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CHEMOTAXONOMICAL COMPARISON OF ASTRAGALUS MEGACARPUS, ASTRAGALUS BECKWITHII AND ASTRAGALUS OOPHORUS IN UTAH

A Thesis

Presented to the

Department of Botany and Range Science

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Marzilla Anderson

April 1975

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INTRODUCTION

The genus Astragalus has been of taxonomic interest for several hundred years. As the New World was explored many new species of Astragalus were collected. The species descriptions were often published simultaneously by several different collectors or botanists and often the same species were given different names in different journals. As a result the number of species of Astragalus has burgeoned to 1,500 species on a worldwide basis. In 1964 Rupert C. Barneby published a definitive work on the taxonomy of Astragalus. It was titled "The Atlas of North American Astragalus." He divided the genus into groups of related species called sections and the related sections into groups called phalanxes. There still has not been sufficient time for workers to unravel all the difficulties in relation to the proper species names. The taxonomic relationships of the section examined in this thesis has not been resolved. In addition, Astragalus oophorus var. caulescens and Astragalus beckwithii var. beckwithii, when in flower, are virtually indistinguishable. Also Astragalus oophorus and Astragalus megacarpus

are very similar when in fruit.

As the West was settled, Astragalus became of particular interest because of its peculiar poisonous effects. Astragalus species were considered to be members of a group of plants known as "loco weeds". These plants were thought to kill animals, particularly horses; or if the plants were eaten in lesser quantities, the animals gained weight slowly, had a wobbly gait, and gave birth to malformed young. The toxin often induced such long-term behavior aberations that the horses could not be ridden or restrained dependably.

Since the 1930's, sporadic efforts have been made by various investigators to determine the toxic principles in Astragalus. The effort has been intensified in the last 20 years with the increasing technology for analyzing plant products. It appears that Astragalus species contain several toxins and many other compounds which may be of value in determining the taxonomic relationships or may simply aid in identification of particular species.

This thesis investigated the relationship of lipid compounds, toxic principles, and other plant compounds, to the taxonomy of three Astragalus species; Barneby's section megacarpi (Barneby 1964) which occur in Utah.

LITERATURE REVIEW

Classical Taxonomy

The present species Astragalus megacarpus Astragalus oophorus, and Astragalus beckwithii have not always been named as they are now. These names come from the latest revision of the genus by R. C. Barneby. Astragalus megacarpus was first named Phaca megacarpa by Nuttall and published in Torrey and Gray's Flora of North America (Tidestrom 1925). It was changed to Tragacantha in 1891 by O. Kunze, to Astragalus by M. E. Jones in 1923, back to Phaca by P. A. Rydberg in 1929 and finally back to Astragalus by Barneby in 1964. At one time or another A. megacarpus has had three varieties; they are variety parryi by A. Gray in 1876, variety prodigus by Sheldon in 1894, and variety caulescens by Jones in 1895. Jones later realized that his variety was really A. oophorus and made the change in 1923 when he wrote his revision of the genus. Varieties prodigus and parryi have been recognized as synonyms by later writers (Barneby 1964).

Astragalus oophorus was changed to Astragalus

artipes by A. Gray in 1878, to Phaca artipes in 1905 by P. A. Rydberg, back to Astragalus oophorus by M. E. Jones in 1923. Using the original species name, Rydberg made it Phacamenes oophorus in his revision in 1929. It became Astragalus oophorus again through Barneby in 1964. There are presently four varieties of A. oophorus, but only the variety caulescens is considered in this investigation. Its history was discussed with A. megacarpus (Jones 1923, Tidestrom 1925, Rydberg 1929, Barneby 1964).

Astragalus beckwithii was first described and published by Torrey and Gray in 1864. The genus was changed to Tragacantha in 1891 by O. Kuntze (Jones 1923). Jones changed it back to Astragalus, Rydberg made it Phaca in 1929, and Barneby published it as Astragalus beckwithii in 1964. He described the flowers as being close to A. megacarpus. There are four varieties of A. beckwithii, of which only the variety beckwithii occurs in Utah. It was first published in 1964 by Barneby to distinguish it from weiseriensis, a similar but larger variety (Jones 1923, Rydberg 1929, Barneby 1964).

Chemotaxonomy

There are few published accounts of chemotaxonomic

work done on the genus Astragalus. When the amino acids of 120 species were analyzed two groups of species contained seleno-amino acids. The other common amino acids were also present in all species (Dunnill and Lowden 1967). In a survey of flower pigments of 18 European species of Astragalus, rubixanthan was the common pigment in all. The investigators speculated that this pigment might be characteristic for the genus (Neamter and Bodea 1969). Twenty-nine samples of Astragalus seeds tested for oil, protein, and nitrogen-free gum content on a dry weight basis gave 4.9% seed oil, 41.4% seed protein, and 13.7% nitrogen-free gum. These values were ranked with 59 other genera from Leguminosae but the ranking did not follow taxonomic groups (Harborne et al. 1971).

Chemical investigations have been conducted on Astragalus species in conjunction with other investigations; generally the analytical work has been done on only one or a few species of Astragalus at a time. These analyses have been done by different workers on diverse species.

Alkaloids and other nitrogenous compounds that have been reported are as follows: Astragalus (smernoveine) (Harborne et al. 1971), alpha and beta earleines

in Astragalus earlei, and betaine, choline and trigonelline in Astragalus wootonii (Henry 1949).

Lipid compounds and elements such as "phytosteroids", flavonoids, tocopherol, selenium, coumarin, and phytocoumarin have been extracted from Astragalus dasyanthus (Khoro'ko and Yashchenko 1968). Flavonoid glycosides and coumarin compounds were found in a drug survey done on European Astragalus (Blinova and Blankova 1968). A glycoside is reported as the basic structure of miserotoxin, a substance which is reported to release 3-nitro-1-propanol into the rumin of sheep, cattle and other animals. This compound is reported to be present in Astragalus miser var. serotinus (Hahn 1970, Mosher et al. 1971a, 1971b, Stermitz et al. 1971a, 1971b, Williams et al. 1969 and 1970).

Astragalus gummifer and other Mediterranean species have been used pharmaceutically for millennia in that area. In the United States gum tragacanth is used both pharmaceutically and industrially (Claus 1961). In China, researchers report that Astragalus membranaceus has a desirable diuretic and hypotensive effect similar in action to amino-phylline and hydrochlorothiazide but with no toxic effects or side effects (Hou-p'ing Huang

et al. 1965).

Toxicology

There appears to be some taxonomic significance in the toxic effects of Astragalus species. All the species reported to be toxic to animals in the last forty years fall into Barneby's Phalanx B (the Homaloboid Astragali) or Phalanx E (the Piptoloboid Astragali). The section Megacarpi is in Phalanx E. All the species reported to be selenium accumulators and those reported to contain more than 150 ppm selenium are of the Homaloboid type. Of these, the species reported to be indicators are in the section Bisulcati or its relative, Pectinati. The non-accumulators are in sections Preussiani and Ocreati. (Astragalus michauxii is reported to be toxic, but there is no data on its selenium content. It is in Phalanx B in section Michauxi) (Beath et al. 1939, Barneby 1964, Draize and Beath 1935, Lewis et al. 1967, Martin et al. 1971, Nigam et al. 1969, Nigam and McConnell 1973, Shrift et al. 1966). Astragalus species which owe their toxicity to some other factor than selenium are of the Piptoloboid type. All these species occur in different sections and the symptoms of poisoning vary.

Astragalus miser var. serotinus whose toxic principle is miserotoxin, is reported to cause ulcers and hemorrhage in the intestines and stomach of cattle and sheep; also, it causes a loss of equilibrium (staggered gait), arching of the back, and increase in glutamic oxaloacetic transaminase and serum isocitrate dehydrogenase blood levels (Hahn 1970, Mosher et al. 1971a and 1971b, Stermitz et al. 1971a and 1971b, Williams et al. 1970). Astragalus miser var. serotinus is in section Genistodei.

Astragalus mollissimus, section Mollissimi, is reported to cause a loss of weight, gait alterations, hyperexcitability or depression, gastric ulcers and pyloric edema, kidney tubule damage, liver necrosis and a general decrease in neuronal tissue when sheep and cattle graze on it (Duncan et al. 1955 and Oheme et al. 1968).

Astragalus michauxii, section Michauxi, is reported to cause a loss of weight, anorexia, hyperexcitability or irritability, decreased circulation to the extremities, decreased body temperature and liver damage when fed to chickens (Duncan et al. 1955).

Astragalus pubentissimus, section Inflati, and A. flavus candicans, section Ocreati, are reported to be habit forming, i.e., they are "loco weeds", and cause

congenital malformations of lambs (James and Binns 1967).

Astragalus lentiginosus, section Dyphysi, has been reported as both a selenium accumulator (Beath et al. 1939) and a non-accumulator (Ulrich and Shrift 1968). Several workers have fed unspecified varieties to animals and the results have been reported for blood values, glandular changes, reproductive system effects, teratological malformations and physical appearance changes. The blood values for serum glutamic oxaloacetic transaminase and serum isocitrate dehydrogenase and blood proteins increased, which indicated tissue damage (James and Van Kampen 1971). Thyroid and parathyroid glands were enlarged, as were the seminal vesicals, and the epithelium of the epididymus. The kidneys and liver were discolored and reduced in size. Vacuolation increased in the cells of the pancreas, kidneys, liver, reproductive systems of both sexes, and nervous tissue (James and Van Kampen 1971, James et al. 1968, James et al. 1971, Van Kampen et al. 1970). Abortions increased 56%, and fetal malformations included pastern joint contraction or overextention, carpel joint flexure with lateral rotation of the forelimbs, osteoporosis, bone fragility, and brachygnathia (Keller et al. 1971).

MATERIALS AND METHODS

Collection Data

The plants used in this study were collected from the following sites: Astragalus megacarpus (A. Gray) was collected from a sandy red outcropping of aropean shale in the Morrison formation 0.4 miles northeast of the junction of Pigeon Creek Canyon and Chicken Creek Canyon roads. This junction is 1.7 miles east of Utah Highway 28 in Levan, Juab Co., Utah. Astragalus oophorus variety caulescens (Jones) Jones was collected on the Morrison Formation from an overgrazed sheep pasture 0.3 miles east on a graded road which intersects U. S. Highway 91, one mile south of Nephi, Juab Co., Utah. Astragalus beckwithii (Torr & Gray) variety beckwithii was collected 100 yards north of Provo city water tank at the east end of a parking lot for a junk yard 1.9 miles south of the Provo Cemetery along U. S. Highway 89. Astragalus lentiginosus variety araneosus (Sheld) Barneby, used for rat assay series number one, was collected at several places in road cuts along U. S. 6 and 50, south of Eureka to Jerico, Juab Co., Utah. Astragalus sabulosus (Jones) was collected from Bromley's ridge in the LaSal

Mountains, 15 miles east of U. S. Highway 163 along a dirt road which is located on the southern border of the airport 10 miles south of Moab, Grand County, Utah. All plants were collected in anthesis or fruit or both, during the summers of 1971 and 1972. The plants were identified by S. L. Welch of the BYU herbarium during the summer of 1971 and subsequently by the author with the aid of The Atlas of North Astragalus.

Pollen Analysis Methods

Entire keels were collected of A. megacarpus, A. oophorus var caulescens, and A. beckwithii var beckwithii. The keels from about 10 flowers of each species in early anthesis were placed in new, labeled glass vials. The pollen grains were shaken out of the keels onto electron microscope grids which had been previously coated with about 200 angstroms of a gold-paladium alloy. The extine of the pollen grains were judged to be sufficiently hard and stable to permit the omission of the usual coating of carbon and platinum. Pollen was observed with an SSM-2 Hitachi Scanning Electron Microscope at an electron acceleration voltage of 10,000 volts. About 5-7 micrographs were selected from each species at magnifications of 1400X and 3500X. The pictures

were taken on type 55PN, 4"x5" polaroid film with a polaroid camera attached to the viewing screen. The film was developed with no fixatives. The microscope was operated by J. V. Allen and the photographic technique was also suggested by him. Measurements made of the micrographs were used to compare the relative lengths, widths, suture length, and pitting characteristics of the extine. The measurements were made with a metric ruler and converted to microns.

Morphological Parameters

Measurements were taken of pressed plants in the Utah State University, University of Utah, and Brigham Young University herbaria. Only those plants which had both fruits and flowers were measured to assure correct identification. Measurements were made of plants' height, leaf length, leaflet length and width, calyx tube length, pod length and width, pedicel length, and gynophore length. The measurements were taken with a metric ruler and sometimes with the aid of binocular microscope. Observations on growth habits under controlled environment conditions were made on Astragalus species potted and grown in the BYU Department of Botany and Range Science greenhouses.

Six-Channel Autoanalyzer

Water soluble components from five grams of powdered seeds and pods from the three Astragalus species were extracted with 100 ml of tris-glycine buffer. The buffer was made from six grams of tris /tris (hydroxymethyl) amino-methane Sigma Chemical Company/ and 28 grams of glycine. The three extractions were filtered and stored in acid-washed culture tubes at 4°C until they could be transported to Salt Lake City. The six tests were for chlorine, glucose, urea nitrogen, potassium, sodium, and soluble phosphorous. The samples were analyzed on the autoanalyzer by T. E. Northstrom.

Lipid Analysis Methods

Plant material was collected at each collecting site in large plastic bags and transported to the laboratory where they were separated, labeled and dried on newsprint paper in a drying oven at 40-50°C for 8-12 hours. The dried tissue was ground in a Wiley Mill using a gauge 20 screen. The ground plant material was stored in new glass jars with aluminum cap liners. An extraction process proceeded as follows: 600 gm of whole plant powder from each species was placed in a two-liter flask

containing 600 ml of spectrogra e heptane. The flask was covered and set on a slide warmer for one hour. The heptane extract was collected and the tissue was further extracted with another 200 ml of heptane. The extracts were combined and concentrated in a flash evaporator at 40°C. The concentrated sample was stored in a freezer at -20°C. The remaining plant residue was further extracted with petroleum ether with a boiling point of 30-60°C in a similar manner, concentrated under a stream of compressed nitrogen on the slide warmer and stored in a freezer -20°C.

These extracts were then spotted on thin layer chromatography (TLC) plates. The TLC plates were 20 cm by 20 cm and were prepared by washing with detergent and rinsing with methanol. After drying, the plates were coated with silica gel G (1 gram of silica gel: 2 ml water). The thin layer plates were activated in a small oven at 100°C for two hours and stored in a dessicator chamber until ready for use. The solvent system used consisted of heptane, chloroform, and methanol (4/2/1 v/v). The previously prepared plates were spotted with about 400 microliters of the combined heptane and ether extracts in a line about 2 cm from the bottom of the plates. The plates were allowed to dry and then placed on a rack

designed to hold two thin layer plates in an upright position. The rack and plates were placed in the solvent inside the equilibrated chamber until the solvent had moved about 1-2 cm from the top of the plate. The plates were removed and placed in a potassium iodide chamber to detect any lipids. (Lipids turn brown to yellow upon exposure to potassium iodide.) Upon removal from the iodide chamber the TLC plates contained 10-12 horizontal bands, the 12 bands were marked with a dissecting needle. Some bands were missing in some species (Figure 1).

