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ALKALOIDS ISOLATED FROM *CRINUM VIVIPARUM* (LAM.) R. ANSARI & V. J. NAIR AND THEIR BIOLOGICAL ACTIVITIES

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Summary

Five known alkaloids, lycorine (**1**), 2-*O*-acetyllycorine (**2**), (+)-vittatine (**3**), 8-demethyl-3-oxomaritidine (**4**), and (-)-maritinamine (**5**) were isolated from the Vietnamese medicinal plant *Crinum viviparum* (Lam.) R.Ansari & V.J.Nair. Their structures were identified by spectroscopic methods and by comparison with literature data. Except for lycorine, the remaining compounds were reported for the first time from Vietnamese *C. viviparum*. All five alkaloids were evaluated for nitric oxide (NO) suppression and cytotoxicity. Compounds **3** and **4** moderately inhibited NO production, while compounds **1**, **2**, and **4** showed cytotoxic activity against the HepG2 and LNCaP cell lines (IC_{50} = 7.02 – 72.25 μM). These results highlight *C. viviparum* as a promising source of bioactive alkaloids with potential anti-inflammatory and anticancer properties.

Keywords: *Crinum viviparum; Amaryllidaceae alkaloids; Anti-inflammatory activity; Cytotoxicity.*

1. Introduction

Crinum viviparum (Lam.) R.Ansari & V.J.Nair, commonly known as "*Náng lá gươm*" or "*Náng hoa đỏ*" in Vietnamese. According to the World Flora Online, *C. defixum* Ker is listed as a synonym of the *C. viviparum* [1]. It is a bulbous herb extensively utilized in traditional medicine. For instance, the leaf extract is used to treat pimples, body aches, leprosy, fever, and diarrhea. The bulb is used to cure burns, itching, whitlows, and carbuncles, as well as nauseant, emollient, and diaphoretic conditions [2]. Pharmacological studies have demonstrated that *C. viviparum* possesses multiple biological activities, including free radical scavenging [3]; analgesic and wound healing effects [4]; anti-cancer activity (in KB cells) [5]; antibacterial, thrombolytic, antidiarrheal, and anti-inflammatory effects [4],[6]. *C. viviparum* contains a variety of secondary metabolites, including alkaloids, tannins, flavonoids, steroids, and terpenoids, according to earlier chemical analyses [6]. Lycorine is effective against several tumors both *in vivo* and *in vitro*, as well as against some drug-resistant cancer cells, at low concentrations and with high selectivity [7]. Given these promising findings, further phytochemical and pharmacological investigations into the alkaloid constituents of *C. viviparum* are warranted. In the particular study, five alkaloids (**1-5**) from *C. viviparum* were isolated and structrurally clarified. They were also subjected to primary evaluations for their ability to inhibit the NO production and cytotoxicity against two human cancer cell lines and human hepatocellular carcinoma (HepG2) and human prostate carcinoma (LNCaP).

2. Materials and methods

2.1. General experimental procedures

Compound structures were elucidated using modern spectroscopic techniques, including a Bruker Avance NEO spectrometer (500 or 600 MHz) (Bruker, Karlsruhe, Germany) and a Chirascan™ CD spectrometer (Applied Photophysics Limited, UK). All measurements were performed at the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). Optical rotation was measured on a JASCO P-2000 polarimeter of the Institute of Chemistry (VAST). Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} or RP-18 $F_{254}S$ plates. Open column chromatography (CC) was conducted using *silica gel* (40–63 or 63 –200 μm) and RP-18 gel (40–63 μm) from Merck (Germany). TLC spots were visualized under UV light (254 and 365 nm) and by spraying with Dragendorff's reagent followed by

heating. Commercially available solvents were used without further purification.

