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NMR analysis of a new canthin-6-one alkaloid from *Simaba bahiensis* (Simaroubacae)

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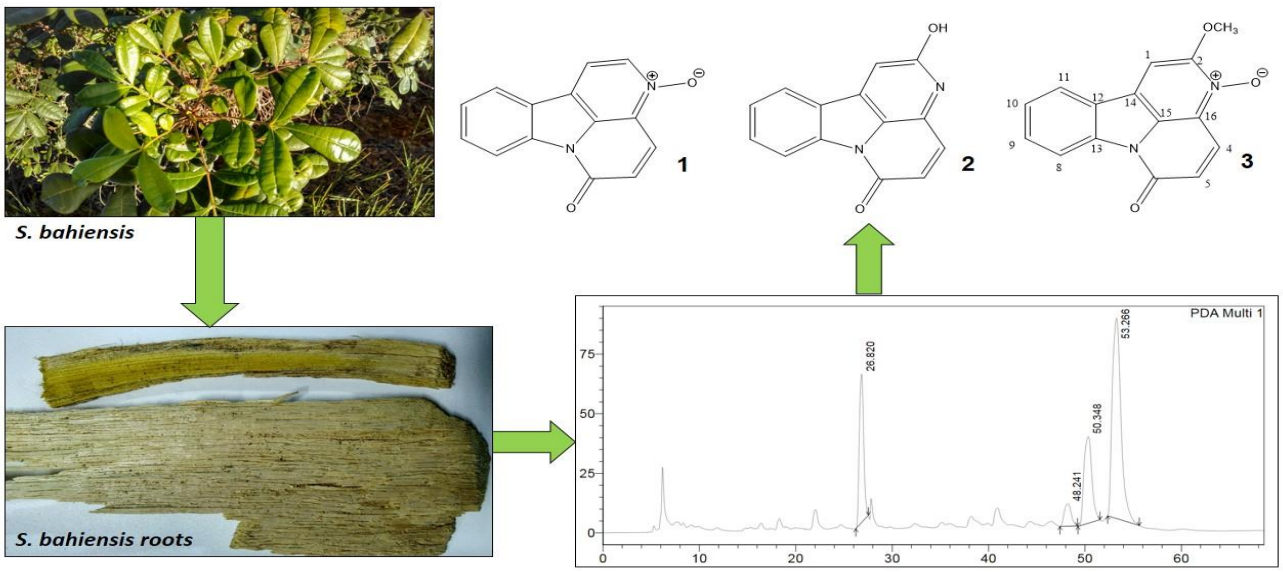
Graphical Abstract



Abstract.

Alkaloids of the canthi-6-one type have been reported in differents natural sources. These alkaloids have showed a wide range of pharmacological properties including cytotoxic, antibacterial, antifungal, antiparasict, antiviral, anti-inflammatory and beside it, some of them show excellent photophysical properties that gives an interesting use as fluorescent dye probe in florescent cellular microscopy. Because it, new studies have been carrying out in an attempt to identify new alkaloids with pharmacological properties. Some researchers have been trying to synthesize new derivatives or identify new compounds in natural sources. Among the >60 canthin-6-one alkaloids already reported in natural sources, more the half are present in plants of the family Simaroubaceae. In the *Simaba* genus, up to the present, only eighteen alkaloids was described in seven species, but other plants of the genus that occur in the brazilian's flora haven't been studied yet. In this work we describe the first phytochemical study of the specie *Simaba bahiensis* (Simaroubaceae), collected at the city of Camaçari, Bahia State, Brazil. Also, it's related complete structural determination using differents NMR experiments and HRMS of a new canthin-6-one alkaloid in addition to two others already known

Introduction

Canthi-6-one alkaloids occur in plants from families Rutaceae, Simaroubareace, Amaranthaceae, Caryophyllaceae and Zygophylaceae [1-3]. More than 60 members of this type of alkaloid were isolated from natural sources since first report in 1952 [4]. These alkaloids have shown a wide range of pharmacological properties including cytotoxic, antibacterial, antifungal, antiparasict, antiviral, anti-inflammatory and beside it, some of them show interesting photophysical properties that gives an interesting use the fluorescent dye probe in florescent cellular microscopy. New studies have been carring out in an attempt to identify new alkaloids with pharmacological properties by synthesis or isolation from different natural sources [2]. In family Simaroubaceae more than 30 canthi-6-one alkaloids have already been described with different substitution patterns and it shows that the investigation of new alkaloids in this family it's an important way to search new chemical entities [2, 3]. In this work we describe the first phytochemical study of *Simaba bahiensis* (Simaroubaceae), collected at the city of Camaçari, Bahia State, Brazil. Also, it's related complete structural determination using differents NMR experiments and HRMS of a new canthin-6-one alkaloid and two others already known.

