SECONDARY PLANT METABOLITES WITH ANTIFEEDING ACTIVITY AND THEIR EFFECTS ON SOME STORED PRODUCT INSECTS

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Abstract

The results of antifeeding activity tests of ten different sesquiterpene lactones carried out on selected stored-product pests were compared with the activity of azadirachtin and with that of about 60 other sesquiterpenes, lignans and other aromates tested previously. The ten most effective substances against adults of Sitophilus granarius and Tribolium confusum and larvae of Trogoderma granarium and Tribolium confusum were (in descending order): helenalin, linifolin A, alantolactone, mexicanin J, bakkenolide A, bisabolangelone, yatein, cubebin, rotenone and phellopterine. Some of them caused morphogenetic changes in, and a toxic effect on the larvae of T. granarium and T. confusum; others decreased absorbtion of the digested food and caused hypoglycemia in the larvae of Tenebrio molitor. Work was carried out also on the use of some antifeedants for protection of wrapping materials against perforation by the storage pest Rhyzopertha dominica.

Introduction

The chemical ecology of insects is concerned with all chemical components which influence the development and behaviour of insects. It concerns mainly secondary metabolites of insects and chemicals of plant origin since plants are the most frequent hosts of insects. Negatively acting substances of this kind can serve as insectistatics and thus maintain the populations of species considered as pests at such a level as does not cause economic and other damage to agriculture, forestry and the food industry, particularly to stored products. One of these extensively investigated groups of compounds are antifeedants or food deterrents. These are molecules, ions, or radicals which when perceived, reduce or prevent feeding. In the last decade a number of good and well presented articles have been published on this theme (Schoonhoven, 1982; Bernays, 1983; Norris, 1986; Jermy, 1983). They deal mainly with secondary metabolites of plants which are the hosts of insects. This specially concerns research on the natural defence of plants against herbivorous insects. Plant substances as interspecific messengers in chemical ecology, which are adaptively advantageous to the emitter or releaser - in this case to the plant - are termed allomones. The same substances can be exploited as kairomones (advantageous to the perceiving organism) by insects searching for food or looking for a place to oviposit.

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Grains and seeds of some cultured plants which are the main food of stored product pests, do not contain typical metabolites which could function as allomones. Recent investigations show that these products only contain inhibitors of digestive enzymes that protect the consumed food from exploitation by the insects (Yetter et. al., 1979). Therefore, the search for active antifeedants against storage pests has been focussed on those kinds of plants that are known for their effect on insects. More than 50 sesquiterpenes, 10 lignans and several rotenoids, benzofurans and furokumarins have been tested and investigated.

Food deterrents

So far, more than 6 000 sesquiterpenes are known, among them 2 500 sesquiterpene lactones with a broad spectrum of biological activities, such as antibacterial, fungistatic, antiprotozoal, cytotoxic, anticancerous, and mutagenous. Well-known and intensively investigated is the food deterrent activity of these substances on insects and vertebrates, as well as growth-regulating activity on insects. Also, they have a heterogenous effect in human pharmocology (Picman, 1986).

For the study of food-deterrent activity a standard test method has been devised. Rings of wheat wafer discs, 1 cm in diameter and about 1 mm thick, were dipped into an alcoholic solution at 10 mg/ml concentration. Then the rings were weighed and put in boxes (two in each box) thus giving a no-choice test, and also together with an untreated disc offering a choice test. The amount of food consumed in 5 days has been the basis for calculating coefficients of deterrent activity. A coefficient of 150 to 200 indicates a high deterrent activity. Test insects were adults of *Sitophilus granarius* and *T. confusum* and also larvae of *Trogoderma granarium* Ev. and *Tribolium confusum* Duv. (Nawrot et. al., 1982, 1986).

In addition to the 42 sesquiterpenes investigated earlier (Nawrot et. al., 1986) another 11 compounds of this type (Fig. 1) have been studied. Of the total of 68 chemical plant substances examined, a strong deterrent activity has been proved for 10 of them (Fig. 2).

Activities of the chemical substances given in Figure 1, under the conditions of the standard test, are equal to, or higher than the activity of azadirachtine which is the most active antifeedant known so far. Rotenone has even shown an absolute activity (total coefficient) of 200 (Fig. 2).

When comparing chemical structure and activity relationships in the results of our tests it follows that natural substances are generally more efficient than their chemically modified derivatives. More effective are those components that contain two or more chemically reactive groups in their molecule. For substances with the same molecular structure but with different substitutes no unambiguous

