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Extraction and Identification of Alkaloids of the *Ipomoea fistulosa* (Aguapei or Mandiyurá) of Argentina

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Abstract: The classical toxic effect produced by this *Ipomoea fistulosa* is due to the accumulation of oligosacharides in various tissues cell cytoplasm, mainly nervous, liver and lymphatic tissues, that leads to the cell vacuolitation. These effects are attributed to the presence of substances called swansonina and calisteginas in the vegetables, which produce inhibition of enzymes liposomales responsible for the metabolic carbohydrates. In natural conditions, the plant is not consumed by the animals. Its consumption occurs alone in determined times of year, for lack of sufficient pasture and it is more frequent in young animals. Since standard literature does not register any data on the chemical analysis of the kind *Ipomoea*, we report here the results on the extraction, isolation and spectroscopic identification of the present alkaloids in this plant.

Key words: Alkaloids, *Ipomoea fistulosa*, IR spectrum

Introduction

The intoxication due to the consumption of toxic plants in animals of different species represents a constant economic risk for the people devoted to the production of agricultural goods and cattle raising tasks. The toxicity of a plant changes according to several factors: vegetable species and variety, time of year, phase of growth, type of ground where it grows, fertilisations, use of herbicides, etc *Ipomoea fistulosa* is a plant that belongs to the *Convolvulaceae* family, which is conformed by a large number of species. It is world-wide distributed, being found in Latin America from Colombia to Paraguay, Bolivia and Argentina. It is also found in Africa (de Balogh *et al*., 1999) and India (Nath and Pathak, 1995). In Argentina 6 species have been identified in the northeast region and *Ipomoea fistulosa* is a very abundant species. It is known in this region with the vulgar names of aguapié or mandiyurá (O'Donell, 1959). In Brazil, *I. fistulosa* is known as algodao brave, manjorana or canudo (Lorenzi, 1991). The same species in India produces allergic reactions in human beings, since its pollen constitutes a powerful allergen (Amal *et al*., 1998).

At the north zone of Argentina the Ipomea genus is found in a vast portion of the region but up to now the basic toxic components of this genus have not been studied in detail. Then, people do not know the corresponding risks when bovine cattle and other animals feed themselves when there is scarcity of pastures, giving rise to an economical expense for the bovine cattle producer.

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The mandiyurá o aguapié is a plant that is found in large groups at the rivers and lake shores. It is a bush with a height up to 3 m, few ramified; thick stems, fistulouses finely striated. Its flowers are light violet coloured, of great size and with a shape of bellflower, grouped in bunches of 2 or 3 units, with very bright colours. Their leaves are intense green coloured, in the shape of an arrowtip. The fruit is a capsule that is opened spontaneously when it is nature, it possesses few seeds, covered with silky, long hairs, that facilitate their dispersion by the wind (Austin, 1977).

The polyhydroxy alkaloids were isolated from the leaves, flowers and seeds of the *Ipomoea carnea* and characterized by gas chromatography-mass spectrometry. Affected animals were ataxic, with head tremors and nystagmus (de Balogh *et al*., 1999). The leaves were soaked in methanol for 16 h in Soxhet apparatus and the alkaloids fraction was purified by ion-exchange chromatography. Cromatographic separation of the leaf extract resulted in the isolation of swansonine, 2-epi-lentiginosine, calystegine B_1, B_2, B_3 and C_1 and N-methyl-trans-4-hydroxy-1-proline (de Balogh *et al*., 1999; Haraguchi *et al*., 2003). The swansonine y calystegine B_2 have been identified as constituents of the seeds from *Ipomoea calobra* by gas chromatography-mass spectrometry (Molyneux *et al*., 1995).

Swansonina is a potent inhibitor of the α-mannosidase, whereas calystegine B1, B2 and C1 are potent inhibitors of the β-glucosidase (Ikeda *et al*., 2003).

Ipomea species produces a neurological disorder when consumed by livestock (Molyneux *et al*., 1995). Different organs from envenoming livestock were histolgically characterized by vacuolated cells (Hueza *et al*., 2005).

Chronic exposure of livestock to *Ipomoea carnea* is clinically manifested by CNS signs, abnormal endocrine and gastrointestinal functions, alterations of the immune system and abnormal embryogenesis (Hueza *et al*., 2003).

In natural conditions, the plant is not consumed by the animals. Its consumption occurs alone in determined times of year, for the lack of sufficient pasture, being this more frequent in young animals (Méndez and Rief-Correa, 2000).

The continuous Soxhlet extractive method employed by authors is a method based on the decomposition of the alkaloid pyrrolidinic rings, so that we propose a methodology that improves the yielding without producing the decomposition of the alkaloids.

