Flavonoids and Isoflavonoids with Antiplasmodial Activities from the Root Bark of *Erythrina abyssinica*

**Abstract**

From the root bark of *Erythrina abyssinica* a new pterocarpene [3-hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpene] and a new isoflav-3-ene [7',4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene] were isolated. In addition, the known compounds 3,3-dimethylallyl, licoagrocalchone A, octacosyl ferulate and tricatonyl 4-hydroxycinnamate were identified. The structures were determined on the basis of spectroscopic evidence. The crude extract and the flavonoids and isoflavonoids obtained from the roots of this plant showed antiplasmodial activities.

**Key words**

*Erythrina abyssinica* · Leguminosae · root bark · pterocarpene · isoflav-3-ene · 3-hydroxy-9-methoxy-10-(3,3-dimethylallyl) pterocarpene · 7',4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene · antiplasmodials

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**Introduction**

Malaria remains a serious parasitic disease killing over one million people annually. With the emergence of drug-resistant *Plasmodium falciparum*, many of the currently prescribed antimalarial drugs are becoming less effective [1]. Most of the mortality associated with malaria is present in developing areas of the world where inexpensive prophylaxis and treatment alternatives are needed. In Kenya, chloroquine is no longer effective and the current use of sulfadoxine-pyrimethamine is now in jeopardy with the increase in resistance to this combination treatment [2], [3].

Worldwide, *Erythrina* species (Leguminosae) are used in traditional medicinal practice to treat infectious diseases [4]. In Kenya there are five *Erythrina* species, *E. abyssinica*, *E. burtii*, *E. excelsa*, *E. melanocantha* and *E. sacleuxii* [5]. Flavonoids and isoflavonoids have been reported from *E. abyssinica* [6], [7], [8], *E. burtii* [9], [10] and *E. sacleuxii* [11], [12]. Among these, *E. abyssinica* is the most widely used to treat microbial infection and malaria in traditional medicinal practice [13]. Whereas flavonoids and isoflavonoids are reported to be responsible for the traditional antimicrobial uses of this plant [6], the antimalarial properties have not been investigated. Here we report the structure elucidation and antiplasmodial (against *Plasmodium falciparum*) activities of two new isoflavonoids (a pterocarpene, 1 and an isoflav-3-ene, 2) along with some known flavonoids and isoflavonoids isolated from the root bark of *E. abyssinica*.

**Materials and Methods**

**General**

Analytical TLC: Merck pre-coated silica gel 60 F$\text{_{254}}$ plates. CC on oxalic acid impregnated silica gel 60 (70 – 230 mesh) and Sephadex LH-20. EIMS: direct inlet, 70 eV on a SSO 710, Finnigan MAT 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. HMBC and HMQC spectra were acquired using the standard Bruker software.
Plant material
The root bark of *Erythrina abyssinica* DC was collected near Thika town, Kenya, in December 2001. The plant was identified by S. G. Mathenge of the Herbarium, Botany Department, University of Nairobi, where a voucher specimen (AY-SCM-2001-09) is deposited.

Extraction and isolation
Air-dried and ground root bark of *E. abyssinica* (500 g) was extracted with acetone by percolation at room temperature. After removing the solvent under reduced pressure, a dark brown extract (34 g) was obtained. Twelve grams of the extract were subjected to column chromatography on oxalic acid-impregnated silica gel (120 g) and eluted with hexane containing increasing amounts (5, 10, 15, 20, 25, 30 and 40%) of acetone. A total of seven fractions was collected. Crystallization of a fraction containing acetone (25% of hexane in elution volume 250 ml) afforded triacontyl 4-hydroxycinnamate (9 mg) as a white amorphous powder. The fraction eluted with 50% of acetone (elution volume 500 ml) was subjected to column chromatography on 50 g of Sephadex LH-20 (eluting with 400 ml of MeOH/CH₂Cl₂, 1:1) followed by PTLC (hexane/EtOAc, 9:1) to afford 1 (24 mg, Rf 0.37). The 20% eluent (elution volume 800 ml) was purified by column chromatography (on 50 g of deactivated silica gel, eluted with hexane/acetone, 9:1; three major fractions each containing 200 ml) were collected) to give ericyrstagallin (330 mg), abbyssinode-IV (6 mg) [6] and erythryssin-II (54 mg) [6]. The 25% eluent (elution volume 900 ml) was applied on Sephadex LH-20 (50 g, eluting with 400 ml of MeOH/CH₂Cl₂, 1:1) followed by PTLC (hexane/acetone, 4:1), which gave further amounts of abbyssinode-IV (30 mg, Rf 0.28). The 30% eluent (elution volume 900 ml) gave licoagrolachone A (49 mg), while the 40% eluent (elution volume 500 ml) gave 2 (235 mg).