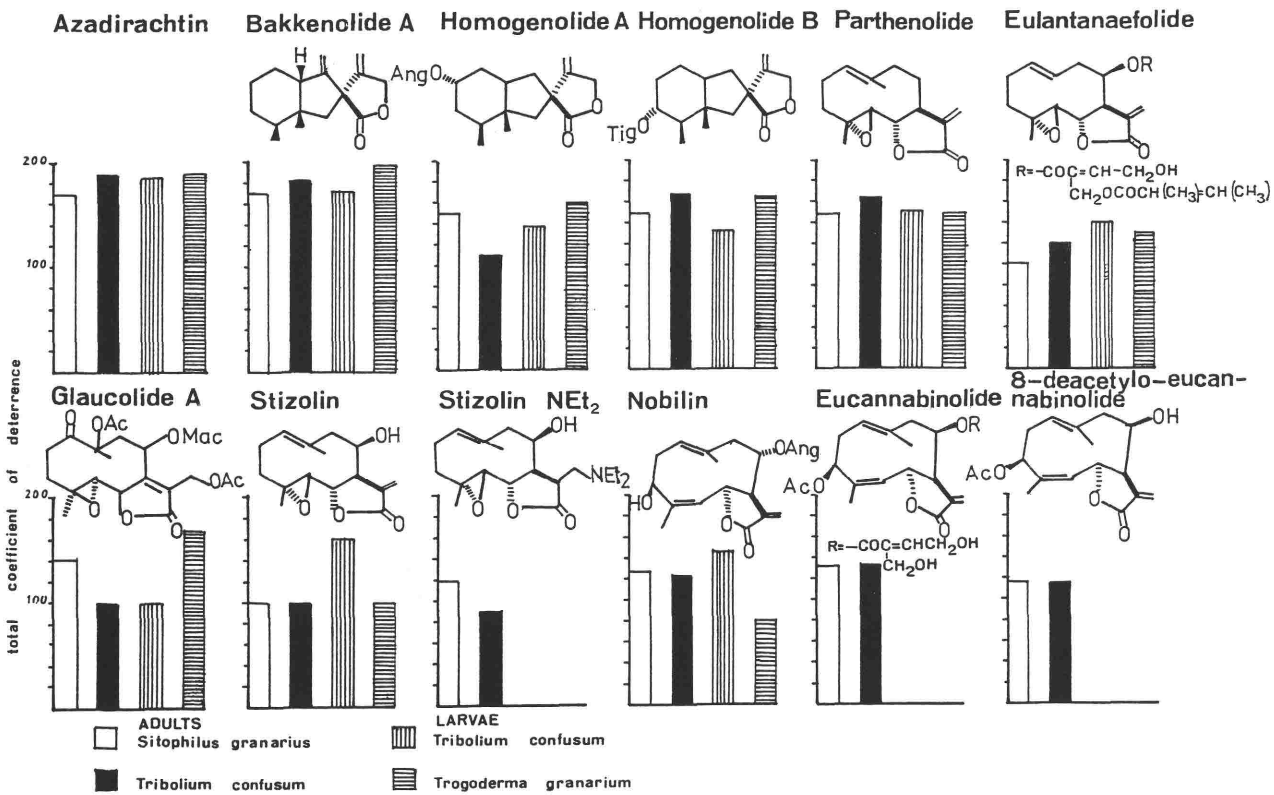
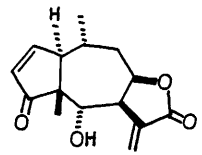
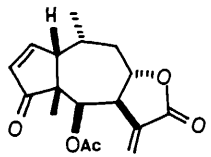


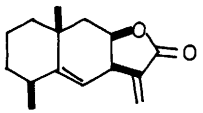
Fig. 1. Antifeeding activity of some sesquiterpene lactones



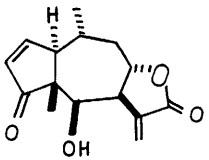
Helenalin
(Helenium aromaticum)



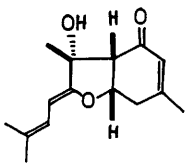
Linifolin A
(Helenium aromaticum)



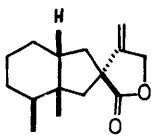
Alantolactone
(Locopholea heterophylla)



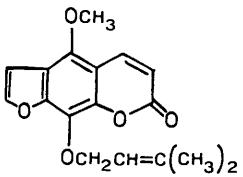
Mexicanin J
(Helenium aromaticum)



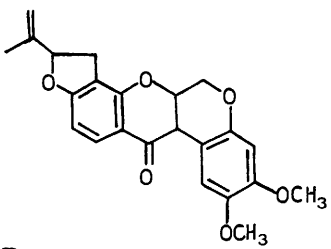
Bisaboloangelone
(Angelica silvestris)



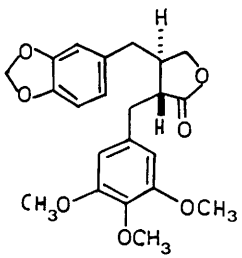
Bakkenolide A
(Homogyne alpina)



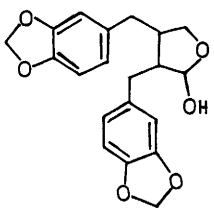
Phellopterine
(Angelica silvestris)



Rotenon
(Derris eliptica)



Yatein
(Libocedrus yateensis)



Cubebin
(Piper cubeba)

Fig.2. Chemical structures of the strongest antifeedants and their plant sources

influence of these substitutes on activity has been observed. The often discussed chemical mechanisms of activity, such as the ability to form Michael aducts, or oxido-reductive changes (Norris, 1986) cannot be in the case of our substances, the only explanation of the biological activity mechanism.

