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MALFORMINS FROM *ASPERGILLUS FICUUM*, *A. AWAM ORI* AND *A. PHOENICIS**

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Abstract—Malformins were isolated from culture filtrates of *Aspergillus ficuum*, *A. awamori* and *A. phoenicis*. They were characterized as malformin A (a mixture of malformins A_1 and A_2) on the basis of biological activity, chromatographic behavior, i.r. and mass spectrum, optical rotation and amino acid analysis.

INTRODUCTION

THE MALFORMINS, which were first isolated from culture filtrates of the fungus *Aspergillus niger*,[1] are plant growth regulators which induce malformations on the stems and petioles of *Phaseolus vulgaris* and curvatures in the roots of *Zea mays*.[2] Malformin A was isolated. from *A. niger* strain 58-883 or 56-39 and malformin B from strain 56-30. The structure of malformin A was cyclo-L-isoleucyl-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl.[3] Subsequently, it was shown that malformin A was a mixture of two cyclic pentapeptides, A_1 (88 per cent) and A_2 (12 per cent).[4] The structure of malformin A_1 was identical to that of malformin A of the earlier report. Malformin A_1, $C_{23}H_{39}O_5N_5S_2$ (M.W. 529), contains the amino acids cys:val:leu:ileu (2:1:1:1), and A_2, $C_{22}H_{37}O_5N_5S_2$ (M.W. 515), contains cys:val:leu or ileu (2:2:1). Malformin B was an approximately equal mixture of B_1 and B_2.[4]

Malformin-like activity was detected in ether extracts of the culture filtrates of *A. ficuum*, *A. awamori*, *A. phoenicis* and *Byssochlamys nivea*, and preliminary evidence suggested that the active compound produced by *A. awamori* was malformin A.[5] We describe here the isolation and characterization of malformins from three of these fungi.

RESULTS AND DISCUSSION

Malformin from Aspergillus ficuum

From 40 l. of culture filtrate of *A. ficuum* approximately 700 mg of malformin was isolated as described.[1] The optimum concentration (0·1 ppm) for corn root curvature was identical with that of authentic malformin A isolated from culture filtrate of *A. niger*. Following TLC, using silica gel H as adsorbent, water-saturated ethyl acetate as solvent, and iodine vapours

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[2] R. W. CURTIS, *Plant Physiol.* **36**, 37 (1961).
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[4] S. TAKEUCHI, M. SENN, R. W. CURTIS and F. W. MCLAFFERTY, *Phytochem.* **6**, 287 (1967).
[5] R. W. CURTIS and H. TANAKA, *Appl. Microbiol.* **15**, 1519 (1967).

for detection, both authentic malformin A and *A. ficuum* malformin separated into two compounds with R_fs of 0·82 (A_1) and 0·77 (A_2). The i.r. (nujol mull) and mass spectra (Hitachi RMU-6A) of malformin A and *A. ficuum* malformin were essentially identical. Major ion peaks were *m/e* 30, 41, 43, 55, 69, 72 and 86. Ion peaks at *m/e* 72 and 86 are indicative of valine and leucine, respectively.[6] The mass spectrum of *A. ficuum* malformin had molecular ion peaks at *m/e* 529 due to A_1 and *m/e* 515 due to A_2 (intensity ratio of $A_1:A_2=90:10$). For *A. ficuum* malformin, $[\alpha]_D^{18}=-28{\cdot}7°$ (c=0·76, methyl cellosolve); malformin A, $[\alpha]_D^{18}=-26{\cdot}6°$ (c=0·68, methyl cellosolve) (Perkin–Elmer Model 141 Polarimeter). Amino acid analyses following hydrolysis with 6 N HCl in sealed tubes for 20 hr at 120° are given in Table 1.

TABLE 1. MOLAR RATIO OF AMINO ACIDS IN MALFORMIN PRODUCED BY FUNGI*

Aspergillus species	1/2 cys	Val	Leu	Ileu	Leu *plus* ileu	$A_1:A_2$
A. niger	1·260	1	0·864 (0·851)	0·685 (0·704)	1·549 (1·555)	82·6:17·4
A. ficuum	1·591	1	1·010 (0·909)	0·730 (0·818)	1·740 (1·727)	90:10
A. awamori	1·549	1	1·126 (0·870)	0·485 (0·739)	1·611 (1·609)	85:15
A. phoenicis	1·398	1	0·972 (0·935)	0·840 (0·869)	1·812 (1·804)	93:7

* Valine=1. Calculated values are shown in parentheses.

Malformin from Aspergillus awamori

From 40 l. of *A. awamori* culture filtrate approximately 80 mg of malformin was isolated. On the basis of biological activity, TLC, i.r. and mass spectrum, we concluded that the active material was malformin A. From the mass spectrum the ratio of $A_1:A_2=85:15$. $[\alpha]_D^{18}=-28{\cdot}0°$ (c=0·7, methyl cellosolve). The amino acid analysis is given in Table 1.

