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Enantioselective Synthesis of (S) 3-Methyl-4-Octanol as Insect Pheromone Using Polymeric Asymmetric Reagent

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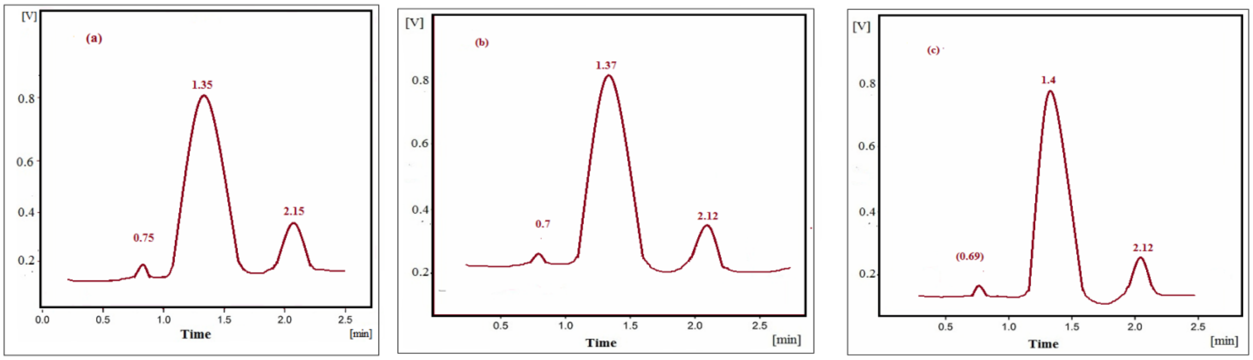
ABSTRACT

In pheromone science, molecular asymmetry is important both chemically and biologically. Techniques for the preparation and determination of the absolute configuration of pheromones are discussed. Enantio selective synthesis of pheromones is the central part of the research on molecular asymmetry in pheromone science. Synthesis of palm weevil pheromone is given as an example in this area. Molecular asymmetry governs the biodiversity of pheromone perception as illustrated by the detailed discussion on the relationships between absolute configuration and pheromone activity.

Graphical Abstract: *In pheromone science, molecular asymmetry is important both chemically and biologically. In this research we prepare and determine the absolute configuration of palm weevil pheromone. Molecular asymmetry governs the biodiversity of pheromone perception by the detailed discussion on the relationships between absolute configuration and pheromone activity.*



The HPLC Chromatogram of the diastereomeric amides using MMA linear polymer which metalated in THF.

Keywords: Pheromone, Absolute configuration, Enantioselective, Palmweevil.

INTRODUCTION

The ever-increasing use of conventional pesticides leads to resistant pests, severely alters natural ecology, damages the environment and, ultimately, affects the economy adversely. The number of insect and other species developing resistance to pesticides is growing steadily, forcing chemical companies to develop novel pesticide formulas. In response to the problems caused by the increased use of conventional pesticides, the concept of integrated pest management (IPM) was developed. IPM combines chemical, biological and agro technical approaches to achieve pest control at a reasonable cost while minimizing damage to the environment. The first step in IPM is effective monitoring by the use of pheromones [1]. Pheromones are substances which occur in Nature and are used for chemical communication between animals. The term pheromone (from the Greek pherein = to transfer and hormone = to excite), coined by Karlson and Luscher [2]. Is a substance which is secreted by an individual and received by a second individual of the same species, in which it releases a specific reaction, for example, a specific behavior or a development process. The main ways of exploiting pheromones in pest control are: monitoring, mating disruption and mass trapping [3]. Such pheromone applications provide significant cost reduction and environmental benefits to the farmer, to the consumer and to the society [4]. Chemically identified releaser pheromones are of three basic types: those which cause sexual attraction, alarm behavior, and recruitment. Sex pheromones release the entire repertoire of sexual behavior. Thus a male insect may be attracted to and attempt to copulate with an inanimate object that has sex pheromone on it. It appears that most insects are rather sensitive and selective for the sex pheromone of their species. Insects show far less sensitivity and chemo specificity for alarm pheromones. Alarm selectivity is based more on volatility than on unique structural features. Recruiting pheromones are used primarily in marking trails to food sources. Terrestrial insects lay continuous odor trails, whereas bees and other airborne insects apply the substances at discrete intervals.

