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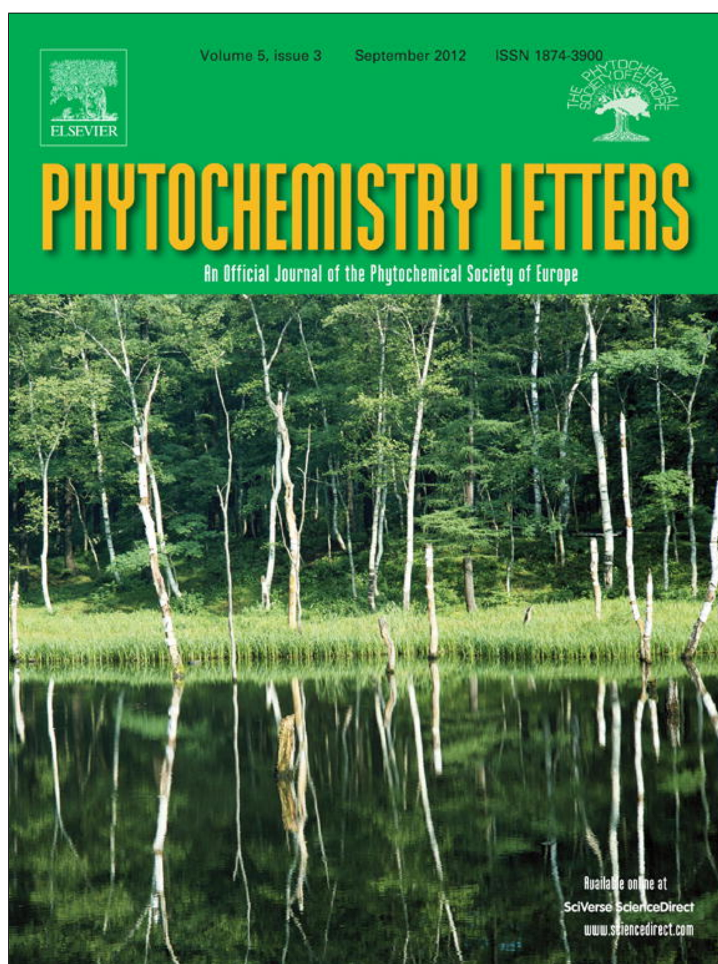

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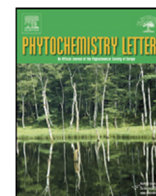

Naphthoquinones from the roots of *Aloe secundiflora*

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ABSTRACT

Two new naphthoquinones, 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione, were isolated from the roots of *Aloe secundiflora* together with the known compounds chrysophanol, helminthosporin, isoxanthorin, ancistroquinone C, aloesaponarins I and II, aloesaponols I and II, laccaic acid D methyl ester and asphodelin. The structures were elucidated based on spectroscopic evidence. This appears to be the first report on the occurrence of naphthoquinones in the genus *Aloe*. Aloesaponarin I and 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione showed anti-bacterial activity against *Mycobacterium tuberculosis* with MIC values of 21–23 μg/mL in the Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA); 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione also showed cytotoxicity against the Vero cell line (IC_{50} = 10.2 μg/mL).
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1. Introduction

The genus *Aloe* (sub-family Alooideae, family Asphodelaceae), comprising about 600 species, is indigenous to Eastern and Southern Africa and Madagascar (Okamura et al., 1996). It has also been introduced to the West Indies and many other tropical countries (Mabberly, 1987; Demmisew, 1996). About 83 *Aloe* species occur in East Africa, of which 60 grow naturally in drylands of Kenya with 26 of them being endemic (Carter, 1994).

Aloe secundiflora is widely distributed in parts of Kenya and Tanzania and extends northwards into Southern Ethiopia and the Southern Sudan (Reynolds, 1966). *A. secundiflora* has long been used in traditional medicine practice for the treatment of various ailments including malaria, rheumatism, diarrhea, dysentery and skin infections (Kokwaro, 2009). The leaf exudate of *A. secundiflora* has also found traditional use in ethno-veterinary medicine in the treatment of bacterial diseases, ectoparasites and in the management of some viral diseases. The crude extract of the leaves of *A. secundiflora* has been shown to inhibit the growth of *Candida albicans* (Msoffe and Mbilu, 2009). To date, the phytochemical information on *A. secundiflora* is limited to TLC (Reynolds, 1985) and HPLC-MS (Waihenya et al., 2003) analyses of the leaves, revealing the presence of anthraquinones, anthrones, pyrones and chromones. We report here the isolation and structural elucidation of two new naphthoquinones (Fig. 1) along with known quinones from the roots of *A. secundiflora*. The anti-bacterial activities against *Mycobacterium tuberculosis* of some of the compounds are also reported.

