“BABES-BOLYAI” UNIVERSITY, CLUJ-NAPOCA

FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

CONTRIBUTIONS TO THE SYNTHESIS AND THE APPLICATIONS OF THE PHEROMONES OF SOME LEPIDOPTERA SPECIES

PhD Thesis Abstract

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Kewords: semiochemical compounds, olefinic pheromones, conjugated diene systems, C-alkylation reactions, cross-coupling reactions, organometallic derivatives, structural analysis by GC-MS, IR şi RMN, ecomonal products, ecological agriculture

INTRODUCTION

The importance of the biologically active natural products is continuously growing in our country as well as in the whole world, due to their use in several fields such as: medicine, pharmaceutics, cosmetics or environment protection. As a results of the efforts of several laboratories from various countries the structure of several biological activity compounds with was determined by isolating them from vegetal materials, animals, or by total synthesis. In the last few years a growth was observed in the interest for the use of these biologically active compounds in the environment protection through for pest control.

The restrictions which appear through the requirements which the European Union imposes in the use of products destined to protection of plant, poses serious questions to the management of pests.

In the nowadays society, in which the practices of sustainable and friendly development towards the surrounding environment have become key requirements, the reduction or replacement of chemical methods for controlling pests with alternative, non-polluting means represents a major preoccupation of research in the field.

The first step in the integrated pest management (IPM) is the effective monitoring, by using pheromones, launching the **third generation of insecticides**.

Pheromones are substances secreted outside the body of an individual and received by another individual from the same specie, by which they emerge a specific reaction, a definite behaviour or a development process.

This doctoral thesis approaches the study of the pheromones of some of the Lepidoptera species, the development of some selective and effective pheromonal product with impact in the ecological control of the oriental fruit moth, *Grapholita molesta*, plum fruit moth, *Grapholita funebrana*, green budworm moth, *Hedya nubiferana*, peach twing borer, *Anarsia lineatella*, leafminer moth *Cameraria ohridella*, invasive pest of ornamental horse chestnut and establishment of the "attract and kill" biotechnique in monitoring the *Cydia pomonella* and *Lobesia botrana* species.

Through the topic approached, the work presents original results in the **synthesis of pheromones with mono and diene structure** offering a concrete, selective and non-polluting solution of monitoring and control of the ofere mentioned species.

The same time the work joins the traditional concerns of the Natural Products Laboratory from *“Raluca Ripan*” Institute for Research in Chemistry, Cluj-Napoca.

1. SEMIOCHEMICAL COMPOUNDS

For a long time the pests control, was made by mechanical ways, followed then by chemical ways. The **first insecticides** were arsenic, lead and antimony derivations, high toxicity inorganic compounds. The real breakthrough in this field was the discovery that certain organic synthesis substances action preferentialy are insects, afecting less “hot blooded animals”, moment when a **second generation of insecticides** appeared.

The long and intensive use of these inecticides revealed a series of negative aspects. A major inconvenient was the lack of selectivity as second generation insecticides distroyed pests as well as using insects. The remarcable insect capacity to adapt to new, induced new insect population resistance to insecticides, which to be efficient had to be applied in higher doses. The toxicity of the second generation insecticides also had to be reconsidered.

Under the pressure of these facts and also helped by the development of some high sensitivity and efficiency methods, researchers made the **third insecticide generation**, wich was ment to the deficiences of the previous generation. This insecticide generation includes **endo- and exo insect hormones**, of which of major interest are exohormones. Insects, from which the major agricultural pests are recruted, are a grup which depends of chemical signals by exohormones, substances spread in the surrounding environment wich have as a purpose making sure that there is a permanent flow between individuals which send and receive informations. When this information circulate between individuals from the same specie, the involved substances are called **pheromones**. [1]

In 1971 F.E. Regnier and J. H. Law proposed the term semiochemicals compounds, for chemical compounds which mediate interactions between organisms.

Semiochemicals compounds are classified in two important groups: allelochemical compounds and **pheromones**, the classification is being made based on the nature of the inter or intraspecific interaction. [2] (Figure1)

Of special practical importance are the sexual pheromones of insects, because of their help we can set up efficient of those from the previous generation.

Comunication made with the help of the **pheromone** has thre basic caracteristics which show the extremly important role of the pheromones at population level in the ecosystems:

- messages are transmited efficiently, as for the transmision of an information only small amounts of substance are used
- the answer of the receptor organism is completely preprogramed, as insects are not capable of modifying their answer by taking account of the other informations simultaneously received from by the physical chanel. Males when receiving the sexual atractive pheromone are not capable to distinguish if it was a call from a female or from an artificial source. This is

the main characteristic in the use of the pheromonal lures to catch the males of the pest species from the habitat

- each pheromone strictly transmites the intraspecific message, the information contained is able to be received and unwrapped only by individuals from the same specie. There are times when substances contained in the pheromone are received by individuals from other species but the transmited information is unwropped or has different meanings. This characteristic confers ecologic value of pheromone treatments which will strictly affect the specie under “treatment”, leaving the rest of the ecosystem unharwed. [8,9]

The begining of the pheromone chemistry was marked by their isolation and synthesis, as they were succesfuly used in the ecological control of the pest insects [3, 32].

1.1 The chemical structure and the synthesis of the sexual pheromones of Lepidoptera

Lepidoptera is a vast order of insects which includes the moths and the butterflies.

The sexual pheromones of the *Lepidoptera* represent the largest class of chemical substances studied until the time of writing this document. 46]

The quite diverse pheromones, which were identified at the female moths for over 530 species all around the world, are classified according to the chemical structure into pheromones of type I (75%), type II (15%) and various structures (10%) [52, 53]. In addition, there are also pheromones produced by male moths and butterflies. Type I pheromones are unsaturated chemical compounds (with one or multiple double bonds), like the acetates, linear chain aldehydes and alcohols, containing 10 to 18 carbon atoms. Type II pheromones are polyunsaturated hydrocarbons and derived cis-epoxy with a chain containing 17 to 23 carbon atoms.

The techniques for the isolation, identification and the synthesis of the chemical compounds depend on their source and nature.

In the last 5 years, sexual pheromones form new species of Lepidoptera have been identified using analysis methods, GC-EAD, GC-MS, LC-MS and RMN, semi-chemical compounds creating an interesting research domain for organic chemistry, ecology and applied etymology.

1.1.1 Pheromones with monoenic structure

An important class of substances in the organic chemistry are the alkenes. A great variety of the sexual pheromones of moths and butterflies contain a double bond, with *Z* or *E* geometry.

In the relevant bibliography in the field several methods for the synthesis of monoenic pheromones are described. [54-58]

❖ ***Monoenic Pheromones with E Geometry***

Regarding the methods for preparing monoenic pheromones with *E* geometry, the following is described: carbon-carbon coupling reactions through the alkylation of alkynes and the use of metalo-organic compounds, followed by the reduction of the triple bond with sodium in liquid ammonic or $LiAlH_4$, the condensation of aldehydes with malonic acid, the inversion of *Z*-alkynes

❖ ***Monoenic Pheromones with Z Geometry***

Regarding the methods for preparing monoenic pheromones with *Z* geometry, the following is described: carbon-carbon coupling reactions through the alkylation of alkynes and the use of metalo-organic compounds, catalytic hydrogenation of the alkynes, Wittig reaction, hydroboration of the alkynes, the inversion of *E*-alkynes

Many species of insects rarely have a single isomer as a pheromone, usually for an optimal attraction the pheromones being well-determined mixtures of Z and E isomers.

1.1.2 Pheromones With a Diene Structure

The chemical structure of many of the sexual pheromones of insects contains a functionalized hydro carbonated chain (alcohols, acetates, aldehydes) as well as diene systems which can have cumulated double bonds, conjugate or isolated. The geometry of the double links in these systems can be of *EE, EZ, ZE or ZZ* type [82-83].

The major significance of the forming reactions in the carbon-carbon bond in the organic synthesis has lead to arduous investigations in the recent years [84-86]. Many transitional metals are effective promoters and catalysts for the cross-coupling reactions, including palladium [93-96], nickel [87-93], copper [94-95] and iron [96-97]. Numerous methods describe stereo selective syntheses of compounds with diene system, among which are the following:

- Syntheses of compounds with diene system through Wittig condensations
- The partial reduction of dienes obtained through coupling reactions between alkynes and brominated alkyne compounds
- The elongation of the carbon chain of an already formed diene system which has a allyl function that can be replaced by a Grignard reactive

- Alkhenyl-alkenyl couplings of vinilorganoboranic compounds and alkinyl lithium, cross-coupling reactions of organoborans with halo alkynes, catalyzed by compounds with palladium

- Cross -coupling reactions catalyzed by palladium compounds of free acetylenes with (*E*-) or (*Z*-) haloalkynes

- Direct coupling of two groups of alkynes is the most frequently used in cross-coupling reactions catalyzed by palladium between: Grignard reactives and halo alkynes, organo-zinc or organo-aluminum compounds and halogens of alkynes, organo-boranic and halogens of alkenyl, cross-coupling reactions of copper dialkenyls

A limitation of the methods for synthesizing diene pheromones is that compounds that contain mixtures of *EE, EZ, ZE, ZZ* type isomers are obtained in different proportions, several additional purification stages being necessary for obtaining the desired isomer. In most cases, the preparation of diene pheromones requires several stages, controlled reaction conditions and specific catalysts.

1.2 The Use of Pheromones in the bio-monitoring of Some Species of Pests

By trapping the pheromonal lure males researchers were able to study the tracking and supervising of populations, the prognoses of the pests appearing and the warning treatments. [122, 123]

The pheromonal control of the pest insects can be made by:

- **Glued traps containing pheromonal baits for** monitorization of the density of population so as to determine the optimum time of insecticide spraying
- **Technique of mass capture of the males („mass trapping”)** The principle of this method consists of capturing with the help of the pheromonal traps of the males before copulation, in a sufficient proportion as the totality or at least majority of the females in habitat remain uncopulated. The direct consequence is succesors decrease and by repetition, the progressive reduction of the biological reserve of the pest under the level of pesting.
- **Technique of male desorientation („mating disruption”)**. The principle of this method is impregnation of the environment with great doses of synthetical pheromone with the aim of interrupting the mate behaviour chemically mediated, respectively to disorient and reduce to incapacity the male localisation of females to mate.
- A recent approach to the direct control of pests by using semiochemical compounds is the “**attract and kill” bio-technique**, which consists of a substrate with controlled release,

combined with the synthetic pheromone and an insecticide which has the role of eliminating the males that come into contact with the drop of attractant [124-129].

The rapid development of this field of study has had as consequence the accelerated growth of the number of specialists that show interest for these problems and a purpose for various establishments of research around the world.

2. CONTRIBUTIONS

2.1 Contributions to the synthesis of (*Z*)-8- and (*E*)-8- dodecen-1-yl acetates, pheromone components of some species of Lepidoptera with economic relevance

2.1.1 Literature research

The (*Z*)-8- and (*E*)-8- dodecen-1-yl acetates are part of a pheromone composition of several species of the *Lepidoptera* order, some of which have economic importance.

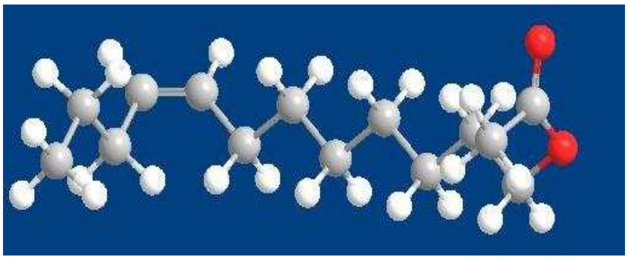


FIGURE 2.1-1 (Z)-8-dodecen-1-yl acetate

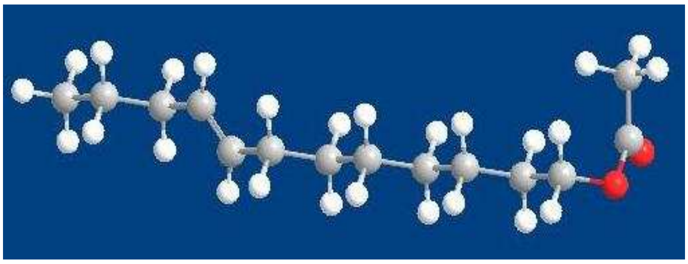


FIGURE 2.1.-2 (E)-8-dodecen-1-yl acetate

This type of pests have a significant importance, their attack being easily noticed and treatments being in order. For our country, the pests with economic relevance are the *Grapholita molesta*, oriental fruit moth, *Grapholita funebrana*, plum fruit moth and the *Hedya nubiferana*, green budworm mmoth, species.

Grapholita molesta Busk *(Lepidoptera, Tortricidae)* is a serious stone fruit pest. Discovered in 1902 in plantation of peach and pear, in NE China and Japan, it has spread to most areas of the globe. In Romania it was identified in 1964 in some plantations of peach and plum in SV the country [166]. This species infests all stones fruit (peaches, nectarines) plus apple and pear. Late ripening peach cultivars are particulary to attak oriental fruit moth. Infested shoot and terminal leaves wilt and bend over. Flagging injuri stimulates lateral growth below the point of injury. This can inhibit good scaffold formation in young trees, and provides wound sites for pathogens [169-171]

Grapholita funebrana Treitschke *(Lepidoptera, Tortricidae),* plum fruit moth, is common to the temperate climate, frequently seen in the prune, cherry, peach and apricot orchards. [176, 177] In our country, when preventive actions are not taken, the pest produces major damages from an economical point of view. The damages are produced by the larvae, reaching 50-80% of the production.

First generation larvae attack the not yet ripped fruits, eating-through holes and consuming the raw pulp. A larva infests 2-5 fruit. The attacked fruits fall. Second generation larvae damage the fruits when they are ripping and the first fruits; they eat-through holes and similarly consume the pulp around the kernel. The attacked fruits rot away. [180, 181]

Green budworm moth – Hedya nubiferana Haw (*Lepidoptera, Tortricidae*) is spread across Europe, including Romania. Even if it is polyphagia, being able to live on the pear, apple, prune, cherry, apricot, almond tree, medlar, gooseberry, rose bush, willow, oak, ash, birch tree it especially attacks the apple tree, which it can seriously damage by destroying the buds, the primary flowers and foliage right after blossom and by eating up the leafs.

The insect is polyphagia, infecting the apple tree, the prune tree, the apricot tree, the pear tree, the cherry tree, the gooseberry, the strawberry fields, but it prefers the apple tree orchards. The larvae wrap the leafs and buds in silky threads, forming a sort of nest or fascicle that shelters them while eating the tender leafs and the buds.

The literature in the field presents several methods for preparing the (*Z*)-8-**128** and (*E*)-8-dodecen-1-yl acetates (**129**), either by acetylene ways, or through the Wittig method, achieving a careful stereo control of the reaction in order to obtain the desired isometric ratio. [189-197]

2.1.2 Studies on the synthesis of the (*Z*)-8- and (*E*)-8-dodecen-1-yl acetates, pheromone components of some *Lepidoptera* species

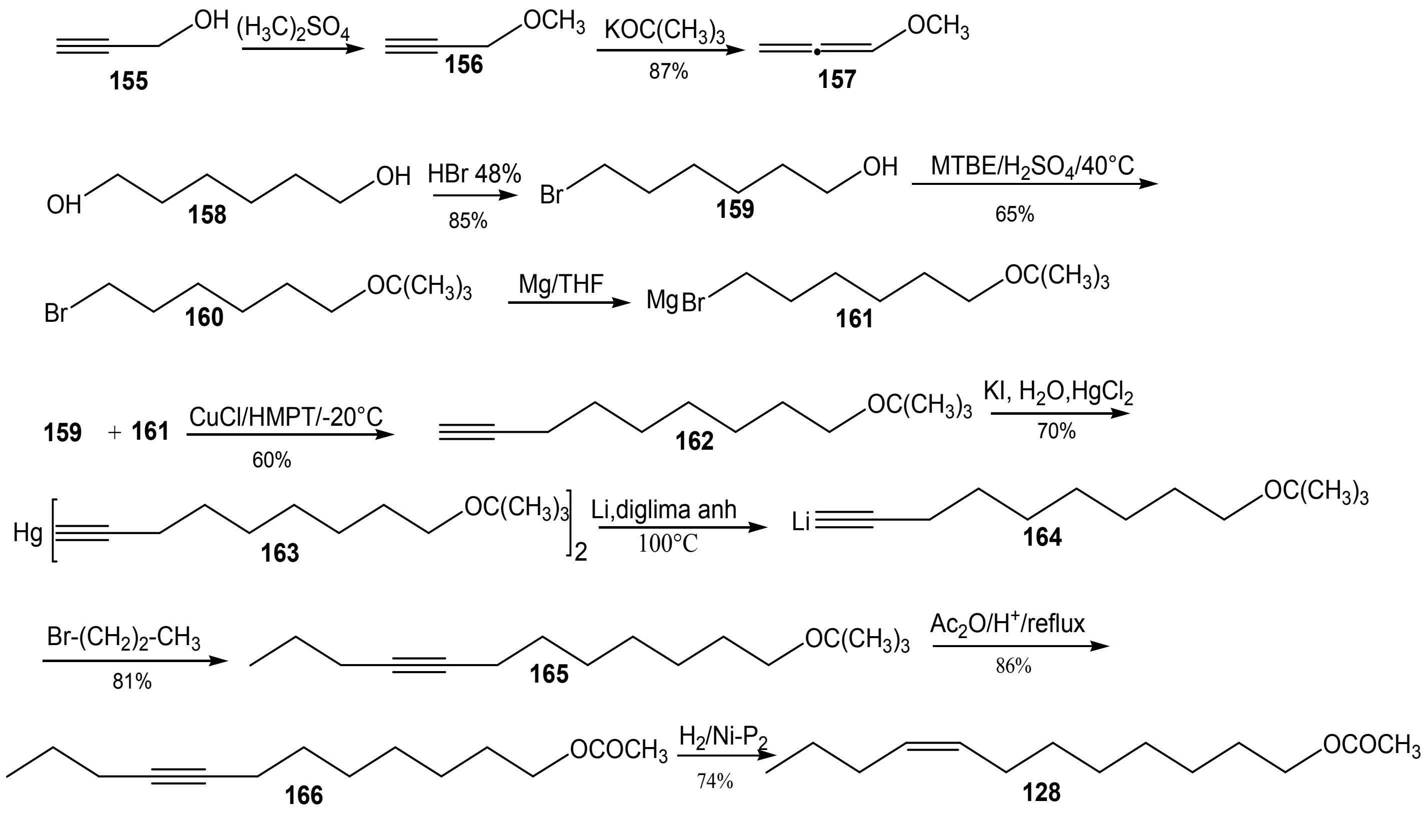
2.1.2.1 The synthesis of the (*Z*)-8-dodecen-1-yl acetate

The synthesis of the (*Z*)-8- and (*E*)-8-dodecen-1-yl acetates was achieved in Natural Products Laboratory from "*Raluca Ripan*" Institute for Research in Chemistry.

