Mechanistic Studies on the Biosynthesis of the Aurovertins using ^{18}O-Labelled Precursors

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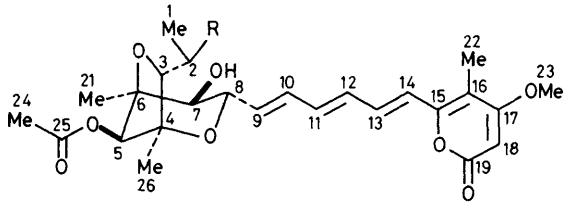
A mechanism is proposed for the formation of the 2,6-dioxabicyclo[3.2.1]octane moiety of the aurovertins, metabolites of *Calcarisporium arbuscula*, based on the observed ^{18}O isotope shifts in the proton-decoupled ^{13}C n.m.r. spectra of aurovertin B and D derived from [1-^{13}C,$^{18}O_2$]acetate and $^{18}O_2$ gas.

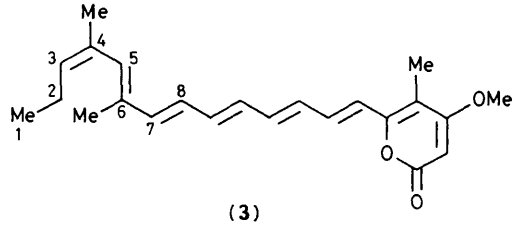
The aurovertins, a group of toxic metabolites isolated from cultures of *Calcarisporium arbuscula* (Preuss),[1] are potent inhibitors of oxidative phosphorylation in mitochondrial enzyme systems.[2] Structural evidence for aurovertin B (**1**) was deduced mainly from its ^1H and ^{13}C n.m.r. spectra.[3] The relative configuration of the substituted 2,6-dioxabicyclo[3.2.1]octane moiety present in the aurovertins is based on the observed proton–proton coupling constants and nuclear Overhauser effect experiments.[3] The structure and relative configuration of aurovertin B were confirmed by *X*-ray crystallography[4] and the absolute configuration followed when Mulheirn[5] established the (7*S*) chirality using the method of Helmchen.[6] Extensive incorporation studies with ^{13}C-labelled precursors have established the operation of two apparently independent pathways (a and b), distinguishable by the different origins of C-1—C-3, in the biosynthesis of aurovertin B. Pathway a involves the methylation of a β-ketoacyl thioester formed from acetyl- and malonyl-CoA units, followed by the loss of the acetate starter unit; C-1 in aurovertin B is thus derived from methionine and C-2 and C-3 from malonate. In contrast, pathway b involves a propionate starter unit and eight malonate units; C-1—C-3 are thus derived from propionate.[7] In this paper we report the results of ^{18}O isotope incorporation experiments, which, in conjunction with the known absolute configuration of aurovertin B, allow us to propose a detailed mechanism for the formation of the substituted 2,6-dioxabicyclo[3.2.1]octane moiety.

Sodium [1-^{13}C,$^{18}O_2$]acetate (8.5 mmol) (50.6% $^{13}C^{18}O_2$, 41.0% $^{13}C^{18}O$, 8.4% $^{13}C^{16}O$), admixed with sodium acetate (17.0 mmol) was added to cultures of *C. arbuscula*, NRRL 3705 (500 ml) over days 5—13 before isolation and purification of aurovertin B (**1**) (12 mg) and D (**2**) (43 mg) on day 14 as previously described.[7] The incorporation of ^{18}O was detected by the presence of isotopically shifted resonances in the proton-decoupled ^{13}C n.m.r. spectra (see Table 1).[8] Only the C-15, C-17, C-19, and C-25 resonances exhibited upfield isotope shifts indicating that the corresponding carbon–oxygen bonds had remained intact throughout the biosynthetic pathway. Similar results have been observed in the biosynthesis of the structurally related metabolites citreoviridin[9] and asteltoxin.[10] The magnitude of the ^{18}O isotope shift (0.037 p.p.m.) observed for the C-25 resonance shows that the sp^2 bonded oxygen of the acetoxy group, but not the sp^3 bonded oxygen atom, is derived from acetate.