When the iodine had evaporated, the bands were scraped with a clean razor blade into acid washed beakers. Bands 1-3 contained the hydrocarbons, bands 4-9 contained the chlorophylls, e.g. band 4 contained chlorophyll a. Band 12 was located at the origin and bands 11 and 10 were near the bottom. About five plates of each species were needed to obtain about 30 g of the lipid-impregnated silica gel. The lipid compounds were removed from the silica gel by placing the mixture in a scintered-glass filter funnel attached to a suction flask which was attached to a water vacuum pump. Water-saturated ether was poured onto the silica gel and collected in a test tube in the suction flask. About 25 ml of water-saturated ether was used to

extract the silica gel in combination with the following solvents: heptane, 25 ml, was used on bands 1-3; benzene, 25 ml, on bands 4-9, benzene, 25 ml, followed by methanol, 25 ml, on bands 10-12. The volume of the 36 (12 from each of the three species) lipid samples were reduced by placing the beakers on a slide warmer at 50°C under a stream of compressed nitrogen until the volume reached 3-5 ml. The samples were then poured into 5 ml acid washed, dry, glass vials covered with aluminum foil, labeled and frozen until used. Before injection of the sample into the gas chromatograph. Packard gas chromatographs with flame ionization detectors were used. All samples were analyzed using a temperature program in which the column oven temperature started at 50°C and then increased 3°C per minute until a temperature of 250°C was reached. The inlet oven temperature was 240°C and the detector oven was set at 250°C. The columns were capillary steel 0.003" x 30' or glass 1/8" x 6' long, coated with SE-30. The flow rates for the gases were as follows: nitrogen for carrier, 40 cc/min., hydrogen for flame ionization detector, 35-40 cc/min. and compressed air, for flame detector, 350-400 cc/min. From 2-5 ul of each sample was injected into the injection port with a 10 ul syringe. Chromatograms of a

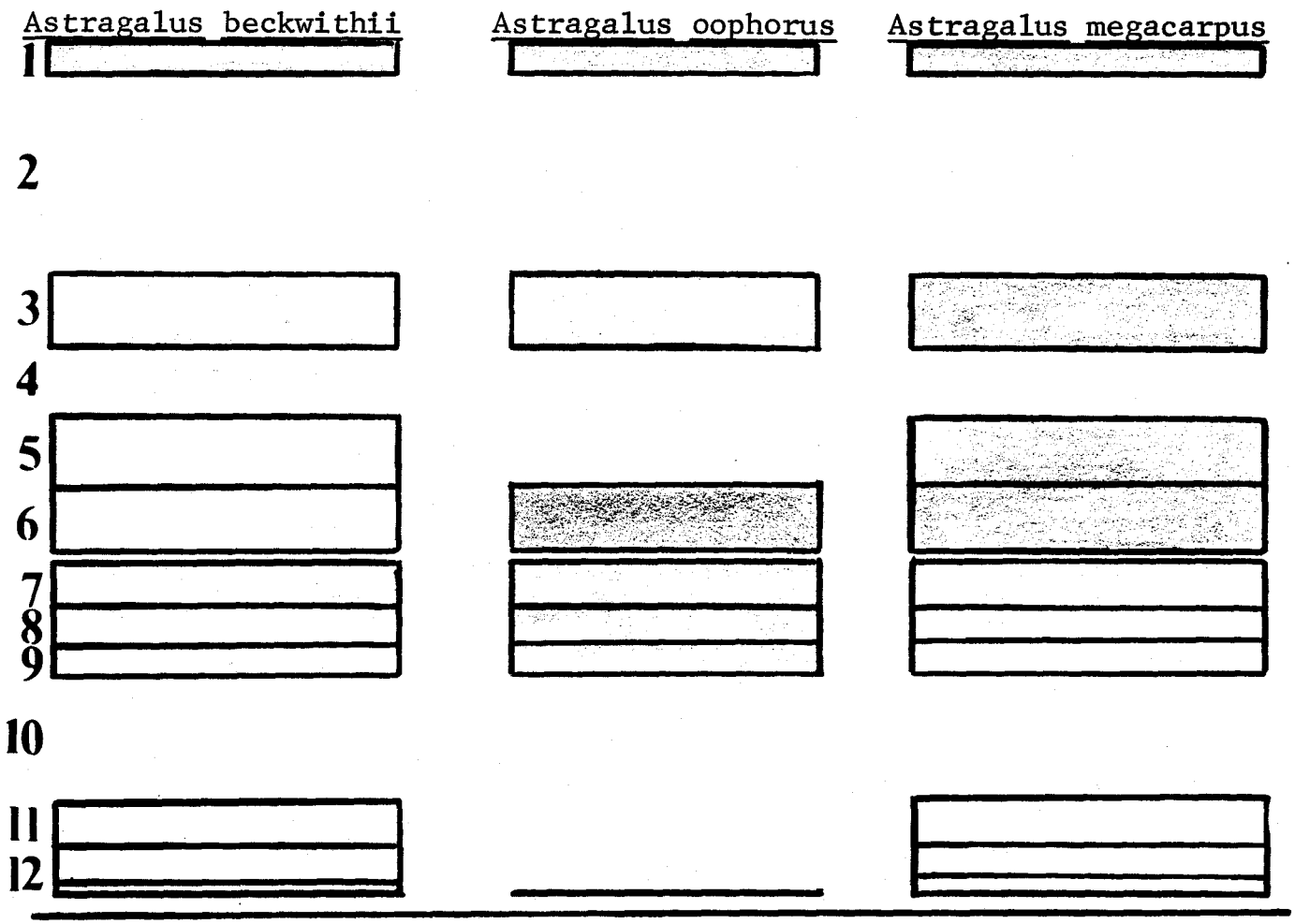


Figure 1. Thin layer chromatograph of heptane-ether extracts of Astragalus beckwithii, Astragalus oophorus, and Astragalus megacarpus. Development solvent was heptane, chloroform and methanol (4/2/1 v/v). Bands visualized with iodine vapor.

standard mixture of known hydrocarbons separated on the same column were obtained by the same procedure. Bands showing one or more large peaks were then injected into a Varian-MAT 111 GC-Mass spectrometer, fitted with a 3' x 1/8" stainless steel or glass column containing 1% SE-30, OU-1, or Pentasil or Chromasorb Q and run on a program of 50°C increasing 6°C per minute to 150°C then increasing 4°C per minute to 250°C. Bands B-1, O-1, and M-1, because of the many compounds located thereon, were run with a much slower program with only 4°C or 2°C increases per minute. Mass spectrograms of the major peaks were obtained from an oscillographic recorder using UV sensitive paper (Kodak Co.). Compounds were identified with the help of D. J. Weber and the NIH computer search at Bethesda, Maryland. The settings for the GC-Mass spectrometer were EID range 32, 80 EV ion chamber, mass range 600, gain 3-5, and inlet line temperature 200°C. All Spectra were calibrated by comparison to a mass spectra of perflourokerosine, and appropriate hydrocarbon spectra.

Rat Assay Series I

Ground, dry plant material was obtained according to the previously described method. In the first series

of toxic assays, five rats feeding experiments were completed. They were conducted as follows: sample fractions of various Astragalus species were weighed and mixed with a commercial rat chow preparation to be fed to 30-day old white male rats obtained from a commercial company in Madison, Wisconsin. In rat assay series number 1, experiment 1, fifty-eight rats were divided into groups and fed according to the manner shown in Table 1.

The rats were separated into groups of two rats per cage, except the controls in larger cages were in groups of three or four rats per cage. They were fed, weighed and watered daily. Their diets were arranged so that they received 10-15 gm of food daily, and water as needed. They were weighed by groups and the weights averaged per treatment.

The first experiment lasted 12 days. At the conclusion the remaining rats were sacrificed with anhydrous ether, examined, photographed and samples of their livers fixed in glutaraldehyde for electron microscopic examination under W. M. Hess's direction. Hematocrits were taken of blood samples collected by R. Henniger. Color photographs were taken of coat color, relative size, and viscera of all treatments.

In the second experiment of series 1 rats were fed fresh foliage and pods from Astragalus beckwithii and A. megacarpus; the controls were fed unmixed rat chow. The fresh plants were collected from near Provo and Levan, Utah. They were stored in plastic bags and kept at 5°C until weighed and fed to the rats. The rats were given 10-15 gm daily; the rats eating the fresh foliage were given 20-50 gm daily or as much as they would eat.

The remaining three experiments were used to assay for the isolation of the toxic factor(s). In the third experiment of the first series, A. megacarpus was treated with several solvents in an attempt to extract the toxic factor(s). The plants were dried whole in a hood for 24-36 hours then ground in a Wiley Mill with a gauge 20 screen, stored in glass jars at 5°C. One liter of benzene with 1% 7N NH_4OH v/v they received 10-15 grams of food daily, and water as needed. They were weighed by groups and the weights averaged per treatment.

The second experiment lasted eight days. On the third day the percentage of pods to the rats eating A. beckwithii was increased to three times the normal amount. At the conclusion of the experiment, the rats were sacrificed in the same manner as before except the hematocrits

TABLE 1

EXPERIMENTAL DESIGN FOR TESTING EFFECT OF FEEDING *ASTRAGALUS SABULOSUS*, *ASTRAGALUS BECKWITHII* AND *ASTRAGALUS LENTIGINOSUS* TO WHITE RATS

Number	Amount	Species	Proportion Wt/Wt
4	800	*sabulosus*	1%
4	800	*sabulosus*	10%
4	800	*sabulosus*	50%
6	1200	*beckwithii*	1%
6	1200	*beckwithii*	10%
6	1200	*beckwithii*	50%
6	1200	*lentiginosus*	1%
6	1200	*lentiginosus*	10%
6	1200	*lentiginosus*	50%
10	2200	control	100%

were omitted.

The remaining three experiments were used to assay for isolation of the toxic factor(s). In the third experiment of the first series, A. megacarpus was treated with several chemical solvents in an attempt to extract the toxic factor(s) (See Figure 2). The plants were dried whole in a hood for 24-36 hours then ground in a Wiley Mill with a 20 gauge screen, stored in glass jars at 5°C. One liter of benzene and 1% 7N NH_4OH v/v was used to extract about 600g of ground A. megacarpus. After the plants soaked in the benzene mixture for an hour, the extract was decanted and the residue washed three times with 0.5 liters benzene until 1.5 liters had been used. The extract was concentrated in a flash evaporator to about 600 ml and then added to 600 gms of rat chow. The mixture was air dried until no odor of benzene remained. The air dried residue was mixed with an equal amount of rat chow feed. The treatments for experiment three were as follows:

5 rats fed unmixed rat chow

5 rats fed the benzene extract mixed with rat chow

5 rats fed benzene residue mixed with rat chow

5 rats fed A. megacarpus foliage as in the previous experiment

5 rats fed A. megacarpus pod as in the previous experiment

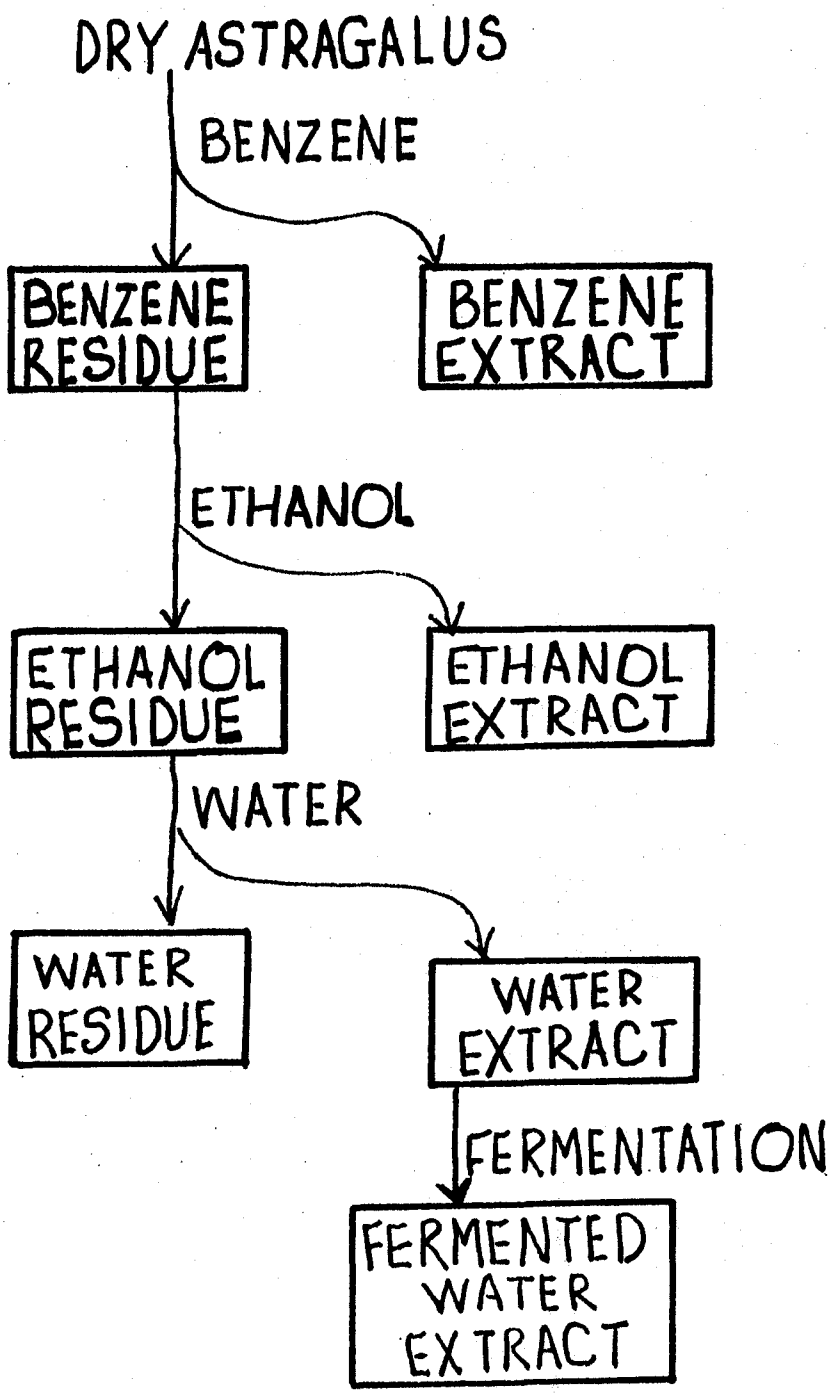


Figure 2. Outline of extraction procedure used for obtaining fractions for assaying for toxic factor(s) in experiment III, IV, and V in rat assay series I.

Seven days later the rats were harvested as before with one exception; one rat eating foliage was reserved and given a choice of A. megacarpus foliage in one feed box and unmixed rat feed in another. The rat was maintained on this mixture for three days, after which he was sacrificed as above.

The livers, spleens, kidneys, and intestines were removed as before and photographed. The tissue was air-dried and stored at 5°C in plastic vials.

The fourth experiment used the remaining benzene residue from the previous extraction. It was extracted with 80% ETOH, for one hour, then washed with additional 0.5 liter portions in a Buchner funnel on a suction apparatus. The extract was concentrated in a flash evaporator and added to rat feed as before, air-dried and fed to five rats for 11 days. Two other groups were used for control, one group was fed unmixed rat feed and the other group was fed dried foliage plus rat feed (1:1 ratio).

