Uptake of Alkaloids by Latex Vesicles and Isolated Mesophyll Vacuoles of *Chelidonium majus* (Papaveraceae)

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Latex vacuoles of *Chelidonium majus* (Papaveraceae) naturally sequester protoberberine and benzophenanthridine alkaloids. Isolated vesicles take up a number of other heterologous alkaloids, such as 9,10-dihydroergocryptine, vinblastine, strychnine, nicotine, colchicine, lupanine, and 13-hydroxylupanine, with a strong preference for the indole alkaloids and for nicotine. Kinetic data clearly show that the uptake proceeds *via* simple diffusion and not by carrier-mediated transport. The alkaloids are concentrated in the vesicles against a concentration gradient. MgATP has no influence on alkaloid uptake. It is likely that alkaloids are trapped in the vesicles by chelidonic acid (concentration in latex 58 mM, in vesicles 661 mM) which complexes these alkaloids and thus prevents their rediffusion out of the vesicles. Uptake of these alkaloids by leaf protoplasts and vacuoles of *Ch. majus*, by vacuoles of suspension-cultured cells of *Lupinus polyphyllus* and by rabbit erythrocytes was studied for comparison.

Introduction

A characteristic feature of higher plants is their capacity to produce an intriguing large number of natural products, the so-called secondary metabolites. These allelochemicals are not waste products or otherwise functionless molecules but seem to be important for the fitness of the plants producing them: Besides minor metabolic roles, the main function of these compounds are that of chemical defence against microorganisms (viruses, bacteria, fungi) and against herbivores (nematodes, insects, molluscs, vertebrates). In addition some of the natural products, especially essential oils and coloured compounds, may serve in attracting pollinating or seed-dispersing animals [1–6].

Because of their ecological role many secondary compounds have substantial biological activities and may even be poisonous or deleterious to the plants that produce them. A prerequisite for the exploitation of secondary metabolites by plants as defence compounds is: 1. a storage site that is separated from the cytoplasm to prevent autotoxicity and 2. the capacity to accumulate large amounts of these active compounds at concentrations which are sufficiently high to repel/deter or kill potential enemies

It is well-established that many secondary products are stored in the vacuole [7, 8]. Most of these compounds are synthesized outside the vacuole in the cytoplasm or in organelles [9]. In order to be stored in the vacuole, secondary products have to pass the tonoplast membrane and have to be accumulated against a concentration gradient.

For passage of secondary metabolites across the tonoplast simple diffusion [10–12] or carrier-mediated transport [13–17, 19, 20] have been described. Trapping mechanisms (*e.g.* ion-traps, phenolics) or ATP-dependent proton-antiport systems have been postulated [10–17] as driving force for membrane passage and accumulation against a concentration gradient.

Chelidonium majus is an old medicinal plant known for its antimicrobial and cholagogenic alkaloids of the protoberberine and benzophenanthridine type. As many other species of the Papaveraceae *Chelidonium majus* has laticifers and latex which contain many small latex vesicles (size less than 1 μm), commonly referred to as latex vacuoles or latex vesicles. This latex has a bright yellow-orange colour because the coloured alkaloids are predominantly sequestered [10, 21] there. It has been shown by Matile [10, 18] that these vesicles are able to take up and concentrate alkaloids such as sanguinarine which is an endogenous alkaloid of *Ch. majus*.

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Earlier work on alkaloid accumulation by latex vesicles of *Chelidonium majus* had been explained by simple diffusion in combination with an ion-trap mechanism [10, 18]. Since specific transport systems have been described recently [13–17, 19, 20], it should be interesting to study whether carrier-mediated transport can be ruled out in this system. We have not re-evaluated the uptake of a homologous *Chelidonium* alkaloid because it was not possible for us to obtain radioactively labelled chelidonine, chelerythrine or sanguinarine. Instead, we have studied the uptake of heterologous alkaloids (which were available as radioactive compounds) by latex vacuoles of *Ch. majus* in comparison to vacuoles isolated from *Ch. majus* mesophyll cells and from cell suspension cultures of *Lupinus polyphyllus*.

