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Pyrrolizidine alkaloid level in *Senecio bicolor* (Willd.) Tod, ssp. *cineraria* (DC.) from Middle Europe

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From *Senecio bicolor*, ssp. *cineraria*, cultivated in middle Europe, seven pyrrolizidine alkaloids (PA) were isolated and their structures elucidated by spectroscopical methods. Besides the already known senecionine, integerrimine, seneciphylline, jacobine, jacoline and jaconine the jacobine-acetate was found. On account of structure toxicity relationship all PA show toxic side-effects.

1. Introduction

Senecio bicolor (Willd.) Tod., ssp. *cineraria* (DC.) (syn.: *Senecio cineraria*; *Cineraria maritima*) (Asteraceae) is a plant originally widespread on rocky and sandy places in the western and central parts of the Mediterranean region in Europe (Flora Europaea, 1976). Meanwhile it is naturalized in northern and central parts of Europe, where it is found as an ornamental plant. Besides this *S. cineraria* is cultivated due to its use as a medicinal plant: in traditional medicine the plant juice is used for treating migraine, menstrutional problems and several eye diseases (Grieve 1995–2005). Homoeopathically *S. bicolor* is used as *Cineraria maritima* for curing cataract (Boericke 1927). In Germany the dose for external and internal usage is ongoing from D4 whereas in the Netherlands the homoeopathical preparations (only internally) are used from D6 and higher.

As this plant contains pyrrolizidine alkaloids (PA) it is of importance to know the concrete structures (structure-toxicity-relationship) as well as the amount of toxic PA contained. PA are mainly hazardous for man and domestic animals leading after consumption to severe hepatic diseases up to cancer. This toxicity is strongly related to three structural aspects: toxic PA show a double bond in position 1,2 of the basic moiety (necine), non-substitution in α-position to the nitrogen and an esterification of the necine hydroxy groups. In the literature five toxic PA are described to occur in *S. cineraria* from mediterranean origin: senecionine, retrorsine, seneciphylline, jacobine and otosenine (Dvorackova et al. 1978; Habib 1974; Klasek et al. 1975). Therefore, plant material cultivated in middle Europe is reinvestigated with respect to its content of PA to evaluate a possible toxic risk when using it medicinally.

Besides the already mentioned senecionine, seniciphylline and jacobine we succeeded in the isolation of integerrimine, jacoline, jaconine and jacobine-acetate; retrorsine and otosenine could not be detected in our plant material.

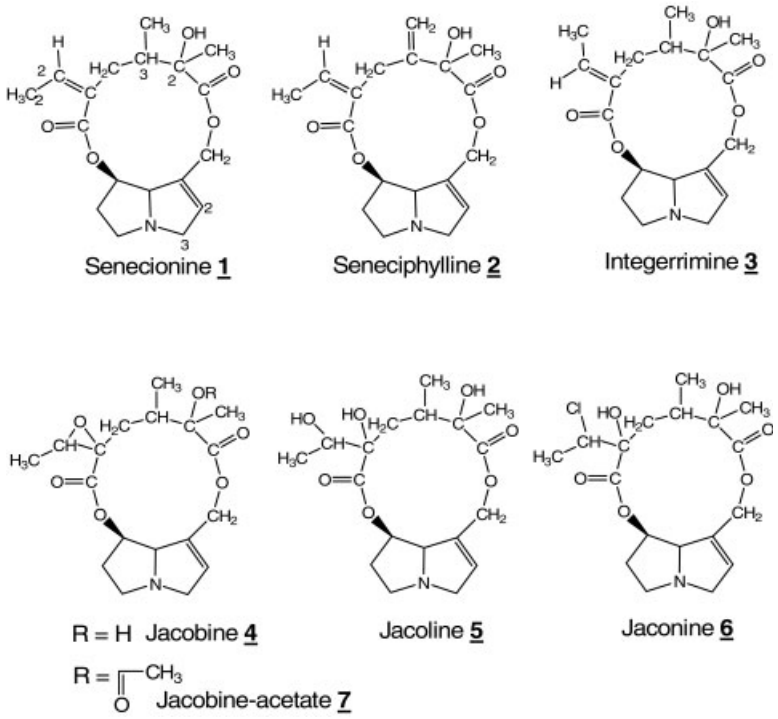
2. Investigations, results and discussion

In our investigation aerial parts of *S. bicolor*, ssp. *cineraria* (syn. *Senecio cineraria, Cineraria maritima*) were extracted as already described (Roeder and Wiedenfeld 1977; Wiedenfeld and Roeder 1979). The resulting alkaloidal extract (RE) was used for the isolation of PA **1**–**7**.

