Critical Reviews in Biochemistry and Molecular Biology, 34(4):253–264 (1999)

Occurrence, Biosynthesis, and Putative Role of Ecdysteroids in Plants

John H. Adler and Robert J. Grebenok

CONTENTS

I. INTRODUCTION

Ecdysteroids are the active principles in arthropod molting or ecdysis and are the subject of extensive literature.[1–4] These molecules are involved in ecdysis of insects (Insecta), spiders and ticks (Chelicerata), and crustaceans (Crustacea) and appear likely to be the molting hormone of all arthropods.[5] Various ecdysteroids may also be isolated from non-arthropods, including certain roundworms (Nematoda), mussels and snails (Mollusca), and soft coral (Cnidaria).[6] In the soft coral *Lobophytum*[7] and the zooanthid *Gerardia*,[8] the high levels of ecdysteroids isolated would argue against their function as a hormone. Recently, in the Pycnogonids (Arthropoda, Pycnogonida) 20-hydroxyecdysone, 20-hydroxyecdysone-2-acetate, and other ecdysteroids were identified at high levels in cuticular glands.[9] Upon mechanical disturbance these animals secrete ecdysteroids from the gland to the environment and, since these compounds have a strong antifeedant effect, the pycnogonids avoid being consumed by crabs (Crustacea).[9]

Among the plants, the ecdysteroid 20-hydroxyecdysone was identified in the mid-1960s, and was demonstrated to be structurally identical to that isolated from insects.[3] Plant ecdysteroids are often present at 10^{-3}%–10^{-1}% of the dry weight of the plant, which is 2–5 orders of magnitude greater than that reported in arthropods.[9] These phytoecdysteroids do not appear to have any appreciable hormonal function within the plant,[10,11] as tested in several plant bioassays. Phytoecdysteroids, however, were proposed to be nontoxic feeding deterrents to certain insects[12,13] and to affect growth and development upon ingestion from artificial diets[14–18] by certain insects.

The biosynthesis of ecdysteroids in plants and their distribution within the plant is not well understood. Yet the control of these processes is of importance if we are to understand the role of phytoecdysteroids as defense molecules against nonadapted insect herbivores. The function of this review is to bring together the limited information available on these processes, and to attempt a synthesis and an integration of our knowledge of plant biosynthetic capabilities with the assumed function of these phytoecdysteroids as defense compounds against insect herbivory.

II. STRUCTURE

Phytoecdysteroids have a varied chemical structure and appear to be derived from C_{27}, C_{28} or C_{29} sterols. Several modifications of the sterol structure are required to produce ecdysteroids. The major transformations are conversion of the trans A/B ring juncture in sterols to a cis A/B ring juncture in the ecdysteroid, the introduction of a 7-en-6-one chromophore and the introduction of 14α-hydroxy group.[4] Additional hydroxylations at C-2, C-20, C-22, and C-25 are required to produce the most commonly

identified phytoecdysteroid, 20-hydroxyecdysone (20E), Figure 1, with the addition of a C-5 β-hydroxy group producing the often reported Polypodine B (5,20E). Additional sites and actions of metabolism are identified in Figure 1. Metabolic modifications of the basic structure have led to the identification of over 100 phytoecdysteroid compounds, which are well reviewed.[3,19] These phytoecdysteroids may exist in either free form or as polar conjugates of glucosides, sulfates, and phosphates or nonpolar conjugates as acetates or benzoates.[19,20] In addition to these true ecdysteroids, plants often make ecdysteroid-related compounds which are loosely defined[19] based upon their structure and biological activity. The reader should note that in older studies 20E was referred to as ecdysterone.

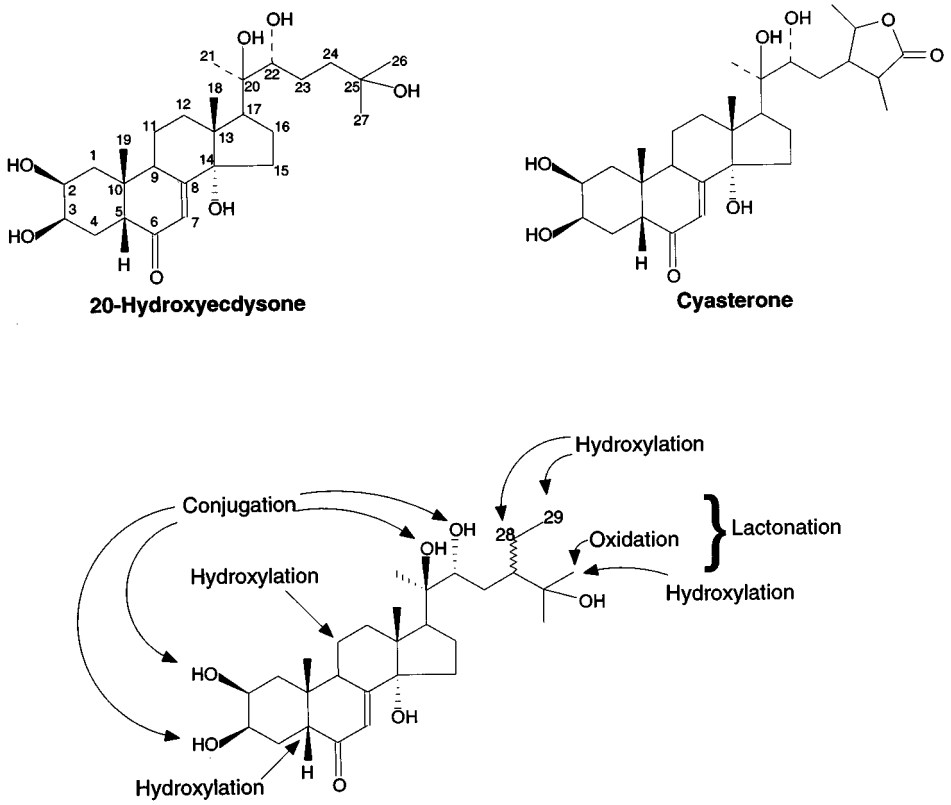

Figure 1 Structure and sites of biochemical modification of phytoecdysteroids. The structures of the most commonly reported phytoecdysteroid, 20-hydroxyecdysone, and a 24-alkylphytoecdysteroid, cyasterone. Some common sites of biochemical modifications reported for phytoecdysteroids.

III. DISTRIBUTION OF PHYTOECDYSTEROIDS

A. PHYLOGENETIC OCCURRENCE

Within the vascular plants, phytoecdysteroids are characterized from evolutionarily older plant families as well as more recently evolved plants. Among the evolutionarily older groups of plants, phytoecdysteroids are reported in 20 families of ferns (Polypodiophyta), one species each from both the whisk-ferns (Psilophyta) and the club-mosses (Lycopodiophyta), and 9 families of gymnosperms (Pinophyta). Within the flowering plants phytoecdysteroids are identified in plants derived from evolutionarily ancestral families (e.g., Magnoliaceae and Ranunculaceae) as well as those more recently evolved (Asteraceae and Liliaceae). The recent review by Lafont and Horn[19] provides a concise table on this occurrence within plants. Overall the flowering plants are reported to possess phytoecdysteroids in various species within 78 families.[19] Some of these reports represent sampling of only one species within a family, whereas other families were extensively examined.

