G. Charalambous (Ed.), Food Flavors: Generation, Analysis and Process Influence

THE APPLICATION OF DEUTERATED SEX PHEROMONE MIMICS OF THE AMERICAN COCKROACH (*Periplaneta americana*, L.), TO THE STUDY OF WRIGHT'S VIBRATIONAL THEORY OF OLFACTION.

Barry R. Havens, and Clifton E. Meloan

Department of Chemistry, Kansas State University, Manhattan, Kansas 66506.

ABSTRACT

A test of Wright's multiple vibrational frequency theory of olfaction was made in the infrared region from 1000 to 200 cm^{-1}. This was done by deuterating (+) and (-) bornyl and isobornyl acetate in twelve combinations at the 1,2,8,9,10, and acetate positions. Deuterium was used to shift vibrational frequencies without changing the geometry, thus preserving Amoore's stereochemical theory. (+)-bornyl acetate is a synthetic sex attractant for the American cockroach, the (-)-bornyl acetate much less, and the isobornyl acetates are ineffective. Electroantennagram responses of these compounds plus four nondeuterated compounds were measured and correlated to the infrared spectra by a PLS pattern recognition program. The 225-265 (C-1 deformation) and 600-675 cm^{-1} regions of (+)-bornyl acetate correlated with insect activity. The 600-675 region contained four bands assigned to the in plane and out of plane bendings and the symmetric stretches of the quaternary carbons at C-1 and C-7.

INTRODUCTION

In the last century, many theories have been proposed to explain the basis of olfaction. However, most have not withstood rigorous investigation, and consequently only a few of the theories are presently considered. Theimer summarizes and reviews the various theories (1).

Our interests currently focuses on Wright's fundamental vibration theory (2-10). In principle, Wright proposes that if an incoming odor molecule has selected fundamental vibrational frequencies that match those of the receptor site, then a resonance condition can exist that results in a transfer of energy to initiate a signal. Wright later proposed that specific frequencies must also be absent even if the required frequencies were present. In addition to Wright's theory, Amoore's site geometry theory (11) which suggests that odor depends upon the shape of the molecule, is also of interest since the requirements of Amoore's theory serves as a prerequisite for the study of Wright's fundamental vibration theory.

In previous work with insect bioassays, Meloan and his group (12-16) have utilized the strategy of selectively deuterating molecules to shift the fundamental vibrational frequencies of the molecule without altering the geometry, and thus preserving the requirements of Amoore's theory. Much of the work centers around the deuteration of (-)-bornyl acetate and its structural and stereoisomers. It has been shown that (-)-bornyl acetate and (+)-bornyl acetate are sex pheromone mimics for the male American

Cockroach, (*Periplaneta Americana* L.) (17) and both induce the typical sexual behavior, (characterized by wing flutter, abdominal extension and attempted copulation), that is observed upon exposure of the insects to the true sex pheromone, *Periplanone B*.

Similar work by Doolittle (19) in 1968 involving the deuteration of "Cue Lure" [4-(p-hydroxyphenyl)-2-butanone acetate], a melon fly attractant, indicated that the deuterium substitution did not significantly affect the attractiveness of the compound. However, Wright (9) indicated that "the overall frequency-patterns were not changed enough to make a change in the biological response at all probable". Kuo (14), working under the direction of Meloan, reported small significant differences in the attractiveness of (-)-bornyl acetate and six selectively deuterated analogs using a live insect behavioral bioassay. The behavioral bioassay, however, suffered from several disadvantages. Good responses to the test compounds were limited to the evening. Also, males had to be kept isolated from the females, and required isolation once again after exposure to the sex pheromone mimics as their sexual activity was lost for several days. It was decided that an assay was needed that could improve the sensitivity and quantitation aspects of the behavioral bioassay, and eliminate the long delays necessary for the male cockroaches to regain their sexual activity.

Scriven (15), continuing Kuo's work, investigated 15 additional deuterated compounds employing an electrophysiological experiment known as the electroantennagram, or EAG, first developed by Schneider (20) in 1957. The tested compounds were deuterated analogs of (+) and (-)-bornyl acetate, and also (+) and (-)-isobornyl acetate (structural isomers of (-) and (+)-bornyl acetate, respectively). The isobornyl acetates have been shown to stimulate only the "generalist" receptors on the antenna of the male cockroach and possess none of the sex pheromone mimic properties (17). However, (+) and (-)-bornyl acetate stimulate both the "generalist" and the "specialist" receptors for the sex pheromone and certain other compounds and therefore effect greater EAG responses. It was felt that the isobornyl acetate compounds would serve as useful controls for the experiment due to their wealth of physical similarities with bornyl acetate, combined with their inability to elicit a behavioral response from the male cockroaches. Scriven (15), found small, but significant differences in response within the sets of the deuterated bornyl and isobornyl acetates as well as relatively large differences between the sets. All of the bornyl acetates exhibited a larger response than the isobornyl acetates as expected.

According to Wright's theory, relatively large frequency shifts (>10 cm^{-1}) in the low-frequency Infrared region are necessary in order to establish a significant change in response (2). It was felt that since many of the bands in the low frequency region of bornyl and isobornyl acetate can be assigned to skeletal vibrations (16), that it would be most beneficial to deuterate the molecules in positions that would likely bring about the largest frequency shifts in the skeletal vibrations. Intuitively, it appeared that deuteration in the 8, 9, and 10 positions would produce the largest frequency shifts, and therefore the largest changes in insect response. Unfortunately, these positions also appeared to be the most difficult to deuterate, and required syntheses involving many steps.

Kim (16) successfully synthesized (+)-bornyl- 10,10,10-d_3 acetate, (+)-bornyl-10-d_3 acetate, and (-)-isobornyl- 10,10,10-d_3 acetate, however, the compounds were never evaluated with the electroantennagram technique. Deuteration of bornyl and isobornyl acetates at the 8 and 9 positions has now been achieved through a synthesis route which employs *trans*-isoketopinic acid as a common intermediate step. These compounds and 9 other com-

pounds previously synthesized by Kim have been tested using the EAG technique (21). Subsequently, the data have been examined for the possible correlation of EAG response with changes of the fundamental vibrational frequencies in the far infrared region of the spectrum. The examination involved both visual inspection upon assignment of the IR bands, and use of a Partial Least Squares algorithm as a pattern recognition technique.

SYNTHESIS

For the details for the preparation of each compound, reference spectra and references see Havens (21,22). Only a summary is presented here. Figure 1 shows the overall synthesis scheme of *trans*-isoketopinic acid which is the starting material for the other compounds.

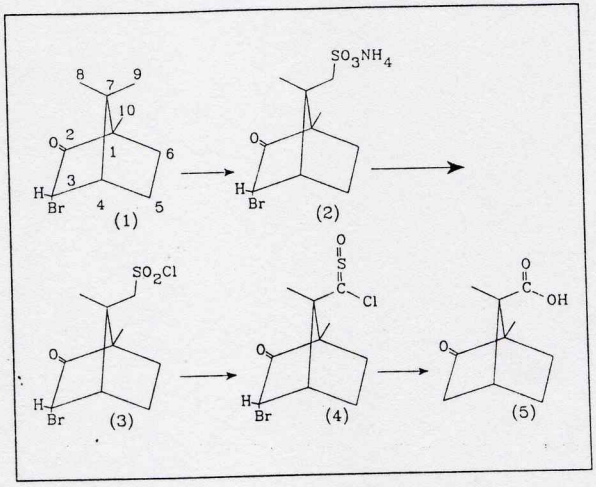

Figure 1. Synthesis scheme of *trans*-isoketopinic acid.

The synthesis began with (+)-3-endo-bromocamphor [1] which was converted to the 9-ammonium bromocamphor sulfonate [2], and then to the 3-bromo-camphor-9-sulfonyl chloride [3]. The 3-bromo-camphor-9-sulfonyl chloride was then converted to 3-bromo-9-chlorosulfoxide [4] by a pyridine-toluenesulfonyl chloride procedure. Finally, *trans*-isoketopinic [5] acid was prepared by oxidative hydrolysis of [4] followed by debromination utilizing zinc dust and acetic acid.

Synthesis scheme of (-)-Bornyl-8,8,8-d_3 acetate and (+)-isobornyl-8,8,8-d_3 acetate

The steps required for the preparation of (-)-bornyl-8,8,8-d_3 acetate and (+)-isobornyl-8,8,8-d_3 acetate are outlined in Figure 2. Isoketopinic acid [6] was first reduced with sodium borohydride to form (-)-9-hydroxy isoborneol [7] and then subsequently converted to the lactone [8] by the reflux of [7] in sulfuric and trifluoroacetic acids. Reduction of [8] by lithium aluminum deuteride afforded (+)-8-hydroxy-8,8,2-d_3 isoborneol [9]. This transformation via the lactone [8] effectively converts a C-9 hydroxy into a C-8 hydroxy, however, the transformation also results in an inversion of the stereochemistry.

In order to achieve the next desired product, 8-hydroxy-8,8-d_2 camphor

[10], a selective oxidation of the diol [9] was performed with a solution of sodium hypochlorite in acetic acid). Conversion of [10] into 8-bromo-8,8-d_2 camphor [11] was accomplished by refluxing in bromobenzene with phosphorus tribromide and quinoline. The third deuterium was introduced at the eight position by the catalytic deuterolysis of [11] effected over 10% palladium on activated carbon in a solution of ethanol-*d* and potassium deuteroxide to form 8,8,8-d_3 camphor [12]. Reduction of the deuterated camphor by borane-t-butylamine resulted in approximately a 60% / 40% mixture of (+)-8,8,8-d_3 isoborneol and (-)-8,8,8-d_3 borneol [13] as determined by capillary gas chromatography. The two isomers were separated by flash chromatography) and then individually esterified with acetyl chloride to form (+)-isobornyl-8,8,8-d_3 acetate [14] and (-)-bornyl-8,8,8-d_3 acetate [15]. Flash chromatography was employed once more to purify the esters.

