Industrial Scale Isolation, Structural and Spectroscopic Characterization of Epiisopiloturine from *Pilocarpus microphyllus* Stapf Leaves: A Promising Alkaloid against Schistosomiasis

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Abstract

This paper presents an industrial scale process for extraction, purification, and isolation of epiisopiloturine (EPI) (2(3H)-Furanone, dihydro-3-(hydroxypheynyl)methyl)-4-[1-methyl-1H-imidazol-4-yl]methyl]-[35-[3a(R®)-4b]], which is an alkaloid from jaborandi leaves (*Pilocarpus microphyllus* Stapf). Additionally for the first time a set of structural and spectroscopic techniques were used to characterize this alkaloid. EPI has shown schistomicidal activity against adults and young forms, as well as the reduction of the egg laying adult worms and low toxicity to mammalian cells (*in vitro*). At first, the extraction of EPI was done with toluene and methylene chloride to obtain a solution that was alkalinized with ammonium carbonate. The remaining solution was treated in sequence by acidification, filtration and alkalinization. These industrial procedures are necessary in order to remove impurities and subsequent application of the high performance liquid chromatography (HPLC). The HPLC was employed also to remove other alkaloids, to obtain EPI purity higher than 98%. The viability of the method was confirmed through HPLC and electrospray mass spectrometry, that yielded a pseudo molecular ion of m/z equal to 287.1 Da. EPI structure was characterized by single crystal X-ray diffraction (XRD), 1H and 13C nuclear magnetic resonance (NMR) in deuterated methanol/chloroform solution, vibrational spectroscopy and mass coupled thermal analyses. EPI molecule presents a parallel alignment of the benzene and the methyl imidazol ring separated by an interplanar spacing of 3.758 Å indicating a π-π bond interaction. The imidazole alkaloid melts at 225 °C and decomposes above 230 °C under air. EPI structure was used in theoretical Density Functional Theory calculations, considering the single crystal XRD data in order to simulate the NMR, infrared and Raman spectra of the molecule, and performs the signals attribution.

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Introduction

Alkaloids are organic compounds found in plant kingdom, fungus, bacteria and animals. The majority of these natural substance exhibits an alkaline character related particularly to the presence of basic amino groups (frequently heterocyclic) in their chemical structure. The word *alkaloid* is derived from the Latin “alkai” (“alkali”) with the suffix “-oid” (“like”). In the literature, many authors claim that a true alkaloid is composed by one or more basic nitrogen atoms in a heterocyclic ring, beside the intense biological activity in the presence of living organisms [1]. Additionally, alkaloids are known to be used as therapeutic agents for anesthetics, analgesics, and psycho-stimulant, among other pharmacological activities.

Over the past one hundred and fifty years, thousands of alkaloids have been isolated [2], and several high standard techniques can now be employed to evaluate the pharmacological and toxicological activities of these substances. Thus, new applications of centenarians alkaloids have been discovered, as the case of Pilocarpine, which has been isolated in 1875 and applied for decades in the treatment of glaucoma and, recently, also used for in the treatment of xerostomy [3,4].

Several alkaloids have been isolated from the *Pilicarpus genus*, but many of them are still in analysis to evaluate their therapeutic
This work reports the industrial extraction, purification and isolation of EPI from jaborandi leaves, and also its physicochemical characterization by single crystal X-ray diffraction, $^1$H and $^{13}$C nuclear magnetic resonance (NMR) in deuterated methanol/chloroform solution, vibrational infrared (FT-IR) and Raman (FT-Raman) spectroscopies, and mass coupled thermal analyses. The spectroscopic characterization data are further supported by computational calculations performed in the framework of the Density Functional Theory (DFT).

Materials and Methods

Industrial extraction, pre-purification and isolation of alkaloid from jaborandi leaves

The initial step is based on the alkalinization of 1,500 kg of jaborandi leaves with a solution of potassium carbonate, followed by the extraction of all alkaloids (solid-liquid extraction) with toluene and methylene chloride solvent (Figure 1). The organic phase was submitted to liquid-liquid extraction with an aqueous solution of sulfuric acid. Hereafter, 250 L of the aqueous solution with a mean content of 2% (m/v) of EPI in the sulfate salt form was cooled, alkalinized with ammonium hydroxide solution and treated with activated carbon and diatomaceous sand.

