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Review Article

Some Aspects of Insect Chemistry

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THE INSECT, until recently, has been relatively neglected by the natural product and medicinal chemist. During the period since the end of World War II this fertile area of research for the chemist, biochemist, physiologist, and even the pharmacologist has received an ever-increasing amount of recognition. The chemistry of these hexapods is unique and exciting.

This discussion will be divided into three parts, (*a*) effects of plant chemical factors on the insect; (*b*) effects of insect chemical factors on the insect environment, and (*c*) unique chemistry of the insect.

It should be recognized that the above categories represent only a part of the total subject of insect chemistry and that it will be impossible, in the space alloted, to cover all of the factors reported in the recent literature. As a limited review, it will also be impossible to discuss in detail the chemistry cited. However, a generous sprinkling of interesting information will be given in an attempt to entice the medicinal biologist and medicinal chemist to seek further application of and knowledge in the field of insects and their environment.

EEFECTS OF NATURAL CHEMICAL FACTORS FROM PLANTS ON INSECTS

The complex interactions between phytophagous insects and their host plants are controlled by factors which are responsible for host selection by the insect and resistance to the insect by the plant. The chemical factors responsible for attracting an insect to a specific host initially, have a "recognition" or "orientation" effect. Once the insect has been attracted to the plant, it then orients to different parts of the plant for oviposition or feeding (1). Thus, the recognition or orientation attractants produce chemoreceptive and/or visual stimuli. Some time ago volatile chemicals were indicated to emanate from foliage to bring an insect to a host (2). Ilse (3) showed that *Pieris brassicae* (Linnaeus) butterflies were attracted to green and blue-green substrates, where they would display a preovipositional behavior; yellow substrates did not cause such behavior. Many other reports of color attraction to the host plant have been recorded (4–9). The chemistry associated with color discrimination in insects will be covered later.

Undoubtedly after arriving at the prospective host plant the insect responds to stimuli, releasing the subsequent components of the ovipositional behavior pattern. Insects characteristically deposit their eggs on selected plant parts. Physical characteristics of the appropriate ovipositional site coupled with the ovipositional attractant have been shown to be necessary for oviposit.

The corn earworm, *Heliothis zea*, requires a villous substrate on which to oviposit, in addition to appropriate chemostimuli (10). The need for a fibrous surface was accounted for on the basis that the moth must maintain a firm grip during egg deposition. The eggs of the diamond-

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back moth, *Plutella maculipennis* (Curtis), were laid preferentially in small cavities and crevices on host plants that contained isothiocyanates (11). This moth could be induced to deposit eggs on nonhosts that had been treated with allyl isothiocyanate (I).

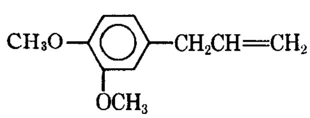
$$CH_2{=}CH{-}CH_2N{=}C{=}S$$

I

Yamamoto and Fraenkel (12) found two plant factors to be involved in the oviposition by the tobacco hornworm, *Protoparce sexta* (Johannson), on the tomato plant. The moths were attracted to the host plant by an unidentified steam-distillable factor, but would deposit eggs only in response to a material extracted from the tomato plant with alcohol. Neither factor had an effect as a feeding stimulant.

Ammonia, amines, sulfides, and fatty acids have been reported to attract many species (13). It can be assumed that these compounds, products of decomposing organic matter, represent a source of food to the insect. Many insects instinctively oviposit in the vicinity of these chemicals (14–17).

Amino acids have been tried as attractants but in general have proved to be poor agents. Essential oils and other plant extracts with reasonable vapor pressures would be expected to attract insects to a plant or to a specific part of a plant. Methyleugenol (II), a constituent of citronella, is a powerful attractant for the



II

oriental fruit fly (18). The male flies are attracted by this compound and will feed to engorgement (19). A mixture of eugenol and geraniol attracts the Japanese beetle (20).

Feeding involves the initial recognition and orientation discussed previously, followed by a biting or piercing, a maintenance of feeding, and finally a cessation of feeding and dispersal (21). Attempts by innumerable workers to extract, purify, and identify various components of plants known to be relatively free from attack by insects have led to factors which fall into the category of antifeeding compounds. A discussion of synthetic antifeeding compounds for insects is beyond the scope of this discussion, but offers an interesting approach to crop damage control (22).

