Gc-ms Determination of Pyrrolizidine Alkaloids in Four Senecio Species

Mark E. Stelljes, Ronald B. Kelley, Russell J. Molyneux, and James N. Seiber

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GC-MS DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN FOUR *SENECIO* SPECIES

MARK E. STELLJES,*

Harding Lawson Associates, 7655 Redwood Blvd., Novato, California 94948

RONALD B. KELLEY,

Facility for Advanced Instrumentation, University of California-Davis, Davis, California 95616

RUSSELL J. MOLYNEUX,

Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California 94710

and JAMES N. SEIBER

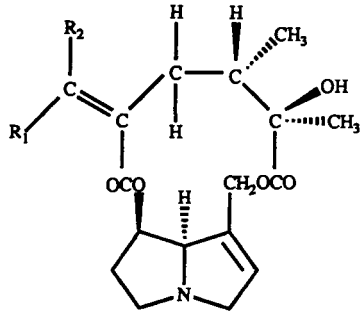
Department of Environmental Toxicology, University of California-Davis, Davis, California 95616

ABSTRACT.—Four *Senecio* species were analyzed by gc-ms for their pyrrolizidine alkaloid content. This is the first published report of pyrrolizidine alkaloids in *Senecio serra, Senecio dimorphophyllus*, and *Senecio hydrophyllus*. Previously undetermined pyrrolizidine alkaloids from *Senecio mikanioides* were also tentatively identified. Gc-ms analyses showed the presence of the stereoisomers senecionine [**9**] and integerrimine [**11**] in *S. dimorphophyllus* and complex mixtures of monoester and diester pyrrolizidine alkaloids in the other three species. Tentative structural identifications were made of ten new pyrrolizidine alkaloids. These new alkaloids are isomers of triangularine, sarracine [**13**], 7-angelylplatynecine [**5**], 7-angelylretronecine [**2a**], and acetylated macronecine-type alkaloids. All three species contained both saturated and unsaturated pyrrolizidine alkaloids, and all had at least ten individual alkaloids. The mass spectral features and gc elution order of these compounds are discussed and the pyrolizidine alkaloid profiles of the plants are compared. The potential toxicity of the three species to range animals is discussed.

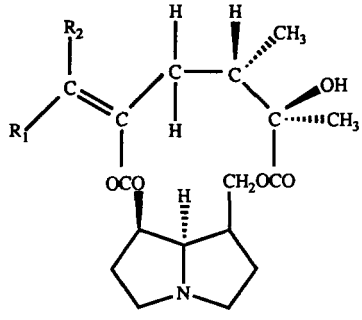
Our previous paper reported the presence of a mixture of saturated and unsaturated pyrrolizidine alkaloids (PAs) in *Senecio mikanioides* Otto. (Asteraceae) (1). Subsequently, a more sensitive gc-ms system using a medium-low polarity DB-17 column was designed to provide tentative identifications of complex mixtures of PAs that are difficult and time-consuming to separate adequately for nmr analysis. Only those compounds that are not adequately identified by gc-ms would then be separated from the mixture for nmr analysis. Both saturated and unsaturated necine bases, monoesters, diesters, and macrocyclics were examined by this system, the results of which are reported elsewhere (2). This method provides a quick characterization of the types of PAs present in a matrix and can provide much information regarding the structural features of these PAs. Others have recently developed gc-ms systems for similar quick characterization uses (3). Re-examination of *S. mikanioides* allowed identification of several PAs not noted with the previous analytical system. The absolute configurations of the PAs discussed herein are based mostly on a combination of comparisons of mass spectra with those in the literature, and known gc behavior of PAs. This methodology is not designed to provide absolute configurations of all detected compounds, but allows tentative identifications without the need for nmr or special gc enantiomeric columns. This new gc-ms system was also applied to three previously uncharacterized species of *Senecio: Senecio serra* Hook., *Senecio hydrophyllus* Nutt., and *Senecio dimorphophyllus* Greene. These were chosen because of their differences in growth forms and habitat requirements compared with *S. mikanioides*, in an effort to determine the spectrum of PAs present in a variety of *Senecio* species. The results show that, in spite of the dramatic differences in natural history, the PA profiles of the plants are remarkably similar.

RESULTS AND DISCUSSION

PA PROFILES.—*S. dimorphophyllus*.—The only PAs found in detectable levels were senecionine [**9**] and integerrimine [**11**] (Figure 1). Senecionine comprised 92% of the total PA content of 0.17% dry wt (Table 1). The mass spectra of these two isomers are indistinguishable; identifications are based on relative gc retention times and comparisons with authentic standards of each compound.



9 R_1=Me, R_2=H
11 R_1=H, R_2=Me



10 R_1=Me, R_2=H
12 R_1=H, R_2=Me

FIGURE 1. Macrocyclic pyrrolizidine alkaloid diesters present in the species discussed in the text.

S. serra.—Ten PAs were found in detectable levels from the recently collected sample (Table 2). The major component, based on comparison with a known standard, was 9-angelylplatynecine [**7**], which comprised just over half (51%) of the total PA content of 0.12% dry wt (Table 1). The dry wt percentage is based on a dry-to-wet-wt ratio of 0.14 as determined for *S. mikanioides*. Only one unsaturated PA was found in the sample: 9-tiglylretronecine [**3b**] was present in low concentration (Table 2, Figure 2). A

TABLE 1. Summary of the Total Pyrrolizidine Alkaloid (PA) Abundance in the Plants Examined in the Current Study.

Species	Dry wt (g)	Total PA (g)	Percent PA (g/g)	Percent PA Unsaturated	Percent PA Saturated
Senecio serra	315	0.39	0.12	3	97
Senecio hydrophyllus	467	0.57	0.12	56	43
Senecio mikanioides	267	0.11	0.04	22	78
Senecio dimorphophyllus	19.5	0.03	0.17	100	0

TABLE 2. Pyrrolizidine Alkaloid (PA) Profile for *Senecio serra* as Determined by Gc-ms.

