FULL PAPER

(+)-*N*-Formylnorglaucine Rotamers from *Unonopsis stipitata* Diels

by **Felipe M. A. da Silva***[a]), **Francinaldo A. da Silva Filho**[a]), **Bruna R. de Lima**[a]), **Richardson A. de Almeida**[a]), **Dácio M. Mendonça**[b]), **Raimundo C. Pereira Junior**[a]), **Lívia M. Dutra**[c]), **Andersson Barison**[c]), **Hector H. F. Koolen**[d]), **Afonso D. L. de Souza**[a]), and **Maria Lúcia B. Pinheiro**[a])

[a]) Department of Chemistry, Federal University of Amazonas, Manaus-AM, 69077-000, Brazil (phone: +55 92 982517341; e-mail: felipemas@ufam.edu.br)

[b]) Amazon Biotechnology Center, Manaus-AM, 69075-351, Brazil

[c]) NMR Center, Federal Universisty of Paraná, Curitiba-PR, 81531-990, Brazil

[d]) DeMpSter Mass Spectrometry Group, Amazonas State University, Manaus-AM, 69050-010, Brazil

(+)-*N*-formylnorglaucine (**1**), an aporphine alkaloid containing a formyl group linked to the heterocyclic nitrogen, was isolated from the leaves of *Unonopsis stipitata*, an Amazon medicinal plant. The chemical structure was characterized based on 1D- and 2D-NMR spectroscopy and HR-ESI-MS. NMR spectra revealed that **1** is composed of two rotamers (**1a** and **1b**) in a ratio of approximately 2:1. In addition, the fragmentation behavior of **1** displayed an unusual fragmentation pattern compared to regular aporphine alkaloids. Thus, this compound is reported for the first time as a natural product in this study.

Keywords: *Unonopsis stipitata*, Aporphine alkaloid, Rotamers, (+)-*N*-Formylnorglaucine, Annonaceae.

Introduction

Unonopsis (Annonaceae) is a neotropical genus with broad distribution through the Amazon region [1]. Some species of *Unonopsis* have been described due to its medicinal applications [2][3], including *Unonopsis stipitata* whose pulverized leaves are employed in the treatment of brain disorders [3]. A recent study indicated that *U. stipitata* is a promising source of aporphine alkaloids [4], which are extensively explored in synthetic studies due to promising biological activities observed for several aporphinoid structures [5 – 8].

Besides its attractive biological properties, the fast recognition and dereplication of this class in complex matrices by mass spectrometry (MS) has accelerated the research for new compounds [9 – 11]. This favorable situation is possible due to the advances on the gas-phase chemistry of aporphines through key fragmentation recognition via collision-induced dissociation (CID) experiments [12].

Aiming the discovery of new alkaloids from Amazon medicinal plants, we carried out a chemical investigation of the leaves of *U. stipitata*, which resulted in the isolation of a new natural aporphine compound. In the present work, we chemically characterized this substance through 1D- and 2D-NMR techniques and MS. In addition, the fragmentation behavior by CID was investigated through high-resolution MS.

Results and Discussion

Structure Elucidation

Compound **1** was obtained as a yellow amorphous solid with molecular formula $C_{21}H_{24}NO_5$ (*m*/*z* 370.1627), as determined by HR-ESI-MS. The ^{1}H-NMR spectrum indicated duplicate aromatic, CH, methoxyl, and CH_2 signals in $CDCl_3$ (*Tables 1* and *2*) at a ratio of approximately 2:1, suggesting the presence of two rotameric isomers (**1a** and **1b**) [13 – 15]. This ratio was estimated based on the integration of aromatic signals. Therefore, each signal of the two rotamers was individually assigned.

