Studies of Polyketide Chain-assembly Processes: Origins of the Hydrogen and Oxygen Atoms in Colletodiol

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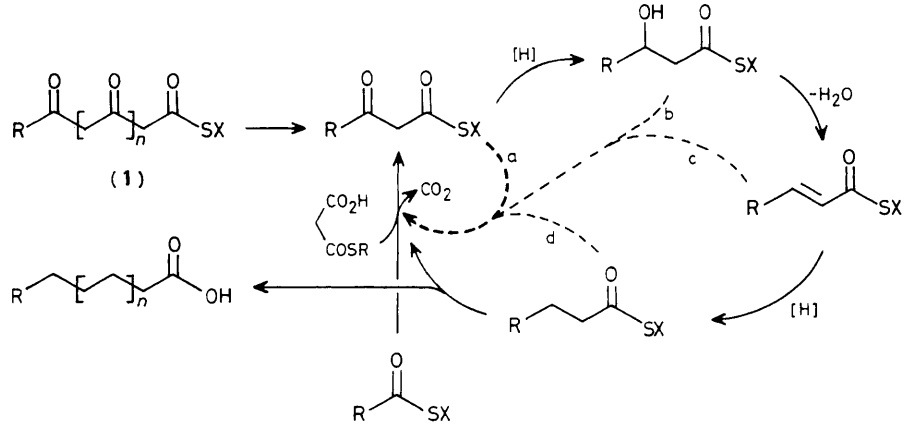
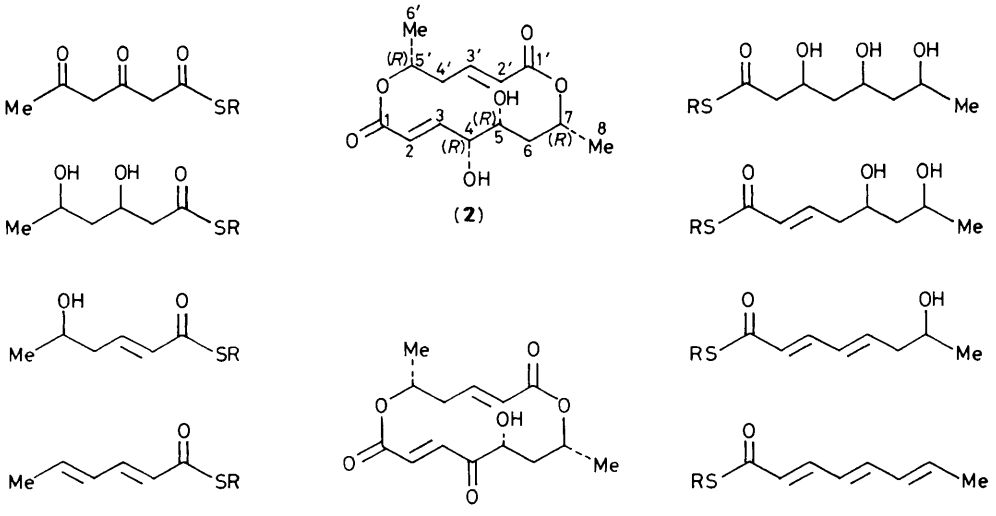
The origins of all the oxygen and hydrogen atoms in colletodiol (**2**) have been elucidated by incorporation of label from [1-^{13}C,$^{18}O_2$]- and [1-^{13}C,$^{2}H_3$]-acetate and $^{18}O_2$ gas into (**2**) in cultures of *Cytospora sp.* (ATCC 20502); from the resultant labelling pattern the structures of the enzyme-bound precursors can be deduced and information obtained on the processes occurring during the early stages of polyketide chain-assembly.

