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Two lignans derivatives and two fusicoccane diterpenoids from the whole plant of *Hypoestes verticillaris (L.F.) Sol. Ex roem. & schult*

Ruth Anyango Omole[a,b,*], Mainen Julius Moshi[a], Matthias Heydenreich[c], Hamisi Masanja Malebo[d], Jeremiah Waweru Gathirwa[e], Sharon Alice Ochieng'[f], Leonida Kerubo Omosa[f], Jacob Ogweno Midiwo[f]

[a] Institute of Traditional Medicine, Muhimbili University of Health & Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania
[b] Department of Chemical Science and Technology, Technical University of Kenya, P.O. Box 52428- 00200, Nairobi, Kenya
[c] Institut für Chemie, Universität Potsdam, OT Golm, Haus 25, D/0.19 (Labor E/0.06-0.08), Karl-Liebknecht-Str. 24-25, D-14476, Potsdam, Germany
[d] Department of Traditional Medicine Research, National Institute for Medical Research, P.O. Box 9653, Dar es Salaam, Tanzania
[e] Centre for Traditional Medicine & Drug Research, Kenya Medical Research Institute (KEMRI) P.O Box, 54840-00200, Nairobi, Kenya
[f] Department of Chemistry, University of Nairobi, P.O. Box 30197, 00100, Chiromo Road, Nairobi, Kenya

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ABSTRACT

Bioassay-guided screening of *Hypoestes verticillaris* whole plant CH_2Cl_2:MeOH (1:1) extract for anti-plasmodial activity yielded four new compounds: two lignans 2, 6-dimethoxysavinin (**1**), 2,6-dimethoxy-(7*E*)-7,8-dehydroheliobuphthalmin (**2**); and two fusicoccane diterpenoids: 11(12)-epoxyhypoestenone (**3**) and 3(11)-epoxyhypoestenone (**4**). The chemical structures were determined using various spectroscopic techniques: UV–vis, IR, CD, 1D, 2D and MS. Two fractions (RAO-43B and RAO-43D) and the isolated compounds were tested for activity against CQ susceptible (D6) and resistant (W2) *Plasmodium falciparum* parasite strains, *in vitro* and the IC_{50} values determined. While the whole extract and some resultant fractions displayed moderate activity, the isolated compounds exhibited mild anti-plasmodial activity against the both strains ranging from IC_{50} value of 328 μM in **1** to 93 μM in **3** against W2 strain.

1. Introduction

Malaria is a major global public health problem with 216 million cases and 445,000 deaths reported in 91 countries in 2016. This showed that there was an increase of malaria cases by 5 million as compared to 2015. Most malaria cases (90%) and deaths (91%) are recorded in Africa and in pregnant women and children under 5 (WHO, 2018a). Anti-malarial drug resistance has emerged as the major challenge facing malaria control in the endemic zones leading to the spread of malaria to new zones and reemergence of the disease in areas where it has been previously controlled (Antony and Parija, 2016; Noedl et al., 2009). Artemisinin resistance has been recently reported in Equatorial Guinea (Lu et al., 2017) while resistance to artemisinin combination therapy (ACTs) has been detected in Southeast Asia (Hanboonkunupakarn and White, 2016; WHO, 2018b), thus heightening the need for newer antimalarial drugs with different modes of action. Medicinal plants have been used traditionally to treat malaria among other ailments for several centuries and have provided several drugs in use and/or drug templates (Mojab, 2012). Several anti-malarial drugs in clinical use that can be traced back to plants justify the potential of folk medicine as sources of and/or leads to novel anti-malarial drugs (Mojab, 2012). Bioassay-guided screening of medicinal plants and isolation of the bioactive constituents may lead to the discovery of new anti-malarial drugs with new modes of action.

Hypoestes verticillaris (Acanthaceaea) has been used traditionally for the treatment of tuberculosis, pneumonia, wound healing, chest pain, malaria and dry coughs (Amuka et al., 2014; Kipkore et al., 2014; Okello et al., 2008). In Madagascar and Saudi Arabia, it is used for treatment of gonorrhea, heart diseases and to manage hypertension (Al-Rehaily et al., 2002; Wu et al., 2016). The genus *Hypoestes* has been found to possess cytotoxic, anti-leishmanial, antimicrobial, anti-malarial, antioxidant, antifungal and anti-trypanosomal bioactivities (Al Haidari, 2018; Almehdar et al., 2012; Mothana et al., 2012). In our previous investigations, we found that solvent extracts of *Hypoestes verticillaris* exhibited moderate anti-plasmodial activity (IC_{50} 5.46–7.04 μg/mL) against the CQ susceptible and resistant strains. We report the isolation, identification and bioassay of two new lignan derivatives: 2,6-dimethoxysavinin (**1**) and 2,6-dimethoxy-(7*E*)-7,8-

* Corresponding author at: P.O. Box 52428, 00200, Nairobi, Kenya.
E-mail address: oruth2002@yahoo.com (R.A. Omole).

