

I N S E C T P H E R O M O N E S

THE SYNTHESIS OF THE SEX PHEROMONE

OF

CRYPTOPHLEBIA LEUCOTRETA MEYR.

AND

RELATED COMPOUNDS

BY

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SUMMARY

The concept of pheromones is briefly expounded leading on to a discussion of the identified sex pheromones in the order Lepidoptera. The relationship between the structures of these compounds and their biological activity is reviewed. A brief introduction to the sexual biology of the false codling moth (Cryptophlebia Leucotreta Meyr.) is given.

The description of the synthesis of the female sex pheromone trans-dodec-7-en-1-yl acetate is preceded by a short review of the syntheses of the known moth sex pheromones. Synthesis of the pheromone was effected by the reaction of 1-hexyne with 2-(6-bromohexyl-oxy) tetrahydropyran to give 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran. Chemical reduction of this acetylenic intermediate afforded only 2-(trans-dodec-7-en-1-yl-oxy) tetrahydropyran. Also, reduction, using quinoline-poisoned palladium catalyst, yielded the cis form which contained only trace amounts of trans compound. Refluxing these cis and trans compounds, individually, in glacial acetic acid with molar quantities of acetyl chloride afforded the cis and trans acetates of dodec-7-en-1-ol.

Similar acylations, allowed preparation of the propoxy and butoxy homologues. A number of analogous compounds have been prepared by way of the various reaction intermediates.

The biological testing of the synthetic pheromone and the related compounds, in the laboratory, is described. The synthetic pheromone was found to elicit full sexual response in male moths at a concentration level of 0.01 μ g/disc. while its geometric isomer at the same level

showed no activity. The propoxy homologue was found to stimulate some sexual response at a level of 0.1 μ g/disc. Conversely, no response was shown at the same level, by the butoxy compound.

All the other compounds were tested at similar concentration levels and found to be inactive.

Finally, it was shown that the cis isomer of the sex pheromone did not inhibit pheromone response in male moths.

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Chapter 1INSECT SEX PHEROMONES1. INTRODUCTION: The Concept of Pheromones.

The phenomenon of chemical communication between insects was studied and reported upon by Fabre in the last century. The chemical studies of these potent biological compounds had to await the development of micro-technique and sophisticated physical methods for the separation and identification of chemical compounds.

These chemical 'messengers' are commonly known as pheromones derived from the Greek word 'pherein' (to carry) and 'horman' (to excite). (Karlson and Lüscher, 1959). The term pheromone is given to any compound secreted by an animal into the outside environment and which induces a behavioral or physiological response in another member of the same species. Novak, (1966), has characterised pheromones into two categories on the basis of the effect they produce in the receiving animal. The first, a primer effect is caused by neurosecretorily active scents which act through the senses and induce a physiological change in the individual. An example of this type of scent is the queen substance of the honey bee (Apis Mellifera) trans-9-oxodex-2-enoic acid, which exerts a caste control in the hive. The second, a releaser effect, is induced by pheromones such as the sex attractants and trail laying substances.

The exact nature of insect olfaction is, as yet, not known. Various theories have been put forward and a comprehensive review of them has been written by Jacobson, (1965).

The many cases in which it has been observed that insects, deprived of their antennae, have been rendered incapable of responding to the pheromone involved, have indicated the antennae to be the principle site of olfaction. Two comprehensive reviews, (Schneider, 1963; Boeckh, Kaissling and Schneider, 1965) and a recent publication by Schneider, (1969), have dealt with the role of antennal sensilla in sex pheromone perception and the electrophysiological methods employed to study them.

Of the many different types of insect pheromones known, the most studied have been the sex pheromones. These pheromones have been the subject of a comprehensive review by Jacobson (1965) and an excellent recent review by Shorey, Gaston and Jefferson, (1968). In the light of these reviews it will suffice to mention merely the different ways in which sex pheromones are utilised. Shorey, et. al., in their review, consider sex pheromones to be "chemicals that directly facilitate mating". These chemicals may be employed by a member of one species to attract a sexually responsive partner over fairly long distances. For instance, Ouye and Butt, (1962), have shown that female pink bollworm moths are capable of attracting males over a distance of two hundred meters. Alternatively a compound may be employed to stimulate or sexually orientate a male or female towards copulation. For example, Pliske and Eisner, (1969), have shown that male queen butterflies (Danaus gilippus berenice) are able to inhibit the flight response of a female, causing her to land and orientate herself towards copulation.

This is achieved by the male overtaking the female in flight and brushing her antennae with two extrudable brushlike "hairpencils" containing the sex pheromone, 2,3-dihydro-7-methyl-1-(H)-pyrrolizin-1-one. Any premating communication system may be dependent on several different pheromones, for example, both attraction and close-range orientation pheromones may be required for actual mating to occur. Also, although this has not been shown, a mixture of pheromones in a set ratio to each other may be required.

Sex pheromones have also been shown to cause aggregation of the sexes. These chemicals are released by members of one sex only, but attract members of both sexes towards the pheromone source. Species responding in this way include the bark beetle (Ips confusus). Once a male has established itself in a suitable host tree, it produces a pheromone which causes flight of other members of the same species towards the tree. Shorey et. al., (1969), have questioned the validity of calling such compounds, sex pheromones, in that they might equally be considered food source pheromones and only indirectly facilitate mating by bringing males and females together. This is a feasible observation, as the possible use of another pheromone as a close-range stimulant for the female has not been disproved.

The wide use of sex pheromones amongst insect species is evident from the many orders in which a sexual communication system is found. Of these, the order Lepidoptera contains by far the most number of documented cases. Jacobson, (1965), lists the number

of species in which the female attracts the male as being one hundred and eleven or more. He has also listed forty species in which the male attracts the female.

2. SEX PHEROMONES IN THE ORDER LEPIDOPTERA.

The two classes of insects found in the order Lepidoptera are the butterflies and the moths. Butterflies are largely day-time fliers and it appears that their premating communication system is primarily one of visual recognition with the sex pheromone limited to close-range courtship only. Most moths, however, are twilight or nocturnal fliers and thus the emphasis in the premating communication systems is on the successful attraction of one partner to another. Indeed, in some moth species, for example, the wattle bag worm (Acanthopsyche junodi), the female is entirely wingless and immobile, thus mating is dependent on a highly developed lure-and-receptor system.

Only a few of the known sex pheromones in this order have been isolated. structurally identified and synthesised. The major reasons for this could be summarised as follows:-

- (i) The difficulty in obtaining enough members of the insect species involved to give a substantial crude extract of the sex pheromone. Butenandt (1961), for example, obtained only 12 mg. of pure pheromone from 500,000 female

silkworm moths. In this laboratory research on the sex pheromone of the false codling moth, (Read 1968), necessitated the rearing of 1,700,000 moths. The rearing or collection of such large numbers is a limiting factor.

- (ii) The possibility of disease destroying large numbers of the insect species involved, be they raised or collected. An artificially raised supply of moths is normally isolated from this possibility due to sterilisation procedures effected during the rearing stage. For instance, in the artificial rearing of the false codling moth the eggs are first sterilised by contact with formaldehyde before placement on the sterilised artificial media. If artificial rearing is impractical then the large numbers of moths required necessitate field collection. Field collection is normally conducted during the pupal stage of the insect and the large numbers collected are sexed and stored until the moths emerge. During this storage period the possibility of viral or fungal infection is high due to the high concentration of pupae. Work in this laboratory on the pine emperor moth (Nudaurelia Cytherea) has suffered such an experience. On this occasion the entire population of

pupae collected was destroyed by a fungus. Subsequently, a recurrence of this has been prevented by storing the pupae in such a way that contact between them is eliminated. Even when thus stored, about 50% of the population is still destroyed by a polyhedral whilt

- (iii) The prerequisite of a biological assay to monitor the isolation and chemical procedures. Every biological assay requires live male or female members of the species. If the insects can be raised artificially then this does not present a problem as there will always be a continuous supply. If field collection is necessary, however, then the seasonal appearance of many species is a delaying factor. The biological assay itself may present problems eg., in some cases the sexual behaviour of the insect species involved is not displayed under artificial laboratory conditions, necessitating natural field conditions. This type of assay is usually based on the in-flight orientation of the responding insects to the pheromone source and is, of necessity, dependent on the environmental conditions which regulate normal mating behaviour. There are other problems which arise but these are beyond the scope of this thesis.

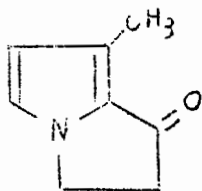
- (iv) The necessity for team work. As can be imagined the successful identification of a sex pheromone does not involve only the efforts of an organic chemist. For instance, an entomologist is required in setting up a bio-assay procedure, in location of the sex pheromone gland. to conduct any field trapping experiments and so on. If field collection is necessary, staff is required to collect, sex and care for the pupae. Also, constant supervision surrounds any artificial rearing programme and the successful launching thereof. The importance of efficient team work is noticed most particularly if the insect species involved is seasonal in appearance and ~~requires~~ field collection. The short period in which males or females are available for bio-assay procedures necessitates quick and accurate work.
- (v) The difficulty of separation of the pheromone from other inactive compounds which are similar in structure. This problem has largely been overcome by the use of gas-liquid chromatography, which has been employed as a final purification step in the isolation of all the identified moth sex pheromones.

The reasons discussed are by no means complete as each situation has its own problems. What the author has attempted to do is discuss the major problems likely to be encountered in any sex pheromone work.

The sex pheromones in the order Lepidoptera, which have been identified, have received wide recognition and reference. It will suffice, therefore, to mention them briefly.

3. STRUCTURE AND SYNTHESSES.

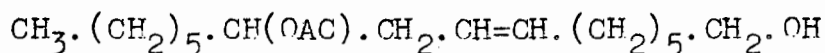
(i) Queen Butterfly (Danaus gilippus berenice).



2:3-Dihydro-7-methyl-1 [H]pyrrolizin-1-one ... (I)

The sex pheromone present in the hairpencil secretion of the queen butterfly (I) was identified and synthesised by Schneider, (1969). It was found to have the same structure as the major component of the hairpencil secretion of the neotropical danaid butterfly (lycorea ceres ceres) identified by Meinwald (1966).

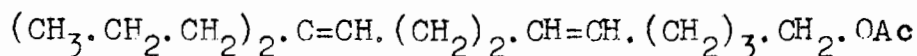
(ii) Gypsy Moth (Porthetria dispar L.).



(+)-10-Acetoxy-1-hydroxy-cis-hexadec-7-en-1-ol .. (II)

Almost thirty years of investigation at the Entomological Research Division, Beltsville, Maryland led to the identification and synthesis of the major sex pheromone of the female Gypsy moth (II) by Jacobson, Beroza and Jones in 1960. The synthesis was effected, (Chart 1), giving an overall yield of 0.2%.

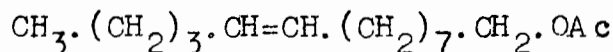
(iii) Pink Bollworm Moth (Pectinophora gossypiella),



10-Propyl-trans-trideca-5,9-dien-1-ol acetate ..(III)

The pink bollworm is one of the most destructive pests in the cotton growing areas of the world. The isolation, identification and synthesis of the pheromone was reported in 1966, by Jones, Jacobson and Martin. The synthesis entailed a twelve-step procedure, (shown in Chart 2) giving the pheromone in poor yield of 0.2%. An alternative synthesis leading to a mixture of isomers of (III) has recently been reported by Eiter, Truschiet and Boness (1967). Their material was, however, biologically inactive. A very recent synthesis by Pattenden, (1968), is superior to the others in having less steps and a higher yield, 18%.

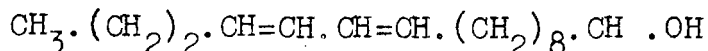
(iv) Fall Army Worm Moth (Spodoptera frugiperda).



cis-Tetradec-9-en-1-ol acetate (IV)

The presence of a substance in female army worm moths capable of exciting male moths was demonstrated by Sekul and Cox (1965). Its isolation, identification and synthesis has recently been published by Sekul and Sparks, (1967), (Chart 3). From a total of 135,000 female moths they were able to isolate 900 μ g. of pure pheromone.

(v) Silk Worm Moth (Bombyx mori L).



trans-cis-Hexadec-10,12-dien-1-ol (V)

Twenty years of investigation and research was climaxed in 1959 by identification of the sex pheromone of the female silk worm moth (Butenandt, Beckmann, Stamm and Hecker). The pheromone designated "bomkykol" was first thought to possess conjugation with a cis, trans configuration. Subsequent synthesis (Chart 4a, 4b) of the four possible geometric isomers (Butenandt and Hecker, 1961) showed the pheromone to possess the trans-10-cis-12-configuration.

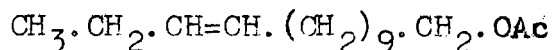
(vi) Cabbage Looper Moth (Trichoplusia ni).



cis-Dodec-7-en-1-yl acetate (VI)

The isolation identification and synthesis of the female sex pheromone was reported in 1966 by Berger (Chart 5). Recently Green, Jacobson, Henneberry and Kishaba, (1967), have reported an individual synthesis of both the cis and the trans forms.

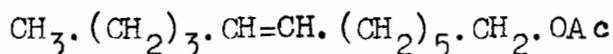
(vii) Red-banded Leaf Roller moth (Argyrotaenia velutinana)



cis-Tetradec-11-en-1-yl acetate (VIII)

The red-banded leaf roller moth is an apple pest and the isolation, identification and synthesis of the female sex pheromone has recently been published by Roelofs and Arn (1968). Working on a crude extract of 40,000 abdominal tips they were able to isolate 200 μ g. of pure pheromone. Once identified, the geometrical configuration was assigned by synthesising both cis and trans-tetradec-11-enyl acetates, (Chart 6).

