Structure-response Relationships in Noctuid Sex Pheromone Reception

An Introductory Report

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Pheromones, Olfactory Receptors, Structure-response Relationships, Lepidoptera

Electroantennogram (EAG) data reflecting response spectra of male pheromone receptors have been analyzed for 16 species of Noctuidae (Lepidoptera). The test compounds included 100 pheromone analogues, altered in chain length, in position and configuration of double bond(s), and in the functional end groups. On comparison of amounts of substance required to elicit an equivalent EAG response, a single compound was determined to be most effective on a given species; these structures, either known or proposed as the natural sex pheromones of the species, were *cis*-7-dodecen-1-yl acetate, *cis*-7-tetradecen-1-yl acetate, *cis*-9-tetradecen-1-yl acetate, *trans*-9-tetradecen-1-yl acetate, *cis*-11-hexadecen-1-yl acetate, *cis*-9, *trans*-12-tetradecadien-1-yl acetate, *cis*-9-tetradecen-1-yl formate, and *cis*-9-tetradecen-1-ol, respectively. Elongation (shortening) of the chain by 1 or 2 methylene groups, the movement of a double bond 1 carbon from the optimum, a change to the opposite geometrical isomer, or the introduction of a second double bond invariably reduced EAG responses to 1.8 to 56 times below that observed with the most stimulating compound, in all 16 species. Further alterations in chain length or in double bond position caused even greater reduction in activity, as did certain changes in end group (Tables I and II).

A set of distinct rules could be derived from these structure-response relationships; one of these rules concerns the optimum position of the double bond(s) in relation to chain length, and another one the ratios in activity values produced by end group variations, irrespective of chain length. The same rules described here for 16 noctuid species held also for the structure-response relationships observed within various additional groups of Lepidoptera.

From EAG values determined in this study, an attempt has been made to calculate physicochemical propteries of underlying acceptor structures.

The Noctuidae comprise about 25000 species. In this family of moths, sex attractant pheromones produced by the female have thus far been identified for ten species; the reported main component is *cis*-7-dodecen-1-yl acetate for two species of Plusiinae [1, 2], *cis*-11-hexadecenal for two species of Heliothidinae [3–5], and *cis*-9-tetradecen-1-yl acetate, *cis*-9, *trans*-11-tetradecadien-1-yl acetate, *cis*-9, *trans*-12-tetradecadien-1-yl acetate, and *trans*-9,11-dodecadien-1-yl acetate, respectively, for six species of Amphipyrinae [6–11]. It is likely that related structures will be found in other noctuid species, considering results of field attraction studies [12–16]. We have concentrated upon the Noctuidae in studying specificity of pheromone receptors in male Lepidoptera.

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Differences among species in properties of these receptors should become evident from testing, electrophysiologically, the same appropriate set of compounds under the same conditions of stimulation. It seemed reasonable to start such a study by recording the electroantennogram (EAG) [17], a slow potential assumed to arise from many receptor cells. EAGs were recorded from approx. 200 noctuid species [18] representing the 15 subfamilies [19] which occur in Central Europe, and a few extra-European representatives of the family. The test compounds were derived from the six structures mentioned above; they varied in chain length, chain branching, the configuration and position of the double bond(s), and the nature of the functional end groups. In addition to the synthetic compounds, excised pheromone glands (or their extracts), containing the natural pheromones of the test species, were included in the EAG measurements.

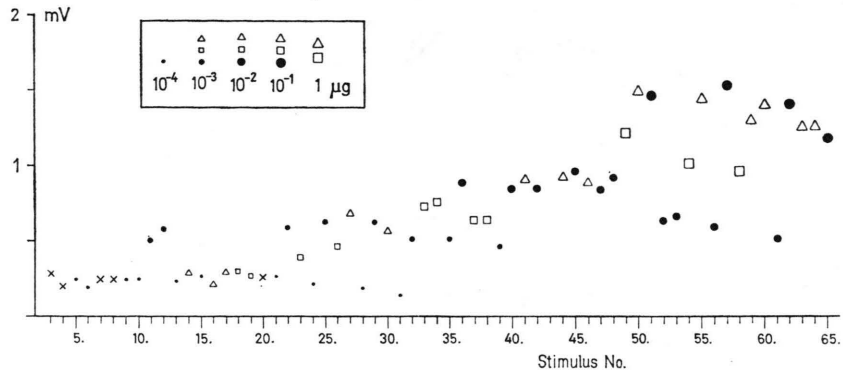

Fig. 1. *Trichoplusia ni* ♂. Sequence of electroantennogram (EAG) responses to indicated amounts of female sex pheromone, *cis*-7-dodecen-1-yl acetate (●) and two analogues, *cis*-8-dodecen-1-yl acetate (△) and *cis*-9-dodecen-1-yl atetate (□). The size of the symbols (four for each compound) reflects the stimulus amounts given in the inset; × represents control stimulation without pheromone. The 63 stimuli were presented in this sequence at 25—45 sec intervals. The responses to 10^{-4} µg ● (stimulus No.'s 5, 6, 9, 10, 13, 15, 21, 24, 28, 31) and to 10^{-3} µg of both △ and □ (No.'s 14, 16, 17, 18, 19) are within the control range (No.'s 3, 4, 7, 8, 20). From No.'s 21 through 65, individual responses to 10^{-2} µg, 10^{-1} µg and 1 µg of △ or □ are compared with the immediately preceeding and following reference stimuli ● (*e. g.*, 37, 38, 10^{-1} µg □ with 35, 39, 10^{-3} µg ● and 36, 40 10^{-2} µg ●). Although, for the chosen antennal preparation, the responsiveness has decreased approx. 10 fold in a short time (61, 0.1 µg ● has fallen to the level of 39, 0.01 µg ●), the test substances can in this manner be adequately classified (see Fig. 2).

During the course of these studies, the fact was discovered that differences between species in chemical structure-response relationships were strictly governed by certain *rules*, applying to all the species investigated. This is illustrated here by considering the responses of a total of sixteen species of Noctuidae with regard to two groups of pheromone analogues: (1) fifty aliphatic acetates, varying in chain length and in position, configuration, and number of the double bonds (Table I); and (2) fifty *cis*-9-monounsaturated compounds, differing in their end groups (Table II).

