Incorporation of Amino Acids into the Cyclohexadepsipeptide, Monamycin

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Summary Studies utilising ^{14}C-labelled amino acids provide evidence relating to the precursors of piperazic acids, *trans*-4-methylproline, and *N*-methyl-leucine residues in the monamycins, cyclohexadepsipeptide antibiotics.

THE amino acid residues in the congeners of the monamycins, antibiotics produced by *Streptomyces jamaicensis*,[1] include several unusual molecular fragments. In view of this we have investigated the incorporation of relevant ^{14}C-labelled amino acids. An earlier study[2] utilising [^{14}C]-*allo*-isoleucine and [^{14}C]-isoleucine has been described. We now report investigations relating to the incorporation of piperazic acid and leucine residues.

The Table summarises the results of measurements of radioactivity in the mixture of ^{14}C-monamycins obtained by harvesting a culture of *S. jamaicensis* growing in the presence of particular ^{14}C-labelled acids. The ^{14}C-levels of the major and minor products of hydrolysis (12N-HCl at 110 °C for 12 h) were determined by separating the mixture by both two-dimensional t.l.c. and high voltage electrophoresis, followed by quantitative radio-active scanning

(Actigraph 4π scanner). All measurements were in duplicate.

The specifically labelled [2-^{14}C]piperazic acid was evidently incorporated into the monamycins as the intact residue and as the 5-hydroxy- and 5-chloro-derivatives; there was some randomization reflected in the identification of valine, isoleucine, and 4-methylproline containing low levels of radioactivity. Ornithine was not incorporated and is not, therefore, a precursor of piperazic acid. The preferential incorporation of glutamine and glutamic acid into piperazic acid residues is notable but further investigation is required to account for this, particularly since there is substantial randomization reflected in incorporation into valine, isoleucine, and 4-methylproline.

Particular interest attaches to the high level of incorporation of leucine into 4-methylproline. This suggests that leucine is converted directly into 4-methylproline by *S. jamaicensis*, presumably by an oxidative cyclisation. The incorporation of leucine into the *N*-methyl-leucine residue of monamycin, although *N*-methyl-leucine is not itself incorporated, and the derivation of the *N*-methyl group from [*Me*-^{14}C]-L-methionine, is in accord with observations of others concerning the origin of *N*-methyl-amino acid residues in peptides produced by micro-organisms.[3] The insignificant levels of incorporation of [*Me*-^{14}C]-L-methionine into 4-methylproline indicates that specific methylation is not involved in this case.

TABLE. Incorporation of ^{14}C labelled amino acids into monamycin and activity of amino acids after hydrolysis[a]

Amino acid	Activity of a.a./μCi	% Incorporation into monamycin	A.a. in hydrolysate (A.a. activity/ d.p.m. mmol^{-1})
[2-^{14}C]-DL-Pip	0·05	7·8	Pip ($3{\cdot}1 \times 10^4$); HOPip ($1{\cdot}6 \times 10^4$); ClPip ($1{\cdot}5 \times 10^4$); [MePro, Val, Ile $< 1 \times 10^3$]
[*U*-^{14}C]-L-Gln	40	0·72	Pip + HOPip + ClPip ($2{\cdot}5 \times 10^6$) MePro ($5{\cdot}9 \times 10^4$); Val ($1{\cdot}2 \times 10^5$); Ile ($4{\cdot}8 \times 10^5$)
[*U*-^{14}C]-DL-Glu	6·25	0·48	Pip + HOPip + ClPip ($3{\cdot}7 \times 10^6$) Ile (7×10^5), Val (7×10^5)
[*U*-^{14}C]-DL-Orn	5·0	0·16	Pip + HOPip + ClPip (2×10^4) Ile = Val = MePro = 5×10^3
[*U*-^{14}C]-L-Leu	6·25	19·0	MePro ($1{\cdot}2 \times 10^6$); *N*MeLeu ($0{\cdot}8 \times 10^6$)
[*U*-^{14}C]-*N*Me-L-Leu	0·93	0·06	—
[^{14}C-Me]-L-Met	10	11·4	*N*MeLeu ($1{\cdot}5 \times 10^6$); Ile ($1{\cdot}3 \times 10^5$); Val ($2{\cdot}0 \times 10^5$); MePro (3×10^4)

[a] A.a. = amino acid; Pip = piperazic acid; HOPip, ClPip = 5-hydroxy- and 5-chloro-piperazic acid respectively; MePro = *trans*-4-methylproline.

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