Sequestration, N-Oxidation and Transformation of Plant Pyrrolizidine Alkaloids by the Arctiid Moth *Tyria jacobaeae* L.

Adelheid Ehmke, Ludger Witte, Andreas Biller, and Thomas Hartmann

Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Mendelssohnstraße 1, D-3300 Braunschweig, Bundesrepublik Deutschland

Z. Naturforsch. **45c,** 1185–1192 (1990); received July 31/August 29, 1990

Tyria, Arctiidae, Pyrrolizidine Alkaloids, Callimorphine, N-Oxidation

Larvae of the arctiid moth *Tyria jacobaeae* reared on *Senecio jacobaea* or *S. vulgaris* take up and store pyrrolizidine alkaloids (PAs) from their host plants. Individual PAs are taken up without preference. The PA patterns found in the insect bodies correspond to the PA composition of their host plants. Like plants the insects store PAs as N-oxides, and larvae as well as pupae are specifically able to N-oxidize any tertiary PA. Callimorphine (O^9-(2-methyl-2-acetoxybutanoyl)-retronecine), an insect PA well known from several arctiids, was found in pupae and imagines of *Tyria* which as larvae had been fed on *S. jacobaea*. It is accompanied by small amounts of its isomer O^7-(2-methyl-2-acetoxybutanoyl)-retronecine named isocallimorphine. The callimorphines may well account for 45% of total PAs found in the insect. Only small amounts of callimorphine were detected in pupae of *Tyria* which as larvae had been fed on *S. vulgaris*. [^{14}C]Callimorphine N-oxide was isolated and identified from *Tyria* pupae which as larvae received [^{14}C]retronecine. It is suggested that *Tyria* is able to esterify retronecine, derived from hydrolysis of ingested plant PAs with a necic acid produced by the insect. During metamorphosis the formation of callimorphine is restricted to the early stage of pupation.

Introduction

Larvae of the arctiid *Tyria jacobaeae* L. feed almost exclusively on the tansy ragwort (*Senecio jacobaea* L.) and the groundsel (*Senecio vulgaris* L.). It is well known from early studies of Aplin and Rothschild [1, 2] that the larvae sequester pyrrolizidine alkaloids (PAs) of their host plants and that these alkaloids are also found in the pupae and imagines. Larvae and imagines are brightly coloured. This aposematic warning colouration advertise their unpalatability to potential predators. In fact, *Tyria* is not eaten by most insectivores.

In continuation of our studies concerning the physiology and biochemistry of PAs as potential plant defense chemicals in *Senecio* species, we became increasingly interested in functional aspects of this example of plant-insect relationship. In *Senecio* PAs are synthesized in the roots [3] and are translocated *via* the phloem into shoots where they are channelled to the inflorescences, the major sites of PA accumulation [4, 5]. PAs are synthesized as N-oxides and PA-N-oxides are the specific forms of translocation and cellular, *i.e.* vacuolar storage of the alkaloids within plant tissues [5, 6].

The objective of this paper is to study in more detail the metabolism of plant derived PAs in *Tyria jacobaeae*. Particularly the following questions should be answered: (1) is there evidence for a selective uptake of certain PAs from the host plants? (2) Do PA-N-oxides play a similar role in insects as they do in plants? (3) What is the biogenetic origin of the "insect metabolite" which was identified as callimorphine [7], a PA which has not been reported to occur in plants so far?

Materials and Methods

Insects and host plants

Larvae of *Tyria jacobaeae,* feeding on *Senecio jacobaea,* were collected near Domburg (Holland) at a dune habitat close to the sea-shore. The larvae were continued to rear von *S. jacobaea* or *S. vulgaris*. *S. jacobaea* was collected at wild habitats in the vicinity of Braunschweig, *S. vulgaris* was grown in the medicinal plant garden of the institute. Generally fully developed, flowering specimens were offered as food plants. In any case the alkaloid composition of the food plant material was determined. Since the larvae prefered the inflorescences, whole inflorescences were analyzed.

