Sensory Efficacy of Alkyl-Branched Pheromone Analogues in Noctuid and Tortricid Lepidoptera *

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The effect of introducing alkyl substituents (methyl to pentyl) to the chain segment n in long-chain alkenyl acetates, $CH_3(CH_2)_nCH=CH(CH_2)_m$-O-$COCH_3$ (**I**), was investigated in pheromone receptors of Noctuidae, Tortricidae, and Cochylidae species. The receptor types studied were maximally responsive to (*Z*)-7- or (*Z*)-9-dodecen-1-yl acetate, (*Z*)-7-, (*Z*)-9-, or (*Z*)-11-tetradecen-1-yl acetate, or (*Z*)-11-hexadecen-1-yl acetate, representing key compounds with $n=1$, $n=3$, or $n=5$ methylene groups.

In terms of the relative stimulus amounts required to elicit equivalent EAG amplitudes, the efficacy of the alkyl-branched derivatives was between 1/1000 to 300 times that of the unbranched chain of the same length and double bond position. The effects of branching were specific to the type of receptor, the length and double bond position of the parent chain, and the kind and position of the alkyl substituent. The most pronounced increase observed occurred with receptors for $n=1$ type pheromones, (*Z*)-9-dodecen-1-yl acetate and (*Z*)-11-tetradecen-1-yl acetate when an α-methyl group was introduced to elongated derivatives; whereas the greatest decreasing effects were obtained on receptors for the $n=5$ type compound, (*Z*)-7-tetradecen-1-yl acetate. The results show basic differences in structure-response relationships between the Noctuidae *vs* Tortricidae receptors studied.

Various possible effects of the substituent groups during sensory transduction are considered. The data should contribute to further elucidation of interaction mechanisms of unbranched alkenyl acetate pheromones (**I**) with insect olfactory receptors.

With few exceptions, chemically identified sex pheromones in female Lepidoptera are long-chain, olefinic alcohols, aldehydes, or esters. So far, the known variations in these compounds occur in terms of chain length, functional end groups, and the number, position, and configuration of double bonds. The present study continues investigations aimed at elucidating the mechanisms of reception of these messenger compounds by sensory cells.

Previously [1] it was shown for receptors maximally responsive to certain alkenyl acetate pheromones of the general formula

$$CH_3(CH_2)_nCH=CH(CH_2)_m-O-COCH_3 \quad (\mathbf{I})$$

that the electrophysiologically-determined efficacy of stimulant compounds generally is reduced more by a variation of the number of methylene groups in the apolar end alkyl portion of the chain (segment n in **I**) than between the olefinic double bond and the ester group (segment m in **I**). This relationship applies, without exception, to male receptor responses in test species from Noctuidae [1] and certain other lepidopteran families.

As a consequence, the end alkyl part in these esters was subjected to additional, stepwise modifications, appropriate to permit further conclusions on mechanisms of interaction with receptor sites. Here we report on the comparative effects of introducing *alkyl side chains* (of varying size and position) to the n part of these molecules (**I**). The following results refer in particular to receptors specialized for alkenyl acetate pheromones with respectively, 1, 3, or 5 methylene groups in the end alkyl part (**I**, $n=1$, 3, or 5).

Test Species and Receptor Types

The major results of this study are described by the use of six pairs of test species, each pair representing receptors maximally responsive to the same alkenyl acetate component (Table I). These

* Pheromones XIV (for XIII see ref. 8).

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Table I. Test species (N, Noctuidae; C, Cochylidae; T, Tortricidae) and receptor key compounds. Species where the key compound has been reported as the major component of the female sex pheromone are indicated by the appropriate reference.

Trichoplusia ni Hbn.[2] (N) *Autographa pulchrina* Haw. (N)	(*Z*)-7-dodecen-1-yl acetate (*Z*7-12:Ac)
Clysia ambiguella Hbn.[3] (C) *Paralobesia viteana* Clem.[4] (T)	(*Z*)-9-dodecen-1-yl acetate (*Z*9-12:Ac)
Syngrapha variabilis Pill. (N) *Amathes candelarum* Stgr. (N)	(*Z*)-7-tetradecen-1-yl acetate (*Z*7-14:Ac)
Cucullia umbratica L. (N) *Polia pisi* L. (N)	(*Z*)-9-tetradecen-1-yl acetate (*Z*9-14:Ac)
Argyrotaenia velutinana Walk.[5] (T) *Choristoneura rosaceana* Harr.[6] (T)	(*Z*)-11-tetradecen-1-yl acetate (*Z*11-14:Ac)
Mamestra configurata Walk.[7] (N) *Monima gracilis* Schiff. (N)	(*Z*)-11-hexadecen-1-yl acetate (*Z*11-16:Ac)

six 'key compounds', all acetates, have alcohol moieties of 12, 14, or 16 carbons, and a single (*Z*) double bond at position 7, 9, or 11; *viz.* (*Z*)-7- and (*Z*)-9-dodecen-1-yl acetate, (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-tetradecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate (Table I). For *T. ni*, *C. ambiguella*, *P. viteana*, *A. velutinana*, *C. rosaceana*, and *M. configurata*, the indicated compound is the reported major component of the female sex pheromone[2–7], whereas this identity is based so far on indirect evidence for the other species[1]. The twelve species belong to three families, the Tortricidae (*P. viteana*, *A. velutinana*, and *C. rosaceana*), the Cochylidae (*C. ambiguella*), and the Noctuidae (remaining species).

Additional test species, using either the same or structurally related acetate pheromones, will be mentioned in the text.

Test Compounds

The structural analogues considered in this report were all derived from the six alkenyl acetates listed in Table I. For the three series of double bond position, (*Z*)-7, (*Z*)-9, and (*Z*)-11, modifications were restricted to the apolar end alkyl part (*n* part in **I**) of the chain. These modifications comprise methyl, ethyl, n-propyl, and n-pentyl side chains in positions α, β, and/or γ relative to the (*Z*) double bond (Table II); and a few analogues in which the end alkyl part had been replaced by a cyclohexyl

Table II. Test compounds. Alkyl-branched analogues of (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-alkenyl acetates.

Substituent	Formula	Name of compound	Abbreviation
α-methyl	$CH_3>CH-CH=CH-(CH_2)_{8,10}-OAc$ CH_3	11-methyl-(*Z*)-9-dodecen-1-yl acetate	11me-*Z*9-12:Ac
		13-methyl-(*Z*)-11-tetradecen-1-yl acetate	13me-*Z*11-14:Ac
α-methyl	$CH_3-CH_2>CH-CH=CH-(CH_2)_{8,10}-OAc$ CH_3	(*R,S*)-11-methyl-(*Z*)-9-tridecen-1-yl acetate	11me-*Z*9-13:Ac
		(*R,S*)-13-methyl-(*Z*)-11-pentadecen-1-yl acetate	13me-*Z*11-15:Ac
α-methyl	$CH_3-(CH_2)_2>CH-CH=CH-(CH_2)_{6,8,10}-OAc$ CH_3	(*R,S*)-9-methyl-(*Z*)-7-dodecen-1-yl acetate	9me-*Z*7-12:Ac
		(*R,S*)-11-methyl-(*Z*)-9-tetradecen-1-yl acetate	11me-*Z*9-14:Ac
		(*R,S*)-13-methyl-(*Z*)-11-hexadecen-1-yl acetate	13me-*Z*11-16:Ac