The method for preparing the main component, the (*Z*)-8-dodecen-1-yl acetate (**128**), is based on C-alkylation reactions, using as intermediates allenic derivatives, organomagnesium derivatives and mercury derivatives of some 1-alkyne ω-fuctionalised.

The novelty of the method consists in forming the mercury salts on terminal alkyne ω-fuctionalised, transmetallation with metal lithium reaction, followed by coupling reaction with halo-derivatives. [198]

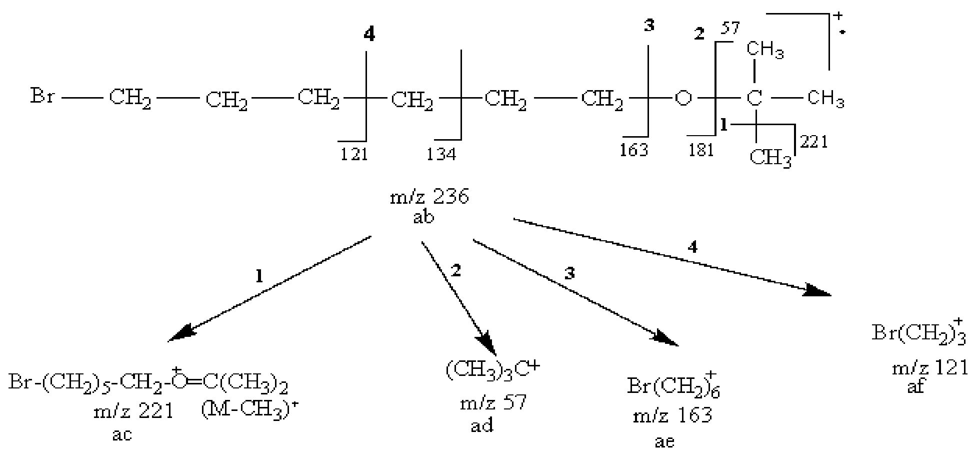
The 12 carbon atom chain is make according to the coupling scheme $C_3+C_6=C_9$ and $C_9+C_3=C_{12}$, the starting materials being the 2-propyn-1-ol and 1, 6- hexanediol. Scheme 2.1-7



The methoxyallene (**157**) was prepared from methyl propargyl ether (**156**) which was treated with potassium *tert*-butoxide. After distillation from the reaction mixture the methoxyallene **(157)** was obtained with 87 yield.

Methyl-*tert*-butyl ether was used under acid catalysis to protect the –OH function of the 6-bromo-hexane-1-ol (**159**), yield 65%.

The mass spectrum of 1-*tert*-butoxy-6-bromo-hexane (**160**) show characteristic fragmentation of the tert-butoxy group and isotopic peaks separated with 2um because of the presence of bromine. Scheme 2.1-8



SCHEME 2.1-8

The C-alkylation reaction of methoxyallene (**157**) with Grignard reagent of 1-*tert*-butoxy-6-bromo-hexane (**160**) in the presence of monovalent copper in anhydrous diethyl ether, -20°C , gave 1-*tert*-butoxy-non-8-yne. (**162**). The reaction occurs by the SN2 nucleophylic substitution type of methoxy group with alkyl group. After distillation at 75°C/2mmHg 1-*tert*-butoxy-non-8-yne **(162)** was obtained with purity 95%, yield 60%.

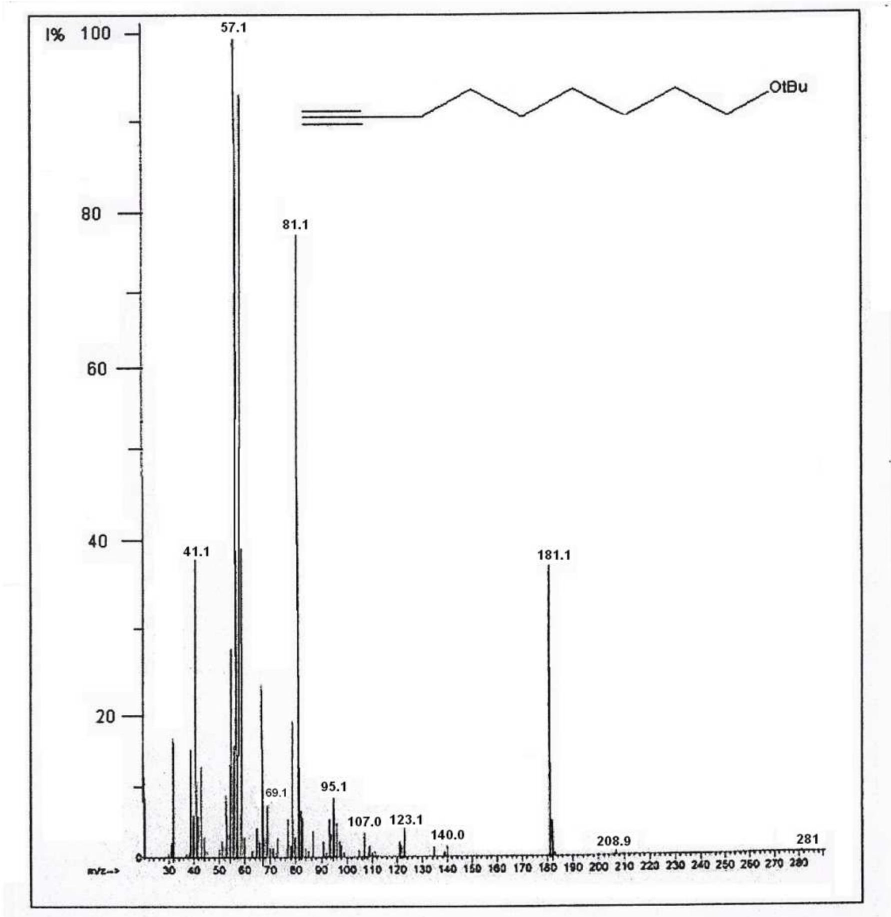


FIGURE 2.1-11 Mass spectrum of 1-*tert*-butoxy-8-nonyn

The mercury derivative of 1-*tert*-butoxy-non-8-yne **(162)** was achieved with potassium iodide dissolved in aqueous solution of mercury chloride and 10% sodium hydroxide solution. After filtration and drying was obtained di (tert-butoxy-non-8-yne (**163**), yield 70%

The second coupling reaction consisted in directly lithiated (100-110°C) of the mercury compound 163 and then alkylated with propyl bromide obtaining 1-*tert*-butoxy-dodec-8-yne (165), yield (81%). This reaction is the key step of synthesis.

The chemical structure of the compounds **165** was confirmed by ^{1}H-NMR and ^{13}C-NMR spectrum (Figure 2.1-13, Figure 2.1-14)

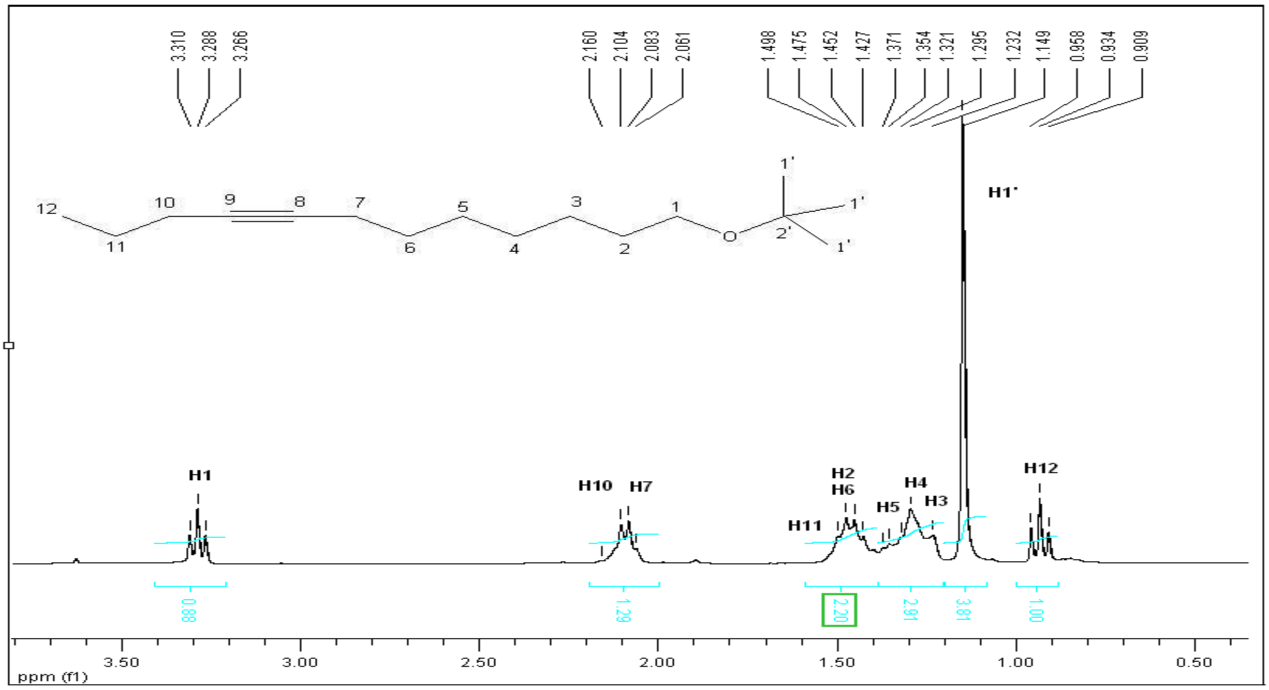


FIGURE 2.1-13 ^{1}H-RMN spectrum (300 Mz, $CDCl_3$) of 1-tert-butoxy-dodec-8-yne

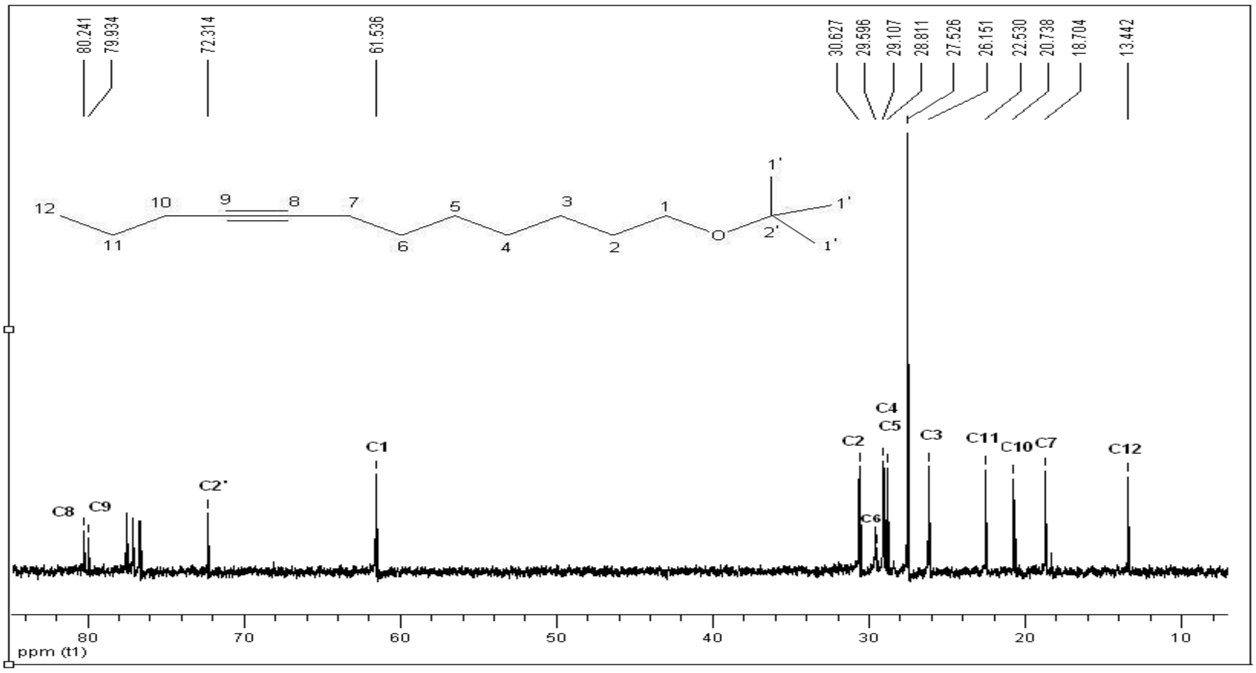


FIGURE 2.1-14 ^{13}C-RMN spectrum (75 Mz, $CDCl_3$) of 1-tert-butoxy-dodec-8-yne

After acetylation and stereoselective reduction of 1-*tert*-butoxy-dodec-8-yne (**165**) in the presence of NiP-2 catalyst, gave (*Z*)-8-dodecen-1-yl acetate with 98% isomeric purity, yield 74%.

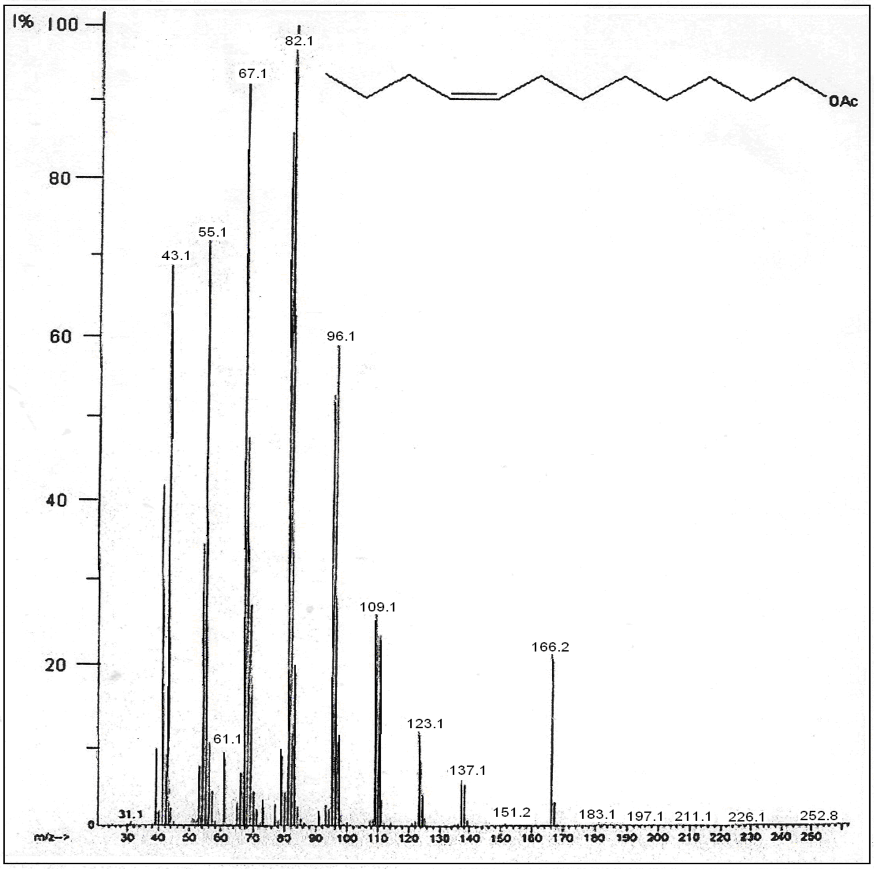
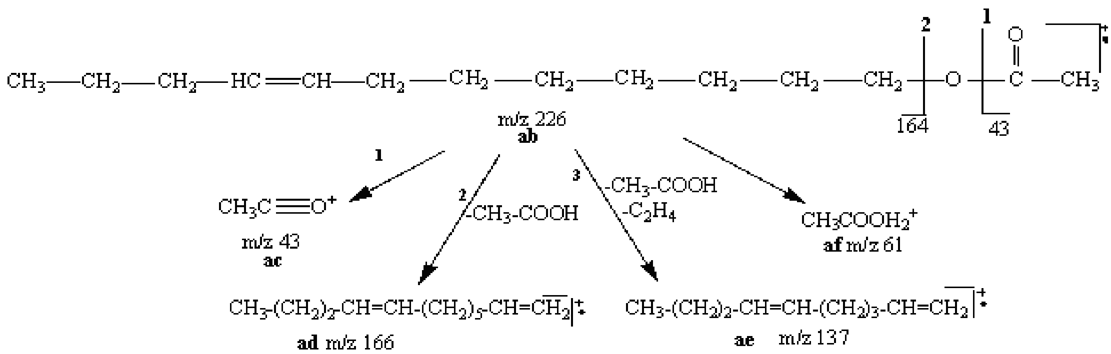