After treatment with carbon and diatomaceous sand, the impure EPI was dissolved in an aqueous solution containing hydrochloric acid and filtered on a pressure lentil filter (filter medium: cloth/two filter papers/cloth) under reduced pressure. The filtrate containing EPI hydrochloride was alkalinized with ammonium hydroxide solution to precipitate the EPI neutral form, and then the solution was filtered under reduced pressure [8]. The aim of the previous step was to remove the impurities such as carbon and diatomaceous sand in order to submit EPI to further purification process by high performance liquid chromatography (Figure 1) [all details from this steps is in patently process – 000121-INPI, Brazil].

The crude EPI (Figure 1) at a concentration of 10 mg/mL was dissolved in the mobile phase with potassium phosphate 5% (v/v), filtered by a membrane of 0.45 μm and set to the preparative high performance liquid chromatography – HPLC (SHIMADZU Prominance, AUTOSAMPLER SIL-10AF, CTO-20A, DGU-20A5, LC-6AD, CBM-20A, SPD-20A, Tokyo, Japan).

The preparative chromatographic conditions set were performed in a column of LiChroPrep 60 RP Select B (250×25 mm, 5 μm). The mobile phase was 367.59 mM potassium phosphate adjusted at pH 2.5 with a flow rate of 10 mL/min for a time run of 90 min. The detection was done using a UV detector at a wavelength of 216 nm and the column oven was set to 50°C.

The injection volume was 1000 μL and 500 mL fractions were collected with a concentration of 100 mg/L of crude EPI. The solution obtained after preparative HPLC was alkalinized between pH 9 to 9.5 and subjected to liquid-liquid extraction with industrial chloroform. The organic phase was submitted to liquid-liquid extraction with an aqueous solution containing sulfuric acid. Hereafter, 250 L of the aqueous solution was acidified with hydrochloric acid solution to precipitate the EPI neutral form, and then the solution was filtered under reduced pressure [8].

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For fine analyses an analytical HPLC was employed to verify each process step, with the LiChroPrep 60 RP Select B (250×4.6 mm, 5 μm), using external standard (EPI standard at 20 µg/mL and pilocarpine standard at 50 µg/mL, Merck, Darmstadt, Germany). The mobile phase was 367.59 mM potassium phosphate adjusted at pH 2.5. The flow rate was 1 mL/min, the column oven was set to 50°C for a time run of 50 min with UV detection at a wavelength of 216 nm.

The molecular mass confirmation was performed by mass spectrometry (AmaZon SL, Bruker Daltonics, Bremen – Ger-
Figure 2. Analytical HPLC used LiChrospher 60 RP column and eluted with potassium phosphate. (A) Standard EPI (20 μg/mL), (B) Standard pilocarpine (50 μg/mL), (C) “cultivated jaborandi leaves” solution, resulted from first extraction step, (D) “cultivated jaborandi acid” solution, obtained EPI under salt form, (E) Solution of “crude EPI” with some impurities as pilocarpine and other alkaloids, (F) last step of isolation showing EPI >98% purity.

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Figure 3. Mass spectrum obtained from ESI+/Ion Trap. (A) free EPI with a pseudo molecular ion m/z 287.1 Da [M+H]^+, (B) MS^2 with characteristic fragment at m/z 269.1 Da [M – H_2O + H]^+, (C) MS^3 with fragments at m/z 251.0 Da [M – 2H_2O + H]^+ and 168.06 Da with proposed chemical structure.

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The capillary voltage was – 1,800 V, temperature at 250°C, and the mass spectra were acquired in mass range of m/z 160 – 300 Da. MS/MS was carried out in manual mode with fragmentation of the precursor ion by collision induced dissociation (CID) using helium (He) as the collision gas. Precursor ions were selected within an isolation width of 2 u and scans were accumulated with variable RF signal amplitudes. The m/z scale of the mass spectrum was calibrated using the external calibration standard G2421A electrospray ‘tuning mix’ from Agilent Technologies (Santa Rosa, USA).

