Toxic Nitro Compounds in Species of *Astragalus* (Fabaceae) in Argentina

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Abstract

Thirty species of *Astragalus* from Argentina were analyzed for toxic aliphatic nitro compounds. Twenty-seven species, including 7 species known to poison livestock, synthesized nitro compounds that hydrolyzed to highly toxic 3-nitro-l-propanol (3-NPOH). Nitro compounds in *Astragalus palenae* (Phil.) Reiche hydrolyzed to both 3-NPOH and 3-nitropropionic acid (3-NPA). *Astragalus palenae* is the only species of *Astragalus* so far examined that synthesized both compounds. Analyses of leaves of the 30 species for cyanogenic glycosides were negative. Species of *Astragalus* from Argentina that synthesize nitro compounds should not be introduced into the United States because of their potential hazard to livestock.

The genus *Astragalus* consists of over 2,000 species world-wide. Although most species occur in the temperate regions of the Northern Hemisphere, 105 species are indigenous to western and southern South America from Ecuador to Argentina. Sixty-four species are native to Argentina.

Several species of *Astragalus* are known to poison livestock and have caused losses on rangelands and pastures in Argentina. Gómez-Sosa (1979) reported that *Astragalus bergii* Hieron., *A. chamissonis* (Vog.) Reiche, *A. distinens* Macloskie, *A. pehuenches* Niederl. and *A. vesiculosus* Clos. caused livestock losses in Patagonia (the five southern provinces of Argentina). Monticelli (1938) reported a large loss of animals to *Astragalus bergii* in 1931 in San Luis Province in central Argentina. Gómez-Sosa was told by farmers in Tucumán Province in northwestern Argentina that *Astragalus cryptobotrys* Johnst. caused both addiction and death in their livestock. Elsewhere in Argentina, *A. garbancillo* Cav. has caused livestock losses (Burkart 1952). *Astragalus arequipensis* Vog. was reported toxic to livestock in Bolivia (Burkart 1952).

Evidence existed that species of *Astragalus* indigenous to Argentina synthesized highly toxic nitro compounds. Nitro compounds are particularly toxic to ruminants because microflora in the rumen hydrolyze them to 3-nitro-1-propanol (3-NPOH) or 3-nitropropionic acid (3-NPA) (Williams et al. 1970, Williams 1982). While both compounds are toxic, animals are poisoned orally at lower concentrations of 3-NPOH because it is more rapidly absorbed into the circulatory system.

Of the species reported poisonous in Patagonia by Gómez-Sosa (1979), *Astragalus bergii, A. distinens,* and *A. vesiculosus* were known to synthesize nitro compounds (Williams 1981) and the latter 2 species synthesized 3-NPOH (Williams 1982). The toxic principle in the other 2 species, *A. pehuenches* and *A. chamissonis,* was unknown. Four other nitro-bearing species of *Astragalus* also occur in Patagonia: *A. domeykoanus* (Phil.) Reiche, *A. cruckshanksii* (Hook. & Arn.) Griseb., *A. palenae* (Phil.) Reiche, and *A. patagonicus* (Phil). Speg. (Gómez-Sosa 1984). These data suggested that nitro compounds might be the toxic principle in other poisonous species of *Astragalus* in Argentina.

Cooperative research between personnel at the Instituto de Botanica Darwinion, San Isidro, Argentina, and the Poisonous Plant Research Laboratory, Logan, Utah, was initiated to: determine the presence or absence of aliphatic nitro compounds in species of *Astragalus* in Argentina; identify the type of nitro compound present; recommend methods to reduce livestock losses from nitro-bearing species; and prevent the exportation of poisonous species of *Astragalus* which, if introduced or released on grazing lands, might cause livestock losses in other countries.

Methods

Thirteen species of *Astragalus* were collected in Argentina in the field during 1984 from the provinces of San Juan and Tucumán and from Mendoza in 1985. San Juan and Mendoza Provinces border Chile in west central Argentina. Plants were in flower to pod stage of growth. Leaves of the remaining species of *Astragalus* were sampled from herbaria at the Instituto de Botanica Darwinion, San Isidro, Argentina (SI), the National University of La Plata, Argentina (LP), Royal Botanic Gardens, Kew, England (K), The New York Botanical Garden, Bronx, New York, USA (NY), and from specimens at the Biological Control of Weeds Research Laboratory, U.S. Dept. of Agriculture, ARS, Buenos Aires, Argentina. The latter source has been designated as Herbarium Cordo. All species were personally identified by Edith Gómez-Sosa except *Astragalus domeykoanus* and *A. micranthellus* Wedd. Leaves of these species were sampled at K and NY and the presence of nitro compounds in them was reported previously (Williams 1981, Williams 1982). Samples of the other 34 species of Argentine *Astragalus* were not available for study. All samples were sent to the Poisonous Plant Research Laboratory, Logan, Utah, for analysis.

