Evidence for a Mono-oxygenase Mechanism in the Biosynthesis of Austdiol

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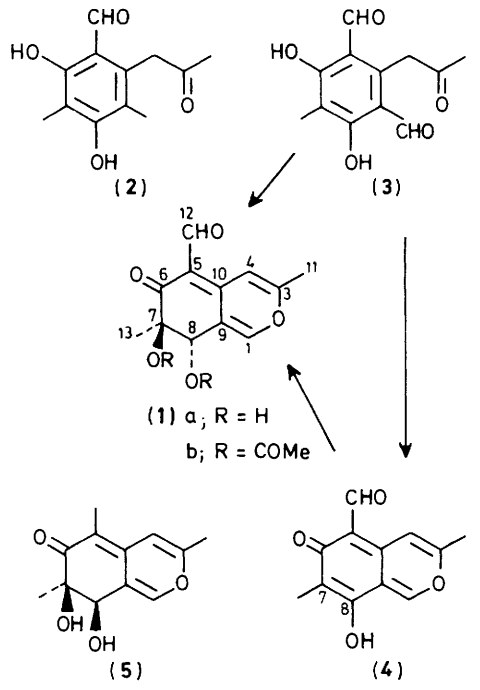
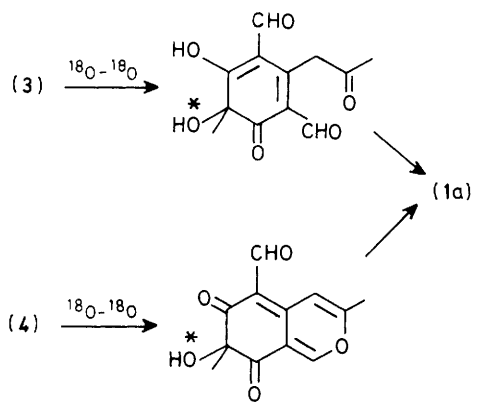
Interpretation of the incorporation data of $^{18}O_2$ into austdiol in *Aspergillus ustus* suggests a mono-oxygenase mechanism.

The biosynthetic pathway leading to austdiol (**1a**),[1] the main toxic metabolite from *Aspergillus ustus*, may involve intermediates such as (**2**) and (**3**).[2] The final step in the biosynthesis of (**1a**) is the oxidation of the C(7)–C(8) double bond of the quinone-methide intermediate (**4**), derived from the dialdehyde (**3**) by dehydration, or of the dialdehyde (**3**) itself (Scheme 1).

To investigate the type of mechanism involved in the oxidation step, it was necessary to determine the origin of the oxygen atoms. Accordingly, we carried out the fermentation



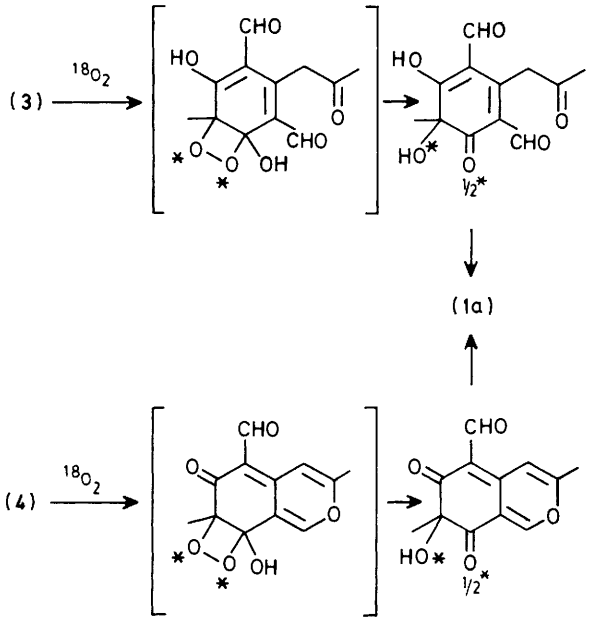
Scheme 1



Scheme 2. * = ^{18}O.

in an atmosphere consisting of nitrogen (70%), $^{16}O_2$ (18%), and $^{18}O_2$ (12%) (v:v). The composition of the atmosphere in the fermentation flask was kept constant by feeding, under constant pressure, the isotopically enriched oxygen mixture. The sites of ^{18}O enrichment were determined directly by ^{13}C n.m.r. spectroscopy by taking advantage of the isotope shifts of the resonance of the ^{13}C nuclei attached to ^{18}O atoms. In the 100.62 MHz ^{13}C n.m.r. spectrum of ^{18}O-derived austdiol diacetate (**1b**),[2] measured in a mixture of $(CD_3)_2SO$–$CDCl_3$ 1:3, only the peaks from C(7) and one acetate carbonyl group appeared, after Gaussian resolution enhancement,[3] as a pair of signals corresponding to the ^{13}C–^{16}O and ^{13}C–^{18}O species. These resonances were shifted 0.03 and 0.015 p.p.m. upfield, respectively, consistent with the type of C–O bond.[4]

The aldehyde carbon C(12) of (**1b**) is also thought to be derived from oxidation of a methyl group. However, interestingly, no isotopically shifted signal was detected for C(12). If the aldehyde group of (**1b**) were ^{18}O-labelled, observation of the corresponding carbon signal should be straightforward at 100.62 MHz. The width at half-height of the C(12) resonance was 1.5 Hz and the model ^{18}O-labelled benzaldehyde is known to exhibit an isotopic shift of 0.043 p.p.m.[5] However, biosynthetic studies carried out[6] using ^{18}O-labelled precursors



Scheme 3. * = ^{18}O.

showed that carbonyl groups could undergo ready oxygen exchange.

The above results are in agreement with an oxidation mechanism involving addition of an activated oxygen molecule to the C(7)–C(8) double bond of the quinone-methide (**4**) or the dialdehyde (**3**), mediated by a mono-oxygenase. In the subsequent step, reduction of the C(8) carbonyl group should occur to provide the *trans*-vicinal diol system (Scheme 2).

Moreover, *Aspergillus ustus* produces, in addition to austdiol (**1a**), minor amounts of dihydrodeoxy-8-*epi*-austdiol (**5**).[7] We assume the *cis*-vicinal arrangement of the hydroxy groups in (**5**) can also be explained by the above mechanism; however the C(8) carbonyl group is reduced to the (8*R*) configuration, opposite to that in austdiol. The same mechanism could be operating in the biosynthesis of the azaphilones[8] whose C(8) carbonyl group remains unaffected. We can therefore exclude an alternative mechanism involving a dioxygenase (Scheme 3) because this mechanism would afford austdiol (**1a**) containing two oxygen atoms, on C(7) and C(8), incorporated from the same molecular species of $^{18}O_2$.

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