Reprint requests to Prof. Dr. T. Hartmann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/1100–1185 $ 01.30/0

The larvae were reared on their food plants until analysis or pupation. Pupae were kept in sand at *ca.* 20 °C. The sand was kept slightly wet using an atomizer from time to time. The moths which emerged about 10 months later were killed by freezing about 24 h after emerging from pupae.

Alkaloid extraction

Plant material (6–10 g fr. wt.; inflorescences) was homogenized in 20 ml 0.05 M H_2SO_4 for 3–4 min (Ultra-turrax) and the homogenate left to stand for 30 min. After centrifugation half of the supernatant was made basic with NH_4OH and was extracted by liquid-solid extraction using Extrelut columns (Merck) [3]. PAs were eluted with CH_2Cl_2 (6 ml/g Extrelut). This eluate contains all tertiary PAs. The remaining half of the acidic supernatant was adjusted to 0.25 M H_2SO_4 and mixed with Zn dust in excess. The mixture was stirred for 5 h. The solution was made basic and was further processed as given above; it constitutes the total PA fraction (PAs + PA-N-oxides). After evaporation of the solvent the residues were redissolved in MeOH for GLC analysis.

Single insects (larvae, pupae or imagines) were ground with 2.0 ml acidic MeOH (1% HCl) and quartz sand in a mortar for 10 min. The homogenate was suspended in a total volume of 5.0 ml acidic MeOH and after centrifugation the supernatant was divided into two aliquots; the MeOH was evaporated. One aliquot was dissolved directly in dilute NH_4OH and applied to an Extrelut column, the second aliquot was redissolved in 0.1 M H_2SO_4 and reduced in the presence of Zn dust. Both aliquots were further treated as given above to provide the fraction of tertiary PAs and total PAs.

Alkaloid analysis

PAs were separated and evaluated quantitatively by capillary GLC (Perkin-Elmer, Sigma 2B) on quartz columns (WCOT, 15 m or 30 m × 0.25 mm; DB-1, J&W scientific CA) [10]. Conditions: injector, 250 °C; temp. progr., 150–300 °C, 6 °C/min; split ratio, 1:20; injection vol., 1–2 µl; carrier gas, He 0.75 or 1.2 bar, respectively. Detection: flame ionization and nitrogen detectors. Atropine was used as internal standard. Retention indices (RIs) were calculated from cochromatographed hydrocarbon standards according to [11]. GC-MS: A Carlo Erba Mega 5160 gas chromatograph equipped with a quartz column (30 m × 0.32 mm) specified as given above, was directly coupled to a quadrupole mass spectrometer Finnigan MAT 4515. GLC conditions were as given above.

Radioactive tracers

[^{14}C]Senecionine (1.07 GBq·mmol^{-1}) and its N-oxide were prepared biosynthetically from [1,4-^{14}C]putrescine (4.4 GBq·mmol^{-1}) using a root culture of *S. vulgaris* according to [6]. [^{14}C]Retronecine was prepared from labelled senecionine by hydrolysis (10% NaOH, 2 h at 100 °C). The solution was adjusted to pH 10–11 with HCl and purified *via* an Extrelut column. [^{14}C]Retronecine was eluted with CH_2Cl_2:MeOH (95:5, by vol.). Recovery 65 to 85%. [1,4-^{14}C]Putrescine was obtained from Amersham (Braunschweig, F.R.G.), N-methyl^{3}H-labelled atropine (2.9×10^3 GBq·mmol^{-1}) from Du Pont (Boston, U.S.A.).

