possible to study samples up to 25 mm in length and width. Samples consisted of ultrasonically cleaned, dried, pinned insects (Fig. 1A), mammal bones (Fig. 1B), and fragments of insects from late Pleistocene deposits (Fig. 1C).

Satisfactory to very good results were obtained with such specimens, but a number of problems were encountered in the process. It is difficult to maintain the integrity of the beam with a low accelerating voltage (1.5 to 2.5 kv) without an unacceptable loss of resolution or a large amount of distortion. The type of specimen, the angle of the surface scanned, and contaminants in the chamber all produce varying effects on resolution and distortion. With nonconductive samples low accelerating voltages are essential if surface-charging is to be held at an acceptable level (*2*). The use of low accelerating voltages slows the buildup of "hot spots" but does not eliminate them. Moreover, the longer a specimen is subject to the scanning beam before the picture is taken, the greater the possibility of charging. The rapidity with which a specimen can be positioned in the viewing chamber, therefore, is as important a factor as the low accelerating voltage. If charging occurs during the time necessary for focusing, it is often possible to "bleed off" the charge by reducing the intensity of the beam or shutting it down for 5 or 10 minutes.

Some dried insects, even with rapid handling and a low accelerating voltage, charge rapidly. In many cases, if the specimens are placed in a humidor for 24 hours or if the surface is made wet, some of the charging may be eliminated. Brody and Wharton (*3*) found a mixture (by weight) of glycerol (96.6 percent), potassium chloride (0.05 percent), and water (3.35 percent) useful for reducing charging in mites. In a few cases (for example, very convex, smooth, leaf-feeding beetles, Chrysomelidae) we found a conductive coating, such as gold or gold-palladium, essential. According to Echlin (*4*), SEM pictures of uncoated botanical specimens can be obtained in a similar manner, particularly if fresh specimens are used.

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3 July 1972

Caste-Specific Compounds in Male Carpenter Ants

Abstract. *Three caste-specific substances new to arthropod glandular secretions occur in the mandibular glands of male ants of five species in the genus* Camponotus. *These volatile compounds, which are not found in alate females or workers, have been identified as methyl 6-methyl salicylate, 2,4-dimethyl-2-hexenoic acid, and methyl anthranilate. The free acid has not been described previously.*

Coordinating the pairing and mating of the two sexes of an insect species is an obvious fundamental necessity. Many species of termites and ants exhibit a synchronized swarming of male and female alates from many nests, ensuring that large populations of reproductives are airborne at the same time. This swarming behavior and flight coordination of males and females of the carpenter ant, *Camponotus herculeanus*, has been described by Hölldobler and Maschwitz (*1*), who concluded that the simultaneous swarming from a large number of nests is dependent upon season, temperature, and time of day. However, it was also convincingly demonstrated that the mandibular gland secretion of the males is a critical factor in initiating the activity of the females before swarming. This secretion is used to scent the area immediately surrounding the nest entrance and to entice the females to swarm from the nest when the male flight is at a maximum (*1*). This releaser activity of the male-derived exudate would appear to constitute a new function for a caste-specific secretion.

We have identified the major volatile components in the mandibular glands of males of *Camponotus novebora-censis, C. pennsylvanicus, C. nearcticus, C. rasilis*, and *C. subbarbatus* as the initial part of a program investigating factors governing the swarming behavior of certain species of ants. Three new arthropod natural products have been chemically characterized from among these species and found to be methyl 6-methyl salicylate, 2,4-dimethyl-2-hexenoic acid, and methyl anthranilate. The free acid has not been described.

Heads of male *C. nearcticus* were crushed in *n*-pentane; the resulting pentane extract was subjected to gas chromatographic–mass spectrometric analysis (*2*). Of the three peaks detected, the first and major showed a molecular ion (M) at *m/e* 166. This compound appeared to be aromatic (*m/e* 77 and 78), containing both a $COOCH_3$ group (loss of CH_3OH and $HCOOCH_3$ from *m/e* 166) and a phenolic hydroxyl (conversion to an *O*-acetate, M^+ 208; and a slow reaction with CH_2N_2 forming a methyl ether, M^+ 180). Both the retention time and the mass spectrum of methyl 6-methyl salicylate (*3*) correspond to those of this peak in the male heads, whereas the retention times of methyl 5-methyl salicylate and methyl 3-methyl salicylate were distinctly different.

The second peak from this extract is an unsaturated acid with a molecular ion at *m/e* 142 (conversion to a methyl ester, M^+ 156). Reduction of the methyl ester provided a dihydro derivative (M^+ 158) whose fragmentation pattern showed it to be an ester of an α-methyl substituted acid (intense peaks at *m/e* 88 and 101). Ozonolysis of the acid yielded 2-methylbutanal, suggesting the structure 2,4-dimethyl-2-hexenoic acid. This acid was synthesized by slow hypochlorite oxidation of the known 3,5-dimethyl-3-hepten-2-one (*4*) and also from hydrolysis of the product of the Wittig reaction between 2-methylbutanal and the ylid derived from triphenylphosphine and methyl 2-bromopropionate (*5*). The resulting acids had retention times and mass spectra identical to those of the natural product. The geometry of the double bond and the configuration of the asymmetric center in the natural substance are unknown. This relatively simple compound has not heretofore been described.

