Colorado, gave advice on interpretation of x-ray patterns (including those for SUI 34972, 34973, and 35303 and UCGM 40606 through 40608 and 40610).
6. T. W. Bloxam, Department of Geology, University College of Swansea, Wales, chemically analyzed specimens for this study.
7. P. Sandberg, Department of Geology, University of Illinois, made possible amino acid analyses of our material.
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18. H. Mutvei, Naturhistoriska Riksmuseet, Stockholm; R. Batten, American Museum of Natural History, New York; and H. Lowenstam, California Institute of Technology, Pasadena, provided information on invertebrate shell histology.
19. Contribution No. 131 of the Department of Geology, University of Cincinnati.

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Cyclopentyl Ketones: Identification and Function in Azteca Ants

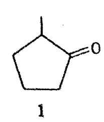
Abstract. *The anal gland secretions of dolichoderine ants in the genus* Azteca *are fortified with cyclopentyl ketones. Since these compounds, 2-methylcyclopentanone,* cis-*1-acetyl-2-methylcyclopentane, and 2-acetyl-3-methylcyclopentene, release sustained alarm behavior in ant workers, they constitute a new chemical class of insect pheromones. The ketones probably also function as defensive compounds and thus are part of the ants' alarm-defense system.*

Ants in the subfamily Dolichoderinae have yielded several novel natural products (*1*) related to nepetalactone, a characteristic compound of the catnip plant (*2*). Five of these exocrine products have been identified from ant workers in a variety of dolichoderine genera (*1*). Also, several acyclic ketones, of both terpenoid (*3*) and nonterpenoid (*4*) origin, are produced by these ants. Whereas these ketones function as both defense materials (*4*) and releasers of alarm behavior (*5*), the cyclopentanoid monoterpenes are reported to be utilized either for defense (*6*) or as fixatives for the more volatile ketones (*7*). All of these exocrine products are synthesized in the anal glands (*8*), structures which are limited to species in the subfamily Dolichoderinae and appear to have arisen de novo to function as social organs.

The dolichoderine genus *Azteca* constitutes one of the most conspicuous ant taxa in the American tropics. Many species have an obligatory relationship with myrmecophytes in the moraceous genus *Cecropia*, whereas others construct large carton nests in a variety of trees and shrubs (*9*). When disturbed, workers in the populous colonies swarm all over the intruder. Although they lack a functional sting, an emphatic deterrent message is conveyed through painful bites administered by the aggressive workers.

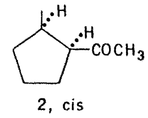
Disturbed workers of many species of *Azteca* are characterized by a pungent sweet-sour odor which, to our noses, differed clearly from that of any other dolichoderine genus with which we were familiar. Consistent with their distinctive organoleptic properties, three cyclopentyl ketones which have not been previously detected in ants

m/e (relative intensity):



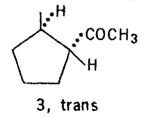
98(50), 83(10), 70(10), 69(30), 56(10), 55(60), 42(100), 41(50)

1



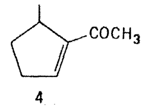
126(20), 111(9), 83(57), 71(83), 55(88), 43(100)

2, cis



126(22), 111(34), 83(60), 71(42), 55(88), 43(100)

3, trans



124(38), 109(42), 81(100), 79(24), 53(19), 43(85)

4

Fig. 1. Mass spectra of cyclopentyl ketones.

or any other biological source and possess this odor have been identified as anal gland products (*10*). Furthermore, since these compounds function as alarm pheromones, they constitute a new chemical class of releasers of social behavior (*11*) and may also be utilized as defensive compounds.

Methylene chloride extracts of two species of *Azteca* which had constructed carton nests in a spiny palm (*Astrocaryum standleyanum*) and a cashew tree (*Anacardium occidentale*) were prepared for chemical analyses. The palm species was determined as *Azteca* nr. *velox* and the cashew population as *Azteca* nr. *nigriventris* (*12*). Combined gas chromatography–mass spectroscopy (*13*) of the extracts indicated three previously unreported major volatile components with molecular weights of 98 (**1**), 126 (**2**), and 124 (**4**) (see Fig. 1) in *A.* nr. *nigriventris*, whereas the 124 component was not detected in *A.* nr. *velox*. A computer search of numerous mass spectra (*14*) indicated similarities of **1** to 2-methylcyclopentanone, and examination of authentic samples of positional isomers (*15*) confirmed the presence of the 2-isomer (**1**) in both species of *Azteca*.