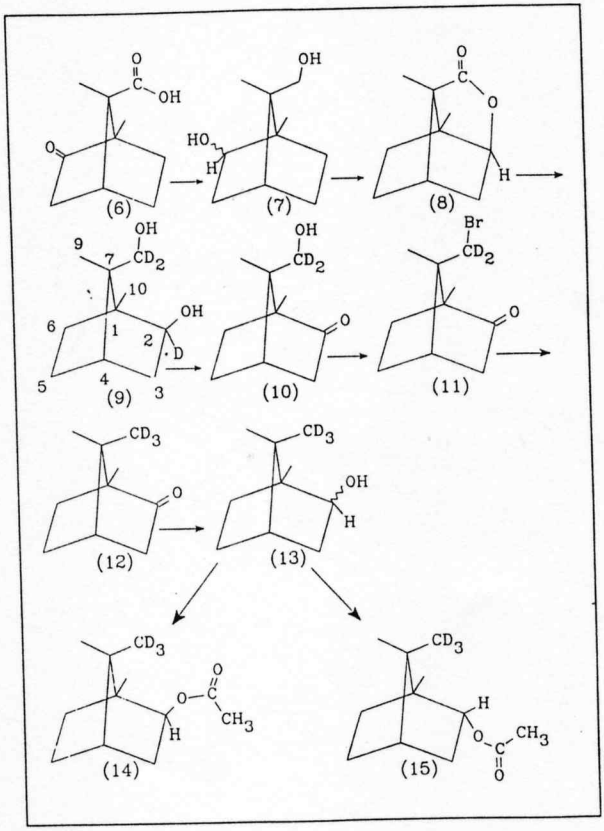

Figure 2. Synthesis scheme of (-)-bornyl-8,8,8-d_3 acetate and (+)-isobornyl-8,8,8-d_3 acetate.

Synthesis scheme of (+)-bornyl-9,9,9-d_3 acetate and (-)-isobornyl-9,9,9-d_3 acetate

The overall synthesis scheme for the preparation of (+)-bornyl-9,9,9-d_3 and (-)-isobornyl-9,9,9-d_3 acetate is shown in Figure 3. Starting with

trans-isoketopinic acid [6], methyl isoketopinate [16] was prepared by reaction with diazomethane. [16] was converted to a mixture of 9-hydroxy-3,9,9-d_3 isoborneol and 9-hydroxy-3,9,9-d_3 borneol [17] by reduction with lithium aluminum deuteride in tetrahydrofuran. The mixture was reacted with 1.5 equivalents of *p*-toluenesulfonyl chloride in pyridine to produce the 9-*p*-toluenesulfonates [18]. 9,9,9,2-d_4 isoborneol and 9,9,9,2-d_4 borneol [19] were then prepared by reduction of [18] with lithium aluminum deuteride in tetrahydrofuran. A nitroxide-catalyzed oxidation with *m*-chloroperoxybenzoic acid was carried out to convert the alcohol mixture [19] to 9,9,9-d_3 camphor [20]. The 9,9,9-d_3 camphor was then reduced with borane *t*-butylamine to yield (+)-9,9,9-d_3 borneol and (-)-9,9,9-d_3 isoborneol [21]. This second oxidation followed by reduction was necessary to obtain a larger quantity of the deuterated borneol and to remove the deuterium from carbon 2. (Reduction of camphor by lithium aluminum hydride yields about 90% isoborneol and only 10% borneol. Borane *t*-butylamine affords roughly 40% borneol.) Separation of the isomers was conducted by flash chromatography. The separated isomers were then esterified with acetyl chloride to produce (-)-isobornyl-9,9,9-d_3 and (+)-bornyl-9,9,9-d_3 acetates [22,23] which were purified by flash chromatography.

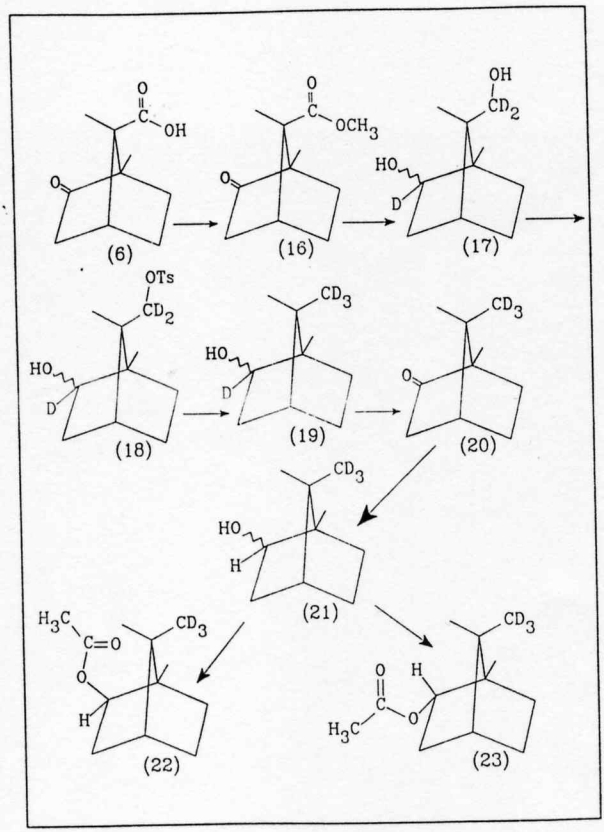

Figure 3. Synthesis scheme of (+)-bornyl-9,9,9-d_3 acetate and (-)-isobornyl-9,9,9-d_3 acetate

ELECTROANTENNAGRAM BIOASSAY

Sixteen selectively deuterated analogs of bornyl and isobornyl acetate were evaluated using the electroantennagram (EAG) technique. A schematic diagram of the electroantennagram experimental setup is shown in Figure 4.

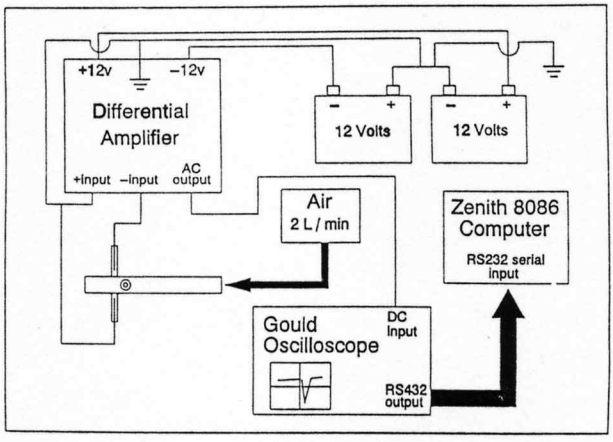

Figure 4. Schematic Diagram of the EAG Experimental Setup

Clean air at a flow rate of 2 L per minute was passed through the EAG apparatus at all times. The air source was a compressed air tank fitted with a needle valve and a scrubber tube (1.5 cm x 7 cm in length) to remo organic impurities. It was packed with activated carbon and located down-stream from the regulator. The chlorided silver wire recording electrodes were located inside the capillary tubes of the Pyrex EAG apparatus and were connected by means of shielded coaxial cable to the differential amplifier. Detailed drawings of the Pyrex EAG apparatus are shown in Figures 5-7.

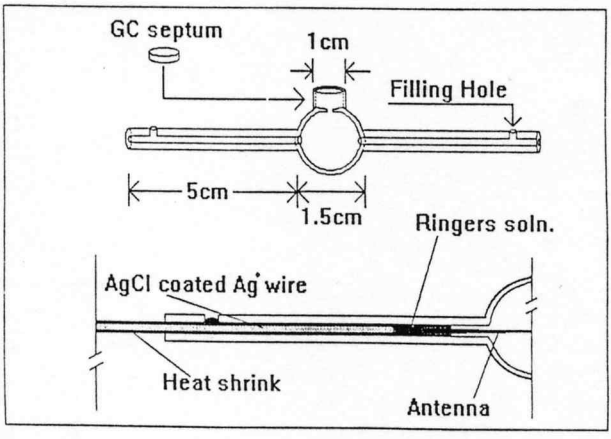

Figure 5. Top: Front View of the EAG Apparatus
Bottom: Blow-up of Capillary Tube

Electrical connection of the antenna to the recording electrodes was provided by means of an electrolytic (Ringer's) solution. Stable, noise free power for the differential amplifier was provided by two 12 volt car batteries wired in series. Amplified EAG responses were recorded on the storage oscilloscope and the data files were subsequently transferred to the Zenith computer hard disk drive for storage.

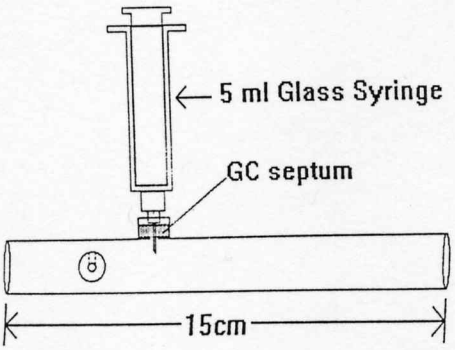

Figure 6. EAG Apparatus - Side View

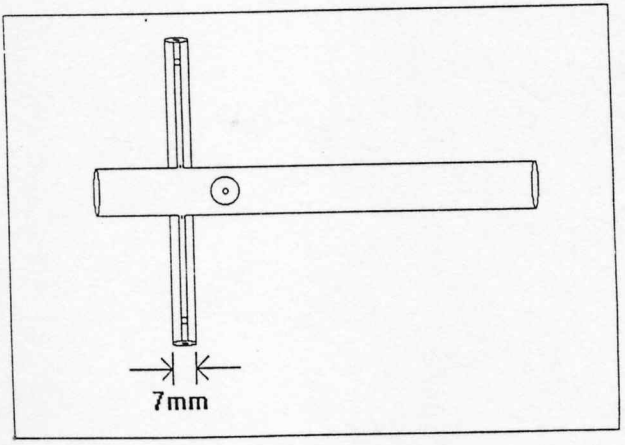

Figure 7. EAG Apparatus - Top View

APPARATUS

EAG The electroantennagram apparatus used was hand blown from Pyrex glass. The final design was a modified version of the apparatus described by Scriven (15).

