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4′-Prenyloxyderrone from the stem bark of *Millettia oblata* ssp. *teitensis* and the antiplasmodial activities of isoflavones from some *Millettia* species

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ABSTRACT

The CH_2Cl_2/MeOH (1:1) extract of the stem bark of *Millettia oblata* ssp. *teitensis* showed antiplasmodial activity (IC_{50} = 10–12 μg/mL) against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Chromatographic separation of the extract led to the isolation of a new isoflavone, 4′-prenyloxyderrone (**1**), together with known isoflavones (8-*O*-methylretusin, durmillone, maximaisoflavone B, maximaisoflavone H and maximaisoflavone J), a rotenoid (tephrosin) and a triterpene (lupeol). Similar investigation of *Millettia leucantha* resulted in the identification of the isoflavones afromosin and wistin, and the flavone chrysin. The identification of these compounds was based on their spectroscopic data. Five of the isoflavones isolated from these plants as well as 11 previously reported compounds from *Millettia dura* were tested and showed good to moderate antiplasmodial activities (IC_{50} = 13–53 μM), with the new compound, 4′-prenyloxyderrone, being the most active (IC_{50} = 13–15 μM).

1. Introduction

Flavonoids and isoflavonoids are known for wide variety of biological activities including antiplasmodial (Batista et al., 2009; Bero et al., 2009; Kaur et al., 2009), antioxidant (Arredondo et al., 2004), antimicrobial (Yenesew et al., 2005) and cancer chemoprevention (Walle, 2007). Whereas flavonoids are ubiquitous, the distribution of isoflavonoids is rather restricted to the subfamily Papilonoideae of the family Leguminoseae with sporadic occurrence in few other families (Lapčík, 2007).

In our interest on the antiplasmodial activities of Kenyan plants belonging to the family Leguminoseae, we have identified several prenylated flavonoids and isoflavonoids with antiplasmodial activities from the genera *Erythrina* (Yenesew et al., 2012), *Tephrosia* (Juma et al., 2011; Muiva et al., 2009) and *Millettia* (Yenesew et al., 2003b). The genus *Millettia* is a rich source of isoflavonoids, especially isoflavones and rotenoids (Derese et al., 2003; Yenesew et al., 1996, 1997, 1998, 2003a,b). In this study we report the isolation of a new isoflavone along with known compounds from an endemic Kenyan *Millettia* species, *Millettia oblata* ssp. *teitensis* (J.B. Gillett). Similar investigation of *Millettia leucantha* (Vatke) only gave known compounds. In addition, the antiplasmodial activities of some of the compounds isolated from these plants as well as those previously isolated from *Millettia dura* (Dunn) (Derese et al., 2003; Yenesew et al., 1996, 1997) are reported.

2. Results and discussion

The dried and ground stem bark of *M. oblata* ssp. *teitensis* was extracted with CH_2Cl_2/MeOH (1:1). The extract showed antiplasmodial activity against the chloroquine-resistant Indochina 1 (W2) and chloroquine-sensitive Sierra Leone 1 (D6) strains of *Plasmodium falciparum* (Table 1). Chromatographic separation of the extract yielded a new isoflavone (**1**) along with seven known compounds.

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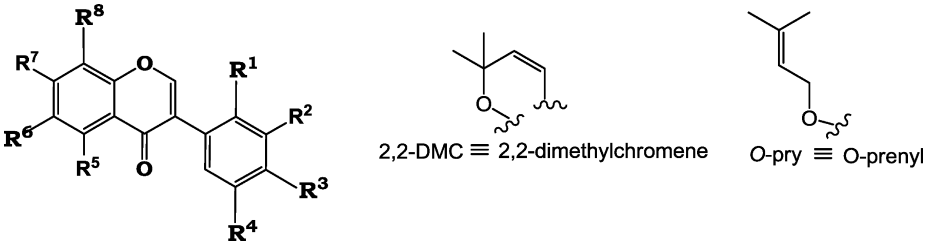
Table 1
In vitro antiplasmodial activities of (a) crude extracts and (b) isoflavones of *Millettia* species against the W2 and D6 strains of *Plasmodium falciparum*.