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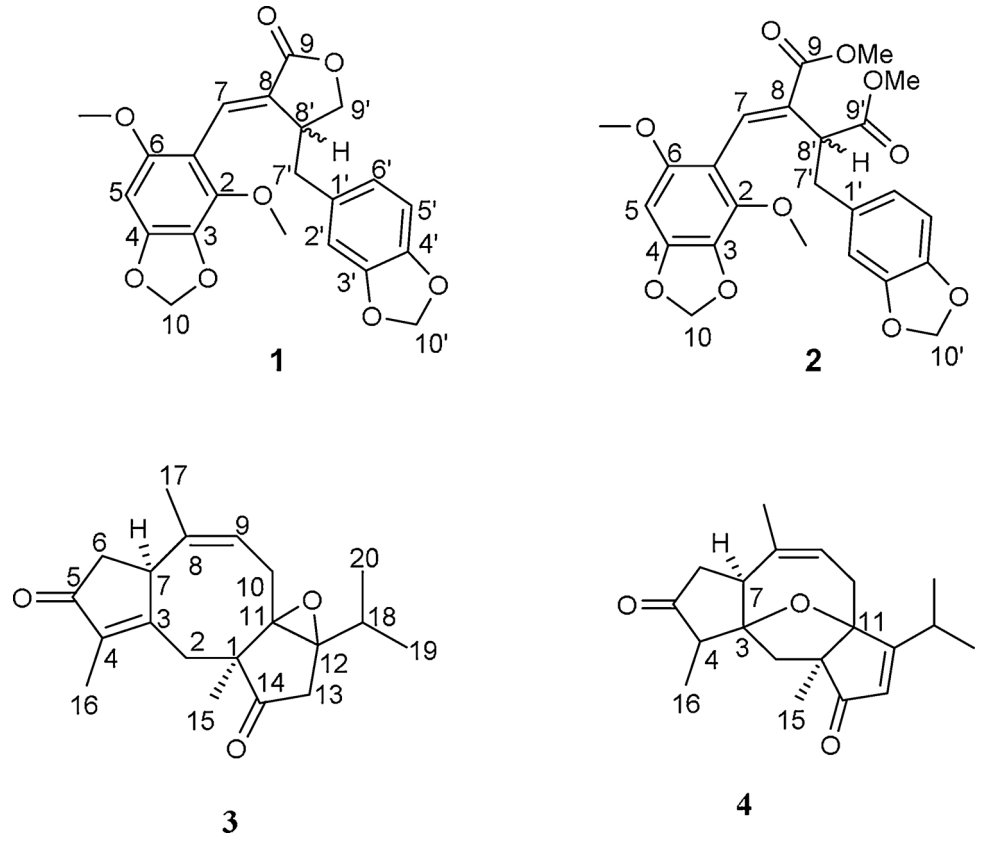

Fig. 1. Chemical structures of compound **1**, **2**, **3** and **4**.

dehydroheliobuphthalmin (**2**); and two new fusicoccane diterpenoids: 11(12)-epoxyhypoestenone (**3**) and 3(11)-epoxyhypoestenone (**4**) from *Hypoestes verticillaris* the whole plant.

2. Results and discussion

2.1. Structure elucidation of new compounds

Compound **1** (Fig. 1) was obtained as a light yellow solid. HR-ESIMS data revealed a quasi-molecular ion peak at *m/z* 413.1190 $[M+H]^+$ (calcd 413.1192) suggesting the molecular formula $C_{22}H_{20}O_8$ and thirteen degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) showed signals for four aromatic protons. The three signals at δ_H 6.58 (d, J = 7.9 Hz, H-5′), δ_H 6.44 (*dd*, J = 7.9, 1.7 Hz, H-6′), δ_H 6.47 (d, J = 1.7 Hz, H-2′) were consistent with a disubstituted benzene ring and a singlet at δ_H 6.23 (H-5). An olefinic proton observed at δ_H 7.58 (d, J = 2.8 Hz, H-7) was assigned to δ_C 130.7 (C-7). The COSY correlation showed a cross peak between H-5′ and H-6′. The other diagnostic ^{1}H-^{1}H COSY correlations for compound **1** are shown in Fig. 2.

