response attenuation with stimulus repetition (*1*, *2*). However, while sensory stimulation can trigger brief responses in these units, PRF neurons exhibited sustained bursts of unit discharge only in conjunction with specific motor activities. Indeed, discharge rates in PRF neurons were undiminished after the elimination of identified sensory inputs.

These findings suggest a parsimonious explanation of many conditioning, sensory, and sleep cycle studies of PRF neurons. The apparent selectivity of PRF discharge for "noxious" stimuli (*5*), the very long latency and duration of certain sensory responses (*1*, *2*, *5*), and the changes in PRF activity during conditioning (*2*, *9*) may all reflect specific motor discharges. Only careful monitoring and control of motor activity can determine if sensory or conditioned influences on unit firing are separable from the motor changes that accompany them. The motor-related discharge in PRF cells in waking is consistent with the discharge of these neurons in REM sleep (*10*), a time of intense activation of motor systems (*11*). Our observations in the unrestrained cat indicate that discharge is not selective for REM sleep, but rather for motor activation.

Pontine animals have been shown to be capable of exhibiting a wide variety of complex motor behaviors (*12*), and must therefore retain sufficient neuronal substrate for the regulation of complex movements. The PRF's medial zone, whose unit activity is reported here, is the principal source of pontine reticular projections to the spinal cord; more than half of its neurons send their axons directly into the ventral, motor areas of the cord (*13*). Many of these neurons also receive monosynaptic input from the cerebellum and other areas related to motor control (*14*). Therefore, the anatomy and physiology of this region are compatible with the behavioral data reported here, which suggest a major role for PRF neurons in the regulation of motor output.

JEROME M. SIEGEL
DENNIS J. MCGINTY
Neurophysiology Research, Veterans Administration Hospital, Sepulveda, California 91343, and *Brain Research Institute* and *Department of Psychology, University of California, Los Angeles*

References and Notes

1. V. E. Amassian and R. V. Devito, *J. Neurophysiol.* **17**, 575 (1954); W. E. Bradley and C. J. Conway, *Exp. Neurol.* **16**, 237 (1966); O. Pompeiano and C. D. Barnes, *J. Neurophysiol.* **34**, 709 (1971); M. Glickstein, J. Stein, R. A. King, *Science* **178**, 1110 (1972); F. Motokizawa, *Exp. Neurol.* **44**, 135 (1974); B. W. Peterson, M. Filion, L. P. Felpel, C. Abzug, *Exp. Brain Res.* **22**, 335 (1975); S. Nagano, J. A. Myers, R. D. Hall, *Exp. Neurol.* **49**, 653 (1974); J. B. Hornby and J. D. Rose, *ibid.* **51**, 363 (1976).
2. M. Scheibel, A. Scheibel, A. Mollica, G. Moruzzi, *J. Neurophysiol.* **18**, 309 (1955); P. Bach-Y-Rita, *Exp. Neurol.* **9**, 327 (1964); M. E. Scheibel and A. B. Scheibel, *Arch. Ital. Biol.* **103**, 279 (1965); J. P. Segundo, T. Takenaka, H. Encabo, *J. Neurophysiol.* **30**, 1221 (1967); B. W. Peterson, J. I. Franck, N. G. Pitts, N. G. Daunton, *ibid.* **39**, 564 (1976).
3. J. M. Siegel and D. J. McGinty, *Science* **193**, 240 (1976).
4. The pontine gigantocellular tegmental field as defined by A. L. Berman in *The Brain Stem of the Cat* (Univ. of Wisconsin Press, Madison, 1968) includes parts of the reticularis pontis oralis and caudalis nuclei described in earlier nomenclature.
5. Responses of PRF units to noxious stimuli have been examined in acute and chronic studies including: K. L. Casey, *Science* **173**, 77 (1971); K. L. Barnes, *Exp. Neurol.* **50**, 180 (1976); J. K. Nyquist and J. H. Greenhoot, *Brain Res.* **70**, 157 (1974); D. W. Young and K. M. Gottschaldt, *Exp. Neurol.* **51**, 628 (1976); and C. L. Sun and G. B. Gatipon, *ibid.* **52**, 1 (1976).
6. We tested 31 cells for somatic, 44 for vestibular, 25 for auditory, 32 for visual, and 25 for polysensory responses.
7. Socket nuts were imbedded in the head-plug acrylic cement. During restraint, socket wrenches were fitted into the nuts and fixed in a stereotaxic head holder.
8. A total of 12 units were subjected to the stimulus reduction procedure. With the cat unrestrained, 10 of the 12 gave maximal responses to vestibular stimuli, and 2 to somatic stimuli. In the somatic cells, the 3-minute postrestraint period was drawn from the first waking period occurring 4 hours after the restraint had ended, to allow for dissipation of the local anesthetic agents. The procedure reduced discharge in only one unit (by 30 percent) relative to the rate before restraint. Six of the units had higher rates during the stimulus reduction period than after. Phasic electromyographic activity was greatest during and after the procedure.
9. N. Yoshii and H. Ogura, *Med. J. Osaka Univ.* **11**, 1 (1960); R. P. Vertes and N. E. Miller, *Brain Res.* **103**, 229 (1976).
10. J. A. Hobson, R. W. McCarley, R. T. Pivik, R. Freedman, *J. Neurophysiol.* **37**, 497 (1974); D. J. McGinty and J. M. Siegel, presented at the Conference on the Neurobiology of Sleep and Memory, Mexico City, 17–20 March 1975, and in *Neurobiology of Sleep and Memory*, R. Drucker-Colin and J. McGaugh, Eds. (Academic Press, New York, in press).
11. REM motor activity can be seen in cats with lesions blocking peripheral atonia [see K. Henley and A. R. Morrison, *Acta Neurobiol. Exp.* **34**, 215 (1974)] and can be recorded in intact animals in a variety of motor systems; see, for example, E. V. Evarts, *J. Neurophysiol.* **27**, 152 (1964); J. Pellet, M. F. Tardy, F. Harlay, S. Dubrocard, J. C. Gilhodes, *Brain Res.* **81**, 75 (1974); Y. Lamarre, M. Filion, J. P. Cordeau, *Exp. Brain Res.* **12**, 480 (1971).
12. P. Bard and M. B. Macht, in *The Neurological Basis of Behavior*, G. E. W. Wohlstenholme, Ed. (Little, Brown, Boston, 1968); K. E. Bignall, *Exp. Neurol.* **42**, 566 (1974).
13. A. Torvik and A. Brodal, *Anat. Rec.* **128**, 113 (1957).
14. J. C. Eccles, R. A. Nicoll, D. W. F. Schwarz, H. Taborikova, T. J. Willey, *J. Neurophysiol.* **38**, 513 (1975); G. N. Orlovskii, *Biophysics* **15**, 761 (1970).
15. We thank S. M. Breedlove. Supported by the Medical Research Service of the Veterans Administration and PHS grant MH 10083.

