The Biosynthesis of Gliotoxin; Possible Involvement of a Phenylalanine Epoxide

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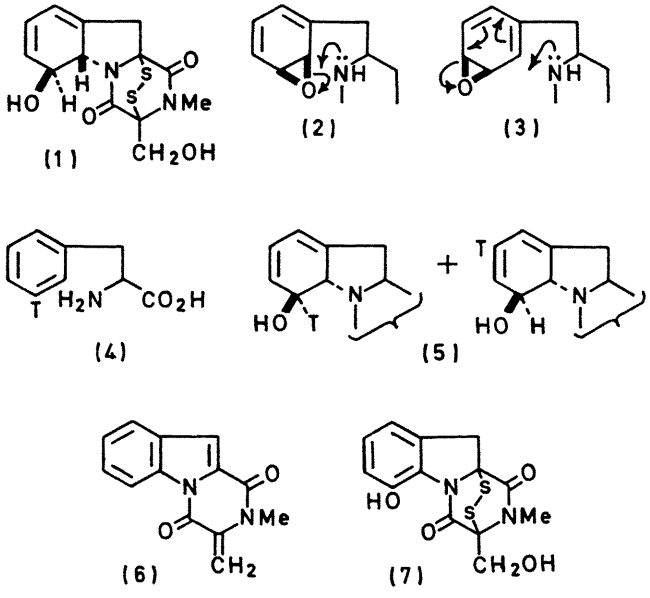
Summary Phenylalanine is incorporated, by *Trichoderma viride*, into gliotoxin *ca.* 100 times more efficiently than is *m*-tyrosine: incorporation of DL-[3-^{3}H]phenylalanine occurs without loss or migration of tritium suggesting the involvement of an arene oxide intermediate.

PHENYLALANINE is well established[1-3] as a biosynthetic precursor for the fungal toxin, gliotoxin (**1**). Also, Winstead and Suhadolnik[2] reported that generally tritiated *m*-tyrosine was incorporated considerably more efficiently (30·8 and 44·3%) than phenylalanine (14·2 and 17·6%) into this metabolite. The biological hydroxylation of phenylalanine to give *m*-tyrosine might, in principle, involve an arene oxide[4] intermediate [(**2**) or (**3**)] having the appropriate functionality to cyclise directly to the unusual dihydrobenzenoid system of gliotoxin. Either intermediate could give the required *trans*-stereochemistry in the end product. We report a reinvestigation of the status of *m*-tyrosine as a gliotoxin precursor.

Radio-labelling of DL-*m*-tyrosine† was effected in tritiated 5N-HCl at 100° for 1 h. A control experiment in DCl showed (n.m.r.) that nuclear exchange had occurred, at least predominantly, *ortho* and *para* to the phenolic hydroxy-group. DL-[2,4,6-$^3H_3$]-*m*-Tyrosine was fed to *Trichoderma viride* (strain NRRL-1828) under prescribed[1] conditions. Only a small (0·0095%) incorporation of tritium into gliotoxin was observed (see Table) although no tritium from the site of attack. DL-[3-^{3}H]Phenylalanine (**4**), prepared from 3-bromotoluene *via* [3-^{3}H]toluene, was



Incorporation of phenylalanine and m-*tyrosine into gliotoxin in* Trichoderma viride

Precursor; wt. (mg);		^{3}H : ^{14}C	% Incorp.	Wt. of (**1**) (mg)	^{3}H : ^{14}C Ratios in (**1**)	(**6**)	(**7**)
DL-[2,4,6-$^3H_3$]-*m*-Tyrosine 20	11·5	4·9	233	*ca.* 0·1		
DL-[1-^{14}C]Phenylalanine .. .. 0·07						
DL-[2,4,6-$^3H_3$]-*m*-Tyrosine .. .. 9·6	11·9	1·6	159	*ca.* 0·1		
DL-[1-^{14}C]Phenylalanine .. .. 9·3						
DL-[2,4,6-$^3H_3$]-*m*-Tyrosine .. .. 8·6		$9·5 \times 10^{-3}$	215			
DL-[1-^{14}C,3-^{3}H]Phenylalanine 13	7·07	3·9	189	7·51	7·51	3·91
DL-[1-^{14}C,3-^{3}H]Phenylalanine 17	6·51	2·1	215	6·73	6·79	3·59

significant loss of tritium from the precursor occurred under the same conditions in the absence of the organism. In a more decisive experiment, a mixture of DL-[2,4,6-$^3H_3$]-*m*-tyrosine and DL-[1-^{14}C]phenylalanine (^{3}H : ^{14}C ratio, 11·5) was incubated with *T. viride*. Tritium was barely detectable in the derived gliotoxin (^{3}H : ^{14}C ratio, *ca.* 0·1)‡ although good incorporation (4·9%) of ^{14}C was observed. A similar result was obtained when the relative weights of the two amino-acids were changed *ca.* 300 fold. It appeared therefore unlikely that, under the conditions of our experiments, *m*-tyrosine could be an obligatory intermediate in the conversion of phenylalanine into gliotoxin. However, a further test was made. Hydroxylation of phenylalanine to give *m*-tyrosine must involve loss or migration[5] of mixed with DL-[1-^{14}C]phenylalanine and the double-labelled specimen incubated with *T. viride*. No loss of tritium occurred during conversion into gliotoxin [labelling pattern (**5**)]. Dehydration and desulphurisation§ gave the derivative (**6**) again without loss of tritium. Finally, dehydrogenation[6] gave dehydrogliotoxin (**7**) with retention of 52% of the tritium. Repetition of the entire experiment gave essentially the same results which are fully consistent with a biosynthetic intermediate of the type (**2**) or (**3**). Circumstantial evidence for this possibility is provided by the structures of the aranotins which contain an oxepin ring plausibly attributed[7] to valence tautomerism of a phenylalanine epoxide.

Bu'Lock and Ryles[8] have recently published independent

† The purity and identity of commercially available (Koch–Light Ltd.) *m*-tyrosine was checked by chromatography, n.m.r. spectroscopy, and conversion, with dimethyl sulphate and alkali, into *m*-methoxycinnamic acid.

‡ Accurate measurement of small tritium activities in the presence of ^{14}C is not possible.

§ Effected with grade I, neutral alumina in dry benzene at room temperature (*cf.* ref. 3).

observations leading to the same conclusions. In particular they showed that *m*-tyrosine was poorly incorporated (highest value, 0·053%) into gliotoxin, and phenylalanine, fully deuteriated in the aromatic ring, gave [$^2H_5$]-gliotoxin as the major deuteriated metabolite.

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