PLAMED-2011-02-0169-OP (pmV0169) Satz Ziegler + Müller
PDF 1 25. 8. 2011 Herst. K. Grohe
eFirst n.a. Datum 25.08.2011

Antiplasmodial Quinones from *Pentas longiflora* and *Pentas lanceolata*

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Key words
- *Pentas longiflora*
- *Pentas lanceolata*
- Rubiaceae
- anthraquinone
- 5,6-dihydroxydamnacanthol
- pyranonaphthoquinone
- malaria

received February 19, 2011
revised July 21, 2011
accepted July 25, 2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1280179
Planta Med 2011; 77: 1–5

ISSN 0032-0943

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Abstract

The dichloromethane/methanol (1 : 1) extracts of the roots of *Pentas longiflora* and *Pentas lanceolata* showed low micromolar (IC_{50} = 0.9–3 µg/mL) *in vitro* antiplasmodial activity against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum*. Chromatographic separation of the extract of *Pentas longiflora* led to the isolation of the pyranonaphthoquinones pentalongin (**1**) and psychorubrin (**2**) with IC_{50} values below 1 µg/mL and the naphthalene derivative mollugin (**3**), which showed marginal activity. Similar treatment of *Pentas lanceolata* led to the isolation of eight anthraquinones (**4–11**, IC_{50} = 5–31 µg/mL) of which one is new (5,6-dihydroxydamnacanthol, **11**), while three – nordamnacanthal (**7**), lucidin-ω-methyl ether (**9**), and damnacanthol (**10**) – are reported here for the first time from the genus *Pentas*. The compounds were identified by NMR and mass spectroscopic techniques.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedic

Introduction

According to the estimates of the World Health Organization, almost one million deaths are caused by malaria each year in Africa alone, of which most are children under the age of five [1]. In addition, this mosquito-borne disease has a serious economic impact due to loss of commercial and labor outputs, predominantly in countries with tropical and subtropical climates. Over 300 000 000 people worldwide are infected, and each year nearly one-third of these exhibit acute manifestations of the disease [2]. While awaiting the development of a malaria vaccine, millions of lives are still dependent upon treatment with chemotherapeutic agents. Since most of the available drugs are becoming increasingly ineffective due to the rapid emergence of resistant *Plasmodium falciparum* strains [3], there is an urgent need for novel antimalarial agents. Because of the high cost of the few still effective antimalarial drugs [4], traditional medicine remains an important source of treatment in developing countries.

Pentas longiflora Oliver (Rubiaceae) is an important medicinal plant of Tropical East Africa [5]. In Kenya, a decoction of its roots mixed with milk is taken as a cure for malaria [6]. Although its leaves have previously been tested for *in vitro* antimalarial activity, no attempts were made to isolate and identify the antiplasmodial constituents [7]. *Pentas lanceolata* (Forsk.) is mostly found in the highlands of Kenya and was reported to exhibit micromolar *in vitro* antiplasmodial activity against *P. falciparum* [8]. Although extracts of these plants have been assayed against a range of diseases [8, 9], their constituents have not been investigated for antiplasmodial activity. Motivated by the traditional uses and the preliminary screening reports [7–9], we performed isolation, characterization, and an antiplasmodial investigation of naphthoquinones and anthraquinones found in the extracts of the roots of *P. longiflora* and *P. lanceolata*.

Materials and Methods

General experimental procedures

Column chromatography was performed on oxalic acid impregnated silica gel [the silica gel was deactivated by mixing 2 kg of silica gel 60 (70–230 mesh) with 3% oxalic acid (30 g in 1 L water) and allowed to stand for 30 min, filtered and dried in an oven (100 °C) for 45 min]. TLC was done using silica gel 60 F_{254} (Merck) precoated

