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Isolation and characterization of sodium 2-fluoroacetate from *Mascagnia rigida* using chromatography and infrared spectroscopy

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ABSTRACT

Sodium monofluoroacetate was first identified in *Dichapetalum cymosum*, a South African plant that can cause livestock poisoning and death. After, several other plants also showed to contain this toxin, which leads to the "sudden death". *Mascagnia rigida*, a well identified poisonous plant, commonly found in northeast of Brazil also cause sudden death in cattle, which shows clinical signs similar to those produced by the ingestion of plants that contain monofluoroacetate. Our aim was to identify the toxic compound present in the aqueous extract of *M. rigida*. For this purpose, the dried and milled plant was extracted; the extract was lyophilized and submitted to successive chromatographic process, until the desired purity of the active compound was achieved. The study of this material by planar chromatography and by infrared spectrometry indicated that the toxin can be a mixture of mono, di and trifluoroacetate.

1. Introduction

Since 1961, naturally occurring cases of poisoning in cattle, sheep and goats, by *Mascagnia rigida*, (Malpighiaceae) were reported throughout the Brazilian northeastern and southeastern regions. This plant, also known as "tingui", was firstly found in the municipalities of the States of Pará and Ceará, (Tokarnia et al., 1961) and later in the north of the State of Espírito Santo (Tokarnia et al., 1985), northeast of Minas Gerais State (Tokarnia et al., 2000), State of Rio Grande do Norte (Pacífico da Silva et al., 2008), and in many different regions in the State of Paraíba (Medeiros et al., 2002; Vasconcelos et al., 2008a, b).

In spontaneous outbreaks caused by *M. rigida*, clinical signs were reluctance to move, respiratory distress, muscular tremors, and tachycardia and were observed only when the animals were exercised. The more affected animals, if forced to walk, stagger and fall into convulsions, with death occurring 1 min to 2 h after the first manifestation of poisoning, whereas the less severely affected ones, if not moved, can recover. Histological observation of kidney tissue showed hydropic-vacuolar degeneration (Gava et al., 1998). Therefore, considering the clinical and histopathological characteristics of *M. rigida*, it is classified as belonging to the group of "Plants that cause sudden death", which is responsible for about 60% of cattle losses due to toxic plants, in Brazil (Medeiros et al., 2002).

About 40 species of plants that produce sudden death are spread mainly over the tropical, sub-tropical, and temperate regions of the world like Australia, South Africa and South America (Twigg et al., 1996). It is well established that monofluoroacetic acid or sodium monofluoroacetate

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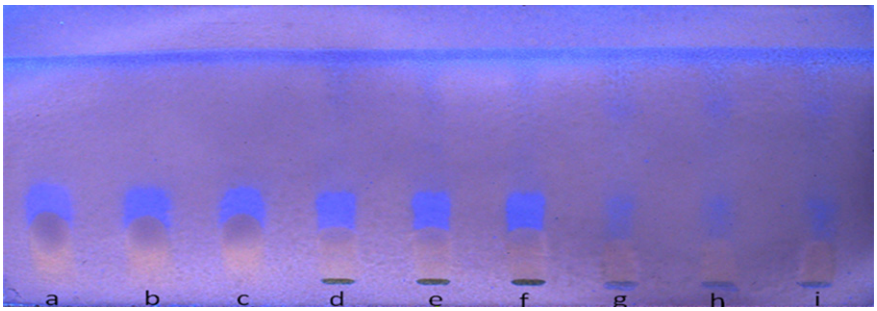

Fig. 1. Cellulose plate chromatogram of: a, b, c – SMFA standard; d, e, f –*Mascagnia rigida* aqueous extract; g, h, i –*Palicourea marcgravii* aqueous extract. Mobile phase: ethanol/ammonium hydroxide/pyridine/water (95:3:1:1, v/v/v/v); derivatization reagent: Nile Blue.

