McClendon, L. T., in "Trace Substances in Environmental Health", VIII, Hemphill, D. D., Ed., University of Missouri, Columbia, 1974, p 334.
McClendon, L. T., *J. Radioanal. Chem.* **42**, 85 (1978).
Mertz, W., *Physiol. Rev.* **49**, 165 (1969).
Mertz, W., *J. Am. Diet. Assoc.*, in press (1979).
Mertz, W., Wolf, W., Alvarez, R., unpublished data (1977).
Mertz, W., Anderson, R. A., Wolf, W. R., Roginski, E. E., in "Trace Element Metabolism in Man and Animals", Vol. III, Kirchgessner, M., Ed., Institut fur Ernahrungsphysiologie, Techniche Universitat Munchen, Freising-Weichenstephan, 1978, p 272.
Murakami, Y., Suzuki, Y., Yamagata, T., Yamagata, N., *J. Radiat. Res.* **6**, 105 (1965).
Parr, R., in "Trace Element Metabolism in Man and Animals", Vol. III, Kirchgessner, M., Ed., Institut fur Ernahrungsphysiologie, Technische Universitat Munchen, Freising-Weichenstephan, 1978, p 622.
Rook, H. R., Wolf, W., in "Trace Substances in Environmental Health", XI, Hemphill, D. D., Ed., University of Missouri, Columbia, 1977, p 342.
Schelenz, R., *J. Radioanal. Chem.* **37**, 539 (1977).
Schroeder, H. A., Balassa, J. J., Tipton, J. H., *J. Chronic Dis.* **15**, 946 (1962).
Scott, K., *Analyst (London)* **103**, 754 (1978).
Shapcott, D., Dhoury, K., Demers, P. P., Vobecky, J., Vobecky, J., *Clin. Biochem.* **10**, 178 (1977).
Toepfer, E. W., Mertz, W., Roginski, E. E., Polansky, M. M., *J. Agric. Food Chem.* **21**, 69 (1973).
Tuman, R. W., Bilho, J. T., Doisy, R. J., *Diabetes* **27**, 49 (1978).
Versieck, J., Hoste, J., Barbier, F., Steyaert, H., De Rudder, J., Michaels, H., *Clin. Chem. (Winston Salem, N.C.)* **24**, 303 (1978).
Wolf, W., Greene, F. E., in "Accuracy in Trace Analysis: Sampling Sample Handling, Analysis I", La Fleur, P. D., Ed., NBS Publ. No. 422, U.S. Government Printing Office, Washington, DC., 1976, p 605.
Wolf, W., Mertz, W., Masironi, R., *J. Agric. Food Chem.* **22**, 1037 (1974).

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Chemistry of Toxic Range Plants. Determination of Pyrrolizidine Alkaloid Content and Composition in *Senecio* Species by Nuclear Magnetic Resonance Spectroscopy

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The pyrrolizidine alkaloid and corresponding *N*-oxide contents of small samples of *Senecio longilobus*, *S. riddellii*, *S. jacobaea*, and *S. vulgaris* have been measured using nuclear magnetic resonance spectroscopy. The percentages of the individual alkaloids seneciphylline, senecionine, riddelliine, and retrorsine in the total alkaloid mixture have also been determined from the same NMR spectra. Exceptionally high total alkaloid contents were found for *S. longilobus* and *S. riddellii* relative to most *Senecio* sp. The technique provides a rapid, facile method for examination of plant samples in order to evaluate the hepatotoxicity hazard to animals and humans and for regulation of dose rate of pyrrolizidine alkaloids in animal feeding experiments.

The hepatotoxic pyrrolizidine alkaloids (PAs) present in *Senecio* and several other plant species have been implicated in the poisoning of animals and, on occasion, humans in many parts of the world (Bull et al., 1968; Mattocks, 1972). *Senecio* species present a toxicity hazard to cattle, horses, and, to a lesser extent, sheep on ranges and pastures in time of drought or overgrazing when other food is scarce or after spring rains when these plants exhibit lush growth that precedes growth of more palatable and nutritious species. In addition, concern has arisen that the pyrrolizidine alkaloids may enter the human food supply in milk (Johnson, 1976; Dickinson et al., 1976), in honey (Deinzer et al., 1977), and through contamination of grains by seeds.

