Why Does the Grasshopper Not Eat Spinach

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Spinasterol, ecdysones, tebufenozide, supernumerary molts.

Plants produce chemical compounds to protect themselves. Molting hormones could lead to supernumerary molts in insects. Studies on such substances have led to new environmentally safe insecticides.

Is there an answer to the question posed in the title? What is the connection, if any, between the grasshopper and spinach? Does spinach in some way keep the grasshopper away? A typical grasshopper is shown below (*Figure* 1). Grasshoppers and locusts are closely related to each other. The former is of a solitary nature, while the latter are more social, gregarious and move as swarms destroying large areas of green vegetation. (See *Box* 1).When they gather together, often for food, they bump their hind legs against each other over crowding. This triggers metabolic changes inside their body because of which they transform into gregarious, multi-coloured locusts which form swarms with millions (or even billions) of them moving together. (*Figure* 2). Locust swarms are shown in *Figure* 3.

The famous cartoon character Popeye's (*Figure* 4) favorite food is spinach. This sailor seems to have gained a lot of muscle power and strength by eating spinach. Is this really possible? About 100 years ago, it was reported that spinach is rich in iron. Many a mother has insisted that her child consume lot of spinach, even if

Box 1. Possible Locusts Attack During Beijing Olympics

"If you thought locusts were a problem only in the Old Testament times, think again. In 2002, the pests devoured 3.7 million acres of farmland in northern and central China. The insects are now eating their way through Inner Mongolia just in time for the start of the games. The last time the locusts reached the capital, locals snagged the protein-filled insects for midsummer snacks. International athletes unaccustomed to Chinese diet might not be pleased to find one in their mouth during the completition. There are 50% chances of the attack.**"** (*Times of India*, July 6, 2008)

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3a

3b

2a

2b

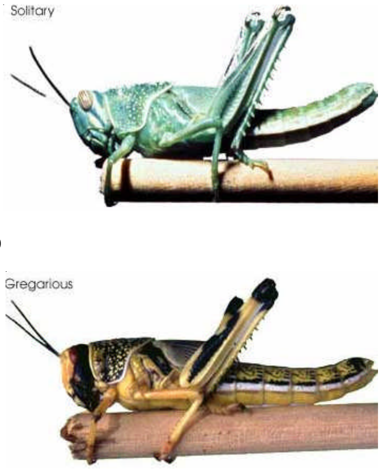

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Figure 1. A typical grasshopper.

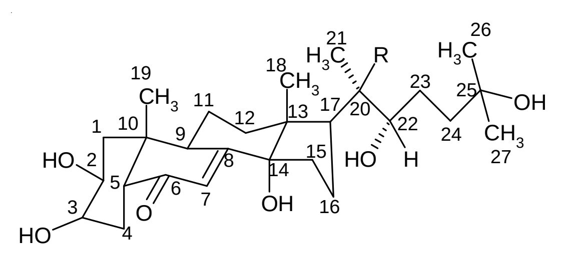
Figure 2. (a) A green solitary newly hatched grasshopper (nymph). (b) A multi- coloured gregarious nymph. (http://upload.wikimedia.org/wikipedia/commons/8/83/DesertLocust.jpeg)

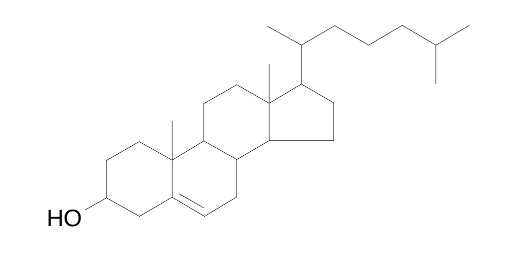
Figure 3. (a) A desert locust swarm. (http://aycu37.webshots.com/image/47916/2003862006509574063_rs.jpg4)
(b) A locust swarm. (http://blogs.tnr.com/tnr/blogs/environmentandenergy/locusts.jpg)

Figure 4. Popeye – the sailor man.

the child resisted it. A year later it was shown to be a wrong report. However, it has continued to stay in the public mind. The question whether spinach is beneficial to human beings remains as yet unanswered.