(SMFA) is the toxic principle of the *Dichapetalum cymosum* (Marais, 1944), and the Australian plants *Acacia georginae*, *Oxylobium parviflarum* and *Gastrolobium grandiflorum* (Aplin et al., 1983); on the other hand, considering plants from Brazil that cause sudden death, it was identified this substance as the toxic principle only in *Palicourea marcgravii* (Oliveira, 1963; de-Moraes-Moreau et al., 1995) and *Arrabidaea bilabiata* (Krebs et al., 1994).

SMFA is also a pesticide; thus, it has been used also as a rodenticide since 1940's in many countries such as United States, Australia and New Zealand (Beasley, 2002), leading to many cases of accidental poisoning in man and pets (Harris, 1975; Eason, 2002).

The mechanism of action of SMFA is well known, hence, this compound requires the transformation into fluorocitrate within cell mitochondria, which blocks the action of the enzyme aconitase, interrupting the Krebs cycle and ATP production in the intoxicated organism leading to the "lethal synthesis" (Peters, 1963). Thus, this interruption in ATP production results in strong toxic effect on the functioning of the heart and the central nervous system. A predominance of cardiac or nervous symptoms depends on the animal species affected, thus in cattle predominantly affects heart (Tokarnia et al., 2002), while in dogs the SNC is the main system affected (Goh et al., 2005).

Taking into account that animals intoxicated by *M. rigida* presents the same clinical symptoms described by plants that contain SMFA and previous studies conducted in our laboratory revealed positive results of a prior planar chromatographic study, comparing crude *M. rigida* samples with a SMFA standard, the aim of the current study was to confirm the identity of *M. rigida*'s toxic principle, by isolating, purifying and chemically characterizing this active compound.

2. Material and methods

2.1. Plant material

M. rigida was collected in Cabeceiras, Paraíba, Brazil and authenticated (voucher specimen # HCSTR 1294) and deposited in the Centro de Saúde e Tecnologia Rural Herbarium – CSTR.

SMFA standard was of analytical grade, Pestanal®, 99,7% and was obtained from Sigma. Microcrystalline cellulose plates 20 × 20 cm, analytical grade solvents, and reagents were from Merck®. XAD-2 was treated prior to use according to manufacture's instructions, and was dry-packed into a glass column (inner diameter 2.5 mm, height 30 cm).

2.2. Plant extraction and characterization of toxic compound by planar chromatographic

The air-dried plant (200 g) was ground in a Marconi MA 048 mill and extracted with deionized water (4x). The extract was filtered, frozen and lyophilized; the lyophilized powder was suspended in milli-Q water (w/v 1:1) before submitting to comparative planar chromatography in cellulose plates for investigating the presence of SMFA. Ethanol/ammonium hydroxide/pyridine/water (95:3:1:1, v/v/v/v) were employed as the mobile phase and Nile Blue as a post-derivatization agent (Vickery et al., 1973). All planar chromatographic posterior analyses were developed under the conditions described here. As a chemical proof, it was used *P. marcgravii* aqueous extract, obtained in the same way as the *M. rigida* extract.

2.3. Isolation of toxic principle

In sequence, this extract (13 g) was loaded into a XAD-2 column and then subjected to gradient elution with solvents of decreasing polarity: H_2O (**F1**); H_2O/MeOH (80:20, v:v) (**F2**); H_2O/MeOH (40:60, v:v) (**F3**); and MeOH 100% (**F4**). **F1** was lyophilized and the others fractions (**F2** – **F4**) were concentrated under vacuum until complete elimination of the organic solvent and subsequently lyophilized. All dried-fractions plus an authentic sodium 2-fluoroacetate sample were analyzed by planar chromatography. The band at the same R_f as the standard SMFA was extracted with deionized water, lyophilized, and chromatographically compared to the standard SMFA.

2.4. Infrared spectroscopy

Powder infrared spectra of the lyophilized extracted band and of the SMFA standard were recorded at temperature room, over the 50- to 4000 cm^{-1} range, on a Perkin Elmer FT-IR Spectrum™ Spectrophotometer, equipped with a KBr beam splitter and a standard DTGS detector. Both simplex were prepared as KBr pellets.