The effect upon animals of consumption of *Senecio* is generally chronic, characterized by a cirrhosis-like condition of the liver. Signs of intoxication are frequently slow to appear, occurring weeks or even months after ingestion of the plant, resulting in poisoning being falsely attributed to infectious diseases or to toxic plant species most visible on the range at the time the symptoms are observed. Moreover, the insidious and irreversible nature of the hepatotoxicity results in unexpected fatality when the animals are subjected to any of a variety of severe, subsequent stresses.

On ranges in the western United States, three *Senecio* species are of particular concern: *S. jacobaea* (tansy ragwort), an introduced plant which presents a problem in coastal areas of the Pacific Northwest, *S. longilobus* (threadleaf groundsel), and *S. riddellii* (Riddell's groundsel), native species in semidesert areas of the Southwest. In connection with animal experiments designed to evaluate the effect of sublethal doses of the latter two species on cattle and synergism with other toxic plants such as locoweed which may be consumed concurrently, it became essential to determine the level of PAs present in a large number of plant samples. In addition to measuring the total level of PAs, it was considered necessary to determine the amount existing in the *N*-oxide form, since the relative toxicities of the alkaloid vs. *N*-oxide are not known, and also to obtain the relative proportions of at least the major individual alkaloids present.

To date, the only satisfactory method for estimation of PAs in biological materials has been a spectrophotometric method (Mattocks, 1967, 1968; Bingley, 1968) based upon the Polonovsky reaction. In this method the alkaloidal

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material is oxidized to the *N*-oxide, dehydrogenated with acetic anhydride to the pyrrole, and the latter condensed with 4-dimethylaminobenzaldehyde (Ehrlich reagent), giving a magenta color with an absorbance maximum ca. 560 nm. Although very sensitive to small quantities of PAs, this approach was found to suffer from a number of serious deficiencies when applied to examination of plant extracts. The oxidation, dehydrogenation, and color formation reactions must be very carefully regulated; a blank sample must be run for estimation of background and the molar absorptivities of the individual alkaloids, a few of which have quite low values, must be known. Most seriously, in our hands the spectrophotometric method gave results consistently lower than the actual percentage of alkaloid extracted and recrystallized from large samples of material.

We have therefore developed a fairly rapid, small-scale analytical method which provides a measurement of total PA content and in some cases the relative proportions of individual alkaloids in the total extract. This technique has been used in order to screen plant samples for animal feeding experiments and to study variation in alkaloid level with the stage of plant growth, part of plant, and growing season.

EXPERIMENTAL SECTION

Apparatus. Nuclear magnetic resonance (NMR) spectra were obtained using a Varian HA-100 or EM-390 spectrometer. Samples were run as solutions in $CDCl_3$ with addition of sufficient Me_2SO-d_6 to dissolve all material in the sample. Tetramethylsilane (Me_4Si) was used as an internal standard.

Sample Preparation. Plant samples were thoroughly air-dried and ground in a Wiley mill, and all material passing through a 1-mm screen was collected. The ground material was thoroughly mixed before sampling to ensure homogeneity.

The weighed plant material (typically 10–20 g) in a Soxhlet thimble (33 × 94 mm) was extracted with MeOH (300 mL) overnight and the extract evaporated to dryness at reduced pressure. The residue was taken up in 2 N HCl, washed with ether to remove nonbasic material, and divided into equal portions. The first portion was basified with dilute NH_4OH and extracted with $CHCl_3$ (4 × 100 mL), and the combined extracts were dried ($MgSO_4$) and evaporated to dryness. The second portion was reduced with Zn dust for 30 min, filtered, and treated in the same manner. If the amount of extract was such that an excessive amount of solvent was required to dissolve the sample for NMR analysis, the material was dissolved in $CHCl_3$ and a suitable aliquot taken. A precisely weighed amount of *p*-dinitrobenzene (5–10 mg) was added to the residue and the entire sample dissolved in $CDCl_3$, DMSO-d_6 for NMR spectroscopic analysis. Integration of signals was carried out by scanning in both directions and the average of these values measured.

