($CHCl_3$); IR $\nu_{max}^{CHCl_3}$: 1730 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$); δ0.85 (3 H, *t*, *J* 7 Hz, 21-H_3), 1.53 (2 H, *m*, 20-H_2), 1.53 (3 H, *s*, 18-H_3), 2.06 (3 H, *s*, NMe), 2.10 (1 H, *m*, 14-H_a), 2.20 (1 H, *m*, 6-H), 2.25 (1 H, *m*, 15-H), 2.43 (1 H, *m*, 6-H), 2.65 (1 H, *m*, 5-H), 2.80 (1 H, *m*, 14-H_b), 2.85 (1 H, *m*, 5-H), 3.20 (1 H, *br d*, J_{gem} 18 Hz, 3-H), 3.44 (1 H, *br d*, J_{gem} 18 Hz, 3-H), 3.66 (1 H, *br s*, 0-H), 4.46 (1 H, *br d*, J_{gem} 11 Hz, 9-H), 4.81 (1 H, *t*, *J* 3 Hz, 7-H), 5.09 (1 H, *d*, J_{gem} 11 Hz, 9-H), 5.10 (2 H, *d*, *J* 6 Hz, 19-H_2), and 6.02 ppm (1 H, *br s*, 2-H). ^{13}C NMR (50 MHz, $CDCl_3$); δ12.0 (C-21), 26.5 (C-20), 28.5 (C-18), 36.1 (C-14), 37.5 (C-6), 40.2 (NMe), 46.9 (C-15), 53.2 (C-5), 58.6 (C-9), 66.6 (C-3), 75.2 (C-12), 77.2 (C-7), 117.9 (C-19), 131.8 (C-2), 135.7 (C-1), 146.5 (C-13), 174.7 and 177.6 (C-11 and -16) and 191.5 ppm (C-8); MS (probe) 70 eV, *m/z*: 365 $[M]^+$ 337, 321, 306, 168, 151, 125, 110, 96, 53 and 43. (Found: M^+, 365.1825. $C_{19}H_{27}NO_6$ requires 365.1838).

Acknowledgements—We are grateful to the staff at the Botanic Gardens, Glasgow, for growing and identifying *Emilia flammea*. We thank the SERC and MRC for a CASE award (to RHB).

REFERENCES

1. Robins, D. J. (1982) *Fortschr. Chem. Org. Naturst.* **41**, 115.
2. Wunderlich, J. A. (1967) *Acta Crystallogr., Sect. C* **23**, 846.
3. Atal, C. K. Culvenor, C. C. J., Sawhney, R. S., and Smith, L. W. (1967) *Aust. J. Chem.* **20**, 805.
4. Kohlmuenzer, S., Tomczyk, H., and Saint-Firmin, A. (1971) *Diss. Pharm. Pharmacol.* **23**, 419.
5. Tomczyk, H. and Kohlmuenzer, S. (1971) *Herba Pol.* **17**, 226.

Phytochemistry, Vol. 26, No. 8, pp. 2431–2433, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

PYRROLIZIDINE ALKALOIDS FROM *SENECIO LONGILOBUS* AND *SENECIO GLABELLUS*

ALLEN C. RAY, HOWARD J. WILLIAMS* and JOHN C. REAGOR

Texas Veterinary Medical Diagnostic Laboratory and *Departments of Chemistry and Entomology, Texas A&M University, College Station, TX 77843, U.S.A.

Key Word Index—*Senecio longilobus*; *Senecio glabellus*; Compositae; capillary GC and GC-MS; ^1H NMR; pyrrolizidine alkaloid; integerrimine; retrorsine; seneciphylline; senecionine.

Abstract—TLC, capillary GC, packed column and capillary GC-MS, and ^1H NMR were used to characterize pyrrolizidine alkaloids from *Senecio longilobus* and *S. glabellus*. *S. glabellus* contained senecionine and integerrimine, and *S. longilobus* contained senecionine, integerrimine, seneciphylline and retrorsine, all present predominantly as *N*-oxides. Alkaloid content varied greatly in collections of *S. longilobus*. This is the first report of integerrimine in these plants.