(viii) False Codling Moth: (Cryptophlebia leucotreta
Meyr. = Agyroploce
leucotreta Meyr.)



trans-Dodec-7-en-1-yl acetate (VII)

Seven years of work in this laboratory led, finally, to the isolation and identification of the sex pheromone of the female false codling moth as trans-dodec-7-en-1-yl acetate by Read, Hewitt and Warren, (1968).

The isolation procedure and the identification of the pheromone will be discussed in section (2.4). The synthesis which is the subject of this thesis will also be discussed later.

4. STRUCTURE-ACTIVITY RELATIONSHIP.

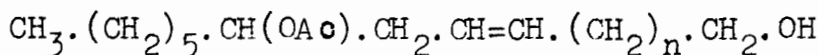
Evidence for the correlation between chemical structure and sexual activity is very limited at the present time. It is felt that this field would be less intractable to investigation should the mechanism of sex pheromone perception be established. What evidence there is has been obtained from biological testing of, (a) the hydrogenated sex pheromones, (b) their geometrical isomers and (c) compounds analogous to the sex pheromones. It is important, then, to review this evidence and list the various compounds synthesized.

Each of the seven sex pheromones identified contain one or two double bonds. Butenandt, Hecker and Zayed, (1963), found that hydrogenation of the silkworm pheromone reduced drastically its attractiveness to male moths. Similar results were obtained by Jacobson, Beroza and Jones, (1961), on hydrogenation of the gypsy moth pheromone and by Shorey and Gaston (unpublished work) for the sex pheromone of the cabbage looper moth. Roelofs and Feng, (1967), found that hydrogenation of the red-banded leaf roller moth sex pheromone destroyed completely its attractiveness to male moths in laboratory assays. Identical results were obtained in this laboratory on hydrogenation of the sex pheromone of the false codling moth by Read, Hewitt and Warren, (1968). Thus, it would seem that sex pheromone activity is dependent on the presence of unsaturation in the molecule.

With regard to the geometrical configuration surrounding this unsaturation, Truschiet, Eiter, Butenandt and Hecker, (1962), found a great decrease in the attractiveness of bombykol to male silkworm moths when changing the configuration of the molecule from 10-trans-12-cis to 10-cis-12-trans. Jacobson, (1969), found the cis form of propylure to be far less attractive to male pink bollworm moths than the trans form in laboratory tests. Jacobson and Jones, (1962) showed that although the trans form of gyplure was less active in the laboratory than the cis form, it showed no activity in field tests. Testing in this laboratory has shown the cis isomer of the false codling moth pheromone to be unattractive to male moths. Similar results were obtained by Roelofs and Arn. (1968). in field tests on the trans isomer of the red-banded leaf roller moth pheromone. They found the trans form attracted no males to traps hung in apple orchards while traps baited with cis form did. Clearly then, sexual response in male moths is dependent on the olefin configuration shown by the natural pheromone.

The effect of an increase in chain length of the pheromones on the sexual response of male moths can be gauged from the activity of the various analogues which have been synthesised. Jacobson and Jones, (1962), synthesised (+)d-12-acetoxy-cis-octadec-9-en-1-ol, (1Xb), designated "gyplure," which represents a two carbon increase in the chain length between the double bond and the hydroxyl group, and found it to be as attractive to male gypsy moths as the natural pheromone (1Xa). A further increase by two carbons

to give the C₂₀ homologue (1Xc) produced a decrease in attractiveness.

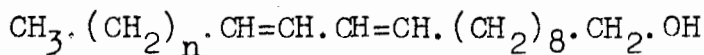


1Xa n = 5 (Natural).

1Xb n = 7 (Gyplure).

1Xc n = 9.

Butenandt's group, (Truschiet, et. al., 1962), have synthesised two isomeric analogues of the silk worm moth pheromone. These are trans-cis-tetradeca-10,12-dien-1-ol (Xb), which shows a two carbon decrease in chain length and trans-cis-octadeca-10,12-dien-1-ol (Xc) which shows a two carbon increase in chain length. On testing these compounds they found that a decrease in chain length caused a vast drop while an increase produced only a slight decline in activity.



cis trans

Xa n = 2 (Natural)

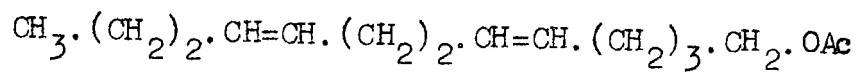
Xb n = 0

Xc n = 4.

It would seem, therefore, that sexual responsiveness in males is less affected by an increase than by a decrease in chain length.

Sexual responsiveness in male pink bollworm moths has been shown by Jacobson and Jones (1967), to be dependent on propyl branching. These workers synthesised the four geometric isomers of trideca-5,9-dienyl acetate (X1) as analogues of the sex

pheromone lacking a propyl group on carbon 10,
They found them to be completely inactive in
both field and laboratory tests.



XI

Finally, very little evidence for the dependence
of sexual responsiveness on the hydroxyl or acetyl
function in each of the pheromones is available.

Chapter IIFALSE CODLING MOTH

(Crytophlebia Leucotreta = Argyroploce Leucotreta Meyr.)

1. INTRODUCTION

The false codling moth (Crytophlebia leucotreta) is a pest indigenous to South Africa which attacks and destroys many types of fruit. Paramount among these types are apples, pears, leechies, walnuts and especially the citrus fruits. The moth is destructive in the sense that the larvae, as soon as they emerge from eggs deposited on the fruit, bore inwards and commence eating the pulp. This attacked fruit then soon discolours and drops off.

The larval stage lasts approximately one month depending on the particular season. On reaching maturity the larvae leave the fruit and enter the ground where a fibrous cocoon is spun and pupation takes place. These cocoons are difficult to locate as they are small ($\frac{1}{4}$ ") and expertly camouflaged by particles of sand.

The pupal stage lasts three weeks whereupon the moths emerge. The females do not mate immediately but commence calling two days after emergence. Analogously, males under two days of age are not capable of sex pheromone detection. A mated female commences egg laying three days after copulation and normally lays between 10 - 50 eggs. The female is able to lay her eggs over a relatively long period and this leads to overlapping of the generations of which there may be 5 - 6 per year.

Both male and female false codling moths are dark, splotchy grey in colour with no really prominent

markings save for a distinct white tuft found dorsally on the thorax. The moths are approximately $\frac{3}{8}$ " in length with a wingspan of slightly less than $\frac{1}{2}$ ". The female moth has a full rounded abdomen terminating in an almost sharp point. Situated ventrally on the terminal segment is the pheromone gland. The gland is halfmoon in shape, pale orange in colour and entirely scaleless. Immediately above the gland is the ovipositor. The male differs in having a thinner abdomen showing slight tapering towards the last segment. Attached ventrally on the base of the last segment are two claspers. These claspers are covered on the outside by an immense layer of orange scales, while the inside is smooth and bears on the peripheral edge a row of stiff hairs. It is with these claspers that the male grips the abdomen of the female during copulation.

2. SEXING OF MALES AND FEMALES

To obtain enough crude extract of the female sex pheromone for isolation and identification work Read, (1968), was fortunate in being able to extract two-day old mixed populations and so circumvent the laborious task of sexing the large numbers required. The bio-assay procedure, however, used by Read and by the author calls for two to five day old males only. Separation of males and females is done in the pupal stage by examination of the pupae, individually, under magnification. Male pupae have, situated ventrally on the terminal segment, two distinct bumps lying side by side (fig. 1a). These bumps are replaced in the female by a minute longitudinal dark line. Pupal size may serve as a very rough guide, as the females tend to be larger than the males.



FEMALE



MALE

Fig. 1a. Difference between male and female pupae.

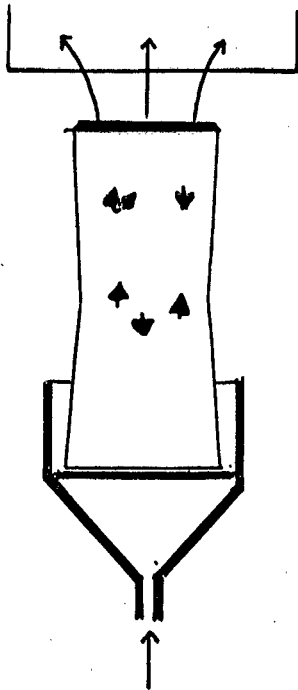


Fig. 1b. Apparatus used in the bio-assay procedure.

3. BIO-ASSAY PROCEDURE

The bio-assay procedure used by the author is a semi-quantitative adaptation of the quantitative method used by Shorey and Gaston, (1964), for the bio-assay of the sex pheromone of Tricoplusia ni. Ten male moths, 2 - 5 days old are housed in a tubular plastic cage, 8 inches in length, diameter of 2.5 inches and which is sealed at both ends by cotton gauze. Ten such cages are used for each test run. A portion of the extract to be tested is spotted onto a circular sheet of filter paper and the solvent allowed to evaporate. The impregnated filter paper is then placed in a büchner funnel (fig. 1b), which is connected to an air supply at a velocity of 5 litres/min. Each cage is then placed in the funnel for a period of 30 seconds, and during this period a visual count of males responding in each cage is made. The response to the sex pheromone is one of extreme wing vibration and circular movement of the body with flexing of the abdomen in attempts to copulate. It is thus clearly discernable from any other form of response and cannot be mistaken. Other types of response noticed during testing procedures give the impression of agitation. Typical of this is a continuous moving around the cage interspersed very occasionally by flight or frantic flitting to and fro. On one occasion the author was able to trace the cause of this to traces of acetic acid in the extract tested. Washing the extract with sodium bicarbonate solution was found to eliminate this behaviour.

The response to any extract tested was recorded in tabular form and expressed as a percentage of males responding to the total number of males tested.

Suitable testing conditions and procedures were found to be:

1. Time of testing; maximum response was obtained between the hours 7.00 - 11.00 p.m. under 0.2 ft-c. illumination (Read, 1968).
2. Age of caged males; maximum response was obtained from moths 2 - 5 days old, (Read, 1968).
3. Temperature and humidity; males responded within a range of 17 - 22°C and relative humidity of 45 - 75%, (Read, 1968). Unfortunately, no facilities for humidity control were available.
4. Place of testing; it was essential for testing to be done in a room entirely free from contact with synthetic pheromone. It was also found that clothes worn in the laboratory, and thus exposed to the synthetic pheromone, could not be worn in the testing room. In addition, facility for removal of contaminated air issuing from the büchner funnel was essential. This was accomplished by the positioning of a suction fan immediately above the büchner funnel, allowing space for free handling of the plastic cages.
5. Care of moths; regular feeding was found to be necessary. This was done just after dark by placing a plug of cottonwool, saturated with a 10% sucrose solution on top of each cage. While testing, it was found that gentle handling of the cages was essential as any undue knocking caused the moths to fly around and thus give misleading responses.

4. SEX PHEROMONE; ISOLATION AND IDENTIFICATION

The isolation and identification was achieved in this laboratory by Read, Hewitt and Warren, (1968), and it will suffice to review briefly the procedures involved.

Whole male and female moths, two days old were homogenised in 5 times their weight of ethanol and filtered. The filtrate was concentrated and held at 0°C for twelve hours. The precipitated fats were removed by filtration and an equal volume of half saturated sodium sulphate added to the filtrate. The whole was extracted with petroleum ether until this phase appeared colourless. The combined petroleum extracts were dried over anhydrous sodium sulphate and concentrated. This crude concentrate showed no biological activity. Column chromatography (Woelm Alumina Grade 1 Neutral) and elution with benzene: ethylacetate, (1:1 v/v), gave an active fraction which was concentrated and steam distilled. The distillate was extracted with light petroleum and the combined extracts dried and concentrated to 10,000 ME. /ML.

Gas-liquid chromatography on this crude concentrate revealed the presence of four major components, one of which elicited sexual response in the male moth. Catalytic hydrogenation and alkaline hydrolysis of this component destroyed all activity. Acetylation of the hydrolysed product was found to regenerate activity. A comparison of the g.l.c. retention times of the active component, on polar and non-polar stationary phases, with those times given by known esters and acetates, substantiated by the above

chemical evidence, gave results indicative of a C₁₂ unsaturated acetate.

A sample of the synthetic sex pheromone of the cabbage looper moth, cis-dodec-7-en-1-yl, obtained from Dr. R.S. Berger (Auburn University, Alabama), gave the same retention time as the active component on both non-polar and polar stationary phases. Identical results were obtained for the trans isomer obtained from Dr. M. Jacobson (U.S. Department of Agriculture). The mass spectrum of an isolated active fraction from g.l.c. had fragments m/e 43 (base peak) 61, 82, 166 and 266 (M+) with similar relative intensities as the peaks in the mass spectra of the synthetic acetates. Oxidation of the active component and the synthetic trans isomer with periodate/permanganate followed by esterification of the resulting acids, gave, in each case, on g.l.c. analysis, methyl pentanoate and identical chromatograms.

Finally, bio-assay of the purified cis and trans isomers (thin layer chromatography on silicic acid impregnated with silver nitrate, 25%) showed that the trans and not the cis form was the active isomer.

It was, therefore, concluded that the sex pheromone of the false codling moth was trans-dodec-7-en-1-yl acetate.

Chapter III

SYNTHESES OF trans-DODEC-7-EN-1-YL ACETATE

1. OTHER SYNTHESSES

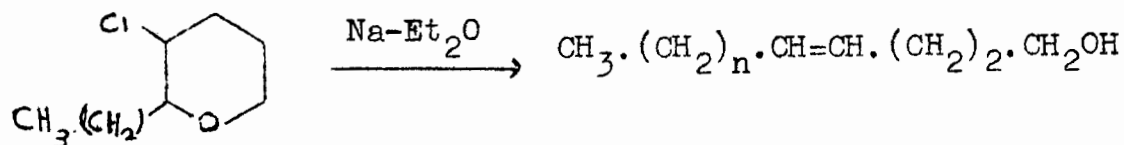
The seven identified sex pheromones are essentially similar in structure in that they are all simple aliphatic compounds, one of which shows propyl branching. They are 14 - 18 carbons in length, show unsaturation and possess a primary or secondary hydroxyl group which is either free or acetylated. Before synthesising trans-dodec-7-en-1-yl acetate it was, therefore, pertinent to review the syntheses of these compounds and the reactions employed.

