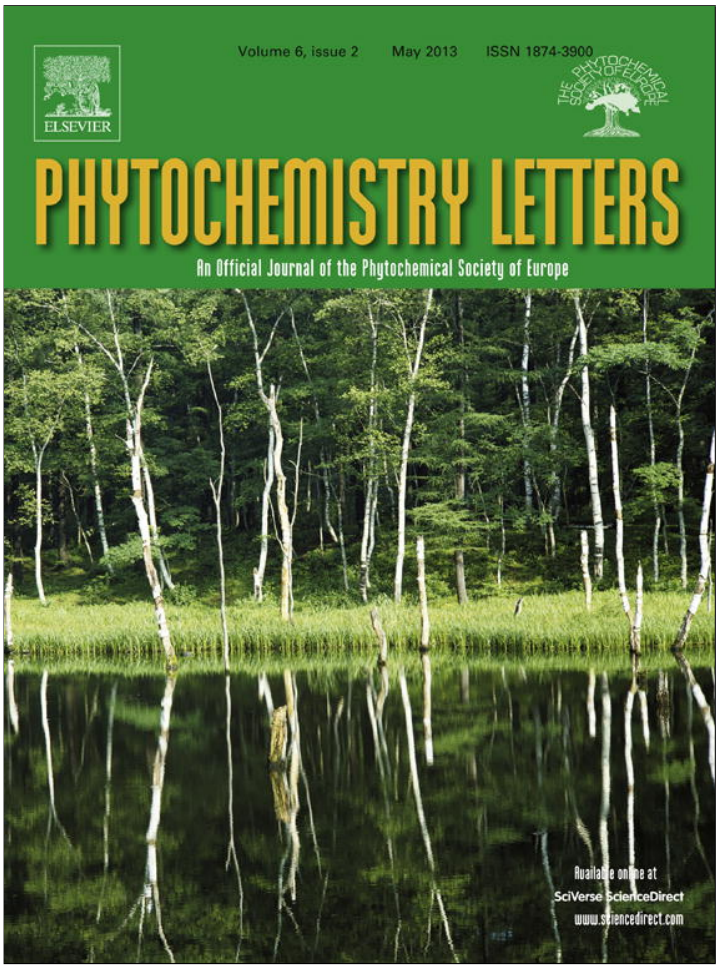

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Knipholone cyclooxanthrone and an anthraquinone dimer with antiplasmodial activities from the roots of *Kniphofia foliosa*

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ABSTRACT

A new phenylanthrone, named knipholone cyclooxanthrone and a dimeric anthraquinone, 10-methoxy-10,7′-(chrysophanol anthrone)-chrysophanol were isolated from the roots of *Kniphofia foliosa* together with the rare naphthalene glycoside, dianellin. The structures were determined by NMR and mass spectroscopic techniques. The compounds showed antiplasmodial activities against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* with 10-methoxy-10,7′-(chrysophanol anthrone)-chrysophanol being the most active with IC_{50} values of 1.17 ± 0.12 and 4.07 ± 1.54 μg/ml, respectively.

1. Introduction

Despite reduction in malaria cases leading to death (WHO, 2011), malaria remains a public health problem because of the increased resistance toward the available antimalarial drugs (Ginsburg and Deharo, 2011). The search for antimalarial lead compounds from plants, especially from those with documented traditional uses, has gained momentum since the discovery of the most recent and effective antimalarial compound artemisinin, from the Chinese medicinal plant *Artemisia annua* (Asteraceae) (Balint, 2001).

The genus *Kniphofia* (sub-family Asphodeloideae, family Asphodelaceae) comprises 70 species mainly confined to Africa with the center of diversity being South Africa (Dahlgren et al., 1985). Fifteen *Kniphofia* species have been recorded in Eastern Africa, of which seven occur in Ethiopia, including *Kniphofia foliosa* Hochst (Whitehouse, 2002; Demissew and Nordan, 2010). *K. foliosa* is a perennial herb which grows on road sides, overgrazed areas with scattered trees and is commonly distributed in the mountainous regions of central and northern Ethiopia (Marias, 1974; Demissew and Nordan, 2010). The roots of *K. foliosa* have been used for the treatment of abdominal cramps and for wound healing (Abate, 1989). The plant is also used to remove endoparasites in cattle (Demissew and Nordan, 2010).

