bilayers can come into close contact, allowing the subsequent fusion to take place. The calcium ions are effective in establishing a tight contact between adjacent chromaffin granules with fusion of the external membrane leaflets, resulting in a pentalaminar structure. Similar membrane formations have also been observed between adjacent secretory granules of mast cells (*15*) and of pancreatic islet cells (*16*). It appears that additional factors are involved in the completion of the fusion process beyond the stage of reversibility.

In some systems where exocytotic release involves preformed sites on the inner surface of the cell membrane, the frequency of exocytotic figures as seen in freeze-fracture preparations can be increased by various stimuli: in neuromuscular junction, electrical stimulation in the presence of calcium (*17*); in central synapses of lamprey, electrical stimulation and potassium depolarization in the presence of calcium (*18*); in central synapses of rat, lack of anesthesia (*19*); in mossy fiber endings of rabbit hippocampus, epileptic convulsion (*20*); in neurohypophysis, electrical stimulation or exposure to cold (*21*); and in *Paramecium*, ionophores in addition to calcium (*22*).

While all these examples represent intact biological systems, the identification of specific fusion factors will require further biochemically defined in vitro experiments.

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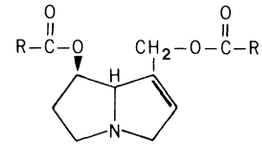
Pyrrolizidine Alkaloids: Their Occurrence in Honey from Tansy Ragwort (*Senecio jacobaea* L.)

Abstract. *The hepatotoxic alkaloids known to occur in tansy ragwort* (Senecio jacobaea *L.) are also present in honey produced from the nectar of this species. These alkaloids, which include senecionine, seneciphylline, jacoline, jaconine, jacobine, and jacozine, are potentially carcinogenic, mutagenic, and teratogenic and may pose health hazards to the human consumer.*

The hepatotoxic pyrrolizidine alkaloids present in local tansy ragwort (*Senecio jacobaea* L.) have been demonstrated conclusively to be present in honey produced from the nectar of this plant. Certain liver ailments and other diseases in humans in developing nations have been attributed to the consumption of foods and herbal medicines prepared from pyrrolizidine alkaloid–containing plants. We report here that human exposure to the pyrrolizidine alkaloids through food products is a very real possibility in the United States.

Tansy ragwort is a weed introduced to maritime regions of both western and eastern North America from Europe (*1*, *2*). The toxicity of *S. jacobaea* is well known and is due to a mixture of pyrrolizidine alkaloids which include senecionine, seneciphylline, jacobine, jaconine, jacoline, and jacozine (*2–5*). All six of these alkaloids are cyclic diesters of the 1,2-dehydropyrrolizidine ring system (**1**). Values for the median lethal dose (LD_{50}) of the alkaloids in tansy ragwort are around 100 mg/kg on the basis of animal experiments (*2*).

The consumption of foods and herbal medicines contaminated with pyrrolizidine alkaloids results in acute veno-occlusive lesions which progress to liver cirrhosis (*5*). The Budd-Chiari syndrome, which is manifested by hepatic vein occlusions in native South African populations is apparently also related to the consumption of bread containing *Senecio* flour (*5*). More important, however, are the animal experiments that have shown that certain pyrrolizidine alkaloids are carcinogenic (*6*), mutagenic (*2*), and teratogenic (*8*).



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Blooming of *S. jacobaea* occurs from the middle of July through September in western Oregon and Washington. During this time there is a general dearth of nectar and pollen in other entomophilus species, and tansy ragwort is actively foraged upon by honey bees (*Apis mellifera* L.).

We attempted to discover whether the endogenous alkaloids in tansy ragwort are shunted through the nectar secretory process and ultimately deposited in the

Table 1. Percentage of tansy ragwort pollen and concentration (expressed as parts per million) of pyrrolizidine alkaloids found in honey samples from the Pacific Northwest.

Honey sample	Geographical source	Average tansy ragwort pollen (%)*	Concentration of pyrrolizidine alkaloids (ppm)†
1	Elma, Washington	2.6 ± 0.7	1.1 and 1.4
2	Beaverton, Oregon	0.8 ± 0.1	0.3 and 0.4
3	Toledo, Oregon	1.9 ± 0.4	1.2 and 2.2
4	Salem, Oregon	0.7 ± 0.4	3.2 and 3.9
Control	Corvallis, Oregon	0.0	0.0

*Average of three replicates. †Uncorrected; two separate determinations.

honey. Four samples of suspected ragwort honey were provided by three beekeepers in western Oregon and one beekeeper from western Washington (*9*). A fifth honey, free of ragwort nectar, served as a control. All honeys were produced during the late summer of 1975 in the Coast Range mountains of Oregon and Washington. Samples were not filtered or heated, and were stored at ambient room temperature, about 20°C, until analysis.

