The Biosynthesis of the Tryptophan-derived Mould Metabolites Roquefortine and Aszonalenin

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L-[2,4,5,6,7-$^2H_5$]Tryptophan has been incorporated into roquefortine and aszonalenin, by *Penicillium roqueforti* and by *Aspergillus zonatus* respectively, with retention of five deuterium atoms; the 5a-hydrogen of each of these metabolites is derived from the 2-hydrogen of tryptophan, contrary to an earlier report.

A considerable degree of interest has been shown during the last two decades in the biosynthesis of tryptophan-derived metabolites that contain a 1,1-dimethylallyl substituent.[1,2] In this connection Barrow *et al.* have reported that isotopic label from [2,4,5,6,7-$^2H_5$]tryptophan was incorporated by a tryptophan-auxotrophic mutant of *Penicillium roqueforti* into the benzenoid ring of roquefortine **1**, but not into the 5a-hydrogen of the latter. It was concluded that the 5a-hydrogen did not derive from the 2-hydrogen of tryptophan.[3] The use of ^1H NMR in that study, for assay of deuterium by difference, is expected to be insensitive to the presence of biosynthetically significant traces of deuterium at the 5a-position of roquefortine. We have investigated the incorporation of [$^2H_5$]tryptophan into both roquefortine **1** and the related mould metab-

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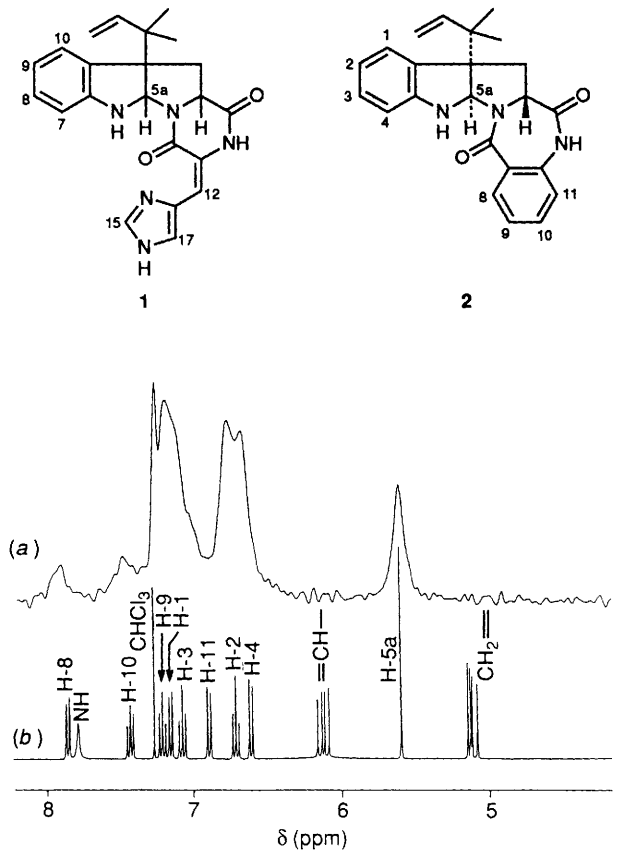

Fig. 1 (*a*) Resolution-enhanced proton-noise decoupled ^2H NMR spectrum of [^2H]aszonalenin in $CHCl_3$ (56 000 transients) recorded at 55.3 MHz. The positions and areas of the recorded bands are consistent with the following distribution of the ^2H-label in **2**: H(8), H(9), H(10), H(11) *ca.* 5% each; H(1), H(2), H(3), H(4) *ca.* 17% each; H(5a) *ca.* 12%. The resonance at δ 7.25 is due to C^2HCl_3 at natural abundance. (*b*) ^1H NMR spectrum of **2** in C^2HCl_3 recorded at 360.1 MHz. Critical assignments were confirmed by NOE difference spectroscopy

olite aszonalenin **2**, with direct assay of deuterium by ^2H NMR, and now report that the 5a-hydrogen atoms of both metabolites are derived from the 2-hydrogen of tryptophan, contrary to the earlier claim.

L-[2,4,5,6,7-$^2H_5$]Tryptophan[4]‡ (153 mg) was supplied to a 5 day-old surface culture of *P. roqueforti* grown on a 2% yeast extract medium (2500 ml) supplemented with sucrose (15%). The mycelium was harvested after growth for a further 21 days at 24 °C. The mass spectrum of the roquefortine (25 mg) that was isolated revealed the presence of a [$^2H_5$]-species (*ca.* 8%). The 55.3 MHz ^2H NMR spectrum of a $CHCl_3$ solution of this sample of roquefortine consisted of a sharp resonance at δ 7.25 for C^2HCl_3 at natural abundance, superimposed upon a broad envelope, *ca.* δ 6 to 8, due to the four overlapping aromatic resonances, and a broad ($w_{\frac{1}{2}}$ *ca.* 22 Hz) partially resolved resonance at δ 5.6. Comparison with the 360 MHz ^1H NMR spectrum of roquefortine revealed that the latter resonance could only be assigned to the deuterium-labelled 5a-proton, notwithstanding the poor resolution achieved in the ^2H NMR spectrum.

We have also studied the incorporation of L-[2,4,5,6,7-$^2H_5$]tryptophan into aszonalenin[5] **2** by *Aspergillus zonatus*. Mass spectrometry showed that the [^2H]aszonalenin that was isolated contained both [$^2H_4$]- and [$^2H_5$]-species (*ca.* 3% of each). Furthermore, comparison of the ^2H NMR spectrum of the [^2H]aszonalenin (Fig. 1*a*) with the ^1H NMR spectrum (Fig. 1*b*), revealed that the 5a-proton of the labelled aszonalenin is enriched with deuterium. It is also noteworthy that the 8- and 10-protons were weakly labelled and that the extent of deuterium enrichment at the 5a-position of aszonalenin is only about 70% of the average for positions 1 to 4; these observations may be accounted for by the metabolism of some of the [$^2H_5$]tryptophan to [$^2H_4$]anthranilic acid, which could furnish protons 8 to 11 of aszonalenin, or which could be re-incorporated into tryptophan and thence furnish [1,2,3,4-$^2H_4$]aszonalenin. A similar sequence might also explain the earlier report that the 5a-proton of roquefortine is not derived from the 2-proton of tryptophan.[3] It may be significant that the [$^2H_5$]tryptophan in the latter study was supplied at the start of the fungal culture, thus increasing the opportunity for its degradation to [$^2H_4$]anthranilic acid before the onset of roquefortine biosynthesis.

Our observation that the 5a-proton of both roquefortine and aszonalenin derives from L-tryptophan has important implications for the mechanism of introduction of the 1,1-dimethylallyl substituent during the biosynthesis of these compounds. In particular this result precludes the involvement of a free 2-substituted indole[2,3] as an intermediate in the biosynthesis of either metabolite. Furthermore the suggestion of an intermediate enzyme-bound 2-substituted indole[6] now appears implausible in the absence of a mechanism which could account for overall retention of the 2-proton of tryptophan.

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‡ The extent of deuterium enrichment at positions 2, 4, 5, 6 and 7 in the labelled L-tryptophan was estimated as 97, 86, 99, 96 and 93% respectively by integration of residual protium in the ^1H NMR spectrum.

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