The structures were determined by the interpretation of the GC-MS and NMR data. (see 3.4.)

The quantification of the PA content in plants and preparations was done using a GC-method according to Roeder et al. (1990). The GC-spectrum of the crude plant extract from 2002 is shown in Fig. 1. It becomes obvious that jaconine (60%) followed by jacobine (31%) and integerrimine (4%) are the main constituents within the alkaloidal fraction. In 2004 this changed in the way that these PA are the main constituents, too (41%, 23% and 9%, respectively) but the other PA are occurring at a higher rate: senecionine 14%, seneciphylline 6%, jacoline 7%. In contrary to this in the homeopathic tincture (GC: see Fig. 2) jacobine (53%) is still the main PA; but senecionine (25%), seneciphylline and integerrimine (9% each) are occurring in high amounts; jacoline and jacobine-acetate are not found. A comparison of those data is given in Fig. 4. These data show that there is a correlation between the mode and the medium of plant extraction: whereas a maceration procedure with ethanol, 86%, (= Homeopathic Pharmacopoeia, rec. 2A) leads to a higher content of the more lipophylic PA (senecionine and jacobine) the soxhlet extraction with methanol affects a more equal extraction of all PA contained in the plant material.

The total PA content of the homeopathic tincture (64.43 g) was found to be 1502.5 μg (= 23.34 ppm). Referring to the German requirements concerning the use of PA containing drugs and preparations (the daily uptake of toxic PA has to be less than 1 μg internally and 100 μg externally (Bekanntmachung 1992)) 42 mg of this tincture can be applied daily. Respecting the homeopathic dosages (D4 Germany; D6 Netherlands) there should be no toxic risk and no limitations for medicinal use.



