Structure-activity relationships for analogues of (Z)-5-decenyl acetate, a sex pheromone component of the turnip moth, Agrotis segetum

Synthesis and conformational analysis

Bengtsson, Marie

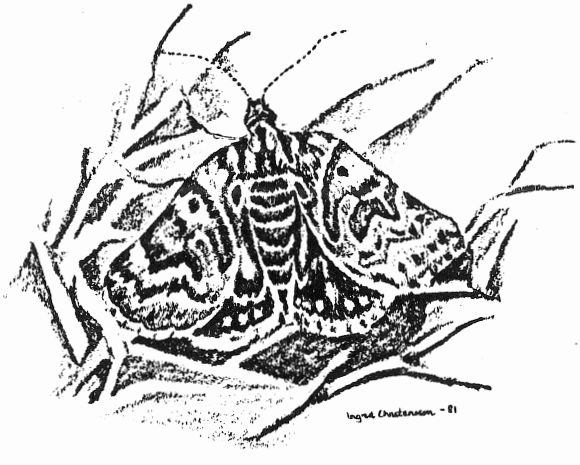
1988

Document Version:
Publisher's PDF, also known as Version of record

STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANALOGUES OF (Z)-5-DECENYL ACETATE, A SEX PHEROMONE COMPONENT OF THE TURNIP MOTH, *Agrotis segetum.*

Synthesis and conformational analysis.

Marie Bengtsson



LUND 1988

STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANALOGUES OF (Z)-5-DECENYL ACETATE, A SEX PHEROMONE COMPONENT OF THE TURNIP MOTH, *Agrotis segetum.*

Synthesis and conformational analysis.

Marie Bengtsson, fil. kand., Sm.

Akademisk avhandling som för avläggande av filosofie doktorsexamen vid matematisk-naturvetenskapliga fakulteten vid universitetet i Lund kommer att offentligen försvaras å hörsal F, Kemicentrum, lördagen den 6 februari 1988, kl 10.15.

Lund 1988

STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANALOGUES OF (Z)-5-DECENYL ACETATE, A SEX PHEROMONE COMPONENT OF THE TURNIP MOTH, *Agrotis segetum*.

Synthesis and conformational analysis.

Marie Bengtsson, fil. kand., Sm.

Lund 1988

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION
	Date of issue February 1988
Chemical Center	CODEN: LUNK DL/NKOK-1007/1-139(1988)
Author(s) Marie Bengtsson	Sponsoring organization

Title and subtitle STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANALOGUES OF (Z)-5-DECENYL ACETATE, A SEX PHEROMONE COMPONENT OF THE TURNIP MOTH, AGROTIS SEGETUM. Synthesis and conformational analysis.

Abstract

Structure-activity relationships for 28 analogues of (Z)-5-decenyl acetate, a sex pheromone component of the turnip moth Agrotis segetum, have been studied.

Synthesis and conformational analysis of chain-elongated, chain-shortened, alkyne, alkenyne, diene and cyclic analogues, and configurational isomers are presented.

A conformational-energy model for the interaction of a pheromone component with its receptor cell has been developed and tested.

The model is shown to correlate measured electrophysiological single-cell activities with conformational energies calculated by the molecular mechanics (MM2/MMP2) method.

A biologically active conformation of the natural pheromone component, (Z)-5-decenyl acetate, is suggested.

Key words Structure-activity relationships, conformational analysis, Molecular mechanics, Agrotis segetum, sex pheromone, receptor interaction, analogues of (Z)-5-decenyl acetate.

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language English

ISSN and key title

ISBN

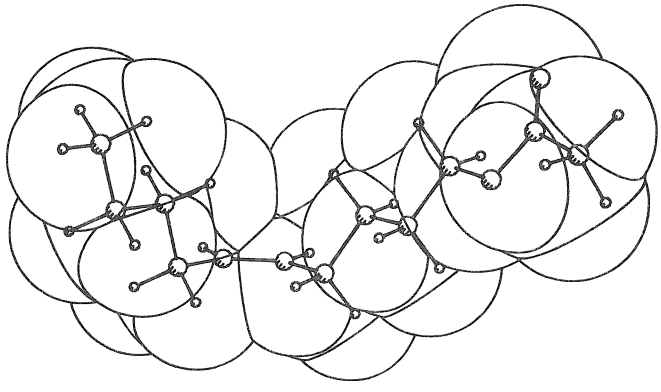
Recipient's notes

Number of pages

Price

Security classification

Distribution by (name and address) Organic Chemistry 3, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden



The wonder is not that it dances well-
but that it dances at all.

Acknowledgements :

I wish to express my deepest gratitude to my supervisor, Dr. **Tommy Liljefors** for his skilled guidance and assistance throughout this project. His never-failing enthusiasm and his constant encouragement have been of the utmost importance for the progress of this work.

I am very grateful for the congenial athmosphere provided by **all of my friends and colleagues** at the Chemical Center, especially **Bernt Thelin**, with whom I shared laboratory space during my first years and **Anders Sundin**; **Jonas Palm** who introduced me to the Macintosh computer.

I am also very happy about the good contacts we have developed with collaborators at the department of Animal Ecology, University of Lund: **Bill S. Hansson**; Dr. **Jan Van der Pers** (present adress; Hilversum, The Netherlands), who made all the electrophysiological measurements on my compounds and kindly allowed me to use some of their drawings; Dr. **Christer Löfstedt**; Dr. **Jan Löfqvist** who contributed with interesting discussions and valuable suggestions. Dr. **Eric Hallberg**, Department of Structural Zoology, is also gratefully acknowledged for supplying the photographs.

I also wish to thank Dr. **Weston Pimloth**, Department of Medicinal and Physical Chemistry, University of Göteborg, for kindly providing me with high resolution mass spectroscopy.

Special thanks to the laboratory staff at the Chemical Center - **Lars Trege, Rolf Servin, Jan Glans, Einar Nilsson, Kerstin Pettersson** and **Ove Anderberg** - for kind technical assistance.

I am very indebted to Dr. **Robert E. Carter** for linguistic criticism and for maintaining the facilities of the Computer Graphics Laboratory for Organic Chemistry.

I am grateful to **Ingrid Christensson-Lindström** who made the cover drawing.

CONTENTS:

CHAPTER 1

INTRODUCTION.

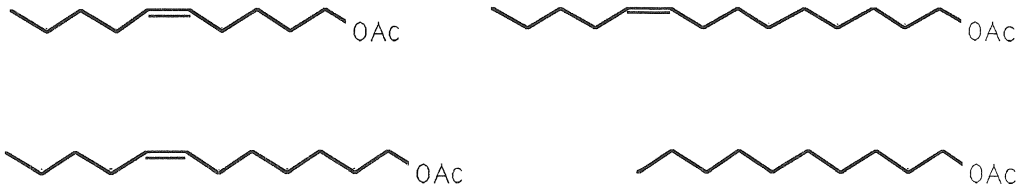
In 1959 Adolf Butenandt et al.[1] identified the chemical structure of the sex-stimulating signal substance released by the female silk moth, *Bombyx mori*. At the same time the word **pheromone**[2], from the Greek roots *pherein* (to carry) and *hormon* (to excite), was introduced and accepted for the concept of a chemical signal between individuals of the same species, releasing or preparing a behavioural response. Examples are sex pheromones, alarm or alerting pheromones and territory-marking pheromones.

In the past thirty years or so, great advances have been made in our understanding of the way in which chemicals control insect behaviour[3-16]. Pheromone blends have been identified for numerous species and extensive behavioural studies have been carried out to elucidate the detailed biological roles of different compounds. These studies have of course been of great scientific interest in themselves, but apart from this, the important possibilities for insect pest control by the use of synthetic pheromones have certainly increased the effort expended by both universities and industries.

Straight chain mono-olefinic acetates with a (Z)-double bond constitute by far the largest class of known pheromone components of noctuid moths[17].

The male moth perceives the pheromone blend produced by the female moth by highly specialized receptor cells in antennal sensilla. Although it has been shown that the receptors are very selective for a certain component in the pheromone blend, it has also been found that analogous compounds **may** substitute for the natural pheromone component to some extent. This observation opens up the possibility to perform structure-activity studies on the problem of how a pheromone component interacts with its receptor cell. Depending on the degree of biological activity of different analogues, important information about the receptor and the biologically active conformation of the native pheromone component may be obtained.

The sex pheromone components of the turnip moth, *Agrotis segetum*, mainly responsible for the biological activity, have been identified as (Z)-5-decenyl acetate, (Z)-7-dodecenyl acetate, (Z)-9-tetradecenyl acetate and decyl acetate[18].



Three different receptor cells sensitive to (Z)-5-decenyl acetate, (Z)-7-dodecenyl acetate or (Z)-9-tetradecenyl acetate respectively, have been identified[18].

In the present thesis, 28 different analogues of (Z)-5-decenyl acetate have been synthezised and investigated (see Figure 1). The analogues belong to 6 different structural classes: chain-elongated, chain-shortened, alkyne, alkenyne, diene and cyclic analogues, and configurational isomers.

In this thesis a quantitative model - **the conformational energy model** - for the interaction between analogues of (Z)-5-decenyl acetate and its receptor is developed (Chapter 4). The model uses conformational energies calculated by the molecular mechanics method (Chapter 2, part 2.2), to rationalize measured biological activities (Chapter 2, part 2.1) of pheromone component analogues. A direct correlation is obtained between calculated conformational energies required to mimic spatial relationships of the natural pheromone component and measured activities when the model is applied to several of the different structural classes of analogues (Chapter 6).

(Z)-5-Decenyl acetate is a very flexible molecule with a large number of low energy conformers, all of which are reasonable candidates for the biologically active conformation. By the study of the conformationally restricted analogues, the biologically active conformation of different parts of the molecule is evaluated (Chapter 6, parts 6.2 and 6.4).

The results of the work presented in this thesis suggest a conformation as a strong candidate for the biologically active conformation of (Z)-5-decenyl acetate (Chapter 6).

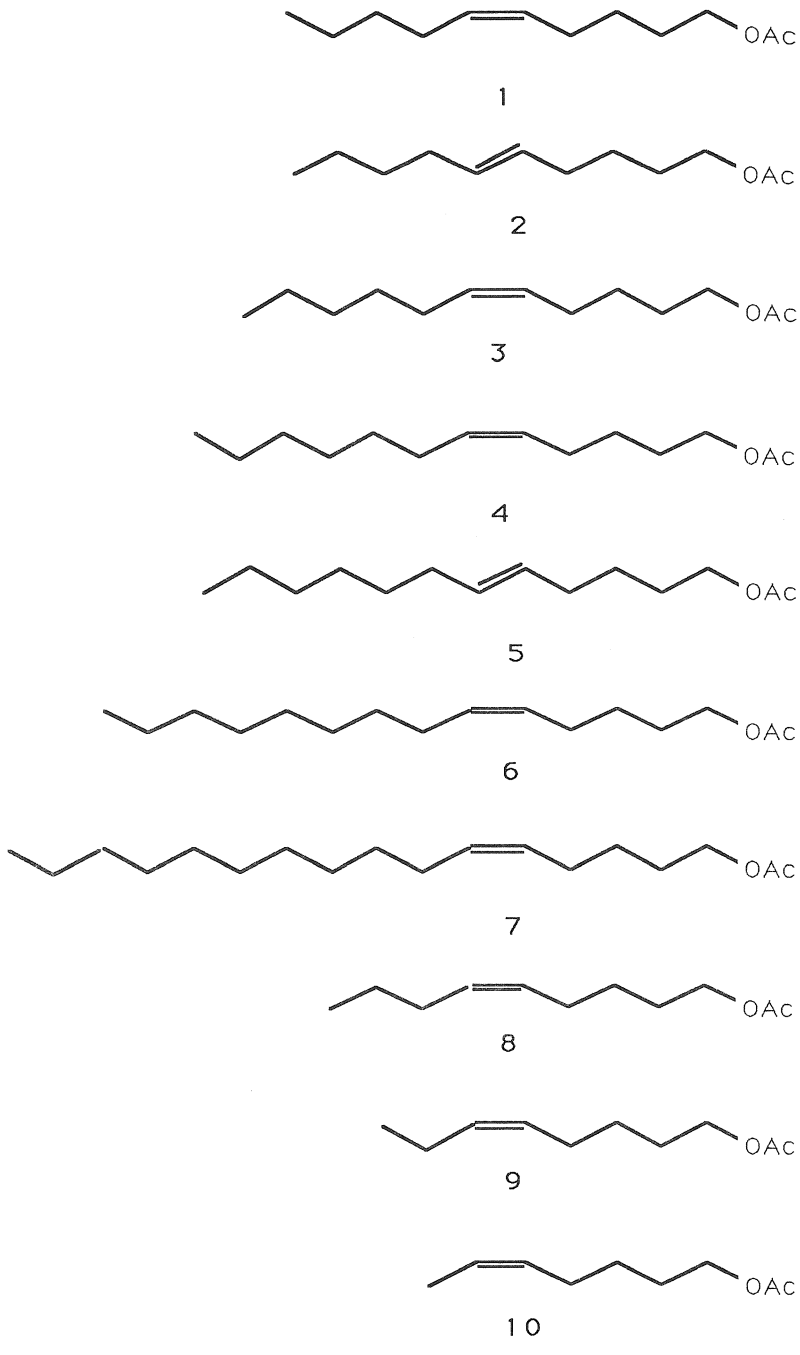


Figure 1. Compounds studied.

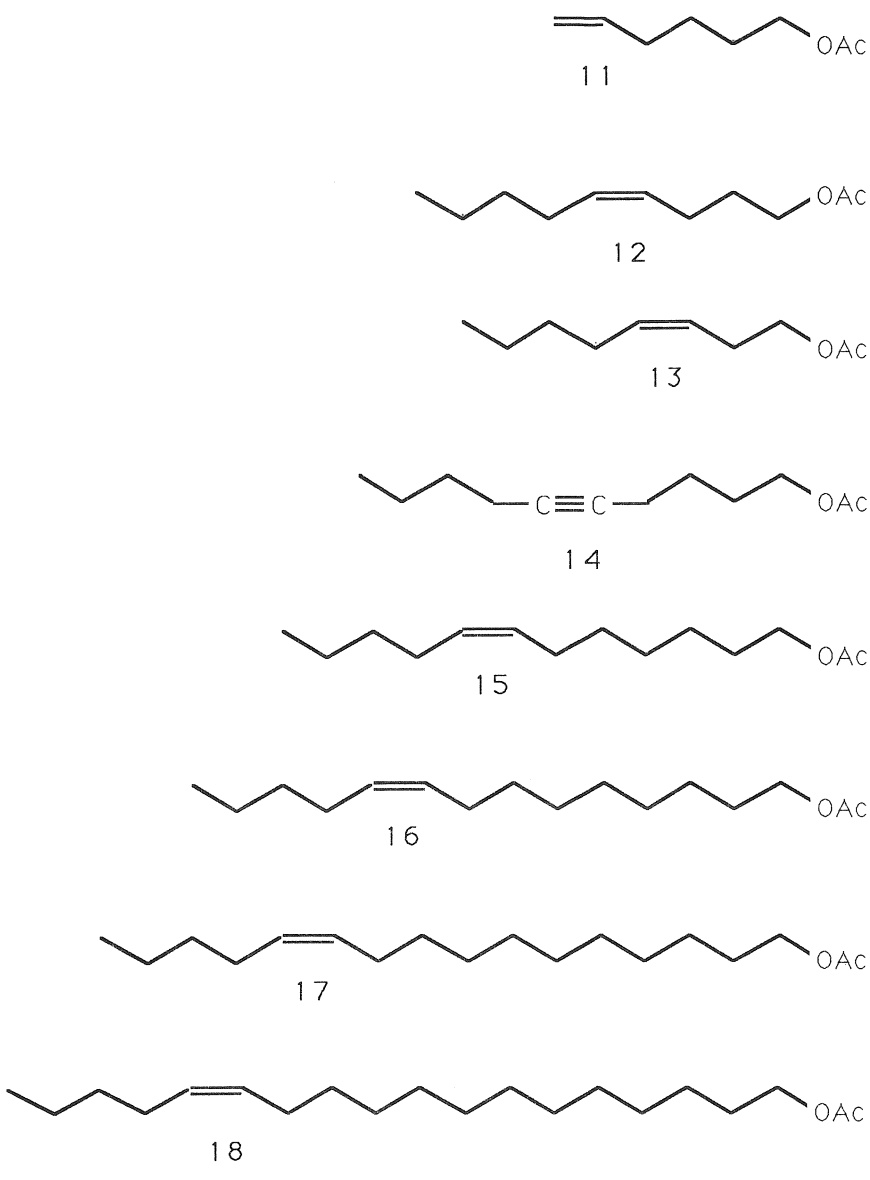
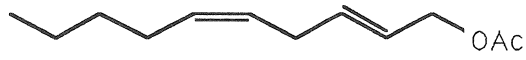
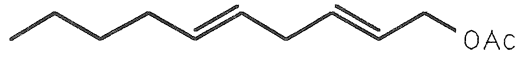


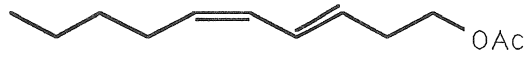
Figure 1. (continued)



19

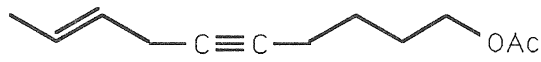


20



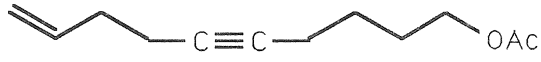
21

22

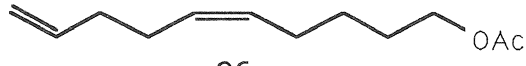


23

24

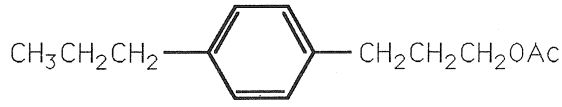


25

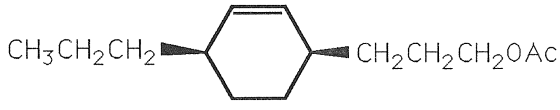


26

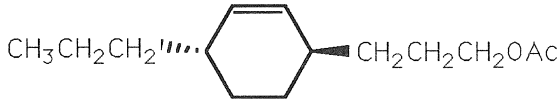
Figure 1. (continued)



27



28



29

Figure 1. (continued)

The following parts of this thesis have been summarized in manuscripts already published or accepted for publication:

Chapter 5.1 (in part)

Bengtsson M. and Liljefors T., "DMPU - an alternative to HMPT in moth sex pheromone synthesis", *Synthesis*, in press.

Chapter 5.2.1, 5.2.2 and 6.2

Bengtsson M., Liljefors T. and Hansson B.S., "Dienic analogues of (Z)-5-decenyl acetate, a pheromone component of the turnip moth, *Agrotis segetum*: Synthesis, conformational analysis and structure-activity relationships", *Bioorg. Chem.*, **15**, 0000 (1987).

Chapter 6.1 and 6.3

Liljefors T., Bengtsson M. and Hansson B.S., "Effects of double-bond configuration on interaction between a moth sex pheromone component and its receptor", *J. Chem. Ecol.*, **13**, 2023 (1987).

REFERENCES

1. Butenandt A., Beckmann R., Stamm D. and Hecker H., *Z. Naturforsch.*, **14 b**, 283 (1959).

2. Karlson P. and Lüscher M., *Nature, Lond.*, **183**, 55 (1959).

3. *"Olfaction and Taste"*, Vol. **VII**, Ed. van der Starre H., IRL Press Ltd, London (1980).

4. *"Recent developments in Chemistry of Natural Carbon Compounds"*, Eds. Bognár R., Bruckner V. and Szántay Cs., Volume **IX**, Akadémiai Kiadó, Budapest (1979).

5. *"Techniques in Pheromone Research"*, Eds. Hummel H.E. and Miller T.A., Springer-Verlag New York Inc. (1984).

6. *"Chemical Ecology : Odour Communication in Animals"*, Ed. Ritter F.J., Elsevier/North-Holland Biomedical Press, Amsterdam (1979).

7. Kullenberg B. and Bergström G., *Endeavour*, **34**, 59 (1975).

8. Wood W.F., *J. Chem. Educ.*, **60**, 531 (1983).

9. Silverstein R.M., *Science*, **213**, 1326 (1981).

10. Bestmann H.J. and Vostrowsky O. in *"Olfaction and Endocrine Regulation"*, Ed. Breipohl W., IRL Press Ltd, London, p. 253 (1982).

11. Baker R. and Evans D.A., *Chem. in Britain*, **16**, 412 (1980).

12. *" Structure-Activity Relationships in Chemoreception"*, Ed. Benz G., IRL Press Ltd, London (1976).

13. a) Schneider D., *Nova acta Leopoldina*, **51**, 249 (1980).
    b) Schneider D., *Science*, **163**, 1031 (1969).

14. Silverstein R.M., *Pure & Appl. Chem.*, **54**, 2479 (1982).

15. Bestmann H.J. and Vostrowsky O., *Naturwissensch.*, **69**, 457 (1982).

16. Jacobson M., *"Insect Sex Pheromones"*, Academic Press, New York (1972).

17. Steck W., Underhill E.W. and Chisholm M.D., *J. Chem. Ecol.*, **6**, 203 (1982).

18. Löfstedt C., Van der Pers J.N.C., Löfqvist J., Lanne B.S., Appelgren M., Bergström G. and Thelin B., *J. Chem.Ecol.*, **8**, 1305 (1982).

CHAPTER 2

METHODS.

2.1 Measurements of biological activity. Male moth antennae possess thousands of long olfactory hairs, *sensilla trichodea*, which contain receptorcells that respond to the female pheromone components[1,2] (Figure 1).

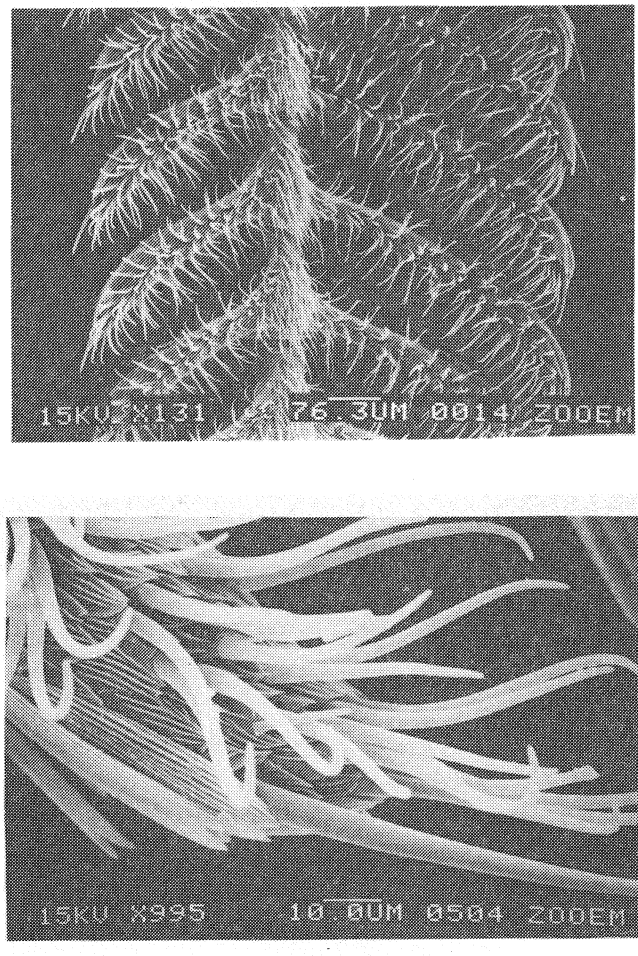


FIGURE 1. Photo: Eric Hallberg.

Each of these hairs contains as a rule at least two olfactory cells, each highly specialized to respond to a particular key molecule[3a] (Figure 2).

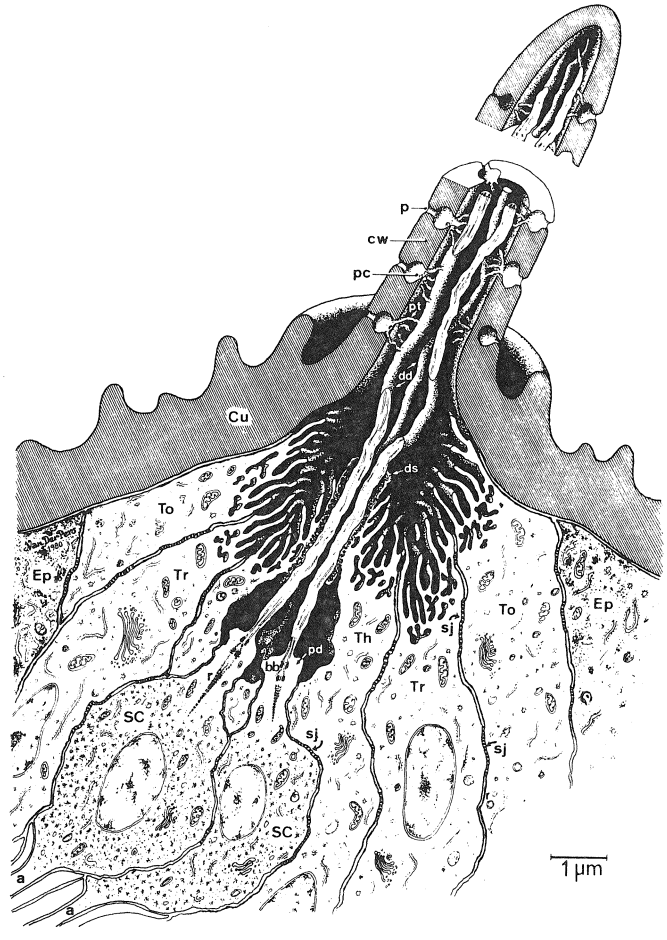


FIGURE 2. Schematic representation of antennal olfactory sensillum trichodeum in Lepidoptera. Drawing made by Jan Van der Pers[3b].

Slow olfactory potentials may be recorded from an isolated antenna positioned between two microelectrodes connected to an amplifier and a recording instrument[4]. An

electroantennogram (**EAG**) is essentially the sum of many olfactory receptor potentials recorded simultaneously[5-9] (see Figure 3). The amplitude of the EAG signal depends on the concentration and chemical structure of the stimulus.

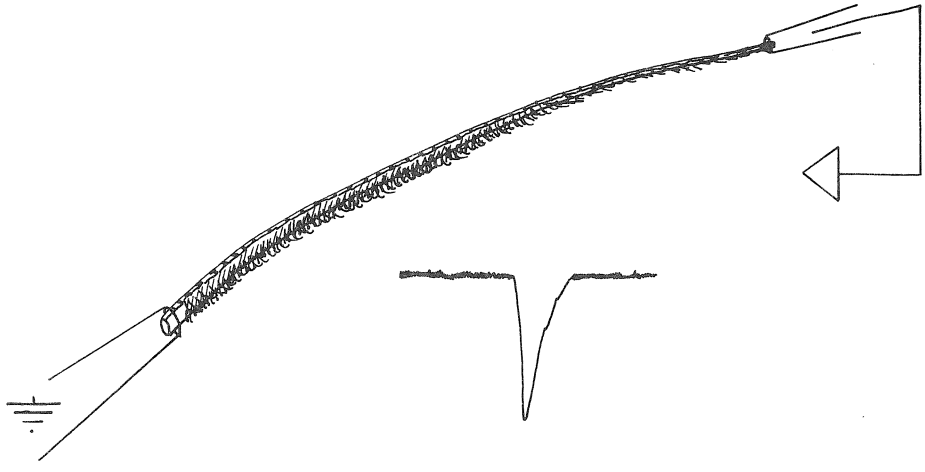


FIGURE 3. EAG recording technique. Schematic representation made by Bill S. Hansson.

The use of **EAG** data for structure-activity studies is limited due to

i) overlap of the responses of more than one receptor-type to the same compound[10]; and
ii) the involvement of responses from other receptors than pheromone receptors[10], for example mechano-receptors.

Recordings of the action potentials generated by the receptor cells associated with **individual** sensilla are called the **single sensillum** technique. This method is better suited for structure-activity investigations and the biological data for the studies performed in this thesis were obtained by this technique.

Microelectrodes were applied to the antennal base and to the tip of one olfactory hair[11] (Figure 4).

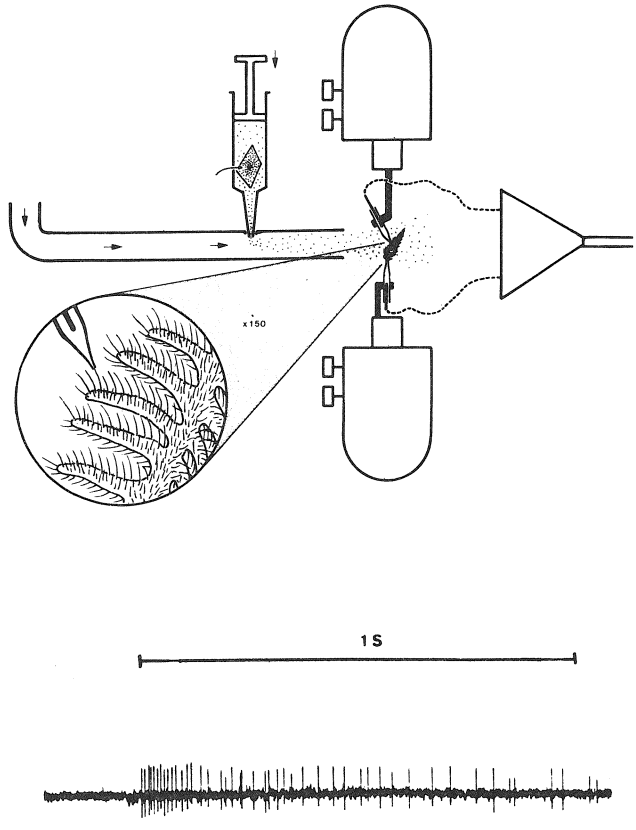


FIGURE 4. Single sensillum recording technique. Schematic representation by Jan Van der Pers.

The stimulus was loaded on a piece of filter paper in amounts usually ranging from 10^{-3} to 10^{2} µg. These filter papers were placed in plastic syringes and two ml of the gaseous contents of each syringe was injected into an airstream flushing the antennae. Ten replicates were recorded with each different stimulus[12].

The single sensillum response was measured as the number of spikes per second, and depends upon the concentration and chemical structure of the stimulus molecule. To evaluate the biological activity of a certain compound, the responses due to stimulation with a number of different concentrations of the compound were recorded. From these data a dose-response curve was constructed (Figure 5).

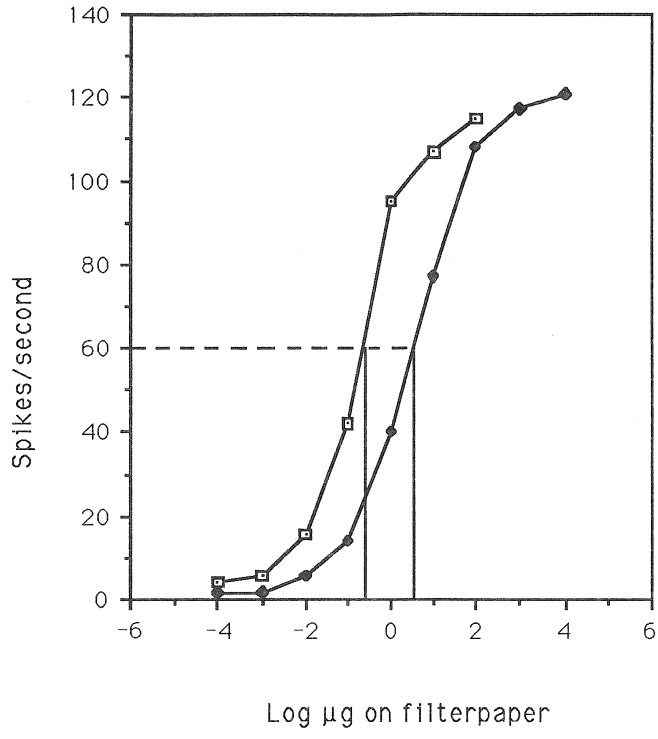


FIGURE 5. Dose-response curves.

When the biological activity of two stimulus are compared, the amounts of the stimulus giving rise to equal number of spikes per second are measured on the parallel parts of their dose-response curves[13] (see Figure 5).

In *Agrotis segetum*, three different cells sensitive to (Z)-5-decenyl acetate, (Z)-7-dodecenyl acetate or (Z)-9-tetradecenyl acetate respectively, have been identified[14-16]. Their relative abundances were estimated to be 68%, 28 % and 2 % [14]. (The remaining 2 % of the sensilla were of the "silent type", giving no response). The sensillum sensitive to (Z)-5-decenyl acetate also responded to (Z)-5-decenol and (Z)-8-dodecenyl acetate, but with different amplitudes of the signal.

The relative electrophysiological activities, compared to (Z)-5-decenyl acetate **1** for all the compounds investigated in this thesis, were expressed as the reciprocal of the relative quantities of the different amounts of substances required to elicit the same response from the receptor cell.

All the electrophysiological measurements on the compounds discussed in this thesis were done by HE **B.S. Hansson** and Dr. **J.N.C. Van der Pers** (chain-elongated analogues), Department of Animal Ecology, University of Lund.

The dose-response curves from the single-sensillum measurements reflect the amounts of compounds loaded on the filter papers versus the number of spikes generated. To make it possible to compare the biological activities between compounds studied, it is necessary to make corrections, so the measured values reflect the amounts of the compounds actually reaching the antennae. This may be achieved by the use of the vapour pressures of the compounds. It has been shown[17] that for a certain amount of substance loaded on the filter-paper, the relative amounts in the vapour phase may be calculated from the relative vapour pressures.

This has been done for the analogues studied in this thesis, either by using experimentally determined vapour pressures for the actual pheromone analogues[18] or by estimating the vapour pressures from tabulated data for related molecules[19,20].

Another possibility to estimate vapour pressures is the use of a recently published method[21], which was shown to give a correlation of retention times on a liquid crystal capillary column with reported vapour pressures for (Z)-5-decenyl acetate and a number of related pheromone compounds.

2.2 The molecular mechanics method. Molecular mechanics (MM)[22-25] is currently the method of choice for calculations of molecular geometries and conformational energies of medium-sized and large molecules. The MM method is empirical and is based on a large volume of experimental data such as bond lengths, bond angles, conformational energies, energy barriers and heats of formation. These data must exist for a given class of compounds before the method can be applied to any compound in that class. In the MM method, the molecule is considered as a collection of atoms held together by elastic or harmonic forces. Bond lengths and angles tend to have certain natural or ideal values. A deformation of a molecular structure will result in an energy change which is possible to calculate if the necessary force-laws and constants involved are known. These force-laws and constants constitute the force field used in the MM calculations. The total energy **E** (equation 2.1) of a molecule, sometimes called the steric energy, is approximated as a sum of the stretching energy ($E_{stretch}$), the bending energy (E_{bend}), the sum of the energy due to the van der Waals interactions (E_{VdW}), the torsional energy (E_{tors}), the electrostatic energy (E_{el}) and cross terms.

$$\mathbf{E} = E_{stretch} + E_{bend} + E_{vdW} + E_{tors} + E_{el} + \text{cross terms} \qquad [2.1]$$

In the force field, the stretching energy ($E_{stretch}$) and the bending energy (E_{bend}) for a molecule may be defined as in equations [2.2] and [2.3].

$$E_{stretch} = \sum_{bonds} \frac{k_s}{2} (l - l_0)^2 \qquad [2.2]$$

$$E_{bend} = \sum_{angles} \frac{k_\theta}{2} (\theta - \theta_0)^2 \qquad [2.3]$$

l_0 , θ_0 = reference bond length and bond angle.

k_s , k_θ = force constants.

These equations are valid only for small deformations. When larger changes are made, it is necessary to add cubic, quartic, etc. terms[25].

A function used to calculate the van der Waals interaction between two atoms is shown in equation [2.4].

$$E_{vdw} = \varepsilon \left[-c_1 \left(\frac{r^*}{r} \right)^6 + c_2 \exp\left(-c_3 \frac{r}{r^*} \right) \right] \qquad [2.4]$$

ε is an energy parameter, r is the interatomic distance and r^* is generally the sum of the van der Waals radii of the two interacting atoms; c_1-c_3 are constants.
Finally, the torsional energy term, (E_{tors}), is generally written as a Fourier series as shown in equation [2.5].

$$E_{tors} = \sum_{angles} \left[\frac{V_1}{2} (1 + \cos\omega) + \frac{V_2}{2} (1 - \cos 2\omega) + \frac{V_3}{2} (1 + \cos 3\omega) \right] \qquad [2.5]$$

ω is the dihedral angle and V_i are energy parameters.

After the initial steric energy of the molecule has been calculated, the geometry is optimized, i.e. the total energy **E** is minimized. Many different procedures have been developed to perform this operation[22,25].

The energy-minimized geometries and conformational energies presented in this thesis were calculated using the molecular mechanics programs MM2 and MMP2 developed by Allinger et al.[24,25]. Starting structures for the energy-minimization program were constructed using the molecular modeling system MIMIC[26,27]. This system was also used for the superimposition studies

REFERENCES

1. Altner H., in "*Olfaction and Taste*", Vol. **VI**, Eds. Le Magnen J., MacLeod P., IRL Press Ltd, London, p. 351 (1971).

2. Hansson B.S., Löfstedt C., Löfqvist J. and Hallberg E., *Naturwissensch.*, **73**, 269 (1986).

3. a) Schneider D. and Steinbrecht R.A., *Symp. Zool. Soc.*, London, **23**, 279 (1968).
   b) Van der Pers J.N.C., "*Olfactory receptors in small ermine moths (Lepidoptera:Yponomeutidae): Electrophysiology and morphology*", Ph. D. Thesis (1980), University of Groningen, The Netherlands.

