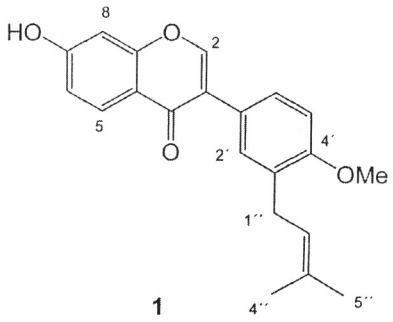
Antiplasmodial Flavonoids from *Erythrina sacleuxii*

Andrew W. Andayi[1, 2], Abiy Yenesew[1], Solomon Derese[1], Jacob O. Midiwo[1], Peter M. Gitu[1], Ogoche J. I. Jondiko[2], Hosea Akala[3], Pamela Liyala[3], Julia Wangui[3], Norman C. Waters[3], Matthias Heydenreich[4], Martin G. Peter[4]



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Abstract

The acetone extracts of the root bark and stem bark of *Erythrina sacleuxii* showed antiplasmodial activities against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Chromatographic separation of the acetone extract of the root bark afforded a new isoflavone, 7-hydroxy-4′-methoxy-3′-prenylisoflavone (trivial name 5-deoxy-3′-prenylbiochanin A) along with known isoflavonoids as the antiplasmodial principles. Flavonoids and isoflavonoids isolated from the stem bark of *E. sacleuxii* were also tested and showed antiplasmodial activities. The structures were determined on the basis of spectroscopic evidence.

Some *Erythrina* species of Kenya, including *E. sacleuxii* are used traditionally for the treatment of microbial infections and malaria [1], [2]. It is established that flavonoids and isoflavonoids are responsible for the traditional antimicrobial uses of *Erythrina* species [3], [4]. Recently we reported antiplasmodial flavonoids and isoflavonoids isolated from the root and stem bark of *E. abyssinica* [5], [6]. Here we report the identification and antiplasmodial activity of a new isoflavone (**1**) along with other flavonoids from *E. sacleuxii*.

HR-MS analysis of compound **1** showed a molecular ion peak at *m/z* = 336.1347 corresponding to the molecular formula $C_{21}H_{20}O_4$. The UV (λ_{max} = 263 nm), ^{1}H- (δ = 8.14 for H-2) and ^{13}C-NMR (δ = 154.0 for C-2, 125.9 for C-3 and 176.4 for C-4) spectra indicated an isoflavone skeleton. The presence of a prenyl, a hydroxy and a methoxy substituent was evident from the mass, ^{1}H- and ^{13}C-NMR spectra. For the A-ring, the ^{1}H-NMR spectrum showed an AXY spin system at δ = 8.06 (d, J = 8.7 Hz for H-5), 6.98 (1H, dd, J = 2.1, 8.7 Hz, for H-6) and 6.90 (d, J = 2.1 Hz, for H-8) indicating that C-7 is oxygenated as expected biogenetically. In the MS, the fragment ion at *m/z* = 137 resulting from retro-Diels-Alder fragmentation of the C-ring suggested that the A-ring contains one hydroxy group, viz at C-7, and hence the methoxy and the prenyl groups should be located in the B-ring. For this ring, the ^{1}H- and ^{13}C-NMR spectra are similar with those of 3′-prenylbiochanin A, an isoflavone earlier reported from the stem bark of this plant [7], and suggesting identical substitution pattern. Thus in this ring, an ABX spin system at δ = 7.40 (d, J = 2.1 Hz for H-2′), 6.99 (d, J = 8.4 Hz, for H-5′) and 7.43 (dd, J = 2.1, 8.4 Hz, for H-6′) is in agreement with the placement of the methoxy at C-4′ and the prenyl at C-3′. The HMBC spectrum showed correlations of the methylene protons (δ = 3.34) of the prenyl group with C-4′(δ = 158.7) and C-6′(δ = 129.3), confirming the substitution pattern in the B-ring. Thus this compound was characterized as 7-hydroxy-4′-methoxy-3′-prenylisoflavone for which the trivial name 5-deoxy-3′-prenylbiochanin A is suggested. This compound has been reported as a synthetic derivative [8]. However this is the first report on its occurrence in nature.

