Phytochemistry, Vol. 22, No. 8, pp. 1719–1720, 1983.
Printed in Great Britain.

0031–9422/83$3.00 + 0.00

INCORPORATION OF RADIOACTIVE PRECURSORS INTO BEAUVERICIN PRODUCED BY *PAECILOMYCES FUMOSO-ROSEUS*

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(*Revised received* 14 *January* 1983)

Key Word Index—*Paecilomyces fumoso-roseus*; fungi; beauvericin; biosynthesis; cyclodepsipeptide antibiotic; entomopathogenic fungus.

Abstract—The biosynthesis of the depsipeptide antibiotic beauvericin was studied by feeding submerged cultures of *Paecilomyces fumoso-roseus* with [^{14}C]-labelled precursors. L-Phenylalanine, hydroxyisovaleric acid, L-valine and methyl-labelled methionine were efficiently incorporated into beauvericin, whereas no radioactive depsipeptide was formed with L-leucine and L-tyrosine. [^{14}C]Phenylalanine was found to label exclusively the *N*-methylphenylalanine residues of beauvericin, the *N*-methylgroups, thereof, were derived from methionine. Beauvericin synthesized in the presence of DL-[2-^{14}C]hydroxyisovaleric acid or L-[^{14}C]valine was specifically labelled in its hydroxy valeryl moieties.

INTRODUCTION

Beauvericin is a cyclodepsipeptide antibiotic with insecticidal properties [1–3] produced by several fungi. The structures of beauvericin and the closely related enniatins A–C are shown in Fig. 1. This class of depsipeptide antibiotics is characterized by a ring system consisting of alternating D-2-hydroxyisovaleric acid (D-HYIV) and *N*-methylated amino acid residues.

The biosynthesis of enniatins could be elucidated [4–6]. It proceeds via a thiotemplate mechanism analogous to the biosynthesis of peptide antibiotics in bacteria of the genus *Bacillus* [7]. In this communication we present feeding experiments *in vivo* which indicate that the biosynthesis of beauvericin by the fungus *Paecilomyces fumosos-roseus* resembles that of enniatin in *Fusarium oxysporum*.

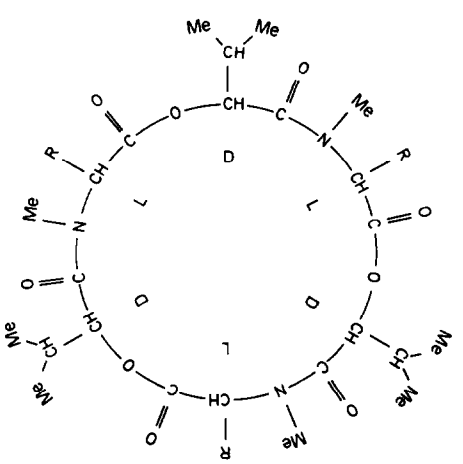

Fig. 1. Beauvericin, R = –CH_2–C_6H_5; enniatin A, R = –CH(Me)CH_2Me; enniatin B, R = –$CHMe_2$; enniatin C, R = –CH_2CHMe_2.

RESULTS AND DISCUSSION

Paecilomyces fumoso-roseus grown in submerged culture in the modified NRRL cornsteep molasses medium produced beauvericin after *ca* 48 hr. Beauvericin concentration reached a constant level of *ca* 5 mg/l. after *ca* 80 hr of fermentation. Despite the low titer of depsipeptide formed, the incorporation of presumed precursors into the molecule was very efficient under these conditions. In contrast, the higher yielding (100–150 mg/l. beauvericin) galactose-medium or a medium used by Hamill *et al.* [1] were not suitable for incorporation studies.

L-[^{14}C]Phenylalanine, DL-[^{14}C]HYIV, L-[^{14}C]valine and L-[methyl-^{14}C]methionine were readily incorporated into beauvericin (Table 1). The radioactively labelled beauvericins were analysed after acid hydrolysis by PC and TLC. When [^{14}C]phenylalanine was fed to the cultures, only one radioactive spot with the chromatographic behaviour of *N*-methylphenylalanine (solvent systems I and II) appeared. When L-[^{14}C]valine or [^{14}C]HYIV was used as the radiolabel, only one spot, with the R_f of HYIV, could be detected in the same solvent systems. Incorporation of L-[^{14}C-methyl]methionine into beauvericin yielded only one spot of *N*-methylphenylalanine after acid hydrolysis.

Table 1. Incorporation of radioactive precursors into beauvericin*

Precursor	% radioactivity incorporated	Incorporation (nmol)
L-[^{14}C]Valine	6.4	0.112
DL-[^{14}C]Hydroxyisovaleric acid	10.7	0.666
L-[^{14}C]Phenylalanine	16.0	0.17
L[methyl-^{14}C]-Methionine	5.6	0.56

*0.5 μCi of the individual compound was used.

