AN ABSTRACT OF THE THESIS OF

Douglas Goeger for the degree of Master of Science in

Animal Science presented on Oct. 9, 1979

Title: Determination of the Transfer of Pyrrolizidine Alkaloids into Goats Milk by Biological Assay.

Abstract approved: Redacted for privacy

Peter R. Cheeke

The transfer of pyrrolizidine alkaloids, the toxins of Senecio jacobaea, into goats milk was investigated by biological assay using rats and bull calves. The effect of chronic low dietary administration of Senecio jacobaea on the rat was studied for comparison with the assays. The susceptibility of the dairy goat to Senecio jacobaea was also investigated.

Dairy goats were fed a pelleted diet containing 25% Senecio jacobaea. Due to the unpalatability of the ration, this was diluted with a control pellet, containing alfalfa instead of the Senecio, at a level for each goat that would be acceptable. The milk from one doe was freeze-dried and incorporated at a level of 80% in a ration for rats. This was fed, as was a control ration containing commercial powdered milk, to rats for three and six months. Two rats from the three month goat milk group were sacrificed at the end of the three months and examined

histologically. The remaining rats were sacrificed at the end of a year from the start of the study. The rats examined at the end of three months had no lesions attributable to pyrrolizidine alkaloid poisoning. One rat had consumed 832 g of milk while the other consumed 1039 g. The dry matter of the goat milk, determined by freeze-drying, was 12%. Of the six surviving rats from the three month goat milk group, three had multiple necropurulent foci scattered throughout the liver sections examined. The remaining three had some scattered focal cytoplasmic vacuolization of the hepatocytes. In the seven controls on a similar feeding regime five had cytoplasmic vacuolization. The remaining had no significant lesions. Of the rats consuming the milk rations for six months and examined six months later, all rats of both groups had swollen hepatocytes. In the three from the control group, these were limited to a few foci and were as a result of cytoplasmic vacuolization. Of the four rats in the goat milk group, the swollen hepatocytes were of centrilobular distribution. One rat also had biliary hyperplasia.

Rats were fed Senecio jacobaea at levels of 0%, 1.0%, 0.1%, 0.01% and 0.001% for six months, followed by a control diet for 225 days. Hepatic lesions were found in the four Senecio groups ranging from moderate in the 1.0% group to mild in the 0.001% group. The pyrrolizidine alkaloid content of the 0.001% ration would equal 0.18 ppm based on an alkaloid content of 0.18% in the Senecio

jacobaea. All but one of the eight rats in the 1.0% group died. One control rat had swollen vacuolated hepatocytes primarily of centrilobular distribution.

Two day-old bull calves were given milk from five goats consuming the Senecio jacobaea ration. The total milk consumed in the four month feeding trial was 549 L. At six months of age, histological examination revealed hepatic lesions similar to those of the rats fed the chronic low levels of Senecio. These were characterized by swollen hepatocytes with irregular clear vacuoles distributed throughout the sections examined. The nuclei in some cells were absent or chromophobic while in others they appeared slightly pyknotic.

Five lactating does, three wether kids and three doe kids were fed Senecio jacobaea. One wether kid and one doe kid died after consuming 148% and 404% of their initial body weights in Senecio jacobaea respectively. Both had the typical hepatic lesions seen in cattle dying from chronic pyrrolizidine alkaloid poisoning. The doe died 296 days after the last exposure to the Senecio while the wether died during the feeding trials. Two lactating does developed diarrhea which progressed, three days later, to central nervous system disorders, manifested as opisthotonos and muscular incoordination. These goats were euthanized when they were unable to stand. This was 348 days after their last exposure to Senecio jacobaea. One goat had megalocytosis of the hepatocytes after consuming

125% of her initial body weight in Senecio jacobaea over a 152 day period. The other had fatty changes of the hepatocytes, but this could have resulted from hypoxia due to verminous pneumonia which was also present. This goat consumed 141% of her initial body weight in Senecio jacobaea over a 388 day period. The etiology of the central nervous system disorders was not determined. The remaining goats are in apparent good physical health after consuming from 9% to 305% of their initial body weights in Senecio jacobaea. Most have eaten near 50%. The goat is therefore more resistant to pyrrolizidine alkaloid poisoning than are cattle and horses.

The lesions exhibited by rats and calves consuming milk from goats fed Senecio jacobaea suggest that possible milk transfer of pyrrolizidine alkaloids should be further studied in view of potential public health consideration.

Determination of the Transfer of Pyrrolizidine Alkaloids into Goats Milk by Biological Assay

by

Douglas Eugene Goeger

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June, 1980

APPROVED:

Redacted for privacy

Professor of Animal Science in charge of major

Redacted for privacy

Head of Department of Animal Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented Oct. 9, 1979

Typed by Clara Homyer for Douglas Eugene Goeger

ACKNOWLEDGEMENT

I wish to extend my sincerest appreciation to those who helped me complete this program. Those who deserve a special note of thanks are my major professor, Dr. P. R. Cheeke, the members of my graduate committee and Dr. D. R. Buhler.

Those who devoted countless hours to this project are: Mary E. Rasmusson, for the collection of the large quantities of tansy ragwort used in these studies, Dr. J. A. Schmitz and the Veterinary Diagnostic Laboratory for the histopathological work, and R. A. Swick and J. C. Throckmorton for help in the animal management. My gratitude is extended to the Department of Poultry Science for the chickens and facilities for the chicken experiment and to Dr. S. Nicholson of the Louisiana Cooperative Extension Service for supplying the Senecio glabellus. Special thanks go to Marvin and Glenn Ufford of the Ufford Dairy in Lebanon for the donation of the calves and to Holyoke Adams for his help in locating them.

I would like to thank the Department of Animal Science for the financial support by way of the graduate research assistantship. I am also in debt to my parents for the financial and moral support in accomplishing this goal in my life, and Mary Pierson for her helpful advice.

TABLE OF CONTENTS

LIST OF TABLES

DETERMINATION OF THE TRANSFER OF PYRROLIZIDINE ALKALOIDS IN GOATS MILK BY BIOLOGICAL ASSAY

INTRODUCTION

Senecio jacobaea, more commonly known as tansy ragwort in the Pacific Northwest, has caused extensive livestock losses in susceptible species. A common weed in Great Britain, it has spread to New Zealand, Australia, Canada and the United States. Its toxicity was suspected by farmers as early as 1787, however it was not confirmed until the beginning of the twentieth century. The disease, known by different names in the various countries where it occurs, causes progressive and irreversible liver damage which may be fatal months after the toxic dose has been consumed. The toxic factor is a group of alkaloids of the pyrrolizidine type which are also found in other genera of plants. Though non toxic themselves, conversion to the pyrrolic metabolite by the liver is responsible for the insidious poisoning.

Of more recent concern is the possibility that these alkaloids could gain access to the human food chain by way of food products from animals consuming the toxic plants. They have been suspected of causing death and illness when present as contaminates of grain or when the plants are brewed into teas. The purpose of the following study was to investigate the transfer of

pyrrolizidine alkaloids in goats milk. This was done by biological assay using rats and bull calves. The effect of chronic administration of low dietary Senecio jacobaea on the rat was investigated for comparison with result obtained from the assays. Due to the limited published work on the susceptibility of goats to Senecio jacobaea, this also was examined.

LITERATURE REVIEW

Overview of *Senecio jacobaea* and Pyrrolizidine Alkaloids

Tansy ragwort (*Senecio jacobaea*) is a toxic plant found throughout the world in temperate to subtropical zones (Muth, 1968). Indigenous to Europe and the British Iles (Muth, 1968; Snyder, 1972), it was first observed in Oregon in 1922. Since the early 1950's, *Senecio jacobaea* poisoning of livestock has been on the increase (Snyder, 1972). Presently in Oregon, there are more livestock deaths precipitated by this plant than all other toxic plants combined. Livestock most commonly affected are horses and cattle.

Although unpalatable and not normally consumed, it may be ingested in overgrazed pastures where it constitutes the principal vegetation. Consumption may also occur in pastures so heavily infested that it is inadvertently consumed or when it is a contaminate of fodder.

Sheep are more resistant to its toxic principal, and may consume large quantities without exhibiting clinical signs of poisoning (Muth, 1968). Due to their resistance, the use of sheep for control of *Senecio jacobaea* has been suggested (Dollahite, 1972; Mosher, 1979).

The poisonous principal in *Senecio jacobaea* is a

group of pyrrolizidine alkaloids (Kingsbury, 1964; Bull et al., 1968). Six have been isolated and identified; they are jacobine, jacoline, jaconine, jacozine, senecionine, seneciphylline (Bull et al., 1968). Pyrrolizidine alkaloids were first isolated in the early 1900's by Watt (Schoental, 1957) and were thought to be characteristic only of the Senecio species (Bull et al., 1968). Since then, they have been isolated from other genera. Several of the more common are Crotalaria, Heliotropium, Amsinckia, Echium, and Trichodesma (Appendix Table 3). There are representative plants in eight families. These include Boraginaces (Heliotropium), Compositae (Senecio) and Leguminosae (Crotalaria). Over 100 alkaloids have been isolated and structural configuration determined. The structures of those mentioned in the text are given in Appendix Table 4.

The pyrrolizidine alkaloids are unique in that they are the only hepatoxic alkaloids known (Stillman et al., 1977). Although suspected of being carcinogenic, teratogenic, and possessing some pharmocological properties (McLean, 1970), their hepatoxicity cannot be disputed. While exhibiting extrahepatic lesions in some species, prolonged exposure usually results in a cumulative irreversible cirrhosis of the liver in susceptible species. This condition often results in death with the absence of clinical symptoms except in the agonal stages (Bull et al.,

1968). Another characteristic of these toxins is that death can occur many months after the lethal dose has been consumed. This results from disturbance or loss of hepatic function.

Human poisoning has occurred from consumption of grains contaminated with the seeds of the toxic plants. Herbal teas, brewed as folk remedies from plants containing pyrrolizidine alkaloids, have also resulted in poisoning (Stillman et al., 1977). Case histories have been reported of such poisonings, some of which will be discussed later.

The possibility that these toxins may contaminate food of animal origin, when the plants are ingested by the animal, has raised much concern. One possibility would be the transfer of the alkaloids into milk. Other food products have been viewed as possible sources of contamination. This will also be considered in more detail in subsequent sections.

Pathology Due to Pyrrolizidine Alkaloids

The principal organ affected by pyrrolizidine alkaloid poisoning is the liver, and death results from the disturbance or loss of its function (Bull et al., 1968). Death might also result from infections or stress to which the alkaloids have decreased the animals tolerance. There can be variations in the pathological lesions produced in a given species as a result of dosage and time involved in

production of the lethal effect. Variations in lesions are also noted between certain susceptible species (Schoental, 1968; Selzer and Parker, 1951).

The lesions as a result of chronic intoxication are characterized by enlargement of the parenchymal hepatocytes, small atrophic lobules, bile duct proliferation, firboris and collagen formation (Bull et al., 1968). These can result from the exposure to small levels of the alkaloid over an extended length of time or a larger dose which does not have an immediate lethal effect.

The enlargement of the parenchymal cells and nuclei (megalocytosis), is the most characteristic change (Bull et al., 1968; Culvenor et al., 1962). This results from the antimitotic effect of the alkaloids which produces a polyploid cell which becomes larger with time. All hepatocytes are susceptible to high doses of the alkaloids, but the contrilobular cells are the most vulnerable (Bull et al., 1968). Accordingly, the lesions are primarily of centrilobular distribution.

In acute or subacute poisoning, the primary lesions are that of centrilobular or zonal necrosis, and hemorrhagic extravasation (Bull et al., 1968; Culvenor et al., 1962). Little time has elapsed prior to death for the development of the enlarged hepatocytes, fibrosis, or bile duct proliferation. Peracute doses given experimentally,

often cause convulsions prior to death (Schoental and Mattocks, 1960). This is suggestive of neurologic envolvement.

Due to variations in the chemical structures, there are differences in the secondary pathological effects and in cell specificity (Culvenor et al., 1962). Although not characteristic of pyrrolizidine alkaloids, extrahepatic lesions occur and are generally limited to the kidney, lung, and vascular system (Bull et al., 1968). The changes in the kidney are generally those of enlarged epithelial cells of the nephron. Lung lesions are characterized by edema, fibrosis, foci of consolidated proliferative tissue, or epithelization. The blood vascular system is susceptible to high concentrations of pyrrolizidine alkaloids and results in hemorrhagic extravasation in the liver, peritoneal cavity and elsewhere.

Extrahepatic and variations in lesions are often characteristic responses for certain susceptible species (Schoental, 1968a). The lesions of the kidney and lung are common in the poisoning of horses and pigs (Bull et al., 1968). In primates, the vascular system of the liver is generally involved (Schoental, 1968a). This is manifested as occlusion of the lumina of the central and sublobular hepatic veins by fine fibril deposition, or by thickening of the innermost coat in the larger hepatic veins (Selzer

and Parker, 1951). Hill and Rhodes (1953) described it in humans as a gross hepatic fibrosis beginning as an edema, followed by deposition of a coagulum, giving way to invasion by fibroblasts.

The rat is the experimental animal of choice used in laboratory pyrrolizidine alkaloid research (Bull et al., 1968). It is used not only for economic and management reasons, but also because the lesions developed resemble those of livestock. However, they often exhibit a less fibrotic liver than cattle or horses. Because of its extensive use, much is known about the lesions produced under various conditions of poisoning.

Acute poisoning in the rat results in the typical centrilobular necrosis and frequently hemosiderin deposits in the kidney (Bull et al., 1968). This is apparently from red blood cell destruction, and can be the cause of death.

When exposed to repeated small doses (0.1 of the LD_{50}) of some alkaloids, rats show the expected irreversible additive lesions (Bull et al., 1968). At death, the total chronic lethal dose may be 5 times that of the 72 hour LD_{50}. With repeated doses of 0.02 of the LD_{50}, survival time is increased over that of the 0.1 LD_{50} and the liver lesions are much more pronounced. At this low level, complete inhibition of growth and division of the hepatocytes may not result, and some focal regeneration may be evident.

Concurrent with liver lesions, small doses also cause a progressive lung lesion which may prove fatal (McLean, 1970).

The bovine calf is very susceptible to pyrrolizidine alkaloid poisoning. This is shown by the experimental feeding by Dollahite (1972) of Senecio longilobus to 12 calves. They received a total of 0.25-1.0% of their body weight in dried plant in 1-3 days. Ten of the calves died between 1 and 281 days. All had typical acute or chronic liver lesions. The acute lesions were exhibited by 6, which succumbed before 43 days. The bovine often shows edema of the omentum, mesentery, and of the folds of the mucosa (Bull et al., 1968). There is also an increase of fluid in the serous cavities, especially of the peritoneal.

Fowler (1968) reported on the clinical findings of calves poisoned by hay contaminated with Amsinckia intermedia and Senecio vulgaris. These calves had tenesmus with rectal prolapse. Blackish liquid feces was evident in the former, but absent in the latter. They exhibited a goose-stepping gait, and often bumped into obstacles. There was edema of the intestinal mesentery and associated lymph nodes. The liver exhibited a whitish mottling which, upon histological examination, revealed megalocytosis and diffuse fibrosis.

Thorpe and Ford (1968) fed 5 calves varying levels of pellets containing 20% Senecio jacobaea and produced the

characteristic edema, parenchymal megalocytosis, and fibrosis. They also found anorexia and occlusion of some veins of the liver in all calves.

Serum changes have been studied as a diagnostic aid in pyrrolizidine alkaloid poisoned animals. Ford et al. (1968) studied serum changes displayed by the preceding 5 calves. These were primarily of protein production, bilirubin excretion, and enzyme release. There was no definite change in total protein, but a fall in albumin and a rise in globulin concentrations was seen in two calves. The albumin/globulin ratio generally falls in chronic liver disease. There was no change in bilirubin removal except just prior to or during the agonal stages. Elevation of enzyme levels occurred in all calves but at different times and duration. They occurred initially when there was minimal cellular changes. Levels were generally lower or near normal just prior to death.

The enzymes which showed elevation were glutamate-oxaloacetate transaminase, glutamate dehydrogenase, sorbitol dehydrogenase, and ornithine carbamyl transferase (Ford et al., 1968). Glutamate pyruvate transaminase was also measured, but did not show any significant changes in any of the calves.

Dickinson et al. (1976) studied enzyme changes in lactating dairy cattle poisoned with Senecio jacobaea.

