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6α-Hydroxy-α-toxicarol and (+)-tephrodin with antiplasmodial activities from *Tephrosia species*

Lois Muiva-Mutisya [a], Bernard Macharia [a], Matthias Heydenreich [b], Andreas Koch [b], Hoseah M. Akala [c], Solomon Derese [a], Leonidah K. Omosa [a], Amir O. Yusuf [a], Edwin Kamau [c], Abiy Yenesew [a,*]

[a] Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya
[b] Institut für Chemie, Universität Potsdam, Karl-Liebknecht-Str. 24-25, D-14476 Potsdam, Germany
[c] Global Emerging Infections Surveillance (GEIS) Program, United States Army Medical Research Unit-Kenya (USAMRU-K), Kenya Medical Research Institute (KEMRI) – Walter Reed Project, Kisumu and Nairobi, Kenya

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ABSTRACT

The CH_2Cl_2/MeOH (1:1) extract of the roots of *Tephrosia villosa* showed good antiplasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 3.1 ± 0.4 and 1.3 ± 0.3 μg/mL, respectively. Chromatographic separation of the extract yielded a new rotenoid, 6α-hydroxy-α-toxicarol, along with five known rotenoids, (rotenone, deguelin, sumatrol, 12a-hydroxy-α-toxicarol and villosinol). Similar treatment of the extract of the stem of *Tephrosia purpurea* (IC_{50} = 4.1 ± 0.4 and 1.9 ± 0.2 μg/mL against D6 and W2 strains of *P. falciparum*, respectively) yielded a new flavone having a unique substituent at C-7/C-8 [trivial name (+)-tephrodin], along with the known flavonoids tachrosin, obovatin methyl ether and derrone. The relative configuration and the most stable conformation in (+)-tephrodin was determined by NMR and theoretical energy calculations. The rotenoids and flavones tested showed good to moderate antiplasmodial activities (IC_{50} = 9 – 23 μM). Whereas the cytotoxicity of rotenoids is known, the flavones (+)-tephrodin and tachrosin did not show significant cytotoxicity (IC_{50} > 100 μM) against mammalian African monkey kidney (vero) and human larynx carcinoma (HEp2) cell lines.

1. Introduction

The genus *Tephrosia* (Leguminoseae-Papilionidae) is a large tropical and subtropical genus estimated to contain more than 300 species (Chang et al., 2000), of which about 30 occur in Kenya (Tarus et al., 2002). In East African traditional medicinal practice *Tephrosia* species are widely used to treat various infectious diseases (Kokwaro, 2009). The genus is rich in flavonoids and isoflavonoids (Andrei et al., 1997, 2000), showing antiplasmodial (Juma et al., 2011; Muiva et al., 2009), antibacterial and antifungal (Chang et al., 2000), insecticidal, piscicidal and insect repellent properties (Carlos et al., 2001). In our continued search for antiplasmodial agents from *Tephrosia* species found in Kenya (Juma et al., 2011; Muiva et al., 2009), the isolation, characterization and antiplasmodial activities of a new rotenoid (**1**, Fig. 1) from the roots of *T. villosa*; and a new flavone (**2**, Fig. 1) from the stem of *Tephrosia purpurea* along with known flavonoids is reported.

2. Results and discussion

The CH_2Cl_2/MeOH (1:1) extracts from different parts of *T. villosa* were tested for *in vitro* antiplasmodial activities against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* (Table 1). The roots of *T. villosa* showed the highest activity with IC_{50} values of 3.1 ± 0.4 μg/mL (against D6) and 1.3 ± 0.3 μg/mL (against W2). Chromatographic separation of the roots extract afforded six rotenoids, of which one is new (**1**). The known rotenoids of this plant were identified as sumatrol (Dagne et al., 1989), 12a-hydroxy-α-toxicarol (Andrei et al., 1997), villosinol (Krupadanam et al., 1977), rotenone (Dagne et al., 1989) and deguelin (Andrei et al., 1997) by comparison of their spectroscopic data with literature.

* Corresponding author. Tel.: +254 733 832576; fax: +254 20 4446138.
E-mail addresses: ayenesew@uonbi.ac.ke, abiyenesew@yahoo.com (A. Yenesew).