Beck and co-workers (23–30) have studied the feeding behavior of the larval European cornborer, *Ostrinia nubilalis*, over a number of years. Although this insect will feed and grow on a wide variety of plants, the larvae are found on corn plants mainly because of the ovipositional behavior of the moths. However, newly hatched larvae move from their hatching site on the corn leaves down into the plant whorl or into the spaces between the stem and leaf sheath or ear husks. This orientation has been shown to result from the insects thigmotactic (stereotaxis) and phototactic responses (24). Under laboratory conditions, the borer larvae were attracted to dietary substrates containing foliage extracts (26). In the absence of chemical attractants, the borer larvae only encounter food by chance. In a study by Matsumoto and Sugiyama (31) on the host plant determination of the leaf-feeding insects, the feeding attractant for the vegetable weevil has been shown to be the widely occurring leaf alcohol, 2-hexene-1-ol (III). The corresponding aldehyde, 2-hexenal

$$CH_3CH_2CH_2CH{=}CH{-}CH_2OH$$

III

$$CH_3CH_2CH_2CH{=}CH{-}CHO$$

IV

(IV) also has been shown to be present and to have attractant properties.

Since plant leaves are the only food source of many insects and the principal source for others, they must contain all of the nutrient materials required by these insects. However, insects never feed on all plants; on the contrary, they are somewhat selective in their choice of food plants. They may feed on only one plant specie, on a few closely related species, or on a somewhat larger group of plants usually confined to a certain plant family. The basic nutritional requirements of all insects seem to be related and much like those of higher animals. A tenable case exists for supposing that the food preferences are partly the outcome of the discrimination of nutritionally advantageous features.

It has been demonstrated that olfactory attraction by specific chemicals found in food plants can orient certain insects to food (32, 33). Since the distances over which this attraction functions was never more than a few centimeters, it has been suggested that the effect was probably more concerned in inhibiting the insects from leaving the host after having encountered the plant by chance. A marked controversy exists in this area, and recent experiments indicate olfaction might help to locate distant food if air currents are flowing over the food toward the insect (34). Hoppers of the desert locust usually walked downwind when an air stream was passed through a special channel, but when grass was placed in the air current, they moved toward the odor source. If the plant material was crushed before it was placed in the current, the response increased. Moisture alone and coumarin, a common constituent of many grasses, had no effect.

There is a paucity of definitive chemical work in the area of feeding stimulants and attractants and a plethora of interesting and economically important problems to be investigated. As an example, work is underway to isolate, purify, and identify the feeding stimulant for the elm beetle (35).

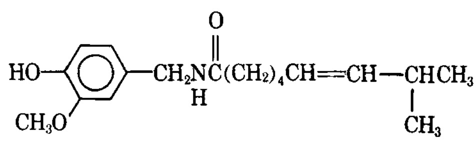
Since 1930, the red elm, together with most of the other species of elm, has been a point of interest in the United States because of its affliction with Dutch elm disease (36). By 1960, it was estimated that $25,000,000 had been spent just on removing diseased trees in an attempt to control this disease, with millions more being spent on other methods of control (37). The causative organism of the disease is the fungus *Ceratocystis ulmi* (38) which grows as a saprophyte in the wood of dead trees. As a plant pathogen, it produces a vascular wilt by the formation of toxins. These toxic substances can be produced in culture (39).

The spread of the disease, in the form of the spores of the fungus, may take place through root unions with neighboring trees; however, the most important mode of dissemination is by an insect vector. A number of insects have been shown to be vectors, but in the U. S. the European bark beetle, *Scolytus multistriatus* (Marsh.), and the native elm bark beetle, *Hylurgopinus rufipes* (Eich.), are the only known carriers of the disease.

To determine if there is a substance in elm bark responsible for attack by elm beetles, Professors Beck and Norris, University of Wisconsin, prepared aqueous extracts of elm bark (40). These extracts were diluted with a neutral material and a portion placed in a pile, called a "station," on a Petri dish, along with control stations composed of neutral material. European elm bark beetles were randomly distributed on the plate and their behavior observed. After a short time, the beetles aggregated at the station containing the extract, thus indicating that chemical compound may be responsible for attack of elm trees by elm bark beetles. This general approach can be used in searching for similar host factors.

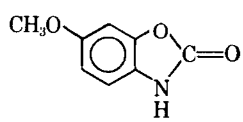
The general topic of plant-produced insect repellents and toxicants is not new in the relative sense. Nicotine, pyrethrins, and citronella have been used for many years. The subtlety of the repellent effects has been overlooked until recently. It is well known that nicotine is synthesized in the root of the tobacco plant. If a tobacco plant is grafted to a potato root, it will grow readily and be free of nicotine and will be eaten by the potato beetle. If, conversely, the potato plant is grafted on a tobacco root, the plant becomes totally resistant (41).

Extensive work in the area of plant resistance factors has been reported from many laboratories. Kuhn and his co-workers (42) have obtained glycoalkaloids from the family *Solanaceae* and have demonstrated their ability to render the plant repellent or toxic. These workers never devised experiments to separate toxic from repellent effects. Thus, the actual role of these substances in host plant resistance has not been clarified. Capsaicine (V) was isolated from *Solanum capsicum* and reported by Schreiber (43) to be the active resistance agent of that plant, but his evidence appears to be inconclusive.