**Physical measurements**

**Nuclear Magnetic Resonance (NMR).** 10 mg of EPI was dissolved in 2 mL of CD_{3}OD/CDCl_{3} 1:1 mixture. Afterward 600 µL of this solution was transferred to a 5 mm NMR sample tube. For the measurements of NMR data, standard parameter sets created for the Bruker CMC-se (complete molecular confidence-structure elucidation) program were uniformly employed. Gradient COSY (Correlation Spectroscopy) (2 scans per increment) and H-13C HMBC (Heteronuclear Multiple-Bond Correlation spectroscopy) (8 scans per increment) were acquired using 4 k complex data points in F2 and 512 points in F1 dimension. A 1H-15N HMBC was acquired with 32 scans per increment with a time domain of 256 in F1 and 2 k points in F2. The multiplicity edited gradient HSQC (Heteronuclear Single Quantum Correlation) (2 scans per increment) was acquired with 2k data points in F2 and 400 points in the F1 dimension. The instrument used was an AVANCE III 600 MHz NMR spectrometer equipped with a 5 mm TXI probe head (Bruker Biospin, Rheinstetten, Germany). The NMR data acquired were processed according to the general experimental procedures.

The Bruker structure elucidation package CMC-se (Topspin 3.1) was used to get the peak and multiplet lists in a fully...
automated way. The multiplet lists are collected into a correlation table. The automatic step is usually followed by short visual inspection of the results. The SELU module contains combined display linking the correlation table and the spectra display. This allows fast inspection and correction of generated HSQC, HMBC and COSY multiplet lists. After the correlation table is populated and inspected, the structure generator calculates structures which are consistent with the NMR data acquired. Finally an independent $^{13}$C chemical shift prediction method is used to validate the result.

**Single crystal X-ray diffraction (XRD).** The X-ray diffraction data were collected at room temperature using a KAPPA-CCD Diffratometer with MoK$_\alpha$ radiation ($\lambda = 0.71073$ Å). The cell refinements were performed using the software Collect

### Table 1. EPI bond distances obtained through x-ray diffraction (Experimental) and DFT results (Calculated).

| Atom   | Experimental (Å) | Calculated (Å) | Difference (Experimental -Calculated) (Å) |
|--------|------------------|----------------|------------------------------------------|
| O1–C9  | 1.419 (3)        | 1.429          | –0.010                                   |
| O1–H1  | 0.82             | 0.963          | –0.143                                   |
| C1–N1  | 1.314 (3)        | 1.312          | 0.002                                    |
| C1–N2  | 1.341 (3)        | 1.364          | –0.023                                   |
| C1–H1A | 0.93             | 1.080          | –0.150                                   |
| N1–C3  | 1.379 (3)        | 1.379          | 0.000                                    |
| C3–C2  | 1.359 (3)        | 1.374          | –0.015                                   |
| C3–C4  | 1.481 (3)        | 1.496          | –0.015                                   |
| N2–C2  | 1.369 (3)        | 1.381          | –0.012                                   |
| N2–C16 | 1.452 (3)        | 1.453          | –0.001                                   |
| C2–C16 | 1.511 (3)        | 1.520          | –0.009                                   |
| C9–C8  | 1.536 (3)        | 1.543          | –0.007                                   |
| C9–H9  | 0.98             | 1.098          | –0.118                                   |
| C5–C4  | 1.531 (3)        | 1.546          | –0.015                                   |
| C5–C6  | 1.532 (3)        | 1.539          | –0.007                                   |
| C5–C8  | 1.537 (3)        | 1.539          | –0.002                                   |
| C5–H5  | 0.98             | 1.090          | –0.110                                   |
| C2–H2  | 0.93             | 1.078          | –0.148                                   |
| C10–C11| 1.383 (3)        | 1.396          | –0.013                                   |
| C10–C15| 1.390 (3)        | 1.398          | –0.008                                   |
| C4–H4A | 0.97             | 1.095          | –0.125                                   |
| C4–H4B | 0.97             | 1.095          | –0.125                                   |
| C8–C7  | 1.515 (3)        | 1.533          | –0.018                                   |
| C8–H8  | 0.980            | 1.092          | –0.112                                   |
| O3–C7  | 1.345 (3)        | 1.353          | –0.008                                   |
| O3–C6  | 1.446 (3)        | 1.448          | –0.002                                   |
| C16–H16A| 0.960            | 1.092          | –0.132                                   |
| C16–H16B| 0.960            | 1.089          | –0.129                                   |
| C16–H16C| 0.960            | 1.092          | –0.132                                   |
| O2–C7  | 1.207 (3)        | 1.199          | 0.008                                    |
| C11–C12| 1.381 (3)        | 1.394          | –0.013                                   |
| C11–H11| 0.930            | 1.083          | –0.153                                   |
| C15–C14| 1.382 (3)        | 1.392          | –0.010                                   |
| C15–H15| 0.930            | 1.086          | –0.156                                   |
| C6–H6A | 0.970            | 1.090          | –0.120                                   |
| C6–H6B | 0.970            | 1.090          | –0.120                                   |
| C13–C12| 1.371 (4)        | 1.392          | –0.021                                   |
| C13–C14| 1.376 (4)        | 1.394          | –0.018                                   |
| C13–H13| 0.930            | 1.084          | –0.154                                   |
| C12–H12| 0.930            | 1.084          | –0.154                                   |
| C14–H14| 0.930            | 1.085          | –0.155                                   |

