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Antiplasmodial Alkaloids from *Desmos rostrata*

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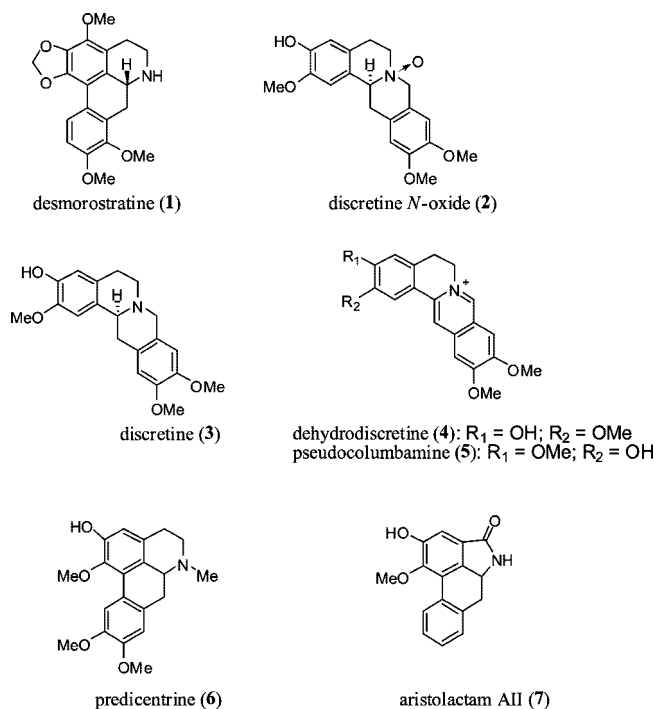
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Two new alkaloids, desmorostratine (**1**) and discretine *N*-oxide (**2**), were isolated from the stem bark of *Desmos rostrata*, together with five known alkaloids, discretine (**3**), dehydrodiscretine (**4**), pseudocolumbamine (**5**), predicentrine (**6**), and aristolactam AII (**7**). The structures were established on the basis of spectroscopic data, including mass spectrometry and 2D-NMR. Compound **1** was cytotoxic against KB cells (IC₅₀ 2.4 μ M), while **2**, **3**, and **4** inhibited *Plasmodium falciparum* (IC₅₀ of 4.2, 1.6, and 0.9 μ M, respectively).

The genus *Desmos* is comprised of about 59 species and subspecies.¹ Previous studies of several species of this genus reported the presence of flavonoids² and alkaloids.³ In the course of a screening program of Vietnamese plants, an extract of *Desmos rostrata* (Merr. & Chun) P.T.Li [basionym: *Dasymaschalon rostratum*, (Annonaceae)] was selected for fractionation, as it inhibited *Plasmodium falciparum* (40% inhibition at 10 μ g/mL) and was high in alkaloid content. Herein, we describe the isolation and structural elucidation of two new alkaloids, **1** and **2**, along with five known alkaloids, **3**–**7**, from the stem bark. These compounds were evaluated for cytotoxicity on KB cells and for antiplasmodial activity on *P. falciparum*.

A methanol extract of dried stem bark of *D. rostrata* (1.0 kg) was partitioned successively with *n*-hexane and then with dichloromethane. The dichloromethane solubles were purified by repeated open column chromatography over silica gel to give compounds **1**–**7**.

Compound **1** was obtained as a colorless solid (mp 153–155 °C) and was optically active ([α]_D²⁰ –22.9, *c* 0.5, CHCl₃). In its HRESI mass spectrum, the protonated molecular ion [M + H]⁺ was observed at *m/z* 356.1499 [M + H]⁺ (calcd for C₂₀H₂₂NO₅, 356.1498), suggesting the molecular formula C₂₀H₂₁NO₅. The 1D-NMR spectra (¹H and ¹³C) indicated the presence of four methylenes, three methines, 10 sp² quaternary carbons, and three methoxy groups. The ¹H–¹H COSY spectrum of **1** showed three sets of correlations as follows: CH₂-4 (δ _H 2.81) with CH₂-5 (δ _H 2.99 and 3.54), CH-6a (δ _H 3.96) with CH₂-7 (δ _H 2.49 and 3.50), and between two sp² protons H-10 (δ _H 6.85) and H-11 (δ _H 7.74). In the HMBC spectrum, correlations of H-10 with C-8 (δ _C 145.9), C-9 (δ _C 151.7), and C-11a (δ _C 124.6) were observed. Cross-peaks of H-11 with C-11a and C-7a (δ _C 128.1) were also noted. These data suggested the presence of a 1,2,3,4-tetrasubstituted benzene ring (**d**-ring). On the other hand, ³J-HMBC correlations of CH₂-7 (δ _H 2.49 and 3.50) with C-8 and C-11a and of H-11 with C-1a (δ _C 110.8) were observed, revealing the linkages of C-1a to C-11a and C-7 to C-7a. The H-6a signal (δ _H 3.96) was correlated to C-3a (δ _C 118.2), CH₂-5 (δ _C 42.1), and C-7a (δ _C 128.1). The chemical shifts of CH-6a and CH₂-5 suggested their linkages to a nitrogen atom. This depicted the bonding of C-1b to CH-6a, which was linked to CH₂-5 via the nitrogen atom N-6. Moreover, the methylene CH₂-4 (δ _H 2.81) showed cross-peaks with C-1b and C-3 (δ _C 139.8), suggesting its linkage to C-3a. The methylenedioxy (δ _H 5.91 and 6.06) was