Physical and spectroscopic data
1-Hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpane (1): Amorphous powder. UV (MeOH): λ \text{max} = 224, 266, 272, 314, 340 nm. 1H-NMR (acetone-d₆, 500 MHz): δ = 7.34 (1H, d, J = 8.3 Hz, H-1), 6.52 (1H, dd, J = 2.4, 8.3 Hz, H-2), 6.44 (1H, d, J = 2.3 Hz, H-4), 5.56 (1H, s, CH₂-6), 7.26 (1H, d, J = 8.5 Hz, H-7), 6.97 (1H, d, J = 8.5 Hz, H-8), 3.61 (2H, J = 7.4 Hz, H-1'), 5.33 (1H, m, H-2'), 1.82 (3H, s, Me-4'), 1.66 (3H, s, Me-5'), 3.90 (3H, s, OMe). 13C-NMR (acetone-d₆, 125 MHz): δ = 121.9 (C-1), 109.7 (C-1a), 109.5 (C-2), 159.5 (C-3), 104.5 (C-4), 155.8 (C-4a or C-9 or C-10a), 66.0 (C-6), 106.6 (C-6a), 120.5 (C-6b), 116.8 (C-7), 108.8 (C-8), 155.1 (C-9 or C-4a or C-10a), 114.5 (C-10), 156.3 (C-10a or C-4a or C-9), 148.2 (C-11a), 23.4 (C-1), 122.9 (C-2), 132.2 (C-3), 17.9 (C-4'), 25.9 (C-5'), 56.9 (OMe); EIMS: m/z (rel. int.) = 336 [M]+ (100), 278 (20), 168 (9), 125 (11).

Acetylation of 1 (10 mg) with acetic anhydride and pyridine gave the mono-acetate (6 mg). Oil. 1H-NMR (CDCl₃, 200 MHz): δ = 2.30 (3H, s, OAc); EIMS: m/z (rel. int.) = 378 [M]+ (60), 363 (100).

7,4'-Dihydroxy-2,5'-dimethoxyisoflav-3-one (2): Amorphous powder. UV (MeOH): λ \text{max} = 220, 244, 254, 300 nm. 1H-NMR (acetone-d₆, 500 MHz): δ = 4.92 (1H, d, J = 1.2 Hz, CH₂-2), 6.56 (1H, br.s, H-4), 6.94 (1H, d, J = 8.3 Hz, H-5), 6.41 (1H, d, J = 2.4, 8.2 Hz, H-6), 6.33 (1H, d, J = 2.3, H-8), 6.57 (1H, s, H-3'), 6.95 (1H, s, H-6'), 3.77 (3H, s, 2'-OMe), 3.84 (3H, s, 5'-Ome), 7.76 (1H, s, 4'-OH), 13C-NMR (acetone-d₆, 125 MHz): δ = 69.0 (C-2), 129.8 (C-3), 121.6 (C-4), 117.2 (C-4a), 128.4 (C-5), 109.4 (C-6), 159.1 (C-7), 103.4 (C-8), 155.8 (C-8a), 119.4 (C-1'), 153.2 (C-2'), 101.3 (C-3'), 148.4 (C-4'), 142.4 (C-5'), 113.5 (C-6), 56.4 (2'-Ome), 57.2 (5'-Ome); EIMS: m/z (rel. int.) = 300 M+ (100), 286 (84), 269 (64), 241 (15), 147 (23), 143 (32).

