylsulphonyl substitution, while significantly activating, did not override the regiochemical directing effects of carbonyl functions.[56] Therefore, as shown in equation (6), siloxy diene (**53**) was to be treated with β-phenylsulphonylbutenolide (**56**) to give the phthalide (**57**). α-Bromo-γ-butyrolactone was readily dehydrohalogenated to afford the butenolide (**62**),[57] which reacted with thiophenol and a catalytic amount of sodium hydride to give β-phenylthio-γ-butyrolactone (**63**). The double bond was reintroduced by reaction with *N*-chlorosuccinimide (NCS) to




Scheme 6.

give compound (**64**) which readily underwent oxidation to afford sulphone (**56**) (Scheme 6).

Reactions of β-phenylsulphonylbutenolide (**56**) with siloxydiene (**53**) under a variety of conditions were disappointing. 20—25% Yields of the phthalide (**57**) could be obtained from reaction at reflux in benzene or at room temperature in chloroform, accompanied by dark polymer and decomposition of the diene to ethyl acetoacetate. Reasoning that these latter undesired reactions were caused by the liberated benezenesulphinic acid, we tested a range of hindered bases and acid scavengers but their use resulted in instantaneous polymerization of the extremely electrophilic butenolide (**56**).

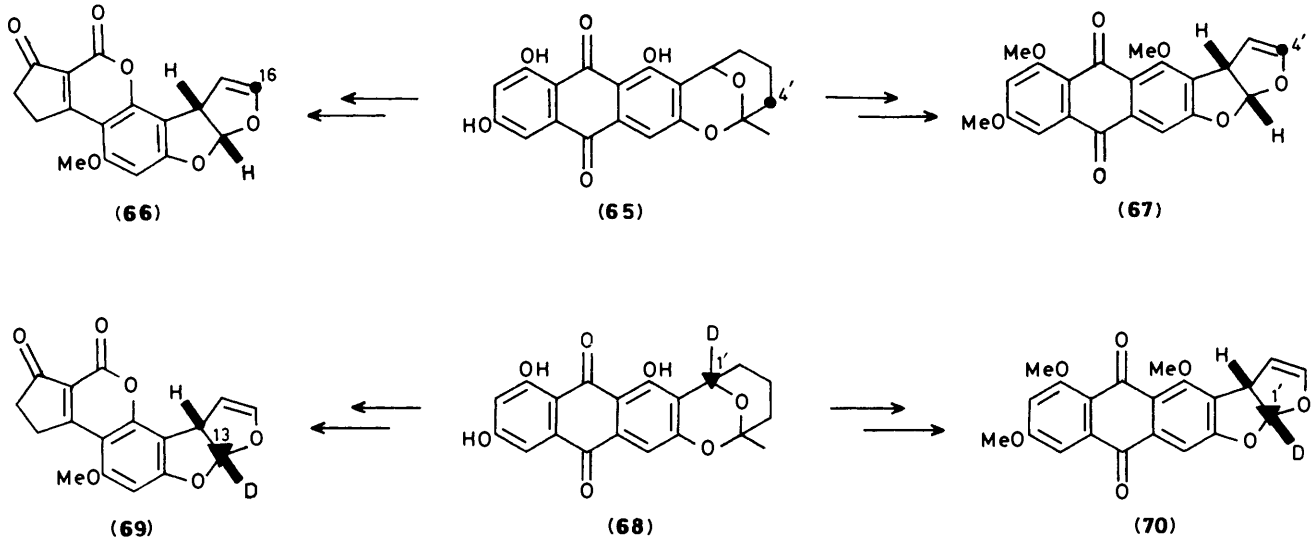
While these studies were underway, a parallel line of investigation was based on the known[54] reaction of DMAD (**48**) with diene (**53**; R = Me). In our hands the closely related case of (**53**; R = Et) gave 55—60% yields of phthalate (**58**; R = Et:H, 2:1). The *O*-ethyl group could be easily removed by reaction of the product mixture with boron trichloride.[58] As outlined in equation (7), our initial thoughts were to convert diester (**58**) into the phthalic anhydride (**59**) (*e.g.* R′ = MOM) and to take advantage of the electron-releasing substituents on the aromatic ring to achieve a selective reduction[59] to, for example in labelled form, 5,7-bis(methoxymethoxy)[4,5-$^{13}C_2$]phthalide (**60**). However, further consideration of the envisioned biosynthetic experiment led us to destroy the inherent asymmetry of intermediate (**58**) by convenient and well precedented saponification–decarboxylation[60] to 3,5-dihydroxybenzoic acid, which could be trivially elaborated to, for example in labelled form, 3,5-bis(methoxymethoxy)[2,3-$^{13}C_2$]benzaldehyde (**61**). From this material a 1:1 mixture of [5,6-$^{13}C_2$]- and [8,11-$^{13}C_2$]-averufin (**71**) could be prepared (route B, Scheme 3). This isomeric mixture of doubly labelled averufins lends considerably greater deductive force to the planned incorporation experiment over that based on a simpler labelling scheme from, *e.g.*, compound (**60**). This argument will be set out in the following section.

Condensation of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) and [1,2-$^{13}C_2$]acetyl chloride followed by ethanolysis gave ethyl [3,4-$^{13}C_2$]acetoacetate[61] in 68% yield after distillation. Stepwise silylation[54] then afforded 1-ethoxy-1,3-bis(trimethylsiloxy)[3,4-$^{13}C_2$]buta-1,3-diene (**53**; R = Et). Reaction with DMAD (**48**) gave the dimethyl [5,6-$^{13}C_2$]-phthalate derivative (**58**) as a mixture of the 3-ethoxy and 3-hydroxy compounds. Treatment of the mixture with boron trichloride[58] readily afforded the dimethyl [5,6-$^{13}C_2$]phthalate (**58**; R = H), which was converted in a straightforward manner into 3,5-bis(methoxymethoxy)[2,3-$^{13}C_2$]benzaldehyde (**61**; R′ = MOM).* Through the reactions established in Scheme 4, doubly labelled benzaldehyde (**61**) gave a 1:1 mixture of (±)-[5,6-$^{13}C_2$]- and [8,11-$^{13}C_2$]-averufin (**71**).

*Incorporation of Averufin into Aflatoxin $B_1$, Versicolorin A, and Versiconal Acetate.*—Incorporations of averufin into aflatoxin were carried out with washed mycelial pellets[62] of a wild-type aflatoxigenic strain of *Aspergillus parasiticus* (SU-1)† suspended in a replacement medium.[63] Labelled specimens of averufin were administered in a small amount of acetone. After an incubation period of 48 h, the aflatoxin $B_1$ produced was isolated from the medium, and a smaller amount was usually extracted from the filtered mycelia (<10%). The results of the

* Details of these steps are reported in the Experimental Section. In the course of this work numerous observations of long-range $^{13}C$–$^1H$ coupling constants were made that have been noted as well. Detailed discussion may be found in the doctoral dissertation of S. G. Davis, The Johns Hopkins University, 1985.

† The SU-1 strain used is equivalent to NRRL A16,462 or ATCC 56775. Reference in earlier publications from this laboratory incorrectly cited strain ATCC 15517, which is equivalent to SU-7.



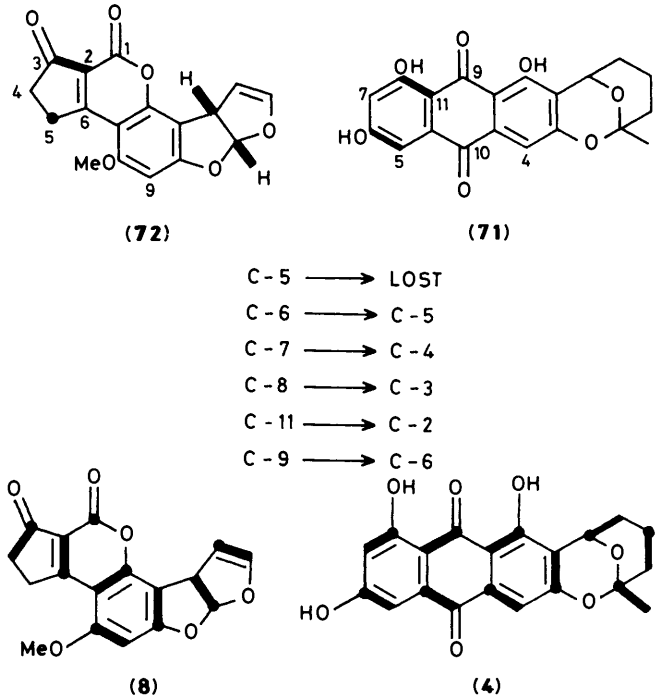

Scheme 7.

incorporation experiments were then assessed by n.m.r. spectroscopy of the toxin after purification by silica gel chromatography and crystallization.

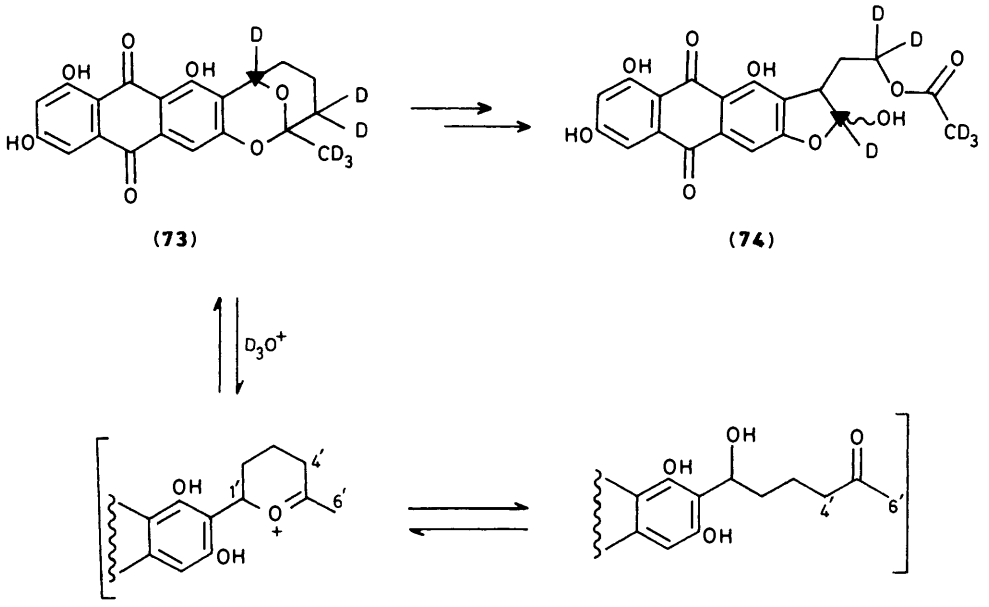
The $^{13}C$-{$^1H$} n.m.r. spectrum of (±)-[4′-$^{13}C$]averufin (**65**) in 1:1 $CDCl_3$-[$^2H_6$]$Me_2SO$ gave an intense singlet at $\delta_C$ 35.3 in accord with earlier assignments.[67] On incorporation into aflatoxin $B_1$ (**66**) (Scheme 7), a single strong signal at $\delta_C$ 145.1 was observed indicating a >20% specific incorporation of label at C-16.[65,66] The site of enrichment was confirmed in the n.o.e.-enhanced proton-coupled spectrum where two-bond coupling to 15-H ($^2J_{CH}$ 11.6 Hz) and three-bond coupling to 13-H and 14-H ($^3J_{CH}$ 4.5 Hz) were observed in each branch of the major doublet ($^1J_{CH}$ 197 Hz).

An analogous incorporation experiment with (±)-[1′-$^{13}C$,$^2H$]averufin (**68**) showed incorporation of the anthraquinone precursor to label C-13 exclusively in aflatoxin (**69**) (Scheme 7).[65,66] In spectra published earlier,[17] deuterium label in compound (**68**) (90% $^2H_1$, *vide supra*) was evident in the $^{13}C$-{$^1H$} n.m.r. spectrum as a slightly upfield triplet ($\delta_C$ 65.9, $^1J_{CD}$ 22.6 Hz) superimposed on a singlet ($\delta_C$ 66.2) corresponding to C-1′ with a directly bound hydrogen.[64] Of considerable mechanistic importance was the observation that the singlet: triplet ($\delta_C$ 113.3; 113.0, $^1J_{CD}$ 28.5 Hz) intensities in the $^{13}C$-{$^1H$} n.m.r. spectrum of the aflatoxin (**69**) were unchanged when compared with that of the precursor (**68**), hence indicating no loss of deuterium label during the overall transformation to bisfuran in which the oxidation state at this carbon is raised.

Subsequent incorporations of compounds (**65**) and (**68**) gave versicolorin A,[67] the first proposed bisfuran-containing intermediate of the pathway (Scheme 1), which when analysed similarly by $^{13}C$-{$^1H$} n.m.r. spectroscopy as the more soluble tri-*O*-methyl ether indicated completely parallel incorporations of label as shown in structures (**67**) and (**70**) (Scheme 7). When compared with the [1,2-$^{13}C_2$]acetate incorporation experiments (**4**) and (**8**), it can be seen that three intact acetate units are used to construct the $C_6$ side-chain of averufin (**4**). Two of these remain in the bisfuran of aflatoxin $B_2$ (**8**). Inspection of the results summarized in Scheme 7 and structures (**4**) and (**8**) reveals that it is the innermost of the three side-chain acetate units in averufin (**4**) that becomes the central unit of the bisfuran, the central unit that becomes the outer unit of the bisfuran; and last, it is the terminal pair of carbons in averufin that must be lost in the course of bisfuran formation.




The $^{13}C$-{$^1H$} n.m.r. spectrum (1:1 $CDCl_3$–[$^2H_6$]$Me_2SO$) of A-ring-labelled averufin containing a 1:1 mixture of [5,6-$^{13}C_2$]- and [8,11-$^{13}C_2$]averufin (**71**) gave two pairs of strongly enhanced doublets, one centred at $\delta_C$ 107.4 and 163.7 ($^1J_{CC}$ 62.7 Hz) corresponding to C-5 and C-6, respectively; and the other centred at $\delta_C$ 163.0 and 107.0 ($^1J_{CC}$ 62.6 Hz) corresponding to C-8 and C-11, respectively.[64] On incorporation into the aflatoxin $B_1$ (**72**) and $^{13}C$-{$^1H$} n.m.r. analysis, an enhanced singlet at $\delta_C$ 28.9 was observed, indicating 6—8% enrichment at C-5 in compound (**72**).[65,66] In addition, a pair of doublets was observed at $\delta_C$ 117.3 and 201.2 ($^1J_{CC}$ 57.1 Hz), which may be assigned to C-2 and C-3, respectively.[65,66] As was possible for the side-chain carbons of averufin, a one-to-one mapping of the anthraquinone A/B-ring carbons into aflatoxin $B_1$ could now be made by recognizing that the [5,6-$^{13}C_2$]-labels in structure (**71**)



(73) (74)

correspond to a doubly labelled acetate pair in averufin (**4**) while the [8,11-$^{13}C_2$]-labels in (**71**) do not correspond to an intact pair from [1,2-$^{13}C_2$]acetate. Turning to aflatoxin (**72**) and comparing aflatoxin (**8**), it can be seen that enrichment of C-5 is common to both incorporation experiments whereas the labelled C-2/C-3 pair in compound (**72**) is derived uniquely from labelled averufin (**71**); that is, C-8 and C-11 in averufin map to C-3 and C-2 of aflatoxin, respectively. Therefore, it may be concluded that C-6 in averufin, derived from C-1 of acetate, must correlate to C-5 in aflatoxin. Atom C-5 of averufin, therefore, must be one of the two carbons lost from the anthraquinone nucleus during the formation of aflatoxin. By following a similar logic, the further correlations shown in Scheme 7 and structures (**4**) and (**8**) can be made unambiguously.[18]

The side-chain-labelled averufin incorporation experiments clearly demonstrate that the terminal pair of carbons is lost *en route* to the bisfuran. The structure of versiconal acetate (**5**) suggests that this cleavage process might arise by a Baeyer–Villiger-like oxidation of a methyl ketone derived from opening of the averufin (**4**) side-chain. This is an appealing hypothesis for the adroit insertion of oxygen in the midst of a carbon chain preparatory to generation of the second furan ring. However, given the uniform level of labelling of versiconal acetate (**5**) (Scheme 1) from [1,2-$^{13}C_2$]acetate, it is also entirely possible that the *O*-acetyl function arises by simple acylation by endogeneous acetylCoA. To test for the intramolecularity implicit in a Baeyer–Villiger reaction, [1′-$^{13}$C,$^{2}$H]averufin (**68**) was exchanged to completion at C-4′ and C-6′ to give the multi-labelled compound (**73**) by heating with deuterium chloride in THF–deuterium oxide for 17 days. Control experiments, based on careful n.m.r. assignments of the averufin side-chain hydrogens, showed that exchange of the diastereotopic C-4′ hydrogens was rapid compared with that of the hydrogens at C-6′. Full exchange was confirmed by a strong $M + 7$ ($m/z$ 375) ion in the mass spectrum of compound (**63**).

