Biosynthesis of the Indolizidine Alkaloid, Cyclizidine

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The ^1H and ^{13}C n.m.r. spectra of cyclizidine (**1**) have been completely assigned and incorporation experiments with ^{13}C-labelled sodium acetate and propionate and sodium [3-$^2H_3$]propionate have revealed that the entire carbon skeleton of (**1**) is derived from these precursors by a polyketide-type biosynthetic pathway, with the cyclopropyl ring being derived from a single propionate unit.

Cyclizidine (**1**), which was isolated recently from a new *Streptomyces* species, NCIB 11649,[1] and the subsequently reported indolizomycin (**2**),[2] are the only indolizidine alkaloids known to be produced by *Streptomyces* species. Cyclizidine is especially unusual because of the cyclopropyl ring at the chain terminus which is unique among natural products. The biosynthetic pathway to cyclizidine is therefore a matter of particular interest.

The biosynthetic route determined for simple indolizidines in fungi is from lysine which is the precursor of the piperidine moiety.[3] However this derivation of piperidine rings has not been observed in *Streptomyces*. The alkaloids nigrifactin (**3**)[4] and dihydrolatumcidin (**4**)[5] are known to be biosynthesized from acetate in a polyketide fashion and pyrindicin (**5**)[6] is derived in a similar way from five acetates and one propionate unit. For cyclizidine, therefore, the first task was to determine the origin of the carbon skeleton, whether it was derived from lysine, or acetate + methionine, or acetate + propionate, or even conceivably by an isoprenoid pathway.

When [1-^{14}C]acetate was fed to the *Streptomyces* strain, the cyclizidine isolated showed a specific incorporation of 2.4%, sufficient to attempt incorporation experiments with stable isotopes. The growth conditions were however critical, as when the shaking speed was reduced from 250—300 r.p.m. to *ca.* 150 r.p.m., both the yield of cyclizidine and the specific incorporation of [1-^{14}C]acetate (0.12%) were very much reduced. [Me-^{14}C]Methionine showed negligible incorporation.

The ^1H and ^{13}C n.m.r. spectra of cyclizidine were totally assigned by standard two-dimensional techniques (COSY) and decoupling and the ^{13}C assignments were confirmed by ^{13}C–^{13}C connectivity. The assignments, which are shown in Table 1, extend the previously published ones[1] and differ from the tentative ^{13}C assignments at C-3, 7, 8, and 8a.

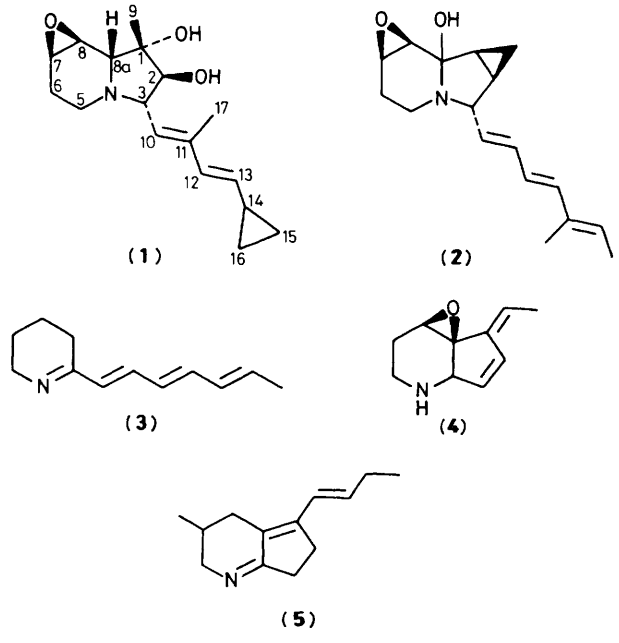
On incorporation of [1-^{13}C]acetate, a ^{13}C enrichment (*ca.* 1.5%) was observed for four carbons, C-2, 5, 7, and 12. This shows that the piperidine ring is not derived from lysine but is most probably of polyketide origin. If C-5 is assumed to be the carboxy terminus of the polyketide chain then the intact acetate units are C-5–C-6, C-7–C-8, C-2–C-3, and C-12–C-13 as illustrated in Scheme 1. This arrangement of acetate units has subsequently been confirmed by incorporation of [1,2-$^{13}C_2$]acetate. There remain three isolated three-carbon units to be accounted for. Therefore the next experiment was incorporation of [1-^{13}C]propionate. The ^{13}C n.m.r. spectrum of the cyclizidine from this experiment showed a strong (*ca.* 7%) enrichment of three carbons, C-8a, C-10, and C-14. This is exactly as predicted for the polyketide pathway, which therefore accounts for the origin of the entire carbon skeleton (see Scheme 1).

