Suggested Pharmacophagy of the African Bushhopper *Phymateus leprosus* (Fabricius) (Pyrgomorphidae, Orthoptera)

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The bushhopper *Phymateus leprosus* (Fabricius) in the field shows a special appetite for the milkweed *Asclepias fruticosa*. Asclepiadaceae, like Apocynaceae and Scrophulariaceae, contain cardiac glycosides. Raw and purified extracts of these plants phagostimulate larval and adult *P. leprosus*. We also screened natural and half-synthetic compounds found in those plant extracts. While saponins and sapogenins did not stimulate the animals, many cardiac glycosides and aglycones, offered on filter paper, proved to be phagostimulants.

Introduction

Various butterflies, grasshoppers and aphids feed on Asclepiadaceae, Euphorbiaceae, Scrophulariaceae, Apocynaceae, or Solanaceae which contain secondary compounds, like pyrrolizidine alkaloids (PAs), cardenolides (CAs) and cardiac glycosides (CGs) which are noxious to many vertebrates. The compounds are stored, either chemically unchanged or enzymatically converted, in tissues and special glands of the insects, protecting them from predators. Typically, these poisonous insects show aposematic colouration (Brower, 1969; Duffey and Scudder, 1972; Rothschild and Reichstein, 1976; Malcolm, 1989; Frick and Wink, 1995). The CGs from the defense glands of the insects and the secondary compounds of the plants they eat are of similar chemical structures. The CAs obtained from the insect bodies and from their secretions show physiological effects similar to compounds found in some arrow poisons of African natives (Whitman, 1990; Neuwinger, 1994).

It has been assumed that also pyrgomorphid grasshoppers of the genus *Phymateus* store noxious substances and become unpalatable to insectivorous mammals and birds (Ebner, 1914; Swynnerton, 1915; Carpenter, 1938; Uvarov, 1977). The well-known bush locust or bush hopper *P. leprosus* is called "Bosstink Springkaan" in South Africa because of its repulsive smell. Larvae and adults of this species eat many different (including cultivated) plants (Taylor, 1956; Smit, 1964, Bishop, 1940; Kevan, 1949; Steyn, 1962; Annecke and Moran, 1982), but particularly they like to feed on *Asclepias* milkweeds and some other plants that contain CAs, CGs as well as genins (Abisch and Reichstein, 1962; Hegnauer, 1964).

Cardenolides are common in plants of the families Asclepiadaceae, Apocynaceae, and Scrophulariaceae, which all seem to be eaten by some aposematic locusts and grasshoppers (Watt and Breyer-Brandwijk, 1962; Bernays and Chapman, 1978; Roth *et al.*, 1994; Neuwinger, 1994). The CAs are glycosides of steroidgenins (aglycones, AGLs) with 23 C-atoms, but only a few special AGLs are basic compounds to all the CAs that frequently occur in tropical and subtropical noxious plants. CAs of African noxious plants and insects have been isolated and analyzed by various authors (Hesse and Reicheneder, 1936; Hesse *et al.*, 1949; Reichstein, 1967; von Euw *et al.*, 1967) who identified as main components two glycosides derived from the CAs of calotropagenin: calotropin and calactin; smaller amounts of calotoxin, uscharin, uscharidin, and voruscharin were also found. According to Reichstein *et al.* (1968), Rothschild and Reichstein (1976), calotropin and calactin found in butterflies and grasshoppers stem from *Calotropis procera* and related Asclepiadaceae.

Abbreviations: CA, cardenolide; CG, cardiac glycoside; AGL, aglycone = genin; PA, pyrrolizidine alkaloid.

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Feeding noxious substances from plants has been named "Pharmacophagy" (Haase 1892, 1893) and redefined recently by Boppré (1984) and Boppré *et al.* (1984) as a feeding behaviour that aims at specific secondary plant substances which are then incorporated as drugs for special purposes.

To test for pharmacophagy of *P. leprosus*, we examined the feeding reactions of larvae and adults towards (1) different plants, particularly those containing CAs, and towards filter papers that were impregnated (2) with plant extracts, (3) with fractions of those extracts, and (4) with pure CAs. As controls we used filter papers that were empty or carried the solvents alone.