Several families of flowering plants in the order Caryophyllales were examined for phytoecdysteroids. Individual species in 9 out of the 12 families in this order, based on the classification by Cronquist,[21]

contain phytoecdysteroids. These are well documented from multiple species in the families Amaranthaceae, Caryophyllaceae, and Chenopodiaceae.[19] The families Aizoaceae, Basellaceae, Cactaceae, Nyctaginaceae, Phytolaccaceae, and Portulacaceae are all reported to possess at least one species containing ecdysteroids.[19] There has been no systematic examination of this order for phytoecdysteroids in spite of the general agreement among taxonomists as to the relatedness of these families.[21] Several other orders contain these compounds in several families.[19] These include the Ranunculales (4 families), Urticales (3 families), Malvales (4 families), Violales (7 families), Capparales (3 families), Rosales (3 families), Sapindales (5 families), Polemoniales (3 families), Scrophulariales (3 families), and Liliales (5 families). Thus, 51 of the 78 families of flowering plants reported to possess ecdysteroids are found in only 11 orders of plants. In spite of the fragmentary distribution data for phytoecdysteroids reported within the flowering plants, several orders appear to maintain this biosynthetic capacity. Assuming that the presence of phytoecdysteroids in the plant is of some defensive advantage, the strategy has been maintained over an evolutionarily diverse array of plants.

The presence of phytoecdysteroids within a plant family will likely be genus as well as species dependent. Examination of species in the family Chenopodiaceae by Dinan[22,23] has provided some insight into this occurrence. The family Chenopodiaceae is composed of two subfamilies, the Chenopodioideae and the Salsoloideae,[24] with no species in the latter found to be ecdysteroid-positive in its seed extract. Among the six tribes of the subfamily Chenopodioideae, the Salicorniae is ecdysteroid negative, whereas representatives of the other five tribes possess ecdysteroids. However, not all species within each genus possess ecdysteroids. High levels of ecdysteroids were detected in species of the tribes Chenopodieae and Atripliceae, whereas species in the Camphorosmae and Corispermae possess low levels. Thus, the ability to produce phytoecdysteroids, although related to phylogeny, is not predictable at the species level. The specific genetics and environmental conditions from and in which a species evolves are likely the major factors that control or influence this biosynthetic capability.

B. ONTOGENETIC OCCURRENCE

The distribution of phytoecdysteroids within an individual plant is related to the organ type (e.g., leaf) and position of organ on the plant as well as the state of development of the organ. The presence of phytoecdysteroids within a specific organ may fluctuate over the time course of growth, depending upon cycles of biosynthesis and transport. In general the growth strategy that the plant has adopted, i.e., whether perennial or annual, appears to provide a basis for sorting out the physiological distribution data reported in the literature.

Many of the early isolations of phytoecdysteroids were made from perennial plants, and often the perennial structures of these plants were analyzed. Thus, the rhizomes of ferns, as well as bark and perennial root structures of other plants, were reported to possess significant quantities of these compounds.[3] Levels of ecdysteroid range as high as 2% dry weight from the rhizome of the fern *Polypodium vulgare*.[25,26] Among the fern species, six families are known to produce significant quantities of phytoecdysteroids with 8 of the 18 compounds identified not reported in higher plants.[27,28] The gymnosperms, specifically those in the families Podocarpaceae and Taxaceae, were also reported to possess high levels in the dry bark and moderate levels in leaves.[3]

Few analyses of the fluctuation of ecdysteroids between plant parts were reported in the early period of phytoecdysteroid discovery, since the major emphasis was on isolation and structural characterization. Recent work in the gymnosperm *Taxus cuspidata* (Taxaceae) demonstrated that the phytoecdysteroid levels fluctuated in the vegetative shoots at different developmental stages during shoot growth.[29] The new vegetative shoots, one to four weeks old, possessed 20-hydroxyecdysone (20E) at 50 mg kg^{-1} fresh weight with only trace levels of ponasterone A present. As these shoots matured (18–37 weeks old) the 20E level increased to 120 mg kg^{-1}, whereas the ponasterone A level was 20 mg kg^{-1}. In 52- to 104-week-old leaves, i.e., 2-year-old leaves, the level of 20E returned to 45 mg kg^{-1} and ponasterone A was present at 20 mg kg^{-1}. These data suggest a dynamic control of ecdysteroid accumulation within the leaves of this plant. The 20E never dropped below 45 mg kg^{-1} fresh weight over the life of the leaf, and ponasterone A levels, once they reached 20 mg kg^{-1}, were maintained in spite of a decrease in 20E content between 37 and 52 weeks. Upon shoot emergence 20E transport from older leaves to young emerging leaves would explain the drop observed in older leaf 20E content. Developmental onset of ecdysteroid biosynthesis produces the high levels of 20E and ponasterone A observed at 18–37 weeks. These observations in the gymnosperm *Taxus* appear to follow a pattern of transport and biosynthesis recently observed in the annual spinach.[30]

Among the flowering plants, woody and herbaceous perennials were also screened for ecdysteroids early in the history of phytoecdysteroid discovery.[3] Most of these reports belong to species of the families and orders listed earlier. The genera that served as good sources of phytoecdysteroids are *Achyranthes* and *Cyathula* (Amaranthaceae), *Helleborus* (Ranunculaceae), and *Ajuga* (Lamiaceae). Early reports by different investigators quantitated the ecdysteroid content from entire plants of the same species, e.g., *Ajuga decumbens*, at substantially different levels.[31,32] Subsequently, studies on the distribution of ecdysteroids during the growth of herbaceous perennials have demonstrated a general pattern of ecdysteroid cycling within some perennial plants. In *Lychnis chalcedonica* (Caryophyllaceae) the roots maintain a relatively constant level of 0.27–0.33% dry weight of ecdysteroid during the growing season.[33] The aerial parts, however, possess 0.79% dry weight in the spring, and decrease the ecdysteroid level to 0.18% dry wt during seed ripening. *Rhaponticum carthamoides* (Asteraceae), also known as *Leuzea carthamoides*, possesses the highest levels of ecdysteroids in newly developed shoots in spring, and appears to cycle these from the subsurface parts to the developing shoots.[34,35] *Ajuga reptans* (Lamiaceae) maintains high concentrations of ecdysteroids ($1.5–31 \times 10^3$ μg g^{-1} dry weight) in the roots throughout the growing season. These *Ajuga* plants maintain a concentration gradient of ecdysteroids throughout their aerial parts, with the most distal leaves having the highest concentration and the proximal leaves the lowest concentration. The profile of the six phytoecdysteroids produced did, however, vary depending upon organ and growth conditions of the plant.[36] Two perennial species in the Chenopodiaceae were recently examined for ecdysteroid content, *Rhagodia candolleana*, a slow growing woody perennial, and *Beta patellaris*, an herbaceous perennial.[22] All members of the genus *Rhagodia* examined were reported to possess high levels of ecdysteroids,[22] whereas the majority of species in the genus *Beta* do not contain ecdysteroids. The *Rhagodia candolleana* had the highest levels of ecdysteroids in newly developing side-shoots, reaching a 2- to 4-fold increase in ecdysteroid concentration compared to that found in the leaves of the main shoot or root. In *Beta patellaris* the highest levels of ecdysteroids were present in the older, larger leaves lower on the stem and the apical-most leaves. The root concentrations are not reported. Both of these perennials maintain a concentration gradient of ecdysteroids, *Rhagodia* between young side shoots and central shoots, and *Beta* between oldest and youngest leaves and those centrally located on the stem. Both species concentrate ecdysteroids in young, developing tissues and *Beta* also maintains a high concentration in its oldest leaves, perhaps because this is the site of ecdysteroid biosynthesis, as observed in some annuals. These two species should be studied over a seasonal time course to determine if the levels of ecdysteroids increase in young tissues with growth flushes and decrease with senescence of various organs as observed with other perennials. The high levels of ecdysteroids in the side-shoots of *Rhagodia* could be a manifestation of the growth state of the plant, as in many plants the side-shoots have delayed growth, post growth of the main plant axis.