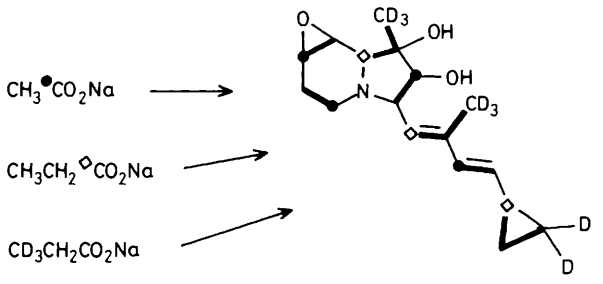
The major difference between cyclizidine (**1**) and the other known indolizidine from *Streptomyces*, indolizomycin (**2**), is that the cyclopropyl group of (**1**) has been replaced by a four-carbon acyclic unit in (**2**). In view of this intriguing difference, and the fact that a cyclopropyl ring at the end of a chain has not been observed in any other natural product, it was important to establish that all three carbons are indeed derived from propionate. In order to do this sodium [3-$^2H_3$]propionate was synthesized by alkylation of the anion from t-butyl acetate with CD_3I, followed by hydrolysis of the

Table 1. N.m.r. data for cyclizidine (**1**).

Position	δ(^1H)	δ(^{13}C)
1	—	78.1
2	3.58	86.4[a]
3	2.80	68.3
5	2.57	41.9[a]
	ca. 1.98	
6	2.08	26.1
	ca. 1.98	
7	3.23	51.1[a]
8	3.31	51.6
8a	2.30	69.2[b]
9	1.34[c]	16.9
10	5.21	127.7[b]
11	—	138.1
12	6.18	131.8[a]
13	5.17	133.9
14	1.43	14.1[b]
15	0.76[c]	7.2
16	0.40[c]	7.2
17	1.76[c]	13.1

[a] Enriched from [1-^{13}C]acetate. [b] Enriched from [1-^{13}C]propionate. [c] Enrichment in ^2H n.m.r. from [3-$^2H_3$]propionate.





Scheme 1

ester, and was fed to the organism. ^2H N.m.r. spectroscopy of the cyclizidine produced revealed a large incorporation of deuterium into both methyl groups (δ 1.76 and 1.43) and into two cyclopropyl positions (δ 0.76 and 0.40) as expected for the presence of two deuterium atoms on one carbon of the three-membered ring (see Scheme 1). This result is consistent with derivation of the entire cyclopropyl ring from a single propionate unit.

The biosynthesis of indolizomycin (**2**) has not been studied but its structural similarity to cyclizidine (**1**) makes it probable that it has a similar biosynthesis. However the branched four-carbon unit of (**2**) which replaces the cyclopropyl group of (**1**) cannot be directly produced by a polyketide route. By analogy with the biosynthesis of cyclizidine it is conceivable that these four carbons are derived from two acetate units by a rearrangement involving an intermediate cyclopropane ring.

The experiments described above have elucidated the biosynthetic origin of the carbon skeleton of cyclizidine as a prelude to further studies into the stereochemistry and mechanisms of the various processes involved in the biosynthesis, especially the ring-forming reactions. It is interesting to note the alternation of propionate and acetate units that is found in this alkaloid. Similar interspersion of acetate and propionate (and butyrate) units can be found[7] in the polyethers and macrolides which are much more widespread among *Streptomyces* species. The present example shows that this is an underlying assembly mechanism available for the biosynthesis of alkaloids as well as the other classes of secondary metabolites in this genus.

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