Analogue Biosynthesis in *Trichoderma viride*: the Formation of 3a-Deoxygliotoxin

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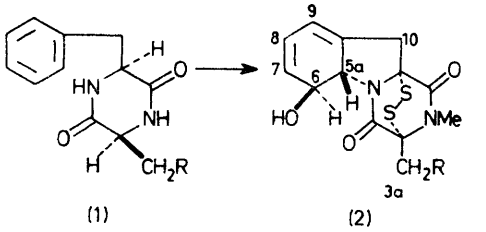
Summary *Cyclo*-(L-alanyl-L-phenylalanyl) is converted in cultures of *Trichoderma viride* (*Gliocladium deliquescens*) into the gliotoxin analogue, 3a-deoxygliotoxin, with an efficiency similar to that of the corresponding 'natural' process.

COMPLEX natural products, including many of medicinal value, are often more readily obtained from natural sources than by total synthesis. The chemical conversion of natural products into structural analogues, for pharmacological evaluation, is common practice but is necessarily limited in scope by the functionality of the starting material. In principle, the biosynthesis in intact organisms of metabolite analogues from appropriately designed, unnatural precursors is attractive.[1] Ideally, the organism should be easy to grow, the modified precursor simple to synthesise, and the biosynthetic conversion efficient. We report the biosynthesis of a gliotoxin analogue which meets these criteria.

Recently, Bu'Lock and Leigh showed[2] that gliotoxin (**2**; R = OH), a metabolite of phenylalanine,[3] was formed from *cyclo*-(phenylalanylseryl) (**1**; R = OH) in *Trichoderma viride* (NRRL-75). Efficient (21%) conversion of the precursor, fed as a mixture of diastereoisomers, into gliotoxin was observed without appreciable degradation and resynthesis. We have observed the analogous conversion of (**1**; R = H) into the unnatural metabolite (**2**; R = H) in *Trichoderma viride* (*Gliocladium deliquescens*, NRRL-1828).

^{14}C-Labelled *cyclo*-(L-alanyl-L-phenylalanyl) (**1**; R = H) was prepared by standard methods[4] from L-[*U*-^{14}C]phenylalanine methyl ester hydrochloride and *N*-benzyloxycarbonyl-L-alanine. The precursor, in dimethyl sulphoxide (16 mg per ml), was added to one-day old cultures of *T. viride* (1·0—8·0 mg per 100 ml of culture medium) and, after 3 days, the chloroform-soluble metabolites were isolated and found to contain 20—40% of the original



(1) (2)

radioactivity. The higher figure was observed when the precursor was fed at the lower (1 mg per 100 ml) concentration. Radio scanning and autoradiography of thin-layer chromatograms showed that radioactivity was very largely confined to a single component running faster than gliotoxin.† The new metabolite was isolated by preparative

† The gliotoxin band contained very little radio-activity. Hydrolysis of (**1**; R = H) and incorporation of liberated phenylalanine into gliotoxin had not, therefore, occurred to any appreciable extent. This agrees with Bu'Lock and Leigh's observations[2] with the natural precursor (**1**; R = OH).

t.l.c. and identified as 3a-deoxygliotoxin (**2**; R = H), m.p. 185—186 °C (from MeOH), $[\alpha]_D^{20}$ − 262° (*c* 0·63 in $CHCl_3$), λ_{max} 269 nm (ϵ 5680 in EtOH). The composition, $C_{13}H_{14}N_2O_3S_2$, was established by accurate mass measurement of the characteristically weak molecular ion and the intense ($M - S_2$) fragment ion and by elemental analysis. The pattern of multiplets, arising from protons at positions 5a, 6, 7, 8, 9, and 10, in the n.m.r. spectrum (100 MHz, in $CDCl_3$) of (**2**; R = H) was almost identical with that of gliotoxin (**2**; R = OH). The *N*-methyl singlet of (**2**; R = H) appeared upfield (τ 6·93) from the corresponding signal (τ 6·80) of gliotoxin and a new, methyl, singlet (τ 8·00) replaced the 3a-methylene quartet (τ 5·60 and 5·76, *J* 12 Hz) of the natural metabolite. These data, taken with the large, negative optical rotation (*cf.* $[\alpha]_D^{25}$ − 255° for gliotoxin[5]) established the gross structure and absolute stereochemistry of (**2**; R = H).

3a-Deoxygliotoxin appears to be a new metabolite of *T. viride* formed in response to administration of the precursor (**1**; R = H). No detectable amounts of (**2**; R = H) were formed when the organism was grown on the normal culture medium; moreover, the specific activity of ^{14}C-labelled (**2**; R = H) was identical, within experimental error, with that of its precursor (**1**; R = H) showing that appreciable dilution with endogenous material has not occurred. Pure, crystalline (**2**; R = H) was isolated in *ca.* 10% yield [based on (**1**; R = H)] from high-concentration experiments (*ca.* 20% incorporation of ^{14}C) without optimisation of culture conditions or isolation procedures. The 'unnatural' transformation, (**1**; R = H) → (**2**; R = H), appears therefore to proceed about as efficiently as the natural process despite a significant structural change, OH→ H, close to the site of two subsequent enzymic reactions, *N*-methylation and sulphur insertion. The ability of *T. viride* to effect the chemically demanding[6] late stages in the synthesis of gliotoxin analogues is being explored further.

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[6] Gliotoxin itself has not been synthesised; for the synthesis of (±)-5a,6-didehydrogliotoxin see Y. Kishi, T. Fukuyama, and S. Nakatsuka, *J. Amer. Chem. Soc.*, 1973, **95**, 6492, and for a review see P. G. Sammes in 'Progress in the Chemistry of Organic Natural Products,' eds. W. Herz, H. Grisebach, and G. W. Kirby, Springer-Verlag, Wien and New York, 1975, Vol. 32, p. 51.