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CHEMOECOLOGY : THE LEGACY LEFT BY TONY SWAIN

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Key Word Index—Polyhydroxyalkaloids; phenolics; terpenoids; non-protein amino acids; insect-plant interactions.

Abstract—The role of plant-derived compounds in chemoecology, especially herbivory, was of great interest to the late Tony Swain. This short review gives examples of polyhydroxyalkaloids, diterpenoids, limonoids, phenolics and non-protein amino acids that modify the feeding behaviour of insects.

INTRODUCTION

Tony Swain played a leading role in establishing research into the ecological importance of plant-derived compounds [1]. When working at the Royal Botanic Gardens, Kew, he stimulated many natural product chemists to evaluate the role of plant-derived compounds on the feeding behaviour of herbivores. His legacy remains, as the role of plant- and fungal-derived compounds on the feeding behaviour of insects is still a major interest of the natural product chemists and entomologists working at Kew. This brief review reports on the chemoecological importance of some compounds that Tony had an interest in.

Polyhydroxy alkaloids

When Tony Swain left Great Britain to work in America, his group at Kew, funded by the Agricultural and Fisheries Research Council, was closed. However, Kew realised the value of his work and appointed Linda Fellows to run his laboratory, a former potting shed built in 1899, which Tony had equipped with kitchen fittings to serve as a biochemistry laboratory.

Linda Fellows, in collaboration with the then Director of Kew, Arthur Bell, initiated a detailed study into the distribution in plants of a group of compounds called imino sugars or polyhydroxy alkaloids (PHAs). Emphasis was initially on the role of these compounds as characters for taxonomic studies in legumes [2]. However, when the compounds were shown to have a range of biological activities the emphasis of the studies changed [3]. These nitrogen containing compounds mimic the structure of simple sugars and their pharmaceutical and ecological activities are usually associated with their ability to inhibit glycosidases [4]. One of their important ecological functions could be to modulate herbivory. For example, 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP) isolated from the legume *Derris elliptica* Bentham [5] acts as an antifeedant against Lepidoptera and locusts [6] and is also toxic to bruchids [7] and nematodes [8].

A range of PHAs were tested for their ability to deter insects from feeding [9] and to inhibit glycosidases [10, 11]. Swainsonine, castanospermine, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB-1) and DMDP were the most potent antifeedants of the eleven PHAs tested against larvae of the African Leaf Worm *Spodoptera littoralis* (Boisd.) [9]. DMDP was the only PHA to deter all four species of the Lepidoptera tested from feeding, and castanospermine deterred *Heliothis virescens* (F.) and *Helicoverpa armigera* (Hubner), 2(*S*)-carboxy-3(*R*), 4(*R*), 5(*S*)-trihydroxypiperidine (BRI) was active against *Spodoptera frugiperda* (J. E. Smith) and *H. virescens*, and DAB-1 was active against *H. armigera*. In the majority of cases the PHAs, when dissolved in an electrolyte, stimulated a dose-dependent neural response from neurones in the styloconic taste sensilla on the mouthparts of the caterpillars. These sensilla contain neurones with receptors that are responsive to plant compounds [12, 13]. When stimulated these neurones produce action potentials and it is the ratio of action potentials from the different neurones that is used by an insect to assist it to discriminate between host and non-host plants [12–14]. Electrophysiological experiments with *S. littoralis* showed that when the PHAs were tested in

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combination with sugars there was a decrease in the total neural input from the sensilla. This phenomenon has been called "peripheral interaction" [15, 16]. Initially after a sensillum had been stimulated with a PHA the response to a wide range of sugars are inhibited by up to 91% [9]. Whether the PHA was interacting with the sugar or alkaloid receptors on the neurones was not known. After an interval of 30 min there was some specificity in the interaction. Ninety minutes after a one-second stimulation with DMDP the neural response to fructose was still lower than normal but the response to sucrose and glucose had recovered. In contrast, castanospermine decreased the response to sucrose and glucose but not to fructose and DAB-1 decreased the response to sucrose but not to fructose or glucose. Swainsonine did not exhibit such a marked peripheral interaction as the other active PHAs, although it did lower the neural responses to glucose. It appears as if the PHAs made the insects unaware to the phagostimulants that usually elicit a feeding response. Behavioural observation showed that the larvae rejected normally accepted plants treated with these PHAs. Further bioassays are currently being undertaken to evaluate the antifeedant activity of glycosides of PHAs [17] and the *nor*tropane PHAs [18] which have recently been isolated from plants.