4. Schneider D., *Z. Vergl. Physiol.*, **40**, 8 (1957).

5. Schneider D. and Hecker E., *Z. für Naturforsch.*, **11**, 121 (1956).

6. Schneider D., *Experientia*, **13**, 89 (1957).

7. Schneider D. in *"Olfaction and taste"*, ed. Zotterman I., Pergamon Press, Oxford (1963).

8. Van der Pers J.N.C., *Exp. Appl.*, **30**, 181 (1981).

9. Roelofs W.L. in *"Techniques in Pheromone research"*, eds. Hummel H.E., Miller T.A., Springer-Verlag New York Inc., p. 131 (1984).

10. Priesner E. in *"Chemical Ecology: Odour Communication in Animals"*, Ed. Ritter F.J, Elsevier/North-Holland Biomedical Press, Amsterdam, p. 57 (1979).

11. Van der Pers J.N.C. and Den Otter C.J., *J. Insect Physiol.*, **24**, 337 (1978).

12. Hansson B.S., Van der Pers J.N.C and Löfstedt C., submitted to *Physiol. Entomol.*

13. Kafka W.A. in *"Chemical Ecology: Odour Communication in Animals"*, Ed. Ritter F.J, Elsevier/North-Holland Biomedical Press, Amsterdam, p. 41 (1979).

14. Löfstedt C., Van der Pers J.N.C., Löfqvist J., Lanne B.S., Appelgren M., Bergström G. and Thelin B., *J. Chem. Ecol.*, **8**, 1305 (1982).

15. Hallberg E., *Cell Tissue Res.*, **218**, 209 (1981).

16. Van der Pers J.N.C. and Löfstedt C., *Physiol. Entomol.*, **8**, 203 (1983).

17. Löfstedt C., Copaja S.V., unpublished results.

18. Olsson A.-M., Jönsson J.-Å., Thelin B. and Liljefors T., *J. Chem. Ecol.*, **9**, 375 (1983).

19. Dykyj J. and Repas M. *"Tlak nasy´tenej pary organicky´ch zlu´cenin"*, Red. Kossaczky´ E., Veda, Vydavatelstvo Slovenskej Akadémie Vied, Bratislava (1979).
20. *Handbook of Chemistry and Physics*, 58th Edition, Ed. Weast R.C., CRC Press, Inc., West Palm Beach (1978).
21. Heath R.R. and Tumlinson J.H., *J. Chem. Ecol.*, **12**, 2081 (1986).
22. Allinger N.L., *Adv. Phys. Org. Chem.*, **13**, 1 (1976).
23. Boyd D.B. and Lipkowitz K.B., *J. Chem. Educ.*, **59**, 269 (1982).
24. Allinger N.L. and Yuh Y.H., *Quantum Chemistry Program Exchange*, **11**, 395 (1980).
25. Burkert U. and Allinger N.L., *"Molecular Mechanics"*, American Chemical Society, Washington D.C. (1982).
26. Liljefors T., *J. Mol. Graphics*, **1**, 111 (1983).
27. Von der Lieth C.W., Carter R.E., Dolata D.P. and Liljefors T., *J. Mol. Graph.*, **2**, 117 (1984).

CHAPTER 3

RECEPTOR-INTERACTION MODELS FOR MOTH SEX PHEROMONE COMPONENTS.

At the time the investigations presented in this thesis were started, four different models attempting to describe the interaction between a pheromone component and its receptor had been presented. In the following, these models are briefly described, followed by critical comments on them. A presentation of an alternative model follows in Chapter 4.

3.1 A qualitative model by Roelofs and Comeau. During extensive field studies on substances related to (Z)-11-tetradecenyl acetate, a sex pheromone component of the red-banded leaf roller, *Argyrotaenia velutinana*, Roelofs and Comeau[1] found that many of the compounds affected the response of the male moths. When certain analogues were mixed with the pheromone itself and subjected to field tests, two different phenomena of modulation of the attractancy of the male moths were observed. Some substances provided a synergistic effect that increased the number of males trapped, others an inhibitory effect with a decrease in trap catches. None of the synergistic chemicals was active as attractants when used alone.

In order to elucidate the molecular features responsible for the observed changes in attractancy and to correlate them with possible receptor-site interactions, a qualitative model of sex pheromone perception was developed by Roelofs and Comeau. The model is based on physical-organic mechanisms similar to those used to describe the actions of enzymes. Three prominent and electron-rich parts of the pheromone molecule were assumed to be essential for the receptor interaction:

i) the acetate group,
ii) the double bond and
iii) the terminal methyl group.

The pheromone molecule was assumed to interact via electrostatic and hydrophobic forces with a set of sites in the receptor cavity, as shown in Figure 1. On interaction, a conformational change of the receptor was assumed to occur. This change was assumed to trigger the nerve impulse.

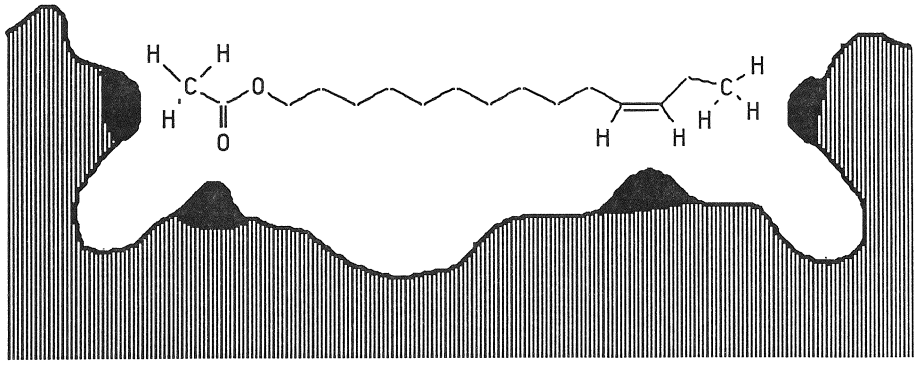


FIGURE 1

The attractancies of forty-six analogues of (Z)-11-tetradecenyl acetate were estimated mainly by field tests, but also by laboratory bioassays. The structures of the analogues were manually fitted to the receptor model in a way that optimally mimicked the assumed interactions of the natural pheromone component. This was achieved by matching electron-rich functional groups or parts of the molecule with the assumed receptor sites. In the study, the pheromone component as well as the analogues were kept in a planar, all-anti conformation, as shown in Figure 1. The authors consider their model capable of correlating the biological activity of various molecules with the possibilities for interactions with postulated receptor-sites.

3.2 The Kafka-Neuwirth model. The Kafka-Neuwirth model is based on an extensive study on relationships between molecular structure and receptor responses in noctuid moths, made by Priesner et al.[2]. The electroantennographic (EAG, see Chapter 2, part 2.1) activities were analyzed for sixteen species, subjected to one hundred pheromone component analogues. At that time, sex pheromone components for only ten species of the family *Noctuidae* had been identified[3-13]. The main components reported for eight of the species were mono- or dienic acetates[3,4,8-13] and for the other two an aldehyde[5-7]. The analogues investigated by Preisner et al. were derived from these structures. One group consisted of aliphatic acetates, varying in chain length or in position, configuration or number of the double bond(s), the other group of (Z)-9-mono-unsaturated compounds with different functional groups. For each of the

species, one of the test compounds was considerably more active than the others. The activities of the other analogues were related to this most potent compound. It was found that they required between 1.8 to 10 000 times higher amounts of stimulus to elicit the same EAG-amplitude as the reference compound. Summarizing their results, the authors[2] suggest general "structure-activity rules" for the interaction between a stimulus molecule and its receptor.

Rule 1: The influence on olfactory activity of any modification in chain length is more pronounced on altering the **n**-chain compared to a corresponding change in the **m**-chain.

Rule 2: For a given species, the EAG activities for analogues with different polar end-groups (esters, aldehydes, alcohols), are in a constant proportion, almost unaffected by variation in chain length.

Based on the EAG data obtained by Priesner et al. (see above), Kafka and Neuwirth[14-15] published a model which in a quantitative way aimed at describing the first step of the olfactory process - the formation of a complex between the odor molecule and a hypothetical receptor. This was the first quantitative approach to the problem, an attempt to directly correlate the molecular characteristics of the pheromone molecule with its receptor binding probability.
The authors define, as in the previous model (part 3.1), three positions of pronounced electron density in moth pheromone components which are necessary for the biological activity. These positions are denoted **PM1**, **PM2** and **PM3** in Figure 2. The pheromone component molecule is assumed to interact with the complementary sites **RS1**, **RS2** and **RS3** in the receptor cavity.

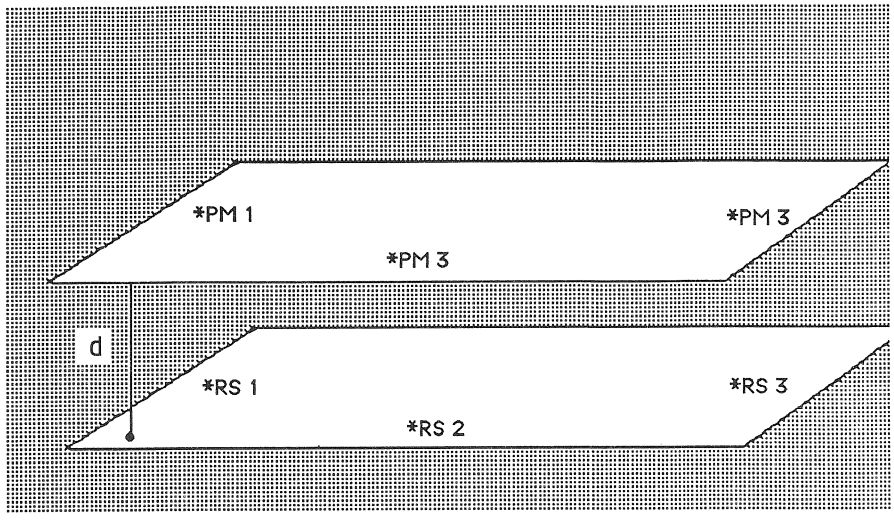


FIGURE 2

The probability for a certain molecule, **s**, to interact with the receptor, in relation to a reference molecule, **m**, was assumed to depend on the relative binding energies ($E_{tot,max}$) between the molecules and the receptor. The relative amounts of compounds **s** and **m** needed to evoke a standard EAG-response was then calculated according to equation [3.1].

$$\frac{C_s}{C_m} = \frac{\exp\left({}^{m}E_{tot,\,max} - {}^{s}E_{tot,\,max} \right)}{kT} \qquad [3.1]$$

C_s	= stimulus quantity of compound **s**, needed to evoke a standard response.
C_m	= stimulus quantity of compound **m** (reference).
$E_{tot,max}$	= The total maximum binding energy between the odor molecule and the receptor.
k	= The Boltzmann constant.
T	= Temperature in K.

The maximum binding energy, $E_{tot,max}$, equation [3.2], is assumed to be a function of three parameters:

i) the distance, d, see Figure 2, between the crucial positions in the stimulus molecule and their complementary receptor sites,
ii) the electron polarisabilities and
iii) the dipole moments of **PM1, PM2, PM3, RS1, RS2** and **RS3**.

$$E_{tot,max} = \sum ({}^{a}E + {}^{p}E) \qquad [3.2]$$

${}^{a}E$ = energy of interaction due to electron polarisation between molecular and receptor site points.

${}^{p}E$ = energy of dipole interaction between molecular and receptor site points.

To evaluate the distances and the angle between the sites **RS1**, **RS2** and **RS3** in the receptor (see Figure 2) the most active stimulus in a planar all-anti conformation was used as a "pattern". The spatial relationships between **RS1**, **RS2** and **RS3** were assumed to be the same as for **PM1**, **PM2** and **PM3**. The polarisabilities and the dipole moments of the hypothetical receptor sites were evaluated using a series of test compounds. By fitting their measured electrophysiological (EAG) activities to binding energies expressed in terms of the physico-chemical parameters to be determined, equations [3.1] and [3.2], these could be evaluated. The electrostatic interactions with all the three pronounced sites in the stimulus molecule, as well as the van der Waals interactions with the carbon chain, were taken into account when the properties of each of the sites **RS1**, **RS2** or **RS3** in the receptor were evaluated.

However, the model presented above (Figure 2) could not account for activities obtained for analogues elongated in the **n**-chain. It was found necessary to include a spatial restriction on the receptor model in the form of a perpendicular wall on one side[15] (see Figure 3). Furthermore, one of the assumed receptor sites, **RS1**, had to be moved to a position on this side wall, away from the receptor plane. These refinements of the model made it possible to determine a set of values of electron polarisabilities and dipole moments for the receptor points which could very well account for the EAG activities of all the investigated test compounds. With the values thus determined,

Kafka and Neuwirth suggest that it is possible to predict the activities of other molecules on the same receptor. As an example, the method was used for a series of monounsaturated acetates differing in chain length and position of the (Z)-double bond. The electrophysiologically determined activities of these compounds were consistent with calculated binding probabilities.

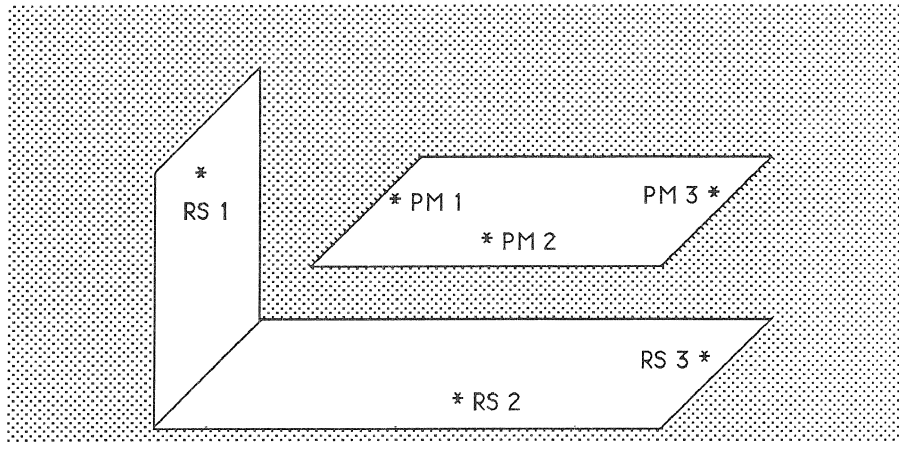


FIGURE 3

3.3 A "zipper"- model approach. Another approach to the problem of relationships between structure and activity for moth sex pheromone components was made by Bestmann and Vostrowsky[16]. Their model is similar to the "zipper" model[17], which has been suggested for the interaction of flexible molecules, such as enzyme substrates with macromolecules. The structures of numerous naturally occurring pheromone components were synthetically varied and their EAG activities measured[2]. From the results, a set of structure-activity rules was formulated, similar to those suggested by Preisner et al.[2] (see part 3.2). The authors conclude that the activity of a pheromone analogue is more sensitive to changes in the **n**-chain than in the **m**-chain. The **n**-alkyl part of the pheromone component molecule was subjected to further investigations by the introduction of branches of different chain-lengths in the alpha, beta, or gamma positions relative to the double bond[16]. The introduction of a methyl

group in the alpha position lowered the EAG activity of the analogue by a factor of 10 to 30 compared to the pheromone molecule itself. However, substituting the alpha-methyl group with an ethyl or propyl group did not cause any substantial effects on the EAG response. For chain-elongated pheromone analogues, the opposite effect was noticed in some cases. An activating effect was observed on introducing an alpha-branch. The resulting increase of efficacy was a factor of 3 compared to the unbranched analogue. Based on these structure-activity investigations, Bestmann and Vostrowsky suggest that the interaction between the pheromone molecule and its receptor is achieved by a flexible insertion of the pheromone molecule into the whole receptor region (see Figure 4).

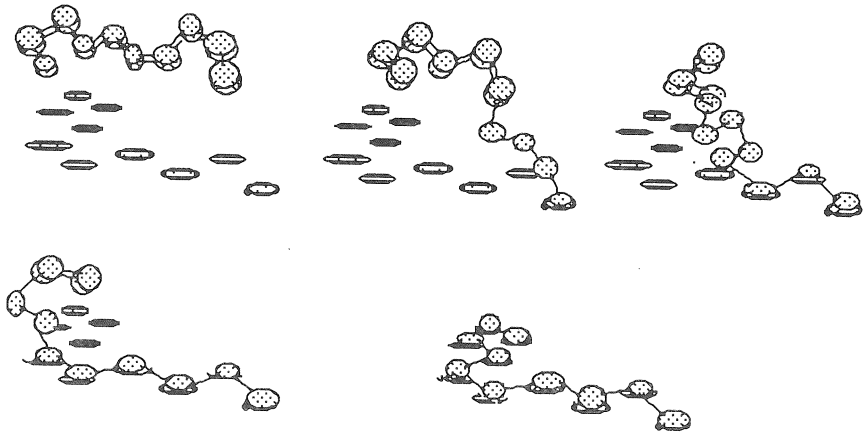


FIGURE 4. Drawing made by Stig Jönsson.

The study also includes EAG measurements on both enantiomers of some of the chiral, branched pheromone analogues. The test was performed with two moth species which both use the achiral molecule (Z)-11-tetradecenyl acetate as their main

pheromone component. Both species were able to differentiate between the (R)- and (S)-forms of the different analogues in all the cases investigated. The activity value for the corresponding racemate was always in between the enantiomer values. According to the authors, this must indicate that the receptor cavity contains some elements of chirality and that a prochiral pheromone molecule can only properly be adjusted from one of the two enantiotopic sides. The results throughout the investigation were only discussed in a qualitative way. No attempts were made to extend the model to quantitatively rationalize the results.

3.4 The bifunctional unit model. In 1975 Kikuchi[18] suggested that the activity of a pheromone component or an analogue may be due to the probability of existence of a range of distances between a specific pair of functional groups (bifunctional units) in the molecules. This suggestion was based on a study performed on bombykol, (E)-10,(Z)-12-hexadecadien-1-ol, a sex pheromone component of *Bombyx mori*, and eleven related derivatives. Two functional groups and the π-bond(s) in these molecules were defined as functional regions which were assumed to be of importance for the biological activity. These three regions were combined into three possible types of bifunctional systems (see Figure 5):

1. A proton acceptor, **A** (double- or triple bond) - proton donor, **D** (OH-group) system.
2. A proton acceptor - methyl group, **Me**, system.
3. A proton donor - methyl group system.

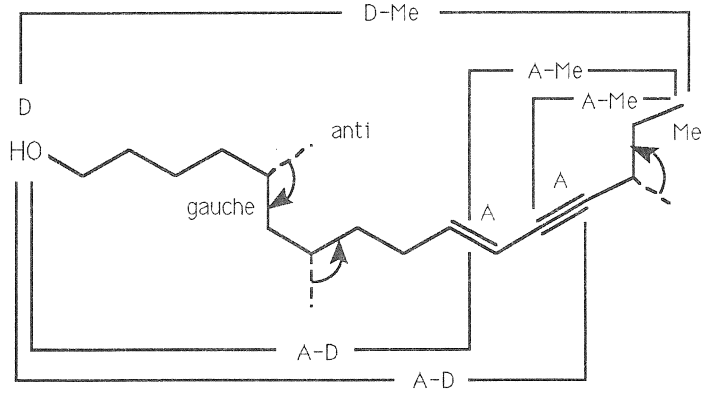


FIGURE 5

With the help of mechanical models(!), the geometries of 100 conformers for each of the compounds under study were generated. The distances between the functional groups in the different bifunctional systems were measured for all these conformers, and a frequency distribution of distances was calculated. The biological activities, defined as the minimal amount of odor sources required to evoke nerve impulses in single cell recordings, were available for four moth species. Bifunctional units were in each case determined by fitting the probability of the occurrence of such a unit to the measured activity.

The author concludes that it is possible to rationalize the biological activities in the four moth species by the presence of one or two bifunctional units in the stimulus molecule. Some of them were assumed to play a favorable role in eliciting the activity, while others showed the opposite action. Thus, in some cases it was necessary to postulate two types of receptor-cells for one pheromone component.

3.5 Comments on the receptor models. The different approaches to the problem of structure-activity relationships described above, and their deficiencies, constitute the background to the substrate-receptor interaction model developed and tested in the present thesis. Even if the previous models and structure-activity rules in some cases could rationalize the activities or attractancies obtained for the compounds investigated , all of them have drawbacks and/or defiencies.

1) The model by Roelofs and Comeau (part 3.1). Data on structure-activity relationships can be obtained by studying behavioral responses in the field and in the laboratory or by electrophysiological methods. Since the probability of interference by unknown factors is high in the first method, which is the one used by Roelofs and Comeau, it is less reliable in this context. Furthermore, in field studies it may only be possible to estimate the attractancies for rather potent compounds. Under a certain threshold value, the attractancies probably will be very low for all compounds. At the time the investigation by Roelofs and Comeau was performed, the entire pheromone blend of the red-banded leaf-roller was not identified. Some of the compounds used as analogues have later been shown to be pheromone components[19]. This makes the conclusions drawn by the authors on inhibitory and synergistic effects invalid.

The model has not been used for quantitative correlations and has not been further developed.

2) The model by Kafka and Neuwirth (part 3.2). This model is based on EAG data obtained by Priesner et al[2]. Since an EAG response in general reflects the combined activity of several different types of olfactory cells on the antennae, this method introduces a considerable amount of uncertainty into the biological data. The single-cell recording technique (see Chapter 2, part 2.1) offers the most direct method in studies of structure-activity relationships and is the method used for all the measurements in this thesis.

In the Kafka/Neuwirth model, the interacting molecules are considered to be planar. The authors claim that this is the thermodynamically most stable conformation for these compounds. As will be shown later in this thesis, this is not true. The planar conformation is not even an energy minimum!

The strength of the interactions between receptor sites and the stimulus molecule are determined by moving the planar stimulus molecule over the receptor surface until a position of the molecule is found which gives a maximum binding energy. The possibility for an analogue to rearrange conformationally and thereby interact more favourably with the receptor is not taken into account by the model. As a result of this, the model is only applicable to analogues with a (Z)-double bond, no estimations about activities for (E)-analogues can be done.

To account for the activities obtained for analogues elongated in the **n**-chain, it was necessary to incorporate a spatial restriction in the form of a wall into the model. A much greater flexibility is allowed for the double bond and the acetate group. However, the position and properties of the acetate group have been shown to be exceedingly important for the biological activity[20]. This must inevitably result in similar problems for the model to account for analogues elongated in the **m**-chain, as was found to be the case for the **n**-chain.

3) The "zipper"- model due to Bestmann (part, 3.3). This model, like the one formulated by Roelofs and Comeau, is conceptually qualitative. However, the flexibility of the pheromone component molecules under study is taken into account, but the model has not been used to directly rationalize biological data.

4) The bifunctional unit model by Kikuchi (part 3.4). This model is an interesting approach and the only one among those presented above which in a quantitative way tries to account for molecular flexibility. Conformational analysis is performed on the pheromone components and analogues under study, but unfortunately the analysis is very rudimentary. This results in an invalid population analysis. Concepts obtained from conformational analysis of simple acyclic hydrocarbons such as n-butane and n-pentane are used for the analysis of the eleven mono- or

diunsaturated alcohols. The alcohols are divided into "three-bond systems" such as C-C-C-C, H-O-C-C, O-C-C-C or C=C-C-C which are treated as butane systems with respect to rotations around carbon-carbon single bonds. As a result, all "three-bond systems" are considered to be anti and planar in their most stable conformation, even if they contain carbon-carbon double bonds or oxygen atoms. This is definitely not true for C=C-C-C where the planar anti conformation is not even an energy minimum!

The correlations obtained between the results from the conformational analysis and statistical treatment and the single-cell measurements are not possible to interpret in terms of a specific molecular conformation necessary for biological activity. For some of the species investigated, Kikuchi found it to be necessary to suggest two types of receptor-cells working against each other to account for the results. There are no experimental data indicating that this should be the case.

Furthermore, common for all the models discussed above is that they do not take into account the differences in volatility between the pheromone components and analogues under study. Determination of the vapour pressures of moth sex pheromone components[21] show variations according to chain length and position of the double bond. To make an adequate comparison of the biological activities of analogues differing in this respects, it is necessary to make corrections for their different volatilities (see Chapter 2, part 2.1).

REFERENCES

1. Roelofs W.L. and Comeau A., *J. Insect Physiol.*, **17**, 435 (1971).

2. Priesner E., Jacobson M. and Bestmann H.J., *Z. Naturforsch.*, **30 c**, 283 (1975).

3. Berger R.S., *Ann. Entomol. Soc. Amer*, **59**, 767 (1966).

4. Tumlinson J.H., Mitchell E.R., Browner S.M. and Lindqvist D.A., *Entomol.*, **1**, 466 (1972).

5. Sparks A.N., *Entomol. Soc. Amer. Nat. Meeting*, Paper No. 63 (1973).

6. Tumlinson J.H., Hendricks D.E., Mitchell E.R. and Doolittle R.E., *Chem. Engin. News*, **20** (1974).

7. Roelofs W.L., Hill A.S., Cardé R.T. and Baker T.C., *Life Sci.*, **14**, 1555 (1974).

8. Sekul A.A. and Sparks A.N., *J. Econ. Entomol.*, **60**, 1270 (1967).

9. Jacobson M., Redfern R.E., Jones W.A. and Aldridge M.H., *Science*, **170**, 542 (1970).

10. Brady U.E. and Ganyard M.C., *Ann. Entomol. Soc. Amer*, **65**, 898 (1972).

11. Nesbitt B.F., Beevor P.S., Cole R.A., Lester R. and Poppi R.G., *Nature New Biol.*, **244**, 208 (1973).

12. Tamaki Y., Noguchi H. and Yushima T., *Appl. Entomol. Zool.*, **8**, 200 (1973).

13. Tamaki Y. and Yushima T., *J. Insect Physiol.*, **20**, 1005 (1974).

14. Kafka W.A. and Neuwirth J., *Z. Naturforsch.*, **30 c**, 278 (1975).

15. Neuwirth J., *"Multiple-Site Wechselwirkung zwischen Duftmolekülen und Akzeptoren"*, Diplomarbeit, Tech. Universität, München (1973).

16. Bestmann H.J. and Vostrowsky O. in *"Olfaction and Endocrine Regulation,* IRL Press Ltd, London, p. 253 (1982).

17. Burgen A.S.V., Roberts G.C.K. and Feeney J., *Nature*, **253**, 753 (1975).

18. Kikuchi T., *Proc. Nat. Acad. Sci. USA*, **72**, 3337 (1975).

19. Bjostad L., Linn C., Roelofs W.L. and Du J.-W. in *"Semiochemistry:Flavors and Pheromones"*, Walter de Gruyter & Co., Berlin, p. 223 (1985).

20. Liljefors T., Thelin B. and Van der Pers J.N.C., *J. Chem. Ecol.*, **10**, 1661 (1984).

21. Olsson A.-M., Jönsson J.-Å., Thelin B. and Liljefors T., *J. Chem. Ecol.*, **9**, 375 (1983).

CHAPTER 4

AN ALTERNATIVE MODEL: THE CONFORMATIONAL ENERGY MODEL.

4.1 The pilot model. The straight-chain mono-olefinic acetates that constitute the great majority of sex pheromone components for noctuid moths are very flexible molecules. With the expense of only a few kcal/mol, a large number of conformations may be achieved by simple rotations around carbon-carbon single bonds. All of them are reasonable candidates for "a biologically active conformation". The model developed and tested by Liljefors et al.[1] is based on the action of (Z)-5-decenyl acetate **1**, one of the pheromone components of *Agrotis segetum*, on its receptor.

As pointed out above (Chapter 3, part 3.1), three parts of the pheromone molecule have been found to be crucial for full biological activity: the terminal methyl group, the double bond and the polar functional group. Compounds in which any of these three parts are missing or changed all show a decrease in biological activity.

The model assumes geometrically well-defined receptor sites complementary to these three molecular parts. The spatial relationships between the different receptor sites are defined by the relative positions in space of the corresponding molecular parts in (Z)-5-decenyl acetate, the natural pheromone component. It is assumed that the interactions between the receptor and the pheromone component take place with (Z)-5-decenyl acetate in a fully extended all-anti conformation, which is one of the thermodynamically most stable ones. An example of such a conformation is shown in Figure 1. In such a conformation the interactions between the alkyl chains and the receptor should be optimal.

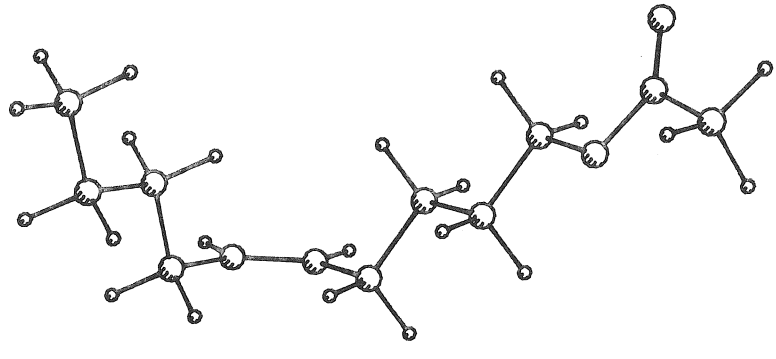


FIGURE 1

Furthermore, it is assumed that in order to be biologically active, an analogue must be capable of interacting with the receptor with the terminal methyl group, the double bond and the acetate group in the relative positions in space already defined. This may be accomplished by a conformational rearrangement of the analogue from its thermodynamically most stable conformation, to a conformation which has these crucial parts in the required positions in space (see Figure 2).

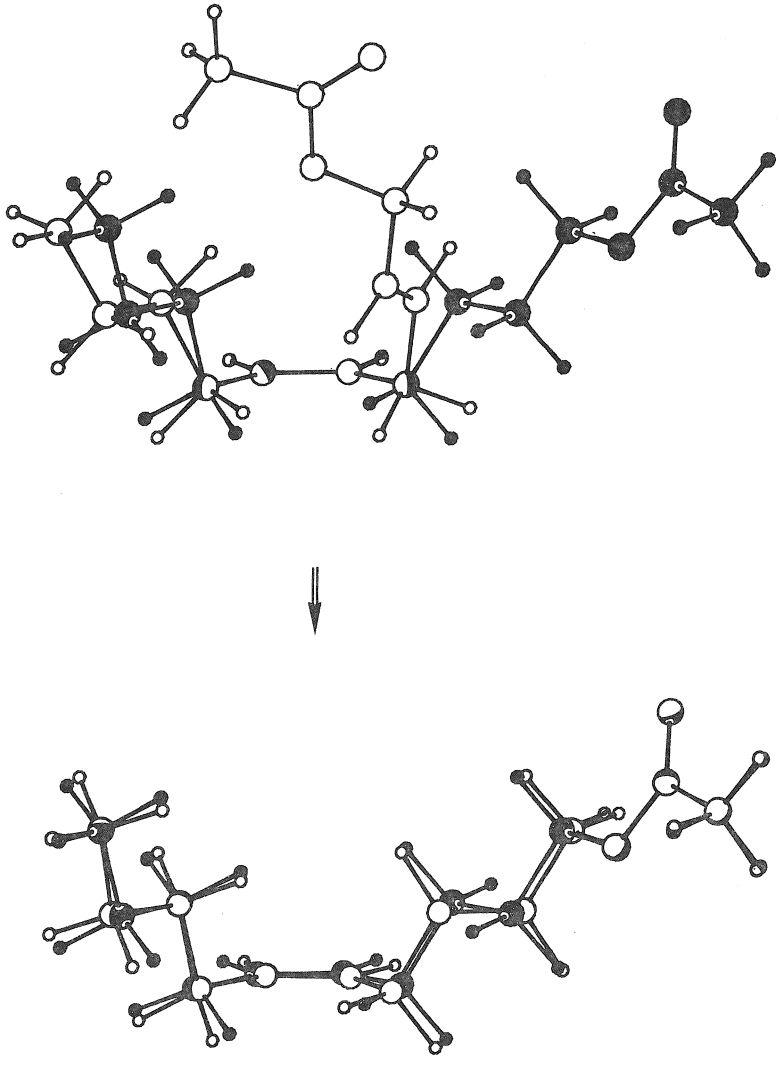


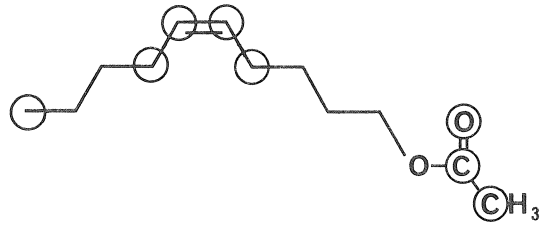
FIGURE 2. Superimposition of (Z)-5-decenyl acetate (filled atoms) and an analogue (**19**) in its thermodynamically preferred conformation (upper part) and after conformational rearrangement (lower part).

The conformational rearrangement of the analogue will require energy; how much depends on how severely the molecule must be perturbed.

At the present stage of development, the model is only applicable to compounds analogous to the natural pheromone component and which have the ability to position the crucial molecular parts, as defined above, in the required positions. Thus, compounds like chain-shortened analogues, compounds **8-13**, cannot directly be fitted to the model and its assumed interaction sites.

The **conformational energy model** implies that all analogues in their biologically active conformations have closely similar interaction energies with the receptor sites. The different abilities of the analogues to bind to the receptor, or rather to form an "activated complex" with the receptor are, according to the model, due to different conformational energies of their "biologically active conformations". A high conformational energy should correspond to a low probability to form an activated complex and consequently to a low biological activity and vice versa.

The conformational energy for the "biologically active conformation" may be calculated from molecular mechanics calculations. Computationally this is accomplished by restricting the encircled atoms to fixed positions during the energy-minimization procedure, while the remaining alkyl-chain atoms and other non-restricted atoms are allowed to find positions that minimize the total energy of the molecule. As mentioned above, the "fixed positions" are defined by spatial relationships in the natural pheromone component.



In a second calculation the global unconstrained energy minimum of the same molecule is calculated. The conformational energy of the calculated biologically active conformation may then be evaluated by taking the calculated energy difference between the lowest energy conformationally rearranged structure that fits the model and the thermodynamically most stable one.

In a first attempt to test the model, calculations were made on chain-elongated

analogues[1]. To facilate the calculations, the entire molecule was not used. Instead, attention was paid only to the parts of the molecule that were changed. This was accomplished by the use of two model compounds, (Z)-5-heptenyl acetate and (Z)-2-heptene.

4.2 Refinements of the conformational energy model. The pilot conformational energy model, discussed in part 4.1, was successfully used to rationalize the effects of chain-elongation on observed electrophysiological single-cell activities of homologues of (Z)-5-decenyl acetate **1**[1]. To include other effects as well, for example those of a change of the double bond configuration, an extension of the model was necessary. The pilot model was found to be too rigid, resulting in very great differences in energy for analogues, depending on whether the changes were made in the **n**- or in the **m**-chain of the pheromone component molecule. The model was able to rationalize changes within the **n**-chain **or** within the **m**-chain, but it was not possible to compare the conformational energies of analogues with changes made in different chains. Furthermore, the model gave very high conformational energies for (E)-analogues, predicting very low biological activities for such compounds, which is not in agreement with experimental data[2]. An analysis of the results obtained by the pilot model suggests that the difficulties are due to overly severe constraints imposed on the molecule by holding the double bond in an fixed position in space. The additional features of the refined model compared to the pilot model are:

i) the use of the entire pheromone component molecule **1** in the construction of the model instead of using only part of the molecule,

ii) the use of the complete structure of an analogue in the calculations of conformational energies, and

iii) addition of flexibility to the model with respect to the required location of the double bond (see Computational procedure section below).

In the calculations performed in this thesis, the complete molecules are used (**i, ii** above). However, this introduces a complication since it is now necessary to take the conformational properties of the natural pheromone component **1** fully into account.

Conformational analysis of (Z)-5-decenyl-acetate 1. Compound **1** is a very flexible molecule with a large number of conformers within 1 kcal/mol of the

energy of the thermodynamically most stable one. It is assumed that the alkyl chains of **1** should have an all-anti conformation in the biologically active state (see above). This reduces the problem to the conformations with respect to the C=C-C-C fragments. It is then implicitly assumed that one of the stable conformers in this respect corresponds to the biologically relevant structure of compound **1**.

A calculated conformational energy map, fully energy minimized by MM2, for the rotation about the vinylic bonds in (Z)-4-octene, used as a model for the olefinic part of a mono-olefinic pheromone component, is shown in Figure 3. The energy map shows a degenerate double-minimum for the **cisoid** conformation, and a single minimum for the **transoid** one with an energy difference of 0.11 kcal/mol, favouring the **transoid** conformation. The populations of the two types of conformations are thus very similar, in agreement with experimental data on other (Z)-olefins. An electron diffraction study on (Z)-3-hexene indicates the presence of both types of conformations with comparable populations[3]. This has also been concluded from infrared data on liquid (Z)-3-hexene[4]. Raman spectra of crystals of (Z)-mono-olefinic fatty acids show the molecules to have either the **cisoid** or the **transoid** conformation depending on the crystalline modification[5]. The energy barrier between the two **cisoid** conformers in (Z)-4-octene is calculated to be very low, 0.12 kcal/mol. The interconversion between the two **cisoid** forms may thus be described as a large amplitude torsional motion. The energy barrier for the **cisoid** to **transoid** interconversion is calculated to be 0.84 kcal/mol, in good agreement with the experimental value 0.60 +/- 0.06 kcal/mol for the analogous barrier in (Z)-2-pentene[6].

The above analysis of (Z)-4-octene implies that there are three conformers of the natural pheromone component **1** which are candidates for the biologically active structure within the context of the model. The energy-minimized geometries of these conformers are shown in Figure 4.