It is, therefore, very appropriate to have a closer look at the relationship between plants and insects. This study has in fact yielded not only information about insect physiology, growth and development but also about constituents, both volatile and non-volatile, present in plants which





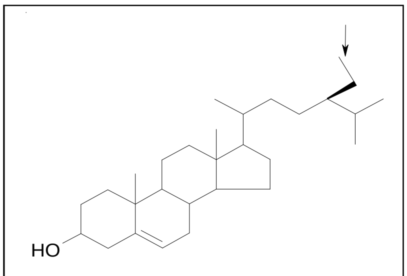
Structure 1 (left). 20-Hydroxy ecdysone.

Structure 2 (right): Cholesterol.

affect insects. The study has led to the development of new environmentally-safe insecticides, which are already commercially available in the international market. The importance of such studies leading to other benefits to human beings and to studies on environment and ecology is also highlighted here.

Insect growth and development involves many stages of shedding its hard outer cuticle – a process termed as metamorphosis (*ecdysis*). The hormone controlling this process has been therefore called *ecdysone*. These steroidal hormones were isolated and characterized by A. Butenandt and P Karlson in 1954 from *Bombyx mori* (silkworm moth). From 500 kg of this female insect, they isolated 25 mg of the hormone. Ecdysone is today known as a pro-hormone, as the real hormone 20-hydroxy ecdysone (*Structure* 1) is formed inside the insect's body by a stereo-specific hydroxylation at position 20 in the steroid skeleton. Cholesterol (*Structure* 2) is converted in the insect's body to these hormones. However, insects lack the ability to make cholesterol and they must necessarily take it from outside. They get it from the plants by breaking down molecules like β-sitosterol (*Structure* 3).

Plants like spinach contain phytoecdysones (plant-based ecdysones). Suprisingly, 1 kg of spinach can contain up to 20 to 30 g of such steroids. K Nakanishi, now at Columbia University, extracted 25 milligrams of 20-hydroxy ecdysone from mere 2.5 grams of a dried rhizome of the fern, *Polypodium vulgare*. But what are insects molting hormones doing in plants? This is obviously the plant's way of warning the insect that large quantities of its own hormone are present on its (spinach) leaves. This could bring about multiple molts in the insect. The insects could be mal-formed and their gregarious phytophagous (excessive plant eating) stages interrupted. Thus locusts don't eat the plant bugleweed, *Ajuga remora*. It has been shown that when plant extracts of *Ajuga remora* are applied, the pupa of Fall



Structure 3. β-Sitosterol (Arrow indicates structural differences from cholesterol).

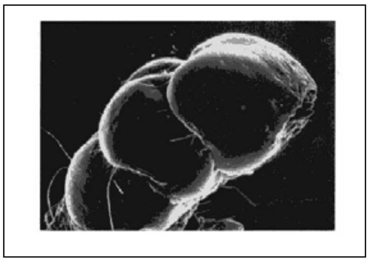
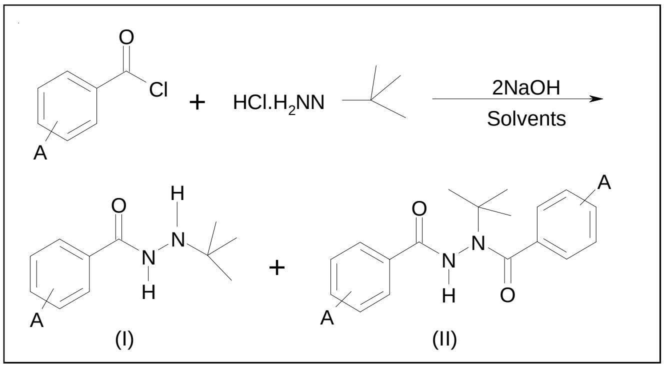