Table II (continued)

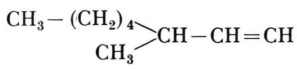
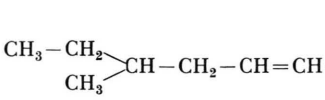
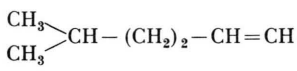
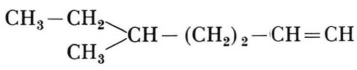
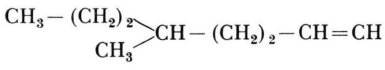
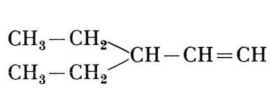
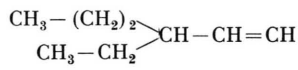
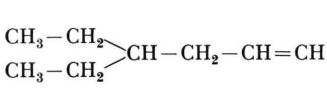
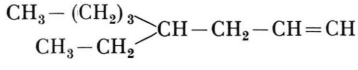
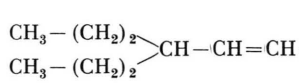
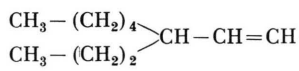
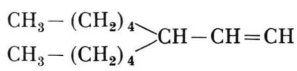
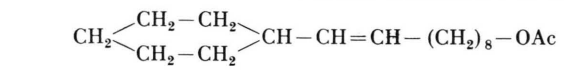
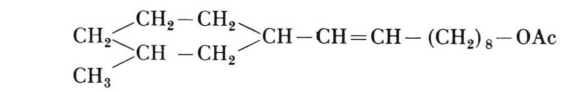
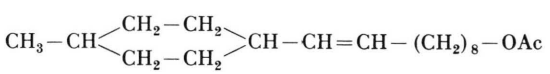
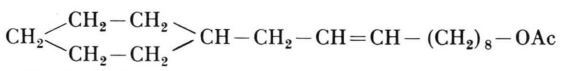
Substituent	Formula	Name of compound	Abbreviation
α-methyl		(*R,S*)-11-methyl-(*Z*)-9-hexadecen-1-yl acetate	11me-Z9-16:Ac
β-methyl		(*R,S*)-10-methyl-(*Z*)-7-dodecen-1-yl acetate	10me-Z7-12:Ac
		(*R,S*)-12-methyl-(*Z*)-9-tetradecen-1-yl acetate	12me-Z9-14:Ac
		(*R,S*)-14-methyl-(*Z*)-11-hexadecen-1-yl acetate	14me-Z11-16:Ac
γ-methyl		11-methyl-(*Z*)-7-dodecen-1-yl acetate	11me-Z7-12:Ac
		13-methyl-(*Z*)-9-tetradecen-1-yl acetate	13me-Z9-14:Ac
		15-methyl(*Z*)-11-hexadecen-1-yl acetate	15me-Z11-16:Ac
γ-methyl		(*R,S*)-13-methyl-(*Z*)-9-pentadecen-1-yl acetate	13me-Z9-15:Ac
γ-methyl		(*R,S*)-11-methyl-(*Z*)-7-tetradecen-1-yl acetate	11me-Z7-14:Ac
		(*R,S*)-13-methyl-(*Z*)-9-hexadecen-1-yl acetate	13me-Z9-16:Ac
α-ethyl		11-ethyl-(*Z*)-9-tridecen-1-yl acetate	11et-Z9-13:Ac
		13-ethyl-(*Z*)-11-pentadecen-1-yl acetate	13et-Z11-15:Ac
α-ethyl		(*R,S*)-11-ethyl-(*Z*)-9-tetradecen-1-yl acetate	11et-Z9-14:Ac
β-ethyl		12-ethyl-(*Z*)-9-tetradecen-1-yl acetate	12et-Z9-14:Ac
		14-ethyl-(*Z*)-11-hexadecen-1-yl acetate	14et-Z11-16:Ac
β-ethyl		(*R,S*)-12-ethyl-(*Z*)-9-hexadecen-1-yl acetate	12et-Z9-16:Ac
α-propyl		9-propyl-(*Z*)-7-dodecen-1-yl acetate	9pr-Z7-12:Ac
		11-propyl-(*Z*)-9-tetradecen-1-yl acetate	11pr-Z9-14:Ac
		13-propyl-(*Z*)-11-hexadecen-1-yl acetate	13pr-Z11-16:Ac
α-propyl		(*R,S*)-11-propyl-(*Z*)-9-hexadecen-1-yl acetate	11pr-Z9-16:Ac
α-pentyl		9-pentyl-(*Z*)-7-tetradecen-1-yl acetate	9pe-Z7-14:Ac
		11-pentyl-(*Z*)-9-hexadecen-1-yl acetate	11pe-Z9-16:Ac

Table III. Test compounds. Cyclohexyl analogues of (*Z*)-9 alkenyl acetates.

Formula	Name of compound
	10-cyclohexyl-(*Z*)-9-decen-1-yl acetate
	10-(3-methylcyclohexyl)-(*Z*)-9-decen-1-yl acetate *
	10-(4-methylcyclohexyl)-(*Z*)-9-decen-1-yl acetate *
	11-cyclohexyl-(*Z*)-9-undecen-1-yl acetate

* Configuration of ring substituents not specified.

ring Table III). For comparison with these branched and cyclic analogues, test results are included for the homologous straight-chain (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-alkenyl acetates with 12 to 16 carbon atoms in the alcohol moiety (dodecenyl to hexadecenyl acetates in Tables IV to VI).

For these straight-chain homologues, syntheses and selected electrophysiological data have been reported [1, 8–10]. The branched and cyclic analogues (Table II and III) have to our knowledge not been prepared previously. They were synthesized by (*Z*)-stereoselective Wittig olefination [10] of branched or cyclic alkylidene-triphenylphosphoranes by ethyl 6-formyl hexanoate, 8-formyl octyl acetate, and 10-undecenal. 8-Formyl octyl acetate gave (*Z*)-9-alkenyl acetates directly; the ethyl (*Z*)-7-alkenoate esters from ethyl 6-formyl hexanoate were reduced and acetylated to give the corresponding (*Z*)-7-alkenyl acetates, and the 1,11-alkadienes derived from 10-undecenal were selectively hydroborated at the terminal double bond, oxidative work-up and acetylation giving the corresponding (*Z*)-11-alkenyl acetates.

The amount of (*E*) isomer was up to 6% for the α-branched compounds, and up to 3% for the β- and γ-branched ones. In consistence with the synthetic route, no detectable trace of unbranched alkenyl acetates could be found in the samples used.

Methods

The techniques of stimulation, recording, and evaluation of the relative efficacies of test compounds were essentially the same as in earlier studies on the same types of receptors [1]. Air currents of 1.0 m/sec were directed on to the antennal preparation for 1.0 sec after they had passed the stimulus source. The amount of substance at source was increased, in log or half log steps, from 10^{-5} μg to 10^{2} μg. Here only the amplitude of summated slow receptor potentials (EAGs) is evaluated as the measure of cell excitation. Amounts of 10^{-3} μg to 10^{-1} μg of the single compound most effective for the respective receptor (see Table I) served as the internal standards; these standards were offered continuously throughout the course of an experiment and were used to correct for inter-individual and time-dependent alterations in responsiveness of the preparations [1]. For a given receptor, a certain analogue was tested between 8 to 30 times (with 2 or 3 steps in concentration) in comparison to the standard compound.

