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GC/MS Characterization of Pyrrolizidine Alkaloids in some Species of Asteraceae

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Introduction

The pyrrolizidine alkaloids (PAs) are a class of compounds occurring in a large variety through a wide range of the flora. Until now more than 200 different PAs have been isolated and identified, mainly in plants of the family *Compositae*. Within the *Compositae* the occurrence of PAs is restricted to just two of the tribes, the *Eupatoriae* and the *Senecioneae* (1). An actual review on the state of research on the PAs is given by *Robins* (2). In cases of accidental intake of certain PA containing plants by humans the alkaloids have shown high hepatotoxicity (3, 4). Similar observations are reported on poisonings of cattle by hay containing *Senecio alpinus* (5). At comparable doses the PAs cause similar symptoms in laboratory animals (mice and rats) (6). In long-time studies at subacute dose levels carcinogenic effects have been observed with the PAs monocrotaline and lasiocarpine (7). Our own investigations have demonstrated that certain medical herbs and their extracts used in medicaments contain PAs (8, 9).

In the present paper we report on the analytical investigation of PAs from different plant species with gas chromatography/mass spectrometry (GC/MS) using electron impact (EI) as well as positive and negative ion chemical ionization (CI). This study is also part of a project on using the PA content and pattern of alpine plants for their chemotaxonomic characterization.

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Experimental

Plant samples

Aerial parts of *Homogyne alpina* (L.) Cass., *Adenostyles Alliariae* (Gouan) Kerner, *Senecio Doronicum* L. and *Senecio Fuchsii* Gmelin (possibly a bastard of *Senecio Fuchsii* and *Senecio nemorensis* which occur frequently in Switzerland) were collected near Einsiedeln in the Swiss prealpine region (samples of these plants are stored in a herbarium by A. B.). *Eupatorium cannabinum* L. (*Herba Eupatorii cannabini conc.*, Dixia AG, CH-9014 St. Gallen, Switzerland) is a commercial sample of unknown origin and botanically not further characterized. Treatment of the plant material included extraction of the PAs, purification and reduction of the N-oxides as described earlier (8, 10).

Gas Chromatography/Mass Spectrometry

Gas chromatograph: Carlo Erba 4160

Glass capillary column: 20 m × 0.30 mm, SE-54 film thickness 0.15 μm

Carrier gas: hydrogen, linear flow velocity 50 cm/s

Injection: 1 μl, split ratio 1:10

Temperature of injector: 250 °C

Temperature programme: injection at 140 °C, 4 °C/min up to 260 °C

Mass spectrometer: Finnigan 4510 with data system Incos

Temperature of ion source: 150 °C

Reagent gas: methane, ion source pressure 0.60 torr

Mass range: m/e 41 to 450, cycle time 1.5 s.

Results and Discussion

Adenostyles Alliariae

The chromatogram shows 5 signals due to PAs (see table 1). The mass spectra of peaks No 1 and 2 have revealed senecionine (IX) and seneciphylline (VI) respectively (fig. 1); identity was confirmed by co-gas chromatography of the two original compounds isolated from *Senecio alpinus* (11); signal No 3 is due to platyphylline (XI, M⁺ 337). The mass spectrum of peak No 4 indicates a molecular ion at m/e 333, but a fragment pattern corresponding to an unidentified seneciphylline isomer, for example spartiodine (VII) (12). Mass spectrum and chromatographic behaviour of peak No 5 agree with the corresponding data of 12-acetyl-seneciphylline, a compound which has already been tentatively identified in *Senecio alpinus* (10). In an earlier investigation seneciphylline and platyphylline have been identified in a sample of *A. Alliariae* of Russian origin (13).