Amplifier - Frederick Haer and Co. Inc. AC differential amplifier. The amplifier was powered by two 12 volt car batteries and was used to amplify the EAG impulses.
Oscilloscope - Gould model 1425 digital storage oscilloscope. The oscilloscope was used to record the amplified EAG impulses from the differential amplifier. A short BASIC program was written to convert the trace files in HPGL format to ASCII XY data pair format and store the files on the hard disk drive of the Zenith 8086 computer.
Electrodes - Chlorided silver wire electrodes were used as the recording electrodes for the EAG setup. These were made from 18 gauge (~ 1mm diameter) silver wire and were plated in sets of 2.
Shielding - Shielding of extraneous electrical noise was provided by wrapping the EAG apparatus with shielding tape and connecting the shielding tape to ground.
Syringes 5 mL ground glass syringes with 20 gauge needles (0.5 inch length) were used to puff the test compounds over the excised antenna. A 0.5 cm disk of Whatman #1 filter paper was placed inside each syringe and 5 uL of the test compound was applied to the filter paper.
Software - Oscilloscope traces were individually stored as each was collected using a home written Quick BASIC program called GOULD2.BAS (21). Upon selection of the plot option on the Gould oscilloscope, the program captures the HPGL file intended for an XY plotter, strips out header and tailer information, converts the file to an ASCII XY data file and stores the file on the hard disk drive of the Zenith 8086 computer. EAG values were measured after the ASCII XY data files were imported into Spectra Calc (Salem NH). Quattro Pro 3 (Borland International Inc.) was used for EAG data workup.
GC - All chromatograms were performed using a Tracor model 360 equipped with a capillary column interface and a flame ionization detector. The column used was a Carbowax 30 m fused silica capillary column with a 0.25 mm inside diameter and helium as a carrier gas.
NMR - Bruker model WM400 400 MHz Fourier transform NMR spectrometer. Deuterochloroform was the solvent employed for all samples. Several of the ^1H NMR's contain acetone as an impurity (singlet ~2.1 ppm) from not having the allowed the NMR tubes to dry well enough between samples.
Mass Spectra - All mass spectra were recorded by direct probe injection and electron impact on a Hewlett Packard model 5989A MS with the exception of (+)-bornyl-$10d_1$-acetate in which the HPLC interface and chemical ionization was used.
Ringers solution - 7.5 g NaCl, 3.5 g KCl, 0.21g $CaCl_2$/L.
Plating solution - 10 g NaCl/L.
Insects - Adult male American cockroaches were purchased from Wards Natural Science (Henrietta, NY). Upon arrival the colony was maintained as described by Scriven (15).
Test compounds - Compounds deuterated in the eight and nine positions were prepared as described. All others were prepared according to the procedures described by Kim (16). Purities of the compounds were checked by proton NMR and gas chromatography employing the same conditions as described in the synthesis of the C-8 and C-9 deuterated compounds. Mass spectra were recorded at a later time for additional characterization information.

PROCEDURE

Nine identical 5 mL syringes with ground glass plungers and needles (1.3 cm, 18 gauge) were cleaned, rinsed with acetone and baked in a drying

oven overnight to remove any organics. The two silver wire recording electrodes were polished, cleaned, plated, placed in the EAG apparatus and the capillary tubes filled with Ringer's solution. A tight fit of the electrodes inside the capillary tubes was assured by applying a short length of heat shrink tubing to the base of each electrode near the solder connection to the coaxial cable, and then wrapping with Parafilm prior to plating. (A tight fit was necessary to avoid motion artifacts in the EAG traces.) It was observed that allowing the electrodes to soak in the Ringer's solution for at least one hour before use decreased the recorded noise.

A 0.5 cm disk of Whatman #1 filter paper was then added to each syringe. With the exception of the blank, 5 uL of the appropriate compound was carefully applied to each of the filter paper disks in the syringes such that the entire aliquot was absorbed by the filter paper. The plungers were set to a volume of 4 mL, then the syringes were capped and allowed to stand for at least one hour before use.

A healthy looking adult male cockroach was selected and then anesthetized by the administration of a small amount of carbon dioxide. An antenna was severed approximately 1 mm above the swivel joint at the base of the antenna. Approximately 2 mm of the distal portion of the antenna was also removed to insure a good electrical connection. The remaining section of antenna was 4 - 4.5 cm in length.

The antenna was placed in the EAG apparatus, and the glass capillary tubes containing the chlorided silver wires were filled with Ringer's solution by the means of a narrow tipped Pasteur pipet to establish electrical connection with the antenna. (Antennae were positioned within the EAG apparatus such that a consistent 2.5 cm exposure region was established ranging from 4 to 29 mm proximal on the antennae.) Electrical tape was used to seal and insulate the filling holes in the capillary tubes. The EAG apparatus was then wrapped with shielding tape and the tape was grounded. Clean air, at a flow rate of 2 L/min was allowed to flow over the antenna for a period of five minutes before starting the experiment.

A syringe containing (-)-bornyl acetate (Aldrich, 97%), prepared as described above, was attached to the apparatus and puffed into the air stream three times at two minute intervals. (Experimentation showed this to be a quick way to adapt the antenna to the bioassay.) At the next two minute interval, the blank syringe, filled with clean air, was puffed, the EAG response recorded on the oscilloscope, and the trace data file transferred to the Zenith computer for storage. The syringe used for the adaptation procedure was puffed once more at the next time interval. Finally, the test compounds were puffed once each in a predetermined sequence at two minute intervals, the sequence repeated and then followed by a second blank as the last puff.

The puffing sequence of the compounds was changed in a rotating fashion for each antenna used. Upon completion of a full rotation, a second full rotation was performed in the reverse order. Rotation of the testing order was necessary to cancel out the decreased response due to the metabolic decay of the antenna with time as the experiment proceeds and to cancel out any "memory" effects associated with previous puffs. EAG's were stored on the Zenith computer and evaluated at a later time in Spectra Calc. An example electroantennagram of (-)-bornyl acetate is shown in Figure 8.

ELECTROANTENNAGRAM RESULTS

The results of the electroantennagram experiment are shown in Table 1 and are descendingly ordered by EAG response.

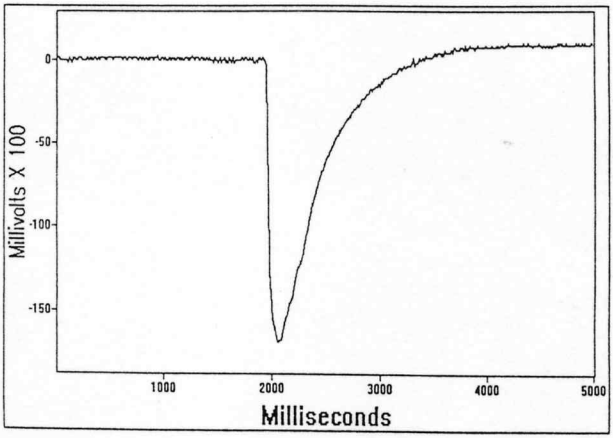

Figure 8. EAG Response for (-)-Bornyl Acetate

Table 1.
Electroantennagram Data For Deuterated Bornyl and Isobornyl Acetates (Mean Values of 180 Repetitions Unless Otherwise Noted)

Compound	mV x 10^2	α = 0.05*
(+)-Bornyl-2-d_1 acetate-d_3 [1]	175.6	a
(+)-Bornyl acetate-d_3	173.0	ab
(+)-Bornyl-2-d_1 acetate	171.2	b
(+)-Bornyl acetate [2]	170.8	b
(+)-Bornyl-9,9,9-d_3 acetate	165.9	cd
(+)-Bornyl-10,10,10-d_3 acetate	165.1	cd
(+)-Bornyl-10-d_1 acetate	163.3	d
(-)-Bornyl acetate	154.4	e
(-)-Bornyl-8,8,8-d_3 acetate	152.6	e
(-)-Isobornyl acetate	123.1	f
(+)-Isobornyl-2-d_1 acetate	119.2	g
(+)-Isobornyl-2-d_1 acetate-d_3	118.0	gh
(+)-Isobornyl acetate	116.7	hi
(+)-Isobornyl-8,8,8-d_3 acetate	116.2	hij
(-)-Isobornyl-9,9,9-d_3 acetate	114.7	j
(-)-Isobornyl-10,10,10-d_3 acetate	112.2	j
Blank	30.4	

[1] 36 repetitions
[2] 108 repetitions
* Compounds with the same letter are not significantly different at the 95% confidence level.

Each compound, except for those marked with an asterisk, was tested a total of 180 times (2 EAG's per compound per antenna x 90 antennas). Compounds marked with an asterisk were tested fewer times due to a lack of sample. The deuterated bornyl acetates and isobornyl acetates were investigated in separate groups. Each group contained seven deuterated compounds, a standard of (-)-bornyl acetate, and a blank.

Because of the large amount of variability in response between antennae, the individual run sequences were scaled to an average value obtained for the entire data set. EAG responses for each sequence were summed. The sum was compared to the average sequence sum obtained for the entire data set and a multiplication factor for the sequence calculated. Multiplying this factor by the individual EAG values within the sequence scaled the values to the average response and furnished a 3-4 fold reduction in the standard deviation.