Material and Methods

Isolation of latex vacuoles

Flowering plants of *Chelidonium majus* were obtained from ruderal sites in the city of Munich during April to June 1987 and 1988 and from the Botanical Garden Heidelberg in April to May 1990. Plants were collected immediately prior to an experiment, since the yellow latex could be best prepared from fresh specimens. Petioles or shoots were cut with a razor blade and immediately transferred to Eppendorf vials (1.5 ml) kept on ice, to collect the latex in 1 ml isotonic vacuole buffer "A" (0.45 M sorbitol, 1 mM L-cysteine, 10 mM MES, adjusted to pH 5.5 with 1 M KOH). In 1987 10 mM citrate (pH 6.7) was substituted for 10 mM MES. The same vial was used to collect the latex of 5–7 petioles. In some experiments (1987) the vacuoles were sedimented by centrifugation (6000 r.p.m. for 2 min) in a Biofuge A (Christ, Osterode) and quickly resuspended in buffer "A".

Uptake of alkaloids into latex vesicles

250 µl buffer "A" containing the latex vacuoles were added to Eppendorf vials kept at room temperature. Uptake experiments were started by adding 2 µl of radioactive alkaloids ([N-methyl-^{3}H]-nicotine, specific activity: 85 Ci/mmol; [2,4,11-^{3}H]-strychnine sulphate, specific activity: 30 Ci/mmol; [G-^{3}H]vinblastine sulphate, specific activity 11.5 Ci/mmol; [ring A-4-^{3}H]colchicine, 5 Ci/mmol; [9,10(n)-^{3}H]9,10-dihydroergocryptine, specific activity 20.7 Ci/mmol) which were obtained from Amersham-Buchler (Braunschweig, F.R.G.) or labelled in our laboratory (*e.g.* lupanine, 13-hydroxylupanine; Wink, in preparation). Radioactivity differed between 0.01 to 1 µCi within different sets of experiments, resulting in a final alkaloid concentration between 0.001 to 0.8 µM. After 30 min 70 µl of the incubation mixture were taken and applied on top of 100 µl silicone oil (a mixture of AP 150/AR 20; 1.4:1) in a small conical centrifuge tube (plastic) (0.5 × 45 mm). After centrifugation (13,000 r.p.m., 1 min) the conical tip of the tubes, which contained the vacuoles that were centrifuged through the silicon oil, were cut off and extracted in 5 ml scintillation cocktail (Roth, Karlsruhe) [15]. The supernatant was processed accordingly. Radioactivity was determined by liquid scintillation counting.

Isolation of vacuoles from mesophyll cells

About 1 g (fresh weight) of 2–3 week old leaves of *Ch. majus* were surface sterilized with 70% ethanol. Then they were immersed in 10 ml 0.45 M sorbitol and cut into segments of about 0.5 cm^2 size. The segments were transferred to 3 petri dishes containing 10 ml buffer "B" (pH 5.5–6; 0.45 M sorbitol, 10 mM $CaCl_2$, 1% cellulase from *Trichoderma viride* (Boehringer Mannheim), 0.5% cellulysine (Calbiochem), and 0.25% pectinase (Roth, Karlsruhe). Incubation was overnight at 22 °C on a rotary shaker (50 r.p.m.). Then the solution was filtered through 1 layer of mull and 1 layer of a nylon membrane (pore size 58 µm) and diluted with 4 volumes of solution "C" (0.45 M sorbitol). Protoplasts were obtained by centrifugation (400 × *g*, 10 min) in a swing-out rotor. Protoplast yield was about 5–15 × 10^7 protoplasts/g leaf.

The protoplast pellet was suspended in 12 ml buffer "D" (0.2 M sorbitol, 10% Ficoll 400, 2 mM DDT, 20 mM EDTA, 5 mM Hepes adjusted to pH 8 with 1 M KOH), and kept at 40 °C for 10 min. Under these conditions the protoplasts were lyzed and released intact vacuoles. In order to obtain purified vacuoles solution "D" was transferred to 2 Correx centrifuge tubes and overlayered with a Ficoll gradient: Step 1: 2 ml of 4% Ficoll (1.4 vol. buffer "D" plus 1 vol. buffer "E" (= buffer "A", but with 10 mM Hepes, pH 7.5 instead of MES). Step 2: 0% Ficoll, 1 ml buffer "E".

Pure vacuoles were concentrated at the interface between 4% and 0% Ficoll after centrifugation at 5500 × *g* for 20 min in a swing-out rotor. The vacuole band was collected with a Pasteur pipette and employed in the uptake experiments. Vacuole yield was between 3 and 15% of the original protoplasts. Their diameter was between 5 and 20 µm.

