Hz, 3.3 Hz, 1 H) 1.80 (m, 2 H); 1.70 (m, 2 H); 1.58 (ddt, 3.5 Hz, 5.1 Hz, 13.8 Hz, 1 H); 1.33–1.07 (m, 5 H); 1.29, 1.25, 1.15, and 0.98 (s, 4 × 3H); 0.94 (br, t, 3.5 Hz, 1 H); and 0.78 (br, dd, 5.3 Hz, 13.1 Hz, 1 H). Unequivocal constitutional identity was established from broad-band decoupled CNMR spectra of the synthetic material[15] and the *A. minimus* compound[16] which revealed shift differences of < 0.1 ppm for each respective resonance. The *A. minimus* natural product produced ^{13}C δ values of 17.6, 22.1, 25.2, 29.0, 29.0, 29.3, 29.6, 31.0, 32.5, 34.8, 41.0, 42.1, 42.8, 72.8, 74.5, and an optical rotation of $[\alpha]_D^{26}$ −34° (c = 0.0104, $CDCl_3$) which differs from the −22° value reported by Baker[12] for both the *A. evuncifer* and the synthetic compound.

The elapsed time from the start of the test until the first *C. californica* worker initiated feeding averaged 35 ± 31 (sec ± 95% CI) for the eudesmane treatments and 7 ± 2 for the controls. Data of the feeding census, summarized in figure 2, show that significantly fewer ants contacted the honey solution when surrounded by a eudesmane residue of $^1/_{10}$ soldier equivalent than compared to untreated control trials. Many ants, upon entering the perceived active space of the eudesmane deposit, reversed direction, and began stereotypical cleaning behavior. Two groups of 10 ant workers were topically treated (abdomen) with 0.8 µg of the eudesmane compound in 0.64 µl acetone or acetone alone. After a 48 h provisioned confinement, no mortality or abnormal behavior was observed in either group. From these findings we conclude that 4,11-epoxy-*cis*-eudesmane is an allomonal repellent probably acting in vapor phase, and that the defensive strategy of *A. minimus* is similar to the one proposed for a sympatric species, *A. wheeleri*[6]. The secretion storage and delivery systems of both species are also congruent.

Unlike *Amitermes* from other regions, colonies of Nearctic species are inconspicuous as they do not build epigeal mounds. Much of the life history, biology, and ecology of Nearctic *Amitermes*, and *A. minimus* in particular, is unknown beyond fragmentary notes from early descriptions[17]. The distribution of *A. minimus* is extensive, ranging from southern California to central Texas, where it occurs with seven congeners[18]. Although their secretions are chemically alike, the mandibles of *A. minimus* soldiers are distinct from *A. evuncifer*[19]. The latter is remarkably similar to *A. wheeleri* which is characterized by bisabolene-secreting soldiers[6]. From Fuller's written description[20], *A. messinae* soldiers more closely resemble those of *A. minimus*.

The status of the zoogeographical origin and course of speciation of *Amitermes* is obscure at best[21]. The isolation of the same complex chemical in *A. minimus* and the 2 African *Amitermes* strongly suggests homologous biosynthetic pathways among these species and, therefore, close phylogenetic relationships. Presumably, the eudesmane-producing phenotype confers increased fitness since speciation retaining this trait has occurred at least 3 times along this *Amitermes* line. Further identification of soldier-specific secretions in *Amitermes* may help clarify the uncertainties regarding the phylogeny and present day distribution of these termites.

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Chiral influence of sex pheromonal substances on responses of the male American cockroach[1]

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Summary. In order to elucidate the roles of optical enantiomers of sex pheromonal substances of the American cockroach, behavioral assays with a single enantiomer and with mixtures of enantiomers of sex pheromone mimics were carried out. Inactive enantiomers [(−)-enantiomers] had no influence on the potency of enantiomers active as sex pheromones [(+)-enantiomers]. By analysis of the results from EAG recordings with single and mixed sample of the enantiomers, it was confirmed that (−)-enantiomers did not react with the sex pheromone receptors which are responsive to (+)-enantiomers.
Key words. Cockroach; *Periplaneta americana;* pheromones, sex; chirality; sexual behavior; enantiomers, inactive.