Methods

About equal parts of this series of analogues were prepared by the laboratories at Beltsville and Erlangen, respectively. The samples were at least 95% pure.

The experimental arrangement used in the electrophysiological measurements was essentially the same as in earlier studies with Bombykol isomers [20, 21]. Air currents (100 cm/sec), passed over known quantities of substance (hereafter referred to as the stimulus source), were directed on the antennal preparation for 1.0 sec. With other experimental conditions constant, the quantities were increased from 10^{-6} to 10^{2} µg during the course of the experiments, which consisted of two successive series (A, B).

A. As the natural sex pheromones were chemically-unknown for most of the test species, it was first necessary to single out, by systematic screening, the molecular structure most effective on male receptors of the respective species. About 600 pheromone analogues were available for these measurements. Evaluations were usually made at 0.1 µg (amount at stimulus source) and the most effective compound defined as the one which elicited the highest EAG amplitude at this stimulus amount.

B. The second series was directed at classification of structural analogues according to their relative effectiveness. As will be pointed out below, *activity classes* are defined in terms of the reciprocals of the half log amounts (µg) required to elicit equivalent EAG responses. There are three features of the measurements which are particularly relevant to the level of confidence of this half log classification:

1. To eliminate errors due to time-dependent changes in the responsiveness of the preparation, reference stimuli were presented throughout the experiment. The known sex pheromone of the test species, or the most effective substance(s) determined under A., served as the reference compounds. Test stimuli (amounts of an analogue) were compared only with the immediately preceeding and following reference stimuli.

2. For each such comparison (test stimulus *vs* reference stimuli), the boundaries between the half log activity classes were determined as follows. Two amounts of the reference compound were presented at the ratio of 1:10; the amplitude difference of the EAG responses was quartered, and the class boundaries were set 1 quarter above the lower and 1

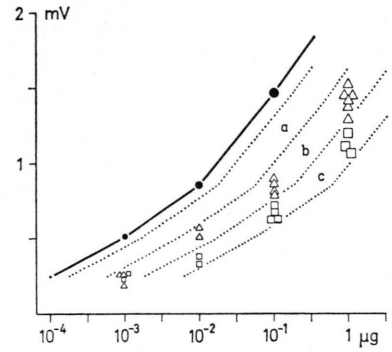

Fig. 2. *Trichoplusia ni* ♂. EAG data for *cis*-8-dodecen-1-yl acetate (□) and *cis*-9-dodecen-1-yl acetate (△) from Fig. 1, corrected for the time-dependent shift in the antennal response. The heavy line is the lower part of the standard dose-response curve [18] for the sex pheromone, *cis*-7-dodecen-1-yl acetate. Each of the 26 values for □ and △ was corrected by that factor by which the reference values ● in Fig. 1 differed from the respective value on the standard curve. The three areas (a, b, c) now indicate the range of EAG amplitudes for substances which are between 1.8 to 5.6 times (a), or 5.6 to 18 times (b), or 18 to 56 times (c) less effective than ● (half log activity classes). *Cis*-8-dodecen-1-yl acetate is approx. 10 times, and *cis*-9-dodecen-1-yl acetate is approx. 30 times less effective than the pheromone (see activity values for *T. ni* in Table I).

quarter below the higher of the two amplitudes. The test stimulus which elicited an EAG response falling in the interval between the two amplitudes was classified accordingly; *i. e.*, as belonging to the class of the higher reference amount, or the class of the lower amount, or the intermediate half log class, respectively. This procedure was executed for each individual test stimulus.

3. For a given analogue, these comparisons were usually conducted at three decadic steps of stimulus amount. The number of measurements necessary to obtain a certain confidence of classification depended on how often the test compound fell into the same class. The desired confidence coefficient of classification of >0.9 was reached, *e. g.*, when 5 out of 6, or 8 out of 10, or 10 out of 15 measurements fell within the same class range.

A typical sequence of measurements and the resulting classification are shown in Figs. 1 and 2.

Throughout this report, the efficacy of a given compound on a given species will be expressed by a single *activity value*. These values represent the half log amounts (μg) of test substance which elicited the same EAG amplitude as 0.001 μg of the most effective (standard) compound. For the sake of convenience, amounts 1.8 to 5.6 times higher than 0.001 μg will be presented as 0.003 μg, those 5.6 to 18 times higher, as 0.01 μg, etc. The total of the activity values for a given species will be referred to as its *response spectrum.*

Results

In each of the test species, only one compound (indicated by the value 0.001 in Tables I and II) was found to elicit the maximum EAG response. Of six hundred structural modifications, the following eight showed this effect with one or more of the sixteen species: *cis*-7-dodecen-1-yl acetate, *cis*-7-tetradecen-1-yl acetate, *cis*-9-tetradecen-1-yl acetate, *trans*-9-tetradecen-1-yl acetate, *cis*-11-hexadecen-1-yl acetate, *cis*-9, *trans*-12-tetradecadien-1-yl acetate, *cis*-9-tetradecen-1-ol, and *cis*-9-tetradecen-1-yl formate. In the case of *Trichoplusia ni* and *Spodoptera (Prodenia) eridania,* this most effective structure (*cis*-7-dodecen-1-yl acetate and *cis*-9, *trans*-12-tetradecadien-1-yl acetate, respectively) is indeed the same as that produced by the female [1, 7]. In *Heliothis zea,* *cis*-9-tetradecen-1-yl formate [22] (a structure unknown as an insect pheromone) is approx. 30 times more effective than the reported pheromone constituent, *cis*-11-hexadecenal [3, 5]. There is so far no report on the identity of the natural pheromones for the thirteen remaining species.

Statements concerning structure-response relationships will always be made relative to the single most potent compound, considering first those species in which this compound is mono-unsaturated.

Isomerism and chain elongation

In all those species, the opposite geometric isomer is between 1.8 to 5.6 times less effective (value 0.003 in Table I). With regard to positional isomers, the efficacy is generally observed to decrease on shifting the double bond from the optimal position; the decrease is between 1.8 to 56 fold when the double bond occurs at a position one carbon from the optimum, and may be, in certain species, as much as 3000 fold for the most extensive shift (*cf. Acosmetia caliginosa* [Amphipyrinae]; Table I). Altering the chain length by two methylene groups reduces EAG activity by approx. 10 to 100 fold, in all the species (Tables I and II). It also seems to be characteristic that the configuration of the double bond found in the compound listed at 0.001 is preferred also at the other double bond positions; thus, *A. caliginosa* prefers *trans* over *cis,* whereas the other species prefer *cis* over *trans,* throughout the response spectrum (Table I).

