Biosynthesis of the Polyether Antibiotic ICI139603† in *Streptomyces longisporoflavus*: Investigation of Deuterium Retention after Incorporation of $CD_3{}^{13}CO_2H$, $^{13}CD_3CO_2H$, and $CH_3CD_2{}^{13}CO_2H$ using ^2H N.M.R. and edited ^{13}C N.M.R. Spectra

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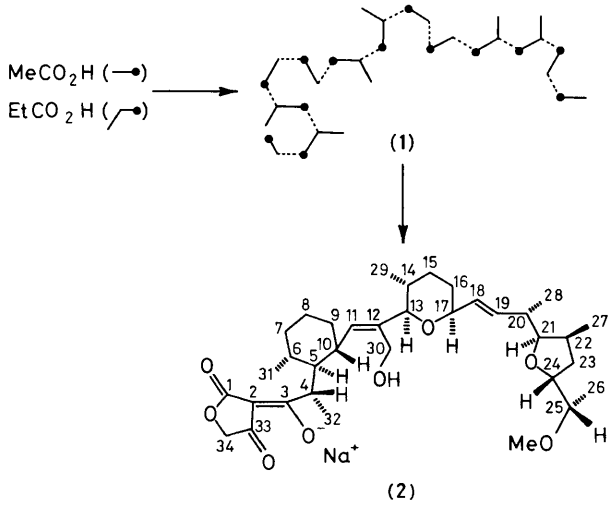
The mechanism of carbocyclic ring formation in the biosynthesis of the polyether antibiotic ICI139603 (**2**) was investigated by deuterium retention studies after incorporation of $CD_3{}^{13}CO_2H$, $^{13}CD_3CO_2H$, and $CH_3CD_2{}^{13}CO_2H$ using ^2H n.m.r. spectroscopy, α- and β-isotopic shifts, and edited ^{13}C n.m.r. spectroscopy.

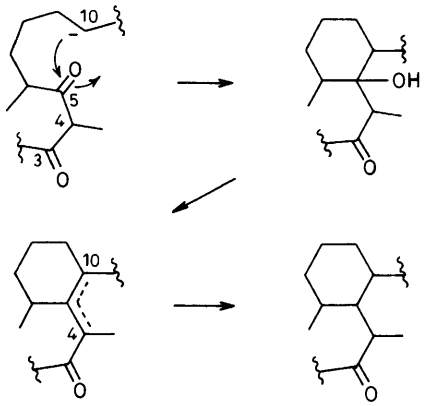
In the preceding communication[1] we reported that the polyether antibiotic ICI139603 (**2**) is labelled by acetate and propionate in a manner typical of polyketide biosynthesis (Scheme 1). On this basis it is possible that an uncyclised intermediate having the skeleton structure (**1**) is formed initially. In the later stages of the biosynthesis, a C_2 unit is added at C-2, and the various rings are formed by cyclisation of suitably functionalised residues.

† Previously named M139603, ref. 1.

Of particular interest is the formation of the unusual cyclohexane ring. By analogy with carbocyclic ring formation in the biosynthesis of aromatic polyketides, it is suggested in Scheme 2 that this cyclisation is achieved by an aldol reaction between a carbanion at C-10 and a carbonyl group at C-5, followed by dehydration to either C-4 or C-10, and reduction. Alternatively, the electrophilic reactivity at C-5 could be provided by a carbon–carbon double bond between C-4 and C-5 conjugated with the carbonyl group at C-3. Structures (**3**), (**4**), and (**5**) show how the proposed carbanion at C-10 could be stabilised by conjugation with a suitably placed carbonyl



Scheme 1



Scheme 2

group either directly or through an adjacent double bond. Another form of nucleophilic reactivity at C-10 could be realised as in (**6**), by a double bond between C-10 and C-11, in which case the cyclisation process would be reminiscent of those taking place in terpene biosynthesis, and might involve a carbonium ion intermediate. All but one of the proposed arrays of functional groups could exist in the initial uncyclised intermediate corresponding to (**1**) as a consequence of standard reactions involved in building the chain on a polyketide synthase. The exception is the aldehyde group at C-30, which would need to be generated at a later stage by oxidation of a methyl group; it is noteworthy that this carbon is hydroxylated in the final metabolite.