Previous phytochemical investigations of this plant have resulted in the isolation of monomeric anthraquinones (Dagne and Steglich, 1984; Berhanu and Dagne, 1984), phenylanthraquinones (Dagne and Steglich, 1984; Dagne and Yenesew, 1993; Yenesew et al., 1994) and dimeric anthraquinones (Dagne et al., 1987; Wube et al., 2005). Further analysis of the roots of *K. foliosa* has resulted in the isolation of two new anthraquinone derivatives (**1** and **2**, Fig. 1) along with known compounds. The new compounds were tested for antiplasmodial activities because related dimeric anthraquinones, such as 10-hydroxy-10,7′-(chrysophanol anthrone)-chrysophanol (**3**) (Wube et al., 2005) and phenylanthraquinones, such as knipholone anthrone (**4**) were reported to be active (Bringmann et al., 1999, 2008), The isolation, characterization and antiplasmodial activities of these compounds are discussed.

2. Results and discussion

Compound **1** was obtained as yellow amorphous solid. Its molecular formula was established as $C_{24}H_{18}O_7$ from HRMS analysis ($m/z = 418.1055$, calculated for 418.1053). The UV spectrum (λ_{max} 284, 354 and 425 nm) suggested the presence of a phenylanthrone moiety as in knipholone anthrone (Dagne and Yenesew, 1993). In fact the ^{1}H NMR spectrum is similar to that of knipholone anthrone with two highly deshielded singlets at δ_H 12.25, and 11.66, indicating intramolecular hydrogen bonded hydroxyl groups, and were assigned to 1-OH and 8-OH of a chrysophanol anthrone moiety. In agreement with this, the ^{1}H NMR spectrum showed three mutually coupled aromatic protons with an ABX spin system at δ_H 7.46 (*d*, *J* = 7.6 Hz), 7.63 (*t*, *J* = 7.9 Hz) and 7.06 (*d*, *J* = 8.3 Hz) which were assigned to H-5, H-6 and H-7, respectively, of ring C of the chrysophanol anthrone part of the molecule. Ring A only showed a singlet at δ_H 7.00 for H-2 with the biogenetically expected aromatic methyl (δ_H 2.42; δ_C 23.7) being at C-3. As in knipholone anthrone, C-4 (δ_C 120.1) is the point of attachment of the acetylphloroglucinol methyl ether unit, whose presence was evident from NMR spectra (Table 1) which showed a downfield shifted singlet at δ_H 15.02 due to the chelated hydroxyl proton, upfield shifted singlet aromatic proton (δ_H 6.38 for H-5′), an acetyl group (δ_H 2.72, δ_C 33.3, 204.2) at C-3′, and methoxy group (δ_H 3.99, δ_C 56.0) at C-4′.

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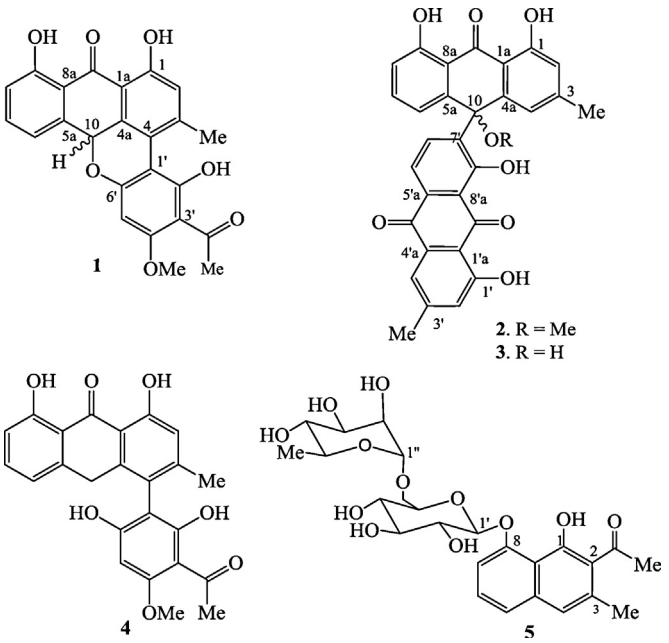

Fig. 1. Structures of compounds from the roots of *Kniphofia foliosa*.