Table 1. Mass spectra of pyrrolizidine alkaloids

Nr.	RT(min)	Amt.	Identification (cc: co-chromatography; ms mass spectrum) or mass spectrum
<i>Adenostyles Alliariae</i>			
1	15.96	++	Senecionine cc, ms
2	16.25	+++	Seneciphylline cc, ms
3	16.73	+	Platyphylline cc, ms
4	16.84	+	M = 333
5	18.60	++	Acetylseneciphylline ms
<i>Homogyne alpina</i>			
1	8.74	++	M = 237
2	22.20	+++	Petasitenine cc, ms
3	25.93	++	Neopetasitenine ms
<i>Senecio Doronicum</i>			
1	4.77	+++	239 (2, M), 140 (21), 139 (24), 109 (9), 108 (100), 84 (9), 83 (30), 82 (4), 81 (4), 80 (12), 70 (15), 68 (4), 55 (23), 53 (5), 43 (6), 42 (7), 41 (9).
2	5.13	++	Macrophylline (9-angelylmacronecine) ms
3	5.53	++	see signal no. 1
4	5.69	++	see signal no. 2
5	10.95	+	see text
6	11.32	++	M = 335
7	11.88	+	M = 337
8	12.07	++	Doronine ms
9	12.27	++	Bulgarsenine ms
10	12.50	+	M = 337
11	12.86	++	M = 337
12	13.03	+	M = 353
13	14.76	+	M = 353
<i>Senecio Fuchsii</i>			
1	10.73	+	7-Angelylretronecine ms
2	11.15	+	see signal no. 3
3	12.00	+++	Senecioylplatynecine, fuchsisenecionine ms
4	19.00	+	M = 319
5	22.31	+	M = 337
6	22.88	+	Platyphylline cc, ms
7	24.35	+++	337 (0.1, M), 237 (12), 222 (12), 140 (40), 139 (29), 138 (100), 123 (30), 122 (52), 121 (7), 120 (5), 108 (6), 97 (8), 96 (32), 95 (35), 83 (29), 82 (70), 80 (6), 55 (43), 53 (12), 43 (7), 41 (19).
8	24.62	++	M = 337
9	24.92	+	see signal no. 9
10	25.22	++	M = 337
11	25.50	+	see signal no. 10
<i>Eupatorium cannabinum</i>			
1	23.75	++	Supinine ms
2	25.69	+++	381 (M), 336 (5), 238 (4), 221 (35), 220 (68), 141 (9), 138 (6), 136 (49), 122 (5), 121 (93), 120 (100), 119 (54), 118 (8), 95 (7), 94 (36), 93 (58), 83 (20), 82 (8), 80 (15), 67 (8), 55 (38), 53 (10), 45 (10), 43 (34), 42 (18), 41 (11).
3	26.09	+++	see signal no. 2
4	26.75	++	see signal no. 2
5	27.08	++	see signal no. 2

