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Lycorine Derivatives Against *Trichomonas vaginalis*

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Six lycorine derivatives were prepared, characterized, and evaluated for their *in vitro* anti-*Trichomonas vaginalis* activity. Compounds bearing an acetyl (2), lauroyl (3), benzoyl (4 and 5), and *p*-nitrobenzoyl (6 and 7) groups were synthesized. The best activity was achieved with lycorine esterified at C-2 position with lauroyl group. Preliminary structure–activity relationship points that unprotected OH group at C-1 and C-2 is not necessary to the antiparasitic activity, and none of the derivative was less active than lycorine. The lycorine structural requisites required to kill this amitochondriate cell seem to be different in comparison with the derivatives most active against other parasites and tumor cell lines, both mitochondriated cells. This result is an important contribution with our ongoing studies regarding the mechanism of action of the Amaryllidaceae alkaloids on *T. vaginalis* cell death opening a new perspective to optimize this innovative pharmacological potential.

Key words: alkaloid, Amaryllidaceae, *Hippeastrum*, lycorine derivatives, *Trichomonas vaginalis*

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Trichomonas vaginalis is a flagellate protozoan that infects the human urogenital tract and causes trichomonosis, the most common non-viral sexually transmitted disease (1). The factors considered to be involved in the pathogenicity of *T. vaginalis* include the ability of trichomonads to adhere to vaginal epithelial cells (2), the cytotoxic effect of the pathogen on host cells, trichomonad proteinase activity (3), and the ability of trichomonads to produce subcutaneous abscess lesions in mice (4). Complications related to trichomonosis include infertility (5), cervical cancer (6), pelvic inflammatory disease (7), birth outcomes, and increase in human immunodeficiency virus (HIV) transmission and acquisition (8).

Metronidazole and tinidazole are two drugs of choice recommended by Food and Drug Administration (FDA, USA) for the treatment of human trichomonosis (9). However, its potential carcinogenicity in rats and mutagenicity in bacteria, when administered at high doses for protracted periods of time (10,11), have been harmful. Reports on metronidazole-resistant isolates of *T. vaginalis* have increased, besides common adverse reactions (12–14). To improve the current chemotherapy of *T. vaginalis* infection, natural products as well as synthetic compounds could be a source of new antiprotozoal drugs with high activity, low toxicity, and alternative mechanisms of action.

Our ongoing studies regarding the anti-*T. vaginalis* activity of natural products, specially on Amaryllidaceae alkaloids, have showed interesting results. Crude extracts of alkaloids and isolated compounds from several Amaryllidaceae species showed antitrichomonas activity (15). The investigation of the mechanism of action implicated, using the alkaloids lycorine and candimine, demonstrated that the extracellular ATP and adenosine levels could be modulated by these compounds, and it could be relevant in increasing susceptibility of *T. vaginalis* to host immune response in the presence of these alkaloids (16). Additionally, we verified that cell death induced by lycorine and candimine involves arrest of the parasite cell cycle and displays an unprecedented group of effects that failed to fulfill the criteria for apoptosis and apoptosis-like death already reported in trichomonads, suggesting some similarities to paraptotic cell death described for multicellular organisms (17,18). To advance in our study on cytotoxicity of Amaryllidaceae alkaloids against *T. vaginalis*, in this work, we report the preparation of six lycorine derivatives, their antiprotozoal activity, and some preliminary conclusions about structure–activity relationships (SAR).

Materials and Methods

Chemical experimental information

All reagents and solvents were purchased from commercial sources. Silica gel GF_{254} was used for thin-layer chromatography and Silica gel 60 G for column chromatography. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker DRX spectrometer using tetramethylsilane as the internal standard and $CDCl_3$ as the solvent; the chemical shifts are reported in ppm (δ).

