J Comp Physiol B (1993) 163:123–132

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Biogenic amines and phenolics characterize the defensive secretion of saturniid caterpillars (Lepidoptera: Saturniidae): a comparative study

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Accepted: 24 November 1992

Abstract. The morphology of the scoli of *Eudia pavonia*, *Saturnia pyri* and *Eupackardia calleta* last instar caterpillars has been clarified. Chemical and biochemical comparisons of scolus secretions and corresponding caterpillar haemolymphs indicate two differing defensive strategies: *Eudia* and *Saturnia* contain phenolic and related compounds, whereas *Eupackardia* additionally synthesizes biogenic amines (e.g. neurotransmitters) in considerable amounts. For most aromatic compounds from the caterpillars a theoretical biogenetic scheme is proposed. Deterrent effects of all secretions and most haemolymphs on particular predatory ants were ascertained.

Key words: Aromatic metabolism – Biogenic amines – Chemical defense – Defensive secretion of caterpillars – Saturniidae

Introduction

Caterpillars of the lepidopteran family Saturniidae (Emperor moths) are favourites of collectors since they produce colourful, often very large moths with big eyespots. Their caterpillars are also attractive, many of them (especially in the subfamily Saturniinae) being covered with yellow, red or blue outgrowths of the integument ("scoli") bearing bristles. It is well known that these caterpillars, especially in the last instar, can excrete a fluid from these scoli on irritation. To date few attempts have been made to examine the exact morphology of the scoli and the chemistry and biological significance of the secretions by means of modern techniques. Recently some morphological details about the scoli of last instar caterpillars of the Palearctic Emperor moth, *Eudia* (= *Saturnia*) *pavonia* (L.) have been published (Deml and Dettner 1990). At the same time the presence of proteins and various aromatic compounds in both secretion and HL has been demonstrated for the first time in this species. Initial biological tests of both body fluids of this species revealed a fumigant and toxic activity of these fluids and several compounds therein.

In order to assess whether aromatic compounds generally characterize chemical defensive secretions and HLs of saturniid caterpillars, we have undertaken further chemical and morphological investigations of two other saturniids, the Palearctic Great peacock moth (*Saturnia pyri* Schiffermüller) and the North American species *Eupackardia calleta* Westwood. The species *E. pavonia* was also investigated in more detail.

It became evident that different evolutionary strategies are realized in saturniid secretions, which include production of either phenolic compounds or additional biogenic amines. Having outlined a hypothetical metabolic scheme for these caterpillar aromatics, we were subsequently able to demonstrate the presence of several missing intermediates. For assessing the biological significance of saturniid GSs and HLs on ants, which seemingly represent major predators of the caterpillars, feeding deterrent effects of both body fluids were determined against the ants *Lasius niger* L. (laboratory experiment) and *Formica pratensis* Retz. (field experiment).

Materials and methods

Insects. Living specimens of *Saturnia pyri, Eupackardia calleta* and *Eudia pavonia* were obtained commercially and the larvae (ex ovo) were fed with only one foodplant species in each case for their whole lives: *E. pavonia* larvae on *Crataegus monogyna* (whitethorn) or *Prunus spinosa* (sloe); *S. pyri* on *Prunus spinosa* and *E. calleta* on *Ligustrum vulgare* (privet). The animals were reared in plastic boxes at 22 °C. Last instar caterpillars were killed by freezing and stored at −20 °C until used. Secretion was obtained by strong mechanical stimulation of living caterpillars or defrosting the frozen larvae and pressing the scoli and underlying integument gently with forceps. Extravasated secretion was sucked up with a glass capillary. To sample the HL a small cut was made with a scalpel in the integu-

Abbrevations: CI, chemical ionisation; EI, electronimpact ionisation; GC-MS, gas chromatography-mass spectrometry; GS, gland secretion; HL, haemolymph; PMSF, phenylmethylsulphonyl fluoride; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, scanning electron microscope;

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ment beyond the scoli and the HL droplets sucked up with a glass capillary.

Morphology of scoli. Scoli were excised from the caterpillar integument, macerated in 5% KOH for 24 h (all species) or in 2% KOH for 5 days *(E. pavonia)*, dried in acetone and prepared for SEM by critical point drying and sputter coating with gold. L2 larvae of *E. pavonia* were not macerated; they were dried in a vacuum desiccator above P_2O_5 for 3 days and sputter coated. SEM was carried out with a Stereoscan 90 scanning electron microscope (Cambridge Instruments).

SDS–PAGE of caterpillar body fluids. Immediately after taking the body fluids PMSF was added to prevent protease activity. After SDS-treatment caterpillar secretion and HL were centrifuged at 14,900 × *g* for 15 min and the supernatant diluted with TRIS/HCl buffer (pH 8.0) to 1 : 10 prior to electrophoresis. Electrophoresis was carried out using the PhastSystem and PhastGel Gradient 8–25 (both Pharmacia). For reference a LMW Electrophoresis Calibration Kit (Pharmacia) solution was run on the same gel. The gel was developed by staining with Coomassie brilliant blue R 350 dye.

Tyrosinase assay. An observed darkening of secretions within a few minutes indicated the probable presence of tyrosinase and formation of melanin. For enzyme assay secretion was mixed 1 : 1 (v/v) with a saturated solution of *p*-cresol in phosphate buffer (pH 7.0) and colour development followed (Blaich 1978).

Gas chromatography – mass spectrometry (GC–MS). Caterpillar body fluids were analysed by transferring them from the capillary onto a Solid Injektor SI 1 (SGE) syringe and injecting into a Carlo Erba GC 6000 Vega gas chromatograph containing a 12-m or 14-m glass capillary column FS–OV 1701 (Chrompack) coupled to a Finnigan-MAT ITD mass spectrometer. Temperature program: 50–260 °C (10 °C · min^{-1}), 10 min isotemperature, 260–280 °C (5 °C · min^{-1}), 5 min isotemperature; carrier gas: helium. Electron impact ionization (EI) and chemical ionization (CI, reactant gas methanol) mass spectra were obtained in total ion chromatograms and compared to mass spectral data of the NBS library and the mass spectra registries of Stenhagen et al. (1974) and McLafferty and Stauffer (1989). For confirmation of supposed compounds, authentic chemicals were injected and retention times and mass spectra compared.

Derivatizing procedures. For detecting non-volatile compounds in HLs and secretions by GC–MS both fluids were derivatized. For most amines and for polyalcohols the body fluids were acetylated by reaction with a 1 : 1 mixture (v/v) of acetic anhydride and pyridine for 4 h under boiling and reflux conditions; subsequently the reaction mixture was injected directly into the GC. For aromatic acids in *E. pavonia* and *S. pyri* the fluids were treated with diazomethane, the resulting methyl esters dissolved in ethanol and analyzed. For aromatic amino acids in *E. pavonia* and *S. pyri* the body fluids were first treated with diazomethane and subsequently acetylated to reach the necessary volatility. Authentic substances were treated in the same ways.

