Biosynthesis of Penicillin G from D- and L-[^{14}C]- and [α-^{3}H]-Valine

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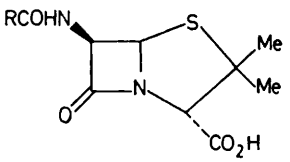
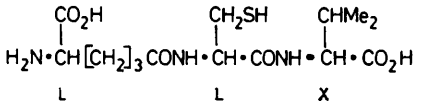
Summary Excellent incorporations of both D-[1-^{14}C]- and L-[*U*-^{14}C]-valine into penicillin G by a high producing strain of *Pencillium chrysogenum* is reported; the label from D-[α-^{3}H]-valine is not incorporated.

As an extension of our systematic investigation[1] of the biosynthesis of penicillin G (**1**) in a commercial high producing strain of *Penicillium chrysogenum*, we now report experiments relating to the origin of the D-valine unit.



(1) R = $PhCH_2$-

(2) R = $H_2N{\cdot}CH[(CH_2)_3]-$ (CO_2H)



(3) X = D

(4) X = L

It is already well established that L-valine is an effective precursor of this portion of (**1**). However, there is conflicting evidence as to the role of D-valine which appears to be incorporated much less readily than the L-isomer[2] and there are reports that it inhibits penicillin production.[3] Similarly penicillin N (**2**) but not cephalosporin C production is inhibited by the addition of D-valine to washed cell suspensions of *Cephalosporium* species.[4] More recently it has been reported[5] that (**3**) and not (**4**) is utilised by protoplasts from *Cephalosporium* for the formation of penicillin N

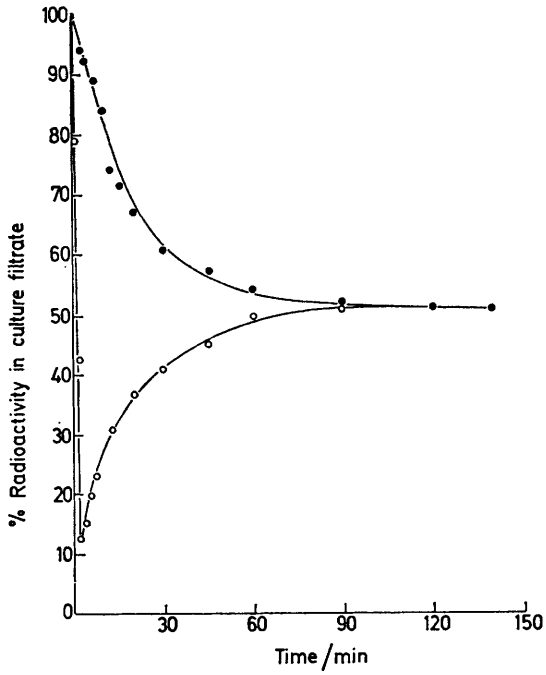


FIGURE. L-valine, ● D-valine.

(**2**), suggesting that the D-centre of the valine group is generated prior to the cyclisation steps.

We have demonstrated that the uptake of ^{14}C-labelled D- and L-valine into the cells of *Penicillium chrysogenum* (Figure)‡ follows a characteristic pattern for each isomer. Although in all the experiments conducted the rate of uptake of D-valine was considerably slower than for the L-isomer, the final amount of radioactivity (*ca.* 50%) within the culture filtrate was practically the same for each isomer. In both cases a similar amount (*ca.* 37%) of the original activity was associated with penicillin G which was subsequently extracted and crystallised to constant activity. These experiments established that the carbon skeleton of both D- and L-valine are equally effective precursors.

In order to investigate the possibility that D-valine is incorporated directly into (**1**), D-[α-^{3}H]valine together with the corresponding L-isomer was prepared by a route similar to that employed for α-tritiated cystine.[1] Incubation of mycelium with D-[α-^{3}H]valine (3 × 5·3 mg; 0·21 μCi/mg) and L-[α-^{3}H]valine (3 × 3·0 mg; 0·40 μCi/mg) afforded (**1**) containing 0·20 and 0·25% activity respectively. Double labelled experiments with both isomers afforded *ca.* 37% incorporation of ^{14}C and again negligible incorporation of tritium.

Clearly D-valine is not incorporated directly into (**1**), but is possibly converted into L-valine either *via* the corresponding α-keto acid or by a mechanism not involving C–N bond cleavage. Alternatively, both D- and L-valine may proceed through a common intermediate.

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‡ Whole broth (*ca.* 300 ml) was transferred from a production fermenter during the phase of linear penicillin G production to a small scale fermenter. Glucose, vegetable oil, and sodium phenylacetate were constantly added to maintain optimum antibiotic production. The broth was equilibrated at pH 7 prior to the addition of precursor; in the case of L-[*U*-^{14}C]valine (0·12 μCi/mg), 3·0 mg was added after 1 h whereas with D-[1-^{14}C]valine (0·27 μCi/mg), 3·0 mg was added after an overnight incubation of *ca.* 18 h.

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