The ^{13}C NMR (Table 1) and HSQC revealed the presence of 22 carbons consisting of 2 methoxy, 4 methylene, 6 methine and 10 quartenary carbon atoms. This included a lactone group at δ_C 172.6 (C-9), δ_C 70.3 (C-9′) and two methoxyl groups at δ_C 59.7 and δ_C 56.3, respectively (2 − OCH_3 and 6 − OCH_3). The HMBC data of compound **1** (Table 1) revealed that δ_H 7.58 (H-7) exhibited $^3J_{H,C}$ correlation to the lactone carbonyl δ_C 172.6 (C-9), δ_C 41.1(C-8′), δ_C 153.7 (C-6) and δ_C 141.6 (C-2). Furthermore, the signal at δ_H 7.58 showed $^2J_{H,C}$ correlation to δ_C 128.8 (C-8). The diastereotopic methylene protons with signals at δ_H 2.68 and 2.34 indicated a $^3J_{H,C}$ correlation to δ_C 70.3 (C-9′), δ_C 128.8 (C-8), δ_C 109.0 (C-2′) and δ_C 121.9 (C-6′). Likewise, the signals at δ_H 2.68 and 2.34 revealed $^2J_{H,C}$ correlation to δ_C 41.1 (C-8) and δ_C 132.3 (C-1′). The observed HMBC correlations confirmed the position of the lactone carbon in the lignan skeleton. The diagnostic HMBC correlations for compound **1** are shown in Fig. 2. The stereochemistry at C-7 and C-8 was found to be *Z* (*Cis*) using NOESY experiments (Fig. 2). A NOESY cross peak was observed between H-7 (δ_H 7.58) and 6 − OCH_3 (δ_H 3.78) indicating they are on the same side. In addition, a cross peak was also observed between H-7 (δ_H 7.58) and H-2′(δ_H 6.47) showing that the two protons are close to each other. The NMR data for this compound is comparable to savinin (hibalactone) apart from the methoxyl groups at C-2 and C-6 (Feliciano et al., 1987; Silva et al., 2005). Compound **1** displayed two negative cotton effects at λ_{max} 303 and 340 nm in the CD spectrum which are (Fig. 3) similar to that of (3R)-hibalactone (Fig. 3) (Burden et al., 1969), suggesting that the configuration of **1** was assigned. Based on spectroscopic data and comparison with information available from the chemical literature compound **1** was identified as benzylidenebenzyl butyrolactone lignan and named 2,6-dimethoxysavinin. However, the stereochemistry at C-8′ remains unresolved.

Compound **2** (Fig. 1) was isolated as white crystals. HR-ESIMS data revealed a quasi molecular ion peak at *m/z* 473.1428 $[M+H]^+$ (calcd 473.1403) suggesting the molecular formula $C_{24}H_{24}O_{10}$ and thirteen degrees of unsaturation. The major differences between compounds **1** and **2** were noted mainly in the ^{1}H NMR, HSQC and HMBC spectra. Like compound **1**, the ^{1}H NMR data of compound **2** (Table 1) revealed the presence of four aromatic protons at δ_H 6.21 (s, H-5), δ_H 6.34 (d, J = 1.8 Hz, H-2′), δ_H 6.56 (d, J = 7.9 Hz, H-5′) and δ_H 6.38 (*dd*, J = 7.9, 1.8 Hz, H-6′). Unlike compound **1**, signals for four methoxyl groups were observed for compound **2** at δ_H 3.87 (s, 2 − OCH_3), δ_H 3.63 (s, 6 − OCH_3), δ_H 3.80 (s, 9 − OCH_3) and δ_H 3.72 (s, 9′ − OCH_3).

The ^{13}C NMR (Table 1) and HSQC for compound **2** revealed the presence of 24 carbon atoms consisting of 11 quarternary, 6 methine, 3 methylene and 4 methoxyl groups unlike the 22 in compound **1**. Two of these were associated with two carbonyls at δ_C 173.4 (C-9′) and 167.1 (C-9). Unlike compound **1**, the HSQC of compound **2** revealed the presence of the signals for the four methoxyl groups in compound **2** at

Table 1
^{1}H and ^{13}C NMR data compound **1** and **2** (δ in ppm and J in Hz).

No.	**1**			**2**		
	δ_C[a]	δ_H[b], *m*, *J*	HMBC (H to C)	δ_C[c]	δ_H[d], *m*, *J*	HMBC (H to C)
1	108.3			108.2		
2	141.6			140.8		
3	130.2			130.0		
4	150.9			150.0		
5	88.4	6.23 *s*	1, 2, 3, 4, 6	88.0	6.21 *s*	1, 4, 3, 6
6	153.7			152.7		
7	130.7	7.58 *d* (2.8)	8′, 8, 2, 6, 9	135.4	7.36 s	8, 8′, 2, 6, 9
8	128.8			131.0		
9	172.6			167.1		
10	100.3	5.88 d (1.5); 5.91 d (1.5)	3, 4	101.1	5.93 (d 1.4); 5.91 d (1.4)	3, 4
1′	132.3			133.4		
2′	109.0	6.47 d (1.7)	7′, 3′, 4′, 6′	108.9	6.34 d (1.8)	7′, 6′, 4′
3′	147.5			147.0		
4′	146.0			145.5		
5′	107.9	6.58 d (7.9)	7′, 3′, 4′, 6′	107.3	6.56 d (7.9)	1′, 3′, 4′
6′	121.9	6.44 *dd* (7.9, 1.7)	7′, 2′, 4′	121.9	6.38 *dd* (7.9, 1.8)	2′, 4′, 7′
7′	37.9	2.68 *dd* (13.8, 5.9) 2.34 *dd* (13.8, 9.0)	9′, 8′, 8, 1′, 2′, 6′	36.1	3.30 *dd* (5.3, 13.9) 2.89 *dd* (9.9, 13.9)	2′, 6′, 8, 9′, 8′
8′	41.1	3.66 *m*	9, 7′, 8	47.4	3.72 *dd* (5.3, 9.9)	7, 8, 9, 1′, 7′, 9′
9′	70.3	4.28 *ddd* (9.0, 8.2, 0.5) 4.00 *dd* (9.0, 4.5)	8, 9, 7′	173.4		
10′	100.9	5.86 d (1.5); 5.85 d (1.5)	3′,4′	100.8	5.89 d (1.4); 5.88, d (1.4)	3′, 4′
2- OCH_3	59.7	4.01 s	2	59.4	3.87 s	2
6- OCH_3	56.3	3.78 s	6	55.8	3.63 s	6
9-OCH_3				51.7	3.80 s	9
9′-OCH_3				51.7	3.72 s	9′