Tracer feeding experiments

Approx. 2.2×10^5 cpm of [^{14}C]retronecine or 4.7×10^5 cpm ^{14}C- or ^{3}H-labelled alkaloid solubilized in MeOH were applied to flower heads or leaves of the respective host plant and were offered to larvae which were kept individually. After complete ingestion of the labelled sample larvae were again fed with untreated host plant material. The frass pellets were collected at time intervals during the first 2 to 4 d following tracer application. Insects were killed by freezing either as larvae or pupae and were stored at −20 °C until chemical analysis. In some experiments the tracer was injected (1 to 2 µl tracer in 70% ethanol) directly into the haemolymph of larvae or into prepupae. Frass and carcass were extracted twice with acidic MeOH. The combined MeOH extracts were analyzed for total radioactivity by liquid scintillation counting. Aliquots were subjected either to TLC of HPLC analysis for identification and quantification of labelled products. MeOH-insoluble residues were chemically digested using Lumasolve (Baker) before scintillation measurements.

Thin-layer chromatography (TLC)

^{14}C-Labelled PAs and ^{3}H-labelled atropine were separated on Silica gel 60 (Merck). Solvent system:

$CHCl_3$:CH_3OH:NH_4OH (25%): *n*-pentane (82:14:2.6:20, by vol.). Detection: radioscan with a TLC multichannel analyzer (Rita-32a, Raytest). Autoradiography: exposure of TLC plates to Agfa-Curix X-ray film for 7 to 14 d.

High pressure liquid chromatography (HPLC)

Reversed-phase ion-pair HPLC [12] was used to separate the PA-N-oxides as described by [13]. ^{14}C- and ^{3}H-labelled compounds were detected with the HPLC radioactivity monitor LB-506D (Berthold) equipped with a 2 ml flow-cell and the split-mixer LB-5034.

Results

PA patterns of Tyria and its host plants

Early instar larvae of *Tyria* fed on *S. vulgaris* and *S. jacobaea* of known PA composition until pupation. The PA patterns of host plants, larvae, pupae and in the case of *S. jacobaea* reared insects, also of moths were evaluated by means of GLC-MS analysis (Tables I and II). PAs of plant origin were identified by their retention indices (RIs), molecular ions and MS fragmentation patterns in comparison to reference compounds. The identity of erucifoline was proved by ^{1}H NMR spectro-

Table I. PA patterns and concentrations in *Tyria jacobaeae* and its larval host plant *Senecio jacobaea*.

Alkaloid	RI	M^+ (m/z)	Alkaloid composition [%] Host plant[a]	Larvae[b,c]	Pupae[c]	Imagines[d]
Callimorphine	1965	297	n.d.	n.d.	24	47
Isocallimorphine	1980	297	n.d.	n.d.	3	3
Senecivernine	2278	335	<1	<1	2	n.d.
Senecionine	2290	335	28	10	24	19
Seneciphylline	2303	333	19	9	19	9
Integerrimine	2350	335	2	2	6	4
Erucifoline	2510	349	44	78	15	18
Retrorsine	2515	351	tr.	1	7	n.d.
O-Acetylerucifoline	2610	391	6	n.d.	n.d.	n.d.
Total PA concentration (mg/g fr. wt.):			0.27	3.24	5.56	2.67
Total PA content (mg/individuum):				0.45	0.74	0.32

[a] Whole inflorescences were analyzed; [b] last instar; [c] $n = 5$; [d] $n = 3$; n.d. = not detectable; tr. = traces.

Table II. PA patterns and concentrations in *Tyria jacobaeae* and its larval host plant *Senecio vulgaris*.

Alkaloid	RI	M^+ (m/z)	Alkaloid composition [%] Host plant[a]	Larvae[b,c]	Pupae[c] 2 d	Pupae[c] 9 d
Callimorphine	1965	297	n.d.	n.d.	3	4
Senecivernine	2278	335	tr.	n.d.	tr.	4
Senecionine	2290	335	48	40	49	50
Seneciphylline	2303	333	24	26	24	20
Spartioidine	2342	333	8	9	6	5
Integerrimine	2350	335	20	18	18	17
Retrorsine	2515	351	tr.	7	n.d.	n.d.
Total PA concentration (mg/g fr. wt.):			0.09	1.11	3.68	3.10
Total PA content (mg/individuum):				0.12	0.37	0.31

[a] Whole inflorescences were analyzed; [b] last instar; [c] $n = 5$; n.d. = not detectable; tr. = traces.

scopy. Two chemotypes of *S. jacobaea* were found to occur [8]. The "jacobine type" shows the "classical" PA pattern with jacobine, jacoline and jacozine as major PAs, whereas the "erucifoline type" is distinguished by erucifoline and its O-acetyl derivative (Table I) [8]. Since the "erucifoline chemotype" is very common in the vicinity of Braunschweig we used this chemotype as food plant. The larvae did not show any preference for one chemotype or the other.

