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Note

High-performance liquid chromatography of various insect pheromones

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Insect pheromones are chemical messages produced and used by insects for communication between individuals of a given species. Isolated pheromones are presently used for surveying insect populations and in a number of cases as part of insect control programs. The pheromones of any given species are often multi-component rather than single compounds.

Gas chromatography is the most widely used purification technique for insect pheromones. Previous reports of high-performance liquid chromatography (HPLC) being used in pheromone purification describe separations of synthetic mixtures containing (*Z*) and (*E*) isomers of specific pheromones[1–5]. This paper describes HPLC techniques that can be used for isolating many of the general classes of known insect pheromones. The method is applied to the resolution of the pheromone components of *Trogoderma anthrenoides*.

EXPERIMENTAL

Apparatus

A Laboratory Data Control (Riviera Beach, Fla., U.S.A.) Model GLC-401 gradient high-performance liquid chromatograph was used. The system included a Model 1204 variable wavelength absorbance detector and a Model 304-40 computing integrator.

Columns. Laboratory Data Control Excalibar columns with LiChrosorb DIOL, Spherisorb C-6, Spherisorb 5 μm ODS and Spherisorb 5 μm silica packings were evaluated.

Mobile phase. Several mixtures of mobile phases were tried with each column. A 20-min gradient of 5%—25% isopropanol—hexane was finally used.

Reagents

UV grade isopropanol and hexane were obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). The pheromone standards were obtained from

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Drs. W. Burkholder and T. Shappas, Dept. of Entomology, Univ. of Wisconsin, and Dr. D. Daves, the Oregon Graduate Center. The pheromone complex of *T. anthrenoides* was isolated by the airation–absorption procedure previously described[6].

Procedure

The pheromone samples were dissolved in hexane before injection. The solvents were degassed under vacuum before using. The UV absorbance at 230 nm was monitored.

RESULTS AND DISCUSSION

To obtain the experimental conditions for the resolution of *T. anthrenoides* components without prior purification, solvent systems, columns and flow-rates were systematically varied. The LiChrosorb DIOL column gave more reproducible retention times than the Spherisorb ODS and silica columns. The LiChrosorb DIOL column was better suited for resolution of the pheromone than the Spherisorb C-6 column because it reduced tailing of the carboxylic acids (Table I). A hexane–isopropanol gradient at 1.0 ml/min gave satisfactory resolution of the *T. anthrenoides* pheromone components (Fig. 1). Identification of the pheromone component peaks was done by co-injection with standards and further substantiated by thin-layer chromatography, gas–liquid chromatography and bioassays[7].

TABLE I

COMPARISON BETWEEN PHEROMONE SEPARATIONS ON DIOL AND C-6 COLUNNS

Pheromone	*Retention time in min*	
	*DIOL**	*C-6***
(*E,Z*)-14-Methyl-8-hexadecenal	3.2	6.1
Methyl(−)-(*E*)-2,4,5-tertradecatrienoate	4.0	5.6
(*E*)-11-Tetradecenyl acetate	4.2	0.3
(*Z*)-6-Henicosen-11-one	4.4	6.1
Hexanoic acid	4.9	16.3
(*E,Z*)-14-Methyl-8-hexadecenol	5.7	8.8
γ-Caprolactone	6.1	13.1
(*Z*)-9-(*E*)-12-Tetradecadienyl acetate	8.0	7.1
(*Z*)-3-Decanoic acid	8.48.4	>25
(*E*)-3-(*Z*)-5-Tetradecadienoic acid	9.6	>25

* 1.0 ml/min.

** 0.5 ml/min.

Detection at 230 nm was used because it was a common wavelength of absorbance for the pheromones and it minimized the baseline shift of the solvent gradient. The detectability of the pheromones at 230 nm ranged from 2 μg of methyl-(*E*)-2,4,5-tetradecatrienoate to 160 μg of 14-methyl-8-hexadecenol for a 50% peak response at 0.2 a.u.f.s. Using a fixed wavelength Laboratory Data Control 214 nm detector and an isocratic solvent system would substantially increase the detectability of specific insect pheromones*.

* We detected 5 ng juvenile hormone I with 10% peak response using an LDC 214 nm detector at 0.01 a.u.f.s.

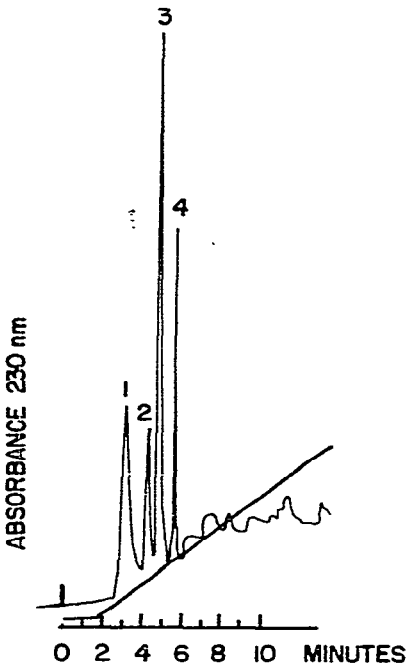

Fig. 1. Separation of pheromone components of *T. anthrenoides* on a LiChrosorb DIOL column. The peaks 1,2,3 and 4 correspond to 14-methylhexadecenal, unknown, hexanoic acid and 14-methylhexadecenol, respectively.

The HPLC procedure described above is a flexible method that can be used for resolving insect pheromones (Table I). This method is ideal for pheromones with low volatility and/or thermal instability. We expect this method to be useful for identification and isolation of pheromone complexes.

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