but with 1% WS about 70% of BEM and RES remained after 5 h of exposure.

The temperature at 30 cm from the sunlamp was approximately 70 °C. Loss due to heat-induced evaporation rather than photodegradation was estimated by placing samples in a drying oven at 70 °C for 5 h. The estimated losses were 0% for BEM and 5% for RES.

PYR formulated with PAN and WS to 0.2% showed a similar but somewhat weaker photostabilization of the four major active constituents ("pyrethrins") (Figure 2). About 10% of cinerin I and 5% of cinerin II and pyrethrins I and II were lost when PYR was exposed to 70 °C (oven) for 2 h. This indicates that some of the losses observed under sunlamp exposure were due to evaporation rather than to photodegradation. PYR formulated with WS and PAN, exposed to the sunlamp and sunlight in small Petri dishes, showed greater photostability for all major pyrethrins than the patented formulation F7076 (Table I). Without antioxidants or UV absorber, cinerin I and pyrethrin I were more stable in mineral oil, but cinerin II was more stable in PAN, while the persistence of pyrethrin II was variable.

Bioassays. The tests of proportions ($\alpha = 0.05$) showed that for all formulations (1) unstabilized, exposed chemicals killed significantly fewer insects than unexposed chemicals and (2) stabilized, exposed chemicals killed significantly more insects than unstabilized exposed chemicals (Table II).

There was no significant difference between the two unexposed, stabilized formulations. After exposure, however, the WS-stabilized formulation caused significantly greater kill ($P < 0.00006$) than F7076. A comparison of the two unstabilized pyrethrum formulations before and after exposure showed that, while there was no difference in unexposed treatments, the exposed, unstabilized F7076 clearly killed more insects than did pyrethrum in PAN ($P < 0.00006$). This finding supports the general belief that mineral oil itself, the carrier solvent in F7076, stabilizes the insecticidally active components of pyrethrum.

LITERATURE CITED

Donegan, L.; Goodin, P. J.; Thain, E. M. *Chem. Ind. (London)* **1962**, *3*, 1420.
Elliott, M.; Janes, N. F.; Potter, C. *Annu. Rev. Entomol.* **1978**, *23*, 443.
Head, S. W. *Pyrethrum Post* **1966**, *8*, 3.
Lehman, E. L. "Nonparametrics: Statistical Methods Based on Ranks"; Holden-Day: San Francisco, 1975; p 217.
LeRosen, A. L.; Moravek, R. T.; Carlton, J. K. *Anal. Chem.* **1952**, *24*, 1335.
Lyon, R. L.; Richmond, C. E.; Robertson, J. L.; Lucas, B. A. *Can. Entomol.* **1972**, *104*, 417.
Miskus, R. P.; Andrews, T. L.; Blair, P. D. U.S. Patent 3 560 613, 1971.
Robertson, J. L.; Gillette, N. L.; Look, M.; Lucas, B. A.; Lyon, R. L. *J. Econ. Entomol.* **1975**, *69*, 99.

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High-Performance Liquid Chromatographic Determination of β-Exotoxin Produced by the Bacterium *Bacillus thuringiensis*

A high-performance liquid chromatography method was developed to measure the concentration of β-exotoxin, a potent insect toxin, produced by some strains of *Bacillus thuringiensis*. The procedure gave a rapid and direct measure of β-exotoxin concentration, and because the neutralized mobile phase of 0.1% trifluoroacetic acid in water was nontoxic to fly larvae, the column effluent could be bioassayed without concentration or extraction.

The endotoxin of *Bacillus thuringiensis* (BT) is well-known for its insecticidal properties and is available in a variety of commercial products. However, some strains of BT also produce one or more other toxins that are potent insecticides. The most studied and best characterized of these is a toxin designated β-exotoxin (Heimpel, 1967).

Vankova (1978) most recently reviewed the production, purification, bioassay, and identification procedures used in β-exotoxin studies. Bioassay procedures have been used to determine the relative efficiencies of various production and purification procedures and have indicated, by differing toxicological properties, that more than one heat-stable exotoxin may exist. Because bioassays often require weeks to complete and are subject to many variables, a rapid, sensitive analytical method that is both quantitative and qualitative is needed for β-exotoxin. We have developed a high-performance liquid chromatography (HPLC) method that fills these needs.

METHODS AND MATERIALS

Solutions to be analyzed were filtered through a Gelman 0.45-μm membrane filter and then injected into a Waters Associates Model 404 high-performance liquid chromatograph. The mobile phase of 0.1% trifluoroacetic acid in water flowed through the 3.9 mm × 30 cm C_{18} μBondapak reverse-phase column (Waters Associates) at the rate of 2 mL/min. The absorbance of eluting compounds was measured at 254 nm.