(i) Unsaturation

The presence of unsaturation in the sex pheromones has caused considerable diversity in their syntheses. The most favoured method of introducing unsaturation into an alkyl chain has been through the formation of the alkyne which, on stereospecific reduction, gives the desired olefin. This method was first employed by Ahmad and Strong, (1948), in the synthesis of unsaturated fatty acids, and has since found wide application. Formation of the alkyne is achieved through coupling of an alkyl halide with the alkali salt of acetylene or substituted acetylenes. The coupling is normally carried out in liquid ammonia but can be done in dioxane (Jacobson and Warthen 1967). The syntheses in which this method has been used, will be discussed later.

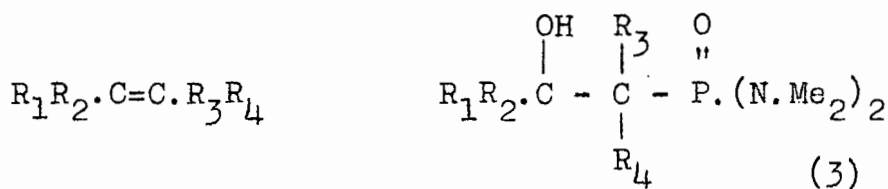
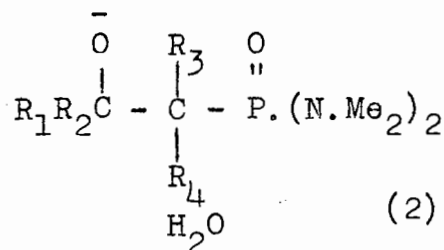
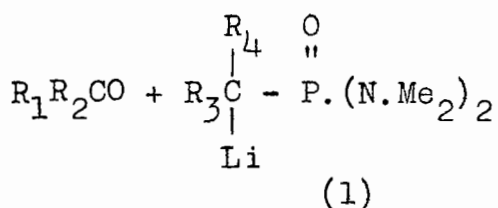
Green, Jacobson, Henneberry and Kishaba, (1967), used a new approach. In their synthesis of trans-dodec-7-en-1-yl acetate, the stereoisomer of the

T.ni. pheromone, these workers prepared trans-non-4-en-1-ol by ring scission of 2-butyl-3-chlorotetrahydropyran. This was based on a finding of Crombie and Harper, (1950), that ring scission of 2-alkyl-3-chlorotetrahydropyrans give only trans alkyl-4-en-1-ols.



In one of their syntheses of "bombykol" Butenandt and Hecker, (1961), used the Wittig reaction in coupling ethyl-10-*al*-decanoate with the corresponding alkyltriphenyl phosphorane. The Wittig reaction has recently gained in popularity due to the discovery of Bergelson and Shemyakin, (1964), that stereospecific control of olefination is possible through the choice of suitable reaction conditions and structural factors. This, however, is conclusive only for the cis isomer and not the trans. Parallel with this reaction an interesting new synthesis of olefins from phosphonic acid bis amides has been reported by Corey and Kwiatkowski, (1968). In essence the reaction of 2-lithio-phosphonamide derivatives (1) with aldehydes or ketones afford β -hydroxy-phosphonamides (3) by carbonyl addition, in excellent yields. Thermal decomposition of these adducts in benzene or toluene solution, at reflux, leads to olefins by elimination of the elements of the corresponding

phosphonic acid amide.



This phosphonamide route is complementary to the Wittig and Emmons-Wadsworth, (1961), reactions, and has certain general advantages which may be relevant in future syntheses. Of importance is the stereospecific cis and trans syntheses, of which various examples have been given. In addition, the scope appears to be broad, since mono-, di-, tri- and tetrasubstituted ethelenic derivatives can be synthesised.

Finally, carbonyl condensation, through the Reformatsky reaction, with an alkyl halide has been employed by Jacobson and Jones, (1962), and Butenandt and Hecker, (1961). This reaction, although it gives a mixture of cis and trans isomers, is of value in extending the carbon chain of an aldehyde.

(ii) Olefin Configuration

In each of the pheromones identified, except that of the silk worm moth, it has been found that only one of the geometric isomers will elicit male response. This has been effective in influencing their syntheses towards reactions giving only one geometrical isomer. It is for this reason that stereospecific reduction of alkynes has had such wide application in the insect pheromone field. Catalytic hydrogenation using Lindlar catalyst (Pd - CaCO₃, poisoned with quinoline) is reported to give only the cis olefin (House 1965). The author, however, on testing cis-dodec-7-en-1-yl acetate, prepared by reduction of the corresponding alkyne found it gave a positive sexual response, indicating, therefore, the presence of some trans isomer. Chemical reduction of the parent alkyne with sodium or lithium in liquid ammonia affords only the trans isomer (House 1965). Where syntheses have afforded mixtures of cis and trans isomers separation has been necessary. Budenandt and Hecker, (1961), in their syntheses of the four geometric isomers of bombykol effected separation of ethyl-trans-hexadec-10-en-12-ynoate from the cis form by trapping the trans form as a urea complex and thus enabling its precipitation from a methanolic solution of the mixture. (This method is discussed in Fieser and Fieser, 1967). In another of their syntheses, cis and trans dodec-10-yn-12-en-1-ols were separated by fractional crystallisation at low temperatures from petroleum ether.

(ii) Hydroxyl function

The hydroxyl group has either been incorporated into the alkyl chain as a protected group in the starting material or by Lithium Aluminium Hydride reduction of the ester of the corresponding acid.

The protection of the hydroxyl group by formation of the tetrahydropyranyl ether has found application in almost all the syntheses and is essential if the formation of alkyl ethers is to be avoided. These ethers are formed by reaction between the alkyl halide and the sodium derivative of the unprotected hydroxyl group.

2. trans-DODEC-7-EN-1-YL ACETATE

The structure of the sex pheromone of the female false codling moth (Cryptophlebia leucotreta Meyr.) through comparison with synthetic acetates obtained from two independent sources, has unequivocally been shown to be trans-dodec-7-en-1-yl acetate. The synthesis of the pheromone was undertaken in this laboratory, not merely as further confirmation of its structure, but to make available synthetic material for use as a standard in biological assay and field tests.

It was felt that any synthesis with this objective should conform to three requirements:

- (a) Be unequivocal. For this the reaction stages must proceed in a definite direction and give high yields so as to avoid large scale purification, procedures. Of particular importance in this respect is the avoidance of the separation of cis and trans isomers in other than trace quantities.
- (b) Be capable of application to large scale production and, therefore, avoid the use of expensive reagents and starting materials.
- (c) Be adaptable to the synthesis of appropriate analogues through variation of the starting compounds.

These requirements invalidated reactions employed in other syntheses such as the Wittig and Reformatsky, as they afford mixtures of cis and trans isomers. Also, in the latter reaction some discrepancy as to the position of the double bond may arise due to its mode of formation. Similarly, formation of the hydroxyl group by reduction of a carboxylic acid was avoided due to the expense involved in large scale reduction.

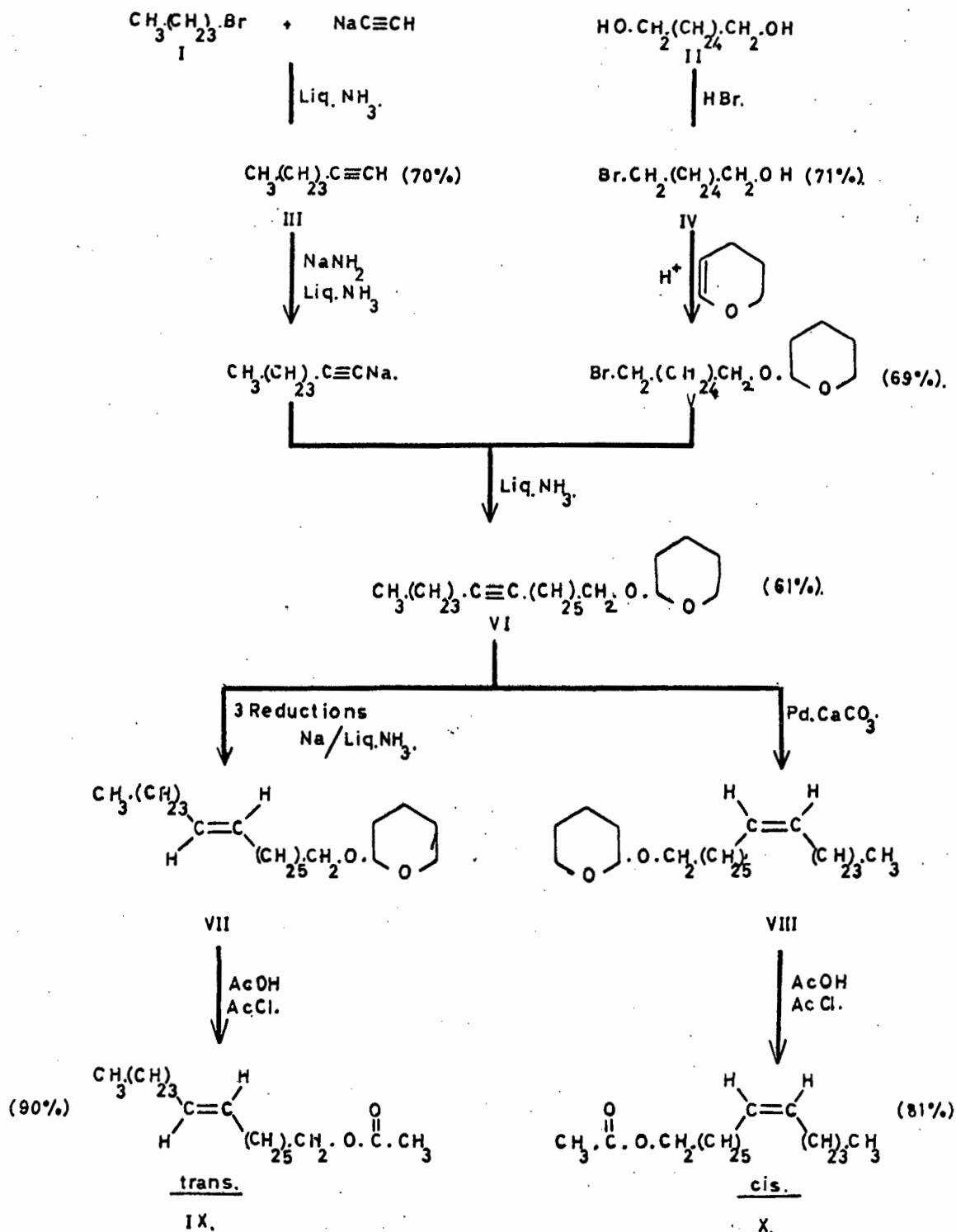
It was decided to effect the synthesis of the pheromone by formation of the acetylenic alcohol, dodec-7-yn-1-ol, which, on hydrogenation and acetylation, would give the desired product as well as its geometrical isomer. The reaction sequence followed is illustrated in Route 1.

Formation of this acetylenic intermediate as its tetrahydropyranyl ether (VI) was effected in good yield by coupling of the inexpensive starting material hex-1-yne (III) and the tetrahydropyranyl ether of 6-bromo-1-hexanol (IV).

Preparation of 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran (VI) leaves no doubt as to the position of unsaturation in the alkyl chain since isomerisation of internal acetylenes occurs only under certain reaction conditions which do not apply here. Chemical reduction with sodium in liquid ammonia allows complete conversion to the trans isomer, thus eliminating the necessity of separation of cis and trans forms. Alternatively, catalytic reduction with Lindlar's catalyst, Pd-CaCO₃, poisoned by quinoline) affords the cis isomer only. The ready reaction of dihydropyran with primary hydroxyls to give tetrahydropyranyl ethers in good yields, allows the economical protection of the hydroxyl group of 6-bromo-1-hexanol (IV) through the coupling and reduction stages. Further, its ready hydrolysis under mildly acid conditions allows almost quantitative conversion to the acetate by refluxing in glacial acetic acid and acetyl chloride.

Economically, this synthesis lends itself to large scale production through the inexpensive starting materials employed, the high yields obtained, the

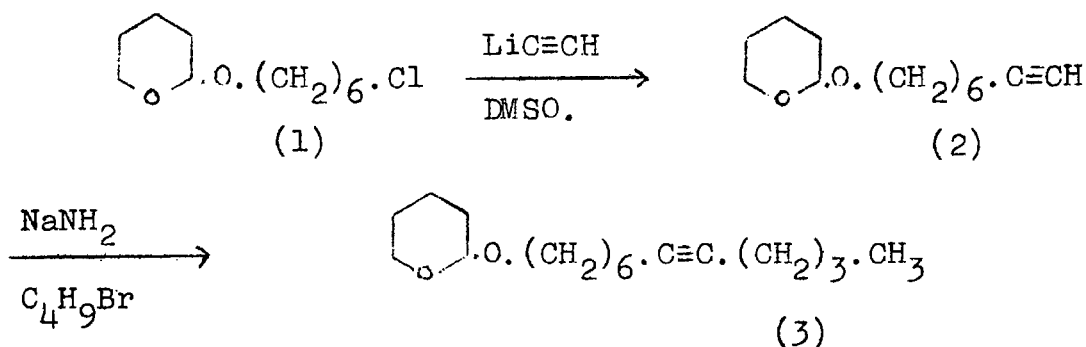
ROUTE 1



cheap reagents involved and the final product of high purity.

