# Cytotoxic and Antimalarial Alkaloids from the Bulbs of Crinum amabile

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# CYTOTOXIC AND ANTIMALARIAL ALKALOIDS FROM THE BULBS OF *CRINUM AMABILE*[1]

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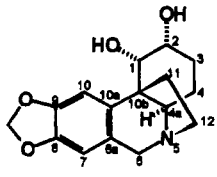
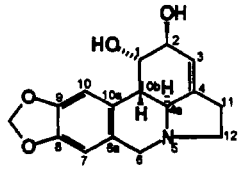
ABSTRACT.—From the bulbs of *Crinum amabile* (Amaryllidaceae), a new alkaloid (−)-amabiline [**1**], together with the known alkaloids (−)-lycorine [**2**], (−)-buphanisine [**3**], (−)-augustine [**4**], and (+)-crinamine [**5**], were isolated. The structural characterization of **1** and the revised $^1$H- and $^{13}$C-nmr assignments of **2** are discussed. Alkaloids **2**, **4**, and **5** were found to be the principal cytotoxic and antimalarial constituents.

Although a wide range of biological activities have been established for the Amaryllidaceae alkaloids, including antitumor, antiviral, and immunostimulant properties (2–4), there have been no reports concerning the antimalarial activity of these compounds. This prompted us to explore the possibility of developing antimalarial drugs from this group of alkaloids, as a part of our ongoing search for antimalarial agents from natural sources (5–7). A previous phytochemical investigation of the bulbs of *Crinum amabile* Donn. described the identification of (−)-lycorine [**2**], (+)-tazettine, (−)-galanthamine, (±)-narvedine, (−)-galanthine, (+)-hippeastrine, and (−)-crinidine, on the basis of interpretation of their mp, uv, ir, and $[\alpha]^{20}$D data (8). Our preliminary biological evaluation of an EtOH extract of the bulbs of *C. amabile* revealed both cytotoxic and antimalarial potential for the plant (1). Bioassay-guided fractionation was then employed to determine the compounds responsible for the biological activities. In a previous report (1), we discussed the structure elucidation and cytotoxic and antimalarial properties of amabiloside, a new glycoside isolated from the non-alkaloid fraction. As a continuation of this work, further separation of *C. amabile* was undertaken, and this led to the isolation of a new alkaloid, which we have named (−)-amabiline [**1**], in addition to the known alkaloids (−)-lycorine [**2**], (−)-buphanisine [**3**], (−)-augustine [**4**], and (+)-crinamine [**5**]. We present, herein, the structure elucidation of the new compound **1**, the revised $^1$H- and $^{13}$C-nmr assignments of **2**, and the cytotoxic and antimalarial activities of the isolates.

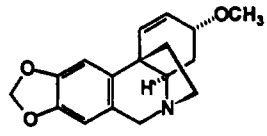


**1**



**2**

[1]Paper XXII in the series "Traditional Medicinal Plants of Thailand." For paper XXI, see Likhitwitayawuid *et al.* (1).

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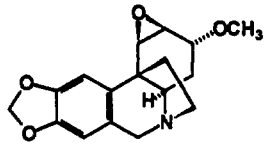


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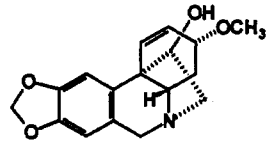


