Table 1. MODIFICATION OF SUNLIGHT INDUCED KILLING AND MUTATION BY LOW TEMPERATURE AND BY FILTERS, OVERALL SIGNIFICANCE OF THESE FACTORS

	Mutation			Survival		
Comparison	July 22	August 17	August 19	July 22	August 17	August 19
Frozen versus unfrozen	VHS	S at 0·1 per cent	HS	VHS	VHS	VHS
Glass versus silica	S at 0·1 per cent	HS	HS	S at 0·1 per cent	VHS	VHS

Analysis of variance: NS, not significant, $P > 5$ per cent; S, significant, 5 per cent $> P > 1$ per cent; HS, highly significant, $P < 0{\cdot}1$ per cent; VHS, very highly significant, $P \ll 0{\cdot}1$ per cent.

half of each figure represents the number of induced mutations per 1×10^7 surviving cells. Table 1 summarizes the statistical significance of these results.

The results of these three separate experiments can be summarized as follows. (1) Exposure of *E. coli* suspended in phosphate buffer to sunlight resulted in about 40 per cent of the cells being killed in 45 min if energy of wavelengths below about 3100 Å were excluded. If, however, the total solar spectrum at the surface of the Earth was permitted access to the bacterial suspension then about 70 per cent of the cells were killed. (2) Exposure of cells frozen at $-60°$ C to similar conditions resulted in 75 per cent of the cells being killed when light below 3100 Å was excluded. When bacteria were exposed to the complete solar spectrum at the Earth's surface then about 98 per cent of the cells were killed. (3) This remarkable increase in the lethal effects of solar irradiation at low temperature was also paralleled in the greatly increased number of mutations after a 45 min exposure at $-60°$ C. On average, there was a fifteen-fold increase in the number of mutations seen after 45 min of solar irradiation in the frozen as compared with the non-frozen state. (4) Although the number was insufficient to establish statistical significance there was a suggestion of an increase in the number of mutations after 45 min of solar radiation in bacteria exposed in the liquid state at 29° C.

The mechanism responsible for the increased killing and mutation after solar radiation of bacteria at low temperature in the frozen state is complex but amenable to analysis. The present experiments were not designed to analyse these complex factors. Enzymatic photoreactivation could not occur in the frozen state. In nature the amount of killing and mutation would be dependent on the interplay of the lethal and mutagenic processes and the repair mechanisms of which photoreactivation is one. Dark repair could not play any part in modifying damage induced during radiation at low temperature, but as soon as the bacteria were thawed then this mechanism could operate. Whatever the nature of the lesions induced by ultra-violet at low temperature and the parts played by the known enzymatic repair processes, the fact remains that there is a greater accumulation of mutations in bacteria surviving solar radiation at low temperatures in the frozen state than in non-frozen conditions.

It is probable that a small fraction of induced mutations is not detrimental to the activities of bacteria and could therefore play a part in speciation. Thus, the interplay of solar radiation and temperature may be of some importance in the development and selection of micro-organisms. It is proposed to continue these experiments in more detail and in a more propitious climate than that of England.

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Cardenolides (Heart Poisons) in a Grasshopper feeding on Milkweeds

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A North African grasshopper (*Poekilocerus bufonius* (Klug 1882) (Pyrgomorphidae)), with warning coloration and which feeds on milkweeds (Asclepiadaceae), contains cardenolides similar to those found in the plant. These heart poisons which, like digitalis, excite nausea and vomiting are found in the insect's body tissues and can also be ejected in solution from its defensive glands. They thus form part of the grasshopper's defence mechanisms.

REPRESENTATIVES of at least eight orders of insects have been found to feed on Asclepiadaceae (milkweeds) and Apocynaceae[1,2]. These related families include many plants containing glycosides resembling digitalis, with poisonous properties[3,4]. An unusually high proportion of this diverse complex of insects have warning colours (that is, they are aposematic). It has often been suggested, without any proof, that many of these species obtain protection by incorporating into their bodies certain toxic substances found in their food plants[5–8].

