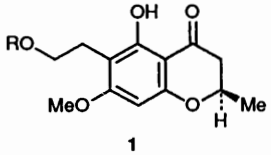
Biosynthesis of LL-D253α, a Polyketide Chromanone Metabolite of *Phoma pigmentivora*: Incorporation of ^{13}C, ^{2}H and ^{18}O Labelled Precursors

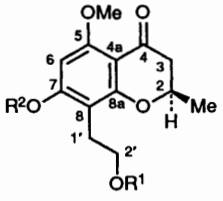
I. Michael Chandler, C. Rupert McIntyre and Thomas J. Simpson *,†
Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, UK

The incorporation of ^{13}C, ^{2}H and ^{18}O labelled acetates and $^{18}O_2$ gas into LL-D253α, a chromanone metabolite of *Phoma pigmentivora* and other fungi, and analysis of the enriched metabolites by ^{13}C and ^{2}H NMR spectroscopy and mass spectrometry indicates its formation *via* two preformed polyketide chains. Evidence is presented for the mechanism of formation of the chromanone ring and a cyclopropyl intermediate is proposed to account for the unexpected randomisation of label observed in the hydroxyethyl side chain. A pathway is proposed to explain the occurrence of the co-metabolites LL-D253β and γ.

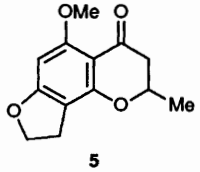
LL-D253α is an antibiotic chroman-4-one metabolite isolated from *Phoma pigmentivora* and other fungi. It was originally assigned the structure **1**, in which the C_2 side-chain was positioned at C-6 on the chromanone skeleton.[1,2] However, on the basis of spectroscopic and synthetic studies, the structure



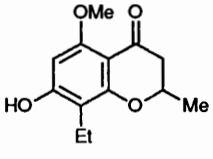
1



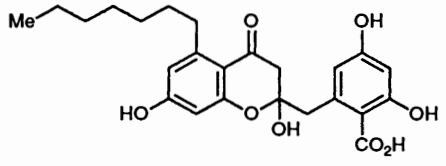
2 $R^1 = R^2 = H$
3 $R^1 = R^2 = Ac$
4 $R^1 = Ac$, $R^2 = H$



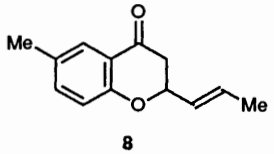
5



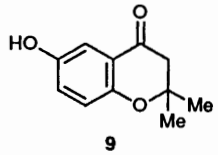
6



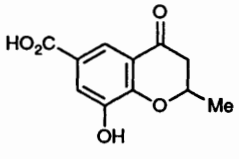
7



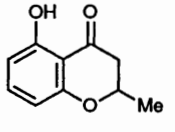
8



9



10

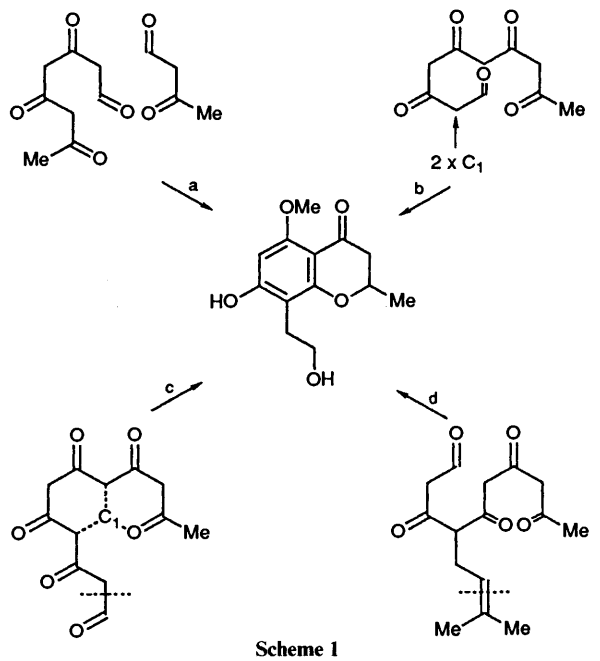


11

has been revised to **2** with the side-chain at C-8 of the chromanone nucleus.[3] Although it has not been explicitly proved, the structures of the known co-metabolites[1,4] of LL-D253α, which have modified C_2 side-chains should be reassigned to structures **4**–**6**. Structural analysis suggests that the chromanone nucleus is polyketide derived but the origin of the unusual hydroxyethyl side-chain is obscure. Naturally occurring chroman-4-ones, in contrast to the related flavanones,[5] appear to comprise a rather small group of compounds, and only a few of these, *e.g.* compounds **7**–**11** are fungal metabolites.[6] Biosynthetic studies of chroman-4-ones are scarce, but incorporation of [1-^{14}C]-acetate into 5-hydroxy-2-methylchroman-4-one **11** in cultures of *Daldinia concentrica* confirmed its polyketide origin.[7]

The carbon skeleton of LL-D253α **2** cannot be formed by simple folding of a single polyketide intermediate. A number of possible biosynthetic origins, for which some precedent exists, can be proposed and some of these are summarised in Scheme 1. The skeleton could be formed by a number of different condensations of two preformed polyketide chains. One possibility is indicated in path a. Two chain pathways have been established in a limited number of cases, *e.g.* radicinin (see below).[8] In path b the side-chain is formed by two successive *C*-methylations of a pentaketide precursor which itself could fold in two possible ways. The 1-hydroxyethyl side-chain of pactamycins[9] and *C*-ethyl groups in steroidal side-chains are known to be formed in this way.[10] A further possibility would be addition of a C_1 unit to a hexaketide chain (path c). Initial attachment could occur on any one of three chain positions. The introduction of a C_1 unit into a carbocyclic ring is uncommon but a similar process is found in the biosynthesis of the mycotoxin diplosporin, in *Diplodia mayis*,[11] and carbons derived from C_1-units are also found in heterocyclic rings, *e.g.* in rotenones and the berberine alkaloids.[12] The oxidation levels of the side-chain suggested a very likely pathway is that illustrated in path d where a prenyl unit is attached to a pentaketide precursor and oxidative cleavage then gives the side-chain. Mycophenolic acid is known to be formed in an analogous manner from 6-farnesyl-5,7-dihydroxy-4-methylphthalide[13] and the two carbons of the furanoid moiety in furanocoumarins are known to be derived from cleavage of a dimethylallyl substituent.[12] 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 precursors to identify the correct pathway. Full details of these studies are now reported.[14]

† *Present address:* School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS.



Scheme 1

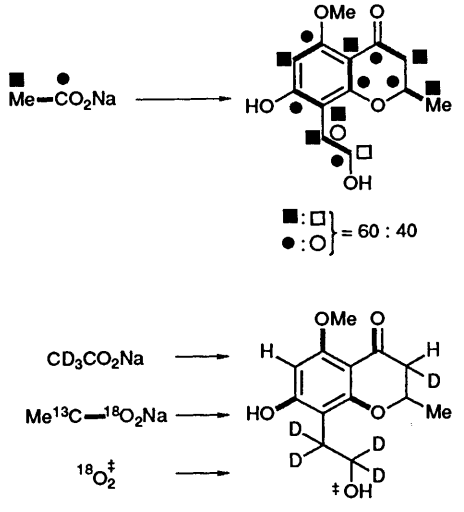


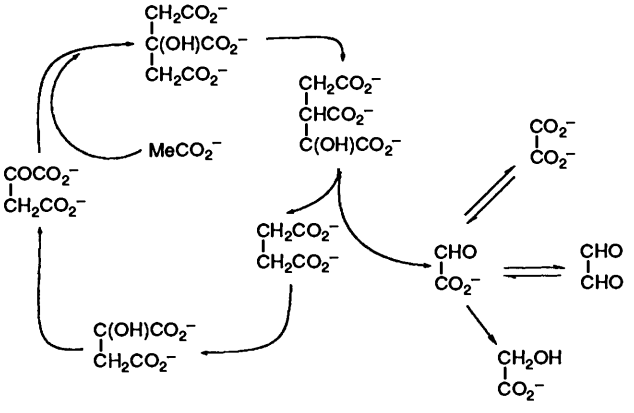
Fig. 1 Labelling patterns in LL-D253α from ^{13}C, ^{2}H and ^{18}O labelled acetates

LL-D253α can be isolated in high yield (200–400 mg dm^{-3}) from shake flask cultures of *P. pigmentivora*. Preliminary experiments with ^{14}C-labelled acetate indicated that high enrichment levels could be readily obtained and that the optimum time of feeding labelled precursors was *ca.* 60 h after inoculation of the cultures. A dilution value of 17 for each labelled site, corresponding to an expected ^{13}C-enrichment of 6% was observed.[15] Therefore [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetates were fed and the proton noise decoupled (PND) ^{13}C NMR spectra of the enriched metabolites were determined. These were initially determined in [$^{2}H_6$]-DMSO solution and a number of problems were immediately apparent. It was evident that high enrichments had been achieved, but the spectra from feeding both singly and doubly labelled acetates showed extensive evidence for *inter* acetate unit ^{13}C-^{13}C couplings. This has been observed in previous studies, *e.g.* of griseofulvin,[16] and is due to inhibition of the *in vivo* production of acetyl CoA by the fungus, so that the endogenous, labelled acetate gives very high specific enrichment. In addition, the spectrum of LL-D253α itself showed a number of overlapping signals, *e.g.* C-4a and C-8 and several ^{13}C-^{13}C couplings were of a similar size which made the identification of intact acetate units problematic. These problems were readily overcome by repeating the feeding experiments with a 1:2 mixture of ^{13}C-labelled and unlabelled acetates and determining the PND ^{13}C NMR spectra of the enriched metabolites as their diacetates. The ^{13}C NMR spectrum of LL-D253α diacetate **3** has been rigorously assigned.[3] The enrichments and ^{13}C-^{13}C couplings observed are summarised in Table 1 and Fig. 1.