The olfactory sense in animals and even in human beings is often influenced by the optical chirality of odorous compounds in estimating the quality and quantity of the odors[2]. In sexual communication with pheromones in insects, the optical chiral property of the pheromones plays an important role in discriminating species and sexes. Adams et al.[3] reported that in males of the American cockroach (*Periplaneta americana* L.) an enantiomer of one of 2 female sex pheromones, periplanone-B[4], was active in inducing their sexual behavior, whereas the other enantiomer was inactive, which demon-

strates the effect of the optical chirality of sex pheromone receptors on the male's antennae. The influence of the inactive enantiomer on the active one, however, has not been mentioned in their report. On account of the difficulties of the isolation[4] and synthesis[5] of the pheromones of this insect, investigation using the natural pheromones may be too difficult for elucidating mutual roles between the enantiomers. Nishino et al.[6–8] recognized several compounds having the verbane skelton (fig. 1) as mimics of the sex pheromone of the American cockroach. It is known from a single cell recording study[9] that the antenna of the male cockroach possesses individual sex pheromone receptors specific for periplanone-A and periplanone-B. Quite recently, differential saturation electro-antennogram (EAG) results showed that the verbane mimics have a strong interaction with the sex pheromone receptors for periplanone-B[10].

In this communication, we report: 1. Sex pheromonal activity of enantiomeric pairs of the verbane type mimics in a behavioral assay, and 2. the influence of behaviorally inactive enantiomers on active ones based on the results of behavioral assay and EAG experiments with single and mixed samples of the optical enantiomers. Since acetates (**2a** and **7a**) and propionates (**3a** and **8a**) of (+)-trans-verbenol (**1a**) and (+)-verbanol (**6a**) have been reported to be sex pheromonally active[7], (−)-enantiomers (**2b**, **7b**, **3b** and **8b**) of the active esters were synthesized, and additionally (−)-enantiomer (**5b**) of inactive (+)-isoverbanyl acetate (**5a**)[8] was prepared. The structures of all enantiomers are shown in figure 1. (−)-trans-Verbenol (**1b**) was commercially obtained (SCM Gliden Organics Co., USA) and was hydrogenated to obtain (−)-isoverbanol (**4b**) in analogy with conversion from **1a** to **4a**[11]. (−)-Verbanol (**6b**) was synthesized from (+)-apoverbenone[11] according to the following synthetic procedures. (+)-Apoverbenone − (H_2O_2/NaOH) → epoxide−($NH_2NH_2H_2O$/AcOH)→(−)-trans-apoverbenol−(MnO_2) → (−)-apoverbenone − (Me_2CuLi/ether) → (−)-verbanone − (Meerwein-Ponndorf reduction and chromatographic separation)→**6b**. Esters were prepared by the usual esterification of the alcohols with acid anhydrides and pyridine. Physical data of all the (−)-enantiomers were identical with those of the (+)-enantiomers[8,12] except for $[\alpha]_D$-values. $[\alpha]_D$-Values of the alcohols and their esters measured in 1% benzene solution are indicated in figure 1.

Based on $[\alpha]_D$-values of optically pure **1a** and **1b** by Mori et al.[13], the optical purity was calculated as 92% for our **1a** and **4a**, and 95% for **1b** and **4b**. To **6a** 92% of optical purity was given, because the starting material, (−)-β-pinene ($[\alpha]_D$ −21° (pure), Aldrich Chemical Co., USA), has the same purity[14]. Accordingly, **6b** has > 90% optical purity taking into account the measuring error for the $[\alpha]_D$-value.