In 1959 Butenandt elucidated the chemical structure of the first insect pheromone, Bombykol is an achiral alcohol isolated as the female produced sex pheromone of the silkworm moth, Bombyxmori. Because it is achiral, bombykol poses no stereo chemical problem except the olefin geometry. Subsequently a number of chiral pheromones such as exo-brevicomin, the pheromone of the western pine beetle, were isolated, which started stereochemical studies in pheromone science. The absolute configuration of a non-racemic and chiral pheromone must be investigated so as to establish the stereostructure of the naturally occurring material and also to clarify the relationships between stereochemistry and pheromone activity [5]. In 1973 Mori demonstrated that the naturally occurring and levorotatory dermestid beetle pheromone was the (R)-enantiomer, since the synthetic (S)-isomer was dextrorotatory [6]. This work was the first success in identifying the absolute configuration of an insect pheromone by its enantiose1ective synthesis [7]. In 1974 three independent groups synthesized both the enantiomers of three different insect pheromones. Silverstein and his coworkers synthesized the enantiomers of the alarm leaf-cutting ant *(Atta texana),* and found the (S)-isomer to be about 400 times more active than the (R)-enantiomer.

MATERIALS AND METHODS

MMA monomer was purchased from fluka, lithium aluminum hydride was purchased from Sigma, butanoic acid was purchased from Fluka, n-butyl lithium and cyclohexyl isopropyl amine were purchased from Fluka, methyliodide was purchased from Sigma, Para-toluene sulphonic acid was purchased from Sigma, triethylamine was purchased from Fluka, ethyl acetate and petroleum ether [High Performance Liquid Chromatography analytical (HPLC) grade, Fisher], benzene (BDH Laboratory), Freshly distilled water was used for the preparation of all our products.

Elemental micro analysis was performed at Cairo university, FT-IR absorption spectra were recorded on a Perkin Elmer 1420 Spectrophotometer by central laboratory at Tanta university, and the spectra were carried out by using KBr disc technique, the samples were dried in oven then mounted on a sample holder with a large cavity , a smooth surface was obtained by pressing the powder samples with a glass paste. The separation of diastereomers were performed by using HPLC using silica gel column of 250×4.6 mm/Si 60-5 Mm, eluents: petroleum ether : ethyl acetate 8:2, flow rate =1 ml/min, detector: UV 254 nm

Synthesis

Synthesis of methylmethacrylate linear polymer: In a 250 mL two neck round bottom flask, (30mL, 28.26g, 282mmol) of methylmethacrylate, 0.5g of benzoyl peroxide, 100 mL of benzene were refluxed in a water bath for 5 h, then the formed polymer was precipitated by petroleum ether, filtrated off, dried under vacuum to give 27 g of dried methylmethacrylate linear polymer (90% yield). IR (KBr): indicate the appearance of the C=O stretching at 1725 cm^{-1} and disappearance of the C=C stretching at 1640 cm^{-1}.Anal. Calcd for $C_5H_8O_2$ (100): C: 60.00, H: 8.00, found: C: 59.30, H: 7.77.

Reduction of methylmethacrylate linear polymer: In a 250 mL two neck round bottom flask suspension of (10g, 100mmol) of methylmethacrylate linear polymer and $LiAlH_4$ (10 g, 263mmol) in 40 mL dry dioxane were refluxed for three days, after destroying the excess of $LiAlH_4$ with (263 mmol of water, 263 mmol of sodium hydroxide, dissolved in 789.47 mmol of water), the beads were filtrated, washed with dilute HCl, water, dried under vacuum to give 7g (97.22%). The IR spectra showed the OH stretching at 3400 cm^{-1} and a complete loss of the C=O stretching at 1725 cm^{-1}.

Synthesis of linear polymeric asymmetric reagent: In a 100mL two neck round bottom flask (5g, 69.4mmol) of reduced methylmethacrylate polymer was swelled overnight in 30 ml benzene then (13 mL, 12.53g, 141.87 mmol) of butanoic acid was added and 1 mL of methane sulphonic acid was added drop wise, the mixture was refluxed for 5 h using dean stark trap. Then the products were cooled and neutralized by 5% sodium carbonate solution. The polymeric asymmetric reagent was filtrated, dried to give 8.5g (72%).IR (KBr): ν:1725(C=O) and a complete disappearances of the OH stretching at 3400 cm^{-1}. Anal. Calcd for $C_8H_{14}O_2$ (142): C: 67.60, H: 9.85, found: C: 67.42, H: 9.77.

