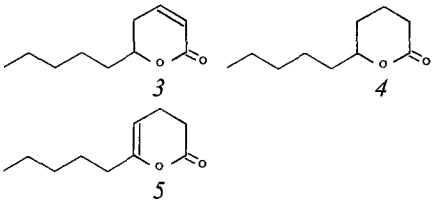
166 an exact mass of 166.1000; for $C_{10}H_{14}O_2$ a mass of 166.0994 would be required.

The proton magnetic resonance spectrum contained signals for a primary methyl group at 0.85 ppm (3H, triplet) for methylene protons at 1.31 ppm (4H, multiplet); 1.65 ppm (2H, multiplet) and 2.45 ppm (2H, triplet), all consistent with an n-pentyl group. The spectrum also contained signals from olefinic protons at 5.94, 6.11, and 7.24 ppm (1H, doublets) corresponding to the three protons of the α-pyrone ring. The α-pyrone ring protons formed an ABX pattern representing those H-atoms attached to the olefinic C-atoms in positions 5, 3, and 4.

In the ^{13}C-NMR spectrum the olefinic C-atoms correspond to signals at 102.5, 112.5, and 143.5 ppm. The NMR spectral properties are in good agreement with those reported for synthetic or for naturally occurring 6-pentyl-α-pyrone [5,6,9].

For additional confirmation of the presence of an α-pyrone ring IR and UV spectra were taken. The IR spectrum showed strong C=O absorption bands at 1710/1720 cm^{-1} (doublet) and C=C stretching bands at 1636 and 1533 cm^{-1}. The UV spectrum gave a well-defined absorption maximum at 301 nm, indicating the presence of an α-pyrone chromophor skeleton.

The coconut-like smelling substance produced by and isolated from *T. koningii* was definitely 6-pentyl-α-pyrone (*1*). There are detailed reports on the formation of saturated and unsaturated δ-lactones by oxydative degradation from related higher fatty acids and glycerides as their precursors [4,10]. The organoleptic properties of some alkyl-substituted α-pyrones have been systematically evaluated. Pittel [9] and Nobuhara [11] reported on general synthesis methods for this group of compounds, in which the position and number of double bonds in the pyrone ring were varied, as well as the length of the alkyl substituent. Hydrogenation of the double bonds in the pyrone ring changed the organoleptic properties as follows: 6-pentyl-α-pyrone (*1*), 5,6-dihydro-α-pyrone(Massoia lactone, *3*) and 3,4,5,6-tetrahydro-α-pyrone(δ-decalactone, *4*) had a strong but pleasant peach-like, coconut, creamy aroma while 3,4-dihydro-α-pyrone (*5*) had the least desirable odor.



3 *4* *5*

A progressive change in the organoleptic properties was reported with increasing length of the 6-alkyl-α-pyrones. The lower homologs (C_3/C_4) were sweet, coumaric, while the higher members of the series (C_6/C_7) were green, fatty, and waxy. 6-Pentyl-α-pyrone was considered to have the most pleasant organoleptic properties of all 6-alkyl-α-pyrone derivatives. Many of these synthesized compounds have been isolated from natural sources. A comparable systematic evaluation of the biological/pharmacological properties of alkyl-substituted α-pyrones has, to our knowledge, not been reported so far, although information on biological activities of many substituted α-pyrones is available [12].

Received July 10, 1990

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Naturwissenschaften 77, 540 – 543 (1990)

Interspecific Flow of Pyrrolizidine Alkaloids

From Plants via Aphids to Ladybirds

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Pyrrolizidine alkaloids (PAs) are a group of typical plant secondary constituents found frequently in some genera of the Asteraceae, Boraginaceae, and Fabaceae. Due to their presumed function as efficient protective agents against herbivores, they received much attention in respect of plant-insect relationships [1, 2]. Some specialized insects, for instance, the European cinnabar moth, *Tyria jacobaeae,* which as larvae feed almost exclusively on *Senecio jacobaea,* are able to sequester considerable amounts of plant PAs in their bodies [3]. These insects are avoided and not eaten by most insectivores. In addition, larvae and imagines of *Tyria* advertise their unpalatability by a bright aposematic warning coloration.