A portion of the 500 MHz $^1$H n.m.r. spectrum of averufin is displayed in the Figure containing resonances for the six methylene hydrogens at C-2′, C-3′, and C-4′ and a singlet ($\delta_H$ 1.51) for the C-6′ methyl. Initially two assumptions were made. First, the major coupling observed at each methylene resonance was taken to the homonuclear spin interaction with its geminal neighbour, which is generally larger in magnitude than vicinal

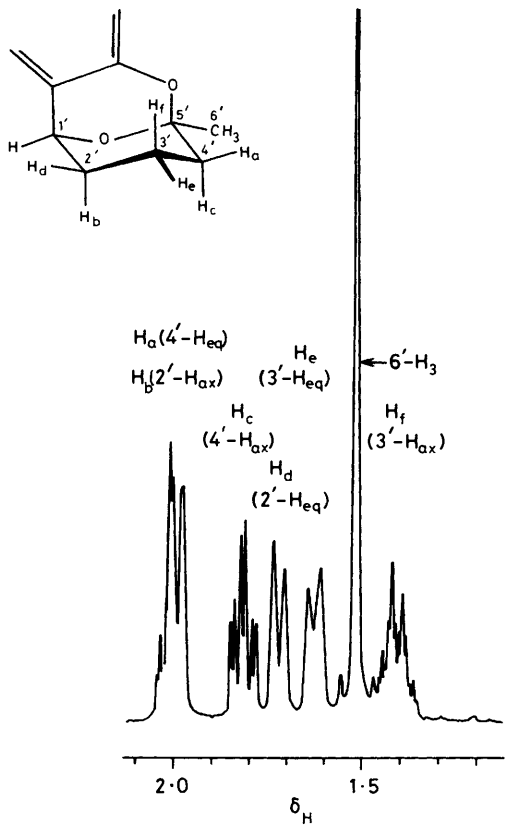



**Figure.** Upfield portion of the 500 MHz $^1$H n.m.r. spectrum of averufin (**4**) in [$^2H_6$]DMSO

coupling in six-membered rings.[68a] Second, the virtually coupled doublets ($H_a$, $H_d$, and $H_e$) were taken to be signals for equatorial hydrogens while the first-order multiplets ($H_b$, $H_c$, and $H_f$) were taken to be axial hydrogens since $^3J_{ax,eq}$ are smaller than $^3J_{ax,ax}$ in six-membered rings.[68a] Homonuclear decoupling experiments beginning with 1′-H (not shown at $\delta_H$ 5.24) led readily to the assignments shown in the Figure and

confirmed the expected[68b] chair conformation for ring E. These assignments were further confirmed in a subsequent $^1H$–$^{13}C$ COSY experiment and by comparison with the $^1H$ n.m.r. spectra of natural averufin that had been exchanged in the standard deuterium oxide solution above for 4 days. The latter showed complete loss of the signals for $H_a$ and $H_c$ and a triplet at $\delta_H$ 1.48 for 6′-H corresponding principally to a single exchange at this centre ($CH_2D$). The resonance for $H_b$ emerged as a clean triplet of triplets and that for $H_f$ as a sharp doublet of triplets. The signals for $H_d$ and $H_e$ remained broad doublets.

A sample of compound (**73**) was administered to mycelial suspensions of the wild-type *A. parasiticus* (SU-1) in the presence of Dichlorvos[12,69] (10 p.p.m.) to afford labelled versiconal acetate (**74**). Analysis of this material by both mass spectrometry and $^2H$-{$^1H$} n.m.r. spectroscopy[17] securely established the distribution and number of labels shown in structure (**74**). Had the terminal acetate unit been derived trivially, *e.g.* by acylation with acetylCoA, deuterium would have been detectable in no more than trace amounts at the acetyl methyl group. The proportions of integrated intensities of deuterium label in structure (**74**) at C-1′:C-4′:C-6′ were 0.9:2:3,[17] in complete accord with their relative levels at the respective centres in the precursor (**73**) (*vide supra*). Analysis of the mass spectrum of compound (**74**) unequivocally demonstrated the intramolecularity of the rearrangement steps from (**73**). An in-beam electron impact spectrum of unlabelled versiconal acetate (**5**) at 210 °C failed to give a good molecular ion, but strong fragments[69] were observed at *m/z* 382 ($M^+ - H_2O$) and 340 ($M^+$ – HOAc). An analogous spectrum of compound (**74**) gave these fragments for unlabelled versiconal acetate but also higher mass clusters at *m/z* 389 ($M + 7 - H_2O$) and 344 ($M + 7 - HOOC^2H_3$) whose intensities indicated *ca.* 20% specific incorporation of compound (**73**).

*Conclusions.*—Two synthetic routes have been developed to prepare samples of averufin bearing one or more labels at selected positions in experimentally useful amounts. Extension of these methods can and has been made to synthesize related materials of potential biosynthetic interest. For the present discussion, however, specific correlations of labelled sites from the A-ring and side-chain of averufin into aflatoxin $B_1$, versicolorin A, and versiconal acetate have been secured. Intact incorporation has, therefore, been established for averufin in the biosynthetic pathway to the mycotoxin.

While the details of the post-bisfuran rearrangement steps remain to be determined, utilization of A-ring-labelled averufin in conjunction with the patterns of [1,2-$^{13}C_2$]acetate incorporation unequivocally establish the folding of the polyketide precursor (**1**) (Scheme 1), readily identifiable in averufin (**4**), through the cleavage and rearrangement steps to aflatoxin $B_1$. The overall manner in which the individual nuclear carbons of averufin map to aflatoxin is consistent with the general proposals of published biogenetic schemes.[3,15,70,71]

The process by which the unusual bisfuran is assembled can now be addressed in greater detail and may be taken to involve migration of the anthraquinone nucleus of averufin from C-1′ to C-2′ with a net change in oxidation state at the origin of migration, C-1′, from that of an alcohol to an aldehyde — but without loss of the bound hydrogen. This observation excludes a Favorski-like rearrangement[72] to account for the transformation, but other chain-branching mechanisms remain viable.[64b,73] An intramolecular cleavage reaction takes place (either before or after the chain-branching step) to sever the last two carbons of the averufin side-chain, presumably as acetate, in a Baeyer–Villiger-like oxidation of a methyl ketone derived from opening of the side-chain. In so doing an oxygen is inserted into the alkyl side-chain to lead on in an incompletely understood manner to the second ring of the dihydrobisfuran. A working hypothesis to account for these observations has been advanced[67,71,74] to serve as a framework for the design of further experiments. Some preliminary reports of studies to test this theory have been published.[75] These and further results will be fully described in due course.

## Experimental

M.p.s were determined in open capillaries using a Thomas-Hoover m.p. apparatus; both m.p.s and b.p.s are uncorrected. U.v. absorption spectra were recorded on a Varian Cary Model 219, and i.r. absorption spectra were recorded on a Perkin-Elmer Model 599B spectrometer. $^1H$ N.m.r. spectra were obtained using a Varian CFT-20 spectrometer fitted with a proton probe to operate at 80 MHz or, as indicated, a Bruker WM-300 or Varian XL-400 spectrometer operating at 300 and 400 MHz, respectively; chemical shifts ($\delta$) are reported downfield from tetramethylsilane internal standard; cm indicates a complex multiplet. $^2H$ N.m.r. spectra were recorded using a Bruker WM-300 spectrometer operating at 46.1 MHz; spectra reported are proton-noise-decoupled, with chemical shifts given from [$^2H_6$]$Me_2SO$ ([$^2H_6$]DMSO) ($\delta_D$ 2.50) as reference standard. $^{13}C$ N.m.r. spectra were recorded using a Varian CFT-20 or, as indicated, a Bruker WM-300 spectrometer operating at 20 or 75.5 MHz, respectively; unless otherwise indicated, spectra are proton-noise-decoupled, with the chemical shifts given from deuteriochloroform ($\delta_C$ 76.89) or [$^2H_6$] DMSO ($\delta_C$ 39.69) as internal reference. Mass spectra were recorded with a Hitachi-Perkin-Elmer RMU-6 spectrometer or a Kratos MS-50 operating at an electron impact ionizing potential of 70 eV. Microanalytical combustion analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

Analytical t.l.c. was performed on Analtech GHLF Uniplate glass plates coated with silica gel (0.25 mm) containing a fluorescent indicator, and using the mobile phase noted. Preparative t.l.c. (p.l.c.) was performed on Merck Silica Gel 60 $F_{254}$ glass plates, using the size, thickness, and mobile phase indicated. Column chromatography was conducted on Merck Silica Gel 60 (70—230 mesh), using the column size and mobile phase indicated. Short-path column chromatography was conducted under 60 mmHg pressure with silica gel 60, size finer than 0.063 mm (E. Merck). Distillations of liquid products were performed with a Büchi Kugelrohr oven at the indicated air-bath temperature and pressure.

Dry THF and dry ether were distilled from sodium benzophenone ketyl prior to use. DMSO, hexamethylphosphoric triamide (HMPA), and DMF were distilled under reduced pressure from calcium hydride and stored over molecular sieves (4 Å) under nitrogen. Tetramethylethylene diamine (TMEDA) was distilled from and stored over sodium under nitrogen. 2,2,6,6-Tetramethylpiperidine (TMP), pyridine, triethylamine, and di-isopropylamine were distilled from potassium hydroxide under nitrogen and stored under nitrogen. Ethylene glycol was distilled under reduced pressure and stored under nitrogen. Dry dichloromethane, chloroform, and ethyl acetate were distilled from phosphorus pentaoxide. Dry methanol was obtained by distillation from magnesium turnings immediately prior to use. 1,2-Dibromoethane, 1,1,1,3,3,3-hexamethyldisilazane (HMDS), DMAD, and chlorotrimethylsilane were distilled and stored under nitrogen. Magnesium chloride and zinc chloride were dried under reduced pressure in a vacuum Abderhalden desiccator over phosphorus pentaoxide until the desiccant ceased absorbing water. All other reagents were of reagent grade or purified further as indicated.

Butyl-lithium in hexane and s-butyl-lithium in cyclohexane were titrated [diphenylacetic acid[76] (220—250 mg, 1.04—1.18 mmol) in dry THF (5 ml) under nitrogen] prior to use. Unless

otherwise indicated, all liquids or solutions used in organolithium reactions were prepared in flame-dried flasks under an inert atmosphere and were added *via* syringe (Hamilton gastight).

Baths used for low-temperature reactions were formulated[77] as follows: −196 °C, liquid nitrogen; −78 °C, propan-2-ol–solid $CO_2$; −30 °C, nitromethane–solid $CO_2$; −15 °C, ethylene glycol–solid $CO_2$; −10 °C, water–ice–NaCl; 0 °C, water–ice.

### *Synthetic Procedures*

*I. Preparation of* 9,10-*Dihydro*-1,3,6,8-*tetramethoxy*-9,10-*dioxoanthracene*-2-*carbaldehyde* (**13**).—*Methyl* 2,4-*Dimethoxy*-3-*methylbenzoate*. A three-necked, 1 l round-bottomed flask equipped with a condenser, gas inlet tube, heating mantle, and magnetic stirring bar was charged with water (425 ml), and carbon dioxide was bubbled through the water for 1 h. The gas inlet tube was removed and potassium hydrogen carbonate (200 g, 2 mol) was added, followed 30 min later by 2-methylresorcinol (45 g, 365 mmol). The mixture was heated at reflux for 5 h, with carbon dioxide bubbled through the solution for the last 1 h of reflux. The mixture was acidified by a 2 h dropwise addition of conc. hydrochloric acid (180 ml) and was then refrigerated overnight. The precipitate collected by filtration was washed well with cold water and recrystallized from water to afford 2,4-dihydroxy-3-methylbenzoic acid (25.7 g, 42%) as off-white needles, m.p. 213—214 °C (decomp.) (lit.,[78] 210—215 °C after sublimation).

A three-necked, 500 ml round-bottomed flask equipped with a condenser with nitrogen inlet, heating mantle, and magnetic stirring bar was charged with anhydrous potassium carbonate (74 g, 536 mmol), anhydrous acetone (175 ml), 2,4-dihydroxy-3-methylbenzoic acid (12.6 g, 75 mmol), and dimethyl sulphate (56.7 g, 450 mmol). The mixture was heated at reflux under nitrogen and, after 8 h, additional dimethyl sulphate (12 ml, 125 mmol) was added. At 23 h, additional dimethyl sulphate (10 ml, 106 mmol) and anhydrous potassium carbonate (10 g, 72 mmol) were added. At 50 h, the mixture was cooled to room temperature, then filtered, and the filtrate was concentrated under reduced pressure. The yellow residue was partitioned between ether and 1M-potassium hydroxide and the ether phase was washed successively with a second aliquot of 1M-potassium hydroxide, once with 1M-hydrochloric acid, then twice with brine, and was dried over anhydrous magnesium sulphate. Filtration and concentration under reduced pressure gave a yellow oil, which was distilled [126—128 °C/*ca.* 0.5 Torr] to afford *methyl* 2,4-*dimethoxy*-3-*methylbenzoate* (12.4 g, 78.5%) as a solid, m.p. 36—37 °C; $\delta_H$ ($CDCl_3$) 7.76 (1 H, d, *J* 8.8 Hz, 6-H), 6.65 (1 H, d, *J* 8.8 Hz, 5-H), 3.88 (3 H, s, OMe), 3.87 (3 H, s, OMe), 3.80 (3 H, s, OMe), and 2.15 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 000m, 2 940s, 2 840m, 1 710s, 1 595s, 1 485s, 1 460s, 1 435s, 1 410s, 1 275s, 1 140s, 1 110s, 1 015m, and 995m $cm^{-1}$; $R_F$ (ether–hexane, 1:1) 0.46 (Found: C, 62.7; H, 6.9. $C_{11}H_{14}O_4$ requires C, 62.85; H, 6.71%).

Ether extraction of the acidified potassium hydroxide phase afforded the intermediate reaction product methyl 2-hydroxy-4-methoxy-3-methylbenzoate (611 mg, 4%), whose disappearance was used to determine completion of the reaction. Physical data for this compound were: m.p. 74—75 °C (lit.,[79] 76—77 °C); $\delta_H$ ($CDCl_3$) 11.07 (1 H, s, exchangeable with $D_2O$, OH), 7.70 (1 H, d, *J* 8.9 Hz, 6-H), 6.44 (1 H, d, *J* 8.9 Hz, 5-H), 3.91 (3 H, s, OMe), 3.87 (3 H, s, OMe), and 2.11 (3 H, s, Me); $R_F$ (ether–hexane, 1:1) 0.57.

2,4-*Dimethoxy*-3-*methylbenzanilide* (**15**; R = Me). A three-necked, 500 ml round-bottomed flask equipped with a condenser with nitrogen inlet, heating mantle, magnetic stirring bar, and a distillation assembly was charged with anhydrous methanol (80 ml), and sodium metal (5.06 g, 220 mmol) was added in small portions during 1 h. Most of the excess of methanol then was removed from the flask by distillation, leaving some methanol (*ca.* 15 ml) containing the sodium methoxide. The distillation apparatus was removed and dry benzene (200 ml) was added, followed by aniline (18.6 g, 200 mmol), and methyl 2,4-dimethoxy-3-methylbenzoate (42 g, 200 mmol), and the mixture was heated at reflux. At 38 h, t.l.c. (ether–hexane, 1:1) indicated a significant quantity of unchanged starting material ($R_F$ 0.46) along with product (**15**) ($R_F$ 0.36). Therefore, at 38 h, and again at 52 h, sodium methoxide (5.9 g, 110 mmol) was added. At 66 h, the mixture was cooled to room temperature, then poured into 10% hydrochloric acid (200 ml), and the precipitate was collected by filtration. The solid was washed successively with cold water and cold hexane and was recrystallized from benzene–hexane to afford *compound* (**15**) (31.5 g, 58%) as white needles, m.p. 149—150 °C; $\delta_H$ ($CDCl_3$) 9.80 (1 H, br s, NH), 8.06 (1 H, d, *J* 8.8 Hz, 6-H), 7.75—6.95 (5 H, cm, NH*Ph*), 6.78 (1 H, d, *J* 8.8 Hz, 5-H), 3.89 (3 H, s, OMe), 3.84 (3 H, s, OMe), and 2.20 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 340w (NH), 3 000m, 2 960w, 2 940w, 1 660s, 1 595s, 1 535s, 1 498m, 1 482m, 1 440s, 1 315m, 1 268s, 1 250w, and 1 105m $cm^{-1}$ (Found: C, 70.9; H, 6.4; N, 5.1. $C_{16}H_{17}NO_3$ requires C, 70.83; H, 6.32; N, 5.16%).

