Phytochemistry, Vol 28, No 6, pp 1635–1638, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00
Pergamon Press plc

PYRROLIZIDINE ALKALOID PATTERNS IN POPULATIONS OF *SENECIO VULGARIS*, *S. VERNALIS* AND THEIR HYBRIDS

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(*Received* 28 *September* 1988)

Key Word Index—*Senecio vulgaris*; *Senecio vernalis*, hybrid *Senecio vulgaris* × *Senecio vernalis*; Asteraceae; pyrrolizidine alkaloids, alkaloid variability

Abstract—Major and minor pyrrolizidine alkaloids (PAs) of the inflorescences of *Senecio vulgaris*, *S. vernalis* and the hybrid *S. vulgaris* × *S. vernalis* were separated and identified by capillary gas liquid chromatography and GC-MS. Variations in the alkaloid composition were found between different populations within the two species. In *S. vulgaris* either senecionine or seneciphylline in *S. vernalis* senecionine or senecivernine were found to be the major alkaloids

INTRODUCTION

Senecio vulgaris is a widespread weed, well known for its content of hepatotoxic pyrrolizidine alkaloids (PAs) [1–4]. Previous reports on the composition of the alkaloid patterns established for *S. vulgaris* in different laboratories displayed great variations. Some authors reported senecionine (**1**) to be the dominating alkaloid [5, 6], whereas others found seneciphylline (**2**) as the main alkaloid [7–9]. We report here on the variation of the alkaloid pattern found in different populations of *S. vulgaris*, the related species *S. vernalis* and their hybrid. In addition, the occurrence of some minor PAs, new to the two species, is described.

RESULTS AND DISCUSSION

In the two *Senecio* species PAs accumulate mainly in the inflorescences where they are stored as *N*-oxides [5]. Alkaloid analysis of single specimens revealed only minor variations between individuals of a given population. Genuine variations in the alkaloid composition of flower heads due to exogenous factors such as time of the day of sampling or extent of flowering were insignificant (less than 10%). Thus the alkaloid patterns established for different *Senecio* populations represent genetically based variations between populations. Prior to analysis the alkaloid *N*-oxides had to be reduced to yield the respective tertiary PAs. GC-MS analysis of PA extracts obtained from different populations of *Senecio vulgaris* showed a total of ca 10 alkaloids (Table 1). Of the three pairs of geometrical isomers identified recently by mixture analysis of alkaloid extracts with ^{1}H and ^{13}C NMR spectroscopy [9], the *Z*-isomer senecionine (**1**) and its *E*-isomer integerrimine (**5**) seneciphylline (**2**) and retrorsine (**8**) [1, 2, 5, 6] were confirmed. Spartioidine (**4**), the *E*-isomer of seneciphylline, previously reported to coelute with integerrimine (**5**) [3] was successfully resolved with a slightly lower RI than integerrimine. Spartioidine (**4**) is a minor PA, detectable in substantial amounts only in plant extracts with high levels of seneciphylline (**2**). We were, however, unable to resolve usaramine (**9**), the *E*-isomer of retrorsine (**8**). Senecivernine (**3**), so far only known from *S. vernalis* [10], is frequently present in *S. vulgaris* as a minor PA accounting usually for $< 5\%$ of total alkaloids. Riddelliine (**6**) which combines the structural features of seniciphylline (**2**) and retrorsine (**8**) was detected in trace amounts in a seneciphylline rich population. It was recently reported to occur in an American *S. vulgaris* population [11]. Finally the *Z*/*E*-isomers platyphylline and neoplatyphylline which in contrast to senecionine/integerrimine possess the saturated necine base platynecine (Table 1) were identified as minor PAs in some populations.

The alkaloid pattern of *S. vernalis* can easily be recognized by the otonecine derivative senkirkine (**7**) [5, 11] which is absent in *S. vulgaris* (Table 1).

Analysis of a number of different *S* vulgaris populations always indicate senecionine (**1**) and seneciphylline (**2**) as main alkaloids, which together account for 75 to 90% of the total alkaloid (Table 2). The proportions of the two alkaloids, however, vary considerably, i.e. from 10:1 to 1:2 in the examples shown in Table 2. *Senecio vulgaris* is self-compatible and strongly self-pollinating [12]. Outbreeding frequency was estimated to be very low and never to exceed 1% in natural populations [13]. This breeding behaviour favours the formation of genetically isolated populations which may preserve their specific chemical characters.

In *S. vernalis* senecionine (**1**) and senecivernine (**3**) are the main PAs found in flower heads; they account for *ca* 75 to 80% of total alkaloids. Their proportions vary between 1 to 1.2 and 5.5 to 1 (Table 2). Senkirkine (**7**) is

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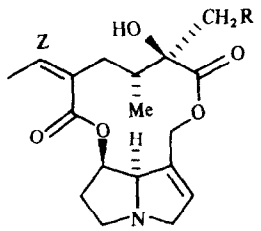

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5 R = H, E
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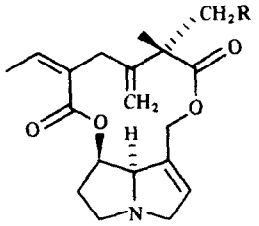
9 R = OH, E



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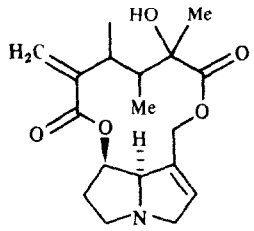
6 R = OH



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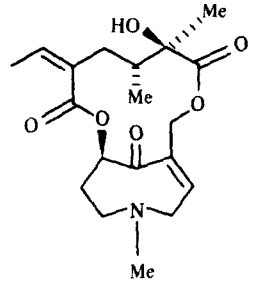

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always detectable. It is a minor alkaloid in the inflorescences but the main alkaloid in leaves and stems [5].

