Integrative Approach Based on Leaf Spray Mass Spectrometry, HPLC-DAD-MS/MS, and NMR for Comprehensive Characterization of Isoquinoline-Derived Alkaloids in Leaves of *Onychopetalum amazonicum* R. E. Fr.

Bruna R. de Lima,a Felipe M. A. da Silva,a,b Elzalina R. Soares,a Richardson A. de Almeida,a Francinaldo A. da Silva-Filho,a Andersson Barison,c Emmanoel V. Costa,b Hector H. F. Koolen,d Afonso D. L. de Souzaa,b and Maria Lúcia B. Pinheiroa,b

—Central Analítica-Centro de Apoio Multidisciplinar (CAM), Universidade Federal do Amazonas (UFAM), 69077-000 Manaus-AM, Brazil
—Departamento de Química, Universidade Federal do Amazonas (UFAM), 69077-000 Manaus-AM, Brazil
Laboratório de RMN, Universidade Federal do Paraná (UFPR), 81531-990 Curitiba-PR, Brazil
Grupo de Pesquisa em Metabolômica e Espectrometria de Massas, Universidade do Estado do Amazonas (UEA), 69050-010 Manaus-AM, Brazil

*Onychopetalum* is an Amazonian botanical genus close-related with *Bocageopsis* and *Unonopsis*, whose species are used in traditional medicine. Although these genera are widely investigated from the phytochemical viewpoint, the knowledge about *Onychopetalum* remains almost unexplored. In order to perform a comprehensive characterization of isoquinoline-derived alkaloids from *Onychopetalum amazonicum*, an integrative approach based on the alkaloid profile analysis by leaf spray mass spectrometry (LS-MS), followed by a robust dereplication through high-performance liquid chromatography coupled with diode array detector and atmospheric pressure chemical ionization tandem mass spectrometry, (HPLC-DAD-APCI-MS/MS) and nuclear magnetic resonance (NMR) analysis was adopted. LS-MS allowed the tentative identification of reticuline, norjuziphine, nornuciferine, *O*-methylisopiline, nuciferine, *N*-methyl-*O*-methylisopiline, anonaine, and *N*-methylanonaine. HPLC-DAD-MS/MS analysis supported the tentative identification of *N*-methylasimilobine, asimilobine, norushinsunine, *N*-methylisopiline, isopiline, liriodenine, and lycicamine. Moreover, the integration of MS and NMR data allowed the additional identification of stepholidine and isocorypalmine. The applied integrative approach proposed herein enabled a comprehensive analysis of the alkaloid content from the *O. amazonicum*, showing to be a useful strategy for the dereplication of isoquinoline-derived alkaloids.

**Keywords:** Annonaceae, dereplication, isocorypalmine, leaf spray mass spectrometry, stepholidine

Introduction

*Onychopetalum*, the smaller Annonaceae genus, comprises only two species (*Onychopetalum amazonicum* R. E. Fr. and *Onychopetalum periquino* (Rusby) D. M. Johnson & N. A. Murray). These species are characterized as trees (8-30 m) and present restricted distribution within the Amazon Region.1 *Onychopetalum*, along with *Unonopsis* and *Bocageopsis* are considered botanically close genera, being this close relationship supported by phylogenetic and phytochemical researches.1,2 Some species belonging to these genera are used in traditional medicine, e.g., *U. stipitata* and *U. veneficiorum*, whose leaves are used to treat age-related cognitive disorders by indigenous people.3 From the phytochemical viewpoint, *Unonopsis* and *Bocageopsis* are promising sources of bioactive isoquinoline-derived alkaloids,4,5 while the alkaloidal composition in *Onychopetalum* remains almost unexplored. For *O. amazonicum*, data about the alkaloid content is scarce, with a single report of the isolation of an...
azafluorenone alkaloid from trunk wood, named onychine.\textsuperscript{6} In addition, its terpene content for leaves, twigs and trunk bark was previously reported.\textsuperscript{2,7}

Key fragmentation recognition of isoquinoline-derived alkaloids (e.g., aporphine, tetrahydroprotoberberine, and benzylisoquinoline skeletons) via collision-induced dissociation (CID) has provided a fast and safe way to access these biologically active compounds in complex matrices.\textsuperscript{4,5} Advances on the gas-phase ion chemistry of isoquinoline-derived alkaloids were recently reported to the oxoaporphine\textsuperscript{8} and \textit{N}-formylaporphine classes,\textsuperscript{9} thus providing complementary information to these alkaloids dereplication.