Synthesis of linear polymeric α- alkylated ester (using THF as solvent) General procedure: In a 100 mL two neck round bottom flask (5g,35.2 mmol) of polymeric asymmetric reagent was swelled in 20 mL THF overnight, then (10.2mL, 59.84 mmol) of CHIPA was added drop wise at -96^oC (using methanol and liquid nitrogen) as cooling bath and at -80^oC (using acetone and liquid nitrogen) and at -63^oC (using chloroform and liquid nitrogen) then (4.928 ml, 59.84mmol) of n-BuLi was added drop wise then the reaction mixture was stirred for 60 min, then 1 mL of TMEDA was added as catalyst and finally (10 mL, 70.4 mmol) of methyl iodide was added drop wise at -40^oC ,then the reaction mixture was stirred overnight, the product was filtrated off, dried under vacuum and weighted to give different yields as we see in table 1.IR (KBr): ν: 2996 (CH_2 chain), and 1715 (C=O)cm^{-1}.Anal. Calcd for $C_9H_{16}O_2$ (156): C: 69.23, H: 10.25 found: C: 69.11, H: 10.13.

Table 1: The yields of polymeric α alkylated esters which prepared at different temperatures using THF as solvent.

RX	temperature	(Practical yield(g)	Yield (%)
MeI	-96^oC	4.85	88.34
MeI	-80^oC	5.1	92.89
MeI	-63^oC	4.9	89.25

Hydrolysis of linear polymeric α- alkylated ester: In a 100 mL round bottom flask (5 g, 32 mmol) of linear polymeric asymmetric reagent was swelled in 20 ml dioxaneovernight ,then (0.5 gm, 2.97 mmol) of P-toluene sulphonic acid was added, the reaction mixture was refluxed for 12 hrs, then pour the reaction mixture in ice water, the reduced polymer was filtrated off and the α-alkylated acid (2- methyl butanoic acid) was extracted by 20 mL methylene chloride , dried by anhydrous sodium sulphate, to give pale yellow liquid. The IR spectra showed absorption at 1725 cm^{-1} which is characterized for C=O group, and at 3415 cm^{-1} which is characterized for OH group.

Synthesis of diastereomeric amides *of 2- methyl butanoic acid:* In round bottom flask (1.11 mL, 10 mmol) of α-alkylated acid (2-methyl butanoic acid) was added to (3.6 mL, 50 mmol) of thionyl chloride, then the reaction mixture was refluxed for two hours, then evaporate the excess of thionyl chloride, wash with dry benzene then evaporate it again, dry and dissolve the acid chloride in 10mL THF. In another 100 mL round bottom flask dissolve (1.5 g, 11 mmol) of amine (phenyl glycinol) in (1.53 mL, 11 mmol) triethylamine and 10 mL THF, cool (ice bath), then the acid chloride was added drop wise, then the liquid mixture was refluxed for 5-6 h (TLC control) using petroleum ether: ethyl acetate 8:2 [$R_{f.p}$ =1.3, $R_{f.s}$ =2.1, UV 254nm] , then the diastereomeric amides of 2-methyl butanoic acid were extracted with 10 ml methylene chloride, washed with 1 N HCl then 1N NaOH, dried over sodium sulphate an hydrous, filtrated off to give yellow liquid.IR (KBr): ν:1725 cm^{-1}(C=O), 3415 (OH), 3369 (NH), 2996 (CH_2 chain) Cm^{-1}.Anal. Calcd for $C_{13}H_{19}O_2N$ (221): C: 70.58, H: 8.59, N: 6.33. found: C: 70.36, H: 8.34, N: 6.19.

Separation of the two diastereomers using HPLC with silica gel column: The diastereomeric amides were dissolved in methylene chloride and separated by HPLC using silica gel column of 250×4.6 mm/Si 60-5 Mm, eluents: petroleum ether : ethyl acetate 8:2, flow rate =1 mL min^{-1}, detector: UV254 nm, t_{R1}=1.35 min, t_{R2}=2.11 min.