2.2. Plant material

The whole plant of *C. viviparum* was collected from Thai Binh province, Vietnam, in March 2022. This plant was identified by Do Thanh Tuan, Ph.D., Thai Binh University of Medicine and Pharmacy. A specimen (TB070122) was deposited in the Center for High Technology Research and Development, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

2.3. Extraction and isolation

The dried powder of the whole plant *C. viviparum* (4 kg) was extracted with methanol (3 times × 20 L) by using a multifunctional ultrasonic extraction (at 50℃, 3 hours each time). The extracts were combined, filtered, and concentrated under reduced pressure to obtain a crude methanol extract (500 g). After this crude extract was scattered in 2.0 L of a mixture of MeOH/H_2O (2/1, v/v), the mixture was acidified to pH = 2 with HCl (36-38%) and subjected to a liquid-liquid distribution with EtOAc (03 times) to give EtOAc extract (CVE, 90 g). Continue to alkalize the remaining aqueous layer was basified to pH 12 –13 with NaOH solution, extracted with CH_2Cl_2 (4 times), and concentrated under reduced pressure to obtain the total alkaloid residue (CVD, 18 g) and water layer. Fraction CVD was put on a *silica gel* chromatography column (CC) and eluted with a gradient solvent of CH_2Cl_2/MeOH (100/1-1/100, v/v) to obtain six fractions (1A–1F). Fraction 1B (2.0 g) was submitted to a *silica gel* CC using a solvent system of CH_2Cl_2/MeOH (80/1 –1/1, v/v) to get three sub-fractions, (2A-2C). Sub-fraction 2B (625.4 mg) was purified by the RP-18 column and eluted with MeOH/H_2O (2/1, v/v) to yield compound **1** (250.0 mg). Sub-fraction 2C (1.5 g) was further separated by using a *silica gel* CC with a gradient solvent of CH_2Cl_2/MeOH/NH_3 (30/1/0.1-1/1/0.1, v/v/v) to afford compounds **2** (10.0 mg). Compounds **3** (8.0 mg) and **4** (9.0 mg) were obtained by purifying fraction 1C (2.0 g) on *silica gel* CC using a solvent system of CH_2Cl_2/MeOH/NH_3 (30/1/0.1, v/v/v) and a reversed phase RP-18 column with MeOH/H_2O (2/1, v/v). Fraction 1E (6.0 g) was placed on a *silica gel* column with a solvent system of CH_2Cl_2/MeOH (15/1-0/1, v/v) to obtain five sub-fractions, (5A –5E). Compound **5** (5.3 mg) was obtained by purification of fraction 5B (500 mg) on an RP-18 column and eluted with MeOH/H_2O (1/1, v/v).

Lycorine (1) - white powder. ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 6.80 (1H, s, H-11), 6.67 (1H, s, H-8), 5.94 (1H, d, J = 1.2 Hz, -OCH_2O-), 5.93 (1H, d, J = 1.2 Hz, -OCH_2O-), 5.36 (1H, brs, H-3), 4.27 (1H, brs, H-1), 4.01 (1H, d, J = 14.0 Hz, H-7α), 3.98 (1H, m, H-2), 3.32 (1H, d, J = 14.0 Hz, H-7β), 3.18 (1H, t, J = 7.2 Hz, H-5α), 2.60 (1H, brd, J = 10.8 Hz, H-11c), 2.51 (1H, m, H-11b), 2.50 (1H, m, H-4α), 2.44 (1H, m, H-4β), and 2.20 (1H, q, J = 8.4, 8.4, 8.4 Hz, H-5β). ^{13}C-NMR (150 MHz, DMSO-d_6): δ_C 145.7 (C-10), 145.2 (C-9), 141.8 (C-3a), 129.8 (C-7a), 129.6 (C-11a), 118.5 (C-3), 107.1 (C-8), 105.1 (C-11), 100.6 (-O$\underline{C}H_2O$-), 71.8 (C-2), 70.2 (C-1), 60.8 (C-11c), 56.7 (C-7), 53.3 (C-5), 40.2 (C-11b), and 28.2 (C-4).

2-*O*-Acetyllycorine (2) - colorless solid. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 6.80 (1H, s, H-11), 6.59 (1H, s, H-8), 5.93 (1H, s, -OCH_2O-), 5.91 (1H, s, -OCH_2O-), 5.47 (1H, brs, H-3), 5.32 (1H, brs, H-2), 4.51 (1H, brs, H-1), 4.15 (1H, d, J = 14.0 Hz, H-7α), 3.52 (1H, d, J = 14.0 Hz, H-7β), 3.36 (1H, m, H-5α), 2.80 (1H, brd, J = 10.5 Hz, H-11c), 2.71 (1H, d, J = 10.5 Hz, H-11b), 2.65 (2H, m, H-4), 2.38 (1H, q, J = 8.5, 8.5, 8.5 Hz, H-5β), and 2.08 (3H, s, -$OCOCH_3$). ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 170.6 (-O$\underline{C}OCH_3$), 146.6 (C-10), 146.4 (C-9), 146.0 (C-3a), 130.1 (C-11a), 127.2 (C-7a), 113.7 (C-3), 107.7 (C-8), 104.7 (C-11), 101.0 (-O$\underline{C}H_2O$-), 73.8 (C-2), 69.3 (C-1), 60.7 (C-11c), 53.7 (C-5), 57.0 (C-7), 41.8 (C-11b), 28.8 (C-4), and 21.2 (-OCO$\underline{C}H_3$).