Materials and Methods

Insects

Larval and adult *P. leprosus* came from Natal, South Africa. Larvae, caught as 3rd or 4th larval stage, were reared in insect breeding cages (30cm × 35cm × 25 cm) in a glasshouse. Adults were observed in a large cage (160 cm × 156 cm × 210 cm). Room temperature was about 30 °C, the relative atmospheric humidity 40–60%. Light period of the day was from 06.00 to 20.00 h, with artificial light period added to the shorter natural daylight. As basic food we used Chinese cabbage, oat flakes and wheat shoots.

Plants offered

Besides *Asclepias*, we offered various other plants (see Table I) that contain secondary substances which are chemically related to the compounds found in the grasshoppers' preferred food plants.

Preparing plant extracts

Immediately after cutting in Africa some of the fresh material was stored in methanol. Most of the material was dried at 20 °C and kept in deep-freezing boxes and drug glasses. Fresh and dried leaves and stems from different *Asclepias* species and (for comparison) *Nerium oleander* from Central America were ground, pulverized and/or deeply frozen for extraction (Stahl and Schild, 1981; Brower *et al.*, 1982; Wagner *et al.*, 1983; Wagner, 1993). Solvents as well as elution techniques were chosen according to the water content of the material. Highly aqueous material was extracted with methanol and/or distilled water. Repeatedly frozen and thawed aqueous material was eluted with polar solvents more efficiently than fresh material. Fresh and highly aqueous plants gave best elution with MeOH and/or with $HCCl_3$. From dried or slightly aqueous materials the highest amounts of CAs were obtained with $HCCl_3$ – MeOH or $HCCl_3$ – EtOH.

Solvents and reagents

Short-chained alcohols are the best solvents for the CAs and to extract them from plant material. Solvents Uvasol and Rotipur were highly pure for analytical and chromatographic purposes. Methanol, ethanol, chloroform, petrol ether (b.p. 30–60 °C), formamide and acetone were obtained from Merck (Darmstadt), Carl Roth (Karlsruhe), and Sigma (Deisenhofen). Na_2CO_3 and Na_2SO_4 (calcined) were used to purify and dry the solvents and extracts. Distilled or deionized water (Aquapurificator, Miele, Gütersloh) was used for applications with aqueous and organic solvent mixtures. Standard solvents for chromatography (TLC) were mixtures of ethylacetate – methanol (97: 3, by vol.) and of chloroform, methanol, formamide (93: 6:1, by vol.).

Due to osmotic interaction, distilled water eluted at least some CAs from intact plant tissue (suggesting that under natural conditions rain will wash out some CAs, even more likely from damaged plants). Aqueous alcohols with 20 to 30% water proved best for working up the CAs from fresh vegetable tissues. High-polar CGs as well as less polar AGLs were eluted particularly well with these solvents. As a disadvantage, MeOH also resolves a variety of other secondary substances. A mixture of $HCCl_3$ and MeOH proved a good solvent for mildly polar CAs. From dried or slightly aqueous materials CAs were best eluted with $HCCl_3$ – MeOH or $HCCl_3$ – EtOH. These molecules dissolve the outer waxy layers of the leaves, penetrate into the inner cell structures and destroy the fat and lipid-enriched membranes (Wagner, 1993).

Extracts of fresh leaves and stems

Asclepias (physocarpa, syriaca, curassavica), N. oleander, and *D. stramonium* contain highly polar

water-soluble glycosides. Plant material was extracted for 7 days at 20 °C (1) with distilled water, (2) with chloroform – methanol ($HCCl_3$- MeOH; 2:1, by vol.), (3) with methanol purissimum (MeOH).

For all batches it was necessary to decant and filter the raw extracts, then to concentrate the clear solutions at 60 °C by vacuum (Rotavapor, Büchi). Thereafter these extracts were kept and stored at 4 °C in flasks.

Extracts of dried leaves and stems

Plant material of *A. physocarpa* (from S. Africa) and *Nerium oleander* was dried at 100 °C for 24 hours in a drying oven (Memmert). Then 10 g of that material was ground to dust (Pulverisette, Mellert) and shaken at 20 °C with 100 ml aqueous MeOH. The suspensions were kept in darkness for 2 days at 20 °C. After filtration under vacuum and washing the residue repeatedly with 1 liter aqueous MeOH the solvent volume was reduced and concentrated exactly to 500 ml.

Preparative purification and fractionation of CAs

The extracts from *A. physocarpa*, *A. syriaca*, and *N. oleander* obtained with different solvents contained a mixture of CAs (Abisch and Reichstein, 1962; Hegnauer,1964; Rothschild *et al.*,1970; Seiber *et al.* 1982). The crude plant extracts obtained with aqueous ethanol contained a variety of other secondary substances.

