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QUANTITATIVE GC ANALYSIS OF SECONDARY ALCOHOL PHEROMONES: DETERMINATION OF RELEASE RATE OF RED PALM WEEVIL, *Rhynchophorus ferrugineus*, PHEROMONE FROM LURES

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Abstract—Aliphatic secondary alcohols are components of several aggregation pheromones of important beetle and weevil pests. Some of these pheromones are used frequently for the monitoring and mass trapping of the relevant insects. We encountered severe difficulties in direct GC quantitative analysis of these compounds. Therefore, we developed a simple GC analysis of secondary alcohols converting them to trifluoroacetyl derivatives and using secondary alcohol acetates as internal standards. This method was applied for the quantitative analysis of several secondary alcohols, including the aggregation pheromone components of the almond bark beetle and the red palm weevil. The release rate of the latter pheromone from commercial lures was also determined.

Key Words—Aggregation pheromones, secondary alcohols, trifluoroacetyl derivatives, GC analysis.

INTRODUCTION

Several aliphatic secondary alcohols are common components of aggregation pheromones of weevils and beetles (Bartelt, 1999; Schlyter and Birgersson, 1999). The use of pheromone lures in pest management requires a quantitative determination of the content in order to measure the release rate of the pheromone and the longevity of the lures. Capillary GC analysis, with an internal standard, is the best

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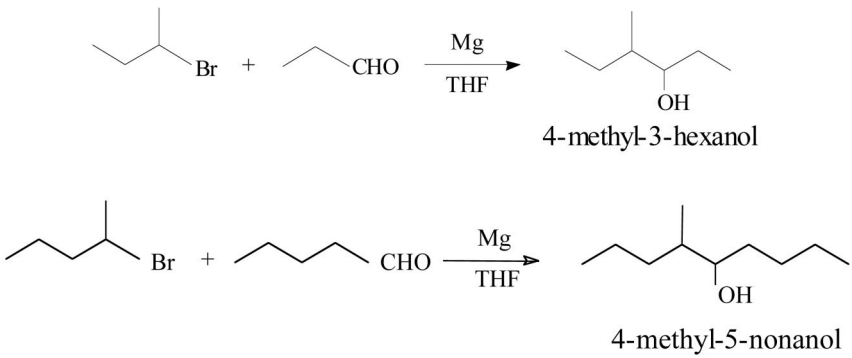
method for quantitation. However, peaks of secondary alcohols have a tendency to broaden and tail on different capillary columns, which makes their direct analysis difficult and inaccurate. The problem is particularly severe when analyses are performed repeatedly on the same column.

In this paper, we present a simple GC method of quantitation of secondary alcohols using their trifluoroacetyl derivatives and appropriate acetates as internal standards. The effect of the mode of injection on the analysis has also been studied. The following alcohols were analyzed: 4-methyl-5-nonanol, 4-methyl-3-heptanol, 4-methyl-3-hexanol, and 2-octanol. These alcohols (except 2-octanol) are pheromone components of two agricultural pest insects, the red palm weevil (*Rhynchophorus ferrugineus*) (Perez et al., 1996) and the almond bark beetle (*Scolytus amygdali*) (Ben-Yehuda et al., 2000), respectively.

METHODS AND MATERIALS

Chemicals. Mixtures of syn/anti 4-methyl-3-hexanol and 4-methyl-5-nonanol were prepared by Grignard reaction (Scheme 1) from 2-bromobutane and propionaldehyde and from 2-bromopentane and valeraldehyde, respectively, using magnesium turnings in THF (dried over Na + benzophenone). Distillation was carried out under reduced pressure (142°C/760 mmHg and 87°C/12 mmHg, respectively) to obtain the pure alcohols (97–100% by GC). The acetate derivatives were prepared from the corresponding alcohols and acetic anhydride in pyridine and purified by chromatography on SiO_2, using hexane and 1% ether as eluent. All other chemicals, including syn/anti (60:40) 4-methyl-3-heptanol, were purchased from Aldrich.



SCHEME 1.

