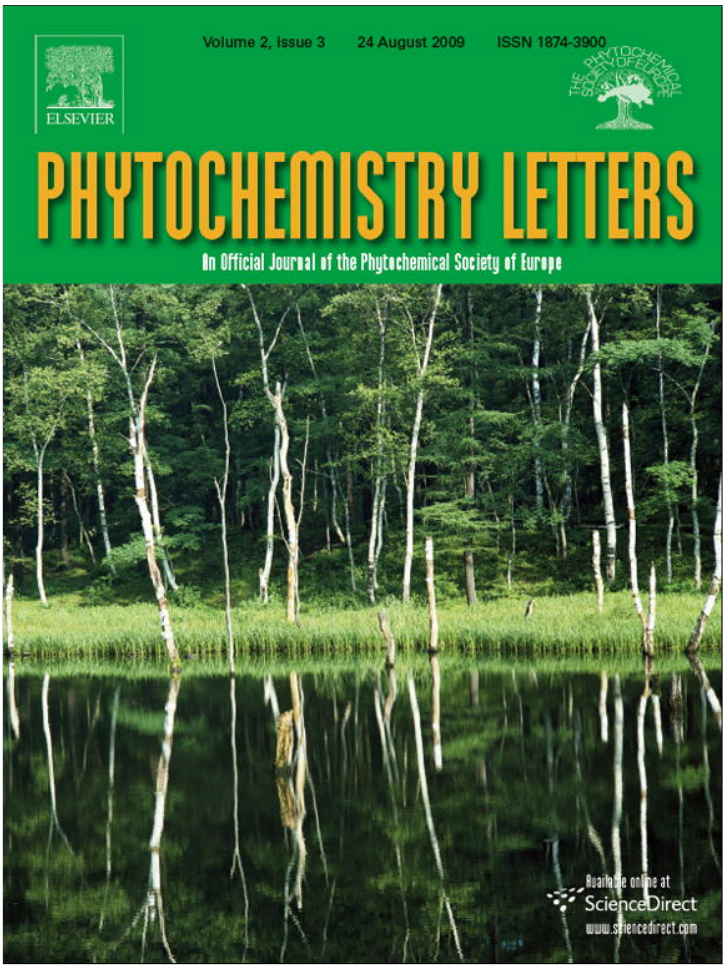

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Antiplasmodial β-hydroxydihydrochalcone from seedpods of *Tephrosia elata*

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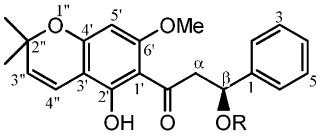
ABSTRACT

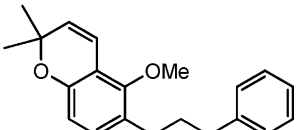
From the seedpods of *Tephrosia elata*, a new β-hydroxydihydrochalcone named (*S*)-elatadihydrochalcone was isolated. In addition, the known flavonoids obovatachalcone, obovatin, obovatin methyl ether and deguelin were identified. The structures were determined on the basis of spectroscopic evidence. The crude extract and the flavonoids obtained from the seedpods of this plant showed antiplasmodial activities. The literature NMR data on β-hydroxydihydrochalcones is reviewed and the identity of some of the compounds assigned β-hydroxydihydrochalcone skeleton is questioned.

1. Introduction

In East Africa *Tephrosia* species (Leguminosae) are used in traditional medicinal practice to treat infectious diseases (Kokwaro, 1993). It is estimated that there are between 300 and 400 *Tephrosia* species, of which some 30 are found in Kenya (Tarus et al., 2002). The genus *Tephrosia* is rich in flavonoids and isoflavonoids including rotenoids (Andrei et al., 1997). Previous phytochemical investigation of the roots of *T. elata* yielded flavanones, a flavone, pterocarpans and rotenoids (Lwande et al., 1985). In our search for compounds with antiplasmodial activity from plants using the new non-radioactive SYBR Green 1 malaria drug assay technique (Smilkstein et al., 2004), we have analyzed the seedpods of *T. elata* and report here the isolation and identification of a new antiplasmodial β-hydroxydihydrochalcone, named elatadihydrochalcone (**1**), along with known flavonoids.



1 R H
1a R Ac



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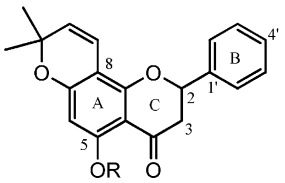

10 R H
11 R Me

2. Results and discussion

HRMS analysis of compound **1** showed a $[M+1]^+$ peak at *m/z* 355.1535 corresponding to the molecular formula of $C_{21}H_{23}O_5$. The

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Table 1
^{1}H (300 MHz) and ^{13}C NMR (75 MHz) along with HMBC Correlations for compounds **1** and **1a**.