When this reaction was conducted on the same scale without removal of most of the excess of methanol, the yield of anilide (**15**) was 43% and methyl 4-hydroxy-2-methoxy-3-methylbenzoate (1.53 g, 13.7%) was obtained as a by-product of the reaction. Physical data for this compound were: m.p. 144—145 °C; $\delta_H$ ($CDCl_3$) 11.1 (1 H, br, exchangeable with $D_2O$, OH), 8.01 (1 H, d, *J* 8.9 Hz, 6-H), 6.77 (1 H,d, *J* 8.9 Hz, 5-H), 3.90 (6 H, s, 2-OMe), and 2.18 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 200br (OH), 2 980w, 2 950w, 2 910w, 2 830w, 1 730s, 1 595s, 1 455m, 1 405m, 1 380s, 1 280s, 1 260s, 1 090s, and 980w $cm^{-1}$; $R_F$ (ether–hexane, 1:1) 0.06.

3-(3′,5′-*Dimethoxphenyl*)-5,7-*dimethoxy*-6-*methylphthalide* (**19**; R = Me). A three-necked, 100 ml round-bottomed flask equipped with an addition funnel, a nitrogen inlet, a rubber septum, and a magnetic stirring bar was flame-dried under nitrogen and was charged with dry THF (30 ml), TMEDA (909 mg, 7.82 mmol), and anilide (**15**; R = Me) (1.00 g, 3.70 mmol). The mixture was cooled to −78 °C and s-BuLi in cyclohexane (6.6 ml, 7.84 mmol) was added dropwise during 3 min to produce a bright orange solution. After a further 7—8 min, a solution of aldehyde (**17**; R = Me) (689 mg, 4.15 mmol) in THF (9 ml) was added dropwise *via* the addition funnel, causing complete disappearance of the orange colour. After 1 h, the mixture was allowed to come to room temperature; a typical t.l.c. (ethyl acetate–hexane, 3:10) profile at this time revealed unchanged aldehyde (**17**; R = Me) ($R_F$ 0.59), a small amount of unchanged anilide (**15**; R = Me) ($R_F$ 0.42), a trace of required phthalide (**19**; R = Me) [$R_F$ 0.27; resulting from hydrolysis and cyclization of (**18**; R = Me) on the t.l.c. plate], and the hydroxy amide (**18**; R = Me) ($R_F$ 0.14). The mixture was acidifed with 3M-hydrochloric acid (30 ml), stirred under nitrogen overnight, and then was poured into water and extracted three times with ether. The extracts were pooled, washed successively with water (2 ×) and brine, dried over anhydrous magnesium sulphate, and filtered, the filtrate was concentrated under reduced pressure. The residue from two such reactions was dissolved in a small amount of chloroform and chromatographed on a column of silica gel (250 g, 50 × 40 cm; ethyl acetate–hexane, 3:10) to afford the anilide (**15**; R = Me) (177 mg, 8.9% recovery) and the *required phthalide* (**19**; R = Me) (2.18 g, 85.9% for the two reactions), which crystallized from toluene–hexane as white needles, m.p. 121—122 °C; $\delta_H$($CDCl_3$) 6.43 (4 H, br s, 2′-, 4-, 4′-, and 6′-H), 6.13 (1 H, s, 3-H), 4.08 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.77 (6 H, s, 2-OMe), and 2.14 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 000w, 2 940w, 2 838w, 1 750s, 1 600s, 1 468s, 1 430m, 1 322m,

1 235m, 1 160s, 1 140s, 1 000m, 940w, 860w, and 840w $cm^{-1}$ (Found: C, 66.4; H, 5.95. $C_{19}H_{20}O_6$ requires C, 66.27; H, 5.85%).

6-(3′,5′-*Dimethoxybenzyl*)-2,4-*dimethoxy*-3-*methylbenzoic acid* (**20**; R = Me). A three-necked, 100 ml round-bottomed flask equipped with a gas inlet tube, condenser, heating mantle, and magnetic stirring bar was charged with glacial acetic acid (50 ml) and the phthalide (**19**; R = Me) (688 mg, 2 mmol). 5% Palladium–carbon (172 mg) was added and a rapid stream of hydrogen was bubbled through the mixture at 65 °C. After 3 h, the mixture was cooled and filtered, and the filtrate was concentrated under reduced pressure to give a yellow oil, which crystallized from aq. ethanol to afford the *acid* (**20**; R = Me) (580 mg, 83.8%) as white needles, m.p. 129—130 °C; $\delta_H$ ($CDCl_3$) 7.80 (1 H, br, exchangeable with $D_2O$, $CO_2H$), 6.48 (1 H, s, 5-H), 6.32 (3 H, m, 2′-, 4′-, and 6′-H), 4.17 (2 H, s, $CH_2$), 3.82 (3 H, s, OMe), 3.76 (3 H, s, OMe), 3.73 (6 H, s, 2-OMe), and 2.13 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 490w (OH), 3 100br (OH), 2 940w, 2 838w, 1 725m, 1 690m, 1 595s, 1 460m, 1 430w, 1 290w, 1 150s, 1 125w, 1 065w, and 835w $cm^{-1}$; $R_F$ (ethyl acetate–hexane, 7:10) 0.08 (Found: C, 65.6; H, 6.3. $C_{19}H_{22}O_6$ requires C, 65.88; H, 6.40%).

1,3,6,8-*Tetramethoxy*-2-*methylanthraquinone* (**22**; R = Me, X = H). A 5 ml round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was charged with the acid (**20**; R = Me) (50 mg, 0.14 mmol) and TFA (2 ml). The mixture was cooled to −10 °C, TFAA (1.49 g, 7.0 mmol) was added, and the mixture was stirred under nitrogen for 15 min. The mixture was allowed to come to room temperature and was then concentrated under reduced pressure to afford a dark green oil, which was used without purification in the next reaction. The oil could be chromatographed on a column of silica gel (30 g, 30 × 1.8 cm; ether–chloroform, 8:2) to provide a *ca.* 1:9 mixture (46 mg) of the anthraquinone (**22**; R = Me, X = H) and the anthracenol (**21a**; R = Me) as a viscous, green-yellow oil, $\delta_H$ ($CDCl_3$) 10.64 (1 H, br, exchangeable with $D_2O$, OH), 6.76 (1 H, br s, 4-H), 6.60 (1 H, br d, *J* 1.8 Hz, 5-H), 6.33 (1 H, d, *J* 1.8 Hz, 7-H), 4.03 (3 H, s, OMe), 3.91 (3 H, s, OMe), 3.90 (3 H, s, OMe), 3.89 (3 H, s, OMe), and 2.27 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 350br (OH), 3 000w, 2 935w, 1 625s, 1 595m, 1 575m, 1 410m, 1 415m, 1 380m, 1 365m, 1 300m, 1 240m, 1 170m, 1 158m, 1 130s, 1 055w, and 860w $cm^{-1}$; $R_F$ (ether–chloroform, 8:2) 0.76 [the $R_F$ of anthraquinone (**22**) was 0.53].

A 10 ml round-bottomed flask containing the crude anthracenol (**21a**; R = Me) was equipped with a magnetic stirring bar and 80% acetic acid (4 ml) was added. The mixture was cooled to 0 °C and a 0.58M stock solution (0.5 ml) of chromium trioxide (580 mg, 5.8 mmol) in 80% acetic acid (10 ml) was added. After 30 min, the mixture was allowed to come to room temperature and more stock solution (2 ml) was added at this time, and again at 2 h and at 3 h. After an additional 1 h, the mixture was poured into water and extracted three times with chloroform. The extracts were pooled, washed successively with water, 5% aq. sodium hydrogen carbonate, and brine, and dried over anhydrous magnesium sulphate. Filtration and concentration under reduced pressure gave a yellow solid, which was recrystallized from ethyl acetate–hexane, to give the anthraquinone (**22**; R = Me, X = H) [25 mg, 50.6% from acid (**20**)] as fine yellow needles, m.p. 191–192 °C (lit.,[21] 192 °C); $\delta_H$($CDCl_3$) 7.48 (1 H, s, 4-H), 7.33 (1 H, d, *J* 2.5 Hz, 5-H), 6.76 (1 H, d, *J* 2.5 Hz, 7-H), 3.98 (3 H, s, OMe), 3.96 (3 H, s, OMe), 3.95 (3 H, s, OMe), 3.92 (3 H, s, OMe), and 2.24 (3 H, s, Me); $\nu_{max.}$($CHCl_3$) 3 000m, 2 965m, 2 940m, 2 840w, 1 665s, 1 610s, 1 590s, 1 450s, 1 325s, 1 265s, 1 165s, 1 135s, 1 065m, and 995s $cm^{-1}$; $R_F$ (ethyl acetate–hexane, 7:10) 0.34.

2-*Bromomethyl*-1,3,6,8-*tetramethoxyanthraquinone* (**22**; R = Me, X = Br). A 100 ml round-bottomed flask equipped with a condenser with nitrogen inlet and a magnetic stirring bar was charged with the anthraquinone (**22**; R = Me, X = H) (250 mg, 0.73 mmol), *N*-bromosuccinimide (NBS) [194 mg of 70% pure material (iodometric assay[80]), 0.76 mmol], and tetrachloromethane–chloroform (10:1) (45 ml; bubbled 30 min with nitrogen). The mixture was irradiated with a Dyna-Lume High Intensity Radiant Heat Projector lamp (Cole-Parmer) for 1 h, cooled, poured into water, and extracted three times with chloroform. The extracts were pooled, washed successively with 10% aq. potassium carbonate (2 ×), and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a yellow solid, which was triturated with ether to afford the title bromide (**22**; R = Me, X = Br) (308 mg, 100%) as a yellow powder, m.p. 222—225 °C (decomp.) (lit.,[21] 225 °C); $\delta_H$($CDCl_3$) 7.53 (1 H, s, 4-H), 7.34 (1 H, d, *J* 2.5 Hz, 5-H), 6.79 (1 H, d, *J* 2.5 Hz, 7-H), 4.68 (2 H, s, $CH_2$), 4.09 (3 H, s, OMe), 4.06 (3 H, s, OMe), 3.98 (3 H, s, OMe), and 3.96 (3 H, s, OMe); $R_F$ (ethyl acetate–hexane, 1:1; plus 10 drops acetic acid per 10 ml) 0.39 [$R_F$ of (**22**; R = Me, X = H) 0.32].

9,10-*Dihydro*-1,3,6,8-*tetramethoxy*-9,10-*dioxoanthracene*-2-*carbaldehyde* (**13**). A 50 ml round-bottomed flask containing bromide (**22**; R = Me, X = Br) (308 mg, 0.73 mmol) was equipped with a reflux condenser with nitrogen inlet and a magnetic stirring bar. Acetic anhydride (15 ml) and sodium acetate (750 mg, 9.15 mmol) were added and the mixture was heated at reflux under nitrogen for 30 min. The mixture was cooled to room temperature, poured into water, and extracted three times with chloroform. The extracts were pooled, washed successively with warm water (2 ×), 10% aq. sodium hydrogen carbonate, and brine, and dried over anhydrous magnesium sulphate. Filtration and concentration under reduced pressure provided acetate (R = Me, X = OAc) 293 mg, 100%), used in the next reaction without purification, as a yellow solid, m.p. 204—208 °C (decomp.) (lit.,[21] 208 °C); $\delta_H$($CDCl_3$) 7.55 (1 H, s, 4-H), 7.35 (1 H, d, *J* 2.5 Hz, 5-H), 6.70 (1 H, d, *J* 2.5 Hz, 7-H), 5.26 (2 H, s, $CH_2$), 4.00 (3 H, s, OMe), 3.98 (6 H, s, 2-OMe), 3.96 (3 H, s, OMe), and 2.07 (3 H, s, Ac); $R_F$ (ether) 0.46 [$R_F$ of (**22**; R = Me, X = Br) 0.59].

A 25 ml round-bottomed flask containing acetate (**22**: R = Me, X = OAc) (293 mg, 0.73 mmol) was equipped with a condenser with nitrogen inlet and a magnetic stirring bar, and absolute ethanol (10 ml) and 0.5M-ethanolic KOH (2.5 ml, 1.25 mmol) were added. The mixture was heated at reflux under nitrogen for 30 min, cooled to room temperature, poured into water, and extracted three times with chloroform. The extracts were pooled, washed successively with water (2 ×), and brine, and dried over anhydrous magnesium sulphate. Filtration and concentration under reduced pressure afforded the alcohol (**22**; R = Me, X = OH) (262 mg, 100%), used in the next reaction without purification, as a yellow solid, m.p. 229—231 °C (lit.,[21] 233 °C); $\delta_H$($CDCl_3$–[$^2H_6$]DMSO, 4:1) 7.54 (1 H, s, 4-H), 7.32 (1 H, d, *J* 2.5 Hz, 5-H), 6.82 (1 H, d, *J* 2.5 Hz, 7-H), 4.77 (2 H, br, d, *J* 5.9 Hz, collapses to s upon addition of $D_2O$, $CH_2$), 4.03 (3 H, s, OMe), 3.99 (6 H, s, 2 OMe), and 3.97 (3 H, s, OMe); $R_F$ (ether) 0.22.

A 25 ml round-bottomed flask containing the alcohol (**22**; R = Me, X = OH) (262 mg, 0.73 mmol) was equipped with a magnetic stirring bar, and dichloromethane (10 ml) and PCC (315 mg, 1.46 mmol) were added. The mixture was stirred vigorously for 3 h, poured into ether (150 ml), and stirred for 10 min. Filtration through Celite and concentration under reduced pressure provided a brown residue, which was chromatographed on a column of silica gel (50 g, 35 × 2.1 cm; ether–chloroform, 8:2). The product was triturated with ether to afford aldehyde (**13**) [221 mg, 84.9% from (**22**; R = Me, X = H)] as a yellow powder, m.p. 212—213 °C; $\delta_H$($CDCl_3$) 10.49 (1 H, s, CHO), 7.57 (1 H, s, 4-H), 7.33 (1 H, d, *J* 2.5 Hz, 5-H), 6.81 (1 H, d, *J* 2.5 Hz, 7-H), 4.06 (6 H, s, 2 OMe), 3.99 (3 H, s, OMe), and 3.97 (3 H, s, OMe); $\nu_{max.}$($CHCl_3$) 3 020w, 3 010w, 2 970w, 2 940w, 2 840(2), 1 695s, 1 670s, 1 598s, 1 580s, 1 460m, 1 438m, 1 385m, 1 325s,

1 265s, 1 165s, 1 140s, and 1 000m $cm^{-1}$; $R_F$ (ether) 0.45; $R_F$ (ether–chloroform, 8:2) 0.76.

*II. Preparation of* (+)-6,8-O-*Dimethylaverufin* (**36**).—2,6-*Bis*-(*methoxymethoxy*)*benzaldehyde* (**22**; R′ = MOM).[33] A three-necked, 250 ml round-bottomed flask equipped with an argon inlet, a rubber septum, and a magnetic stirring bar was flame-dried under argon and charged with dry ether (65 ml), TMEDA (2.62 g, 22.5 mmol), and compound (**23**)[33] (4.01 g, 20.2 mmol). The mixture was cooled to 0 °C and BuLi in hexane (14.3 ml, 22.3 mmol) was added dropwise during 5 min to produce a yellow suspension. After being stirred under argon at 0 °C for 2 h, the mixture was allowed to warm to room temperature, whereupon dry DMF (2.63 g, 40 mmol) was added in one portion. The mixture was stirred for a further 30 min and then was poured into water and extracted three times with ether. The extracts were pooled, washed successively with water (3 ×), saturated aq. ammonium chloride, and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a yellow solid (4.2 g), which was chromatographed on a column of silica gel (320 g, 50 × 4.8 cm; hexane–ether, 5:2) to give *compound* (**24**; R′ = MOM) (3.82 g, 83.5%) as a white solid, m.p. 57—58 °C; $\delta_H(CDCl_3)$ 10.54 (1 H, s, CHO), 7.40 (1 H, split t, $J_{app}$ 8.4 Hz, A of $AB_2$, 4-H), 6.81 (2 H, s and d, $J_{app}$ 8.4 Hz, $B_2$ of $AB_2$, 3- and 5-H), 5.26 (4 H, s, 2 $OCH_2O$), and 3.50 (6 H, s, 2 OMe); $\nu_{max.}(CHCl_3)$ 3 000w, 2 960m, 2 830m, 2 780w, 1 685s, 1 595s, 1 470m, 1 400m, 1 260s, 1 165s, 1 050s, 930m, and 920m $cm^{-1}$; $R_F$ (ether–hexane, 2:5) 0.37 (Found: C, 58.2; H, 6.4. $C_{11}H_{14}O_5$ requires C, 58.40; H, 6.24%).