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Extracts and isoflavones									IC_{50} in μM	
									W2	D6
(a) *Crude extracts*[a]										
Millettia oblata ssp. *teitensis* (stem bark extract)									10.0 ± 2.3	12.0 ± 1.2
M. dura (stem bark extract)									21.4 ± 2.9	23.6 ± 4.1
M. dura (seedpods extract)									18.5 ± 2.6	18.8 ± 2.5
M. dura (seeds extract)									25.4 ± 1.9	22.5 ± 0.9
	R^1	**R^2**	**R^3**	**R^4**	**R^5**	**R^6**	**R^7**	**R^8**	**W2**	**D6**
(b) *Isoflavones*										
7,2′-Dimethoxy-4′,5′-methylenedioxyisoflavone	OCH_3	H	–OCH_2O–		H	H	OCH_3	H	45.6 ± 7.0	47.5 ± 2.1
Maximaisoflavone B	H	H	–OCH_2O–		H	H	O-Pry	H	42.0 ± 4.2	36.0 ± 4.8
Maximaisoflavone J	H	H	OCH_3	H	H	H	O-Pry	H	29.7 ± 6.8	35.7 ± 3.6
Maximaisoflavone H	H	H	OCH_3	H	H	H	–OCH_2O–		38.8 ± 2.0	45.6 ± 5.7
7,3′-Dimethoxy-4′,5′-methylendioxyisoflavone	H	OCH_3	–OCH_2O–		H	H	OCH_3	H	48.4 ± 5.5	37.7 ± 4.9
Mildurone	OCH_3	H	–OCH_2O–		H	OCH_3	OCH_3	H	44.1 ± 6.2	35.9 ± 5.1
Wistin	H	H	OCH_3	H	H	OCH_3	O-β-glu	H	23.2 ± 2.8	22.3 ± 3.2
Nordurlettone	H	H	O-pry	H	H	H	OH	H	28.9 ± 5.3	25.1 ± 4.7
4′-Prenyloxyderrone (**1**)	H	H	O-Pry	H	OH	H	2,2-DMC		14.9 ± 2.2	13.3 ± 2.4
Isoerythrin A 4′-(3-methylbut-2-enyl) ether	H	H	O-Pry	H	H	H	2,2-DMC		21.6 ± 1.5	19.3 ± 2.1
Calopogoniumisoflavone A	H	H	OCH_3	H	H	H	2,2-DMC		51.5 ± 5.7	45.8 ± 3.2
Durmillone	H	H	–OCH_2O–		H	OCH_3	2,2-DMC		25.1 ± 4.2	37.3 ± 4.8
Jamaicin	OCH_3	H	–OCH_2O–		H	H	2,2-DMC		38.6 ± 3.2	41.0 ± 5.6
Isojamaicin	H	OCH_3	–OCH_2O–		H	H	2,2-DMC		38.9 ± 2.1	48.7 ± 2.9
Durallone	H	H	OCH_3	OCH_3	H	OCH_3	2,2-DMC		50.0 ± 6.1	32.7 ± 3.6
6-Methoxycalopogonium isoflavone A	H	H	OCH_3	H	H	OCH_3	2,2-DMC		53.1 ± 4.7	34.8 ± 5.2

O-β-glu = *O*-β-glucopyranoside.
Standard drugs: chloroquine (IC_{50} = 0.09 ± 0.01 μM for W2; 0.008 ± 0.001 μM for D6), quinine (IC_{50} = 0.26 ± 0.01 μM for W2; 0.051 ± 0.001 μM for D6).
[a] IC_{50} values for crude extracts are given in μg/mL.

The known compounds were identified as durmillone (Ollis et al., 1967), 8-*O*-methylretusin (Jurd et al., 1972), maximaisoflavone B (Dagne et al., 1991), maximaisoflavone H (Dagne et al., 1991; Yenesew et al., 1996), maximaisoflavone J (Murthy and Rao, 1985), tephrosin (Ollis et al., 1967; Luyengi et al., 1994) and lupeol (Furukawa et al., 2002).

The new compound (**1**) was isolated as white crystals, m.p. 130–132 °C. The HRMS showed a $[M]^+$ at *m/z* 404.1603 corresponding to the molecular formula $C_{25}H_{24}O_5$. The ^1H (δ 7.93 for H-2) and ^{13}C (δ 152.9 for C-2, 123.8 for C-3 and 181.3 for C-4) NMR spectra (Table 2) indicated that compound **1** is an isoflavone derivative (Yenesew et al., 1996). The presence of a chelated hydroxyl (δ_H 12.94, OH-5), a 2,2-dimethylpyrano and a prenyloxy groups were evident from the ^1H and ^{13}C NMR spectra (Table 2). In the EIMS, the fragment ion at *m/z* 203 (**1a**, Fig. 1), formed through the loss of methyl group and *retro*-diels-Alder (RDA) cleavage of ring-C, showed that the hydroxyl (at C-5) and the 2,2-dimethylpyrano groups are placed in ring-A. In the HMBC spectrum (Table 2), the singlet at δ_H 6.25 (H-6) and the chelated hydroxyl (δ_H 12.94), showed correlation with C-5 (δ_C 162.5), while H-4″ (δ_H 6.71) correlated with C-7 (δ_C 159.4), C-8 (δ_C 101.5) and C-8a (δ_C 152.5), fixing the 2,2-dimethylpyrano ring at C-7/C-8.

