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LITERATURE CITED

AOAC. "Official Methods of Analysis", 13th ed., Association of Official Analytical Chemistry: Washington, DC, 1980; Method 11.011.
Belikov, V. M.; Antonova, T. V.; Bezrukov, M. G. *Nahrung* **1981**, *25*, 97.
Bjarnarson, J.; Carpenter, K. J. *Br. J. Nutr.* **1970**, *24*, 313.
Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911.
Boonvisut, S.; Whitaker, J. R. *J. Agric. Food Chem.* **1976**, *24*, 1130.
Buttkus, H. *J. Food Sci.* **1970**, *35*, 558.
Cecil, R.; McPhee, J. R. *Adv. Protein Chem.* **1959**, *14*, 255.
Connell, J. J. "Symposium on Foods: Proteins and Their Reactions"; Avi Publishing Co.: Westport, CT, 1964; p 255.
Dvorschak, E. *Z. Lebensm.-Unders. -Forsch.* **1970**, *143*, 167.
El-Zeany, B. A.; Pokorny, J.; Smidrkalova, E.; Davidek, J. *Nahrung* **1975**, *19*, 327.
Evans, R. J.; Butts, H. A. *Science (Washington, D.C.)* **1949a**, *109*, 569.
Evans, R. J.; Butts, H. A. *J. Biol. Chem.* **1949b**, *178*, 543.
Finley, J. W.; Lundin, R. E. "Autooxidation in Food and Biological Systems"; Plenum Press: New York, 1979; p 223.
Friedman, M. "The Chemistry and Biochemistry of the Sulfhydryl Groups in Amino Acids, Peptides, and Proteins"; Pergamon Press: New York, 1973; p 1.
Friedman, M.; Grosjean, O.-K. K.; Zahnley, J. C. *J. Sci. Food Agric.* **1982**, *33*, 165.
Furakawa, A.; Tsukahara, H. *Bull. Jpn. Soc. Sci. Fish.* **1966**, *32*, 502.
Gabaudan, J.; Pigott, G. M.; Halver, J. E. *Proc. Annu. Meet—World Maric. Soc.* **1980**, *11*, 424.
Hamm, R.; Hofmann, K. *Nature (London)* **1965**, *207*, 1269.
Itoh, Y.; Yoshinaka, R.; Ikeda, S. *Bull. Jpn. Soc. Sci. Fish.* **1979a**, *48*, 1019.
Itoh, Y.; Yoshinaka, R.; Ikeda, S. *Bull. Jpn. Soc. Sci. Fish.* **1979b**, *45*, 1023.
Itoh, Y.; Yoshinaka, R.; Ikeda, S. *Bull. Jpn. Soc. Sci. Fish.* **1980**, *46*, 621.
Jacobsen, E. E.; Moller, A.; Nielsen, J. J.; Schmidtsdorf, W.; Weidner, K. E., International Association of Fish Meal Manufacturers, 12th Annual Conference, Rome, IAFMM, Potters Bar, 1972, p 1.
Kisza, J.; Rotkiewics, W. *Z. Ernaehrungscwiss.* **1974**, *13*, 172.
Krause, M. V.; Mahan, L. K. "Food Nutrition and Diet Therapy"; W. B. Saunders Co.: Philadelphia, PA, 1979.
Matthews, A. D.; Park, G. R.; Anderson, E. M. In "Advances in Fish Science and Technology, Jubilee Conference"; Connell, J. J., Ed.; Fishing News Books Ltd.; Farnham, Surrey, England, 1980; p 438.
Meinke, W. W.; Finne, G.; Nickelson, R., II; Martin, R. *J. Agric. Food Chem.* **1982**, *30*, 477.
Miller, E. L.; Hartley, A. W.; Thomas, D. C. *Br. J. Nutr.* **1965**, *19*, 565.
Rios Iriarte, B. J.; Barnes, R. H. *Food Technol. (Chicago)* **1966**, *20*, 835.
Saxena, V. P.; Wetlaufer, D. B. *Biochemistry* **1970**, *9*, 5015.
Schnack, U.; Klostermeyer, H. *Milchwissenschaft* 1980, *35*, 206.
Schoberl, A.; Eck, H. *Justus Liebegs Ann. Chem.* **1936**, *522*, 97.
Sedlak, J.; Lindsay, R. H. *Anal. Biochem.* **1968**, *25*, 192.
Skrede, A.; Opstvedt, J. "NJF's subseksjon for pelsdyr."; Kingelv. Stensiltrykk 1979; Mote 10–12, No. 111, p 1.
Snedecor, G. W.; Cochran, W. G. "Statistical Methods"; 6th Ed.; The Iowa State University Press: Ames, IA, 1974.
Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.
Spencer, R. L.; Wold, F. *Anal. Biochem.* **1969**, *32*, 185.
Tinbergen, B. J., Proceedings of the 16th European Meeting of Meat Research Workers, Warna, Golden Sands, Bulgaria, 1970, I, p 576.
Tristram, G. R.; Smith, R. H. "The Proteins"; Academic Press: New York and London, 1963.
Tsuchiya, Y.; Tsuchiya, J.; Matsumoto, J. J. "Advances in Fish Science and Technology"; Fishing New Books, Ltd.: Farnham, Surrey, England. 1979; p 434.
Waibel, P. E.; Cuperlovic, M.; Hurrell, R. F.; Carpenter, K. J. *J. Agric. Food Chem.* **1977**, *25*, 171.
Williams, A. P.; Hewitt, D.; Cockburn, J. E. *J. Sci. Food Agric.* **1979**, *30*, 469.
Wolf, W. J. *J. Agric. Food Chem.* **1970**, *18*, 969.