The root bark of *E. sacleuxii* also afforded the ferulate ester erythrinasinate A [9], the pterocarpan shinpterocarpin [10], the isoflav-3-ene 7,4′-dihydroxy-2′,5-dimethoxyisoflav-3-ene [5], the isoflavanone prostratol C [11], the isoflavones corylin [12] and erysubin F [13]. This is the first report on the occurrence of prostratol C in the genus *Erythrina*.

The acetone extract of the stem bark and the root bark of *E. sacleuxii* showed antiplasmodial activity against the D6 (IC_{50} = 3.8 ± 0.9 μg/mL for stem bark extract and 2.2 ± 0.6 μg/mL for the root bark extract) and W2 (IC_{50} = 6.3 ± 1.4 μg/mL for stem bark and 1.34 ± 0.3 μg/mL for the root bark) strains of *P. falciparum*. This supports the traditional use of this plant to treat malaria in East Africa [2]. The flavonoids and isoflavonoids previously reported from the stem bark of this plant [7], [14], as well as those isolated in this investigation from the root bark were tested for antiplasmodial activities (Table **1**). This plant mainly elaborates isoflavones and all the eight isoflavones tested showed activities with 5′-prenylpratensein being the most active. Prior to this work very little had been reported on the antiplasmodial activities of isoflavones [15]. Activities have also been observed in other subclasses of flavonoids against both strains (Table **1**). The flavanone abyssinone V and the pterocarpan shinpterocarpin are among the most active.

Material and Methods

The root bark of *Erythrina sacleuxii* was collected from the South Coast of Kenya, in July 2003. The plant was identified at the herbarium, Botany Department, University of Nairobi, where a voucher specimen (AY-SGM-2003 – 02) is deposited.

Affiliation: [1] Department of Chemistry, University of Nairobi, Nairobi, Kenya · [2] Department of Chemistry, Maseno University, Maseno, Kenya · [3] United States Army Medical Research Unit – Kenya, MRU 64109, APO, AE 09831–4109, USA · [4] Institut für Chemie, Universität Potsdam, Potsdam, Germany

Correspondence: Abiy Yenesew · Department of Chemistry · University of Nairobi · P.O. Box 30197 · Nairobi · Kenya · Phone: +254-20-444-9004 ext 2170 · Fax: +254-20-4446138 · E-mail: ayenesew@uonbi.ac.ke

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Table 1 Antiplasmodial activities of flavonoids of *Erythrina sacleuxii*

Flavonoids	IC_{50} (μM)*	
	D6	W2
Flavanones		
Abyssinone V [14]	4.9 ± 0.8	6.1 ± 1.3
Abyssinone V 4′-methyl ether [14]	11.3 ± 2.4	11.1 ± 2.1
Sigmoidin B 4′-methyl ether [14]	13.0 ± 2.0	12.7 ± 2.9
Isoflavones		
5-Deoxy-3′-prenylbiochanin A (**1**)	17.6 ± 1.7	22.5 ± 2.1
Corylin [12]	16.6 ± 3.8	19.7 ± 4.3
Erysubin F [13]	12.0 ± 0.5	12.8 ± 0.7
3′-Prenylbiochanin A [7]	23.7 ± 4.3	28.4 ± 4.8
7-Demethylrobustigenin [7]	27.2 ± 3.3	31.7 ± 5.7
5′-Prenylpratensein [7]	6.3 ± 0.3	8.7 ± 1.5
5′-Formylpratensein [7]	21.7 ± 8.6	27.9 ± 6.2
2,3-Dehydrokeivetone [14]	15.1 ± 3.4	12.7 ± 2.3
Isoflavanones		
Prostratol C [11]	17.6 ± 1.7	19.8 ± 3.5
Saclenone [14]	24.2 ± 3.5	22.6 ± 1.4
2,3-Dihydro-7-demethylrobustigenin [14]	28.0 ± 5.3	31.8 ± 6.1
Pterocarpan		
Shinpterocarpin [10, 14]	6.6 ± 1.2	8.3 ± 1.1
Isoflav-3-ene		
7,4′-Dihydroxy-2′,5′-dimethoxyisoflav-3-ene [5]	22.0 ± 2.4	24.9 ± 3.0
Reference drugs		
Chloroquine	0.008 ± 0.002	0.075 ± 0.002
Quinine	0.050 ± 0.02	0.28 ± 0.02

* Values are expressed as mean ± SD, with n = 3.