To quantify the catecholamines the peak areas determined in substance-typical fragment mass chromatograms of acetylated caterpillar body fluids and authentic chemicals were compared.

Ant feeding deterrence test. Forty *Lasius niger* workers were collected from each of five field nests and placed in petri dishes (i.d. 13.7 cm), 20 per dish; these ten dishes were used for all tests (ants total $n = 200$). The edges of the dishes were painted with "fluon" to prevent the ants escaping. A glass slide was added in the middle of the dish and a 12.5-cm filter paper placed on it. Ants were fed only once soon after capture with honey water; subsequently they were starved and given only water. The filter paper was moistened occasionally. Ants were tested only once a day at the same time in a darkened room. Half an hour before testing the filter paper and the slide were taken out of the dish. A 5-µl drop of a control suspension – triturated entrails of *Tenebrio molitor* larvae (meal worms) diluted 1 : 3 with water – was placed on one half of the slide. A 5-µl drop of a mixture (1 : 1, v/v) of the diluted meal worm suspension and the caterpillar body fluid of interest was pipetted onto the other half. The trial was started by returning the slide to the middle of the petri dish. Ants feeding on the drops were counted at 1-min intervals for 10 min. Every caterpillar GS or HL was tested once with all ten dishes. The orientation of the glass slides was changed regularly from dish to dish within each test series to avoid side preference of the ants.

This test was based on the method of Hilker and Schulz (1991) with several alterations. Statistical evaluation was performed for every minute using the Wilcoxon matched-pairs signed-rank test for the two-tailed case (Sachs 1984). Field observations with *Formica pratensis* workers were made to supplement the laboratory tests.

Results

Morphology of scoli

SEM photographs of the scoli surface of *Eudia pavonia*, *Saturnia pyri* and *Eupackardia calleta* caterpillars (Fig. 1a, c, e) show that these body outgrowths are covered by many bristles with open tips. Haffer (1921) described these globular scoli in conjunction with their radially arranged bristles as "Sternwarzen" ("star warts") in several species of Saturniidae. In view of the delivery of secretion, all three kinds of scoli also belong to the type "Sekretstechborstenscolus" [scoli with secreting bristles; Nässig (1989)]. As can be seen under the binocular microscope the secretion flows out from the bristles' openings and may remain as a transparent droplet at their tips, evaporating continuously. Otherwise, the secretion runs down to the base of the bristle so that after the animal has been severely disturbed the whole scolus surface is covered with fluid.

In *E. calleta* (Fig. 1a) the bristles seem to be broken merely by mutual contact of the caterpillars or on rubbing along the foodplant twigs. Many of the scoli of *S. pyri* (Fig. 1e) possess a central hair that differs from the surrounding bristles. It is distinctly elongated and bears a thickened tip without an opening ["Kolbenhaar" or "club hair", Haffer (1921)]. *E. pavonia* L2 caterpillars possess closed bristles which can be broken off under mechanical strain. If such cut bristles are inspected interiorly by means of the SEM without maceration one can discern that they are completely filled with a hardened material (presumably dried protein) which may contain bubbles. SEM photographs of the interior scoli surfaces (Fig. 1b, d, f) show that the bristles are hollow. On moderate KOH maceration *(S. pyri, E. calleta)* one cuticular sac per bristle can be seen to enclose the inner bristle opening (arrows in Fig. 1b, f). The sacs seem to represent a kind of cuticular secretion reservoir located inside the individual gland cells of the scolus.

The central "Kolbenhaare" of *S. pyri* caterpillars differ from other bristles by possessing apparently smaller sac-like internal outgrowths. In *E. pavonia* scoli the reservoirs of gland cells may survive a short maceration; continuous KOH treatment results in a complete decomposition of these soft structures [Fig. 1d; see Deml and Dettner (1990)]. The inner surface of *E. pavonia* scoli also possesses ridges which presumably represent demarcations and/or attachments of the individual gland cells.

