Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. II, 121-127, 1991

METHODOLOGY IN STRUCTURAL DETERMINATION AND SYNTHESIS OF INSECT PHEROMONE

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By means of ethereal washing of insect pheromone glands of female moths, GC-MS detection along with microchemical reactions and electroantennogram (EAG) survey, six economically important insect species were targeted for pheromone identification. The discovery of a natural pheromone inhibitor, chemo-selectivity and species isolation by pheromone will be described.

The modified triple bond migration and triethylamine liganded vinyl cuprate were applied for achiral pheromone synthesis in double bond formation. Some optically active pheromones and their stereoisomers were synthesized through chiral pool or asymmetric synthesis. Some examples of chiral recognition of insects towards their chiral pheromones will be discussed. A CaH_2 and silica gel catalyzed Sharpless Epoxidation Reaction was found in shortening the reaction time.

Key words: insect pheromone – isolation – structure determination – chiral synthesis – chiral recognition

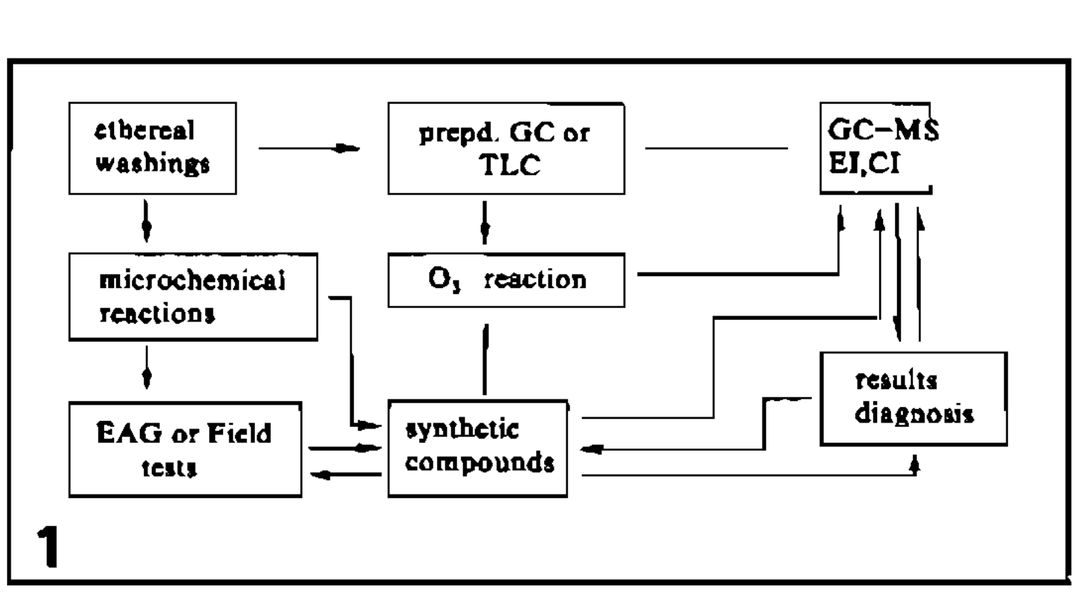
Communication between insects are mediated out by so called pheromones which are among the highest bioactive natural products. Due to the less amount of pheromone available in nature, some technique must be adopted in the scale of 10 to 100 nanogram level for structure determination. On the other hand, insects show critical chemo- and chiral-recognition toward pheromone, therefore it needs stereoselective and enantioselective synthesis either of double-bond formation or of optically active compounds.