In the fifth experiment a water extract of the ETOH residue was made, separated and mixed with the rat feed. Another experiment was included in which the plant tissue was allowed to ferment over the weekend. The

fermented extract was mixed with rat feed and fed to five rats. These groups were compared with the groups that were fed the ETOH residue and the unmixed rat chow. This experiment lasted for 11 days, then the rats were sacrificed and their groups examined as before.

Rat Assay Series No. 2

Another series of rat assays were conducted to compare the effects on rats of Astragalus species of Barneby's section Megacarpi that occur in Utah. The ground plant material was obtained as before, and used in 400 gm lots. Four such lots were used in the course of this 14 day experiment. The extractions were made as follows: 100g plant material from each species, was placed in extraction thimbles, with 50 mg of $CaOH_2$ and wet thoroughly with doubly distilled water. One hundred ml of benzene was poured into a round-bottom flask and recycled with a soxhlet condenser for eight hours. The benzene fraction was then separated from the aqueous fraction with a separation flask. The aqueous portion was diluted with enough water to bring the pH to near eight. This resulted in a volume of 200 ml of aqueous solution. Both fractions were poured over separate lots of rat chow

and mixed in clean plastic trays. The mixtures were stirred and allowed to dry under a fume hood until they were dry to the touch and no odor of solvent could be detected. The resulting feed portion weighed 500g. These were fed to 60 day old white male rats.

The rats were placed three to four per cage. They were fed 10-25g of food per rat, per day and given water ad libitum. They were weighed and fed every two days but given water daily. Their weights were averaged per cage and per treatment and weight was correlated with food eaten.

TABLE 2

EXPERIMENTAL DESIGN FOR ASSAYING EXTRACTED FRACTIONS OF *ASTRAGALUS BECKWITHII*, *ASTRAGALUS OOPHORUS*, AND *ASTRAGALUS MEGACARPUS* USING WHITE RATS

No. of Rats	Amount Fed	Fractions	Species	Comment
8	2000g	Benzene Extract & rat chow	*A. beckwithii*	4 returned to rat chow after 7 days
8	2000g	Benzene Extract & rat chow	*A. oophorus*	4 returned to rat chow after 7 days
8	2000g	Benzene Extract & rat chow	*A. megacarpus*	4 returned to rat chow after 7 days
7	2000g	Water Extract & rat chow	*A. beckwithii*	3 returned to rat chow after 7 days
7	2000g	Water Extract & rat chow	*A. oophorus*	3 returned to rat chow after 7 days
8	2000g	Rat Chow		Control
4	4000g	Benzene only & rat chow		Benzene Control

RESULTS

These data were obtained from the various methods mentioned previously to allow a taxonomic description of each species, keys to the species, evidence of their chemical kinship and evidence of their toxic effect on rats. These data show that Astragalus beckwithii var. beckwithii and Astragalus oophorus var. caulescens, although closely related, are still distinct species. The data reveal differences in the morphology and pollen characteristics, but similarities in the chemical constituents of the two species above. Table 3 is a summary of the results as they relate to the taxonomy of Section Megacarpi.

Plant Descriptions

The following are plant descriptions compiled from field observations and examination of the specimens cited in Appendix A. Maps showing the distribution of these species in Utah are shown in Figures 3, 4, and 5. See Appendix C for keys to these species.

TABLE 3

SUMMARY OF RESULTS AS THEY AFFECT THE TAXONOMY OF BARNEBY'S SECTION MEGACARPI

Characteristic	Species		
	A. oophorus	*A. beckwithii*	*A. megacarpus*
1) Gynophore length in flower	3mm-5mm	1.5mm-3mm	2mm-2.5mm
2) Gynophore length in fruit	5mm (longer than calyx)	3mm	2.5mm
3) Pedicel length in fruit	Shorter than pod length	Shorter than pod length	Length equal to or longer than pod
4) Pod character	Inflated, papery	Reflexed, leathery	Inflated, papery, splits base of calyx
5) Pollen grain length	27-32 μ	25-30 μ	22-25 μ
6) Sextine markings	Large spaces	Intermediate species	Small spaces
7) Pore thickenings	Absent	Present about 40%	Present

(Continued)

Characteristic	Species		
	A. oophorus	A. beckwithii	A. megacarpus
8) Overall shape of the pollen	Long ovoid	Egg-shaped	Spherical
9) Glucose content	65 mg%	80 mg%	100 mg%
10) Rat toxicity of benzene	+	+	0
11) Rat toxicity of water extract	+	+	+
12) Locoism produced	+	+	+
13) Unique lipid compounds	23	2	17

Astragalus beckwithii var.
beckwithii Torr. & Gray

Perennial herb. Root, infrequently branched tap. Stem, aerial caudex with 3-5 (25) aerial stems, one upright, the others ascending to humistrate; hirsute to stigose; upper internodes longer than lower. Leaves 4-15 cm long, 3-10 cm wide; leaflets 11-27, pedunculate, truncate or retuse, entire sub-orbicular, or lanceolate to ovate, 8-13 mm long, 6-8 mm wide, bright green and leathery. Flowers 8-12, ochroleucous in a terminal raceme on a shorter, lateral peduncle; pedicel stipulate, 1-2.5 cm long in anthesis 3-5 cm in fruit; calyx tube cylindrical, tube 4-5 cm long, stigulose with black hairs; lateral calyx tooth 2.5-3.7 mm long; banner 16-17 mm long, reflexes 45-60°, wings, length-width ratio 5.6 to 6.8, cover keel; ovary 2-5 mm long, transparent in immaturity, atigulose to subglabrous, linear, resembling a silique; gynophore 1.5 mm in anthesis, 5 mm in fruit, slightly thickened, jointed; nectary inconspicuous. Fruit, a pod, olive or with red mottling 1.5-3 cm long 7-12 mm wide, reflexed 15-50°, stigose to subglabrous, turgid, coreaceous, fleshy, dorsal inflexion appears as a partial septum.

Astragalus oophorus (Wats)
var. caulescens (Jones) Jones

Perennial herb. Root, infrequently branched tap. Caudex, ground level or above; stems 4-12 (25), humistrate caulescent, (5) 12-30 cm long; upper internode length longer than more basal internodes, pubescence simple and hirsute, leaves 5-15 cm long 2.5-8.5 cm wide; stipules distinct; leaflets 9-21 odd pinnate pedunculate, 3-9 mm wide 6-13 mm long, lanceolate to obovate, entire, acute to retuse, and leathery; pubescence simple, hirsute, hairs more frequent on the upper than the lower surface. Flowers, (4) 8-13, creme to ochroleucous; raceme terminal; peduncle lateral; calyx tube cylindrical to campanulate .4 to 1.2 cm chartuese green hirsute with sparse dark hairs, lateral calyx tooth .2 to 5 mm; wings 11-21 mm long 2.2 to 3.5 mm wide; reflexed 45-90°; ovary and pod glabrous; gynophore; .35 to 1.2 cm; nectary large and products profuse, covering the end of the gynophore. Fruit, a pod, 3-5.5 (6) cm long, 1-2 (3) cm wide, unilocular; valves commonly papery and bladdery inflated, olive green with red mottling.

Astragalus megacarpus (Nutt)
Gray

Perennial Herb. Root, long sparsely branched tap.

Stem subacaulescent, 1-5 cm, low tufted covered with stipules and thatch. Leaves 5-17 cm long 3-12 cm wide, spatulate to sub-globose; leaflets, pedunculate, 5-14 mm long 4-12 mm wide, entire, retuse in more globose forms, lanceolate to obtuse or ovate, bright green, leathery. Stems, leaves and peduncles, calyx hirsute with dark hairs. Flowers large, lavender or purplish; raceme 3-5 (8); peduncles sub-radical .5 to 2.5 (7) cm long; pedical 3.5-5 mm elongates to 5.8 cm in fruit; calyx green and hirsute with dark hairs, 8.5 to 13.5 mm long, cylindrical, teeth 1.9-4.5 mm; petals large, banner reflexed 30-90°, wings at least 16.5 mm long up to 9 mm wide; gynophore 2 mm in anthesis up to 4 mm in fruit, not exceeding the length of the calyx tube; nectary inconspicuous. Fruit, a pod, large 3-6 cm long, 1.5-3.0 cm wide, appears to dehis along both sutures, unilocular, bladdery inflated slightly coreaceous, splitting the calyx tube on the ventral side at maturity.

Pollen Observations

While scanning electron micrographs of pollen are impressive to view, it must be noted that all the electron micrographs are slightly distorted. The vertical axis is

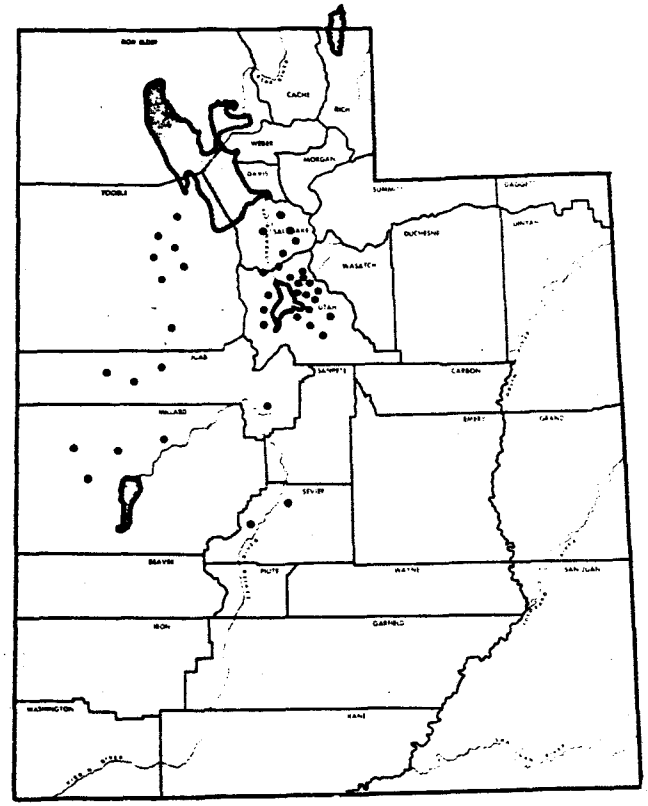


Figure 3. The distribution of Astragalus beckwithii var. beckwithii in Utah.

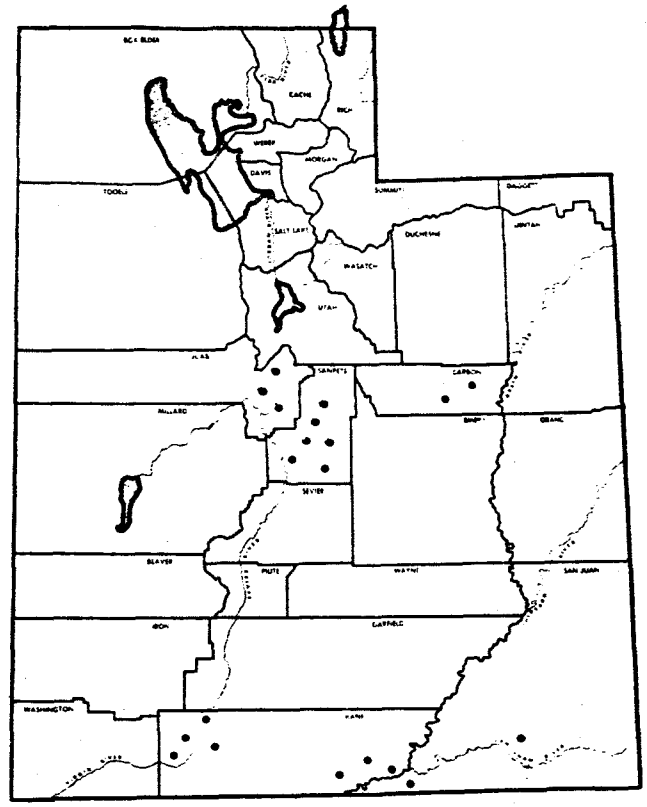


Figure 4. The distribution of Astragalus megacarpus in Utah.

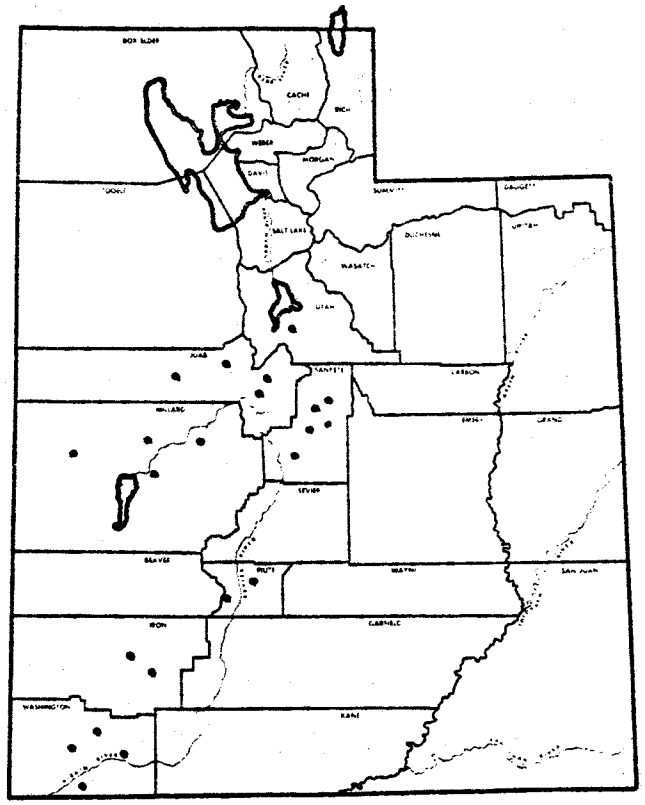


Figure 5. The distribution of Astragalus oophorus var. caulescens in Utah.

shortened while the horizontal axis is elongated. However, these distortions are consistent and uniform so that relative measurements and comparisons of the pollen from the different species are valid.

All the pollen grains appeared superficially like cantalopes. The extine was divided into three large sections by three longitudinal sulci, and the sextine had a reticulate pattern.

The pollen from the three species were different in their appearance, and in the size, shape and pattern of the sextine. Astragalus megacarpus had the smallest size, 22-27 u, the most spherical shape and was three-sulco-porate (Fig. 6). The pores were lolate, from 1/4 to 1/3 the length of the sulcus. The reticulate pattern in the sextine between the sulci had smaller spaces in the equatorial regions with the spaces larger towards the ends. There was a thickened portion of the sextine along the sides of the pores appearing as lips on a mouth, occupying the middle 1/2 of the sulcus length.

The pollen of A. beckwithii was intermediate in size, ranging from 26-31 u in length, with a more elongated appearance (Fig. 7). There were occasional lolate pores in the sulci, accompanied by thickened portions of

the extine as described above; except, the pores were smaller and the thickened portions less conspicuous than in A. megacarpus.

The pollen of A. oophorus was the largest and longest of the three, the sextine ranging from 32-36 u long (Fig. 8). The average length was slightly longer than A. beckwithii. The reticulate pattern in the sextine was much like A. beckwithii in that it was uniform throughout. A. oophorus pollen grains had no apparent pores and only occasional thickened sides of the sulcus.