For each compound and receptor, the relative efficacy is presented by a single numerical value. These *activity values* [1] reflect, in half decadic steps, the stimulus amounts (in μg at source) required to elicit response amplitudes equivalent to 0.001 μg of the most efficacious (standard, key) substance. For this half log scale, compounds required in amounts of 0.0018 to 0.0056 μg will be listed by value '0.003'; those required in amounts of 0.0056 to 0.018 μg, by '0.01'; etc. The total set of the activity values for a single receptor will be referred to as its *response spectrum*.

Sections of response spectra (determined in the same manner) reported earlier for receptors of the same species [1] can be combined directly with the present data, thus extending certain response spectra to more than 200 test compounds. Furthermore, by applying the same absolute scale to all species, any two activity values can be directly compared not only within the same response spectrum but also with respect to the relative effect of the same compound on different species.

Results

Straight-chain homologues

In the upper sections of Tables IV to VI, activity values are given for the homologous (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-alkenyl acetates, respectively, over a range of chain length from $C_{12,13}$ to C_{16} (**I**, $n = 0$ to 7). In these homologous series, starting from the most efficacious structure (value 0.001) the response gradually decreases with stepwise elongation or shortening of the chain, as previously described [1]. The magnitude of this decrease usually is in the order of a factor of 10 to 30 per methylene unit when altering the key molecule, and of a factor of 3 to 10 between the less stimulatory homologues.

The unbranched and the branched compounds listed in the same Table have the same position of the (*Z*) double bond. Changes in stimulatory efficacy due to an alkyl side chain may thus be expressed by the quotient of the activity values between a given branched analogue and the unbranched homologue of same chain length.

Alkyl-branched analogues

In each of the Tables IV to VI, the results of the branched derivatives having the same position of the (*Z*) double bond are presented following the series of homologues. If for all the compounds and species in the Tables we briefly summarize the above quotients of activity values (between a given branched compound and the corresponding unbranched one), it appears that in 21 cases this quotient is close to 1 (same half log activity class), whereas in 110 cases the branched compound is less stimulatory, and in 18 cases more stimulatory, than the unbranched one. These results will be specified as follows with respect to receptor types, the different kinds of alkyl substituents, and the chain length of the test molecules as compared to the key compound (pheromone).

Alkyl branching in the key molecule

For each test species, a single compound, known or suspected to be a component of the female sex pheromone, elicited the standard response at the lowest amount of 0.001 μg. As with various other structural modifications [1], alkyl side chains also reduced the sensory response to a greater extent when applied to this key compound, as compared to the effects of the same substituents on shortened or elongated chains (see following sections).

*Z*9-12:Ac and *Z*11-14:Ac are the two pheromone examples with a single methylene group in their end alkyl part (**I**, $n = 1$). Here, the only alkyl sub-

Table IV. Stimulatory efficacy of alkyl-branched versus straight-chain (*Z*)-7-alkenyl acetates for pheromone receptors of six species of male noctuid Lepidoptera. The values indicate half log amounts [μg] required to elicit the equivalent EAG amplitude.

Test compound	Abbreviation	*Trichoplusia ni*	*Autographa pulchrina*	*Amathes candelarum*	*Syngrapha variabilis*	*Cucullia* * *umbratica*	*Mamestra** *configurata*
(*Z*)-7-dodecen-1-yl acetate	*Z*7-12:Ac	**0.001**	**0.001**	0.03	0.03	0.01	0.03
(*Z*)-7-tridecen-1-yl acetate	*Z*7-13:Ac	0.01	0.01	0.01		0.01	0.03
(*Z*)-7-tetradecen-1-yl-acetate	*Z*7-14:Ac	0.03	0.1	**0.001**	**0.001**	0.01	0.03
(Z)-7-pentadecen-1-yl acetate	*Z*7-15:Ac	0.1	0.3	0.01		0.1	0.1
(*Z*)-7-hexadecen-1-yl acetate	*Z*7-16:Ac	0.3	1	0.1	0.1	0.3	0.3
(*R,S*)-9-methyl-(*Z*)-7-dodecen-1-yl acetate	9me-*Z*7-12:Ac	0.01	0.03	0.3	0.3	0.1	0.3
(*R,S*)-10-methyl-(*Z*)-7-dodecen-1-yl acetate	10me-*Z*7-12:Ac	0.03	0.03	0.3		0.1	0.1
11-methyl-(*Z*)-7-dodecen-1-yl acetate	11me-*Z*7-12:Ac	0.01	0.01	0.1	0.3	0.1	0.1
9-propyl-(*Z*)-7-dodecen-1-yl acetate	9pr-*Z*7-12:Ac	0.03	0.03	1	1	0.3	0.3
(*R,S*)-11-methyl-(*Z*)-7-tetradecen-1-yl acetate	11me-*Z*7-14:Ac	0.1	0.3	0.01	0.03	0.1	0.1
9-pentyl-(*Z*)-7-tetradecen-1-yl acetate	9pe-*Z*7-14:Ac	0.1	0.3	1	1	0.1	0.3

* For key compounds for *C. umbratica* and *M. configurata*, see Tables V and VI.

Table V. Efficacy (as in Table IV) of branched and cyclic versus straight-chain (*Z*)-9-alkenyl acetates for lepidopterous pheromone receptors.