*Tricyclic Ketal* (**26**).[33] A three-necked, 25 ml round-bottomed flask was charged with lithium (2% sodium) dispersion (25% in mineral oil; 440 mg, 15.7 mmol). The flask was equipped with an argon inlet, a rubber septum, and a magnetic stirring bar, and the mineral oil was removed by three washings with pentane under argon. Dry ether (8 ml) was added and the mixture was cooled to −10 °C, whereupon 5-bromopentan-2-one ethylene ketal[32,40] (**11**; X = Br) (938 mg, 4.5 mmol) was added dropwise during 5 min. The mixture was stirred at −10 °C under argon for 3 h, after which time a solution of aldehyde (**24**; R′ = MOM) (1.02 g, 4.5 mmol) in dry THF (5 ml) was added dropwise during 5 min. This mixture was stirred under argon at −10 °C for 1 h, quenched with water (1 ml), poured into brine, and extracted four times with ether. The extracts were pooled, dried over anhydrous potassium carbonate, filtered, and concentrated under reduced pressure. The residue (1.45 g) was chromatographed on a column of silica gel (60 g, 50 × 1.8 cm; ether) to afford compound (**25**; R′ = MOM) (1.43 g, 89.0%) as a viscous oil, $\delta_H(CDCl_3)$ 7.13 (1 H, split t, $J_{app}$ 8.1 Hz, A of $AB_2$, 4-H), 6.71 (2 H, s and d, $J_{app}$ 8.1 Hz, $B_2$ of $AB_2$, 3- and 5-H), 5.20 (4 H, s, 2 $OCH_2O$), 5.15 (1 H, obscured br, 1′-H), 3.90 (4 H, s, $OCH_2CH_2O$), 3.66 (1 H, d, *J* 11.6 Hz, exchangeable with $D_2O$, OH), 3.48 (6 H, s, 2 OMe), 2.0—1.3 (6 H, cm, 2′-, 3′-H, and 4′-$H_2$), and 1.30 (3 H, s, 6′-$H_3$); $\nu_{max.}$); $\nu_{max.}(CHCl_3)$ 3 550(OH), 3 000s, 2 950s, 2 900m, 2 825w, 1 600s, 1 460s, 1 440m, 1 400m, 1 380m, 1 235s, 1 185s, 1 160s, 1 045s, and 925m $cm^{-1}$; $R_F$ (ether) 0.46. The alcohol (**25**) was found to be unstable at room temperature, and so was converted directly into tricyclic ketal (**26**).

A 25 ml round-bottomed flask equipped with a nitrogen inlet and magnetic stirring bar was charged with the alcohol (**25**; R′ = MOM) (280 mg, 0.79 mmol) and 50% aqueous acetic acid (16 ml; degassed for 1 h with nitrogen ebullition) containing conc. sulphuric acid (9 drops per 30 ml). The mixture was stirred in the dark under nitrogen for 26 h, poured into water, and extracted three times with ether. The extracts were pooled, washed successively with water (3 ×), 5% aq. sodium hydrogen carbonate, and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a yellow oil (198 mg), which was chromatographed on a column of silica gel (10 g, 25 × 1.4 cm; pentane–ether, 4:1) to afford the *ketal* (**26**) (132 mg, 67.1%) as a solid, m.p. 46—47 °C; $\delta_H(CDCl_3)$ 7.07 (1 H, t, *J* 8.2 Hz, 5-H), 6.57 (1 H, br d, *J* 8.2 Hz, 6-H), 6.48 (1 H, br d, *J* 8.2 Hz, 4-H), 5.26 (1 H, br d, $J_{app}$ 3.3 Hz, 1′-H), 5.16 (2 H, s, $OCH_2O$), 3.44 (3 H, s, OMe), 2.2—1.2 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.52 (3 H, s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 000m, 2 950s, 2 870w, 2 850w, 2 820w, 2 790w, 1 612s, 1 600s, 1 470s, 1 388s, 1 245s, 1 160s, 1 050s, 945s, and 865s $cm^{-1}$; $R_F$ (ether–pentane, 3:2) 0.84 (Found: C, 67.4; H, 7.4. $C_{14}H_{18}O_4$ requires C, 67.18; H, 7.25%).

*Anilide* (**30**; R′ = MOM). A three-necked, 100 ml round-bottomed flask containing the above ketal (**26**) (750 mg, 3 mmol) was equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dry ether (20 ml) was added and the mixture was stirred under nitrogen as BuLi in hexane (2.3 ml, 3.6 mmol) was added rapidly to produce a yellow solution; within 15 min, a milky white precipitate began to form. When the solution above this precipitate had become virtually colourless (*ca.* 2.5 h), phenyl isocyanate [438 mg, 3.68 mmol; distilled through a 15 cm Vigreaux column under reduced pressure (65 °C, *ca.* 15 Torr) immediately before use] was added in one portion as the neat liquid, thus causing disappearance of the precipitate. After 30 min, the mixture was poured into water and extracted three times with ether. The extracts were pooled, washed successively with water (3 ×) and brine, and dried over potassium carbonate. Filtration and concentration under reduced pressure produced a yellow gum (1.28 g), which was chromatographed on a column of silica gel [320 g, 60 × 4.0 cm; hexane–chloroform–ether, 4:3:1; this amount of silica gel was required to remove a trace impurity ($R_F$ 0.31) from the anilide (**30**; R′ = MOM) ($R_F$ 0.36): the diphenylurea ($R_F$ 0.45) and cyclic trimer ($R_F$ 0.21) by-products could be removed easily using 40% less silica gel if slightly impure aniline were desired] to afford pure *anilide* (**30**; R′ = MOM) (1.085 g, 98.0%) as a foam, $\delta_H(CDCl_3)$ 8.98 (1 H, br s, NH), 7.90 (1 H, d, *J* 8.8 Hz, 6-H), 7.75—6.95 (5 H, cm, NHA*Ph*), 6.74 (1 H, d, *J* 8.8 Hz, 5-H), 5.37 (1 H, br t, $J_{app}$ 3.5 Hz, 1′-H), 5.07 (2 H, AB q, *J* 5.6 Hz, $OCH_2O$), 3.54 (3 H, s, OMe), 2.25—1.25 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.55 (3 H, s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 370w (NH), 3 000w, 2 940w, 1 660s, 1 600s, 1 530s, 1 500m, 1 475m, 1 440s, 1 382w, 1 315s, 1 260s, 1 242s, 1 162s, 1 020m, 920m, and 695w $cm^{-1}$ (Found: C, 68.1; H, 6.5; N, 3.6. $C_{21}H_{23}NO_5$ requires C, 68.28; H, 6.28; N, 3.79%).

*The phthalide* (**31a**). A three-necked, 25 ml round-bottomed flask containing the anilide (**30**; R′ = MOM) (224 mg, 0.61 mmol) was equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dry THF (4.5 ml) and TMEDA (154 mg, 1.32 mmol) were added and the mixture was cooled to −78 °C, whereupon s-BuLi in cyclohexane (1.1 ml, 1.32 mmol) was added dropwise during 5 min to produce a dark orange solution. After 7 min, a solution of 3,5-dimethoxybenzaldehyde (**17a**) (106 mg, 0.64 mmol) in dry THF (3 ml) was added dropwise, causing complete disappearance of the orange colour. The mixture was stirred under nitrogen at −78 °C for 30 min, allowed to warm to room temperature, quenched with water, and extracted three times with ether. The extracts were pooled, washed successively with water (3 ×) and brine (2 ×), and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a yellow gum (340 mg). A typical t.l.c. (hexane–chloroform–ether, 4:3:1) profile of this material revealed a trace of aldehyde ($R_F$ 0.53), a trace of anilide (**30**) ($R_F$ 0.36), a trace of phthalide (**31a**) ($R_F$ 0.31; resulting from hydrolysis and cyclization on the t.l.c. plate), and hydroxy amide (**33a**) ($R_F$ 0.05). A 100 ml round-bottomed flask containing this gum was fitted with a nitrogen inlet and a

magnetic stirring bar. THF (38 ml), water (36 ml), and 2M-sulphuric acid (2.4 ml, 1.2 mmol) were added; the mixture was purged with nitrogen for 30 min and then was stirred under nitrogen for 24 h. The mixture was poured into water and extracted three times with ether. The extracts were pooled, washed successively with water, saturated aq. sodium hydrogen carbonate, and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a gum, which was chromatographed on a column of silica gel (35 g, 30 × 1.8 cm; pentane–ether, 3:1) to afford the *phthalide* (**31a**) (236 mg, 88.0%) as a diastereoisomeric mixture, which crystallized from pentane as cubes, m.p. 114—123 °C; $\delta_H(CDCl_3)$ 6.43 (4 H, br s, 4-, 2″-, 4″-, and 6″-H), 6.15 and 6.12 (1 H, 2 s, 3-H), 5.51 (2 H, s, $OCH_2O$), 5.35 (1 H, br m, 1′-H), 3.77 (6 H, s, 2 OMe), 3.55 (3 H, s, $OCH_2OMe$), 2.25—1.20 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.51 and 1.50 (3 H, 2 s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 000m, 2 940m, 2 840w, 1 750s, 1 610s, 1 598s, 1 458s, 1 382m, 1 360m, 1 335m, 1 300m, 1 160s, 1 135s, 1 060m, 1 020s, 912s, and 840m $cm^{-1}$ (Found: C, 65.05; H, 6.1. $C_{24}H_{26}O_8$ requires C, 65.15; H, 5.92%).

*Diol* (**34a**). A 25 ml round-bottomed flask containing the phthalide (**31a**) (245 mg, 0.554 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dry THF (8 ml) was added and as the mixture was being stirred under nitrogen, lithium aluminium hydride (64 mg, 1.68 mmol) was added to produce a rust-coloured mixture; within 30 min, the colour had become grey. After being stirred for a total of 1 h, the mixture was treated with water (70 μl) (added carefully), followed by 20% aq. sodium hydroxide (70 μl) and water (0.25 ml). Ether (15 ml) and anhydrous potassium carbonate (*ca.* 0.25 g) were added and the mixture was filtered through Celite. Concentration under reduced pressure provided the diol (**34a**) (248 mg, 100%) as a foamy, diastereoisomeric mixture, which was used in subsequent reactions without purification; $\delta_H(CDCl_3)$ 6.58 and 6.37 (1 H, 2 s, 4-H), 6.59 (2 H, d, *J* 2.3 Hz, 2″- and 6″-H), 6.40 (1 H, t, *J* 2.3 Hz, 4″-H), 6.00 and 5.90 [1 H, 2 s, ArC*H*(OH)Ar], 5.14 (1 H, br m, 1′-H), 5.00 (2 H, 3 lines, $OCH_2O$), 4.61 and 4.50 (2 H, 2 s and apparent d, $J_{app}$ 2.8 Hz, Ar$CH_2$OH), 4.10 (1 H, br, exchangeable with $D_2O$, OH), 3.78 (3 H, s, OMe), 3.77 (3 H, s, OMe), 3.61 and 3.60 (3 H, 2 s, $CH_2OMe$), 3.35 (1 H, br, exchangeable with $D_2O$, OH), 2.25—1.25 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.49 and 1.48 (3 H, 2 s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 400br (OH), 3 000m, 2 945m, 2 840m, 1 605m, 1 590m, 1 460m, 1 430m, 1 385m, 1 338m, 1 160s, 1 125m, 1 065m, 1 035m, 1 025m, 970m, 930m, 850m, 840m $cm^{-1}$; $R_F$ (ethyl acetate–hexane, 5:4) 0.38 [$R_F$ of phthalide (**31a**) 0.79].

*The* o-*benzoylbenzaldehyde* (**37a**). A 10 ml round-bottomed flask containing diol (**34a**) (44.6 mg, 0.1 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dichloromethane (1.5 ml), PCC (88 mg, 0.41 mmol), and sodium acetate (7 mg, 0.08 mmol) were added. The mixture was stirred under nitrogen for 1 h, ether (8 ml) was added, the brown mixture was filtered through a pad of Florosil, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of silica gel (2 g, 10 × 1 cm; pentane–ether, 3:1), to give the phthalide (**31a**) (5.4 mg, 12.2%) and the *o*-benzoylbenzaldehyde (**37a**) (31 mg, 70.1%) as an oil, $\delta_H(CDCl_3)$ 9.96 (1 H, s, CHO), 6.92 (2 H, d, *J* 2.3 Hz, 2″- and 6″-H), 6.63 (1 H, t, *J* 2.3 Hz, 4″-H), 6.61 (1 H, s, 3-H), 5.39 (1 H, br s, 1′-H), 5.12 (2 H, s, $OCH_2O$), 3.74 (6 H, s, 2 OMe), 3.51 (3 H, s, $CH_2OMe$), 2.25—1.25 (6 H, m, 2′-, 3′-, and 4′-$H_2$), and 1.54 (3 H, s, 6′-$H_3$); $R_F$ (ethyl acetate–hexane, 5:4) 0.67.

*Pseudoacid* (**35a**). A 100 ml round-bottomed flask containing the diol (**34a**) (248 mg, 0.556 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar. Acetone (25 ml) and potassium permanganate (640 mg, 4 mmol) in 1% aq. sodium hydrogen carbonate (12 ml; pH *ca.* 8.6) were added and the mixture was purged with nitrogen for 30—45 min. The mixture was stirred vigorously under nitrogen in the dark (foil wrap); after 8–10 h, solid potassium permanganate (90 mg, 0.57 mmol) was added. After a total of 24 h, most of the acetone was removed under reduced pressure and then water (24 ml) was added to restore the original solvent volume. The mixture was stirred vigorously as a 1.3M-solution of hydrazine monohydrochloride (1.78 g, 26 mmol) in water (20 ml) was added slowly until all of the brown solid (manganese dioxide) had been reduced to a gelatinous white solid [$Mn(OH)_2$] (11—12 ml, 3.5—4 equiv. per equiv. permanganate used, was required). Hydrochloric acid (1M) then was added dropwise just until all of the white solid had dissolved (pH *ca.* 6), and the mixture was extracted three times with ether. The extracts were pooled, washed successively with water (3 ×) and brine (2 ×), and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure provided crude pseudoacid (**35a**) (270 mg, 106%) as a yellow foam. This compound was found to be unstable and was used in subsequent reactions without purification. However, the compound (135 mg) could be purified by chromatography on a column of acid-washed silica gel (45 g, 35 × 2.1 cm; ether–dichloromethane, 95:5), to afford pure pseudoacid (**35a**) (108 mg, 84.8%) as a diastereoisomeric mixture in the form of a foam, $\delta_H(CDCl_3)$ 6.73 (2 H, d, *J* 2.3 Hz, 2′- and 6′-H), 6.64 (1 H, s, 4-H), 6.45 (1 H, t, *J* 2.3 Hz, 4′-H), 5.45 (2 H, AB q, *J* ~6 Hz, $OCH_2O$), 5.36 (1 H, br m, 1′-H), 4.25 (1 H, br, exchangeable with $D_2O$, OH), 3.78 (6 H, s, 2-OMe), 3.53 (3 H, s, $CH_2OMe$), 2.25—1.25 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.50 (3 H, s, 6′-H); $\nu_{max.}(CHCl_3)$ 3 300br (OH), 3 000w, 2 950m, 2 840w, 1 760s, 1 610s, 1 600s, 1 440s, 1 235s, 1 160s, 1 135s, 1 068m, 1 030m, 990m, 975s, and 842m $cm^{-1}$; $R_F$ (ethyl acetate–hexane, 5:4) 0.12.

6,8-*Di*-o-*methylaverufin* (**36**). A 25 ml round-bottomed flask containing crude pseudoacid (**35a**) (135 mg of *ca.* 80% pure material; 0.236 mmol) was purged with nitrogen for 5 min. TFA (3 ml) and TFAA (1.49 g, 7.08 mmol) were added to produce a dark red solution. After being stirred under nitrogen for 1 h, the mixture was evaporated under reduced pressure, the residue was chromatographed on a column of silia gel (35 g, 30 × 1.8 cm; ether–chloroform, 8:2), and the product was triturated with ether to give 6,8-di-*O*-methylaverufin (**36**) (75 mg, 80.3%) as a yellow-orange powder, m.p. 210—213 °C (lit.,[69] 208—209 °C; lit.,[33] 211.5—212.5 °C); $\delta_H$ 12.55 (1 H, s, exchangeable with $D_2O$, OH), 7.44 (1 H, d, *J* 2.5 Hz, 5-H), 7.20 (1 H, s, 4-H), 6.77 (1 H, d, *J* 2.5 Hz, 7-H), 5.39 (1 H, br d, $J_{app}$ 3.5 Hz, 1′-H), 4.01 (3 H, s, OMe), 3.97 (3 H, s, OMe), 2.25—1.25 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.57 (3 H, s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 400br, (OH), 3 000w, 2 950w, 2 840w, 1 670w, 1 620s, 1 595s, 1 560w, 1 400m, 1 345s, 1 325s, 1 270s, 1 250s, 1 215m, 1 160s, 995m, and 840m $cm^{-1}$; $\lambda_{max.}$(EtOH) (log ε) 224 (4.52), 250 (4.08), 287 (4.43), 310 (3.88), and 440 nm (3.89); $R_F$ (ether–chloroform, 8:2) 0.70 (Found: C, 66.5; H, 5.3. Calc. for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09%).