In order to investigate the substrate specificity of the beauvericin synthetase(s) the strain was fed with substrate analogs of phenylalanine, such as [^{14}C]leucine and [^{14}C]tyrosine. In no case could the formation of any labelled compound be observed, even in long term experiments lasting more than 24 hr.

The efficient incorporation of L-phenylalanine and DL-HYIV into beauvericin clearly indicates that these metabolites are direct precursors of the respective constituents of the beauvericin molecule. The incorporation of radioactivity from L-valine is not at all surprising, since in the case of the structurally related antibiotic, enniatin, labelled L-valine is incorporated into enniatin with equal distribution of radioactivity between the *N*-methylvaline and D-HYIV moiety [4].

The intriguing structural detail of the depsipeptide antibiotics is the occurrence of *N*-methylated peptide bonds. *N*-Methylated valine is not a direct precursor of enniatin, but the *N*-methylation takes place only after activation of L-valine on the enniatin synthetase multienzyme [5]. In the case of beauvericin, it remains unclear whether phenylalanine or *N*-methylphenylalanine serve as direct precursors for the *N*-methylphenylalanine moiety of the final product. Attempts to demonstrate a possible incorporation of *N*-methylphenylalanine failed since, at least under the conditions we employed, this compound was not taken up by the fungal mycelium. On the other hand, it is most likely that methionine serves as the donor of the *N*-methyl group of *N*-methylphenylalanine since beauvericin, analysed after labelling with [^{14}C]methionine contained radioactivity exclusively in its *N*-methylphenylalanine residues. Methionine, in the form of SAM, serves as the methyl donor for the *N*-methylated peptide bonds in the biosynthesis of enniatins [4].

In view of the close structural relationship between enniatin and beauvericin (see Fig. 1), it seems reasonable to assume a similar mechanism for the biosynthesis of both types of depsipeptide antibiotics.

EXPERIMENTAL

Chemicals. Authentic beauvericin was purchased from Bachem (Switzerland), cornsteep liquor was kindly provided by Dr. Rettschlag, Schering AG, Berlin, West Germany. All other chemicals were of highest purity commercially available. Radiochemicals were from Amersham, U.K. 2-(DL-1-^{14}C)HYIV was prepared from DL-(1-^{14}C]valine (60 Ci/mol) according to ref. [8]. Sp. acts. were: L-[U-^{14}C]valine (285 Ci/mol), L-[U-^{14}C]phenylalanine (450 Ci/mol), L-[methyl-^{14}C]methionine (50 Ci/mol).

Growth of organism. Paecilomyces fumoso-roseus was obtained from the Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Biologische Schädlingsbekämpfung, Darmstadt, West Germany. It was grown on agar slants (2% agar, 3% molasses and 1% cornsteep liquor) for 10–14 days at 27° 500-ml Erlenmeyer flasks containing 100 ml medium were inoculated with 0.2 ml of a spore suspension prepared from slant cultures (2×10^8 spores/ml).

The media for submerged cultivation were: (A) 3% molasses, 1% cornsteep liquor; or (B) galactose 50 g, peptone 5 g (from casein, Merck), glucose 2 g, KH_2PO_4 2 g, $MgSO_4 . 7H_2O$ 0.25 g, yeast extract (Difco) 0.1 g, trace element soln 4 ml, H_2O made up to 1 l. The pH of the galactose medium was adjusted to 6.8 with 1 M NaOH before autoclaving. The trace element soln contained (per l. H_2O): $FeCl_3 \cdot 6H_2O$ 0.29 g, $CuSO_4 \cdot 5H_2O$ 0.039 g, $MnCl_2 \cdot 4H_2O$ 0.018 g, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0075 g.

The inoculated flasks were incubated on a rotatory shaker (125 rpm) at 27°.

Feeding experiments. 2 ml 65 hr old cultures were removed, centrifuged and the mycelium pellet was washed twice with tap H_2O. After resuspension in 2 ml tap H_2O the labelled precursors were added (0.5 μCi). After 10 min of incubation at 27° the labelled beauvericin was extracted with 2 ml EtOAc and separated by TLC (solvent system III). It was analysed after acid hydrolysis (6 M HCl, 110°, 18 hr) by means of PC (solvent system I) and TLC (solvent system II) as described previously [4].

Solvent systems used were: system I, *n*-BuOH–HOAc–H_2O (4:1:5) (Whatman 3 mm paper); system II, *n*-BuOH–HOAc–H_2O (5:1:4) (cellulose); system III, EtOAc–MeOH–H_2O (5:2:1) (Si gel).

Acknowledgements—This research was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 9). We are obliged to Dr. G. Zimmermann for supplying the microorganisms.

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