Entomologists interested in this particular problem have chiefly specialized in a study of the butterflies (Rhopalocera). Attention has consequently been centred, so far as the milkweed feeders are concerned, on members of the sub-family Danainae and their various harmless and acceptable "Batesian" mimics and even more numer-

ous, distasteful and noxious "Mullerian" mimics. Although at the time the experiment attracted little attention, it was shown as long ago as 1915 (ref. 9) that vomiting was induced in captive birds by feeding them with *Danaus chrysippus* L. and *Amauris* sp., whereas the female Batesian mimic of the former species, *Hypolymnas misippus* L., which does not feed on Asclepiadaceae, produced no such reaction. In view of the discovery (ref. 10 and Rothschild and Parsons, unpublished work) that both sexes of the imago of the Monarch butterfly (*Danaus plexippus* L.) and *D. chrysippus* contain digitalis-like substances, it may now be safely assumed that the old hypothesis is correct and that these butterflies derive at least some of their protective attributes directly from the milkweeds on which they feed. Related genera of Danainae such as *Amauris* Hübner, *Euploea* Fab., *Ituna* Doubleday, etc., also no doubt obtain cardenolides from their food plants, but it is improbable that all the different species of Lepidoptera feeding on the Asclepiadaceae do so. Thus, for example, the Arctiidae (tigers, etc.) of which about a dozen aposematic species have been recorded feeding on these plants, are distasteful moths, several of which are known to secrete other poisonous substances not derived from their food plants[11–13].

Aposematic insects benefit from the presence of other aposematic insects and also obtain certain advantages from congregating on protected plants, which, for instance, are not eaten by grazing herbivores. Furthermore it has been shown[14] that caterpillars can obtain protection from lizards if their gut temporarily contains distasteful food plants. These factors tend to unite and bring together aposematic insects in both time and space. It is probable that Arctiidae and Ctenuchidae, for example, feed on Asclepiadaceae because these moths are poisonous and aposematic, rather than that they are poisonous and aposematic on account of toxic properties obtained directly from these particular food plants.

There seems little doubt that certain insects are pre-adapted to feed on poisonous plants, possibly by the insensitivity of their enzyme systems, or by the ability to inactivate toxins by conjugation, or to destroy and excrete them with exceptional efficiency. Such mechanisms may be characteristic of species feeding on poisonous foliage which are cryptic (their form and coloration being adapted for concealment) and generally acceptable to predators. The crypsis exhibited by Sphingid (hawk-moth) larvae feeding on such plants as *Nerium oleander* L. and *Nicotiana tabacum* L. indicates that they belong in this category. It is a striking fact that virtually no insects feeding consistently on tobacco are aposematic, suggesting that nicotine is too general a poison to be incorporated into their tissues, and thus does not enable them to exploit the aposematic way of life. *Protoparce sexta* (Johan.) = (*Manduca sexta* Johan.) (Sphingidae) is extremely resistant to nicotine and it is now known[15] that the alkaloid absorbed while the caterpillar is feeding on the tobacco plant is rapidly eliminated when feeding ceases. Other moths (Lepidoptera) such as *Prodenia eridania* (Cram.) and the grasshopper *Melanoplus differentialis* (Thom.) (Acridoidea) can produce a large number of alkaloid metabolites from nicotine[16].

The relationships between both aposematic and cryptic insects and their plant hosts are seen to be varied and any form of generalization about such relationships appears premature. It is necessary to investigate each case as a special situation.

The Grasshopper *Poekilocerus bufonius*

This North African species[17] in nature probably feeds exclusively on Asclepiad plants. Despite its tendency to hide among foliage, or to remain motionless on the ground, it is basically an aposematic insect, dark bluish grey with yellow spots, orange hind-wings and with a leathery and resistant integument. It has a bilobed poison gland[18] the opening of which is situated dorsally (between the first or second terga), both in the hopper (immature stage) and in the adult. The hoppers can eject the contents of this gland in a well directed double jet by arching the body and "firing" over their lowered heads at the objective, up to a distance of 60 cm. In the adult, on the other hand, the secretion flows down a shallow groove between the first and second tergites on either side of the body, until it passes over the opening of the second abdominal spiracle. Air is forced out of the spiracle and, when mixed with the sticky gland secretion, forms a white, milky, rainbow-tinted foam which froths along the sides of the body. In the sunshine this foam is very bright and conspicuous, contrasting well with the bluish black colour of the body and adding to the aposematic effect. The secretion has a pungent odour which contains an element strongly reminiscent of the food plant and presumably has a warning character. It produces an acrid pricking sensation on the tongue. In the hopper (immature) stages the secretion is watery, not milky, and blood cells of varying types are present which clot in the defensive fluid under a coverslip, but such blood cells are lacking in the adult foam.

