unbound oleate concentration was 7.6 ± 2.0. While this agreement is further evidence that the minor component is due to unbound oleate, these results should be interpreted with caution since uptake of the albumin-free oleate may have been flow-limited (single-pass extraction averaged 90 percent).

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Anabaseine: Venom Alkaloid of *Aphaenogaster* Ants

Abstract. *Anabaseine, a tobacco alkaloid, is identified as a poison gland product in* Aphaenogaster *ants, in which it functions as an attractant.*

Collecting *Aphaenogaster* ants by mouth-operated insect aspirator produces a brief burning sensation in the mouth and throat similar to that felt during aspiration of the myrmicine ant *Monomorium pharaonis*. Because dialkylpiperidines, pyrrolidines (*1*), dialkylpyrrolidines (*2*), and an indolizine (*3*) have been found in the poison glands of myrmicine ants, we decided to analyze *Aphaenogaster* species for similar alkaloids. Here we report that the poison glands of *Aphaenogaster fulva* and *A. tennesseensis* (*4*) contain the tobacco alkaloid anabaseine (Fig. 1). We also show that anabaseine serves as an attractant in *A. fulva*.

Methylene chloride extracts of whole ants and abdomens were analyzed by combined gas chromatography–mass spectrometry (*5*). In addition to the usual alkanes and alkenes from Dufour's gland, a major peak was noted which exhibited a molecular ion at mass-to-charge ratio (*m/e*) 160, with additional fragments at *m/e* 159, 156, 145, 131, 104 (base peak), 78, and 51. Extracts of excised poison glands showed that this peak represented the predominant volatile (> 90 percent). The compound was established as a base by its solubility in 5 percent hydrochloric acid, which allowed separation from the hydrocarbons. A proton magnetic resonance spectrum (*6*) of the basic material revealed the following: a chemical shift (δ) of 8.95, singlet, 1 H; 8.6, broad singlet, 1 H; 8.1, doublet, 1 H; 7.3, doublet, 1 H; 3.85, triplet, 2 H; 2.62, triplet, 2 H; 1.85, multiplet, 2 H; and 1.70, multiplet, 2 H. These data are consistent with a 3-substituted pyridine (*7*) in which the substituent has four nonequivalent methylene units. Deuterium oxide exchange indicated that only one of these methylene units was labile, as evidenced by the disappearance of the peak at δ 2.62.

Treatment of the natural produce with chloranil (*8*) gave an aromatic product identical to 2,3′-bipyridyl (*9*). Anabaseine was synthesized by the method of Spath and Mamoli (*10*), giving material whose mass spectrum, proton magnetic resonance spectrum (*11*), and gas chromatographic retention time (isothermal coelution) were identical to those of the natural product. The unusual loss of methyl and ethyl groups (*m/e* 145 and 131) in the mass spectrometer requires rearrangement, but this process is known in tobacco alkaloids (*12*). The peak at *m/e* 156 appears to show dehydrogenation to 2,3′-bipyridyl in the mass spectrometer. Proton magnetic resonance spectra of extracts of dissected poison glands and gas chromatographic comparison with standard solutions indicated that the amount of anabaseine present in each ant is ≤ 1 μg.

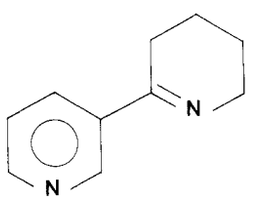

Fig. 1. Structure of anabaseine.

Although anabaseine is a minor alkaloid in tobacco (*12*) and has been identified as a toxin in a nematode (*13*), it has not previously been found in insects. In an effort to establish the chemobehavioral function of anabaseine in *Aphaenogaster* ants, we placed anabaseine-treated paper disks (*14*) in the foraging arena of a laboratory colony of *A. fulva*. Within minutes, six to eight ants appeared in the vicinity of each treated disk. In repeated trials, these disks continued to be attractive for 8 to 10 minutes. Although control disks elicited no noticeable reaction, worker ants approached the treated disks with their heads raised, mandibles open, and antennae outstretched at a 45° angle. They picked up some of the treated disks and carried them into the colony or around the foraging arena. When control and treated disks were placed in a colony of carpenter ants (*Camponotus pennsylvanicus*), both were ignored. Since *Aphaenogaster* and *Camponotus* feed on similar foods, the rejection by *C. pennsylvanicus* and acceptance by *A. fulva* of treated disks indicates that they were reacting to a pheromone and not exhibiting a gustatory response.

To measure the attractant response, we placed control and treated disks in the center of separate 8-cm-diameter circles drawn in the foraging arena of a laboratory colony of *A. fulva*. Ants entered the experimental circles twice as often as the control circles over repeated 15-minute periods.

The data in Table 1 confirms that anabaseine does not elicit a gustatory response and is still an attractant. Mealworm (*Tenebrio molitor*) homogenates (*15*) can serve as food for *A. fulva* and are attractive. Homogenates containing

Table 1. Reactions of *A. fulva* workers to mealworm homogenates containing anabaseine. We added 5 μl of methylene chloride or 5 μl of 0.5 percent anabaseine to 10 μl of mealworm homogenates and placed each mixture in the foraging arena of *A. fulva* colonies. The ants were observed for 5- and 10-minute intervals and their feeding behavior was scored as repelled or attracted. In repelled behavior the ant, after contacting the test solution with its mouthparts, fed for less than 5 seconds. This behavior was frequently followed by the ant backing up very quickly and pausing to clean its mouthparts and antennas. In attracted behavior the ant, after touching the solution with its mouthparts, fed for 5 seconds or longer.

