

CHEMICAL CONSTITUENTS OF THE ROOT WOOD OF *ERYTHRINA SACLEUXII* AND DETERMINATION OF THE ABSOLUTE CONFIGURATION OF SUBRECTIN

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ABSTRACT. Phytochemical investigation on the root wood of *Erythrina sacleuxii* (Leguminosae) led to the isolation of nine secondary metabolites (1-9). Compound **1** was isolated from the genus *Erythrina* for the first time. The pure compounds were identified on the basis of comprehensive spectroscopic and spectrometric analyses, while their absolute configurations were determined based on chiroptical measurements. Compounds **5** and **6** showed weak antifungal activity against *Pyricularia oryzae* with MIC values of 20 µg/mL.

KEY WORDS: *Erythrina sacleuxii*, Leguminosae, Antifungal activity, *Pyricularia oryzae*

INTRODUCTION

The genus *Erythrina*, a member of Leguminosae family, is made up of more than 110 species of red or orange flowered trees or shrubs widely distributed in the tropical and subtropical regions of the world [1-3]. *Erythrina sacleuxii* Hua is a 9-24 m tall tree, endemic to Kenya and Tanzania [4]. Traditionally, the tree is used for the treatment of malaria fever and microbial infections [5, 6]. Previous phytochemical studies on the root and stem bark of the Kenyan specimens of *E. sacleuxii* had yielded flavones, isoflavones, isoflavanones, pterocarpanes, and isoflav-3-enes [7-10], which have attracted interest on the account of their antiplasmodial activity [7]. We have recently reported some flavonoids from the stem bark and twigs of the Kenyan specimens of *E. sacleuxii* with antifungal and cytotoxic activities [11, 12]. As part of our continuing investigation on *E. sacleuxii*, herein, we report the isolation and structural elucidation of nine known compounds from the root wood of *E. Sacleuxii* (Figure 1) and their antifungal activity.

EXPERIMENTAL

General experimental procedures

Melting points were determined on a Stuart melting point apparatus SMP1 (UK) and are uncorrected. Silica gel 60 Å (35–70 µm) from Acros Organics was used for column chromatography. Preparative TLC was performed on 20 cm × 20 cm glass plates precoated with silica gel 60 PF₂₅₄₊₃₆₆ having 0.75 mm layer thickness. The spots and bands were visualized under a UV lamp (254 and 366 nm). Optical rotation measurements were performed on a 241 model polarimeter (Perkin-Elmer, Waltham, MA, USA) at 22 °C. NMR spectra were recorded on an Avance-III spectrometer (Bruker, Karlsruhe, Germany) at 400 and 100 MHz (¹H and ¹³C,

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respectively) equipped with a 5 mm probe head. The spectra were referenced to the residual solvent signal [13, 14]. ESI-HRMS was obtained with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Eschborn, Germany). A Tensor 27 spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a diamond ATR unit was used to record IR spectra. UV and ECD measurements were performed using a J-815 spectropolarimeter (Jasco, Tokyo, Japan) in a quartz glass cuvette with a path length of 1 mm and a spectral range of 400-185 nm at a scan speed of 20 nm/min with 8 repetitions. The concentration of the acetonitrile solution of **1** amounted to 0.25 mmol/L. The baseline was corrected by subtraction of a solvent spectrum obtained with the same parameters.

Plant material

The root wood of *Erythrina sacleuxii* was collected from Mutsengo, Kilifi County, along the Kenyan coast in August 2015. Identification and authentication of the plant species was performed by Prof. S.T. Kariuki of Biological Sciences, Egerton University. A voucher specimen of *E. sacleuxii* was deposited at the Department of Biological Sciences, Egerton University (no. 0136EUH).