Position	**1** ($CDCl_3$)			**1** (acetone-d_6)		**1a** ($CDCl_3$)	
	δ_C	δ_H *m* (*J* in Hz)	HMBC	δ_C	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)
1	143.4			145.6		140.4	
2/6	125.9	7.26–7.44 *m*	C-1,β, 3/5, 4	126.1	7.23–7.48 *m*	126.7	7.27–7.41 *m*
3/5	128.4	7.26–7.44 *m*	C-1,2/6,4	128.3	7.23–7.48 *m*	128.5	7.27–7.41 *m*
4	127.4	7.26–7.44 *m*	C-2/6,3/5	127.2	7.23–7.48 *m*	128.0	7.27–7.41 *m*
1′	105.6			105.7		105.5	
2′	161.9			161.9		161.9	
3′	102.9			102.6		102.9	
4′	160.7			160.6		160.3	
5′	91.4	5.87 *s*	C-1′,3′,4′,6′	91.7	6.00 *s*	91.2	5.88 *s*
6′	163.0			163.0		162.6	
α	52.7	3.45 *dd* (3.0, 18.0)	C═O,β,1	54.0	3.44 *dd* (4.6, 16.2)	50.3	3.44 *dd* (4.5, 17.1)
		3.34 *dd* (9.0, 18.0)	C═O,β,1		3.37 *dd* (7.8, 16.2)		3.62 *dd* (8.7, 17.1)
β	70.2	5.28 *dd* (3.0, 9.0)	C-2/6	70.0	5.29 *dd* (4.6, 7.8)	71.9	6.38 *dd* (4.5, 8.7)
2″	78.3			78.2		78.2	
3″	125.5	5.46 *d* (10.1)	C-3′	125.9	5.56 *d* (10.2)	125.4	5.45 *d* (10.2)
4″	115.8	6.66 *d* (10.1)	C-2″,4′	115.7	6.66 *d* (10.2)	115.9	6.64 *d* (10.2)
2″-$(CH_3)_2$	28.4	1.44 *s*	C-2″,3″	27.9	1.43 *s*	28.4	1.44 *s*
6′-OCH_3	55.7	3.78 *s*	C-6′	55.8	3.91 *s*	55.7	3.83 *s*
2′-OH		13.98 *s*	C-1′, 2′,3′		14.21 *s*		13.99 *s*
C═O	204.2			203.9		200.7	
$OCOCH_3$						21.2	2.03 *s*
$OCOCH_3$						170.0	

UV (λ_{max} = 275, 296 and 308 nm), ^{1}H (δ 3.45, *dd*, 3.0, 18.0 Hz and 3.34, *dd*, *J* = 9.0, 18.0 Hz for CH_2-α; 5.28 *dd*, *J* = 3.0, 9.0 Hz for H-β) and ^{13}C (δ = 204.2 for C═O, 52.7 for C-α, 70.2 for C-β) NMR spectra (Table 1) showed that this compound is a β-hydroxydihydrochalcone derivative (Nel et al., 1999; Chen et al., 2005). The presence of hydroxyl group at the β-carbon was confirmed from ^{1}H NMR spectrum of the mono acetate (**1a**) which showed down-field shift for H-β (Table 1). In agreement with this, the HMBC spectrum of **1** showed correlations between H-β and C-2/6, H-2/6 and C-β, CH_2-α and C═O, CH_2-α and C-1 (Table 1). Furthermore the presence of a chelated hydroxyl (δ 13.98), a methoxyl (δ_H = 3.78, δ_C = 55.7) and a 2,2-dimethylpyran substituents were established from NMR (Table 1) and MS data.

The ^{1}H and ^{13}C NMR spectra (δ_H 7.26–7.44, 5H for H-2, -3, -4, -5, -6; δ_C 143.4 for C-1; 125.9 for C-2/6, 128.4 for C-3/5) showed that ring-B in compound **1** is not substituted. All the substituents are then located in ring-B, with the chelated hydroxyl and the methoxyl at C-2′ and C-6′. The 2,2-dimethylpyrano group could either be adjacent to the hydroxyl group (**1**) or adjacent to the methoxyl group (**2**). The ^{13}C NMR chemical shift value of the methoxyl (δ_C 55.7) is within the normal range and is consistent with structure **1** rather than **2** where the methoxy group being di-*ortho*-substituted is expected to resonate above 59 ppm (Yenesew et al., 1998). In agreement with this, irradiation of the methoxy protons resulted in a nOe enhancement of H-5′. Hence this compound was characterized as 3′,4′-(2″,2″-dimethylpyrano)-2′,β-dihydroxy-6′-methoxydihydrochalcone, for which the trivial name elatadihydrochalcone is suggested. The CD spectrum showed a positive Cotton effect at 350 nm and a negative one at 290 nm which suggested *S*-configuration at the β carbon (Nel et al., 1999).

