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Review Article
Role of Phytoecdysteroids in Insect Pest Management: A Review

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Abstract
Phytoecdysteroids are analogues of insect moulting steroid hormone, ecdysteroid. These are found in different plant groups and provide protection by deterring insects. Phytoecdysteroids have been synthesized in at least 27 families of *Pteridophyta*, 10 families of *Gymnospermae* and 74 families of *Angiospermae*. Chemically, phytoecdysteroids are triterpenoids, the group of compounds that includes triterpene saponins, phytosterols and phytoecdysteroids. These are polar steroids with sugar-like solubility properties. Over 300 phytoecdysteroids analogues have been identified so far and it has been speculated that there are over 1,000 possible structures in nature. These are synthesized from mevalonic acid and cholesterol. Phytoecdysteroids occur in relatively high concentration in many plants and comprise 0.001-3% of the dry weight. These have been isolated from all parts of plants in much higher amounts than those present in insects. Thus, plants are far better sources of ecdysteroids than insects. Different plant parts contain different amounts of ecdysteroids and that ecdysteroid concentration varies with season and geographical distribution of the plant. Ecdysteroids control insect development at all stages of the life cycle. Disruption of normal ecdysteroid level severely impairs insect development. Phytoecdysteroids mimic the insect ecdysteroid by binding to its receptors and eliciting a cascade of effects in insects. Phytoecdysteroids provide protection to plants by altering the normal levels of ecdysteroid hormone in adults and larvae in insects. Therefore, phytoecdysteroids can be an excellent replacement of synthetic insecticides in insect pest management programme.

Key words: Ecdysone, phytoecdysteroides, triterpenoids, moulting hormone, genetic manipulation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

With the beginning of agricultural practices and storage of food grains, insects started damaging standing crops and grains in storage both qualitatively and quantitatively. Several synthetic pesticides have been developed and used to protect crops and grains from insect infestation. The continuous and extensive application of synthetic pesticides has developed the risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenicity in non-target animal species and cross- and multi-resistance in insects[1-4]. These adverse effects have increased public awareness regarding human safety and possible environmental damage diverting attention towards other alternatives especially the use of plant products in insect pest management.

Plant botanicals include plant extracts. These are secondary plant metabolites synthesized by the plant for protective purposes. Some of these compounds are toxic to insects. These plant derived chemicals are called botanical pesticides. These target a broad range of insect pests. Plant insecticides act as toxicants[5-8], as repellents[9-11], as antifeedants[12-15], as oviposition deterrents[9-11,13] and developmental inhibitory activities[9-11,14-17]. Botanical insecticides do not affect the natural enemies of the pests adversely. Botanical pesticides are good alternatives to chemical pesticides as these are cheap, eco-friendly, cost-effective, target-specific and biodegradable. This study work synergistically and it is difficult for insect pests to develop resistance.

Ecdysteroid, insect moulting hormone, was first isolated from *Bombyx mori*[18]. This hormone governs all the aspects of transformation from larva to pupa to adult. A number of ecdysteroid anologues called phytoecdysteroids have been reported from plants. Phytoecdysteroids are a class of chemicals that plants synthesize for defense against phytophagous insects. Insects that ingest phytoecdysteroids and have not adapted to these defense molecules are subject to serious adverse effects, including weight loss, molting disruption and/or mortality[19-21]. Ecdysteroids control insect development at all stages of the life cycle and disruption of normal ecdysteroid level or its action can be expected to impair insect development severely by mimicking the function of insect ecdysteroid[22]. After the discovery of 20-Hydroxyecdysone, active form of ecdysone in the conifer, *Podocarpus nakaii*[23], ecdysteroids have been detected in 27 families of the *Pteridophyta*, 10 families of *Gymnospermae* and 74 families of *Angiospermae*[24]. Since phyotecdysteroids are found in primitive plants such as ferns, it was assumed that these secondary compounds appeared very early in plant evolution. In the angiosperms, 74 families possess ecdysteroid containing species. A majority of these families are within eleven orders: *Liliales*, *Ranunculales*, *Urticales*, *Malvales*, *Violales*, *Capparales*, *Rosales*, *Sapindales*, *Polemoniales* and *Scrophulariales*.