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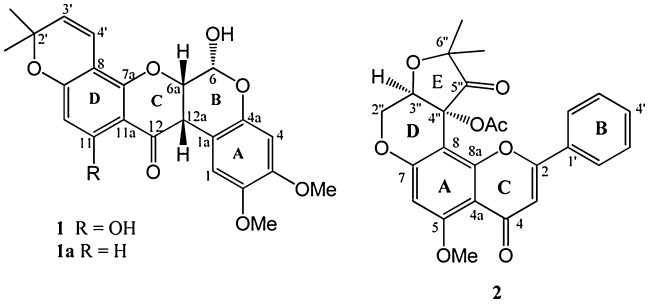

Fig. 1. Structures of the new compounds isolated from *Tephrosia* species.

The new compound (**1**) was isolated as colourless amorphous solid. HRMS showed a molecular ion peak at *m/z* 426.1315 attributed to molecular formula of $C_{23}H_{22}O_8$. The NMR spectral data [δ_H 5.70 (1H, *d*, *J* = 2.6 Hz for H-6; δ_C 90.1); 4.68 (1H, *dd*, *J* = 2.6, 3.8 Hz for H-6a; δ_C 73.3); 3.81 (1H, *d*, *J* = 3.0 Hz for H-12a; δ_C 40.2) and δ_C 194.6 (C-12)] indicated that compound **1** is a 6a-hydroxyrotenoid derivative (Dagne et al., 1989; Luyengi et al., 1994). The presence of a chelated hydroxyl (at C-11, δ_H 12.09), two methoxyl (δ_H 3.64 and 3.69; δ_C 56.6 and 56.1) and a 2′,2′-dimethylpyran (Table 2) substituents were evident from the NMR spectra. In the HMBC spectrum, correlation of H-3′ (δ_H 5.42) with C-8 (δ_C 102.1); H-4′ (δ_H 6.42) with C-9 (δ_C 162.9) and C-7a (δ_C 155.6); OH-11 (δ_H 12.09) with C-10 (δ_C 97.9), C-11 (δ_C 164.8) and C-11a (δ_C 101.9) is consistent with the placement of the 2′,2′-dimethylpyran ring at C-8/C-9 and the hydroxyl group at C-11. The ^{1}H NMR spectrum further showed two singlets at δ_H 6.75 (H-1) and 6.40 (H-4) which allowed the placement of the two methoxyl groups in ring A at C-2 and C-3 (Dagne et al., 1989). The substitution pattern in ring A was confirmed from the HMBC spectrum (Table 2).

The small coupling constant (*J* = 3.8 Hz) observed between H-6a and H-12a is consistent with *cis*-B/C ring junction where both protons are β-oriented (6a-β and 12a-β) as the other rotenoids of this genus (Dagne et al., 1989). The axially oriented H-6a also gave small coupling constant (*J* = 2.6 Hz) with H-6, placing the latter proton in equatorial position (in this case β-oriented). NOE interaction of H-6 with H-6a is in agreement with the placement of these protons on the same face (β-orientation); consequently the hydroxyl at C-6 should have α-orientation as in 6a-hydroxydeguelin (**1a**) reported from *Mundulia serica* (Luyengi et al., 1994). In fact compound **1** is levorotatory ($[\alpha]_D$ − 12.6) as **1a** (Luyengi et al., 1994), and hence **1** should have the same (6*R*,6a*S*,12a*S*) configuration. Based on the above spectroscopic evidence, this new compound was assigned structure **1** for which the trivial name (6*R*,6a*S*,12a*S*)-6-hydroxy-α-toxicarol is given.

Table 1
In vitro antiplasmodial activities of *Tephrosia purpurea* and *T. villosa* against D6 and W2 Strains of *P. falciparum*.