Synthesis of (S) 3-Methyl-4-octanone: In a 100 ml two neck round bottom flask (4.928 ml, 59.84mmol) of n-BuLi was added to (5g,35.2 mmol) of acid chloride (which formed from 2-methyl butanoic acid) at -80°C followed by stirring overnight, then the product was extracted with methylene chloride.

Synthesis of (S) 3-Methyl-4-octanol

1. Fermentation: 39 m mol (13.3g) of sucrose was added to 0.6 mmol (0.041g) of sodium hydrogen phosphate then dissolved in 25 mL warm water followed by addition of 2.6g of yeast, the reaction mixture was heated at 40°C for 1h and finally the product was cooled.
2. Stereo selective reduction: 7.3mmol (1g) of (S) 3-Methyl-4-octanone was added to the product of step (1) and stirred vigorously at room temperature for three days, and then the product was filtered and extracted with methylene chloride.

RESULTS AND DISCUSSION

Many insect semiochemicals are chiral compounds and the biological activities of both enantiomers often differ. Usually, only one of the enantiomers is bioactive; interactions between receptor proteins in insect antenna with one of the enantiomers of a pheromone constituent were shown in palm weevil pheromone. The latest studies show that these receptors respond strongly to one enantiomer ((S) 3-Methyl-4-octanol) but also more weakly to the other enantiomer ((R) 3-Methyl-4-octanol). Sometimes, both enantiomers are biologically active and they may even show a different type of activity. Thus, the ‘‘unnatural enantiomer’’ (i.e., not produced in the insect) can be equally active, less active (but enhancing the activity of the ‘‘natural enantiomer’’), or it inhibits the activity of the natural pheromone. Some insect species use pure enantiomers as pheromones, while others produce a racemic mixture or a mixture of enantiomers in a speciesspecific proportion. In this way, the organisms possess an enormous diversity in communication systems [8].

The reduction of PMMA beads were carried out by treatment with $LiAlH_4$ in dioxane as solvent. The conversion of the ester group was followed by the IR spectra, which showed the OH stretching at 3400 cm^{-1} and a complete loss of the C=O stretching at 1725 cm^{-1}. The conversion was also obtained quantitatively by further chemical modification through esterification by treatment of polymeric alcohol by butanoicacidin presence of methane sulphonic acid and benzene as solvent using dean stark trap. The IR spectra of the polymeric asymmetric reagent indicate the reappearance of the C=O stretch at 1725 cm^{-1} and the complete disappearances of the OH stretch at 3400 cm^{-1} as summarized in figure 1.

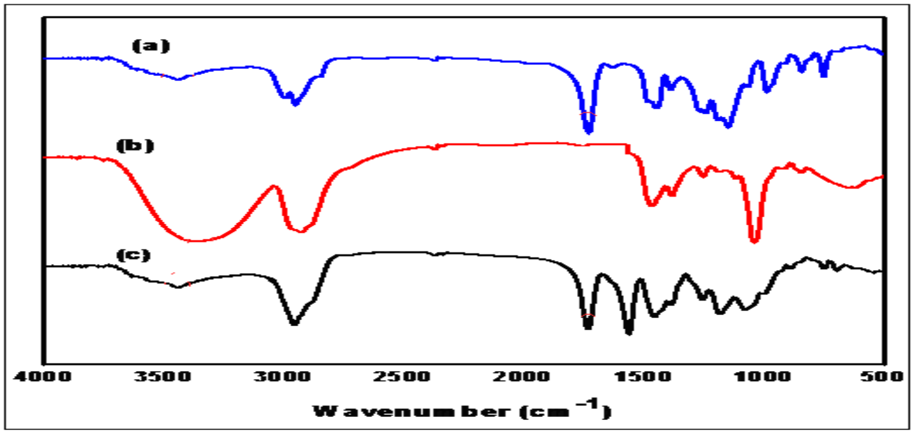
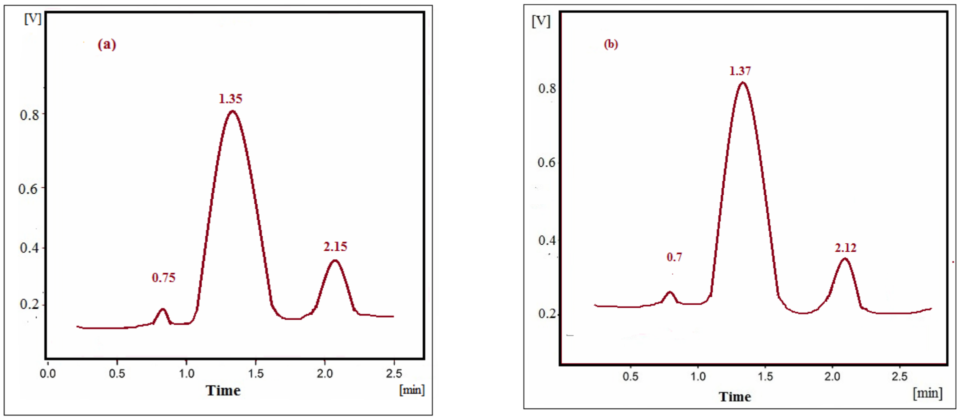


