involving the heptapeptide metabolite and remain almost as active as the heptapeptide in this respect.

We conclude the following: (i) The renin-angiotensin system functions in the physiological control of aldosterone secretion from the rat adrenal cortex, and other investigators were dealing with artifacts of anesthesia and surgical stress. (ii) (The rat adrenal cortex in vivo is at least as responsive to the naturally occurring des-aspartyl-angiotensin II metabolite of angiotensin II as it is to angiotensin II itself. (iii) The angiotensin antagonist P-113 acts in vivo to inhibit the effects of these peptides on the rat adrenal cortex. (iv) In view of these observations in vivo and the studies in vitro (*17*), we suggest that the naturally occurring heptapeptide, des-aspartyl-angiotensin II, be called angiotensin III.

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Defensive Use by an Insect of a Plant Resin

Abstract. *Larvae of the sawfly* Neodiprion sertifer *(Hymenoptera: Diprionidae), when disturbed, discharge an oily oral effluent essentially identical chemically to the terpenoid resin of its host plant* (Pinus sylvestris). *The resin is sequestered by the larva upon feeding, and stored in two compressible diverticular pouches of the foregut. The fluid is effectively deterrent to predators. The defensive use by an insect of a plant resin provides an instance of secondary utilization by a herbivore of the protective chemical weaponry of its host.*

Many animals, when attacked, traumatized, or otherwise "stressed," discharge enteric contents. Insects in particular are prone to regurgitate or defecate when handled, as anyone knows who has collected grasshoppers, caterpillars, or beetles in the field. What seems generally unrecognized is that in some species these discharge mechanisms serve primarily for protection. We here report on the defensive function, chemical nature, and dietary origin of the oral effluent of the larva of the sawfly *Neodiprion sertifer*.

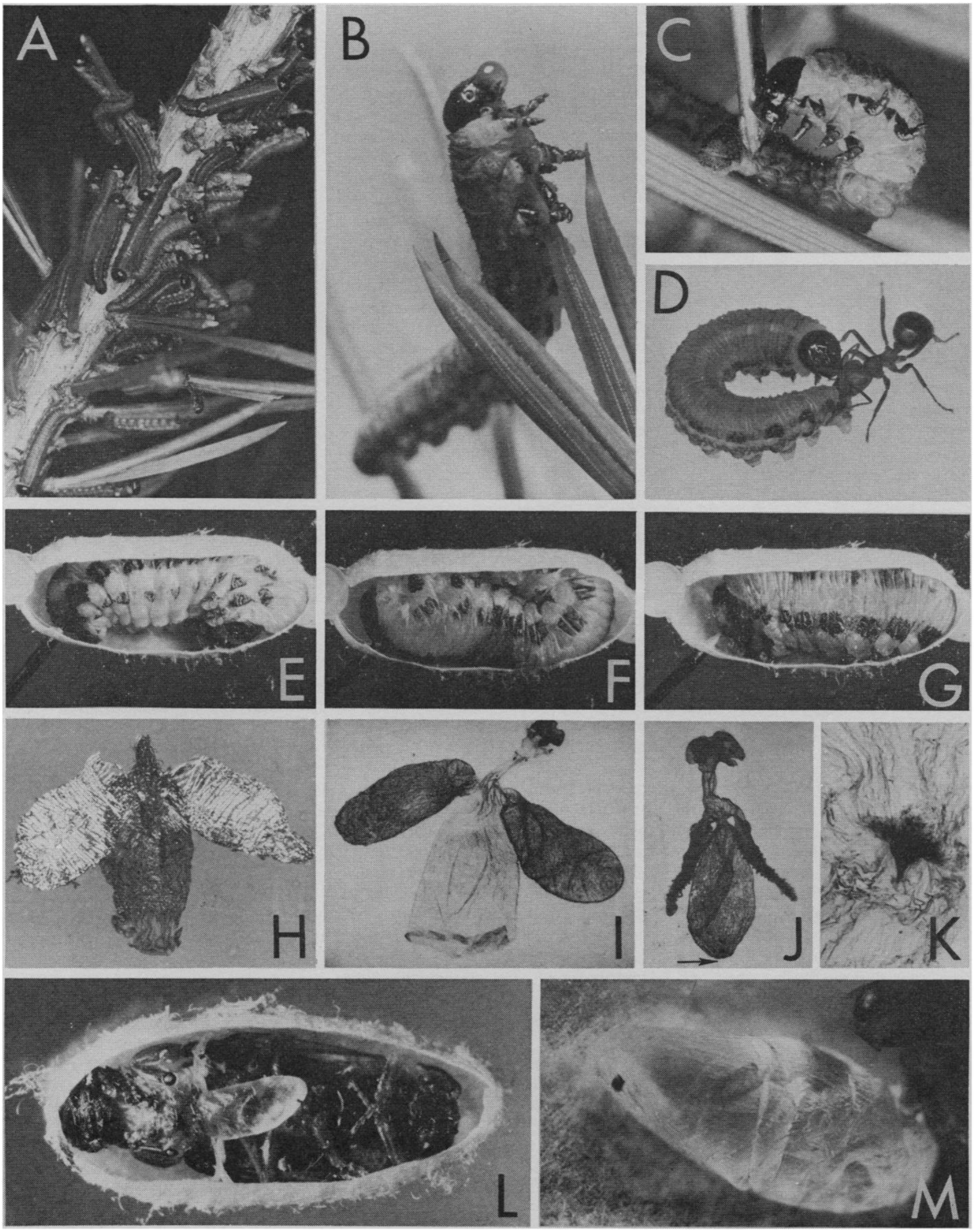
Like some of its relatives, *N. sertifer* is a common pest. Gregarious as a larva (*1, 2*) it feeds on such conifers as Scotch pine (*Pinus sylvestris*), denuding branches of their needles (Fig. 1A). When disturbed, the larva shows a diversity of startle responses (*2*), involving most commonly the rearing of the front end, and the emission from the mouth of a droplet of fluid (Fig. 1B). The droplet is viscous, immiscible with water, and of the consistency and unmistakable odor of *Pinus* resin. When poked or pinched, the larva revolves its body and, in an obvious defensive maneuver, dabs the droplet directly on the offending object (Fig. 1C). The response is quick and accurate, and the larva can reach any part of its back and sides with the mouth, except the region just behind the head.

