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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 erycristagallin, licoagrochalcone A, octacosyl ferulate and triacontyl 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

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. burttii, E. excelsa, E. melanacantha* and *E. sacleuxii* [5]. Flavonoids and isoflavonoids have been reported from *E. abyssinica* [6], [7], [8], *E. burttii* [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_{254} plates. CC on oxalic acid impregnated silica gel 60 (70–230 mesh) and Sephadex LH-20. EIMS: direct inlet, 70 eV on a SSQ 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. HMQC and HMBC spectra were acquired using the standard Bruker software.

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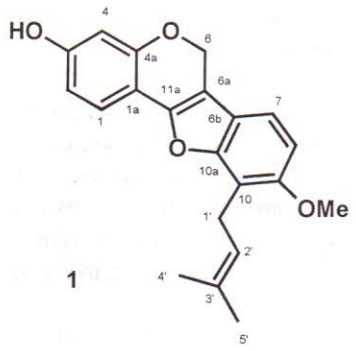

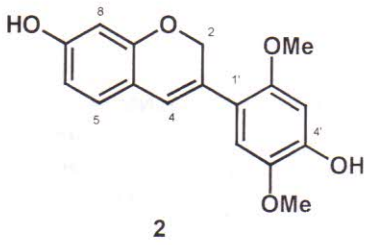

Plant material

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-SGM-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 (from hexane/CH_2Cl_2) of the fraction eluted with 5% acetone in hexane (elution volume 800 mL), gave octacosyl ferulate (38 mg) while the fraction eluted with 10% acetone (elution volume 750 mL) afforded triacontyl 4-hydroxycinnamate (9 mg) as a white amorphous powder. The fraction eluted with 15% acetone (elution volume 850 mL), was subjected to column chromatography on 50 g of Sephadex LH-20 (eluting with 400 mL of MeOH/CH_2Cl_2, 1 : 1) followed by PTLC (hexane/EtOAc, 9 : 1) to afford **1** (24 mg, R_f 0.37). The 20% eluent (elution volume 800 mL) was purified by column chromatography (on 50 gm of deactivated silica gel, eluted with hexane/acetone, 9 : 1; three major fractions each containing 200 mL were collected) to give erycristagallin (330 mg), abyssinone-IV (6 mg) [6] and erythrabyssin-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/CH_2Cl_2, 1 : 1) followed by PTLC (hexane/acetone, 4 : 1), which gave further amounts of abyssinone-IV (30 mg, R_f 0.28). The 30% eluent (elution volume 900 mL) gave licoagrochalcone A (49 mg), while the 40% eluent (elution volume 950 mL) gave **2** (235 mg).

Physical and spectroscopic data

3-Hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpene (**1**): Amorphous powder. UV (MeOH): λ_{max} = 224, 266, 272, 314, 340 sh, 352 nm. ^{1}H-NMR (acetone-d_6, 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*, CH_2–6), 7.26 (1H, *d*, *J* = 8.5 Hz, H-7), 6.97 (1H, *d*, *J* = 8.5 Hz, H-8), 3.61 (2H, *d*, *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*, 9-OMe); ^{13}C-NMR (acetone-d_6, 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. ^{1}H-NMR ($CDCl_3$, 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-ene (**2**): Amorphous powder. UV (MeOH): λ_{max} = 220, 244, 254sh, 310 nm. ^{1}H-NMR (acetone-d_6, 500 MHz): δ = 4.92 (1H, *d*, *J* = 1.2 Hz, CH_2–2), 6.56 (1H, br *s*, H-4), 6.94 (1H, *d*, *J* = 8.3 Hz, H-5), 6.41 (1H, *dd*, *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); ^{13}C-NMR (acetone-d_6, 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).

Acetylation of **2** (20 mg) gave the di-acetate (17 mg). Amorphous powder. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.33 (3H, *s*, OAc), 2.28 (3H, *s*, OAc); ^{13}C-NMR (acetone-d_6, 50 MHz): δ = 169.6, 169.6 (2 MeC = O), 21.4, 20.9 (2 MeC = O); EIMS: *m/z* (rel. int.) = 384 (92, M^+), 342 (75) 300 (100), 286 (63).

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 IC_{50} values. Plates were incubated in a mixed gas incubator for 24 hours. [^{3}*H*]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 unincorporated 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 IC_{50} values. A minimum of three separate determinations was carried out for each sample.