Figure 1 FT-IR spectra for PMMA, reduced PMMA and polymeric asymmetric reagent.

The deprotonation step takes place by the addition of Li - CHIPA to polymeric asymmetric reagents at different temperatures (-96°C, -80°C and -63°C) followed by alkylation using methyl iodide in the presence of TMEDA as catalyst lead to formation of polymeric enantiomerically excess α-alkylated ester. The IR spectra of the product showed the absorption at 2996 cm^{-1} is characteristic for (CH_2 chain) and at 1715cm^{-1} which is characteristic for C=O group. The hydrolysis of this product by Para-toluene sulphonicacid leads to formation of enantiomerically excess α-alkylated acid (2-methyl butanoic acid) as we see in scheme (1). The IR spectra of this acid indicate the appearance of the C=O stretching at 1725 cm^{-1} and the appearances of the OH stretching at 3400 cm^{-1}. The treatment of this acid by thionyl chloride leads to the formation of acid chloride which added to phenyl glycinol to form diastereomeric amides. These diastereomeric amides were separated using HPLC with silica gel column as we see in scheme (2) to give different results. The different results were shown in figure 2 and the data reported were summarized in table 2.

Table 2. Enantiomeric excess and configurations of 2-Methyl butanoic acid at different temperatures

R_1	R_2X	Enantiomeric Excess (%)	Temperature	Configuration
C_2H_5 C_2H_5 C_2H_5	CH_3I CH_3I CH_3I	82 78 70	-96°C -80°C -63°C	S S S