From the ^{13}C NMR spectrum (Table 1) of compound **1**, the presence of only two carbonyl carbon signals (at δ_C 192.2 for C-9 and 204.2 for the acetyl group) was evident which clearly signified the lack of a carbonyl at C-10 as in knipholone anthrone (Dagne and Yenesew, 1993). The difference between compounds **1** and **4** (knipholone anthrone) is that the methylene (CH_2-10) signals in the latter (δ_H 4.06, 2H, *s*; δ_C 32.6) are replaced by an oxymethine signal (δ_H 5.57, 1H, *s*; δ_C 72.2), clearly indicating that compound **1** is knipholone oxanthrone. The HMBC spectrum (Table 1), showed correlation between H-10 and C-6′ which suggested the presence of an unprecedented cyclic ring involving C-10 and C-6′ and allowed the assignment of structure **1** (Fig. 1) for which the trivial name knipholone cyclooxanthrone is suggested. The CD spectrum of compound **1** showed a negative Cotton effect at 273 nm which is in agreement with the compound being chiral having a center of chirality at C-10 with an axis of chirality at C4-C1′. Due to the cyclization involving C-10 and C-6′, there are two possible isomers for this compound *vis-á-vis* (4*M*, 10*S*) or (4*P*, 10*R*); to decide which of these is the absolute configuration for the compound, advanced quantum chemical CD calculations based on time-dependent DFT (TDDFT) and multireference configurational interaction (DFT/MRCI) approaches as described by Bringmann et al. (2007) may be used.

Compound **2** was obtained as yellow amorphous solid. The TOF-HREIMS, did not show the $[M]^+$ peak, instead $[M-OCH_3]^+$ peak (*m*/*z* = 491.0353) was observed. Its UV–vis spectrum showed characteristic absorptions (λ_{max} 265, 295, 393 and 440 nm) of an

Table 1
^1H NMR (600 MHz) spectral data for compounds **1**–**4** and ^{13}C NMR (150 MHz) data together with HMBC correlation of compounds **1** and **2** (in ppm, CD_2Cl_2).

H/C	δ_C	1	HMBC	δ_C	2	HMBC	3	4
		δ_H, *m* (J in Hz)			δ_H, *m* (J in Hz)		δ_H, *m* (J in Hz)	δ_H, *m* (J in Hz)
1	160.8	–		162.9	–		–	–
1a	110.2	–		114.3	–		–	–
2	120.5	7.00, *s*	C-1, C-4, C-1a	119.3	6.77, *d* (1.2)	C-1a, C-4, C-4a	6.70, *d* (1.8)	6.89, *brs*
3	147.4	–		149.0	–		–	–
4	120.1	–		120.9	6.62, *d* (1.2)	C-1, C-1a, C-10	6.53, *d* (1.8)	–
4a	134.8	–		148.1	–		–	–
5	118.7	7.46, *d* (7.6)	C-7, C-10, C-8a	118.1	6.78, *dd* (1.2, 8.4)	C-7, C-8a	6.74, *dd* (1.8, 8.2)	6.82, *brd* (7.4)
5a	138.6	–		143.2	–		–	–
6	136.5	7.63, *t* (7.9)	C-8, C-5a	136.8	7.43, *t* (8.4)	C-5a, C-8	7.34, *t* (8.2)	7.5, *t* (7.4)
7	118.2	7.06, *d* (8.3)	C-5, C-8, C-8a	117.7	6.94, *dd* (1.2, 8.4)	C-5, C-8a, C-9	6.89, *dd* (1.8, 8.2)	6.86, *brd* (7.4)
8	162.8	–		162.6	–		–	–
8a	114.1	–		116.1	–		–	–
9	192.2	–		193.1	–		–	–
10	72.2	5.57, *s*	C-4, C-5a, C-1a, C-6′, C-4a	75.6	–		–	4.06, *s*
1′	106.1	–		158.9	–		–	–
1′a	–	–		115.7	–		–	–
2′	163.0	–		124.6	7.04, *d* (1.2)	C-1′, C-1′a	6.97, *d* (1.6)	–
3′	107.3	–		149.9	–		–	–
4′	163.2	–		121.7	7.61, *d* (1.2)	C-1′a, C-10′, 3′-Me	7.60, *d* (1.6)	–
4′a	–	–		143.6	–		–	–
5′	91.3	6.38, *s*	C-3′, C-4′	119.6	7.95, *d* (8.0)	C-6′, C-7′, C-8′a	8.57, *d* (8.2)	6.31, *s*
5′a	–	–		131.4	–		–	–
6′	162.5	–		133.1	8.66, *d* (8.0)	C-5′a, C-8′	7.94, *d* (8.2)	–
7′	–	–		132.2	–		–	–
8′	–	–		162.4	–		–	–
8′a	–	–		116.2	–		–	–
9′	–	–		191.4	–		–	–
10′	–	–		182.1	–		–	–
COMe	204.2	–		–	–		–	–
3-Me	23.7	2.42, *s*	C-2, C-3	22.3	2.26, *s*	C-2, C-3, C-4	2.19, *s*	2.11, *s*
3′-Me	–	–		22.4	2.35, *s*	C-2′, C-3′, C-4′	2.37, *s*	–
CO*Me*	33.3	2.72, *s*	C-3′, CO,	–	–		–	2.63, *s*
4′-OMe	56.0	3.99, *s*	C-4′	–	–		–	4.00, *s*
10-OMe	–	–		50.7	2.86, *s*	C-10	–	–
1-OH	–	12.25, *s*		–	12.37, *s*		12.35, *s*	12.27, *s*
1′-OH	–	–		–	12.27, *s*		12.26, *s*	–
8-OH	–	11.66, *s*		–	12.07, *s*		12.08, *s*	12.36, *s*
8′-OH	–	–		–	11.72, *s*		11.74, *s*	–
2′-OH	–	15.02, *s*		–	–		–	14.33, *s*