Recently, bisabolangelone, bakkenolide A, and helenalin have been applied to wheat grains at doses of 50 and 250 mg/kg. Used in this way these feeding deterrents secured good protection against larvae of T. granarium and T. confusum which gnaw large shallow excavations in the grain surface. The presence of antifeedants significantly decreased the feeding activity of these larvae and diminished the number of progeny, as well as making population growth more difficult. The antifeedants on the grain had no significant effect on adults of S. granarius, which gnaw at the grain from an angle and after penetrating the outer pericarp they consume the endosperm where they also lay their eggs. Only during the initial phase of grain colonisation was a decrease in feeding intensity observed.

Antifeedants

Active antifeedants were mixed with flour in 10 and 100 mg/kg proportions and after adding dextrin, granules the size of wheat grains were formed. Thus the test material was uniformly present within the granules and insects were in contact with it throughout their time of feeding, namely 30 days. Feeding intensity of the beetles S. granarius and T. confusum as measured by the quantity of dust produced, decreased by 30%, and the number of progeny by 25-50%. On food treated in this way, population development of Oryzaephilus surinamensis was not possible.

Granules treated in this way were also fed to larvae of Tenebrio molitor; after the antifeedant entered the digestive tract, the physiological effect of its activity was observed. Eight compounds administered at 10 mg/kg of diet inhibited larval growth during a seven day feeding period. Analysis of digestion and absorption of the food indicated that the inhibitory effect was not only due to reduced consumption of food but also to decrease of the substrates absorbed into larval tissues. The highest reduction in the process of digestion and absorption of food resulted from the influence of bakkenolide A, bisabolangelone and helenalin. There was a correlation between the impairment of intestinal activity by these three compounds and hypoglycemia induced by them in the larval haemolymph. However, the hypoglycemia was not so intense as after a seven day period of starvation. On the other hand, alatolide, grossheimin and cnicin do not produce hypoglycemia in the haemolymph although they also reduce intestinal activity (Rosinski et. al., 1986).

Insecticidal effect

Further, the insecticidal effect of bisabolangelone, helenalin and bakkenolide A was also investigated. These three compounds were topically applied to larvae, pupae and adults of T. confusum and T.

granarium and to adults of S. granarius. Helenalin and bisabolangelone were more toxic to larvae and caused morphogenetic changes in pupae. Adults treated with these compounds laid less eggs and their longevity decreased. Bakkenolide A had no effect on the test insects (Nawrot et. al., 1984).

This year, in cooperation with Dr. R. Wohlgemuth of the Institute for Stored Products Protection in West-Berlin, experiments have been carried out to evaluate the possibility of protecting packaging materials with antifeedants. The method elaborated by Schmidt (1979) and Noack (1983), was used.

Acetone solutions of 10 different antifeedants were applied at 20 $\mu g/cm^2$ to filter paper circles. Then adults of Rhyzopertha dominica were placed on these circles, and covered with a wire gauze. For seven days these adults were able to move in the arena thus formed and also had the possibility of perforating the filter-paper circle. The experiments were carried out both one day and 30 days after treatment of the filter papers. Results are summarized in Table 1. The best protectants were: linifolin A, helenalin, 9 alpha-hydroxyparthenolide and telekin. Bakkenolide A and cubebin were not active against R. dominica.

Table 1: Penetration by Rhyzopertha dominica adults through filter paper impregnated with acetone solutions of plant antifeedants (20 $\mu g/cm^2$) during 7 day exposure.

	One day after impregnation			30 days after impregnation		
	% mortality	damage slight	damage heavy	% mortality	damage slight	damage heavy
control	34	1	4	22	0	5
bakkenolide A	38	4	1	68	0	5
linifolin A	36	4	1	64	4	1
helenalin	58	4	1	62	5	0
cubebin	30	2	3	74	2	3
asperilin	24	5	0	78	2	3
mexicanin J	44	3	2	78	1	4
cnicin	46	4	1	78	3	2
repin	44	2	3	60	4	1
telekin	38	4	1	50	3	2
9- hydroxypar-thenolide	42	5	0	60	5	0

Conclusions

Our results and those of other workers have shown that secondary plant metabolites have various biological mechanisms of activity, ranging from hormone analogs and growth regulators, to semiochemicals, insecticides and toxins. Food deterrents themselves, as one of the semiochemical types (allomones), can have an extensive range of

efficiency, for example, they influence oviposition, the development of larvae, and morphogenetic changes of pupae. To investigate these substances is more laborious than to search for efficient insecticides but it provides a better opportunity to obtain specific and selective influences on the regulation of insect-pest populations. Although antifeedants are less effective than insecticides, these treatments are ecologically more acceptable for obtaining balanced control between pest and plant products. For the time being, antifeedants are not used on an extensive scale in biorational plant protection, because further information is required concerning their toxicological and pharmacological characteristics, and more economical chemical or biotechnological preparations are required before their formulations can be commercialized.

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