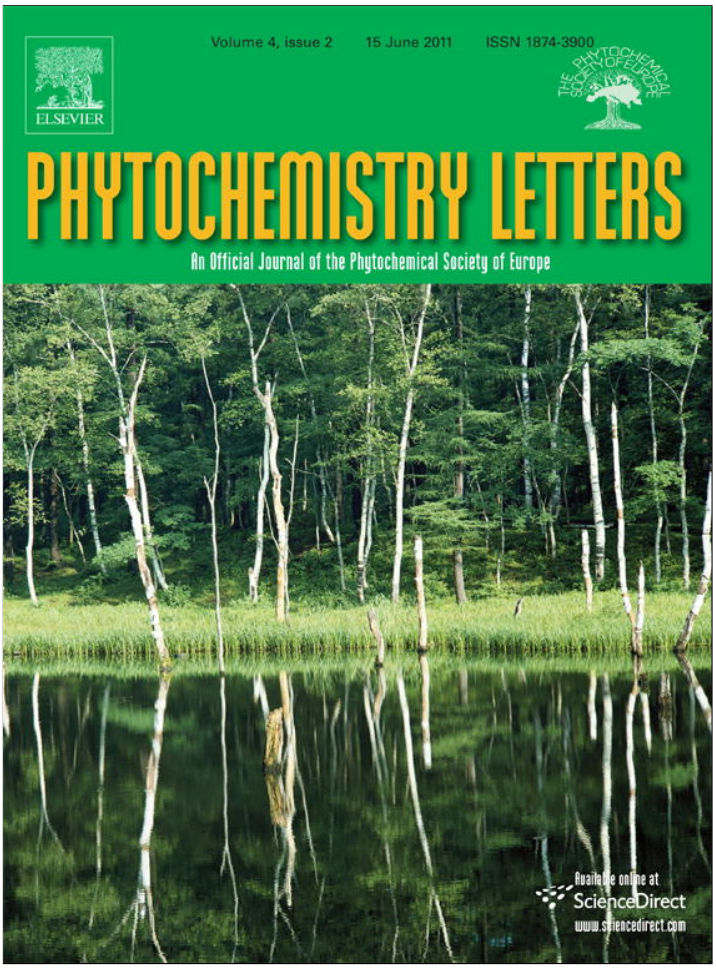

Phytochemistry Letters 4 (2011) 176–178

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Terpurinflavone: An antiplasmodial flavone from the stem of *Tephrosia Purpurea*

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ARTICLE INFO

Article history:
Received 10 September 2010
Received in revised form 16 December 2010
Accepted 23 February 2011
Available online 29 March 2011

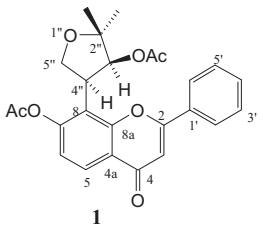
Keywords:
Tephrosia purpurea
Leguminosae
Stem
Flavone
Terpurinflavone
Antiplasmodial

ABSTRACT

The stem extract of *Tephrosia purpurea* showed antiplasmodial activity against the D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of *Plasmodium falciparum* with IC_{50} values of 10.47 ± 2.22 μg/ml and 12.06 ± 2.54 μg/ml, respectively. A new prenylated flavone, named terpurinflavone, along with the known compounds lanceolatin A, (−)-semiglabrin and lanceolatin B have been isolated from this extract. The new compound, terpurinflavone, showed the highest antiplasmodial activity with IC_{50} values of 3.12 ± 0.28 μM (D6) and 6.26 ± 2.66 μM (W2). The structures were determined on the basis of spectroscopic evidence.

1. Introduction

Tephrosia Pers (Leguminosae-Papilionoideae) is a large tropical and sub-tropical genus estimated to contain about three hundred species (Waterman and Khalid, 1980; Abou-Douh et al., 2005) out of which thirty species are found in Kenya (Tarus et al., 2002). The extracts of some *Tephrosia* species have shown various biological activities including antiplasmodial (Muiva et al., 2009), antibacterial (Abou-Douh et al., 2005) anticancer (Santram et al., 2006) and insecticidal activities (Delfel et al., 1970). The taxon *T. purpurea* is among the most widely used *Tephrosia* species in traditional medicine (Damre et al., 2003). Various biological activities including antibacterial (Hegazy et al., 2009; Chinniah et al., 2009), antidiabetic and antioxidant (Pavana et al., 2009), immunomodulatory (Damre et al., 2003), anti-inflammatory (Damre et al., 2003) and cancer chemopreventive activities (Chang et al., 2000) have been reported for extracts and pure compounds from this plant. *T. purpurea.* is rich in prenylated flavonoids including flavones (Hegazy et al., 2009; Pelter et al., 1981), flavanones (Pelter et al., 1981; Gupta et al., 1980), chalcones (Chang et al., 2000; Pelter et al., 1981) and rotenoids (Ahmad et al., 1999). In the search for compounds with antiplasmodial activity from Kenyan plants, the stem of *T. purpurea* has been investigated. This report is on the isolation and characterization of a new prenylated flavone, named terpurinflavone (**1**), with antiplasmodial activity along with three known flavonoids.