1. STRUCTURE DETERMINATION

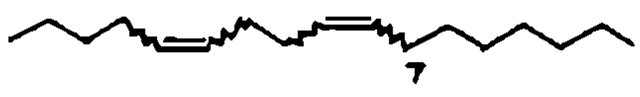
In order to avoid using large quantity of insects for isolation and identification of pheromone, ethereal washings of the sex pheromone gland of female moths and GC-MS detection were applied along with the performance of the microchemical reaction and electroantennogram (EAG) survey (Lin & Zhou, 1988) (Fig. 1).

a. Pink bollworm moth, *Pectinophora gossypiella* – Determination of the isomeric ratio of the sex pheromone of pink bollworm moth, was performed on GC-MS and EAG analysis. It revealed that the calling female moths elicited a mixture of *1*a and *1*b in ratio of 58-55/42-45, other two isomers *1*c, *1*d were undetectable; while female moths after mating produced free OH compounds *2*a and *2*b in the same ratio as that of acetates. *2*a and *2*b were proved to be the pheromone inhibitor (Zhu et al., 1983). With increasing equivalent % of *2*a + b was added to pheromone *1*a + b, catches of male moths, in two nights-field test, were decreased; when 1/3 of 2a + b was added to 1a + b, 98.4% inhibition was recorded.

Incubation of the homogenized pheromone glands and the synthetic pheromone produced *2*a and *2*b indicating that there was a kind of enzyme in the gland, which converted pheromone to its inhibitor OH compound. Thus *2*a, b could be considered as a metabolite of the pheromone (Lin & Li, 1990).



b. *Euproctis similis xanthocampa* – Four components, i.e. Z-7-octadecenyl isovalerate (92%) *3*a, 6-octadecenyl isovalerate (1.4%)



OR

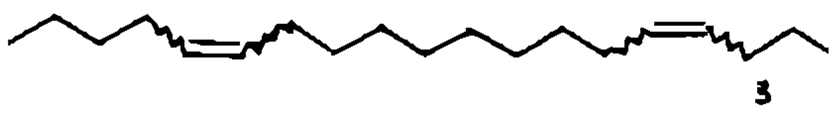
*1*a. *Z,Z* R = Ac
b. *Z, E* R = Ac
c. *E, Z* R = Ac
d. *E, E* R = Ac
2a. Z, Z R = H
b. *Z, E* R = H

$$CH_3(CH_2)_nC(H)=CH(CH_2)_mOAc$$

*4*a. *E*9, m = 8, n = 1
b. *Z*9, m = 8, n = 1
c. *E*7, m = 6, n = 3
d. *Z*7, m = 6, n = 3

$$CH_3(CH_2)_nC(H)=C(H)(CH_2)_mOCCH_2CH(CH_3)_2$$

*3*a, 7z, m = 6, n = 9
b. 7*E*, m = 6, n = 9
c. 6*Z*, m = 5, n = 10
d. 6*E*, m = 5, n = 10
e. (*Z* or *E*) m = 8, n = 7



OR

*5*a. *E, Z* R = H
b. *E, E* R = H
c. *Z, E* R = H
d. *Z, Z*
e. *Z, Z* R = Ac
f. *E, Z* R = Ac

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*3*c or *3*d, 6-octadecenyl n-valerate (1.6%) and 9-octadecenyl isovalerate (5%) *3*e have been identified from the sex pheromone gland washings of this insect, a pest of mulberry tree. The synthetic *3*a was showed to be as active as the virgin female moth both in the field bioassay or on the EAG response. All other isomers *3*b-e and those of the normal valerate were inactive. The isovalerate type of the sex pheromone is discovered in Lymantridae for the first time (Tan et al., 1984) (Fig. 2).

c. *Ancylis sativa L.* – Four components 4a-d were identified from the sex pheromone gland of the female moth *Ancylis sativa L.*, a Chinese date pest. The pheromone amounted to 2.4 μg per female moth. Based upon the field bioassay, combination of *4*a and *4*b demonstrated a strong attraction in an optimum ratio of 4a/4b in 8/2. Both other two isomers *4*c and *4*d were inactive and unlikely produced any inhibitory or synergist effect (Lin et al., 1984).

