

## RESEARCH REPORT

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U. S. Golf Association (USGA)

"CHARACTERIZATION OF THE SEX PHEROMONE OF THE SOUTHERN  
MASKED CHAFER *Cyclocephala lurida* "

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### Summary

Masked chafers are one of the key pests of turfgrasses. White grubs, the larval stage of these beetles, feed and damage the root systems of turfgrasses. Adult females of the southern masked chafer (*Cyclocephala lurida*) uses a potent sex pheromone to attract conspecific males. The males of this beetle are attracted also to conspecific male and female grubs, a very unusual phenomenon. Males are not only attracted, but also attempt to copulate with the grubs. Once this pheromone is characterized, it will be useful in the development of novel strategies to control this pest. Although the pheromone is very potent, the amount of pheromone present per grub is extremely minute. Chemical analysis of solvent extracts made from grubs showed that only about 1-5 nanograms of the pheromone could be obtained from each grub (a nanogram is a millionth of a milligram). Since the pheromone is only a minute component among hundreds of other compounds, isolation and characterization of the pheromone is more difficult than searching for needle in a haystack since we don't even know what the "needle" looks like. However, we have managed to obtain a significant amount of information about the structure of this pheromone. A tremendous collection effort provided us about 7000 grubs. Using extracts made from these grubs, we have determined the molecular formula of the pheromone to be  $C_{17}H_{26}O$ . The compound belongs to a group of chemicals called aldehydes and bears a bicyclic ring structure. Although the complete structure not yet established, we will continue our research, until sufficient amount grubs are collected, and the pheromone is isolated in sufficient quantities, for more sophisticated analytical experiments.

## Introduction

Adult females of the southern masked chafer (*Cyclocephala lurida*) uses a potent sex pheromone to attract conspecific males. The males of this beetle are attracted also to conspecific male and female grubs (Haynes and Potter, 1995). Males are not only attracted, but also attempt to copulate with the grubs, a very unusual phenomenon. Preliminary chemical investigations had established that the grubs also contain the sex pheromone. In addition, a closely related species, *Cyclocephala borealis*, the northern masked chafer appears to share the same compound as its sex pheromone. Our objective is to characterize the pheromone. Once characterized and synthesized, the pheromone will be very useful in the control of these pests of turfgrass.

## Results

The identification of this pheromone is an extremely challenging task since the amount of pheromone present per grub is only about 1-5 ng. Moreover, the pheromone is only a trace component found among many other volatile contaminants or compounds produced by the beetles. By a tremendous collection effort, about 7000 grubs were obtained and soaked in hexane. The hexane extracts were concentrated and fractionated by silica-gel liquid chromatography (Table 1).

**Table 1. LC fractionation of grub extracts.**

Fraction no.	Collected volume	Solvent system
1-2	4.5 mL each	100% hexane
3-4	4.5 mL each	0.1% EtOAc in hexane
5-6	4.5 mL each	0.2% EtOAc in hexane
7-8	4.5 mL each	0.5% EtOAc in hexane
9-10	4.5 mL each	0.7% EtOAc in hexane
11-12	4.5 mL each	1.0% EtOAc in hexane
13-14	4.5 mL each	1.25% EtOAc in hexane
15-25	4.5 mL each	1.5% EtOAc in hexane
26-30	4.5 mL each	2.0% EtOAc in hexane

The pheromone activity of each fraction was tested by a behavioral bioassay using male beetles. From the results, it was evident that the fractions 13-16 contained the pheromone. A small sample of the LC fraction that contains the pheromone was analyzed by capillary gas chromatography. Since the retention time of the pheromone peak was known from GC-behavior and GC-EAD studies, the chromatographic peak for the pheromone could be identified. With the flame detector turned off, the narrow region of the effluent (0.3 min wide) coming out of the GC column at the retention time of the pheromone was collected in a pre-cooled glass tube. By injecting 2.5-3  $\mu\text{L}$  at time, the experiment was repeated about 50 times (each collection cycle takes about 1-2 hrs). The condensed material was extracted into  $\text{C}_6\text{D}_6$  ( $\text{CDCl}_3$  was not used since commercial  $\text{CDCl}_3$  contained many chlorinated impurities that interferes with gas chromatographic analysis).

The purity of the collected pheromone sample was checked by GC-MS (Figure 1B). A comparison of this chromatogram with that obtained from the crude grub extract, in which the chromatographic peak for the pheromone is largely obscured by other volatiles in the mixture (Figure 1A), showed that although extremely labor intensive, the pheromone can be purified in this way.