To test the proposition (*2*) that the oral effluent represents resin from the host plant regurgitated by the larva after ingestion, chemical analyses were made of effluent and of resin obtained from needles and branches of *P. sylvestris*. Effluent was collected by pinching larvae with forceps and placing capillary tubes over their mouths so as to trap the disgorged fluid (90 mg of material was "milked" from 500 medium to full-sized larvae). Branch resin was obtained by scraping the oozings from bark incisions made on needle-bearing twigs comparable to those ordinarily beset by larvae. Needle resin was obtained by extracting whole needles with methylene chloride, or by squeezing freshly transected needles and taking up in microcapillary tubes the droplets that emerged from the resin ducts at the severed surface. The compounds identified, and their distribution in the three samples, are given in Fig. 2 and Table 1. It is clear, in accord with previous findings (*3, 4*), that branch resin differs chemically from needle resin. Both resins contain the two volatile monoterpenes, α-pinene and β-pinene, responsible for the characteristic odor of *Pinus* resin, but only branch resin contains a complex mixture of resin acids. Needle resin has only one major acidic component, pinifolic acid. The oral effluent contains all the identified components.

The analytical procedures were as follows. Components 1 and 2, detected by gas chromatography–mass spectroscopy (GC-MS) of the larval effluent, were identified as α-pinene and β-pinene by GC-MS comparison with authentic samples. The presence of car-

Fig. 1. (A) Cluster of larvae of *Neodiprion sertifer* on twig of *Pinus sylvestris.* (B) "Altered" larva, raising its front end and regurgitating. (C) Larva, pinched in forceps, responding by dabbing regurgitated fluid onto the instrument. (D) Larva dabbing fluid on an attacking ant (*Formica exsectoides*). (E to G) Larva within cocoon responding to prodding of its rear (with pin shown at lower left) by revolving and rotating its body so as to bring its fluid-laden mouthparts to bear upon the pin. (The cocoon has been cut open and covered over with glass, through which the photographs were taken.) (H) Foregut of larva (mouth at upper center) showing the two muscled diverticular pouches in which the resin is stored (muscles appear as bright bands in partially polarized transmitted light). (I) Comparable to preceding, but consisting of the cuticular lining only (muscles and other cellular components have been removed by treatment with hot potassium hydroxide). (J) Comparable to preceding, but of a prepupa. The arrow points to the sealed junction where the foregut formerly opened to the midgut. (K) Enlargement of the sealed foregut-midgut junction [region shown by arrow in (J)] showing the plug that effects the seal. (L) Pupa in cocoon, shedding the larval skin with the attached resin sac (photograph of a specimen that died during the molt). (M) Resin sac such as is characteristically left behind in the cocoon after emergence of the adult.

