

Acyclic Ketones in the Defensive Secretion of a "Daddy Longlegs" (*Leiobunum vittatum*)

(Arachnida/Opiliones)

J. MEINWALD*, A. F. KLUGE*, J. E. CARREL†, AND T. EISNER†

* Department of Chemistry and † Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14850

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ABSTRACT The defensive secretion of the "daddy longlegs" *Leiobunum vittatum* was analyzed and found to contain the acyclic ketones 4-methylheptan-3-one and E-4,6-dimethyl-6-octen-3-one as its major organic components. Although 4-methylheptan-3-one has been found previously as an alarm substance in certain ant genera, the second component, whose structure is confirmed by synthesis, is new.

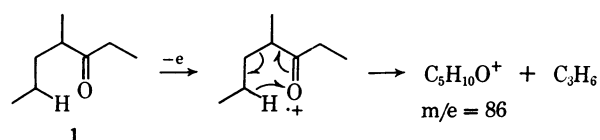
Many terrestrial arthropods are protected by defensive secretions, which they produce and store in integumental glands, and eject when attacked by predators. Much work has been done in recent years on these defensive mechanisms (1), but the studies have been confined largely to insects and millipeds, and have generally ignored species of other classes. We here report on the chemical composition of the defensive secretion of *Leiobunum vittatum*, an arachnid. It is a "harvestman" (order Opiliones), belonging to the group (suborder Palpatores, family Phalangiidae) that includes most of the species commonly known in the United States as "daddy longlegs".

The defensive glands of the animal are a pair of small sacs, opening near the anterolateral border of the carapace (2). When seized, the animals almost invariably discharge, visibly wetting themselves with secretion and releasing a powerful odor. The secretion was collected by grasping the animals in forceps, pinching them slightly, and causing them to discharge into small capillary tubes.

The sample, obtained from 14 individuals of both sexes, amounted to approximately 5.7 μ l of opaque liquid, of which the major constituent was water. The infrared spectrum (CCl_4) of the organic constituents showed aliphatic C-H absorptions and a carbonyl absorption at 1713 cm^{-1} . Analysis by gas-liquid chromatography (GLC) on a 3-m column of 30% Carbowax 20M on Chromosorb W at 125°C showed two major volatile components with retention times of 2.7 and 6.3 min. In addition, there were trace components (<1%) at longer retention times. Further information was obtained by GLC-mass spectrometry.

The mass spectrum of the first eluted component contained the following prominent peaks at 70 eV: m/e (relative intensity) 128(3), 99(13), 86(38), 71(79), 57(100), and 43(89). These data are consistent with a ketone of molecular formula $\text{C}_8\text{H}_{16}\text{O}$. The McLafferty rearrangement peak at m/e = 86 is best rationalized in terms of formula 1 (4-

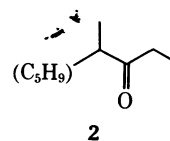
methylheptan-3-one, scheme 1), and an authentic sample of



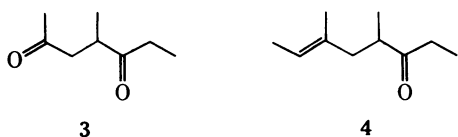
Scheme 1

4-methylheptan-3-one (3) was indistinguishable from this component by GLC-mass spectrometry.

The mass spectrum of the second eluted component contained the following prominent peaks at 70 eV: 154(6), 139(5), 136(4), 125(27), 97(60), 86(24), 69(49), 57(84), and 55(100). The spectrum is consistent with a ketone of molecular formula $\text{C}_{10}\text{H}_{18}\text{O}$. The rearrangement peak at m/e = 86, along with the peaks at M-29, M-57, and 57, indicated that the second component was closely related to 4-methylheptan-3-one. With this assumption, partial structure 2 seemed attractive. Spectral data alone, however, did not permit the assignment of an unambiguous structure to the C_5H_9 residue comprising the distal portion of the molecule.

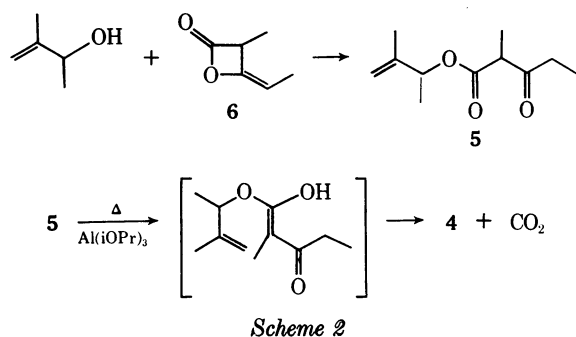


Further information was obtained by microozonolysis. To the natural two-component mixture in 30 μ l of CCl_4 (containing approximately 200 μ g each of 4-methylheptan-3-one and the second component) was added 70 μ l of hexane (Fisher Certified 99 mole %). A stream of ozone-containing oxygen was passed through the solution (-78°C) until a blue color persisted. The reaction vessel was warmed to room temperature as argon was passed through the solution. Triphenylphosphine was added to reduce the ozonide. Analysis by GLC-mass spectrometry (2 m Porapak Q, 80-100 mesh, 160°C) revealed the presence of acetaldehyde. Use of another column (2 m, 5% Carbowax 20M, 60-80 mesh firebrick, 140°C) permitted the characterization of the remaining fragment as $\text{C}_5\text{H}_{14}\text{O}_2$. The mass spectrum (70 eV) [142(3.5), 113(35), 57(92), and 43(100)] is consistent with expectations for the diketone 4-methylheptan-2,5-dione (3). On this basis, the second component can be formulated as 4,6-dimethyl-6-octen-3-one (4).