d. *Paranthrane rabaniformis* R. and Sesiidae family – Pheromone of five related clearwing moth (sesiidae) was examined. *5*a was identified as the sex pheromone of Poplar twig clearwing moth *P. rabiniformis R* (Zhang et al., 1986). Traps baited with 200 μg of *5*a captures more male moths than 2 alive females. Whereas a similar moth, poplar large hornet moth *Sphecia Siningensis Hsu* was structurally proved to use *5*d as sex signal (Guo et al., 1990). In the screening tests, male *Paradoxes prelli L.* mulberry tree borer behaved strong response to *5*f both in field and on EAG biassays (Tan, 1991); the vine tree borer *P. regalis* B was captured by *5*a (Guo, 1990). Synanthedox *castanerora* Y. responded to a mixture of *5*a + *5*d.

2. DOUBLE BOND FORMATION

a. triple bond migration – Most pheromones are unsaturated aliphatic alcohols, their acetates or aldehydes. The double bond was introduced by semi-hydrogenation for cis configuration and LAH reduction for trans form from an appropriate alkyne in which the key step was the generation of alkyne by migration of the triple bond in the middle to terminal position first, than elongation.

A typical example was illustrated by synthesis of sex pheromone of pink bollworm moth, a mixture of *1*a and *1*b (Fig. 3) (Zhong et al., 1982).

Since the pheromone is a mixture of *1*a and *1*b (1:1), a mixture of *8*a and *8*b was subjected to the following sequence of reaction *8*a + b → *9*a + b → *11*a + b → *1*a + b. E-isomer *8*b was easily obtained from its Z-isomer *8*a by olefin isomerization with HNO_3-$NaNO_2$. Thus the equimolecular mixture of *8*a + b could be produced from *8*a with HNO_3-$NaNO_2$ at 50 °C for 30 min (Zhou et al., 1983), *10* was obtained by means of isomerization with

3

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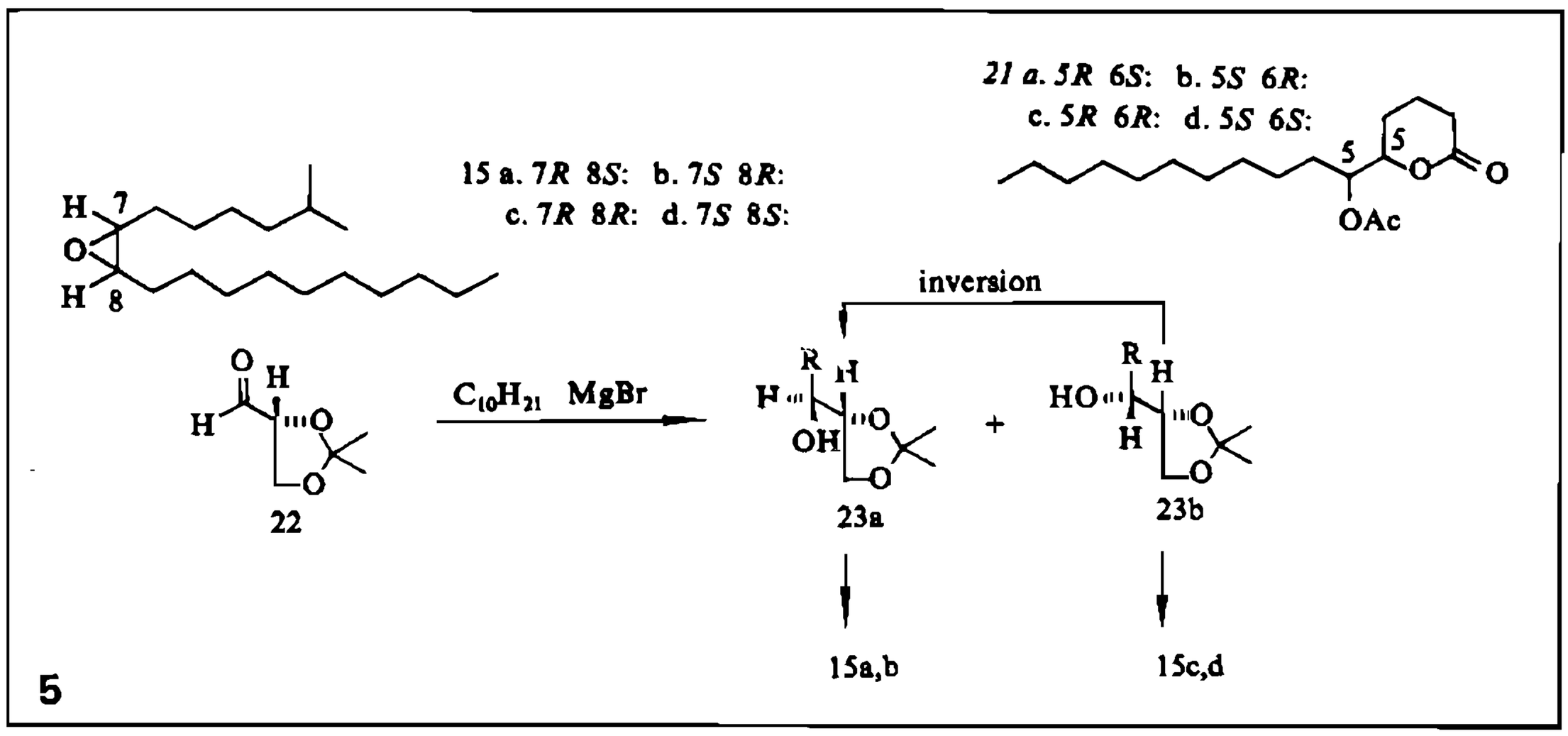
NaNH$(CH_2)_3NH_2$ in high yield. Therefore, both the left half of *1* and the right half of *1* could be prepared from the common starting material *6*. The cheaper reagents 1 2-propylenediamine and 1 2-ethylenediamine were also used to replace 1 3-propylene-diamine (Zhang & Zhou, 1983).

