THE USE OF DOUBLY-LABELED ^{13}C-ACETATE IN THE STUDY OF STREPTOLYDIGIN BIOSYNTHESIS

CEDRIC J. PEARCE* and KENNETH L. RINEHART, Jr.

School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, U.S.A.

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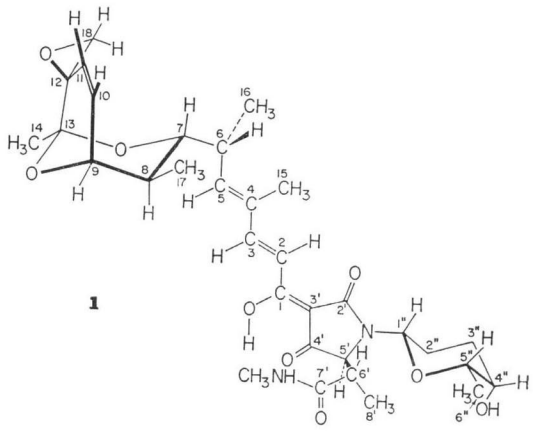
Using [$^{13}C_2$]acetate the biosynthesis of streptolydigin has been investigated. It has been demonstrated that a total of 4 acetate units are incorporated into the antibiotic, 3 into the acyl side chain and 1 into the tetramic acid portion.

The biosynthesis of streptolydigin (**1**)[1], an acyl tetramic acid antibiotic produced by *Streptomyces lydicus*, has been a subject of study for some time in this laboratory[2,3]. We have previously shown that sodium [3-^{14}C]propionate, L-[*methyl*-^{14}C]methionine, D-[*U*-^{14}C]glucose, D,L-[1-^{14}C]glutamic acid**, and [1-^{14}C]acetate are all utilized as biosynthetic precursors to varying extents. Degradation studies on ^{14}C-labeled streptolydigin produced from either [3-^{14}C]propionate or L-[*methyl*-^{14}C]methionine argued that the *C*-methyl groups of the streptolic acid unit (C-15, -16, and -17), as well as C-18, were derived from the former, and the *N*-methyl group from the latter. This was confirmed using ^{13}C-labeled propionate and methionine.

Determining the labeling pattern from acetate was difficult for two reasons. First, there is no available degradation scheme for the potentially labeled areas, thus making the classical approach of introducing ^{14}C-label followed by isolation and analysis of specific portions of the molecule not immediately available. In addition, due to the low incorporation of acetate, it was not possible to demonstrate the exact position of incorporation using singly-labeled ^{13}C-precursor because enrichment of individual carbons was not detectable. We have now overcome this problem by using [$^{13}C_2$]acetate and examining the biosynthetic product from this precursor by looking for ^{13}C-^{13}C coupling in its ^{13}C NMR spectrum.[4]



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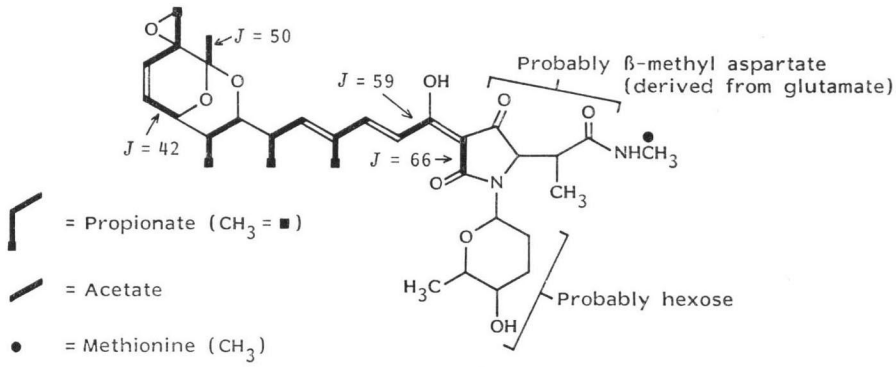
S. lydicus was grown as described previously[3] under conditions previously shown to be optimum for incorporation of [1-^{14}C]acetate into streptolydigin with respect to concentration and time of addition. Sodium [$^{13}C_2$]acetate (11.5 mg) was added to each of forty 500-ml wide-mouthed Erlenmeyer flasks containing 50 ml of a 3-day old *S. lydicus* culture growing at 30°C in a complex streptolydigin production medium (0.25% brewers yeast, 0.6% $CaCO_3$, 3% sucrose, 0.2%

* Present address: School of Pharmacy, University of Connecticut, Storrs, Connecticut 06268, U.S.A.

** Although preliminary experiments demonstrated that glutamate was incorporated relatively poorly into streptolydigin, a more detailed study revealed up to 0.2% incorporation when labeled precursor was added to more advanced cultures.

Scheme 1. Origin of carbon atoms in streptolydigin.



$(NH_4)_2SO_4$, 2% soybean meal, distilled water). Following incubation for a further 24 hours, streptolydigin was isolated from the culture broth using methylene chloride in the usual way[5]. From 2 liters of culture broth, 250 mg of antibiotic was obtained. This was dissolved in deuteriochloroform and a Fourier transform proton-decoupled ^{13}C NMR spectrum was obtained, employing a Varian XL100 spectrometer.

On examination of the ^{13}C NMR spectrum, it was observed that a number of satellite peaks were present which were not present in a similar spectrum obtained from unenriched material. It was apparent from the coupling behavior that the following carbons were enriched: C-2′ and C-3′ (J_{CC}=67.0 Hz and 65.5 Hz), C-1 and C-2 (J_{CC}=58.0 Hz and 59.5 Hz), C-9 and C-10 (J_{CC}=42.0 Hz and 42.5 Hz), C-13 and C-14 (J_{CC}=50.0 Hz and 50.0 Hz).*

These data demonstrate that acetate is incorporated as an intact unit into streptolydigin and is used to provide the carbon pairs 2′, 3′; 1, 2; 9, 10; and 13, 14. The biosynthetic origin of the tetramic acid carbons 2′ and 3′ is, therefore, similar to that of analogous carbons of other tetramic acid-containing antibiotics, such as tenuazonic acid[5], erythroskyrine[6] and malonomicin[7]. These results also demonstrate that the 2-carbon fragments of the acyl side chain which do not arise from propionate are acetate-derived. The remainder of the tetramic acid portion of streptolydigin, carbons 4′ to 8′, probably is derived from β-methyl aspartic acid and it is noteworthy that glutamate, an immediate precursor for β-methyl aspartate[8], seems to be incorporated exclusively into this part of the molecule (C. J. PEARCE & K. L. RINEHART, Jr., unpublished data). Scheme 1 gives a summary of our present knowledge. Work is continuing in this laboratory on the origins of the β-methyl aspartate and the deoxyhexose units.

Acknowledgment

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* The coupling constants given are accurate to within ±1.5 Hz. Although both satellites were not clearly observed for carbons 2′, 13, and 14 due to overlap with signals from other carbons, the coupling constants could be calculated on the assumption that the satellite peaks are symmetrically arranged around the unsplit ^{13}C-signal.

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