Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(2): 36-39
Received: 16-12-2020
Accepted: 18-01-2021

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Alkaloids isolated from *Crinum jagus* L. bulb (Amaryllidaceae) from Côte d'Ivoire

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DOI: https://doi.org/10.22271/phyto.2021.v10.i2a.13694

Abstract
Two alkaloids, 3-O-demethyltazettine (1) and hippadine (2) were isolated by column chromatography. The structures elucidated by different analytical techniques like NMR, IR, UV and mass for the first time from *Crinum jagus* bulb, an Amaryllidaceae from Côte d'Ivoire. This study is intended as a Contribution to its promotion.

Keywords: *Crinum jagus* L., bulb, 3-O-demethyltazettine, hippadine, NMR, Côte d'Ivoire

1. Introduction
Crinum jagus is a bulbous plant of the botanical family Amaryllidaceae [1] which is used in traditional medicine to treat cough, rickets, diabetes, asthma and malaria [1-4]. It is also used as a food additive for certain prescriptions [4]. Several studies have reported its pharmacological properties, such as anti-tuberculosis, anti-diabetic, antihemorrhagic, antioxidant, antibacterial, anti-acetylcholinesterase, healing and hepatoprotective [4-11] which are dependent on its phytochemical composition [4, 9, 10, 11]. A recent study conducted on total alkaloid extracts from *Crinum jagus* bulb from Côte d'Ivoire, indicated coexistence of fifteen alkaloids namely hippadine, lycorine, 3-O-demethyltazettine, ambelline, acetylambelline, acetylcaranine, crinanine acetate, crinine, caranine, O-methylmacronine, 3-epimacronine, crinine acetate, trispheridine, epinorgalanthamine and voacangine in addition to terpenes and coumarins [12] (Figure 1).

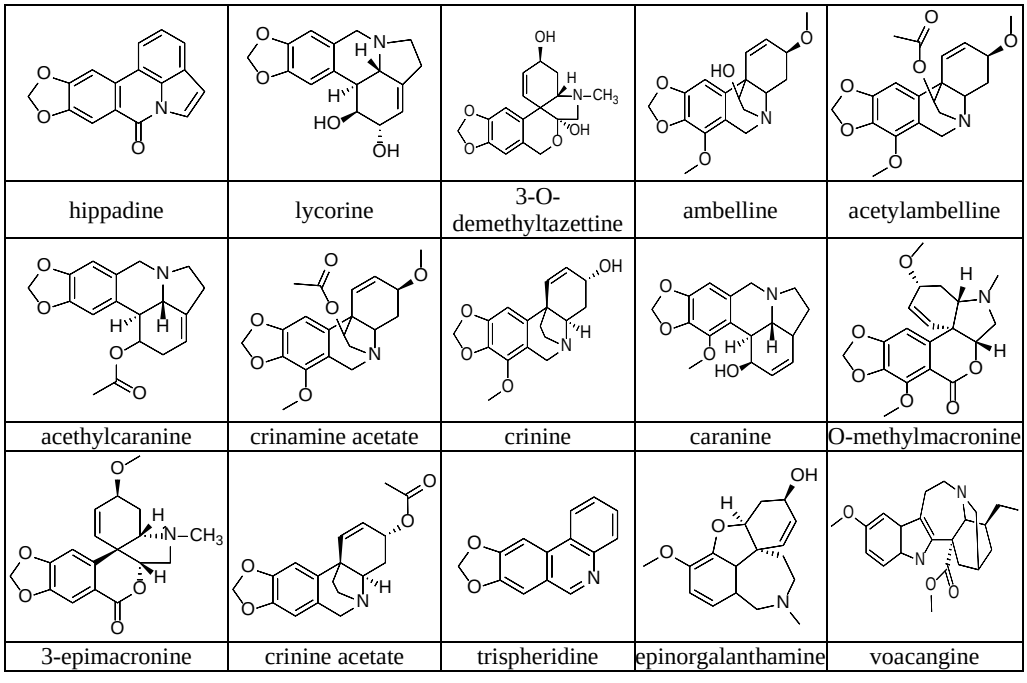

Fig 1: Alkaloids from Côte d'Ivoire *Crinum jagus* bulbs [12]

Hippadine identified as the most abundant alkaloid in *Crinum jagus* bulb [12]. Furthermore, an antioxidant potential substantially close to that of vitamin C has been observed, which would be due to the synergistic action of antioxidant phytoconstituents contained in bulbs [12]. Phytochemical investigations carried out on bulb of Nigerian species, have led to the isolation and characterization of several alkaloids, including hamayne, crinamine acetate, hamayne acetate, hydroxy crinamine, crinamine and lycorine [7]. In addition, two other alkaloids, demethoxybowdensine and bowdensine were isolated from same organ [11]. In research for new non-toxic active ingredients of plant origin to provide a response to recalcitrant pathologies through phytotherapy, the present study focused on the extraction of alkaloids from *Crinum jagus* from Côte d'Ivoire, which, moreover, have not yet been studied.