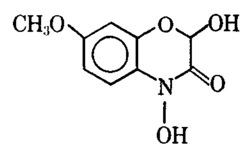


V

In many cases, more than one factor has been reported to be responsible for the total resistance of the host (29). Three different laboratories have reported the isolation of factors from corn (44–46). Two of the factors reported are 6-methoxybenzoxazolinone (VI) and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (VII).



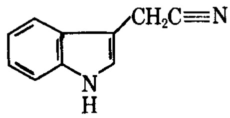
VI



VII

Wahlroos and Virtanen (47) reported that VI arises when corn tissue is injured. This is due to a facile rearrangement of VII to VI with the loss of formic acid. These agents act by inhibiting the growth of the corn borer, and in this action VI is a much more potent agent that VII (48).

The cabbage plant was found to have several insect growth inhibition factors present (49), one of which was shown to be indole-3-acetonitrile (IAN) (VIII) which Jones and co-



VIII

workers (50) had reported earlier to be a highly active neutral auxin. Salicylic acid, isolated from alfalfa, also proved to inhibit the growth of insects (51). Unidentified inhibitors of insect growth have been detected in a large number of plant species and varieties, including barley, potato, oats, alfalfa, wheat, cabbage, kale, and beets (29).

Various techniques for measuring the resistance of a host to its principal parasite have been developed. Jenkins *et al.* have studied the antibiosis of various cotton plants to the boll weevil and report that their technique has application with other insects and crop plants in host plant resistance programs (52). They homogenized fresh or frozen squares, seedlings, or bolls, and then lyophilized the homogenate to a powder. The dried powder could be stored until required and was reconstituted by mixing with agar, sterile water, and an antimicrobial agent. The boll weevil eggs are placed on the agar and the weevils, weighed at emergence and again at the emerging adult stage, and the variance in weight for different varieties of cotton noted.

The most efficient food plants of the tobacco hornworm, *Protoparce sexta,* is the tobacco plant, yet other insects are repelled by the nicotine in tobacco plants. Recently it has been reported (53) that the tobacco hornworm excretes nicotine and other ingested alkaloids before a toxic dose can accumulate. Evidence also is presented that insects feeding on tobacco may possess a metabolic mechanism for the conversion of nicotine to a less toxic substance. Thus, it is evident that certain insects can adapt to the resistance mechanism of the host plant.

From the preliminary studies on the mode of action of 6-methoxybenzoxazolinone (VI), Smissman *et al.* (54) found that the concentration of uric acid in the excreta of *Ostrina nubilalis* decreases when the corn borer is given 6-methoxybenzoxazolinone, 5-chlorobenzoxazolinone,[1] or 5-chloro-2-aminobenzoxazole.[2] The latter is a known uricosuric agent. An excellent correlation exists between the dose required to cause a 50% inhibition of growth (I_{50}) by these agents and the increase in *in vitro* solubilization of uric acid.[3]

[1] Marketed as Paraflex.
[2] Marketed as Flexin.

EFFECTS OF INSECT CHEMICAL FACTORS ON THE INSECT ENVIRONMENT

An equally provocative area of research deals with the ability of an insect to attract or repel other insects, to destroy plants with toxins, to develop antibiotics against microorganisms, and to attack or influence noninsecta. Much attention has been given to pheromones, from the Greek *pherein* (to carry) and *horman* (to excite), substances secreted by one individual to the outside, capable of eliciting specific behavioral or developmental responses in another of the same species.

Insect pheromones include the sex attractants, trail substances of ants and termites, queen substance of honey bees, releasers of alarm behavior in ants, and other varied substances. The area of insect sex attractants has received an unusual amount of publicity during the past decade, with the idea in mind that these compounds could lead to the control of vast insect populations. A review on this subject covers the literature to 1958 (55).

The survival of insects depends on their rapid propagation, which is directly dependent on the ability of opposite sexes to locate each other and mate. This location phenomenon is controlled principally by the insect sex attractants. These attractants have been termed "sex pheromones." A number of insects liberate sex pheromones which attract the opposite sex of the same species and induce mating behavior and copulation. To date, females from more than 150 species of insects and males from over 50 species have been shown to give off attractant excitants (56).

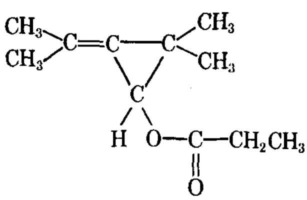
The sex attractant in females is usually found in the tip of the abdomen. Males that have come into contact with these secretions are capable of eliciting sexual activity in another male, although normally males ignore other males. A glass rod or piece of filter paper on which droplets of secretion from the female have been placed, have been shown to attract and excite the male to typical sexual behavior (57, 58). The male's antennae have been implicated as the recipient structure since an antennaless male, or a male whose antennae were coated with paraffin oil, was not attracted to the female or to isolated secretions. A theory of insect sex attraction based on molecular vibration has been proposed by Wright (59). That author, an expert in the science of olfaction, gives an

[3] Preliminary complexation studies were performed by Dr. John Windheuser.

interesting discussion of insect recognition signals in a recent article (60).