Table I. The effect, expressed in half log amounts (μg) to produce the same EAG amplitude, of fifty unsaturated acetates on male pheromone receptors of ten species of Noctuidae.

	Trichoplusia ni	*Pachetra fulminea*	*Syngrapha variabilis*	*Amathes candelarum*	*Panolis flammea*	*Hyssia cavernosa*	*Acosmetia caliginosa*	*Monima gracilis*	*Chersotis multangula*	*Spodoptera eridania*
Dodecenyl acetates										
cis-3	0.03	0.1	0.1	0.1	1	0.3	0.3	10		1
trans-3	0.1	0.3	0.3	0.3	1		0.1	10		1
cis-4	0.1	0.1	0.1	0.3	1	0.3	0.3	10	1	1
trans-4	0.1	0.3	0.3	0.3	1		0.1	10	1	1
cis-5	0.01	0.03	0.03	0.03	0.3	0.1	0.1	3	0.3	1
trans-5	0.03	0.1	0.1	0.1	1	0.3	0.03	10	1	1
cis-6	0.01	0.03	0.1	0.1	0.3	0.1	0.1	3	0.3	1
trans-6	0.03	0.03	0.1	0.3	1	0.3	0.03	10	1	1
cis-7	**0.001**	**0.001**	0.03	0.03	0.1	0.03	0.03	1	0.1	0.1
trans-7	0.003	0.003	0.1	0.1	0.3	0.1	0.01	3	0.3	0.3
cis-8	0.01	0.01	0.1	0.1	0.3	0.1	0.03	3	0.3	0.3
trans-8	0.03	0.03	0.3	0.3	1	0.1	0.03	10	1	0.3
cis-9	0.03	0.03	0.1	0.1	0.1	0.03	0.1		0.3	0.1
Tetradecenyl acetates										
cis-4	0.3	0.3	0.03	0.03	0.3	0.1	3	3	1	0.3
trans-4	0.3	1	0.1	0.1	0.3	0.3	1	3	1	0.3
cis-5	0.1	0.1	0.01	0.01	0.1	0.03	1	1	0.3	0.3
trans-5	0.3	0.3	0.03	0.03	0.3	0.1	0.3	3	1	0.3
cis-6	0.1	0.1	0.01	0.01	0.1	0.03	0.3	1	0.3	0.1
trans-6	0.3	0.3	0.03	0.03	0.3	0.1	0.3	1	1	0.1
cis-7	0.03	0.03	**0.001**	**0.001**	0.03	0.01	0.3	0.3	0.1	0.03
trans-7	0.1	0.1	0.003	0.003	0.1	0.03	0.1	0.3	0.3	0.03
cis-8	0.03	0.03	0.01	0.01	0.03	0.01	0.03	0.1	0.03	0.03
trans-8	0.1	0.1	0.03	0.03	0.03	0.01	0.01	0.3	0.03	0.03
cis-9	0.01	0.01	0.01	0.01	**0.001**	**0.001**	0.003	0.01	0.01	0.01
trans-9	0.03	0.03	0.03	0.03	0.003	0.003	**0.001**	0.03	0.03	0.03
cis-10	0.1	0.03	0.1	0.1	0.01	0.01	0.03	0.1	0.03	0.03
trans-10	0.3	0.03	0.3	0.3	0.03	0.03	0.03	0.3	0.1	0.03
cis-11	0.1	0.03	0.1	0.3	0.03	0.03	0.3	0.3	0.1	0.1
trans-11	0.3	0.1	0.3	1	0.1	0.1	0.1	1	0.3	0.1
cis-12	0.3	0.1	0.3	1	0.3	0.1	1	1	0.3	0.3
Hexadecenyl acetates										
cis-5	1	0.3	0.3	0.3	10	3	10	3	0.3	1
trans-5	1	1	1	1	10	3	3	3	0.3	1
cis-6	1	0.3	0.3	0.3	10	3	10	3	0.3	1
trans-6	1	1	1	1	10	3	3	3	0.3	1
cis-7	0.3	0.1	0.1	0.1	3	1	3	0.3	0.1	0.3
trans-7	1	0.3	0.3	0.3	10	3	3	1	0.3	1
cis-8	0.3	0.1	0.1	0.1	3	1	3	0.3	0.1	0.3
trans-8	1	0.1	0.1	0.3	10	3	1	1	0.3	1
cis-9	0.1	0.03	0.01	0.03	1	0.1	3	0.1	0.03	0.3
trans-9	0.3	0.1	0.03	0.1	3	0.3	1	0.3	0.1	0.3
cis-10	0.3	0.1	0.1	0.3	0.3	0.03	1	0.03	0.01	0.3
trans-10	1	0.3	0.1	1	0.3	0.1	0.3	0.03	0.03	0.3
cis-11	0.1	0.03	0.03	0.1	0.1	0.01	0.3	**0.001**	**0.001**	0.1
trans-11	0.3	0.1	0.1	0.3	0.3	0.03	0.1	0.003	0.003	0.3
cis-12	0.3	0.1	0.1	0.3	0.3	0.03	1	0.03	0.03	0.3
trans-12	1	0.3	0.3	1	1	0.1	1	0.1	0.03	0.3
cis-13	1	0.3	0.3	1	1	0.1	3	0.1	0.1	0.3
cis-7, *trans*-10-dodecadienyl acetate		0.003	0.03	0.03	0.1	0.03	0.1	3	0.1	0.03
cis-9, *trans*-12-tetradecadienyl acetate		0.03	0.03	0.03	0.003	0.003	0.03	0.1	0.03	**0.001**
cis-11, *trans*-14-hexadecadienyl acetate		0.1	0.1	0.3	0.1	0.03	1	0.01	0.003	0.1

Table II. The effect (as in Table I) of fifty *cis*-9-monounsaturated compounds on male pheromone receptors of eight species of Noctuidae.