Six-channel Auto-analyzer

The auto-analyzer was used to determine the concentration of Cl, CO_2, K, Na, and glucose in the three species in this study. All of the samples analyzed were extracted from 10 grams of dried ground plant material. The values obtained for chlorine were all near zero; CO_2, A. beckwithii 55 meg/1, A. oophorus 48 meg/1, A. megacarpus 48 meg/1; potassium, A. beckwithii 35.0 meg/1, A. oophorus 29.5 meg/1, A. megacarpus 33.0 meg/1; sodium, 2.0 meg/1 in A. beckwithii, 1.0 meg/1 in A. oophorus and 1.0 meg/1 in A. megacarpus; urea nitrogen, less than .05 meg/1 in A. oophorus, 0.5 meg/1 in A. beckwithii and 1.5 meg/1

Pollen Micrographs

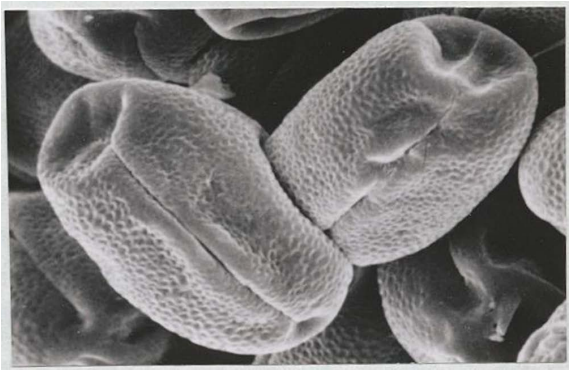


Fig. 6. Micrograph of pollen grains of Astragalus megacarpus X1630.

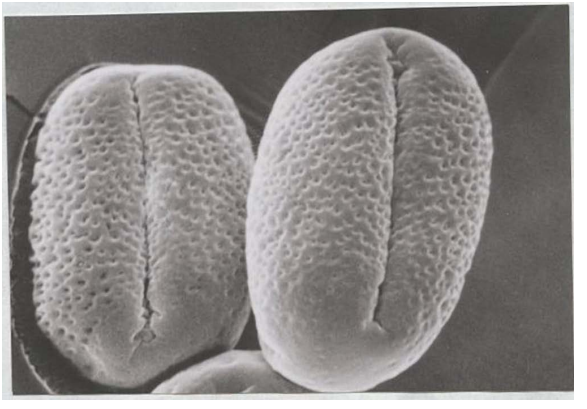


Fig. 7. Micrograph of pollen grains of Astragalus beckwithii var. beckwithii X1630.

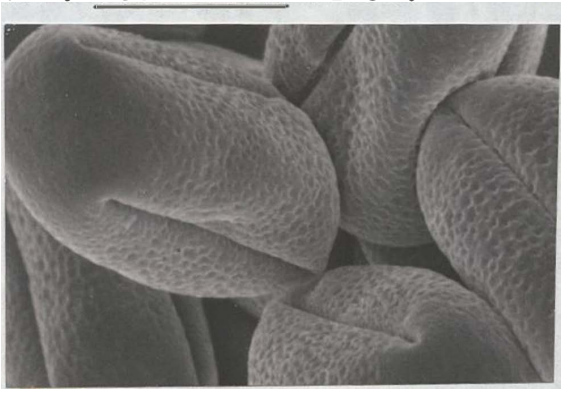


Fig. 8. Micrograph of pollen grains of Astragalus oophorus var. caulescens X1630.

in A. megacarpus; glucose, 60 mg% in A. oophorus,84 mg% in A. beckwithii, and 100 mg% in A. megacarpus.

Atomic Absorption for Selenium

The concentration of selenium was determined with an atomic absorption spectrophotometer. The results of the absorption readings were compared to a curve plotted from six standard solutions of H_2SeO_3. The readings for A. megacarpus, A. oophorus and A. beckwithii were not significantly different from one another. The species all appeared to contain less than 2 ppm selenium. In comparison, another species, A. sabulosus, contained 26.6 ppm selenium.

Rat Assay Series I

In the first assay, rats eating 1% Astragalus sabulosus at first appeared injured then later seemed to recover from the toxic effects. Upon harvesting them, the intestines were slightly bloated, the excretory organs were somewhat swollen, the hair was loose and appeared yellow; but the other viscera appeared to be normal.

Rats eating 10% A. sabulosus showed signs of sickness in that they curled their bodies into balls when

picked up. (Healthy rats squirm about and extend the extremities when picked up.) Those eating 10% A. sabulosus were smaller than those eating 1%. The excretory organs were swollen and the coats were more yellow. One rat of this group had a lower hematocrit and its liver had a "grainy" texture with darker spots. (Healthy livers have a smooth, glistening surface and a uniform color.)

The rats eating 50% A. sabulosus died before they could be effectively examined, but a precursory autopsy disclosed that the small intestine was thin and the lumen was smaller in diameter as compared to the control. The coats were yellowish and rough. Both the groups eating 10% and 50% A. sabulosus were observed to be bloody around the mouth, nose and pinnea of the ears. Occasionally there was a reddish spot on the back of the head. The daily weights of the above rats is summarized in Figure 9.

Rats eating 1% Astragalus beckwithii var. beckwithii in their feed were larger than the controls. The fur appeared normal in color and texture, but the intestines were somewhat bloated and the tails were dirty. The number of droppings increased proportionately as the percentage of A. beckwithii in the food increased, and the number of droppings was greater than the number under any

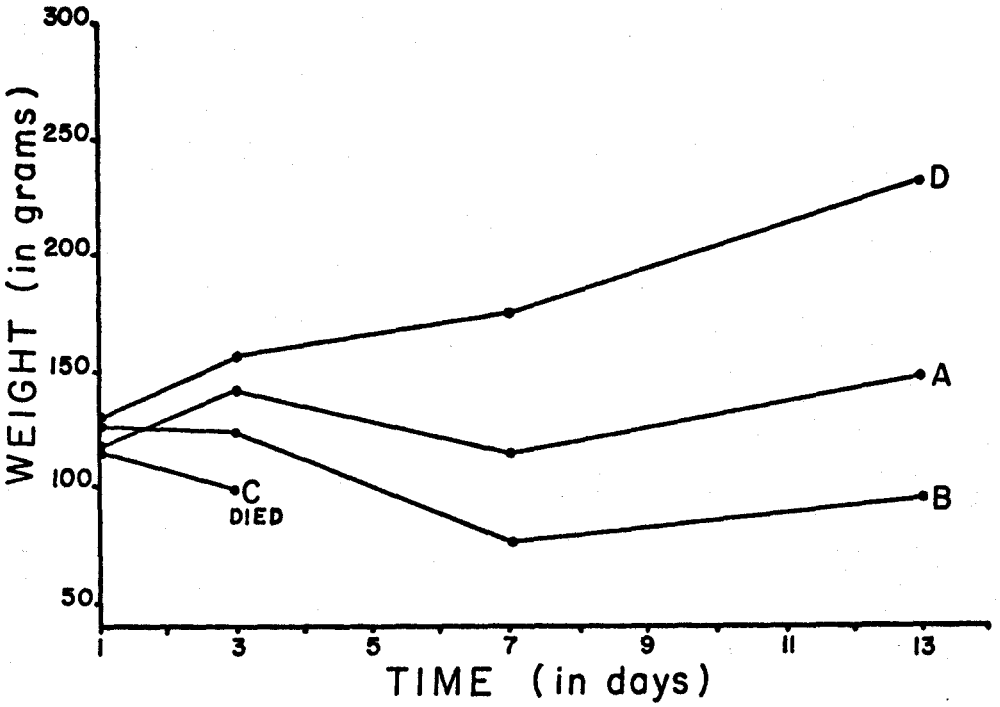


Figure 9. Daily weights of white rats fed 1% (line A), 10% (line B), and 50% (line C) of dry, ground Astragalus sabulosus mixed with a commercial rat chow preparation as compared to rats fed 100% rat chow (line D). Weight values are the average of 4-6 rats.

other treatments.

Those animals eating 10% A. beckwithii were almost as large as the ones eating 1%. The retraction of their eyelids was restricted, giving them the appearance of squinting. Their extremities were somewhat pallid, their tails were dirty and they were less active than the control rats.

Rats eating 50% A. beckwithii were smaller than others eating A. beckwithii, had more squinted eyes, more yellowish extremities, and yellowish hair. They were less active than the rats eating 10% A. beckwithii, but not lethargic. Their excretory organs were swollen, soiled and stained green as were their tails. The hematocrit for one rat was below normal and the clotting time was slow. The daily weights for the above group are shown in Figure 10.

Rats eating 1% Astragalus lentigenosus araneosus in their feed had hair texture that appeared more coarse and the rats were smaller than normal. Two rats died, and their cagemate was smaller than others. Their extremities were somewhat less pink than normal, but the rats were active and alert.

Increasing the amount of A. lentigenosus to 10%

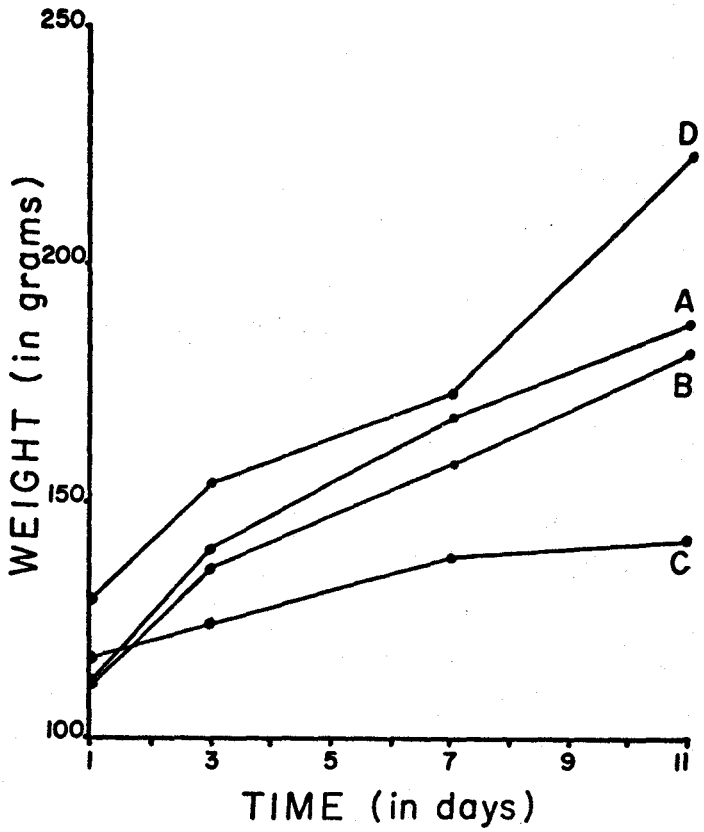


Figure 10. Daily weights of white rats fed 1% (line A), 10% (line B), and 50% (line C) of dry, ground Astragalus beckwithii mixed with a commercial rat chow preparation as compared to rats fed 100% rat chow (line D). Weight values are the average of 4-6 rats.

seemed to increase their irritability and the jaundiced appearance of the extremities. The yellow color of the fur and coarseness seemed to be increased also. They were smaller than controls and less active. The squinting seemed to be worse and the margin of the eyelid was enlarged and red with yellow crusts at the epecanthal fold. The "butting" motion reported by James et al. (1968) was observed in this group.

Rats eating 50% A. lentigenosus appeared near death; they were generally very weak. They were small, yellow, lethargic, irritable, and their eyes excreted tears. They tried to bite other rats and investigators when weighed. Their eyes were slits, the gait was wobbly. The extremities were quite yellow, the fur was rough and coarse. Their excretory organs were swollen and soiled. Their tails were soiled and stained. The daily weights are shown in Figure 11. One rat was observed as it died; there was a generalized convulsion with much head and neck activity. The rat appeared to have great difficulty in breathing; respiration was irregular, deep and noisy.

In the second assay, groups of rats were fed fresh A. beckwithii and A. megacarpus. The objective was to see if rats would eat fresh Astragalus. The rats ate

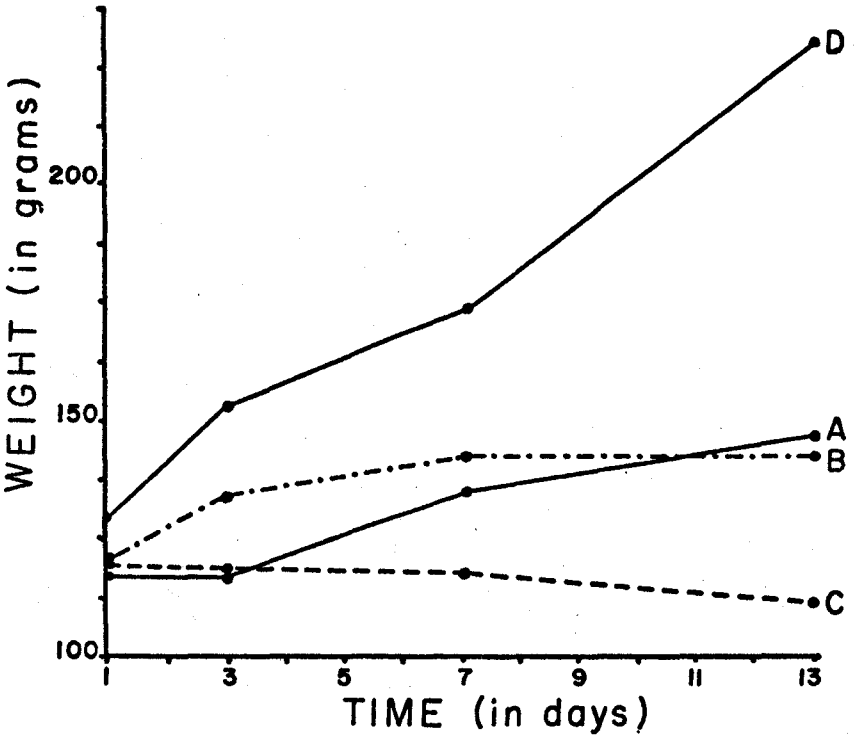


Figure 11. Daily weights of white rats fed 1% (line A), 10% (line B), and 50% (line C) of dry, ground Astragalus lentiginosus mixed with a commercial rat chow preparation as compared to rats fed 100% rat chow (line D). Weight values are the average of 4-6 rats.

fresh material readily, especially the A. beckwithii pods, leaving only the midvein and the seeds. After they had eaten the leaves and pods, and chewed the stems, they made nests out of the remaining material in the back of their cages, sleeping and spending much time on the pile.

The A. beckwithii appeared to still cause bloating, inflamation of the large intestines, and in general the same effect as the dried material. One of the controls was small and appeared sick. The results of the rat weights are shown in Figure 12.