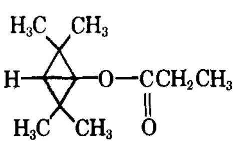
The sex attractants of some species possess a very high activity as demonstrated by the isolated material from the female moths of the silkworm, *Bombyx mori*. The response of 50–100 male moths was elicited by a concentration of 10^{-10} mcg./ml. which represents the activity of about 1000 molecules.

The extent to which sex attractants occur in the insect world is not certain, but to date reports indicate that sex lures may be prevalent among insect species. Recent developments have involved the successful chemical identification of two of these attractants: the gypsy moth, *Porthetria dispar* (L.), attractant (61), and the silkworm, *Bombyx mori* (L.), attractant (62). A third attractant, from the American cockroach, *Periplaneta americana* (L.), was reported to have the structure IX (63). The pure substance was obtained by passing air over approximately 10,000 virgin females continuously during 9 months. The vapors were condensed, extracted with hexane, and chromatographed to yield 12.2 mg. of pure attractant. However, it was synthesized, and the structure (IX) found to be incorrect (64). The investigators who



IX

completed the synthesis of IX suggested X as the correct structure. Jacobson and Beroza (65) have recently refuted X and have stated that the structure remains to be determined.



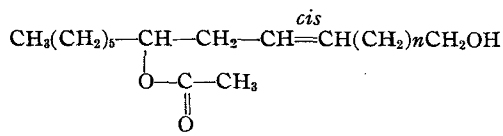
X

The structure of the gypsy moth attractant was determined after 30 years of extremely tedious work (61). Isolation of this substance was effected by saponification of the benzene extract prepared from the last two abdominal segments of 500,000 virgin female moths. The neutral fraction was further separated and the acetone-soluble material chromatographed to yield 20 mg. of a pure colorless liquid. It was identified as *d*-10-acetoxy-*cis*-7-hexadecen-1-ol (XI*a*). The compound was synthesized in an eight-step sequence, resolved (66), and found to be identical with the natural gypsy moth attractant; the *d*, *l*, and *dl* forms possessed equal activity at a level of 10^{-7} mcg. in the field.

TABLE I.—COMPARATIVE ATTRACTANCY OF GYPLURE AND ITS HOMOLOGS TO MALE GYPSY MOTHS (69)

Compd.	Attractancy, mcg. Lab.	Field
XI*a* (natural)	10^{-12}	10^{-7}
XI*b* (gyplure)	10^{-12}	10^{-5}
trans-Gyplure	10^{4}	$>2.5 \times 10^{5}$
XI*c*	10^{-2}	10

The synthesis of some compounds related to the gypsy moth sex attractant was undertaken for comparative purposes (67). Synthesis of (+)-12-acetoxy-*cis*-9-octadecen-1-ol (XI*b*) (gyplure) was effected by reduction of ricinoleic acid to the diol, acetylation to the 1,12-diacetate, followed by preferential hydrolysis of the 1-acetate. Synthesis of (+)-14-acetoxy-*cis*-11-eicosen-1-ol (XI*c*) was accomplished in a similar manner starting with lesquerolic acid (68). The *trans* isomer of XI*b* was obtained by isomerization of the *cis* form using sodium nitrite and nitric acid. (Table I.) The propoxy and



XI

a, $n = 5$
b, $n = 7$
c, $n = 9$

butoxy analogs of gyplure were completely inactive.

From 500,000 glands of the female silkworm moth, *Bombyx mori*, was isolated the *p*-(*p*-nitrophenylazo)benzoate derivative of the active compound. Elementary analysis and ultraviolet spectra indicated the pheromone was a conjugated doubly unsaturated alcohol, $C_{16}H_{38}O$. Infrared spectra and identification of the products of catalytic hydrogenation and of oxidative degradation of the alcohol led to the structure,

$$CH_3(CH_2)_2CH{=}CH{-}CH{=}CH{-}(CH_2)_8CH_2OH$$

XII

10,12-hexadecadien-1-ol, or "bombykol" (XII).

TABLE II.—ACTIVITY OF ISOMERS OF BOMBYKOL

Compd.	Activity, mcg./ml. (67)
Bombykol	10^{-10}
10-*cis*-12-*cis*	1
10-*cis*-12-*trans*	10^{-3}
10-*trans*-12-*cis*	10^{-12}
10-*trans*-12-*trans*	10

Synthesis of the four possible geometric isomers of this unsaturated alcohol revealed that the 10-*trans*-12-*cis* isomer possessed similar physical properties and biological activity; thus, the natural compound probably has the 10-*trans*-12-*cis* geometry (70, 71). (Table II.)

