Short Communications

4,11-Epoxy-*cis*-eudesmane, soldier cephalic secretion of the Nearctic desert termite, *Amitermes minimus* Light (Termitidae: Termitinae)[1,2]

R.H. Scheffrahn, J.J. Sims, L.K. Gaston and M.K. Rust

Department of Entomology and Department of Plant Pathology, University of California, Riverside (California 92521, USA), 27 June 1983

Summary. The soldier cephalic secretion of the Nearctic desert termite, *Amitermes minimus,* consists almost entirely of 4,11-epoxy-*cis*-eudesmane which was previously identified from soldiers of 2 African *Amitermes* species. Soldiers of *A.minimus* each store circa 61 µg of the secretion. Bioassays with the ant, *Crematogaster californica,* indicate a repellent role for the eudesmane compound in termite defense.

Key words. Termite, Nearctic desert; *Amitermes minimus;* soldier cephalic secretion; eudesmane, 4,11-epoxy-cis-; termite defense; allomones; pheromones.

Termite soldier cephalic secretions generally serve as defensive allomones against predators and may concomitantly function as nestmate alarm pheromones[3]. In numerous termite taxa, these diverse compounds have potential application as phenotypic markers for chemotaxonomy and intraspecific variability[4]. The chemistry of soldier secretions in the large cosmopolitan genus *Amitermes* Silvestri includes a variety of compounds including monoterpene[5] and sesquiterpene hydrocarbons[6], methyl ketones[7], and undetermined viscous substances[5,8]. The tricyclic sesquiterpenoid ether, 4,11-epoxy-*cis*-eudesmane, was isolated as the primary component (> 90%) of soldier secretions of the West African termite, *Amitermes evuncifer* Silvestri[9], and the East African species, *A.messinae* Fuller[10], and was reported to be toxic against several ant predators[11]. We now report this compound as the major constituent of the soldier secretion of the nearctic desert termite, *Amitermes minimus,* and suggest its primary role as a defensive repellent.

Materials and methods. A total of 109 *A.minimus* soldiers was collected during April and May 1983, in the chaparral habitat near the University of California, Riverside. The heads of decapitated soldiers were immersed in 1.0 ml CS_2 and crushed. The crude extract was chromatographed on a 2 m × 6 mm ID glass column packed with 10% Silar 10C on Chromosorb W at 155 °C (N_2 = 24 ml/min) which produced a simple trace dominated by a single large peak. The major component was collected as a pure oil by a previous method[6].

Foraging groups consisting of soldiers, workers, and nymphs were unearthed in dead root systems of *Encelia farinosa* Grey that had been internally mined by the termites. Soldiers constituted 1.0% of 6 separate foraging contingents sampled ($\bar{X}$ = 757 termites each, range 84 to 1662). When soldiers were removed from the wood and combined, an unsettling floral odor eminated from the agitated caste assemblage.

To test for intrinsic repellency of the major component, a bioassay was devised using a laboratory colony of *Crematogaster californica* Emery as a representative sympatric ant of *A.minimus.* A 0.5 mm wide circular groove (4.4 cm circ.) inscribed on a 6 cm dia disk of thick aluminium foil was evenly filled with 5 µl acetone solution containing 6.2 µg of the major component or acetone alone. After the solvent evaporated, a 0.7 cm dia drop of dilute honey solution was deposited at the center of the circular groove. The disk was placed in a foraging area of the ant colony which had not been fed for 24 h. The time required for the first ant to begin feeding on the honey solution and the number of ants feeding after each of 5 min from introduction of the disk was recorded. Treatments and controls (10 each) were alternately tested.

Results and discussion. The major component of the soldier head extract constituted 96% (RT = 9.97 min) of the secretion while 3 small peaks at 6.02 (1%), 6.33 (2%), 7.58 (1%) (*β*-caryophyllene standard = 6.13) accounted for the remaining volatiles. The average yield of secretion was 61 µg/soldier or 3.7% of their fresh biomass ($\bar{X}$ = 1.66 mg, n = 24). The main component was identified as 4,11-epoxy-*cis*-eudesmane (fig. 1) from spectral similarities with the known compound[9,12]. Under our conditions[13], EIMS yielded no molecular ion (m/z = 222, $C_{15}H_{26}O$) with the observed fragment ions > 10%: m/z = 81 (14%), 91 (14), 93 (12), 95 (10), 105 (11), 107 (13), 109 (53), 119 (13), 133 (15), 147 (12), 149 (26), 189 (29), 207 (100), 208 (13). The PNMR recording[14] of this compound gave the following data: *δ* 2.27 (dt, 5.3 Hz, 13.6 Hz, 1 H); 2.17 (dq, 13.6