Compound	Gc Retention Time (Min.)	Percent of Total PA
Anhydroplatynecine [**1**]	8.65	14
9-Tiglylretronecine [**3b**]	23.25	3
Unknown C [**21**]	23.95	3
7-Angelylplatynecine [**5**]	24.45	5
9-Angelylplatynecine [**7**]	25.81	51
7-Tiglylplatynecine [**6**]	26.40	4
Unknown D [**22**]	26.99	2
9-Tiglylplatynecine [**8**]	27.97	14
Sarranicine [**14**]	49.19	2
Neosarranicine [**16**]	50.66	2

small amount of 7-tiglylretronecine [**2b**] was also apparently present based on the mass spectrum, but verification was not possible because of coelution of **2b** and **3b**. Identifications of both **2b** and **3b** are based on ion fragmentation patterns obtained from standards from this laboratory as well as comparison with literature spectra (4). The presence of anhydroplatynecine [**1**] is considered to be largely an artifact from heating during Soxhlet extraction or passage through the gc injector port. This compound has been formed during acidification and steam distillation of sarracine from *S. serra* (5). Table 3 lists the base and molecular ions of all PAs found in *S. serra*.

Seven of the ten tentatively identified compounds were monoesters, including all four possible isomers of 7- and 9-angelate and tiglate esters of platynecine. Based on relative retention times, no equivalent senecioyl esters were found in the sample. The angelate isomers were at least as abundant as the tiglate isomers (Table 2). Evidence of a third saturated base esterified at the 7 position with angelic acid that eluted after the other two was also present (Unknown C, Table 3). One PA with an ms fragmentation pattern consistent with a macronecine-type base structure (Table 3) was identified in the sample (Unknown D).

Two diesters were present in the sample, both in low concentrations. These were both determined to be isomeric with sarracine but, based on comparative gc retention times with an authentic standard of sarracine, were assigned tentative structures **14** and **16** (Figure 3). These new compounds are given the trivial names sarranicine and neosarranicine, respectively.

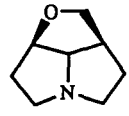
The sample extracted and analyzed in 1979 showed a pattern simpler than the one described above. Only three PAs were positively identified: 7-angelylplatynecine [**5**], 7-tiglylplatynecine [**6**], and sarracine [**13**]. Five other gc peaks were not identified.

S. hydrophyllus.—Twenty-two PAs were found in detectable levels as shown in Table 4. The major component was tentatively identified as 7-senecioylretronecine [**4**], which comprised 35% of the total PA content of 0.12% dry wt (Table 1). 7-Senecioylretronecine has been reported in the literature to be present in *S. triangularis* (6). Two other compounds were also present in large concentrations, 7-tiglylretronecine [**2b**] and 9-angelylplatynecine [**7**]. These components accounted for 13% and 16% of the total PA content, respectively (Table 4). The presence of anhydroplatynecine [**1**] is again considered to be an artifact from heating and isolation of the PAs from the plant (5). Eleven monoesters, four macrocyclics, and six diesters were found in the sample. Both saturated and unsaturated compounds were present in the sample in roughly equal amounts (Table 1). Table 3 lists the base and molecular ions of all PAs found in *S. hydrophyllus*.

TABLE 3. Summary of the Chromatographic Data for the Pyrrolizidine Alkaloids Present in the Species Discussed in the Text Tentatively Identified by Gc-ms.

Compound	Gc Retention Time	Molecular Ion $[M]^+$	Base Ion	Necine Base	Acid Moiety At:			Species Found In[a]
					C-2	C-7	C-9	
Anhydroplatynecine [1]	8.67	139	82	platynecine	H	—	—	1,2
Unknown A [19]	11.45	199	83	macronecine	OH	H	Acetyl	2
Unknown B [20]	12.46	199	83	macronecine	Acetyl	H	H	2
7-Angelylretronecine [2a]	21.63	237	80	retronecine	H	Angelyl	H	2
9-Angelylretronecine [3a]	21.84	237	93	retronecine	H	H	Angelyl	2
7-Tiglylretronecine [2b]	23.24	237	80	retronecine	H	Angelyl	H	2,3
9-Tiglylretronecine [3b]	23.30	237	93	retronecine	H	H	Tiglyl	1,2,3
Unknown C [21]	23.95	239	82	hastanecine	H	H	Angelyl	1
7-Angelylplatynecine [5]	24.37	239	82	platynecine	H	Angelyl	H	1,2,3
7-Senecioylretronecine [4]	25.49	237	80	retronecine	H	Senecioyl	H	2
9-Angelylplatynecine [7]	25.71	239	82	platynecine	H	H	Angelyl	1,2,3
7-Tiglylplatynecine [6]	26.16	239	82	platynecine	H	Tiglyl	H	1,2,3
Unknown D [22]	26.99	239	83	macronecine	OH	H	Tiglyl	1
9-Tiglylplatynecine [8]	27.68	239	95	platynecine	H	H	Tiglyl	1,2,3
Senecionine [9]	43.60	335	136	retronecine	H	—	—	2,4
Platyphylline [10]	45.56	337	82	platynecine	H	—	—	2
Integerrimine [11]	46.71	335	93	retronecine	H	—	—	2,4
Neoplatyphylline [12]	47.22	337	82	platynecine	H	—	—	2
Sarracine [13]	48.59	337	138	platynecine	H	Angelyl	t-Sarracinyl	2,3
Sarranicine [14]	49.25	337	138	platynecine	H	Tiglyl	t-Sarracinyl	1,2,3
Triangularicine [17]	49.68	335	83	retronecine	H	Tiglyl	t-Sarracinyl	2,3
Neosarracine [15]	50.05	337	138	platynecine	H	Angelyl	c-Sarracinyl	2,3
Neotriangularicine [18]	50.39	335	83	retronecine	H	Tiglyl	c-Sarracinyl	2
Neosarranicine [16]	50.68	337	138	platynecine	H	Tiglyl	c-Sarracinyl	1,2,3

[a]1 = *Senecio serra*; 2 = *Senecio hydrophyllus*; 3 = *Senecio mikanioides*; 4 = *Senecio dimorphophyllus*.