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Stability of Pyrrolizidine Alkaloids in Hay and Silage

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The stability of pyrrolizidine alkaloids (PAs) in hay and silage samples with various amounts of *Senecio alpinus* L. was studied. While the PA content in hay remains constant over months, the PAs in silage were found to be destroyed to a great extent, but the degradation of PAs was much less complete in the lower concentration range. A quantitatively important PA-degradation product in silage was identified as retronecine. A second PA-derived compound was tentatively identified by GC–MS as a dehydratation product of retronecine with a $M^+ = 137$. Silage with a *S. alpinus* percentage of 3.5–23 still contained macrocyclic PAs in a concentration of ca. 20 mg/kg wet weight. Such a silage cannot be recommended as a safe fodder for cattle.

Plants containing pyrrolizidine alkaloids (PAs) are responsible for increasing animal health problems in various countries all over the world (Bull et al., 1968; Johnson, 1979; Lüthy et al., 1981). The fate of PAs in silage is a controversial point in literature: Vardiman (1952) reported that the toxicity of silage made from *Senecio ridellii* is greatly reduced or even nontoxic to calves. On the other hand, Donald and Shanks (1956) described a massive outbreak of ragwort poisoning in England with cattle fed on silage containing ragwort. However, in both cases no chemical analysis on PA content was performed.

According to a recent analysis *Senecio alpinus* L. contains nine different PAs with seneciphylline (see Chart I) as the major component and with a total PA content in

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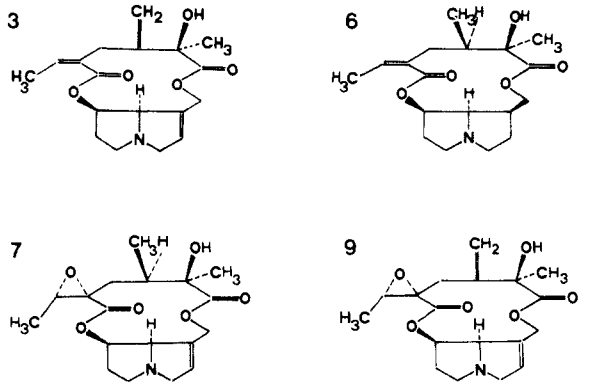
Chart I. Structures of the Most Important Pyrrolizidine Alkaloids Present in Silage Prepared from *S. alpinus*: 3, Seneciphylline; 6, Platyphylline; 7, Jacobine; 9, Jacozine



the range of 0.3–0.45% of the dry weight (Lüthy et al., 1981). In the present paper we report on the stability of PAs in hay and silage samples containing different amounts of *S. alpinus* L.

EXPERIMENTAL SECTION

Materials. Preblooming samples of *Senecio alpinus* were collected in alpine meadows near Sörenberg (Switzerland) in June. One batch of these plants (10% dry matter content) was dried at 60 °C immediately after collecting, milled, and mixed with various amounts of identically prepared grass/clover (16% initial dry matter content). The PA content of these greenfodder samples was determined. A second batch of *S. alpinus* was air-dried to hay for 4 days (79% dry matter content) and further dried at 60 °C, milled, and mixed with various amounts of dried grass/clover.

A total of five different mixtures of *S. alpinus* and grass/clover were ensiled, without additive, in 1.5-L gastight laboratory silos equipped with a valve during 114 days (dry matter content ranging between 19 and 33%). The gas loss of the samples during the silage process varied between 3.9 and 4.5% of the dry matter content. All samples were stored at −24 °C until analyzed.