The PA patterns of host plants and herbivoring caterpillars are very similar, indicating that the plant PAs are taken up by *Tyria* larvae without significant discrimination. O-Acetylerucifoline was the only exception, it could never be detected in the insect (Table I). In the case of larvae which developed on *S. vulgaris* almost identical PA patterns were found in food plants and herbivoring insects (Table II). On the other hand, the PA pattern was significantly different in pupae and adults of *Tyria* which as larvae had been reared on *S. jacobaea* (Table I). This alteration is mainly caused by a decrease in the relative concentration of erucifoline, the major PA found in host plants and herbivoring *Tyria* larvae, and the appearance of callimorphine in pupae. Callimorphine was identified by its MS data (Fig. 1) which are identical with those reported by Edgar *et al.* [7]. Callimorphine is always accompanied by a minor component with the same M^+ and the typical fragmentation pattern of a retronecine 7-O-ester [9] (Fig. 1). This compound was tentatively identified as the isomeric 7-O-ester of callimorphine, named isocallimorphine (Fig. 1). The callimorphines are absent from the host plants and *Tyria* larvae. Only small amounts of callimorphine (less than 5% of total PAs) could be detected in *Tyria* pupae which as larvae had been reared on *S. vulgaris*.

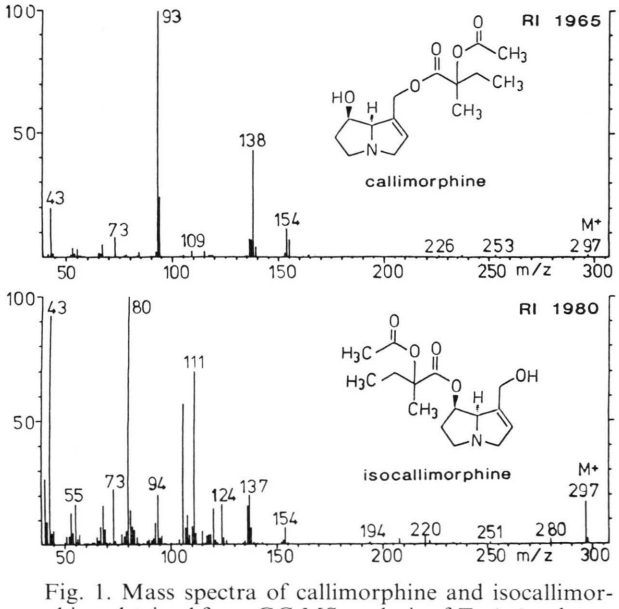

Fig. 1. Mass spectra of callimorphine and isocallimorphine obtained from GC-MS analysis of *Tyria jacobaeae* (pupal stage).

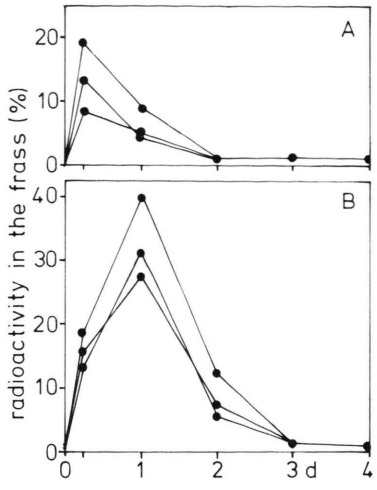

Fig. 2. Time course of occurrence of radioactivity in the frass of *Tyria jacobaeae* following administration of [^{14}C]senecionine (A) and [^{3}H]atropine (B). Tracers were fed orally to the larvae. The curves represent the elimination kinetics of single specimens.