Ambient ionization mass spectrometry (AIMS) techniques, including desorption electrospray ionization (DESI), direct analysis in real time (DART) and leaf spray (LS) have been demonstrated to be rapid and low-cost sampling methods for direct analysis of complex mixtures, without sample preparation, being useful to the screening of organic compounds of plant origin, in special hydrophilic bioactive compounds.\textsuperscript{10,11} Although less sensitive than mass spectrometry (MS), nuclear magnetic resonance (NMR) provides a powerful complementary technique for confirmation of main metabolites in plant extracts.\textsuperscript{12,13} In an integrative way, these techniques enable several analytical possibilities, whether in the prior recognition of complex matrices or seeking pharmacologically active substances.\textsuperscript{14,15}

Thus, the aim of the present work was to perform a comprehensive characterization of isoquinoline-derived alkaloids from leaves of \textit{O. amazonicum}. To achieve this goal, an integrative approach comprising analyses by leaf spray mass spectrometry (LS-MS), high-performance liquid chromatography coupled with diode array detector and tandem mass spectrometry (HPLC-DAD-MS/MS), and nuclear magnetic resonance (NMR) spectroscopy was applied.

**Experimental**

**Plant material**

Leaves of \textit{Onychopetalum amazonicum} R. E. Fr. (Annonaceae) were collected in March 2014 at the Adolpho Ducke Forest Reserve (26 km along the AM-010 highway, in the city of Manaus, Amazonas state, Brazil, 2°59'15.9''S 59°55'35.5''W), from a specimen previously catalogued during the Flora project.\textsuperscript{16} The access to genetic heritage was registered at Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) under the code No. AE0F182. A voucher (No. 218341) was deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA). The material was immediately dried over ambient temperature (ca. 20 °C) during 20 days.

**Leaf spray mass spectrometry (LS-MS) analysis**

The home-built LS-MS interface was achieved through the substitution of the housing for a default Db15 male connector to disable the atmospheric pressure ionization (API) interlocks (Figure 1).\textsuperscript{17} To allow the easy change between different API sources (electrospray ionization-ESI or atmospheric pressure chemical ionization-APCI) and LS, a jumper to bypass the interlock was created between sockets 9 and 10, and a 2 kOhm resistor was attached directly between sockets 7 and 8, generating the source recognition through the ESI source ID read back voltage. This configuration was tested with both, LCQ Fleet (ion trap) and TSQ Quantum Access (triple quadrupole) mass spectrometers (Thermo-Fisher, CA, San Jose).

The MS and MS\textsuperscript{2} measurements of LS were performed with the TSQ Quantum Access mass spectrometer. Mass spectra were acquired in a continuous monitoring mode. Dried leaves were cut into a triangle-shaped piece (base and height of 10 mm each) and held by a metal clip at a distance of 10 mm from the mass spectrometer inlet (Figure 1). High voltage (4.5 kV, positive polarity) was applied directly to the plant tissue after wetting it with approximately 10 µL of MeOH HPLC grade in order to generate a spray of charged droplets carrying endogenous chemicals toward the inlet of the mass spectrometer. MS analytical conditions were: capillary voltage, 20 V; capillary temperature, 200 °C; tube lens, 115 V; scan range: 100-500 \textit{m/z}. Argon (Ar) was used as collision gas with collision energies ranging from 20 to 30 eV.