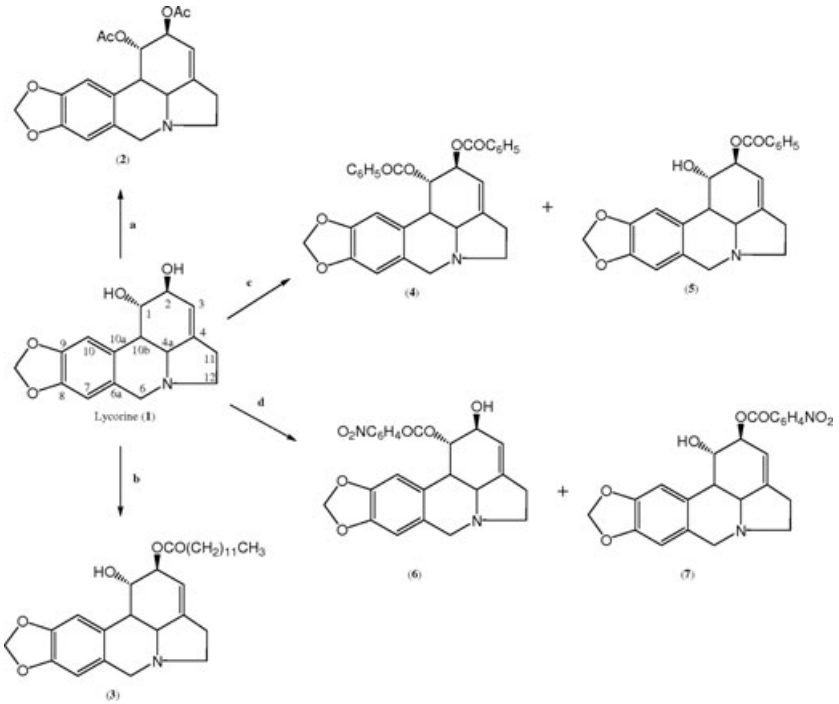
Lycorine (**1**) was obtained as the main alkaloid from the bulbs of *Hippeastrum santacatarina* (Traub) Dutilh (19). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.80 (1H, s, H-7); 6.57 (1H, s, H-10); 5.40 (1H, s, H-3); 5.90 (2H, s, OCH_2O); 4.37 (1H, s, H-1); 4.15 (1H, s, H-2); 4.10 (1H, d, *J* 14.1 Hz, H-6); 3.30 (1H, d, *J* 14.1 Hz, H-6); 3.25 (1H, m, H-12); 2.79 (1H, d, *J* 10.2 Hz, H-4a); 2.65 (1H, d, *J* 10.1 Hz, H-10b); 2.55 (2H, m, H-11); 2.35 (1H, m, H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 149.7, 148.1, 137.9, 130.6, 125.7, 122.9, 108.8, 106.4, 102.8, 71.9, 70.1, 61.8, 54.2, 55.1, 38.2, 30.3. The lycorine derivatives were prepared using the synthetic strategy described in Scheme 1.

1,2-di-*O*-acetyllycorine (**2**) was obtained by reaction of lycorine (0.105 mmol) with acetic anhydride (0.53 mmol) in pyridine (3 mL) in the presence of catalytic amount of dimethylaminopyridine (DMAP). The reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated and extracted with chloroform and water. The organic layer was evaporated under reduced pressure, and the residue was purified by column chromatography using dichloromethane/methanol as eluent furnishing **2** as a white crystal in 62% yield (25 mg, 0.065 mmol). m.p. 217–218 °C; $[\alpha]_D^{25}$ + 10.0 (*c* 0.2, $CHCl_3$); [lit.(20) m.p. 216–217 °C; $[\alpha]_D^{25}$–31.1 (*c* 1.1, $CHCl_3$]; IR ν_{max}/cm: 2731–2954,7 (C-H aliphatic chain); 1728 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.73 (1H, s, H-7); 6.56 (1H, s, H-10); 5.90 (2H, s, OCH_2O); 5.72 (1H, s, H-1); 5.52 (1H, s, H-3); 5.24 (1H, s, H-2); 4.17 (1H, d, *J* 12.4 Hz, H-6); 3.53 (1H, d, *J* 12.4 Hz, H-6); 3.36 (1H, m, H-12); 2.87 (1H, d, *J* 10.3 Hz, H-10b); 2.79 (1H, d, *J* 10.3 Hz, H-4a); 2.64 (2H, m, H-11); 2.41 (1H, m, H-12), 2.07 (3H, s, $OCOCH_3$); 1.94 (3H, s, $OCOCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.1, 169.9, 146.6, 146.5, 146.1, 129.4, 126.7, 114.0, 107.5, 105.2, 101.1, 71.0, 69.4, 61.3, 56.9, 53.7, 40.5, 28.8, 21.3, 21.0.