In rat assay series I, experiment three, twenty rats were divided into four groups of five rats each. Group one was fed a dried mixture of benzene extract and rat chow; group two was fed a mixture of rat chow and dried benzene residue (3:1); group three was fed rat chow mixed with ground A. megacarpus foliage (3:1), and group four served as a control, eating only rat chow. The rats fed the benzene residue mixture lost weight most quickly. Their weight loss pattern most resembled the pattern of the rats eating fresh foliage and pods. Rats eating the foliage mixture lost 1.3 grams per gram eaten, while those eating the extract mixture gained slightly. Control rats gained 0.5 grams for each gram eaten. At the end of the

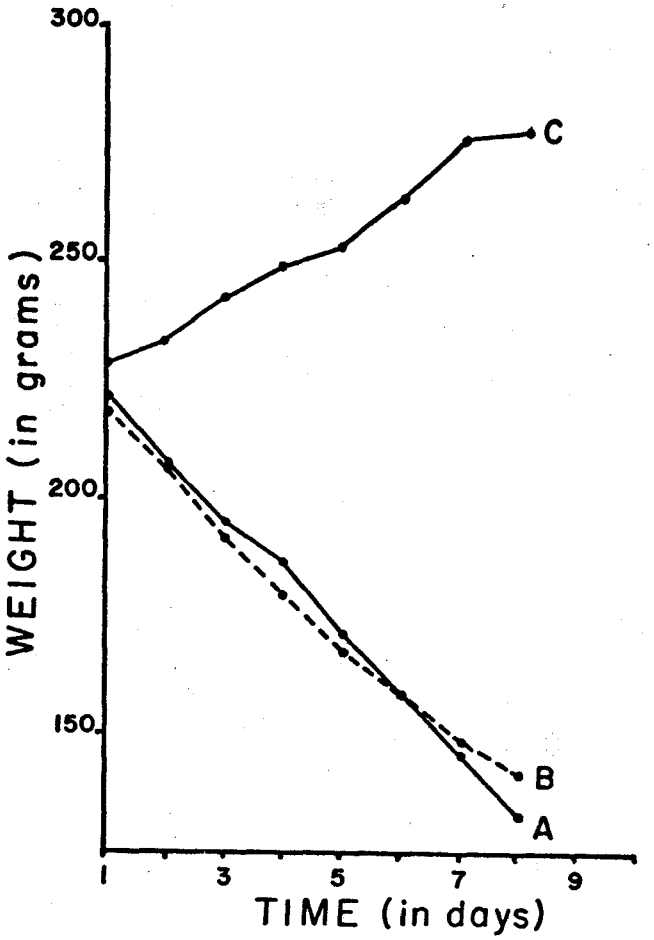


Figure 12. Daily weights of rats fed fresh Astragalus megacarpus (line A), Astragalus beckwithii var. beckwithii (line B) and a commercial rat chow preparation (line C). Weight values are the average of 5 rats.

assay one rat eating the foliage mixture was given a choice of a food bin of rat chow or foliage. For three days 10-17 grams per day of the rat chow was consumed by the rat but the foliage was not eaten by the rat. The daily weights of the above group are shown in Figure 13.

Rat assay series I, experiment 4, was similar to experiment 3 in organization except an ethanol extraction was taken from the benzene extracted residue and mixed with feed. The rats eating foliage were the most active of any group in this experiment. They apparently chewed the pinnae of each other's ears. They lost hair in small tufts or single hairs. They died more rapidly with a similar type of convulsion noted previously. The rats eating the ethanol extract mixture had the head lifting motion similar to those reported in sheep poisoning with Astragalus lentiginosus (James et al. 1967). The rats drank water more rapidly as compared to other rats, and were incessently active while they were observed. They were difficult to constrain during weighing. They bit researchers and each other. They lost hair in small tufts, in larger amounts than those eating the foliage mixture. Daily weights of the above group are summarized in Figure 14.

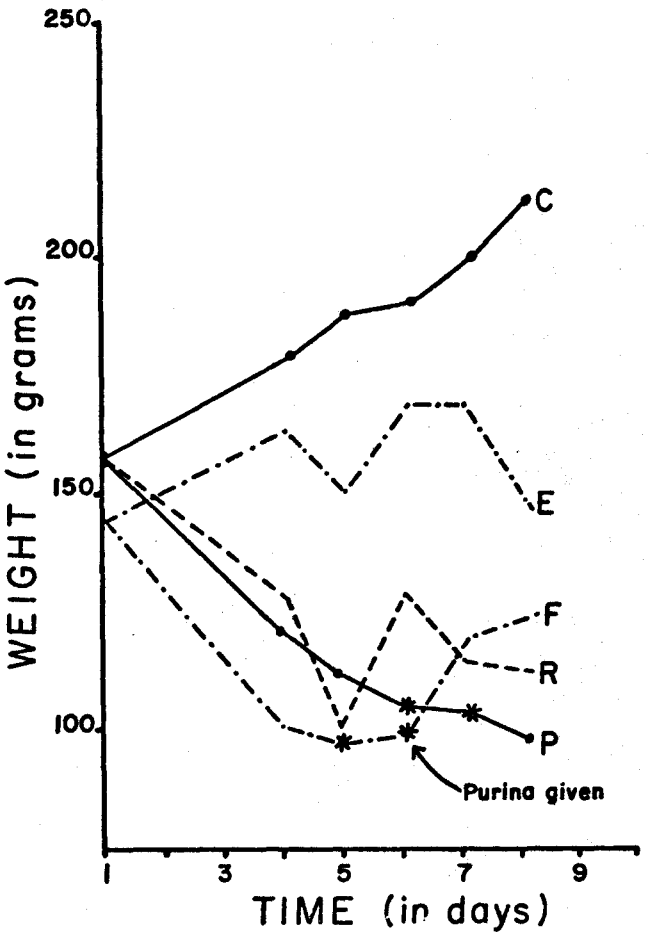


Figure 13. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with the benzene extract of Astragalus megacarpus (line E), dry foliage of A. megacarpus, rat chow mixed with benzene residue of extracted A. megacarpus (line R), and pods of A. megacarpus (line P). Weights are the average of 5 rats.

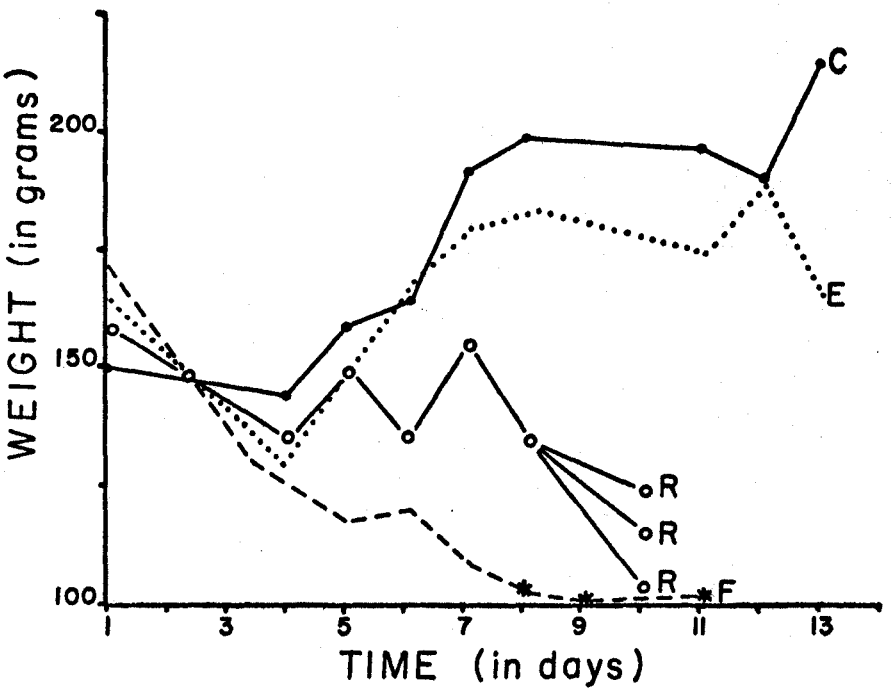


Figure 14. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with ethanol extract of Astragalus megacarpus (line E), dry foliage of A. megacarpus (line F), rat chow mixed with ethanol residue of A. megacarpus (line R). Weight values are the average of 5 rats.

Rat assay series I, experiment 5, continued the extraction process; a water extract was made of the previous ethanol residue. The rats eating the water extract residue lost weight faster than those eating the foliage mixture. All groups but the controls had a net weight loss.

The rats eating the fermented water extract mixture displayed a curious pattern of daily weights: they lost weight rapidly for five days then gained rapidly, regaining their original weight. They displayed no unusual behavior nor showed any pathological physical changes. Their daily weights are shown in Figure 15.

Rat Assay Series II

The water extract mixtures were eaten readily. The mixture made from A. beckwithii and A. megacarpus extract appeared to be the most toxic; they produced the greatest weight loss and had the least efficient food utilization. They also were lethargic, had diarrhea and 3 died. The water extracts in general seemed to produce physical symptoms most like those in rat assay series I.

The rats fed benzene extract mixture seemed to have less appetite for the food they did eat. A. beckwithii and A. oophorus benzene mixtures produced the greatest

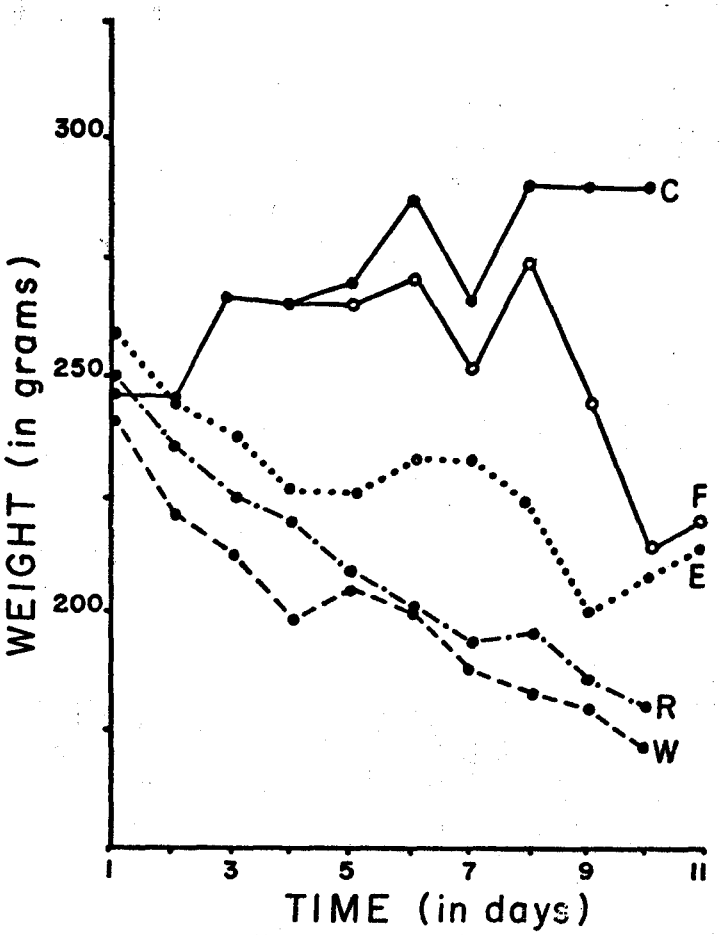


Figure 15. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with water extract of Astragalus megacarpus (line W), dry foliage of A. megacarpus (line F), rat chow mixed with water residue of A. megacarpus (line R), pods of A. megacarpus (line P) and the fermented water extract from A. megacarpus (line FE). Weight values are the average of 5 rats.

weight loss of all the groups but had the increased droppings noted for A. beckwithii previously. While these extracts seemed somewhat toxic, the rats eating A. megacarpus extract gained weight, looked healthy and weighed more than either of the control groups or the rats taken off the benzene extract at day 8 and fed only rat chow for 6 days. The rats eating A. megacarpus extract also gained more weight per gram of food eaten than any other except the group that ate A. beckwithii benzene extract followed by rat chow.

The benzene control group, while not weighing as much as the rat chow control group, did utilize their food more efficiently. The benzene appears to either remove some volatile nutrient(s) or make the food less palatible.

The rats which were fed only rat chow after day 8 all gained weight; those which had been fed A. beckwithii or A. megacarpus benzene extract gained weight and increased in food utilization efficiency dramatically. See Figures 16-21.

Lipid Analysis

Of the compounds present in the extractable lipids separated by layer chromatography, 29 to 34 compounds

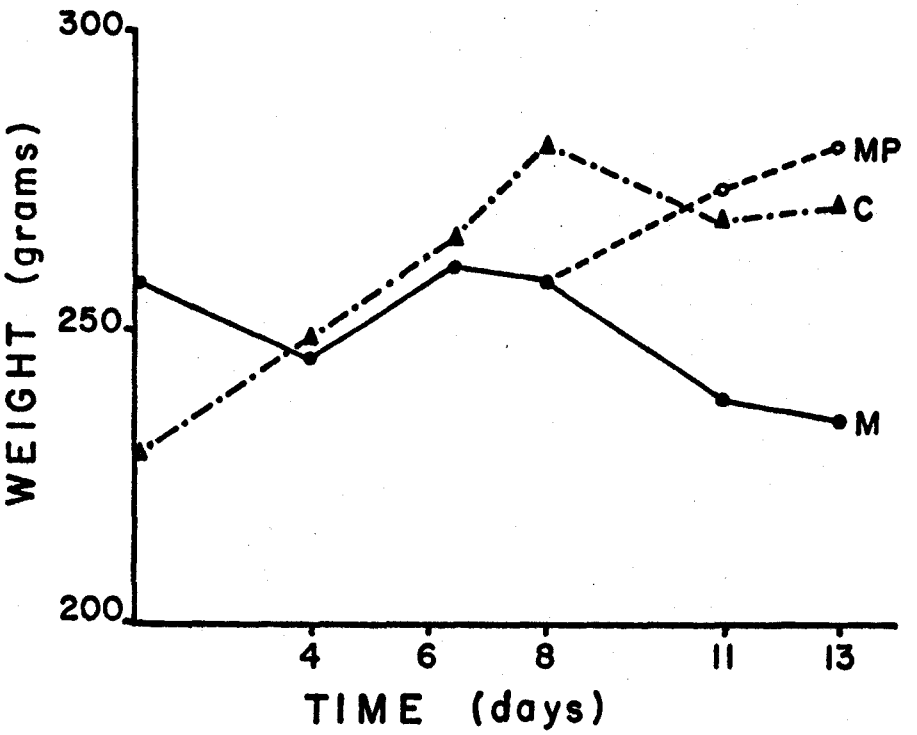


Figure 16. Daily weights of rats fed a commercial rat chow preparation (line C) and rat chow mixed with water extract of Astragalus megacarpus (line M). Four rats were returned from water extract and rat chow after 7 days to just rat chow (line MP). Weight values are an average of 7 rats.

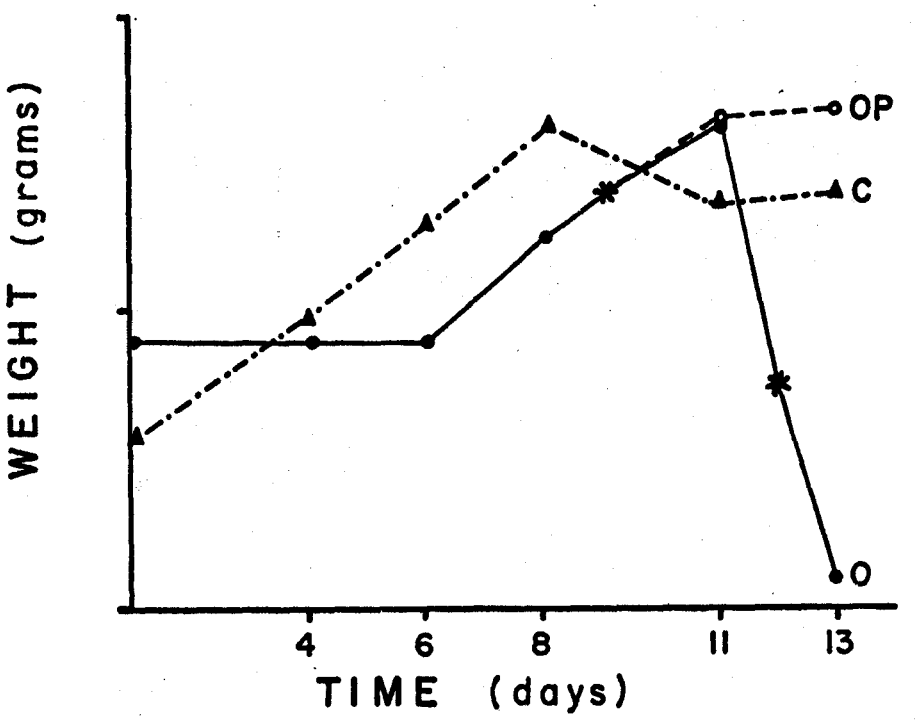


Figure 17. Daily weights of rats fed a commercial rat chow preparation (line C) and rat chow mixed with water extract of Astragalus oophorus (line O). Four rats were returned from water extract and rat chow after 7 days to just rat chow (line OP). Weight values are an average of 7 rats.