Key:
[a] recorded in 150 MHz.
[b] recorded in 600 MHz; NMR solvent is $CDCl_3$.
[c] recorded in 125 MHz.
[d] recorded in 500 MHz; NMR solvent is CD_2Cl_2.

δ_C 59.4 (2 − OCH_3), δ_C 55.8 (6 − OCH_3), δ_C 51.7 (9 − OCH_3) and δ_C 51.7 (9′ − OCH_3) while in compound **1** there were only two such groups. The signal at δ_H 3.72 showed $^{3}J_{H,C}$ HMBC correlation to the ester carbonyl at δ_C 173.4 (C-9′) while the one at δ_H 3.8 showed $^{3}J_{H,C}$ correlation to the other ester carbonyl at δ_C 167.1 (C-9), confirming the presence of two ester groups in compound **2**. In compound **1**, no methoxyl protons exhibited $^{3}J_{H,C}$ correlation to the carbonyl at δ_C 172.6 ruling out the presence of an ester group while confirming the presence of lactone group. H-8′ showed $^{3}J_{H,C}$ correlation to C-9 and $^{2}J_{H,C}$ correlation to C-9′ further confirming the position of carbonyl esters in compound **2** while in compound **1**, H-8′ exhibited $^{3}J_{H,C}$ to C-9 only confirming the presence one carbonyl group. The structure of compound **2** was further confirmed by 2D experiments (^{1}H-^{1}H COSY and HMBC) as indicated in Fig. 2. A related compound without the two methoxyl groups at C-2 and C-6, (7*E*)-7,8-dehydroheliobuphthalmin, has been previously isolated from the stem bark of *Pycnanthus angolensis,* the roots of *Heliopsis helianthoides* var. *scabrathe* and the roots of *Rhinacanthus nasutus* (Abrantes et al., 2008; Hajdu et al., 2014; Jakupovic et al., 1986; Ngoc et al., 2018). Based on structural similarity to (7*E*)-7,8-dehydroheliobuphthalmin, compound **2** was named as 2,6-dimethoxy-(7*E*)-7,8-dehydroheliobuphthalmin. However, the absolute configuration at C-8′ remains unresolved.

Compound **3** (Fig. 1) was isolated as white crystaline substance. HR-ESIMS revaled a quasi-molecular ion peak at *m/z* 315.1943 $[M+H]^+$ (calcd 315.1915), suggesting the molecular formula $C_{20}H_{26}O_3$ and eight degrees of unsaturation. The ^{13}C NMR and HSQC data for compound **3** (Table 2) indicated the presence of 20 carbon atoms consisting of 5 methyl, 4 methylene, 3 methine and 8 quaternary carbon atoms. A tetra-substituted double bond (δ 171.1, C-3; 139.6, C-4) conjugated with a ketone group (δ_C 209, C-5) and a tri-substituted double bond (δ_C 137.8, C-8; 123.3, C-9) (Muhammad et al., 1998) was observed in the ^{1}H NMR (Table 2) revealed the presence of an olefinic proton signal at δ_H 5.63 (t, J = 7.7, Hz, H-9) while the isopropyl moiety was suggested by the peak at δ_H 1.08 (d, J = 7.0 Hz, H-19), 1.13 (d, J = 7.0 Hz, H-20) and 1.98 (*m*, H-18) and confirmed by ^{1}H-^{1}H COSY correlations between H-18, H-19 and H-20. The epoxide at position C-11 and C-12 was confirmed by long range diagnostic (^{3}J) correlations between H-2, H-13 and H-15 to C-11 and H-10, H-19, H-20 to C-12. The complete structural and stereochemical assignments were done through 2D NMR spectroscopy (COSY, NOESY, HSQC and HMBC) as shown in Table 2 and the diagnostic interactions shown in Fig. 2. The NOESY cross peak that was observed between H-7 (δ_H 3.96) and H-15 (δ_H 1.02), suggested that they are on the same side of the tricyclic rings and have close spatial proximity. Furthermore, both H-7 and H-15 showed NOESY cross peaks with the signal at H-2b (δ_H 2.25) confirming that it also has close spatial to both protons. The spectral data of compound **3** showed close similarity to a fusicoccane diterpene, hypoestenone, which had previously been isolated from *Hypoestes forskaleii* (Al-Rehaily et al., 2002; Al Musayeib et al., 2014; Muhammad et al., 1998) with the only difference being the epoxidation of the C-11, C-12 bond. Consequently, compound **3** was named 11(12)-epoxyhypoestenone.

