Biosynthesis of the Polyketide Mellein from CD_3CO_2H and $^{13}CD_3CO_2H$ in *Aspergillus melleus*: Detection of Deuterium by ^2H N.M.R. and Edited ^{13}C N.M.R. Spectra

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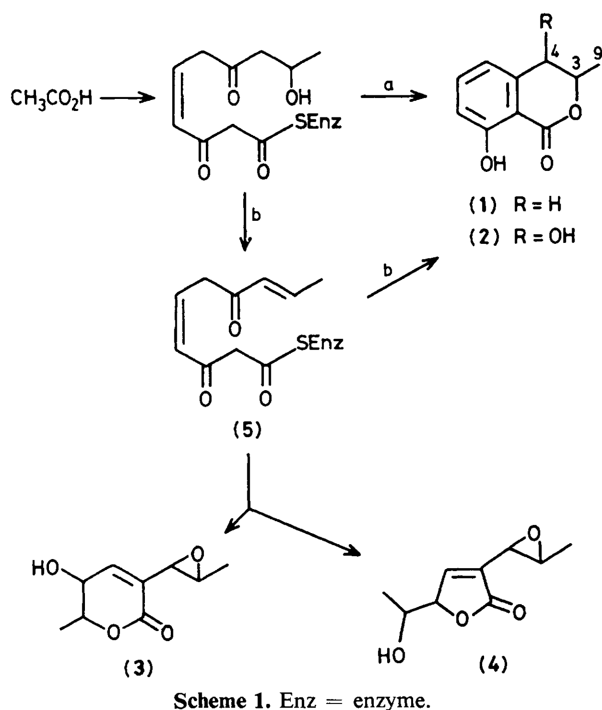
The pattern of incorporation of deuterium into mellein from CD_3CO_2H and $^{13}CD_3CO_2H$ was investigated by ^2H n.m.r. and edited ^{13}C n.m.r. spectra; the deuterium incorporated into the C-4 methylene group was found to be evenly distributed between the two diastereoisotopic positions and molecules were detected which retained two deuteriums at that site.

The fermentation products of *Aspergillus melleus* include the four pentaketides: mellein (**1**), hydroxymellein (**2**), aspyrone (**3**), and asperlactone (**4**). Evidence that (**3**) and (**4**) are probably derived from a common linear polyketide precursor such as (**5**) has been obtained from incorporation experiments using CD_3CO_2H and $^{13}CD_3CO_2H$ as precursors.[1,2] In addition, the origin of the carbon skeleton of mellein has been established by feeding with [1,2-$^{13}C_2$]acetate.[2]

The biosynthetic sequences leading to the pairs of metabolites (**1**) and (**2**), or (**3**) and (**4**) may branch from a common sequence of enzyme-bound intermediates in the early steps. In this paper we report the results of further studies of mellein biosynthesis using [2-$^2H_3$]- and [2-^{13}C,2-$^2H_3$]-acetates which test the possible intermediacy of an arylalkene derived from (**5**) in the biosynthesis of the aromatic compounds (Scheme 1, path b). Quantitative aspects of deuterium retention are studied by the complementary techniques of ^2H n.m.r. spectroscopy and a novel refinement of the α-shift technique which exploits edited as well as standard ^{13}C n.m.r. spectra. In an earlier study[3] the overall distribution of deuterium in mellein derived from CD_3CO_2H was determined by ^2H n.m.r. spectroscopy and the relative distribution in the aryl ring was interpreted in terms of hypothetical general mechanisms of cyclisation on polyketide synthases.[3] Here the point of interest is the distribution of deuterium in the side chain. In particular it was important to know if there are molecules which retain two



Scheme 1. Enz = enzyme.

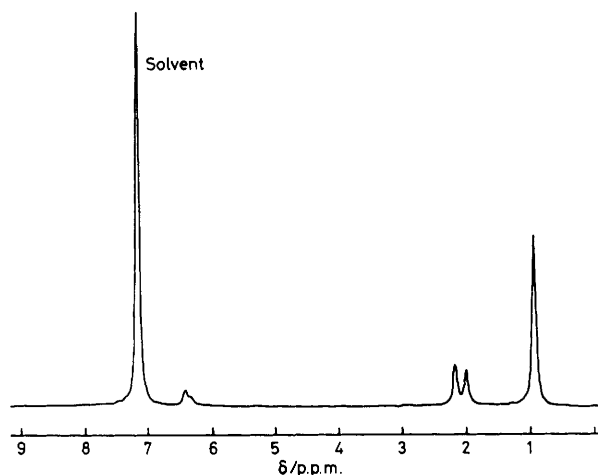


Figure 1. 61 MHz ^{2}H N.m.r. spectrum of the *O*-methyl ether of mellein produced from CD_3CO_2H (solvent C_6H_6).

deuteriums simultaneously at C-4, and also if there is any stereoselectivity in the retention of deuterium in those molecules which retain one deuterium at that site.

