Insect Pheromone Technology: Chemistry and Applications

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Based on a symposium sponsored by the Division of Pesticide Chemistry at the 182nd Meeting of the American Chemical Society, New York, New York, August 25–26, 1981.

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

Those who have followed the pheromone field know that there have been great strides forward in virtually every aspect of pheromone technology, especially in the past decade. Aside from the biological aspects of the problem, which are not directly addressed here, substantial progress has been made in isolation, identification, analysis and synthesis of pheromones, and—most important and recently—in actual applications of insect pheromones to solve difficult pest-control problems.

Although much of the impetus toward pheromone use has been generated by the urgent demands from scientists and the public that pheromones be explored as a means of minimizing pesticide contamination of our food, fiber, wildlife, and the environment generally, interest has been heightened by a number of concurrent developments:

1. new and improved techniques and methods for isolating and identifying ever-decreasing amounts of pheromones;

2. actual identifications, or more complete identifications, of pheromones, especially those of important insect pests;

3. novel syntheses, including asymmetric ones, that were devised following the finding of optically active pheromones;

4. research findings and subsequently demonstrations showing that pheromones can be a safe and viable alternative to insecticides, or at least, a means of reducing insecticide use through integrated pest-management techniques;

5. and, most important, the entry into the market of commercial firms that have developed improved formulations and pheromone products and introduced new and unique equipment to make the use of pheromones practical in many instances.

Despite these advances, most pheromone workers will agree that the pheromone field is only beginning to blossom and that there are many questions requiring answers before the full rewards of this technology can be realized. To respond to some of these questions and to bring the record up to date, we invited leading scientists from the United States and elsewhere to share their latest findings, advances, and thoughts in their respective fields of pheromone technology. Thus, this volume contains their papers, which were presented at the Symposium on Chemistry and Applications of Insect Pheromone Technology at a national meeting of the American Chemical Society in 1981. Previous symposia were held in

1969 and 1975, the latter published as Symposium Series No. 23, "Pest Management with Insect Sex Attractants."

Because progress in pheromone technology has involved the coordinated effort of a wide variety of disciplines, it is anticipated that the subject matter in this volume may be useful to organic, analytical, agricultural, environmental and micro chemists, biochemists, entomologists, pest control operators, chemical manufacturers and formulators, insect physiologists, agriculture extension workers, life scientists, ecologists, university personnel (chemists, entomologists, zoologists, agronomists), state and federal government officials dealing with agriculture or the environment, and engineers designing specialized equipment. With continued cooperation of the various disciplines, we can be optimistic about pheromones becoming important pest-control tools in the future.

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March 1, 1982

CONTENTS

Analysis of Chemical Communications Systems of Lepidoptera

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Recent research has shown that the pheromone mediated behavior of lepidopterous insects is very complex. The chemical components of the pheromones are usually simple molecules, but complex mixtures involving permutations of geometry, functionality, and chain length are often required to elicit the complicated behavioral repertoire that eventually culminates in mating. To elucidate the chemical and behavioral aspects of this communications system, we have used a combination of methods including collection of the volatiles emitted by the female, analysis by high resolution capillary gas chromatography (GC), and the sequential and temporal analysis of the male's behavioral response to the pheromone blend and components thereof. New liquid phases and state of the art techniques have been developed for capillary GC to separate all the components of a pheromone blend. With these methods the chemical communication systems of Heliothis virescens (F.) and H. subflexa (Gn.) have been analyzed and certain aspects have been elucidated.

Numerous investigations of the pheromone communication systems of Lepidoptera have been conducted during the last two decades, probably because Lepidoptera are ubiquitous phytophagous pests and because, superficially, their pheromones and related behavior appear simple. Most of these investigations have involved the chemical identification of the pheromone or pheromone blend obtained from the females and subsequent evaluation of synthesized pheromones to determine whether or not they "work" as trap baits for males, or communication disruptants in the field. However, in the last five years there has been a growing body of evidence that lepidopteran pheromones and pheromone mediated behavior is much more complex than first believed. It is now clear that information regarding the chemical composition of the pheromone and the pheromone elicited behavior

is incomplete for most species. Thus, monitoring systems employing pheromone baited traps do not always give consistent results, representative of population densities, and reduction of mating below the economic threshold is often difficult to achieve by communication disruption with the pheromones that have been identified for a species. The development of effective practical insect control systems based on the use of semiochemicals will depend on the development of a thorough knowledge and understanding of the chemical communications systems of these insects.