Specimens of *P. bufonius* collected in Israel in April 1962 were flown to England, and their glandular secretion was examined for pharmacological activity[19].

The Pharmacology of the Grasshopper

Gland fluid was obtained from hoppers of stages III to IV and from adult grasshoppers by gently nipping in tared filter paper. Each fresh insect typically yielded 100–200 mg of secretion, which was slightly acid (*p*H 5.5–6·0 on narrow-range indicator paper).

The fresh gland fluid and aqueous extracts of whole insects were tested on the isolated guinea-pig ileum, both alone and in the presence of specific inhibitors (mepyramine and hyoscine). Further pharmacological investigation of the nature of active substances in the insect was carried out by testing its effects on the blood pressure and electrocardiogram of anaesthetized cats, and on isolated preparations of the hamster colon, the rat stomach fundus, the frog rectus abdominis muscle, and the frog heart. Chemical tests of the nature of active substances were also applied, by re-assay after boiling with acid and alkali and after incubating with proteolytic enzymes, and by subjecting material to high voltage electrophoresis, paper chromatography and solvent extraction. The methods and results of these experiments will be published in detail elsewhere.

It was found that the secreted fluid contained about 1 per cent by weight of histamine (as the dihydrochloride), both when obtained from insects fed *Asclepias* and when collected from this species reared on Compositae. This is a concentration similar to that found in the venom of bees and wasps[20]. Fluid from insects fed *Asclepias* also contained a digitalis-like toxin—each insect contained about one cat lethal dose. Fluid secreted by the grasshoppers fed on Compositae showed less than one-tenth of this amount of digitalis-like activity; the amount was not estimated accurately. It was found that both the digitalis-like toxin and the histamine retained substantially unaltered activity when dried on filter paper; they could be recovered largely separated, by soxhlet extraction with dichloromethane and ethanol respectively.

In order to determine whether the digitalis-like toxin was confined to glandular fluid, haemolymph was obtained from *Asclepias*-fed grasshoppers by absorption on to slips of filter paper inserted through a small abdominal incision. It proved to contain digitalis-like toxin in concentration of the same order as that in the glandular secretion. Its histamine content, however, was less than one thousandth of that in gland fluid.

A saline extract of whole insects fed *Asclepias* was tested for acetylcholine, 5-hydroxytryptamine, irin-like substances and toxins of high molecular weight, but none of these was found in detectable amounts.

Since *P. bufonius* proved to contain such a high concentration of digitalis-like toxin in the haemolymph, the lethal dose of a cardiac glycoside for this insect was determined by injecting saline solutions of ouabaine through the abdominal wall. It was found that the LD_{50} was 2,000 mg/kg (Parsons, J. A., and Summers, R. J., unpublished work). For comparison, the LD_{50} of ouabaine was similarly determined in *Locusta m. migratorioides* (R. and F.) and in *Schistocerca gregaria* (Forsk.). In both cases it was found to be only 7 mg/kg, showing that *P. bufonius* was about 300 times less sensitive to digitalis than the two locusts.

The cardioactive toxin could be completely extracted from watery solution by dichloromethane. It was shown to be truly digitalis-like by the criteria described elsewhere for toxin from the Monarch butterfly. This extractable toxin was purified by column partition chromatography, using the system dichloromethane/*cyclo*hexane/ethylene glycol on 'Celite'. Parsons[19] has given a preliminary account of the isolation of two components, one of which was obtained crystalline. These were provisionally named poekilocerin *A* and *B*.

Isolation and Identification of the Cardenolides

P. bufonius was reared from the egg in cages in the Department of Zoology, University of Tel-Aviv, Israel. Secretion from adults was collected directly on to filter paper, while hoppers were induced to eject their secretion into watch glasses and the fluid then absorbed on filter paper. It was found that hoppers replenished the volume of fluid in their poison glands within 8–14 days, after which they could be "milked" for a second time. The total amount of fluid obtained during a milking was counted as one discharge. No comparison was made between the cardenolide content of the first and subsequent discharges. Dried filter papers bearing the various secretions were sent together with other material from the insects by air mail to Basle, and were stored (for not longer than 6 months) at 0° C before being worked up.