Test compound	Abbreviation	*Clysia ambiguella*	*Paralobesia viteana*	*Cucullia umbratica*	*Polia pisi*	*Trichoplusia ni* *	*Mamestra configurata* *
(*Z*)-9-dodecen-1-yl acetate	*Z*9-12:Ac	**0.001**	**0.001**	0.03	0.01	0.03	0.1
(*Z*)-9-tridecen-1-yl acetate	*Z*9-13:Ac	0.003	0.01	0.01	0.003	0.03	0.03
(*Z*)-9-tetradecen-1-yl-acetate	*Z*9-14:Ac	0.03	0.03	**0.001**	**0.001**	0.01	0.003
(*Z*)-9-pentadecen-1-yl acetate	*Z*9-15:Ac	1	0.3	0.01	0.01	0.03	0.03
(*Z*)-9-hexadecen-1-yl acetate	*Z*9-16:Ac	1	0.3	0.1	0.1	0.1	0.1
11-methyl-(*Z*)-9-dodecen-1-yl acetate	11me-*Z*9-12:Ac	0.01		0.03	0.01	0.3	0.03
(*R,S*)-11-methyl-(*Z*)-9-tridecen-1-yl acetate	11me-*Z*9-13:Ac	0.03		0.03	0.01	0.3	0.03
11-ethyl-(*Z*)-9-tridecen-1-yl acetate	11et-*Z*9-13:Ac	0.1		0.01	0.01	0.3	0.03
(*R,S*)-11-methyl-(*Z*)-9-tetradecen-1-yl acetate	11me-*Z*9-14:Ac	0.003	0.003	0.03	0.01	0.1	0.03
(*R,S*)-12-methyl-(*Z*)-9-tetradecen-1-yl acetate	12me-*Z*9-14:Ac	0.03	0.03	0.01	0.01	0.3	0.01
13-methyl-(*Z*)-9-tetradecen-1-yl acetate	13me-*Z*9-14:Ac	1	0.3	0.01	0.01	0.3	0.01
12-ethyl-(*Z*)-9-tetradecen-1-yl acetate	12et-*Z*9-14:Ac	1	0.3	0.3	0.3	1	0.3
11-propyl-(*Z*)-9-tetradecen-1-yl acetate	11pr-*Z*9-14:Ac	0.1	0.1	0.003	0.003	0.03	0.01
(*R,S*)-11-methyl-(*Z*)-9-hexadecen-1-yl acetate	11me-*Z*9-16:Ac	0.003	0.003	0.3	0.1	0.1	0.3
(*R,S*)-13-methyl-(*Z*)-9-hexadecen-1-yl acetate	13me-*Z*9-16:Ac	0.03	0.01	0.1	0.1	0.3	0.1
(*R,S*)-12-ethyl-(*Z*)-9-hexadecen-1-yl acetate	12et-*Z*9-16:Ac	0.01	0.01	0.3		0.3	1
(*R,S*)-11-propyl-(*Z*)-9-hexadecen-1-yl acetate	11pr-*Z*9-16:Ac	0.01	0.01	0.3	0.3		1
11-pentyl-(*Z*)-9-hexadecen-1-yl acetate	11pe-*Z*9-16:Ac	1	0.3		0.3	0.3	1
10-cyclohexyl-(*Z*)-9-decen-1-yl acetate		3	1	1	1	3	3
10-(*cis,trans*-3-methyl-cyclohexyl)-(*Z*)-9-decen-1-yl acetate		0.1	0.1	0.3	0.1	1	0.3
10-(*cis,trans*-4-methyl-cyclohexyl)-(*Z*)-9-decen-1-yl acetate		0.01	0.01	0.03	0.03	0.3	0.1
11-cyclohexyl-(*Z*)-9-undecen-1-yl acetate		0.3	0.1	0.3	0.1	1	0.3

* For key compounds for *T. ni* and *M. configurata*, see Tables IV and VI.

stituent at the *n* part not altering the length of the chain is an α-methyl side group. Compared to the key compound, it reduces the measured response approx. 10 fold with *C. ambiguella* and *P. viteana* (see 11me-*Z*9-12:Ac *vs.* *Z*9-12:Ac, Table V), but approx. 100 fold with *A. velutinana* and *C. rosaceana* (13me-Z11-14:Ac *vs.* Z11-14:Ac, Table VI); a similar reduction factor was obtained with additional species (not listed) known to use the Z11-14:Ac as a female pheromone.

Receptors for *Z*7-12:Ac, *Z*9-14:Ac, or Z11-16:Ac, the three key compounds of the type with $n = 3$ methylene groups, are represented by *T. ni* and *A. pulchrina*, *C. umbratica* and *P. pisi*, and *M. configurata* and *M. gracilis*, respectively. Here, in comparison to the key compound, a methyl substituent generally lowered the efficacy between 10 to 30 fold for all six species (values 0.01 or 0.03 in Tables IV to VI), largely irrespective of whether the substituent was at position α, β, or γ relative to the (*Z*) double bond. However, differences between receptor types specialized for either *Z*7-12:Ac, *Z*9-14:Ac, or Z11-16:Ac become apparent when evaluating the influence of longer alkyl side chains:

Table VI. Efficacy (as in Table IV) of branched versus straight-chain (*Z*)-11-alkenyl acetates for lepidopterous pheromone receptors.

Test compound	Abbreviation	*Argyrotaenia velutinana*	*Choristoneura rosaceana*	*Mamestra configurata*	*Monima gracilis*	*Trichoplusia ni* *	*Clysia ambiguella* *
(*Z*)-11-tridecen-1-yl acetate	*Z*11-13:Ac	0.003		0.03		0.1	0.01
(*Z*)-11-tetradecen-1-yl acetate	*Z*11-14:Ac	**0.001**	**0.001**	0.01	0.1	0.1	0.01
(*Z*)-11-pentadecen-1-yl acetate	*Z*11-15:Ac	0.03	0.03	0.01	0.01	0.1	0.3
(*Z*)-11-hexadecen-1-yl acetate	*Z*11-16:Ac	0.3	0.3	**0.001**	**0.001**	0.1	0.3
13-methyl-(*Z*)-11-tetradecen-1-yl acetate	13me-*Z*11-14:Ac	0.1	0.1	0.03	0.1	0.3	0.1
(*R,S*)-13-methyl-(*Z*)-11-pentadecen-1-yl acetate	13me-*Z*11-15:Ac	0.3	0.3	0.03		0.3	0.3
13-ethyl-(*Z*)-11-pentadecen-1-yl acetate	13et-*Z*11-15:Ac	0.3	0.3	0.03	0.03	0.3	
(*R,S*)-13-methyl-(*Z*)-11-hexadecen-1-yl acetate	13me-*Z*11-16:Ac	0.01	0.01	0.01	0.01	0.3	0.3
(*R,S*)-14-methyl-(*Z*)-11-hexadecen-1-yl acetate	14me-*Z*11-16:Ac	0.1	0.03	0.01		1	
15-methyl-(*Z*)-11-hexadecen-1-yl acetate	15me-*Z*11-16:Ac	0.3	0.3	0.01	0.01	1	1
14-ethyl-(*Z*)-11-hexadecen-1-yl acetate	14et-*Z*11-16:Ac	0.03		0.1		1	0.3
13-propyl-(*Z*)-11-hexadecen-1-yl acetate	13pr-*Z*11-16:Ac	0.01	0.01	0.03	0.03	0.3	0.3

* For key compounds for *T. ni* and *C. ambiguella*, see Tables IV and V.

with *C. umbratica* and *P. pisi*, the two species maximally responsive for *Z*9-14:Ac, the introduction of an α-propyl substituent reduced the efficacy only approx. 3 fold (see 11pr-*Z*9-14:Ac *vs.* *Z*9-14:Ac, Table V); whereas with the receptors for *Z*7-12:Ac and *Z*11-16:Ac the comparable modification had a reducing effect of approx. 30 fold (see 9pr-*Z*7-12:Ac *vs.* *Z*7-12:Ac for *T. ni* and *A. pulchrina*, and 13pr-*Z*11-16:Ac *vs.* *Z*11-16:Ac for *M. configurata* and *M. gracilis;* Tables IV and VI). On the other hand, the (symmetrical) ethyl branching at the β position lowered the efficacy 100 to 300 fold for all six species: these β-ethyl branched compounds represent the least effective of the different analogues derived by introducing alkyl side chains in key molecules with **I**, $n = 3$ methylene groups (see values for 12et-*Z*9-14:Ac and 14et-*Z*11-16:Ac, Tables V and VI).