This appears to be the first report on the occurrence of a β-hydroxydihydrochalcone in the genus *Tephrosia*. β-Hydroxydihydrochalcones constitute a small subclass of flavonoids with only few reported in nature (Dictionary of Organic Compounds, 2008). The literature data on the assignments of the ^{1}H and ^{13}C NMR data (Table 2) for the characteristic β-hydroxydihydrochalcone atoms were found to be inconsistent. This prompted us to do comprehensive NMR analysis (Table 1) of compound **1** and its acetate derivative (**1a**). The ^{1}H and ^{13}C NMR data of compound **1** are in close agreement (Table 2) for what have been reported for the β-hydroxydihydrochalcones **3** (Nel et al., 1999) and **4** (Chen et al., 2005), and for the β-methoxydihydrochalcones (e.g. **5**) reported by Tanaka et al. (1992). In contrast, the data reported (Table 2) for **6** (Thuy et al., 1998), **7** (Rafi et al., 2002), **8** (Manners and Jurd, 1979) and **9** (Adinarayana et al., 1982) are significantly different. Such difference could suggest different skeletal structures for the two sets of compounds, compounds **1**, **3**, **4** and **5** as one set and compounds **6**–**9** as a second set.

Interestingly the NMR data for α and β atoms as reported for compounds **6**–**9** are similar (Table 2) to C-ring atoms (CH_2-3 and CH-2 groups) of the flavanones obovatin (**10**) and obovatin methyl ether (**11**), and other flavanones in literature (Tanaka et al., 1992; Andrei et al., 2000; Yenesew et al., 1998). In fact the similarity of NMR data of compounds **8** (Manners and Jurd, 1979) and **9** (Adinarayana et al., 1982) with flavanones have been used by these authors as supporting evidence in assigning β-hydroxydihydrochalcone skeleton for these compounds. However, the NMR data we generated for compound **1** and the literature report for compounds **3**–**5** is distinct from those of the flavanones **10** and **11** (Table 2). Such difference is expected considering that β-hydroxydihydrochalcones have an open chain for α, β and C═O atoms, while flavanones have cyclic system (ring-C).

It follows then that compounds **6** (Thuy et al., 1998), **7** (Rafi et al., 2002), **8** (Manners and Jurd, 1979), **9** (Adinarayana et al., 1982) and other related compounds assigned β-hydroxydihydrochalcone structures in these papers could be flavanones rather than β-hydroxydihydrochalcone derivatives. We therefore suggest re-examination of these compounds by a combination of modern spectroscopic techniques to resolve this issue.

The crude extract ($MeOH/CH_2Cl_2$, 1:1) of the seedpods of *T. elata* showed antiplasmodial activities against chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum* with IC_{50} values of 8.4 ± 0.3 and 8.6 ± 1.0 μg/ml respectively (Table 3). Among the compounds isolated from the seedpods of *T. elata* the new compound (**1**) exhibited good antiplasmodial activity with IC_{50} values of 2.8 ± 0.3 and 5.5 ± 0.3 μg/ml against D6 and W2 strains, respectively. This compound along with the other flavonoids appears to be responsible for the activities observed in the crude extract. The antiplasmodial activities of some flavonoids (Yenesew et al., 2003; Andayi et al.,

Table 2
Comparison of the ^{1}H (a) and ^{13}C (b) NMR data of the α and β atoms of compound **1** with literature for β-hydroxydihydrochalcone derivatives (**3**–**9**) and flavanones (**10** and **11**).