	Trichoplusia ni	*Pachetra fulminea*	*Hyssia cavernosa*	*Polia nana*	*Polia pisi*	*Cucullia umbratica*	*Cucullia lychnitis*	*Heliothis zea*
cis-9-dodecen-1-yl formate	0.3	0.1	0.3	0.3	0.3	0.3	0.3	0.03
cis-9-tridecen-1-yl formate	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.01
cis-9-tetradecen-1-yl formate	0.1	0.03	0.01	0.01	0.03	0.01	0.01	**0.001**
cis-9-pentadecen-1-yl formate	0.3	0.1	0.1	0.1	0.3	0.1	0.1	0.03
cis-9-hexadecen-1-yl formate	1	0.1	0.3	0.3	1	0.3	0.3	0.3
cis-9-undecen-1-yl acetate	0.1	0.1	0.3		0.1	0.3	3	3
cis-9-dodecen-1-yl acetate	0.03	0.03	0.03	0.03	0.01	0.03	0.3	0.3
cis-9-tridecen-1-yl acetate	0.03	0.03	0.01	0.01	0.003	0.01	0.1	0.1
cis-9-tetradecen-1-yl acetate	0.01	0.01	**0.001**	**0.001**	**0.001**	**0.001**	0.01	0.01
cis-9-pentadecen-1-yl acetate	0.03	0.03	0.01	0.01	0.01	0.01	0.1	0.3
cis-9-hexadecen-1-yl acetate	0.1	0.03	0.1	0.1	0.03	0.03	0.3	3
cis-9-heptadecen-1-yl acetate	1	0.1	1		0.3	0.3	3	10
cis-9-octadecen-1-yl acetate	10	1	10		3	3	10	
cis-9-dodecen-1-yl propionate	1	0.3	0.3	1	0.1	0.3	1	1
cis-9-tridecen-1-yl propionate	1	0.3	0.1	0.3	0.03	0.1	0.3	0.3
cis-9-tetradecen-1-yl propionate	0.3	0.1	0.01	0.03	0.01	0.01	0.03	0.1
cis-9-pentadecen-1-yl propionate	1	0.3	0.1	0.3	0.1	0.1	0.3	1
cis-9-hexadecen-1-yl propionate	3	0.3	0.3	1	0.3	0.3	1	3
cis-9-dodecen-1-yl butyrate	3	1	1	3	0.1	1		3
cis-9-tridecen-1-yl butyrate	3	1	0.3	1	0.1	0.3	1	1
cis-9-tetradecen-1-yl butyrate	1	0.3	0.03	0.1	0.03	0.03	0.1	0.3
cis-9-pentadecen-1-yl butyrate	3	1	0.3	1	0.3	0.3	1	3
cis-9-hexadecen-1-yl butyrate	10	1	1	3	1			10
cis-9-dodecen-1-ol	0.3	0.3	0,.3	3	0.3	0.3	0.03	0.3
cis-9-tridecen-1-ol	0.3	0.3	0.1	1	0.1	0.1	0.01	0.1
cis-9-tetradecen-1-ol	0.1	0.1	0.01	0.1	0.03	0.01	**0.001**	0.01
cis-9-pentadecen-1-ol	0.3	0.3	0.1	1	0.3	0.1	0.01	0.3
cis-9-hexadecen-1-ol	1	0.3	0.3	3	1	0.3	0.03	3
methyl *cis*-9-dodecen-1-yl ether	3	0.3	1		0.3	1	0.3	10
methyl *cis*-9-tetradecen-1-yl ether	1	0.1	0.03		0.03	0.03	0.01	0.3
methyl *cis*-9-hexadecen-1-yl ether	10	0.3	1		1	1	0.7	10
ethyl *cis*-9-dodecen-1-yl ether	3	1	1		0.3	1	1	10
ethyl *cis*-9--tridecen-1-yl ether	3	1	0.3	1	0.1	0.3	0.3	3
ethyl *cis*-9-tetradecen-1-yl ether	1	0.3	0.03	0.1	0.03	0.03	0.03	1
ethyl *cis*-9-pentadecen-1-yl ether	3		0.3	1	0.3	0.3	0.3	3
ethyl *cis*-9-hexadecen-1-yl ether	10	1	1		1	1	1	
cis-9-dodecenal	0.3	0.3	1	3	0.3	0.3	0.3	0.3
cis-9-tetradecenal	0.1	0.1	0.03	0.1	0.03	0.01	0.01	0.01
cis-9-hexadecenal	3	0.3	1	3	1	0.3	0.3	1
cis-9- dodecenoic acid	10	3	3	10	3	3	10	10
cis-9-tetradecenoic acid	3	1	0.1	0.3	0.3	0.1	0.3	1
cis-9-hexadecenoic acid	10	3	10	10	10	3	10	
methyl *cis*-9-dodecenoate	1	0.3		3	0.03	0.3		10
methyl *cis*-9-tetradecenoate	0.3	0.1		0.1	0.03	0.03		0.3
methyl *cis*-9-hexadecenoate	3	0.3		3	1			10
ethyl *cis*-9-dodecenoate	1	1	1	3	0.3	0.3	1	10
ethyl *cis*-9-tridecenoate	1	1	0.3	1	0.1	0.3	0.3	3
ethyl *cis*-9-tetradecenoate	0.3	0.3	0.03	0.1	0.03	0.03	0.03	1
ethyl *cis*-9-pentadecenoate	1	1	0.3	1	0.3	0.3	0.3	3
ethyl *cis*-9-hexadecenoate	3	1	3	3	1		1	10

However, even if two species respond maximally to the same compound, there may be a considerable difference in the activity values to other structures, thus giving rise to response spectra which are not identical. This may be inferred, from Table I, with regard to positional isomers (*cf. Monima gracilis*

[Hadeninae] *vs Chersotis multangula* [Noctuinae]) as well as with regard to altered chain length (*cf. Panolis flammea vs Hyssia cavernosa* [both Hadeninae], or *M. gracilis vs Ch. multangula*). On the other hand, species may be found which, although from different subfamilies, not only show maximum response to the same compound but also almost identical response spectra (*Syngrapha variabilis* [Plusiinae] and *Amathes candelarum* [Noctuinae]; Table I).