A decline in plasma albumin concentrations, apparently due to a decreased liver activity, was seen. Also noted was an increase in sorbitol dehydrogenase activity preceding the appearance of megalocytosis and fibroplasia. Persistent diarrhea, weight loss, reduced milk production and anorexia were also observed.

Goats, like sheep, can consume relatively large quantities of Senecio plants before clinical signs, lesions or death result (Dollahite, 1972). These plants are often extensively grazed with no serious losses in regions where cattle losses have occurred. Dollahite (1972) reports on work of Mathews who fed Senecio longilobus to three goats. One animal died after consuming 77% of its body weight in dried plant material, and showed moderate cirrhosis of the liver. The others had consumed 81 and 115% of their body weight when killed for necropsy. The former had no clinical signs, but revealed typical lesions of chronic Senecio poisoning. The latter was anorexic and had moderate cirrhosis of the liver. It was estimated that 20 times as much Senecio is required to poison sheep and goats, on a body weight basis, as that of cattle.

In most respects, the lesions in humans resemble those of Senecio poisoning in rats and horses (Selzer and Parker, 1951). The clinical signs begin with upper abdominal pain and marked ascites. The latter may progress

to the lower limbs (Selzer and Parker, 1951; McGee et al., 1976; Lyford et al., 1976). There is often marked edema of the large intestine (Selzer and Parker, 1951). The liver upon palpation is usually enlarged, extending well below the costal margin. Upon gross examination it is pale, soft and exhibits a mottled appearance (McGee et al., 1976). Microscopic characteristics are markedly distended sinusoids from red blood cell accumulation (McGee et al., 1976; Stillman et al., 1977). There is often an absence of hepatic cells in the centrilobular area (McGee et al., 1976; Selzer and Parker, 1951) which are replaced by red blood cells and Kupffer cells (Selzer and Parker, 1951). This is evident in virtually every lobule. The surviving hepatic cells in the peripheral zones show fatty change, but hepatic necrosis is absent. There is little or no regeneration.

The central and sublobular veins show changes which are characterized by occlusion of the lumina (Selzer and Parker, 1951; McGee et al., 1976; Lyford et al., 1976; Stillman et al., 1977). This is due to fine fibril deposition, or in the larger hepatic veins by thickening of the innermost coat (Selzer and Parker, 1951).

Lyford et al. (1976) report on a case revealing the typical gross and histological symptoms. This patient recovered after eight weeks of hospital treatment. A

liver biopsy taken a year later showed complete resolution of the pathological changes. This is not typical of Senecio poisoning (Bull et al., 1968; Mclean, 1970).

Pyrrolizidine Alkaloid Contamination

Toxic compounds are not necessarily products of the chemical industry, and certain natural weeds, which modern chemicals help to eliminate, may present a considerable hazard to livestock and man (Schoental, 1963). The contamination of both food and feedstuffs with pyrrolizidine alkaloid containing plants has caused poisoning of both humans (Selzer and Parker, 1951; Anonymous, 1978) and livestock (Hooper and Scanlan, 1977; Fowler, 1968). Human intoxication has also occurred from ingestion of herbal teas brewed as folk remedies from plants containing pyrrolizidine alkaloids (Stillman et al., 1977). Plants which are known to contain, or suspected of containing, these hepatoxic alkaloids have been recommended for medicinal and other purposes in different countries (Schoental, 1963). Although more common in the underdeveloped countries, their use is not limited here. In the past, Senecio jacobaea has been sold by herbalists in England. Pyrrolizidine alkaloid poisoning is not confined to the direct contamination of food, but could result indirectly by the excretion of the alkaloids into food

products by animals consuming these toxic plants. Milk has been suspected as a possible source of contamination and will be reviewed in more detail later in this section.

Due to the cumulative toxicity of pyrrolizidine alkaloids, any level of contamination should be considered perilous. In rats, lesions resulting from a small number of doses are qualitatively indistinguishable from those due to a single dose (Schoental, 1963). They may even be more pronounced in the former case.

Selzer and Parker (1951) reported on twelve people exhibiting Senecio poisoning symptoms in South Africa. These were thought to be caused by consumption of bread contaminated with seeds of Senecio species. Although no attempt was made to confirm this, the patients reported that the bread had an abnormal "musty" or bitter taste. Senecio burchelli and Senecio ilicifolius have been reported to contaminate wheat. Six of the patients died and it appeared that males and females were affected with equal frequency with no partiality to any age group.

During 1973 and 1975, an epidemic of a fatal liver disease characterized by centrilobular necrosis and occlusion of the efferent hepatic veins occurred in India (Anonymous, 1978). This resulted from consumption of millet contaminated with seeds of Crotalaria species. A similar outbreak occurred in Western Afghanistan in 1974 by consumption of wheat contaminated with seeds of

Heliotropium plants. Comparative biopsies during the active phase of the disease and after recovery in the latter case, revealed complete disappearance of initial abnormalities.

(a) Herbs and Teas

Consumption of teas brewed from pyrrolizidine alkaloid containing plants represents a unique situation. The practice of making tea from leaves of various plants is well established in Jamaica, and neighboring islands, for the treatment of an assortment of diseases (Bras *et al.*, 1954). *Senecio* plants are also known to be favorite remedies of the African Negro (Schoental, 1954).

Hill and Rhodes (1953) reported on the investigation of 150 cases of liver disease, characterized by occlusion of the hepatic veins, in Jamaican children. They ranged in age from 4 months to 16 years. All were of poor or lower middle class homes where it was common in the neonatal period for most babies to be given "bush tea." This is an infusion of leaves, flowers or seeds of various plants. Also in common with all cases was a diet of low protein, the majority of which was of plant origin. The diet was suspected as being the cause, however toxic involvement was not ruled out. The effects of pyrrolizidine alkaloid poisoning are more pronounced if superimposed on protein deficiency (Schoental, 1968a; Cheeke and Garman, 1974; Stillman *et al.*, 1977).

Similar, but not identical, diseases as those in the Caribbean have been noted in Egypt and South Africa (McGee et al., 1976). In other countries, occlusion of the hepatic veins is very uncommon.

The consumption of bush teas, termed herbal teas, is also practiced in the more developed countries. It is thought that pyrrolizidine alkaloid poisoning may be an "iceberg" disease in the U.S.-Mexican American population (Stillman et al., 1977; Huxtable et al., 1977). This is due primarily from brewing teas with Senecio longilobus, mistaken or substituted for the herb gnaphaluim (gordolobo yerba). Senecio longilobus is indigenous to the deserts of the southwestern U.S. and northern Mexico (Huxtable et al., 1977). The tea is used widely as a gargle and cough medicine by these people. This plant has been found to contain 1.3% by weight of pyrrolizidine alkaloids. Consumption of these preparations may lead to compromised liver or lung function, which may remain subclinical. Stillman et al. (1977) state that children with atypical hepatitis should be suspected of pyrrolizidine alkaloid ingestion.

Fox et al. (1978) reported on a two month-old Mexican American boy who had been given a tea made from gordolobo, later identified as Senecio longilobus. The infant died on the sixth day of hospitalization with

symptoms of acute poisoning. It was calculated that 66 mg. of mixed alkaloid was consumed over a four day period. Stillman *et al.* (1977) investigated a Mexican American infant with a similar case history. In this instance the child survived though consuming between 70 and 147 mg. of alkaloid in a two week period. In Southern Arizona, a 62 year old woman died 6 months after consuming large quantities of gordolobo tea, partially prepared from *Senecio* (Huxtable *et al.*, 1977). On autopsy, a cirrhotic liver was found though she did not consume alcohol.

Poisoning by herbal teas is not limited to the Mexican-American population of the southwestern U.S. In Britain, a 26 year-old woman suffered a fatal episode of *Senecio* poisoning after consuming maté or Paraguay tea over a two year period (McGee *et al.*, 1976). Analysis of the patient's sample revealed the presence of trace amounts of pyrrolizidine alkaloids. None could be detected in material from the "health" food store from where it was obtained. A 35 year-old woman from Ecuador consumed a herbal tea, prepared from *Crotalaria juncea*, for 6 months (Lyford *et al.*, 1976). She survived, and 1 year later revealed complete resolution of pathological changes which were initially exhibited.

(b) Food and Milk

The contamination of food of animal origin, by

pyrrolizidine alkaloids or toxic metabolites, represents a potential danger. Though these levels may not equal those found in contaminated grains or teas brewed from the toxic plants, the cumulative effect of these alkaloids (Bull et al., 1968; McLean, 1970) would make any level pernicious. Investigations have shown that this potential danger is probable with respect to honey and milk.

Bee colony owners have been able to detect the characteristic odor of Senecio jacobaea in honey prepared from areas where this weed was abundant (Dickinson et al., 1976). The blossoms contain the highest concentration of alkaloids in the plant. In samples of honey analyzed, levels up to one ppm were detected. The more contaminated samples had the characteristic odor and were not considered palatable.

Dienzer and Thomson (1977) analyzed four samples of honey from western Oregon and western Washington suspected of pyrrolizidine alkaloid contamination. Small amounts (0.5-2.5%) of Senecio jacobaea pollen were found. Concentrations of alkaloids ranging from 0.3-2.5 ppm were also measured, though these were not considered accurate quantitative figures. All six alkaloids present in the plant were found in the honey. The samples were bitter in taste and off colored so similar samples would probably not be marketable. Due to the low per capita honey consumption

in the U.S. (0.6 kg/year), acute levels of pyrrolizidine alkaloid contaminated honey would probably not be consumed (Dienzer and Thomson, 1977).

Unlike honey, milk is used extensively as a food source. Contamination of milk could cause toxic effects if consumed over long enough periods of time. Milk would constitute a larger portion of the diet in infants and young animals which, in susceptible species, are more sensitive to these alkaloids (Bull et al., 1968: Mclean, 1970).

Schoental (1959) administered solutions of the pyrrolizidine alkaloids lasiocarpine and retrosine, by intraperitoneal injection or stomach tube, to post partum rats. Lasiocarpine was given as a single dose or divided doses at a few days interval in quantities close to the LD_{50}. The young were allowed to nurse their mother. A few of the young died between 4 and 7 weeks with lesions similar to those produced by pyrrolizidine poisoning. Most survived to 6 months at which time examination revealed no abnormalities. Microscopically there was some mild fibrosis and a few enlarged hepatocytes.

Rats were dosed with quantities of retrorsine ranging from 4-10 mg. (Schoental, 1959). The number of doses varied between 1 and 14. Milk production did not appear to be affected by the treatment and some mothers showed no

ill effects. Liver abnormalities of the young were only slight when dying the first days of life. These became increasingly more prominent when death was delayed to about 7 weeks. Others remained in apparent good health for 6-10 months after birth.

The most common hepatic lesion of those animals dying between 18 and 30 days was edematous or fatty vacuolation of the parenchymal cells (Schoental, 1959). Some lobes showed little fatty change but there were areas of hemorrhaging into distended sinusoids. Liver biopsies of the surviving rats had a less abnormal appearance, and showed very few of the large parenchymal cells. Some contained hyperplastic nodules, strands of fibrous tissue and focal bile duct proliferation.

Though mortality was high in the rats receiving the higher doses of retrorsine, at lower doses it was evident that alkaloids can poison the suckling young with no noticeable effect on the mother (Schoental, 1959). The possibility that the toxic factor in the milk was a metabolic oxidation product was not ruled out.

Johnson (1976) administered Senecio jacobaea to 6 lactating cows for 30-84 days. Total intake ranged from 8.5-15% of their body weight. Calves were allowed to nurse their dam, and milk was given by stomach tube to rats either fresh or after being frozen for two years. This was given in 12 ml daily doses for 15-30 days. Of the

50 rats of both groups, all were free of any histological lesions.

All the lactating cows died at about 3 months with typical symptoms of pyrrolizidine alkaloid poisoning (Johnson, 1976). No histological lesions were found in the calves, though elevated serum lactate dehydrogenase and glutamate-oxaloacetate transaminase enzyme levels were found.

Dickinson et al. (1976) administered Senecio jacobaea to 4 lactating cows via rumen cannula at a rate of 0.25-1.0% of their body weight daily. The milk was bucket-fed to four bull calves twice daily. A declining physical state, characterized by weight loss and reduced milk production, was evident in the cows. They received the Senecio over a 14-26 day period. The calves failed to develop any abnormal lesions.

Milk samples were analyzed for alkaloid content by the method of Mattocks (1967). This procedure only gave a 20% recovery rate on samples of milk supplemented with known amounts of alkaloid (Dickinson et al., 1976). Mean concentrations of alkaloid in the experimental milk ranged between 47.0 and 83.5 ug/100 ml with compensation for the 20% recovery rate. Isolation by paper chromatography revealed jacoline as the only alkaloid to be present in the milk.

In a subsequent study, Dickinson and King (1976)

dosed four lactating goats, by rumen cannula, with 1% of their body weight in dried Senecio jacobaea daily. The milk was fed twice daily to their isolated kids for three weeks. Necropsy revealed no gross, biochemical, or histological lesions in either the milk goats or their kids. The mean alkaloid content of the milk samples was 266 ug/100 ml. Gas chromatography revealed jacoline in the highest concentration with a significant amount of jacobine.

Atypical Pathogenesis

Though alkaloids may not be in milk at concentrations high enough to produce short term toxic responses, the possibility of latent reactions cannot be overlooked. There is disagreement about the carcinogenic activity of pyrrolizidine alkaloids (Mclean, 1970; Svoboda and Reddy, 1972) but they have been reported to produce hepatomas in rats and chickens (Mclean, 1970; Schoental, 1968a). Most studies require more than a year after dosing for the tumors to develop (Harris and Chen, 1970; Svoboda and Reddy, 1972; Schoental, 1968a). In the preceding studies on milk transfer, assay animals were sacrificed at relatively short periods of time.

It has also been shown that certain pyrrolizidine alkaloids can cross the placenta and cause liver lesions in the fetus (Sundareson, 1942) and teratogenic changes

(Green and Christie, 1961). Whether ingestion of milk, contaminated with these alkaloids, at a critical time in pregnancy could have similar effects in humans must be expected unless proven otherwise.

(a) Carcinogenic Action

The disagreement in the hepatocarcinogenicity of the pyrrolizidine alkaloids may be due to differencies in the length of the study, alkaloid used and means of administration, strain or species of animal (Svoboda and Reddy, 1972), differences in dosing schedule (Mclean, 1970), and contamination of the diet with aflatoxin (Svoboda and Reddy, 1972; Mclean, 1970). The fact that negative results are not usually reported, and the disagreement among researchers over the morphological definition of hepatomas, further complicate the issue (Mclean, 1970).

The optimal conditions for the production of liver tumors have not been established (Schoental, 1963), though repeated intermittent doses of retrorsine and its N-oxide, isatidine, induced primary liver tumors in rats (Schoental, 1968b). Also, a single oral dose of retrorsine given to weanling rats induced hepatomas in 20% of the animals surviving more than a year. Liver carcinomas in rats are more likely to develop after repeated intermittent administration of small doses of the alkaloids (Schoental, 1963). Even smaller doses are effective when given to

newborn rats (Schoental, 1968b). If these conditions hold true for humans, then periodic consumption of contaminated milk could have similar latent effects.

Harris and Chen (1970) produced hepatomas in rats fed intermittently diets containing Senecio longilobus. The highest incidence occurred with feeding a 0.5% Senecio diet for one week alternated with a Senecio free diet during the intervening week for 54 weeks. Of 50 males and 50 females started, only 47 rats lived more than 200 days. Of these, 14 males and 3 females developed malignant liver tumors between 217 and 470 days. By feeding the Senecio diet for a month alternated with the Senecio free diet for two weeks for a year, fewer hepatomas evolved which took a longer time to develop. It was evident from this study that male rats are more susceptible to the development of liver tumors than females when exposed to pyrrolizidine alkaloids.

Injection of lasiocarpine is capable of inducing malignant tumors of the liver and skin (Svoboda and Reddy, 1972). Twenty-five inbred male Fischer rats were injected twice weekly for 4 weeks followed by weekly injections for 52 weeks with 0.1 of the LD_{50} of lasiocarpine. Dosing was then discontinued. Of 18 rats that survived between 60 and 76 weeks, 16 developed tumors. Liver tumors were present in 61%, and 33% developed squamous cell carcinomas of the

skin. Ten of these developed more than one tumor. The rats received an average total dose of 125 mg of alkaloid. The dosing was discontinued 4-20 weeks before the appearance of most tumors. Control rats given saline injections in lieu of lasiocarpine failed to develop any of the same tumors.