*III. Preparation of* (±)-*Averufin* (**4**).—3,5-*Bis*(*methoxymethoxy*)*benzaldehyde* (**17b**). A 500 ml round-bottomed flask equipped with a nitrogen inlet and magnetic stirring bar was charged with PCC (11.83 g, 54.9 mmol), sodium acetate (1.5 g, 18.3 mmol), and dichloromethane (60 ml). A solution of 3,5-bis(methoxymethoxy)benzyl alcohol[33] (6.07 g, 26.6 mmol) in dichloromethane (10 ml) was added in one portion and the mixture was stirred under nitrogen for 3 h. After this time, ether (300 ml) was added and the brown mixture was filtered through a short pad of Florisil over Celite. Concentration under reduced pressure provided a brown oil, which was chromatographed on a column of silica gel (360 g, 60 × 4.8 cm; pentane–ether, 3:1) to provide the *aldehyde* (**17b**) (5.45 g, 90.6%), which was distilled

under reduced pressure [138—139 °C (*ca.* 0.5 Torr)] and stored under argon; $\delta_H(CDCl_3)$ 9.91 (1 H, s, CHO), 7.22 (2 H, d, *J* 2.3 Hz, 2- and 6-H), 6.97 (1 H, t, *J* 2.3 Hz, 4-H), 5.21 (4 H, s, 2 $OCH_2O$), and 3.49 (6 H, s, 2 OMe); $\nu_{max.}(CHCl_3)$ 3 000m, 2 955m, 2 930m, 2 925m, 2 900m, 2 730w, 1 695s, 1 595s, 1 460s, 1 380s, 1 335m, 1 290s, 1 145s, 1 025s, 925s, and 855m $cm^{-1}$; $R_F$ (hexane–chloroform–ether, 4:3:1) 0.53 (Found: C, 58.2; H, 6.4. $C_{11}H_{14}O_5$ requires C, 58.40; H, 6.24%).

*The Phthalide* (**31b**). A three-necked, 100 ml round-bottomed flask containing the anilide (**30**; R′ = MOM) (919 mg, 2.49 mmol) was equipped with a nitrogen inlet, a rubber septum, and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dry THF (25 ml) and TMEDA (608 mg, 5.23 mmol) were added and the mixture was cooled to −78 °C, whereupon s-BuLi in cyclohexane (4.3 ml, 5.3 mmol) was added dropwise during 5 min to produce a dark orange solution. After 7 min, a solution of aldehyde (**17b**) (578 mg, 2.55 mmol) in dry THF (3 ml) was added dropwise, causing complete disappearance of the orange colour. The mixture was stirred under nitrogen at −78 °C for 45 min, allowed to warm to room temperature, quenched with water, and extracted three times with ether. The extracts were pooled, washed successively with water (3×) and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a light yellow gum (1.54 g). A typical t.l.c. (ether–pentane, 1:1) profile of this material revealed a trace of aldehyde (**17b**) ($R_F$ 0.70), a trace of anilide (**30**) ($R_F$ 0.60), a trace of the phthalide (**31b**) [$R_F$ 0.42; resulting from hydrolysis of compound (**33b**) on the t.l.c. plate], and hydroxy amide (**33b**) ($R_F$ 0.16). A 500 ml round-bottomed flask containing this gum was equipped with a nitrogen inlet and a magnetic stirring bar. THF (115 ml), water (125 ml), and 2M-sulphuric acid (6.2 ml, 3.1 mmol) were added, and the mixture was purged with nitrogen for 30 min stirred under nitrogen for 24 h, poured into water, and extracted four times with ether. The extracts were pooled, washed successively with water (3×), saturated aq. sodium hydrogen carbonate, and brine, and dried over anhydrous potassium carbonate. Filtration and concentration under reduced pressure provided a yellow gum (1.36 g), which was chromatographed on a column of silica gel (136 g, 45 × 3.3 cm; ether–pentane, 1:1) to afford the *phthalide* (**31b**) (1.11 g, 88.6%) as a diastereoisomeric mixture in the form of a gum, $\delta_H(CDCl_3)$ 6.74 (1 H, t, *J* 2.1 Hz, 4″-H), 6.61 (2 H, d, *J* 2.1 Hz, 2″- and 6″-H), 6.46 (1 H, s, 4-H), 6.14 and 6.12 (1 H, 2 s, 3-H), 5.50 (2 H, s, $OCH_2O$), 5.14 (1 H, br m, 1′-H), 5.13 (4 H, s, 2 $OCH_2O$), 3.55 (3 H, s, OMe), 3.46 (6 H, s, 2 OMe), 2.25—1.20 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.52 and 1.51 (3 H, 2 s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 000w, 2 960m, 2 905w, 2 835w, 1 755s, 1 615s, 1 600s, 1 465m, 1 450m, 1 445m, 1 160s, 1 145s, 1 045s, 930m, 920m, and 840m $cm^{-1}$ (Found: C, 62.3; H, 6.1. $C_{26}H_{30}O_{11}$ requires C, 62.14; H, 6.02%).

*Diol* (**34b**). A 100 ml round-bottomed flask containing the phthalide (**31b**) (1.05 g, 2.09 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dry THF (30 ml) was added and as the mixture was being stirred under nitrogen, lithium aluminium hydride (250 mg, 6.58 mmol) was added in small portions during 2—4 min to produce a rust-coloured mixture; within 45 min, the colour had become grey. After the mixture had been stirred for a total of 1 h, water (0.3 ml) was added carefully, followed by 20% aq. sodium hydroxide (0.45 ml) and water (0.9 ml). Ether (60 ml) and anhydrous potassium carbonate (2 g) were added and the mixture was filtered through Celite. Concentration under reduced pressure provided the diol (**34b**) (1.05 g, 99.2%) as a foam, which was used in subsequent reactions without purification. For analysis, a sample (212 mg) from a similar reaction was chromatographed on a column of silica gel (15 g, 30 × 1.8 cm; ether), providing pure *diol* (**34b**) (205 mg, 96.7%) as a foamy diastereoisomeric mixture, $\delta_H(CDCl_3)$ 6.76 (2 H, d, *J* 2.2 Hz, 2″- and 6″-H), 6.68 (1 H, t, *J* 2.2 Hz, 4″-H), 6.55 and 6.39 (1 H, 2 s, 4-H), 6.05—5.80 [1 H, br m, collapses to two singlets (5.99 and 5.90) upon addition of $D_2O$, ArC*H*(OH)Ar], 5.15 (2 H, s, $OCH_2O$), 5.13 (2 H, s, $OCH_2O$), 5.14 (1 H, obscured br, 1′-H), 5.07 (2 H, 3 lines, $OCH_2O$), 4.70—4.40 [2 H, br m, collapses to s (4.60) and apparent d (4.51, $J_{app}$ 2 Hz) upon addition of $D_2O$, Ar$CH_2$OH], 4.06 (1 H, br, exchangeable with $D_2O$, OH), 3.61 and 3.60 (3 H, 2 s, OMe), 3.47 (3 H, s, OMe), 3.46 (3 H, s, OMe), 3.35 (1 H, br, exchangeable with $D_2O$, OH), 2.25—1.25 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.49 and 1.48 (3 H, 2 s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 400br (OH), 3 000m, 2 950m, 2 910m, 2 840w, 1 600m, 1 580m, 1 450m, 1 440m, 1 400m, 1 382m, 1 335m, 1 290m, 1 100s, 1 025s, 970m, 930m, 870m, and 845m $cm^{-1}$; $R_F$ (ethyl acetate–hexane, 5:4) 0.34 [$R_F$ of phthalide (**31b**) 0.75] (Found: C, 61.9; H, 6.9. $C_{26}H_{34}O_{10}$ requires C, 61.65; H, 6.77%).

*The o-benzoylbenzaldehyde* (**37b**). A 10 ml round-bottomed flask containing a diol (**34b**) (50 mg, 0.099 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar, and was purged with nitrogen for 5 min. Dichloromethane (2 ml), PCC (43 mg, 0.198 mmol), and sodium acetate (5.4 mg, 0.66 mmol) were added. The mixture was stirred under nitrogen for 1 h, ether (8 ml) was added, the brown mixture was filtered through a pad of Florisil, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of silica gel (2 g, 10 × 1.0 cm; pentane–ether, 2:1) to give the phthalide (**31b**) (5.1 mg, 10.3%) and the *o*-benzoylbenzaldehyde (**37b**) (40 mg, 80.6%) as an oil, $\delta_H(CDCl_3)$ 9.96 (1 H, s, CHO), 7.08 (2 H, d, *J* 2.3 Hz, 2″- and 6″-H), 6.93 (1 H, t, *J* 2.3 Hz, 4″-H), 6.62 (1 H, s, 3-H), 5.40 (1 H, br m, 1′-H), 5.15 (6 H, s, 3 $OCH_2O$), 3.58 (3 H, s, OMe), 3.46 (6 H, s, 2 OMe), 2.25—1.20 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.56 (3 H, s, 6′-$H_3$); $R_F$ (ethyl acetate–hexane, 5:4) 0.64.

*Pseudoacid* (**35b**). A 250 ml round-bottomed flask containing the diol (**34b**) (1.05 g, 2.07 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar. Acetone (100 ml) and potassium permanganate (2.30 g, 14.5 mmol) in 1% aq. sodium hydrogen carbonate (50 ml; pH *ca.* 8.6) were added and the mixture was purged with nitrogen for 30—45 min. The mixture was stirred vigorously under nitrogen in the dark (foil wrap); after 8—10 h, solid potassium permanganate (475 mg, 3.0 mmol) was added. After a total of 24 h, most of the acetone was removed under reduced pressure and water (100 ml) was added. The mixture was stirred vigorously as a *ca.* 1.3M solution of hydrazine monohydrochloride (4.80 g, 70 mmol) in water (55 ml) was added slowly until all of the brown solid (manganese dioxide) had been reduced to a white solid [$Mn(OH)_2$] (40—50 ml required). Hydrochloric acid (1M) then was added dropwise just until all of the white solid had dissolved (pH *ca.* 6) and the mixture was extracted three times with ether. The extracts were pooled, washed three times with brine, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure provided pseudoacid (**35b**) (1.15 g, 107.5%) as a light yellow foam, which was found to be highly unstable unless stored in a freezer under argon and was used in subsequent reactions without purification. In order to obtain this compound for analysis, a portion (60 mg) of a similar reaction product was chromatographed on a column of acid-washed silica gel (20 g, 30 × 1.4 cm; ether–$CH_2Cl_2$, 95:5) to afford pure *pseudoacid* (**35b**) (48 mg, 86.0%) as a foamy, diastereoisomeric mixture, $\delta_H(CDCl_3)$ 6.90 (2 H, overlapping d, *J* 2.1 Hz, 2″- and 6″-H), 6.72 (1 H, overlapping t, *J* 2.1 Hz, 4″-H), 6.64 and 6.61 (1 H, 2 s, 4-H), 5.48 (2 H, br s, $OCH_2O$), 5.35 (1 H, br m, 1′-H), 5.14 (4 H, s, 2 $OCH_2O$), 4.20 (1 H, br, exchangeable with $D_2O$, OH), 3.53 (3 H, s, OMe), 3.46 (6 H, s, 2 OMe), 2.25—1.20 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.51 (3 H, s, 6′-$H_3$); $\nu_{max.}(CHCl_3)$ 3 260br (OH), 3 000w, 2 950m, 2 830w, 1 755s, 1 605s, 1 455m, 1 435m, 1 385m, 1 230m, 1 150s, 1 030s, 940s, and 840m $cm^{-1}$;

$R_F$ (ethyl acetate–hexane, 5:4) 0.08 (Found: C, 60.3; H, 6.0. $C_{26}H_{30}O_{11}$ requires C, 60.23; H, 5.83%).

(±)-*Averufin* (**4**). A 100 ml round-bottomed flask containing crude pseudoacid (**35b**) (820 mg of *ca.* 80% pure material; 656 mg, 1.26 mmol) was equipped with a nitrogen inlet and a magnetic stirring bar, and was purged with nitrogen for 5 min. TFA (35 ml) and TFAA (7.44 g, 35.4 mmol) were added, and within 15 min an orange precipitate had formed. The mixture was stirred under nitrogen for 2 h, the liquid was removed under reduced pressure, and the residue was chromatographed on a column of silica gel (30 g, 30 × 1.8 cm; chloroform–methanol, 98:2) to afford impure averufin (256 mg). The column was then stripped (chloroform–methanol, 9:1) and the residue (426 mg) was treated as above with TFA (15 ml) and TFAA (5.95 g, 28 mmol). Chromatography of this residue on a column of silica gel (15 g, 25 × 1.4; chloroform–methanol, 98:2) afforded additional material (72.4 mg). The two column fractions were combined, and triturated with chloroform, the mixture was filtered, and the residue was washed well with chloroform, to provide (±)-averufin (**4**) [293 mg, 63.1%; 53.4% from phthalide (**31b**)] as a bright orange solid, m.p. 282—288 °C (decomp.) [(synthetic) lit.,[19] >280 °C (decomp.); (natural) lit.,[32] 280—282 °C (decomp.) lit.,[81] 280 °C (decomp.); lit.,[10] 282—289 °C (decomp.)]. The physical and spectral characteristics of this material were identical with those of authentic material [kindly provided by Professor J. C. Vederas (University of Alberta), Professor D. P. H. Hsieh (University of California, Davis), Professor P. Brassard (Université Laval), and Dr P. S. Steyn (CSIR, Pretoria)]; $\delta_H$ ([$^2H_6$]DMSO) 12.40 (1 H, br s, exchangeable with $D_2O$, 1- or 8-OH), 12.01 (1 H, br s, exchangeable with $D_2O$, 8- or 1-OH), 8.3 (1 H, s, exchangeable with $D_2O$, 6-OH), 7.05 (1 H, d, *J* 2.3 Hz, 5-H), 6.94 (1 H, s, 4-H), 6.53 (1 H, d, *J* 2.3 Hz, 7-H), 5.24 (1 H, br s, 1′-H), 2.2—1.2 (6 H, cm, 2′-, 3′-, and 4′-$H_2$), and 1.53 (3 H, s, 6′-H); $\nu_{max.}$(KBr) 3 400br (OH), 2 975w, 1 670w, 1 620s, 1 570m, 1 400s, 1 315s, 1 270s, 1 255s, 1 160s, 1 025w, and 835w $cm^{-1}$; $\lambda_{max.}$(EtOH) (log ε) 224 (4.48), 254sh (4.17), 264 (4.23), 294 (4.39), 316 (4.36), 450sh (3.92), and 467 nm (3.94) [lit.,[32] $\lambda_{max.}$(EtOH) (log ε) 223 (4.54), 256sh (4.21), 266 (4.26), 286sh (4.43), 294 (4.51), 324 (3.98), and 453 nm (4.03); $R_F$ (hexane–acetone–ether, 7:3:2) 0.38; $R_F$ (chloroform–methanol, 98.2) 0.28.

*IV. Preparation of* (+)-[4′-$^{13}$C]*Averufin* (**65**).—*Ethyl acetoacetate* (**41**; unlabelled). A three-necked, 250 ml round-bottomed flask equipped with an addition funnel with rubber septum, a condenser with nitrogen inlet, a heating mantle, and a magnetic stirring bar was flame-dried under argon and was charged with HMDS (26.78 g, 166 mmol) and dry ether (50 ml). BuLi in hexane (105 ml, 168 mmol) was added dropwise *via* the addition funnel at a rate to maintain gentle reflux; a white precipitate formed during the course of this addition. The mixture was heated at gentle reflux for 30 min, the condenser was replaced with a short-path distillation apparatus, and all but *ca.* 10 ml of the solvents were removed by distillation under argon during the course of 2 h. The remaining light yellow mixture was cooled to room temperature, pentane (*ca.* 10 ml; purged 15 min with argon) was added, and the liquid was removed by pipette and discarded. The solid was washed under argon with a second aliquot of pentane (*ca.* 15 ml) to produce a white solid, which was dissolved in dry THF (50 ml) to produce a homogeneous solution (determined by titration to be 1.3M).

