Pergamon

PII: S0031-9422(96)00445-1

Phytochemistry, Vol. 43, No. 6, pp. 1227–1229, 1996

0031-9422/96 $15.00 + 0.00

PYRROLIZIDINE ALKALOID COMPOSITION IN SIX BRAZILIAN *SENECIO* SPECIES*

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(*Received in revised form* 29 *May* 1996)

Key Word Index—*Senecio oxiphyllus*; *Senecio* sp.; Asteraceae; pyrrolizidine alkaloids; quantitative NMR spectroscopy; ^{13}C NMR.

Abstract—Four macrocyclic pyrrolizidine alkaloids (PA) could be identified and quantified in extracts of the Brazilian *Senecio oxiphyllus* by means of proton- and inverse-gated decoupling ^{13}C NMR spectroscopy. Seasonal and local differences were found in PA composition. Reinvestigation of five other *Senecio* species by the same method led to the identification of various retronecine- and otonecine-based PA, exceeding prior results in both qualitative and quantitative respects. In the extract of *S. selloi*, a new alkaloid was found, which was identified as 18-hydroxyjaconine (20-chloro-15,20-dihydro-12,15,18-trihydroxysenecionan-11,16-dione) according to NMR and GC-mass spectrometry data. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

Poisoning by plants of the genus *Senecio* is one of the main causes for losses among livestock in Brazil, Paraguay and Uruguay. In southern Brazil, different species are known to be toxic to cattle, sheep and horses [1], and a few years ago the main pyrrolizidine alkaloids (PA) of *S. brasiliensis*, *S. cisplatinus*, *S. heterotrichus*, *S. leptolobus* and *S. selloi* were isolated and identified [2].

Feeding experiments with *S. oxiphyllus* to calves have shown that this species is one of the most toxic members of the family [3]. It has been known for 40 years [4] but no determination of alkaloid content has been carried out as yet.

In our studies, we investigated the PA composition of *S. oxiphyllus* and the other five species by means of quantitative NMR spectroscopy [5]. This includes ^{1}H NMR of the crude extract with an exact amount of *p*-dinitrobenzene for determination of the total PA content and an integratable inverse-gated decoupling ^{13}CNMR for identification and quantification of the individual alkaloids. The method avoids further purification of the extracts and possible losses of substance through decomposition.

RESULTS AND DISCUSSION

Three different samples of *S. oxiphyllus* were investigated for seasonal and local changes in alkaloid composition. Two samples were collected in July (vegetative stage) and October 1989 (flowering time) in the area of the Universidade Federal de Santa Maria (UFSM); one sample was collected in July on a plateau 50 km outside of Santa Maria, Rio Grande do Sul, South Brazil. The recovery rate of PA in the crude extract was 90% (determined by extraction of *S. oxiphyllus* plant material with an additional exact amount of senecionine). Retrorsine, riddelliine and usaramine were found in all samples (Table 1). The two July extracts showed the same total content but the retrorsine/riddelliine ratio changes from *ca* 10:1 (UFSM) to 3:1 (plateau). The October material, with a 20% increase of total PA, also contained a small amount of seneciphylline.

The other species were collected near Pelotas, Rio Grande do Sul, South Brazil (*S. brasiliensis*: January 1989; *S. cisplatinus*, *S. heterotrichus*, *S. leptolobus*, *S. selloi*: June 1986); the results obtained are shown in Table 2.

The composition of the complex mixture of *S. leptolobus* was verified by means of GC-mass spectrometry. Nevertheless, the quantitative results differ depending on concentration of the sample. Above all, the acetates floresenine and doronine seem to be decomposed, appearing in *ca* half the concentration measured by NMR.

Besides retrorsine, usaramine, senecionine and senecivernine, the ^{13}C NMR spectrum of the extract of *S. selloi* showed a number of peaks indicating the presence of a novel alkaloid, representing *ca* 30% of the PA mixture. The carbon shifts were related to those of jaconine [5], except for differences known between senecionine and its 18-hydroxy-derivative, retrorsine.

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*Part of the Ph.D. thesis of T. Carl, Universität Hannover, 1995.

Table 1. PA composition (%) in *Senecio oxiphyllus*

PA*		UFSM (July)	Plateau (July)	UFSM (Oct.)
Retrorsine	[8]	0.170	0.128	0.198
Riddelliine	[8]	0.016	0.038	0.016
Usaramine	[9]	0.008	0.010	0.011
Seneciphylline	[10]	-	-	0.003
Total†		0.19	0.18	0.23

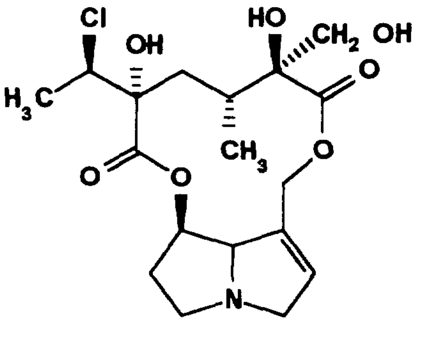
*Carbon shifts in accordance with [Lit.].
†Determined via ^1H NMR.