The route is adaptable to analogue formation by the employment of readily available compounds homologous to the starting materials. It is, therefore, possible to synthesise compounds having an increase or a decrease in the number of carbon atoms or compounds showing a shift in the position of unsaturation.

Green, Jacobson, Henneberry and Kishaba, (1967), in their elegant synthesis of the cabbage looper pheromone, prepared the same compound, 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran (3) by reacting the tetrahydropyranyl ether of 6-chloro-1-hexanol (1) with lithium acetylide - ethylenediamine complex in dimethyl sulphoxide to form 2-(oct-7-yn-1-yl-oxy) tetrahydropyran (2). On alkylation with n-butyl bromide, in liquid ammonia, the desired acetylenic compound was obtained.



In another synthesis of the pheromone from the cabbage looper which was not available to us at the time, Berger and Canerday, (1968), prepared the (dodec-7-yn-1-yl-oxy) tetrahydropyran by reacting the tetrahydropyranyl ether of 6-iodohexanol with the mono-substituted acetylene, hexyne, in sodamide/liquid ammonia. The iodo-alcohol was prepared in 74% yield by refluxing the corresponding chloro compound with sodium iodide in acetone. Interesting in this regard is the considerable decomposition encountered in attempts to convert the tetrahydropyranyl ether of 4-bromobutanol into the iodo derivative.

After careful consideration it was decided that this synthesis satisfied the requirements enumerated above. Each of the reaction stages will now be briefly discussed.

(i) 1-HEXYNE (111)

Preparations of 1-hexyne described in the literature include the following:

1. Addition of n-butyl bromide to a slurry of calcium carbide and potassium hydroxide in dibutyl carbitol, (Lyon and Rultedge, 1956).
2. Reaction of n-butyl bromide with ethynyl magnesium bromide at 80 - 90^o, (Grignard, Lapayre and Faki, 1920).
3. Reaction of alcoholic potassium hydroxide on dibromohexanes (van Risseghem, 1926).
4. Dehydrohalogenation of 2-bromo-1-hexene by sodamide in xylene (Bourguel, 1925).

The preparation of 1-hexyne in this laboratory followed the method of Campbell and Campbell, (1963). This entailed the reaction of n-butyl bromide with sodium acetylide in liquid ammonia. A small portion of sodium was dissolved in the liquid ammonia to give an intense dark blue solution. During addition of further sodium, dry acetylene was continually bubbled through to convert the dissolved metal into colourless sodium acetylide. The addition of the sodium was so regulated that no complete blue colour developed for any length of time. Upon complete conversion into sodium acetylide, n-butyl bromide was added to give the mono-substituted acetylene. Addition of saturated ammonium chloride solution, followed by normal work-up of the product, afforded 1-hexyne in 70% yield, b.p. 71 - 72^o. A drawback of this procedure, especially on a large scale, derives from the exothermic nature of the reaction; the consequent splashing tends to coat the inside of

the reaction vessel limiting visibility. A further disadvantage is the conversion of one third of the acetylene to ethylene. Another method of forming sodium acetylide depends on the conversion of a liquid ammonia solution of sodium into sodamide before addition of acetylene. This method is based on the discovery of Vaughn, Vogt and Nieuwland, (1934), that the rate of reaction of sodium and liquid ammonia to form sodamide is accelerated markedly by the presence of catalytic amounts of ferric nitrate. This sodamide then undergoes ready reaction with acetylene to furnish sodium acetylide. This would seem to be a preferable method, as the loss of acetylene by reduction to ethylene is eliminated. Also, there are definite indications that the sodium acetylide thus obtained is more reactive than that produced in the first procedure. This is, no doubt, due to the presence of colloidal iron derived from the catalyst.

Loss of the product, 1-hexyne, does occur through entrainment by escaping ammonia vapours, but this can be eliminated by the use of a dry ice-acetone condenser. A condenser, however, is not essential since this effect can be minimised by fairly quick addition of the n-butyl bromide and working up of the product approximately an hour later. It would probably have been equally as efficient to have prepared 1-hexyne by reaction of the n-bromobutane and sodium acetylide in an organic solvent mixture of xylene and dimethyl formamide in 35.40 volume percent, (Rutledge, 1959). No apparatus, however, was available in this laboratory for preparation of the sodium particle size of 25-50 microns required in the formation

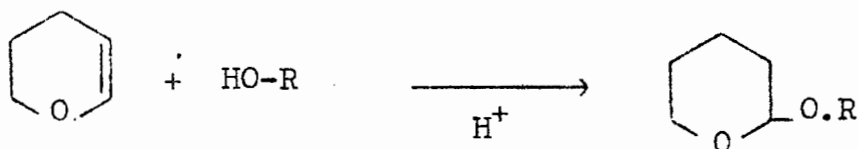
of the sodium acetylide.

The coupling of sodium acetylide with alkyl halides in liquid ammonia is restricted to primary halogeno-compounds containing the group $-\text{CH}_2-\text{CH}_2\text{X}$; secondary and tertiary compounds undergo dehydrohalogenation by the reagent, sodamide, to give ethylenic compounds. Reaction rates of these halogeno-compounds with the metallic acetylide decrease in the order iodide > bromide > chloride; chlorides are rarely used for preparative purposes, bromides being the reagents of choice. Iodides, although they undergo smooth reaction, prove fairly expensive in large scale work.

Jacobs, (1949), and Campbell and Campbell, (1950), have shown that yields of monoalkylacetylenes prepared in liquid ammonia range from 50-80% when the halides used vary in length from methyl to n-decyl. Yields obtained for the higher analogues are low due to the decrease in their solubility in liquid ammonia. This may be an important point to remember in future syntheses. Recently, use of a lithium acetylide-ethylenediamine complex has found application in the preparation of terminal acetylenes through reaction with alkyl halides. This complex, a dry powder, can be used in organic solvents such as diglyme, dioxane, dimethylformamide and dimethyl sulphoxide, thus eliminating the use of liquid ammonia as coupling medium. Also, employment of this reagent provides the only means of obtaining high yields of mono-substituted acetylenes from chloro-compounds.

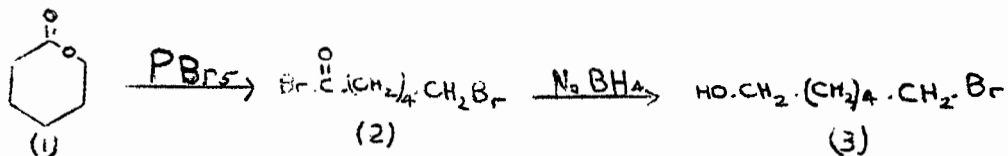
(ii) 2-(6-BROMOHEXYL-OXY) TETRAHYDROPYRAN (V)

Formation of the tetrahydropyranyl ether of 6-bromo-1-hexanol was achieved in good yields by slow addition of 2,3 dihydropyran to the alcohol containing 5 ml. concentrated hydrochloric acid as catalyst. After this addition, removal of the acid by addition of sodium bicarbonate followed by filtration and distillation of the filtrate at reduced pressure gave the desired product. The exothermic nature of these reactions necessitates cooling of the reaction flask during the addition of the dihydropyran, as high temperatures may be reached leading to possible decomposition of the reaction materials.



The protective role of dihydropyran has been extended to carboxyl groups, (Bowman and Fordham, 1952), sulphhydryl groups, (Parham and DeLaitch, 1954) and the imadazole hydrogen in purines (Robins, Godefroi, Taylor, Lewis and Jackson, 1961). In each case the active hydrogen compound is regenerated by hydrolysis with dilute mineral acid.

It was originally planned to prepare 6-bromo-1-hexanol (3) by reaction of caprolactone (1) with phosphorous pentabromide according to the method of Linstead, (1934).



The resulting 5-bromo hexynyl bromide, (2), on reduction with sodium borohydride, would give the required bromo alcohol (3) in good yields. A drawback of this method was realised to be the expense involved in large scale reduction. This procedure was, therefore, rejected and preparation of 6-bromo-hexanol effected following the method of Degering and Boatright, (1950). This preparation entailed the continuous extraction of a heated (80°) mixture of hexane-1,6-diol and aqueous hydrogen bromide, (48%), by high boiling petroleum ether (90-100°), over a period of twenty four hours. The preferential solubility of the resulting bromo alcohol in the petroleum ether afforded its continual removal from the mixture. Distillation of the extracted product under reduced pressure gave 1,6-dibromohexane in 10% yield and 6-bromo-1-hexanol in 69% yield. The reaction was carried out in a liquid-liquid extraction apparatus. Attachment of a gas dispersion disc to the end of the extraction stem, as in the original procedure would have increased the dispersion of the petroleum through the mixture, thus lowering the extraction time from twenty four to twelve hours. (The extraction time employed by Degering and Boatright).

(iii) 2-(DODEC-7-YN-1-YL-OXY) TETRAHYDROPYRAN (VI)

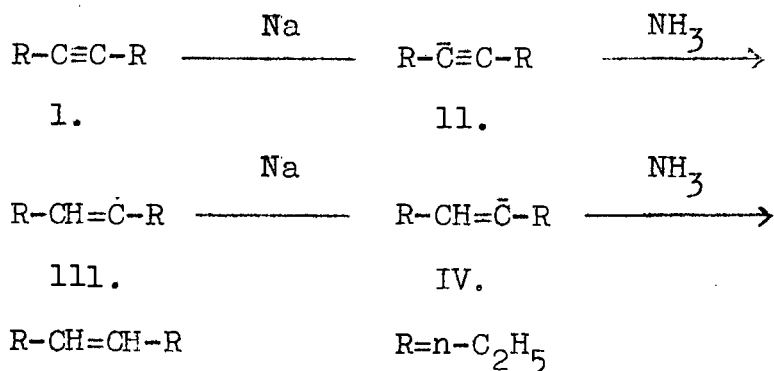
Formation of 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran was achieved by reaction of the lithium salt of 1-hexyne with 2-(6-bromohexyl-oxy) tetrahydropyran in liquid ammonia. A solution of lithium in liquid ammonia was converted, in the presence of colloidal iron catalyst, into lithamide. Complete conversion was indicated by

the replacement of the blue colour of dissolved lithium, by the grey-black of colloidal iron. 1-Hexyne was immediately added and converted into its lithium salt by reaction with the preformed lithamide. Slow addition of the bromo ether at this stage resulted in the formation of the acetylenic intermediate. Addition of saturated ammonium chloride solution, followed by normal work-up procedures and distillation under reduced pressure, gave the unsaturated ether (VI) in 69% yield.

(iv) 2-(DODEC-7-EN-1-YL-OXY) TETRAHYDROPYRAN:

(a) Chemical reduction

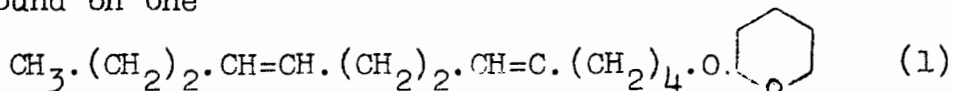
The partial reduction of acetylenes to trans olefins was first reported by Campbell and Eby in 1941. This method has since found application in synthesis of unsaturated compounds due to its stereospecificity in forming only the trans isomer. The reaction is presumed to proceed by the addition of an electron to the acetylenic bond to form an anion radical (II) which is protonated to give (III).



Subsequent electron transfer, to form the anion (IV) and protonation, gives the trans olefin. Preference for the formation of the trans isomer could be the rapid formation of the second anion (IV) and its protonation to give the olefin, allowing no inversion of the vinylic radical or of the vinylic carbanion to proceed. Alternatively, the addition of the first proton and the second electron could be concerted processes leading directly to the formation of the anion (IV) from the anion radical (11).

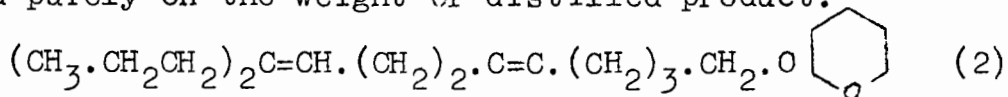
This chemical reduction, in liquid ammonia, is not applicable to terminal acetylenes unless the reaction is carried out in the presence of ammonium sulphate, (Henne and Greenlee, 1943). This arises by virtue of the negative charge which develops on the ethynyl carbon atom in the metallic acetylide formed.

The yields obtained for these sodium/liquid ammonia reductions when carried out at -35° (the boiling point of liquid ammonia) and at atmospheric pressure, vary considerably. Jacobson and Warthen, (1967), show 80% conversion of 2-(cis-9-tridecen-5-yn-1-yl-oxy) tetrahydropyran (1) to the 5 trans compound on one

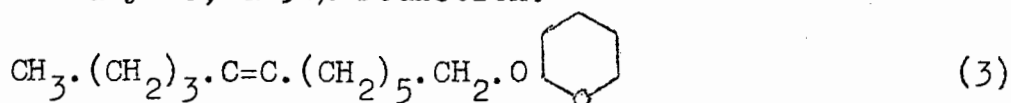


reduction. Also they report a 72% conversion to the olefin on reduction of the 5-cis, 9-yn-tetrahydropyranyl ether. Both products were assumed pure by appearance of only one peak on g.l.c. analysis. Jones, Jacobson and Martin, (1966), report 85% reduction of 10-propyl-2-(9-tridecen-5-yn-1-yl-oxy) tetrahydropyran, (2), a yield

based purely on the weight of distilled product.



Berger and Canerday, (1968), on reduction of 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran (3) obtained only 25% conversion to the trans olefin. Three further reductions gave a product which showed, on g.l.c. analysis, a 90% reduction.