The incorporation of [1,2-$^{13}C_2$]acetate indicated that the skeleton of LL-253α was derived from six intact acetate units as shown in Fig. 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 two carbons of the side-chain are derived from an intact acetate unit. However, the results of incorporating [1-^{13}C]- and [2-^{13}C]-acetates were particularly interesting. As expected for a polyketide origin, C-2, C-4, C-5, C-7 and C-8a were all enriched to a similar extent (mean enrichment of 1.5%). However, both C-1′ *and* C-2′ were enriched, C-1′ somewhat more so than C-2′, but the combined enrichment at these two sites was equal to the average enrichment at the other sites labelled by [1-^{13}C]acetate. Exactly analogous results were obtained from [2-^{13}C]acetate, but now C-2′ was more highly enriched than C-1′.

These results clearly rule out pathways b and c in Scheme 1 which require partial derivation of the skeleton from the C_1-pool. Our initially favoured route, path d, was also ruled out from results of an incorporation experiment with [5-^{14}C]-mevalonate which showed negligible incorporation of label (Table 2), so making derivation of the side-chain from cleavage of a prenyl residue unlikely.

The partial randomisation of acetete-derived label in the side-chain was unexpected and difficult to rationalise. Although the overall level of acetate incorporation in the side-chain was identical with that in the chromanone nucleus, one possible explanation for the randomisation of labelling and retention of coupling was the involvement of a symmetrical C_2 unit derived from glyoxylate *via* the glyoxylic acid cycle. This is an anaplerotic pathway within the tricarboxylic acid cycle and only occurs in plants and microorganisms.[17] Isocitrate is split into succinate and glyoxylate in such a way that the glyoxylate is derived from an intact acetate unit (Scheme 2). Reduction of glyoxylate to glyoxal or oxidation to oxalate would give a symmetrical intermediate which might be the source of the hydroxyethyl group. This would not in itself completely explain the *partial* randomisation, but if the immediate precursor, although itself not symmetrical, *e.g.* glycollate or glyoxylate, was in equilibrium with a symmetrical species such as oxalate, then an explanation would be available. Thus the incorporation of both [1-^{14}C]glycollate and [^{14}C]oxalate was examined. Although the former gave negligible incorporation (Table 2) oxalate was incorporated with high efficiency (4.2%). However,



Scheme 2

Table 1

Carbon	$\delta(2)^a$	$\delta(3)^d$	Enrichments	J_{CC}/Hz[g]	$\Delta\delta^h(^{18}O:^{16}O)$	$\Delta\delta^h$
2-CH_3	20.5	20.0	1.6[e]	40.0		26.9, 54.0, 81.1[l]
2	73.3	73.6	1.5[f]	40.0		4, 8, 13 (50:11:20:19)[k]
3	45.2	44.9	1.4[e]	40.9		32.6, 36.9[l]
4	188.5	189.4	1.5[f]	41.1	4.4 (94:6)[i]	
4a	104.5[b]	108.8	1.4[e]	64.9		
5	159.9[c]	159.3	1.2[f]	69.0	1.7 (83:17)[i]	
6	92.7[b]	98.5	1.7[e]	68.9		29.8[l]
7	162.0[c]	154.5	1.2[f]	73.4	2.1 (81:19)[i]	
8	104.7[b]	110.2	1.5[e]	72.6	1.6 (83:17)[i]	
8a	162.7[c]	161.5	1.1[f]	64.9		
1′	26.7	22.7	0.8,[f] 0.5[e]	37.6		27.8, 55.7[l]
						9, 18 (44:18:38)[k]
2′	60.1	62.2	0.5,[f] 1.1[e]	37.6	2.9 (85:15)[j]	30.4, 61.1[l]
						5, 11 (54:17:29)[k]
OCH_3	55.4	55.5				
$COCH_3$		168.0, 170.2				
$COCH_3$		20.2, 20.3				

[a] [$^2H_6$]-DMSO. [b,c] Assignments may be interchanged. [d] In $CDCl_3$. [e,f] From [2-^{13}C]- and [1-^{13}C]-acetate respectively, expressed in atom % (see ref. 15). [g] From [1,2-$^{13}C_2$]acetate diluted with unlabelled acetate. [h] Isotope-induced shifts expressed in ppm × 100; figures in parentheses indicate relative intensities of natural abundance and isotopically shifted signals. [i] From [1-^{13}C,$^{18}O_2$]acetate. [j] From $^{18}O_2$. [k] From [1-^{13}C,$^2H_3$]acetate. [l] From [2-^{13}C, $^2H_3$]acetate.

Table 2 Incorporation of ^{14}C-labelled precursors into LL-D253α

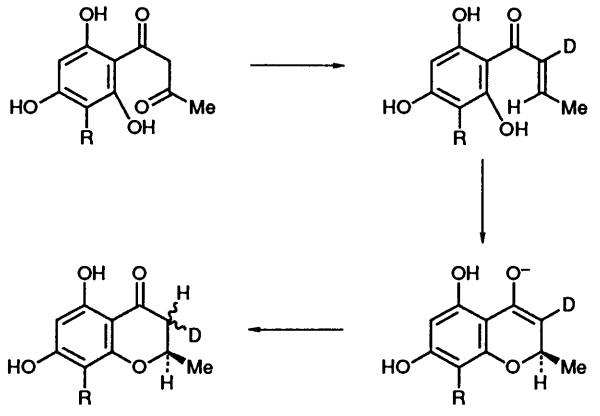
Precursor	Dilution	% Incorporation
[1-^{14}C]Acetate	2.8×10^5	7.2
[1-^{14}C]Glycollate	2.0×10^5	0.14
[U-^{14}C]Oxalate	1.3×10^5	4.2
[5-^{14}C]Mevalonate	1×10^7	0.01

all the label was shown to be present in the 5-methoxy group as demethylation of the enriched LL-D253α by boron trichloride resulted in loss of 98% of the total activity. It is known that oxalate can be decarboxylated to formate which will enter the C_1 pool and ultimately methionine.[18]

In order to obtain more information on the biosynthesis of LL-D253α the origins of the hydrogen and oxygen atoms were studied by the incorporation of [2-$^2H_3$]-, [1-^{13}C,2-$^2H_3$]-, [2-^{13}C,2-$^2H_3$]- and [1-^{13}C,1-$^{18}O_2$]-acetates. The isotope-induced shifts observed in the ^{13}C NMR spectra of the metabolites enriched by the doubly labelled acetates are shown in Table 1 and summarised in Fig. 1. Incorporation of [1-^{13}C,$^{18}O_2$]acetate results in isotopically shifted resonances for C-4, C-5, C-7 and C-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. The intensities of the isotopically shifted resonances for C-8a, C-5 and C-7 were essentially identical, but that for C-4 was much lower. Chemical exchange *via* the hydrated form of the carbonyl is probably responsible for the much greater loss of ^{18}O label from acetate seen at this position.

