Biosynthesis of Solanapyrone A, a Phytotoxin of *Alternaria solani*

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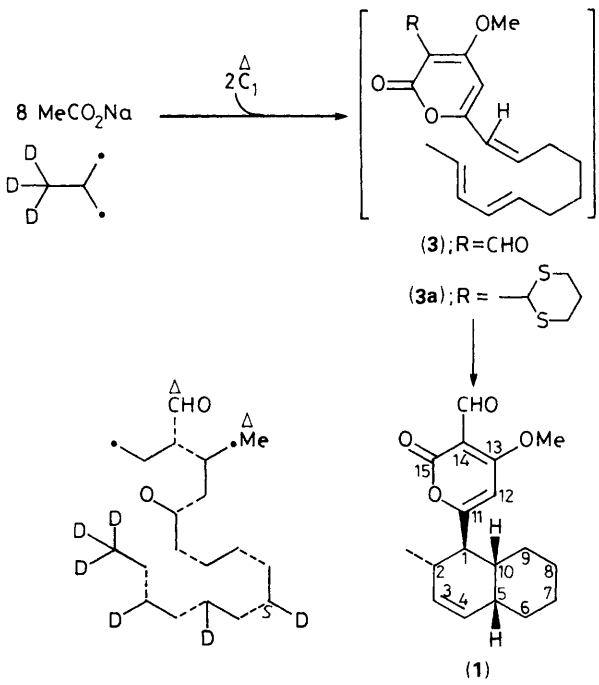
The building blocks and the origin of hydrogen and oxygen atoms in solanapyrone A (**1**) were determined by incorporation experiments employing sodium [1-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C, $^{18}O_2$]-, [1-^{13}C, $^{2}H_3$]-, [2-^{13}C, $^{2}H_3$]acetates and [*S*-$^{13}CH_3$]methionine; the labelling pattern supports the hypothesis that (**1**) is biosynthesized *via* the intramolecular Diels–Alder reaction.

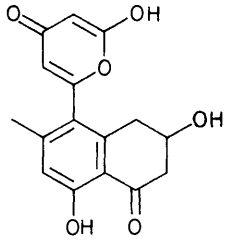
Recently, attention has focussed on possible intermediates in the construction of biologically active reduced polyketides by micro-organisms.[1—3] Among such metabolites, the production of decalin-based compounds such as betaenones[4] and mevinolin[5] may involve a Diels–Alder reaction during the biosynthesis. In the present work we report labelling experiments which support the intermediacy of such a process for yet another metabolite.

Solanapyrone A (**1**) is a phytotoxin produced by a pathogenic fungus, *Alternaria solani*. Its structure was deduced by spectroscopic analysis and chemical degradation,[6] and has been confirmed by the total synthesis.[7] The absolute configuration was established by the c.d. exciton chirality method with a dibenzoate derived from (**1**).[8]

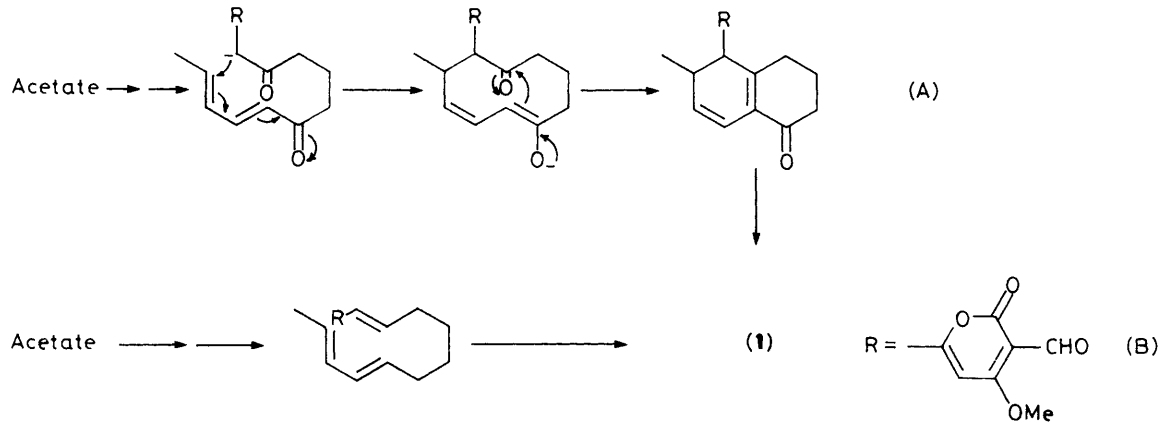
The assignment of the ^{13}C n.m.r. spectrum of (**1**) employed combined analysis of the normal ^{1}H n.m.r., ^{1}H {^{13}C} correlation, and ^{1}H COSY spectra as well as consideration of chemical shifts. This allowed unambiguous identification of all signals except those of C-11, C-13, and C-15 in the pyrone ring. These could be assigned from couplings to known carbons in the sample derived biosynthetically from [1,2-$^{13}C_2$]acetate (see below). For incorporation experiments,



Scheme 1



(2)



Scheme 2

Table 1. N.m.r. spectral assignment of solanapyrone A (**1**) and incorporation experiments.

