would also not be as readily split by a Lactobacillus or other gut bacteria as are the monoglycosides, although in time it too should respond to a microbial treatment process. The reduction in levels of toxicants III + IV by *L. acidophilus* 629 was proportional to the reduction in simmondsin levels.

The intertwining of toxicity and palatability factors in jojoba meal add complexity to interpretations of animal feeding data. The possibility that there is a bitter cyano polyglycoside present that is relatively intractable to solvent extraction and chemical and microbial treatment should be considered. Structural studies on the minor toxicants and other components in jojoba seeds are continuing.

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Pyrrolizidine Alkaloids of *Senecio alpinus* L. and Their Detection in Feedingstuffs

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Senecio alpinus L. was recently discovered as the cause of pyrrolizidine alkaloidosis in livestock in Switzerland. A GC–MS analysis of this plant and of hay and silage containing *S. alpinus* revealed the presence of nine different alkaloids with seneciphylline as the main constituent and senecionine, integerrimine, jacozine, jacobine, jaconine, and the unsaturated analogue of jaconine as minor constituents. The structure of the two other alkaloids are discussed on the basis of their mass spectra.

In the past 80 years poisoning of domestic animals with pyrrolizidine alkaloids (PA) have been reported from various countries all over the world, the main cause of these incidents being plants belonging to one of the three genera *Senecio*, *Crotalaria*, and *Heliotropium* (Bull et at., 1968). Besides their high acute hepatotoxicity, many PA tested so far were found to be mutagenic and carcinogenic and are therefore of considerable interest to toxicologists (IARC, 1976).

Senecio alpinus, a widespread plant in alpine meadows, was recently discovered as the cause of pyrrolizidine alkaloidosis in three herds of dairy cattle in Switzerland (Pohlenz et al., 1980). Klasek et al. (1968) were able to isolate seneciphylline and a small amount of jacozine from a sample of *S. alpinus* L. of Swiss origin. In the present paper we report a GC–MS analysis of the alkaloids derived from *S. alpinus* and the detection of such compounds in hay and silage, both having been used as feed for cattles.

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EXPERIMENTAL SECTION

Apparatus. The separation and identification of the alkaloids was achieved on a Finnigan Model 4021 GC–MS system. GC was performed on a 20-m SE 54 capillary column, with He as the carrier gas. The temperatures were injector 250 °C and column 100 °C for 1 min and 10 °C/min until 220 °C. The mass spectrometer had an electron energy of 70 eV and ion source temperature of 250 °C. CI conditions were source pressure 0.30 torr, and source temperature 200 °C. Quantitation of the alkaloids were made by peak integration.

Materials. Preblooming samples of *S. alpinus* L. were collected near Einsiedeln, Maloja, and Sörenberg (Switzerland) in June. Samples of hay and silage from the area of Einsiedeln were taken in January and stored at −24 °C until analyzed. Authentic samples of seneciphylline, senecionine, and integerrimine were kindly provided by Dr. C. C. J. Culvenor, Parkville, Australia, and Dr. M. H. Benn, Calgary, Canada.

Procedure. The samples (50–100 g wet weight) were extracted extensively with methanol in a Soxhlet apparatus, and the extracts evaporated to dryness under reduced

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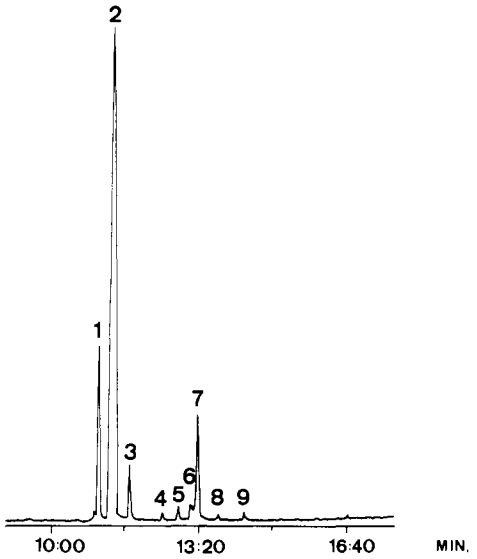

