ANALYSIS OF BIFURCATE HAIRED ASTRAGALUS SPECIES FROM IRAN FOR TOXIC NITRO COMPOUNDS

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Ebrahimzadeh, H., Maassoumi, A. A. & Niknam, V. 2000 08 01: Analysis of bifurcate haired *Astragalus* species from Iran for toxic Nitro compounds. *-Iran. Journ. Bot. 8 (2): 213-222.* Tehran.

Leaflets of 111 specimens including 82 bifurcate haired *Astragalus* species in 13 sections were taken from the herbarium specimens of Research Institute of Forests and Rangelands (TARI). They were analyzed quantitatively for toxic aliphatic nitro compounds. The catabolites of nitro compounds, 3-nitro-1-propanol and 3-nitropropionic acid, are especially toxic to cattle and sheep. Nitro compounds are also important from chemotaxonomic point of view. These nitro-toxins were detected in 4 of 82 species.

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Key Words. Fabaceae, Astragalus, bifurcate hairs, nitro compounds, poisonous plants, Iran.

بررسی ترکیبات ازت دار سمّی در گونهای کرک دوشاخه ایران

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برگچه‌های ۱۱۱ نمونه هرباریومی از گونهای کرک دوشاخه ایران، شامل ۸۲ گونه از ۱۳ بخش، به منظور تشخیص ترکیبات ازت‌دار آلیفاتیک سمّی مورد بررسی قرار گرفتند. کاتابولیتهای ترکیبات ازت‌دار، ۳-نیترو ۱-پروپانول و اسید ۳- نیترو پروپیونیک، برای احشام سمّی هستند. بعلاوه این ترکیبات در طبقه بندی گیاهان نیز حائز اهمیت می‌باشند. وجود این نیتروتوکسین‌ها در ۴ گونه از ۸۲ گونه مذکور گزارش می‌شود.

Introduction

The nitro toxins 3-nitropropionic acid (3-NPA) and 3-nitro-1-propanol (3-NPOH), are found in many Leguminous plants (Anderson & al. 1993) and are known to be the principal poisons associated with *Astragalus*. More than 465 species and varieties of *Astragalus* synthesize these compounds (Williams 1981; Williams & Barneby 1977; Ebrahimzadeh, Maassoumi & Niknam 1999). Aliphatic nitro compounds are alsosynthesized in the genera *Coronilla, Lotus* and *Indigofera* of the family *Leguminosae* (Williams 1981a; Williams 1981b; Williams 1983; Williams 1985; Niknam, Ebrahimzadeh & Maassoumi 1999).

Analysis of *Astragalus* for these compounds from herbarium specimens identify the species that are nitro-bearing and therefore might be poisonous to livestock. Examination of the chemotaxonomic relationships among the nitro-bearing species suggests, that related species might be nitro-bearing even if specimens are not available for analysis (Williams, 1981a). Because *Astragalus* may be grazed by livestock or fed as fodder, these species require toxicological investigation. Moreover, the correct identification of the species is a major problem. The genus *Astragalus* is a complex and is under constant revision. Species may be renamed, trasferred to different sections, reduced to subspecies or varieties, or several species may be combined into one. Nitro bearing sections as well as species may be chemotaxonomically related. Analysis for nitro compounds can be used in correct identification and classification of nitro-bearing species as well as synonymy Problems.

This paper identifies 4 nitro-bearing species of *Astragalus* from Iran.

Materials and Methods

About 25 mg samples of leaves of 111 specimens includig 82 bifurcate haried species removed from the herbarium specimens at the herbarium of Research Institute of Forests and Rangelands (TARI). The leaflets were analyzed quantitatively for toxic nitro compounds (Williams & Barneby 1977). For details see Ebrahimzadeh, Maassoumi & Niknam (1999).

Results and Discussion

Nitro compounds were found in 4 species out of 111 specimens including 82 species (4.88% of the species examined) (table 1).

Table 1. Nitro concentration in bifurcate haired *Astragalus* species from Iran; the approximate concentration (mg NO_2 / g dry weight) represented by the scores are: -=0; 1=4 to 8; 2=9 to 13; 3=14 to 19; 4=20 to 25.