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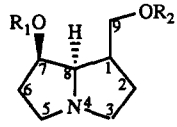
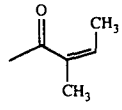
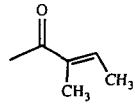
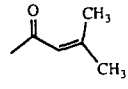
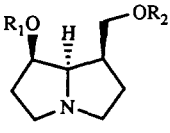


2a R_1=  , R_2=H

2b R_1=  , R_2=H

3a R_1=H, R_2=Angelyl
3b R_1=H, R_2=Tiglyl

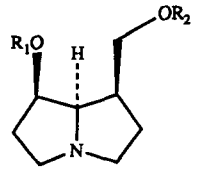
4 R_1=  , R_2=H

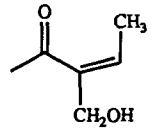


5 R_1=Angelyl, R_2=H
6 R_1=Tiglyl, R_2=H
7 R_1=H, R_2=Angelyl
8 R_1=H, R_2=Tiglyl

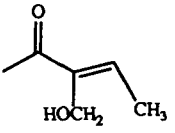
FIGURE 2. Structures of pyrrolizidine alkaloid monoesters tentatively identified in the species discussed in the text.

The monoesters determined from the gc-ms analysis show the presence of three different necine bases: platynecine, retronecine, and macronecine-type. Macronecine-type PAs are rarely encountered necine bases with a very characteristic ms fragmentation pattern much different from that of the isomeric platynecine (Table 3). Two acetylated derivatives of macronecine were postulated to be present: one acetylated on the 9 position (**19**) and one on the 2 position (**20**) (Figure 4). The relative retention times of these compounds were determined based on previous studies in this laboratory regarding gc behavior of PAs (2). All four possible isomers of 7- and 9-angelate and tiglate esters of platynecine and retronecine were found in the sample (Figure 2). Current assignments are based on comparison with previous studies in this laboratory (2). Interestingly, while the platynecine-based isomers all separated on this system, the 7- and 9-isomers of retronecine apparently coeluted to a large extent, based on the mass spectra obtained.

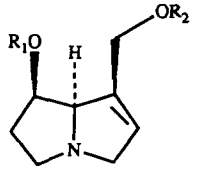


13 R_1=Angelyl, R_2= 

14 R_1=Tiglyl, R_2=t-Sarracinyl

15 R_1=Angelyl, R_2= 

16 R_1=Tiglyl, R_2=c-Sarracinyl



17 R_1=Tiglyl, R_2=t-Sarracinyl

18 R_1=Tiglyl, R_2=c-Sarracinyl

FIGURE 3. Structures of the pyrrolizidine alkaloid diesters tentatively identified in the species discussed in the text. Positions on C-7 esters are, by convention, designated "double prime" (i.e., 1″). Positions on C-9 esters are designated "prime" (i.e., 1′).

7-Senecioylretronecine [**4**] did not coelute with the other 7-isomers of retronecine, but was significantly delayed on gc.

Four macrocyclics, constituting two isomeric pairs, were found in the sample. These were all present in relatively low amounts, although senecionine [**9**] and platyphylline [**10**] were more abundant than most of the diesters present. The two pairs of isomers differed only in the degree of saturation of the necine base; senecionine [**9**] and integerrimine [**11**] are based on retronecine while platyphylline [**10**] and neoplatyphylline [**12**] are based on platynecine (Figure 1).

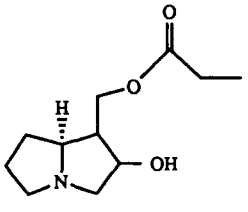
Six diesters were found in the sample, four saturated isomers and two unsaturated isomers. The saturated compounds were sarracine [**13**] and its three isomers, the latter three of which are new compounds. These have been tentatively assigned the trivial names sarranicine [**14**], neosarracine [**15**], and neosarranicine [**16**] (Figure 3). Although these four compounds have the same molecular weight as platyphylline [**10**] and neoplatyphylline [**12**], the ms fragmentation patterns are significantly different.

TABLE 4. Pyrrolizidine Alkaloid (PA) Profile for *Senecio hydrophyllus* as Determined by Gc-ms.

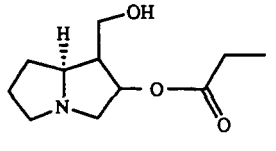
Compound	Gc Retention Time (min)	Percent of Total PA
Anhydroplatynecine [**1**]	8.61	4
Unknown A [**19**]	11.45	1
Unknown B [**20**]	12.46	1
7-Angelylretronecine [**2a**]	21.63	2
9-Angelylretronecine [**3a**]	21.84	1
7-Tiglylretronecine [**2b**]	23.24	13[a]
9-Tiglylretronecine [**3b**]	23.30	13[a]
7-Angelylplatynecine [**5**]	24.45	9
7-Senecioylretronecine [**4**]	25.48	35
9-Angelylplatynecine [**7**]	25.62	16
7-Tiglylplatynecine [**6**]	26.07	2
9-Tiglylplatynecine [**8**]	27.49	2
Senecionine [**9**]	43.60	4
Platyphylline [**10**]	45.56	2
Integerrimine [**11**]	46.71	0.4
Neoplatyphylline [**12**]	47.22	0.4
Sarracine [**13**]	48.55	1
Sarranicine [**14**]	49.21	3
Triangularicine [**17**]	49.59	0.4
Neosarracine [**15**]	50.04	0.2
Neotriangularicine [**18**]	50.39	0.8
Neosarranicine [**16**]	50.63	0.8

[a]Compounds coeluted. This number refers to the sum of both compounds.

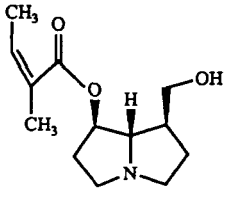
The two unsaturated diesters are also new compounds and appear to be the pair of isomers complementary to triangularine and neotriangularine. This is a situation similar to that for sarracine and neosarracine; thus the two new structures have been assigned the names triangularicine [**17**] and neotriangularicine [**18**]. These compounds were tentatively identified based on comparison with authentic standards of triangularine and neotriangularine.