A sex attractant has been demonstrated to be present in the honeybee and can be extracted from the mandibular glands of queens (72). It is most likely that the queen substance (*trans*-9-keto-2-decenoic acid) is not the true lure since it is only active at 0.1 mg. per assay tube in the attraction of drones. Sex attractants are usually much more potent than this substance.

Recently, the sugarcane borer moth, *Diatraea saccharalis* (F.), has been shown to possess a powerful attractant (73). However, as with many of the female insects, the attractant is much more prevalent in the virgin than in the mated female. The virgin female pine sawfly, *Diprion similis* (Hartig), is capable of attracting exceptionally large numbers of males; one caged female placed in a field attracted over 11,000 males. At a distance of 200 ft., 4×10^{-3} mcg. of an impure substance extracted from the females attracted almost 1000 males within 5 min. The material could be obtained by bathing the abdomen of the virgin sawfly in methylene chloride (74).

Recently, an unusual report was issued stating that the cause of the rapid population decline of the Virginia pine sawfly, *Neodiprion pratti pratti* (Dyar), is attributed to the loss of sex attractiveness by the female (75). During 1960, males were strongly attracted to the females and a high percentage mated; whereas in 1963, the females were not attractive to the males and only 2.5% mated. Mated females deposited 88% of the total eggs in the ovaries, but unmated females deposited very few eggs.

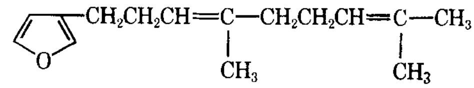
A sex attractant has been extracted from female cabbage loopers, *Trichoplusia ni* (Hübner) (76). The intensity and regularity of the response demonstrated that a lure was present and could be extracted with methylene chloride. The attractant is fairly volatile and has a relatively low molecular weight.

Recently, a report of a sex pheromone in the housefly, *Musca domestica* (L.), was issued (77). This could be a socially and economically important discovery. The presence of the material, a volatile chemical, was demonstrated in female houseflies. The behavior modification elicited was in the nature of an attraction to a source of the pheromone, or an excitation of mating-behavior patterns. The material, which is benzene-soluble and relatively stable, was shown to be sex-related as well as species-related since extracts of the closely related *Musca autumnalia* (Debeer) did not affect the behavior of male houseflies.

The above examples should suffice to indicate the generality of these attractants; reports of similar factors are entering the literature at an ever-increasing rate.

Since ancient times formic acid has been isolated from all species of ants in the sub-family *Formicinae*. This is probably the oldest known insect protective factor. The venom, stored in the anal glands, is described by Heim (78). Beard has reviewed the topic of insect toxins and venoms with emphasis on their physiological effects (79).

In 1957, Quilico *et al.* reported the isolation and structure of an odoriferous oil from the mandibular glands of the *Formicinae* ants (80). The substance, dendrolasin (XIII), while not toxic to *Lasium fuliginosus*, the ant from which



XIII

it is isolated, is toxic to six other species of ants.

A water-insoluble, nonproteinaceous, nitrogen-containing venom has been isolated from the fire ant, *Solenopsis saevissima richteri* (81). Other ant venoms also have been isolated, but their chemistry has not been reported (79).

The chemical defenses of insects against predators has been reviewed by Roth and Eisner (82). The structures of these defense chemicals are extremely varied.

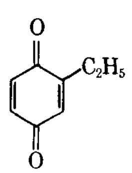
If certain species of insects are disturbed, they discharge an offensive odor. When the pentatomids (stink bugs) are attacked by their main enemies, the ants, or experimentally pinched with forceps, they defend themselves by attempting to spray a secretion on the aggressor. A number of pentatomids direct the secretion unilaterally over a wide range, while others can direct an unaimed long-range spray and an aimed short-range spray (83). Eisner *et al.* (84) observed similar reactions with carabids, cockroaches, and whip scorpions. Certain insects (imagines of mirids, larvae of certain lygaeids, and pyrrhocorids) moisten the mesotarsus or metatarsus with secretion and then brush the poison on the aggressor (83).

The secretion of the stink bugs is a contact poison, causing paralysis in ants and other insects. Chemical analysis indicates this secretion is composed of various saturated and unsaturated aldehydes (C_6–C_{10}) and *n*-tridecane (85). A general discussion of the scent glands of land bugs and their biological function explains the mode of action of these insect secretions (83).

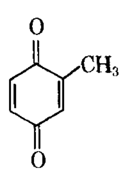
Modern scientists have shown sporadic interest in antimicrobial substances found in insects. The use of insects and insect-derived products in medicine dates from early Chinese and Greco-Roman cultures (86). Scorpions were said to be beneficial in skin diseases and malignant boils, while wood lice were used to treat malaria. The scarab or dung beetle found use as an amulet to ward off disease. Powdered horse flies snuffed into the nose supposedly cured recurve eyelashes, while the long-horn beetle was used to control convulsions in infants. Ancient insect remedies constitute an endless list, because each locale utilized those insects native to the specific area. Moffet in 1634 compiled the "Theratrum Insectrum" which listed applications of insect therapy and over 50 medicinally useful insects.