Only incomplete results on effects of chain branching are available for *A. candelarum* and *S. variabilis*, the two species listed as maximally responding to *Z*7-14:Ac (the only key compound in this report with **I**, $n = 5$ methylene groups). Nonetheless it is apparent that with this type of receptor the response is more drastically reduced by longer alkyl side groups (at the *n* part), as compared to the receptor types mentioned before. In comparison to *Z*7-14:Ac, the symmetrically α-pentyl branched analogue, 9pe-*Z*7-14:Ac, is 1000 times less stimulatory for both species (Table IV): this is the greatest loss in efficacy due to an alkyl side group observed during these investigations, in marked contrast to the small changes in efficacy by symmetrically α-propyl or α-pentyl branching that were obtained on the types of receptors mentioned before (see discussion, p. 990).

Branched chains shorter than the pheromone

Some of the alkyl branched (*Z*)-9 and (*Z*)-11 unsaturated analogues listed are shorter than the single key compound by 1 to 3 carbon units. Compared to the unbranched compounds of the same length, these shortened, branched analogues in most cases fall into the same half log activity class, and in a few cases, into the next lower or higher class (see 11me-*Z*9-12:Ac *vs.* *Z*9-12:Ac, or 11me-*Z*9-13:Ac and 11et-*Z*9-13:Ac *vs.* *Z*9-13:Ac for *C. umbratica* and *P. pisi*, Table V; and 11me-*Z*11-14:Ac

vs. Z11-14:Ac, or 13me-Z11-15:Ac and 13et-Z11-15:Ac *vs.* Z11-15:Ac for *M. configurata* and *M. gracilis*, Table VI). Even by extending these studies to additional test species (not listed), with receptors for Z9-14:Ac or for Z11-16:Ac no example so far could be found in which a chain shorter than the optimum (pheromone) was altered in efficacy by more than half a decadic step due to an alkyl substituent.

However, with *A. candelarum* and *S. variabilis*, both maximally responsive to Z7-14:Ac (type **I**, $n = 5$), the lowered response to the shortened homologue, Z7-12:Ac, is 10 to 30 fold further reduced by methyl or propyl substituents. This again demonstrates the greater influence of alkyl side groups on this type of receptor (see also p. 987 and 988).

Branched chains longer than the pheromene

With the exceptions of *M. configurata* and *M. gracilis*, which respond maximally to Z11-16:Ac (the longest chain considered in this report), for each of the other ten species one or more of the branched analogues listed have chains 1 to 4 carbons longer than the single most stimulatory compound. These elongated, branched analogues comprise impressive examples of up to 100 fold decrease as well as up to 300 fold increase in efficacy due to an alkyl side group. The latter effects are found within the four species which respond maximally to Z9-12:Ac or Z11-14:Ac, the two pheromones with a single methylene group in the end alkyl part (**I**, $n = 1$).

For *C. ambiguella* and *P. viteana*, in the series of homologues from Z9-12:Ac (natural pheromone) to Z9-16:Ac the efficacy decreases, stepwise, over a 1000 fold range (Table V). With certain alkyl side groups the low response to longer chains is further reduced (see 13me-Z9-14:Ac or 12et-Z9-14:Ac, Table V). However, by the introduction of a methyl group at the 11 (α) position, the efficacy for both the Z9-14:Ac and the Z9-16:Ac is restored to a level which differs from the Z9-12:Ac by only half a decadic step (see values 0.003 for 11me-Z9-14:Ac and 11me-Z9-16:Ac, Table V).

Surprisingly, a pronounced enhancement of the efficacy of the Z9-16:Ac is also observed with certain other alkyl substituents, such as an 11-propyl, 12-ethyl, or 13-methyl side chain (see 11pr-Z9-16:Ac, 12et-Z9-16:Ac, or 13me-Z9-16:Ac for *C. ambiguella* and *P. viteana*, Table V). Comparable effects were obtained in the (*Z*)-11 series with certain other species:

With *A. velutinana* and *C. rosaceana*, from Z11-14:Ac (natural pheromone) to Z11-16:Ac the response drops approx. 300 fold (Table VI). Analogously to the above results on (*Z*)-9 compounds, with both *A. velutinana* and *C. rosaceana* the low efficacy of the Z11-16:Ac is strongly restored by a 13-methyl, 14-methyl, 13-propyl, or 14-ethyl substituent (see also next section). Similar results were consistently found also with other species known to use Z11-14:Ac as major female pheromone, including additional Tortricidae species as well as members from other families, such as the European Corn Borer, *Ostrinia nubilalis* (Phycitidae).

In contrast to these results on receptors for the key molecules with $n = 1$ methylene group, Z9-12:Ac and Z11-14:Ac, *no* example of marked increase in response to elongated chains due to introducing an alkyl side group could so far be found for the two species maximally responding to Z9-14:Ac, *C. umbratica* and *P. pisi* (Table V), or within the series of (*Z*)-7 compounds (Table IV). Rather, the moderate to low responses by these species to elongated chains were up to 10 fold further reduced by alkyl substituents (Tables IV and V).

Branched analogues of altered double bond position

In each of the Tables IV to VI, the compounds listed have the same position of the (*Z*) double bond. Of the six species in each Table, the first four respond maximally (by value 0.001) to a certain compound of that Table. For these species all the structures listed in this Table thus have the (*Z*) double bond at the position as in the maximally stimulatory (key) compound. Results concerning these species and compounds have been treated above.

Included in each Table, however, are two further species for which the most efficacious compound (value 0.001) is found not at the same but in one of the other two Tables (*e.g.*, *T. ni* shows its maximum response to Z7-12:Ac [Table IV] but is listed again in Tables V and VI; *C. umbratica* and *M. configurata* are also included in Table IV; etc.). These few examples are directed to the question whether similar relationships in the response to branched versus straight-chain compounds, as described above for the optimum position of the

double bond, may also be observed when the single (*Z*) double bond is not at this position.

In fact, there seem to be certain structure-response relationships mutually observable for chains of different double bond positions (segments *m* in **I**), for receptors specialized for pheromones which have the same number (*n*) of methylene groups in their end alkyl segments. With *T. ni*, *A. pulchrina*, *C. umbratica*, *P. pisi*, *M. configurata*, and *M. gracilis* (the species examples for key molecules of type **I**, $n = 3$), the introduction of a methyl substituent to the *n* part lowered the efficacy approx. 10 to 30 fold for the key molecule as well as for the derivative(s) with $n = 3$ methylene groups but different chain segments *m* (see 9me-*Z*7-12:Ac, 10me-*Z*7-12:Ac, and 11me-*Z*7-12:Ac *vs.* *Z*7-12:Ac, Table IV; 11me-*Z*9-14:Ac, 12me-*Z*9-14:Ac, and 13me-*Z*9-14:Ac *vs.* *Z*9-14Ac, Table V; and 13me-*Z*11-16:Ac, 14me-*Z*11-16:Ac, and 15me-*Z*11-16:Ac *vs.* *Z*11-16:Ac; for these species).