H	**1** ($CDCl_3$)	**1** (acetone-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** (pyridine-d_5)	**7** ($CDCl_3$)	**8** (acetone-d_6)	**9** (DMSO-d_6)	**10**[a] ($CDCl_3$)	**11**[a] ($CDCl_3$)
(a) ^{1}H (*J* in Hz)											
α	3.45 *dd* (2.9,18.3)	3.44 *dd* (4.6,16.2)	3.40 *dd* (3.3,18.1)	3.37 *dd* (8.8,17.4)	3.55 *dd* (9,17)	2.93 *dd* (2.9,16.3)	2.69 *dd* (3.1,16.3)	2.77 (8,15)	2.76 *dd* (8,15)	2.81 *dd* (3.3,16.7)	2.75 *dd* (3.4,16.5)
	3.34 *dd* (9.0,18.3)	3.37 *dd* (7.8,16.2)	3.30 *dd* (9.1,18.1)	3.29 *dd* (3.2,17.4)	3.15 *dd* (5,17)	3.27 *dd* (12.8,16.3)	3.06 *dd* (13.0,16.3)	3.01 (5,15)	3.00 *dd* (5,15)	3.10 *dd* (12.5,16.7)	2.95 *dd* (12.5,16.5)
β[a]	5.28 *dd* (3.0, 9.0)	5.29 *dd* (4.6, 7.8)	5.21 *dd* (3.6, 8.7)	5.34 *dd* (3.2,8.8)	4.74 *dd* (5,9)	5.52 *dd* (2.9,12.8)	5.38 *dd* (3.0,13.0)	5.21 *m*	5.07 *m*	5.45 *dd* (3.3,12.5)	5.39 *dd* (3.4,12.6)
C	**1** ($CDCl_3$)	**1** (acetone-d_6)	**3** ($CDCl_3$)	**6** (pyridine-d_5)			**7** ($CDCl_3$)	**9** (DMSO-d_6)		**10**[a] ($CDCl_3$)	**11**[a] ($CDCl_3$)
(b) ^{13}C NMR data											
α	52.7		54.0	52.45		46.1	45.24	40.1		43.19	45.52
β	70.2		70.0	70.41		79.5	81.33	73.3		78.97	78.84

3 = β-Hydroxy-4,4′-dimethoxy-2′-*O*-methoxymethyldihydrochalcone (Nel et al., 1999). **4** = 3′-Methoxy-2′,4′,β-trihydroxydihydrochalcone (Chen et al., 2005). **5** = Ponganone VIII (2′,5′,β − Trimethoxy-3,4-methylenedioxy-6″,6″-dimethylpyrano[2″,3″:4′,3′]dihydrochalcone (Tanaka et al., 1992). **6** = 4,2′,4′,β-Tetrahydroxy-6′-methoxy-α,β-dihydrochalcone (Thuy et al., 1998). **7** = 2′,4,4′,β-Tetrahydroxydihydrochalcone (Rafi et al., 2002). **8** = Gliricidol (4-metoxy-β,2′,3,4′,5-pentahydroxydihydrochalcone (Manners and Jurd, 1979). **9** = Pterosupin (3′-β-D-glucopyranosyl-2′,4,4′,β-tetrahydroxydihydrochalcone (Adinarayana et al., 1982). **10** = Obovatin (Andrei et al., 2000). **11** = Obovatin methyl ether (Andrei et al., 2000).
[a] For flavanones **10** and **11**, α = C-3 and β = C-2.

Table 3
In vitro IC_{50} values of the crude extract and flavonoids from the seedpods of *Tephrosia elata* against W2 and D6 strains of *Plasmodium falciparum*.

Compounds	IC_{50} (μg/ml)	
	D6	W2
Seedpods extract	8.4 ± 0.3	8.6 ± 1.0
Elatadihydrochalcone (**1**)	2.8 ± 0.3	5.5 ± 0.3
β-Acetoxyelatadihydrochalcone (**1a**)	9.6 ± 2.1	12.6 ± 0.5
Obovatin (**10**)	4.9 ± 1.7	6.4 ± 1.1
Obovatin methyl ether (**11**)	3.8 ± 0.3	4.4 ± 0.6
Deguelin	6.3 ± 1.8	8.9 ± 2.0
Chloroquine	0.008 ± 0.004	0.051 ± 0.010
Mefloquine	0.042 ± 0.008	0.015 ± 0.002

2006), especially of chalcones (Li et al., 1995; Liu et al., 2001), have been reported. However this is the first report on the antiplasmodial activity of a β-hydroxydihydrochalcone.

3. Experimental

3.1. General experimental procedures

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC on silica gel 60 (70–230 mesh). EIMS: direct inlet, 70 eV, on SSQ 710, Finnigan MAT mass spectrometer. ^{1}H-NMR (300 or 200 MHz) and ^{13}C-NMR (75 MHz) on Bruker or Varian-Mercury spectrometers using TMS as internal standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

3.2. Plant material

The seedpods of *Tephrosia elata* were collected from Kilungu hills in Makueni district, Kenya, in August, 2007. The plant was identified by Mr. Patrick C. Mutiso of the University Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen (Mutiso-027/August 2007) is deposited.