The modern era was ushered in with Nicholl's (87) report that the secretion of the blowfly larvae, *Sarcophaga*, was active against *Staphylococcus aureus*. Blowfly larvae have been used medicinally to clean lesions associated with osteomyelitis. The growth-promoting and antibiotic actions are attributed to allantoin, produced as a nitrogenous excretion product. Whether the latter action is correct has not been proved. The blowfly exhibits activity against *Clostridium welchii*, *Salmonella typhosa*, *Brucella abortus*, and hemolytic *Streptococci*.

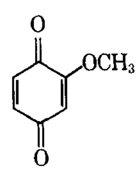
Antibiotic activity has been detected in wax-moth larvae, *Galleria mellonella* (88), and in *Tribolium castasneum* (89), where a quinone is believed to be responsible for the activity. From the confused flour beetle, *Tribolium confusum*, Alexander and Barton (90) isolated and identified ethylquinone (XIV) as the active component. Toluquinone (XV) and methoxyquinone (XVI) have also been identified in the flour beetle.



XIV

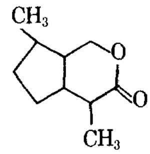


XV

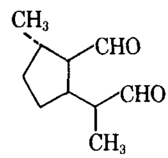


XVI

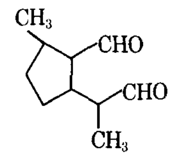
Investigations into the chemistry of ants by Pavan and others have uncovered several biologically potent but weakly antibacterial compounds. Iridomyrmecin (XVII) was isolated from the Argentine ant, *Iridomyrmex humilis*, while biogenetically related dolichodial (XVIII) and iridodial (XIX) have been identified in other ant species (91–93).



XVII



XVIII



XIX

Frings and co-workers (94) reported antibiotic activity against *Staphylococcus aureus* and one strain of *Bacillus subtilis* in the blood of the milkweed bug, *Oncopeltus fasciatus*. The water-soluble principle was active at a dilution of 1 part in 10,000, stable to boiling for 30 min., but destroyed by autoclaving. In this laboratory evidence has been obtained to indicate that the active material has the generalized formula XX (95) which is similar to sphingosine.

$$\underset{\text{OH}}{\text{R—CH}}\text{—}\underset{\text{NH}_2}{\text{CH}}\text{—}\underset{\text{OH}}{\text{CH}_2}$$

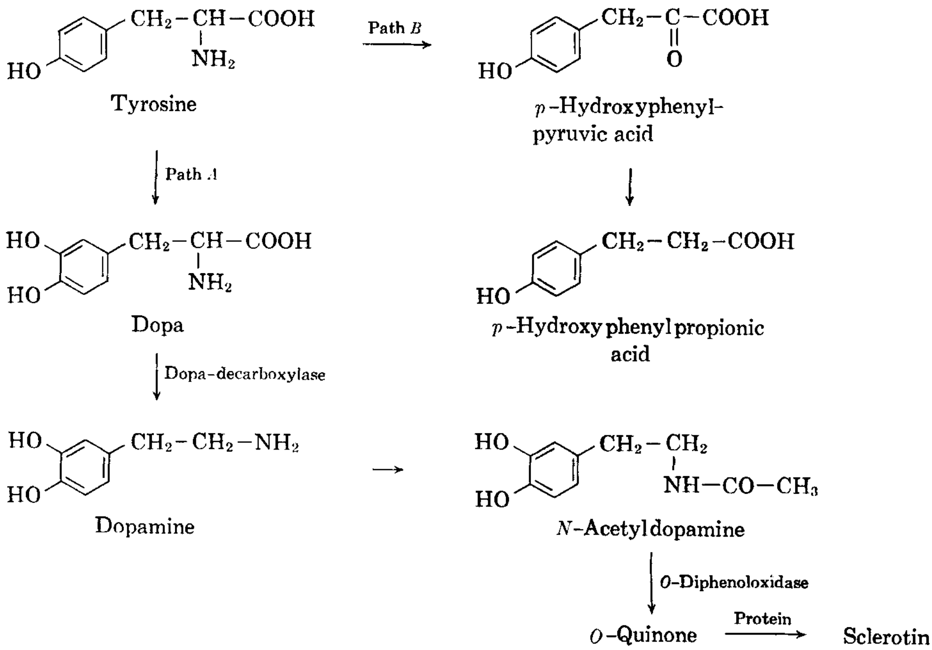
XX

UNIQUE CHEMISTRY OF THE INSECT

The study of the biochemistry of insects is presently in a revolutionary phase. A comprehensive survey of selected topics in this field is found in a series of books recently initiated to serve as a guide to recently acquired knowledge and as a stimulus for further advances (96). Gilmour (97) in his book on insect biochemistry affords a general review of intermediate metabolism, nutrition, energy production, and other topics.