2. Experimental

2.1. General experimental procedures

The alkaloids (compounds **1** and **2**) were characterized based on the experimental results and by comparison with data published in the literature [13, 14, 15]. Several methods were used, including NMR where different sequences (^{1}H, ^{13}C, COSY, HSQC and HMBC) were executed on Shimadzu NMR spectrometers whose frequencies are 600 MHz and 150 MHz. Chemical shift data are given in δ ppm calibrated with $CDCl_3$: 7.26 ppm for ^{1}H and 77.1 ppm for ^{13}C at 25 °C. Coupling constants *(J)* are in hertz (Hz). As for IR and UV analyses, they were carried out respectively on Perkin Elmer spectrum 100 FT-IR and HP model 8453 (191 to 1100 nm) spectrophometers. Mass determination was possible using an HPLC-ESI-Q-TOF-MS/MS spectrometer. Finally, melting points were detected using a Stuart® SMP50 melting point meter.

2.2. Plant material

Crinum jagus bulb is the focus of our study. The place and time of harvest as well as its identification and authentication were reported in a previous study [12].

2.3. Extraction and isolation

2.3.1. 3-O-déméthyltazettine

375.5 mg of a chloroform concentrate of total alkaloids were placed in an open chromatographic column (2 cm × 40 cm). 20 g of silica gel (40-63 µm) was used as the stationary phase. The eluent used was the solvent gradient AcOEt / MeOH (9/1, v/v). 74 ml of fractions (F_{33} - F_{70}) were grouped according to their chromatographic profiles revealed with the Dragendorff reagent at different wavelengths (254 and 365 nm). 44.6 mg of product obtained after removal of the eluent by rotary evaporation at 45 °C (vacuum 474 mm Hg), were subjected to flash chromatography (column: 37 mm ×157 mm; silica gel (15-70 µm)), with a gradient of AcOEt / MeOH eluents (95/5 and 90/10). Fractions F_{14}-F_{23} were collected on the basis of their molecular fingerprints revealed by TLC. After concentration in the rotary evaporator at 45 °C, a solid compound (6.5 mg) was obtained.

2.3.2. Hippadine

288 mg of a cyclohexane concentrate of total alkaloids were fractionated on an open chromatographic column (2 cm × 40 cm), containing 15 g of silica gel (40-63µm), with a C_6H_{12}/CH_2Cl_2 (2/6) as eluent gradient. 64 ml of fractions (F_{58}-F_{90}) were combined on the basis of their TLC profiles. After concentration in the rotary evaporator at 45 °C (vacuum 474 mm Hg), a dry product (35.3 mg) resulting was chromatographed on a preplate and eluted with CH_2Cl_2. Fractions (F'_{58} - F'_{79}) were collected and dried to give a solid (10.3 mg).