Phytoecdysteroids occur in relatively high concentration in many plants and comprise 0.001-3% of the dry weight and have been isolated from all parts of plants in much higher amounts than those present in arthropods[19,20]. They are far better source of ecdysteroids than arthropods[25]. It is important to note that different plant parts contain different amounts of ecdysteroids and that ecdysteroid concentration varies with season and geographical distribution of the plant[26]. Presence of the moulting hormone analogues in plants is suggestive of their role as allelochemicals, protecting them against insect predation by acting as antifeedants and/or disrupting the endogenous endocrine level of insects[27]. These phytoecdysteroids provide little protection against specialized herbivores, which have evolved efficient detoxification mechanisms but represent a consistent barrier against non-adapted herbivores. Low concentrations (2-25 ppm) of phytoecdysteroids deter some insects, while other insects are resistant to very high concentrations (400-1000 ppm).

The role of phyotecdysteroids in plants is not clearly understood, as there are conflicting data supporting ecdysteroids as plant hormones (i.e., signaling molecules produced in plants) which travel to targeted locations, regulating specific cellular responses. Although initial reports show that ecdysteroids do not elicit any of the classical responses of known plant hormones, there are also data supporting ecdysteroids being physiologically active in plants[28]. Phytoecdysteroids may play a role in plant defense. Production of 20-Hydroxyecdysteroids in spinach is elicited by both mechanical wounding and insect feeding. Phytoecdysteroids protect spinach from plant-parasitic nematodes and may confer a mechanism for nematode resistance[29]. Although not conclusive, ecdysteroid's role in plants may be similar to the defensive role of lignins, whose production increases in response to pathogens. Application of these phytoecdysteroids on insects has been reported to result in marked growth and developmental disruption[30-34]. In this review, sources, structures and applications of phytoecdysteroids have been discussed. Phytoecdysteroids provide protection to plants by altering the normal levels of ecdysteroid hormone in adults and larvae of insects. Therefore, phytoecdysteroids can be applied against insects causing economic injury and can be proved an excellent replacement of synthetic insecticides in insect pest management programme.

CHEMISTRY OF PHYTOECDYSTEROIDS

Chemically, phytoecdysteroids are triterpenoids, a group of compounds that includes triterpene saponins, phytosterols and phytoecdysteroids. Ecdysteroids are polar steroids with sugar-like solubility properties[33]. Animals and humans also synthesize some of the same products from the mevalonate pathway, such as ubiquinone and sterols. They can not, however, synthesize ecdysteroids. Phytoecdysteroids universally lack the polyhydroxylated side chain characteristic of ecdysteroids and are, therefore, more lipophilic[33]. Unlike invertebrates, which are unable to synthesize ecdysteroids and must consume dietary phytosterols that are then converted into ecdysteroids, plants can synthesize ecdysteroids from mevalonic acid and cholesterol[35]. Plants synthesize phytoecdysteroids from mevalonic acid in the mevalonate pathway in cell using acetyl-CoA as a precursor.