Carbon atom	δ_C	J_{CC} /Hz[a,f]	Enrichment	Isotope shift (p.p.b.) ^2H $\alpha^{c,i}(\beta)^{b,h}$	Isotope shift (p.p.b.) $^{18}O^{a,g}$
2-CH_3	20.28	35.8		299, 590, 888	
C-1	47.94	49.9			
2	35.16	35.7	★[e]	(82, 157, 211)	
3	130.02	68.5		338	
4	131.53	68.5	★	(136)	
5	36.77	33.3		504	
6	29.72	32.8	★	(96)	
7	25.86	32.8		428	
8	21.00	33.3	★	(96)	
9	28.37	34.3			
10	36.03	34.2	★		
11	176.39	50.4	★		
12	95.86	58.7			
13	173.65	58.7	★		22
14	101.80	78.3			
15	162.28	77.8	★		36
CHO	186.60		o[d]		
OCH_3	57.72		o		

[a,b] Obtained in $CDCl_3$ at 67.9, and 125 MHz on a Jeol JNM-GX270, and a Bruker AM500 spectrometer, respectively. [c] Obtained in $CDCl_3$ (C_6F_6 lock) at 100 MHz on a Bruker WH400 spectrometer. [d,e,f,g,h,i] Sample of (**1**) derived from [*S*-$^{13}CH_3$]methionine, [1-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C, $^{18}O_2$]-, [1-^{13}C, $^2H_3$]-, and [2-^{13}C, $^2H_3$]acetate, respectively. ★ = Isotope labelled.

solutions of labelled precursors were added in equal amounts to two ten day old cultures of *A. solani* (150 ml per flask). Fermentation was continued for another 15 days. The ethyl acetate extract of the medium was chromatographed on a silica gel column to give (**1**) (yield: 15—30 mg per 300 ml medium). Administration of sodium [1-^{13}C]acetate enhances the resonances for C-2, C-4, C-6, C-8, C-10, C-11, C-13, and C-15 of (**1**). Similarly, the signals of aldehyde and methoxy carbons were enhanced by [*S*-$^{13}CH_3$]methionine. In the incorporation experiment of [1,2-$^{13}C_2$]acetate, the ^{13}C n.m.r. spectrum of enriched (**1**) shows eight pairs of coupled signals (Table 1). These results establish the basic building blocks of the carbon skeleton in (**1**) (Scheme 1), and confirm the assignment of all signals. A feeding experiment with sodium [1-^{13}C, $^{18}O_2$]acetate defines the origin of the oxygen atoms. The ^{13}C n.m.r. spectrum of the resulting sample of (**1**) displays upfield shifted signals for C-13 (+22 p.p.b.) and for C-15 (+36 p.p.b.). This result excludes the possibility that the C-16 polyketide chain is derived from a longer precursor by oxidative scission, and suggests that C-15 is the terminal carbon of the polyketide. Resolution of an isotopically shifted signal for C-11 was not possible owing to the natural broadness of the unenriched resonance.

The fate of hydrogen atoms of acetate could be ascertained by incorporation of [1-^{13}C, $^2H_3$]- and [2-^{13}C, $^2H_3$]-acetate. Upfield shifted signals due to the β- and α-isotope shifts of deuterium were easily visible. Shifted signals of C-2, C-4, C-6, and C-8 in the former experiment, and of 2-Me, C-3, C-5, and C-7 for the latter incorporation, indicate retention of deuterium at 2-Me, C-3, C-5, and C-7 (Table 1). To determine the stereochemical course of the polyketide enoyl thiol ester reductase,[9] assignment of diastereotopic methylene hydrogens at C-7 was necessary. Nuclear Overhauser enhancement (n.O.e.) studies together with chemical shift correlation and extensive decoupling experiments allowed assignment of the signals for the four methylene groups. Thus, the axial proton at C-7 appears at δ_H 1.27 whereas the corresponding equatorial hydrogen resonates at δ_H 1.73. In the ^2H decoupled ^1H {^{13}C} shift correlation spectrum of (**1**) derived from sodium [2-^{13}C, $^2H_3$]acetate, the isotopically shifted (CHD) carbon resonance for C-7 at δ_C 25.9 gives only one cross peak which correlates with the equatorial hydrogen (7-H_{eq}). This result demonstrates that the retained acetate deuterium resides only in the *pro-S* position on the growing polyketide chain, in agreement with results reported for other fungal polyketides.[9]

Initially several biogenetic paths to the decalin system appeared reasonable, and two representative routes are depicted in Scheme 2. However, the retention of deuterium from acetate at C-5 clearly rules out pathway A, as well as any involvement of aromatic intermediates [*e.g.* compounds related to mutactin (**2**)[10]]. The biological Diels–Alder reaction depicted in pathway B is especially attractive because it is consistent with the stereochemistry at C-1, C-2, C-5, and C-10, and explains the location of the double bond. In addition, the stereochemistry of the acetate deuteriums at C-5 and C-7, and the concurrence of the diastereoisomer of (**1**),[11] support this hypothesis. Although certain alternative routes based on anionic condensation cannot be rigorously excluded, simplicity and potential efficiency strongly favour the biological Diels–Alder route. In addition, frequent occurrence of polyene–pyrone systems in fungal metabolites[12] provides

circumstantial evidence for the intermediacy of triene (**3a**). Additional evidence for this biosynthetic proposal is discussed in the following Communication.

We thank Dr. A. Tajimi, Hokkaido National Agricultural Experimental Station, Sapporo, for the gift of the Strain *Alternaria solani*. Studies at the University of Alberta were supported by the Natural Sciences and Engineering Research Council of Canada.

Received, 22nd March 1989; Com. 9/01254K

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