ISSN:2321-1156
International Journal of Innovative Research in Technology and Science

# SAPONINS IN FOOD, FEEDSTUFFS AND MEDICINAL PLANTS

Sanjeev Urmaliya, Research Scholar, S. S. L. Jain College, Vidisha, M.P., India1
Dr S. P. Sharma, Professor, S. S. L. Jain College, Vidisha, M.P., India2

## Introduction

Herbivorous insects exploit the main primary metabolites of plants as nutrients and many further chemical constituents that have a rich structural and quantitative variability. These constituents, characterised most often as secondary metabolites, possess various biological activities and functions. Insects frequently utilise such compounds to their benefit, i.e., as kairomones. However, in an ecological sense, plants defend themselves again with a wide variety of secondary metabolites that negatively affect in- sects, i.e., allomones. These positively or negatively acting substances are often specified concerning functions or species and classified in many ways. One example is shown in the following table:

Table 1:Bio-active plant substances with effects on insects

| Group name | Types of activity |
|---|---|
| Kairomones (With appositive effect) | attractants, oviposition stimulants, feeding stimulants, essential nutrients, specific nutrients, hormone analogues, pheromone components or precursors |
| All omones (With negative effect) | repellents, deterrents of reproduction, anti- feed ants, attack inhibitors, antihormonal substances, toxins or insecticides |

The scale of specific chemical interactions between plants and insects is pervasive and complex, as complex and diverse as their co-evolution. Insects respond to many plants' chemical components, inducing various changes in behaviour, growth, development, and reproduction. These represent a great variety of biological ef- facts (Table 2), but the variety of chemical structures responsible for these effects is even more incredible. Regard- ing the chemical structures, nearly all types of low molecular weight natural substances can be found as interaction regulators, e.g., aliphatic substances, terpenoids, steroids, alkaloids, lignans and related phenylpropanoids, or phenolics, as well as some derivatives of these compounds: most frequently simple ethers, esters, glycosides or saponins. These derivatives or other more complicated conjugates can act directly or be activated on release.

**A. Behaviour regulators (within formation effect)**

1. Kairomones–attractants, arrests, stimulants
2. Allomones–repellents, deterrents, antifeedants
3. Plant components and precursors of pheromones

**B. Growth, development and reproduction regulators (with physiological effect)**

1. Juvenoids – plant analogues of juvenile insect hormone
2. Phytoecdysones–plant analogues of insect moulting hormone
3. Antihormonal substances and chemosterilants
4. Plant toxins and insecticides

The incredible complexity of chemical structures requires the application of an extensive range of separation techniques, analytic procedures, methods of structural analysis, chemical transformations and synthetic preparations of analogues. These are needed to elucidate structure-activity relations and modes of action, solve synergy problems, and differentiate the active compounds from their artefacts. Our research interest in this field mainly concerns areas of plant-derived insect feeding deterrents and ecdysone analogues or ecdysis inhibitors of plant origin. Phytoecdysones, as well as any moulting modifiers or antihormonal active plant substances, occupy an important place in the wide variety of compounds effective in plant-insect interaction (Table 2). Phytoecdysones are specific steroid analogues of the moulting hormone, Ecdysone, with relatively limited structural characteristics (see Fig.1), but with a considerably widespread occurrence in a large number of taxonomically unrelated plants. Many of the phytoanalogues of Ecdysone perform the same hormonal activity in insects as the moulting hormone itself, but this is not the case for their acylic or glycosidic conjugates. Several ecdysteroid derivatives and conjugates whose biological activity remains unknown have been found in plants. Although only concrete ecdysteroid structures are hormonally active, there are various natural or synthetic agonists and antagonists, which are effective directly on the binding site of the ecdysone receptor. These and several other indications stimulate a long-lasting discussion concerning the ecological significance of phytoecdysteroids and ecdysis modifiers in plant-insect chemical interactions. One of the questions is why the concentration of ecdysteroids in plants is much higher than the physiologically active hormonal dose or even higher than the effective dietary concentration in oral assays. At the same time, their polar character pre- vents them from being active topically, as they cannot penetrate the insect cuticle. It is also the case with the less polar acyl conjugates. Our attention has also been ex- tended to the ecdysteroid glycosides, which often occur in plants, but not in insects. Two ways of obtaining such glycosides led us to two different results. The synthetic approach yielded a series of required 20-hydroxyecdysone (=ecdysterone) glycosides, and the phytochemical approach led us to identify steroid saponins as active sub-stances with a new and unique mode of action in plant-insect chemical interactions.