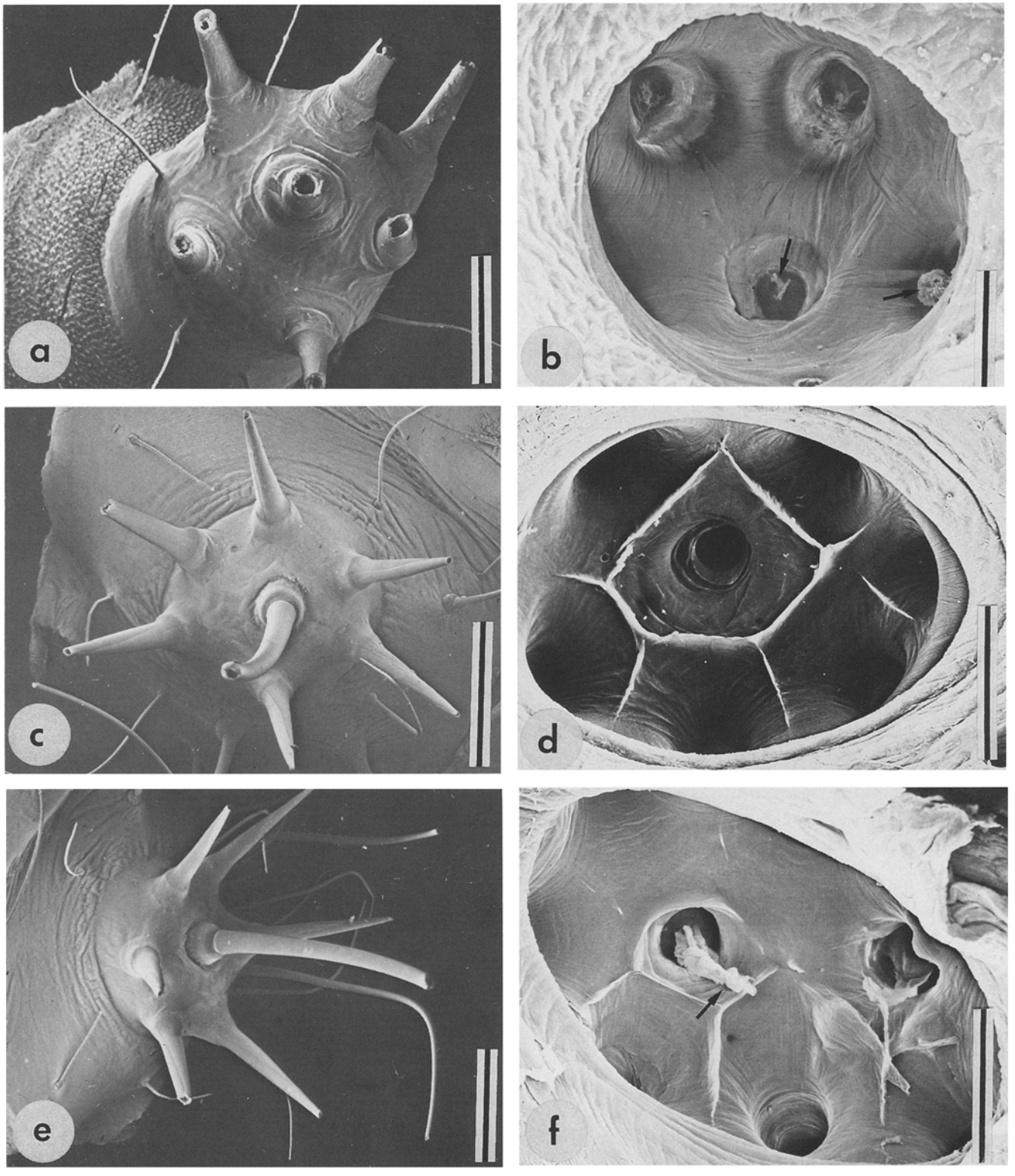

Fig. 1. SEM photographs of "Sternwarzen" (scolus + bristles) of *Eupackardia calleta* **(a, b),** *Eudia pavonia* **(c, d)** and *Saturnia pyri* **(e, f)** caterpillars. *Left row:* surface views; *Right row:* inside views after maceration. *Arrows* indicate the cuticular layers of the gland cells inside the scoli. *Scales:* 500 µm **(a, c, e),** 250 µm **(b, d, f)**

SDS–PAGE and tyrosinase assay

Electropherograms (Fig. 2) of *S. pyri* and *E. calleta* caterpillar secretions and HLs distinctly show qualitative and quantitative differences between the polypeptide contents of the two body fluids, as previously described in *E. pavonia* (Deml and Dettner 1990). In *S. pyri* several polypeptides appear exclusively in the secretion (Fig. 2B:

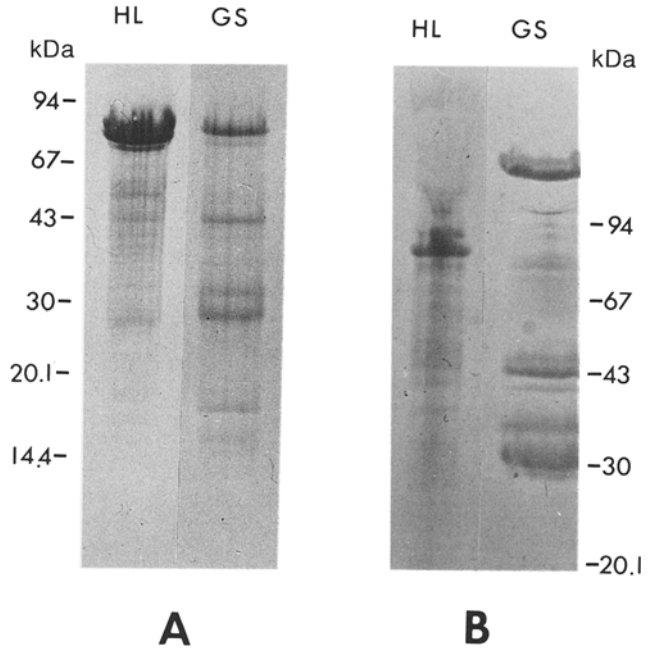

Fig. 2. SDS–PAGE of *Eupackardia calleta* **(A)** and *Saturnia pyri* **(B)** gland secretions *(lanes GS)* and haemolymphs *(lanes HL)*, dilution of each body fluid 1 : 10, applied volume 1 µl. Reference proteins are phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa)

band with about 100 kDa; bands with masses below 38 kDa). Otherwise, secretion polypeptides may be more concentrated than in the HLs (*E. calleta,* Fig. 2A: bands with masses of 43 and 27 kDa; *S. pyri,* Fig. 2B: band with mass 43 kDa) or conversely (*E. calleta,* Fig. 2A: HL band at 80 kDa; *S. pyri,* Fig. 2B: HL band at 80 kDa). Significant differences have been identified on comparison of the polypeptide patterns of the three different species investigated.

The qualitative tyrosinase assay with the secretions revealed a clearly visible red coloring of the reaction mixture, which indicates a high tyrosinase activity and gives evidence of the formation of melanin as well as the presence of tyrosine in the secretion.

GC–MS analyses

Chemical compositions of *E. pavonia, S. pyri* and *E. calleta* caterpillar GSs and HLs (Table 1) revealed remarkable quantitative and qualitative differences both between the two fluids and among the three species. HL and GS of all species contain many aromatic compounds along with other substances such as glycerol and trehalose which represent the main compounds. Several substances (especially trace constituents) could be found in only some of the individuals examined or were present in all individuals of a species in varying amounts.

In searching for hypothetically postulated precursors of most of these compounds the presence of phenylglycine, a possible precursor of benzonitrile, could be identified. Benzoic acid amide, another possible precursor of benzonitrile (T. Hartmann, personal communication), could not be found in any of the fluids examined.

All caterpillar compounds were identified by comparing their mass spectra and retention times with authentic compounds. For interpretation of mass spectra mainly the works of Budzikiewicz et al. (1967) and Budzikiewicz (1980) were used.

Toluene (M^+ 92) and 2-xylene (M^+ 106) both show the same base peak at m/z 91 (tropylium ion) just as the authentic references. The presence of 3- and 4-xylene could be excluded. Phenol (M^+ 94) and hydroquinone (M^+ 110) exhibit typical main fragments at M–28 and M–29 (release of −CO and −CHO), whereas 2-cresol (M^+ 108) produces fragments at m/z 107 (M–H), 90 ($M–H_2O$), 89, 79 (M–CHO) and 77. Based on their retention times and mass spectra 3- and 4-cresol could be excluded. Similarly 3,5-dimethylphenol (M^+ 122) possesses fragments at m/z 121, 107 ($M–CH_3$), 91, 79 and 77. Retention times and mass spectra show distinct differences from isomeric dimethyl- as well as the ethylphenols. Benzaldehyde (M^+ 106) shows release of H and CHO at m/z 105 and 77 (base peak), whereas phenylacetaldehyde (M^+ 120) is characterized by a base peak at m/z 91 (loss of CHO). Benzylamine (M^+ 107) produces prominent fragments at m/z 106 and m/z 91. Benzonitrile (M^+ 103) could be identified especially due to its typical fragment at m/z 76 (M–HCN).

From the acetylated catecholamines an M+1 peak (protonated amino group) could be found only in dopamine. However, dopa gave an M^+peak, whereas norepinephrine and epinephrine were characterized by M–17 fragments (release of H_2O from M+1). Due to a release of acetyl-functions, which is determined by the number of free amino and hydroxy groups, dopamine and dopa further produced consecutive M-($n \times 42$) or M-($n \times 43$) fragments (n: 1–4) and a corresponding base peak at m/z 43. In norepinephrine and epinephrine consecutive loss of 43 amu started from M–17.