In the ^{1}H-NMR spectrum, signals of eight deshielded H-atoms were observed at δ(H) 6.62 (*s*, H–C(3)), 6.80 (*s*, H–C(8)), 8.14 (*s*, H–C(11)), and 8.26 (*s*, N–CHO), which are relative to the main rotamer **1a**, and at δ(H) 6.65 (*s*, H–C(3′)), 6.77 (*s*, H–C(8′)), 8.15 (*s*, H–C(11′)), and 8.39 (*s*, N–CHO') for the minor rotamer **1b**. Besides, four MeO groups were observed at δ(H) 3.68 (*s*, MeO–C(1)), 3.91 (*s*, MeO–C(2)), 3.91 (*s*, MeO–C(10)), and 3.92 (*s*, MeO–C(9)) for **1a**, whereas MeO group signals at δ (H) 3.67 (*s*, MeO–C(1′)), 3.91 (*s*, MeO–C(2′)), 3.92 (*s*, MeO–C(10′)), and 3.94 (*s*, MeO–C(9′)) were attributed to **1b**. A typical CH signal was observed at *δ*(H) 4.92 (*dd*, *J* = 14.2, H–C(6a)) for **1a** and δ(H) 4.49 (*dd*, *J* = 14.4, 4.5, H–C(6a′)) for **1b**, suggesting that **1** has an aporphine skeleton [10][13 – 15]. Through HSQC experiments, it

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Table 1. Assignment of ^{1}H- and ^{13}C-NMR data for (*Z*)-rotamer **1a**. δ in ppm, *J* in Hz

Position	δ(H)[a])	δ(H)[a])[b])	HMBC[a])
1	–	145.3	–
1a	–	–	–
2	–	152.6	–
3	6.62 (*s*)	110.8	31.2, 124.8, 145.3, 152.6
3a	–	–	–
3b	–	124.8	–
4	2.74 – 2.78 (*m*), 2.89 – 2.95 (*m*)	31.2	–
5	3.38 – 3.44 (*m*), 3.81 – 3.85 (*m*)	42.4	–
6a	4.92 (*dd*, *J* = 14.2, 4.0)	49.9	–
7	2.72 – 2.76 (*m*), 3.07 (*dd*, *J* = 13.6, 4.4)	33.7	49.9, 111.5, 124.9, 129.3
7a	–	129.3	–
8	6.80 (*s*)	111.5	33.7, 124.1, 147.8
9	–	148.4	–
10	–	147.8	–
11	8.14 (*s*)	112.0	148.4, 129.3
11a	–	124.1	–
1-MeO	3.68 (*s*)	60.3	145.3
2-MeO	3.91 (*s*)	56.1	152.6
9-MeO	3.92 (*s*)	56.1	148.4
10-MeO	3.91 (*s*)	56.1	147.8
N-CHO	8.26 (*s*)	162.3	49.9

[a]) 600 MHz for ^{1}H, 150 MHz for ^{13}C in $CDCl_3$. [b]) Protonated C-atoms assigned using an HSQC experiment and quaternary C-atoms assigned using the HMBC experiment.

Table 2. Assignment of ^{1}H- and ^{13}C-NMR data for (*E*)-rotamer **1b**

Position	δ(H)[a])	δ(H)[a])[b])	HMBC[a])
1′	–	145.2	–
1a′	–	–	–
2′	–	152.6	–
3′	6.65 (*s*)	111.0	29.6, 124.2, 145.2
3a′	–	–	–
3b′	–	124.2	–
4′	2.72 – 2.74 (*m*), 2.80 – 2.85 (*m*)	29.6	–
5′	3.16 – 3.21 (*m*), 4.43 (*dt*, *J* = 12.9, 4.0)	36.7	–
6a′	4.49 (*dd*, *J* = 14.4, 4.5)	53.8	–
7′	2.71 – 2.73 (*m*), 3.07 – 3.12 (*m*)	37.6	–
7a′	–	128.7	–
8′	6.77 (*s*)	111.2	37.6, 124.3, 148.0
9′	–	148.5	–
10′	–	148.0	–
11′	8.15 (*s*)	112.0	128.7, 148.5
11a′	–	124.3	–
1-MeO′	3.67 (*s*)	60.3	145.2
2-MeO′	3.91 (*s*)	56.1	152.6
9-MeO′	3.94 (*s*)	56.1	148.5
10-MeO′	3.92 (*s*)	56.1	148.0
N-CHO′	8.39 (*s*)	162.3	–