**Active Substances in the Leek – Leek Moth Chemical Interaction**

Our interest in searching for ecdysis influencing compounds, especially those active in feeding assays, brought us to a collaboration with French entomologists specialising in the leek-moth biological and chemical interactions Acrolepiopsisassectella with its host plant: the leek Allium porrum. They analysed sulphur-containing chemical sub-stances with kairomone properties, typical for Allium plants that stimulated insect behaviour, growth, or egg-laying. On the other hand, they found that leek flowers contain allelopathic substances with a toxic or development restraining effect, indicating ecdysis-disturbing properties. Our participation in this research involved phytochemical analysis and elucidation of the chemical basis of the mode of action.

**Identification of the Spirostanol Saponin Aginosid as the Main Leek Active Constituent**

At the beginning of the phytochemical analysis, it was already known that the leek moth Acrolepiopsisassectellais an insect herbivore specialist, is living on Allium plants and is the only Lepidoptera attacking leek Allium porrum. It is leek's most specialised insect consumer and strictly depends on its development and reproduction. It can damage crops sufficiently to cause economic problems as an actual pest insect. However, it avoids feeding on leek flowers. Experimentally was proven that it suffers from digestive dysfunction, intoxication and inhibition of ecdysis when fed on a semisynthetic diet containing flowers or flower extracts. Isolation of the active compound was performed by bio-activity controlled and guided extractions and fractionations. The dried flowers were successively extracted with ethyl acetate (no activity), methanol (low activity), and with a methanol-water (1:1) mixture. After evaporating a significant part of methanol, the last extract was extracted again by n-butanol (the active fraction). Further fractionation was performed by column chromatography on silicagel eluted with chloroform-methanol- water in a gradient mode (6:1:0 – 14:6:1). Final purification was carried out by repeated crystallisation checked by RP-HPLC eluting with methanol-water (7:3).

The structural elucidation was performed by a combination of spectroscopic and chemical methods. FAB-MS data revealed molecular weight 1066 ([M+1]+ 1067). Further fragmentation indicated the elimination of three hexoses and one pentose: 1067– (3 x 162) – 132, and the aglycone: m/z 448. The glycosidic nature of the compound was confirmed by hydrolysis, which yielded the aglycone with [M]+. 448 and with elemental composition$C_{27}H_{44}O_5$. Carbohydrate components of the acid hydrolysate were analysed by HPLC and determined as glucose, galactose, and the leek-moth larvae were similar to that produced by leek flowers.

The same effect was observed with digitonin after isolating a sufficient amount of aginosid for bioassays. The lowest mortality and the fastest development of leek-moth larvae occurred with the dried leaves. The mortality was significantly enhanced with the dried flowers or flower extract. In the latter case, the mortality and the ecdysial failures changed dose-dependent. However, the addition of cholesterol or □-sitosterol to the diet during the test obviated the effect of both the dried flowers and aginosid. The obtained results corresponded with the reviewed data [59] on activities and the role of saponins in the resistance of plants to insects and initiated us to pro- pose a mechanism of action.

**Mode of action of saponins on insects**

The formation of an insoluble complex of sterols with digitonin is well known, and it has been used for a long time for the precipitation of sterols. Cholesterol forms such a complex not only with digitonin but also with several other structurally related saponins. Cholesterol or dealkylated phytosterols are precursors in the insect moulting hormone ecdysone biosynthesis. The only source of these sterols for most insect species is their food. If all cholesterol in the food is bound to saponins, the in- sects cannot utilise it.