correlated to C-1 (δ _C 143.9) and C-2 (δ _C 135.4) in the HMBC spectrum, indicating its bonding to these carbons. Finally, methoxy groups (δ _H 4.01, 3.81, and 3.89) were bonded to C-3, C-8 (δ _C 145.9), and C-9 (δ _C 151.7), as determined by their ³J-HMBC correlations, respectively. Complete analysis of the HMBC spectrum permitted assignment of the planar structure of **1** as shown (Figure 1).

In the ¹H spectrum, H-6a appeared as a doublet of doublets with coupling constants of 14.5 Hz (*anti*) and 5.0 Hz (*gauche*). Strong interaction of H-6a with H-5_{ax} was observed in the NOESY spectrum. The H-6a proton was thus axial on both **b**- and **c**-rings. Comparing the optical rotation of **1** ([α]_D²⁰ –22.9, *c* 0.5, CHCl₃) with that of similar structures such as (–)-nordicentrine ([α]_D²⁰ –34, *c* 0.2, MeOH)⁴ and (+)-*O*-methylcassayifline ([α]_D²⁰ +16.4, *c* 1.0, CHCl₃)⁵ suggested the *R*-configuration for the C-6a chiral center. This new aporphine alkaloid (**1**) was named desmorostratine.

Compound **2** was colorless, amorphous, and optically active ([α]_D²⁰ –185.4 (*c* 0.5, CHCl₃)). The HRESI mass spectrum displayed the protonated molecular ion [M + H]⁺ at *m/z* 358.1656 (calcd for C₂₀H₂₄NO₅, 358.1654), in agreement with the molecular formula C₂₀H₂₃NO₅. Signals of four sp³ methylenes, five methines (1 sp³

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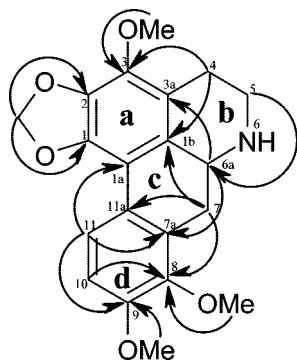


Figure 1. Selected HMBC correlations of 1.

Table 1. Cytotoxicity and Antiplasmodial Activity of Compounds 1–4, 6, and 7

compound	KB cells, IC ₅₀ (μM)	<i>P. falciparum</i> (FcB1), IC ₅₀ (μM)
1	2.4	3.6
2	>28	4.2
3	>29	1.6
4	>29	0.9
6	>29	27.8
7	>37	>37
Taxotere	0.0002	

and 4 sp²), and eight sp² quaternary carbons in the 1D-NMR spectra (¹H and ¹³C), together with three methoxy groups were observed. Analysis of 2D-NMR spectra of **2** indicated that it had the same skeleton as discretine (**3**). The main differences between them were the NMR signals of CH₂-6, CH₂-8, and CH-13a. The chemical shifts at δ_H 3.79 and 3.71 (CH₂-6), 4.82 and 4.34 (CH₂-8), and 4.83 (CH-13a) were assigned for **2**, whereas the values of 3.06 and 2.66 (CH₂-6), 3.96 and 3.66 (CH₂-8), and 3.62 (CH-13a) were observed for discretine (**3**). Taking into account the molecular mass observed in the HRESI mass spectrum suggested that compound **2** was discretine *N*-oxide. Since (–)-discretine (**3**) {[α]_D²⁰ –287.6 (c 0.5, CHCl₃); lit.⁶ –296.6 (c 1.04, CHCl₃)} was also found from *D. rostrata*, the 9*S*-configuration was thus proposed for (–)-discretine-*N*-oxide (**2**).