Another example for the interspecific role of PAs in plant-insect relationships will be given here. Previous studies in our laboratory have shown that in *Senecio* species PAs are present in the form of their N-oxides which are syn-

thesized in the roots [4, 5]. From roots the PA-N-oxides are translocated into shoots via the phloem [6]. Phloem transport of the PA-N-oxides is a specific process which assures that the alkaloids are channeled to the inflorescences, the major sites of PA-N-oxide accumulation in *Senecio* [6, 7]. One method to prove whether or not a compound is phloem-mobile is to employ phloem feeders as "analysts". An aphid specialist on *Senecio* is *Aphis jacobaeae* Schrank (Aphididae). We found this aphid on three species. Aphid colonies on *S. jacobaea* and *S. inaequidens* were frequently found to be infested by ladybirds (*Coccinella septempunctata* L., Coccinellidae). Host plants, aphids, and concomitantly occurring ladybirds were analyzed for their PA contents by means of capillary gas chromatography/mass spectroscopy (GC-MS) [5]. The PA patterns of the host plants, shown in Table 1, were characterized as follows:

Senecio jacobaea L: Two chemotypes of *S. jacobaea* were found to exist (unpublished results). The "jacobine type" (Table 1) shows the "classical" PA pattern of *S. jacobaea* with jacobine (major PA) and related structures such as jacozine, jacoline, and jaconine as typical alkaloids [8, 9]; erucifoline and its O-acetyl derivative are missing or detectable in trace amounts only. The "erucifoline type" (Table 1) is distinguished by erucifoline and acetylerucifoline, whereas jacobine and its relatives are absent or present in trace amounts only. The erucifoline type was found to be rather common in the vicinity of Braunschweig. The alkaloids were identified by their retention indices (RIs), molecular ions (M^+), and MS fragmentation patterns in comparison to reference compounds. In addition to their MS data [5], the identities of erucifoline and jacobine were verified by ^{1}H-NMR spectroscopy.

Senecio inaequidens DC: The four major alkaloids (Table 1) were identified by their MS data in comparison to reference compounds. They are known from this species [10, 11]. In addition, we found small amounts of otonecine derivatives which recently have been reported for *S. inaequidens* [12].

Senecio silvaticus L: Triangularine, sarracine, and O-7-angeloylretronecine are known alkaloids from this plant [13]. They were identified together with two isomeric angeloyl-platynecines by their RIs, molecular ions, and characteristic fragmentation patterns.

PA extracts from *Aphis jacobaeae* feeding on *Senecio* species showed the same PA patterns as their host plants (Table 1). Although the PA patterns may vary considerably between *Senecio* populations from different habitats, the PA composition of the patterns found in the aphids corresponds well to the patterns of the respective host plant populations. This was particularly true for the different chemotypes of *S. jacobaea*. Relating to quality, only two deviations were observed: acetylerucifoline (*S. jacobaea*) and the otonecine derivatives mentioned as minor components of *S. inaequidens* were never detected in the insects. It remains an open question whether these compounds are absent from the phloem of the host plant or whether they are discriminated by the aphids' uptake system. Analysis of the ho-