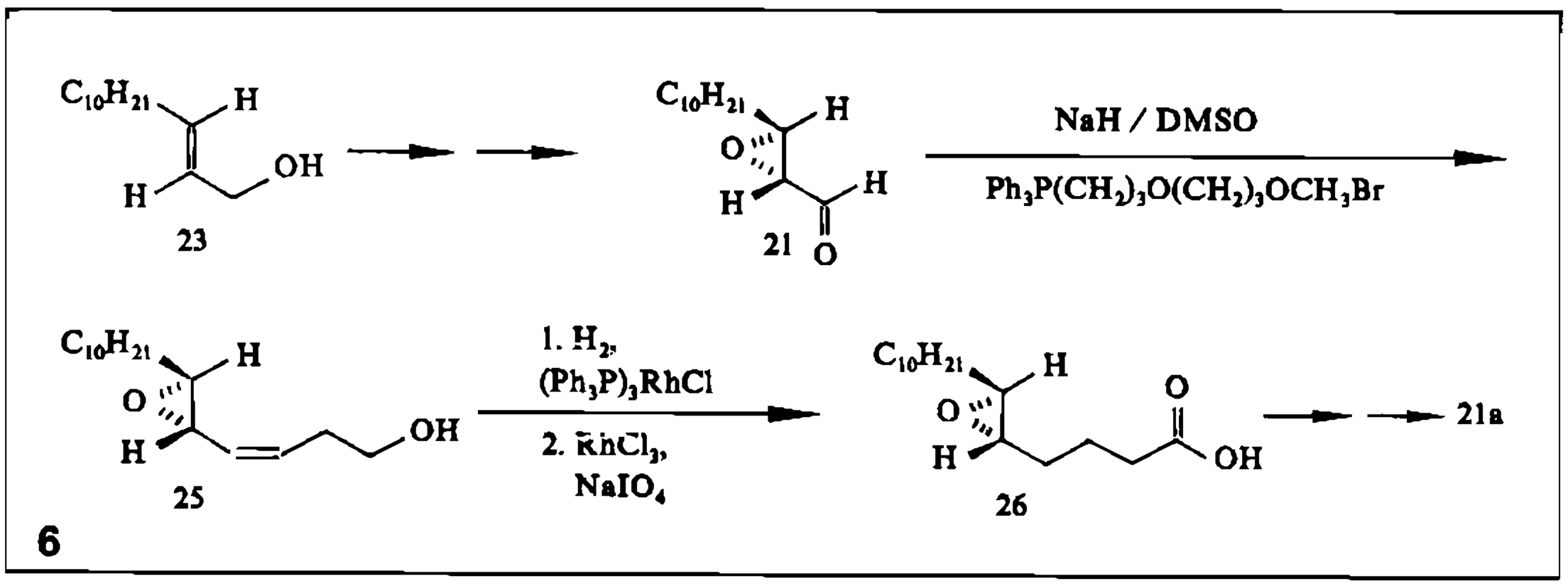
b. Synthesis of olefin with high cis purity by application of triethylamine liganded vinyl cuprate – Triethyl amine was used to replace the toxic and stinking triethylphosphine in the synthesis of the major components of the sex pheromone of old world bollworm moth *Heliothis armigera*. *11*, *12* and gypsymoth *Porthetric dispar 13* with high Z purity up to 99.9% (Fig. 4) (Liu & Lin, 1987).

