



Alkaloids and volatile constituents from the stem of *Fusaea longifolia* (Aubl.) Saff. (Annonaceae)

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ABSTRACT: A phytochemical study of the ethanol extract and an extraction of the volatile compounds, performed by means of Clevenger apparatus were carried out with the stem of *Fusaea longifolia* (Aubl.) Saff. (Annonaceae). The ethanol extract yielded *O*-methylmoschatoline, isolated for the first time in this species, and stepholidine, reported for the first time in genus *Fusaea*. The structural identification of the alkaloids was made based on the analysis of their NMR spectra. Through the use of GC and GC-MS, two sesquiterpenoids, α -cadinol (12.5%) and spatulenol (12.0%) were identified as the major constituents of the essential oil.

Keywords: *Fusaea longifolia*, Annonaceae, alkaloids, volatile constituents.

INTRODUCTION

The family Annonaceae, created by Jussieu in 1789 (Hutchinson, 1973), is comprised by 2.300 species distributed in approximately 130 pantropical genera (Maas et al., 2001). The plants of this family are trees and some times climbing plants are also possible to be found (Hegnauer, 1964). Besides their fruits being most of them edible, the plants are also very used in the folk medicine (Correa, 1984).

Circa 170 alkaloids of the types isoquinoline, protoberberine, aporphine and others were isolated from different genera of the Annonaceae (Leboeuf et al., 1982). From the genus *Fusaea*, comprised by four species commonly found in the Amazon region (Brummitt, 1992), alkaloids of the types aporphine and oxoaporphine were isolated. The species *Fusaea longifolia* (synonyms: *Annona longifolia* Aubl., *A. rhombipetala* Ruiz & Pav. ex G. Don, *Duguetia longifolia* (Aubl.) Baill., *Fusaea decurrens* R. E. Fr.), popularly known in Northern Brazil as "envira", "envireira" is a bush or small tree found in the Brazilian states of Acre, Amapá, Amazonas, Maranhão, Mato Grosso and Pará (Chatrou, 1998). A previous phytochemical study with this species reported the occurrence of a mixture constituted by β -sitosterol and stigmasterol and the alkaloids fuseine and liriodenine (Braz-Filho et al., 1976). The present study reports the isolation of the alkaloids stepholidine (**1**) and *O*-methylmoschatoline (**2**), as well as the analysis of the composition of the essential oil from the stem of this species.

MATERIAL AND METHODS

Plant material

The botanic material was collected in the Mocambo Reserve in the city of Belém, state of Pará, Brazil August 2002. It was identified by Jorge Oliveira, from the Museu Paraense Emilio Goeldi. A voucher specimen is deposited at the Herbarium (MPEG) in Belém, Pará, Brasil under the number 165435.

Extraction and isolation of the alkaloids

The stem was dried in an oven with circulating air at the temperature of 40 °C for three days. Afterwards it was grounded and yielded 1800 g of the stem powder. This material was humidified with 5% NH₄OH and subjected to extraction by maceration with 95% ethanol for 72 hours. The extractive solution was concentrated under vacuum yielding 200 g of the crude ethanol extract (CEE). The CEE was treated under mechanical stirring with 3% HCl and filtered over Celite. The filtrate (acid solution) was made alkaline with NH₄OH (to pH 9-10) and extracted with CHCl₃ until obtaining a negative result with Dragendorff's reagent. The chloroform phase after concentration under vacuum yielded a residue weighing 2.0 g which was called Total Tertiary Alkaloids Fraction (TTAF). TTAF was fractionated on a chromatography column over silica gel using hexane, CHCl₃ and methanol, pure or as binary mixtures in crescent polarity gradient as eluents. From this procedure stepholidine (0.00034%) (**1**) and *O*-methylmoschatoline (0.00080%) (**2**) were obtained (Figure 1).

Table 1. NMR data (^1H : 200 MHz; ^{13}C : 50 MHz, CDCl_3 , δ) of the alkaloids **1** and **2**

