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N-OXIDATION AND DEGRADATION OF PYRROLIZIDINE ALKALOIDS DURING GERMINATION OF *CROTALARIA SCASSELLATII*

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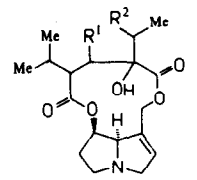
Key Word Index—*Crotalaria scassellatii*; Fabaceae; pyrrolizidine alkaloids; *N*-oxides; degradation.

Abstract—The pyrrolizidine alkaloids (axillaridine, axillarine, desoxyaxillarine) found in the seeds of *Crotalaria scassellatii* occur exclusively as tertiary alkaloids. However, they are rapidly converted into the respective *N*-oxides during the first days of seedling development. *N*-Oxidation is followed by a progressive degradation of the seed alkaloids; five-week-old seedlings are almost depleted of alkaloids. Dry seeds contain *ca* 2% alkaloids which account for less then 2% of total seed nitrogen and *ca* 20% soluble nitrogen. Mature plants accumulate pyrrolizidine alkaloids exclusively in the form of *N*-oxides in all tissues. It is suggested that the polar pyrrolizidine *N*-oxides are the preferred storage compounds in vacuolated tissues, whereas the desiccated seeds accumulate the lipophilic tertiary alkaloids.

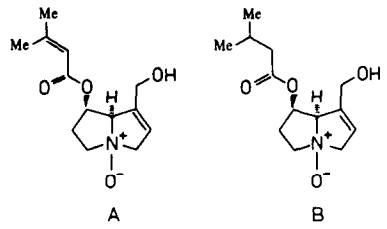
INTRODUCTION

It has long been known that in plants, pyrrolizidine alkaloids occur as mixtures of the tertiary alkaloids and the respective *N*-oxides [1–3]. In *Senecio* species (Asteraceae) *N*-oxides are the dominating alkaloid forms found in the various plant tissues [4]. Root cultures of *Senecio* species synthesize the *N*-oxides without occurrence of detectable amounts of the respective tertiary form as an intermediate [5, 6]. Furthermore, the *N*-oxides are selectively taken up by cell suspension cultures originated from pyrrolizidine alkaloid producing species [7]. Evidence has been presented, that the very polar, salt-like *N*-oxides are translocated via a specific carrier mediated process into the cell vacuole, where they are stored [8, 9]. Thus, in *Senecio* the *N*-oxides appear to represent the molecular form by which the pyrrolizidine alkaloids are synthesized, translocated and accumulated.

For *Crotalaria* (Fabaceae) it has frequently been reported that the ratios of tertiary alkaloids to *N*-oxides can vary between different parts of the plant. Thus, in *C. retusa* [10, 11] and *C. spectabilis* [12] the tertiary alkaloids were found to dominate in the seeds whereas *N*-oxides accumulated in the vegetative tissues. We wanted to know, whether the seed alkaloids of *Crotalaria* represent an exception to the dominating role of the *N*-oxides established for *Senecio*. In this communication we report that seeds of *Crotalaria scassellatii*, which were previously shown to accumulate axillaridine, axillarine and desoxyaxillarine as major alkaloids [13], accumulate these as tertiary alkaloids, but rapidly *N*-oxidize and degrade the alkaloids during the first stages of germination.



R^1	R^2	
OH	H	Axillaridine
OH	OH	Axillarine
H	OH	Desoxyaxillarine



A B

N-Oxide of:
A: O^7-Senecioylretronecine
B: O^7-Dihydrosenecioylretronecine

RESULTS AND DISCUSSION

Axillaridine, axillarine and desoxyaxillaridine could be identified as main alkaloids by GC/MS. They display characteristic fragmentation patterns, which are identical to those described previously (Table 1). Two minor alkaloids which were detectable by GC in extracts of young *C. scassellatii* seedlings (Table 2) were tentatively identified as O^7-senecioylretronecine and its dihydrode-

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Table 1. Pyrrolizidine alkaloids identified from *Crotalaria scassellatii* by GC/MS