Gas Chromatography. Analyses were performed on an HP6890 gas chromatograph equipped with an HP5 column (30 m × 0.25 mm ID; 0.25 μm), flame

ionization detector, and a split/splitless injector. Helium was used as the carrier gas with a flow rate of 1.5 ml/min. The analysis was performed in the split mode with a ratio of 10:1. The column was kept at 50°C for 3 min and then programmed at a rate of 20°C/min to 100°C for the analysis of 4-methyl-3-hexanol, 4-methyl-3-heptanol, and 2-octanol and kept at 60°C for 2 min, and then programmed at a rate of 20°C/min to 130°C for the analysis of 4-methyl-5-nonanol. The injector and detector were maintained at 220°C.

Procedure of Analyses. Solutions of 4-methyl-5-nonanol, 4-methyl-3-heptanol, 4-methyl-3-hexanol, and 2-octanol were prepared in hexane at a concentration of 1 mg/ml ($= 1\ \mu g/\mu l$). Representative aliquots of each solution (1, 5, 10, 50, 75, 100, 150, and 200 μl) were placed in a 4-ml vial containing 1 ml of hexane. Then, an appropriate amount of trifluoroacetic anhydride (50–100 μl) was added, and the vial was shaken thoroughly. The reaction mixture was shaken occasionally for 1 hr at room temperature and then cooled in an ice-bath. The derivatization was terminated by addition of 1 ml of 5% NH_4OH (aq) and 100 μl of a hexane solution containing 100 μg of the appropriate acetate derivative (stock solution of 1 mg/ml). Fifty micrograms of the acetates' internal standard was used for the small amounts of analyzed alcohols (1–10 μg). The mixture was shaken thoroughly, and the organic phase was separated with a pipet. Aliquots of approximately 2 μl of the organic phase were injected (three replicates) into the gas chromatograph. A representative analysis of 100 μg of 4-methyl-5-nonanol with 50, 75, and 100 μg of the corresponding acetate as internal standard was injected several times and gave the same results.

Application of the Method with Red Palm Weevil Pheromone Lures. Pheromone lures of the red palm weevil were purchased from ChemTica (Costa Rica). They contained 750 ± 5 mg pheromone of which 90% is 4-methyl-5-nonanol and 10% is 4-methyl-5-nonanone enclosed in a plastic bag with a membrane (Hallett et al., 1999). The lures were placed in weevil bucket traps in a date plantation in the Eden ranch, Jordan Valley. The traps also contained a mixture of plant material (molasses sugar and dates) in water and a bottle of ethyl acetate. Every 10 d, three lures were removed from the orchard and kept at 4°C until analyzed. The plastic bag containing the pheromone was cut into pieces and immersed in 20 ml of dichloromethane (CH_2Cl_2) to extract the pheromone. The samples were kept in an ultrasound bath for 30 min and left overnight at 4°C. The plastic material was not affected by the solvent. For GC analysis, samples were diluted by a factor of 100. Then, an aliquot of 100 μl was further diluted with 1 ml hexane and 50 μl of the appropriate acetate standard were added.

RESULTS AND DISCUSSION

It is well known that quantitative GC analysis of secondary alcohols is difficult and inaccurate due to peak broadening and tailing (Krupcik et al., 1975).

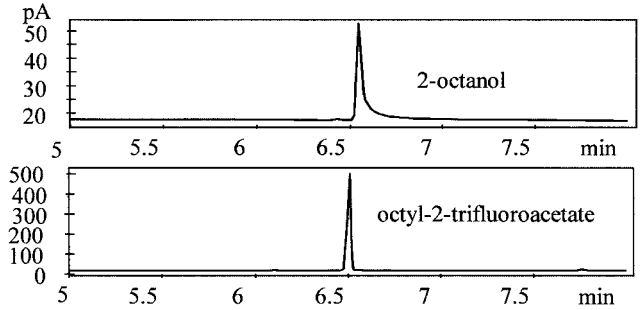


FIG. 1. GC profiles of 2-octanol versus octyl-2-trifluoroacetate. HP5 column was kept at 50°C for 3 min and then programmed at a rate of 20°/min to 100°C. Split ratio 10:1.