Liquid-Liquid partition of CAs from A. physocarpa

The crude extract of 10 g of dry material (powdered leaves, stems and branches with about 1% of blossoms, seeds, and seed capsules) was dissolved in 500 ml MeOH -H_2O (4:1, by vol.) and evaporated *in vacuo*, the dry residue taken up with 200 ml acetone, MeOH, and H_2O (2:2:1, by vol.).

The solution was extracted exhaustingly 5 times with 100 ml petrol ether (b.p. 40–60 °C). This liquid-liquid partition removed the fats and lipid compounds from the aqueous solution almost completely. Then the petrol ether-fraction was re-extracted with 50 ml aqueous MeOH. The main fraction of MeOH-H_2O was eluted 4 times with 100 ml $HCCl_3$. Extraction was finally completed with 100 ml $HCCl_3$ – EtOH (3:2, by vol.). Washing with 40 ml of 1 M Na_2CO_3 solution removed all acidic components from the $HCCl_3$ extract. After washing with 50 ml water and drying with 20 g Na_2SO_4, the purified extract contained most of the CAs.

Feeding tests

The following substances were tested:
AGLs of Scrophulariaceae: digitoxigenin, digoxigenin, gitoxigenin, 3-deoxy-digitoxigenin, gitoxigenin-3-acetate;
AGLs of Apocynaceae: oleandrigenin and ouabagenin.
CGs of Scrophulariaceae: digitoxin, digoxin, gitoxin and acetyl-digitoxin.
CGs of Apocynaceae: oleandrin, acovenoside A, ouabain, helveticoside, neriifolin, convallatoxin and strophanthidin.
Control Sapogenins and Saponins were: diosgenin, yamogenin, hecogenin, gitogenin, digitonin, hederacoside C, ginsenoside Rb1, escinol (Merck and Roth).

Test compounds were obtained from pharmaceutical companies that isolated CGs and AGLs from plants.

The prepared plant extracts were dropped onto 4 cm^2 large filter papers. The impregnated papers were colour-labeled and fixed to branches and brushwood in the test cages, easily accessible for the animals. The number of animals available for the tests varied. The animals were constantly observed for some hours and then controlled daily, and the feeding results recorded.

During feeding tests, wheat shoots where continuously offered *ad lib.* to ensure that the bushhoppers never had to feed from test papers due to hunger. Empty control papers as well as papers with solvents alone remained untouched, proving that the animals needed phagostimulants and did not consume filter paper per se. Test substances that were not eaten were regarded as not to stimulate feeding.

Results and Discussion

As the test substances do not evaporate, the insects possibly could not smell, and choose from a distance. They instead had to respond to any substance upon encounter. Direct observation proved

that they did not serially visit and memorize several papers to finally return to the most promising one. Instead, the animals seemed to move randomly in the cage. In doing so, 90.4% of the test papers had clearly been visited between day 1 and 7 of exposure (Fig. 1). Untouched papers remained exposed to the animals for up to 70 days. A test substance was considered to be accepted and thus active as a phagostimulant if at least 15% of the respective test paper had been consumed.

Accepted food plants

Our caged larvae and adults accepted all the plants offered, though not all parts of them readily (see Table I). *Nerium oleander* was rarely eaten, probably due to the hardness of the leaves. The animals just nibbled at *Hedera helix* and *Taxus baccata*. Both in the field and in our cages, the animals did not exclusively feed from plants that contained noxious secondary substances. (The

Table I. Feeding response of *P.leprosus* towards various plants.
* = greenhouse grown; + = accepted; P = only nibbled.