The overall pattern of phytoecdysteroid distribution in the herbaceous perennials would allow for a generalization of ecdysteroid cycling from perennial portions of the plant to the deciduous organs. Presumably, these ecdysteroids are recovered by transport back to the perennial structures prior to shedding the deciduous parts with perhaps the exception of seeds or fruits, which may maintain a high level of ecdysteroids. This cycling of ecdysteroids with periods of biosynthesis on a yearly basis would help explain the high levels of ecdysteroids present in the perennial structures of these plants. These high levels of ecdysteroids are the accumulation of many years of ecdysteroid production. As the plant size increases with age, the ecdysteroids present in the newly emerging organs (usually aerial parts) would be maintained at levels sufficient to act as a feeding deterrent for non-adapted herbivores. With the increase in plant biomass, accumulated during the current year's carbon fixation process, an appropriate amount of new ecdysteroid biosynthesis would occur, providing adequate levels for the current year's growth and the next year's early growth flush of aerial parts.

The distribution of ecdysteroids in annual flowering plants is similar in many ways to the patterns observed in perennial plants. The major differences are that a) the only stored ecdysteroid available at the onset of seed germination is that stored in the seed as compared to perennial organ storage and b) the plant's ability to complete its reproductive cycle (i.e., set seed) within the growth period is paramount for species survival as compared to the long-term growth and survival strategy of a perennial, i.e., perennial plants as a species can survive a bad seed-producing year. The best characterized annual plant for ecdysteroid content and distribution during ontogeny is spinach (*Spinacia oleracea* L.).[30] Spinach seeds possess 17 μg ecdysteroid per seed embryo whereas only 1 μg ecdysteroid is present in the seed coat. Young plants redistribute this ecdysteroid from the seed and at 11 days of age possess 8 μg ecdysteroid in the cotyledons and 6 μg ecdysteroid in the first true leaves. This ecdysteroid mass is concentrated in the first leaves, which constitute only 20% of the plant biomass and thus possess an effective dose level

to a herbivore of 120 μg ecdysteroid g^{-1} fresh weight of leaf.[30] As leaves are initiated on the plant the ecdysteroid level in these new leaves is high, peaks, and then drops as the next apical leaf set develops.[30] Onset of ecdysteroid biosynthesis occurs between 11 and 20 days after seed planting (see Section IV). The spinach plant concentrates the ecdysteroids (20-hydroxyecdysone and polypodine B) in its apical-most leaves through ontogeny.[30] Dinan recently confirmed this distribution profile using radioimmunoassay in *Spinacia oleracea*, *Atriplex oblongefolia*, *Chenopodium ficifolium*, and *Chenopodium album*, all annual plants in the Chenopodiaceae.[22,23,37] Flowers of *Chenopodium album* accumulate ecdysteroids in the anthers,[23] and *Kochia scoparia* (Chenopodiaceae)[38] accumulates intermediate levels in the flower. It is argued that protection of wind-blown pollen during its development in the anthers of *C. album* is an important feature for the ecdysteroid concentrating there.[23] The pollen grains themselves have <3% of the ecdysteroid present in the anther and the anthers contain 4 to 5 times the level (500 μg g^{-1} fresh weight) of ecdysteroid reported in the other flower parts. Interestingly, three chenopod species that are presumed to be insect pollinated by virtue of the small amount of pollen produced, have low levels of ecdysteroids in their growing tips (0–3 μg g^{-1} fresh weight).[23] With regards to seed production, spinach has a high level of ecdysteroid (90 μg g^{-1} fresh weight) in its one-seeded fruits, with 95% of the ecdysteroid in the seed,[30] whereas *Kochia scoparia* accumulates intermediate levels.[38] Both annual and perennial plants are known to possess ecdysteroids in their seeds and fruits.[3,30] Presumably ecdysteroid sequestered in such seeds is used to protect young seedlings from herbivores as the ecdysteroid is redistributed in the developing seedling, as is the case in spinach.[30]

IV. BIOSYNTHESIS AND REGULATION

Unlike insects, the plants possess a complete sterol and ecdysteroid biosynthetic capability. Insects are unable to biosynthesize sterols; thus, they require dietary sterols for membranous sterol requirements and as precursors to ecdysteroids.[39] The insects have evolved several biosynthetic pathways to metabolize typical C_{28} and C_{29} phytosterols to the C_{27} sterol required for insect metabolism to ecdysone and 20-hydroxyecdysone.[4] A series of studies prior to 1985 on plant biosynthetic capacities supported the conversion of mevalonic acid and cholesterol to ecdysteroids, and provided evidence that the mechanism of A/B ring inversion is different from that observed in insects.[40–45] Recent studies in spinach, *Spinacia oleracea*, have demonstrated that phytoecdysteroid biosynthesis is both vigorous and a highly controlled pathway as demonstrated by the incorporation of [2-^{14}C] mevalonic acid, MVA, and other putative ecdysteroid biosynthetic intermediates.[20,30,46,47] In the germinating spinach plant prior to the onset of ecdysteroid biosynthesis, the ecdysteroid to sterol ratio is 1:1, but after the onset of biosynthesis in the first leaves the ratio can reach 10:1 on a microgram of steroid/per gram fresh weight basis.[48] Thus, the synthesis of ecdysteroids represents a major metabolic sink for sterols produced by these leaves. The biosynthesis and regulation of ecdysteroids in spinach proceeds via the scheme presented in Figure 2. From radiolabel incorporation studies, the C_{27} reduced side chain sterol, lathosterol, is the proposed intermediate to the 24-desalkyl-ecdysteroids found in spinach.[46] All the sterols in spinach are Δ^7-sterols as compared to the Δ^5-sterols found in many higher plants (e.g., sitosterol).[39] Subsequently, ecdysone is formed and is present at 0.004% of the total free ecdysteroid, yet contains 6% of the total radioactivity from [2-^{14}C] mevalonic acid incorporation.[20] Ecdysone is converted to ecdysone-3-phosphate and then to 20-hydroxyecdysone. The C-20 hydroxylase is putatively a cytochrome P-450-based enzyme (Grebenok, unpublished). Biosynthesis of ecdysteroids proceeds in the leaves immediately subtending the most apical leaf set early in plant development. The apical-most leaves contain the highest levels of ecdysteroid up to 800 μg ecdysteroid g^{-1} fresh weight in spite of their inability to biosynthesize ecdysteroids. These apical leaf ecdysteroids are transported from leaves lower on the plant that are biosynthetically active.[30] These young apical-most leaves are incapable of converting [2-^{14}C] MVA into either lathosterol or ecdysteroid.[30] Lathosterol is undetectable by mass, or by radiolabel from [2-^{14}C] MVA, even when cold-carrier lathosterol is added. Presumably, the Δ^{24}-reductase is not active in these apical leaves at this stage of ontogeny, and all sterol biosynthesis is toward the 24-alkylsterols. In biosynthetically active leaves [4-^{14}C] cholesterol, the Δ^5 analog of lathosterol and a nonindigenous sterol to spinach, can be converted to ecdysteroids, but only after a substantial time course of incubation, suggesting that cholesterol is not an optimal substrate.