Compound **4** (Fig. 1) was isolated as a transparent solid. HR-ESIMS revealed a quasi-molecular ion peak at *m/z* 315. 1923 $[M+H]^+$ (calcd 315.1915) suggesting the molecular formula $C_{20}H_{26}O_3$ and eight degrees of unsaturation. The ^{1}H NMR of compound **4** (Table 2) indicated the presence of five signals due to methyl groups at δ_H 0.85 (d, J = 6.9 Hz, H-16), 1.15 (d, J = 6.8 Hz, H-19), δ_H 1.18 (d, J = 6.8 Hz, H-20), δ_H 1.85 (t, J = 1.4 Hz, H-17) and δ_H 1.28 (s, H-15). The two observed at δ_H 5.52 (*ddd*, J = 6.5, 2.7, 1.4 Hz, H-9) and δ_H 5.80 (d, J = 1.1 Hz, H-13). The major differences between this compound and compound **3** were observed on the methyl group attached to C-4 which appeared as a doublet in compound **4** and the additional olefinic proton which was observed at C-13. These observations suggested the reduction of the C-3, C-4 bond and oxidation of C-12, C-13 bond. The ^{13}C

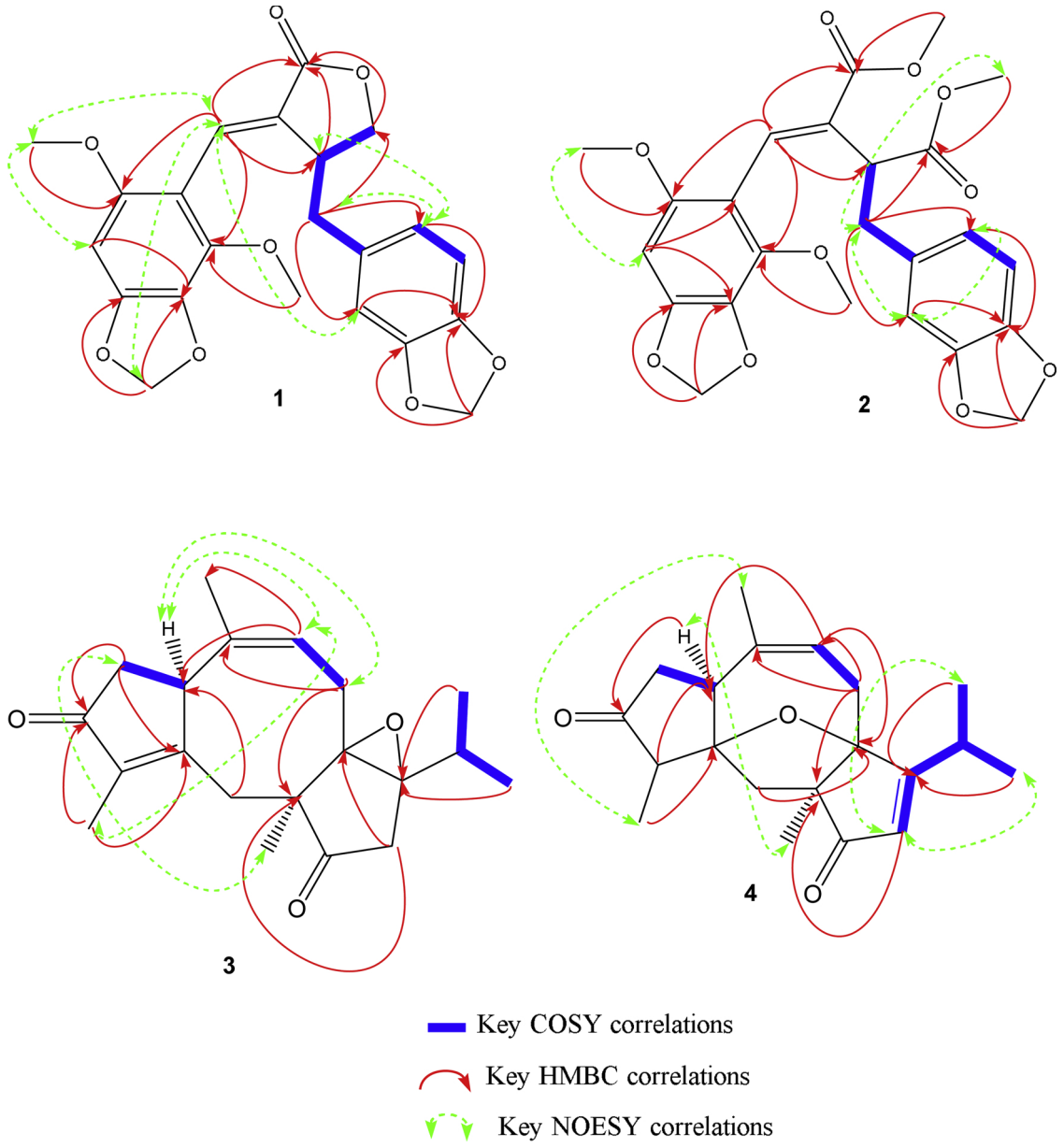

Fig. 2. Key ^{1}H-^{1}H COSY, NOESY and ^{3}J HMBC correlations of compound **1**, **2**, **3** and **4.**