DMDP along with three other PHAs, 2,6-dideoxy-2,6-imino-D-glycero-L-gulo-heptitol (HNJ), 1,5-dideoxy-1,5-imino-D-mannitol (DMJ) and 1,5-dideoxy-1,5-imino-D-glucitol (DNJ) occur in the foliage of *Omphalea diandra* L. (Euphorbiaceae) which is host to a day flying moth *Urania fulgens* Walker (Lepidoptera, Uraniidae) [19, 20]. The larvae are able to tolerate the PHAs in the leaves and two of them, DMDP and HNJ, are accumulated into the adult stage [21]. *Alcides metaurus* (Hopffer), another uraniid moth from Australia was also able to accumulate these PHAs from its food plant *Omphalea queenslandia* [22]. Recent studies revealed that other polyphagous insects consume leaves of *O. diandra*; however, none of these insects accumulate the PHAs to the same extent as *U. fulgens* [23]. The gut glycosidases of the specialist insects were more tolerant of the PHAs than the glycosidases in the generalist insects, which suggests that these enzymes in the specialist insects have adapted to the PHAs. For example, DMDP at 3.3×10^{-3} M did not inhibit the hydrolysis of trehalose or sucrose in the specialists *U. fulgens* and *A. metaurus* but it did inhibit hydrolysis of these sugars in the generalists *Panthiades ballus* (Reakirt) (Lepidoptera), *Theope virgilius* (Fab.) (Lepidoptera), *Rhabdopterus fulvipes* (Jac.) (Coleoptera) and a non-*Omphalea* feeding insect, *Spodoptera littoralis* [23]. Trehalase in *U. fulgens* was, however, inhibited by HNJ and a HNJ glucoside, the latter being the most abundant of the PHAs found in *O. diandra*. Although HNJ was accumulated in the adult moths its glycoside is not. The accumulation by *U. fulgens* of the aglycone HNJ but not its glycoside appears at first to be unusual as insects usually accumulate glycosides but not aglycones [24]. This is because glycosides are usually less toxic than their corresponding aglycones [25]. It appears that within the PHAs, glycosides are more active than their aglycones. For example, with *U. fulgens* the HNJ glucoside (ED_{50} 2.4×10^{-7} M) was a more active inhibitor of trehalase than the aglycone HNJ (ED_{50} 1.5×10^{-4} M) [23], which could explain why the aglycone was accumulated but not the glycoside.

More work needs to be undertaken to establish the evolutionary significance of PHAs in chemoecology and whether insects such as *U. fulgens* use the compounds as stimuli to identify host plants. The information currently available suggests that PHAs give some protection to plants from herbivory by generalists. They could also provide those insects that accumulate them with protection from predators as glycosidases in birds are inhibited by PHAs, such as HNJ [21]. This later hypothesis still needs to be tested. In fact, we know nothing about the effect of PHAs on tritropic interactions.

Phenolics

A number of plant phenolics have been tested for their effects on insects [26–29]. They were thought for many years to have non-specific activity as antinutritional compounds because many decreased the utilisation of nutrients in leaves by binding leaf proteins into indigestible complexes [30]. Levels of phenolics in plants show seasonal variations [31] and can increase in plants after they have been damaged. The role of phenolics in the induced responses of plants has advanced in the last 20 years from a topic often trivialised by its association with the term "talking trees" to an established science with molecular biologists genetically manipulating plants to produce defence compounds when damaged [32].

