Application of ^{18}O Induced Isotope Effects in ^{13}C N.M.R. Spectroscopy to the Biosynthesis of Asteltoxin

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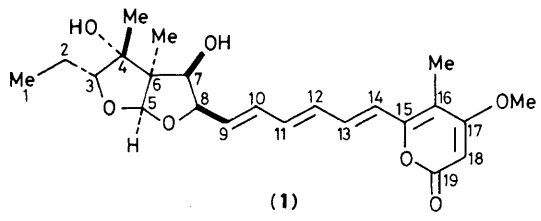
Observation of ^{18}O induced isotope shifts in the ^{13}C n.m.r. spectra of asteltoxin derived from [1-^{13}C,$^{18}O_2$]acetate and $^{18}O_2$ gas established the origin of the oxygen atoms and clarified the mechanism of the formation of the 2,8-dioxabicyclo[3.3.0]octane moiety.

Asteltoxin (**1**), a mycotoxin isolated from toxic maize cultures of *Aspergillus stellatus* Curzi, strain MRC 641,[1] and structurally related to citroviridin[2,3] and aurovertin B,[4,5] is a potent inhibitor of bacterial ATPase.[6] Extensive incorporation studies with ^{13}C-labelled precursors have revealed that asteltoxin can be formed by two biosynthetic pathways (A and B) which are distinguishable by the different origins of C-1—C-3.[7] 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, and C-1 in asteltoxin 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 addition a 1,2-bond migration occurs in the course of the biosynthesis to generate a branched aldehyde which is subsequently utilised in the formation of the 2,8-dioxabicyclo[3.3.0]octane moiety.[7] To investigate the mechanism of this 1,2-shift and the stereochemistry of the intermediate transformations leading to the asteltoxin structure it was necessary to determine the origin of the oxygen atoms in the metabolite. In this paper we report the results of ^{18}O-isotope incorporation experiments, which in conjunction with the known relative configuration of asteltoxin,[1] allows us to propose a detailed mechanism for the formation of the subsituted 2,8-dioxabicyclo[3.3.0]octane moiety.



(**1**)

Sodium [1-^{13}C,$^{18}O_2$]acetate (12.0 mmol) (50.6% $^{13}C^{18}O_2$, 41.0% $^{13}C^{18}O$, 8.4% $^{13}C^{16}O$), admixed with sodium acetate (24.0 mmol) was added to cultures of *Emericella variecolor*,[7] strain NHL 2881 (500 ml) over days 6—17 before isolation and purification of asteltoxin (**1**) (30 mg). The sites of ^{18}O-enrichment were determined by ^{13}C n.m.r. spectroscopy by taking advantage of the ^{18}O induced isotope shifts observed for the resonances of ^{13}C nuclei attached to ^{18}O.[8] The proton-decoupled ^{13}C n.m.r. spectrum of the enriched asteltoxin exhibited ^{18}O-isotope shifts for the C-15, C-17, and C-19 resonances (see Table 1) indicating that the corresponding carbon–oxygen bonds had remained intact throughout the biosynthetic pathway. No ^{18}O was present at either C-3, C-5, or C-7 as the resonances at δ_C 90.10, 112.98, and 80.19, respectively appeared as enhanced singlets. The lack of ^{18}O-labelling at C-4 and C-8 is to be expected as these carbon atoms are derived from C-2 of acetate.[7]