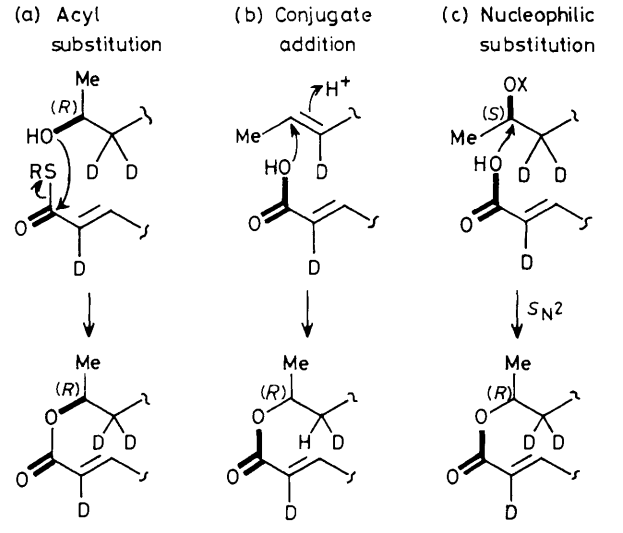
The polyketide pathway is one of the major pathways of secondary metabolism, but despite much effort over the 30 years since the recognition of the pathway,[1] little is known of the exact nature of the intermediates involved in the early stages of polyketide chain-assembly. At its simplest, it is thought that poly-β-ketide intermediates (**1**) are built up by a cyclic process (Scheme 1) analogous to fatty acid biosynthesis[2] but omitting the reduction–elimination–reduction sequence responsible for the loss of acetate oxygen. While some aromatic metabolites do retain the full oxygen content of intermediate (**1**) most metabolites show varying degrees of reduction and/or deoxygenation and an increasing body of evidence suggests that this occurs by processes analogous to fatty acid biosynthesis before the initial release of metabolites or intermediates from the chain-assembly enzymes. Thus, path a in Scheme 1 would simply produce poly-β-ketides but by invoking paths b, c, and d intermediates with varying degrees of reduction may be formed. There has been little progress in enzymatic or other direct methods of observing these early intermediates but recent developments in n.m.r.-based methods[3] (*viz.* ^{2}H and ^{18}O isotope-induced shifts in ^{13}C n.m.r., and ^{2}H n.m.r. spectroscopy) which facilitate determination of the biosynthetic origins of hydrogen and oxygen enable significant indirect evidence for the nature of the