Studies on the carcinogenic effect of the pyrrole form of some pyrrolizidine alkaloids have been done. This is believed to be the principal toxic metabolite of the alkaloids (Mattocks, 1968), which is produced by the hepatic microsomal enzyme system (Mattocks, 1971). The pyrrole will react very rapidly with water, soluble thiols, some amino acids, as well as nucleophilic groups in macromolecules (Mattocks, 1972). They can be prepared as solutions in non-toxic non-aqueous solvents, but will be expected to react as soon as they meet the aqueous phase of the blood and tissues (Butler et al., 1970).

Allen et al. (1975) produced highly malignant tumors of the striated muscle at the site of subcutaneous injection with dehydroretronecine (pyrrole synthesized from monocrotaline). These rats were given 20 mg/kg body weight biweekly for 4 months followed by 10 mg/kg biweekly for the succeeding 8 months. The repeated localized exposure may have been the reason for the tumor development (Allen et al., 1975). This would not be expected with

natural ingestion of the alkaloids, however the pyrrole produced in the liver could have similar effects on hepatic tissue. Ten percent of another group of rats developed tumors after biweekly injections of 5 mg/kg body weight of monocrotaline for 12 months (Allen et al., 1975). Three percent were hepatocellular carcinomas. Hooson and Grasso (1976) also produced injection site sarcomas with weekly injections of 60 ug and 30 ug of the monocrotaline pyrrole in tricaprylin. There was no statistical difference between controls injected with tricaprylin alone. The slight increase in numbers of sarcomas in the pyrrole group, over controls, was thought to be due to the monocrotaline pyrrole enhancement of the tumorigenic effect of the oil. Whether pyrrolizidine alkaloids can enhance the effect of other carcinogenic food contaminates, or vice versa, is a subject which should receive further attention.

(b) Teratogenic and Fetotoxic Effects

It has been shown that pyrrolizidine alkaloids possess teratogenic properties (Green and Christie, 1961) and can diffuse in either direction across the placental barrier producing toxic effects in both the mother and fetuses (Sundareson, 1942; Newberne et al., 1968).

Teratogenic changes are alterations in the formation of cells, tissues, and organs resulting from physiological and biochemical changes (Casarett and Doul, 1975).

Factors responsible for these changes can be physical, nutritional, hereditary or chemical in nature. Once the fetal tissue has formed, a teratogenic response can no longer occur. At this stage of gestation, the fetus is liable only to toxic changes which can affect any anatomical, physiological or biochemical system.

A total dose of 15 mg of senecionine was given in 3 equal doses to rats in their third trimester of pregnancy (Sundareson, 1942). This resulted in centrilobular necrosis and hemorrhagic exudates in necrotic areas. In the fetuses, there was cytoplasmic vacuolization and swelling of the hepatocytes. Blood vessels and sinusoids were markedly distended with blood. Five fetuses of one rat were injected with 15 mg senecionine and 4 of another with 12.5 mg at day 19 of pregnancy (Sundareson, 1942). In the former, the mother died and had extensive degeneration and necrosis around the central vein. In the latter, the mother was killed on the second day following the injections and revealed the same type of degenerative changes, but to a lesser degree. Injection of rats biweekly with senecionine starting at different times from the twelfth day of gestation onward, resulted in a high mortality rate within litters (Sundareson, 1942). There were a number of premature litters. Histological appearance was similar to those already mentioned. Repeated administration to mated

rats before the twelfth day of gestation frequently resulted in failure to implant.

Heliotrine was injected as a single dose between 15 and 300 mg/kg body weight in rats during their second week of gestation (Green and Christie, 1961). Litters of those injected with less than 50 mg/kg were normal. In rats dosed with 100 mg/kg, there was a high intrauterine mortality rate. These fetuses were subnormal in size and weight, and one of 6 litters had skeletal deformities. In the higher doses, there was a greater incidence in malformities with severity proportional to dose. The most common abnormality was dwarfism, with a high frequency of musculoskeletal defects (Green and Christie, 1961). These were predominantly of the ribs. Cleft palate was also noted in a number of cases. The possibility that these abnormalities could have resulted from disturbances of the vasculature was considered. This is a characteristic of many of the pyrrolizidine alkaloids (Green and Christie, 1961; Mclean, 1970).

Newberne et al. (1968), in testing the effect of control or low lipotrope diets on pyrrolizidine alkaloid toxicity, dosed pregnant rats with lasiocarpine by gastric intubation. This was given at the rate of 75 or 100 mg/kg body weight, or as a total dose of 70 mg/kg in two equal doses, 4 days apart. Lasiocarpine at 100 mg/kg resulted

in the death of approximately half of the mothers which exhibited liver necrosis. These lesions were more severe in the low lipotrope group. Fetal weights were reduced in the low lipotrope group which also exhibited a higher incidence of liver necrosis. The 75 mg/kg group had a better survival rate of both the mothers and their fetuses which resulted in more marked lesions than the preceding groups. In the 70 mg/kg two-dose group, both the mothers and fetuses were considerably less affected when compared to the two higher dose rates. There were fewer stillborns, and fetal weights were decreased only in the low lipotrope group. There was some liver deterioration initiated in utero. Offsprings killed seven weeks after birth showed bile duct proliferation and some enlargement of parenchymal cell nuclei in the periportal and centrilobular zones. These changes were more severe in the periportal area of the low lipotrope treated animals.

Whether these teratogenic or fetotoxic responses are from the parent alkaloid, the pyrrole, or another toxic metabolite remains to be shown. The parent alkaloids have been reported to be low in toxicity until being converted to the pyrrolic form by the liver microsomes (Mattocks, 1968). In the fetus or newborn, these enzyme systems are several times lower than in the adult (Fouts, 1959). Due to the high reactivity and short half life of pyrrole

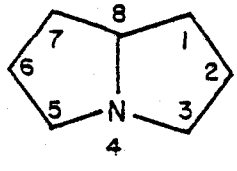
metabolites in aqueous environments,the possibility of pyrroles diffusing through the placenta to exert a toxic action on the fetus seems remote. The placenta is a very active site in biotransformation of both exogenous and endogenous substances (Casarette and Doul, 1975) and could be the source of toxic metabolites.

These studies reviewed on the effects of pyrrolizidine alkaloids to the developing fetus deal with innoculations of relatively large doses of pure solutions. This would not necessarily parallel the consumption of contaminated staples. There have been field cases which relate more closely to this. Fowler (1968) reported on calves which died who were born to cows fed hay contaminated with Senecio vulgaris during pregnancy. The calves were maintained on Senecio free hay from birth which suggests poisoning in utero. Hooper and Scanlan (1977) reported on a piggery which observed a high prevalence of congenital abnormalities, particularly cleft palates. These were of piglets born to sows fed grain contaminated with 0.1% Crotalaris retusa seeds for three weeks. This was followed by an additional week of grain contaminated with 0.05% Crotalaria.

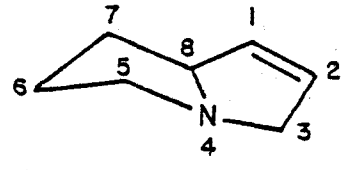
Structure In Relation To The Characteristics Of And Pathology Due To Pyrrolizidine Alkaloids

An understanding of the characteristics and pathological lesions of specific pyrrolizidine alkaloids can aid in the evaluation of responses produced by unknown alkaloids in biological assays. Differences are due, in part, to the specific structures of the alkaloids. This may influence the metabolism of the pyrrolizidine alkaloid to the reactive pyrrole derivitives, and the chemical reactivity of the pyrrole metabolite itself (Mattocks, 1978). This may result by influencing the proportion and rate of alkaloid converted. The acute toxicity of various alkaloids can vary by a factor of 10 depending on their structure (Schoental, 1968a).

The pyrrolizidine alkaloids are derivatives of the pyrrolizidine nucleus (I). It is composed of 2 fused five membered rings inclined to each other (Schoental, 1968b) like the wings of a butterfly. The rings are nearly planar when unsaturated and buckled in an exo or endo sense if saturated (II) (Culvenor and Willette, 1966).



I

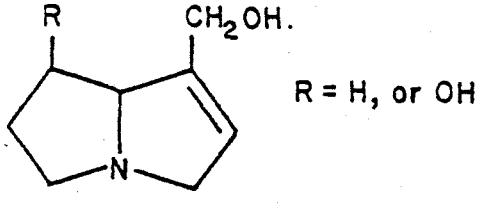


II

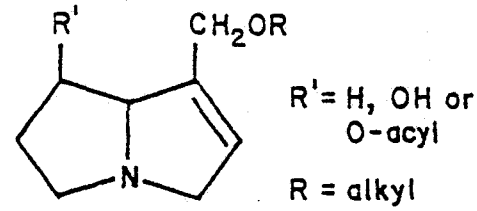
A nitrogen atom is at a cis ring junction between the two rings, designated position four.

All of the toxic alkaloids (IV) are derivatives of the amino alcohol I-hydroxymethyl-1:2 dehydropyrrolizidine (III) (Mclean, 1970). Due to the presence of an asymmetrical carbon atom, the amino alcohols can exist in several stereochemical forms (Schoental, 1968b). There are five from which the hepatoxic alkaloids are derived (Culvenor, 1978). These will be discussed in more detail in the amino alcohol section.

The toxic alkaloids are allylic esters of the basic amino alcohols with branched chain acids (Schoental, 1968a). These can be as a mono ester at the 1-methoxy group, or as an open or cyclic diester involving the 7-hydroxy position and the 1-methoxy group. Non-ester alkaloids found in the plants are not likely to be hepatoxic but could cause other pathological effects.

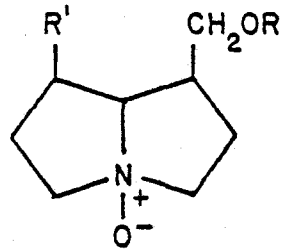


III



IV

The pyrrolizidine alkaloids are often present in plants as their N-oxides (V) (Schoental, 1957). These are especially prominent during the growing and flowering period, and may constitute the predominant part of the alkaloidal fraction (Schoental, 1963). The reduced and oxidized form are readily interconvertible depending on the redox-potential. The N-oxides resemble the parent alkaloids in their chronic hepatoxic action, but are considered more palatable. The acute and chronic effects of retrorsine N-oxide are qualitatively the same as retrorsine, but when given interperitoneally, it is only one-fifth as toxic as when given by stomach tube (Mattocks, 1972).



V

R' = H, OH or O-acyl

R = alkyl

Although toxic when produced in the body, they are considered to be detoxification products which, being highly water soluble, are rapidly excreted (Mattocks, 1972). The hepatic mixed function oxidase system of the liver is involved in N-oxide production.

The features of the pyrrolizidine alkaloids required for hepatoxicity are a double bond in the pyrrolizidine moiety at the 1-2 position, and the esterification of a

branched chain acid at the positions previously mentioned (Schoental, 1957). Alkaloids without the double bond, such as platyphylline and saracine, show no hepatoxicity (Culvenor et al., 1962). However, platyphylline, like many of the toxic alkaloids, is capable of inhibiting longitudinal tonus and antagonising acetyl choline in rat's ileum (McKenzie, 1958). Its activity is greater than the alkaloids of Senecio jacobaea and is estimated to be one-fifth to one-twentieth that of atropine. Whether this pharmacologic activity could affect absorption of the alkaloids by decreasing passage rate of ingesta is not known.

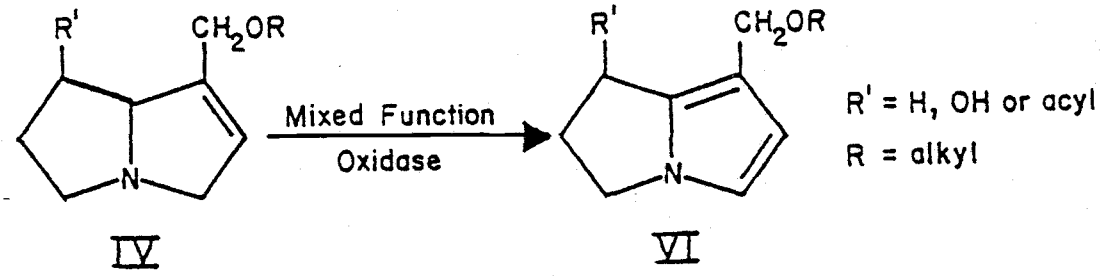
The amino-alcohols and acids derived from the toxic alkaloids by hydrolysis are not hepatoxic (Culvenor et al., 1962; Schoental, 1968a). Therefore, the intact ester is essential for toxicity.

The chemical reactivity of the parent pyrrolizidine alkaloids is usually low, limited to hydrolysis of the ester linkages and to readily reversible N-oxide formation (Mattocks, 1968). Culvenor et al. (1969) state that they do have weak alkylating reactivity, but far less than the corresponding pyrrole form.

Mattocks (1968) proposed a hypothesis for the toxic action of the alkaloids. The alkaloids themselves are not hepatoxic, but a portion is dehydrogenated by the mixed function oxidase system of the liver to a pyrrole like

derivative (VI). This possesses one or more electrophilic centers in addition to its pyrrole like properties. This metabolite reacts rapidly with certain nucleophilic tissue constituents, both soluble and insoluble, forming bound pyrroles. In this state, the high reactivity is lost but the pyrrole function remains. The soluble bound pyrroles are either eliminated in the urine or possibly absorbed on local macromolecular sites.

The pyrrole can be reacted with modified Ehrlich reagent (4-dimethylaminobenzaldehyde) (Mattocks, 1968) and its presence can then be quantitatively determined spectrophotometrically (Mattocks, 1967; Mattocks and White, 1970). The presence of the unsaturated alkaloids can be



IV VI

determined by first converting them to the N-oxide (Mattocks, 1967). This is then reacted with acetic anhydride to produce pyrrole like derivatives which then can be reacted with the modified Ehrlich reagent. Neither the parent alkaloid nor its N-oxide will react with this reagent (Mattocks, 1968).

These *in vitro* pyrroles were determined not to be the same as the metabolic pyrroles (Mattocks, 1968). This was based on differences between the absorption spectra of the two at 450-650 mμ. The curves were always similar but there was a shift to the higher wave length with the metabolic pyrroles.

By using the Ehrlich test, pyrrole-like metabolites can be detected in urine and tissues of rats dosed with pyrrolizidine alkaloids (Mattocks, 1968). These metabolites are not formed by incubating urine with the alkaloids. Metabolites from certain alkaloids can be demonstrated in the liver and other tissues after the alkaloids themselves have ceased to be detectable. The excretion of the unchanged alkaloids, and of their N-oxides, after intraperitoneal injection is almost complete after 24 hours. However, bound pyrroles can be detected in the liver, and at lesser levels in the lung and other tissues, up to 48 hours after treatment. In the liver, these are found bound to the solid debris and microsomal fraction.

The pyrrolizidine alkaloids form water-soluble salts at the physiological pH (7.4), and thus are partially excreted in the urine (Schoental, 1963). This depends on the pK_a of the alkaloids. Being weak bases, a pH below the pK_a would result in the alkaloid being predominately in the ionized, water soluble, state. At a pH above the pK_a, it

would be predominantly in the unionized, lipid soluble, form.

The pK_a is a measurement of physical data relevant to mechanisms of biological action and structure activity relationship (Culvenor and Willette, 1966). This is of importance in metabolic distribution of the pyrrolizidine alkaloids and milk transfer. Certain compounds may penetrate membranes in their unionized, lipid soluble state (Sisodia and Stowe, 1964).

When an unionized compound reaches a particular concentration in a body fluid, then its concentration should be the same on both sides of a membrane (Sisodia and Stowe, 1964). If the compound exists partly in the unionized state and partly in the ionized state, only the unionized fraction will obtain equilibrium. If there is a pH difference across the membrane, as is the case with plasma and milk, then the ionized concentration of the compound may differ markedly in the two compartments. Since the total concentration equals the sum of both the ionized and unionized forms, this pH difference can act as a "trap." The milk to plasma ratio is independent of the plasma concentration and the volume of milk secreted (Stowe and Plaa, 1968).

Only a few pyrrolizidine alkaloids are sufficiently soluble in water for pK_a measurements (Culvenor and Willette, 1966). Culvenor and Willette (1966) obtained

values by using a 80% methyl cellosolve and water mixture. These values were usually lower than those measured in water alone. In general, the amino alcohols are stronger bases than monoesters, which are stronger than diesters. This pattern results from the relative base weakening effects of substituents on the pyrrolizidine ring (Bull et al., 1968). These are approximately constant for a given substituent separated by the same number of carbon atoms from the basic nitrogen. The amino alcohol supinidine and its esters are stronger bases than the corresponding derivatives of heliotridine and retronecine (Culvenor and Willette, 1966).