Another feature common to these six species was that, of all the different substituents investigated for *Z*9-14:Ac, the α-propyl side chain was consistently the most potent one, relatively speaking, and the β-ethyl group the least potent one (with a lowering of the efficacy by approx. half a decadic step and by 2 to 3 decadic steps, respectively; see 11pr-*Z*9-14:Ac and 12et-*Z*9-14:Ac *vs.* *Z*9-14:Ac, Table V). This correspondance between the six species, which have pheromones of different chain length (C_{12}, C_{14}, and C_{16}) but the same end alkyl part (**I**, $n = 3$) is even more noteworthy when one considers that in the same six species the α-propyl and β-ethyl substituents did *not* reveal comparable effects when introduced in *Z*7-12:Ac, or in *Z*11-16:Ac (see 9pr-*Z*7-12:Ac, 13pr-*Z*11-16:Ac, and 14et-*Z*11-16:Ac, for these species; Tables IV and VI). The extreme difference in response to 11pr-*Z*9-14:Ac *vs.* 12et-*Z*9-14:Ac (but not 13pr-*Z*11-16:Ac *vs.* 14et-*Z*11-16:Ac) was similarly registered with various additional Noctuidae species (not listed), from five subfamilies (Agrotinae, Hadeninae, Cuculliinae, Amphipyrinae, Plusiinae), representing further receptors for the same three compounds (*Z*7-12:Ac, *Z*9-14:Ac, and *Z*11-16:Ac) as well as those for certain structurally related (*e.g.*, doubly unsaturated) acetate pheromones. This correspondence in the relative effects of *Z*9-14:Ac, 11pr-*Z*9-14:Ac, and 12et-*Z*9-14:Ac can thus be considered as a *rule*, apparently applicable to chemical structure-response relationships in many species of Noctuidae, in addition to rules previously described[1] for species of this family (see also discussion, p. 990).

Considering the receptors for the two pheromones with the short end alkyl part of $n = 1$ methylene group, *viz.* *Z*9-12:Ac and *Z*11-14:Ac, we have mentioned above (p. 986) the strong compensation for the low efficacy of elongated chains by an α-methyl substituent, as illustrated in the responses of *C. ambiguella* and *P. viteana* to 11me-*Z*9-14:Ac or 11me-*Z*9-16:Ac, and of *A. velutinana* and *C. rosaceana* to 13me-*Z*11-16:Ac (see p. 986). However, after altering the position of the (*Z*) double bond, for the same receptors the α-methyl substituent no longer had this effect; see 13me-*Z*11-16:Ac *vs.* *Z*11-16:Ac, and 9me-*Z*7-12:Ac *vs.* *Z*7-12:Ac, for *C. ambiguella* or *P. viteana*; and 11me-*Z*9-16:Ac *vs.* Z9-16:Ac for *A. velutinana* and *C. rosaceana* (Tables IV to VI). Thus, with these receptors, the compensatory effect of the α-methyl side group seems to be specific to the positions of the (*Z*) double bond (chain segment *m* in **I**) found in the natural pheromone.

A somewhat different situation seems to exist with the increase in response by *A. velutinana* and *C. rosaceana* due to longer (ethyl, propyl) substituents in the C_{16} chain. Although more pronounced in the (*Z*)-11 series (see 13pr-*Z*11-16:Ac and 14et-*Z*11-16:Ac, Table VI), these effects are evident for the same species also with (*Z*)-9 compounds (*e.g.*, 12et-*Z*9-16:Ac and 11pr-*Z*9-16:Ac *vs.* *Z*9-16:Ac; data not listed in Tables). According to all available results, these strange effects are restricted to the C_{16} chain where they are found with ethyl and propyl substituents at varying positions, and also for analogues with the double bond remote from the optimum (see discussion, p. 990). However, after shortening the distance between double bond and the acetate group from *Z*11-16:Ac to *Z*9-14:Ac, the analogous ethyl or propyl substituents at the end alkyl part no longer showed these effects (not specified in Tables).

Receptors for *Z*7-14:Ac, the pheromone example with $n = 5$ methylene groups, were represented by *A. candelarum* and *S. variabilis*. As has been pointed out (p. 985), this type of receptor is remarkable for the extreme reduction in stimulatory efficacy of (*Z*)-7 unsaturated acetates due to longer (propyl, pentyl) α side chains. Here, also after

elongating the distance between double bond and ester group from Z7-14:Ac to Z9-16:Ac, α-propyl and α-pentyl side chains again drastically reduced the efficacy (see 11pr-Z9-16:Ac and 11pe-Z9-16:Ac *vs.* Z9-16:Ac, Table V). This may provide an example of a structure-response relationship, resulting from alkyl branching at the *n* part of the molecule (**I**), and retained after altering by two methylene groups the *m* part.

Cyclohexyl analogues

In Table V, in addition to the alkyl branched analogues, four (*Z*)-9 unsaturated compounds are listed in which the end alkyl part of the pheromone molecule was replaced by a cyclohexane ring, either adjacent to the (*Z*) double bond or separated from it by 1 methylene group. These few examples are taken from ongoing investigations aimed at collecting, by use of these analogues with less flexible end segments, further data on the possible nature of the receptor site (see discussion, p. 991).

Here it should be briefly mentioned that for all the twelve test species the 10-cyclohexyl-(*Z*)-9-decen-1-yl acetate (the structural derivative with the non-substituted cyclohexane ring adjacent to the double bond; see Table III) was consistently the least effective of all the compounds included in this report. With the same species, the 11-cyclohexyl-(*Z*)-9-undecen-1-yl acetate (the derivative with 1 methylene group between double bond and cyclohexyl ring) was 3 to 10 times more stimulatory than the above 10-cyclohexyl analogue. It seems worthy to note that, with the same twelve species, the substitution of a methyl group on the ring increased the very low efficacy of the 10-cyclohexyl analogue between 3 to 300 fold, the 4-methyl substituent generally being more effective than 3-methyl (see examples in Table V).

Relatively high responses to certain of these cyclic analogues were consistently obtained with receptors specialized for Z9-12:Ac (see Table V) or for Z11-14:Ac, whereas the lowest ones occurred with those for Z7-14:Ac. This again indicates basic differences between receptors for pheromones with short ($n = 1$) versus long ($n = 3, 5$) end alkyl parts (see discussion, p. 990).

Discussion

The present structure-activity study investigated the effect on sensory responses of introducing alkyl side chains, varying in size and position, to the end alkyl part of long-chain alkenyl esters. Lepidopteran species, mainly Noctuidae and Tortricidae, were studied where the male receptors respond maximally to Z7-12:Ac, Z9-12:Ac, Z7-14:Ac, Z9-14:Ac, Z11-14:Ac, or Z11-16:Ac, the known or presumed major female sex pheromones of the species (Table I).

The relative efficacies of test compounds have been presented by single values which indicate, in half decadic steps, the stimulus amounts (μg at source) required to elicit electroantennogram (EAG) responses equivalent to 0.001 μg of the most efficacious (key) compound, in all species. By using systematically varied test compounds, the response spectrum composed of these activity values can be taken as the measure of the chemical specificity of a given receptor. For long-chain ester pheromones of the type considered here, several hundreds of stepwise modifications may be required [1] to describe receptor specificities in sufficient detail to recognize the degree of similarities and differences between species, to derive rules in structure-response relationships, and to deduce information on mechanisms of acceptor interaction. For six of the twelve test species, the data for the novel branched analogues presented here further broaden the response spectra reported earlier. On the other hand, the range of test compounds in this report was selected such that most of the relationships can be discussed by using the listed compounds only, without close reference to earlier results on other analogues. Also, as had been pointed out previously [1], a variety of conclusions may be reached by way of activity values of the kind presented here; whereas for other cases these values would have to be corrected with respect to the actual molecular concentrations of active components reaching the antennal receptors in the test situation (see below).

