Biosynthesis of the Polyether Antibiotic Monensin A: Incorporation of a Polyketide Chain Elongation Intermediate

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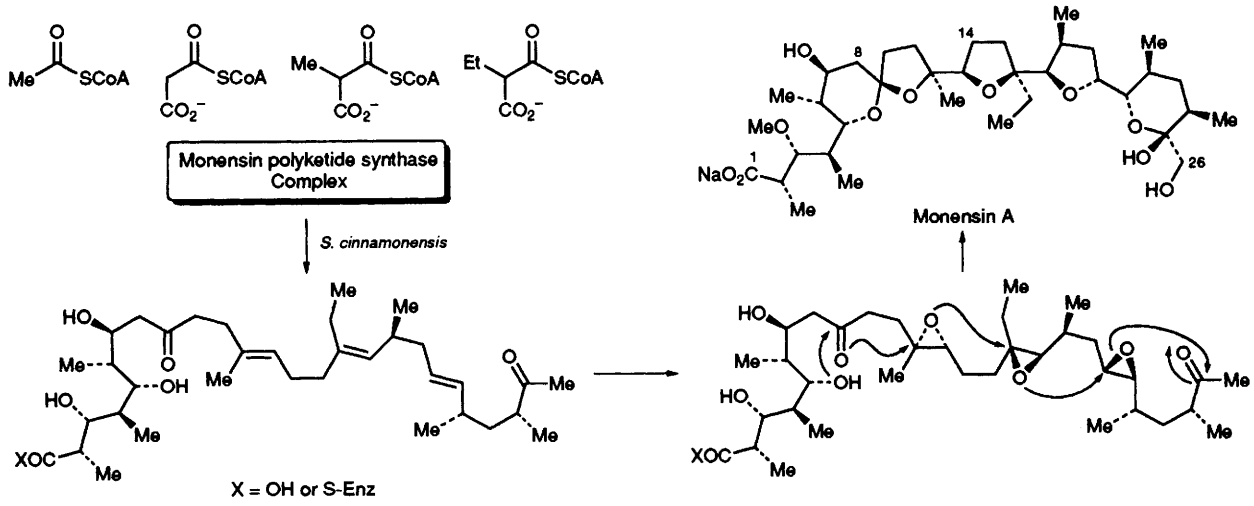
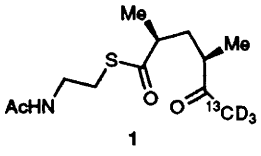
Evidence that the polyether antibiotic monensin A is constructed by a processive strategy of polyketide assembly has been obtained by the specific incorporation of labelled 5-oxo-2,4-dimethylhexanoate, activated as an acetylcysteamine thioester, into the antibiotic in shake cultures of *Streptomyces cinnamonensis*: crucial to the successful incorporation are the presence of 2,6-*O*-dimethyl-β-cyclodextrin and a β-oxidation inhibitor during the fermentation.

The polyethers are an important class of polyketide antibiotics produced mainly by *Streptomyces* species. Biosynthetic interest in these metabolites was greatly stimulated by labelling experiments[1] which supported a mechanism for ether ring formation involving a possible cascade of cyclisations on a putative polyepoxide intermediate,[2] as illustrated for monensin A[1a] in Scheme 1. Unfortunately, direct support for this hypothesis is still lacking. One important question concerns the strategy of polyether backbone assembly. In the biosynthetically related, and well studied, class of macrolide antibiotics, strong evidence in favour of the processive strategy of polyketide assembly has accumulated.[3] We report here the first evidence that a similar strategy is followed during polyether biosynthesis, through the specific incorporation of a polyketide chain elongation intermediate into the polyether monensin A.

Polyketide backbone assembly involves a series of decarboxylative condensations, much like those known to occur during fatty acid biosynthesis. Following each condensation, however, fatty acid synthases catalyse a complete cycle



Scheme 1 A possible biosynthetic pathway to monensin A



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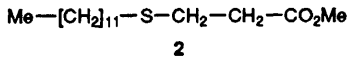

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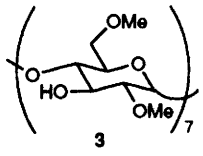

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consisting of a ketoreduction, dehydration and enoylreduction on the β-keto group of the growing fatty acid chain, whereas polyketide synthases, active for example in macrolide biosynthesis,[4] seem to introduce chemical functionality into the product by omitting or curtailing this cycle, after some or all condensation steps. Support for this processive strategy of assembly during monensin biosynthesis was sought here through the incorporation of the acyl chain of substrate **1** into monensin in cultures of *Streptomyces cinnamonensis*.

The substrate **1** was synthesised by methods[5] described previously. It is activated as a thioester to facilitate transfer of the acyl chain onto the polyketide synthase, and contains a [^{13}C,$^{2}H_3$]-labelled methyl group at C-6. Numerous early feeding experiments, in which this precursor (in admixture with the corresponding [6-$^{14}CH_3$] labelled material) was added at different times to shake cultures of *S. cinnamonensis*, gave monensin A without detectable incorporation of label. However, when **1** was administered along with the β-oxidation inhibitor[6] **2** and 2,6-*O*-dimethyl-β-cyclodextrin **3** to *S. cinnamonensis*, not only was monensin production strongly stimulated[7] by the presence of **3**, but the labelled substrate was now efficiently and specifically incorporated.