Figure 5. Multiple heads developed in fall armyworm pupa when it was treated with extracts of Ajuga remora. (http://www.uky.edu/~garose/link100.htm)

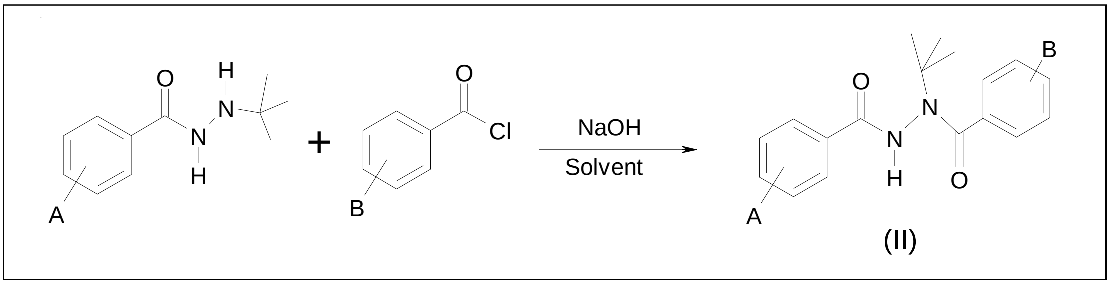


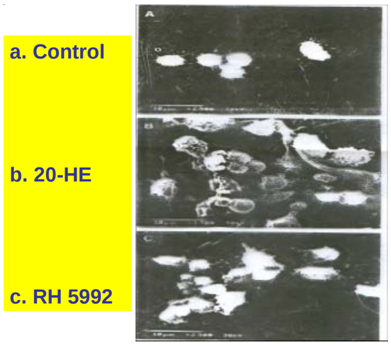
Scheme 1. A chance observation which led to the synthesis of substituted tert-butyl dibenzoyl hydrazides.

armyworm develops multiple heads (*Figure* 5). Thus, sufficient food does not reach any of the brains. Could such plants or plant extracts be used as natural insecticides which are safe to man, as man does not molt and only insects do? Studies have shown that the ecdysones are very easily metabolized by the insects and thus cannot serve as insecticides.

During the attempted preparation of the organic compounds mono benzoyl hydrazides, a chance observation led to substituted tertiary butyl dibenzoyl hydrazides (*Scheme* 1). The latter were then synthesized by carrying out the di-benzolylation of tert-butyl hydrazines (*Scheme* 2). These dibenzoyl derivatives showed activity, similar to the insect-moulting hormone 20-hydroxy ecdysone and thus served as ecdysone agonists. This included puffing of chromosomes of the K_c cells in *Drosophila* species which resembled similar observations using 20-hydroxy ecdysone (*Figure* 6). It was obvious that the three-dimensional structure of this small molecule carrying a bulky tertiary butyl substituent resembled that of the insect-molting hormone itself. The binding sites where weak and strong bonding occurs in the hormone with its receptor sites are shown in *Figure* 7. The difference between the two is that the binding of the real hormone is fully reversible while that of the synthetic compound is completely irreversible. The activity of the synthetic

Scheme 2. Synthesis of substituted tert-butyl dibenzoyl hydrazides.