Figure 1. GC–MS (total ion current) of the alkaloids from *S. alpinus* L.

pressure. The residues were taken up in 2 N sulfuric acid, washed with petroleum ether and ether to remove chlorophyll and lipids, and divided into equal portions. The first portion was basified and extracted with chloroform. The extract was dried over anhydrous sodium sulfate and evaporated to dryness (free alkaloids). The second portion was reduced with zinc dust overnight, filtered, and treated in the same manner (reduced *N*-oxides). The residues were dissolved in an appropriate amount of ethanol to give a final concentration of ~2 mg of extract/mL. Aliquots of these solutions (0.5, 1, or 2 μL) were used for GC–MS.

RESULTS AND DISCUSSION

A GC–MS analysis of *S. alpinus* is shown in Figure 1. The indicated nine peaks exhibited a EI–MS fragmentation pattern typical for pyrrolizidine alkaloids (Bull et al., 1968). Additionally, CI–MS with methane as the reactant gas allows rapid recognition of the $M^+ + 1$ ion. Figure 2 shows a comparison between the EI- and CI–MS of peak 7, jacozine.

Using these techniques we were able to identify the following compounds: 1, senecionine (18% of the total alkaloids) (Figure 3a); 2, seneciphylline (65%) (Figure 3b); 3, integerrimine (7%) (Figure 3c); 4, $M^+ = m/e$ 337 (0.5%) (Figure 3d); 5, jacobine (0.8%) (Figure 3e); 6, $M^+ = m/e$ 375 (0.8%) (Figure 3f); 7, jacozine (7%) (Figure 2); 8, jaconine (0.4%) (Figure 3g); 9, the unsaturated analogue of jaconine (0.5%) (Figure 3h). The reference compounds senecionine, seneciphylline, and integerrimine displayed the same retention times and mass spectra as peaks 1, 2, and 3, respectively. The mass spectra of jacobine, jacozine, and jaconine are described in the literature (Klasek et al., 1968; Segall, 1978; Segall and Krick, 1979) and are identical with the MS of peaks 5, 7, and 8, respectively. The MS of peak 4 (Figure 3d) shows some characteristic differences: the fragments 93–95, 119–121, and 136–138 (120 as the base peak), all typical for the retronecine part in the molecule, are shifted to 95, 96, 122, 123, 138, and 140 with m/e 82 as the base peak. This fragmentation is typical for saturated PA and, indeed, the MS of peak 4 is almost identical with the mass spectra of the three closely related compounds platyphylline, neoplatyphylline, and hastacine, described by Culvenor et al. (1968), all with a molecular weight of 337. Peak 6 (Figure 3f) with a molecular ion (M^+) at mass 375 and prominent fragments at m/e = 316 ($M^+ - 59$, CH_3CO_2), 288 ($M^+ - 87$, $CH_3CO_2 + CO$), and 272 ($M^+ - 103$, $CH_3CO_2 + CO_2$) could be the hitherto unknown