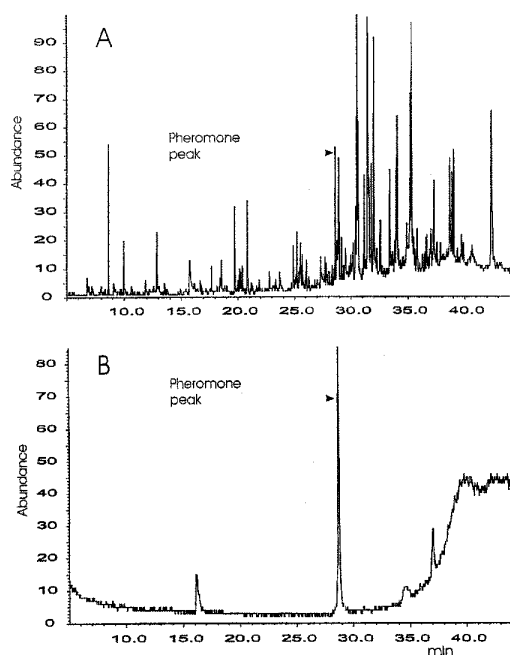
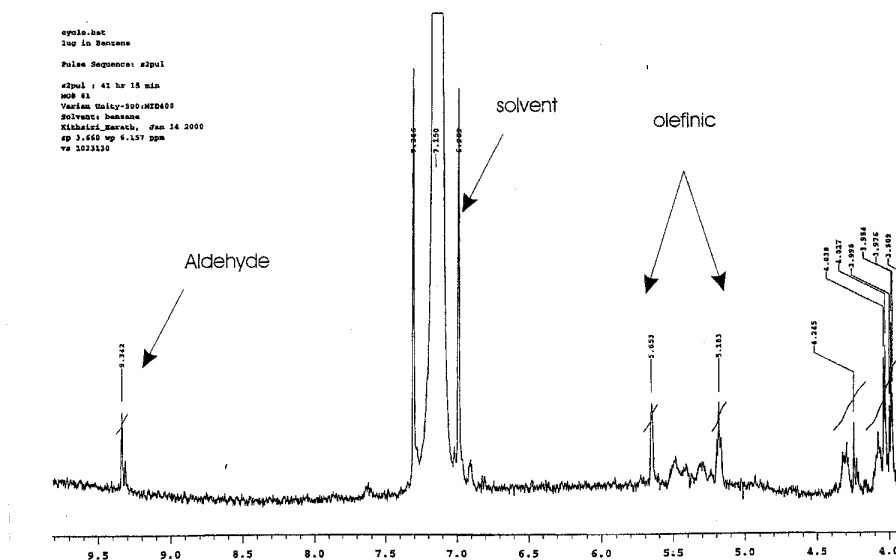


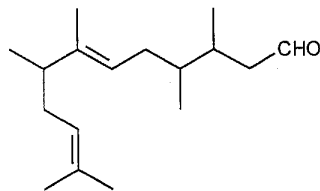
Figure 1. Gas chromatograms obtained from crude (A) extract, and purified pheromone (B)(DB-5 capillary column 60 for 1 min, 5/min to 250  $^{\circ}\text{C}$ ).

We estimated that total amount of purified sample obtained was about 700 ng. This sample in  $C_6D_6$  was examined by 500 MHz NMR spectroscopy. Although a peak for the aldehyde proton, and two peaks for olefinic protons could be observed, unfortunately the amount of sample was insufficient for a complete analysis.



**Figure 2.** A section of the 500 MHz  $^1H$  NMR spectrum of the pheromone.

Although we had established the molecular formula of the pheromone by accurate mass determinations to be  $C_{17}H_{26}O$ , we were not sure how many carbon-carbon double bonds and rings are present in its structure. Usually, a simple hydrogenation experiment followed by GC-MS of the product provides this information. We tested the reliability of our hydrogenation procedure by using the following compound.