The oxygen atoms of the 2,6-dioxabicyclo[3.2.1]octane moiety are therefore derived from molecular oxygen or water from the incubation medium. The fermentation of cultures of *C. arbuscula* (500 ml) in a closed system in which the normal atmosphere was replaced by one containing $^{18}O_2$ (50.0 atom % ^{18}O) and the simultaneous addition of [1-^{13}C]acetate (8.5



(1) R = H (aurovertin B)
(2) R = OH (aurovertin D)



(3)

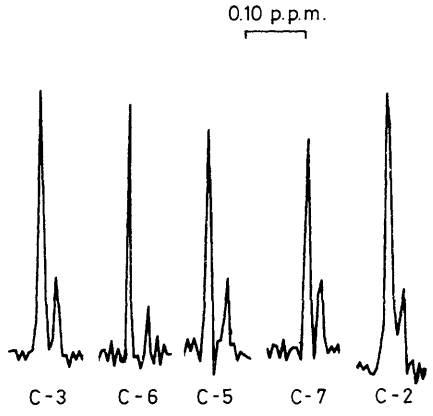
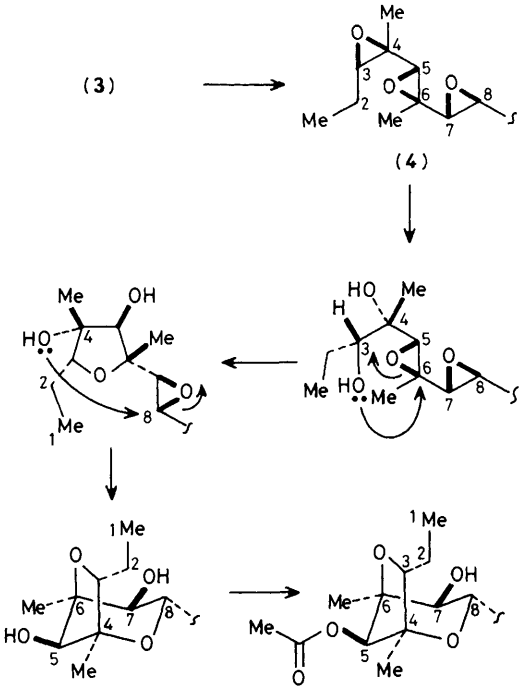
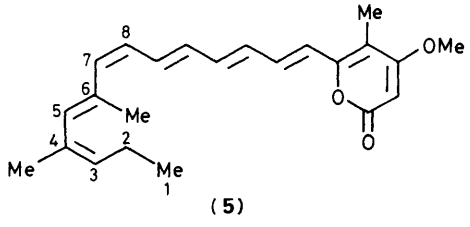


Figure 1. ^{18}O Isotope shifts observed in the ^{13}C n.m.r. spectrum† of aurovertin D (**2**) derived from $^{18}O_2$ gas. The intensity of each carbon resonance is not drawn to the same scale.



Scheme 1. Proposed mechanism for the formation of the 2,6-dioxabicyclo[3.2.1]octane moiety of the aurovertins.



(5)

Table 1. ^{18}O Isotopically shifted resonances observed in the 125.76 MHz ^{13}C n.m.r. spectra of the aurovertins.[a]