The pK_a values for some alkaloids, including those of Senecio jacobaea, in 80% methyl cellosolve and some corresponding values in water are given in Table 1.

The pH of cow and goat milk is approximately 6.7 and 6.4 respectively (Altman and Dittmer, 1968). This is appreciably lower than that of plasma at 7.4. If simple diffusion alone determines the transfer of these alkaloids into milk, most of those from Senecio jacobaea should obtain significant milk to plasma ratios due to the "pH trap." This would be even more likely if the 80% methyl cellosolve values are lower than those obtained in water.

TABLE 1. pK_a VALUES FOR SOME PYRROLIZIDINE ALKALOIDS

Alkaloid	80% MCS[a]	H_2O	Reference
A) *Senecio jacobaea*			
jacoline	6.73 ± 0.03	--	Culvenor, 1966
senecionine	6.73 ± 0.04	--	"
seneciphylline	6.20	--	Bull, 1968
jacobine	6.04	--	"
jacozine	5.96	--	"
B) Other Alkaloids			
heliotrine	7.82 ± 0.04	8.52 ± 0.03	Culvenor, 1966
monocrotaline	6.93 ± 0.03	7.08 ± 0.05	"
fulvine	6.81 ± 0.04	--	"
lasiocarpine	6.55 ± 0.02	7.46 ± 0.02	"

[a]80% MCS = 80% methyl cellosolve/water

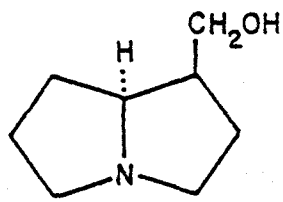
The isolation of jacoline from cow's and goat's milk by Dickinson *et al.* (1976; 1978 respectively) is suggestive of this occurrence. Jacoline, with the highest ionization constant, would be expected to have a larger proportion in the unionized state than the other alkaloids. After diffusing through the mammary gland to the more acidic milk, a larger portion would be converted to the non-diffusable ionized state. However, senecionine was not found, which

has a comparable pK_a value. Whether transfer of pyrrolizidine alkaloids is governed by some other phenomenon, or whether there was metabolic conversion of senecionine to jacoline is not known.

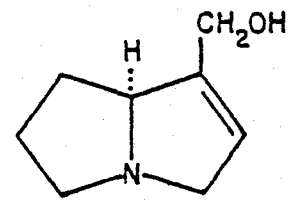
The weaker base jacobine was also found in goat's milk (Dickinson et al., 1978). This could be explained by the lower pH of goat's milk compared to that of cow's. Higher concentrations of both alkaloids were also found in the goat's milk possibly due to a more effective "pH trap" from this lower pH.

(a) The Amino Alcohols

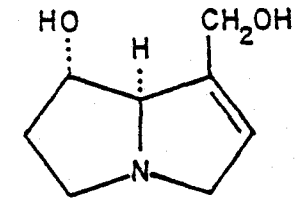
The amino alcohols are known in many stereochemical forms (Culvenor, 1978). They can be diastereoisomers or simple derivatives of 1-hydroxymethyl-pyrrolizidine (VII). They may have 1, 2 or 3 hydroxyl groups, and a 1,2-double bond. Those with the 1, 2-double bond, supinidine (VIII), heliotridine (IX), retronecine (X), otonecine (XI) and crotanecine (XII), when esterfied with the appropriate aliphatic acid, form the hepatoxic alkaloids.



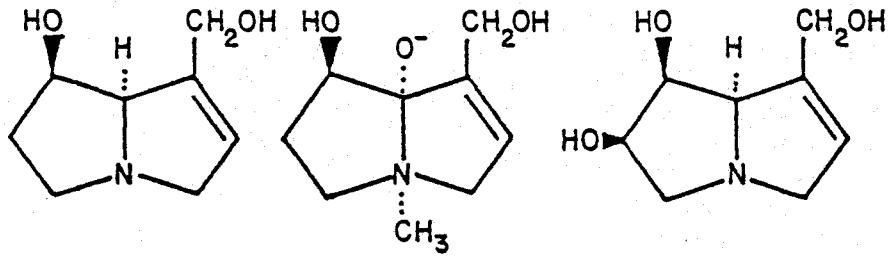
VII



VIII



IX



X XI XII

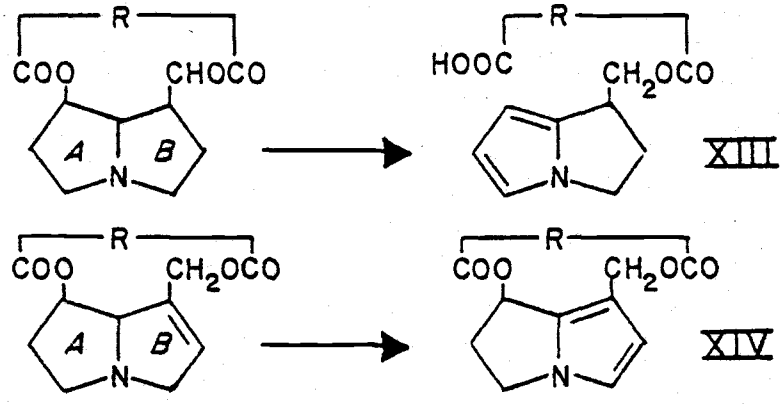
Dotted and thickened lines denote alpha (towards observer) and beta (away) orientation of bonds respectively.

The alkaloids of crotanecine are probably less toxic than corresponding alkaloids of retronecine (Schoental, 1968b). This would result from the 6-hydroxy group, which increases water solubility and the rate of excretion.

The alkaloids of the saturated pyrrolizidine ring, such as platyphylline and rosmarinine, though not toxic do give large amounts of pyrrole derivatives (Mattocks and White, 1971). Evidence shows that the "A" ring is dehydrogenated giving pyrrole like derivatives (XIII). This is unlike the dehydrogenation of ring "B" in the toxic alkaloids (XIV). Both ester groups in XIV are highly labile due to conjugation with the nitrogen atom, whereas the ester of XIII is relatively unreactive.

In the open di- and monoester alkaloids of the bases heliotridine, retronecine and supinidine, it seems that the stereochemistry of the amino alcohol is partly responsible for the differences in toxicity (Schoental, 1968b). The alkaloids of heliotrine are more active than

those of retronecine. In retronecine, both the 1-hydroxymethyl and 7-hydroxyl groups face the same side. As a result, the esterifying mono-carboxylic acids probably interfer with each other and the acid esterfying the 7-beta-hydroxyl position is often readily lost with a resulting loss of activity.



In the course of isolation, these and other unstable alkaloids may undergo decomposition so that the isolated alkaloid may not represent the true toxicity of the plant (Schoental, 1968b). This illustrates the importance of using the whole plant in toxicological studies for evaluating hepatoxicity and any other pathological effects that the plant may possess.

(b) Acid Moiety

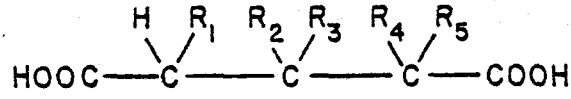
Although the free acids obtained by hydrolysis of the pyrrolizidine alkaloids are not toxic (Schoental, 1960), they are essential for the alkylating ability (Mattocks, 1970). This is exemplified by the fact that the chemically prepared pyrrole of the amino alcohol retronecine does not

exhibit toxic effects. However, when acetylated at both hydroxyl groups, it produces lesions in the liver and lung similar to pyrroles of pyrrolizidine alkaloids. This also shows that the type of acid is not significant at the site of reaction. The acetate moiety is not a branched chain, which is required for toxicity in the parent alkaloids (Schoental, 1968a). Since pyrrole derivitives of various alkaloids are the same when applied directly to the organ, the differences in toxicity of these parent alkaloids are influenced by the acidic moiety.

The esterified acid may contain certain features which can favor alkylation or detoxication (Culvenor et al., 1962). A high degree of substitution of the alpha carbon sterically hinders ester hydrolysis. This is likely a major detoxication mechanism (Mattocks, 1978; Schoental, 1968b).

The acids may be present as monoesters, or open or cyclic diesters (Schoental, 1957). The cyclic diesters can be further grouped according to number of carbons. Those containing 10 or more are very effective in inducing liver damage. Those containing nine, such as monocrotaline and fulvine, also have a pronounced effect on the lungs (Schoental, 1957; Mattocks, 1970). Retronecine, containing diesters of glutaric acid (XV) derivatives, also can induce lung and vascular lesions (Schoental, 1968b).

In general, the acids contain 5-10 carbon atoms and show great variation in structure (Schoental, 1968b). They are branched, often hydroxylated and unsaturated. They may contain substituents which give rise to optical or positional isomers. These substituents may be hydrophobic such as methyl, methylene, methoxy, ethyl, ethylene, isopropyl, isopropylene, etc. Hydrophilic groups such as hydroxy and carboxy, would increase water solubility and the rate of excretion with a resulting decrease in toxicity.



XV

Alkaloids with the same basic moiety esterified with different acids have widely different toxic effects (Mattocks, 1970). This influences the different amounts which are metabolized to the pyrrole derivitives, the stability of the reactive metabolite in the liver cells, distribution of the alkaloid, binding affinity to metabolizing enzymes, and ease of hydrolysis. The alkaloids are apparently not metabolized by the lungs, so for damage to occur here, sufficient metabolites must be transported from the liver to the lungs. The monocrotaline pyrrole is less reactive than the retrorsine pyrrole, so would be more likely to reach the lungs.

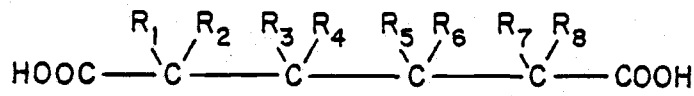
The chemical modification of alkaloids can cause

large changes in their toxicity. The acetylation of the three hydroxyl groups of indicine increases the toxicity more than 8-fold on a molar basis (Mattocks, 1972). The proposed reasons for this are: (1) improved access of the alkaloid to lipophilic metabolizing sites, (2) decreased detoxication by hydrolysis of the original ester group of indicine, (3) altered chemical reactivity or an increased half life of the pyrrolic metabolite, (4) or a combination of these effects.

The stereochemistry of the acid moiety may also have a pronounced effect on toxicity (Mattocks, 1971). This is seen by comparing pyrrole formation of senecionine and isosenecionine. Both are esters of retronecine with (+) and (-) senecic acids respectively. Isosenecionine gives 74% of the pyrrole form as that of senecionine in vitro after one hour.

All six alkaloids of Senecio jacobaea are cyclic di-esters of retronecine (Bull et al., 1968) with derivatives of adipic acid (XVI) (Schoental, 1968b). Cyclic di-esters of retronecine are considered the most toxic. They all contain ten carbon atoms, and from 1-3 hydroxyl groups in the acidic moieties (Hull et al., 1968). Although jacoline contains 3 hydroxyl groups, its presence in goat's milk (Dickinson and King, 1978) suggests that its possible high water solubility would not prevent its secretion

in the mammary gland.



XVI

Jacozine and senecionine contain a double bond in the acidic moiety (Bull et al., 1968). Seneciphylline contains two. The carcinogenic activity of retrorsine and its N-oxide is thought to be as a result of the double bonds in which they too possess (Schoental, 1968b). These could act as a multifunctional agent which would become more firmly bound to the liver than alkaloids which do not contain such a bond. It is likely that Senecio jacobaea could thus be carcinogenic.

EXPERIMENTAL

Whether or not pyrrolizidine alkaloids are transferred into the milk of dairy animals is of importance in areas where plants containing these alkaloids are found. Due to the widespread occurrence of such plants (Bull et al., 1968), this knowledge would be relevant to virtually every part of the world. If these alkaloids do have the capacity to contaminate milk, then management practices could be employed to minimize such an occurrence.

The following studies were conducted to investigate this phenomenon in dairy goats fed Senecio jacobaea. This was done by biological assay using rats and bull calves. The apparent high tolerance of goats to pyrrolizidine alkaloid poisoning (Dollahite, 1972; Dickinson et al., 1978) would allow a long term study without the loss of animals. The relative high susceptibility of rats and calves would permit detection of transfer by presence of lesions typical of pyrrolizidine alkaloid poisoning. For comparative purposes, a group of rats fed very low levels of Senecio jacobaea for an extended length of time was included. Due to the limited work on effect of Senecio jacobaea on goats, their susceptibility to chronic Senecio toxicity was determined.

Materials and Methods

The *Senecio jacobaea* used in these studies was collected in the vicinity of Corvallis, Oregon, while in full bloom, dried and ground to a suitable size. It consisted primarily of flowers, though a portion was made up of leaves. An alkaloid content of 0.18% dry weight has been reported by Buckmaster *et al.* (1976) and Dickinson *et al.* (1976) on whole plant collected in this area and Olympia, Washington, respectively.

(a) Transfer of Pyrrolizidine Alkaloids in Goat Milk

Goat Diet and Feeding

The *Senecio jacobaea* was fed to goats by incorporating it into an alfalfa-barley based ration, and pelleted into a 0.5 cm pellet (Table 2). A control pellet was also used, which consisted of the same ration with alfalfa in place of the *Senecio jacobaea*. The 25% *Senecio* pellet proved to be initially unacceptable due to its unpalatability. Goats that were to be fed this diet were started at a level which they would accept by diluting the ration with the control pellet. The *Senecio jacobaea* level was gradually increased at a rate tolerable for each individual goat.

Goats were fed the diets *ad libitum* with daily consumption being recorded. They were also given grass

hay free choice.

TABLE 2. PERCENT COMPOSITION OF GOAT DIET

Ingredient	Percent
Senecio jacobaea	25.0
Alfalfa	27.0
Barley	30.0
Soybean meal	10.0
Molasses	5.0
Bentonite	2.0
Trace mineral salt	0.3
Dicalcium phosphate	0.5

Rat Assay

A Saanen doe, freshened approximately three months earlier, was fed the Senecio diet. Milking was done twice daily, in the morning and evening, with total collection being recorded. The milk was frozen, freeze-dried (Thermovac) and incorporated at a level of 80% into a diet for rats (Table 3).

Twenty-eight Long Evans weanling male rats were divided into two equal groups according to weight. They were housed individually in hanging wire cages. Temperature and lighting were controlled. One group received the goat milk diet, and the other a control diet ad libitum. The control diet contained commercial non-fat powdered milk

with lard added to obtain a lipid level comparable to that of the goat milk (Table 3). Feed consumption and body

TABLE 3. PERCENT COMPOSITION OF MILK-CONTAINING RAT DIETS

Ingredient	Percent Test	Control
Goat milk	80.0	--
Powdered milk	--	67.0
Lard	--	20.0
Starch	14.5	8.5
Cellulose	3.0	3.0
Corn oil	1.0	--
Vitamin mix[a]	1.0	1.0
Trace mineral salt	0.5	0.5

a Cheeke et al. (1977).

weights were recorded. After three months, the two groups were divided into two subgroups. One subgroup, consisting of six rats, was continued on its respective milk diet. The other subgroup was switched to the OSU pelleted rat diet. At this time, two rats from the goat milk group were sacrificed and samples of lung, liver, spleen and kidney were obtained for histological examination. Tissues were fixed in 10% buffered formalin, and stained with hematoxylin-eosin. The two milk subgroups were continued on these diets for an additional three months, at which

time they were switched to OSU rat pellets. At the end of one year from the beginning of the study, all surviving rats were sacrificed and submitted for histological examination.

Rat Chronic Low Level Toxicity Experiment

As a basis for comparison of any histological changes which may be present, a similar study using Long Evans weanling male rats fed low dietary levels of Senecio jacobaea was conducted. Forty rats were divided into five groups of eight rats each according to weight. The rats were housed under similar conditions as those in the preceding study. One group received a corn-soy control diet (Table 4), and the other groups received Senecio jacobaea incorporated into the corn-soy diet at levels of 1.0%, 0.1%, 0.01% and 0.001%. The three lowest levels were prepared by dilution of the 1.0% ration with the control diet.

The five groups were fed ad libitum their respective diets for 180 days with feed consumption and body weights being recorded. At this time, all rats were switched to the pelleted OSU rat diet for an additional 227 days. At the end of this period, all surviving rats were sacrificed and submitted for histological examination. Tissues were fixed in buffered 10% formalin, and stained with hematoxylin-eosin. Tissues from rats dying before the end

of the study were also examined.