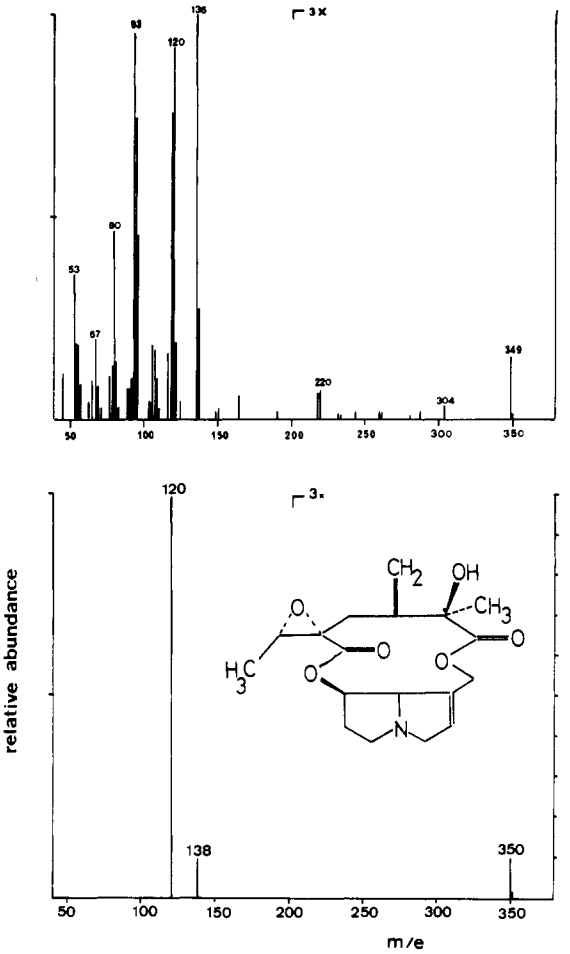

Figure 2. EI–MS (above) and CI–MS with methane as reactant gas of peak 7, identified as jacozine.

Table I. Occurrence of Pyrrolizidine Alkaloids in *S. alpinus* (As Identified in This Paper) and *S. jacobeae* [Estimated from Figure 1 in Segall and Krick (1979)]

compd	occurrence[a] in *S. alpinus*	occurrence[a] in *S. jacobeae*
seneciphylline	+++	+++
senecionine	++	++
jacozine	++	++
integerrimine	++	–
jacobine	+	+
$M^+ = 375$	+	–
$M^+ = 337$	+	–
$M^+ = 385$	+	+++
jaconine	+	+
jacoline	–	+

[a] (+++) More than 30% of the total alkaloids; (++) 5–30% of the total alkaloids; (+) less than 5% of the total alkaloids.

O-acetylseneciphylline. The recently isolated neopetasitenine (Yamada et al., 1976), with an analogous acetyl group at C-12 of the petasitenecic acid, also showed a fragment at $m/e = M^+ - 59$. Further examples of C-12 acetylated PA are ligularidine (Hikichi et al., 1979) and *O*-acetylsenkirkine (Briggs et al., 1965). The MS of peak 9 ($M^+ = m/e$ 385) is identical with the published MS data of the unsaturated analogue of jaconine, recently isolated from *Senecio jacobeae* (Segall, 1978). Freshly collected *S. alpinus* from different origins showed a total PA content in the range of 0.3–0.4% of the dry weight with a highly variable ratio of free alkaloids/*N*-oxides. The amounts of alkaloids found in hay and silage samples were 15 and 4 mg/kg fresh weight, respectively. Qualitative comparison