Extraction and isolation

The root wood of *E. sacleuxii* (1.03 kg air-dried powder) was extracted with EtOAc (10 L) at room temperature for 72 h (3 times). The extract was concentrated under vacuum to afford a brown residue (10 g). The EtOAc extract was subjected to silica gel column chromatography using *n*-hexane with increasing proportions of EtOAc (0-100%) to yield 11 fractions (A-K). Fraction D was chromatographed over silica gel eluting with CHCl₃-MeOH (10:0.5) to afford two sub-fractions D₁ and D₂. Further purification of D₁ by preparative TLC with *n*-hexane-EtOAc (4:1) yielded cristacarpin (**2**, 2.2 mg) and phaseollidin (**3**, 2.0 mg), while purification of D₂ by silica gel column eluting with CH₂Cl₂-EtOAc (5:0.5) afforded *p*-hydroxybenzaldehyde (**4**, 5.4 mg). Fraction E was separated by silica gel column chromatography using *n*-hexane-acetone (5:1.5) and CH₂Cl₂-EtOAc (10:2.5) to give prostratol C (**5**, 2.8 mg). Fraction F was purified by repeated silica gel column chromatography using *n*-hexane-acetone (3:2) to give orobol (**6**, 2.1 mg). Repeated column chromatographic purification of fraction G led to isolation of daidzein (**7**, 2.3 mg). Purification of fraction H by silica gel column chromatography using CH₂Cl₂-MeOH (5:0.5) afforded 3'-methoxy coumestrol (**8**, 4.8 mg) and coumestrol (**9**, 1.3 mg). Fraction K was subjected to silica gel column chromatography eluting with CHCl₃-MeOH (4:1) to afford 2 sub-fractions K₁ and K₂. Further purification of K₂ by preparative TLC using CH₂Cl₂-MeOH (5:1.5) yielded suberectin (**1**, 13 mg).

Suberectin (1)

Brown powder; mp (168-170 °C); $[\alpha]_D^{22}$ -4.0° (*c* 0.1, MeCN); IR (ATR): 3352, 1655, 1616, 1504, 1471, 1279, 1152 cm⁻¹; UV/Vis λ_{\max} (MeCN) nm (log ϵ): 200 (4.70), 235 (4.32), 272 (4.11), 330 (3.76); ¹H NMR, COSY (400 MHz, acetone-*d*₆): 5.33 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-2), 2.99 (1H, *dd*, *J* = 16.8, 13.0 Hz, H-3a), 2.67 (1H, *dd*, *J* = 16.8, 3.0 Hz, H-3b), 7.24 (1H, *s*, H-5), 3.81 (3H, *s*, 6-OCH₃), 6.45 (1H, *s*, H-8), 7.05 (1H, *d*, *J* = 2.1 Hz, H-2'), 6.86 (2H, *d, m*, H-5' and H-6'); ¹³C NMR (100 MHz, acetone-*d*₆): 79.6 (C-2), 43.5 (C-3), 190.2 (C-4), 106.9 (C-5), 143.3 (C-6), 154.5 (C-7), 103.4 (C-8), 158.1 (C-9), 112.7 (C-10), 131.0 (C-1'), 113.7 (C-2'), 144.9 (C-3'), 146.2 (C-4'), 115.1 (C-5'), 118.2 (C-6'), 55.5 (6-OCH₃); ESI-HRMS: *m/z* [M + H⁺] calcd. for C₁₆H₁₄O₆: 303.0863; found: 303.0863.

RESULTS AND DISCUSSION

Phytochemical investigation on the root wood of *E. saclexii* led to the isolation and identification of nine known compounds. The known compounds were identified as suberectin (**1**) [15], cristacarpin (**2**) [16], phaseollidin (**3**) [17], *p*-hydroxybenzaldehyde (**4**) [18], prostratol C (**5**) [19], orobol (**6**) [20], daidzein (**7**) [21], 3'-methoxy coumestrol (**8**) [22] and coumestrol (**9**) [23]. Their chemical structures were established by comparison of their spectroscopic and spectrometric data with the literature data. Whereas compounds (**2-9**) have been reported from the genus *Erythrina* before, this is the first report of the existence of suberectin (**1**) in the genus *Erythrina*.