2. Results and discussion

The air-dried and ground roots of *A. secundiflora* were extracted with CH_2Cl_2/MeOH (1:1). The concentrated extract was partitioned between ethyl acetate and water. The ethyl acetate fraction was then subjected to column chromatography on oxalic acid impregnated silica gel to yield twelve compounds, of which two naphthoquinones are new natural products.

Compound **1** was obtained as orange crystals which upon exposure to ammonia turned reddish-brown. TOF-HREIMS showed a *pseudo*-molecular ion peak at *m*/*z* 271.0592, corresponding to

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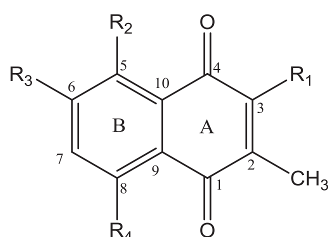

1 $R_1 = R_3 = OCH_3$, $R_2 = OH$, $R_4 = H$
2 $R_1 = OCH_3$, $R_2 = R_4 = OH$, $R_3 = H$
3 $R_1 = OH$, $R_2 = R_3 = OCH_3$, $R_4 = H$

Fig. 1. Naphthoquinones of the roots of *Aloe secundiflora*.

molecular formula of $C_{13}H_{12}O_5Na$. In agreement with this the ^{13}C NMR spectrum showed the presence of thirteen non-equivalent carbon atoms. The UV (λ_{max} 280, 336, 390, 430 nm) and ^{13}C NMR (δ_C 188.4 and 185.1 for two carbonyl groups) spectra suggested a 1,4-naphthoquinone skeleton (Bringmann et al., 2008). Furthermore, from the ^1H and ^{13}C NMR spectra (Table 1), the presence of a chelated hydroxyl (δ_H 12.15), two methoxyls (δ_H 4.08 and 3.97; δ_C 62.1 and 57.4) and an aromatic methyl (δ_H 2.03; δ_C 10.2) substituents on a 1,4-naphthoquinone skeleton were evident.

Ring A in this compound is substituted with methoxyl and methyl groups at C-2 and C-3 of the 1,4-naphthoquinone system. This was clear from the ^{13}C NMR chemical shift value of the methoxyl resonance (δ_C 62.1) which requires that it is di-*ortho*-substituted allowing its placement at C-3 or C-2. HMBC correlation of the methyl protons (δ_H 2.03) with C-1 (δ_C 185.1), C-2 (δ_C 135.6) and C-3 (δ_C 159.0) is in agreement with the placement of the methyl at C-2 and hence the methoxyl (δ_C 62.1) should be at C-3. In ring B, the ^1H NMR spectrum showed, two *ortho*-coupled protons at δ_H 7.31 and 7.57 (J = 8.4 Hz), the latter showing HMBC correlation with C-1 (δ_C 185.1), allowing its assignment to H-8 and hence its coupling partner to H-7. With the chelated hydroxyl group being at C-5 the second methoxyl (δ_H 3.97, δ_C 57.4) in this compound was then placed at C-6. The chemical shift values of C-5 (δ_C 153.7) and C-6 (δ_C 155.4) are typical of *ortho*-di-oxygenated aromatic carbon atoms. This new compound was therefore characterized as 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione (**1**, Fig. 1).

The UV (λ_{max} 270, 345, 462 nm) spectrum of compound **2** (M^+ *m/z* 234.0533, $C_{12}H_{10}O_5$) also suggested a 1,4-naphthoquinone skeleton (Bringmann et al., 2008). The ^{13}C NMR spectrum (Table 1) revealed the presence of 12 carbon atoms, ten of which belonging to the naphthoquinone skeleton, while the remaining two carbon atoms corresponded to methoxyl (δ_H 4.03; δ_C 61.6) and an aromatic methyl (δ_H 2.01, δ_C 9.0) substituents. Ring A of **2** is substituted as in **1** with methyl at C-2 and methoxyl at C-3. HMBC correlation (Table 1) of the methyl protons (δ_H 2.01) with C-1 (δ_C 188.9), C-2 (δ_C 133.6) and C-3 (δ_C 158.4) is again in agreement with this substitution pattern in this ring. In ring B, from the ^1H and ^{13}C NMR spectra (Table 1), it was evident that there are two chelated hydroxyl groups (δ_H 12.19 and 12.59) and these are placed at C-5 and C-8 with two *ortho*-coupled protons at δ_H 7.12 and 7.15 (J = 9.4 Hz) assigned to H-6 and H-7. The substitution pattern of ring B was further confirmed from the HMBC spectrum (Table 1). Therefore this compound was characterized as 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**, Fig. 1). This is the first report of the occurrence in nature of compound **2** having previously been reported as a synthetic product (Kuroda, 1944).