TABLE 4. PERCENT COMPOSITION OF THE CONTROL RAT DIET

Ingredient	Percent
Ground corn	58.5
Soybean meal	30.0
Sucrose	5.0
Corn oil	3.0
Mineral mix[a]	3.0
Vitamin mix[b]	0.5

a Jones and Foster (1942).

b Cheeke et al. (1977).

Calf Assay

Four lactating does freshened 1-3 months previously, plus the doe from the rat assay, were fed the Senecio ration. These were grade does predominately of Alpine, Toggenberg, or Nubian breeding. Milking was done in the morning and evening. Individual milk production was measured, after which it was pooled and fed by nursing bottle to two Jersey bull calves (day old). The calves were also given the control pellets along with grass hay. The hay was examined for possible Senecio contamination. One calf received milk for 124 days and the other for 109 days.

Plasma samples, using oxalate as the anticoagulant, were taken at approximately two week intervals from the goats and calves. These were analyzed for glutamic-oxalacetic transaminase (Sigma Method No. 505)[a], glutamic-pyruvic transaminase (Sigma Method No. 505)[a], lactate dehydrogenase (Sigma Method No. 500)[a], total protein (Sigma Method No. 540)[a] and albumin (Sigma Method No. 630)[a]. At about six months of age, both calves were sacrificed and submitted for examination.

(b) Effect of Senecio jacobaea on Goats

Three does and three wethers of about three months of age were used along with the five lactating does to assess the effect of Senecio jacobaea on goats. The kids were predominately of Alpine and Saanen breeding. Plasma samples were taken and analyzed as stated previously. One doe kid of three months of age was maintained on a Senecio free diet for comparative enzyme levels. These values, along with the initial enzyme levels of each goat, were used to assess any changes.

Results and Discussion

The 25% Senecio ration proved to be very unpalatable for goats. By diluting it with the control pellet, it was

[a]Sigma Chemical Company, St. Louis, Missouri.

possible to get them to accept the ration. The initial level was as low as 10% Senecio pellets for some goats.

For most goats, a 5% increase in Senecio pellets often resulted in a temporary reduction in feed intake. After an adequate length of time, some goats would accept the undiluted diet.

Rat Assay

The milk freeze-dried for the rat diet totaled 168 l. This was collected over a 236 day period, during which time the doe consumed 29 kg of Senecio jacobaea, or 59% of her body weight. Dried milk consumed and pertinent data of the various groups of rats is summarized in Table 5. The dry matter content of the goat milk was 12%, determined by freeze-drying. A high mortality rate is shown in the control milk group, but at least one in the six month group was attributed to a respiratory ailment. One death in the goat-milk group was precipitated by malocclusion.

TABLE 5. MORTALITY AND FEED CONSUMPTION OF MILK-FED RATS

Group	Period of Milk Consumption	Number of Rats Started	Number of Rats Dying	Total Milk Consumed (kg)
Goat Milk	3 months	8	1	7.26
	6 months	6	1	12.85
Control Milk	3 months	8	1	5.37
	6 months	6	3	8.49

The only significant lesions found in the two rats examined after consuming goat milk for three months were mild to moderate lymphoid proliferation of the white pulp in the spleen, and a moderate amount of extramedullary hematopoiesis within the red pulp. The lung had focal peribronchial lymphoid accumulation in a patchy distribution. This is suggestive of mycoplasma infection, common to laboratory rats. These lesions were not attributable to toxic involvement. The two rats consumed a total of 1871 g of milk, 832 g being consumed by one rat and 1039 g by the other.

The rats consuming the milk rations for three months consisted of seven controls and six from the goat milk group. Two rats in the control group had no hepatic lesions. The remaining five had cytoplasmic vacuolization of the hepatocytes. In one, this was not a definite cellular change and may have been an artifact of fixation or processing. In another, this was a mild change. The remaining three had mild changes but were more pronounced. In two of these, the vacuolization was primarily of centrilobular distribution. In one, this was limited to a few foci which also contained swollen hepatocytes. The outstanding lesion of the three month goat milk group was multiple necropurlent foci exhibited by three rats. These were scattered throughout the sections examined and were characterized by necrosis of the hepatocytes with

infiltration of leukocytes, principally neutrophils, and the accumulation of small amounts of plasma. These were small in two of the rats but rather large in the other. The latter also exhibited some focal cytoplasmic vacuolization and hepatocytic swelling scattered irregularly through the sections. The remaining three rats had cytoplasmic vacuolization of the hepatocytes. In one, this was only mild and was not representative of a true degenerative change. In the remaining two, it was a more marked and widespread change. This was evident throughout the parenchyma in one rat with some sparing of the periportal regions. There were a few foci in which the hepatocytes appeared swollen with clear large vacuolated cytoplasm. In most areas the vacuoles were small, round and sometimes several per cell. This is suggestive of fatty metamorphosis. In the third rat, there was hepatocytic swelling along with vacuolization involving all areas of the sections examined. This was predominantly of midzonal distribution; however scattered discrete foci of affected cells were evident.

In the two six month subgroups, all surviving rats had swollen hepatocytes. However, of the three rats in the control subgroup, these were limited to one focus in the two sections of each rat examined. This was due to cytoplasmic vacuolization and was not a widespread change. In one rat, there were individual enlarged hepatocytes scattered through the parenchyma in some areas. Of the four

rats in the goat milk subgroup, the swollen hepatocytes were primarily of centrilobular distribution. One rat, which had several tiny foci of swollen hepatocytes, also had several portal areas with multiple bile ducts, some of which were quite large. One rat had some nuclei which appeared slightly enlarged while another had some which appeared degenerative. The latter also had several well-defined foci of vacuolated swollen hepatocytes with granular cytoplasm.

Though both subgroups had swollen hepatocytes, the distribution between the two groups was not the same. The focal distribution of the control group was unlike the centrilobular distribution of the goat milk group. The latter is more characteristic of pyrrolizidine alkaloid poisoning (Bull et al., 1968). The biliary hyperplasia of the one rat in this group was also suggestive of pyrrolizidine alkaloid involvement. Since the milk was consumed over a six month period, if these lesions are as a result of alkaloid contamination it is probably at low concentrations.

Rat Chronic Low Level Toxicity Experiment

Table 6 shows the data obtained from the rats fed chronic low levels of Senecio jacobaea. One rat in the control group died the first week of the study, leaving only seven rats in this group. There was a high

TABLE 6. LONG TERM CHRONIC TOXICITY OF LOW LEVELS OF *SENECIO JACOBAEA* FED TO RATS

Observation	Dietary *Senecio jacobaea* Level 0%	1.0%	0.1%	0.01%	0.001%
Number of rats started	8	8	8	8	8
Number of survivors	6	1	8	6	8
Average survival time (days) of dead rats	299	198	-	313	-
Mean *Senecio* intake of survivors (g)	0	33.14	4.20	0.43	0.04
Estimated alkaloid intake of survivors (mg)[a]	0	39.77	5.04	0.52	0.05
Number of rats with swollen hepatocytes	1	2	8	1	4
Number of rats with megalocytosis	0	4	0	0	0
Number of rats with cytoplasmic vacuolization	1	0	7	1	5
Number of rats with biliary hyperplasia	0	7	1	0	1
Number of rats with fibrosis	0	7	0	1	1

[a]Estimated concentration of 0.18% of the dry weight of *Senecio jacobaea* (Buckmaster *et al.*, 1976; Dickinson *et al.*, 1976).

mortality rate in the 1.0% group with seven of the eight rats exhibiting lesions typical of chronic *Senecio* poisoning. Only one rat of this group survived until the end of the study. Seven of the rats had both biliary

hyperplasia and fibrosis with four also having megalocytosis. Two also exhibited swollen hepatocytes. These lesions were slight to moderate. One rat did not show any lesions; however it died at 75 days after consuming only 11 g of Senecio jacobaea. Malnutrition due to inadequate food intake was suspected as the cause of death. All other rats in this group consumed between 30.5 and 42.3 g of Senecio in the six month feeding period.

The control group had one rat with both swollen hepatocytes and cytoplasmic vacuolization, primarily of centrilobular orientation, in some lobules. Of the two rats dying before the end of the study, only one had any significant lesions of the liver. This rat died at 191 days and had extreme vascular congestion, with widely dilated sinusoids filled with blood. There were areas in which the hepatocytes were in various stages of degeneration. In one section, there was mild leukocytic infiltration of the portal areas with sinusoids containing moderate numbers of leukocytes. However, these lesions were not characteristic of those generally seen with pyrrolizidine alkaloid toxicity in rats. The degenerative changes may have been due to anoxia resulting from vascular congestion or stasis.

All rats of the 0.1% group survived the total 407 days of the study and showed slight to moderate swelling of the hepatocytes. Seven of these also had cytoplasmic vacuolization. These lesions were generally multifocal

and primarily of centrilobular distribution. In one rat, large portions of the parenchyma were involved in some lobules. One rat exhibited extensive biliary hyperplasia and fibrosis of a localized nature. Some of the bile ducts in these areas contained a purulent exudate which suggests a possible infectious or parasitic etiology to this localized degeneration.

The 0.01% group was spared from most of the hepatic lesions, with only one rat exhibiting multiple tiny foci of swollen hepatocytes with cytoplasmic vacuolization. This was not considered to be as a result of a low Senecio intake since the 0.001% group had over half the rats exhibiting hepatic lesions. Five of these had slight to mild cytoplasmic vacuolization of which four also had slight to moderate swelling of the hepatocytes. The distribution of these changes ranged from occasional scattered cells to widespread involvement of the lobules. However, they were generally limited to occasional foci with no discernible zonal distribution. Another rat exhibited slight biliary hyperplasia and fibrosis. The reason for the absence of lesions in the 0.01% group is not known. It appears as though this group may have detoxified the alkaloids thus being spared the toxic effects. This could be as a result of an enzyme system induced by this level of alkaloids but not by the lower level. At the higher dose groups, this system could have been saturated resulting in

an "overflow" of alkaloids available to be converted to the toxic pyrrole. This is only speculative with no experimental evidence to support it.

Lesions were produced in this study from the lowest average total dose of 0.04 mg of pyrrolizidine alkaloids per rat. If the goat milk were contaminated at a level sufficient to result in an equal or greater dose in the six month rat assay, then lesions would be expected. The average total intake of dried milk in this group was 2.14 kg. If contamination was at an average level of 233 ug/100 ml whole milk, as reported by Dickinson and King (1978), then the total alkaloid intake would average 42 mg per rat at a 12% dry matter content. This level is greater than the dose received by the 1.0% Senecio group. The milk group did not show any megalocytosis or fibrosis which was predominant in the 1.0% group. However, the average daily Senecio intake, on a body weight basis, of the goat used in this study was 0.25%. A daily dose of 1.0% was used by Dickinson and King (1978). Therefore, a comparable transfer rate would not be expected. Jacoline was also reported to be the predominant alkaloid transferred in goats (Dickinson and King, 1978). No data is available on the toxicity of this alkaloid to rats, but it cannot be expected to produce the same degree of lesions as the mixed alkaloids of Senecio jacobaea at equal doses. The lesions exhibited in the six month milk group correlate with those

of the 0.1% *Senecio* group. Both groups had swollen hepatocytes, primarily of centrilobular distribution. This is unlike the extensive degeneration of the 1.0% *Senecio* group, or the randomly scattered foci of swollen hepatocyptes of the 0.01% and 0.001% *Senecio* groups. If hepatic lesions of the six month goat milk group were as a result of pyrrolizidine alkaloid contamination, then this level would be comparable in toxicity to a total intake of approximately five mg of mixed alkaloid in six months. This would be representative of a contamination rate of 28 ug/100 ml of whole milk.

There were no discernible differences in the lesions exhibited by the three month control and goat milk groups. The one exception would be the necropurulent foci seen in the three rats consuming the goat milk. Hepatic necrosis in pyrrolizidine alkaloid poisoning is generally limited to the centrilobular areas and is more characteristic of acute poisoning (Bull *et al.*, 1968). Since neither type of necrosis was found in the six month goat milk group or in any of the rats consuming the low levels of *Senecio*, it is doubtful that this is as a result of pyrrolizidine alkaloid contamination. The presence of hepatic lesions in the two control milk subgroups was not anticipated. The presence of toxins at low levels in the milk or lard used in the diet could have resulted in lesions at the levels fed.

Calf Assay

Table 7 shows the data obtained from the calves fed goat milk. Calf #1 was two weeks older than calf #2 so consumed the milk two weeks longer. The final body weights were taken the day before being sacrificed, at which time the calves were approximately six months old. Both animals ate well, gained weight and maintained a healthy appearance throughout the course of study.

TABLE 7. MILK CONSUMPTION AND BODY WEIGHTS OF CALVES FED MILK FROM GOATS ADMINISTERED SENECIO JACOBAEA

Calf Number	Initial Body Wt.	Final Body Weight	Number of Days on Milk	Total Milk Consumed
1	25.5 kg	149.1 kg	124	298 L
2	22.3 kg	144.5 kg	109	251 L

Table 8 shows the amount of milk contributed by each goat, and total Senecio jacobaea consumed during the collection period. Two-thirds were given by NUB and DD which also ate the greatest quantities of Senecio. A total of 551 l was collected and 129 KG of Senecio was consumed.

The significant lesions of the calves were limited to the liver and lungs. The hepatic lesions were similar to those noted in the rat chronic toxicity study, however in this instance the changes were minimal and indistinctive.

TABLE 8. CONTRIBUTION OF MILK FROM EACH GOAT AND AMOUNT OF *SENECIO JACOBAEA* CONSUMED

Goat	Milk Collected (l)	Percent Of Pool	*Senecio* Consumed (kg)
NUB	197	16	52
DD	190	34	29
ALP	70	13	19
NEL	64	12	4
KEL	30	5	25

but probably early degenerative changes resembling cloudy swelling. These changes were characterized by swollen hepatocytes with irregular clear vacuoles or spaces which, in some cells, follow the cell border. In these cells, the cytoplasmic organelles and cytosol were present as irregular eosinophilic granules. The nuclei were absent or chromophobic in some of the cells, while in others they appeared slightly pyknotic. In some lobules, the hepatic cords in the affected areas were disorganized or disassociated. This was especially prominent near the central veins. In calf #2, these lesions were slightly more definite and were more uniformly distributed throughout the sections examined. Approximately half of the hepatocytic parenchyma was involved in this calf. In both calves, there was a lobular pattern of partial atelectasis

in the lungs. Prominent lymphatic hyperplasia was present with calf #1 also showing proliferation and accumulation of alveolar macrophages in some alveoli. This calf also had goblet cell hyperplasia of the bronchiolar epithelium. In both cases, there was an infusion of proteinacious fluid into the alveoli. These pneumonic changes were not considered to be related to pyrrolizidine alkaloid poisoning.

Effect of Senecio jacobaea on goats

The data obtained from all goats consuming Senecio jacobaea is shown in Table 9; there was one death, three others were euthanized in morbid conditions. The death was that of an Alpine wether, PH-1, which consumed 148% of his initial body weight in Senecio over a 261 day period. Approximately a week before death this goat became anorexic which hastened an already declining body weight. A few days before death, he became lethargic which became more pronounced with time. This progressed to the state where the animal would stand motionless with his head in a corner of the pen. In this condition, the animal was oblivious to activity in the surrounding area. He became comatose just prior to death. Gross examination revealed a firm pale liver. The gall bladder was fully extended. Edema of tissues or any excess peritoneal fluid was absent. The feces were fluid and the intestine generally flaccid. Histologically, the lesions of the liver were similar to

TABLE 9. *SENECIO JACOBAEA* CONSUMPTION OF GOATS

Goat	*Senecio* Consumed (kg)	Period of Time (Days) That *Senecio* Was Consumed	Intake of *Senecio* as Percent of Initial Body Weight	Deaths
Milking Does				
KEL	69.1	388	141	+
NUB	57.4	152	125	+
DD	33.5	162	68	--
ALP	19.5	114	49	--
NEL	4.4	43	9	--
Wethers				
PH-1	29.8	261	148	+
PH-2	10.7	155	57	--
SAT	11.4	155	61	--
Does				
LW	71.5	372	404	+
BW	67.9	389	305	--
1-I	4.5	155	40	--

cattle stricken by pyrrolizidine alkaloid poisoning. This consisted of severe hepatocellular degeneration characterized by vacuolization, megalocytosis, nuclear chromatin clumping, swelling of hepatocytes and bile duct proliferation. Extrahepatic lesions consisted of mild congestion in

the lungs, scattered thickening of the alveolar septa, due to accumulation of fibrin, and a few inflammatory cells. The kidneys exhibited acute nephrosis with increased protein leakage into the lobules and glomeruli. Some glomerular tufts contained excess inflammatory cells and fibrin.

