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Short communication

Gas chromatographic analysis of indole alkaloids from *Tabernaemontana hilariana*

Claudia A.L. Cardoso, Wagner Vilegas*, Gilberto L. Pozetti

Instituto de Química de Araraquara-Unesp, C.P. 355, CEP 14800-900 Araraquara-SP, Brazil

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Abstract

A fast and efficient procedure was elaborated to identify the alkaloid constituents from *Tabernaemontana hilariana* (Apocynaceae). The strategy based on fractioning of the crude alkaloid fraction in small silica cartridges followed by thin-layer chromatography (TLC), capillary gas chromatography–flame ionization detection as well as high-resolution gas chromatography–mass spectrometry afforded voacangine, coronaridine, ibogamine, voacangine pseudoindoxyl, voacangine hydroxyindolenine, 3-hydroxycoronaridine and 3-(2-oxopropyl)coronaridine. © 1997 Elsevier Science B.V.

Keywords: *Tabernaemontana hilariana*; Alkaloids

1. Introduction

Tabernaemontana hilariana Muell Arg. (Apocynaceae) is a small tree that grows wild in São Paulo State, Brazil. The leaves are supposed to be lethal to cattle [1]. This genus is chemically characterized mainly by its indole alkaloids, which are biologically active [2,3].

Previous chemical investigations of *T. hilariana* extracts by traditional phytochemical processes of fractionation using adsorption column chromatography have failed, leading to complete decomposition of the substances. In fact, Madinaveitia et al. [4] report the air oxidation of a sample of voacangine – an indolic alkaloid usually found in plants of the *Tabernaemontana* genus – dissolved in $CHCl_3$ by solar radiation within two weeks. Among the available techniques for the identification of indole alkaloids, capillary gas chromatography coupled to flame ionization detection (GC–FID) has shown to be a very efficient system in separating complex indole alkaloid mixtures, due to its speed and resolution [5]. In the search for a rapid and efficient technique to separate and identify the *T. hilariana* alkaloids, we have tested an approach based on fractionation of the crude alkaloid fraction in silica cartridges followed by thin-layer chromatography (TLC), capillary GC–FID and high-resolution (HR) GC–mass spectrometry (GC–MS) analysis.

2. Chromatographic instruments and conditions

GC analyses were performed using a Shimadzu GC–14B gas chromatograph equipped with a fused-silica capillary CBP-5 (25 m×0.33 mm I.D., film thickness 0.5 μm) interfaced with FID. H_2 was used as carrier gas (60 kPa). The injection split ratio was 1:30, and injection temperature was 250°C. The column temperature was programmed to rise from

*Corresponding author.

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100°C for 2 min, then to 280°C at 10°C/min; this temperature was held for 10 min. The detector temperature was 280°C. The integrator used was a Shimadzu C-R6A Chromatopack. Samples of 1 μl were injected with a 10 μl Hamilton syringe.

The HRGC–MS analyses were performed on an HP 5970 mass-selective detector (MSD) (Hewlett-Packard), with electron impact ionization (70 eV), coupled to an HP 5890 GC. The column used was a 25 m×0.25 mm I.D. HP-1 (cross-linked methylsilicone 0.3 μm film thickness). Samples of 1 μl were injected using the split mode (split ratio 1:30), with injector temperature and interface both at 280°C. The temperature program used was the same as described above. Hydrogen was used as carrier gas (100 kPa). The MS scan range was 50–500 u. Data were processed on a HP 7946/HP 9000-300 CPU (Hewlett-Packard).

3. Results and discussion

Green fruits and root bark of *T. hilariana* were collected at Boa Esperança, SP, Brazil, by one of us (G.L.P.) in 1984. A 500-g amount of each part was separately dried (60°C), powdered and extracted (Soxhlet) first with 2 l benzene and then with 2 l ethanol. The solvents were evaporated under vacuum. 100 mg of each extract was submitted to acid–base extraction affording the crude alkaloidal fractions [6]. A 10-mg amount each of the four crude alkaloidal samples was fractionated on silica cartridges (Sep-Pak Classic, Millipore, 690 mg, 55–105 μm, eluted with (1) 15 ml $CHCl_3$ (F1), (2) 15 ml $CHCl_3$–CH_3OH (9:1) (F2), (3) 20 ml CH_3OH (F3). The solvents were evaporated under a nitrogen stream and the masses were weighed. The recovery of ca. 90% from the initial mass fractionated was considered good, showing that there was no significative loss of alkaloids.