Biosynthesis of ecdysteroids from various plants using plant tissue culture techniques is reported from callus cultures of *Achyranthes* (Amaranthaceae),[49] *Trianthema* (Portulacaceae),[50] *Ajuga turkestaneca* (Lamiaceae),[51] and *Agrobacterium rhizogenes* transformed hairy root cultures of *Ajuga reptans* var. *atropurpurea*.[52] Not all tissue cultures from ecdysteroid producing plants, however, actively biosynthesize

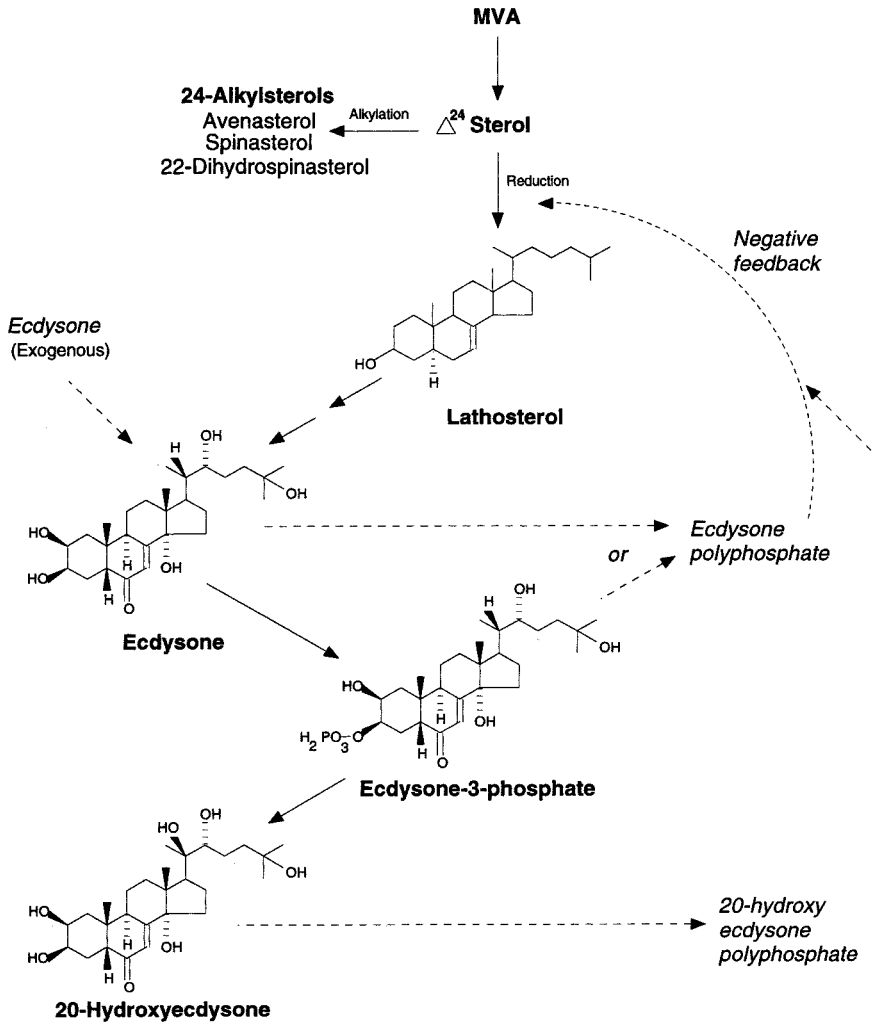

Figure 2 The pathway of biosynthesis and putative regulation for ecdysteroids in spinach. The *in vivo* incorporation of radiolabeled mevalonic acid (MVA), solid lines, produces radiolabeled 20-hydroxyecdysone, ecdysone, and ecdysone-3-phosphate. Ecdysone incorporation in mass, dashed lines, produces ecdysteroid polyphosphates, ecdysone polyphosphate, and 20-hydroxyecdysone polyphosphate, and causes inhibition of MVA incorporation into ecdysteroids (see text).

ecdysteroids. When callus cultures from *Ajuga reptans* leaves and roots were examined, no phytoecdysteroids were detected with a lower detection limit of 5 ppm dry weight.[53] The capacity to produce phytoecdysteroids appeared to reside in differentiated cells organized into tissues of *A. reptans*.[36] Thus, *in vitro* root cultures were capable of phytoecdysteroid biosynthesis, whereas leaf cultures were not.[53,54] Similarly, callus and cell cultures of *Serratula tinctoria* (Asteraceae) possessed phytoecdysteroids with quantities variable depending upon growth conditions.[53] Thus, the biosynthesis of phytoecdysteroids is expressed in different organs, and the organization of tissues in these appears to influence expression.

Our understanding of the regulation of phytoecdysteroid biosynthesis is fragmentary at best. In spinach the intermediacy of phosphorylated intermediates in the ecdysteroid pathway appears to be important to the overall regulation scheme. Ecdysone and ecdysone-3-phosphate can be isolated from leaves actively biosynthesizing ecdysteroids[20] with the sequence of the pathway suggested in Figure 2. However, when exogenous carrier ecdysone was added to actively biosynthesizing leaves, all of the ecdysone and 20-hydroxyecdysone mass dissappeared from the ecdysteroid reverse phase high performance liquid chromatograph profile. This ecdysteroid could eventually be recovered following wheat germ acid phosphatase treatment of the intact butanol fraction.[20] The ecdysteroids had been converted to ecdysteroid

polyphosphates which were cleaved by the enzyme action. A time course of disappearance and reappearance of free ecdysteroids following their entry into the polyphosphate pool was also observed in intact leaves.[20] The presence of other putative ecdysteroid precursors, 2-deoxy-20-hydroxyecdysone, polypodine B, and 22,25-dideoxyecdysone, also demonstrated a similar regulatory response.[20] The production of the polyphosphorylated end product from these nonconjugated ecdysteroids also appeared to downregulate the incorporation of [2-^{14}C] MVA into end-product ecdysteroids,[20] thus providing a negative feedback (Figure 2). In addition, a search for nonconjugated ecdysteroids in spinach, following [2-^{14}C] MVA incorporation, was able to detect only the presence of three ecdysteroids, ecdysone, 20-hydroxyecdysone, and polypodine B. The other endogenous intermediates, which must exist, are perhaps present in conjugated forms such as the ecdysone-3-phosphate. The data, to date, provide a rather intriguing picture of how the overall regulation of ecdysteroids may be accomplished within plants (Figure 2). Ecdysteroid phosphates are known to accumulate within some insect tissues, particularly ovaries and eggs, and are potentially biosynthetic precursors to insect ecdysteroids.[4] Plants may use ecdysteroid phosphates in regulating the endogenous biosynthesis of ecdysteroids from the sterol precursor.

