Potent Antioxidant and Lipoxygenase Inhibitory Flavanone and Chalcone from *Erythrina mildbraedii* Harms (Fabaceae) of Cameroon

Muhammad Shaiq Ali[a], Muhammad Imran Ali[a], Ghafoor Ahmed[a], Nighat Afza[b], Mehreen Lateef[b], Lubna Iqbal[b], Alain F. K. Waffo[a,c], and Zeeshan Ahmed[a]

[a] H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi-75270, Pakistan
[b] Pharmaceutical and Research Center, Pakistan Council for Scientific & Industrial Research Laboratories Complex, Karachi, Pakistan
[c] Organic Chemistry Department, University of Douala, Douala, Cameroon

Reprint requests to Prof. Dr. Muhammad Shaiq Ali. E-mail: shaiq303@hotmail.com

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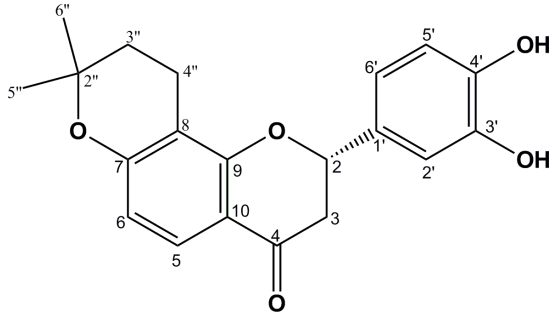
In continuation of our search for new secondary metabolites from African *Erythrina* species, *E. mildbraedii* of Cameroon was investigated. As a result of this study, a new flavanone (mildbone, **1**) and a new chalcone (mildbenone, **2**) have been obtained and characterized by spectroscopic means. Both the compounds exhibited significant antioxidant and moderate lipoxygenase inhibitory activities. Compounds **1** and **2** showed potent antioxidant activity even stronger than the positive control. Among both the compounds, mildbone (**1**) was found to be potent in both the assays.

Key words: Erythrina mildbraedii, Mildbone, Mildbenone, Antioxidant Activity, Lipoxygenase Inhibition

Introduction

The genus *Erythrina* (Fabaceae) comprises approximately 100 species distributed in the tropical and subtropical regions [1]. Many *Erythrina* species are used for the treatment of diseases such as stomach pain and gonorrhea [2]. Several *Erythrina* species are reported to contain flavones [3], isoflavones [4], isoflavanones [5], isoflavans [6], coumarins [7], triterpenoids [8], alkaloids [9], and pterocarpans [10]. Some of the *Erythrina* secondary metabolites are known for their antimicrobial [11], antifungal [12], anti-inflammatory [13], cytotoxic [14], and radical scavenging [11] properties. *Erythrina mildbraedii* (Synonyms: *E. altissima*, *E. klainei* and *E. problematica*) is found in the forests of Nigeria and Cameroon and in village communities of Cameroon, and parts of this plant are popular for the treatment of various types of inflammations [15].

In continuation of our search for new secondary metabolites from unexplored African plants [16 – 18], particularly *Erythrina* species [19 – 20], *E. mildbraedii* of Cameroon was investigated. This article describes the isolation and characterization of a new flavanone (mildbone, **1**) and a new chalcone (mildbenone, **2**) from *E. mildbraedii*. Both the compounds showed



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significant antioxidant and moderate lipoxygenase inhibitory properties. Compound **1** was found to be more potent in both the assays.

Results and Discussion

Mildbone (**1**): The methanolic extract of dried and chopped roots of *E. mildbraedii* afforded **1** as a yellowish amorphous powder. The UV spectrum showed a λ_{max} at 242 ($\log \varepsilon$ = 3.84) and 286 ($\log \varepsilon$ = 3.92) nm typical for a flavanone skeleton [21]. The IR spectrum exhibited strong absorption bands at 3290, 1674 and 1515 cm^{-1} due to the hydroxyl, α,β-unsaturated

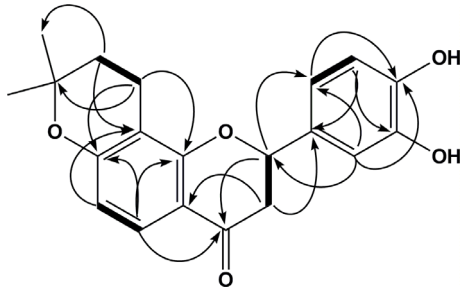


Fig. 1. Important HMBC (H→C) and COSY (▬) correlations in **1**.

ketone and aromatic C=C functionalities, respectively, in the molecule.