A three-necked, 100 ml round-bottomed flask equipped with an argon inlet, a rubber septum, and a magnetic stirring bar was flame-dried under argon and as charged with the freshly prepared solution of LiHMDS in THF (19.8 ml; 25.74 mmol). Dry THF (5.2 ml) was added to produce a 1M solution and the mixture was cooled to −78 °C. Dry ethyl acetate (1.12 g, 12.8 mmol; distilled from $P_2O_5$ under argon) was added dropwise during 5 min and the mixture was stirred under argon at −78 °C for 15 min. Acetyl chloride [1.005 g, 12.8 mmol; distilled through a 15 cm Vigreaux column from *N,N*-diethylaniline (*ca.* 1 ml per 5 ml acetyl chloride) under argon immediately before use] was added dropwise during 5—8 min, and the mixture was stirred for 30 min and then allowed to come to room temperature. The flask was then equipped with a short-path distillation apparatus (receiving flask cooled to −78 °C) and the solvents were removed at room temperature under reduced pressure (*ca.* 160 Torr at the start, gradually reduced to *ca.* 15 Torr) to produce a dry, light yellow powder. This powder was subjected to high vacuum (*ca.* 0.5 Torr) for 10—15 min, and carefully acidified to pH 4—5 with 3M-hydrochloric acid (*ca.* 4.3 ml), and the mixture was poured into brine and extracted four times with ether. The extracts were pooled, washed once with brine, and dried over anhydrous magnesium sulphate. When all but *ca.* 20 ml of the ether had been removed by rotary evaporation (*ca.* 15 Torr) at room temperature, the vacuum was reduced (to *ca.* 160 Torr) and further solvent was evaporated off (15 min) to produce a yellow oil. Kugelrohr distillation [*ca.* 120 °C (120 Torr)] provided pure ethyl acetoacetate (**41**; no label) (1.416 g, 85.0%); $R_F$ (ether–hexane, 1:1) 0.50.

*5-Bromopentan-2-one ethylene ketal* (**11**; X = Br) (**40**; unlabelled). A 50 ml round-bottomed flask equipped with a condenser with nitrogen inlet and a magnetic stirring bar was charged with anhydrous potassium carbonate (5.34 g, 38.7 mmol), dry acetone (17.5 ml; distilled from anhydrous potassium carbonate under nitrogen shortly before use), ethyl acetoacetate (1.26 g, 9.65 mmol; distilled under reduced pressure and stored under nitrogen), and 1,2-dibromoethane (6.32 g, 33.65 mmol; distilled and stored under nitrogen). The mixture was heated at reflux for 24—26 h, allowed to cool to room temperature, and poured into ether (*ca.* 200 ml); the solution was dried over anhydrous potassium carbonate and filtered through Celite. When all but *ca.* 20 ml of the solvents had been removed by rotary evaporation (*ca.* 15 Torr) at room temperature, the vacuum was reduced (to *ca.* 160 Torr) and more solvent was evaporated off (20—25 min) to provide a crude product mixture (4.0 g) that was used directly in the subsequent reaction. A portion (6.30 mg) of the crude product mixture (4.255 g) from a similar reaction was purified by p.l.c. (20 × 20 cm × 2 mm; pentane–ether, 4:1; developed twice) to give the cyclopropane (**42**; unlabelled) (145 mg, 65.0%), $\delta_H$($CDCl_3$) 4.20 (2 H, q, *J* 7.1 Hz, $CO_2CH_2$), 2.46 (3 H, s, COMe), 1.46 (4 H, s, $CH_2CH_2$), and 1.28 (3 H, t, *J* 7.1 Hz, $CO_2CH_2Me$); $R_F$ (ether–hexane, 1:1) 0.71.

A 25 ml round-bottomed flask containing the crude alkylation-product mixture (4.0 g) was equipped with a nitrogen inlet and a magnetic stirring bar. Hydrobromic acid (48%; 11 ml) was added and the mixture was stirred under nitrogen in the dark (foil wrap) for 48 h. The mixture then was poured into brine and extracted three times with ether. The extracts were pooled, washed once with brine, dried over anhydrous magnesium sulphate, and filtered. When all but *ca.* 10 ml of the ether had been removed by rotary evaporation (*ca.* 15 Torr) at room temperature, the vacuum was reduced (to *ca.* 160 Torr) and more solvent was evaporated off (20—25 min) to provide an orange mixture (2.5 g), found by $^1$H n.m.r. (80 MHz) spectroscopy to consist of 1,2-dibromomethane and the bromo ketone (**39**; unlabelled) [molar ratio 1:1, corresponding to 46% by weight of (**39**) (1.17 g, 7.08 mmol)],* $\delta_H$($CDCl_3$) 3.45 (2 H, t, *J* 6.3 Hz, 5-$H_2$), 2.64 (2 H, unsymmetrical t, $J_{app}$ 7.0 and 6.5 Hz, 3-$H_2$), 2.17 (3 H, s, 1-$H_3$), and 2.16 (2 H, split m, $J_{app}$ 6.5 and 1.2 Hz, 4-$H_2$); $R_F$ (ether–hexane, 1:1) 0.58 (iodine stain).

* In two other reactions of this type, ethanol (*ca.* 15% by weight) also was present in the crude product mixture.

A 25 ml round-bottomed flask containing this mixture was equipped with a Dean–Stark distillation assembly (2 ml capacity). Dry benzene [15 ml; distilled and stored under nitrogen over molecular sieves (4 Å)], toluene-*p*-sulphonic acid hydrate (PTSA) (one tiny crystal), and ethylene glycol (445 mg, 7.17 mmol; distilled under reduced pressure and stored under nitrogen over 4 Å molecular sieves) were added and the mixture was heated at reflux under nitrogen with azeotropic removal of water for 2 h. The mixture then was allowed to cool to room temperature, poured into saturated aq. sodium hydrogen carbonate, and extracted three times with ether. The extracts were pooled, washed once with brine, dried over anhydrous potassium carbonate and filtered. When all but *ca.* 10 ml of the solvents had been removed by rotary evaporation (*ca.* 15 Torr) at room temperature, the vacuum was decreased (to *ca.* 160 Torr) and more solvent was evaporated off (20—25 min). A 15 ml round-bottomed flask containing this mixture was equipped with a Claisen-type distillation apparatus containing a Vigreaux column (37 × 8 mm), a vacuum distillation adapter wrapped with $CH_2Cl_2$-saturated cotton (no condenser), and a receiver with four 3.0 ml receptacles. After complete removal of the 1,2-dibromoethane by room-temperature distillation at reduced pressure (*ca.* 15 Torr), the mixture was heated and product (**40**; unlabelled) (870 mg, 43.2% from ethyl acetoacetate) was collected [78—80 °C (*ca.* 15 Torr)] in pure form. Once distillation had ceased, the heating was stopped and the assembly was brought to atmospheric pressure by introduction of argon. The contents of the Claisen-head were rinsed with ether into a separate flask and the ether was removed under reduced pressure to afford additional (unlabelled) product (**40**) (120 mg, 49.1% total yield from ethyl acetoacetate) as an oil, $\delta_H(CDCl_3)$ 3.94 (4 H, s, $OCH_2CH_2O$), 3.43 (2 H, t, *J* 6.4 Hz, 5-$H_2$), 2.15—1.65 (4 H, cm, 3- and 4-$H_2$), and 1.32 (3 H, s, 1-$H_3$); $R_F$ (ether–hexane, 1:1) 0.60 (iodine stain).

(±)-[4′-$^{13}$C]*Averufin* (**65**). The procedure reported for the preparation of ethyl acetoacetate was followed using ethyl [2-$^{13}$C]acetate (1.45 g, 16.4 mmol) as supplied (Prochem), LiHMDS in THF (33.7 ml; 33.7 mmol) and acetyl chloride (1.36 g, 1.73 mmol).

The procedure reported for the preparation of the cyclopropane (**42**) was followed using the impure labelled ester (**41**) (2.062 g, *ca.* 78% pure), anhydrous potassium carbonate (9.8 g, 71.0 mmol), dry acetone (30 ml), and 1,2-dibromoethane (10.55 g, 56.2 mmol) to provide the crude alkylation-product mixture (9.91 g) containing labelled cyclopropane (**42**), $\delta_H(CDCl_3)$ 4.20 (2 H, q, *J* 7.1 Hz, $CO_2CH_2$), 2.46 (3 H, d, $^3J_{CH}$ 1.2 Hz, COMe), 1.46 (4 H, d, $^3J_{CH}$ 2.4 Hz, $CH_2CH_2$), and 1.29 (3 H, t, *J* 7.1 Hz $CO_2CH_2$*Me*).

The procedure recorded for the preparation of bromo ketone (**39**) was followed using hydrobromic acid (48%; 25 ml) and the crude alkylation-product mixture (9.91 g) containing labelled compound (**42**). The crude product mixture (6.98 g) was found by $^1$H n.m.r. spectroscopy to consist of 1,2-dibromoethane (56.9% by weight), ethanol (26.3% by weight), and labelled product (**39**) (16.8% by weight; 1.17 g, 7.07 mmol).

The procedure described for the preparation of bromo ketal (**40**) was followed using the crude product mixture (6.98 g) containing labelled bromo ketone (**39**) (1.17 g, 7.07 mmol), dry benzene (25 ml), PTSA (one small crystal), and dry ethylene glycol (479 mg, 7.71 mmol). Fractional distillation of the crude product mixture (4.23 g) provided pure bromo ketal (**40**) (751 mg, 3.58 mmol) and, from rinsing of the distillation column, a crop of slightly impure compound (**40**) (578 mg, 2.75 mmol), $\delta_H(CDCl_3)$ 3.94 (4 H, s, $OCH_2CH_2O$), 3.43 (2 H, d t, *J* 6.8, $^3J_{CH}$ 4.1 Hz, 5-$H_2$), 2.2—1.78 (2 H, cm, 4-$H_2$), 1.99 [0.2 H, obscured, 3-H; and 1.8 H, cm, $^1J_{CH}$ ~128 Hz, $^{13}$C(3)-H], and 1.32 (3 H, d, $^3J_{CH}$ 2.7 Hz, 1-$H_3$).

The procedure reported for the preparation of the alcohol (**25**; R′ = MOM) was followed using lithium (2% sodium) dispersion (25% in mineral oil; 449 mg, 16.2 mmol) in dry ether (4.2 ml), pure bromo ketal (**40**) (751 mg, 3.58 mmol), and aldehyde (**24**; R′ = MOM) (810 mg, 3.58 mmol) in dry THF (4 ml) to provide the alcohol [4′-$^{13}$C](**25**; R′ = MOM) (1.17 g, 91.5%).

Likewise, this procedure was followed using lithium (2% sodium) (25% dispersion in mineral oil; 340 mg, 12.25 mmol) in dry ether (4 ml), slightly impure compound (**40**) (578 mg, 2.75 mmol) in dry THF (3 ml); chromatography of the product residue on a column of silica gel (25 g, 30 × 1.5 cm; ether–hexane, 4:1) provided the alcohol [4′-$^{13}$C](**25**) (131 mg, 13.3%), $\delta_H(CDCl_3)$ 7.13 (1 H, split t, $J_{app}$ 8.1 Hz, A of $AB_2$, 4-H), 6.79 (2 H, s and d, $J_{app}$ 8.1 Hz, $B_2$ of $AB_2$, 3- and 5-H), 5.20 (4 H, s, 2 $OCH_2O$), 5.16 (1 H, obscured m, 1′-H), 3.91 (4 H, s, $OCH_2CH_2O$), 3.60 (1 H, d, *J* 11.8 Hz, exchangeable with $D_2O$, OH), 3.48 (6 H, s, 2 OMe), 2.0—1.3 (4 H, cm, 2′- and 3′-$H_2$), 1.66 [0.2 H, obscured, 4′-H; and 1.8 H, broadened t, *J* ~8, $^1J_{CH}$ 126.2 Hz, $^{13}$C(4′)-H], and 1.30 (3 H, d, $^3J_{CH}$ 2.3 Hz, 6′-$H_3$).

The procedure reported for the preparation of ketal (**26**) was followed using [4′-$^{13}$C](**25**) (1.30 g, 3.64 mmol) and 50% acetic acid (73 ml, containing 27 drops conc. sulphuric acid per 90 ml). The crude product (926 mg) was chromatographed on a column of silica gel (50 g, 35 × 2.1 cm; pentane–ether, 4:1) to give pure ketal (**43**) (481 mg, 52.6%; 11.7% from ethyl [2-$^{13}$C]acetate), $\delta_H(CDCl_3)$ 7.08 (1 H, t, *J* 8.2 Hz, 5-H), 6.57 (1 H, dd, *J* 8.2 and 0.8 Hz, 6-H), 6.47 (1 H, dd, *J* 8.2 and 0.8 Hz, 4-H), 5.26 (1 H, d, $J_{app}$ 3.1 Hz, 1′-H), 5.16 (2 H, s, $OCH_2O$), 3.45 (3 H, s, OMe), 2.20—1.20 (4 H, cm, 2′- and 3′-H), 1.92 [0.2 H, obscured, 3′-H; and 1.8 H, br m, $^1J_{CH}$ 128.4 Hz, 4′-$H_2$], and 1.52 (3 H, d, $^3J_{CH}$ 3.0 Hz, 6′-$H_3$); $\delta_C(CDCl_3)$ 36.07 ($^1J_{CH}$ 128.5 Hz).

[4′-$^{13}$C]Tricyclic ketal (**43**) was then elaborated to give (±)-[4′-$^{13}$C]averufin (**65**) in *ca.* 40% overall yield using the route described in Part III above.

*V. Preparation of* (±)-[1′-$^{13}$C,$^2$H]*Averufin* (**68**).—2,6-*Bis*-(*methoxymethoxy*)[carbonyl-$^{13}$C]*benzaldehyde* (**44**). Bis(*O*-methoxymethyl)resorcinol (**23**) was metallated as above for the preparation of the unlabelled aldehyde (**24**) and was treated with *N*,*N*-dimethyl[1-$^{13}$C]formamide (1.0 g, 13.5 mmol; Merck of Canada, 90% $^{13}$C enrichment). Aqueous work-up as before and chromatography on silica gel (ether–hexane, 1:1) gave compound (**44**) (2.17 g, 69.8%), $\delta_H(CDCl_3)$ 10.54 (1 H, d, $^1J_{CH}$ 181 Hz), 7.40 (1 H, split t, $J_{app}$ 8 Hz, $AB_2$), 6.81 (2 H, d, $J_{app}$ 8 Hz, $AB_2$), 5.26 (4 H, s), and 3.50 (6 H, s).

*The* [1′-$^{13}$C,$^2$H]*tricyclic ketal* (**46**). A 100 ml three-necked flask fitted with an addition funnel and a reflux condenser was flame-dried under nitrogen. The reaction vessel was then charged with THF (23 ml), potassium (1.88 g, 48.1 mmol), and anhydrous magnesium chloride (2.38 g, 25.0 mmol) which had been rigorously dried as described previously.[82] The reaction mixture was then heated to reflux for 2 h, then cooled to room temperature, and treated with 5-bromopentan-2-one ethylene ketal (**11**; X = Br) (2.56 g, 12.24 mmol)[30] added by syringe to give a highly exothermic reaction.[33] This mixture was stirred for 1.25 h, after which time a solution of 2,6-bis(methoxymethoxy)-[*carbonyl*-$^{13}$C]benzaldehyde (**44**) (2.17 g, 9.56 mmol) in THF (8 ml) was added dropwise. This mixture was stirred for an additional 20 min. The reaction was quenched by the addition of water until a white solid had precipitated and the reaction mixture was otherwise clear. The solution was filtered through Celite and the residue was washed with ether (*ca.* 75 ml). Water (35 ml) was added to the filtrate and the organic layer was separated. The aqueous layer was then extracted with ether (2 × 35 ml). The combined organic layers were washed with brine (2 × 35 ml), dried over anhydrous potassium carbonate, and concentrated under reduced pressure. The crude alcohol (**45**; R′ = H) was used without further purification.

A 250 ml three-necked flask was fitted with an addition funnel and was charged with dichloromethane (125 ml) (which had been passed through a short column of neutral alumina), pyridine (9.37 g, 113.40 mmol), and chromium(VI) trioxide (5.66 g, 56.6 mmol). The [1′-$^{13}$C]alcohol (**45**) was transferred to the addition funnel in dichloromethane (17 ml). After 20 min, ether (300 ml) was added and the resulting solution was washed successively with 5% aq. sodium hydroxide until nearly colourless (6 × 190 ml), saturated aq. ammonium chloride (2 × 150 ml), saturated aq. sodium hydrogen carbonate (2 × 150 ml), and brine (2 × 150 ml). The solution was dried over anhydrous potassium carbonate and the solvent was removed under reduced pressure. Benzene (20 ml) was added to the round-bottomed flask, and was then promptly removed under reduced pressure. The process was repeated five times to afford the [1′-$^{13}$C]ketone free from pyridine contamination. This product was used without further purification.