Most phytoecdysteroids possess a (cyclopentano-perhydrophenanthrene carbon skeleton) cholest-7-en-6-one carbon skeleton (C27), derived biosynthetically from cholesterol and phytosterols, often with a hydroxyl group in the 14-position[36]. The carbon number can vary between C19-C29[35]. The anellations of the rings are characteristic: The C/D ring junctions are generally trans, while the A/B ring junction is generally cis (5β-H) and only rarely trans (5α-H). Essential structural elements of phytoecdysteroids are a 7-en-6-one chromophore in ring B and a β-side chain at C-17. Phytoecdysteroids are highly hydroxylated with 2-8 hydroxyl groups. The common hydroxylated sites are 2β, 3β, 14α, 20R, 22R and 25th position which together give rise to the highly biologically active ponasterone A (25-Deoxy-20-hydroxyecdysone)[19,20]. The structural variations among different phytoecdysteroids include number, position and orientation of hydroxyl groups. Ecdysteroids hydroxylated at C-9, C-12, C-17, C-19, C-21, C-24, C-28 and C-29 occur only in plants. Further possibilities are additional/alternative double bond at one of locations of 4-5, 8-9, 9-11, 12-13, 14-15, 24-25, 25-26 or 24-28. Second oxogroup can occur in one of the positions of C-2, C-3, C-12, C-17, C-20 or C-22 (Fig. 1). Among the natural steroids the cardenolides, bufadienolides, sterines and bile acids have A/B cis anellation. Further common characteristic is the C-11-hydroxylation (in addition to C-1-, C-3-, C-5- and C-14-hydroxylations), which is usually frequent at ecdysteroids as well as for cardenolides and bufadienolides.

Although the most common ecdysteroid found in plants is 20-Hydroxyecdysone, over 300 ecdysteroid analogues have been identified so far in plant kingdom and it has been speculated that there are over 1,000 possible structures in

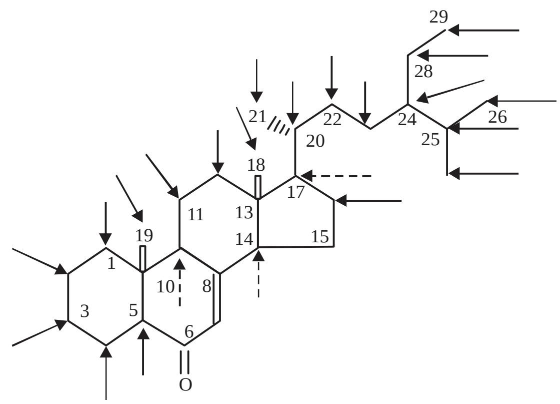


Fig. 1: General structure of ecdysteroid (heavy line makes the position of hydroxyl group in β-orientation, solid arrows show position of hydroxyl group in α or β-orientation and dotted arrows show position of hydroxyl group in α-orientation. Numbers show carbon number in the ecdysteroid skeleton

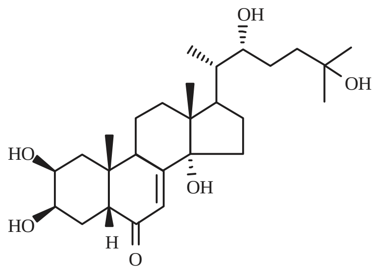
nature[19,20] (Fig. 2). The various analogues differ in the number and site of hydroxylations, as well as the length and structure of the carbon side chain. Glycosylated and acetylated ecdysteroids have been described both in nature and in the laboratory[37]. Many more plants have the ability to 'turn on' the production of phytoecdysteroids when under stress, animal attack or other conditions[19].

The term phytoecdysteroid can also apply to ecdysteroids found in fungi, even though fungi are not plants. Fungi that produce phytoecdysteroids include *Achyranthes bidentata*[38], *Tinospora cordifolia*[39], *Pfaffia paniculata*[40], *Leuzea carthamoides*[41], *Rhaponticum uniflorum*[42], *Serratula coronate*[43], *Cordyceps* and *Asparagus*[20].

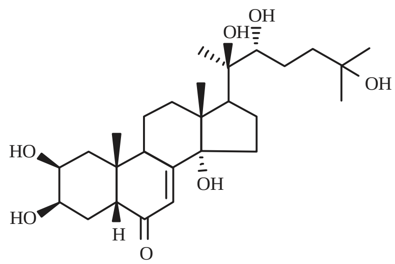
During discovery for anti-cancer compounds in plant extracts, Nakanishi *et al.*[23] isolated ponasterone A, a C27 polyhydroxy steroid closely related to the insect moulting hormone, from the plant *Podocarpus nakaii*. Soon after the isolation of ponasterone A, 20-Hydroxyecdysone, the major ecdysteroid of arthropods was reported in a conifer, *Podocarpus elatus*[44], *Polypodium vulgare* (fern)[45] and the angiosperm, *Achyranthes fauriei*[46]. Later, both ecdysone and 20-Hydroxyecdysone were isolated from the ferns, *Pteridium aquilinum*[47] and *Polypodium vulgare*[48].

