Phytochemistry, 1978, Vol 17, p. 147. Pergamon Press. Printed in England

SHORT REPORTS

TABTOXININE-β-LACTAM FROM *PSEUDOMONAS TABACI*

RICHARD D. DURBIN, THOMAS F. UCHYTIL, JOHN A. STEELE and RAUL DE L. D. RIBEIRO
A.R.S., U.S.D.A., Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, U.S.A.

(*Received* 25 *April* 1977)

Key Word Index—*Pseudomonas tabaci*; Pseudomonadaceae; tabtoxins; tabtoxinine-β-lactam; amino acid.

Pseudomonas tabaci (Wolf and Foster) Stevens No. 11528 was obtained from the American Type Culture Collection, Rockville, MD. This bacterium, as well as several closely related species, produce toxins that induce chlorosis in plants. These toxins are dipeptides containing a tabtoxinine-β-lactam residue linked to the amino group of either threonine (tabtoxin) or serine ([Ser^2]-tabtoxin) [1]. They readily translactamize to yield the corresponding biologically inactive isotabtoxins which contain tabtoxinine in the more stable δ-lactam form. This amino acid (3-amino-methyl-6-carboxy-3-hydroxy-2-piperidone) has been found free in the culture medium [1], and it has been synthesized [2]. Acid hydrolysis of either tabtoxins or isotabtoxins yields tabtoxinine (5-amino-2-aminomethyl-2-hydroxyadipic acid), threonine and serine. This paper describes the isolation of a new form of the toxin, namely the β-lactam. The bacterium was grown in Woolley's medium [3], modified by substituting ferric for ferrous sulfate, for 5 days at 24° on a rotary shaker. The cells were removed by centrifugation and filtration (Millipore 0.45 μ). Five volumes of cold ethanol were added and the solution left for 1–2 hr at 3°, then centrifuged. The volume was reduced *in vacuo* at 30° and the concentrate chromatographed on Beckman PA 28 ion-exchange resin (1.2 × 54 cm; 0.2 M citrate buffer, pH 3.12). The headspace was filled with 0.2 M citrate buffer pH 3.55, and elution started at 30° using the former buffer. The column temperature was increased to 60° starting after 60 min. Eight ml aliquots were collected and the biological activity of each was determined using tobacco leaves [4]. The tabtoxins eluted between 88–96 ml. In addition, a new ninhydrin-positive, biologically active substance was found eluting at 40–48 ml. A GC–MS analysis was done by converting a dried sample to its TMS derivative (60° for 30 min in dimethyl formamide–*bis*-silyl-trifluoroacetamide, 1:1). GC used a 1m column of 3% OV-1 on 100–120 mesh Gas Chrom Q with temperature program of 6°/min from 150 to 270°. The unknown gave a MS 70eV *m/e* (rel. int.): 476 M^+ (8); 461 (M^+-Me, 14); 359 (M^+-COOSiMe, 100); 258 (M^+-CH_2·CHNHSiMe_3·COOSiMe_3, 8); 244 (M^+-$(CH_2)_2$·CHNHSiMe_3, 100). This spectrum is consistent with tabtoxinine-β-lactam [2-amino-4-(3-hydroxy-2-oxo-azacyclobutan-3-yl)-butanoic acid] as reported by Taylor *et al.* for the tabtoxins [1]. The equivalent δ-lactam gives a major fragment at *m/e* 375 but lacks *m/e* 359 and 244. The 90 MHz NMR spectrum had a 2 proton multiplet at 3.3 ppm (J = 6 Hz) and a 4 proton singlet at 1.9 ppm. These results also are not consistent with either a δ-lactam in which the ε protons would be equivalent, or tabtoxinine [1]. After hydrolysis (6N HCl, 100° for 1 hr) the unknown cochromatographed with authentic tabtoxinine on PA 28 resin, and the Me_3Si derivative gave a GC–MS identical to that of authentic (Me_3Si)$_5$-tabtoxinine [1]. The β-lactam structure is also supported by the IR spectrum (KBr pellet) which has a strong absorption maximum at 1745 cm^{-1} indicative of a strained ring carbonyl.

This is the first report of the occurrence of free tabtoxinine-β-lactam. Its capacity to induce chlorosis shows that it contains all the structural features of the tabtoxins required for biological activity.

REFERENCES

1. Taylor, P. A., Schnoes, H. K. and Durbin, R. D. (1972) *Biochim. Biophys. Acta* **286**, 107.
2. Lee, D. L. and Rapoport, H. (1975) *J. Org. Chem.* **40**, 3491.
3. Woolley, D. W., Schaffner, G. and Braun, A. C. (1955) *J. Biol. Chem.* **215**, 485.
4. Sinden, S. L. and Durbin, R. D. (1970) *Phytopathol.* **60**, 360.