Tested samples	IC_{50} (μg/mL, or μM)	
	D6	W2
Crude extracts		
Tephrosia purpurea (stem)[a]	4.1 ± 0.4	1.9 ± 0.2
Tephrosia purpurea (leaves)	7.0 ± 0.8	11.8 ± 2.2
Tephrosia purpurea (seeds)	4.2 ± 0.6	2.8 ± 0.6
Tephrosia purpurea (roots)	6.7 ± 1.2	3.6 ± 0.6
Tephrosia villosa (stem)	12.5 ± 0.5	7.1 ± 1.7
Tephrosia villosa (leaves)	5.9 ± 1.0	12.7 ± 2.1
Tephrosia villosa (seed pods)	8.4 ± 0.4	6.8 ± 1.5
Tephrosia villosa (seeds)	5.5 ± 0.4	2.8 ± 0.3
Tephrosia villosa (roots)	3.1 ± 0.4	1.3 ± 0.3
Pure compounds[a]		
6α-Hydroxy-α-toxicarol (**1**)	7.97 ± 0.5	12.2 ± 2.6
(+)-Tephrodin (**2**)	14.0 ± 1.5	18.0 ± 2.4
Tachrosin	12.5 ± 1.3	20.4 ± 3.0
Deguelin/Rotenone mixture	9.6 ± 2.0	22.6 ± 5.0
Obovatin methyl ether (Muiva et al., 2009)	11.3 ± 0.9	13.1 ± 1.7
Standard[a]		
Chloroquine	0.013 ± 0.01	0.237 ± 0.03
Mefloquine	0.031 ± 0.02	0.011 ± 0.01

[a] IC_{50} in μg/mL for crude extracts; in μM for pure compounds and standards).

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

Position	δ_H, *m* (*J* in Hz)	δ_C	HMBC (H → C)
1	6.75, *s*	110.8	C-1a, C-2, C-3, C-4a, C-12a
1a		105.1	
2		144.6	
3		150.4	
4	6.40, *s*	102.0*	C-1a, C-2, C-3
4a		144.7	
6	5.70, *d* (2.6)	90.1	C-4a, C-6a, C-12a
6a	4.68, *dd* (3.8, 2.6)	73.3	C-1a
7a		155.6	
8		102.1*	
9		162.9	
10	5.83, *s*	97.9	C-6, C-8, C-9, C-11, C-11a
11		164.8	
11a		101.9*	
12		194.6	
12a	3.81, *d* (3.8)	40.2	C-1(w), C-1a, C-4a, C-11a, C-12
2′		78.6	
3′	5.42, *d* (10.1)	127.1	C-2′, Me-2′, C-8
4′	6.42, *d* (10.1)	115.3	C-2′, C-9, C-7a, C-11a
MeO-2	3.64, *s*	56.6	C-2
MeO-3	3.69, *s*	56.1	C-3
Me-2′$_{ax}$	1.26, *s*	28.2	C-2′, C-3′, Me-2′
Me-2′$_{eq}$	1.34, *s*	28.6	C-2′, C-3′, Me-2′
11-OH	12.09, *s*		C-10, C-11, C-11a

* Assignment may be interchangeable.

The extract of the stem of *T. purpurea* also showed good *in vitro* antiplasmodial activity with IC_{50} values of 4.1 ± 0.4 μg/mL (against D6) and 1.9 ± 0.2 μg/mL (against W2). Chromatographic separation of the extract yielded four compounds; HR–MS analysis of one of these (**2**) gave a molecular ion peak at *m/z* 450.1293 ($C_{25}H_{22}O_8$). A flavone skeleton was deduced for this compound from the UV (λ_{max} 297, 328 nm), ^{1}H NMR (δ_H 6.77, 1H, *s*, for H-3); ^{13}C NMR (δ_C 161.4 for C-2, 109.1 for C-3 and 177.2 for C-4) spectra (Juma et al., 2011). The ^{1}H NMR spectrum (δ_H 8.23, 2H, *m*, for H-2′/6′ and 7.52, 3H, *m*, for H-3′/4′/5′) further revealed that ring B of the flavone is unsubstituted; while ring A is trisubstituted, with the only aromatic proton appearing at δ_H 6.46 (*s*) for H-6. The NMR spectra also showed signals for a methoxyl group (δ_H 3.97, δ_C 56.5) which was placed at C-5 of ring A from its NOE correlation with H-6 (δ_H 6.46). This was further supported by HMBC spectrum showing correlation of the methoxyl protons with C-5 (δ_C 161.7) and C-6 (δ_C 96.9); H-6 (δ_H 6.46) with C-4 (δ_C 177.2), C-4a (δ_C 110.7), C-5 (δ_C 161.7), C-7 (δ_C 159.7) and C-8 (δ_C 94.3).