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plates. NMR analyses were carried out on Varian 800, 600, 500 and 200 MHz spectrometers. Structural assignment was performed based on gCOSY, gTOCSY, gNOESY, gHSQC, gHMBC, and gH2BC spectra. ESI LC-MS was performed on a Perkin Elmer PE SCIEX API 150 EX instrument equipped with a Turbolon spray ion source and a Gemini 5-mm C18 110 Å HPLC column using a water-acetonitrile gradient (80 : 20 to 20 : 80). High-resolution mass spectral analysis (Q-TOF-MS) was performed at Stenhagen Analyslab AB, Gothenburg, Sweden. The compound purity was determined by NMR and HPLC. Analytical HPLC was run on a Hewlett Packard Series 1050 HPLC using the software Chromulan (Pikron Ltd.), a Gemini 5-mm C18 110 Å HPLC column and a methanol-water mixture as the eluent.

Plant material

The roots of *Pentas longiflora* were collected from Nandi East district, Kenya (Nandi Hills-Chebarus location) in August 2009. The roots of *Pentas lanceolata* were collected from Ngong forest in December 2009. The plant materials were identified by Mr. Patrick Chalo Mutiso, School of Biological Sciences, University of Nairobi. Specimens are deposited at the Herbarium, School of Biological Sciences, University of Nairobi, under voucher numbers MEA 2009/001 (*Pentas longiflora*) and MEA 2009/002 (*Pentas lanceolata*).

Extraction and isolation

The dried and grounded roots of *Pentas longiflora* (1.1 kg) were extracted by cold percolation with CH_2Cl_2:MeOH (1 : 1) three times for 24 hrs in each case. The extract was concentrated using a rotary evaporator to yield a brownish crude extract (50 g, 4.54%). A 35-g portion of the crude extract was subjected to column chromatography (80 cm length and 80 mm diameter column size, 350 g oxalic acid impregnated silica gel) with an increasing gradient of acetone in *n*-hexane. Two hundred fractions (each ca. 200 mL) were collected. Fractions 15–17 (2% acetone in *n*-hexane) were purified by Sephadex LH-20 (eluent CH_2Cl_2:MeOH; 1 : 1) to give mollugin (**3,** 34 mg). Fractions 18–25 (3% acetone in *n*-hexane) were purified by column chromatography on oxalic acid impregnated silica gel (eluent, 2% acetone in *n*-hexane) to give pentalongin (**1**, 40 mg). Fractions 90–112 (20% acetone in *n*-hexane) were combined and purified by Sephadex LH-20 (eluent, CH_2Cl_2/MeOH; 1 : 1) to give psychorubrin (**2**, 150 mg). The ground roots (1.4 kg) of *Pentas lanceolata* were extracted with CH_2Cl_2:MeOH (1 : 1) and then with methanol three times for 24 hrs in each case. The extracts were concentrated using a rotary evaporator to yield a brownish crude extract (57 g, 4.8% and 100 g, 7.1%, respectively).

A 54-g portion of the crude CH_2Cl_2:MeOH (1 : 1) extract was subjected to column chromatography (80 cm length and 80 mm diameter, 420 g oxalic acid impregnated silica gel) with an increasing gradient of ethyl acetate in *n*-hexane. A total of 550 fractions (each 200 mL) were collected. Fractions 10–13 (2% ethyl acetate in *n*-hexane) were combined and purified on Sephadex LH-20 (eluent, CH_2Cl_2:MeOH; 1 : 1) to give tectoquinone (**4**, 40 mg). Fractions 15–25 (eluent, 3% ethyl acetate in *n*-hexane) were combined and purified by Sephadex LH-20 (eluent, CH_2Cl_2:MeOH; 1 : 1) to give rubiadin (**5**, 680 mg) and rubiadin-1-methyl ether (**6**, 50 mg). Fractions 30–35 (5% ethyl acetate in *n*-hexane as the eluent) were combined and purified by column chromatography to give damnacanthal (**8,** 320 mg). Fractions 53–65 (7% ethyl acetate in *n*-hexane) were combined and purified on Sephadex LH-20 with CH_2Cl_2:MeOH; (1 : 1) as the eluent to give nordamnacanthal (**7**, 20 mg). Fractions 131–135 (18% ethyl acetate in *n*-hexane) were combined and purified using column chromatography on oxalic acid impregnated silica gel (increasing gradient of ethyl acetate in *n*-hexane) to give lucidin-ω-methyl ether (**9**, 50 mg). Fractions 400–405 (50% ethyl acetate in *n*-hexane) were combined and purified by MPLC (increasing gradient of ethyl acetate in *n*-hexane as the eluent; flow rate of 30 mL/min) to give damnacanthol (**10,** 50 mg).

