A Stereospecific Exchange Reaction at the β-CH_2 of Phenylalanine, competing with Gliotoxin Biosynthesis

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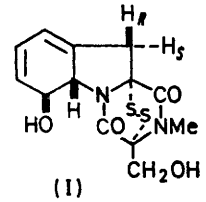
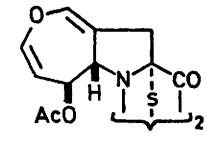
Summary Gliotoxin biosynthesis in *Trichoderma viride* proceeds with the retention of both hydrogens of the CH_2 group of phenylalanine, but in competition with a stereospecific reaction of the phenylalanine in which the 3'-*S*-hydrogen is exchanged.

IN our study of the biosynthesis of gliotoxin (I) we established that the five aromatic protons of phenylalanine are incorporated intact into (I) and so excluded certain types of possible intermediate from further consideration.[1] Independently, Brannon *et al.*[2] showed that in a strain of *Aspergillus terreus* producing the related acetylaranotin (II), [$^2H_8$]phenylalanine contributed 7 or 14 hydrogens to the molecule of (II), confirming the parallel with (I) and further showing that the β-CH_2 of phenylalanine is also incorporated intact in this case. Since indole derivatives, lacking one of these protons, have sometimes been considered as possible gliotoxin precursors, we felt it desirable to repeat this demonstration for gliotoxin. DL-[3',3'-$^2H_2$]Phenylalanine was prepared by the acetamidomalonate route from $PhCD_2Cl$ (*Cf.* Swan *et al.*[3]); the product contained not less than 97% of the $^2H_2$ species.

The labelled amino-acid, fed as in previous work[1] to *Trichoderma viride*, gave (I) which from its mass spectrum was enriched some 43% in mono-deuteriated species. Moreover, the n.m.r. spectrum of the product showed that one deuterium was specifically located: the relevant methylene protons in (I) are non-equivalent, giving rise to doublets centred at δ 3·77 (*J* 18Hz) and 2·99 (*J* 18 Hz); the former (lower-field) signal is ascribed to the *S*-hydrogen which is deshielded by the adjacent bridge-sulphur.[4] In the n.m.r. spectrum of the deuteriated product the doublet at δ 2·99 due to the *R*-hydrogen was of reduced intensity, while that at δ 3·77 was partly collapsed to a singlet with the total integral unchanged—*i.e.*, the *R*-substituent was substantially deuterium and the deuterium loss was from the *S*-substituent.



(I)



(II)

A more careful examination of the mass-spectroscopic data nevertheless reduced the apparent discrepancy between our results for (I) and those of Brannon *et al.* for (II). In the case of (I) the observed enrichments for the $^2H_1$ and the $^2H_2$ species (*e.g.* for the readily observed $M^+ - 2S$ ion) were 43% and 4%; the latter figure is by no means negligible and it demonstrates that gliotoxin can be formed without the loss of either deuterium. Equally in the case of (II) the published data[2] show, *e.g.* 88% enrichment on the $M^+ - 2S + 7$ species but also 31% enrichment of M^+

$-2S+6$, *i.e.*, in this case too there is quite a substantial loss of one deuterium even though incorporation without this loss predominates. We have since confirmed this using DL-[1′-^{14}C-3′,3′-$^{2}H_2$]phenylalanine in cultures of an *A. terreus* kindly supplied to us by Dr. Brannon.

It therefore appeared likely that some process quite independent of gliotoxin synthesis was causing the observed loss of one of the deuteriums of the phenylalanine. To study this, DL-[1′-^{14}C-3′-^{3}H]phenylalanine was fed to *T. viride* at inoculation; after 22 h the mycelium was filtered off, dried, and extracted with hot water, and the extract was evaporated with carrier phenylalanine which was then recovered and purified. The $^{3}H/^{14}C$ ratios of the precursor fed, the recovered phenylalanine, and the extracted mycelial residues were, respectively 5·52, 4·13, and 3·59, equivalent to a loss of up to 35% of the ^{3}H (*i.e.* up to 70% of the ^{3}H in one specific position) during the 22 h incubation, in processes quite unconnected with the biosynthesis of (I) (which is in fact negligible during the experimental period).

As a further test of the stereospecificity of the exchange process, samples of L-3′*R*-[3′-$^{2}H_1$]- and DL-3′*S*-[3′-$^{2}H_1$]-phenylalanine (*cf.* Kirby and Michael[5]) were similarly fed to *T. viride*, with added DL-[1′-^{14}C]phenylalanine as an internal check. With the 3′-*R*-$^{2}H_1$ species the dilution of ^{14}C in the recovered (I) was × 5·1 while the dilution of ^{2}H was rather more, × 5·8; with the 3′-*S*-$^{2}H_1$ species the dilutions were × 3·3 for ^{14}C and × 19 for ^{2}H. The (M^+-2S+1) enrichments were 21% for the 3′-*R* case and 6% for the 3′-*S*. This confirms our initial observations and the conclusion that the exchange of the 3′-H is not an obligatory step in the biosynthesis of (I). Quantitatively the comparison may be qualified by the proviso that the stereochemical purity of the monodeuterio-phenylalanines may not be absolute,[5] but this does not alter the main conclusion. Moreover, since there is no reason to suppose that any change in configuration at C-3′ occurs in the biosynthesis of (I), and the 3′-*R*-hydrogen of the phenylalanine is retained in a corresponding configuration in (I), we conclude that the process by which the 3′-*S*-hydrogen of phenylalanine is exchanged occurs without inversion at this centre. Unlike the well-known lability of the α-H of amino-acids *in vivo*, this is an unsuspected reaction, which could give rise to misinterpretations in biosynthetic studies. However, Oshima and Tamija[6] showed that all the hydrogens of L-alanine are exchangeable in $^{2}H_2O$ incubations with pyridoxal phosphate and alanine-glutamate transaminase; phenylalanine, in which the β-protons are nonequivalent, might be expected to undergo such an exchange at least as readily and with stereospecificity. The exchanging species is conceivably an enzyme-complexed pyridoxamine derivative of the α-keto-acid.[6]

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[1] J. D. Bu'Lock and A. P. Ryles, *Chem. Comm.*, 1970, 1403.
[2] D. R. Brannon, J. A. Mabe, B. B. Molloy, and W. A. Day, *Biochem. Biophys. Res., Comm.*, 1971, **43**, 588.
[3] F. Binns, J. A. G. King, A. Percival, N. C. Robinson, and G. A. Swan, *J. Chem. Soc.* (*C*), 1970, 1134.
[4] R. Nagarajan, L. Huckstep, D. H. Lively, D. C. Delong, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, 1968, **90**, 2980.
[5] G. W. Kirby and J. Michael, *Chem. Comm.*, 1971, 187.
[6] T. Oshima and N. Tamija, *Biochem. J.*, 1961 ,**78**, 116.