For these reasons we decided to conduct an in-depth study of the chemical communication systems of certain lepidopteran species. We chose to first investigate Heliothis virescens (F.) (Lepidoptera: Noctuidae) because it is an important economic pest and because of our previous experience with it (1). Additionally, males of this species can be mated with Heliothis subflexa (Gn.) females to produce sterile hybrid males (2). This phenomenon is the basis of genetic strategies for control of H. virescens. It also provides an opportunity to study the production and perception of pheromones by hybrids.

Our initial goal was to accurately define the chemical composition of the pheromone produced by H. virescens females and to analyze and describe the male behavior elicited by the pheromone and components thereof. Ultimately we plan to delineate the chemical communication systems of both species and to analyze pheromone production and male behavior of hybrids and backcrosses. Hopefully the results of the latter part of the investigation will provide useful correlations with biochemical genetic investigations being conducted on these hybrids by other scientists.

Thus far our investigations have been focused on the chromatographic analysis of pheromones produced and emitted by females and analysis of male behavior evoked by these pheromones. The methods developed to conduct these investigations, using H. virescens and H. subflexa as models, are presented here. These methods are directly applicable to similar investigations of other species.

Capillary Gas Chromatography

Although, with a few exceptions, the chemical components of lepidopteran pheromones are simple molecules, complex mixtures that include permutations of geometry, functionality, and chain length are often produced and emitted by females. Analysis of these pheromones requires a system capable of separating compounds differing in geometry and position of an olefinic bond and resolving the mixtures produced by the females. Additionally, sensitivity sufficient to detect nanogram or smaller

quantities of these compounds is required to analyze the pheromone produced by only one or a few females. The only method having the capabilities required for these analyses is capillary GC with high resolution glass or fused-silica columns. This method has the added advantage that the capillary columns can be coupled to a mass spectrometer and a great amount of information concerning the identity of each eluted compound can be obtained. The mass spectral data plus retention indices of a compound on two or three capillary columns that separate compounds based on different characteristics provide complete and accurate identification of most compounds.

The resolution (Rs) of compounds on capillary GC columns is a function of the column efficiency (N effective), the ability of the stationary phase to separate the compounds (separation factor, α), and the ratio of the amount of time the compounds spend in the stationary phase vs. the time the compounds spend in the carrier gas phase (partition ratio, k'). Resolution of two compounds can be defined by the equation:

$$Rs = 1/4 \frac{(\alpha-1)}{(\alpha)} \frac{(k')}{(k'+1)} N^{1/2}$$

Improvements in resolution on a capillary column after it has been prepared can be made only by the adjustment of retention time, which alters the partition ratio of the compounds, and by the optimization of the carrier gas used. Increases in retention time which result in a partition ratio of greater than 5 afford very little improvement in resolution and are done at the expense of analysis time. Similarly, the use of nitrogen instead of helium as a carrier gas results in an increase in resolution of 1.14 at the expense of doubling the analysis time.

The greatest change in resolution of components is obtained through the use of a stationary phase that results in an increase in the separation factor (α) (see later). The amount of resolution required is dependent on the need to analyze and quantitate minor components that elute close to a major component of the pheromone blend. Figure 1 shows the effect that the reduction in column efficiency (peak B), and the introduction of peak asymmetry (peak C) have on the separation of a 1% minor component eluting before and after a major component peak. The accurate determination of the 1% component peak which is possible in Figure 1A is severely limited in 1B and 1C. The detection of a 10% component (Figure 1D) is still possible with the reduced column efficiency. A column coated with a stationary phase that improves α by ca. 0.01 as illustrated in Figure 1E is capable of providing an adequate determination of 1% components even at reduced column efficiency.