Section	Specific epithet	Voucher specimens	NO2 scores
Ammodendron	*podolobus* Boiss. & Hohen.	Wendelbo & Assadi, 29436	-
Ammodendron	*squarrosus* Bunge	Assadi, 23123	-
Ammodendron	*squarrosus* Bunge	Assadi & Mozaffarian 35828	-
Ammodendron	*oligophyllus* Boiss.	Rechinger, 46315	-
Ammodendron	*oligophyllus* Boiss.	Babakhanlou & Amin., 15471	-
Ammodendron	*ammodendroides* Bornm.	Wendelbo & Assadi, 18298	1
Ammodendron	*macrobotrys* Bunge	Rajamand & Bazargan, 31773	-
Ammodendron	*macrobotrys* Bunge	Maassoumi, Pakravan & Nasseh, 72347	-
Ammodendron	*ahmad-adlii* Bornm., & Gauba	Runemark & Varnecke, 25456	-
Ammodendron	*acutifolius* Benth. ex Bunge	Assadi & Shahmohammadi, 60015	-
Ammodendron	*kavirensis* Freitag	Freitag & Jadidi, 29103	-
Ammodendron	*brevipetiolatus* Sirj. & Rech. f.	Assadi & Mozaffarian, 35692	-
Ammodendron	*tarumensis* Sirj. & Rech. f.	Mozaffarian, 46844	-
Asciocalyx	*asciocalyx* Bunge	Wendelbo & Assadi 29692	-
Caraganella	*stocksii* Benth. ex Bunge	Runemark & Sardabi, 22343	-
Cystium	*mazandaranus* Bunge	Assadi & Mozaffarian, 33037 B	2
Cystodes	*dendroproselius* Rech. f.	Forghandust, 36132	-
Erioceras	*djenarensis* Sirj. & Rech. f.	Assadi & Maassoumi 55574	-
Erioceras	*pentanthus* Boiss.	Wendelbo & Assadi, 16875	-
Erioceras	*harsukhianus* Rech. f.	Assadi & Mozaffarian, 35835	-
Erioceras	*anacamptus* Bunge	Assadi & Mozaffarian, 33282	-
Erioceras	*argyroides* G. Beck	Rechinger, 50283	-
Erioceras	*argyroides* G. Beck	Takestan, 28776	-
Erioceras	*argyroides* G. Beck	Hewer, 3757	-

Table 1. (continued).

Section	Specific epithet	Voucher specimens	NO2 scores
Erioceras	*argyroides* G. Beck	Bonvan, 9699	-
Erioceras	*triqueter* Bornm. & Gauba	Babakhanlou & Amin, 23515	-
Incani	*robustus* Bunge	Assadi & Mozaffarian, 30445	1
Incani	*robustus* Bunge	Maassoumi, 64815	-
Incani	*ulothrix* G. Beck	Rechinger, 50423	-
Incani	*cinereus* Willd.	Wendelbo & Cobham, 13452	-
Incani	*curvirostris* Boiss.	Wendelbo & Forughi 17784	-
Incani	*demavendicus* Boiss. & Buhse	Moaffarian, 49029	-
Incani	*abnormalis* Rech. f.	Riazi, 6243	-
Incani	*thionanthus* Bornm.	Assadi, 60474	-
Incani	*campylosema* Boiss.subsp. *campylosema*	Maassoumi & Abouhamzeh, 56930	-
Incani	*campylosema* Boiss.subsp. *campylosema*	Maassoumi & Abouhamzeh, 56868	-
Incani	*ackerbergensis* Freyn.	Rechinger, 4590	-
Incani	*ackerbergensis* Freyn.	Assadi & Maassoumi, 50075	-
Incani	*rostratus* C. A. Meyer	Maassoumi & Abouhamzeh, 56955	-
Incani	*choicus* Bunge	Maassoumi & Abouhamzeh, 57009	-
Incani	*supervisus* Sheld.	Sanii, 11246	-
Incani	*fridae* Rech. f.	Assadi, 56636	-
Incani	*zangelanus* Grossh.	Mozaffarian & Nowroozi 35070	-
Incani	*quinquefoliatus* Bunge	Wendelbo & Foroughi, 17869	-
Incani	*micrancistrus* Boiss. & Haussk.	Wendelbo & Assadi, 16919	-
Incani	*cyclophyllon* G. Beck	Assadi, 60580	-
Incani	*cyclophyllon* G. Beck	Foroughi & al., 12508	-
Incani	*fedorovii* Tackt.	Runemark & Foroughi, 19613	-
Incani	*refractus* C. A. Meyer	Mozaffarian & Nowroozi, 34118	-
Incani	*gudrunensis* Boiss.	Mozaffarian & Maassoumi, 48021	-
Incani	*jolderensis* Fedtsch.	Mozaffarian, 48704	-

Table 1. (continued).