NMR (Table 2) revealed the presence of twenty carbon atoms consisting of 5 methyl, 3 methylene, 5 methine and 7 quaternary groups. The ketonic signals at δ_C 216.1 was assigned to C-5 and the one at δ_C 212.5 to C-14. The HSQC showed that olefinic proton at δ_H 5.52 had a cross peak with δ_C 120.7 while the one at δ_H 5.80 to δ_C 124.5. The position of the epoxide at C-3 and C-11 was determined using HMBC experiments. The signal at δ_C 91.5 (C-3) showed a cross peak with H-6 and H-16 while that at δ_C 94.7 (C-11) showed cross peaks with H-2, H-9, H-13, H-15 and H-18. The stereochemistry at C-1, C-4 and C-7 was determined by NOESY experiments where a cross peak was observed between H-4 and H-15 indicating that these protons are on the same side of the ring with close spatial proximity and therefore the methyl group at C-15 and C-16 are in *trans* orientation with respect to each other. A cross peak was also observed between H-7 and H-15 showing that they are on the same side. The stereochemistry and complete spectral data assignments for compound **4** were done using 2D NMR (COSY, NOESY, HSQC and HMBC) (Table 2) through the diagnostic interactions shown in Fig. 2. Comparison of the spectral data of compound **4** to the available chemical literature information revealed close similarity to a fusicoccane diterpene, hypoestenone, which had previously been isolated from *Hypoestes forskaleii* (Al-Rehaily et al., 2002; Al Musayeib et al., 2014; Muhammad et al., 1998). The only difference between hypoestenone and this compound was the presence of the epoxide ring at C-3 and C-11. Consequently, compound **4** was named 3(11)-epoxyhypoestenone.

2.2. Anti-plasmodial activity of whole extract, fractions and isolated compounds

The *in vitro* anti-malarial activity of fractions and compounds from *Hypoestes verticillaris* were evaluated against D6 and W2 *Plasmodium falciparum* strains (Table 3). Two fractions, RAO-43B and RAO-43D exhibited good antiplasmodial activities against the two strains of *P. falciparum* with IC_{50} values of 7.309 ± 1.2 µg/ml (W2), 10.15 ± 3.1 µg/ml (D6) and 13.44 ± 0.6 µg/ml (W2) and 8.6 ± 2.5 µg/ml (D6), respectively. However, the bioactivity reduced with fractionation and the isolated compounds showed lower anti-plasmodial activity against both strains of *P. falciparum*. 2,6-Dimethoxysavinin (**1**) exhibited the lowest IC_{50} at 327.8 µM while 11(12)-

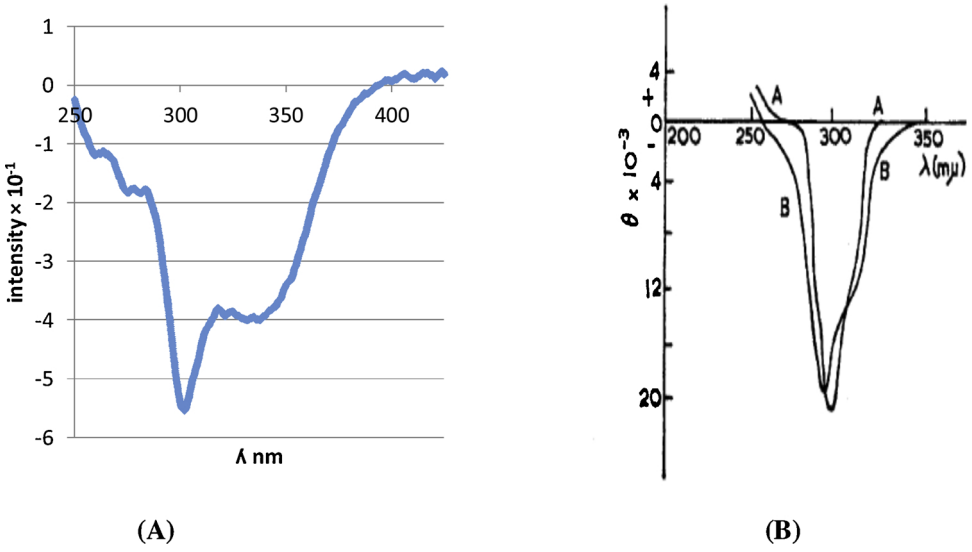

Fig. 3. (A), C.D data for compound **1**; **(B)**, savinin.

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **3** and **4** in $CDCl_3$ (δ in ppm and J in Hz).