Hybrids of the two *Senecio* species can easily be recognized and distinguished morphologically from their parents by short ray florets. As expected hybrids show intermediate alkaloid patterns which combine the chemical characters of the parents: senecionine (**1**) and seneciphylline (**2**) are the main alkaloids, senecivernine (**3**) and senkirkine (**7**) are always present (Table 2)

Table 1 Pyrrolizidine alkaloids of *Senecio vulgaris* and *S. vernalis*, respectively, separated by capillary GC and identified by GC-MS

Alkaloid	Ret index	M^+ (*m/z*)	Necine base*	*Senecio vulgaris*	*Senecio vernalis*†
1 Unknown	2260	333	Ret	(+)	(+)
2 Senecivernine (**3**)	2280	335	Ret	+	+ + +
3 Senecionine (**1**)	2290	335	Ret	+ + +	+ + +
4 Seneciphylline (**2**)	2305	333	Ret	+ + +	+ + +
5 Spartioidine (**4**)	2335	333	Ret	+	+
6 Platyphylline	2340	337	Pla	(?)	(+)
7 Integerrimine (**5**)	2350	335	Ret	+ + +	+ + +
8 Neoplatyphylline	2400	337	Pla	(+)	(+)
9 Riddelliine (**6**)	2440	349	Ret	(+)‡	–
10 Senkirkine (**7**)	2470	365	Oto	–	+ + +
11 Retrorsine (**8**)	2510	351	Ret	+ + +	+

*Ret = retronecine moiety, typical mass fragments: *m/z* 93, 119, 136; Pla = platynecine (1,2-dihydroretronecine) moiety *m/z* 82 (base peak), 122, 138, 140; Oto = otonecine moiety *m/z* 110, 123, 151, 168

† + + + = main alkaloid (always detectable); + = minor alkaloid (frequently detectable), (+) = minor alkaloid (occasionally detectable), – = not detectable.

‡Identified in one population with seneciphylline as main alkaloid

Table 2. Composition of the alkaloid patterns found in flower heads of different populations of *Senecio vulgaris*, *S. vernalis* and the hybrid *S. vulgaris* × *S vernalis*

Population	Alkaloid composition (%)*						Total alkaloid (mg/g fr. wt)
	Sev	Sen	Sph	Int	Sek	Ret	
Senecio vulgaris							
H16 Bs	3	83	8	5	—	< 1	0 739
H11 Danmark	3	59	26	9	—	3	0 467
H14 Danmark	6	56	27	8	—	3	0.970
H18 Bs	—	52	30	11	—	7	0.664
H17 Bs	—	58	32	7	—	3	0.645
H15 HH	9	31	36	15	—	9	0.996
H10 Danmark	2	39	42	10	—	7	0.558
H21 Canada	—	37	57	3	—	3	3.384†
H13 Bs	—	37	59	2	—	2	0.631
H19 HH	—	25	58	8	—	8	0.686
Senecio vernalis							
H5 Bs	41	34	7	7	7	—	1.103
H2 Bs	24	50	9	5	11	< 1	0.948
H4 Bs	23	52	16	4	4	—	1.438
H7 Bs	22	56	15	3	4	—	1 188
H8 Bs	16	63	15	3	3	—	1.813
H6 Bs	13	70	8	4	5	—	1.114
H3 Bs	12	67	14	4	3	—	1 144
S vulgaris × *S. vernalis*							
H1 Bs	14	43	33	8	2	—	0.735
H9 Bs	7	53	13	19	2	6	0.832
H12 Bs	13	47	35	5	<1	—	1.406

*Total alkaloid = 100%
†Calculated on dry weight basis
— = not detectable with the methods applied
Sev = senecivernine (**3**), Sen = senecionine (**1**); Sph = seneciphylline (**2**);
Int = integerrimine (**5**), Sek = senkirkine (**7**); Ret = retrorsine (**8**)
H1 – H19 = Different populations from which samples were collected;
Bs = vicinity of Braunschweig; HH = Hamburg

EXPERIMENTAL

All plants were collected at their natural habitats, weighed and freeze-dried as fast as possible. PA-*N*-oxides were extracted, reduced with Zn dust to give the respective tertiary alkaloids, and prepurified as described previously [14]. GLC and GC-MS analysis were performed according to ref. [15]. A fused silica column (30 × 0.25 mm, DB – 1 J & W) was used to resolve the tertiary PAs.

Senecivernine (**3**), senecionine (**1**), seneciphylline (**2**), integerrimine (**5**), senkirkine (**7**), and retrorsine (**8**) were identified by their RIs and mass spectra in comparison to reference compounds previously isolated from *S. vernalis* [5]. Spartioidine (**4**) shows a mass spectrum identical to seneciphylline (**2**), its retention time is well in agreement with a recent report [16]. Platyphylline and neoplatyphylline show the typical fragmentation pattern of the saturated platynecine moiety (see Table 1). The RI of platyphylline and its MS fragmentation pattern were found to be identical with a PA detected in alkaloid extracts of *Adenostylis alliariae* which was used as a reference. *Adenostylis alliariae* was recently shown to contain platyphylline [17]. Neoplatyphylline, which displayed an identical MS fragmentation, was identified by its delayed RI [16] (Table 1)

Acknowledgements—This study was supported by the Deusche Forschungsgemeinschaft and by Fonds der Chemischen Industrie

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