Each of the six receptor types (corresponding to the six key compounds) has been presented by two species examples (Table I). In some of these pairs of species the response spectra towards the listed compounds can be classified as 'identical' within the experimental limits [1], such as between *A. velutinana* and *C. rosaceana*, which share the same set of half log activity values for nine of the twelve compounds listed in Table VI. Also within the other pairs of species the spectra are very similar,

coinciding for at least two thirds of the test compounds, and differing by not more than half a decadic step for any of the rest. No separation between the species of the same pair will therefore be made in the following discussions. This close similarity is especially noteworthy in cases such as *C. ambiguella* and *P. viteana*, which belong to different families (Cochylidae and Tortricidae, respectively) and must have convergently evolved the same major pheromone component, Z9-12:Ac.

Here, as with certain other types of pheromone receptors [11–13], each measured response spectrum is assigned to a homogeneous population of olfactory acceptors. The graded stimulus amounts represented by the activity values (see p. 982) of a given response spectrum are thus viewed as reflecting the probabilities for the different analogues to activate a single species of molecular acceptor, located at the dendritic membrane of the receptor cell.

The value obtained for a given compound and receptor may formally be considered as having resulted from several factors determined by the structure of the stimulant molecule, some of which have lowered while others have increased the sensory response. Illustrating this concept are the responses shown by *T. ni* towards the four straight-chain (Z)-11-alkenyl acetates listed in Table VI, all of which reached the same activity value of 0.1. This may be ascribed to the balance in the contribution of two opposing major parameters – the chain length of the molecule, which from Z11-16:Ac to Z11-13:Ac is optimized stepwise; and the length of the apolar end alkyl part (n in **I**) which, starting from $n = 0$ (Z11-13:Ac), in the Z11-16:Ac reaches the same number of $n = 3$ methylene groups as in the natural pheromone, Z7-12:Ac.

Analogously, for the alkyl-branched derivatives treated here, a variety of influences by the substituent group during the sequence of acceptor interaction must be taken into account. Factors possibly increasing or decreasing the measured responses include:

(i) Rotational barriers to changes in conformation, which may stabilize distinct conformers of the end alkyl parts at positions favourable or unfavourable to acceptor interaction; (ii) altered flexibility of chain end(s) in the n part; (iii) competition for a binding site between favourable and unfavourable chain segments when the branches are different; (iv) increased probability for interaction of larger alkyl ends when branched symmetrically; (v) reduced probability for interaction of a single, terminal methyl group in end-methyl branched chains; (vi) facilitated interaction of a methyl side group when offered at a position corresponding to the terminal methyl group of the pheromone; (vii) steric hindrance, by the substituents, to the access of essential parts of the stimulant molecule to sensitive acceptor positions; (viii) interaction of the alkyl substituent(s) with acceptor areas other than the sensitive binding positions, preventing acceptor activation (allosteric inhibition) or further sensory events; (ix) allosteric activation of the acceptor by the substituent(s); (x) non-specific binding interaction of the substituent groups, possibly prolongating the presence of the ligand molecule in the acceptor field; (xi) altered balance between polarity of the stimulant molecule and lipophilicity of its end alkyl part.

The lack of pronounced branching effects which was repeatedly observed during this study (*e. g.*, with all the chains shorter than the key molecule, see p. 985) may in fact be due to an interplay of several of these factors, which would of course render great difficulties in the interpretation of many of the activity values. Here we will, therefore, rely primarily on those examples of pronounced alteration in the sensory efficacy by an alkyl substituent, which we consider to be attributable to one major parameter.

The most pronounced increase in efficacy observed during these studies was the strong compensation for chain elongation by an α-methyl side group, obtained on the receptors for Z9-12:Ac presented by *C. ambiguella* and *P. viteana* (see p. 986). The result could suggest interaction of the 11me-Z9-14:Ac and the 11me-Z9-16:Ac at these receptors in a conformer in which, relative to other chain segments, the α-methyl group occupies a position corresponding to that of the terminal methyl group of the natural pheromone, Z9-12:Ac. One of the many conformations to be considered for the Z9-12:Ac molecule is presented (Fig. 1 a). For the (*R*) isomer of 11me-Z9-16:Ac, Fig. 1 c shows a conceivable conformation meeting the above requirement, *i. e.*, the α-methyl group accepts about the same position (relative to chain segment m) as the terminal methyl group in Fig. 1 a. Such a conformer might possibly allow for optimum

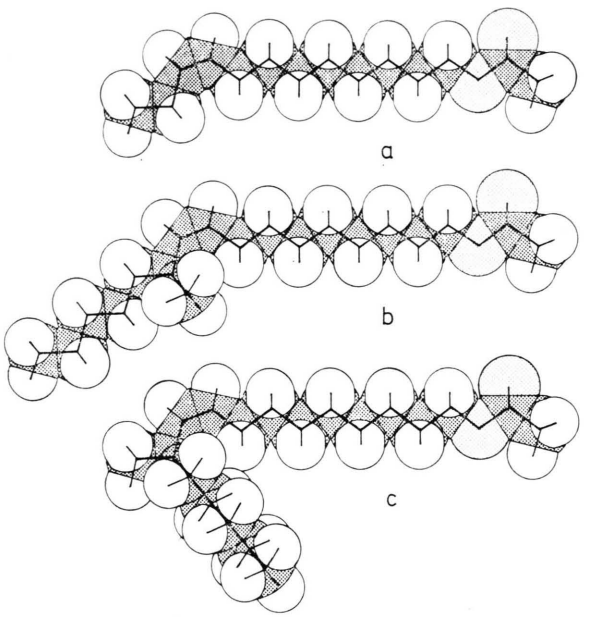


Fig. 1. Drawings of models of (a) (*Z*)-9-dodecen-1-yl acetate (*Z*9-12:Ac), and two conformers (b, c) of respectively (*S*)- and (*R*)-11-methyl-(*Z*)-9-hexadecen-1-yl acetate (11me-*Z*9-16:Ac).

interaction, unhindered by the longer chain end, of the 11me-*Z*9-16:Ac molecule with acceptors for *Z*9-12:Ac. – This view is, of course, highly speculative and we emphasize that it represents only one of the lines we are following in the interpretation of the experimental data. – The analogous statement applies to the extraordinary effect of 13me-*Z*11-16:Ac on receptors for *Z*11-14:Ac, as seen with certain other species (p. 986).

In general, to acceptors for pheromones with a single *n* methylene group, there seems to be accessibility for molecules with bulky groups in their end alkyl parts, as further indicated by the high responses of the respective receptors to certain cyclohexyl analogues (p. 988). – The other extreme apparently is represented by receptors for key molecules of type **I**, n = 5, such as *Z*7-14:Ac. Here, longer alkyl side chains reduced the efficacy up to 1000 fold, and cyclic analogues were almost completely ineffective. These marked influences suggest severe hindrance to the access of essential segments of these molecules to sensitive positions within this acceptor, and lead us to suspect a more specifically structured active site, compared to the receptors for pheromones with short ($n = 1$) end alkyl parts, treated above.