The only goats to consume more Senecio than the wether were two does, LW and BW. This was greater than twice as much in both cases. Liver biopsies were taken from these two goats at a point when they had consumed approximately 90% of their total intake. This revealed an early mild reversible form of hydrophic degeneration of the hepatocytes which were subtle in BW and mild but definitive in LW. In BW, the hepatocytes were uniformly swollen to mild degree due to vacuolization of the cytoplasm. The vacuoles were generally small but irregular in size. They contained lipids, representing a mild generalized fatty metamorphosis. Some hepatocytes scattered through the parenchyma contained nuclei that were one and a half or two times larger than most of the adjacent hepatocytic nuclei. These nuclei had somewhat more prominent deeper staining chromatin granules, and often had multiple nucleoli. In these cells, the vacuolization of the cytoplasm was not definitely lipoid and may represent intracellular edema. If so, this would constitute a form of hydrophic swelling, or degeneration somewhat analogous to cloudy swelling.

In LW, the hepatocytes in the centrilobular areas exhibited marked fatty metamorphosis characterized by the presence of large well demarcated vacuoles. These were dispersed irregularly through the cytoplasm. Hepatocytes of the periportal zones were slightly swollen and had somewhat vacuolated cytoplasm. The vacuoles were irregularly sized, poorly demarcated and distributed uniformly throughout the cytoplasm. The enlarged hepatocytes with large nuclei described in BW were also scattered through the parenchyma with no discernible zonal pattern. Also noted were mild leukocytic infiltrates in the portal areas consisting of both neutrophils and lymphocytes.

Approximately 2.5 months after the liver biopsies were taken, LW became anorexic which initiated a decline in body weight during the ensuing eight months. This began one month after she was switched to a Senecio free diet. During this period, her body weight dropped from 49 kg to 30 kg at which time she was euthanized in a morbid state. Clinical features were a rough coat with alopecia on face, legs, chest and abdomen. A pasty feces was noted two weeks before termination. This was 296 days after the last exposure to Senecio jacobaea. Gross necropsy showed the lungs to be edematous and the blood to be watery. Fibrin was present on the serosa of liver, rumen, spleen and kidneys. Both liver and kidneys were pale, nodular and

fibrotic, the latter also being shrunken. Microscopic examination of the liver revealed moderate centrilobular fatty changes, megalocytosis, fibrosis and proliferation of bile ducts. The kidneys showed chronic interstitial nephritis characterized by a markedly fibrotic cortex with numerous dilated tubules and foci of plasma cells and lymphocytes in the interstitium. In many areas of the lungs, the alveoli contained proteinaceous fluid.

The lesions of the liver were those of chronic toxic hepatitis due to pyrrolizidine alkaloid poisoning. It is of interest to note the degenerative changes of the kidneys in this goat and that of the wether, PH-1. Renal lesions are generally not seen in pyrrolizidine alkaloid poisoning except in sheep that have developed hemoglobinemia, pigs and occasionally horses (Bull et al., 1968). When present, they are most commonly manifested as megalocytosis occurging usually in the epithelial cells of the proximal tubules and the thick loops of Henle (Hooper, 1978). Petechial hemorrhages have also been observed in poisoning of sheep by Crotalaria mucronata (Laws, 1968) and pigs by Crotalaria retusa (Hooper, 1977). Neither lesion was found in the present study, however the degenerative changes and fibrosis found in both cases cannot be overlooked.

At 348 days after their last exposure to Senecio jacobaea, KEL and NUB developed diarrhea. Three days

later, they had opisthotonos and were unable to stand. At this point, both goats were euthanized. Gross examination revealed a few pale foci in the dorsal lung lobe of NUB and congested lungs in KEL. These goats consumed 125% and 141% of their initial body weights in Senecio jacobaea in 152 and 388 days respectively. No significant bacterial isolates were found in the brain of either goat or in the lung of KEL. NUB had marked megalocytosis of the hepatocytes. KEL had centrilobular fatty changes in the liver, but this was probably from hypoxia resulting from verminous pneumonia which was also present. The etiology of the central nervous system disorders was not determined. Presently, all surviving goats are on a Senecio free diet and are maintaining a healthy appearance.

The plasma enzyme levels, total protein and albumin to globulin ratios of the goats consuming Senecio jacobaea, and calves receiving the goat milk, are given in Table 10. The control goat values are from 24 samples obtained from the doe on a Senecio free ration and the initial values of the goats before being fed the Senecio. The values given for PH-1 are those from a sample taken the day of his death. The initial calf values were obtained from plasma samples taken before calves were given the goat milk. None of the goats showed any substantial deviations from the control values for enzymes, total protein or albumin to globulin ratios. One exception would be the maximum albumin to

globulin ratio of 2.98 shown by LW compared to the control maximum of 1.78. However, the ratio generally falls in chronic liver disease (Ford et al., 1968). This was also from a sample taken early in the five month sampling period. Afterwards, the values returned to the normal range. The range of values in the calves did not deviate substantially from their initial levels. Since the initial values were only made up of two samples, this cannot be assumed to represent the normal range. However, they do give a reference for comparisons. The maximum albumin to globulin ratios of both calves are appreciably higher than their initial values. This is not assumed to be of significance in this study due to reasons previously mentioned.

The enzymatic values obtained here cannot be directly compared to studies by others, (Ford et al., 1968; Dickinson et al., 1976; Johnson, 1976, and Dickinson and King, 1978) since values were not expressed in similar units. The Sigma Chemical Company (Sigma technical bulletin No. 505) warns against converting values to International Units for comparative reasons. When different assay values are converted to International Units, they may be different even when enzymatic activity is the same. However, when the enzyme levels in these other studies were elevated due to chronic liver damage from pyrrolizidine alkaloid poisoning it was usually several fold over normal values. In the present study, no elevation of any enzyme measured was of this magnitude.

TABLE 10. RANGE OF VALUES FOR PLASMA ENZYME, TOTAL PROTEIN AND ALBUMIN TO GLOBULIN RATIO OF GOATS FED *SENECIO JACOBAEA* AND CALVES CONSUMING MILK FROM GOATS FED *SENECIO JACOBAEA*

Animal Identification	LDH[a]	GOT[b]	GPT[b]	TP[c]	A/G
Goat control	290-670	38-109	0-11	5.04-7.63	0.76-1.78
LW	310-670	48-115	3-10	5.78-7.11	0.76-2.98
BW	400-820	38-59	0-11	5.71-6.37	0.90-1.51
DD	420-590	36-84	4-11	6.74-7.63	1.09-1.44
KEL	460-800	38-128	0-10	6.00-6.81	1.14-1.62
ALP	290-590	46-64	0-14	6.52-7.33	0.76-1.48
NUB	460-670	66-109	4-14	6.52-7.33	0.98-1.17
SAT	420-880	40-91	0-8	6.07-7.11	0.80-1.78
PH-1	700	58	8	6.32	1.30
PH-2	310-530	50-95	3-10	5.56-6.67	0.97-1.48
1-I	380-630	52-91	0-8	5.93-6.67	0.92-1.31
NEL	350-590	38-70	0-8	6.07-7.19	1.30-1.48
Calf initial	820-1600	32	0	5.63-6.74	0.72-0.75
Calf #1	780-1330	29-58	0-42	4.95-5.63	0.75-2.48
Calf #2	720-1600	14-55	0-14	5.09-6.74	0.72-2.19

[a]Values given in Berger-Broida units/ml.

[b]Values given in Sigma-Frankel units/ml.

[c]Values given in grams protein/100 ml.

CONCLUSIONS

Results show that the transfer of pyrrolizidine alkaloids in goat milk is not at a level that would produce short term toxic responses to susceptible species consuming the milk. Rats consuming the freeze-dried milk for six months had hepatic lesions suggestive of those caused by pyrrolizidine alkaloids or some other hepatoxin. Due to the lack of characteristic lesions such as megalocytosis, fibrosis, or biliary hyperplasia, usually seen in pyrrolizidine alkaloid poisoning, it cannot be definitely concluded that pyrrolizidine alkaloid induced lesions occurred. However, these characteristic lesions were found only in the 1.0% Senecio group of the low level chronic toxicity study. The lower levels, though exhibiting hepatic lesions, were unlike those due to higher doses of pyrrolizidine alkaloids. The resemblance of these lesions to those of the rats of the six month goat milk group and the calves consuming the goat milk indicate the necessity of further research in this area. The effect of very low chronic administration of pyrrolizidine alkaloids to susceptible species should also receive further attention. This would be more typical of consumption of milk contaminated with pyrrolizidine alkaloids at low levels.

The reason for hepatic lesions in the control milk

groups was not determined. The capability of milk to acquire or accumulate drugs or toxins is well established. The possibility of low level contamination of the milk or other ingredients used in the diet with an unknown toxin could have resulted in these changes when consumed at the levels and duration in which they were. These lesions could also have been a senile change.

The goats proved not to be resistant to poisoning by Senecio jacobaea. However, they are much more resistant to pyrrolizidine alkaloid poisoning than are horses and cattle. It appears that over 100% of the body weight is required for lethal effects. The difficulty in getting the animals to consume the Senecio suggests that consumption of similar levels in the field would not be likely. Though they have been observed to browse the lush portions of the plant, consumption of quantities fed experimentally would not be likely unless it constituted the only available vegetation. It should be pointed out that the feeding trials lasted much longer than the seasonal availability of the plant.

LITERATURE CITED

Allen, J. R., I. C. Hsu and L. A. Carstens. 1975. Dehydroretronecine-induced rhabdomyosarcomas in rats. Cancer Res. 35:997-1002.

Altman, P. L. and D. S. Dittner. 1968. Metabolism Federation of American Societies for Experimental Biology. Bethesda, Maryland.

Anon. 1978. Epidemics of veno-occlusive disease in India and Afghanistan. Nutrition Reviews. 36:48-49.

Bras, G., D. M. Berry and P. György. 1957. Plants as aetiology factor in veno-occlusion disease of the liver. Lancet. 1:960-962.

Buckmaster, G. W., P. R. Cheeke and L. R. Shull. 1976. Pyrrolizidine alkaloid poisoning in rats: Protective effects of dietary cysteine. J. Animal Science 43:464-473.

Buckmaster, G. W. 1978. Nutritional Influences Affecting Senecio Toxicity in Laboratory Animals. Master of Science Thesis, Oregon State University, Corvallis.

Bull, L. B., C. C. J. Culvenor and A. T. Dick. 1968. The Pyrrolizidine, Alkaloids. John Wiley and Sons, Inc., New York.

Butler, W. H., A. R. Mattocks and J. M. Barnes. 1970. Lesions in the liver and lung of rats given pyrrole derivatives of pyrrolizidine alkaloids. J. Path. 100:169-175.

Campbell, J. G. 1956. An investigation of the hepatotoxic effects in the fowl of ragwort, with special reference to the induction of liver tumours with seneciphylline. Proc. Roy. Soc. Edinburgh Sect. B. Biol. 66:111-130.

Casarett, L. J. and J. Doull. 1975. Toxicology: The Basic Science of Poisons. Macmillan Publishing Co., Inc. New York. 313-332.

Cheeke, P. R. and G. R. Garman. 1974. Influence of dietary protein and sulfur amino acid levels on the toxicity of Senecio jacobaea (Tansy ragwort) to rats. Nutr. Rep. Int. 9:197-207.

Culvenor, C. C. J., A. T. Dann and A. T. Dick. 1962. Alkylation as the mechanism by which the hepatatoxic pyrrolizidine alkaloids act on cell nuclei. Nature (London) 195:570-573.

Culvenor, C. C. J. and R. E. Willette. 1966. Ionization constants of pyrrolizidine alkaloids. Aust. J. Chem. 19:885-889.

Culvenor, C. C. J., D. T. Downing and J. A. Edgar. 1969. Pyrrolizidine alkaloids as alkylating and antimitotic agents. Ann. N.Y. Acad. Sci. 163:837-847.

Culvenor, C. C. J. 1978. Pyrrolizidine alkaloids-occurrence and systemic importance in angiosperms. Bot. Notiser 131:473-486.

Dickinson, J. O. 1976. Pyrrolizidine alkaloids in honey. West. Vet. 14:11-13.

Dickinson, J. O., M. P. Cooke, R. R. King, P. A. Mohamed. 1976. Milk transfer of pyrrolizidine alkaloids in cattle. J. Am. Vet. Med. Assn. 169:1192-1196.

Dickinson, J. O. and R. R. King. 1978. Effects of Poisonous Plants on Livestock. Academic Press. Inc. New York, San Francisco, London. 201-208.

Dienzer, M. L. and P. A. Thomson. 1977. Pyrrolizidine alkaloids: their occurrence in honey from Tansy ragwort (Senecio jacobaea L.) Science 195:497-499.

Dollahite, J. W. 1972. The use of sheep and goats to control Senecio poisoning in cattle. The Southwest Veterinarian. Spring: 223-226.

Emmel, M. W. 1937. The Pathology of Crotoloria spectabilis 10th seed poisoning in the domestic fowl. J. Am. Vet. Med. Assn. 90:627-634.

Ford, E. J. H., H. E. Ritchie and E. Thorpe. 1968. Serum changes following the feeding of ragwort (Senecio jacobaea) to calves. J. Comp. Pathol. 78:207-219.

Fouts, J. R. 1959. Drug metabolism in the newborn rabbit. Science. 129:897-898.

Fowler, M. F. 1968. Pyrrolizidine alkaloid poisoning in calves. J. Am. Vet. Med. Assn. 152:1131-1137.

Fox, D. W., M. C. Hart, P. S. Bergeson, P. B. Jarrett, A. E. Stillman and R. J. Huxtable. 1978. Pyrrolizidine (Senecio) intoxication mimicking reye syndrome. J. Pediat. 93:980-982.

Gopinath, C. and E. J. H. Ford. 1977. The effect of ragwort (Senecio jacobea) on the liver of the domestic fowl (Gallus domesticus): a histopathological and enzyme histochemical study. Br. Poult. Sci. 18:137-141.

Green, C. R. and G. S. Christie. 1961. Malformations in foetal rats induced by the pyrrolizidine alkaloid, heliotrine. Bri. J. Exp. Cathol. 42:369-378.

Harris, P. N. and K. K. Chen. 1970. Development of hepatic tumors in rats following ingestion of Senecio longilobus. Cancer Res. 30:2881-2886.

Hill, K. R., K. Rhodes, J. L. Stafford and R. Aub. 1953. Serous hepatosis:a pathogenesis of hepatic fibrosis in Jamaican children. Bri. Med. J. 1:117-122.

Hooper, P. T. and W. A. Scanlan. 1977. Crotalaria retusa poisoning of pigs and poultry. Aust. Vet. J. 53:109-114.

Hooper, P. T. 1978. Effects of Poisonous Plants On Livestock. Academic Press, Inc. New York, San Francisco, London. 161-176.

Hooson, J. and P. Grasso. 1976. Cytotoxic and carcinogenic response to monocrotaline pyrrole. J. Path. 118:121-129.

Huxtable, R.,A. Stillman and D. Ciaramitaro. 1977. Characterization of alkaloids involved in human Senecio (pyrrolizidine) poisoning. Proc. West. Pharmacol. Soc. 20:455-459.

Johnson, A. E. 1976. Changes in calves and rats consuming milk from cows fed chronic lethal doses of Senecio jacobaea (Tansy ragwort). Am. J. Vet. Res. 37:107-110.

Jones, J. H. and C. Foster. 1942. A salt mixture for use with basal diets either high or low in phosphorus. J. Nutr. 24:245-256.

Kingsbury, J. M. 1964. Poisonous Plants of the United States and Canada. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Laws, L. 1968. Toxicity of Crotalaria mucronata to sheep. Aust. Vet. J. 44:453-455.

Lyford, C. L., G. G. Vergara and D. D. Moeller. 1976. Hepatic veno-occlusive disease originating in Ecuador. Gastroenterology. 70:105-108.

Mattocks, A. R. 1961. Extraction of heat-labile alkaloids from plants. Nature 191:1281-1282.

Mattocks, A. R. 1967. Spectrophotometric determination of unsaturated pyrrolizidine alkaloids. Anal. Chem. 39:443-447.