2-*O*-lauroyllycorine (**3**) was obtained by reaction of lycorine (0.105 mmol) with lauroyl chloride (0.800 mmol) in a mixture of dichlorometane (1 mL) and pyridine (3 mL) in the presence of catalytic amount of DMAP. The reaction mixture was stirred for 5 days at room temperature. The solvent was evaporated under reduced pressure and extracted with chloroform and water. After evaporation at reduced pressure of the organic layer, the residue was purified by column chromatography using dichlorometane/methanol as eluent furnishing **3** as a pale yellow oil in 40% yield (18 mg, 0.04 mmol); $[\alpha]_D^{25}$ + 85.0 (*c* 0.4, $CHCl_3$); IR ν_{max}/cm: 3421 (O-H); 2853–2959 (C-H aliphatic chain); 1732 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.80 (1H, s, H-7); 6.61 (1H, s, H-10); 5.92 (2H, s, OCH_2O); 4.50 (1H, s, H-1); 5.31 (1H, s, H-3); 5.48 (1H, s, H-2); 4.12 (1H, d, *J* 14.3 Hz, H-6); 3.68 (1H, d, *J* 14.3 Hz, H-6); 3.37 (1H, m, H-12); 2.70 (4H, m, H-4a, H-10b and H-11); 2.32 (1H, m, H-12), 1.24 ($C_{12}H_{25}$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.5, 147.0, 146.6, 145.2, 130.7, 129.3, 114.5, 107.9, 104.9, 101.2, 73.4, 69.1, 60.6, 56.5, 53.9, 41.2, 29.7, 22.8, 14.3.

1,2-*O*-dibenzoyllycorine (**4**) and 2-*O*-benzoyllycorine (**5**) were obtained by reaction of lycorine (0.105 mmol) with benzoyl chloride (0.21 mmol) in pyridine (2 mL) and in the presence of catalytic amount of DMAP. The reaction mixture was stirred for 2 days at room temperature. The solvent was evaporated under reduced pressure followed by extraction with chloroform and saturated aqueous solution of sodium bicarbonate. The organic layer was concentrated, and the residue was purified by column chromatography using dichlorometane/methanol as eluent yielding compounds **4** (0.046 mmol, 23 mg, 47%) and **5** (0.023 mmol, 9 mg, 23%).

Compound **4** was obtained as a white crystal; m.p. 90–93 °C; $[\alpha]_D^{25}$ + 14.3 (*c* 0.14, $CHCl_3$); IR ν_{max}/cm: 3036–3037 (O-H); 2775–2924 (C-H aliphatic chain); 1718 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.06 (2H, d, *J* 7.2 Hz, H-2′, H-6′), 7.90 (2H, d, *J* 7.2 Hz, H-2′, H-6′), 7.62 (4H, m, H-3′, H-5′), 7.30 (2H, m, H-4′), 6.57 (1H, s, H-10); 6.86 (1H, s, H-7); 6.14 (1H, s, H-2); 5.68 (2H, s, H-1, H-3); 5.85 (2H,



Scheme 1: Reagents and conditions: (a) Ac_2O, DMAP, py; (b) $CH_3(CH_2)_{10}COCl$, DMAP, py; (c) C_6H_5COCl, DMAP, dichlorometane, py; (d) *p*-$NO_2C_6H_4COCl$, DMAP, dichlorometane, py. DMAP, dimethylaminopyridine

s, OCH_2O); 4.21 (1H, d, *J* 14.2 Hz, H-6); 3.60 (1H, d, *J* 14.2 Hz, H-6); 3.44 (1H, m, H-12); 3.17 (1H, d, *J* 10.3 Hz, H-4a); 3.04 (1H, d, *J* 10.3 Hz, H-10b); 2.63 (2H, m, H-11); 2.53 (1H, m, H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.5, 165.4, 146.7, 146.5, 146.4, 133.3, 131.3, 130.0, 128.5, 128.5, 114.2, 107.5, 105.3, 101.1, 71.2, 69.8, 61.8, 57.0, 53.8, 41.1, 29.8.

Compound **5** was obtained as a white crystal; m.p. 96 °C; $[\alpha]_D^{25}$ + 100.0 (*c* 0.1, $CHCl_3$); IR ν_{max}/cm: 3412 (O-H); 2851–2957 (C-H aliphatic chain); 1713 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.02 (2H, d, *J* 7.0 Hz, H-2′, H-6′), 7.46 (1H, m, H-4′), 7.43 (2H, m, H-3′, and H-5′), 6.84 (1H, s, H-7); 6.64 (1H, s, H-10); 5.88 (2H, s, OCH_2O); 5.65 (1H, s, H-2); 5.62 (1H, s, H-3); 4.71 (1H, s, H-1); 4.22 (1H, d, *J* 13.8 Hz, H-6); 3.78 (1H, d, *J* 13.8 Hz, H-6); 3.48 (1H, m, H-12); 3.22 (1H, d, *J* 10.3 Hz, H-4a); 2.97 (1H, d, *J* 10.3 Hz, H-10b); 2.76 (3H, m, H-11 and H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.0, 146.8, 145.3, 133.4, 133.1, 130.2, 130.0, 128.6, 128.4, 114.6, 108.0, 105.0, 101.3, 73.9, 69.2, 60.5, 56.2, 53.7, 41.1, 29.1.