PLANT FAMILIES PRODUCING ECDYSTEROIDS

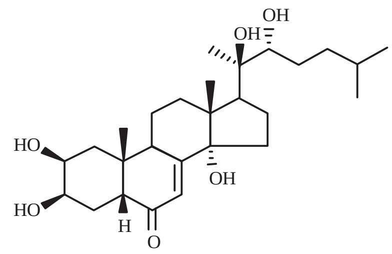
Approximately 6% of all plant species synthesize phytoecdysteroids[19-20] (Table 1). Among plant species, highest concentration of phytoecdysteroid is found in spinach, *Spinacia oleracea* (Chenopodiaceae) containing 50 μg g^{-1}



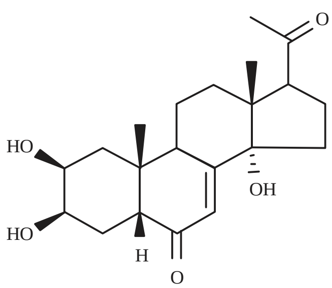
Ecdysone



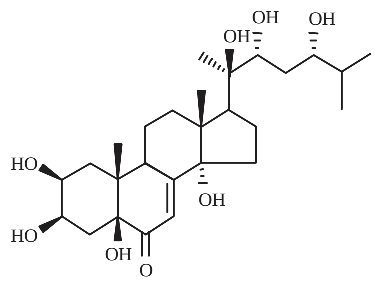
20-Hydroxyecdysone



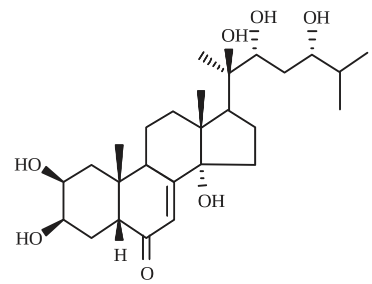
Ponasterone A



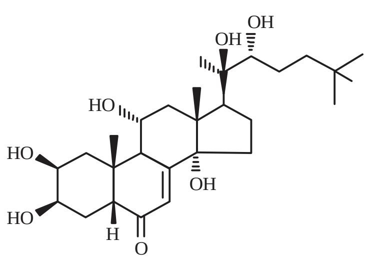
Poststerone



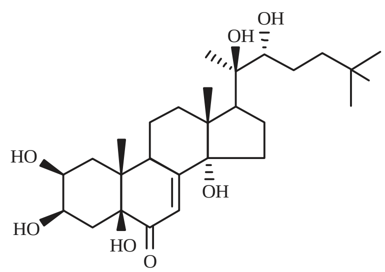
Poststerone C



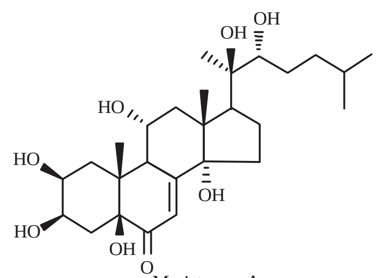
Pterosterone



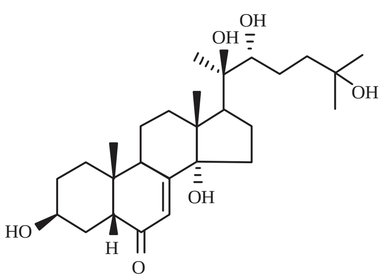
Ajugasterone C



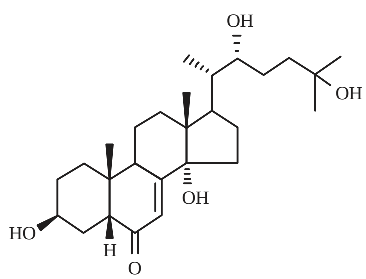
Polypodine B



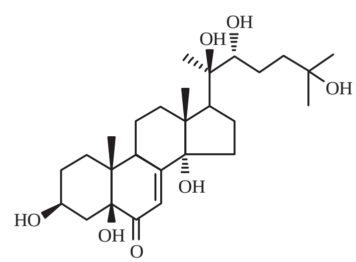
Muristerone A



