Insect antifeedant properties of an iridoid glycoside: ipolamiide

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Summary. A novel iridoid glycoside, ipolamiide from *Stachytarpheta mutabilis* was identified and found to inhibit feeding by several insect species. It is active at concentrations well below those occurring naturally.

Iridoid glycosides are cyclopentanoid derivatives of terpene origin[2] which have been found in the leaves, fruit, seeds, bark and roots of a number of plants of the following families: Verbenaceae, Labiatae, Callitrichaceae, Buddlejaceae, Scrophulariaceae, Bignoniaceae, Globulariaceae, Acanthacaceae, Pedaliaceae, Martyniaceae, Gesneriaceae, Orobranchaceae, Lentibulariaceae, Myoporaceae, Plantaginaceae, Menyanthaceae, Oleaceae, Hippuridaceae and Loasaceae which all belong to the Tubiflorae[3], together with Rubiaceae, Apocynaceae and Gentianaceae[4] and now, as recently found, Phytolaccaceae[5].

These glycosides all have a bitter taste to humans, which may account for the use of the plants which contain them in traditional medicine. It has also been suggested that they play a role as plant defence compounds[6], though there is no direct evidence of this.

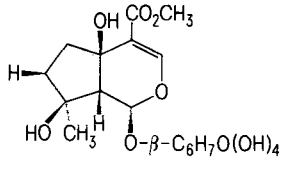
A typical iridoid glycoside from an easily available source was chosen for further study. This compound, ipolamiide, was obtained from the south American *Stachytarpheta mutabilis* (family Verbenaceae), where it occurs at a concentration of 1%. Its structure is shown in the figure.

Tests for antifeedant effects were carried out with the polyphagous caterpillar *Spodoptera littoralis*, the polyphagous acridid *Schistocerca gregaria*, and the oligophagous acridid *Locusta migratoria*.

Methods. The product was presented for feeding tests either on the surface of leaf discs or impregnated in an artificial test disc impregnated with sucrose. For leaf disc experiments, leaves of either *Sorghum bicolor* (for *Locusta migratoria*) or of *Brassica chinensis* (for the other 2 species) were cut out to a uniform size of 2 cm diameter with a corkborer and dipped in solutions of different concentrations of ipolamiide. These were placed in a cool air stream until the solvent had evaporated and then used immediately. Control discs were treated in a similar manner with the solvent only. Amounts added to the leaf discs were estimated by weighing some discs immediately before and after dipping.

The 2 acridids were tested with the artificial discs also. These have the advantage of being more easily standardized. Glass fiber filter papers (GF/A) of 4.5 cm diameter were first prepared by adding known quantities of sucrose solutions to give either 5% or 12% dry weight of sucrose. After drying, discs were treated with known amounts of ipolamiide in solution. On this filter paper 0.4 ml just saturates the disc and the amount of ipolamiide added was calculated to give concentrations of 0.02, 0.1, 0.2, 1.0 and 2.0% dry weight. After evaporation of the solvent, these were checked by weight measurements. The variation was approximately ± 1% of the calculated value.

All tests were carried out on well fed insects approximately half way through the final larval instar. Each insect was placed in a clear plastic box 27 × 15 × 10 cm high with a choice of 2 discs - 1 test and 1 control. The tests were run in the dark at 28 °C for approximately 3 h. At each concentration with each species there were 10–15 replicates. After the test the area of each disc remaining was measured with a Li-Cor electronic area measurer and the amounts of each disc eaten by each insect calculated.



Results and discussion. The most sensitive insect was *Locusta migratoria* which totally rejected the ipolamiide discs at the higher concentrations, and had a significant effect at 0.1% dry weight (table 1). This is not very surprising as this insect is normally graminivorous and is deterred by many secondary plant compounds in families of plants other than the Gramineae[8]. *Schistocerca gregaria* was least sensitive, but it is nevertheless deterred from feeding at the higher concentrations (table 2). *Spodoptera littoralis* was found to feed poorly on the artificial test material so that only tests with leaf discs were carried out. These showed that concentrations above 0.1% dry weight inhibited feeding usually by almost 100%.

The other components present in *Stachytarpheta* leaves have not antifeedant effects, since the raw extract of the plant, containing 10% of the iridoid, is almost 10-fold less active than pure ipolamiide.

In all these cases therefore ipolamiide was found to be a feeding deterrent. Although its effectiveness varied with species of insect, it is likely that the natural concentrations of ipolamiide in *Stachytarpheta mutabilis* would ensure that

Table 1. Inhibition of feeding of *Locusta migratoria* when different concentrations of ipolamiide is added to different feeding substrates

GF/A + 12% sucrose		GF/A + 5% sucrose		Sorghum leaf discs	
Ipolamiide (%)	Inhibition of feeding (%)	Ipolamiide (%)	Inhibition of feeding (%)	Ipolamiide (%)	Inhibition of feeding (%)
2	100	2	100	1.7–2.5	100
1	100	1	100	-	-
0.2	84	0.2	100	0.15–0.24	97
0.1	62	0.1	79	-	-
0.02	0	0.02	51	0.02 (approximately)	29

Table 2. Inhibition of feeding of *Schistocerca gregaria* when different concentrations of ipolamiide is added to different feeding substrates

GF/A + 12% sucrose		GF/A + 5% sucrose		Sorghum leaf discs	
Ipolamiide (%)	Inhibition of feeding (%)	Ipolamiide (%)	Inhibition of feeding (%)	Ipolamiide (%)	Inhibition of feeding (%)
2	96	2	100	1.4–2.1	100
1	52	1	82	-	-
0.2	0	0.2	48	0.18–0.2	43
0.1	0	0.1	11	-	-
0.02	0	0.02	0	0.02 (approximately)	0

this plant is non-preferred or indeed untouched in the field by such insects. Its potential as an applied antifeedant in crop protection requires further work on other insect species, on possible systemic uptake by plants and on its stability in field conditions. It is possible that other iridoids exhibit similar activity as they are structurally very similar.

