Uresiphita reversalis (Lepidoptera: Pyralidae): Carrier-Mediated Uptake and Sequestration of Quinolizidine Alkaloids Obtained from the Host Plant *Teline monspessulana*

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Larvae of *Uresiphita reversalis* feed almost exclusively on legumes of the tribe Genisteae, whose characteristic secondary metabolites are quinolizidine alkaloids (QA). Aposematic larvae store host plant-derived QA in their integument, while the pupae are almost alkaloid-free. In the last instar larvae, alkaloids were concentrated in the larval head, possibly in the silk glands. About 80% of the alkaloids were transferred to the cocoon silk and 19% were lost with larval exuviae.

The larval alkaloid pattern was characterized by capillary GLC and GLC-MS and compared to that of the host plant, *Teline monspessulana*. Whereas the host plant contained mainly epiaphylline, dehydroaphylline and aphylline, larvae selectively accumulated N-methylcytisine, a relatively minor component of the plant QA; the faeces contained mainly epiaphylline and dehydroaphylline. Thus uptake and sequestration must be selective processes. Uptake by isolated larval midguts was time-, pH- and temperature-dependent and displayed an activation energy between 50 and 80 kJ/mol. Furthermore the *in vitro* uptake of 13-hydroxylupanine was competitively inhibited by cytisine. All these data provide evidence that QA uptake does not proceed by simple diffusion but instead with the aid of a carrier mechanism; this could explain the differential alkaloid uptake observed in living larvae.

Introduction

Larvae of the pyralid moth, *Uresiphita reversalis* (Guenée), feed on legumes of the genera *Lupinus, Genista, Laburnum* and *Cytisus*. In California the main host plant is *Teline monspessulana* (synonyms: *Cytisus monspessulanus, C. candicans, Genista monspessulana*) although, given the choice, *Lupinus arboreus* is preferred [1, 2]. These plants produce quinolizidine alkaloids, which appear to be useful in their chemical defence [3, 4]. *Uresiphita reversalis* is a specialized herbivore and is closely adapted to the secondary chemistry of legumes [1, 2, 24]. The QA cytisine and sparteine are phagostimulants for last instar (L 5) larvae and influence larval growth positively. Larvae, which feed on young leaves that have an elevated alkaloid content, sequester QA in the integument and can exploit them as acquired defence compounds. Larvae are aposematic and distasteful for predators. At pupation most alkaloids are lost; thus the inconspicuous adult moths are almost alkaloid-free and readily eaten by predators.

In lupins, quinolizidine alkaloids, synthesized in leaf chloroplasts, are translocated throughout the plant *via* the phloem and accumulate predominantly in peripheral tissues, such as epidermis. Alkaloid concentrations in epidermis are 20–50 mM but can reach 200 mM. QA are stored in the vacuole, into which they are selectively transported with the aid of a carrier system. The driving force for the uphill concentration seems to be a proton – alkaloid antiport mechanism [5–9]. Since larvae of *U. reversalis* store dietary QA [1, 2], we sought to determine whether the alkaloids are taken up by simple diffusion or whether they are resorbed from the food with the aid of a specific carrier system as in lupin vacuoles.

In this communication we have further analyzed QA storage in *U. reversalis* in comparison to that

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of the host plant by capillary GLC and GLC-MS and have performed biochemical transport studies with isolated larval guts, providing the first evidence for a QA carrier mechanism.

Materials and Methods

Larvae were reared in an insectary on foliage of *T. monspessulana* as described in [1, 2]. Larvae were transported by air to Germany, where they were fed with *Genista tinctoria*.

Alkaloid analysis

Larvae or dissected organs were stored at −20 °C until processing. Tissues were ground in a mortar in 0.5 M HCl. After 1 h the homogenates were made alkaline with 6 M NaOH and poured onto standard Chem elut columns for liquid-solid extraction. Alkaloids were eluted with CH_2Cl_2. Eluates were concentrated *in vacuo*. Alkaloid extracts from plants and insects described in Table II were performed as described in [1]. All alkaloid extracts were analyzed by capillary GLC, using a Varian instrument which was equipped with both FID and a nitrogen specific detector. Column: DB-1, 30 m × 0.3 mm; 1 µm film (J&W, Scientific). Oven temperature: 150 °C 1 min; 150–300 °C with 6 or 20 °C/min, then 15 min isothermal. Injector: split injection (1:30), 250 °C; detector: 300 °C. Helium was used as a carrier gas. Sparteine and cytisine were used as external standards. For GLC-MS a Carlo Erba (5160) instrument was combined with a quadrupol mass spectrometer Finnigan MAT 4515. GLC-MS measurements were performed as described previously [6, 10].

