A New \( \beta \)-Carboline Alkaloid From the Aerial Part of \textit{Hedyotis capitellata}

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Abstract

A new \( \beta \)-carboline alkaloid, 10-hydroxy-capitelline (I) together with three known anthraquinones, hedanthroside B (2), hedanthroside C (3), and rubiadin (4) were isolated from the aerial part methanol extract of \textit{Hedyotis capitellata}. Their structures were established by spectroscopic data (one-dimensional, two-dimensional nuclear magnetic resonance (1D, 2D-NMR) and high-resolution-electrospray ionization-mass spectrometry, HR-ESI-MS) and comparison with those reported in the literature. The anti-inflammatory activity of the isolated compounds is evaluated by their inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. At a concentration of 20 \( \mu \)M, compounds 1 to 4 showed weak inhibitory effects on NO production with inhibitory values ranging from 1.2\% to 23.9\% compared to a value of 74.5\% for the positive control compound, \( N^\beta \)-monomethyl-\( L \)-arginine (L-NMMA).

Keywords

\textit{Hedyotis capitellata}, \( \beta \)-carboline alkaloid, anthraquinone, 10-hydroxy-capitelline, anti-inflammatory activity

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Introduction

\textit{Hedyotis} is a genus of the flowering plants belonging to the family Rubiaceae.\(^1\) The \textit{Hedyotis} plants are widely distributed in tropical and subtropical regions with about 500 different species.\(^1\) The previous studies showed that they have diverse chemical constituents with mainly secondary metabolite classes of alkaloids,\(^2-5\) flavonoids,\(^6-7\) lignans,\(^9\) iridoids, and triterpenoids.\(^7\) The diversity in chemical composition has brought various important pharmacological activities such as antibacterial, anti-diabetic, antioxidant, anti-inflammatory, and anticancer.\(^6,11,12\) In Vietnam, \textit{Hedyotis capitellata} is a typical species of the genus \textit{Hedyotis} and is a traditional medicine as diuretic, anti-toxicity, and anti-inflammatory for the stomach, tongue, and throat.\(^10\) In this study, we reported the isolation and structure elucidation of a new \( \beta \)-carboline alkaloid and three known anthraquinones from the methanol extract of the aerial part of \textit{H capitellata}. The inhibition of nitric oxide (NO) production in lipopolysaccharide-stimulated RAW 264.7 cells by compounds 1 to 4 was also evaluated.

Results and Discussion

Compound 1 was obtained as a yellowish-brown colloid, which reacted positively with the Dragendorff reagent, suggesting an alkaloid compound. Its molecular formula was deduced to be \( C_{20}H_{24}N_2O_3 \) from the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) quasimolecular ion peak at \( m/z \) 341.1862 (calcld for [\( C_{20}H_{23}N_2O_3 \]+, 341.1855, \( \Delta = -0.9 \) ppm), indicating nine degrees of unsaturation (see Supplemental Figure S1). The proton nuclear magnetic resonance (\( ^1 \)H NMR) spectrum of I exhibited signals that are characteristic of a \( \beta \)-carboline skeleton (Table 1).\(^4,13\) The two ortho-coupled signals at \( \delta_1 = 8.20 \) (1H, \( d, J = 5.5 \) Hz) and 7.84 (1H, \( d, J = 5.5 \) Hz) were assigned to H-5 and H-6, respectively. One ABX-coupled olefinic proton pattern at \( \delta_1 = 7.51 \) (d, \( J = 2.0 \) Hz), 7.12 (dd, \( J = 8.5, 2.0 \) Hz), and 7.44 (d, 8.5 Hz), one olefinic proton at \( \delta_1 = 6.68 \) (s), one methine carbinol proton at \( \delta_1 = 3.44 \) (dd, \( J = 10.5, 1.5 \) Hz), three methyl singlets at 1.22, 1.25, and 1.96 (each 3H) were identified. The carbon nuclear magnetic resonance (\( ^{13} \)C NMR) spectrum of I indicated the presence of 20 carbon signals, including eight quaternary, seven methine, two methylene, and three methyl carbons, classified

