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TOXIC AMINES AND ALKALOIDS FROM *ACACIA RIGIDULA*

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Abstract—*Acacia rigidula* Benth., blackbrush, is a shrub found growing on rocky ridges in west and southwest Texas and northern Mexico. Consumption of blackbrush and a related species guajillo, *Acacia berlandieri* Benth., has been associated with a locomotor ataxia known as "limber leg". In an effort to identify the mechanism of this toxicity, blackbrush was subjected to rigorous chemical analysis. In addition to the four previously detected amines, *N*-methyl-β-phenethylamine, tyramine, *N*-methyltyramine, and hordenine, 40 other alkaloids and amines were isolated and identified by GC-MS. These alkaloids and amines included nicotine, *N*,*N*-dimethyltryptamine, mescaline, several tetrahydroisoquinoline alkaloids, and four amphetamines. A significant increase in the number and relative quantities of these compounds was observed in late season foliage.

INTRODUCTION

Acacia rigudula Benth., blackbrush, is a shrub found growing on rocky ridges in southwest and west Texas, and in the northern states of Mexico, including Tamaulipas, Nuevo Leon, Chihuahua, San Luis Potosi, and Jalisco [1]. The leaves are bipinnate and predominately short 15–25 mm long, pinnae 1–2 pairs that are 6–15 mm long, with 2–4 pairs (rarely 5 pairs) of elliptic to oblong leaflets 4–12 mm long and 6–8 mm wide. Blackbrush is heavily defended with long sharp spines and, if left unchecked, will form virtually impenetrable thickets. In addition to its physical defences, *A. rigidula* has been shown to contain appreciable levels of toxic alkaloids. Sheep and goats grazing on a related species, *Acacia berlandieri* Benth., guajillo, during periods of drought in the Rio Grande Plains of Texas have developed a locomotor ataxia referred to as "guajillo wobbles" or "limber leg" [2]. Previous analysis of blackbrush had detected and identified four amines, *N*–methyl-β-phenethylamine (NMPEA), tyramine, *N*-methyltyramine, and hordenine, which were also found in guajillo [3].

The locomotor ataxia developed in the early *A. berlandieri* feeding trials [4] was not observed in adequately fed animals injected with NMPEA or tyramine [5]. A review [6] of previous work has shown that many plant species endemic to southwest Texas and northern Mexico contain a wide variety of aromatic monoamines. A need to more fully explain the results of earlier studies, together with advances in GC-MS technology, led to an intensive chemical analysis of *A. berlandieri* to identify other amines and alkaloids present in the leaves [7]. As a result of this study, an intensive chemical analysis of *A. rigidula* Benth. was undertaken to identify the amines and alkaloids present in the leaves and stems that would comprise browse material.

RESULTS AND DISCUSSION

Leaves and stems that would comprise browse material were packed into an extraction thimble, placed in a Soxhlet apparatus and extracted with methanol followed by extraction with chloroform. This extraction procedure was compared with the more traditional methods of extraction by soaking the plant material in acid solution [2, 8] and found to produce a cleaner, more complete isolation of the amines and alkaloids present in the sample. The initial extract was fractionated by extraction with aq. acid, the pH of the acid extract was adjusted to ca pH 10 prior to re-isolation of the alkaloids by extraction with organic solvents. The pH of the aq. fraction was routinely checked between extractions and adjusted (dil. NaOH added) as necessary. It was found that when the pH exceeded 11, a large portion of the phenol containing alkaloids remained in the aqueous solution as their corresponding phenolate salts. Particular care was taken to maintain the extracts and isolates under

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an inert atmosphere. Left unprotected, the isolated amines and alkaloids readily decomposed.

Forty-four amines and alkaloids, including the four previously encountered amines, *N*-methyl-β-phenethylamine, tyramine, *N*-methyltyramine, and hordenine, were identified by GC-MS. Both splitless injection and dedicated on-column injection systems were employed for GC-MS analysis. The on-column injection is a much milder method of sample introduction. This technique, although it produced the most complex chromatograms, was best for the detection of the phenol containing components of the mixture which tended to be more thermally labile. No derivatization was performed on the analytes, therefore GC-MS analysis would only be expected to detect the volatile amines and alkaloids present in the sample.