Samples were analyzed qualitatively for the presence of nitro compounds (Williams and Barneby 1977), type of nitro compound (Williams 1982), and for cyanogenic glycosides (AOAC 1980). Cyanogenic glycosides were reported in *Astragalus* in Argentina (Giusti 1938).

Leaves from 3 accessions of *Lotus tenuis* Waldst. & Kit. ex Willd. and 1 of *L. wrightii* (A. Gray) Greene collected between 1970 and 1980 were sampled from the Intermountain Herbarium at Utah State University, Logan, Utah, and tested for HCN. These species were known to synthesize cyanogenic glycosides (M.C. Williams, unpubl. data). The *Lotus* leaves were analyzed for HCN to determine if this compound could be detected in dried leaves that were 5 to 15 years old and might therefore be detected in dried leaves of *Astragalus* if present.

Results and Discussion

Twenty-seven species and 1 variety of *Astragalus* tested positive for nitro compounds (Table 1). Only 3 species, *A. ruiz-lealii* Johnst., *A. monticola* Phil., and *A. joergensenii* Johnst., tested negative. The nitro compounds in all other species yielded 3-NPOH upon hydrolysis. The nitro compounds in *A. palenae* yielded both 3-NPOH and 3-NPA. Heretofore, nitro-bearing species of *Astragalus* examined have synthesized either 3-NPOH or 3-NPA but not mixtures of the two. *Astragalus palenae* is the first species of *Astragalus* found to contain both compounds. The origin of the specimen of *A. domeykoanus* sampled at Kew is

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obscure. However, the species is found throughout Patagonia from the provinces of Neuquén to Santa Cruz.

Qualitative analysis of *A. bergii* and *A. distinens* yielded a nitro content of approximately 9 mg NO_2/g of leaf. Quantitative analysis of leaves of 1 sample of *A. vesiculosus* yielded 9.2 mg NO_2/g. The concentration of 3-NPOH in these species was equal to that found in *A. emoryanus* var. *emoryanus* (Rydb.) Cory which caused devastating losses in cattle and sheep in New Mexico in 1975 (Williams et al. 1979). *A. palenae* contained the same amount of NO_2, but as a mixture of 3-NPOH and 3-NPA.

All *Lotus* species tested positive for HCN. Analyses for HCN in the Argentine species of *Astragalus* tested in this study were all negative. Cyanogenic glycosides have not previously been implicated as toxic compounds in the genus *Astragalus*.

Cyanogenic glycosides, saponins, and selenium have been suggested as the toxic principle in Argentine species of *Astragalus*. Giusti (1938) found cyanogenic glycosides in *Astragalus bergii*. Awschalom (1928) extracted saponins from *Astragalus garban-*

Table 1. Presence and type of nitro compound in species of *Astragalus* in Argentina.