boxylic acids was suggested by the infrared spectrum of the effluent. Components 3 to 8 were isolated chromatographically following esterification by diazomethane treatment. Tentative structural assignments resulting from infrared, nuclear magnetic resonance, and mass spectrometric examination were confirmed by comparison with authentic samples. Component 9 was isolated from needle extracts by chromatography following esterification with diazomethane, and shown to have a mass spectrum identical to that reported for dimethyl pinifolate (*4*). Free



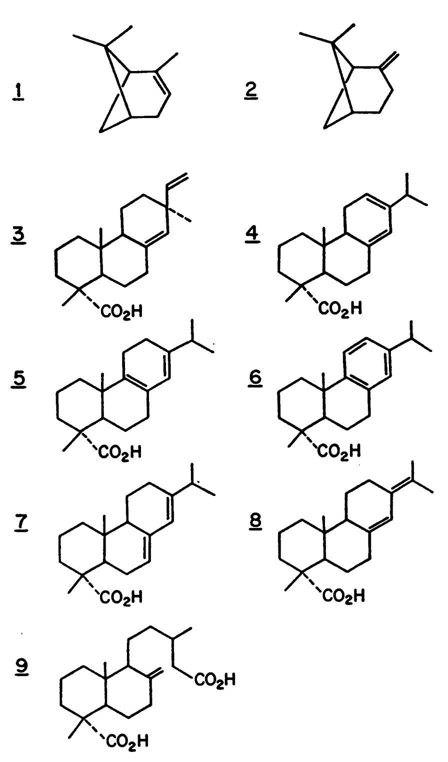


Fig. 2. Compounds found in *Pinus* resin and in the oral effluent of *Neodiprion*. The distribution and names of the compounds are given in Table 1.

pinifolic acid was readily crystallized from the needle extract.

Judging from observations of *Neodiprion* in the field and on potted *Pinus* in the laboratory, all larvae, except the youngest, have feeding habits that ensure their ingestion of resin of both kinds. Such larvae consume not only the entire shafts of the needles, which provide them with needle resin, but also commonly some of the basal fascicled portions of the needles, which are said to be penetrated by lateral extensions from resin canals of the branches (*5*). The presumption that the oral effluent is therefore a mixture of both resins was supported by the finding that its quantitative composition (*6*) is essentially similar to that of branch resin, except for a higher content (about 3.5-fold) of pinifolic acid. First-instar larvae confine their niblings to the shafts of the needles, without chewing into their bases. Predictably, a sample of effluent milked from several dozen first-instar larvae proved to be comparable to needle resin.

Dissection of larvae confirmed the claim (*2*) that effluent stems from a pair of large diverticular pouches of the foregut (Fig. 3). The pouches are filled with viscous water-immiscible oil, unlike the remainder of the gut, which is densely packed with pieces of pine needle and other solid debris in aqueous suspension. Elaborate compressor muscles surround the pouches (Fig. 1H) and evidently serve to effect the discharge. Methylene chloride extracts of excised pouches (full-grown larvae) showed a chromatographic profile identical to that of the oral effluent. Replete pouches taken from larvae killed by a procedure (slow freezing) that does not cause them to regurgitate yielded about 1 mg of oil per animal. Since oral deliveries from pinched larvae averaged only 0.2 mg, it is clear that single discharges do not necessarily deplete the pouches. Chromatographic analyses of extracts of midgut contents and of day-old fecal pellets revealed no trace of pinenes or resin acids. Whatever the mechanism that shunts the ingested resin to the pouches, it must operate with considerable efficiency. The pouches, like all parts of an insect foregut, are lined with an impervious cuticular membrane (Fig. 1I). The larva therefore never really "swallows" the resin. It sequesters it and stores it within insulated confines, without exposing its own potentially sensitive tissues to the material. That the resin is never passed to the midgut, not even for nutritive purposes under conditions of stress, was shown by the finding that starved larvae with totally depleted alimentary canals still had fully replete pouches.