anthrone-anthraquinone dimer (Alemayehu et al., 1993). In agreement with such moiety, the ^{1}H NMR spectrum showed the presence of four highly deshielded singlets resonating at δ_H 12.37, 12.27, 12.07 and 11.73 due to the presence of four chelated hydroxyl groups as in 10-hydroxy-10,7′-(chrysophanol anthrone)-chrysophanol (**3**) (Alemayehu et al., 1993). In addition, the presence of two aromatic methyl protons which resonated at δ_H 2.26 (C-3) and 2.35 (C-3′) supported that the compound is a dimeric anthraquinone/anthrone derivative. One-half of the molecule showed *meta*-coupled protons at δ_H 6.77 (*d*, *J* = 1.2 Hz) and 6.62 (*d*, *J* = 1.2 Hz). These signals were assigned to H-2 and H-4, respectively, with the biogenetically expected methyl (δ_H 2.26; δ_C 22.3) being at C-3. In addition, an ABX spin system was observed for three aromatic protons which resonated at δ_H 6.78 (*dd*, *J* = 8.4, 1.2 Hz), 7.43 (*t*, *J* = 8.4 Hz) and 6.94 (*dd*, *J* = 8.4, 1.2 Hz) and were assigned to at H-5, H-6 and H-7 of the chrysophanol anthrone moiety, leaving C-10 (δ_C 75.6) as the point of attachment to the other half of the molecule.

The ^{1}H NMR spectral pattern of the other half of the molecule showed that it is that of a chrysophanol moiety where the ABX pattern in chrysophanol is replaced by a pair of de-shielded *ortho*-coupled protons with AX pattern at δ_H 7.95 (*d*, *J* = 8.0 Hz) and δ_H 8.66 (*d*, *J* = 8.0 Hz) and these were readily assigned to H-5′ and H-6′ respectively. This indicated that the point of attachment in this half of the molecule is at C-7′ (δ_C 132.2). Therefore, this compound is composed of chrysophanol and chrosophanol oxanthrone units similar to compound **3** (10-hydroxy-10,7′-(chrysophanol anthrone)-chrysophanol), a compound previously isolated from *K. foliosa* (Wube et al., 2005), *Senna longiracemosa* (Alemayehu et al., 1993) and *Asphodelus ramosus* (Lanzetta et al., 1990). The only difference between compounds **2** and **3** is that the 10-hydroxyl group in **3** has been replaced with methoxyl group (δ_H 2.86) in **2**. This was confirmed by ^{1}H–^{13}C HMBC experiment (Table 1) where the methoxyl protons correlated with C-10. The upfield chemical shift (due to anisotropic effect) of the methoxy group (δ_H 2.86) is an indication of its attachment to the sp^3 carbon, C-10 (δ_C 75.6). The compound was therefore characterized as 10-methoxy-10,7′-(chrysophanol anthrone)-chrysophanol (**2**). Although the absolute configuration of this compound has not been determined, it is worth reporting that the CD spectrum showed a positive Cotton effect at 272 nm. This is the first report of the occurrence in nature of compound **2** having previously been reported as a synthetic derivative (Yagi et al., 1978).