In plants and faeces two alkaloids (*i.e.* epiaphylline, dehydroaphylline) were present in larger quantities. These alkaloids were separated and isolated by layer chromatography and analyzed by ^{13}C NMR (Bruker, 360 MHz).

Uptake experiments

Larvae were dissected in a modified ringer solution, which consisted of: 800 mg NaCl, 200 mg KCl, 1000 mg glucose, 1000 mg MES, pH 5 in 1000 ml distilled water. Guts were excised and slit open to remove the peritrophic membrane and the gut contents. Midguts were cut into pieces of 1 mm length, which were immediately used for the uptake experiments. For each data point, two or three gut pieces were added to 200 µl buffer in Eppendorf vials and left there for equilibration for 15 min. Experiments were started by adding 10 µl ^3H-13-hydroxylupanine (*ca.* 150,000 cpm). Experiments were terminated by taking out the gut segments and washing them in "cold" incubation buffer for 2 min. Then they were individually transferred to Eppendorf vials containing 100 µl 1 M HCl. Tissues were homogenized with a small glass rod. Homogenates were quantitatively given into scintillation vials containing 10 ml scintillation cocktail. The radioactivity was determined in a scintillation counter. In addition, the radioactivity of the incubation buffer was measured for the calculation of the percentage of radioactivity taken up by the gut segments. As a control, gut segments were incubated with tritiated water and were processed in the same way as in the alkaloid experiments.

Results and Discussion

Identification of alkaloids

Complex mixtures of QA were analyzed by capillary GLC and GLC-MS as the method of choice (Fig. 1). Kovats retention indices and MS fragmentation data are available for more than 100 QA which allow the identification even of minor components in most instances (for review [22]).

Of the main QA from *T. monspessulana* and *U. reversalis*, 15 could be unequivocally identified according to their MS spectra and their Kovats retention indexes (Table I, Fig. 2), which have previously been recorded in our laboratories [5, 6, 10, 21]. A major QA of foliage with the RI of 2110 is clearly a QA belonging to the aphylline-type alkaloids. The molecular ion of m/z 246 indicates the presence of a double bond. Aphyllidine, a known alkaloid with a double bond in the 5,6-position displays a different mass spectrum [22]. We have isolated this alkaloid and analyzed its ^{13}C NMR spectrum: In aphyllidine the signals at C 5 and C 6 are at 102.4 and 139.3 ppm [23], in our compound the relevant carbon atoms have values of 109.21 and 124.4 ppm. Therefore, we can assume that this QA is indeed a dehydroaphylline, which is being further analyzed at present (data will be presented elsewhere). Due to limited material a few minor