The larva can still make defensive use of its resin once it has crawled to the ground and spun its cocoon. It retains the larval condition for at least several weeks within the enclosure before pupating, and during this time responds to tapping or scraping of the cocoon by revolving its body so as to bring the mouth toward the site, and by regurgitating on the offending object if the cocoon is pierced (Fig. 1, E to G). Not until pupation occurs is the resin finally relinquished. It is then extricated from the body of the larva (Fig. 1L), neatly packaged within the shed cuticular lining of the foregut, which is discarded as part of the larval skin. In anticipation of this molt a plug develops at the rear of the foregut (Fig. 1, J and K) that seals the formerly gaping valvular junction with the midgut, thereby ensuring that the cuticular sac that encloses the resin is leakproof when extricated from the animal. Old sacs recovered from cocoons that had yielded adults 3 years earlier were found to be replete with resin that was still clear and unhardened. Even volatile pinenes were still detectable by gas chromatography in this aged material, indicating that the cuticular investiture of the sac, which in the living larva lines all parts of the foregut and shields the larva from systemic exposure to the resin, is a highly impermeable barrier.

Table 1. Distribution of substances identified in needle and branch resin of *Pinus* and in oral effluent of *Neodiprion*. Numbers correspond to formulas in Fig. 2; +, present; −, not present.

Compound	Oral effluent	Resin	
		Needle	Branch
1. α-Pinene	+	+	+
2. β-Pinene	+	+	+
3. Pimaric acid	+		+
4. Levopimaric acid	+	−	+
5. Palustric acid	+	−	+
6. Dehydroabietic acid	+	−	+
7. Abietic acid	+	−	+
8. Neoabietic acid	+	−	+
9. Pinifolic acid	+	+	+

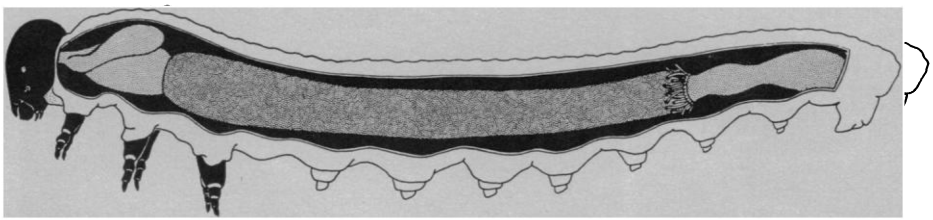


Fig. 3. Diagram of digestive tract of larva of *Neodiprion sertifer*. The foregut is shown as the lightly stippled anterior portion of the tract. Only the left diverticular pouch of the foregut is shown.

Earlier studies had shown that the oral effluent of *Neodiprion* is an effective deterrent to birds, as well as possibly to certain entomophagous parasites (*2*). We have found the fluid to be effective against ants and spiders. Larvae (medium sized) confined with small groups of ants in corralled subdivisions of the foraging arena of a laboratory colony of the ant *Formica exsectoides* were promptly attacked by ants, but the larvae administered oral effluent onto them (Fig. 1D) and caused them to turn away and flee, often noticeably hindered by the viscous contaminant. For prolonged periods thereafter the ants engaged in intensive and characteristic (*7, 8*) preening activities. Even the vapors from the effluent were repellent: wetted mouthparts of larvae previously attacked or alerted were usually avoided just short of actual contact by the approaching ants. Tests with lycosid spiders, caged singly and starved for several days before being offered individual larvae, gave comparable results. The spiders pounced on the larvae, but desisted and began cleaning themselves as soon as they were dabbed with effluent. Only one of eight larvae tested received visible (and eventually fatal) injury from the bite of a spider.