The majority of the isolated alkaloids, 29 of the 44 identified, were related to the parent compound β-phenethylamine. These compounds generally varied in the degree of *N*-methylation, α-methylation (amphetamine family), and in oxygenation of the aromatic ring (tyramine, dopamine, and mescaline families). The 2-cyclohexylethylamine and the *N*-cyclohexylethyl-*N*-methylamine are the saturated analogs of the phenethylamine and NMPEA respectively. Tryptamine, *N*-methyltryptamine, and *N*,*N*-dimethyltryptamine were also isolated from blackbrush. Tryptamine and *N*,*N*-dimethyltryptamine were also detected in the related species guajillo [7], albeit at relatively low levels. Early season levels of tryptamine in *A. berlandieri* range from 90 to 124 ppb while late season levels range between 287 and 334 ppb. *N*,*N*-Dimethyltryptamine was only detected in late season guajillo at levels ranging between 75 and 115 ppb. It is interesting to note that a similar animal ataxia called "Phalaris staggers" occurs in Australia and is associated with consumption of various *Phalaris* species which are known to contain *N*,*N*-dimethyltryptamine [9]. Other noteworthy alkaloids found in blackbrush include nicotine, nornicotine, and four tetrahydroisoquinoline alkaloids, anhalamine, anhalidine, anhalonidine, and peyophorine. The amides of the amino acids pipecolic acid and *p*-hydroxypipecolic acid were also detected. It should be noted that no other amino acid esters or amides were detected in the extract.

Initial identification of the alkaloids present within the plant extracts was based upon library comparison of their MS fragmentation patterns with the final confirmation of identification made by direct comparison with spectra of authentic samples. Quantification of the levels of each compound was based upon standard curves generated with authentic samples. Leaves, petioles and unlignified stems were collected twice, a first growth sample collected early in the spring and a late season sample collected before frost in the autumn. As was previously found with NMPEA the foliage collected in the autumn contained higher quantities of amines and alkaloids [10]. There was also a distinct increase in the number and quantity of methylated analogs present (Table 1).

Several as yet unidentified amine-containing compounds have been detected but have not yet been identified. It is also probable that several biosynthetic precursors are present in the mixture but as yet are unresolved and are below the threshold of GC-MS detection.

Phenolic amines, as a group, impact the hypothalamic–pituitary–adrenal axis [11]. The consequent release of ACTH and cortisol results in symphathomimetic action. The number of phenolic amines reported in Table 1 and their concentrations in the plant indicate a substantial toxic load to animals consuming blackbrush. The toxicity of nicotine and nornicotine has been well established [12], as has the psychoactivity of mescaline and its derivatives. None of the compounds identified appear to have been implicated in locomotor ataxia. However the presence of the amphetamines suggests the possibility for a reduction of monoamine oxidase activity [13].

EXPERIMENTAL

GC-quadrupole MS (EIMS at 70 eV) were obtained on Hewlett Packard Model 5988A and Model 5970C systems each having both splitless heated injection port and dedicated on-column injection capabilities. Grade 0 He was used as the carrier gas and the transfer line was maintained at 280°. Heated injection conditions include: head pressure 5 psi; injection volume 1 μl; injection port 200°; splitless time 1 min; purge flow 60 ml/min; initial time 1 min; ramp 3°/min; final temp. 270°; final time 20 min. On-column injection conditions include: head pressure 5 psi; injection volume 1 μl; initial temp. 60°; initial time 1 min; ramp 1.5°/min; final temp. 270°; final time 35 min. WCOT cross-linked methyl silicone 12 (only used on HP 5970 system) and 36 m, 0.2 mm i.d., 33 μ coating in thickness capillary columns were used. Mass spectral data was collected as the total ion chromatograms in the operating range of 35–800 amu. The GC-MS operating system including the Wiley mass spectral library and the NBS mass spectral library of standards. Preliminary identification was made by library comparison, final identification was made by direct spectral comparison with the spectra of an authentic sample obtained from the GC-MS. Authentic samples were either purchased or prepared by known chemical procedures.

GC-MS quantification was made by using the area under a selected *m*/*z* peak for each compound and comparing this to a standard curve generated by injection of a series of known standards. Care was taken to make sure that the detector response was linear in the concentration range being run.

Collection of leaves and stems

Samples of *A. rigidula* Benth. were collected from plants growing on a southwest-facing slope in Zavala

Table 1. Amines and alkaloids from *A. ridigula* Benth.