The isotope shifts observed in the spectrum of [1-^{13}C,2-$^2H_3$]acetate enriched LL-D253α indicate that mainly three deuterium atoms are incorporated at the 2-Me and that C-1′ and C-2′ may each carry two acetate-derived deuteriums. This result confirms that highly oxidised species such as glyoxylate or oxalate cannot be involved in the biosynthesis of the hydroxyethyl group. No isotope shift was apparent for incorporation of deuterium into the aromatic ring and C-4 showed a weak *downfield* shoulder which indicated some incorporation of deuterium at C-3. These results were confirmed by ^{2}H NMR analysis of the same enriched metabolite. The apparent lack of acetate-derived deuterium at C-6 was at first surprising. However, hydrogen label can be readily lost at such positions by enolisation at the polyketide intermediate stage.[19] In order to obtain information on the mechanism of formation of the chromanone ring it was important to know whether one or both hydrogens were acetate derived. It has been shown in previous studies that carbonyl groups are unsatisfactory for the observation of ^{2}H β-shifts (as result from the incorporation of [1-^{13}C,2-$^2H_3$]acetate).[20] Such shifts may be downfield or even zero in contrast to the upfield shifts normally observed. This can be overcome by incorporation of [2-^{13}C,2-$^2H_3$]acetate and observation of the resulting α-shifts.[21] Incorporation of [2-^{13}C,$^2H_3$]acetate into LL-D253α gave the shifts shown in Table 1. These were in agreement with those observed for [1-^{13}C,$^2H_3$]acetate. In this experiment a very small isotopically shifted signal was observed on C-6 to indicate that a small amount of acetate derived deuterium is retained in the aromatic ring. However, C-3 now showed two similar isotopically shifted signals which suggested that both the axial and equatorial hydrogens were labelled. No shift was observed corresponding to the *simultaneous* incorporation of two deuteriums at C-3. The oxygen and hydrogen labelling results are summarised in Fig. 1.

These indicate that the chromanone ring can be formed by conjugate addition of a phenolic hydroxy group to the corresponding αβ-unsaturated ketone as indicated in Scheme 3. As LL-D253α has the *R* configuration at C-2, the ring closure is stereospecific at C-2 and presumably enzyme mediated. Protonation of the intermediate enolate might be expected also to be stereospecific as in the closely analogous chalcone to flavanone ring closure where addition occurs with overall *syn* stereochemistry.[22] In order to clarify the stereospecificity of the ring closure with respect to C-3, the ^{2}H NMR spectrum of [$^2H_3$]acetate-enriched LL-D253α diacetate has been examined in detail. The axial and equatorial hydrogens at C-3 have almost coincident chemical shifts and even at highfield strengths form part of a complex ABX system. Fig. 2 shows the ^{1}H NMR spectrum of LL-D253α diacetate and the effect of adding the lanthanide shift reagent, Eu(fod)$_3$. At 0.3 mol equiv. of Eu(fod)$_3$, the C-3 hydrogens are well resolved, appearing at 3.58 ppm (dd, *J* 16.5, 2.5 Hz) and 3.94 ppm (dd, *J* 16.5, 13.5 Hz). These couplings are only consistent with a conformation in which the 2-methyl group occupies the equatorial position. As the 3β hydrogen possesses a *trans*-diaxial relationship to the 2α-hydrogen, it is assigned to the downfield signal at 3.94 ppm which shows the large vicinal coupling of 13.5 Hz.



Scheme 3

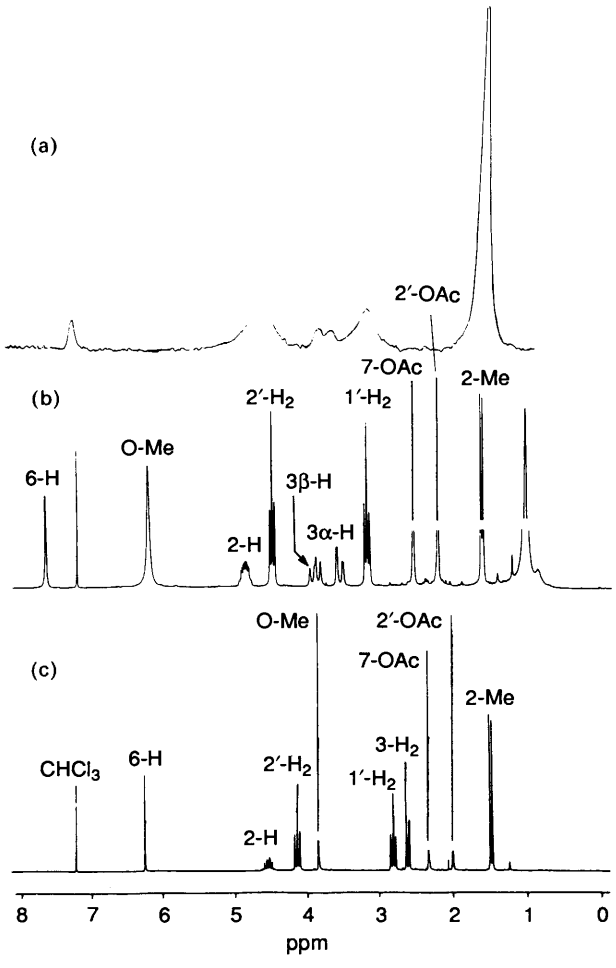
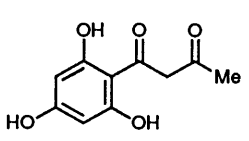


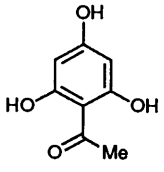
Fig. 2 200 MHz ^{1}H NMR spectra of LL-D253α diacetate (a) in $CDCl_3$ and (b) in the presence of 30 mol % Eu(fod)$_3$ (c) 55.3 MHz ^{2}H NMR spectra of LL-D253α diacetate enriched from [$^2H_3$]acetate in $CHCl_3$ in the presence of 30 mol % Eu(fod)$_3$

A generally deuterium-enriched sample of LL-D253α was prepared by the simple expedient of growing *P. pigmentivora* in a medium in which 5% of the water had been replaced by ^2H_2O. The ^{2}H NMR spectrum of the diacetate, also obtained after the addition of *ca.* 0.3 equiv. of Eu(fod)$_3$ confirmed that the two C-3 hydrogen resonances could also be resolved in the ^{2}H NMR spectrum. Finally, repetition of the experiment with a sample of LL-D253α diacetate enriched from [$^2H_3$]acetate indicated (Fig. 2) that both the 3α and 3β hydrogens were labelled by deuterium. This confirms that the protonation of the intermediate enolate must be non-stereospecific.

To answer the original problem of how the carbon skeleton of LL-D253α is assembled a number of pieces of evidence require to be considered. Firstly, the skeleton appears to be entirely polyketide, being derived from six intact acetate units. This is supported by the equal levels of labelling throughout the carbon framework by single-labelled ^{13}C acetates. Secondly, the mode of incorporation of [1,2-$^{13}C_2$]acetate indicates that intermediates containing a symmetrically substituted phloroglucinol ring, *e.g.* **12** or **13** are unlikely. In all instances so far examined,



12



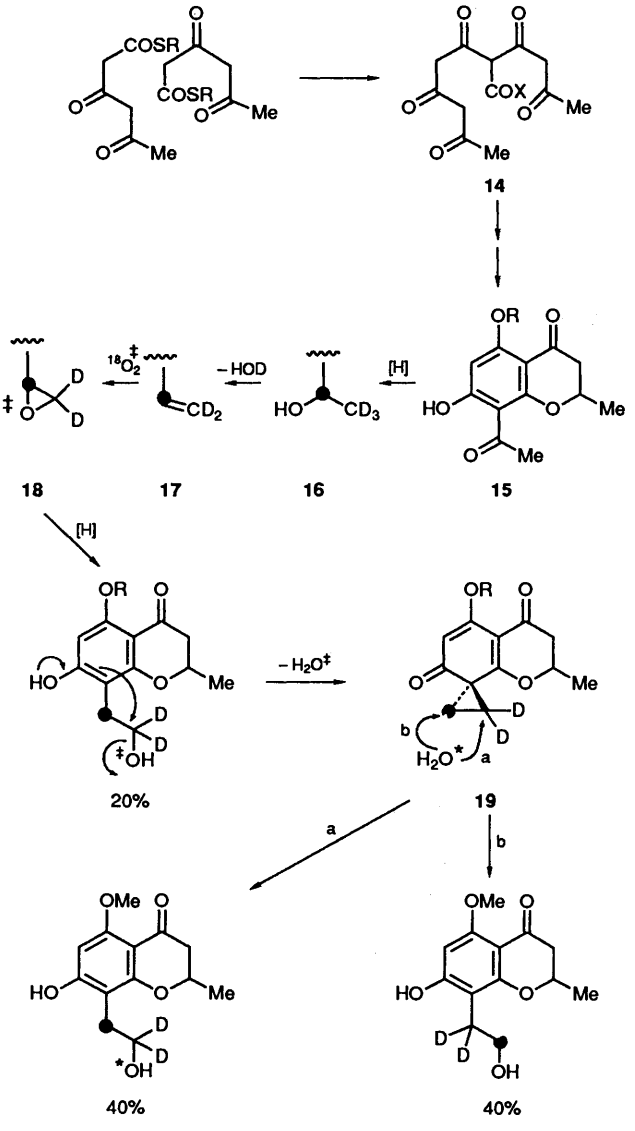
13

the involvement of such symmetrical intermediates results in randomisation of labelling in the phloroglucinol (or similar resorcinol) rings.[23] Thirdly, the partial randomisation of label between C-1′ and C-2′ indicates that these carbons *may* become equivalent during the course of biosynthesis. Comparisons of enrichment levels between these two positions in both ^{13}C and ^{2}H labelling experiments give a reasonably consistent 60:40 ratio of label distribution between them. Fourthly, the results of both α and β shift experiments with ^{2}H and ^{13}C-labelled acetates preclude intermediates in which the hydroxyethyl carbon originally from C-2 of acetate possesses less than two acetate derived hydrogens.