Compound **5** displayed identical NMR spectral data (section 3.6.) with those reported for the naphthalene glycoside, dianellin (Cichewicz et al., 2002). The glycoside moiety was placed at C-8 on the basis of a three-bond HMBC correlation of the anomeric proton at δ_H 5.17 (1H, *d*, *J* = 7.8 Hz, H-1′) with C-8 (δ_C 156.4). The HMBC correlations between the CH_2-6′ signals (δ_H 4.12,1H, *dd*, *J* = 2.0, 11.0 Hz, H-6′a; δ_H 3.68, 1H, *dd*, *J* = 7.2, 11.0 Hz, H-6′b) and C-1″ (δ_C 102.5), and also between H-1″ (δ_H 4.80, 1H, *d*, J = 1.4 Hz) and C-6′ (δ_C 68.5) established a 1 → 6 linkage between the D-glucopyranosyl and L-rhamnopyranosyl moieties. The configuration at the anomeric center of the D-glucopyranosyl group (C-1′) was determined to be β from the big coupling constant (*J* = 7.8 Hz) as the result of 1,2-diaxial relationship between H-1′ and H-2′; whereas the configuration at the anomeric center (C-1″) of L-rhamnopyranosyl moiety, having the more stable $^1C_4$ conformation, was deduced to be α from the big $^1J_{C,H}$ (168 Hz) coupling constant between H-1″ and C-1″, with the expected value for the β-anomer being in the range 152–160 Hz (Bock and Pedersen, 1974; El Ashry et al., 2008). Therefore this compound was identified as 2-acetyl-3-methyl-1-hydroxyl-8-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-naphthalene, trivial name dianellin (**5**). This is the first report of the isolation of compound **5** from the family Asphodelaceae; having been reported from *Dianella* (Batterham et al., 1961; Dias et al., 2009) and *Hemerocallis* species (Cichewicz et al., 2002). It is worth to point out that, in the structure given for dianellin by Cichewicz et al. (2002), the rhamnopyranoside was drawn as α-D-rhamnopyranoside in the $^4C_1$ conformation (instead of α-L-rhamnopyranoside in the $^1C_4$ form as in **5**, Fig. 1), whereas acid hydrolysis gave L-rhamnose as one of the products as reported in the same manuscript.

Our investigation of the roots of *K. foliosa* has also resulted in the re-isolation of chrysophanol (Dewick, 2002), 10-hydroxy-10,7′-(chrysophanol anthrone)-chrysophanol (Alemayehu et al., 1993; Lanzetta et al., 1990; Wube et al., 2005), knipholone (Dagne and Steglich, 1984), knipholone anthrone (Dagne and Yenesew, 1993) and isoknipholone (Yenesew et al., 1994).

Compounds **1**, **2** and **5** were evaluated for their *in vitro* antiplasmodial activities against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. All the three compounds showed antiplasmodial activities (Table 2), with compound **2** exhibiting the highest activity against chloroquine-resistant (W2) strain of *P. falciparum* with an IC_{50} value of 1.17 ± 0.12 μg/ml. The results support the previous assertion that anthraquinone–anthrone dimers and phenylanthraquinones are emerging classes of antiplasmodial compounds (Wube et al., 2005; Bringmann et al., 1999).