2-*O*-*p*-nitrobenzoyllycorine (**6**) and 1-*O*-*p*-nitrobenzoyllycorine (**7**) were obtained by reaction of lycorine (0.105 mmol) with *p*-nitrobenzoyl chloride (0.30 mmol) in pyridine (2 mL) and in the presence of catalytic amount of DMAP. The reaction mixture was stirred for 4 days at room temperature. The solvent was evaporated under reduced pressure followed by extraction with chloroform and saturated aqueous solution of sodium bicarbonate. The organic layer was concentrated, and the residue was purified by column chromatography using dichlorometane/methanol as eluent yielding compounds **6** (0.016 mmol, 7 mg, 17%) and **7** (0.017 mmol, 8 mg, 18%).

Compound **6** was obtained as a colorless solid; m.p. 85–87 °C; $[\alpha]_D^{25}$-1520.0 (*c* 0.1, $CHCl_3$); IR ν_{max}/cm: 3425 (O-H); 2851–2956 (C-H aliphatic chain); 1724 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.22 (4H, m, H-2′, H-3′, H-5′ and H-6′), 6.77 (1H, s, H-7); 6.58 (1H, s, H-10); 5.89 (2H, s, OCH_2O); 5.59 (2H, s, H-2; H-3); 4.65 (1H, s, H-1); 4.16 (1H, d, *J* 14.5 Hz, H-6); 3.55 (1H, d, *J* 14.5 Hz, H-6); 3.37 (1H, m, H-12); 2.83 (2H, m, H-11); 2.66 (1H, d, *J* 10.5 Hz, H-4a and H-10b); 2.40 (1H, m, H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.9, 150.9, 147.5, 146.8, 136.3, 131.2, 131.1, 123.8, 123.7, 114.2, 107.7, 104.9, 101.2, 72.0, 70.7, 61.7, 57.0, 53.8, 41.1, 29.9.

Compound **7** was obtained as a colorless solid; m.p. 107–108 °C; $[\alpha]_D^{25}$ + 31.3 (*c* 0.1, $CHCl_3$); IR ν_{max}/cm: 3431 (O-H); 2850–2960 (C-H aliphatic chain); 1728 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 8.20 (4H, m, H-2′, H-3′, H-5′ and H-6′), 6.77 (1H, s, H-7); 6.58 (1H, s, H-10); 5.90 (1H, s, H-1); 5.89 (2H, s, OCH_2O); 5.62 (1H, s, H-3); 4.35 (1H, s, H-2); 4.23 (1H, d, *J* 13.3 Hz, H-6); 3.62 (1H, d, *J* 13.3 Hz, H-6); 3.43 (1H, m, H-12); 3.08 (1H, d, *J* 10.7 Hz, H-4a); 2.97 (3H, d, *J* 10.7 Hz, H-10b, H-11); 2.52 (1H, m, H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.5, 165.4, 146.7, 146.5, 146.4, 133.3, 131.3, 130.0, 128.5, 128.5, 114.2, 107.5, 105.3, 101.1, 71.2, 69.8, 61.8, 57.0, 53.8, 41.1, 29.8.

Biological experimental information

The *T. vaginalis* isolate 30236 (from the American Type Culture Collection) was used in this study. Trichomonads were cultured axenically *in vitro* (21) and incubated at 37 °C (±0.5). Organisms in the logarithmic phase of growth and exhibiting motility and normal morphology were harvested, centrifuged, and resuspended in new culture medium for cytotoxic assays. Parasites with cellular density of 5.0×10^5 trophozoites/mL were treated with lycorine and their derivatives at final concentrations of 125 and 250 μM. Importantly, DMSO at 0.6% (final percentage) was used for solubilization of the samples according to the previous studies (22). All the results were expressed as the percentage of living organisms compared to untreated parasites after 24 h of incubation, considering motility, normal morphology, and exclusion of trypan blue dye. All experiments were performed in triplicate and with at least four independent cultures (n = 4).

Statistical analysis

Results were expressed as means (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test. Statistical significance was considered at $p \leq 0.05$.

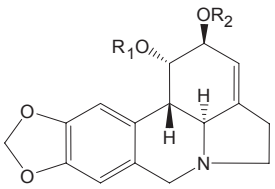
Results and Discussion

The aromatic and aliphatic ester derivatives of lycorine (**1**), compounds **2**–**7**, were obtained by treatment of **1** with acetic anhydride or acid chlorides, in the presence of pyridine and DMAP (Scheme 1). To our knowledge, compounds **3–7** are new lycorine derivatives, so far. Previous studies in this regard have highlighted the difficulties attending the differential functionalization of the hydroxyl groups present within the lycorine series (23) because it is known that ring C of lycorine is aromatized by light, oxygen, or heat (24). However, C-1 and C-2 positions could be considered a key point to the discussion on lycorine SAR enabling comparisons with several activities previously investigated.

