Phytochemistry, Vol. 20, No. 4, pp. 815–816, 1981.
Printed in Great Britain.

0031-9422/81/040815-02 $02.00/0

SHORT REPORTS

NITROGEN-CONTAINING MINOR METABOLIC PRODUCTS OF *BEAUVERIA BASSIANA*

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(*Received* 19 *July* 1980)

Key Word Index—*Beauveria bassiana*; Hyphomycetes; piperazine-2,5-diones; fatty acid amides.

The cyclotetradepsipeptides beauverolides A–F and Ba, Ca, Ea, Fa, Ja and Ka have been isolated [1, 2] from the mycelium of a strain, UICP 22, of the insect pathogen *Beauveria bassiana* (Bals.) Yuill. We have also isolated a number of biologically inactive *N*-containing minor metabolic products of this strain. After separation of the beauverolide fractions by column chromatography [1, 2], more polar eluants furnished a mixture of palmitamide and stearamide, readily resolved into its components by reverse phase HPLC, followed by the piperazine-2,5-diones cyclo-(L-Ile-L-Val), cyclo-(L-Ile-L-Ile) and cyclo-(L-Ala-L-Pro). With the exception of cyclo-(L-Ile-L-Ile), a known metabolic product of *Ustilago cynodontis*[3], none of these compounds has previously been recorded as a microbial product. Palmitamide occurs naturally in the seed of *Casimiroa edulis*[4], and cyclo-(L-Ala-L-Pro) has been isolated from pupae of the silkworm *Bombyx mori*[5]. None of these minor metabolic products showed significant insecticidal activity against the blowfly *Calliphora erythrocephala*[6].

Although simple piperazinediones are known to occur in acid-hydrolysed casein [7, 8], none were present in the sample used to prepare the medium for the *B. bassiana* fermentations.

EXPERIMENTAL

Mps were taken on a Kofler hot-stage apparatus and are corr. Optical rotations were measured in EtOH and IR spectra were determined on mulls in Nujol. MWs and compositions were obtained from mass spectra recorded with a Varian CH5D (double focusing) mass spectrometer coupled to a Varian 620L computer. Merck silica gel HF_{254} was used in TLC and the R_f values quoted are for $CHCl_3$–MeOH (19:1). An account of the extraction procedure and column chromatography has already been given [1,2]. A Brownlee RP18 column (25 × 4.6 mm i.d.) packed with Lichrosorb-C_{18} bonded phase was used in the HPLC separation. The column was used with a Waters 6000A pump, UK6 injector and 401 differential refractometer detector, with MeOH at 1.0 ml/min (2500 p.s.i.) as eluant. Repetitive injections (250 μg) of the mixture in $CHCl_3$ (20 μl) were made and the separated components were accumulated, recovered, and crystallised from MeOH. Acidic hydrolysis of the piperazinediones was conducted in sealed tubes at 110° for 3 days with 6 M HCl, and the resulting amino-acids were identified and estimated quantitatively on a Locarte instrument.

Isolation and identification of metabolites. After elution with benzene–EtOAc (1:9) of beauverolides Ja and Ka [2], further elution of the column with the same solvent (2 × 100 ml) followed by EtOAc (2 × 200 ml) gave gums: (i) 35 mg, (ii) 85 mg, (iii) 56 mg, (iv) 43 mg, which were triturated with MeOH yielding, respectively, solid products: (i) 6 mg, mp 95–100°, ν_{max} 3390, 3280, 3190, 1645 cm^{-1}. Purification of this material by HPLC gave hexadecanamide (palmitamide) (retention time 5.7 min), mp 102°, ν_{max} 3390, 3180, 1642 cm^{-1}, (Found: M 255.2559. $C_{16}H_{33}NO$ requires 255.2562) R_f 0.26; and octadecanamide (stearamide) (retention time 8.2 min), mp 110°, ν_{max} 3390, 3185, 1642 cm^{-1} (Found: M 283.2875. $C_{18}H_{37}NO$ requires 283.2875) R_f 0.28, identified by comparison with authentic specimens. (ii) 3 mg, subl. 250° without melting; ν_{max} 3195, 3050, 1665 cm^{-1}; $[\alpha]_D^{22}$ −40, *c* 0.098; R_f 0.15; Ile:Val, 1.15:1; of (3 *S-cis*)-3-(1-methylethyl)-6-(1-methylpropyl) piperazine-2,5-dione [cyclo-(L-Ile-L-Val)] identified by the mass spectrum [8]. (iii) 3 mg, subl. 250° without melting; ν_{max} 3180, 3045, 1665 cm^{-1}; $[\alpha]_D^{22}$ −47, *c* 0.09; R_f 0.13; amino-acid analysis: Ile only; of (3 S-*cis*)-3,6-*bis*(1-methylpropyl) piperazine-2,5-dione [cyclo-(L-Ile-L-Ile)] (lit. [3], subl. 253°). (iv) 5 mg, mp 162–6°; ν_{max} 3280, 1657 cm^{-1}; $[\alpha]_D^{22}$ − 85, *c* 0.17; R_f 0.10; Ala:Pro, 1:1; of (3 *S-cis*)-hexahydro-3-methylpyrrolo [1, 2] pyrazine-1,4-dione [cyclo-(L-Ala-L-Pro)] (Found: M 168.0904. $C_8H_{12}N_2O_4$ requires M 168.0898) (lit. [5], mp 163–4°, $[\alpha]_D^{22}$ −86).