The second point was investigated by methylating the mellein derived from CD_3CO_2H and then running the ^{2}H n.m.r. spectrum of the product, *O*-methylmellein, in benzene. Under these conditions the diastereoisotopic hydrogens at C-4 are nonequivalent and the signals are well resolved (2.0 and 2.2 p.p.m.). The distribution of deuterium over the two sites was equal (Figure 1). This distribution of deuterium, together with the relatively high retention at the C-4 methylene compared with the chain starter methyl group (3.5 : 7.3), is consistent with a significant proportion of molecules being doubly-labelled with deuterium in the methylene group. This information is crucial since molecules doubly-labelled at that site would not be expected from path b (Scheme 1) in which (**5**) would be a precursor of mellein.

To provide a rigorous test of this possibility an experiment based on the α-shift technique was carried out. In this, deuteriums attached to ^{13}C nuclei are detected by isotopically shifted resonances in the ^{13}C n.m.r. spectra. The aliphatic region (3000 Hz spectral width) of the {^{1}H,^{2}H} decoupled ^{13}C n.m.r. spectrum of mellein derived from $^{13}CD_3CO_2H$ is shown in Figure 2(a). Note that in addition to the ^{13}C–H signals (1 and 4, δ 34.4 and 19.0 p.p.m.) there are two isotopically shifted resonances for C-4 (2 and 3, 0.4 and 0.7 p.p.m. upfield) and three for C-9 (5, 6, and 7, 0.2, 0.5, and 0.7 p.p.m. upfield). The shifted resonances for C-4 establish that there are molecules bearing one and two deuteriums, respectively, in the methylene group.

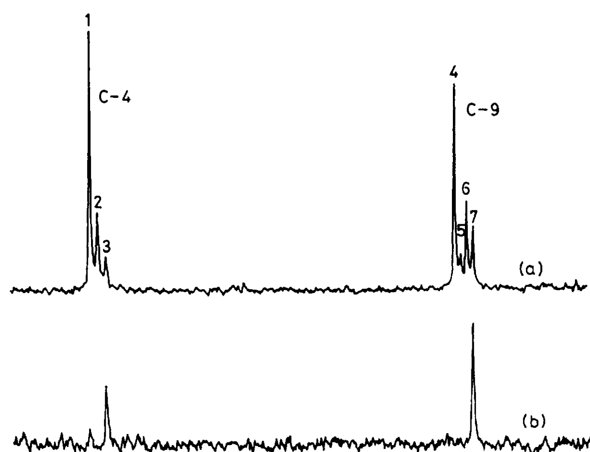


Figure 2. 100 MHz {^{1}H,^{2}H} Decoupled ^{13}C n.m.r. spectrum of mellein derived from $^{13}CD_3CO_2H$ in C_6F_6 (3000 Hz spectral width, δ values measured relative to C_6F_6 at δ 138.3 p.p.m.); (a) normal spectrum, (b) edited spectrum.

Peak 3 in Figure 2(a) was assigned to a CD_2 group on the basis of its isotopic shift (0.7 p.p.m.). It is now possible to confirm this assignment (and that of peak 7 to a CD_3 group) by running an edited spectrum using one of our recently published pulse sequences to suppress signals from protonated carbons.[4] The resultant subspectrum is shown in Figure 2(b). As expected only two resonances are observed, one for the CD_2 group at C-4 and the other for the CD_3 group at C-9. This demonstrates the feasibility of using spectral editing techniques for aiding the detection and assignment of isotopically shifted ^{13}C signals in deuteriated molecules at the level of isotopic enrichment attainable in a typical biosynthetic experiment.

The following conclusions can be drawn from the fact that in some molecules two deuteriums are retained at C-4. First, the carbon destined to become C-4 cannot have been part of a carbon–carbon double bond. Therefore (**5**) is not an intermediate in mellein biosynthesis and if there are intermediates in common from the early stages in the biosynthesis of all four metabolites (**1**)—(**4**), the branch point must occur before (**5**) (*e.g.* as in Scheme 1, path a). Secondly, the formation of the C-3–C-4 carbon–carbon bond in the chain building process on the polyketide synthase must take place with concomitant decarboxylation of the malonate unit undergoing addition. This is true of fatty acid biosynthesis[5] and a similar conclusion can be drawn for chain building steps in the biosynthesis of brefeldin A[6] and asperlactone.[1] In addition the finding that the deuterium label was equally distributed over the two diastereoisotopic sites at C-4 is noteworthy in the light of earlier suggestions that the distribution of deuterium in an equivalent malonate derived methylene of brefeldin A might be uneven and therefore that there might have been stereospecific exchange of deuterium from the methylene of malonate prior to its being added to the enzyme-bound polyketone chain.[6a] There is no evidence of this in mellein biosynthesis.

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