

Biosynthesis of Gliotoxin

By JOHN D. BU'LOCK* and COLIN LEIGH

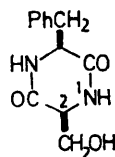
(Weizmann Microbial Chemistry Laboratory, University of Manchester, Manchester M13 9PL)

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

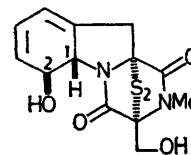
In a recent note, MacDonald and Slater¹ report that although the diketopiperazine (1) is taken up by mycelium of *Penicillium terlikowskii* it is not incorporated into the gliotoxin (2) which the latter produces. This observation is contrary to our own observation that (1) is an efficient precursor of (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-³H]phenylalanine and DL-[1-¹⁴C]serine and the product, m.p. 226–229 °C, was a mixture of (1) and its epimer at C-2, with 4.7×10^{10} d.p.m. mol⁻¹ of ¹⁴C and a ¹⁴C: ³H ratio of 1:13 ± 0.8. The labelled material was added to a gliotoxin-producing culture at the level of 1.5 ± 0.2 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 (¹⁴C) of 9.2×10^8 d.p.m. mol⁻¹ (incorporation, $21 \pm 3\%$; dilution $\times 59 \pm 7$), and the ¹⁴C: ³H ratio was 1:12.3 ± 0.7, 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.

(Received, 2nd June 1975; Com. 624.)

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