3. Experimental

3.1. General

Column chromatography was performed on oxalic acid impregnated silica gel (70–230 mesh). UV spectra were recorded on a Specord S600, Analytik Jena AG, Germany. High resolution mass spectral analysis (HREI-MS) was done on a Micromass GC–TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). CD spectra were measured using JASCO J-710 Spectropolarimeter. Analytical TLC was performed on Merck pre-coated silica gel 60 F_{254} plates. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker Avance III 600 spectrometer using the residual solvent peaks as a reference. HSQC and HMBC spectra were obtained using the standard Bruker software. Optical rotations were measured with a Perkin Elmer polarimeter 341 (Shelton, CT).

Table 2
In vitro antiplasmodial activities (against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *P. falciparum*) of compounds from *K. foliosa*.

Test sample	IC_{50}/μg/ml^{-1}	
	Chloroquine-resistant (W2)	Chloroquine-sensitive (D6)
Crude extract	11.28 ± 0.01	8.92 ± 1.50
knipholone cyclooxanthrone (**1**)	6.13 ± 1.59	3.96 ± 0.70
10-methoxy-10,7′-(chrysophanol anthrone)-chrysophanol (**2**)	1.17 ± 0.12	4.07 ± 1.54
Dianellin (**5**)	3.28 ± 0.19	5.47 ± 1.20
Chloroquine	0.22 ± 0.03	0.01 ± 0.001
Mefloquine	0.03 ± 0.02	0.003 ± 0.001

3.2. Plant material

The roots of *K. foliosa* were collected from Gedo, Ethiopia 193 Km from Addis Ababa on the way to Fincha sugar factory in September, 2011. The plant material was identified by Prof. Sebsebe Demissew, Department of Biology, Addis Ababa University, Ethiopia where a voucher specimen (voucher number NA-03) has been deposited.

3.3. Extraction and isolation

The air dried roots (1.1 kg) of *K. foliosa* were extracted initially with ethyl acetate by cold exhaustive percolation and then with methanol (3 × 3 l) for 24 h. The methanol extract was partitioned between ethyl acetate and water. Based on TLC profile, the organic (ethyl acetate) layer was combined with the initial ethyl acetate extract and the combined extract was concentrated on a rotary evaporator to give 60 g of a residue. The crude extract (58 g) was adsorbed on silica gel and subjected to column chromatography on oxalic acid impregnated silica gel (450 g), which was eluted with *n*-hexane containing increasing amounts of ethyl acetate. Fractions eluted with 5% ethyl acetate in *n*-hexane gave chrysophanol (23 mg). Fractions eluted with 10–15% of ethyl acetate in *n*-hexane was applied on Sephadex LH-20 (eluent: dichloromethane/methanol, 1:1) followed by purification using column chromatography (eluent: increasing gradient of ethyl acetate in *n*-hexane) and gave **2** (4.2 mg), **3** (21.0 mg) and **1** (3.6 mg). Fractions eluted with 20–25% ethyl acetate in *n*-hexane gave isoknipholone (6.5 mg), knipholone (24.2 mg) and knipholone anthrone (14.3 mg) after purification by preparative thin layer chromatography (silica gel, 30% ethyl acetate in *n*-hexane). The fractions eluted with 30–40% ethyl acetate in *n*-hexane were further purified by column chromatography on Sephadex LH-20 (eluent: dichloromethane/methanol, 1:1) to give **5** (56.0 mg).

3.4. Knipholone cyclooxanthrone (1)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 284, 354, 425. CD ($CHCl_3$): $\Delta\varepsilon_{273} = -3.8$ cm^2 mol^{-1}. ^{1}H and ^{13}C NMR (Table 1). EIMS *m*/*z* (70 eV, rel. int.): 418 (29, $[M]^+$), 403 (20), 139 (33), 125 (50), 111 (73), 97 (100), 83 (80), 71 (81), 57 (97), 43 (94). TOF HRMS *m*/*z* = 418.1055, $[M]^+$ $C_{24}H_{18}O_7$ (calculated for 418.1053).

3.5. 10-Methoxy-10,7′-(chrysophanol anthrone)-chrysophanol (2)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 284, 354, 425. CD ($CHCl_3$): $\Delta\varepsilon_{272} = +4.1$ cm^2 mol^{-1}. ^{1}H and ^{13}C NMR (Table 1). EIMS *m*/*z* (70 eV, rel. int.): 491 (43, $[M-OMe]^+$), 490 (29), 392 (42), 391 (53), 353 (43), 294 (59), 265 (72), 196 (100). TOF HRMS *m*/*z* = 491.0353, $[M-OMe]^+$ $C_{31}H_{22}O_8$.