In the EI-MS the molecular ion peak was observed at m/z = 340, and the formula of this peak was found as $C_{20}H_{20}O_5$ in the high-resolution mass spectrum (observed 340.1328; calcd. 340.1310) with eleven degrees of unsaturation in the molecule. The other fragments in the EI and HR-EI mass spectra are given in the Experimental Section.

The ^{13}C NMR spectrum of **1** displayed characteristic signals of a flavanone skeleton at δ = 192.0 due to an α,β-unsaturated ketone, a methylene carbon resonance at δ = 43.7 (C-3) and an oxymethine carbon signal at δ = 79.3 (C-2) [21]. In the proton NMR spectrum, a pair of double-doublets at δ = 2.88 (J = 16.8, 12.9 Hz) and 2.66 (J = 16.8, 2.7 Hz) due to H-3a/H-3b and an oxy-methine proton signal at δ = 5.24 (dd, J = 12.9, 2.7 Hz, 2-H) further attested the flavanone skeleton [21].

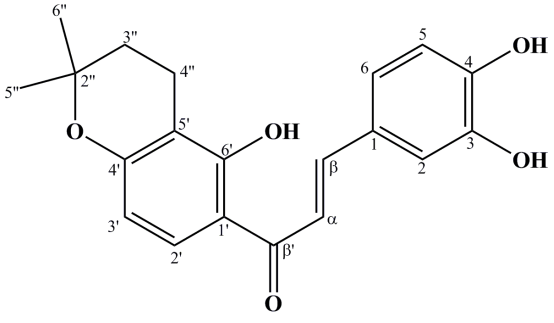
In addition to the characteristic signals due to the flavanone skeleton, the ^{1}H NMR spectrum of **1** displayed two methyl singlets at δ = 1.25 and 1.23 together with a pair of mutually coupled triplets (J = 6.6 Hz) due to the methylenes of a prenyl moiety at δ = 2.57 and 1.68, confirming the presence of a saturated cyclized prenyl moiety in the molecule. This was further confirmed *via* ^{13}C NMR spectroscopy. The carbons associated with the prenyl moiety appeared at δ = 75.6 (C-2″), 16.8 (C-3″), 31.7 (C-4″), 26.9 (C-5″), and 26.3 (C-6″). The mode/site of attachment of this moiety onto the flavanone skeleton was depicted with the aid of HMBC experiments (Fig. 1).

A pair of *ortho*-coupled doublets (J = 8.7 Hz) due to ring A at δ = 7.57 and 6.37 and their corresponding carbon signals at δ = 125.7 and 111.9 in the NMR spectra attested for H-5/C-5 and H-6/C-6, respectively. The proton signals due to ring B resonated as two broad singlets at δ = 6.85 (H-2′) and at δ = 6.75 (2H, H-5′ and H-6′). The expected splitting of signals due to ring B was not observed due to the fact that the signals of H-5′ and H-6′ have the same chemical shift. Signals of both the protons (H-5′ and H-6′) appeared as a common broad singlet with the integartion of two protons in the ^{1}H NMR spectrum. There are many natural flavanones reported in the literature with hydroxyl functions on ring B at C-3′ and C-4′ with the same splitting pattern as observed by us in **1** [21 – 25]. Thus the substitution arrangement at ring B in **1** was attested. The carbon signals associated with these protons appeared at δ = 113.2 (C-2′), 115.1 (C-5′) and 118.3 (C-6′) in the ^{13}C NMR spectrum.

Table 1. NMR data of mildbone (**1**) and mildbenone (**2**) in $CDCl_3$.