The **cisoid 1** and **cisoid 2** conformers differ mainly in the torsional angles about the (C=C)-(C-C) bonds. In the **cisoid 1** structure the C=C-C-C dihedral angles are calculated to be 116.1 and -87.2 degrees for the **n**- and **m**-chains, respectively. The corresponding values for the **cisoid 2** conformer are 88.4 and -114.9 degrees. The difference in calculated conformational energy between the three conformers is less than 0.06 kcal/mol. As there are presently no data from which it is *a priori* possible to choose which of these conformers to use as a model for the biologically active conformation of **1**, all three were used in turn and all calculations were done for each of the three models. These models will be denoted **cisoid 1**, **cisoid 2** and **transoid** according to Figure 4. The use of all three models in the calculations gives a good

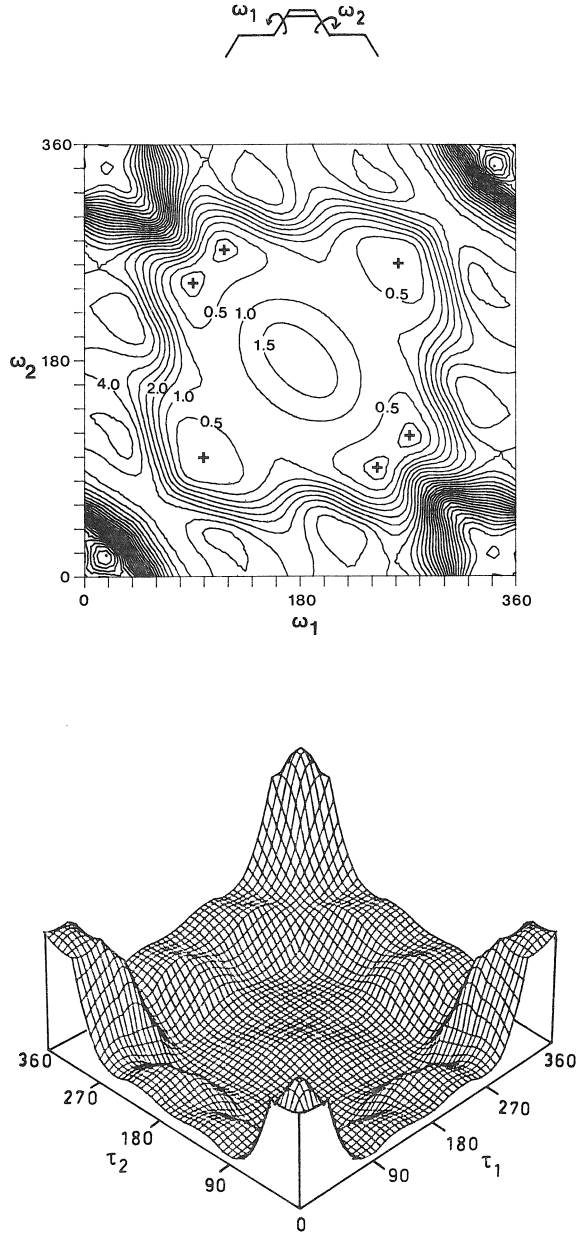


FIGURE 3. Conformational energy map (kcal/mol) for the rotation about the vinylic bonds of (Z)-4-octene. Local minima are denoted by +.

check of the sensitivity of the calculated results to the precise geometry of the substrate-receptor interaction model.

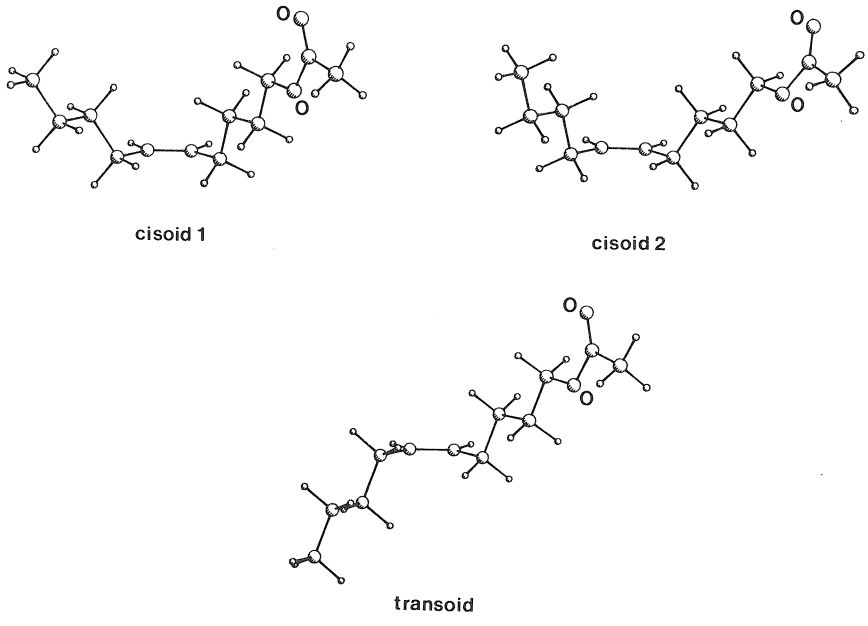
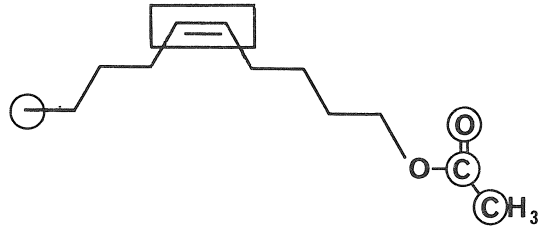


FIGURE 4

Computational procedure. The pheromone component analogues studied were assumed to interact with the receptor as described for the pilot model (see part 4.1). However, in the pilot model the double bond and the vinylic carbons were also restricted to fixed positions. To add more flexibility to the model the double bond and the vinylic carbon atoms were allowed to move during the energy minimization process in the plane defined by C-C=C-C fragment in the reference molecule **1**. The restriction to a common plane ensures that the π-orbitals have the same direction in all molecules studied, and thus may interact with the corresponding binding site in a similar way.



Before energy minimization, the double bond in the studied analogue was placed in a position as close to the double bond in the reference molecule **1** as possible. With these restrictions, the molecules were energy-minimized with respect to all remaining degrees of freedom. A large number of starting structures were employed and the lowest energy structure after energy minimization was used in the further analysis.

The calculated conformational energies correspond to enthalpies. The entropy terms have not been explicitly considered in this thesis but differences in the conformational entropy contributions for the molecules in the series investigated should largely be compensated by differences in hydrophobic binding[1].

REFERENCES

1. Liljefors T., Thelin B., Van der Pers J.N.C. and Löfstedt C., *J. Chem. Soc. Perkin Trans. II*, 1957 (1985).

2. Bengtsson M., unpublished results.

3. Van Hemelrijk D., Van den Enden L. and Geise H.J., *J. Mol. Struct.*, **74**, 123 (1981).

4. Shimanouchi T., Abe Y. and Alaki Y., *Polymer J.*, **2**, 199 (1971).

5. Koyama Y. and Ikeda K., *Chem. Phys. Lipids*, **26**, 149 (1980).

6. Van Eijk B.P., *J. Mol. Spectrosc.*, **85**, 189 (1981).

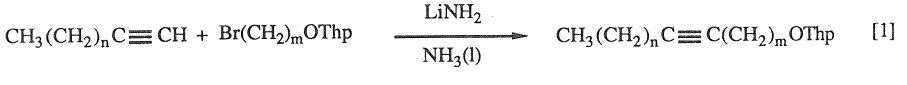
CHAPTER 5

SYNTHETIC PART.

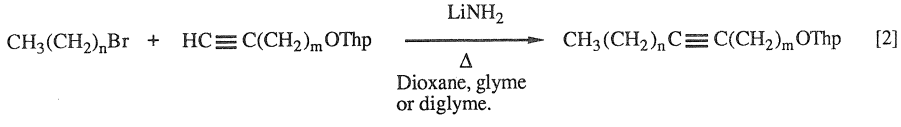
In studies on structure-activity relationships, it is of decisive importance that the compounds examined are of very high stereochemical purity. Even small amounts of impurities may have serious consequences for the measured biological activities. Therefore, in the synthesis of the pheromone component (Z)-5-decenyl acetate **1** and the set of analogues, compounds **2-29**, synthetic routes as stereospecific as possible have been chosen, even if it has sometimes been at the expense of the yields.

The final products have been subjected to several purification methods (Chapter 8, part 8.2) and careful analysis (part 5.3 and Chapter 8, part 8.2) to ensure high purity before their single-cell activities were recorded.

5.1 The natural pheromone component (Z)-5-decenyl acetate 1, E/Z-isomers, chain-elongated and chain-shortened analogues. One of the most frequently used synthetic routes to mono-enic moth sex pheromone components[1] is outlined in Schemes 1, 3 and 4. The key step is a coupling reaction between an alkyne or functionalized alkyne and an alkyl halide or functionalized alkyl halide (reactions [1] and [2], Scheme 1).



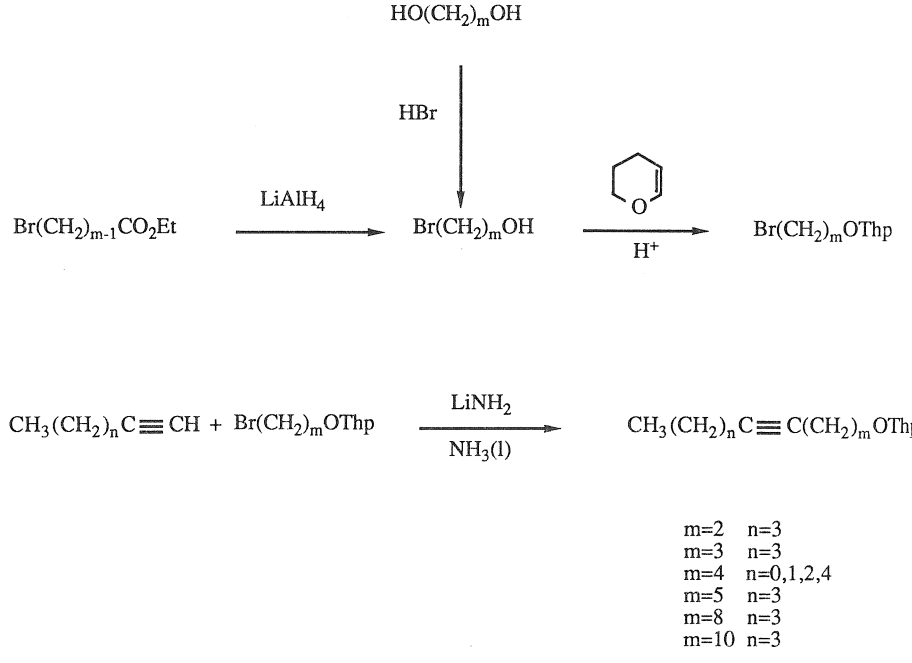
[1]



[2]

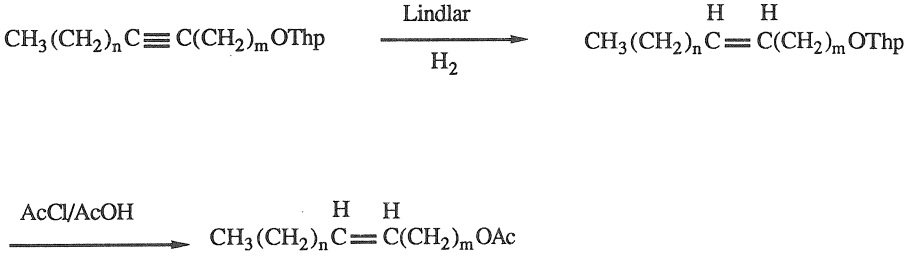
SCHEME 1

Alkylation of unfunctionalized alkynes, [1] , proceeds in almost quantitative yields in liquid ammonia/ether media, with lithium amide as the base[2]. This method was used to prepare (Z)-5-nonenyl acetate **8**, (Z)-5-octenyl acetate **9**, (Z)-5-heptenyl acetate **10**, (Z)-4-nonenyl acetate **12**, (Z)-3-octenyl acetate **13**, (Z)-9-tetradecenyl acetate **16** and (Z)-11-hexadecenyl acetate **17** (Scheme 2).

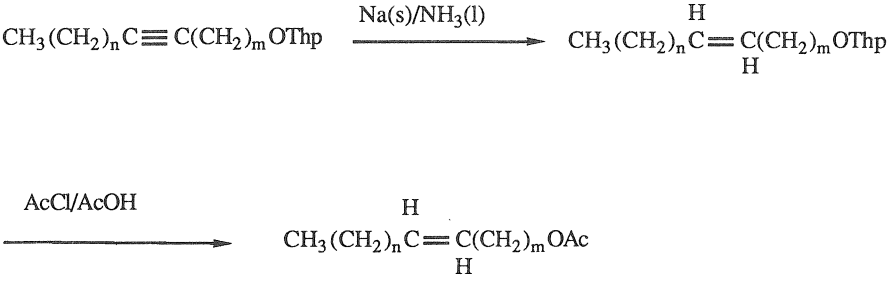


SCHEME 2

The resulting protected alkynols are then readily reduced to the corresponding (Z)- or (E)-monoenes. (Z)-Olefins are obtained by reduction at atmospheric pressure with Lindlar catalyst (palladium on calcium carbonate, poisoned with lead) poisoned with quinoline[3] (Scheme 3). The (E)-isomers are obtained by reduction with Na(s) in liquid ammonia[4] (Scheme 4). Both reductions are highly stereoselective. Only small amounts of the undesired isomer are generated in these reactions. Finally, the protecting group is converted to the corresponding acetate, by treatment with acetyl chloride (excess) in acetic acid[5].

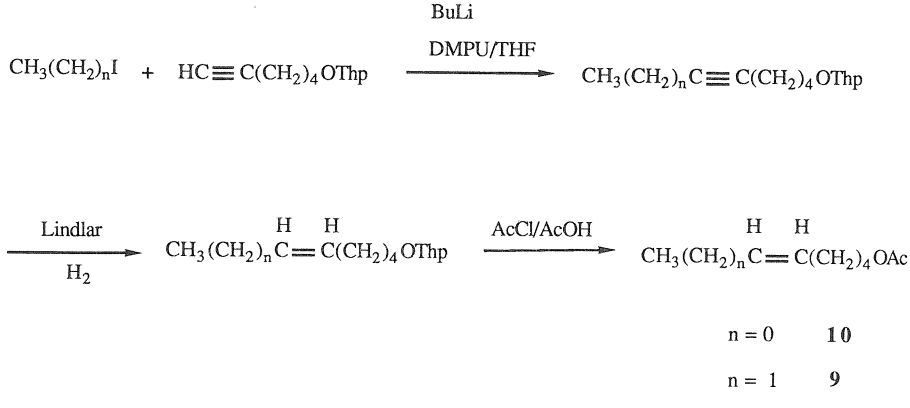


SCHEME 3



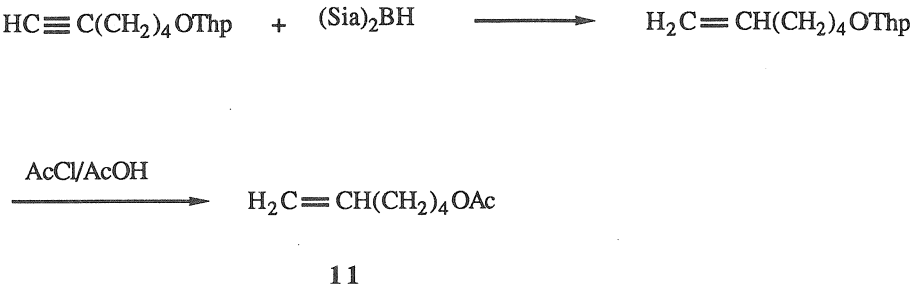
SCHEME 4

However, with functionalized alkynes (reaction [2], Scheme 1) one often faces problems with the solvation of the acetylenides, which results in low yields or even no reaction at all. This is especially pronounced as the length of the $(CH_2)_m$-chain increases[6]. These problems may be overcome by carrying out the synthesis in boiling dioxane[7], glyme[8] (see part 5.2.1) or diglyme[9]. For low-boiling alkyl halides this method is not practical; it is then often necessary to use a large excess of the halide. Instead, a very mild and efficient procedure resulting in high yields is the use of HMPT (hexamethylphosphorictriamide) as a cation complexing agent, in dry THF with butyllithium as the base[5,10-12]. However, in recent years HMPT has been shown to be highly carcinogenic in animal tests, even at low exposures comparable with those in laboratory-scale synthesis work[13,14]. In the search for a safe substitute, DMPU (1,3-dimethyl-2-oxo-hexahydropyrimidine) was shown to have properties similar to those of HMPT in a number of different reactions, for example the Wittig olefination and the Michael addition[15]. The preparation of (Z)-5-octenyl acetate **9** and (Z)-5-heptenyl acetate **10** (Scheme 5) shows the feasibility of replacing HMPT with DMPU with retained good yields, also in alkyne alkylation reactions. The tetrahydropyranyl ethers were obtained in more than 90 % yield.



SCHEME 5

5-Hexenyl acetate **11** was prepared as outlined in Scheme 6. 1-(2-Tetrahydropyranyloxy)-5-hexyne was reduced with disiamyl borane according to the procedure described by Brown et al.[16] and the resulting alkene acetylated, in the usual way. The reduction was also performed with Lindlar catalyst, but this resulted in high yields of the corresponding alkane, which indicates that this is not a suitable method for the reduction of terminal alkynes.

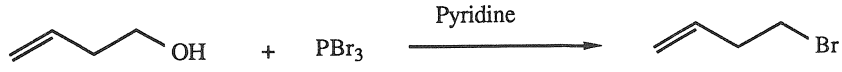


11

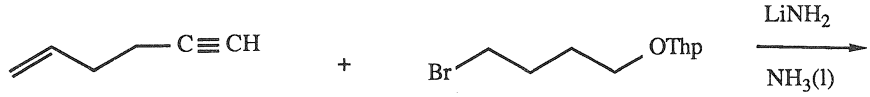
SCHEME 6

5.2 Diene analogues. The synthetic procedures used to prepare the diene (and enyne) analogues **19-26** can be divided into two main groups: procedures suitable for non-conjugated and conjugated dienes.

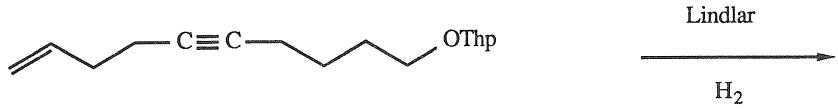
5.2.1 Non-conjugated diene analogues. Similar alkylation processes of alkynes as were used to prepare the monoenic analogues (part 5.1) are also convenient for the preparation of non-conjugated diene compounds. The difference is the presence of an additional double bond in either the alkyl halide or in the alkyne. (Z)-5,9-Decadienyl acetate **26**, and the corresponding enyne **25**, were prepared as shown in Scheme 7. The terminal enyne **30** was prepared by a coupling reaction between 3-

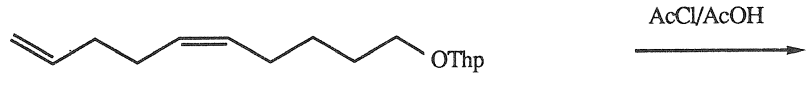


30



30



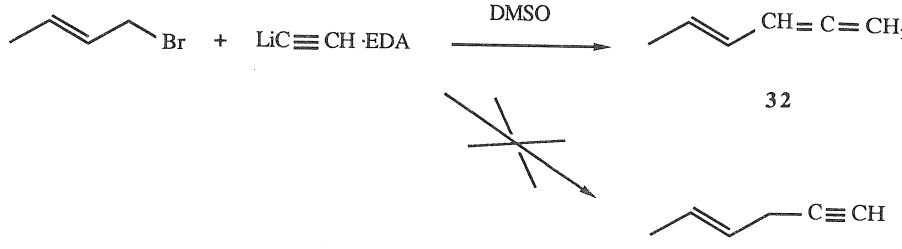


26

SCHEME 7

butenyl bromide and lithium acetylide, ethylenediamine complex in dry DMSO[12,17]. The product was then alkylated in the usual way in liquid ammonia.

It was not possible to use the same method to obtain (Z)-5,(E)-8-decadienyl acetate **24** (and the enyne **23**). Attempts to synthesize the required enyne, hex-4-en-1-yne **31**, by reacting (E)-2-butenyl bromide with lithium acetylide, ethylenediamine complex (Scheme 8) were unsuccessful. The main product obtained in this reaction was the allene **32.**

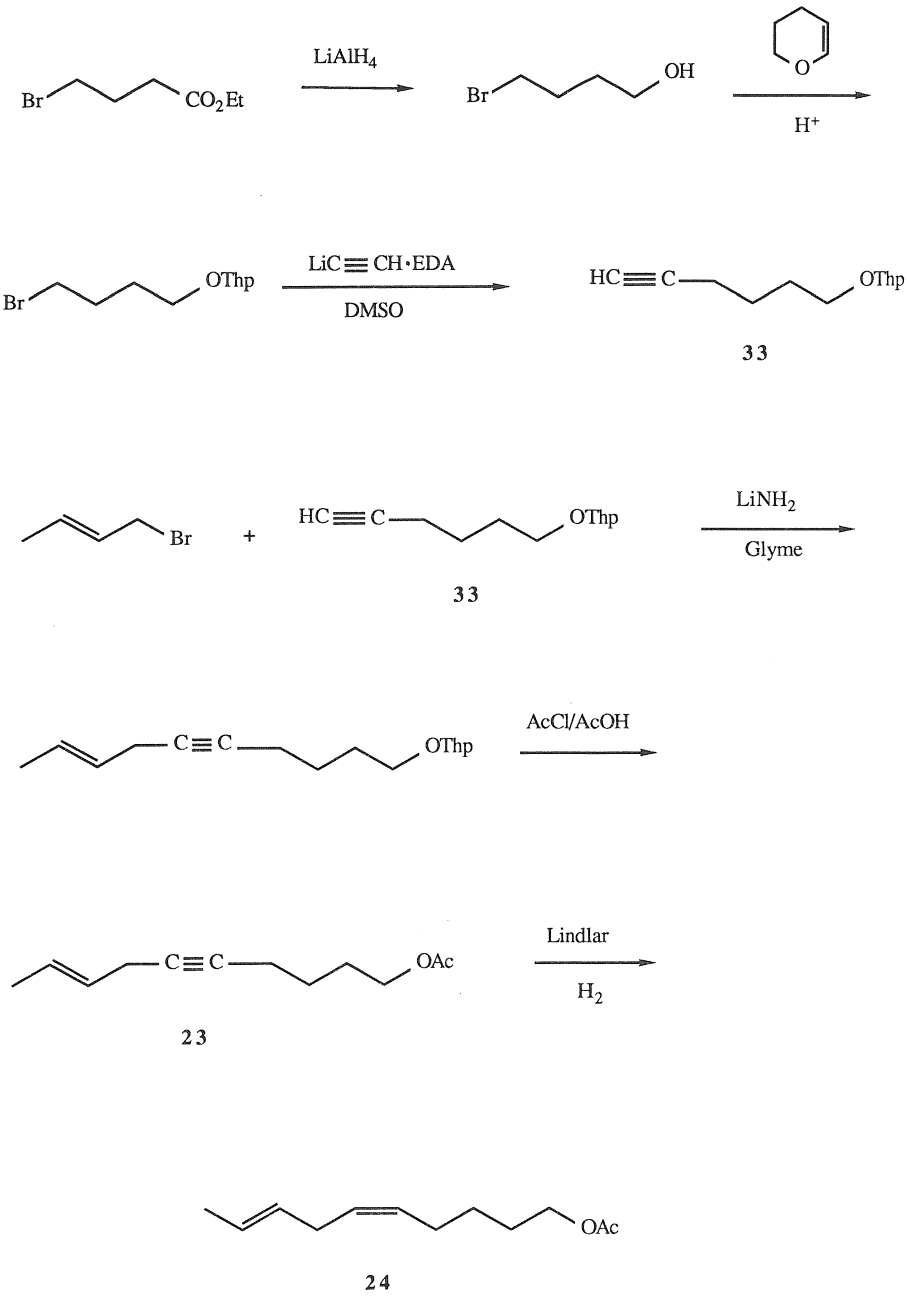


32

31

SCHEME 8

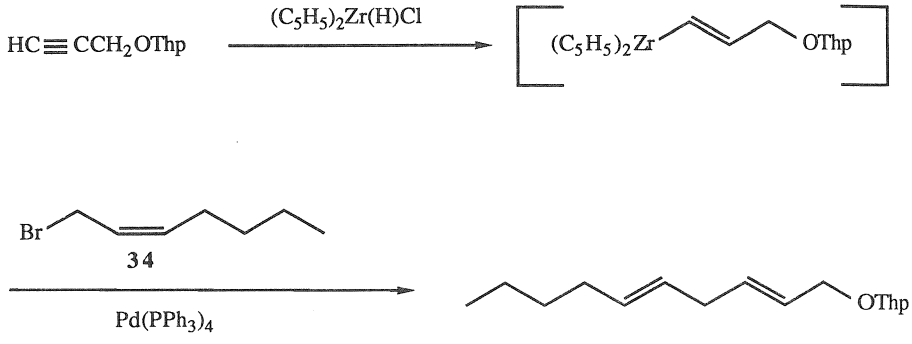
Instead, **24** (and **23**) were synthesized according to Scheme 9. These reactions were performed before our observation that it is an advantage to use DMPU as solvent (see part 5.1). Therefore 5-hexyne-1-ol tetrahydropyranyl ether **33** was coupled with (E)-2-butenyl bromide in boiling dry glyme with lithium amide as the base to give the product in 55% yield. With the addition of one equivalent of the lithium complexing crown-ether, 12-crown-4, the yield was increased to 80%.


LiAlH4
Br
CO2Et
OH
O
H+
OThp
LiC≡CH·EDA
DMSO
HC≡C
33
LiNH2
Glyme
AcCl/AcOH
OAc
Lindlar
H2
23
24

SCHEME 9

For (E)-2,(Z)-5-decadienyl acetate **19**, it was not possible to use the same type of coupling reaction between an alkyne and an alkyl halide as described above. Many different attempts were made according to [1] and [2] (see Scheme 1), but all of them resulted in very low yields and some gave large amounts of by-products. The reason for this is unknown to us, but it seems that the allylic position of the double or triple bond in relation to the tetrahydropyranyloxo group in the different compounds employed in the reactions, caused the problems.

Instead, a recently published synthetic method was tried, including the addition of bis(π-cyclopentadienyl)-zirconium hydrido chloride (Schwarz's reagent $(C_5H_5)_2Zn(H)Cl$) to the alkyne to perform a regioselective coupling reaction[18,19]. 2-Propyne-1-ol tetrahydropyranyl ether was hydrozirconated in benzene to the (E)-zirconium intermediate, Scheme 10.



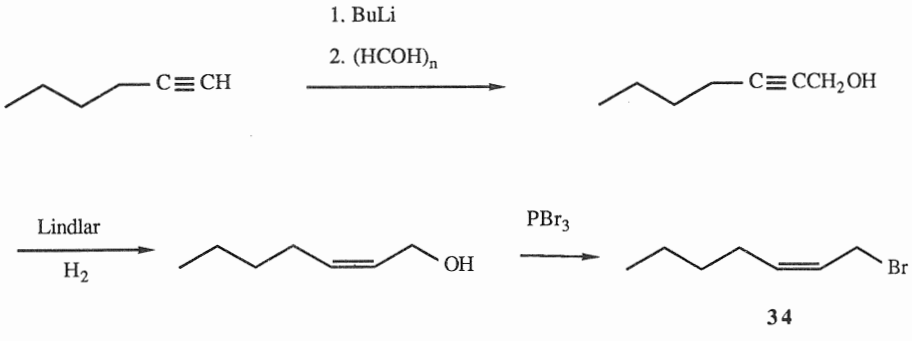
SCHEME 10

The intermediate was coupled with (Z)-2-heptenyl bromide **34** using tetrakis-(triphenylphosphine)palladium as a catalyst. The product obtained was not the expected

(E)-2,(Z)-5-decadien-1-ol tetrahydropyranyl ether. A complete isomerisation of the (Z)-double bond had been achieved to give the (E)-2,(E)-5-decadien-1-ol, tetrahydropyranyl ether in 90% crude yield. After acetylation this gave the diene analogue **20.**

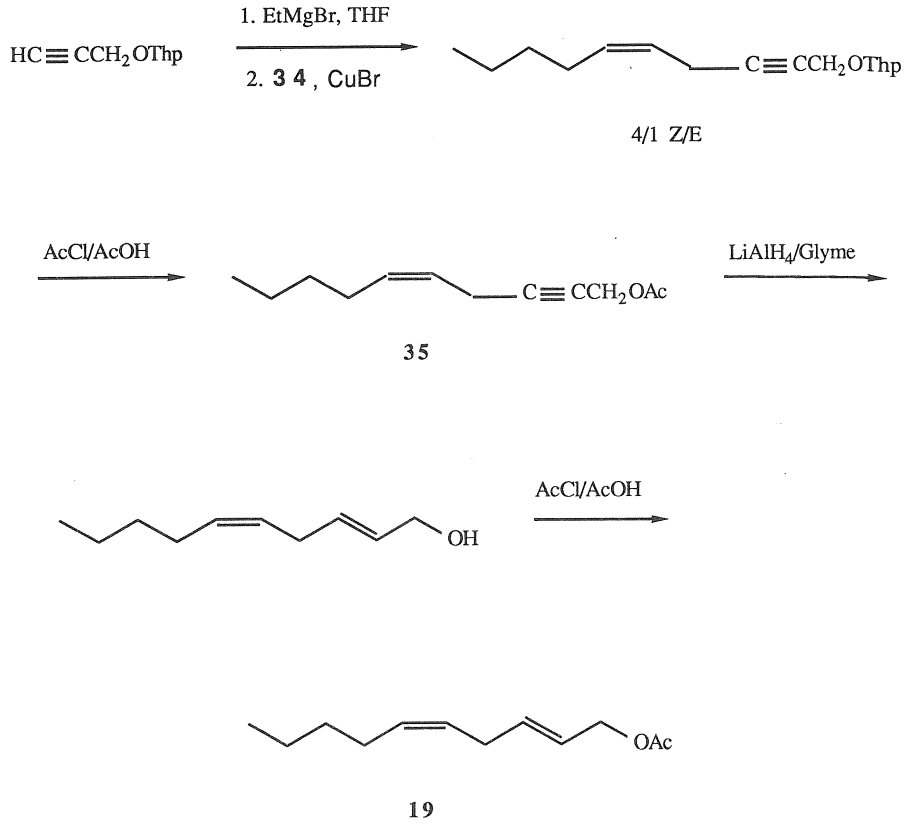
Finally, the dienic analogue **19** was prepared as outlined in Scheme 11, by a coupling reaction between the Grignard reagent prepared from 2-propyne-1-ol tetrahydropyranyl ether and (Z)-2-heptenyl bromide **34** in the presence of cuprous bromide[20]. The product obtained was a 4:1 mixture of (Z)- and (E)-enyne, which required careful argentation liquid chromatography to obtain the final product.

(E)-2-Alkenols are known to be conveniently prepared by reduction of the corresponding 2-alkynols with lithium aluminium hydride in dry diethylether[21,22]. Nevertheless, when this method was employed on the enyne acetate **35**, no signs of reduction were obtained, even with such drastic conditions as refluxing over night. When the reduction was instead carried out in boiling dry glyme, only traces of the starting acetylenic compound remained after 3 hours.



34

SCHEME 11. To be continued...



SCHEME 11. (continued)

5.2.2 Conjugated diene analogues. A stereospecific Wittig-type reaction was used for the preparation of the conjugated dienes **21** and **22**. Since the discovery of the Wittig reaction in 1953[23], a great many synthetic variations and improvements of the method have been reported. Investigations on the mechanism of the reaction have been carried out and opened up ways to construct double bonds in a stereoselective

way[24-26]. This makes the Wittig reaction an alternative to the partial hydrogenation of alkynes discussed above, and today it represents one of the key reaction steps in the preparation of natural compounds.

By affecting the equilibrium between the two diastereomeric forms of the betaine intermediate (see Figure 1), the (Z/E)-isomer ratio of the product formed may be controlled[24].

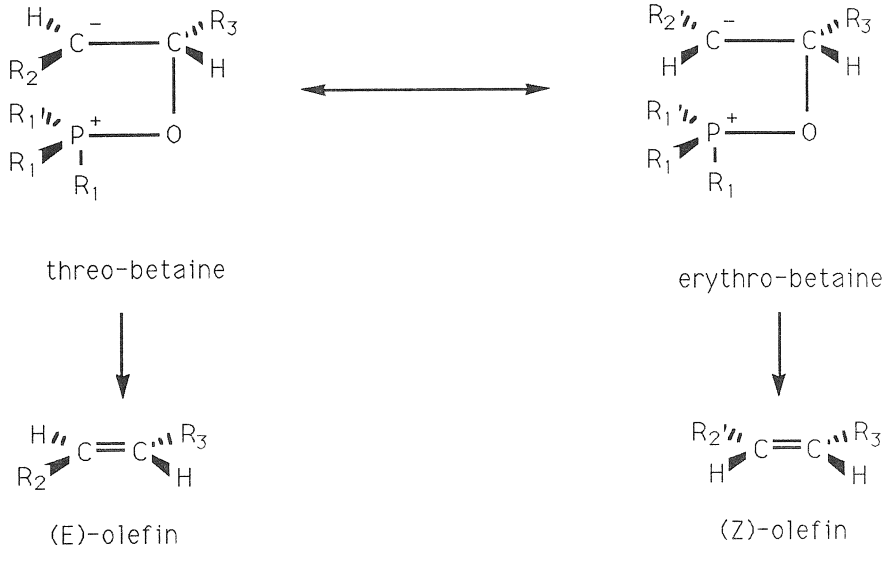


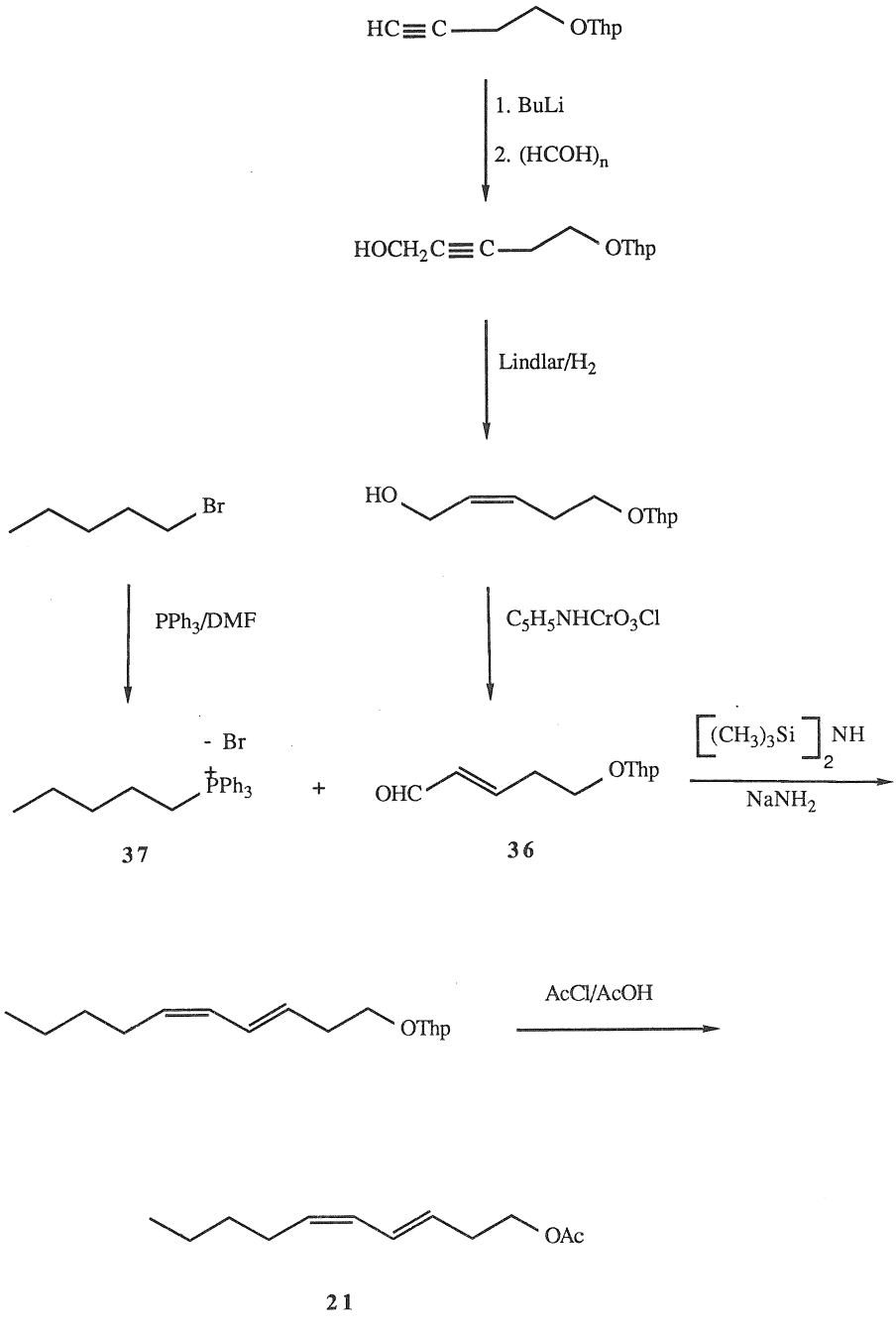
FIGURE 1

(Z)-Alkenes are generated from the erythro-betains, under kinetic control, obtained with unstable alkylidenephosphoranes and "lithium-salt free" ylide solutions. (E)-Alkenes are formed from the threo-betains, which are favored by resonance-stabilized ylids, protic solvents and the formation of associates between the betaine and lithium salts.