In the behavioral assay[15], when the typical sexual display of males[16], which is characterized by wing flutter, extension of the abdomen and attempted copulation among the males, was observed with 200 µg of an ester [direct application of pure liquid of a compound to a rectangular filter paper (0.5 × 2.5 cm)], 100, 50 and 20 µg of the ester were assayed successively [applying 10, 5 and 2 µl respectively form *n*-hexane solution (10 mg/l ml) of an ester to the filter paper]. In mixing enantiomers, the specified quantity of enantiomers (see table) was individually taken on the same filter paper. The assay was performed in a special controlled-environment room (26 °C, 40% relative humidity, and 7/17-h day and night photoperiod) using a testing cage (24 × 30 × 9 cm) equipped with filter paper shelters. In the cage, 25 adult males had been kept for at least 1 month. The assay was initiated at 21.00 h under dim light. The number of males showing sexual display was counted within 3 min.

The behavioral assay data are shown in the table. In the assay with single enantiomers, (+)-enantiomers exhibited significant activities at 20 µg, while the corresponding (−)-enantiomers were all inactive despite the large dose used quantity (200 µg). It should be noted that when the (+)-enantiomer (*5a*) is inactive, the (−)-enantiomer also has no activity. The assay results indicate the effect of the optical chirality of sex pheromone receptors on the antennae of the male American cockroach, since the verbane type sex pheromone mimics have been proved to be associated with the sex pheromone receptors for periplanone-B[10], and the results coincide with the result of

Behavioral assay results with single and mixed samples of enantiomers

Compound	Quantity (µg)	Repetition	Activity*
2a	20**	20	9 ± 3
2b	200***	5	Inactive
3a	20**	20	15 ± 4
3b	200***	5	Inactive
3a + 3b	20 + 10****	10	15 ± 3
	20 + 20	10	15 ± 6
	20 + 30	10	15 ± 3
5a	200***	5	Inactive
5b	200***	5	Inactive
7a	20**	20	15 ± 6
7b	200***	5	Inactive
7a + 7b	20 + 10****	10	15 ± 4
	20 + 20	10	16 ± 4
	20 + 30	10	17 ± 3
8a	20**	20	18 ± 3
8b	200***	5	Inactive
8a + 8b	20 + 10****	10	17 ± 3
	20 + 20	10	19 ± 3
	20 + 30	10	19 ± 2

* Average number of cockroaches showing typical sexual display within 3 min in a group containing 25 males ± SD. ** In this case, 2 µl of a solution in which 10 mg of a compound was dissolved into 1 ml of *n*-hexane was impregnated into a rectangular filter paper (0.5 × 2.5 cm). *** From pure liquid of a compound, 200 µg were directly applied to the filter paper. **** In the mixing test, the specified quantities were taken from the solutions of single enantiomers to mix them on the same filter paper. The 3 kinds of dose samples (20 + 10, 20 + 20 and 20 + 30 µg) were made individually.

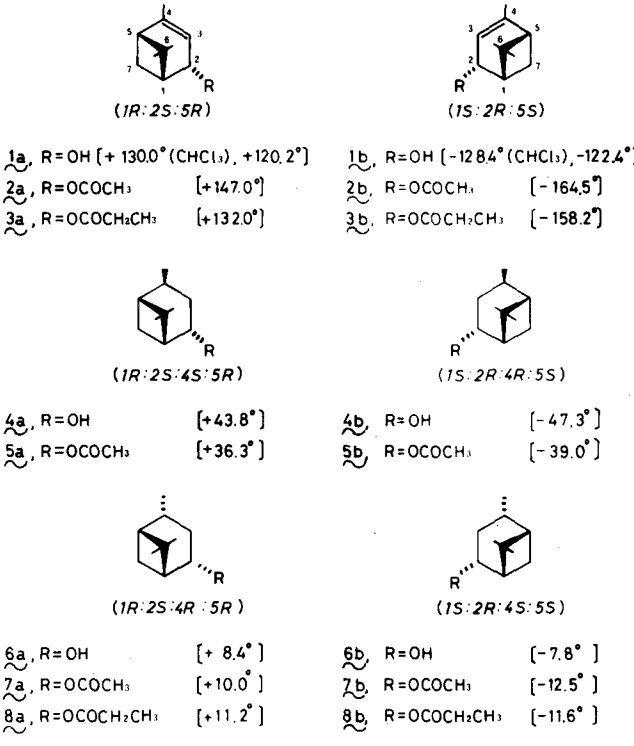

Figure 1. The structures of verbanyl type enantiomers. (+)-Enantiomers were synthesized for the present study. Values in brackets indicate $[\alpha]_D$-value measured in 1% benzene solution, unless otherwise stated.

