Biosynthesis of Aspyrone and Asperlactone, Pentaketide Metabolites of *Aspergillus melleus*. Incorporation Studies with [1-^{13}C,$^{18}O_2$]Acetate and $^{18}O_2$ Gas

Salman A. Ahmed,[a] Thomas J. Simpson,*[a] James Staunton,*[b] Andrew C. Sutkowski,[b] Laird A. Trimble,[c] and John C. Vederas*[c]

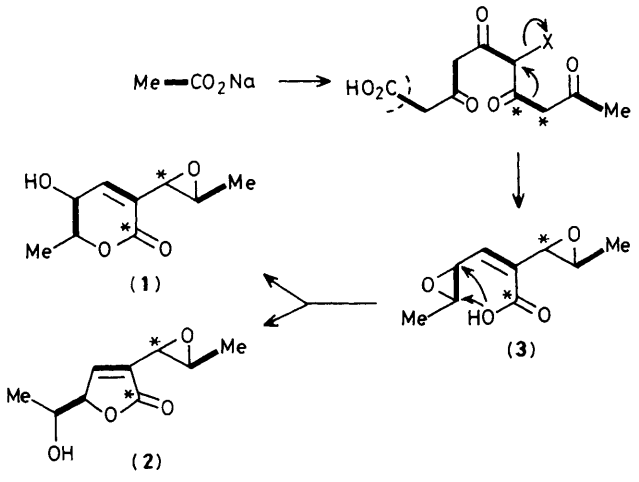
[a] *Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, Scotland, U.K.*
[b] *University Chemistry Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.*
[c] *Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*

Incorporation of [1-^{13}C,$^{18}O_2$] acetate and $^{18}O_2$ gas into aspyrone (**1**) and asperlactone (**2**) by cultures of *Aspergillus melleus* and observation of ^{18}O isotope-induced shifts in the ^{13}C n.m.r. spectra of the enriched metabolites establish the origins of all the oxygen atoms and suggest a biosynthetic pathway involving epoxide-mediated rearrangement and ring closure reactions.

Aspyrone (**1**)[1] and asperlactone (**2**)[2] are closely related metabolites of *Aspergillus melleus*. Their biosynthesis has been extensively studied,[3] and as a result of incorporation studies with isotopically labelled acetates and malonate and potential advanced intermediates the intermediacy of aromatic precursors could be ruled out; a pathway was proposed *via* decarboxylation and Favorski-type rearrangement of a linear pentaketide intermediate as shown in Scheme 1. In support of this, incorporations of [1,2-$^{13}C_2$] acetate resulted in two-bond ^{13}C–^{13}C couplings being observed between C-2 and C-8 in aspyrone[4] and asperlactone.[2] The stereochemistry of (**1**) and (**2**) has been established[5] and this is consistent with their derivation from a common intermediate, *e.g.* (**3**), *via* alternative ring openings of the epoxide by the carboxy function. In order to obtain evidence for this proposal and to obtain information on the nature of intermediates on the pathway we have studied the incorporation of [1-^{13}C,$^{18}O_2$]-acetate and $^{18}O_2$ gas into aspyrone and asperlactone.

[1-^{13}C,$^{18}O_2$]Acetate was fed to cultures of *A. melleus* and the aspyrone and asperlactone isolated from separate experiments were analysed by ^{13}C n.m.r. spectroscopy at 100.6 MHz. High levels of ^{13}C-enrichment (*ca.* 5 atom %) were observed at C-2, -4, -6, and -9 but surprisingly no ^{18}O isotope-induced shifts were apparent indicating that *no* acetate-derived oxygen was incorporated into the metabolites. On carrying out a fermentation in the presence of $^{18}O_2$ gas† the ^{13}C n.m.r. spectrum shown in Figure 1 was obtained for aspyrone. The isotope shifts observed (Table 1) for C-5, C-8, and C-9 indicated that the epoxide and alcohol oxygens were both highly and equally enriched. In addition C-2 shows *two* isotopically shifted signals and C-6 shows one. Within

† Fermentations were carried out in a closed system under an atmosphere composed of N_2, $^{18}O_2$, and $^{16}O_2$ (80 : 10 : 10) for aspyrone and (80 : 6 : 14) for asperlactone.