Section	Specific epithet	Voucher specimens	NO2 scores
Incani	*dilutulus* Bornm.	Wendelbo & Assadi, 16874	-
Incani	*schirkuhicus* Bornm.	Manoochehri & al., 54	-
Incani	*askius* Bunge	Wendelbo & Shirdelpur, 11697	-
Incani	*latifolius* Lam.	Assadi & Mozaffarian, 30190	-
Incani	*latifolius* Lam.	Assadi & Mozaffarian, 29944	-
Incani	*borschensis* Bornm.	Wendelbo & Assadi, 16495	-
Incani	*rostratus* C. A. Meyer	Assadi & Sardabi, 24000	-
Incani	*punctatus* Bunge	Wendelbo & Assadi, 28014	-
Leucocercis	*cornu-caprae* Sirj. & Rech. f.	Wendelbo & Foroughi, 11462	-
Leucocercis	*curviflorus* Boiss.	Zehzad & al., 66937	-
Leucocercis	*curviflorus* Boiss.	Riazi, 5914	-
Leucocercis	*mucronifolius* Boiss.	Mozaffarian & al., 30372	-
Leucocercis	*mucronifolius* Boiss.	Froughi, 10819	-
Leucocercis	*mucronifolius* Boiss.	Assadi & Sardabi, 42234	-
Leucocercis	*ovoideus* Sirj. & Rech. f.	Assadi, 22782	-
Leucocercis	*semnanensis* Bornm. & Rech. f.	Wendelbo & Foroughi, 11462	-
Leucocercis	*talimansurensis* Sirj. & Rech. f.	Mozaffarian, 63273	-
Leucocercis	*talimansurensis* Sirj. & Rech. f.	Assadi & Abouhamzeh, 38855	-
Onobrychoidei	*aduncus* Willd.	Assadi, 11936	-
Onobrychoidei	*argyricus* Bunge	Akbarzadeh & Sardabi, 41113	-
Onobrychoidei	*brevidens* Freyn. & Sint.	Vafaee, 281	-
Onobrychoidei	*cancellatus* Bunge	Assadi & Mozaffarian, 30509	-
Onobrychoidei	*effusus* Bunge	Nowroozi, 4517	-
Onobrychoidei	*lilacinus* Boiss.	Assadi & Mozaffarian, 33157	-
Onobrychoidei	*lilacinus* Boiss.	Mozaffarian & Mohammadi, 49172	-
Onobrychoidei	*lunatus* Pallas	Assadi, 20233	-
Onobrychoidei	*mossulensis* Bunge	Hamzehee & Lashkarbolouki, 589	-

Table 1. (continued).

Section	Specific epithet	Voucher specimens	NO2 scores
Onobrychoidei	*pseudocancellatus* Grossh.	Yousseſi, 7559	-
Onobrychoidei	*strictipes* Bornm.	Maassoumi & Assadi, 100504	-
Onobrychoidei	*tehranicus* Boiss. & Hohen.	Dini & Azarm, 1972	-
Onobrychoidei	*tehranicus* Boiss. & Hohen.	Mousavi & Amin, 15020	-
Ornithopodium	*brachyodontus* Boiss.	Assadi & Mozaffarian, 27566	-
Ornithopodium	*brevipes* Bunge	Borhan, 313	-
Ornithopodium	*glochideus* Boiss.	Maassoumi & Abouhamzeh, 56871	-
Ornithopodium	*ornithopodioides* Lam.	Wendelbo & Assadi, 27860	-
Ornithopodium	*schistosus* Boiss. & Hohen.	Wendelbo & Assadi, 27666	-
Ornithopodium	*stevenianus* DC.	Siami, 4118	-
Ornithopodium	*stevenianus* DC.	Mozaffarian & Nowroozi, 34522	-
Ornithopodium	*stevenianus* DC.	Wendelbo & Assadi, 28016	-
Ornithopodium	*stevenianus* DC.	Foroughi, 6032	-
Ornithopodium	*stevenianus* DC.	Foroughi, 6030	-
Trachycercis	*durandianus* Aitch. & Baker	Assadi & Maassoumi, 21074	-
Uliginosi	*odoratus* Lam.	Abouhamzeh, 57015	2
Xiphidium	*angustatus* Boiss.	Mozaffarian, 54425	-
Xiphidium	*argyroides* G. Beck	Rechinger, 50283	-
Xiphidium	*argyroides* G. Beck	Pabot, 28776	-
Xiphidium	*argyroides* G. Beck	Hewer, 3757	-
Xiphidium	*argyroides* G. Beck	Bonvan, 9699	-
Xiphidium	*eburneus* Bornm. & Gauba	Assadi & Sardabi, 41764	-
Xiphidium	*eburneus* Bornm. & Gauba	Mozaffarian, Banihashemi & Shahinzadeh, 1982	-
Xiphidium	*lorinserianus* Freyn.	" " " , 2664	-
Xiphidium	*sitiens* Bunge	Wendelbo & Foroughi, 11270	-
Xiphidium	*sumbari* Popov	Maassoumi & al., 72305	-

Table 1. (continued).