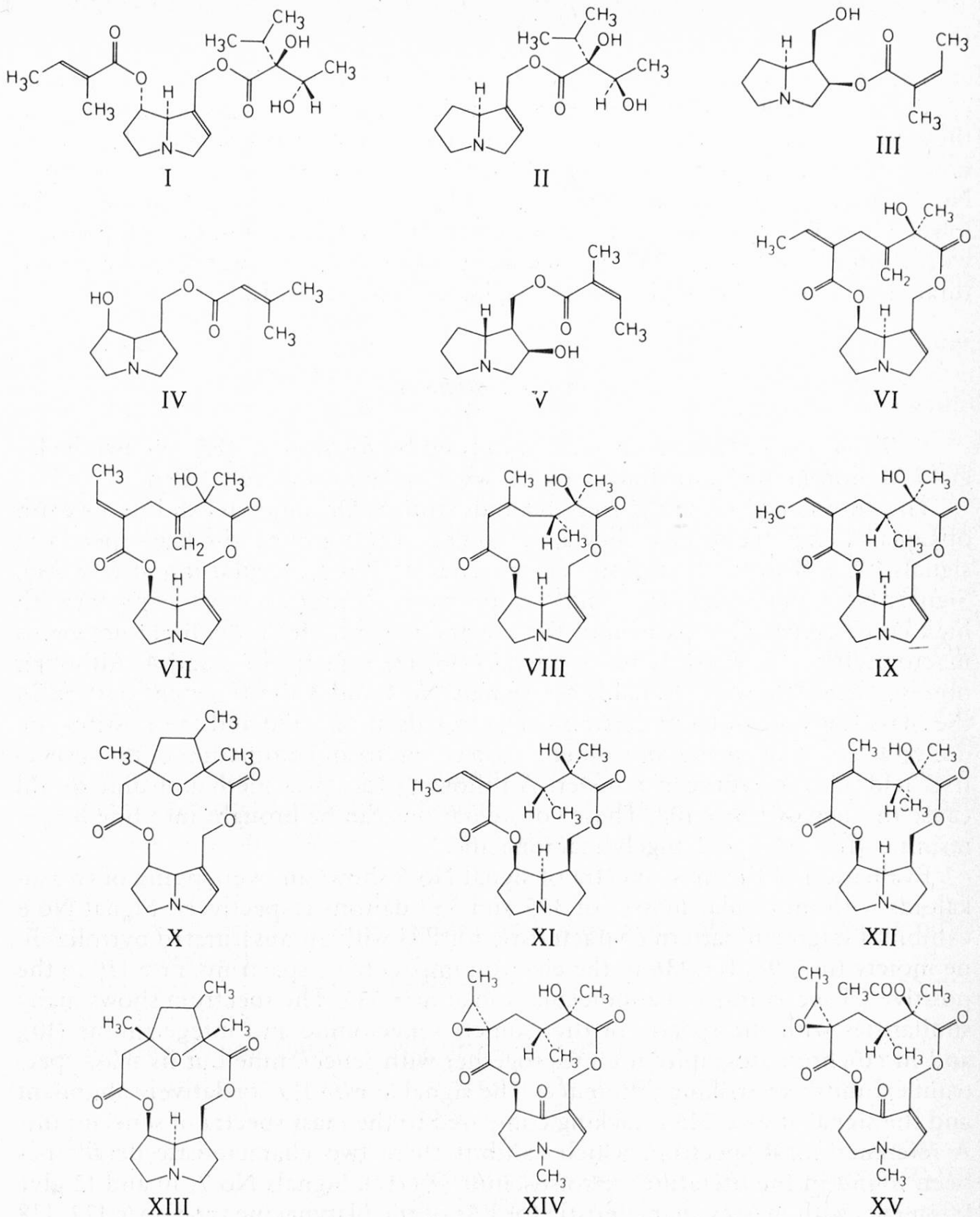


Fig. 1. Molecular formulas of pyrrolizidine alkaloids. I = 7-angelylheliotridine-viridiflorate, II = supinine, III = petasinine, IV = fuchsisenecionine, V = macrophylline, VI = seneciphylline, VII = spartiodine, VIII = doronenine, IX = senecionine, X = retroisosenine, XI = platyphylline, XII = bulgarsenine, XIII = nemorensine, XIV = petasitenine, XV = neopetasitenine

Homogyne alpina

The chromatogram shows two main signals which correspond to petasitenine (XIV, signal No 2) and neopetasitenine (XV, signal No 3). The mass spectra of these two signals are identical with the spectra given by Yamada et al. (14) for petasitenine and neopetasitenine from *Petasites japonicus*. The identity of signal No 2 was ensured by co-chromatography together with native petasitenine. The lower temperature range of the chromatogram contains a minor signal (No 1) with a molecular mass of 237 daltons due to the retronecyl ester of a monounsaturated C₅-carboxylic acid (angelic, tiglic or senecic acid).

Senecio Doronicum

S. Doronicum has already been investigated by Röder et al. (15): the two isologenic PAs doronenine and bulgarsenine were isolated and identified.