Elsner and Paul, (1953), on treatment of oct-3-yne with sodium in liquid ammonia found no reduction to the trans olefin. However, when carried out under pressure, at room temperature in a tilting autoclave almost quantitative conversion to the trans olefin was obtained. Similar conversion of heptadec-8-yn-1-oate to elaidic acid under pressure was reported by Dear and Pattison, (1963), in 95% yield.

It would appear that reduction at room temperature, possible through the use of high pressure apparatus, is inductive to high yields of trans olefin. Unfortunately in this laboratory, no autoclave large enough to cope with macro quantities of material, was available. A cylindrical metal container of 1.5l. internal capacity was therefore made (Fig. 2). A thick metal disc was welded to one end of a 12" length of water piping having an internal diameter of 4" and a wall thickness of $3/16$ ". The other end was adapted to receive a heavy metal disc which could be bolted down thus creating a sealed unit capable of withstanding fairly high pressures. The removable disc was fitted with a brass

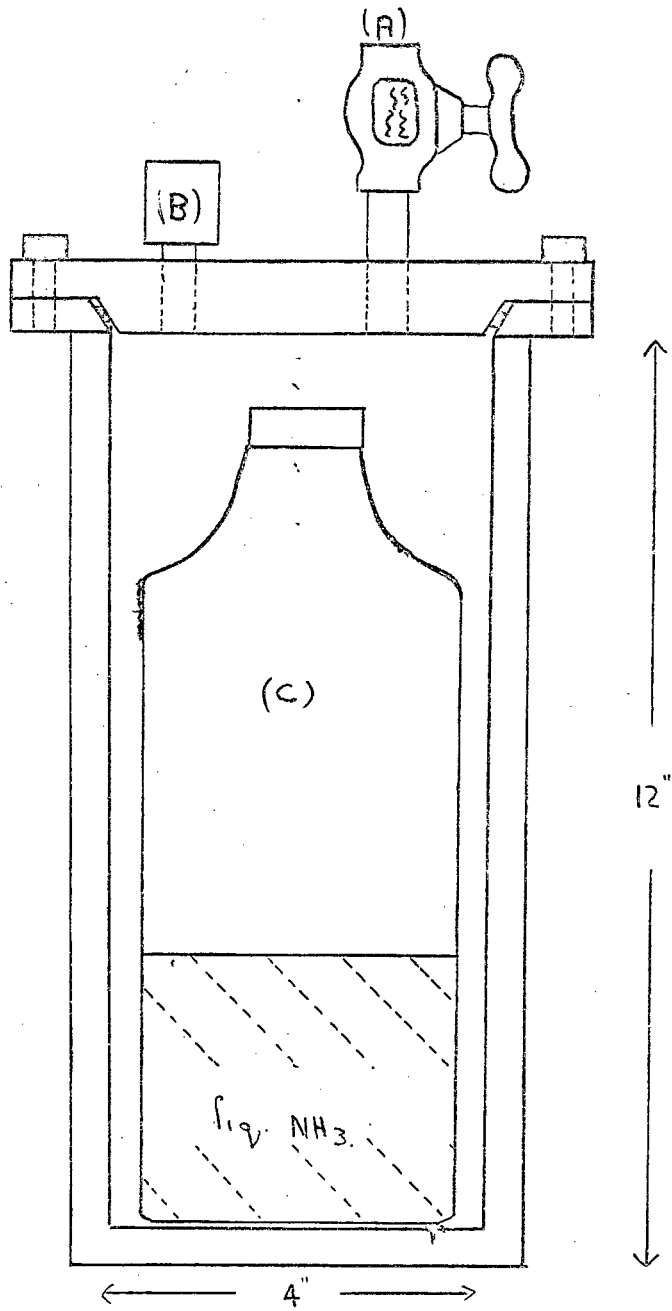


Fig. (2). Cross-section of reduction apparatus.

tap (A) and a safety valve (B), set to blow at a pressure of 200 p.s.i. The safety valve setting was achieved by bolting the disc in position and pumping water into the cylinder, through the brass tap, until a pressure of 200 p.s.i. was reached. Slow adjustment of the safety spring until the valve opened, gave the desired pressure setting.

Reduction of 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran was achieved by charging the insulated glass bottle (c) with liquid ammonia and adding the calculated amount of sodium in small portions, with stirring, over a period of five minutes. The acetylenic compound was, by means of a pipette, run into the bottle immediately below the surface of the liquid ammonia. This was necessary as dropping of the alkyne into the liquid ammonia caused considerable splashing thus coating the sides of the glass vessel with unreduced material. The wadding and stirrer were immediately removed and the bottle sealed in the metal container where the ammonia solution was allowed to attain room temperature. At this temperature liquid ammonia shows a vapour pressure of 151 p.s.i. (10.2 atmospheres). The apparatus was allowed to stand for sixteen hours whereupon the internal pressure was slowly released and the remaining ammonia allowed to evaporate. Work-up of the product and subjection to argentation chromatography on silica gel, impregnated with silver nitrate 25% showed approximately 70% reduction of the starting material. Two further reductions gave a product which showed no unreduced material on thin-layer chromatography. Unfortunately, no means of agitation of the reaction mixture in the sealed unit, could be employed.

It is felt that had this been possible, higher yields would have been obtained.

The silica gel used for TLC work was impregnated with silver nitrate by dissolving the crystalline solid in the water before preparation of the adsorbent paste. The plates used were spread to a thickness of 0.3mm. and air dried in the dark. It was found that plates stored in the dark showed signs of oxidation only after four days. Nevertheless, no plates older than two days were used and each plate was activated for 1 hour at 110° immediately before use. The chromatograms were developed in a spectroscopically pure cyclohexane and peroxide free ether solvent system (9:1), reported by Read, (1968), to give minimal blackening of the thin layer plates. In subsequent work the author has found that a benzene:petroleum ether (80:20) solvent system can be employed as the blackening produced is almost negligible.

Visualisation of the developed chromatograms was achieved by a light spraying with a 1% solution of dichlorofluorescein in ethanol. Subjection of the subsequent pink plate to ultra violet radiation revealed vivid green spots on a purple background.

(b) Catalytic Hydrogenation:

Catalytic hydrogenation of 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran with Lindlar's catalyst afforded the cis olefin in high yield. Conversion of the cis tetrahydropyranyl ether into the corresponding acetate and subsequent biological assay revealed the presence of some trans isomer, as male moths showed a positive sexual response. Infrared spectrum of the acetate

also indicated some trans isomer by showing slight absorption at 970 cm^{-1} . Argentation chromatography, however, did not reveal any trans isomer showing only the cis acetate and a small quantity of unreduced alkyne.

Complete catalytic hydrogenation of acetylenic compounds to the fully saturated substance is a simple process. Palladium, Platinum and Raney nickel catalysts have all been employed. Distribution of the first two catalysts on substrates such as carbon, starch or the carbonates and sulphates of calcium and barium allows partial reduction of acetylenic compounds to olefins. The most generally useful catalyst for this process is palladium.

(v) trans-DODEC-7-EN-1-YL ACETATE (X)

Formation of the final product, trans-dodec-7-en-1-yl acetate was achieved in 90% yield by the one step hydrolysis of 2-(dodec-7-en-1-yl-oxy) tetrahydropyran and acetylation of the resulting alcohol through reflux in glacial acetic acid and acetyl chloride. The reaction contents were allowed to reflux for a period of six hours, during which time a dense black colour developed and then poured onto ice and worked up by normal procedures to give the acetate in high yield.

Subjection of the distilled acetate to gas-liquid chromatography (Aerograph A700, column Chrom. W. E.G.A.) revealed the presence of approximately 5% of an unidentified compound. Mass spectra showed a base peak of m/e 43 and a mass peak of 166.

This compound in no way affected the sexual response of male moths to the synthetic acetate in laboratory tests, so no further purification procedures were considered.

An infrared spectrum of the synthetic acetate showed absorption at 2950cm^{-1} (CH), 1750 (primary acetate) and 970cm^{-1} (trans -CH=CH.)

A sample of the synthetic acetate was purified by g.l.c. and submitted for mass spectroscopy. The spectrum showed a mass peak at m/e 266 and other peaks at m/e 43 (base peak) 67, 82, 96, 100 and 166. Similar peaks of relative intensities were shown by the spectrum of a sample of the natural C. Leucotreta pheromone (Fig. 3) isolated by Read, (1968). On biological testing, the synthetic acetate was found to elicit full sexual response in male false codling moths. This response in no way deviated from the response shown by male moths to an extract of ten virgin female moths.

(vi) cis-DODEC-7-EN-1-YL ACETATE (IX)

Similarly reflux of 2-(cis-dodec-7-en-1-yl-oxy) tetrahydropyran in glacial acetic acid with molar quantities of acetyl chloride followed by normal work-up procedures afforded cis-dodec-7-en-1-yl acetate in high yield. The presence of some trans isomer was shown by an infrared spectrum (slight absorption at 970cm^{-1}). Biological testing added confirmation as male moths showed positive sexual response. No trans isomer, however, could be shown by argentation chromatography.

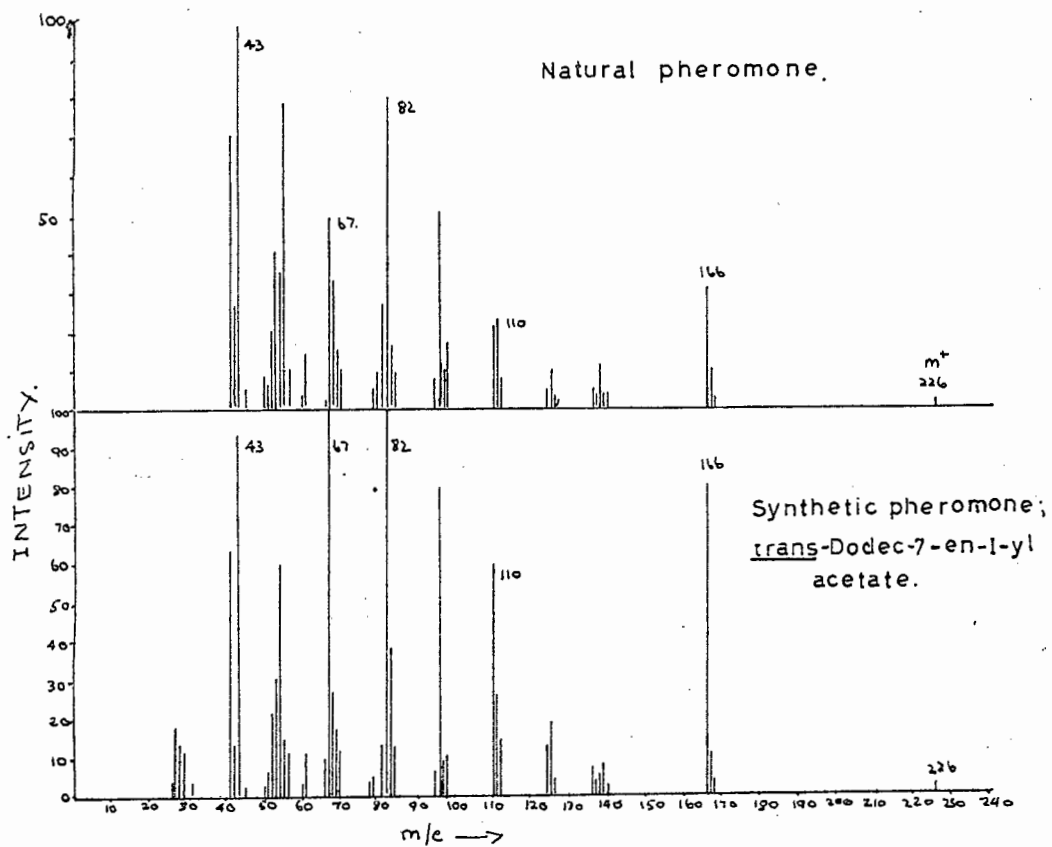
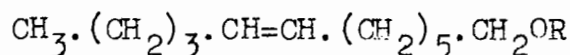


Fig. 3. Mass spectra of synthetic and natural C. leucotreta pheromone.

3. SYNTHESIS OF RELATED COMPOUNDS

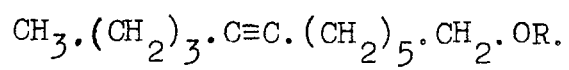
Two homologues of the false codling moth sex pheromone have been synthesised. These are trans-dodec-7-en-1-yl propionate 1(c) and trans-dodec-7-en-1-yl butyrate 1(d). The former was prepared through hydrolysis and acylation of the parent compound, 2-(dodec-7-en-1-yl-oxy) tetrahydropyran 1(a), by refluxing it in propionic acid with molar quantities of propionyl chloride. The latter was likewise prepared by refluxing 1(a), in dry butyric acid with molar quantities of butyryl chloride.



- 1(a). 7-trans, R = tetrahydropyran.
 1(b). 7-trans, R = .CO.CH₃ (Natural).
 1(c). 7-trans, R = .CO.CH₂.CH₃ (Propionate).
 1(d). 7-trans, R = .CO.(CH₂)₂.CH₃ (Butyrate).
 1(e). 7-cis, R = .CO.CH₃.
 1(f). 7-trans R = H.

Other compounds, analogous to the sex pheromone, have been prepared from the various reaction intermediates. These are:

1. Dodec-7-yn-1-yl acetate (IIb) which was prepared in one step by hydrolysis and acetylation of (IIa) by refluxing in glacial acetic acid and acetyl chloride.
2. Dodec-7-yn-1-ol (IIc), prepared by hydrolysis of (IIa) in 3N hydrochloric acid.
3. trans-Dodec-7-en-1-ol (If), prepared by hydrolysis of (Ia) in 3N hydrochloric acid.



IIa. R = tetrahydropyran.

IIb. R = $\cdot\text{CO}\cdot\text{CH}_3$

IIc. R = H.