Treatment	Test series 1			Test series 2		
	0 to 5 minutes observation	5 to 10 minutes observation	Total	0 to 5 minutes observation	5 to 10 minutes observation	Total
Anabaseine						
Repelled	16	28	44	15	20	35
Attracted	2	3	5	3	5	8
Control						
Repelled	1	3	4	2	1	3
Attracted	12	14	26	10	15	25

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approximately 0.1 percent anabaseine (by volume) initially attract more ants to the area. However, the presence of anabaseine then prevents feeding. The ants are attracted to the vicinity of the test solution, touch the treated homogenate with their mouthparts, and then quickly back up. Such behavior, rarely observed at the control homogenates, might be interpreted as a mild alarm response and suggests that anabaseine is a gustatory repellant when contacted orally or ingested.

Nitrogenous compounds released from the venom gland of myrmicine ants have been demonstrated to elicit trail-following behavior in *Atta* (*16, 17*) and *Monomorium* (*2*). Anabaseine, tested according to the method of Moser and Blum (*18*), elicited no such behavior in *A. fulva*. Although nitrogenous compounds are not common exocrine products in ants (*19*), several have been identified in the poison glands of myrmicine genera. We believe that the poison gland secretions of myrmicine ants will prove to be a rich source of such alkaloids.

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4. *Aphaenogaster fulva* (Roger) and *A. tennesseensis* (Mayr) were collected from rotten logs on the Howard University property in Beltsville, Md., and on the Marine Training Base in Quantico, Va.
5. We used a Finnigan 3200 combined gas chromatograph–mass spectrometer with 1.6-m 3 percent OV-17 and 10 percent SP-1000 columns on Supelcoport 60/80, programmed at 10°C per minute from 40°C to 200°C (300°C for OV-17).
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14. Five microliters of a 0.5 percent (by volume) solution of anabaseine in methylene chloride was applied to an 8-mm-diameter Whatman filter paper disk. The methylene chloride was allowed to evaporate for 30 seconds before testing was begun. Five microliters of methylene chloride was applied to an 8-mm-diameter Whatman filter-papeated six times.
15. Mealworms were homogenized in 0.1 ml of water. The test solution consisted of 10 μl of mealworm homogenate and 5 μl of anabaseine standard (*14*). Control solutions consisted of 10 μl of mealworm homogenate and 5 μl of methylene chloride. Each solution was placed on a slide and allowed to evaporate for 30 seconds before testing was begun.
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Living Tissue Formed in vitro and Accepted as Skin-Equivalent Tissue of Full Thickness

Abstract. *Living skin-equivalent grafts consisting of fibroblasts cast in collagen lattices and seeded with epidermal cells were successfully grafted onto the donors of the cells. The grafts were vascularized, did not evoke a homograft reaction, inhibited wound contraction, filled the wound space, and persisted.*

We previously showed that a collagen lattice seeded with skin fibroblasts contracts into a tissue and that this phenomenon is dependent on the concentration of cells and protein (*1*). We also reported that suspensions of epidermal cells, applied to the contracted lattices in vitro, cover them rapidly and differentiate thereon (*2*). The epidermis becomes multilayered and undergoes keratinization. We now find that such living tissue complexes, fabricated in vitro, can be grafted onto the animals that provide the cells. These grafts become well vascularized and are incorporated into the host much as are autografts of full thickness. Further, the incorporated graft inhibits wound contraction so that the final area occupied by the graft is essentially unchanged.

To prepare a skin-equivalent graft, fibroblasts are isolated from a skin biopsy from a rat donor, cultured, harvested, and used to populate a collagen lattice in vitro (*1*). The cells used to populate the lattice are taken from the potential graft recipient. The proliferation of cells in vitro permits the fabrication of a tissue of any required size. Five days to 1 week later an epidermal biopsy is taken from the back of the same animal with a dermatome, dissociated, and applied as a cell suspension to the contracted, dermal-equivalent lattice containing the fibroblasts. The graft is fitted into an open skin wound made on the back of the donor rat, sutured in place, and bandaged. For long-term grafts the bandages are removed after 10 days.

We applied a total of 52 grafts measuring 6 to 8 cm^2 each; no rejections were observed. The oldest graft was retained for 68 days, at which time it was excised for examination. In a more recent series of experiments, the oldest grafts have been in place for 13 months at this writing (*3*). To ascertain the degree to which the host is sensitized by the graft, second grafts were given to animals that had received grafts previously. Cell stocks from original biopsies were used again to make up the lattices; fresh biopsies were made to obtain the epidermal cells. We found that the rats were not sensitized by the first graft. For example, graft 29 was in place for 43 days, at which time it was removed. Later, graft 29′ was applied and left in place for an additional 35 days, giving a total of 78 days of acceptance by the same animal.

About 80 percent of the grafts retained their original size and shape, thus inhibiting wound contraction (Fig. 1, a and b). The grafts were hairless and smooth (Fig. 1c) and, therefore, readily distinguishable from the surrounding tissue. Examined from beneath, the grafts, like autografts of full thickness, appeared somewhat thinner than the surrounding tissue. Not all the grafts took well; about 20 percent became partly dislodged and showed signs of inflammation where contact with adjacent skin was interrupted. We attribute these failures to inadequate immobilization of the grafts during the early period after grafting.

Four days after epidermal cells are seeded onto the lattice, the lattice can be observed to be partly covered with poorly organized epidermis. There is little evidence of a coherent basal layer, and the epidermal cells are irregularly distributed. After 5 weeks, however, a fairly regular basal layer is present with

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