Uptake of alkaloids by isolated vacuoles

To 150 µl of a purified vacuole suspension we added 3.5 µl radioactive alkaloids (0.35 µCi) and incubated them for 30 min at room temperature. In most experiments 10 mM MgATP and 40 mM KCl were employed additionally. In order to determine the amount of alkaloids taken up the vacuoles were separated from the incubation medium by silicone oil centrifugation (see above): 70 µl of the incubation mixture was layered on top of 100 µl silicone oil (1.4 vol. AP 150:1 vol. AR 20) and centrifuged at 13,000 r.p.m. for 1 min. Radioactivity measurements were as described above. Values were corrected for the simple diffusion of the radioactive alkaloids into the silicone oil.

Volume determinations

The volume of vesicles, protoplasts, and vacuoles was measured in medium containing tritiated water (specific activity 5 mCi/ml; Amersham-Buchler). After 30 min cells or vacuoles were separated from the medium by silicone oil centrifugation and the radioactivity inside the cells/vacuoles was compared to that left in the medium.

Isolation of vacuoles from cell suspension cultures of Lupinus polyphyllus

Heterotrophic cultures which form only small aggregates (LPSP 3) were taken at day 3 or 4 after subcultivation for the isolation of protoplasts [15]. About 10 g cells were added to 50 ml of filter-sterilized solution "F" (0.5 M mannitol, 0.1 M $CaCl_2$, 1% pectinase (Roth), 1% hemicellulase (Sigma), 1% cellulase (Boehringer), 10 mM MES, pH 5.5). Incubation was overnight at room temperature on a rotary shaker (150 r.p.m.). Then the solution was filtered through 0.58 µm gauze and distributed to 2 Correx tubes. After centrifugation the protoplast pellet was washed two times with 0.6 M mannitol and 5 mM MES (pH 5.5) and centrifuged at 390 × *g* for 10 min. Protoplast yield was $1-2 \times 10^8$ protoplasts/experiment.

Protoplasts were suspended in 10 ml lysis medium (10% Ficoll, 5 mM Hepes/KOH, pH 8, 20 mM EDTA, 2 mM DTT and 0.2 M mannitol) and kept at 40 °C for 5 min. Lysis was controlled by light microscopy.

Lyzed vacuoles were transferred to Correx tubes and overlayered with 2 ml 4% Ficoll in 1 vol. lysis medium and 1.5 vol. 0.5 M mannitol, 10 mM Hepes, pH 7.5, and as a second step with 1 ml 0.5 M mannitol and 10 mM Hepes (pH 7.5). Centrifugation in a swing-out rotor was 5000 × *g* for 20 min. Vacuoles were harvested from the interface between 0 and 4% Ficoll with a yield of about 5% (as referred to the protoplasts).

Partition of alkaloids in octanol/water

In order to determine the lipophilicity of alkaloids in relation to their pK values we determined the partition of alkaloids in mixtures of octanol and buffers of different pH values: 500 µl octanol and 500 µl buffer (10 mM MES or Hepes buffer) were filled into 1.5 ml Eppendorf vials. 5 µl of radioactive alkaloids were added (1 µCi) and the vials mixed thoroughly on a whirly mixer. Then the tubes were centrifuged at 13,000 r.p.m. for 5 min to separate the phases. 50 µl aliquots were collected from the octanol and the buffer phases and their radioactivity was determined by liquid scintillation counting.

Determination of chelidonic acid

Chelidonic acid produces a yellow colour under alkaline conditions (pH 13) with a maximum at 355 nm, allowing its photometrical quantitation: 20 µl latex were suspended in 1 ml buffer A (solution I). After centrifugation (1 min at 12,000 r.p.m.) the supernatant was transferred to a fresh Eppendorf vial (solution II). The pellet was dissolved in 1 ml buffer A (solution III). 50 µl aliquots of solution I–III were suspended in 4 ml 1 M NaOH and measured after 10 min at 355 nm. For background control 50 µl aliquots were added to 4 ml water and analyzed at the same wavelength. Chelidonic acid (Fluka) was used to set up a calibration curve.