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## RESULTS AND DISCUSSION

The molecular formula of the new alkaloid (−)-amabiline [**1**] was deduced to be $C_{16}H_{19}NO_4$ (calcd 289.1314 amu) from its molecular ion at *m/z* 289.1305 in the hreims. A crinane structure was evident from its uv absorptions at 234 and 292 nm (3), and the presence of a methylenedioxy group at C-8 and C-9 was indicated from the methylene proton signals at δ 5.89 and 5.91 (1H each, s) and the aromatic proton resonances of H-7 at 6.51 (s) and H-10 at δ 6.79 (s) in the $^1$H-nmr spectrum. Analysis of the $^{13}$C-nmr and APT spectra of **1** revealed the presence of five methylene carbons (δ 25.26, 26.36, 37.67, 50.28, and 61.04), three methine carbons (δ 62.95, 67.37, and 68.52), and a quaternary carbon (δ 49.30) in the aliphatic region which could be assigned to C-10b. The most upfield methine carbon resonance (δ 62.95) was tentatively assigned to C-4a. It could be inferred from their chemical shifts that the two remaining methine carbons were hydroxylated. In order to locate these carbinol carbons, the homonuclear COSY, NOESY, and HETCOR experiments were performed. From the HETCOR spectrum of **1**, the carbon at δ 68.52 was correlated to the proton at δ 4.32 (br s), whereas the carbon at δ 67.37 showed one-bond coupling to the proton at δ 3.88 (br d, $J$=11.2 Hz). Vicinal coupling between these two protons (δ 4.32 and 3.88) was observed in the COSY spectrum, suggesting a glycol partial structure for **1**. In the NOESY spectrum, since the proton at δ 4.32 showed an nOe with H-10 (δ 6.79), it must be located at C-1 and have an equatorial orientation; hence, the C-1 OH group must have an axial orientation. These structural features necessitated the placement of the other OH group at C-2 (δ 67.37), and the proton resonance at δ 3.88 was consequently assigned to H-2. The nOe between H-1 (δ 4.32) and H-2 (δ 3.88) in the NOESY spectrum substantiates these attributions. Further examination of the COSY, NOESY, and HETCOR spectra allowed for the assignment of all of the remaining proton and carbon resonances of **1** (Table 1). The proton resonance at δ 3.05 (dd, $J$=12.1, 5.7 Hz) was correlated to C-4a (δ 62.95) in the HETCOR spectrum and was therefore assigned to H-4a. This H-4a proton displayed an nOe with the proton at δ 4.13 (d, $J$=16.6 Hz), which consequently was assigned to H-6α. From the COSY spectrum, H-6α showed geminal coupling ($J$=16.6 Hz) with H-6β at δ 3.59. An nOe between these two methylene protons was also observed in the NOESY spectrum. In addition, H-6β displayed an nOe with the proton at δ 2.62 (ddd, $J$=14.2, 6.1, 5.9 Hz), which was therefore assigned to H-12β; from the COSY spectrum, H-12β was coupled to H-12α at δ 3.13. The nOe observed between these two methylene protons in the NOESY spectrum confirmed these assignments. These C-12 methylene

TABLE 1. $^{1}H$- and $^{13}C$-nmr Spectral Assignments of (−)-Amabiline [**1**].[a]

| Position | $^{1}H$ | $^{13}C$ |
|---|---|---|
| 1 | 4.32 (br s) | 68.52 |
| 2 | 3.88 (br d, 11.2) | 67.37 |
| 3α | 1.48 (m) | 26.36 |
| β | 1.61 (m) | |
| 4α | 1.56 (m) | 25.26 |
| β | 1.25 (dddd, 12.7, 12.1, 12.1, 0.8) | |
| 4a | 3.05 (dd, 12.1, 5.7) | 62.95 |
| 6α | 4.13 (d, 16.6) | 61.04 |
| β | 3.59 (d, 16.6) | |
| 6a | | 125.97 |
| 7 | 6.51 (s) | 105.85 |
| 8 | | 144.74 |
| 9 | | 145.31 |
| 10 | 6.79 (s) | 105.16 |
| 10a | | 138.81 |
| 10b | | 49.30 |
| 11α | 2.06 (ddd, 16.4, 5.9, 5.6) | 37.67 |
| β | 1.67 (m) | |
| 12α | 3.13 (m) | 50.28 |
| β | 2.62 (ddd, 14.2, 6.1, 5.9) | |
| $OCH_2O$ | 5.89 and 5.91 (each s) | 100.22 |

[a]Chemical shifts are reported in ppm (δ) from TMS in DMSO-$d_6$ at 300 MHz for $^{1}H$ and 75.6 MHz for $^{13}C$; signal multiplicity and coupling constants are in parentheses.

protons were shown to be coupled to the C-11 methylene protons at δ 2.06 and 1.67. The former signal, showing a nOe with H-2 (δ 3.88), was assigned to H-11α, and the latter should therefore be assigned to H-11β. More importantly, the nOe between H-2 (δ 3.88) and H-11α (δ 2.06) established that H-2 had a β-axial orientation, and since H-1 (δ 4.32) displayed a nOe with H-2, it must have a β-equatorial orientation. In the NOESY spectrum, H-4a displayed an nOe with another proton at δ 1.56 (m), which was therefore assigned to H-4α. The proton at δ 1.25 (ddd, $J$=12.7, 12.1, 0.8 Hz), which was geminally coupled to and displayed an nOe with H-4α (δ 1.56), was assigned to H-4β.

