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THE INSECTICIDAL ACTIVITY OF BEAUVERICIN AND THE ENNIATIN COMPLEX

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Abstract

The insecticidal activity of a complex mixture of enniatins, cyclohexadepsipeptides produced by *Fusarium lateritium*, a pathogen of the scale insect *Hemiberlesia rapax*, is compared with that of enniatin A and beauvericin in two bioassays against *Calliphora erythrocephala* and *Aedes aegypti*.

Introduction

The enniatins, produced by several plant pathogenic *Fusarium* sp., including *F. lateritium*, are a group of cationophoric hexadepsipeptides whose phytotoxic[4] and antibacterial[12] properties are well documented. Although the naturally-occurring enniatins were originally thought to be homogeneous regular cyclic hexadepsipeptides made up from D-α-hydroxyisovaleryl-N-methyl-L-isoleucyl (a) (enniatin A, 3a) or D-α-hydroxyisovaleryl-N-methyl-L-valyl (b) units (enniatin B, 3b), reexamination of these materials by high resolution mass spectroscopy[10] showed them to be solid solutions (the enniatin complex) of enniatins A or B with enniatin A_1 (2a + b) and B_1 (a + 2b).

The enniatins are analogues of beauvericin, a regular cyclic hexadepsipeptide containing only D-α-hydroxyisovaleryl-N-methyl-L-phenylalanyl units and produced by the insect pathogens *Beauveria bassiana*[7] and *Paecilomyces fumoso-roseus*[1] and by the plant pathogen *Polyporus sulphureus*[3]. Although beauvericin was claimed[7] to have insecticidal properties, little detailed information has been published[9 13]. The enniatins have not, to our knowledge, been examined for insecticidal activity either alone or in comparison with beauvericin, although many comparisons of their antimicrobial and cationophoric properties have been made.[5, 8, 11]

Materials and methods

Tests for insecticidal activity

The tests were carried out as described previously[6] except that the solvent used for injection in the *Calliphora* bioassay was acetone – Ringer's solution (1 : 1).

The fermentation medium

Fermentation medium contained Difco Bactopeptone (10 g), glucose (10 g) and sodium chloride (5 g) in water (1 litre). Minor element concentrate containing $FeCl_3 \cdot 6H_2O$ (0.03 g), $CuSO_4 \cdot 5H_2O$ (0.004 g), $MnCl_2 \cdot 4H_2O$ (0.002 g) and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.0007 g) in water (100 ml) was added at a rate of 10 ml/l.

Fermentations and isolation of secondary metabolites

Conical flasks (250 ml) containing the medium (100 ml) were inoculated with a spore suspension of the *F. lateritium* strain and shaken on a rotary shaker for 4 days at 25°. After harvesting in the usual way, the culture filtrate was extracted with light petroleum b.p. 60–80°. With strain 113, recovery and crystallisation from aqueous methanol[2] afforded crystals (135 mg/l), m.p. 115–125°, $[\alpha]_D^{25}$ –99° of a solid solution of enniatins B, B_1 and A_1 γ_{max} 1738, 1670 cm^{-1} (Found: *M* 639.4074, 653.4230, 667.4385. Calc. for $C_{33}H_{57}O_9N_3$, $C_{34}H_{59}O_9N_3$ and $C_{35}H_{61}O_9N_3$: 639.4094, 653.4251, 667.4407). Analysis based on the relative intensities of the molecular ions in the low resolution mass spectrum gave an approximate composition of 5 : 3.5 : 1.

The culture filtrates from strains 112 and 114 yielded similar complexes with approximate composition B : B_1 : A_1 : A of 2 : 4 : 3 : 1 and 4 : 5 : 3.5 : 1 respectively.

The powdered dried mycelium (10.7 g) from the culture fluid (1 l.) of strains 113 and 114 was extracted in a Soxhlet

apparatus with light petroleum b.p. 60–80°. Recovery and purification as described above gave crystals (740 mg), m.p. 110–120° γ_{max} 1738, 1670 cm^{-1} of a solid solution of enniatins B, B_1, A_1, and A of composition approximately 3 : 4 : 3 : 1 (Found, for enniatin A: *M* 681.4561. Calc. for $C_{36}H_{63}O_9N_3$: 681.4563).

Results and discussion

Through the courtesy of L.N. Ferguson, Entomology Division, DSIR, New Zealand, we have examined three strains (numbers 112–114 in our collection) of *F. lateritium*, pathogenic to the scale insect *Hemiberlesia rapax*, for the formation of insecticidal secondary metabolites. These strains when grown on a glucose-peptone medium produced, like the plant pathogenic strains, enniatin complexes in which the proportions of the component enniatins varied slightly, both from strain to strain, and according to whether the complex was obtained by extraction of the culture filtrate or the dried mycelium. Although enniatin B is known to be less soluble than enniatin A in light petroleum, we found that a solid solution consisting mainly of enniatins B and B_1 and containing no enniatin A was readily extracted by this solvent from the culture filtrate of strain 113. Enniatin A was a relatively minor component of the complex from the mycelium of this strain and from the culture filtrates of strains 112 and 114.