V. ECDYSTEROID FUNCTIONS IN INSECTS

Ecdysteroids are endogenously synthesized steroid hormones used by insects to regulate growth, development, and reproduction. Insect embryonic and postembryonic maturation is defined periodically by molting episodes that are initiated by the production of ecdysteroids, the specifics of which are well reviewed.[1,2,56,57] General conclusions regarding the physiological function of ecdysteroids can be drawn from these data. The ecdysteroid, 20-hydroxyecdysone, appears to be the molting hormone utilized by arthropods to regulate the physiological events surrounding the molting cycle. A myriad of tissue types are known targets for ecdysteroid action. Ecdysteroids exert their effects by a receptor-mediated process similar to that described for steroid responses in vertebrates, in which the steroid-receptor complex binds to nuclear chromatin and alters gene transcription, and finally, insect development occurs in a highly ordered cascade that depends upon the timely cycling of ecdysteroid titers.[58] At the molecular and cellular levels, ecdysteroids are responsible for tissue-specific increases and repression of ribosomal and nonribosomal RNA synthesis, induced DNA synthesis, and cellular morphology changes during insect developmental processes, e.g., epidermal molts and imaginal disc development.

The insect cuticular molting process involves the rise and fall of ecdysteroids, which coordinate various cellular events. The cellular responses are initiated by the binding of ecdysteroid to external cellular receptors, with subsequent movement of the receptor complex to the chromatin of the nucleus. These receptor sites appear to be tissue specific and transient in their ecdysteroid binding capacity, suggesting that morphological changes in insect development are dependent on both ecdysteroid titers and receptor availability.[59] Examination of the molting process under artifically modified ecdysteroid titers demonstrates several titer and time-dependent considerations.[60] Prolonged maximal ecdysteroid titers during cuticle formation cause abnormal cuticle morphology and delay further pigmentation and sclerotization.[61,62] Additionally, elevated ecdysteroid titers inhibit the activation of secreted molting fluid that prevents endocuticular breakdown and stalls ecdysis.[63] The highly regulated temporal and titer-dependent processes of the epidermal molt may expose insects to potential fluctuations of hemolymph ecdysteroid titer from exogenous sources, with significant morphological consequences. During metamorphosis of the larva to the pupa and then to the adult form, the imaginal discs, clusters of undifferentiated epidermal cells, differentiate, producing numerous adult cuticular structures, e.g., wings, legs and eyes.[64] Imaginal disc differentiation is induced by the synthesis of ecdysteroids, which may be accompanied by the reduction of juvenile hormone titers. The mechanism of ecdysteroid action is similar to that observed for the epicuticular molting process.[65,66] Although the metamorphosis of imaginal disc tissue is directly mediated through ecdysteroids,[62,65] the disc tissue does not have the ability to metabolize ecdysteroid structures. Various ecdysteroids with divergent structures have varied inductive activities.[67] 20-Hydroxyecdysone demonstrates approximately 450-fold greater stimulatory activity of imaginal disc differentation than does ecdysone, whereas ponasterone A, a phytoecdysteroid, demonstrates a 20-fold greater activity than 20-hydroxyecdysone.[68] These results suggest the ability of various ecdysteroid (and phytoecdysteroid) structures to have a profound impact on growth and development of imaginal disc-derived tissues.

Insects contain an open circulatory system in which the hemolymph carries molecules (e.g., protein, lipids, and carbohydrates) between organs and tissue types. The nature of the circulatory system allows for dietary compounds to be passed from the midgut to numerous carrier proteins that were identified

for several hemolymph constituents (e.g., fatty acids, vitamins, and steroid hormones). Feyereisen[69,70] demonstrated the occurrence of the ecdysteroid carrier protein that appears to regulate free ecdysteroid levels in the hemolymph. The regulation of ecdysteroid-mediated developmental effects by the carrier protein complex is not well understood. It is likely that the carrier protein complex removes excess ecdysteroid from active participation in developmental processes or, conversely, only ecdysteroid carrier protein complexes are useful in mediating ecdysteroid-dependent developmental responses. Ecdysteroids from numerous plant sources were observed to pass into the hemolymph and act upon target receptor sites, creating inappropriate physiological responses during insect development.[13–15] However, the titer of carrier protein complexes or the level of tissue-specific ecdysteroid receptors during these responses has not been addressed. It appears clear from the susceptibility of major developmental processes (e.g., molting and imaginal disc differentiation) that unmetabolized phytoecdysteroids (e.g., 20-hydroxyecdysone and ponasterone A) absorbed into the hemolymph could possibly have major repercussions on several aspects of insect development.

VI. INSECT AND PHYTOECDYSTEROID INTERACTIONS

Physiologically, non-adapted herbivorous insects appear susceptible to dietary levels of phytoecdysteoids, as discussed above. However, if phytoecdysteroids are to be considered defensive compounds, ecological examples of plant and insect interactions should be available to support this premise. Insect pest populations are well documented for numerous crop species.[71,72] The ecdysteroid content of spinach dynamically cycles ecdysteroid (20-hydroxyecdysone and polypodine B) throughout its development with apical leaf concentrations reaching 800 mg ecdysteroid/kg fresh weight of plant. Although these ecdysteroid titers appear dramatically high in some tissues, several insect species utilize spinach as a food source, the spinach leaf miner *Pegomya hyoscyamia*, the spinach aphid *Myzus persicae*, and the alfalfa looper *Autographa gamma californica*.[71,72] The precise feeding locations and plant ecdysteroid content of those locations would necessarily have to be investigated to determine if these insects specifically avoid or tolerate plant parts with elevated ecdysteroid content. However, few other insects have been observed utilizing spinach as a primary food source.[71,72] In comparison, the major agronomic crops, corn and cotton, which are not known to produce high levels of phytoecdysteroids, have been observed to host some 20 different major insect pests.[71,72] In particular the fall armyworm (*Spodoptera frugiperda*), which is a major crop pest of both cotton and corn, appears not to prefer spinach as a primary host. Purified 20-hydroxyecdysone, when administered to the fall armyworm larvae in artificial diets, elicits significant physiological abnormalities during maturation,[73,74] suggesting that phytoecdysteroids potentially play a defensive role and limit the number of primary insect pests in plants such as spinach.

However, for ecdysteroids to function as defensive compounds in higher plant systems, not only must plants contain appropriate structures (e.g., 20-hydroxyecdysone, ponasterone A) but the plant also must produce sufficient levels so that insects encountering the plant could either ingest a physiologically significant dose or be deterred from feeding. Of equal importance, however, insects must also be ingesting or absorbing the phytoecdysteroid at a developmental state at which the insect is sensitive to ecdysteroid action. Although few observational experiments utilizing intact phytoecdysteroid-producing plant organs and known insect pest species have been attempted, several isolated phytoecdysteroids have been tested in artificial diets for antifeedant and hormonal responses in several insect species.[13] Results from several studies present a varied picture of ecdysteroid action (Table 1).