The separations of Δ7- and Δ9-tetradecen-1-ol acetates on 4 capillary columns coated with different stationary phases are compared in Figure 2. The nonpolar OV-1 phase and the

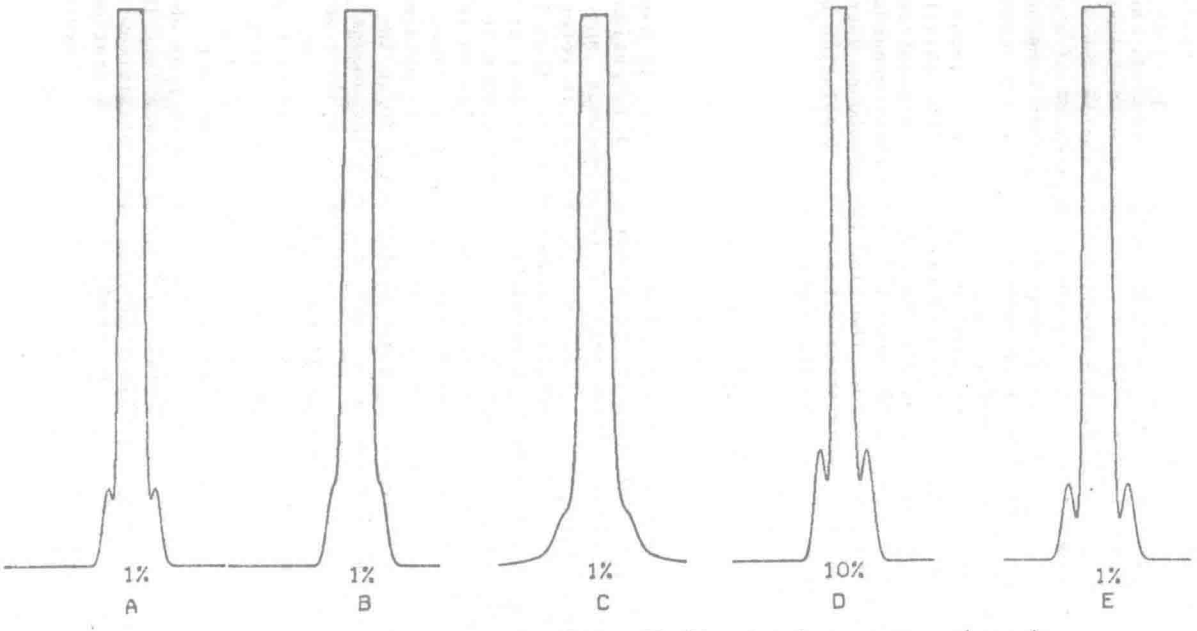

Figure 1. Effect of separation (α), column efficiency (N eff.), and peak asymmetry on the resolution and accurate quantitation of minor peaks. Key: A, α = 1.04, N eff. = 2000/M, R_s = 1.87; B, α = 1.04, N eff. = 1600/M, R_s = 1.66; C, α = 1.04, N eff. = 2000/M, peak assym. = 10%, R_s = 1.66; D, α = 1.04, N eff. = 1600/M, R_s = 1.66; and E, α = 1.05, N eff. = 1600/M, R_s = 2.11.