Compound **1** was isolated as a brown powder and assigned the molecular formula $C_{16}H_{14}O_6$ determined on the basis of ESI-HRMS data ($[M + H]^+ m/z$ 303.0863, calcd. 303.0863) and NMR analyses. The 1H NMR spectrum of compound **1** displayed signals characteristic for a flavanone skeleton with an oxymethine proton resonance at δ_H 5.33 (13.0, 3.0 Hz, H-2) coupled to methylene protons resonance at δ_H 2.99 (16.8, 13.0 Hz, H-3ax) and δ_H 2.67 (16.8, 3.0 Hz, H-2eq) indicating the presence of axial and equatorial protons in ring C of the flavanoid [24]. Two *para*-oriented aromatic proton singlets at δ_H 7.24 and δ_H 6.45 were assigned to H-5 and H-8, respectively, based on the HMBC cross-peaks of H-5 with C-4 (δ_C 190.2), C-6 (δ_C 143.3), C-7 (δ_C 154.5), C-8 (δ_C 103.4), C-9 (δ_C 158.1), C-10 (δ_C 112.7) and H-8 with C-4 (δ_C 190.2), C-5 (δ_C 106.9), C-7 (δ_C 154.5), C-9 (δ_C 158.1), and C-10 (δ_C 112.7). The methoxyl group (δ_H 3.81, δ_C 55.5) was placed at C-6 based on its HMBC correlation to C-6 (δ_C 143.23). The signals of aromatic protons of ring B comprised a doublet at δ_H 7.05 (1H, 2.1 Hz, H-2') and a multiplet at δ_H 6.86 (2H, *m*, H-5'/6'). The lack of splitting between H-5' and 6' could be due to dynamic process as a result of tautomerism between flavanone and chalcone. Furthermore, the small specific rotation value as a result of partial racemization due to inter-conversion between flavanone and chalcone suggests a free -OH at C-4'.

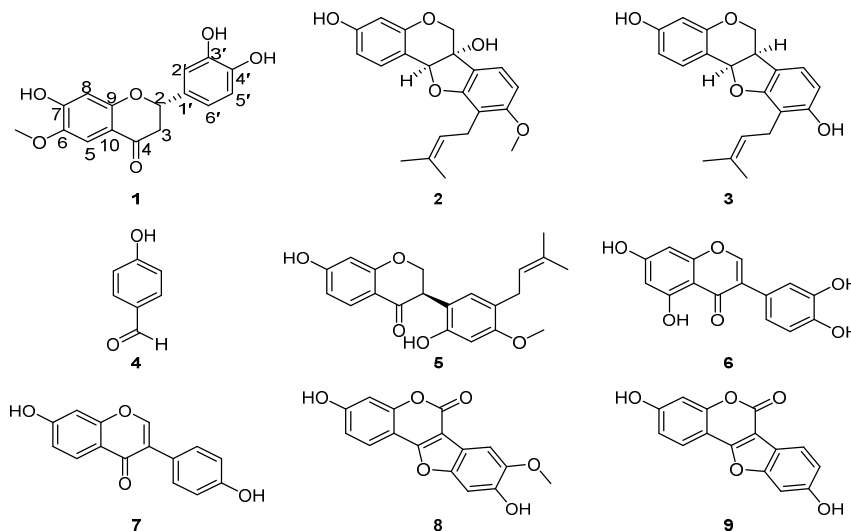


Figure 1. Structures of compounds **1-9**.

The absolute configuration of (-)-suberectin which is reported for the first time was determined by comparing the experimental ECD spectrum with a TDDFT calculated ECD

spectrum of (-)-suberectin (Figure 2). The experimental ECD spectrum of (-)-suberectin exhibited a positive Cotton effect at around $\lambda = 340$ nm as well as a negative Cotton effect at around 310 nm (Figure 2). Such observations in an ECD spectrum according to Gaffield, suggest that the compound should have an absolute configuration of (2*S*) [25].