Table 1. Comparison of the GC-MS-evaluated PA patterns found in three *Senecio* species (host plants), *Aphis jacobaeae* feeding on these plants, and *Coccinella septempunctata* feeding on the aphids. *Sample preparation:* Plant PAs were extracted from fresh material by homogenization (Ultra-turrax) in 0.1 *M* HCl and liquid-solid extraction using Extrelut columns (Merck) according to [4]. Insect material was extracted with acidic MeOH (1% HCl) in a mortar for 10 min. After centrifugation and evaporation of the solvent the residue was dissolved in dilute NH_4OH and applied to an Extrelut column and treated further as given in [4]. PA-N-oxides were reduced prior to liquid-solid extraction by stirring the acidic crude extracts with Zn dust in excess for 5 h [4]. *GLC-MS:* A Carlo Erba Mega 5160 gas chromatograph equipped with a quartz column (WCOT, 30 m x 0.32 mm; DB-1, J&W scientific CA) was directly coupled to a quadrupole mass spectrometer Finnigan MAT 4515. Conditions: injector 250 °C; temp. prog., 100–300 °C, 6 °C/min; split ratio 1:20; carrier gas He 0.5 bar. Retention indices (RI) were calculated from cochromatographed hydrocarbon standards [20]. For quantification atropine was used as internal standard

Alkaloids	RI	M^+ (m/z)	Relative abundance [%]		
			Plant	*Aphis jacob.*	*Coccinella septempunc.*
S. jacobaea[a] "jacobine type"					
Senecivernine	2278	335	2	4	2
Senecionine	2290	335	5	20	26
Seneciphylline	2303	333	26	25	30
Integerrimine	2345	335	4	9	6
Jacobine	2432	351	46	33	36
Jacozine	2460	349	17	2	tr
Jacoline	2485	369	tr	1	tr
Erucifoline	2510	349	tr	6	–
S. jacobaea[a] "erucifoline type"					
Senecivernine	2278	335	tr	tr	tr
Senecionine	2290	335	28	46	48
Seneciphylline	2303	333	18	13	15
Integerrimine	2345	335	3	9	8
Erucifoline	2510	349	45	32	29
Acetylerucifoline	2610	391	6	–	–
S. inaequidens[a]					
Senecivernine	2278	335	16	15	26
Senecionine	2290	335	9	22	37
Integerrimine	2345	335	14	18	24
Retrorsine	2525	351	61	45	13
S. silvaticus[a]					
O-7-Angeloylretronecine	1792	237	3	12	
O-7-Angeloylplatynecine	1815	239	10	2	
O-9-Angeloylplatynecine	1850	239	7	2	
Triangularine	2375	335	24	31	
Sarracine	2400	337	56	53	

[a] Selected populations found in habitats in the vicinities of Braunschweig and Hannover

neydew of *Aphis jacobaeae* feeding on *Senecio jacobaea* revealed the typical plant PA pattern. In addition to physiological studies [6], excretion into honeydew as well as sequestration of PAs by *A. jacobaeae* provide further evidence that in *Senecio* species long-distance translocation of PAs occurs via the phloem. It is interesting to note that the aphids preferentially infest the branching inflorescences which are particularly rich in PAs. Sequestration of alkaloids by phloem-feeding aphid specialists and thus proof of the phloem mobility of the respective alkaloids have been demonstrated for quinolizidine alkaloids in *Cytisus scoparius* [14], the indolizidine alkaloid swainsonine in *Astragalus lentiginosus* [15], and diterpenoid alkaloids of *Aconitum* species [16].

Ladybirds feeding on aphids colonizing *S. jacobaea* and *S. inaequidens* were found to sequester considerable amounts of PAs. The PA patterns of the ladybirds again correspond closely to the respective aphid and host plant patterns (Table 1). The PA concentrations found in aphids may reach levels up to 3.5 mg/g fresh weight, those of ladybirds reach even 4.9 mg/g fresh weight (Table 2). This clearly indicates that the two insects must be able to actively sequester PAs. Ladybirds, like other members of the Coccinellinae, produce their own alkaloids (coccinellines) as deterrents [17]. We assayed the precoccinellines together with the PAs. They are present at a mean concentration of 10.5 mg/g fresh weight. Thus the sequestered PAs reach 10 to almost 50% of the insects' endogenous alkaloid levels (Table 2). It is interesting to note that both aphids and ladybirds store their PAs preferentially as tertiary alkaloids, whereas other PA insects, e.g. *Tyria, Arctia* [18], or *Creatonotos* [19] not only store PAs as N-oxides but are able to N-oxidize tertiary PAs. In the host plant species PAs are exclusively present as N-oxides.