Astragalus species	Qualitative analysis[1]	Nitro compound	Voucher number	Herbarium	Province	Year collected
A. arequipensis Vog.	1	3-NPOH	Gómez-Sosa & Múlgura 142	SI, UTC	Tucumán	1984
A. arnottianus (Gill.) Reiche	1	3-NPOH	J. Hunziker et al. 11189	SI, UTC	Mendoza	1985
A. bergii Hieron.	2	3-NPOH	Cordo 77-D-28	Herb. Cordo[2]	Córdoba	1977
A. bustillossii Clos.	1	3-NPOH	Gómez-Sosa & Múlgura 168	SI, UTC	Tucumán	1984
A. carinatus (Hook. & Arn.) Reiche	1	3-NPOH	Cordo & Ferrer 77-D-12	Herb. Cordo	Mendoza	1977
A. chamissonis (Vog.) Reiche	1	3-NPOH	Gerling 259	SI	Mendoza	1907
A. cruckshanksii (Hook. & Arn.) Griseb.	1	3-NPOH	Marques & Gómez-Sosa 54	SI	Mendoza	1985
A. crypticus Johnst.	Trace	3-NPOH	Gómez-Sosa & Múlgura 232	SI, UTC	Tucumán	1984
A. cryptobotrys Johnst.	1	3-NPOH	Gómez-Sosa & Múlgura 161	SI, UTC	Tucumán	1984
A. cuyanus Gómez-Sosa	1	3-NPOH	Cordo & Deloach ------	Herb. Cordo	Mendoza	1978
A. distinens Macloskie	2	3-NPOH	Troncoso & Bacigalupo 3059	SI	Entre Ríos	1981
A. domeykoanus (Phil.) Reiche	1	3-NPOH	None	K	South America[3]	1927
A. famatinae Johnst.	1	3-NPOH	Cordo & Runnacles 77-D-39	Herb. Cordo	Jujuy	1978
A. flavocreatus Johnst.	1	3-NPOH	Gómez-Sosa & Múlgura 193	SI, UTC	Tucumán	1984
A. garbancillo Cav.	Trace	3-NPOH	Kiesling 4428	SI	San Juan	1984
A. hypsogenus Johnst.	1	3-NPOH	Cabrera et al. 27426	SI	Jujuy	1976
A. joergensenii Johnst.	0	None	Fabris 6312	LP	Jujuy	1966
A. micranthellus Wedd.	1	3-NPOH	Ledingham & Cabezas	NY	Jujuy	1966
A. monticola Phil.	0	None	Cabrera et al. 31166	SI	San Juan	1979
A. palenae (Phil.) Reiche	2	3-NPOH & 3-NPA	Cabrera et al. 33089	SI	Chubut	1981
A. palenae var. *grandiflora* Speg.	1	3-NPOH	Ruiz-Leal 23914	SI	Neuquén	1965
A. parodii Johnst.	Trace	3-NPOH	Burkart 10307	SI	Córdoba	1977
A. patagonicus (Phil.) Speg.	Trace	3-NPOH	Roig, TBPA 2474	SI	Santa Cruz	1977
A. pauranthus Johnst.	1	3-NPOH	Cordo & Runnacles 77-D-54	Herb. Cordo	Catamarca	1977
A. pehuenches Niederl.	1	3-NPOH	Marques & Gómez-Sosa 58	SI, UTC	Mendoza	1985
A. peruvianus Vog.	Trace	3-NPOH	Gómez-Sosa & Múlgura 166	SI, UTC	Tucumán	1984
A. ruiz-lealii Johnst.	0	None	Covas 2140	SI	Mendoza	1943
A. sanctae-crucis Speg.	Trace	3-NPOH	Nicora 8411	SI	Chubut	1982
A. tarijensis Wedd.	1	3-NPOH	Gómez-Sosa & Múlgura 227	SI	Tucumán	1984
A. uniflorus DC.	Trace	3-NPOH	Gómez-Sosa & Múlgura 212	SI, UTC	Tucumán	1984
A. vesiculosus Clos.	2	3-NPOH	J. Hunziker et al. 11304	SI, UTC	Mendoza	1985

[1]Qualitative analysis: Trace equals 2 to 3 mg NO_2/g of leaf
1 equals 4 to 8 mg NO_2/g of leaf
2 equals 9 to 13 mg NO_2/g of leaf

[2]Herbarium at the Biological Control of Weeds Research Laboratory, U.S. Dept. Agr., ARS, Buenos Aires, Argentina.

[3]*Astragalus domeykoanus* is indigenous in the four southern provinces of Argentina: Neuquén, Rio Negro, Chubut, and Santa Cruz.

cillo (sub *A. unifultus* L.Heritier) that were toxic by intravenous, intracardiac, and intraperitoneal injection to laboratory animals. Neither compound had previously been reported in toxic levels in *Astragalus*. Saponins have been isolated in *Astragalus glycyphyllos* L. and *A. ammodytes* Pall. (Gibbs 1974) but neither is known to be poisonous. Analyses for selenium, an element found in toxic concentrations in more than 20 species of North American *Astragalus*, have proved negative in Argentina (Burkhart 1952).

Several cases of poisoning by *A. pehuenches* in Chubut Province in central Patagonia were ascribed to selenium accumulation by the plant (unpubl. medical reports of the Minister of Economy, Services and Public Works, Chubut Province, Argentina). The diagnosis of selenium poisoning was derived by comparing the toxic signs observed or described by local farmers with those described in the literature. No analyses of the plants were made to determine if selenium was in fact present, nor plants analyzed determine the presence or concentration of 3-NPOH, which is known to be present in *A. pehuenches*.