C 1/2	**1** δ_H ppm (mult., J in Hz) (at 300 MHz)	**1** δ_C ppm (mult.) (at 75 MHz)	**2** δ_H ppm (mult., J in Hz) (at 300 MHz)	**2** δ_C ppm (mult.) (at 75 MHz)
2/β	5.24 (dd, 12.9, 2.7)	79.3 (d)	7.45 (d, 16.0)	142.0 (d)
3a / α	2.88 (dd,16.8,12.9)	43.7 (t)	7.40 (d, 16.0)	125.1 (d)
3b	2.66 (dd,16.8, 2.7)	–	–	–
4 / β'	–	192.0 (s)	–	192.1 (s)
5/2′	7.57 (d, 8.7)	125.7 (d)	7.44 (d, 8.5)	129.8 (d)
6/3′	6.37 (d, 8.7)	111.9 (d)	6.33 (d, 8.4)	106.5 (d)
7/4′	–	161.0 (s)	–	156.7.2 (s)
8/5′	–	113.6 (s)	–	108.6 (s)
9/6′	–	160.9 (s)	–	159.3 (s)
10/1′	–	109.2 (s)	–	117.8 (s)
1′/1	–	131.1 (s)	–	128.1 (s)
2′/2	6.85 (br.s)	113.2 (d)	7.03 (d, 2.0)	114.6 (d)
3′/3	–	144.8 (s)	–	144.6 (s)
4′/4	–	144.6 (s)	–	146.8 (s)
5′/5	6.75 (br.s)	115.1 (d)	6.77 (d, 8.0)	115.2 (d)
6′/6	6.75 (br.s)	118.3 (d)	6.97(dd, 8.0, 2.0)	121.1 (d)
2″	–	75.6 (s)	–	74.9 (s)
3″	1.68 (t, 6.6)	16.8 (t)	1.77 (t, 7.0)	17.0 (t)
4″	2.57 (t, 6.6)	31.7 (t)	2.64 (t, 7.0)	31.7 (t)
5″	1.25 (s)	26.9 (q)	1.32 (s)	26.7 (q)
6″	1.23 (s)	26.3 (q)	1.32 (s)	26.7 (q)

Assignments of various protons (Table 1) were made with the aid of COSY experiments (Fig. 1), and their associated carbon atoms (Table 1) were correlated *via* HMQC experiments. Finally, all the assignments were cross-checked by HMBC connectivities (Fig. 1). The absolute configuration at C-2 was assigned '*S*' on the basis of the CD spectrum which showed a similar Cotton effect as reported in the literature for related flavonoids [26 – 28]. In the light of the obtained spectral data and comparison with the data of similar compounds [3, 29], the structure of the above described compound is elucidated as **1** and named mildbone. This compound is a new addition in the series of natural prenyl flavanones from *E. mildbraedii*.



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Mildbenone (**2**): The second compound was obtained with the elution of 18 % ethyl acetate in hexane during silica gel column chromatography as a brownish amorphous solid. The UV spectrum of **2** exhibited λ_{max} at 241 ($\log\varepsilon$ = 3.67) and 337 ($\log\varepsilon$ = 3.66) nm. The IR spectrum displayed two prominent absorptions at 3210 and 1640 cm^{-1} due to the hydroxyl and α,β-unsaturated ketone functionalities in the molecule.

The mass spectra of both the compounds were found to be quite similar. The molecular ion peak was found in the EI-MS at $m/z = 340$, and the formula of this peak was depicted as $C_{20}H_{20}O_5$ in the HRMS (EI) showing exactly the same observed and calculated mass (m/z = 340.1311) with eleven degrees of unsaturation. The other fragments in the EIMS and HRMS (EI) are given in the Experimental Section.

Again the NMR data of **2** were found to be very similar to those of **1** except for a few signals (Table 1). In the NMR spectra of **1** and **2**, a drastic change in chemical shifts was observed in the ^{13}C NMR signals of positions C-2/β, C-3/α, C-5/2′, C-6/3′, C-7/4′, C-8/5′, and C-10/1′. Similarly, a significant change was noticed in the proton signals of positions 2/β and 3a/α. These changes in the NMR spectra indicated that **2** had a chalcone skeleton [30]. However, a similar pattern was

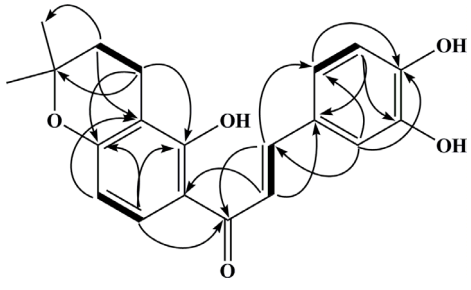

Fig. 2. Important HMBC (H→C) and COSY (▬) correlations in **2**.

Table 2. Antioxidant and lipoxygenase (LOX) inhibition activities of **1** and **2**[a].

Compound	DPPH scavenging activity IC_{50} (μM)	Lipoxygenase inhibition IC_{50} (μM)
1	20.2 ± 0.14	41.8 ± 0.19
2	28.5 ± 0.11	59.7 ± 0.21
BHA	44.2 ± 0.12	–
Baicalein	–	22.6 ± 0.08

[a] Butylated hydroxyanisole (BHA; for DPPH scavenging activity) and baicalein (for lipoxygenase inhibition) were used as positive controls, and each bioassay was performed in triplicate.

observed for the prenyl moiety in the NMR spectra of both the compounds.