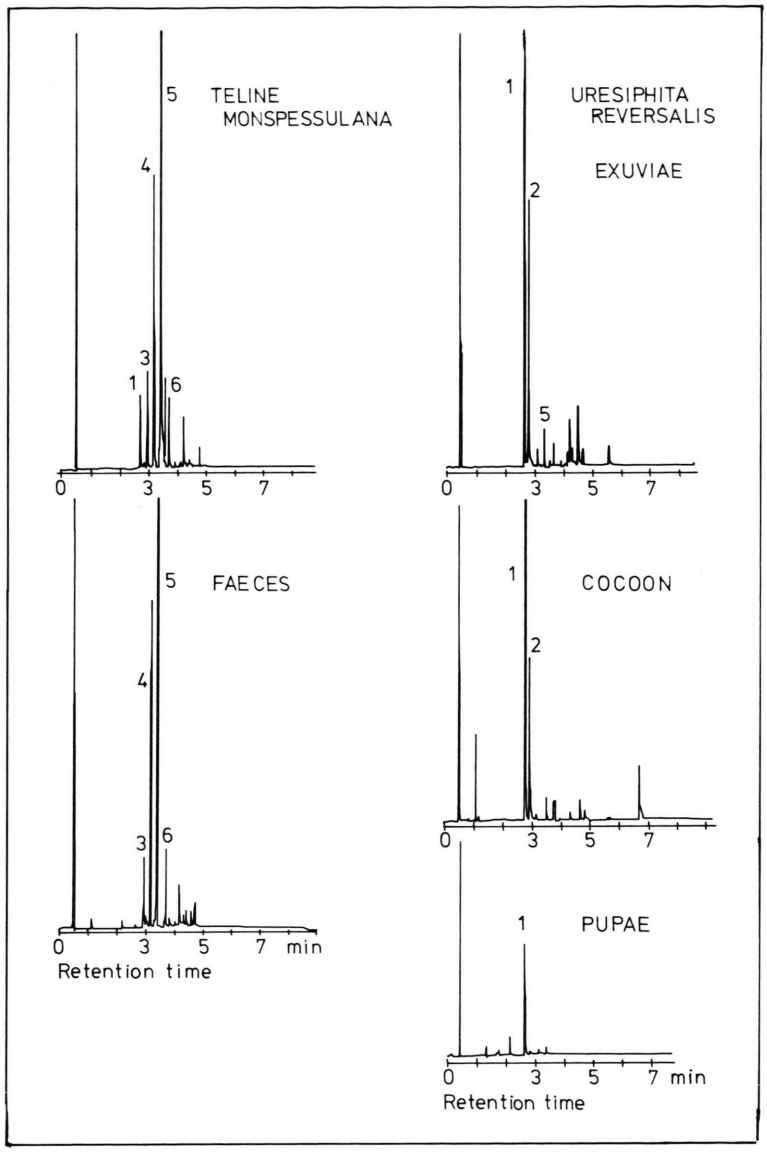

Fig. 1. Separation of alkaloid extracts from *T. monspessulana,* and *U. reversalis* (cocoon, larval exuviae, pupae; faeces) by capillary GLC. Detection with a nitrogen-specific detector. 1 = N-methylcytisine, 2 = cytisine, 3 = virgiboidine, 4 = epiaphylline, 5 = dehydroaphylline, 6 = aphylline.

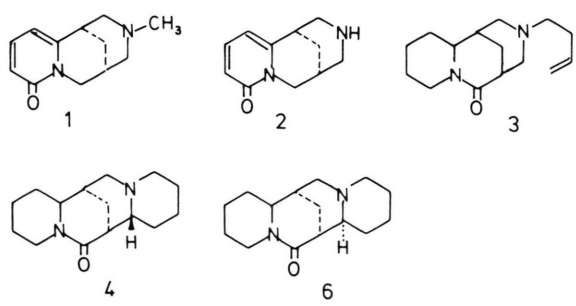

Fig. 2. Structures of the main quinolizidine alkaloids from *T. monspessulana* and *U. reversalis*. 1 = N-methylcytisine, 2 = cytisine, 3 = virgiboidine, 4 = epiaphylline, 6 = aphylline.

Table I. Mass spectral data and GLC-performance of alkaloid extracts from *U. reversalis* A DB1-30W (J&W Scientific) column was used; temperature program: 150–300 °C, 6 °C/min.