Analytical Methods. The methods used (an exhausting extraction with methanol, separation, and estimation of the alkaloids by TLC, GC-FID, and GC–MS, respectively) have been already described (Lüthy et al., 1981; Brauchli et al., 1982). The amounts of free alkaloids and *N*-oxides, after reduction with zinc dust, were determined separately.

Apparatus. For the gas chromatography a GC Carlo Erba Fractovap 4160 was used with a temperature programmer, LT Model 400, and a 20-m SE-54 capillary column with hydrogen as the carrier gas. The temperature program was injector 250 °C and column 200 °C for 1 min and 5 °C/min until 250 °C.

GC–MS was performed on a Finnigan Model MAT 4500, with the Incos data system, using the same GC system as before, but with He as the carrier gas. The temperature conditions for the detection of retronecine were as follows: injector, 260 °C; column, 140 °C for 1 min and 6 °C/min until 260 °C. The mass spectrometer had an ionizing voltage of 70 eV and an ion source temperature of 120 °C. An authentic sample of seneciphylline was used as a standard for the quantitation of the alkaloids.

RESULTS AND DISCUSSION

The amounts of PAs found in hay, greenfodder, and silage samples in relation to the percentage of *S. alpinus* are shown in Tables I and II. It is obvious that PAs in hay and dried greenfodder remain fully preserved where

Table I. PA Content in Dried Greenfodder and Hay (Dry Matter) Prepared with Various Amounts of *S. alpinus* L.

sample	% of *S. alpinus* in dry matter	amount of total PAs, mg/kg dry matter
greenfodder	100	4000 ± 1360
greenfodder	50	2630 ± 530
hay	50	3180 ± 320
greenfodder	30	1750 ± 630
greenfodder	10	640 ± 103
hay	10	485 ± 120
greenfodder	5	316 ± 63
greenfodder	3	197 ± 30
greenfodder	1	78 ± 24
greenfodder	0.5	26 ± 3
greenfodder	0.2	13 ± 1

Table II. PA Content of Silage Samples Prepared with Various Amounts of *S. alpinus* L.

	amount of total PAs		
% of *S. alpinus*	mg/kg wet weight	mg/kg dry weight	% of initial PAs
100	35 ± 7[a]	183 ± 35[a]	4.5
41	39 ± 7	149 ± 25	9.1
23	23 ± 4	79 ± 13	8.6
7	17 ± 2	55 ± 8	19.6
3.5	21 ± 4	64 ± 13	45.7
0	0	0	

[a] A GC–MS analysis of the PAs in this sample is shown in Figure 1.

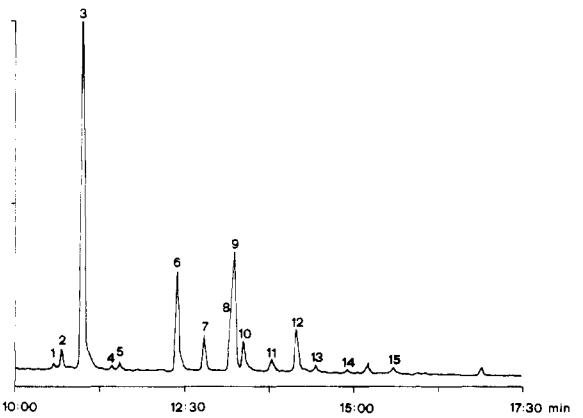


Figure 1. Separation of an alkaloid extract from a silage sample prepared from *S. alpinus* L. by capillary GC–MS. Injector 250 °C; column 100 °C for 1 min and 10 °C/min until 220 °C. Numbers of the peaks correspond to those in Table III and Chart I. Peaks 4, 10, and 12–15 were not and peak 6 was in trace amounts only detected in samples of fresh *S. alpinus* (Lüthy et al., 1981).

as the PAs in silage are destroyed to a great extent during the fermentation process. However, the degradation is much less complete in the lower concentration range. The ratio of free alkaloids/*N*-oxides is generally >1 in silage and <1 in hay and dried greenfodder. The relatively large errors of the PA determinations are due to the inhomogenicity of the analyzed material as well as the chosen analytical procedure.