Section	Specific epithet	Voucher specimens	NO2 scores
Xiphidium	*sumbari* Popov	Assadi & Maassoumi, 50363	-
Xiphidium	*sumbari* Popov	Mozaffarian, 48700	-
Xiphidium	*tolgorensis* Sirj. & Rech. f.	Assadi & Maassoumi, 50276	-
Xiphidium	*triqueter* Bornm. &. Gauba	Babakhanlou & Amin, 1990	-
Xiphidium	*xiphidioides* Freyn. & Sint.	Maassoumi & al., 72354	-
Xiphidium	*xiphidioides* Freyn. & Sint.	Mozaffarian, 67604	-
Xiphidium	*xiphidioides* Freyn. & Sint.	Mozaffarian & Abouhamzeh, 59063	-

The positive species are *A. (Ammodendron) ammodendroides* Bornm., *A. (Cystium) mazandaranus* Bunge, *A. (Incani) robustus* Bunge, *A. (Uliginosi) odoratus* Lam. The presence of nitro toxins in the first three species is reported for the first time. The presence of these compounds in *A. odoratus* Lam. has been reported previously (Williams 1981a). Nitro compounds were not found in sections *Asciocalyx* Bunge, *Caraganella* Bunge, *Cystodes* Bunge, *Erioceras* Bunge, *Leucocercis* Bunge, *Onobrychoidei* DC., *Ornithopodium* Bunge, *Trachycercis* Bunge and *Xiphidium* Bunge.

As it can be seen in table 1, one out of 10 species in section *Ammodendron* Bunge is nitro bearing. In section *Cystium* Bunge only one species was analyzed and this species was nitro positive. In section *Incani* DC., one out of 28 species was nitro positive.

According to a previous study (Williams 1981a), one species, *A.quinquejugus* in section *Ammodendron* Bunge was ranked 5 in nitro content, except for a trace amount previously reported in *A. igniarius*, the other species tested in section *Ammodendron* Bunge were negative for nitro compounds.

Based on the present study only one species in setion *Incani* DC. is nitro-bearing and the other species tested were negative for these compounds.

Thus nitro bearing species of *Astragalus* occasionally occur atypically in otherwise nitro-free sections. This is in accordance

with the results of Williams (1981a). Many atypical nitro-bearing species may be improperly identified or classified within sections (Williams 1981a).

According to the present study and our previous paper (Ebrahimzadeh, Maassoumi & Niknam, 1999) nitro-bearing species are more common among simple haired *Astragalus* species than bifurcate haired species.

Analysis of nitro compounds can be used in correct identification and classification of nitro-bearing species as well as to resolve synonymy problems. Species of *Astragalus* studied to date synthesize either 3-NPOH or 3-NPA compounds but not mixtures of the two (Stermitz & Yost 1978). Thus, the presence of 3-NPOH or 3-NPA further restricts a species to certain taxonomic sections. Nitro-bearing species within otherwise nitro-free sections should be reexamined for proper identification and sectional classification.

For chemical formula and metabolism of 3-NPOH and 3-NPA see Ebrahimzadeh, Niknam & Maassoumi (1999).

Species that synthesize 3-NPOH at any level are highly poisonous to cattle and sheep and should not be considered for use as forage or other purposes. Species that synthesize metabolites of 3-NPOH usually fall within the range of 1 to 3. Nitro-toxins that are synthesized at level 4 and 5 are catabolized to 3-NPA in the digestive tracts of ruminants (Williams & Braneby 1977). Finally, Although nitro compound are relatively stable, they may decompose slowly over several decades. Some problems were encountered in the use of herbarium specimens of *Astragalus* for nitro detection. The identification of many species is based on the examination of mature fruits. When fruits are mature, leaves may have begun to bleach or lose green colour. Nitro levels in *Astragalus* leaves drop rapidly with the onset of senescence, and nitro compounds are rarely detected in completely bleached leaves. If plants are collected and dried properly during flowering to early pod when nitro levels are highest, nitro compounds can usually be detected for a century or more (Williams 1981a).

Acknowledgments

Financial support for this work was provided by Research Council of University of Terhan. The authors wish to express their deep appreciation to Dr. M. Coburn Williams, 1427 East 800 North Logan,

Utah 84321, USA, for his scientific articles and continuous helpful communications.

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