The following materials were examined: (1) Dried fluid from 197 discharges from adult animals, which had been fed on *Calotropis procera* L. and *Asclepias curassavica* L. (2) Dried fluid from 1,150 discharges from hoppers (stages III–V) reared on the same Asclepiadaceae. (3) Dried fluid from 217 discharges from hoppers (stages III–V) which were fed only on non-poisonous plants: *Portulaca oleracea* Hook., *Lactuca scariole* L., *Crepis aspera* L., and *Daucus carota* L. (*P. bufonius* feeds on these plants in captivity, but only when the Asclepiad species are not available.) (4) Dried fluid from 200 discharges from hoppers (stages I–II) fed only on non-poisonous plants, the parents of which had also been reared on a non-poisonous diet. (5) 19·8 g of eggs (equivalent to about 1,900 eggs) and sixteen whole pods (secretion mixed with sand adherent to the pods) were also sent to Basle. The eggs, from which the secretion and sand were removed by sieving, were sealed *in vacuo* in a glass ampoule and stored at 0° C until required. (6) The sand, together with the dry secretion, which was separated from the eggs by sieving (weight 29·8 g). (7) 5·6 g of exuviae (cast skins) from various age groups. The exact number could not be determined.

Following the procedures described previously[21] with certain minor modifications, each sample of material was extracted with a mixture of water and methanol. The eggs (sample 5) were homogenized with methanol. The methanol was evaporated *in vacuo* from each crude extract and the aqueous suspensions successively extracted with organic solvents of increasing polarity: *a*, petroleum ether; *b*, diethyl ether; *c*, chloroform; *d*, mixtures of chloroform and ethanol.

Table 1 shows the weight of solid material contained in each extract and the results of testing for cardenolides by the reaction with tetranitrodiphenyl[22], by paper chromatography and by thin layer chromatography.

The bulk of the cardenolides were present in extracts *b* and *c*. Extracts of type *d* contained small quantities of highly polar cardenolides, which probably included products formed through autoxidation. Only extracts *b* and *c* were further separated on a preparative scale. Paper and thin-layer chromatography revealed that both contained, preponderantly, two cardenolides. These were isolated by column chromatography on silica gel. The less polar of the two was obtained in pure crystalline form from all samples except number 6. It was identical with "poekilocerin *B*" and could further be identified with the known substance calactin[23,24]. The second, slightly more polar substance, was isolated in pure crystalline form from samples 2, 3, 4 and 7. Amorphous concentrates, identical on paper and thin-layer chromatography with those from 2, 3, 4 and 7, were obtained from the other samples. The crystallization of this second cardenolide from concentrates is more difficult and success is somewhat a matter of chance. Chromatography showed it to be identical with "poekilocerin *A*" and it could further be identified with the known substance calotropin[23–25]. Traces of other cardenolides (not, so far, definitely identified) were obtained in crystalline form from the eggs and cast skins (exuviae). The amounts of calactin and calotropin obtained are listed in Table 1. A rough estimate of the quantity in each discharge is also given, as well as the probable total content of the cast skins (exuviae).

The properties of the two pure cardenolides isolated from *P. bufonius* are given in Table 2. The crystals contained solvent of crystallization. Their melting points are therefore in reality decomposition points