The other two volatiles both appeared to be related methyl ketones from their mass spectra (base peak *m/e* 43). Comparison of **2** with acetylcyclohexane (*16*) and of **4** with acetylcyclohexene (*15*) suggested that the two unknowns might be methylcyclopentyl (or pentenyl) methyl ketones. A mixture of *cis*- and *trans*-1-acetyl-2-methylcyclopentane was synthesized by the method of Hopff (*17, 18*), and *cis*-1-acetyl-2-methylcyclopentane was prepared by catalytic hydrogenation of 1-acetyl-2-methylcyclopentene (*19, 20*). The retention time and mass spectrum of the ant volatile of *m/e* 126 (**2**) were identical to those of *cis*-1-acetyl-2-methylcyclopentane. Although synthetic *cis* and *trans* (**2** and **3**) are separable on the column used, *trans* (**3**) was not detected in either *Azteca* species. The mass spectra of the isomeric 1-acetyl-1-methylcyclopentane (*21*) and 1-acetyl-3-methylcyclopentane (*22*) were sufficiently different to distinguish them from **2**.

The mass spectral fragmentation pattern of **4** was similar to those of 1-acetyl-2-methylcyclopentene (*23*) and 3-acetyl-2-methylcyclopentene (*23*), but the relative intensities differed significantly. Ozonolysis of 2,4-dimethylcyclohexene obtained by acid dehydration of 2,4-dimethylcyclohexanol (*15*) followed by treatment with potassium *tert*-butoxide gave 2-acetyl-3-methylcyclopentene, which was identical with the third volatile from *A.* nr. *nigriventris.* All three ketones were also found in extracts of three other *Azteca* species but were lacking in two others (*24*).

The communicative functions of the cyclopentyl ketones were evaluated on strong colonies of the *Azteca* species in both the palm and cashew trees. Both species had strong foraging populations on well-developed trails. Droplets (10 μl) of the synthetic ketones were placed directly on trails 30 cm from the nests, and the reactions of the workers were observed for up to 2 hours.

All of the ketones produced typical alarm reactions in both *Azteca* species. Large aggregations of excited ants formed above and below the ketonic droplet, and the alarm reaction appeared to spread concentrically from these masses of workers. Many workers appeared to be recruited to the point of application of the droplet so that traffic along the odor trail increased at least two- to fourfold almost immediately. At the same time some workers moved away from the aggregations and as a consequence the upper portion of the tree was covered with ants moving in a rapid but nonoriented manner. Typical alarm behavior sometimes persisted for more than 30 minutes. For *A.* nr. *velox* ketone **1** was considerably more active than **2** in releasing alarm behavior. Ketone **4** was the most active alarm releaser for workers of *A.* nr. *nigriventris,* and **1** and **2** were approximately equal in activity.

Fighting often resulted when the workers of either species were exposed to a *cis-trans* mixture of 1-acetyl-2-methylcyclopentane (**2**, **3**) (*25*). Ants attracted to the ketonic emission source frequently challenged their sister workers with spread mandibles and the struggling combatants often fell from the trees (*26*).

An especially aggressive ant species, the imported fire ant *Solenopsis invicta,* was used to determine whether the ketones were effective repellents. Droplets (10 μl) of each ketone were applied to the thoraxes of freshly killed crickets, which were then placed on the foraging platform of a laboratory colony. Excited workers approached the crickets but rapidly moved away from them without making contact. Untreated crickets, on the other hand, were quickly overrun by the ant workers (*27*).