We sought to investigate these mechanisms by determining, for key sites in (**2**), the extent to which deuterium is incorporated from [2-$^2H_3$]acetate and [2-$^2H_2$]propionate, using both the α- and β-shift approaches.[2,3] Precursors (500 mg) were fed to a culture of *Streptomyces longisporoflavus* (300 cm^3) over days 2 to 6 before isolation and purification of (**2**) on day 7 as described previously.[1]

A ^2H n.m.r. spectrum of (**2**) after incorporation of $CD_3{}^{13}CO_2H$ gave evidence for deuterium incorporation at C-11 and C-19, and at other sites in the aliphatic region. The incorporation of deuterium at C-11 and C-19, which are derived from the carboxy group of propionate, was at first sight surprising. It can be explained by the reduction of carbonyl groups by deuteride transfer from nicotinamide

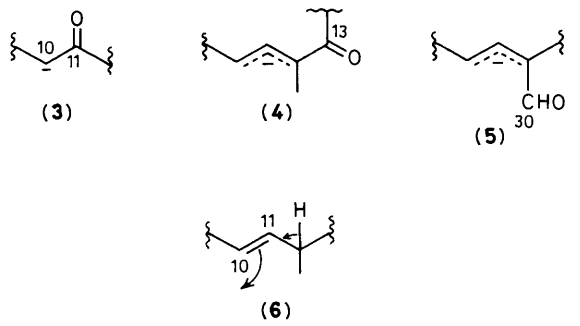


Table 1. Relative proportions of ^{13}C labelled species incorporated from $^{13}CD_3CO_2H$ into propionate-derived carbon atoms of ICI139603, (**2**).

Carbon	$^{13}CH_3$	$^{13}CH_2D$	$^{13}CHD_2$
27	0.5	0.1	0.4
28	0.4	0.3	0.3
29	0.5	0.1	0.4
30[a]	0.4	0.3	0.3
31	0.4	0.3	0.3
32	0.5	0.2	0.3

[a] For C-30 the labelled species were $^{13}CH_2OH$, $^{13}CHDOH$, and $^{13}CD_2OH$.

coenzymes which have themselves been labelled with deuterium from C-2 of acetate. This has also been noted by White in studies of fatty acid biosynthesis from CD_3CO_2H in *Escherichia coli*.[4] The signals in the aliphatic region could not be assigned to specific sites because of inadequate resolution. It was apparent, however, that there was no deuterium at C-18 or C-10, both sites being derived from the methyl group of acetate. The ^{13}C n.m.r. spectrum was similarly uninformative because no β-shifted peak could be detected corresponding to retention of deuterium in any of the acetate units at sites derived from the methyl group of acetate. Similarly, in an equivalent experiment, with $CH_3CD_2{}^{13}CO_2H$ as precursor, there was no evidence for a β-shifted peak at C-3 corresponding to deuterium retention at C-4; nor was there evidence for retention at any other site derived from C-2 of propionate.

It was reasoned that the α-shift technique combined with spectral editing might be more successful in locating the aliphatic sites into which the deuterium was incorporated from the methyl group of acetate. Accordingly, $^{13}CD_3CO_2H$ was administered to the organism and sites of deuterium labelling were determined from α-shifted peaks in a $\{^1H,^2H\}$ decoupled ^{13}C n.m.r. spectrum. Again, ^{13}C was incorporated efficiently (5%) in all the acetate derived C_2 units, but none of these sites, not even the presumed 'starter unit' methyl, C-26, gave rise to the isotopically shifted peaks which would be expected if one or more deuterium atoms had been retained. Similar results have also been obtained for lasalocid A biosynthesis by Hutchinson *et al.*,[5] on incorporating the same precursor.

Fortunately, even though there was no evidence in any of the experiments described so far for deuterium retention at C-4 and C-10, the $^{13}CD_3CO_2H$ feeding did yield useful information about the closure of the carbocyclic ring, because there was a substantial incorporation of ^{13}C label into all three carbons in each of the six propionate units. This may arise from conversion of the labelled acetate to succinate, which is in turn isomerised to methylmalonyl-coenzyme A prior to incorporation into the C_3-units. These units did retain

significant amounts of deuterium in their methyl groups. Thus in the ^{13}C n.m.r. spectrum, the signals corresponding to C-27, C-28, C-29, C-30, C-31, and C-32 each showed two significantly strong, isotopically-shifted peaks corresponding to molecules with one and two attached deuterium atoms respectively (see Table 1).

The identity of these isotopically shifted peaks was then confirmed using edited ^{13}C n.m.r. spectroscopy employing the pulse sequences described previously.[6] The subspectrum (quaternary only), generated by the pulse sequence which identifies carbons without directly attached hydrogens, showed the presence of a signal for C-30, and so proved that a significant proportion of the molecules retained two deuterium atoms at that site. This would not be expected if this site had been oxidised to an aldehyde group at any stage in the biosynthesis (the degree of multiple labelling was too high to be explained by chance re-incorporation of deuterium from deuteriated nicotinamide-coenzyme, following the precedent set at C-11 and C-19). Thus any mechanism for carbocyclic ring closure relying on part structure (**5**) can be ruled out. Incidentally, the absence in this subspectrum of signals corresponding to the C-methyl groups confirms that these groups retain at most two deuterium atoms, as would be expected for an incorporation of the label *via* succinate.

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