

INSECT SEX ATTRACTANTS

K. EITER

*Bayer AG ZB-ZF-Wissenschaftliches Hauptlaboratorium,
509-Leverkusen, GFR*

ABSTRACT

The sex attractants described in the literature for certain pests have been incorrectly classified. The corresponding synthetic products, inasmuch as they show any biological activity, are therefore not identical with the genuine pheromones and, in comparison with, for example, attractant units of 'Bombycol', represent at most pheromone mimics. All the inhibition-, masking- and synergism-effects so far described with such artefacts, cannot therefore be regarded as proof of any such biological effects.

For those sex attractants which have recently been isolated from pink bollworm moth, (*Pectinophora gossypiella* S.), and which have also in fact been found in *Sitotroga cerealella*, such as 7Z, 11Z-hexadecadienyl acetate, we were able to develop a new synthesis of three geometrical isomers. By investigating their gas chromatographic behaviour, we have demonstrated the difficulty of separating such isomers (in contrast to previously published results), thus illustrating the problems involved in determining hundreds or thousands of distinct sex attractant molecules in a mixture.

The sex attractant of the silk moth *Bombyx mori* was discovered by Butenandt *et al.*¹ in 1959. The total synthesis of Bombycol (10E, 12Z-hexadecadien-1-ol) was first achieved in our laboratory² and established the complete constitution unambiguously. Since then we have seen that this new class of natural products (designated by P. Karlson as pheromones) is made up of substances with a clearly reproducible biological activity in concentrations which had never been observed prior to the discovery, isolation and total synthesis of Bombycol.

The respective values (*Figure 1*) of 10^{-12} and 10^{-13} $\mu\text{g/ml}$ (say, 10^{-18} and 10^{-19} g/ml) given by Butenandt *et al.*¹ and by Truscheit and Eiter² as an attractant unit (AU) for Bombycol were derived from a test which, according to Butenandt, allowed the quantitative determination of the attractant unit in the laboratory. In this procedure an accurately weighed sample of 10E, 12Z-hexadecadien-1-ol, or one of its three theoretically possible geometrical isomers (10Z, 12E-; 10Z,12Z-; and 10E,12E-) which were synthesized simultaneously, dissolved in low-boiling light petroleum ether, and a series of dilutions prepared. When a glass rod, after being dipped into the petroleum ether solution, was then held 5 cm away from the antennae of 150 male silk moths, each resting individually on the bottom of a covered beaker, 75 insects (50 per cent) had to show definite buzzing reactions. This test method, admittedly far from satisfactory from the scientific viewpoint, did, however,



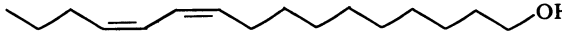
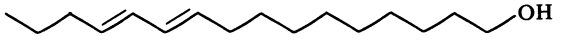
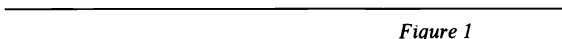
10,12-Hexadecadien-1-ol		A. Butenandt H. Hecker M. Hopp W. Koch	E. Truscheit K. Eiter
		LE(AU) $\mu\text{g/ml}$	
(1) Natural Bombycol		10^{-10}	
(2) 10-E, 12-Z		10^{-12}	10^{-13} Mol. wt 238.42 252 molecules
(3) 10-Z, 12-E		10^{-3}	10^{-5}
(4) 10-Z, 12-Z		1	10^{-1}
(5) 10-E, 12-E		10	10^2

Figure 1

have one decisive advantage; with exact formulation of the dilution series perfectly reproducible results were possible.

As shown in the table for the four 10,12-hexadecadien-1-ol isomers (Figure 1), AU values could in this way be obtained for the individual geometrical isomers which showed the 10E,12E-isomer to be the least active compound.

Even discounting the numerous sources of possible error which are to be expected with a numerical activity of approximately 250 molecules/ml, the information contained in this table is still not conclusive unless isomers can be determined in minute amounts in the main constituent by independent analysis. Since these biological activities, as already mentioned, can represent concentrations of a few hundred molecules, this situation calls for methods of analysis with a sensitivity hitherto only seen in nuclear chemistry (tracer methods).

Neither gas chromatography, gas-liquid chromatography nor ^{13}C -spectroscopy permit an exact and conclusive determination either of homogeneity or of the presence of trace isomers. This inevitably raises the question whether the activities measured with isomers of Bombycol actually originate from the isomers themselves or from molecules formed by spontaneous rearrangement, that is to say whether a simulation of biological activities has occurred.

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The electroantennogram (EAG) method of determination was developed by Schneider and his school³ in an attempt to solve the Bombycol problem. This seemed to provide a procedure which allowed the measurement of typical potentials at the antennae of the experimental insects after the attractant molecules had made contact, whereby by plotting the so-called⁴ EAG-characteristic curves it was thought that erroneous interpretations would be avoided. However, with a series of attractants it became evident that many substances with a chemical structure similar to that of the genuine attractant molecule of the experimental insect show a 'response' in the EAG. For such reasons the EAG determinations led to completely incorrect interpretations for the insects *Lymantria dispar* (*Porthetria dispar*, gypsy moth, nun), *Periplaneta americana* (American cockroaches), *Pectinophora gossypiella* Saunders, pink bollworm moth, *Carpocapsa pomonella*, *Laspeyresia pomonella* (codling moth), *Adoxophyes orana*, *Heliothis virescens*, *Argyroplote leucotreta*, and *Spodoptera frugiperda*. By means of an EAG investigation, with evaluation of the characteristic curves, the actual sex attractants peculiar to the species can be identified, but not necessarily conclusively. This assertion will now be explained and substantiated by reference to the examples just quoted.