In the ^1H NMR spectrum, the presence of an AA′XX′ spin system centered at δ_H 7.44 and 6.96 (*d*, *J* = 9.0 Hz) indicated that ring-B is

Table 2
^1H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **1** in CD_2Cl_2.

Position	δ_C	δ_H (m, J in Hz)	HMBC (^2J, ^3J)
2	152.9	7.93 (*s*)	C-3, -8a, -4, -1′
3	123.8		
4	181.3		
4a	106.3		
5	162.5		
6	100.2	6.25 (*s*)	C-4a, -5, -7, -8
7	159.4		
8	101.5		
8a	152.5		
1′	123.1		
2′/6′	130.4	7.44 (*d*, 9.0)	C-1′, -3, -4′, -2′/6′
3′/5′	114.9	6.96 (*d*, 9.0)	C-1′, -3′, -4′
4′	159.8		
2″	78.4		
2″-Me_2	28.2	1.47 (*s*)	C-2″
3″	127.8	5.62 (*d*, 10.2)	C-2″, -8, 2″-Me_2
4″	114.7	6.71 (*d*, 10.2)	C-2″, -7, -8, -8a
1‴	65.1	4.55 (*d*, 6.6)	C-2‴, -3‴, -4′
2‴	119.9	5.49 (*d*, 6.6)	
3‴	138.4		
4‴-Me	18.2	1.80 (*s*)	5‴-Me
5‴-Me	25.7	1.76 (*s*)	4‴-Me
5-OH		12.94 (*s*)	C-4a, -5, -6

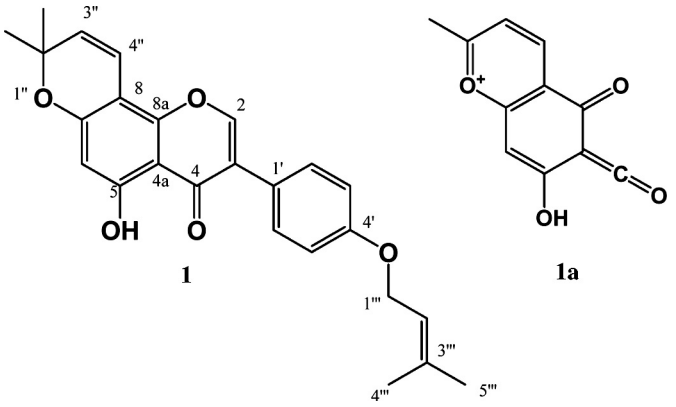

Fig. 1. Structure of compound **1** and its RDA fragment ion (**1a**).

substituted at C-4′ with the prenyloxy group, and its placement was confirmed by the HMBC correlation of CH_2-1‴ with C-4′. Therefore, the new compound (**1**) was characterized as 5-hydroxy-2″,2″-dimethylpyrano[5″,6″:7,8]-4′-prenyloxyisoflavone for which the trivial name 4′-prenyloxyderrone is suggested (see Fig. 1).

Similar investigation of the methanol extract of the root bark of *M. leucantha* led to the isolation of the isoflavone afrormosin (Gong et al., 2009), the 7-β-D-*O*-glucoside of afrormosin, wistin (Kaneko et al., 1988), and the flavone chrysin (Wolfman et al., 1994). Wistin and chrysin are reported here for the first time in the genus *Millettia*.

The *in vitro* antiplasmodial activities of flavonoids and isoflavonoids are well documented (Batista et al., 2009; Bero et al., 2009; Kaur et al., 2009); with some chalcones (Ziegler et al., 2004; Batista et al., 2009; Bero et al., 2009), flavones (Bero et al., 2009; Juma et al., 2011), flavanones (Batista et al., 2009; Bero et al., 2009), isoflav-3-enes (Yenesew et al., 2012), and biflavonoids (Batista et al., 2009; Bero et al., 2009; Kaur et al., 2009) showing IC_{50} values less than 10 μM. The chalcone lichochalcone A, besides showing *in vitro* antiplasmodial activity (IC_{50} 5.6 ± 0.6 μM), it also markedly decreased parasitemia in mice infected with *Plasmodium yoelii*, and hence identified as a lead structure for antimalarial drug development (Ziegler et al., 2004). Furthermore, licochalcone A (as well as some other flavonoids) also shows synergistic effects with artemisinin against *P. falciparum*, providing the other dimension where flavonoids can be used in malaria control (Mishra et al., 2009; Ferreira et al., 2010).