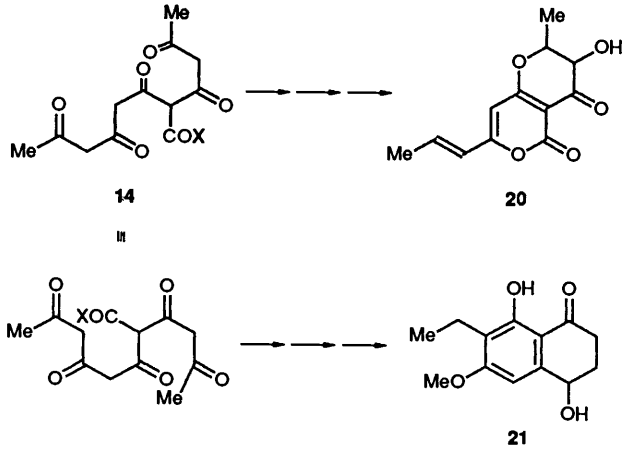
To accommodate these observations, the pathway shown in Scheme 4 is proposed. Two preformed polyketide chains are condensed to give the intermediate **14** which after aromatisation and chromanone ring formation gives the 8-acetylchroman-4-one **15** (R = H). No information is available on the nature of the two preformed polyketides. Two C_6 or triketide intermediates are shown but *a priori* one cannot exclude other combinations, C_2 plus C_{10}, or C_4 plus C_8. However, it is likely that the two chains are combined before any aromatisation occurs as this would necessarily produce potentially symmetrical phenolic intermediates. Such two-chain pathways are known, *e.g.* radicinin **20**. It has been firmly established by ^{2}H-labelling studies that both methyl groups in radicinin carry three acetate-derived hydrogens and therefore two acetate 'starter' units are present.[8] The intermediate required for radicinin biosynthesis is identical with that proposed here for LL-D253α. One of the few other polyketide metabolites to possess a C_2 side chain is *O*-methylasparvenone **21**. This is also a hexaketide and it is noteworthy that the carbon skeleton of **21** could also be derived from a common intermediate **14** as indicated in Scheme 5. *O*-Methylasparvenone has been shown to be derived from six intact acetate units with an assembly pattern consistent with this proposal.[20]

Reduction and dehydration of the 8-acetyl intermediate **15** would give the 1′-hydroxyethyl and vinyl intermediates **16** and **17**. Direct reduction of this double bond would produce the known[4] co-metabolite **6** (in *Phoma violacea*) of LL-D253α. ^{2}H-Labelling studies have established that the ethyl side chain in *O*-methylasparvenone is also formed by a similar reduction, dehydration reduction sequence on an acetyl side-chain after aromatisation of the intermediate polyketide.[20]

A mono-oxygenase mediated introduction of oxygen onto **17** to give the epoxide **18**, followed by reductive opening of the ring would produce LL-D253α in which the oxygen of the hydroxyethyl group was entirely atmospherically derived. The partial

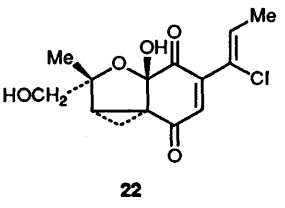


Scheme 4

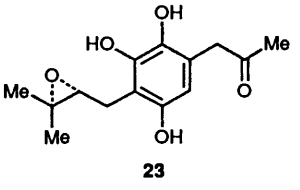


Scheme 5

randomisation of carbon label can be rationalised by a subsequent process whereby the phloroglucinol ring participates in displacing the 2′-hydroxy, perhaps after appropriate activation (see below), to give a spirocyclopropyl intermediate **19**. Hydrolytic opening of this unstable species could occur at either



22



23

methylene of the cyclopropane, in once case (a) retaining the original carbon labelling pattern and in the other case (b) reversing it. Any molecules of LL-D253α which had undergone these reactions would, therefore, possess water-derived oxygen at C-2′.

The formation of phenonium ions and related cyclopropylcyclohexadienone structures by participation of phenyl rings is well known. The solvolysis reactions of β-arylethyl systems was studied in relation to the 'non-classical ion' problem and their existence has been demonstrated by NMR spectroscopy.[24] A similar randomisation has been observed during the synthesis of chirally labelled tyramines.[25] The aryl participation was suppressed by using the electron-withdrawing methylsulfonyl group in place of the methyl group as protection for the phenol function. The fungal metabolite mikrolin **22** contains a cyclopropyl ring attached to a cyclohexadienone-derived ring. A biosynthetic pathway *via* the epoxide **23** has been demonstrated.[26]

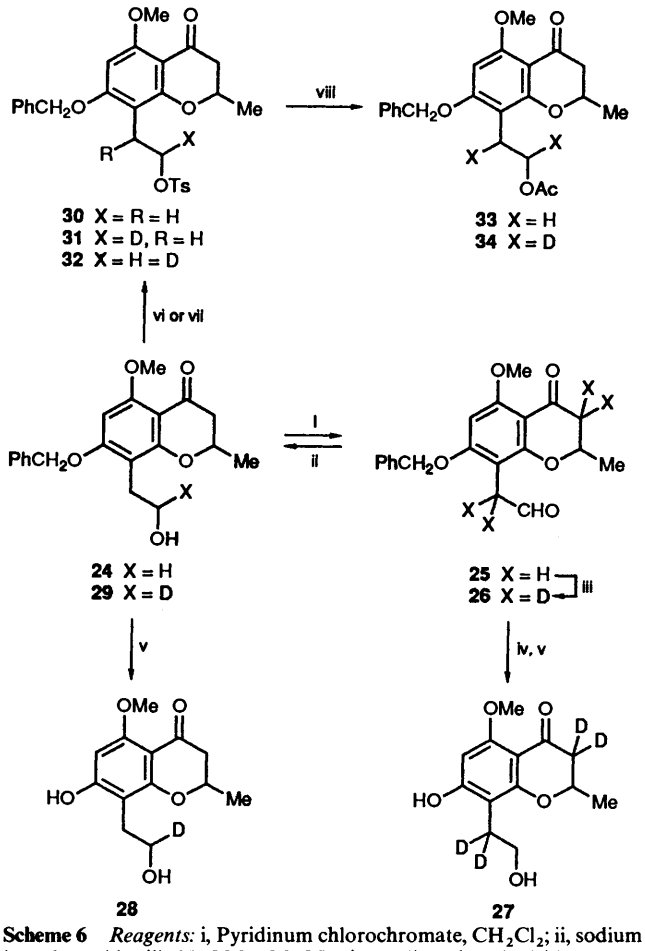
Support for the proposed randomisation mechanism was provided by carrying out a fermentation of *P. pigmentivora* under an atmosphere containing $^{18}O_2$ gas. The mass spectrum of the resultant LL-D253α showed an M + 2 peak which mass matched for $^{12}C_{13}H_{16}{}^{16}O_4{}^{18}O_1$. After allowing for the effect of the natural abundance of ^{13}C, this peak was seen to comprise about 15% of the total molecular ion currents for M, M + 1 and M + 2. On conversion into the diacetate, only the ^{13}C NMR resonance due to C-2′ showed (Table 1) an isotopically shifted signal which had an intensity of *ca.* 20% that of the natural abundance resonance.

The results indicate that *ca.* 80% of the molecules undergo the randomisation process. It is not clear whether this randomisation is an *in vivo* or *in vitro* process. In order to test this, specifically deuteriated sample of LL-D253α have been prepared by PCC oxidation of the benzyl ether **24** to give the aldehyde **25**. Base-catalysed exchange gave the tetra-deuteriated product **26** which after borohydride reduction and hydrogenolysis gave labelled LL-D253α **27**. When this was heated with a catalytic quantity of toluene-*p*-sulfonic acid, ^2H NMR analysis showed that *ca.* 60% of the ^2H label was lost from C-3 but no randomisation of label from C-1′ to C-2′ had occurred.