Uptake kinetics and storage of ^{14}C-labelled alkaloids

In order to study the efficiency of PA uptake and storage, flower heads of *S. jacobaeae* treated with a defined amount of [^{14}C]senecionine were offered to *Tyria* larvae (last instar). The insects generally consumed the treated flower heads within 2 to 3 h and were then allowed to continue feeding on untreated host plant material. The frass pellets were collected at time intervals and analyzed for radioactivity. The results of three representative experiments are shown in Fig. 2A. Considerable amounts of radioactivity are rapidly excreted with the frass pellets collected 6 h after tracer feeding. Within two days the elimination of radioactivity

decreased to a "noise level" of less than 1% per day. The total amount of radioactivity eliminated by individual larvae fed during the last instar was between 16 and 33% (Table III). The larvae were allowed to pupate and the respective pupae were analyzed 11 days after tracer application. The percentage of MeOH extractable radioactivity recovered from the pupae was in the range of 33 to 50% of total radioactivity applied (Table III). Essentially the same results were obtained if [^{14}C]senecionine N-oxide was offered instead of [^{14}C]senecionine. Analysis of the tissue distribution of PAs in larvae revealed the integument to be the major site of PA storage (about 75% of total PAs). The remaining alkaloid was found in the haemolymph.

For comparative reasons the feeding experiment was repeated using [^{3}H]atropine as tracer (Fig. 2 B and Table III). Most of the radioactivity is rapidly eliminated during the first two days and only a small amount of radioactivity was ingested by the larvae and transmitted to the pupae.

N-Oxidation capacity

PAs extracted from the different developmental stages of *Tyria* are present as N-oxides. With our extraction procedure generally more than 95% of total PAs are recovered as N-oxides. Furthermore larvae and pupae are able to N-oxidize tertiary PAs. If [^{14}C]senecionine is offered orally to larvae or is injected directly into prepupae the tertiary PA is rapidly and completely transformed into its N-oxide (Table IV). As already pointed out above both labelled senecionine and senecionine N-oxide were taken up and stored with the same efficiency. In both cases the alkaloid N-oxide is the exclusive form of PA storage. Analysis of MeOH extracts of the frass revealed that [^{14}C]senecionine had already been N-oxidized to a large extent during its gut passage; 18 to almost 70% of radioactivity was recovered as [^{14}C]senecionine N-oxide and less then 5% as tertiary alkaloid. In dead, already decaying insect tissue, however, a considerable proportion of alkaloid is detectable as tertiary alkaloid (Table IV, larva[a]).

Table III. Sequestration and elimination of orally fed [^{14}C]senecionine and [^{3}H]atropine by *Tyria jacobaeae*. Tracers were fed to larvae (last instar). Larvae were allowed to develop to pupae (pupation 3 to 10 d after tracer administration). Extraction of pupae 11 d after tracer application.

	% of radioactivity administered[a]		
	I[b]	II[b]	III[b]
[^{14}C]Senecionine			
frass	16	33	20
pupae	50	43	33
[^{3}H]Atropine			
frass	53	54	73
pupae	3	7	13

[a] MeOH-soluble radioactivity.
[b] I to III represent single specimens.

Table IV. N-oxidation and stability of labelled PAs administered orally or by injection to *Tyria jacobaeae*.

Tracer administration	Developmental stage	Tracer extraction (MeOH) Time [d]	Composition [%] Sen–N–ox	Sen
[^{14}C]Senecionine				
Orally to larva	pupa	9	94	n.d.
Orally to larva	pupa	9	85	tr.
Injected pupa	pupa	9	87	tr.
Injected pupa	pupa	9	91	tr.
Orally to larva	larva	2	90	tr.
Orally to larva	larva	2	93	2
Orally to larva	larva[a]	4	43	45
Orally to larva	frass	1	67	1
Orally to larva	frass	1	57	3
Orally to larva	frass	1	18	3
[^{14}C]Senecionine N-oxide				
Orally to larva	pupa	9	92	tr.
Orally to larva	larva	2	90	n.d.
Orally to larva	frass	2	66	2
Orally to larva	frass	2	88	4

[a] Larva died during the experiment.