Table 1

Series	(1) Material, number of animals and stages, and diet		(2) Petroleum ether extract	(3) Ether extract	(4) Chloroform extract	(5) Chloroform ethanol-(3 : 2) extract	Amounts found (mg)	(6) Calactin	(7) Calotropin
1	Dried secretion, 197 adults on Asclepiadaceae	*a*	54·0	66	131	142	Total	28	Present
		b	−	+ + +	+ + +	(+)		−	−
		c	0·274	0·325	0·666	0·72	Per secretion	(0·2)	(0·2)
2	Dried secretion, 1,150 hoppers III–V on Asclepiadaceae	*a*	103·5	256·5	203	139	Total	138·5	11·5
		b	−	+ + +	+ + +	+		−	−
		c	0·09	0·223	0·177	0·12	Per secretion	(0·14)	(0·06)
3	Dried secretion, 217 hoppers III–V on non-poisonous diet	*a*	34	30	16	72	Total	0·9	0·7
		b	—	+ +	+ +	−		−	−
		c	16	0·14	0·74	0·33	Per secretion	(0·01)	(0·01)
4	Dried secretion, 200 hoppers I–II parents already on non-poisonous diet	*a*	32	7	4	26	Total	0·1	0·05
		b	−	+	+	−		−	−
		c	16	0·035	0·02	0·13	Per secretion	(0·002)	(0·001)
5	19·8 g eggs (*ca.* 1,900) from 16 animals on Asclepiadaceae	*a*	1,530	112	360	110	Total	18	Present
		b	−	+ + +	+ + +	(+)		−	−
		c	0·8*	0·059	0·19	0·058	Per secretion	(0·01)	(0·01)
6	29·8 g sand with secretion from 16 egg pods	*a*	24	10	5	50		Trace	Trace
		b	—	−	+	−			
7	5·6 g cast skins (exuviae)	*a*	19	50	39	64	Isolated crystals (estimated total content)	23·8 (35)	9·5 (25)
		b	−	+ + +	+ + +	+			

* Orange and partly crystalline.

For each batch of material tested the total weight of material contained in each successive extract is given in line *a* and is recalculated as weight per discharge (per animal) or per egg in line *c*. Line *b* shows the results of applying the tetranitrodiphenyl reaction to the extracts.

The amounts of crystalline calactin and calotropin obtained from each batch are shown in columns 6 and 7 on line *a*, while line *c* gives (in parentheses) a rough estimate of the approximate content per animal or per egg. When cardenolides not obtained crystalline were identified only by paper chromatography and thin-layer chromatography they are shown simply as "present".

Table 2. MELTING POINTS (*Kofler* BLOCK CORR.) AND ROTATIONS

Compound	Isolated from	Crystallized from	M.p. (decomposition)	$[\alpha]_D$ Methanol	Crystal shape
Calactin	*P. bufonius*	Acetone-ether	261°–264° C	+64·5° ± 3°	Platelets
Calactin (Hesse)	*Calotropis*	Acetone-ether	262°–267° C	+67·3° ± 3°	Platelets
Calactin	*Pergularia extensa* (ref. 24)	Methanol-ether	270°–272° C	+70·4° ± 3°	Platelets
Calotropin	*P. bufonius*	Methanol-ether	215°–220° C	+65·3° ± 2°	Granules
Calotropin	*Calotropis procera* (ref. 25)	Ethanol (values in literature)	221° C	+55·7° ± (hydrate)	
Calotropin	*Calotropis procera* (ref. 33)	Chloroform (values in literature)	221° C	+66·8°; 65·6°	

and are largely dependent on the method of determination, on the crystal size, and on the solvent from which they were crystallized.

Crout *et al.*[26] doubted the existence of calactin, and Hassal and Reyle[27,28] as well as Crout *et al.*[29,30] have apparently confused it with uscharidin. We therefore include the properties of an original sample of calactin obtained from Hesse, who isolated it from *Calotropis procera* as the chloroform compound[23,25]. From this we obtained the high melting chloroform free form by crystallization from methanol-acetone or methanol-ether. It gave only one spot on paper and thin-layer chromatography (mobilities as in Figs. 1 and 2). The sample was kept sealed *in vacuo* for several years and had remained unaltered. Hesse *et al.*[23] reported for calactin a melting point of 220° C and decomposition at 275° C and $[\alpha]_D = +48°$ in methanol; the rotation value is, apparently, an error.

Identification of our compounds with original material from *Calotropis procera* is, in addition, based on an unchanged mixed melting point (although this has very little confirmatory power in this particular case), paper chromatography (Fig. 1), thin-layer chromatography (Fig. 2), infra-red spectra and colour reactions with sulphuric acid.

The six principal calotropis cardenolides (see later) can easily be distinguished by a combination of paper (Fig. 1) and thin-layer chromatography (Fig. 2).