![Figure 1. Home-built LS-MS interface with a Db15 male connector prepared to defeat the API interlock and promote ESI source recognition (a). \textit{O. amazonicum} leaf cut in triangle-shaped pieces (base and height of 10 mm each) and held by a high voltage connector at 10 mm of distance from the mass spectrometer inlet (b).](image-url)
Alkaloid extraction

The alkaloid fraction was obtained according to a previously reported methodology. After dried, the plant material was powdered with a knife mill. The powdered leaves (2 g) were vigorously stirred using a vortex mixer for 1 min with a mixture of 10% ammonium hydroxide (NH₄OH) aqueous solution (20 mL) and dichloromethane (CH₂Cl₂) (20 mL) in a glass container. The organic phase was transferred to another glass container and vigorously stirred (1 min) with a 10% acetic acid (CH₃CO₂H) aqueous solution (20 mL). After, the aqueous phase was separated and treated with NH₄OH until pH 10 was achieved. Then, this phase was extracted under vigorous stirring (1 min) with CH₂Cl₂ (20 mL). The organic phase was dried over anhydrous sodium sulfate, transferred to a new container and the solvent evaporated to dryness under a nitrogen gas stream, yielding the crude alkaloid fraction (8.2 mg, 0.41%).

HPLC-DAD-MS/MS and high-resolution mass spectrometry (HR-MS) analysis

The alkaloid fraction was diluted to 1 mg mL⁻¹ in MeOH and analyzed (10 µL, injection volume) with an Accela HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with a Phenomenex Luna C18 column (5 µm, 150 × 4.6 mm internal diameter, i.d.) (Torrance, CA, USA). The mobile phase consisted of 0.1% trifluoroacetic acid aqueous solution (A) and MeOH (B). Elution was performed using a flow of 1 mL min⁻¹ and through a gradient of 20% B to 80% B over 14 min, followed by 10 min at 80% B. UV spectra were registered from 200 to 400 nm. The outlet from the diode array detector (DAD) was connected through a split valve (flow of 300 µL min⁻¹) and benzylisoquinoline alkaloids, with base peaks at m/z 328 and 342; Figures S8 and S10, SI section) is in accordance with two methoxyl groups at D ring, while the loss of 164 Da (precursor m/z 328 and 342; Figures S8 and S10, SI section) suggest the presence of methoxyl and hydroxyl groups at A ring (Figure 3a). In addition, the tetrahydroprotoberberine alkaloids containing methoxyl formed via a retro-Diels-Alder (RDA) ring opening 18 for the tetrahydroprotoberberine alkaloids containing methoxyl and a hydroxyl group at the A ring (Figure 3a). In addition, the neutral loss of 150 Da (precursor m/z 328; Figure S8, SI section) suggest the presence of methoxyl and hydroxyl groups at D ring, while the loss of 164 Da (precursor m/z 342; Figure S10, SI section) is in accordance with two methoxyl groups at D ring.

Although the fragmentation patterns observed to the protonated molecules at m/z 328 and 342 have been previously described in Unonopsis and Bocageopsis
species, the exact substitution pattern remained unknown, once for this skeleton different positions with the same substituents will provide the same fragmentation pattern. Similarly, base peak fragment ions at \( m/z \) 192 (precursor \( m/z \) 330; Figure S9, SI section) and 107 (precursor \( m/z \) 286; Figure S4, SI section) have been previously associated with benzylisoquinoline skeletons with and without an \( N \)-methyl group, respectively (Figure 3b). The presence or absence of this group in benzylisoquinoline skeletons directly influences the fragmentation pathway. The ion at \( m/z \) 192 agrees with the presence of a methoxyl and hydroxyl groups at A ring, while the ion at \( m/z \) 107 agrees with a hydroxyl group at C ring. On the other hand, the loss of 138 Da (precursor \( m/z \) 330; Figure S9, SI section) and 179 Da (precursor \( m/z \) 286; Figure S4, SI section) suggest the presence of a methoxyl and hydroxyl groups at C and A ring, respectively. To reinforce this idea, other diagnostic product ions were observed, where \( m/z \) 299, 175, 143, and 137, from \( m/z \) 330, and \( m/z \) 269 and 143, from \( m/z \) 286, were consistent with the benzylisoquinoline alkaloids reticuline (1) and norjuziphine (2), respectively, (Figure 4). The MS\(^2\) spectra of the protonated molecules at \( m/z \) 282, 296, 312 and \( m/z \) 326 presented pathways consistent with aporphine alkaloids with adjacent methoxyl groups at the A ring, and with presence or absence of \( N \)-methyl groups. Consistently, their losses were assigned 17 Da (-NH\(_3\)) (\( m/z \) 282 → 265; Figure S3, SI section, and \( m/z \) 312 → 295; Figure S6, SI section) or 31 Da (-NH\(_2\)CH\(_3\)) (\( m/z \) 296 → 265; Figure S5, SI section and \( m/z \) 326 → 295; Figure S7, SI section), followed by competitive losses of 15 Da (-CH\(_3\)) (\( m/z \) 265 → 250; Figure S3, SI section, and \( m/z \) 295 → 280; Figure S6, SI section) and 31 Da (-OCH\(_3\)) (\( m/z \) 265 → 234; Figure S5, SI section, and \( m/z \) 295 → 264; Figure S7, SI section).