In vitro anti-plasmodial activity
The crude extract and pure compounds were assayed using an automated microdilution technique to determine 50% growth inhibition of cultured parasites [14, 15]. Two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *Plasmodium falciparum* were grown as described in the literature [14, 15]. The samples were serially diluted across the plate to provide a range of concentrations used to determine IC₅₀ values. Plates were incubated in a mixed gas incubator for 24 hours. [3H]Hypoxanthine was then added and parasites allowed to grow for an additional 18 hours. Cells were processed with a plate harvester (TomTec) onto filter paper and washed to eliminate uncirnporated isotope. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter were imported into a Microsoft Excel spreadsheet, which is then imported into an Oracle database program to determine IC₅₀ values. A minimum of three separate determinations was carried out for each sample.
Results and Discussion

The UV, $^\text{1H}$H- ($\delta = 5.56, s$, for methylene protons at C-6) and $^{13}$C- ($\delta = 66.0$ for C-6, 106.6 for C-6a and 148.2 for C-11a) NMR spectra of 1 suggested a pteroecarpen skeleton. Furthermore, the presence of hydroxy (formed a mono-acetate), methoxy and 3,3-dimethylallyl substituents was deduced from the MS (336, M$^+$) and NMR spectra (see Materials and Methods). The $^\text{1H}$-NMR displayed AXY ($\delta = 7.34, d, J = 8.3$ Hz; 6.52, dd, $J = 2.4, 8.4$ Hz and 6.44, d, $J = 2.3$ Hz) and AX ($\delta = 7.26, d, J = 8.5$ Hz and 6.57, d, $J = 8.5$ Hz) spin systems for aromatic protons. With the biogenetically expected oxygenation at C-3 and C-9, the 3,3-dimethylallyl group could be placed either at C-4 or C-10. This was resolved from the HMBC spectrum which showed correlation of the doublet at $\delta = 7.34$ with C-11a ($\delta = 148.2$), allowing the assignment of this doublet to H-1 and hence the AXY spin system corresponded to the A-ring protons (H-1, H-3 and H-4). The AX spin system could then be assigned to the B-ring protons (H-7 and H-8) with the 3,3-dimethylallyl group placed at C-10. HMBC correlation between the methylene protons of 3,3-dimethylallyl group with C-9 and C-10a was in agreement with its placement at C-10. Finally, the methoxy group was located at C-9, as revealed by the NOESY experiment, which showed correlation of the methoxy protons with H-8. Hence, this new compound was identified as 3-hydroxy-9-methoxy-10-(3,3-dimethylallyl) pteroecarpen (1).

The MS of compound 2 showed a molecular ion peak at m/z = 300 corresponding to C$_{17}$H$_{20}$O$_3$. The UV spectrum as well as $^\text{1H}$- ($\delta = 4.92, d, J = 1.2$ Hz, CH$_2$ at C-2 and 6.56 br s for H-4) and $^{13}$C- ($\delta = 69.0$ for C-2, 129.8 for C-3 and 121.6 for C-4) NMR spectra were typical of isoflav-3-enes [10]. Furthermore, the presence of two hydroxy (formed a di-acetate) and two methoxy substituents was evident from the MS and NMR spectra (see Materials and Methods). The $^\text{1H}$-NMR further showed two singlets at $\delta = 6.57$ and 6.95 and an AXY spin system ($\delta = 6.94, d, J = 8.3$ Hz; 6.41, dd, $J = 2.4, 8.2$ Hz and 6.33, d, $J = 2.3$ Hz) for aromatic protons. With the biogenetically expected oxygenation at C-7 and C-4; these data suggested two possible structures differing by the positions of the remaining two oxygenations in this compound, either at C-2’ and C-5’ (as in structure 2, where the two singlets would correspond to H-3’ and H-6’ and the AXY spin system would correspond to the A-ring protons, H-5, H-6 and H-8), or at C-6 and C-2’ (where the two singlets correspond to H-5 and H-8 and the AXY spin system correspond to the B-ring protons, H-6’, H-7 and H-3’).