Before embarking on a critical discussion of a number of inhibition and synergism effects, perhaps the following should first be stated.

Since the investigation (Figure 2) and discovery of 10E, 12Z-hexadecadien-1-ol (Bombycol) in *Bombyx mori*, natural products with AU values as lepidoptera pheromones of 10^{-12} to 10^{-13} $\mu\text{g/ml}$ have been found which induce a biologically relevant reaction, namely stimulation and triggering of mating behaviour. This is observed at concentrations previously regarded as inconceivably small, i.e. with a few hundred molecules at the receptor site. In order to forestall immediately any discussion on this point, the attractant unit (AU) as presently defined should be recognized as an obligatory unit of

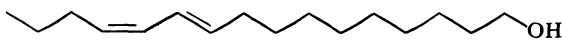
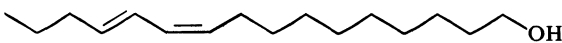
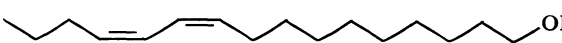
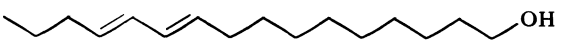
Authentic Bombycol	10^{-10}	Butenandt <i>et al.</i>
	10^{-13}	Butenandt <i>et al.</i>
10 E, 12 Z Hexadecadien-1 ol	10^{-13}	Truscheit and Eiter
	10^{-3}	Butenandt <i>et al.</i>
10 Z, 12 E Hexadecadien-1 ol	10^{-5}	Truscheit and Eiter
	1	Butenandt <i>et al.</i>
10 Z, 12 Z Hexadecadien-1 ol	10^{-1}	Truscheit and Eiter
	10	Butenandt <i>et al.</i>
10 E, 12 E Hexadecadien-1 ol	10^2	Truscheit and Eiter

Figure 2

measurement by all research workers, in view of the difficulties in determining the concentrations of pheromones (that is, their biological activities). Viewed historically, the determination of the AU with the four theoretically possible 10,12-hexadecadien-1-ols which we had then synthesized for the first time, yielded reproducible results. However, these results finally made it clear to us, after several years of disagreement with a large number of newly discovered pheromones with a chemical constitution since recognized as incorrect, that we would never have recognized the true attractant of the silk moth (10E, 12Z-hexadecadien-1-ol) by means of our synthetic procedures in conjunction with biological testing, if the 10,12-hexadecadien-1-ol mixture of isomers originally produced by us had not been recognized as the 10E, 12E and 10Z, 12-E-isomers with an AU of 10^{-2} $\mu\text{g}/\text{ml}$ after separation via the *p*-nitroazobenzoate; in other words, the determination of the attractant unit was of exceptional service in this earliest stage of synthetic pheromone chemistry, although I shall refrain from estimating the usefulness of this method for the biologist in determining the relationship of the receptor site.

Since we have meanwhile learned that many similar aliphatic, unsaturated and polyunsaturated long-chain olefinic alcohols or their esters can act as biological stimulants, the first fact to emerge is that according to confirmed findings with *Bombyx mori*, and also with *Porthetria dispar* and *Apis mellifera*, the genuine pheromone concentrations would have to exist within ranges determinable either by efficient modern analysis, or by methods such as those developed by Shorey *et al.*⁵ Substances which do not reach these 'genuine' concentration ranges, for example only causing impulses and thus stimulation of the sexual behaviour of the experimental insects in substantially higher concentrations, should be designated as *pseudopheromones* or *pheromone mimics*. However, all masking and inhibiting effects discovered with such systems, and caused by the presence of geometrical isomers, or of substances having no connection at all with the class of pheromones under discussion, as well as synergistic effects, should not be regarded as belonging to a single category. One thing has emerged clearly from the world-wide research which has been carried out in the last ten to fifteen years, namely that even in juvenile hormones (*Figure 3*) such as C_{17} - and C_{18} -Coecropia hormone activity is associated with a greater range of structure than is the case with the 4-isomeric 10,12-hexadecadien-1-ols, of which only the 10E,12Z-isomer shows high biological activity.

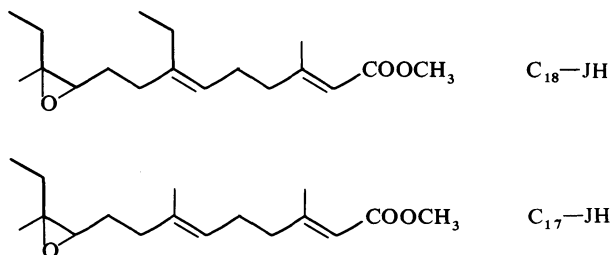


Figure 3

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For the sake of greater clarity, I should like to sketch the currently available published facts regarding the constitution/activity relationships with several sex attractants.

After the isolation¹ of the sex attractant of the silk moth in 1959 and the total synthesis and biological testing of its four possible isomers in 1961², M. Jacobson, N. Beroza and W. A. Jones (*Figures 4*)⁶ reported the isolation, purification, constitution, classification and total synthesis of the sex attractant of *Porthetria (lymantria) dispar* (gipsy moth) (*Figure 4*), D-10-acetoxyhexadec-7Z-en-1-ol, 'gyptol', and an artefact 12-acetoxyoctadec-9Z-en-1-ol, 'gyplure', with an effectiveness equal to the genuine sex attractant of *Lymantria*, namely 10^{-12} µg/ml in the laboratory trial (10^{-7} µg/ml in the field trial). These authors stated that the two synthetic products were identical to the genuine sex attractant with regard to their activity. Sterically homogeneous gyptol and gyplure synthesized in our laboratory by E. Truscheit in 1964 did not show the slightest activity either in the laboratory test, the field test, or in the electrophysiological test. The American authors explained similar observations in their own preparations as inhibition effects or deactivations caused by the presence of traces of the geometrically isomeric E-compounds, as well as of completely different substances such as traces of