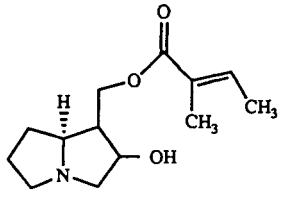
9-Acetylmacronecine **19**



2-Acetylmacronecine **20**



7-Angelylhastanecine **21**



9-Tiglylmacronecine **22**

FIGURE 4. Putative structures of the uncharacterized pyrrolizidine alkaloids present in the species discussed in the text.

S. mikanioides.—Eleven PAs were found in detectable levels as shown in Table 5. The major component was 9-angelylplatynecine [**7**], which comprised 39% of the total PA content of 0.04% dry wt (Table 1). 9-Tiglylretronecine [**3b**] was also present in large amounts but was mixed with lesser amounts of 7-tiglylretronecine [**2b**]. Together, these accounted for 21% of the total PA content (Table 5). The presence of anhydroplatynecine [**1**] is again considered to be an artifact from heating and isolation of the PAs from the plant (5). Five monoesters and five diesters were found in the sample. Both saturated and unsaturated PAs were present, but saturated PAs accounted for roughly 75% of the total alkaloid (Table 1).

All four possible isomers of 7- and 9-angelate and tiglate esters of platynecine were found in the sample (Figure 2), with the angelate isomers at least as abundant as the tiglate ones (Table 5). Only one pair of unsaturated monoesters was found; the angelate esters of retronecine were not detected in the sample.

TABLE 5. Pyrrolizidine Alkaloid (PA) Profile for *Senecio mikanioides* as Determined by Gc-ms.

Compound	Gc Retention Time (min)	Percent of Total PA
Anhydroplatynecine [**1**]	8.79	6
9-Tiglylretronecine [**3b**]	23.26	22
7-Angelylplatynecine [**5**]	24.21	4
9-Angelylplatynecine [**7**]	25.70	39
7-Tiglylplatynecine [**6**]	26.00	4
9-Tiglylplatynecine [**8**]	27.57	8
Sarracine [**13**]	48.62	5
Sarranicine [**14**]	49.35	7
Triangularicine [**17**]	49.77	1
Neosarracine [**15**]	50.05	2
Neosarranicine [**16**]	50.74	4

Four of the five diesters comprise the sarracine isomer group (structures **13–16**). The other was the only unsaturated diester, triangularicine [**17**] (Figure 3).

COMPARATIVE TOXICOLOGICAL IMPLICATIONS.—Many similarities are evident among the various species of *Senecio* examined in the present study. All of the species except *S. dimorphophyllus* contained 9-tiglylretronecine [**3b**], all four possible isomers of 7- and 9-angelate and tiglate esters of platynecine (**4–7**), and two isomers of sarracine (**14** and **16**) (Table 3). This is surprising because few investigations to date have shown the presence of both unsaturated and saturated PAs in the same species (7). Both *S. hydrophyllus* and *S. mikanioides* contained sarracine and triangularine isomers (**13–16**, **17**). The occurrence of saturated and unsaturated diesters differing only in the structure of the necine base has been previously reported only in *S. triangularis* (8). It is especially surprising that these two species should contain such similar PA profiles based on differences in their evolutionary history. *S. mikanioides* evolved as an ivy (hence its common name of German ivy) in areas near the coast of southern Africa, while *S. hydrophyllus* grows as an erect perennial herb in montane areas at high elevations in western North America. The similarity of PAs between these two species suggests that sections of the genus might have a basic template for PA synthesis incorporating both platynecine- and retronecine-based PAs. Recent work on the biosynthesis of PAs with other *Senecio* species has demonstrated a common pathway for the formation of retronecine- and rosmarinine-based PA esters (9). Additional work is needed to show how platynecine and other necine-based esters are incorporated into this biosynthetic scheme. The differences that are found in the PA profiles of various closely related

species of *Senecio* may be due in part to local extrinsic factors, such as soils and weather, as well as the evolutionary development of the particular species. The similarities of PA profiles among various species may serve as an index of systematic affinity and biosynthetic consistency.

S. hydrophyllus and *S. serra* have essentially equal proportions of PAs present in terms of percent dry weight, yet vary considerably in the proportion of saturated to unsaturated compounds (Table 1). This results in *S. hydrophyllus* presenting much more of a toxicity threat to animals than does *S. serra*. This emphasizes the importance of identifying individual PAs in plants, rather than just determining a total alkaloid percent. Although *S. hydrophyllus* and *S. mikanioides* have similar PA profiles, they differ in total PA content. *S. hydrophyllus* contains a larger percentage of PAs than does *S. mikanioides* (Table 1). These comparisons demonstrate the variability of PA content and toxicity potential characteristic of the genus and point out the importance of characterizing each species completely to understand its taxonomy, chemistry, and potential toxicity. Stage of growth, maturity, and regional differences within a species will no doubt be found, but a combination of total PA content and structural identification of individual PAs allows comparison of various species to be made.

The alkaloidal composition of *S. dimorphophyllus* is similar to that seen in *Senecio alpinus* (10), although seven additional macrocyclic PAs were found in the latter species. This plant contained both senecionine [**9**] and integerrimine [**11**] in roughly equal amounts and was shown to be responsible for pyrrolizidine alkaloidosis in livestock. Mass spectra of these compounds are consistent with those reported in the literature (10,11). The total PA content of *S. alpinus* was roughly twice that seen for *S. dimorphophyllus*. This, coupled with the widely dispersed growth pattern typical of *S. dimorphophyllus* and its low prevalence in dry subalpine habitats where livestock graze, should result in little livestock toxicity due to *S. dimorphophyllus*.