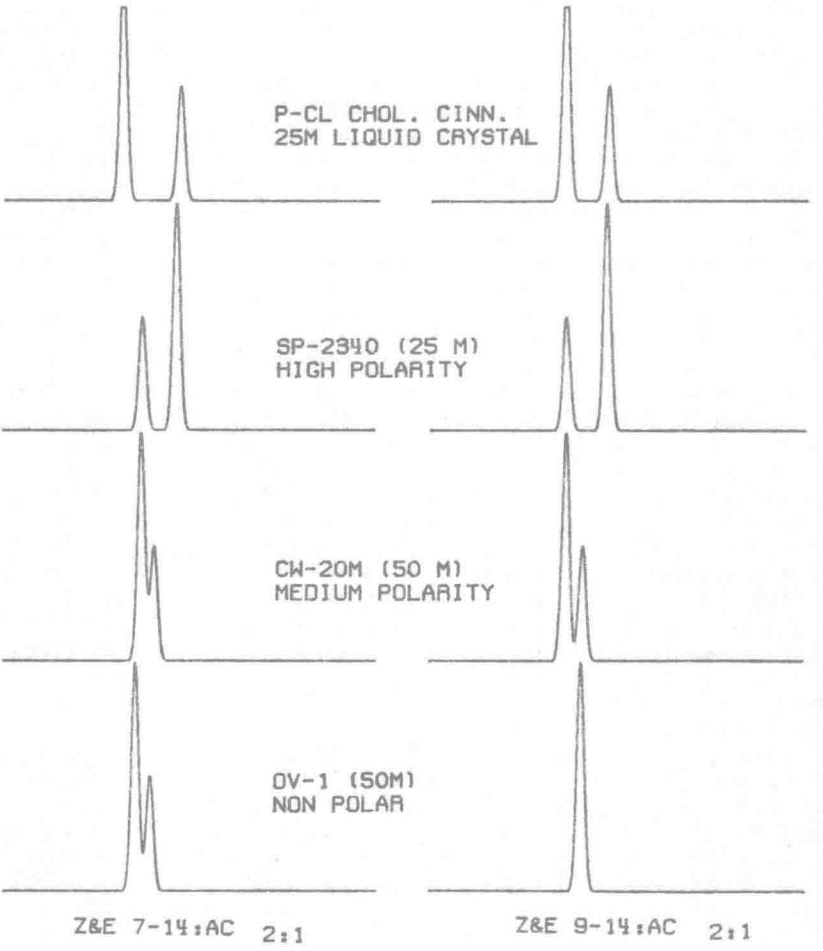

Figure 2. Separation of (Z)- and (E)-7-tetradecen-1-ol acetate (Z & E7-14:Ac) and (Z)- and (E)-9-tetradecen-1-ol acetates (Z & E9-14:Ac) on four different stationary phases on capillary columns. The OV-1 and Carbowax 20M (CW-20M) are coated on fused silica and the SP-2340 and p-chlorocholesteryl cinnamate on glass.

medium polarity Carbowax 20M are commercially available (Hewlett-Packard) fused silica capillary columns. The usefulness of these highly efficient (N effective) fused silica columns is severely limited because the separation factor, α, on Carbowax 20M and OV-1 is insufficient to resolve most positional and geometrical isomers found in the pheromone blends of lepidopteran insects. The high polarity phases containing large amounts of cyano groups such as SP-2340 (Supelco) and Silar 10C (Applied Science) provide good resolution of many positional and geometrical isomers of mono-unsaturated compounds found in lepidopteran insects (3). These high polarity capillary columns are commercially available. Superior resolution of the geometrical isomers of mono and diunsaturated compounds is obtained with liquid crystal phases, although the separation of positional isomers is compromised as the double bond position approaches the middle of the compound in some cases. The comparison of the separations of the analogous series of tetradecen-1-ol acetates on the liquid crystal and cyano phase capillary columns is shown in Figure 3. The E-isomers elute prior to Z-isomers from the cyano phase (SP-2340). However, on the liquid crystal column, the Z-isomers elute first when the olefinic bond is near the middle of the chain. As the double bond is moved toward the hydrocarbon end of the chain Z and E11-14:Ac co-elute and then the elution order reverses for Z and E12-14:Ac. The use of both the liquid crystal columns and the cyano columns, combined with mass spectral data on compounds eluted from these columns, offers the most powerful analytical procedure available for the identification of compounds like those found in lepidopteran blends. Since use of liquid crystal capillary columns for the separation of aliphatic olefinic insect pheromones is a recent development, some discussion of their properties is worthwhile.