Figure 2. LS-MS alkaloid profile (a) from the leaf of *O. amazonicum* with * denoting the even \( m/z \) of possible isoquinoline-derived alkaloid ions. HPLC-MS total ion chromatogram (TIC) from crude alkaloid fraction (b).
section), confirming the two adjacent methoxyl groups (Figure 5). Therefore, the fragmentation patterns observed for the protonated molecules at \( m/z \) 282 and 312 were consistent with the aporphine alkaloids nornuciferine (3) and O-methylisopiline (4), respectively, and \( m/z \) 296 and 326 were as their N-methyl forms, nuciferine (5) and N-methyl-O-methylisopiline (6).

In addition, MS\(^2\) spectra of the protonated molecules at \( m/z \) 266 and 280 presented strong evidence of the aporphine skeletons containing a methylenedioxy group with absence and presence of N-methyl group, respectively. An initial loss of 17 Da (-NH\(_3\)) (\( m/z \) 266 \( \rightarrow \) 249; Figure S1, SI section) or 31 Da (-NH\(_2\)CH\(_3\)) (\( m/z \) 280 \( \rightarrow \) 249; Figure S2, SI section), followed by subsequent losses of 30 Da (CH\(_2\)O) (\( m/z \) 249 \( \rightarrow \) 191; Figures S1 and S2, SI section) and 28 Da (-CO) (\( m/z \) 219 \( \rightarrow \) 191; Figures S1 and S2, SI section), observed in both spectra, reinforced the structural proposal (Figure 6). Therefore, the observed fragmentation patterns are consistent with the aporphine alkaloids anonaine (7) and its N-methyl form, N-methylanonaine (8), respectively. Thus, the initial profile by the positive LS-MS allowed the tentative identification of 8 isoquinoline-derived alkaloids, with tetrahydroprotoberberines and aporphine as the major compounds. However, it should be noted that the recognition of isobaric and minor compounds from direct MS analysis can have been compromised by matrix effect,\(^2^2\) being required HPLC-DAD-MS analysis for a more comprehensive investigation.

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**Figure 3.** Proposed fragmentation pathways for the major product ions observed in LS-MS/MS spectra of tetrahydroprotoberberine (\( m/z \) 328 and 342) (a) and benzylisoquinoline (\( m/z \) 286 and 330) (b) alkaloids.
Figure 4. Tentatively identified isoquinoline-derived alkaloids from the leaves of *O. amazonicum*.

Figure 5. Proposed fragmentation pathways for the major product ions observed in LS-MS/MS spectra of aporphine alkaloids with adjacent methoxyl groups at ring A (m/z 282, 296, 312 and 326).
HPLC-DAD-MS/MS analysis