3.3. Extraction and isolation

The dried and ground seedpods (1.7 kg) of *T. elata* were extracted with dichloromethane-methanol (1:1) by cold percolation (3× 1 L). Removal of the solvent resulted in brown oily extract (53 g). Some 50 g of the extract was subjected to CC on silica gel (1 kg) eluting with *n*-hexane containing increasing amounts of ethyl acetate (3%, 5%, 7%, 10%, 13%, 15%, 20%, and 30% ethyl acetate in *n*-hexane each of *ca.* 1 L). The fraction eluted with 3% EtOAc in *n*-hexane was further separated on Sephadex LH20 column (eluent CH_2Cl_2/MeOH, 1:1) and by Prep TLC on silica gel (*n*-hexane/CH_2Cl_2, 3:2) to yield obovatin methyl ether (**11**) (37.5 mg) (Andrei et al., 2000), obovatin (**10**) (24.8 mg) (Andrei et al., 2000) and obovatachalcone (3.5 mg) (Andrei et al., 2000). The fraction which was eluted with 10% EtOAc in hexane was purified by prep TLC (hexane/acetone, 9:1) and yielded elatadihydrochalcone (**1**) (89.4 mg). The fraction eluted with 13% EtOAc in hexane was also purified by Prep TLC (hexane/CH_2Cl_2/EtOAc, 5:9:1) to give deguelin (32.3 mg) (Andrei et al., 1997).

3.4. Elatadihydrochalcone (1)

Light yellow oil. R_f = 0.2 (hexane/EtOAc, 9:1). $[\alpha]_D^{28.8} = +34.5^\circ$ (c 0.71, $CHCl_3$). UV λ_{max}: 275, 296, 308 nm. CD (MeOH, 0.01): $[\ominus]_{350}$ +3187, $[\ominus]_{290}$ −8346. HRMS $[M+1]^+$ found: 355.1535, calculated for $C_{21}H_{23}O_5$, 355.1545. ^{1}H and ^{13}C NMR (Table 1). EIMS (*m*/*z*, rel. int.): 354 (52, $[M]^+$), 339 (15, $[M-Me]^+$), 336 (15, $[M-H_2O]^+$), 321 (55, $[M-Me-H_2O]^+$), 233 (98, $C_{13}H_{13}O_4$), (217 (100, $C_{12}H_9O_4$), 107 (9, C_7H_7O). Acetylation of **1** (35 mg) with acetic anhydride and

pyridine yielded **1a** (26 mg) after the usual workup and purification. ^{1}H and ^{13}C NMR (Table 1).

3.5. In vitro antiplasmodial activity assay

The crude extract and pure compounds were assayed using a non-radioactive assay technique (Smilkstein et al., 2004) with modifications to determine 50% growth inhibition of cultured parasites. This is an accepted method for assaying *in vitro* drug susceptibility using the fluorochrome called "SYBR Green I", a non-radioactive intercalating DNA marker that accurately depicts *in vitro* parasite replication. This test replaces the older, ^{3}H-hypoxanthine uptake assay, is fully endorsed by the WHO. Briefly, two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *P. falciparum* were grown as described in the literature (Johnson et al., 2007). Concurrently, twofold serial dilutions of the drugs chloroquine (1.953–1000 ng/ml), mefloquine (0.488–250 ng/ml) and test sample (97.7–50,000 ng/ml) were prepared on a 96-well plate. The culture-adapted *P. falciparum* were added on to the plate containing dose range of drugs and incubated in gas mixture (5% CO_2, 5% O_2, and 90% N_2) at 37 °C. The assay was terminated 72 h later by freezing at −80 °C.

After thawing, lysis buffer containing SYBR Green I (1× final concentration) were added directly to the plates and gently mixed by using the Beckman Coulter Biomek 2000 automated laboratory workstation (Beckman Coulter, Inc., Fullerton, CA). The plates were incubated for 5–15 min at room temperature in the dark. Parasite growth inhibition was quantified by measuring the per-well relative fluorescence units (RFU) of SYBR green I dye using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 and 535 nm, respectively, and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating IC_{50}'s for each drug using Prism 4.0 software for Windows (Graphpad Software, San Diego, CA). A minimum of three separate determinations was carried out for each sample. Replicates had narrow data ranges hence presented as mean ± S.D.

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