Malformin from Aspergillus phoenicis

From 32 l. of *A. phoenicis* culture filtrate approximately 3 mg of active material was isolated. On the basis of biological activity, TLC, i.r. and mass spectrum the active material was malformin A. The ratio $A_1:A_2=93:7$. The amino acid analysis is given in Table 1.

Malformin-like Activity in Culture Filtrate of Byssochlamys nivea

Culture filtrate of *B. nivea* contains a neutral, ether-soluble substance which induces corn root curvatures and malformations of *P. vulgaris*.[5] Because the active substance was eluted from columns of alumina and silica-gel by solvents that do not remove malformins, and chromatographed differently than known malformins by TLC, we concluded that neither malformin A nor B were responsible for the activity in the culture filtrate. The active substance formed an insoluble, resinous substance after drying and further characterization was not attempted.

Malformins produced by *A. ficuum*, *A. awamori* and *A. phoenicis* appear to be malformin A as judged by biological activity, TLC, i.r. and mass spectrum. However, the amino acid

[6] B. J. MILLARD, *Tetrahedron Letters* 3041 (1965).

analyses (Table 1), especially that of *A. awamori* malformin, differ somewhat from that of authentic malformin A. When authentic malformin A is considered to be a mixture of 82·6 per cent A_1 (cys:val:leu:ileu = 2:1:1:1) and 17·4 per cent A_2 (cys:val:leu = 2:2:1), the relative amount of valine, leucine and isoleucine in the mixture may be calculated as 82.6 (from A_1) + 17·4 × 2 (from A_2), 82·6 (from A_1) + 17·4 (from A_2), and 82·6 (from A_1) respectively. The molar ratio of val:leu:ileu = 1·0:0·851:0·704. These values are in close agreement with the ratio obtained from amino acid analysis (1·0:0·864:0·685). From the ratio of A_1:A_2 in the malformins produced by *A. ficuum*, *A. awamori* and *A. phoenicis*, the ratios of val:leu:ileu were calculated similarly (Table 1). The calculated amino acid ratios of *A. ficuum* and *A. phoenicis* malformins are roughly consistent with the experimentally determined ratios. Although the calculated and experimentally determined ratios of amino acids of *A. awamori* malformin are not in agreement, the ratios of val:leu *plus* ileu agree (calc. 1·0:1·609; found, 1·0:1·611). If the A_1 fraction of *A. awamori* malformin contains a second species of malformin containing only leucine (cys:val:leu = 2:1:2), the high leucine and low isoleucine values are understandable and would not alter the ratio of val:leu *plus* ileu. The slight deviations in calculated and experimentally determined ratios of leucine and isoleucine of the other malformins may be similarly explained.

EXPERIMENTAL

Production of Culture Filtrate

All fungi were grown for 5 days in New Brunswick Model FS 314 fermentation jars containing 10 l. of corn steep-dextrose medium as described.[1] At the end of the fermentation period the mycelium was removed by filtration and discarded.

Isolation of Malformins

Malformins were isolated from culture filtrates of *A. niger* van Tiegh. strain 58-883, *A. ficuum* (Reich.) Hennings and *A. awamori* Nakazawa as described.[1] Culture filtrate, 32 l., of *A. phoenicis* (Cda.) Thom was concentrated *in vacuo* to 3 l., adjusted to pH 3 with H_2SO_4, and extracted with EtOAc (5 × 1·5 l.). The combined extracts were washed with saturated $NaHCO_3$ and evaporated to dryness *in vacuo*. The residue was dissolved in acetone, 20 ml, mixed with silica gel, 30 g, dried *in vacuo*, suspended in water, and poured on top of a column consisting of a mixture of activated charcoal, 50 g, and celite, 50 g. The column was eluted with water and then with increasing concentrations of acetone. Most of the active fraction, determined by the corn root curvature test,[2] was eluted with 50 to 70% acetone. Active fractions were combined, evaporated to remove acetone, and the aqueous residue, 500 ml, extracted with EtOAc (5 × 200 ml). The combined extracts were concentrated to approximately 5 ml, adsorbed on a column of silica gel, and the column eluted with benzene, benzene containing increasing amounts of EtOAc, EtOAc, and EtOAc containing 2% ethanol. The active fraction was eluted by the last two solvents. The active fractions were combined and evaporated to dryness. The residue was dissolved in acetic acid, streaked onto TLC plates (20 × 20 cm) coated with silica gel, developed with EtOAc (H_2O saturated), and dried. Malformin, approximately 3 mg, was extracted with EtOAc (H_2O saturated) from the band of silica gel between R_f 0·7 to 0·9.

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