Statistical significance of the differences in response between the test compounds was assessed by performing a Fisher's Least Significant Difference test on the scaled data at the 95% confidence level. All of the data workups were performed using Quattro Pro 3.

Conclusion In all cases, bornyl acetates gave greater responses than did the isobornyl acetates, and within each group of compounds, the response for the (+) isomers was greater than the response for the (-) isomers with the exception of (-)-Isobornyl acetate. Deuteration of the acetate group or deuteration at carbon 2 resulted in slightly larger EAG responses than the corresponding non-deuterated compounds and the differences were not significant at the 95% confidence level in all cases. Deuteration at carbon 8 had no significant effect on EAG response while deuteration at the 9 and 10 positions decreased the magnitude of the EAG signal by 0.05 - 0.10 mv. No significant difference was found in EAG response between deuteration at the 9 or 10 positions, however the order was 9 ≥ 10.

CORRELATION OF ACTIVITY WITH INFRARED SPECTRA

Infrared spectra of the selectively deuterated bornyl and isobornyl acetate compounds were examined for any type of correlation present that would indicate a relationship between insect response (EAG) and frequency. The region between 1000 and 200 cm^{-1} was chosen as the spectral window for several reasons:

1. Wright indicates that the far infrared is the important spectral region for examination since it would be this low energy region of the spectrum where fundamental modes of vibration would be populated to a significant level at ambient temperature (2).

2. Deuteration creates changes in the vibrational frequencies that are more likely to be apparent in this region of the IR spectrum.

3. The vibrational modes for many of the compounds tested had been previously assigned by S. B. Kim covering the region from 1000 - 200 cm^{-1} (16).

Spectra were recorded for each of the compounds studied and the normal modes of the newly synthesized compounds were assigned. The spectral data was then subjected to examination by both visual inspection and computer assisted means. A Partial Least Squares (PLS) algorithm was implemented as the computer assistance because of its widespread use in molecular spectroscopy as a powerful pattern recognition technique. The PLS algorithm is well known and a comprehensive discussion of the PLS technique with references is provided by Havens (21).

Infrared - Perkin Elmer model 1330 dispersive infrared spectrophotometer. Spectra were recorded on chart paper over the region from 1000-200 cm^{-1} using a CsI liquid cell with a 0.1 mm Teflon spacer at the 48 minute scan setting. The instrument default slit program was also used. The spectra were then digitized and converted into an ASCII XY data pair format for use in Spectra Calc and PLS Plus.
Computer - Zenith 386SX IBM PC clone equipped with 2 MB of RAM and a math coprocessor.
Plotter - Hewlett Packard model 7475A was used in the process of digitizing the IR spectra.
Software - The spectra were recorded on chart paper, photocopied, and then digitized using UN-PLOT IT (Silk Scientific, Orem, Utah). All spectral manipulation and PLS correlation work was performed with Spectra Calc and PLS Plus (Galactic Industries Corp., Salem, NH).

The IR bands were assigned for the compounds deuterated in the 8 and 9 positions by comparison with spectra previously recorded and assigned by S. B. Kim and Dr. R. M. Hammaker. Since no Raman spectra were available, the assignment was not as complete or as rigorous as Kim's (16). Assignments were made in a very empirical manner based on the information available and not all bands were assigned. Vibrational modes of bands that could not be easily justified by inspection of the spectra were left unassigned.

The spectra, recorded on chart paper, were photocopied onto 8.5" x 11" white paper to enable digitizing on the HP 7475A XY plotter. The Un-Plot It digitizing system was connected to the RS-232 serial ports on the Zenith computer and the XY plotter. The main program for the system, UPIP.EXE, was executed and communications between the digtizer, plotter and computer were tested. The light pen was placed in the pen holder in place of the plotter pen, the coordinate system was set up and the contrast adjusted on the light sensor by following the instructions displayed on the screen.

Digitization was then performed at the highest resolution setting. Upon completion, the digitized spectra were tranformed within Un-Plot It using its linear interpolation routine to yield data files for the region 945 - 210 cm^{-1} with 1471 equally spaced points (1 point every 0.5 wave-numbers). The Un-Plot It files created were equivalent to an ASCII XY data pair format file with the exception of an additional line at the beginning of the file which was the number of data points in the file.

In order to import the files into Spectra Calc one of two operations had to be performed. The first option was to edit the data files with a text editor and delete the first line so that the file could be imported into Spectra Calc using the ASCII XY format option. A second alternative, which was later used, was an Array Basic application program called SILK.AB, provided by Galactic for Spectra Calc that enabled the direct importation of the Un-Plot It files into Spectra Calc. SILK.AB was executed from the ARITHMETIC/DOPROGRAM menu within Spectra Calc and it automatically imported and converted the files into Galactic's .SPC spectral file format.

Much of the manipulation of the spectra was also performed using the ARITHMETIC menu selections. Absorbance spectra are typically used in PLS calibrations, so it was necessary to convert the original transmission spectra. This conversion was performed using the selection ABSORB from the ARITHMETIC menu. ABSORB converts a single beam transmission spectrum into an absorbance spectrum. In order to perform the operation ABSORB also requires a reference spectrum. Since the imported spectra were recorded on a double beam instrument, it was necessary to create a 100% T reference spectrum. The reference spectrum was created using the FUNCTIONS selection

from the ARITHMETIC/OTHER menu. Using FUNCTIONS, one of the spectra was first multiplied by zero, and then a value of 100 was added to it to yield a 100% T spectrum with the same number of data points. The file was used as the reference spectrum to convert all of the files to absorbance units. The baselines of the spectra were adjusted by the use of the MINIMUM_ZERO selection from the ARITHMETIC/OTHER/OFFSET menu. This procedure forced the minimum value of the current spectrum to equal a value of 0 absorbance units. Once the baseline corrections had been performed, the baseline corrected spectra files were saved.

Although all of the spectra were recorded in a liquid cell with a 0.1 mm spacer, upon inspection, it was apparent that the path length was not uniform for all of the samples. This probably resulted from the ease at which the CsI windows were distorted over time by pressure after assembly of the cell. It was decided that normalization of the spectra would be necessary to obtain a better PLS calibration and to avoid "transparent" pre-treatment algorithms prior to calibration which did not allow the treated data to be observed.

The first step in the normalization process was to establish a feature in the spectral window from which a ratio could be calculated. Inspection revealed that the intensity of the deformation of the quaternary carbon at C-1 ($\tilde{}$ 237 cm^{-1}) was the best choice. The intensity of the deformation at C-1 was measured for each of the spectra, and factors were calculated to arbitrarily assign the band in each spectrum an intensity value of 0.3 absorbance units. The spectra were operated on using FUNCTIONS where each of the spectra were multiplied by the factors. Correctness of the normalization was checked and the spectra saved. While not perfect, this process greatly decreased the apparent intensity variations between spectra. An example showing an original spectra (Figure 9) and one after a full treatment (Figure 10) is shown for (+)-Bornyl-9,9,9-d_3-acetate.

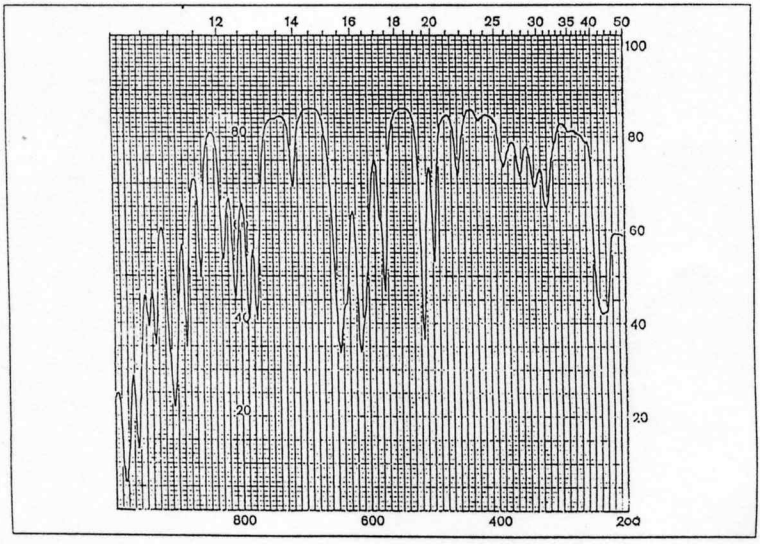

Figure 9. %T IR spectrum of (+)-bornyl-9,9,9-d_3 acetate recorded on chart paper

In order to perform a computer pattern recognition program to correlate the insect activity to a region of an infrared spectrum it was necessary to digitize the %T spectrum, then linearize it, convert it to absorbance, baseline correct it and then normalize it. Figure 10 shows this for the spectrum in Figure 9.

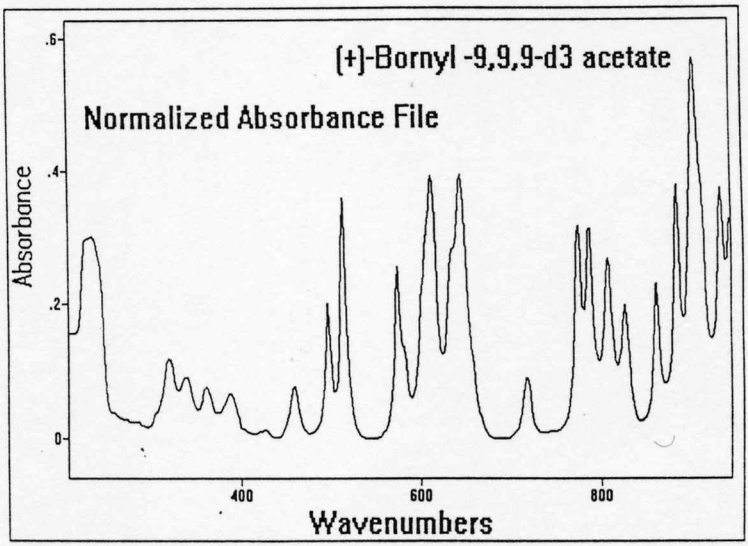

Figure 10. Absorbance IR spectrum of bornyl-9,9,9-d_3 acetate - digitized, linearized, baseline corrected and normalized.