Methylated and acetylated phenylglycine showed no M^+ peak but fragments at m/z 192 (4%; $M–CH_3$), 148 (28%; $M–COOCH_3$), 106 (100%; $M–COOCH_3–COCH_2$), 79 (34%), 77 (17%), 51 (10%) and 43 (78%; acetyl residue). Due to its additional CH_2 group phenylalanine gave (X+14) fragments corresponding to those of phenylglycine. But both compounds show fragments at m/z 43 and phenylalanine produces no fragment at m/z 93. The fragment at m/z 91 from phenylalanine represents a tropylium ion. Coumarin (M^+ 146) gave fragments at m/z 118 (base peak), 90, 89 and 63. Pyrazine (M^+ 80) only showed an important fragment at m/z 53.

Free acetylcholine and choline produce no mass spectra because both compounds decompose within the ion source chamber of the mass spectrometer or in the GC injection system, to produce tertiary amines by *N*-demethylation (Johnston et al. 1968). Both amines could be indirectly determined by recording mass spectra of their typical decomposition products, which may at the same time represent biogenetic precursors. The simultaneous presence of both quaternary amines and their demethylated counterparts could be verified by the broad peaks

Table 1. Detected compounds from crude and derivatized samples of haemolymph (HL) and gland secretions (GS) of *Eudia pavonia, Saturnia pyri* and *Eupackardia calleta* caterpillars

	Eudia pavonia (*Crataegus monogyna*)		*Eudia pavonia* (*Prunus spinosa*)		*Saturnia pyri* (*Prunus spinosa*)		*Eupackardia calleta* (*Ligustrum vulgare*)	
	HL	GS	HL	GS	HL	GS	HL	GS
Toluene	−	−	−	−	+	−	−	−
2-Xylene	−	−	−	−	+	−	−	−
Veratrole	−	−	−	−	−	+	−	−
Phenol	+	+	+	+	?	−	−	?
2-Cresol	−	−	−	−	+	+	−	−
3,5-Dimethylphenol	+	+	?	+	+	+	+	+
Hydroquinone	+	−	+	?	−	?	−	?
Benzaldehyde	+	+	−	+	+	?	?	−
Phenylacetaldehyde	++	++	++	++	++	−	++	++
Phenylglyoxylic acid	−		−	−	−	−		
Phenylpyruvic acid	−		−	−	−	?		
Phenylacetic acid	−		−	−	−	−		
Phenylglycine	?		+	−	?	−		
Phenylalanine	++		++	+	++	−		
Benzylamine	−	−	−	−	−	−	−	+
Benzonitrile (Cyanobenzene)	+	++	+	?	−	−	−	++
3,4-Dihydroxyphenylalanine (Dopa)	−	−	−	−	−	−	+	−
Dopamine	−	−	−	−	−	−	+	−
Norepinephrine (Noradrenalin)	−	−	−	−	−	−	+	−
Epinephrine (Adrenalin)	−	−	−	−	−	−	++	++
Coumarin	−	+	−	−	−	−	−	−
Pyrazine	−	−	−	−	+	−	−	−
Nicotinamide	−	−	−	−	+	−	−	−
Trehalose	−	−	−	−	−	−	+++	++
Glycerol	+++	+++	+++	+++	+++	+++	+++	+++
Acetylcholine	−	−	−	−	−	−	−	+++
2-(Dimethylamino)ethylacetate	−	−	−	?	−	−	−	++
2-(Methylamino)ethylacetate	?	−	−	−	−	−	−	−
Aminoethylacetate	?	?	?	−	?	?	−	−
Choline	−	−	−	+	−	+	++	++
2-(Dimethylamino)ethanol	−	−	−	−	−	−	?	++
2-(Methylamino)ethanol	−	−	−	−	−	−	−	−
2-Aminoethanol	−	−	−	−	−	−	−	?
4-Aminobutyric acid (GABA)	++	−	−	−	−	−	+	+

Food plants are given in parentheses. Semiquantitative data (+/−) are each based on peak areas of three to five GC–MS total ion chromatograms (+++ = main compound, ++ = minor compound, + = trace, − = not detectable, ? = authentic mass spectrum only partially to be found in the body fluid at corresponding retention time)

of choline and acetylcholine in the total ion current chromatogram on which are superimposed higher, narrow peaks that correspond to dimethyl aminoethanol and dimethyl aminoethylacetate.

The tertiary, secondary and primary choline precursors (aminoethanols) all gave M+1 peaks as typical for many amines in biological materials and produced distinct M–17 fragments (loss of H_2O). M/z 58 (base peak) in dimethyl aminoethanol is probably due to the formation of $(CH_3)_2N^+=CH_2$, and the intensive fragment at m/z 44 in all three amines might correspond to an ethanol residue. The acetylated ethanolamines (acetylcholine precursors) also gave peaks for M+1, M–18 (M–H_2O) except for dimethyl aminoethylacetate, and the following important fragments: m/z 87, 72, 58 (base peak) and 43 (tertiary amine); m/z 86, 76, 74, 58, 56, 44 (base peak) and 43 (secondary amine); m/z 73, 72, 62, 60 and 43 (base peak; primary amine). Acetylated GABA gave no M^+ peak but main fragments at m/z 128, 99, 86 and 43 (base peak; acetyl residue).

Particularly poisonous chemicals seem to be confined to the secretions. *E. calleta* especially differs from other species by containing catecholamines and other aliphatic biogenic amines which may also represent potent neurotransmitters in other taxa. Measured amounts of catecholamines in *E. calleta* caterpillar fluids are: HL: dopa 90–110 ng · µl^{-1}, dopamine 15–20 ng · µl^{-1}, norepinephrine <40–100 ng · µl^{-1}, epinephrine 600–800 ng · µl^{-1}; GS: epinephrine 600–800 ng · µl^{-1}. Other common biogenic amines such as serotonin (5-hydroxytryptamine), histamine, octopamine, tyramine, epinine and ephedrine could not be detected at all in the three species of Saturniidae examined.

Ant feeding deterrence test

The results of the feeding test with *Lasius niger* (Fig. 3) for the most part indicate a highly significant feeding deterrence of the three saturniid caterpillar GSs, especial-