Adams[3] who used enantiomeric periplanine-B. In the mixing tests with (+)- and (−)-enantiomers, pheromone activities induced by 20 µg of (+)-enantiomers were not affected basically by adding various quantities of (−)-enantiomers.

The ways in which optical enantiomers of insect pheromones may influence each other's activity can be listed as follows: 1. Pheromonally inactive enantiomers inhibit the activity of active enantiomers[17]. 2. Inactive enantiomers do not influence the potency of active enantiomers[18]. 3. All enantiomers are active[19]. 4. All enantiomers are inactive unless they are mixed[20]. Our mixing tests demonstrate that inactive enantiomers have no influence on the activity of (+)-enantiomers, i.e., case 2 applies our experiments. Among the 4 cases, the mutual influence between optical enantiomers on the activity may be due to competitive or cooperative occupation by the enantiomers of the same receptor in the cases, 1, 3 and 4. In the case it is assumed that chiral enantiomers are not recognized by the same receptor. In order to elucidate this further, we applied the EAG technique, and stimulated the male antennae with single and mixed samples of (+)- and (−)-enantiomers.

Both ends of an excised adult male antenna were inserted into recording and indifferent electrodes. The antenna was stimulated with a mixture of an odorous compound on a filter paper (1.3 cm diameter), applied by a 10-ml syringe in a continuous air-stream (30 ml/sec), by pressing the syringe plunger. The filter paper was impregnated with a specified quantity [10, 20, 50 or 100 µg for single enantiomers and 5 + 5, 10 + 10, 25 + 25 or 50 + 50 µg for sample of (+)- and (−)-enantiomers], using the hexane solution used in the behavioral assay. The EAG responses were amplified with microelectrode and biophysical amplifiers. EAG amplitudes were measured from the amplified responses on an oscilloscope. Five tests were performed for each quantity. For the control, the same procedure was repeated without the compound (for detailed method, see Nishino et al.[15]).

As shown in figure 2, average amplitudes from 5 repetitions for each quantitiy were plotted against the logarithms of the quantities for the single (+)- or (−)-enantiomer. In the mixed sample, plotting was based on the quantity of one enantiomer, i.e., in the case of 50 + 50 µg mixture, the EAG amplitude produced by this mixture was plotted at the 50 µg point of the abscissa. Subsequently, linear regressions were drawn for the single and mixed enantiomers [the following correlation coefficients (r) were obtained. r = 1.00 (**3a** + **3b** and **8a**), r = 0.99 (**3a** and **8a** + **8b**), r = 0.98 (**7a**, **7b**, **7a** + **7b** and **8b**), r = 0.97 (**3b**)]. We selected the quantity of 25 + 25 µg of enantiomeric mixture for analysis of the EAG result by considering significant pheromonal activity at 20 µg of (+)-enantiomers (table), and carried out the analysis as follows: as an example, the 25 + 25 µg mixture of **8a** + **8b** is cited here (fig. 2C). a) The 25 + 25 µg mixture shows 0.79 mV on the regressive line (x in fig. 2C). b) This amplitude corresponds to 28.2 µg of (+)-enantiomer (**8a**) (x'). c) The x' (0.79 mV) is regarded as EAG amplitude comprised of amplitudes due to the original 25 µg of **8a** and (−)-enantiomer (**8b**) corresponding to 3.2 µg (28.2.–25) of **8a**. d) Accordingly, EAG amplitude at 3.2 µg of **8a** is read using the line (0.15 mV, y'). e) The y' is substituted on the line of **8b** (y''), and the quantitiy of **8b** causing the amplitude y'' is determined (25 µg). Thus, it was revealed that the EAG amplitude induced by the 25 + 25 µg mixture was made up of amplitudes elicited by 25 µg of **8a** and 25 µg of **8b**. This implies that the EAG amplitude caused by the mixture is composed of independent contributions by an equivalent quantity of either enantiomer, which strongly suggests that the (+)- and (−)-enantiomers

