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LYCORINE ALKALOIDS FROM *HYMENOCALLIS LITTORALIS**

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Key Word Index—*Hymenocallis littoralis*; Amaryllidaceae; lycorine alkaloids; littoraline; structure determination; biological evaluation

Abstract—From *Hymenocallis littoralis*, one new alkaloid, named littoraline, together with 13 known lycorine alkaloids and one lignan, were isolated. The structure and NMR assignments of this new alkaloid were determined by 1D and 2D NMR techniques. Littoraline showed inhibitory activity of HIV reverse transcriptase, and lycorine and haemanthamine showed potent *in vitro* cytotoxicity.

INTRODUCTION

During our investigations of HIV reverse transcriptase inhibitory, cytotoxic and antimalarial compounds from plants, the alkaloid extract of *Hymenocallis littoralis* (Amaryllidaceae) showed *in vitro* cytotoxic activity. Amaryllidaceae alkaloids are well known to possess interesting biological activities [1, 2]; we therefore studied this extract further and isolated one new alkaloid, littoraline (**1**), along with 13 known lycorine alkaloids, tazettine (**2**) [3, 4], pretazettine (**3**) [3, 4], macronine (**4**) [3–5], lycorine (**5**) [3, 4], homolycorine (**6**) [6, 7], lycorenine (**7**) [3, 4], *O*-methyllycorenine (**8**) [3, 7], hippeastrine (**9**) [3, 4], lycoramine (**10**) [3, 4], demethylmaritidine (**11**) [3, 4], haemanthamine (**12**) [3, 4], vittatine (**13**) [3, 4] and 5,6-dihydrobicolorine (**14**) [8], together with a known lignan, secoisolariciresinol (**15**) [9]. The structure elucidation of the new alkaloid was accomplished by a range of one- and two-dimensional NMR techniques, such as COSY, CSCM 1D [10] and selective INEPT [11, 12]. Some of the isolated alkaloids showed cytotoxicity and HIV reverse transcriptase inhibitory activity. In this paper, we present the isolation and biological evaluation of the above compounds, and the structure determination of the new alkaloid.

RESULTS AND DISCUSSION

Compound **1**, a white powder, $C_{18}H_{21}NO_5$ (HRMS), showed ^1H, ^{13}C and APT NMR spectral signals for one methoxyl, one *N*-methyl, one OCH_2O group, four olefinic protons, two aliphatic methines, three methylenes, four olefinic quaternary carbons, and two aliphatic quaternary carbons, suggesting that **1** was a tazettine-type lycorine alkaloid. Furthermore, both alkaloids have the same molecular formula, the same type of functional groups, and very similar ^1H and ^{13}C NMR data. However, the COSY spectra of **1** and **2** showed some different correlation contours: for **1**, there were clear correlation contours between H-6a and each of the H-6 methylene protons, but no proton showed correlation with the H-4 protons; for **2**, clear correlations were noted between H-4a and each of the H-4 protons, but no proton showed a correlation with the H-6 protons. This fact suggested that the hydroxyl function of **1** was located at the C-4a position, which was proved by the selective INEPT NMR technique. Selective INEPT irradiation of the *N*-Me (δ2.43) enhanced C-6 (δ63.5) and a quaternary carbon signal at δ93.0, which should be C-4a. Analysis of the ^1H NMR data for **1**, **2**, **3** and **4** indicated that the C-3 methoxyl group of **1** is also in a β-configuration, as in alkaloids **2** and **3**, but different from **4**, which has a 3-OMe in an α-configuration, with the H-4 proton signals of **4** at δ1.72 (*ddd*, J = 2.0, 9.5, 11.0 Hz) and 2.55 (*m*). Furthermore, comparison of the chemical shift and coupling constants of H-6a with H-6A and H-6B of **1** and **4**, indicated that the H-6a of **1** could be deduced to be in the β-configuration. Computer modelling calculations by the PCMODEL program [13] also suggested that the 3-OMe, H-6a and C4a-OH were each in a β-configuration (calc. $J_{3\alpha,2}$ = 2.68 Hz, $J_{3\alpha,4A}$ = 10.84 Hz, $J_{3\alpha,4B}$ = 4.8 Hz, $J_{6a,6A}$ = 10.06 Hz, and $J_{6a,6B}$ = 6.99 Hz; obsd $J_{3\alpha,2}$ = 2.0 Hz, $J_{3\alpha,4A}$ = 9.5 Hz, $J_{3\alpha,4B}$ = 6.5 Hz, $J_{6a,6A}$ = 9.5 Hz, and $J_{6a,6B}$ = 7.5 Hz). Computer modelling calculations