3. CHRIAL SYNTHESIS AND CHIRAL RECOGNITION

With the increasing number of identified chiral pheromone compounds, more attention has been paid on the topic of stereochemistry-activity relationship among pheromones. Generally, two approaches of chiral synthesis is employed as follows.

a. Through chiral pool – 2, 3-O-isoprolidine-(+)-(R)-glyceraldehyde *22*, easily prepared from





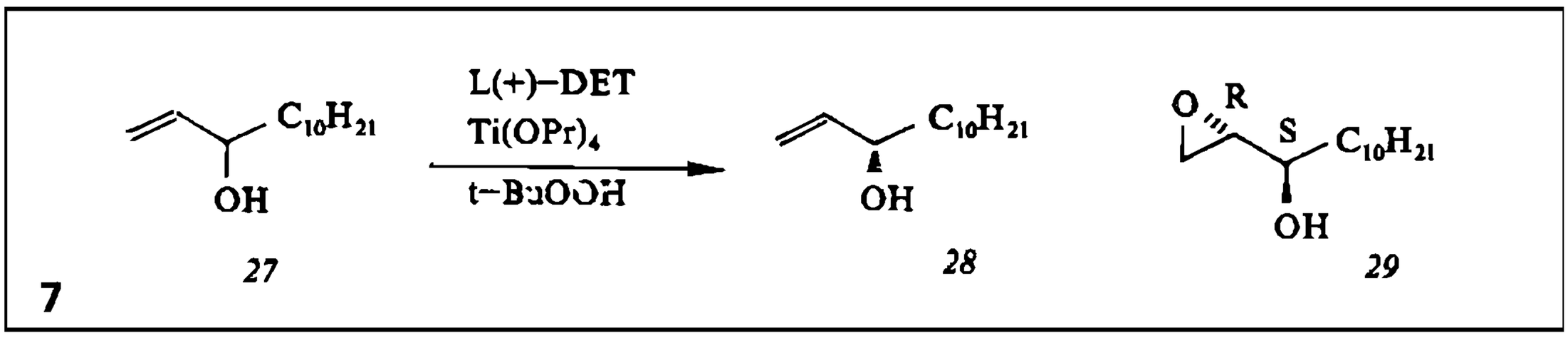
(+)-mannitol according to known document (Baer & Fisher, 1938) was employed to synthesize all the four stereoisomers *15*a-d (Fig. 5) (Lin et al., 1984). Only *15*a was active in the field bioassay, other isomers *15*b-d were ineffective, indicating that male gypsy moths showed chiral recognition (Wu, 1983).