A 100 ml three-necked flask was fitted with an addition funnel and was charged with THF (35 ml) and lithium aluminium deuteride (860 mg, 22.64 mmol; Fluka, 99 atom % deuterium). The ketone was transferred to the addition funnel in THF (12 ml) and this solution was then added dropwise to the reaction mixture. After being stirred for 15 min the reaction mixture was quenched by the dropwise addition of water (9 ml) and 15% aqueous sodium hydroxide (9 ml). This was followed by filtration through Celite and washed with ether (*ca.* 100 ml). Water (75 ml) was added and the organic layer was separated. The aqueous layer was extracted with ether (2 × 45 ml) and the combined extracts were washed with brine (2 × 50 ml), dried over potassium carbonate, and concentrated under reduced pressure. The crude [1′-$^{13}$C,$^{2}$H]alcohol (**45**; R′ = D) was cyclized as described previously[33] in water–acetic acid (1:1) (100 ml) containing conc. sulphuric acid (30 drops). The crude product was purified by column chromatography [70—230 mesh silica gel (120 g); 1:1 ether–hexane] to afford the [1′-$^{13}$C,$^{2}$H]bicyclic ketal (**46**) [1.23 g, 51.1% from aldehyde (**44**)], $\delta_H$($CDCl_3$) 7.07 (1 H, t, *J* 8 Hz), 6.57 (1 H, b d, *J* 8 Hz), 6.48 (1 H, br d, *J* 8 Hz), 5.16 (2 H, s), 3.44 (3 H, s), 1.52 (3 H, s), and 1.2—2.2 (6 H, cm); $\delta_C$ (100 MHz) 154.5, 152.5, 128.1, 111.8, 108.7, 104.3, 98.9, 94.1, 67.2 (enhanced s), 66.9 (enhanced t, $^{1}J_{CD}$ 23.5 Hz), 56.1, 36.8, 28.3, 28.2, and 16.1; *m/z* (rel. int.) 250 (1.3), 251 (21.6), 252 (100), 253 (14.3), and 254 (1.8). For unlabelled tricyclic ketal (**26**), *m/z* 250 (100), 251 (16.0), and 252 (2.0). From these data it can be shown that the contributions of unlabelled, monolabelled, and dilabelled materials are 1, 18, and 81%, respectively. These distributions are satisfied by average $^{2}$H and $^{13}$C contents of 90%.

(±)-[1′-$^{2}$H,$^{13}$C]*Averufin* (**68**). The [1′-$^{13}$C,$^{2}$H]Tricyclic ketal (**46**) was treated according to the procedures outlined in Part III above to provide (±)-[1′-$^{13}$C,$^{2}$H]averufin (**68**) in 36% overall yield from (**46**).

*VI. Preparation of* (±)-[1′-$^{13}$C,1′,4′,4′,6′,6′,6′-$^{2}H_6$]*Averufin* (**73**).—A 50 ml round-bottomed flask equipped with a nitrogen inlet and a magnetic stirring bar was flame-dried under nitrogen. The flask was charged with freshly sublimed (*ca.* 15 Torr) phosphorus pentachloride (2.0 g, 9.6 mmol) and the nitrogen inlet was replaced with a rubber septum using a needle as nitrogen inlet. The flask was cooled to 0 °C and deuterium oxide (8.0 ml; 99.8 atom % $^{2}$H) was added very slowly and carefully. The mixture was allowed to come to room temperature and stored under nitrogen. This standard acid solution was *ca.* 6M in deuterium chloride and *ca.* 1.2M in deuteriophosphoric acid.[20]

A 50 ml round-bottomed flask equipped with a condenser with nitrogen inlet and a magnetic stirring bar was flame-dried under nitrogen and charged with the labelled averufin (**68**) (150 mg, 0.405 mmol), dry THF (15 ml), deuterium oxide (12 ml; 99.8 atom % D) and the standard acid solution (3.0 ml). The mixture was heated at reflux under nitrogen for 60 h, when further dry THF (0.5 ml) and standard acid solution (1.0 ml) were added; after 120 h, more dry THF (3 ml) and standard acid solution (1.5 ml) were added. After a total of 17 days at reflux under nitrogen, the mixture was allowed to cool to room temperature, then was poured into water and extracted three times with ether. The extracts were pooled, washed successively with water (5 ×) and brine, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure provided an orange solid, which was triturated with deuteriochloroform to afford the title compound (**73**) (145 mg, 96.6%) as an orange solid, m.p. 284—290 °C (decomp.); $\delta_H$ ([$^{2}H_6$]DMSO) 7.08 (1 H, d, *J* 2.3 Hz, 5-H), 7.00 (1 H, s, 4-H), 6.56 (1 H, d, *J* 2.3 Hz, 7-H), and 2.15—1.30 (4 H, cm, 2′- and 3′-$H_2$); $\delta_D$(DMSO) 6.00—4.50 (0.9 $^{2}$H, br d, 1′-$^{2}$H), 2.45—1.00 (2 $^{2}$H, cm, 4′-$^{2}H_2$), and 1.43 (3 $^{2}$H, s, 6′-$^{2}H_3$); *m/z* (rel. int.) 373 (2.7), 374 (16.2), 375 (55.5), 376 (27.8), and 377 (7.4); for comparison, *m/z* (rel. int.) of (**4**): 366 (4.38), 367 (1.63), 368 (55.17), 369 (12.14) and 370 (2.42).

*VII. Preparation of* 3-*Phenylsulphonylbut-2-en-4-olide* (**56**).—β-*Phenylthio-γ-butyrolactone* (**63**).[83] A 10 ml three-necked flask was fitted with an addition funnel and charged with THF (3.5 ml), thiophenol (223 mg, 2.03 mmol), and a catalytic amount of sodium hydride. But-2-en-4-olide (**62**)[57] was then added dropwise as a solution in THF (3 ml). The reaction mixture was stirred for 1.5 h and then quenched with water (15 ml). This mixture was then extracted with ethyl acetate (3 × 10 ml). The extracts were combined, washed with brine (2 × 10 ml), dried over anhydrous magnesium sulphate, and concentrated under reduced pressure. The residue was purified by column chromatography [silica gel (8 g); 1:1 either–hexane] to give the sulphide (**63**) as a clear oil (315.2 mg, 80.2% isolated yield), $\delta_H$($CDCl_3$) 7.36 (5 H, m), 4.54 (1 H, dd, *J* 6.6, 9.6 Hz), 4.22 (2 H, cm), 2.91 (1 H, dd, $J_{gem}$ 17.8, *J* 7.5 Hz), and 2.48 (1 H $J_{gem}$ 17.8, *J* 6.1 Hz).

3-*Phenylthiobut-2-en-4-olide* (**64**).[84] A 250 ml round-bottomed flask was charged with dichloromethane (125 ml), δ-phenylthio-γ-butyrolactone (**63**) (4.21 g, 21.2 mmol), and NCS (2.90 g, 21.7 mmol). After 20 min the solvent was removed under reduced pressure, and $CCl_4$ (74 ml) was added. The insoluble succinimide was filtered off, and the solvent was removed under reduced pressure. Purification of the residue by column chromatography [silica gel (100 g); ether–hexanes, 1:1] gave the product (**64**)[84] as a clear oil (2.71 g, 70%), $\delta_H$($CDCl_3$) 7.52 (5 H, m), 5.51 (1 H, t, *J* 1.5 Hz), 4.76 (2 H, d, *J* 1.5 Hz); $\nu_{max.}$($CHCl_3$) 3 015w, 3 005w, 1 780s, 1 745s, 1 570, 1 475w, 1 440w, 1 345w, 1 260w, 1 155m, and 1 030m $cm^{-1}$.

3-*Phenylsulphonylbut-2-en-4-olide* (**56**). A 25 ml round-bottomed flask was charged with dichloromethane (11 ml) and 3-phenylthiobut-2-en-olide (**64**) (230 mg, 1.20 mmol). To this solution was added *m*-chloroperbenzoic acid (4.0 g, 2.50 mmol). The mixture was then stirred for 15 h, after which time it was filtered, washed successively with 5% aq. potassium carbonate (3 × 5 ml) and brine (2 × 5 ml), and dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to give a white, crystalline solid (191 mg, 71.2 %). This could be recrystallized from acetone–hexane to give *compound* (**56**) as bright, white crystals, m.p. 134.5—136 °C; $\delta_H$($CDCl_3$) 7.61—8.04 (5 H, m), 6.55 (1 H, t, *J* 2.1 Hz), and 4.97 (2 H, d, *J* 2.1 Hz); $\nu_{max.}$($CHCl_3$) 3 110w, 3 015w, 1 785s, 1 750m, 1 450m, 1 350m, 1 335m, 1 170s, 1 145m, 1 065m, 810s, and 770m $cm^{-1}$ (Found: C, 53.55; H, 3.75; S, 14.9. $C_{10}H_8O_4S$ requires C, 53.56; H, 3.59; S, 14.30%).

*VIII. Preparation of* [5,6-$^{13}C_2$]- *and* [8,11-$^{13}C_2$]*Averufin* (**71**).—*Ethyl* [3,4-$^{13}C_2$]*Acetoacetate*.[61] A 50 ml three-necked flask was charged with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (1.88 g, 13.04 mmol) and dichloromethane

(11 ml). The solution was cooled to 0 °C with an ice-bath, pyridine (2.06 g, 26.1 mmol) was added dropwise by syringe, and the mixture was stirred for 20 min. [1,2-$^{13}C_2$]Acetyl chloride (1.00 g, 12.4 mmol; Merck of Canada, 92% $^{13}C$ enrichment) was added to the mixture by syringe. The residue in the vial, which contained the labelled material, and syringe was transferred by washing both with dichloromethane (3 × 1.0 ml). The temperature of the mixture was kept at 0 °C for 1 h, after which time the ice-bath was removed and the mixture was stirred for a further 1 h at room temperature. Then, dichloromethane (85 ml) was added. This solution was washed successively with 0.4M-hydrochloric acid (4 × 35 ml) and brine (1 × 35 ml), and dried over anhydrous magnesium sulphate, and the solvent was removed under reduced pressure. The residue was transferred to a 100 ml round-bottom flask. Absolute ethanol (53 ml) was added to the round-bottom flask and the mixture was refluxed for 2 h. The solvent was then removed under reduced pressure and the residue was distilled by Kugelrohr (90 °C; 95—100 mmHg) to give ethyl [3,4-$^{13}C_2$]acetoacetate (1.0684 g, 65.1%).

*Ethyl 3-Trimethylsiloxy*[3,4-$^{13}C_2$]*but-2-enoate.*[54,85] A 25 ml three-necked flask fitted with an addition funnel was charged with triethylamine (1.89 g, 18.7 mmol) and a catalytic amount of anhydrous zinc chloride (dried in a vacuum Abderhalden desiccator with refluxing xylene over $P_2O_5$). The above labelled ethyl acetoacetate (10.69 g, 8.08 mmol) was transferred to the addition funnel as a solution in benzene (2.5 ml) which was added to the reaction mixture dropwise. To ensure complete transfer, the round-bottomed flask and addition funnel were washed with benzene (2 × 0.3 ml). After the mixture had been stirred for 30 min, chlorotrimethylsilane (2.03 mg, 18.8 mmol) was added dropwise by syringe to give a cloudy, viscous solution. The mixture was stirred for another 8 h. The reaction was quenched by the addition of pentane to precipitate all of the triethylammonium chloride. The reaction mixture was then passed through Celite into a round-bottomed flask, and the residue was marked with adequate pentane to ensure complete transfer of the product. The solvent was carefully removed on a rotary evaporator (and the process above was repeated if necessary to remove all of the precipitate) to give a light yellow liquid (1.35 g, ~82%). The product, containing traces of pentane, was used without further purification.

1-*Ethoxy*-1,3-*bis*(*trimethylsiloxy*)[3,4-$^{13}C_2$]*buta*-1,3-*diene* (**53**; R = Et).[52–54] A 50 ml three-necked flask was fitted with an addition funnel, charged with THF (9.6 ml) and di-isopropylamine (816 mg, 8.08 mmol), and then cooled to −78 °C. To this mixture was added butyl-lithium in hexanes (4.96 ml, 8.09 mmol) and the mixture was stirred for 10 min. The previously mentioned labelled butenoate (1.35 g, 6.62 mmol) was then transferred to the addition funnel with THF (5 ml) and was added dropwise to the reaction mixture. The mixture was stirred for 25 min after which time chlorotrimethylsilane (882 mg, 8.10 mmol) was added dropwise by syringe. The cold bath was removed and the mixture was stirred for another 40 min. Pentane (10 ml) was added to precipitate lithium chloride and the solution was filtered as above. After removal of the solvent on a rotary evaporator, further pentane (3 ml) was added and the solution was filtered through a disposable pipette with a cotton plug. The solvent was removed, but it was necessary to repeat the process once again to remove all of the lithium chloride. This procedure finally yielded a yellow liquid (1.68 g, ~92%) containing a small amount of pentane. The product was used without further purification.

*Dimethyl* 3,5-*Dihydroxy*[5,6-$^{13}C_2$]*phthalate* (**58**; R = H). A 25 ml round-bottomed flask was charged with the doubly labelled diene (**53**; R = Et) (1.68 g, 6.08 mmol) and toluene (7.1 ml). DMAD (953 mg, 6.68 mmol) was added by syringe with an ensuing exothermic reaction. The reaction mixture was then brought to reflux and maintained at this temperature for 7 h. After this time the oil-bath was removed and, after the mixture had cooled to ambient temperature, 6M-hydrochloric acid (2 drops) was added and the entire mixture was stirred for another 8 h. Water (25 ml) and then ethyl acetate were added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 10 ml). The combined organic extracts were washed with brine (2 × 10 ml), dried over anhydrous magnesium sulphate, and concentrated under reduced pressure. The resulting brown oil was purified by short-path column chromatography [silica gel (90 g); ether–hexanes, 1:1] to give dimethyl 3,5-dihydroxy[5,6-$^{13}C_2$]phthalate (**58**; R = H) (147.4 mg, ~10%) and dimethyl 3-ethoxy-5-hydroxy[5,6-$^{13}C_2$]-phthalate (**58**; R = Et) (516.7 mg, ~33% recovery).

Removal of the ethyl protecting group was accomplished as follows. Compound (**58**; R = Et) (516.7 mg, 2.02 mmol) was placed in a 50 ml three-necked flask and dichloromethane (17.3 ml) was added. The mixture was cooled to −78 °C and a 1.0M-solution of boron trichloride in dichloromethane (4.04 ml, 4.04 mmol) was added by syringe. Upon completion of the addition the cold-bath was removed and the reaction mixture was stirred for 1.5 h. Water (20 ml) was used to quench the reaction and the organic phase was separated while the aqueous phase was extracted with dichloromethane (2 × 15 ml). The combined phases were washed with brine (2 × 10 ml), dried over anhydrous magnesium sulphate, and concentrated under reduced pressure. The residue was combined with the previously isolated dimethyl 3,5-dihydroxy[5,6-$^{13}C_2$]phthalate and purified by short-path column chromatography [silica gel (28 g); ether–hexanes, 1:1] to give dimethyl 3,5-dihydroxy-[5,6-$^{13}C_2$] phthalate (**58**; R = H) (422 mg) as a white solid, m.p. 124—126 °C (lit.,[54] 124.5—126.5 °C); $\delta_H(CDCl_3)$ 11.02 (1 H, d, *J* 1.8 Hz, 3-OH), 6.52 (1 H, distorted dd, $^2J_{CH} \simeq 11$, $^3J_{CH} \simeq 1.0$ Hz, 5-OH), 6.51 (1 H, td, $^4J_{HH}$ 2.4, $^2J_{CH}$ 2.4, and $^3J_{CH}$ 0.9 Hz, 4-H), 6.46 (1 H, dt, $^1J_{CH}$ 164.4, $^2J_{CH}$ 2.4, and $^4J_{HH}$ 2.4 Hz, 6-H), 3.93 (3 H, s), and 3.92 (3 H, s); $\delta_C$ 161.6 (d, *J* 64.6 Hz, C-5) and 108.2 (d, *J* 64.6 Hz, C-6).