The other known compounds **4**, **5**, **6**, and **7** were isolated from this plant and identified as dehydrodiscretine,⁷ pseudocolumbamine,⁸ predicecentrine,⁹ and aristolactam AII,¹⁰ respectively. Their structures were determined by comparison of their spectroscopic data (IR, MS, and NMR) with those reported in the literature.

The isolated compounds were evaluated for cytotoxicity on KB cells and antiplasmodial activity on *P. falciparum* (FcB1 strain). The results showed that the most active components on *P. falciparum* were **2**, **3**, and **4**, with IC₅₀ values of 4.2, 1.6, and 0.9 μM, respectively, while they showed weak cytotoxic activity on KB cells (Table 1). Thus, the antiplasmodial activity of **2**, **3**, and **4** should not be due to their cytotoxicity. On the other hand, desmorostratine (**1**) was moderately toxic to KB cells (IC₅₀ of 2.4 μM), and this value was 3.6 μM when tested on *P. falciparum*. Thus, the antiplasmodial activity of desmorostratine (**1**) could result from its cytotoxicity.

Experimental Section

Plant Material. Stem bark of *D. rostrata* was collected in North Vietnam (Hatinh Province) in March 2004, and a specimen (VN 1273) was deposited at the Institute of Ecology and Natural Resources, Vietnam Academy of Science and Technology, Vietnam.

Extraction and Isolation. Dried and ground stem bark (1.0 kg) of *D. rostrata* was extracted with MeOH (3 × 4 L) at room temperature. The solvent was removed under diminished pressure. The crude extract was suspended in the water and extracted successively with *n*-hexane and then with CH₂Cl₂. The CH₂Cl₂ solution was concentrated under diminished pressure, and the residue was purified by open column

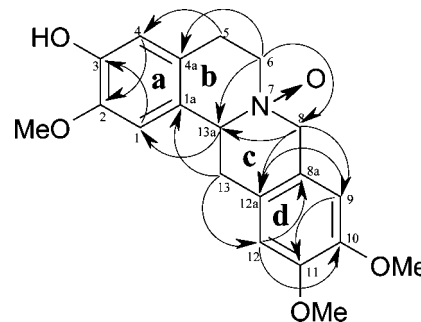


Figure 2. Key HMBC correlations of 2.

chromatography over silica gel, eluted with a mixture of CH₂Cl₂/MeOH (99:1 to 0:100) to afford eight fractions (1–8). Fraction 3 was subjected to column chromatography, eluting with CH₂Cl₂/MeOH (95:5) to yield discretine (**3**, 7 mg) and predicecentrine (**6**, 10 mg). Fraction 4 was purified by preparative TLC (5% of MeOH in CH₂Cl₂), affording aristolactam AII (**7**, 16 mg). Fractions 5 and 6 were combined and purified by column chromatography, eluting with CH₂Cl₂/MeOH (90:10) to give desmorostratine (**1**, 15 mg) and discretine *N*-oxide (**2**, 6 mg). Fraction 7 was subjected to column chromatography, using CH₂Cl₂/MeOH (85:15), yielding dehydrodiscretine (**4**, 8 mg) and pseudocolumbamine (**5**, 12 mg).

Desmorostratine (1): Colorless solid; mp 153–155 °C; [α]_D²⁰ –22.9 (c 0.5, CHCl₃); UV (CHCl₃) λ_{max} (log ε) 240 (3.13), 285 (3.17); IR (KBr) ν_{max} 3426, 1640, 1562, 1411, 1265, 1082, 655 cm^{–1}; ¹H NMR (CDCl₃, 500.13 MHz) δ 7.74 (1H, d, *J* = 8.5 Hz, H-11), 6.85 (1H, d, *J* = 8.5 Hz, H-10), 5.91, 6.06 (each 1H, d, *J* = 1.5 Hz, O-CH₂-O), 4.01 (3H, s, OCH₃-3), 3.96 (1H, dd, *J* = 5.0, 14.5 Hz, H-6a), 3.89 (3H, s, OCH₃-9), 3.81 (3H, s, OCH₃-8), 3.54 (1H, ddd, *J* = 4.0, 4.0, 12.5 Hz, H-5_{eq}), 3.50 (1H, dd, *J* = 5.0, 14.5 Hz, H-7_{eq}), 2.99 (1H, ddd, *J* = 6.5, 11.5, 12.5 Hz, H-5_{ax}), 2.81 (2H, m, CH₂-4), 2.49 (1H, dd, *J* = 14.5, 14.5 Hz, H-7_{ax}); ¹³C NMR (CDCl₃, 125.76 MHz) δ 151.7 (C-9), 145.9 (C-8), 143.9 (C-1), 139.8 (C-3), 135.4 (C-2), 128.1 (C-7a), 126.2 (C-1b), 124.6 (C-11a), 122.6 (C-11), 118.2 (C-3a), 110.8 (C-1a), 110.5 (C-10), 100.7 (O-CH₂-O), 60.7 (OCH₃-8), 59.5 (OCH₃-3), 55.8 (OCH₃-9), 52.8 (C-6a), 42.1 (C-5), 28.4 (C-7), 22.6 (C-4); ESIMS (*m/z*): 356.1499 [M + H]⁺ (calcd for C₂₀H₂₂NO₅, 356.1498).