RESULTS AND DISCUSSION

Analysis for Total Alkaloid and *N*-Oxides. The PAs may be divided into four main types, namely nonester, monoester, diester, and macrocyclic diester. In *Senecio* species the alkaloids are generally 12-membered ring macrocyclic diesters of closely related structure which appear to exhibit greater hepatotoxicity than the noncyclic diesters and monoesters, while PAs lacking unsaturated heterocyclic rings are regarded as being nonhepatotoxic (Mattocks, 1972). Thus, *S. longilobus* has been reported to contain seneciphylline (**1**), retrorsine (**2**), senecionine (**3**), and riddelliine (**4**) (Adams and Govindachari, 1949), while *S. riddellii* contains only the latter alkaloid (Adams et al., 1942) and *S. vulgaris* contains the first three above-mentioned compounds but no riddelliine (Tschu Shun et al., 1960). On the other hand, in addition to seneciphylline and senecionine, four additional alkaloids, jacobine (**5**), jacoline (**6**), jaconine (**7**), and jacozine (**8**) have been isolated from *S. jacobaea* (Bradbury and Culvenor, 1954; Adams and Gianturco, 1956).

The NMR spectra of the PAs are rather complex, most of the signals due to the pyrrolizidine nucleus (Culvenor et al., 1965) being obscured by signals derived from the esterifying necic acids and demonstrating quite considerable shifts with variation in solvent. The only signal of the basic ring system which can be easily identified is the vinyl H-2 proton which is seen as a broadened singlet (actually a multiplet), centered at ca. δ 6.2 in the macrocyclic diesters and ca. δ 5.8 in the noncyclic diesters and monoesters when the spectra are run in $CDCl_3$ solution. This signal occurs at the lowest field relative to all other signals in the spectra, and since it is due to a structural feature common to all the major toxic PAs present in *Senecio* species, it is the only signal which could potentially be used for estimation of total alkaloids.

We have found that measurement of the intensity of this signal, relative to the intensity of the signal due a known weight of a standard added to the NMR sample, provides an accurate measurement of the amount of alkaloid present. After consideration of a number of possible standards, *p*-dinitrobenzene (DNB) was chosen since it is readily soluble in $CDCl_3$, it exhibits a signal well removed (δ 8.46) from the signals due to the alkaloids and produces sharp, four-proton singlet on addition of a relatively small amount of material. Moreover, the alkaloid present in an analysis sample can be readily separated from the *p*-dinitrobenzene by extraction with dilute acid. The amount of alkaloid present in a sample can be calculated using the formula

$$(\text{wt of PA}) = (\text{mol wt of PA}) \times \frac{A(\text{Hh2})}{0.25A(\text{DNB})} \times \frac{\text{wt of DNB}}{\text{mol wt of DNB}}$$

where mol wt of PA is the molecular weight of the major PA present or preferably a weighted average of the various PAs in the extract, if known; A(H-2) is the integrated amplitude of the signal due to the proton at the 2 position of the pyrrolizidine ring; A(DNB) is the integrated amplitude of the singlet due to the four protons of *p*-dinitrobenzene; wt of DNB is the weight of *p*-dinitrobenzene added to the analysis sample and; mol wt of DNB is the molecular weight of the *p*-dinitrobenzene (168).

The formula can thus be reduced to

$$(\text{wt of PA}) = (\text{mol wt of PA}) \times \frac{A(\text{Hh2})}{A(\text{DNB})} \times \frac{\text{wt of DNB}}{42}$$

In order to test the accuracy of this method, analyses were carried out using known weights of alkaloid. Two samples of pure riddelliine, isolated from *S. riddellii* and recrystallized several times, were examined and excellent agreement was obtained between the known and calculated amounts of alkaloid (Table I). In addition, two samples of recrystallized alkaloid mixture obtained from *S. longilobus*, which were known to contain seneciphylline, senecionine, retrorsine and riddelliine were examined, and the results obtained using a weighted average molecular weight found to give the closest agreement with the known weight, although using either an average molecular weight or the molecular weight of the major alkaloid (seneciphylline) gave acceptable values (Table I).