The syndromes of selenium and nitro poisoning are similar and both are characterized by loss of motor control, depression, grating of teeth, salivation, foaming at the nostrils, rapid and labored respiration, frequent urination, and impaired vision which causes the animal to stumble over small objects or to run into objects in its path (Rosenfeld and Beath 1964).

Giusti (1934) fed *A. bergii* as part of the diet to horses, pigs, sheep, and goats. Horses and pigs showed no toxic signs in 5 months of feeding, but 9 of 14 sheep died within 25 days and 6 of 8 goats died within 24 hours. *A. bergii* contains 3-NPOH at levels that would produce these results, and this compound is much more toxic to ruminants than nonruminants.

Gallo (1979) reported toxic signs observed in livestock poisoned by *A. pehuenches* (sub *A. bergii*) were: nervousness, rigidity in walk, loss of sense of direction, lack of gregariousness, muscular incoordination, weight loss, general weakness, animals leaned against objects to keep from falling, and once the animal collapsed, it could not arise. Kidneys and liver were discolored but of normal consistency, and there was a decrease in percent hemoglobin.

The above toxic signs are similar to those described for poisoning by nitro-bearing species of *Astragalus* in North America (James et al. 1980) and an increase in methemoglobin is a constant finding.

Nitro compounds are very stable and the presence and type of compound can be determined with as little as 10 mg of leaf from herbarium specimens up to 150 years old (Williams 1981). Nitro compounds occur primarily in the leaflets of *Astragalus*. The concentration of these compounds is usually highest when the plant is in bloom to early pod stage of growth, then drops as the plant approaches senescence (Williams and Norris 1969). Once the leaves turn brown and die, nitro concentration falls to nontoxic levels. Therefore, nitro levels for some species listed in Table 1 might have been higher had the plants been collected under optimum conditions.

Approximately 90% of the Argentine species examined synthesized nitro compounds. By contrast, only about 12% of the Old World and 52% of the North American species of *Astragalus* examined are known to be nitro-bearing (Williams 1981, Williams and Barneby 1977).

Livestock should be prevented from grazing nitro-bearing *Astragalus*. Medical treatment is largely ineffective. NO_2 complexes with ferrous hemoglobin and prevents its reoxygenation. The methemoglobin concentration in the blood of fatally poisoned animals may exceed 30%. Prompt administration of methylene blue alleviates methemoglobinemia, but this treatment does not counteract the effects of 3-NPOH or 3-NPA which affect the portion of the brain that controls motor and automatic responses (Williams et al. 1969).

The LD_{50} of 3-NPOH, given orally in a single dose, is approximately 50 mg NO_2/kg of body weight for sheep and 25 mg NO_2/kg for cattle (Williams and James 1975, Williams et al. 1969). Sublethal doses of nitro compounds affect the brain, lungs, spinal cord, heart, kidneys, and liver. Many of these changes are irreversible and once toxic signs have become pronounced, animals will never recover normal health even if they are removed to wholesome forage. Therefore, plants should be avoided until they have dried up, or they should be controlled chemically or mechanically. Herbicides that cause the plants to desiccate hasten their detoxication (Williams 1970).

Argentina lies between latitudes 22° South and 55° South, approximately the same distance in north latitude between Cuba and Hudson Bay. This area includes mountains, subtropic and temperate zones, and cold deserts similar to those found in the United States. Many species of *Astragalus* from Argentina, if introduced into the United States, might be adapted to similar ecological sites, particularly on the cold deserts, foothills, and mountains of the West. Because of the high incidence of species that synthesize nitro compounds that hydrolyze to 3-NPOH, all species of *Astragalus* from Argentina should be carefully evaluated for toxicological properties before any research and development leading to their introduction and release is contemplated. Species that synthesize 3-NPOH should be excluded from introduction for any purpose because of their potential hazard to livestock.

Summary and Conclusions

The data indicate that aliphatic nitro compounds that hydrolyze to 3-NPOH are present in 27 species and 1 variety of *Astragalus* in Argentina. HCN was not detected in any species. We therefore conclude that 3-NPOH is the principal if not the sole toxic compound in poisonous species since (1) all species known to cause livestock losses synthesize toxic levels of 3-NPOH; (2) the toxicological and pathological signs described are similar to those described for nitro-bearing species of *Astragalus* in western North America; and (3) *Astragalus bergii, A. distinens,* and *A. vesiculosus,* species often associated with livestock losses in Argentina, synthesized 3-NPOH at the same concentration as *A. emoryanus,* a species that is known to cause extensive livestock losses in the United States.

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