(+)-Vittatine (3) - colorless needles. $[\alpha]_D^{25}$ + 26 ° (c = 0.5, $CHCl_3$). ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.95 (1H, s, H-10), 6.63 (1H, d, J = 10.2 Hz, H-1), 6.57 (1H, s, H-7), 5.95 (1H, ddd, J = 1.2, 5.4, 10.2 Hz, H-2), 5.893 (1H, d, J = 1.8 Hz, -OCH_2O-), 5.890 (1H, d, J = 1.8 Hz, -OCH_2O-), 4.40 (1H, d, J = 16.2 Hz, H-6α), 4.29 (1H, m, H-3), 3.87 (1H, d, J = 16.2 Hz, H-6β), 3.46 (1H, dd, J = 4.2, 13.2 Hz, H-12α), 3.37 (1H, m, H-4a), 3.40 (1H, d, J = 4.2 Hz, H-12β), 2.19 (1H, m, H-11α), 2.00 (1H, m, H-11β), 1.93 (1H, m, H-4α), and 1.81 (1H, td, J = 4.8, 13.2 Hz, H-4β). ^{13}C-NMR (150 MHz, CD_3OD) δ_C 148.0 (C-9), 147.5 (C-8), 139.4 (C-10a), 132.2 (C-1), 128.7 (C-2), 126.3 (C-6a), 107.9 (C-7), 104.0 (C-10), 102.2 (-O$\underline{C}H_2O$-), 64.4 (C-4a), 64.1 (C-6), 62.8 (C-3), 53.9 (C-12) 45.6 (C-11), 44.8 (C-10b) and 33.4 (C-4). CD (MeOH): Δε (nm) + 2.37 (294), - 0.27 (244).

8-Demethyl-3-oxomaritidine (4) - colorless crystals. ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.96 (1H, d, J = 10.2 Hz, H-1), 7.09 (1H, s, H-10),

6.56 (1H, s, H-7), 6.10 (1H, dd, J = 0.6, 10.2 Hz, H-2), 4.37 (1H, d, J = 16.8 Hz, H-6α), 3.90 (3H, s, -OCH_3), 3.82 (1H, d, J = 16.8 Hz, H-6β), 3.71 (1H, dd, J = 6.6, 12.6 Hz, H-4a), 3.56 (1H, m, H-12α), 3.06 (1H, m, H-12β), 2.62 (2H, m, H-4), 2.39 (1H, m, H-11α) and 2.29 (1H, m, H-11β). ^{13}C-NMR (150 MHz, CD_3OD): δ_C 200.0 (C-3), 152.2 (C-1), 148.0 (C-9), 146.8 (C-8), 135.0 (C-10a), 129.1 (C-2), 125.8 (C-6a), 115.0 (C-7), 107.3 (C-10), 70.0 (C-4a), 61.9 (C-6), 56.8 (-O<u>C</u>H$_3$), 54.5 (C-12), 46.1 (C-10b), 45.3 (C-11) and 40.7 (C-4).