No	**3**			**4**		
	δ_C	δ_H, *m*, *J*	HMBC (H to C)	δ_C	δ_H, *m*, *J*	HMBC (H to C)
1	53.8			58.1		
2	28.7	3.15 d (15.6) 2.25 d (15.6)	1, 3, 4, 7, 11, 14	40.9	2.50 *d* (13.6) 1.78 *d* (13.6)	1, 3, 7, 11, 15
3	171.1			91.5		
4	139.6			53.0	2.23 *m*	7, 3,16
5	209			216.1		
6	37.3	2.59 *dd* (3.1, 18.6) 2.52 *dd* (6.1, 18.6)	3, 5, 7, 8	42.2	2.68 *dd* (1.1, 8) 2.23 *m*	3, 7, 8
7	43.3	3.96 *br s*	3, 8, 9	53.7	2.56 *m*	6, 9, 8,17
8	137.8			135.3		
9	123.3	5.63 *t* (7.7)	7, 10, 16	120.7	5.52 *ddd* (6.5, 2.7, 1.4)	7, 10, 11, 17
10	22.9	2.17 *dd* (14.3, 7.7) 2.91 *dd* (14.3, 8.3)	1, 8, 9, 11, 12	32.9	2.89 *dd* (17.6, 2.7, 1.4) 2.26 *m*	1, 8, 9, 11
11	74.8			94.7		
12	72.6			183.8		
13	35.6	2.69 *d* (18.4) 2.41 *d* (18.4)	1, 11, 12, 14	124.5	5.8 *d* (1.1)	1, 11, 12, 18
14	211.9			212.5		
15	18.5	1.02 *s*	1, 2, 11, 14	20.4	1.28 *s*	1, 2, 7, 11, 14
16	8.8	1.81 *t* (1.6)	3, 4, 5	5.9	0.85 *d* (6.9)	3, 4, 5
17	18.8	1.59 *d* (1.0)	7, 9, 8	27.2	1.85 *t* (1.4)	7, 8, 9
18	28.0	1.98 *m*	13, 12, 19, 20	28.3	2.64 *m*	11, 12, 13, 19, 20
19	18.9	1.08 *d* (7.0)	2012, 18,	21.9	1.15 *d* (6.8)	12, 18, 20
20	18.4	1.13 *d* (7.0)	19, 12	21.5	1.18 *d* (6.8)	12, 18, 19

epoxyhypoestenone (**3**) was the most active at of 93.3 μM against W2 strain (CQ resistant). The higher anti-plasmodial activity of the fractions may be attributed to the synergistic effects between different constituents of the whole extract/fractions. From previous studies, the synergism between different constituent compounds in whole extract and/or fractions has been invoked as a vital factor in the efficacy of many plant extracts in comparison to the single individual isolated

Table 3
In vitro antiplasmodial activity (IC_{50}) of fractions and isolated compounds from *Hypoestes verticillaris*.

Fraction/compound	D6 IC_{50} (M ± SD) μg/mL	W2 IC_{50} (M ± SD)
RAO-43B	10.15 ± 3.1 μg/mL	7.309 ± 1.2 μg/mL
RAO-43D	8.6 ± 2.5 μg/mL	13.44 ± 0.6 μg/mL
1	328 μM	328 μM
2	NT	NT
3	93 μM	93 μM
4	NT	NT
Chloroquine	0.0179 μg/mL	0.121 μg/mL

Key: fractions from crude extract, RAO-43B, RAO-43D; 2,6-dimethoxysavinin (**1**); 2,6-dimethoxy-(7E)-7,8-dehydroheliobuphthalmin (**2**); 11(12)-epoxyhypoestenone (**3**) 3(11)-epoxyhypoestenone (**4**); D6 chloroquine susceptible *Plasmodium falciparum* strain; W2 chloroquine resistant *Plasmodium falciparum* strain; NT, not tested.

compounds. This phenomenon may be responsible for the low potency of many compounds isolated from active medicinal plant extracts (Abrantes et al., 2008). Fusicoccanes are diterpenes with the dicyclopenta [α,δ]-cyclooctane ring system (Muhammad et al., 1997) which occur in liverworts, fungi, bacteria and higher plants like *Hypoestes* species (Banerji et al., 1976; De Boer and de. V. van Leeuwen, 2012). Hypoestenonol A and hypoestenone isolated from *Hypoestes forskalei* (Acanthaceae) showed weak anti-plasmodial activity with IC_{50} values at 18 μM and 25 μM against *P. falciparum* K-1 strain, respectively (Al Musayeib et al., 2014). Lignans have been reported to have a wide range of biological activities including but not limited to anti-viral, anti-fungal, anti-cancer amongst others (Abrantes et al., 2008; Kernan et al., 1997; Ngoc et al., 2018; Suzuki and Umezawa, 2007).