The structure of **1** was further confirmed by a series of selective INEPT experiments (9,10). This also led to the unambiguous $^{13}C$-nmr assignments of **1**. Polarization transfer from H-10 (δ 6.79) resulted in the enhancements of the carbon resonances at δ 49.30, 125.97, and 144.74, which could be assigned to C-10b, C-6a, and C-8, respectively. Selective INEPT irradiation of H-7 (δ 6.51) enhanced the signals of C-6 (δ 61.04), C-9 (δ 145.31), and C-10a (δ 138.81). The signals of C-10a (δ 138.81) and C-1 (δ 68.52) were enhanced when H-11α was irradiated, and this, therefore, confirmed the presence of an OH group at C-1. When H-6β (δ 3.59) was irradiated, the resonances of C-4a at δ 62.95 and C-10a at δ 138.81 were enhanced. Magnetization transfer from H-1 (δ 4.32) to C-3 (δ 26.36), C-4a (δ 62.95), and C-10a (δ 138.81) was observed when H-1 was selectively irradiated. Selective INEPT irradiation of H-6α (δ 4.13) elicited the three-bond couplings between H-6α and C-12 (δ 50.28), and between H-6α and C-10a (δ 138.81). Enhancements of C-4a (δ 62.95), C-6 (δ 61.04), and C-10b (δ 49.30) were observed on selective INEPT irradiation of H-12β. When the H-4β was irradiated, the enhancements of C-2 (δ 67.37) and C-10b (δ 49.30) were observed.

(−)-Lycorine [**2**] has been the subject of various nmr studies as a result of its

antitumor and antiviral properties (3,4). However, although the $^{1}H$- and $^{13}C$-nmr spectra of (−)-lycorine in DMSO-$d_6$ (11) and $CD_3OD$-$CD_3COOD$ (3:1) (12) have been extensively analyzed, complete $^{1}H$- and unambiguous $^{13}C$-nmr assignments have not been reported. In the present study, a combination of several nmr techniques consisting of the homonuclear COSY, NOESY, APT, HETCOR, and selective INEPT experiments was applied, and the results are summarized in Table 2. It should be noted that the previous assignments of 1-OH, 2-OH, C-1, C-2, C-8, and C-9 (11) were revised.

Compounds **1–5** were then evaluated for their antimalarial and cytotoxic potential according to established protocols (7), and the results are summarized in Tables 3 and 4. Among the 5,10b-ethanophenanthridines (crinane type), (−)-augustine [**4**] appeared to be the most active alkaloid, demonstrating a significant cytotoxic response in all cell lines tested except drug-resistant KB (KB-V1) and antimalarial activity in both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. These activities were probably due to the presence of the epoxide functionality, which can result in the formation of adducts with nucleophiles in biological systems, leading to nonselective toxicity. This is reflected in the generation of a poor selectivity index (Table 3). Structures lacking the oxiran ring between C-1 and C-2, (−)-amabiline [**1**] and (−)-buphanisine [**3**], were not active. However, (+)-crinamine [**5**], differing from **1**, **3**, and **4** in its absolute configuration, demonstrated strong cytotoxic and moderate antimalarial activities. Thus, the epoxide functionality is not essential for activity, and other elements of structure may come into play. This is also suggested by the pyrrolophenanthridine, (−)-lycorine [**2**], which was found to be very cytotoxic in all cancer cell lines tested (Table 3). Crinamine [**5**] also showed moderate antimalarial activity, but, as for compounds **2** and **4**, very low selectivity indices were obtained in comparison to the values observed with the antimalarial control compounds (Table 4).