Fermentation of cultures of *E. variecolor* (500 ml) in a

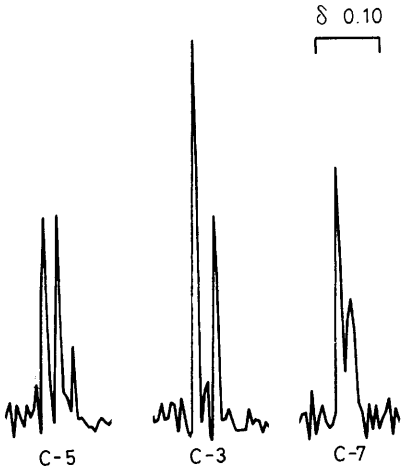
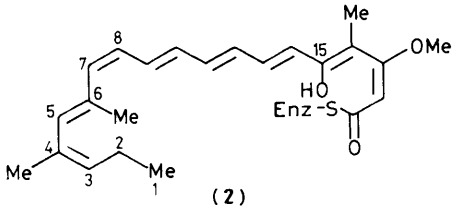
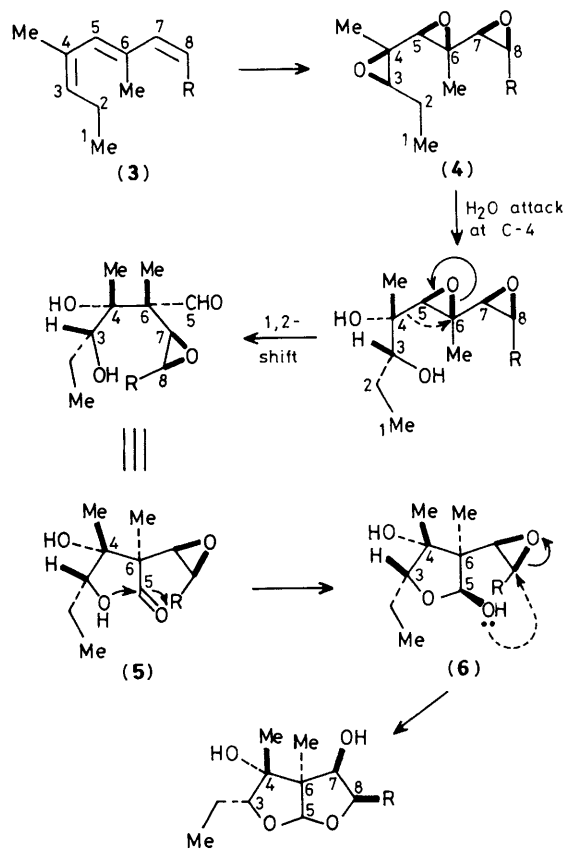


Figure 1. ^{18}O Isotope shifts observed in the ^{13}C n.m.r. spectrum of asteltoxin (**1**) derived from $^{18}O_2$ gas. Spectroscopic parameters were as follows: the spectrum was accumulated at 303 K in a 5 mm bore tube using a sweepwidth of 21739 Hz with a 64 K data block, 14336 scans, pulse angle 90°, and an acquisition time of 1.507 s. For resolution enhancement a line broadening factor of −2.0 was applied together with a Gaussian multiplier of 0.4 prior to Fourier transformation, 0.66 Hz/data point.





Scheme 1. Proposed mechanism for the formation of the 2,8-dioxabicyclo[3.3.0]octane moiety of asteltoxin.

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

Carbon	δ_C	Δδ (p.p.m. × 100)
C-3	90.10 d	3.2[b]
C-4	80.93 s	—[b,c]
C-5	112.98 d	2.1, 2.1, 4.2[b]
C-7	80.19 d	2.1[b]
C-8	84.80 d	—[b,d]
C-15	154.80 s	3.2[c]
C-17	171.06 s	2.1[c]
C-19	162.79 s	3.2[c]

[a] Recorded on a Bruker WM-500 spectrometer; solvent [$^2H_6$]acetone. [b] Enriched by $^{18}O_2$. [c] Enriched by [1-^{13}C,$^{18}O_2$]acetate. [d] Not detected.

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 (15.0 mmol, 99.0 atom % ^{13}C) admixed with unlabelled acetate (15.0 mmol) to the medium from day 6 to 17 produced labelled asteltoxin (7 mg) whose proton decoupled ^{13}C n.m.r. spectrum (see Figure 1) demonstrated the origin of the C-7 and the 2,8-dioxabicyclo[3.3.0]octane ring oxygens from oxidative processes. ^{18}O-Isotope shifts were observed for the C-3 (Δδ −0.032 p.p.m.), C-7 (Δδ −0.021 p.p.m.), and C-5 resonances (see Table 1). For the C-5 resonance, two isotopically shifted signals appeared due to species having ^{18}O in (a) both tetrahydrofuran rings (Δδ −0.042 p.p.m.) and (b) in either of the two tetrahydrofuran rings (Δδ −0.021 p.p.m.). The presence of the two isotopomers mentioned under (b) was deduced from the intensity of the ^{18}O shifted signal (Δδ −0.021 p.p.m.). The ratio of $^{13}C^{16}O$: $^{13}C^{18}O$ for this signal is twice the ratio of that of the C-3 and C-7 resonances (Figure 1). The low intensity of the C-8 resonance precluded the observation of an ^{18}O-isotope shift. The fact that no ^{18}O-isotope shift is observed for C-4 in either of the experiments implies that the C-4 oxygen atom is derived from water.