Some of the classical hormones of vertebrate physiology, such as progesterone and pregnenolone, have been shown to be present in the insect (98); however, their function is unknown. Castrations of young insects and transplantations of the testes and ovaries into the opposite sex by early workers gave uniformly negative results. The regulation of secondary sex characteristics by the sex hormones is recognized in vertebrates, but this particular type of hormone action does not appear to operate in insects. Insect hormones act primarily on developmental processes. Karlson has given an excellent discussion of the recent chemistry and biochemistry of the insect hormones (99) and Wigglesworth of their physiology (100).

If an insect egg hatches to give a young insect



Scheme I

which resembles the adult as it grows and proceeds to the adult stage without an intervening resting stage (pupa), it is said to have incomplete metamorphosis. While it is in the growing stage it is known as a nymph. Butterflies, houseflies, bees, beetles, etc., have complete metamorphosis. Their eggs hatch into creatures which do not resemble the parents, known as larvae. All of their growth is made in the larval stage. At the last molt they assume a resting stage which may last from several days to months. During this period, while inactive outwardly, the transformation to an adult is occurring, and with the final imaginal molt, the adult is liberated.

The growth of insects is always cyclical, the development being punctuated by a series of molts, each preceded by a period of active growth and followed by a period in which true growth may be entirely absent. Inadequate nutrition, causing a prolonged larval period, may increase the number of molts. In such cases molting can take place without growth. In most insects molting ceases when the body is fully grown. Molting is also involved with changes in form. Metamorphosis is characterized by molting to the pupa or in some cases to a nymph and to the imago (adult). Numerous experiments have led to the recognition that these molts are induced by hormones.

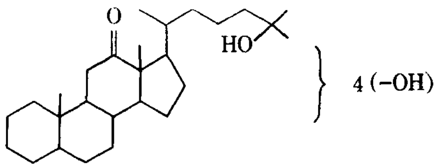
Molting is controlled by several hormones. It is initiated by a hormone produced in the neurosecretory cells of the brain, which acts on a subordinate gland, the prothorax. This gland is stimulated by the "prothoracotropic hormone" to produce its own hormone, the actual molting hormone, ecdysone. In the larva, ecdysone induces molting, but whether the molt thus initiated results in larva, pupa, or imago is determined by the activity of another endocrine gland, the corpus allatum (101).

Current literature on the chemical characteristics of this brain hormone is contradictory. From brains of pupae an oily substance was isolated which caused imaginal molting in pupae (102). Kobayashi (103) and Schneiderman (104) found brain hormone activity in lipid extracts from insect tissues, while Ishikawa (105) found activity not in the lipid-soluble portion but in the water-soluble portion of the extract.

Much of the work concerning ecdysone was performed by Karlson and his co-workers at the University of Munich. Chemical experiments began with the observation that puparium formation can be induced in ligated larvae of *Calliphora erythrocephala* by blood transfusion (106). Later a quantitative bioassay was developed. Using this bioassay method, 25 mg. of crystalline ecdysone was isolated by extraction of 500 Kg. of silkworm pupae, *Bombyx mori* (107).

Studies on the structure of ecdysone have led to the tentative formula XXI (108).

Karlson proposed a hypothesis for the mode



XXI

of action of ecdysone. According to this hypothesis, ecdysone, which produces a definite step in the development of the insect, simultaneously summons all the genetic information needed at that time by acting at one of the sites on the chromosome to which the requisite information has been passed. A messenger ribonucleic acid (RNA) is formed at this site which combines with the ribosomes and there directs the synthesis of certain proteins (including enzyme proteins) (99). A suggestion of the action of ecdysone in bringing about gene-controlled *de novo* synthesis of some enzyme proteins by ribosomes was obtained during the study of the process of sclerotization.

The basic chemical processes involved in sclerotization are shown in Scheme I (109–112) (path *A* in older larvae and path *B* in younger larvae).

Karlson and Sekeris found that in ligated abdomens as well as in intact animals decarboxylase activity was enhanced by injection of ecdysone (113). Horowitz and Fling have shown that the phenol oxidase of flies exists in an inactive form as a proenzyme and is converted into active phenol oxidase by means of an activator enzyme (114). It was found that in permanent larvae, produced by destruction of the ring gland, the activator concentration remained constant at first and finally decreased sharply. After injection of ecdysone, the activator concentration returned to its normal level (115). Enzyme production in both cases is very probably due to activation of certain gene loci by ecdysone, by which means the protein synthesis is stimulated.

Another hormone, the juvenile hormone, appears to be widely distributed in insects and vertebrate organs (116–119). The most important function of the juvenile hormone is to guarantee the persistence of larval characteristics during larval molting. The exact chemical nature of juvenile hormones is unknown. However, known compounds have been shown to possess the same activity. Starting from the finding that *Tenebrio* feces contain juvenile hormone, Schmialek detected farnesol and farnesal in the extract (120). These compounds were shown to possess definite juvenile hormone activity. These findings were extended to include a wide variety of alcohols, including alcohols from 8 to 15 carbon atoms, both saturated and unsaturated, in straight or branched chains. Ether derivatives of several of the alcohols further enhanced the activity.