Pollen spectral analyses were conducted to demonstrate the presence of *S. jacobaea* in the honeys (*10*). The samples were diluted with distilled water and, after centrifugation, the supernatant was discarded and the pellet dispersed on a slide for microscopic examination. In all suspected honey samples there were small amounts of ragwort pollen. The expressed percentage of ragwort pollen was derived after three replicate counts of 1200 pollen grains.

The concentration of alkaloids was determined by extracting an ammoniacal solution of the honey with chloroform. The concentrated extract was analyzed spectrophotometrically according to Mattocks' procedure (*11*) with the temperature and solvent modifications of Bingley (*12*). All samples that had been shown to contain ragwort pollen developed color, and the concentration of alkaloids in these samples is reported in Table 1.

The alkaloids tend to concentrate in the flowers of the plant (*3*) and, from our experience, constitute 0.15 to 0.30 percent of the dried flowers. However, nothing is known about possible metabolites and other products in honey, to which our detection methods are not sensitive, nor are we fully satisfied that our recoveries are quantitative. For these reasons it is not possible to relate the alkaloid content with the percentage of pollen found.

In a separate experiment, contaminated honey sample No. 1 was diluted with water and acidified with hydrochloric acid. This solution was extracted with chloroform. Centrifugation was necessary to break the emulsion. The aqueous portion was made basic with ammonium hydroxide and extracted with chloroform, and the cycle was repeated to remove all of the waxes present in the honey. The final chloroform extract was concentrated and analyzed by combined gas chromatography and mass spectrometry. Figure 1 shows the reconstructed chromatogram of the pyrrolizidine alkaloids present in the extract.

All of the alkaloids present in local tansy ragwort were found in the honey sample. The mass spectra of each of these alkaloids showed typical fragmentation patterns (*13*) with abundant ions at mass to charge ratios (*m/e*) of 136, 121, 120, 94, and 93 (Fig. 2). The reconstructed chromatogram and mass spectra suggest there may be an additional unidentified pyrrolizidine alkaloid present with a molecular ion of 305.

The presence of plant toxins in honeys is not new (*14*). Nectars from the Ericaceae plant family (*Rhododendron, Azalea, Andromeda*, and *Kalmia*) containing grayanotoxins (*14, 15*), a mixture of diterpenes, are the most common sources of toxic honeys. The presence of pyrrolizidine alkaloids in honey, however, may present new health hazards. Our results suggest that an individual would proably not consume enough honey to suffer acute effects, because of the low per capita honey consumption in the United States (0.6 kg per year) (*16*). Furthermore, ragwort honey samples are very bitter in taste and are off-color compared to high-quality honeys, and are probably not often marketed. It is common practice among beekeepers in tansy ragwort areas to use ragwort honey as winter food for bee colonies.

However, the long-term consumption of food contaminated by chemical carcinogens, even when present in only trace amounts, must be viewed with much greater caution. The pyrrolizidine alkaloids in particular are known to form active metabolites and bind irreversibly to sites on the liver and other vital organs (*4*), and their effects are accumulative. Thus, honey samples and other agricultural food products contaminated with such low concentrations of these alkaloids that they are still palatable cannot necessarily be considered safe without further experimental work.

Pyrrolizidine alkaloids are endemic throughout the world, and are found in a

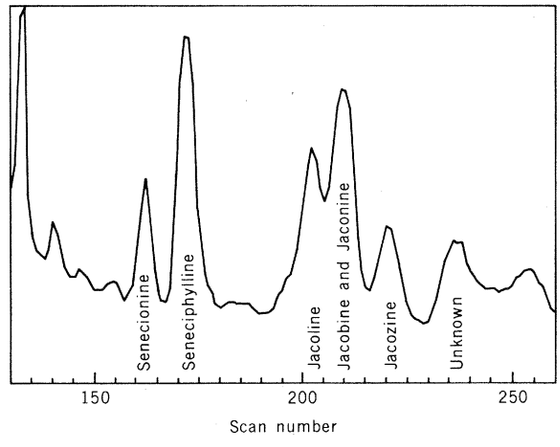

Fig. 1. Total ion chromatogram of pyrrolizidine alkaloids extracted from honey sample No. 1.