3.6. Dianellin (5)

Viscous yellow oil. $[\alpha]_D^{25}$ −104.5° (*c* 0.25, MeOH). UV λ_{max} ($CHCl_3$) nm: 266, 340. ^{1}H NMR (acetone-d_6) δ_H 9.63 (1H, 1-OH), 7.45 (1H, *dd*, *J* = 1.2, 8.0 Hz, H-5), 7.42 (1H, *dd*, *J* = 8.0, 8.2 Hz, H-6), 7.42 (1H, *dd*, *J* = 1.2, 8.2 Hz, H-7), 7.18 (1H, *s*, H-4), 5.17 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.80 (1H, *d*, *J* = 1.4 Hz, H-1″), 4.12 (1H, *dd*, *J* = 2.0, 11.1 Hz, H-6′a), 3.91 (1H, *dd*, 3.4, 1.5 Hz, H-2″), 3.78 (1H, *ddd, 9.6, 7.3, 2.1 Hz*, H-5′), 3.74 (1H, *dd*, *J* = 3.4, 9.3 Hz, H-3″), 3.68 (3H, *m*, H-6′b, H-5″and H-2′), 3.63 (1H, *t*, *J* = 8.9 Hz, H-3′), 3.49 (1H, *dd*, *J* = 8.8, 9.6 Hz, H-4′), 3.43 (1H, *t*, *J* = 9.4 Hz, H-4″), 2.55 (3H, *s*, H-12), 2.30 (3H, *s*, H-13), 1.21 (3H, *d*, *J* = 6.2 Hz, H-6″). ^{13}C NMR (acetone-d_6) δ_C 204.8 (C-11), 156.4 (C-8), 152.8 (C-1), 138.1 (C-10), 135.0 (C-3), 128.9 (C-6), 127.0 (C-2), 124.2 (C-5), 121.2 (C-4), 115.3 (C-9), 112.5 (C-7), 104.7 (C-1′), 102.5 (C-1″), 78.7 (C-3′), 77.9 (C-5′), 75.4 (C-2′), 74.4 (C-4″), 73.2 (C-3″), 72.6 (C-2″), 72.1 (C-4′), 70.0 (C-5″), 68.5 (C-6′), 32.9 (C-12), 20.5 (C-13), 18.8 (C-6″). EIMS *m*/*z* (70 eV, rel. int.): 525 (5, $[M+1]^+$), 216 (35), 201 (34), 198 (12), 91 (15), 73 (16), 64 (40), 46 (100). TOF HRMS *m*/*z* = 525.1970, $[M+1]^+$ $C_{25}H_{33}O_{12}$ (calculated for 525.1972).

3.7. Antiplasmodial assays

Two strains of *P. falciparum*, the Indochina W2 (chloroquine-resistant) and the Sierra Leone D6 (chloroquine-sensitive), 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 (Juma et al., 2011). The reference antimalarial drugs chloroquine and mefloquine were tested along with test compounds isolated from the roots of *K. foliosa*.