Optimum position of the $-C=C-$ *double bond*

A rule can be deduced from the results of chain shortening and elongation: Generally, the loss in activity is least when the alkyl portion beyond the $-C=C-$ double bond remains constant.

This becomes evident by comparing the most effective position of the double bond in an altered chain with the position of the double bond for the compound listed at 0.001 in Table I: In order to elicit maximal responses following elongation of the chain by two methylene groups, it was necessary to shift the double bond from position 7 to 9 with *Trichoplusia ni, Pachetra fulminea, Syngrapha variabilis,* and *Amathes candelarum,* and from position 9 to 11 with *Panolis flammea, Hyssia cavernosa,* and *Acosmetia caliginosa.* Following reduction of the chain by two methylene groups, the most effective double bond position is found to be shifted from carbon 11 to carbon 9 with *Monima gracilis* and *Chersotis multangula.* Tridecen-1-yl acetates and pentadecen-1-yl acetates (not included in Table I) generally were most effective when the position of their double bond was intermediate between the optimum positions for the two adjacent even-numbered acetates [18]. Thus, defining n as the number of methylene groups between the terminal methyl group and the $-C=C-$ double bond, and m as the number of methylene groups between the $-C=C-$ double bond and the ester group

$$CH_3-(CH_2)_n-CH=CH-(CH_2)_m-O-Ac, \quad (1)$$

the influence on olfactory activity of any modification in chain length generally was more pronounced on altering n rather than m.

This rule was found, during these studies, to be applicable to a number of lepidopterous families (including Notodontidae, Cymatophoridae, Drepanidae, Cochlidiidae, Zygaenidae, Cossidae, Aegeriidae, Yponomeutidae, Gelechiidae, Pyralididae, Geometridae, Saturniidae, or Lasiocampidae) [18] in addition to the Noctuidae; it does not appear to have been noted before.

In view of this principle it is noteworthy that sex pheromone structures of closely-related species often appear to differ only in the m portion of the chain. Within various noctuid genera including *Chersotis* (Noctuinae), *Cosmia* (Cuculliinae), *Polia* and *Orthosia* (Hadeninae), or *Apamea, Oligia,* and *Arenostola* (Amphipyrinae), some species were found to be most responsive to *cis*-9-tetradecen-1-yl acetate whereas other species of the same genus preferred *cis*-11-hexadecen-1-yl acetate [18]. This suggests that a change of two methylene groups at m, with n constant, has occurred repeatedly during the course of evolution of this moth family.

Introducing a second $-C=C-$ *double bond*

Of the six structures thus far reported as major components of female pheromones of Noctuidae species (see page 283), three are doubly unsaturated. Thus, a mono-unsaturated compound can only be regarded as the most potent for a given species after the effect of double unsaturation has been carefully investigated. This was attempted by keeping chain length, end group, and one $-C=C-$ double bond constant and introducing a second $-C=C-$ double bond, either *cis* or *trans,* at varying positions.

This kind of study is about to produce increasing evidence that, for the majority of the 200 noctuid species investigated, more than 1 double bond will be required in order to duplicate the outstanding EAG effect shown by the natural sex pheromone (as derived from the female gland). A 10 fold increase in EAG activity due to an additional *trans* double bond is seen, *e. g.*, in the response of *Spodoptera eridania* (Amphipyrinae) to *cis*-9, *trans*-12-tetradecadien-1-yl acetate (Table I), the major component of its female sex pheromone [7].

With the 15 other species listed (Tables I and II), however, introducing a second $-C=C-$ double bond consistently lowered the EAG response.

Replacement of the $-C=C-$ *double bond*

Whereas the conversion to the opposite geometric isomer reduced EAG activity only between 1.8 to 5.6 fold (see above), replacement of the double bond by, *e. g.*, a $-C\equiv C-$ triple bond, a $-C-C-$ single bond, or the $-\overset{O}{\overset{/\backslash}{C}}-C-$ epoxide resulted in

a 100 to 3000 fold decrease in EAG activity with the noctuid species considered here. This is the same order of magnitude that had been observed after maximally shifting the double bond from the optimum position (see above).

Side chains

These effects were investigated by keeping chain length, double bond(s), and end group constant and introducing, at varying positions, methyl, ethyl, or propyl branches. The observed reduction in EAG activity was consistently small; *e. g.*, introducing a methyl or ethyl side group at carbons 11, 12, or 13 of a *cis*-9-monounsaturated acetate usually reduced its potency by only 1.8 to 5.6 fold [18]. No noctuid species was found for which a branched chain was more effective than the unbranched chain of same length.

Varying the functional end group

All the compounds listed in Table I are acetates, the structure eliciting the highest EAG responses with all the species mentioned thus far. The effects of formates, propionates, butyrates, alcohols, ethers, aldehydes, acids, and acid esters, in comparison with the corresponding acetate, are presented for eight Noctuidae species (Table II). In these compounds, the double bond is constant at *cis*-9, whereas the chain length is varied between 12 (11) and 16 (18).

On comparison of any two of these end group variations, the activity values shown by a given species can be observed to be in a constant proportion, almost unaffected by variations in chain length. *E. g.*, in *Polia pisi* (Hadeninae), the acetate: formate ratio remains at approx. 30, and the acetate: propionate ratio at approx. 10, when n in Eqn (1) (*i. e.*, the number of methylene groups beyond the $-C=C-$ double bond) is varied from 1 to 5 (Table II). For the eleven structures and eight species listed, these ratios range between 1 and 1000 (Table II).

Four of these species (*H. cavernosa, P. nana, P. pisi, C. umbratica*) responded maximally to *cis*-9-tetradecen-1-yl acetate. Of these, *Hyssia cavernosa* (Hadeninae) and *Cucullia umbratica* (Cuculliinae) show an almost identical set of activity values in regard to the varied end groups (Table II). More commonly, however, among species maximally responsive to the same compound these patterns differed appreciably; *e. g.*, the acetate: alcohol ratio is 10 in *H. cavernosa* and *C. umbratica*, but 30 in *P. pisi*, and 100 in *P. nana* (Table II). Thus, in addition to alterations of the hydrocarbon chain, as seen in Table I, varying the end group is another means of disclosing subtle receptor differences among species which seem to share the same pheromone.