Air-dried and powdered root bark of *E. sacleuxii* (524 g) was extracted with acetone (2.5 L×3) by percolation at 25 °C to yield 52 g of a brown sticky extract after concentration under vacuum. A portion of the extract (50 g) was chromatographed on oxalic acid-impregnated silica gel (280 g, 5×50 cm) eluting with hexane containing increasing amounts of acetone (**1**, 2, 3, 5 and 7% acetone in hexane). Five major fractions, each ca. 1 L, were collected and labelled A to E. Erythrinasinate A (40 mg, R_f = 0.52, hexane/CH_2Cl_2, 3:7) precipitated from fraction A (eluted with 1% acetone in hexane). Fraction B (2% acetone in hexane) was purified on Sephadex LH-20 (5×50 cm, eluted with CH_2Cl_2/MeOH, 1:1) and preparative TLC (silica gel, hexane/CH_2Cl_2, 3:7, multiple development) to give shinpterocarpin (980 mg, R_f = 0.31, hexane/CH_2Cl_2, 3:7). Fraction C (3% acetone in hexane) was further purified by CC (oxalic acid-impregnated silica gel, 2×50 cm, elution with CH_2Cl_2/EtOAc, 1:1) to give prostratol C (120 mg, R_f = 0.45, CH_2Cl_2). Fraction D (5% acetone in hexane) was separated by CC on Sephadex LH-20 (2×50 cm, elution with CH_2Cl_2/MeOH; 1:1), and preparative TLC (silica gel, 1% MeOH in CH_2Cl_2, multiple development) to give 7,4′-dihydroxy-2′,5′-dimethoxyisoflav-3-ene (8 mg, R_f = 0.35, 1% MeOH in CH_2Cl_2) and **1** (6 mg, R_f = 0.28, 1% MeOH in CH_2Cl_2). Fraction E (eluted with 7% acetone in hexane) was treated as above and afforded corylin (6 mg, R_f = 0.25, 1% MeOH in CH_2Cl_2) and erysubin F (91 mg, R_f = 0.18, 1% MeOH in CH_2Cl_2).

5-Deoxy-3′-prenylbiochanin A (**1**): Needles (CH_2Cl_2), m.p. 190–192 °C; UV (MeOH): λ_{max} (log ε) = 263 (4.3) nm; ^{1}H NMR (acetone-d_6, 500 MHz): δ = 8.14 (1H, s, H-2), 8.06 (1H, d, *J* = 8.7 Hz, H-5), 6.98 (1H, dd, *J* = 2.1, 8.7 Hz, H-6), 6.90 (1H, d, *J* = 2.1 Hz, H-8), 7.40 (1H, d, *J* = 2.1 Hz, H-2′), 6.99 (1H, d, *J* = 8.4 Hz, H-5′), 7.43 (dd, *J* = 2.1, 8.4 Hz, H-6′), 3.34 (2H, d, *J* = 7.5 Hz, H-1″), 5.31 (1H, m, H-2″), 1.70 (3H, s, Me-4″), 1.73 (3H, s, Me-5″), 3.88 (1H, s, 4′-OMe); ^{13}C-NMR (acetone-d_6, 125 MHz): δ = 154.0 (C-2), 125.9 (C-3), 176.4 (C-4), 130.9 (C-4a), 129.1 (C-5), 111.6 (C-6), 159.5 (C-7), 103.8 (C-8), 164.2 (C-8a), 126.0 (C-1′), 131.6 (C-2′), 130.9 (C-3′), 158.7 (C-4′), 119.1 (C-5′), 129.3 (C-6′), 31.8 (C-1″), 124.3 (C-2″), 133.2 (C-3″), 18.5 (4″-Me), 26.6 (5″-Me), 56.5 (OMe); EI-MS: *m/z* (rel. int.) = 336 (100) [M$^+$], 137 (76); HR-MS: *m/z* = 336.1347 [M]$^+$; calcd. for $C_{21}H_{20}O_4$: 336.1362.

In vitro antiplasmodial activity: The crude extracts and pure compounds were evaluated for antiplasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* using a [^{3}*H*]hypoxanthine uptake assay as described in [5].

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