Moreover, if larvae feed on a saponin-rich food, the ingested saponins may complex even cholesterol in their body, and thus suspend the biosynthesis of Ecdysone and cause disturbances in ecdysis. In the test, inhibition in moult and characteristic malformations in the larvae development leading to their deaths were diagnosed. It was observed when digitonin or aginosid were added to the standard diet. When a surplus of cholesterol or □-sitosterol was added to the digitonin or aginosid into the test food, the mortality or ecdysial failures decreased to a minimum. The antagonistic effect of these simple sterols in the test demonstrates that the mechanism of aginosid, digitonin and other saponins' activity is based on forming an insoluble sterol-saponin complex, which can suspend the normal process of biosynthesis until an excess number of sterols re-starts it again to exclude other possible considerations, digitonin and aginosid were also tested for their eventual direct effect on the ecdysone receptor, in the B-II assay.

**Antifeeding Activity of Steroid Saponins**

Insect feeding deterrent activity of spirostanol and other related steroid saponins has been observed only in a few cases. For the activity, spirostanesaponins with structures similar to the Allium saponins were responsible but isolated from unrelated plants of Balanitesand Agave species. We have also tested the antifeeding activity of aginosid in a storage insects pest assay. 17 as a part of our screening and search for finding leads in naturally occurring or chemically modified compounds exploitable for a storage insects' control. When comparing results from this test with structurally unrelated cardiotonic glycosides and saponins, the activity of aginosid was low and insignificant (Nawrot and Harmatha, unpublished results). How- ever, the intense deterrent activity of aginosid was found in larvae of the polyphagous lepidopteraPeridromasaucia. Growth inhibition during the feeding test was also observed. The same effect was also found after a topical application on the fourth instar larvae of P. saucia. Certain spirostane glycosides showed larval growth inhibition on a cotton pest: the spiny bollworm Eariasinsulana.

**Conclusions**

Spirostanesaponins serve to protect leek flowers from the herbivory attack of the most specialised insect consumer leek-moth larvae. The mechanism of their activity has been elucidated and depends on the general feature of specific saponins to form insoluble complexes with sterols, in this case with cholesterol, the precursor of biosynthesis of the moulting hormone ecdysone. This type of activity is non-specific and can occur wherever the conditions are suitable for such a mechanism. Appropriate conditions are in plants or their single organs containing many saponins, sufficient to bind a critical number of basic sterols, essential for insect development. In the case of leek, the condition is fulfilled in flowers, which seem to be protected by allelopathic amounts of saponins. It demonstrates an excellent strategy to avoid damage in those parts of plants that cannot regenerate and are essential for reproduction. Higher content of saponins in the flowers or fruits of Allium plants than in the leaves or bulbs was observed quite frequently. Similar observations also came from other plant families containing different saponins, e.g., triterpene saponins. The content and distribution of spirostanesaponins in Allium species exhibit re- markable chemotaxonomic similarities, and the above-discussed defence mechanism seems to be more generally efficient. Saponin - cholesterol complex formation is not restricted only to spirostanolsaponins, so the exact mechanism could be even more common in nature.

The ecdysis disturbing effect of saponins substitutes the general absence of phytoecdysteroids in Allium plants. The dietary effects of phytoecdysones tested in the same feeding assay on the leek-moth larvae were similar to the previously observed effect of saponins but with a different mode of action. The direct effects of our leek flower spirostanesaponins and some selected spirostanols, including the aglyconeagigenin, were tested in the B-II bioassay. They did not show significant agonistic or antagonistic effects on the ligand-binding site of the modelDrosophila ecdysteroid receptor (Harmatha and Dinan, unpublished results). It is in accord with our explanation of the assumed non-specific mode. The observed effects of saponins and the explained mechanism may be utilised in plant protection strategy. Insect pests could be controlled by making plants' natural defence system more efficient, for example, by breeding varieties of plants with a higher saponin content, especially in their reproductive organs; of course, resistance development or selection pressure to find new food sources cannot be excluded here.