Chapter IVBIOLOGICAL TESTING1. BIO-ASSAY OF trans-DODEC-7-EN-1-YL ACETATE AND RELATED COMPOUNDS

Investigation into the structure-activity relationships of the known sex pheromones has revealed the steric requirements for activity to be fairly rigid. This has been discussed earlier in section (1.4) and it will suffice to mention briefly that these steric requirements have been shown to be dependent on (i) unsaturation, either cis or trans or both, (ii) a hydroxyl function either free or acetylated (iii) propyl branching in one case and (iv) chain length.

Although the effect of increasing or decreasing the carbon chain of trans-dodec-7-en-1-yl acetate, in attractiveness for male false codling moths, has not been investigated it has been shown in this laboratory that sexual response in the male is dependent on unsaturation with a trans configuration and on the presence of an acetoxy group.

It is important at the outset to note that all analogues and homologous compounds assayed for activity have been investigated in the laboratory only, using the biological assay procedure discussed previously. It does not necessarily follow that the same results would have been obtained in field trapping experiments, as a field assay would be based on the attraction of males to the material source and not just the sex behavioural dance which is the criterion in laboratory tests. Jacobson and Jones, (1962), for example, found that although the trans form of gyplure was attractive to male gypsy

moths in laboratory tests, it remained unattractive to males in field tests even up to a concentration of 250 milligrams per trap.

No calculations on the rate of evaporation of any compounds from the filter paper substrate have been done. Such data would have made possible the testing of the various compounds at concentrations which allow a constant number of molecules to impinge on the moth antennae per second. Such a criterion is perhaps the only valid means of comparing the attractiveness of various compounds. It can be seen for example, that the rate of evaporation of trans-dodec-7-en-1-yl butyrate would be slower than that of the corresponding acetate due to it being four carbons longer. Evaporation of the trans alcohol may also be slower than the trans acetate due to the alcohol being more strongly held to the substrate through its hydroxyl group. All tests have, therefore, been done by applying the same quantity of compound to the filter paper discs and comparisons, where made, have been based on this initial concentration.

All compounds assayed for activity were purified before testing by gas-liquid chromatography on a Varian A700 Autoprep instrument and made up as stock solutions in petroleum ether. These stock solutions were freshly prepared before each test run and handled in such a way that no mutual contamination was possible. Prior to any compounds or extracts being tested, a petroleum blank response was recorded to establish whether the petroleum used

in the making of the stock solutions gave any response. A blank response was also recorded after the testing of any active extract or compound, to ensure no carry over of the active material through contamination of the buchner funnel. For comparison purposes, a synthetic pheromone response was recorded at the beginning and end of each test run.

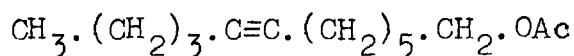
The number of moths responding in each cage was recorded in tabular form and the total response to each extract recorded as a percentage of the total number of moths tested. Two types of response were shown. Numbers designated (+) represent sexual response while others represent merely flight response. This flight response listed here consisted of occasional flitting to and fro and not the frantic flight mentioned earlier.

When tested at a concentration of $0.01\mu\text{g}$ per filter paper disc, the propionate and butyrate analogues of the false codling moth pheromone showed, respectively, a 14% and 12% flight response with no sexual activity (Table I). (The concentration level of $0.01\mu\text{g}$ per disc was chosen because it was found that at this level synthetic sex pheromone elicited the same percentage response as a crude extract of 10 virgin female moths). When tested again, however, at a concentration level of $0.1\mu\text{g}$ per disc, 10 times that previously tested, it was found that the propionate elicited a 31% sexual response while the butyrate gave only a 1% flight response (Table II). Thus, extension of the acetate group, by two carbons, produces a marked drop in

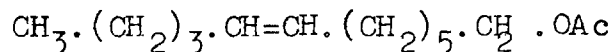
activity.

To investigate further the steric requirements surrounding the acetoxy group trans-dodec-7-en-1-ol was tested and found to give, at a concentration of 0.01 μ g per disc, a 5% flight response only. (Table II). It appears, then, that activity in male moths in the laboratory at a low concentration of compound is dependent on the presence of an acetoxy group.

In investigating the steric requirements surrounding unsaturation in the sex pheromone, compounds assayed for activity were dodec-7-yn-1-yl acetate, (I) and cis-dodec-7-en-1-yl acetate, (II).



I



II

The former at a concentration of 0.1 μ g disc was found to elicit neither sexual nor flight response (Table III), whilst the latter, also at a concentration of 0.1 μ g. disc, gave a 28% sexual response (Table II). However, purification of the cis isomer by Thin Layer Chromatography (Silica gel, silver nitrate), and retesting showed only a 6% flight response (Table III). Although dodecan-1-ol acetate has not been tested, hydrogenation of the sex pheromone which gives this compound,

has been shown to inactivate it (Read 1968). Sexual activity in male moths is also, therefore, dependent on the presence of unsaturation having a trans configuration. The effect of a shift in position of this unsaturation on response in male moths has not been investigated. Likewise the effect of an increase or a decrease in the number of methylene groups, on either side of the double bond, on male response has not been determined.

TABLE I

Numbers of male moths responding when exposed to different compounds in a cage of ten moths. The figures marked (+) indicate sexual response; others indicate short flight. Tested in order of columns with 10 mins. between tests.

Cage No.	BL	A	B	C	A	10 Females
I	1	9+	3	1	8+	7+
II	0	7+	3	1	3+	5+
III	0	8+	1	3	8+	7+
IV	1	7+	0	1	5+	7+
V	0	5+	1	2	8+	5+
VI	0	9+	0	0	9+	6+
VII	0	8+	2	1	5+	8+
Total	3	53+	12	9	46+	45+
%	4	75	14	12	65	64

Code: A = trans-Dodec-7-en-1-ol acetate (0.01 μ g./disc).
 B = trans-Dodec-7-en-1-ol propionate (0.01 μ g./disc).
 C = trans-Dodec-7-en-1-ol butyrate (0.01 μ g./disc).

BL = Petroleum ether.

TABLE II

Numbers of male moths responding when exposed to different compounds in a cage of 10 moths. The figures marked (+) indicate sexual response; others indicate short flight. Tested in order of columns with 10 mins. between tests.

Cage No.	BL	A	BL	B	C	BL	D	E	A
I	0	7+	0	2	4+	2	2+	0	7+
II	0	7+	1	0	3+	2	4+	0	7+
III	0	6+	2	2	2+	0	2+	0	6+
IV	0	6+	1	0	3+	1	5+	1	6+
V	0	4+	0	1	1+	0	5+	0	4+
VI	0	5+	1	1	3+	1	5+	0	5+
VII	0	5+	1	0	4+	0	2+	0	6+
VIII	0	4+	0	0	2+	1	3+	0	4+
Total	0	44+	6	6	22+	7	28+	1	45+
%	0	56	5	5	28	6	31	1	56

Code: A = trans-Dodec-7-en-1-ol acetate (0.01 μ g./disc).
 B = trans-Dodec-7-en-1-ol (0.01 μ g./disc).
 C = cis-Dodec-7-en-1-ol acetate (0.1 μ g./disc).
 D = trans-Dodec-7-en-1-ol propionate (0.1 μ g./disc).
 E = trans-Dodec-7-en-1-ol butyrate (0.1 μ g./disc).

BL = Petroleum ether.

TABLE III

Numbers of male moths responding to different compounds in a cage of 10 moths. Figures marked (+) indicate sexual response; others indicate short flight. Tested in order of columns with 10 mins. between tests.

Cage No.	BL	A	BL	B	C	A
I	2	8+	1	1	0	8+
II	1	5+	1	0	0	8+
III	1	7+	1	0	0	7+
IV	1	5+	1	0	0	5+
V	0	4+	0	0	0	5+
VI	2	5+	0	0	0	6+
VII	0	9+	1	2	0	8+
VIII	0	6+	1	3	0	6+
Total	7+	49+	6	6	0	53+
%	8	52	7	7	0	56

Code: A = trans-Dodec-7-en-1-ol acetate (0.01 μ g./disc).

B = cis-Dodec-7-en-1-ol acetate (0.1 μ g./disc)

C = Dodec-7-yn-1-ol acetate. (0.1 μ g./disc).

BL = Petroleum ether.

2. BIOLOGICAL MASKING OF SEX PHEROMONE ACTIVITY

The biological masking of a sex pheromone by its geometrical isomer has been reported on more than one occasion. Berger, (1966), has shown that sexual activity in male cabbage looper moths is masked when the female sex pheromone, cis-dodec-7-en-1-yl acetate is mixed in equal proportions with the trans isomer. Jacobson, (1963), has shown that gyplure, a synthetic sex attractant for male gypsy moths, is completely inactivated by admixture with 20 percent of its trans isomer. Similarly, Jacobson, (1969), has shown that the natural sex pheromone, "propylure", is completely inactivated by admixture with 15 percent of its trans isomer. This phenomenon accounts for the biological inactivity of synthetic propylure prepared by Eiter et. al., (1967), which contained a mixture of the cis and trans isomers.

The masking effect of the cis isomer on sexual response, in male false codling moths, to the female sex pheromone, trans-dodec-7-en-1-yl acetate, has been investigated and found to be non-existent, when admixed at equal concentrations with the synthetic pheromone (Table IV). The possible masking of activity by the cis isomer at higher concentrations has not been investigated.

The possible masking by trans-dodec-7-en-1-ol and dodec-7-yn-1-yl acetate has also been found to be non-existent when these compounds were tested as equal mixtures with the synthetic pheromone (Table IV).

This masking phenomenon as a factor in sex pheromone inhibition or inactivation by admixed contaminants has been noted in various other species. Jacobson lists these as introduced pine sawfly (Diprion similis), corn earworm (Heliothis zea, Boddie), tobacco budworm (H virescens), American cockroach (Periplaneta americana), cynthia moth (Samia cynthia) and omnivorous leaf roller (Platyrota stultana). The mechanism through which masking occurs has not been established but it is presumed to act peripherally by blocking the receptor site, for the pheromone, in the sensilla of the antennae or at some higher nervous centre.

A masking phenomenon has been noticed in this laboratory by Read, (1968), and by the author. A mixed extract of equal numbers of whole male and female false codling moths when bio-assayed evokes no response in male moths. Column chromatography of this inactive extract, however, yields an active fraction. Re-combination of all the eluted fractions gives a drop in attractiveness but not total inactivity. This phenomenon has not been investigated further and is a field which should be tackled in future work.

TABLE IV

Numbers of male moths responding to equal mixtures of three compounds with synthetic pheromone. Numbers marked (+) represent sexual response; others represent short flight only.

Cage No.	BL	A	A+B	A+B	A+B	A
I	0	6+	2+	5+	4+	2+
II	0	5+	8+	7+	7+	8+
III	0	5+	5+	4+	3+	5+
IV	0	2+	2+	4+	2+	5+
V	0	4+	4+	7+	5+	4+
VI	0	3+	6+	6+	5+	5+
VII	1	7+	5+	5+	3+	5+
Total	1	32+	32+	38+	29+	34+
%	1	46	46	54	41	48

Code: A = trans-Dodec-7-en-1-yl acetate. 0.01 μ g./disc.

B = cis-Dodec-7-en-1-yl acetate. 0.01 μ g./disc.

C = trans-Dodec-7-en-1-ol. 0.01 μ g./disc.

D = Dodec-7-yn-1-yl acetate. 0.01 μ g./disc.

BL = Petroleum ether.

EXPERIMENTAL

1-Hexyne: A two litre, four necked flask was fitted with an efficient stirrer, a gas inlet tube reaching the bottom of the flask, a 12" length of flexible iron wire capable of being dipped into the flask or withdrawn as required and an outlet tube for the escaping ammonia. In addition, the flask was insulated by wrapping around it a layer of cottonwool (note 1).

The flask was transferred to the fume cupboard and charged with liquid ammonia (1.5 l.) (note 2). The stirrer was started and purified acetylene, (note 3), bubbled through (approximately 5 bubbles per second) for five minutes to saturate the liquid ammonia. Sodium (43.59 g., 1.89 mole), to be added, was divided into approximately 4 g. portions. One of the portions was attached to the iron wire and slowly lowered beneath the surface of the liquid ammonia. The flow of acetylene was increased to approximately 10 bubbles per second. The dissolving sodium formed a continuous blue streak which disappeared with the formation of sodium acetylide. Addition of the sodium was done at a rate such that the whole solution did not turn blue. Occasionally, however, this occurred as small pieces of the main portion broke away. At such times the wire was withdrawn and the sodium suspended above the surface until the blue colour was discharged (note 4). After complete addition and conversion of the sodium into sodium acetylide the acetylene was shut off and the bubbler replaced by a drying tube containing potassium hydroxide pellets (note 5). The outlet tube was

connected to a length of plastic tubing enabling the escaping ammonia to be directed through an ether/water trap. This ensured the retention of 1-hexyne which would otherwise be lost due to entrainment.

Redistilled n-bromobutane (259 g., 1.58 mole) was added dropwise to the flask over a period of 40 minutes. Stirring was continued for a further 2 hours whereupon saturated ammonium chloride (200 ml.) was cautiously added. Water (800 ml.) was added and the contents of the flask transferred to a separating funnel and the organic layer removed. The aqueous layer was extracted with ether (2x100 ml.) The combined ether extracts, organic layer and ether layer from the ether/water trap were washed once with water, once with 6N hydrochloric acid (note 6), twice with 5% aqueous sodium bicarbonate solution (2x100 ml.) and dried over anhydrous sodium sulphate. Fractional distillation through a Vigreux column gave, after a small forerun, 1-hexyne (107.5 g., b.p. 83-84^o) in 71% yield. Infrared spectra showed peaks at 3570 cm^{-1} (terminal $-\text{C}\equiv\text{C}-$), 2950 ($-\text{CH}$) and 2120 ($-\text{C}=\text{C}-\text{H}$).