The amounts of PAs sequestered by *Aphis jacobaeae* and *Coccinella septempunctata* are comparable to those found in typical PA insects such as *Tyria jacobaeae* [3]. *Tyria* larvae sequester about 1 to 4 mg PA/g fresh weight [18]. It seems reasonable to assume that the high PA level may protect the aphids against predators, particularly birds. Birds are reported to re-

Table 2. PA concentrations found in *Aphis jacobaeae,* its host plants, and *Coccinella septempunctata* feeding on the aphids

Host plant species	Alkaloid [mg/g fresh weight]		
	Host plant	*Aphis jacobaeae*	*Coccinella septempunctata*[a]
S. jacobaea	0.7 – 1.8	1.3 – 3.5	0.3 – 1.4
S. inaequidens	0.5 – 1.2	1.1 – 2.7	0.9 – 4.9
S. silvaticus	0.3 – 0.9	0.8 – 2.8	

[a] The mean concentration of isomeric precoccinellines was found to be 10.5 mg/g fresh weight

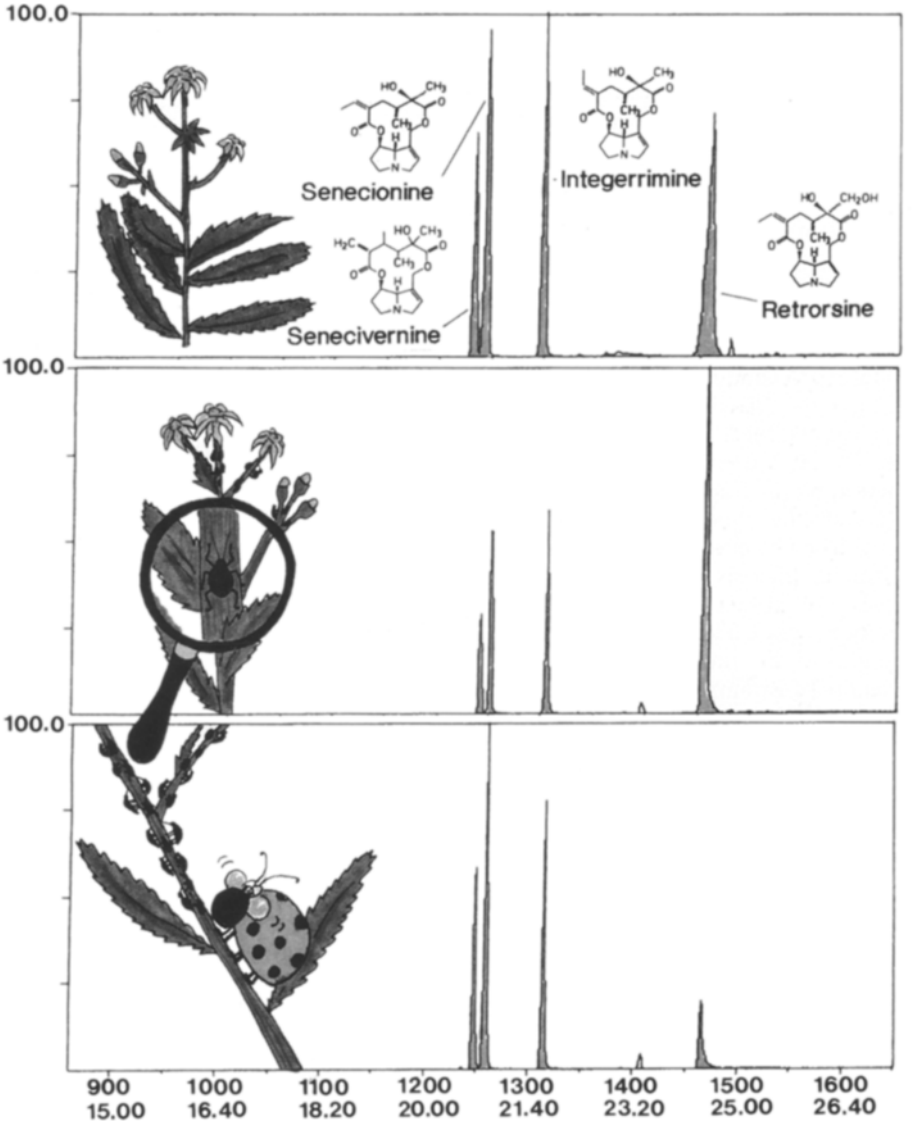

Fig. 1. GLC profiles of PAs found in *Senecio inaequidens* (host plant), *Aphis jacobaeae* (first consumer), and *Coccinella septempunctata* (second consumer). GLC conditions see legend to Table 1

ject food after PA contamination [2]. Whereas *Aphis jacobaeae* is a specialist adapted to its PA-carrying host plants and obviously able to sequester the alkaloids for its own benefit, the situation is quite different with *Coccinella*. Ladybirds generally feed on aphids which are feeding on a great variety of plants. To our knowledge this is the first report that ladybirds were found to sequester defense chemicals from their prey. As already mentioned they synthesize their own defensive alkaloids, the coccinellines, which have been evolved to function as deterrents for a variety of vertebrate and invertebrate predators. In their ability to sequester PAs ladybirds resemble the garden tiger moth *Arctia caja* L. (Arctiidae). Larvae of *Arctia* feed on almost any shrub or herbaceous plant. If they, however, feed on *Senecio jacobaea* or related species they are able to store PAs to the same extent as the monophagous *Tyria jacobaeae* restricted to the *Senecio* species. Figure 1 illustrates this fascinating example of interspecific flow of presumed defense chemicals of plant origin via two successive consumers, both of which apparently sequester these chemicals for their own benefit.