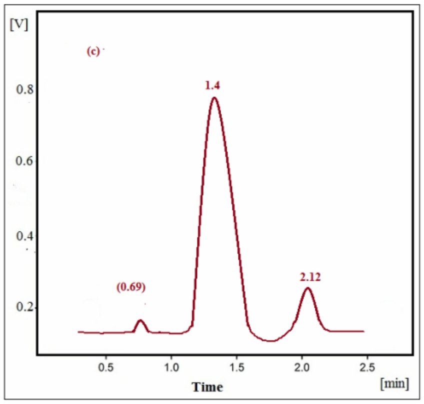
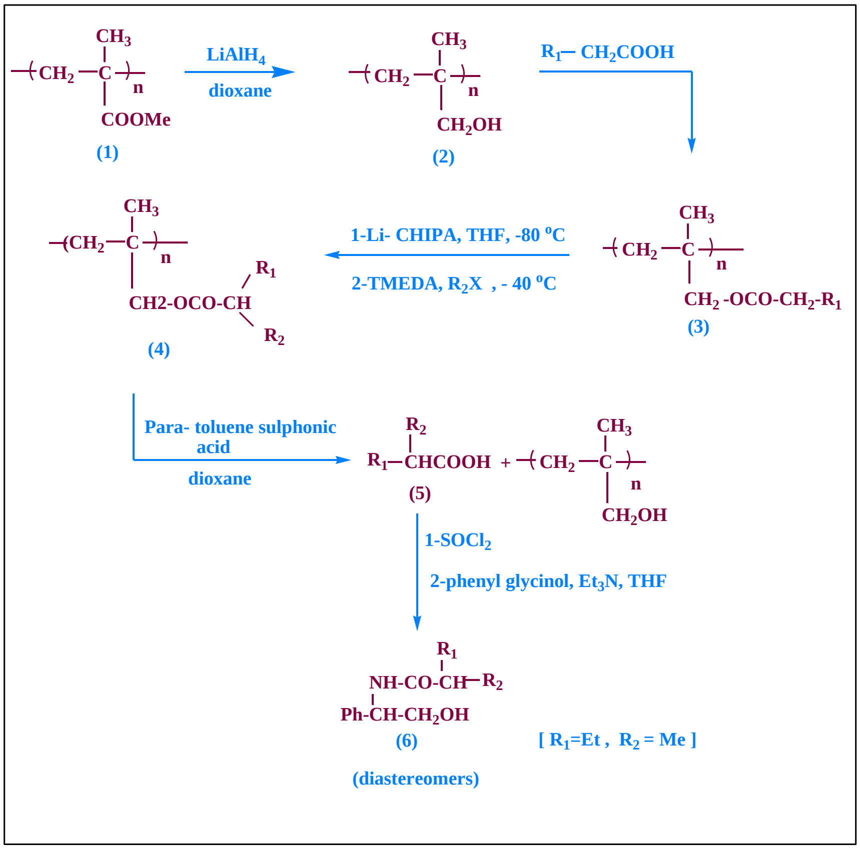
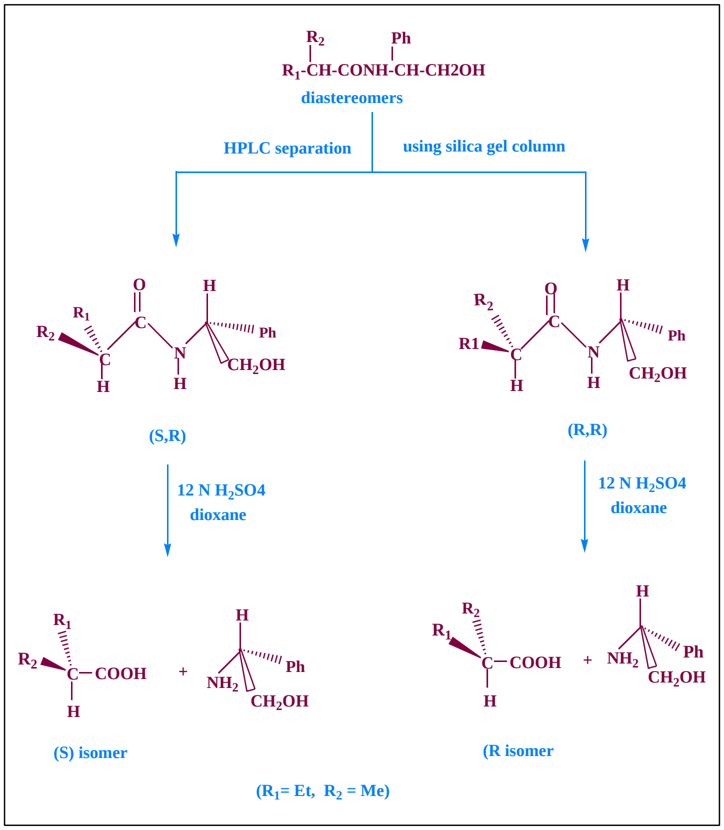


Figure 2 The HPLC Chromatogram of the diastereomeric amides using MMA linear polymer which metalated in THF



Scheme 1 General scheme for the synthesis of enantiomeric excess α- alkylated acid