MASS SPECTRAL AND GAS CHROMATOGRAPHIC BEHAVIOR OF PAs.—The PA profile for *S. serra* (Table 2) showed the presence of ten PAs potentially with four different necine bases. *Senecio doronicum* has been shown to contain PAs with three necine bases (platynecine, retronecine, and macronecine) (12). The additional necine base tentatively identified to be present in *S. serra* was hastanecine, which only differs from platynecine in the orientation of the hydrogen at C-8. The mass spectrum of unknown C [**21**] was identical to that of 7-angelylplatynecine [**5**], but the former eluted before the latter. Based on previous studies in this laboratory (2), the gc retention time of the necine bases increases with symmetry of orientation at C-7, C-8, and C-9. For example, heliotridine, which has the same orientation at both C-7 and C-8, elutes after retronecine, which has opposite orientations at C-7 and C-8. This suggested a more symmetric orientation of the necine base for unknown C, so the compound was tentatively identified as 7-angelylhastanecine [**21**].

The 22 PAs in *S. hydrophyllus* contained the same three necine bases as the thirteen PAs found in *S. doronicum* (12). This species was also found to contain saturated monoesters as well as saturated and unsaturated diester congeners with molecular ions of *m/z* 335 and 337. The authors did not identify these diesters, but suggested that the mass spectra were characteristic of PAs with platynecine and retronecine. A similar range of eleven PAs was found in *Senecio fuchsii*, and the authors identified platyphylline and three unknown diesters with a molecular ion of *m/z* 337 with spectra consistent with sarracine (12). Both of these species apparently contained platyphylline and sarracine isomers plus triangularine or similar PAs. The discovery of these compounds in *S. hydrophyllus* is therefore consistent with other *Senecio* species. The current study is the first report of a species in the United States demonstrated to contain such a wide range of PAs, and contains more PAs than any other reported species.

The presence of sarracine and newly identified isomers in *S. hydrophyllus* as well as in *S. serra* and *S. mikanioides* was established based on comparisons with a known standard of sarracine and ms fragmentation patterns. The mass spectra were consistent with that of sarracine determined previously by this laboratory (1,2) as well as elsewhere (13–15). The three isomers eluted after sarracine, establishing their configurations as **14**, **15**, and **16** based on the known chromatographic behavior of tiglate and angelate esters on gc (2).

Examination of the gc elution order of the diesters found in *S. hydrophyllus* (Table 4) reveals some patterns that govern their behavior on gc. The macrocyclic compounds **9–12** elute before the isomeric open-chain diesters **13–18**. Among the macrocyclic compounds, the ones with a methyl group cis to the carbonyl (senecionine and platyphylline) elute before isomers with a methyl group trans to the carbonyl (integerrimine and neoplatyphylline). This difference is more pronounced for the retronecine-based isomers than for the platynecine-based isomers. Also, saturated compounds are retained on gc longer than the unsaturated congeners.

A similar situation exists with the open-chain diesters. Sarracine [**13**], with both methyl groups cis to the carbonyls, elutes first, whereas neosarranicine [**16**], with both methyl groups trans to the carbonyls, elutes last. Sarranicine [**14**], which contains a methyl group trans to the carbonyl at 1″, elutes before neosarracine [**15**], which contains a methyl group trans to the carbonyl at 1′ (Figure 3). The effect of this configuration on the retention time of these compounds is fairly predictable and regular (Table 3). Moving the methyl group at 2″ from a cis to a trans position (**13** to **14**) retards the compound by 0.66 min. The situation is almost identical for the 2′ position (**15** to **16**), resulting in a 0.63 min difference. The unsaturated pair differed by 0.71 min (**17** and **18**), which again illustrates that these effects are more pronounced for unsaturated bases than for saturated bases.

The effect is more pronounced, but still proportional, when the shift involves the hydroxylated methyl group. Neosarracine [**15**], which has a hydroxymethyl group trans to the carbonyl at 1′, is delayed 1.46 min over sarracine [**13**], which has its hydroxymethyl group cis to the 1′ carbonyl. For the 1″ congeners, the difference is 1.43 min (**14** to **16**). Much information can be obtained concerning the probable structures of these compounds by examining the gc elution patterns in this manner. Senecioate esters were discounted based on these considerations since they should have been retarded on gc longer than these results indicate.

The presence of all four possible isomers of 7- and 9-angelate and tiglate esters of platynecine (**5–8**) in the same plant has not yet been demonstrated in the literature. The current study has shown the presence of all four isomers in *S. serra, S. hydrophyllus,* and *S. mikanioides*. The gc elution pattern of these isomers is analogous to that for the sarracine isomers. The tiglate esters are delayed on gc relative to the angelate esters, and the C-9 esters are delayed over the C-7 isomers. This is due to the trans configuration of the carbonyl and methyl groups on the tiglate esters versus the cis configuration on the angelate esters. This difference results in a larger retention time effect than that for the diesters; a shift from angelate to tiglate retards the compound by 1.79 min for the C-7 esters and 1.97 min for the C-9 esters (Table 3). The C-7 esters elute about 1.5 min earlier than the corresponding C-9 isomers. No senecioyl esters were found in these plant samples based on these considerations.

It was reported (13) that 7- and 9-angelylplatynecine have identical mass spectra, but this is clearly not the case (Figure 5). The major diagnostic ion is *m/z* 95. This ion rivals the abundance of the base peak for 9-angelyl derivatives but is almost absent from the spectra of 7-angelyl compounds. Several other differences in their mass spectra are apparent from Figure 5, including the abundant ions at *m/z* 139 and 156 for 7-angelyl