FIGURE 2.1-16 Mass spectrum of (Z)-8-dodecen-1-yl

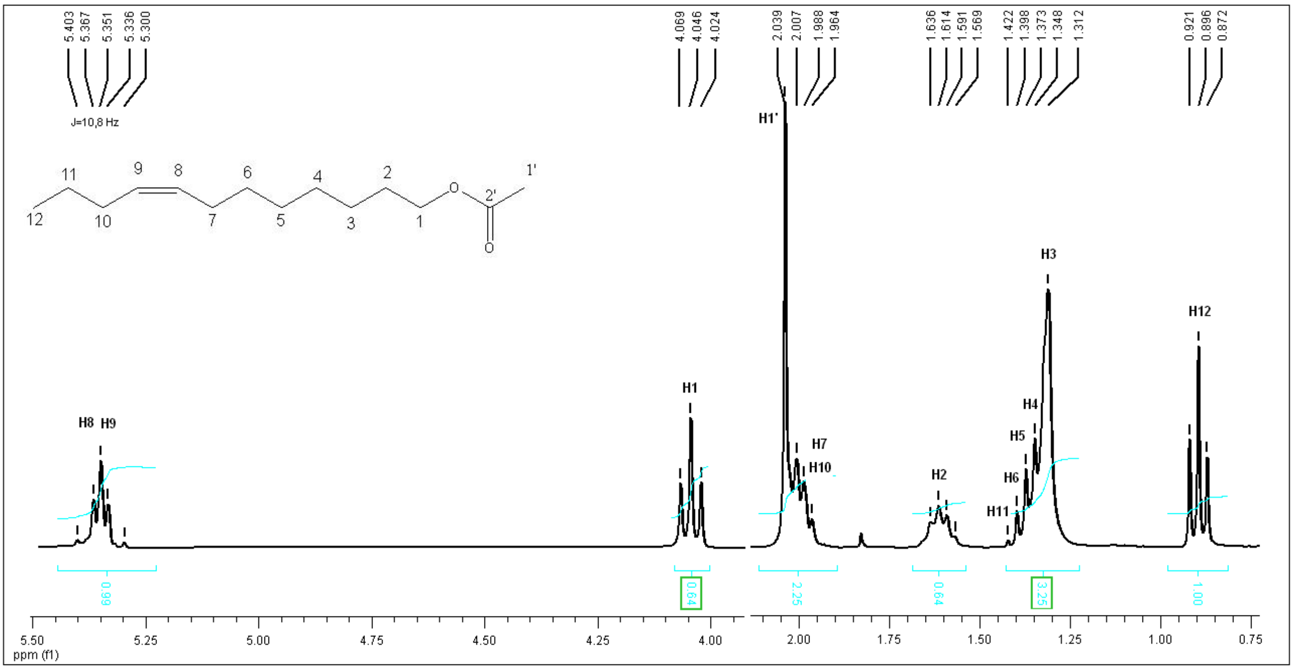
The characteristic fragmentations of (*Z*)-8-dodecen-1-yl acetate (**128**) are shown in scheme 2.1-11.



Scheme 2.1-11

In the mass spectrum of the (*Z*)-8-dodecen-1-yl acetate (**128**) was identified the molecular peak to m/z=226. Other very important fragment appears at m/z=43(CH_3CO^+) as base peak.

^{1}H-NMR spectrum (Figure 2.1-17) of (*Z*)-8-dodecen-1-yl acetate (**128**) includes the following signals: triplet at δ=0.96 ppm corresponding to the protons of the methyl group from position 12 (3H, J=7,5Hz), multiplet (δ=1.31-1.42 ppm) for metthylene protons from positions 3,4,5,6 and 11 (10H), multiplet at δ=1.60 ppm (2H), for methylene protons from position 2, triplet (δ=1.96-2.01ppm) for methylene protons from position 7 and 10 (4H), triplet at δ=4.04 ppm corresponding to the protons of the methylene group from position 1 (2H), singlet at δ=2.03 ppm for metyl group protons from position 1’ (3H) and multiplet δ=5.35 ppm for the olefinic group protons (2H, J=10,8Hz)



,*FIGURE 2.1-17 ^{1}H-RMN spectrum (300 Mz, $CDCl_3$) of (Z)-8-dodecen-1-yl acetate*

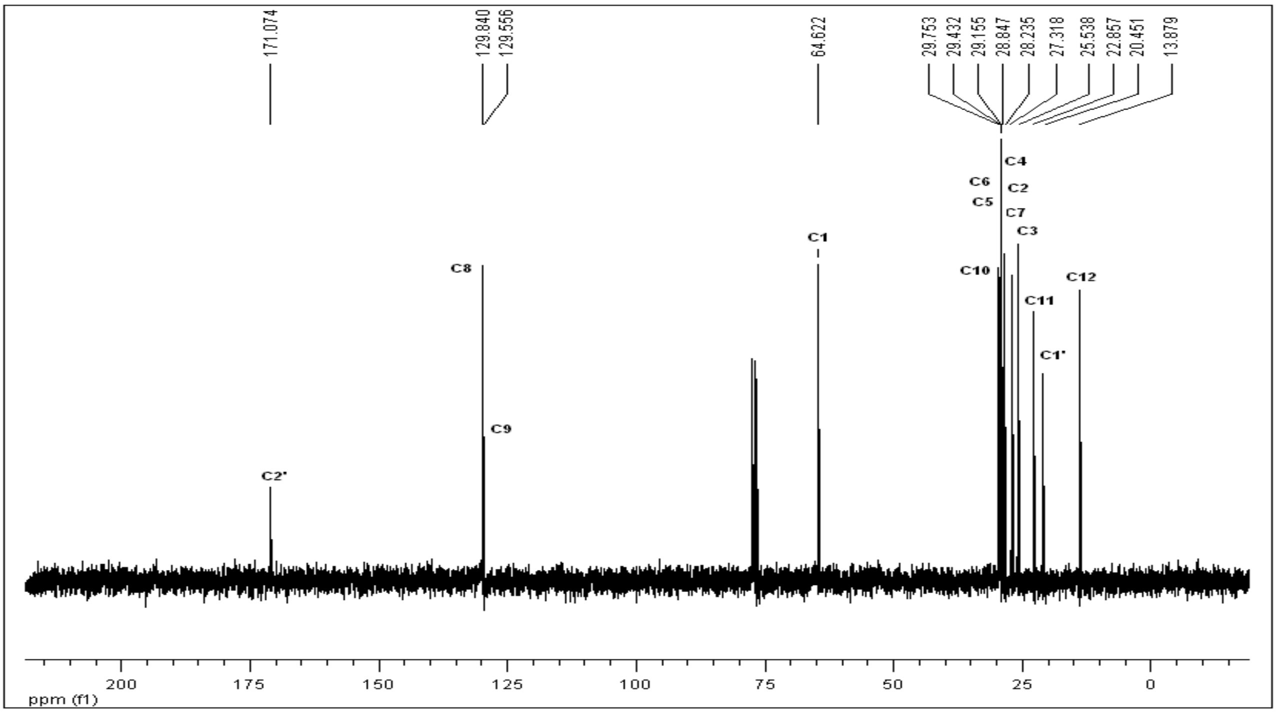
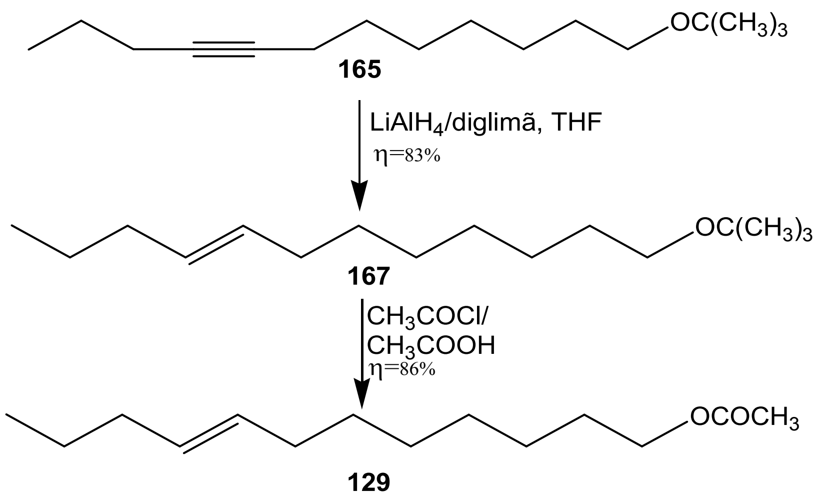


FIGURE 2.1-18 ^{13}C-RMN spectrum (75 Mz, $CDCl_3$) of (Z)-8-dodecen-1-yl-acetate

^{13}C-NMR spectrum, confirms the existence of *Z*- configuration, the chemical shifts of allylic carbons (C_7 and C_{10}) according with data obtained from a series of related compounds with 12 C-atoms in the molecule [201, 202], values of the chemical shifts being δ = 27.3 ppm for C_7 and 29. 4 ppm for C_{10}. The acetoxy group from compound **128** was confirmed by δ = 171.1 ppm for C2 'and δ = 20.4 ppm for C1'.

2.1.2.2 Synthesis of the (*E*)-8-dodecen-1-yl acetate

The preparation of (*E*)-8-dodecen-1-yl acetate (**129**) was achieved by selective reduction of triple bonds from 1-*tert*-butoxy-dodec-1-yne (**165**) with $LiAlH_4$ [203]



SCHEME 2.1-12

The reaction was controlled by TLC. The 1-*tert*-butoxy-(*E*)-8-dodecen (**167**) was obtained with a gas chromatography purity of 93%.

By acetylation of the compounds **167** with a mixture of acetic acid: acetyl chloride=10:1, gave (*E*)-8-dodecen-1-yl acetate (**129**), with 98% isomeric purity.

The mass spectrum of (*E*)-8-1-yl-dodecen acetate (**129**) is identical with the mass spectrum of (*Z*)-8-dodecen-1-yl acetate (**128**) by mass spectrometry the double bond geometry not be established . (Figure 2.1-19)

The ^{1}H-NMR spectrum of *E*-(8) -dodecen-1-yl acetate (**129**) shows a triplet at δ = 3.98 ppm ,assigned of the methylene group protons bearing of acetoxy group, a multiplets at δ = 5.30 ppm showing presence of olefinic bond with trans geometry. The singlet at δ =2.01 ppm corresponding of the methyl group protons from acetoxy group and the multiplets (δ = 1.25 - 1.35 ppm) for methylene protons from the center of the molecule. Figure 2.1-20

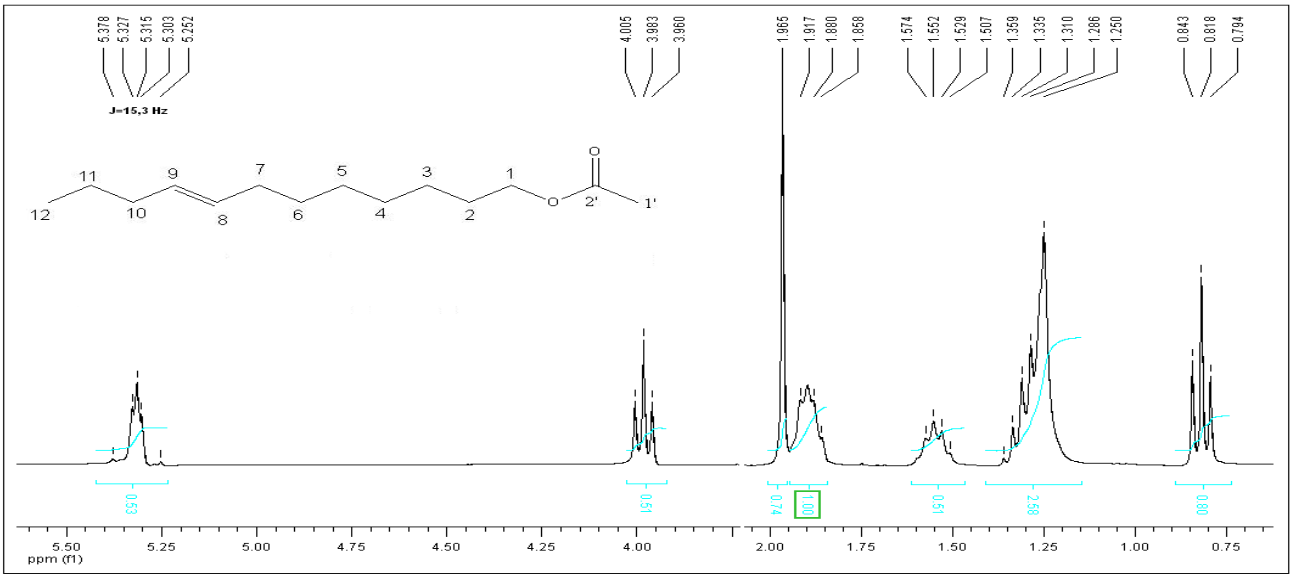


FIGURE 2.1-20 ^{1}H-RMN spectrum (300 Mz, $CDCl_3$) of (E)-8-dodecen-1-yl acetate

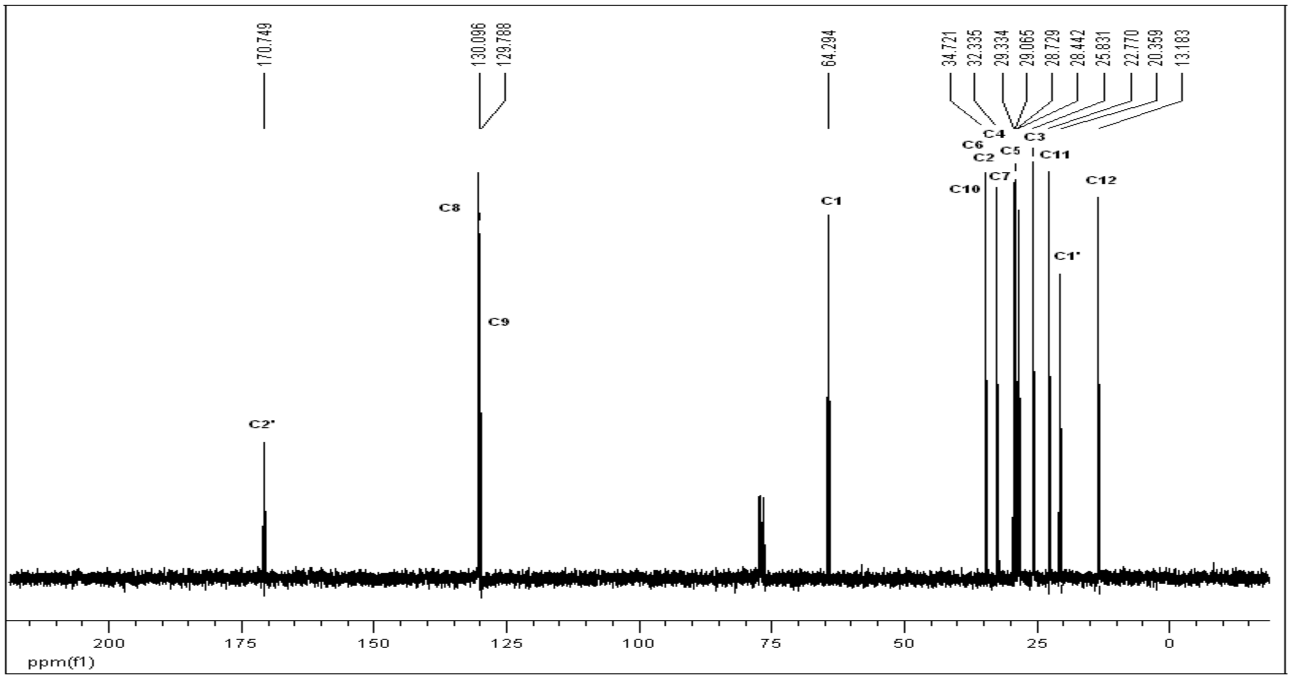


FIGURE 2.1-21 ^{13}C-RMN spectrum (75 Mz, $CDCl_3$) of (E)-8-dodecen-1-yl-acetate

^{13}C-NMR spectrum, confirms the existence of *E*- configuration, the chemical shifts of allylic carbons (C_7 and C_{10}) according with data obtained from a series of related compounds with 12 C-atoms in the molecule [201, 202], values of the chemical shifts being δ = 32.3 ppm for C_7 and 34.7 ppm for C_{10}.

2.1.2.3 Cromatographyc separation of (*Z*)-8- and (*E*)-8-dodecen-1-yl acetate

Figure 2.1-22 shows gas chromatography analysis of a sample of mixture of (*Z*) -8- and (*E*)-8-dodecen-1-yl-acetate (**128, 129**) separated on a capillary column HP-5ms 30m x 0, 25 mm.

