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Anti-plasmodial flavonoids from the stem bark of *Erythrina abyssinica*

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Abstract

The ethyl acetate extract of the stem bark of *Erythrina abyssinica* showed anti-plasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 7.9 ± 1.1 and 5.3 ± 0.7 μg/ml, respectively. From this extract, a new chalcone, 2′,3,4,4′-tetrahydroxy-5-prenylchalcone (trivial name 5-prenylbutein) and a new flavanone, 4′,7-dihydroxy-3′-methoxy-5′-prenylflavanone (trivial name, 5-deoxyabyssinin II) along with known flavonoids have been isolated as the anti-plasmodial principles. The structures were determined on the basis of spectroscopic evidence.

Keywords: *Erythrina abyssinica*; Leguminosae; Stem bark; Chalcone; Flavanone; 5-Prenylbutein; 5-Deoxyabyssinin II; Anti-plasmodial; Malaria; *Plasmodium falciparum*

1. Introduction

There are five *Erythrina* species (Leguminosae) in Kenya (Bentjee, 1994). Among these, *E. abyssinica* is the most widely used in traditional medicine where it is used for treatment of malaria and microbial infections (Kokwaro, 1993). The presence of prenylated flavonoids from the roots (Kamat et al., 1981; Yenesew et al., 2003a) and stem bark (Ichimaru et al., 1996; Moriyasu et al., 1998) has been reported. The anti-microbial (Kamat et al., 1981) and anti-plasmodial (Yenesew et al., 2003a) properties of the roots of this plant have been associated with some of these flavonoids.

In earlier work we have reported new flavonoids and isoflavonoids from the stem bark (Yenesew et al., 1998b, 2003b) and the root bark (Yenesew et al., 2002) of *E. burttii* and also from the stem bark of *E. sacleuxii* (Yenesew et al., 1998a, 2000). Here, we report the isolation and characterization of a new chalcone and a new flavanone, along with known flavonoids as the anti-plasmodial principles of the stem bark of *E. abyssinica*.

2. Results and discussion

The ethyl acetate extract of the stem bark of *E. abyssinica* showed anti-plasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 7.9 ± 1.1 and 5.3 ± 0.7 μg/ml, respectively. Chromatographic separation of the extract led to the

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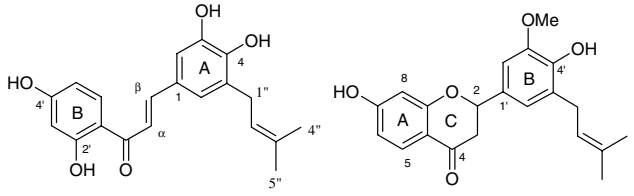
isolation of a new chalcone and a new flavanone along with known flavonoids.

Compound **1** was isolated as yellow amorphous powder. The UV (λ_{max} 375 nm), ^{1}H (δ 7.63, *d*, *J* = 15.4 Hz for H-α; 7.74, *d*, *J* = 15.4 Hz for H-β) and ^{13}C (δ 192.6 for carbonyl; 117.7 for C-α; 145.8 for C-β) suggested that this compound is a chalcone. The presence of four hydroxyl (one of which is chelated) and a prenyl substituent on the chalcone skeleton was evident from the MS (M^+ 340), ^{1}H and ^{13}C NMR spectra (see Section 3.4).

In the B-ring, with the chelated hydroxyl group (δ 13.68) being at C-2′, an AXY spin system in the ^{1}H NMR spectrum (δ 8.05, *d*, *J* = 9.3 Hz for H-6′; 6.46, *dd*, *J* = 9.3, 2.6 Hz for H-5′ and 6.35, *d*, *J* = 2.6 Hz for H-3′) is in agreement with the biogenetically expected oxygenation (hydroxyl) at C-4′. Thus, the remaining two hydroxyl and the prenyl groups should be in A-ring. Two *meta*-coupled protons at δ 7.11 and 7.19 (*J* = 2.0 Hz) were readily assigned to H-2 and H-6 with C-3, C-4 and C-5 being substituted by the two hydroxyl and the prenyl groups. The chemical shift values of the carbon atoms in this ring (δ 127.0 for C-1, 113.6 for C-2, 147.4 for C-3, 145.5 for C-4, 129.4 for C-5 and 123.2 for C-6) are in agreement with the placement of the hydroxyl groups at C-3 and C-4 and the prenyl at C-5. Thus this new compound was identified as 2′,3,4,4′-tetrahydroxy-5-prenylchalcone (**1**) for which the trivial name 5-prenylbutein is assigned.