Species (Family)	Substances contained	Offered parts	Response
Asclepias fruticosa (Asclepiadaceae)*	Gomphoside, Afroside, Calotropin, about 20 Cardenolides	leaves	+
Asclepias syriaca (Asclepiadaceae)*	Syriogenin, Syrioside, Syriobioside about 8 Cardenolides	leaves	+
Convallaria majalis (Convallariaceae)	Convallatoxin, 38 Glycosides from 8 Genins	leaves	+
Cussonia s. spicata (Araliaceae)	Jamaicine – Andirinaglucoside	leaves	+
Vincetoxicum hirundinaria (Asclepiadaceae)	Vincetoxin, α-Amyrin, Triterpene, β-Sitosterin, Sterines, various Cardenolide Glycosides	leaves	+
Digitalis purpurea (Scrophulariaceae)	Purpureaglycoside A + B, about 10 Cardenolides and Glycosides	leaves	+
Eupatorium cannabium (Asteraceae)	Euparin, Echinatin Pyrrolizidine-alkaloids, Eupatoriopicrin	leaves	+
Hedera helix (Araliaceae)	Hederacoside B + C, Germacren, *Hedera*-Saponins, Sesquiterpenes	leaves	P
Nerium oleander (Apocynaceae)	Oleandrin, Digistroside, Stropeside, about 28 Cardenolide glycosides	leaves	+
Polygonatum multiflorum (Convallariaceae)	Homoserinlactone, Chelidonic-acid, Steroid- saponines	leaves	+
Sambucus nigra (Caprifoliaceae)	α-Sambunigrin, cyanogenic Glycosides, Saponines	leaves stems	+ +
Senecio fuchsii (Asteraceae)	Fuchsisenecin, Senecionin, Jacobin, Pyrrolizidine alkaloids	leaves stems	+ +
Senecio nemorensis (Asteraceae)	Senecionin, Jacobin, Pyrrolizidine alkaloids	leaves flowers stems	+ + +
Solidago canadensis (Asteraceae)	Quercetin, Catechine, Tannin, Triterpenic acid, Saponines, Flavonoids	leaves	P
Taxus baccata (Taxaceae)	Taxins, alkaloids	leaves	P
Veratrum album (Melanthiaceae)	Protoveratrin A+B, Germerin, Steroid alkaloids	leaves	+

Table II. Plant extracts accepted for feeding by *P.leprosus* (at least 4 individuals tested).

Solvent	H_2O			MeOH			$CHCl_3$/ MeOH		
Food plant	Dry weight mg/ml	n samples offered	n samples accepted	Dry weight mg/ml	n samples offered	n samples accepted	Dry weight mg/ml	n samples offered	n samples accepted
Asclepias syriaca	0.820	2	2	0.740	26	29	0.630	19	13
Asclepias fruticosa	0.125	2	2	0.105	6	5	0.240	8	8
Asclepias curassavica	0.150	3	2	0.175	5	5	0.205	5	5
Datura stramonium	0.055	2	0	0.035	2	0	0.040	3	1
Nerium oleander	0.530	2	2	0.645	10	10	0.775	6	6

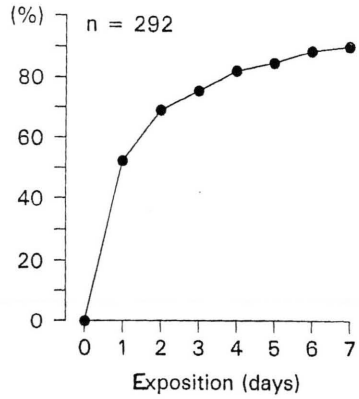

Fig. 1. Cumulative percentages of 292 baited filter paper probes that were accepted (at least 15% consumed) and had thus been visited during exposition days 1 to 7.

plant substances are listed according to Roth *et al.*, 1994). The cabbage tree, *Cussonia s.spicata*, is included in the list because in Transvaal we observed a group of *Phymateus* larvae eating the leaves

Phagostimulants in plant extracts

Secondary substances eluted with distilled water from fresh *A. curassavica*, *A. syriaca*, and *N. oleander*, as well as those substances eluted with MeOH from *A. physocarpa* and *N. oleander* clearly stimulated feeding behaviour. Aqueous as well as methanolic extracts of *Datura stramonium* were not eaten. However, an extract obtained with $HCCl_3$ – MeOH from *Datura* was nibbled at (Table II).

Natural and half-synthetic CAs as phagostimulants

As can be seen from Table III, the CGs and cardiac AGLs offered in various concentrations on filter paper proved to be phagostimulants; exceptions are ouabain, 3-deoxy-digitoxigenin and ouabagenin. None of the saponins and sapogenins were accepted.

Calotropis procera (Asclepiadaceae) contains the following CGs: calotropin, calactin, calotoxin, uscharidin, uscharin, voruscharin, proceroside, and ascleposide. The CAs contained also small amounts of the genins calotropagenin, uzarigenin, coroglaucigenin and corotoxigenin.