Scheme 1

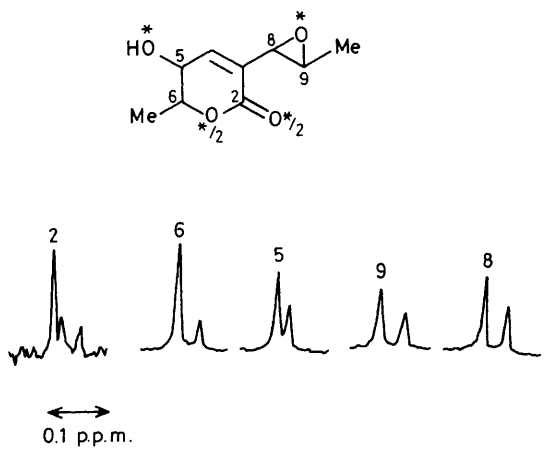
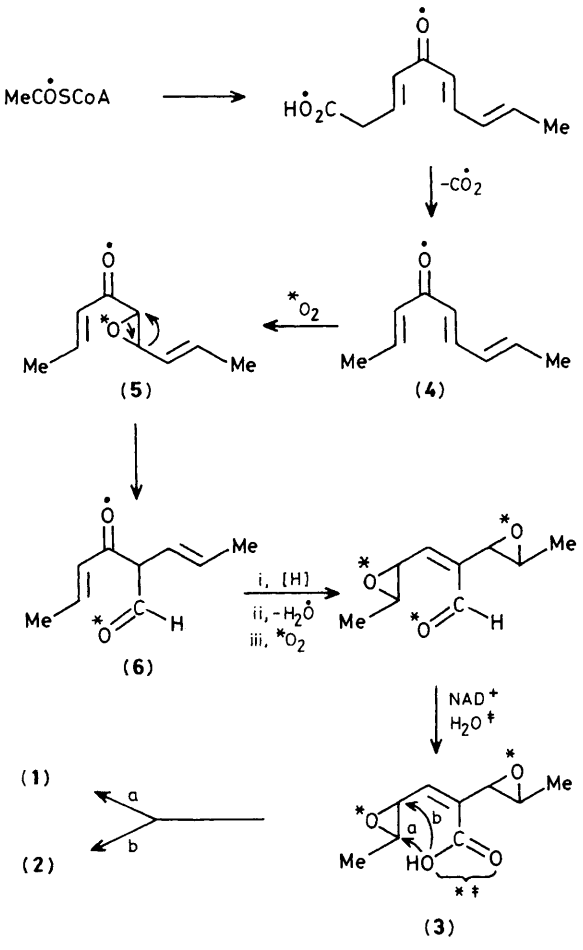


Figure 1. ^{18}O Isotope shifts in the 100 Mz ^{13}C n.m.r. spectrum of $^{18}O_2$-enriched aspyrone (**1**).



Scheme 2

Table 1. ^{18}O Isotopically shifted resonances observed in the 100.6 MHz ^{13}C n.m.r. spectrum[a] of aspyrone (**1**) enriched from $^{18}O_2$ gas.

Carbon	δ	100 Δδ/p.p.m.	$^{16}O:{}^{18}O$[b]
2	163.2	0.9, 3.7	61:20:19
5	67.7	1.6	63:37
6	79.3	3.1	78:22
8	54.6	3.1	62:38
9	59.0	3.4	63:37

[a] For experimental conditions see ref. 9. [b] All values (±2%) were determined using a Du Pont curve analyser.

experimental error, the intensities of these signals are essentially equal to one another but are half those observed at C-5, C-8, and C-9. The most reasonable interpretation of these results is that one oxygen-18 atom has been introduced from the atmosphere onto C-2 and that this labelled atom has been incorporated equally into both the carbonyl and ether oxygens of the lactone moiety. Thus three of the oxygen atoms in aspyrone appear to be derived from the atmosphere and so the remaining oxygen on C-2 must be derived from the medium. Asperlactone, produced in the presence of $^{18}O_2$ gas† in a separate experiment, gave essentially identical results.

To account for these results the pathway shown in Scheme 2 is proposed. Rearrangement of the epoxide (**5**) formed from the trienone (**4**), itself derived from a pentaketide precursor by reduction, dehydration, and decarboxylation reactions, would generate the aldehyde (**6**). This could then be converted into the key epoxycarboxylic acid intermediate (**3**) *via* further epoxidation and NAD^+ mediated oxidation of the aldehyde as indicated. Ring closure to either end of the epoxide moiety would then generate aspyrone or asperlactone with the observed positions and levels of oxygen-18 labelling as indicated. The steps proposed in this pathway bear comparison with the postulated biosynthesis of monensin[6] and other polyether antibiotics[7] where ring closures onto epoxide intermediates have been proposed for the formation of 5- and 6-membered oxygen-containing rings. A further possibility which cannot be rigorously excluded at this stage is that the carboxylate oxygen atom derived from the atmosphere could have been introduced in a final hydroxylation step on an aldehyde intermediate in which the aldehyde oxygen is derived entirely (*e.g.* by exchange) from the medium. The observed retention of two acetate-derived hydrogens on C-7 in both aspyrone[8] and asperlactone[3d] on incorporation of [2-^{13}C,$^2H_3$]acetate rules out pentaketide-derived intermediates having a double bond between the carbons which become C-6 and C-7.

We thank the S.E.R.C., NATO, the Government of Iraq, and the Natural Sciences and Engineering Research Council of Canada for financial support.

Received, 9th August 1985; Com. 1193

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