The NMR spectra (Table 3) further indicated that there is a substituent at C-7/C-8 where a C_5 unit at C-8 in a precursor has cyclized involving methoxyl group at C-7 as the other flavonoids of this plant (Pelter et al., 1981; Juma et al., 2011). In the HMBC spectrum, one of the methylene protons (CH_2-2″; δ_C 63.6) at δ_H 4.62 showed correlations with C-7 (δ_C 159.7), an oxygenated methine (δ_C 72.1 for C-3″ that showed HSQC correlation with δ_H 4.97) and an sp^3 hybridized quaternary carbon at δ_C 75.4 (C-4″). The methine proton (δ_H 4.97) also showed HMBC correlation with the quaternary carbon at δ_C 96.9 (C-8) and 75.4 (C-4″) suggesting that a pyran ring is fused to ring A at C-7/C-8. The signal at δ_H 4.97 also showed HMBC correlation with a quaternary carbon (δ_C 80.9) which in turn correlates with two germinal methyl groups (δ_H 1.57 and 1.08). These methyl groups also correlate with a carbonyl (δ_C 206.6)

Table 3
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for (+)-tephrodin (2) in $CDCl_3$.

Position	δ_H *m* (*J* in Hz)	δ_C	HMBC (H → C)
2		161.4	
3	6.77 *s*	109.1	C-2, C-4a, C-1′, C-4
4		177.2	
4a		110.7	
5		161.7	
6	6.46 *s*	96.9	C-4, C-4a, C-4″, C-7, C-8
7		159.7	
8		94.3	
8a		158.8	
1′		131.2	
2′/6′	8.23 *m*	126.6	C-6′/2′, C-5′, C-4′, C-1,
3′/5′	7.52 *m*	128.8	C-2′/6′, C-4′/5′/3′, C-1′
4′	7.52 *m*	131.3	
2″	4.17 *dd* (12.2, 0.8)	63.6	C-3″, C-6″
	4.62 *dd* (12.2, 2.3)		C-3″, C-7, C-4
3″	4.97 *dd* (2.2, 0.9)	72.1	C-2″, C 7, C-4″, C-6″
4″		75.4	
5″		206.6	
6″		80.9	
MeO-5	3.97 *s*	56.5	C-5, C-6
CH_3-6″-	1.08 *s*	26.5	C-6″, C-5″, Me-6″
CH_3-6″	1.57, *s*	24.8	C-6″, C-5″, Me-6″
$COCH_3$-4″	2.04 *s*	20.9	COMe-4″
$COCH_3$-4″		169.3	

showing that a 2,2-dimethylfuranone group is fused to the pyran ring at C-3″/4″, completing the seven carbon substituent in ring A of the flavone. Finally, the NMR spectra also showed the presence of an acetoxyl group [$COCH_3$ (δ_H 2.04 *s*; δ_C 20.9); COMe (δ_C 169.3)] which could only be attached to one of the bridge carbon atoms between the pyran and furanone rings, i.e. C-4″ (δ_C 75.4). From the above spectral evidence compound **2** has a general structure of (−)-tephrodin, a compound that has been reported from *Tephrosia polystachyoides* (Vleggar *et al.*, 1972). The compound isolated from the stem of *T. purpurea* (**2**), however, is dextrorotatory suggesting that **2** is a stereoisomer of (−)-tephrodin.