Assignments of various protons were made with the aid of COSY experiments (Fig. 2), and their associated carbons were correlated *via* HMQC experiments. Finally, all the assignments were reconfirmed through HMBC connectivities (Fig. 2). On the grounds of the obtained spectral data, the structure of the above discussed compound is characterized as **2** and named mildbenone. This compound is also a new addition in the series of natural chalcones.

*Bioactivities of compounds **1** and **2***

Mildbone (**1**) and mildbenone (**2**) were screened for antioxidant and lipoxygenase (LOX) inhibition properties. Both the compounds exhibited significant antioxidant and moderate LOX inhibition activities. Compounds **1** and **2** displayed potent and even stronger antioxidant activities than butylated hydroxyanisole (BHA). Among both the compounds, **1** was found to be more potent than **2** in both the assays (Table 2).

Experimental Section

General

CD was measured on a JASCO-J-810 instrument. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) and IR spectra on a Shimadzu IR-460 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 spectrometer at 300 and 75 MHz, respectively. Chemical shifts are expressed in δ (ppm) relative to tetramethylsilane (TMS) as an internal standard, and coupling constants are given in Hz. The mass spectra were obtained on a Jeol-JMS HX-110 mass spectrometer.

Plant material

The roots of *E. mildbraedii* were collected from Ngaoundere (Cameroon) in February, 2005 and identi-

fied by Mr. Nana Laurent of the National Herbarium, Yaounde (Cameroon), where a voucher specimen is deposited (49011/HNC).

Extraction and isolation

The shade-dried roots (5.0 kg) were extracted with methanol (8.0 L) at r. t. The obtained crude methanol extract was concentrated by means of evaporation under vacuum distillation (83 g) and subjected to silica gel column chromatography. Hexane, hexane : ethyl acetate and pure ethyl acetate were used as mobile phase. Compound **1** was obtained as a light yellowish amorphous solid (5.0 mg) with the elution of 15 % ethyl acetate in hexane. Further elution with 18 % ethyl acetate yielded **2** as a brownish amorphous solid (5.2 mg).

*Mildbone (**1**)*

$[\alpha]_D^{29}$ = −61.7 (c = 0.093, $CHCl_3$). – CD ($CHCl_3$): $[\theta]_{336}$ = +20.99 and $[\theta]_{304}$ = −40.67. – UV ($CHCl_3$): $\lambda_{max}(\log\varepsilon)$ = 242 (3.84), 286 (3.92) nm. – IR (KBr): ν_{max} = 3290 (OH), 1674 (α,β-unsaturated ketone C=O), 1515 (aromatic C=C) cm^{-1}. – ^1H and ^{13}C NMR: Table 1. – MS(EI): m/z = 340 $[M]^+$, 284, 205, 149, 136. – HRMS ((+)-EI): m/z = 340.1328 (calcd. 340.1310 for $C_{20}H_{20}O_5$, $[M]^+$), 284.0680 (calcd. 284.0685 for $C_{16}H_{12}O_5$), 205.0863 (calcd. 205.0865 for $C_{12}H_{13}O_3$), 149.0232 (calcd. 149.0239 for $C_8H_5O_3$), 136.0527 (calcd. 136.0524 for $C_8H_8O_2$).

*Mildbenone (**2**)*

UV ($CHCl_3$): $\lambda_{max}(\log\varepsilon)$ = 241 (3.67), 337 (3.66) nm. – IR (KBr): ν_{max} = 3210 (OH), 1640 (α,β-unsaturated ketone C=O), 1591 (C=C), 1514 (aromatic C=C) cm^{-1}. – ^1H and ^{13}C NMR: Table 1. – MS ((+)-EI): m/z = 340 $[M]^+$, 284, 205, 149. – HRMS ((+)-EI): m/z = 340.1311 (calcd. 340.1311 for $C_{20}H_{20}O_5$, $[M]^+$), 284.0693 (calcd. 284.0685 for $C_{16}H_{12}O_5$), 205.0871 (calcd. 205.0865 for $C_{12}H_{13}O_3$), 149.0234 (calcd. 149.0239 for $C_8H_5O_3$).

Biological assays

Antioxidant (DPPH scavenging) activity measurements were performed according to the method described by Cotelle *et al.* [31] while LOX inhibiting activity was measured by a modified spectrophotometric method [32] developed by Tappel [33].

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