(-)-Maritinamine (5) - white powder. $[\alpha]_D^{25}$ -20.3° (c = 1.8, MeOH). ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.80 (1H, s, H-10), 6.48 (1H, s, H-7), 4.30 (1H, d, J = 16.8 Hz, H-6α), 4.17 (1H, t, J = 3.0 Hz, H-3), 3.85 (3H, s, -OCH_3), 3.77 (1H, d, J = 16.8 Hz, H-6β), 3.33 (1H, dd, J = 5.4, 12.0 Hz, H-4a), 3.29 (1H, m, H-12α), 2.88 (1H, m, H-12β), 2.24 (1H, dt, J = 3.0, 13.8 Hz, H-1β), 2.11 (1H, td, J = 5.4, 12.6 Hz, H-1α), 2.32 (1H, m, H-11α), 1.90 (1H, m, H-4α), 1.84 (2H, m, H-2), 1.77 (1H, m, H-11β), and 1.47 (1H, td, J = 2.4, 12.0 Hz, H-4β). ^{13}C-NMR (150 MHz, CD_3OD): δ_C 147.9 (C-9), 146.1 (C-8), 140.5 (C-10a), 125.2 (C-6a), 114.0 (C-7), 107.6 (C-10), 66.8 (C-3), 64.3 (C-4a), 62.1 (C-6), 56.6 (-O<u>C</u>H$_3$), 52.6 (C-12), 43.5 (C-10b), 38.2 (C-11), 34.0 (C-4), 28.5 (C-2), and 23.4 (C-1). CD (MeOH): Δε (nm) + 0.55 (294), -0.72 (244).

2.4. Nitric oxide (NO) production inhibition assay

The inhibition of NO production in murine macrophage RAW 264.7 cells was evaluated using the previously reported procedure [8].

2.5. Cytotoxic assay

The cytotoxicity of the tested compounds was evaluated against two human cancer cell lines, LNCaP (prostate carcinoma) and HepG2 (hepatocellular carcinoma), using the Sulforhodamine B (SRB) assay [9],[10]. Cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM.

3. Results and Discussion

Purification of the methanol extract of the whole plant of *C. viviparum* by chromatographic techniques gave five compounds: lycorine **(1)** [11], 2-*O*-acetyllycorine **(2)** [12], (+)-vittatine **(3)** [13], 8-demethyl-3-oxomaritidine **(4)** [14], and (-)-maritinamine **(5)** [15] (Fig. 1).

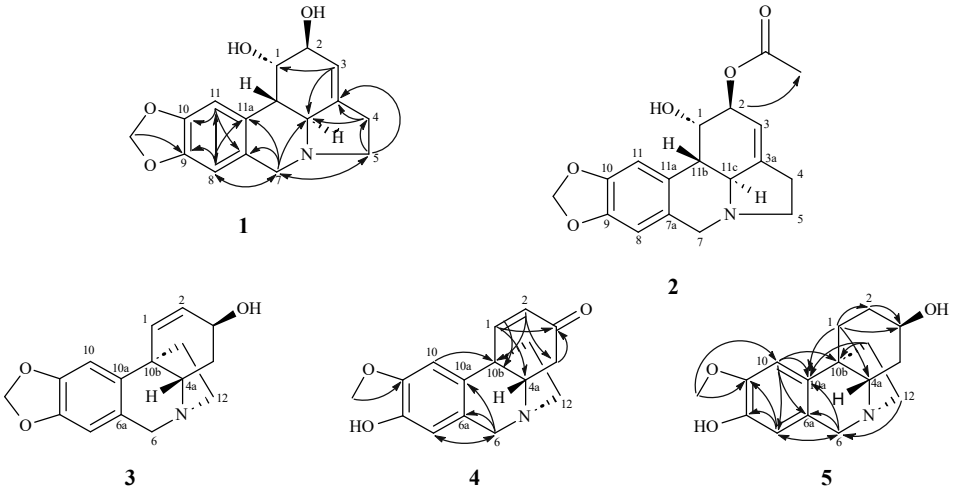

Fig. 1. Chemical structures of compounds **1** –**5** isolated from *C. viviparum* and key HMBC correlations for compounds **1**, **4**, and **5**

Compound **1** was obtained as a white powder. The ^{1}H-NMR spectrum of **1** showed signals of two aromatic protons [δ_H 6.67 (1H, s, H-8) and 6.80 (1H, s, H-11)]; one olefinic proton [δ_H 5.36 (1H, brs, H-3)]; three methylene groups [δ_H 4.01 (1H, d, J = 14.0 Hz, H-7α), 3.32 (1H, d, J = 14.0 Hz, H-7β), 2.50 (1H, m, H-4α), 2.44 (1H, m, H-4β), 3.18 (1H, t, J = 7.2 Hz, H-5α), and 2.20 (1H, q, J = 8.4, 8.4, 8.4 Hz, H-5β)]; two methine protons [δ_H 2.51 (1H, m, H-11b) and 2.60 (1H, brd, J = 10.8 Hz, H-11c)]; two oxygenated methine protons [δ_H 4.27 (1H, brs, H-1) and 3.98 (1H, m, H-2)], and two dioxymethylene protons [δ_H 5.94 (1H, d, J = 1.2 Hz, -OCH_2O-) and 5.93 (1H, d, J = 1.2 Hz, -OCH_2O-)]. The ^{13}C-NMR and HSQC spectrum of **1** demonstrated the