Many insect species demonstrate physiological responses to low levels of dietary ecdysteroid (0.03 to 100 mg ecdysteroid/kg fresh weight) during particular growth phases. Ingestion of ponasterone A at 1.5 and 100 mg/kg fresh weight of diet by the silkworm, *Bombyx mori*, produced accelerated prepupal maturation and fourth instar larval mortality, respectively.[78] Similarly, exposure of 2.5 mg/kg fresh weight of 20-hydroxyecdysone in artificial diet to *Plutella xylostella* produced 80% mortality of first instar larvae and a 95% reduction in oviposition of surviving females (Grebenok, unpublished). Additional insect species, *Ornthodous monbata*, *Drosophila melanogaster*, *Halotimes favicollis*, and *Glossia moritans*, also demonstrate detrimental physiological responses to these phytoecdysteroid titers.[13] Although not as sensitive, *Musca domestica*, *Tribolium confusum*, *Prodenia litura*, *Pieris rapae*, *Leucania separata*, and *Blatella gemanica* all demonstrate detrimental physiological responses to phytoecdysteroids at levels from 100–2000 mg/kg fresh weight of diet.[13] Significantly, all insects listed have a developmental period in which some level of dietary phytoecdysteroid would affect an aspect of growth, development, or reproduction. The literature on the occurrence of various ecdysteroids in plants is extensive, with reported

Table 1 Effect of Ingested Ecdysteroids on Some Herbivorous Insects

Insect	Treatment	Major Effects			Ref.
			In EI_{95}	ppm ED_{50}	
Pectinophora gossypiella Pink bollworm	Artificial diet feeding				
	Ecdysis inhibition (EI_{95})	Ponasterone A	2	1	74
		Ecdysterone	50	35	
	Effective dose (ED_{50})	Cyasterone	40	25	
		Ajugasterone	45	14	
	Growth inhibition	Ajugalactone	N.D.	430	
Spodoptera frugiperda Fall armyworm	Ecdysterone feeding	Failed molting cycle, entrapped organism			74
Bombyx mori Silkworm	Feeding	Unable to feed or locomote			74
Schizaphis graminum Aphid	Feeding	Feeding Inhibition			74
		In ppm	ED_{50}		
		Ajugasterone C	62		
		Ecdysterone	650		
		Cyasterone	2000		
Heliothis complex Corn earworm	Ecdysterone feeding at 3000 ppm	No effect			74
Spodoptera litura Armyworm	Ingestion of 2–6 μg ecdysterone by 5th and 6th instar	1) Abnormal and malformed pupae 2) Decreased fecundity (60%; 90%) and fertility (33%; 60%) females emerging treated (5th; 6th instar)			75
Dacus tryoni Queensland fruit fly	Administered in food	Ecdysterone no effect on fecundity or fertility			76
Epilachna varivestis Mexican bean beetle	Feeding on bean plants dipped or sprayed with 22,25-bisdeoxyecdysone	Precocious molting Emerged females sterile Oviposition stopped			77

concentrations of these compounds ranging up to 2×10^4 mg ecdysteroid/kg fresh weight of plant. Phytoecdysteroid contents of many higher plant species[3,19] significantly exceed the sensitivity levels of the above insects and would suggest that these phytoecdysteroid levels have the potential to disrupt physiological processes of susceptible insect species. Susceptible insect species would absorb such phytoecdysteroids into the hemolymph in an unregulated manner where these would bind to existing receptors and act to produce abnormal physiological situations during insect growth and development. A small alteration in the fitness of the organism to its environment may be all that is necessary to effect a detrimental effect on the insect population.[79] Insects adapted to exogenous ecdysteroids would presumably conjugate and excrete[80] these exogenous compounds in a fashion to minimize developmental alterations. The structure-function relationship of phytoecdysteroids, their obvious fundamental physiological role in insect development, and the documented sensitivity of numerous insect species to purified phytoecdysteroids provides the basis for a reasonable argument for phytoecdysteroid function as a specific defense by numerous higher plant species against herbivorous insects.

VII. SUMMATION

Plants and insects over the course of time have developed a co-evolutionary arms race for survival. In plants the biosynthesis of ecdysteroids, which have a known site of action in insects, provides a structure-function basis for phytoecdysteroid use as putative regulatory agents to alter insect predation. This putative regulation could be either immediate, halted feeding, or long-term, reduced fecundity or fitness of the insect herbivores. Proof of this hypothesis will ultimately require the availability of variants of a plant species that do not biosynthesize ecdysteroids as well as those that do biosynthesize ecdysteroids. The recent developments in our fundamental understanding of the biochemistry, regulation, and transport of ecdysteroids in plants provides a starting point for the experimental design of the requisite investigations. The physiological distribution and apparent cycling of ecdysteroids within perennial plants would likely limit hypotheses testing to genetically modified annuals. Unfortunately, *Arabidopsis thaliana* does not biosynthesize any detectable nonconjugated phytoecdysteroids (Grebenok and Adler, unpublished). Thus, analysis would likely require a different host species. Several potential sites for

controlled alteration of phytoecdysteroid biosynthesis are becoming more evident as our fundamental knowledge of the ecdysteroid pathway is elucidated.