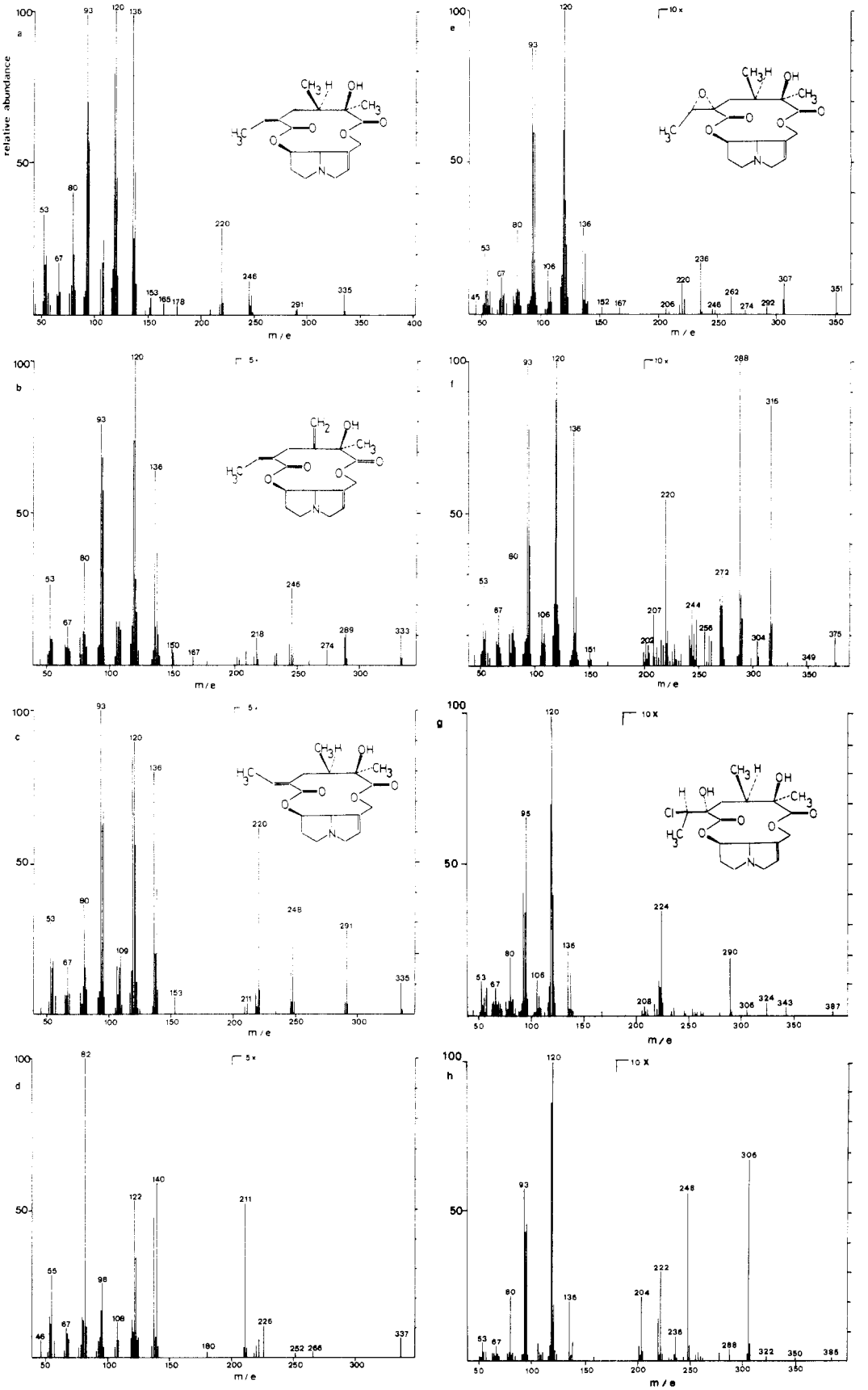

Figure 3. EI–MS of the peaks 1 [(a) senecionine], 2 [(b) seneciphylline], 3 [(c) integerrimine], 4 [(d) M = *m/e* 337]; 5 [(e) jacobine], 6 [(f) M = *m/e* 375], 8 [(g) jaconine], and 9 [(h) the unsaturated analogue of jaconine].

of *N*-oxides and free alkaloids in the silage sample showed seneciphylline to be present mainly in the *N*-oxide form, with senecionine rather as the free alkaloid.

Table I summarizes the identified PA and compares their occurrence in *S. alpinus* and *S. jacobeae*. Six PA occur in both plants, especially the three main constituents seneciphylline, senecionine, and jacozine. Although the ratio of the different PA may vary considerably, the toxicity of the two *Senecio* species appears very comparable. In a feeding study with cattle, Thorpe and Ford (1968)

found that the daily consumption of 1 g of dried *S. jacobeae* (kg of body weight)$^{-1}$ day^{-1} causes illness and death of the animals within a few months. From the PA content of 15 mg/kg of hay, measured in our case, one may calculate ~0.5% *S. alpinus* to be present in the hay. The daily consumption of the cattle was probably around 0.5 g of *S. alpinus* (kg of body weight)$^{-1}$ day^{-1}, an amount which can easily explain the liver damages detected in 10 animals which were described earlier (Pohlenz et al., 1980).