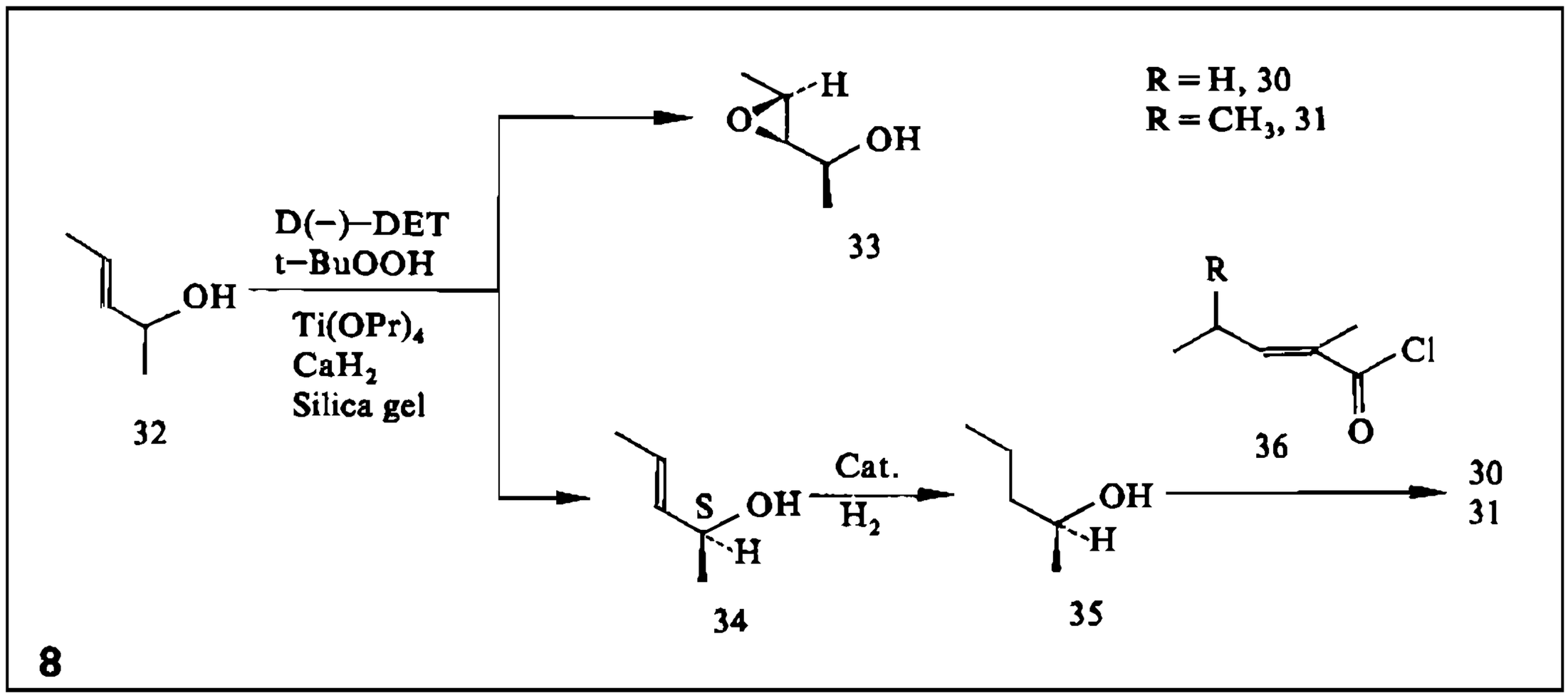
The mosquito oviposition attractant pheromone *21*a was also obtained from *23*a via 11 steps in an overall yield of 15% (Lin et al., 1987).