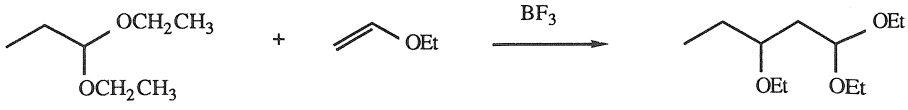
The "lithium-salt free" ylide solution necessary for the formation of (Z)-olefins is

achieved by the use of a base like sodium amide[27], methylsulfinyl carbanion[28], potassium-tert.-butoxide[29] or sodium-bis(trimethylsilyl)amide (the silazide method)[30,31]. In the preparation of **21** and **22**, freshly sublimed potassium-tert.-butoxide and methylsulfinyl carbanion generated by the reaction between sodium hydride (100%) and dry DMSO under a nitrogen atmosphere, were first tried as bases. Both methods failed and gave hardly any yields at all of the desired products. Instead the silazide-method was used with good results. Sodium-bis-trimethylsilyl-amide was generated from hexamethyldisilazane and sodium amide-suspension (50% in toluene) in dry THF under a nitrogen atmosphere. The mixture obtained was directly used in the synthesis.

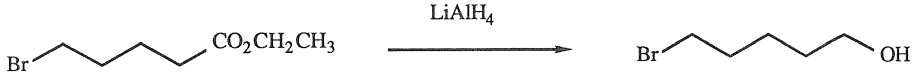
(E)-3,(Z)-5-Decadienyl acetate **21** was prepared as outlined in Scheme 12. The stereoselective Wittig reaction was performed between 5-(2-tetrahydropyranyloxy)-(E)-2-pentenal **36** and (pentyl)-triphenylphosphonium bromide **37** in dry THF at -78° C. To obtain (Z)-5,(E)-7-decadienyl acetate **22**, the same procedure was used, but with (E)-2-pentenal **38** and 5-acetoxypentyl-triphenylphosphonium bromide **39** (Scheme 13). (E)-2-Pentenal was made from propionaldehyde diethyl acetal with boron trifluoride diethyl etherate as catalyst[32]. The Wittig reactions proceeded in 22-30% yields and with excellent stereoselectivity. The corresponding (E)-olefins were formed in less than 2%, as indicated by capillary g.l.c. on a 30 m Supelcowax column. This is in agreement with results reported by Bestmann et al.[31] for similar systems.

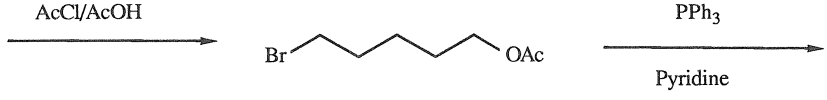

HC≡C—OThp
1. BuLi
2. (HCOH)n
HOCH2C≡C—OThp
Lindlar/H2
Br
HO—OThp
PPh3/DMF
C5H5NHCrO3Cl
- Br
+PPh3
+
OHC—OThp
[(CH3)3Si]2NH
NaNH2
37
36
OThp
AcCl/AcOH
OAc
21

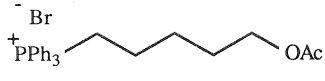
SCHEME 12



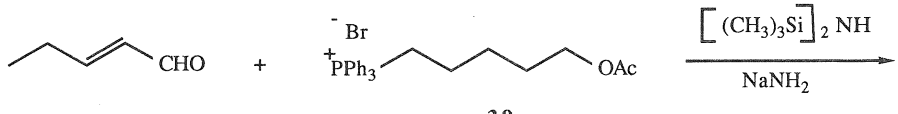
38



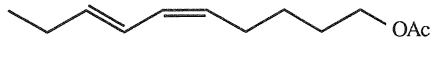




39



38 **39**



22

SCHEME 13

5.3 Use of ^{13}C n.m.r for assigning the configuration at carbon-carbon double bonds.

^{13}C N.m.r has proven very useful in the assignment of the configuration at carbon-carbon double bonds in mono- and dienic pheromone components[33-37]. The olefinic carbons are easily recognized due to their large chemical shifts. However, these signals are of less interpretative value, since their unequivocal assignment is not always possible, and they are sometimes difficult to observe as separate signals.

Instead, the shifts of the carbons in the alpha-position to the double bond are more informative. Depending on the configuration of the double bond, these carbon atoms will show different chemical shifts. Rossi et al.[35] investigated the difference, $\Delta\ \partial$, between:

i) the observed chemical shift value of an allylic carbon atom in a mono- or diunsaturated carbon atom and

ii) the chemical shift value of the carbon in the same position in the corresponding saturated n-alkane chain.

It was found that carbon atoms allylic to a (**Z**)-double bond were shifted **2.4** +/- 0.3 ppm **upfield**, whereas the corresponding value for an (**E**)-double bond was **2.8** +/- 0.4 ppm **downfield**.

For the dienic compounds **19-22**, **24** and **26**, signals of their ^{13}C n.m.r spectra were assigned to individual carbon atoms by comparison with literature data for related compounds[35-37]. The shifts for the allylic carbons were subtracted from the shifts of the corresponding atoms in 1-decyl acetate. The resulting $\Delta\ \partial$ values are shown in Table 1.

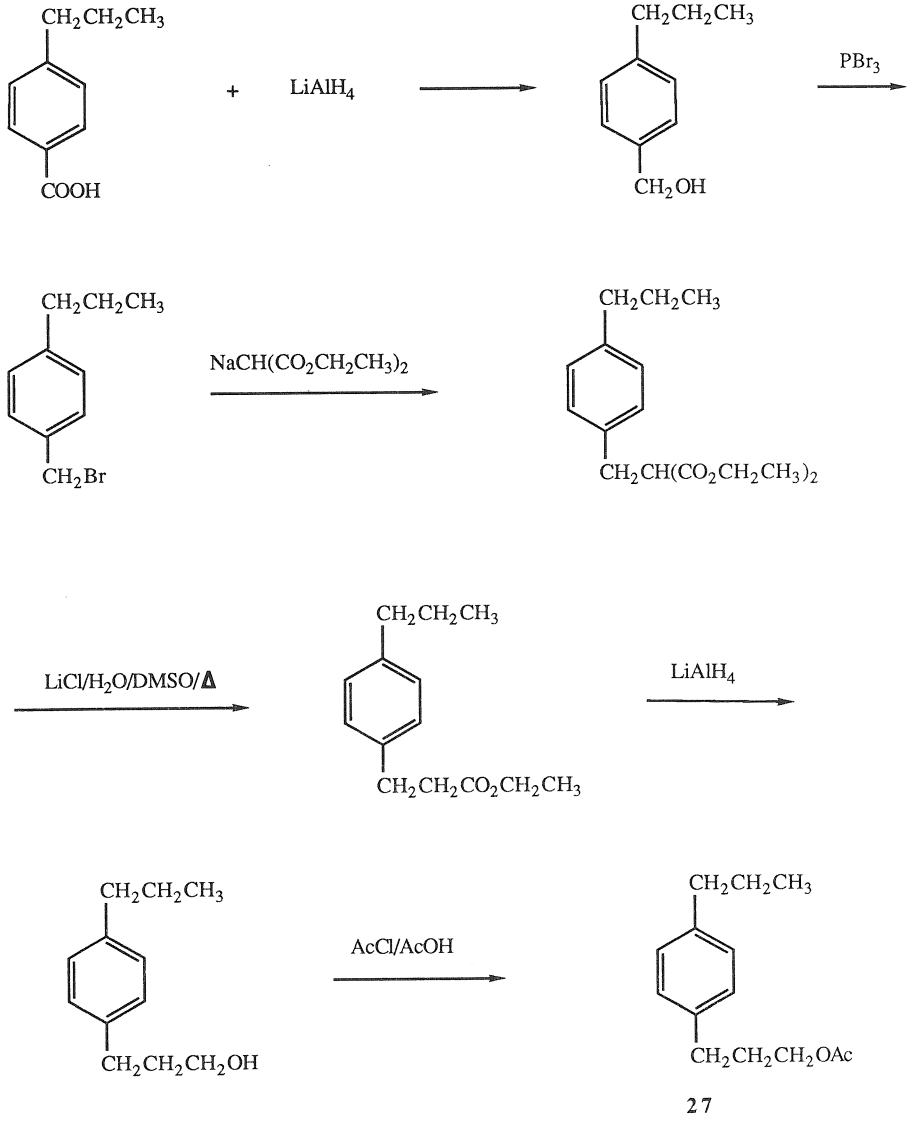
In earlier studies[33-37], there are no examples where the carbon atoms at the ends of the molecule were used for configuration assignments. For compounds **19, 20** and **24** (Table 1) the shift difference values for carbon atoms in positions 1 and 10 are included. The magnitudes of the shift differences for these atoms are not in accordance with those obtained for carbon atoms in the other positions, but the downfield direction is still the one predicted for carbon atoms allylic to an (E)-double bond.

Compound	Position of the allylic carbon atom	Configuration of the C=C bond	Δ ∂
(2E,5Z)-decadienyl acetate	1*	E	+0.6*
19	7	Z	-2.4
(2E,5E)-decadienyl acetate	1*	E	+0.5*
20	7	E	+2.9
(3E,5Z)-decadienyl acetate	2	E	+3.5
21	7	Z	-2.0
(5Z,7E)-decadienyl acetate	4	Z	-2.3
22	9	E	+3.2
(5Z,8E)-decadienyl acetate	4	Z	-2.9
24	10*	E	+4.0*
(5Z,9)-decadienyl acetate	4	Z	-2.7
26			

TABLE 1. * indicates carbon atoms at the ends of the molecule. These have not previously been used for configuration determinations[33-37].

5.4 Cyclic analogues.

5.4.1 A benzene analogue. 3-(p-Propylphenyl)propyl acetate **27** was prepared as outlined in Scheme 14. The reaction step in which the bromide group is substituted by sodium diethyl malonate was first carried out in a similar way as described by Jacobson[38]. Sodium diethyl malonate, as a 2 M solution, was generated from sodium and diethyl malonate in dry ethanol and the bromide was added. However, this method resulted in substantial dialkylation of the malonate, even when the addition of the bromide was slow. By increasing the solvent (EtOH) volume to twice the previous amount, this problem was essentially solved. The traces of dialkylated product produced in spite of all of our efforts were easily removed by flash chromatography[39,40].


CH2CH2CH3
COOH
+
LiAlH4
CH2CH2CH3
CH2OH
PBr3
CH2CH2CH3
CH2Br
NaCH(CO2CH2CH3)2
CH2CH2CH3
CH2CH(CO2CH2CH3)2
LiCl/H2O/DMSO/Δ
CH2CH2CH3
CH2CH2CO2CH2CH3
LiAlH4
CH2CH2CH3
CH2CH2CH2OH
AcCl/AcOH
CH2CH2CH3
CH2CH2CH2OAc

27

SCHEME 14

5.4.2 Cyclohexene analogues. 3-(Cis-4-propylcyclohex-2-en-1-yl)propyl acetate **28** and 3-(trans-4-propylcyclohex-2-en-1-yl)propyl acetate **29** were first planned to be synthesized by a Diels-Alder cyclisation reaction as the key step. The configurations at the double bonds in the starting diene molecule in a Diels-Alder reaction are known to determine the stereochemistry of the cyclohexene product[41]. A cis-substituted cyclohexene system should be obtained from the reaction of a conjugated (E,E)-diene with a dienophile and a trans-substituted cyclohexene system from a corresponding reaction performed with a (Z,E)-diene (see Figure 2).

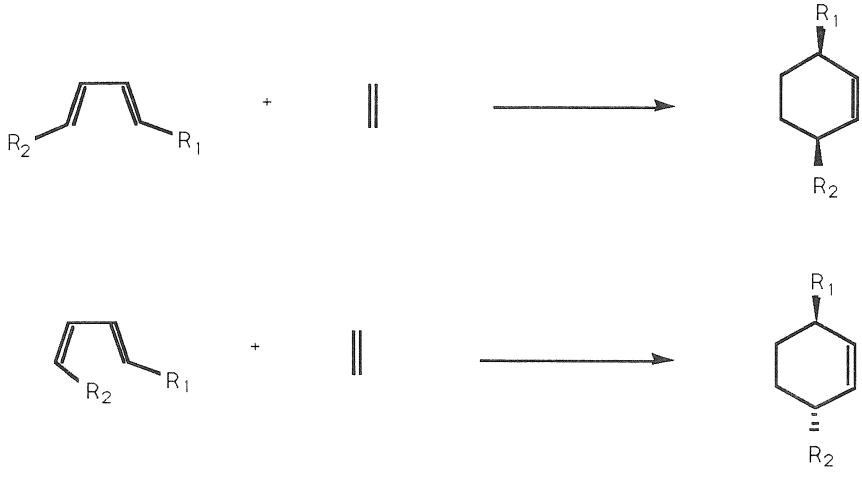
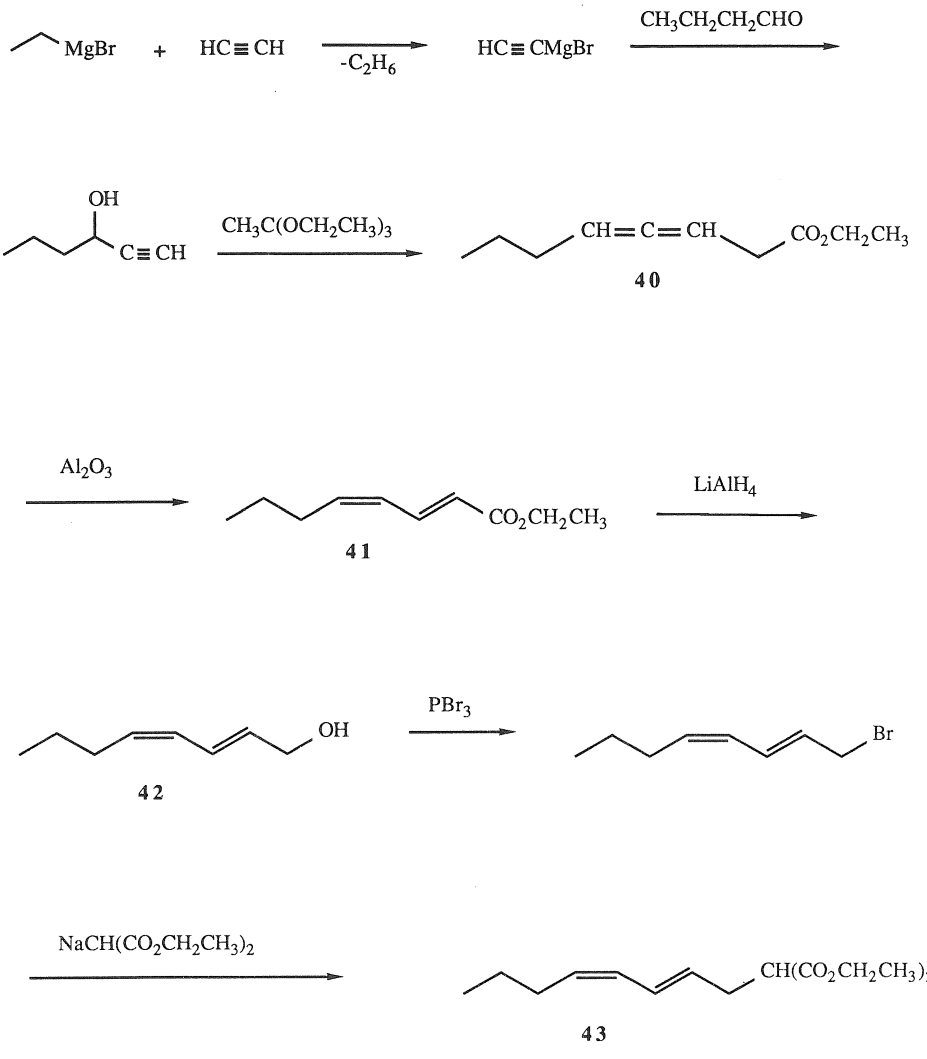
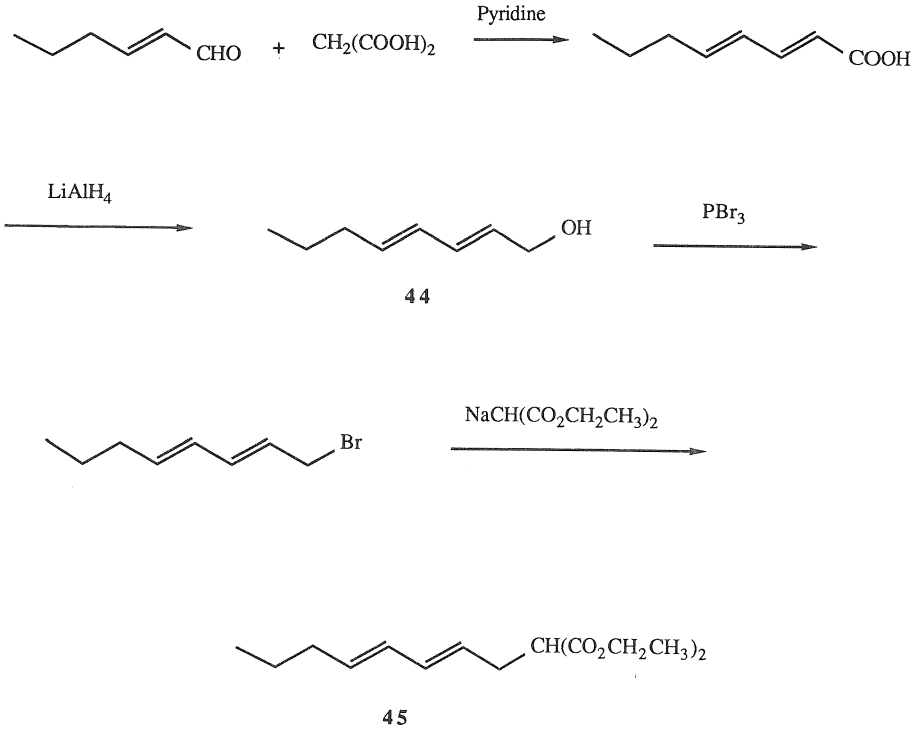


FIGURE 2

The preparation of the two dienic reactants suitable for the Diels-Alder reactions on the route to **28** and **29** is outlined in Schemes 15 and 16. In the synthetic route leading to the diene **43**, Scheme 15, the thermal treatment of the beta-allenic ester **40** with alumina catalyst was found to require substantially longer reaction times than previously reported for this method[42]. Instead of the reported 2-5 hours it was necessary to reflux the reaction mixture for 96 h, with addition of more catalyst after half the reaction time. The reaction was followed by g.l.c on a OV-351 column.


MgBr
+
HC≡CH
-C2H6
HC≡CMgBr
CH3CH2CH2CHO
OH
C≡CH
CH3C(OCH2CH3)3
CH=C=CH
CO2CH2CH3
40
Al2O3
CO2CH2CH3
41
LiAlH4
OH
42
PBr3
Br
NaCH(CO2CH2CH3)2
CH(CO2CH2CH3)2
43

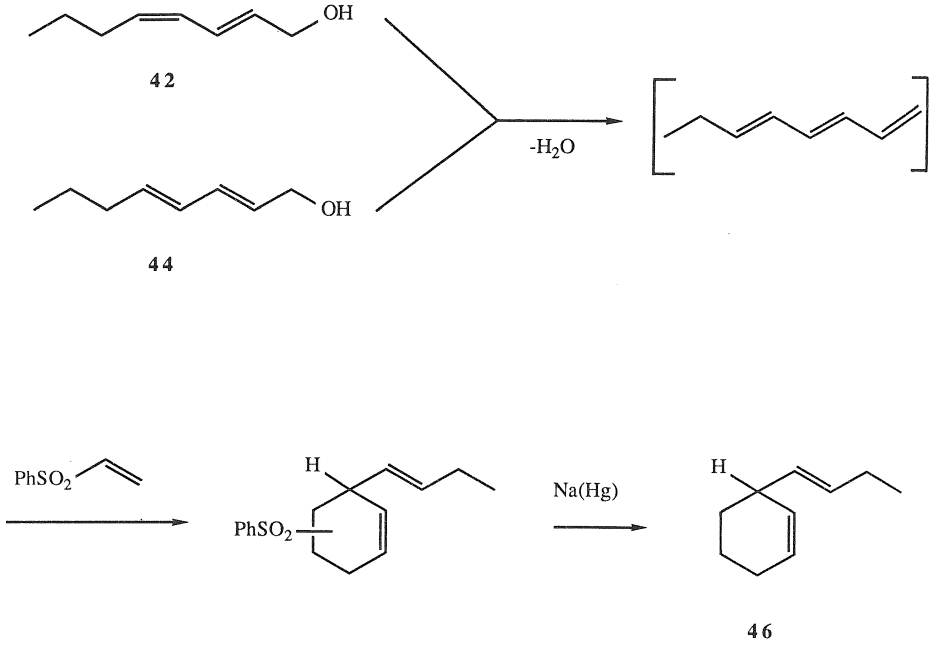
SCHEME 15



SCHEME 16

Since the Diels-Alder reaction steps were assumed to be the most crucial with respect to the yields, it would be advantageous to place this step as early as possible in the synthetic route. Phenyl vinyl sulfone has been shown to serve very conveniently as a reactive ethylene equivalent in [4+2] cycloadditions, and is the dienophile used in these reactions[43]. First, the dienic ester **41** (Scheme 15), was reacted with phenyl vinyl sulfone in dry benzene with some crystals of hydroquinone as a radical scavenger[44]. The reactants were placed in a Parr-bomb at 145° C. The yield, however, was very low even after long reaction times (up to seven days). The reason for this is probably the lack of pi-donor-acceptor complementarity for the diene and dienophile. Instead, the dienic alcohols **42** and **44** were reacted in the same way as the ester. This

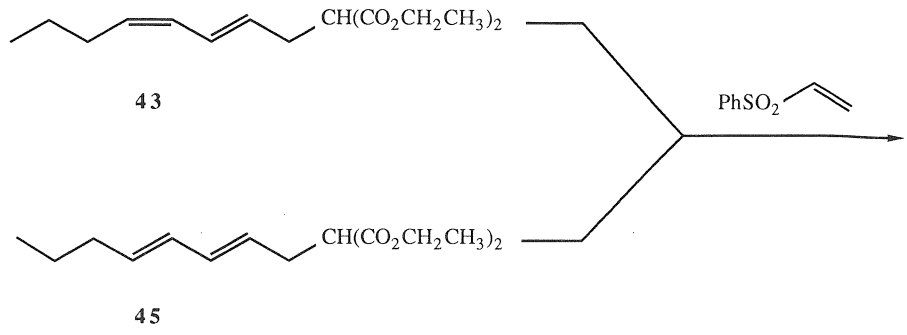
reaction proceeded in much better yields, but the product obtained was not the desired one (Scheme 17).



SCHEME 17

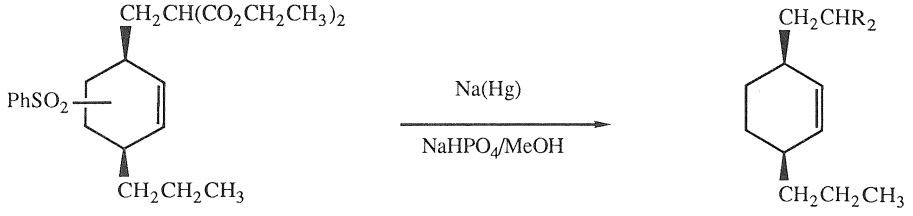
N.m.r and mass spectroscopy indicate that the result of this Diels-Alder reaction, after removal of the phenylsulfonyl group[45], is **46**, possibly generated by initial loss of water from the alcohols before cyclisation. In a new attempt, the two diesters **43** and **45** were used in the Diels-Alder reaction. This afforded cycloaddition products in acceptable yields, but unfortunately another disadvantage with the Diels-Alder method was demonstrated.

It was not possible to obtain the trans-substituted cyclohexene isomer, precursor to **29**, by this method. In both reactions the resulting product was the cis isomer (Scheme 18). Presumably, the diethyl (E)-2,(Z)-4-octadienyl malonate **43** isomerizes under



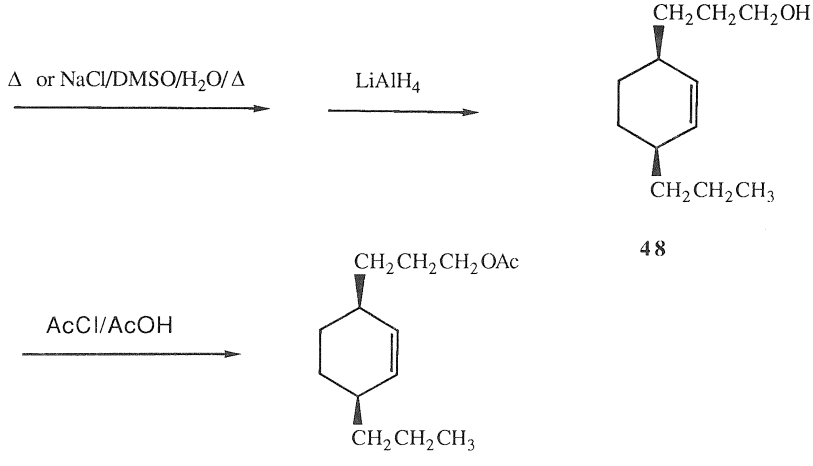
43

45



47

R= -COOH or -CO_2CH_3



48

28

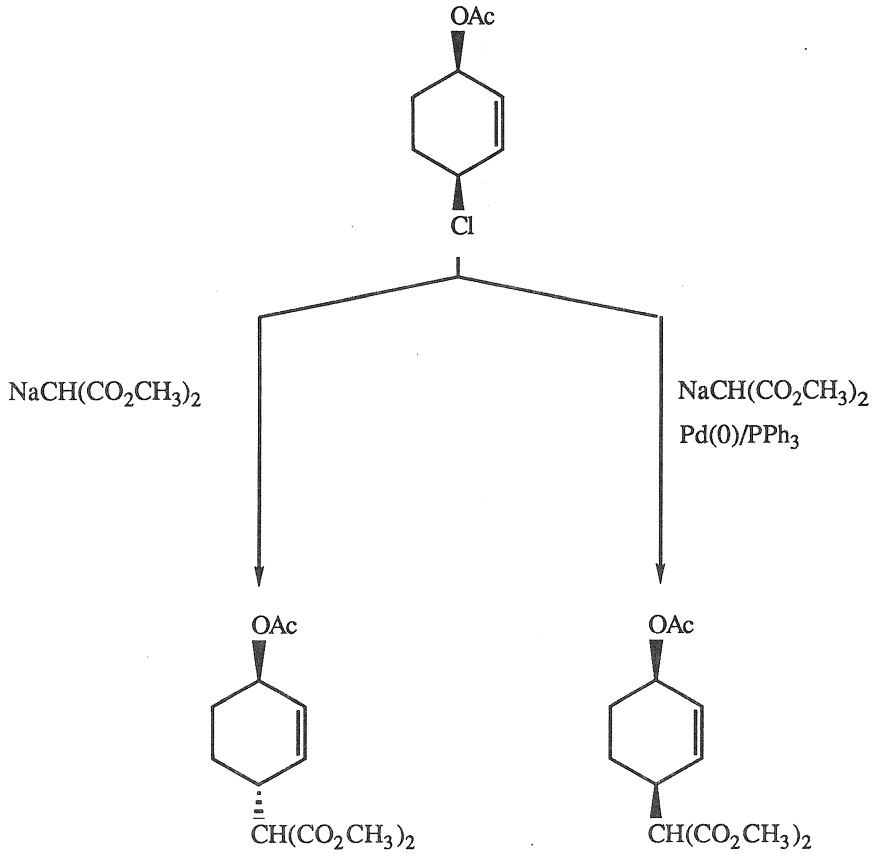
SCHEME 18.

these reaction conditions to the thermodynamically more stable (E),(E)-isomer. This is indicated by the fact that both reactions give approximately **the same product mixture**. Starting from the (E)-2,(Z)-4-isomer **43** (Scheme 18) the isomer ratio of the product **28** is 92% cis and 8 % of another unidentified isomer that **is not** the trans isomer. The corresponding values for the (E)-2,(E)-4-isomer **45**, Scheme 18, are 89% cis and 11% of the same unidentified isomer. Attempts to carry out the reaction with **43** at lower temperatures, thus achieving kinetic instead of thermodynamic control, gave hardly any yields at all even after long reaction times.

The sulfone **47** produced in the Diels-Alder reactions is easily desulfonated using excess 6% sodium amalgam in dry methanol[45]. The product obtained is a mixture of the diacid (R=COOH) and the dimethylester ($R=CO_2CH_3$) (Scheme 18). Decarboxylation, for the diacid merely by heating[35], for the dimethyl ester by heating with sodium- or lithium chloride in aqueous DMSO[46,47] and reduction gave the alcohol **48** which was acetylated in the usual way.

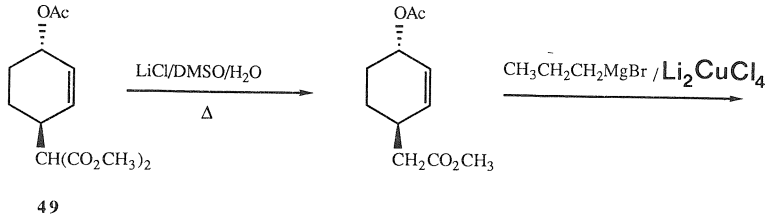
In 1985 Bäckvall et al. published a highly stereospecific synthesis of 1-acetoxy-4-chloro-alk-2-enes[48], including that of cis-1-acetoxy-4-chloro-cyclohex-2-ene. Since it is possible to sequentially and stereospecifically substitute the chloro- and acetoxy groups by two different nucleophiles[48], cis-1-acetoxy-4-chloro-cyclohex-2-ene constitutes a synthon for alternative routes leading to the cyclohexene analogues **28** and **29** . **28** was successfully synthesized with the Diels-Alder method, as described above, but since this is the most central compound of the structure-activity studies carried out in this thesis, it was considered beneficial to measure the biological activity of samples made by different synthetic procedures.

The chloro atom of cis-1-acetoxy-4-chloro-cyclohex-2-ene was substituted with sodium dimethyl malonate either in the classical way or with Pd(0) as a catalyst to yield the two starting materials dimethyl(trans-4-acetoxycyclohex-2-en-1-yl)malonate **49** and dimethyl(cis-4-acetoxycyclohex-2-en-1-yl)malonate **50** (Scheme 19).

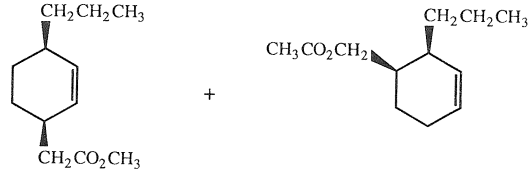


SCHEME 19

Recently, Sellén and Bäckvall extended their method to also include the introduction of non-stabilized carbon nucleophiles[49,50]. After decarboxylation, the acetoxy groups of **49** and **50** were substituted according to this technique. A propyl group was introduced by a Cu(II) catalysed Grignard coupling reaction with inversion of the configuration (Schemes 20 and 21).



49



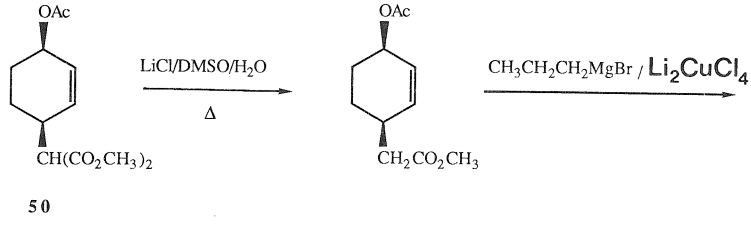
96 %

51

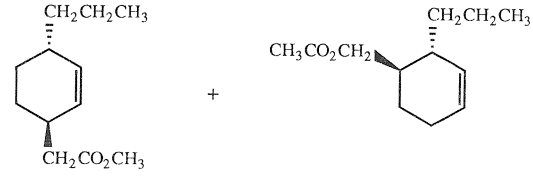
4 %

52

SCHEME 20



50



41 %

53

59 %

54

SCHEME 21

However, this reaction is not regiospecific, especially not for the reaction of the cis-isomer **50**. On substitution of **49** and **50**, in addition to the desired 1,4-substituted cis- and trans-cyclohexenes **51** and **53**, the 1,2-isomers **52** and **54** were produced. For the reaction of the trans-isomer **49**, the product composition was 96% 1,4- and 4% 1,2-isomer, while the corresponding result for the cis-isomer **50** was 41 % and 59 %. The formation of 1,2-isomer has been shown to be suppressed in favor of 1,4-isomer by the use of sterically more bulky nucleophiles than sodium dimethyl malonate[50b]. Therefore, methyl phenyl sulfonyl acetate and bis-(phenyl sulfonyl)-methane were tried under the same reaction conditions, but the yields in these reactions were very low.

The positional isomers are easy to separate by preparative g.l.c or preparative h.p.l.c., but this was only done for the final products. All reactions in Schemes 22 and 23 were carried out on the isomeric mixtures obtained by the Grignard coupling reactions.

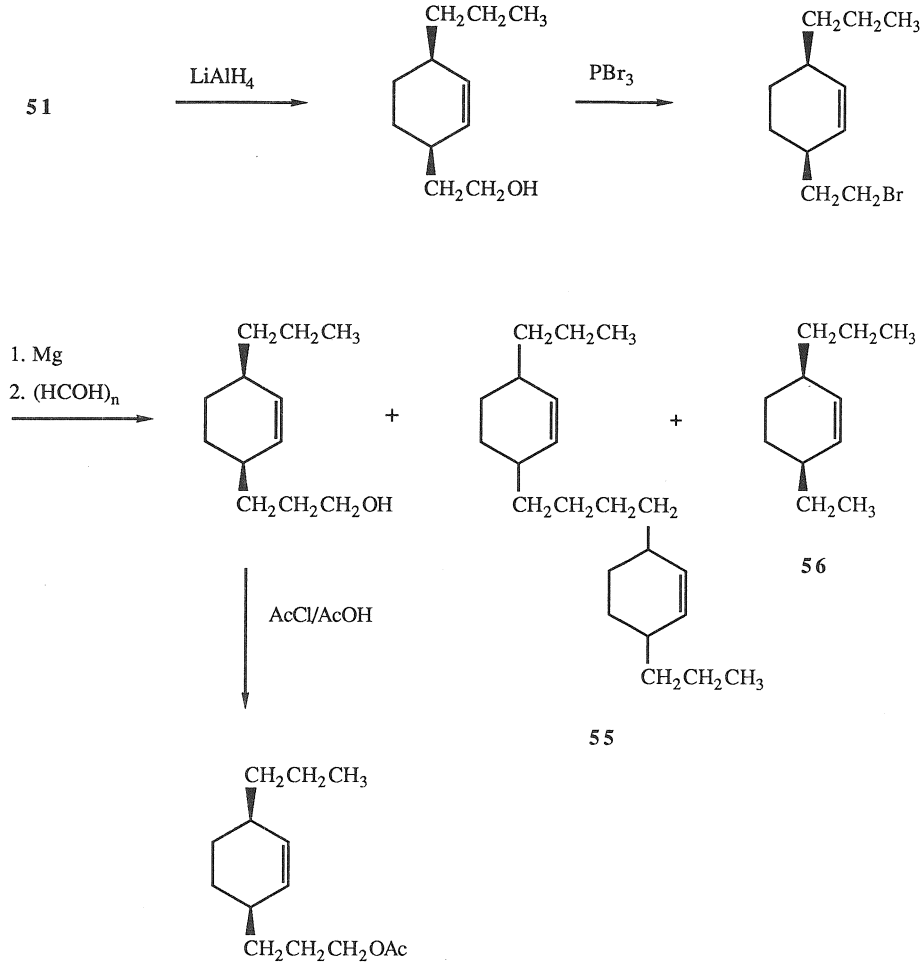
In the synthesis leading to **28**, the attempted one-carbon elongation reaction by a Grignard reaction with paraformaldehyde caused unexpected difficulties (Scheme 22). The reaction resulted in a moderate yield: only 26% of the product alcohol was isolated after flash chromatography. Instead, the two hydrocarbons **55** and **56** were formed to a great extent, indicating

i) problems forming the Grignard reagent and/or

ii) problems for the Grignard reagent to add formaldehyde.