TABLE 2. $^{1}H$- and $^{13}C$-nmr Spectral Assignments of (−)-Lycorine [**2**].[a]

| Position | $^{1}H$ | $^{1}H$[b] | $^{13}C$ | $^{13}C$[b] |
|---|---|---|---|---|
| 1 | 4.27 (br s) | | 70.21 | 71.8 |
| 2 | 3.97 (br s) | | 71.72 | 70.3 |
| 3 | 5.37 (br s) | | 118.48 | 118.5 |
| 4 | | | 141.68 | 141.68 |
| 4a | 2.60 (d, 10.6) | | 60.83 | 60.8 |
| 6α | 3.32 (d, 14.4) | | 56.73 | 56.7 |
| β | 4.02 (d, 14.4) | | | |
| 6a | | | 129.75 | 129.8[c] |
| 7 | 6.68 (s) | 6.60 | 107.01 | 107.1 |
| 8 | | | 145.20 | 145.7 |
| 9 | | | 145.65 | 145.3 |
| 10 | 6.81 (s) | 6.80 | 105.06 | 105.1 |
| 10a | | | 129.57 | 129.7[c] |
| 10b | 2.50 (m) | | 40.18 | 40.3 |
| 11α,β | 2.44 (2H, m) | | 28.13 | 28.2 |
| 12α | 2.19 (ddd, 14.4, 8.6, 1.5) | | 53.31 | 53.4 |
| β | 3.19 (dd, 14.4, 7.5) | | | |
| $OCH_2O$ | 5.94 and 5.96 (each s) | 5.90 (2H, s) | 100.57 | 100.6 |
| 1-OH | 4.79 (br d, 2.9) | 4.80 | | |
| 2-OH | 4.90 (br s) | 4.70 | | |

[a]Chemical shifts are reported in ppm (δ) from TMS in DMSO-$d_6$ at 300 MHz for $^{1}H$ and 75.6 MHz for $^{13}C$; signal multiplicity and coupling constants are in parentheses.

[b]Data are from Ali *et al.* (11).

[c]Assignments are interchangeable.

TABLE 3. Evaluation of the Cytotoxic Activity of Compounds Isolated from *Crinum amabile*.

| Alkaloid | Cell line tested ($ED_{50}$, μg/ml)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BCA-1 | HT-1080 | LUC-1 | MEL-2 | COL-1 | KB | KB-V1 | P-388 | A-431 | LNCaP | ZR-75-1 | U-373 |
| 5,10b-Ethanophenanthridines | | | | | | | | | | | | |
| (−)-Amabiline [1] | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >5 | >20 | >20 | >20 | >20 |
| (−)-Buphanisine [3] | >20 | >20 | >20 | >20 | >20 | >20 | 19.8 | >5 | >20 | >20 | >20 | >20 |
| (−)-Augustine [4] | 2.8 | 1.2 | 3.7 | 3.2 | 2.4 | 0.6 | >20 | 0.6 | 4.9 | 1.7 | 1.8 | 0.6 |
| (−)-Crinamine [5] | 1.4 | 1.3 | 1.4 | 5.0 | 1.0 | 1.0 | 0.6 | 0.7 | 6.9 | 1.5 | 0.8 | 0.9 |
| Pyrrolophenanthridine | | | | | | | | | | | | |
| (−)-Lycorine [2] | 1.6 | 0.5 | 0.9 | 1.6 | 0.8 | 0.3 | 0.4 | 0.9 | 1.3 | 0.5 | 0.9 | 0.3 |

[a]BCA-1=human breast cancer, HT-1080=human fibrosarcoma, LUC-1=human lung cancer, MEL-2=human melanoma, COL-1=human colon cancer, KB=human oral epidermoid carcinoma, KB-V1=vinblastine-resistant KB, P-388=murine lymphoid neoplasm, A-431=human epidermoid carcinoma, LNCaP=hormone-dependent human prostatic cancer, ZR-75-1=hormone-dependent breast cancer, U-373=human glioblastoma.