Table 2. PA composition (%) in five *Senecio* species

PA*		$[M]^+$	(RI)	*S. bras.*	*S. cis.*	*S. het.*	*S. lept.*	*S. sell*
Senecionine	[9]	335	(2)	0.07	–†	0.55	0.006	0.004†
Integerrimine	[9]	335	(5)	1.18†	–	0.16†	0.084†	–
Retrorsine	[8]	351	(12)	0.18†	0.131†	0.21†	traces	0.036†
Usaramine	[9]	351	(19)	0.04	0.013†	–	0.007	0.012
Senecivernine	[10]	335	(10)	–	–	–	0.004	0.003
Riddelliine	[8]	349		–	traces	–	–	–
18-Hydroxy-jaconine		403	(15)	–	–	–	–	0.025
Senkirkine	[10]	365	(8)	–	–	–	0.007	–
Neosenkirkine	[11]	365	(6)	–	–	–	0.045†	–
Otosenine	[12]	381	(10)	–	–	–	0.020	–
Florosenine	[12]	423	(30)	–	–	–	0.009†	–
Doronine	[13]	459	(20)	–	–	–	0.012	–
Total‡				1.47	0.14	0.92	0.19	0.08

*^{13}C shifts in accordance with [lit.].
†PA isolated in lit. [2].
‡Determined via ^1H NMR.

Another clue was given by the ^1H NMR spectrum where a significant peak appearing at δ 5.14 was comparable with the H-7 signal of jaconine (δ 5.13). According to these results we assume that this alkaloid is a 18-hydroxyjaconine. GC-mass spectrometry of the extract gave one fraction with mass peaks confirming the NMR data; the fragmentation pattern corresponded with published data for jaconine [6].



18-Hydroxyjaconine

EXPERIMENTAL

Material. All samples originate from the state Rio Grande do Sul, South Brazil and were identified by Prof. C. S. L. de Barros, UFSM. *S. oxiphyllus* was collected in July and October 1989 in the UFSM area and on a plateau 50 km outside of Santa Maria. *S. brasiliensis, S. cisplatinus, S. heterotrichus, S. leptolobus* and *S. selloi* were collected in 1986 and 1989, respectively, near Pelotas. Aerial parts were dried, ground and stored under cool conditions until extraction.

Extraction. Plant material (200–300 g) was extracted for 24 hr with MeOH in a Soxhlet apparatus. After evapn, the extract was treated with 1N H_2SO_4 and extracted several times with Et_2O. The aq. soln was stirred with Zn dust, to reduce native or artificial *N*-oxides, filtered, made alkaline with NH_3 to pH 10–11 and extracted with CH_2Cl_2, until no more PA could be detected (TLC, silica gel, CH_2Cl_2–MeOH–NH_3, 85:14:1, detection by Dragendorff's reagent). The combined organic frs were dried (Na_2SO_4) and evapd to dryness. The whole process was repeated to yield an amorphous or crystalline alkaloid extract which could be analysed without further purification.

NMR. Spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C. For ^{1}H NMR, the crude extract (20–30 mg, precisely weighed) was dissolved in 1 ml of a 0.3% (w/v) soln of *p*-DNB in CDCl$_3$–TMS. ^{13}C NMR were recorded from a solution of *ca* 150 mg crude extract in CDCl$_3$–1% TMS. Spectra were measured and evaluated according to ref. [5]. Alkaloids were identified via comparison with ref. NMR data [7–13].

GC-MS. Spectra were recorded on a quadrupole instrument using a 25 m capillary GC tube (Hewlett Packard HP 1, $\varnothing$0.32 mm, 0.52 μm methylsilicone gum), carrier He (36 cm s^{-1}). A temp. prog. according to ref. [14] was used.

18-*Hydroxyjaconine*. ^{13}C NMR (75 MHz, CDCl$_3$): δ 14.4 (C-19), 18.2 (C-21), 34.2 (C-6), 35.3 (C-13), 37.5 (C-14), 53.5 (C-5), 60.7 (C-9), 63.2 (C-20), 63.4 (C-3), 66.6 (C-18), 76.6 (C-7), 77.5 (C-8), 81.3 (C-12), 82.5 (C-15), 131.1 (C-1), 135.5 (C-2), 173.2 (C-16), 175.4 (C-11). MS (70 eV) *m/z* (rel. int.): 403 ([M]$^+$, 15), 322 (8), 306 (12), 299 (9), 254 (7), 223 (17), 193 (8), 155 (7), 136 (25), 120 (100), 106 (22), 93 (47), 80 (21).

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