	Early season (ppm)	Late season (ppm)
2-Cyclohexylethylamine	0.8	35.2
N-2-Cyclohexylethyl-*N*-methylamine	1.2	47.1
Phenethylamine	872.3	1135.7
N-Methylphenethylamine	2314.6	5264.8
N,*N*-Dimethylphenethylamine	123.6	724.5
Amphetamine	6.7	11.8
Methamphetamine	nd	12.4
N,*N*-Dimethyl-α-methylphenethylamine	57.6	394.2
p-Hydroxyamphetamine	2.1	6.9
p-methoxyamphetamine	nd	15.7
Tyramine	459.1	1699.2
N-Methyltyramine	237.4	1237.6
Hordenine (anhaline)	6.4	533.8
Dopamine	8.9	36.1
N-Methyldopamine	0.5	8.2
N,*N*-Dimethyldopamine	11.2	44.6
3-Methoxytyramine	1.8	12.9
N-Methyl-3-methoxytyramine	3.4	28.4
3-Hydroxy-4-methoxyphenethylamine	15.8	163.2
N-Methyl-3-hydroxy-4-methyoxyphenethylamine	19.2	184.7
3,4-Dimethoxyphenethylamine	1.3	6.5
N-Methyl-3,4-dimethoxyphenethylamine	7.6	28.3
3,4,5-Trihydroxyphenethylamine	1.6	12.4
N-Methyl-3,4,5-trihydroxyphenethylamine	0.3	1.9
Mescaline	3.4	27.5
N-Methylmescaline	1.8	35.3
Trichocereine	0.2	13.8
3,5-Dimethoxytyramine	1.6	21.6
3,4-Dimethoxy-5-hydroxy-β-phenethylamine	15.6	57.1
β-Methoxy-3,4-dihydroxy-5-methoxy-β-phenethylamine	4.6	22.1
3,4-Dimethoxy-α-methyl-5-hydroxy-β-phenethylamine	5.3	61.4
Tryptamine	0.8	21.2
N-Methyltryptamine	4.6	54.9
N,*N*-Dimethyltryptamine	323.8	568.4
Nicotine	45.8	152.4
Nornicotine	23.4	84.3
Anhalamine	9.6	48.7
Anhalidine (*N*-methylanhalamine)	5.6	51.2
Anhalonidine	2.3	15.7
Peyophorine	3.8	43.4
Pipecolamide	872.8	978.2
p-Hydroxypipecolamide	241.6	353.1
1,4-Benzezediamine	104.8	129.6
4-Methyl-2-pyridinamine	341.5	567.3

County, Texas. Early season collection was performed in the spring after vigorous new growth appeared. Late season collection was performed in the late fall prior to the first front and before color change was detected. Leaves, petioles, and attached tender stems were collected until ca 500 g of fr. wt had been gathered. The material was sealed in waterproof bags and placed on ice immediately. Samples were then frozen at -20° and stored until extraction. Voucher specimens were collected and stored at the Texas A & M University Agricultural Research and Extension Center, Uvalde, Texas.

Soxhlet extraction procedure

Still frozen *A. rigidula* was packed into Whatman single thickness cellulose extraction thimbles (ca 50 g/thimble, 200 g total) and extracted continuously for 24 h with MeOH. The MeOH was removed and replaced with $CHCl_3$ and the extraction continued for an additional 24 h. A pilot study established that the extracts could be safely concentrated by rotary evaporation (water aspirator). The MeOH extract was concentrated and the residue dissolved in 100 ml of $CHCl_3$. This solution was extracted $\times 3$ with 50 ml

portions of 10% aq. HCl. The acid fractions were combined, the pH adjusted to ca 10.3 by addition of aq. NaOH, and the resulting solution was first extracted $\times 3$ with 50 ml portions of $CHCl_3$ followed by extraction $\times 3$ with 50 ml portions of EtOAc. These organic extracts were combined, dried with $MgSO_4$, filtered, concentrated under vacuum, and stored under argon prior to analysis by GC-MS. The $CHCl_3$ fraction from the Soxhlet extraction was handled in an identical fashion to the MeOH extract with the exception that it was not first concentrated prior to aq. acid extraction. The MeOH and $CHCl_3$ extracts from the Soxhlet extraction were not combined.

Acid extraction of plant material

Acacia rigidula, 100 g, was placed into a 1 l Erlenmeyer flask and mixed with 500 ml of 10% aq. acid (HCl and HOAc were both used). The suspension was stirred under argon at 60° overnight. The darkened suspension was filtered through glass wool covered with a 2 cm bed of sand. The filtrate was extracted $\times 2$ with 100 ml portions of EtOAc followed by $\times 3$ extraction with 100 ml portions of $CHCl_3$. The pH of the filtrate was adjusted to ca 10 by addition of NaOH pellets. This solution was extracted $\times 3$ with 150 ml portions of $CHCl_3$ followed by extraction $\times 3$ with 150 ml portions of EtOAc. The organic extracts were combined, dried over $MgSO_4$, filtered, concentrated under vacuum and stored under argon prior to analysis by GC-MS.

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