The mixture of **1** (50 mg), **2** (50 mg) and **3** (100 mg) was administered in five equal portions at 8 hourly intervals to a shake culture (60 ml) of *S. cinnamonensis*, during the period of peak antibiotic production, in a chemically defined medium.[8] The monensin A (16 mg) isolated from this culture showed only one additional signal in the $^{13}C\{^{1}H,^{2}H\}$ NMR spectrum, 0.68 ppm upfield of the normal C-26 resonance due to the isotope shift,[9] which collapsed to a barely observable multiplet when deuterium decoupling was removed [Fig. 1(*b*)]. More dramatically, however, the $^{2}H\{^{1}H\}$ NMR spectrum of this labelled monensin showed (only) two doublets at δ 3.12 and 4.04 [Fig. 1(*c*)], the former slightly upfield† (by 0.14 ppm) and the latter slightly downfield† (by 0.09 ppm) of the normal ^{1}H chemical shifts[1d] of the diastereotopic protons at C-26 in monensin A, and these doublets collapsed to two singlets in the $^{2}H\{^{1}H,^{13}C\}$ spectrum, as the ^{13}C-^{2}H coupling of 19 Hz was removed [Fig. 1(*d*)]. The C-26 position is, therefore, labelled as an intact $^{13}C^{2}H_2$ group, which is only possible if the precursor is specifically incorporated at the beginning of chain assembly, without prior degradation to labelled acetate. In previous work,[10] feeding [^{13}C,$^{2}H_3$]acetate to *S. cinnamonensis* led to incorporation of ^{13}C label only (no deuterium) at C-26, whereas all the other methyl groups formally derived from C-3 of propionate were also efficiently labelled with intact $^{13}C^{2}H_3$ groups. This labelling pattern was not observed upon incorporation of the acyl chain of **1** into monensin.

† Deuterium at each diastereotopic position on C-26 has deuterium as a neighbour rather than protium, so a small isotope-induced change in the ^{2}H chemical shift, relative to the normal ^{1}H chemical shift, is to be expected.

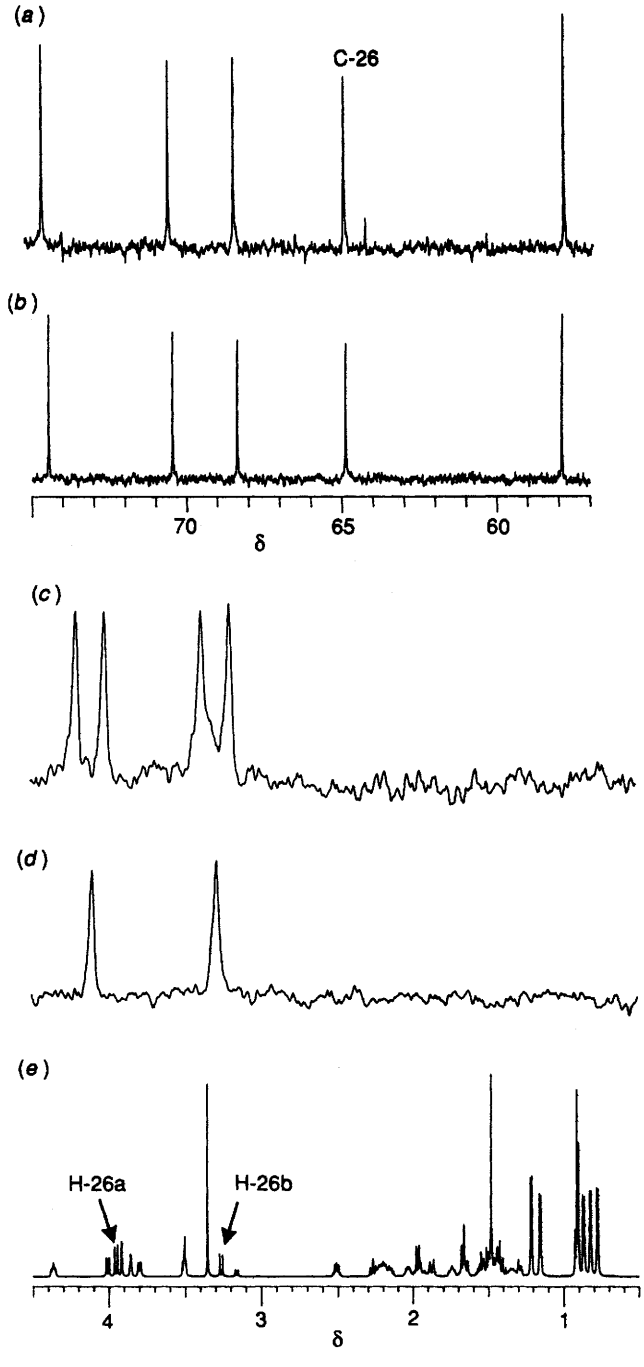

Fig. 1 Sections from (*a*) $^{13}C\{^{1}H, ^{2}H\}$ and (*b*) $^{13}C\{^{1}H\}$ NMR spectra of the labelled monensin A. (*c*) The $^{2}H\{^{1}H\}$, (*d*) $^{2}H\{^{1}H, ^{13}C\}$ (at 92 MHz) and (*e*) ^{1}H NMR spectra (at 600 MHz) of the labelled monensin A.

In the absence of **3**, the solubility and stability of **1** towards hydrolysis of the thioester, in the fermentation, were greatly reduced. The cyclodextrin **3** when added alone stimulates monensin production, and may, conceivably, also stimulate the uptake of **1** into the cells. These problems of solubility, stability and uptake of the precursor, combined with a voracious β-oxidation activity in *S. cinnamonensis*, are likely reasons for the failure of previous incorporation attempts, problems which appear to have been solved by cofeeding **1** with **2** and **3**.

In summary, the intact incorporation, reported here, of the acyl chain of **1** into monensin A provides the first evidence that a processive strategy of assembly is operative during polyether biosynthesis. Moreover, the observation that 2,6-*O*-dimethyl-

β-cyclodextrin can stimulate the uptake and incorporation of this intermediate may prove generally useful in future studies of polyether biosynthesis.

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