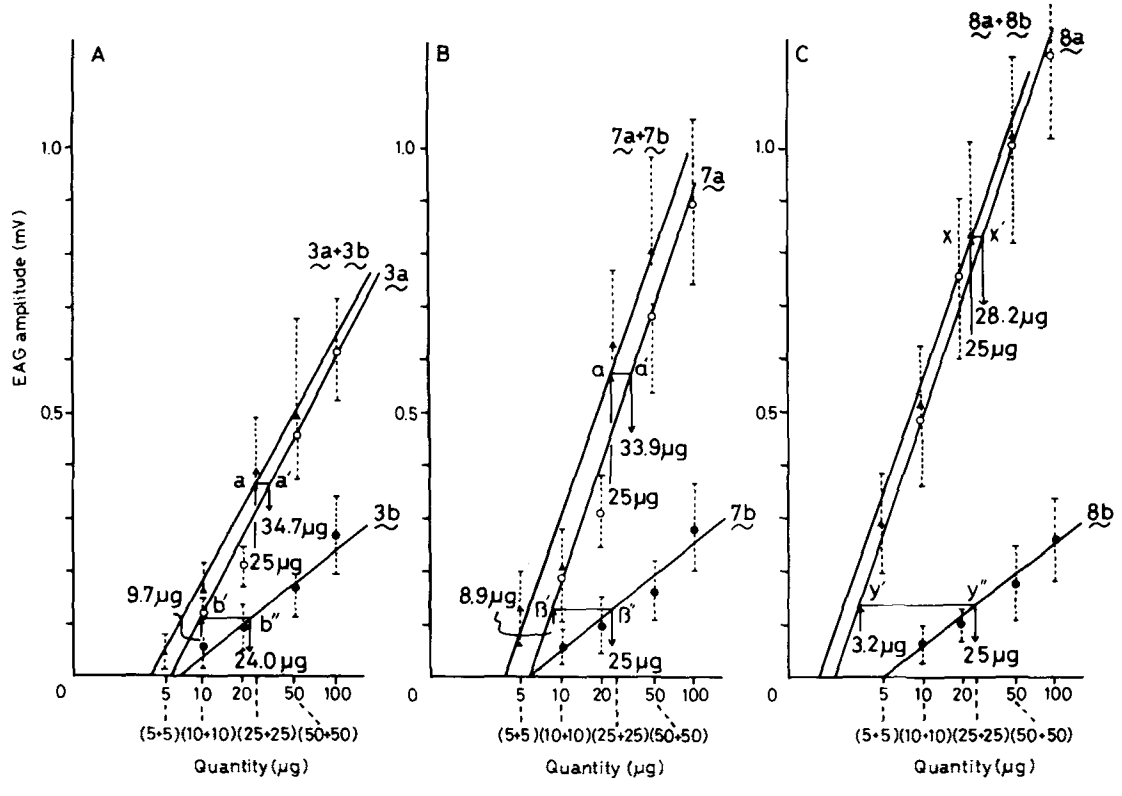

Figure 2. EAG amplitudes of single and mixed samples of (+)- and (−)-enantiomers of the verbane type sex pheromone mimics [(+)-transverbenyl propionate (A), (+)-verbanyl acetate (B) and (+)-verbanyl propionate (C)]. Plottings are made of average amplitudes from 5 repetitions at 10, 20, 50 and 100 µg of (+)-enantiomers (○; **3a**, **7a** and **8a**) and (−)-enantiomers (●; **3b**, **7b** and **8b**). Plots for the mixed samples (▲; **3a** + **3b**, **7a** + **7b** and **8a** + **8b**) are also based on average amplitudes from 5 repetitions with mixtures of equivalent quantities (5 + 5, 10 + 10, 25 + 25 and 50 + 50 µg) of (+)- and (−)-enantiomers. Ordinate: EAG amplitude in mV. Abscissa: logarithmic scale of quantity; in the case of a mixed sample the quantity of 1 component [(+)- or (−)-enantiomer] is adjusted to the unit of quantity on the scale.