3. Results and Discussion

Compound 1 (Figure 2) was isolated from a chloroformic extract of *Crinum jagus* bulbs, as a light yellow powder, with a frontal ratio of 0.31 and melting point of 196.2ºC. Its mass spectrum in the ESI-Q-TOF-MS/MS showed a molecular peak at m/z 318.13 $[M+H]^+$ (Calculated mass ($[M+H]^+$): 318.1341 g/mol), compatible with the molecular formula $C_{17}H_{19}NO_5$. ^{1}H NMR has revealed fourteen massifs of different signals, the most characteristic of which are: two aromatic protons [δ_H 6.59 (1H, s, H-13) and 6.51 (1H, s, H-10)]; N-methyl group [δ_H 2.45 (3H, s, H-17)] to distinguish between tazettine-type alkaloids and those of the crinine and hemanthine type [16]; methylenedioxy group [δ_H 5.90 (2H, s, *J* = 0.7 Hz, H-16 and H-16')] [16] and two olefinic protons [δ_H 6.38 (1H, dd, *J* = 10.1 and 5.3 Hz, H-2) and 5.71 (1H, dd, *J* = 10.3 and 1.4 Hz, H-1)]. Several proton-proton correlations were observed with the COSY spectrum. Thus the proton H-4 correlates with the protons H-4' and H-3. H-6 and H-8 couple respectively with H-6' and H-8'. H-3 correlates with H-2, which in turn correlates with H-1. We also note a correlation between H-5 and H-4. ^{13}C NMR identified five quaternary carbons (C-11; C-12; C-14; C-15 and C-7) whose chemical displacements are respectively 146.7 ppm, 146.8 ppm, 125.2 ppm, 49.9 ppm and 102.6 ppm. HSQC sequence allowed to identify the methylene groups [δ_H 5.00 (1H, dd, *J* = 14.9 Hz, H-8') and 4.70 (1H, dd, *J* = 14.9 Hz, H-8) / C-8 (δ_c 62.21 ppm) and δ_H 3.44 (1H, d, *J* = 11.2 Hz, H-6') and 2.70 (1H, d, *J* = 11.2 Hz, H-6) / C-6 (δ_c 64.25 ppm)] and methines [δ_H 4.14 (t, H-5) / C-5 (δ_c 63.3 ppm) and δ_H 3.05 (d, H-3) / C-3 (δ_c 70.54 ppm)]. The HMBC spectrum mentions long-range heteronuclear correlations between the protons H-2, H-3, H-4, H-13 and H-6 with quaternary carbon C-15 (δ_C 49.9); H-8, H-8', H-1 and H-6 with C-7 (δ_C 102.6); H-4 with C-2 (δ_C 132.8); H-16 with C-12 (δ_C 146.8); H-1 with C-6 (δ_C 64.2); H-3 with C-4 (δ_C 27.3); and finally between the protons H-6' and H-6 and the carbon of the N-methyl group C-17 (δ_C 42.6). The infrared spectrum was used to confirm the presence of various characteristic groups. Thus, the thin bands at 1483, 1244 and 1031 cm^{-1} respectively show the presence of a double bond (C=C) on an aromatic ring, an ether oxide group (-O-) between C-7 and C-8 and methylenedioxy group (-O-CH_2-O-). In addition, a thin band at 1056 cm^{-1} could correspond to the presence of N-methyl (N-CH_3). Hydroxyl groups linked to C-7 and C-3 carbons were visualized respectively in regions located at 1381 and 3207 cm^{-1}. Finally, the double bond (C=C) of cyclohexene was identified at 1508 cm^{-1}. Based on the above spectral data, compound 1 was identified as 3-O-demethyltazettine.