PRELIMINARY VISUAL CORRELATION

Tables 2 and 3 list the frequency assignments for (+)-bornyl acetate as an example. The rest are given by Havens (21). The first step in the comparison was to look at the band assignments and determine which of the bands had been shifted an appreciable amount in at least one of the deuterated compounds. A threshold value of 10 wave numbers was arbitrarily chosen and each frequency shifted 10 or more wave numbers from the non-deuterated value was noted. The second step consisted of looking within the groups of compounds to see if there were any trends that related shifted bands to the magnitude of the EAG response.

Upon inspection of the largest group of compounds, the (+)-bornyl acetates, some general trends could be seen. The trends seemed to encompass five of the assigned bands, and all bands were related to the acetate functionality of the molecule. The normal modes involved were assigned to the COC bending, the C-O out of plane bending, the C-O in plane bending, the carbonyl out of plane bending and the carbonyl in plane bending. Their respective frequencies for the non-deuterated (+)-Bornyl acetate were 341, 462, 507, 608, and 635 cm^{-1}.

The trends observed were an increased EAG response with a decrease in the frequency of the vibrational mode. The shifts seen are in the range of -14 to -80 cm^{-1}. Wright's theory would indicate that the bands were shifted

to occupy a more favorable region of the spectrum for establishing a resonance transfer of energy with the receptor site. Also for these compounds, an increase in the frequency of the vibrational modes generally yielded a

Table 2
Frequency assignment table for (+)-bornyl acetate compounds

Compound	(+)-Bornyl Acetate	(+)-Bornyl-2d1-Acetate-d3	(+)-Bornyl Acetate-d3	(+)-Bornyl-2d1-Acetate
EAG in mV*100	171	176	173	171
Deform. of quat. carbon at C-1	239	238	237	236
Torsion about ester C-O bond	269	267	266	268
Ring twisting	321	318	326	318
COC bending	341	326	326	340
CCO bending	360	362	365	358
Deform. of quat. carbon at C-7	397	394	389	396
C-O out of plane bending	462	445	450	460
C-O in plane bending	507	486	493	502
Deform. of quat. carbon at C-7	519	513	512	517
Deform. of quat. carbon at C-1	569	563	568	566
Deform. of quat. carbon at C-1	585	581	586	581
COOR skeletal	600	603		598
Carbonyl out of plane bending	608	525	529	605
Carbonyl in plane bending	635	603	613	630
Sym.stretch of quat carbon at C-1	635	641	645	630
Sym.stretch of quat carbon at C-7	650	641	645	643
Bicyclic ring bending	741	736	741	740
Bicyclic ring methylene rock	784	762	776	770
C-C stretch of acetyl group	819	803	842	787
CD3 rocking of acetyl group		803	796	
Bicyclic ring breathing	819	833	832	835
Bicyclic ring breathing	832	833	842	817
2-CD bending		780		

decreased EAG response with only a few exceptions. However, the compounds that revealed a decreased EAG response had in all cases frequency shifts 10 wave numbers or less from those of the nondeuterated (+)-bornyl acetate for the bands in question, and consequently the significance of this latter judgement is justifiably questionable. No other trends were as apparent in the other groups of compounds, but given the smaller numbers of compounds in each of the groups, this was not thought to be a very surprising conclusion.

Another approach was also investigated as a means of identifying important spectral frequencies. If Wright's theory is valid, then a group of compounds which all elicit a similar biological response should share common spectral characteristics. Therefore, if the spectrum is divided up into small spectral windows, and a frequency (of occurrence) histogram is constructed which indicates the number of compounds within a group that have one or more peaks within each of the spectral windows, then the highest frequency (of occurrence) values should indicate the spectral windows which contain important spectral features. Windows with lower frequency

Table 3
Frequency assignment tables for (+)-Bornyl acetate compounds

Compound	(+)-Bornyl -9,9,9- d3-Acetate	(+)-Bornyl- 10,10,10- d3-Acetate	(+)-Bornyl- 10- d1-Acetate
EAG in mV*100	166	165	163
Deform. of quat. carbon at C-1	235	215, 241	240
Torsion about ester C-O bond	262	273	272
Ring twisting	320	317	324
COC bending	339	350	347
CCO bending	362	350	365
Deform. of quat. carbon at C-7	387	395	397
C-O out of plane bending	460	455	461
C-O in plane bending	497	503	508
Deform. of quat. carbon at C-7	514	519	521
Deform. of quat. carbon at C-1	573	577	577
Deform. of quat. carbon at C-1	581	555	588
COOR skeletal		600	610
Carbonyl out of plane bending	612	609	610
Carbonyl in plane bending	634	636	637
Sym.stretch of quat carbon at C-1		636	656
Sym.stretch of quat carbon at C-7	645	636	640
Bicyclic ring bending			722
Bicyclic ring bending	717	713	742
Bicyclic ring methylene rock	775	780	786
C-C stretch of acetyl group	787	799	824
Bicyclic ring breathing	808	828	824
Bicyclic ring breathing	827	828	847
10-CD bending			806
10-CD3 rocking		762	

values would be considered to be of little importance, or to be responsible for subtle differences between compounds within the group. Regions possessing a frequency of zero may also be considered important as Wright has indicated that certain regions must also be absent of peaks in order to elicit a response.

Separate histograms of this type were constructed for the bornyl acetates and isobornyl acetates as groups, on the assumption that each of the compounds within a group elicit similar responses. No distinctions were made regarding the stereochemistry of the individual compounds since (+) and (-) isomers had identical IR spectra. For the cases of the non-deuterated bornyl and isobornyl acetates, where spectra of both stereoisomers were available, only one was included in the group so that there were no duplications.

The spectra were divided up into spectral windows 20 cm^{-1} in width (+/- 10 wavenumbers about a center frequency). Complete peak tables were generated (all of the peaks were used in this case, even those that could not be assigned) for each of the compounds and then the tables were examined to count the peaks contained within each spectral window. Information related to Raman spectral bands was not included because Raman spectra were not available for all of the compounds. If a single compound contained more than 1 peak within a spectral window, that compound was only given a count of 1 for the window so that the frequency never exceeded the total number

of samples that contained peaks. This situation was encountered only a small number of times. The histograms are shown in Figures 11 and 12. The center frequency for each of the histogram bars is indicated by the tick mark at the left edge of the bar.

Copies of the histograms were overlaid on a light table and examined for similarities and differences. If Wright is correct, then regions of

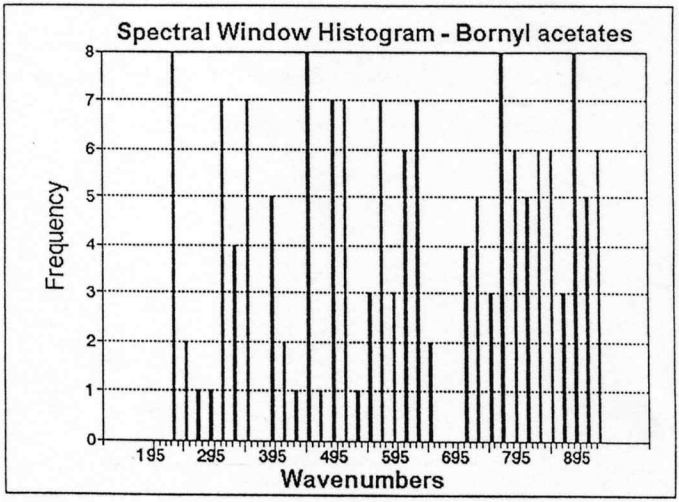

Figure 11. Spectral window histogram - Bornyl acetates 20 cm^{-1} spectral windows

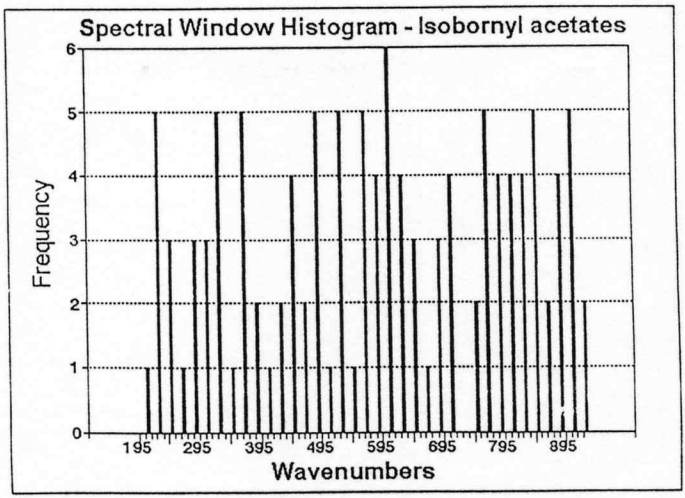

Figure 12. Spectral window histogram - Isobornyl acetates 20 cm^{-1} spectral windows

similarity between the two histograms should be areas of little importance in relation to the biological response differences. Regions with marked differences then are possible regions of importance.

In general, the peak distributions were very similar with a few notable regions of difference. Spectral windows which contained a number of peaks equal to the number of compounds within the group (8 for the bornyl acetates and 6 for the isobornyl acetates) were all found to fall within the regions of similarity. Regions with center frequencies of 345 and 505 cm^{-1} each had 7 peak occurrences within their respective windows for the bornyl acetates while only one peak occurrence observed in the vibrational mode frequency assignment tables mentioned previously. The modes are the C-O in plane and C-O out of plane bending vibrations.