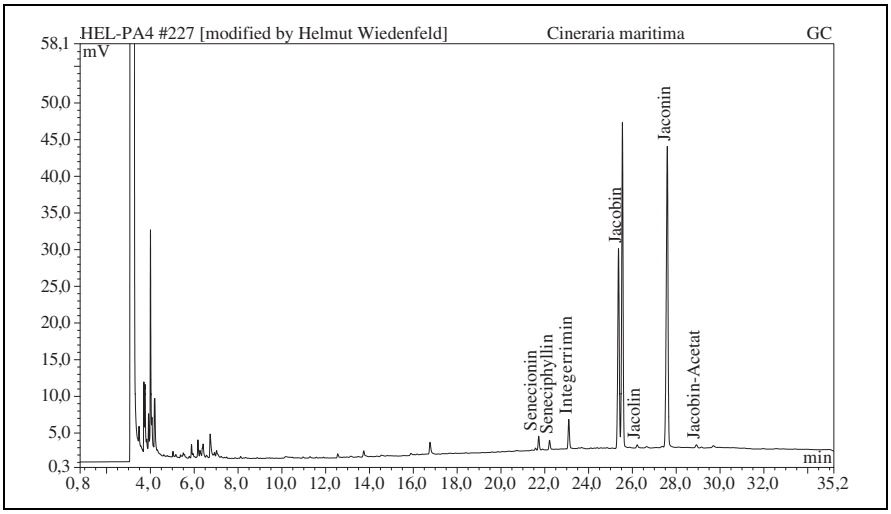


Fig. 1:
GC of the resulting alkaloidal extract from 2002

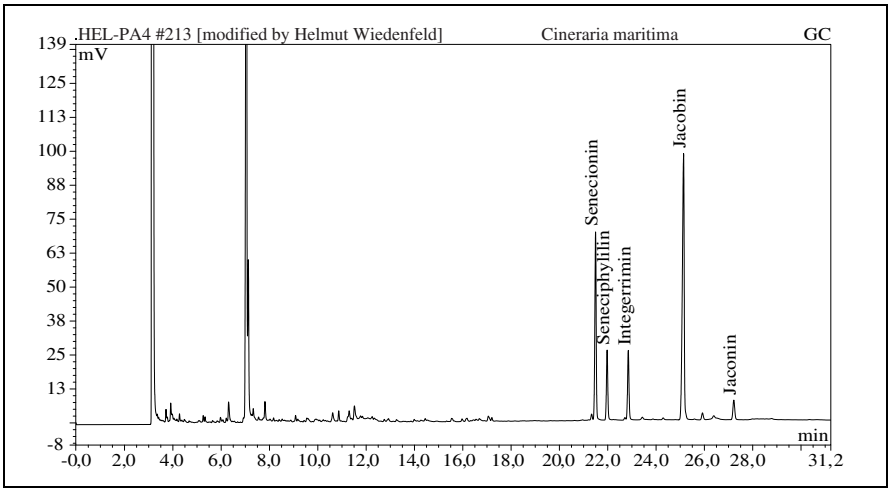


Fig. 2:
GC from Cineraria maritima, homeopathic tincture ⊘

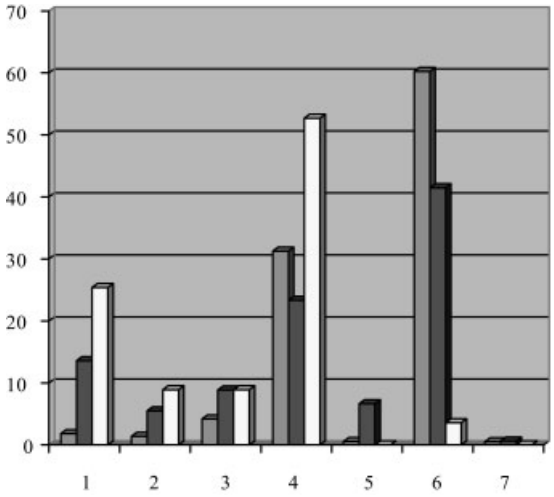


Fig. 3: Relative PA-distribution (% of total alkaloids) in the plant extracts and in the homeopathic tincture ⊘
■ Extr. 2002 ■ Extr. 2004 □ Homeop.

3. Experimental

3.1. General procedure

NMR-spectra (Bruker AC-400) were measured in $CDCl_3$/D_6-DMSO at 400 and 100 MHz, respectively. GC (Hewlett Packard 5890, Series II): 180 °C (10 min)−280 °C (10°/min; 15 min), Inj. + det.: 300 °C; R_t: **1**: 21.8 min, **2**: 22.2 min, **3**: 23.1 min, **4**: 25.3 min, **5**: 26.2 min, **6**: 27.6 min; **7**: 28.9 min; GC-MS: GC: 150° (5 min) −250 °C, 10°/min; HP-1, 25m × 0.32 mm; Inj.: 250 °C, det.: 280 °C; MS: 220 °C; interface: 250 °C; 2000 emV. TLC: silica gel 60_{F254}, 0,25 cm (Macherey & Nagel).

3.2. Plant material

Plants were cultivated in the plant gardens of VSM Geneesmiddelen bv, Alkmaar, The Netherlands. For homoeopathic use the whole plant is harvested during flowering (July and August) according to the German Homeopathic Pharmacopoeia. While the fresh material is immediately used for further processing, in 2002 and 2004 one part (500 g each) was collected for the phytochemical analysis.

3.3. Extraction and isolation

Extraction of plant material (aerial parts; 500 g, dried and pulverized) was carried out as described earlier (Roeder and Wiedenfeld 1977; Wiedenfeld and Roeder 1979) resulting in 2.8 g alkaloid extract (RE 2002) and 3.7 g (RE 2004). Prep. TLC (CH_2Cl_2−MeOH−NH_4OH (25%), 75 : 24 : 1) yielded the seven alkaloids.

3.4. Characterization of the compounds

3.4.1. Senecionine (**1**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{25}NO_5$ 335 (8.0); $C_{17}H_{24}NO_3$ 290 (4.0); $C_{15}H_{20}NO_2$ 246 (51.1); $C_{13}H_{18}NO_2$ 220 (60.3); $C_8H_{10}NO$ 136 (75.1); $C_8H_{10}N$ 120 (84.5); C_6H_8N 94 (100); C_5H_6N 80 (29.5).
NMR (δ = ppm): C-9: 5.48 (1 H, d, 11.7 Hz, H_α-9) + 4.03 (1 H, ddd, 11.7; 1.4; 0.5 Hz, H_β-9)/60.33; C-14: 38.15; C-20: 5.70 (1 H, qd, 7.2; 1.6 Hz, H-20)/133.92; further values corresponding to Wiedenfeld and Roeder (1979).

3.4.2. Seneuphylline (**2**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{23}NO_5$ 333 (8.0); $C_{17}H_{24}NO_3$ 290 (4.0); $C_{15}H_{20}NO_2$ 246 (51.1); $C_{13}H_{18}NO_2$ 220 (60.3); $C_8H_{10}NO$ 136 (75.1); $C_8H_{10}N$ 120 (84.5); C_6H_8N 94 (100); C_5H_6N 80 (29.5).
NMR (δ = ppm): C-9: 5.40 (1 H, d, 11.6 Hz, H_α-9) + 4.01 (1 H, ddd, 11.6; 1.8; 0.5 Hz, H_β-9)/60.85; C-13: 146.19; C-14: 37.27; C-19: 5.23 (1 H, d, 2.3 Hz, H_α-19) + 5.04 (1 H, dd, 2.3; 1.2 Hz, H_β-19)/114.07; C-20: 5.84 (1 H, qdd, 7.0; 1.6; 1.2 Hz, H-20)/135.86; further values corresponding to the literature (Roeder and Bourauel 1992; Roeder and Bourauel 1993).