Mattocks, A. R. 1968. Toxicity of pyrrolizidine alkaloids. Nature. 217:723-728.

Mattocks, A. R. 1970. Role of the acid moieties in the toxic actions of pyrrolizidine alkaloids on liver and lung. Nature 228:174-175.

Mattocks, A. R. and I. W. H. White. 1970. Estimation of metabolites of pyrrolizidine alkaloids in animal tissues. Anal. Biochem. 38:529-535.

Mattocks, A. R. and I. W. H. White. 1971. The conversion of pyrrolizidine alkaloids to N-oxides and to dihydropyrrolizidine derivatives by rat-liver microsomes in vitro. Chem.-Biol. Interact. 3:383-396.

Mattocks, A. R. 1972. Acute hepatotoxicity and pyrrolic metabolites in rats dosed with pyrrolizidine alkaloids. Chem.-Biol. Interact. 5:227-242.

Mattocks, A. R. 1978. Recent studies on mechanisms of cytotoxic action of pyrrolizidine alkaloids. Effects of Poisonous Plants on Livestock. Academic Press, Inc. New York, San Francisco, London.

McGee, J. O'D., R. S. Patrick, C. B. Wood and L. H. Blumgart. 1976. A case of veno-occlusive disease of the liver in Britain associated with herbal tea consumption. J. Clin. Pathol. 29:788-794.

McKenzie, J. S. 1958. Some pharmacological properties of pyrrolizidine alkaloids and their relationship to chemical structure. Aust. J. Exp. Biol. 36:11-22.

McLean, E. K. 1970. The toxic actions of pyrrolizidine (Senecio) alkaloids. Pharmacol. Reviews. 22: 429-483.

Morton, J. F. 1958. Ornamental plants with poisonous properties. Proc. Florida State Hort Soc. 71:372-380.

Mosher, W. 1979. Use of sheep in control of tansy ragwort. Tansy Ragwort Symposium. Oregon State University.

Muth, O. H. 1968. Tansy Ragwort (Senecio jacobaea) a potential menace to livestock. J. Am. Vet. Med. Assn. 153:310-312.

Newberne, Paul M., Robert Wilson and Adrianne E. Rogers. 1971. Effects of a low-lipotrope diet on the response of young male rats to the pyrrolizidine alkaloid, monocrotoline. Toxic. Appl. Pharmacol. 18:387-397.

Schoental, R. 1954. Senecio alkaloids and liver cancer. Br. Med. J. ?:335-336.

Schoental, R. 1957. Hepatotoxic action of pyrrolizidine (Senecio) alkaloids in relation to their structure. Nature. 179:361-363.

Schoental, R. 1959. Liver lesions in young rats suckled by mothers treated with the pyrrolizidine (Senecio) alkaloids, lasiocarpine and retrorsine. J. Bact. Pathol. 77:485-495.

Schoental, R. and A. R. Mattocks. 1960. Hepatotoxic activity of semi-synthetic analogues of pyrrolizidine alkaloids. Nature. 185:842-843.

Schoental, R. 1963. Liver disease and "natural" hepatotoxins. Bull. Wld. Hlth. Org. 29:823-833.

Schoental, R. 1968a. Toxicology and carcinogenic action of pyrrolizidine alkaloids. Cancer Res. 28: 2237-2246.

Schoental, R. 1968b. Chemical structures and pathological effects of pyrrolizidine alkaloids. Israel J. Med. Sci. 4:1133-1145.

Selzer, G. and R. G. F. Parker. 1951. Senecio poisoning exhibiting as Chiari's syndrome. Am. J. Pathol. 29:885-900.

Shull, L. R., G. W. Buckmaster and P. R. Cheeke. 1976. Factors influencing pyrrolizidine (Senecio) alkaloid metabolism: species, liver sulfhydryls, and rumen fermentation. J. Animal Science 43: 1247-1253.

Sisodia, C. S. and C. M. Stowe. 1964. The mechanism of drug secretion into bovine milk. Ann. N.Y. Acad. Sci. 11:650-661.

Snyder, S. P. 1972. $1,200,000 poisoning of livestock. Oregon Cattleman. 21:22.

Stillman, A. E., R. J. Huxtable, P. Consroe. 1977. Hepatic veno-occlusive disease due to pyrrolizidine (Senecio) poisoning in Arizona. Gastroenterology 73:349-352.

Stowe, C. M. and G. L. Plau. 1968. Extrarenal excretion of drugs and chemicals. Ann. Rev. Pharmacol. 8:337-356.

Sundareson, A. E. 1942. An experimental study on placental permeability to cirrhogenic poisons. J. Pathol. Bacteriol. 54:289-298.

Svoboda, D. J. and J. K. Reddy. 1972. Malignant tumors in rats given lasiocarpine. Cancer Res. 32: 908-912.

Thomas, E. F. 1934. The toxicity of certain species of Crotalaria seed for the chicken, quail, turkey and dove. J. Amer. Vet. Med. Assn. 85:617-622.

Thorpe, E. and E. J. H. Ford. 1968. Development of hepatic lesions in calves fed with ragwort (Senecio jacobaea). J. Comp. Path. 78:195-205.

APPENDIX

EVALUATION OF THE CHICK AS A BIOASSAY ANIMAL IN *SENECIO JACOBAE* TOXICITY STUDIES

In using animals as bioassay organisms, they should meet certain requirements. They should (1) be sensitive to the substance being tested and exhibit characteristic reproduceable lesions or responses, (2) be small and easily maintained so that large numbers can be used for statistical evaluations, (3) and be readily available and inexpensive.

Previous work has shown the chicken to be very sensitive to ingested pyrrolizidine alkaloid containing plants (Thomas, 1934; Emmel, 1937; Campbell, 1956; Gopinath and Ford, 1977; Buckmaster, 1978). They have also been shown to be susceptible to hepatomas as a result of poisoning by these alkaloids (Campbell, 1956). In acute poisoning, they die from one to ten days but in chronic poisoning, they may survive for months (Thomas, 1934). Grossly, the liver is usually smaller and dull gray in color with rounded borders. Petechial hemorrhages in the serous membranes and visceral fat are seen in acute poisoning by *Crotalaria spectabilis* seeds, whereas chronic poisoning results in necrotic enteritis and ascites (Emmel, 1937). Histologically, the nuclear enlargement of liver cells, commonly seen in calves poisoned by pyrrolizidine alkaloids (Thorpe and Ford, 1969), is also seen, which develops more

rapidly than that of cattle (Gopinath and Ford, 1977). The veno-occlusion, common to cattle and horses, is not seen and the degree of fibrous tissue proliferation is less in chicks than in other species.

The purpose of the following study was to assess the value of the chick as a bioassay organism in Senecio jacobaea toxicity studies. The minimum lethal dose will be determined by feeding Senecio jacobaea to day old Leghorn male chicks for varying lengths of time.

Materials and Methods

Two replicates of 10 Leghorn male day old-chicks were fed 5% Senecio jacobaea for one of the following number of days: 0, 2, 4, 8, 16 and 20. After this period, they were switched to a Senecio free (control) ration. Another group was maintained on the control ration throughout the course of study. The Senecio was fed via the OSU starter diet and offered ad libitum. The chicks were housed in temperature controlled batteries. The room was equipped with forced-air ventilation and 24 hour lighting. Mortalities were recorded as were group weights, for the first 43 days, and Senecio intake.

Results and Discussion

The data collected are shown in Appendix Table 1. The high susceptibility of chicks to Senecio jacobaea was

not evident as determined by mortality. As a result, the chicks outgrew the batteries so all groups were terminated at 67 days except the two control and the two continuous Senecio replicates. The latter were fed their respective diets for an additional 28 days at which time they too were terminated. A total of six chicks died in the two continuous Senecio replicates by the end of the study. The total elapsed time was 95 days. Gross examination of the livers showed them to be small with rounded edges and grey in color as was previously described (Thomas, 1934). The body weights of this group were substantially lower than that of the controls, which has also been reported as a characteristic of Senecio jacobaea poisoning (Campbell, 1956).

In the studies by Campbell (1956) and Gopinath and Ford (1977), Senecio jacobaea was fed at 7% of the diet. Campbell (1956) found this level to be too toxic and cut it by one-half. In the former study, chicks of six to eight weeks of age were used, and week old male chicks were used in the latter. It has been shown that males are more susceptible to the toxic effects of pyrrolizidine alkaloids (Schoental, 1968a; Campbell, 1956) as are the young (Schoental, 1968a) in susceptible species. In the present study, day old male chicks were fed Senecio jacobaea at 5% of the diet which should show high susceptibility in view of previous results. This was not established through mortality, though it may be shown by histological studies

similar to those of Campbell (1956) and Gopinath and Ford (1977).

Conclusion

Though the high susceptibility of the chick to pyrrolizidine alkaloids may be shown through serial histological studies, the relative susceptibility of the rat and extensive published pathological work in this area would support its continued use as the bioassay animal of choice in pyrrolizine alkaloid toxicity studies.

APPENDIX TABLE 1. EFFECT OF 5% DIETARY *SENECIO JACOBAEA* ON CHICKS

Number Days on *Senecio*	Replicate	Avg. Starting BW(g)	Avg. 43 Day BW(g)	Number Chicks Dying Before 43 Days	Survival Time (Days) of Chicks That Died	Avg. *Senecio* Consumed of Survivors	Percent of Initial BW Consumed
0	I	29.7	487.1	0	--	0	0
	II	31.5	472.8	0	--	0	0
2	I	30.3	445.7	0	--	0.3	0.1
	II	31.5	540.3	1	8	0.3	0.1
4	I	29.2	436.1	0	--	0.8	2.7
	II	28.8	488.2	1	6	1.0	3.5
8	I	30.3	414.5	0	--	2.8	9.2
	II	29.9	407.4	0	--	2.9	9.7
12	I	31.3	393.0	1	16	4.7	15.0
	II	31.8	380.2	1	4	5.0	15.7

APPENDIX TABLE 1 (continued)

Number Days on *Senecio*	Replicate	Avg. Starting BW(g)	Avg. 43 Day BW(g)	Number Chicks Dying Before 43 Days	Survival Time (Days) of Chicks That Died	Avg. *Senecio* Consumed of Survivors	Percent of Initial BW Consumed
16	I	31.3	369.8	0	--	7.2	23.0
	II	29.0	353.7	0	--	6.3	21.7
20	I	30.4	317.8	2	14, 22	9.8	32.2
	II	29.5	318.1	2	22, 33	8.6	29.1
95	I	31.6	243.8	2	1, 35	136	430
	II	30.9	165.1	1	38	124	401

COMPARISON OF THE TOXICITIES OF *SENECIO JACOBAE*, *SENECIO VULGARIS* AND *SENECIO GLABELLUS* IN RATS

Introduction

There are more than 1200 species of *Senecio* found throughout the world (Kingsbury, 1964). There are 50 species alone in North America of which seven have been proven or suspected of being toxic. *Senecio vulgaris*, also known as common groundsel, is an annual commonly found in gardens and cultivated areas. The pyrrolizidine alkaloids which it is known to contain are senecionine, seneciphylline, and retrorsine. These are found at concentrations of 0.21% of dried plant material (Buckmaster *et al.*, 1976). For comparison, *Senecio jacobaea* contains senecionine, seneciphylline, jacobine, jacoline, jaconine and jacozine at concentrations of 0.18% (Buckmaster *et al.*, 1976; Dickinson *et al.*, 1976). Although *Senecio vulgaris* does not cause as much economic loss as *Senecio jacobaea*, it has been implicated in the deaths of dairy cattle (Fowler, 1968). There has been little work reported on the toxicity of *Senecio glabellus* (*Senecio lobatus*). Commonly known as bitterweed, it is found in wet soils of the southeastern United States north to Illinois and North Carolina (Kingsbury, 1964). The only pyrrolizidine alkaloid that it is reported to contain is senecionine (Bull *et al.*, 1968). This plant has been suspected

of poisoning cattle in Florida (Morton, 1958).

The purpose of the following study is to compare the toxicity of Senecio jacobaea, Senecio vulgaris and Senecio glabellus by feeding trials with rats. The alkaloid extracts were also compared by gas chromatography.

Materials and Methods

Three groups of eight rats each were individually housed in hanging wire cages in temperature and light controlled rooms. Average individual weights were 153 g. Each group was fed ad libitum one of the Senecios at 10% of a corn-soy basal ration (Table 4) for 18 days. They were then fed the OSU pelleted rat diet ad libitum for 51 days. Following this period they were again given the Senecio diets at the 5% level for 14 days followed by the OSU pelleted diet till the termination of the study at 121 days. Water was furnished ad libitum. Feed consumption and weight gains were recorded as were mortalities.

Isolation and gas chromatography-mass spectrometric analysis of alkaloids was conducted by H. Ramsdell, Department of Agricultural Chemistry, Oregon State University. The gas chromatography-mass spectrometry analyses were done on a Varian CH-7 mass spectrometer equipped with a Systems Industries Model 150 data system. A ten foot Ni-200 column packed with 3% OV-101 on Chromsorb-HP-W (80/100 mesh) was used with helium as the carrier gas and the column held

at 235°C. The effluent was split between a flame ionization detector and the separator inlet and an ionization potential of 70 eV was utilized. Samples were injected as methanol solutions. Isolation of alkaloids were done at room temperature using the Dowex 50 procedure (Mattocks, 1961). This method allows a purer preparation to be obtained compared to the alternative Soxhlet method since many extractable compounds, other than bases, are not bound to the resin. In addition, the alkaloids are not exposed to the elevated temperature of boiling methanol which could result in degradation of the alkaloids or formation of artifacts as they may be by the latter method.

Results and Discussion

Appendix Table 3 shows the survival time of the rats dying and amount of *Senecio* consumed. All eight rats of the *Senecio vulgaris* group died before the termination of the study at 121 days. This is to be expected since it contains the three most toxic alkaloids of the seven contained in the three plants. The LD_{50} in the rat of these are: retrorsine 38 mg/kg, seneciphylline 77 mg/kg and senecionine 85 mg/kg (Bull *et al.*, 1968). *Senecio glabellus*, which contains the comparatively toxic senecionine, killed seven of the eight rats. The LD_{50} of the group of alkaloids from *Senecio jacobaea* is approximately 140 mg/kg for the rat (Shull *et al.*, 1976), which

APPENDIX TABLE 2. TOXICITY OF DIETARY S. JACOBAEA, S. GLABELLUS AND S. VULGARIS TO THE RAT

Plant	Rats Started	Rats Dead At 121 Days	Avg. Survival[a] Time (Days) of Dead	Avg. Senecio[a] Intake (g)	Senecio As % of Initial Body Weight
S. jacobaea	8	5	32 ± 1	32.1 ± 2.2	21
S. glabellus	8	7	65 ± 11	30.7 ± 3.0	20
S. vulgaris	8	8	78 ± 9	35.1 ± 2.4	23

[a]Means ± SEM

would be less than the other two plants in view of the LD_{50} of the alkaloids in which they contain. This is also evident from the fewer deaths from this plant than the others.

The gas chromatograms of the pyrrolizidine alkaloids isolated from the three plants are shown in Figures 1 and 2. Jaconine of Senecio jacobaea and retronecine of Senecio vulgaris were not evident from the chromatograms. It appears that there may be another alkaloid (F) in Senecio glabellus in addition to senecionine which it has been previously reported to contain. This corresponds in retention time to peaks (F) in Senecio jacobaea and Senecio vulgaris. However, mass spectrometric analysis of this

peak showed it to be identical with senecionine (Figure 3). The difference in retention time could be as a result of two different isomeric forms. Senecionine is known to exist as isosenecionine; the only difference between the two are esterification of the retronecine nucleus with cis and trans senecic acids respectively (Schoental, 1968a).

Conclusion

The toxicities of three Senecios to the rat was evaluated in feeding trials. The toxicities starting with the most toxic are: Senecio vulgaris, Senecio glabellus and Senecio jacobaea. This can in part be explained by the relative toxicities of the pyrrolizidine alkaloids in which each plant contains.

APPENDIX TABLE 3. SOME PLANT SPECIES CONTAINING PYRROLIZIDINE ALKALOIDS

Family	Subdivision	Genus	Species
Boraginaceae	Heliotropiodeae	Heliotropium	europaeum
	Boraginoideae		
	Tribe Eretrichieae	Amsinckia	intermedia
	Tribe Echieae	Echium	
	Tribe Cynoglosseae	Trichodesma	
Compositae	Senecioneae	Senecio	jacobaea
		Senecio	longilobus
		Senecio	vulgaris
		Senecio	ilicifolius
		Senecio	glabellus
Gramineae			
Leguminosae	Tribe Genistae		
	Series Simplicifoliae		
	Subseries Erectae	Crotalaria	retusa
		Crotalaria	spectabilis
	Subseries Eriocarpae	Crotalaria	juncea
	Series Digitatae		
	Subseries Polyspermae	Crotalaria	mucronata

Bull et al. (1968).

