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ON THE MODE OF INCORPORATION OF LEUCINE SAMPLES LABELLED AT POSITIONS 5 AND 2 WITH ^{3}H AND ^{14}C INTO THE ISOPRENYLATED TRYPTOPHAN DERIVATIVE ECHINULINE FROM ASPERGILLUS AMSTELODAMI

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Summary Feeding experiments with (4S) [5-^{3}H;5-^{14}C]L-leucine (1a) and with the (4S) [5-^{3}H;2-^{14}C]-isomer (1b) show that incorporation into the isoprenylated tryptophan derivative echinuline (3), produced by Aspergillus amstelodami, involves, to a large extent, loss of the fragment enbodying the carbon atom at position 2 of the fed amino acid.

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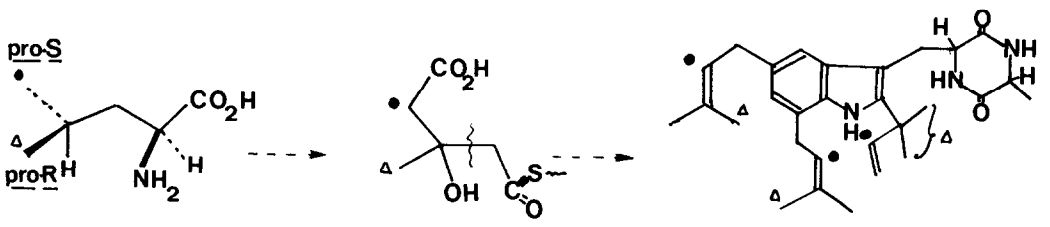
In a study on the stereochemical course of the enzymic reactions of the biological degradation of the amino acid L-leucine (1) we observed[1] using leucine samples asymmetrically labelled with ^{13}C in the isopropyl moiety[2], as major incorporation pattern into the mevalonate-derived C_5 isoprenoid chains of echinuline (3), produced by A. amstelodami, the one shown in the Scheme, accompanied, however by some scrambling of the labels.

The above results have been tentatively explained through the operation of a cleavage of the intermediate β-hydroxy-β-methylglutarylCoA (2) to acetylCoA and acetoacetate, incorporating regioselectively the labels of the fed amino acid, followed by intact incorporation of this C_4 moiety as acetoacetylCoA into mevalonate, thus accounting for the observed labelling pattern. This picture requires, however, confirmation from complementary experiments showing the fate of the C_2 unit enbodying the carbon atoms at positions 2 and 3 of leucine (1) during the biosynthetic process leading to (3).



(1) (2) (3)

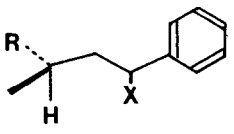
Scheme

We here describe the results of these experiments. A doubly labelled leucine sample was first required, carrying a ^{14}C label at position 2 and a reference label somewhere in the molecule

against which to measure any loss of ^{14}C activity due to the carbon atom at position 2 of (1), because of the lack of suitable degradation procedures enabling us to determinate the labelling pattern of the biosynthetic echinuline (3).

Thus, (4S) [5-^{3}H] L-leucine was prepared by a modification of the sequence previously[1] developed in the ^{13}C series. Accordingly, (2R) [1-^{3}H] 2-methyl-4-phenylbutan-1-ol (4) was obtained upon $NaB^{3}H_4$ reduction of the corresponding aldehyde, and converted, without tritium loss, via compounds (5) and (6), followed by ozonolysis, into N-acetyl (4S) [5-^{3}H] D,L-leucine. Enzymic hydrolysis (hog kidney acylase) of the latter gave (4S) [5-^{3}H] L-leucine, which, mixed with the 2-^{14}C-isomer, yielded doubly labelled (4S) [5-^{3}H;2-^{14}C] L-leucine (1b). This material was fed to A. amstelodami cultures under conditions identical to those used in the reported ^{13}C series. Incorporation (1.2%, based on ^{3}H) into echinuline (3) was accompanied by a raise in the ^{3}H:^{14}C ratio from 1.25 to 3.75, which clearly shows a considerable loss of the ^{14}C label.

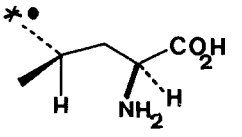
However, a proper interpretation of the above results required the determination of the exact value of the retention in (3) of the tritium originally present in the 5 pro-S methyl group of the fed precursor. To this end, (4S) [5-^{14}C] L-leucine was prepared using $Ba^{14}CO_3$, as reported in the ^{13}C series, mixed with (4S) [5-^{3}H] L-leucine, and the doubly labelled material (1a) was incorporated into echinuline (3). The incorporation took place with ca. 30% tritium loss.



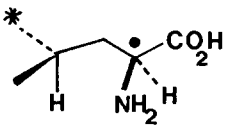
(4) R= CTHOH; X= H

(5) R= CTHOTs; X= H

(6) R= CTH_2; X= $NHCOCH_3$



(1a)



* = ^{3}H; • = ^{14}C

(1b)

Although in the absence of a direct knowledge of the labelling pattern of echinuline (3) biosynthetized from (1b), the present results and those previously obtained with ^{13}C labelled leucine indicate that in A. amstelodami the more important pathway leading from (1) to the mevalonate derived C_5 chains of (3) involves, at some stage, the loss of the fragment enbodying the carbon atom at position 2 of (1).

In ref. 1 a mistake has been made in the assignement of the names of the ^{13}C asymmetrically labelled leucine samples, which has to be reversed as here indicated

from CIS, Gif-sur-Yvette

Reference

1 R.Cardillo, C.Fuganti, D.Ghiringhelli, P.Grasselli and G.Gatti, J.C.S.Chem.Comm.,1977,474

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