stimulate independently different kind of olfactory receptors. The same analysis was performed for the other compounds (a→a′ and b′→b″ in **3a–3b** series (fig. 2A), and $\alpha \rightarrow \alpha'$ and $\beta' \rightarrow \beta''$ in **7a–7b** series (fig. 2B)], and the independent contributions of the optical isomers to the EAG amplitudes were elucidated in these enantiomeric pairs as well as the case of **8a–8b** series.

It is reasonable to expect that the pheromonally inactive enantiomers interact with receptors other than sex pheromone receptors which are assumed to interact with active (+)-enantiomers[10]. Thus, it has been shown by behavioral and physiological methods that in the American cockroach pheromonally inactive enantiomers of optical chiral pheromonal substances possess no influence on the potency of active enantiomers.

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Rapid characterization and partial purification of various animal amine oxidases[1]

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Summary. The use of chromatofocusing to obtain a rapid characterization of tissue amine oxidases from various mammals is proposed. This technique yields partially purified enzymes well suited for immunological studies. Chromatofocusing can be also used in a three-step purification of pig kidney diamine oxidase.
Key words. Amine oxidases, purification; chromatofocusing.

Amine oxidases are widespread throughout living organisms[2–5]. They catalyze the oxidative deamination of various amines according to the equation:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$$

Circulating and intracellular amine oxidases have been described both in plants and animal tissues[2–6]. The intracellular enzymes are more active on the diamines, particularly putrescine and cadaverine; histamine is often oxidized at an even greater rate[5]. In view of the increasing interest in putrescine as the precursor of polyamines, the level of which has been related to various cellular events and particularly to cellular proliferation, the role of diamine oxidases is at present actively being investigated. The mechanism of action of these enzymes and their localization have been studied[7]. Their presence in various tissues of the same organism, and in different organisms, prompted us to start a thorough investigation on the immunological relationships between some amine oxidases[8,9].
In the present paper we describe a rapid purification procedure using a chromatofocusing technique that has been successfully applied to a number of mammalian tissues. This procedure allows us to obtain purified amine oxidase preparations suitable for comparative kinetic and immunological studies.

Materials and methods. All chemicals used were of analytical grade and used without further purification. Putrescine was purchased from Sigma, St. Louis, Mo., USA. Polybuffer exchanger 94 (PBE 94), polybuffer 74 (PB 74) and Sepharose 4B from Pharmacia, Uppsala, Sweden. Bio-Rad protein assay kit from Bio-Rad Laboratories, Richmond, Cal., USA; 1,4-^{14}C-Putrescine by The Radiochemical Centre, Amersham, England.
Enzyme assay. Protein was determined either with a biuret method[10] or with the Bio-Rad protein assay. Diamine oxidase activity was tested according to Okuyama and Kobayashi[11] by using ^{14}C-putrescine as substrate. After 1 h incubation at 37 °C, the reaction mixture was stopped and extracted twice with toluene. The toluene containing ^{14}C-Δ'-pyrrolidine so formed was mixed with 3 ml of Lipoluma (LUMAC, Swiss) and counted in a liquid scintillation counter.
Antibody production. New Zealand rabbits were injected with diamine oxidase from pig kidney, purified according to Rinaldi et al.[12], in complete Freund's adjuvant. After several boosters, the animals were bled and the antisera pooled. The gamma-globulin fraction was purified by 33% ammonium sulphate precipitation and extensive dialysis against borate-NaCl buffer, pH 8.4. Antigen-antibody reaction was tested either by double diffusion[13] or with a Behring Laser Nephelometer (Behring-