Biosynthesis of Monocerin. Incorporation of ^{2}H-, ^{13}C-, and ^{18}O-Labelled Acetates by *Drechslera ravenelii*

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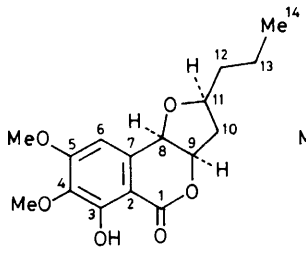
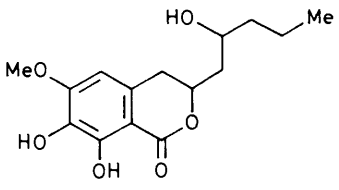
Incorporation of ^{2}H-, ^{13}C-, and ^{18}O-labelled acetates into monocerin (1) by cultures of *Drechslera ravenelii* and analysis of the enriched metabolites by ^{2}H and ^{13}C n.m.r. spectroscopy indicate a heptaketide origin; observation of ^{2}H and ^{18}O isotope shifts in the ^{13}C n.m.r. spectrum allows the fate of acetate-derived hydrogen and oxygen on incorporation into monocerin to be followed and conclusions on the mechanism of formation of the fused furobenzopyrone ring system to be drawn.

Monocerin (**1**) was first isolated as a compound active against powdery mildew of wheat from *Helminthosporium monoceras*.[1] It was subsequently isolated, along with the fusarentins [*e.g.*, (**2**)], a group of related compounds with insecticidal activity, from *Fusarium larvarum*;[2] and from *Readeriella mirabilis*.[3] We have also isolated monocerin from *Drechslera ravenelii* in the course of biosynthetic studies on ravenelin.[4] Monocerin had previously been assigned the 8*R*,9*R* configuration but the configuration at C-11 was uncertain.[2] Analysis of the 360.13 MHz ^{1}H n.m.r. spectrum, Figure 1(a), and difference n.O.e. studies have allowed a full assignment of the spectrum and indicate the *S* configuration at C-11.† The structure of monocerin suggests a polyketide origin and we now report incorporation studies with ^{13}C-, ^{2}H-, and ^{18}O-labelled acetates which confirm a heptaketide origin, suggest a mechanism of formation of the fused furobenzopyrone system, and provide information of more fundamental significance on reduction and deoxygenation processes in polyketide biosynthesis.

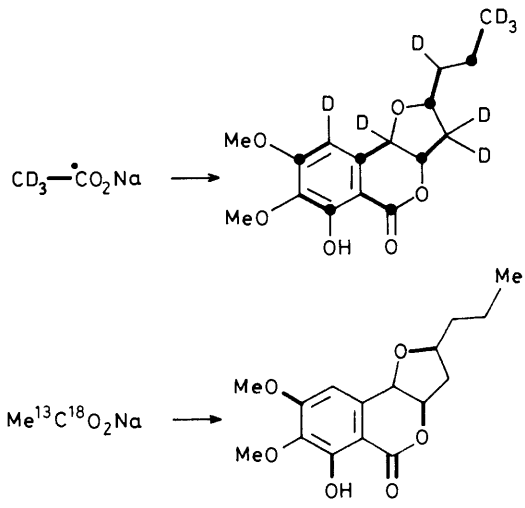


(1)