This entire procedure was repeated with a second ampule of [1,2-$^{13}C_2$]acetyl chloride (1.00 g, 12.4 mmol) to afford dimethyl 3,5-dihydroxy[5,6-$^{13}C_2$]phthalate (405.7 mg) which was combined with the previously synthesized batch to give doubly labelled material (824.2 mg, 3.61 mmol) for an overall yield of 23% from the starting [1,2-$^{13}C_2$]acetyl chloride.

3,5-*Dihydroxy*[2,3-$^{13}C_2$]*benzoic acid.* To a 100 ml round-bottomed flask containing dimethyl 3,5-dihydroxy[5,6-$^{13}C_2$]-phthalate (**58**; R = H), was added 15% aq. sodium hydroxide[60] (35 ml). The reaction mixture was brought to reflux for 3 h. After cooling to room temperature the solution was acidified to pH 1 with conc. hydrochloric acid and was then extracted with ethyl acetate (3 × 30 ml). The combined extracts were washed with brine (2 × 25 ml), and dried over anhydrous magnesium sulphate, and the solvent was evaporated under reduced pressure to give a brown solid (567 mg, 100%). This material was used without further purification.

*Methyl* 3,5-*Dihydroxy*[2,3-$^{13}C_2$]*benzoate.* To a 25 ml round-bottomed flask containing 3,5-dihydroxy[2,3-$^{13}C_2$]benzoic acid (567 mg, 3.61 mmol) were added anhydrous methanol (8.4 ml) and conc. sulphuric acid (0.343 ml). The reaction mixture was brought to reflux and stirred for 2 h, quenched by the addition of water (20 ml), and extracted with ethyl acetate (3 × 20 ml). The combined extracts were then dried over anhydrous magnesium sulphate and the solvent was removed under reduced pressure. Purification was achieved by short-path column chromatography [silica (14 g); hexanes–dichloromethane–ethyl acetate, 1:2:1] to give methyl 3,5-dihydroxy[2,3-$^{13}C_2$]benzoate (510 mg, 83%), m.p. 165 °C (lit.,[86] 165 °C).

*Methyl* 3,5-*Bis*(*methoxymethoxy*)[2,3-$^{13}C_2$]*benzoate.* Methyl

3,5-dihydroxy[2,3-$^{13}C_2$]benzoate (510 mg, 3.00 mmol) was converted into the title compound (560 mg, 72.4%) as described earlier.[33] The product had $\delta_H(CDCl_3)$ 7.41 (1 H, dm, $^3J_{CH}$ 5.0, $^4J_{HH}$ 2.3 Hz), 7.41 (1 H, dm, $^1J_{CH}$ 164.0, $^4J_{HH}$ 2.3 Hz), 6.96 (1 H, quin, $^4J_{HH}$ 2.3, $^2J_{CH}$ 2.3, and $^3J_{CH}$ 2.3 Hz), 5.24 (2 H, d, $^3J_{CH}$ 5.5 Hz), 5.24 (2 H, s), 3.94 (3 H, s), and 3.53 (6 H, s).

3,5-*Bis(methoxymethoxy)*[2,3-$^{13}C_2$]*benzaldehyde* (**61**; R′ = MOM). The labelled ester obtained directly above was reduced with lithium aluminium hydride[34] to give 3,5-bis(methoxymethoxy)[2,3-$^{13}C_2$]benzyl alcohol which was in turn oxidized with PCC[29] as in the preparation of compound (**17b**) above (section III) to afford the labelled aldehyde (**61**; R′ = MOM) in 79.7% yield for the two steps.

(±)-[5,6-$^{13}C_2$]- *and* (±)-[8,11-$^{13}C_2$]-*Averufin* (**71**). 3,5-Bis(methoxymethoxy)[2,3-$^{13}C_2$]benzaldehyde (**61**; R′ = MOM) was treated with the anilide (**30**) and the product was further elaborated to give a 1:1 mixture of [5,6-$^{13}C_2$]- and [8,11-$^{13}C_2$]-averufin (**71**) by the route described in Part III.

### *Microbiological Procedures*

The wild-type aflatoxigenic strain of *A. parasiticus* used was SU-1* obtained from Professor J. W. Bennett (Tulane University). Stock cultures were maintained on slants of potato-dextrose agar (Difco) containing 0.5% yeast extract (Difco). Plates subcultured from these slants were grown for 7 days in the dark before use.

The growth medium used for cultivation of the organisms in submerged culture in shaken flasks was the minimum mineral medium (MM) of Adye and Mateles.[87] The replacement medium (RM) was the nitrogen-free resting cell medium of Hsieh and Mateles[62] containing various amounts of glucose.

Shaken cultures were incubated in cotton-stoppered Erlenmeyer flasks in the dark at 28 °C and at the indicated revolutions $min^{-1}$ in a New Brunswick Scientific model G-25-K gyratory incubator shaker. For inoculation of liquid cultures, a 7-day-old plate culture was flooded with 0.85% saline solution (9 ml) containing 0.5% Tween 80 (1 ml), and a 5 mm inoculating loop was used to generate a suspension of spores; the suspension was transferred to a tube containing the saline solution (9 ml) and was mixed well to produce a suspension of *ca.* $10^6$ conidiospores per 1 ml.

All broths, slants, and equipment were sterilized at 125 °C/20 lb $in^{-2}$ for 20 min prior to inoculation.

*Isolation of Versiconal Acetate* (**5**) *from SU*-1.—Four portions (500 ml) of MM contained in 1 l Erlenmeyer flasks were inoculated with SU-1 and incubated at 175 r.p.m. for 48 h. The mycelial pellets were collected on cheesecloth and washed with RM (36 g $l^{-1}$ glucose[88]). The mycelial pellets (50 g wet weight from each flask) were resuspended in RM (500 ml; 36 g $l^{-1}$ glucose) contained in 4 $l^{-1}$ Erlenmeyer flasks, an acetone solution (3 ml) of Dichlorvos® (2 mg/ml of solution) was added to each, and incubation was continued for 48 h. The mycelial pellets were collected by filtration, washed with water, and exhaustively extracted with acetone. The extract was concentrated under reduced pressure to *ca.* 300 ml, poured into water, and the mixture was extracted with dichloromethane–ether (4:1) until the organic layer was colourless. These latter extracts were pooled, washed twice with water, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure provided an oily mixture, that was chromatographed on a column of silica gel (50 g, 35 × 2.1 cm; chloroform–methanol, 96:4) and the product was triturated with chloroform to afford versiconal acetate (**5**) (68 mg) as an orange solid, m.p. 222—226 °C [lit.,[89] 216—220 °C; lit.[88] 219—225 °C (decomp.)]. This compound existed in [$^2H_6$]DMSO solution as a mixture of the open aldehyde form (A, *ca.* 10%), the hemiacetal to the C-3 hydroxy (B, *ca.* 45%), and the hemiacetal to the C-1 hydroxy (C, *ca.* 45%); $\delta_H$ (400 MHz; [$^2H_6$]DMSO) 13.37, 12.43, 12.11, 11.33, 11.24, and 11.03 (0.45 H each, 6 s, ArO*H* of B and C), 13.38, 12.74, 12.07, and 11.20 (0.1 H each, 4 s, ArO*H* of A), 9.61 (0.1 H, s, CHO of A), 8.02, 7.86, and 7.78 (0.2 H, 0.36 H, and 0.36 H, 3 br s, 1′-OH of B and C), 7.26 and 7.11 (0.45 H each, 2 s, 4-H of B and C), 7.24 (0.1 H s, 4-H of A), 7.11 and 7.05 (0.5 H each, obscured and d, *J* 2.4 Hz, 5-H of A, B, and C), 6.58 and 6.55 (0.5 H each, 2 d, *J* 2.0 Hz, 7-H of A, B, and C), 6.21 (0.1 H, d, *J* 1.5 Hz, 2′-H of A), 6.00 (0.9 H, br s, 1′-H of B and C), 4.10 (2 H, AB q, *J* 6.8 Hz, 4′-$H_2$ of A, B, and C), 4.25—3.90 (0.9 H, obscured m, 2′-H of B and C), 2.25—1.87 (2 H, cm, 3′-$H_2$ of A, B, and C), and 1.99 (3 H, s, 6′-$H_3$ of A, B, and C); $R_F$ (chloroform–methanol, 96:4) 0.19.

*General Feeding Experiment.*—The indicated number of portions of MM (500 ml) contained in Erlenmeyer flasks was inoculated with *ca.* $10^6$ conidiospores. After incubation in the dark for 48 h, at 175 r.p.m. and 28 °C, the mycelial pellets were collected on cheesecloth and washed with the indicated RM (300 ml used for the contents of each flask). A measured wet weight of pellets was resuspended in the indicated RM (100 ml) contained in each of the indicated number of Erlenmeyer flasks. Labelled material was added and the incubation was continued at 175 r.p.m. and 28 °C for the indicated time. The mycelial pellets were collected on cheesecloth and were washed well with distilled, deionized water. Compounds were isolated as indicated.

*Incorporation of* [4′-$^{13}$C]*averufin* (**65**) *into* [16-$^{13}$C]*AFB*$_1$ (**66**) *by SU*-1. The general feeding experiment was followed, inoculating 6 portions of MM contained in 1 l flasks with SU-1. [4′-$^{13}$C]Averufin (**65**) (40 mg) in acetone (20 ml) was distributed equally to 10 replacement cultures (10 g each) in RM (1.62 g $l^{-1}$ glucose[63]) contained in 250 ml flasks and incubation was continued for 24 h. The mycelial pellets were steeped in acetone (300 ml) for 20 min, filtered off, and washed with acetone until the washings were colourless. The filtrate was concentrated under reduced pressure to *ca.* 100 ml, poured into water, and extracted with chloroform until the chloroform and water layers were colourless. The extracts were pooled, washed successively with water (3 ×) and brine, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure afforded the mycelial extract, which consisted mostly of labelled averufin but contained a small amount of labelled aflatoxin (< 10% of the total amount of aflatoxin produced). Meanwhile, the medium was extracted four times with chloroform (until the chloroform layer contained no aflaxtoxin by t.l.c.). The extracts were pooled, washed successively with water (2 ×) and brine, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure afforded the mycelial extract, which consisted almost entirely of labelled aflatoxin (> 90% of the total aflatoxin produced) but contained a trace of labelled averufin. The medial and mycelial extracts were combined (80 mg) and subjected to p.l.c. (20 × 20 cm × 2 mm; hexane–acetone–ether 7:6:4; 3 plates) to afford [4′-$^{13}$C]-averufin (**65**) (36 mg) and [16-$^{13}$C]aflatoxin (**66**) (30 mg, *ca.* 3:1 $AFB_1$:$AFG_1$, trace of $AFB_2$ and $AFG_2$). The aflatoxin mixture was chromatographed on a column of silica gel (15 g, 20 × 1.4 cm; chloroform–methanol, 97:3) to afford [16-$^{13}$C]-$AFB_1$ (**66**) (12 mg), containing a trace of $AFB_2$, as a light yellow solid, $\delta_C(CDCl_3)$ 145.1 ($^1J_{CH}$ 197, $^2J_{CH}$ 11.6, and $^3J_{CH}$ 4.5 Hz, C-16) [lit.,[66] $\delta_C$ (25.1 Hz; $CDCl_3$) 145.4 ($^1J_{CH}$ 196, $^2J_{CH}$ 12, and $^3J_{CH}$ 4 Hz, C-16)].

*Incorporation of* [1′-$^{13}$C,$^2$H]*averufin* (**68**) *into* [13-$^{13}$C, $^2$H]*AFB*$_1$ (**69**) *by SU*-1. The procedure reported for administr-

* See footnote † on page 8.

ation of labelled averufin (**65**) to SU-1 was followed exactly except that [1′-$^{13}$C, $^{2}$H]averufin (**68**) (40 mg) in acetone (20 ml) was adminstered to the replacement cultures and incubation was continued for 40 h. The combined medial and mycelial extracts (129 mg) were subjected to p.l.c. (20 × 20 cm × 2 mm; hexane–acetone–ether, 7:6:4; 4 plates) to afford labelled averufin (**68**) (35 mg) and the aflatoxin mixture (60 mg). The aflatoxin mixture was chromatographed on a column of silica gel (25 g, 30 × 1.5 cm; chloroform–acetone, 9:1) to afford labelled $AFB_1$ (**69**) (16 mg, free from $AFB_2$) as a faint yellow solid, $\delta_C(CDCl_3)$ 113.3 [s, C-13(H)] and 113.0 [t, $^1J_{CD}$ 28.5 Hz, C-13(D)] {lit.,[66] $\delta_C$ (25.1 MHz; $CDCl_3$) 113.6 [C-13(H)]}.

Chloroform–acetone (9:1) provided much cleaner separation of $AFB_1$ from the other aflatoxins: $R_F$ (hexane–acetone–ether, 7:3:1) averufin, 0.40; all aflatoxins, 0.18; $R_F$ (chloroform–methanol, 97:3) averufin, 0.55; $AFB_1$ and $AFB_2$, 0.52; $AFG_1$ and $AFG_2$, 0.44; $R_F$ (chloroform–acetone, 9:1) $AFB_1$, 0.54; $AFB_2$ and averufin, 0.50; $AFG_1$, 0.40; $AFG_2$, 0.35.

*Incorporation of labelled averufin* (**71**) *into labelled AFB* (**72**) *by SU*-1. This experiment was carried out like those above except that labelled averufin (20 mg) was used and the incubation was carried out for 48 h. See text for discussion of $^{13}$C n.m.r. results.

*Incorporation of* [1′-$^{13}$C,1′,4′,4′,6′,6′,6′-$^{2}H_6$]*averufin* (**73**) *into* [1′-$^{13}$C,1′,4′,4′,6′,6′,6′-$^{2}H_6$]*versiconal acetate* (**74**) *by SU*-1. The general feeding experiment was followed, inoculating 8 portions of MM contained in 1 l flasks with SU-1. An acetone solution (1 ml) of Dichlorvos® (1 mg/ml of solution) was added to each of 21 replacement cultures (10 g, each) in RM (5.4 g $l^{-1}$ glucose[88]) contained in 250 ml flasks before labelled averufin (**73**) (63 mg) in acetone (21 ml) was distributed equally to the cultures; incubation was continued for 40 h. The mycelial pellets were steeped in acetone (500 ml) for 20 min, filtered off, and washed with acetone until the acetone washings were colourless. The filtrate was concentrated under reduced pressure to *ca.* 200 ml, poured into water, and extracted with dichloromethane until the organic and water layers were colourless; the medium was extracted in the same way. All organic extracts were pooled, washed successively with water (3×) and brine, and dried over anhydrous sodium sulphate. Filtration and concentration under reduced pressure provided an oily mixture (800 mg), which was chromatographed on a column of silica gel (35 g, 30 × 1.8 cm; chloroform–acetone, 9:1) to afford labelled averufin (**73**) (56 mg) and impure labelled versiconal acetate (**74**). The product was rechromatographed on a column of silica gel (30 g, 30 × 1.8 cm; dichloromethane–acetone, 9:1) and the product was triturated with chloroform to afford labelled versiconal acetate (**74**) (19 mg) as an orange solid, $\delta_D$([$^2H_6$]DMSO) 7.0—5.5 (0.9 $^2$H, br, 1′-$^2$H), 4.09 (2 $^2$H, br s, 4′-$^2H_2$), and 1.90 (3 $^2$H, s, 6′-$^2H_3$); *m/z* (rel. int.) 391 (0.2), 390 (1.4), 389 (4.7), 388 (1.4), 384 (1.2), 383 (6.2), 382 (20.0), 346 (0.3), 345 (1.8), 344 (5.0), 343 (2.2), 342 (2.8), 341 (9.0), and 340 (23.1).

*Incorporation of* [4′-$^{13}$C]*averufin* (**65**) *and* [1′-$^{13}$C,$^{2}$H]-*averufin* (**68**) *into* [4′-$^{13}$C]- *and* [1′-$^{13}$C,$^{2}$H]-*versicolorin A* (**67**) *and* (**70**). These experiments have been described previously.[67,74]

## Acknowledgements

We are deeply grateful to Professor J. W. Bennett for providing laboratory space and experienced help with the microbiological aspects of this work. The National Institutes of Health (ES 01670), the Alfred P. Sloan Foundation, and the Camille and Henry Dreyfus Foundation are thanked for financial support. The high-field n.m.r. spectrometers were acquired with the assistance of major instrumentation grants (Bruker WM-300; NIH, GM 27512), (Varian XL-400; NIH, RR 01934, NSF, PCM 82-03176). The Middle Atlantic Mass Spectrometry Laboratory (Johns Hopkins) provided key mass spectral analyses, and the 500 MHz $^1$H n.m.r. assignments of the averufin side-chain were carried out at the Northeast Regional NMR Facility (Yale).

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*Received* 23*rd March* 1987; *Paper* 7/514