The methanol extract (70 g) was subjected to column chromatography on oxalic acid impregnated silica gel (80 cm length and 80 mm diameter, 500 g oxalic acid impregnated silica gel) eluting with an increasing gradient of methanol in dichloromethane. A total of 100 fractions (each ca. 200 mL) were collected. Fractions 5–11 (100% dichloromethane) were combined and purified on Sephadex LH-20 (eluent, CH_2Cl_2:MeOH; 1 : 1) to give rubiadin (**5,** 20 mg) and rubiadin-1-methyl ether (**6,** 18 mg). Fractions 21–25 (eluent, 1% of methanol in CH_2Cl_2) were combined and further purified on Sephadex LH-20 (eluent, CH_2Cl_2:MeOH; 1 : 1) to give damnacanthol (**10,** 15 mg). Fractions 87–90 (5% MeOH in CH_2Cl_2) were combined and purified using Sephadex LH-20 (eluent, CH_2Cl_2/MeOH; 1 : 1) to give 5,6-dihydroxydamnacanthol (**11,** 40 mg).

Drugs

The reference antimalarial drugs, chloroquine and mefloquine, having well-documented IC_{50} values, were tested alongside test samples of pyranonaphthoquinones and a naphthalene derivative isolated from the roots of *Pentas longiflora*; anthraquinones were isolated from the roots of *Pentas lanceolata* as described above.

Drug susceptibility testing

Two laboratory clones of *Plasmodium falciparum,* the Sierra Leone D6 chloroquine-sensitive and the Indochina W2 chloroquine-resistant strains, were maintained in continuous culture to attain replication robustness prior to assays. Drug susceptibility was tested by the Malaria SYBR Green I-based *in vitro* assay technique, described in Juma et al. [10].

Supporting information

Cytotoxicity assay: Experimental details are given in the Supporting Information.

NMR spectra for compound 11 are given. 1D and 2D spectra of all additional, known compounds are available upon request.

Please check/expand.

Replacement ok? **5,6-Dihydroxydamnacanthol (11).** Red solid. UV (CH_3OH) λ_{max} 218 nm, 274 nm, 308 nm, 424 nm. *ok?* ^{1}H NMR (**Table 2**). ^{13}C NMR (**Table 2**).

HRMS (ESI): m/z = 317.0659 $[M + H]^+$, calcd. 317.06558.

Results and Discussion

In our experience, the root extracts of *P. longiflora* and *P. lanceolata* showed significant antiplasmodial activities (**Table 1**). From the root extract of *P. longiflora,* the naphthoquinone derivatives pentalongin (**1**) [11–13], psychorubrin (**2**) [14], and mollugin (**3**) [13, 15] were chromatographically isolated, identified, and tested for antiplasmodial activities. The major constituents **1** and **2** showed good to moderate activities (IC_{50} < 1 µg/mL), whereas **3** had marginal inhibition against the W2 chloroquine-

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Table 1 *In vitro* antiplasmodial activity and cytotoxicity of crude extracts and pure compounds.