REFERENCES

1. **Sridhara, S., Nowock, J., and Gilbert, L. I.,** Biochemical endocrinology of insect growth and development, in *Biochemistry and Mode of Action of Hormones*, Part 2, *Int. Rev. Biochem.* Vol. 20, Rickenberg, H. V., Ed., University Park Press, Baltimore, 1978, 133.
2. **Riddiford, L. M.,** Insect endocrinology: actions of hormones at the cellular level, *Annu. Rev. Physiol.*, 42, 1980, 511.
3. **Horn, D. H. S. and Bergamasco, R.,** Chemistry of ecdysteroids, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 7, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 6.
4. **Rees, H. H.,** Biosynthesis of ecdysone, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 7, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 7.
5. **Spindler, K. D.,** Hormonal roles of ecdysteroids in Crustacea, Chelicerata and other Arthropods, in *Ecdysone, From Chemistry to Mode of Action*, Koolman, J., Ed., Thieme Medical Publishers, New York, 1989, chap. 29.
6. **Franke, S. and Kauser, G.,** Occurrence and hormonal role of ecdysteroids in non-arthropods, in *Ecdysone, From Chemistry to Mode of Action*, Koolman, J., Ed., Thieme Medical Publishers, New York, 1989, chap. 30.
7. **Tursch, B., Hootele, C., Kaisen, M., Losman, D., and Karlson, P.,** Chemical studies on marine invertebrates XVI Structure and absolute configuration of lobosterol, a novel polyoxygenated sterol from the Alcyonacean *Lobophytum pauciflorum* (Coelentorata octocorallia), *Steroids*, 27, 137, 1976.
8. **Sturaro, A., Guerriero, A., DeClauser, R., and Pietra, F.,** A new, unexpected marine source of a molting hormone: isolation of ecdysone in large amounts from the zoanthea *Gerardia savaglia*, *Experentia*, 38, 1184, 1982.
9. **Tomaschko, K. H.,** Ecdysteroids from *Pycnogonum litorale* (Arthropoda, Pantopoda) act as chemical defense against *Carcinus maenas* (Crustacea, Decapoda), *J. Chem. Ecol.*, 20, 1445, 1994.
10. **Dreier, S. I. and Towers, G. H. N.,** Activity of ecdysterone in selected plant growth bioassays, *J. Plant Physiol.*, 132, 509, 1988.
11. **Machackova, I., Vagner, M., and Slama, K.,** Comparison between the effects of 20-hydroxyecdysone and phytohormones on growth and development in plants, *Eur. J. Entomol.*, 92, 309, 1995.
12. **Ma, W. C.,** Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input a behavioral, ultrastructural and electrophysiology study, *Mede-Landbouwhogesch. Wageningen Ned.*, 72, 1, 1972.
13. **Jones, C. G. and Firn, R. D.,** The role of phytoecdysteroids in bracken fern, *Pteridium aquilinum* (L.) Kuhn as a defence against phytophagous insect attack, *J. Chem. Ecol.*, 4, 117, 1978.
14. **Robbins, W. E., Kaplanis, J. N., Thompson, M. J., Shortina, T. S., Cohen, C. F., and Joyner, S. C.,** Ecdysones and analogs: effects on development and reproduction of insects, *Science*, 161, 1158, 1968.
15. **Robbins, W. E., Kaplanis, J. N., Thompson, M. J., Shortina, T. J., and Joyner, S. C.,** Ecdysones and synthetic analogs: molting hormone activity and inhibitive effects on insect growth, metamorphosis and reproduction, *Steroids*, 16, 105, 1970.
16. **Riddiford, L. M.,** Hormonal interactions between plants and insects, in *Chemical Ecology*, Sondheimer, E. and Simone, J. B., Eds., Academic Press, New York, 1970, chap. 6.
17. **Singh, P. and Russell, G. B.,** The dietary effects of 20-hydroxyecdysone on the development of the housefly, *J. Insect Physiol.*, 26, 139, 1980.
18. **Singh, P., Russell, G. B., and Fredericksen, S.,** The dietary effects of some ecdysteroids on the development of the housefly, *Entomol. Exp. Appl.*, 32, 7, 1982.
19. **Lafont, R. and Horn, D. H. S.,** Phytoecdysteroids: structures and occurrence, in *Ecdysone, From Chemistry to Mode of Action*, Koolman, J., Ed., Thieme Medical Publishers, New York, 1989, chap. 4.
20. **Grebenok, R. J., Venkatachari, S., and Adler, J. H.,** Biosynthesis of ecdysone and ecdysone phosphates in spinach, *Phytochemistry*, 36, 1399, 1994.
21. **Cronquist, A.,** An Integrated System of Classification of Flowering Plants, Columbia University Press, New York, 1981, 231.
22. **Dinan, L.,** Distribution and levels of phytoecdysteroids within individual plants of species of the Chenopodiaceae, *Eur. J. Entomol.*, 92, 295, 1995.
23. **Dinan, L.,** The association of phytoecdysteroids with flowering in fat hen, *Chenopodium album*, and other members of the Chenopodiaceae, *Experientia*, 48, 305, 1992.
24. **Blackwell, W. H.,** The subfamilies of the Chenopodiaceae, *Taxon*, 26, 395, 1977.
25. **Jizba, J., Herout, V., and Sorm, F.,** Isolation of ecdysterone (crustecdysone) from *Polypodium vulgare* L. rhizomes, *Tetrahedron Lett.*, 18, 1689, 1967.
26. **Jizba, J., Herout, V., and Sorm, F.,** Polypodine B — a novel ecdysone-like substance from plant material, *Tetrahedron Lett.*, 51, 5139, 1967.
27. **Hikino, H., Okuyama, T., Jin, H., and Takemoto, T.,** Screenings of Japanese ferns for phytoecdysones. I, *Chem. Pharm. Bull.*, 21, 2292, 1973.
28. **Yen, K. Y., Yang, L. L., Okuyama, K., Hikino, H., and Takemoto, T.,** Screenings of Formosan ferns for phytoecdysones. I, *Chem. Pharm. Bull.*, 22, 805, 1974.

29. **Ripa, P. V., Martin, E. A., Cocciolone, S. M., and Adler, J. H.,** Fluctuation of phytoecdysteroids in developing shoots of *Taxus cuspidata*, *Phytochemistry*, 29, 425, 1990.
30. **Grebenok, R. J. and Adler, J. H.,** Ecdysteroid distribution during development of spinach, *Phytochemistry*, 30, 2905, 1991.
31. **Imai, S., Toyosato, T., Sakai, M., Sato, Y., Fujioka, S., Murata, E., and Goto, M.,** Isolation of cyasterone and ecdysone from plant materials, *Chem. Pharm. Bull.*, 17, 340, 1969.
32. **Koreeda, M. and Nakavishi, K.,** Ajugalactone, an insect moulting inhibitor as tested by the Chilo dipping method, *J. Am. Chem. Soc.*, 92, 7512, 1970.
33. **Zibareva, L. N. and Sviridova, T. P.,** Dynamics of ecdysterone contents in *Lychnis chalcedonica* L. grown in Siberian botanical garden in Tomsk, *Rastit. Resur.*, 25, 561, 1989.
34. **Varga, E., Szendrei, K., Hajdu, Z., Hornok, L., and Csaki, G.,** Study of the compounds contained in Hungarian grown *Leuzea carthamoides* D.C. (Asteraceae) with special regards to ecdysteroids, *Herba Hung.*, 25, 115, 1986.
35. **Vereskovskii, V. V., Chekalinskaya, I. I., and Pashina, G. V.,** Dynamics of ecdysterone content in *Rhaponticum* Ludw. species, *Rastit. Resur.*, 19, 60, 1983.
36. **Tomos, J., Camps, F., Claveria, E., Coll, J., Mele, E., and Messenguer, J.,** Composition and location of phytoecdysteroids in *Ajuga reptans in vivo* and *in vitro* cultures, *Phytochemistry*, 31, 1585, 1992.
37. **Dinan, L.,** The analysis of phytoecdysteroids in single (preflowering stage) specimens of fat hen, *Chenopodium album*, *Phytochem. Anal.*, 3, 132, 1992.
38. **Dinan, L.,** Phytoecdysteroids in *Kochia scoparia*, *J. Chromatogr.*, 658, 69, 1994.
39. **Nes, W. R. and McKean, M. L.,** *Biochemistry of Steroids and Other Isopentenoids*, University Park Press, Baltimore, 1977, chap. 10.
40. **Boid, R., Rees, H. H., and Goodwin, T. W.,** Studies on insect-moulting hormone biosynthesis biosynthesis of cyasterone in the plant *Cyathula capitata*, *Biochem. Physiol. Pflanzen.*, 168, 27, 1975.
41. **Cook, I. F., Lloyd-Jones, J. G., Rees, H. H., and Goodwin, T. W.,** The stereochemistry of hydrogen elimination from C-7 during biosynthesis of ecdysones in insects and plants, *Biochem. J.*, 136, 135, 1973.
42. **Davies, T. G., Lockley, W. J. S., Boid, R., Rees, H. H., and Goodwin, T. W.,** Mechanism of formation of the A/B cis ring junction of ecdysteroids in *Polypodium vulgare*, *Biochem. J.*, 190, 537, 1980.
43. **Hikino, H., Jin, H., and Takemoto, T.,** Steroids 22: Biosynthesis of inokosterone and ecdysone from cholesterol and mevalonic acid in *Achyranthes*, *Yakugku Zasshi*, 95, 590, 1975.
44. **Lloyd-Jones, J. G., Rees, H. H., and Goodwin, T. W.,** Biosynthesis of ecdysterone from cholesterol in *Taxus baccata*, *Phytochemistry*, 12, 569, 1973.
45. **Lockley, W. J. S., Boid, R., Lloyd-Jones, J. G., Rees, H. H., and Goodwin, T. W.,** Fate of the C-4 hydrogen atoms of cholesterol during its transformation into ecdysones in insects and plants, *J. Chem. Soc. Chem. Commun.*, 346, 1975.
46. **Grebenok, R. J. and Adler, J. H.,** Ecdysteroid biosynthesis during the ontogeny of spinach leaves, *Phytochemistry*, 33, 341, 1993.
47. **Adler, J. H. and Grebenok, R. J.,** Biosynthesis and distribution of insect molting hormones in plants, *Lipids*,30, 257, 1995.
48. **Grebenok, R. J., Ripa, P. V., and Adler, J. H.,** Occurrence and levels of ecdysteroids in spinach, *Lipids*, 26, 666, 1991.
49. **Hikino, H., Jin, H., and Takemoto, T.,** Occurrence of insect-moulting substances ecdysterone and inokosterone in callus tissues of *Achyranthes*, *Chem. Pharm. Bull.*, 19, 438, 1971.
50. **Ravishankar, G. A, and Metha, A. R.,** Control of ecdysone biogenesis in tissue culture of *Trianthema portulacastrum*, *J. Nat. Prod.*, 42, 152, 1971.
51. **Lev, S. V., Zakirova, R. P., Saatov, Z., Gorovits, B., and Abubakirov, N. K.,** Ecdysteroids from *Ajuga turkestanica* tissue and cell culture, *Khim. Prir. Soedin.*, 51, 1990.
52. **Matsumoto, T. and Tanaka, N.,** Production of phytoecdysteroids by hairy root cultures of *Ajuga reptans* var. *atropurpurea*, *Agric. Biol. Chem.*, 55, 1019, 1991.
53. **Tomas, J., Camps, F., Coll, J., Mele, E., and Messeguer, J.,** Phytoecdysteroid production by *Ajuga reptans* tissue culture, *Phytochemistry*, 32, 317, 1993.
54. **Camps, F. and Coll, J.,** Insect allelochemicals from *Ajuga* plants, *Phytochemistry*, 32, 1361, 1993.
55. **Corio-Costet, M. F., Chapuis, L., Mouilett, J. F., and Delbecques, S. P.,** Sterol and ecdysteroid profiles of *Serratula tinctoria* (L.): plant and cell cultures producing steroids, *Insect Biochem. Mol. Biol.*, 23, 175, 1993.
56. **Sridhara, S.,** Macromolecular changes during insect metamorphosis, in *Metamorphosis, A Problem in Developmental Biology*, Gilbert, L. I. and Frieden, E., Eds., Plenum Press, New York, 1981, 177.
57. **Richards, G.,** Insect hormones in development, *Biol. Rev.*, 56, 501, 1981.
58. **O'Connor, J. D.,** Ecdysteroid action at the molecular level, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 8, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 3.
59. **Riddiford, L. M.,** Hormone action at the cellular level, in *Comprehensive Insect Physiology Biochemistry and Pharmacology*, Vol. 8, Kerkut, G. A, and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 2.
60. **Reynolds, S. E.,** Hormonal control of cuticle mechanical properties, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 8, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 10.