The third naphthoquinone isolated from this plant was readily identified (Table 1) as 3-hydroxy-5,6-dimethoxy-2-methylnaphthalene-1,4-dione (**3**, Fig. 1), a compound previously isolated from the callus cultures of *Ancistrocladus abbreviatus* under the trivial name ancistroquinone C (Bringmann et al., 2008). This is only the second report on the occurrence of this compound in nature. The other known compounds were identified as the anthraquinones chrysophanol, helminthosporin, isoxanthorin (Yagi et al., 1977), aloesaponarins I and II, laccaic acid D methyl ester, the preanthraquinones aloesaponols I and II (Yagi et al., 1974) and the anthraquinone dimer asphodelin (Yagi et al., 1978; Adinolfi et al., 1991).

The anthraquinones and pre-anthraquinones isolated in this study are commonly found in the roots of *Aloe* species and are of little chemotaxonomic value at the intrageneric level. On the other hand, this is the first report on the occurrence of naphthoquinones from the genus *Aloe*. According to Reynolds (1966), *A. secundiflora* has been placed in group 14 along with species with secund flowers. However the phylogenetic relationship among the members of this group has been disputed, but still close affinity between the taxa *A. brandhamii* and *A. secundiflora* has been suggested. In support of this Viljoen and van Wyk (2000) gave chemotaxonomic evidence which showed that *A. brandhamii* and *A. secundiflora* along with *A. leachii* are closely related. In light with our finding on the first occurrence of naphthoquinones in the genus *Aloe*, it will be worthwhile to conduct directed investigation on the presence or absence of naphthoquinones on members of group 14, more so on *A. brandhamii* and *A. leachii* in order to assess the chemotaxonomic significance of these naphthoquinones.

Table 1
^1H (600 MHz), ^{13}C (150 MHz) and HMBC spectral data for compounds **1**–**3**.

Carbon	**1** ($CDCl_3$)			**2** (CD_2Cl_2)			**3** (CD_3OCD_3)	
	δ_H (J in Hz)	δ_C	HMBC	δ_H (J in Hz)	δ_C	HMBC	δ_H (J in Hz)	δ_C
1		185.1			188.9			185.2
2		135.6			133.6			119.6
3		159.0			158.4[a]			156.3
4		188.4		–	183.9		–	181.2
5		153.7	C-6, C-7, C-9, C-10		158.4[a]			151.0
6		155.4	C-5, C-6, C-8, C-10	7.12 *d* (9.4)	128.8[b]	C-5, C-8		159.6
7	7.31 *d* (8.4)	117.3		7.15 *d* (9.4)	130.0[b]	C-5, C-8	7.43 *d* (8.4)	118.2
8	7.57 *d* (8.4)	121.2			157.6[a]		7.87 *d* (9.0)	124.5
9		125.5		–	111.4[c]			125.1
10		116.1		–	111.8[c]			
3-OCH_3	4.08 *s*	62.1	C-3	4.03 *s*	61.6	C-3		
5-OCH_3							3.98 *s*	61.7
6-OCH_3	3.97 *s*	57.4	C-6				3.82 *d*	57.4
2-CH_3	2.03 *s*	10.2	C-1, C-2, C-3	2.01 *s*	9.0	C-1, C-2, C-3	1.97 *s*	9.2
3-OH							9.15 *s*	
5-OH	12.15 *s*		C-5	12.19 *s*		C-5, C-6, C-10		
8-OH				12.59 *s*		C-7, C-8, C-10		

[a,b,c] May be interchangeable.

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Table 2
Anti-microbial activity (against *Mycobacterium tuberculosis*) and cytotoxicity of quinones of *A. secundiflora*.

Compound	MIC (μg/mL) (% inhib. at highest conc.)		Cytotoxicity IC_{50} (μg/mL)
	MABA	LORA	Vero cell
1	23.5	23.1	10.2
Aloesaponarin I	22.8	21.1	40.2
Aloesaponarin II	>50 (83%)	>50 (74%)	9.9
Aloe-emodin	>50 (87%)	>50 (80%)	10.1
Laccaic acid D methyl ester	>20 (60%)	>20 (71%)	>20 (22%)
Rifampicin (RMP)	0.05	1.4	151.3

Four of the isolated compounds were tested for antimicrobial activity against *Mycobacterium tuberculosis* (Table 2). Aloesaponarin I showed moderate activity with MIC values of 22.8 and 21.1 μg/mL in the Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA) respectively; while 5-hydroxy-3,6-dimethoxy-2-methylnaphthoquinone-1,4-dione (**1**), showed MIC of 23.5 (MABA) and 23.1 (LORA) μg/mL respectively. Compound **1** also exhibited cytotoxicity against the Vero cell line (IC_{50} = 10.2 μg/mL). No significant antimicrobial activity was observed for the other compounds, though aloesaponarin II and aloe-emodin showed cytotoxicity (Table 2).

