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RESEARCH ARTICLE

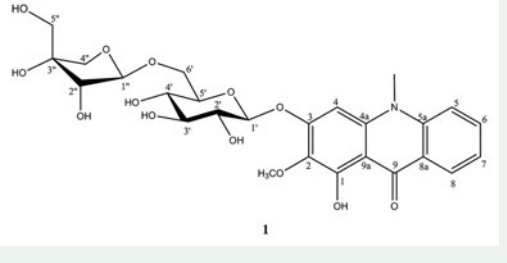
Acridone alkaloids from the rhizomes of *Luvunga scandens* (Roxb.) Buch. Ham.

Nguyen Minh An Tran[a], Thi Hong Tuoi Do[b], Luu Hong Truong[c], Dung Tien Le[d], Minh Nhat Phan[e], Nguyen Kim Tuyen Pham[f], Dinh Tri Mai[e,g] and Tan Phat Nguyen[e,g]

[a]Faculty of Chemical Engineering, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Viet Nam; [b]Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam; [c]Southern Institute of Ecology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam; [d]Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Viet Nam; [e]Institute of Chemical Technology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Viet Nam; [f]Faculty of Environmental Science, Sai Gon University, Ho Chi Minh city, Viet Nam; [g]Faculty of Chemistry, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Viet Nam

ABSTRACT

The ethyl acetate extract of the rhizomes of *Luvunga scandens* (Roxb.) Buch. - Ham. ex Wight & Arn (Rutaceae) delivered one new acridone alkaloid named Luvungaside A (**1**) together with three known acridone alkaloids, namely 1,3-dihydroxy-2-methoxy-10-methyl-9-acridone (**2**), arborinine (**3**) and 1-hydroxy-3-methoxy-10-methyl-9-acridone (**4**). Compounds were reported for the first time from the species *L. scandens* applying various chromatography methods. Chemical structures were elucidated by IR, UV, HR-ESI-MS, NMR 1D & 2D experiments and comparison with the literature. The cytotoxicity and hepatoprotective activity of compounds **1**–**4** in human hepatoma cell line HepG2 was measured by MTT assay. At 10–100 μM, compounds expressed significant hepatoprotective effect with prevention percentage ranging from 81.1% to 194.3%, compared to the positive control quercetin displaying 49.0%.



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Luvunga scandens; Luvungaside A; alkaloid; cytotoxicity; hepatoprotective activity

CONTACT Tan Phat Nguyen ntphat@ict.vast.vn

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1. Introduction

L. scandens has been used traditionally in Vietnam for the treatment of cirrhosis, rheumatism, scorpion bite, hepatitis, sinusitis and pain in bone joints and muscles (Luu 2007). Chemical constituents farnesyl acetate, farnesol, carvotanacetone, methyl eugenol, caryophyllene oxide, myristicin, 1,8-cineole, carvone, *a*-pinene, camphene, phellandrene, terpinolene, *a*-terpineol, camphor, caryophyllene, β-sitosterol and sesamin were reported from the essential oil of the berries as well as cytotoxic, antioxidative, antiinflammatory and antibacterial properties of stem extracts (Aggarwal et al. 1985; Sirinut et al. 2014). Furthermore, the tirucallane triterpenes flindissol and 3-oxotirucalla-7,24-dien-21-oic-acid were identified from the dichloromethane extract of the stem showing potent cytotoxicity against MCF-7 cell line with IC_{50} values of 13.8 μM and 27.5 μM, respectively. (Al-Zikri et al. 2014). As part of our continuing study on hepatoprotective activity of bioactive compounds from plant origin (Nguyen, Mai, et al. 2017; Nguyen, Tran, et al. 2017) and phytochemical constituents from the rhizomes of *L. scandens* (Nguyen, Phan, et al. 2017), we report herein the isolation and structure elucidation of one new acridone alkaloid and three known ones which indentified for the first time from the rhizomes of *L. scandens* collected in Ta Kou Mountain, Binh Thuan province, Viet Nam.