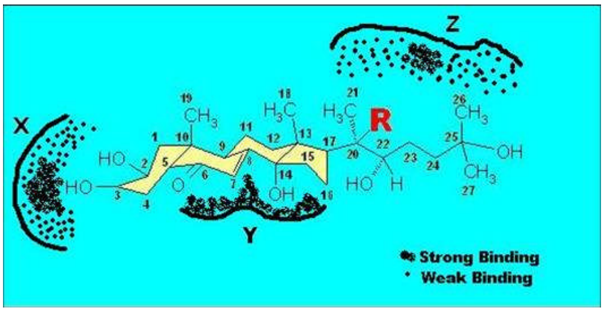


Figure 6 (left). Puffing of polytene chromosomes. SEM of Drosophila K_c cells.
(From *Science*, Vol.241, pp.467–469, 22 July 1988. Reprinted with permission from AAAS.)
Figure 7 (right). The binding of 20-hydroxy ecdysone with its receptor sites.
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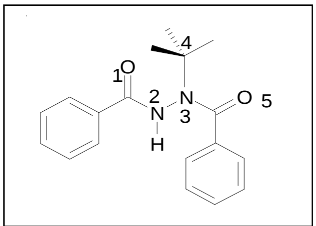
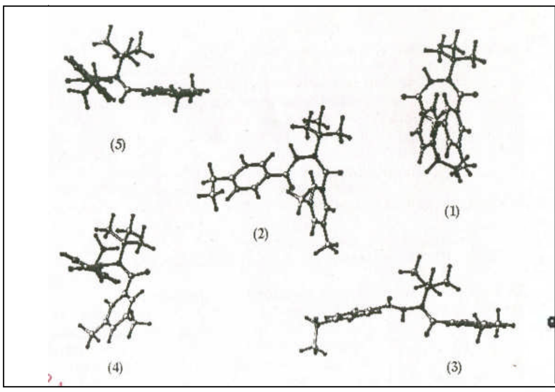
compound, Tebufenozide RH-5992 (*Structure* 4) has been shown in armyworm, *Spodoptera exigua.* The application of tebufenozide leads to the formation of a second cuticle (*Figure* 8). Indeed, the insect struggles hard even to emerge out of its original cuticle. The formation of a second cuticle now makes this struggle even more difficult for the insect. This work was carried out in Rohm and Haas Company, USA where the molecular modelling of this compound was also carried out. Different conformations, i.e., folded conformer (1), T-shaped conformer (2), extended conformer (3), hooked conformer (4), hooked conformer with a twist (5), of this new compound are shown in *Figure* 9. The T-shaped (2) conformation was shown to be the same as that obtained by X-ray diffraction studies (*Figure* 10). Quantitative structure activity relationship (QSAR) studies led to the successful development of new insecticides for spruce budworm, *Choristoneura*



Structure 4. Tebufenozide RH-5992.

Figure 8. The synthetic compound tebufenozide leads to double cuticle formation in armyworm, Spodoptera exigua (marked with arrows).



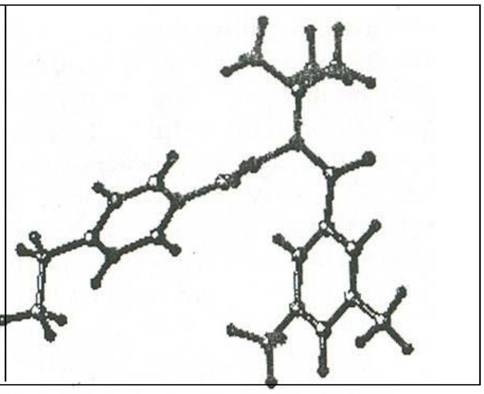


Figure 9 (left). Different conformations of Tebufenozide RH-5992. The structure (Figure 10) obtained by X-ray diffraction studies resembles the T-shaped conformation (2).

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Figure 10 (right). X-ray structure of RH-5992. (Reprinted with permission from *Phytochemicals for Pest Management,* Chapter 16, ACS Symposium series 658. Copyright 1997 American Chemical Society)

fumiferana. Ten compounds, with different substituents were selected and tested against the 6th instar, a developmental stage in the life cycle of the insect. Seven of these showed 100% mortality, feeding reduction more than 90% and LD_{50} values (LD_{50} stands for the lethal dose required to kill 50 % of the population of the insects) ranging from 0.120 to 1.003 μg/larvae (*Figure* 11).