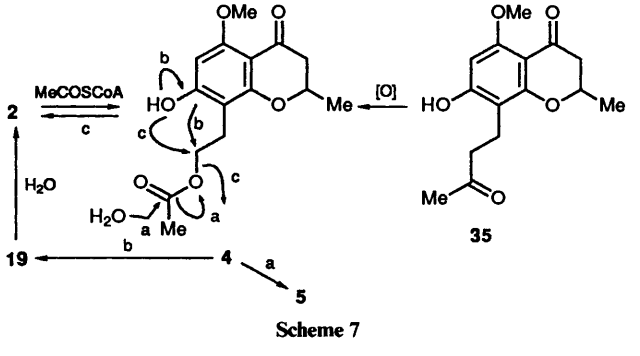
LL-D253α specifically labelled with one deuterium at C-2′ has also been prepared by reduction of the aldehyde **25** with sodium borodeuteride. On deprotection, the labelled LL-D253α **28** was obtained. This was fed to cultures of *P. pigmentivora*, 60 h after inoculation and reisolated a further 24 h later. As expected, the ^2H label had been diluted by endogenously produced LL-D253α but ^2H NMR analysis demonstrated that all the label remained at C-2′. Thus it can be seen that the randomisation is not a facile *in vitro* process occurring during the fermentation or isolation.

The randomisation has been induced to occur under more forcing conditions. When the labelled benzyl ether **29** was treated under reflux with an excess of toluene-*p*-sulfonic acid in benzene, the tosylate **31** was prepared with no randomisation of label by treatment of **29** with toluene-*p*-sulfonyl chloride in pyridine. When heated in acetic acid it gave the acetate **34** in which complete randomisation of label was shown by ^2H NMR analysis.

On the basis of these studies it is likely that the observed partial randomisation of labelling in the side-chain of LL-



Scheme 6 *Reagents:* i, Pyridinum chlorochromate, CH_2Cl_2; ii, sodium borodeuteride; iii, NaOMe, MeOD; iv, sodium borohydride; v, H_2, Pd(C); vi, TsOH, C_6H_6, heat; vii, TsCl, pyridine; viii, AcOH, heat



Scheme 7

D253α is due to reactions occurring during the biosynthesis. An alternative pathway which would account for this and the co-occurrence of the co-metabolites LL-D253β **4** and LL-D253γ **5** is shown in Scheme 7, where it proposed that LL-D253β is the key intermediate. The acetoxy group would be a better leaving group than hydroxy and so it could be converted into LL-D253α either (a) by direct hydrolysis or (b) *via* the cyclopropyl intermediate **19**. LL-D253γ could be formed from **4** by intramolecular displacement (path c) of the acetoxy group by the phenolic hydroxy group on C-7. LL-D253β could itself be formed from an intermediate **35** with a *four* carbon side-chain on C-8. Baeyer–Villiger-type oxidation of the ketone would give LL-D253β directly. Some precedent for such a pathway is provided by the aflatoxin pathway where it is known that the conversion of averufin into versiconal acetate *via* versicolorone involves a similar Baeyer–Villiger-type process.[27]

An alternative explanation is that the randomisation may be mediated by a kinase which phosphorylates the 2′-hydroxy to give a good leaving group and again competition occurs between cyclopropyl ring formation and direct hydrolysis. It may be that the observed biological activity of LL-D253α is due to such activation of the molecule *in vivo* with resultant formation of the cyclopropylcyclohexadienone which could then function as an alkylating agent.

The resolution of these questions awaits further studies with advanced intermediates which are in progress.

Experimental

General experimental details and culture conditions are described in the previous paper.[3]

Radioactivity was determined using a Beckman LS7000 liquid scintillation counter, operating on program 4 without automatic quench correction. Counting efficiency was determined using both standard channels ratio and H-number quench curves. The scintillant was butyl-PBD (10 g dm^{-3}) in methanol–toluene (50:50). Samples for radiocounting were purified to constant activity by recrystallisation and dissolved in either methanol or toluene.

All NMR analyses of biosynthetically enriched samples of LL-D253α were carried out on the derived diacetate **3**, which was prepared as previously described.[3]

Incorporation of Sodium [2-^{14}C]*Acetate into LL-D253α.*—*Phoma pigmentivora* was grown as previously described in 24 flasks. 12 Hours after inoculation, a sterile solution of sodium [2-^{14}C]acetate (500 mg, 10 μCi, sp.act. = 3.64×10^6 dpm $mmol^{-1}$) in distilled water (12 cm^3) was distributed evenly by syringe among 6 flasks. Parallel experiments, feeding at 57 h and 105 h, were conducted concurrently. Six flasks were retained as controls. All flasks were incubated for a total of 174 h. Isolation and purification of LL-D253α followed. The yield of LL-D253α from the control flasks was 176 mg dm^{-3}; the 12 h feed gave 362 mg dm^{-3} (4.3×10^5 dpm $mmol^{-1}$, 8.5 dilution); the 57 h feed gave 311 mg dm^{-3} (1.3×10^6 dpm $mmol^{-1}$, 2.8 dilution); and the 105 h feed gave 218 mg dm^{-3} (4.5×10^5 dpm $mmol^{-1}$, 8.1 dilution).

Incorporation of Sodium [1-^{13}C]-, [2-^{13}C]- *and* [1,2-$^{13}C_2$]-*Acetates into LL-D253α (no Dilution of Labelled Precursor).*—*Phoma pigmentivora* was grown as described in 8 flasks. 57.5 Hours after incubation, a sterile solution of sodium [1-^{13}C]-acetate (200 mg, 92.6 atom % ^{13}C) in distilled water (8 cm^3) was distributed evenly by syringe between 2 flasks. Parallel experiments, feeding with sodium [2-^{13}C]acetate (200 mg, 97.5 atom % ^{13}C) and sodium [1,2-$^{13}C_2$]acetate (200 mg, 91.6, 91.5 atom % ^{13}C) again at 57.5 h, were conducted concurrently. All the flasks were incubated for a total of 126 h after which the LL-D253α (93–107 mg dm^{-3}) was isolated.

Incorporation of Sodium [1-^{13}C]-, [2-^{13}C]- *and* [1,2-$^{13}C_2$]-*Acetate into LL-D253α (with Dilution of Labelled Precursor).*—*Phoma pigmentivora* was grown as described in 25 flasks. 58 Hours after inoculation, a sterile solution of sodium [1-^{13}C]acetate (200 mg at 91.7 atom % ^{13}C; 400 mg at natural abundance) in distilled water (20 cm^3) was distributed evenly by syringe among 4 flasks. Parallel experiments, feeding with sodium [2-^{13}C]acetate (200 mg at 90.6 atom % ^{13}C; 400 mg at natural abundance) and sodium [1,2-$^{13}C_2$]acetate (200 mg at 91.8, 90.6 atom % ^{13}C; 400 mg at natural abundance), were conducted concurrently. All the flasks including 13 control flasks were incubated for a total of 126 h. LL-D253α was isolated as usual, 317, 437, 413 and 453 mg cm^{-3} being isolated from the control flasks, the [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]-acetate feeds respectively.

Incorporation of Sodium [1-^{14}C]*Acetate, Sodium* [1-^{14}C]*Glycollate,* [U-^{14}C]*Oxalic Acid and Sodium* [1-^{13}C,$^{18}O_2$]*Acetate into LL-D253α.—Phoma pigmentivora* was grown as described in 20 flasks. 71 Hours after inoculation the following compounds were administered as sterile solutions in distilled water. (1) Sodium [1-^{14}C]acetate (58.2 mCi mmol^{-1}, 0.08 μCi cm^{-3}); 2 cm^3 to each of 5 flasks. (2) Sodium [1-^{14}C]glycollate (5 mCi mmol^{-1}, 1 μCi cm^{-3}); 2 cm^3 to each of 5 flasks. (3) [U-^{14}C]oxalic acid (98 mCi mmol^{-1}, 1 μCi cm^{-3}); 2 cm^3 to each of 5 flasks. (4) Sodium [1-^{13}C, $^{18}O_2$]acetate (150 mg at 90 atom % ^{13}C, 81 atom % $^{18}O_2$, 18 atom % ^{18}O; 300 mg at natural abundance together in 9 cm^3); 3 cm^3 to each of 3 flasks. The remaining 2 flasks were retained as controls and 20 flasks were incubated for a total of 148 h. LL-D253α was then isolated as usual in yields of 189, 368, 363 and 382 cm^3 dm^{-3} from experiments (1)–(4) respectively.

