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Mosquito larvicidal activity of alkaloids from *Zanthoxylum lemairei* against the malaria vector *Anopheles gambiae*

Ferdinand M. Talontsi [a], Josphat C. Matasyoh [b,*], Rostand M. Ngoumfo [c], Regina Chepkorir [b]

[a] Institute of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany
[b] Department of Chemistry, Egerton University, P.O. Box 536, Egerton 20107, Kenya
[c] Department of Organic Chemistry, University of Yaounde I, Box 812, Yaounde, Cameroon

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ABSTRACT

Four alkaloids, 10-*O*-demethyl-17-*O*-methylisoarnottianamide **1**, 6-acetonyl-*N*-methyl-dihydrodecarine **2**, nitidine **3**, and chelerythrine **4** were isolated from the plant *Zanthoxylum lemairei* (Rutaceae) and evaluated for mosquito larvicidal activity against the malaria vector *Anopheles gambiae*. The mortalities of the larvae were determined after 24 h. The results of the larvicidal tests demonstrated that compounds **1** and **2** were the most potent with mortality rates of 96.7% and 98.3% at a concentration of 250 mg/L, respectively. Compound **3** was less potent with a mortality of 28.3% at the same concentration. The percent mortality of 100% was observed at a concentration of 500 mg/L. The least potent of the four alkaloids was compound **4**, which achieved 100% mortality at 1000 mg/L. These findings could be useful in the research for newer more selective, biodegradable and natural larvicidal compounds or can be used as lead compounds for the development of larvicides.

1. Introduction

Zanthoxylum lemairei is in the family of Rutaceae. The genus *Zanthoxylum* (Rutaceae) occurs in tropical and subtropical regions and comprises of about 250 species [1]. These species have been used in traditional medicine for the treatment of a wide range of disorders, including toothache, urinary and venereal diseases, rheumatism and lumbago [2]. Mosquitoes are the major vectors for the transmission of malaria, dengue fever, yellow fever, filariasis, and several other diseases [3]. Mosquitoes also cause allergic responses on humans that include local skin and systemic reactions such as angioedema [4]. Most of the widely used vector interruption methods are synthetic insecticide-based. These synthetic insecticides not only affect the non-target population but can also constantly increase mosquito resistance to the insecticide [5]. Therefore, the development of techniques that would provide more efficient insect control, not have any ill effects on the non-target population, and are easily biodegradable is important [6].

In recent years, the emphasis to control the mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes [7–9]. One of the strategies of the WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. Malaria is a parasitic disease from which more than 300 million people suffer yearly throughout the world. It is one of the main causes of infant and young child mortality [10].

Interest in the control of *Anopheles gambiae* lies in the fact that it is one of the major vectors of malaria especially in sub-Saharan Africa. We describe here the isolation, structure elucidation and larvicidal activity of four alkaloids from *Z. lemairei* namely; 10-*O*-demethyl-17-*O*-methylisoarnottianamide **1**, 6-acetonyl-*N*-methyl-dihydrodecarine **2**, nitidine **3**, and chelerythrine **4**.

2. Experimental

2.1. General experimental procedures

The NMR spectra were measured on a Bruker AMX 300 (300.135 MHz), a Varian Unity 300 (75.145 MHz) and a Varian Inova 600 (150.820 MHz) spectrometer. Optical rotation was measured on a Perkin-Elmer polarimeter, model 241. UV/VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrometer. ESIMS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). EIMS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for HREIMS. Flash chromatography was carried out on silica gel (230–400 mesh). R_f-values were measured on Polygram SIL G/UV_{254} (Macherey–Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

* Corresponding author. Fax: +254 51 2217942.
E-mail address: josphat2001@yahoo.com (J.C. Matasyoh).

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2.2. Plant material

Fresh roots of *Z. lemairei* were collected in August 2005 at Barumbi Camp, Kumba, Centre province of Cameroon. The plant was identified by Mr. Victor Nana, a botanist at the National Herbarium of Cameroon, where a voucher specimen (HNC No. 10672/SFR/CAM) has been deposited.

2.3. Extraction and isolation

The air-dried powdered roots of *Z. lemairei* (1.8 kg) were exhaustively extracted with methanol (MeOH) at room temperature for 48 h. The filtrate was concentrated to dryness under reduced pressure to afford 87.4 g of brown crude extract. About 67.0 g was fractionated by flash chromatography over silica gel, eluting with CH_2Cl_2/MeOH of increasing polarity to yield seven major fractions (from A1 to A7).