[a]) 600 MHz for ^{1}H, 150 MHz for ^{13}C in $CDCl_3$. [b]) Protonated C-atoms assigned using an HSQC experiment and quaternary C-atoms assigned using the HMBC experiment.

was observed that the H-atoms at δ(H) 8.26 (N–CHO) and 8.39 (N–CHO′) present J^1 correlation with the same C-atom signal at δ(C) 162.3. These spectroscopic characteristics were previously reported to *N*-formyl aporphine structures [13 – 15]. *N*-formyl functionality has been reported to allow the observation of the rotameric phenomenon for different natural compounds in NMR spectroscopy [16][17].

The multiplicity pattern observed for the aromatic H-atoms agree with tetrasubstituted aporphine skeletons at positions 1, 2, 9, and 10 [10][13 – 15]. This hypothesis was confirmed through characteristic correlations

observed for **1a** at 2D-spectra. For the most shielded aromatic H-atom (H–C(3)), J^1 correlation with the C-atom at δ(C) 110.8 (C(3)) was observed in the HSQC experiment, and ^2J or ^3J correlations with C-atoms at δ(C) 31.2 (C(4)), 124.8
(C(3b)), 145.3 (C(1)), and 152.6 (C(2)) for HMBC. The influence of the *N*-formyl group over C(4) was noticed through its deshielded chemical shift in comparison to regular C(4) aporphine shifts [10][13 – 15]. Besides C(4), CH_2 C-atoms at C(5) (δ(C) 42.4) and C(7) (δ(C) 33.7) are fundamental for the structural determination of this kind of skeleton. In general, C(4) is the most shielded C-atom in aporphine skeletons, followed by C(7) and C(5) [10] [13 – 15].

The substitution pattern for the *A* ring was confirmed through the J^1 correlations for the MeO H-atoms at δ(H) 3.68 and 3.91 with C-atoms at δ(C) 60.3 (MeO–C(1)) and 56.1 (MeO–C(2)), and the ^3J correlations with C-atoms at δ(C) 145.3 (C(1)) and 152.6 (C(2)), respectively. The signal at δ(H) 6.80 was assigned to H–C(8) after the observation of ^3J correlations with C(7), the second most shielded C-atom, C(11a) (δ(C) 124.1) and C(10) (δ(C) 147.8). For the H-atom at δ(H) 8.14 (H-11), ^3J correlations were observed with C-atoms at δ(C) 129.3 (C(7a)) and 148.4 (C(9)). These observations reinforced that **1a** has a 9,10-disubstituted ring *D*, which was confirmed through the correlations for the MeO H-atoms at δ(H) 3.91 and 3.93 with C-atoms at δ(C) 147.8 (C(10)) and 148.4 (C(9)), respectively. Thus, the structure was established as *N*-formylnorglaucine (*Fig. 1*). Considering that the biosynthetic pathway of *N*-formylnorglaucine from its precursor norglaucine, compound previously reported from *U. stipitata* [4], does not affect the configuration of C(6a), the (*S*)-configuration was suggested to this stereocenter. This proposal was supported through the observation of similar optical rotation sign between these two compounds [18].

The spectral data for **1b** were consistent with those described for **1a**. The conformation of each formyl group was deduced by the 1D-NOESY correlations (*Fig. 2*). For the formyl H-atoms at δ(H) 8.26 (**1a**) and 8.39 (**1b**) were observed spatial correlations with the CH_2 and CH H-atoms at δ(H) 3.83 (H–C(5′)) and 4.49 (H–C(6a)), respectively. Through these observations, the main rotamer **1a** was assigned as a (*Z*)-rotamer, which was in accordance with the aporphine literature data [13 – 15].