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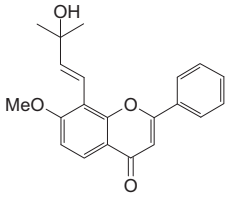

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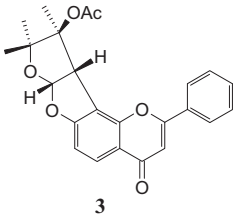

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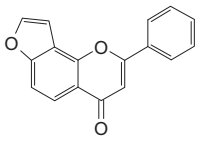

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doi:10.1016/j.phytol.2011.02.010

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with an R_f value of 0.45 in n-hexane/ethyl acetate (3:2). It showed $[M+H]^+$ peak at m/z 437.1593 in its positive electrospray ionization time of flight mass spectrum (ESI–TOF-MS) constituting the molecular formula $C_{25}H_{24}O_7$. The presence of a flavone skeleton was deduced from the UV (λ_{max} 295, 325 nm), ^{1}H (δ 6.79s for H-3) and ^{13}C (163.9 for C-2, 108.5 for C-3 and 177.6 for C-4) NMR spectroscopic data (Table 1). The presence of unsubstituted ring-B was clearly shown in ^{1}H (δ 7.63m for H-3′/4′/5′, δ 8.18m for H-2′/6′) and ^{13}C (δ 127.9 for C-2′/6′, δ 130.7 for C-3′/5′, δ 133.1 for C-4′ and 133.6 for C-1′) NMR spectra. In ring-A, an AX protons which are *ortho*-coupled at δ 8.00 and 6.94 (J = 8.5 Hz) were assigned to H-5 and H-6, respectively, with C-7 and C-8 being substituted with an acetoxy (at C-7) and a tetrahydrofuran ring (at C-8) derived from a modified prenyl group as in tephrorin B (Chang et al., 2000). The HMBC spectrum showed correlation of H-4″ (δ 4.44) with C-5″ (79.1), C-3″ (78.7), C-2″ (84.0), C-7 (167.9), C-8 (116.0) and C-8a (155.9) confirming that the tetrahydrofuran ring is placed at C-8. The presence of a second acetate group was also evident from the NMR spectra (Table 1) and placed at C-3″ of the tetrahydrofuran group based on the HMBC spectrum which showed correlation of H-3″ (δ 5.35) with acetoxy carbon (δ 170.3) and the two methyl carbon atoms (δ_C 22.4 and 24.4) at C-2″.

The ^{1}H and ^{13}C NMR chemical shift values of the tetrahydrofuran ring of **1** were quite similar to those reported for tephrorin B (Chang et al., 2000). The coupling constant (J = 8.5 Hz) between H-3″ and H-4″ indicated that the relative orientation of these two protons is *cis* as in tephrorin B. In the NOESY spectrum, NOE interaction of H-3" with H-4" supported the *cis* geometry. The new compound was therefore characterized as 7-acetoxy-8-[3″-acetoxy-2″,2″-dimethyltetrahydro-4″-furanyl]flavone (**1**) for which trivial name terpurinflavone was assigned. The isolation of this new compound once again demonstrated the unique capacity of *T. purpurea* to oxidize the C-7 methoxyl group in compound **2** and cyclize it with the adjacent 2-hydroxy-2-methylbut-1-enyl group into complex C-8 substituted flavonoids (Pelter et al., 1981).

The EtOAc fraction of the CH_2Cl_2/MeOH (1:1) extract of *T. purpurea* showed moderate antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2), strains of *Plasmodium falciparum* with IC_{50} values of 10.47 ± 2.22 μg/ml and 12.06 ± 2.54 μg/ml, respectively. The pure compounds isolated from this plant were also tested with the new compound terpurinflavone (**1**) exhibiting the highest activity with IC_{50} values of 3.12 ± 0.28 μM and 6.26 ± 2.66 μM against D6 and W2 strains of *P. falciparum*, respectively. Lanceolatin A (**2**) also showed significant activity with IC_{50} values of 11.36 ± 2.97 and 14.97 ± 3.07 μM against D6 and W2 strains of *P. falciparum*, respectively (Table 2). The activity of the crude extract could be due to these compounds, especially that of compound **1** which showed the highest activity. Recently, the *in vitro* and *in vivo* antiplasmodial activities of synthetically modified flavones have been reported (Auffret et al., 2007). It is therefore worthwhile to isolate and test the other flavones of this plant.

3. Experimental

3.1. General

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 (500 or 200 MHz) and ^{13}C-NMR (125 or 50 MHz) on Bruker or Varian-Mercury spectrometers using TMS as internal standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data along with HMBC correlations for compound **1** in acetone-d_6.