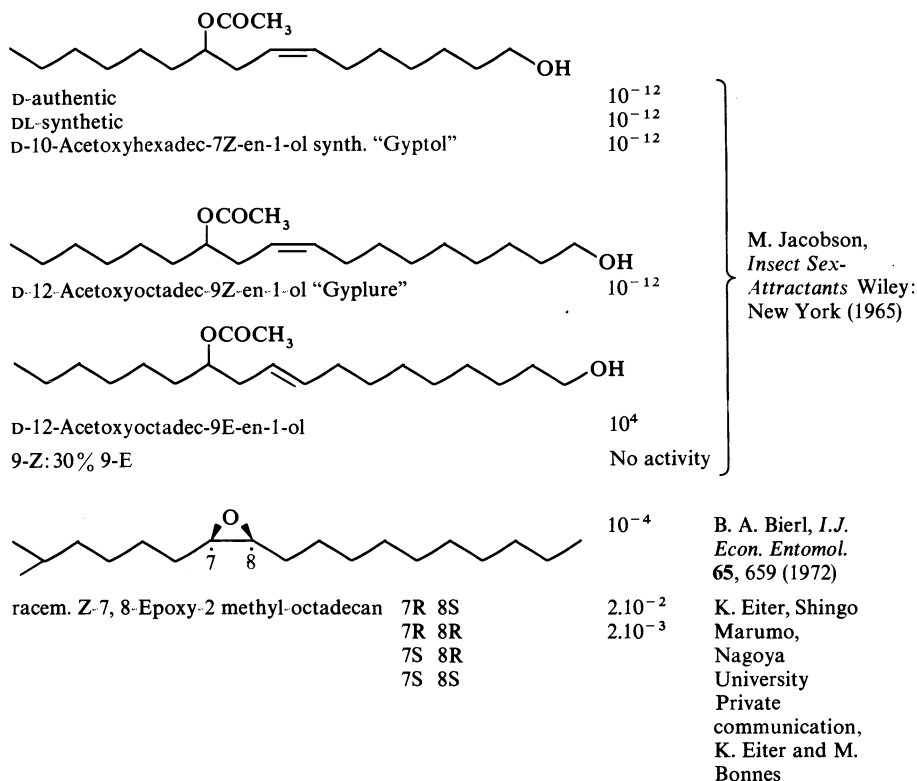


Figure 4

solvent or impurities originating from the synthesis. These explanations, are still quoted today especially by biologists, but also by chemists, even though in our *Annalen-Arbeit 1967*² we pointed out that experiments in which our synthetic products were mixed with the genuine attractant from *Porthetria* did not show any inhibition or masking effects either in the EAG or later in the laboratory test.

In amplifications of the expression 'sterically homogeneous', originating from 1964, it must be stated that the analytical methods available at the time, such as the gas chromatographic, infra-red, nuclear magnetic resonance, mass spectroscopic and thin-layer chromatographic methods, like the GLC method also developed for these problems, have a value which is as limited in 1974 as it was then; in the case of olefins¹³C-spectroscopy, which does have benefits, only permits the estimation of Z/E-ratios of 97:3 at most. It can thus be readily seen that inhibition and also synergism effects cannot be scientifically established with certainty in this concentration range and must therefore temporarily remain within the domain of biological theology.

The structures shown in *Figure 4* were very carefully synthesized by us to achieve steric homogeneity in 'gyptol' and 'gyplure'. We clearly recognized that no efficient method of analysis exists which would have permitted an exact determination of geometrical isomers present to the extent of between one and ten per cent in the main product. GC investigations performed at the time on mixtures of certain isomeric 10,12-hexadecadien-1-ols using the customary commercial polar and non-polar columns of 1-3 metres in length revealed no useful separation. Although this method is repeatedly quoted in the literature, the result of injecting approximately 100 per cent 7Z-gyptol and 100 per cent 7E-gyptol as a mixture into the gas chromatograms is not stated. Only one possibility remained for us to disprove concerning the inhibition and masking effects, namely that our synthetic product was mixed with genuine material in the ratio 9:1 and that a definite response was observed in the EAG.

In this period there were many publications which were intended to prove that small admixtures of geometrical isomers, e.g. 30 per cent of the 9E-compound with 70 per cent 9Z-gyplure, meant an absolute loss of activity. Thus M. Jacobson *et al.*⁶ determined an AU of 10^{-12} µg/ml, an activity value in the same range as that of Bombycol. It was clear to us at the time, however, not only from our experience with *Bombyx mori*, but also from experiments with benzene extracts from the abdominal segments of *Porthetria dispar* that the attractant could not be 10-acetoxy-7Z-hexadecen-1-ol.

We were extremely pleased that the negative investigations of this period resulted in the isolation in 1972 by B. A. Bierl *et al.*⁷ of a new substance as the pheromone of the gipsy moth which proved to be Z-7,8-epoxy-2-methyl-octadecane. We synthesized this compound on a scale of 200 g and immediately demonstrated a qualitative activity in *Lymantria*; the quantitative investigation, however, carried out by Prof. Shingo Marumo⁸ of Nagoya University in Japan, only yielded AU values of 2×10^{-2} to 2×10^{-3} µg/ml.