Discretine *N*-oxide (2): Colorless, amorphous; [α]_D²⁰ –185.4 (c 0.5, CHCl₃); UV (MeOH) λ_{max} (log ε) 235 (3.87), 283 (4.11); IR (KBr) ν_{max} 3320, 1620, 1572, 659 cm^{–1}; ¹H NMR (CD₃OD, 500.13 MHz) δ 6.96 (1H, s, H-12), 6.89 (1H, s, H-1), 6.77 (1H, s, H-9), 6.69 (1H, s, H-4), 4.83 (1H, m, H-13a), 4.82 (1H, m, H-8_{ax}), 4.34 (1H, d, *J* = 14.5 Hz, H-8_{eq}), 3.90 (3H, s, OCH₃-2), 3.87 (3H, s, OCH₃-11), 3.84 (3H, OCH₃-10), 3.79 (1H, ddd, *J* = 3.5, 11.5, 11.5 Hz, H-6_{ax}), 3.71 (1H, br dd, *J* = 5.0, 11.5 Hz, H-6_{eq}), 3.63 (1H, m, H-5_{ax}), 3.62 (1H, dd, *J* = 12.3, 16.5 Hz, H-13_{ax}), 3.32 (1H, dd, *J* = 4.5, 16.5 Hz, H-13_{eq}), 2.77 (1H, br dd, *J* = 3.5, 16.5 Hz, H-5_{eq}); ¹³C NMR (CD₃OD, 125.76 MHz) δ 150.0 (C-11), 149.6 (C-10), 148.4 (C-2), 147.3 (C-3), 126.2 (C-8a), 126.0 (C-4a), 124.4 (C-1a), 122.1 (C-12a), 116.0 (C-4), 113.0 (C-12), 111.2 (C-9), 110.4 (C-1), 71.0 (C-8), 69.3 (C-13a), 65.1 (C-6), 56.7 (OCH₃-2), 56.6 (OCH₃-10), 56.5 (OCH₃-11), 30.9 (C-13), 24.9 (C-5); ESIMS (*m/z*): 358.1656 [M + H]⁺ (calcd for C₂₀H₂₄NO₅, 358.1654).

Antiplasmodial Activity Assay. Antiplasmodial activity of the extracts was determined against the chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum* (IC₅₀ chloroquine = 0.1 μM) as previously described.¹¹ Compounds at the concentration of 10 μg/mL in DMSO were diluted with culture medium to the expected concentrations in 96-well microplates, asynchronous parasite cultures were then added (1% parasitemia and 1.5% final hematocrite), and plates were maintained for 24 h at 37 °C in a candle jar. [³H] hypoxanthine (0.5 μCi) was subsequently added to each well, and parasites were maintained for an additional 24 h. After freezing and thawing, the cells were harvested from each well onto glass fiber filters, and the dried filters were counted in a scintillation spectrometer. The growth inhibition for each well was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture maintained on the same plate. The antiplasmodial activity was further assessed, by determining the concentration inhibiting 50% of parasite growth (IC₅₀) according to Desjardins.¹²

Cytotoxic Activity Assay. KB cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine

(2 mM), penicillin G (100 UI/mL), streptomycin (100 µg/mL), and gentamicin (10 µg/mL). Stock solutions of compounds were prepared in DMSO/H₂O (1:9), and the cytotoxicity assays were carried out in 96-well microtiter plates against human nasopharynx carcinoma KB cell lines (3×10^3 cells/mL) using a modification of the published method.¹³ After 72 h incubation at 37 °C in air/CO₂ (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC₅₀ value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control.

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Supporting Information Available: NMR and MS spectra of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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