# Biosynthesis of Gliotoxin

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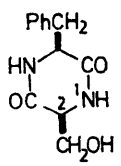
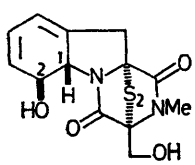
*Summary* Biosynthesis of gliotoxin (**2**) from the doubly-labelled diketopiperazine (**1**) proceeds efficiently and without alteration in the labelling ratio.

IN a recent note, MacDonald and Slater[1] report that although the diketopiperazine (**1**) is taken up by mycelium of *Penicillium terlikowskii* it is not incorporated into the gliotoxin (**2**) which the latter produces. This observation is contrary to our own observation that (**1**) is an efficient precursor of (**2**) in '*Trichoderma viride*' (probably *Gliocladium* sp.) strain NRRL-75.

Doubly-labelled (**1**) was prepared by condensation of L-*N*-benzyloxycarbonylphenylalanine and DL-serine methyl ester hydrochloride (EtO.COCl, *N*-Me morpholine) followed by hydrogenolysis; labelling was from L-[*Ar*-$^3$H]phenylalanine and DL-[1-$^{14}$C]serine and the product, m.p. 226—229 °C, was a mixture of (**1**) and its epimer at C-2, with $4{\cdot}7 \times 10^{10}$ d.p.m. mol$^{-1}$ of $^{14}$C and a $^{14}$C : $^3$H ratio of $1 : 13 \pm 0{\cdot}8$. The labelled material was added to a gliotoxin-producing culture at the level of $1{\cdot}5 \pm 0{\cdot}2$ mg per 100 ml, from which 30 mg of (**2**) was isolated 72 h later. After recrystallisation to constant specific activity the gliotoxin had a specific activity ($^{14}$C) of $9{\cdot}2 \times 10^8$ d.p.m. mol$^{-1}$ (incorporation, $21 \pm 3\%$; dilution $\times$ $59 \pm 7$), and the $^{14}$C : $^3$H ratio was $1 : 12{\cdot}3 \pm 0{\cdot}7$, *i.e.* effectively unchanged. Thus, intact (**1**) is incorporated into (**2**).




(1)




(2)

The discrepancy between our own observations and those of MacDonald and Slater might be ascribed to the use of different micro-organisms but it is more likely to be due to important differences in their experimental arrangement. The levels of (**1**) supplied to the cultures in their work were

in excess of the amounts of (**2**) being produced [20 mg (**1**) fed, 15—20 mg (**2**) isolated] and consequently in very considerable excess of any pool of (**1**), or its enzyme-bound equivalent, in the mycelium; relative to the yields of (**2**) being produced, our precursor was fed at *ca.* 1/25 of the level they used. If, as they suggest, the true intermediates in the biosynthesis of (**2**) are enzyme-bound, it would appear that the high level of (**1**) added in their experiments was sufficient to block equilibration of the exogenous material with the biosynthetic intermediates (though perhaps not completely, since they did observe *ca.* 0·3% incorporation). Their comparison of the dilution of label from (**1**) with that observed when labelled phenylalanine was fed is particularly misleading since the latter was supplied at a level of 800 mg per 18 mg of (**2**) produced, which was clearly sufficient to block the endogenous synthesis of phenylalanine altogether: hence the observed dilution, 1·0, which corresponds to *ca.* 1% incorporation.

It cannot be too strongly emphasized that the use of labelled precursors in testing biosynthetic hypotheses is a *tracer* technique, and that the use of levels of exogenous materials in vast excess over endogenous substrate is liable to generate misleading results.

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[1] J. C. MacDonald and G. P. Slater, *Canad. J. Biochem.*, 1975, **53**, 475.