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC: on oxalic acid impregnated silica gel 60 (70–230 mesh). Gel filtration on Sephadex LH-20. UV spectra were recorded on a Specord S600, Analytik Jena AG, Germany. EI-MS: direct inlet, 70 eV on Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker Avance 600 spectrometer using the residual solvent peaks as reference. For known compounds ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) was recorded on a Varian-Mercury 200 spectrometer. HSQC and HMBC spectra were acquired using the standard Bruker software.

3.2. Plant material

The plant material of *A. secundiflora* was collected from Ndaragua, Central province of Kenya in September 2008. The plant was identified by Mr. Simon Mathenge of the Herbarium, Botany Department, School of Biological Sciences, University of Nairobi, Kenya, where a voucher specimen has been deposited.

3.3. Extraction and isolation

The air-dried and ground roots of *A. secundiflora* (2 kg) were extracted by cold percolation with CH_2Cl_2/MeOH (1:1) (3× 3 L) to give a dark brown residue. The extract was partitioned between ethyl acetate and water. The ethyl acetate layer (31 g) was fractionated on column chromatography using oxalic acid impregnated silica gel (400 g) whilst eluting with n-hexane containing increasing gradients of ethyl acetate to give a total of 55 fractions each of *ca.* 200 mL.

The fraction eluted with 100% n-hexane was further separated by passing over Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) which gave chrysophanol (12 mg) and helminthosporin (9 mg); while the fraction eluted with 0.5% EtOAc in n-hexane was subjected to Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) which gave 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**2**, 9 mg). The fraction eluted with 2% EtOAc in n-hexane was subjected to Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) which yielded isoxanthrin (7 mg), 5-hydroxy-3,6-dimethoxy-2-methyl naphthalene-1,4-dione (**1**, 14 mg). ancistroquinone C (10 mg). Preparative TLC (using 100% dichloromethane) of the fraction eluted with 3% EtOAc in n-hexane yielded asphodelin (12 mg). Crystallization (from dichloromethane) of fraction eluted with 4% EtOAc in n-hexane yielded aloesaponarin II (45 mg). The fraction eluted with 5% EtOAc in n-hexane was subjected to Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) to give aloesaponarin I (26 mg). Finally, the fraction eluted with 30% EtOAc in n-hexane was subjected to Sephadex LH-20 (eluent, CH_2Cl_2/CH_3OH; 1:1) which yielded laccaic acid D methyl ester (16 mg) and aloesaponols I (23 mg) and II (9 mg).

3.4. 5-Hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione (1)

Orange crystals. UV λ_{max} (MeOH) nm: 280, 336, 390, 430. ^{1}H and ^{13}C NMR (Table 1). EIMS *m/z* (70 eV, rel. int.): 248 (100, $[M]^+$), 233 (45, $[M-Me]^+$), 230 (18), 205 (38), 177 (17), 151 (18). TOF HRMS *m/z*: 271.0592, $[M]^+$ $C_{13}H_{12}O_5Na$ (calculated for 271.0582).

3.5. 5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2)

Red amorphous solid. UV λ_{max} (MeOH) nm: 270, 345, 462. ^{1}H and ^{13}C NMR (Table 1). EIMS *m/z* (70 eV, rel. int.): 234 (100, $[M]^+$), 216 (23), 204 (17), 191 (22), 189 (25), 188 (19). HRMS *m/z*: 234.0533, $[M]^+$ $C_{12}H_{10}O_5$ (calculated for 234.0528).

3.6. Anti-microbial assay

The anti-bacterial assay was carried out using a Tuberculosis strain H37Rv (ATCC #27294). The MICs of test samples against *Mycobacterium tuberculosis* were determined by the Microplate Alamar Blue Assay (MABA) as described by Falzari et al. (2005) and by the Low Oxygen Recovery Assay (LORA) as described by Cho et al. (2007). Rifampicin was used as a standard drug with MIC values of 0.05 and 1.4 μg/mL in the MABA and LORA tests respectively.

3.7. Cytotoxicity

The cytotoxicity test was carried out on the Vero cells as described by Falzari et al. (2005). Rifampicin did not show significant cytotoxicity (IC_{50} value of 151.3 μg/mL).

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