Feeding was stimulated by: (1) Most AGLs of Scrophulariaceae and Apocynaceae. (The hydroxyl group at C-3 position seems important for the stimulating effect of AGLs and CGs. The only exception found is 3β-acetyl-strophanthidin.) The CGs of Asclepiadaceae, Scrophulariaceae and Apocynaceae were as stimulating as their AGLs.

(2) A mixture of the glycosides from *Convallaria majalis* as well as pure convallatoxin; also the pure glycosides of Asclepiadaceae, Scrophulariaceae and Apocynaceae.

The sapogenins and saponins as well as the terpenoid escinol did not stimulate feeding in *P. leprosus*.

The results satisfy Haase's (1893) criterion of pharmacophagy for *Phymateus leprosus*. Still un-

Table III. Cardiac glycosides, half-synthetic cardiac aglycones, saponins and their genins (offered in various concentrations) accepted for feeding by *P. leprosus*.

Sec. plant substances	Compounds (µg / 4 cm² filter paper)	µg / 4 cm² filter paper	Formula	Molecular weight	n samples offered	n samples accepted*)
Cardiac glycosides	Acovenoside A	50; 1000	$C_{30}H_{46}O_9$	550.7	2	2
	Convallatoxin	5–1000	$C_{29}H_{42}O_{10}$	550.6	42	42
	Digitoxin	50–1000	$C_{41}H_{64}O_{13}$	764.9	12	12
	α/β-Acetyl-digitoxin	1–500	$C_{43}H_{66}O_{14}$	807.0	8	8
	Digoxin	100–200	$C_{41}H_{64}O_{14}$	780.9	3	2
	Gitoxin	200–500	$C_{41}H_{64}O_{14}$	780.9	3	3
	Helveticoside	100; 200	$C_{29}H_{42}O_9$	534.6	2	2
	Neriifolin	50–500	$C_{30}H_{46}O_8$	534.7	5	5
	Oleandrin	50–1000	$C_{32}H_{48}O_9$	576.7	8	8
	Ouabain	50–1000	$C_{29}H_{44}O_{12}$	584.6	6	0
	K-Strophantoside	100; 1000	$C_{42}H_{64}O_{19}$	873.0	2	2
	CAs (*Calotropis procera*)	5–1000			22	22
	CAs (*Convallaria majalis*)	5–1000			8	6
Cardiac aglycones	Digitoxigenin	50–1000	$C_{23}H_{34}O_4$	374.5	9	8
	3-Deoxy-digitoxigenin	200–500	$C_{23}H_{34}O_3$	358.5	3	0
	Digoxigenin	250; 500	$C_{23}H_{34}O_5$	390.5	2	2
	3β,12β-Diacetyl-digoxigenin	250; 500	$C_{27}H_{38}O_7$	474.6	2	2
	Gitoxigenin	200–500	$C_{23}H_{34}O_5$	390.5	3	1
	3β-Acetyl-gitoxigenin	250; 500	$C_{25}H_{36}O_6$	432.6	2	1
	Oleandrigenin	5–250	$C_{25}H_{36}O_6$	432.6	9	7
	Ouabagenin	50–1000	$C_{23}H_{34}O_8$	438.5	6	0
	Strophanthidin	500; 1000	$C_{23}H_{32}O_6$	404.5	2	2
	3β-Acetyl-strophanthidin	100; 250	$C_{25}H_{34}O_7$	446.5	2	2
Saponins & Sapogenins	Diosgenin	10–1000	$C_{27}H_{42}O_3$	414.6	6	0
	Yamogenin	10–1000	$C_{27}H_{42}O_3$	414.6	6	0
	Hecogenin	10–1000	$C_{27}H_{42}O_4$	430.6	6	0
	Gitogenin	10–1000	$C_{27}H_{44}O_4$	432.6	6	0
	Ginsenoside Rb1	10–1000	$C_{54}H_{92}O_{23}$	1109.3	6	0
	Hederacoside C	10–1000	$C_{59}H_{96}O_{26}$	1221.6	6	0
	Digitonin	50; 2000	$C_{56}H_{92}O_{29}$	1229.3	2	0
	Escinol	10–1000	$C_{54}H_{84}O_{23}$	1101.2	6	0

*) Acceptance was not related to the compound concentration.

published data (by Kasang *et al.*) show that the various cardenolides offered were in fact incorporated by the animals into their repugnatorial secretion. They are thus pharmacophageous according to the criteria outlined by Boppré (1984).

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