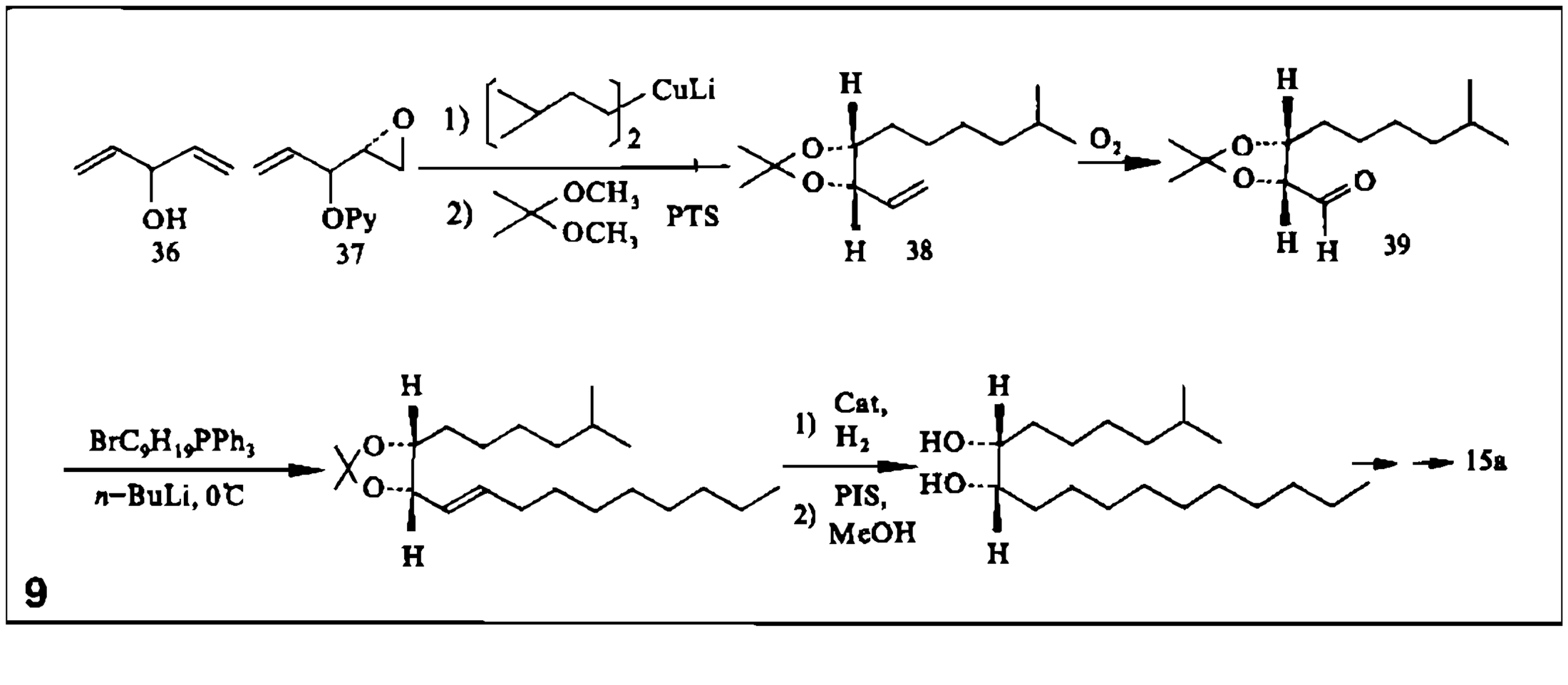
b. Through asymmetric synthesis – All the four stereoisomers of 6-acetoxy-5-hexadecanolide *21*a-d, the mosquito oviposition attractant pheromone were synthesized via Sharpless Asymmetric Epoxidation (for instance *21*a, Fig. 6) (Lin et al., 1985).

The activity of *21*a-d on mosquito was bioassayed. Only (–)-(5R6S)-*21*a was active in attracting *Culex pipiens fatigens* females for oviposition at dosage of 0.5 μg/100 ml H_2O. Other *21*b-d were inactive. *21*a was 1/100 times less ovipositionally acttractive to *C. tarsalis* and inactive to *Aedes aegypti L.* and *Anopheles quadrimacutates*, indicating genus specific of the pheromone (Hwang et al., 1987).