Materials and Methods

Roots of *S. bahiensis* was collected in Arembepe, Bahia State, Brazil (S lat: -12.800833 long: -38.214444 WGS84 alt. 10m). Professor MSc Maria Lenise Silva Guedes performed the botanical identification and a voucher was deposited at Herbário Alexandre Leal Costa (ALCB) in Biology Institute of Federal University of Bahia with identification 1235018a.

The yellower parts of *S. bahiensis* roots (17,57g), called roots deposits (RD) was manually separated and extracted with MeOH (750 mL) at ultrasound for 10 minutes. The methanol RD extract (0,77g) was produced by evaporation of alcoholic solution. The separation of RD extract was performed by preparative HPLC using: C_{18} column 250 mm; 21.0 mm; 5 μm; 100 °A, gradient elution with solvents A (H_2O) and B (methanol), 0–35min 80 to 50% A, 35-70 min 50%A, flow 8,0 mL.min^{-1}, diode array detector (DAD), injection volume was 100 μL, the total injection volume was 1,5 mL. Three fractions were collected, evaporated and then analyzed by NMR and HRMS.

Results and Discussion

The figure 1 shows the chromatogram of HPLC separation and the collected peaks. NMR ^{1}H spectrum, of collected peak $R_{t(min)}$ = 53.266, shows signals related to aromatic systems and one singlet that corresponds to methoxyl group. The ^{1}H pattern observed for this substance indicates the existence of a canthi-6-one alkaloid substituted by a methoxyl. Four hydrogens (δ_H 8.62, 7.97, 7.69 and 7.50) of ring A of the canthi-6-ones were assigned considering multiplicity and chemical shifts. Hydrogens at positions 4 and 5 (δH = 7.74 and 6.93, J = 9.8 Hz) are present, the latter signal being a singlet for a hydrogen at ring C, which could be at position 1 or 2, another position would be the methoxyl group.

The irradiation of hydrogen δ_H 7.30, by NOEDIFF experiment, resulted in an increase of the doublet signal δ_H 7.97. The signals at δ_H 7.50 and δ_H 7,30 were increased when δ_H 7.97 was irradiated, indicating the proximity between the H-11 position in ring A and the hydrogen δ_H 7,30 present in the C-ring. NOEDIFF experiments suggest that the bound of the methoxyl group in this substance occurs in position 2. Irradiation of the hydrogens of the methoxyl group in δ_H 4.20 increased the signal of the H-4 δ_H 7.75. All NOE experiments was compared with previous reported data [5].

These NMR data led to 2-methoxy-canthi-6-one alkaloid, but HRMS shows ion $[M + H]^+$ = 267.0733 m/z with molecular formula $C_{15}H_{10}N_2O_3$, and diverged 16 m/z from the proposed alkaloid 2-

methoxy-canthi-6-one. According to the NMR and MS data, the only possibility that justify this mass difference is the presence of an $N\text{-}O^-$ group at the structure.

The IR analysis confirms the $N\text{-}O^-$ bond in the intense absorption at 1303 cm^{-1}. It is a first report of spectroscopic data that shows the presence of alkaloid 2-methoxy-canthi-6-one N-oxide (**3**). NOESY, HMBC and HMQC were performed and the results are described at table 1 and figures 2 and 3. Peaks that corresponds to compounds **1** and **2** were analyzed by NMR and HRMS all data were compared to previous described data [6, 7].

Other canthi-6-on alkaloids have been reported in the literature, among the structures most have substitutions at ring A, structural varieties with substitutions at the position 2 on ring C are uncommon. Although the substitution pattern is easily identified by ^1H NMR and NOE experiments can allow unambiguous determination of the substitution pattern in ring A and C [5], the complementary spectroscopic techniques as IR and HRMS allow the determination of the presence of N-oxide groups.

Conclusions

The first phytochemical study of *S. bahiensis* were possible identify 3 canthi-6-one alkaloids and the new one 2-methoxy-canthi-6-one N-oxide, previous studies shows the occurrence of alkaloids with N-oxide but without substitution at position 2.

Substitution pattern is easy to identify by ^1H NMR, but is necessary use different experiments to correctly determine the position of substituents at rings A and C, since studies of structure relationships have determined that different substitution patterns in these rings influence the pharmacological activity of these substances.

References

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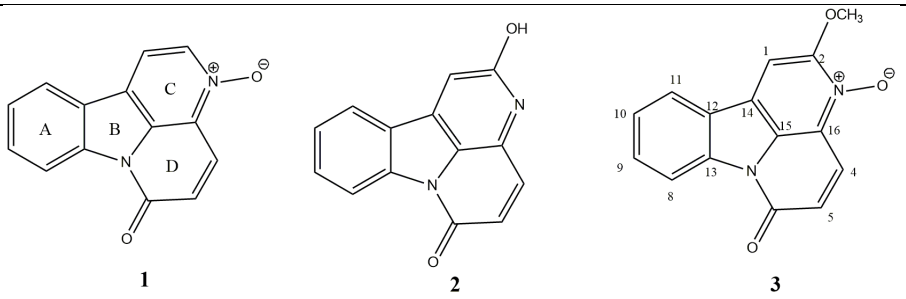

