Biosynthesis of the Peptide Antibiotic Etamycin. Origin of the 3-Hydroxypicolinyl and Amino-acid Fractions

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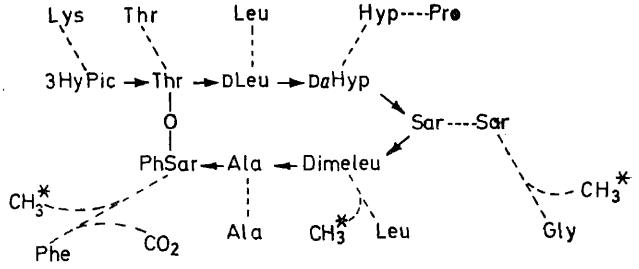
Summary Radiotracer experiments have established the biosynthetic origin of the components of etamycin and have shown that the 3-hydroxypicolinic acid fraction, unlike that of pyridomycin, is derived from L-lysine.

AMONG the peptidolactone antibiotics of *Streptomyces*, pyridomycin,[1] etamycin,[2] and the complex represented by staphylomycin S[3] all contain 3-hydroxypicolinic acid. Biosynthetic studies on pyridomycin have shown[4] that radioactivity is incorporated into the 3-hydroxypicolinic acid fraction from L-[U-^{14}C]aspartic acid, [1-^{14}C]glycerol, and sodium [2-^{14}C]pyruvate, but not from L-[U-^{14}C]lysine, DL-α-amino[1-^{14}C]adipic acid, nor from DL-[*Ar*-^{14}C]tryptophan. This evidence for the mode of pyridine-ring biosynthesis by *Streptomyces* suggests a route from 3- and 4-carbon precursors similar, if not identical, to that used for niacin synthesis in bacteria and higher plants.[5]

Our work on the biosynthesis of etamycin in cultures of *Streptomyces griseoviridus* ATCC 04955 provides evidence for a second route. Radioactivity from L-[U-^{14}C]lysine was efficiently incorporated (30%) into the antibiotic and, after acid hydrolysis,[2] was located predominantly (95·9%) in the 3-hydroxypicolinic acid residue. Labelled carbon from DL-[1-^{14}C]- and -[4-^{14}C]-aspartic acid and from L-[U-^{14}C]-alanine was incorporated less efficiently (2·8, 5·7, and 12%, respectively) and its distribution in the products of hydrolysis was consistent with indirect entry to 3-hydroxypicolinic acid *via* lysine. [1,3-^{14}C]Glycerol (specific incorporation 0·5%) was a non-specific precursor of etamycin constituents, and no radioactivity was incorporated from DL-[*Ar*-^{14}C]tryptophan.

As observed for pyridomycin,[4] [G-^{3}H]-3-hydroxypicolinic acid specifically labelled the 3-hydroxypicolinic acid fraction, suggesting that it is synthesized as an intermediate before incorporation into the peptidolactone. Parallel results were obtained with other etamycin constituents (Scheme). L-Threonine, L-alanine, and sarcosine were each preferentially labelled by the appropriate ^{14}C-amino-acid. As reported earlier by Perlman and his co-workers,[6] D-leucine and *N*,β-dimethyl-L-leucine were specifically labelled by L-[U-^{14}C]leucine but not by L-[U-^{14}C]isoleucine. L-



SCHEME. *Origin of the amino-acid components of etamycin.* 3HyPic = 3-hydroxypicolinic acid, Hyp = 4-hydroxy-L-proline, DaHyp = *allo*-4-hydroxy-D-proline, Sar = sarcosine, DimeLeu = *N*,β-dimethyl-L-leucine, PhSar = α-L-phenylsarcosine, CH_3* = active-methyl derived from L-methionine.

[Me-^{14}C]Methionine labelled the methyl groups of sarcosine, *N*,β-dimethyl-leucine, and α-phenylsarcosine. The carbon skeleton of the last amino-acid was derived from phenylalanine. Radioactivity from the [α-^{14}C], [β-^{14}C], and [*ring*-1-^{14}C] labelled amino-acid was efficiently incorporated and was found mainly in the phenylsarcosine residue of etamycin. Recoveries of 0·05, 99·0, and 99·3%, respectively, in benzoic acid obtained by dichromate oxidation indicate that the ring, α, and β carbons were incorporated without rearrangement of the carbon skeleton. However, a small but specific incorporation of label from L-[*carboxy*-^{14}C]phenylalanine (1·5% compared with 89% for DL-[*Ar*-^{14}C]phenylalanine in a direct comparison) suggests that the carboxy-group of an intermediate may equilibrate with radioactive carbon dioxide. [G-^{3}H]-4-Hydroxy-L-proline as

well as L-[U-^{14}C]proline were specific precursors of the *allo*-hydroxy-D-proline fraction of etamycin. Generation of the D-configuration in leucine and *allo*-hydroxyproline by α-epimerization after activation and attachment to an enzyme is consistent with the known mechanisms of peptide antibiotic biosynthesis.[7]

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