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# Non-Fatty Acyl Polyketide Starter in the Biosynthesis of Vicenistatin, an Antitumor Macrolactam Antibiotic

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**Abstract**

A biosynthetic pathway of an antitumor antibiotic vicenistatin was investigated by feeding experiments of [1-$^{13}$C] and [1,2-$^{13}C_2$]acetate, [1-$^{13}$C]propionate, [2,3,3-$^{2}H_3$]glutamate, and D-[6,6-$^{2}H_2$]glucose. The elongating units of the aglycon of vicenistatin are derived from standard acetate-propionate, whereas the starter unit appears to be derived from a 3-amino-2-methylpropionate equivalent through the glutamate mutase reaction. 

*Keywords:* antibiotics; biosynthesis; labelling; amino acids and derivatives

The structure of vicenistatin, an antitumor antibiotic produced by a strain of *Streptomyces*, is unique in that an aminosugar vicenisamine is attached to a 20-membered lactam, which is distinct from the classical macrolide and ansamycin antibiotics. Its biological activity is also intriguing since antitumor activity has been particularly shown against a xenographed model of certain human colon cancers [1].

We have been interested in the biosynthesis of vicenistatin because, while the lactam aglycon is partially derived from the polyketide pathway, the branched starter portion appears to be irrelevant to the acetate-propionate theory. The same is actually true in most of the macrolactam antibiotics and related microbial metabolites including ansamycins [2], hitachimycin [3], lankacidins [4,5], and the cytochalacin group of antibiotics [6,7]. Hitachimycin is known to contain β-phenylalanine as a starter unit [3], cytochalasins include an aromatic amino acid starter [6,7], and glycine is known to be the starter unit of lankacidins [4,5].

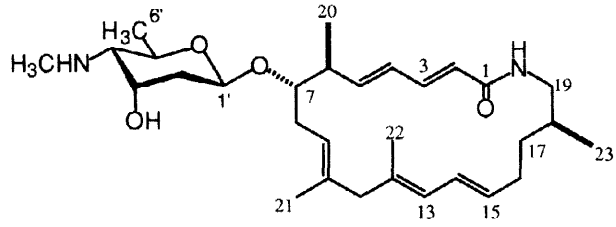


Figure 1. Structure of vicenistatin

At first look at the structure of vicenistatin, the elongating units seemed to be derived from acetate-propionate or acetate-methionine precursors of a rather standard polyketide pathway, and this was in fact proved by a group of Abbott laboratories [8], but the starter appeared to be irregular and unknown. The precursor of the starter unit was anticipated to be related to an amino acid origin.

We undertook chase-experiments to elucidate the precursor-product relationship in the biosynthesis of vicenistatin. Standard supplementation culture either with [1-$^{13}$C]acetate or

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[1-$^{13}$C]propionate was carried out and the labeled vicenistatin was purified as reported [1]. The $^{13}$C-NMR spectra were then analyzed and the results are summarized in Table 1. The labeling pattern with propionate was just as anticipated. Thus, a high degree of incorporation was found at the C-5, 9, and 11. In the case of acetate, while C-1, 3, 7, 13, and 15 were labeled efficiently, again as anticipated, the C-17 was also labeled with [1-$^{13}$C]acetate. The latter observation was not in accord with the standard polyketide pathway. No incorporation was observed both at C-19 and C-23 from propionate and acetate. It thus appears that vicenistatin is, in fact, derived from a modified polyketide with an unknown starter unit.

In order to look more closely into the acetate incorporation, a feeding experiment with [1,2-$^{13}C_2$]acetate was next pursued. Incorporation into C-1,2, C-3,4, C-7,8, C-13,14, and C-15,16 supported the regular polyketide pathway for these positions. A particularly important observation was the incorporation of an intact acetate unit into C-17 and C-18 ($J$= 35.1 Hz) as shown in Figure 2. This fact further implied that C-17 cannot be derived from any regular precursor of a polyketide starter. Instead, the starter unit may well be derived from an amino acid origin; perhaps, a 3-amino-2-methylpropionate equivalent is the most plausible. However, a methyl-branched amino acid is not well established as an efficient metabolic intermediate.

Connection between the acetate metabolism and amino acid metabolism is found in the TCA cycle. An acetate unit is known to be incorporated into the C-4 and C-5 position of glutamate through acetyl-CoA, citrate-isocitrate and 2-oxoglutarate. Structural differences between glutamate and the plausible starter unit, 3-amino-2-methyl-propionate, are the methyl branching and decarboxylation. A plausible scenario for the formation of the starter unit may not be so complex. The methyl group extrusion is well predictable, since glutamate mutase is known to convert L-glutamate into 3-methylaspartate with the aid of vitamin $B_{12}$ coenzyme [9,10], though the stereochemistry at C-18 is opposite to that of abundant (2*S*,3*S*)-3-methylaspartate. Decarboxylation of α-amino acid is ubiquitous so that 3-methylaspartate may well be converted into 3-amino-2-methylpropionate unit.