Blank extraction of casein hydrolysate. Casein hydrolysate (B.D.H. 5.2 g) was extracted with MeOH (150 ml) in a Soxhlet apparatus for 7 hr and the product was then re-extracted batchwise with EtOAc (6 × 100 ml). The recovered extract (160 mg), in benzene, was chromatographed as described above and previously [1, 2] for the *B. bassiana* extracts. Trituration with MeOH of the gummy fractions (1–2 mg, total 29 mg) gave no solid products.

Acknowledgements—We thank Dr. F. A. Mellon for the mass spectra and P. Dew for amino-acid analysis.

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Phytochemistry, Vol. 20, No. 4, pp. 816–818, 1981.
Printed in Great Britain.

0031–9422/81/040816–03 $02.00/0

CARBOHYDRATE COMPOSITION AND PHYSICAL PROPERTIES OF TOBACCO PHOSPHODIESTERASE

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(*Received* 25 *July* 1980)

Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; cell culture; glycoprotein; phosphodiesterase.

Abstract—Some physical and chemical properties of phosphodiesterase from cultured tobacco cells were studied. The enzyme contained *ca* 50% carbohydrate consisting of residues of arabinose, glucose, glucosamine, galactose, mannose and xylose. Analyses showed that the enzyme had a sedimentation coefficient, $s_{20,w}$, of 16 S, a Stokes' radius of 5.7 nm and a calculated specific volume of 0.66 ml/g. The MW of the enzyme was calculated to be 300 000 from these values.

INTRODUCTION

Previously we reported the purification and properties of a new phosphodiesterase isolated from cultured tobacco cells [1, 2]. The enzyme also shows pyrophosphatase activity, but it does not hydrolyse DNA or RNA. The enzyme preferentially cleaves the pyrophosphate bond of the 5′-terminal methylated blocked structure in eukaryotic *m*RNA without splitting a main chain in the RNA molecule. Thus the enzyme has been used for studies on the identification and biological function of the blocked terminal structure in a nucleic acid molecule [3–5].

The results of the previous investigations indicated that the enzyme is a tetrameric glycoprotein [2, 6]. The purposes of the present study were to characterize its chemical and physical properties further.

RESULTS AND DISCUSSION

The results of amino acid and carbohydrate analyses indicate that essentially all of the sample weight (98%) is accounted for by amino acid and carbohydrate residues, of which 48% is represented by amino acid residues and 50% by carbohydrate. The results show that the neutral sugar content of the enzyme is much more than that previously reported [2]. The previous value was estimated by the orcinol–sulfuric acid method using mannose as a standard.

Table 1. Carbohydrate composition of tobacco phosphodiesterase

Carbohydrate	Residues g/100 g	Residues Number/g subunit MW (MW = 72000)
Arabinose	27.2	148
Xylose	0.5	3
Mannose	1.1	5
Galactose	3.1	14
Glucose	13.3	59
N-Acetylglucosamine	4.5	16

Analytical determination of individual sugar residues is shown in Table 1. Arabinose and glucose are the major neutral sugar residues. The hexosamine in the enzyme was identified as glucosamine by GC; no galactosamine was found.

Arabinose and glucose are not usually found in oligosaccharides linked via an *N*-glycosidic bond of *N*-acetylglucosamine to asparagine in the polypeptides [7].