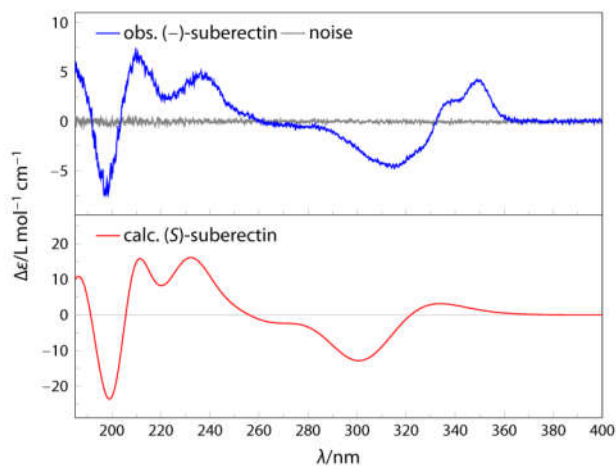


Figure 2. Observed ECD spectrum of (-)-suberectin in acetonitrile (top, blue) and calculated ECD spectrum of (S)-suberectin (bottom, red).

To further confirm the absolute configuration of (-)-suberectin as (2*S*), its UV and ECD spectra were calculated using TDDFT at the TD- ω B97XD/6-311G++(d,p)/IEFPCM//B3LYP/6-311G(d,p)/IEFPCM level of theory. Comparison of the observed and theoretical spectra was performed using the procedure established by Bringmann and co-workers [26-28]. The calculated ECD spectrum of (S)-suberectin showed a similarity of 85.2% to the experimental one, whereas the calculated ECD spectrum of (R)-suberectin yielded only 1.23% similarity. The difference value (also called enantiomeric similarity index, ESI) therefore amounts to 83.97%. This observation further confirmed that (-)-suberectin has an absolute configuration of (2*S*). It is worth noting that (-)-suberectin can adopt four relevant conformations which only differ in the orientation of the hydroxyl groups of the phenyl substituent. The two most important conformers yield very similar ECD spectra (Figure 3). For the lowest energy conformer, natural transition orbitals were calculated. The positive Cotton effect at approx. $\lambda = 340$ nm seems to be caused by $n \rightarrow \pi^*$ transition in the chromanone unit, whereas the negative Cotton effect at 310 nm is due to $\pi \rightarrow \pi^*$ transition. At around 260 nm (scaled wavelength), two ECD bands appear to cancel each other. The positive Cotton effect at around 240 nm is predicted to originate from another chromanone $\pi \rightarrow \pi^*$ transition.

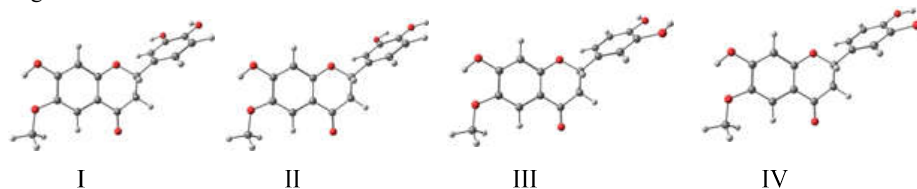


Figure 3. Conformations of (S)-suberectin.

Biological activity

The *in vitro* antifungal activity of the isolated compounds were tested against a panel of pathogens, including *Botrytis cinerea*, *Candida albicans*, *Eremothecium coryli*, *Penicillium notatum*, *Pyricularia oryzae*, and *Rhizomucor miehei* as agar diffusion assays as per the method described previously [29] with minor modifications. Compounds **5** and **6** showed weak activity against *P. oryzae* with MIC values of 20 µg/mL. The rest of the compounds did not show any appreciable activity. The positive control, ciclopirox, had an MIC of 5 µg/mL.

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