Compound	RI	Mass spectral data M^+ m/z (relative abundance)					CI-MS
Ammodendrine	1865	208(55)	191(50)	165(100)	136(60)	123(60)	
N-Methylcytisine	1955	204(15)	160(4)	146(5)	58(100)		205(100)
Cytisine	1990	190(60)	160(20)	147(80)	146(100)	138(25)	191(100)
Virgiboidine	2005	248(1)	207(100)	136(2)	84(45)	58(60)	249(100)
Epiaphylline	2060	248(25)	247(30)	220(25)	136(100)	96(50)	249(100)
Angustifoline	2075	234(1)	193(100)	112(70)	84(20)	55(10)	235(100)
Dehydroaphylline	2110	246(100)	218(12)	164(15)	136(100)	96(75)	247(100)
Isolupanine	2105	248(50)	149(50)	136(100)	98(30)		
5,6-Dehydrolupanine	2130	246(25)	148(8)	134(8)	98(100)	97(35)	
Rhombifoline	2155	244(1)	203(80)	160(20)	146(10)	58(100)	245(100)
Lupanine	2165	248(40)	219(8)	149(50)	136(100)	98(30)	249(100)
Aphylline	2180	248(25)	247(30)	220(30)	136(100)	84(30)	249(100)
Dehydrolupanine	2190	246(48)	148(30)	134(55)	97(20)	85(50)	
11-Allylcytisine	2240	230(0.1)	189(100)	160(10)	146(25)	134(10)	
17-Oxolupanine	2340	262(45)	234(8)	150(100)	136(10)	110(40)	
Anagyrine	2380	244(30)	160(10)	146(12)	136(10)	98(100)	245(100)
Dehydrobaptifoline	2610	258(100)	147(95)	146(90)	112(90)	84(50)	
Baptifoline	2630	260(25)	152(10)	146(15)	114(100)	96(10)	
A 1	1835	206(80)	192(20)	162(100)	94(45)	82(50)	
A 2	1966	232(85)	136(40)	124(60)	98(40)	84(100)	233(100)
A 3	1980	220(5)	190(10)	162(4)	146(5)	58(100)	
A 4	2020	246(35)	163(10)	148(10)	98(100)	97(70)	247(100)
A 5	2027	244(50)	215(10)	164(20)	136(100)	83(40)	
A 6	2030	246(1)	205(100)	134(2)	82(15)	58(10)	247(100)
A 7	2080	222(45)	193(10)	164(10)	150(100)	84(50)	
A 8	2102	278(10)	263(5)	246(15)	218(70)	136(100)	279(100)
A 9	2145	262(10)	246(25)	236(5)	218(50)	136(100)	
A 10	2150	264(15)	246(25)	218(50)	136(100)	97(50)	265(100)
A 11	2168	262(35)	245(15)	136(20)	98(100)	84(20)	
A 12	2220	278(30)	262(20)	247(20)	218(50)	136(100)	
A 13	2225	246(90)	217(15)	164(15)	136(100)	96(90)	247(100)
A 14	2250	294(15)	262(20)	245(70)	136(100)	97(60)	295(20) 263(100)
A 15	2253	276(30)	261(100)	245(30)	136(40)	98(50)	
A 16	2260	262(90)	245(70)	136(70)	96(90)	84(100)	263(100)
A 17	2270	264(30)	247(25)	236(15)	136(100)	97(40)	
A 18	2300	264(35)	236(20)	136(100)	97(65)	82(20)	265(100)
A 19	2310	262(15)	245(15)	236(20)	152(100)	138(10)	263(100)
A 20	2332	262(30)	246(30)	218(100)	152(20)	132(40)	
A 21	2350	242(100)	227(25)	148(25)	134(45)	120(30)	
A 22	2365	262(40)	242(40)	234(20)	132(100)	82(45)	
A 23	2390	298(1)	263(10)	245(100)	136(45)	97(45)	
A 24	2420	264(30)	236(30)	207(20)	152(100)	84(40)	
A 25	2467	262(100)	234(10)	205(15)	152(100)	136(40)	

alkaloids could be identified tentatively only. The double bond in dehydrolupanine is probably in the 11,12-position according to the MS fragmentation pattern (*i.e.* m/z 134). The alkaloid with the M^+ 258 (RI 2610) has not been described and could be a dehydrobaptifoline, because of the close similarities to the respective fragmentation pattern of baptifoline. A 3 is related to N-methylcytisine, which is indicated by the characteristic m/z 58 fragment. The mass difference between N-methylcytisine (M^+ 204) and M^+ 220 indicates the presence of a hydroxyl group; its position needs to be elucidated. Many of the alkaloids presented in Table I seem to be related to the aphylline skeleton and have not been described so far. A thorough analysis of *T. monspessulana* is certainly necessary to

elucidate all the new alkaloids present. This analysis was, however, out of scope of the present project.

Alkaloid contents and alkaloid patterns of insects and host plants

We have analyzed the alkaloid patterns and alkaloid contents of larvae, larval exuviae, silk cocoons, pupae and of adult moths of *U. reversalis* by capillary GLC and GLC-MS (Fig. 1, Table II and III).

Pupae, and presumably adult moths, have been reported to be almost alkaloid-free [1, 2]. In this study, larvae stored between 26 and 120 µg/per animal (Table III). Differences in absolute values reflect the fact that we have analyzed different "batches" of *U. reversalis,* which were raised at different times and whose feeding history differed. QA levels in insects are positively related to those in the food plant; the latter vary widely over time, and between plants [1]. Larvae from Exp. IV were laboratory cultures raised in California, whereas those of Exp. I–III were sent to Germany, where *T. monspessulana* was not available. We offered *Genista tinctoria* as food instead.