The analytical method was then applied to samples of

Table I. NMR Analysis for Total Pyrrolizidine Alkaloid Content in Samples Containing Known Amounts of Pure or Mixed PAs

sample	wt of PA, mg	mol wt of PA	H(2) proton integral	DNB proton integral	wt of DNB, mg	calcd wt of PA, mg
riddelliine	34.5	349	24	47	8.2	34.8
riddelliine	16.2	349	20.5	103	9.7	16.0
alkaloid extract from *S. longilobus*	44.1	333[a]	64	114	9.8	43.6
	44.1	342[b]	64	114	9.8	44.8
	44.1	339[c]	64	114	9.8	44.4
alkaloid extract from *S. longilobus*	39.0	339[c]	28	57	10.2	40.4

[a] Molecular weight of seneciphylline. [b] Average molecular weight of seneciphylline, senecionine, retrorsine, and riddelline. [c] Weighted average molecular weight calculated for 65% (seneciphylline + senecionine) and 35% (retrorsine + riddelline).

various *Senecio* species (Table II). The portion of the acidic extracts treated with zinc dust gave a measure of the "total alkaloid" content since the *N*-oxides are thereby reduced to the corresponding PAs, whereas the unreduced portion of the extract gave only the "free alkaloid" content. The *N*-oxide content can thus be calculated by the difference between the total and free alkaloid contents.

S. jacobaea collected during 1970–1972 showed fairly consistent levels of total alkaloid, ranging from 0.09 to 0.18%, and a sample of *S. vulgaris* collected in 1977 showed a total alkaloid level in the same range (0.16%). In contrast, *S. longilobus* collected in 1976–1978 showed much higher levels of total alkaloid, generally over 1%, with one sample, consisting of leaves and small stems, showing a content of 3.55%. A single sample of *S. riddellii* collected in the same area of New Mexico in 1977 had a total alkaloid level of 2.96%, while a sample gathered in 1978 contained the exceptional level of 9.66%, and a third sample gathered in Nebraska in 1978 contained 2.44%. The relative quantities of alkaloid *N*-oxide vs. total alkaloid showed considerable variation with collection date, ranging from 45 to 95% in the *S. longilobus* samples.

Although the number of plant samples analyzed to date is too limited to provide totally reliable information regarding annual variation in alkaloid content, certain preliminary conclusions can be drawn. In *S. longilobus* there appears to be a higher concentration of total alkaloid in the leaves and small stems which are most likely to be eaten by grazing animals than in the woody stems. There is also appreciable variation in the relative amounts of free alkaloid vs. *N*-oxide, the level of the latter being much higher in young plants than in mature plants. Although the *N*-oxides might be considered a priori to be less toxic than the free alkaloids due to their high water solubility and consequent rapid excretion, little information is available regarding their reduction to the parent alkaloids or conversion to the hepatotoxic pyrroles in the digestive tract, particularly in ruminant animals (Mattocks, 1972).

A significant point in regard to the analyses of *S. longilobus* and *S. riddellii* is the very high levels of total alkaloid which may occur in the plant, although such levels may be intermittent, depending upon climatic factors, or localized, depending upon soil nutrients. The results obtained for *S. riddellii*, albeit limited, indicate that this species is a potent pyrrolizidine alkaloid producer and, with levels which may approach 10%, might be expected to give rise to immediate rather than delayed toxicity if consumed. In any event, the amounts of alkaloid recorded in the 1977–1978 plant collections are exceptionally high and appear to be the highest ever recorded for pyrrolizidine alkaloid-containing plants. Typical levels reported for *Senecio* species are ca. 0.1–0.2%, corresponding to the results obtained for *S. jacobaea* and *S. vulgaris*, with many species containing even smaller amounts.

In view of the fact that *S. longilobus* is consumed directly by humans in the southwestern United States as the herbal tea "gordolobo yerba" (Huxtable et al., 1977) and has been implicated in infant hepatic veno-occlusive disease and death (Stillman et al., 1977), levels of total alkaloid as high as 2–3% should be of very great concern, whether normal or intermittent. Previous work concerned with possible hazards to the human population due to indirect consumption of PAs via agricultural products has been restricted to *S. jacobaea* (Deinzer et al., 1977). Since this species is fairly localized in distribution and has now been shown to have a relatively low alkaloid content, more attention should be focused upon the indigenous species, particularly *S. longilobus* and *S. riddellii* which are far more widespread than *S. jacobaea* on rangelands in the western United States.