Biosynthesis of callimorphine

The metabolic origin of callimorphine is unknown. It has been termed a "metabolite" without suggestions concerning its mode of formation [1, 2, 7]. L'Empereur *et al.* [9], who recently found callimorphine in the arctiid *Gnophaela,* regarded a direct conversion of the host plant PAs into callimorphine for chemical reasons as most unlikely, and instead suggested hydrolysis of plant PAs and reesterification of the resulting necine base as a possible mechanism.

To test this suggestion we fed [^{14}C]retronecine to *Tyria* larvae (last instar) and analyzed the PA ex-

tracts after pupation. Orally fed retronecine is efficiently taken up by larvae, 11 to 40% of total radioactivity could be recovered in MeOH extracts of individual pupae 6 to 12 days after tracer application. TLC separation of crude MeOH extracts revealed one major labelled component which in three independent feeding experiments accounted for 23 to 52% of soluble radioactivity (Fig. 3A). By comparison to reference compounds the labelled compound was tentatively identified as callimorphine N-oxide. Zn/H^+ reduction of the crude MeOH extract and rechromatography in the same solvent system showed a labelled spot which displayed the same R_f value as authentic callimorphine. To prove the identity of the two compounds the labelled spots were thoroughly localized by autoradiography (Fig. 3), scraped off the TLC plates, extracted with MeOH and after reduction of the N-oxide the two extracts were submitted to GLC-MS analysis. The two samples were shown to contain only one substance, which was found to be identical with callimorphine in respect to RI value, molecular ion and fragmentation pattern.

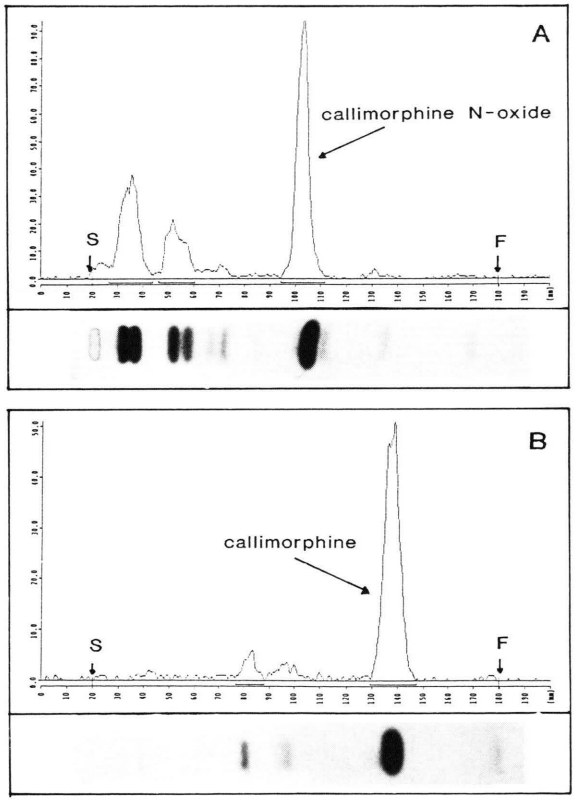

Fig. 3. Transformation of [^{14}C]retronecine into [^{14}C]callimorphine N-oxide by *Tyria jacobaeae*. The tracer (2.2×10^5 cpm) was fed orally to larvae (last instar). Pupae (age 3 d) were extracted and the crude MeOH extracts were applied to TLC separation. A: Detection of callimorphine N-oxide in the crude MeOH extract by TLC radioscanning and autoradiography. B: Detection of callimorphine by TLC radioscanning and autoradiography following reduction and prepurification by solid phase chromatography (Extrelut) of the crude MeOH extract. Solvent in A and B: CH_2Cl_2/MeOH/NH_3 (82:15:3); S: start; F: front.