Results

Latex vesicles

In our experiments which involved 7 heterologous alkaloids of various structures (Fig. 1) and physicochemical properties we tested whether and how these alkaloids are sequestered by latex vesicles of *Chelidonium majus*. As can be seen from Table I, three indole alkaloids, *i.e.* vinblastine, 9,10-dihydroergocryptine, and strychnine and the pyridine alkaloid nicotine were readily sequestered and accumulated to a substantial degree against a concentration gradient. The other 3 alkaloids were subjected to silicone oil centrifugation. The radioactivity was determined in the supernatant and the vacuole pellet. Uptake (in %) was calculated as

$$\text{uptake [\%]} = \frac{(\text{cpm pellet} - \text{cpm diffusion}) \times 100}{(\text{cpm supernatant} + \text{cpm pellet})}.$$

Uptake data for ^3H_2O can be used to determine the volume and stability of the vacuoles. For the calculation of accumulation rates of the alkaloids, the uptake (%) of ^3H_2O was set as 1. Any value higher than 1 means an accumulation against a concentration gradient. Data were corrected for background radioactivity and the simple diffusion of alkaloids into the silicone oil.

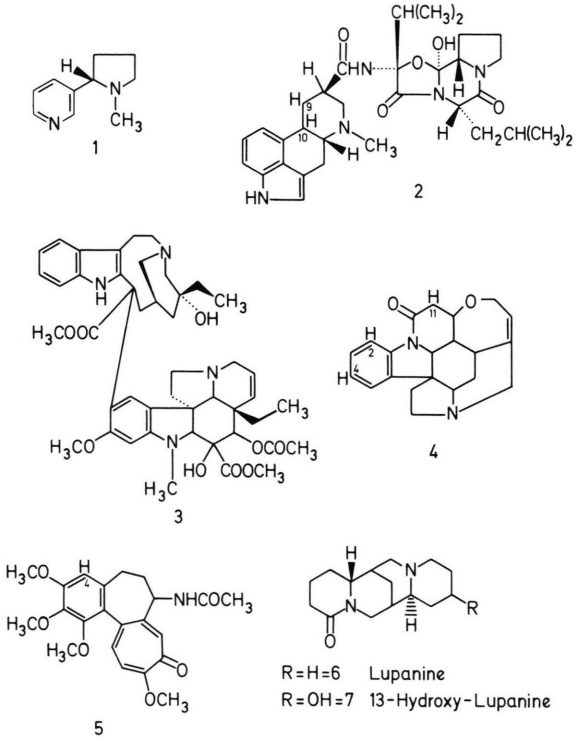

Fig. 1. Structures of the alkaloids tested. **1,** nicotine; **2,** vinblastine; **3,** 9,10-dihydroergocryptine; **4,** strychnine; **5,** colchicine; **6,** lupanine; **7,** 13-hydroxylupanine.

Table I. Time course of alkaloid uptake by latex vesicles of *Chelidonium majus* at different temperatures. Latex vacuoles were incubated in buffer "A" at the given temperature. For each compound and temperature an independent assay was performed. The reaction was started by adding the respective radioactive compounds. At the intervals given 2 aliquots of 70 µl each were taken and

A. Conditions: 4 °C, pH 6.7

Compound	Rate of accumulation Time [min] 5	10	15	60
^3H_2O	1	1	1	1
(uptake %)	1.3	1.0	0.9	1.5
Nicotine	2.5	2.2	1.9	2.3
Dihydroergocryptine	25.2	29.9	18.2	16.9
Vinblastine	10.5	21.0	12.0	11.7
Strychnine	4.4	2.9	2.8	3.6
Colchicine	1.5	1.8	1.4	0.9
Lupanine	1.8	2.3	1.2	1.1
13-Hydroxylupanine	1.2	1.0	2.3	1.5

B. Conditions: 22 °C, pH 5.5

Compound	Rate of accumulation Time [min] 6	30	60	120
^3H_2O	1	1	1	1
(uptake %)	0.18	0.53	0.20	0.29
Nicotine	5.0	1.1	4.5	5.9
Dihydroergocryptine	31.0	21.5	28.0	17.2
Vinblastine	29.4	9.1	31.5	21.4
Strychnine	11.1	1.1	4.5	2.7
Colchicine	2.6	0.6	1.7	2.5
Lupanine	2.1	0.6	1.2	0.9
13-Hydroxylupanine	1.8	1.2	0.9	2.3

C: Conditions: 37 °C, pH 6.7

Compound	Rate of accumulation Time [min] 5	10	15	60
^3H_2O	1	1	1	1
(uptake %)	0.6	0.5	0.5	0.4
Nicotine	5.7	3.8	4.8	8.0
Dihydroergocryptine	43.2	63.0	51.8	111.5
Vinblastine	45.3	56.2	51.0	76.0
Strychnine	7.3	10.2	7.2	12.3
Colchicine	1.2	2.0	1.8	2.5
Lupanine	1.8	2.4	2.4	6.8
13-Hydroxylupanine	1.3	1.2	1.0	1.3

concentrated in the vesicles by a factor of 1–2 only, as compared to the uptake of ^3H_2O (= 1), which easily and completely equilibrates with the vacuolar content. The alkaloid uptake proceeded rapidly and was almost complete after 5 or 6 min (Table I). The vesicles were rather stable for at least 1 to 2 h as can be seen from the ^3H_2O values (% uptake) given in Table I.