We discovered that addition of a catalytic amount of CaH_2 and silica gel to Sharpless reagent greatly reduced the reaction time (Wang et al., 1985). For example, reaction time of Z- and E-2-tridecen-1-ol (*23* and its E isomer) in the presence of 5-10% equivalent of CaH_2 and 10-15% equivalent of Silica gel with the sharpless reagent can be reduced from 96 h and 72 h to 8 and 6 h, respectively while both chemical and optical yields of the epoxide products remained unchanged. Reaction time of (+)-1-tridecen-3-ol with our modified Sharpless Condition via kinetic resolution can be reduced from 360 h to 25 h. The chiral epoxide *29* was used to prepared *15*a in e.e of 92% (Fig. 7) (Lin et al., 1985).





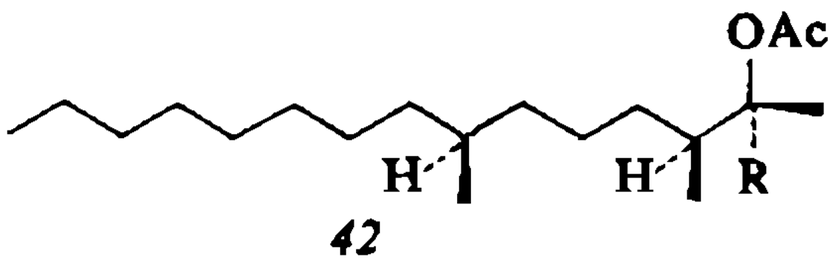


Another example is the preparation of *30* or *31* (Liu & Lin, 1989), which are aggregation pheromone of *Rhyzopetha dominica*, lesser grain borer, *30* and *31* were prepared from the common starting material *32* in an overall yield of 30% in 90% e.e. (Fig. 8).

Very high enantiomeric excess can be obtained by employing the epoxidation of divinyl carbinol *32*, a way of combination of enantiotropic group and diastereotropic face selectivity (Schreiber et al., 1987). In our case, we got more than 99.7% e.e in yield of 86% over two steps from *36* to *37* (Where Pg was trimethylsilanyl). *37* was subjected to preparation of *15*a (Fig. 9) (Lin & Chung, 1991).

Chiral α-alkylalkanoic acid is usefull building block in synthesis of natural product such as the pheromone of pine sawfly *Neodiprion lecontei 42*.

Both R and S forms of α-alkylalkanoic acid were prepared by asymmetric alkylation of the dianion of *43* which served as an *auxiliary* reagent (Fig. 10) (Lin et al., 1984).



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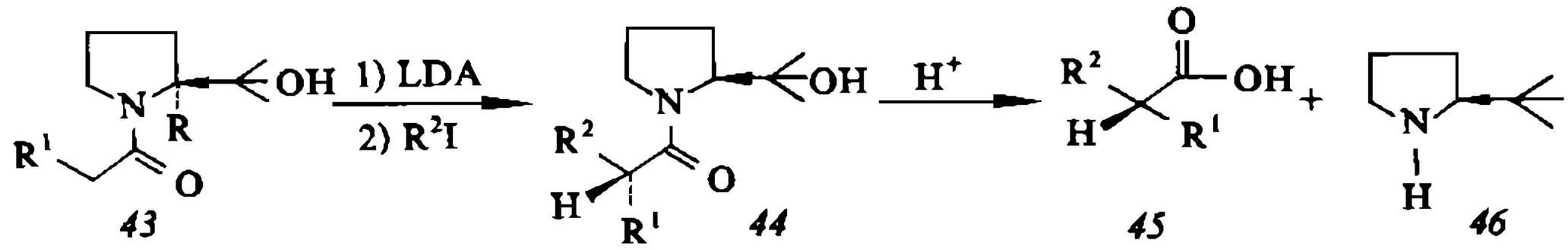

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