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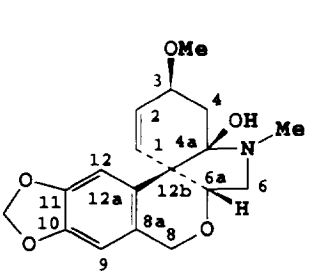

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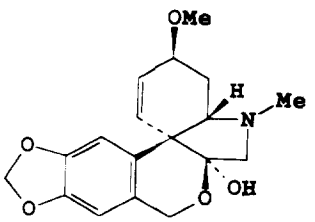

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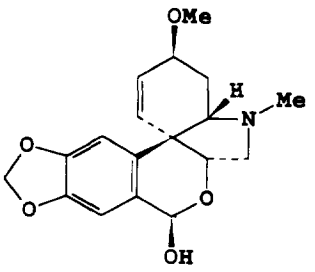

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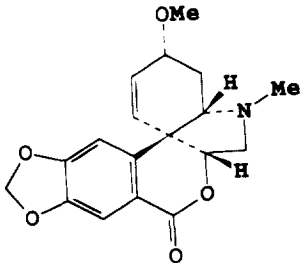

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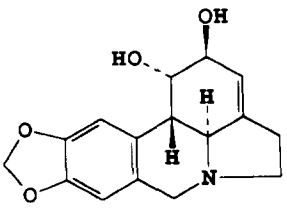

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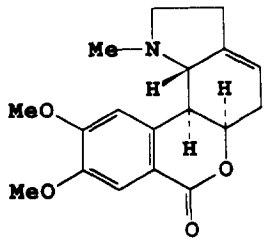

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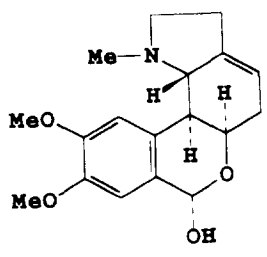

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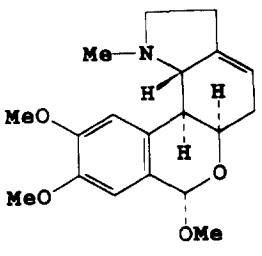

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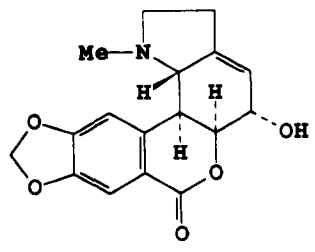

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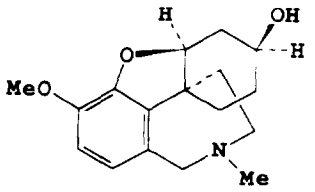
gave the coupling constants for different, alternative combinations of the configurations of the C-3-OMe and C-4a-OH, i.e. β-C-3a-OMe and α-C-4a-OH ($J_{3\alpha,2}$ = 4.75 Hz, $J_{3\alpha,4A}$ = 8.61 Hz and $J_{3\alpha,4B}$ = 1.58 Hz), α-C-3-OMe and β-C-4a-OH ($J_{3\alpha,2}$ = 4.85 Hz, $J_{3\alpha,4A}$ = 4.13 Hz and $J_{3\alpha,4B}$ = 2.67 Hz) and α-C-3-OMe and α-C-4a-OH ($J_{3\alpha,2}$ = 3.37 Hz, $J_{3\alpha,4A}$ = 9.52 Hz and $J_{3\alpha,4B}$ = 2.14 Hz), but none of them gave J values closer to the observed data. Therefore, this hydroxyl function is most probably in a β-configuration. However, due to a lack of material for derivatization or signal crystal analysis, the stereochemistry of the C-4a hydroxyl function is not absolutely proved.