Tebufenozide RH 5992 is commercially available as 'Confirm' and 'Mimic' and is safe to human beings and mammals as it only interferes with molting in insects. For this work Rohm and Haas Company has been awarded the Designing Greener Chemicals Award, 1998. In 1993, $MIMIC^R$ was introduced in Japan and Europe. It was used as $CONFIRM^R$ in 1994 for controlling army worm, *Spodoptera exigua,* on 7,50,000 acres of cotton in Mississippi. It has been used as an insecticide in apples, sugarcane, citrus, rice, vegetables, sugar beets and forests. It is safe for predatory mites, wasps, spiders, and beetles and effective for Integrated Pest Management (IPM) to reduce the total amount of insecticide use. It is safe for mammals. Structures of four

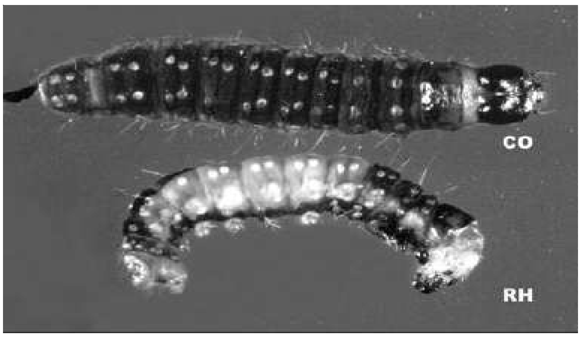
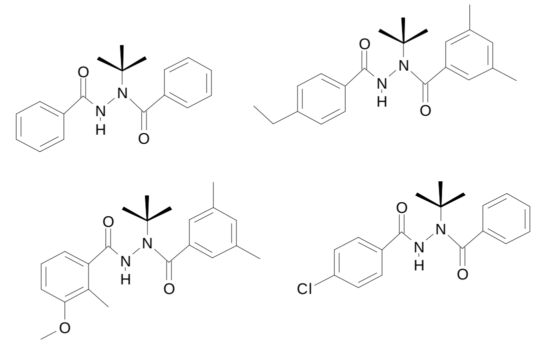


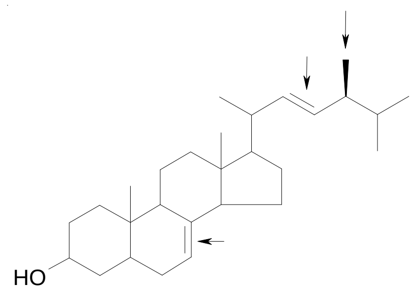
Figure 11. Spruce budworm sixth instar larvae treated with tebufenozide versus control larvae. (Note the reduction in size of treated insect as compared to the control).

(Arthur Retnakaran *et al. Pest Management Science,* Vol.57, pp. 951-957, 2001, © Society of Chemical Industry. Reproduced with permission.)



Methoxyfenozide **Halofenozide**

Figure 12. Four new Insecticides.



Structure 5. Spinasterol Arrows indicate structural differences from cholesterol.

such new analogs developed are shown in *Figure* 12.

While insects like grasshoppers can break down β-sitosterol into cholesterol, they are unable to break down spinasterol (*Structure* 5) which is present in spinach. Thus it is termed as an unsuitable sterol. Spinasterol contains double bonds at position at 7 and 22 and a methyl group at 23 which makes it difficult for grasshoppers to break it down. Thus, grasshoppers don't prefer to eat spinach.

Application of ecdysones leads to synchronous maturing of cocoons thus allowing mechanically harvesting (collection using machines) them. This helps in silk production. Spinach has been shown to be hepato protective for human liver and helps in protection from diabetes and cancer. Reports suggested that it cuts down risks due to heart attacks. Athletes have been known to use spinach extracts as performance boosters instead of the banned anabolic steroids..

A recent report from Rutgers University published in *New Scientist* and quoted by a local newspaper this year clearly established its positive role in muscle building in rats. It stated that the people who introduced Popeye, the Sailor Man, with strong biceps in 1919, were indeed ahead of their times. Taking a cue from Popeye, we human beings should learn to include spinach in our food even if the grasshopper is reticent to do so.

Suggested Reading

[1] Behmer, Elias and Bernays, Post-ingestive feedbacks and associative learning regulate the intake of unsuitable sterols in a generalist grasshopper, *The Journal of Experimental Biology* Vol.202, pp. 739–748, 1999.

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