Found: C, 87.6., H, 12.2.

Calcd. for C_6H_{10} : C, 87.7., H, 12.3%.

NOTES:

1. Insulation is not essential as a layer of ice soon forms and the flask is effectively insulated.
2. A protective hood should also be worn to avoid exposure to the ammonia.
3. The acetylene was purified by passage through a cold trap (acetone/dry ice), simple mercury

- trap to prevent suck-back, two bubblers of conc. sulphuric acid and finally through a soda lime column ($\text{CaCl}_2 + 10\% \text{NaOH} + 12\% \text{water}$).
4. Removal of some wadding allowed a look into the flask. If no wadding is used a portion of the ice layer can be removed by pouring ethanol over the outside. It was found, however, that internal splashing covered the inside of the flask, hindering vision to a large extent.
 5. This is not absolutely necessary as the escaping ammonia keeps out appreciable amounts of moisture.
 6. The aqueous layer must be tested and if found to be basic then a further washing with 6N hydrochloric acid is essential.

6-Bromo-1-hexanol: In a liquid-liquid extraction apparatus, immersed in an oil bath thermostatically kept at 80° , was placed hexane-1, 6-diol (90 g., 0.76 mole) and aqueous 48% hydrobromic acid (128g., 0.8 mole). Petroleum ether, b.p. $90 - 100^{\circ}$, was allowed to percolate through the mixture for 24 hours, (note 1), whereupon it was removed and concentrated by rotary evaporation to give a reddish brown liquid.

Distillation under reduced pressure (1mm.) gave, after a small forerun, 1,6-dibromohexane (10 g., b.p. 65°) and 6-bromo-1-hexanol (95 g., b.p. $80-81^{\circ}$), in 69% yield.

Infrared spectra showed peaks at 3400 cm^{-1} (-OH), 2950 cm^{-1} (CH), 1050 cm^{-1} (Primary hydroxyl) and 730 cm^{-1} ($-(\text{CH}_2)_4-$).

Found: C, 39.7., H, 7.3., Br, 41.9., 43.6.
Calcd. for $\text{C}_6\text{H}_{13}\text{BrO}$: C, 39.8., H, 7.2., Br, 44.4%.

NOTES:

1. Further experiments have shown that more effective percolation can be achieved by attaching a gas dispersion disc to the end of the extraction stem. This reduces extraction time considerably.

2-(6-bromohexyl-oxy) tetrahydropyran: In a 500 ml., two-necked, round-bottomed flask, fitted with a dropping funnel and a stirrer, was placed 6-bromo-1-hexanol (200 g., 1.1 mole) and concentrated hydrochloric acid (0.5 ml.). To this was added, dropwise with stirring, 2,3-dihydropyran (note 1) (110 g., 1.3 mole) over a period of 1 hour. The flask was immersed in an ice bath and at no stage during the addition was the temperature allowed to exceed 30°. Stirring was continued for a further 30 minutes, whereupon sodium bicarbonate (0.5 g.) was added and stirring continued for a further 90 minutes.

Filtration and fractional distillation of the mixture at reduced pressure, (1mm.) gave, 6-bromo-1-hexanol (67 g., b.p. 80-81°), and, after a small forerun, 2-(6-bromohexyl-oxy) tetrahydropyran (190 g., b.p. 102-105°), in 71% yield.

Found: C, 50.7., H, 8.2., Br, 29.8., 27.6., 28.8.
Calcd. for C₁₁H₂₁BrO: C, 49.8., H, 7.9., Br, 30.2%.

NOTES:

1. Dihydropyran can be prepared by dehydration-rearrangement of tetrahydrofurfuryl alcohol over alumina (Sawyer and Andrus, 1955).

2-(Dodec-7-yn-1-yl-oxy) tetrahydropyran: Lithium metal (6.2 g., 0.88 mole) was added, with stirring, in small portions (0.25 g.) to liquid ammonia (800 ml.) in a two-necked, two litre, round-bottomed flask, equipped with a dropping funnel and a stirrer. After addition of the first two portions, powdered ferric nitrate (0.2 g.) was added. After 20 minutes the conversion of lithium into lithamide was judged to be complete due to the disappearance of the blue colour of dissolved lithium. 1-Hexyne (70 g., 0.88 mole) was then added dropwise over a period of one hour, stirring continued for a further one hour and the ammonia lost during the preparation replenished. 2-(6-Bromohexyl-oxy) tetrahydropyran (200 g., 0.75 mole) was added dropwise over a period of one hour. The solution was stirred for 3.5 hours and set aside overnight when the ammonia evaporated. Water (800 ml.) was cautiously added and the whole extracted three times with light petroleum. The combined extracts were washed with water until the washings were neutral, dried over sodium sulphate and the solvent removed. Distillation at reduced pressure (1mm.) gave 2-(6-bromohexyl-oxy) tetrahydropyran (51 g.) and 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran (142 g. b.p. 120 - 125°) in 61% yield. Infrared spectra showed strong absorption at 2950 cm^{-1} (-CH) and 1200-1000 cm^{-1} (tetrahydropyran).

Found: C, 76.8., H, 11.3.

Calcd. for $\text{C}_{17}\text{H}_{30}\text{O}_2$: C, 76.8., H, 11.2%.

2-(trans-Dodec-7-en-1-yl-oxy) tetrahydropyran:

To liquid ammonia (500 ml.) in a cylindrical vessel, was added lithium (5.6 g., 0.8 mole) with stirring over a period of 5 minutes. 2-(Dodec-7-yn-1-yl-oxy) tetrahydropyran (100 g. 0.377 mole) was immediately added to the lithium/liquid ammonia, stirred, and the whole sealed in a metal container, set to blow at 200 p.s.i. The contents of the reaction vessel were allowed to attain room temperature and then set aside for 24 hours. After slow release of the internal pressure the apparatus was dismantled, water (500 ml.) cautiously added to the reaction mixture, and the whole extracted three times with light petroleum. The combined extracts (700 ml.) were washed with water until the washings were neutral and the solvent removed. Thin layer chromatography on silica gel, impregnated with 25% silver nitrate, showed approximately 70% reduction to the trans olefin. A further two reductions gave complete formation of the olefin.

The chromatograms were developed in cyclohexane/ether (9/1), (note 1), and visualised by spraying with 1% dichlorofluorescein in ethanol. Subjection to ultraviolet radiation yielded green spots on a purple background. The infrared spectrum showed strong absorption at 970 cm^{-1} (trans olefin).

Found: C, 75.9., H, 12.1.

Calcd. for $\text{C}_{17}\text{H}_{32}\text{O}_2$: C, 76.1., H, 12.0%.

NOTES:

1. Cyclohexane, pure enough for spectroscopic purposes, and peroxide free ether were used as they gave minimal blackening of the plate (Read, 1968).

trans-Dodec-7-en-1-yl acetate: 2-(trans-Dodec-7-en-1-yl-oxy) tetrahydropyran (90 g., 0.339 mole) and acetyl chloride (32 g., 0.41 mole) were refluxed in glacial acetic acid (200 ml.) for 8 hours in a 500 ml. flask fitted with a condenser closed by a calcium chloride drying tube. The contents were poured onto ice, diluted to 500 ml. with saturated sodium chloride solution and extracted three times with light petroleum. The combined extracts were washed twice with 5% aqueous sodium bicarbonate, three times with water and dried over anhydrous sodium sulphate.

Removal of the solvent and distillation at reduced pressure, (1mm.), gave, after a small forerun, trans-dodec-7-en-1-yl acetate, (81 g., b.p. 105 - 106°) in 90% yield. The infrared spectrum showed peaks at 2930 cm^{-1} (CH), 1730 and 1230 cm^{-1} (primary acetate) and 970 cm^{-1} (trans olefin).

Found: C, 74.3., H, 11.4.

Calcd. for $\text{C}_{14}\text{H}_{26}\text{O}_2$: C, 74.3., H, 11.5%

trans-Dodec-7-en-1-yl propionate:

2-(trans-Dodec-7-en-1-yl-oxy) tetrahydropyran (2.0 g., 0.008 mole) and propionyl chloride (0.8 g., 0.009 mole) were refluxed for five hours in dry propionic acid (15 ml.). After refluxing, the whole was poured onto ice, diluted to 75 ml. with water and extracted three times with petroleum ether (3x20 ml.). The combined ether extracts were washed three times with 5% aqueous sodium bicarbonate solution and twice with water and dried over anhydrous sodium sulphate. Removal of the solvent and distillation under reduced pressure, (2mm.), gave, after a small forerun, trans-dodec-7-en-1-yl propionate (1.05 g., b.p. 113 - 115°). The infrared spectrum showed peaks at 2950 cm^{-1} (CH).

1200 cm^{-1} (propionate) and 970 cm^{-1} (trans olefin).

A sample (0.5 ml.) was purified by Gas-liquid chromatography (Varian A700 Autoprep, column, Silicone S.E. 30 on Chromosorb 40-60) and used in attractancy tests.

Found: C, 75.0., H, 11.8.

Calcd. for $\text{C}_{15}\text{H}_{28}\text{O}_2$: C, 74.9., H, 11.7%.

trans-Dodec-7-en-1-yl butyrate: 2-(trans-Dodec-7-yn-1-yl-oxy) tetrahydropyran (2.0 g. 0.008 mole) and butyryl chloride (0.95 g., 0.009 mole) were refluxed for five hours in dry butyric acid (15 ml.). After refluxing the whole was poured onto ice, diluted to 75 ml. and extracted three times with petroleum ether (3x20 ml.). The combined extracts were washed three times with 5% aqueous sodium bicarbonate solution, twice with water and dried over anhydrous sodium sulphate. Removal of the solvent and distillation under reduced pressure (1mm.) gave, after a small forerun, trans-dodec-7-en-1-yl butyrate (0.9 g., b.p. 117 - 120°). Infrared spectra showed absorption at 2950 cm^{-1} (CH), 1190 cm^{-1} (butyrate) and 970 cm^{-1} (trans olefin). A sample was purified by Gas-liquid chromatography (Varian A700 Autoprep, column, silicone S.E. 30 on Chromosorb 40 - 60) and used in attractancy tests.

Found: C, 75.5., H, 11.9.

Calcd. for $\text{C}_{16}\text{H}_{30}\text{O}_2$: C, 75.5., H, 11.8%.

cis-Dodec-7-en-1-yl acetate: A hydrogenation flask was charged with 2-(dodec-7-yn-1-yl-oxy) tetrahydropyran (8.0 g., 0.03 mole), Lindlar catalyst, (0.3 g.) (note 1), and quinoline (1 ml.). The flask was attached to a hydrogenation apparatus and flushed out with hydrogen. Hydrogenation was effected

under slight positive pressure with agitation by a shaker. When the uptake of hydrogen ceased, the flask was removed and the catalyst recovered by filtration. The filtrate was freed of quinoline by washing three times with dilute hydrochloric acid and dried over anhydrous sodium sulphate. Thin layer chromatography on silica gel, impregnated with 25% silver nitrate showed only trace amounts of unreduced material and no trans compound.

2-(cis-Dodec-7-en-1-yl-oxy) tetrahydropyran (7.5 g., 0.028 mole) and acetyl chloride (2.56 g., 0.032 mole) were refluxed for five hours in glacial acetic acid (20 ml.). The contents were poured onto ice and diluted to 300 ml. with cold water. The organic layer was removed and the aqueous layer extracted three times with petroleum ether. The combined organic layer and petroleum extracts were washed twice with 5% aqueous sodium bicarbonate solution, once with water and dried over sodium sulphate. Removal of the solvent and distillation under reduced pressure, (1mm.), gave, after a small forerun, cis-dodec-7-en-1-yl acetate (5.1 g., 108 - 109°) in 81% yield. The infrared spectrum showed absorption at 2950 cm^{-1} (CH), 1730 and 1230 cm^{-1} (primary acetate), 780 cm^{-1} (cis olefin).

Found: C, 74.0., H, 11.1.

Calcd. for $\text{C}_{14}\text{H}_{26}\text{O}$: C, 74.2., H, 11.5%.

NOTES:

1. The catalyst used was prepared according to a slight modification of Lindlar's original procedure, (Fieser and Fieser, 1967).

Dodec-7-yn-1-ol: 2-(Dodec-7-yn-1-yl-oxy) tetrahydropyran (2.0 g., 0.008 mole) was refluxed for five hours in 3N hydrochloric acid (20 ml.) and ethanol (5 ml.). Water (100 ml.) was added and the whole extracted three times with petroleum ether. The combined ether extracts were washed twice with 10% aqueous sodium bicarbonate solution, once with water (20 ml.) and dried over anhydrous sodium sulphate. Removal of the solvent and distillation under reduced pressure (2mm.) gave dodec-7-yn-1-ol (1.01 g., b.p. 135 - 140^o). The infrared spectrum showed absorption at 3350 and 1150 cm.⁻¹ (OH) and 2950 cm.⁻¹ (CH). A small sample (0.5 ml.) was purified by Gas-liquid chromatography (Varian A700 Autoprep, Column Silicone S.E. 30 on Chromosorb 40 - 60) and used in attractancy tests.

Found: C, 79.7., H, 12.4

Calcd. for C₁₂H₂₂O: C, 79.1., H, 12.2%.

Dodec-7-yn-1-yl acetate: 2-(Dodec-7-yn-1-yl-oxy) tetrahydropyran. (2.0 g., 0.008 mole) and acetyl chloride (0.7 g., 0.009 mole) were refluxed in glacial acetic acid (15 ml.) for 5 hours. Water (80 ml.) was added to the mixture and the whole extracted three times with petroleum ether. The combined ether extracts were washed twice with water and dried over anhydrous sodium sulphate. Removal of the solvent and distillation under reduced pressure (1mm.) gave dodec-7-yn-1-yl acetate (1.21 g., b.p. 88 - 90^o) in 80% yield. The infrared spectrum showed absorption at 2930 cm.⁻¹ (CH), 1730 and 1230 cm.⁻¹ (primary acetate). A small sample (0.5 ml.) was purified by Gas-liquid chromatography (Varian A700 Autoprep, column Silicone S.E. 30 on Chromosorb 40 - 60) and used in attractancy

tests.