Compound **2** was isolated as amorphous solid. The UV (λ_{max} 275 and 312 nm), ^{1}H (δ 5.42, *dd*, *J* = 2.7, 13.2, for H-2; 2.71, *dd*, *J* = 2.7, −17.0 Hz for H-3α; 3.04, *dd*, *J* = 13.2, −17.0 Hz for H-3β) and ^{13}C (δ 79.5 for C-2; 43.2 for C-3 and 189.1 for C-4) NMR spectra were consistent with a flavanone skeleton. In addition, the presence of two hydroxyl, a methoxyl and a prenyl substituents were evident from the MS (M^+ 354, $C_{21}H_{22}O_5$) and NMR data (see Section 3.5). In the MS, the presence of a fragment ion at *m/z* 137 ($C_7H_5O_3$), resulting from a retro-Diels–Alder cleavage of C-ring, would place one hydroxyl group in A-ring. In the ^{1}H NMR spectrum an AXY spin system at δ 7.73 (*d*, *J* = 8.5 Hz for H-5), 6.58 (*dd*, *J* = 8.5, 2.5 Hz, for H-6) and 6.42 (*d*, *J* = 2.5 Hz, for H-8) is consistent with the placement of the biogenetically expected oxygenation (hydroxyl) at C-7 of A-ring. The B-ring should then contain hydroxyl, methoxyl and prenyl substituents. In addition to the biogenetically expected oxygenation at C-4′, the ^{13}C NMR chemical shift values of the oxygenated carbon atoms in this ring (δ 146.4 and 143.5) require that C-3′ should be oxygenated. In the ^{1}H NMR spectrum, two *meta*-coupled protons at δ 7.06 (*d*, *J* = 2.0 Hz) and 6.92 (*d*, *J* = 2.0 Hz) could then be assigned to H-2′ and H-6′ with the prenyl being at C-5′. The placement of the prenyl at C-5′ was confirmed from HMBC spectrum where the methylene protons (δ 3.35) of the prenyl group correlate with C-4′ (δ 143.5). Finally, the position of the methoxy group was fixed at C-3′, from NOE (where irradiation of the methoxyl protons resulted in enhancement of the doublet at δ 7.06 for H-2′) and HMBC (where the methoxyl protons correlates with C-3′) experiments. The ^{13}C NMR chemical shift value of the methoxyl group (δ 54.8) is consistent with it being at C-3′ rather than C-4′ (Yenesew et al., 1998b). Therefore, this new compound was characterized as 4′,7-dihydroxy-3′-methoxy-5′-prenylflavanone (**2**) for which the trivial name 5-deoxyabyssinin II is assigned, by relating it to abyssinin II, a compound which has been isolated from this plant earlier (Ichimaru et al., 1996). This compound was isolated as a racemic mixture ($[\alpha]_D = 0°$) as other flavanones with free phenolic group at C-4′ (Ichimaru et al., 1996).



The known compounds, licoagrochalcone A (Yenesew et al., 2003a), homobutein (La Duke, 1982), octacosyl ferulate (Yenesew et al., 2003a), 3-hydroxy-9-methoxy-10-prenylpterocarpene (Yenesew et al., 2003a), 7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene (Yenesew et al., 2003a) and sigmoidin E (Promsattha et al., 1988) have also been identified for the first time from the stem bark of this plant.

The flavonoids described in this study and the compounds already reported from the stem bark of this plant (Ichimaru et al., 1996; Moriyasu et al., 1998) were tested for anti-plasmodial activities against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum*. Activities have been observed for chalcones, flavanones and two isoflavonoid derivatives (Table 1), and these compounds appear to be responsible for the observed anti-plasmodial activities of the crude ethyl acetate extract of the stem bark of this plant. All the three chalcones tested were active against both strains of *P. falciparum* and the anti-plasmodial activities of chalcones are well established (Liu et al., 2001).