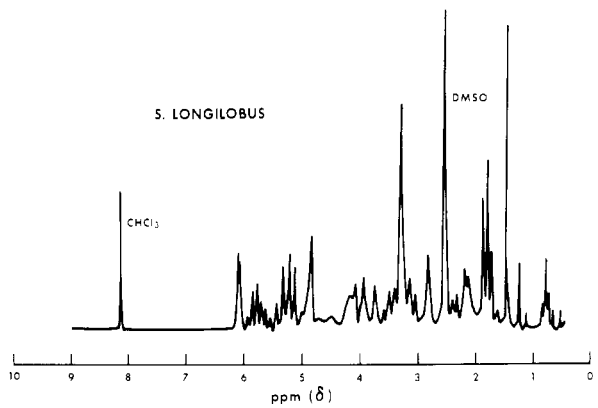

Figure 1. NMR spectrum of PAs from *S. longilobus*.

Analysis for Composition of Individual Alkaloids. Although all PAs of the macrocyclic diester type having a double bond in the 1,2 position appear to be hepatotoxic with little variation in pathological effects in experimental animals, there can be appreciable differences in dose rate with structural variation of the esterifying acids. This being the case, it is of interest to know not only the total alkaloid content but also the relative percentages of at least the major PAs in a given plant sample.

In *S. longilobus*, *S. riddellii*, and *S. vulgaris* these percentages can readily be determined from the NMR spectra used to calculate the total alkaloid level. For these species, the alkaloids of concern are seneciphylline (**1**), retrorsine (**2**), senecionine (**3**), and riddelliine (**4**), and although the superimposed spectra of these alkaloids are quite complex, it is possible by a differential process to pick out characteristic signals for each alkaloid in such a way that the percentages of each, relative to the total alkaloid content, can be calculated.

A typical NMR spectrum of the alkaloidal extract from *S. longilobus* is shown in Figure 1. The H-2 proton signals occur as a broad signal at δ 6.14 which represents the total

Table II. NMR Analysis for Total PA Content, *N*-Oxide Content, and Individual PAs in Various *Senecio* Species

plant species	collection date and location	plant part[a] and maturity	total PA, %	free PA, %	PA *N*-oxide (by difference), %	individual PAs,[b] %
S. longilobus	9/16/76 Tatum, NM	whole plant	0.63			
	10/5/77 Hobbs, NM	leaves and small stems, prebud	1.68	0.17	1.51	Sph. 49 Sen. 17 Rid. 13 Ret. 21
		coarse stems, prebud	0.58	0.08	0.50	Sph. 47 Sen. 21 Rid. 8 Ret. 24
	10/5/77 Tatum, NM	leaves and small stems, prebud	3.55	0.16	3.39	Sph. 55 Sen. 12 Rid. 16 Ret. 15
	12/28/78 Hobbs, NM	leaves and small stems, mature	1.17	0.62	0.55	Sph. 54 Sen. 12 Rid. 17 Ret. 17
	1/4/78 Tatum, NM	leaves and small stems, mature	1.03	0.57	0.46	Sph. 56 Sen. 9 Rid. 19 Ret. 16
	3/5/78 Tatum, NM	whole plant, young	1.18	0.54	0.64	Sph. 54 Sen. 10 Rid. 21 Ret. 15
	3/5/78 Tatum, NM	whole plant, mature	1.47	0.61	0.86	Sph. 55 Sen. 10 Rid. 20 Ret. 15
	4/26/78 Hobbs, NM	leaves and small stems, late flower	0.90	0.21	0.69	Sph. 51 Sen. 15 Rid. 20 Ret. 13
		flowers and some seeds, late flower	1.43	0.32	1.11	Sph. 65 Sen. 12 Rid. 12 Ret. 6
S. riddellii	10/5/77 Eunice, NM	whole plant, early bud	2.96	0.38	2.58	Rid. 97 Ret. 3
	6/9/78 Scottsbluff, NB	whole plant, young	2.44	0.46	1.98	Rid. 100 Ret. 0
	6/8/78 Eunice, NM	whole plant, prebud	9.66	2.02	7.64	Rid. 96 Ret. 4
S. vulgaris	12/16/77 Albany, CA	whole plant, late flower	0.16	0.14	0.02	Sph. 69 Sen. 21
S. jacobaea	6/23/70 Oregon City, OR	whole plant, early flower	0.09			
	9/16/70 Tillamook, OR	flowers and seeds, mature	0.09			
		stems, mature	0.15			
	6/30/71 Tillamook, OR	whole plant, bud stage	0.18			
	6/28/72 Tillamook, OR	whole plant, early bud stage	0.18			

[a] "Whole plant" indicates total above-ground plant with exception of coarse woody stems, where present. [b] Calculated on total PA extract. Sph. = seneciphylline; Sen. = senecionine; Rid. = riddelliine; Ret. = retrorsine.