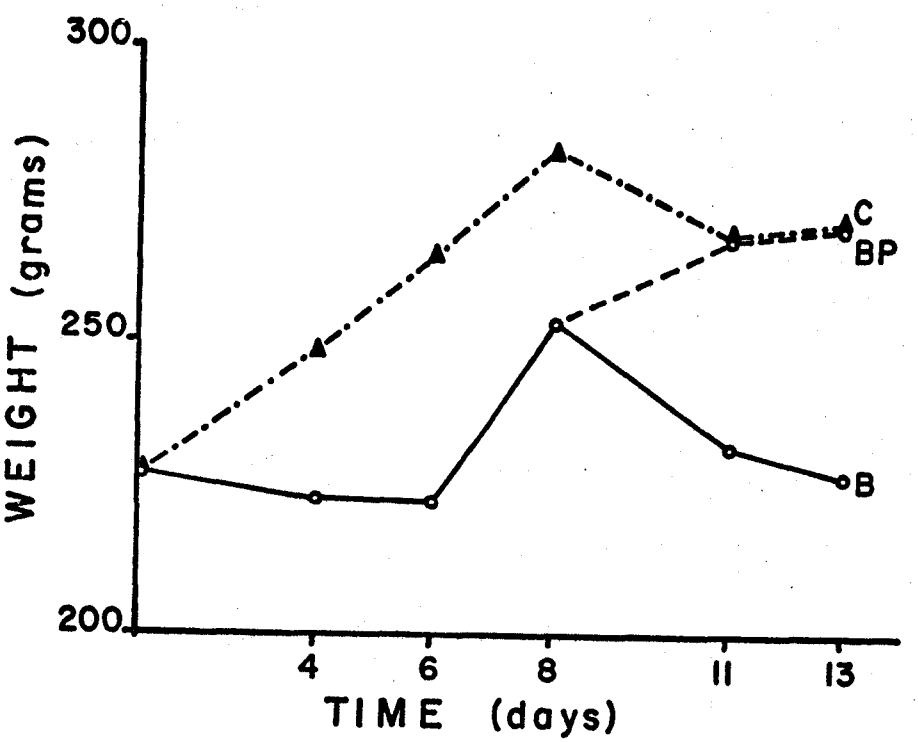


Figure 18. Daily weights of rats fed a commercial rat chow preparation (line C) and rat chow mixed with water extract of Astragalus beckwithii (line B). Four rats were returned from water extract and rat chow after 7 days to just rat chow (line BP). Weight values are an average of 7 rats.

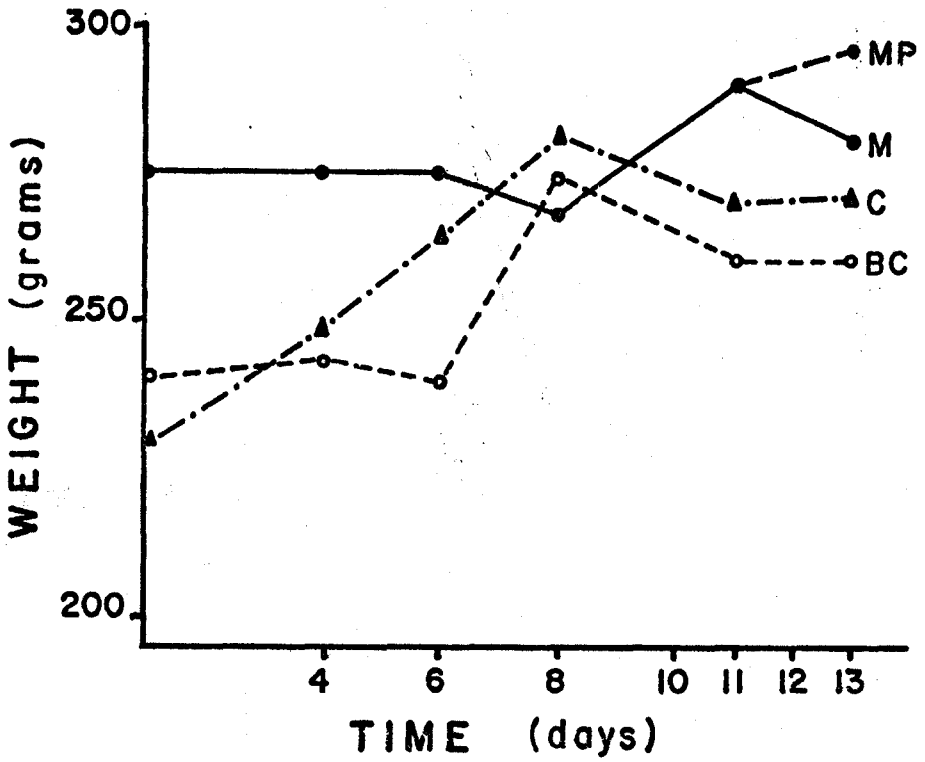


Figure 19. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with benzene extract of Astragalus megacarpus (line M) and rat chow soaked in benzene (line BC). Four rats were returned from benzene extract and rat chow after 7 days to just rat chow (line MP). Weight values are the average of 7 to 8 rats.

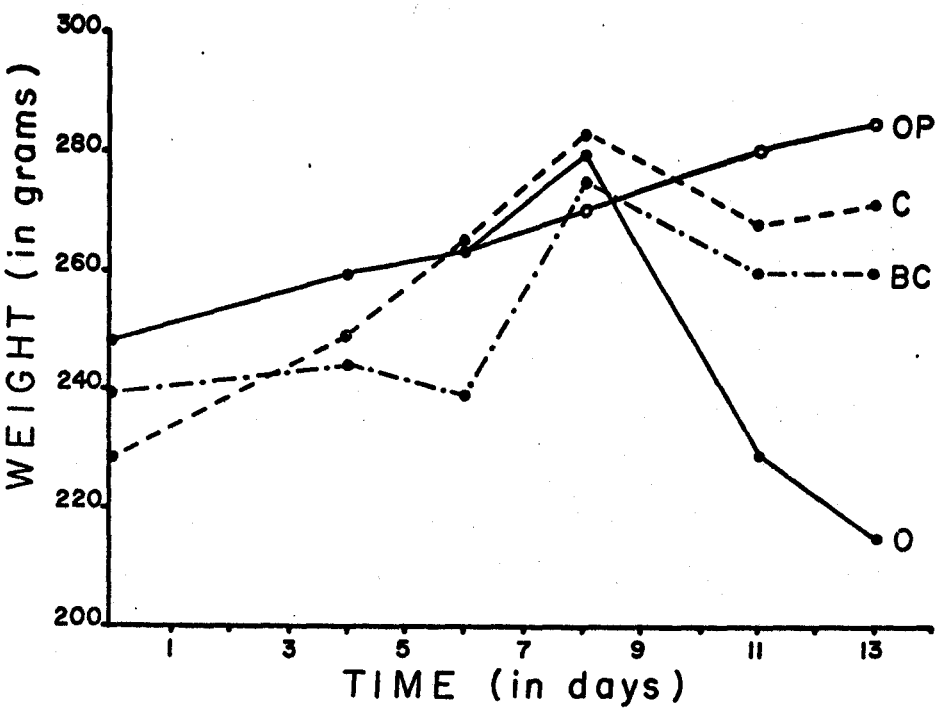


Figure 20. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with benzene extract of Astragalus oophorus (line O) and rat chow soaked in benzene (line BC). Four rats were returned from benzene extract and rat chow after 7 days to just rat chow (line OP). Weight values are the average of 7 to 8 rats.

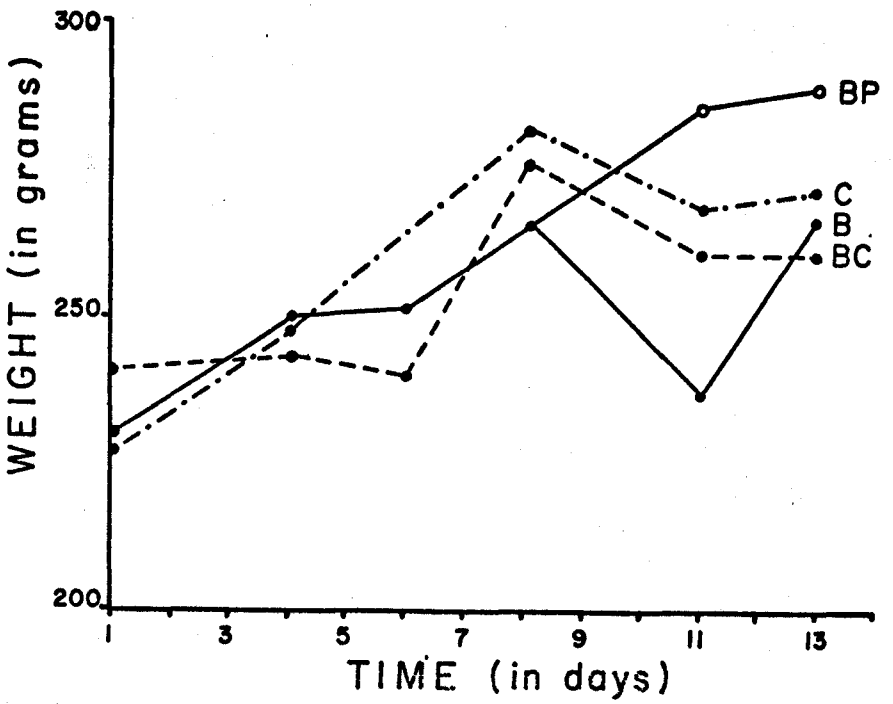
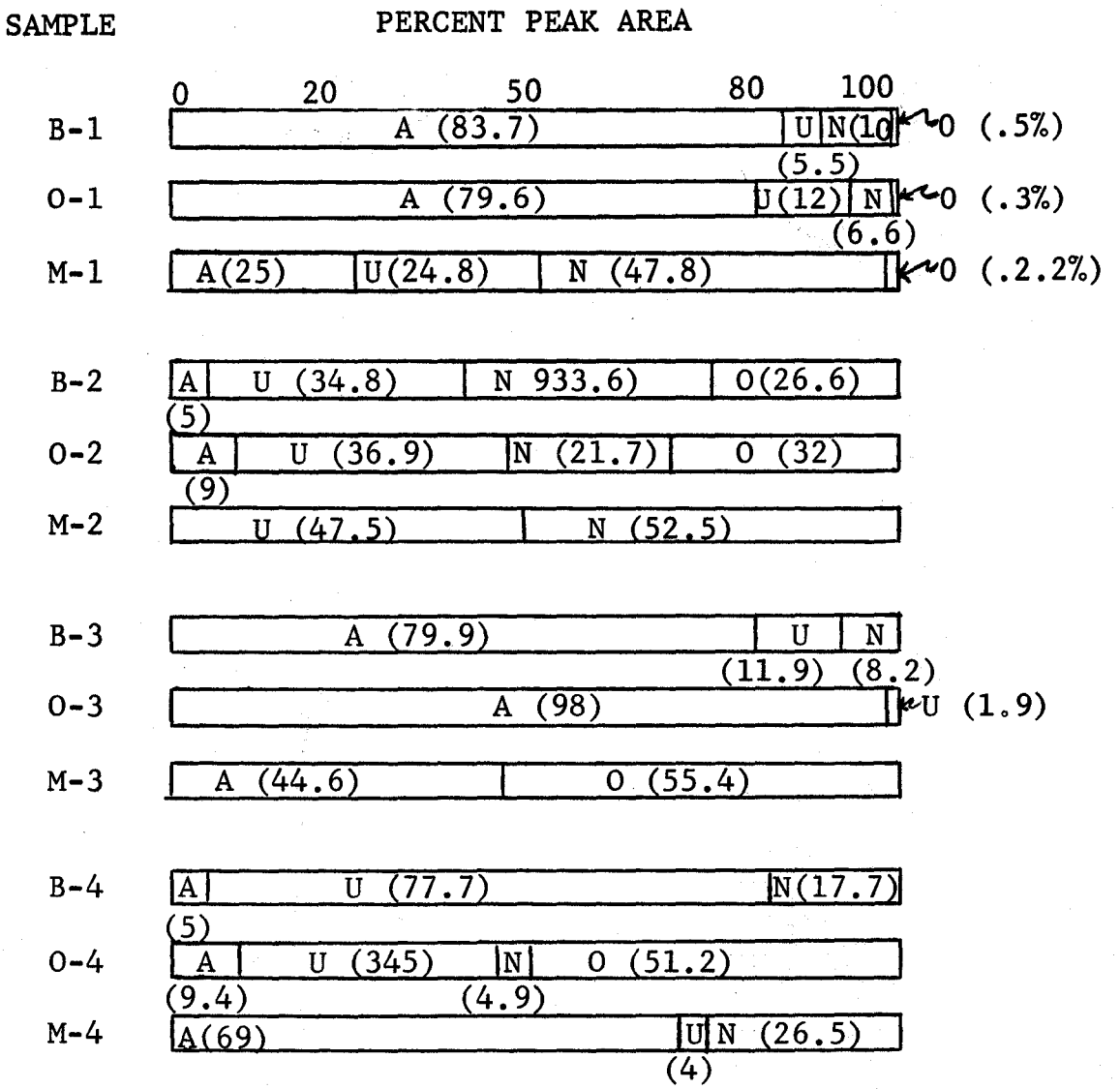


Figure 21. Daily weights of rats fed a commercial rat chow preparation (line C), rat chow mixed with benzene extract of Astragalus beckwithii (line B) and rat chow soaked in benzene (line BC). Four rats were returned from benzene extract and rat chow after 7 days to just rat chow (line BP). Weight values are the average of 7 to 8 rats.

occur in the top-most band (band one on the silica-gel plate). From the data obtained from thin layer chromatography, gas chromatography and GC-mass spectrometry it is not always possible to determine the exact elemental composition or molecular formula, but some statements can be made about the nature and type of compound present. Almost half of the compounds detected were identified. The results of the analysis are summarized in Appendix B and Figure 22.

Band one from Astragalus megacarpus contained 29 compounds, 25% of which (based on peak areas) were alkanes; 25% were unsaturated hydrocarbons, including an alkene, a heterocyclic compound, a cyclic alcohol and several long chain alkenes or dienes. The largest percentage of the peak area, 48%, were nitriles. Two percent of the peak area were phthalates which could be contaminants but may have been part of the plant lipids.

Band one from Astragalus oophorus contained 34 compounds, eight percent of which (peak areas) were alkanes, both normal and branched; most of these were long chain normal alkanes. Nitriles made up 7% of the peak area and phthalates were 3%. The unsaturated hydrocarbons were an interesting mixture of phytanes, terpene-like compounds, and alkanes and possible amides. The top band of A.