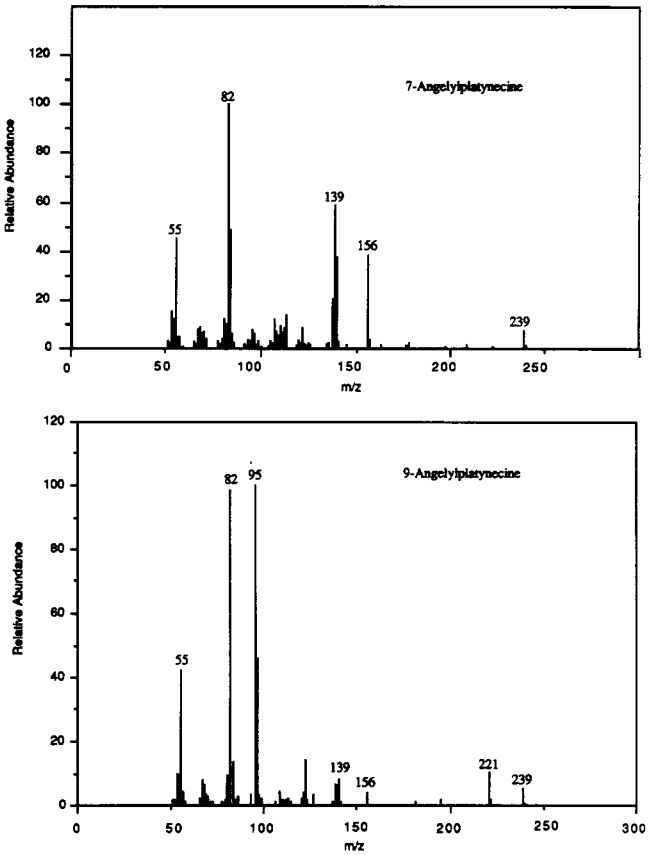


FIGURE 5. Ei mass spectra of 7- and 9-angelylplatynecine.

derivatives. The formation of the *m/z* 95 ion for 9-angelyl esters has been described (16). Spontaneous isomerization from angelyl to tiglyl esters occurs for these compounds, as demonstrated by comparing a sample extract of *S. serra* analyzed over time. When first analyzed in 1978, only traces of 7-tiglylplatynecine [6] were seen. Reanalysis of the same extract kept frozen until 1989 showed almost equal amounts of both (Table 2). Angelyl and tiglyl esters both exist naturally in numerous PA-containing species, but the relative amounts of tiglyl and angelyl isomers of a PA ester can be affected by storage, sunlight, and other factors. It is not known to what extent the relative amounts of tiglyl and angelyl isomers are artifactual in these *Senecio* species, but both appear to exist naturally in *S. serra*, as well as in *S. hydrophyllus* and *S. mikanioides*.

7-Senecioylretronecine is the most abundant PA found in *S. hydrophyllus* (Table 4). This compound has been reported from *S. triangularis* (6). 7- and 9-Angelyl and tiglyl esters of retronecine were also present in *S. hydrophyllus*, although the 7- and 9- isomers coeluted. The reported mass spectra of these compounds are significantly different. 7-Angelylretronecine has a base ion of *m/z* 80 and an *m/z* 93 ion of no more than 25 percent of this base ion (17). The *m/z* 94 ion is larger than the ion at *m/z* 93. Other significant diagnostic ions are at *m/z* 111 and 106. 9-Angelylretronecine has a base ion of *m/z* 93 with a small *m/z* 80 ion. Ions at *m/z* 111 and 106 are very weak if present. Another diagnostic ion is present at *m/z* 154 (17). These have been supported by fragmentation patterns from standards in our own laboratory. Examination of the mass spectra obtained in this study show a mixture of these peaks. Identifications and relative abundances were based on a comparison of the relative heights of the *m/z* 80 and 93 ions.

The elution order still followed the trend of the saturated congeners with the angelate esters preceding the tiglate esters by 1.5 min. The senecioyl ester was retained for an additional 2.2 min over the 9-tiglate isomers. This relationship allows one to make conclusions with a high degree of confidence as to the presence or absence of these isomers in mixtures.

Unknown D [**22**], which has mass spectral features consistent with a 2,9-OH saturated angelyl macronecine-type PA (Table 3), was identified in *S. serra* based on comparison with literature mass spectral fragmentation patterns of macrophylline (12,18) and an isomer (19). Macrophylline is 9-angelylmacronecine, which should have a mass spectrum identical to that of 9-tiglylmacronecine. Macrophylline, identified only in *S. macrophyllus* from the Soviet Union (7), and petasinine, or 2-angelylmacronecine, identified from *Petasites japonicus* (20), have fragmentation patterns consistent with **22**. The tentative structure **22** shown in Figure 4 is based on the relative abundance of tiglyl esters in *S. hydrophyllus* as compared with angelate esters. Without nmr analysis the actual configuration of the ester cannot be determined.

Two additional macronecine-type PAs (**19**, **20**) are present in *S. hydrophyllus*. No comparable spectra could be found in the literature, but the molecular ion of *m/z* 199 and the ion at *m/z* 156 $[M-43]^+$ are consistent with the tentative structures of acetyl macronecine-type isomers. Very few fragment ions are evident, which indicates that the compounds are quite stable. It cannot be determined what stereo-orientation is present based on existing information. Further analysis is required for structural identification of these compounds.

Spontaneous isomerization of sarracine from angelate to tiglate forms was demonstrated when a sample of *S. serra* was chromatographed over time. When first analyzed in 1978, the only apparent diester present was sarracine. Reanalysis of the same extract kept frozen until 1989 showed the absence of sarracine but the presence of sarranicine [**14**] and neosarranicine [**16**]. This was also observed in *S. mikanioides* over the period of approximately one year. An earlier paper (1) reported the presence of sarracine [**13**] in this plant, but over the course of several months the amount of sarracine declined and the amounts of the three isomers increased. The percent of total PA reported for these isomers represents the apparently stable outcome of these isomerizations. The spontaneous conversion of angelate to tiglate forms in sarracine is apparently much more rapid than that for the comparative monoesters.

The presence of both platyphylline and triangularine isomers in *S. hydrophyllus* has also been reported in other species (8,12). These were present with sarracine as well in at least one other species (12). Platyphylline and neoplatyphylline were identified based on mass spectral fragmentation patterns consistent with reported literature (8,15,21). New triangularine isomers were tentatively identified based on comparative elution order with co-chromatographed standards of triangularine and neotriangularine, as well as ms fragmentation patterns identical to those standards. The situation is similar to that for sarracine and its isomers. The only difference in the structures is that the sarracine isomers are based on platynecine and the triangularine isomers are based on retronecine. It should not be surprising that all four possible isomers of triangularine exist in plants. The trivial names assigned to structures **17** and **18** maintain consistency with equivalent isomers of the sarracine group.