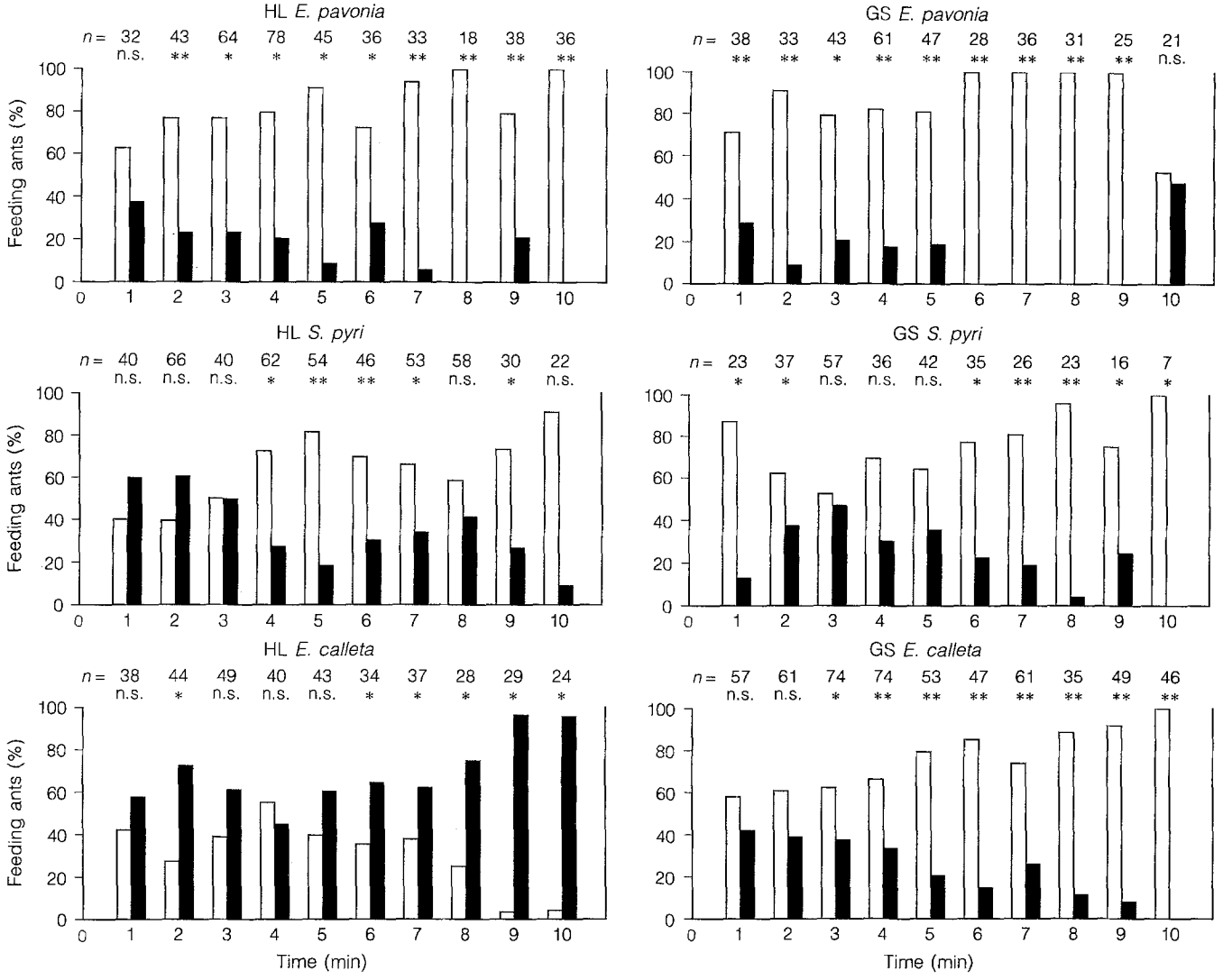

Fig. 3. Feeding test of the haemolymphs (HL; *left*) and corresponding secretions (GS; *right*) of three saturniid caterpillars against *Lasius niger* workers. Time-dependent percentage of ants feeding on two droplets (each 5 µl) containing either suspension of *Tenebrio molitor* larvae mixed with caterpillar body fluid (1:1, v/v; *black columns*) or larval suspension of *T. molitor* as control (*clear columns*) is indicated. n = total number of ants feeding on both droplets (= 100%), recorded at consecutive 1-min time intervals. **: $\alpha = 0.01$; *: $\alpha = 0.05$; n.s.: not significant (Wilcoxon matched pairs signed rank test, two-tailed)

ly of *E. pavonia*, compared to the control. Also, the HLs of *S. pyri* and mainly *E. pavonia* larvae act as repellents towards *Lasius niger*, whereas *E. calleta* HL has a significantly attractant effect.

These results are partially supported by field observations with the large *Formica pratensis* workers. If caterpillars of all three species were placed beside an ant trail they were attacked by *F. pratensis*, but the ants were hardly able to climb onto the caterpillars. This is because the larvae assume a sphinx-like or curled-in posture, they perform whipping movements, and *E. pavonia* and *S. pyri* larvae possess a very smooth integument. *F. pratensis* workers that succeeded in climbing onto the caterpillars could only fix themselves by biting into the scoli. After that the ants immediately left the caterpillar, ran away, wiped their mandibles on the ground and showed cleaning behaviour. They hid themselves for a while between stones as if to avoid contact with other ants before they ran away. No caterpillar was injured seriously. The best and fastest deterrent effect was achieved by *E. pavonia* caterpillars. If the defensive secretion was removed from defrosted caterpillars and these cut larvae were subsequently presented to the ants, *F. pratensis* avidly licked up the extravasating HL of all three species and preferred *E. calleta*.

Discussion

Morphology of scoli

The structure of the saturniid scolus apparatus seems to support several functions. Inside the scolus cavity hang large gland cells (separated by cuticular walls in *Eudia pavonia*) which produce the secretion and store it in the intracellular cuticular reservoirs. Such intracellular reser-