12 October 1976

Sesquiterpene Progenitor, Germacrene A: An Alarm Pheromone in Aphids

Abstract. *Germacrene A, the elusive biogenetic "parent" of many sesquiterpenes, has been isolated from the spotted alfalfa aphid and identified as a new intrageneric aphid alarm pheromone.*

When attacked by a predator, certain aphids secrete droplets of fluid from their cornicles (Fig. 1). This secretion contains an alarm pheromone, which in the manner of a dying gasp, signals danger to other aphids nearby. The response of aphids is to walk, fall, or leap away from the plant.

Since the phylogenetic relationships of aphids at the subfamily level have been difficult to determine on morphological grounds, we have tried to utilize the aphid alarm pheromones as unique chemical taxonomic characters. The discovery of (*E*)-β-farnesene as a broadly interspecific alarm pheromone in the subfamilies Aphidinae and Chaitophorinae (*1*) demonstrated their apparent close relationship, whereas their relationship to the subfamily Drepanosiphinae remains unclear and has not been resolved on morphological grounds (*2*). We were therefore anxious to investigate the alarm pheromone chemistry of representative species in the Drepanosiphinae. Our cross-reaction tests revealed that the sweetclover aphid, *Therioaphis riehmi* (Börner), and the spotted alfalfa aphid, *Therioaphis maculata* Buckton, both drepanosiphins, did not respond to (*E*)-β-farnesene but demonstrated strong alarm responses to injured siblings, an indication of the presence of a new alarm pheromone.

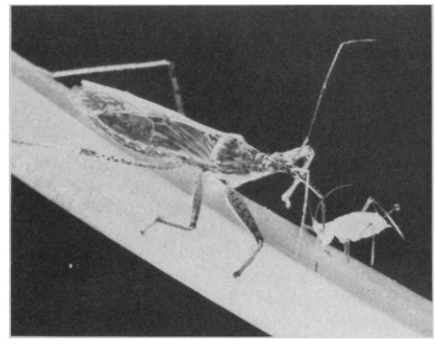


Fig. 1. After attack by a nabid predator, the aphid secretes a droplet of fluid from the cornicles which contain the alarm pheromone.