Compound	RI	$[M]^+$ m/z	Characteristic ions m/z (relative abundance)	Ref.
Retronecine	1420	155	111 (60), 94 (20), 80 (100)	[15]
O^7-Dihydrosenecioylretronecine	1720	239	221 (1), 154 (4), 137 (50), 80 (100)	
O^7-Senecioylretronecine	1740	237	219 (2), 154 (37), 137 (71), 80 (100)	[16, 17]
Monocrotaline	2280	325	236 (72), 136 (54), 120 (100)	[15]
Axillaridine	2420	353	250 (38), 223 (3), 136 (41), 120 (100)	[18, 19]
Desoxyaxillaridine	2450	353	309 (16), 223 (4), 136 (58), 120 (100)	[19]
Axillarine	2570	369	250 (86), 223 (8), 136 (62), 120 (100)	[18–20]

Table 2. Changes in alkaloid content and composition during the development of *Crotalaria scassellatii* seedlings

Seedling age (days)	Alkaloid abundance (%)				Total alkaloids	
	Axilla-ridine	Axilla-rine	Desoxy-axillarine	†Sen-ret/ †Sen-ret · H_2	per plant (mg)	per fr wt (mg/g)
0*	67	24	9	nd	2.3	25.5
1	53	35	12	nd	2.1	10.5
3	47	34	14	5	1.1	5.8
6	43	31	15	11	1.2	3.2
13	46	12	21	21	0.7	1.6
35	tr	tr	nd	nd	tr	nd

*Dry seed.

nd = not detectable; tr = traces.

†Sen-ret = O^7-senecioylretronecine; Sen-ret · H_2 = O^7-dihydrosenecioylretronecine.

rivative. The mass spectra indicate esterification at C-7 (Table 1). The fragmentation pattern is identical with that described in the literature [16, 17]. The isomeric O^7-angelylretronecine which would result in the same fragmentation pattern [17, 21], could be excluded by its RI of 1790 which is significantly higher than that of the compound described here. An alkaloid extract of *Senecio silvaticus* which recently was shown to contain the angeloyl ester [22], was chosen for comparison. Additional support in favour of the O^7-senecioyl esters comes from the observation that substantial amounts of the two esters are detectable only during the stage of rapid breakdown of the macrocyclic alkaloids (Table 2). This may characterize the two esters as degradation products, which, of course, should then carry the senecioyl moiety. Free retronecine, the necine base of the ester alkaloids found in *C. scassellatii*, as well as monocrotaline were present in trace amounts only. They were identified by comparison of their retention data and mass spectra with reference material.

Dry seeds of *C. scassellatii* contain pyrrolizidine alkaloids only in the tertiary form; *N*-oxides were virtually absent. However, during the very first stage of germination a rapid *N*-oxidation of the tertiary alkaloids occurred (Fig. 1). On the third day almost all alkaloid was present as *N*-oxide. At this stage the radicle had just appeared and contained 21% of total seedling alkaloids (Fig. 1A) and this was solely detectable as *N*-oxide. At later stages of seedling development all plant tissues contain the alkaloids exclusively as *N*-oxides.

During seedling development *N*-oxidation is followed by a progressive decrease in the total alkaloid content per plant. (Fig. 1B). *Ca* five weeks after germination only traces of alkaloids were detectable in the seedling. Since during the early stages of growth the alkaloid *N*-oxides are obviously exported from the storage cotyledons, we have to assume that they are not only broken down in the storage tissue but also in the newly developing tissues. The three main alkaloids are degraded more or less independently, without indication for interconversion (Table 2). The detection of the senecioyl and dihydrosenecioyl esters at the third day and their increasing abundance during the later stages, points to their role as intermediates in the degradation of the macrocyclic pyrrolizidines. Again it should be noticed that the senecioyl esters are present exclusively as *N*-oxides.

Although the five-week-old seedlings are almost depleted of alkaloids (Fig. 1), mature plants again accumulate pyrrolizidine alkaloids in all tissues. Green-house grown specimens as well as preserved samples of *C. scassellatii* harvested in its natural habitat, showed alkaloid concentrations in the range 1.5 and 3.6 mg/g tissue fr wt of roots, leaves or stems. The highest amounts were always found in the shoot apices. The alkaloid pattern was comparable to that established for the seeds.

