Biosynthesis of Averufin in *Aspergillus parasiticus* from [^{13}C]-Acetate

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Summary The ^{13}C n.m.r. spectrum of averufin has been completely assigned, providing unambiguous proof of its structure; the ^{13}C n.m.r. spectra of averufin enriched with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate by *Aspergillus parasiticus* established its formation from ten intact acetate units and the mode of folding of the precursor decaketide chain.

AVERUFIN (**1**), an orange pigment, is elaborated by *Aspergillus versicolor*,[1] *Aspergillus ustus*,[2] and *Aspergillus parasiticus*.[3] It is regarded as a pivotal step in the biosynthesis of aflatoxin B_1 owing to its efficient conversion into the aflatoxins by *A. parasiticus* ATCC 15517.[4] Mutations of this parent strain of *A. parasiticus* were induced by treatment of the conidia with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to give *A. parasiticus* ATCC 24551 in which aflatoxin production was impaired.[3] This mutant accumulated large quantities of averufin in the mycelium and was employed in this study.

The directional mode of polyketide folding (a) to generate the fused carbocyclic structure of (**1**) was deduced by us[5] from the assembly pattern of acetate units in aflatoxin B_1. Seto *et al.*[6] predicted the alternative arrangement (b) based on a ^{13}C n.m.r. study of the related sterigmatocystin. Some crucial carbon signals were, however, interchanged in their ^{13}C n.m.r. assignments.[5] Turner[7] favoured arrangement (b) for averufin and other anthraquinones. The use of [1,2-$^{13}C_2$]-acetate was mandatory to clarify this biosynthetic ambiguity.

Conidia of *A. parasiticus* were inoculated into the low salts medium[8] and incubated without shaking as surface cultures at 27 °C. In the enrichment experiments, the growing organism was pulsed every 24 h from day three to day six with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate and harvested after ten days. The solvent of choice for the ^{13}C n.m.r. study of averufin was $CDCl_3$–$(CD_3)_2SO$ (1 : 1).

The complete assignment of the natural-abundance ^{13}C n.m.r. spectrum of (**1**) derived from coupled, proton noise decoupled (p.n.d.), single frequency off-resonance proton decoupled, and selective proton decoupled spectra is given in the Table. Our findings verified the assignments of Fitzell *et al.*[9] for the six aliphatic and the two carbonyl carbon atoms. By correlating the residual splittings in off-resonance decoupled spectra with the known proton chemical shifts[10] the signals at δ 107·6, 109·1, and 108·0 p.p.m. could be assigned to C(4), C(5), and C(7), respectively. The assignment of the quaternary carbon atoms is based on selective proton decoupling and from the multiplicities arising from long-range carbon-proton couplings [^3J(CH) 4—8 Hz, ^2J(CH) 1—4 Hz, and ^4J(CH) *ca.* 1 Hz].[11] The four carbon atoms directly bound to oxygen resonated between δ 158·1 and 164·9 p.p.m. Selective decoupling of the H(1') changed the resonance at δ 158·1 p.p.m. to a singlet and that at 159·9 p.p.m. to a doublet. Irradiation of H(7) led to the collapse of the resonances at δ 164·1 and 164·9 p.p.m. to a singlet and doublet, respectively. These assigned the above four resonances as given in the Table. The signals of the three quaternary carbon atoms adjacent to oxygen

appeared at δ 108·2, 108·6, and 115·8 p.p.m. Upon irradiation of H(1′) the quartet at δ 115·8 p.p.m. collapsed to a triplet (J 5 Hz), whereas selective decoupling of H(7) led to the removal of a 6 Hz coupling from the resonance at δ 108·6 p.p.m. which then appeared as a doublet (J 6 Hz). These findings assign the above-mentioned three resonances to C(13), C(12), and C(2), respectively. The signals at δ 132·9 and 134·6 p.p.m. are characteristic for aromatic carbon atoms *meta* to two oxygen functions as found in (**1**). The peaks at δ 134·6 and 132·9 p.p.m. were assigned to C(11) and C(14), respectively from the carbon—carbon coupling constants observed in averufin enriched with [1,2-$^{13}C_2$]-acetate.

