Biosynthesis of the Polyketide Polyether Antibiotic ICI 139603 in *Streptomyces longisporoflavus* from ^{18}O-Labelled Acetate and Propionate

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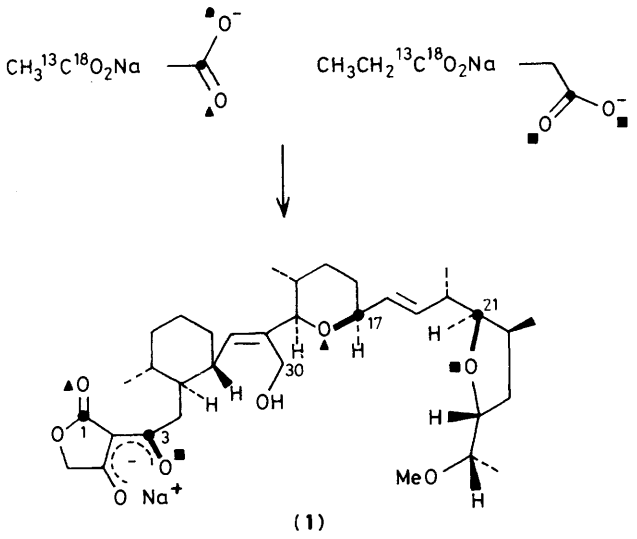
The polyether antibiotic ICI 139603 retains oxygen from acetate at C-1 and C-17, and from propionate at C-3 and C-21, after incorporation of [1-^{13}C,1-$^{18}O_2$]acetate and [1-^{13}C,1-$^{18}O_2$]propionate, and on this basis the tetrahydropyran and cyclohexane rings may be formed concertedly in a novel biosynthetic cyclisation.

Recently we have reported results which determined the sequence of biosynthetic building blocks in the polyketide polyether antibiotic ICI 139603 (**1**).[1] In a preliminary investigation of the reactions which generate the four rings in the antibiotic using deuterium labelled precursors, we were able to rule out any mechanism of carbocyclic ring closure which involved a carbonyl group at C-30, and we suggested other possible mechanisms.[2] In this paper we report results of oxygen isotope incorporations, again from simple precursors, which have enabled us to propose detailed mechanisms of ring closure for both the carbocyclic ring and the reduced furan and pyran rings.

In separate experiments ICI 139603 (**1**) was labelled by incorporation of [1-^{13}C,1-$^{18}O_2$]acetate and [1-^{13}C,1-$^{18}O_2$]-propionate. Each precursor (200 mg) was fed to a culture of *Streptomyces longisporoflavus* (300 ml) over days 2—6 before isolation and purification of (**1**) on day 7 as previously described.[1] Incorporation of ^{18}O was detected by the presence of isotopically shifted signals in the ^{13}C n.m.r. spectra.[3] The spectra gave evidence of incorporation of intact ^{13}C–^{18}O units at C-1 and C-17 from acetate, and at C-3 and C-21 from propionate. These results are summarised in Scheme 1.

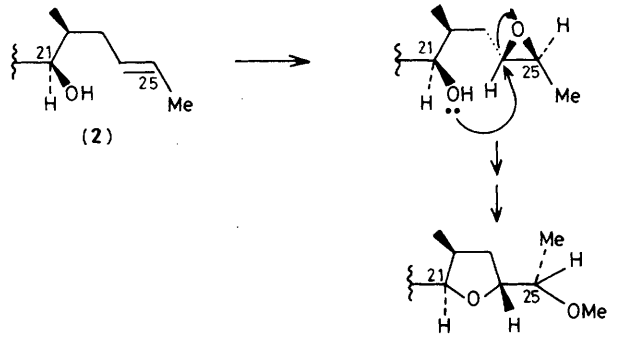
Some years ago it was suggested that the biosynthesis of polyketide macrolide and polyether antibiotics may take place on enzymes similar to those involved in fatty acid biosynthesis,[4] and recent results, notably those of Hutchinson[5] and of Cane *et al.*,[6] have supported this view. In the following discussion of the implications of these results we suggest possible uncyclised precursors, each of which contains an array of functional groups which might reasonably be generated by standard reactions used in fatty acid biosynthesis.

The retention of oxygen from propionate at C-21, and the lack of retention of oxygen from acetate at C-25, strongly suggest that the tetrahydrofuran ring is formed from an initial



(1)

Scheme 1. Only retention of intact ^{13}C–^{18}O units is shown.