The two pinenes are in themselves "obnoxious" to arthropods. Droplets of chromatographically purified α- and β-pinene, applied to the abdomen of decapitated cockroach nymphs (*Periplaneta americana*), elicited prompt scratching with the hind legs (*9*), and capillary tubes filled with either terpene and held close to the proboscises of tethered flies (*Lucilia cuprina*) induced reflex-cleaning of the proboscis (*10*). Pieces of filter paper (2 by 2 mm) impregnated with either pinene and placed in the midst of ants (*Pogonomyrmex occidentalis*) feeding at a bait (crushed grasshopper thorax) beside the entrance to their natural nest, induced quick dispersal of even those ants which had merely come into proximity with the papers. Ants directly contacted cleaned themselves vigorously. No comparable effects were elicited with papers impregnated with resin acids alone (*11*). Papers impregnated with oral effluent, or with an approximate replicate of the effluent (*12*), also had a dispersing effect. Moreover, the effectiveness of such papers always outlasted that of counterparts soaked in pinenes alone, indicating that the viscous matrix of resin acids has a retarding effect on the evaporative loss of pinenes. The resin acids may therefore act only physically, as viscid topical contaminants, and as fixatives for the pinenes. Interestingly, α- and β-pinene are also known from the defensive spray of certain termites (*13*). This fluid, a glandular product seemingly produced by the termites themselves, is also resinous in character, but the chemistry of the resinous base is unknown.

Despite its proven effectiveness, the chemical defense of *Neodiprion* has its shortcomings. A number of enemies successfully parasitize the larvae, or prey upon them, both before and after the larvae have spun their cocoons (*2, 14*), and these enemies must have ways of tolerating or circumventing the defense.

The utilization by an animal of an unaltered product of exogenous origin has implications of interest. The resin of *Pinus* is to be viewed as a chemical defense of the tree itself. It has repellent qualities and serves for wound healing, and hence must contribute in a number of ways toward reducing the vulnerability of the plant. What *Neodiprion* has evolutionarily achieved is to "crash" through the chemical defenses of its host, without detoxifying or otherwise inactivating the weaponry involved. It simply appropriates intact the resin of the plant, thereby obviating the need for metabolic production of an alternative defensive agent of its own. One wonders to what extent the defensive enteric discharges of animals generally derive their effectiveness from unaltered dietary components. In fact, the whole question of transmission along food chains of nonnutritive substances (*15*), and of the use to which these substances are put as they are relayed with or without alteration from one link to another in a chain, is worthy of increased consideration.

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5. These lateral extensions of branch resin canals are said to end blindly in the base of the needles without merging with the resin canals of the needles [V. Mozheiko, *For.-Chem. Ind. (U.S.S.R.)* **2**, 44 (1933)]. Our finding that branch resin and needle resin differ chemically is in line with this claim. Older larvae also chew sporadically into the bark of twigs, and are sometimes seen with their mouthparts dipped in the resin that bleeds from such injuries, as if drinking from the outflow.
6. The quantitative assays involved gas chromatographic comparisons with standardized solutions of authentic samples of the terpenes.
7. Such cleansing activities, as induced in ants under comparable experimental circumstances, have been described elsewhere (*8*).
8. T. Eisner, J. Meinwald, A. Monro, R. Ghent, *J. Insect Physiol.* **6**, 272 (1961).
9. Such scratch reflexes are a useful criterion for evaluation of irritancy of topically applied materials (*8*).
10. The flies responded by extending the proboscis, emitting a droplet of regurgitate onto the labellum, and wiping the labellar tip against the substrate or other available surface. This cleansing response is also elicited by benzoquinones, phenols, short-chain aliphatic acids, aldehydes, and ketones, and by other "noxious" volatile substances (T. Eisner, unpublished).
11. Pimaric, levopimaric, dehydroabietic and abietic acids were individually tested.
12. The artificial effluent had the following composition: 1 percent α-pinene, 1 percent β-pinene, 68 percent levopimaric acid, 14 percent pimaric acid, 14 percent abietic acid, and 2 percent dehydroabietic acid.
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Pheromone-Regulated Anemotaxis in Flying Moths

Abstract. *Certain male moths flying upwind toward a scent-producing female appear to be guided anemotactically by optomotor reactions to the ground pattern. Loss of the odor stimulus changes the anemotactic angle from into wind to across wind with left-right reversals.*

For some years it has been generally held that insects steer toward a distant odor source not chemotactically but anemotactically, by turning into the wind when they receive an odor stimulus (*1*). In flying insects this anemotactic orientation has been assumed to depend on optomotor reactions to the apparent movement of the ground pattern. However, as far as sex pheromones are concerned, no experimental evidence for optomotor anemotaxis exists, and a chemotactic guidance mechanism has recently been proposed