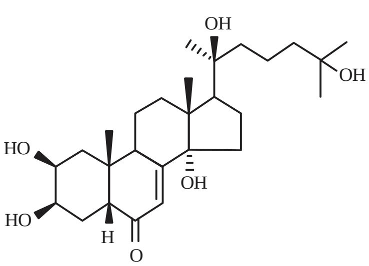
2-Deoxy-20-hydroxyecdysterone



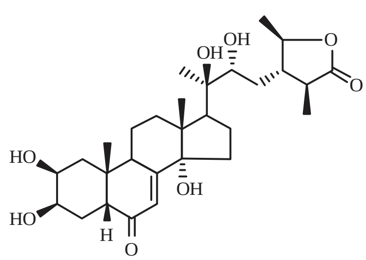
2-Deoxyecdysone



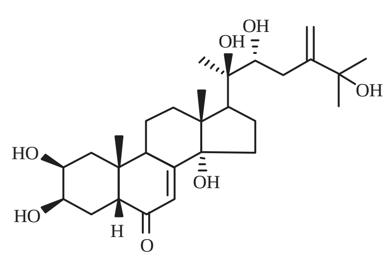
2-Deoxy polypodine



22-Deoxy-20-hydroxyecdysone



Cyasterone



24(28)-Dehydro makisterone A

Fig. 2: Continue

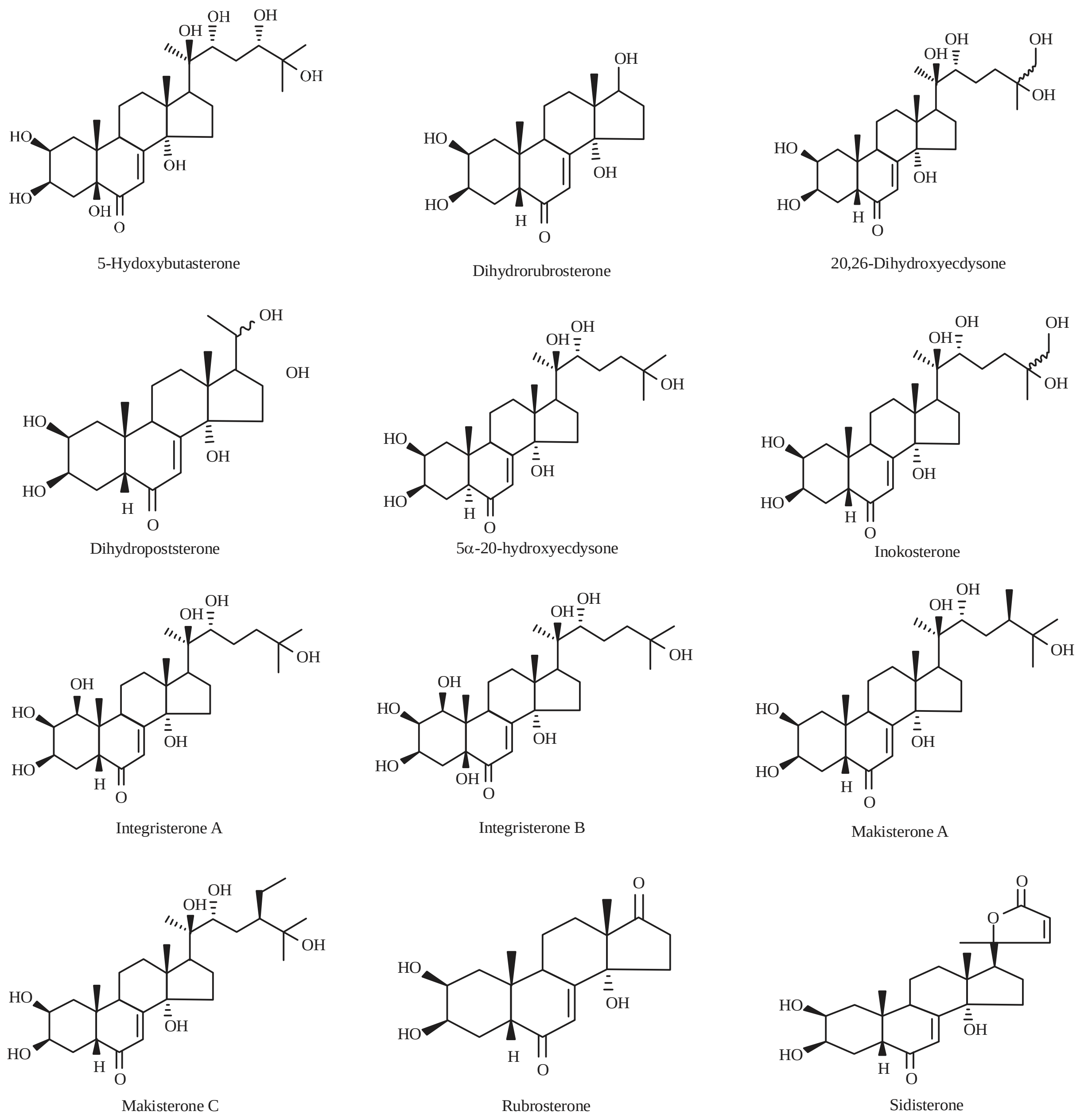


Fig. 2: Diversity of phytoecdysteroid structures

fresh weight[49]. Phytoecdysteroids obtained from plants are ajugasterone in *Ajuga* (Lamiaceae) and *Vitex* (Verbenaceae); leuzeasterone in *Leuzea carthamoides* (Asteraceae), 2-Deoxy-20-hydroxyecdysone 3-Glucoside and 3-Epi-2-deoxy-20-hydroxyecdysone in *Tinospora cordifolia* (Menispermaceae)[39], ecdysterone and inokosterone in *Achyranthes bidentata* (Amaranthaceae), polypodine B in *Leuzea carthamoides* (Asteraceae)[41] and ecdysterone, ajugasterone C, ajugasterone C-20, 22-monoacetonide in *Rhaponticum uniflorum* (Asteraceae)[42]. To date, 300 different phytoecdysteroids have been identified[50]. Phytoecdysteroids differ in the number of C-atoms present (24C-29C), the number, position and location of hydroxyl and keto groups on the steroid skeleton. The most commonly occurring phytoecdysteroid is 20-Hydroxyecdysone, followed by polypodine B. Typically, a phytoecdysteroid-containing species will contain 1-3 major ecdysteroids, which together make up 95% of the total ecdysteroid, together with plenty of minor ecdysteroids, forming a diverse cocktail of ecdysteroid structural analogues. Although most of the plant families have at least some species accumulate ecdysteroids, less than 2% of the world's flora has been investigated for the presence of ecdysteroids[19,51].

