Biosynthesis of Colletodiol and Related Polyketide Macrodiolides in *Cytospora* sp. ATCC 20502: Synthesis and Metabolism of Advanced Intermediates

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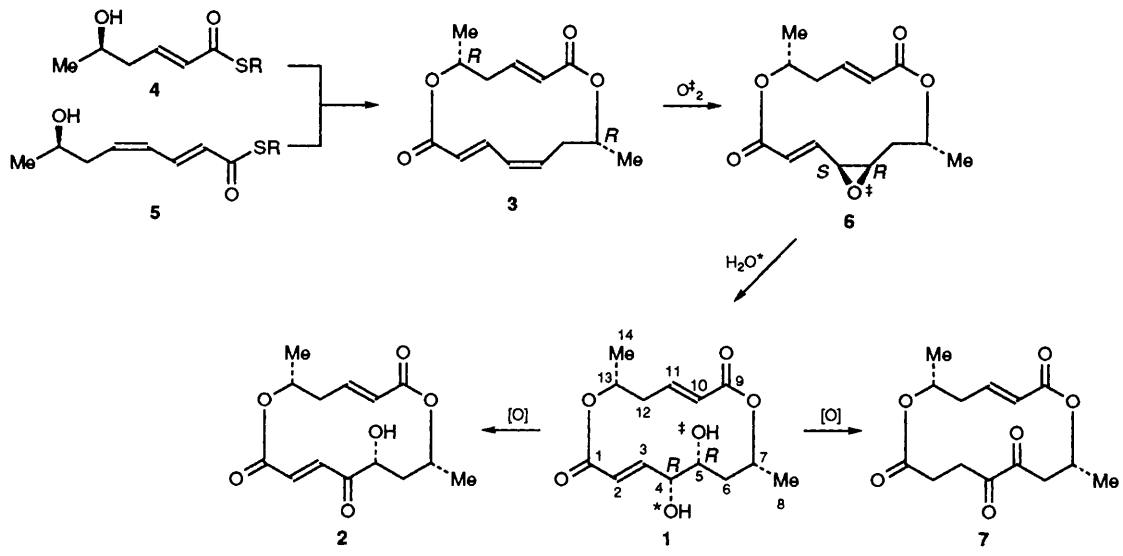
The triene **3** and epoxide **6** have been synthesised in specifically labelled form and their incorporation into the macrodiolide, colletodiol **1** demonstrated in cultures of *Cytospora* sp. ATCC 20502; results of a biomimetic reaction indicate that both colletoketol **2** and grahamimycin A_1 **7** may be biosynthesised in a single oxidation step from colletodiol.

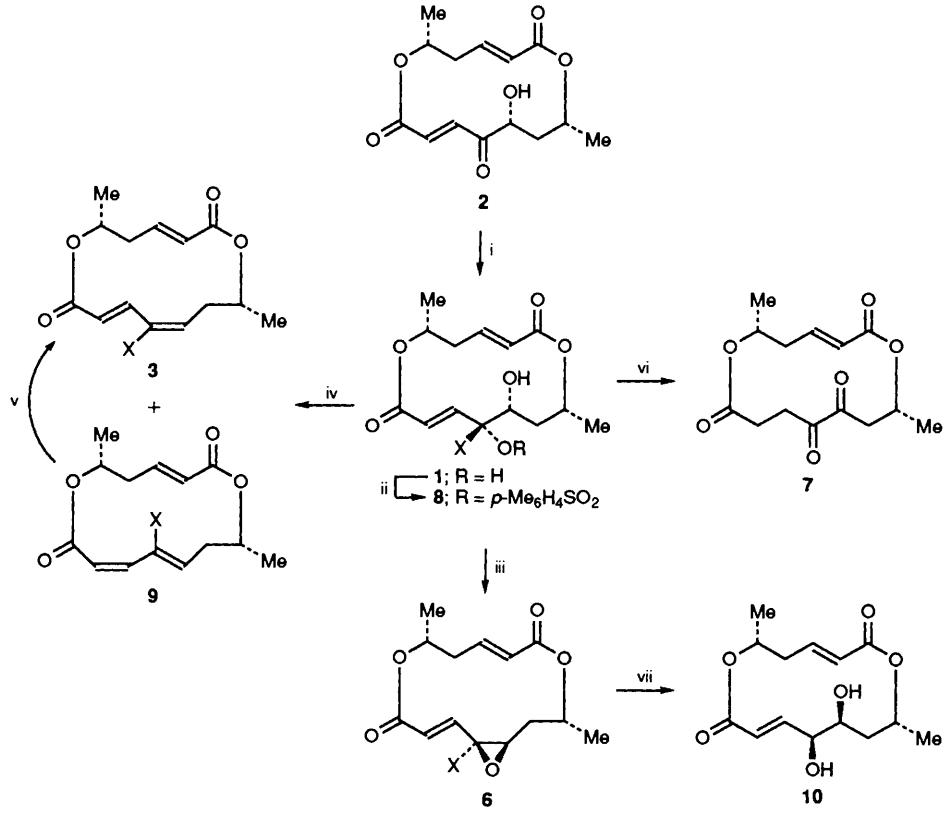
Colletodiol **1** and colletoketol **2** (Scheme 1) are macrocyclic dilactones (macrodiolides) first isolated from the plant pathogenic fungus, *Colletotrichum capsici*.[1] Colletoketol (as grahamimycin A) was subsequently isolated from *Cytospora* sp. ATCC 20502.[2] A number of related macrodiolides have been isolated from both organisms and these are now seen to belong to a larger group of natural macrodiolides, including pyrenophorin,[3] vermiculin[4] and eliaphylin,[5] which have been isolated from both fungi and actinomycetes.[6] A number of these compounds and their synthetic analogues have been reported to show activity against a range of microorganisms.[7] The macrodiolides have been the target of a number of synthetic studies in recent years.[8]