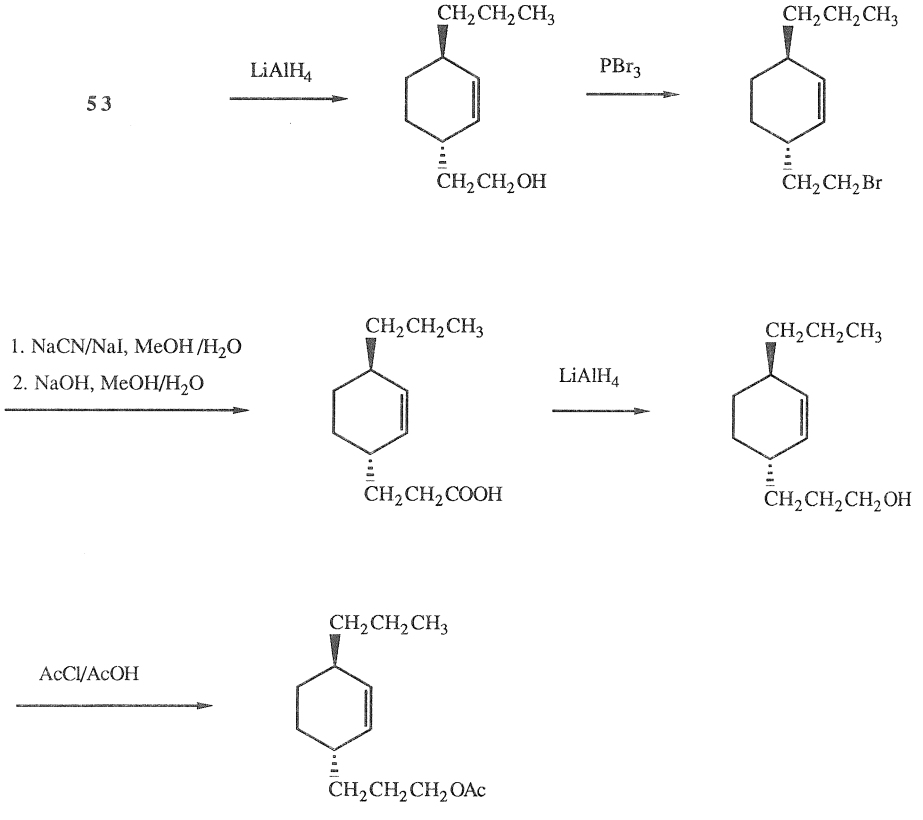
In order to overcome these problems, activation of the magnesium by ultrasonic radiation was attempted[51,52]. The flask containing the magnesium turnings in ether was immersed in an ultrasonic cleaning bath at 50° C 10 min. prior to the addition of the bromide, and then kept under these conditions during the reaction. Furthermore, the paraformaldehyde was thermally de-polymerized before addition to the Grignard reagent. However, these changes did not change the product composition to any great extent. Instead, highly reactive magnesium metal, prepared from anhydrous magnesium chloride and potassium, with the addition of sodium iodide to even further increase the reactivity, was used[53]. Also dried CO_2(g) was tried instead of paraformaldehyde. Even these conditions did not significantly influence the product formation.

Finally, the method of choice was to convert the cyclohexene bromide to the corresponding nitrile with sodium cyanide in aqueous methanol[54,55]. The resulting nitrile was then hydrolyzed without isolation, to afford the corresponding acid[56]. This procedure was applied in the synthesis of **29**, as shown in Scheme 23.


51
LiAlH4
CH2CH2CH3
CH2CH2OH
PBr3
CH2CH2CH3
CH2CH2Br
1. Mg
2. (HCOH)n
CH2CH2CH3
CH2CH2CH2OH
+
CH2CH2CH3
CH2CH2CH2CH2
CH2CH2CH3
+
CH2CH2CH3
CH2CH3
56
AcCl/AcOH
55
CH2CH2CH3
CH2CH2CH2OAc
28

SCHEME 22

The (trans-4-propylcyclohex-2-en-1-yl)-3-propanoic acid was obtained in 51% yield. Compounds **28** and **29** were purified from their corresponding 1,2-isomers by h.p.l.c. on a semi-preparative 500 mm Polygosil 60-5 μ C18-column with methanol/water 9/1 as eluant.



SCHEME 23

REFERENCES.

1. Sonnet P.E. in *"Techniques in Pheromone Research"*, Springer-Verlag New York Inc., p. 371 (1984).

2. Berger R.S. and Canerday T.D., *J. Econ. Entomol.*, **61**, 452 (1968).

3. Lindlar H., *Helv. Chim. Acta*, **35**, 446 (1952).

4. Warthen, Jr. J.D. and Jacobson M., *Synthesis*, 616 (1973).

5. Schwartz M. and Waters R.M., *Synthesis*, 567 (1972).

6. Kochansky J., Tette J., Taschenberg E.F., Cardé R.T., Kaissling K.-E. and Roelofs W.L., *J. Insect Physiol.*, **21**, 1977 (1975).

7. Warthen D. and Jacobson M., *J. Med. Chem.*, **11**, 373 (1968).

8. Chisholm M.D., Steck W. and Underhill E.W., *J. Chem. Ecol.*, **6**, 203 (1980).

9. Struble D.L. and Swailes G.E., *Envir. Entomol.*, **4**, 632, (1975).

10. Sonnet P.E., *J. Org. Chem.*, **39**, 3793 (1974).

11. Brattesani D.N. and Heathcock C.H., *Synth. Comm.*, **3**, 245 (1973).

12. Rossi R., Carpita A., Gaudenzi L. and Quirici M.G., *Gazz. Chim. Ital.*, **110**, 4 (1980).

13. Sax N.I. in *"Dangerous properties of industrial materials"*, Van Nostrand Reinhold Company Inc., New York, p. 1522 (1982).

14. Spencer H., *Chem. Ind.*, 728 (1979).

15. Mukhopadhyay T. and Seebach D., *Helv. Chim. Acta*, **65**, 385 (1982).

16. Brown H.C. and Zweifel G., *J. Amer. Chem. Soc.*, **83**, 3834 (1961).

17. Smith W.N. and Beumel, Jr. O.F., *Synthesis*, 441 (1974).

18. Ando T., Vu M.H., Yoshida S. and Takahashi N., *Agric, Biol. Chem.*, **46**, 717 (1982).

19. Schwartz J., *J. Organometal. Chem. Library*, **1**, 461 (1976).

20. Rossi R., Carpita A. and Quirici M.G., *Gazz. Chim. Ital.*, **111**, 173 (1981).

21. Rossi R. and Carpita A., *Synthesis*, 561 (1977).

22. a) Attenburrow J., Cameron A.F.B., Chapman J.H., Evans R.M., Hems B.A., Jansen A.B.A. and Walker T., *J. Chem. Soc.*, 1094 (1952).
b) Riemschneider R., Kasang G. and Böhme C., *Mh. Chem.*, **96**, 1766 (1965).

23. Wittig G. and Geissler G., *Liebigs Ann. Chem.*, **580**, 44 (1953).

24. Bestmann H.J., *Pure Appl. Chem.*, **51**, 515 (1979).

25. Bestmann H.J. and Vostrowsky O. in *"Selected Topics of Current Chemistry"*, Springer-Verlag, Berlin, **109**, 86 (1983).

26. Schlosser M., Tuong H.B. and Schaub B., *Tetr. Lett.*, **26**, 311 (1985).

27. a) Bestmann H.J. and Zimmermann R., *Fortschr. Chem. Forsch.*, **20**, 1 (1971).
b) Bestmann H.J., *Angew. Chem.*, **77**, 612 (1965).

28. Greenwald R., Chaykovsky M. and Corey E.J., *J. Org. Chem.*, **28**, 1128 (1963).

29. Goto G., Shima T., Masuya H., Masuoka Y. and Hiraga K., *Chem. Lett.*, 103, (1975).

30. Wannagat U. and Niederprum H., *Chem. Ber.*, **94**, 1540 (1961).

31. Bestmann H.J., Stransky W. and Vostrowsky O., *Chem. Ber.*, **109**, 1694 (1976).

32. Hall D., personal communication..

33. Disselnkötter H., Eiter K., Karl W. and Wendisch D., *Tetrahedron*, **32**, 1591 (1976).

34. Barabás A., Botar A.A., Gocan A., Popovici N. and Hodosan F., *Tetrahedron*, **34**, 2191 (1978).

35. Rossi R., Carpita A., Quirici M.G. and Veracini C.A., *Tetrahedron*, **38**, 639 (1982).

36. Ando T., Kusa K., Uchiyama M., Yoshida S. and Takahashi N., *Agric. Biol . Chem.*, **47**, 2849 (1983).

37. Ando T., Kurotsu Y., Kaiya M. and Uchiyama M., *Agric. Biol. Chem.*, **49**, 141 (1985).

38. Jacobson M., *J. Am. Chem. Soc.*, **78**, 5084 (1956).

39. Taber D.F., *J. Org. Chem.*, **47**, 1351 (1982).

40. Taber D.F., Supplementary material to *J. Org. Chem.*, **47**, 1351 (1982).

41. Martin J.G. and Hill R.K., *Chem. Rev.*, **61**, 537 (1961).

42. a) Tsuboi S., Masuda T. and Takeda A., *J. Org. Chem.*, **47**, 4478 (1982).
b) Tsuboi S., Masuda T., Makino H. and Takeda A., *Tetr. Lett.*, **23**, 209 (1982).

43. a) Carr R.V.C. and Paquette L.A., *J. Am. Chem. Soc.*, **102**, 853 (1980).
b) Snyder H.R., Anderson H.V. and Hallada D.P., *J. Am. Chem. Soc.*, **73**, 3258 (1951).

44. Azovskaya V.A., Prilezhaeva E.N. and Stepanyants A.U., *Izv. Akad. Nauk SSSR, Ser. Khim.*, **3**, 662 (1969); C.A., **71**, 254 (1969).

45. Trost B.M., Arndt H.C., Strege P.E. and Verhoeven T.R., *Tetr. Lett.*, **39**, 3477 (1976).

46. Krapcho A.P., *Synthesis*, 805 (1982).

47. Blum Z., Ekström M. and Wistrand L.-G., *Acta Chem. Scand. B*, **38**, 297 (1984).

48. Bäckvall J.-E., Nyström J.-E. and Nordberg R.E., *J. Am. Chem. Soc.*, **107**, 3676 (1985).

49. Sellén M., Bäckvall J.-E., Abstract from "*Organikerdagarna*", Stockholm, Sweden (1986).

50. a) Bäckvall J.-E. and Sellén M., *J. Chem. Soc., Chem. Commun.*, 827 (1987).
b) Sellén M., personal communication.

51. Suslick K.S., Schaffner K. and Demuth M., in *"Modern Synthetic Methods 1986"*, Ed., Scheffolg R., Springer Verlag, Berlin Heidelberg, p.1.

52. Sprich J.D. and Lewandos G.S., *Inorg. Chim. Acta*, **76**, L241 (1983).

53. Rieke R.D. and Bales S.E., *J. Amer. Chem. Soc.*, **96**, 1775 (1974).

54. Vogel A.I. in *"A text-book of practical organic chemistry including qualitative organic analysis"*, Longmans, Green and Co, London, p. 401 (1951).

55. Holan G. and O´Keefe D.F., *Tetr. Lett.*, 673 (1973).

56. Vogel A.I. in *"A text-book of practical organic chemistry including qualitative organic analysis"*, Longmans, Green and Co, London, p. 353 (1951).

CHAPTER 6

RESULTS OF THE STRUCTURE-ACTIVITY STUDIES.

6.1 Chain-elongated analogues. The new model described in Chapter 4 was used to recalculate the conformational energies required for the previously studied[1] chain-elongated analogues **4, 6, 7, 15-18** to acquire their "biologically active conformations". For details of the previously obtained results using the pilot model (Chapter 4, part 4.1), see Liljefors et al.[1] The purpose of this recalculation was to study the performance of the refined model in relation to the old one and to investigate if the calculated results are dependent on the choice of biologically relevant conformer for the natural compound **1** (**cisoid 1, cisoid 2** or **transoid**; see Chapter 4, part 4.2). The results are shown in Figure 1. All the relative activities are corrected for differences in volatility.

The calculated energies are clearly not very dependent on the conformer assumed to be the biologically active one for the natural pheromone component **1**. The observed minima in activity for compounds **6** and **16** correspond to calculated conformational energy maxima in all three cases, and the results are qualitatively similar to those previously obtained[1] . However, very satisfactorily, the refined model now makes it possible to put the results for chain-elongation on either side of the double bond on the same energy scale. The pilot model clearly exaggerated the conformational energies required for the analogues chain-elongated between the double bond and the acetate group, by as much as 8-10 kcal/mol[1] . This was due to the overly severe constraints imposed on the molecule in the pilot model. Thus, the added flexibility of the refined model resolves this problem.

From the results in Figure 1 it is possible to calculate the conformational energy corresponding to a decrease of the biological activity by a factor of 10. On the average this becomes 1.7, 1.7 and 1.6 kcal/mol for the **cisoid 1**, **cisoid 2** and **transoid** models, respectively. The similar results obtained with this series of compounds for the three different models precludes the possibility to select one of them as the most probable biologically active structure of the natural pheromone component **1**. For all three models, and for all seven analogues, the maximum calculated deviation from these numbers is 0.5 kcal/mol. The refined model thus significantly improves the results for the chain-elongated analogues **4, 6, 7, 15-18**, and gives an essentially quantitative relationship between calculated conformational energy and observed biological activity.

As an example of the structure of a calculated biologically active conformation, a

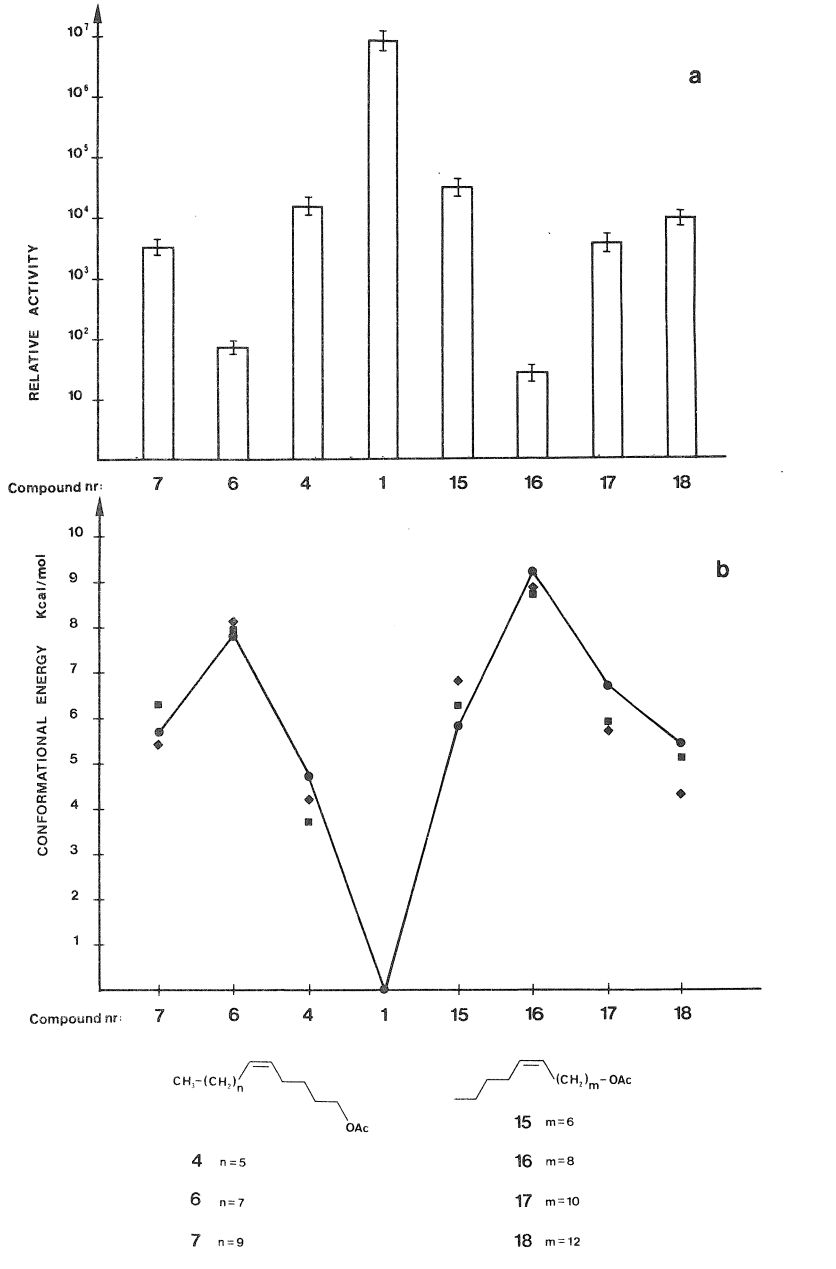


FIGURE 1. a) Experimental single-cell activities (from Liljefors et al.[1]) and
b) calculated conformational energies for the biologically active
conformations of chain-elongated analogues;
◆ **cisoid 1** model; ● **cisoid 2** model; ■ **transoid** model.

superimposition of the calculated "active structure" of compound **4** and the natural pheromone component **1** in its **cisoid 2** conformation is shown in Figure 2. To fit the geometrical requirements of the receptor-interaction model, **4** is forced to adopt gauche conformations about two adjacent bonds.

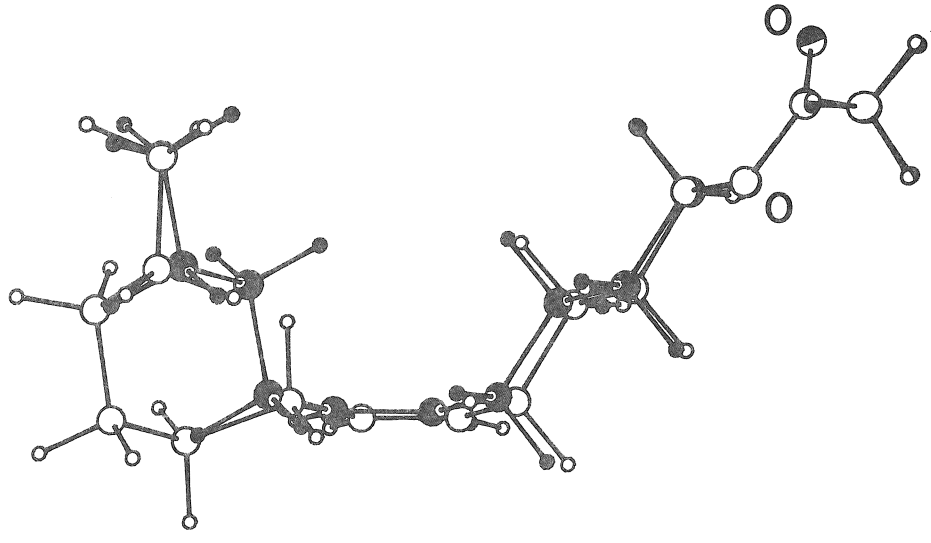


FIGURE 2. Superimposition of the **cisoid 2** conformer of **1** (filled atoms) and the calculated biologically active conformation of compound **4**.

The difference in double bond positions of **1** and **4** is quite small. The distance between the midpoints of the double bonds in **1** and **4** in the superimposition shown is 0.6 Å.

The calculated conformational energy of **4** in this "biologically active" conformation relative to the lowest energy one is 4.7 kcal/mol, which may be compared to the corresponding value using the pilot model, 5.8 kcal/mol. For compounds **15-18** the difference between the calculated energies using the old and new models is even larger, as much as 5-10 kcal/mol. This implies that a substantial reduction of the conformational energy may be achieved with a modest adjustment of the double bond position in the cavity of the receptor "active site". It should be noted that the calculated biologically active conformations of **4, 6, 7, 15-18** do not correspond to local energy minima of the unconstrained "free" molecules.

6.2 Dienic analogues. The measured electrophysiological single-cell activities of compounds **19, 21, 22, 24** and **26** relative to the natural pheromone component **1**, are shown in Figure 3a.

The replacement of a CH_2-CH_2 unit by an (E)-double bond in any of the investigated positions around the (Z)-double bond of **1** results in a decrease of the biological activity. However, depending on the position of the introduced additional double bond, this decrease in activity differs significantly.

It is of particular interest to note that the movement of the (E)-double bond the same number of steps in the two possible directions with respect to the (Z)-double bond, causes very different changes of the observed activity (Figure 3a). While the (E)-3,(Z)-5 diene, E(**i**-2) compound **21**, (**i** is the position of the (Z)-double bond in the natural component and in the dienes) has almost 100 times **higher** activity than the (Z)-5,(E)-7 analogue, E(**i**+2), compound **22**, the (E)-2,(Z)-5 diene, E(**i**-3), compound **19**, has about 50 times **lower** activity than the (Z)-5,(E)-8 diene, E(**i**+3), compound **24**. The second case is in line with field trapping results obtained by Chisholm et al[2]. It was observed that three moth species that use mono-olefinic acetates and alcohols as sexual pheromone components were attracted to pheromone blends in which a mono-olefinic component was replaced by a di-olefinic analogue with an additional (E)-double bond in the **i**+3 position. However, in this field study, conjugated diene analogues corresponding to compounds **21** and **22** were found to be totally devoid of attraction for male moths. This result is probably due to the limited possibility in field studies to observe attractancy of compounds which have low activity in electrophysiological recordings. Considering the results shown in Figure 3a, it seems reasonable to suggest that biologically significant signals for the male moths in field tests are obtained only from compounds with an electrophysiological single-cell activity not less than about one tenth of the activity of the natural pheromone component. Using this assumption,

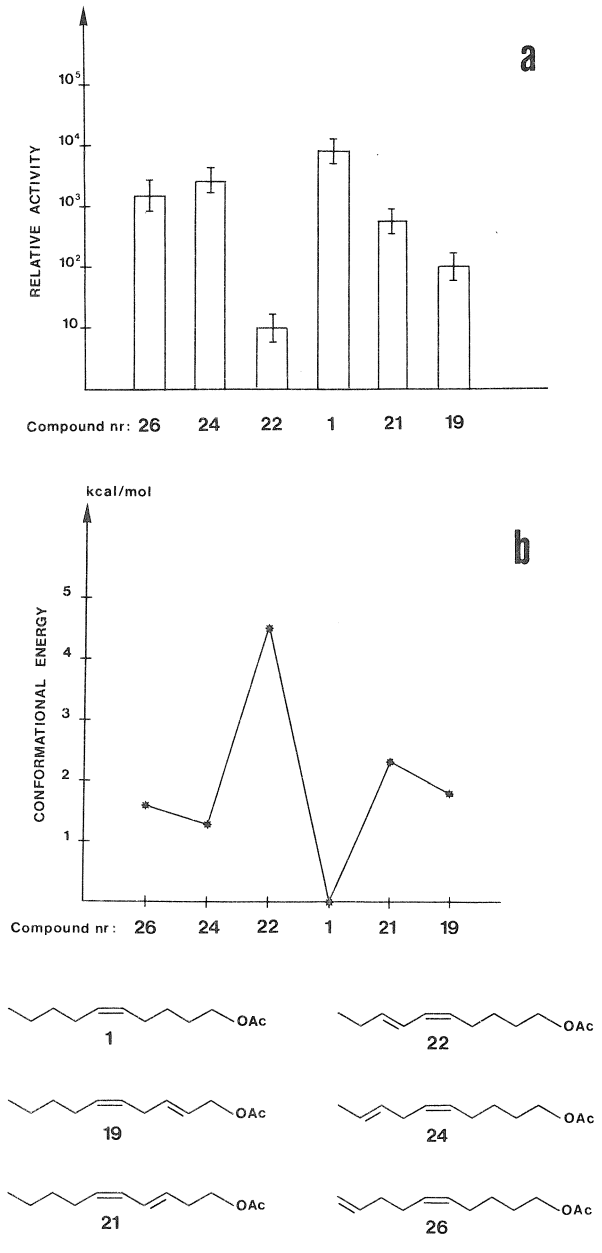


FIGURE 3. a) Experimental electrophysiological single-cell activities for compounds **19, 21, 22, 24** and **26.**

b) Calculated conformational energies (**cisoid 2** model) for the biologically active conformations of **19, 21, 22, 24** and **26.**

the field trapping results reported in reference 2 are in complete agreement with the electrophysiological results shown in Figure 3a.

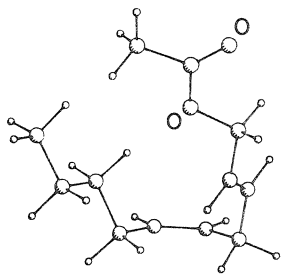
A similar effect was observed by Priesner et al. in an EAG screening of the antennal response of several species of *Noctuidae*[3]. For five of the species, di-olefins with an (E)-double bond in the **i**+3 position were shown to have an activity of only 1.8-5.6 times less than that of the monoenic natural pheromone component. No other diene analogues were investigated in this work.

One of the few studies on moth single cell responses to dienic acetates was performed on the tortricid moth, *Adoxophyes orana*. On stimulating a receptor cell selective for (Z)-9-decenyl acetate with di-olefinic compounds containing an additional (E)-double bond in the **i**+2 or **i**+3 positions, the activities observed were 100 and 10 times less, respectively, than that obtained on stimulation with (Z)-9-tetradecenyl acetate. The E(**i**-2) analogue was shown to have about one thirtieth of the activity of (Z)-11-tetradecenyl acetate when receptor cells specific for this compound were stimulated[4]. These results are also in line with the electrophysiological data in Figure 3a.

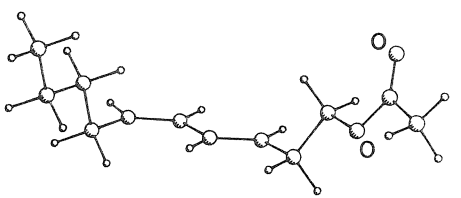
It should be borne in mind that these other studies referred to were performed with other moth species than the one studied in the present thesis. However, different noctuid moth species seem to respond very similarly to structural variations of a natural stimulus molecule[5].

Bestmann et al.[6] conclude from extensive EAG investigations of noctuid moths that structural variations of the alkyl parts of a pheromone component produce a more drastic loss of activity if the end alkyl part (the $(CH_2)_n$-part) is varied, than when the equivalent changes are made in the $(CH_2)_m$-part, see Chapter 3.

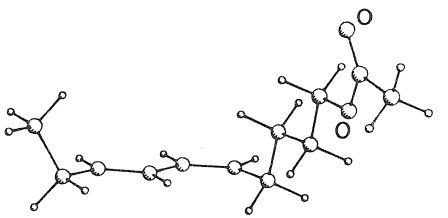
This "structure-activity rule" is not in agreement with the results in Figure 3a. It is found that the loss of activity due to the (E)-double bond in one case is more drastic for the $(CH_2)_n$-part (compounds **21** and **22**) but less drastic in another case (compounds **19** and **24**).



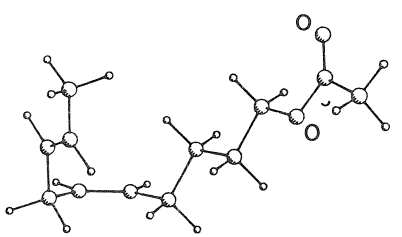
19



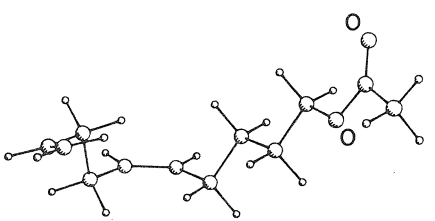
21



22



24



26

FIGURE 4. Calculated lowest energy conformers for compounds **19, 21, 22, 24** and **26**.

Calculated structures and conformational energies. The calculated thermodynamically most stable conformers of the dienes **19, 21, 22, 24** and **26** are shown in Figure 4.

In all cases the alkyl chains prefer a **cisoid** arrangement, but there are several other conformers, both **cisoid** and **transoid,** within 1 kcal/mole above the energy of the most stable one. Interestingly, the conformation of the alkyl chains in compound **19** seems to be determined by attractive van der Waals interactions between almost parallel chains. This conformer is favoured by 0.74 kcal/mol compared to the next most stable one. A van der Waals space-filling representation of **19** is shown in Figure 5.

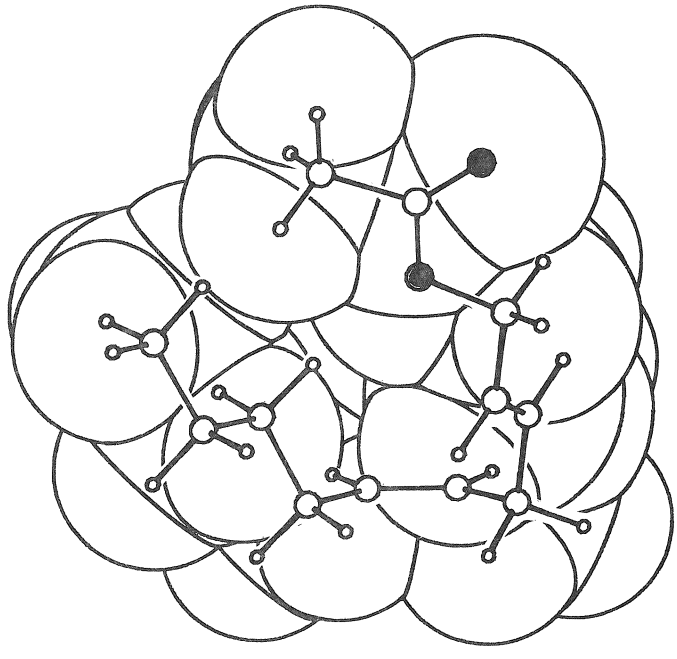


FIGURE 5

The calculated difference in conformational energy between the lowest energy structure suitable for receptor interaction according to our model and the global energy

minima are given in the Table for each of the diene analogues and for each of the three models of the biologically active conformation of **1**, according to Chapter 4:

Compound	Relative energy in kcal/mol ; Cisoid 1	Cisoid 2	Transoid
19	2.9	1.8	1.9
21	4.6	2.3	4.2
22	2.2	4.5	4.9
24	1.0	1.3	1.1
26	0.8	1.6	1.6

The most striking feature of the results in the Table, is the great difference in the calculated energies for the two conjugated dienes **21** and **22** with respect to the **cisoid 1** and **cisoid 2** models. Only for the **cisoid 2** model is there a correspondence between the calculated conformational energy for the biologically active conformation and the observed electrophysiological activity. The calculations for the **cisoid 1** model predict that compound **21** should be much less active than compound **22** in disagreement with the experimental results. The **transoid** model predicts equal biological activity for the two conjugated dienes **21** and **22**.

The calculated conformational energies assuming the **cisoid 2** model are summarized in Figure 3b. A comparison of Figures 3a and 3b clearly shows that there is a close correlation between calculated conformational energies using this model and the observed activities. Very satisfactorily, the relative activities of compounds **19** and **24** E(i-3) and E(i+3), respectively) are correctly calculated. The calculated conformational energy of compound **26** with a terminal double bond is also in agreement with the observed activity. This indicates that the interaction between the receptor and a methylene group is very similar to the corresponding interaction with a methyl group.

The calculated conformational energies correspond to enthalpies. However, since the translational, rotational and conformational entropies should be very similar for compounds **19, 21, 22, 24** and **26**, the calculated energies may be treated as free energies and may thus be directly used in comparisons with the experimental data.

Superimpositions of compound **1** in the **cisoid 2** conformation and the calculated biologically active structures of compounds **19, 21, 22, 24** and **26** are shown in Figure 6. It should be noted that the calculated biologically active conformations are not local minima for the unconstrained "free" molecules.

The reason for the difference in the calculated energies for the biologically active conformations of the conjugated dienes **21** and **22** are to be found in the difference in the C=C-C-C dihedral angles for the two alkyl chains in compound **1**. In the **cisoid 2** conformation of **1** the dihedral angles of the **m** and **n** alkyl chains are calculated to be -114.9 and 88.4 degrees, respectively (see Chapter 4, part 4.2). In the preferred conformers of **21** and **22** the diene systems are planar (Figure 4). When the diene structures are forced to mimic the natural pheromone component **1**, rotation about the (C=)C-C(=C) bond is necessary, with a concomitant increase of the conformational energy (see Figures 6b and c). To fit the geometrical requirements of the receptor-interaction model, the dihedral angle with the (E)-double bond belonging to the **n**-chain (corresponding to compound **22**) has to be significantly more perturbed from the preferred 180 degrees than is the case for the dihedral angle with the (E)-double bond belonging to the **m**-chain (compound **21**). The necessary C=C-C=C rotations are calculated to be 12.9 and 42.1 degrees for compounds **21** and **22**, respectively, leading to a higher conformational energy (and a lower biological activity) for compound **22**. The reverse is true for the **cisoid 1** model. In this case the corresponding dihedral angles for the **m** and **n** chains of **1** are calculated to be -87.2 and 116.1 degrees, respectively.

Since the replacement of a CH_2-CH_2 unit with an (E)-double bond in a carbon chain simulates an anti-conformation about that bond, this study strongly supports the assumption that the all-anti conformation of (Z)-5-decenyl acetate **1** is the biologically active conformation. For bond positions 3 and 8 this may be inferred directly from the experimental data shown in Figure 3a, considering the high activities of compounds **21** and **24**. The close correlation between conformational energy and observed electrophysiological activity assuming all-anti alkyl chains in **1** makes it highly probable that bonds 2 and 7 also have anti conformations in the biologically active structure of **1**.

The structure-activity results presented above (part 6.1) for chain-elongated analogues of **1**, were shown to be largely independent of the choice of model for the biologically active conformation of **1** with respect to conformations about the vinylic bonds (**cisoid 1, cisoid 2** or **transoid;** see Chapter 4). However, only the **cisoid 2**

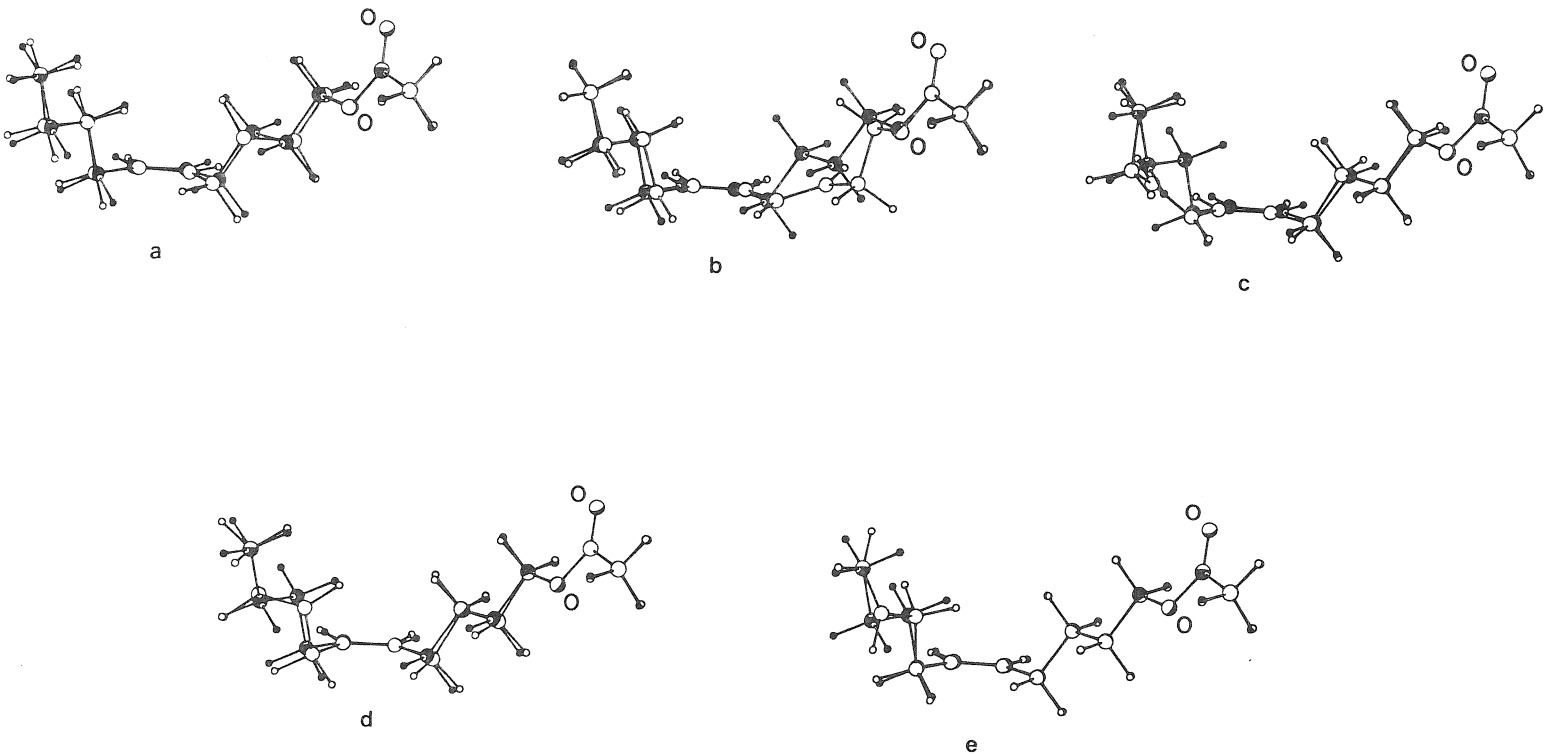


FIGURE 6. Molecular superimpositions of the **cisoid 2** conformer of **1** (filled atoms) and the calculated biologically active conformations of a) **19** b) **21** c) **22** d) **24** and e) **26**.

model is fully compatible with the experimental data for the dienic analogues studied in the present work.

In the previous structure-activity studies on chain-elongated analogues (part 6.1) it was found that there is a fairly constant conformational energy increase, 1.7 kcal/mol, corresponding to a 10-fold decrease of the observed electrophysiological activity. For the dienes **19, 21, 22, 24** and **26** the corresponding number is calculated to be 1.9 +/- 0.4 kcal/mol.

6.3 Double bond configurational isomers. The measured electrophysiological single-cell activities for compounds **2, 5, 19** and **20** relative to the natural pheromone component **1** are shown in Figure 7a.

The (E)-isomer **2** is a factor of 100 times less active than the corresponding natural (Z)-isomer **1**. This result is in good agreement with earlier structure-activity studies. In an EAG screening of noctuid species, Priesner el al.[3] report that the (E)-isomer is 1.8-5.6 times less active than the (Z)-isomer. Single-cell measurements show up to 10-fold less activity for the (E)-isomer in noctuid species[5]. In tortricid species this difference is usually about 100-fold [4,5,7].

Chain elongation by two methylene units (compound **5**) further lowers the activity by about a factor of 100 (including corrections for differences in vapour pressure). This may be compared to the relative activity for (Z)-5-dodecenyl acetate **4** with respect to **1**, which is ca 1000 (part 6.1, Figure 1). The loss of activity due to chain-elongation is thus somewhat less in the (E)-series than in the corresponding (Z)-series.