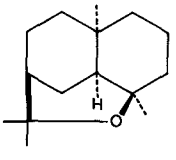

Figure 1. The principle component of the *Amitermes minimus* soldier secretion, 4,11-epoxy-*cis*-eudesmane.

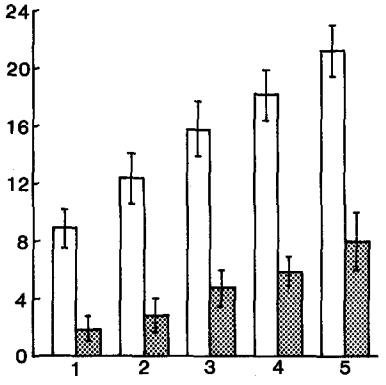

Figure 2. Repellent activity of 6.2 µg deposits of 4,11-epoxy-*cis*-eudesmane (patterned bars) against foraging *Crematogaster californica* workers. Control treatments shown by open bars. The vertical axis represents the number of ants feeding on the honey solution. The horizontal axis indicates time in minutes when counts were taken after bioassay disks had been introduced. Confidence intervals are at the 95% level.

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.

1 Classification as proposed by Sands, W.A., Bull. Br. Mus. nat. Hist., Ent., suppl. *18* (1972).

2 Supported by Sigma Xi Grant-in-Aid of Research and California Statewide Critical Applied Research grant.

3 Prestwich, G.D., J. chem. Ecol. *5* (1979) 459.
4 Prestwich, G.D., Sociobiology *4* (1979) 127.
5 Moore, B.P., J. Insect Physiol. *14* (1968) 33.
6 Scheffrahn, R.H., Gaston, L.K., Sims, J.J., and Rust, M.K., J. chem. Ecol. *9* (1983) 1293.
7 Prestwich, G.D., Proc. IUSSI Symp., p. 149. Dijon 1975.
8 Noirot, Ch., Biology of Termites, vol. 1, p. 89. Eds K. Krishna and F.M. Weesner. Academic Press, New York 1969.
9 Wadhams, L.J., Baker, R., and Howse, P.E., Tetrahedron Lett. *1974* (1974) 1697.
10 Meinwald, J., Prestwich, G.D., Nakanishi, K., and Kubo, I., Science *199* (1978) 1167.
11 Howse, P.E., Proc. IUSSI Symp., p. 23. Dijon 1975.
12 Baker, R., Evans, D.A., and McDowell, P.G., J. chem. Soc. chem. Commun. *1977* (1977) 111.
13 EIMS was conducted with a Nicolet FT/MS-1000 instrument at 70 eV. CIMS using methane and isobutane as reaction gases failed to render a molecular ion.
14 A 500 MHz proton recording with resolution enhancement (CCl_4+10% D_6-acetone) was obtained using a Bruker WM-500 spectrometer operated at the California Institute of Technology under NSF grant No. CHE-79-16324.
15 Baker, R., by personal communication for which our sincere gratitude is offered.
16 A CNMR spectrum ($CDCl_3$) was recorded on a Jöel FX-200 instrument at 25 MHz.
17 Light, S.F., Calif. Univ. Publ. Ent. *5* (1932) 355.
18 Weesner, F.M., Biology of Termites, vol. 2, p. 477. Eds K. Krishna and F.M. Weesner. Academic Press, New York 1970.
19 Silvestri, F., Boll. Lab. Zool. gen. agr. Portici *9* (1915) 1.
20 Fuller, C., S. Afr. J. nat. Hist. *3* (1922) 70.
21 Emerson, A.E., Fieldiana, Zool. *37* (1955) 465.

0014-4754/84/101136-02$1.50 + 0.20/0

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

C. Nishino, S. Manabe and H. Takayanagi

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194 (Japan), 30 June 1983

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-