In Argentina, there is no data on the chemical analysis of the kind *Ipomoea*, so the purpose of this work is to report the experimental results on the extraction, isolation and identification of the present alkaloids in this plant. The last task has been accomplished via an IR spectroscopic study in order to give the main vibrational mode assignments.

The characterization of the components of *Ipomea fistulosa* in corrientes is very essential because determine the actual cause the dead of goat.

Materials and Methods

Extraction and Isolation

The *Ipomea fistulosa* leaves were collected from the cultivation made in the University Campus of the North-Est National University during Autumn. The species identification was made by technical people belonging the the Ivonne Institute, located at the Agrary Sciences Faculty belonging to this University.

The vegetable material was dried in stove to 60°C to constant weight and then it was pulverised and sifting to obtain a constant granulometry (sieve 30-40) degree. We weighed 2 g of vegetable material (leaves), we placed them in an Erlenmeyer and we added 100 mL of the solvent sulfuric ether. Then, we alkalinised with 10% ammonia (NH_3) leaving the mixture in soaking for 12 to 24 h and agitating it occasionally. After this time, we filtered the sample by a vacuum equipment and we placed it in a blister of decanting to carry out a series of washes with 2% sulfuric acid (H_2SO_4).

Once the acid solution was obtained, we alkalinised with 10% ammonia (NH_3). The final task was to add ether to obtain the organic solvent on the one hand and the acid solution on the other. Finally, we left to evaporate the solvent organic, precipitating the alkaloid that was dried and purified.

After obtaining the mixture of alkaloids, we proceeded to its identification by infrared spectrophotometry, using an infrared spectrum trade mark Nicolet. The IR spectrum was determined among the range 400-4000 cm^{-1}, using the technique of diffuse refractancy with BrK as solvent.

Results and Discussion

In our investigation aerial parts of *Ipomea fistulosa* were extracted as already described (Wiedenfeld and Roeder, 1979). From the crude alkaloidal extracts the swansonine, calystegine B1, B2 and C1 were isolated (Fig. 1).

The alkaloids content of *Ipomea fistulosa* collected from differents regions is the same in all the sample plants. References do not exist in our country about the components of the *Ipomea fistulosa* nor of its danger or toxicity, either to the human or the cattle. In this work it was possible to isolate in the extracts, the alkaloids swansonine, calystegine B_1, B_2 and C_1 that were identified in other species of Ipomea, in other countries. This important information will put in alert the rural producer to eradicate these weeds in the pasture.

Vibrational Analysis

In Fig. 2 we display the IR spectrum obtained experimentally for the extracted sample. In the analyzed sample, it contains all the alkaloids. In the first stage of this study it was not possible to proceed to the separation of each one of them. In Table 1, we present experimental IR spectra as well the corresponding assignment of the different vibrational modes of the extracted sample.

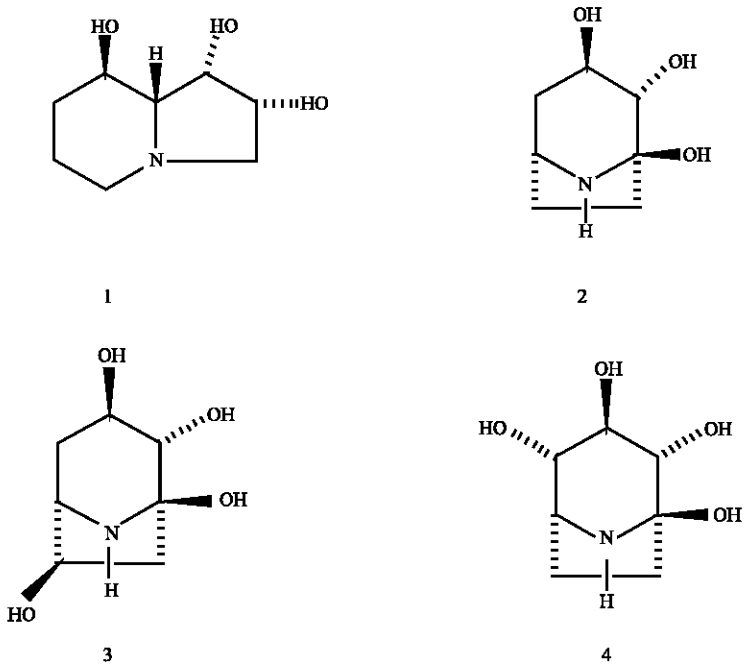

Fig. 1: Structure of alkaloids present in *Ipomea fistyulosa*: Swainsonine (1); Calistegines A_3 (2), B_1 (3), B_2 (4)

