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Biosynthesis of Aurovertin B. The Rôle of Methionine in the Formation of the Ethyl Side-chain

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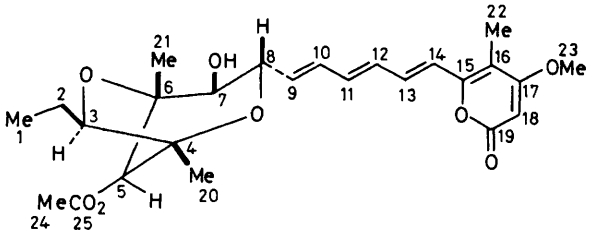
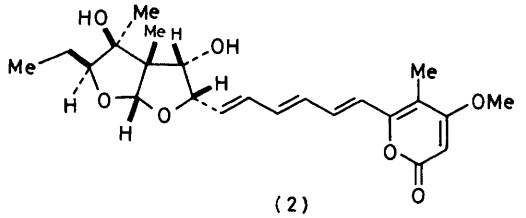
Summary Aurovertin B, a metabolite from *Calcarisporium arbuscula* is formed from a C_{18}-polyketide precursor; the C(1) carbon atom is derived by the introduction of a methyl group from the C_1-pool onto the methyl carbon atom of the chain-initiating acetate unit.

Aurovertin B (**1**), a metabolite from *Calcarisporium arbuscula*[1,2] is a member of a group of toxic substances



(1)

which act as inhibitors of ATP-synthesis and ATP-hydrolysis catalysed by mitochondrial enzyme systems.[3] Our isolation of asteltoxin (**2**), a related metabolite from



(2)

Aspergillus stellatus[4] as well as recent reports on the unusual incorporation of propionic acid into aurovertin[5] prompted us to investigate the biosynthesis of aurovertin B.

Plausible mechanisms, although without firm precedent in polyketide biosynthesis, can be formulated for the biosynthesis of aurovertin B. The involvement of a C_{18}-polyketide precursor requires the introduction of a methyl group from the C_1-pool onto the methyl carbon of the chain-initiating acetate unit. Alternatively, a C_{20}-polyketide origin necessitates the loss of the methyl carbon atom of the chain-initiating acetate unit. The reported incorporation of propionic acid[5] can be accommodated by a third biosynthetic postulate in which propionic acid is utilized as a chain-initiating unit, a process more common in bacteria *e.g. Streptomyces* species.

The assignment of the natural-abundance ^{13}C n.m.r. spectrum of aurovertin B (**1**) derived from coupled, proton noise decoupled (p.n.d.), single frequency off-resonance proton decoupled and selective proton decoupled spectra and selective population inversion experiments[6] is given in the Table and differs from that in the literature.[2] The signal at δ 137·0 p.p.m. was assigned to C(11) on the basis of results obtained with [1-^{13}C]acetate-derived aurovertin B.

Cultures of *C. arbuscula* NRRL A-12139 were grown in the dark at 23 °C in stationary culture on F14 medium at pH 6·2.[7] The optimum time and yields for incorporation studies with the fungus were determined. Preliminary experiments with [1-^{14}C]acetate and 2*S*-[methyl-^{14}C]-methionine indicated that both precursors were efficiently incorporated (0·3% and 4·9%, respectively) into aurovertin B and that dilution of the precursor was minimal

TABLE

^{13}C n.m.r. data for aurovertin B.

	δ_C[a]/p.p.m.			δ_C[a]/p.p.m.	
C(1)	11·7Q	Δ	C(14)	119·3D[b]	
C(2)	20·1T		C(15)	154·1S	●
C(3)	85·3D	●	C(16)	107·9S	
C(4)	82·6S		C(17)	170·4S	●
C(5)	80·6D	●	C(18)	88·6D	
C(6)	83·4S		C(19)	163·4S	●
C(7)	76·2D	●	C(20)	16·4Q	Δ
C(8)	77·6D		C(21)	15·0Q	Δ
C(9)	134·3D	●	C(22)	8·8Q	Δ
C(10)	131·7D[b]		C(23)	56·1Q	Δ
C(11)	137·0D	●	C(24)	20·7Q	
C(12)	131·2D[b]		C(25)	169·6S	●
C(13)	135·5D	●			