We selected a complex containing enniatins B, B_1 and A_1 in the ratio of approximately 5 : 3.5 : 1 and compared it with enniatin A and beauvericin for toxicity, by injection, to adults of the blowfly *Calliphora erythrocephala* (Table 1), and to larvae of the mosquito *Aedes aegypti* (Table 2). Enniatin A and the enniatin complex were less active than beauvericin against mosquito larvae but more active than beauvericin in the assay against adult blowflies where, however, the low solubility of the substances prevented the determination of LD50 values. Beauvericin compared unfavourably with its co-metabolite bassianolide (4c), the regular octadepsipeptide analogue of enniatin C (3c) and composed of D-α-hydroxyisovaleryl-N-methylleucyl (c) units, when tested against silkworm larvae both by oral administration and by injection[9]. Even against mosquito larvae it was an order of magnitude less active than carbaryl.

In most tests for biological activity the more lipoid soluble enniatin A has been found more active than enniatin B, and this generalisation seems to hold true for the bioassay against mosquito larvae. However, in some tests, mixtures of enniatins A and B have been more active than the individual purified components and the *Calli-*

Table 2. Larvicidal activity of enniatin and beauvericin to *Aedes aegypti*.

Compound	*Concentration* (μg/ml)	*Mortality* (%) *Hrs*		
		18	*48*	*72*
Enniatin complex	25	1	3	
	44	4	15	31
	75*	20	63	71
Enniatin A	10	11	10	
	20	25	37	35
Beauvericin	10	10	39	64
	20	58	86	
1-Naphthylmethylcarbamate (carbaryl)	1	54	67	73
Control		0	0	0

* Saturated solution.

Table 1. Insecticidal activity of enniatin and beauvericin to *Calliphora erythrocephala*.

Compound	*Dose* (μg/fly)	*Knockdown* (% Flies down) *Hrs*					*Toxicity* (% Mortality) *Days*	
		0	1	2	3	18	1	2
Enniatin complex**	5	66	44	38	38	28	28	39
	10*	90	75	75	75	55	60	60
Enniatin A	5*	62	26	12	9	7	15	32
Beauvericin	5*	60	8	6	0	2	8	15
Control		0	0	0	0	0	0	1

* saturated solution.

** the enniatin complex was inactive by topical application at 50 μg/fly.

phora bioassay appears to be another example in this category.

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References

1. Bernardini, M, A. Carilli, G. Pacioni & B. Santurbano. 1975. Isolation of beauvericin from Paecilomyces fumosoroseus. Phytochem. 14: 1865.
2. Cook, A.H., S.F. Cox & T.H. Farmer. 1949. Production of antibiotics by fungi. Part IV. Lateritiin I, Lateritiin II, Avenacein, Sambucinin and Fructigenin. J. Chem. Soc. 1022–1028.
3. Deol, B.S., D.D. Ridley & P. Singh. 1978. Isolation of cyclodepsipeptides from plant pathogenic fungi. Aust. J. Chem. 31: 1397–1399.
4. Gaumann, E., S. Naef-Roth & H. Kern. 1960. The phytotoxic effectiveness of the enniatins. Phytopathol. Zeit. 40: 45–51.
5. Gorneva, G.A., T.S. Chumburidze, L.A. Fonina, A.V. Evstratov, I.D. Ryabora, V.T. Ivanov & Y.A. Ovchinnikov. 1976. Ionophoric properties and the mode of antimicrobial action of valinomycin, enniatins and their synthetic analogs. Bioorg. Khim. 2: 1165–1173.
6. Grove, J.F. & M. Pople. 1977. The insecticidal activity of flavensomycinoic acid and some analogues. J. Antibiotics 30: 980–982.
7. Hamill, R.L., C.E. Higgins, H.E. Boaz & M. Gorman. 1969. The structure of beauvericin, a new depsipeptide antibiotic toxic to Artemia salina. Tetrahedron Letters 4255–4258.
8. Ivanov, V.T., A.V. Evstratov, L.V. Sumskaya, E.I. Melnik, T.S. Chumburidze, S.L. Portnova, T.A. Balashova & Y.A. Ovchinnikov. 1973. Sandwich complexes as a functional form of the enniatin ionophores. Febs. Letters 36: 65–71.
9. Kanaoka, M., B. Isogai, S. Murakoshi, M. Ichinoe, A. Suzuki & S. Tamura. 1978. Bassianolide, a new insecticidal cyclodepsipeptide from Beauveria bassiana and Verticillium lecanii. Agric. Biol. Chem. 42: 629–635.
10. Kiryushkin, A.A., B.V. Rozynov & Y.A. Ovchinnikov. 1968. A mass spectrometric study of natural mixtures of enniatin antibiotics. Khim. Prir. Soedin. 4: 182–186.
11. Prince, R.C., A.R. Crofts & L.K. Steinrauf. 1974. A comparison of beauvericin, enniatin and valinomycin as calcium transporting agents in liposomes and chromatophores. Biochem. Biophys. Res. Comm. 59: 697–703.
12 Shemyakin, M.M., Y.A. Ovchinnikov, V.T. Ivanov, A.A. Kiryushkin, G.L. Zhdanov & I.D. Ryabora. 1963. The structure-antimicrobial relation of depsipeptides. Experientia 19: 566–568.
13. Vey A., J.M. Quiot & C. Vago. 1973. Mise en évidence et étude de l'action d'une mycotoxine, la beauvericine, sur des cellules d'insectes cultivées in vitro. Compt. rend (D) 276: 2489–2492.