Atom labels accordingly to Figure 4.

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|                  | Experimental (°) | Calculated (°) | Difference(Experimental – Calculated) (°) |
|------------------|-----------------|----------------|------------------------------------------|
| C9–O1–H1         | 109.50          | 108.21         | 1.29                                     |
| N1–C1–N2         | 112.37 (19)     | 112.32         | 0.05                                     |
| N1–C1–H1A        | 123.80          | 125.88         | −2.08                                    |
| N2–C1–H1A        | 123.80          | 121.80         | 2.00                                     |
| C1–N1–C3         | 105.62 (17)     | 105.54         | 0.08                                     |
| C2–C3–N1         | 108.60 (18)     | 109.82         | −1.22                                    |
| C2–C3–C4         | 130.68 (19)     | 129.22         | 1.46                                     |
| N1–C3–C4         | 120.60 (18)     | 120.95         | −0.35                                    |
| C1–N2–C2         | 106.14 (17)     | 106.27         | −0.13                                    |
| C1–N2–C16        | 126.60 (20)     | 126.76         | −0.16                                    |
| C2–N2–C16        | 127.24 (19)     | 126.96         | 0.28                                     |
| O1–C9–C10        | 112.88 (16)     | 112.61         | 0.27                                     |
| O1–C9–C8         | 107.18 (16)     | 106.45         | 0.73                                     |
| C10–C9–C8        | 111.68 (16)     | 113.75         | −2.07                                    |
| O1–C9–H9         | 108.30          | 109.79         | −1.49                                    |
| C10–C9–H9        | 108.30          | 108.25         | 0.05                                     |
| C7–C8–C5         | 103.81 (17)     | 103.80         | 0.01                                     |
| C9–C8–C5         | 114.85 (17)     | 116.96         | −2.11                                    |
| C7–C8–H8         | 109.20          | 107.96         | 1.24                                     |
| C9–C8–H8         | 109.20          | 107.18         | 2.02                                     |
| C5–C8–H8         | 109.20          | 111.91         | −2.71                                    |
| C7–O3–C6         | 109.86 (17)     | 110.48         | −0.62                                    |
| N2–C16–H16A      | 109.50          | 110.89         | −1.39                                    |
| N2–C16–H16B      | 109.50          | 110.70         | −1.2                                     |
| H16A–C16–H16B    | 109.50          | 108.64         | 0.86                                     |
| N2–C16–H16C      | 109.50          | 108.87         | 0.63                                     |
| H16A–C16–H16C    | 109.50          | 109.10         | 0.4                                      |
| H16B–C16–H16C    | 109.50          | 108.57         | 0.93                                     |
| O2–C7–O3         | 121.60 (20)     | 122.96         | −1.36                                    |
| O2–C7–C8         | 126.90 (20)     | 127.01         | −0.11                                    |
| O3–C7–C8         | 111.48 (18)     | 110.03         | 1.45                                     |
| C12–C11–C10      | 121.60 (20)     | 120.53         | 1.07                                     |
| C8–C9–H9         | 108.30          | 105.75         | 2.55                                     |