Introduction of an (E)-double bond in the 2-position of the natural pheromone component (compound **19**) has approximately the same effect as the Z/E configurational change (Figure 7a); the observed activity is reduced by a factor of ca 100. Surprisingly, no further significant change in the activity was observed for the (E)-5-isomer **20** of this diene. The close similarity of the electrophysiological response of the two compounds is demonstrated in Figure 8, which shows the dose-response curves for the natural pheromone component **1** and the two diene analogues **19** and **20**.

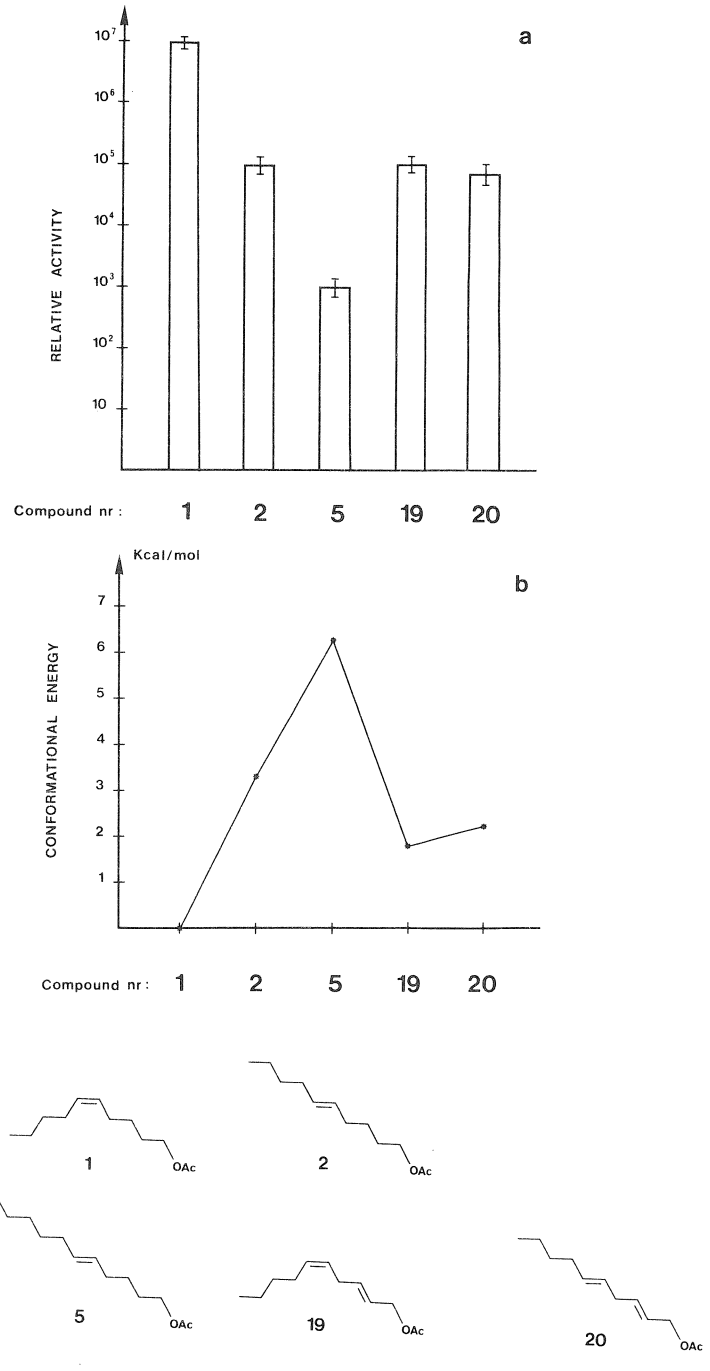


FIGURE 7. a) Experimental single-cell activities for compounds **1, 2, 5, 19** and **20**.
b) Calculated conformational energies for the biologically active conformations (**cisoid 2** model) of compounds **1, 2, 5, 19** and **20.**

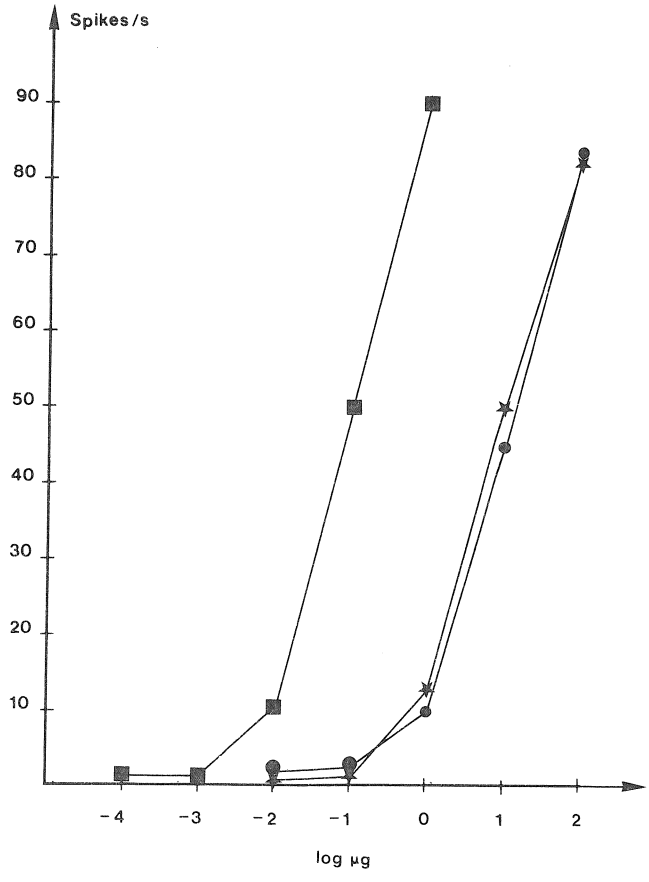


FIGURE 8. Dose-response curves for compounds **1**: ■ , **19**: ★ , **20**: ● obtained by electrophysiological single-cell recordings.

The effect of configurational change is thus not additive, but depends on the properties of the entire molecule.

The conformational energies required for compounds **2, 5, 19** and **20** to acquire their biologically active conformations, according to the receptor-interaction model described in Chapter 4, were calculated and the results are shown in Figure 7b. (The results for the **cisoid 2** model according to Chapter 4 are shown. The results for the other two models are very similar in this series).

The calculated conformational energies are clearly very closely correlated with the

measured electrophysiological activities. The calculated conformational energy for compound **2** is 3.3 kcal/mol, which corresponds to 1.65 kcal/mol for each power of ten of decrease in activity compared to **1**. This is in agreement with the numbers obtained above (parts 6.1 and 6.2), for the chain-elongated and the dienic analogues.

A superimposition of compound **2** in its calculated "biologically active conformation" and compound **1** is shown in Figure 9.

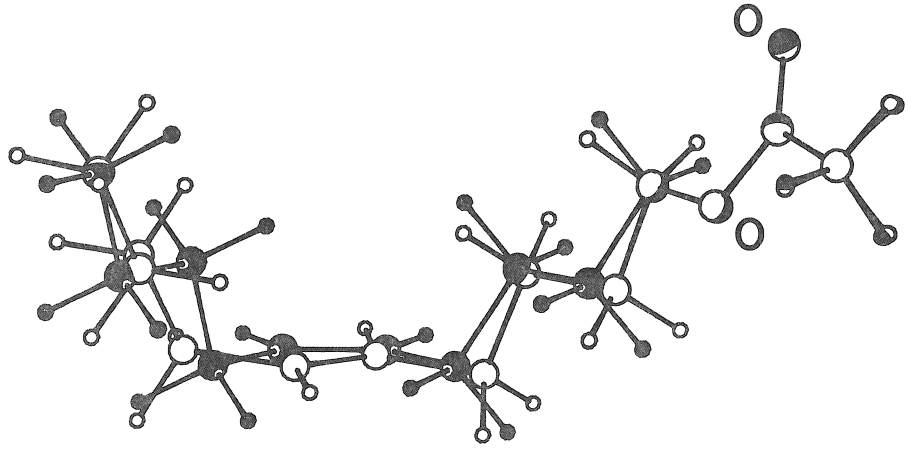


FIGURE 9

The (E)-isomer is forced to adopt a gauche-anti conformation of the alkyl chain connecting the double bond and the methyl group. The position of the (E)-double bond in **2** is sufficiently close to that of the (Z)-double bond in **1** to assure similar interactions with the binding site.

The chain-elongated (E)-isomer **5** is calculated to have a conformational energy requirement of 6.3 kcal/mol to reach its "biologically active conformation". Using the value 1.65 kcal/mol for a tenfold decrease in activity as obtained above, this should give a reduction of the activity by a factor of ca 100 compared to that of **2**, which is also observed (Figure 7a). Furthermore, comparing compounds **2** and **5**, the conformational energy for reaching the biologically active conformation is increased by

3.0 kcal/mol due to chain-elongation by two methylene groups in the (E)-series. This may be compared to the corresponding value of 4.7 kcal/mol in the (Z)-series (part 6.1, Figure 1b, compound **4**). The calculations thus also reproduce the observation that chain elongation in the (E)-series leads to a smaller decrease of the activity than in the (Z)-series.

Very gratifyingly, the unexpected similarity of the activities of compounds **19** and **20** is also well calculated. After conformational rearrangements which increase the energy by 1.8 kcal/mol (Figure 7b) the (E)-2,(Z)-5 diene **19** becomes an extremely good "mimic" of the natural pheromone component **1**, as is demonstrated by the superimposition of the two molecular structures in Figure 10a.

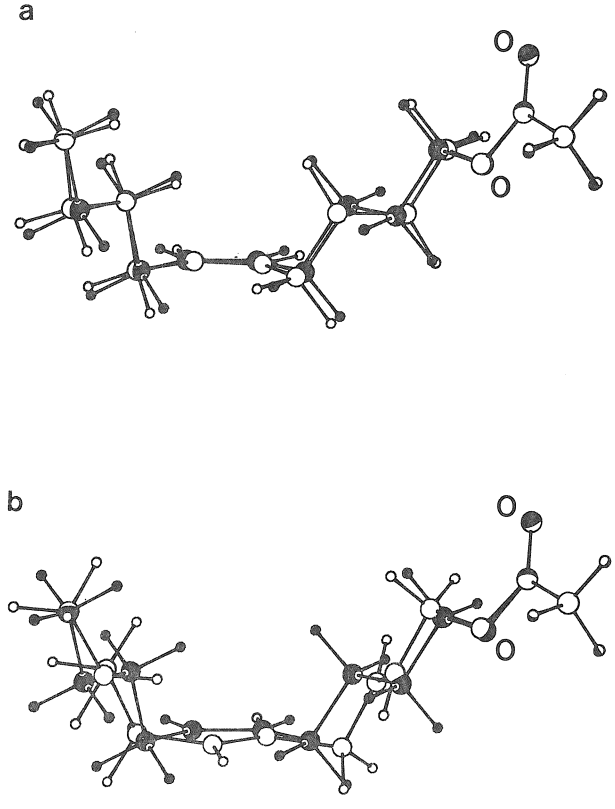


FIGURE 10. Superimposition of the **cisoid 2** conformer of **1** (filled atoms) and the calculated biologically active conformations of a) compound **19** and b) compound **20**.

The (E)-double bond in **19** closely mimics the anti-conformation of the corresponding saturated fragment in **1**. The (E)-2,(E)-5 diene **20** requires a conformational energy of 2.2 kcal/mol, only slightly higher than that for **19**, to mimic the molecular shape of **1**, as can be seen in Figure 10b.

As mentioned above, in the structure-activity studies on chain-elongated analogues (part 6.1), dienic analogues (part 6.2), and double bond configurational isomers (part 6.3) to **1**, a fairly constant conformational energy increase corresponding to a decrease in the observed electrophysiological activity was found. Thus, for fifteen analogues of **1** belonging to three different structural classes, there is a good linear relationship between calculated conformational energies and experimental activities. This relationship is shown in Figure 11. The correlation coefficient for the regression line is 0.965. This correlation suggest that the **cisoid 2** conformer or a "nearby" conformation may be the biologically active one for the natural pheromone component.

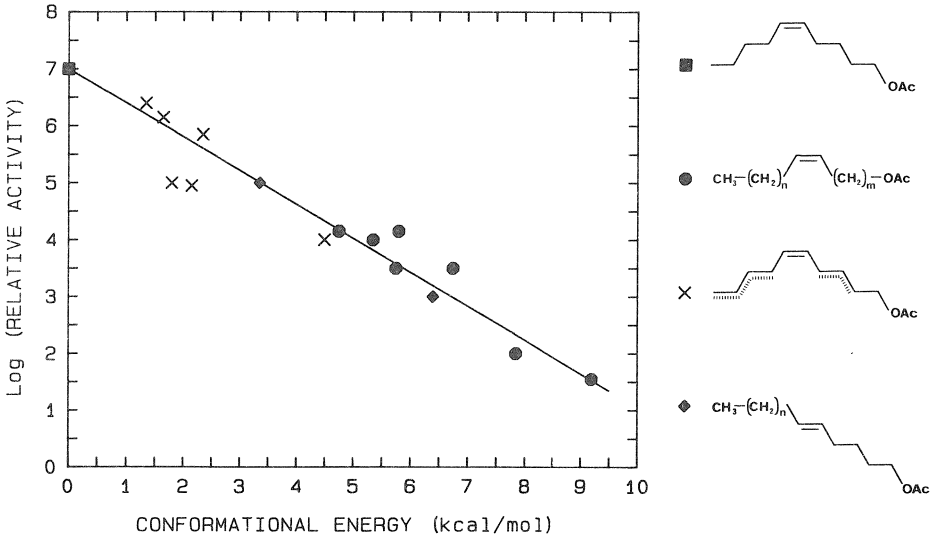


FIGURE 11

6.4 Cyclic analogues. The cyclic analogues - the benzene analogue **27**, the cis-cyclohexene and trans-cyclohexene analogues **28** and **29** - were designed and synthesized to test certain central predictions about the biologically active conformation of the pheromone component **1**:

i) the biologically active conformation is planar.
ii) the biologically active conformation is the **cisoid 2** conformer.

In some of the models on pheromone action discussed in Chapter 3, a planar conformation is implied as the biologically active one for the pheromone component and its analogues (Parts 3.1, 3.2 and 3.3). The benzene analogue **27** can achieve a planar conformation with no great increase in conformational energy, and therefore constitutes a test of this prediction.

The results obtained from the studies of the dienic analogues (part 6.2) strongly indicate that the **cisoid 2** conformer or a "nearby" conformation may be the biologically active one for the natural pheromone component **1**. Furthermore, the **cisoid 2** model could also rationalize the electrophysiological activities for several other analogues, as summarized in Figure 11 (Part 6.3). The cis-cyclohexene analogue **28** and the trans-cyclohexene analogue **29** were designed and synthesized to test this prediction. Since the alkyl chains are incorporated into a cyclic structure (see Figure 12), compounds **28** and **29** provide a possibility to **separately** test the **cisoid** and **transoid** conformations of the pheromone component **1**.

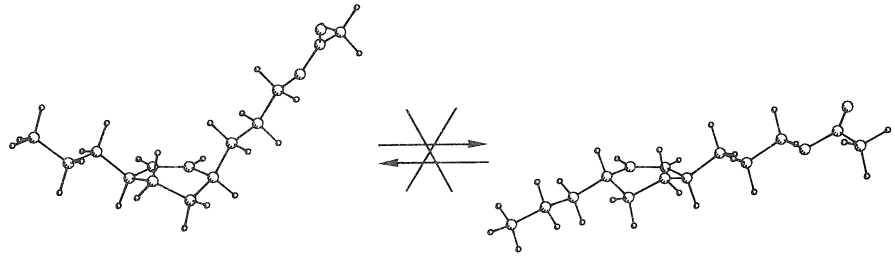


FIGURE 12

In the electrophysiological single-cell measurements, compound **27** was shown to be essentially inactive. This total lack of activity strongly indicates that the biologically active conformation of **1 is not a planar one.**

The conformationally restricted analogues **28** and **29** are of course not perfect mimics of (Z)-5-decenyl acetate. Energy will be required for these analogues to rearrange to conformations similar to the low-energy ones of (Z)-5-decenyl acetate **1**. (This is a problem of general nature in studies of conformationally restricted analogues). Furthermore, **28** and **29** do not have the same possibilities to fit into the three models, **cisoid 1**, **cisoid 2** and **transoid** (Chapter 4) as the analogues previously studied in this Chapter. Analogue **28** may only be applied to the **cisoid 1** and **cisoid 2** models, while analogue **29** may be applied only to the **transoid** model.

The calculated conformational energies assuming the **cisoid 1** and **cisoid 2** models for the cis-cyclohexene analogue **28** and the **transoid** model for the trans-cyclohexene analogue **29** are given below:

Compound	**Cisoid 1**	**Cisoid 2**	**Transoid**	
28	1.79	1.88		kcal/mol
29			4.65	kcal/mol

In the previous structure-activity studies presented in this Chapter, a fairly constant conformational energy increase of 1.7 kcal/mol was found to correspond to a 10-fold decrease of the observed electrophysiological activity (part 6.3). If this is assumed to also be valid for the analogues **28** and **29**, the theoretical predictions of the conformational-energy model are as shown in Figure 13.

Thus, the model predicts two possible results from the interaction of **28** and **29** with the receptor cell for the pheromone component **1**:

i) The biologically active structure of **1** is **cisoid**. As a result, the cis-compound **28** will have a high activity, about a factor of 10 less than that of the pheromone component **1**, while the trans-compound **29** will be inactive.

ii) The biologically active structure of **1** is **transoid**. As a result, the trans-compound **29** will have a moderate activity, about a factor of 1000 less than that of the pheromone component **1**, while the cis-compound **28** will be inactive.

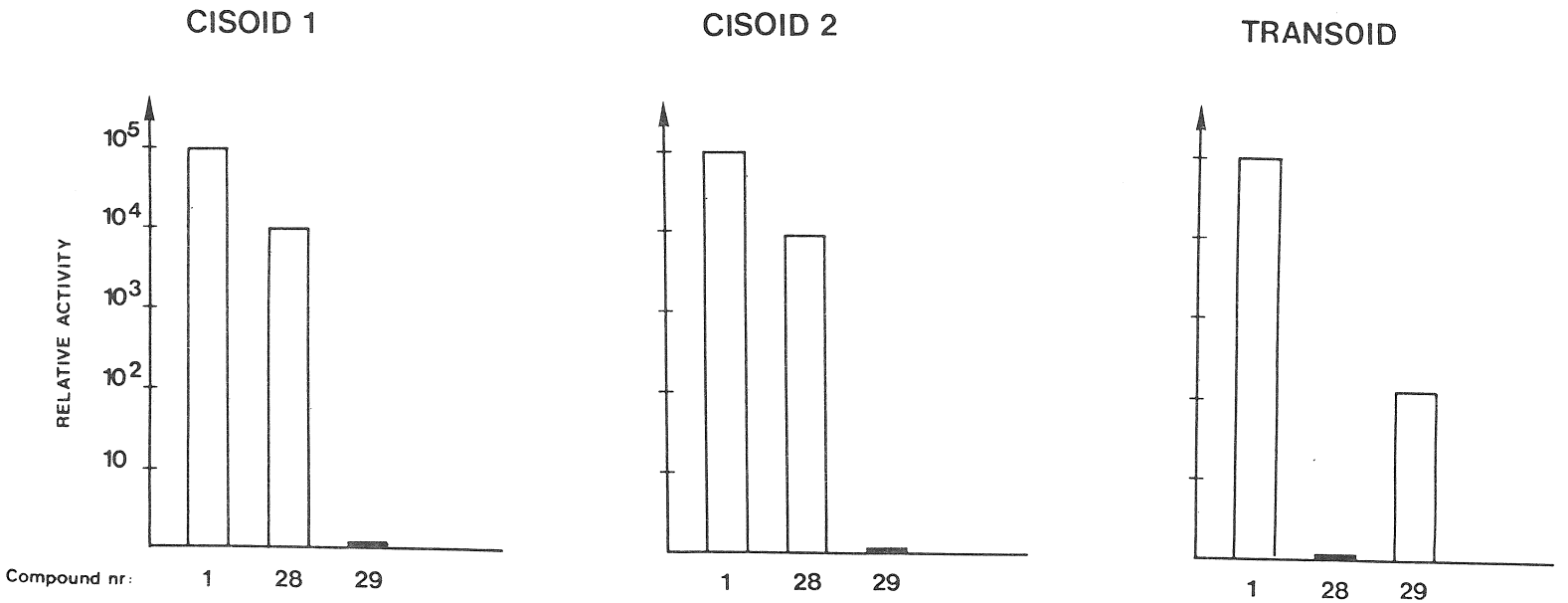

CISOID 1
CISOID 2
TRANSOID
RELATIVE ACTIVITY
10^5
10^4
10^3
10^2
10
Compound nr:
1
28
29
1
28
29
1
28
29

FIGURE 13

These theoretical predictions, obtained by the model calculations, reveal the possibility to distinguish between a **cisoid** or **transoid** type of conformation as the biologically active one of **1**. However, it will not be possible to distinguish between the **cisoid 1**- and **cisoid 2**-type conformations of **1**.

The measured electrophysiological single-cell activities of compounds **28** and **29**, including corrections discussed in the following section, are shown in Figure 14.

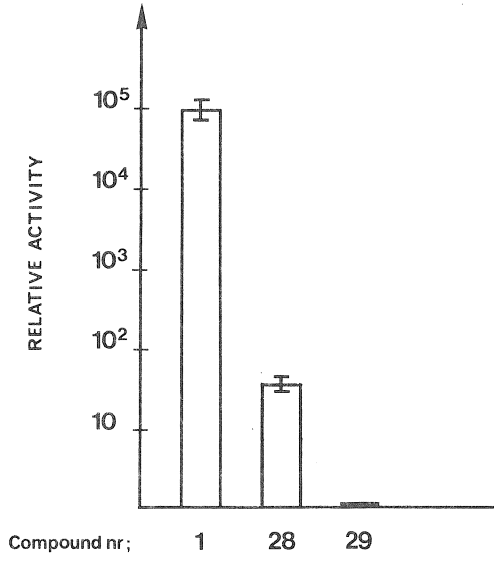


FIGURE 14. Experimental single-cell activities for compounds **28** and **29**.

The activities of the cyclohexene analogues are corrected for differences in volatility compared to the natural pheromone component **1**. The ratio of the vapour pressures was assumed to be the same as the ratio between cis-2-butene[8] and cyclohexene[8], which was estimated to be a factor of about 18.

Furthermore, the introduction of a cyclic structure into (Z)-5-decenyl acetate makes it necessary to take certain other aspects into account. The cyclic structure introduces two chiral carbon atoms that generate two enantiomers of compounds **28** and **29**. It has been suggested by Chapman et al.[9] that receptors for pheromone components in

Lepidoptera are chiral. This was concluded after studies on the racemate and on the optically pure enantiomers of chiral analogues of (Z)-11-decenyl acetate, a pheromone component of the European corn borer, *Ostrinia nubilalis* and the redbanded leafroller, *Argyrotaenia velutinana*. Similar results were obtained by Bestmann and Vostrowsky[6,13]. If, in accordance with these results, an element of chirality is assumed in the receptors of *Agrotis segetum,* the result is that only **one** of the enantiomers of **28** and **29** can interact with the receptor cell. This makes it necessary to correct the measured activity by a factor of 2.

Furthermore, the cyclic structure of **28** introduces a significant energy barrier between its two low-energy conformations of **cisoid 1**- and **cisoid 2**-type. A conversion between these two conformations of compound **28** implies an inversion of the cyclohexene ring (see Figure 15).

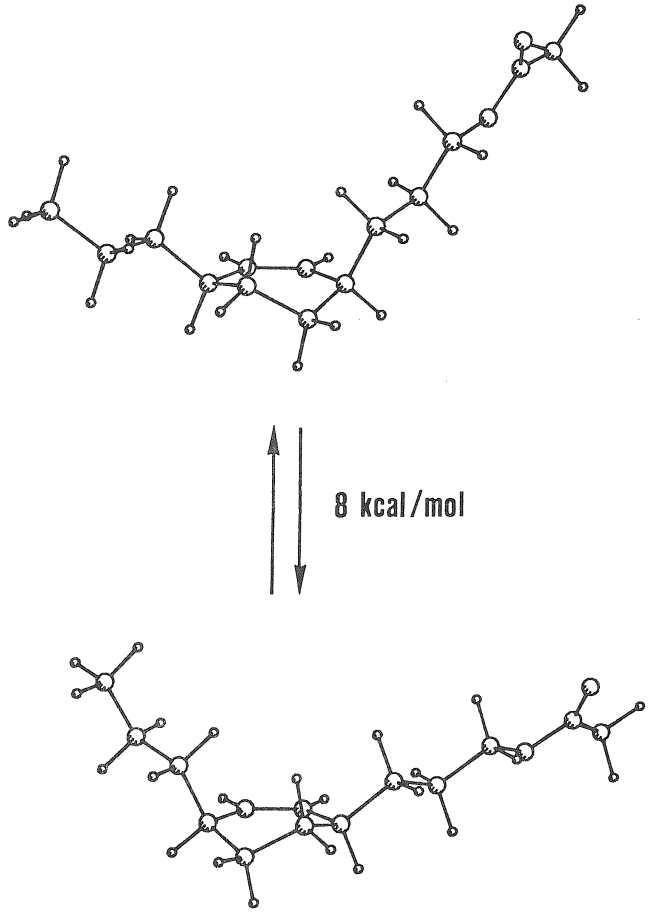


FIGURE 15

The barrier for this ring inversion was calculated to be 8.0 kcal/mol. Since the arrangement in space of the alkyl chains in the **cisoid 1** and **cisoid 2** type conformations are very different, it is not likely that both conformations may successfully interact with the receptor cell. This makes it necessary to adjust the measured activity by a factor of 2. Altogether these corrections increase the measured activities by a factor of 72.

If the two theoretical predictions **i)** and **ii)** obtained by the model are compared with the experimentally measured biological activities (Figure 14), it is obvious that **only** prediction **i)**, suggesting a **cisoid** structure of the pheromone component as the biologically active one, is in agreement with experimental data. This is also in agreement with the results previously obtained for fifteen other analogues (part 6.3).

However, the quantitative prediction for the activity of compound **28** obtained by the model, is not very well correlated with the experimentally obtained value. The model predicts compound **28** to have an activity only a factor of 10 less than the pheromone component **1**. Even when the conformational properties of the cyclohexene analogue **28** are taken into account, the observed biological activity of **28** is still a factor of about 100 less than that predicted by the model.

A possible explanation may be that the cyclohexene ring of **28** to some extent prevents a favourable interaction with the receptor cell. In the thermodynamically stable conformation of compound **28**, one of the axial hydrogen atoms in the cyclohexene ring has a position in space that may affect the interaction of the postulated receptor site for the (Z)-double bond and the double bond of the cyclohexene ring (see Figure 16).

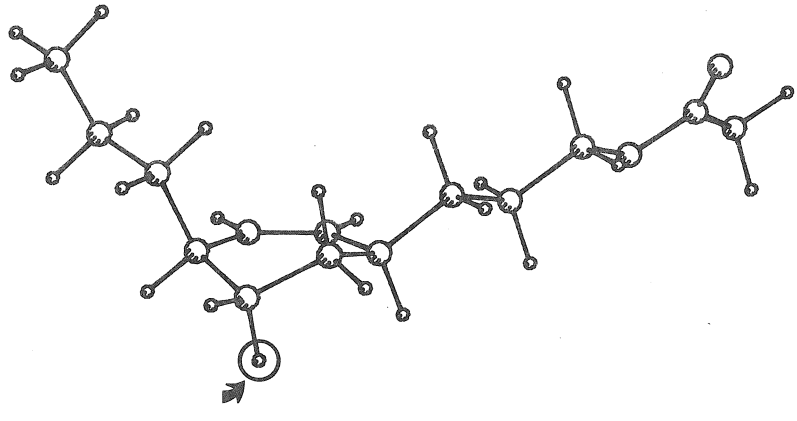


FIGURE 16

Furtermore, if the biologically active conformation of **1** is a "nearby" conformation to **cisoid 2**, the cyclic analogues **28** and **29** will require significantly more energy to acquire such a conformation, than the natural pheromone component **1.** This would also result in a lower activity than that predicted for the cyclic analogues.

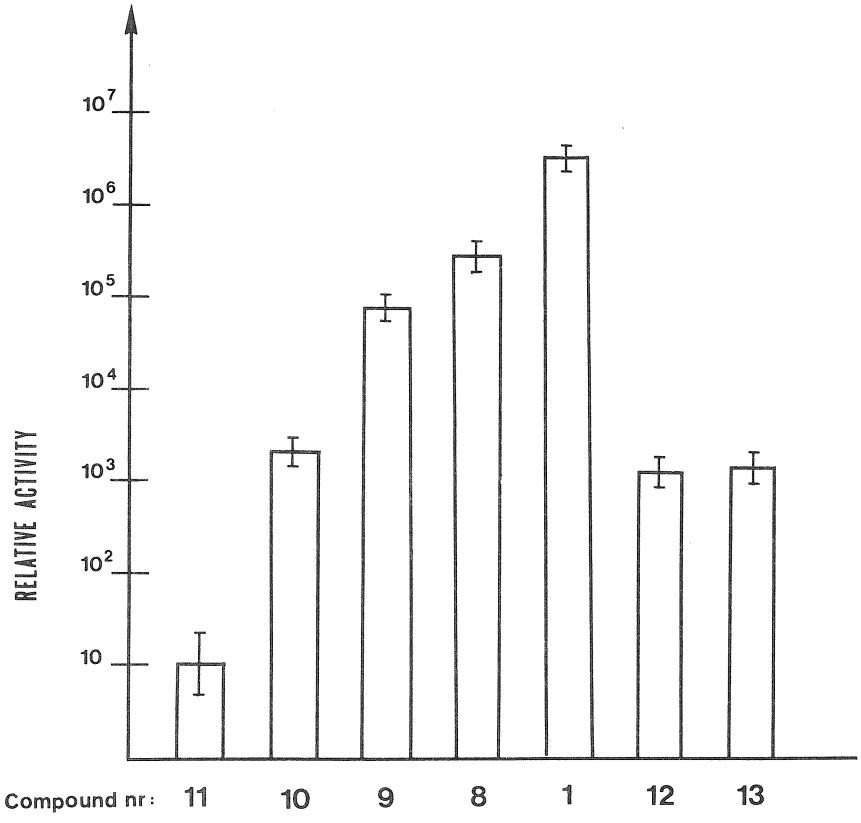
These hypotheses may be tested by the synthesis and investigation of analogues with other ring sizes, for example five- or seven-membered rings. A five-membered ring has no hydrogen atoms in the assumed critical position in space. A seven-membered ring is more flexible than the six-membered ring investigated, and could therefore be assumed to be less sensitive to small changes in the conformation. This would decrease the influence on the results for the case of a "nearby" conformation to **cisoid 2** as the biologically active one of **1**.

6.5 Chain-shortened analogues. As mentioned above (Chapter 4), the chain-shortened analogues can not be directly fitted into the conformational energy model, because they lack the possibility to optimally interact with all three assumed interaction sites. However, these compounds may give information important for the further development of the receptor-interaction model.

The measured electrophysiological single-cell activities for compunds **8-13** are shown in Figure 17. All the chain-shortened analogues show a decrease of the biological activity, compared to the pheromone component **1**. For the analogues chain-shortened in the **n**-chain, a loss of activity is found for each additional CH_2-group removed (compounds **8-11**).

The loss of activity is about a factor of 10 for each of the first two CH_2-groups (compounds **8** and **9**), and increases with further shortening of the chain, finally giving the essentially inactive compound **11**. Thus the **n**-alkyl chain retains possibilities to interact with the end-methyl receptor site to a certain extent, even when the methyl group in position 10 of compound **1** is missing. This indicates that this receptor site interacts with parts of the **n**-alkyl chain and not only with the end-methyl group.

When the entire **n**-alkyl chain is removed (compound **11**), the electrophysiological activity is essentially lost. This shows the necessity for a compound to interact - at least



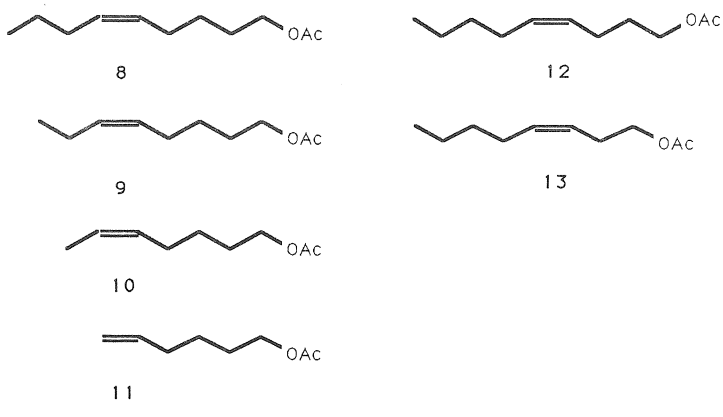


FIGURE 17. Experimental single-cell activities for compounds **8-13.**

to some extent - with **all three** postulated receptor sites, to achieve electrophysiological activity.

These results are in line with structure-activity studies performed on some moth and other lepidopteran species. In EAG screenings of several species of *Noctuidae* and *Tortricidae*[5,10], Priesner observed that, for 5 of the species, analogues chain-shortened by one (CH_2)-unit in the **n**-chain had an activity 5.6-18 times less than that of the natural pheromone component. With further **n**-chain-shortening, the activity continued to decrease. The analogues chain-shortened by two and three (CH_2)-groups were found to have 18-180 and 180-560 times less activity, respectively.

During field studies, Struble and Byers[11] observed that the darksided cutworm, *Euxoa messoria,* for which the pheromone components were identified as (Z)-7- and (Z)-11-hexadecenyl acetate, was attracted to pheromone blends in which (Z)-7-hexadecenyl acetate was replaced by chain-shortened analogues. A mixture of (Z)-7-pentadecenyl acetate and (Z)-11-hexadecenyl acetate attracted 75 % as many males as the mixture of both the pheromone components, while the corresponding mixtures including (Z)-7-tetradecenyl acetate and (Z)-7-tridecenyl acetate attracted 55 % and essentially 0 %, respectively.

Even though recordings from single receptor cells is the method of choice for structure-activity relationships, very few studies on moth species have been done. The sexual pheromone of the tortricid moth, *Adoxophyes orana*, is a blend of (Z)-9- and (Z)-11-tetradecenyl acetate. On stimulation of a male receptor cell selective for (Z)-9-tetradecenyl acetate with (Z)-9-dodecenyl acetate, the activity observed was 30 times less than that obtained on stimulation with (Z)-9-tetradecenyl acetate[4].

When chain-shortening is performed in the **m**-chain (compounds **12** and **13**, Figure 17), the loss of electrophysiological activity is more dramatic. The removal of one or two CH_2-units from the **m**-chain results in a substantially greater decrease of the biological activity, than when corresponding changes are made in the **n**-chain. This is another example of disagreement with the structure-activity rule[3,6], which predicts that the activity is less sensitive to changes in the **m**-chain than in the **n**-chain (see Chapter 3). As mentioned above (part 6.2), neither do the results on the dienic analogues support this rule.

Furthermore, the data show that essentially the same loss of activity is achieved whether one or two CH_2-units are lost from the **m**-chain. The analogues **12** and **13** lack the possibility to interact properly with the receptor-site for the double bond and the methyl group **or** with that for the acetate group. The great loss of activity indicates very strict requirements for the position of the acetate group. This is in agreement with previous results on acetate group analogues[12].

In the EAG screenings by Priesner mentioned above[5,10], some analogues chain shortened in the **m**-chain were also included. Although the magnitude of decrease in activities for these alterations varied very much between the different species, it is interesting to note that within one species chain-shortening by one or two (CH_2)-units caused approximately the same decrease in activity. Also the single sensillum investigation mentioned above[4] includes two **m**-chain shortened analogues. On stimulating the cell specific for (Z)-9-tetradecenyl acetate with (Z)-7-dodecenyl acetate, the activity was 100 times less than that obtained on stimulation with (Z)-9-tetradecenyl acetate. The corresponding value when the cell specific for (Z)-11-tetradecenyl acetate was stimulated with (Z)-9-dodecenyl acetate was 30.

6.6 Alkyne and alkenyne analogues. The measured electrophysiological single-cell activities of compounds **14**, **23** and **25** relative to the natural pheromone component **1** are shown in Figure 18a. The replacement of the original (Z)-double bond with a triple bond causes a dramatic decrease in activity (Figure 18a). If, in addition, a CH_2CH_2-unit in the **i**+3 or **i**+4 position is replaced by an (E)-double bond, the decrease in activity is less! While dec-5-ynyl acetate **14** is about 50 000 times less active than compound **1**, (E)-8-decen-5-ynyl acetate **23** and 9-decen-5-ynyl acetate **25** are only about 5 000 and 1 000-times less active, respectively. These results are very surprising if compared with those obtained for the dienic analogues (part 6.2). When a CH_2CH_2-unit in different positions in (Z)-5-decenyl acetate is replaced with a (E)-double bond, the result is in all cases a **decrease** in activity.

The calculated conformational energies for the biologically active conformations according to the **cisoid 2** model are shown in Figure 18b. Satisfactorily, the results show that the model is capable of reflecting the observed fact that the alkenynic analogues are more active than the alkyne analogue. However, it is difficult to quantitatively account for these differences.