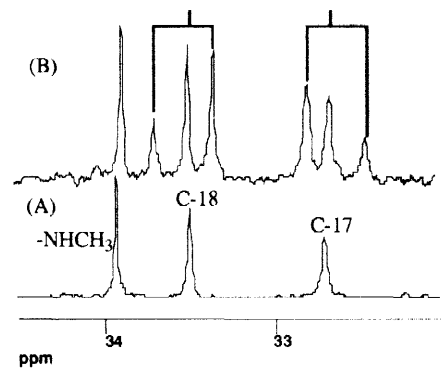
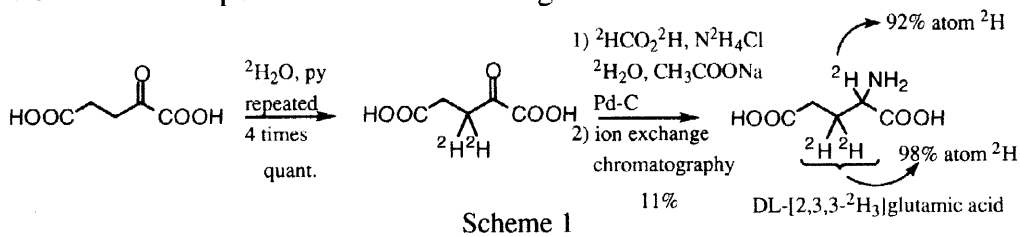



Figure 2. Partial $^{13}$C NMR spectra (100 MHz, pyridine-$d_5$) of (A); non-labeled vicenistatin and (B); labeled vicenistatin by [1,2-$^{13}C_2$]acetate.

Since the above scenario seemed promising, we undertook a feeding experiment of DL-[2,3,3-$^2H_3$]glutamate, which had been prepared by us as depicted in Scheme 1. The feeding experiment was carried out as described above, and the resulting labeled vicenistatin was analyzed with $^2$H-NMR spectrum as shown in Figure 3.[1]




Scheme 1

Apparently, deuterium was incorporated into all methyl groups of the vicenistatin aglycon despite the fact that the deuterated substrate has no methyl group in it. Clearly, all the methyl groups were formed through structural rearrangement at least in the case of the

glutamate substrate. The deuterium incorporation into the C-23 methyl group strongly supports that the above scenario was in fact involved in the formation of the polyketide starter. Incorporation of deuterium into C-20, C-21, and C-22 methyl groups is also rationalized straightforwardly. Glutamate may be deaminated into 2-oxoglutarate, which in turn is decarboxylated into succinyl-CoA in the TCA cycle. The well-established rearrangement pathway, again vitamin $B_{12}$ being a cofactor, from succinyl-CoA into methylmalonyl-CoA gives rise to the elongating $C_3$ unit [9,11], which is equivalent to an intermediate from propionate. No incorporation of deuterium at C-2 of glutamate into C-19 was observed. This may be due to equilibrium between glutamate and 2-oxoglutarate.

Although the origin of C-19 and the amide nitrogen atom have not determined unequivocally, a decarboxylated 3-methylaspartate is the most plausible intermediate of the starter unit in the macrolactam biosynthesis. With regard to the vicenisamine moiety, incorporation of D-[6,6-$^2H_2$]glucose was studied. Labeling of deuterium at the 6'-position was clearly secured as also shown in Figure 3. We have not examined the origin of the *N*-methyl carbon, but it may obviously be derived from the $C_1$ pool. Methionine is the most likely precursor.

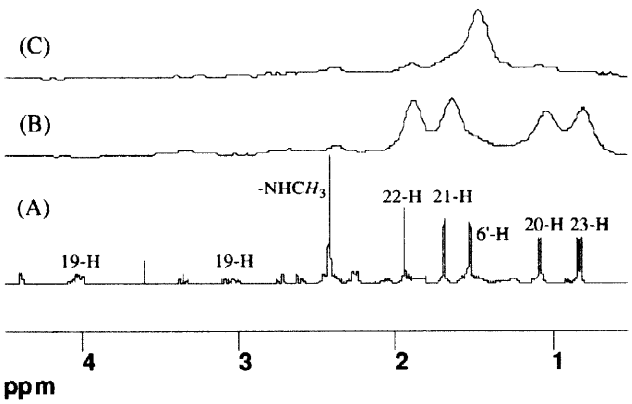



Figure 3. Partial $^1H$ and $^2H$ NMR spectra of non-labeled and deuterated vicenistatins: (A); $^1H$ NMR spectrum (400 MHz, pyridine-$d_5$) of non-labeled vicenistatin, (B); $^2H$ NMR spectrum (60 MHz, pyridine) of labeled vicenistatin with [2,3,3-$^2H_3$]-glutamate, (C); $^2H$ NMR spectrum (60 MHz, pyridine) of labeled vicenistatin with [6,6-$^2H_2$]-glucose.

In summary, the elongating units of the aglycon of vicenistatin appear to be derived from standard polyketide, acetate-propionate, and the starter unit was proposed to be derived from a 3-amino-2-methylpropionate equivalent through the glutamate mutase reaction. The plausible biosynthetic pathway of vicenistatin is summarized in Figure 4.