In the HR-ESI-MS/MS spectrum of the ion at *m*/*z* 370.1627 $[M + H]^+$ were observed competitive initial neutral losses of NH_2COH (−45.0241 u, *m*/*z* 370.1627 → 325.1386) and MeOH (−32.0285 u, *m*/*z* 370.1627 → 338.1342), besides subsequent losses of carbon monoxide (CO) (−27.9994 u, *m*/*z* 338.1342 → 310.1349) and NH_3 (−17.0255 u, *m*/*z* 310.1349 → 293.1094). Generally, aporphine skeletons when subjected to fragmentation investigations present well-defined losses of *N*-linked groups (typically –H or –Me), followed by losses of the peripheral substituents. The effect of substituents groups, such as MeO, MeO with OH groups in adjacent positions and methylenedioxy bridge groups, over fragmentation patterns has been widely investigated [9 – 12]. For **1**, the presence of *N*-formyl seems to affect the expected fragmentation behavior. The loss of the *N*-linked group is discrete and competes with a proposed protonation of the peripheral MeO group, which results in unusual losses of MeOH, followed by CO and NH_3 (*Fig. 3*). This unusual pattern can be related to the partial C=C bond character assumed by C–N bond through resonance, as supported by the NMR observations.

Conclusions

In summary, the chemical investigation of an Amazon medicinal plant resulted in the isolation of a *N*-formyl aporphine compound. To the best of our knowledge, this is the first report of *N*-formylnorglaucine as a natural product. The unusual fragmentation pattern observed by high-resolution MS increases the knowledge about aporphine dissociation. This may be useful to support the fast recognition of this class in complex matrices, allowing the prioritization of the isolation of substances not yet identified, dismissing known compounds and simplifying the phytochemical studies.

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Experimental Part

General

All solvents used for chromatographic and MS experiments were HPLC grade and were purchased from *Tedia* (Fairfield, OH, USA), and water was purified using a *Milli-Q* system (*Millipore*, Bedford, MA, USA). Semi-prep. HPLC: *Shimadzu* (Columbia, MD, USA) *UFLC* system (*LC-6 AD* pump; *DGU-20A5* degasser; *SPD-20AV* UV detector; rheodyne injector; *CBM-20A* communication module). Optical rotations: *343* polarimeter (*PerkinElmer*, Wellesley, MA, USA). 1D- and 2D-NMR spectra: *AVANCE III 600* NMR (*Bruker*, Karlsruhe,

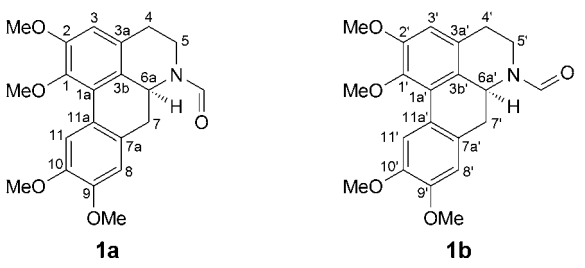

Fig. 1. Structures of **1a** and **1b** rotamers of *N*-formylnorglaucine.