Bradbury and Willis (1956) studied the degradation of PAs from *Senecio jacobaea* L. under acidic conditions (conditions similar to those during the silage fermentation) and found that jacobine, jaconine, and jacoline are hydrolyzed to retronecine and the corresponding dicarboxylic acids. Mattocks (1982) has reported an enzymatically by esterase-catalyzed hydrolysis of PAs to retronecine as an important detoxification mechanism. GC–MS of extracts

Table III. Alkaloid Composition of a Silage Sample Prepared from *S. alpinus* As Determined by Capillary GC–MS

peak no.	compound	M^+	rel. abundance, %
1	isomer of senecionine[a]	335	0.9
2	senecionine	335	2.2
3	seneciphylline	333	47.6
4	platyphylline or isomer[a]	337	0.5
5	integerrimine	335	0.9
6	platyphylline or isomer[a]	337	11.9
7	jacobine	351	3.8
8	*O*-acetylseneciphylline	375	
9	jacozine	349	17.7
10	jacoline	369	4.8
11	jaconine	387	2.0
12	water adduct of jacozine[a]	367	6.2
13	unknown[a]	381	0.9
14	acetate adduct of jacobine[a]	411	0.2
15	acetate adduct of jacozine[a]	409	0.9

total macrocyclic PAs: 35 mg/kg wet weight
retronecine: 11 mg/kg wet weight

[a] Tentative identification.

of silage samples containing *S. alpinus* indeed showed a peak with the same retention time and the same fragmentation pattern as retronecine: *m/e* (rel intensity) 156 (2), 155 (18), 130 (7), 111 (50), 107 (9), 97 (8), 94 (16), 83 (10), 80 (100), 68 (18), 56 (38), and 53 (12) (Neuner-Jehle et al., 1965). A second PA-derived compound was tentatively identified by GC–MS as a dehydration product of retronecine with $M^+ = 137$. Both compounds were not found in silage prepared without *S. alpinus*. A GC–MS of the macrocyclic PAs remaining in silage prepared from 100% *S. alpinus* is shown in Figure 1 and Table III. Quantitatively most important are seneciphylline (47.6% of the total PAs) and jacozine (17.7%). The peaks not seen when analyzing the fresh plants are no. 10 (jacoline), 11, and 14, which are likely formed from jacobine, and the probably jacozine-derived peaks no. 12 and 15.

Data presented do not suggest the safety of silage containing ca. 20 mg of PAs/kg wet weight. A 600-kg calf eats about 30 kg of silage/day; from these data one may calculate a daily intake of ca. 1 mg of PAs/kg body weight. Johnson (1979) found in an extensive feeding study with *S. jacobaea* that the minimum lethal dose for cattle is between 1 and 2 mg of PAs (kg body weight)$^{-1}$ day^{-1} when fed for several weeks. Unfortunately, no exact determination of the individual PAs has been performed in that study. Earlier reports have shown that the major PAs in *S. jacobaea* are jacobine, seneciphylline, senecionine, and jacozine (Roitman et al., 1979, and references cited therein). Even with the assumption that the toxicity of the PA-degradation products in silage is negligible, the safety margin is not high enough to recommend the use of silage containing even only a few percent *Senecio* plants as fodder for cattle.

Registry No. Senecionine, 130-01-8; seneciphylline, 480-81-9; platyphylline, 480-78-4; integerrimine, 480-79-5; jacobine, 6870-67-3; *O*-acetylseneciphylline, 90341-45-0; jacozine, 5532-23-0; jacoline, 480-76-2; jaconine, 480-75-1; retronecine, 480-85-3.

LITERATURE CITED

Bradbury, R. B.; Willis, J. B. *Aust. J. Chem.* **1956**, *9*, 258.
Brauchli, J.; Lüthy, J.; Zweifel, U.; Schlatter, Ch. *Experientia* **1982**, *38*, 1085.
Bull, L, B.; Culvenor, C. C. J.; Dick, A. T. "The Pyrrolizidine Alkaloids"; North Holland: Amsterdam, 1968.
Donald, L. G.; Shanks, P. L. *Br. Vet. J.* **1956**, *112*, 307.
Johnson, A. E. In "Symposium on Pyrrolizidine (Senecio) Alkaloids: Toxicity, Metabolism, and Poisonous Plant Control Measures"; Cheeke, P. R., Ed.; The Nutrition Research Institute: Corvallis, OR, 1979.
Lüthy, J.; Zweifel, U.; Karlhuber, B.; Schlatter, Ch. *J. Agric. Food Chem.* **1981**, *29*, 302.
Mattocks, A. R. *Toxicol. Lett.* **1982**, *14*, 111.
Neuner-Jehle, N.; Nesvadha, H.; Spiteller, G. *Monatsh. Chem.* **1965**, *96*, 321.
Roitman, J. N.; Molyneux, R. J.; Johnson, A. E. In "Symposium on Pyrrolizidine (Senecio) Alkaloids: Toxicity, Metabolism, and Poisonous Plant Control Measures"; Cheeke, P. R., Ed.; The Nutrition Research Institute: Corvallis, OR 1979.
Vardiman, P. H. *J. Am. Vet. Med. Assoc.* **1952**, *121*, 397.

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