Table 1: Plant families and species containing phytoecdysteroids

Plant species	Family
Achyranthes aspera	Amaranthaceae[38]
Achyranthes bidentata	Amaranthaceae[38]
Ajuga turkestanica	Lamiaceae[71]
Cassia tora	Caesalpiniaceae[72]
Cyanotis somaliensis	Commelinaceae[50]
Cyathula achyranthoides	Amaranthaceae[50]
Cyathula officinalis	Amaranthaceae[50]
Dianthus deltoides	Caryophyllaceae[73]
Leuzea carthamoides	Asteraceae[73]
Melandrium firmum	Caryophyllaceae[74]
Melandrium sachalinense	Caryophyllaceae[74]
Pfaffia iresinoides	Amaranthaceae[75]
Polypodium aurea	Polypodiaceae[75]
Polypodium glycyrrhiza	Polypodiaceae[50]
Polypodium vulgare	Polypodiaceae[50]
Rhaponticum uniflorum	Asteraceae[42]
Saponaria bellidifolia	Caryophyllaceae[73]
Serratula coronata	Asteraceae[76]
Spinacia oleracea	Chenopodiaceae[49]
Tinospora cordifolia	Menispermaceae[39]

MECHANISM OF ACTION OF ECDYSTEROIDS

Hydroxyecdysone is a naturally occurring ecdysteroid which controls the ecdysis and metamorphosis in insects. Ecdysteroid is derived from enzymatic modification of cholesterol by p450 enzymes. The primary sources of 20-Hydroxyecdysone in larvae are the prothoracic gland, ring gland, gut and fat bodies in larvae. These tissues convert dietary cholesterol into 20-Hydroxyecdysone[52]. In the adult female, ovary is a source of 20-Hydroxyecdysone production[53]. In adult males, fat body is the source of hydroxyecdysone. Its anologues called phytoecdysteroids are produced by various plants where these disrupt the development and reproduction of insect pests. This hormone is hydrophobic in nature, thus, it traverses lipid membranes and permeates the tissues of an organism. In insects, 20-Hydroxyecdysone acts through the ecdysone receptor. The ecdysone receptor is a nuclear receptor (a ligand-activated transcription factor), which controls development and contributes to other processes such as reproduction[54,55]. The ecdysone receptor is a non-covalent heterodimer of two proteins viz., the EcR protein and ultraspiracle protein (USP). The ecdysteroid-binding pocket is located in the EcR subunit, but EcR must be dimerised with a USP for high-affinity ligand binding.The binding of ecdysone to receptor leads to the activation of ecdysone responsive genes and many other genes causing puffing of polytene chromosomes at over a hundred sites. Sites of puffing represent the position of genes to be extressed and thus, position of transcription. The DNA-binding domains of EcR and USP recognise specific short sequences in DNA and mediate the binding of the heterodimer to ecdysone response elements (ECREs) in the promoters of ecdysone-responsive genes. The first gene to be activated are called early response genes. This is a code for proteins that are themselves gene regulatory factors either alone or in combination with hormones. They activate late-response genes that code for the proteins that actually cause structural changes, cell differentiation or apoptosis (programmed cell death). Ultimately, the activation cascade causes physiological changes that result in ecdysis (moulting)[56]. The ecdysone receptor also binds to and is activated by phytoecdysteroids. Thus, phytoecdysteroids can mimic 20-Hydroxyecdysteroid of insects, bind insect ecdysone receptors and can elicit the same responses. These phytoecdysteroids induced responses at inappropriate time and stage causes feeding detterency, abnormal development, altered pattern of ecdysis and finally death.

PHYTOECDYSTEROIDS AS INSECT GROWTH REGULATORS

Ecdysteroides inhibit the feeding in *Pieris brassicae* and *Mamestra brassicae* larvae when given at 200 mg kg^{-1} fresh weight in sucrose solution [57] and inhibit drinking in *Dysdercus koenigii*, *D. fulvoniger* and *Spiloslethus pandrus* adult at 100 mg kg^{-1} concentration[58]. Jones and Firn[59] reported that ecdysone and 20-Hydroxyecdysone deter feeding in *P. brassicae* when incorporated above 5 mg kg^{-1} diet. *Chilo partellus*, *Phyllobius pyri* and *P. argentatus* were deterred from feeding at concentration of 20-Hydroxyecdysone above 50-70 mg kg^{-1}, whereas, *Schistocerca gregaria*, *Hyponomeuta euonymella* and *Spodoptera littoralis* were not deterred at this concentration[59]. Hydroxyecdysone and polypodine B regulate the length of feeding periods in larval instars and silk production when added to the diet of *B. mori*[60]. Methanolic extract of *Ajuga remota* leaves and roots containing cyasterone and ecdysterone disrupt the moulting cycle in *B. mori*, *Peclinophora gossypiella* and *Spodoptera frugiperda*[61].