Of special importance to the consumer is the fate of these compounds in the animal. In the urine of a cow from the same area we were able to detect ~0.5 mg of PA metabolites/L, giving a positive Mattocks (1967) reaction. The distribution in the body and the chemical and toxicological properties of these PA metabolites are presently under study.

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Structure–Taste Correlations for Flavans and Flavanones Conformationally Equivalent to Phyllodulcin

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Preparation of a conformationally defined series of compounds related to phyllodulcin allows more accurate correlations of structural features with taste. Four flavans (3,4-dihydro-2*H*-1-benzopyrans) and their parent flavanones (2,3-dihydro-4*H*-1-benzopyran-4-ones) were prepared from chalcones derived from 2-hydroxy-, 2,4-dihydroxy-, 2,6-dihydroxy-, or 2,4,6-trihydroxyacetophenone and isovanillin (3-hydroxy-4-methoxybenzaldehyde). These compounds can exist as both semiplanar and bent conformers, equivalent to those of phyllodulcin, permitting a close comparison of structural features with taste. Semiplanar conformations were established for phyllodulcin and the analogous compounds by ^{1}H NMR. Evidence for bent conformations was lacking. Flavans derived from 2-hydroxy- and 2,4-dihydroxyacetophenone were sweet, the latter intensely so, whereas the 2,6 analogue was intensely bitter. Comparisons with phyllodulcin and derivatives demonstrated the effects of nonaromatic ring heteroatom location and A-ring hydroxylation and carbonyl group effects.

Investigators seeking intensely sweet analogues of natural compounds have tended to focus on the effects of specific molecular functional groups on taste, and simple compounds containing various groups have been synthesized. Such research has succeeded in producing several sweet compounds structurally related to phyllodulcin (Yamato et al., 1972a–c, 1973, 1974a,b, 1975, 1977a–d, 1978; Yamoto and Hashigaki, 1979) and an extensive literature relating similar structures of dihydrochalcone derivatives to taste.

Assessments of how tastant molecules and their specific structural features interact with receptors to produce or modify perceived tastes are much less complete. While the above dihydrochalcones and substituted 1,2-diphenylethanes are sweet, they differ markedly from the parent model in terms of taste qualities, intensities, and predicted abilities to undergo conformational alterations when binding to a receptor. There is no way to predict or demonstrate that they achieve equivalent conformations in the bound state or that their structural features interact with identical sensitive areas of the taste receptors. The well-known proposal by Kier (1972) that the AH–B sites (Shallenberger and Acree, 1967) and a third, distant, hydrophobic site must achieve critical geometric relationships with one another for production of intense sweetness underscores the need to determine solution-average conformations for phyllodulcin analogues and to predict their abilities to achieve the Kier (1972) requirements when bound to taste receptors.

For the present, however, only the solution-average conformations of molecules possessing the structural features for intense sweetness can be established. For molecules that do not meet the Kier (1972) requirements in solution, investigators are limited to model studies and subjective evaluations of bond flexibilities for predictions of bound conformers. In the absence of any firm understanding of how sweet molecules are bound within or onto a receptor, variation of structural characteristics within relatively limited conformational arrangements could lead to an improved understanding of receptor–tastant interactions. Compounds that mimic the size and structure of phyllodulcin are attractive, in that the basic structural featues associated with AH–B and distant bonding have been proposed (Yamato et al., 1972a,b, 1973, 1974b, 1977a,c,d; Yamoto and Hashigaki, 1979).

Further, the molecules can serve as vehicles for varying positions of structural features not believed necessary for

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