Antiplasmodial Alkaloids from *Desmos rostrata*

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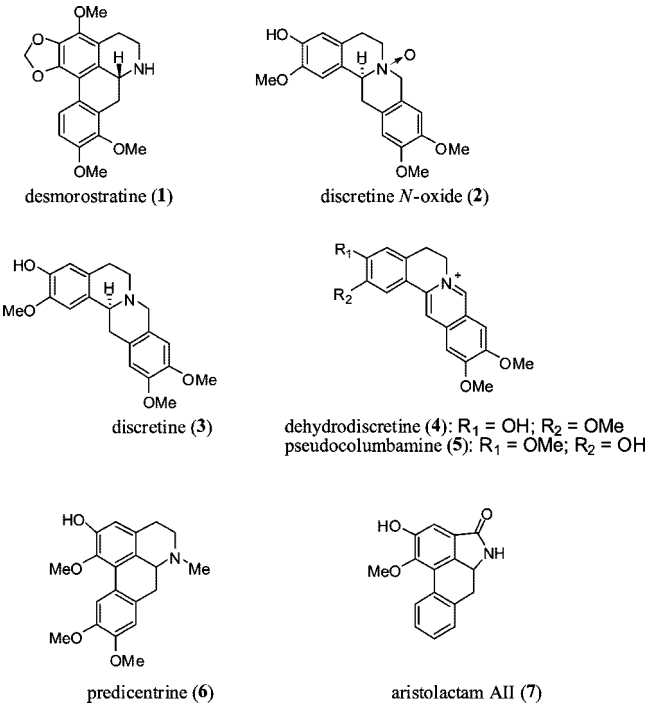
Two new alkaloids, desmorostratine (**1**) and discretine *N*-oxide (**2**), were isolated from the stem bark of *Desmos rostrata*, together with five known alkaloids, discretine (**3**), dehydrodiscretine (**4**), pseudocolumbamine (**5**), predicentrine (**6**), and aristolactam AII (**7**). The structures were established on the basis of spectroscopic data, including mass spectrometry and 2D-NMR. Compound **1** was cytotoxic against KB cells (IC_{50} 2.4 μM), while **2**, **3**, and **4** inhibited *Plasmodium falciparum* (IC_{50} of 4.2, 1.6, and 0.9 μM, 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 flavonoids[2] 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 μg/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 cytotoxity 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 ($[\alpha]_D^{20}$ −22.9, *c* 0.5, $CHCl_3$). In its HRESI mass spectrum, the protonated molecular ion $[M + H]^+$ was observed at *m/z* 356.1499 $[M + H]^+$ (calcd for $C_{20}H_{22}NO_5$, 356.1498), suggesting the molecular formula $C_{20}H_{21}NO_5$. The 1D-NMR spectra (^1H and ^{13}C) indicated the presence of four methylenes, three methines, 10 sp^2 quaternary carbons, and three methoxy groups. The $^1H-^1H$ COSY spectrum of **1** showed three sets of correlations as follows: CH_2-4 (δ_H 2.81) with CH_2-5 (δ_H 2.99 and 3.54), CH-6a (δ_H 3.96) with CH_2-7 (δ_H 2.49 and 3.50), and between two sp^2 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 CH_2-7 (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), CH_2-5 (δ_C 42.1), and C-7a (δ_C 128.1). The chemical shifts of CH-6a and CH_2-5 suggested their linkages to a nitrogen atom. This depicted the bonding of C-1b to CH-6a, which was linked to CH_2-5 via the nitrogen atom N-6. Moreover, the methylene CH_2-4 (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** ($[\alpha]_D^{20}$ −22.9, *c* 0.5, $CHCl_3$) with that of similar structures such as (−)-nordicentrine ($[\alpha]_D^{20}$ −34, *c* 0.2, MeOH)[4] and (+)-*O*-methylcassyfiline ($[\alpha]_D^{20}$ +16.4, *c* 1.0, $CHCl_3$)[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 ($[\alpha]_D^{20}$ −185.4 (*c* 0.5, $CHCl_3$). The HRESI mass spectrum displayed the protonated molecular ion $[M + H]^+$ at *m/z* 358.1656 (calcd for $C_{20}H_{24}NO_5$, 358.1654), in agreement with the molecular formula $C_{20}H_{23}NO_5$. Signals of four sp^3 methylenes, five methines (1 sp^3

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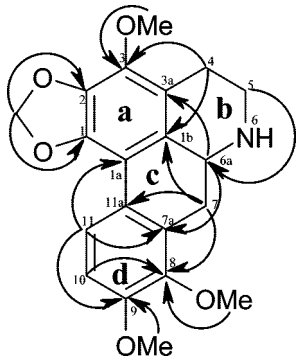

Figure 1. Selected HMBC correlations of **1**.

