ALKALOIDS OF Astragalus kahiricus DC. PLANT ROOTS

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By
I. I. Mohamed, H.E. Hassan*, S. A. Ahmed** and S. M. Bahaa

Medicinal and Aromatic Plants Department, Desert Research Center, El-Matareya, Cairo, Egypt.
*Department of Botany, Faculty of Science, El-Monofiya University, Egypt.
**Department of Applied Organic Chemistry, National Research Center, Giza, Egypt.

ABSTRACT
While looking for the toxic principle of Astragalus kahiricus DC., a herb highly toxic in livestock especially in small ruminants, hypaphorine, the N,N,N-trimethyl tryptophan betaine was isolated. This alkaloid was identified in Astragalus kahiricus, using NMR and MS methods. It was synthesized in sufficient amount and tested for potential toxicity in mice and lambs. In this study it is shown, with good evidence, that hypaphorine could not be held responsible for Astragalus kahiricus poisonings in small ruminants.

Key words: Astragalus kahiricus, hypaphorine, small ruminants, toxicity.

1. INTRODUCTION
Astragalus kahiricus (Fabaceae) is found in some Mediterranean countries, where it was reported to be toxic for livestock, especially in Spain, Portugal and North Africa (Rodriguez et al., 1990). In Egypt, this plant is frequently responsible of intoxication in sheep and goats, especially in young animals (Abdennebi et al., 1998).

The poisonous species of Astragalus may be classified, on the basis of their toxic principles, into three main groups: (a) The group of the species termed locoweeds which is known to contain the alkaloid swainsonine (Stegelmeier et al., 1995). (b) The group of those containing the nitro compound: miserotoxin (Williams and Norris, 1981). (c) The group of species that are selenium accumulators (Davis, 1972).

The toxic A. kahiricus in Egypt does not contain neither swainsonine nor miserotoxin, and contains only very low concentrations of selenium (Abdennebi and Lammaouer, 1999). However, the clinical signs and lesions produced in intoxicated animals are dominated by epileptic crisis and cytoplasmic vacuolization in brain and other tissues (Moyano et al., 1989), and are similar to those caused by locoweeds (James et al., 1970).

Knowing that swainsonine is highly water soluble, it was found in the aqueous extract of the plant compound, which gives with Erlich reagent a purple color on TLC like swainsonine. This compound was isolated and identified as hypaphorine. In the literature, this alkaloid was the subject of conflicting reports on its toxicity. On one hand it is considered as neurotoxic agent (Merck Index, 1976) and on the other hand it was reported to have only little pharmacological activity (Folkers and Koniuszy, 1939).

In order to verify if hypaphorine plays a role in the toxicity of Astragalus kahiricus, it was synthesized in sufficient amount and administered at relatively high doses to mice and lambs.

2. MATERIALS AND METHODS
2.1. Plant materials
Astragalus kahiricus was collected in December 2007 in the region of El Sheikh Zuwyid, North Sinai, Egypt. In this area, frequent animal poisoning, by this plant species, are recorded each year in sheep herds.

2.2. Isolation and purification of hypaphorine
One kg of fresh plant root material was macerated in 2 liters of water, under continuous shaking, for 24 h. The filtrate was evaporated under reduced pressure to yield 30 g of a dry extract. Six grams of the extract were dissolved in a small volume of water and chromatographed on reversed phase Silica gel RP2 (0.063-0.2 mm Merck), eluted
with water and water methanol mixture. Thirty 
fractions were obtained, and controlled using TLC 
under UV light and after spraying with the Ehrlich 
reagent. The fraction number 14 contains a major 
compound X, which shows a purple color with 
Ehrlich reagent. It was purified by preparative TLC 
using: Chloroform: methanol: ammonia (7/2.6/0.4) 
as a solvent system and identified as hypaphorine 
(21mg), which is colorless, mp 254-255 °C (Ghosal 
and Srivastava, 1974).

