Incorporation of [α-^{2}H]- and [α-^{3}H]-L-Cystine into Penicillin G and the Location of the Label using Isotope Exchange and ^{2}H Nuclear Magnetic Resonance

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Summary The feeding of L-[α-^{2}H]- and L-[α-^{3}H]-cystine to *Penicillium chrysogenum* affords [6-^{2}H]- and [6-^{3}H]-penicillin G, thus confirming that an αβ-dehydrocysteine residue is not involved in the biosynthetic pathway.

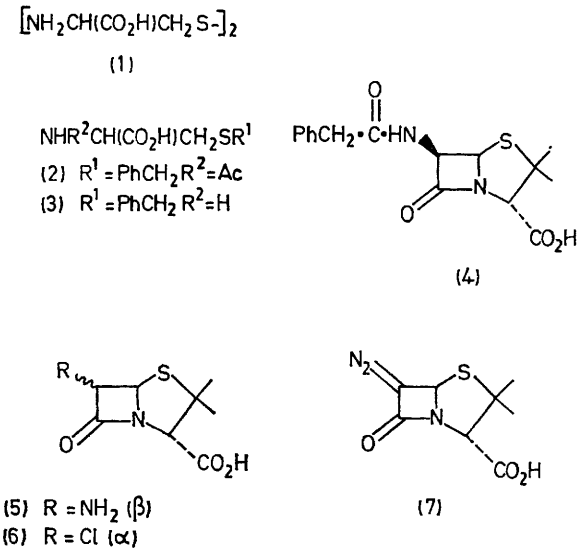
THERE is circumstantial evidence to suggest that the biosynthesis of penicillin proceeds through a peptide intermediate derived initially from L-cysteine and L-valine.[1] The sequence of subsequent oxidation steps has been the subject of much speculation.[2] Recent work has led to a greater understanding of the fate of the valine unit during its incorporation into penicillins and cephalosporins,[3] but the role of cysteine has not been so well defined.

The natural occurrence of a considerable number of microbial peptides containing structural units formally derived from αβ-dehydrocysteine residues, together with our interest in dehydroamino-acid chemistry,[4] prompted us to investigate the possibility that a dehydrocysteine system is involved as an intermediate in the penicillin biosynthetic pathway. Earlier work[5] on the incorporation of DL-[α-^{3}H]cystine had not in our opinion completely excluded this possibility.

L-[α-^{3}H]Cystine (**1**) was synthesised as follows. Treatment of DL-(**2**) with acetic anhydride–$MeCO_2{}^3H$ in 1,2-dimethoxyethane under reflux resulted in the specific exchange of the α-proton with tritium. Resolution using hog renal acylase 1 afforded L-[α-^{3}H]-(**3**). The benzyl group was removed with anhydrous hydrogen fluoride[6] and the product was oxidised to give L-[α-^{3}H]-(**1**). This method provides a general labelling procedure for the α-position of amino-acids.

L-[*U*-^{14}C, α-^{3}H]cystine hydrochloride (^{3}H/^{14}C ratio, 7·0) was incubated with the mycelium of a high-producing strain of *Penicillium chrysogenum*. 40% of the ^{14}C-label and 34% of the ^{3}H-label were incorporated into penicillin G (**4**) (^{3}H/^{14}C ratio, 5·9). The small reduction in the isotope ratio probably reflects the loss of the α-proton from the carbon skeleton of cystine in primary metabolic processes.

In a separate experiment L-[α-^{3}H]cystine hydrochloride was incorporated into (**4**). The [^{3}H]-(**4**), diluted with unlabelled material, was hydrolysed to 6-APA (**5**) ($1·25 \times 10^5$ d.p.m./mM) using a resin-bound acylase from *E. coli*. The recovered (**5**) was treated with $NaNO_2$ in 1N-HCl to give the chloro-compound (**6**) *via* the diazo-intermediate (**7**).[7] Purification of (**6**) as the Me ester ($2·16 \times 10^3$ d.p.m./mM) showed that more than 98% of the label was lost in the reaction. The tritium atoms were lost solely from the 6-position, since when (**5**) was treated with $NaNO_2$ in 1N-DCl, deuterium was only incorporated into the 6-position, as determined by ^{2}H n.m.r. spectroscopy.



L-[α-^{2}H]Cystine, prepared according to the above procedure was incorporated into (**4**). The location of the label in the α-position of (**1**) and the 6-position of (**4**) has been confirmed by ^{2}H n.m.r. spectroscopy. Thus the incorporation of L-cystine into penicillin occurs without loss of the α-proton, and consequently αβ-dehydrocysteine-containing intermediates must be excluded from consideration as intermediates in penicillin biosynthesis.

(*Received, 29th November,* 1974; *Com.* 1447.)

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