Biosynthesis of the Polyketide Antibiotic ICI139603† in *Streptomyces longisporoflavus*: Assignment of the ^{13}C N.M.R. Spectrum by Two-dimensional Methods, and Determination of the Origin of the Carbon Atoms

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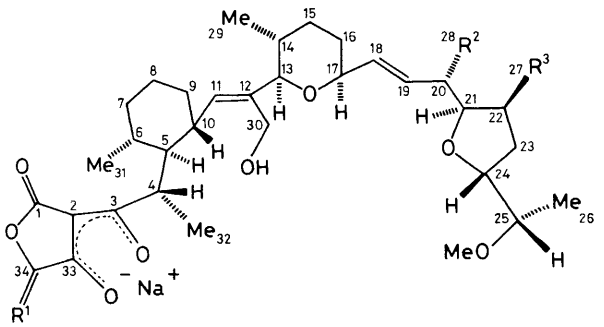
The carbon skeleton of the polyether antibiotic ICI139603 (**1**) is shown to consist of a polyketide chain, derived from seven acetate units and six propionate units, combined with a C_2-unit of unknown origin.

The polyether antibiotic ICI139603 (**1**) has an unusual structure,[1] which contains a tetronic acid moiety and a six membered carbocyclic ring. These features are also found in the structurally similar tetronomycin (**2**), which, intriguingly from a biosynthetic point of view, has the opposite stereochemistry at all ten chiral centres.[2] From inspection of the structure it seemed likely that the carbon skeleton is derived from a linear combination of acetate and propionate units. It is not clear whether the starter unit is C-26 + C-25 or C-34 + C-33, however, and so the chain could in principle run in either direction. We are presently studying the biosynthesis of (**1**) in order to determine the mode of assembly of the chain, and also to determine the mechanisms of ring closure and stereocontrol in the biosynthesis of this and related polyether antibiotics.

In order to determine the origin of all the carbon atoms we first sought to assign unambiguously the ^{13}C n.m.r. spectrum of which only one signal had been previously assigned.[3] This was accomplished by a combination of ^1H–^{13}C and ^{13}C–^{13}C two dimensional correlation spectroscopy, after finding the multiplicities of the signals in the ^{13}C n.m.r. spectrum using DEPT.[4] In the ^{13}C n.m.r. spectrum one of the two ketone carbon resonances shows long range coupling to two protons; it must, therefore, be assigned to C-33, the other being assigned to C-3. The remaining carbonyl resonance was accordingly assigned to the lactone carbon C-1. In the ^1H n.m.r. spectrum, the signals due to the three vinylic protons, H-11, H-18, and H-19, were then assigned on the basis of their couplings. From these the signals for H-10, H-20, H-21, and H-22, which were clearly resolved, could also be unambiguously assigned. Having made these proton assignments the carbon atoms to which they were bonded were assigned using ^1H–^{13}C two dimensional correlation spectroscopy.[5] All the remaining unassigned carbon signals were then assigned using the two dimensional INADEQUATE technique for ^{13}C–^{13}C correlation spectroscopy.[6] The complete assignment is given in Table 1. The ^1H n.m.r. spectral assignments were essentially the same as those for (**2**).[2,3]



(**1**) $R^1 = H_2$, $R^2 = R^3 = Me$
(**2**) $R^1 = CH_2$, $R^2 = R^3 = H$

The stereochemistry shown is that for (**1**).