The stem bark of *E. abyssinica* mainly elaborates flavanones with an unmodified A-ring (**2** and abyssinone IV lack oxygenation at C-5), differing on the substitution pattern in B-ring. The flavanones with free phenolic group at C-4′ were isolated as racemic mixture, while the others are levorotatory with *S* absolute configuration at C-2. All the 10 flavanones tested were active against both strains (Table 1). Prior to this report, very

Table 1
In vitro IC_{50} values of flavonoids against W2 and D6 strains of *P. falciparum*

Flavanoids	IC_{50} (μM)	
	D6	W2
Chalcones		
5-Prenylbutein (**1**)	10.3 ± 1.3	11.2 ± 1.9
Homobutein	15.0 ± 2.8	16.1 ± 2.1
Licoagrochalcone A	12.7 ± 3.2	12.0 ± 2.6
Flavanones		
5-Deoxyabyssinin II (**2**)	13.6 ± 0.9	13.3 ± 1.5
Abyssinin III	5.8 ± 1.1	5.2 ± 1.7
Abyssinone IV	5.4 ± 1.5	5.9 ± 1.8
Abyssinone V	4.9 ± 0.8	6.1 ± 1.3
Abyssinone V-4′-methyl ether	11.3 ± 2.1	11.1 ± 2.4
Sigmoidin A	5.8 ± 0.6	5.9 ± 1.1
Sigmoidin B	8.1 ± 2.2	9.3 ± 2.7
Sigmoidin B-4′-methyl ether	13.0 ± 2.0	12.7 ± 2.9
Sigmoidin C	17.8 ± 3.6	15.8 ± 3.9
Sigmoidin E	9.1 ± 2.3	11.8 ± 2.5
Isoflavonoids		
3-Hydroxy-9-methoxy-10-prenylpterocarpene	18.2 ± 3.6	20.3 ± 3.2
7,4′-Dihydroxy-2′,5′-dimethoxyisoflav-3-ene	22.0 ± 2.4	24.9 ± 3.0
Reference drugs		
Chloroquine	0.009 ± 0.002	0.08 ± 0.003
Quinine	0.04 ± 0.01	0.21 ± 0.01

little had been reported on the anti-plasmodial activities of flavanones (Schwikkard and van Heerden, 2002).

3. Experimental

3.1. General

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

3.2. Plant material

The stem bark of *E. abyssinica* was collected near Thika town, Kenya, in December 2001. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen (AY-SGM-2001-09) is deposited.

3.3. Extraction and isolation

Air-dried and ground stem bark (5.2 kg) of *E. abyssinica* was extracted with ethyl acetate by cold percolation. Removal of the solvent under reduced pressure afforded a brown gummy extract (380 g). A 100-g portion of the extract was fractionated by CC on silica gel (1.2 kg) eluting with *n*-hexane containing increasing amounts (10%, 20%, 30%, 40%, 60%, 80% and 100%) of dichloromethane and combined into seven fractions and labelled A to G, respectively. Crystallization (from *n*-hexane/dichloromethane) of fraction A (eluted with 10% dichloromethane in *n*-hexane) gave octacosyl ferulate (48 mg). Subsequently CC of fraction B on oxalic acid impregnated silica gel (eluting with 2% ethyl acetate in hexane) and PTLC purification (5% acetone in hexane) gave 3-hydroxy-9-methoxy-10-prenylpterocarpene (42 mg), sigmoidin E (54 mg) and 7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene (72 mg). Fraction C was also purified by CC (on oxalic acid impregnated silica gel, eluting with 3% ethyl acetate in hexane) to give **2** (34 mg); while fraction D was separated by CC on oxalic acid impregnated silica gel (eluting with 4% ethyl acetate in hexane) and PTLC (20% acetone in hexane) to give **1** (24 mg), 3-methylbutein (42 mg) and licoagrochalcone A (38 mg). The isolation procedure for the other compounds from the stem bark of *E. abyssinica* has been described (Ichimaru et al., 1996; Moriyasu et al., 1998).