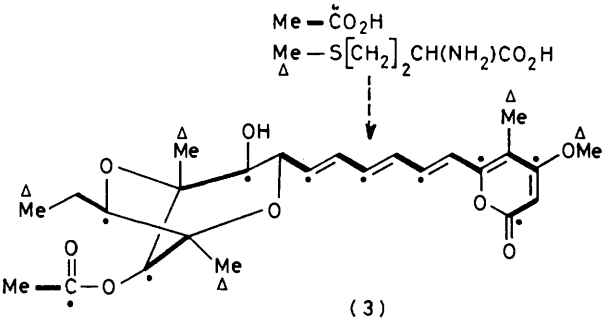
[a] Relative to internal Me_4Si. [b] May be interchanged. ● Derived from [1-^{13}C]acetate. Δ Derived from [methyl-^{13}C]methionine.

(3·2 and 2·3, respectively). No inhibitory effect was noticed for methionine.

The p.n.d. ^{13}C n.m.r. spectrum of [1-^{13}C]acetate-derived aurovertin B showed enhancement of the signals of ten carbon atoms *viz.* C(3), C(5), C(7), C(9), C(11), C(13), C(15), C(17), C(19), and C(25) thereby pointing to a C_{18}-polyketide origin for the skeleton of the metabolite. The p.n.d. ^{13}C n.m.r. spectrum of aurovertin B derived from 2*S*-[methyl-^{13}C]methionine showed a five-fold enhancement of the signals attributed to C(1), C(20), C(21), C(22), and C(23).

The yield of aurovertin B derived from [1,2-^{13}C]acetate was poor owing to the deterioration of the fungus and this resulted in some difficulties in observing the ^{13}C–^{13}C coupling satellites. This difficulty was compounded by the severe overlap of ^{13}C signals and the appearance of the ^{13}C–^{13}C couplings as AB spin systems owing to the similarity in the chemical shift of some of the coupled carbon atoms. The p.n.d. ^{13}C n.m.r. spectrum of the [1,2-^{13}C]acetate-derived aurovertin B clearly indicated, however, that the signals from C(2) [^1J(CC) 40·0 Hz] and C(3) [^1J(CC) 40·2 Hz) exhibited ^{13}C–^{13}C spin–spin coupling and thus were derived from an intact acetate unit. A similar result was obtained for C(4) and C(5) by analysis of the observed AB spin system. A disparity in the enrichment of the carbon atoms of the *O*-acetate group, C(24) [^1J(CC) 60·2 Hz] and C(25) [^1J(CC) 59·8 Hz] was observed as the signals of both the C(24) and C(25) carbon atoms exhibited a three-fold enhancement over those of the other acetate-derived carbon atoms. This phenomenon was also evident for C(25) in the p.n.d. ^{13}C n.m.r. spectrum of aurovertin B derived from [1-^{13}C]acetate.

The above results indicate that aurovertin B, and most probably asteltoxin, are derived from a C_{18}-polyketide precursor with the introduction of a methyl group from the C_1-pool onto the methyl carbon atom of the chain-initiating acetate unit as indicated in (**3**). This particular involvement of methionine in fungal polyketide biosynthesis has been demonstrated in the biosynthesis of barnol[8] and is probably also indicated for the biosynthesis of stellatin.[9]



(3)

An alternative interpretation involves the *C*-methylation of a C_{20}-polyketide precursor at C(18) followed by the loss of the chain-initiating acetate unit through deacylation (retro-Claisen cleavage) to give the C_{18}-polyketide. This aspect of aurovertin B biosynthesis is receiving attention.

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Note added in proof. The reported incorporation of propionic acid into aurovertin (ref. 5) has been verified using both [1-^{14}C]- and [1-^{13}C]-propionate. In the p.n.d. ^{13}C n.m.r. spectrum of aurovertin B derived from [1-^{13}C]-propionate only the signal assigned to C(3) (δ 85.3 p.p.m.) was enhanced (about fourteen-fold). The foregoing results indicate that C(1)–C(3) can originate either from acetate–methionine or from propionate. This finding is unique amongst fungal secondary metabolites.

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