Fraction A3 (10.4 g) was subjected to successive column chromatography over silica gel, eluting with hexane–EtOAc mixture to yield 10-*O*-demethyl-17-*O*-methylisoarnottianamide **1** (67.3 mg) and chelerythrine **4** (34.0 mg). Fraction A5 (200.0 mg) was repeatedly chromatographed on silica gel with CH_2Cl_2/MeOH of increasing polarity and then Sephadex LH-20 eluting with MeOH to afford 6-acetonyl-*N*-methyl-dihydrodecarine **2** (25.0 mg), and nitidine **3** (70.1 mg).

10-*O*-demethyl-17-*O*-methylisoarnottianamide **1**: Brown amorphous powder, mp 229–230 °C. ^{1}H (300 MHz, $CDCl_3$ + CD_3OD) and ^{13}C (75 MHz, $CDCl_3$ + CD_3OD) NMR spectroscopic data, see Table 1; HR-ESIMS *m/z*: 382.1284 $[M+H]^+$ 382.1198 $[M+H]^+$ (calcd. for $C_{21}H_{20}NO_6$).

6-acetonyl-*N*-methyl-dihydrodecarine **2**: Brown amorphous powder, m.p. 175 °C, $[\alpha]^{20}D$ – 11 (0.12, MeOH), ^{1}H (300 MHz, DMSO-d_6) and ^{13}C (75 MHz, DMSO-d_6) NMR spectroscopic data, see Table 1; HR-ESIMS *m/z*: 392.1492 $[M+H]^+$ (calcd. for $C_{23}H_{21}NO_5$).

2.4. Structure elucidation

The isolated alkaloids **1**–**4** showed strong fluorescence under UV (254 and 365 nm) on silica gel TLC plates, as well as positive reactions with Dragendorff's reagent. Compound **1** was isolated as a brown amorphous powder. Its HR-ESIMS of $[M+H]^+$ ion at *m/z* 382.1198 gave the pseudomolecular formula $C_{21}H_{20}NO_6$. The ^{1}H NMR spectrum (Table 1) displayed in the aromatic region, four singlets at 7.02 (1H, s), 7.43 (1H, s), 6.61 (1H, s), and 6.58 (1H, s), a pair of *ortho*-coupling doublets at δ_H 7.80 (1H, d, J = 8.6 Hz) and 7.30 (1H, d, J = 8.6 Hz), one *N*-methyl (δ_H 2.91, 3H, s) and two methoxyl group in the higher-field region at δ_H 3.76 (3H, s) and 3.64 (3H, s). Moreover, the ^{1}H NMR spectrum also exhibited a singlet at δ_H 6.18 (2H, s), typical of a methylenedioxy group signal. The protons signals δ_H 2.91 (3H, s) and 8.02 (1H, s) were attributed to *N*-methyl formamide of which the corresponding carbons appeared at δ_C 31.5 and δ_C 165.8 on ^{13}NMR spectrum. The methylenedioxy was located on C-2 (δ_C 149.9) and C-3 (δ_C 148.2), the two *O*-methyl groups in *para* position on C-11 (δ_C 150.0) and C-7 (δ_C 141.2) and the hydroxyl in C-10 (δ_C 148.1). The above evidences and comparison of the data with the related secobenzo[c]phenantridine [11] led to conclusion that **1**, 10-*O*-demethyl-17-*O*-methylisoarnottianamide, is identical to Turraeanthin A, isolated from *Turraeanthus africanus* (*Meliaceae*) [12] and reported here for the first time from *Z. lemairei*.

Compound **2** showed in HR-ESIMS a peak at 392.1492 corresponding to a pseudomolecular elemental composition of $C_{23}H_{21}NO_5$. Apart from the general features of a dihydrobenzophenanthridine skeleton, the ^{1}H, ^{13}C, and DEPT NMR spectra of **2** showed signals for one methoxy groups at δ_H 3.85 (3H, s)/δ_C 60.0 and one methylenedioxy group at δ_H 6.10 (2H, s)/δ_C 101.0. These data suggested the partial structure of **2** to be an 8-substituted dihydrodecarine moiety. The remaining data in the ^{1}H, ^{13}C and DEPT NMR spectra (Table 1) indicated signals of a methyl group at δ_H 2.05 (3H, s)/δ_C 30.0, an methine at δ_H 4.90 (1H, dd, 3.7, 15.3)/δ_C 54.3, an methylene group at δ_H [2.20 (1H, dd, J = 3.7, 15.3 Hz), 2.30 (1H, dd, J = 3.7, 15.3 Hz)]/δ_C 47.2, and carbonyl group at δ_C 206.1. All of the above structural characteristics were confirmed by analysis of the HMBC spectrum of **2**, which showed correlations of the proton at δ_H 4.90 with the methylene carbon at δ_C 47.2, the carbonyl at δ_C 206.1. Thus, the structure of **2** was established as 6-acetonyl-*N*-methyl-dihydrodecarine, isolated recently