A new endocrine function has been shown to play a role in insect morphogenesis (121). The hormone named proctodone is secreted by specialized cells of the hindgut and appears to affect the brain, resulting in the activation of the brain hormone-producing system. In *Ostrina nubilalis*, proctodone plays a part in both photoperiodism and diapause. Diapause, as a state of arrested growth, is considered to be caused by an endocrine failure, usually that of neurosecretion. With resumption of neurosecretion, the diapause state is terminated.

Studies in the mode of action of royal jelly in honeybee development have uncovered such potent factors as queen substance, 9-keto-2-decenoic acid (XXII), which is produced by the queen honeybee to inhibit queen rearing by

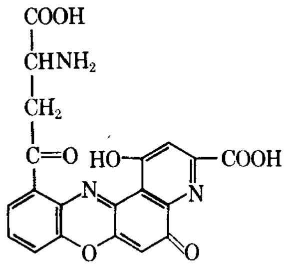
$$CH_3\underset{\underset{O}{\|}}{C}CH_2CH_2CH_2CH_2CH_2CH{=}CHCOOH$$

XXII

her workers (122). The mode of action as an endocrine function has been reported by Schaller (123), Lukoschus (124), and more recently by Shuel and co-workers (125).

As a result of differential nutrition during the larval period, female honeybee larvae of common genetic endowment develop into either queens or workers. This dichotomy is initiated during the first 3 days and is characterized at this early stage by differences in respiratory metabolism, tissue composition, and reproductive development. After the fourth molt, the queen develops much faster than the worker. Endocrine control over the phases of metamorphosis has been demonstrated, but whether the brain is a source of activating hormone which diffuses to the sites of activity in the honeybee is still an open question.

The screening pigments of the insect eye play an important role in isolating optically the ommatida of the compound eye from each other and in enhancing visual acuity. These pigments are called ommochromes and have been classified by Becker into two groups: (*a*) the low molecular weight ommatines and (*b*) the high molecular weight ommines (126). Butenandt has been concerned with the isolation and characterization of xanthommatin (XXII), one of the ommatines (127).



XXII

An excellent presentation on color discrimination in insects and the visual pigments in insects has been written by Burkhardt (95).

Any review of current research in the chemistry and biochemistry of insects is incomplete if it fails to deal with the excellent work presently being reported in the areas of cuticular chemistry, blood chemistry, vitellogenesis, nutrition, tissue culture, differentiation, energy production, and intermediary metabolism. Thus, it is obvious that this author has selected only a minute portion of the over-all picture. It is left for the reader to travel further into the many facets of the chemistry of insects. Over a million species of insects are known, and they represent an exceptional amount of chemistry which is unknown.

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Research Articles

Dissolution Rates of Polyphase Mixtures

By W. I. HIGUCHI, N. A. MIR, and S. J. DESAI

The theory for the dissolution rate of polyphase mixtures has been investigated and applied to several situations. Physical models involving simultaneous diffusion and rapid equilibria have led to relationships that describe experimental data rather well for the benzoic acid–salicylic acid, the benzocaine–caffeine, and the benzoic acid–trisodium phosphate mixtures.

PHYSICAL models involving the use of simultaneous diffusion and rapid equilibria have been successful (1) in describing diffusion-controlled kinetics of dissolution in reactive media. Most of these methods have been applied to single pure phases dissolving, although in a few instances the effects of surface-precipitating new phases have been examined (2).

In many practical situations one often deals with the simultaneous dissolution of more than one phase. It appeared, therefore, that it would be worthwhile to study the dissolution rate behavior of polyphase mixtures. Several situations have now been studied, and these are reported here.

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SIMPLE CASE OF TWO NONINTERACTING PHASES: BENZOIC ACID—SALICYLIC ACID

General Theory.—When a uniform, intimate, nondisintegrating mixture of two crystalline compounds, A and B, is exposed to a solvent, both phases should initially begin to dissolve at rates proportional to their solubilities and diffusion coefficients according to the Noyes-Whitney law. After a short period of time, usually one of the phases would become depleted in the solid–liquid interface region of the solid mass because N_A/N_B may not necessarily be equal to $(D_AC_A^0)/(D_BC_B^0)$, where N_A and N_B are the amounts of A and B in the mixture, D_A and D_B are the respective diffusion coefficients, and C_A^0 and C_B^0 are the solubilities. We are, of course, assuming diffusion-controlled rates. As a result of this situation, a surface layer is formed made of only one of the phases.

The three possible cases after time, t, > 0 are illustrated in Fig. 1. Most mixture ratios would lead to cases A or B. Only for the critical mixture ratio,