INTRODUCTION

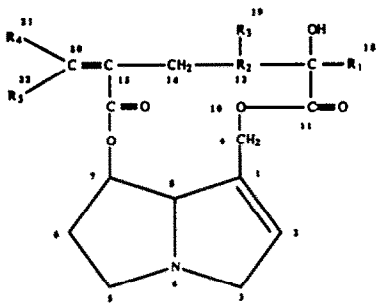
The chronically hepatotoxic pyrrolizidine alkaloids are common in a number of genera of plants, including *Senecio*, *Crotalaria*, *Heliotropium* and *Amsinckia* [1]. These compounds are responsible for worldwide livestock losses, and have been implicated in public health concerns, such as carcinogenicity, cirrhosis and milk transfer in cattle [1–3].

A large number of pyrrolizidine alkaloids have been isolated and characterized. *Senecio longilobus* (threadleaf groundsel) and *S. glabellus* (butterweed), two species common in Texas, have been reported to contain riddelliine, retrorsine, senecionine and seneciphylline [4, 5] and senecionine, respectively [6] (Fig. 1). Isolates from these plants were studied using TLC, GC-MS, capillary GC and GC-MS, and ^1H NMR to compare the alkaloids present in local species to literature reports, and to estimate the prevalence of *N*-oxides.

RESULTS

EtOH was superior to $CHCl_3$, Me_2CO, dilute H_2SO_4 and EtOAc in extracting the alkaloids. Extraction with EtOH at room temperature produced better yields than extraction using Soxhlet apparatus.

Examination of plant extracts by TLC was useful in acquiring qualitative information about pyrrolizidine alkaloids concerning *N*-oxide occurrence, relative abundance, and base strengths and polarities. As evidenced by the detection system developed by Mattocks [7], which is specific for unsaturated pyrroline rings and is designed to differentiate between *N*-oxides and free bases, essentially all of the alkaloids were present in the plants as *N*-oxides (i.e. unreduced extracts contained no free bases). *N*-Oxides were readily extractable from alkaline solutions, the best yield being obtained at pH 8. The free base yield, from Zn-reduced extracts, was also best at pH 8. Qualitative and quantitative differences in the alkaloid content of the



	R_1	R_2	R_3	R_4	R_5
Senecionine	$-CH_3$	-CH-	$-CH_3$	-H	$-CH_3$
Integerrimine	$-CH_3$	-CH-	$-CH_3$	$-CH_3$	-H
Seneciphylline	$-CH_3$	-C-	$=CH_2$	-H	$-CH_3$
Retrorsine	$-CH_2OH$	-CH-	$-CH_3$	-H	$-CH_3$

Fig. 1. Pyrrolizidine alkaloids from *Senecio longilobus* and *S. glabellus*.

various plant types and collections were apparent from comparison of TLC profiles.

Preliminary screening of plant extracts by packed column GC-MS corroborated the information obtained by TLC analysis, but also showed that small amounts of free base alkaloids were present in the plants (i.e. in unreduced extracts). *N*-Oxide forms of the alkaloids were not eluted from the column. Mass spectral data [5, 8] indicated the presence of senecionine in *S. glabellus* and seneciphylline and integerrimine (Fig. 1) in *S. longilobus*. Other peaks were present in too low concentration for identification.

Extracts were analysed by capillary GC and capillary GC-MS to improve resolution and sensitivity. The presence of senecionine and integerrimine in *S. glabellus* and senecionine, seneciphylline, and integerrimine in *S. longilobus* was confirmed by comparison with authentic standards (Table 1). Small amounts of retrorsine were detected in two collections of *S. longilobus* based on MS data, particularly the molecular ion at 351 *m/z*, and peaks at 307 and 320 *m/z* [5]. The alkaloid content of all collections is summarized in Table 1. An unidentified alkaloid in *S. glabellus* extracts was observed as a shoulder on the senecionine peak. Low abundance and incomplete resolution prevented identification of this compound, but a small mass peak at 335 *m/z* suggested that it was an isomer of senecionine. No riddelliine was detected in any sample. *N*-Oxides were not eluted from the capillary columns.