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References

Abate, G., 1989. In: Demissew, S. (Ed.), Etse Debdabe: Ethiopian Traditional Medicine. Addis Ababa University Press, Addis Ababa, pp. 99–183.
Alemayehu, G., Abegaz, B., Snatzke, G., Duddeck, H., 1993. Bianthrones from *Senna longiracemosa*. Phytochemistry 32, 1273–1277.
Balint, G.A., 2001. Artemisinin and its derivatives: an important new class of antimalarial agents. Clin. Pharmacol. Ther. 90 , 261-165.
Batterham, T., Cooke, R.G., Duewell, H., Sparrow, L.G., 1961. Colouring matters of Australian plants VII. Naphthalene derivatives from *Dianella* species. Aust. J. Chem. 14 (14), 637–642.
Berhanu, E., Dagne, E., 1984. Aloe emodin acetate, an anthraquinone derivative from the leaves of *Kniphofia foliosa*. Planta Med. 50, 523–524.
Bock, K., Pedersen, C., 1974. A study of 13CH coupling constants in Hexopyranoses. J. Chem. Soc. Perkin Trans. II 293–297.
Bringmann, G., Menche, D., Bezabih, M., Abegaz, B.M., Kaminsky, R., 1999. Antiplasmodial activity of knipholone and related natural phenylanthraquinones. Planta Med. 65, 757–758.
Bringmann, G., Maksimenka, K., Mutanyatta-Comar, J., Knauer, M., Bruhn, T., 2007. The absolute axial configurations of knipholone and knipholone anthrone by TDDFT and DFT/MRCI CD calculations. Tetrahedron 63, 9810–9824.
Bringmann, G., Mutanyatta-Comar, J., Knauer, M., Abegaz, B.M., 2008. Knipholone and related 4-phenylanthraquinones: structurally, pharmacologically, and biosynthetically remarkable natural products. Nat. Prod. Rep. 25, 696–718.
Cichewicz, R.H., Lim, K., Kerrow, J.H., Nair, M.G., 2002. Kwanzoquinones A– G and other constituents of Hemerocallisfulva 'Kwanzo' roots and their activity against the human pathogenic trematode Schistosoma mansoni. Tetrahedron 58, 8597–8606.
Dagne, E., Berhanu, E., Steglich, W., 1987. New bianthraquinone pigments from *Kniphofia* species. Bull. Chem. Soc. Ethiopia 1, 32–35.
Dagne, E., Steglich, W., 1984. Knipholone: a unique anthraquinone derivative from *Kniphofia foliosa*. Phytochemistry 23, 1729–1731.
Dagne, E., Yenesew, A., 1993. Knipholone anthrone from *Kniphofia foliosa*. Phytochemistry 34, 1440–1441.
Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The Families of the Monocotyledons. Springer-Verlag, Berlin.
Demissew, S., Nordan, I., 2010. Aloes and Lilies of Ethiopia and Eritrea. Addis Ababa University, Addis Ababa 113-125.
Dewick, P.M., 2002. Medicinal Natural Products: A Biosynthetic Approach. John wiley & Sons Ltd., England 35.
Dias, D.A., Silva, C.A., Urban, S., 2009. Naphthalene aglycones and glycosides from the Australian medicinal plant, *Dianella callicarpa*. Planta Med. 75, 142–145.
El Ashry, E.S.H., Rashed, N., Ibrahim, E.S.I., 2008. Challenges in the stereocontrolled synthesis of β-rhamnosides. Tetrahedron 64, 10631–10648.
Ginsburg, H., Deharo, E., 2011. A call for using natural compounds in the development of new antimalarial treatments. Ginsburg and Deharo Malaria J. 1–7.

Juma, W.P., Akala, H.M., Eyase, F.L., Muiva, L.M., Heydenreich, M., Okalebo, F.A., Gitu, P.M., Peter, M.G., Walsh, D.S., Imbuga, M., Yenesew, A., 2011. Terpurinflavone: An antiplasmodial flavone from the stem of *Tephrosia Purpurea*. Phytochem. Lett. 4, 176–178.
Lanzetta, R., Parrilli, M., Adinolfi, M., Aquila, T., Corsaro, M., 1990. Bianthrone C-glycosides: three new compounds from Asphodelus ramosus tubers. Tetrahedron 46, 1287–1294.
Marias, W., 1974. A revision of the tropical species of *Kniphofia* (Liliaceae). Kew Bull. 28, 465.
Whitehouse, C., 2002. Asphodelaceae. In: Beentje, H.J. (Ed.), Flora of Tropical East Africa. Balkema Publishers, Roterdam.
WHO. World Malaria Report. 2011. Geneva. 73.
Wube, A.A., Bucar, F., Asres, K., Gibbons, S., Rattray, L., Croft, S.L., 2005. Antimalarial compounds from *Kniphofia foliosa* roots. Phytother. Res. 19, 472–476.
Yagi, A., Makino, K., Nishioki, I., 1978. Studies on the constituents of *Aloe saponaria* HAW. I. The structure of bianthraquinoid pigments. Chem. Pharm. Bull. 26, 1111–1116.
Yenesew, A., Dagne, E., Muller, M., Steglich, W., 1994. An anthrone, an anthraquinone and two oxanthrones from *Kniphofia foliosa*. Phytochemistry 37, 525–528.

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