Ingested 20-Hydroxyecdysone causes death without moulting, death following completion of promoted moulting or death during moulting in larvae of *B. mori*. Comparison of hydroxyecdysone with other phytoecdysteroids in *B. mori* and *P. gossypiella* shows ponasterone A as more potent effect[62]. Effective ecdysis inhibition has been observed in *P. gossypiella* with ponasterone A[63]. Phytoecdysteroids like ecdysterone, polypodine B and ponasterone A when applied in 25-250 ppm in the diet induce ecdysial failure associated with the appearance of larvae having two head capsules and developmental anomalies during metamorphosis in larvae of *Acrolepiopsis assectella*[30]. Slama *et al.*[64] have reported hormonal activity of ecdysteroids in the larvae of *Galleria mellonella*, *Dermestes* sp. and *Sarcophaga bullata* and found cyasterone as the most effective lepidopteran ecdysteroid and turkesterone as the dipteran and coleopteran specific ecdysteroid[64]. Kubo[65] conducted artificial diet feeding assay with the green bug, *Schizaphis graminum* and showed ajugasterone C to have 10 fold more potency in feeding deterrancy than 20-Hydroxyecdysone and 30 fold more potency than cyasterone[65].

Ecdysone in artificial diets of *B. mori* induces ultranumerary larval ecdyses[32]. Tanaka[66] has reported altered sensitivity of epidermis to 20-Hydroxyecdysone at 300 ppm of ecdysone. Only a very limited number of insect species have been examined for their susceptibility to dietary ecdysteroids. Most of the species tested belong to order *Lepidoptera* which includes phytophagous pests. Among the tested lepidopteran species some are highly susceptible to dietary 20-Hydroxyecdysone such as *A. assectella*, *Aglaisurticae, B. mori*, C. *partellus*, *Inachisio* and *P. gossypiella*, some are semi-tolerant like *Cynthia cardui* and *Tyria jacobaeae* while others such as *A. convolvuli*, *Helicoverpa* spp., *M. brassicae*, *Manduca sexta* and *S. littoralis* are highly resistant[67]. The specific effects of ecdysteroids and their low toxicity formed the basis of application of phytoecdysteroids as safe insecticides[68]. However, the phytoecdysteroids have limited application in the control of insect pest because of their environmental instability[69].

Ecdysteroids are compounds with low mammalian toxicity. The LD_{50} values in mice are 6.4 and >9 g kg^{-1}, using intraperitoneal and oral administration of 20-Hydroxyecdysone, respectively. The 0.1 g kg^{-1} dose of intravenous administration of 20-Hydroxyecdysone to rabbits has not been followed with any toxic reaction and sub-acute treatment of rats with 2 g kg^{-1} day^{-1} treatment neither gave toxic symptoms[70].

CONCLUSION

Phytoecdysteroids are toxic compounds that defend plants against phytophagus insects. These can be used as insecticidal tool in pest management programme, as these are eco-friendly, economic, target-specific and biodegradable.

SIGNIFICANCE STATEMENTS

Phytoecdysteroids are plant derived steroid hormones analogues to insect moulting hormone. These deter predation by non-adapted insect predators. This property of phytoecdysteroids can be exploited to develop new strategies for insect pest control. These plant derived ecdysteroids can be used as lead molecules for developing insect controlling agents. New varieties of plants of economic interest can be developed with altered ecdysteroid levels/profiles through plant breeding or genetic manipulation.

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