2.3. Synthesis of hypaphorine

Hypaphorine was synthesized according to the 
method of (Von Romburgh and Barger, 1911). The 
L(-)-S-tryptophane 2.04 g (1 mmol) was dissolved 
in 25 ml of methanol and added to a solution of 0.4 
g of sodium hydroxide in 2 ml of water. Then 8.52 g 
of methyl iodide (6 mmol) were added to the 
mixture under magnetic stirring at 20-25 °C. The 
solution was maintained alkaline, by addition of a 
small portion of sodium hydroxide (0.85 g) in 2 ml 
of water, during 24 h. After evaporation of methyl 
iodide (bp. 42.5°C), the mixture was heated at 65°C 
for 1h to hydrolyze the methyl ester to an acid. The 
solution was then acidified by addition of HCl 
(10%). The hypaphorine chloride precipitate was 
was washed with methanol, dissolved in methanol-
ammonia and then chromatographed on Sephadex 
LH20 (30 g), eluted with methanol. The crystals 
formed in the first fractions are pure hypaphorine 
(1.8g): mp 252-255°C. [α]D +140°C (C l, H2O), 
MS (Electro-spray): m/z 247 [MH]+, 1H and 
13C NMR (Table 1). These data agreed with those in the 
literature for this compound (Sarragiotto et 
al.,1981).

2.4. Animals

Three local breed lambs were used. They were 
purchased from the animal market and housed in a 
sheep box in the barn of the Faculty of Veterinary 
Medicine, Cairo University. They were fed good 
quality forage and water ad libitum. After one week 
of acclimatizing, they received one administration 
of hypaphorine solution in water (100 mg/ml) by 
oral route.

Sixteen week old balb-C mice were kindly 
provided by the Pharmacology Laboratory. They 
were housed 2 per cage in polyethylene cages and 
fed pellet food (Cicalim n°47) and water ad libitum. 
After 1 week of acclimatation they were given 
hypaphorine solution. The solution of hypaphorine 
administered to mice by ip injection was prepared at 
concentration of 25 mg/mL in PBS solutions and 
sterilized by filtration through a membrane (porosity 
0.22 µm) and administered by oral route. This was 
at a concentration of 100 mg/ml in tap water.

3. RESULTS AND DISCUSSION

3.1. Identification of the compound

The ESI-TOFMS spectrum of the compound X 
showed the protonated molecular ion [MH]+ at m/z 
247 suggesting formula C11H18N2O2 (M=246) in 
agreement with 13C NMR data (Table1). The J-
modulated 13C spectrum presented 9 CH or CH3 
carbons, one CH2 and 4 quaternary carbons.

Bidimensional NMR spectra 1H-13C HSQC and 1H-
13C COSY, assigned the 4 aromatic vicinal CH at 
ᵦ 7.50, 7.02, 7.05, and 7.35 of benzene ring, 3H in - 
CH2-CH- system at ᶦ 3.20 and 3.72 and 3CH3 in 
singulet at ᶦ 3.07. The HMBC spectrum showed 
long-range correlations of 3 quaternary sp2 carbons 
at ᶦ 108.96 with H11; 128.27 H10 and H-8 and 
138.04 with H-2, H-5 and H-7, indicating the 
presence of the indolic skeleton and one remaining 
quaternary carbon at ᶦ 171.62 assigned to carboxylic 
group.

Table (1): 1H and 13C NMR data of compound x (CD3OD).

<table>
<thead>
<tr>
<th>No</th>
<th>13C δ (ppm)</th>
<th>1H δ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>NH</td>
</tr>
<tr>
<td>2</td>
<td>125.11</td>
<td>CH</td>
</tr>
<tr>
<td>3</td>
<td>108.96</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
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<td>119.05</td>
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<tr>
<td>6</td>
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<td>CH</td>
</tr>
<tr>
<td>7</td>
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<td>CH</td>
</tr>
<tr>
<td>8</td>
<td>112.45</td>
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<tr>
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<td>C</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>11</td>
<td>80.56</td>
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</tr>
<tr>
<td>12</td>
<td>171.62</td>
<td>COO-</td>
</tr>
<tr>
<td>13</td>
<td>52.66</td>
<td>N(CH3)3</td>
</tr>
</tbody>
</table>

The structure of compound X was thus identified 
to that of hypaphorine, N,N, N-trimethyl 
tryptophanium betaine (Figure 1).