In order to establish the relative configuration and the most stable conformation in **2**, the energy was calculated theoretically and gave a global energy minimum of the *gauche–gauche* conformation between H-3″ and both H-2″ (Fig. 2a) for the *cis* linked *R**R** diastereomer. The next local energy minimum ($\Delta\Delta G$ = 1.83 kcal/mole) for the *R**R** diastereomer was found also to have a *gauche-gauche* conformation between H-3″ and both H-2″ (Fig. 2b), that differs only in rotational angle of the acetyl group. The conformation with a local energy minimum for an *antiperiplanar–gauche* conformation between H-3″ and both H-2″ is shown in Fig. 2c; the energy difference to the local minimum is 5.13 kcal/mole. Considering the Boltzmann distribution, this energy is too high to be found by NMR in solution. The *trans*-linked *R**S** diastereomer did not give any local minimum with a *gauche–gauche* conformation, all calculations went to the global minimum with an *antiperiplanar–gauche* conformation between H-3″ and both protons at C-2″ which is shown in Fig. 2d. In the NMR spectrum of **2**, the coupling constants observed between H-3″ and both protons at C-2″ (*J* = 2.2 and 0.9 Hz) allow only *gauche–gauche* conformation. Thus, it can be assumed that **2** is one of the two *R**R** stereoisomers, and since the optical rotation observed for compound **2** (dextrorotatory) is opposite to the one reported for (−)-tephrodin isolated from *Tephrosia polystachyoides* (Vleggar et al., 1972), **2** must be the other *R**R** stereoisomer.

The known compounds isolated from this plant were identified as the flavones tachrosin (Waterman and Khalid, 1980; Vleggar et al., 1972), the flavanone obovatin methyl ether (Andrei et al., 2000) and the isoflavone derrone (Chibber and Sharma, 1980). The co-occurrence of (+)-tephrodin and tachrosin in this plant supports the previous suggestion that (+)-tephrodin is formed by picking yet another carbon from C-7 methoxyl group of tachrosin forming ring D. Whereas there are some examples of flavonoids of *T. purpurea* (and few related taxa) containing a C_6

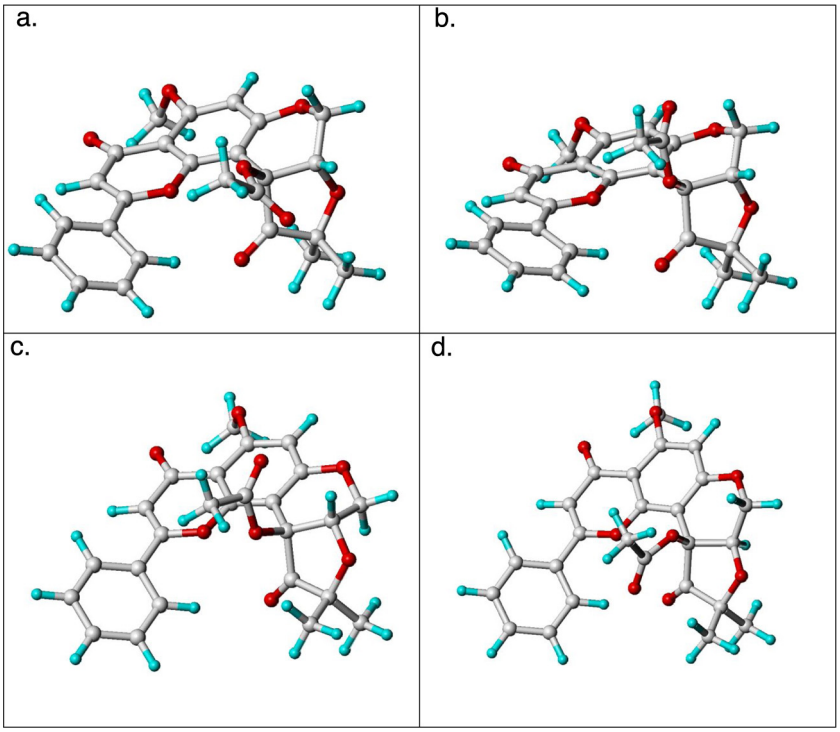

Fig. 2. 3D-structures of possible configurations and conformations in compound **2**: 2a. Global energy minimum of (3″*S*,4″*S*)-**2**; 2b. Local energy minimum of (3″*S*,4″*S*)-**2** ($\Delta\Delta G$ = 1.83 kcal/mole); 2c. Local energy minimum of (3″*S*,4″*S*)-**2** with an *antiperiplanar–gauche* conformation ($\Delta\Delta G$ = 5.13 kcal/mole); 2d. Global energy minimum of (3″*R*,4″*S*)-**2** with an *antiperiplanar–gauche* conformation.

substituent at C-7/C-8 (Chen et al., 2014), tephrodin appears to be the only known flavonoid that contain a C_7 substituent through incorporation of two methyl groups into a C_5 chain.