[It is to be noted that the occurrence of the McLafferty rearrangement in the mass spectrum of the second eluted component led to the formulation of partial structure 2. The absence of a hydrogen atom γ to the carbonyl oxygen atom in 4,6-dimethyl-6-octen-3-one does not vitiate the proposed structure in light of the facility of hydrogen migrations in mass spectrometry (4).]

E-4,6-Dimethyl-6-octen-3-one (4) was prepared by the aluminum isopropoxide-catalyzed Claisen rearrangement of 2-methyl-1-buten-3-yl 2-methyl-3-oxopentanoate (5), obtained by the addition of 2-methyl-1-buten-3-ol to methylketenedimer 6 (Scheme 2; for an analogous preparation, see



ref. 5). Compound 5 was heated at 210°C for 15 hr while gas evolution was monitored. The reaction was carried to approximately 75% conversion, as shown by GLC and infrared spectroscopy. The product, 4, bp 206–210°C (80%), was distilled directly from the reaction vessel. Further purification was effected by preparative GLC (3 m \times 1.0 cm, 7% Carbowax 20M on 60–80 mesh Chromosorb W, 150°C).

Analysis: Calculated for $C_{10}H_{18}O$: C, 77.86; H, 11.76. Found: C, 78.09; H, 11.73.

The infrared spectrum (CCl_4) of 4 showed the expected carbonyl absorption at 1714 cm^{-1} . The NMR spectrum (CCl_4) contained the following absorptions: δ 0.96 (3, d, $J = 6.5$ Hz, CH_3-CHCO), 0.92 (3, t, $J = 7.3$ Hz, CH_3-CH_2CO), 1.42–1.66 (6, overlapping allylic methyl groups), 1.8–2.82 (5,

complex), and 5.17 (1, m, $CH_3-HC=$). Ozonolysis of 4 gave acetaldehyde and 3. E-4,6-Dimethyl-6-octen-3-one was identical with the second eluted component of the *L. vittatum* secretion according to GLC-mass spectrometry.

Approximately 6% of the product mixture obtained from the Claisen rearrangement of 5 was the Z isomer (4.75 min as against 6.3 min for the E isomer on a 3-m 30% FFAP column at 130°C). That the two components were geometric isomers was shown by their having identical mass spectra and by the fact that ozonolysis of the mixture yielded solely acetaldehyde and 3. The assignment of the more stable E stereochemistry to the major isomer is made by analogy to the known thermodynamic course of the Claisen rearrangement (6).

The presence of various aliphatic ketones in arthropod secretions has been noted previously (7). 4-Methylheptan-3-one itself has been reported as an alarm substance in ants of the genera *Pogonomyrmex* (8) and *Atta* (9). Some of these ants coexist with *L. vittatum* through part of its range, and one wonders how this "daddy longlegs" might fare when attacked by them. Would its defensive secretion have the potentially disastrous effect of alarming and drawing the attention of the ants rather than repelling them? Or would the secretion be unambiguously defensive because of the second ketone that it contains?

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1. Reviews by Eisner, T., in *Chemical Ecology*, ed. E. Sondheimer and J. B. Simeone (Academic Press, New York, 1970); Eisner, T., and J. Meinwald, *Science*, **153**, 1341 (1966); Weatherston, J., *Quart. Rev. Chem. Soc.*, **21**, 287 (1967).
2. Kaestner, A., *Invertebrate Zoology* (Interscience Publishers, New York, 1967), Vol. 1.
3. DuBois, J. E., and R. Luft, *Bull. Soc. Chim. Fr.*, 1153 (1954).
4. See, for example, Budzikiewicz, H., C. Djerassi, and D. H. Williams, *Mass Spectrometry of Organic Compounds* (Holden-Day, San Francisco, 1967).
5. *Chem. Abstr.*, **60**, 13147e (1964).
6. Rhoads, S. J., in *Molecular Rearrangements*, ed. P. de Mayo (John Wiley and Sons, New York, 1963).
7. Review by Weatherston, J., and J. E. Percy in *Chemicals Controlling Insect Behavior*, ed. M. Beroza (Academic Press, New York, 1970).
8. McGurk, D. J., J. Frost, E. J. Eisenbraun, K. Vick, W. A. Drew, and J. Young, *J. Insect. Physiol.*, **12**, 1435 (1966).
9. Blum, M. S., F. Padovani, and E. Amante, *Comp. Biochem. Physiol.*, **26**, 291 (1968).