Sample (purity in %)	Antiplasmodial activity IC_{50}* (µg/mL)		Cytotoxicity LD_{50}§ (µg/mL)	Selectivity index	
	W2 clone (CQ-R)	D6 clone (CQ-S)		W2	D6
Pentas longiflora (root extract)	0.93 ± 0.16	0.99 ± 0.09			
Pentalongin (**1**, ≥ 98 %)	0.27 ± 0.09	0.23 ± 0.08	0.80	2.96	3.48
Psychorubrin (**2**, ≥ 98 %)	0.91 ± 0.15	0.82 ± 0.24	0.89	0.98	1.09
Mollugin (**3**, ≥ 95 %)	10.22 ± 1.37	7.56 ± 1.13	20.0	1.96	2.65
Pentas lanceolata (root extract)	2.55 ± 0.30	1.33 ± 0.15			
Tectoquinone (**4**, ≥ 98 %)	10.78 ± 1.33	6.74 ± 1.73	> 100	> 9.27	> 14.83
Rubiadin (**5**, ≥ 98 %)	8.36 ± 2.19	5.47 ± 0.70	53.0	6.34	9.69
Rubiadin-1-methyl ether (**6**, ≥ 98 %)	18.91 ± 0.39	12.08 ± 2.28	64.0	3.38	5.30
Nordamnacanthal (**7**, ≥ 99 %)	9.33 ± 2.98	9.29 ± 0.00	51.0	5.47	5.49
Damnacanthal (**8**, ≥ 99 %)	10.88 ± 2.09	7.67 ± 0.36	73.0	6.71	9.52
Lucidin-ω-methyl ether (**9**, ≥ 98 %)	13.19 ± 2.15	12.08 ± 3.69	> 100	> 7.58	> 8.28
Damnacanthol (**10**, ≥ 98 %)	31.42 ± 2.32	16.07 ± 1.15	> 100	> 3.18	> 6.22
5,6-Dihydroxydamnacanthol (**11**, > 99 %)	19.33 ± 6.36	15.02 ± 4.28	> 100	> 5.17	> 6.66
Chloroquine	0.07 ± 0.01	0.01 ± 0.01			
Mefloquine	0.004 ± 0.38	0.06 ± 0.04			

* Data are the mean of at least 3 independent experiments. § The mean value of at least 6 independent experiments are given; 95 % confidence interval and dose-response curves are presented in Supporting Information

Table 2 NMR spectroscopic data (DMSO-d_6) for 5,6-dihydroxydamnacanthol (**11**).

Position	δ_H (*J* in Hz)	δ_C	HMBC (^2J, ^3J)
1		161.7	–
1a		115.8	–
2		125.4	–
3		161.7	–
4	7.55, s	109.6	C-1a, 2, 3, 4a, 10
4a		135.2	–
5		150.0	–
5a		118.2	–
6		151.2	–
7	7.20, d (8.2)	120.4	C-5, 6, 8, 8a
8	7.57, d (8.2)	121.2	C-6, 7, 9, 8a
8a		129.5	–
9		178.7	–
10		188.5	–
11	4.55, s (2H)	52.2	C-1, 2, 3
12	3.83, s (3H)	62.4	C-1
5-OH	12.4, s	–	C-5a, 6
11-OH	4.55	–	C-11

resistant and D6 chloroquine-sensitive strains of *P. falciparum* (**Table 1**). Although these compounds were previously reported [11, 12] and studied for antibacterial [16], antifungal [17], and antiviral [18] properties, their antiplasmodial activities are reported here for the first time.

Chromatographic separation of the dichloromethane/methanol (1 : 1) extract of the roots of *P. lanceolata* resulted in the isolation of seven known anthraquinones (**Fig. 2**), spectroscopically (NMR and MS) identified as tectoquinone (**4**) [15], rubiadin (**5**) [19], rubiadin-1-methyl ether (**6**) [19], nordamnacanthal (**7**) [20], damnacanthal (**8**) [19], lucidin-ω-methyl ether (**9**) [26, 29], and damnacanthol (**10**) [21]. Three of these (**7, 9,** and **10**) are reported here for the first time from the genus *Pentas*. In agreement with previous investigations of rubiadin-1-methyl ether (**6**), damnacanthal (**8**), and lucidin-ω-methyl ether (**9**) [22], the anthraquinones isolated from the roots of *P. lanceolata* showed moderate antiplasmodial activities (**Table 1**).