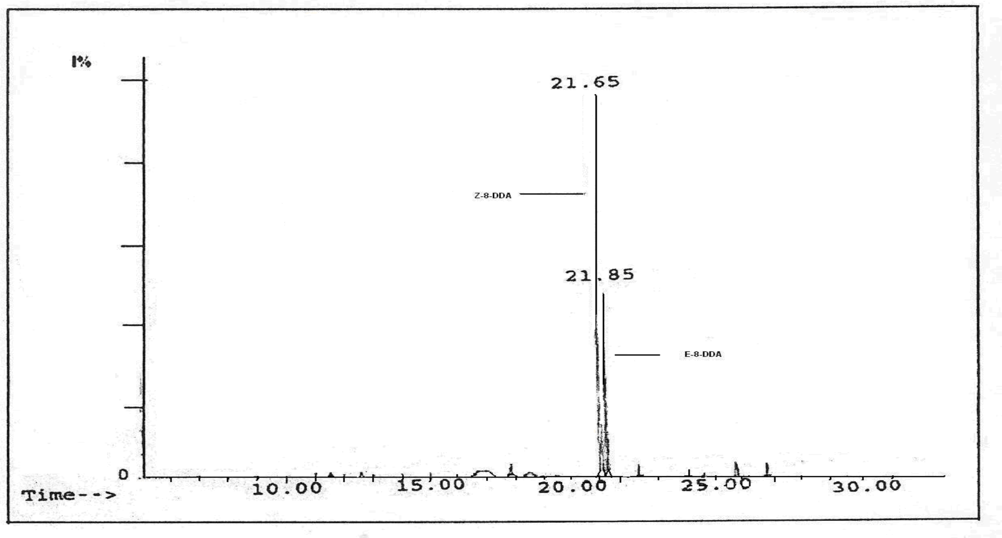


FIGURE 2.1-22, GC-analysis of (Z)-8- and (E)-8-dodecen-1-yl acetate

The separation of (*Z*)-8-dodecen-1-yl- acetate (**128**) and (*E*)-8-dodecen-1-yl-acetate (**129**) was performed by thin layer chromatography, silica gel plates Merck 60, eluent , benzene: ether = 23:2, detected with sulfuric acid (d = 1.28). (Figure 2.1-22)

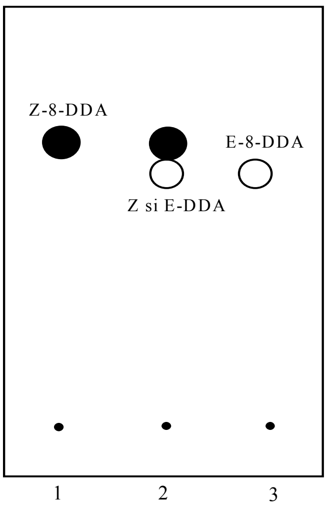


FIGURE 2.1-22, separation of (Z)-8- and (E)- 8-dodecen- 1-yl-acetate by TLC

2.2 Contributions to the synthesis of the sexual pheromone of the peach twig borer, *Anarsia lineatella*

2.2.1 Literature Research

The peach has a large number of diseases and pests that attack the fruit and other organ of the trees. [204]

Because the two key pests, *Grapholita molesta* (oriental fruit moth) and *Anarsia lineatella* (peach twig borer), peach cultivation is difficult to occur even in good conditions and a large quantity of insecticide. New directions in research emphasisez ecological alternativeto control pest and diseases encouraging organic peach production. [170, 205]

The peach twig borer, *Anarsia lineatella* produces damage with an economic importance in the peach orchards, scarcer in the apricot or prune orchards. The damage is produced by the larvae which have hibernated, which affect the buds and the newly formed offshoots and also the damage is produced by the first and second generation larvae, which attack the fruit. The damaged fruit remain small and ripe prematurely and fall off. The ecologic control methods through mass trapping of males with sexual pheromone has produced good results.

The evidence of specific sexual pheromone was described for the first time by Anthon and all. [210]. This was identified as a mixture of two components, (*E*)-5-decen-1-yl acetate and (*E*)-5-decen-1-ol (Figure 2.2-4, Figure 2.2-5)

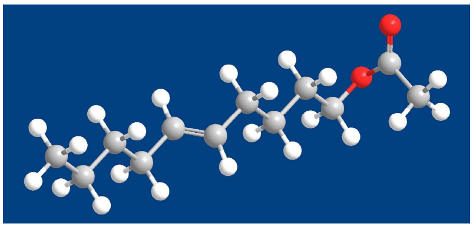


FIGURE 2.2-4 (E)-5-decen-1-yl acetate

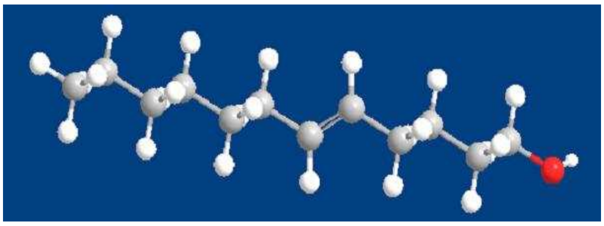
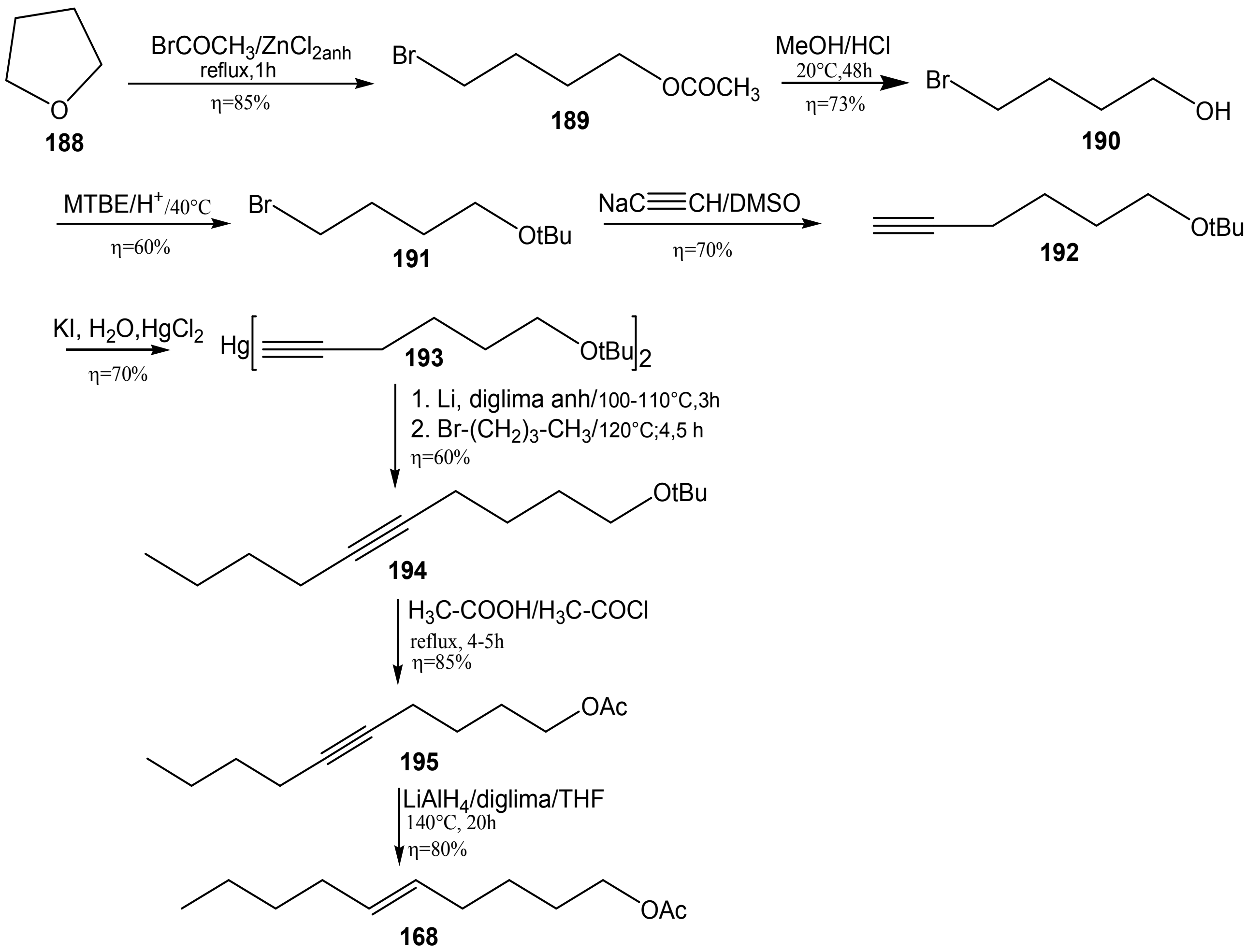


FIGURE 2.2-5 (E)-5-decen-1-ol

In the literature various methods of the preparation of (*E*)-5-decen-1-yl acetate (**168**), the main component of the sexual pheromone of peach twig borer, *Anarsia lineatella,* are mentioned.

2.2.2 Studies on the synthesis of (*E*)-5-decen-1-yl acetate

The preparation of the (*E*)-5-decen-1-yl- acetate (**168**) was achieved in Natural Products Laboratory from "Raluca Ripan" Institute for Research in Chemistry, after original synthesis pathway (**C_4+C_2=C_6; C_6+C_4=C_{10}**), using as intermediates the oranomercury derivative of an 1-alkyne ω-functionalised. Scheme 2.2-7



Scheme 2.2-7

The preparation of 4-bromo-butane-1-yl acetate (**189**) is achieved for ring opening of tetrahydrofuran by the action of acetyl bromide in the presence of zinc chloride (yield 85%). By acid hydrolysis (HCl/MeOH) at room temperature of compound **189,** gave 4-bromo-butan-1-ol (**190**), yield 75%.

Methyl *tert*-butyl ether was used under acid catalysis to protect –OH fuction of the 4-bromo-butane-1-ol (**190**), obtaining 1-*tert*-butoxy-4-bromo-butane (**191**), yield 65% and purity 95% .

The first coupling reaction took place between monosodium acetylene obtained in situ, and 1-*tert*-butoxy-4-bromo-butane, the metallation reagent being dimsyl sodium in DMSO.

1-*tert*-Butoxy-hex-5-yne (**192**) was isolated from the reaction mixture by extraction with petroleum ether, yield 75%, 80% gas chromatography purity.

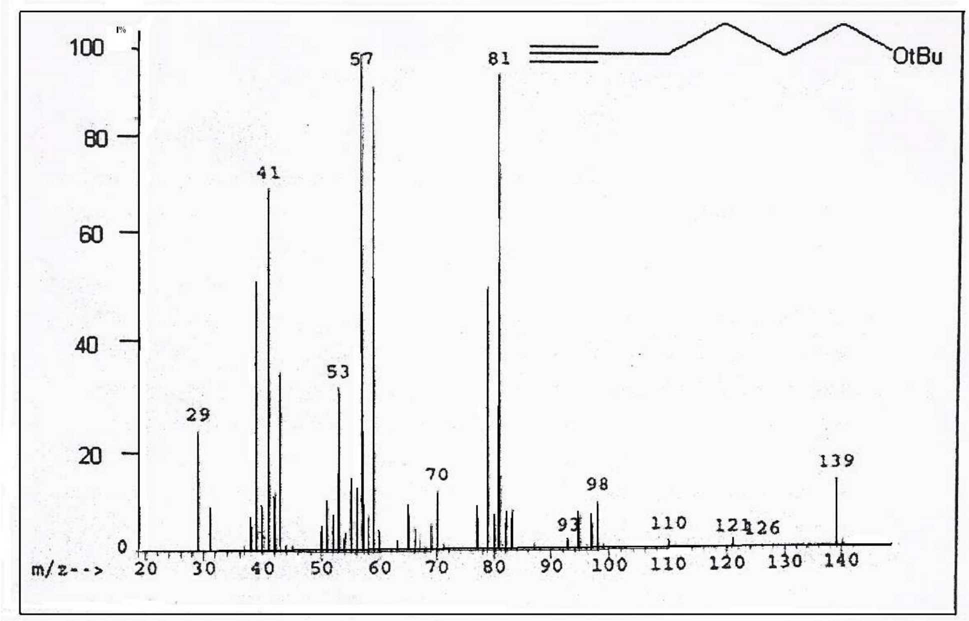
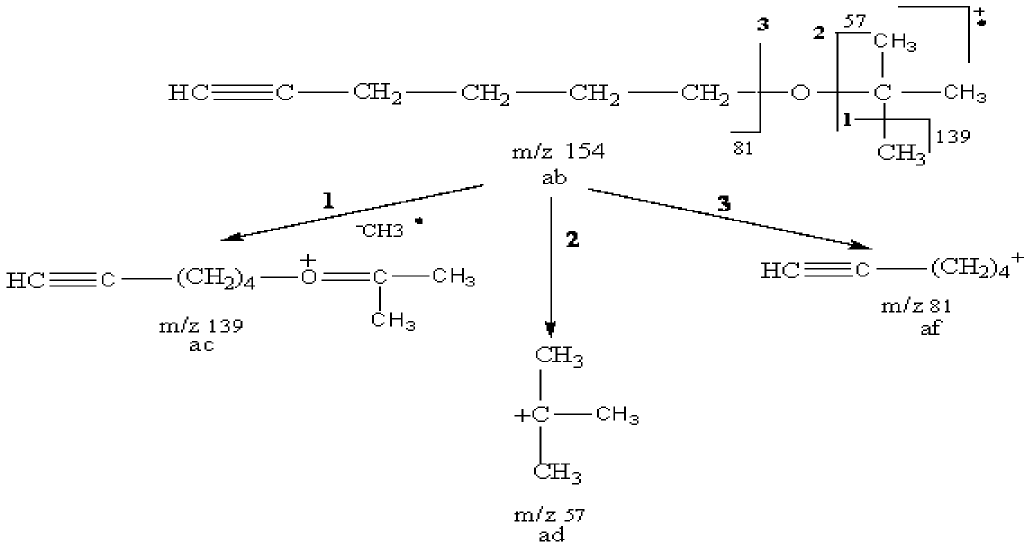


FIGURE 2.2-9 Mass spectrum of 1-tert-butoxy-hex-5-yne

The molecular peak of **192** was not identified, the mass spectrum presenting only characteristic fragmentations. It is necessary to mention the base peak in the spectrum (m/z=59), the peak m/z 57 which is generated by very stable tertiary carbocation $+C(CH_3)_3$, the peak corresponding to the cleavage of a methyl group from the branch part (m/z=139) and of a neutral molecule of isobutylene (m/z=98). Scheme 2.2-8.



SCHEME 2.2-8

1-*tert*-Butoxy-hex-5-yne (**192**) was checked by means of IR spectrum, (film, cm $^{-1}$): 895 m, 1110 vs, 1220 vs, 2160 w -C ≡ C-, 3280 s ≡CH, which presents the vibration of terminal triple bond and the mass spectrum characteristic to a *tert*-butyl ether.

1-*tert*-Butoxy-hex-5-yne (**192**) was converted in mercury derivative **193** by precipitation with Nessler reagent, prepared from mercury chloride, potassium iodide and sodium hydroxide 10%, yield 70%.

The use of di(tert-butoxy-hex-5-yne) mercury (193) as intermediate in the synthesis of the sexual pheromone *Anarsia lineatella* species was not reported in the literature.

The second metallation reaction consisted in directly lithiated of the mercury compound **193** and then alkylated with butyl bromide obtaining 1-*tert*-butoxy-dec-5-yne (*194*), yield 60%.

By acetylation of 1-*tert*-butoxy-dec-5-yne (**194**) with a mixture of glacial acid acetic : acetyl chloride = 10:1 and selective reduction of triple bond from compound **195** in the presence of $LiAlH_4$ gave (*E*)-5-decen-1-yl acetate (**168**) with 98% isomeric purity.