The use of a liquid crystal as a GC stationary phase was first reported in 1963 (4). The application of ordered phases to pheromone research did not occur until 1978 when Lester reported the separation of conjugated dienes with diethyl-4,4'-azoxydicinnamate (a smectic liquid crystal) on packed columns (5). Subsequently we coated cholesteryl cinnamate (a cholesteric liquid crystal) on capillary columns which resulted in coupling the separating power of the liquid crystal phases with the high resolving capability of wall-coated open tubular columns (6). Several liquid crystal properties must be considered when using this type of phase in GC (7). As illustrated in Figure 4, the use of a liquid crystal such as cholesteryl cinnamate below the temperature required for liquification of the phase is of no utility. At its mesophase transition temperature (temperature required for the phase to go from crystalline to ordered liquid), which is ca. 158°C for cholesteryl cinnamate, good separation of the geometrical isomers of tetradecen-1-ol acetate is observed. Increase in the temperature of the phase to its isotropic point (temperature at which

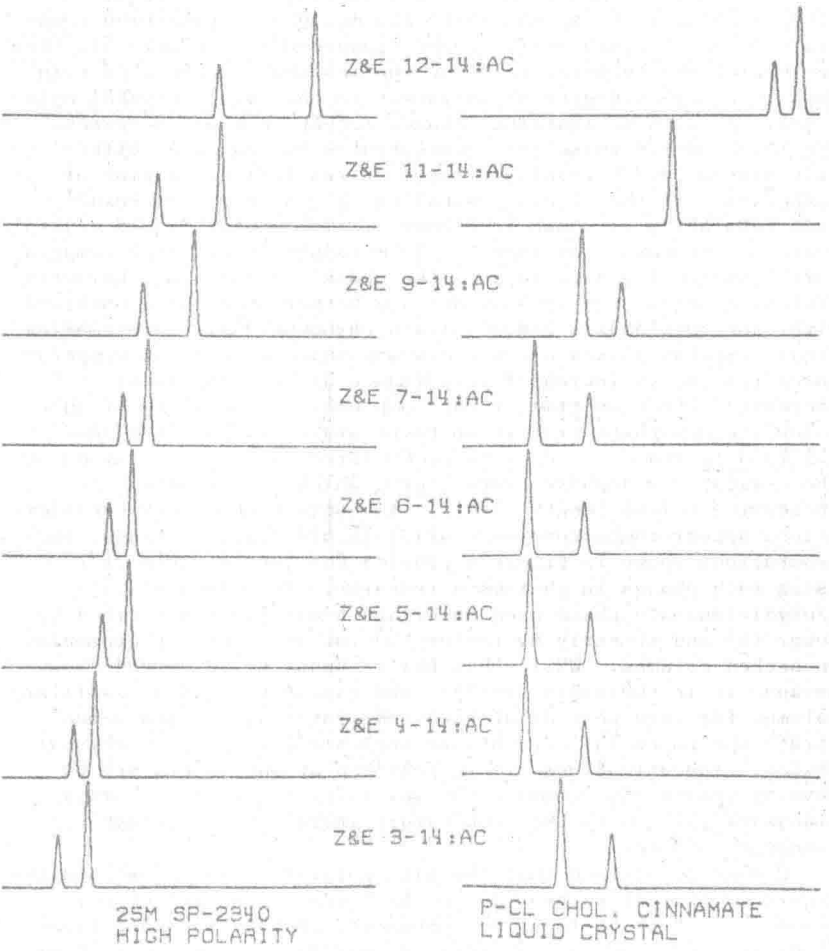

Figure 3. Separation of the (Z)- and (E)-isomers of tetradecen-1-ol acetates on SP-2340 and p-chlorocholesteryl cinnamate liquid crystal capillary columns. The ratio of Z:E is 2:1 in each set.