The methanol extract yielded further amounts of (**4**), **5, 10,** and a new compound **11** (**Fig. 3**) isolated as a red solid. The Q-TOF-MS spectrum provided the exact mass at *m/z* 317.0659 $[M + H]^+$, suggesting a molecular formula of $C_{16}H_{12}O_7$. The UV-VIS absorption maxima at 218, 274, 308, and 424 nm suggests a 9,10-anthraquinone skeleton [23]. Its ^{1}H NMR spectrum (**Table 2**) revealed an aromatic singlet, a pair of *ortho*-coupled aromatic protons, a methoxy, and an oxymethylene substituent as well as three solvent accessible and one chelated (δ_H 12.40) hydroxyl groups. Two carbonyl functionalities were indicated by ^{13}C-NMR. The HMBC correlation of the methoxy protons with C-1 and the oxymethylene protons with C-1, C-2 and C-3 (**Table 2**) are consistent with the methoxy, oxymethylene, and a hydroxyl substitution in ring A. The high chemical shift of the methoxyl group (δ_C 62.4 ppm) is indicative of di-*ortho* [24] substitution allowing its placement at C-1 rather than C-3. Hence, in similarity to previously identified anthraquinones of the Rubiaceae family [25], ring A of **11** is oxygenated at C-1 and C-3 and has the oxymethylene at C-2. The aromatic singlet at δ_H 7.55 ppm (H-4) showed an HMBC correlation with the C-10 carbonyl (δ_C 189.2 ppm), indicating their *peri* position. The high chemical shift of this carbonyl is indicative of a *peri*-hydroxyl group at C-5, which is further confirmed by the HMBC correlation of the aromatic doublet at δ_H 7.57 ppm (H-8) to the carbonyl at δ_C 178.7 ppm (C-9), but not with the one at δ_C 188.5 ppm (C-10). These three bond heteronuclear correlations confirm the dihydroxy substitution at C-5 and C-6 in ring C. Therefore, compound **11** was characterized as 3,5,6-trihydroxy-1-methoxy-2-hydroxymethyl-9,10-anthraquinone (**Fig. 3**) for which the trivial name 5,6-dihydroxydamnacanthol is proposed. Our assignation is in good agreement with that of the recently reported and closely-related 2-hydroxymethyl-1-methoxy-3,5,6-trihydroxyanthraquinone-3-O-β-glycopyranoside, isolated from *Putoria calabrica* (L. fil, Rubiaceae) [26]. An additional evidence for the biosynthetic route in the family Rubiaceae [25] yielding compound **11** is the presence of the 2-ethoxyl-derivative of **11**, 2-ethoxymethyl-3,5,6-trihydroxy-1-methoxyanthraquinone, in the extract of *Putoria calibrica* (L. fil) [27].

Based on the biosynthesis of anthraquinones of the Rubiaceae family, most of these compounds are substituted with hydroxyl,

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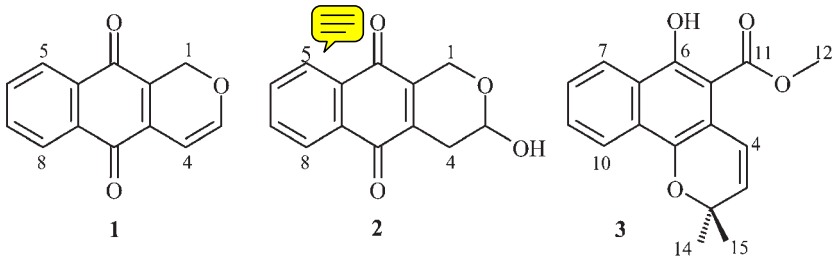
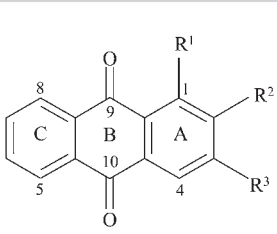


Fig. 1 Compounds isolated from the roots of *Pentas longiflora*.



	R^1	R^2	R^3
4.	H	CH_3	H
5.	OH	CH_3	OH
6.	OCH_3	CH_3	OH
7.	OH	CHO	OH
8.	OCH_3	CHO	OH
9.	OH	CH_2OCH_3	OH
10.	OCH_3	CH_2OH	OH

Fig. 2 Structures of known compounds isolated from the roots of *Pentas lanceolata*.