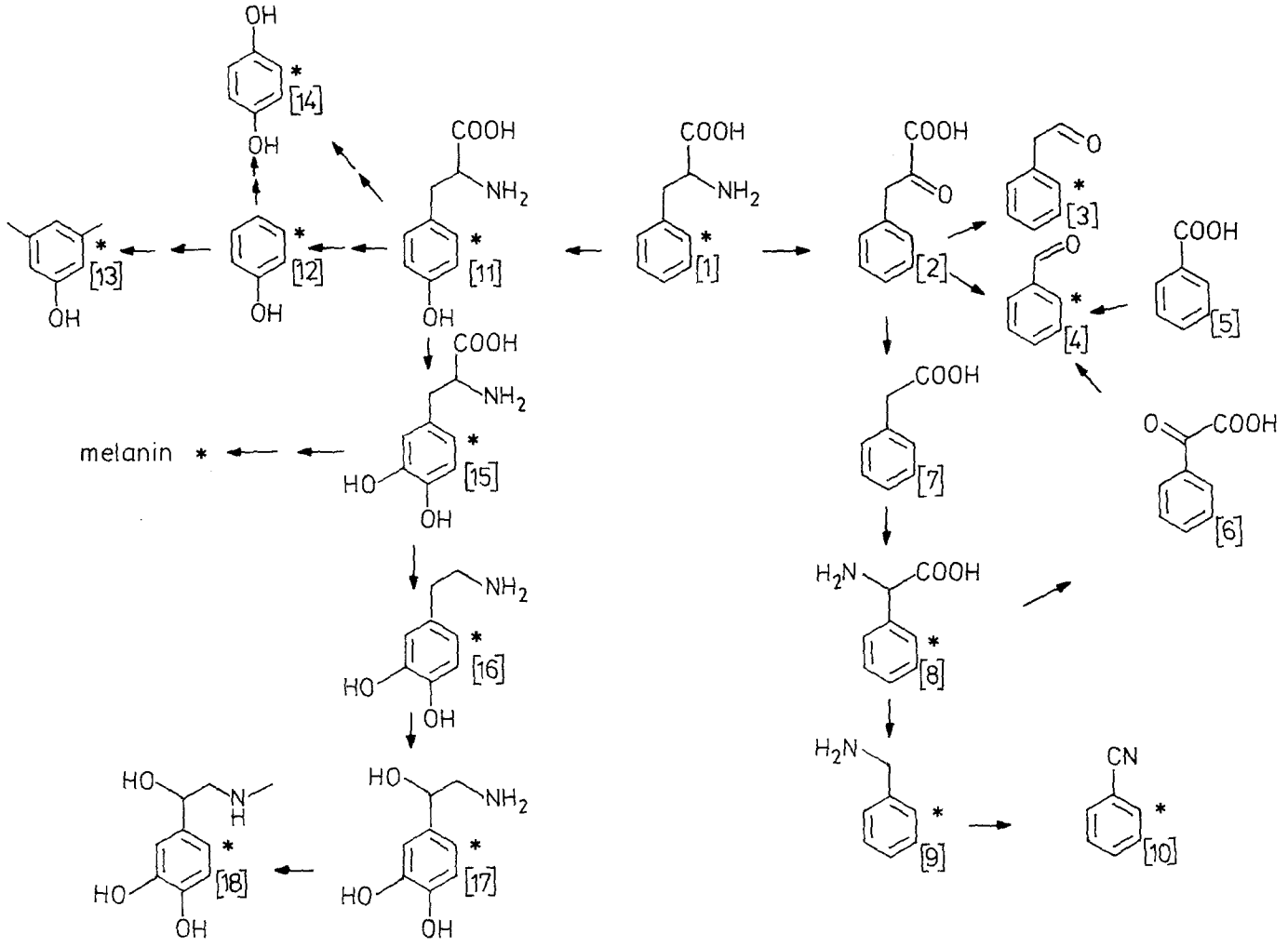

Fig. 4. Theoretical metabolic pathway scheme for most of the aromatic compounds detected in the secretions and haemolymphs of *Eudia pavonia*, *Saturnia pyri* and *Eupackardia calleta* caterpillars. An *asterisk* indicates the detected compounds. 1 = phenylalanine, 2 = phenylpyruvic acid, 3 = phenylacetaldehyde, 4 = benzaldehyde, 5 = benzoic acid, 6 = phenylglyoxylic acid, 7 = phenylacetic acid, 8 = phenylglycine, 9 = benzylamine, 10 = benzonitrile (cyanobenzene), 11 = tyrosine, 12 = phenol, 13 = 3,5-dimethylphenol, 14 = hydroquinone, 15 = 3,4-dihydroxyphenylalanine (dopa), 16 = 3,4-dihydroxyphenylethylamine (dopamine), 17 = norepinephrine (noradrenalin), 18 = epinephrine (adrenalin)

voirs are a common means of achieving self-protection in diverse insects producing chemical defensive compounds. They prevent contact between the cytoplasm and the poisonous compounds of the secretions (Dettner 1989). Each of the intracellular reservoirs in the Saturniidae is associated with one of the hollow bristles. On irritation the liquid secretion is pressed outwards through the bristles and adheres as a sticky droplet at the open tip or runs down to the scolus roof. The hard bristles serve as syringe cannulas, possibly for injecting the secretion into the skin or mucous membranes of attacking predators. In *E. pavonia* L2 caterpillars the tips of the bristles must be broken off, apparently by mechanical contact (predator skin or biting mandibles?) before secretion can flow out. As the SEM photographs indicate, the whole bristle might be filled previously with secretion in this caterpillar stage. Even apart from the irritant secretion, the sharp bristles represent effective stinging weapons for defensive purposes and splinters of the hairs (especially of *Eupackardia calleta*) could cause long lasting irritations of the skin.

Delivery of the larval secretion normally only occurs following strong pressure on the scoli from outside or on the caterpillars' vehement whipping of the forebody in defense. It remains uncertain whether the larvae perceive irritating external pressure by means of their whole body or by specific sensory cells which may be located inside or near the scolus. A possible afferent innervation of the scoli is suggested by the presence of the peculiar "Kolbenhaare" on *Saturnia pyri* caterpillars. Following Haffer (1921) these structures have been considered to represent sensory hairs. In addition, saturniid scoli are often surrounded by a circle of distinctly smaller, probably sensory, hairs. It is not known whether the delivery of secretion is brought about only by increasing the HL pressure of the caterpillar or if there is a direct innervation of the gland cells. Further morphological and histological studies will answer these questions.

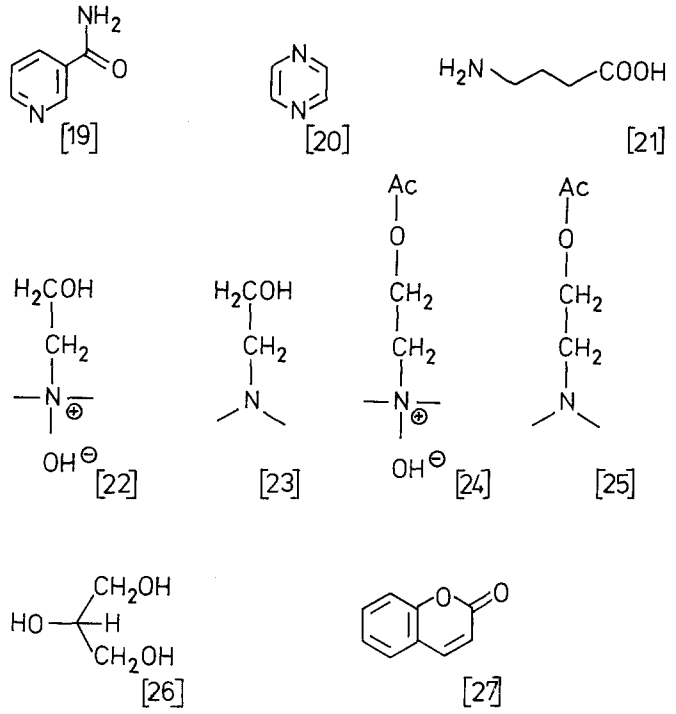

Fig. 5. Some unusual compounds found in the secretions and/or haemolymphs of *Eudia pavonia* (21, 22, 26, 27), *Saturnia pyri* (19, 20, 22, 26) and *Eupackardia calleta* (21, 22, 23, 24, 25, 26) caterpillars by GC–MS analyses. 19 = nicotinamide, 20 = pyrazine, 21 = γ-aminobutyric acid (GABA), 22 = choline, 23 = 2-(dimethylamino)ethanol, 24 = acetylcholine, 25 = 2-(dimethylamino)ethylacetate, 26 = glycerol, 27 = coumarin

Biochemical properties of caterpillar body fluids

The electropherograms of the three saturniid caterpillar species definitely show (Fig. 2) that the fluid produced by the scolus is not HL. Every species has a typical secretion polypeptide pattern differing from its own HL and from other species' body fluids. There might be two main ways of interpreting different polypeptide patterns of the haemolymph and secretion samples within a given species:

(1) Secretion proteins could not possess any defensive function at all or could be responsible for an external self-protective decomposition of the low-molecular-weight constituents of the secretion after its delivery onto the larva's integument. In this respect the biological significance of the high tyrosinase activity found especially in the naturally black coloring secretion is not clear.