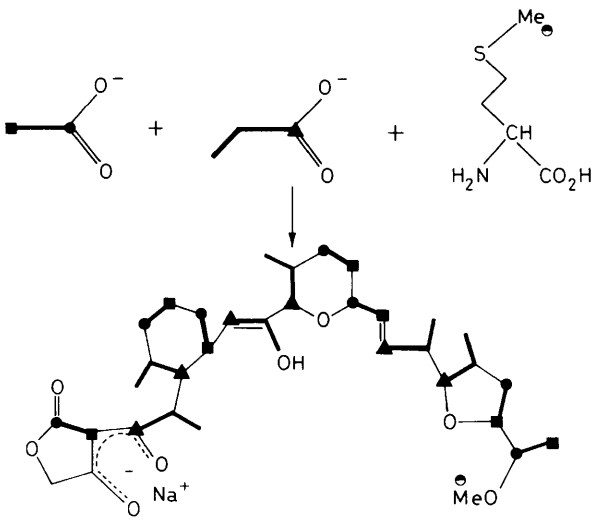
After the ^{13}C n.m.r. spectrum had been assigned, biosynthetic experiments were carried out in which (**1**) was labelled by incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate, [1-^{13}C]propionate, and [Me-^{13}C]methionine using the following regimen. Each precursor (500 mg) was fed to a culture of *Streptomyces longisporoflavus* (500 ml) over days 3, 4, and 5 before isolation of (**1**) on day 6 by methanol extraction of the mycelium and purification by ion-exchange chromatography. The ^{13}C n.m.r. spectra of the samples of labelled (**1**) identified the building blocks which make up the carbon skeleton as indicated in Scheme 1. The [1,2-$^{13}C_2$]acetate, in addition to labelling seven intact C_2-units (enrichment 7%), also labelled each of the six propionate derived C_3-units with a lower enrichment (1.5%). Similarly the [1-^{13}C]acetate labelled C_2-units as expected (enrichment 7%) and also labelled the carbon atoms derived from C-1 of propionate (enrichment 2%). One explanation for these results is that succinyl-coenzyme A (-CoA), labelled by incorporation of the acetate *via* the citric acid cycle, is isomerised to methylmalonyl-CoA by a methylmalonyl-CoA mutase,[7] before incorporation into the C_3-units of the antibiotic. The labelling of the carbon atoms derived from C-1 of propionate by [1-^{13}C]acetate would then be explained by interconversion of [4-^{13}C]- and [1-^{13}C]-

Table 1. Assignment of the ^{13}C n.m.r. spectrum of ICI139603, (**1**), for a 0.8 M solution in CD_2Cl_2 at 62.9 MHz.

Carbon	δ/p.p.m.[a]	Carbon	δ/p.p.m.[a]
1	177.44	18	131.17
2	96.51	19	140.51
3	201.07	20	39.48
4	43.25	21	86.32
5	48.03	22	34.54
6	34.34	23	35.67
7	35.98	24	79.63
8	26.13	25	79.12
9	35.24	26	10.75
10	36.58	27	13.79
11	141.11	28	16.68
12	130.98	29	18.27
13	91.02	30	56.60
14	33.02	31	19.80
15	32.60	32	8.63
16	32.38	33	192.87
17	79.19	34	70.26
		-OMe	57.25

[a] Chemical shifts relative to CD_2Cl_2 (53.68 p.p.m.).

† Previously named M139603, ref. 1.



Scheme 1

succinyl-CoA *via* succinate before conversion into methylmalonyl-CoA.

This sequence of chain assembly and the resulting stereochemistry at the various chiral centres are not consistent with either of the stereochemical prototypes recently proposed by Cane *et al.*[8] ICI139603 may represent a new stereochemical class of polyether antibiotics.

Two carbon atoms (C-33 and C-34) of the tetronic acid ring remained unlabelled in all the above feeding. Previous studies on the biosynthesis of tetronic acids have shown that the ring carbon atoms can be derived entirely from acetate, *e.g.* in multicolic acid.[9] Alternatively, they may be produced by the 'phenylpropanoid' pathway, *e.g.* in the pulvinic acids[10] or derived from acetate and a C_4 citric acid cycle intermediate (probably oxalacetate), *e.g.* in carolic acid.[11] In the biosynthesis of geldanamycin, Rinehart *et al.* have reported that two C_2 units both originate from glycollate and glycerate.[12] More recently, the precursor C_2 unit to C-3 and C-4 of leucomycin A_3 has been shown by Omura *et al.*[13] to be glycollate (derived from glycerol). Preliminary studies with ^{14}C labelled precursors indicate that both glycollate and glycerate are incorporated efficiently into (**1**).

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