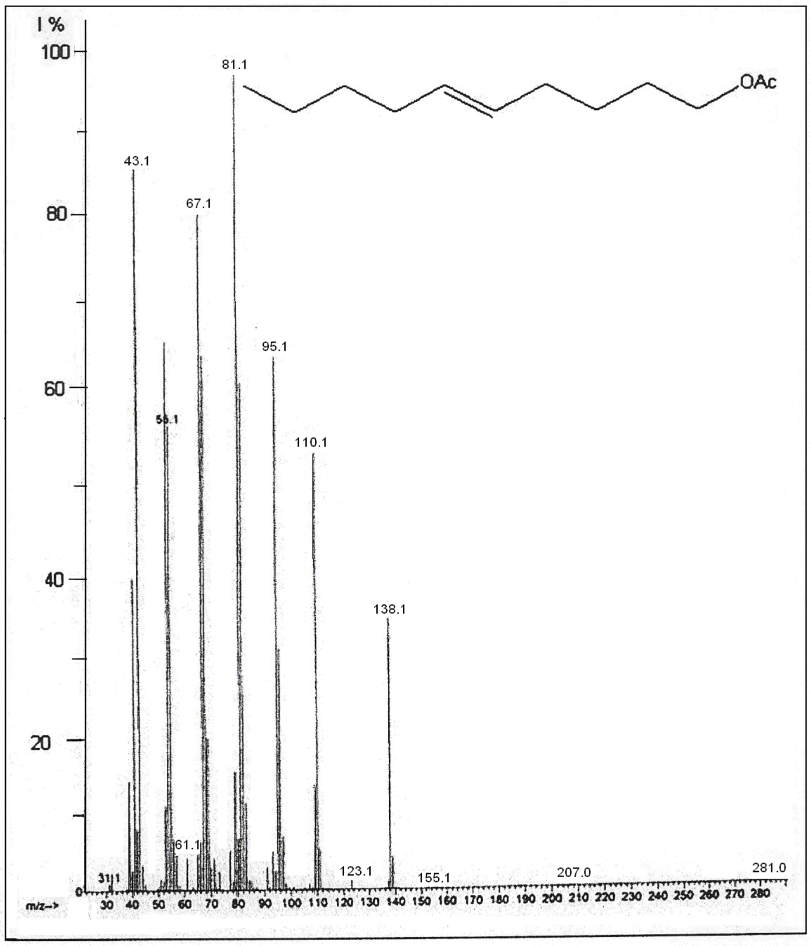
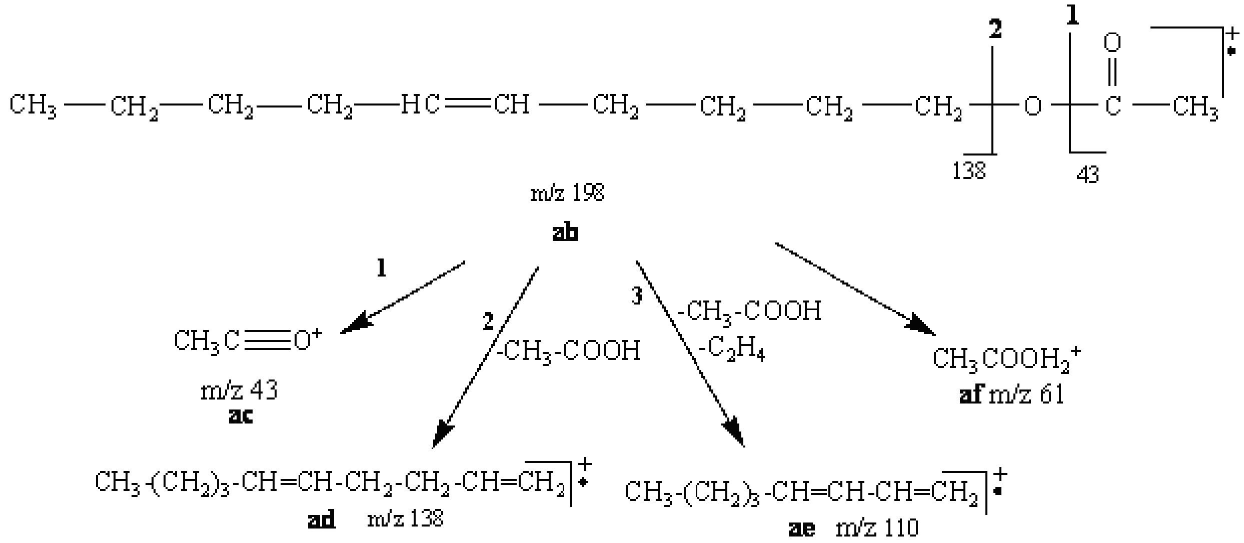


FIGURE 2.2-11 Mass spectrum of (E)-5-decen-1-yl acetate

The characteristic fragmentations of the (*E*)-5-decen-1-yl acetate (**168**) a presented in scheme 2.2-9



SCHEMA 2.2-9

The mass spectrum is characteristic to an alkenol acetate, showing of base peack at m/z 43 (CH_3CO^+), a peak at m/z 61 generated by double transposition ion $CH_3COOH_2^+$.

^{1}H-NMR spectrum of the (*E*)-5-decen-1-yl acetate (**168**) presents a triplet at δ=4.01 ppm assigned to methylene group bearing the acetoxy group, a multilplets at δ=5.36 ppm at showing presence of olefinic bounds, a singlet at δ=2.01 ppm coresponding to the methyl group from acetoxy group and a multiplets at δ=1.26-1.30 ppm for methylene protons in the center of the molecule. Figure 2.2-12

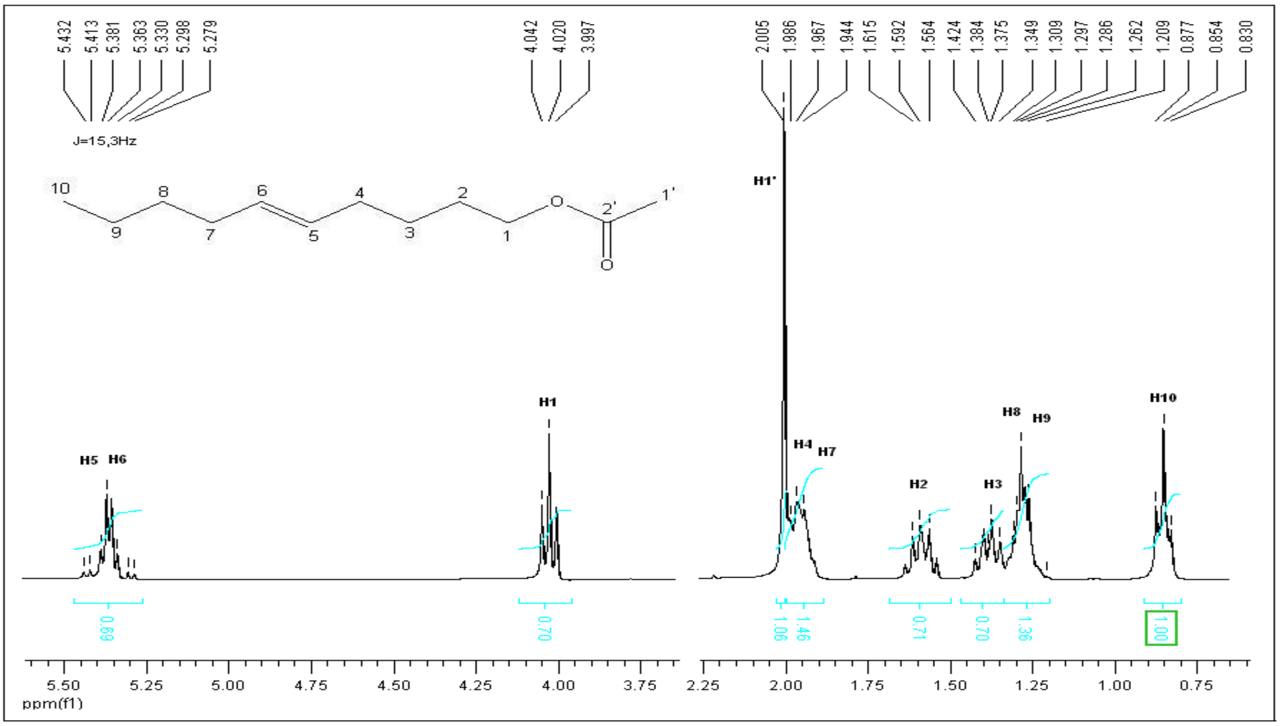


FIGURE 2.2-12 ^{1}H-NMR spectrum(300 Mz, $CDCl_3$) of (E)-5-decen-1-yl acetate

In figure 2. 2-13 is shows ^{13}*C-NMR spectrum* of the compounds **168.**

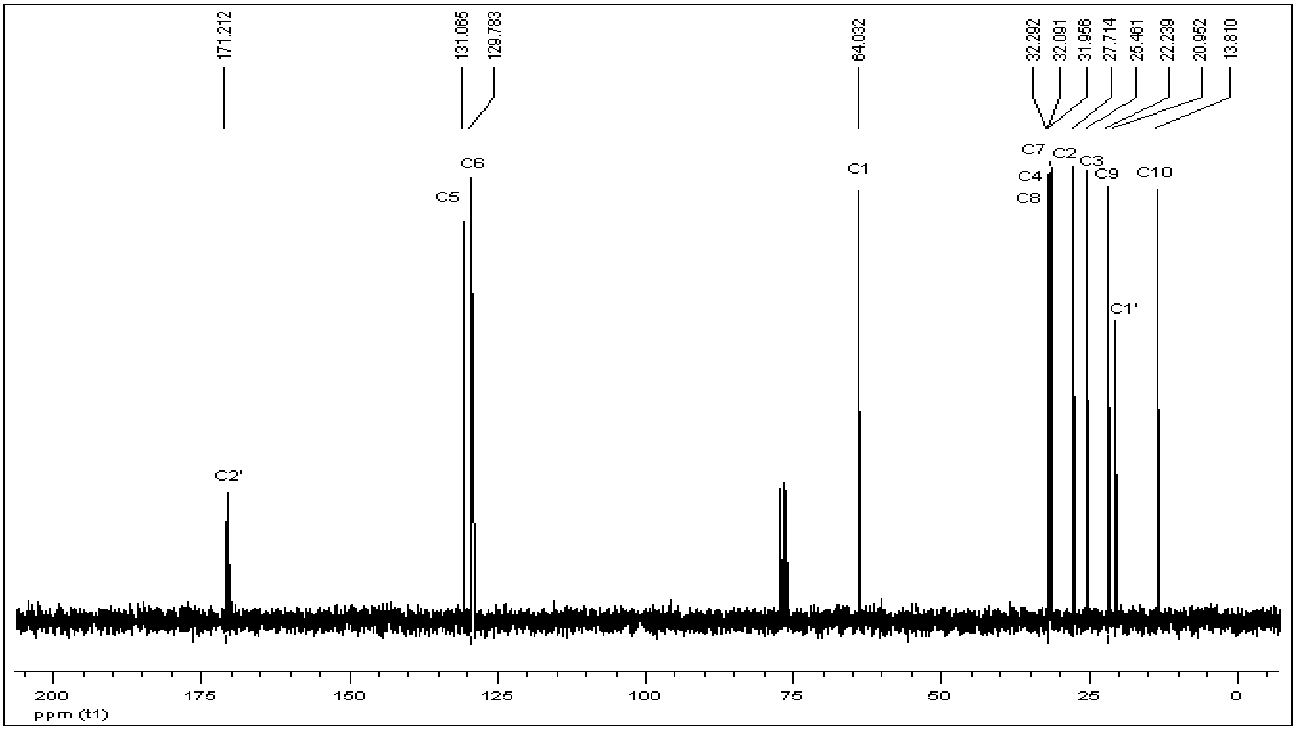


FIGURE 2.2-13 ^{13}C-NMR spectrum (75 Mz,$CDCl_3$) of (E)-5-decen-1-yl acetate

2.3 Contributions to the synthesis of the sexual pheromone of the leafminer species, Cameraria ohridella

2.3.1 Literature research

The biocenosis balance of the ornamental chesnut tree was disturbed during last years by the presence of a new pest –**the leaves mining moth *Cameraria ohridella* (Deschka-Dimić, 1986)**, [224] recently seen in the European countries entomofauna, including in Romania.

In our country, during the last years it rapidly spread creating protection problems of the common ornamental chesnut-tree *Aesculus hippocastanum L*. In the ecological conditions in our country *Cameraria ohridella* species can have up to four generation per a year and infested trees are usually completely defoliated before theend of the season.

The chemical control is difficult because subsequent to eclosion, the larvas penetrates immediately between the folio epidermis in which it starts feeding gnowing a gallery. The pheromonal method is the only efficient one in prognosis, warning and pest control of the *Cameraria ohridella* species and consists in mass trapping by means of traps with sexual synthetic pheromone before copulation. In our country, during the last years it spread with great rapidity creating protection problems of the common ornamental chesnut-tree *Aesculus hippocastanum L*.

This chapter relates to a new synthesis pathway of the sex pheromone of the leaves mining moth *Cameraria ohridella*, the main dangerous pest of the ornamental horse chesnut, used as bio-insecticide in ecological control of the species.

The identification and synthesis of the specific sex pheromone of species *Cameraria ohridella* has been made in 1998 and was an important step towards biological control of this dangerous. [251]

The earlier studies using analytical methods (for example GC-EAD and GC-MS coupling of the extract obtained by introduction in hexane of the attacked lives with *Cameraria ohridella*) demonstrated that the chemical structure of the pheromone is (*8E,10Z*)-8,10-tetradecadien-1-al. Figure 2.3.4

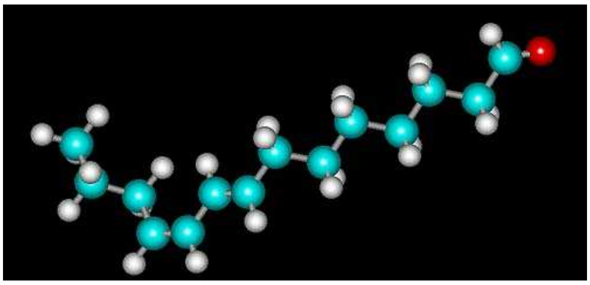


FIGURE 2.3.4 (8E,10Z)-8,10-tetradecadien-1-al

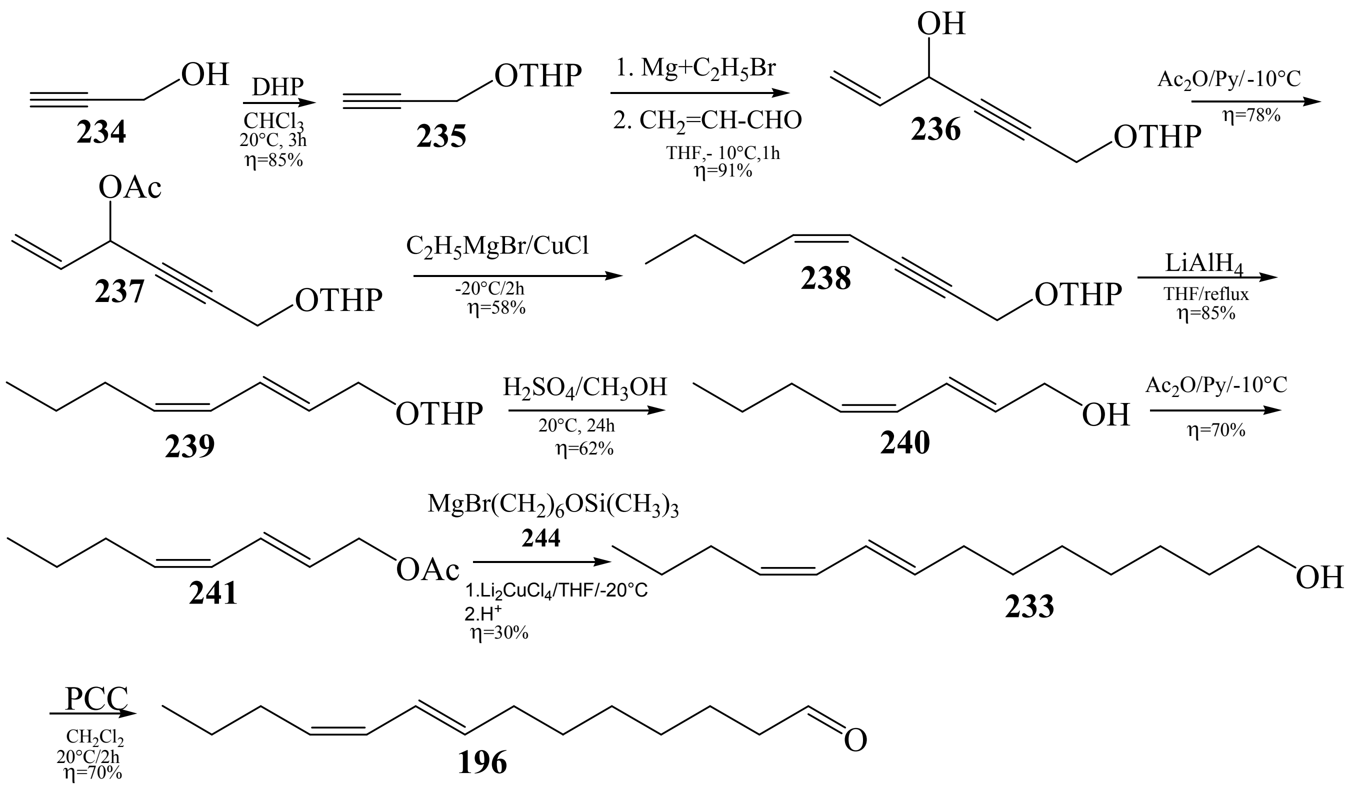
This compound exists in four geometrical isomers (EZ, ZE, EE, ZZ) and among them, the EZ isomer presents biological activity higher than the other isomers.

The literature presents different methods of synthesis of (8E,10Z)-8,10-tetradecadien-1-al [254-260]

The five methods of the preparation of (8E,10Z)-8,10-tetradecadien-1-al (**196**), the sex pheromone of the horse chestnut leafminer *Cameraria ohridella*, invasive pest of ornamental chestnut, reported in the literature are based on cross-coupling reactions catalyzed by palladium complexes or Wittig reactions.

2.3.2 Studies on the synthesis of (8E,10Z)-8,10-tetradecadien-1-al, the sex pheromone of *Cameraria ohridella* species

The synthesis was based on a $C_3+C_3+C_2=C_8$ şi $C_8+C_6=C_{14}$ coupling scheme. Scheme 2.3.6



SCHME 2.3-6

The synthesis of (8E,10Z) -8,10- tetradecadien-1-al, the sex pheromone of *Cameraria ohridella* species Was achieved in a research project funded by the National Research Development and Inovation Programme, BIOTECH Programme, Project 4556, in Laboratory for Natural Product at the "*Raluca Ripan* " Institute for Research in Chemistry,

The method takes after the original way of synthesis (Scheme 2.3-6) is based on Grignard-Schlosser cross-coupling reactions, using the catalytic activity of monovalent copper. [261-264]

2.3.2-1 Synthesis of the (2E, 4Z)-2,4-octadien-1-yl acetate

2,3-dihydro-2H-Pyran was used to protect the –OH function of 2-propyn-1-ol **(234).** [265-269]

Mass spectrum of 1 - (tetrahydropyranyl)-oxy-2-propyne (**235**) is shown in Figure 2.3-7.