A third notable difference was observed at the center frequency of 525 cm^{-1}, where the isobornyl acetates had 5 peak occurrences while the bornyl acetates had only one. Consequently, this could be a region that is of little importance to a response, or possibly even a region of inhibitory action.

While there was some overlap of spectral features between the groups for these three spectral windows, this was not felt to be of great importance. Examination of the data showed that the majority of the discrepancies resulted from peaks at the edge or just beyond the edge of a window. Since the center frequencies and width of the spectral windows were arbitrarily assigned their values, they would not be expected to correctly match the important spectral regions in all cases. Histograms were also constructed with 10 cm^{-1} spectral windows, and were found to reveal very similar information but were more difficult to interpret since none of the spectral windows had a frequency equal to the total number of samples within the group.

Three more spectral windows were also of interest because of their complete absence of peaks. The center frequencies for these windows were 665 and 685 cm^{-1} for the bornyl acetates and 725 cm^{-1} for the isobornyl acetates. The suggestion here once again is that the regions may be unimportant, or in some way may be required for, or inhibitory to, a response. Assuming the latter is true, then peaks within the windows centered at 665 and 685 cm^{-1} could possibly inhibit a response while peaks within the 725 cm^{-1} window might play a role in the initiation of a response.

COMPUTER CORRELATION

Due to the complexity of attempting to correlate the spectra with the EAG response by visual inspection, it was decided that a Partial Least Squares (PLS) pattern recognition program would be implemented to investigate possible correlations in the spectral data set. PLS is a popular factor based method of analysis. As a factor based method, PLS assumes the variance in the training set data is responsible for the concentration or "response" data. PLS is similar to Principle Component Regression (PCR) which determines the factors ("principle components") by computing the orthogonal direction of the maximum variance in the training set spectra.[1]

In PLS, however, the concentration information is also used in the determination of the factors (23,24). Spectra with the highest concentrations (responses) are considered to be more important and are weighted more heavily than those with lower concentrations in the factor computation step. The effect is that a greater amount of information directly related to the concentration is forced into the first few factors when compared to PCR. Absorbance spectra are typically used in the training set so that

intensity and concentration are linearly related.

The appropriateness and predictive power of the PLS model is determined by an extensive cross validation algorithm known as the prediction residual error sum of squares or PRESS. The PRESS calculation itself is a multi-step process. Simply stated, each sample spectrum is left out of the training set one at a time, and a PLS calibration performed with the remaining spectra. Prediction errors for each number of factors are determined by predicting the concentration of the left out sample spectrum for the PLS model generated with successive numbers of factors.

Once this information has been obtained for all sample spectra, a sum of squares for the prediction errors of each number of factors is calculated. The PRESS values are plotted versus the number of factors. The minimum for the PRESS plot generally indicates the number of factors that will yield the best prediction results. Plots of the actual versus the predicted concentration values obtained from the PRESS rotation also serve as useful diagnostic indicators of any correlations present.

Overview

The compounds were grouped by type and the entire spectrum of each was correlated with insect activity. No type had a significant correlation. The spectra were then divided into four regions and each correlated. One type, (+)-bornyl acetate, had two regions that were significant. These two regions were then subdivided and additional correlations made.

While all of the spectra were grouped into four sets: (+)-bornyl acetates, (-)-bornyl acetates, (+)-isobornyl acetates and (-)-isobornyl acetates, only that of the (+)-bornyl acetates are presented (21). The spectra were grouped in this manner because of the relatively large EAG response differences between the stereoisomers. Also, if Wright's theory was to be tested in an objective manner, site geometry variables would need to be held constant within the groups.

The spectra used covered the region from 210 - 945 cm^{-1}. The group of (+)-bornyl acetates was the largest with a total of seven spectra. The next largest group was the (+)-isobornyl acetates with only four. The (-)-bornyl acetates and the (-)-isobornyl acetates contained two and three, respectively. It was felt that the most useful information would be obtained by performing a PLS calibration on the individual groups, but due to their size limitations, the only group this was practical for was the (+)-bornyl acetates. Fortunately, (+)-bornyl acetate was also the best sex pheromone mimic.

A full spectrum calibration was attempted first on the group of (+)-bornyl acetates. The resulting model was deemed very poor. The largest correlation coefficient (R^2) value obtained was 0.16 with a single factor model. Increasing the number of factors decreased the R^2 value to zero. Due to the lack of success with full spectrum calibration, it was decided to divide the spectrum into four smaller regions and perform a calibration for each region. It was felt that this would also provide a better indication as to which region or regions of the spectrum were most important if any correlations were found. The four regions chosen were 210-400 cm^{-1}, 401-600 cm^{-1}, 601-800 cm^{-1} and 801-945 cm^{-1}. Two of the calibration regions produced noteworthy results. The two regions are 210-400 cm^{-1} and 601-800 cm^{-1}.

DISCUSSION, 210-400 cm^{-1} REGION

The PRESS plot and the Actual versus Predicted plots for these two

regions are presented. The diagnostic results for the 210-400 cm^{-1} region are shown in Figures 13 and 14. PRESS results indicate a minimum value was reached at three factors, the maximum number allowed by PLS Plus for seven samples. Also, the PRESS had only decreased by approximately one-third of its initial value at the third factor, indicating a large prediction error for one or more of the training samples. Predicted versus Actual EAG values reveal that with a two factor model, five of the samples can be predicted quite well, while two of the samples, (+)-bornyl-10-d_1-acetate and (+)-bornyl-9,9,9-d_3-acetate, lie a considerable distance from the actual values. This results in a very low correlation coefficient value (R^2=0.11). Bornyl-10d_1 acetate was the largest outlier. The correlation coefficient is improved with the addition of a third factor (R^2=0.30), however the predicted values for the same two compounds were still far enough away from the actual values that a relatively low R^2 value was obtained.

The factors provided qualitative information related to the specific regions in the spectrum that were most important to the calibration. It is inherent to the spectral decomposition process of the PLS algorithm that the factors are ordered in terms of their degree of importance to the model. Therefore, the first few factors should contain the greatest amount of qualitative information. The degree of importance of a spectral region to the calibration is generally directly proportional to its deflection from zero at that region for mean centered data. The first factor for the region 210-400 cm^{-1} displayed that a large portion of the region was of significant importance to the calibration. This was subsequently proven by a number of failed attempts to improve the calibration by a more careful choice of the spectral region. With the exception of the portion from approximately 370-400 cm^{-1}, removal of any portion of the region from the calibration produced markedly worse results. In addition, no improvement was seen as the calibration region was extended beyond 400 cm^{-1}.

Removal of (+)-bornyl-10d_1-acetate

A single factor model produced the best results. Scatter of the data points was more uniform about the regression than for the previous calibration line and an improved correlation coefficient value was obtained (R^2=0.54). Once again (+)-bornyl-10d_1-acetate was the furthest from the regression line. Because of these discrepancies, attention was focused on the sample for possible explanations.

While casual inspection of the IR spectrum of (+)-bornyl-10d_1-acetate did not reveal any gross differences, the history of the spectrum is unique. An insufficiently small quantity of the compound was available to fill the CsI liquid cell and consequently no IR spectrum was recorded for (+)-bornyl-10d_1-acetate at the time the rest of the spectra were recorded. The spectrum was instead reproduced from S.B. Kim's Ph. D. dissertation (16). A Hewlett Packard model 130 dispersive IR spectrophotometer was used to record the original spectrum and the instrument parameters were unknown as no original remains. The digitization process for (+)-bornyl-10d_1-acetate was carried out in an identical manner to that of the other spectra in the training set, nevertheless, it was necessary to photo-enlarge the thesis figure to several times its original size prior to the digitization. Therefore, it was possible that distortions from the photo-enlargement and instrumental differences were responsible for the large errors. Because of this possibility, (+)-bornyl-10d_1-acetate was excluded from the training set, and the calibrations were repeated with the six remaining spectra.

No discernible improvements were apparent for a full spectrum calibration with the new training set. Calibrations performed on the smaller regions indicated that the same two regions of 210-400 and 601-800 cm^{-1}

merited further study. Dramatic improvements were seen in the correlation coefficients and other diagnostic results used to determine the quality of the models for these regions. It was felt the dramatic improvements provided further evidence that (+)-bornyl-10d_1-acetate spectrum was not representative, especially when the small size of the training set was considered.

The diagnostics for the 210-400 cm^{-1} region for the new six spectrum training set are shown in Figures 13 and 14.

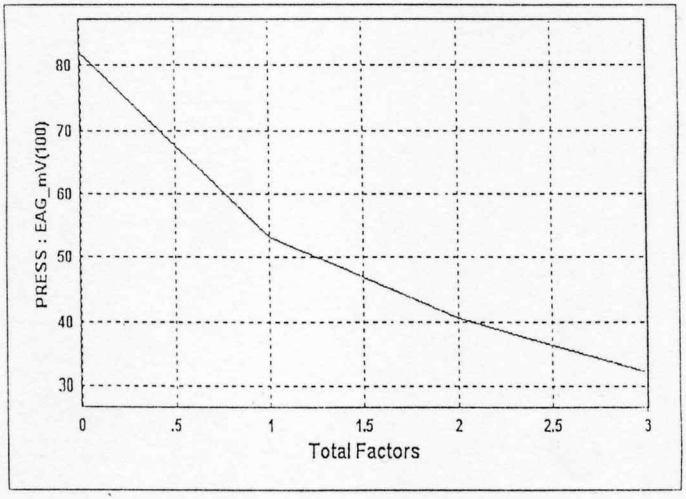

Figure 13. PRESS Plot - (+)-bornyl acetates 210 - 400 cm^{-1}, six spectrum training set.