2. Results and discussion

The ethyl acetate extract from the dried rhizomes of *L. scandens* was subjected to column chromatography over silica gel normal-phase and reversed-phase RP-18 to give one acridone alkaloid, named Luvungaside A (3-*O*-β-[D-apiofuranosyl-(1→6)]-β-D-glucopyranosyl-1,3-dihydroxy-2-methoxy-10-methyl-9-acridone) (**1**), together with three known acridone alkaloids, 1,3-dihydroxy-2-methoxy-10-methyl-9-acridone (**2**) (Ngoumfo et al. 2010), arborinine (**3**) (Bergenthal et al. 1979), and 1-hydroxy-3-methoxy-10-methyl-9-acridone (**4**) (Ngoumfo et al. 2010) (Figure 1).

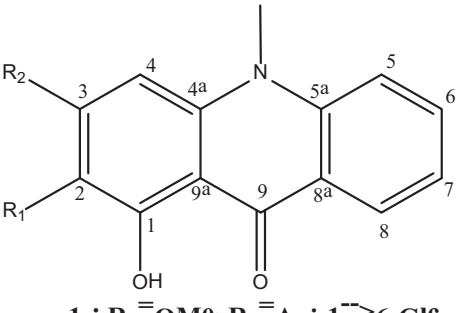

Figure 1. Structure of compounds 1–4.

Compound **1** was obtained as a yellow amorphous powder. The molecular formula was established as $C_{26}H_{31}NO_{13}$ by HR-ESI-MS data ($[M+H]^+$ *m/z* 566.1821, calcd. 566.1864). The IR spectrum of **1** showed absorptions of hydroxyl (3446 cm^{-1}) and carbonyl (1641 cm^{-1}) groups. The ^{13}C-NMR and DEPT spectrum showed twenty six carbons including one carbonyl carbon, seven quaternary aromatic carbons, five methine aromatic carbons, two anomeric carbons, one oxygenated quaternary carbon, five oxygenated methine carbons, three oxygenated methylene carbons, one oxygenated methyl carbon and one nitrogenated methyl carbon (Table S1). The presence of one carbonyl carbon at δ_C 180.2 (C-28), two nitrogenated aromatic carbons at δ_C 139.8 (C-4a), 142.0 (C-5a), two quaternary aromatic carbons at δ_C 105.4 (C-9a), 119.6 (C-8a), three oxygenated aromatic carbons and five methine aromatic carbons indicated an acridone aglycone (Ngoumfo et al. 2010; Wouatsa et al. 2013). Thus **1** was identified as an acridone alkaloid bearing two methyl groups and two sugar units. The ^{1}H-NMR spectrum showed two methyl groups at δ_H 3.91 (*s*, N-CH_3), 3.78 (*s*, O-CH_3), five aromatic protons at δ_H 6.82 (*s*, H-4), 7.85 (*m*, H-5, H-6), 7.38 (td, 2.0, 8.0, H-7) and 8.32 (*d*, 8.0, H-8), respectively (Table S1). Moreover, two anomeric protons at δ_H 5.24 (*d*, 7.5, H-1′), 4.81 (*d*, 3.5, H-1″) corresponding to two anomeric carbons at δ_C 100.1 (C-1′), 109.1 (C-1″) were assigned to β-D-glucose (Glc) and β-D-apiose (Apio) units, respectively. The COSY, HSQC and ROESY spectrum allowed analysis of their spin systems and assignment of their proton resonances to determine sugar units. Moreover, acidic hydrolysis and TLC comparision of the hydrolysate with authentic sugars verified sugar moieties as D-glucose and D-apiose, see also under 3.5. Acid hydrolysis, Experimental part. In the HMBC spectrum, one methyl group at δ_H 3.91 (N-CH_3) correlated with two carbons at δ_C 139.8 (C-4a), 142.0 (C-5a) and one methyl group at δ_H 3.78 (O-CH_3) correlated with carbon at δ_C 129.8 (C-2) (Figure S1). In addition, the HMBC spectrum showed correlations between anomeric proton at δ_H 5.24 (H-1′) of Glc and carbon at δ_C 157.3 (C-3) of acridone skeleton; between anomeric proton at δ_H 4.81 (H-1″) of Apio and carbon at δ_C 67.9 (C-6′) of Glc; between oxygenated methylene proton at δ_H 3.96 (*d*, 10.0, H-6′a) and acetal carbon at δ_C 109.1 (C-1″). The connectivity was further confirmed by correlations observed in the ROESY spectrum between H-6′b (δ_H 3.44) of Glc and H-1′ (δ_H 4.81) of Apio (Figure S1).