Attempted Incorporation of (3R)-[5-^{14}C]*Mevalonate into LL-D253α.*—To a benzene solution (0.5 cm^3, 50 μCi ml^{-1}) of (3*R*)-[5-^{14}C]mevalonic acid lactone (57 mCi mmol^{-1}) was added aqueous potassium hydroxide (0.1 mol dm^{-3}, 1 cm^3). The mixture was stirred at 50–60 °C for 1 h while the benzene was removed by a stream of nitrogen; the aqueous residue was then diluted to 10 cm^3 with distilled water. This solution of potassium mevalonate was sterilised and distributed evenly by syringe among 5 flasks of a 48 h culture of *Phoma pigmentivora.* The flasks were incubated for a total of 166 h after which time the LL-253α (357 mg dm^{-3}) was isolated as usual.

Although highly active after the initial chromatographic purification, after two recrystallisations the mevalonate-labelled sample had a specific activity of only *ca.* 1 × 10^4 dpm mmol^{-1}. Based on the quantity of LL-D253α initially isolated (134 mg) and on the final activity, the percentage incorporation was *ca.* 0.01%.

Incorporation of Sodium [1-^{13}C,$^2H_3$]*Acetate and* [2-^{13}C, $^2H_3$]*Acetate into LL-D253α.*—To a 60 h culture of *Phoma pigmentivora* was fed a sterile solution of sodium [1-^{13}C, $^2H_3$]acetate (250 mg at 90 atom % ^{13}C, 98 atom % ^{2}H; 500 mg at natural abundance) in distilled water (20 cm^3), distributed evenly by syringe among 5 flasks. After a total incubation time of 144 h, LL-D253α (55 mg) was isolated. Similarly, feeding sodium [2-^{13}C,$^2H_3$]acetate (204 mg at 90 atom % ^{13}C, 95 atom % ^{2}H; 402 mg at natural abundance) gave LL-D253α (158 mg).

Demethylation of LL-D253α by Boron Trichloride.—LL-D253α (50 mg, 0.198 mmol) was suspended in dry methylene dichloride (15 cm^3) and the mixture was chilled to −50 °C. Boron trichloride (0.5 cm^3, 0.7 g, 6 mmol), also chilled to −50 °C, was added and the flask was immediately sealed. The LL-D253α dissolved instantly and the solution became yellow. After 30 min at room temperature the flask was rechilled to −50 °C and opened. The excess of boron trichloride was removed by attaching the flask to the rotary evaporator (without heating) for 15 min: the residual solution was diluted (to 50 cm^3) with further methylene dichloride, washed with water (4 × 20 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to yield 5,7-*dihydroxy*-8-(2′-*hydroxyethyl*)-2-*methylchroman*-4-*one* (40 mg, 0.168 mmol, 85%) as a white crystalline solid. Recrystallisation of this from methanol–ethyl acetate gave white triangular plates, m.p. 171–173 °C (sublim.) (Found: M$^+$ = 238.0870. $C_{12}H_{14}O_5$ requires *M*, 238.0841); λ_{max}(MeOH)/nm (log ε), 329 (3.48), 290 (4.21) and 213 (4.26); ν_{max}(KBr)/cm^{-1} 3310m br, 1642s, 1630s, 1613s, 1348m, 1305m and 1162m; δ_H[200 MHz; $(CDCl_3)_2CO$] 1.48 (3 H, d, *J* 6.3, CHC*H*$_3$), 2.56–2.79 (2 H, AB of ABX, J_{AB} 17.2, 3-CH_2), 2.84 (2 H, 't', sharpened by D_2O, *J* 7.4, ArCH_2), 3.71 (2 H, 't', sharpened by D_2O, *J* 7.4, CH_2O*H*), 4.50–4.68 (1 H, m, 2-H), 5.96 (1 H, s, ArH) and 12.16 (1 H, s, ex, 5-OH); δ_C[50 MHz; $(CD_3)_2CO$] 19.3 (q), 25.1 (t), 41.9 (t), 61.0 (t), 73.3 (d), 95.3 (d), 101.5 (s), 104.4 (s), 159.8 (s), 161.7 (s), 164.3 (s) and 196.1 (s); *m/z* (%), 238 (M$^+$, 38), 207 (100), 165 (76) and 69 (14).

Demethylation of [^{14}C]*Oxalate-labelled LL-D253α.*—LL-D253α (88 mg, 0.349 mmol, 1.7 × 10^6 dpm mmol^{-1}) labelled from [U-^{14}C]oxalic acid was demethylated by boron trichloride as described above to yield 5,7-dihydroxy-8-(2′-hydroxyethyl)-2-methylchroman-4-one (63 mg, 0.265 mmol, 76%). This was recrystallised to constant activity: 3.2 × 10^4 dpm mmol^{-1}). Thus 98% of the activity had been lost.

Incorporation of Sodium [$^2H_3$]*Acetate into LL-D253α.*—To a 57.5 h culture of *Phoma pigmentivora* was fed a sterile solution of sodium [$^2H_3$]acetate (1.00 g, 99 atom % ^{2}H) in distilled water (20 cm^3), distributed evenly among 10 flasks. After 264 h of incubation, LL-D253α (283 mg, 377 mg dm^{-3}) was isolated.

Preparation of Generally Deuteriated LL-D253α.—Phoma pigmentivora was grown as previously described in 7 flasks. In 5 of these, 5% of the water had been substituted by D_2O. The flasks were incubated for 192 h and the LL-D253α (325 mg dm^{-3}) was isolated as usual.

Incorporation of $^{18}O_2$ *into LL-D253α.—Phoma pigmentivora* was grown, as previously described, in 4 flasks. Two were fitted with foam rubber bungs as usual and retained as controls. The other two were attached to the 'constant pressure' apparatus[28] for measuring the uptake of oxygen and grown under an atmosphere of $^{18}O_2$–$^{14}N_2$ (20:80). After 123 h both pairs of flasks were worked up and LL-D253α was isolated as usual. A total of 1.66 dm^3 of $^{18}O_2$ was consumed. There was no significant difference in yield of LL-D253α between the $^{18}O_2$ incorporation flasks (253 mg dm^{-3}) and the control flasks (240 mg dm^{-3}).

Preparation of [1′,3-$^2H_4$]*LL-D253α-Benzyl Ether* **27**.—7-Benzoyloxy-5-methoxy-2-methyl-8-(2′-oxoethyl)chroman-4-one **25** (47 mg, 0.138 mmol)[3] was dissolved in CD_3OD (0.5 cm^3) in a 5 mm NMR tube. The 60 MHz ^{1}H NMR spectrum was complex and, in particular, the aldehyde signal which, in $CDCl_3$, occurs at 960 ppm was very small: this suggested that the substrate was present largely in hemiacetal and/or acetal forms. The sample was left at room temperature for 60 h after which a further ^{1}H NMR spectrum was obtained. No change was indicated so two drops of $NaOCD_3$ solution (Na, 9 mg in CD_3OD, 0.25 cm^3) were added. The clear solution in the NMR tube rapidly adopted a yellow colour. After 40 min at room temperature, a further ^{1}H NMR spectrum indicated the absence of any signals attributable to methylene groups adjacent to carbonyls. The aldehyde signal was now completely absent. The solution was taken up in ethyl acetate (20 cm^3) washed with water (3 × 5 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to give [1,3-$^2H_4$]-7-benzyloxy-5-methoxy-2-methyl-8-(2′-oxoethyl)chroman-4-one **26** as a pale brown gum (40 mg, 0.116 mmol, 84%). A ^{1}H NMR spectrum (60 MHz; $CDCl_3$) showed that the signals, at 2.60 and 3.67 ppm in the unlabelled material, due, respectively, to the 3-methylene and benzylic methylene groups, were absent. The aldehyde signal at 9.60 ppm, previously a triplet, had been replaced by a singlet. This material was not purified but was reduced using sodium borohydride as previously described.[3] The resulting [1′,3-$^2H_4$]LL-D253α-benzyl ether was purified by TLC, also as previously described, to give a clear gum (29 mg, 0.084 mmol, 72%). A ^{1}H NMR spectrum (200 MHz; $CDCl_3$) showed only vestigial signals at 2.68 and 2.95 ppm. Integration indicated that the compound was *ca.* 90% deuteriated at the positions

corresponding to these signals. The resonances at 3.77 and *ca.* 4.5 ppm, a triplet and a multiplet respectively in the spectrum of the unlabelled compound, showed here as a singlet and quartet. A ^2H NMR spectrum (55.3 MHz; $CHCl_3$) displayed two broad singlets at 2.56 and 2.91 ppm. Mass spectrometry gave a predominant parent-ion peak at m/z = 346 (M + 4).