Table 1
NMR data of 10-*O*-demethyl-17-*O*-methylisoarnottianamide (**1**, $CDCl_3$ + CD_3OD) and 6-acetonyl-*N*-methyl-dihydrodecarine (**2**, DMSO-d_6).

1			**2**		
No.	δ_C	δ_H	No.	δ_C	δ_H
1	98.7	7.02 (s)	1	99.3	7.35 (s)
2	149.9		2	146.5	
3	148.2		3	147.5	
4	105.2	7.43 (s)	4	104.0	7.30 (s)
5	127.9	7.80 (d, 8.6)	5	123.6	7.56 (d, 8.7)
6	127.8	7.30 (d, 8.6)	6	119.5	7.78 (d, 8.7)
7			7		
8	165.8	8.02 (s)	8	54.3	4.90 (dd, 3.7, 15.3)
9	115.0	6.61 (s)	9	149.5	
10	148.1		10	143.8	
11	150.0		11	116.0	6.90 (d, 8.5)
12	101.9	6.58 (s)	12	118.7	7.48 (d, 8.5)
13	134.2		13	127.4	
14	135.6		14	137.8	
15	128.1		15	123.1	
16	131.2		16	126.4	
17	141.2		17	121.3	
18	116.5		18	130.1	
11-OMe	55.4	3.76 (s)	9-OMe	60.0	3.85 (s)
17-OMe	56.5	3.64 (s)	N–CH3	42.4	2.52 (s)
–OCH_2O–	102.0	6.18 (s)	–OCH_2O–	101.0	6.10 (s)
N–Me	31.5	2.91 (s)	–$COCH_3$	206.1	
			–CH_3CO–	30.0	2.05 (s)
			–CH_2–	47.2	2.20 (1H, dd, J = 3.7, 15.3 Hz)
					2.30 (1H, dd, J = 3.7, 15.3 Hz)

from the roots of *Z. riedelianum* [13]. This compound is reported and described here for the first time from *Z. lemairei*.

The alkaloids **3** and **4** are already reported to have been isolated from *Z. lemairei* [14] and therefore no detailed spectral description is given here. ^{1}H NMR spectroscopic data of the two alkaloids showed characteristics of a benzophenanthridine skeleton. The major characteristic difference in ^{1}H NMR spectra of **3** and **4** is the substitution pattern of the two methoxyl groups on the aromatic rings. The spectra show four and two aromatic singlets for **3** and **4**, respectively. The molecular formulae of these compounds were the same and established as $C_{21}H_{18}NO_4$ on the basis of ESIMS.

2.5. Larvicida assays

The compounds were solubilized in dimethyl-sulphoxide (DMSO, analytical reagent, Lobarchemi) and diluted to the required concentration with spring water. The concentration of DMSO was kept below 1%. The bioassay experiments were conducted mainly according to standard WHO procedure [15] with slight modifications. The bioassays were conducted at the Kenya Medical Research Institute (KEMRI), Centre for Disease Control (CDC), Kisumu, Kenya, where the insects were reared in plastic and enamel trays in spring river water. They were maintained and all experiments were carried out at 26 ± 3 °C and the humidity ranged between 70% and 75%. The bioassays were performed with third instar larvae of *A. gambiae* and carried out in triplicate using 20 larvae for each replicate assay. The replicates were run simultaneously yielding a final total of 60 larvae for each dosage. The larvae were placed in 50 ml disposable plastic cups containing 15 ml of test solution and fed on tetramin fish feed during all testing. Mortality and survival was established after 24 h of exposure. Larvae were considered dead if they were unrousable within a period of time, even when gently prodded. The dead larvae in the three replicates were combined and expressed as the percentage mortality for each concentration. The negative control was 1% DMSO in spring river water while the positive control was the pyrethrum based larvicide based larvicide pylarvex® (Pyrethrum Board of Kenya).