In last instar larvae, QA were not evenly distributed within the insects as analyzed with dissected insect parts (Table III). Hemolymph and gut tissue (without food remains) do not store QA (Table III, Exp. I, III). The remaining tissue, from which the fat body was removed by scraping, mainly consists of the integument. The integument, to which the larval head was still attached, contained more than 92% of the alkaloids stored. In order to find out whether the alkaloids were evenly distributed in the integument we separately analyzed the head, terminal abdominal segments, and the other segments. Exp. I–III (Table III) clearly showed that the alkaloids were concentrated (76–88% of total alkaloids) in the head of the larvae. Since almost 80% of the larval alkaloids were transferred to the cocoon (Table III, Exp. IV) where they were deposited in the silk, we tentatively assume that the alkaloids were previously stored or associated with the larval silk glands that are located in the head segment. About 17% of the larval alkaloids were removed with the larval exuviae, which probably reflects the amount of QA that is present in the integument (Table III; Exp. I–IV). A surface extract of 100 late-instar larvae was made by swirling them in chloroform for 1 min [24]. This procedure released about 1% of the alkaloids stored by the insects (Table III, Exp. IV), indicating that the QA deposited in the integument are readily available for chemical defence [24].

Table II. Patterns of main alkaloids (total alkaloid = 100%) in organs of the host plant *T. monspessulana* and in developmental stages of *U. reversalis*.
+ trace amounts, n.d. = not detected.

Alkaloid	Plant Aerial parts	Young parts	Root	Flowers	Pods	Seeds	Insect Larval exuviae	Surface extract	Cocoon	Pupae	Faeces
Alkaloid abundance [%]											
N-Methylcytisine	4.9	+	95	52.3	1.8	6.3	84.2	50.5	80.5	96	+
Cytisine	n.d.	n.d.	+	1.6	n.d.	62.0	8.5	n.d.	9.0	+	n.d.
Virgiboidine	6.6	1.6	n.d.	3.4	14.6	1.1	n.d.	n.d.	n.d.	n.d.	6.6
Epiaphylline	20.1	27.1	+	6.1	65.6	25.2	+	13.5	n.d.	+	29.3
Dehydroaphylline	33.0	45.6	+	12.8	5.5	0.7	7.2	n.d.	1.2	n.d.	41.8
Aphylline	21.0	13.5	n.d.	4.6	n.d.	0.3	+	n.d.	+	n.d.	4.5
Virgiline	1.3	1.0	n.d.	0.7	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A-8	0.9	+	n.d.	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5
A-12	4.8	2.7	+	7.5	9.4	0.4	+	36.4	1.0	n.d.	4.7
A-19	0.9	1.3	n.d.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5
Other alkaloids	**										***
Total alkaloids [µg/g d.w.]	4145	2401	344	4214	900	4242					3590

Other alkaloids detected by GC-MS (traces) include for ** A 1–A 25, angustifoline, rhombifoline, lupanine, anagyrine and 17-oxolupanine; and for *** A 1, A 2, A 4–A 8, A 13, A 17–A 19, A 23, A 24.

Table III. Distribution of QA in different organs or fractions of *U. reversalis*. In experiments I–III the larvae obtained *T. monspessulana* first (in U.S.A.) and then *Genista tinctoria* (in Germany). In experiment IV, the larvae were reared entirely on *T. monspessulana*. Except for II and III all animals were from different "batches", which explains the absolute difference in alkaloid content between experiments. Larvae were dissected as outlined. The fraction "integument" also includes some of the fat body which was scraped off the integument.

Organ	Alkaloid content µg/animal
Exp. I:	
Larvae (complete)	26.0
Integument plus head	24.0
Integument minus head	4.7
Head	19.2
Gut	1.0
Hemolymph	1.1
Pupae	1.2
Cocoon plus larval exuviae	31.1
Imagines	0.8
Exp. II:	
Larvae	
Head	59.0
Last larval segment ("tail")	0.8
Segments between head and tail	7.1
Exp. III:	
Larvae	
Head	24.0
Gut plus contents	13.0
Integument minus head	7.4
Exp. IV:*	
Cocoon (without pupae)	88.8**
Larval exuviae	18.0
Pupae	2.2
Surface extract	0.9
Faeces [µg/g d.w.]	3590

* Extracted according to [1].
** Cocoons were from a separate batch of insects and were extracted according to methods in this paper.