(2) Alternatively, the secretion polypeptides could actually have an important function in creating an effective defensive secretion. Such proteins could be the anabolizing enzymes (or proenzymes) of compounds belonging mainly or exclusively to the secretion (such as for biogenesis of epinephrine, acetylcholine or benzonitrile). However, this kind of enzyme is usually localized within the cytoplasm or the cell membrane of the gland cells.

The secretion polypeptides could be enzymes or zymogens that produce damaging effects such as destruction of tissues in attacking predators after injection. Such functions are well known from bee and snake venoms (Habermehl 1987; Urich 1990) and from caterpillars with poisonous spines (Rothschild 1985; Teuscher and Lindequist 1988). The formation of thread-like structures on contact with air as observed in *E. pavonia* secretion is also due to the presence of secretion proteins (Deml and Dettner 1990). This process is similar to blood clotting and the network generated presumably serves as glue to make the secretion more adherent to target organisms. Further separation of polypeptides and exact assignment of singular bands to specific enzymes and isozymes is to be conducted.

Comparative chemistry of caterpillar secretions and haemolymphs

The distinctly different composition (Table 1) of the GC–MS-analyzed caterpillar HLs and GSs corroborates the electrophoretic finding that the secretion is not pure HL. Deml and Dettner (1990) supposed that the secretion of *E. pavonia* might represent a kind of HL filtrate. Among its constituents are compounds found exclusively in the secretion, where they are obviously synthesized. Other constituents may be found in the GS in higher concentration than in the HL, which suggests that active transport structures (carriers) must be present in the gland cell membranes.

The larval compounds of the three species investigated are biosynthetically similar and show specific patterns; therefore, we assume common metabolic pathways for most of the aromatic compounds. These ideas and suggestions are summarized in a new metabolism scheme (Fig. 4), mainly based upon data in the literature (Meister 1972; Goodwin 1976; Dettner 1990). Some missing intermediate compounds in this scheme were detected afterwards by defined derivatizations, e.g. the amino acids [1, 8]. Moreover, a completely new way of anabolizing cyanobenzene [10] from phenylglycine [8] (H. Geiger, D. Schlee, personal communication) and benzylamine [9] has been suggested. Some compounds (e.g. [3], [8], [10]) are very erratically distributed in both insects and other organisms.

Formation of melanin also gave evidence of the presence of tyrosine [11] and led to the discovery of catecholamines in *E. calleta* caterpillars. The unusually high concentrations of adrenalin [18] in both body fluids is particularly striking. Presumably this biogenic amine is formed in the fat-body or other internal organs of the caterpillar. After delivery of secretion the original elevated amount of adrenalin in the gland cells could be rapidly restored by osmosis or facilitated permeation through the cell walls. The absence of detectable amounts of dopa [15] in the secretion applies only to free dopa, whereas fixed dopa might be present for melanin formation (Urich 1990).

There are two biochemical branches within the Saturniidae investigated: *E. pavonia* and *S. pyri* mainly produce phenolic and related compounds, whereas *E. calleta* in addition synthesizes biogenic, highly effective aromatic amines. This finding is strengthened by the occurrence of aliphatic amines like acetylcholine and several of its precursors in *E. calleta* caterpillars (Table 1, Fig. 5 [22]–[25]).

Some of the amines are known to act as transmitter substances in the nervous systems of vertebrates (Reichert 1990) and insects (Pichon and Manaranche 1985) but have also been identified in non-nervous tissues and venoms of several moths and other insects (Morley and Schachter 1963; Dazzini and Finzi 1974; Rothschild 1985). From the relatively high concentrations of acetylcholine in certain non-nervous tissues, Morley and Schachter (1963) inferred that this biogenic amine and related compounds could have additional functions such as stimulating (gland) cell metabolism or facilitating membrane transport.

Apart from acetylcholine and its precursors several other compounds (e.g. the neurotransmitter GABA [21]) have been detected in the three species of Saturniidae (Fig. 5). The presence of huge amounts of glycerol [26] within the secretions is remarkable. Usually this trihydroxy alcohol serves as an anti-freezing agent in the HL of several diapausing insects (e.g. Somme 1964, 1965). It has been found previously in some saturniid pupae but could not be detected in the larvae of the same species (Wyatt and Meyer 1959). We suggest that glycerin in the three saturniid larval fluids examined might instead function as a solvent. The presence of this alcohol within both compartments of the larvae may be due to the fact that it can easily pass cell membranes (Hochachka and Somero 1980).

Increased trehalose titres in the larval secretion of *Eupackardia* (Table 1) are especially peculiar. Whether toxic compounds in secretions could exist as harmless glucosides ("trehalosides") has to be investigated. Detection of trehalose from HL samples is not unusual since this compound is the most widely distributed insect blood sugar (review: Urich 1990). However, HL trehalose in these larvae could be bound to toxic metabolites, which might therefore be stored in the HL. This is a common means of detoxication in insects (Weber and Weidner 1974).

Comparison of body fluids of *E. pavonia* caterpillars reared ex ovo on different foodplants (whitethorn and sloe; Table 1) reveals only slight differences. Only a few substances, such as benzonitrile, GABA and phenylglycine, show distinct qualitative and quantitative variations, but analysis of three to five individuals per foodplant revealed considerable individual variability, especially with respect to trace constituents. Therefore, the influence of larval foodplants on the composition of HLs and GSs cannot be sufficiently clarified as yet. Forthcoming studies dealing with synthetic caterpillar diets and analysis of foodplant chemistry will show which caterpillar compounds are synthesized de novo by the caterpillars and which are derived from the foodplant.

Biological significance of caterpillar gland secretions

The chemical similarity of all three saturniid species examined and comparisons of caterpillars reared on two different foodplants indicate that excretion is not a main task of the gland cells inside the scolus.

Deml and Dettner (1990) have tested some aromatic compounds found in *Eudia pavonia* caterpillars for their topical irritancy and mortality effects on last stage *Calliphora vomitoria* larvae; no compound showed any irritancy, but benzonitrile in particular exhibited high toxic effects. The biological activity of the compounds was also assessed in the gas phase by using a fumigant test with *Drosophila melanogaster* adults (Dettner et al. 1992). Benzonitrile and other liquid aromatics exhibited the best fumigant activity. This high fumigant effect of the secretion could help to deter some parasitoids that search by olfaction before egg-laying. Nevertheless, many caterpillars of *E. pavonia* and *S. pyri* caught in the field are parasitized.

Feeding tests and field observations with two ant species are reported which show a clearly repellent effect of the three tested saturniid secretions. Some saturniid HLs also act as deterrents to susceptible ants. It should be noted that ants are assumed to be the main arthropod predators of caterpillars and therefore an ant-deterring fumigant effect would be of very great importance for the survival of the saturniid caterpillars. Indeed, ants are distinctly repelled by the secretion of the relatively small and therefore most endangered *E. pavonia,* as well as the much larger *S. pyri* caterpillars in the laboratory feeding test. This result was also obtained in the field, although no visible amounts of secretion were delivered, but *Formica pratensis* might have had contact with low amounts of secretion for cleaning their mandibles.