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Observations on salicyl hydroxamic acid, an experimental trypanocide

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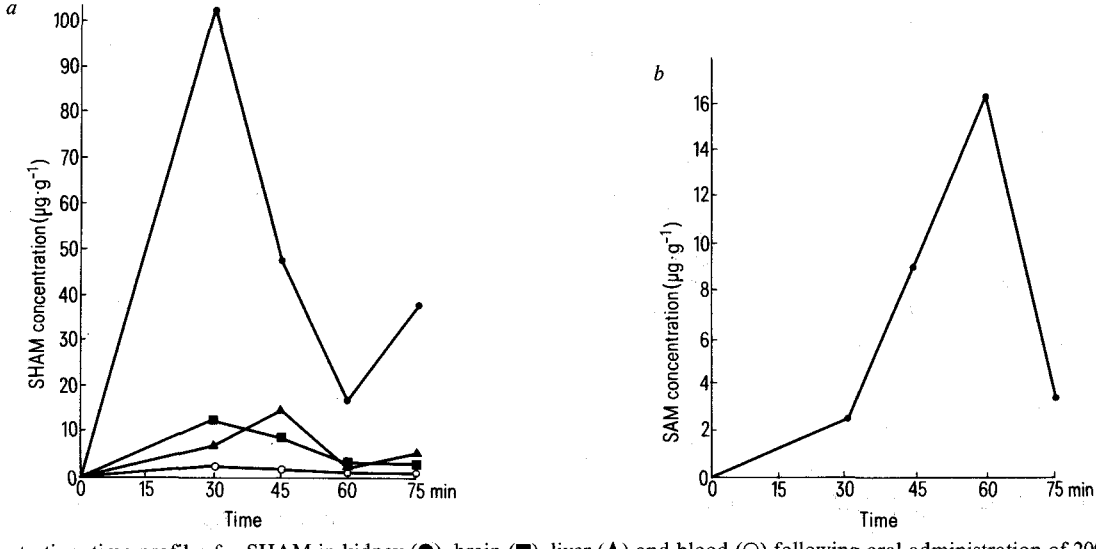
Summary. The inferior in vivo efficacy of salicyl hydroxamic acid against trypanosomiasis may be explained by its short half-life, high degree of protein binding and low tissue levels.

Salicyl hydroxamic acid (2-hydroxybenzhydroxamic acid; SHAM) has been used as an experimental trypanocide since it is a potent inhibitor of the respiration of bloodstream forms of *Trypanosoma brucei* in vitro[1]. Its therapeutic effect in infected rats is less marked[2], both as a single agent and in combination with glycerol, another potent in vitro inhibitor of anaerobic glycolysis in *T. brucei*. Permanent cures in rats infected with *T. brucei* were obtained using a dosage regime which was just sublethal[3]. The plasma concentration of SHAM has been shown to fall rapidly after stopping the administration of the drug[2,3]. We describe here some aspects of the pharmacokinetics and tissue levels of SHAM in mice.

SHAM (a gift from Prof. T. Urbanski, Technical University, Warsaw, Poland) was administered to mice in a dose of 200 mg/kg as a solution (in isotonic saline, pH 7.5) by gavage tube. 5 female, SAS/ICI mice were killed at each of the time intervals 30, 45, 60 and 75 min after administration and samples of blood, liver, kidney and brain taken. Tissue metabolism was halted with 10% trichloroacetic acid and the tissues weighed and homogenized by ultrasonication forming a homogenate at a concentration of 1:4 w/v. 0.3-ml aliquots of these homogenates were extracted and analyzed by the method of Barnicoat et al.[4]. This method of assaying SHAM and its major metabolite, salicylamide (SAM), uses high pressure liquid chromatography and UV-absorbance detection and is very sensitive, accurate and reproducible with a coefficient of variation of 4–6.6% over the concentration range 1–50 µg/ml and a minimum level of detection of 0.1 µg/ml from a 0.1-ml sample for both SHAM and SAM.

Tissue levels of SHAM following oral administration are shown in the figure. Observations from the organs of control animals treated with saline only showed that in the absence of SHAM there are no interfering peaks in the chromatograms. SAM was detected in all samples of tissues



a Concentration, time profiles for SHAM in kidney (●), brain (■), liver (▲) and blood (○) following oral administration of 200 mg/kg to mice (each point is the mean of 5 individual animals). *b* Concentration, time profile for SAM in kidney following oral administration of 200 mg/kg SHAM to mice (each points is the mean of 5 individual animals).