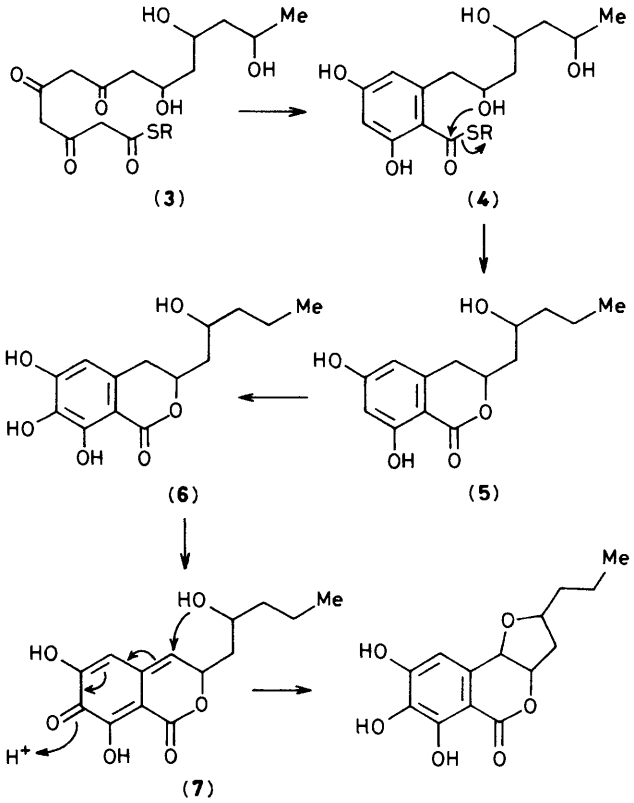


(2)

† Full details of the ^{1}H and ^{13}C n.m.r. spectral assignments and isotope shifts will be given in the full paper.



Scheme 1



Scheme 2

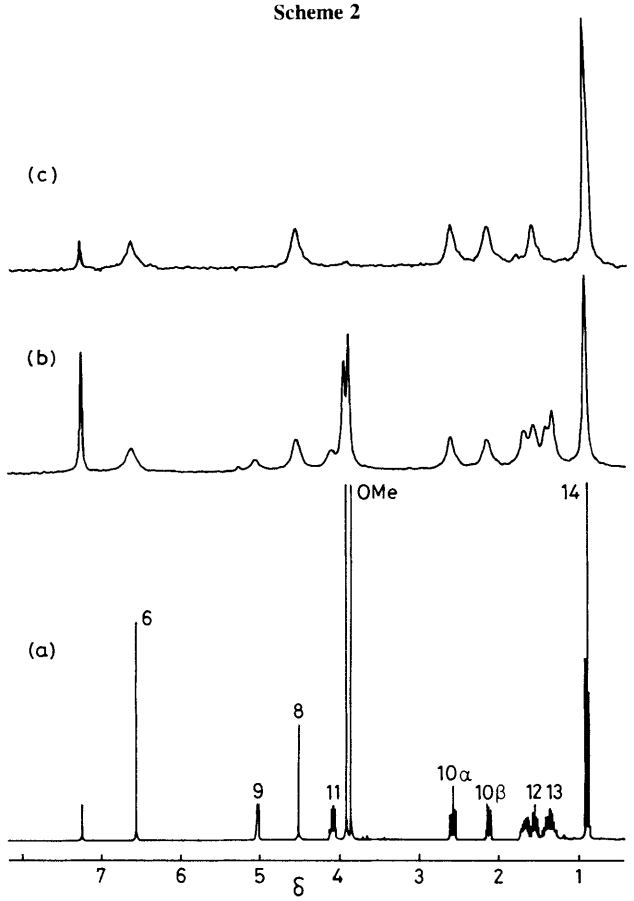


Figure 1. (a) 360.13 MHz ^{1}H N.m.r. spectrum of monocerin; (b) 55.28 MHz ^{2}H n.m.r. spectrum of universally ^{2}H enriched monocerin; and (c) 55.28 MHz ^{2}H n.m.r. spectrum of [1-^{13}C,$^{2}H_3$]acetate-enriched monocerin.

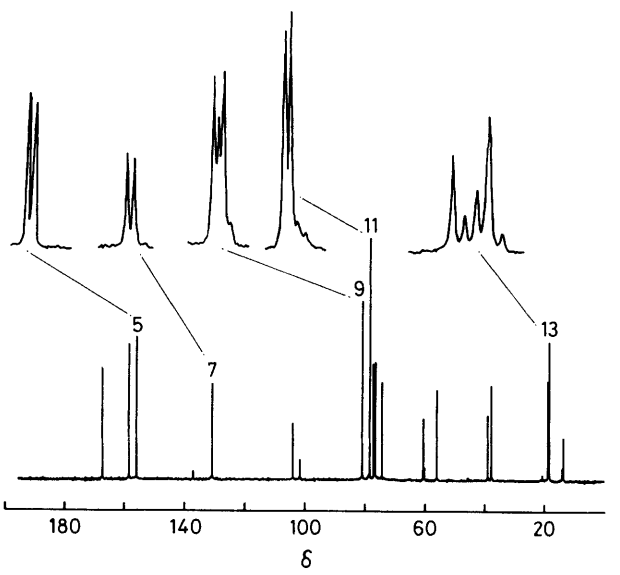


Figure 2. 90.56 MHz Proton noise-decoupled ^{13}C n.m.r. spectrum of [1-^{13}C,$^{2}H_3$]acetate-enriched monocerin in $CDCl_3$.

Incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]-acetates into monocerin by cultures of *D. ravenelii* and analysis of the ^{13}C n.m.r. spectra of the enriched metabolites showed that acetate was incorporated with high efficiency to give the labelling pattern summarised in Scheme 1. The 55 MHz ^{2}H n.m.r. spectrum of [U-^{2}H]monocerin‡ showed, Figure 1(b), that all the ^{2}H resonances including the diastereotopic hydrogens on C-10, C-12, and C-13 were resolvable. Incorporation of [1-^{13}C,$^{2}H_3$]acetate into monocerin and determination of the ^{2}H n.m.r. spectrum showed, Figure 1(c), that ^{2}H was incorporated into the 14-methyl group and at an essentially equal level at H-6, H-8, both H-10α and H-10β, and only one of the diastereotopic C-12 hydrogens. The equal incorporation at both diastereotopic positions on C-10 strongly suggests that the molecules are doubly labelled, but as retention of two acetate-derived hydrogens at a methylene is an unusual observation in polyketide biosynthesis[5] we have used the β-shift method[6] to confirm this.

The ^{13}C n.m.r. spectrum of the [1-^{13}C,$^{2}H_3$]acetate-derived metabolite (Figure 2) shows isotopically shifted resonances on C-13, C-11, C-9, C-7, and C-5 indicating respectively the retention of up to three acetate-derived hydrogens on C-14 thereby confirming it as a 'starter' unit;[7] it also shows two acetate-derived hydrogens on C-10, and one acetate-derived hydrogen on C-12, C-8, and C-6. Incorporation of [1-^{13}C,$^{18}O_2$]acetate and ^{13}C n.m.r. analysis of the enriched metabolite showed isotopically shifted resonances[8] for C-1, C-3, C-5, C-9, and C-11 (Δδ, 0.03, 0.01, 0.01, 0.03, and 0.03 p.p.m. respectively) indicating that the corresponding carbon–oxygen bonds had remained intact throughout the biosynthetic pathway. These ^{2}H and ^{18}O labelling patterns are summarised in Scheme 1.

The above results enable us to make the following conclusions about the main events occurring during the biosynthesis of monocerin. (a) The retention of two acetate-derived hydrogens at C-10 suggests that reduction of the β-ketoacyl intermediates to the corresponding β-hydroxyacyl intermediates takes place during chain assembly before significant loss through exchange processes can occur,[5a] *i.e.* the trihydroxy moiety (**3**) is a likely enzyme-bound polyketide precursor. In agreement with observations in fatty acid biosynthesis[9] and

‡ Prepared by producing the metabolite in a medium supplemented with 5% $^{2}H_2O$.[12]

mellein biosynthesis[5c] the carbon–carbon bond formation in the chain assembly process probably occurs with concomitant decarboxylation of the malonyl CoA unit which is added. (b) The loss of oxygen from C-13 presumably occurs by an elimination-reduction sequence analogous to fatty acid biosynthesis.[10] Since only one of the diastereotopic hydrogens on C-12 is labelled the process is clearly stereospecific, but the absolute stereochemistry of the process is as yet uncertain. (c) The benzopyrone ring must be formed by nucleophilic attack at the terminal carboxy moiety by a hydroxy group on C-9. It is likely that the cyclisation takes place on the thioester (**4**), to give (**5**) as the first enzyme-free intermediate. (d) The retention of the carbon–oxygen bond at C-11 indicates that the tetrahydrofuran ring is formed by attack of a C-11 hydroxy function at C-8. A mechanism for this consistent with the observed ^{2}H and ^{18}O labelling would be nucleophilic addition onto a quinonemethide intermediate (**7**) formed (Scheme 2) by oxidation of (**6**), the hydroxylated derivative of (**5**). A similar ring closure mechanism has been proposed in granaticin biosynthesis,[11] and is supported by the co-occurrence of monocerin and fusarentin methyl ether (**2**) in *F. larvarum*.[2]

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