Our own investigations, performed by Dr Boneß at our Biological Institute, yielded values of approximately 10^{-4} µg/ml, values which differ by 8 or 9 powers of ten from those known for Bombycol and those described for gyplure by the American authors. Thus at the time we were only able to establish that

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a very large difference exists between *Bombyx* and *Lymantria* with regard to the activities of their sex attractants. Some biologists consider that a difference might well exist between the so to say domesticated *Bombyx mori* and the *Porthetria* or *Lymantria dispar* still living in natural surroundings. This difference might take the form of reduced values in the biological activities of their sexual pheromones, although the phrase *natura non facit saltus* which is to be found in Linné and Leibniz, gives me considerable cause for thought, particularly with reference to this area of biology. The situation became even more suspect when a paper published shortly afterwards by M. Beroza *et al.*⁹ advanced the view that the precursor of *Z*-7,8-epoxy-2-methyloctadecane, namely the olefinic compound 7*Z*-2-methyloctadecene, exerts a strong inhibiting effect on the epoxide attractant (*Figure 5*).

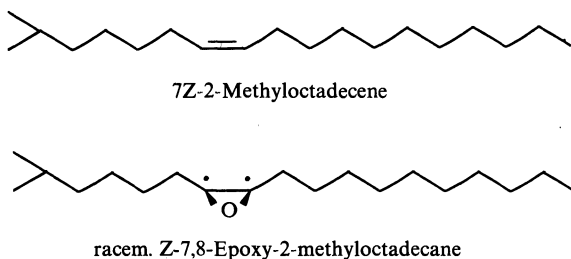


Figure 5

D. Schneider *et al.*¹⁰ on the other hand do not find any masking effect of the 7*Z*-olefin in EAG experiments, although neither Beroza nor Schneider established the geometrical purity of the 7*Z*-olefin they used. In the absence of such evidence, descriptions of biological experiments remain of dubious significance. No clear conclusions can be drawn from experiments using inhomogeneous substances.

We have not verified these facts for the pair of compounds 7*Z*-2-methyloctadecane and *Z*-7,8-epoxy-2-methyloctadecane. We can only establish that neither precursors, admixtures of geometrical isomers, impurities originating from the synthesis process, solvent addition, nor admixtures of organic salts, had the slightest effect in the case of Bombycol. This is, in our opinion,

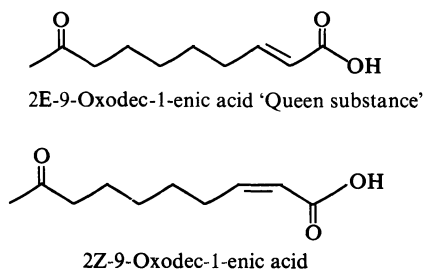
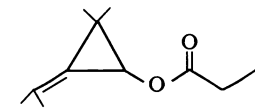


Figure 6

understandable for a genuine attractant since it will always be found in the insect in the natural biotope in association with other sex attractants, feeding attractants and scents of various origins, yet it will nonetheless still excite and attract the males of the same primary species specifically. We were able to observe and confirm this principle with regard to pheromone activity in the pheromone of the honey-bee *Apis mellifera*, 2E-9-oxodec-1-enic acid, the 'queen substance'. We synthesized both the natural 2E- and the 2Z-form, stereochemically pure for practical trials in 1961 (Figure 6).

We observed no inhibition (Figure 6) effects on the geometric isomers, either from solvents or other substances



1-Propionyloxy-2,2-dimethyl-3-isopropylidene-cyclopropane
Periplaneta americana

10^{-12} M. Jacobson and
L. A. Smalls
J. Econ. Entomol.
59, 414 (1966).

Figure 7

When in 1966 the sex attractant of the American cockroach *Periplaneta americana* was reported by M. Jacobson *et al.*¹², its constitution was given as 1-propionyloxy-2,2-dimethyl-3-isopropylidene-cyclopropane (Figure 7). We like many other university and industrial laboratories, set about synthesizing this cyclopropane derivative. It seemed a very tempting prospect to be able to attract and decimate this abundant insect for which scarcely any practically applicable insecticide existed.

The objections regarding the inhibition or deactivation of such pheromones advanced in the literature at the time were the reason that the laborious syntheses of the cyclopropane compound through carbene reactions in our laboratory were not published. In such reactions mixtures were always obtained which could only be separated to a certain extent by means of gas chromatography. As the biological tests of such mixtures were completely negative, we entertained some doubt regarding the spectroscopic interpretation of our reaction products, until Whiting and Day¹³ in their well-known investigation synthesized the compound, that is to say separated it from the reaction mixture obtained, and were able to characterize it by n.m.r. spectroscopy. No biological activity at all was detected. The authors who originally discovered the cockroach attractant thereupon withdrew their paper, stating with regret that they had obviously been misled by an inexplicable trace admixture of active material. Thus a *tabula rasa* was made with regard to the pheromone activity of this cyclopropane derivative.

At the IUPAC Congress in Stockholm in 1966 M. Jacobson reported¹⁴ on the structural elucidation and total synthesis of propylure (Figure 8), the sex attractant of the pink bollworm *Pectinophora gossypiella* Saunders. The synthetic 1-acetoxy-10-propyl-5E-9-tridecadiene was claimed to have the same effect as the natural attractant.