A - Alkanes
U - Unsaturated hydrocarbons
N - Nitriles, nitrogen containing compounds
O - Others; contaminants, phthalates, ethers etc.

Figure 22. Histograms of Lipid Classes in Astragalus beckwithii var. beckwithii (B-1, B-2, B-3, B-4), A. oophorus (O-1, O-2, O-3, O-4), and A. megacarpus (M-1, M-2, M-3, M-4). The percent of the individual lipid classes are based on peak area obtained by the gas chromatograph. Lipid classes were characterized by a GC-mass spectrometer.

beckwithii contained 31 compounds, 84% of which (peak areas) were alkanes, of which 80% were long chain normal alkanes. Ten percent were nitriles; most of which were aliphatic nitriles but half the volume of nitriles were two long chain alcoholic nitriles. The 6% unsaturated hydrocarbons seemed to be mostly alkanes. A phthalate represented 1% of the peak area.

Band two of A. beckwithii contained 10 peaks. The greatest peak area (35%) was taken up by unsaturated hydrocarbons, alkenes and alkyldienes. Nitriles made up 34% of the peak area, most appeared to be aliphatic but one large peak appeared to be a cyclic nitrile.

Band two of A. oophorus contained nine peaks. Of these 37% of the peak areas were unsaturated hydrocarbons; one compound was an alkane, two were alkyldienes. The remaining peak area was a possible nitrile (22%), phthalates attached to or at least associated with a C_{19} alkane (32%) and a C_{36} alkane (9%).

Band two of A. megacarpus contained only two peaks but two spectra were obtained from the last peak. The first peak is a possible diene and the last two are nitriles. The percentage of peak area is about equal in the two peaks.

Third band contained 19 compounds total from all three species. Some of these compounds were present only in very small quantities, so that it was difficult to get good spectra in order to identify them. Astragalus beckwithii had one nitrile and three alkanes. There was a small amount of a possible fatty acid. Band four of A. oophorus was 98% alkanes but there was more contamination in that band than any other. Band three of A. megacarpus contained only a C_{13} alkane and silicon contaminants.

The fourth band of each species made up nearly half of the sample volume. Astragalus beckwithii contained 78% unsaturated hydrocarbons of which alkenes and a possible ether were large contributors. Nitrogenous compounds made up 18% of the peak area with nitriles being the largest portion. Astragalus oophorus contained one alkane, a diene, and 51% were other compounds; mainly silicon compounds and phthalates. The phthalates were separated into five peaks but four had possibly the same molecular weight and very similar spectra. Band four from A. megacarpus contained normal and branched alkanes and four close but separate peaks. The four appear to be nitriles with very similar spectra.

The remaining bands' spectra became more

complicated than the knowledge and skill of the author. She does not feel competent to discuss them.

The above data is summarized in Figure 22.

DISCUSSION

The purpose of this investigation was to consider the possible taxonomic relationships in this group. The fundamental question that needs to be answered is whether Astragalus beckwithii var. beckwithii is one type of hybrid or intergressory product from Astragalus oophorus var. caulescens and Astragalus megacarpus. Since these species are not treated in the literature in any chemical investigation, any information about their chemical constituents or toxicity should contribute to the solution of this question.

The taxonomic relationship question arises immediately when A. beckwithii and A. oophorus are viewed in anthesis: they are remarkably similar in habit and every aspect of their flowers. Yet when the pods appear, A. oophorus is sometimes difficult to distinguish from A. megacarpus, while A. beckwithii is not like either of them; but resembles some A. lentiginosus species.

The following data tend to support the theory that A. beckwithii is a type of intermediate; the nectary is inconspicuous and the flower size and dimensions resemble

A. megacarpus nectaries and flower parts. The gynophore is similar to A. oophorus but shorter truly intermediate between the latter and A. megacarpus. The hairy covering is most like A. megacarpus but the stems and leaves are in some cases indistinguishable from A. oophorus. When the glucose content is compared between A. beckwithii and A. oophorus the glucose values are very nearly the same, differing by only 15 percent. The selenium content data places them all in the non-indicator or non-accumulator group. When the pollen grains are compared, A. beckwithii falls into the intermediate portion in every category measured; i.e. length, length-width ratio, reticulations and thickenings of the extine and pore size and position. The lipid assay reveals 12 unique compounds found in A. megacarpus, 23 unique compounds found in A. oophorus, but only 2 unique compounds in A. beckwithii. Compounds occurring in all three species numbered only 3. These calculations were taken from 69 compounds excluding solvents which occurred 178 times in 12 extractions. In Figure 22 and Table 4 comparison can be made of the percentage of peak area occupied by various classes of compounds. Table 4 is a summary of differences of these percentages. The smaller the numbers, the more similar the percentage of

TABLE 4

COMPARISON OF DIFFERENCES IN LIPID CLASSES OF A. BECKWITHII, A. OOPHORUS, A. MEGACARPUS BASED ON PERCENT PEAK AREAS AS DETERMINED BY GAS CHROMATOGRAPHY. LIPID CLASSES CHARACTERIZED BY GC-MASS SPECTROMETRY

	TLC Band 1	TLC Band 2	TLC Band 3	TLC Band 4
Alkanes				
B-O	(1) *4.11	(1) *3.80	(1) *18.19	(1) *4.20
O-M	(2) 58.61	(2) 5.20	(2) 35.30	(2) 64.10
B-M	(3) 54.50	(3) 9.00	(3) 53.49	(3) 59.90
Unsat. Hydrocarbons	(B1)	(B2)	(B3)	(B4)
B-O	(1) *8.06	(1) *2.00	(1) 17.99	(1) 43.20
O-M	(2) 19.32	(2) 12.72	(2) 19.9	(2) 73.60
B-M	(3) 11.26	(3) 10.62	(3) 1.9*	(3) 30.40*
Nitriles	(B1)	(B2)	(B3)	(B4)
B-O	(1) *3.47	(1) *11.9	(1) 17.2	(1) 17.76
O-M	(2) 37.70	(2) 18.89	(2) 17.2	(2) 8.74*
B-M	(3) 41.17	(3) 30.79	(3) 0.0*	(3) 26.5
Other	(B1)	(B2)	(B3)	(B4)
B-O	(1) .29*	(1) 5.80*	(1) 0.0*	(1) 51.2
O-M	(2) 1.716	(2) 26.6	(2) 55.4	(2) 0.0*
B-M	(3) 2.006	(3) 32.4	(3) 55.4	(3) 51.2

B-O = % Lipid class of A. beckwithii substracted from % lipid class of A. oophorus.

O-M = A. oophorus - A. megacarpus.

B-M = A. beckwithii - A. megacarpus.

* Represents group with smallest difference in lipid peak area.

that lipid class; this similarity is noted with an asterick (*). It can be seen that in 16 comparisons A. beckwithii is most similar to A. oophorus in 11 cases, and is most similar to A. megacarpus in 3 cases and A. oophorus and A. megacarpus are most similar in 2 comparisons. The second rat assay graphs of weight and food efficiency show A. beckwithii closer to A. oophorus in water and benzene weights and benzene extract efficiency. Plants grown in the greenhouse show similarities in growth habit; A. beckwithii and A. megacarpus are indistinguishable.

The evidence against A. beckwithii being a hybrid product between A. oophorus and A. megacarpus is contained in the following data: In nature A. beckwithii has much longer stems than the other two species and the pod characteristics are decidedly different. The pods of A. beckwithii resemble those of A. lentiginosus. Plants grown in the greenhouse show similarities in growth habit; A. beckwithii and A. megacarpus are indistinguishable.

While these species of Astragalus did produce a "locoism" in rats, the severity varied and the physical damage was unpredictable. The second rat assay graphs place A. beckwithii near A. megacarpus in the efficiency of the rats eating the water extracts and the ones eating

the water extracts and the ones eating water extracts and later returned to rat chow.

The Astragalus species in this study did produce a "locoism" in rats used as a biological assay. It appeared that they were affected in proportion to the percentage of Astragalus in their food. We obtained many of the same results as other workers using other Astragalus species such as wobbly gait, weakness, coat luster and texture change, intestinal damage, behavioral changes, and weight gain interference. Some had the same sort of liver damage done by other species. The mass spectral data indicates that as much as 30% of the volume of observed lipid compounds contained nitrogen in a possible nitrile combination. This may account for at least some toxicity and aberrant behavior since aliphatic nitriles are quite toxic to digestive tracts and sub-lethal doses of cyanide have produced local brain lesions in experimental animals. Perhaps this may account for the intestinal damage we noted and the change in behavior. The solubility of these nitrogenous compounds is interesting. Most nitriles are connected to sugar molecules in plants and thus are water soluble. This may account for the toxicity evidenced in A. megacarpus water extracts. This species has the most

nitriles. But aliphatic nitriles are ether soluble with other lipids. Also, because no alkaloids were made evident by the extractions, is no reason to conclude that none are present. One researcher in Albuquerque, New Mexico indicated that it was necessary to concentrate extract from about 300 pounds of dry material to obtain 1 mg of alkaloid. Our plant samples were quite a bit more diluted.

CONCLUSION

While the weight of the data obtained seems to indicate that Astragalus beckwithii var. beckwithii is some hybrid or intergressory product of Astragalus oophorus var. caulescens and Astragalus megacarpus, there is some data to the contrary. It would be premature to suggest combining A. beckwithii and A. oophorus into A. beckwithii because only those varieties in the state have been studied, and data on the remaining three varieties may lead to other conclusions.

These Astragalus species appear to produce "locoism" in rats similar to that produced in horses, cattle and sheep by other Astragalus species. The toxin may prove to be another nitrogenous compound like the nitrogenous compounds of miserotoxin or cyanogenic ones. Three conditions account for this conclusion: (1) They are easily ether soluble but not as soluble in benzene or alcohol so the compounds may have remained in the residue; (2) There is a possible metabolic pathway for creating cyanide from aliphatic nitriles and alcoholic nitriles, in vivo; (3) The effects of sub-lethal doses of cyanide are not well-

documented, but one textbook mentions minute brain lesions and intestinal irritation and thinning, in addition to other organ damage. The brain lesions are of particular interest in that they may account for the perception and motor changes and the irritability shown by various animals tested. Perhaps nitrile poisoning is what is being observed and reported with Astragalus.

APPENDIX A

Specimens Examined

BRY - Utah County	S. L. Welsh & Glen Moore	1933	
	C. S. Schoener		
	S. L. Welsh	1229	
	S. L. Welsh	1730	
	B. F. Harrison	9073	
Juab	M. Wright	71	
	C. S. Schoener		
	S. L. Welsh	1730	
	M. Wright	327	
	Arnold Standing	126	
Sanpete	Brent Christensen	(No)	
	S. L. Welsh	1717	
Salt Lake	Mary Carlquist	64/13-20	
Washington	Larry C. Higgins	G55	
Millard	R. H. Foster	409	
(No County)	K. E. Weight	13-31/20	
	E. P. Sheldon	1880-Frisco	
	S. Flowers	21	
	Rose E. Cottom	(No)	
UT - Utah County	R. E. Coombs	151	
	S. L. Welsh & Glen Moore	1933	
	Larry Eyre	34	
	S. Flowers	(No)	
	A. O. Garrett	3862	
	S. Flowers	May 20, 1880	
	S. Walsh	4-26-31	
Salt Lake	M. E. Jones	1892	
	A. O. Garrett	1736	1906
	O. Howard	1885	
	O. Howard	1880	
	Jerry B. Anderson	211	
	K. Brizzee	7772	
Kane	O. S. Walsh	103	
	Hayle Buchanan	214	
	K. E. Weight	13-31/12	
	Hayle Buchanan & W. P. Cottom	134	
	Hayle Buchanan	21	
Juab	M. Wright & Schoener	71	
	Marzilla Wright	394	

	Box Elder	S. J. Pruce, Jr.	883
		Jack H. Berryman	14
		Jack H. Berryman	43
	Weber	S. Flowers	445 (1925)
	Iron	W. P. Cottom	6718
	Millard	A. Perry Plummer	176
	Tooele	S. Flowers	21
UTC - Utah County		Duane Isley	8668
		T. H. Vansell	May 1940
		Marcus & Jones	6 20
		Thomas Jensen	489
		T. H. Vansell	May 25, 1940
		L. A. Stoddart	June 8, 1940
		L. G. Andrews	52
		S. L. Welsh	
		Noel Holmgren	
		Dwight Isley	
	Juab	Dan Burton	3396
		S. L. Welsh	
		Dwight Isley	
	Millard	Noel Holmgren	
		Dan Burton	3396
		Alma Esplin	Spring 1935
		G. D. Pickford	67782
		Bassett Mcguire	25092
		Arthur H. Holmgren	
		A. D. Ripley &	
		R. C. Barneby	9245
		Bassett Mcguire	June 1941
		Duane Isley	
		S. L. Welsh	8736
		Dwight Isley	1880
	Sanpete	Dwight Isley	
		S. L. Welsh	
		Duane Isley	8672
		Thomas Jensen	532
		Thomas Jensen	562
		B. F. Harrison	11851
		T. H. Vansell	May 25, 1940
		Reed C. Collins	2800
	Salt Lake	Jerry B. Anderson	May 19, 1959
	Tooele	G. H. Vansell	Summer 1940
		G. H. Vansell	May 21, 1940
		Bassett Mcguire	20726
		Bassett Mcguire	June 15, 1941

	Kane	Arthur Cronquist	10127
		Duane Isley	
		S. L. Welsh	
		Noel Holmgren	
	Sevier	Loren C. Anderson	683
	Washington	N. H. Holmgren	3431
		Dan Burton	
	Iron	Dwight Isley	8689
		Duane Isley	3689
		S. L. Welsh	
		Dwight Isley	
	Piute	Dwight Isley	8681
		S. L. Welsh	
		Dwight Isley	
	Carbon	Noel H. Holmgren	1957
		James L. Reveal	
UTC -	Carbon	Charles La France	1957
		H. D. Ripley &	
		R. C. Barneby	
		C. L. Porter	4509
	(No County)	Duane Isley	8736

APPENDIX B

MASS-SPECTRAL DATA USED FOR LIPID ANALYSIS

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
0-1 Peak Numbers						
1	112	.6	21.6	184	$C_{13}H_{28}$	Tridecane
2	120	1.7	24.8		$C_{11}H?N$	Nitrile
3	121	.93	25.6	142	$C_{10}H_{22}$	Decane
4	125	3.2	28.5		$C_{14}H_{30}$	6-methyl Tetradecane Alkane
5	127	2.6	29.2	194	$C_{14}H_{26}$	Phytane Fragment?
6	127	3.1	30.5	194	$C_{14}H_{26}$	Tetradecane
7		1.2	31.6	184	$C_{13}H_{28}$	Tridecane
8		.92	33.6	198	$C_{14}H_{30}$	Tetradecane
9		.43	38	212	$C_{15}H_{32}$	Pentadecane
10		.52	41.5	212	$C_{15}H_{32}$	Pentadecane
11	152	.6	48.5	170	$C_{12}H_{26}N?$	Nitrile
12	157	.32	52.5	182	$C_{13:1}H_{26}N?$	Nitrile
13	165	.87	56.7	182	$C_{13:1}H_{26}$	Tridecene
14	168	.22	60	212	$C_{15}H_{32}N$	Pentadecane & Amide Bran Ch
15		.67	63.2	210	$C_{15:1}H_{30}$	Unsat - 12, Pentadecene
16		.78	64.0	213	$C_{15}H_{32}$	Pentadecane
17		.27	67.0	235	C_{18} or C_{19}	Terpene
18	183	.7	71	243	$C_{17}H$ N	Nitrile
19	188	.36	74.7	243		Unsat - contaminated Nitrile