References

[1].Lafont, R. and Horn, D.H.S. (1989) Phytoecdysteroids: structures and occurrence, in J. Koolman (ed.), Ecdysone. From Chemistry to Mode of Action, Georg Thieme Verlag, Stuttgart, pp. 39-64.

[2].Harmatha, J. and Dinan, L. (1997) Biological activity of natural and synthetic ecdysteroids in the B- II Bioassay, Archives Insect Biochem. Physiol. 35, 219-225.

[3].Píš, J., Harmatha, J. and Sláma, K. (1991) Action of some new synthetic derivatives of phytoncide-sones on insects, in J. Hrdý (ed.), Insect Chemical Ecology, Academia, Prague and Acad. Publishing, The Hague, pp. 227-234.

[4].Dinan, L., Whiting, P., Girault, J.-P., Lafont, R., Dhadialla, T.S., Cress, D.E., Mugat, B., Antoniewski, Ch. and Lepesnt J.-A. (1997) Cucurbitacins are insect steroid hormone antagonists acting at the ecdysteroid receptor, Biochem. J. 327, 643-650.

[5].Arnault, C. and Sláma, K. (1986) Dietary effects of phytoecdysones in the leek-moth, Acrolepiop-sisassectella, J. Chem. Ecol. 12, 1979-1986.

[6].Píš, J., Hykl, J., Budešínský, M. and Harmatha, J. (1994) Regioselective synthesis of 20-hydroxy-ecdysone glycosides, Tetrahedron, 50, 9679- 9690.

[7].Harmatha, J., Mauchamp, B., Arnault, C. and Sláma, K. (1987) Identification of a spirostane-type saponin in leek flowers with inhibitory effects on growth leek-moth larvae, Biochem. Syst. Ecol. , 113-116.

[8].Auger, J., and Thibout, E. (1983) Spécificité des substances non volatiles des Allium responsables de la ponte de la teigne de poireau, Acrolepiop-sisassectella, Entomol. Exp. Appl. 34, 71-77.

[9].Thibout, E. and Auger, J. (1996) Behavioural events and host constituents involved in oviposition in the leek moth Acrolepiopsisassectella, Entomol. Exp. Appl., 80, 101-104.

[10]. Arnault, C. (1975) Influence des organesflo-rauxd´Alliumporrumsur le développment de la teigne du poireau (Acrolepiopsisassectella), C. R. Acad. Sc. Paris D, 2477-2480.

[11]. Arnault, C. (1979) Influence de substances de la plante-hôte sur le développmentlarvaired´Acro-lepiopsisassectellaen alimentation artificielle, Entomol. Exp. Appl., 25, 64-74.

[12]. Arnault, C., Harmatha, J., Mauchamp, B. and Sláma, K. (1987) Influence of allelochemical sub-stances of the host plant (Allium porrum) on de-velopment and moulting of Acrolepiopsisassectella. Their role as selective factors, in V. Labeyrie, G. Fabres, D. Lachaise (eds.) Insects – Plants, Dr W. Junk Publishers, Dordrecht, pp. 249- 255.

[13]. Samek, Z. and Budešínský, M. (1979) In situ reactions with trichloroacetic isocyanate and their

application to the structural assignment of hydroxy compounds by 1H-NMR spectroscopy A general comment, Collect. Czech. Chem. Com- mun., 44, 558- 588.

[14]. Kelginbayev, A.N., Gorovitz, M.B., Gorovitz, T.T. and Abubakirov, N.K. (1976) Allium steroid saponins and sapogenins. IX - Structure of aginosid, Khim. Prir.Soedin.480-486.

[15]. Hostettmann, K. and Marston, A. (1995) Sapo- nins, Cambridge Univ. Press, Cambridge.

[16]. Kawashima, K., Minaki, Y., and Sashida, Y. (1991) Steroidal saponins from Allium giganteumand Alliumaflatunense, Phytochemistry30, 3063-3067.

[17]. Ismailov, A.I., Tagiev, S.A. and Rasulov, E.M. (1976) Steroidal saponins and sapogenins from Allium rubella and A. albanum, Khim. Prir.Soedin., 550-551.