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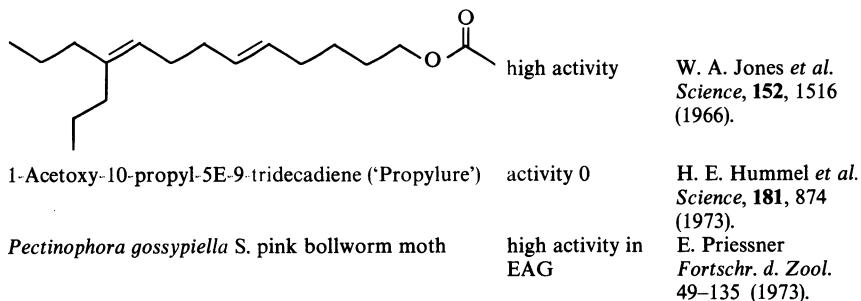


Figure 8

We had carried out a synthesis which was conceived in such a way that steric homogeneity was not attempted from the very start. 1-Acetoxy-10-propyl-5E-9-tridecadiene was only present in the reaction mixture in a form allowing satisfactory determination. This was in fact the case, but no biological activity at all has yet been observed.

At our request the American authors informed us that propylure was inhibited just as much by admixtures of up to fifteen per cent of the 5Z-compound as by traces of solvents, and that meta-toluic acid diethylamide (a well-known insect repellent: Deet, Autan) had proved to be an exceptionally effective synergist!

In the meantime, Dr Pattenden¹⁵ in Cambridge and Dr M. Stoll and I. Flament¹⁶ of Firmenich in Geneva, as well as a number of other authors¹⁷, had synthesized sterically homogeneous 5E-propylure which they sent to Dr Jacobson for testing.

These compounds were found to be biologically fully active, although Dr Pattenden kindly placed at my disposal both the i.r. spectra of his synthetic product (Figure 9) as well as that of Dr Jacobson's (Figure 10).

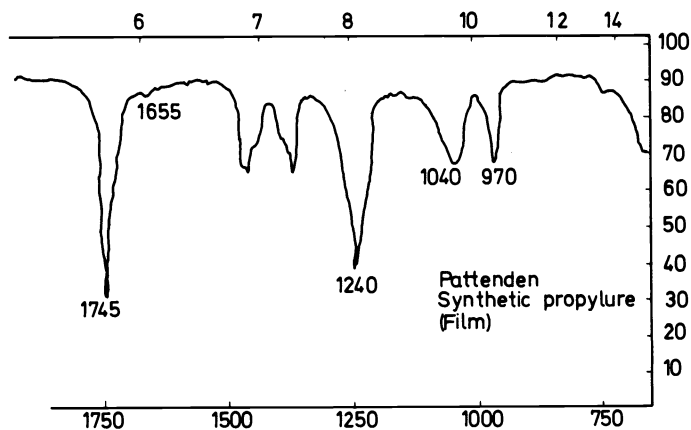


Figure 9

It can be clearly seen from this that the synthetic product of Jacobson—as was to be anticipated on the basis of this published synthesis—contains a considerable proportion of Z-compound. This indicated to me that the propylure synthesized by Jacobson could also hardly possess any biological activity. This assumption indirectly received confirmation when a compound more effective than propylure was found in the form of the so-called 'hexalure', 7Z-hexadecenylacetate, by J. C. Keller, Sheets, Breen and Jacobson (Figure 11)¹⁸.

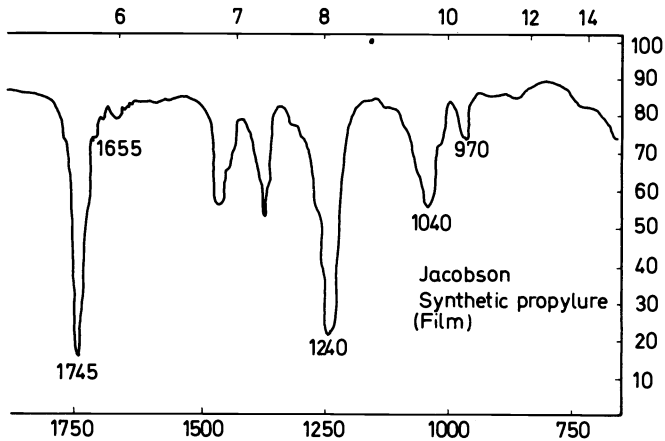


Figure 10




Hexalure, showing AU values of 10^2 to 10^3 $\mu\text{g}/\text{ml}$, is a pheromone mimic.

M. Jacobson¹⁹ has repeated our synthesis, isolated the pure 5E-propylure fraction, and reported it to be biologically fully active. However, H. E. Hummel *et al.*²⁰, in 1973, concluded:

- (1) propylure according to Jacobson does not possess any detectable biological activity, not even when mixed with *m*-tolyl acid diethylamide (Deet, Autan).
- (2) The sex attractant of *Pectinophora gossypiella* S. pink bollworm moth contains a mixture of two isolated 7,11-hexadecadienyl acetates, namely 7Z, 11Z and 7Z, 11E, in the ratio 75:25, a 50:50 mixture of the two isomers (gossypure) having the highest activity in the laboratory.

In a very recent paper entitled 'Attraction of the male pink bollworm moth under laboratory and field conditions', M. Jacobson and W. A. Jones²¹ repeat their statements about the identity of the sex attractants from pink bollworm moth with 10-*n*-propyl-5-*trans*-9-tridecadienyl acetate and their findings that Deet (Autan) (*m*-tolyl acid diethylamide) is a synergist for 'propylure', a substance 'which was detected in pink bollworm moth (wild females) obtained from widely separated geographical locations'.

