

## THE BIOSYNTHESIS OF EPIDITHIODIOXOPIPERAZINES

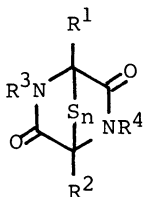
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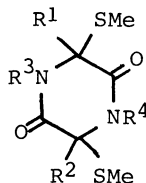
**Abstract** - Studies on the biosynthesis of gliotoxin (3), the first known member of the epidithiodioxopiperazine group of fungal metabolites, are reviewed. Phenylalanine is incorporated into gliotoxin, without loss or migration of aryl-hydrogen, by a pathway which may involve an arene oxide. cyclo-(L-Phenylalanyl-L-seryl) is identified as an intermediate and is efficiently (ca. 50%) converted into gliotoxin in Trichoderma viride. Structural analogues of cyclo-(L-Phe-L-Ser) are also efficiently metabolised and their products provide insight into the nature, and possibly sequence, of transformations occurring in the organism.

### INTRODUCTION

The epipolythiodioxopiperazines (1) and related dethiodi(methylthio)-derivatives (2) constitute a family of ca. 40 toxic, fungal metabolites.

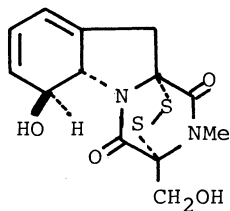


(1)

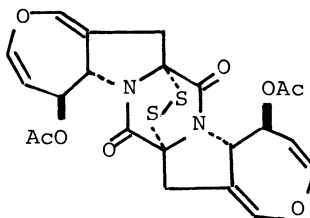


(2)

To date, biosynthetic studies have been confined to representatives of the gliotoxin (3) (Refs. 1-7), aranotin [e.g. acetylaranotin (4)] (Refs. 4, 8), sporidesmin (5) (Ref. 9), and sirodesmin [e.g. sirodesmin A (6)] (Ref. 10) groups of metabolites. Discussion here will be restricted to gliotoxin (3), the first known and most intensively studied member of the epidithiodioxopiperazines.



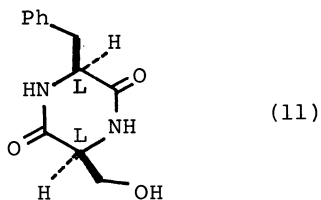
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(4)

## DIOXOPIPERAZINES AS INTERMEDIATES

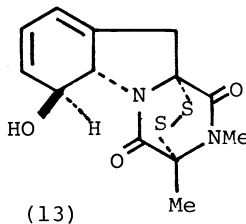
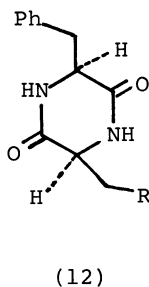
Nearly 20 years elapsed between the original feeding experiments with phenylalanine and the first identification of an intermediate on the pathway to gliotoxin. MacDonald and Slater (Ref. 5) fed labelled cyclo-(L-phenylalanyl-L-seryl) (11) to Penicillium terlikowskii but observed only a low incorporation into gliotoxin despite efficient uptake of the cyclo-



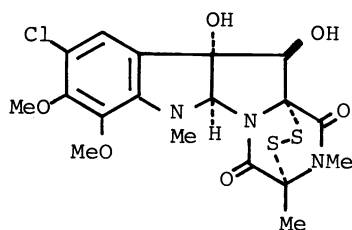
dipeptide into the mycelium. They concluded that (11) could not be a free intermediate. In contrast, Bu'Lock and Leigh (Ref. 6) observed a high incorporation (21%) of activity into gliotoxin from a mixture of radiolabelled cyclo-(L-phenylalanyl-L-seryl) and cyclo-(L-phenylalanyl-D-seryl) in Trichoderma viride (probably a Gliocladium sp.). The mixture was prepared from L-[Ar-<sup>3</sup>H]phenylalanine and DL-[1-<sup>14</sup>C]serine and, significantly, the same isotope ratio was observed in the precursor and metabolite. Recent experiments (Ref. 7) with all 4 stereoisomers of (11) in T. viride have shown that only the LL-isomer is incorporated efficiently (upto ca. 50%) into gliotoxin and, further, that this isomer may be detected in cultures of the organism by radiodilution techniques. The efficient metabolism of (11) has encouraged further experiments with dioxopiperazine derivatives aimed at elucidating the sequence of changes leading to the elaboration of the final product (3).

