Biosynthesis of Citromycetin: Incorporation of [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]Acetates

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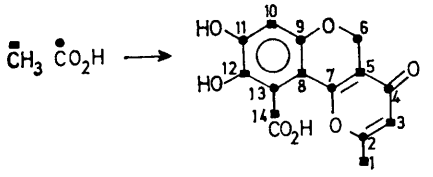
Summary Citromycetin is derived from seven intact acetate units linked head to tail.

ON the basis of earlier experiments[1] with [1-^{14}C]acetate it has been proposed that citromycetin (**1**) incorporates seven units of acetate as shown in Scheme 1. This labelling

pattern shows an unusual and interesting feature for a polyketide metabolite in having a branch in the polyketide chain at C(5). Unfortunately, however, relatively few degradations are known for citromycetin and so the degree of incorporation was determined by direct measurement only for C(6) and certain carbon atoms located in the pyrone ring. For the remainder of the molecule only minimal support was obtained for the proposed labelling pattern. In further experiments[2] it was found that citromycetin



(1)

SCHEME 1

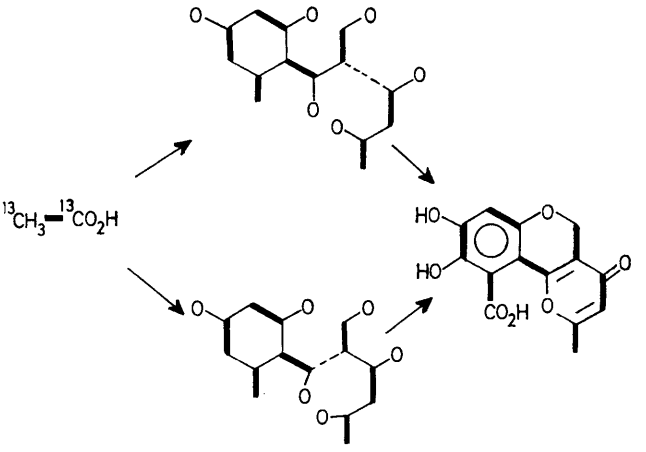
incorporated activity from [2-^{14}C]malonate to produce a non-uniform distribution of activity in which C(1) and C(14) each carried less activity than the remaining labelled carbons. On this basis it has been proposed that two initial chain units are involved, with C(2) and C(13) being the corresponding carboxy-derived carbon atoms but a complementary experiment with [1-^{14}C]malonate has not been carried out to test this assumption.

TABLE ^{13}C n.m.r. data for citromycetin

Carbon	Carbon chemical shifts/p.p.m. (rel. to Me_4Si)	^{13}C–^{13}C coupling in (1) labelled with [1,2-$^{13}C_2$]acetate/Hz
1	19·3[a]	51·3
2	167·3[b]	51·1
3	114·2[a]	56·2
4	178·2[b]	56·4
5	112·6[a]	47·9
6	63·4[b]	48·3
7	158·2[b]	67·4
8	105·2[a]	67·3
9	153·7[b]	72·5
10	105·9[a]	72·7
11	152·9[b]	70·2
12	141·7[a]	70·6
13	117·9[b]	72·0
14	170·0[a]	72·0

[a] Enhanced in intensity after incorporation of [1-^{13}C]acetate.
[b] Enhanced in intensity after incorporation of [2-^{13}C]acetate.

In view of the interesting nature of these results we have decided to re-examine the pattern of incorporation of acetate using ^{13}C-labelled precursors and have obtained the results as shown in the Table. The assignments of signals in the ^{13}C n.m.r. spectrum are based on calculated values of



SCHEME 2

chemical shifts and are supported by off-resonance-decoupling experiments, pulse relaxation techniques,[3] and the observed ^{13}C–^{13}C couplings in the spectrum of citromycetin derived from [1,2-$^{13}C_2$]acetate. The complementary experiments with [1-^{13}C]acetate and [2-^{13}C]acetate (Table) establish rigorously and completely the pattern of incorporation shown in Scheme 1. The experiment with [1,2-$^{13}C_2$]acetate shows that all the C_2 units are intact and that the direction of the polyketide chain at the key branching point at C(5) is as shown in Scheme 2: thus this carbon forms an intact C_2 unit with C(6) rather than C(7). Taken together with the earlier evidence for two initial chain units these results point to a biosynthesis along one of the two pathways[4] shown in outline in Scheme 2.

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