Table 1. The cytotoxicity activity of **1**–**4** in HepG2 cell line.

Sample	Concentration (μM)	OD570 nm ± SD	Percentage of control (%)
1	10	0.566 ± 0.029	79.6
	50	0.610 ± 0.009	90.5
	100	0.765 ± 0.033	124.2
2	10	0.632 ± 0.015	88.9
	50	0.690 ± 0.025	102.4
	100	0.760 ± 0.039	123.3
3	10	0.698 ± 0.029	98.2
	50	0.668 ± 0.015	99.0
	100	0.578 ± 0.036	93.7
4	10	0.672 ± 0.021	94.5
	50	0.740 ± 0.029	109.7
	100	0.782 ± 0.080	126.9
	10	0.711 ± 0.037	
Control	50	0.674 ± 0.019	
	100	0.616 ± 0.011	
Paclitaxel	10	0.317 ± 0.023	44.6

Table 2. The hepatoprotective activity using HepG2 of **1**–**4**.

Sample	Concentration (μM)	OD570 ± SD	Prevention percentage (%)
1	10	0.346 ± 0.026	−41.5
	50	0.314 ± 0.014	−37.5
	100	0.379 ± 0.039	4.4
2	10	0.735 ± 0.036	**109.3**
	50	0.831 ± 0.016	**159.8**
	100	0.587 ± 0.007	**88.2**
3	10	0.759 ± 0.027	**118.5**
	50	0.750 ± 0.029	**128.8**
	100	0.569 ± 0.032	**81.1**
4	10	0.954 ± 0.021	**194.3**
	50	0.911 ± 0.029	**190.3**
	100	0.775 ± 0.031	**163.7**
DMSO	10	0.711 ± 0.037	
	50	0.674 ± 0.019	
	100	0.616 ± 0.011	
	10	0.453 ± 0.037	
Control	50	0.412 ± 0.019	
	100	0.368 ± 0.011	
Quercetin	10	0.579 ± 0.009	49.0

Based on HR-ESI-MS, 1D, 2D-NMR data and comparision with published data, the structure of **1** was identified as 3-*O*-*β*-[D-apiofuranosyl-(1→6)]-*β*-D-glucopyranosyl-1,3-dihydroxy-2-methoxy-10-methyl-9-acridone and named Luvungaside A.

The cytotoxicity and hepatoprotective effect of compounds (**1-4**) on human hepatoma cell line HepG2 was measured by MTT assay (Table 1–2). While at 10-100 μM cell growth was not affected, a significant hepatoprotective effect on CCl_4-induced hepatotoxicity in HepG2 cells was observed, with prevention percentage ranging from 81.1% to 194.3% compared to the positive control quercetin displaying 49.0%.

In summary, one new acridone alkaloid (**1**) together with three known acridone alkaloids (**2**-**4**) were isolated from the ethyl acetate extract of the rhizomes of *L. scandens*, the latter three reported here for the first time from this species. Compounds **1**-**4** exhibited significant hepatoprotective activity, surmounting that of the positive control quercetin. Structures were elucidated by intensive spectroscopic and spectrometric analyses.

3. Experimental part

3.1. General experimental procedures

The high resolution electrospray ionisation mass spectroscopy (HR-ESI-MS) was recorded on a Bruker MicrOTOF-QII spectrometer. The ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), DEPT, COSY, HSQC and HMBC spectra were recorded on a Bruker AM500 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard. Column chromatography was carried out using Merck Silica gel normal-phase (230–240 mesh) and reversed-phase C_{18} (Merck). Analytical TLC was carried on in silica gel plates (Merck DC-Alufolien 60 F_{254}). Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min.