Hydrogenolysis of [1′,3-$^2H_4$]*LL-D253α-Benzyl Ether.*—This experiment was carried out in the same manner as previously described. The benzyl ether **27** (24 mg, 0.069 mmol) thus yield [1′,3-$^2H_4$]LL-D253α **27** (11 mg, 0.043 mmol, 62%). The ^1H NMR spectrum (200 MHz; CD_3OD) was compared with one of natural LL-D253α (80 MHz; CD_3OD). Two signals in the latter spectrum: the AB of ABX pattern between 2.58 and 2.68 ppm and the triplet centred at 2.92, assigned, respectively, to the 3-methylene and benzylic methylene groups, were only present to the extent of 10% of their natural intensity in the spectrum of the labelled material. The multiplet at around 4.6 ppm and the triplet at 3.72 ppm were simplified to a quartet and a singlet in the spectrum of the labelled compound.

Attempted Acid-catalysed Rearrangement of [1′,3-$^2H_4$]*LL-D253α.*—[1′,3-$^2H_4$]LL-D253α **27** (11 mg, 0.043 mmol) was heated under reflux in dry benzene (10 cm^3) with toluene-*p*-sulfonic acid (1 mg) for 16 h. Precautions were taken to exclude moisture. The benzene was removed under reduced pressure and the resulting brown gum was examined by ^1H NMR spectroscopy (80 MHz; CD_3OD). The signal at 2.92 ppm was still absent (see above) but that between 2.58 and 2.68 ppm was present to some extent, although still reduced relative to the corresponding signal in a spectrum of natural LL-D253α. A ^2H NMR spectrum (55.3 MHz; CH_3OH) of the product confirmed that deuterium was still present at the 3-methylene position (2.6 ppm) but to only *ca.* 40% of the level at the benzylic methylene group (2.9 ppm).

[2′-$^2H_1$]-7-*Benzyloxy*-8-(2-*hydroxyethyl*)-5-*methoxy*-2-*methylchroman*-4-*one* **29**.—7-Benzyloxy-5-methoxy-2-methyl-8-(2-oxoethyl)chroman-4-one **25** (35 mg, 0.10 mmol) in dry tetrahydrofuran (1.5 cm^3) was reduced by sodium borodeuteride (98% ^2H) (10 mg, 0.24 mmol). The product, a pale brown oil, was purified by preparative TLC to give the *title compound*, almost pure, as a white solid (10 mg, 29%); δ_H(80 MHz; $CDCl_3$) 1.46 (3 H, d, *J* 6.3, C-CH_3), 2.59 (2 H, m, AB of ABX, *J* 7.6, 1.0, 3-H_2), 2.93 (d, *J* 7.5, ArCH_2CHD), 2.94 (t, ArCH_2CH_2), 3.74 (m, ArCH_2CHD), 3.84 (3 H, s, OCH_3), 4.50 (1 H, dq, *J* 7, 1 $CHCH_3$), 5.15 (2 H, s, PhCH_2), 6.15 (1 H, s, ArH) and 7.38 (5 H, s, C_6H_5); $\delta_{^2H}$ (55 MHz; $CHCl_3$) 3.73; m/z (%) 343 (M^+, 3), 311 (6), 287 (4), 256 (5), 255 (7), 221 (7), 94 (97) and 91 (100).

[2′-$^2H_1$]-7-Hydroxy-8-(2-*hydroxyethyl*)-5-*methoxychroman*-4-*one* (*LL-D253α*) **28**.—A stirred, degassed solution of the chroman-4-one **29** (38 mg, 0.11 mmol) in dry ethyl acetate containing a suspension of palladium–charcoal (10:90 w/v; 20 mg), was hydrogenated at ambient temperature. The reaction was monitored by analytical TLC [chloroform–methanol (96:4)]. As starting material (R_f 0.5) diminished in intensity, a spot corresponding to the anticipated product (R_f 0.35) appeared. After 3.5 h the product was degassed and filtered through Celite and concentrated under reduced pressure to give a clear gum (36 mg) which was purified by preparative TLC [chloroform–methanol (96:4)] to yield the title compound a white solid (26 mg, 93%); δ_H[80 MHz; $(CD_3)_2CO$] 1.4 (3 H, d, *J* 6, $CHCH_3$), 2.5 (2 H, d, *J* 7, 3-H), 2.8 (2 H, d, *J* 6, ArCH_2CHD), 3.6 (ArCH_2C*H*D), 3.75 (3 H, s, OCH_3), 6.2 (1 H, s, ArH); $\delta_{^2H}$[55 Hz; $(CH_3)_2CO$] 3.72.

7-*Benzyloxy*-5-*methoxy*-2-*methyl*-8-[2-(p-*tolylsulfonyloxy*)-*ethyl*]*chroman*-4-*one* **30**.—7-Benzyloxy-8-(2-hydroxyethyl)-5-methoxy-2-methylchroman-4-one (LL-D253α-7-*O*-benzyl ether) (118 mg, 0.345 mmol) and toluene-*p*-sulfonic acid (85 mg, 0.49 mmol) were stirred together in refluxing benzene (25 cm^3) with the exclusion of moisture. After 15 h the brown solution was allowed to cook and concentrated under reduced pressure. After preparative TLC [chloroform–methanol (98:2)] the most intense band (R_f 0.2), was isolated, to give the title compound **30** which recrystallised from ethyl acetate as white needles, m.p. 83–85 °C (Found: C, 65.5; H, 5.58. $C_{27}H_{28}O_7S$ requires C, 65.3; H, 5.68%); λ_{max}/nm (log ε) 318 (3.62), 287 (4.22) and 2.44 (4.04); ν_{max}(Nujol)/cm^{-1} 1669s, 1586s, 1567s, 1302m, 1289m, 1262m, 1254m, 1205m, 1177s, 1172s, 1143m and 1111s; δ_H(80MHz; $CDCl_3$) 1.33 (3 H, d, *J* 6.2, C-CH_3), 2.34 (3 H, s, ArCH_3), 2.38–2.49 (2 H, m, AB of ABX, 3-H), 2.94 (2 H, t, *J* 6.8, ArCH_2), 3.79 (3 H, s, OCH_3), 4.10 (2 H, t, *J* 6.8, ArCH_2CH_2), 4.28–4.43 (1 H, m, X of ABX, $CHCH_3$), 5.05 (2 H, s, PhCH_2), 6.05 (1 H, s, ArH), 7.14 (2 H, d, *J* 8.5, ArH), 7.34 (5 H, s, ArH) and 7.58 (2 H, d, *J* 8.4, ArH); m/z (%) 496) (M^+, 8), 234 (26), 192 (20), 107 (28), 92 (15) and 91 (100).

[1′,2′-$^2H_1$]-7-*Benzyloxy*-5-*methoxy*-2-*methyl*-8-[2-(p-*tolyl-sulfonyloxy*)*ethyl*]*chroman*-4-*one* **31**.—The chromanone **29** (15 mg, 0.04 mmol) and toluene-*p*-sulfonic acid (9 mg, 0.05 mmol) were dried thoroughly and stirred in refluxing benzene with the exclusion of moisture. Analytical TLC [chloroform–methanol (98:2)] after 13 h showed a major spot (R_f 0.4) plus starting material (R_f 0.25). Further toluene-*p*-sulfonic acid (16 mg, 0.08 mmol) was added. Analytical TLC after a further 8 h indicated that little starting material remained. The solution was concentrated under reduced pressure to give a brown gum (68 mg) which was purified by preparative TLC [Merck glass-backed silica plates; chloroform–methanol (96:4)]. The main band (R_f 0.8) was isolated to give the *title compound* **31**, as a clear gum (10 mg, 46%); δ_H(80 MHz, $CDCl_3$) 1.37 (3 H, d, *J* 6.25, C-CH_3), 2.98 (d, *J* 7.0, ArCH_2CHD), 2.98 (t, *J* 7.0, ArCH_2CH_2), 3.83 (3 H, s, OCH_3), 4.13 (d, *J* 7.1, ArCHDCH_2), 4.14 (t, *J* 7.0, ArCH_2CH_2), 4.25–4.50 (1 H, m, X of ABX, $CHCH_3$), 5.08 (2 H, s, PhCH_2), 6.07 (1 H, s, ArH), 7.18 (2 H, d, *J* 8.6, ArH), 7.38 (ArH) and 7.62 (2 H, d, *J* 6.7, ArH); $\delta_{^2H}$(55 MHz, $CDCl_3$) 2.97 and 4.13.