toxic PA content of the extract. The skewed triplet centered at δ 0.81 is actually a pair of overlapping doublets due to the methyl groups attached to C(3′) in retrosine and senecionine, the latter alkaloid giving rise to the lower field doublet (δ 0.87) as shown by the NMR spectrum of a sample of the pure alkaloid. From the latter spectrum, the singlet at δ 1.27 can be assigned to the CH_3(1′) group of senecionine and, therefore, the singlet at δ 1.49 must arise from the methyl group at the same position in seneciphylline. The relative intensities of these two signals can thus be determined, and by subtraction of the integral for the δ 1.27 signal (senecionine only) from the δ 0.81 signal (senecionine + retrorsine), the corresponding intensity of the signal due to retrorsine may be obtained. A similar pattern is observed for *S. vulgaris*, with variations in intensity of the individual signals (Figure 2).

Of the four major alkaloids only the contribution due to riddelliine therefore remains to be determined. This presents a more difficult problem since this alkaloid has no methyl groups absorbing at high field in the NMR spectrum and thus unobscured by signals from other alkaloids. However, a characteristic grouping in riddelliine

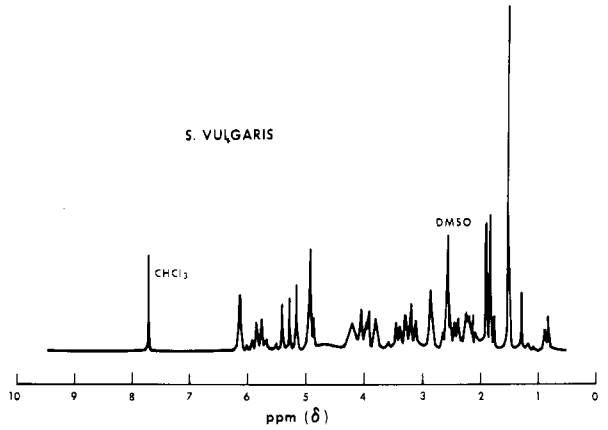

Figure 2. NMR spectrum of PAs from *S. vulgaris*.

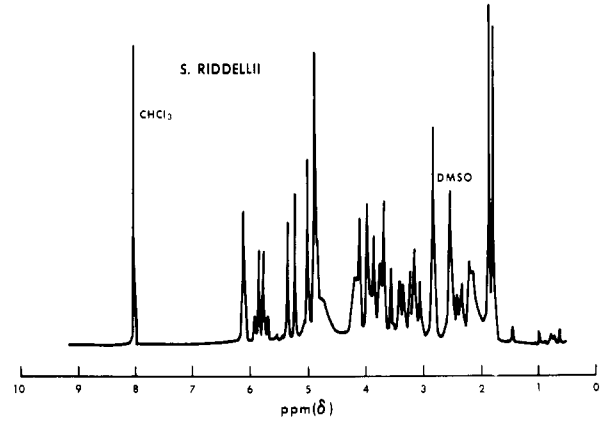

Figure 3. NMR spectrum of PAs from *S. riddellii*.

is the methylene group attached to C(3′) which also occurs in seneciphylline. Fortunately, the signal from the latter occurs as a well-separated pair of doublets, the low-field resonance appearing at δ 5.16, whereas the high-field signal is obscured by the pyrrolizidine ring C(7) proton, as are both protons of the C(3′) methylene group in riddelliine, the combined resonances giving rise to a broad signal at δ 4.8–5.1. Subtraction of the contribution to this signal of the C(7) proton of all the alkaloids and one of the C(3′)=CH_2 protons of seneciphylline provides the relative intensity of both C(3′)=CH_2 protons due to riddelliine. The signals of concern can be seen clearly in the NMR spectrum of an extract from *S. riddellii* which consists almost entirely of riddelliine (Figure 3).