2.6. Statistical analysis

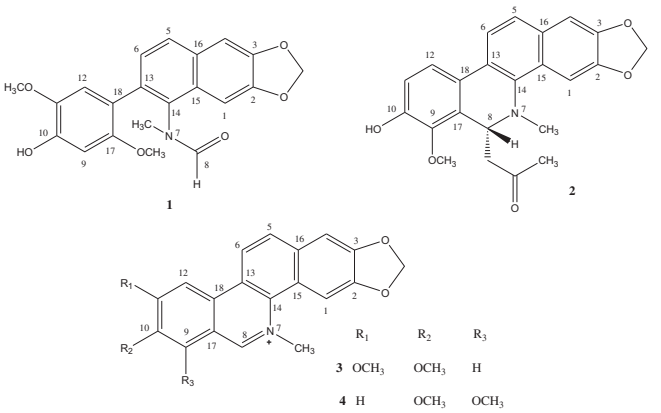
The standard errors were calculated using the statistical software package SPSS version 12.0.

3. Results and discussion

Comparison of the NMR spectroscopic data with those reported in literature indicated that the four alkaloids are 10-*O*-demethyl-17-*O*-methylisoarnottianamide **1**, 6-acetonyl-*N*-methyl-dihydrodecarine **2**, nitidine **3** and chelerythrine **4**. Compound **1** was reported [12] to have been isolated from the stem bark of *T. africanus* (*Meliaceae*) while **2** was recently isolated from the roots of *Z. riedelianum* [13]. This is the first report of isolation of compounds **1** and **2** from *Z. lemairei*. Nitidine **3** and chelerythrine **4** we previously reported from *Z. lemairei* [14].

To evaluate the mosquito larvicidal activities of these alkaloids, third instar larvae of the malaria mosquito *A. gambiae* were used. Table 2 summarizes the percentage mortality after 24 h for the compounds. The most potent compounds were compounds **1** and **2** with mortality rates of 96.7% and 98.3% at a concentration of 250 mg/L, respectively. Compound **3** was less potent with a mortality of 28.3% at the same concentration. Experimental observation also indicated that most of the larvae died within the first few hours.

The least potent of the four alkaloids was compound **4** which achieved 100% mortality at 1000 mg/L. From the comparisons of these mortality values, compounds **1** and **2** showed relatively good toxicities against the larvae of *A. gambiae*. Larvicidal activity of the compounds was proportional to the dosage indicating a dose-dependent effect on mortality. As adult mosquitoes transmit diseases, the critical concentrations of the compounds that lead to high mortalities of the treated larval population, therefore, preventing them from emerging into adults are more meaningful. There are few reported studies of the larvicidal activity of pure compounds on *A. gambiae*. A himachalene sesquiterpenoid isolated from *Hugonia busseana* showed moderate activity against this mosquito after 24 h at a concentration of 237 mg/L [16]. Two triterpenoids and nimocinol also isolated from *Azadirachta indica* are also reported to show larvicidal activity against *Aedes aegypti* [17].



4. Conclusion

Results of this study suggest that these alkaloids (**1**–**4**) are potential natural mosquito larvicides. Moreover, these findings could be useful in the research for newer more selective, biodegradable and natural larvicidal compounds or can be used as lead compounds for the development larvicides. The findings also offer an opportunity for developing alternatives to rather expensive and environmentally hazardous inorganic insecticides.

Table 2
Larvicidal activity of compounds **1**, **2**, **3** and **4**.

Compounds	% Mortality ± SE					
	1000	500	250	125	62.5	0 mg/L
1	100 ± 0.00	90 ± 5.00	96.7 ± 2.89	90 ± 5.00	0.0 ± 0.00	0.0 ± 0.00
2	100 ± 0.00	100 ± 0.00	98.3 ± 2.89	26.7 ± 41.93	0.0 ± 0.00	0.0 ± 0.00
3	100 ± 0.00	100 ± 0.00	28.3 ± 14.50	1.70 ± 2.89	0.0 ± 0.00	0.0 ± 0.00
4	100 ± 0.00	11.7 ± 5.77	10.0 ± 5.00	0.0 ± 0.00	1.7 ± 2.89	0.0 ± 0.00
Pylarvex (100 mg/L) 100 ± 0.00[a]						

[a] Positive control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pestbp.2010.11.003.

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