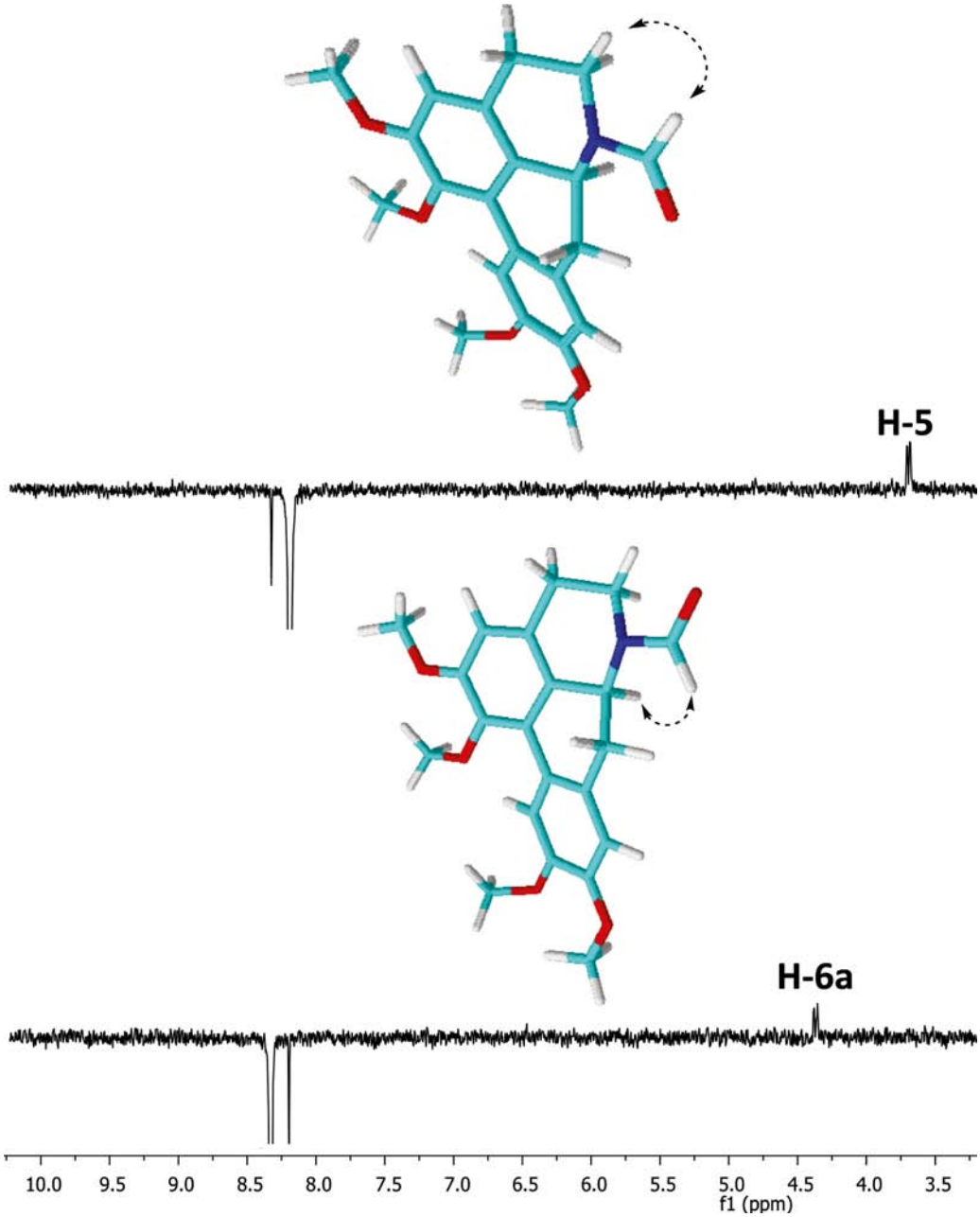

Fig. 2. 1D-NOESY correlations observed for *N*-formyl H-atoms.

Germany) spectrometer, operating at 14.1 T, observing ^{1}H at 600 and ^{13}C at 150 MHz; in $CDCl_3$ (*Cambridge Isotope*, Tewksbury, MA, USA); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *micrOTOF-Q II* (*Bruker*, Billerica, MA, USA) mass spectrometer; in *m*/*z*.

Plant Material

Leaves of *U. stipitata* were collected at the campus of the Federal University of Amazonas (UFAM), in September 2012. The specimen was identified by Dr. *Antonio Carlos Webber* from the Biology Department from UFAM. A voucher specimen was deposited with the Herbarium of UFAM under registration number 8164.

Extraction and Isolation

The botanic material was dried over ambient temp. (*ca.* 20 °C) and powdered. The crude alkaloid fraction was obtained according to a previously reported method [11], as follows: the leaf powder (300 g) was extracted with a solution 10% NH_4OH (2 l) and CH_2Cl_2 (2 l) at ambient temp. (*ca.* 20 °C) for 72 h, being the material mixed daily by a gentle inversion. The recovered org. phase was transferred to a new container and manually