The chromatogram of the free alkaloids from *S. Doronicum* exhibits a wealth of signals being due to PAs; they are arranged in two groups: the mass spectra of signals No 1–4 in the first group correspond in their molecular ion at m/e 239. Signals No 1 and 3 and No 2 and 4 respectively exhibit each mass spectra with high coincidence. Comparison of the spectra with data from the literature yields macrophylline (V, 9-angelylmacronecine) (16) for signals No 2 and 4. Although no reference data were available for signals No 1 and 3 the fragment pattern in the mass spectra can be understood. The signals at m/e 140 and 139 possibly are due to a loss of an angelyl or isomeric moiety and to an elimination of the respective acid after a hydrogen transfer. A following loss of a methanol unit would cause the signal at m/e 108. These considerations can be brought into line by petasinine (III) (17) or 2-angelylmacronecine.

Evaluation of the mass spectra of signal No 5 shows an overlapping of two alkaloids with molecular masses of 335 and 337 daltons respectively. Signal No 6 exhibits a fragment pattern characteristic for PAs with an unsaturated pyrrolizidine moiety (m/e 93, 119, 136 in the electron impact mass spectrum, m/e 120 in the positive CI spectrum) and a molecular ion at m/e 335. The spectrum shows many similarities with the spectra of the isomers senecionine and integerrimine (10), and in co-chromatography it elutes together with senecionine but its mass spectrum exhibits two striking differences: the signal at m/e 119 is relatively abundant and the signal at m/e 246 is lacking compared to the mass spectra of senecionine. A reference mass spectrum which exhibits these two characteristic details has been found in the literature: retroisosenine (X) (17). Signals No 7, 10 and 12 give fragments with masses characteristic for PAs of the platynecine type (m/e 122, 138 and 140) and molecular ions at m/e 337. The mass spectrum of signal No 8 corresponds with the mass spectrum of doronenine (VIII) (15) and signal No 9 gives a spectrum which is assigned to bulgarsenine (XII) (18). For the spectra of the remaining signals in the chromatogram no references could be found in the literature. The last signal in the chromatogram (No 13) gives a mass spectrum which

points to a PA containing a platynecine moiety, for example a hydroxibulgarseni-
ne isomer.

Senecio Fuchsii

The PA content of *S. Fuchsii*, a species of the group of *S. nemorensis* has already been investigated by several authors (see table 2).

The main signal in the chromatogram (No 7) is due to an alkaloid not reported in the literature until now. Its mass spectrum indicates a molecular mass of 337 daltons, and the fragment pattern points to a saturated PA with a platynecine moiety (m/e 96, 122, 138, 140).

Comparison with nemorensine (23) from *S. nemorensis* (co-chromatography, mass spectra) revealed a different behaviour. The reference mass spectra of other isobar PAs as neoplathyphylline (24), hastacine (24) and bulgarsenine (18) exhibit differing fragment patterns. Signals No 8 and 9 give identical spectra indicating isomers of the unknown PA. Signals No 5 and 6 again show the same molecular ions M^+ 337). Signal No 6 corresponds to platyphylline, whereas for signal No 5 no reference mass spectrum was available. Signals No 10 and 11 contain very similar spectra of apparently isomeric compounds with a molecular mass of 353 daltons. Evaluation of the fragment pattern in the lower mass region leads to a saturated PA, tentatively an epoxy or hydroxy analogue of the unknown PA in signal No 7. The main PA in the lower temperature range of the chromatogram (No 3) corresponds to 7-senecioylplatynecine (fuchsisenecionine (IV) (20). Signal No 1 exhibits a molecular ion at m/e 237, with a mass spectrum corresponding to the spectrum of 7-angelylretronecine reported by *Pedersen et al.* (25). Due to its mass spectrum signal No 2 corresponds to an isomer of fuchsisenecionine. Although reference data from the literature are lacking a platynecine esterified with angelic (or an isomeric) acid is supposed.