Position	δ_H (J in Hz)	δ_C	HMBC (^2J, ^3J)
2		163.9	
3	6.79 s	108.5	C-1′, 2, 4, 4a
4		177.6	
4a		119.9	
5	8.00 d (8.5)	129.3	C-4, 7, 8a
6	6.94 d (8.5)	110.1	C-4a, 8
7		167.9	
8		116.0	
8a		155.9	
1′		133.6	
2′/6′	8.18 m	127.9	C-1′, 2
3′/5′	7.63 m	130.7	C-2′/6′, 4’
4′	7.63 m	133.1	
2″		84.0	
3″	5.35 d (8.5)	78.7	C-2″, 5″, *C*OMe-3″
4″	4.44 ddd (2.0, 8.0, 8.5)	42.3	C-2″, 3″, 5″, 7, 8, 8a
5″	4.84 dd (8.0, 9.5)	79.1	C-3″
	5.02 dd (2.0, 9.5)		C-4″, 8
*C*OMe-3″		170.3	
CO*Me*-3″	1.61 s	20.9	*C*OMe-3″
Me-2″	1.76 s	22.4	C-2″, 3″, Me-2″
Me-2″	1.61 s	24.4	C-2″, 3″, Me-2″
*C*OMe-7		170.8	
CO*Me*-7	2.00 s	23.0	*C*OMe-7

3.2. Plant material

T. purpurea was collected from Kilifi district, Coast province, Kenya in August, 2007. The plant was identified by Mr. Patrick C. Mutiso of the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen (Mutiso-520-August 2007) is deposited.

3.3. Extraction and isolation

Air dried and ground stems of *T. purpurea* (2 kg) were extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature (3 × 1.5 L). The extract was filtered and the solvent removed under vacuum using a rotary evaporator at 35 °C. This gave dark oily extract that was partitioned between water and ethyl acetate. The organic layer (36 g) was subjected to CC on silica gel (400 g) eluting with n-hexane containing increasing percentages (2%, 4%, 6%, 8%, 10%, 12.5%, 15%, 17.5%, 20%, 25%, 30%, 40%, 50%, 75% and 100%) of ethyl acetate and gave 15 fractions each of 1.5 L. The fraction eluted with 6% ethyl acetate in n-hexane was crystallized from n-hexane-dichloromethane mixture to yield lanceolatin B (**4**, 317 mg) (Pelter et al., 1981). The fractions eluted with 10% and 12.5% ethyl acetate in n-hexane were mixed and further purified by CC on Sephadex LH-20 (CH_2Cl_2–MeOH; 1:1) and crystallization from acetone gave semiglabrin (**3**, 87 mg) (Waterman and Khalid, 1980; Abou-Douh et al., 2005; Pelter et al., 1981). The fraction eluted with 15% ethyl acetate was separated on

Table 2
In vitro IC_{50} values of pure compounds isolated from *T. purpurea* against the D6 and W2 strains of *P. falciparum*.

Sample	IC_{50} (μM ± SD)	
	D6	W2
Terpurinflavone (**1**)	3.12 ± 0.28	6.26 ± 2.66
Lanceolatin A (**2**)	11.36 ± 2.97	14.97 ± 3.09
Semiglabrin (**3**)	25.77 ± 6.08	35.58 ± 5.41
Lanceolatin B (**4**)	27.02 ± 2.65	35.99 ± 4.24
Mefloquine	–	0.013 ± 0.002
Chloroquine	0.035 ± 0.003	–

Sephadex LH-20 (CH_2Cl_2–MeOH; 1:1) to give terpurinflavone (**1**, 34 mg). The fraction that was eluted with 25% ethyl acetate was crystallized from hexane/dichloromethane mixture to yield lanceolatin A (**2**, 106 mg) (Waterman and Khalid, 1980; Abou-Douh et al., 2005; Pelter et al., 1981).

3.4. Terpurinflavone (1)

White amorphous powder. $R_f = 0.45$ (n-C_6H_{14}/EtOAc, 3:2), melting point 144–145 °C. $[\alpha]_D^{25.3} = +58.24$ (c 0.9, MeOH). UV λ_{max} (MeOH) nm: 295, 325 nm. ^1H and ^{13}C NMR (Table 1). ESI–TOF-MS: $[M+H]^+$): 437.1593 for $C_{25}H_{24}O_7$ calculated for 437.1600. EIMS *m/z* (rel. int.): 437 (8 $[M]^+$), 376 (15), 317 (55), 316 (100), 264 (27), 265 (75).

3.5. 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. This method uses the fluorochrome called "SYBR Green I", a non-radioactive DNA dye that accurately depicts *in vitro* parasite replication. Briefly, the parasites were cultured as described in literature (Johnson et al., 2007), to establish replication robustness and attain a 3–8% parasitemia. 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 reconstituted to 1% parasitemia and added on to the plate containing dose range of the reference drugs and test samples 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 for 24 h.

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 1 dye using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 nm and 535 nm, respectively, and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating 50% inhibition concentration (IC_{50}'s) for each drug using Prism 4.0 windows software (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 ±SD.

Acknowledgements

We acknowledge the Deutche Forschungemeinschaft (DFG), Germany and the Germany Federal Ministry for Economic Cooperation and Development (BMZ) within the DFG/BMZ Programme "Research Cooperation with Developing Countries", for supporting the study. Mr. P.C. Mutiso is thanked for the identification of the plant material.

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