Analysis of *S. mikanioides* demonstrated the presence of eleven PAs while an earlier study (1) showed only four PAs plus unknowns to be present. This report updates the previous study and provides tentative identification of the unknown PAs. These include the monoesters **6**, **7**, and **8** and the diesters **14–17**. The increased sensitivity of the current analytical system allowed for tentative identification of these minor alkaloids in this species. This is the first report of the presence of these compounds in *S. mikanioides*.

EXPERIMENTAL

GENERAL CHROMATOGRAPHY PROCEDURES.—All chromatography was by glc using an HP5890A gas chromatograph equipped with a model 5970 mass selective detector (MSD). The chromatographic system consisted of a 20 m DB-17 0.18 mm i.d. capillary column with 0.3 μm film thickness. A one-meter uncoated retention gap was inserted in front of the analytical column and an eight-meter uncoated retention gap was inserted behind the column. Helium was the carrier gas at 1.0 ml/min, and the oven temperature was programmed from 50° to 184° at 15°/min, then from 184° to 270° at 2°/min, with a 13 minute final hold. Ei mass spectra were obtained using the mass selective detector at 70 eV energy. Quantification was based on total ion current and assumed that all PAs ionize equally.

ISOLATION PROCEDURES.—*S. serra* (2.25 kg wet wt) was collected in Cache County, Utah four miles off US 89 on the road to Tony Grove Resort (elevation about 2500 m) on July 27, 1989, in a moist subalpine meadow and identified by R.B. Kelley. A voucher specimen (collection number 286) is in the University of California–Davis Department of Botany herbarium. Plant material was stored frozen until extraction. The plants were ground in a Waring blender and Soxhlet-extracted with MeOH for 72 h. The MeOH extract was acidified with 0.5 N H_2SO_4 to pH 3, and the MeOH was evaporated under reduced pressure. Zinc dust was added to the acidic aqueous solution to convert all *N*-oxides to the parent alkaloids, and the extract was allowed to stand overnight at 4°. The mixture was filtered, made basic to pH 9 with 6 N NH_4OH, and extracted with 7 × 50 ml of $CHCl_3$ following saturation of the aqueous layer with NaCl. The resulting organic extract was concentrated under reduced pressure and transferred to a vial with EtOAc, and the volume was adjusted for analysis. A more complete extraction scheme is published elsewhere (1).

An additional sample (4.09 kg dry wt) was collected nearby in 1978 by one of us (RM) and analyzed in July of that year. The extraction scheme for this sample is described elsewhere (22).

S. dimorphophyllus (19.5 g dry wt) was provided by F.R. Stermitz, Colorado State University. This sample was collected on July 8, 1976 by T.R. Suess in Larimer County, Colorado near highway 14 east of Cameron Pass in an open area with sandy soil and identified by D.H. Wilken, Department of Biology, Colorado State University. The sample was stored dry until extraction. A voucher specimen is in the Colorado State University herbarium. The extraction procedure was identical to that for *S. serra* described above.

S. hydrophyllus (467 g dry wt) was provided by F.R. Stermitz. This sample was collected on July 28, 1977 by T.R. Suess in Jackson County, Colorado near highway 14 ten miles north of Gould in a wet ditch and identified by D.H. Wilken. The sample was stored dry until extraction. A voucher specimen is in the Colorado State University herbarium. The extraction procedure was identical to that for *S. serra* described above.

S. mikanioides (1.915 kg wet wt) was collected, non-flowering, at Natural Bridges State Park in Santa Cruz, California on June 5, 1989, on a slope at elevation 15 m within 0.25 km of the Pacific Ocean and identified by R.B. Kelley. A voucher specimen is in the University of California–Davis Department of Botany herbarium. This sample was frozen for 1 week, oven dried (266.65 g dry wt), and extracted as above.

DATA FOR INDIVIDUAL ALKALOIDS.—*Anhydroplatynecine* [**1**].—Gc Rt = 8.67 min; eims *m/z* (rel. int.) $[M]^+$ 139 (29), 138 (24), 122 (4), 120 (5), 110 (9), 96 (15), 95 (9), 82 (100), 55 (21).

7-Angelylretronecine [**2a**].—Gc Rt = 21.63 min; eims *m/z* (rel. int.) $[M]^+$ 237 (8), 154 (5), 138 (48), 137 (39), 136 (21), 124 (13), 111 (34), 106 (37), 94 (59), 93 (99), 80 (100), 55 (40).

9-Angelylretronecine [**3a**].—Gc Rt = 21.84 min; eims *m/z* (rel. int.) $[M]^+$ 237 (2), 193 (3), 154 (3), 138 (49), 137 (36), 136 (15), 124 (9), 111 (10), 108 (6), 106 (10), 94 (46), 93 (100), 80 (44), 55 (27).

7-Tiglylretronecine [**2b**].—Gc Rt = 23.24 min; eims *m/z* (rel. int.) $[M]^+$ 237 (3), 191 (2), 154 (9), 138 (20), 137 (32), 136 (23), 124 (17), 111 (23), 108 (7), 106 (34), 94 (44), 93 (61), 80 (100), 55 (36).

9-Tiglylretronecine [**3b**].—Gc Rt = 23.30 min; eims *m/z* (rel. int.) $[M]^+$ 237 (2), 193 (5), 154 (17), 138 (37), 137 (36), 136 (20), 124 (10), 111 (9), 108 (9), 106 (15), 94 (43), 93 (100), 80 (49), 55 (38).

7-Angelylplatynecine [**5**].—Gc Rt = 24.37 min; eims *m/z* (rel. int.) $[M]^+$ 239 (7), 221 (1), 156 (38), 140 (37), 139 (59), 138 (20), 122 (8), 114 (12), 96 (6), 95 (7), 83 (48), 82 (100), 55 (45).

7-Senecioylretronecine [**4**].—Gc Rt = 25.47 min; eims *m/z* (rel. int.) $[M]^+$ 237 (5), 193 (2), 154 (11), 138 (13), 137 (36), 136 (21), 124 (19), 111 (24), 108 (7), 106 (36), 94 (39), 93 (65), 80 (100), 55 (31).