Table 2. PA content of some species and subspecies of *S. nemorensis* (after *Robins (2)*)

<i>Senecio nemorensis</i> L.	
Jacquiniiana (Rchb.) Durand	Nemorensine (XIII) [23]
bulgaricus (Vel.) Stoj. et Stef.	Nemorensine (XIII), bulgarsenine (XII), retroisosenine (X) [18, 23]
subdecurrens Griseb.	Nemorensine (XIII), oxynemorensine (N-oxide of nemorensine), retroisosenine (X), bulgarsenine (XII) [16, 22, 23]
Fuchsii Gmelin	Senecionine (IX), fuchsisenecionine (IV) [20, 21, 23]
Fuchsii Gmelin	Two unknown isomeric PAs, fuchsisenecionine (IV), bulgarsenine (XII), and minor PAs (this paper)
nova (Zlatnik)	Nemorensine (XIII) [23]

A comparison of these results with the data on the investigation of *S. nemorensis* from the literature is presented in table 2. Three different alkaloid patterns have been established for *S. Fuchsii*. Klasek and coworkers (23) have shown the presence of nemorensine, which is also a constituent in other subspecies and varieties of *S. nemorensis*. Röder and coworkers identified fuchsisenecionine as main constituent and senecionine in *S. Fuchsii* (20, 21, 26). In a later publication the same authors reported the g. l. c. behaviour of PAs from some *Senecio* species (27). They found that fuchsisenecionine was eluted after senecionine. This is in strong contrast to the elution sequence observed in the chromatogram of the present paper (see table 1). A possible explanation for this contradiction is that the retention of fuchsisenecionine in (27) resembles much more the retention of a PA with a higher molecular mass like the main PA in the present paper (signal No 7) than the relatively small fuchsisenecionine.

Eupatorium cannabinum

For *E. cannabinum*, another representative of the family *compositae* the PA constituents echinatine and supinine are reported by Pedersen et al. (28). Our own investigation of a Swiss sample of this plant revealed some supplementary results.

Signal No 1 in the chromatogram gives a mass spectrum with the molecular ion and the fragment pattern characteristic for supinine (II), which could be verified by co-chromatography with native supinine. Four apparently isomeric compounds with a molecular mass of 381 daltons and practical identical fragmentation pattern in the mass spectra were detected and established with chemical ionisation (signals No 2–5). They are supposed to correspond to isomers of 7-an-gelylheliotridine-viridiflorate (I). A mass spectrum corresponding to echinatine as found by Pedersen is lacking in our results.

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Summary

The occurrence of pyrrolizidine alkaloids in the plant species *Adenostyles Alliariae*, *Homogyne alpina*, *Senecio Doronicum*, *Senecio Fuchsii*, and *Eupatorium cannabinum* was studied using GC/MS. EI and CI mass spectra allow a rapid identification of the alkaloids in most cases or can give important indications to the structures at least.

Zusammenfassung

Das Vorkommen von Pyrrolizidinalkaloiden in den Pflanzenarten *Adenostyles Alliariae*, *Homogyne alpina*, *Senecio Doronicum*, *Senecio Fuchsii* und *Eupatorium cannabinum* wurde mittels GC/MS studiert. EI- und CI-Massenspektren der zahlreich gefundenen Alkaloide erlauben in den meisten Fällen deren rasche Identifizierung oder geben zumindest wichtige Hinweise zur Struktur.

Résumé

La présence d'alkaloïdes pyrrolizidiniques dans les sortes de plantes suivantes *Adenostyles Alliariae*, *Homogyne alpina*, *Senecio Doronicum*, *Senecio Fuchsii* et *Eupatorium cannabinum* a été étudiée à l'aide de la GC/MS. Les spectres de masse EI et CI des nombreux alcaloïdes trouvés ont permis dans la plupart des cas de les identifier rapidement ou nous donnent pour le moins des indications importantes quant à leur structure.

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