The preferred end group is subject to successive evolutionary change. The often-observed [18] acetate-alcohol transition is exemplified by two species of *Cucullia* (Table II). Formates, not previously reported as insect pheromones, proved in this study to be most effective on various noctuid species including the corn earworm, *Heliothis zea* (Heliothidinae) (Table II). Certain other structures such as ethers and acid esters (Table II), or ketones (not listed), all unknown as lepidopterous pheromones, elicited moderate EAG responses with many test species; however, no species was found in which one of these structures was the most effective. Acids, on the other hand, were least potent with all test species. Summarizing the end group preferences found with Noctuinae, Hadeninae, Amphipyrinae, Cuculliinae, Plusiinae, and Heliothidinae (the six noctuid subfamilies considered here), the acetate was the most common structure, followed by primary alcohols, aldehydes, formates, certain other esters, and olefins, successively [18].

It should be noted that although the above remarks concerned only the *cis*-9-unsaturated analogues, about the same ratios of activity values among the end group structures were observed [18] within the series unsaturated at the *cis*-7, the *cis*-8, the *cis*-10, or the *cis*-11 position, respectively.

When elongating the acid moiety of the alcohol esters by one-carbon units, each step changed EAG activity between 1.8 to 56 fold (*cf.* the values listed for formates, acetates, propionates, and butyrates; Table II). This is the same order of magnitude which was observed (see above) after the hydrocarbon part of the test compounds had been elongated by 1 methylene group.

Discussion

Electroantennogram (EAG) data reflecting the response of pheromone receptor cells have been presented for males of sixteen species of Noctuidae (Lepidoptera).

In each species, only a single compound was maximally effective; the other compounds required between 1.8 to approx. 10000 times higher amounts, to elicit the same EAG amplitude at the same conditions of stimulation (values 0.003 through 10 in Tables I and II). It seems reasonable to ascribe this outstanding effect shown by a single molecular structure to an optimum combination of submolecular features (such as chain length, double bond position, or end group). Thus, by changing one of these parameters systematically while keeping others constant, we attempted to assess its contribution to the total effect. By this procedure, EAG response spectra were produced, sections of which are shown here (Tables I and II).

Discussion will focus on the use of such data a. for disclosing structural properties of as yet unidentified pheromones, b. for describing phenomenologically the similarities and differences in receptor responses among species, and c. for calculating physical properties of underlying acceptor structures, Also, the relationships between EAG activity and behavioural activity will be briefly considered.

The most effective compound

In the Lepidoptera Heterocera (commonly referred to as moths), all EAG studies thus far reported support the conclusion that certain male receptors are maximally responsive for the pheromone of the conspecific female; this refers to cross-checking female gland extracts [21, 23] as well as to comparing a synthetic pheromone with its structural derivatives. Consequently, EAG responses to altered compounds offer valuable indications to the structure of as yet unidentified pheromones, a tool successfully employed by Roelofs and his coworkers with various species of Tortricidae [13, 16]. This method has proved equally fruitful in the present family, the Noctuidae.

Once the identity of such a structure has been suggested, it may then be further corroborated, or eventually disproved, by a variety of techniques. Here again the EAG provided a promising new method; it is based on exposing, in parallel, both the candidate chemical and the natural pheromone extract to the antennal preparations of an array of moth species, thus using the discriminatory powers of the different receptors for detecting differences in the composition of the two stimuli. Indeed, by this method, most of the suggested identities were easily disproved. With regard to the sixteen species mentioned in this report, it is noteworthy that the compound listed as most effective (value 0.001 in Tables I and II) and the respective natural female pheromone both elicited roughly the same pattern of EAG responses [18]. Moreover, when two or more species responded maximally to the same compound (Tables I and II), it was satisfying to find that their natural pheromones showed full interspecific EAG effectiveness when cross-checked quantitatively [21, 23]. Nonetheless, we emphasize the distinction between a pheromone structure thus suggested, and one identified by chemical analysis.

Differences and similarities in EAG response spectra

By modifying, stepwise, the most effective compound, response spectra were obtained (Tables I and II).

When considering these EAG activity values, it must be borne in mind that they reflect amounts of substance at source (over which a defined air current was passed onto the antenna). The values thus do not indicate relations in amounts leaving the source, adsorbed on the antennae, or conducted to the target areas at the sensory cell membrane. These factors must be considered when using the data for an attempt such as to derive underlying acceptor properties (see below). However, as the stimulatory arrangement was identical with all the test species, several statements may be made on the basis of these activity values.

As can be seen, a comparable structural modification (such as the shifting of the double bond by 1 carbon) affects EAG activity within the same order of magnitude, in all the test species. Moreover, rules governing structure-response relationships could be attributed to all species using a similar type of pheromone (such as a monounsaturated ester); one of these rules concerned the optimum position of the double bond in relation to chain length (see page 288), and another one the ratios in responses to varied end groups (see page 289). Such rules, all reported here for the first time, suggest that a homogeneous population of olfactory acceptors (as opposed to a heterogeneous one) determines the EAG response, in each species (see last chapter). Governed by these rules, two categories of response differences among species could be defined; the one includes species which use

different pheromones whereas the other category is of species for which the same compound is most effective.

The first category is well known and implies that any change in the main component of the female pheromone is concomitant with a change in the maximum responsiveness of certain male receptors. Not hitherto recognized is the second category. Here, species respond maximally to the same compound, yet differ appreciably in their response spectra (see page 287 and 289). This indicates that in category two the pheromone acceptors are also species specific.

Electroantennogram response and behavioural activity

When presented under appropriate conditions, the single synthetic compound producing the highest EAG usually is found to elicit behavioural responses in the male typical of the natural pheromone, *i. e.* increased motor activity, attraction towards the stimulus source, and/or precopulatory behaviour. There are noctuid species in which all of these behavioural steps can be activated by increasing amounts of the same single compound, such as *cis*-7-dodecen-1-yl acetate in the male cabbage looper (*Trichoplusia ni*)[1, 24, 25]. With certain other noctuid species, secondary (synergistic) pheromonal components are required in order to elicit the full sequence of behavioural events. Even in species in which such tests have not yet been undertaken, the EAG data often suggest that the species would fit into a pattern already known for a taxonomically closely-related species: *e. g.* in *Amathes candelarum* (a European species), the most effective compound, *cis*-7-tetradecen-1-yl acetate (Table I) is the same as reported[12, 13] to be specifically attractive for certain North American representatives of this genus; the same holds true for certain European and American species of *Cucullia, Polia,* and *Apamea* in response to *cis*-9-tetradecen-1-yl acetate and *cis*-11-hexadecen-1-yl acetate, respectively.