3.4.3. Integerrimine (**3**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{25}NO_5$ 335 (4.8); $C_{17}H_{25}NO_3$ 291 (11.1); $C_{15}H_{22}NO_2$ 248 (21.8); $C_{13}H_{18}NO_2$ 220 (42.43); $C_8H_{10}NO$ 136 (53.3); $C_8H_{11}N$ 121 (96.0); C_6H_8N 94 (100); C_5H_6N 80 (29.4).
NMR (δ = ppm): C-9: 5.40 (1 H, d, 11.8 Hz, H_α-9) + 4.09 (1 H, ddd, 11.8; 1.2; 0.6 Hz, H_β-9)/60.95; C-14: 29.55; C-20: 6.50 (1 H, qd, 7.0; 0.8 Hz, H-20)/135.22; further values corresponding to Roeder and Bourauel (1992).

3.4.4. Jacobine (**4**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{25}NO_6$ 351 (2.4); $C_{17}H_{25}NO_4$ 307 (5.2); $C_{13}H_{18}NO_3$ 236 (9.4); $C_8H_{10}NO$ 136 (13.5); $C_8H_{10}N$ 120 (100); C_6H_8N 94 (75.1); C_5H_6N 80 (19.3).
NMR (δ = ppm): C-9: 5.54 (1 H, dd, 11.6; 1.4 Hz, H_α-9) + 4.02 (1 H, dt, 11.6; 1.4 Hz, H_β-9)/60.56; C-14: 35.98; C-15: 64.15; C-20: 2.91 (1 H, q, 5.4 Hz, H-20)/56.05; C-21: 1.19 (3 H, d, 5.4 Hz, H_3-21)/13.62; further values corresponding to Asada et al. (1985).

3.4.5. Jacoline (**5**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{27}NO_7$ 369 (4.8); $C_{18}H_{25}NO_6$ 351 (0.9); $C_{16}H_{23}NO_6$ 325 (23.1); $C_{17}H_{26}NO_4$ 308 (9.2); $C_{13}H_{18}NO_3$ 236 (24.3); $C_8H_{12}NO$ 138 (27.8); $C_8H_{10}N$ 120 (100); C_6H_8N 94 (63.5); C_5H_6N 80 (24.0).
NMR (δ = ppm): C-9: 5.50 (1 H, dd, 11.7 Hz; 1.2 Hz, H_α-9) + 4.03 (1 H, ddd, 11.7 Hz; 1.4 Hz; 1.2 Hz, H_β-9)/60.79; C-14: 36.74; C-15: 81.63; C-20: 3.61 (1 H, q, 6.3 Hz, H-20)/72.45; C-21: 1.14 (3 H, d, 6.6 Hz, H_3-21)/17,21; further values corresponding to the literature (Pieters et al. 1989; Segall 1978).

3.4.6. Jaconine (**6**)

GC-MS m/z (rel. int): $[M]^+$ $C_{18}H_{26}NO_6Cl$ 387 (2.8); $C_{17}H_{26}NO_4Cl$ 343 (5.2); $C_{16}H_{22}NO_6$ 324 (2.3); $C_{17}H_{24}NO_3$ 290 (15.9); $C_8H_{12}NO$ 138 (11.1); $C_8H_{10}N$ 120 (100); C_6H_8N 94 (49.2); C_5H_6N 80 (11.1).
NMR (δ = ppm): C-9: 5.48 (1 H, d, 11.8 Hz, H_α-9) + 4.03 (1 H, d, 11.8 Hz, H_β-9)/60.41; C-14: 37.70; C-15: 81.12; C-20: 3.94 (1 H, q, 6.6 Hz, H-20)/63.26; C-21: 1.44 (3 H, d, 6.6 Hz, H_3-21)/18.16; further values corresponding to Pieters et al. (1989).

3.4.7. Jacobine-acetate (**7**)

GC-MS m/z (rel. int): $[M]^+$ $C_{20}H_{27}NO_7$ 393 (4.1); $C_{17}H_{23}NO_4Cl$ 340 (6.1[1]); $C_{17}H_{24}NO_4$ 308 (20.3); further data corresponding to compound **4**.

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