Biosynthesis of Citrinin in *Penicillium citrinum:* Advanced Precursor Studies using ^{2}H Nuclear Magnetic Resonance Spectroscopy

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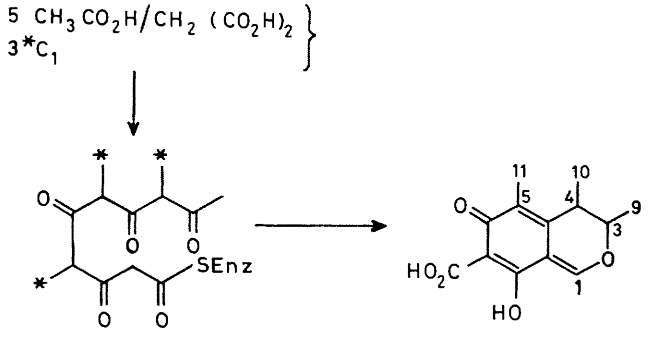
Summary Specific incorporation of 4,6-dihydroxy-3,5-dimethyl-2-(1-methyl-2-oxopropyl)-benzaldehyde into citrinin is demonstrated using ^{2}H n m r spectroscopy

The pentaketide antibiotic citrinin (**1**) is biosynthesized by the fungus *Penicillium citrinum* from $5C_2$ and $3C_1$ units (Scheme 1)[1,2] The isocoumarin (**2**) is specifically incorporated into citrinin[3] though to a very small extent (0 05%), but the related compound (**3**) is not, implying that methylation of the supposed poly-β-ketone chain takes place prior to its cyclization

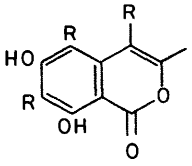
When the fungus was grown on D_2O with unlabelled glucose as the carbon source,[4] protium derived from the C–H bonds of glucose was incorporated, to a significant extent, at all the normally protonated carbon atoms of the



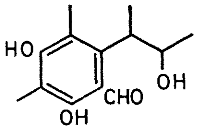
(1)

Scheme 1

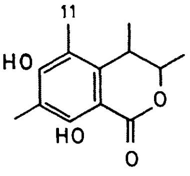
metabolite. The presence of protium at C(4) proved that, despite its specific incorporation, (**2**) cannot be part of the normal biosynthetic pathway to citrinin.



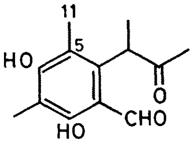
(2) R = Me
(3) R = H



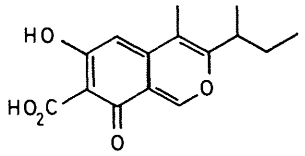
(4)



(5)



(6)

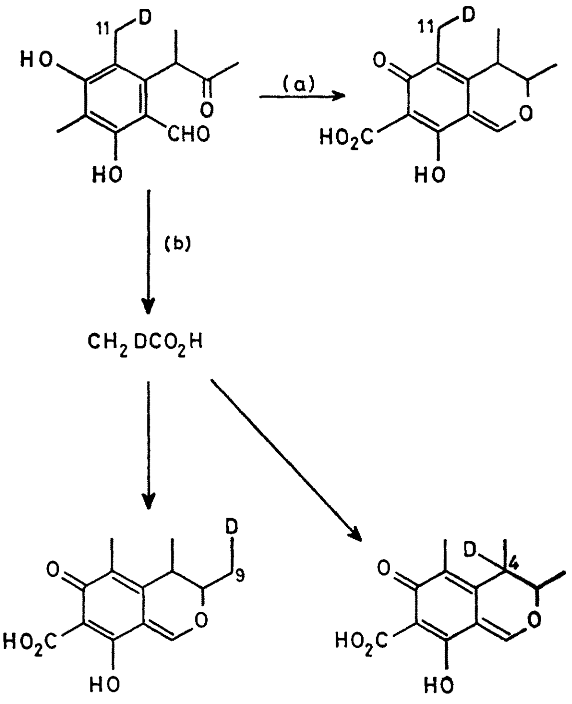


(7)

In the same experiment incorporation of protium at C(1) and C(3) was 45 and 34%, respectively (measured by comparison with the fully protonated hydroxy-positions). We have now shown that this result is reproducible and is therefore highly significant. If the aldehydo-alcohol (**4**) were the first enzyme-free intermediate it would be expected that the hydrogen atoms at C(1) and C(3) would be derived from a common pool of nicotinamide co-enzyme, giving rise to the same protium : deuterium ratio at the two positions. Although the possibility of differing isotope effects in the reduction of the two positions cannot be ruled out, this result suggests that the two reductions may be carried out by different enzymes at different stages in the biosynthesis and possibly in different parts of the cell.

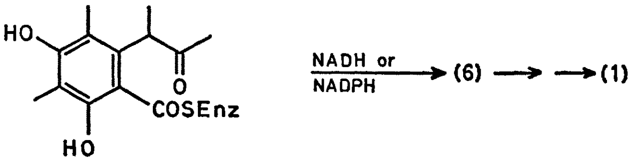
Hence, the prime candidates for consideration as the first enzyme-free intermediate in the biosynthesis of citrinin are the lactone (**5**), in which reduction has occurred at C(3) but not at C(1) and the ketoaldehyde (**6**), in which C(1) but not C(3) is reduced.

Both compounds were prepared with a single deuterium label in the aromatic methyl group, C(11), and were separately administered to 3 week-old cultures of *Penicillium citrinum*. Citrinin was harvested after a further 10 d. A ^2H n.m.r. spectrum of the citrinin derived from (**5**) showed no deuterium signal other than that due to the natural abundance. However, the citrinin derived from the feeding of (**6**) shows a strong peak at δ 2·0, corresponding to deuterium enrichment at C(11) of citrinin. Comparison of the intensity of the signal with that due to the natural abundance level of deuterium in the chloroform solvent shows an enrichment of 1·6% which corresponds to a specific incorporation of 6·5% of the precursor.



Scheme 2.

Although most of the deuterium is introduced by specific incorporation [path (a) in Scheme 2], there are small peaks corresponding to deuterium at C(9) and/or C(10) (the signals overlap), and also at C(4), indicating that some minor scrambling of the label has occurred. We suggest that enzymatic degradation [path (b) of Scheme 2], takes place in competition with specific incorporation, so that C(11) and C(5) of (**6**) produce some CH_2DCO_2H, which is then incorporated into citrinin giving rise to the observed labelling pattern.[5] The ease with which this minor pathway was detected strikingly demonstrates the advantage of deuterium over other isotopes hitherto used routinely for advanced precursor studies.[6]



Scheme 3.

We conclude that (**6**) is the first enzyme-free intermediate in the biosynthesis of citrinin, and suggest that it is produced by reductive cleavage of an enzyme-bound thioester (Scheme 3). These results compare interestingly with recently published work on the hexaketide ascochitine (**7**)[7] in which incorporation studies also implicate a ketoaldehyde as the first enzyme-free intermediate, though this is perhaps less surprising since there is no subsequent reduction of the ketone

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