For the analogues discussed above (parts 6.1-6.4), the interaction energies with the three postulated receptor sites are assumed to be equal to that of (Z)-5-decenyl acetate (see Chapter 4). This can not be assumed for the alkyne and enynic analogues. Since the analogues **14**, **23** and **25** have a triple bond instead of a (Z)-double bond, they do not have the same interaction with all three postulated receptor sites as (Z)-5-decenyl acetate. The interaction energy between a triple bond and the receptor site for the double bond, is presumably different from that between the site and a (Z)-double bond. This is reflected in the calculated conformational energies. For the chain-elongated analogues (part 6.1), the dienic analogues (part 6.2) and the double bond configurational isomers (part 6.3), it was found that there is a fairly constant conformational energy increase of 1.7-1.9 kcal/mol corresponding to a 10-fold decrease

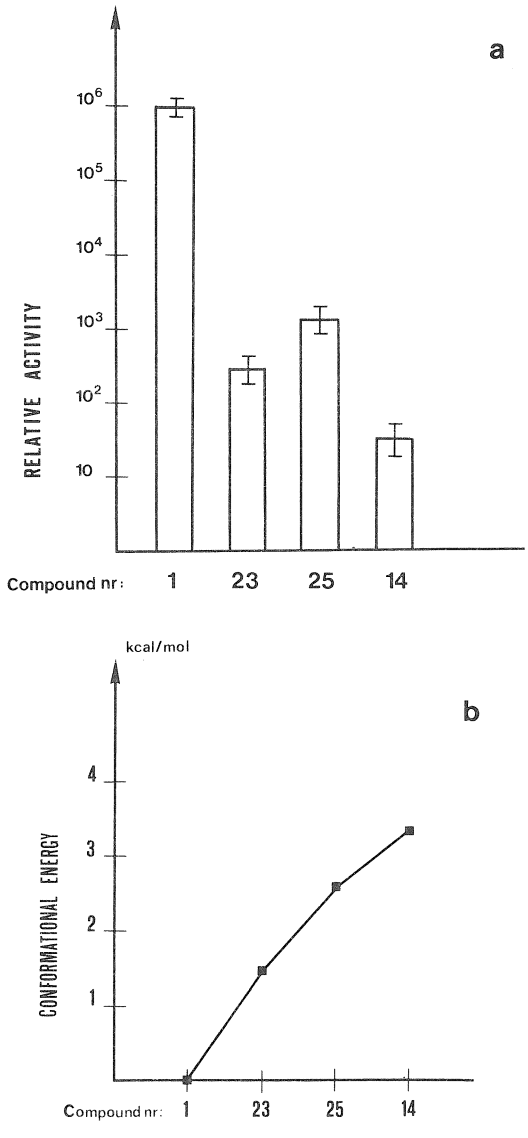


FIGURE 18. a) Experimental single-cell activities and b) calculated conformational energies for the biologically active conformations of the alkyne and enyne analogues.

of the observed electrophysiological activity. If these results are applied to the analogues **14**, **23** and **25**, 2-4 kcal/mol are left unaccounted for (see Figure 19). This energy may reflect the difference in interaction energy between a (Z)-double bond and a triple bond with the receptor site and may give valuable information about the nature of this interaction.

To fit the geometrical requirements of the model, the alkyne and alkenyne analogues adopt conformations with the midpoints of the triple bonds at much greater distances from the midpoint of the double bond in (Z)-5-decenyl acetate, than any of the other classes of analogues investigated. This is a more serious problem for compound **25** than for compounds **14** and **23**. To make a consistent treatment of all analogues and to improve the quantitative estimations for the interactions of alkyne and enynicanalogues, it is necessary to develop the model further.

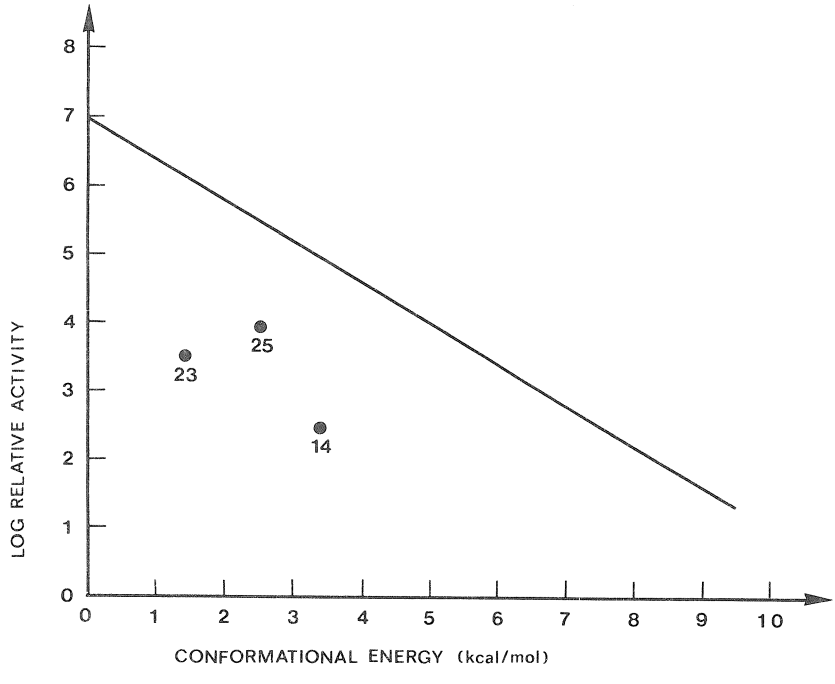


FIGURE 19. Compounds **14**, **23** and **25** in comparison with the linear relationship obtained between calculated conformational energies and experimental activities for fifteen other analogues to **1** (see part 6.3)

REFERENCES

1. Liljefors T., Thelin B., Van der Pers J.N.C. and Löfstedt C., *J. Chem. Soc. Perkin Trans II* , 1957 (1985).

2. Chisholm M.D., Steck W. and Underhill E.W., *J. Chem. Ecol.*, **6**, 203 (1980).

3. Priesner E., Jacobson M. and Bestmann H.J., *Z. Naturforsch.*, **30 c**, 283 (1975).

4. Priesner E., *Z. Naturforsch.*, **38 c**, 874 (1983).

5. Priesner E., *Ann. Zool. Ecol. Anim.*, **11**, 209 (1979).

6. Bestmann H.J. and Vostrowsky O. in *"Olfaction and Endocrine Regulations"*, ed. Breipohl W., IRL Press Ltd, London, p. 253 (1982).

7. Priesner E. in *"Neurobiology and Insecticide Action (Neurotox 1979)*. Society Chem. Ind., London (1980).

8. Dykyj J. and Repas M. *"Tlak nasy´tenej pary organicky´ch zlu´cenin"*, Red. Kossaczky´ E., Veda, Vydavatelstvo Slovenskej Akadémie Vied, Bratislava (1979).

9. a) Chapman O.L., Mattes K.C., Sheridan R.S. and Klun J.A., *J. Am. Chem. Soc.*, **100**, 4878 (1978).
   b) Chapman O.L., Klun J.A., Mattes K.C., Sheridan R.S. and Maini S., *Science*, **201**, 926 (1978).

10. Priesner E. in: *"Chemical Ecology: Odour Communication in Animals"*, Ed. Ritter F.J., Elsevier/North-Holland Biomedical Press, Amsterdam, p.57 (1979).

11. Struble D.L. and Byers J.R., *J. Chem. Ecol.*, **13**, 1187 (1987).

12. Liljefors T. Thelin B. and Van der Pers J.N.C., *J. Chem. Ecol.*, **10**, 1661 (1984).

13. Bestmann H.J.,Hirsch H.L., Platz H., Rheinwald M. and Vostrowsky O., *Angew. Chem.*, **92**, 492 (1980).

CHAPTER 7

SUMMARY AND CONCLUSIONS.

- The observed differences in biological (electrophysiological) activity of the compounds studied in this thesis are concluded to be determined by the conformational energies required to mimic spatial relationships in the natural pheromone component, assumed to be critical for a productive receptor interaction.

- The **conformational-energy model** for the interaction between a pheromone component and its receptor developed and tested in this thesis, gives an **essentially quantitative** correlation for most of the analogues investigated, between the measured electrophysiological single-cell activities and conformational energies calculated by the molecular mechanics method. However, at the present stage of development, the model can not fully account for the measured activities of alkenyne and cyclic analogues.

- Of three different low-energy conformers of (Z)-5-decenyl acetate **1** - **cisoid 1**, **cisoid 2** and **transoid** - employed as possible biologically active conformations, **only one** is fully compatible with the experimental data presented in this thesis. This conformer - **cisoid 2** - or a "nearby" conformation, is therefore suggested to be the biologically active one for compound **1**.

- Furthermore, the results obtained strongly indicate that the biologically active conformation of the alkyl chains in **1** is **all-anti**.

SUGGESTIONS FOR FUTURE WORK.

- To improve the quantitative treatment of alkenynic and cyclic analogues, it is necessary to further develop the conformational-energy model. This work will include synthesis and investigations of key compounds that may provide additional information

about the receptor topology and binding sites.

For the cyclic analogues, it will also be of great interest to prepare the enantiomerically pure compounds and investigate their biological activities in order to evaluate the importance of elements of chirality in the receptor cell.

Up till now, the conformational energy model has only been applied to one of the pheromone components of the turnip moth, (Z)-5-decenyl acetate. To investigate how general the model is, it should be of interest to apply it to some of the other sex pheromone components of the turnip moth and, even more interesting, to pheromone components of other species.

CHAPTER 8

PREPARATIVE PART.

8.1 General Procedures. Some basic synthetic procedures were used in a similar way in the preparation of several of the pheromone analogues. These procedures are described in a general way in this section. References to studies in which these methods have been used to prepare pheromone or pheromone-like compounds are given.

Reduction of esters and acids to alcohols[1-5] . To a stirred mixture of lithium aluminium hydride (1.52 g, 0.04 mol for esters, 3.34 g, 0.088 mol for acids) and dry ether (125 ml) at -30°C under an argon atmosphere, the ester/acid (0.065 mol) dissolved in dry ether (100 ml) was slowly added. The resulting mixture was stirred for an additional 2.5 hrs and then allowed to slowly warm up to room temperature. The reaction was followed by g.l.c., and when all the starting material was consumed, the mixture was cooled in an ice bath and carefully quenched with water (1.5 ml), 15 % NaOH solution (1.5 ml) and water (4.5 ml). After stirring over night, the mixture was filtered and the ether solution dried over $MgSO_4$. Removal of the solvent gave the alcohol in about 90 % yields.

Conversion of alcohols to tetrahydropyranyl (THP) ethers[6-9]. The alcohol (0.06 mol) was dissolved in dry ether (100 ml) in a flask equipped with a $CaCl_2$-drying tube. Dihydropyran (0.09 mol) and para-toluenesulfonic acid (0.05 g) were added and the solution stirred at room temperature over night. The ether solution was washed 3 times with satd. K_2CO_3 solution and dried over $MgSO_4$. Removal of the solvent gave the crude product in 85-95 % yields. G.l.c. analyses were performed on a Carbowax 20 M column with an injector temperature below 200° C, since the tetrahydropyranyl ethers are thermally unstable. If necessary, the products were distilled under reduced pressure (1 mmHg, with nitrogen inlet), all glassware being base-washed (dil. KOH solution) and with a few pellets of KOH placed in the distillation pot[6].

Conversion of alcohols to bromides[10]. The alcohol (0.047 mol) was dissolved in dry ether (40 ml) and pyridine (0.55 ml) and the solution was cooled to -30° C. Phosphorous tribromide (0.019 mol) was slowly added and the resulting mixture stirred for 0.5 hours, then allowed to warm up to room temperature and stirred over night. (Occasionally, the mixture was refluxed for 2 hours, but this did not seem to have any effect on the yields.) The mixture was poured into water, and extracted three times with ether; the combined ethereal phase was washed with 5% $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvent gave the crude bromides in yields typically around 60-80%.

Monobromination of diols[6,11,12]. The diol (0.15 mol), 47 % HBr (100 ml) and water (50 ml) were mixed in an apparatus for continuous extraction. Heptane (270 ml) was added and the mixture was refluxed for about 90 h. After cooling, the heptane phase was worked up, affording the bromoalcohol in yields around 90 %.

Conversion of alcohols and tetrahydropyranyl (THP) ethers to acetates[13]. AcOH/AcCl, 10/1, (14 ml), was added to the alcohol or THP ether (0.015 mol) and the solution was refluxed for 3 hours. The resulting dark brown oil was poured into 75 ml ice/saturated NaCl solution and extracted four times with ether. The ethereal layer was neutralized by repeated washing with 5% $NaCO_3$ solution, and then dried over $MgSO_4$. After solvent removal and flash chromatography, the products were obtained in more than 90% yields.

Alkylation of alkynes in liquid ammonia[9,12a]. To a solution of liquid ammonia (200 ml) and dry ether (20 ml) at -78° C under a nitrogen atmosphere, lithium amide (1.72 g, 0.075 mol) was added in portions. The mixture was stirred for 20 min., and the alkyne (0.059 mol) was added. After stirring for an additional 45 min., the THP-protected bromo alcohol dissolved in dry ether (25 ml) was slowly added. The resulting mixture was stirred for 2 hrs at -78°C and then allowed to slowly warm up to room temperature while the ammonia evaporated. Water (100 ml) was added and the resulting solution was extracted three times with hexane or heptane. The combined organic layers were washed with satd. NaCl solution and dried over $MgSO_4$. Removal of the solvent gave the crude products in almost quantitative yields.

Reduction of alkynes to (Z)-alkenes with Lindlar catalyst[12b,14-17]. The alkyne (0.01 mol) dissolved in absolute ethanol (10 ml) containing 1 drop of quinoline, was hydrogenated at room temperature and atmospheric pressure over Lindlar catalyst (0.09 g). Absorption of hydrogen ceased after approximately 1.5 h. The catalyst was filtered off and the solvent removed, affording the product in nearly quantitative yields. The isomeric purity of the products was more than 98 % (Z), as determined by g.l.c. on an OV-275 or OV-351 column.

Reaction of lithium acetylide, ethylenediamine complex with haloalkanes[18-20]. Lithium acetylide, ethylenediamine complex (7.2 g, 0.078 mol) was dissolved with stirring in dry DMSO (40 ml) under an argon atmosphere. The flask was cooled in an ice/water bath and the haloalkane (0.074 mol) was added at such a rate that the temperature was kept around 8° C. After the addition, the dark brown mixture was stirred at room temperature for 1 h, and water (15 ml) was then slowly added, maintaining the temperature below 35° C. Unfunctionalized alkynes containing 5-7 carbon atoms were distilled directly from the reaction mixture, employing a Dean-Stark trap. For higher alkynes and functionalized alkynes, the reaction mixture was poured into twice the volume of water and extracted with hexane. The combined hexane phases were washed with satd. NaCl solution and dried over K_2CO_3 or $MgSO_4$, affording the products in yields typically around 60-70 %.

8.2 Synthesis of compounds 1-29. Compounds **3, 4, 6, 7, 14** and **18** were a gift from Dr. Christer Löfstedt, Department of Ecology, University of Lund, and compounds **1, 2, 5** and **15** were a gift from FK. Bernt Thelin, Department of Organic Chemistry 3, University of Lund. The synthetic procedures used to prepare the other compounds are described below.

Products were purified by flash chromatography[21,22] on TLC-Silica gel 60 H supplied by Merck, argentation chromatography[23] and/or preparative g.l.c. on an 6 m OV-351 column. Capillary g.l.c. was run on a Supelcowax 30 m or DB-Wax 30 m column.

Mass spectra were recorded on a Finnigan 4021 mass spectrometer, high resolution mass spectroscopy on a ZAB-HF, VG Analytical, 11-250 mass

spectrometer, ^{1}H and ^{13}C N.m.r spectra on a Varian XL-300, a Nicolet 360 WB or a Jeol FX-60 spectrometer. N.m.r. spectra were recorded on $CDCl_3$ solutions with Me_4Si as internal reference. For some of the products, the ^{13}C signal for the carbonyl carbon was of too low intensity to be unambiguously identified.

Attempts to analyze the elemental composition of the acetate analogues gave inconsistent results. Analyses were attempted by two independent analytical laboratories. For the compounds discussed in this thesis, their unambiguous identification is established by spectroscopical methods.

Aluminium oxide 90 was purchased from Merck (basic, activity I). Before use it was dried in a desiccator at 10 mmHg at 200° C over night.

Glyme and diglyme was dried by refluxing over night over CaH_2, then distilling at reduced pressure over fresh CaH_2.

Paraformaldehyde was dried in a desiccator at 1 mm Hg over night prior to use.

DMPU was purchased from Fluka AB. Immediately before use, it was distilled over CaH_2 at reduced pressure and kept over 4 Å molecular sieves under an argon atmosphere.

(Z)-5-NONENYL ACETATE 8.

Ethyl 4-bromobutyrate was reduced with lithium aluminium hydride, the resulting bromoalcohol was protected with dihydropyran (8.1), and the product was distilled at reduced pressure in base-washed glassware, b.p. 66-73° C/0.2 mmHg.
4-(2-Tetrahydropyranyloxy)-1-bromobutane was coupled with pentyne in liquid ammonia with lithium amide as the base (8.1). Reduction with Lindlar catalyst (8.1), followed by acetylation (8.1) of the tetrahydropyranyl ether gave **8**.

∂_H(300 mHz) 0.90 (t,3H,Me), 1.31-1.46 (m,4H,CH_2CH_2), 1.59-1.69 (m,2H,CH_2-C-O), 1.96-2.10 (m,4H,CH_2C=), 2.04 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.34-5.40 (m,2H,J_{AB} 10.5 Hz,CH=CH).

∂_C(75.4 MHz) 13.8, 20.9, 22.8, 26.0, 26.7, 28.2, 29.1, 64.5, 129.2, 130.3, 171.2.

m/e ; 124 (M^+-60; 7%), 109 (1), 96 (24), 81 (35), 67 (37), 61 (2), 54 (45), 43 (100).

(Z)-5-OCTENYL ACETATE 9.

1-(2-Tetrahydropyranyloxy)-5-hexyne 33. 4-(2-Tetrahydropyranyloxy)-1-bromobutane (17.6 g, 0.074 mol) was reacted with lithium acetylide, ethylenediamine complex (8.1), affording 14.4 g (62 %) of the product **33**.

Butyllithium (6 ml of a 1.44 M solution in hexane) was slowly added to 1-(2-tetrahydropyranyloxy)-5-hexyne **33** (1.5 g, 8.2 mmol), dissolved in dry THF (8 ml). The solution was stirred at room temperature for 2 h; the colour turned dark red. Ethyl iodide (2.0 g 0.013 mol) dissolved in DMPU (14 ml, freshly distilled from CaH_2) was added at such a rate that the temperature did not exceed 25° C. During the addition the colour of the solution disappeared. The solution was stirred for 3 h, then poured into ice water (50 ml) and extracted with hexane. The combined hexane layers were washed with saturated NaCl solution and dried over $MgSO_4$. After removal of the solvent, 1.69 g (92 %) of crude 1-(2-tetrahydropyranyloxy)-5-octyne was obtained. After hydrogenation with Lindlar catalyst (8.1) and acetylation (8.1) , **9** was obtained. The product was purified by flash chromatography and argentation chromatography.

∂_H(300 MHz) 0.95 (t,3H,Me), 1.38-1.46 (m,2H,CH_2CH_2), 1.59-1.68 (m,2H,CH_2-C-O), 2.01-2.10 (m,4H,CH_2C=), 2.04 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.28-5.41 (m,2H,J_{AB} 10.7 Hz,CH=CH).

∂_C(75.4 MHz) 14.3, 20.5, 21.0, 26.0, 26.6, 28.1, 64.4, 128.4, 132,1, 171.2.

m/e ; 110 (M^+-60; 9%), 95 (6), 82 (40), 67 (52), 61 (2), 55 (19), 43 (100).

(Z)-5-HEPTENYL ACETATE 10.

10 was prepared from 1-(2-tetrahydropyranyloxy)-5-hexyne **33** and methyl iodide, according to the same procedure as described for **9.**

∂_H(300 MHz) 1.36-1.47 (m,2H,CH_2CH_2), 1.58-1.69 (m,5H,MeC=,CH_2-C-O),

2.03-2.11 (m,2H,CH_2C=), 2.05 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.32-5.52 (m,2H,J_{AB} 10.6 Hz,CH=CH).

∂_C(75.4 MHz) 12.7, 21.0, 25.8, 26.3, 28.1, 64.5, 124.3, 130.0, 171.3.

m/e ; 96 (M^+-60, 11%), 81 (20), 68 (64), 61 (3), 55 (27), 43 (100).

5-HEXENYL ACETATE 11.

1-(2-Tetrahydropyranyloxy)-5-hexyne **33** was reduced with disiamyl borane according to the method described by Brown and Zweifel[24] and acetylated (8.1). The product was purified by flash chromatography.

∂_H(300 MHz) 1.37-1.48 (m,2H,CH_2CH_2), 1.56-1.66 (m,2H,CH_2-C-O), 2.00-2.09 (m,2H,CH_2C=), 2.01(s,3H,Me-COO), 4.03 (t,2H,CH_2-OCO), 4.91-5.02 (m,2H,C=CH_2), 5.70-5.83 (m,1H,CH=C).

∂_C(75.4 MHz) 20.9, 25.1, 28.0, 33.2, 64.4, 114.8, 138.3, 171.2.

m/e ; 82 (M^+-60, 13 %), 73 (2), 67 (29), 61 (2), 54 (35), 43 (100).

$C_8H_{14}O_2$	calc.	C	67.6	H 9.9
(142.2)	found	C	67.3	H 9.9

(Z)-4-NONENYL ACETATE 12.

3-(2-Tetrahydropyranyloxy)-1-bromopropane (prepared from 1-bromo-3-propanol

and dihydropyran) was coupled with hexyne in liquid ammonia (8.1), hydrogenated with Lindlar catalyst (8.1) and acetylated (8.1). Purification as above.

∂_H(300 MHz) 0.90 (t,3H,Me), 1.28-1.35 (m,4H,CH_2CH_2), 1.63-1.73 (m,2H,CH_2-C-O), 1.99-2.15 (m,4H,CH_2C=), 2.05 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.31-5.43 (m,2H,J_{AB} 11.2 Hz, CH=CH).

m/e ; 124 (M^+-60; 9%), 109 (1), 95 (15), 81 (38), 68 (49), 61 (1), 54 (40), 43 (100).

(Z)-3-OCTENYL ACETATE 13.

2-(2-Tetrahydropyranyloxy)-1-bromoethane (prepared from 1-bromoethanol and dihydropyran) was coupled with hexyne in liquid ammonia (8.1), hydrogenated with Lindlar catalyst (8.1) and acetylated (8.1).

∂_H(300 MHz) 0.90 (t,3H,Me), 1.30-1.36 (m,4H,CH_2CH_2), 2.01-2.06 (m,2H,CH_2C=), 2.04 (s,3H,Me-CO), 2.36-2.41 (m,2H,O-C-CH_2-C=), 4.06 (t,2H,CH_2-OCO), 5.32-5.38 (m,1H,J_{AB} 11.2 Hz,CH=C), 5.46-5.52 (m,1H,J_{AB} 11.2 Hz,CH=C).

m/e ; 110 (M^+-60; 8%), 95 (3), 81 (17), 73 (2), 68 (20), 54 (46), 43 (100).

(Z)-9-TETRADECENYL ACETATE 16.

8-(2-Tetrahydropyranyloxy)-1-bromooctane (prepared by mono-bromination of 1,8-octandiol, followed by protection with dihydropyran; part 8.1) was coupled with hexyne in liquid ammonia (8.1), hydrogenated with Lindlar catalyst (8.1) and acetylated (8.1).

∂_H(60 MHz) 0.9 (t,3H,Me), 1.2-1.8 (m,16 H,CH_2CH_2), 1.8-2.3 (m,4H,CH_2C=), 2.0 (s,3H,Me-COO), 4.1 (t,2H,CH_2-O-C-O), 5.2-5.5 (m,2H,CH=CH).

IR (film) : ν = 2930, 2860, 1745 cm^{-1}.

m/e ; 194 (M^+-60; 8 %), 138 (4), 124 (7), 110 (19), 96 (46), 82 (64), 67 (58), 61 (9), 55 (89), 43 (100).

(Z)-11-HEXADECENYL ACETATE 17.

10-(2-Tetrahydropyranyloxy)-1-bromodecane (prepared by mono-bromination of 1,10-decandiol, followed by protection with dihydropyrane, part 8.1) was coupled with hexyne in liquid ammonia (8.1), hydrogenated with Lindlar catalyst (8.1) and acetylated (8.1).

∂_H(60 MHz) 0.9 (t,3H,Me), 1.1-1.7 (m,20H,CH_2CH_2), 1.8-2.2 (m,4H,CH_2C=), 2.0 (s,3H,MeCOO), 4.0 (t,2H,CH_2OCO), 5.2-5.5 (m,2H,CH=CH).

IR (film) ; ν = 2920, 2845, 1740 cm^{-1}.

m/e ; 222 (M^+-60; 3 %), 138 (4), 124 (7), 110 (14), 96 (43), 82 (63), 67 (53), 61 (13), 55 (100).

(E)-2,(Z)-5-DECADIENYL ACETATE 19 (5.2.1, Scheme 11).

(Z)-2-Heptenyl bromide 34. 2-Heptyne-1-ol (2.1 g, 0.018 mol), prepared

according to the method described by Smith and Beumel for a similar compound[18], was reduced by Lindlar catalyst (8.1), affording 1.9 g (92 %) of (Z)-2-heptene-1-ol. The alcohol (4.3 g, 0.038 mol) was treated with phosphorous tribromide (8.1), affording 4.9 g (73 %) of **34**.

(Z)-5-decen-2-ynyl acetate 35. Ethylmagnesium bromide was prepared according to standard procedures from magnesium turnings (0.33 g, 0.014 mol), ethyl bromide (1.7 g, 0.015 mol) and dry THF (18 ml). After cooling, 1-(2-tetrahydropyranyloxy)-2-propyne (1.8 g, 0.013 mol) in dry THF (4 ml) was slowly added and the solution was refluxed for 1 h, according to a method previously described by Rossi et al.[5] for similar compounds. After cooling, cuprous bromide (0.11 g, 0.8 mmol) was added, followed by (Z)-2-heptenyl bromide **34** (2.3 g, 0.013 mol) in dry THF (3 ml). The mixture was refluxed for 1 h, then stirred at room temperature over night, quenched with 10 % NH_4Cl solution and extracted with ether. The ether solutions were washed with satd. NaCl solution and dried over $MgSO_4$. Removal of the solvent afforded 2.7 g (91 %) of the crude product as a 4:1 mixture of the (Z)- and (E)-enynes. The (Z)-5-decen-2-yne-1-ol tetrahydropyranyl ether (1.2 g, 5.1 mmol), was acetylated (8.1), and the product purified by flash chromatography, affording 0.61 g (76 %) of **35.**

(Z)-5-Decen-2-ynyl acetate **35** (1.0 g, 5.2 mmol) dissolved in dry glyme, was added with stirring to a mixture of lithium aluminium hydride (0.44 g, 0.012 mol) in dry glyme (30 ml) under a nitrogen atmosphere. The mixture was refluxed until all of the starting material was consumed, as determined by g.l.c. on an OV-351 column. After cooling, the mixture was quenched with H_2O (0.5 ml), 15 % NaOH solution (0.5 ml) and H_2O (1.5 ml), dried over $MgSO_4$ and filtered. Removal of the solvent afforded 0.79 g (99 %) of crude (E)-2,(Z)-5-decadienol. The alcohol (0.79 g, 5.1 mmol) was acetylated with acetyl chloride (excess) in acetic acid, as described in part 8.1, affording 0.86 g (86 %) of the crude product. Pure **19** was obtained by flash chromatography and argentation chromatography.

∂_H(300 MHz) 0.89 (t,3H,Me), 1.30-1.36 (m,4H,CH_2CH_2), 1.98-2.07 (m,2H,CH_2C=), 2.06 (s,3H,MeCOO), 2.78-2.83 (m,2H,=CCH_2C=), 4.52 (dd,2H,CH_2OCO), 5.31-5.52 (m,2H,J_{AB} 10.7 Hz,CH=CH), 5.53-5.65 (m,1H,J_{AB} 15.2 Hz,CH=CH), 5.71-5.82 (m,1H,J_{AB} 15.2 Hz,CH=CH).

∂_C(75.4 MHz) 14.0, 21.0, 22.3, 26.9, 30.0, 31.8, 65.2, 124.0, 126.0, 131.6, 134.6, 170.9.

m/e ; 136 (M^+-60; 8%), 107 (2), 93 (9), 79 (42), 70 (3), 67 (12), 55 (13), 43 (100).

(E)-3,(Z)-5-DECADIENYL ACETATE **21** (5.2.2, Scheme 12).

5-(2-Tetrahydropyranyloxy)-(Z)-2-penten-1-ol. 5-(2-Tetrahydropyranyloxy)-2-pentyn-1-ol, prepared according to a method described by Jäger[25] for similar compounds, was reduced with Lindlar catalyst (8.1), affording the product in quantitative yield.

5-(2-Tetrahydropyranyloxy)-(E)-2-pentenal, 36. 5-(2-Tetrahydropyranyloxy)-(Z)-2-penten-1-ol (3.1 g, 0.017 mol) dissolved in dry dichloromethane (25 ml), was added to pyridinium chlorochromate (PCC), (5.5 g, 0.026 mol) in dichloromethane (25 ml) buffered with sodium acetate (0.42 g, 6 mmol) according to a method described for similar compounds[6,26,27]. The mixture was stirred at room temperature for 2 h and then used directly without isolation of the product, in the synthesis of **21** (see below).

(E)-3,(Z)-5-decadienyl acetate, 21. Hexamethyldisilazane (2.5 g, 0.017 mol) was added with stirring under an argon atmosphere to sodium amide (50 % suspension in toluene, 1.20 g, 0.015 mol), and dry THF (5 ml) according to the method described by Wannagat and Niederprum[28] and Bestmann et al.[29]. The mixture was refluxed for 6.5 h, and after cooling dry THF (5 ml) was added, followed by (pentyl)-triphenylphosphonium bromide **37** (7.0 g, 0.017 mol), prepared according to the method described by Bestmann et al.[30], in portions. The mixture was stirred 0.5 h at room temperature and was then refluxed for 1 h. The resulting red mixture was cooled to -78° C, then 5-(2-tetrahydropyranyloxy)-(E)-2-pentenal **36** (0.017 mol), prepared as described above, was added and the mixture stirred for 1 h and then at room temperature over night. The solvent was removed and the residue extracted repeatedly with hexane. The combined hexane solutions were washed with 5 % $NaHCO_3$ and dried over $MgSO_4$. After removal of the solvent, the product was directly acetylated (8.1), affording 0.54 g (18 %) of the product, which was purified by flash chromatography, argentation chromatography and preparative g.l.c.

∂_H(360 MHz) 1.02 (t,3H,Me), 1.40-1.50 (m,2H,CH_2Me), 1.60-1.70 (m,2H,CH_2CH_2, 2.04 (s,3H,MeCOO), 2.08-2.26 (m,4H,CH_2C=), 4.07 (t,2H,CH_2-OCO), 5.23-5.32 (m,1H,J_{AB} 10.9 Hz,CH=CH), 5.66-5.76 (m,1H,J_{AB} 14.6 Hz, CH=CH), 5.93-6.01 (m,1H,J_{AB} 10.9 Hz,CH=CH), 6.23-6.33 (m,1H,J_{AB} 14.6 Hz, CH=CH).

∂_C(15.03 MHz) 13.9, 20.9, 22.2, 27.4, 31.8, 32.1, 63.7, 128.0,128.0,128.6, 131.4.

m/e ; 136 (M^+-60; 8%), 107 (2), 93 (14), 79 (48), 67 (17), 55 (5), 43 (100).

(Z)-5,(E)-7-DECADIENYL ACETATE 22 (5.2.2, Scheme 13).

(E)-2-Pentenal, 38. 1,1,3-Triethoxypentane (96.1 g, 0.46 mol), H_2O (107 ml), oxalic acid (78 g, 0.87 mol) and some crystals of hydroquinone were heated to reflux with nitrogen bubbled through the mixture, according to the method described by Hall et al.[6] and Crombie and Williams[31]. After refluxing for 15 min., H_2O (107 ml) was added and the mixture was steam distilled, affording 20.2 g (52%) of the product.

∂_H(60 MHz) 1.1 (t,3H,Me), 2.1-2.6 (m,2H,CH_2), 5.9-6.3 (m,H,CH=C), 6.7-7.2 (m,H,CH=C), 9.5 (d,H,*J* 7 Hz,CHO).

5-Acetoxypentyl-triphenylphosphonium bromide 39. 5-Bromopentyl acetate (11.7 g, 0.056 mol) and triphenyl phosphine (13.2 g, 0.05 mol) were dissolved in acetonitrile (15 ml) according to the method described by Bestmann et al.[30] for similar compounds. The mixture was refluxed for 48 h. After removal of the solvent, the remaining thick oil was crystallized by scratching it in small portions with a glass rod. The resulting white crystals were washed repeatedly with dry ether and dried in a vacuum desiccator, affording 23.1 g (87 %) of **39**.

(Z)-5,(E)-7-Decadienyl acetate 22. Hexamethyldisilazane (4.8 g, 0.03 mol), sodium amide, 50 % suspension in toluene (2.3 g, 0.03 mol), dry THF (10 ml), 5-acetoxypentyl-triphenylphosphonium bromide, **39** (14.0 g, 0.03 mol) and (E)-2-pentenal (2.5 g, 0.03 mol) were reacted in the same way as described above for the synthesis of (E)-3,(Z)-5-decadienyl acetate, **21**, affording 1.3 g (22 %) of the product. The product was purified in the same way as **21**.

∂_H(360 MHz) 0.84 (t,3H,Me), 1.20-1.35 (m,4H,CH_2CH_2), 1.98 (s,3H,MeCOO), 2.06-2.13 (m,2H,CH_2C=), 2.32-2.40 (m,2H,CH_2C=), 4.04 (t,2H,OCH_2), 5.25-5.34 (m,1H,J_{AB} 10.9 Hz,CH=CH), 5.48-5.58 (m,1H,J_{AB} 15.4 Hz,CH=CH), 5.83-5.92 (m,1H,J_{AB} 10.9 Hz,CH=CH), 6.27-6.37 (m,1H,J_{AB} 15.4 Hz, CH=CH).

∂_C(91.0 MHz) 13.6, 21.0, 25.9, 26.0, 27.2, 28.2, 64.4, 124.4, 129.0, 29.2, 136.6, 171.1.

m/e ; 196 (M^+; 1%), 136 (9), 121 (4), 108 (19), 107 (19), 93 (29), 79 (100), 67 (50), 61 (1), 55 (40), 45 (1).

(Z)-5,(E)-8-DECADIENYL ACETATE 24 (5.2.1, Scheme 9).

(E)-8-Decen-5-ynyl acetate 23. To a stirred, refluxing mixture of lithium amide (0.25 g, 0.01 mol) and dry glyme under an argon atmosphere (25 ml; solvent used by Chisholm et al.[32] for similar reactions), 12-crown-4 (1.76 g, 0.01 mol) was added followed by 1-(2-tetrahydropyranyloxy)-5-hexyne **33** dissolved in dry glyme (15 ml). The mixture was refluxed for 3.5 h, then cooled to room temperature and 2-butenyl bromide (1.17 g, 8.7 mmol), prepared from 2-butene-1-ol and phosphorous tribromide (8.1), was added. The mixture was refluxed over night and after cooling water (19 ml) was added. The usual workup with heptane and satd. NaCl solution afforded 1.8 g (88 %) of the crude 1-(2-tetrahydropyranyloxy)-(E)-8-decen-5-yne. This enyne was acetylated (1.8 g, 7.7 mmol) with acetyl chloride (excess) (8.1). The product was purified by flash chromatography, affording 0.85 g (57 %) of pure (E)-8-

decen-5-ynyl acetate **23.**

∂_H(360 MHz) 1.45-1.58 (m,2H,CH_2CH_2), 1.59-1.72 (m,5H,CH_2CH_2, MeC=), 1.98 (s,3H,Me-COO), 2.10-2.19 (m,2H,$CH_2C\equiv$), 2.77-2.82 (m,2H,=$CCH_2C\equiv$), 4.02 (t,2H,CH_2-OCO), 5.30-5.40 (m,1H,J_{AB} 15.2 Hz), 5.52-5.66 (m,1H,J_{AB} 15.2 Hz,CH=C).

m/e ; 194 (M^+; 0.3 %), 151 (10), 133 (4), 119 (24), 105 (31), 94 (27), 91 (100), 79 (43), 67 (14), 55 (41), 45 (9).

(Z)-5,(E)-8-Decadienyl acetate 24. (E)-8-Decen-5-ynyl acetate **23** (0.85 g, 4.4 mmol) was hydrogenated with Lindlar (8.1), affording 0.80 g (93 %) of crude **24.** The product was purified by flash chromatography and argentation chromatography.

∂_H(360 MHz) 1.30-1.40 (m,2H,CH_2CH_2), 1.50-1.62 (m,2H,CH_2CH_2),1.58 (d,3H,Me), 1.98 (s,3H,MeCOO), 1.98-2.05 (m,2H,CH_2C=), 2.62-2.68 (m,2H,=CCH_2C=), 3.99 (t,2H,CH_2-OCO), 5.27-5.38 (m,2H,J_{AB} 11 Hz,CH=CH), 5.30-5.42 (m,2H,J_{AB} 15.5 Hz,CH=CH).

∂_C(15.03 MHz) 17.9, 20.9, 25.9, 26.6, 28.2, 30.4, 64.4, 125.1, 128.2, 129.5, 129.5.

m/e ;196 (M^+; 0.5%), 136 (27), 121 (16), 107 (32), 93 (46), 79 (100), 68 (81), 61 (5), 55 (56), 45 (2).

(Z)-5,9-DECADIENYL ACETATE 26 (5.2.1, Scheme 7).