[2′-$^2H_1$]-7-*Benzyloxy*-5-*methoxy*-2-*methyl*-8-[2-(p-*tolylsulf-onyloxyethyl*)*chroman*-4-*one* **32**.—The chromanone **29** (15 mg, 0.044 mmol) and toluene-*p*-sulfonyl chloride (27 mg, 0.142 mmol) were stirred in pyridine (10 drops) at ambient temperature with the exclusion of moisture. After 5 h the product was acidified (2 mol dm^{-3} hydrochloric acid) to give a white precipitate, which was taken up in chloroform (2 × 20 cm^3). This was dried ($MgSO_4$) and ^1H NMR spectroscopy indicated that the desired product was present, with minor impurities. After preparative TLC [chloroform–methanol (97:3)], the most intense band (R_f 0.7) was isolated to give the *title compound* **32** as a clear gum, (18 mg, 83%); δ_H(80 MHz; $CDCl_3$) 1.37 (3 H, d, *J* 6.2, C-CH_3), 2.38 (3 H, s, ArCH_3), 2.45–2.55 (2 H, m, AB of ABX, 3-H), 2.98 (d, *J* 7.0, ArCH_2CHD), 2.98 (t, *J* 7.0, ArCH_2CH_2), 3.82 (3 H, s, OCH_3), 4.13 (br t, *J* 7.0, ArCH_2C*H*D–), 4.25–4.50 (1 H, m, X of ABX, 2-H), 5.08 (2 H, s, PhCH_2), 6.07 (1 H, s, ArH), 7.18 (2 H, d, *J* 9.2, Ar*H*CH_3), 7.37 (5 H, s, Ph*H*) and 7.62 (2 H, d, *J* 8.3, Ar*H*); $\delta_{^2H}$(55 MHz; $CHCl_3$) 4.13.

8-(2-*Acetoxyethyl*)-7-*benzyloxy*-5-*methoxy*-2-*methyl-chroman*-4-*one* **33**.—The chroman-4-one **30** (75 mg, 0.22 mmol) was heated in glacial acetic acid at 60 °C for 62 h. The yellow solution was concentrated under reduced pressure to give a brown gum. After preparative TLC [chloroform–methanol (96:4)], the two main bands were isolated. The material with the

lower R_f (0.7) corresponded to an authentic sample of starting material. That with the higher R_f (0.8) was the expected product, the *title compound* **33** (49 mg, 58%). Recrystallisation from ether afforded clear blocky plates, m.p. 99–101 °C (Found: C, 68.9; H, 6.35. $C_{22}H_{24}O_6$ requires C, 68.7; H, 6.29%); λ_{max}/nm (log ε) 318 (3.72), 284 (4.35) and 240 (4.10); ν_{max}(Nujol)/cm^{-1} 1749s, 1672s, 1594s, 1238m, 1219s, 1141m, 1128s, 1078m and 814m; δ_H(80 MHz; $CDCl_3$) 1.44 (3 H, d, *J* 6.3, C-CH_3), 1.96 (3 H, s, $COCH_3$), 2.55 (2 H, m, AB of ABX, 3-H), 2.95 (2 H, t, *J* 7.0, $ArCH_2$), 3.81 (3 H, s, CH_3), 4.16 (2 H, t, *J* 7.0, $ArCH_2CH_2$), 4.45 (1 H, m, X of ABX, 2-H), 5.13 (2 H, s, $PhCH_2$), 6.11 (1 H, s, ArH) and 7.36 (5 H, s, ArH).

[1′,2′-$^2H_1$]-8-(2-*Acetoxyethyl*)-7-*benzyloxy*-5-*methoxy*-2-*methylchroman*-4-*one* **34**.—The chroman-4-one **32** (10 mg, 0.020 mmol) was stirred in glacial acetic acid at ambient temperature with the exclusion of moisture for 15 h. Difficulty was encountered in monitoring the reaction by analytical TLC, as the presence of acetic acid caused streaking on the silica plate; however it was clear that some starting material remained. The reactants were stirred at 60 °C for 20 h.

The mixture was concentrated under reduced pressure and taken up in chloroform (15 cm^3). This solution was then washed with water (3 × 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure to yield a pale red gum. Preparative TLC (chloroform) yielded starting material (3 mg) and the expected *title compound* **34** (4 mg, 50%), as a clear gum, with identical analytical TLC properties to the unlabelled material; $\delta_{^2H}$(55 MHz; $CHCl_3$) 2.98 and 4.18.

Attempted in vivo *Scrambling of Deuterium Label in LL-D253α*.—Five 250 cm^3 Erlenmeyer flasks, each containing 75 cm^3 of medium were inoculated with a mycelial suspension of *Phoma pigmentivora* as described previously, and incubated at 26 °C. The chroman-4-one **28** (20 mg) was taken up in ethanol (0.5 cm^3) and sterile water (5 cm^3), and added to one flask, after growth for 60 h. 10 Days after initial inoculation the flask's contents were worked up as described previously, to give a brown oil (207 mg), whose components were separated by preparative TLC [chloroform–methanol (90:10) and then acetone–chloroform (20:80)] to give a white solid (8 mg), partially deuteriated 7-hydroxy-8-(2-hydroxyethyl)-5-methoxy-2-methylchroman-4-one (LL-D253α); $\delta_{^2H}$[55 MHz; $(CH_3)_2CO$, 76 555 scans] 3.68.

Acknowledgements

We thank SERC for Studentships (I. M. C. and C. R. M.) and Lederle laboratories for samples of LL-D253α, β and γ.

References

1 W. J. McGahren, G. A. Ellested, G. O. Morton and M. P. Kunstmann, *J. Org. Chem.*, 1972, **37**, 1636.
2 C. Takahashi, S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, H. Jurata, M. Enomotu, K. Ohtsubo, M. Umeda and M. Saito, *Chem. Pharm. Bull.*, 1973, **21**, 2286.
3 I. M. Chandler, C. R. McIntyre and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, preceding paper.
4 G. C. Crawley and C. J. Strawson, unpublished results quoted by W. B. Turner and D. C. Aldridge in *Fungal Metabolites II*, Academic Press, London, 1983, p. 98.
5 F. M. Dean, *Naturally Occurring Oxygen Ring Compounds*, Butterworths, London, 1963.
6 S. T. Saengchantora and T. W. Wallace, *Nat. Prod. Reports*, 1986, **3**, 465.
7 D. C. Allport and J. D. Bu'lock, *J. Chem. Soc.*, 1960, 654.
8 B. Tal, G. Goldsby, B. A. Burke, A. J. Aasen and D. J. Robeson, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1283.
9 D. D. Weller and K. L. Rinehart, *J. Am. Chem. Soc.*, 1978, **100**, 6757.
10 T. W. Goodwin, *Biochem. Soc. Trans.*, 1977, **5**, 1252.
11 A. A. Chalmers, C. P. Gorst-Allman, P. S. Steyn, R. Vleggaar and D. B. Scott, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1481.
12 R. B. Herbert, *The Biosynthesis of Secondary Metabolites*, 2nd edn., Chapman and Hall, London, 1989.
13 L. Colombo, C. Gennari, D. Potenza, C. Swlastico, F. Aragozzini and R. Gualandris, *J. Chem. Soc., Perkin Trans. 1*, 1982, 365.
14 Preliminary communication, C. R. McIntyre, T. J. Simpson, L. A. Trimble and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1984, 706.
15 T. J. Simpson, *Chem. Soc. Rev.*, 1975, **4**, 497.
16 T. J. Simpson and J. S. E. Holker, *Phytochemistry*, 1977, **16**, 229.
17 E. E. Conn and P. K. Stumpf, *Outlines of Biochemistry*, 3rd edn., Wiley, New York, 1972, p. 316.
18 G. Michael, *Biochemical Pathways*, Boehringer Mannheim GmbH., West Germany, 1976.
19 C. Abell, M. J. Garson, F. J. Leeper and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1982, 1011.
20 T. J. Simpson and D. J. Stenzel, *J. Chem. Soc., Chem. Commun.*, 1982, 1074.
21 T. J. Simpson: ^{13}C-*NMR in Metabolic Studies*, in *Modern Methods of Plant Analysis*, eds. H. F. Linskens and J. F. Jackson, Springer-Verlag, Berlin, 1986, p. 1.
22 R. A. Dixon, P. M. Dey and L. M. Whitehead, *Biochem. Biophys. Acta*, 1982, **715**, 25.
23 See for example, E. Bardshiri and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1981, 195.
24 G. A. Olah and R. D. Porter, *J. Am. Chem. Soc.*, 1971, **93**, 6877.
25 A. R. Battersby, E. J. T. Chrystal and J. Staunton, *J. Chem. Soc., Perkin Trans. 1*, 1980, 31.
26 K. K. Chexal and C. Tamm, *Helv. Chim. Acta*, 1978, **61**, 2002.
27 C. A. Townsend, P. R. D. Whittamore and S. W. Brobst, *J. Chem. Soc., Chem. Commun.*, 1988, 736.
28 C. R. McIntyre, F. E. Scott, T. J. Simpson, L. A. Trimble and J. C. Vederas, *Tetrahedron*, 1989, **45**, 2307.

Paper 2/02778J
Received 27*th May* 1992
Accepted 12*th June* 1992