In rejecting Hummel's paper, Jacobson and Jones report that many authors who had worked on propylure describe a high biological activity for their synthetic substances; all the synthetic samples of 10-propyl-*trans*-9-tridecadienyl

Sex attractants*	AU $\mu\text{g/ml}$	Authors
	No activity: inhibition of 5E-product when present up to min 15%	M. Jacobson <i>Science</i> , 163 , 3863 (1969).
5Z-Propylure	10^2-10^3	I. C. Keller, <i>et al.</i> <i>I. J. Econ. Entomol</i> 62 , 1520 (1969).
Hexadecen-(7Z)-yl acetate 'Hexalure'	25×10^3	K. W. Vick <i>et al.</i> <i>Experientia</i> , 30 , 18 (1974).
	75:25 authentic 50:50 'gossypure'	H. E. Hummel, <i>et al.</i> <i>Science</i> , 181 , 874 (1973).
7Z, 11Z-Hexadecadienyl acetate, $25-10^{-3}$ <i>Sitotroga cerealella</i>	Unknown	Unknown
	Unknown	Unknown
7E, 11Z-Hexadecadienyl acetate	Unknown	Unknown

* NOTE ADDED IN PROOF: All four isomeric 7,11-hexadecadienyl acetates have been synthesized in pure form by H. Disseln-Kötter and K. Eiter; unpublished results.

Figure 11

acetate from G. Pattenden, M. Stoll and I. Flament, J. C. Stowell, A. I. Mayers and E. W. Collington¹⁷ cited in the last paper by Jacobson and Jones were tested for biological activity in Beltsville, Maryland.

Accordingly, all reports on propylure as a sex attractant are suspect, even if in 1973 E. Preissner⁴ still inexplicably described propylure as a highly active substance in his EAG experiments. In the border areas between organic chemistry and biology the investigator testing biological activity must inform himself about the homogeneity and nature of the substance before constructing a theory. Similarly the natural product chemist, who studies this relationship between structure and activity with his synthetic substances, must be clear as to the methods of the relevant biological test.

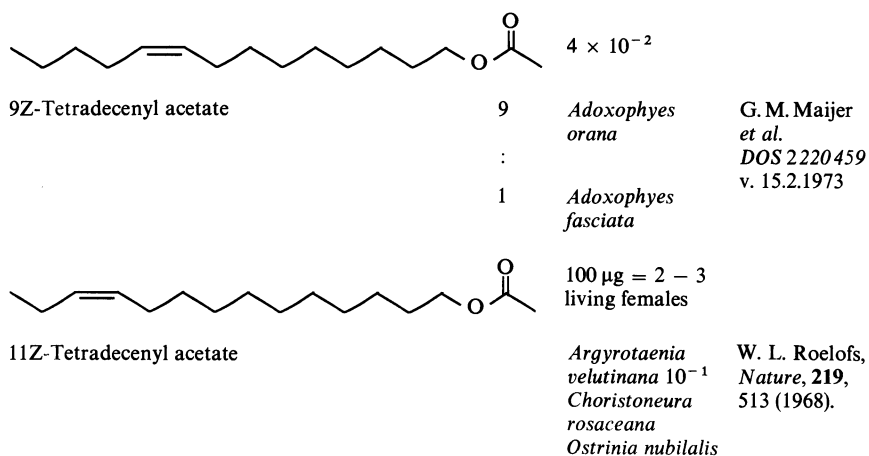


Figure 12

K. W. Vick *et al.*²² have described the 7Z,11E-hexadecadienyl acetate as the only active product in the Angoumois grain moth *Sitotroga cerealella*, with a biological activity of $25 \times 10^{-3} \mu\text{g/ml}$. It is this compound which H. E. Hummel has also determined in gossyplure. Particular importance is again attached here to the high concentrations needed for activity, as well as the presence of only one stereoisomer.

In the case of gossyplure, it remains scarcely comprehensible that the individual components are completely without biological activity, and that genuine gossyplure is effective as a 75:25 mixture, although optimum activity was only observed in a laboratory test with a 50:50 mixture. According to this finding, the pink bollworm moth would still appear to have a thing or two to learn, a consideration which surely does not reach the heart of the matter. The objection that similar relationships have already been described in other species, e.g. *Adoxophyes orana*²³ (9Z- and 11Z-tetradecenyl acetate), are not very convincing. The activities determined are ridiculously low in relation to Bombycol for instance, i.e. the active concentration is very high (*Adoxophyes orana* $4 \times 10^{-2} \mu\text{g/ml}$) (Figure 12).

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14 m glass column 2.3 mm ϕ
4% DEGS Chromosorb WAW
60-80 mesh 175° isotherm
7Z, 11Z-Hexadecadien-
1-ylacetate

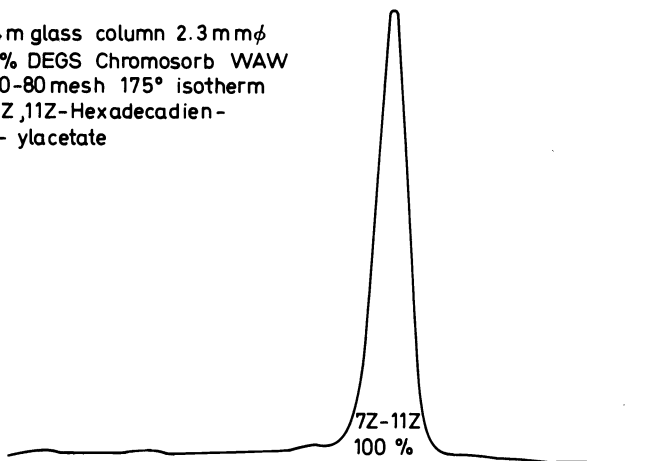


Figure 13

As long as all four geometric isomers of gossyplure have not been synthesized in a pure form, via crystallized precursors, and the biological activities have not been determined on individual compounds and mixtures according to a convincing test method (Butenandt or Shory), no statements can be made regarding this phenomenon. The 7,11-hexadecadienyl acetates synthesized by us, namely the 7Z, 11Z-, 7E, 11E- and 7Z, 11E-forms, cannot be separated according to conventional methods, using gas chromatography with the usual capillary columns.