A large amount of QA that is present in the foliage was apparently not sequestered but eliminated with the faeces (Table II and III). It is likely that the capacity to store QA is limited to the 26–120 µg per animal, any surplus of alkaloids is thus excreted. QA levels in the faeces have been shown to the positively correlated with levels in the host plant [1].

The alkaloid patterns found in the insects (larvae, cocoon, exuviae and pupae) are surprisingly different from those of the host plant *T. monspessulana* (Fig. 1, Table II). In the foliage of *T. monspessulana* QA of the aphylline-type are dominant and α-pyridone alkaloids, such as N-methylcytisine and cytisine are present to a minor degree only (Fig. 2, Table II). In contrast to the alkaloid pattern of foliage, N-methylcytisine is the major alkaloid in larvae, amounting to more than 90% of total alkaloids (Table I and II). This trend can be confirmed for larval exuviae, cocoon silk and for pupae, which contain between 80.5 and 96% N-methylcytisine. In roots, flowers, pods and seeds, which are not consumed by *U. reversalis* larvae (with the exception of flowers, which are eaten to some extent), QA such as cytisine and N-methylcytisine are major alkaloids.

If we compare the alkaloid pattern of the faeces with that of the foliage (Table II), it is apparent that the α-pyridone alkaloids are removed whereas the aphylline-type alkaloids are still present. The quantitative shift from aphylline to dehydroaphylline might reflect a biotransformation in the larval guts. From these data we conclude that larvae process dietary QA in a very selective manner, in that they selectively resorb N-methylcytisine and cytisine (which are supposed to be more toxic than the aphylline-type alkaloids) but eliminate the others *via* the faeces.

Uptake experiments

When feeding QA to larvae of *Syntomis mogadorensis* (Lepidoptera: Ctenuchidae) or *Creatonotos transiens* (Lepidoptera: Arctiidae), we found that the alkaloids were not taken up but eliminated with the faeces [11], indicating that QA do not diffuse unspecifically into moth larvae. Since QA are strong bases which are protonated under physiological conditions, a rapid and simple diffusion is apriori unlikely. In contrast, *U. reversalis* larvae selectively resorb N-methylcytisine in preference to the aphylline-type alkaloids (see Table II). Therefore, we hypothesized that these larvae possess a specific mechanism for selective alkaloid uptake. A carrier-mediated uptake mechanism for QA in the midgut would explain the phenomena observed. In order to test this hypothesis, we analyzed how QA are taken up by midguts of *U. reversalis* larvae.

Isolated midgut segments of late-instar larvae of *U. reversalis* were incubated with tritiated 13-hydroxylupanine (other radioactive QA were not available to us) for different lengths of time.

As shown in Fig. 3 uptake was time-dependent and almost linear for 1 h. In a previous experiment with *Creatonotos transiens* midguts [15] (which do not resorb 13-hydroxylupanine *in vivo*), we could not detect a significant time-dependent uptake of tritiated 13-hydroxylupanine. We therefore conclude that the uptake observed with *U. reversalis* larvae is species-specific.

13-Hydroxylupanine was resorbed against a concentration gradient, which can be seen from parallel experiments with triated water. About 0.8% of the 13-hydroxylupanine that was offered in the incubation mixture was accumulated, compared to only 0.2% of the water (Fig. 3). Tritiated water will equilibrate rapidly between cells and incubation medium. Any value which exceeds that of water uptake thus indicates an accumulation against a concentration gradient.

QA uptake depended on the hydrogen ion concentration of the incubation medium and increased towards alkaline pH. For physiological reasons (higher hydrogen or hydroxyl ion concentrations could kill the cells) we tested the pH-dependence only between pH 4 and 9 (Fig. 4). The higher uptake activity at pH 9 can also be seen in the experiments shown in Fig. 5 and 6. Although we have not measured the pH of the *U. reversalis* gut milieu, caterpillar guts generally tend to be alkaline, with pH of 8–10 being usual.

Alkaloid uptake is temperature dependent and displays an optimum of 24 °C (Fig. 5). The data of temperature dependence can be transformed in an Arrhenius plot to calculate the activation energy. For both pH 4 and 9 we obtained activation energies of 55–78 kJ/mol which clearly indicates that the uptake is a catalyzed process (free diffusion shows values below 20 kJ/mol) (Fig. 5). Furthermore, the uptake of 13-hydroxylupanine was competitively inhibited by adding another QA, *i.e.* cytisine, to the incubation mixture (Fig. 6). A simple diffusion process would not be influenced by such an addition.