Following the good to moderate antiplasmodial activities of the crude roots extract of *T. villosa* (Table 1), the new compound (**1**) along with rotenone and deguelin were tested against D6 and W2 strains of *P. falciparum*, and these rotenoids showed good to moderate activities with IC_{50} values of 7–23 μM (Table 1). With reported cytotoxicity of rotenoids (Nianbai and John, 1998), it is likely that the observed *in vitro* antiplasmodial activities of these rotenoids is due to cytotoxicity.

The two flavones isolated from the stem of *T. purpurea* were also tested for *in vitro* antiplasmodial activities (Table 1) where (+)-tephrodin (IC_{50} = 14.0 ± 1.5 μM against D6 and, 18.0 ± 2.4 μM against W2 strains) and tachrosin (IC_{50} = 12.5 ± 1.3 μM against D6, and 20.4 ± 3.0 μM against W2 strains) showed good-to-moderate *in vitro* antiplasmodial activities, against *P. falciparum*. The cytotoxicity of these flavones were evaluated against the mammalian African monkey kidney (vero) and the human larynx carcinoma (HEp2) cell lines, and no significant cytotoxicity was observed (IC_{50} > 100 μM) showing that the observed antiplasmodial activity of these flavones is not due to general cytotoxicity. The *in vivo* and *in vitro* antimalarial activities of some flavones have already been reported (Juma et al., 2011; Auffret et al., 2007).

3. Experimental

3.1. General

The ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were acquired with a Bruker AVANCE III NMR spectrometer using TMS as the internal standard. COSY, HSQC, HMBC and NOESY spectra were obtained using the standard Bruker software (TopSpin). EI-MS direct inlet, 70 eV on micromass GC-TOFmicro mass spectrometer (micro mass Wythenshawe, Waters Inc. UK). MS direct inlet, on Q-TOF (time of flight mass spectrometer equipped with an electrons pray ionization (ESI)). UV–vis spectra were recorded using a SPECORD S600 Spectophotometer. The CD spectra were obtained using a JASCO J-715 spectropolarimeter.

3.2. Plant materials

The roots of *Tephrosia villosa* were collected from Manyani, Taita Taveta County in August 2009. The stems of *T. purpurea* were collected from Rea Vipigo, along Mombasa–Malindi road, Kilifi County, in June 2013. The plants were identified by Mr. Patrick Mutiso of the School of Biological Sciences, Herbarium, University of Nairobi, where voucher specimens (voucher number: Mutiso-029/August 2009 for *T. villosa* and voucher number Mutiso-853b/June 2013 for *T. purpurea*) were deposited.

3.3. Extraction and Isolation of compounds from the roots of T. villosa

The air-dried and ground roots of *T. villosa* (2 kg) were extracted with dichloromethane/methanol (1:1) by cold percolation (3 × 1.5 L). The solvent was removed under reduced pressure to afford brown oily crude extract (246 g). A portion (100 g) of the extract was subjected to column chromatography on silica gel (500 g), eluting with *n*-hexane containing increasing amounts of ethyl acetate. The fractions eluted with 3% EtOAc in *n*-hexane were combined and purified by column chromatography on Sephadex LH-20 (eluent:CH_2Cl_2/MeOH, 1:1) and PTLC on silica gel (*n*-hexane/CH_2Cl_2/acetone, 8:1:1) to afford a mixture (20 mg) of rotenone and deguelin. Fractions 126–181 (5% EtOAc in *n*-hexane) were combined and purified on Sephadex LH-20 (eluent:CH_2Cl_2/MeOH, 1:1) and subjected to further column chromatography over silica gel (20 g, eluting with increasing gradient of acetone in *n*-hexane) to yield sumatrol (18 mg), 12a-hydroxy-α-toxicarol (20 mg), and villosinol (36 mg). Fractions 300–313 (eluted with 7% EtOAc in *n*-hexane) were combined and further purified on Sephadex LH-20 (eluent:CH_2Cl_2/MeOH, 1:1) and purified by PTLC (*n*-hexane/EtOAc, 7:3) to yield 6α-hydroxy-α-toxicarol (**1**, 60 mg).