Complete NMR data assignments of **1** were obtained from analysis of COSY, CSCM 1D and selective INEPT spectra. The COSY spectrum also showed correlation contours at H-1/H-2, H-2/H-3, H-3/H-4A, H-3/H-4B, H-4A/H-4B, H-6A/H-6B, H-6A/H-6a and H-6B/H-6a. The one-dimensional heteronuclear correlation (CSCM 1D) spectra were obtained by the irradiation of one of the satellites of each proton signal in order to enhance its corresponding, directly attached carbon signal. Selective INEPT irradiation of H-1 enhanced C-3, C-4a, C-6a and C-12a, irradiation of H-2 enhanced C-4 and C-12b, and irradiation of H-3 enhanced C-1, C-4a and the carbon signal of the methoxyl group. Irradiation of H-9 enhanced C-8, C-11 and C-12a, and irradiation of H-12 enhanced C-8a, C-10 and C-12b. In the same way, irradiation of each of the remaining proton signals determined most of their corresponding carbons at three bonds away, which led to the complete and unambiguous assignment of the ^{1}H and ^{13}C NMR reasonances.

The isolated alkaloids were evaluated for their cytotoxic, antimalarial and HIV-1 reverse transcriptase inhibitory activities [14–16]. No antimalarial activity was observed and only **1** weakly inhibited HIV-1 reverse transcriptase (p66/p51) (IC_{50} = 142.0 μg ml^{-1}). Compounds **5** and **12** showed *in vitro* cytotoxicity against a variety of cultured cells (see Table 1). Another known antitumour lycorine alkaloid, **3**, was one of the major alkaloids of this plant and also should be responsible, in part, for the cytotoxicity demonstrated with the extracts of this plant [1–4]. However, due to its rapid transformation into **2** during separation and purification [1–4] a pure sample was not isolated and tested in this investigation.



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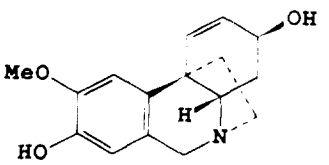

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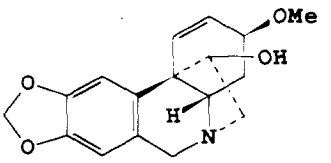

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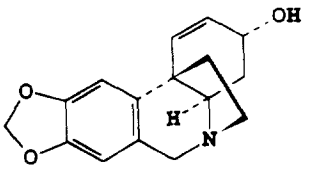

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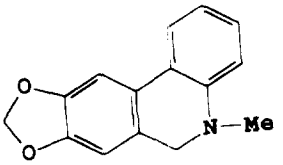

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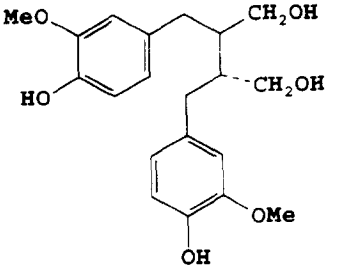

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Table 1. Evaluation of the cytotoxic activity of lycorine (5) and haemanthamine (12)

Cell line* (ED_{50}, $\mu g\ ml^{-1}$)	5	12
BCA-1	0.4	0.7
HT-1080	0.5	0.3
LUC-1	3.6	3.6
COL-2	0.4	0.6
KB	0.4	0.7
KB-V	0.7	1.3
P-388	0.5	5.0
A-431	0.4	1.3
LNCaP	0.7	0.6
ZR-75-1	0.2	0.5
U-373	> 20	3.5

*BCA-1 = human breast cancer; HT-1080 = human fibrosarcoma; LUC-1 = human lung cancer; COL-2 = human colon cancer; KB = human oral epidermoid carcinoma; KB-V = vinblastine-resistant KB; P-388 = murine lymphoid neoplasm; A-431 = human epidermoid carcinoma; LNCaP = hormone-dependent human prostatic cancer; ZR-75-1 = hormone-dependent human breast cancer; U-373 = human glioblastoma.

EXPERIMENTAL

Mps (uncorr.) were determined on a Kofler hot-stage apparatus. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded in MeOH on a Beckman DU-7 spectrometer. IR spectra were recorded in a KBr pellet on a MIDAC FT-IR interferometer. Solns in $CDCl_3$ or DMSO-d_6 were used for all the NMR studies. ^1H NMR and COSY spectra were recorded at 500.1 MHz with a GE OMEGA 500 instrument, using standard GE programs; ^{13}C NMR, DEPT and selective INEPT spectra were recorded on a Nicolet NT-360 spectrometer operating at 90.8 MHz. For selective INEPT irradiations, $J = 8$ or 6 Hz was used for the irradiation of aromatic protons, and $J = 6$ Hz was used for that of aliphatic protons. EI mass spectra and high resolution mass spectra were recorded on a Finnigan MAT-90 instrument.