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by the heteronuclear single quantum coherence (HSQC) spectrum (Table 1). The NMR data of 1 were similar to that of capitelline (1a), previously isolated from *H capitellata*,\(^4\) except that there were some significant changes at C-9, C-10, and C-11 (Table 1), suggesting 1 was 10-hydroxycapitelline. This was further confirmed by the heteronuclear multiple bond correlation (HMBC) correlations as shown in Figure 1. The other two hydroxyl groups were at C-5 and C-6 confirmed by the HMBC correlations from both H-7’ and H-8’ to the quaternary carbon at \(\delta_C 73.9\) and the methine carbonyl carbon at \(\delta_C 78.9\), as well as by the \(^1H-^1H\) correlation spectroscopy (COSY) cross peaks of H-3’/H-4’/H-5’. The double bond was at C-1’/C-2’ as indicated by the HMBC correlations from H-9 to C-1’/C-2’/C-3’ and from H-1’ to C-2 and C-3. In addition, the nuclear overhauser effect spectroscopy (NOESY) correlations of H-9/ H-6 and the HMBC correlations of H-9/C-7 and H-6/C-8 further confirmed the suggested structure of 1. The NOESY cross peaks between H-3’ (\(\delta_H 2.65\) and 2.43) and H-1 (\(\delta_H 6.68\)) were observed, suggesting for E-geometric configuration of the C-1’/C-2’ double bond. The optical rotation of 1 was zero as well as no Cotton effects were observed in the electronic circular dichroism (ECD) spectrum, suggesting that 1 was racemic. From the above evidence, 1 was determined to be a new compound 10-hydroxy-capitelline (Supplemental Figures S1 to S7).

Compounds 2 to 4 were identified to be hedanthroside B, hedanthroside C,\(^8\) and rubiadin,\(^14\) respectively, by the consistent \(^1H\) and \(^13C\) NMR data with those reported in the literature (Supplemental Figures S8 to S24).\(^14\) The assignments of these data were confirmed by 2D NMR spectroscopic analysis (see Tables S1 and S2 in the Supplemental Material). Capitelline was previously isolated from *H. capitellata* (the sample was collected in March 1995 in Ninh Binh Province). However, we have not isolated this compound (the sample collected in August in Nam Dinh Province), which may be due to the differences of geographical location and sampling times.\(^4\)

\(\beta\)-Carboline alkaloid and anthraquinone compounds have been known for many interesting biological activities including anti-inflammatory activity.\(^6,11,12\) So that, compounds 1 to 4 were then evaluated for their effects on NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Each compound was assessed at a concentration of 20 \(\mu\)M. Compounds 1 to 4 exhibited weak inhibitory effects on NO production with inhibitory values ranging from 1.2% to 23.9% compared to a value of 74.5% for the positive control compound, \(N^\prime\)-monomethyl-L-arginine (L-NMMA) (see Table S3 in the Supplemental Material). Therefore, compounds 1 to 4 were not further evaluated for their half-maximal inhibitory concentration (IC\(_{50}\)) values.

### Material and Methods

#### General Experimental Procedures

Optical rotation was measured on a Jasco P-2000 polarimeter (589 nm). Circular dichroism (CD) spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics). NMR spectra were studied on a Bruker 500 MHz spectrometer, and HR-ESI-MS on an Agilent 6530 Accurate Mass quadrupole time-of-flight liquid chromatography/mass spectrometry (Q-TOF LC/MS). Flash column chromatography was performed using either silica gel or reversed phase (RP-18, mesh) resins as adsorbent. Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F254 and/or RP-18 F254S plates. TLC plates were visualized under ultraviolet irradiation (254 and 365 nm) or by spraying with \(\text{H}_2\text{SO}_4\) solution (5%) followed by heating with a heat gun. High-performance liquid chromatography (HPLC) was carried out using an AGILENT 1100 HPLC system.

#### Plant Material

The plant *H capitellata* Wall. ex G. Don was collected in Nam Dinh in August 2018 and identified by Dr. Nguyen The Guong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (HC08.2018-1) was deposited at the Lab of Pharmaceutical Chemistry, VNU University of Science, Hanoi.

### Table 1. \(^1H\) NMR and \(^13C\) NMR Data for Compounds 1 and Capitelline (1a).

| Pos. | \(\delta_C\) type | \(\delta_H\) (mult., J in Hz) | \(\delta_C\) type |
|------|------------------|-----------------------------|------------------|
| 1    | 136.5, C         | –                          | 134.5, C         |
| 2    | 143.0, C         | –                          | 141.6, C         |
| 3    | 137.2, CH        | 8.20 (d, 5.5)              | 137.5, CH        |
| 4    | 114.1, CH        | 7.84 (d, 5.5)              | 113.0, CH        |
| 5    | 129.9, C         | –                          | 128.7, C         |
| 6    | 123.2, C         | –                          | 121.5, C         |
| 7    | 106.7, CH        | 7.51 (d, 2.0)              | 121.6, CH        |
| 8    | 152.2, C         | –                          | 119.6, CH        |
| 9    | 111.9, CH        | 7.12 (dd, 8.5, 2.0)        | 128.2, CH        |
| 10   | 113.5, CH        | 7.44 (d, 8.0)              | 111.8, CH        |
| 11   | 137.0, C         | –                          | 140.6, C         |
| 1’   | 121.0, CH        | 6.68 (s)                   | 120.6, CH        |
| 2’   | 146.8, C         | –                          | 144.9, CH        |
| 3’   | 38.5, CH\(_2\)   | 2.65 (m) and 2.43 (m)      | 37.4, CH\(_2\)   |
| 4’   | 30.5, CH\(_2\)   | 2.03 (m) and 1.63 (m)      | 29.2, CH\(_2\)   |
| 5’   | 78.9, CH         | 3.44 (dd, 10.5, 1.5)       | 77.6, CH         |
| 6’   | 73.9, C          | –                          | 73.2, C          |
| 7    | 26.0, CH\(_3\)   | 1.25 (s)                   | 26.1, CH\(_3\)   |
| 8    | 24.8, CH\(_3\)   | 1.2 (s)                    | 23.4, CH\(_3\)   |
| 9    | 18.6, CH\(_3\)   | 1.94 (s)                   | 18.1, CH\(_3\)   |