| C4–C5–C6         | 113.10 (17)     | 111.44         | 1.66                                     |
| C4–C5–C8         | 112.45 (18)     | 112.38         | 0.07                                     |
| C6–C5–C8         | 102.70 (17)     | 101.97         | 0.73                                     |
| C4–C5–H5         | 109.50          | 108.19         | 1.31                                     |
| C6–C5–H5         | 109.50          | 110.75         | −1.25                                    |
| C8–C5–H5         | 109.50          | 112.07         | −2.57                                    |
| C3–C2–N2         | 107.27 (17)     | 106.04         | 1.23                                     |
| C3–C2–H2         | 126.40          | 132.04         | −5.64                                    |
| N2–C2–H2         | 126.40          | 121.80         | 4.6                                      |
| C11–C10–C15      | 117.60 (20)     | 118.84         | −1.24                                    |
| C11–C10–C9       | 122.62 (18)     | 121.49         | 1.13                                     |
| C15–C10–C9       | 119.79 (18)     | 119.67         | 0.12                                     |
| C3–C4–C5         | 112.38 (16)     | 113.42         | −0.04                                    |
| C3–C4–H4A        | 109.10          | 108.57         | 0.53                                     |
| C5–C4–H4A        | 109.10          | 109.84         | −0.74                                    |
| C3–C4–H4B        | 109.10          | 109.96         | −0.86                                    |
The final unit cell parameters were obtained on all reflections. Data reduction was carried out using the software Denzo-SMN and Scalepack. The structure was solved by Direct Methods and anisotropically refined with full-matrix least-squares on F² using SHELXL97. The hydrogen atoms bonded to C and N atoms were positioned geometrically and refined with riding constraints with distance restraints of N-H = 0.86 Å, aromatic C-H = 0.93 Å and with Uiso(-H) = 1.2 Ueq(N,C). The crystallographic data were deposited at the Cambridge Crystallographic Data Center under the numbers CCDC 915132. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk.

Vibrational FT-IR and FT-Raman. FT-IR spectrum of EPI sample diluted in KBr was recorded in the 4000–400 cm⁻¹ range on a Bomen spectrophotometer, model MB-102, with a coupled diffuse reflectance accessory (Pike Technologies, Inc.). FT-Raman spectrum was recorded in a FT-Raman Bruker FRS-100/S spectrometer using 1064 nm exciting radiation (Nd:YAG laser Coherent Compass 1064–500 N) and a Ge detector.

Mass coupled thermal analyses (TGA-DSC-MS). The thermal analyses were recorded on a Netzsch thermoanalyser model TGA/DSC 490 PC Luxx coupled to an Arolos 403 C mass spectrometer, using a heating rate of 10°C/min and under synthetic air flow of 50 mL/min.

Computational analysis

The Density Functional Theory (DFT) [14] in the Kohn-Sham (KS) scheme [15] was used to investigate the electronic structure, vibrational properties and NMR $^{13}$C and $^1$H isotropic chemical shifts for the isolated EPI alkaloid molecule. The B3LYP [16] exchange correlation functional and the 6–311++G** basis set were used as implemented in the Gaussian 09 computational package [17]. Gauge Including Atomic Orbitals (GIAO) method [18–20] and diffuse functions in the basis set were applied for the calculation of the C and H NMR spectra. All simulations were carried out in vacuum conditions and T = 0 K.