liquid crystal becomes unordered) results in decreased retention time (k') observed with isotropic phases. The decrease of the alignment of the liquid crystal molecules as the temperature approaches the isotropic point also results in decreased α, effective plates, and resultant resolution. If the liquid crystal phase is first raised to its mesophase transition temperature and then gradually cooled (supercooling, Figure 4), then decreasing the temperature below the mesophase transition point imparts a higher degree of alignment to the liquid crystal molecules. As with an isotropic phase, retention time increases. The more ordered phase also demonstrates increased α, effective plates, and a resultant improved resolution. A plot of temperature of the liquid crystal vs. k', α, N/m, and resultant resolution is shown in Figure 5. The use of liquid crystal phases at temperatures above their mesophase transition temperature dramatically deteriorates the phase's separation characteristics; however, the separations are better than those obtained with isotropic phases like OV-1 and Carbowax 20M. Supercooling liquid crystal phases below their mesophase transition temperatures results in increased performance at the expense of increased retention time of the compounds. A decrease of 20°C below the mesophase transition temperature results in a ca. 1.5-fold increase in resolution, compared with that obtained at the crystal's mesophase temperature, which is equivalent to increasing column length 2-fold. Although temperature considerations appear cumbersome when using liquid crystal phases, the separations shown in Figure 6 provide the justification for using such phases in pheromone research. The diethyl-4,4'-azoxydicinnamate phase used in Figure 6 was first described by Dewar (8) and recently by Lester (5) for separating pheromones on packed columns. While this phase cannot be recommended because it is thermally unstable and cannot be used on capillary columns for long periods at high temperatures, it does demonstrate the potential separations possible using liquid crystal phases. Investigations are in progress at our laboratory to develop phases that combine the resolving power of the azoxycinnamate phase with the temperature stability of cholesteryl cinnamate phases.

Having determined that the high polarity cyano phase and the liquid crystal phase provide the best separation of the compounds likely to be found as components of the pheromone blend of H. virescens and H. subflexa, we analyzed a complex mixture of positional and geometrical isomers of 16 carbon aldehydes, alcohols, and acetates on these two columns (Figure 7). As noted earlier, the elution order of Z- and E-isomers is opposite on the two phases. Aldehydes elute first on both phases. The alcohols are retained more than the acetates on the high polarity cyano phase, but the elution order of alcohols and acetates is reversed on the liquid crystal phase. While neither phase separated all of the synthetic mixture, the combination of separations obtained on both columns enabled us to pursue the

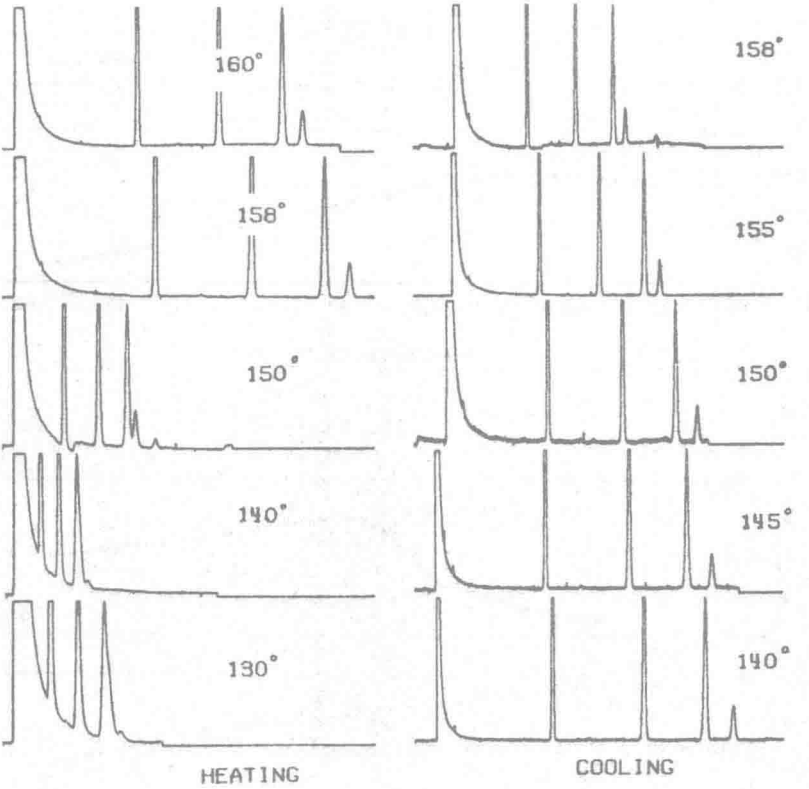

Figure 4. Effect on the separation of isomers on a liquid crystal column (20 m cholesteryl cinnamate) when increasing column temperature to mesophase transition temperature and beyond (left), and then gradually cooling (right) below mesophase transition. Peaks represent, in increasing retention time, hexadecane, heptadecane, (Z)-, and (E)-9-tetradecen-1-ol acetate.