# The Biosynthetic Origin of D-Isoleucine in the Monamycins

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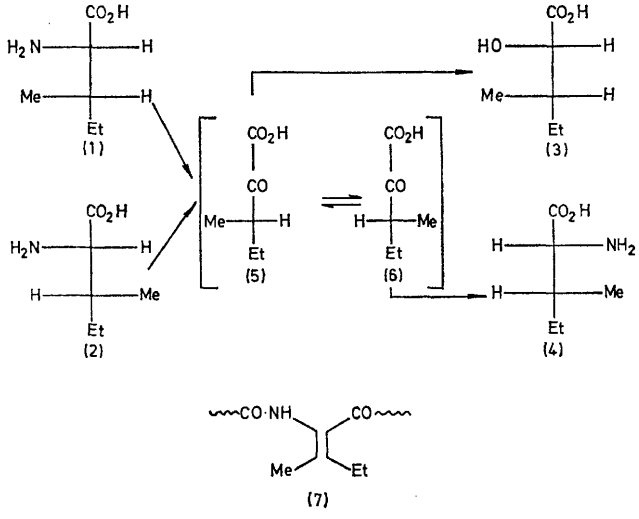
*Summary* $^{14}$C-Labelling studies have established that both L-isoleucine and L-alloisoleucine may serve as precursors of the D-isoleucine residues in members of the family of cyclohexadepsipeptide antibiotics known as the monamycins; possible pathways of biosynthesis of D-isoleucine from these precursors are discussed.

THE identification of D-isoleucine (**4**)[1] as a constituent residue of particular monamycin congeners[2] was the first observation to be regarded as an exception to the α-epimerisation concept formulated by Bodanszky and Perlman.[3] This, together with subsequent studies[4,5] relating to the origin of D-amino-acid residues in metabolites of micro-organisms, has led us to investigate the nature of precursors of D-isoleucine in monamycins. The results of experiments with $^{14}$C-labelled amino-acids are summarised in the Table.

The samples of the $^{14}$C-labelled compounds were added to 20 h cultures of *Streptomyces jamaicensis* which were harvested 28 h later. The monamycins from these cultures were purified, crystallised, and then hydrolysed with 6M hydrochloric acid to give the constituent hydroxy- and amino-acids.[6] The individual amino-acids were separated as fractions eluted from the analytical column of a Beckman 120C amino-acid analyser. Each fraction was tested for radioactivity. In all these experiments no trace of the alloisoleucine diastereoisomer was found in the hydrolysate; this included the cases where it was fed to the organism.

[*U*-$^{14}$C]-L-Isoleucine (**1**) was selectively incorporated into both the D-isoleucine (**4**) and the L-isoleucic acid (**3**) components of the monamycins. Although the alloisoleucines




are not of wide occurrence in nature,[3,7] it was of interest to determine whether the organism could use them for conversion into D-isoleucine. The L-alloisoleucine was incor-

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TABLE

| Materials | Activity | % Incorporation into monamycin | Activity in D-isoleucine (d.p.m./mmol) | Activity of the amino acids in hydrolysate for comparison (d.p.m./mmol) | Activity of isoleucic acid[a] (d.p.m./mmol) |
|---|---|---|---|---|---|
| [*U*-$^{14}$C]-L-Ile .. .. .. | 50 $\mu$C | 6·3 | 2·1 × $10^6$ | MePro 3·5 × $10^4$<br>Val 2·3 × $10^4$ | 2·8 × $10^6$ |
| [3,3′,4,5-$^{14}$C]-DL-alloIle .. | 20 $\mu$C | 2·4 | 1·5 × $10^6$ | Val 2·6 × $10^4$<br>Pip 1·7 × $10^4$ | Not measured |
| [2-$^{14}$C]-3-methylpent-2-enoic acid[b] | 5·1 $\mu$C | 0·02 | 1·8 × 10 | MeLeu 1·2 × 10<br>HyPip 1·9 × 10<br>Val 2·1 × 10<br>MePro 2·6 × 10 | 1·9 × 10 |
| [3,3′,4,5-$^{14}$C]-D-alloIle .. .. | 4·2 $\mu$C | 0·41 | 1·4 × $10^5$ | Not measured | 1·9 × $10^5$ |
| [3,3′,4,5-$^{14}$C]-L-alloIle .. .. | 5·3 $\mu$C | 2·8 | 8·5 × $10^5$ | Not measured | 9·6 × $10^5$ |

[a] Based on partially purified hydroxy-acid fraction. [b] Synthesised from [2-$^{14}$C]-malonate *via* the dehydrobromination of [2-$^{14}$C]-2-bromo-3-methyl-pentanoic acid (ref. 9). Pip = piperazic acid, HyPip = 5-hydroxypiperazic acid.

porated selectively, as was D-alloisoleucine, but to a lesser degree than the L-isomer. Since [2-$^{14}$C]-3-methylpent-2-enoic acid is not incorporated, it is unlikely that a deamination step resembling that observed for L-phenylalanine[8] is involved in our case.

Bycroft proposed[4] that a combined form of a dehydroamino-acid such as (**7**) derived from the corresponding L-amino-acid residue, might be reduced stereoselectively *in vivo*, to the D-isomer; this would account readily in our case for the changes in chirality at the two adjacent centres. However, this does not explain for this study, the incorporation of L-isoleucine (**1**) into both D-isoleucine, and L-isoleucic acid. Rather, our incorporation studies suggest that there is a common intermediate, derivable from either L-isoleucine (**1**) or L-alloisoleucine (**2**) for both L-isoleucic acid (**3**) and D-isoleucine (**4**). The most likely intermediates to satisfy these requirements are the α-keto-acids [(**5**), (**6**)] which through interconversion by inversion at C-3[7,10] could serve as the sources of L-isoleucic acid or a D-isoleucine. Recent studies by Lipmann[11] on the biosynthesis of gramicidin S and the tyrocidins are in accord with this suggestion that D-amino-acid units are incorporated directly into monamycin.

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