Some phenolics show selectivity in their activity as antifeedants [33] and growth disrupters [34], whereas others have broader activity in crop resistance to pests [35]. For example, the levels of caffeoylquinic acid esters (e.g. chlorogenic acid) in *Arachis* spp. have been shown to be high in accessions and wild relatives of groundnut that are resistant to attack by *Spodoptera litura* Fabricius [35]. Until recently, our knowledge of the role of chlorogenic acids and tannins in plant resistance was mainly based on information gained from experiments that incorporated these compounds into artificial diets [36]. Results from these experiments can show discrepancies when compared with results from insects feeding on plants. The potency of chlorogenic acid is usually expressed after it has been oxidised by plant and insect polyphenoloxidases or peroxidases to quinones [37, 38]. Therefore results from laboratory experiments using artificial diets do not reflect the actual potential of this compound [39]. In contrast, enzymes in plants can oxidize condensed tannins reducing their potency. For example, levels of

tannins in cotton leaves did not correlate with resistance to *Heliothis virescens* [40], whereas the correlation was positive when the tannins were incorporated in a diet used to rear the larvae [41].

Other phenolics, such as the apigenin-*C*-glycosides, schaftoside and isoschaftoside, may play an important role in the resistance of rice to attack by the brown planthopper, *Nilaparvata lugens* [42]. These compounds occur in high levels in the phloem of cultivars of rice that show resistance to feeding by *N. lugens*. When fed to adults in an artificial diet, schaftoside caused dose-dependent mortality [43]. Field experiments indicate that *N. lugens* probes more but does not feed on resistant plants, thus the mortality of insects observed in the laboratory experiment could result from the antifeedant activity of the compounds causing the insects to starve to death. Whether apigenin-*C*-glycosides show resistance to other rice feeding pests has yet to be established.

The roles of phenolics in chemoecology are usually investigated by extracting phenolics from ground plant material but these compounds can occur on the plant surfaces and could influence the acceptance or rejection behaviour of insects when they contact a plant. The flavonoid glycosides luteolin-7-β-D-glucoside and luteolin-7-*O*-(6″-*O*-malonyl)-β-D-glucoside occur on the surface of *Daucus carota* leaves and are thought to be contact oviposition cues for the swallowtail butterfly, *Papilio polyxenes* [44], as is chlorogenic acid [45]. Whether these interact with the monoterpenes, sabinene hydrate or 4-terpineol that also influence host selection has not been established [46].

Laboratory experiments with *Helicoverpa armigera* have shown adults are able to discriminate among substrates coated with different phenolics and that compounds such as rutin, kaempferol-7-neohesperidoside and catechol stimulated oviposition, dose-dependently [47]. These compounds were extracted from the surface of host plants such as sunflowers, chick peas and pigeon peas which were susceptible to attack by *H. armigera* [47]. Results of behavioural experiments suggest that rutin could be an important stimulant, but tarsal taste sensilla used by the female moth to discriminate among compounds did not respond to rutin in electrophysiological experiments. It could be that rutin stimulates responses from neurones in sensilla on the ovipositor, sensilla not yet studied, or that by chance ''non'' responding sensilla were stimulated. Other studies have shown that the responses of these tarsal sensilla to stimulants correlate with the host acceptance behaviour of the adults [48]. The role of these compounds in host selection therefore needs further study.

Chemotaxonomic studies have shown that lipophilic flavonoid aglycones occur on the surface of some plants [49]. For example, a study of 16 accessions of *Ocimum basilicum* L. showed the presence of 12 flavone aglycones on the surface of leaves [50]. The proportions of the flavones varied among the accessions, although the levels of nevadensin and salvigenin were high in most accessions. These variations in flavone levels, along with differences in the profiles of essential oils [51] could explain why accessions of *O. basilicum* differ in their potency as botanical insecticides or medicinal plants.