3.2. In vivo toxicity assay

The ip administration of hypaphorine to mice at 
doses of 0.5 and 1 g /kg body wt (Table 2), did not 
produce any sign of toxicity in animals. They 
tolerated even high doses of 2 g/kg by oral route 
without any apparent toxic effect and survived until
they were given an euthanasia solution 3 months later.

**Fig. (1): Structure of Hypaphorine.**

<table>
<thead>
<tr>
<th>Lamb ID #</th>
<th>Body weight (Kg)</th>
<th>Hypaphorine Dose (g/Kg)</th>
<th>Total amount administered (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1.5</td>
<td>9</td>
</tr>
</tbody>
</table>

Table (2): Toxicity assay in mice of hypaphorine solution by ip and oral routes.

Table (3): Toxicity assay in lambs of hypaphorine solution by oral route.
It should be noted that a derivative of hypaphorine, the methyl á-dimethylamino-á-(3-indole)-propionate methiodide has an LD50 of 450 mg/kg in mice (Folkers and Unna, 1938).

On the basis of this historical data, it was decided to test hypaphorine in mice by injection of high doses of 500 mg/kg up to 2000 mg/kg. No toxic effects were observed.

Being aware of any species response variation, hypaphorine was also tested in lambs. Despite the oral administration of relatively high doses of pure alkaloid (0.5; 1 and 1.5 g/kg) compared to lethal dose of the whole plant (5 g/kg dry weight) (Abdennebi et al., 1998), these doses didn't produce any toxic effect. Thus, by testing hypaphorine in lambs, the most sensitive animals to A. lusitanicus poisoning, in the field and experimental conditions, this study presents good evidence that hypaphorine could not be responsible for the toxicity of this plant species. Moreover, it can be suggested that the affirmation saying that hypaphorine is a convulsive agent, as it is stated in the Merck Index (1976), should be reconsidered. It may also be concluded that the confusion about the toxicity of hypaphorine came from the fact that hypaphorine part in erysophorine and erysodinophorine molecules (Figure 2), makes these alkaloids more water soluble and thus enhances their curare-like activity in the frogs.

However, free hypaphorine produced no toxicity, whereas the other parts of erysophorine and erysodinophorine were toxic. Thus, the results showing that hypaphorine has no toxic effect in mice and lambs are in agreement with the literature data.

**Fig. (2): Some toxic alkaloids**

4. REFERENCES


Alkaloids of Astragalus Kahiricus DC……………………………………………………………………………………………

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Astragalus kahiricus

قلويات جذور نبات

إيناس إبراهيم محمد حسن الطنطاوي حسن* - سيد عبد الحميد أحمد** - سارة محمد بهاء الدين***

مركز بحوث الصحراء - القاهرة - * قسم النبات - كلية العلوم - جامعة المنوفية

قسم كيمياء المواد الدابغة - المركز القومي للبحوث – الدقي – الجيزة**

ملخص

نبات خالي السمية في الثروة الحيوانية وخاصة في الحيوانات المجترة الصغيرة. تم عزل مادة الهيبافورين وهى عبارة عن N-N-trimethyl tryptophan betaine وتم التعرف على هذا المركب القلويدي بطرق التحليل الطيفي المختلفة مثل الرنين المغناطيسي وطيف الكتلة لتحديد ماهية التركيب وقد تم تحليل هذه المادة كمية كافية واختبار سميتها في الفئران والحملان. أظهرت هذه الدراسة أن مادة الهيبافورين في نبات Astragalus kahiricus لايمكن أن تكون مسئولة عن حالات التسمم في الحيوانات المجترة الصغيرة.