Previous biosynthetic studies[9] have shown that label from [$^2H_3$]acetate is retained at C-4 of colletodiol so that colletoketol must be formed *via* colletodiol and not *vice versa*. These studies also suggested that colletodiol may be biosynthesised *via* the macrocyclic triene **3** which itself would be formed by cyclisation of the thio-ester intermediates **4** and **5** (Scheme 1). Examination of molecular models suggested that the triene **3** would adopt a conformation similar to that established for colletodiol in both the solid state and in solution.[10] In this conformation, epoxidation should occur from the more accessible 4*Re*,5*Re* face of the alk-4-ene to give the 4*S*,5*R*-epoxide **6**, which on hydrolysis would give colletodiol with the correct 4*R*,5*R*-diol stereochemistry and the observed[9] origins for the hydroxy oxygens. We now report studies which confirm the late stages of colletodiol biosynthesis.

Epoxide **6** and triene **3** were prepared from colletodiol as shown in Scheme 2. Both colletodiol and colletoketol are readily isolated in quantity from fermentation of *Cytospora* sp (ATCC 20502). Selective tosylation of the 4-hydroxy of **1** gave **8** which on treatment with sodium hydride gave the desired 4*S*,5*R*-epoxide **6**. X-Ray crystallographic studies[11] not only confirmed the stereochemistry of the epoxide **6**, but also that the overall conformation of the molecule was consistent with the molecular modelling studies discussed above. Colletodiol was converted in one step into a mixture of trienes by



Scheme 1 Biosynthesis of colletodiol **1** and related metabolites in *Cytospora* sp. ATCC 20502



Scheme 2 *Reagents and conditions*: i, $NaBH_4$ (X = H) or $NaBD_4$ (X = D); ii, *p*-$MeC_6H_4SO_2Cl$, Et_3N; iii, NaH, 18-crown-6, tetrahydrofuran; iv, Ph_3P, imidazole, I_2; v, PhMe, I_2 heat; vi, dimethyl sulfoxide, *p*-$MeC_6H_4SO_2Cl$, Et_3N; vii, $BF_3{\cdot}OEt_2$, C_6H_6; $NaHCO_3$ (aq)

treatment with triphenylphosphine, imidazole and iodine in toluene.[12] NMR analysis of the product showed it to be a 1 : 2 mixture of the desired 2*E*,4*Z*,10*E*-triene **3** and the 2*Z*,4*E*,10*E*-isomer **9**. This mixture was separated by preparative direct phase HPLC to give **3** and **9** in isolated yields of 15 and 38%, respectively. Treatment of **9** with iodine in toluene converted it into a 2 : 3 mixture of **3** and **9** to allow further pure **3** to be isolated.

Reduction of colletoketol with sodium borodeuteride gave [4-^{2}H]colletodiol (**1**, X = D) and repeating the above syntheses with this labelled colletodiol gave the epoxide and triene specifically labelled at C-4 for metabolic studies. Each compound was separately fed to 3 day old cultures of *Cytospora* sp. and the colletodiol produced was isolated after a further 24 h. ^{2}H NMR analysis of both samples showed a single peak at δ 4.0 corresponding to 4-H of colletodiol. Mass

spectrometric analysis of the enriched metabolites indicates an enrichment level of less than 1%. There was no evidence of any indirect incorporation of label *via* degradation of the labelled precursors to acetate. In order to test whether any of the observed incorporation of label from the epoxide was due to *in vitro* hydrolysis of epoxide **6** to colletodiol, a number of control experiments were carried out. Unlabelled epoxide was incubated with uninoculated culture medium and also with a 3 day old culture of *Cytospora* sp ATCC 20502 which had been sterilised by autoclaving. Extraction of both incubations gave essentially quantitative recoveries of the epoxide in both cases. In contrast, addition of unlabelled epoxide (15 mg) to a growing 3 day old culture and extraction after a further 24 h, showed that only a small amount of epoxide (4 mg, 24%) remained. In addition, colletodiol (14 mg) and colletoketol (6 mg) were isolated. Finally, hydrolysis of the epoxide ($BF_3{\cdot}OEt_2$ in benzene, then aq $NaHCO_3$) gave, not the expected colletodiol, but the 4*S*,5*S*-diol **10** whose formation requires nucleophilic attack of water at C-5.

These studies firmly establish the intermediacy of both the triene **3** and epoxide **6** on the biosynthetic pathway to colletodiol and therefore that epoxidation and subsequent enzyme-mediated hydrolysis to the diol occur after formation of the macrodiolide ring. Attempted (biomimetic) oxidation of colletodiol to colletoketol under modified Pfitzer–Moffatt conditions[13] surprisingly gave the dione grahamimycin A_1 **7** as the only product. Presumably this is formed by oxidation of the non-allylic 5-hydroxy group of **1** to the 5-ketone followed by rearrangement of the double bond into conjugation with the ketone and subsequent tautomerisation to the 4,5-dione. The biosynthetic interrelationships suggested by these studies are summarised in Scheme 1.

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