When caterpillar species (mainly *E. pavonia*) are bitten on their legs, they are further protected by their deterrent HL from certain aggressing ant species (e.g. *Lasius niger*). Edible HL such as is found in *Eupackardia* might be exceptional because it can even contain ant-attracting substances.

Saturniid caterpillars spend most of their lives hanging head-down on bushes and trees. Moreover, they defend themselves with sharp bristles, a very smooth integument and behaviours such as whipping, curling-in and sphinx-like posture. Furthermore, the largest scoli (with the largest amounts of secretion) are located dorsally on the front and the rear ends (thoracic and last abdominal segments). Caterpillars are exposed to a greater danger from ants (and other predators) while running on the ground and looking for a place to spin their silken cocoons. As indicated by field observations, caterpillars are well protected in this situation. They seem to be helpless only when they are spun to a branch before every moulting, and immediately afterwards when the new soft skin has not yet hardened in the air.

The occurrence of various biologically active biogenic amines, especially in *E. calleta* caterpillars which are most poorly protected from ants, suggests a compromise in this species: worse chemical defence against ants but better facilities for repulsing other enemies, believed to be vertebrates, especially birds. Other peculiarities of all saturniid caterpillars examined, such as the warning colour of the scoli and the camouflage by a cryptic green, partially counter-shaded coloration of the three species, imply that birds are also predators on *Eudia* and *Saturnia*. An observation of Nässig and Oberprieler (1990) is

of interest in this regard; that is, certain caterpillars of the Saturniidae with another scolus type containing presumed toxins ["Stechborstenscolus"; Nässig (1989)] are cryptically coloured, which is very unusual in venomous insects. These findings should be extended to the three species dealt with here and to the scolus type "Sekretstechborstenscolus".

The real importance of saturniid caterpillars to vertebrates remains to be tested. Numerous other species of Saturniidae must also be investigated to learn more about the distribution of the characteristics described here within this family.

Acknowledgements. This investigation was carried out within the program of the Bayreuth Institute of Terrestrial Ecosystem Research (BITÖK) and was supported by the German Federal Ministry for Science and Technology (BMFT) under grant no. OEF 2029.

References

Blaich R (1978) Analytische Elektrophoreseverfahren. Thieme, Stuttgart

Budzikiewicz H (1980) Massenspektrometrie. Eine Einführung. 2. Aufl. Taschentext 5. Chemie–Physik Verlag, Weinheim

Budzikiewicz H, Djerassi C, Williams DH (1967) Mass spectrometry of organic compounds. Holden-Day, San Francisco Cambridge London Amsterdam

Dazzini MV, Finzi PV (1974) Chemically known constituents of arthropod defensive secretions. Atti Accad Naz Lincei – Mem. S VIII, 7:109–146

Deml R, Dettner K (1990) Chemical defense of *Eudia (Saturnia) pavonia* caterpillars. Naturwissenschaften 77:588–590

Dettner K (1989) Insektenabwehrstoffe – Produktion, Speicherung, Abgabe und Anwendung im Biologischen Pflanzenschutz. UWSF–Z Umweltchem Ökotox 3:46–53

Dettner K (1990) Chemische Abwehr bei der ursprünglichen Käferfamilie der Amphizoidae. Ein Beitrag zur Evolution der Pygidialdrüse der Hydradephaga. Mitt Dtsch Ges Allg Angew Ent 7:519–526

Dettner, K, Fettköther R, Ansteeg O, Deml R, Liepert C, Petersen B, Haslinger E, Francke W (1992) Insecticidal fumigants from defensive glands of insects – a fumigant test with adults of *Drosophila melanogaster*. J. Appl Entomol 113:128–137

Goodwin BL (1976) Handbook of intermediary metabolism of aromatic compounds. Chapman & Hall, London

Habermehl GG (1987) Gift-Tiere und ihre Waffen. 3. Aufl. Springer, Berlin Heidelberg New York London Paris Tokyo

Haffer O (1921) Bau und Funktion der Sternwarzen von *Saturnia pyri* Schiff. und die Haarentwicklung der Saturnidenraupen. Arch Naturgesch A 87:110–166

Hilker M, Schulz S (1991) Anthraquinones in different developmental stages of *Galeruca tanaceti* (Coleoptera, Chrysomelidae). J Chem Ecol 17:2323–2332

Hochachka PW, Somero GN (1980) Strategien biochemischer Anpassung. Thieme, Stuttgart New York

Johnston GAR, Triffett ACK, Wunderlich JA (1968) Identification and estimation of choline derivatives by mass spectrometry. Anal Chem 40:1837–1840

McLafferty FW, Stauffer DB (1989) The Wiley/NBS registry of mass spectral data. Wiley, New York Chichester Brisbane Toronto Singapore

Meister A (1972) Biochemistry of the amino acids, vol 2, 2nd edn., 3rd pr., Academic Press, New York London

Morley J, Schachter M (1963) Acetylcholine in non-nervous tissues of some Lepidoptera. J Physiol (London) 168:706–715

Nässig W (1989) Wehrorgane und Wehrmechanismen bei Saturniidenraupen (Lepidoptera, Saturniidae). Verh Westdeutsch Entom Tag 1988:253–264

Nässig WA, Oberprieler RG (1990) Mimetischer oder aposematischer Habitus – ein Vergleich larvaler und imaginaler Strategien bei Saturniinen (Lepidoptera, Saturniidae). Verh Dtsch Zool Ges 83:655

Pichon Y, Manaranche R (1985) Biochemistry of the nervous system. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology, vol 10. Pergamon, Oxford New York Toronto Sydney Paris Frankfurt, pp 417–450

Reichert H (1990) Neurobiologie. Thieme, Stuttgart New York

Rothschild M (1985) British aposematic Lepidoptera. In: Heath J, Maitland Emmet A (eds) The moths and butterflies of Great Britain and Ireland, vol. 2. Harley Books, Colchester, pp 8–62

Sachs L (1984) Angewandte Statistik 4. Aufl. Springer, Berlin Heidelberg New York Tokyo

Somme L (1964) Effects of glycerol on cold-hardiness in insects. Can J Zool 42:87–101

Somme L (1965) Further observations on glycerol and cold-hardiness in insects. Can J Zool 43:765–770

Stenhagen E, Abrahamsson S, McLafferty FW (1974) Registry of mass spectral data. Wiley, New York London Sydney Toronto

Teuscher E, Lindequist U (1988) Biogene Gifte. Akademie-Verlag, Berlin

Urich K (1990) Vergleichende Biochemie der Tiere. Fischer, Stuttgart New York

Weber H, Weidner H (1974) Grundriß der Insektenkunde. 5. Aufl. Fischer, Stuttgart New York

Wyatt GR, Meyer WL (1959) The chemistry of insect hemolymph. III. Glycerol. J Gen Physiol 42:1005–1011