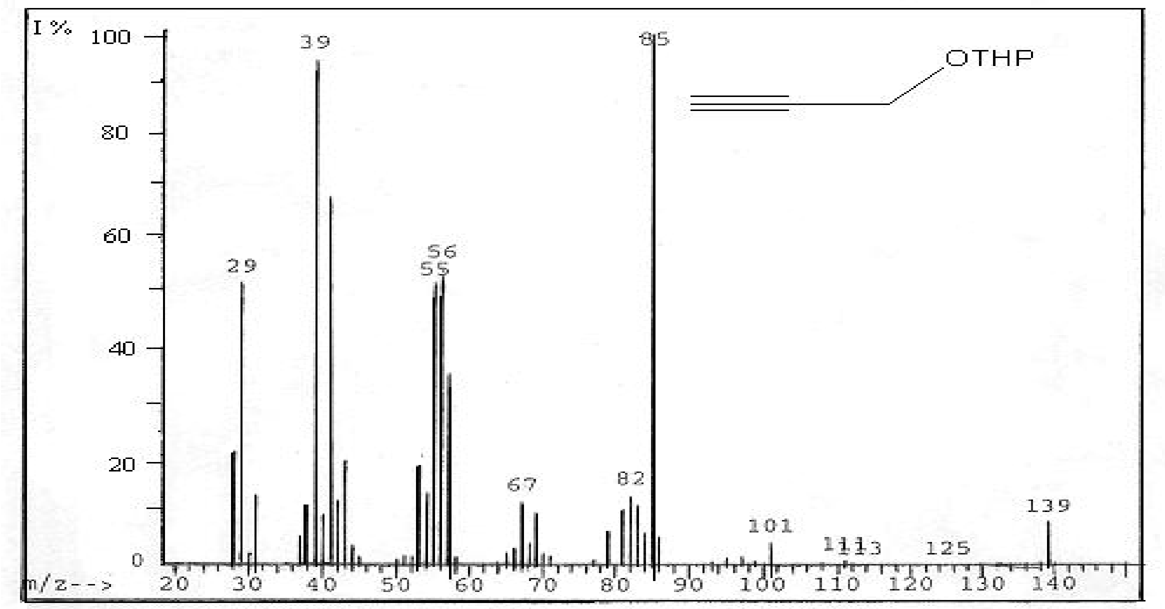
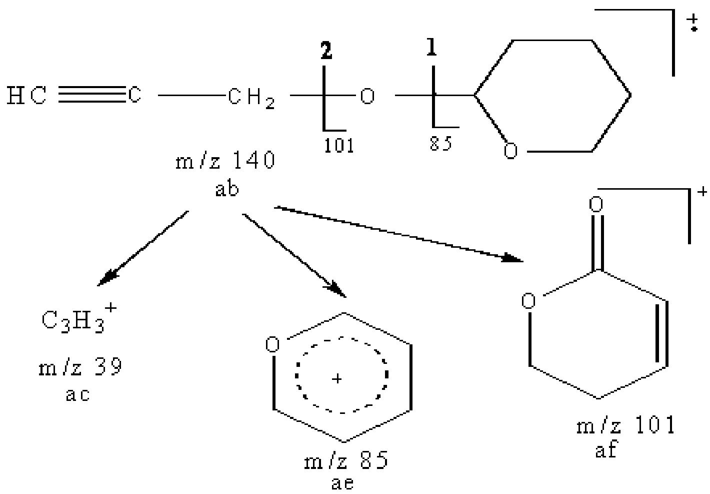


FIGURE 2.3-7 Mass spectrum of 1 - (tetrahydropyranyl)-oxy-2-propyne



SCHEME 2. 3-8

The preparation of 1- (tetrahydropyranyl)-oxy-hex-5-en-2-yn-4-ol (**236**) is presented in the following scheme:

$$C_2H_5Br + Mg \xrightarrow{THF} C_2H_5\text{-}MgBr$$

$$C_2H_5\text{-}MgBr + HC{\equiv}C\text{-}CH_2\text{-}OTHP \ (\mathbf{235}) \xrightarrow{20°C, 1h} BrMgC{\equiv}C\text{-}CH_2\text{-}OTHP$$

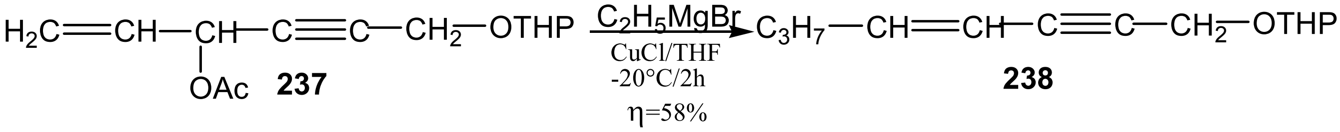
$$BrMgC{\equiv}C\text{-}CH_2\text{-}OTHP + H_2C{=}CH\text{-}CHO \xrightarrow[\eta=91\%]{THF,\ -10°C, 1h} H_2C{=}CH\text{-}CH(OH)\text{-}C{\equiv}C\text{-}CH_2\text{-}OTHP \ (\mathbf{236})$$

SCHEME 2.3-9

1-(tetrahydropyranyl)-oxy-2-Propyne **(235)** throught the Grignard reagent was added to acroleine forming compound **236** [270, 271] which was transformed in corresponding acetate **237.**

Acetylation of 1-(tetrahydropyranyl)-oxy-hex-5-en-2-yn- 4-ol (**236**) was achieved with a mixture of acetic anhydride and pyridine. Yield from 1-(tetrahydropyranyl)-oxy-2-Propyne was 78%.

The second coupling reaction of 1-(tetrahydropyranil)-oxy-4-acetoxy-hex-5-en-2-yne (**237**) with C_2H_5MgBr in THF, in the presence of monovalent copper gave compound **238.** This coupling reaction took place through allylic rearrangement with formation of double bond having Z geometry in ratio of 80%.



SCHEME 2.3-12

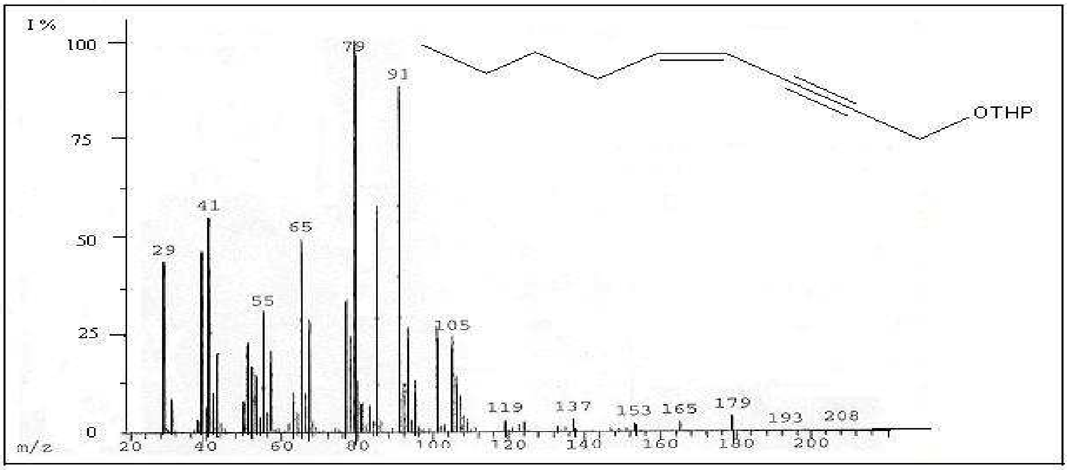
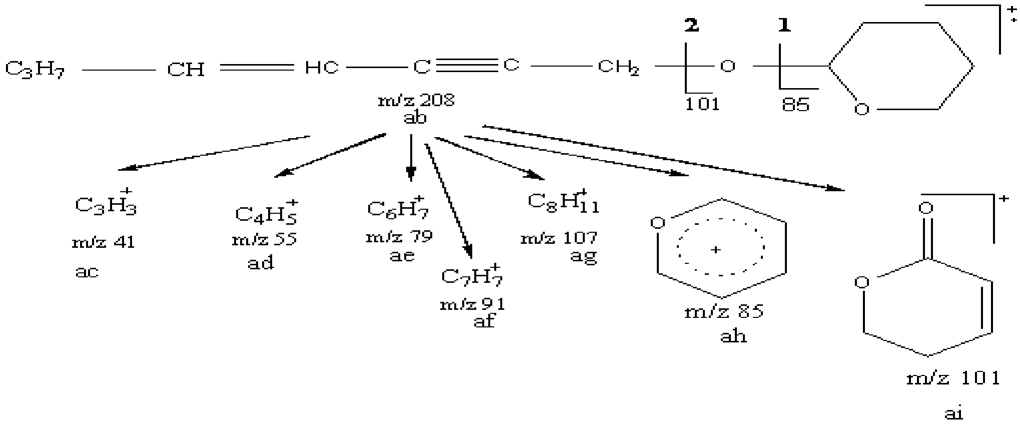


FIGURE 2.3-11 Mass spectrum of 1-(tetrahydropyranyl)-oxy-oct-4-en-2-yne



SCHEME 2.3-13

Mass spectrum of 1-(tetrahydropyranyl)-oxy-oct-4-en-2-yne (**238**) presents of peaks spaced by 14 um features by olefins and acetilenes and ions m/z 41, 55, 79, 91, 107, corresponding to the formula CnH2n-1. Scheme 2.3-13 [272]

The other double linkage of the (*E,Z*) dienic conjugated system was obtained by reduction of the triple bond of compoud **238** with $LiAlH_4$. The molecular peak of the reduction product (**239**) shows 2 um in addition to the compound **238**, which indicates reduced triple double links.(Figure 2.3-12)

1-(tetrahydropyranil)-oxy-Oct-4-en-2-yne **(239)** was deprotected and acetylated obtaining (2E,4Z)-2,4-octadien-1-yl acetate **(241).**

In the mass spectrum of the (2E, 4Z)-2,4-octadien-1-yl acetate (**11**) was identified the molecular peak to m/z=168. Other very important fragment appears at m/z=43 (CH_3CO^+) as base peak, Figure 2.3-14. Scheme 2.3-17

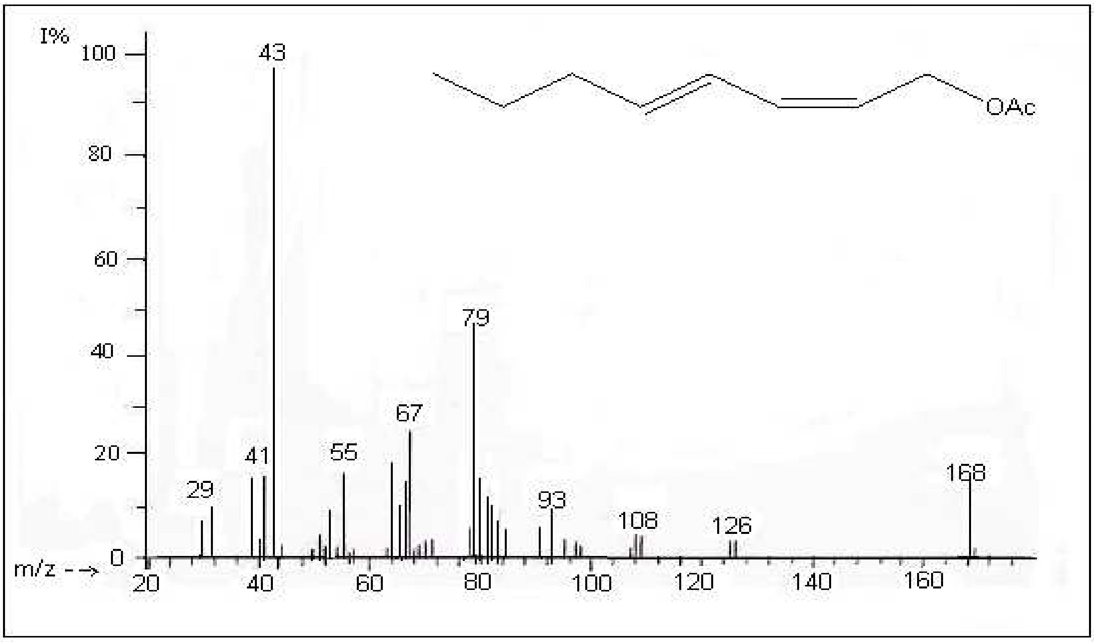
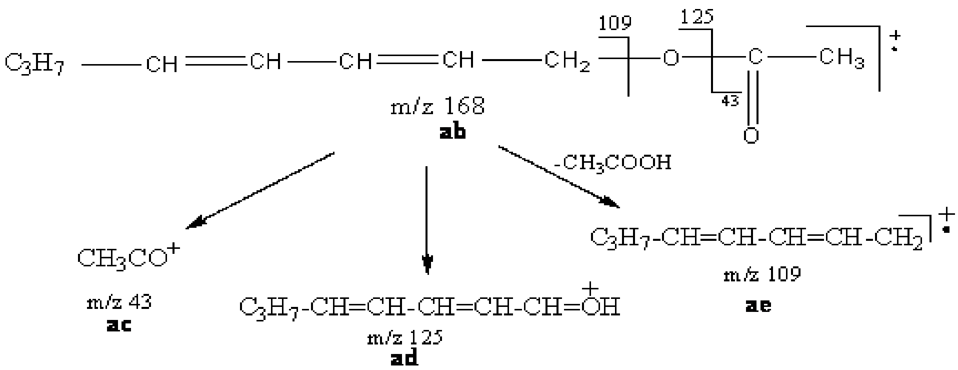


FIGURE 2.3-14 Mass spectrum of (2E, 4Z)-2,4-octadien-1-yl acetate



SCHEME 2.3-17

(2E,4Z)-2,4-Octadien-1-yl acetate (**11**), the key synthon in the synthesis, was checked by means of IR spectrum (film, cm^{-1}): 1600 w (C=C), 3000 m (=C-H), 1000 s (-C-H), 1768 vs (C=O), 1250 s (C-O-C), 1460 m ($O-CO-CH_3$) which contains the vibrations characteristic to conjugated diene system and acetoxy group.

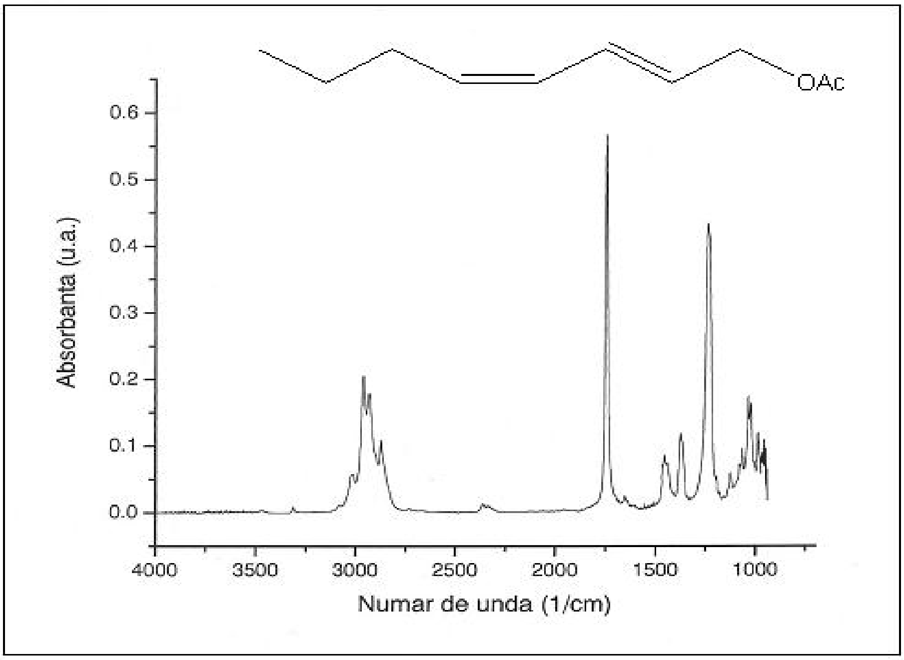


FIGURE 2.3-15 IR Spectrum of (2E, 4Z)-2,4-octadien-1-yl acetate

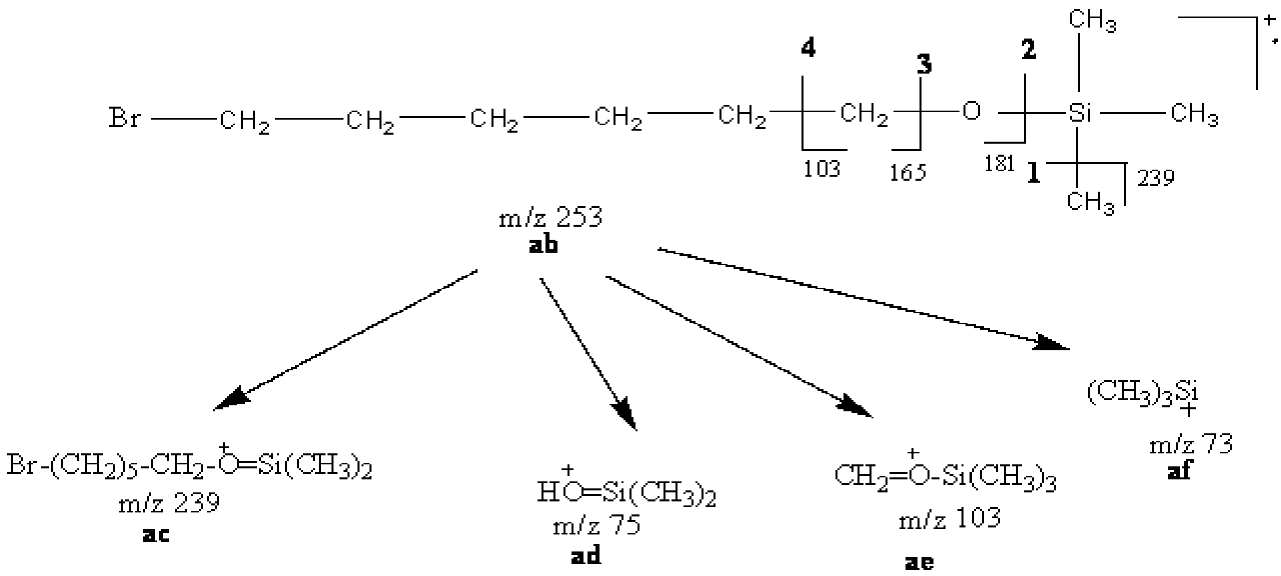
The chemical structure of the (2E, 4Z)-2,4-octadien-1-yl acetate (**241**) was confirmed by ^{1}H-NMR and ^{13}C-NMR spectrum.