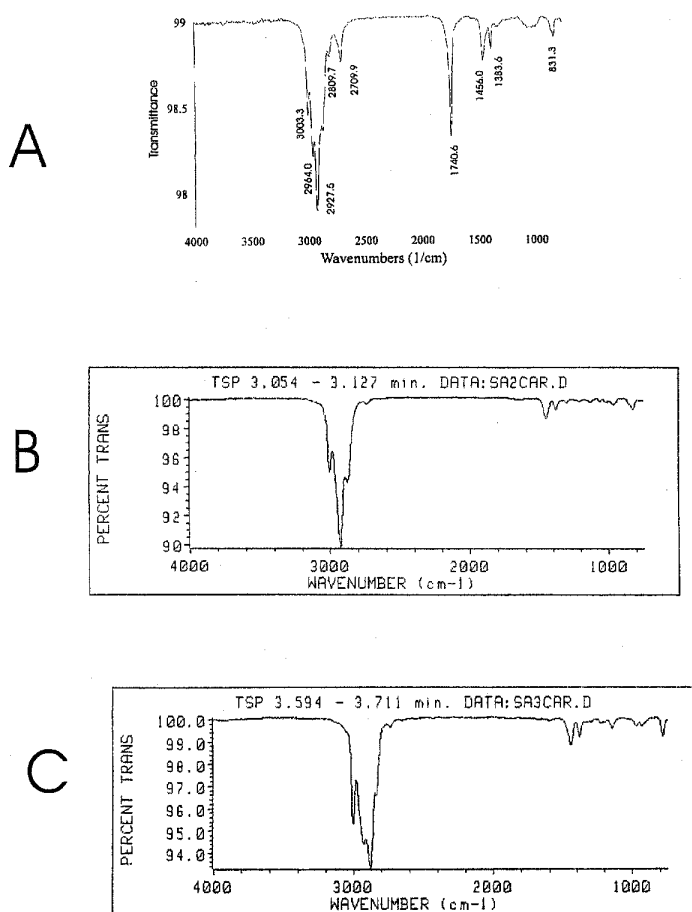


tetramorene II

This compound of molecular formula  $C_{17}H_{30}O$  ( $m/z$  250) upon hydrogenation gave a product of molecular mass 254 ( $C_{17}H_{30}O$ ) indicating it bears two carbon-carbon double bonds. However, we were unable to obtain a hydrogenated product from the pheromone.

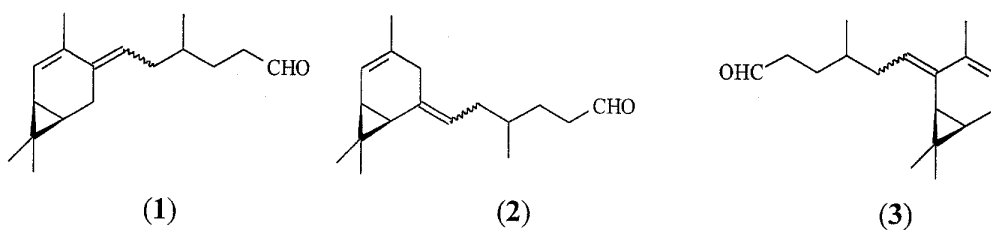
Many different methods and catalysts were tried. With comparable amounts of material, we always obtained the expected result from tetramorine II, but not from the pheromone. Finally, we concluded, that hydrogenolysis is taking place with the pheromone to produce low molecular weight products. Thus, it seemed unlikely that the pheromone has an open structure similar to that of tetramorene II.

The infrared spectrum of the pheromone shows an intense peak at  $3003\text{ cm}^{-1}$ . Such an intense peak in this region is rather unusual (Figure 3). A comparison of this spectrum with those in our collection of gas phase infrared spectra indicated that the pheromone could be structurally related to either 2- or 3-carene.

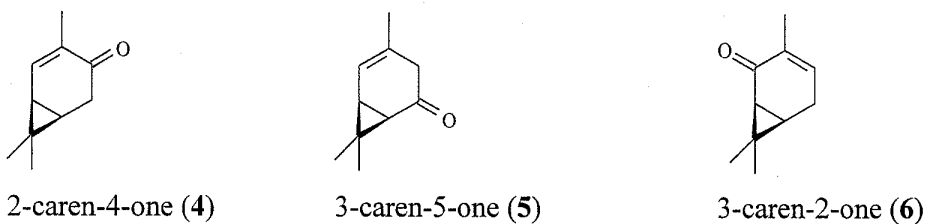


**Figure 3.** Gas phase infrared spectrum of the pheromone (A), 2-carene (B), and 3-carene (C).

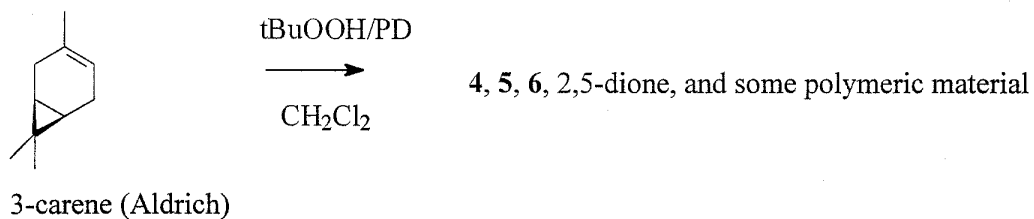
Thus based on IR evidence, and the other structural information we had previously, the following structures were suggested for the pheromone.



At this stage, we decided to synthesize some model compounds. Hydrocarbon analogs of the proposed compounds can be synthesized from following ketones as starting materials.