The third peak exhibits a molecular ion at *m/e* 151, suggesting that it contains one nitrogen atom. It appeared to be aromatic (doubly charged ions, in-

Table 1. Volatile substances in the mandibular glands of males of five *Camponotus* species (++ denotes major component, + denotes minor component, − denotes not detected).

Species	Methyl 6-methyl salicylate	2,4-Dimethyl-2-hexenoic acid	Methyl anthranilate
C. nearcticus	++	+	+
C. rasilis	−	+	++
C. subbarbatus	++		−
C. noveboracensis	++	−	−
C. pennsylvanicus	++	−	−

tense M^+ ion, and the like) and lost CH_3OH and $COOCH_3$, suggesting it to be an aminobenzoic acid methyl ester. This structure was confirmed by the formation of an *N*-acetate (M^+ 193) that lost both the elements of ketene and C_2H_2O plus CH_3OH. The methyl ester of *p*-aminobenzoic acid gives a loss of CH_3O, rather than CH_3OH, and the retention times of both the para and the meta isomers are incorrect for this third peak. However, the ortho isomer, methyl anthranilate, has an identical retention time and mass spectrum to those of the natural product.

The extract of *C. nearcticus* male heads also contained several long chain fatty acids having α-methyl branching and unsaturation. Of the other species investigated, males of *C. rasilis* produce both the 2,4-dimethyl-2-hexenoic acid and methyl anthranilate, whereas males of *C. pennsylvanicus* and *C. noveboracensis* apparently produce only methyl 6-methyl salicylate as a major component. The mandibular glands of *C. subbarbatus* (*6*) males yield methyl 6-methyl salicylate in addition to several other components, one of which may be either geranic or nerolic acid. These results are summarized in Table 1. Excision of the mandibular glands from the males established that all compounds were present in these exocrine structures. None of these substances could be detected in the heads of either alate females or workers of any of these species.

As the mandibular gland secretion of males of *C. ligniperda* was shown to cause an identical response in females of *C. herculeanus* during swarming (*1*), this exudate cannot be regarded as species-specific or as a species isolating mechanism. Both of these European *Camponotus* species belong in the subgenus *Camponotus*, as do the two North American species, *C. pennsylvanicus* and *C. noveboracensis*, and this investigation has established that these latter two species contain the same major volatile compound, methyl 6-methyl salicylate, in their mandibular glands. The three other species studied, *C. nearcticus*, *C. rasilis*, and *C. subbarbatus*, belong in the subgenus *Myrmentoma*, and all have considerably more complex secretions. In spite of the fact that we have analyzed only five species in a genus with more than 600 species (*7*), it appears that some species have the same major volatile substance, whereas others may have a blend distinctive of the species.

In *C. herculeanus*, it is the male mandibular gland secretion which stimulates the females and induces them to fly off. In contrast, this same secretion of another formicine, *Lasius niger*, elicits an indifferent response from females of its species and, instead, excites the males themselves and causes them to fly off from the nest (*1*). This gross difference in their behavior may not be surprising as *Camponotus* and *Lasius* are not closely related formicine genera. While heads of males of *L. alienus*, *L. neoniger* and *Acanthomyops claviger* contain an indole, possibly skatole, which does not occur in the heads of workers, both workers and males of *L. alienus* and *A. claviger* contain appreciable quantities of volatile substances (*8*). Our investigation of these five *Camponotus* species has shown that it is only the males that produce detectable quantities of volatile substances. In at least the five *Camponotus* species studied these compounds are therefore truly caste-specific. The identification of these novel and caste-specific compounds may provide the means for comprehending the function and significance of these exocrine products in the mandibular glands of many male *Camponotus* species.

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31 August 1972

Pituitary Gonadotrophs: Nuclear Concentration of Radioactivity after Injection of [^{3}H]Testosterone

Abstract. *Gonadotrophs and castration cells in the male rat pituitary showed nuclear concentration of radioactivity 1 hour after [1,2,6,7-^{3}H]testosterone injection. Thyrotrophs and acidophils did not retain radioactivity; also the cells of the intermediate and posterior lobes did not accumulate radioactivity. The autoradiographic results suggest a direct and selective action of androgen on gonadotrophs, which contrasts with the action of estradiol which was shown earlier to bind not only to basophils but to acidophils and chromophobes as well.*

It is well established that androgen feedback control of gonadotrophin secretion is mediated through the hypothalamus (*1*). However, it is still debated whether androgen feedback exists at the pituitary level (*1*), since intrapituitary implants of testosterone have yielded controversial results. More recent studies suggest that both estrogen and androgen can act directly on the pituitary (*2–4*). While the pituitary has been shown to selectively concentrate radioactivity after the injection of labeled testosterone (*5–7*), incubation of anterior pituitary homogenate did not result in a nuclear binding of [^{3}H]-testosterone or a radioactive metabolite of it (*8*). By use of the dry-mount auto-