THE BIOGENETIC ORIGIN OF THE N-METHYL-γ-METHYL-L-ISOLEUCINE RESIDUE OF ETAMYCIN

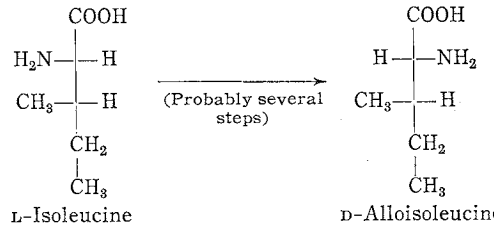
Sir:

In earlier publications[1,2] we proposed that the presence of L-isoleucine and D-alloisoleucine in many peptide antibiotics and the absence of D-isoleucine or L-alloisoleucine in such peptides can be considered as evidence that D-alloisoleucine results from epimerization of L-isoleucine at the α-carbon (Fig. 1). This "rule of α-epimerization" supports the view that the D-amino acid constituents of microbial peptides are derived from L-amino acids. More recently BEVAN *et al.*[3] reported that monamycin, a depsipeptide, contains D-isoleucine residues. The same authors also called attention to the presence of N-methyl-γ-methyl-L-alloisoleucine in etamycin[4] and in triostin C.[5] This prompted us to investigate the biogenetic origin of this alloisoleucine derivative.

Streptomyces griseoroseus (ATCC 12125)[6] was grown in 250 ml Erlenmeyer flasks each of which contained 100 ml of a medium of the following composition: soybean meal, 1 g; glucose, 1 g; NaCl, 0.5 g; $CaCO_3$, 0.1 g; tap water to 100 ml. After 24-hour incubation at 30°C on a rotary shaker (280 r.p.m., 2.54 cm (1 inch), 5 μC L-leucine-U-^{14}C was added and the flask incubated for an additional 48 hours. The whole culture was then extracted with an equal volume of methyl-iso-butylketone, the extract dried with anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness. The crude etamycin was further purified by thin-layer chromatography on silica gel with the solvent system $CHCl_3$ - CH_3OH (98 : 2, v/v). Approximately 0.25 % of the added radioactivity was recovered in the material corresponding to etamycin eluted from the chromatogram. This material was hydrolyzed in a sealed tube in 6 N HCl for 18 hours at 110°C. Examination of the hydrolyzate showed that approximately one-half of the radioactivity of the etamycin was present in the N-methyl-γ-methyl-L-alloisoleucine and the other half in the D-leucine component of the hydrolyzate (the components being separated by paper ionophoresis (pH 1.8, 60 v/cm, 1.5 hour) followed by paper chromatography in the perpendicular direction in butan-1-ol-acetic acid-water (4 : 1 : 4, by vol.). None of the other components of the hydrolyzate contained appreciable radioactivity. When the experiment was repeated using L-isoleucine-U-^{14}C as the supplement none of the added radioactivity was found in the etamycin.

Fig. 1*

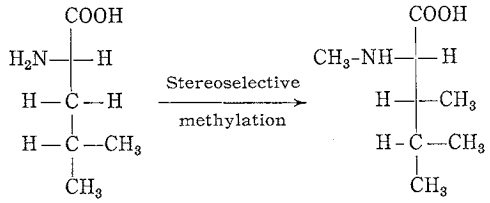
(α-Epimerization)



L-Isoleucine D-Alloisoleucine

Fig. 2*

Biogenesis of N-methyl-β-methyl-L-leucine



L-leucine N-Methyl-β-methyl-L-leucine (N-methyl-γ-methyl-L-alloisoleucine)

* FISCHER Projections

We conclude from this study that N-methyl-γ-methyl-L-alloisoleucine residue of etamycin is produced by methylation of L-leucine at the β-carbon atom (with the formation of a second center of asymmetry) and by methylation of the nitrogen (Fig. 2). Whilst in a formal sense the former amino acid can be described as a derivative of alloisoleucine, the designation N-methyl-β-methyl-leucine used earlier[4] is to be preferred on biogenetic grounds. Obviously, the presence in etamycin of such an L-alloisoleucine derivative cannot be looked upon as evidence against the "rule of α-epimerization". Incidentally, our experiment also showed that the D-leucine residue in etamycin in common with many other D-amino

acid residues in antibiotics[2] is derived from the corresponding L-isomer.

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