Despite the availability of improved capillary GC columns today, secondary alcohols display erratic behavior, particularly with aging columns. The columns are too expensive to be replaced after a short usage. Periodical weighing has been recommended as an alternative method in order to determine release rates of such pheromones from lures (Vickers et al., 1998; A.C. Oehschlager, personal communication). However, loss of weight is not specific to the loss of the pheromone alone. In addition, it gives erratic results due to absorption of dust and moisture. GC–FID analysis, with an appropriate internal standard, is the most accurate method, provided that peaks are sharp and the analysis is consistent.

During our work on mass trapping of the red palm weevil and the almond bark beetle with pheromone lures, we were in need of a reliable quantitative analysis of their respective main pheromone components, 4-methyl-5-nonanol and 4-methyl-3-heptanol. Direct GC analysis of the alcohol gave inaccurate results and, therefore, we looked for an alternative route and decided to use a derivatization method. We chose trifluoroacetylation because this derivatization of alcohols is easy to perform. It is quantitative and the formed trifluoroacetates can be readily analyzed by capillary GC-FID (Krupcik et al., 1994). Peaks are symmetrical and sharp (Figure 1). Therefore, sensitivity is higher as compared with the parent alcohols. The method has been employed for the analysis of alcohols and sugars (Krupcik et al., 1994; Hisatomi et al., 2000). However, as far as we know, trifluoroacetylation has not been used for quantitative analysis of secondary alcohols by GC. The FID response of the acetates and the trifluoroacetates is practically identical (GC-FID factor >0.98), and the latter always elute before their corresponding acetates (Representative example is in Scheme 2 and Figure 2).

4-methyl-3-heptyltrifluoroacetate (Analyte)

4-methyl-3-heptylacetate (Internal standard)

SCHEME 2.

The method was tested with various amounts of 4-methyl-3-hexanol, 4-methyl-3-heptanol, 2-octanol, and 4-methyl-5-nonanol in the range of 1–200 μg. Representative results of 4-methyl-3-heptanol and 4-methyl-5-nonanol, presented in Figures 3 and 4, indicate an excellent correlation (>99%) between the amount of secondary alcohol inserted and detected by the GC method. The use of the acetate of the same alcohol as the internal standard is not essential. For example, 4-methyl-3-hexanol and 2-octanol were analyzed with 4-methyl-3-heptylacetate as the internal standard, and the results were accurate. For better peak shape and resolution, we recommend performing the GC analysis in the split mode and programing the oven temperature so that both the analyte and the standard will be analyzed isothermally. The trifluoroacetylation reaction is quantitative, and no

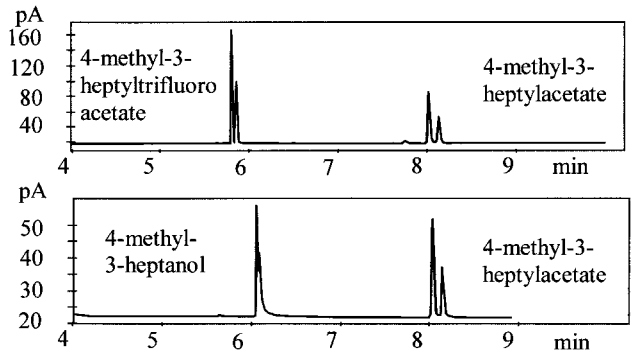


FIG. 2. GC profiles of the analysis of syn/anti (60:40) 4-methyl-3-heptanol with its acetate derivative as internal standard versus analysis of 4-methyl-3-heptyltrifluoroacetate using the same standard. HP5 column was kept at 50°C for 3 min and then programmed at a rate of 20°/min to 100°C. Split ratio 10:1

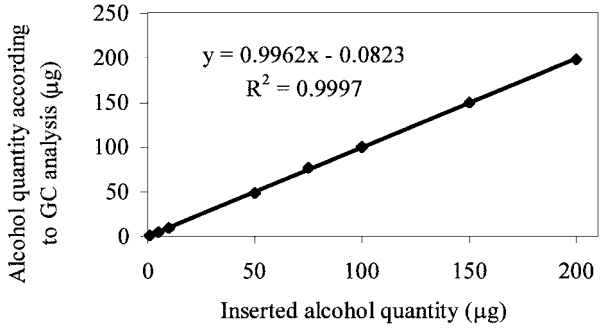


FIG. 3. GC quantitative analysis of 4-methyl-3-heptanol. Each point is the average of three anlyses.

residual alcohol was detected by GC in all cases. The use of varying amounts of alcohols or internal standard does not introduce any significant error, as shown by the excellent reproducibility.