3.4. 5-Prenylbutein (*1*)

Amorphous powder. UV λ_{max} (MeOH) (log ε) nm: 375 (4.3). ^1H NMR (acetone-d_6, 500 MHz): δ 7.63 (1H, *d*, *J* = 15.4 Hz, H-α), 7.74 (1H, *d*, *J* = 15.4 Hz, H-β), 6.35 (1H, *d*, *J* = 2.6 Hz, H-3′), 6.46 (1H, *dd*, *J* = 2.6, 9.3 Hz, H-5′), 8.05 (1H, *d*, *J* = 9.3 Hz, H-6′), 7.11 (1H, *d*, *J* = 2.0 Hz, H-2 or H-6), 7.19 (1H, *d*, *J* = 2.0 Hz, H-6 or H-2), 3.36 (2H, *d*, H-1″), 5.37 (1H, *t*, *J* = 7.2 Hz, H-2″), 1.65, 1.72 (6H, 2X *s*, Me-4″ and Me-5″), 13.68 (1H, *s*, OH). ^{13}C NMR (acetone-d_6, 50 MHz): δ 192.6 (C=O), 117.7 (C-α), 145.8 (C-β), 127.0 (C-1), 113.6 (C-2), 147.4 (C-3), 145.5 (C-4), 129.4 (C-5), 123.2 (C-6), 114.6 (C-1′), 167.4 (C-2′), 103.6 (C-3′), 165.4 (C-4′), 108.5 (C-5′), 133.0 (C-6′), 28.6 (C-1′′), 123.8 (C-2″), 132.5 (C-3″), 17.6 (C-4″), 25.8 (C-5″). EIMS *m/z* (rel. int.): 340 (4, $[M]^+$), 282 (7), 226 (8), 191 (9), 137 (21). HRMS *m/z* 341.1318 $[M + 1]^+$ (calcd for $C_{20}H_{21}O_5$ 341.1389).

3.5. 5-Deoxyabyssinin II (*2*)

Amorphous powder. $[\alpha]_D = 0°$ (MeOH, *c* 0.01). UV λ_{max} (MeOH) (log ε) nm: 275 (4.0), 312 (4.8). ^1H NMR (acetone-d_6, 500 MHz): δ 5.42 (1H, *dd*, *J* = 2.7, 13.2 Hz, H-2), 2.71 (1H, *dd*, *J* = 2.7, −17.0 Hz, H-3α), 3.04 (1H, *dd*, *J* = 13.2, −17.0 Hz, H-3β), 7.73 (1H, *d*, *J* = 8.5 Hz, H-5), 6.58 (1H, *dd*, *J* = 2.5, 8.5 Hz, H-6), 6.42 (1H, *d*, *J* = 2.5 Hz, H-8), 7.06 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.92 (1H, *d*, *J* = 2.0 Hz, H-6′), 3.35 (2H, *d*, H-1″), 5.35 *t*, *J* = 7.2 Hz, H-2″), 1.70, 1.71 (6H, 2X *s*,

Me-4″ and Me-5″), 3.89 (3H, *s*, OMe). ^{13}C NMR (acetone-d_6, 125 MHz): δ 79.5 (C-2), 43.2 (C-3), 189.1 (C-4), 127.9 (C-5), 106.9 (C-6), 163.0 (C-7 or C-9), 102.1 (C-8), 163.7 (C-9 or C-7), 113.6 (C-10), 129.3 (C-1′), 109.6 (C-2′), 146.4 (C-3′), 143.5 (C-4′), 126.7 (C-5′), 119.5 (C-6′), 27.1 (C-1″), 122.0 (C-2″), 131.0 (C-3″), 16.3 (Me-4″), 24.3 (C-5″), 54.8 (OMe). EIMS *m*/*z* (rel. int.): 354 (85, $[M]^+$), 218 (15), 205 (44), 163 (17), 137 (22). HRMS *m*/*z* 355.1532 $[M + 1]^+$ (calcd for $C_{21}H_{23}O_5$ 355.1545).

3.6. In vitro anti-plasmodial activity

The crude extract and pure compounds were tested for anti-plasmodial activities based on the inhibition of [^{3}H]hypoxanthine uptake as described in Yenesew et al. (2003a). IC_{50} values were calculated from a triplicate experiment.

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