Pyrrolizidine alkaloids are generally regarded as protective chemicals for the producing plant [23, 24]. This may be especially true for the seeds of some *Crotalaria* species which accumulate toxic pyrrolizidine alkaloids at concentrations from 0.5 to 5% [25, 26]. The complete

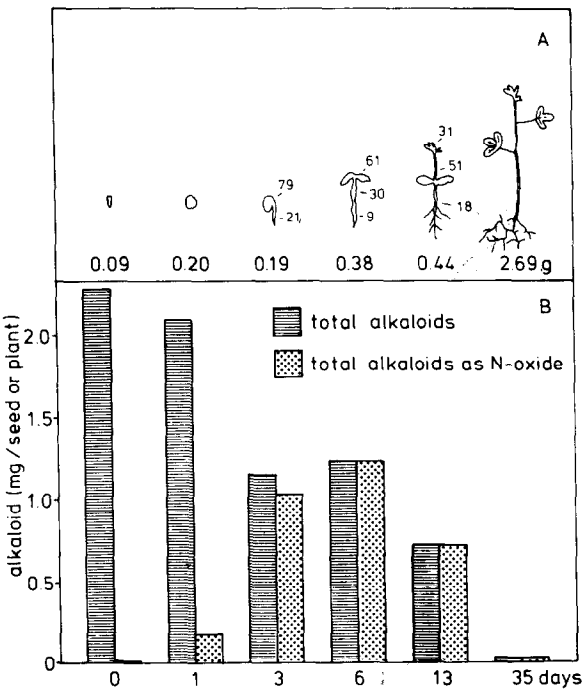

Fig. 1. *N*-oxidation and degradation of the pyrrolizidine alkaloids during seedling development of *Crotalaria scassellatii*. A: Developmental stages (morphology and fr wt). B: Total alkaloids in comparison to *N*-oxides.

Table 3. Total nitrogen and soluble nitrogen committed to pyrrolizidine alkaloid (PA) storage in seeds of *Crotalaria scassellatii*

Seed PA content (% dry wt)	2.00
Seed N content (% dry wt)	4.17
Soluble N content (% total N)	10.25
N stored in PA (%)	1.90
Soluble N stored in PA (%)	19.96

breakdown of the *C. scassellatii* seed alkaloids during germination implies that the alkaloid nitrogen is mobilized and reused in primary metabolism. In this respect the seed alkaloids would be N storage compounds, although they account for not more than 1.9% of total N and 20% of soluble N stored in the seeds (Table 3). Herbivory may have provided the selection pressure to divert some of the N into toxic alkaloids, and concomitantly the ability to remobilize this N during germination may have evolved. Thus, the 'secondary function' as chemical protective and the 'primary function' as N storage compound are combined within the structure of the seed alkaloid. The seed pyrrolizidine alkaloids of *C. scassellatti* may be compared to the toxic nonprotein amino acids, such as cannavanine, which not only represents an effective chemical barrier to predation, but also a readily available source of stored N for the germinating seed [27].

In *Senecio* the pyrrolizidine alkaloids are synthesized, translocated and stored as *N*-oxides [4–9]. At first glance, the *Crotalaria scassellatii* seed seems to contradict this general role of the *N*-oxides. However, it is just the dry seed which contains the pyrrolizidines as tertiary alkaloids, whereas all other plant tissues store the respective *N*-oxides. Immediately after imbibition and seed swelling and during the first days of germination the tertiary alkaloids are completely converted into the *N*-oxides. We suggest that the more lipophilic tertiary alkaloids may be better suited to be stored in the desiccated seed, than the very polar *N*-oxides, which in turn are the adapted forms of storage in the vacuolated tissues.

EXPERIMENTAL

Plant material. Seeds of *C. scassellatii* Chiov. were collected in Kenya, East Africa. The seeds were soaked in aerated H_2O for *ca* 24 hr and then germinated and grown in a 1:1-mixture of vermiculite and sand. *Ca* 5 to 8 weeks after germination the plants were fertilized with dil. Hoagland's soln to overcome the critical stage when the plants are changing from heterotrophic (seed supply) to autotrophic growth.

Alkaloid analysis. Alkaloid extraction and analysis of tertiary alkaloids and alkaloid *N*-oxides as carried out as described previously [5]. The alkaloids were sepd and evaluated quantitatively as tertiary alkaloids by capillary GC on fused silica columns (WCOT, 15 m × 0.25 mm; DB-1, J & W). Conditions: inj. 250°, temp prog. 100–300°, 6°/min; split ratio 1:50; inj. vol. 1–2 μl; carrier He 0.7 bar; detectors: FID, PND. RI values were calcd according to ref. [14]. For GC/MS a fused silica column as given above was coupled via an open split connection. MS were recorded at 24 eV. Monocrotaline was obtained from Aldrich. Retronecine was prepared from monocrotaline by hydrolysis.

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