The degree by which exogenous alkaloids were sequestered varied largely with the developmental stage of the plants: At the beginning (*i.e.* March and early April) and at the end (*i.e.* July, August) of the vegetation period of *Ch. majus* uptake rates were very low. High values were obtained for plants at the onset and during flowering. Since our experiments involved many different specimens, which were collected during April to July 1987 and 1988, some variation in the absolute accumulation rates was encountered in Tables I, III, IV and V although the qualitative trends remained more or less identical.

Sequestration was temperature-dependent with substantially higher uptake rates for all alkaloids at 37 °C (Table II). Arrhenius plots were used to calculate the activation energy of this process (Table II).

The uptake was influenced by the hydrogen ion concentration of the incubation buffer (Table III). For nicotine, lupanine and 13-hydroxylupanine sequestration was enhanced at pH values higher than pH 7. For the other alkaloids the opposite trend was recorded, with highest accumulation rates at pH 5 and lowest at pH 9. Vesicles seem to be stable over the pH range tested, as could be seen from the ^3H_2O values.

Table II. Activation energy for alkaloid uptake into latex vesicles of *Chelidonium majus*. Experiments were those of Tab. I. Initial uptake values for 5 or 6 min, respectively were used to calculate the activation energies (Arrhenius plots).

Compound	Activation energy [kJ/mol]
Nicotine	16.8
9,10-Dihydroergocryptine	10.4
Vinblastine	28.9
Strychnine	10.9
Colchicine	4.1
Lupanine	1.2
13-Hydroxylupanine	1.5

Table III. Influence of the hydrogen concentration in the incubation buffer on alkaloid uptake by latex vesicles of *Chelidonium majus*. Experiments were performed as described in Table I. Reactions were terminated after 30 min. Buffer salts: pH 5: 10 mM citrate/NaOH; pH 6: 10 mM MES/KOH; pH 7: 10 mM Hepes/NaOH; pH 8: 10 mM Hepes/NaOH; pH 9: 10 mM Tris/HCl.

Compound	Rate of accumulation pH				
	5	6	7	8	9
^3H_2O	1	1	1	1	1
Nicotine	3.8	1.2	2.7	4.5	19.2
9,10-Dihydroergocryptine	45.9	33.4	26.0	24.4	0.8
Vinblastine	38.9	11.3	14.6	11.6	2.9
Strychnine	19.2	7.6	14.8	18.2	14.4
Colchicine	8.0	2.1	3.4	0.5	0.4
Lupanine	4.3	1.4	2.6	4.3	5.8
13-Hydroxylupanine	1.9	1.7	2.8	3.6	2.0

Data on the concentration dependence of alkaloid uptake generally showed linearity and no saturation kinetics (data not shown). Alkaloid sequestration in vesicles was not enhanced by addition of Mg-ATP or K^+ in the incubation mixture (Table IV); on the contrary, although the vesicles were stable, the accumulation rates were substantially lower after ATP treatment.

Chelidonic acid is a typical constituent of *Ch. majus* latex [10, 18]. Its distribution in latex, especially in latex vesicles (which amount to 10–20% of the latex volume) is shown in Table VIII: the concentration is highest in vesicles with a mean of 661 mM, whereas it is 58 mM in the surrounding cell sap. Preliminary data on the partitioning of protoberberine alkaloids in latex indicate a similar trend, with almost equimolar concentrations in vesicles as compared to chelidonic acid. Chelidonic acid apparently forms complexes with the alkaloids present which can no longer diffuse across the vesicle membrane.

To study the potential of chelidonic acid as a trapping agent, we have added this compound to the external incubation buffer. Indeed, alkaloid sequestration was substantially decreased in these uptake experiments (Table V), especially at 20 mM chelidonic acid.

Protoplasts and vacuoles

For comparison we have isolated protoplasts of leaf mesophyll cells and vacuoles of *Ch. majus*. In both systems we observed an alkaloid sequestra-

tion, with significant accumulation rates for vinblastine, 9,10-dihydroergocryptine, strychnine, and nicotine. In vacuoles also lupanine and 13-hydroxylupanine were accumulated. Alkaloid uptake was mostly independent of Mg-ATP activation (Table IV), except for vinblastine and dihydroergocryptine in *Chelidonium* vacuoles.