Anti-*T. vaginalis* bioactivity assay was performed on lycorine (**1**) and six ester derivatives (**2–7**), at 125 and 250 μM (Table 1). Lycorine (**1**) did not display a significant difference at both concentrations tested, and around 60% of the parasites remained viable after 24 h. The ester derivatives **2**, **4,** and **5** at both concentrations and **7** at 125 μM presented anti-*T. vaginalis* activity equivalent to **1**. The results from the diesterified compounds **2** and **4** showed that no matter the aliphatic or aromatic nature of the substituent and even C-1 and C-2, lycorine-substituted derivatives are active.

Compound **7** at 250 μM showed a discreet improve on activity: Just 40% of the trophozoites remain viable. Compound **6** showed a good activity where at 125 μM was equally active than **7** and at 250 μM was observed just 21% of viable trophozoites. In comparison, both the *p*-nitrobenzoyl esters (C-1 or C-2 substituted) were more active than lycorine, and C-2 unsubstituted seems it to be favorable to antiprotozoal activity. Nitroimidazoles are the drugs of choice to treat *T. vaginalis,* and they possess a nitro group that is involved on the generation of free radicals and on the effectiveness of the drug. We could hypothesize that similar event could be occur on lycorine nitrobenzoyl derivatives as an additional cytotoxic requisite of the molecule. It is reinforced by observing the results of the ben-

Table 1: Anti-*Trichomonas vaginalis* activity of lycorine (**1**) and their ester derivatives



Compound	R1	R2	Anti-*T. vaginalis* activity (% viable trophozoites) 125 μM	250 μM
1	H	H	70[a]	60[b]
2	Acetyl	Acetyl	77[a]	81[b]
3	H	Lauroyl	16*[a]	3[b]
4	Benzoyl	Benzoyl	68	66[b]
5	H	Benzoyl	51*[a]	65
6	H	*p*-Nitrobenzoyl	40*[a]	21[b]
7	*p*-Nitrobenzoyl	H	80*[a]	42[b]

Symbols represent statistical significance by comparing *both dosages for the same compound: [a]lycorine with derivatives at 125 μM; [b]lycorine with derivatives at 250 μM. Data were analyzed by ANOVA followed by Tukey's test ($p \leq 0.05$).

zoyl derivatives where C-2 benzoyl lycorine was less active than C-2 nitro benzoyl lycorine.

The better results were obtained with 2-*O*-lauroyllycorine **3** where, at both concentrations 125 and 250 μM, it was better than lycorine with just 16% and 3.5% of remaining viable trophozoites, after 24 h, respectively. Considering the amitochondriated parasite *T. vaginalis,* the lipophilicity could be indicated as a facilitator for cytotoxicity of lycorine, and it may be related to a better cellular penetration of the alkaloid.

Lycorine structural requirements necessary to anti-*T. vaginalis* activity constitute important differences in comparison with the chemical characteristics optimized regarding other parasites. For example, C-1 and C-2 disubstituted lycorine derivatives showed less antiplasmodial and antitrypanosomal activities than **1** or its monosubstituted derivatives. Among the C-1 and C-2 disubstituted derivatives, the larger alkyl chain contributed to improve the activity, as well as against *T. vaginalis*. The C-1 esterified derivatives were equally antiplasmodial no matter the chain length while compound bearing a larger chain possesses more antitrypanosomal activity in comparison with lycorine. Finally, C-2-substituted derivatives were worse as antiplasmodial but better as antitrypanosomal (25,26). Additionally, it is highlighted that the presence of OH groups at C-1 and C-2 positions is not necessary to cell death lycorine induced in *T. vaginalis*, in opposite to mitochondriated eukaryotic cells where 1,2 diol is necessary to induce apoptosis (27).

Overall, the anti-*T. vaginalis* activities of lycorine derivatives showed that the esterification with fatty acids could be a starting point for the preparation of a new series of lycorine derivatives with the aim of improving the biological activity. The SAR requirement of the lycorine against an amitochondriate cell respects a particular structural logic that must be carefully drawn despite the instability and the synthetic difficulties related to lycorine. Finally, the findings reported here are in agreement with our previous results because the structural requirements necessary to induce apoptosis in tumor cell lines were not accomplished by the most active anti-*T. vaginalis* derivative.

Acknowledgments

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