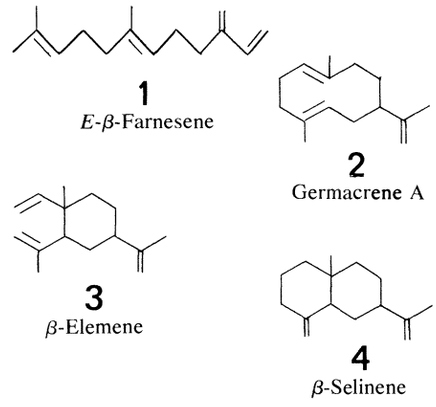
From approximately 2 liters of the closely related spotted alfalfa aphid, *Therioaphis maculata* Buckton, we isolated, by column chromatography over Florisil and silica gel, 9 mg of a biologically active, but highly unstable hydrocarbon. This compound was active against both sweetclover and spotted alfalfa aphids (*3*). Mass spectral analysis (*4*) of this

compound gave a molecular ion of 204 which, allowing for its hydrocarbon composition, is satisfied by the molecular formula $C_{15}H_{24}$, including four degrees of unsaturation. Our progress with structural elucidation was greatly hampered by the extremely labile nature of the compound. In our hands, analysis by vapor phase chromatography (VPC) was invariably accompanied by the appearance of at least one degradation product. Prolonged contact with silica gel promoted decomposition although column chromatography over silica gel performed without delay did not result in noticeable breakdown. Exposure to temperatures over 40°C was avoided, and storage in solution (petroleum ether) at freezer (−15°C) temperatures minimized decomposition.

The infrared spectrum of the isolated pheromone showed a typical hydrocarbon absorption with trisubstituted double bonds (1780, 1650, and 850 cm^{-1}) and terminal methylene absorption (3060, 1650, and 880 cm^{-1}).

The nuclear magnetic resonance spectrum (NMR) in $CDCl_3$ was especially instructive (*5*). A broad singlet corresponding to two vinyl methyls appeared at 1.52 ppm and a two olefinic proton multiplet at 4.75 to 5.25 ppm revealed the presence of two trisubstituted double bonds. The presence of an isopropenyl group was indicated by the appearance of a sharp doublet at 1.73 ppm (J = 1.5 hertz) (methyl group), and broad singlets at 4.59 and 4.66 ppm (terminal methylene).

Although several structures might be proposed from these data, the likely sesquiterpenoid nature of the pheromone suggested structure **2**. This compound is the often proposed biogenetic precursor of most mono- and bicyclic sesquiterpenic hydrocarbons and has been designated germacrene A (*6*, *7*). Although germacrene A has been referred to as a progenitor of many sesquiterpenes, its lability to heat and oxidation conspired to prevent its isolation until Weinheimer *et al.* (*8*) isolated (−)-germacrene A from a gorgonian coral, *Eunicea mammesa* Lamouroux. Doubtless, a survey of plants containing obviously derived sesquiterpenes by techniques designed to minimize isomerization will reveal other sources of germacrene A. Indeed, Weinheimer confirmed the lability of germacrene A and effected its isomerization to β-elemene (**3**) and β-selinene (**4**). Similarly, we treated our isolated pheromone with silica gel in hexane for 8 hours (*7*) and obtained a quantitative yield of the expected β-selinene (*9*). The optical rototatory dispersion of the pheromone (in CCl_4) gave the negative sign plain curve



indicating that the pheromone is the (−) enantiomer.

Finally, the infrared and NMR spectra of our isolated alarm pheromone were compared with the same spectra of Weinheimer *et al.* (*8*) for (−)-germacrene A and found to be identical. It is clear that our alarm pheromone, isolated from the spotted alfalfa aphid, is indeed (−)-germacrene A.

In addition to the spotted alfalfa aphid, the sweetclover aphid also responds with strong alarm reaction to germacrene A. Vapor phase chromatography analysis of the hydrocarbon fraction of *T. riehmi* reveals a peak with a retention time coincident with that of germacrene A isolated from *T. maculata*. These data, taken together with the positive cross-reaction of these aphids to each other and to germacrene A, leave little doubt that germacrene A is indeed the alarm pheromone of both species. Unlike the broadly interspecific (*E*)-β-farnesene, germacrene A does not function as an alarm pheromone for aphids outside the genus *Therioaphis* (*10*).

It seems reasonable for aphids to use a molecule like germacrene A as an alarm pheromone. It is rare in nature and would not act as a plant-feeding deterrent, while its lability ensures that the alarm pheromone would break down soon after the predator has moved on. Aphids could then reinfest the feeding site.