Carbons	Alkaloids			
	$\delta_{\text{C 1}}$	$\delta_{\text{C 2}}$	$\delta_{\text{H 1}}$	$\delta_{\text{H 2}}$
C				
1	-	156.6	-	-
2	145.0	147.5	-	-
3	143.8	148.6	-	-
3a	-	131.3	-	-
4a	130.5	-	-	-
6a	-	145.7	-	-
7	-	182.4	-	-
7a	-	131.6	-	-
8a	128.1	-	-	-
9	141.4	-	-	-
10	143.9	-	-	-
11a	-	134.7	-	-
11b	-	115.9	-	-
11c	-	123.0	-	-
12a	121.1	-	-	-
13b	126.0	-	-	-
CH				
1	110.5	-	6.80 (s)	-
4	111.4	119.3	6.57 (s)	8.21 (d, $J = 5.2$ Hz)
5	-	144.8	-	8.99 (d, $J = 5.2$ Hz)
8	-	129.1	-	8.56 (dd, $J = 7.7; 1.6$ Hz)
9	-	128.4	-	7.55 (dt, $J = 7.7; 0.8$ Hz)
10	-	134.5	-	7.76 (dt, $J = 7.7; 2.8$ Hz)
11	108.9	127.8	6.70 (d, $J = 8.4$ Hz)	9.12 (dd, $J = 7.7; 0.6$ Hz)
12	119.3	-	6.62 (d, $J = 8.4$ Hz)	-
13a	59.1	-	3.25 (m)	-
CH₂				
5	29.1	-	3.15; 2.58 (m)	-
6	51.5	-	3.17; 2.65 (m)	-
8	53.5	-	4.20; 3.48 (d, $J = 15.6$ Hz)	-
13	36.2	-	3.47; 2.57 (m)	-
OCH₃				
1	-	61.2	-	4.02 (s)
2	-	61.6	-	4.09 (s)
3	56.1	62.0	3.85 (s)	4.12 (s)
9	55.0	-	3.84 (s)	-

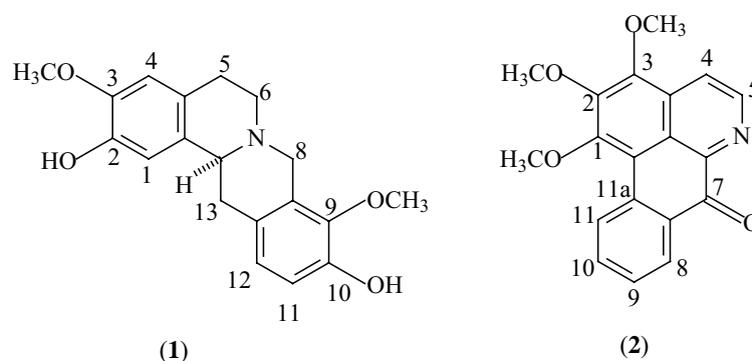


Figure 1. Alkaloids isolated from *Fusaea longifolia*

Extraction and analysis of the essential oil

The essential oil was extracted by distillation in a Clevenger-type (Andrade et al., 2003), and analyzed by GC and GC-MS. The volatile components were identified in accordance with their retention indexes (Kovats index) and their mass spectra in comparison with the data bank in the system library (Adams, 2001).

RESULTS AND DISCUSSION

The ^{13}C -APT NMR (50 MHz) obtained in CDCl_3 of **1** showed a total of 19 signals which led to the recognition of the hydrogenation pattern of each carbon atom (8 non hydrogenated, 4 methylene, 5 methyne and 2 methoxy). These data suggest a carbon skeleton of a tetrahydroprotoberberine type alkaloid. The ^1H NMR (200 MHz) obtained in CDCl_3 showed a signal at δ_{H} 4.20 compatible with the equatorial hydrogen H-8 of this type of alkaloids. All the NMR data in comparison with those found in the literature (Brakuni; Gupta, 1975) led to the conclusion that the substance was indeed stepholidine, which is being reported here for the first time in the genus *Fusaea*. The complete ^1H and ^{13}C NMR data of stepholidine can be found in Table 1.

The ^{13}C -APT NMR (50 MHz) of **2** obtained in CDCl_3 showed a total of total de 19 signals. Nine were attributed to non hydrogenated carbons, 6 to methyne carbons, 3 to methoxy groups and one at δ_{C} 182.4 attributed to a carbonyl carbon. This hydrogenation pattern is compatible with an oxoaporphine type alkaloid. The ^1H NMR spectrum (200 MHz) obtained in CDCl_3 showed a signal at δ_{H} 9.12, which agrees with the literature data for the hydrogen H-11 of this type of alkaloids. These data in comparison with the ones from literature (Harrigan et al., 1994), allowed to propose that substance 2 was *O*-methylmoschatoline, which is being reported here for the first time The complete NMR data of *O*-methylmoschatoline can be found in Table 1.

The GC-MS analysis of the essential oil obtained

from the stem of *F. longifolia*, revealed that its main components were sesquiterpenes: [α -cadinol (12.5 %), spathulenol (12.0 %), δ -elemene (4.2 %), β -elemene (3.8 %), germacrene D (3.7 %) and germacrene B (2.9 %)].

The results confirm that *Fusaea longifolia* is a typical member of the family Annonaceae (Jumana et al., 2000) and show relative chemotaxonomic significance for the genus *Fusaea*. Since it is the first time that the alkaloid stepholidine is being reported as isolated from one of its plants.

ACKNOWLEDGEMENTS

The authors are grateful to Vicente rlos de Oliveira Costa, Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba for acquisition of the NMR spectra and to CNPq for the financial support. Sincere thanks are also due to NAPRALERT.

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