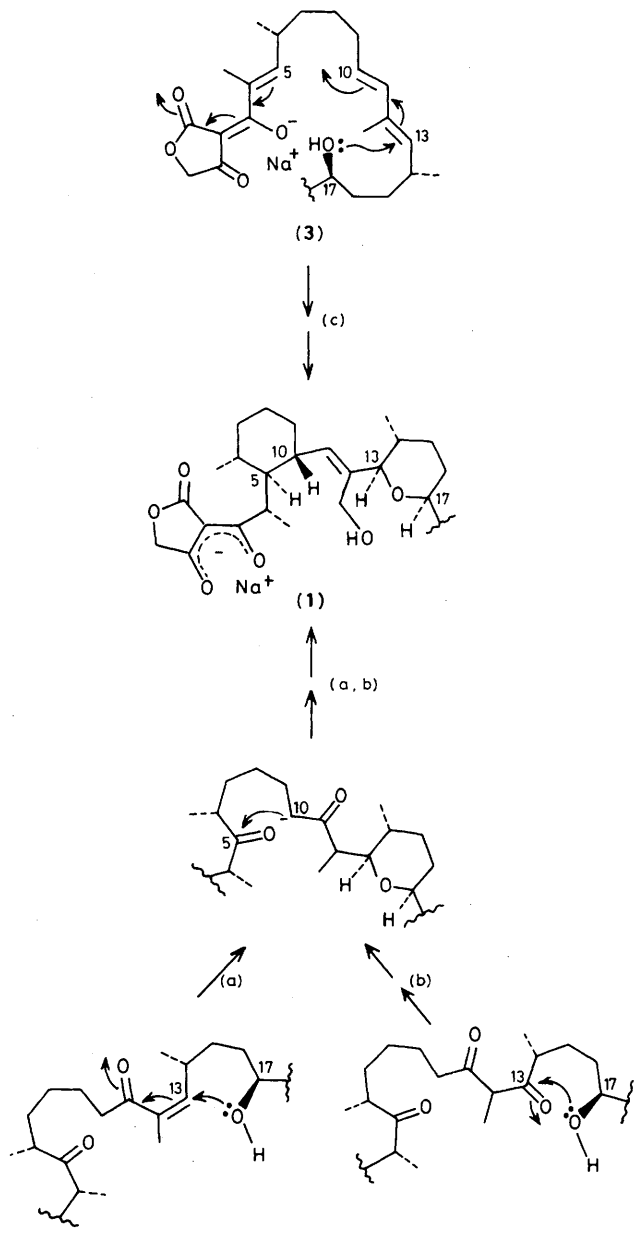
(2)

Scheme 2

intermediate containing the residue (**2**) shown in Scheme 2. The reactions in this scheme are similar to those suggested for the biosynthesis of lasalocid A,[7] asperlactone and aspyrone,[8] and monensin.[6]

On the basis of the retention of oxygen from acetate at C-17, and our earlier results from deuterium isotope retention studies,[2] several mechanisms of ring closure for the tetrahydropyran ring can be proposed. In pathways (a) and (b) of Scheme 3 the tetrahydropyran ring is formed by cyclisation of a hydroxy group at C-17 onto either a carbonyl group or an enone; subsequently the carbocyclic ring may be formed by attack of an anion at C-10 on an electrophilic centre at C-5, possibly a carbonyl group as shown, or the double bond of an enone as we suggested earlier.[2] All variants of these two pathways require one or more unexceptional dehydrative and reduction steps to generate the structural residues present in the antibiotic, following the steps shown. In contrast, the alternative proposal in pathway (c) leads directly from a plausible precursor with the part structure (**3**) to the equivalent part structure of the antibiotic (**1**), with possibly concerted generation of both tetrahydropyran and cyclohexane rings.

Inspection of a Corey–Pauling–Koltun (CPK) space-filling model of (**3**) shows that it can readily fold with the diene in the *transoid* conformation to bring both the electrophilic carbon at C-5 and the nucleophilic hydroxy at C-17 into the required orientation for a concerted *syn* addition at C-10 and C-13, respectively. Moreover, this conformation of (**3**) strongly



Scheme 3

resembles that adopted by (**1**) in its ionophore complex, and it might therefore be favoured by folding around a metal ion such as Na^+, Mg^{2+}, or Zn^{2+} especially in molecules where there is a suitable array of oxygen substituents at C-21, C-24, and C-25 (for examples see Scheme 2). It is possible, therefore, that a true biosynthetic intermediate with the part structure (**3**) might undergo the proposed cyclisation without the aid of an enzyme *in vitro*, or even *in vivo*, given catalysis by a suitable cation.

On the basis of this proposal the observed stereochemistries at C-4, C-5, C-10, C-11, C-12, and C-13 of (**1**) would be dictated by stereoelectronic or steric factors operating in the cyclisation process, and both the cyclohexane and tetrahydropyran rings would be formed in chair conformations with all groups equatorial. Variants of the proposed cyclisation process are possible involving different conformations of (**3**), or certain relatives of (**3**) which are isomeric at one or more of the double bonds.

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