Among the isoflavonoids, the isoflavones are the most abundant; however, the report on their antiplasmodial activities is limited to few (Kraft et al., 2000; Andayi et al., 2006; Kaur et al., 2009). In order to expand the information, five isoflavones isolated in this study along with eleven previously reported from *M. dura* (Derese et al., 2003; Yenesew et al., 1996, 1997) were tested for their antiplasmodial activities against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* (Table 1). In this test the new compound, 4′-prenyloxyderrone (**1**), showed good activity (IC_{50} = 13–15 μM), while the rest of the isoflavones had IC_{50} values between 20 and 53 μM, which are classified as moderate, based on the criteria proposed by Batista et al. (2009). Whether these isoflavones also have *in vivo* antiplasmodial activities, or synergistic effects with antimalarial drugs, is yet to be established.

3. Experimental

3.1. General

UV/VIS spectra were recorded using a Pye-Unicam SPS-150 Spectophotometer. HR-EIMS was done on a Micromass GC-TOF micromass spectrometer (Micromass, Wythenshawe, Waters Inc., UK). ^{13}C NMR (125 or 50 MHz) and ^{1}H NMR (500 or 200 MHz) were run on Bruker or Varian-Mercury spectrometers using residual solvent signals as reference. COSY, NOESY, HMBC and HMQC spectra were acquired using standard Bruker software.

3.2. Plant materials

The stem barks of *M. oblata* ssp. *teitensis* were collected from Taita Hill forest, Coast province, Kenya in July 2009; *M. leucantha* was collected at Kavingo shopping center, Makueni District, Kenya, December 2008; *M. dura* was collected in January, 2000, from and around Chiromo campus, University of Nairobi. The plants were identified by Mr. Patrick C. Mutiso of the University Herbarium, School of Biological Sciences, University of Nairobi, where voucher specimen are deposited.

3.3. Extraction and isolation of compounds from the stem bark of M. oblata ssp. teitensis

Air dried and ground stem bark of *M. oblata* ssp. *teitensis* (450 g) was extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The extract was evaporated under reduced pressure to yield a brown extract (23 g). A 20 g portion of the extract was subjected to column chromatography on silica gel (200 g) eluting with n-hexane containing increasing amounts of ethyl acetate and some 23 fractions each *ca* 1 L were collected.

Crystallization (from dichloromethane/methanol; 1:1) of the fraction eluted with 3% EtOAc in n-hexane gave 4′-prenyloxyderrone (**1**, 56 mg). Similarly crystallization of the combined fractions eluted with 7–12% EtOAc gave durmillone (400 mg). Crystallization (from dichloromethane/methanol; 1:1) of the fraction eluted with 15% EtOAc gave lupeol (40 mg). Similar treatment of the fraction eluted with 20% EtOAc gave a mixture of maximaisoflavone B and maximaisoflavone J (180 mg). The fraction eluted with 25% EtOAc was subjected to CC over Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH; 1:1) and gave maximaisoflavone H (10 mg). The 30% EtOAc eluent was subjected to CC on silica gel (27 g, eluting with n-hexane containing increasing amounts of ethyl acetate) and afforded tephrosin (78 mg) and 8-*O*-methylretusin (5 mg).

3.4. Extraction and isolation from the root bark of M. leucantha

Dried and ground root bark (500 g) of *M. leucantha* was extracted with CH_2Cl_2/MeOH (1:1) to give 22 g of crude extract. Part of the extract (10 g) was subjected to CC on silica gel (100 g) eluting with dichloromethane containing increasing amounts of methanol and some 15 fractions each *ca* 1 L were collected. The fraction eluted with 1.5% MeOH after purification using PTLC (2% MeOH in CH_2Cl_2) gave afrormosin (6 mg); 4% MeOH gave crystals of chrysin (10 mg); and 8% MeOH was purified by CC over Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH; 1:1) yielding wistin (55 mg).

3.5. Extraction and isolation from M. dura

The isolation and identification of isoflavones from the stem bark (Derese et al., 2003) and seed pods (Yenesew et al., 1996, 1997) of *M. dura* has already been described.

3.6. 4′-Prenyloxyderrone (*1*)

White crystals; m.p. 130–132 °C. UV λ_{max} (MeOH) nm: 226, 264. ^{1}H NMR (Table 2). ^{13}C NMR (Table 2). EIMS *m/z* (rel. Int.) 404 (20, $[M]^+$, $C_{25}H_{24}O_5$), 336 (25, $[C_{20}H_{16}O_5]^+$), 321 (100, $[C_{19}H_{13}O_5]^+$), 203 (9, $[C_{11}H_7O_4]^+$), 69 (36, $[C_5H_9]^+$). HR-EIMS $[M]^+$: found *m/z* 404.1603 $C_{25}H_{24}O_5$ (calcd. mass 404.1618).

3.7. In vitro *antiplasmodial activity*

The crude extracts and pure compounds were assayed using a non-radioactive assay technique as described by Smilkstein et al. (2004) with modifications (Juma et al., 2011; Yenesew et al., 2012).

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