Since the various signals thus utilized to measure the relative amounts of the four alkaloids are due to the groups bearing different numbers of protons, it is necessary to normalize the integrals so that the measured intensities correspond to the contribution of each alkaloid on a molecular basis. This is most conveniently achieved by adjusting all integrated intensities to a three-proton basis. Thus, the integrals for -CH and =CH_2 signals are multiplied by 3 and 1.5, respectively. The relative amounts of each alkaloid can then be calculated as follows:

$$\text{total PA} = 3A(\delta\ 6.14)$$

$$\text{retrorsine + senecionine} = A(\delta\ 0.73\text{–}0.90)$$

$$\text{senecionine} = A(\delta\ 1.27)$$

$$\text{retrorsine} = A(\delta\ 0.81) - A(\delta\ 1.27)$$

$$\text{seneciphylline} = A(\delta\ 1.49)$$

$$\text{riddelliine} = 1.5[A(\delta\ 4.8\text{–}5.1) - A(\delta\ 5.16) - A(\delta\ 6.14)]$$

From these values the percentage contribution of each of the four major alkaloids to the total PA content can be calculated.

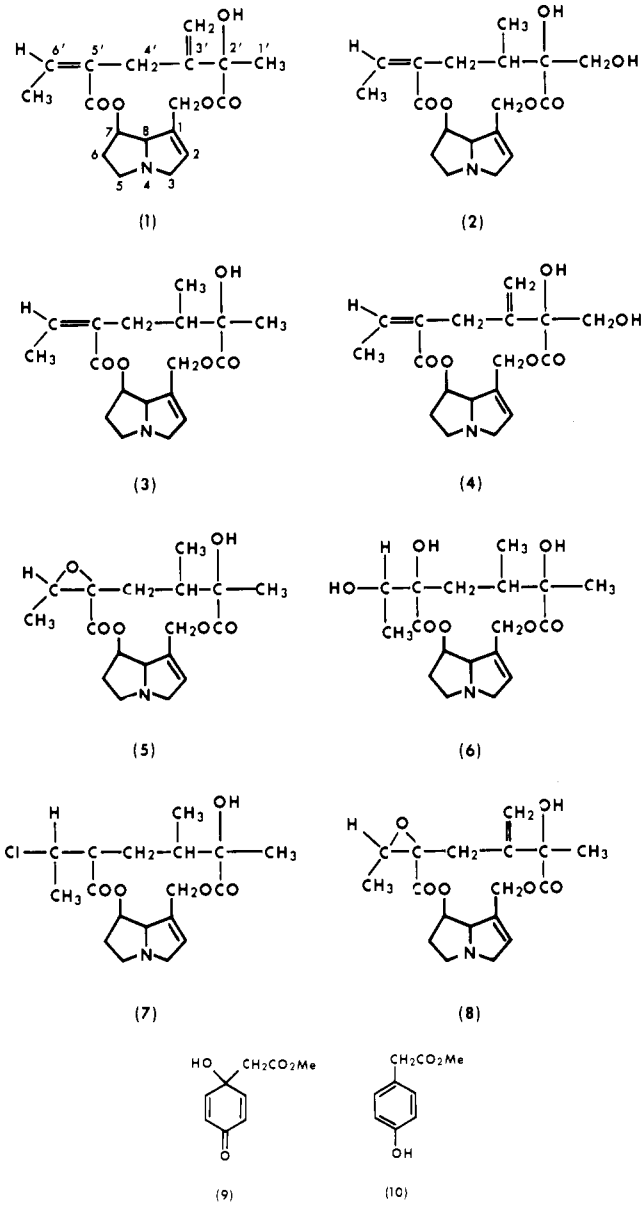

Figure 4. Structures of macrocyclic PAs occurring in *Senecio* sp.

Percentages of individual alkaloids for extracts of plant material collected in 1976–1978 are shown in Table II. The samples of *S. longilobus* showed some variation in the relative proportions of the individual alkaloids but were fairly consistent considering that the samples were collected at a variety of growth stages from different locations and composed of different plant parts. In general, seneciphylline is the major alkaloid of this species making up 50% or more of the total PA content while senecionine is the minor alkaloid, a result which has been confirmed by high-pressure liquid chromatography (HPLC) (Segall and Molyneux, 1978). It is interesting to note that comparison of percentages of the seneciphylline–senecionine pair of monohydroxyl alkaloids vs. the retrorsine–riddelliine pair of diol alkaloids shows remarkable consistency exhibiting values of 64–68% vs. 31–36%, respectively. In view of the fact that the quantity of alkaloid present in these samples showed considerable variation, the consistency of the seneciphylline–senecionine/retrosine–riddelliine ratio suggests that this is a genetically fixed characteristic of the

species.