On the other hand, no generalization with respect to behavioural effects can be made for the various analogues which elicit high although not maximal EAG responses. In certain non-noctuid species not only were such compounds found to activate behavioural responses similar to the natural pheromone, but also the amounts necessary to produce a defined behavioural event roughly corresponded to those used in EAG and single cell measurements; examples include the silk moth (*Bombyx mori*)[20, 21] (Bombycidae) and the gypsy moth (*Porthetria dispar*)[26–31] (Lymantriidae). This pattern has so far not been found in any noctuid species. Here, usually several pheromone analogues were also effective behaviourally but to a much lesser degree than would be expected from the EAG data (*i. e.*, to give an equivalent response, a certain analogue may require, say, 10 times the amount of the natural pheromone in the electrophysiological but 1000 times in the behavioural test). This is the pattern reported, *e. g.*, for the cabbage looper (*Trichoplusia ni*)[32–37]. It indicates that equally high EAG amplitudes must not necessarily imply equal sensory input.

Moreover, there is evidence that not all types of olfactory receptor cells responding to pheromone stimuli contribute to the EAG. In *Trichoplusia ni*, the female sex pheromone (*cis*-7-dodecen-1-yl acetate) consistently elicits the highest EAG whereas the corresponding alcohol (not reported as being produced by the female) required approx. 10 times higher loads to give an equivalent EAG response (Table II). A mixture of the two at the ratio of 95:5 elicits male EAGs not statistically different from those produced by pure *cis*-7-dodecen-1-yl acetate[39]. During field tests, however, the same mixture attracted only 3% of the males in comparison to the same amount of pure acetate[39–41], indicating sensory information in addition to that reflected by the EAG. In fact, during the course of single cell recordings from sensilla trichodea in male *T. ni* (to be published), the most abundant cell type showed a response spectrum consistent with the EAG, whereas another type of cell ('alcohol receptor') was more excited by the attraction-inhibiting alcohol, than by the pheromone[18, 41, 42]. (It is noteworthy that this alcohol is the reported main product of enzymatic degradation of the pheromone by male antennal proteins[39, 43, 44].)

This is similar to data recently found in the gypsy moth (*Porthetria dispar*). The sex pheromone of this species is 2-methyl-*cis*-7,8-epoxy-octadecane[45]. Also present in female gland extracts is the corresponding olefine, 2-methyl-*cis*-7-octadecene, the presumed pheromone precursor[45–48]. There was close correspondence between the EAG and the single cell responses to 55 related epoxides[26–30]. For most cells, this correspondence was also found with the

olefine; however, a few cells ('olefine receptor') were detected [29] for which the olefine was even more excitatory than the pheromone. As in the case of the behaviourally-inhibiting alcohol in the cabbage looper (see above), the olefine by itself elicited almost no observable behavioural response, yet strongly reduced male attraction when traces of it were added to the pheromone [28, 45–47].

The above examples illustrate some of the pitfalls one may encounter when trying to predict pheromonal behaviour from limited electrophysiological information.

Pheromone molecule-acceptor interactions

The electrophysiological data reported here provide a basis for calculating physico-chemical properties of acceptor structures underlying the response.

Several tentative conclusions on the possible mode of action of the test compounds may be made already on the basis of a phenomenological examination of the activity values. Although only a single compound was maximally effective in a given species, the majority of the other structures showed equivalent efficacy after the amounts were increased only 1.8 to 56 fold (values 0.003, 0.01 and 0.03 in Tables I and II); this points to a broad mutual affinity for the acceptors involved. Also, it is obvious that certain kinds of structural modifications (*e. g.*, the conversion to the opposite geometric isomer) affect EAG activity to about the same extent in different species, whereas for certain other kinds of modifications (*e. g.*, positional isomers) the activity ratios are subject to variation among species. To determine the exact role of the spatially-arranged electron densities and mobilities in producing the activities measured electrophysiologically, electronic properties of test compounds had to be related to those of a hypothetical counterpart, using simplified models of pheromone molecule-acceptor interaction.

Kafka and Neuwirth have applied [49, 50], to compounds and receptors used in the present study, a mathematical formulation appropriate to describe the electrophysiological activities in terms of binding probabilities. Their formulation is based on a three-point attachment [51, 52] in which, in each species, the spatial arrangement of three acceptor positions corresponds to electronically pronounced positions (*i. e.*, the terminal CH_3 group; the $-C=C-$ double bond; the functional end group) of the most effective compound (values 0.001 in Tables I and II) in its thermodynamically most favoured conformer. The values for electron polarisabilities and dipole moments at these three acceptor positions were then determined by fitting, for a set of test compounds, Boltzmann statistics with electrophysiological activities. Mathematical procedures and acceptor data are presented in their paper [50]. The method was able to predict activity values for various additional compounds in full correspondence with the experimental (electrophysiological) data.

Although their procedure rendered acceptor values of a magnitude reasonable for functional groups [30, 49, 50], the Kafka-Neuwirth model must be considered as a simplified, abstract approach to the actual acceptor structures. Nonetheless, it seems appropriate in that it reduces the redundancy apparent in the electrophysiological data, delineating species differences in these data (Tables I and II) as species differences in a few spatially and electronically defined positions in a hypothetical binding partner.