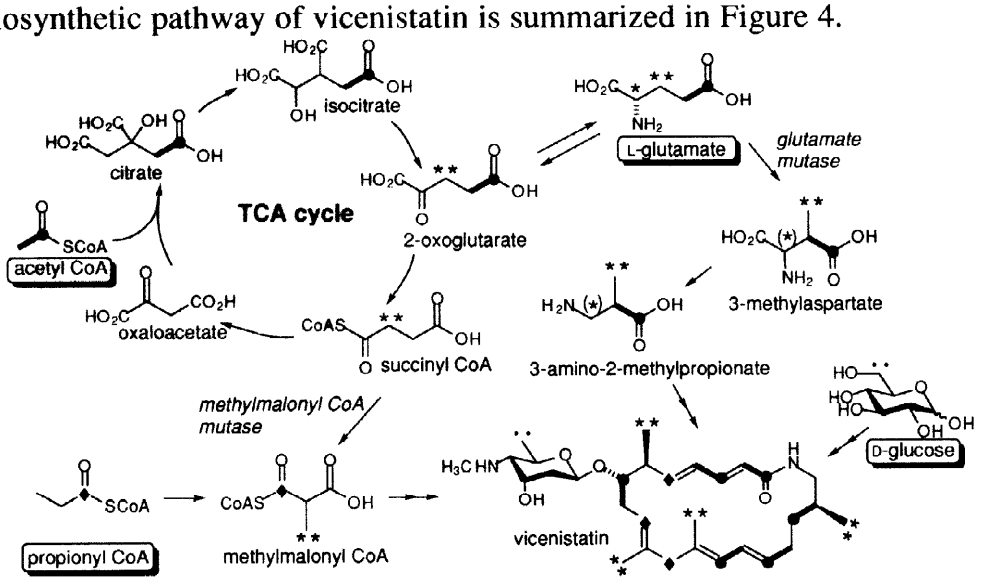



Figure 4. Plausible Biosynthetic Pathway of Vicenistatin.

Table 1. $^{13}C$ NMR Results from the Incorporation of $^{13}C$-Labeled Precursors.[a)]

| Carbon atom | Chemical shift | Relative $^{13}C$ intensities[b)] [1-$^{13}C$]Acetate | [1-$^{13}C$]Propionate | Coupling constant (Hz) [1,2-$^{13}C_2$]Acetate |
|---|---|---|---|---|
| 1 | 166.3 | 3.6 | 1.0 | 64.5 |
| 2 | 124.5 | 0.6 | 0.9 | 64.5 |
| 3 | 140.3 | 4.0 | 1.4 | 55.4 |
| 4 | 128.4 | 0.7 | 1.0 | nd[c)] |
| 5 | 143.3 | 1.5 | 8.2 | |
| 6 | 46.4 | 0.7 | 1.1 | |
| 7 | 86.1 | 3.8 | 1.4 | 38.9 |
| 8 | 36.7 | 0.9 | 1.2 | 38.9 |
| 9 | 121.9 | 1.2 | 6.1 | |
| 10 | 135.0 | 1.1 | 1.6 | |
| 11 | 49.2 | 1.3 | 6.2 | |
| 12 | 134.0 | 1.1 | 1.0 | |
| 13 | 128.1 | 3.2 | 1.1 | nd[c)] |
| 14 | 128.4 | 0.7 | 1.0 | nd[c)] |
| 15 | 132.5 | 4.3 | 1.4 | 43.4 |
| 16 | 27.5 | 1.2 | 1.5 | 43.4 |
| 17 | 32.6 | 2.7 | 1.0 | 35.1 |
| 18 | 33.5 | 0.6 | 1.0 | 35.1 |
| 19 | 43.0 | 0.7 | 1.2 | |
| 20 | 18.7 | 0.6 | 1.0 | |
| 21 | 17.9 | 0.7 | 0.8 | |
| 22 | 17.4 | 0.9 | 0.9 | |
| 23 | 17.6 | 0.7 | 1.0 | |
| 1' | 100.8 | 0.7 | 1.2 | |
| 2' | 39.4 | 0.8 | 1.1 | |
| 3' | 63.1 | 0.8 | 1.1 | |
| 4' | 65.0 | 1.0 | 1.5 | |
| 5' | 70.4 | 1.1 | 1.3 | |
| 6' | 19.5 | 0.8 | 1.1 | |
| 4'-$NHCH_3$ | 33.9 | 1.0 | 1.0 | |

a) measured in pyridine-$d_5$. b) peak intensities were normalized to the carbon signal of 4'-NH$\underline{C}H_3$. c) nd: not determined due to signal overlapping.

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Footnote

1. A similar scenario appears to be operative in the biosynthesis of the 14-membered lactam aglycon of furuvirucin antibiotics [12]. While the authors did not mention it, the reported labeling pattern with $^{13}C$ acetate was in good accord with the plausible starter being a decarboxylated aspartic acid, *i.e.*, β-alanine.