This work was supported by grants of the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie to T. H. We thank C. Theuring for technical assistance and Dr.R. Teschner (Zoologisches Institut, T. U. Braunschweig) for identifying *Aphis jacobaeae*.

Received May 2 and Juli 5, 1990

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Naturwissenschaften 77, 543 – 545 (1990)

Mechanisms of Electrostimulated Uptake of Macromolecules into Living Cells

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The phenomenon of the reversible electrical breakdown of cell membranes (discovered more than 15 years ago) is now widely used for the injection of membrane-impermeable substances of low and high molecular weight into living cells without deterioration of cellular and membrane functions (see reviews in [1 – 3]). The mechanism of electric field-induced uptake of compounds is still unclear. The general belief is [4 – 6] that pores are generated in the membrane in response to breakdown which facilitates solute diffusion. However, there is considerable evidence [1,7] that at least the uptake of macromolecules (DNA, RNA, and proteins) and organelles into the cell interior occurs by mechanisms other than passive diffusion through the field-generated membrane pores. In this communication we present evidence that endocytotic processes in the plasma membrane, which are indirectly induced by the breakdown pulse, are partially involved in the uptake of macromolecules after electroinjection.

Electroinjection of fluorescence-labeled bovine serum albumin into mouse L-cells [8] and of the plasmid pADH 040-2 [9] into yeast protoplasts [10] was performed at room temperature in strongly hypo-osmolar and iso-osmolar sorbitol solutions, respectively. Hypo-osmolar conditions favor electroinjection (and electrofusion) in mammalian cells [2]. For electroinjection the Biojet MI (manufactured by Biomed, Theres, FRG) was used (for field conditions, see figure legends). The macromolecules were added either before or at certain time intervals after the application of the breakdown pulse(s) to the