Results and Discussion

The results, here presented, describe a methodology for extraction, purification and isolation of EPI from jaborandi leaves. The HPLC (Figure 2) and LC/MS ESI+ / Ion Trap (Figure 3) techniques have demonstrated the purity and initial characterization of each process step. For industrial scale, 1,500 kg of jaborandi leaves have been used through all steps described in Figure 1, what lead to obtain around 2 kg of pure EPI, used in the structural and spectroscopic characterization described in this work.

The MS/MS analysis showed a pseudomolecular ion with m/z 287.1 Da [M + H]$^+$ and a MS² fragment with m/z 269.1 Da [M – H₂O + H]$^+$. This “molecular fingerprint” of EPI has been previously reported in the literature[6]. Structural and spectroscopic characterizations were also performed to assure the integrity of the EPI molecule.

The EPI molecular structure is shown in Figure 4, created by the ORTEP [21] software. An interesting feature of this molecule is the parallel alignment of the benzene and the methyl imidazole.
Figure 6. Epiisopiloturine FT-IR spectra: A) experimental and B) calculated.
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Figure 7. Epiisopiloturine FT-Raman spectra: A) experimental and B) calculated.
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| Experimental | Calculated | Assignment |
|-------------|------------|------------|
| IR          | Raman      |            |
| 412         | 407        | \(\delta\) (all structure) |
| 433         | 438        | \(\delta\) (all structure) |
| 498         | 499        | C-C-C out of plane bending (benzene) |
| 526         | 525        | C-C-C out of plane bending (benzene) |
| 567         | 568        | C-C-C in-phase bending (benzene) |
| 621         | 620        | ring puckering (imidazole) |
|             | 628        |            |
| 644         | 657        | sc (O3-C7-C8) |
| 663         | 664        | C-C-C in-phase bending (benzene) |
| 712         | 711        | C-C-C in-phase puckering (benzene) |
| 729         | 717        | C-H out-of-plane in-plane (benzene) |
| 758         | 730        | C-H out of plane bend (imidazole) |
| 775         | 783        | C-H out-of-plane in-phase (benzene) |
| 800         | 796        | ring puckering (imidazole) |
| 831         | 821        | C-H out of plane bend (imidazole) |
| 846         | 854        | r CH\(_2\) (C4) |
| 893         | 900        | r CH\(_2\) (C4), r CH\(_2\) (C6) |
| 910         | 923        | C-H out of plane (benzene), r CH\(_2\) (C4) |
| 932         | 944        | r CH\(_2\) (C4), \(\beta\) (lactone) |
| 984         | 983        | \(\beta\) (lactone) |
| 1004        | 1016       | C-C-C trigonal bending |
| 1022        | 1038       | \(\beta\) (lactone), r CH\(_2\) (C4) |
| 1046        | 1048       | C-H in plane bending (benzene) |
| 1063        | 1076       | w CH\(_3\) (C16) |
| 1088        | 1078       | w CH\(_3\) (C16), C-H in plane bending (benzene) |
| 1105        | 1116       | t CH\(_2\) (C4), \(\gamma\) (lactone) |
| 1148        | 1145       | w CH\(_3\) (C16) |
| 1169        | 1165       | t CH\(_2\) (C4), \(\gamma\) (lactone) |
| 1184        | 1182       | \(\beta\) (lactone) |
| 1207        | 1198       | C-H in plane bending (benzene), t CH\(_2\) (C4) |
| 1197        | 1210       | C-H in plane bending (benzene) |
| 1236        | 1220       | t CH\(_2\) (C4), t CH\(_2\) (C6) |
| 1254        | 1244       | \(\beta\) (lactone) |
| 1254        | 1256       | t CH\(_2\) (C6), C-H in plane bending (imidazole) |
| 1263        | 1266       | t CH\(_2\) (C4), t CH\(_2\) (C6), C-H in plane bending (imidazole) |
| 1286        | 1292       | w CH\(_2\) (C4) |
| 1312        | 1315       | C-C stretching (benzene) |
| 1330        | 1318       | C-N stretching (imidazole) w CH\(_3\) (C4) |
| 1348        | 1349       | C-C stretching (benzene) |
| 1362        | 1354       | w CH\(_3\) (C4) |
| 1385        | 1376       | sc(C9-C1-H), vas(C9-C9-C10) |
| 1423        | 1418       | vs(N1-C1-N2), w CH\(_3\) (C16), vas(C16-N2-C1) |
| 1450        | 1455       | w CH\(_3\) (C16) |
| 1472        | 1484       | C-C stretching (benzene) |
| 1494        | 1486       | sc CH\(_3\) (C16) |
| 1508        | 1520       | sc CH\(_3\) (lactone), w CH\(_3\) |
| 1524        | 1535       | vas N-C-N (imidazole) |
ring separated by an interplanar spacing of 3.758 Å, indicating a π-π bond interaction. Another feature of the crystalline packing is the presence of a hydrogen bond between hydroxyl H group, from one molecule, and the N1 of the methyl imidazole ring of another, forming in this way a continuous chain of hydrogen bonded molecules, as can be seen in Figure 5.