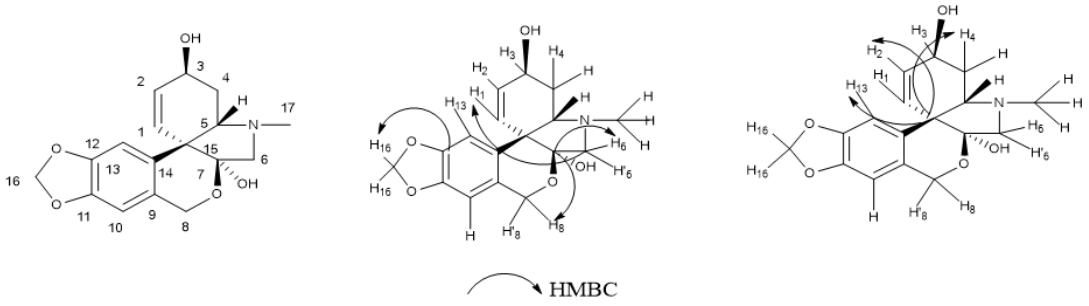

Fig 2: Structure of 3-O-demethyltazettine and its different HMBC correlations

Compound 2 (Figure 3) is a pale yellow powder of formula $C_{16}H_9NO_3$ as suggested by its molecular ion at m/z 264 g/mol (calculated Mass ($[M+H]^+$) : 264.07 g/mol) in ESI-Q-TOF-MS/MS. Its melting temperature and frontal ratio are 195.9 °C and 0.42 respectively. Analysis of NMR 1H data indicated the presence of nine signals with chemical shifts between 8.03 and 6.16 ppm, which are all CH except for the singlet centred on 6.16 ppm which is a CH_2 corresponding to both protons of carbon 10 (C-10). Except for the latter, all signals obtained can be grouped into two groups of signals coupling with each other. The first system consists of two protons leading to two doublets centred on 8.03 and 6.89 ppm. Their coupling constant is 3.5 Hz, which is compatible with an *ortho* coupling constant in a five ring cycle. Similarly, in the second system, the three protons of cycle D are present as a three spin system with respective chemical displacements of 7.90 ppm (doublet), 7.46 ppm (triplet) and 7.74 ppm (doublet) as respective multiplicities for protons H-1 to H-3. The coupling constant is also that of an *ortho* coupling as shown by its value of 7.6 Hz. NMR ^{13}C mentioned an intense signal at 102.4 ppm, which would correspond to the only grouping methylene (CH_2) present on the molecule. Moreover, this chemical shift is compatible with the acetal function. The seven CH signals observable in ^1H NMR were also detected in ^{13}C NMR. The HSQC direct correlation spectrum allowed each carbon to be assigned to its corresponding proton: C-10 (δ_c 102.4 ppm) to H-10 **(**δ_H 6.70 ppm); C-4 (δ_c 110.9 ppm) to H-4 **(**δ_H 6.89, *J* = 3.5 Hz); C-2 (δ_c 124.1 ppm) to H-2 (δ_H 7.46, *J* = 7.6 Hz); C-5 (δ_c 123.7 ppm) to H-5 (δ_H 8.03, *J* = 3.5 Hz); C-3 (δ_c 122.8 ppm) to H-3 (δ_H 7.74, *J* = 7.6 and 0.6 Hz); C-8 (δ_c 108.2 ppm) to H-8 (δ_H 7.97); C-1 (δ_c 118.5 ppm) to H-1 (δ_H 7.90, *J* = 7.6 Hz) and C-12 (δ_c 101.9 ppm) to H-12 (δ_H 7.64). A thin band at 1670 cm^{-1} was observed in the infrared spectrum, which could correspond to the presence of carbonyl group (C=O). The spectrum also shows a band at 1618 cm^{-1} (C=C) for aromatic rings. The methylenedioxy group (-O-CH_2-O-) was revealed with a thin band at 1023 cm^{-1}. The appearance of thin bands at 930-632 cm^{-1} indicates the double bond (C=C) between C-4 and C-5. Thus, compound **2** could be elucidated as the hippadine.

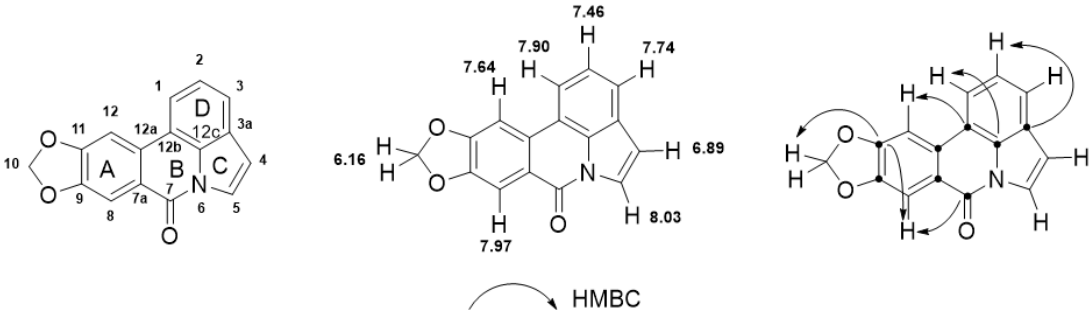

Fig 3: Structure of hippadine and its different HMBC correlations

The presence of these alkaloids has already been reported by Kouadio *et al.* [12]. According to the literature, 3-O-demethyltazettine has been isolated from *equestrian Hippeastrum* (Amaryllidaceae) [14]. Hippadine, a lycorine alkaloid, was isolated at the first time from *Crinum macowanii* (Amaryllidaceae) [15]. From this information, it seems plausible that these alkaloids are specific to the botanical family Amaryllidaceae. The pharmacological properties of the isolated phytoalkaloids could provide justification for the use of *Crinum jagus* in traditional medicine. Indeed, the effectiveness of bulbs against arterial hypertension would be due to hippadine which would be a hypotensor [17]. In addition, tazettine-type alkaloids are known for their antimalarial, antiviral, anticancer and antiproliferative properties [16, 18].

4. Conclusion

This work, carried out for the first time on the bulb of *Crinum jagus* from Côte d'Ivoire, made it possible to show the presence, after separation on a chromatographic column, of two alkaloids: 3-O-demethyltazettine and hippadine, compounds isolated respectively chloroform and cyclohexane fractions of total alkaloids. The chemical structure of these two compounds has been elucidated by analytical techniques such as NMR (^1H, ^{13}C, COSY, HSQC and HMBC), IR, UV and mass. This study of existence could be a chemotaxonomic marker of botanical families of plants.

5. Acknowledgment

The authors would like to thank the "Ministère de l'Enseignement Supérieur et de la Recherche Scientifique de Côte d'Ivoire (MESRSCI)" through the "Direction des Bourses (DOB)" for the financial support granted to KOUADIO Assohoun Tigoli Gildas for his doctoral internship in the "Architectures Moléculaires et Matériaux Nanostructurés" of Institut Charles Gerhardt at "Ecole Nationale Supérieure de Chimie de Montpellier (France)".

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