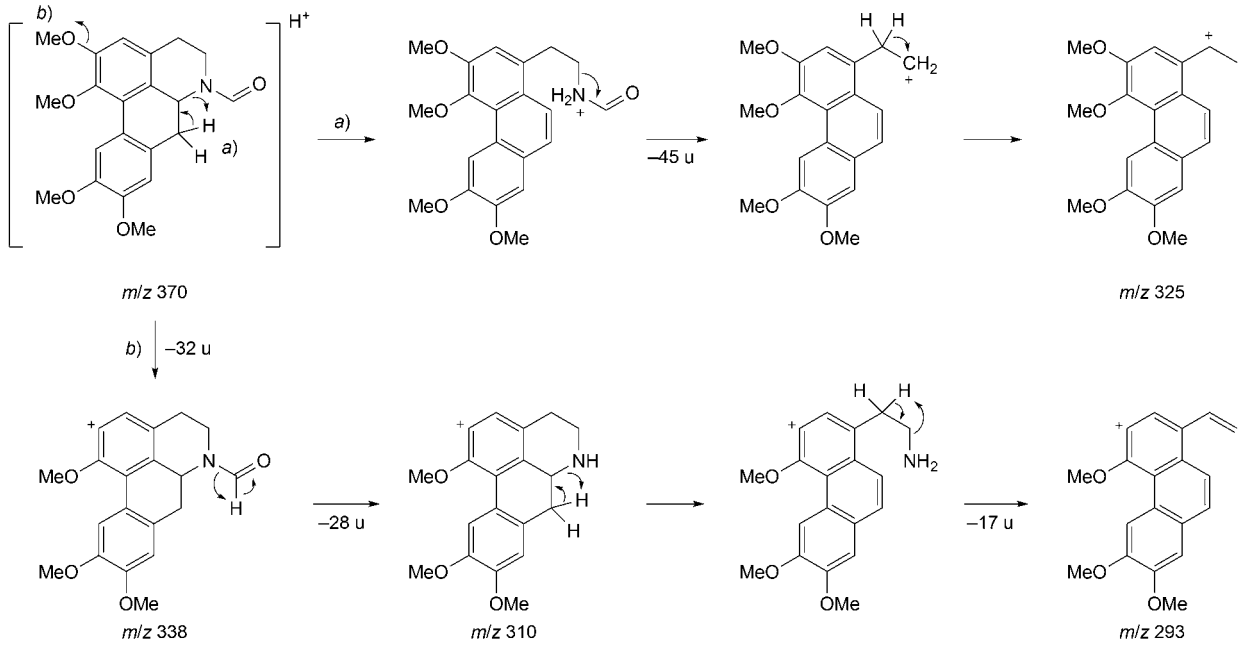

Fig. 3. Fragmentation proposal for the product ions observed in the collision-induced dissociation (CID) experiment of *m*/*z* 370.

shaken in a separatory funnel with 10% AcOH (2 l) during 10 min. Subsequently, the acidic aq. phase was transferred to another container and its pH elevated to 10 using NH_4OH and extracted with CH_2Cl_2 (300 ml, 2×). The CH_2Cl_2 phase was dried (Na_2SO_4) and evaporated at reduced pressure until dryness, resulting in the crude alkaloid fraction (668 mg). An aliquot of the crude alkaloid fraction of the leaves (100 mg) was chromatographed on a semi-prep. HPLC using a *Phenomenex C-18* column (250 × 15.00 mm, 5 μm) (Torrance, CA, USA) and a binary mobile phase constituted by aq. TFA (*A*; 0.01%) and MeOH (*B*). The gradient elution was as follows: 0 – 14 min, 30 – 80% *B*; 14 – 24 min, 80% *B*, at a flow rate of 6 ml/min. The HPLC separation yielded **1** (1.3 mg) (fraction 29) as a yellow amorphous solid.

(+)-*N*-Formylnorglaucine (= **(6a*S*)-4,5,6a,7-Tetrahydro-1,2,9,10-tetramethoxy-6*H*-dibenzo[*de*,*g*]quinoline-6-carbaldehyde**; **1**). $[\alpha]_D^{25}$ = +301 (*c* = 0.001, MeOH). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-ESI-MS: 370.1627 ($[M + H]^+$, $C_{21}H_{24}NO_5^+$; calc. 370.1648).

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