61. **Fristrom, J. W., Doctor, J., Fristrom, D. K., Logan, W. R., and Silvert, D. J.,** The formation of the pupal cuticle by Drosophila imaginal discs *in vitro*, *Dev. Biol.*, 91, 337, 1982.
62. **Ferkovich, S. M., Oberlander, H., Leach, C. E., and Van Essen, F.,** Hormonal control of chitin biosynthesis in imaginal disks, in *Invertebrate Systems In Vitro*, Kurstak, E., Maramorosch, K., and Dubendorfer, A., Eds., Elsevier/North-Holland, Amsterdam, 1980, 209–216.
63. **Truman, J. W., Rountree, D. E., Reiss, S. E., and Schwartz, L. M.,** Ecdysteroids regulate the release and action of eclosion hormone in tobacco hornworn, *Manduca sexta*, *J. Insect Physiol.*, 19, 195, 1983.
64. **Oberlander, H.,** The imaginal discs, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 2, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 3.
65. **Doctor, J. S. and Fristrom, J. W.,** Macromolecular changes in imaginal discs during postembryonic development, in *Comprehensive Insect Physiology Biochemistry and Pharmacology*, Vol. 2, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 5.
66. **Hill, R. J., Watt, P., Yund, M. A., and Fristrom, J. W.,** The effects of 20-hydroxyecdysone on synthesis of chromosomal and cytosol proteins in imaginal discs, *Dev. Biol.*, 90, 340, 1982.
67. **Chihara, C. J., Petri, W. H., Fristrom, J. W., and King, D. S.,** The assay of ecdysones and juvenile hormones on *Drosophila* imaginal discs *in vivo*, *J. Insect Physiol.*, 18, 115, 1977.
68. **Fristrom, J. W. and Yund, M. A.,** Characteristics of the action of ecdysones on *Drosophila* imaginal discs cultured *in vitro*, in *Invertebrate Tissue Culture Research Applications*, Maramorosch, K., Ed., Academic Press, New York, 1976, 161.
69. **Feyereisen, R.,** Ecdysteroid protein interactions: specificity of the ecdysteroid binding protein from *Locusta migratoria* haemolymph, in *Progress in Ecdysone Research*, Hoffman, J. A., Ed., Elsevier/North Holland, Amsterdam, 1980, 325.
70. **Feyereisen, R.,** 20-Hydroxyecdysone binding protein from locusta hemolymph, *Methods Enzymol.*, 111, 425, 1985.
71. **Schwartz, P. H. and Klassen, W.,** Estimate of losses caused by insects and mites to agricultural crops, in *Handbook of Pest Management in Agriculture*, Vol. 1, Pimentel, D., Ed., CRC Press, Boca Raton, 1981, 15.
72. **Metcalf, C. L. and Flint, R. L.,** *Destructive and Useful Insects: Their Habits and Controls*, McGraw-Hill, New York, 1951, 607.
73. **Kubo, I. and Klocke, J. A.,** Chemical methods for isolating and identifying phytochemicals biologically active in insects, in *Insect-Plant Interactions*, Miller, J. B. and Miller, T. A., Eds., Springer Verlag, New York, 1986, chap. 8.
74. **Kubo, I. and Klocke, J. A.,** Isolation of phytoecdysones as insect ecdysis inhibitors and feeding deterrents, in *Plant Resistance to Insects*, Hedin, P. A., Ed., American Chemical Society, Washington, D.C., 1983, 329.
75. **Ahmad, S. and Khan, M. A.,** Effect of beta-ecdysone ingestion on 5th and 6th instar larvae of the armyworm, *Spodoptera litura* (Fabr.) (Lepidoptera: Nocturidae), *Proc. Indian Natl. Sci. Acad.*, B48, 481, 1982.
76. **Smart, P., Armati, P., Pirie, C., and Gilmour, D.,** Chemosterilization of the Queensland fruit fly, *Dacus tryoni*, *Aust. J. Agric. Res.*, 27, 85, 1976.
77. **Walker, W. F. and Thompson, M. L.,** 22,25-B is deoxyecdysone. Pathological effects on the Mexican bean beetle and synergism with juvenile hormone compounds, *J. Econ. Entomol.*, 66, 64, 1973.
78. **Kubo, I. and Klocke, J.,** Insect ecdysis inhibitors, in *Natural Resistance of Plants to Pests*, Green, M. B. and Hedin, P. A., Eds., American Chemical Society, Washington, D.C., 1986, 206.
79. **Harborne, J. B.,** *Introduction to Ecological Biochemistry*, 3rd ed., Academic Press, London, 1988, chap. 4.
80. **Koolman, J. and Karlson, P.,** Regulation of ecdysteroid titer: degradation, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 7, Kerkut, G. A. and Gilbert, L. I., Eds., Pergamon Press, Oxford, 1985, chap. 9.