The red palm weevil, *Rhynchophorus ferrugineus* (RPW), is a serious pest of dates. The major component of its aggregation pheromone is (*S*,*S*)-4-methyl-5-nonanol (Perez et al., 1996). Commercial lures, containing racemic syn/anti 4-methyl-5-nonanol, are being used in the Far and Middle East for mass trapping (Hallett et al., 1999; Vidyasagar et al., 2000). The determination of the release rate of the pheromone is essential in order to evaluate the longevity of the lures. Direct analysis of 4-methyl-5-nonanol produced erratic results. We applied our method

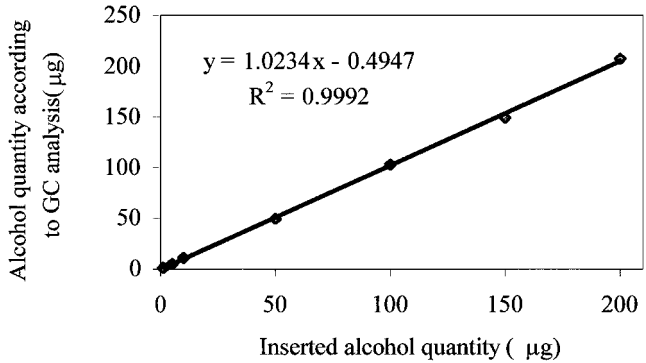


FIG. 4. GC quantitative analysis of 4-methyl-5-nonanol. Each point is the average of three anlyses.

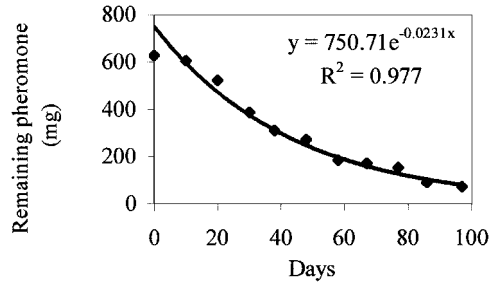


FIG. 5. Release rate of 4-methyl-5-nonanol from the ChemTica lures under field conditions.

and obtained consistent results. The behavior of the lure for the whole period can be represented by an exponential curve (Figure 5). However, the release rate of about 8 mg/day is linear for 58 days, and then the release rate decreases to 3–4 mg/day for another 40 days (Figures 5 and 6). This behavior corresponds with the manufacturer's specifications (release rate of 3–10 mg/day) and the temperature dependence of the release rate. The average temperatures in the plantation for the first period (0–58 d) of the field test was 23.5–36.6°C, and of the second one (58–98 d) was 17–29.8°C.

In conclusion, the quantitation of secondary alcohol aggregation pheromones is of practical importance and is not merely an analytical exercise. Several beetle and weevil pheromones containing such components are being used for mass trapping in Integrated Post Management programs. The method presented in this study will facilitate the evaluation of such lures and will enable the determination of their longevity and release rate profiles.

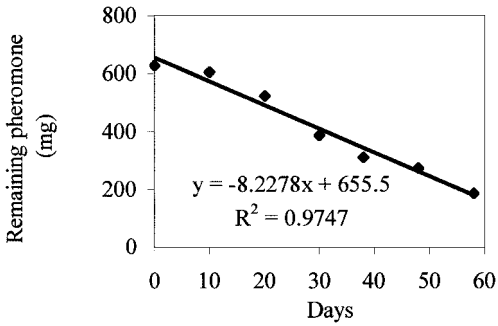


FIG. 6. Release rate of 4-methyl-5-nonanol from the ChemTica lures for the first period of 58 d of the field test.

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