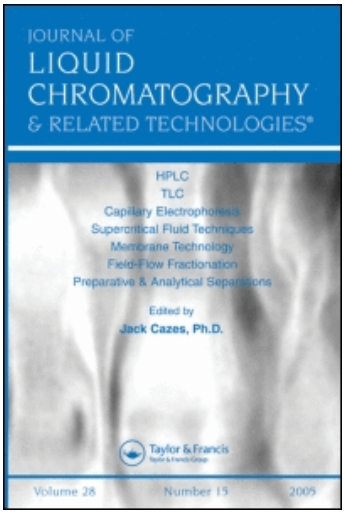

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An Improved Analysis of the Pheromone 3-Methyl-2-Cyclohexen-1-One in a Controlled Release Formulation by Using Liquid Chromatography

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AN IMPROVED ANALYSIS OF THE PHEROMONE 3-METHYL-2-CYCLOHEXEN-1-ONE IN A CONTROLLED RELEASE FORMULATION BY USING LIQUID CHROMATOGRAPHY

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ABSTRACT

A method was developed for estimating the quantity of the pheromone 3-methyl-2-cyclohexen-1-one in a controlled release formulation. The thermal method measured the amount of releasable pheromone remaining in the formulation by using liquid chromatography with a variable wavelength ultraviolet detector. This method is 150 to 250 times more sensitive than the liquid chromatographic method used previously.

INTRODUCTION

During the evaluation of a controlled release formulation of 3-methyl-2-cyclohexen-1-one (MCH), the antiaggregation pheromone of the bark beetle *Dendroctonus pseudotsugae* Hopkins, it was necessary to estimate the quantity of the pheromone available for releasing that remained in the formulation. An accelerated release method was needed to remove and collect the remaining formulated pheromone for analysis. The pheromone was estimated previously by using a method of high performance liquid chromatography (1). The method, however, was not sufficiently sensitive because of a limitation imposed by a fixed wavelength ultraviolet detector at 254 nm that did not allow for a minimum of MCH less than 1 μg.

This paper reports on a thermal method for rapid release of the formulated pheromone and the analysis of it by high performance liquid chromatography in two modes, by using a variable wavelength ultraviolet detector. The method was developed for estimating quantities of MCH in the nanogram range.

EXPERIMENTAL

Apparatus

A Waters Associates (Milford, Massachusetts) (2) ALC 200 liquid chromatographic instrument equipped with a model U6K injector was used. The detector was a Tracor Instrument (Austin, Texas) model 970, set from 224 to 235 nm. The columns used were 2 mm x 61 cm packed with Corasil-1 (Waters Associates) and 3.9 mm x 30 cm packed with μ-Bondapak C_{18} (Waters Associates). A pre-column (Altex #255-56, Altex Scientific Inc., Berkeley, CA) was used in the reverse-phase column. The apparatus was operated at ambient temperature.

The heating apparatus for the formulation was a glass tube that was fitted with a nitrogen gas inlet at one end and connected with a piece of Tygon^T tubing at the other end to a smaller glass tube. The smaller glass tube was stuffed with glass wool at the outlet end and contained from 1.5 to 2 g of an absorbing medium. The larger tube was wrapped with a heating tape, and was connected together with a thermocouple to a recording temperature controller (Figure 1).

Materials

3-Methyl-2-cyclohexen-1-one was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wisconsin as 98% MCH. The controlled release MCH formulation was on a polyamide matrix, as described by Furniss (3). The absorbing medium was Porapak-QS/80-100 mesh (Waters Associates). The solvents used were dioxane (Mallinckrodt 4951), trimethylpentane (Mallinckrodt 6051), methanol (MCB MX684), and distilled water.

Procedure

A small quantity (0.5 g) of the controlled release formulation of MCH estimated to contain about 2% of the pheromone was placed in a porcelain combustion boat. The boat was placed in the heating tube, the tube heated to 150-160°C, and nitrogen was swept through the tube at 25 to 50 cm^3/min. Heating and sweeping were continued for 12 hours, an interval found to be sufficient for removing all of the releasable pheromone at the prescribed temperature. The Porapak containing tube was removed and eluted with either dioxane-

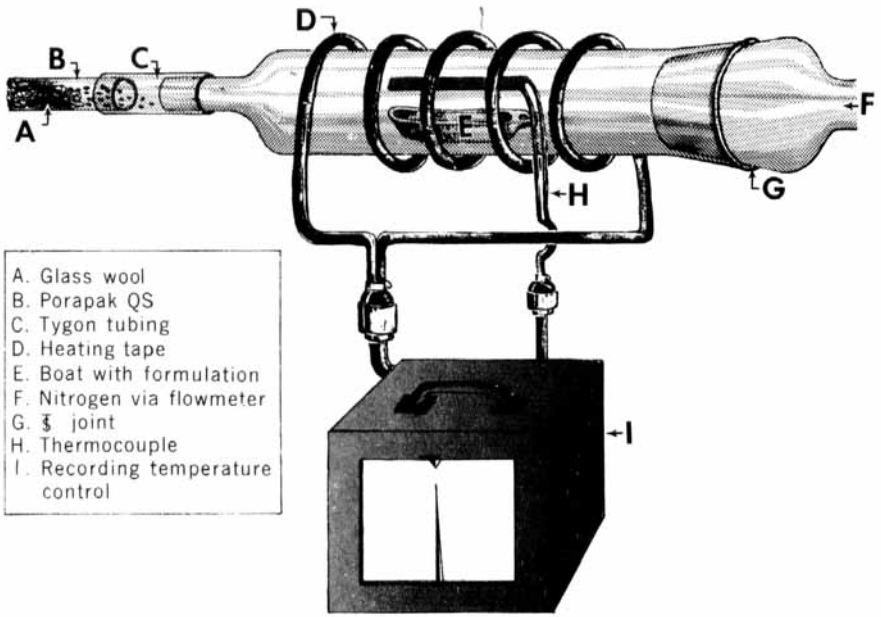

FIGURE 1. The thermal elution apparatus used to heat the controlled release formulation.