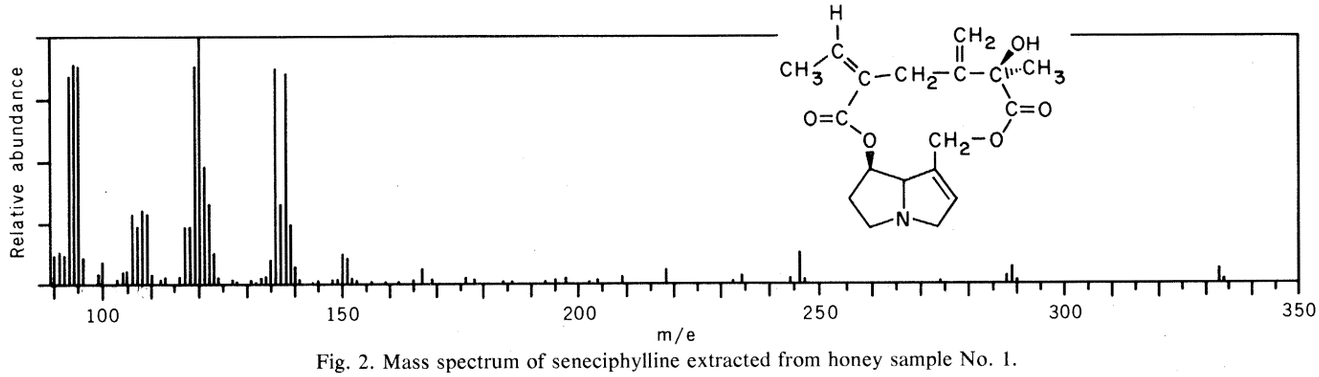

Fig. 2. Mass spectrum of seneciphylline extracted from honey sample No. 1.

wide variety of plant species in genera including *Senecio, Crotalaria, Heliotropium, Trichodesma, Amsinckia*, and others. Within these species at least 100 different pyrrolizidine alkaloids have been identified and their structures elucidated (*2*). Livestock poisoning by consumption of *S. jacobaea* and other pyrrolizidine alkaloid–containing plants is a major problem in Oregon (*17*) and other parts of the United States and the world (*18*). The transmission of these alkaloids or their metabolites to the consumer through meat and dairy products from exposed animals must also be considered possible.

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Primate Model for Long-Term Study of Intraventricularly or Intrathecally Administered Drugs and Intracranial Pressure

Abstract. *Meaningful pharmacokinetic investigations require animal systems which approximate the human situation. This report describes a primate model in which silicone catheters are placed into the fourth ventricle and the spinal subarachnoid space and connected to subcutaneous cerebrospinal fluid reservoirs. This model permits sterile access to ventricular cerebrospinal fluid without tissue damage, provides mixing of injected drugs with lateral ventricular cerebrospinal fluid, enables spinoventricular perfusion, and permits ventricular cerebrospinal fluid sampling over extended periods in unanesthetized rhesus monkeys. This animal system may provide intraventricular pressure recordings and pharmacokinetic data similar to that obtained in man.*

Rational approaches to the treatment of central nervous system malignancies demand precise knowledge of the pharmacokinetic behavior of chemotherapeutic agents in cerebrospinal fluid (CSF) (*1, 2*). Meaningful pharmacokinetic investigations require animal systems which approximate the human situation. Unfortunately, currently available animal models have been plagued by technical problems and have not provided long-term access to ventricular CSF with any reliability.

Most model systems require immobilization of the animal for long periods or anesthesia, and passage of cannulas through the cerebrum. These techniques do not provide experimental physiologic conditions and are associated with tissue damage (*3, 4*), infection, and catheter obstruction (*5*). Moreover, repetitive cisternal or lumbar punctures yield fluid that only simulates ventricular CSF and do not promote mixing of injected drugs with ventricular CSF.

The recently expressed need for suitable animal models (*4, 6*) prompted our development of a primate system which avoids these shortcomings. This model is based on an adaptation of the subcutaneous Ommaya CSF reservoir (*7*) developed for the treatment of central nervous system neoplasms in man (*1*). Preliminary physiologic and pharmacokinetic evaluations with this animal system demonstrate a close correlation with similar investigations in man.

Rhesus monkeys were anesthetized during the surgical implantations. A midline posterior incision was made over the occipitocervical junction and the posterior margin of the foramen magnum was removed (*8*). After midline dural incision, the foramen of Magendie was dilated to enable the insertion into the fourth ventricle of a silicone Pudenz catheter (2 mm in outside diameter). Each catheter tip was placed no less than 3 mm from the exit of the aqueduct of Sylvius to prevent aqueductal obstruction. After the dura was closed and made watertight with fine silk sutures, a 2.5-cm side-armed Ommaya CSF reservoir was connected to the catheter and secured subcutaneously over the occipital bone (Fig. 1, left). Intraoperative magnification is not necessary for the performance of this procedure.

Immobilization of the animal in a chair permitted access to the reservoir without anesthesia. The physiologic characteristics of our animal model were documented by the injection of isotopically labeled serum albumin into the ventricle, by positive contrast ventriculography, and by intracranial pressure recordings (*8*). At the conclusion of each evaluation period, the animals were returned to their cages and thus did not require long-term immobilization.

That the CSF of the fourth ventricle mixed with that of the lateral ventricle and flowed against the natural circulation pattern was verified by isotope injection and positive contrast ventriculography. After the injection of technetium 99m–labeled serum albumin, radioactivity migrated from the reservoir into the fourth ventricle and cisterna magna immediate-