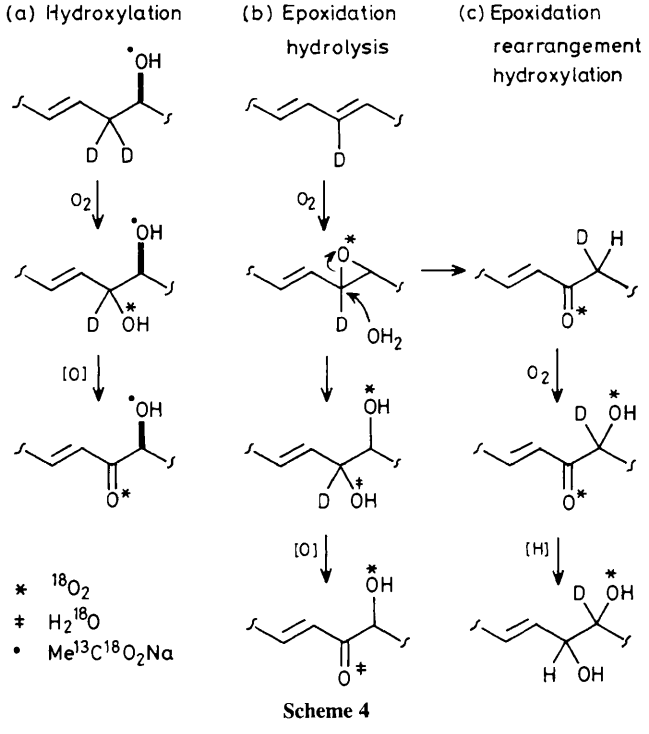
Scheme 1



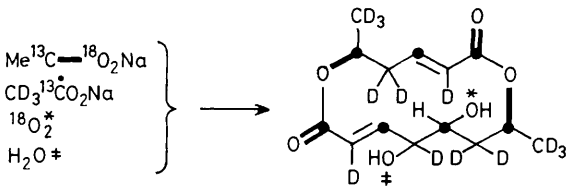
Scheme 2



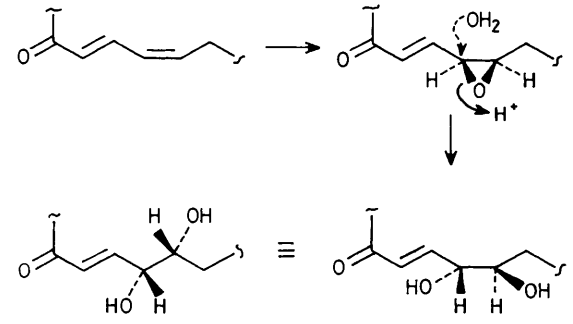
Scheme 3



Scheme 4



Scheme 5



Scheme 6

Table 1. ^2H and ^{18}O isotope-induced shifts observed in the 90.56 MHz ^{13}C n.m.r. spectrum of colletodiol (**2**).

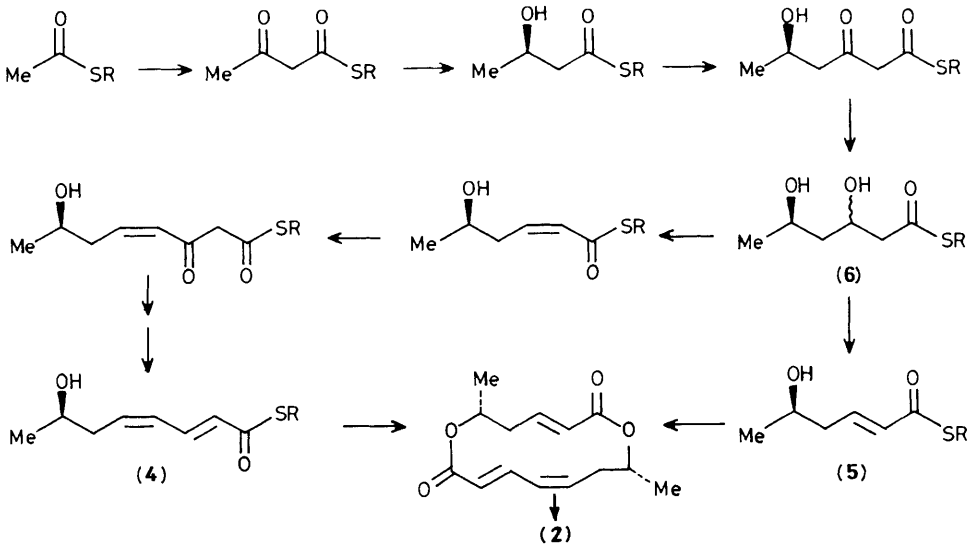
	δ_C	$\Delta\delta \times 100$	$^{16}O:{}^{18}O$	$^1H:{}^2H$
C-1	166.3	3.4[a]	69:31	
C-1′	164.9	3.2[a]	70:30	
C-3	146.4	9.0[c]		95:5
C-3′	143.9	4.4,4.2[c]		53:19:28
C-5	71.7	2.2[b]	54:46	
		4.7,4.6[c]		53:20:27
C-5′	68.6	3.9[a]	79:21	
		4.1,4.3,4.3[c]		30:10:22:38
C-7	67.9	3.7[a]	72:28	
		4.2,4.2,4.3[c]		27:9:23:41

[a] [1-^{13}C,$^{18}O_2$]acetate-enriched. [b] $^{18}O_2$-enriched. [c] [1-^{13}C,$^2H_3$]acetate-enriched.

intermediates to be obtained. We now report ^2H and ^{18}O labelling studies on colletodiol (**2**) designed to obtain information on the processes occurring during the early stages of polyketide chain-assembly.