Despite the knowledge that phenolics can modulate insect behaviour there are very few comparative studies on specific compounds or experiments that have established structure-activity relationships. One study compares the gustatory responses of a polyphagous and oligophagous species of *Spodoptera* to a range of flavanones, flavanes and chalcones isolated from species of *Lonchocarpus* and *Tephrosia* [52]. In an antifeedant bioassay in which the compounds were applied in combination with sucrose to glass-fibre discs, the oligophagous *S. exempta* was responsive to more of the compounds (14 out of 20) than the polyphagous *S. littoralis* (7 out of 21 compounds). Of the 7 chalcones tested, derricin was antifeedant against both species, the 2 flavanes (methylhildgardtol A and B) were active against *S. exempta* but not *S. littoralis* and of the 12 flavanones tested, 5 were active against both species (isolonchocarpin, 7-*O*-methyl-8-prenylflavanone,5-hydroxyisoderricin,7-*O*-methyl-8-(3-methyl-butadienyl)-flavanone and 5-methoxyisolonchocarpin). However, when a selection of the compounds were applied to leaf material, 7-*O*-methyl-8-prenylflavanone was the only compound to retain activity against both species. This compound stimulated the deterrent neurone in the medial styloconic sensilla of *S. exempta* and *S. littoralis*. There was no clear structure-function relationship, although the flavanones were more active than the chalcones and compounds with methoxy substitutions at C-7 were generally more active than those with other substitutions at C-7 [52]. In another study *Heliothis zea* was used to evaluate the growth inhibitory activity of 42 flavonoids [34]. The activity of the compounds was dependent on the substitutions and stereochemistry of the groups on C-5 and C-6 as well as C-3′ on the B ring. The potential importance of these compounds in different aspects of host selection justfies further investigation.

Terpenoids

Monoterpenoids. The role of monoterpenoids in chemoecology has been well established [26–29]. This partly reflects advances in analytical techniques, especially in the availability of capillary GC-MS and the potential use of monoterpenoids in insect traps. Monoterpenoids can have profound effects on insects : pulegone, limonene and linalool have insecticidal activity when applied to flies, cockroaches and the western corn rootworm [53]. These compounds also deter insects from feeding [54]. Many plants are cultivated for their insecticidal monoterpenoids. For example, the monoterpenoids in the essential oils of *Ocimum basilicum* have activity as deterrents and toxicants. The major constituents in essential oils of 16

accessions of *O. basilicum* were linalool, methyl chavicol, eugenol, methyl eugenol, geraniol, geranial and neral [51]. The additive and synergistic activities of these compounds along with the flavones [50] from *O. basilicum* are currently being evaluated in the search for ovicides to control bruchid beetles and to act as deterrents against flies.

Diterpenoids. The diterpenoid ajugarin 1, isolated from *Ajuga remota* Wall. and Benth. [55], attracted a great deal of attention as a potent antifeedant and in the last two decades natural product chemists have isolated many diterpenoids from labiates [56]. These compounds often occur in very low concentrations and phytochemists have isolated enough to determine the structure of the compound but not enough for bioassays that require over 50 mg. Bioassays currently used to assess the antifeedant and insecticidal activities of compounds only require small amounts. Samples of 1–2 mg would enable a compound to be tested against 2–4 species in at least 2 bioassays.

To date, diterpenoids from *Ajuga*, *Teucrium*, *Scutellaria* and *Salvia* have received the most attention. In one study extracts from 24 of the 100 species of *Teucrium* were tested for antifeedant activity and 14 species showed potent antifeedant activity against *S. littoralis* and *S. frugiperda* [54]. The activity of only a few of these plants could be explained by the diterpenoids that had already been isolated from them. Extracts from these active species of *Teucrium* contained diterpenoids but as yet they have not been isolated. Many of the original samples came from plants growing in their natural habitats and when attempts were made to bulk the samples under glasshouse conditions the antifeedant activity was lost.

Of the diterpenoids from *Teucrium* tested for antifeedant activity, 12-epi-teucrin was one of the most potent [54]. In another comparative study the neoclerodanes Jodrellin A and Jodrellin B were isolated from *Scutellaria galericulata* [57]. For some years, these were the most potent diterpenoids antifeedant to have been isolated from the Labiatae. Recently, however, another neo-clerodane scutalpin C has been isolated from *Scutellaria alpina* subsp. *javalambrensis* Pau which is as potent as the Jodrellins against *S. littoralis* when tested at 100 ppm [58]. Other diterpenoids isolated from species of *Salvia* have antifeedant activity but they are not as potent as the Jodrellins [59]. To date, over 100 diterpenoids have been tested for antifeedant activity. However, there appears to be no clear structure-function relationship and responses to specific compounds will differ among insects.