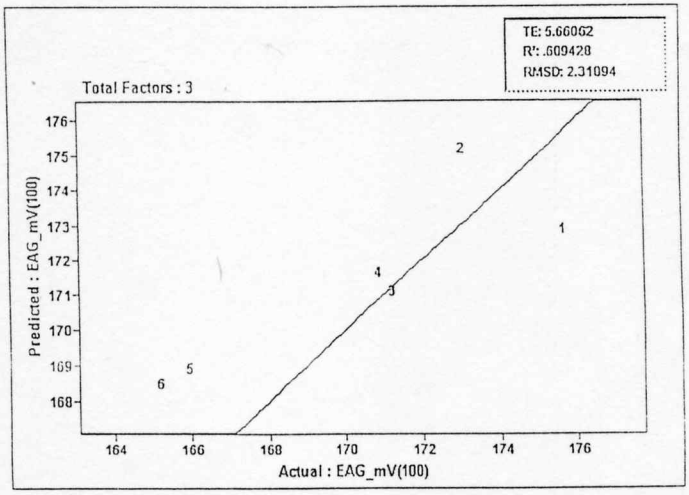

Figure 14. Predicted versus Actual EAG values - 3 factors, 210 - 400 cm^{-1}, six spectrum training set.

three factors. The three factor model also had the highest correlation coefficient, R^2=0.60, a large increase over the value obtained for the seven spectrum training set. Examination of the factors revealed little difference between the first factors of the six and seven spectrum training sets, but the differences became greater as the number of factors increased. The F statistic for the PRESS reported that the decrease in PRESS value from the second to the third factor may not have been significant, implying that there is a possibility that the three factor model might be "overfitting" the data, but given the circumstances of the experiment (ie. no other compounds with known EAG values within the range of the calibration), an empirical determination could not be performed. In any case, the overall quality of the correlation did not appear to suffer drastically when a two factor model was used.

A number of calibrations were performed to see if an optimum calibration region could be empirically determined. This optimum region was found to be 225-265 cm^{-1}. PRESS, Predicted versus Actual and factor plots are shown below in Figures 15 and 16.

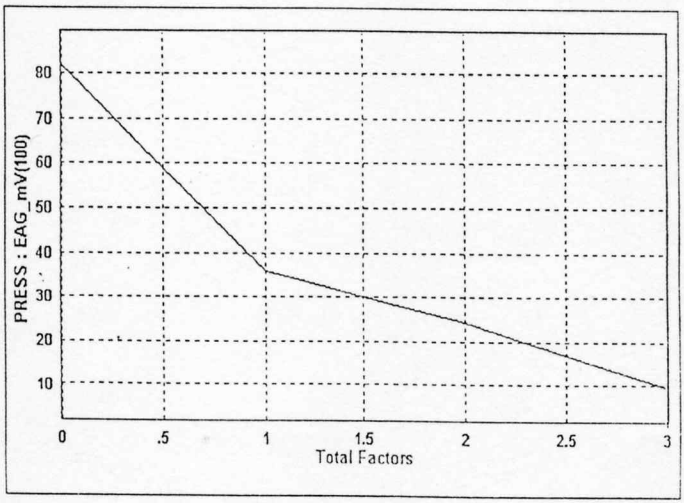

Figure 15. PRESS Plot - (+)-bornyl acetates, 225 - 265 cm^{-1}, six spectrum training set.

The PRESS minimum was once again achieved in three factors, however, the F statistic indicated that the reduction of the PRESS value from the second to the third factor was significant, and consequently a three factor model would be the best. A correlation coefficient of 0.88 was obtained with a three factor model, a marked improvement over the value for the 210-400 cm^{-1} window.

Only a single peak is observed in the 225-265 cm^{-1} region, and that peak is assigned to the deformation of the quaternary carbon at C-1. Factor 1 shows that the region of greatest importance to the calibration is centered at approximately 243 cm^{-1}. As can be seen from the training set spectra, there is an apparent shift and/or broadening of the band into this region as the EAG value decreases.

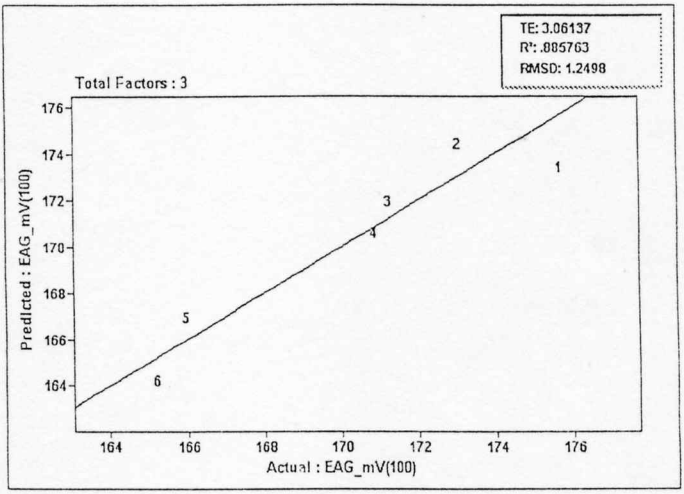

Figure 16. Predicted versus Actual EAG values, 3 factors, 225 - 265 cm^{-1}, six spectrum training set.

DISCUSSION, 601-800 cm^{-1} REGION

The PRESS results, Predicted versus Actual values and factors for the 601-800 cm^{-1} calibration region are shown in Figures 17 and 18.

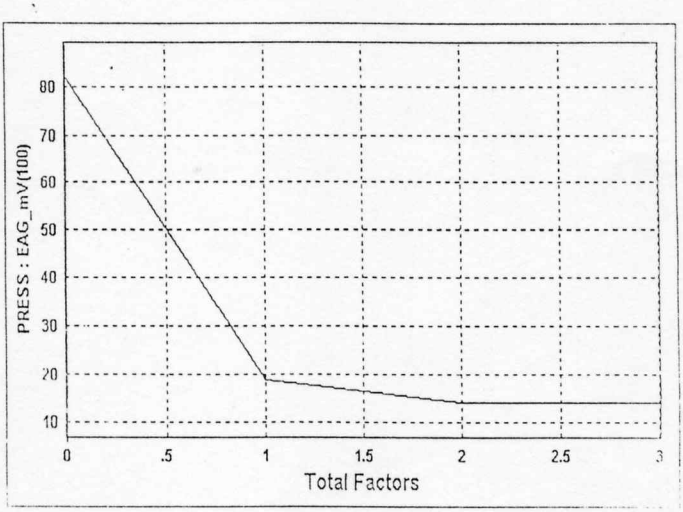

Figure 17. PRESS Plot - (+)-bornyl acetates 601 - 800 cm^{-1}, six spectrum training set.

A minimum in the PRESS was achieved for this calibration region for a three factor model, however the difference between the two factor and the three factor model was highly insignificant as the PRESS value decreased by only 0.13 out of a total of 82.04. The correlation coefficient was once again dramatically improved over the seven spectrum training set, achieving a value of 0.82 for a two factor model. When factors from the two training sets were compared the first two were found to be very nearly identical, indicating that the model had been changed very little by elimination of (+)-bornyl-10d_1-acetate from the training set. Much larger differences were apparent in the third factors, however in both cases the third factor is unimportant and would not be used in order to avoid overfitting.

Upon examination of the factors, two distinct regions of importance are apparent at either end, separated by a region of little importance from approximately 675-700 cm^{-1}. The central region of little importance corresponds to a region where there are no peaks present within any of the spectra.

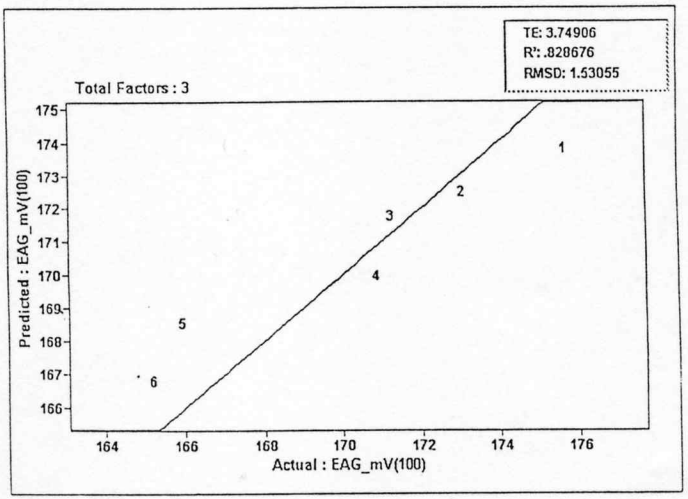

Figure 18. Predicted versus Actual EAG values, 3 factors, 601 - 800 cm^{-1}, six spectrum training set

A number of additional calibrations were performed to see if the correlation could be improved by more careful choice of the spectral region. It was found that the region from 600-675 cm^{-1} yielded the best results. Inclusion of any of the region from 700-800 cm^{-1} was detrimental, as was any extension of the calibration region below 600 cm^{-1}. The diagnostics for the 600-675 cm^{-1} region are shown in Figures 19 and 20.

The PRESS decreased to a smaller value than for the 600-800 cm^{-1} region indicating better predictive ability for this model. The PRESS minimum was achieved in three factors, although the F test indicated that the third factor was insignificant and not necessary. A correlation coefficient of 0.92 was achieved with 2 factors, the best results of any of the calibrations. For the sample with the largest prediction error, (+)-bornyl-9,9,9-d_3-acetate, the predicted and actual values only differed by 0.0019 mv, a 1.2 % error. In addition, factors were essentially unchanged for the

corresponding spectral region of the 600-800 cm^{-1} calibration.