Plant material. The plant material of *H. littoralis* was collected in Bangkok, Thailand, during April 1993, and identified by one of the authors (N.R.). A living specimen was planted in the Medicinal Plant Garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and separation. The freshly minced bulbs (8.5 kg) of *H. littoralis* were exhaustively extracted with 95% EtOH (15 and 10 l, × 2). The filtrates were pooled an evapd after filtration to give a syrupy residue (125 ml). Lyophilization of the syrupy residue afforded a crude extract (56 g), which was repeatedly triturated with 1% HCl until the extract showed a weak reaction with Dragendorff's reagent. The acidic soln, after extraction with $CHCl_3$ (2 l × 5), was basified to pH 9 with NH_3 soln, and extracted with $CHCl_3$ (1 l × 5). The combined $CHCl_3$ extracts were washed (× 2) with H_2O, dried over Na_2SO_4, and evapd to dryness to yield a crude base (300 mg). This extract was subjected to CC, eluted by $CHCl_3$ and $CHCl_3$–MeOH mixts of increasing polarity (1, 2, 5, 10, 20, 50 and 100% MeOH), and finally 1% HCl

Table 2. ^1H and ^{13}C NMR spectral data for 1*

	^1H	^{13}C
1	5.89 (*br d*, 10.0)	128.4
2	6.12 (*d*, 10.0, 2.0)	128.9
3	3.94 (*m*)	78.7
4A	1.86 (*dd*, 9.5, 13.0)	29.2
4B	2.20 (*ddd*, 1.5, 6.5, 12.5)	
4a		93.0
NMe	2.43 (*s*)	31.9
6A	2.78 (*dd*, 8.0, 10.0)	63.5
6B	3.25 (*dd*, 7.5, 9.5)	
6a	4.33 (*dd*, 9.5, 7.5)	75.1
8A,8B	4.63 (AB system)	60.0
8a		124.7
9	6.44 (*s*)	104.1
10		146.9
11		146.7
12	6.90 (*s*)	107.0
12a		132.1
12b		51.2
OMe	3.38 (*s*)	56.1
OCH_2O	5.89 and 5.92 (*d*, 2.0)	100.9

*Recorded in $CDCl_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^1H and 90.8 MHz for ^{13}C; signal multiplicity and coupling constants (Hz) are shown in parentheses.

soln, monitored by TLC, and the combined frs were sepd by prep. TLC, using cyclohexane–EtOAc–diethylamine (6:4:1) or $CHCl_3$–MeOH (49:1 or 9:1) to yield **1** (3.5 mg, 0.4 ppm), **2** (45 mg, 5 ppm), **3** (1.5 mg, 0.2 ppm), **4** (2.5 mg, 0.3 ppm), **5** (18 mg, 2 ppm), **6** (7 mg, 0.8 ppm), **7** (4 mg, 0.5 ppm), **8** (5 mg, 0.6 ppm), **9** (4 mg, 0.5 ppm), **10** (7 mg, 0.8 ppm), **11** (8 mg, 0.9 ppm), **12** (11 mg, 1.2 ppm), **13** (6 mg, 0.7 ppm), **14** (5 mg, 0.6 ppm) and the lignan **15** (4 mg, 0.4 ppm). The known alkaloids **2**, **3**, **5**, **6**, **7**, **10** and **12** were identified by direct comparison with authentic samples through their ^1H NMR data and R_f values in TLC, respectively. The remaining known compounds **4**, **8**, **9**, **11**, **13**, **14** and **15** were identified by comparison of their physical and spectral data with the those in refs [3 9, 17].

Littoraline (**1**). Alkaloid **1** was obtained as a powder with a purity of *ca* 90%. UV (MeOH) λ_{max} (log ε): 243 (4.10) and 282 (3.81) nm; IR ν_{max}: 3450, 2970, 1610, 1500 and 1235 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel. int. %): 331 (M^+, 13), 270 (17), 258 (20) 257 (50), 256 (19), 240 (42), 239 (24), 238 (100), 229 (16) 227 (14), 226 (15), 225 (29), 224 (13), 210 (12), 199 (11) and 44 (11); HRMS: obsd 331.1404 for $C_{18}H_{21}NO_5$, calc. 331.1420.

Cytotoxicity, antimalarial and HIV-1 RT inhibitory assays. The biological evaluation for cytotoxic, antimalarial and HIV-1 RT inhibitory activities of these compounds were carried out according to established protocols [14–16]

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