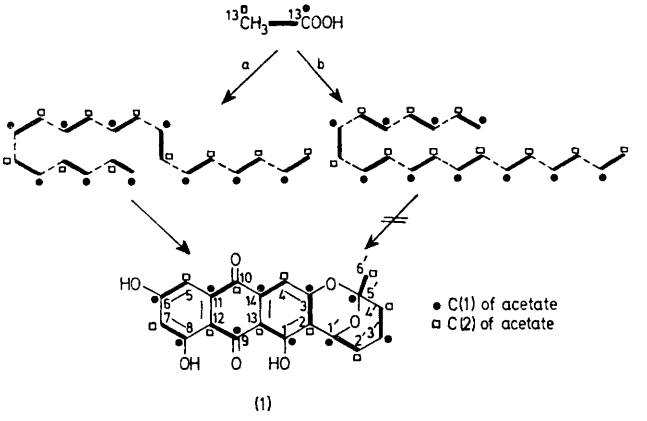
TABLE

^{13}C Chemical shifts, directly bonded [^1J(CH)] and long-range [$^{>1}J$(CH)] ^{13}C–^{1}H coupling constants of averufin (**1**), and ^{13}C–^{13}C coupling constants [^1J(CC)] of [1,2-$^{13}C_2$]-acetate enriched averufin

Carbon	δ/ppm[a]	^1J(CH)/Hz	$^{>1}J$(CH)/Hz	^1J(CC)/Hz
1	158·1 Sd	—	2	64
2	115·8 Sq	—	5	65
3	159·9 St	—	3	65
4	107·6 D	168	–	65
5	109·1 Dd	166	5	62
6	164·9 St	—	2	63
7	108·0 Dd	162	4	70
8	164·1 Sd	—	3	70
9	188·7 S	—	–	58
10	180·7 St	—	5	54
11	134·6 S	—	–	54
12	108·6 St	—	6	58
13	108·2 Sd	—	7	64
14	132·9 S	—	–	65
1′	66·3 Dm	156	–	35
2′	27·1 Tm	128	–	35
3′	15·6 Tm	130	–	32
4′	35·5 Tm	127	–	32
5′	100·9 Sm	—	–	49
6′	27·5 Q	128	–	49

[a] Relative to internal Me_4Si. S = singlet, D or d = doublet, T or t = triplet, Q or q = quartet, m = multiplet. (capital letters refer to the pattern resulting from directly bonded protons and small letters to long-range ^{13}C–H coupling).

The data obtained from the single labelled acetate precursors supported the head-to-tail assembly of the acetate units with the labels occupying alternating positions in the molecule as shown in the Figure. The p.n.d. ^{13}C n.m.r. spectrum of the [1,2-$^{13}C_2$]-derived sample showed intense satellite resonances for all of the carbon signals. The observed spin–spin coupling data (Table) established that C(6′)–C(5′), C(4′)–C(3′), C(2′)–C(1′), C(2)–C(3), C(4)–C(14), C(10)–C(11), C(5)–C(6), C(7)–C(8), C(12)–C(9), and C(13)–C(1) originated from ten intact acetate units. This confirms folding pattern (a), substantiates the product–precursor relationship of averufin and aflatoxin B_1, and evidenced against the intermediacy of a C_{18} naphthacene precursor[12] or a formal C_4-unit' which is linked to a preformed C_{14} anthraquinone[13] in the biosynthesis of aflatoxin B_1. In the spectrum of the averufin derived from [1,2-$^{13}C_2$]-acetate all the carbon signals except C(6′) showed small symmetrically placed satellite pairs due to multiply labelled species.



(1)

FIGURE. Alternative arrangement of acetate units in the biosynthesis of averufin.

The study on the biosynthetic architecture of averufin provides pertinent information on the acetate–malonate biosynthesis of the related decaketides, *e.g.* averantin, averythrin, norsoloronic acid, and hydroxyaverufin. All these metabolites are produced by *A. versicolor*[7] and probably have mostly a common biosynthetic precursor.

Our chemical shift data together with the established arrangement of intact acetate units in (**1**) clearly differentiate between the two previously postulated structures for averufin.[1,14] These data support structure (**1**) as proposed by Holker *et al.*[14] and subsequently confirmed by Roffey *et al.*[15]

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