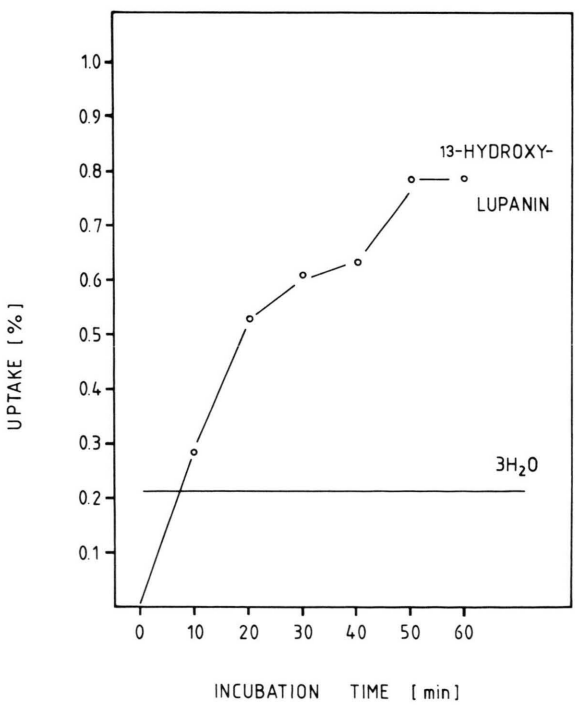

Fig. 3. Time course of the uptake of 13-hydroxylupanine by isolated gut segments of *U. reversalis*. Experiments were performed at pH 5 and 24 °C.

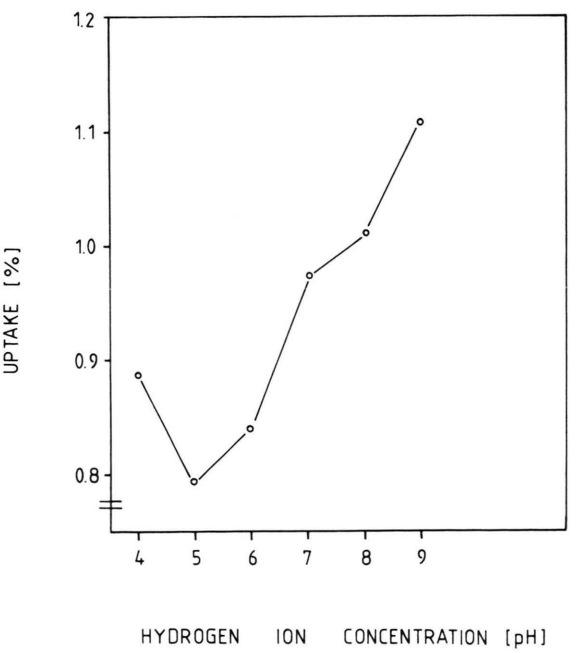

Fig. 4. Influence of hydrogen ion concentration for the uptake of 13-hydroxylupanine by midgut segments of *U. reversalis*. Experiments were performed at 24 °C, reaction time 50 min.