TABLE 4. Evaluation of the Antimalarial Activity of Compounds Isolated from *Crinum amabile*.

| Compound | *Plasmodium falciparum* strain ($ED_{50}$, ng,ml)[a] | | | |
|---|---|---|---|---|
| | D-6 | | W-2 | |
| 5,10b-Ethanophenanthridines | | | | |
| (−)-Amabiline [**1**] | >10000 | | >10000 | |
| (−)-Buphanisine [**3**] | 3500 | | 5340 | |
| (−)-Augustine [**4**] | 140 | (4)[b] | 180 | (3) |
| (+)-Crinamine [**5**] | 2180 | (0.4) | 2520 | (0.4) |
| Pyrrolophenanthridines | | | | |
| (−)-Lycorine [**2**] | 320 | (1) | 300 | (1) |
| Controls | | | | |
| Chloroquine | 1.3 | (13380) | 11.2 | (1550) |
| Quinine | 9.4 | (>2130) | 24.5 | (>820) |
| Mefloquine | 7.3 | (480) | 1.6 | (2190) |
| Artemisinin | 0.6 | (>33300) | 0.5 | (>40000) |

[a]Chloroquine-sensitive (D-6) and chloroquine-resistant (W-2) strains of *Plasmodium falciparum*.

[b]Numbers in parentheses are the ratio of the $ED_{50}$ in KB cells to the $ED_{50}$ in the parasites (selectivity index) (5–7).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Uv spectra were obtained on a Beckman Du-7 spectrometer, and the ir spectra were measured on a Nicolet MX-1 FT-IR (KBr) interferometer. $^1$H-nmr, homonuclear COSY, $^{13}$C-nmr, APT, and HETCOR spectra were recorded in $CDCl_3$, with TMS as internal standard, employing a Varian XL-300 instrument. Standard Varian pulse sequences were used. Selective INEPT experiments were performed at 90.8 MHz using a Nicolet NMC-360 spectrometer. Data sets of 16K covering a spectral width of 10 MHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% $C_6D_6$ ($^1J$=6.7 Hz) in a 5-mm nmr tube. The radiofrequency field strength for the soft proton pulse was on the order of 25 Hz for these experiments. Eight Hz was used as $^3J_{CH}$ for aromatic protons, and 6 and 3 Hz for aliphatic protons. The ms was obtained with a Varian MAT 112S instrument operating at 70 eV. Unless stated otherwise, preparative tlc was performed on Si gel plates.

PLANT MATERIAL.—The bulbs of *C. amabile* were collected in Bangkok, Thailand, in January 1990. Authentication was achieved by comparision with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—The minced fresh bulbs of *C. amabile* (5.5 kg) were extracted with EtOH (12 liters). The EtOH extract, after removal of the organic solvent, was dried in a lyophilizer to give a brownish residue (20 g). This residue was triturated in petroleum ether (500 ml) and filtered. Evaporation of the solvent from the filtrate gave fraction A (136 mg). The petroleum ether-insoluble materials were then partitioned between $H_2O$ (500 ml) and EtOAc (1.5 liters). The EtOAc phase was evaporated under reduced pressure and dried in vacuo to yield fraction B (1.2 g). The $H_2O$ phase was basified with $NaHCO_3$ to pH 9 and then treated with EtOAc (1 liter). Removal of the solvent from the organic fraction gave a residue designated as fraction C. The $H_2O$ phase was then partitioned by shaking with *n*-BuOH (300 ml). The residues obtained from the *n*-BuOH and $H_2O$ fractions, after removal of the solvents, were designated as fractions D and E, respectively. Each fraction was then evaluated for cytotoxic and antimalarial activities. Fractions B ($ED_{50}$ 0.1 and 0.4 μg/ml in P-388 and KB; 1570 and 4160 ng/ml in D-6 and W-2 strains of *Plasmodium falciparum*), C (0.02 and 1.1 μg/ml; 590 and 1940 ng/ml), and D (0.2 and 1.6 μg/ml; 770 and 880 ng/ml) were selected for further investigation.