presence of sixteen carbon signals, consisting of six aromatic carbons [δ_C 129.8 (C-7a), 107.1 (C-8), 145.7 (C-9), 145.2 (C-10), 105.1 (C-11), and 129.6 (C-11a)]; two olefinic carbons at [δ_C 118.5 (C-3) and 141.8 (C-3a)]; two oxygenated methine carbons [δ_C 70.2 (C-1) and 71.8 (C-2)], two methine carbons [δ_C 40.2 (C-11b) and 60.8 (C-11c)], four methylene carbons [δ_C 28.2 (C-4), 53.3 (C-5), 56.7 (C-7), and 100.6 (-OCH_2O-)]. Information for all of the functional groups and their locations in the molecule was obtained from the heteronuclear multiple-bond correlation spectrum (HMBC) (Fig. 1). Significant HMBC correlations between signals at δ_H 6.80 (H-11) and δ_C 145.2 (C-9), δ_H 6.67 (H-8) and δ_C 145.7 (C-10), as well as between those at δ_H 5.93, 5.94 (-OCH_2O-) and δ_C145.2 (C-9), confirmed the presence of one dioxymethylene group at C-9 and C-10, as shown in Fig. 1. In combination with reference [11], compound **1** was identified as lycorine.

Compound **2** was isolated as a colorless solid. Analysis of ^{1}H-, ^{13}C-NMR of **2** and **1** revealed a high similarity between the two compounds, the only difference being the presence of an acetyl group at C-2 [δ_H 2.08 (3H, s, -$OCOCH_3$)], two carbon signals at δ_C 170.6 (-O<u>C</u>OCH_3) and 21.2 (-OCO<u>C</u>H_3) in the structure of **2** instead of a hydroxy group at **1**. This was confirmed by the HMBC correlation of H-2 to δ_C 170.6 (-O<u>C</u>OCH_3). In combination with reference [12], compound **2** was identified as 2-*O*-acetyllycorine.

Compound **3** was obtained as colorless needles. The 1D-NMR spectral analysis of **3** revealed that compound **3** had a framework of crinine-alkaloid. The ^{1}H-NMR spectrum of **3** showed the presence of two aromatic protons [δ_H 6.57 (1H, s, H-7) and 6.95 (1H, s, H-10)]; two olefinic protons [δ_H 6.63 (1H, d, J = 10.2 Hz, H-1) and 5.95 (1H, ddd, J = 1.2, 5.4, 10.2 Hz, H-2)]; two dioxymethylene proton [δ_H 5.893 (1H, d, J = 1.8 Hz, -OCH_2O-) and 5.890 (1H, d, J = 1.8 Hz, -OCH_2O-)], two methylene groups that bound to nitrogen [δ_H 4.40 (1H, d, J = 16.2 Hz, H-6α), 3.87 (1H, d, J = 16.2 Hz, H-6β) and 3.46 (1H, dd, J = 4.2, 13.2 Hz, H-12α), 3.40 (1H, d, J = 4.2 Hz, H-12β)]; two methylene groups [δ_H 1.81 (1H, td, J = 4.8, 13.2 Hz, H-4α), 1.93 (1H, m, H-4β) and 2.19 (1H, m, H-11α), 2.00 (1H, m, H-11β)], one methine proton [δ_H 3.37 (1H, m, H-4a)], and one oxymethine group [δ_H 4.29 (1H, m, H-3)]. The ^{13}C-NMR spectrum of **3** showed signals of sixteen carbons, consisting of six aromatic carbons [δ_C 126.3 (C-6a), 107.9 (C-7), 147.5 (C-8), 147.6 (C-9), 104.0 (C-10), and 139.4 (C-10a)], two olefinic carbons [δ_C 132.2 (C-1) and 128.7 (C-2)], four methylene carbons [δ_C 33.4 (C-4), 64.1 (C-6), 45.6 (C-11), and 53.9 (C-12)], one oxygenated methine [δ_C 62.76 (C-3)], one methine carbon [δ_C 64.4 (C-4a)], one quaternary carbon [δ_C 44.8 (C-10b)], and one dioxymethylene carbon [δ_C 102.1 (-OCH_2O-)]. Besides, the coupling constant (J = 5.4 Hz) of the protons H-2 and H-3, and the absence of a coupling constant between the allylic protons H-1 and H-3, suggest that the proton H-3 has an α-orientation [15],[16]. Additionally, the CD spectrum of **3** showed a positive Cotton effect at 294 nm ($\Delta\varepsilon$ + 2.37) and a negative Cotton effect at 244 nm ($\Delta\varepsilon$ − 0.27), allowing us to determine the 5,10b-ethano bridge of the structure of **3** as α-form [17]. In combination with reference [13], compound **3** was identified as (+)-vittatine.