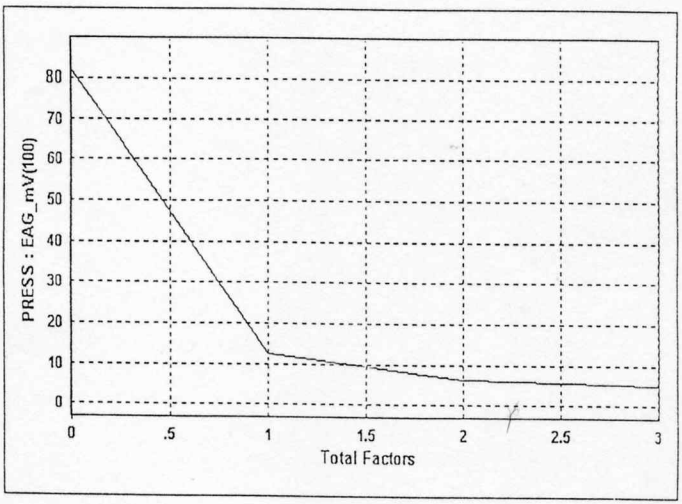

Figure 19. PRESS Plot - (+)-bornyl acetates 600 - 675 cm^{-1}, six spectrum training set.

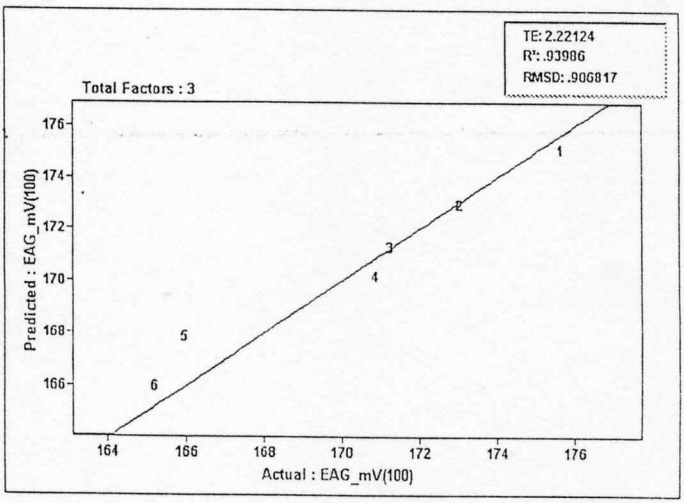

Figure 20. Predicted versus Actual EAG values, 2 factors, 600 - 675 cm^{-1}, six spectrum training set

For the 600-675 cm^{-1} region there are four readily observable IR bands, which have been assigned to the carbonyl out of plane bending, the carbonyl in plane bending, the symmetric stretch of the quaternary carbon

at C-1 and the symmetric stretch of the quaternary carbon at C-7. All of the bands fall within the spectral window with the exception of the carbonyl out of plane bending, which is shifted outside of the region for (+)-bornyl-2d1-acetate-d3 and (+)-bornyl acetate-d3. The factors indicate that the regions of greatest importance are centered about 609 and 638 cm^{-1}. A general trend can also be seen in the training set spectra. EAG values appear to be inversely proportional to the absorbance intensity between ~ 630-650 cm^{-1}.

It would have been desirable to extensively test the predictive ability of the PLS model with a large number of deuterated (+)-bornyl acetates having known EAG values within the range of the EAG values of the training set samples. Obviously, this was not practical with the limited number of compounds available. However it was practical to predict (+)-bornyl-10-d_1-acetate and (-)-bornyl-8,8,8-d_3-acetate since they were not included in the training sets.

(+)-Bornyl-10-d_1-acetate was excluded from the training set because the history of the spectrum was different and it was subsequently considered suspect. As expected, the predicted value for (+)-bornyl-10-d_1-acetate was quite different from the known value. Nevertheless, the predicted values for the two models were very similar: 1.696 mv for the 225-265 cm^{-1} calibration region, and 1.695 mv for the 600-675 cm^{-1} calibration region.

Since the IR spectra are identical for (+) and (-) isomers, (-)-bornyl-8,8,8-d_3-acetate was predicted to see if the EAG value for (+)-bornyl-8,8,8-d_3-acetate could be estimated even though the actual value was unknown. The results for this compound were in less agreement for the two models. An EAG value of 1.717 mv was obtained for the 225-265 cm^{-1} region, while 1.665 mv was obtained for the 600-675 cm^{-1} region.

As an additional test of the validity of the apparent correlations, the same two best regions in the training set spectra were analyzed by another factor-based method known as Principle Component Regression, (PCR). PCR is similar to PLS except the factors describe only the maximum variance between samples, and are not weighted to the component concentrations (23). In both cases, the results obtained were very similar to those obtained by PLS.

CONCLUSION

While the visual inspection, frequency histograms and PLS modeling each yielded different information regarding possible spectral regions of importance in support of Wright's theory, some of the information was common to all three examinations of the data. Nearly all of the possible important regions were predominated by bands related to the acetate functionality of the compounds.

For the visual inspection, the frequencies for the carbonyl in plane bending and the carbonyl out of plane bending modes followed a general trend where their frequencies were inversely proportional to the magnitude of the EAG response. The histograms revealed a region containing no peaks adjacent to the region occupied by these modes. Finally, PLS modeling displayed a high degree of correlation between EAG values and the infrared spectrum for a region which overlapped both the carbonyl in plane and out of plane bending modes, and the "featureless" region observed in the histograms.

The best PLS calibrations were found at the edges of regions where there were no visible peaks present. Either the edges of these "featureless" regions are important, or they are simply regions which are much less

spectrally complex, and therefore can yield good calibrations for a small number of training set spectra. In either case, the 600-675 cm^{-1} had the highest degree of correlation, and was the only one of the regions which corresponded with regions of possible importance indicated by the manual methods.

REFERENCES

1 Theimer, E.T. Ed. "Fragrance Chemistry, The Science of the Sense of Smell," Academic Press, New York, (1982) 635 pp.

2 Wright, R.H., "Odour and Molecular Vibrations. I. Quantum and Thermo dynamic Considerations," J. Applied Chem. 4 (1954) 611-621.

3 Wright, R.H., "Odour and Chemical Constitution," Nature, 172 (1954) 183.

4 Wright, R.H., "Molecular Vibration and Insect Sex Attractants," Na ture, 198 (1963) 455.
Ent., 103 (1971) 627.

5 Wright, R.H., Chambers, D.L., and Keiser, I., "Insect Sex Attractants, Anti-attractants, and Repellents," Can. Ent., 103 (1971) 627.

6. Wright, R.H. and Brand, J.M., Nature, 239 (1972) 225-226.

7. Wright, R.H., "Correlation of Ant Pheromone Activity with Molecular Vibration," Nature, 239 (1971) 225.

8. Wright, R.H., and Stavrakis, G.N., "Molecular Vibration and Insect Attraction: Dacus Oleae (*Diptera Tephritidae*)," Can. Ent., 106 (1974) 333.

9 Wright, R.H., "The Sense of Smell," CRC Press (1982).

10 Wright, R.H., "Molecular Vibration and Odour Blending," Chem Sens., 8 (1) (1983) 103.

11 Amoore, J.E., "Molecular Basis for Odor,", Charles C. Thomas Publisher, (1970).

12 Meloan, C.E., Wang, V.S., Scriven, R., and Kuo, C.K., "Testing Wrights Theory of Olfaction with Deuterated Compounds," 5th International Flavors Conference, Porto Carras, Greece (1987) Elsevier Publ. Co. G. Charalambous Ed.

13 Meloan, C.E., Conry, M., and Jarvis, J., "Computer Assisted Correlation of Infrared Spectra With Insect Behaviour as a Test of Wright's Theory of Olfaction," 6th International Flavors Conference, Rethymnon, Crete, Greece (1989) Elsevier Publ. Co. G. Charalambous Ed. 471-484.

14 Kuo, C.K., "The Effects of Deuterating an Attractant of the American Cockroach, *Periplaneta Americana*, L., as a Test for the Frequency Theory of Olfaction," M.S. Thesis, Kansas State University, Manhattan, KS 66506 (1984)

15 Scriven, R., "Studies of Natural and Synthetic Repellents of the American Cockroach, *Periplaneta Americana*, L.," Ph.D. Thesis, Kansas State University, Manhattan, KS 66506 (1984).

16 Kim, S.B., "An Assignment for Infrared and Raman Spectra of (-)-Isobornyl and (+)-Bornyl Acetates, Their Deuterated Derivatives and Related Compounds (200 to 900 cm^{-1})," Ph.D. Thesis, Kansas State University, Manhattan, KS 66506 (1985).

17 Bowers, W.S. and Bodenstein, W.G., "Sex Pheromone Mimics of the Ameri can Cockroach," Nature, 232 (1971) 259.

18. Nishino, C., Tobin, T.R., and Bowers, W.S., Appl. Ent. Zool. 12(3) (1977) 287.

19 Doolittle, R.E., Beroza, M., Keiser, I., and Schneider, E.L., "Deuteration of the Melon Fly Attractant, Cue Lure, and its Effect on Olfactory Responses and Infrared Absorption," J. Insect Physiol., 14 (1968) 1697.

20 Schneider, D., "Eletrophysiologish Untersuchungen Von Chem-und Mechanorezeptoren der Antenne des Seiden Spinners *Bombex Mori* L., Z. Vergl. Physiol. 40 (1957) 8-41.

21 Havens, B.R., "The Applications of Deuterated Sex Pheromone Mimics of the American Cockroach (*Periplaneta Americana*, L.) to the Study of Wright's Vibrational Theory of Olfaction.

22 Havens, B.R., Meloan, C.E., Freeman, R.D. and Buszek, K.R., "The Synthesis of (+) and (-) Bornyl and Isobornyl-8,8,8-D_3 and 9,9,9-D_3 Acetate and Related Compounds," Accepted: Chemical Synthesis

23 Fuller, M.P., Ritter, G.L., and Draper, C.S., Applied Spec., 42 (1988) 217-236.

24 Beebe, K.R., Kowalski, B.R., Anal. Chem., 59 (1987) 1007A.