Abbreviations: \(^1H\) NMR, proton nuclear magnetic resonance; \(^13C\) NMR, carbon nuclear magnetic resonance.

\(^{a}\)Measured at 125 MHz.

\(^{b}\)Measured at 500 MHz.

\(^{c}\)Measured in deuterated methanol.

\(^{d}\)Measured in deuterated dimethyl sulfoxide.
Extraction and Isolation

The dried and powdered aerial parts of *H capitellata* (3.6 kg) were ultrasonically extracted with hot methanol at 50°C, (each batch: 0.6 kg, 2L MeOH, for 30 min, three times). After filtering, the filtrate was evaporated at 60°C to give the methanol extract (HC, 170 g). This extract was suspended in water (2 l) and successively partitioned with hexane, CH₂Cl₂, and EtOAc to obtain corresponding extracts HCH (26.8 g), HCD (1.9 g), HCE (19.9 g), and the water layer. The HCE fraction (19.5 g) was chromatographed on a silica gel column (500 g, mesh) eluting with CH₂Cl₂-EtOAc (1:1.5) to obtain five subfractions (HCE1-HCE5). The HCE4 (2.1 g) was chromatographed on a silica gel column (70 g, mesh) eluting with CH₂Cl₂-MeOH (7:1) to yield compound 4 (22 mg). HCE5 (3.7 g) was chromatographed on a silica gel column (100 g, mesh) eluting with CH₂Cl₂-MeOH (6:1) to yield compound 1 (26 mg). The water fraction was chromatographed on a Diaion HP-20 column, eluted with a MeOH-H₂O gradient system (25:75, 50:50, 75:25, 100:0, each 1.5 L) to give four fractions, HCW1-HCW4, respectively. Fraction HCW2 (9.33 g) was separated on a silica gel column chromatography eluting with CH₂Cl₂:MeOH:H₂O (10:1:0.1) to give five smaller fractions, HCW2A-HCW2E. Fraction HCW2D (3.5 g) was separated by an RP-18 column (mesh) eluting with MeOH:H₂O (3:1) to obtain four fractions, HCW2D1-HCW2D4. Fraction HCW2D3 (0.8 g) was chromatographed on a silica gel column (30 g, mesh) eluting with CH₂Cl₂:MeOH:H₂O (3:1:0.2) to obtain compounds 2 (21.0 mg) and 3 (19.5 mg).

10-Hydroxy-Capitelline ($\text{I}$). A yellowish-brown colloidal. $[\alpha]_D^{25}: 0.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{	ext{max}}$ nm: 235, 267, 314; IR cm$^{-1}$ (KBr): 3428, 2936, 1651, 1452. HR-ESI-MS $m/z$ 341.1862 [M+H]$^+$ (calcd. for C$_{20}$H$_{25}$N$_2$O$_3$, 341.1865, $\delta$ = −0.9 ppm). $^1$H-NMR (CD$_3$OD, 500 MHz) and $^{13}$C-NMR (CD$_3$OD, 125 MHz): see Table 1.

Conclusions

In summary, this report describes the isolation and structural elucidation of a new β-carboline alkaloid named 10-hydroxy-capitelline ($\text{I}$) and three known anthraquinones, hedanthroside B ($\text{2}$), hedanthroside C ($\text{3}$), and rubiadin ($\text{4}$) from the aerial part methanol extract of *H capitellata*. Compounds $\text{I}$ to $\text{4}$ showed weak inhibitory effects on NO production with inhibitory values ranging from 1.2% to 23.9%...
compared to a value of 74.5% for the positive control compound, L-NMMA.

Author Contributions
Isolation and research idea, LT Huyen, NTT Hau, VH Son, NTL Huyen; Structure elucidation and writing, BHTai, NX Nhiem, PV Kiem.

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Ethical Approval
Our institution does not require ethical approval for reporting individual cases or case series.

Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

Supplemental Material
Supplemental material for this article is available online.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

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