Compound **4** was isolated as colorless crystals. The ^{1}H- and ^{13}C-NMR spectra data of compound **4** showed that this compound has the framework of a crinine-alkaloid. Compared with compound **3**, it was found that the 1D-NMR spectrum of **4** has many similarities; however, the oxymethine group at C-3 [δ_H 4.29 (1H, m, H-3), δ_C 62.8 (C-3)] of **3** is replaced by a ketone group at C-3 [δ_C 200.0] of **4**. The HMBC correlation between proton H-1 [δ_H 7.96 (1H, d, J = 10.2 Hz)] and C-3 (δ_C 200.0) supported this finding. Another difference is that one dioxymethylene group [δ_H 5.95 (2H, s, -OCH_2O-), δ_C 100.8 (-OCH_2O-)] in compound **3** is also replaced by one methoxy group [δ_H 3.90 (3H, s, -OCH_3), δ_C 56.8 (-OCH_3)] in **4**, and the location of the methoxy group at C-9 was demonstrated by the HMBC correlation between proton (-OCH_3) to C-9 (δ_C 148.0). Moreover, in the HMBC spectrum, correlations of proton H-2 with C-4 (δ_C 40.7)/C-10b (δ_C 46.1) and of proton H-4 with C-3 (δ_C 200.0)/ C-4a (δ_C 70.0)/C-10b indicated the *meta*-position of C-2 and C-4; correlations of proton H-6 with C-4a/C-12 (δ_C 54.5)/C-6a (δ_C 125.8)/C-10a (δ_C 135.0)/C-7 (δ_C 115.0) fixed the position of C-6; correlations of proton H-7 with C-10a/C-9 (δ_C 148.0), and of proton H-10 with C-6a/ C-8 (δ_C 146.8)/ C-10b fixed C-7 and C-10 at *para*-position. Key HMBC correlations are shown in Fig. 1. Combined comparison with reference [14], compound **4** was identified as 8-demethyl-3-oxomaritidine.

Compound **5** was acquired as a white powder. The 1D-NMR data of **5** had a high similarity with that of **3**, however, the NMR data of **5** did not show signals of a double bond at C-1 and C-2, which was demonstrated by the upfield chemical

shifts of C-1 (δ_C 23.4) and C-2 (δ_C 28.5) in **5**, compared to C-1 (δ_C 152.2) and C-2 (δ_C 129.1) in **3**. This was further confirmed through the HMBC interaction of protons H-1 [δ_H 2.24 (1H, dt, J = 3.0, 13.8 Hz, H-1α) and 2.11 (1H, td, J = 5.4, 12.6 Hz, H-1β)]) with C-2 (δ_C 28.5)/C-3 (δ_C 66.8)/C-4a (δ_C 64.3)/C-10a (δ_C 140.5), H-2 [δ_H 1.84 (2H, m, H-2)] with C-4 (δ_C 34.0)/C-11 (δ_C 43.5). Another difference is that one dioxymethylene group [δ_H 5.95 (2H, s, -OCH_2O-), δ_C 100.8 (-OCH_2O-)] in compound **3** is also replaced by one methoxy group [δ_H 3.85 (3H, s, -OCH_3), δ_C 56.6 (-OCH_3)] in **5**, and the location of the methoxy group at C-9 was demonstrated by the HMBC correlation between proton (-OCH_3) to C-9 (δ_C 147.9)/C-10 (δ_C 107.6). Key HMBC correlations are shown in Fig. 1. The CD spectrum of **5** displayed a negative Cotton effect at 244 nm ($\Delta\varepsilon$ − 0.72), and a positive Cotton effect at 294 nm ($\Delta\varepsilon$ + 0.55), testifying to the absolute configuration shown in **5**. Compared with previously published references [15], compound **5** was identified as (−)-maritinamine.