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

compound	KB cells, IC_{50} (μM)	*P. falciparum* (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 sp^2), and eight sp^2 quaternary carbons in the 1D-NMR spectra (^1H and ^{13}C), 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 CH_2-6, CH_2-8, and CH-13a. The chemical shifts at δ_H 3.79 and 3.71 (CH_2-6), 4.82 and 4.34 (CH_2-8), and 4.83 (CH-13a) were assigned for **2**, whereas the values of 3.06 and 2.66 (CH_2-6), 3.96 and 3.66 (CH_2-8), 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**) {$[\alpha]_D^{20}$ −287.6 (*c* 0.5, $CHCl_3$); lit.[6] −296.6 (*c* 1.04, $CHCl_3$)} 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] predicentrine,[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 IC_{50} 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 (IC_{50} 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 CH_2Cl_2. The CH_2Cl_2 solution was concentrated under diminished pressure, and the residue was purified by open column chromatography over silica gel, eluted with a mixture of CH_2Cl_2/MeOH (99:1 to 0:100) to afford eight fractions (1−8). Fraction 3 was subjected to column chromatography, eluting with CH_2Cl_2/MeOH (95:5) to yield discretine (**3**, 7 mg) and predicentrine (**6**, 10 mg). Fraction 4 was purified by preparative TLC (5% of MeOH in CH_2Cl_2), affording aristolactam AII (**7**, 16 mg). Fractions 5 and 6 were combined and purified by column chromatography, eluting with CH_2Cl_2/MeOH (90:10) to give desmorostratine (**1**, 15 mg) and discretine *N*-oxide (**2**, 6 mg). Fraction 7 was subjected to column chromatography, using CH_2Cl_2/MeOH (85:15), yielding dehydrodiscretine (**4**, 8 mg) and pseudocolumbamine (**5**, 12 mg).

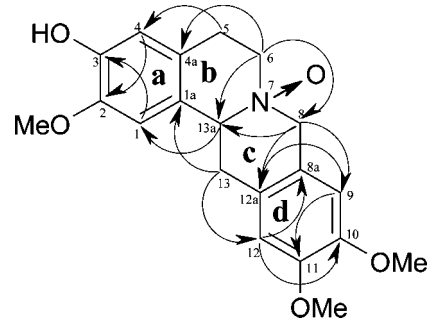

Figure 2. Key HMBC correlations of **2**.

Desmorostratine (1): Colorless solid; mp 153−155 °C; $[\alpha]_D^{20}$ −22.9 (*c* 0.5, $CHCl_3$); UV ($CHCl_3$) 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 ($CDCl_3$, 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-CH_2−O), 4.01 (3H, s, OCH_3-3), 3.96 (1H, dd, J = 5.0, 14.5 Hz, H-6a), 3.89 (3H, s, OCH_3-9), 3.81 (3H, s, OCH_3-8), 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, CH_2-4), 2.49 (1H, dd, J = 14.5, 14.5 Hz, H-7_{ax}); ^{13}C NMR ($CDCl_3$, 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-CH_2−O), 60.7 (OCH_3-8), 59.5 (OCH_3-3), 55.8 (OCH_3-9), 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 $C_{20}H_{22}NO_5$, 356.1498).

Discretine *N*-oxide (2): Colorless, amorphous; $[\alpha]_D^{20}$ −185.4 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 235 (3.87), 283 (4.11); IR (KBr) ν_{max} 3320, 1620, 1572, 659 cm^{-1}; ^1H NMR (CD_3OD, 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, OCH_3-2), 3.87 (3H, s, OCH_3-11), 3.84 (3H, OCH_3-10), 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}); ^{13}C NMR (CD_3OD, 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 (OCH_3-2), 56.6 (OCH_3-10), 56.5 (OCH_3-11), 30.9 (C-13), 24.9 (C-5); ESIMS (*m*/*z*): 358.1656 $[M + H]^+$ (calcd for $C_{20}H_{24}NO_5$, 358.1654).

Antiplasmodial Activity Assay. Antiplasmodial activity of the extracts was determined against the chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum* (IC_{50} 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. [^{3}H] 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 (IC_{50}) according to Desjardin.[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/H_2O (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/CO_2 (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 IC_{50} 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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