	Aurovertin B (**1**)		Aurovertin D (**2**)	
	δ_C	$\Delta\delta \times 100$	δ_C	$\Delta\delta \times 100$
C-2	20.53(t)	-	65.48(d)	2.6[b]
C-3	85.81(d)	2.6[b]	87.30(d)	2.1[b]
C-4	83.16(s)	-	83.45(s)	-
C-5	81.11(d)	3.2[b]	81.56(d)	3.2[b]
C-6	83.87(s)	2.9[b]	83.91(s)	3.2[b]
C-7	76.86(d)	2.1[b]	76.78(d)	2.1[b]
C-8	78.22(d)	-	78.29(d)	-
C-15	154.58(s)	2.6[c]	154.54(s)	2.6[c]
C-17	170.99(s)	2.1[c]	171.01(s)	2.1[c]
C-19	163.47(s)	3.2[c]	163.54(s)	3.2[c]
C-25	170.15(s)	3.7[c]	170.14(s)	3.7[c]

[a] Recorded on a Bruker WM-500 spectrometer; solvent CD_2Cl_2. [b] $^{18}O_2$-enriched. [c] [1-^{13}C,$^{18}O_2$]acetate-enriched.

† Spectroscopic parameters were as follows: the spectrum was accumulated at 303 K in a 5 mm bore tube using a sweepwidth of 21 739 Hz with a 64 K data block, 2368 scans, pulse angle 90° and an acquisition time of 1.507 s. For resolution enhancement a line broadening factor of −3.0 was applied together with a Gaussian multiplier of 0.4 prior to Fourier transformation, 0.66 Hz/data point.

mmol, 99.0 atom % ^{13}C) admixed with unlabelled acetate (17.0 mmol) to the medium produced ^{18}O-labelled (**1**) (31 mg) and (**2**) (44 mg). The presence and location of ^{18}O was deduced from the ^{18}O-induced isotope shifts observed for the C-3, C-5, C-6, and C-7 resonances in the proton-decoupled ^{13}C n.m.r. spectra of both aurovertins and also, in the case of (**2**), C-2 (see Table 1 and Figure 1). The fact that no ^{18}O isotope shift is observed for either C-4 or C-8 implies that the C-4–O–C-8 oxygen atom is derived from water.

The above experiments established the origin of the oxygen atoms in the aurovertins. Analysis of the results affords considerable insight into the stereochemistry and mechanism of the biosynthetic processes by which these metabolites are elaborated. The postulated first enzyme-free intermediate, the (3*Z*,5*E*,7*E*,9*E*,11*E*,13*E*)-polyene (**3**) with the 4-s-*cis*,6-s-*cis*,8-s-*trans*,10-s-*trans*,12-s-*trans*,14-s-*trans* conformation, can be formed by two biosynthetic pathways (a and b). Thus methylation of a β-ketoacyl thioester by *S*-adenosyl methionine introduces the eventual C-2, C-4, C-6, and C-16 methyl groups and generates the C-17 *O*-methyl moiety (pathway a). This methylation is followed by the loss of the starter acetate unit through a retro-Claisen cleavage to give an intermediate which can also be formed from a propionate starter unit and malonyl-CoA units (pathway b) by methylation at the appropriate sites. The subsequent loss of oxygen from C-3,

C-5, C-7, C-9, C-11, and C-13 presumably occurs by a reduction–elimination sequence similar to that postulated for citreoviridin[9] and asteltoxin[10] and analogous to that of fatty acid biosynthesis[11] to give, after formation of the pyrone ring, the polyene (**3**). Once released from its polyketide synthetase the polyene (**3**) is postulated to undergo epoxidation by a mono-oxygenase, using molecular oxygen, to give the (3*R*,4*R*,5*R*,6*R*,7*R*,8*S*)-triepoxide (**4**) (Scheme 1). Nucleophilic attack by water at C-4 initiates a cascade of ring closures to generate eventually the 2,6-dioxabicyclo[3.2.1]octane moiety of the aurovertins. Insertion of an oxygen atom derived from molecular oxygen into the C-2 carbon–hydrogen bond of (**1**) or one of its precursors, leads to the formation of (**2**).

An isomer of the polyene (**3**) with the (7*E*) stereochemistry and the 4-s-*cis*,6-s-*trans* conformation (**5**) has been proposed as an intermediate in the biosynthesis of asteltoxin.[10]

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