Limonoids. The antifeedant activity of limonoids such as azadirachtin from the Neem tree *Azadirachta indica* A. Juss and toosendanin from *Melia toosendan* and *Melia azedarach* is well documented [60, 61]. More recently limonoids with antifeedant activity have been isolated from *Swietenia*, *Cedrela* [62] and *Trichilia* [63]. However, as yet azadirachtin remains the most potent limonoid antifeedant, although the responses to azadirachtin vary among insects [60]. In a recent study *S. littoralis* has been shown to be responsive to compounds in Neem that are precursors of azadirachtin [64]. For example, the steroid tirucallol elicited a potent antifeedant as responsive as the liminoid azadirone, although a similar limonoid azadiradione was less active. These liminoids then give rise to the C-seco-limonoids nimbin and salannin, which both elicit antifeedant responses, with nimbin being more active than salannin. It has been proposed that tirucallol is converted into azadirone, then salannin and finally azadirachtin [65]. Salannin often occurs in Neem extracts at 3–4 times the concentration of azadirachtin, but these values can vary [66]. Although these precursors uzadirachtin showed antifeedant activity to *S. littoralis* they did not cause significant levels of mortality [64]. In another study, salannin has been shown to be as active as azadirachtin against *Epilachna varivestis* [67], and more active than azadirachtin against *Pieris brassicae* [68]. Despite these differences in activity azadirachtin remains, overall, one of the most potent anti-insect limonoids showing activity as an antifeedant and toxicant against a range of insects [60]. Other limonoids will deter insects from feeding but few have this dual mode of action. Salannin has been shown to act as an antifeedant and growth disrupter against *S. litura* and *Pericallia ricini* [69], whereas it only had antifeedant activity against *S. frugiperda*, *S. littoralis* and *H. virescens* [70, 71]. These differences in activity could reflect differences in the susceptibility of the species of insects or they could be due to differences in the bioassays used to access activity. The insects treated with salannin in the later studies were exposed to less of the compound [71] than occurred with *S. litura* [69].

A limitation of the use in pest control of compounds with only antifeedant activity is that insects have been shown to be able to habituate to compounds that cause antifeedant responses but are harmless when consumed, such as tannins [72], cucurbitacins, cardenolides and some alkaloids [73]. However, azadirachtin-based insecticides are presently being marketed and are available in many parts of the world and as yet there are few reports of insects habituating or developing resistance to them.

Another problem encountered with azadirachtin was the loss of biological activity when the compound was exposed to sunlight [74]. This problem has been partly corrected by the addition of formulants to registered products. Salannin and nimbin also absorb UV light and a study has been undertaken into the activity of their photo-oxidation products [75]. The oxidation of nimbin and salannin takes place faster than that of azadirachtin, resulting in the formation of isomeric hydroxybutenolides. When these compounds were tested against insects they were found to have dual activities as antifeedants and toxicants against *S. littoralis*. In fact, isosalanninolide and isonimbinolide were as potent antfeedants as azadirachtin and the growth inhibitory activity of isonimbinolide was com-

parable to that caused by azadirachtin when *S. littoralis* were cannulated, injected or topically treated with the compounds [75]. Whether these breakdown compounds play a role in the efficacy of Neem products has not been established.

Non-protein amino acids

The role of non-protein amino acids such as canavanine in protecting plants from herbivory have attracted considerable attention [76]. These nitrogen containing compounds occur in seeds and act as nitrogen reserves for plants as well as plant protectants and can be completely mobilised during germination.

Seeds of the edible Madagascan legume *Lemuropisum edule* H. Perrier are eaten as a delicacy by people in the South West arid areas of Madagascar although they often cause stomach problems and adverse side effects [77]. These seeds contain the non-protein amino acids *trans*-3-hydroxy-L-proline (1–5% dry weight) and L-azetidine-2-carboxylic acid (1–2%). Extracts from the seeds caused significant antifeedant activity against *S. littoralis* and *S. litura* but did not affect the feeding behaviour of *Heliothis virescens* or *Locusta migratoria* [77]. When tested singly 3-hydroxyproline was more active as an antifeedant than azetidine-2-carboxylic acid and there was no additive activity when the compounds were tested together. Whether the hydroxyproline is responsible for the low level of herbivory of the plant observed in the wild still needs to be proven. Both compounds are analogues of proline and have been shown to have adverse effects against mammals [78]; 3-hydroxyproline caused toxicity to human embryo fibroblast cells when tested at 500 μg ml^{-1} [77], whereas azetidine-2-carboxylic acid inhibits skin fibroblasts at 100 μg ml^{-1} [78]. It is possible that both compounds could play a part in the observed toxicity of the seeds to humans.