Dolichoderine anal glands have previously been demonstrated to be an extraordinarily rich source of terpenoid natural products which possess a clear-cut biogenetic relationship (*1*). These cyclopentyl ketones, however, do not appear to be related to the monoterpenes and may be produced from acyclic precursors (such as 2-octanone). Biosynthetic considerations notwithstanding, the presence of cyclopentyl ketones in the anal glands of *Azteca* species suggests that the chemistry of the Dolichoderinae still offers many surprises as testimony to the biosynthetic versatility of these well-developed exocrine organs (*8*). It is difficult to avoid the conclusion that the great success of the dolichoderine ants is highly correlated with the evolution of these social organs which are "committed" to the synthesis of pheromones and defensive products.

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10. Gas chromatographic–mass spectroscopic analyses of extracts of excised anal glands established that these structures were the source of the cyclopentyl ketones.
11. Although 3-methyl-2-cyclohexane-1-one has been reported in bark beetles [G. W. Knizer, A. F. Fentiman, R. C. Foltz, J. A. Rudinsky, *J. Econ. Entomol.* **64**, 970 (1971); J. A. Rudinsky, M. Morgan, L. M. Libbey, R. R. Michael, *Environ. Entomol.* **2**, 505 (1973)] this is the first report of any cyclopentyl ketones in insects.
12. The genus *Azteca* is badly in need of taxonomic revision, and specific epithets cannot be readily assigned to many of the species. We thank P. B. Kannowski, W. F. Buren, and D. F. Janzen for taxonomic aid. Specimens of all species investigated have been deposited in the museum of the Department of Entomology at the University of Georgia.
13. An LKB-9000 combined gas chromatograph–mass spectrometer equipped with a 6-foot (~ 1.8-m) glass column of 10 percent SP-1000 on Supelcoport 80-100 (Supelco, Bellefonte, Pa.) was used. Secretions were also examined on a 6-foot column containing 1 percent OV-17 (Supelco) on the same support. Both columns were programmed from 70°C at 8° per minute. We thank H. M. Fales and W. Comstock of the National Heart and Lung Institute for the use of this instrument.
14. The program was developed by S. R. Heller of the Division of Computer Research Technology, National Institutes of Health. See S. R. Heller, *Anal. Chem.* **44**, 1951 (1972).
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24. Cyclopentyl ketones (but no 2-heptanone) were identified in two *Azteca* species near *instabilis* (from Puerto Viejo, Costa Rica) and a species near *constrictor* (San Vito, Costa Rica). No cyclopentyl ketones were detectable in *A. chartifex* (Taboga, Costa Rica) and *A. parzensis* (Mannaos, Brazil). *Azteca* nr. *velox* showed 48 percent 2-heptanone, 4 percent **1**, and 15 percent **2** in its volatile extract, while *A.* nr. *nigriventris* exhibited 21 percent 2-heptanone, 7 percent **1**, 4 percent **2**, and 20 percent **4**. *Azteca* nr. *instabilis* had 7 percent **1**, 31 percent **2**, and 40 percent **4**.
25. Although the *trans* isomer (**3**) has not been detected in any *Azteca* species, its biological activity was evaluated in admixture with the naturally occurring *cis* isomer (**2**). It was not separately tested.
26. A similar reaction was reported for workers of the dolichoderine *Conomyrma pyramicus* after exposure to hexanoyl chloride, an isostere of its alarm pheromone, 2-heptanone [R. A. Metcalf and R. L. Metcalf, *Ann. Entomol. Soc. Am.* **63**, 34 (1970)].
27. 2-Heptanone, a major constituent of *A.* nr. *velox* and *A.* nr. *nigriventris*, was equivalent to the cyclopentyl ketones as a repellent for fire ant workers. Iridodial, a major constituent in *A.* nr. *velox* (but not present in *A.* nr. *nigriventris*) was not an effective ant repellent, possibly because it rapidly polymerizes in air. Iridodial is not reported to possess demonstrable insecticidal activity and is believed to serve as a fixative for the acyclic ketones produced in anal glands (*7*).
28. J.W.W. and S.L.E. are supported by a grant from the Minority School Biomedical Support Program of the National Institutes of Health to Howard University and by the College Science Improvement Program of the National Science Foundation.

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