F1, F2 and F3 were redissolved in 5 ml of methanol and analysed by laboratory-made TLC plates (0.1 mm thickness, silica gel G, Merck, Art. 7731) eluted with toluene–ethanol–ammonia (95:5:5, v/v/v) and revealed under UV light (254; 366 nm) and with Dragendorff's reagent, iodoplatinate, ferric chloride–perchloric acid (FCPA) and ceric sulphate–sulfuric acid (CSSA) [7]. Standard indole alkaloids were obtained from a collection of our laboratory. The major alkaloids retained in the F1 were coronaridine and voacangine. Ibogamine was found mainly in F2 (see also Table 2). F3 retained polar substances (R_F<0.2), that were not identified.

To test the capability of the GC system to perform the separation and identification of the alkaloids, we injected seven authentic standards of common indole alkaloids (Table 1). Temperature gradient afforded a baseline separation and it was then used for the analysis of the F1 and F2 fractions. Due to its polarity, F3 could not be injected into the GC–FID and HRGC–MS systems. Addition of authentic standards and HRGC–MS analysis were used to confirm the presence of ibogamine, coronaridine and

Table 1
Indole alkaloids from *Tabernaemontana hilariana*

Alkaloid	Extracts							
	Root barks				Green fruits			
	Benzene		Ethanolic		Benzene		Ethanolic	
	F1	F2	F1	F2	F1	F2	F1	F2
3-Hydroxycoronaridine	+	–	–	–	+	–	–	–
Ibogamine	–	++	–	++	–	–	–	–
Coronaridine	+++	+	+	+	+++	++	+	–
Voacangine	++	+	++	+	++	+	+	–
Voacangine hydroxyindolenine	+	–	–	–	+	–	–	–
Voacangine pseudoindoxyl	–	–	–	–	–	–	–	+
3-(2-Oxopropyl)coronaridine	+	–	–	–	–	–	–	–

–: Absent.
Relative abundance: +++: major component; ++: intermediary concentration; +: minor component.

Table 2
Retention times of indole alkaloids

Peak	Alkaloid	M_r	t_R (min)
1	3-Hydroxycoronaridine[a]	354	20.15
2	Ibogamine[a,b]	280	20.91
3	Coronaridine[a,b]	338	21.30
4	Voacangine hydroxyindolenine[a]	384	22.73
5	Voacangine pseudoindoxyl[a]	384	24.05
6	Tabernanthine[a,b]	310	24.24
7	Voacangine[a,b]	368	24.69
8	Vobasine[b]	352	25.09
9	3-(2-Oxopropyl)coronaridine[a]	394	26.39
10	Conopharyngine[b]	398	28.22
11	Iboxygaine[b]	326	29.55

[a] Structure confirmed by MS.
[b] Standard compounds.

voacangine. The presence of the four other minor alkaloids [3-hydroxycoronaridine, voacangine pseudoindoxyl, voacangine hydroxyindolenine and 3-(2-oxopropyl)coronaridine] was deduced on matching with the NBS-REV data bank (with 42 000 compounds) and also by a comparison of their MS-fragmentation pattern with the literature [8].

Table 2 shows that the major compounds coronaridine and voacangine remain mainly in F1 but they are also present in minor amounts in F2. Ibogamine is the major alkaloid in F2. The minor compounds 3-hydroxycoronaridine, and 3-(2-oxopropyl)coronaridine and voacangine hydroxyindolenine are eluted only in F1. Voacangine pseudoindoxyl was found only in F2 from the ethanolic extract of the green fruits.

In the course of this work we observed that if the extracted samples were not kept under low temperature (~0°C), the chromatographic profiles became totally different after a few hours, showing that the substances are readily oxidized. In some cases the substances did not even elute from the GC column due to the degradation leading to polar compounds.

Thus, Sep-Pak silica cartridges in combination with GC–FID and HRGC–MS provide a simple route to the separation and identification of the *T. hilariana* alkaloids, without degradation of the samples. This approach has advantageously overcome the risk of decomposition and the laborious steps of a traditional phytochemical method. Moreover, the sample amount needed for the fractionation and identification of the alkaloids is much smaller than that required in a traditional phytochemical approach. The limitation of this method is that the alkaloids present in the more polar F3 fraction could not be analysed. On the other hand, all the seven alkaloids contained in the F1 and F2 fractions were readily identified by TLC, GC–FID and HRGC–MS.

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