Hesse *et al.*, in a series of investigations, showed that the latex (and leaves, so far investigated) of *Calotropis procera* contains the following six cardenolides:

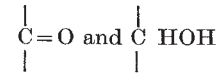
Voruscharin	$C_{31}H_{43}O_8NS$	(refs. 37 and 38)
Uscharin	$C_{31}H_{41}O_8NS$	(refs. 23, 39 and 40)
Uscharidin	$C_{29}H_{38}O_9$	(refs. 23, 32 and 39)
Calactin	$C_{29}H_{40}O_9$	(ref. 23)
Calotropin	$C_{29}H_{40}O_9$	(refs. 23 and 25)
Calotoxin	$C_{29}H_{40}O_{10}$	(refs. 23 and 41)

These six substances are closely related and they all contain the same aglycone (calotropagenin). The first three in this list can be converted into one another[23,38–40]. The conversion of uscharidin into calactin and calotropin has also been reported[23,33].

In addition to *Calotropis procera* our grasshoppers were fed on *Asclepias curassavica* L. The latter species is indigenous to Central and South America but has been introduced into various sub-tropical countries including Israel. From the leaves of this plant (from Brazil) Tschesche *et al.*[42,43] isolated seven crystalline cardenolides, including a trace of calotropagenin, but did not find any of the aforementioned calotropis glycosides. We have, however, recently received from Professor L. P. Brower a portion of dried leaves of *Asclepias curassavica* grown in hothouses from seeds obtained mainly in Trinidad. A botanical (flowering) specimen of this plant was checked by Mr. A. A. Bullock, who confirmed the determination. Santavy *et al.*, in a preliminary analysis to be published elsewhere, were able to isolate from this plant, uscharin, uscharidin, calactin, calotropin and calotoxin in crystalline form, together with a smaller quantity of other cardenolides. We hope to investigate the discrepancies between our own results and those of Tschesche *et al.*, but for the moment it is only necessary to note that *Asclepias curassavica* is a very rich source of calotropis glycosides.

Another indigenous plant eaten by *P. bufonius* in nature is *Pergularia tomentosa* L. This plant has not apparently been investigated chemically. It is interesting, however, that Mittal *et al.*[24] found calactin, calotropin and calotoxin in the closely related *Pergularia extensa* (Jacq.) N.E. Br.

Crout *et al.*[26,29] have suggested the partial formula I for calotropin. If this is correct, calactin too should have this or a very similar partial formula. Presumably they are differentiated from one another only through stereoisomerism at C-3′ or through interchange of



between C-2′ and C-3′, a suggestion which will be discussed elsewhere.

Only calactin and calotropin out of six closely related cardenolides of *Calotropis procera* were found in appreciable quantities in the poison gland of *P. bufonius* fed on this plant and *A. curassavica*. It is possible, therefore, that they are absorbed selectively by the grasshopper and that the other four are rapidly excreted or metabolized. An alternative possibility is that cardenolides such as uscharin, present in the food plant but not in the insect, may be converted *in vivo* into calactin and calotropin, just as Hesse *et al.* demonstrated *in vitro* for voruscharin, uscharin and uscharidin. Although insects with few exceptions[44,45] appear unable to synthesize steroids, they are capable of transforming them[46,47,50] and can even carry out the synthesis of farnesol from mevalonic acid[48,49] which is a step in the known synthetic pathway to steroids.

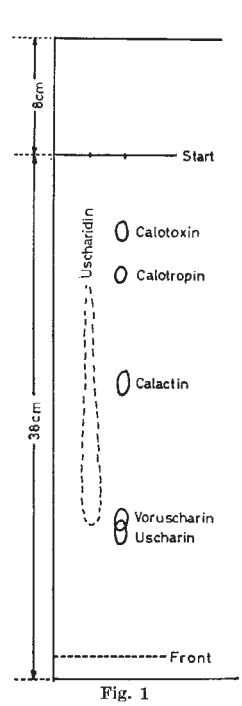


Fig. 1

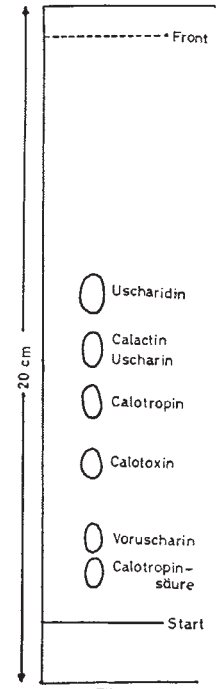


Fig. 2

Fig. 1. Paper chromatogram. Descending, schematic but true to scale. Paper impregnated with 35 per cent formamide[31]. Solvent system: benzene-tetrahydrofurane-(1 : 1). Duration: 2·5 h. Developed with 2,2′,4,4′-tetranitro diphenyl[22]. As Hesse *et al.*[32] emphasize, pure uscharidin always shows severe tailing in formamide systems. (See their figure, ref. 33.)

