Biosynthesis of LL-D253α in *Phoma pigmentivora*. Incorporation of ^{13}C, ^{2}H, and ^{18}O Enriched Precursors

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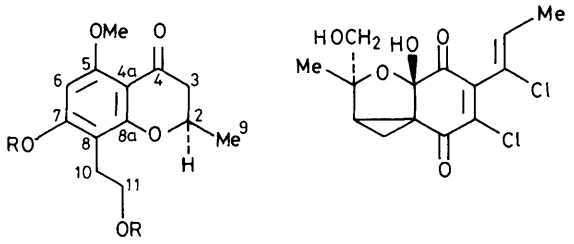
The incorporation of ^{13}C, ^{2}H, and ^{18}O labelled acetates and $^{18}O_2$ gas into LL-D253α (**1**), a chromanone metabolite of *Phoma pigmentivora*, and analyses of the enriched metabolites by ^{13}C and ^{2}H n.m.r. and mass spectroscopy indicate its formation from two preformed polyketide chains; evidence for the mechanism of formation of the chromanone ring is presented, and a cyclopropyl intermediate is proposed to account for the unique randomisation of label observed in the hydroxyethyl side chain.

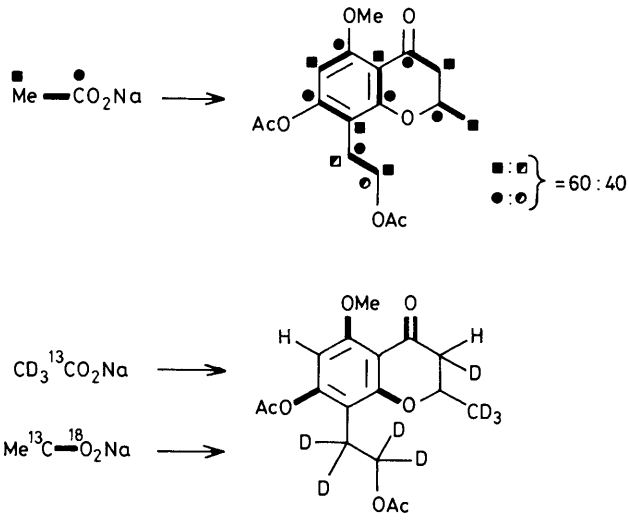
LL-D253α (**1**), a metabolite of *Phoma pigmentivora*, is a chromanone with an unusual hydroxyethyl substituent.[1] Structural analysis strongly suggests a polyketide derivation for the chromanone nucleus but the origin of the C_2-side chain is obscure. Possible routes include: (a) condensation of two preformed polyketide chains;[2] or elaboration of the side chain onto a preformed pentaketide-derived precursor either by (b) *C*-acylation,[3] or (c) stepwise introduction of two C_1 equivalents,[4] or (d) introduction and oxidative cleavage of a prenyl substituent.[5] Since all of these represent unusual routes in fungal polyketide biosynthesis, we have carried out incorporation studies with ^{13}C, ^{2}H, and ^{18}O labelled acetates and $^{18}O_2$ to identify the correct pathway.

Incorporation of [$^{13}C_2$]acetate indicated that all the skeletal



(1) R = H
(2) R = Ac

(6)



Scheme 1

carbons were derived from intact acetate units as shown in Scheme 1. It is noteworthy that no randomisation of labelling was observed in the phloroglucinol ring suggesting that it cannot have been symmetrically substituted at any point in the biosynthetic pathway. The results of incorporating [1-^{13}C]- and [2-^{13}C]-acetates are particularly interesting. As anticipated C-2, C-4, C-5, C-7, C-8a, and C-10 were all highly enriched by [1-^{13}C]acetate. However C-11 also showed significant enrichment, the combined enrichment at C-10 and C-11, and that at C-2 being significantly higher than that at the other enriched sites. Analogous results were obtained with [2-^{13}C]acetate but now C-11 was more highly enriched than C-10.† Incorporation of label from [5-^{14}C]mevalonic acid was negligible thereby rendering route (d), our initially favoured pathway, unlikely.

The fate of acetate-derived hydrogen can be studied by incorporation of [1-^{13}C,^{2}H$_3$]acetate and detection of deuterium-induced β-isotope shifts in the ^{13}C n.m.r. spectrum of the enriched metabolite.[6,7] Figure 1 shows the ^{13}C n.m.r. spectrum of [1-^{13}C,^{2}H$_3$]acetate-enriched LL-D253α diacetate (**2**). Thus C-2 shows isotopically shifted resonances corresponding to the incorporation of one, two, and (mainly) three deuterium atoms at C-9, indicating its origin from an acetate

† The relative enrichments at C-10 and C-11 are consistent with label from C-1 or C-2 of acetate being randomised to the extent of *ca.* 80% between C-10 and C-11, *e.g.* in the [1-^{13}C]acetate feed C-10 has 60% of the label and C-11 40%.

Table 1. ^{18}O Isotopically shifted resonances observed in the 100.6 MHz ^{13}C n.m.r. spectrum of LL-D253α diacetate (**2**).