In the HMBC spectrum, correlation between the doublet at $\delta = 6.94$ with C-4 ($\delta = 121.6$) and between the broad singlet at 6.56 (H-4) with C-5 ($\delta = 128.4$) allowed the assignment of the doublet at $\delta = 6.94$ to H-5. Hence the AXY spin system corresponds to the A-ring protons and the two singlets to the B-ring protons leaving oxygenations at C-2’ and C-5’. Also the chemical shift values of the B-ring carbon atoms ($\delta = 119.4$ for C-1’, 153.2 for C-2’, 101.3 for C-3’, 148.1 for C-4’, 142.1 for C-5’ and 113.5 for C-6’) were consistent with this oxygenation pattern [16]. Finally the positions of the two methoxy groups were assigned to C-2’ and C-5’ from NOEY spectrum. Thus, the methoxy group at C-2’ showed NOE interaction with the methylene protons at C-2’, H-4 and H-3’, while OMe-5’ correlates with H-6’. Therefore this new compound was characterized as 7,4’-dihydroxy-2,5’-dimethoxy-isoflav-3-ene (2).

In addition, by comparison of spectroscopic data with literature reports, erycristagallin [17], licoagrolchrome A [18], octacosyl ferulate [9] and n-triacetyl 4-hydroxyxymannate [19] were identified for the first time from the root bark of E. abyssinica. Licoagrolchrome A has only been reported from root cultures of Glycyrrhiza glabra [18] prior to this report. In the course of this study we have also isolated abyssinone-IV and erythrabysin-II, which have already been reported from the roots of E. abyssinica [6]. Abyssinone-IV was isolated as a racemic mixture ($\alpha L$; 0’); and this has been observed in flavanones with free phenolic group at C-4’ [7]. In the case of erythrabysin-II, high negative optical rotation ($\alpha L$; -250°) was recorded and this is consistent with 6αR:11αR configuration like the other pterocarpen of this genus [9].

The acetone extract of the roots of E. abyssinica showed potent antiplasmodial activities against W2 (chloroquine-resistant) and D6 (chloroquine-sensitive) strains of Plasmodium falciparum, with IC$_{50}$ values of 0.49 ± 0.07 and 0.64 ± 0.06 μg/mL respectively. The activity observed for the crude extract explains the wide traditional uses of the plant to treat malaria in East Africa [13]. Among the compounds isolated from the roots of E. abyssinica, the flavanone abyssinone-IV and the pterocaran erythrabysin-II, showed the highest activities against both strains (Table 1). Some alkaloid, flavonones and isoflavones have been reported as antiplasmodial agents among plant metabolites [20]. Pterocarpenes, isoflav-3-enes and pterocarpan are now identified in this study to represent new sub-classes of isoflavonoids with antiplasmodial activities.

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<table>
<thead>
<tr>
<th>Table 1</th>
<th>In vitro IC$_{50}$ values of flavonoids and isoflavonoids from the root bark of E. abyssinica against W2 and D6 strains of Plasmodium falciparum</th>
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<tbody>
<tr>
<td><strong>Compound</strong></td>
<td><strong>IC$_{50}$ (μM)</strong></td>
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<tr>
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<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>20.6 ± 3.2</td>
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<tr>
<td>2</td>
<td>27.7 ± 1.8</td>
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<tr>
<td>Erycristagallin</td>
<td>20.1 ± 3.6</td>
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<tr>
<td>Licoagrolchrome A</td>
<td>12.8 ± 2.5</td>
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<tr>
<td>Abyssinone-IV</td>
<td>7.7 ± 1.6</td>
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<tr>
<td>Erythrabysin-II</td>
<td>6.5 ± 0.6</td>
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<tr>
<td>Octacosyl ferulate</td>
<td>&gt; 50</td>
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<tr>
<td>Chioroquine</td>
<td>0.093 ± 0.005</td>
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<tr>
<td>Quinine</td>
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References