In addition to retention times on GC and capillary GC systems, senecionine and integerrimine could be differentiated by subtle variances in electron impact mass spectra [8]. Both alkaloids have representative molecular ion peaks (335 *m/z*), but the senecionine spectrum has a large 136 *m/z* ($[C_8H_{10}NO]^+$) peak (from loss of the esterifying dicarboxylic acid and subsequent ring fission) and a consistently less abundant 248 *m/z* peak ($[C_{15}H_{22}NO_2]^+$, from cleavage and rearrangement of the macrocyclic diester moiety) as compared to 246 *m/z* ($[C_{15}H_{20}NO_2]^+$). The integerrimine spectrum has a consistently less abundant 136 *m/z* peak, and a 248 *m/z* peak more abundant than 246 *m/z*.

Integerrimine and senecionine were further purified by preparative GC for comparison of ^1H NMR spectra with those of authentic standards and literature values [1, 9, 10]: agreement of spectral data was excellent. Attempts to determine coupling constants for pyrrolizidine ring protons were unsuccessful due to line broadening, thought to be caused by conformational changes [1]. Attempts to freeze conformation by cooling to $-30°$ and $-80°$ were also unsuccessful, no significant line narrowing being observed. Sufficient integerrimine was collected for ^{13}C NMR spectroscopy, the spectra being identical to that of an authentic sample.

DISCUSSION

Capillary GC is a valuable method for resolving and identifying pyrrolizidine alkaloids in plant extracts. Occurrence of the alkaloids in *S. glabellus* and *S. longilobus* predominantly as *N*-oxides is not unprecedented [1], but contrasts with an earlier report [5]. However, this is the first report of the detection of integerrimine in both plants. The variability of alkaloid content is somewhat surprising, since these results differ qualitatively from earlier reports [4–6]. More surprising, however, is the variability noted among different collections of *S. longilobus* in this study. No alkaloids were detected in the plants collected from Brewster County.

Table 1. Retention times and relative abundances of pyrrolizidine alkaloids from *Senecio longilobus* and *Senecio glabellus* using capillary gas chromatography

		Relative abundance			
Standards*	Retention times (min)	*S. longilobus* Presidio County	*S. longilobus* Brewster County	*S. longilobus* Pecos County	*S. glabellus* Brazos County
Senecionine	16.36	±	ND	±	+ +
Seneciphylline	16.50	+ +	ND	+ + + +	ND
Integerrimine	16.73	+ + + +	ND	+ + +	±
Retrorsine	17.97	±	ND	±	ND

ND = None Detected.
*For structures, see Fig. 1.

The toxicity of *S. longilobus* is well documented, but the findings described here (Table 1) suggest that inconsistencies might be observed in experimental and natural ingestion. *S. glabellus* probably contains sufficient alkaloids to be an additional causative agent of hepatotoxic disease observed in cattle in eastern Texas. Quantitation was not attempted because authentic standards were available only in very small quantities and, in some cases, at less than 100% purity.

EXPERIMENTAL

Plant collection. *S. glabellus* was collected in Brazos County (East Texas). Three collections of *S. longilobus* were made in Presidio, Brewster, and Pecos Counties (West Texas). Plants were collected in the bloom stage, air dried and ground in a Wiley mill. Authority for identification was Dr. Barron Rector, Department of Range Science, Texas A&M University.