The biological activities of compounds **1–5**, including NO production inhibition and cytotoxicity against HepG2 and LNCaP cell lines, are summarized in Table 1, with L-NMMA and ellipticine used as positive controls, respectively. Data are expressed as mean ± SEM.

Table 1. Bioactivities of compounds **1–5**: inhibition of NO production and cytotoxicity

Compound	IC_{50} (μM)		
	Inhibition of NO production	**HepG2**	**LNCaP**
1	–	7.02±0.59	8.58±0.42
2	–	15.07±1.06	16.50±0.85
3	76.61±1.06	–	–
4	51.35±2.14	72.25±3.33	57.84±2.96
5	–	–	–
L-NMMA	7.80±0.36	–	–
Ellipticine	–	0.43±0.03	0.38±0.02

Note: *Statistical analysis was done with Microsoft Excel. All experimental results are expressed as the mean ± standard deviation (SD) or standard error (SE) of at least three independent experiments. The IC_{50} value (concentration that inhibits 50% of growth) for the tested samples was determined by curve-fitting the dose-response data using TableCurve 2Dv4 computer software (Jandel Scientific). – mean inactivity.*

In LPS-stimulated RAW 264.7 cells, compound **4** showed the strongest NO inhibitory effect (IC_{50} = 51.35 ± 2.14 μM), followed by compound **3** (IC_{50} = 76.61 ± 1.06 μM), though both were less potent than L-NMMA. Importantly, the cell viability assays indicated that these compounds maintained high percentages of living cells (>95% at 100 μM for compound **3** and >100% for compound **4**), suggesting that their inhibitory effects were not attributable to cytotoxicity. In contrast, compounds **1**, **2**, and **5** markedly reduced cell viability at 100 μM (ranging from ~ 44% to 65%), implying potential cytotoxic effects at higher concentrations. The α-5,10b-ethano bridge and C1-C2 double bond in crinine-type alkaloids appear relevant to activity, with enhanced effects upon C-3 oxidation.

Compounds **1**, **2**, and **4** exhibited cytotoxicity against HepG2 and LNCaP cells (IC_{50} = 7.02 ± 0.59 to 72.25 ± 3.33 μM). Replacing the hydroxyl group at C-2 in compound **1** with an acetyl group (compound **2**) reduced cytotoxicity. Preliminary SAR suggests the crinine (**4**) scaffold confers lower cytotoxicity than the lycorine-type. Other compounds showed no significant activity.

To date, the biological activities of compound **4** have not been previously reported, whereas the remaining compounds have shown consistent activities in prior studies [18],[19],[20],[21],[22].

4. Conclusions

This study elucidated the chemical structures of five alkaloids (**1-5**) isolated from *C. viviparum* through comprehensive spectroscopic analysis and comparison with previously reported data. Except for compound **1**, compounds **2–5** were isolated for the first time from Vietnamese specimens of *C. viviparum*. Compound **3** displayed moderate inhibition of nitric oxide (NO) production (IC_{50} = 76.61 ± 1.06 μM). Cytotoxic activity was observed for compounds **1** and **2** against HepG2 and LNCaP cancer cell lines, with IC_{50} values ranging from 7.02 ± 0.59 to 16.50 ± 0.85 μM. Notably, compound **4** was

assessed for the first time in this context and exhibited inhibitory effects on NO production (IC_{50} = 51.35 ± 2.14 μM) and cytotoxicity against HepG2 and LNCaP cell lines (IC_{50} = 72.25 ± 3.33 μM and 57.84 ± 2.96 μM, respectively). These findings highlight *C. viviparum* as a promising medicinal plant in Vietnam, particularly for the development of therapeutic agents targeting cancer.

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