Table 1 shows the differences between experimental X-ray diffraction and structural relaxation results obtained through DFT calculations, where we can notice that the largest absolute differences are related with hydrogen atoms. It can be seen, from the bond angle results shown in Table 2, that the maximum distortion difference is 5.64° in the C3-C2-H2 angle.

The EPI was further characterized through 1H and 13C NMR spectroscopy. The standard way of chemical shift assignments [22] was compared with theoretical DFT calculations for the isolated molecule and can be seen in Supporting Information S1. The 1H NMR data obtained in this work are in conformity with seminal results reported by Voigtlander et al [6].

Measured and calculated FT-IR and FT-Raman EPI molecule spectra are shown in Figure 6 and Figure 7. The vibrational band assignments, presented in Table 3, were proposed based on general literature about organic molecules [22–25] and a study reported about an isomer of EPI named episopilosine [26]. The vibrational assignments were supported by DFT calculations performed in this work as can be seen in Table 3. The very strong band at 1769 cm⁻¹ is assigned to C = O stretching of the lactone ring, in the FT-IR spectrum shown in Figure 6, and corresponds to the medium intensity band at 1758 cm⁻¹ in the FT-Raman spectrum, shown in Figure 7. The FT-Raman spectrum reveals three strong bands of EPI around 1602–1568 cm⁻¹ (Figure 7) characteristic of the C-C stretching vibrational mode of benzene and imidazole rings; these bands are weak in the FT-IR spectrum. On the other hand, the bands around 1524–1494 cm⁻¹, attributed to modes related to imidazole and lactone groups (see Table 3) are present in the FT-IR spectrum but practically absent in the FT-Raman spectrum. Well-defined band at 1305 cm⁻¹ assigned to the scissoring C9-O1-H vibrational mode of the secondary alcohol group attached to the organic molecule is observed in both spectra. The strong band at 1004 cm⁻¹ (FT-Raman) is attributed to the C-C-C trigonal bending of benzene,

### Table 3. Cont.

| Epiosopiloturine Assignment | Experimental IR | Experimental Raman | Calculated IR | Calculated Raman |
|----------------------------|----------------|--------------------|--------------|-----------------|
| Experimental                | Calculated     | Assignment         |              |                 |
| IR                         | Raman          |                   |              |                 |
| 1568                       | 1568           | 1592               | ν C-C (imidazole) |
| 1587                       | 1586           | 1627               | ν C-C (benzene) |
| 1602                       | 1647           | ν C-C (benzene)    |              |
| 1769                       | 1758           | 1849               | ν(C = O lactone) |

Calculated vibrational wavenumbers (cm⁻¹) for the isolated EPI molecule. A tentative assignment of the observed vibrational modes is also shown. See text for theoretical details. ν = stretching, δ = bending, β = bending in plane, γ = bending out of plane, r = rocking, τ = twist, sc = scissoring, α = wagging, vs = symmetric stretching, va = antisymmetric stretching, sh = shoulder.