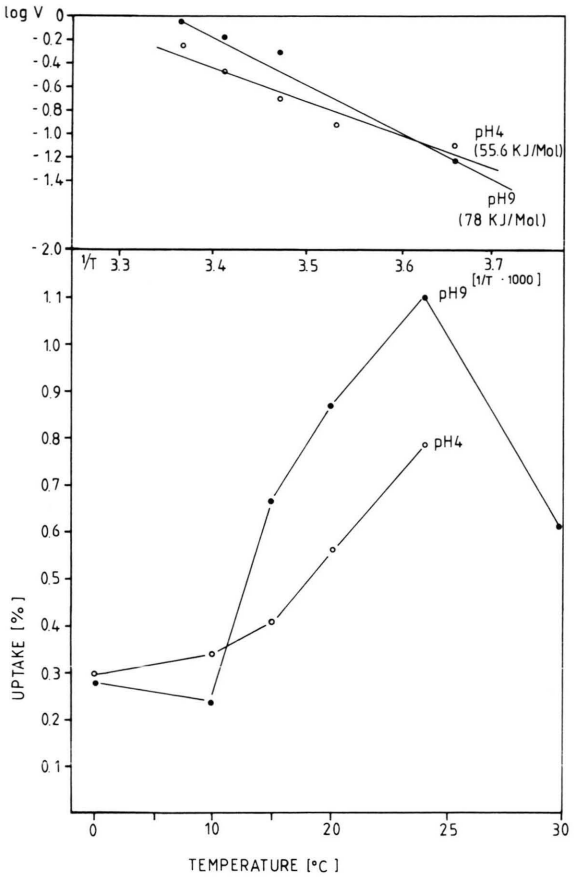

Fig. 5. Temperature dependence of alkaloid uptake in relation to hydrogen ion concentration (pH 4 and pH 9). A. Temperature kinetics, B. Arrhenius plot. Reaction time: 50 min.

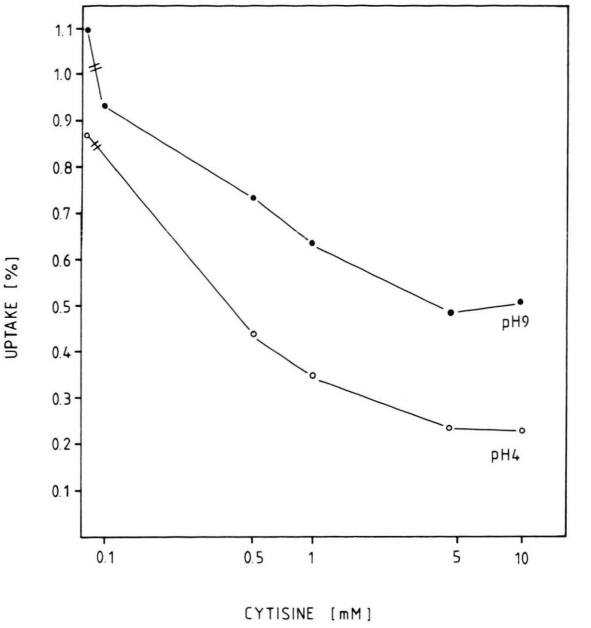

Fig. 6. Competitive inhibition of the uptake of 13-hydroxylupanine by cytisine (final concentrations between 0.1 and 10 mM). Reactions were performed at 24 °C for 50 min both at pH 4 and pH 9.

These preliminary experiments clearly indicate that simple diffusion cannot be responsible for the resorption of N-methylcytisine and cytisine in *U. reversalis* larvae. Instead we have the first evidence for the presence of a carrier mechanism for QA, which probably involves transport proteins in the gut epithelia. Similar transport systems are better known for the uptake of primary metabolites (sugars, amino acids) or ions into living cells of bacteria, fungi, plants or animals [12]. For plant vacuoles specific carrier systems have been demonstrated in a few instances for plant secondary metabolites, *e.g.* isoquinoline alkaloids, indole alkaloids, quinolizidine alkaloids, pyrrolizidine alkaloids, flavonoids, coumaroyl glycosides, and cardiac glycosides (review [13]).

Recently we have shown that similar uptake systems can be found in those insects which sequester secondary metabolites of plant origin. Pyrrolizidine alkaloids (PA) are selectively taken up and stored by larvae of the arctiid moth, *Creatonotos transiens* [11, 14]. The Ctenuchid moth, *Syntomeida epilais* is a *Nerium oleander* specialist and stores dietary cardenolides [11]. In both instances we were able to provide experimental evidence for the presence of transport systems in midgut epithelia for pyrrolizidine alkaloid N-oxides or cardenolides, respectively [15, 16]. The uptake of QA in *U. reversalis* is thus another example of the intricate adaptation of a specialized herbivore to host plant chemistry. Since carriers are proteins, they must be encoded by genes. This would mean that a specialized herbivore must undergo a series of mutations to eventually generate genes which code for these carrier proteins. In addition, further provisions must be made to avoid autotoxicity [17]. The

exact biochemical mechanisms which lead to storage are not well known, but it is remarkable that plant-derived toxins are often deposited in the integument: PA in Arctiid moths [11, 14]; cardenolides in *Danaus* [18], *Syntomeida* [16] and *Oncopeltus* [19]; cyanogenic glycosides in Zygaenidae [20]; and QA in *U. reversalis* [1, 2, this study].

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