## METABOLISM OF UNNATURAL DIOXOPIPERAZINES

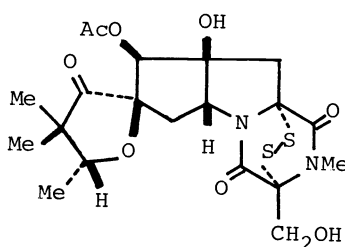
Providing that the enzymes catalysing the reactions of a secondary metabolite pathway do not have high substrate specificities, then feeding experiments with structural analogues of a natural intermediate may lead to the formation of 'unnatural' end products. This was found to be true in T. viride (Ref. 12). The deoxy-analogue (12; R = H) of (11) was converted



with surprising efficiency (ca. 40%) into the new metabolite, 3a-deoxygliotoxin (13). The structure and absolute configuration (13) was established by spectroscopic and c.d. (Ref. 13) measurements and the metabolite was shown to be spectroscopically indistinguishable from a synthetic racemate (Ref. 14). The metabolism of other analogues of (11) has been investigated (Ref. 15). Of special interest is (12; R = Me). Three products are formed reproducibly in substantial amounts. At present, only tentative structures can be advanced. One product appears to be the expected ethyl analogue of (13). Another is probably related to the di(methylthio)-derivative (14), a minor, natural metabolite (Ref. 16) of T. viride, and the third, which lacks an N-methyl group, has an n.m.r. spectrum consistent with the structure (15). It is possible that changes in the structure of the precursor (10) may alter significantly the relative rates of the various, enzyme catalysed processes and cause accumulation of structural types which are present only as minor, undetected, components of the natural metabolite mixture.

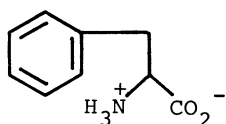


(5)

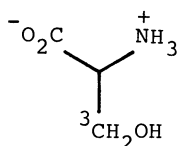


(6)

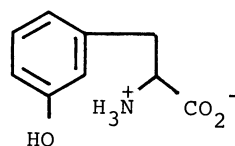
Early experiments by Suhadolnik *et al.* (Refs. 1, 2) showed that phenylalanine (7) rather than tryptophan provided the reduced indole nucleus of gliotoxin in *Trichoderma viride*. The incorporation of serine (8) was complicated by metabolic transfer of the methylene carbon (C-3) into the C<sub>1</sub> pool of the organism. Thus, DL-[3-<sup>14</sup>C]serine gave gliotoxin containing 25% of its activity in the *N*-methyl group (Ref. 2). The suggestion (Ref. 2) that



(7)

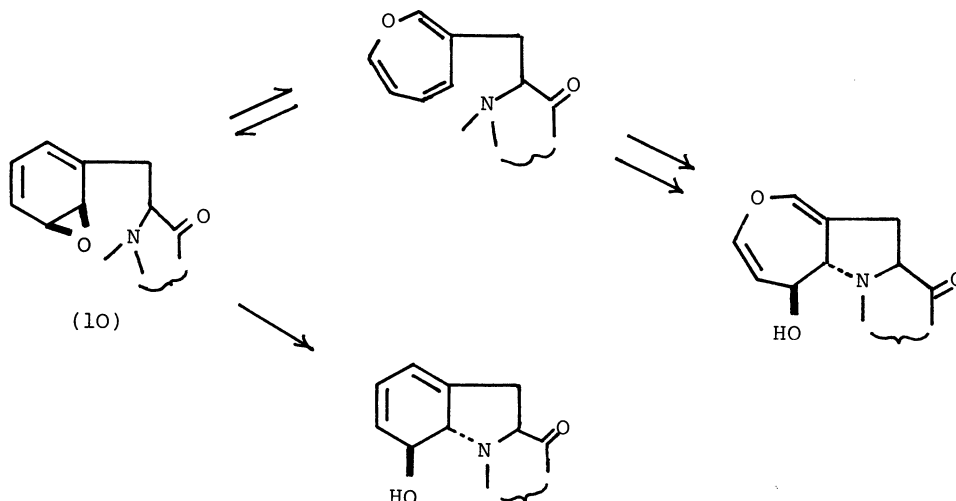


(8)

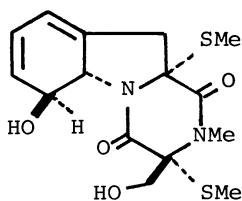


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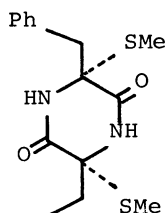
*m*-tyrosine (9) was an intermediate on the biosynthetic pathway may now be discounted in the light of more recent findings (Ref. 4). In particular, the *ortho*-, *meta*-, and *para*-hydrogens of (7) are retained, without migration, during gliotoxin biosynthesis. The formation of a cyclohexadienol ring in gliotoxin (3) and the dihydro-oxepin rings in the arantins [e.g. (4)] may involve an arene oxide of the type (10) (Refs. 4, 8, 11).



(10)



(14)



(15)

Experiments of this kind may provide clues to the sequence of the three major processes, introduction of sulphur, N-methylation, and cyclisation, leading from cyclo-(L-Phe-L-Ser) to gliotoxin and thus facilitate selection of probable intermediates for synthesis and feeding in radiolabelled form.

Acknowledgement - This work was supported by the Science Research Council (U.K.) and the Carnegie Trust.

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