The HPLC-MS total ion chromatogram (TIC) of the crude alkaloid fraction (Figure 2b) corroborated with the LS-MS data. Thereby, the most intense ions of the LS-MS experiment, tetrahydroprotoberberine alkaloids at \( m/z \) 328 and 342 were the main peaks I (retention time \( t_R \) 6.19 min) and IV \( t_R \) 7.80 min) at the HPLC-MS analysis (Table 1), respectively. The protonated non-\( N \)-methylated aporphine alkaloids at \( m/z \) 282, 266 and 312 were observed at the peaks X, XI and XIII \( t_R \) 10.61, 10.91 and 11.84 min, respectively, while their \( N \)-methyl homologs at \( m/z \) 296, 280 and 326 were observed at the peaks VIII, IX and XII \( t_R \) 10.23, 10.43 and 11.38 min, respectively, suggesting that \( N \)-methyl aporphine alkaloids present lower retention in C-18 column compared to their non-\( N \)-methylated homologues. Benzylisoquinolines were also observed at the TIC, where \( m/z \) 330 (peak II, \( t_R \) 6.79 min) and 286 (peak V, \( t_R \) 8.59 min) presented chromatographic behavior similar to tetrahydroprotoberberines.

The HPLC-MS analysis also displayed the presence of minor isobaric alkaloids related to values recorded by LS-MS at \( m/z \) 342 (peak III, \( t_R \) 7.30 min), 282 (peaks V, 8.59 min and VII, \( t_R \) 9.56 min), and 312 (peak IX). MS\(^2\) spectrum of the \( m/z \) 342 ion (peak III) recorded fragments at \( m/z \) 192 (base peak) and 165 (Figure S34, SI section), suggesting a benzylisoquinoline derivative. MS\(^2\) spectrum of the \( m/z \) 282 ion (peak V), suggested the presence of an \( N \)-methyl aporphine structure with vicinal hydroxyl and methoxy groups. The initial loss of 31 Da (-NH\(_2\)CH\(_3\)) (\( m/z \) 282 \( \rightarrow \) 251), followed by subsequent losses of 32 Da (-CH\(_3\)OH) (\( m/z \) 251 \( \rightarrow \) 219) and 28 Da (-CO) (\( m/z \) 219 \( \rightarrow \) 191) (Figure S37, SI section) reinforced this proposal (Figure 6). This fragmentation pattern was consistent with the compound \( N \)-methylasimilobine (9), the \( N \)-methylated form of asimilobine (10), which was observed at peak VI (\( m/z \) 268, \( t_R \) 9.10 min). The latter was also confirmed by its fragmentation pattern (\( m/z \) 268 \( \rightarrow \) 251 \( \rightarrow \) 219 \( \rightarrow \) 191; Figure S38, SI section), which is in agreement with previous data.\(^4\)\(^5\) The third isobaric alkaloid at \( m/z \) 282 (peak VII) presented in its MS\(^2\) spectrum an initial loss of 18 Da (-H\(_2\)O) (\( m/z \) 282 \( \rightarrow \) 264), followed by subsequent losses of 30 Da (-CH\(_3\)OH) (\( m/z \) 264 \( \rightarrow \) 234) and 28 Da (-CO) (\( m/z \) 234 \( \rightarrow \) 206) (Figure S39, SI section). This uncommon fragmentation is in coherence to the aporphine alkaloid norushinsunine (11), a 7-hydroxyaporphine derivative.

Figure 6. Proposed fragmentation pathways for the major product ions observed in LS-MS/MS and HPLC-MS/MS spectra of aporphine alkaloids with adjacent hydroxyl and methoxyl groups at ring A \( (m/z \) 268 and 282) (a) and methylenedioxy bridge at ring A \( (m/z \) 266 and 280) (b).
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containing a methylenedioxy group, whose loss of water and a subsequent 1,2-hydride shift resulting in a more stable fragment ion (Figure 7). This observation suggests that a substitution at C-7 in aporphine skeletons has substantial influence over the first loss, but not the subsequent. The confirmation of this substance was performed through comparison of the MS2 spectrum with standard previously isolated and identified by the authors. 21