The capability of the insect to synthesize callimorphine from [^{14}C]retronecine is restricted to the larvae-prepupae transition. Not even traces of labelled callimorphine could be detected in larvae (last instar) which had been fed with [^{14}C]retronecine 1 to 10 days prior to analysis. In any case labelled callimorphine was detectable in prepupae and it does not seem to increase in concentration during maturation of pupae. Injection of [^{14}C]retronecine into prepupae or pupae does not result in the formation of labelled callimorphine.

Discussion

Are PAs taken up selectively by *Tyria* larvae? Are PAs stored in insects as N-oxides as they are in plants? These were the two questions we asked at the beginning, and which can now clearly be answered. Larvae of *Tyria jacobaeae* take up PAs from their host plants without significant discrimination or preference. The PA patterns found in the larvae are comparable to those found in the host plants. Only O-acetylerucifoline, which was never been found in insect PA extracts, appears to be an exception. It still remains open whether this compound is discriminated or deacetylated prior resorption. The conclusion of Aplin and Rothschild [2]: "that *T. jacobaeae* is selectively storing senecionphylline which, in the ragwort *(S. jacobaea)* is present mainly as the N-oxide", needs to be modified. We know that in *Senecio* species PAs are exclusively present as N-oxides [3–5]. These N-oxides are taken up and stored by *Tyria* larvae. This is in agreement with early studies of Mattocks [14] who found that more than 85% of the PAs extracted from *Tyria* larvae are N-oxides. It must be recalled that PA N-oxides are less stable than the respective tertiary PAs and are easily reduced spontaneously during alkaloid extraction in the

presence of even weak biological reducing agents such as cysteine [3]. Thus the contradictory reports concerning the proportion of N-oxides in PA extracts frequently depend on the extraction procedures used. Furthermore, in dead insects the N-oxides are rapidly converted into the tertiary form (see Table IV). Thus, special care is needed during sample preparations to prevent any spontaneous reactions in autolysing biological materials. Our tracer experiment definitely show that in *Tyria* both the PAs of plant origin as well as the insect PA callimorphine are present exclusively in the form of their N-oxides. Furthermore, larvae and pupae of *Tyria jacobaeae* possess the capability to N-oxidize tertiary senecionine which had been administered orally or by injection (see Table IV). The ability to N-oxidize tertiary PAs has recently been observed for the arctiid *Creatonotos transiens* which as larvae fed on an artificial diet containing heliotrine [15], retronecine [16] or mixtures of tertiary PAs [17]. In this respect the two arctiids behave like PA plants which, in contrast to non-PA plants, are also able to N-oxidize tertiary PAs [5].

Uptake and storage of PAs by *Tyria* larvae is a specific process. Other alkaloids such as atropine are accumulated in trace amounts only (see Table III). The elimination kinetics of excess PA-N-oxide taken up with the food is similar to that recently observed with *Melanoplus sanguinipes,* a grasshopper which feeds on PA plants without sequestering PAs [19]. About 48 h after pulse feeding of ^{14}C-labelled senecionine to *Tyria* larvae the elimination of excess radioactivity had been declined to almost zero (<1% per day). A proportion of 30–50% of the fed [^{14}C]senecionine is stored in the bodies as N-oxide and can be recovered from pupae. This efficient sequestration of PAs predicts specific mechanisms for PA uptake and storage. First experimental evidence for a specific carrier mediated uptake of PA-N-oxides has recently been obtained with isolated guts of *Creatonotos transiens* [20].