2.3.2.2 Synthesis of the (8E, 10Z)-8,10-tetradecadien-1-al

The preparation of 6-bromo-hexane-1-ol (**159**) was achieved by selective bromination with a hydrobromic acid 47% of the 1, 6-hexanediol (**242**).

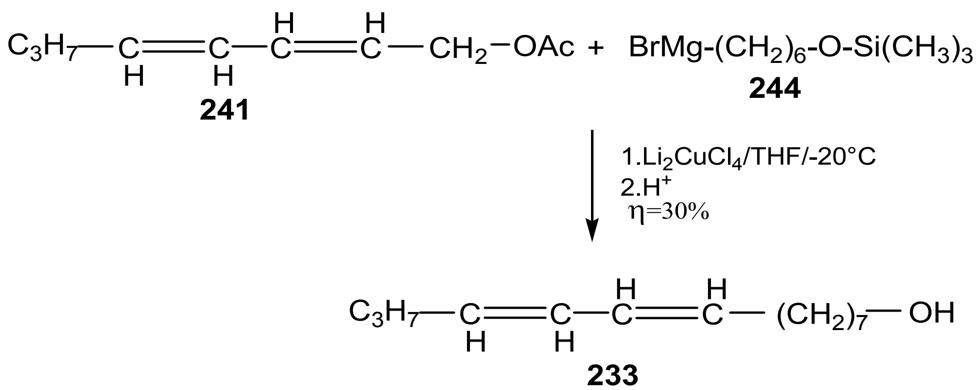
For the preparation of 1-(trimethylsilyl)-oxy-6-bromo-hexane (**243**) was used the trimethylchlorosilane in petroleum ether, and hydrochloric acid as the base for capturing triethylamine. [277-279]

The mass spectrum of 1-(trimethylsilyl)-oxy-6-bromo-hexane (**243**) (Figure 2.3-9) show characteristic fragmentation of trimethylsilyl - ethers [272] and spaced isotope peaks with 2um to the presence of bromine.



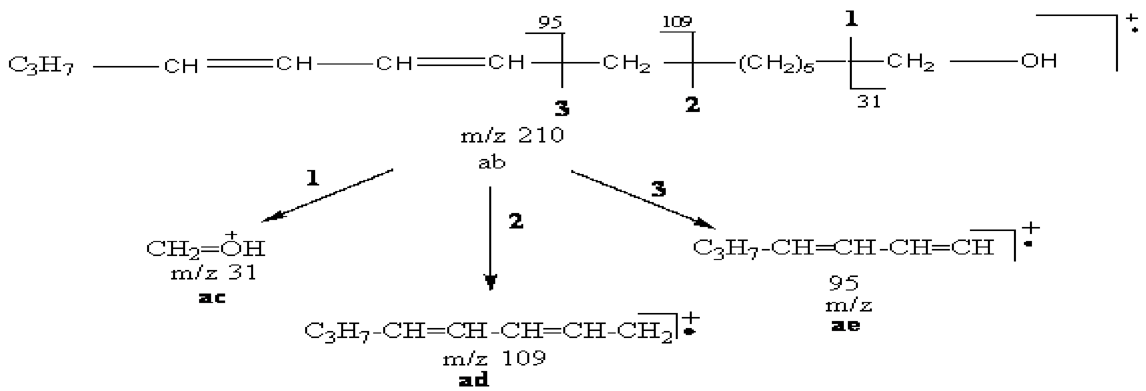
SCHEME 2.3-20

By cross-coupling reaction of (*2E,4Z*)-2,4-octadien-1-yl acetate (**241**) with Grignard reagent of 1-(trimethylsilyl)-oxy-6-bromo-hexane (**244**) in the presence of Li_2CuCl_4 was obtained (8E,10Z)-8,10-tetradecadien-1-ol (**233).** Scheme 2.3-21



SCHEME 2.3-21

Carcteristic fragmentations in mass spectrum of (*8E, 10Z*) -8,10-tetradecadien-1-ol (**233**) are shown in Scheme 2.3-22



SCHEME 2.3-22

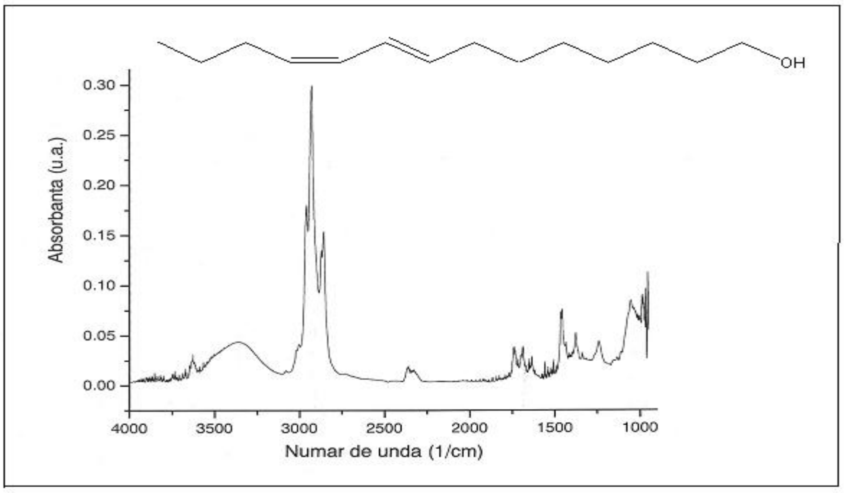
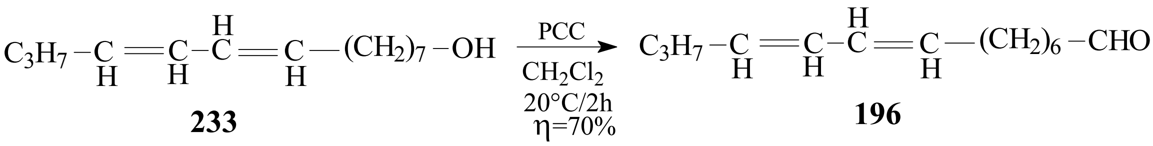


FIGURE 2.3- 21 IR spectrum of (8E,10Z)-8,10-tetradecadien-1-ol

IR spectrum (film, cm-1) of (*8E,10Z*)-8,10-tetradecadien-1-ol (**233**) shows bands characteristic of conjugated diene systems to 1600 w(C = C), 3000 m (= CH), 1000 s (-CH). In the spectrum spectrum is observed an intense broad band at 3200-3400 (OH) characteristic of alcohols. [272-274]

The oxidation of (*8E,10Z*)-8,10-tetradecadien-1-ol (**13)** with PCC gave (*8E,10Z*)-8,10-tetradecadien-1-al (**1**) with 87% isomeric purity and yield of 70%



233 **196**

SCHEMA 2.3-23

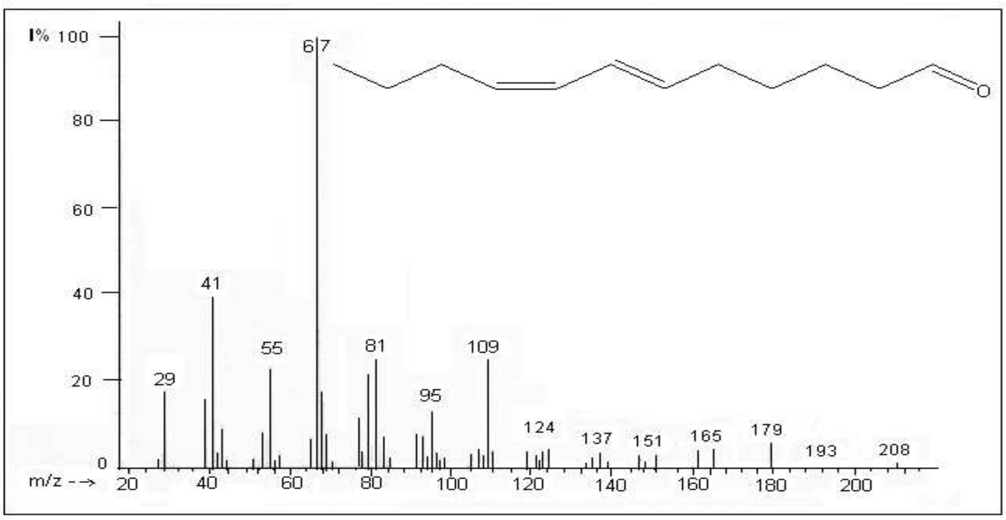
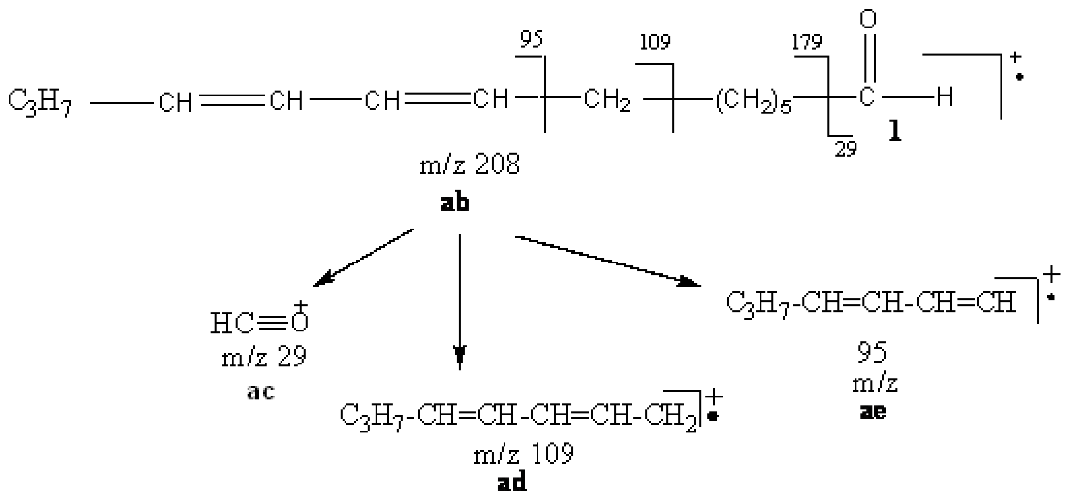


FIGURE 2.3-22 Mass spectrum of (8E,10Z)-8,10-tetradecadien-1-al



SCHEME 2.3-24

Specific aldehydes in mass spectrum of (*8E, 10Z*) -8, 10-tetradecadien-1-al (**196**) was observed the presence of ion m/z 29. Peaks (m/z 179, 165 151 137 124) are the result of simple C-C cleavage. Scheme 2.3-24

The chemical structure of the (*8E,10Z*)-8,10-tetradecadien-1-al (**196**) was confirmed by ^{1}H-NMR and ^{13}C-NMR spectrum

The method use commercially available starting materials.

2.4 Experimental results from the monitoring of the populations of some harmful species using by unconventional biotehniques

2.4.1 Applying the ATRACAM product in monitoring the leafminer *Cameraria ohridella*, the major pest of the ornamental horse chesnut

The use of pheromones in pest control as a non-polluting alternative to using insecticides is part of the durable agriculture.

(*8E,10Z*)-8,10-tetradecadien-1-al, the sexual pheromone of the horse-chestnut leaf miner, *Cameraria ohridella*, prepared at the Natural Products Laboratory of the “*Raluca Ripan*” Institute for Research in Chemistry, was tested by the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (UASVM) and by the Station of Reasearch-Development for Fruit Growing Cluj-Napoca.

In 2005, the testing of the synthesized pheromone was done in the dendrological park, which is part of UASVM, two parameters being monitored.

The **attractiveness** of the pheromone, parameter which makes it usable in the monitoring of the pest, and especially usable in the control of the pest through the method of mass capturing the males.

The **specificity** of the pheromone, meaning the selectiveness of the product to avoid capturing other species of insects, especially those that are part of the useful fauna. The sexual attractant pheromone's selectivity is an extremely important parameter because in many ecosystems the useful fauna (predators and natural parasites) can contribute to maintaining the population of the pest species under the economic pest threshold .

For this reason, on the 27th of April, 2005, before the beginning of the flight of the adults of the hibernating generation, 20 traps with pheromone bait were installed. The traps were checked weekly. The pheromone capsule was changed every other 6 weeks, this task being done on the 8th of June, 20th of July and the 31st of August. The inferior part of the traps was changed whenever necessary, for each trap individually, in order to maintain the trap functional.

The results obtained in 2005, in what concerns the number of captured individuals per week with the help of the traps using the afore mentioned sexual attractant pheromone, are shown in table 2.4-3. [294-297]

TABELUL 2.4-3

The Cameraria ohridella number of butterflies captured with the aid of traps with specific sexual pheromones (Cluj-Napoca, 2005)

Hibernating generation (G-3)			The first generation(G-1)			The second generation (G-2)	
Spaced	Number of butterflies captured		Spaced	Number of butterflies captured		Spaced	Number of butterflies captured
27.04 - 4.05	6057		06.07 –13.07	931		31.08 – 07.09	4370
04.05 – 11.05	7392		13.07 – 20.07	5960		07.09 – 14.09	6210
11.05 – 18.05	9243		20.07 – 27.07	9732		14.09 –21.09	389
18.05 - 25.05	12190		27.07 – 03.08	15930			
25.05 – 01.06	10369		03.08 – 10. 08	14858			
01.06 – 08.06	8113		03.08 – 17.08	12042			
08.06 –15.06	5880		17.08 – 24.08	9417			
15.06 – 22.06	3231		24.08 – 31.08	3820			
22.06 – 29.06	983						
29.06 – 06.07	420						
Total/ generation	63878			72690			10969
Total number of butterflies captured 147537							

During the flight of the adults of the *Cameraria ohridella* species, with the 20 pheromone traps were captured 147537 de males, which means an average number of 7376,85 adults/traps.

Testing the attractiveness and the selectivity of the pheromone baits ATRACAM for the control of the horse-chestnut leaf miner, *Cameraria ohridella*, was done at the Fruit-Growing Research Complex of Cluj-Napoca between 1st of May and the 20th of September, in a horse-chestnut nursery, 10 years old. The longevity of the capacity to attract was monitored, in field conditions, and also the selectivity of the synthetic sexual pheromone was monitored.

The duration of the capacity to attract of the pheromone bait, given the conditions of 2005, was approximately 6 weeks, the traps showing significant captures also after the 6 weeks use. The number of adults captured drops along time with approximately 50-75% in the case of the traps which are older than 4-6 weeks, compared to the fresh bait. The optimal duration for using a pheromone trap is of 4 weeks.

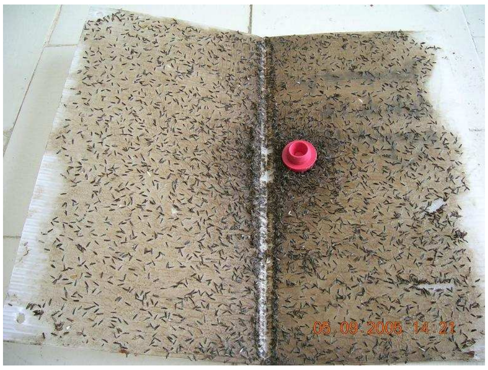


FIGURE 2.4-1 The pheromonal trap *The inferior part of a trap*

Given the conditions of 2005, the *Cameraria orhidella* had about 3 generations, the first adult being captured on the 1st of May and the last one on the 16th of September 2005. The flight chart based on the captures is shown in figure 2.4-2.

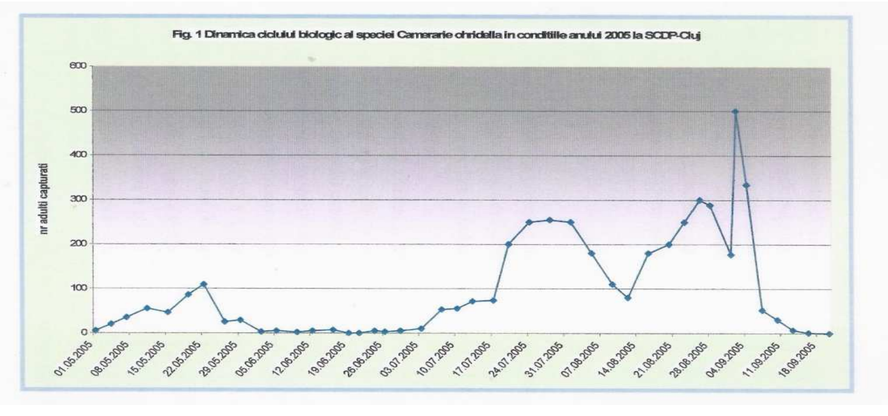


FIGURA 2.4-2 The diagram of the captures for Cameraria ohridella males

The synthetic sexual pheromone of the *Cameraria orhidella* species, formulated in the **ATRACAM product** corresponds to all international standards imposed to synthetic pheromones from the point of view of the power of attraction, longevity and selectivity of the attraction, being successfully used in the monitoring campaigns of the bio-ecology of the species, prognosis and signaling the measurements of limiting the effects to the population.