Fraction B was chromatographed over a Si gel column, using several combinations of $CHCl_3$ and MeOH with increasing polarity. Twenty-five fractions (40 ml each) were collected. Fractions with similar tlc behavior were combined to give three major fractions designated fractions B.1, B.2, and B.3. Fraction B.1 was further purified by preparative tlc eluting with $CHCl_3$-MeOH (95:5) to afford (−)-augustine [**4**] (14 mg). Preparative tlc of fraction B.2, using toluene-MeOH (9:1) as the solvent, afforded buphanisine [**3**]

(10 mg). Fraction B.3 was further purified by preparative tlc, using $CHCl_3$-MeOH (9:1) as the eluent to give (+)-crinamine [**5**] (10 mg). Fraction C was dried and recrystallized from EtOH to give (−)-lycorine [**2**] (1.1 g). Fraction D was suspended in MeOH (200 ml) and filtered. The collected precipitates were identified as lycorine [**2**] (1.0 g). The filtrate was evaporated under reduced pressure and dried to give a residue (200 mg) (Fraction F). This residue was subjected to cc, using Si gel as the adsorbent and $CHCl_3$-MeOH-$NH_4OH$ (7:2:1) as the eluting agent. Twenty-five 30-ml fractions were collected. Fractions 11 and 12 were combined and dried to give (−)-amabiloside (12 mg) (1). Cc of fraction F was continued, and fractions 22 and 23 were combined, dried, and further purified by preparative tlc, eluting with $CHCl_3$-MeOH-$NH_4OH$ (7:2:1), and then recrystallized from MeOH to give (−)-amabiline [**1**] (20 mg).

(−)-*Amabiline* [**1**].—Mp 210° (dec); $[\alpha]^{20}D$ −32° (*c*=0.3, EtOH); uv λ max (EtOH) 209 (log ε 4.13), 234 (3.49), 292 (3.61) nm; ir ν max (KBr) 3488, 1483, 1235, 1132, 1042 $cm^{-1}$; $^1H$ and $^{13}C$ nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 289 (100), 272 (11), 245 (15), 216 (11), 202 (23), 201 (13), 190 (11); hreims 289.1305 (calcd for $C_{16}H_{19}NO_4$, 289.1314).

(−)-*Lycorine* [**2**].—Mp 250°; $[\alpha]^{20}D$ −62° (*c*=0.1, EtOH); uv λ max (MeOH) 207 (log ε 4.59), 234 (3.88), 292 (3.96) nm; ir ν max (KBr) 3324, 2865, 1503, 1486, 1356, 1312, 1262, 1238, 1038, 1001 $cm^{-1}$; $^1H$ and $^{13}C$ nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 287 (56), 286 (35), 268 (36), 250 (18), 228 (14), 227 (82), 226 (100), 112 (26). These physical and spectral properties were consistent with those of (−)-lycorine (11,12).

(−)-*Buphanisine* [**3**].—Mp 118°; $[\alpha]^{20}D$ −26° (*c*=0.1, EtOH); uv λ max (EtOH) 206 (log ε 4.31), 236 (3.37), 294 (3.56) nm; ir ν max (film) 2932, 1483, 1316, 1235, 1092, 1040 $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$) δ 6.81 (1H, s, H-10), 6.58 (1H, d, *J*=10.0 Hz, H-1), 6.45 (1H, br s, H-7), 5.95 (1H, dd, *J*=10.0, 5.3 Hz, H-2), 5.86 and 5.85 (each 1H, s, $OCH_2O$), 4.38 (1H, d, *J*=16.8 Hz, H-6α), 3.80(1H, m, H-3), 3.76 (1H, d, *J*=16.8 Hz, H-6β), 3.34 (3H, s, 3-OMe); $^{13}C$ nmr (75.6 MHz, $CDCl_3$) δ146.06 (C-9), 145.66 (C-8), 138.33 (C-10a), 132.84 (C-2), 126.07 (C-6a), 125.34 (C-1), 106.85 (C-7), 102.92 (C-10), 100.69 ($OCH_2O$), 72.57 (C-3), 63.05 (C-4a), 62.26 (C-6), 56.46 (3-OMe), 53.49 (C-12), 44.33 (C-10b), 44.13 (C-11), 28.70 (C-4); eims *m/z* (rel. int.) $[M]^+$ 285 (100), 270 (23), 254 (22), 230 (24), 216 (17), 215 (64), 201 (16), 198 (11), 185 (13), 172 (14), 157 (13). These data were identical with published values for (−)-buphanisine (11).