Further test systems were vacuoles isolated from cell suspension cultures of *Lupinus polyphyllus* and rabbit erythrocytes (Tables VI, VII). In lupin vac-

Table IV. Uptake of alkaloids by latex vesicles, by protoplasts and isolated vacuoles of leaf mesophyll cells of *Chelidonium majus* and the influence of Mg-ATP/KCl [15]. Experiments were performed as described in Table I. A reaction tube contained: 150 µl vacuoles in respective buffers, 3.5 µl radioactive compounds, 7.5 µl 0.2 M Mg-ATP (final concentration 10 mM), 6.0 µl 1 M KCl (final concentration 40 mM). Reactions were performed at 22 °C for 30 min. C = control.

Compound	Rate of accumulation					
	Protoplasts		Vacuoles		Vesicles	
	C	ATP	C	ATP	C	ATP
^3H_2O	1	1	1	1	1	1
Nicotine	5.4	3.1	13.3	13.5	0.2	0
9,10-Dihydroergocryptine	23.6	18.7	16.5	31.1	16.2	9.2
Vinblastine	25.8	8.9	19.5	32.0	15.0	10.0
Strychnine	10.7	6.2	27.2	19.4	5.5	2.2
Colchicine	1.3	0.7	1.7	0.9	0.8	0.7
Lupanine	1.9	0.7	12.5	8.4	0.9	0.7
13-Hydroxylupanine	0.4	0.2	7.3	2.3	0.7	0.1

Table V. Influence of external chelidonic acid on the uptake of alkaloids by latex vacuoles of *Chelidonium majus*. Experiments were performed as described in Table I. Chelidonic acid was added to the incubation buffer at the concentrations given.

Compound	Rate of accumulation					
	no chelidonic acid		10 mM chelidonic acid		20 mM chelidonic acid	
	30 min	100 min	30 min	100 min	30 min	100 min
^3H_2O	1	1	1	1	1	1
Nicotine	15.4	8.2	1.6	1.4	0	0
9,10-Dihydroergocryptine	94.1	98.8	92.3	93.3	14.2	19.8
Vinblastine	99.5	91.9	86.0	86.0	17.2	23.1
Strychnine	68.7	75.9	19.1	19.1	1.4	9.2
Colchicine	9.5	8.4	7.3	7.1	1.4	1.5
Lupanine	12.3	10.3	3.4	4.8	0.3	2.8
13-Hydroxylupanine	4.7	8.6	1.8	2.4	0.7	0.3

Table VI. Uptake of alkaloids by vacuoles isolated from suspension-cultured cells of *Lupinus polyphyllus*. Two independent experiments (with Mg-ATP/KCl) were performed as described in Material and Methods.

Compounds	Uptake [%]		Relative uptake Lupanine = 1	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nicotine	1.6	4.4	0.57	2.93
9,10-Dihydroergocryptine	6.9	1.6	2.45	1.07
Vinblastine	2.9	0.3	1.04	0.20
Strychnine	0.6	0.1	0.21	0.07
Colchicine	0.4	0.3	0.14	0.20
Lupanine	2.8	1.5	1.00	1.00
13-Hydroxylupanine	0.3	0.3	0.20	0.20

Table VIII. Distribution of chelidonic acid in latex and vesicles of *Chelidonium majus*. Means and their range (in brackets) of 4–7 independent experiments.

	Concentration of chelidonic acid [%]	mM
Complete latex	2.9 (1–3.7)	157 (54–201)
Latex without vesicles	1.1 (0.5–1.5)	58 (16–108)
Vesicles	12.1 (4.3–24)	661 (236–1323)

Table VII. Uptake of alkaloids by rabbit erythrocytes. Blood was collected from a rabbit ear vein into a tube containing heparine to prevent coagulation. To 140 µl blood kept on ice 1 µl of the radioactive compound was given and mixed thoroughly. Aliquots of 40 µl were taken after 3, 15 and 30 min and processed by silicone oil centrifugation. Radioactivity in the supernatant and erythrocyte pellet were determined by liquid scintillation counting.