Fractions from the chromatograph were collected, adjusted to pH 7.0 by the addition of NaOH, and bioassayed for toxicity to immature horn flies, *Haematobia irritans* (L.). Larval medium was prepared by adding 4 parts of neutralized eluant or water to 6 parts of fresh bovine manure. Fifteen grams of this medium was placed in a 5-cm Petri dish and inoculated with 10 horn fly eggs. The

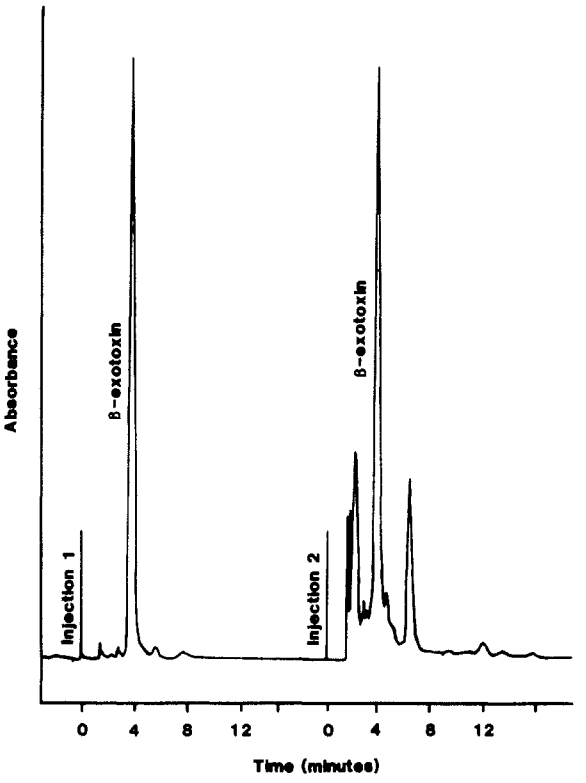

Figure 1. High-performance liquid chromatograms of (1) 5 μg of β-exotoxin and (2) a mineral casein broth growing medium in which *B. thuringiensis* (var. *darmstadiensis*) had been incubated at 30 °C for 48 h.

inoculated medium was kept at 27 °C and 80% relative humidity for 6 days to allow any developing larvae to pupate. The pupae, if any, were washed from the larval medium, dried, and allowed to develop. The number of adult flies produced was counted and used as a basis to determine percentage mortality among the immature insects exposed to the fractions. Volumes of the mobile phase equal to the volumes of the collected fractions were used as controls. An aliquot of the solution being analyzed equal to the injected volume was also bioassayed so that the activity of each fraction could be compared with the total activity of the sample.

Verification of the coincidence of biological activity with the absorbance peak was determined in two different samples by collecting and bioassaying a 4-mL fraction eluting immediately ahead of the suspected β-exotoxin peak, a 4-mL fraction under the peak, and a 4-mL fraction eluting immediately after the peak. Solutions used in this verification were prepared from a 20% β-exotoxin sample, IMC 1001, provided by Sandoz, Inc., and from a β-exotoxin sample of unspecified concentration provided by Roger Bellon Laboratories (France). Fifty milligrams of each sample was dissolved in separate 50-mL portions of the HPLC mobile phase.

Table I. Mortality of Immature Horn Flies Reared in Media Fortified with β-Exotoxin Samples or Chromatography Fractions of β-Exotoxin Samples

	% corrected mortality			
		chromatography fractions[a]		
sample	std[b]	1[c]	2[d]	3[e]
IMC 1001	98	17	100	11
Roger Bellon	100	0	100	9

[a] All fractions were 4 mL. [b] Equal to the quantity of sample injected into the chromatograph. [c] Fraction immediately preceding the absorbance peak. [d] Fraction containing the absorbance peak. [e] Fraction immediately following the absorbance peak.

RESULTS AND DISCUSSION

The major absorption peak, detected 4 min after injection of a β-exotoxin sample, contained virtually all the biological activity of the sample (Table I). A 45% full-scale deflection was obtained when 20 μL of the standard solution, 1 mg/mL IMC 1001, was injected into the chromatograph, and the detector attenuation was set at 0.2 absorbance unit full scale (Figure 1). The detector response was linear between concentrations of 0.01 and 1.0 mg/mL; however, β-exotoxin slowly degrades in acidic solution, and a fresh standard must be prepared daily. The mechanism of this separation is not clear, but trifluoroacetic acid is an ion-pairing reagent very useful for separation of peptides.

This liquid chromatography system is very well suited for the study of the BT exotoxins. For example, growing medium can usually be analyzed simply by injecting it directly into the chromatograph after centrifugation for 10 min at 12000*g* and filtration through a 0.45-μm filter (Figure 1). Fractions can be collected and bioassayed without first evaporating the solvent because the mobile phase has low toxicity to insects.

LITERATURE CITED

Heimpel, A. M. *Annu. Rev. Entomol.* **1967**, *12*, 287.
Vankova, J. *Folia Microbiol. (Prague)* **1978**, *23*, 162.

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