The fourth minor isobaric at \( m/z \) 312 (peak IX) presented MS2 fragmentation consistent with an \( N \)-methyl aporphine derivative with adjacent methoxyl groups (\( m/z 312 \rightarrow 281 \rightarrow 266 \rightarrow 250 \); Figure S43, SI section), which is compatible with the alkaloid \( N \)-methylisopiline (12). Its non-\( N \)-methylated homolog was observed at \( m/z 298 \) (peak X) (\( m/z 298 \rightarrow 281 \rightarrow 266 \rightarrow 250 \); Figure S46, SI section) and attributed to the aporphine alkaloid isopiline (13). The confirmation of this proposal was performed through comparison of the MS2 spectrum with a standard previously isolated and identified by the authors. 20 Additionally, two protonated oxoaporphine alkaloids observed at \( m/z \) 276 (peak VII) and 292 (peak XIII) were tentatively identified as liriodenine (14) and lycisamine (15), respectively, through comparison of their MS2 spectra with those reported in the literature. 8

To reinforce the tentative identification of the proposed isoquinoline-derived alkaloids, the DAD spectrum of the main chromatographic peaks were compared among themselves. Initially, it was observed the strong spectral similarity between the tetrahydroprotoberberine alkaloids present at the peaks I and IV (Figure 8a), pointing to the same substitution pattern, which reinforces that the protonated alkaloid at \( m/z \) 342 (peak IV) is a methylation product of the alkaloid at \( m/z \) 328 (peak I), as evidenced by MS2 analysis. Similar behavior was also observed for the aporphine derivatives, where the peaks VIII and X, IX and XI, and XII and XIII shared similar UV spectra (Figures S26-S31, SI section). Discreet difference between the peaks IX and XI may be due to the co-elution effect of \( N \)-methylanonaine and \( N \)-methylisopiline in peak IX, and anonaine and isopiline in peak XI (Figure 8b). The absence of significant hypsochromic or bathochromic shifts for the other peaks agrees with the inclusion of a methyl group at the heterocyclic nitrogen. Since aporphine and noraporphine alkaloids present invariably substitution at positions 1 and 2, with prominent peaks at 270-280 nm and a shoulder or a smaller peak at 310-320 nm in UV spectra when positions 10 and 11 are unsubstituted, 23 it is plausible the C-3 substitution proposals by MS2 for the aporphine

| Peak | Compound                        | \( t_r \) / min | Class | \([M + H]^+\) | MS/MS | \( \lambda_{max} \) / nm |
|------|---------------------------------|-----------------|-------|---------------|-------|--------------------------|
| I    | stepholidine (16)               | 6.19            | T     | 328           | 178*, 151 | 227, 283                 |
| II   | reticuline (1)                  | 6.79            | B     | 330           | 299, 192*, 175, 143, 137 |
| III  | unknown                         | 7.30            | B     | 342           | 192*, 165   |                          |
| IV   | isocorypalmine (17)             | 7.80            | T     | 342           | 178*, 165, 151 | 232, 282             |
| V    | norjuaizamine (2)               | 8.59            | B     | 286           | 269, 237, 143, 107*      |
| VI   | \( N \)-methylanonaine (8)      |                 | A     | 282           | 251, 219, 191*          |
| VII  | asimilobine (10)                | 9.10            | A     | 268           | 251, 219, 191*          |
| VIII | norushinsuxine (11)             | 9.56            | A     | 282           | 264, 234, 206*          |
| IX   | \( N \)-methylasimilobine (9)   |                 | O     | 276           | 248*, 220              |
| X    | nuciferine (5)                  | 10.23           | A     | 296           | 265, 250, 234*          |
| XI   | \( N \)-methylanonaine (8)      | 10.43           | A     | 280           | 249*, 219, 191          |
| XII  | \( N \)-methylisopiline (12)    |                 | A     | 312           | 281, 266, 250*          |
| XIII | \( O \)-methylisopiline (4)     | 11.38           | A     | 326           | 295, 280*, 264          |
| XIV  | anonaine (7)                    | 11.84           | A     | 312           | 295, 280*, 264          |
| XV   | lycisamine (15)                 |                 | O     | 292           | 277*, 248              |

*Retention time; *aporphine (A), benzylisoquinoline (B), tetrahydroprotoberberine (T) or oxoaporphine (O); *main product ions obtained in QqQ-MS/MS analyses with * denoting the base peak; \( \lambda_{max} \) between 200-400 nm.
alkaloids N-methylisopiline (12), isopiline (13), N-methyl-O-methylisopiline (6), and O-methylisopiline (4). Thus, the HPLC-DAD-MS/MS analysis allowed additionally to LS-MS the tentative identification of seven isoquinoline-derived alkaloids, besides to access additional information such as chromatographic behavior of analogous alkaloids and the UV spectra profile, providing a more comprehensive investigation of the O. amazonicum leaf alkaloid content. At this point, the exact substitution pattern of the major tetrahydroprotoberberine alkaloids present at peaks I and IV remained unknown, being the crude alkaloid fraction subjected to 1D and 2D NMR analysis for the unequivocal assignment of the major compounds.