With respect to changes in efficacy due to introducing longer alkyl substituents to chain segment *n*, the receptors for Z7-12:Ac, *Z*9-14:Ac, and *Z*11-16:Ac (the pheromones of type **I**, $n = 3$) showed a pattern intermediate between the above extremes ($n = 1$ or 5). Common to these receptors (all represented here by Noctuidae species) was the lack of pronounced reducing effects of the α-propyl substituent in the *Z*9-14:Ac, but not in *Z*7-12:Ac or *Z*11-16:Ac. This may suggest hindered access, by this substituent, to certain parts of the receptor site but not to others. Although common throughout the Noctuidae, this relationship (for 11pr-*Z*9-14:Ac *vs.* *Z*9-14:Ac) has never been observed for any Tortricidae species. It may thus be taken as one of several structural relationships which suggest possible basic differences in the architecture between noctuid and tortricid receptor sites [15].

Another distinct relationship apparently specific to certain higher taxonomic groups seems to be the strong enhancement of responces to C_{16} acetates by ethyl or propyl side chains, described for Tortricidae and Cochylidae species (p. 986). It was similarly recorded with various additional species of these and certain other families (*e. g.*, Phycitidae or Yponomeutidae), but never observed with any of the Noctuidae test species. As pointed out (p. 987), these effects were noted only at C_{16} chains although not strictly dependent on a specific position of the (*Z*) double bond or of the alkyl substituent. The results could indicate unspecific, allosteric activation of the hydrophobic region of tortricoid type acceptors by these alkyl groups.

It has been argued above that a certain, preferred conformer of the 11me-*Z*9-16:Ac (similar to Fig. 1 c) could be the relevant one for interaction with receptors specialized for *Z*9-12:Ac (type **I**, $n = 1$). The same compound also elicited relatively high responses (not specified in the Tables) in receptors for *Z*7-14:Ac ($n = 5$). Here, interaction is suggested in a conformation in which the terminal methyl group of the 11me-*Z*9-16:Ac can accept the same (unknown) position as the terminal methyl group of the *Z*7-14:Ac during interaction with this acceptor; one of the many possibilities is illustrated by Fig. 1 b. Analogously, different conformers of 11me-*Z*9-14:Ac might be responsible for the responses to this compound obtained from receptors for *Z*9-12:Ac ($n = 1$) and from those for *Z*9-14:Ac ($n = 3$). However, for most of the

branched molecules studied here, calculations concerning energy differences between distinct conformers will have to await the results of ongoing conformational analyses (see below). – Also, no statement can yet be made on the specific *optical isomer*(s) (*R* or *S*) of the asymmetrically-branched compounds active for the receptors studied here. Investigations in this direction should be of great interest for the elucidation of chiral properties of pheromone acceptors in these species of moths.

We should refrain from drawing at present more detailed conclusions about acceptor interaction, on three major grounds: (i) The actual *molecular concentrations* of stimulant compounds at the antennal receptors are as yet unknown, but they are being determined at present by the use of labelled analogues, as described for certain other types of lepidopterous pheromones [14, 16, 17]. (ii) A program of *quantitative conformational analysis* of the test compounds by the use of force field calculations is in progress. These calculations are based on the classical mechanics approach [18] using the force field parameters of N. L. Allinger [19], which allow comparisons of the energies of conformers and the estimation of rotational barriers to changes in conformation. (iii) Further, specific test results on the effects of *cyclic analogues*, including differential responses to *cis* and *trans* ring substituents, are available for the same receptors (in preparation) and should be included in further, detailed discussion.

With *C. ambiguella*, the 11me-Z9-14:Ac and 11me-Z9-16:Ac at stimulus source amounts only 2 to 3 times greater than for the natural pheromone (Z9-12:Ac) elicited a similar pattern of nerve impulse discharges in the same receptor cells of male sensilla trichodea. Moreover, in behaviour tests, where all straight-chain C_{14} and C_{16} acetates were inactive, these two α-methyl branched compounds evoked male sexual behavioural responses in the laboratory, and attracted numbers of males in the field [20]. The above-mentioned 2 to 3 fold difference in equipotent stimulus amounts referred to the stimulus source (over which an air current was directed to the antennal preparation). Considering corrections as to the actual molecular concentrations reaching the antenna, and the possibility that only one of the two chiral antipodes of the racemate optimally activated the receptor, these α-methyl branched analogues may attain, or even exceed, the stimulatory efficacy of the natural pheromone (Z9-12:Ac). Structural modifications of the kind studied here thus may lead to a new group of extremely potent pheromone analogues.

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[1] E. Priesner, M. Jacobson, and H. J. Bestmann, Z. Naturforsch. **30 c**, 283 [1975].
[2] R. S. Berger, Ann. Entomol. Soc. Amer. **59**, 767 [1966].
[3] H. Arn, S. Rauscher, H. R. Buser, and W. L. Roelofs, Z. Naturforsch. **31 c**, 499 [1976].
[4] W. L. Roelofs, J. P. Tette, E. F. Taschenberg, and A. Comeau, J. Insect Physiol. **17**, 2235 [1971].
[5] W. L. Roelofs and H. Arn, Nature **219**, 513 [1968].
[6] W. L. Roelofs and J. P. Tette, Nature **226**, 1172 [1970].
[7] M. D. Chisholm, W. F. Steck, A. P. Arthur, and E. W. Underhill, Canad. Entomol. **107**, 361 [1975].
[8] H. J. Bestman, I. Kantardiew, P. Rösel, W. Stransky, and O. Vostrowsky, Chem. Ber. **110** (in press).
[9] H. J. Bestmann, W. Stransky, O. Vostrowsky, and P. Range, Chem. Ber. **108**, 3582 [1975].
[10] H. J. Bestmann, W. Stransky, and O. Vostrowsky, Chem. Ber. **109**, 1694 [1976].
[11] W. A. Kafka, Ann. N. Y. Acad. Sci. **237**, 115 [1974].
[12] K. E. Kaissling, Biochemistry of Sensory Functions (L. Jaenicke, ed.), p. 243, Springer-Verlag, Berlin-Heidelberg-New York 1974.
[13] W. A. Kafka, Structure-Activity Relationships in Chemoreception (G. Benz, ed.), p. 123, ECRO Symposium, Wädenswil 1975.
[14] D. Schneider, W. A. Kafka, M. Beroza, and B. Bierl, J. comp. Physiol. **113**, 1 [1977].
[15] E. Priesner, Olfaction and Taste VI (J. LeMagnen, ed.), in press.
[16] G. Kasang, Z. Naturforsch. **23 b**, 1331 [1968].
[17] K. E. Kaissling and E. Priesner, Naturwissenschaften **57**, 23 [1970].
[18] For the use of force field calculations for conformational analyses, see E. M. Engler, J. P. Andose, and P. v. R. Schleyer, J. Amer. Chem. Soc. **95**, 8005 [1973].
[19] N. L. Allinger and J. T. Sprague, J. Amer. Chem. Soc. **94**, 5734 [1972].
[20] H. Arn, personal communication.