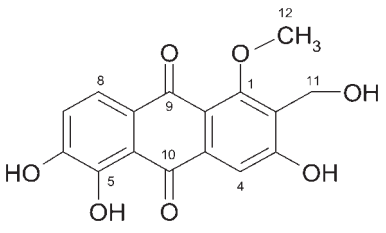


Fig. 3 Structure of 5,6-dihydroxydamnacanthol (**11**).

methoxyl, and/or methyl groups in ring A (**Fig. 2**) [25], and some carry additional hydroxyl or alkoxyl groups in ring C, mainly at positions 5 and 6 [25,28]. These latter oxygen atoms are introduced at a late stage of the biogenesis [25], which is shown, for example, for morindone, as reported from the cell cultures of *Morinda citrifolia* [29] and for putorinoside A, isolated from *Putoria calábrica* [27]. As a consequence of the biosynthetic pathway, most, if not all, anthraquinones carry a carbon substituent at position 2 in ring A [25]. One of the rare exceptions from the above rule is 2-ethoxy-1-hydroxyanthraquinone isolated from *Morinda citrifolia* [30], a compound lacking carbon (CH_2, CHO, CH, etc.) substitution at C-2. We would like to emphasize that if a carbon substitution is present in an anthraquinone derived from the Rubiaceae family, based on biogenetics [25], the currently accepted nomenclature, it is placed unambiguously at position 2 in ring A. Not following the above convention [31] may be perplexing in the evaluation of biosynthetic routes and bioactivities. Hence, the compounds named 1,2-dimethoxy-6-methyl-9,10-anthraquinone and 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone [31] should be correctly named as 5,6-dimethoxy-2-methyl-9,10-anthraquinone and 6-hydroxy-5-methoxy-2-methyl-9,10-anthraquinone. Since complete and correctly assigned spectroscopic characterization was not available for several anthraquinones described here, detailed MS, and ^1H and ^{13}C NMR analysis (based on homo- and heteronuclear correlation spectra providing unambiguous assignment) is reported (Supporting Information).

Despite their promising activity against the W2 and D6 strains of *Plasmodium falciparum*, the comparably high cytotoxicity (**Table 1**) of **1** and **2** makes their direct application as antimalarial agents virtually impossible. The anthraquinones isolated from *Pentas lanceolata*, **4**-**11**, show low cytotoxicity indicating the safer applicability of the anthraquinone containing an indigenous decoction of *P. lanceolata* as compared to that of the pyranonaphtoquinone containing *P. longiflora*.

In conclusion, the pyranonaphthoquinones and some of the anthraquinones isolated from the roots of *P. lanceolata* and *P. longiflora* showed good to moderate antiplasmodial activities against the W2 and D6 strains of *Plasmodium falciparum*, and an overall low cytotoxicity for anthraquinones. Careful analysis of their structure-activity relationship followed by rational synthetic modifications has the potential for identifying more applicable agents in the fight against malaria.

Acknowledgements

M. Endale is thankful to the German Academic Exchange Service (DAAD) and the Natural Products Research Network for Eastern and Central Africa (NAPRECA) for a Ph.D. Scholarship. J.P. Alao and P. Sunnerhagen are thankful to the Chemical Biology Platform at the University of Gothenburg. M. Erdelyi is thankful for the financial support from the Swedish Research Council (VR2007–4407) and the Royal Society of Arts and Sciences in Gothenburg. ■ *spelled as in affiliations*

Conflict of Interest

Herewith we declare the absence of any conflict of interest, financial or personal, for all authors.

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■ Please add cross-reference to **Fig. 1** in the text.

■ **Table 2** is mentioned in the text before **Table 1**, please insert an earlier reference to Table 1, if possible..

■ Please note: In the supporting information file, the tables had a mix of points (e.g., 7.2) and commas (e.g., 7,2). All commas were changed to "point". Ok?■