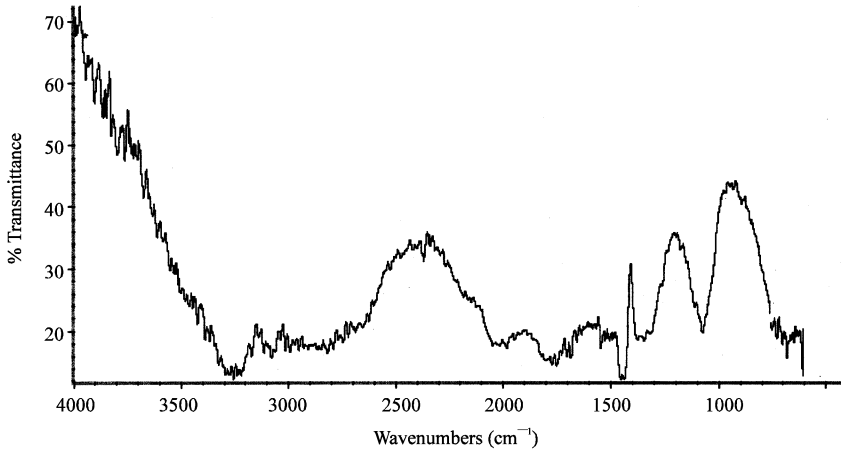

Fig. 2: Vibrational spectrum of the extracted sample

Table 1: Experimental vibrational frequencies and vibrational assignments

Frequency	Assignment
602	Sym ring def. Piridine
649	Bending out of plane OH (H bond)
664.44	Wagging of NH
702.30	Bending out of plane ring of tetra substitute piridine tetrasustituido, bending out of plane of CH
706.68	Def out of plane of ring trisubstitute piridine
714.07	Bending of ring trisubstitute piridine Rocking CH_2
752.83	Bending sec amine NH bending ring pyrrol bending out of plane of CH and OH
1320	Streching terc CN
1428.18	Bending in plane OH streching ring of piridine and pyrrol
1432.16	streching ring of piridine and pyrrol
1448.60	Def CH_2
1453.93	Def CH_2 Bending CH_2
1458.77	Def CH_2
1465.05	Def CH_2 Bending CH_2
1473	Def CH_2
1506	bend NH (sec amine)
1545	bend de acoplamiento NH, Stretching CN
1634.91	Streching ring of piridine and pyrrol Bending NH
2847.95	Asym stretching CH_2
2816.23	Asym stretching CH_2
2993.81	Stretching CH_2, stretching CH
3015.09	Stretching CH
3059.11	Stretching CH
3066.48	Stretching CH
3074.66	Stretching CH
3078.47	Stretching CH
3113.72	Stretching CH stretching CH_2
3219.84	Stretching NH
3222.18	Stretching NH
3222.24	Stretching NH
3291.04	Sym stretching NH
3332.93	Stretching OH
3363.23	Asym stretching NH

The 3363.23 cm^{-1} mode corresponds to the asymmetric stretching of NH of a secondary amine and the 3332.93 cm^{-1} band corresponds the stretching of the OH group. Bands between 3291.04 and 3219.84 cm^{-1} correspond to symmetrical stretching NH of a secondary amine.

In the range of 3215.03-2999.81 cm^{-1} we have the asymmetric stretching of the CH_2. In the range 3113.72-2993.81 cm^{-1} appears the asymmetric stretching of the CH group. The band 2847.95 cm^{-1} corresponds to the symmetrical stretching of CH_2 group.

The asymmetric stretching of the ring of heterocyclic, pyridine and pyrrol appears at 1634.91 cm^{-1}. The coupling between the NH and the OH is located at 1545.09 cm^{-1}, like the stretching of CN group belonging to a secondary amine.

Bands at 1448.60-1473.32 cm^{-1} are assigned to CH bending and deformation of the CH_2 group. Band at 1320 cm^{-1} corresponds to the stretching of the tertiary amine. The symmetrical stretching of the ring of heterocyclic, pyridine and pyrrol appears at 1432.16 cm^{-1}. Band at 1428.18 corresponds to the bending (deformation) in the plane of the OH group.

Within the range between 752.83-702.30 cm^{-1} appear the characteristic groups enabling to identify to the alkaloids and they are deformation of the ring of pyrrol, deformation of the pyridine ring tetra and trisubstituted and deformation out the plane of CH and OH groups. Band at 664.44 cm^{-1} corresponds to the wagging of the NH group and the band appearing at 649.19 cm^{-1} corresponds to the deformation out the plane of the OH group.

Conclusions

Experimentally, we can conclude that we have found the alkaloids Calistegina A_3, B_1 and B_2 and Swansonina, using the method of extraction by soaking. Besides, we have determined the IR spectrum of the extracted sample and we have presented an assignment of the vibrational normal modes. In a second phase of this study, the four alkaloids will be separated by chromatography in column. Besides, each of them will be suitably characterized. Results will be presented elsewhere in the forthcoming future.

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