Fig. 2. Thin-layer chromatogram, ascending[34,35] on lined glass[36] with silica gel *HF* 254. Grain size 2-25μ. Solvent system: ethyl acetate stored for about 4 weeks after distillation. Developed by spraying with 20 per cent *p*-toluene sulphonic acid in ethanol followed by heating at 120° C.

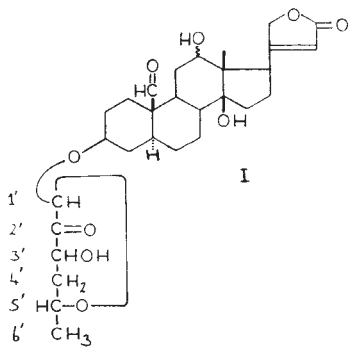


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Fig. 3. Calotropin : hypothetical formula.

Our experiments indicate that *P. bufonius* obtains the cardenolides in its poison glands directly from the food plant. Although the secretions of hoppers reared on a non-poisonous diet (Table 1) contained cardenolides, the quantity present was approximately ten times less than in those reared on Asclepiad plants. A second generation of hoppers descended from animals reared on a non-poisonous diet had a level further reduced by a factor of approximately seven (an exact comparison is not possible because only secretions of stages I–II of these hoppers were available). If no synthesis occurs in the insect the amounts present in the fluid of the defensive gland of both the first and second generation of hoppers reared on non-Asclepiad plants are unexpectedly high, even allowing for an exceptionally efficient storage mechanism from the egg to the hopper stage. These grasshoppers were not, however, reared in isolation. They are known to consume one another, and eat each other's cast skins (exuviae), which are rich in cardenolides, and it is therefore possible that the II–IV instar survivors which were tested by us had added to their original store of cardenolides by cannibalism and scavenging.

Feeding experiments with captive predators (refs. 18, 51, and Rothschild, M., unpublished work) have shown that *P. bufonius* has at least four separate lines of defence apart from its warning coloration: (1) the ejection of a jet or foam of defensive fluid containing cardenolides and histamine; (2) a penetrating and disagreeable odour which can be perceived (by the human observer) from a distance of several metres; (3) an acrid prickling sensation when the fluid is brought into contact with the "human" tongue; (4) cardenolides in the tissues of the body.

It was found that certain inexperienced predators could be repelled by the first, second or third of these defence mechanisms (ref. 18 and Rothschild, M., unpublished work) whether the grasshoppers were reared on *Asclepias* or on other plants. On the other hand, some species, such as white mice and the jay (*Garrulus glandarius* L.), learned to avoid *P. bufonius* only after eating specimens reared on *Asclepias* and apparently experiencing disagreeable after-effects, which in the case of the jay included vomiting[51]. Certain other insectivorous predators, for example, the European hedgehog (*Erinaceus europaeus* L.), are comparatively insensitive to cardenolides and various other toxins (Parsons, J. A., and Summers, R. J.) and unhesitatingly ate one to three whole adult *P. bufonius*, reared on *Calotropis*, daily (for 7 days) without apparently experiencing any ill effects.

It is known that the vomiting caused by digitalis or other cardenolides after absorption into the blood stream is due principally to an action on the central nervous system. Another important extracardiac action of digitalis is to contract intestinal smooth muscle and to increase its rhythmic activity. A local action of this type may account for the fact that vomiting was observed a few minutes after birds ingested *Danaus plexippus* and *D. chrysippus* (ref. 9 and Rothschild, M., unpublished work). Some species may be more sensitive to the local than to the central action of cardenolides.

Although the cardenolides can prove lethal if ingested, vomiting usually intervenes before this stage of intoxication is reached. It would seem, therefore, that the presence of cardenolides in the body tissues provides an excellent protection for the aposematic insect. After a single experience a predator, even if not initially deterred by the defensive spray, or evil smell and taste of the defensive gland secretion, would learn to associate them with the after-effects of ingesting the poisons.

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