Compound	Rate of accumulation Time [min] 3	15	30
^3H_2O	1	1	1
Nicotine	0.43	1.10	0.56
9,10-Dihydroergocryptine	2.13	2.27	1.99
Vinblastine	2.00	2.67	2.72
Strychnine	0.56	0.66	0.63
Colchicine	0.69	0.88	0.67
Lupanine	0.39	0.83	0.58

uoles, uptake of all alkaloids was rather low as compared to vacuoles from *Ch. majus* latex and leaf mesophyll tissue (Table IV). However, the uptake of the lupin homologous alkaloid lupanine and that of 3 heterologous alkaloids (9,10-dihydroergocryptine, vinblastine, nicotine) was remarkably enhanced (Table VI, see [15]). In rabbit erythrocytes the uptake of nicotine, strychnine, colchicine and lupanine was lower than that of ^3H_2O, *i.e.* they were not accumulated against a concentration gradient. Only 9,10-dihydroergocryptine and vinblastine were concentrated in the erythrocytes by a factor of 2 (Table VII).

Lipophilicity of alkaloids

In order to determine their lipophilicity (as a measure for membrane permeability) the test alkaloids were partitioned in H_2O/octanol. As can be seen from Fig. 2 9,10-dihydroergocryptine, colchicine and vinblastine are very lipophilic compounds which are easily dissolved and concentrated in the octanol phase, irrespective of whether they are charged molecules or not (this does not apply for colchicine, which does not react as a true alkaloid because of its amide nitrogen). Strychnine, nico-

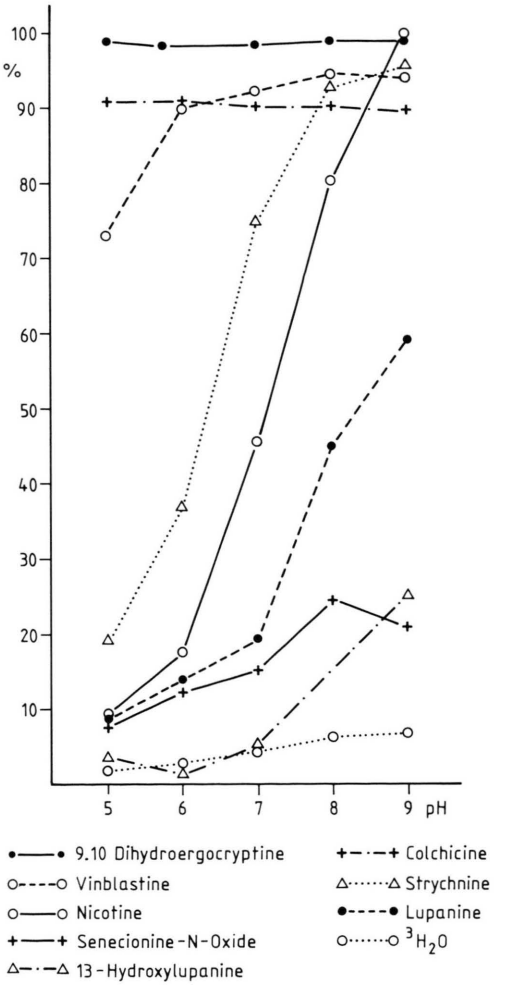

Fig. 2. Partition of alkaloids in octanol/water as dependent on hydrogen ion concentration. Experimental details in Materials and Methods.

tine and to a lesser degree lupanine are hydrophilic compounds as charged species (*e.g.* under acidic conditions) and lipophilic ones as the free bases. Therefore, strychnine and nicotine are found nearly completely in the octanol phase at pH values higher than pH 9 (Fig. 2). The homologous *Chelidonium* alkaloids do not dissolve in octanol/water to a substantial degree (data not shown).

Discussion

Of the 7 heterologous alkaloids tested, especially 9,10-dihydroergocryptine, vinblastine, strychnine and nicotine are sequestered against a concentration gradient by latex vacuoles of *Ch. majus* at a remarkable degree. How do these alkaloids pass the tonoplast and what is the driving force for the apparent "uphill" accumulation?

Simple diffusion or transport system?

Criteria for a transport system would be time, pH, temperature and concentration dependence and selectivity.

Uptake of these alkaloids into latex vesicles was rapid and completed within the initial 5–6 min (Table I), which is in contrast to the kinetics observed when carrier-mediated transport across the tonoplast is involved: in *Lupinus, Catharanthus* or *Fumaria* vacuoles [13, 15, 16] alkaloid uptake reached its maximum after 30 to 90 min.