Forage crops are important dietary constituents of dairy and beef cattle, and the use of insecticides to control pest insects on these crops is complicated by the possibility of toxic residues appearing in the meat and milk (*11*). The use of aphid alarm pheromones to stop or minimize aphid damage should offer unparalleled safety since both (*E*)-β-farnesene and germacrene A are not only extremely labile in the environment but are already present as natural constituents of aphids on the crops requiring protection. In reality, the natural alarm pheromones break down within a few minutes when sprayed on plant surfaces and would therefore be of limited use in plant protection. We have approached this problem through the successful synthesis of several simple analogs of (*E*)-β-farnesene which are biologically active (*12*) and considerably more stable than the natural pheromones. It may also be possible to develop active analogs of germacrene A. The spotted alfalfa aphid is a serious pest of alfalfa and its feeding damage is quite toxic to the plants. If synthetic alarm pheromones can be developed, their use to repel aphids from forage crop plants merits serious study.

WILLIAM S. BOWERS
CHIKAO NISHINO
Department of Entomology, New York State Agricultural Experiment Station, Geneva 14456
MICHAEL E. MONTGOMERY
LOWELL R. NAULT
Department of Entomology, Ohio Agricultural Research and Development Center, Wooster 44691
MERVIN W. NIELSON
U.S. Department of Agriculture, Forage Insects Research Laboratory, Tucson, Arizona 85791

References and Notes

1. W. S. Bowers, L. R. Nault, R. E. Webb, S. R. Dutky, *Science* **177**, 1121 (1972); L. R. Nault and W. S. Bowers, *Entomol. Exp. Appl.* **17**, 455 (1974).
2. C. Börner and K. Heinze, in *Handbuch der Pflanzenkrankheiten*, P. Sorauer, Ed. (Parey, Berlin, 1957), vol. 5, part 2, p. 5; W. R. Richards, *Mem. Entomol. Soc. Can.* **44** (1965); V. F. Eastop, *Aust. J. Zool.* **14**, 399 (1966); O. E. Heie, *Spolia Zool. Mus. Hauniensis* **26**, 225 (1967).
3. The hydrocarbon dissolved in methanol was applied to filter paper triangles which were saturated by 1 μl of solution. When triangles were held within 1 cm of aphids for 1 minute, more than 90 percent of *T. maculata* and *T. riehmi* responded to 0.5 μg of hydrocarbon by walking or jumping away. Nonhydrocarbon fractions and methanol produced no response.
4. The mass spectrum was measured on a Bendix model 12 mass spectrometer.
5. The NMR spectrum was obtained at 100 Mhz on a Varian HA-100; tetramethylsilane was used as internal standard.
6. G. H. Kulkarni, G. R. Kelkar, S. C. Bhattacharyya. *Tetrahedron* **20**, 1301 (1964).
7. K. Morikawa and Y. Hirose, *Tetrahedron Lett.* (No. 22), 1799 (1969).
8. A. J. Weinheimer, W. W. Youngblood, P. H. Washecheck, T. K. B. Karns, L. S. Ciereszko, *ibid.* (No. 7), 497 (1970).
9. The rearranged product was identical with an authentic sample of β-selinene by VPC analysis and by the infrared and NMR spectra. β-Selinene is inactive in the alarm pheromone bioassay.
10. When tested as described (*3*), the following aphids did not exhibit alarm behavior to germacrene A. Aphidinae: *Acyrthosiphon pisum, Hyadaphis erysimi, Macrosiphum euphorbiae, Myzus persicae*, and *Schizaphis graminum*; Chaitophorinae: *Chaitophorus populicola* and *Sipha kurdjumovi;* Drepanosiphinae, *Callaphis* sp., *Eucallipterus tilliae, Myzocallis walshii*, and *Stegophylla quercina*.
11. L. L. Rusoff, R. S. Temple, R. G. Meyers, R. G. Newsom, L. D. Burns, E. C. Barthel, W. F. Corley, A. Allsman, *J. Agric. Food Chem.* **11**, 289 (1963); R. J. Moubry, G. R. Myrdal, A. Sturges, *Pestic. Monit. J.* **2**, 72 (1968).
12. C. Nishino, W. S. Bowers, M. E. Montgomery, L. R. Nault, *Appl. Entomol. Zool.* **11**, 340 (1976); *Agric. Biol. Chem.* **40**, 2303 (1976).

16 August 1976; revised 7 December 1976