Callimorphine has been detected in several arctiid moths, in addition to *Tyria* these are: *Arctia caja* [2], *Callimorpha dominula* [7], *Creatonotos transiens* [16, 18], *Gnophaela latipennis* [9]. The transformation of dietary [^{14}C]retronecine into labelled callimorphine proves the ability of *Tyria* to synthesize this alkaloid by metabolic esterification of a necine base. Recently, creatonotine and isocreatonotine, two novel insect PAs, were isolated and identified from *Creatonotos transiens* moths which as larvae had received retronecine as the only source of alkaloids [16]. The creatonotines represent the O^9- and O^7-esters of retronecine with 2-hydroxy-3-methylpentanoic acid, they are accompanied by trace amounts of callimorphine. Furthermore, it was shown that *Creatonotos* is not only able to esterify dietary retronecine but also to degrade complex ester alkaloids of plant origin and reesterify the resulting necine base. The respective necic acid is an intermediate of insect metabolism [16]. Identification of callimorphine as an insect PA synthesized by esterification of retronecine derived from ingested plant PAs explains the occurrence of this PA in different arctiids feeding on host plants known to contain PAs of quite different chemical structures. Furthermore, it shows the suggestion of L'Empereur *et al.* [9] to be right. The observation that only small amounts of callimorphine are formed in *Tyria* reared on *S. vulgaris* indicate quantitative differences in the capability of the insect to hydrolyze PAs of different structures. In fact, such differences were found in comparative feeding experiments with *Creatonotos* [17].

In conclusion our studies proved that *Tyria* larvae take up and store PAs from their host plants without significant discrimination. PAs are stored exclusively as N-oxides. The long known insect PA callimorphine is produced by the insect by "partial biosynthesis", *i.e.* by esterification of a necine base of plant origin with a necic acid which most likely is a product of insect metabolism.

Acknowledgements

This work was supported by grants of the Deutsche Forschungsgemeinschaft and of the Fonds der Chemischen Industrie to T. H. We thank Claudine Theuring for skilful technical assistance.

[1] R. T. Aplin, M. H. Benn, and M. Rothschild, Nature **219,** 747 (1968).
[2] R. T. Aplin and M. Rothschild, in: Toxins of Animal and Plant Origin (A. de Vries and E. Kochva, eds.), p. 579, Gordon and Breach, London 1972.
[3] T. Hartmann and G. Toppel, Phytochemistry **26,** 1639 (1987).
[4] T. Hartmann and M. Zimmer, J. Plant Physiol. **122,** 67 (1986).
[5] T. Hartmann, A. Ehmke, U. Eilert, K. v. Borstel, and C. Theuring, Planta **177,** 98 (1989).
[6] A. Ehmke, K. v. Borstel, and T. Hartmann, Planta **176,** 83 (1988).
[7] J. A. Edgar, C. C. J. Culvenor, P. A. Cockrum, L. W. Smith, and M. Rothschild, Tetrahedron Lett. **21,** 1383 (1984).
[8] L. Witte, A. Ehmke, and T. Hartmann, Naturwissenschaften (in press).
[9] K. L'Empereur, Y. Li, and F. R. Stermitz, J. Nat. Prod. **52,** 360 (1989).
[10] G. Toppel, L. Witte, B. Riebesehl, K. v. Borstel, and T. Hartmann, Plant Cell. Rep. **6,** 44 (1987).
[11] A. Wehrli and E. Kovats, Helv. Chim. Acta **42,** 2709 (1955).
[12] J. Wagner, C. Danzin, and P. Mamont, J. Chromatogr. **227,** 349 (1982).
[13] T. Hartmann, H. Sander, R. Adolph, and G. Toppel, Planta **175,** 82 (1988).
[14] A. R. Mattocks, Xenobiotica **1,** 451 (1971).
[15] A. Engelhaaf, K. Cölln, B. Schmitz, M. Buck, M. Wink, and D. Schneider, Z. Naturforsch. **45c,** 115 (1990).
[16] T. Hartmann, A. Biller, L. Witte, L. Ernst, and M. Boppré, Biochem. Syst. Ecol. (in press).
[17] M. Boppré, A. Biller, L. Witte, and T. Hartmann, unpublished.
[18] M. Wink, D. Schneider, and L. Witte, Z. Naturforsch. **43c,** 737 (1988).
[19] A. Ehmke, P. Proksch, L. Witte, T. Hartmann, and M. B. Isman, Naturwissenschaften **76,** 27 (1989).
[20] M. Wink and D. Schneider, Naturwissenschaften **75,** 524 (1988).