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
20	193	1.86	78.7	242		Nitrile - contaminated
21		.27	80.5		C_{19}	Hydrocarbon
22		.52	86.3		C_{19}	Hydrocarbon
23		1.32	92.0	276	$C_{21}H_{44}$	Heneicosane
24	216	.52	96.3	296	$C_{19}H_{37}CN$	Nitrile
25	220	5.5	98.7	310	$C_{22}H_{46}$	Docosane
26	228	2.8	106	327	$C_{23}H_{48}$	Tricosane
27	235	12.2	112	352	$C_{25}H_{52}$	Pentacosane
28	249	4.6	117.5	364	$C_{26:1}H_{52}$ at C_{21}	4, Hexacosene
29		9.7	123	380	$C_{27}H_{56}$	Heptacosane
30	258	2.8	127	378	$C_{27:1}H_{54}$ at C_{21}	5, Heptacosene Hydrocarbon
31	265	16.3	135	408	$C_{29}H_{60}$	Nonacosane
32	272	4.7	138	406	$C_{29:1}H_{58}$	20, Nonacosene
33	275	16.3	144	436	$C_{31}H_{64}$	Hentriacontane
34	275	.75	154	450	$C_{32}H_{66}$ at C_{20}	Dotriacontane
						11, 12-Dimethyl triacontane
0-2 Peak Number						
1		14.9		102	C_7H_{18}	Solvent Cont.
2				102	C_7H_{18}	Solvent
3		14.9		268	$C_{19}H_{40}$	Nonadecane
4		8.4		268	Contains $NC_{19}H_{40}$	Nitrile ?
5		23.0		296	$C_{21}H_{44}N$	Possible Nitrile
6		21.7		263	$C_{19:2}H_{35}$	Diene (Nonadediene ?)
7		3.2			$C_{18:1}H?N$	Nitrile
8		18.8		310	$C_{22}H_{46}$	Docosane
9		9.0		341	$C_{23:3}H_{40}C{=}N$	Nitrile
3B		14.9		194	$C_{14:2}H_{26}$	Tetradediene

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
4B		9.4		268	$C_{19}H_{40}$	Nonadecane
5B		23.0		296	$C_{19}H_{40}$	Nonadecane
6B		21.7		263	$C_{18}H_{37}N$	Nitrile
8B		18.8		432	$C_{31:2}H_{60}$	Hentriacontdiene
0-3 Peak Number						
1	77		9		C_7H_{18}	Solvent Heptane
2	158		79.2		C_7H_{18}	Solvent Heptane
3	202	8.13	101		C_8H_{18} or $C_{10}H_{22}$	Octane or Decane
4	209	1.36	104			Unsat Hydrocarbon
5	214	.38	107	184	$C_{13}H_{28}$	Tridecane
6	218	.9	109		$C_{13}H_{28}$ or $C_{15}H_{32}$	Tridecane or Pentadecane
7		27.1				Background
8	224	27.1	112	188	$C_{15}H_{32}$	Pentadecane
9	230	1.15	115			Contaminate
10	240	60.43	120	240	$C_{17}H_{36}$	Heptadecane
11	275 hold		158	282	$C_{20}H_{42}$	Eicosane
0-4 Peak Number						
1	48	12.4				Solvent
2	40	7.4	28.4		C_6H_6 or $C_{10}H_{22}$	Benzene or Decane

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
3	135	.22	68.5	?		
4	151	6.0	115	?		
5	169		127.8	?		
6	181	7.8	141	250	$C_{18:2}$ H_{34}	Octadecdiene
7	191	29.2	144.5			Silicon Lubricant
8	209	21.2	156			Phthlate
9	213	3.9	165.5			Phthlate
B-1 Peak Number						
1	55		3.6	84	C_6H_6	Benzene
2	56		4.8	84	C_6H_6	Benzene
3	57.5		5.4			Blank
4	59		7.8			Blank
5	100	.79	27	142	$C_{10}H_{22}$	Decane
6	117		31.2		C_{13}	Phthlate
7	120	.47	36.2	183	$C_{12}H_{26}$	Dodecane
8	128	.16	36		?	
9	144	.42	39.6			Alkane
10	146	.42	40.3	184	$C_{13}H_{28}$	Tridecane
11	154	.47	44.4	184	$C_{13}H_{28}$	Tridecane
11b	156	2.75	45		C_{12} or C_{14}	Nitrate or Alkane
12	164	1.3	49.2	197	$C_{14}H_{30}$	Tetradecane
13	170	.88	51.6	197	$C_{14}H_{30}$	Tetradecane
14	172	2.6	54.0	204	$C_{12}H_{17}OHCN$?	Alcoholic Nitrile
15	175	.7	57.0	204	$C_{12}H_{17}OHCN$	Alcoholic Nitrile
16	180	2.2	58.8	223	$C_{14}H_{29}CN$	Nitrile
17	184	1.1	61.1	242-243		Nitrile

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
18	188	.4	65.5	242-3		
19	194	2.8	67.8		C_{19} or C_{15}	Unsat Hydrocarbon
20	196	2.9	70.3			Alkane
21	203	18.9	75.5			Alkane
22	210	3.36	81.0	296	$C_{12}H_{26}$	Branched Dodecane
23	217	17.3	87	296	$C_{19}H_{37}CN$	Nitrile
24	224	1.8	91.7		$C_{29}H_{52}$	Pentacosane
24b	224		94.5		$C_{27}H_{56}$	Heptacosane
25	232	20.2	96	352	$C_{25}H_{52}$	Pentacosane
26	238	12.5	102	436	$C_{27}H_{56}$	Heptacosane
27	250	2.8	110	250		
27b	258	2.2	116.3	394	$C_{29}H_{60}$	Nonacosane
28	278	.5	128.3	407	C_{31} or C_{32}	Long Chain Alcoholic Nitrile
29	285	2.9	134			
30				436	$C_{31}H_{64}$	Hentriacotane
B-2 Peak Number						
1	89		5.4			Heptane
2	172	.76	34.8	167	$C_{12}H_{26}$	Dodecane
3	181	79.7	43.2	209	C_{15} or $C_{14}N$	Nitrile
3b	188		56.4	171		Nitrile
4	205		63.6	219-220		?
5	220		67.8		C_{16} or C_{20}	Unsat Hydrocarbon
6	238	4.2	76.2	224	$C_{16}H_{34}$	Hexadecane
7	246	.3	78.6			?
8	253	7.8	82.8			Poor spectra
9	267	7.0	85.8	372		Cyclic Nitrile
10	278	.24	90	254	$C_{18}H_{38}$	Octadecane

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
B-3 Peak Number						
1	157	4.6	46.5			Solvent
2	160	6.9	54			
3	177	6.9	59.4			
4	186	12.7	1.6	196	C_{14}	Nitrile
B-4 Peak Number						
1	65		14.4			Heptane
2	72	2.1	31.2			Heptane
3	135	3.1	65.2		$C_{10}H_{22}$	8 Methyl Nonane
4	150	6.3	67.6	221	$C_{14:2}H_{25}C:N$	Nitrile
5	165		72.8			Phthlate
6	177	11.46	78	242	$C_{15}H_{32}C:NC$	Heterocyclic HCN
7	200	37.1	80	274	$C_{18}H_{32}C:N$	
8	210	39.9	81	301-2		
M-1 Peak Number						
1	60		1.8	100	C_7H_{16}	Heptane
1b	65		.3			
2	70		7.8			
2a	80		1.3			
3	96		23.8		$C_{17}N_4C_{14}$	Cycloalkyl or Alkane without 2 Ether O & N
3b	96.4		3.4		$C_{13:1}$	Tridecene with an Alkane Branch
4	104		30.6	212	$C_{15}H_{32}$	Pentadecane

Sample		% Area	Retention Time	Parent M Weight	Formula	Tentative Name
5	110	2.2	34.8			Alkane Ring
5b	110	2.2	5.8			
6	115	.67	38.4	206	$C_{16:1}N$	
6b	115		6.4			
7	122	.2	42	183	$NC_{15:1}$	Cycloether or 2 DB or Rings Strong Ether Emphasis
7b	120.7		7.0			
8	125	.7	43.7		C Thalate	
8b	125		7.3			
9	127	2.2	46		C_{12} or C_{13} H_{24} or $36^{3}CN$	
9b	127		7.7			
10	135	.22	51	241	$C_{20}H$	Ring or Branched
10b	142		95			
11	142	8.3	55.7	217	$C_{18:1}H?N$	Possible Nitrile
11b	145		9.3			
12	149	6.4	61.7		$C_{18}H_{38}$ or $C_{14}H_{27}OHCN$	Octadecane or Alcoholic Nitrile
12b	150		10.3			
13	155	1.7	67		$C_{19}C_{14}$?	
13B	152		11.2			
14	157	3.4		262-295	B = $C_{23.1}H_{46}$ A = $C_{22}HNO$?	Tricosene Nitrile
14b	155		11.5			
15	165	3.8	73	293	$C_{20}H_{25}N_2$	2 Rings
15b	160		12.2			
16	172	1.1	78.5	243	$C_{18}HC:N$	
16b	165					
17	183	5.4	87	306	$C_{22}H_{42}$	Docosane
17b	175		14.5			
18	191	2.5	93	338	$C_{24}H_{50}$	Tetracosane

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
18b	180					
19	201	19.3	98.5	350	$C_{25:1}H_{50}$	Pentacosene
20	204	19.3	102	352	$C_{25}H_{52}$	Pentacosane
21	206	19.3	103.5	320	$C_{23:2}H_{44}$	Tricodiene ?
22	208	1.0	105.5	362	$C_{24}H_{48}CN$	Nitrile
23	217	15.4	110	327	C_{25}:HC:N	Nitrile ?
24	228	167	120	362	C_{26} or C_{24}	
25	241	0.076	129		Phthalate	
1	92		1.8 cm 7.2 min	84	C_7H_{16}	Solvent Heptane
2	180	47.52	26.6 cm 1 hr 47 min	226	$C_{16}H_{34}$	Hexadecane
3a	250	52.49	39.4 cm 2 38 min	282	$C_{20}H_{42}N$	Nitrile
3b	250	52.49	39.4 cm 2 38 min	250	$C_{23}H_{46}N$?	Nitrile ?
M-3 Peak Number						
1	60		6.8	86	C_7H_6	Heptane
2	122	44.6	85.2	226	$C_{16}H_{34}$	Hexadecane
3	175	55.4	95.6		$C_{16}H_{34}$	Hexadecane
4	183		101.2		C_{11} or C_6	Silicon Lubricant
5	196		139.2		$C_{20}H_{42}$	Eicosane
M-4 Peak Number						
1	85		4.4		C_7H_{16}	Solvent Heptane

Sample	Temp.	% Area	Retention Time	Parent M Weight	Formula	Tentative Name
2	102		12.8		C_4 branched	Branched Alkane
3	106		14.8			Heptane
4	111		17.6			Heptane
5	117	50.9	20	142	$C_{10}H_{22}$	Decane
6	123	13.2	20.8	142	$C_{10}H_{22}$	Decane
7	150	5.2	33.2	155	$C_{11}H_{24}$	5, Methyl-decane
8	195	Background	57.2			Background (Clean)
9	233	1.9	76.4	281	C_{20}	Possible Nitriles
10	245	2.2	82	281	C_{20}	Possible Nitriles
11	247	20.5	84	281	C_{20}	Possible Nitriles
12	250	6.0	88.4	281	C_{20}	Possible Nitriles
Standard						
1	60	40	.5	142	$C_{10}H_{22}$	Decane
2	65	6	.9	170	$C_{12}H_{26}$	Dodecane
3	116	8.2	6.2	198	$C_{14}H_{30}$	Tetradecane
4	122	10.1	7.0	226	$C_{16}H_{34}$	Pentadecane
5	138	7	9.1	254	$C_{18}H_{38}$	Octadecane
6	155	3	11.2	282	$C_{20}H_{42}$	Eicosane
7	157	3.2	11.7	310	$C_{22}H_{46}$	Docosane
8	172	2.7	13.5	338	$C_{24}H_{50}$	Tetracosane
9	176	3	13.9	?	$C_{28}H_{60}$	Silicon Lubricant
10	191	7.7	15.8	?	$C_{32}H_{68}$	Contaminant Plastic

APPENDIX C

KEY TO *ASTRAGALUS* SPECIES - IN FLOWER ONLY

Flowers purplish, or white, purplish veins and keel; not yellowish. Leaves in a basal tuft.

A. megacarpus (Nutt.) Gray

Flowers yellowish, creme, or ochroleucous, if any purple, on the keel tip of a yellow flower. At least one cauline leaf.

Gynophore 1.5 to 5 mm; if longer than 3 mm, then the ovary with sutures parallel, appearing linear. Nectary inconspicuous.

A. beckwithii (Torr. and Gray) var. *beckwithii*

Gynophore 3 to 12 mm; the maturing ovary, brownish and with a bulge in the ventral suture. The proximal end of the gynophore covered with nectar from the nectary.

A. oophorus (Wats.) var. *caulescens* (Jones) Jones

KEY TO *ASTRAGALUS* SPECIES - IN FRUIT ONLY

Pod 1.5 to 3 cm long, dorsoventrally flattened, leathery, curved upward at an angle of 45-90°.

A. beckwithii (Torr. and Gray) var. *beckwithii*

Pods 3 to 6 cm long, not dorsoventrally flattened; inflated, and papery.

Gynophore longer than the calyx tube so that it is visible and pod is free of the calyx. Peduncle not exceeding the length of the pod; at least one leaf obviously cauline.

A. oophorus (Wats.) var. *caulescens* (Jones) Jones

Gynophore not longer than the calyx tube, pod appearing inserted into the calyx tube and splitting it along the ventral suture. Peduncle to 8 cm; at least the length of the pod or longer. Leaves in a basal tuft.

A. megacarpus (Nutt.) Gray

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CHEMOTAXONOMICAL COMPARISON OF ASTRAGALUS MEGACARPUS, ASTRAGALUS BECKWITHII AND ASTRAGALUS OOPHORUS IN UTAH

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ABSTRACT

This investigation was concerned with the taxonomic relationship between Astragalus beckwithii (Torr. & Gray) var. beckwithii, Astragalus oophorus (S. Wats) var. caulescens (Jones) Jones, and Astragalus megacarpus (A. Gray). An attempt was made to relate some chemical constituents and the toxic effects on rats to the taxonomy. A number of parameters were used; rat toxicity, lipid analysis, selenium atomic absorption, various salts and glucose percentages, as well as the usual morphological measurements taken from herbarium specimens, field observations, and pollen measurements.

While the weight of the data indicates that A. beckwithii is a type of intermediary or intergressory product of A. oophorus and A. megacarpus, and that it very closely resembles A. oophorus, it would be premature to suggest combining A. beckwithii and A. oophorus until the related varieties in each species are also studied. Evidence shows definite symptoms of locoism in the white rats tested. A hypothesis was made that the toxic principles in these plants are associated with the nitrile compounds.