The knowledge on the origin of the oxygen atoms in asteltoxin, in conjunction with our previous ^{13}C-labelling studies,[7] allows us to deduce the stereochemistry and mechanisms of the events by which the metabolite is elaborated. Thus, alkylation of a β-ketoacyl thioester by *S*-adenosyl methionine introduces the eventual C-2, C-4, C-6, C-16 methyl groups and generates the C-17 *O*-methyl moiety. 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 by methylation at the appropriate sites. The loss of oxygen from C-3, C-5, C-7, C-9, C-11, and C-13 presumably occurs by a reduction–elimination sequence analogous to that of fatty acid biosynthesis[9] to generate the (3*Z*,5*E*,7*Z*,9*E*,11*E*,13*E*)-polyene with the 4-s-*cis*,6-s-*trans*,8-s-*trans*,10-s-*trans*,12-s-*trans*,14-s-*trans* conformation (**2**). The exact timing of the methylation step and the reduction–elimination sequence is not known but these reactions do not have to involve a C_{18} β-ketoacyl thioester and could occur at an earlier stage of the β-ketoacyl chain assembly. The subsequent formation of the pyrone ring proceeds by

nucleophilic attack of the C-15 enolic hydroxy group on the thioester carbonyl group.

If we assume that water elimination in the reduction–elimination sequence occurs in a *syn* fashion, as is the case in fatty acid biosynthesis,[9] then *E* and *Z* double bonds can be formed only if the steric course of the reductions which generate the chiral secondary alcohols occur in the opposite sense. This difference in stereospecificity is consistent with these reductions occurring at different active sites on distinct reductases. Alternatively, water could be eliminated either by a *syn* or an *anti* mechanism from the same chiral secondary hydroxy group.

Once released from its polyketide synthetase, the polyene (**3**) (Scheme 1) is postulated to undergo epoxidation by a mono-oxygenase, using molecular oxygen, to give the (3*R*,4*R*,5*R*,6*R*,7*S*,8*S*)-triepoxide (**4**) or its enantiomer. Nucleophilic attack by water at C-4 would initiate the formation of the 2,8-dioxabicyclo[3.3.0]octane system as shown in Scheme 1. The 1,2-shift occurs with retention of configuration at C-4 and inversion at C-6 to generate the branched aldehyde (**5**). Formation of the lactol (**6**) by nucleophilic attack of the C-3 hydroxy group on the carbonyl group of the aldehyde and subsequent ring closure generates the bicyclic ring system. The conversion of the triepoxide into the 2,8-dioxabicyclo[3.3.0]octane moiety must be a concerted process as no apparent loss of ^{18}O, present in the aldehyde group, occurs through exchange with water of the medium.

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References

1 G. J. Kruger, P. S. Steyn, R. Vleggaar, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1979, 441.
2 N. Sakabe, T. Goto, and Y. Hirata, *Tetrahedron Lett.*, 1964, 1825; *Tetrahedron*, 1977, **33**, 3077.
3 P. S. Steyn, R. Vleggaar, P. L Wessels, and M. Woudenberg, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2175.
4 L. J. Mulheirn, R. B. Beechey, D. P. Leworthy, and M. D. Osselton, *J. Chem. Soc., Chem. Commun.*, 1974, 874.
5 P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1298.
6 M. Satre, *Biochem. Biophys. Res. Commun.*, 1981, **100**, 267.
7 P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Chem. Commun.*, 1984, 977.
8 J. C. Vederas, *Can. J. Chem.*, 1982, **60**, 1637 and references cited therein; P. E. Hansen, *Annu. Rep. NMR Spectrosc.*, 1983, **15**, 105.
9 B. Sedgwick and C. Morris, *J. Chem. Soc., Chem. Commun.*, 1980, 96.