Only capillary columns of 14-50 m length permitted a separation of certain isomeric, 7,11-hexadecadienyl acetates; in this way, a mixture prepared from

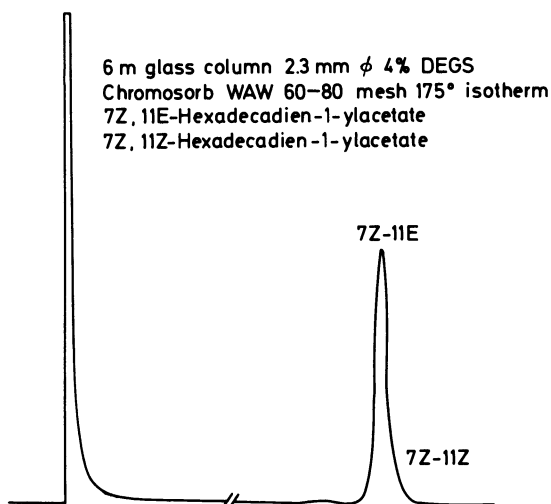


Figure 14

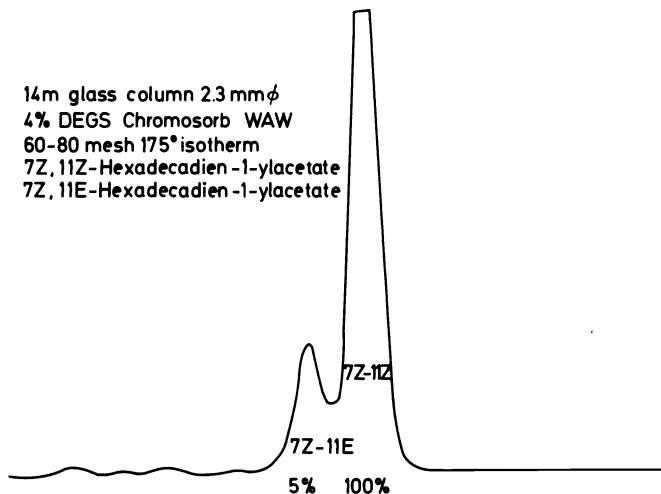


Figure 15

the 7,11-bisacetylene compound, and which according to ^{13}C -spectroscopy consisted of 86 per cent 7Z, 11Z and 14 per cent of a mixture of the other possible isomers, was not resolved.

Polar and non-polar columns of 1 to 6 m in length, repeatedly described in the literature, were ineffective in practice and only showed a high degree of homogeneity in the mixture of 7Z, 11E- and 7Z, 11Z-hexadecadienyl acetate (Figure 14).

In contrast the 14 m glass column gives a distinctly sharp peak on injecting approximately five per cent of the 7Z, 11E-isomer with 7Z, 11Z-hexadecadienyl acetate (Figure 15).

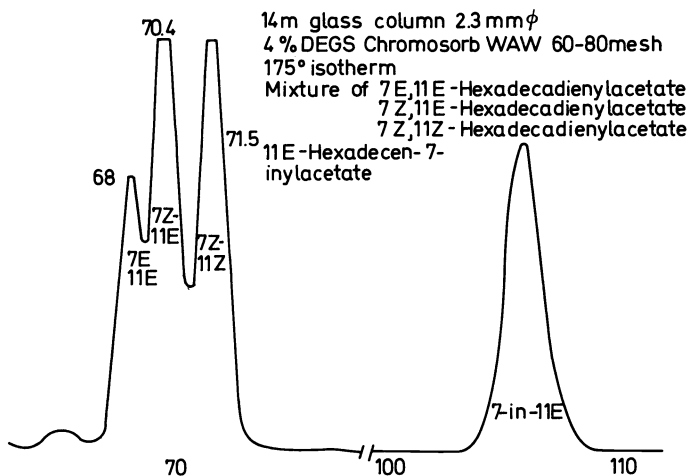


Figure 16

INSECT SEX ATTRACTANTS

The next *Figure 16* demonstrates the separation of a mixture of 7E, 11E-7Z, 11E- and 7Z, 11Z-hexadecadienyl acetates by gas chromatography. The fourth compound, namely one of the synthetic precursors of 7,11-hexadecadienyl acetate—the 11E-hexadecen-7-yn-1-yl acetate, is shown as a single peak well resolved from the other peaks.

In addition Hummel has shown that silica gel loaded with silver nitrate should not be used as a column material, because any biological activity of the natural pheromone is lost in such columns.

A very recent paper by B. A. Bierl, M. Beroza, R. T. Staten, P. E. Sonnet and V. E. Adler²⁴ deals with the sex attractant of the pink bollworm. These authors too, referring to the publications of Hummel *et al.*, state that they found the mixture of 7Z, 11Z- and 7Z, 11E-hexadecadienyl acetates to be active. Their tests were not performed on their own synthetic substances, however, but with products prepared by the Farchan Research Laboratory. The conclusion that the mixtures are identical in their composition of geometric isomers (95 per cent ZZ, 5 per cent ZE) is, in view of the above remarks, dubious. It is also remarkable that, contrary to the findings of Hummel, all four isomers were said to show 'response' in the EAG. The only conclusion which can be drawn is that the EAG method is valueless, or that the substances tested are impure and also heterogeneous, or that the laboratory and field tests provide no acceptable information.