Carbon	δ (p.p.m.)	Δδ (p.p.m. × 100)	Ratio ^{16}O : ^{18}O[c]
4	190.1[a]	4.1	— (90 : 8)
8a	162.1[a]	1.6	80 : 20 (80 : 20)
5	160.0[a]	1.7	78 : 22 (75 : 25)
7	155.1[a]	2.1	84 : 16 (83 : 17)
11	62.8[b]	2.7	86 : 14 (85 : 15)

[a] [1-^{13}C,^{18}O$_2$]Acetate-enriched. [b] ^{18}O$_2$-Enriched. [c] Values in parentheses are from DuPont Curve Resolver.

'starter' unit;[8] C-4 shows one downfield shifted resonance,[7] corresponding to the incorporation of one deuterium atom at C-3; and finally both C-10 and C-11 each showed two isotopically shifted resonances corresponding to the incorporation of two deuterium atoms at C-11 and C-10 respectively. All these results are summarised in Scheme 1.

On incorporation of [1-^{13}C,^{18}O$_2$]acetate, the ^{13}C n.m.r. spectrum of (**2**) showed isotopically shifted resonances for C-4, -5, -7, and -8a, indicating that the oxygens attached to these carbons are acetate-derived, and therefore that the corresponding carbon–oxygen bonds had remained intact throughout the course of the biosynthesis (Table 1).[9] These results and the incorporation of only one deuterium at C-3, indicate that the chromanone ring is formed by conjugate addition of a phenolic hydroxy group to the corresponding α,β-unsaturated ketone. LL-D253α has the 2*R* configuration[10] so the ring closure process is stereospecific with respect to C-2. To examine the stereospecificity with respect to C-3 the ^{2}H n.m.r. spectrum of [^{2}H$_3$]acetate-enriched LL-D253α diacetate was examined. The axial and equatorial hydrogens at C-3 have almost coincident chemical shifts and even at high field strengths form part of a complex ABX system. However on addition of Eu(fod)$_3$ (fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionate), the induced shifts in the 360 MHz ^{1}H n.m.r. spectrum allow the resonances to be resolved and assigned from their coupling constants to the axial and equatorial hydrogens. Determining the ^{2}H n.m.r. spectrum of [U-^{2}H]-LL-D253α‡ under the same conditions confirmed that the corresponding ^{2}H resonances could also be resolved. Finally repeating the experiment with the [^{2}H$_3$]acetate-enriched compound showed that both positions were enriched to equal extents so that protonation of the intermediate enolate must proceed with equal facility from both sides of the molecule (Scheme 2). This contrasts with the corresponding chalcone to flavanone ring closure which has been shown to be completely stereospecific.[11]

The observed randomisation of label between C-10 and C-11 indicates that these carbons may become equivalent during the biosynthesis. To account for this and the lack of randomisation of label in the phloroglucinol ring we propose the pathway shown in Scheme 3. Two preformed polyketide chains, either a C_4 plus C_8 as shown, or C_6 plus C_6, or C_2 plus C_{10}, probably condense before aromatisation of ring A. The transposition of oxygen in the C_2-side chain and randomisation of label can then be explained by reduction and elimination (*cf.* fatty acid biosynthesis) to give the vinyl

‡ Prepared by fermentation of *P. pigmentivora* in a medium supplemented with 5% $^{2}H_2O$.[13]