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[1] R. S. Berger, Ann. Entomol. Soc. Amer. **59**, 767 [1966].
[2] J. H. Tumlinson, E. R. Mitchell, S. M. Browner, and D. A. Lindqvist, Envir. Entomol. **1**, 466 [1972].
[3] A. N. Sparks, Entomol. Soc. Amer. Nat. Meeting 1973, Paper No. 63.
[4] J. H. Tumlinson, D. E. Hendricks, E. R. Mitchell, and R. E. Doolittle, Chem. Engin. News, May 20, 1974.
[5] W. L. Roelofs, A. S. Hill, R. T. Cardé, and T. C. Baker, Life Sci. **14**, 1555 [1974].
[6] A. A. Sekul and A. N. Sparks, J. Econ. Entomol. **60**, 1270 [1967].
[7] M. Jacobson, R. E. Redfern, W. A. Jones, and M. H. Aldridge, Science **170**, 542 [1970].
[8] U. E. Brady and M. C. Ganyard, Ann. Entomol. Soc. Amer. **65**, 898 [1972].
[9] B. F. Nesbitt, P. S. Beevor, R. A. Cole, R. Lester, and R. G. Poppi, Nature New Biol. **244**, 208 [1973].
[10] Y. Tamaki, H. Noguchi, and T. Yushima, Appl. Entomol. Zool. **8**, 200 [1973].
[11] Y. Tamaki and T. Yushima, J. Insect Physiol. **20**, 1005 [1974].
[12] W. L. Roelofs and A. Comeau, J. Econ. Entomol. **63**, 969 [1970].

[13] W. L. Roelofs and A. Comeau, Pesticide Chemistry, **Vol. III** (A. S. Tahori, ed.), p. 91, Gordon & Breach, New York 1971.
[14] D. G. Campion, Misc. Rep. No. 4, Centre Overseas Pest Res., London 1972.
[15] R. S. Kaae, H. H. Shorey, S. U. McFarland, and L. K. Gaston, Ann. Entomol. Soc. Amer. **66**, 444 [1973].
[16] W. L. Roelofs and R. T. Cardé, Frontiers of Biology, **Vol. XXXII** (M. C. Birch, ed.), p. 96, North-Holland Publ. Co., Amsterdam-London 1974.
[17] D. Schneider, Z. vergl. Physiol. **40**, 8 [1957].
[18] E. Priesner, unpublished results.
[19] W. Forster and Th. A. Wohlfahrt, Die Schmetterlinge Mitteleuropas, **Vol. III**, Franckh'sche Verlaghandlung, Stuttgart 1971.
[20] D. Schneider, B. Block, J. Boeckh, and E. Priesner, Z. vergl. Physiol. **54**, 192 [1967].
[21] E. Priesner, Fortschritte Zool. **22**, 49 [1973].
[22] M. Jacobson, B. J. Landis, D. E. Hendricks, E. Priesner, and H. J. Bestmann, Chem. Engin. News, Dec. 19, 1972.
[23] E. Priesner, Z. vergl. Physiol. **61**, 263 [1968].
[24] H. H. Shorey, Annu. Rev. Entomol. **18**, 349 [1973].
[25] M. S. Mayer, Ann. Entomol. Soc. Amer. **66**, 1191 [1973].
[26] V. E. Adler, M. Beroza, B. A. Bierl, and R. Sarmiento, J. Econ. Entomol. **65**, 679 [1972].
[27] R. Sarmiento, M. Beroza, B. A. Bierl, and J. G. R. Tardif, J. Econ. Entomol. **65**, 665 [1972].
[28] D. Schneider, R. Lange, F. Schwarz, M. Beroza, and B. A. Bierl, Oecologia **14**, 19 [1974].
[29] W. A. Kafka, D. Schneider, M. Beroza, and B. A. Bierl, in preparation.
[30] W. A. Kafka, Ann. N. Y. Acad. Sci. **237**, 115 [1974].
[31] W. A. Kafka and J. Scheffler, in preparation.
[32] R. S. Berger and T. D. Canerday, J. Econ. Entomol. **61**, 452 [1968].
[33] M. Jacobson, H. H. Toba, J. Debolt, and A. N. Kishaba, J. Econ. Entomol. **61**, 84 [1968].
[34] M. Jacobson, N. Green, D. Warthen, C. Harding, and H. H. Toba, Chemicals Controlling Insect Behavior (M. Beroza, ed.), p. 3, Academic Press, New York-London 1970.
[35] H. H. Toba, N. Green, A. N. Kishaba, M. Jacobson, and J. W. Debolt, J. Econ. Entomol. **63**, 1048 [1970].
[36] L. K. Gaston, T. L. Payne, S. Takahashi, and H. H. Shorey, Olfaction and Taste, **Vol. IV** (D. Schneider, ed.), p. 167, Wiss. Verlagsges., Stuttgart 1972.
[37] M. Jacobson, Insect Sex Pheromones, Academic Press, New York-London 1972.
[38] T. L. Payne, H. H. Shorey, and L. K. Gaston, Ann. Entomol. Soc. Amer. **66**, 703 [1973].
[39] M. S. Mayer, J. Insect Physiol. **19**, 1191 [1973].
[40] J. H. Tumlinson, E. R. Mitchell, S. M. Browner, M. S. Mayer, N. Green, R. Hines, and D. A. Lindqvist, Envir. Entomol. **1**, 354 [1972].
[41] J. R. McLaughlin, E. R. Mitchell, D. L. Chambers, and J. H. Tumlinson, Envir. Entomol. **3**, 677 [1974].
[42] M. S. Mayer, in preparation.
[43] S. M. Ferkovitch, M. S. Mayer, and R. R. Rutter, Nature **242**, 54 [1973].
[44] S. M. Ferkovitch, M. S. Mayer, and R. R. Rutter, J. Insect Physiol. **19**, 2231 [1973].
[45] B. A. Bierl, M. Beroza, and C. W. Collier, Science **170**, 87 [1970].
[46] B. A. Bierl, M. Beroza, and C. W. Collier, J. Econ. Entomol. **65**, 659 [1972].
[47] R. T. Cardé, W. L. Roelofs, and C. C. Doane, Nature **241**, 474 [1973].
[48] G. Kasang, D. Schneider, and M. Beroza, Naturwissenschaften **61**, 130 [1974].
[49] W. A. Kafka, Biochemistry of Sensory Functions (L. Jaenicke, ed.), p. 275, Springer-Verlag, Berlin-Heidelberg-New York 1974.
[50] W. A. Kafka and J. Neuwirth, this issue.
[51] L. H. Easson and E. Stedman, Biochem. J. **27**, 1257 [1933].
[52] P. S. Portoghese, Annu. Rev. Pharmacol. **10**, 51 [1970].