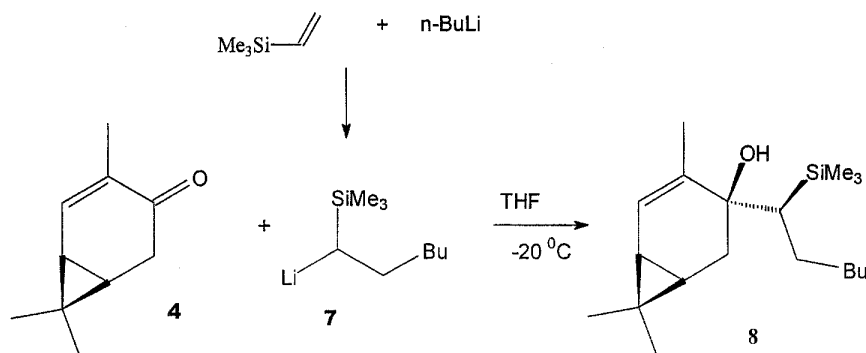


A mixture of 4, 5, and 6 was synthesized by the oxidation of (-)- $\Delta^3$ -carene using pyridiumdichromate/*tert*-butyl hydroperoxide mixture.

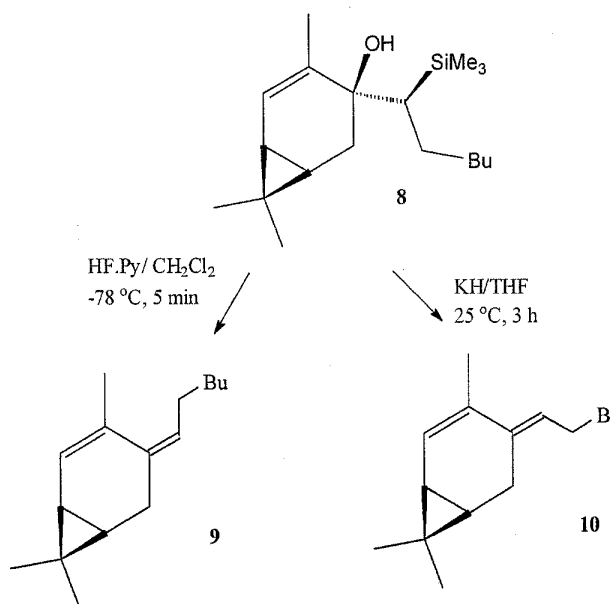


Several olefination methods of were attempted on the carenones obtained in this way. However, most of attempts (Wittig, Wittig-Horner and Reformatski reactions along with "Knochel" 1,1-bimetallic compounds; Grignard coupling with *n*-octylmagnesium chloride) were unsuccessful due to low stability of the starting materials and/or products under reaction conditions used.

The only successful approach was a modified Peterson olefination using 1-trimethylsilyl-1-dichlorocerium organometallic compound. In this way compound **8** was synthesized.



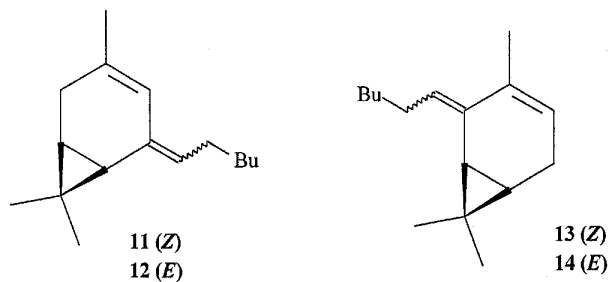
In a similar way, from the ketones **5** and **6** the corresponding silyl alcohols were prepared in preparative yield. These compounds were isolated and characterized by spectrometric methods ( $^1\text{H}$  and  $^{13}\text{C}$  NMR). Interestingly, the reaction produced only one diastereoisomer. The silyl alcohol **8**, when treated with either base or acid provided the expected model compounds **9** and **10** in high isomeric purity.



In a similar manner, starting with ketones **5** and **6**, the following compounds were synthesized. Compounds **9-14** were examined using diverse spectral methods. The  $^1\text{H}$



NMR ( $D_6$ -benzene) spectra of compounds **13** and **14** showed peaks very similar to those observed in the olefinic region in the spectrum of the pheromone.



### Future Research

A new sample of 9000 grubs has been collected during the summer of 2000. We will isolate the pheromone from these samples and combine with the material from previous years and attempt to obtain better NMR data. Once we have a tentative structure, we will attempt to synthesize it.

### References

- K. F. Haynes, and D. A. Potter. Sexual responses of a male scarab beetle to larvae suggest a novel evolutionary origin for a pheromone. *American Entomologist* 41 (1995) 169-175.