3.2. Chemicals

Eagle's Minimum Essential Medium (EMEM), fetal calf serum (FCS) and trypsin-EDTA were purchased from Gibco, USA; L-glutamine, penicillin-streptomycin, phosphate

buffer, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), paclitaxel, quercetin and carbon tetrachloride (CCl_4) from Sigma-Aldrich, USA and dimethylsulfoxide (DMSO) as well as isopropanol from Merck, Germany. All chemicals met cell culture standards.

3.3. Plant material

The rhizomes of *Luvunga scandens* were collected in Ta Kou Mountain, Binh Thuan province, Viet Nam and identified by Dr. Hong Truong Luu, Southern Institute of Ecology, Vietnam Academy of Science and Technology. A voucher specimen (No LS-128) was deposited in Bioactive Compounds Laboratory, Institute of Chemical Technology, Vietnam Academy of Science and Technology, Viet Nam.

3.4. Extraction and isolation

Powdered rhizomes of *L. scandens* (5.0 kg) were extracted three times with 30 L of 96% EtOH each at room temperature. The residue was filtered and solvents evaporated under low pressure to obtain the crude extract (350 g). The crude extract was dissolved in water and successively partitioned into *n*-hexane (120 g), $CHCl_3$ (80 g), EtOAc (30 g) and the aqueous portion (110 g). The CHCl3 extract (80 g) was fractionated on silica gel with n-hexane–EtOAc (50:1) increasing the amount of EtOAc to 100% to give seven fractions. Fraction C.III (12 g) was eluted with n-hexane–EtOAc (50:1→1:1, v/v) to give three subfractions C.III.1–C.III.3. Subfraction C.III.2 (4.6 g) was chromatographed repeatedly over a silica gel column using n-hexane–EtOAc (10:1) to afford **3** (48 mg) and **4** (8 mg). Subfraction C.III.3 (3.0 g) was chromatographed over silica gel column and eluted with $CHCl_3$ MeOH (10:1) to obtain compound **2** (10 mg). The EtOAc extract (30 g) was subjected to silica gel column chromatography and eluted with a gradient solvent system of chloroform - methanol (50:1-1:1) to afford fractions M1-M5. Fraction M5 (5,3 g) was chromatographed over silica gel column and eluted with $CHCl_3$-MeOH (5:1→1:1) to give four subfractions M5.1-M5.4. Subfraction M5.2 was further purifed by RP-18 with MeOH-H_2O (1:2, v/v) to give compound **1** (12 mg).

3.4.1. Luvungaside A (1)

Yellow amorphous powder; IR ν_{max} (KBr): 3446, 1641, 1506, 1253 cm^{-1}; UV (MeOH) λ_{max}: 231 and 274 nm; HR-ESI-MS: *m/z* 566.1821 $[M+H]^+$ (calcd for $C_{26}H_{32}NO_{13}$, 566.1864); ^1H and ^{13}C NMR data (DMSO-d_6), see Table S1.

3.5. Acid hydrolysis

Compound **1** (2 mg) was refluxed with 2 N aq. CH_3COOH (5 mL) for 2h at 100 °C. After extraction with $CHCl_3$ (3 × 5 mL), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel (MeCOEt–isoPrOH–Me_2CO–H_2O 20:10:7:6) by comparison with authentic sugars of D-apiose *Rf* 0.47 and D-glucose *Rf* 0.40 according to Nguyen et al. 2015, 2016.

3.6. The cytotoxicity and hepatoprotective assay

HepG2 cells were seeded and cultured in EMEM containing 10% FCS, 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin at 5% CO_2 at 37 °C to attain confluency, then harvested and seeded into 96-well-plates at 2.5×10^4 cells/cm^2. Cells were treated with EMEM containing 2 mM CCl_4 and compounds alone or combined at different concentrations. Cell viability was evaluated as mitochodrial succinate dehydrogenase activity, a marker of viable cells using MTT test according to Nguyen, Mai, et al. 2017; Nguyen, Tran, et al. 2017. Doxorubicin was used as positive control for cytotoxicity and quercetin for hepatoprotective effect.

Disclosure statement

No potential conflict of interest was reported by the authors.

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