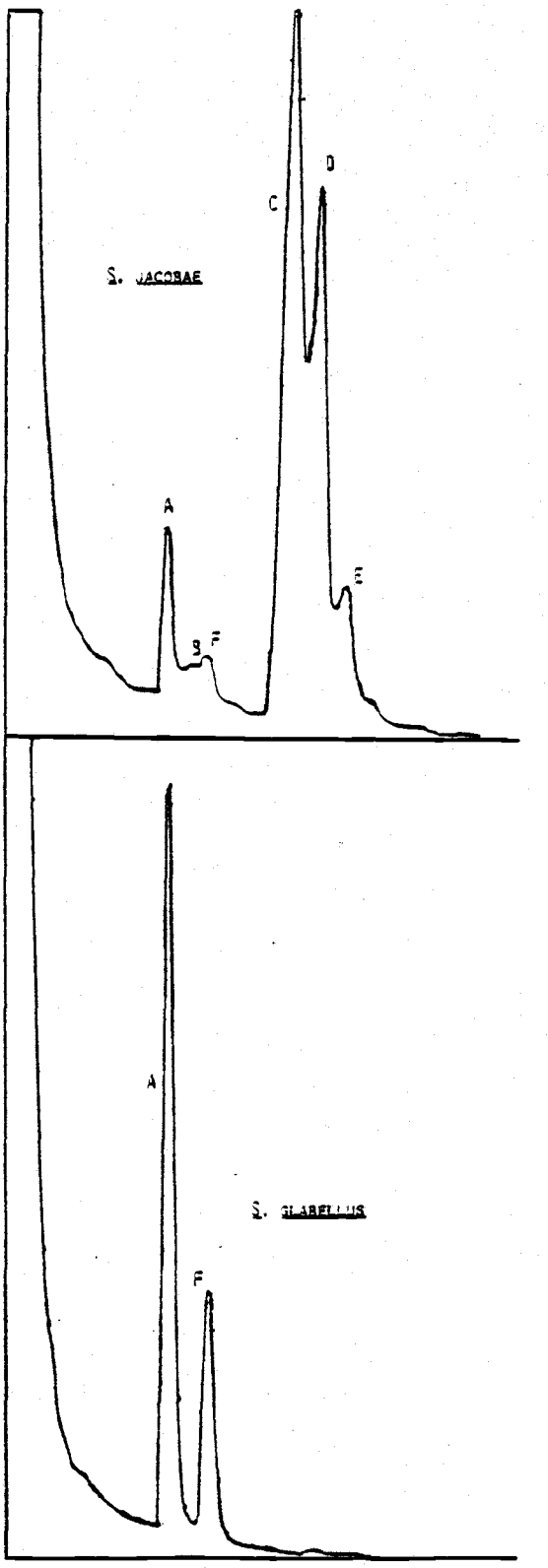


Figure 1. Gas chromatograms of S. jacobaea and S. glabellus alkaloid preparations. Alkaloid peaks designated by letters are: (A) senecionine, (B) seneciphylline, (C) jacobine, (D) jacozine, (E) jacoline, and (F) unidentified.

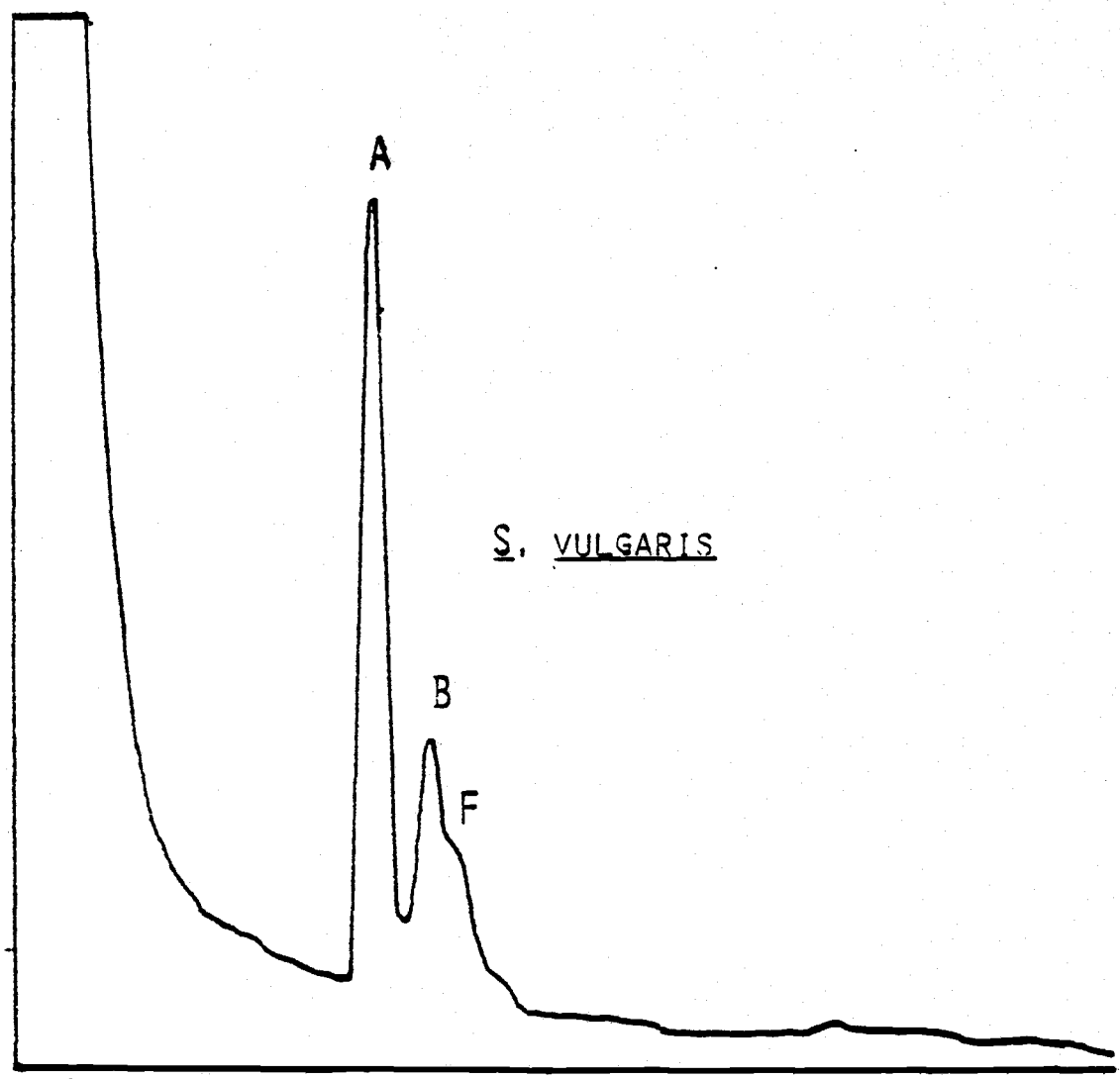


Figure 2. Gas chromatogram of S. vulgaris alkaloid preparation. Alkaloid peaks designated by letters are: (A) senecionine, (B) seneciphylline, and (F) unidentified.

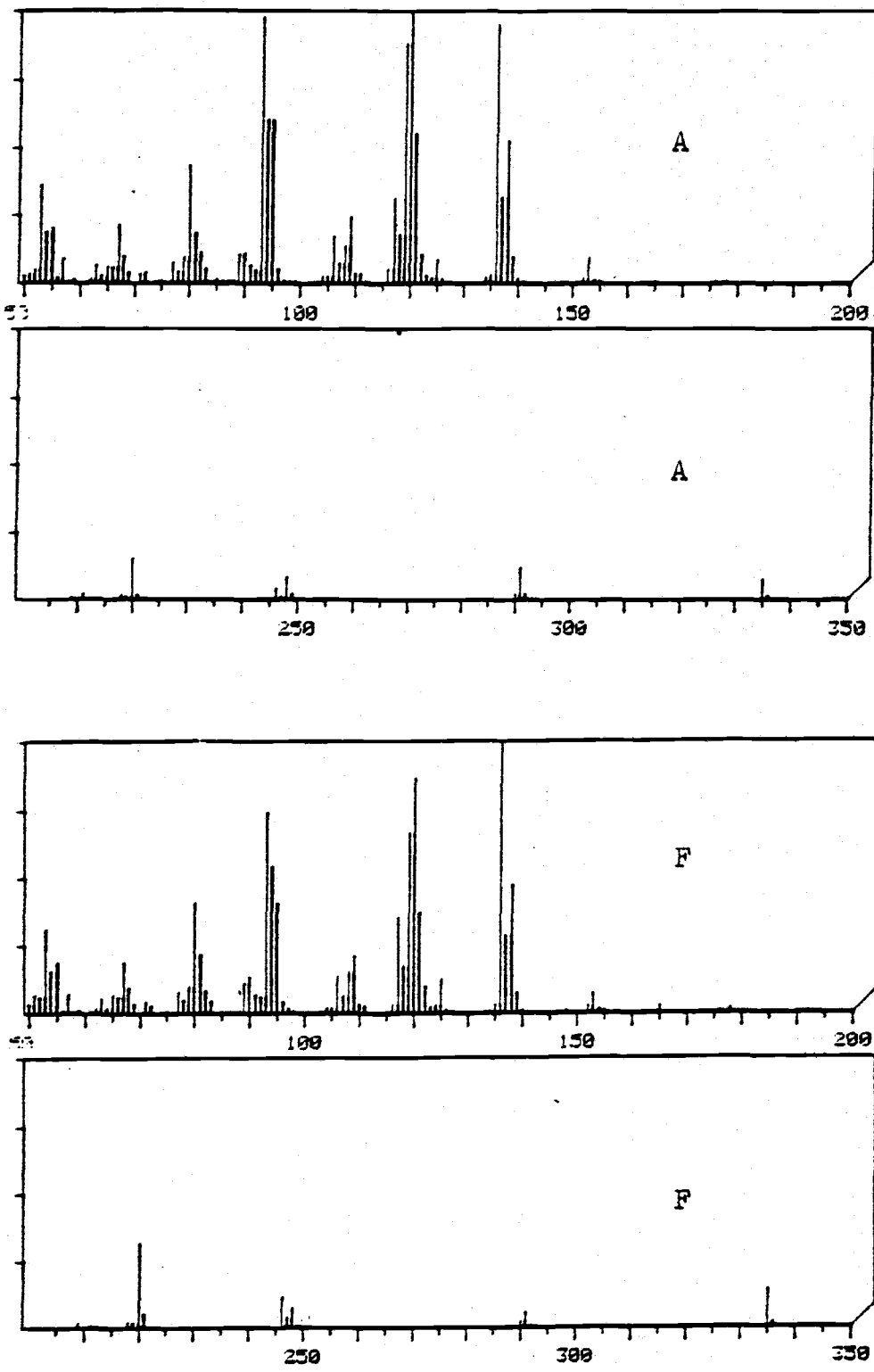
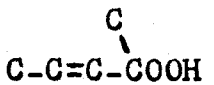
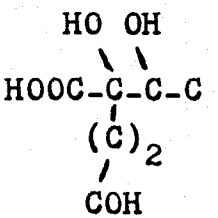
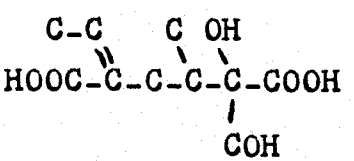
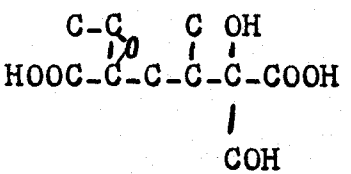
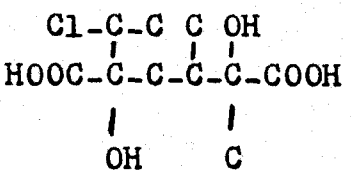
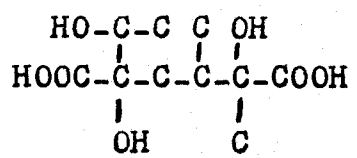
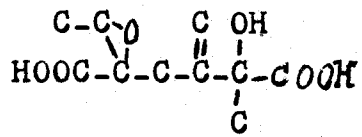
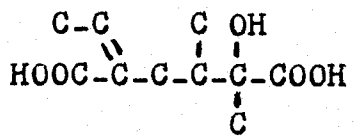
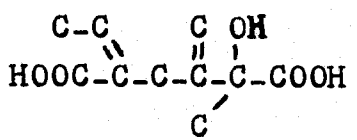
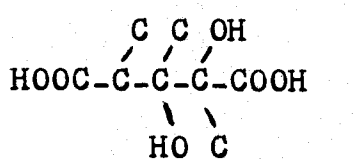


Figure 3. Mass-spectrometric analysis of alkaloid peaks A (senecionine) and F (unidentified) of S. glabellus.

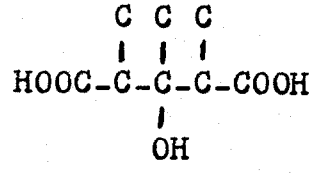
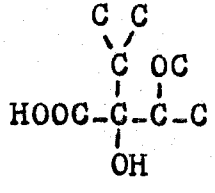
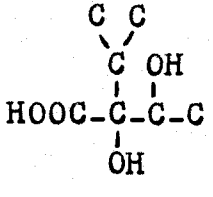
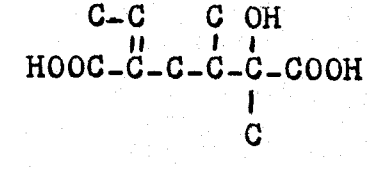
APPENDIX TABLE 4. TYPE AND STRUCTURE OF SOME PYRROLIZIDINE ALKALOIDS[a]

Alkaloid	Type	Amino Alcohol[b]	Acid	Acid Structure[c]
Lasiocarpine	open diester	heliotridine	7-angelic	
			l-lasiocarpic (butyric derivative)	
Retrorsine	closed diester	retronecine	isatinecic (adipic derivative)	
Jacobine	closed diester	retronecine	jacobinecic (adipic derivative)	
Jaconine	closed diester	retronecine	jaconinecic (adipic derivative)	

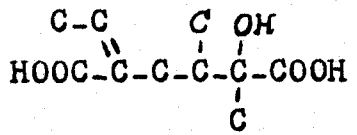
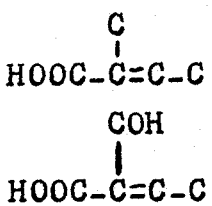
APPENDIX TABLE 4 (Continued)

Alkaloid	Type	Amino Alcohol[b]	Acid	Acid Structure[c]
Jacoline	closed diester	retronecine	jacolic (adipic derivative)	
Jacozine	closed diester	retronecine	jacozic (adipic derivative)	
Senecionine	closed diester	retronecine	senecic (cis)(adipic derivative)	
Seneciphylline	closed diester	retronecine	seneciphyllic (adipic derivative)	
Monocrotaline	closed diester	retronecine	monocrotalic (glutaric derivative)	

APPENDIX TABLE 4 (Continued)

Alkaloid	Type	Amino Alcohol[b]	Acid	Acid Structure[c]
Fulvine	closed diester	retronecine	fulvinic (glutaric derivative)	
Heliotrine	monoester	heliotrine	heliotric (butyric derivative)	
Indicine	monester	retronecine	(-) trachelanthic (butyric derivative)	
Isosenecionine	closed diester	retronecine	senecic (trans) (adipic derivative)	

APPENDIX TABLE 4 (Continued)

Alkaloid	Type	Amino Alcohol[b]	Acid	Acid Structure[c]
Platyphylline	closed diester	platynecine (7 beta hydroxy-1 beta-methoxy 8-alph pyrrolizidine	senecic (adipic derivative)	
Sarracine	open diester	platynecine (7 beta hydroxy-1 beta-methoxy 8-alpa pyrrolizidine	7-angelic 1-sarracinic (butyric derivative)	

[a]Information obtained from Bull et al. (1968) and Schoental (1968a).

[b]Structures of amino alcohols given on pages 40, 41.

[c]Carbon hydrogens omitted for clarity.