2.4.2 Applying the MESAJ CP product in monitoring the *Cydia pomonella* species (codling moth) using the „attract and kill” biotechnique

The codling moth, small moth, *Cydia pomonella*, whose larva is the destructive apple worm. Of European origin, it is now found wherever apples are grown. The adult moth is gray with brown markings and has a wingspan of about 3/4 in. (1.8 cm). The 3/4 -in. larva is pinkish, with a brown head. There are several generations a year; the early eggs are deposited on leaves and the later ones directly on the developing fruit. The larvae feed inside the fruit and pupate on the bark of the tree. Apple worms also attack pears, quinces, and English walnuts.

The level of population at this specie, frequently overwelmes the value of the economical thresshold of damaging and so in the fruit growing plantation there are signalled important loss.

To reduce this pest population, it is necessary to establish a strategy for controling, strategies in wich you want the inclusion of alternative methods to reduce pesticide residues in fruit consumption.

The identification **(8E,10E)-8,10-dodecadien-1-ol** as a specific sex pheromone of species *Cydia pomonella* has been an important step towards biological control of this dangerous. [251].

Use of atracticides is part of the biological methods of protection of environment, assuringthe maintenance of the ecological equillibrum in the supervised systems. The implementation of this system allows the protection of the ecosystems through the elimination of the unconventional insecticides.

The **MESAGE CP** product, prepared in prepared in Laboratory for Natural Products, at the "*Raluca Ripan*" Institute for Research in Chemistry and application "**attract and kill**" biotechnique in monitoring of the apple worm *Cydia pomonella* was done in partnership with the discipline of Entomology of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca and plant protection laboratories in within research units (Station Research-Development for Fruit Growing, Cluj-Napoca, Institute Research-Development for Fruit Growing Pitesti-Maracineni, Institute Research-Development for Plant Protection Bucharest) in a research project funded by the National Management Programme, Programme CEEX M1 -BIOTECH, C 25, 2005-2008. [312, 313]

The **MESAJ CP** product consists of a viscous formulation containing specific apple worm pheromone (8E,10E)-8,10-dodecadien-1-ol) and a pyrethroid. The product was applied manually with a specially applicator ranging the drops uniformly per hectare per application (400g) dispensed on the apple tree branches at approximately 1,5 m high.

Experimental Results

The experimental field, organized in the fruit growing plantation of the partner institutions for accomplishing the proposed objective, 3 experimental variants were placed.

- V1**-** version „attract and kill",

  In the estic part of the experimental field, was delimitated an area of 0,4 ha, on which werw applied 140 of the experimental product **MESAJ CP**. The product was used in 3 phases: once in the proximity of the adult flight starting from the hibernating generation and then twice, at the adult flight from the summer-autumn generation, this one beeing more long –drawn.

- V2- version standard „ the chemical treatment with insecticides"

  In this variant, which has an area of 1,1 ha, were applied 4 treatments (2 treatments per generation). The chemical treatment was applied on warning, in according to the homologated methodology. For control was used the product Reldan.

- V3-the untreat control

  This version was placed in the westic part of the experimental field, on a surface of 0,3 ha. In this version weren't applied any method of control angainst the *Cydia pomonella* species

The evaluation of the experimental results was made by two means:

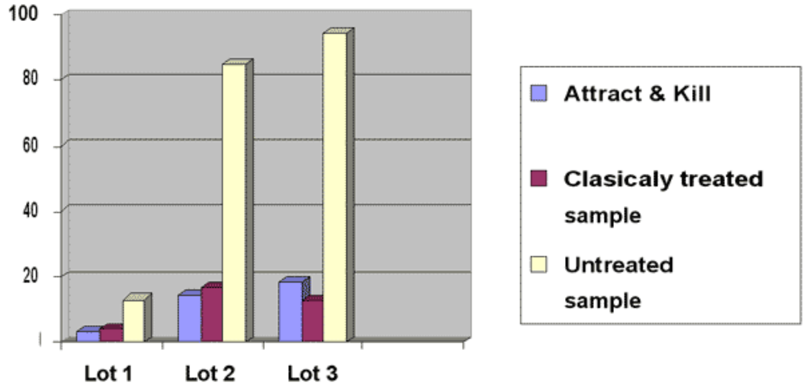
- *Thru monitorising the flight activity of the adults, with the sexually atractant pheromon ATRAPOM,* placed in Tetratrap traps. In every experimental variant were placed 3 traps, on which were made 3 readings per week
- *The attacked fruit frequency analyse in every version, in the fall, at the crop.*

The biological efficacy of the **MESAJ CP** product in „**attract and kill**" biotechnique was assessed by frequency with which it attacks the *Cydia pomnella* pest on fruit and was compared with untreated control, respectively with version standard „the chemical treatment with insecticides"

The biological efficacy of the **MESAJ CP** product in „**attract and kill**" biotechnique evaluated thru the attacked fruits frequency is show in table 2.4-1and figure 2.4-1

Table 2.4.1

	No. variant		Variant	%attacked fruits	Efficiency %
Station Research-Development for Fruit Growing, Cluj-Napoca	1		**Mesaj CP**	21,7	65,3
	2		Standard	4,7	97,65
	3		Untreated lot	83	0
University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca	1		**Mesaj CP**	14	79,71
	2		Standard	3	95,65
	3		Untreated lot	69	0
Institute Research-Development for Plant Protection Bucharest	1		**Mesaj CP**	4,1	**89,05**
	2		Standard	3,5	**84,45**
	3		Untreated lot	33,1	0
Institute Research-Development for Fruit Growing Pitesti-Maracineni	Fallen fruits	1	**Mesaj CP**	30,1	73,6
		2	Standard	18,9	96,3
		3	Untreated lot	78,3	0
	Fruits from the trees	1	**Mesaj CP**	9,7	81,4
		2	Standard	4	92,3
		3	Untreated lot	52	0
	fallen fruits + fruits from the trees	1	**Mesaj CP**	27,2	72,6
		2	Standard	10,3	95,5
		3	Untreated lot	73,6	0



The high attac degree for untreated samples, together with the increase of the population in the 3 lots influenced the efficiency of the treatment and the optimal time of applying the treatment to standard sample

„**Attract & Kill**" method has a good efficiency, suitable for integrated systems against bugs in the apple orchards; the degree of specificity of the pheromon's action only against the target apple worm, allows the evolution of entomofag populations. That's why this methods protects both human and environment.

The pheromone-pesticide combination has an immediate effect for contact action; the pheromone attracts the males, and the pesticide kill them, diminishing reproduction. The lower the level of the population, the higher efficiency obtained.

2.4.3 Applying the PRELUDIU LB product in monitoring *Lobesia botrana* species (grapevine moth), using „attract and kill" biotechnique

The European grapevine moth, *Lobesia botrana* is a major pest of vineyard in Europe, Asia and Africa. active at night, the moth can fly several hundred meters and looks for dry places. In our country prefers warm and dry climate in Dobrogea, Bărăgan, southern Oltenia, Banat and southern Moldova. During the invasion, moths can cause damage of 30-50% in all three generations, starting with flowering vines and grapes to harvest. [314, 315, 46].

Currently, grapevine moth control relies primarly on conventional spray applications. the greapevine moth is successfully controlled with pheromone, but the most common use of pheromones is still for prognosis.

This chapter presents the results of the studies carried out during 2006-2007 in 3 vineyards situated in the representative wine growing centres of Romania (**Research Development Institute for Viticulture Valea Calugareasca, Research Development Station for Viticulture for Murfatlar and Research Development Station for Viticulture for Iasi**) with a product **PRELUDIU LB** used in "attract and kill" biotechnique, as an alternative for environmental monitoring of the *Lobesia botrana* species.

The product obtained at the Natural Products Laboratory of the "*Raluca Ripan*" Institute for Research in Chemistry, Cluj-Napoca, consists of a viscous formulation containing specific grapevine moth (*Lobesia botrana*) pheromone **(*7E,9Z*)-7,9-dodecadien-1-yl acetate** (active substance) and a pyretroid [317-319]

The formulation was applied uniformly on the branches of the vine , by hand (400g/ha), 1-2 time in the season, depending of the infestation level

Produsul este plasat într-o pompă dozatoare şi se aplică manual (400g/ha) prin pulverizare pe butucul de viță de vie, 1-2 aplicații pe sezon, depinzând de nivelul de infestare.

In 2006 was applied 2 treatments (first time after the first moths in pheromones traps and the second one about six weeks later) and in 2007applied only one treatment at the beginning of the second generation because of high temperature that influenced the development of the pest and the attack level.

The field experience was made in 3 sections with on area 0,5 ha for each loction. In the first section, the „attract and kill" treatment with **PRELUDIU LB** was apllied**,** the second variant was the commercial treatment (standard) and the third ,without treatment against target pest, constitutes control.

In the parcels with the pheromone mixture were done unitary for both control units. The product was applied at approximatley the beginning of the flight for each generation, distributed uniformly on the branches from the first level of the strings, in the area of the grapes, o a 3-4 cm at lot Valea Calugareasca and 10 cm at lot Murfatlar. The distance between the treated vines was of 8 m2 at lot Valea Calugareasca and 64 m2 at lot Murfatlar.

The quantity of applied product for hectar was of 200 g of gel\generation. On the 5000 m2 area, approximately 600 vines were treated at lot Valea calugareasca and 120 at lot Murfatlar .

The efficacy estimation of the product **PRELUDIU** LB used in „*attract and kill*" treatment was made by the pheromone traps, monitoring the grapevine moth flight activity in those variants (the catches of LB males were cheched two time /week and sticky inserts were changed in case of need). Figura 2.4-14

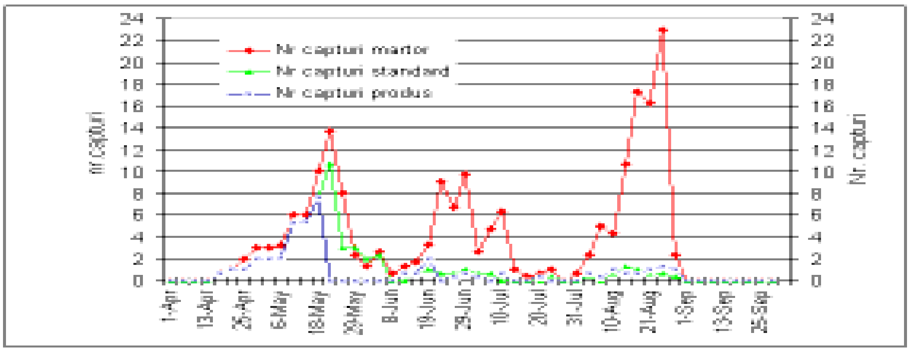


Figura 2.4-14, Number of capture of experimantal lots

The preliminary observations were drawn to establish the intensity of the attack before applying the treatments, by counting the glomerules formed on the young inflorescences. From each group 400 were taken, from approximately 40 vines.

The observation from the verification consist of determining the frequency and intensity of the attack to the inflourescences, by counting the glomerules formed for G1, by counting the pierced grapes for G2 and by counting the alive larva for G3.

The damage was established percentualy by the attack degree and by determining the lones for the crop, using the folowing relation:

GD= (1-b\s)*100, where

s – how much the vines treated with pheromone produced

b – production for the sample and standard parcels.

The efficiency of **PRELUDIU LB** to control the grapevine moth in 2006, Valea Calugareasca and Murfatlar. Table 2.4-14

Table 2.4-14

	Product	Attacked inflorescence (G1)	Attacked grape		Damage average GA (%)	E %
			G2	G3		
Valea Călugareasca	**PRELUDIU LB**	0,7	1,46	2,72	1,62	**81,37**
	Karate zeon	0,4	0,8	1,81	1,08	**87,58**
	Control	5,2	7,3	13,6	8,7	0
Murfatlar	**PRELUDIU LB**	2,5	5,2	3,1	3,6	**73**
	Karate zeon	1,0	1,3	0,5	0,93	**93**
	Control	9,6	15,8	14,8	13,4	0

The efficiency of **PRELUDIU LB** to control the grapevine moth in 2007, Valea Calugareasca and Iasi. Table 2.4-15

Table 2.4-15

	Product	Dose l, kg/ha	Attacked grape	E %
			G3	
Valea Calugarească	**PRELUDIU LB**	0,500	1,67	**61,2**
	Vantex 1,8 % LC	0,80	0,34	**92,1**
	control	-	4,3	
Iasi	**PRELUDIU LB**	0,500	0,36	**74,20**
	Decis WG	0,03	0,17	**87,8**
	control	-	1,4	

I can observe that product **PRELUDIU LB** controlling grapevine moth had the efficacy ranging between 61,2% and 81,37%, less than chemical standard (87,57%-93,0%). In cane observe the lower level of the attack in 2007 when the critical temperature influenced the life cycle of grapevine moth and his potential of attack.

Another reason to recommend this method is the decrease od the yield injuries. Table 2.4-18

TABLE 2.4-18

Variant	Grape yield kg/ vine	GD % of loss comparative with untreated	GD % of loss comparative with standard
PRELUDIU LB	1,1	28	25
Standard	1,35	33	
Martor	0,9		

The table 2.4-18 presents yield losses caused by grapevine moth, the loss of yield was less with 25% compared with standard and with 28% compared with untreated plot.

Even the efficacy of the studied product was less than chemical standard this method can be a good alternative for prapevine moth control in integrated wine growing respectively.

4. CONCLUSIONS

- The (*Z*)-8- and (*E*)-8-dodecen-1-yl acetates are pheromone components of some Lepidoptera species, *Grapholita molesta*, oriental fruit moth, *Grapholita funebrana*, the plum fruit moth, and *Hedya nubiferana*, the green budworm moth, being economically relevant for our country

- Preparing the (Z)-8- and (E)-8-dodecen-1-yl acetates is done according to the coupling scheme C_3+C_6=C_9 and C_9+C_3=$C_{12,}$ the starting material being the propargylic alcohol and the 1,6- hexanediol

- The novelty of the method consists in forming of mercury salts on terminal alkynes ω-functionalized, with 9 carbon atom chain, the transmetallation reaction with metal lithium, followed by the coupling reaction with holo-derivatives

- The (*E*)-5-decen-1-yl acetate (**168**), the sexual pheromone of the peach twig borer, *Anarsia lineatella*, was synthesized using the C_4+C_2=C_6, C_6+C_4=C_{10} coupling scheme.

- The key stage in preparing the (E)-5-decen-1- yl acetate (**168**) is the transmetalltion of the di(1-*tert*-butoxy-hex-5-yne)mercury (**193**) with lithium metal in diglyme. Next, the lithium salt was alkylated with propyl bromide, obtaining the 1-*tert*-butoxy-dec-5-yne with an efficiency of 60%.

- Due to the steric impediment of the tert-butyl group in forming the lithium salt, in the case of ω-functionalized mercury derivatives with 6 carbon atom chain the efficiency of the transmetallation and coupling reaction is lowewr than in the case of ω-functionalised mercury derivatives with 9 carbon atom chains (60% compared to 85%).

- The synthesis of the sexual pheromone of the *Cameraria ohridella* species was achieved, a major pest of the wild chestnut tree, using an original synthesis method, based on cross-coupling reactions using the catalytic activity of monovalent copper.

- (*8E, 10Z*)-8, 10-tetradecadien-1- alul, the sexual pheromone of the *Cameraria orhidella* species was prepared according to the C_3+C_3+C_2=C_8; C_8+C_6=C_{14} coupling scheme, the coupling reaction taking place between the Grignard reactive of the 1-(trimethilsily)-oxy-6-bromo-hexane and the (*2E, 4Z*)-2,4-octadien-1-yl acetate in the presence of $Li_2CuCl_{4.}$

- The synthetic sexual pheromone of the *Cameraria orhidella* species, formulated in the **ATRACAM product** corresponds to all international standards imposed to synthetic pheromones from the point of view of the power of attraction, longevity and selectivity of the attraction, being successfully used in the monitoring campaigns of the bio-ecology of the species, prognosis and signaling the measurements of limiting the effects to the population.

- A more recent approach in the direct control through semiochemical compounds is the "*attract and kill*" bio-technique, which implies a substrate with controlled emission combined with the synthetic pheromone and an insecticide. Compared with the conventional chemotherapy, both atracticides and the pheromone disorientation are advantageous because of the lack of pesticide residues on the fruit.

- The active substance was released in hydrophobic gelling to which a pyrethroid was added. The product was placed in a pump that dosed it and it was distributed to the partners of the project for testing its efficiency. The product branding is **PRELUDIU LB** for the grape vine moth, *Lobesia botrana* and **MESAJ CP** for the apple worm, *Cydia pomonella .*

- The purity of the (8E, 10E)-8, 10-dodecadien-1-ol, the sexual pheromone of the apple worm, *Cydia pomonella*, was 90%, and the isomeric purity of the (*7E, 9Z*)-7, 9-dodecadien-1-yl acetate, the sexual pheromone of the grape vine moth, *Lobesia botrana*, was 87%.

- The efficiency of the **MESAJ CP** product in monitoring the *Cydia pomonella* species had values between 78.8-90.6%. in the untreated control sample, the frequency of attacks on the fruits was 94.4%.

- From the results obtained at Valea Calugareasca, Murfatlar and Iasi, the "*attract and kill*" bio-technique for the *Lobesia botrana* species is efficient in the case when the population is vast, being over the economic threshold for pests.

- The scientific activity performed during the elaboration of the doctoral thesis results in 6 papers, 2 patents, 1 book, 14 scientific sessions, local and abroad, as well as in participating in 10 research and development projects financed by MECTS.

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