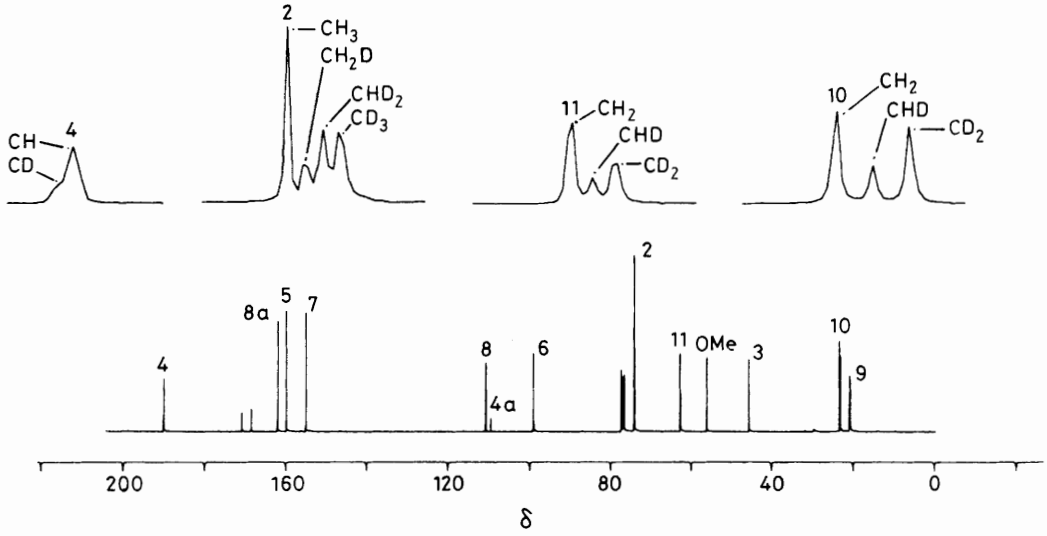
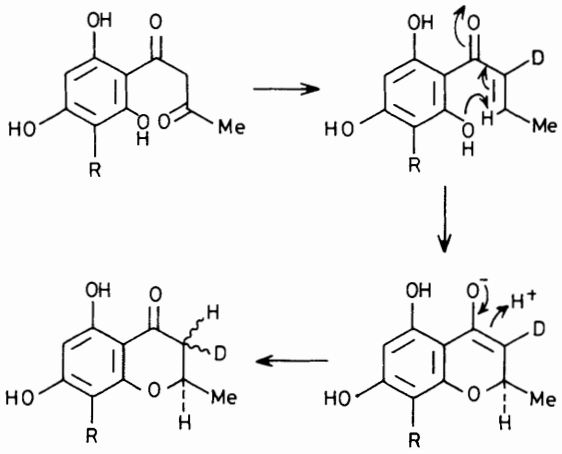
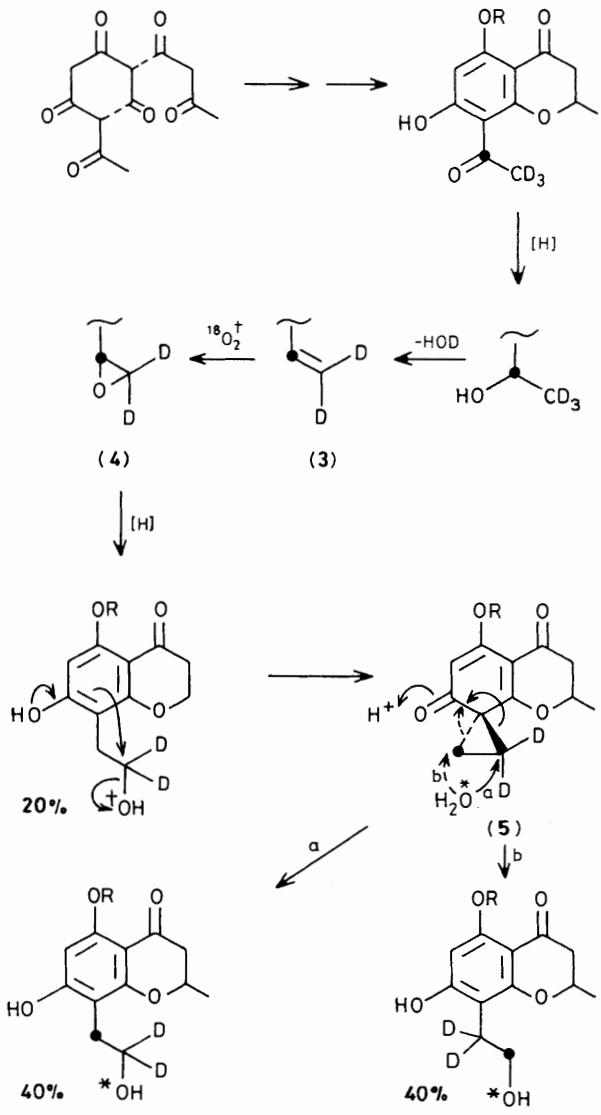


Figure 1. 90.56 MHz Proton noise-decoupled ^{13}C n.m.r. spectrum of [1-^{13}C,$^{2}H_3$]acetate-enriched LL-D253α diacetate in $CDCl_3$.



Scheme 2

intermediate (**3**). Epoxidation and reductive opening of the epoxide (**4**) would furnish the hydroxyethyl moiety directly. To account for the observed 80% randomisation of label between C-10 and C-11 we propose the involvement of a cyclopropyl intermediate (**5**) which would be formed by participation of the phloroglucinol ring in expulsion of the hydroxy group at C-11. Hydrolytic opening of the resulting cyclopropyl ring at either the α or β carbons would then produce the observed randomisation of labelling between C-10 and C-11 in (**1**). An analogy for this process is found in the mould metabolite mikrolin (**6**) which contains a cyclopropyl ring fused to a cyclohexadienone moiety.[12] The observed degree of randomisation requires that 20% of the natural product is derived directly from reduction of the epoxide (**4**). Support for this was provided by carrying out a fermentation of *P. pigmentivora* under an atmosphere of $^{18}O_2$. The mass spectrum of the derived LL-D253α shows an $M + 2$ peak, corresponding to 15% of the metabolite, which mass matches for $C_{13}H_{16}{}^{16}O_4{}^{18}O$. In addition, examination of the ^{13}C n.m.r. spectrum for isotope shifts determined that, within experimental error, all of the ^{18}O was located at C-11 (Table 1). At present we cannot say with certainty whether the randomisation is an *in vitro* or *in vivo* process. However [10-$^{2}H_2$]-LL-D253α has been prepared and no randomisation



Scheme 3

of label between C-10 and C-11 is observed on either mild acid or mild base treatment.

We thank the S.E.R.C., NATO, and the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support.

Received, 5th March 1984; Com. 296

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