Non-protein amino acids occur in many food crops. For example, they occur in species of *Lathyrus* which are part of the staple diet of populations in the Indian subcontinent, Ethiopia and China [79]. The consumption of seeds from these plants can cause a chronic neurophysiological disease called neurolathyrism and the non-protein amino acid 2-amino-3-oxalyaminopropanoic acid (β-ODAP) is thought to be the active compound [79]. Efforts are currently being made to develop a cultivar of *Lathyrus* which has low levels of β-ODAP for human consumption as well as a fodder crop [80]. Investigations were carried out to see if β-ODAP was deterrent to insects and whether lowering the level of the compound in plants might make them more susceptible to predation [81]. The other non-protein amino acids in *L. latifolius* included homoserine (HS), *O*-oxalylhomoserine (OHS), 2-amino-4-oxalylaminobutanoic acid (γ-ODAB) and 2,4-diaminobutanoic acid (DAB). The levels of these non-protein amino acids in different parts of the plant were calculated and then the compounds were tested singly and in combinations. The concentration of the compounds tested reflected the levels found in young and old stems and leaves as well as roots.

Finally, an experiment was undertaken to assess the importance of each non-protein amino acid to the activity of a mixture by re-testing the mixture when only one of the non-protein amino acid had been removed.

All six non-protein amino acids elicited dose-dependent behavioural responses from larvae of *Spodoptera littoralis* [81]. Three of them, OHS, γ-ODAP and DAB, were antifeedants, the latter only at the highest concentration tested (0.5% dry weight). β-ODAB stimulated feeding. The mixtures containing the compounds in levels found in young and old stems and leaves all caused an antifeedant response, whereas the root mixture was inactive. The mixture of non-protein amino acids in the young leaves elicited the greatest antifeedant response and was selected as the mixture to use for the evaluation of the importance of each of the compounds to the activity of the mixture. When OHS and DAB were removed, the antifeedant activity of the mixture was lost, despite the fact that when OHS was tested on its own, at the concentration present in the mixture, it showed potent antifeedant activity. As β-ODAB had potent phagostimulantry activity it was surprising to find that when the compound was removed from the mixture the antifeedant activity of the mixture decreased [81]. These results illustrate very clearly that it is difficult to predict how the addition or removal of a compound from a mixture will influence its activity. These results indicate that removal of the neuro-toxin β-ODAB might make the plant more susceptible to predation. However, it is difficult to extrapolate these findings to determine what might happen with vertebrates such as rabbits and hares that browse on the foliage of these plants.

Taxonomic surveys of plants for antifeedants: why select a species for further study

Since 1985 over 5000 different species of plants have been evaluated for antifeedant activity at Kew. Of these 250 justify further study [82, 83] because the activity cannot be explained by reference to published work on the compounds isolated from these species or related species. The role of antifeedants in chemoecology is still in its infancy as is the understanding of the use of antifeedants in pest control. If the aim of a study is to isolate novel antifeedants then it is important to avoid working on plants that might contain known antifeedants. The selection of the "right" plant is therefore important. This selection process is helped if one has a sound background knowledge of phytochemistry and access to computised databases. In the past the knowledge about phytochemistry was filed in the memories of people like Tony Swain.

Tony Swain returned to work at Kew in 1986 and the author was lucky to have shared an office with him for a few months before his tragic death in 1987. A

quick chat with Tony was the equivalent of spending a day searching through a database. He was a challenging individual to work with and would almost always ask the question "why" : why study that plant? Why do you think that compound has an ecological role? He often helped provide the answers. He has been sadly missed.

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