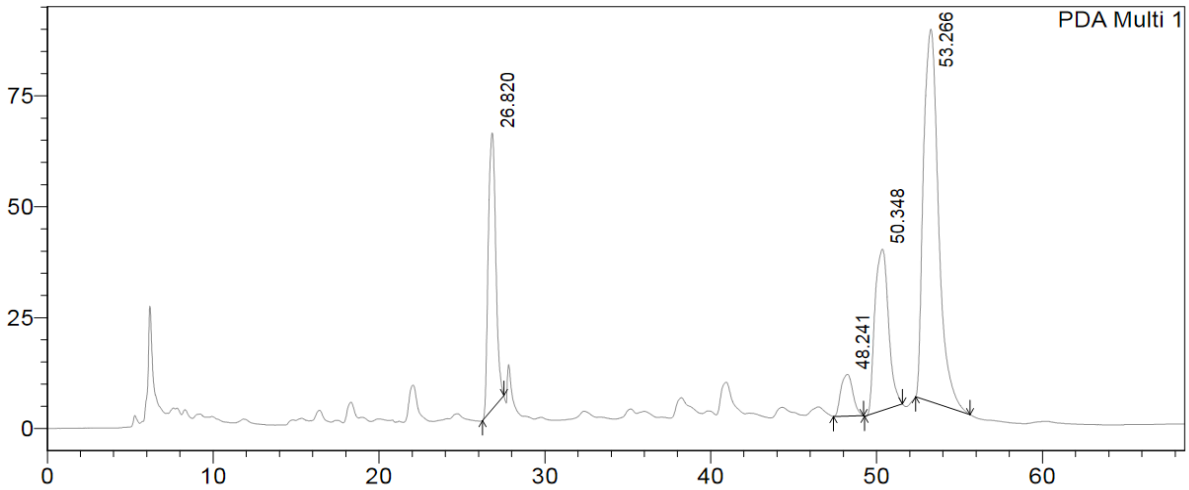

Figure 1: Chromatogram (254 nm) of RD methanol extract obtained by preparative scale. Compound **1** ($\mathbf{R_{t(min)}}$ = 48.241) compound **2** ($\mathbf{R_{t(min)}}$ = 50.348) and compound **3** ($\mathbf{R_{t(min)}}$ = 53.266).

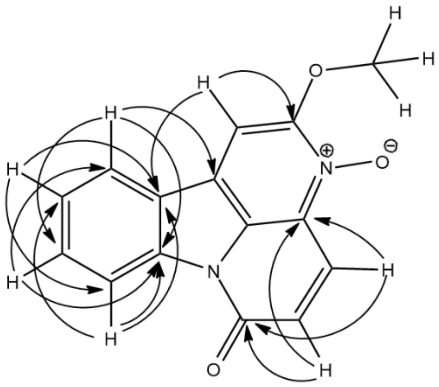

Figure 2: HMBC (400MHz) observed correlations (arrows) of compound **3**.

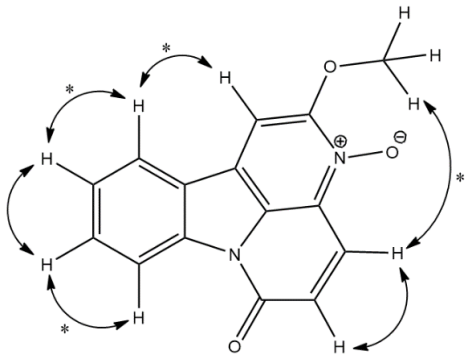

Figure 3: NOESY (400MHz) and (*) NOEDIFF (500MHz) observed correlations (arrows) of compound **3**.

Table 1: NMR data 2-methoxy-N-oxide-canthi-6-one.

	^{1}H δ(ppm), J(Hz)	^{13}C δ(ppm)
1	7,29 (1H, *s*)	114,13 [a]
2	----	157,64 *[b]
4	7,74 (1H, *d*, *J* = 9,8)	125,48 [a]
5	6,93 (1H, *d*, *J* = 9,8)	127,85 [a]
6	---	157,78 *
8	8,62 (1H, *dl*, *J* = 8,0)	117,39 [a]
9	7,69 (1H, *ddd*, *J* = 8,8; 8,0 ;1,2)	132,23 [a]
10	7,50 (1H, *ddd*, *J* = 8,8;7,6 ;1,2)	126,41 [a]
11	7,97 (1H, *dl*, *J* = 7,6)	123,92 [a]
12	---	123,33 [b]
13	---	142,15 [b]
14	---	135,73 [b]
15	---	---
16	---	122,84 [b]
-OCH_3	4,20 (3H, *s*)	64,60 [a]

a = values supported by HMQC b = values supported by HMBC ; * = values may be changed