Whereas clear pH optima were found for carrier transport in other plants [13], this feature was lacking in the latex system: For lupanine, 13-hydroxylupanine, and nicotine the uptake increased with increasing alkalinity of the medium and this was correlated with the hydrophobicity of these alkaloids as determined by partitioning into the octanol phase (Fig. 2): The more alkaline the medium, the higher the concentrations of the lipophilic free base, that can easily diffuse across biomembranes. On the other hand, the indole alkaloids, which had high partition coefficients in octanol at all pH-values (and were thus likely to cross biomembranes readily by diffusion), accumulated in the vesicles to a much higher degree.

Catalyzed transport is characterized by activation energies above 20–30 kJ/mol. Our experiments clearly show (Tables I, II) that the activation energies for the uptake of alkaloids into latex vesicles usually are below 20 kJ/mol and in case of vinblastine below 29 kJ/mol, indicating that crossing of the tonoplast involves simple diffusion only and is not a catalyzed process.

Another indication for simple diffusion is the low selectivity of biomembranes for the alkaloids studied. Lipophilic alkaloids, such as vinblastine, 9,10-dihydroergocryptine, strychnine and nicotine were not only accumulated by latex vesicles but also by protoplasts, mesophyll vacuoles (Table IV), lupin vacuoles (Table VI) and some even by rabbit erythrocytes (Table VII), further indicating that they pass biomembranes unspecifically by simple diffusion. Since these alkaloids are present neither in *Chelidonium majus* and nor in the other systems tested, the existence of a specific uptake system would be *a priori* unlikely.

Our experimental data thus suggest that the uptake of the heterologous alkaloids by latex vesicles proceeds *via* simple diffusion and not by a carrier system. Matile and coworker came to a similar conclusion for the uptake of the endogenous sanguinarine by *Chelidonium* latex vesicles [10, 18], although the quaternary *Chelidonium* alkaloids are hydrophilic compounds which should not diffuse through membranes easily. On the other hand there is good experimental evidence that the uptake of some alkaloids into vacuoles may involve a specific carrier system: vindoline in *Catharanthus* [13], of reticuline in *Fumaria* [14], of senecionine-N-oxide in *Senecio* [19], of lupanine in *Lupinus* [15], of atropine in *Atropa* [15], and morphine in *Papaver* [20]. Contrary to the findings in [14], nicotine uptake in most systems studied so far proceeds by simple diffusion, however [11, 12].

It is not meaningful to assume a general mechanism for the uptake of alkaloids or other natural products by plant cells, since their structures and thus their physicochemical properties differ dramatically. For some compounds it can be simple diffusion, for others specific transport or sometimes even both processes may coexist [15], in any case, each system has to be studied and considered for its own.

What is the driving force for alkaloid uptake?

Some of the alkaloids tested are enriched in the latex vesicles to a high degree, especially vinblastine and 9,10-dihydroergocryptine which in some experiments were concentrated about 90- to 100-

fold (Table V). In other systems the same alkaloids obviously pass the biomembranes by diffusion, but are not accumulated to a similar degree (see lupin vacuoles or rabbit erythrocytes, Tables VI, VII), indicating the presence of a specific accumulating principle in *Chelidonium* latex vesicles.

In the case of vacuoles isolated from *Lupinus, Fumaria,* and *Senecio* [14, 15, 19] it was demonstrated that the alkaloid uptake was dependent on Mg-ATP. It was suggested that a proton translocating ATPase pumps protons into the vacuole and that the uptake of alkaloids is achieved by a proton-alkaloid antiport system [14, 15]. Since Mg-ATP did not stimulate alkaloid uptake in latex vesicles, a proton gradient is unlikely to represent the driving force for the uphill transport observed. Matile [10, 18] had postulated that alkaloids are trapped in the vacuole by ions or other molecules. Such an interpretation was recently reappraised by Guern and coworkers [11, 12]. Our data indicate that this assumption seems to be correct for latex vacuoles of *Chelidonium:* chelidonic acid is present in the latex vesicles at high concentrations (Table VIII). Table V indicates that exogenous chelidonic acid efficiently complexes alkaloids, such as vinblastine strychnine, or 9,10-dihydroergocryptine in the external medium so that they are no longer diffusible. The same applies if chelidonic acid is inside the vacuoles in high concentrations: Alkaloids which are complexed in such a way are trapped and thus enriched in the vacuoles and we explain the apparent uphill transport by such simple systems. It does not rule out that other more elaborate trapping mechanisms exist in *Ch. majus* latex additionally [12].

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