Tetrahydroprotoberberine 1D and 2D NMR analysis

Ratifying the LS-MS analysis, in which the main alkaloids in the leaf sample were from the tetrahydroprotoberberine class (m/z 328 and 342), the 1H NMR spectrum of the total alkaloid fraction exhibited as the major signals typical doublet signals at δH 6.79 (d, J 8.5 Hz) and 6.73 (d, J 8.5 Hz), an indicative of an ortho-substituted system, as well singlet signals at δH 6.75 (s) and 6.67 (s), characteristic of para-substituted tetrahydroprotoberberine alkaloids, and two methoxyl signals at δH 3.82 (s) and δH 3.80 (s). Correlations of these signals with the carbons at δC 125.2, 116.3, 112.3, 112.9, 56.3 and 60.2, respectively, were observed in the heteronuclear single quantum coherence (HSQC) map. The confirmation of the ortho pattern at D ring was reached through the heteronuclear multiple bond correlation (HMBC) experiment. In this, J3-couplings for the proton at δH 6.79 (H-12) with carbons at δC 36.2 (C-13), 128.3 (C-8a), and 148.7 (C-10), and for the proton at δH 6.73 (H-11) with carbons at δC 126.7 (C-12a) and 144.8 (C-9) evidenced our proposal. This was reinforced by the J3-coupling between the methoxyl protons at δH 3.80 (s) and the C-9 carbon. Thus, the structure of the main tetrahydroprotoberberine alkaloid was established as stepholidine (16) and supported by comparison of the NMR data with those existing in the literature. This proposal is in accordance with the molecular formula C19H22NO4 (m/z 328.1539, Figure S59, SI section), as determined by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). Likewise, another set of aromatic protons, analog to that observed to the stepholidine’s ortho-substituted system, was observed at δH 3.80 (s) and the C-9 carbon. Thus, the structure of the main tetrahydroprotoberberine alkaloid was established as isocorypalmine (17), which is consistent with the literature data. This proposal is also in accordance with the molecular formula C20H23NO4 (m/z 342.1697, Figure S60, SI section), as determined.
by HR-ESI-MS. Several biological activities have been described for these major alkaloids, highlighting the stepholidine potential in the treatment of schizophrenia and Parkinson’s disease, and the potential of both alkaloids as an antipsychotic drug with dual behavior D1 agonist and D2 antagonist receptors.

Conclusions

The integrative approach based on the alkaloid profile analysis by LS-MS, a rapid and low-cost sampling methods for direct analysis of complex mixtures, followed by a complementary and robust dereplication through HPLC-DAD-MS/MS and NMR analysis showed to be a useful strategy for dereplication of isoquinoline-derived alkaloids. Tetrahydroprotoberberine, aporphine and analogue alkaloids were highlighted as major compounds in the leaves of *O. amazonicum*, while benzylisoquinoline and oxoaporphine presented discrete distribution. Our findings in the phytochemical study of *O. amazonicum* reinforce the chemotaxonomic significance of isoquinoline-derived alkaloids for Annonaceae family, particularly, to the *Onychopetalum* and close-related genera.

Supplementary Information

Supplementary information (Figures S1-S60 and Table S1) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

The authors are grateful to Prof Richard N. Zare and Prof Boniek Gontijo Vaz for their help with the home-built LS-MS interface, Central Analítica (UFAM) for MS and NMR analysis, and CAPES, CNPq, FINEP, FAPEAM and FAPESP (Proc. 2014/50244-6) for the financial support.

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