Found: C, 74.7., H, 10.7.

Calcd. for $C_{12}H_{24}O$: C, 74.7., H, 10.7%.

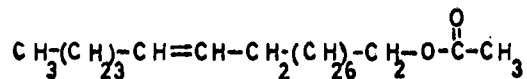
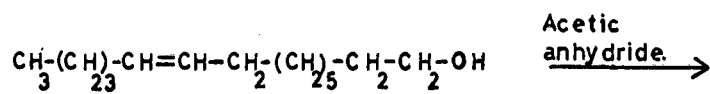
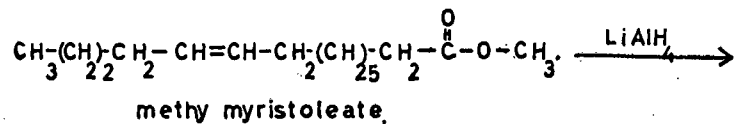
A P P E N D I X

SYNTHESES OF THE SEX PHEROMONES IN THE ORDER LEPIDOPTERA

CHART 3.

Synthesis of the Fall Armyworm pheromone.

cis-9-TETRADECEN-1-OL, ACETATE.

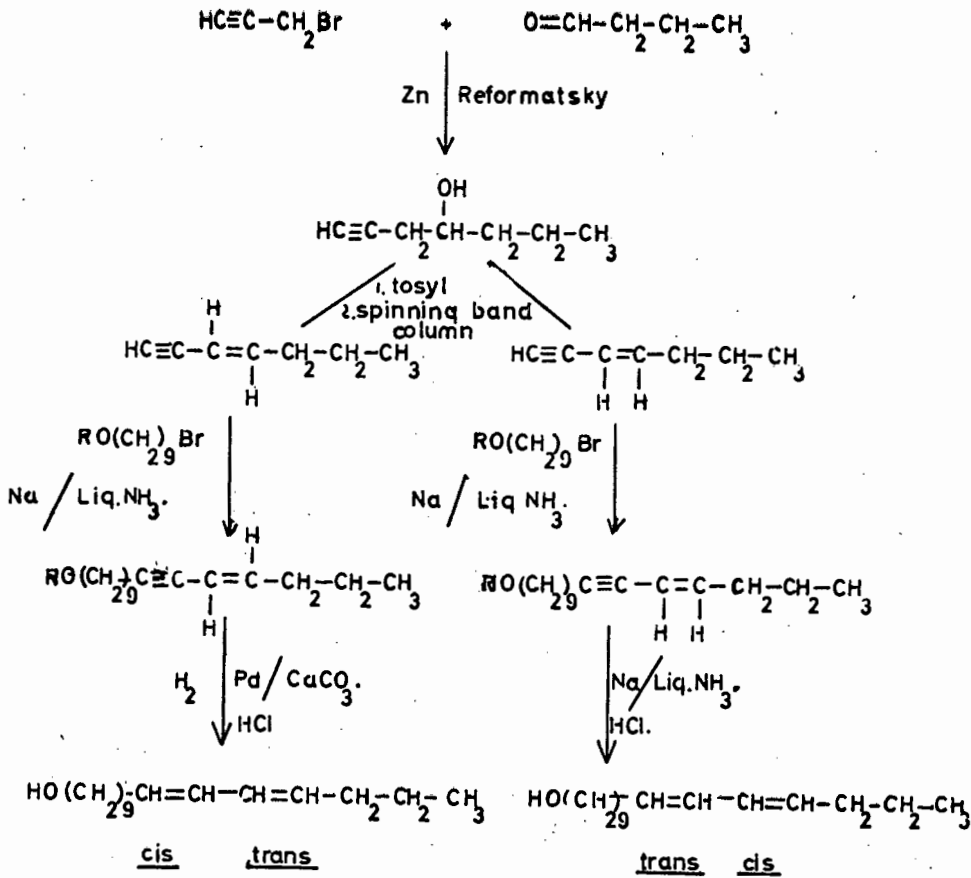


REF. Sekul and Sparks, (1967).

CHART 4b.

Synthesis of Bombykol, pheromone of the Silkworm Moth

Trans-10-12-HEXADECADIEN-1-OL



Ref: Butenandt and Hecker, (1961).

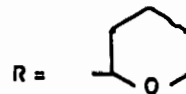
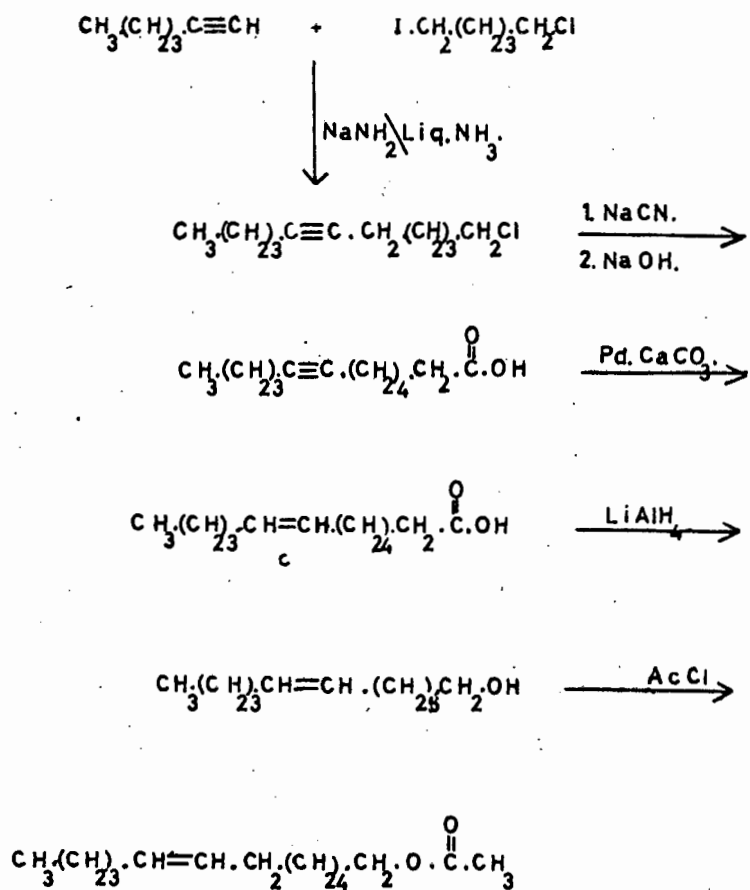


CHART 5.

Synthesis of the Cabbage Looper sex pheromone.



REF. Berger (1966).

REFERENCES:

- Adlung, K.G. (1964) *Angew. Entomology* 54, 304.
- Ahmad, K. and Strong, F.M. (1948) *J. Am. Chem. Soc.* 70, 1699.
- Bergelson, L.D. and Shemyakin, M.M. (1964) *Angew. Chem. Intern. Ed.* 3, 250.
- Berger, R.S. (1966) *Ann. Entomol. Soc. Am.* 59, 767.
- Berger, R.S. and Canerday, T.D. (1968) *J. Econ. Entomol.* 61, 452.
- Beroza, M. (1960) *Agric. Chem.* 15(7), 37.
- Boeckh, J., Kaissling, K.E. and Schneider, D. (1965) *Cold Spring Harbour Symposia Quant. Biol.* 30, 263.
- Borguel, B. (1925) *Ann. chim. (10)*, 3, 222, 380.
- Bowman, R.E. and Fordham, W.D. (1952) *J. Chem. Soc.* 3945.
- Butenandt, A., Beckmann, R., Stamm, D. and Hecker, E. (1959) *A. Naturforsch.* 14b, 283.
- Butenandt, A. and Hecker, E. (1961) *Angew. Chem.* 73, 349.
- Butenandt, A., Hecker, E., Hop, M. and Koch, W. (1962) *Ann. Chemie.* 685, 39.
- Butenandt, A., Hecker, E. and Zayed, S.M.A.D. (1963) *A. Physiol. Chem.* 333, 114.
- Campbell, K. and Campbell, B.K. (1963) *Org. Syn. Coll. vol. IV*, 117.
- Campbell, K.N. and Campbell, B.K. (1950) *Org. Syn.* 30, 15.
- Campbell, K.N. and Eby, L.T. (1941) *J. Am. Chem. Soc.* 63, 216, 2683.
- Corey, E.J. and Kwiatkowski, G.T. (1968) *J. Am. Chem. Soc.* (90:24), 6816.
- Crombie, L. and Harper, S.H. (1950) *J. Chem. Soc.* 1707.
- Dear, R.E.A. and Pattison, F.L.M. (1963) *J. Am. Chem. Soc.* 35, 622.
- Degering, E.F. and Boatright, L.G. (1950) *J. Am. Chem. Soc.* 72, 5138.

- Elsner, B.B. and Paul, P. (1951) J. Chem. Soc. 893.
- Emmons, W.D. and Wadsworth, W.S. (1961) J. Am. Chem. Soc. 83, 1733.
- Fieser, L.F. and Fieser, M. (1967) "Reagents for Organic Syntheses", John Wiley and Sons, Inc., New York. 1262.
- Fieser, L.F. and Fieser, M. (1967) *ibid.* 566.
- Fiter, K., Truscheit, E. and Boness (1967) Justus Liebigs Ann. Chem. 709, 29.
- Green, N., Jacobson, M., Henneberry, T. and Kishaba, A. (1967) J. Med. Chem. 10, 533.
- Grignard, Lapyre and Faki. (1928) Compt. rend. 187, 519.
- Henbest, H.B., Jones, E.R. and Walls, M.S. (1949) J. Chem. Soc. 2696.
- Henne, A.L. and Greenlee, K.W. (1943) J. Am. Chem. Soc. 65, 2020.
- House, H.O. (1965) "Modern Synthetic Reactions," Benjamin, Inc., New York 17.
- Jacobs, T.L. (1949) Organic Reactions 5, 1.
- Jacobson, M., Beroza and Jones, W.A. (1960) Science 132, 1011.
- Jacobson, M. and Jones, W.A. (1962) J. Org. Chem. 27, 2523.
- Jacobson, M. and Warthen, D. (1967) J. Med. Chem. 10, 1190.
- Jacobson, M. (1963) Advan. Chem. 41, 1.
- Jacobson, M. (1969) Science 163, 190.
- Jones, W.A., Jacobson, M. and Martin, D.F. (1966) Science 152, 1516.
- Karlson, P. and Luscher, N. (1959) Nature 183, 55.
- Lindlar and Dubuis, (1952) Helv. Chim. Acta. 35, 446.
- Linstead, M. (1934) J. Am. Chem. Soc. 935.
- Lyon and Rutledge, (1956) C.A., 50 4193. U.S. Pat. 2724,008.
- Meinwold, J. and Meinwold, Y.C. (1966) J. Am. Chem. Soc. 88, 1305.
- Morris, L.J., Wharry, D.M. and Hammond, E.W. (1967) J. of Chrom. 31, 69.

- "
Noller, R.C. and Adams, R. (1926) J. Am. Chem. Soc.
48, 1074.
- Novak, V.J.A. (1966) "Insect hormones" 3rd Ed. Methuen,
London.
- Ouye, M.T. and Butt, B.A. (1962) J. Econ. Entomol.
55, 419.
- Parham, W.E. and DeLaitch, D.M. (1954) J. Am. Chem.
Soc. 76, 4962.
- Pattendem, G. (1968) J. Chem. Soc. (C), 2385.
- Read, J.S. (1968) Thesis is, "Insect Sex Pheromones:
The Sex Pheromone of *Argyroplote leucotreta*, Meyr."
- Read, J.S. Hewitt, P. and Warren F.L. (1968) Chemical
Communications, 792.
- Rienschneider, R. and Kasang, G. (1963) Z. Naturforsch
18b, 646.
- Robins, B.K., Godefroi, E.F., Taylor, E.C., Lewis, R.L.
and Jackson, A (1961) J. Am. Chem. Soc. 83, 2574.
- Roelofs, W.L. and Arn. H. (1968) Nature 219, 513.
- Roelofs, W.L. and Feng, K.C. (1967) Ann. Entomol. Soc.
Am. 60, 1199.
- Rutledge, R. (1963) J. Org. Chem. 24, 840.
- Sawyer, R.L. and Andrus, D.W. (1955) Org. Syn. Coll.
Vol. 3, 276.
- Schneider, D. and Hecker, E. (1956) Naturforsch 11b, 121.
- Schneider, D. (1963) "Olfaction and Taste" Y. Zotterman.
Ed. Macmillan, New York.
- Schneider, D. (1969) Science 164, 1174.
- Schneider, D. (1969) Science 163, 1031.
- Sekul, A.A. and Cox, H.C. (1965) BioScience 15, 670.
- Sekul, A.A. and Sparks, A.N. (1967) J. Econ. Entomol.
60, 1270.
- Shorey, H.H. and Gaston, L.K. (1964) J. Econ. Entomol.
57, 252.
- Shorey, H.H., Gaston, L.K. and Jefferson, R.A. (1968)
Adv. in Pest Control. Res. 56.
- Shorey, H.H. and Gaston, L.K. Unpublished data. Cited
by Shorey et. al. (1968).

Truscheit, E., Eiter, K., Butenandt, A. and Hecker, E.
Ger. Patent 1,138,037 (Oct. 18, 1962).

van Risseghem, (1926) cited by Campbell and Campbell(1963).

Vaughn, T.H., Vogt, R.R. and Nieuwland, J.A. (1934)
J. Am. Chem. Soc. 56, 2120.