9-Angelylplatynecine [**7**].—Gc Rt = 25.71 min; eims *m/z* (rel. int.) $[M]^+$ 239 (6), 221 (11), 156 (2), 140 (9), 139 (4), 138 (5), 122 (14), 96 (46), 95 (99), 83 (13), 82 (100), 55 (40).

7-Tiglylplatynecine [**6**].—Gc Rt = 26.16 min; eims *m/z* (rel. int.) $[M]^+$ 239 (1), 221 (1), 156 (27), 140 (6), 139 (53), 138 (22), 122 (5), 114 (11), 96 (8), 95 (12), 83 (20), 82 (100), 55 (29).

9-Tiglylplatynecine [**8**].—Gc Rt = 27.68 min; eims *m/z* (rel. int.) $[M]^+$ 239 (0.2), 221 (15), 156 (1), 140 (5), 139 (3), 138 (4), 122 (6), 96 (14), 95 (100), 83 (11), 82 (74), 55 (30).

Senecionine [**9**].—Gc Rt = 43.60 min; eims *m/z* (rel. int.) $[M]^+$ 335 (12), 291 (3), 246 (18), 220 (38), 139 (11), 138 (41), 137 (24), 136 (100), 120 (92), 119 (67), 109 (24), 108 (17), 106 (16), 95 (41), 94 (76), 93 (72), 81 (21), 80 (43).

Platyphylline [**10**].—Gc Rt = 45.56 min; eims *m/z* (rel. int.) $[M]^+$ 337 (5), 226 (7), 222 (4), 220 (4), 211 (42), 180 (6), 156 (4), 140 (92), 139 (14), 138 (73), 124 (8), 123 (44), 122 (77), 121 (9), 120 (11), 108 (15), 96 (36), 95 (17), 94 (8), 83 (14), 82 (100), 81 (16), 80 (15).

Integerrimine [**11**].—Gc Rt = 46.71 min; eims *m/z* (rel. int.) $[M]^+$ 335 (5), 291 (13), 248 (14), 220 (23), 139 (10), 138 (46), 137 (24), 136 (99), 120 (100), 119 (90), 109 (25), 108 (18), 106 (19), 95 (58), 94 (78), 93 (85), 81 (19), 80 (42).

Neoplatyphylline [**12**].—Gc Rt = 47.22 min; eims *m/z* (rel. int.) $[M]^+$ 337 (1), 226 (5), 222 (6), 220 (5), 211 (22), 180 (10), 156 (5), 140 (68), 139 (14), 138 (62), 124 (8), 123 (38), 122 (66), 121 (15), 120 (19), 108 (17), 96 (32), 95 (25), 94 (14), 83 (23), 82 (100), 81 (16), 80 (22).

Sarracine [**13**].—Gc Rt = 48.59 min; eims *m/z* (rel. int.) $[M]^+$ 337 (0), 237 (20), 222 (17), 139 (35), 138 (100), 123 (29), 122 (54), 121 (15), 97 (9), 96 (37), 95 (36), 83 (38), 82 (65), 55 (57).

Sarranicine [**14**].—Gc Rt = 49.25 min; eims *m/z* (rel. int.) $[M]^+$ 337 (0.4), 237 (16), 222 (12), 139 (30), 138 (100), 123 (27), 122 (41), 121 (16), 97 (6), 96 (29), 95 (43), 83 (26), 82 (58), 55 (46).

Triangularicine [**17**].—Gc Rt = 49.68 min; eims *m/z* (rel. int.) $[M]^+$ 335 (0), 237 (43), 236 (7), 235 (5), 220 (21), 219 (11), 141 (14), 137 (16), 136 (66), 121 (33), 120 (46), 119 (37), 118 (14), 106 (15), 95 (43), 94 (64), 93 (68), 83 (100), 81 (23), 80 (35).

Neosarracine [**15**].—Gc Rt = 50.05 min; eims *m/z* (rel. int.) $[M]^+$ 337 (0), 237 (18), 222 (18), 139 (38), 138 (100), 123 (34), 122 (62), 121 (17), 97 (9), 96 (42), 95 (45), 83 (37), 82 (78), 55 (54).

Neotriangularicine [**18**].—Gc Rt = 50.39 min; eims *m/z* (rel. int.) $[M]^+$ 335 (6), 237 (58), 236 (9), 235 (7), 220 (24), 219 (14), 141 (11), 137 (16), 136 (96), 121 (27), 120 (43), 119 (46), 118 (15), 106 (12), 95 (21), 94 (79), 93 (90), 83 (100), 81 (17), 80 (27).

Neosarranicine [**16**].—Gc Rt = 50.68 min; eims *m/z* (rel. int.) $[M]^+$ 337 (0), 237 (19), 222 (17), 139 (38), 138 (100), 123 (36), 122 (53), 121 (21), 97 (6), 96 (36), 95 (56), 83 (34), 82 (76), 55 (53).

Unknown A [**19**].—Gc Rt = 11.45 min; eims *m/z* (rel. int.) $[M]^+$ 199 (6), 168 (4), 156 (6), 126 (2), 124 (6), 122 (3), 96 (4), 84 (9), 83 (100), 82 (18), 55 (37).

Unknown B [**20**].—Gc Rt = 12.46 min; eims *m/z* (rel. int.) $[M]^+$ 199 (4), 168 (5), 156 (8), 126 (3), 124 (7), 122 (4), 96 (4), 84 (10), 83 (100), 82 (17), 55 (36).

Unknown C [**21**].—Gc Rt = 23.95 min; eims *m/z* (rel. int.) $[M]^+$ 239 (2), 221 (1), 156 (40), 140 (17), 139 (61), 138 (20), 122 (7), 114 (13), 96 (7), 95 (11), 83 (29), 82 (100), 55 (39).

Unknown D [**22**].—Gc Rt = 26.99 min; eims *m/z* (rel. int.) $[M]^+$ 239 (9), 140 (62), 139 (13), 138 (9), 122 (10), 111 (14), 110 (7), 108 (5), 98 (5), 96 (6), 84 (10), 83 (100), 82 (30), 70 (10), 55 (51).

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