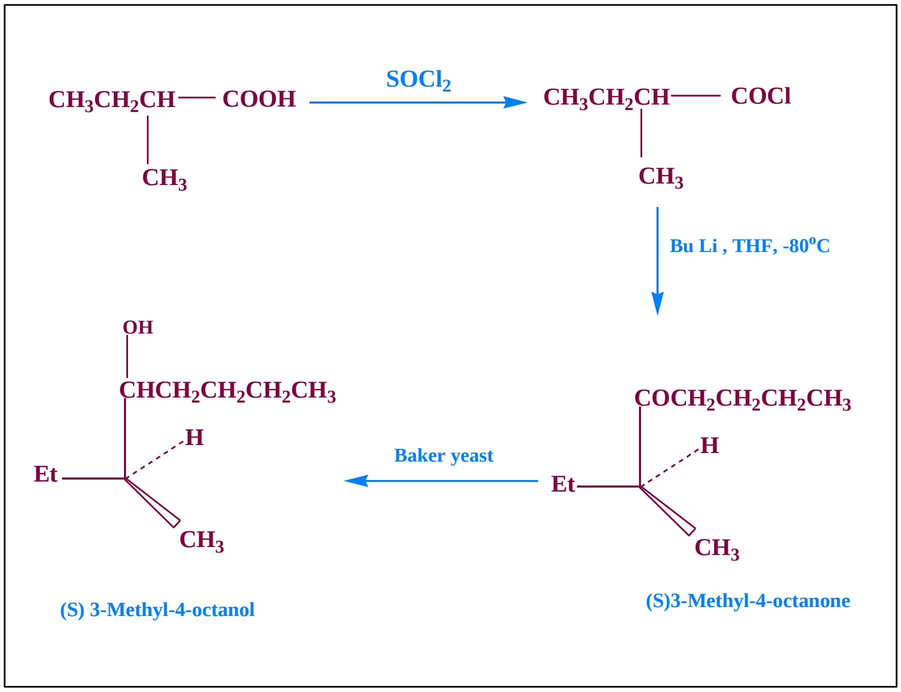


Scheme 2 General scheme for separation of the two diastereomeric amides

Also, the treatment of acid chloride with n-butyl lithium at -80°C in THF as solvent leads to formation of (S) 4-Methyl-3-heptanone as we see in scheme 3.The IR spectra of this product indicate the appearance of the C=O stretching at 1725 cm^{-1} and disappearances of the OH stretching. Further chemical modification of (S) 3-Methyl-4-octanone through stereoselective reduction with Baker's yeast leads to formation of (S) 3-Methyl-4-octanol (palm weevil pheromone). The IR spectra of this product indicate the reappearance of OH stretching at 3400 cm^{-1} and disappearances of C=O stretching.

APPLICATIONS

Antimicrobial activity: The microorganisms used in this study included bacteria *Bacillus subtilis*, *Staphylococcus Aureus*, (Gram positive bacterium), *Pseudomonas Aeruginosa*, *Escherichia coli* (Gram negative bacteria) and *Candida albicans*(fungi). The strains were obtained from the culture collection of Bacteriology Laboratory (Bacteriology Unit, Department of Botany, Faculty of Science, Tanta University, and Tanta, Egypt). An aliquot of 0.1 mL of each bacterial strain was inoculated and spread on nutrient agar while 0.1 mL of the yeast was spread on sabaroud agar slopes. Antimicrobial activities of the synthesized compounds were tested *in vitro* using the cut- plug method according to Pridham et al. [9] The assay plates were inoculated with 100 mL containing the diluted inoculums (107 CFU mL^{-1}) of each tested organism that were spread on the corresponding media. After solidification, the wells were made of dyes (10 mg) were dissolved in DMSO (1 mL) and inserted in the wells. Nutrient agar plated was incubated at 37 °C for 24 h while plates were incubated at 25 °C for 48 h. The inhibition zones around the wells were measured and the average based on reply was recorded. For reference drug 100 mg mL-1 of Ampicillin was used as antibacterial and antifungal drugs.



Scheme 3 Synthesis of (S) 3-Methyl-4-octanone and (S) 3-Methyl-4-octanol

Table 3 Diameters of inhibition zones (mm) of some synthesized compounds against different test bacteria and fungi on nutrient agar at 30^0C after 24 h.

Sample	**Inhibition zone diameter (mm / Sample)**					
	Bacterial species				**Fungi**	
	(G^+)		**(G^-)**			
	Bacillus subtilis	***Staphylococcus Aureus***	***Escherichia Coli***	***Pseudomonas Aeruginosa***	***Aspergillusflavus***	***Candida albicans***
(S) 2-methyl butanoic acid	14	17	13	13	0.0	12
(S) 3-Methyl-4-octanone	9	9	9	9	0.0	0.0
(S) 3-Methyl-4-octanol	10	9	9	9	0.0	9

CONCLUSIONS

In this research we prepare (S) 3-Methyl-4-octanol (the palm weevil pheromone) with high enantiomeric purity using polymeric asymmetric reagent depending on different factors, the first one is the effect of temperature to increase our enantiomeric purityandthe second one is the effect of order of addition to control our configuration, finally we study the biological activity of some of these products.

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