3. Experimental

3.1. General procedures

UV spectra were recorded using Specord S600-212C242 spectrophotometer (Analytik Jena AG). IR spectra were obtained using FTIR-600 spectrometer from Biotech Engineering, UK. Silica gel (70–230 mesh) and sephadex LH-20 (Merck) were used in column chromatography as the stationery phases. The solvents used for column chromatography were double distilled. Thin layer chromatography (TLC) was done using aluminium pre-coated silica gel plates. The TLC plates were visualized under UV light at 254 or 366 nm for UV active

compounds, followed by placing the plate in the iodine tank. The ^{1}H and ^{13}C NMR spectra of the pure isolated compounds were obtained using Varian–Mercury 200 MHz and/or Bruker–Avance 500 and 600 MHz spectrometers. The 2D (COSY, HSQC, HMBC and NOESY) spectra of the compounds were obtained using standard Bruker software. Chemical shifts were measured in ppm relative to the internal standard tetramethylsilane (TMS). CD_2Cl_2 and $CDCl_3$ were used as NMR solvents. The melting points were recorded on Stuart SMP10 apparatus and are uncorrected. The circular dichroism (CD) spectra data were recorded using spektrapolarimeter (*J*-815).

3.2. Plant material and solvent extraction

The whole Hypoestes *verticillaris* (Acanthaceaea) plant was collected in May 2015 in Mau Forest, Nakuru County of Kenya with the aid of a Botanist Patrick Mutiso. The voucher specimen (RO2015/03) is deposited at the University of Nairobi Herbarium. The dried and ground plant sample (3900 g) was extracted using 50% methanol (MeOH) in dichloromethane (CH_2Cl_2) for 24 h and repeated three times. The filtered extract was concentrated under reduced pressure using a rotary vapor to yield a crude extract which was stored in a freezer at -4 °C for further biological assay and/or chemical analysis.

3.3. Isolation

The crude extract (40 g) of the whole *Hypoestes verticillaris* plant was fractionated into two major fractions RAO-43B (900 mg) and RAO-43D (1.2 g) by column chromatography using silica gel and a mixture of *n*-C_6H_{14} and ethyl acetate (EtOAc) in increasing polarity (100:0, 0:100). One fraction, RAO-43B, was subjected to further purification by column chromatography using EtOAc and petroleum ether (3:97 to 30: 70) to obtain compound **1** (60 mg). The other fraction, RAO-43D, was run on Sephadex LH-20 using CH_2Cl_2: MeOH (1:1) to remove chlorophyll followed by column chromatography in silica gel using CH_2Cl_2/EtOAc in increasing polarity to obtain **2** (23 mg), **3** (11 mg) and fraction RAO-$43D_6$ (63 mg) which was further purified by column chromatography using silica gel a mixture of *n*-C_6H_{14} and EtOAc in increasing polarity to obtain compound **4** (27 mg).

3.3.1. 2, 6-Dimethoxysavinin (1)

A light yellow solid; $[\alpha]_D^{24}$ -499 (c = 2; CH_2Cl_2); UV; λ_{max} (log Ɛ) 258 (0.7), 293 (0.76), 340 (0.60) nm; IR (υ_{max}) 1748, 1625, 1479, 1198 and 1041 cm^{-1}; NMR (Table 1); HR-ESIMS *m/z* 413.1190 $[M+H]^+$, (C_{22} H_{20} O_8 requires *m/z* 413.1192), 412 $[M]^+$ (40), 277 (100), 135 (20).

3.3.2. 2,6-Dimethoxy-(7E) -7,8-dehydroheliobuphthalmin (2)

White crystals; m.p 141–143 °C; $[\alpha]_D^{23}$ -184 (c = 0.1; CH_2Cl_2); IR (KBr) υ_{max}; 1744, 1699, 1622, 1492, 1452, 1240, 1196, 1173, 1124, 1024, 1052 and 930 cm^{-1}; NMR (Table 1); HR-ESIMS *m/z* 473.1428 $[M+H]^+$, (C_{24} H_{24} O_{10} requires *m/z* 473.1403), 472 $[M]^+$ (70), 337 (100), 305 (30).

3.3.3. 11(12)-Epoxyhypoestenone (3)

White needle like crystals; m.p 134–136 °C; $[\alpha]_D^{24}$ +64 (c = 0.55; CH_2Cl_2); UV; λ nm (log Ɛ) 235 (0.88) nm; IR (KBR) υ_{max} 1766, 1698, 1642 and 1412; NMR (Table 2); HR-ESIMS *m/z* 315.1943 $[M+H]^+$, (C_{20} H_{26} O_3 requires *m/z* 315.1915), 314 $[M]^+$ (100), 299 (20), 243 (25), 201 (85), 187 (78), 173 (90), 159 (80), 91 (40), 43 (50).

3.3.4. 3(11)-Epoxyhypoestenone (4)

A transparent solid; $[\alpha]_D^{23}$ +74 (c = 0.5; CH_2Cl_2); UV; λ_{max} (log Ɛ) 230 (1.09) nm; NMR (Table 2); HR-ESIMS *m/z* 315.1923 $[M+H]^+$, (C_{20} H_{26} O_3 requires *m/z* 315.1915), 314 $[M]^+$ (100), 216(10), 162(30), 150 (25).

3.4. In vitro anti-plasmodial assay

The *in vitro* antimalarial assay was done using *Plasmodium falciparum* D6 (CQ-susceptible) and W2 (CQ-resistant) strains according to the established procedures (Desjardins et al., 1979).

Disclosure statement

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.02.019.

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