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Figure 8. Epiosopiloturine TGA-DSC (A) and DTG-MS (B) curves under air atmosphere.
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while the bands around 990–930 cm\(^{-1}\) are assigned to the bending of the lactone ring, in both FT-IR and FT-Raman spectra. Several bands attributed to the vibration of C-H group of benzene and imidazole rings can be observed in the 900–730 cm\(^{-1}\) range, as listed in Table 3. The low frequency region is dominated by bands associated to vibrations involving C-C-C of benzene, imidazole (600–500 cm\(^{-1}\)) and also to all the EPI structure bending modes (430–400 cm\(^{-1}\)).

The TGA-DSC-MS curves of the isolated EPI are shown in Figure 8, where three events can be observed. The first event (an endothermic process) occurs at 225°C and can be attributed to the melting process of the imidazole alkaloid (Figure 8A). Parly according to Voigtlander et al [6], the melting point of EPI is about 218–219°C when measured using a copper block. In line with the DTG-MS curves (Figure 8B), EPI is decomposed around 230–350°C (ca. 87 wt. %), in air atmosphere, producing water (m/z = 18), carbon monoxide (m/z = 28) and carbon dioxide (m/z = 44) molecules. The third event observed in the temperature range between 360–695°C (12 wt.% is related to the decomposition of the remaining organic molecule, producing H\(_2\)O and CO\(_2\). Under the experimental conditions used in this work, no other released gases were identified. A residual product of 0.5 wt.% can be attributed to some impurities from the raw material and/or introduced during the process of EPI isolation.

**Conclusion**

This work describes, for the first time, an industrial process to obtain EPI in high purity. The treatment with low-polarity solvents combined with HPLC technique allowed the isolation of the EPI alkaloid from jaborandi leaves. The technique of ESI/Ion Trap allowed attesting that its purity is higher than 98%, besides fragment characteristics of imidazole alkaloids produced by MS\(^{3}\).

Single crystal X-ray diffraction data has shown the structure of the EPI molecule as well as its arrangement in solid state. The \(^1\)H and \(^13\)C NMR, IR and Raman spectroscopy data were supported by DFT simulations. Each assay had their contribution to characterize the EPI, allowing the interpretation of the experimental data which shows the integrity of the molecule isolated by the procedures of extraction and purification presented in this paper. According to TGA-DSC data, EPI melts at 225°C, and undergoes decomposition mainly in the 230–350°C range under air atmosphere.

The results presented in this work contribute to the advance of the isolation of EPI and provide a set of structural, spectroscopic and thermal properties of the alkaloid molecule. This study supports efforts to develop EPI as a new antiparasitic agent.

**Supporting Information**

**Figure S1** Experimental (black) and theoretical (red) \(^1\)H NMR EPI spectra. (TIF)

**Figure S2** Experimental (black) and theoretical (red) \(^13\)C NMR EPI spectra. (TIF)

**Supporting Information S1** Chemical Shift Assignments. (DOCX)

**Table S1** EPI \(^1\)H and \(^13\)C NMR chemical shifts. Atom labels accordingly to Figure S1. (DOC)

**Author Contributions**

Conceived and designed the experiments: LMCV JRSAL VRRC DFL PRPS LGV RVLC YPM JRSAL. Wrote the paper: LMCV VRRC FCDAL VYS FCDAL DFL PSCJ JAE PRPS MG HMP VRLC YPM. Analyzed the data: LMCV YDMC MAG MMV VRRC VYS FCDAL DFL PSCJ JAE PRPS MG HMP VRLC YPM JRSAL. Performed the experiments: LMCV JRSAL VRRC DFL PRPS LGV RVLC YPM JRSAL. Wrote the paper: LMCV LGV RVLC YPM JRSAL. Wrote the paper: LMCV LGV RVLC YPM JRSAL. Performed the experiments: LMCV JRSAL VRRC DFL PRPS LGV RVLC YPM JRSAL. Wrote the paper: LMCV LGV RVLC YPM JRSAL.

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