Thus it is clear that only a highly developed analytical technique, combined with the subtlest chemical techniques, both for the isolation and the synthesis of the pheromones, will prove to be the *conditio sine qua non* for gaining a clear overall view in this veritable jungle of alleged facts which has grown to such huge proportions. No matter how tempting this method may seem of preparing an extract from abdominal segments of a few female insects, splitting it up into individual peaks using one of the current analytical methods and leading these to the antennae of male insects in order to detect response, and to draw conclusions regarding the constitution on the basis of the on-line mass spectrometer, experience has unfortunately provided grounds for serious misgivings.

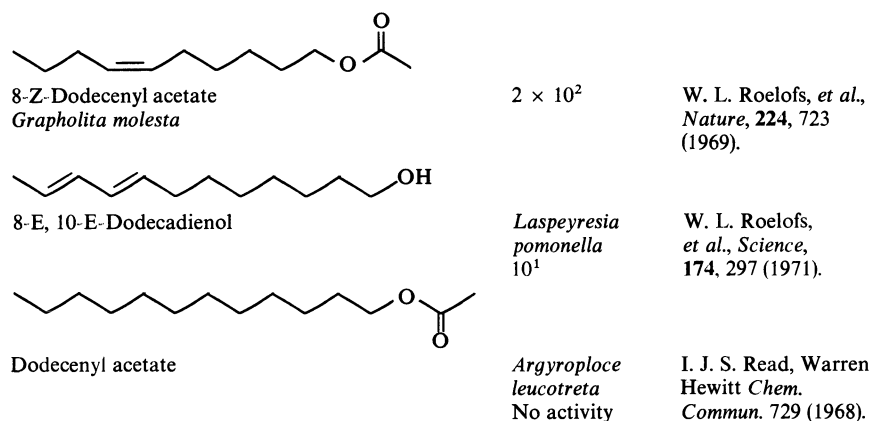
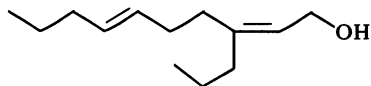
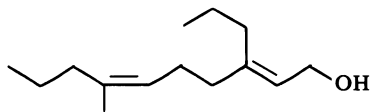


Figure 17

This certainly applies to *Laspeyresia pomonella* just as much as to *Adoxophyes orana* or *Argyrotaenia velutinana* etc. However, when the E,E-8,10-dodecadien-1-ol originally discovered by means of gas chromatography²⁵ was not found at all in thorough follow-up investigations, but rather²⁶ 2Z, 6E-7-methyl-3-propyl-2,6-decadien-1-ol on the basis of n.m.r. spectroscopic investigations and comparisons with sterically homogeneous synthetic material, and when Manning P. Cook Jr²⁷ considers the inverse 2E, 6Z-product to be the most probable constitution of the pheromone of *Laspeyresia pomonella*, it must be concluded that this compulsive approach has contributed greatly to the premature publicity which has brought this field of research into disrepute²⁸. In periods of feverish search for new methods of pest control, this has only performed a disservice to environmental questions and problems of ecology.



2-Z,6-E-7-Methyl-3-propyl-2,6-decadien-1-ol

*Laspeyresia pomonella*L. M. McDonough, *et al.*, *Science*, **177**, 177 (1972).

2-E,6-Z-7-Methyl-3-propyl-2,6-decadien-1-ol

Suggested structure
*Laspeyresia pomonella*Manning P. Cooke Jr., *Tetrahedron Letters*, **15**, 1281 (1973).

Figure 18

If only the development initiated by A. Butenandt, which required more than twenty years for the isolation of one pheromone, had been pursued with the methods available today, there would have been a corresponding saving of time! The flood of papers which have since been published is completely out of proportion to the information obtained, so that especially from the viewpoint of the chemical industry, the hope expressed by Butenandt himself, of a specific form of pest control based on the use of their own sex attractants, still remains unfulfilled.

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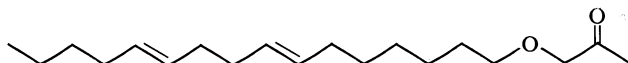
CORRIGENDA

Readers are asked to note the following corrected items which were inadvertently printed wrongly in *Pure and Applied Chemistry*, Vol. 41, Nos 1-2 (1975) pages 211-216:

Page 211

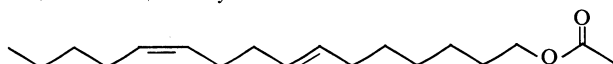
Sex attractants

Authors



Unknown*

7E, 11E-Hexadecadienyl acetate



Unknown*

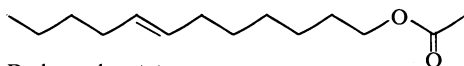
7E, 11Z-Hexadecadienyl acetate

* NOTE ADDED IN PROOF: All four isomeric -7,11-hexadecadienyl acetates have been synthesized in pure form by H. Disselnkötter, K. Eiter and D. Wendisch, *Tetrahedron*, in press.

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Accordingly, all reports on propylure as a sex attractant are suspect, even if in 1973 E. Priesner⁴ still inexplicably described propylure as a highly active substance in his EAG experiments. In the border areas between organic chemistry and biology the investigator testing biological activity must inform himself about the homogeneity and nature of the substance before constructing a theory. Similarly the natural product chemist, who studies this relationship between structure and activity with his synthetic substances, must be clear as to the methods of the relevant biological test.

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Dodecanyl acetate

Argyroploce leucotreta Meyr. (now *Cryptophlebia leucotreta* Meyr.)

J. S. Read, F. L. Warren and P. H. Hewitt *Chem. Commun.* 792 (1968).

No activity

D. A. Evans and C. L. Green, *Chem. Soc. Reviews*, 2(1), 80 (1973).

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⁴ E. Priesner, *Fortsch. d. Zool.* 22(1), 49-135 (1973).