(−)-*Augustine* [**4**].—Mp 170°; $[\alpha]^{20}D$ −62° (*c*=0.2, EtOH); uv λ max (EtOH) 206 (log ε 4.25), 233 (3.50), 294 (3.57) nm; ir ν max (film) 2953, 1485, 1237, 1112, 1038 $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$) δ 6.87 (1H, s, H-10), 6.46 (1H, br s, H-7), 5.87 (2H, br s, $OCH_2O$), 4.35 (1H, d, *J*=16.7 Hz, H-6α), 3.95 (1H, m, H-3), 3.75 (1H, d, *J*=1.5 Hz, H-1), 3.70 (1H, d, *J*=16.7 Hz, H-6β), 3.40 (3H, s, 3-OMe), 3.24 (1H, m, H-2), 3.16 (1H, m, H-12β), 3.10 (1H, dd, *J*=13.2, 3.5 Hz, H-4a), 2.79 (1H, m, H-12α), 2.37 (1H, m, H-11β), 1.99 (1H, m, H-11α), 1.73 (1H, m, H-4α), 1.37 (1H, ddd, *J*=13.7, 13.7, 2.9 Hz, H-4β); $^{13}C$-nmr (75.6 MHz, $CDCl_3$) δ 146.21 (C-9), 145.78 (C-8), 137.57 (C-10a), 126.34 (C-6a), 107.09 (C-7), 102.51 (C-10), 100.85 ($OCH_2O$), 74.77 (C-3), 62.12 (C-6), 61.58 (C-4a), 57.61 (3-OMe), 55.01 (C-2), 53.73 (C-1), 52.23 (C-12), 41.64 (C-10b), 39.09 (C-11), 25.01 (C-4); eims *m/z* (rel. int.) $[M]^+$ 301 (100), 286 (6), 272 (6), 270 (6), 268 (6), 256 (10), 228 (35), 227 (12), 226 (18), 187 (13), 175 (45), 173 (13), 143 (10), 115 (22). The physical and spectral properties of **5** were in excellent agreement with those reported earlier for (−)-augustine (11,13).

(+)-*Crinamine* [**5**].—Mp 188°; $[\alpha]^{20}D$ +96° (*c*=0.1, EtOH); uv λ (EtOH) 208 (log ε 4.22), 235 (3.42), 294 (3.54) nm; ir ν max (film) 2930, 1481, 1238, 1107, 1037 $cm^{-1}$; $^1H$ nmr (300 MHz, $CDCl_3$) δ 6.78 (1H, s, H-10), 6.46 (1H, s, H-7), 6.23 (2H, s, H-1 and H-2), 5.87 (2H, s, $OCH_2O$), 4.29 (1H, d, *J*=16.9 Hz, H-6α), 3.67 (1H, d, *J*=16.9 Hz, H-6β), 3.38 (3H, s, 3-OMe); $^{13}C$ nmr (75.6 MHz, $CDCl_3$) δ 146.50 (C-9), 146.22 (C-8), 135.99 (C-2), 135.32 (C-10a), 126.55 (C-6a), 123.60 (C-1), 106.87 (C-7), 103.18 (C-10), 100.87 ($OCH_2O$), 79.99 (C-11), 76.02 (C-3), 66.10 (C-4a), 63.44 (C-6), 61.18 (C-12), 55.80 (3-OMe), 50.26 (C-10b), 30.14 (C-4); eims *m/z* (rel. int.) $[M]^+$ 301 (2), 269 (100), 268 (30), 240 (31), 225 (16), 224 (18), 211 (17), 181 (40), 153 (10), 115 (11). The physical and spectral data were superimposable with those of (+)-crinamine (14).

CYTOTOXICITY AND ANTIMALARIAL ASSAYS.—The biological evaluations for cytotoxic and antimalarial activities of the isolates were carried out according to established protocols (7).

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