Colletodiol (**2**) and colletoketol (**3**) are macrocyclic dilactonic metabolites originally isolated from the plant pathogen, *Colletotrichum capsici*.[4] More recently grahamimycin A was isolated as a broad spectrum antibiotic from a species of *Cytospora* and was subsequently shown to be identical to colletoketol.[5] All four chiral centres in colletodiol have the (*R*) configuration.[4,6] Incorporation studies with singly ^{13}C-labelled acetates have confirmed the acetate-origin of colletodiol in *C. capsici*.[7] These metabolites can be seen to be derived by combination of C_6 and C_8, moieties and *a priori* one can postulate a number of triketide- and tetraketide-derived moieties as the actual enzyme-bound precursors. Some of these are shown in Scheme 2. Depending on the nature of the actual intermediates a number of mechanisms can be proposed for the formation of the lactone functions. These are summarised in Scheme 3 along with the possible stereochemical outcome and the predicted origins of the associated oxygen and hydrogen atoms. Similarly a number of different mechanisms can be proposed for the formation of the 1,2-diol and α-ketol systems found in colletodiol and colletoketol respectively. These are shown in Scheme 4 and again they may be differentiated, as indicated, by appropriate ^2H and ^{18}O labelling experiments.

In our hands *Cytospora sp.* (ATCC 20502) has produced colletodiol as the major metabolite and only minor amounts of grahamimycin A. Fermentations were carried out in the presence of [1-^{13}C,$^2H_3$]- and [1-^{13}C,$^{18}O_2$]-acetate and under an atmosphere of $^{18}O_2$. The ^2H and ^{18}O isotope shifts observed in the proton noise decoupled ^{13}C n.m.r. spectra of colletodiol isolated in each case are summarised in Table 1. No ^2H isotope-induced shifts could be observed for C-1 or C-1′ in the ^{13}C n.m.r. spectrum of the [1-^{13}C,$^2H_3$]acetate-enriched colletodiol. However carbonyl groups are known to be poor 'reporter' groups for ^2H shifts[8] and the presence of ^2H label at both C-2 and C-2′ was shown by ^2H n.m.r. analysis of the



Scheme 7

enriched metabolite. The labelling pattern resulting from these experiments is summarised in Scheme 5.

The retention of acetate-derived oxygen on both the carbonyl and ether oxygens of the lactone functions indicates that ring closure must proceed by mechanism (a) in Scheme 3 and so the enzyme-bound intermediates must retain the oxygen of the acetate 'starter' units as hydroxy functions with the (*R*) configuration.

Considering the formation of the 1,2-diol system, a low but significant level of acetate-derived hydrogen is retained at C-4. This means that colletoketol cannot be the precursor of colletodiol, and route (c) in Scheme 4 is ruled out. The oxygen labelling results indicate that the 5-hydroxy group is derived from the atmosphere *i.e. via* an oxidative process, whereas the 4-hydroxy group must be derived from the medium *cf.* route (b), Scheme 4. A mechanism consistent with the observed labelling and the (*R*) configuration at both centres is shown in Scheme 6; epoxidation of a (*Z*)-alkene from the β-face is followed by hydrolytic ring opening by attack of water from the α-face at C-4.

On the basis of these results, the thioesters (**4**) and (**5**) can be proposed as the actual enzyme-bound precursors for colletodiol. These may be built up by the sequence shown in Scheme 7 where the diol (**6**) in which the C-3 stereochemistry is uncertain, is proposed as a common intermediate, *trans*-elimination of water giving rise to the C_6 precursor directly, whereas *cis*-elimination followed by addition of a further C_2 unit produces the C_8 precursor. The relative timing of the diol formation step is not yet known but it may occur after lactonisation and release from the enzyme surface as indicated.

Recieved, 22nd August 1985; Com. 1250

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