3.4. Extraction and Isolation of compounds from the stem of T. purpurea

The air-dried and ground stems of *T. purpurea* (1.3 kg) were extracted with CH_2Cl_2/MeOH (1:1) by cold percolation for 24 h (3 × 3 L). The solvent was removed under vacuum to afford 106 g of a brown crude extract. A portion of the extract (100 g) was subjected to CC over silica gel (700 g) and eluted with *n*-hexane containing increasing amounts of acetone (1%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 50%, 100%). The fraction eluted with 3% acetone in *n*-hexane on further purification with Sephadex LH-20 using MeOH/EtOAc (1:1) followed by PTLC using *n*-hexane/acetone (4:1) yielded obovatin methyl ether (19.5 mg). The fractions eluted with 5% and 10% acetone in *n*-hexane contain three minor spots and were not followed at this stage. The fraction eluted with 15% acetone in *n*-hexane was purified using Sephadex LH-20 (CH_2Cl_2/MeOH 1:1) to yield yellow crystals of **2** (19 mg). Crystallization (from methanol) of the fraction eluted with 25% acetone in *n*-hexane afforded tachrosin (24 mg).

3.5. (6R,6aS,12aS)-6-Hydroxy-α-toxicarol (*1*)

Yellow oil; UV (MeOH) λ_{max}: 252, 268, 297, 312 nm; $[\alpha]_D$ − 12.6 (*c* 0.08, MeOH); ^1H and ^{13}C NMR (Table 2); EIMS (*m/z*, rel. int.): 426 (48, M^+), 411 (43), 293 (29), 219 (33), 203 (58), 191 (100); HRMS found *m/z* 426.1315 (calcd. for $C_{23}H_{22}O_8$, 426.1315).

3.6. (+)-Tephrodin (2)

Yellow amorphous solid. UV (MeOH) λ_{max} nm: 297, 328 nm; $[\alpha]_D^{20}$ + 4.7 (*c* 0.55, MeOH); CD (*c* 0.05, MeOH) λ_{max} nm ($\Delta\varepsilon$; $M^{-1}\,cm^{-1}$): $(+25.1)_{330}$, $(-17.8)_{301}$, $(+7.2)_{267}$, $(-2.9)_{243}$; ^1H NMR (Table 3); ^{13}C NMR (Table 3); EIMS (*m/z*, rel. int.): 450 (100, M^+), 322 (80), 293 (29), 219 (15), 163 (13); HRMS found *m/z* 450.1293 for (calcd. for $C_{25}H_{22}O_8$, 450.1315).

3.7. In vitro antiplasmodial activity assay

Antiplasmodial activities of crude extract and pure compounds against chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strain of *P. falciparum* was tested using a non-radioactive assay technique (Smilkstein et al., 2004) with modifications as described in Juma et al. (2011).

3.8. Cytotoxicity assays

Rapid colorimetric assay on the flavones (+)-tephrodin and tachrosin was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) on the mammalian cell lines, African monkey kidney (vero), and human larynx carcinoma (HEp2) as described in literature (Mosmann, 1983; Prayong et al., 2008).

3.9. Theoretical calculations

All geometries of compound **2** were optimized without any restrictions using the density-functional theory (DFT) (Hohenberg and Kohn, 1964; Kohn and Sham, 1965; Parr and Yang, 1989) approaches. The DFT scheme utilized Becke's three-parameter

functional (B3LYP) (Becks, 1993; Lee et al., 1988) and the split-valence triple zeta basis set 6-311G** including polarization functions (Hehre et al., 1986). The Gaussian 09 program package (Frisch et al., 2009) were performed for all of the computations. The 3D structures were handled using SYBYL7.3 (2007) molecular modelling software.

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