1-Hexen-5-yne 30. 3-Butene-1-ol (9.5 g, 0.132 mol) was reacted with phosphorous tribromide (8.1). The product was distilled at atmospheric pressure, affording 11.4 g (64 %), b.p. 97-98^o C of pure 3-butenyl bromide. The bromide (7.55

g, 0.057 mol) was reacted with lithium acetylide, ethylenediamine complex (8.1). The product was distilled off the reaction mixture at atmospheric pressure, affording 0.8 g (17 %) of 1-hexen-5-yne.

(Z)-5,9-Decadienyl acetate 26. 1-Hexen-5-yne **30** (0.38 g, 4.7 mmol) was alkylated with 4-(2-tetrahydropyranyloxy)-butyl bromide (0.73 g, 3.1 mmol) (8.1). The product was purified by flash chromatography, affording 0.54 g (74 %) of 1-(2-tetrahydropyranyloxy)-9-decene-5-yne. The enyne (0.54 g, 2.3 mmol) was hydrogenated with Lindlar catalyst, then acetylated (8.1). The crude product was purified by flash chromatography and argentation chromatography to give pure **26**.

∂_H(360 MHz) 1.36-1.46 (m,2H,CH_2CH_2), .59-1.69 (m,2H,CH_2CH_2), 2.04 (s,3H,Me-COO), 2.03-2.14 (m,6H,CH_2C=), 4.06 (t,2H,CH_2-OCO), 4.93-5.06 (m,2H,C=CH_2), 5.32-5.42 (m,2H,J_{AB} 11 Hz,CH=CH), 5.75-5.88 (1H,m,CH=CH_2).

∂_C(15.03 MHz) 20.9, 25.8, 26.6, 28.1, 33.7, 64.4, 114.4, 129.4, 29.4, 138.2.

m/e ; 196 (M^+; 0.5%), 136 (2), 121 (2), 108 (3), 95 (17), 79 (14), 67 (47), 61 (2), 55 (17), 43 (100).

1-(2-Tetrahydropyranyloxy)-9-decen-5-yne was acetylated (8.1) to give **25.**

∂_H(360 MHz) 1.50-1.62 (m,2H,CH_2CH_2), 1.68-1.78 (m,2H,CH_2CH_2), 2.05 (s,3H,Me-COO), 2.16-2.25 (m,6H,$CH_2C\equiv$,CH_2C=), 4.08 (t,2H,CH_2-OCO), 4.99-5.10 (m,2H,C=CH_2), 5.79-5.92 (m,1H,CH=C).

m/e ; 134 (M^+-60; 1 %), 119 (5), 105 (8), 91 (30), 77 (16), 67 (10), 61 (2), 55 (7), 52 (6), 43 (100).

(E)-2,(E)-5-DECADIENYL ACETATE **20** (5.2.1, Scheme 10).

(E)-2,(E)-5-Decadienyl acetate 20. To a stirred mixture of bis(π-cyclopentadienyl)-zirconium hydrido chloride ($(C_5H_5)_2Zr(H)Cl$) (2.2 g, 8.6 mmol) and dry benzene (10 ml), prepared in a dry box under an argon atmosphere, 1-(2-tetrahydropyranyloxy)-2-propyne (1.2 g, 8.6 mmol) was added , according to the method described by Ando et al. for similar compounds[33]. The mixture was stirred over night, whereupon the benzene was removed with an argon stream, and the residue was diluted with dry THF (7 ml). (Z)-2-Heptenyl bromide **34** (1.5 g, 8.6 mmol; for preparation see synthesis of **19**), and $Pd(PPh_3)_4$ (0.25 g) dissolved in dry THF (10 ml) were slowly added. The mixture was stirred over night, and then poured into hexane (100 ml) with vigorous stirring. After filtering off the yellow precipitate, the solvent was removed and the residue diluted with hexane. The hexane solution was washed with water and satd. NaCl solution and dried over $MgSO_4$. Removal of the solvent afforded 1.9 g (94 %) of the crude product. The 1-(2-tetrahydropyranyloxy)-(E)-2,(E)-5-decadiene was acetylated (8.1), and was purified by flash chromatography and argentation chromatography.

∂_H(300 MHz) 0.89 (t,3H,Me), 1.28-1.36 (m,4H,CH_2CH_2), 1.98-2.04 (m,2H,CH_2C=), 2.06 (s,3H,MeC=O), 2.72-2.78 (m,2H,=CCH_2C=), 4.52 (dd,2H,OCH_2), 5.33-5.51 (m,2H,J_{AB} 15.2 Hz,CH=CH), 5.51-5.62 (m,1H,J_{AB} 15.2 Hz, CH=CH), 5.72-5.83 (m,1H,J_{AB} 15.2 Hz,CH=CH).

∂_C(15.03 MHz) 14.0, 21.0, 22.2, 31.6, 32.2, 35.2, 65.1, 124.1, 126.9, 132.3, 134.9, 170.9.

m/e ; 136 (M^+-60; 5%), 107 (1), 93 (7), 79 (32), 67 (10), 55 (11), 43 (100).

3-(p-PROPYLPHENYL)PROPYL ACETATE 27 (5.4.1, scheme 14).

Diethyl p-propylbenzyl malonate. 4-n-Propyl benzoic acid (5.0 , 0.03 mol) was reduced with lithium aluminium hydride (8.1), affording 3.2 g (71 %) of p-propylbenzyl alcohol, which was treated with phosphorous tribromide (8.1), affording 3.5 g (82 %) of p-propylbenzyl bromide. The bromide (2.1 g, 0.01 mol) was reacted with sodium diethyl malonate in a way similar to that described by Jacobson[1]. The product was purified by flash chromatography, affording 2.6 g (62 %) diethyl p-propylbenzyl malonate.

Ethyl 3-(p-propylphenyl)-propanoate. A mixture of diethyl p-propylbenzyl malonate (2.6 g, 8.9 mmol), lithium chloride (0.38 g, 8.9 mmol), water (0.16 g, 8.9 mmol) and DMSO (18 ml) was heated to reflux until the gas evolution ceased, according to methods described by Krapcho[34] and Blum et al.[35] for similar systems. After cooling, water was added and the mixture was extracted with ether. The combined ether phase was washed with satd. NaCl solution and dried over $MgSO_4$. Removal of the solvent afforded 2.4 g of a colourless oil (93 %).

3-(p-Propylphenyl)-propyl acetate 27. Methyl 3-(p-propylphenyl)-propanoate (2.5 g, 0.011 mol) was reduced with lithium aluminium hydride (8.1), affording 1.4 g (72 %) of 3-(p-propyl)-phenyl propanol. The alcohol (1.0 g, 6 mmol) was acetylated (8.1), affording 1.25 g (98 %) of the product, which was purified by flash chromatography.

∂_H(300 MHz) 0.94 (t,3H,Me), 1.57-1.69 (m,2H,CH_2CH_2), 1.90-2.00 (m,2H,CH_2CH_2), 2.06 (s,3H,CH_3CO), 2.53-2.58 (m,2H,CH_2Ph), 2.64-2.69 (m,2H,CH_2Ph), 4.09 (t,2H,CH_2O), 7.10 (s,4H,Ph).

∂_C(75.4 MHz) 13.87, 21.00, 24.62, 30.24 31.75, 37.65, 63.91, 128.23, 128.34, 128.37, 128.51, 138.35, 140.34, 171.19.

m/e ; 160 (M^+-60; 38%), 147 (3), 131 (98), 117 (48), 105 (9), 91 (36), 77 (5), 69 (1), 65 (3), 55 (3), 43 (100).

DIETHYL (E)-2,(Z)-4-OCTADIENYL MALONATE 43.

(5.4.2, Scheme 15).

Ethyl (E)-2,(Z)-4-octadienoate 41. A mixture of 1-hexyn-3-ol, (15.0 g, 0.15 mol), prepared according to a method described by Skattebøl et al.[37] for a similar compound, triethyl orthoacetate (146.5 g, 0.90 mol), and propionic acid (13 drops) was heated to 130-150° C for 30 h, according to the method described by Tsuboi et al.[4,36]. During the reaction, the ethanol produced was distilled off. Distillation of the residue gave 16.1 g (64%) of ethyl (3,4)-octadienoate **40**, b.p. 104-105° C/15 mm Hg. The ß-allenic ester **40** (16.1 g, 0.096 mol) and dry aluminium oxide were mixed in dry benzene (400 ml). The mixture was refluxed until the reaction was complete, as determined by g.l.c on an OV-351 column. The reaction time was usually between 48-96 hours, and it was necessary to add more aluminium oxide after half the reaction time. The reaction mixture was filtered and evaporated, affording 14.6 g (91%) of the product. Spectroscopic data were in agreement with those presented in the literature.

(E)-2,(Z)-4-Octadienyl bromide. Ethyl (E)-2,(Z)-4-octadienoate **41** (5.2 g, 0.031 mol) was reduced with lithium aluminium hydride (8.1), affording 3.7 g (95 %) of (E)-2,(Z)-4-octadienol **42**. The alcohol (4.8 g, 0.038 mol) was reacted with phosphorous tribromide (8.1), affording 5.3 g (73%) of the bromide.

Diethyl (E)-2,(Z)-4-octadienyl malonate 43. To absolute ethanol (45 ml) under a nitrogen atmosphere, freshly cut sodium (0.75 g, 0.031 mol) was added. After stirring for 0.5 h, diethyl malonate (5.4 g, 0.031 mol) was added and the resulting solution was refluxed for 1.5 h. After cooling, (E)-2,(Z)-4-octadienyl bromide (5.0 g, 0.02 mol) was added, the mixture was stirred for 0.5 h at room temperature and then refluxed for 3 h. Most of the alcohol was distilled off, and 30 ml of water was added. The solution was extracted with ether and the combined ether solutions were washed with satd. NaCl solution and dried over $MgSO_4$. After removal of the solvent, the product was purified by flash chromatography giving 4.1 g (67 %) of the pure product.

∂_H(300 MHz) 0.9 (t,3H,Me), 1.2 (t,6H,Me), 1.3-1.5 (m,2H,CH_2CH_2), 2.0-2.2 (m,2H,CH_2C=), 2.6-2.7 (m,2H,CH_2C=), 3.4 (t,1H,CH), 4.2 (q,4H,CO_2CH_2), 5.30-5.42 (m,1H,*JAB* 11.2 Hz,CH=C), 5.52-5.64 (m,1H,J_{AB} 15.1 Hz,CH=C), 5.88-5.98 (m,1H,J_{AB} 11.2 Hz,CH=C), 6.32-6.46 (m,1H,J_{AB} 15.2 Hz,CH=C).

∂_C(75.4 MHz) 13.7, 14.1, 22.4, 31.9, 34.6, 52.1, 61.4, 126.4, 129.8, 133.4, 134.1, 168.9.

DIETHYL (E)-2,(E)-4-OCTADIENYL MALONATE 45 .

(5.4.2, scheme 16).

(E)-2,(E)-4-Octadienoic acid. Malonic acid (50.0 g, 0.48 mol) was added to dry pyridine (80 ml) under a nitrogen atmosphere with vigorous mechanical stirring, according to the method described by Jacobson[1]. The mixture was cooled with ice/water, and (E)-2-hexenal (50.0 g, 0.51 mol) was slowly added. The mixture was stirred at room temperature for 72 h and then heated on a steambath for 1 h. After cooling, the solution was poured into 170 ml of water. The oily layer was separated and poured into 165 ml of ice-cold 25 % HCl with vigorous stirring. The oily solid that separated was filtered off and washed with cold H_2O and then with cold hexane. Recrystallisation from petroleum ether gave 19.1 g (27 %) of light yellow crystals, m.p. 75.5-76.0° C, lit. 76° C.

Diethyl (E)-2,(E)-4-octadienyl malonate 45. (E)-2,(E)-4-Octadienoic acid (18.4 g, 0.13 mol) was reduced with lithium aluminium hydride (8.1), affording 15.6 g (95 %) of (E)-2,(E)-4-octadienol **44.** The alcohol (15.5 g, 0.123 mol) was reacted with phosphorous tribromide (8.1), affording 12.7 g (55 %) of (E)-2,(E)-4-octadienyl bromide. The bromide (1.1 g, 6 mmol) was reacted with sodium diethyl malonate in the same way as described above for its isomer diethyl (E)-2,(Z)-4-octadienyl malonate **43.** The crude product was purified by flash chromatography, affording 1.3 g (82 %) of pure **45.**

∂_H(300 MHz) 0.9 (t,3H,Me), 1.2 (t,6H,Me), 1.3-1.5 (m,2H,CH_2CH_2), 1.9-2.1 (m,2H,CH_2C=), 2.6-2.7 (m,2H,CH_2C=), 3.4 (t,1H,CH), 4.2 (q,4H,CO_2CH_2), 5.42-5.66 (m,2H,J_{AB} 15.1 Hz, J_{AB} 14.9 Hz,CH=C), 5.90-6.14 (m,2H,J_{AB} 14.6 Hz,J_{AB} 14.4 Hz,CH=C).

3-(CIS-4-PROPYLCYCLOHEX-2-ENYL)PROPYL ACETATE 28 .

(5.4.2, scheme 18).

(Phenylsulfonyl), cis-2(5)-propyl-5(2)-(2,2-bis-ethoxycarbonyl)etyl cyclohex-3-ene 47. (E)-2,(Z)-4-Octadienyl malonate **43** or (E)-2,(Z)-4-octadienyl malonate **45** (0.86 g, 3.1 mmol), phenyl vinyl sulfone (0.55 g, 3.1 mmol) and a few crystals of hydroquinone[38] were mixed in dry benzene (0.86 ml), in a way similar to that described by Carr and Paquette[39a] and Snyder et al.[39b]. The mixture was placed in a teflon cup that was inserted into a Parr bomb. The reaction was run at 140° C and followed by g.l.c. on a 0.5 m Dexil column. After seven days, all of the dienic diester was consumed, but between 15-25 % of the phenyl vinyl sulfone remained unreacted, indicating that some of the diene is consumed in side reactions. The benzene was evaporated, leaving a dark-brown oil (1.3 g) which was desulfonylated (see below) without purification.

Methyl 1-methoxycarbonyl-3-(cis-4-propylcyclohex-2-enyl) propanoate and 2-(cis-4-propylcyclohex-2-enyl) 1,1-ethandioic acid. The crude product from the above Diels-Alder reaction (1.3 g) and anhydrous disodium hydrogen phosphate (1.6 g, 0.012 mol) was dissolved in dry methanol (31 ml) under a nitrogen atmosphere. The mixture was cooled to -20° C, and pulverized 6% sodium amalgam (4.6 g) was added, according to the desulfonylation method described by Trost et al.[40]. The mixture was stirred for half an hour, allowed to slowly warm up to room temperature, and then stirred until the desulfonation was complete (g.l.c., Dexil 0.5 m), which required several hours. The mixture was poured into water and extracted several times with ether. After the usual workup, 0.23 g of the crude dimethyl ester was isolated. The aqueous phase was made acidic with dilute HCl and then extracted again with ether. This gave 0.36 gram of the diacid. Total yield: 76% (dimethyl ester and diacid), calculated from the Diels-Alder step.

In another experiment, starting from the crude product from the Diels-Alder step (2.6 g), anhydrous disodium hydrogen phosphate (3.1 g), dry methanol (61 ml) and 6% sodium amalgam (9.1 g), the product was entirely the diacid, 1.4 g (96 % crude yield). Only traces of the dimethylester could be detected in this experiment.

3-(Cis-4-propylcyclohex-2-enyl) propanol 48. i) 2-(Cis-4-propylcyclohex-2-enyl) 1,1-ethandioic acid (1.4 g, 6 mmol) was heated in an oil bath at 180° C for 3 h, according to the method described by Jacobson[1] . The resulting viscous, dark-brown oil was purified by flash chromatography, giving 0.85 g (70 %)

of the mono-acid.

∂_H(300 MHz) 0.90 (t,3H,Me), 1.18-1.44 (m,6H,CH_2CH_2), 1.56-1.78 (m,4H,CH_2C=), 1.90-2.12 (m,2H,CH), 2.40 (t,2H,CH_2COO), 5.51-5.67 (m,2H,CH=C). The acidic proton could not be detected.

m/e ; 196 (M^+; 3%) 178 (6), 160 (3), 149 (8), 136 (24), 123 (16), 107 (18), 93 (46), 81 (64), 73 (12), 67 (86), 55 (92), 41 (100).

Reduction of the mono acid (0.28 g, 1.3 mmol) with lithium aluminium hydride (8.1) afforded 0.23 g (99 %) of the product (see spectroscopical data in **ii** below).

ii) Methyl 1-methoxycarbonyl-3-(cis-4-propylcyclohex-2-enyl) propanoate (0.26 g, 1 mmol), sodium chloride (0.03 g, 0.5 mmol), water (0.5 g, 28 mmol) and DMSO (4 ml) were heated at 130° C in an oil bath for 8 h, as described for other diesters by Krapcho[34] and Blum et al.[35]. After cooling, the light-brown solution was extracted with hexane. The combined hexane solutions were washed several times with water to remove all DMSO, and were then dried over $MgSO_4$. Removal of the solvent gave 0.16 g (80%) of the methyl ester. Reduction with lithium aluminium hydride (8.1) afforded the product.

∂_H(300 MHz) 0.89 (t,3H,Me), 1.25 (s,1H,OH), 1.20-1.45 (m,8H,CH_2CH_2), 1.46-1.76 (m,4H,CH_2C=), 1.90-2.08 (m,2H,CH), 3.64 (t,2H,CH_2O), 5.57-5.60 (m,2H,CH=C).

m/e ; 182 (M^+; 3%), 164 (2), 149 (1), 135 (16), 121 (32), 109 (16), 93 (46), 79 (75), 67 (100), 55 (53), 41 (82).

3-(Cis-4-propylcyclohex-2-enyl)propyl acetate 28. 3-(Cis-4-propylcyclohex-2-enyl) propanol **48** (0.23 g, 1.3 mmol) was acetylated as described in part 8.1, affording a quantitative yield of the product. The product was purified by flash chromatography and by argentation chromatography. The resulting product was more than 98.5% pure, as determined by capillary g.l.c. and n.m.r., and consisted of an isomeric mixture of 89-92 % of the cis-form and 8-11 % of another unidentified isomer (**not** the trans-isomer).

∂_H(300 MHz) 0.90 (t,3H,Me), 1.20-1.44 (m,8H,CH_2CH_2), 1.58-1.72 (m,4H,CH_2CH_2), 1.98-2.06 (m,2H,CH), 2.04 (s,3H,Me-COO), 4.05 (t,2H,CH_2-OCO), 5.53-5.64 (m,2H,J_{AB} 10.0 Hz,CH=C).

∂_C(75.4 MHz) 14.28, 20.35, 20.99, 25.95, 26.01, 26.34, 32.18, 34.63, 34.64, 38.28, 64.81, 130.75, 132.22, 171.22.

m/e ; 164 (M^+-60; 17%), 149 (2), 136 (33), 121 (42), 107 (15), 93 (40), 79 (46), 73 (2), 67 (40), 61 (1), 55 (19), 43 (100).

3-(CIS-4-PROPYLCYCLOHEX-2-EN-1-YL)PROPYL ACETATE 28

(5.4.2, Schemes 20, 22).

Methyl 2-(cis-4-propylcyclohex-2-en-1-yl)ethanoate 51, Scheme 20. Dimethyl(trans-4-acetoxycyclohex-2-en-1-yl)malonate[41] (3.1 g, 0.11 mol) was decarboxylated with lithium chloride in DMSO/H_2O , according to methods described by Krapcho[34] and Blum et al.[35]. The crude product was purified by bulb-to-bulb distillation, affording 1.9 g (81 %) of methyl 2-(trans-4-acetoxycyclohex-2-en-1-yl)ethanoate.

Propylmagnesium bromide was prepared according to standard procedures from magnesium turnings (0.23 g, 9.4 mmol) and propyl bromide (1.23 g, 10 mmol) in dry THF (20 ml). After stirring at room temperature, the Grignard solution was slowly added through a double-tipped needle to methyl 2-(trans-4-acetoxycyclohex-2-en-1-yl)ethanoate (1.0 g, 4.7 mmol) and dilithium tetrachloro cuprate (0.94 ml of 1 M solution in THF) in dry THF (16 ml) at -30° C, according to the method described by Bäckvall et al[42,43] for similar systems. The solution was stirred for 0.5 h, then allowed to slowly warm up to room temperature and stirred over night. satd. NH_4Cl solution was added and the mixture was extracted with ether. The combined ethereal layers were washed with satd. NaCl solution and dried over $MgSO_4$. After removal of the solvent, the product was purified by flash chromatography, affording 0.75 g (81 %). The product was a 24:1 mixture of the two positional isomers methyl 2-(cis-4-propylcyclohex-2-en-1-yl)ethanoate **51** and methyl 2-(cis-2-propylcyclohex-3-en-1-yl)ethanoate **52**.

Small amounts of the corresponding propyl esters due to transesterfication could be isolated, but this was of no importance since the following reaction step was a reduction of the ester group.

51 ∂_H (300 MHz) ; 0.89 (t,3H, CH_3), 1.22-1.39 (m,6H,CH_2CH_2), 1.64-1.74 (m,2H,CH_2CH_2), 1.99-2.06 (m,1H,$CHCH_2$), 2.29 (m,2H,CH_2CO_2), 2.53-2.60 (m,1H,$CHCH_2COO$), 3.67 (s,3H,MeOCO), 5.51-5.67 (m,2H,J_{AB} 9.9 Hz,CH=CH).

51 m/e ; 196 (M^+; 2%), 164 (7), 153 (2), 136 (3), 122 (100), 107 (11), 93 (53), 79 (96), 67 (38), 59 (14), 55 (24), 41 (54) 33 (3).

52 m/e ; 196 (M^+; 4%), 164 (5), 147 (1), 136 (12), 122 (80), 107 (9), 93 (39), 79 (100), 67 (21), 59 (10), 54 (16), 41 (41).

Methyl 2-(cis-4-propylcyclohex-2-en-1-yl)ethanoate (mixture of **51** and **52**), Scheme 22 was reduced with lithium aluminium hydride, and then treated with phosphorous tribromide, (8.1). The resulting bromide was purified by flash chromatography.

(1,4-isomer) ∂_H(300 MHz) ; 0.90 (t,3H,Me), 1.22-1.43 (m,6H,CH_2CH_2),1.62-1.72 (m,2H,CH_2CC=), 1.76-1.95 (m,2H,CH_2C-Br), 2.01-2.06 (m,1H,CHC=), 2.17-2.28 (m,1H,CHC=), 3.46 (dt,2H,CH_2Br), 5.52-5.68 (m,2H,J_{AB} 10.1 Hz,CH=CH).

(1,4-isomer) m/e ; 232, 230 (M^+; 3%), 189 (9), 187 (9), 151 (4), 12 (32), 109 (12), 107 (17), 95 (29), 91 (15), 81 (100), 67 (85), 55(32), 41 (74).

Magnesium turnings (0.08 g, 3 mmol) and dry ether (3 ml) were placed in a flask that was immersed in an ultrasonic cleaning bath at 50° C, according to a method described by Sprich and Lewandos[44] and Suslick et al.[45]. When the ether started to reflux, 2-(cis-4-propylcyclohex-2-en-1-yl)ethyl bromide (0.64 g, 2.7 mmol) dissolved in dry ether (3 ml) was slowly added. The mixture was kept in the ultrasonic bath with reflux for an additional 5 h. After cooling, dried paraformaldehyde (0.15 g, 5 mmol) was depolymerized by heating and added to the Grignard reagent in a slow current of dry

nitrogen, according to the method described by Gilman and Catlin [46]. The mixture was stirred over night, and then refluxed for 1 h; after cooling, it was poured into satd. NaCl solution. The solution was extracted with ether, the combined ethereal layers washed with satd. NaCl solution and dried over $MgSO_4$. After removal of the solvent, the crude product was purified with flash chromatography. Yield: 0.13 g (26 %). (The by-products formed in this reaction are discussed in part 5.4.3).

(1,4-isomer) ∂_H(300 MHz) ; 0.90 (t,3H,Me), 1.29-1.41 (m,8H,CH_2CH_2), 1.45 (s,1H,OH), 1.59-1.65 (m,4H,CH_2CC=), 2.02-2.04 (m,2H,CHC=), 3.64 (t,2H,CH_2OH), 5.46-5.66 (m,2H,CH=CH).

(1,4-isomer) m/e ; 182 (M^+; 5%), 164 (3), 136 (16), 121 (49), 107 (15), 93 (53), 79 (92), 67 (95), 55(55), 41 (100).

The alcohol was finally acetylated to give a quantitative yield of **28**. Pure **28** was obtained by semi-preparative h.p.l.c. on a 500 mm Polygosil 60-5 μ C_{18}- column with methanol/water 9/1 as eluant.

(1,4-isomer) ∂_H (300 MHz) ; 0.90 (t,3H,Me), 1.20-1.42 (m,8H,CH_2CH_2), 1.57-1.72 (m,4H,CH_2CC=), 2.01-2.05 (m,2H,CHC=), 2.05 (s,3H,Me-COO), 4.05 (t,2H,CH_2-OCO), 5.53-5.64 (m,2H,J_{AB} 10.2 Hz,CH=CH).

(1,4-isomer) ∂_C(75.4 MHz) ; 14.30, 20.35, 21.02, 25.98, 26.01, 26.34, 32.17, 34.64, 34.65, 38.29, 64.82, 130.77, 132.24, 171.23.

(1,4-isomer) m/e ; 164 (M^+-60; 29%), 149 (3), 136 (45), 121 (64), 107 (26), 93 (54), 79 (71), 73 (3), 67 (43), 55 (20), 43 (100).

(1,4-isomer) High resolution MS(EI) m/z ;

$[M^+]_{calc}$ = 224.1776

$[M^+]_{found}$ = 224.1777 +/- 5 ppm

$[M^+-1]_{found}$ = 222.1610 +/- 5ppm

3-(TRANS-4-PROPYLCYCLOHEX-2-EN-1-YL)PROPYL ACETATE 29

(5.4.3, Scheme 21 and 23).

Methyl 2-(trans-4-propylcyclohex-2-en-1-yl)ethanoate 53, Scheme 21. Dimethyl(cis-4-acetoxycyclohex-2-en-1-yl)malonate **50**[41] (4.5 g, 0.017 mol) was decarboxylated with lithium chloride and DMSO/H_2O, according to the method described by Krapcho[34] and Blum et al.[35], affording 2.5 g (70 %) of pure methyl 2-(cis-4-acetoxycyclohex-2-en-1-yl)ethanoate after bulb-to-bulb distillation. This compound (1.5 g, 7 mmol) was substituted with propyl magnesium bromide as described for the corresponding trans-isomer in the synthesis of **28** above, affording 1.04 g (74 %) of the product after flash chromatography.
The product was a 1:1.4 mixture of the positional isomers methyl 2-(trans-4-propylcyclohex-2-en-1-yl)ethanoate **53** and methyl 2-(trans-2-propylcyclohex-3-en-1-yl)ethanoate **54**.

53 m/e ; 196 (M^+; 2%), 164 (7), 136 (2), 122 (100), 107 (11), 93 (53), 79 (92), 67 (31), 59 (12), 55 (20), 41 (48), 33 (2).

54 m/e ; 196 (M^+; 4%), 164 (6), 147 (1), 136 (13), 122 (73), 107 (10),93 (48), 79 (100), 67 (29), 59 (12), 54 (23) 41 (53), 33 (3).

Methyl 2-(trans-4-propylcyclohex-2-en-1-yl)ethanoate (mixture of **53** and **54**) Scheme 23, was reduced with lithium aluminium hydride, and then treated with phosphorous tribromide (8.1). The resulting bromide was purified with flash chromatography.
2-(Trans-4-propylcyclohex-2-en-1-yl)ethyl bromide (0.5 g, 2.1 mmol), sodium cyanide (0.12 g, 2.3 mmol) and sodium iodide (0.33 g, 2.1 mmol) were dissolved in methanol (0.7 ml) and water (0.3 ml), according to the method described by Holan and O´Keefe[47] and Vogel[48], for similar systems. The mixture was refluxed with stirring until the reaction was complete, as determined by capillary g.l.c. on a DB-Wax column. After cooling, sodium hydroxide (0.16 g, 4 mmol), water (0.5 ml) and ethanol (0.5 ml) were added[49], and the mixture was then refluxed over night. After cooling, the methanol/ethanol was removed at reduced pressure and the residue diluted with water. The water phase was washed twice with ether, carefully acidified with 5 % HCl and then extracted repeatedly with ether. The ethereal layers were washed with satd. NaCl

solution and dried over $MgSO_4$ to yield 0.21 g (51 %) of pure (trans-4-propylcyclohex-2-en-1-yl)-3-propanoic acid.

(1,4-isomer) m/e ; 196 (M^+; 4 %), 178 (8), 160 (4), 153 (12), 136 (35), 123 (17), 107 (31), 93 (100), 81 (67), 73 (8), 67 (70), 55 (51), 45 (55), 41 (92).

(1,2-isomer) m/e ; 196 (M^+; 7 %), 178 (1), 153 (7), 136 (35), 123 (22), 107 (26), 93 (90), 81 (77), 73 (6), 67 (82), 54 (64), 41 (100).

The acid was reduced with lithium aluminium hydride, and then acetylated (8.1). Pure **29** was obtained by semi-preparative h.p.l.c. under the same conditions as for **28**.

(1,4-isomer) ∂_H(300 MHz) 0.90 (t,3H,Me), 1.10-1.44 (m,8H,CH_2CH_2), 1.59-1.72 (m,2H,CH_2CC=), 1.78-1.86 (m,2H,CH_2CC=), 2.02-2.06 (m,2H,CHC=), 2.05 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.48-5.58 (m,2H,J_{AB} 10.3 Hz,CH=C).

(1,4-isomer) ∂_C (75.4 MHz) 14.27, 19.91, 21.00, 25.90, 29.10, 29.11, 32.60, 35.47, 35.53, 38.78, 64.80, 130.96, 132.41, 171.20.

(1,4-isomer) m/e ; 164 (17), 149 (1), 136 (43), 121 (65), 107 (26), 93 (67), 79 (83), 67 (53), 55 (20), 43 (100).

(1,4-isomer) High resolution MS(EI) m/z ;

$[M^+]_{calc} = 224.1776$

$[M^+]_{found} = 224.1778$ +/- 5ppm

$[M^+-1]_{found} = 223.1680$ +/- 5ppm

(1,2-isomer) ∂_H(300 MHz) 0.90 (t,3H,Me), 1.20-1.79 (m,12H,CH_2CH_2,CHC=), 1.92-2.06 (m,2H,CH_2C=), 2.05 (s,3H,Me-COO), 4.06 (t,2H,CH_2-OCO), 5.55-5.68 (m,2H,J_{AB} 10.1 Hz,CH=C).

(1,2-isomer) ∂_C(75.4 MHz) 14.44, 19.94, 21.03, 23.50, 25.29, 26.25, 29.38,

36.76, 36.84, 39.90, 64.93, 126.19, 130.90, 171.24.

(1,2-isomer)m/e ; 164 (9), 149 (1), 135 (12), 121 (68), 107 (11), 93 (40), 79 (57), 67 (53), 54 (34), 43 (100).

REFERENCES

1. Jacobson M., *J. Am. Chem. Soc.*,**78**, 5084 (1956).

2. Ferdinandi E.S. and Just G., *Can. J. Chem.*, **49**, 1070 (1971).

3. Fieser & Fieser, "*Reagents for Organic Synthesis*", Vol. **1**, John Wiley & Sons, Inc., New York, p. 584 (1967).

4. Tsuboi S., Masuda T. and Takeda A., *J. Org. Chem.*, **47**, 4478 (1982).

5. Rossi R., Carpita A. and Quirici M.G., *Gazz. Chim. Ital.*, **111**, 173 (1981).

6. D. Hall, personal communication.

7. Green N., Jacobson M., Henneberry T.J. and Kishaba A.N., *J. Med. Chem.*, **10**, 533 (1967).

8. Ando T., Vu M.H., Yoshida S. and Takahashi N., *Agric. Biol. Chem.*, **46**, 717 (1982).

9. Berger R.S. and Canerday T.D., *J. Econom. Entomol.*, **61**, 452 (1968).

10. Vig O.P., Sharma M.L., Verma N.K. and Malik N., *Ind. J. Chem.*, **19B**, 950 (1980).

11. a) Campell K.N. and Sommers A.H. in "*Organic Syntheses*", Coll. Vol. **III**, John Wiley & Sons Inc., New York, p. 446.
    b) Degering E.F. and Boatright L.G., *J. Amer. Chem. Soc.*, **72**, 5137 (1950).

12. a) Henrick C.A., *Tetrahedron*, **33**, 1845 (1977).
    b) Maurer B. and Grieder A., *Helv. Chim. Acta*, **60**, 1155 (1977).

13. Schwartz M. and Waters R.M., *Synthesis*, 567 (1972).

14. Lindlar H., *Helv. Chim. Acta*, **35**, 446 (1952).

15. Leznoff C.C. and Fyles T.M., *Can. J. Chem.*, **55**, 1143 (1977).

16. Marvell E.N. and Li T., *Synthesis*, 457 (1973).

17. Wong J.W., Palaniswamy P., Underhill E.W., Steck W.F. and Chisholm M.D., *J. Chem. Ecol.*, **10**, 1579 (1984).

18. Smith W.N. and Beumel O.F., *Synthesis*, 441 (1974).

19. Rossi R., Carpita A. Gaudenzi L. and Quirichi M.G., *Gazz. Chim. Ital.*, **110**, 237 (1980).

20. Jäger V. in *"Methoden der Organischen Chemie (Houben-Weyl)"*, **V/2a**, Georg Thieme Verlag, Stuttgart, p. 497 (1977).

21. Taber D.F., *J. Org. Chem.*, **47**, 1351 (1982).

22. Taber D.F., Supplementary material to *J. Org. Chem.*, **47**, 1351 (1982).

23. Houx N.W.H., Voerman S. and Jongen W.M.F., *J. Chrom.*, **96**, 25 (1974).

24. Brown H.C. and Zweifel G., *J. Amer. Chem. Soc.*, **83**, 3834 (1961).

25. Jäger V. in *"Methoden der Organischen Chemie (Houben-Weyl)"*, **V/2a**, Georg Thieme Verlag, Stuttgart, 517 (1977).

26. Corey E.J. and Schmidt G., *Tetr. Lett.*, 399 (1979).

27. Corey E.J. and Suggs J.W., *Tetr. Lett.*, 2647 (1975).

28. Wannagat U. and Niederprum H., *Chem. Ber.*, **94**, 1540 (1961).

29. Bestmann H.J., Kantardjiew I., Rosel P., Stransky W. and Vostrowsky O., *Chem. Ber.*, **111**, 248 (1978).

30. Bestmann H.J., Koschatzky K.H., Schatzke W., Suss J. and Vostrowsky O., *Liebigs Ann. Chem.*, 1705 (1981).

31. Crombie L. and Williams J.C., *J. Chem. Soc.*, 2449 (1962).

32. Chisholm M.D., Steck W. and Underhill E.W., *J. Chem. Ecol.*, **6**, 203 (1980).

33. Ando T., Vu M.H., Yoshida S. and Takahashi N., *Agric. Biol. Chem.*, **46**, 717 (1982).

34. Krapcho A.P., *Synthesis*, 805 (1982).

35. Blum Z., Ekström M. and Wistrand L-G., *Acta Chem. Scand.*, **B 38**, 297 (1984).

36. Tsuboi S., Masuda T., Makino H. and Takeda A., *Tetr. Lett.*, **23**, 209 (1982).

37. Skattebøl L., Jones E.R.H. and Whiting M.C. in *"Organic Syntheses"*, Coll. Vol. **IV**, John Wiley & Sons Inc., New York, p. 792.

38. Azovskaya V.A., Prilezhaeva E.N. and Stepanyants A.U., *Izv. Akad. Nauk SSSR, Ser. Khim.*, **3**, 662 (1969); *C.A.*, **71**, 254 (1969).

39. a) Snyder H.R., Anderson H.V. and Hallada D.P., *J. Amer. Chem. Soc.*, **73**, 3258 (1951).
b) Carr R.V.C. and Paquette L.A., *J. Amer. Chem. Soc.*, **102**, 853 (1980).

40. Trost B.M., Arndt H.C., Strege P.E. and Verhoeven T.R., *Tetr. Lett.*, **39**, 3477 (1976).

41. Bäckvall J.-E., Nyström J.-E. and Nordberg R.E., *J. Am. Chem. Soc.*, **107**, 3676 (1985).

42. Sellén M., Bäckvall J.-E., Abstract from *"Organikerdagarna"*, Stockholm, Sweden (1986).

43. a) Bäckvall J.-E. and Sellén M., *J. Chem. Soc., Chem. Commun.*, 827 (1987). b) Sellén M., personal communication.

44. Sprich J.D. and Lewandos G.S., *Inorg. Chim. Acta*, **76**, L241 (1983).

45. Suslick K.S., Schaffner K.and Demuth M.in*"Modern Synthetic Methods 1986"*, Ed. Scheffolg R., Springer Verlag, Berlin Heidelberg, p.1.

46. Gilman H. and Catlin W.E. in *"Organic Syntheses"*, Coll. Vol. I, John Wiley & Sons Inc., New York, p. 188

47. Holan G. and O´Keefe D.F., *Tetr. Lett.*, 673 (1973).

48. Vogel A.I. in *"A text-book of practical organic chemistry including qualitative organic analysis"*, Longmans, Green and Co, London, p. 401 (1951).

49. Vogel A.I. in *"A text-book of practical organic chemistry including qualitative organic analysis"*, Longmans, Green and Co, London, p. 353 (1951).

Lunds Universitet
Reprocentralen, 1987