trimethylpentane (3:7) or methanol, depending on the phase type of the column to be used. Sufficient solvent was passed through the tube to fill a 25-ml volumetric flask.

For normal-phase chromatography, samples of 1 to 25 µl were injected into the Corasil-I column and were eluted with a mixture of dioxane-trimethylpentane (3:7) at a flow rate of 2 ml/min. The quantities of MCH were estimated from a calibration on the basis of peak height. Analyses of 30 to 200 ng MCH per injection were made at an absorbance unit, full scale (AUFS) setting of 0.04 at 235 nm. When analyzing smaller amounts of MCH, the detector was set at 224 nm by using a calibration curve at this wavelength.

For reverse-phase chromatography, samples of 1 to 25 µl were injected into the µ-Bondapak C_{18} column and were eluted with a mixture of methanol-water (1:1) at a flow rate of 1 ml/min. The

MCH was estimated from a calibration curve on the basis of peak height. From 50 to 250 ng MCH were analyzed at an AUFS setting of 0.08 at 235 nm. Smaller quantities were analyzed at lower settings.

RESULTS AND DISCUSSION

Unlike the MCH released from the polyamide matrix at ambient temperature (1), the pheromone released at elevated temperatures was accompanied by interfering peaks in the normal phase (Figure 2). These peaks probably resulted from a decomposing matrix, or excess monomers in the polymer, or both. The peaks were not noticed in reverse-phase chromatography where a precolumn was used (Figure 3). Reverse-phase chromatography was also less critical of solvent impurities than was a normal phase. Both methods were equally sensitive: at an AUFS of 0.01 at 224 nm, a 4 ng-sample gave a peak

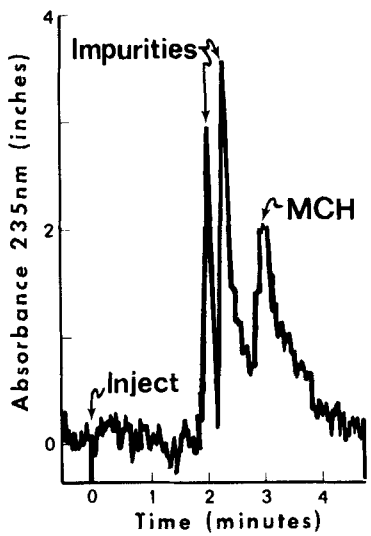

FIGURE 2. Chromatogram of MCH released at elevated temperature from polyamide matrix. UV detector at 0.04 AUFS. Mobile phase: dioxane-trimethylpentane (3:7). Flow rate: 1 ml/min. Corasil-1 2 mm x 61 cm column. 10-inch, 10-millivolt recorder at 0.5 inch/min.

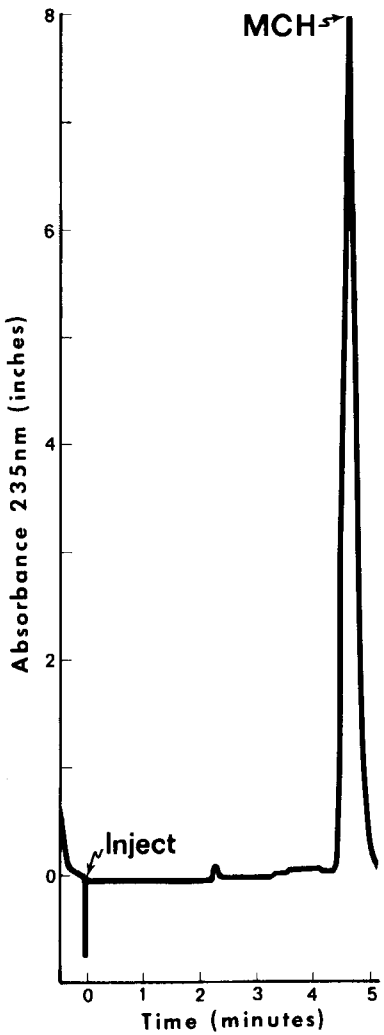

FIGURE 3. Chromatogram of MCH released at elevated temperature from polyamide matrix. UV detector at 0.08 AUFS; mobile phase: methanol-water (1:1); flow rate:/ml/min.; μ-Bondapak C_{18} 3.9 mm x 30 cm column; quantity MCH 0.55 μg; recorder: 10-inch, 10 millivolt at 0.5"/min.

of 2 cm on a 25-cm, 1-millivolt recorder. The signal-to-noise ratio was *ca.* 4:1.

This method of removing and estimating the quantity of MCH from a controlled release formulation compares favorably with that of the solvent extraction method developed by another laboratory. The results were within 0.05% (typical analysis of MCH in matrix was 1.3%) of each other (4).

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