Extraction. An adaptation of the procedure described by Culvenor and Smith [11] was used in this study. Fifty g of plant material were steeped overnight in 500 ml EtOH, then stirred for 4 hr. After filtration, the soln was evapd to dryness and the alkaloids dissolved in 100 ml of 0.2 N H_2SO_4, washed with 50 ml Et_2O and equally divided into parts A and B. Part A was adjusted to 2 N in H^+ by the addition of conc. H_2SO_4, and stirred for 2 hr in the presence of excess Zn dust. Part B was a control. Both solutions were adjusted to pH 8 with 20% Na_2CO_3 and extracted with 2 × 50 ml $CHCl_3$. The $CHCl_3$ solns were dried (Na_2SO_4) and concd to 1 ml by evapn. Residual alkaline solns were adjusted to pH 11 with 20% NaOH, extracted as before with two portions of $CHCl_3$, dried and concd as described for the pH 8 extractions.

Thin layer chromatography. The developing system for TLC (silica gel G) was $CHCl_3$-$NHEt_2$ (9:1) with detection as described by Mattocks [7].

Gas chromatography and gas chromatography-mass spectrometry. Capillary GC (FID) and capillary GC-MS (EI, 70 eV), both with 25 m fused silica BP1 methyl silicone columns and on-column injection systems were used for assay of $CHCl_3$ extracts (1 μl), programmed 60° (hold 1 min) to 300° at 15°/min. The carrier gas was He at a column pressure of 20 PSIG. Initial screening of extracts was done on GC/MS (EI, 70 eV), using a 1.83 m × 2 mm i.d. column packed with 3% OV 1, and using He carrier gas (30 cc/min). Integerrimine and senecionine were further purified for NMR by preparative GC (FID) using a 1.83 m × 4 mm i.d. column packed with 3% OV 101. Conditions included an isothermal run at 190° and a flow rate of 60 ml N_2/min. An all glass splitter and a collection system designed by Brownlee *et al.* [12] were used.

Proton NMR. ^1H NMR spectra were recorded in $CDCl_3$ or acetone-d_6 using a 1.7 mm C/H probe at 90 MHz or a 5 mm H probe at 300 or 500 MHz. ^{13}C NMR spectra were recorded at 22.8 MHz using a 1.7 mm C/H probe.

Acknowledgements—We thank Dr. C. C. J. Culvenor, CSIRO, Melbourne, Australia, for providing pyrrolizidine alkaloid standards. We thank Dr. Ray Allen, Marfa, Texas, and Mr. B. H. Reagor, Ozona, Texas, for collecting specimens of *Senecio longilobus*.

REFERENCES

1. Bull, L. B., Culvenor, C. C. J. and Dick, A. T. (1968) *The Pyrrolizidine Alkaloids*. American Elsevier, New York.
2. Schoental, R. (1968) *Cancer Res.* **28**, 2237.
3. Dickinson, J. O., Cooke, M. P., King, R. R. and Mohamed, P. A. (1976) *J.A.V.M.A.* **169**, 1192.
4. Adams, R. and Govindachari, T. R. (1949) *J. Am. Chem. Soc.* **71**, 1180.
5. Segall, H. J. and Molyneux, R. J. (1978) *Res. Comm. Chem. Path. Pharmacol.* **3**, 545.
6. Adams, R. and Van Duuren, B. L. (1953) *J. Am. Chem. Soc.* **75**, 4631.
7. Mattocks, A. R. (1967) *J. Chromatogr.* **27**, 505.
8. Lüthy, J., Zweifel, U., Karlhuber, B. and Schlatter, C. (1981) *J. Agric. Food Chem.* **29**, 302.
9. Segall, H. J. and Dallas, J. L. (1983) *Phytochemistry* **22**, 1271.
10. Culvenor, C. C. J. and Woods, W. G. (1965) *Aust. J. Chem.* **18**, 1625.
11. Culvenor, C. C. J. and Smith, L. W. (1955) *Aust. J. Chem.* **8**, 556.
12. Brownlee, R. G. and Silverstein, R. M. (1968) *Analyt. Chem.* **40**, 2077.