In contrast to *S. longilobus*, no detectable amounts of seneciphylline or senecionine were present in *S. riddellii*, the alkaloid mixture consisting almost entirely of riddelliine (97%) with a small quantity of retrorsine (3%), a result also confirmed by HPLC, (Molyneux, 1978). On the other hand, *S. vulgaris* showed a preponderance of seneciphylline (69%) and senecionine (21%) with only small amounts of retrorsine (7%) and riddelliine (3%). In the case of *S. jacobaea*, the alkaloid mixture consisting of six or more alkaloids gives an NMR spectrum which is too complex to be used for estimation of the individual alkaloids, and the problem is exacerbated by the presence in the alkaloidal extract of methyl 1-hydroxy-4-oxo-2,5-cyclohexadien-1-acetate (**9**) which has been reported in other *Senecio* species (Bohlmann and Suwita, 1976) and is apparently not readily extracted from aqueous acidic solutions of the alkaloids by organic solvents. During the treatment with zinc dust for total alkaloid estimation, however, the cyclohexadiene (**9**) is reduced to methyl (4-hydroxy)phenyl acetate (**10**) which can be removed from the acidic solution by repeated extraction with methylene chloride.

In certain of the NMR spectra of the alkaloidal extracts there are indications of trace amounts of alkaloids other than seneciphylline, senecionine, retrorsine, and riddelliine which are probably stereoisomers of these compounds. In fact, small quantities of spartioidine, a stereoisomer of seneciphylline, and senkirkine have been isolated from an extract of *S. longilobus* (Roitman, 1978). However, in the plant samples so far examined, these minor alkaloids constitute less than 1% of the total alkaloid content.

The availability of the NMR technique for total, and in certain cases, individual alkaloid composition measurement provides a method whereby the variation of content with season, climate, and plant part can be followed so that correlations of such factors with periods of high or low plant toxicity can be developed. Additional experiments are in progress to obtain such data and to expand the method to nonmacrocyclic PAs.

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LITERATURE CITED

Adams, R., Gianturco, M., *J. Am. Chem. Soc.*, **78**, 398 (1956).
Adams, R., Govindachari, T. R., *J. Am. Chem. Soc.* **71**, 1180, 1956 (1949).
Adams, R., Hamlin, K. E., Jelinek, C. F., Phillips, R. F., *J. Am. Chem. Soc.* **64**, 2760 (1942).
Bingley, J. B., *Anal. Chem.* **40**, 1167 (1968).
Bohlmann, F., Suwita, A., *Chem. Ber.* **109**, 2014 (1976).
Bradbury, R. B., Culvenor, C. C. J., *Aust. J. Chem.* **7**, 378 (1954).
Bull, L. B., Culvenor, C. C. J., Dick, A. T., in "The Pyrrolizidine Alkaloids", North-Holland Publishing Co., Amsterdam, 1968.
Culvenor, C. C. J., Heffernan, M. L., Woods, W. G., *Aust. J. Chem.* **18**, 1605 (1965).
Deinzer, M. L., Thompson, P. A., Burgett, D. M., Isaacson, D. L. *Science* **195**, 497 (1977).
Dickinson, J. O., Cooke, M. P., King, R. R., Mohamed, P. A., *J. Am. Vet. Med. Assoc.* **169**, 1192 (1976).
Huxtable, R., Stillman, A. E., Ciaramitaro, D., *Proc. West. Pharmacol. Soc.* **20**, 455 (1977).
Johnson, A. E., *Am. J. Vet. Res.* **37**, 107 (1976).
Mattocks, A. R., *Anal. Chem.* **39**, 443 (1967).
Mattocks, A. R., *Anal. Chem.* **40**, 1749 (1968).
Mattocks, A. R., in "Phytochemical Ecology", Harborne, J. B., Ed., Academic Press, New York, 1972, Chapter 11, pp 179–200.
Molyneux, R. J., unpublished results (1978).
Roitman, J. N., unpublished results (1978).
Segall, H. J., Molyneux, R. J., *Res. Commun. Chem. Pathol. Pharmacol.* **19**, 545 (1978).
Stillman, A. E., Huxtable, R., Consroe, P., Kohnen, P., Smith, S., *Gastroenterology* **73**, 349 (1977).
Tschu Shun, Koluch, J., Santavy, F., *Coll. Czech. Chem. Commun.* **24**, 934 (1960).

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