

## FOUR ISOFLAVONES FROM THE STEM BARK OF *ERYTHRINA* *SACLEUXII*

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**Key Word Index**—*Erythrina sacleuxii*; Leguminosae; stem bark; isoflavones; 7-demethylrobustigenin; 3'-(3-methylbut-2-enyl)biochanin A; 5'-(3-methylbut-2-enyl)pratensein; 5'-formylpratensein.

**Abstract**—From the stem bark of *Erythrina sacleuxii* four new isoflavones were isolated and characterized as 5,7-dihydroxy-2',4',5'-trimethoxyisoflavone (trivial name, 7-demethylrobustigenin), 5,7-dihydroxy-4'-methoxy-3'-(3-methylbut-2-enyl)isoflavone [3'-(3-methylbut-2-enyl)biochanin A], 5,7,3'-trihydroxy-4'-methoxy-5'-(3-methylbut-2-enyl)isoflavone [5'-(3-methylbut-2-enyl)pratensein] and 5,7,3'-trihydroxy-4'-methoxy-5'-formylisoflavone (5'-formylpratensein). The structures were determined on the basis of spectroscopic evidence.  
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### INTRODUCTION

*Erythrina sacleuxii* Hua (Leguminosae) is a tree 9–24 m tall, which is found in Kenya and Tanzania, and is not known elsewhere [1]. There is no prior phytochemical information on this plant; however, the genus *Erythrina* is known to contain *C*-prenylated flavanones, isoflavones, isoflavanones and pterocarpans [2, 3]. From the CHCl<sub>3</sub> extract of the stem bark of *E. sacleuxii*, four new isoflavones were isolated, and the characterization of these compounds is presented here.

### RESULTS AND DISCUSSION

HRMS analysis of **1** showed a molecular ion peak at  $m/z$  344.0896 corresponding to the formula C<sub>18</sub>H<sub>16</sub>O<sub>7</sub>. From the <sup>1</sup>H (δ 8.07 for H-2) and <sup>13</sup>C (δ 155.7 for C-2) NMR and UV spectra, it was evident that this is an isoflavone. The presence of two hydroxyl and three methoxyl substituents were deduced from the <sup>1</sup>H (Table 1) and <sup>13</sup>C NMR (Table 2) spectra. The presence of a chelated hydroxyl group at δ 13.04 and two *meta* coupled aromatic protons at δ 6.29 (H-6) and 6.42 (H-8), and also the fragment ion at  $m/z$  153, resulting from retro-Diels-Alder cleavage of the *C*-ring [4], are consistent with the placement of the two

Table 1. <sup>1</sup>H NMR data for isoflavones isolated from *E. sacleuxii* (300 MHz, acetone-*d*<sub>6</sub>)

H	1	2	3	4
2	8.07 <i>s</i>	8.14 <i>s</i>	8.15 <i>s</i>	8.32 <i>s</i>
6	6.29 <i>d</i>	6.29 <i>d</i>	6.28 <i>d</i>	6.31 <i>d</i>
8	6.42 <i>d</i>	6.41 <i>d</i>	6.41 <i>d</i>	6.45 <i>d</i>
2'		7.38 <i>d</i>	7.04 <i>d</i>	7.48 <i>d</i>
3'	6.81 <i>s</i>			
5'		6.98 <i>d</i>		
6'	6.98 <i>s</i>	7.39 <i>dd</i>	6.89 <i>d</i>	7.52 <i>d</i>
1''		3.33 <i>d</i>	3.37 <i>d</i>	
2''		5.32 <i>t</i>	5.31 <i>t</i>	
4''		1.72 <i>s</i>	1.74 <i>s</i>	
5''		1.70 <i>s</i>	1.71 <i>s</i>	
5-OH	13.04 <i>s</i>	13.04 <i>s</i>	13.02 <i>s</i>	12.88 <i>s</i>
OH	9.69 <i>br</i>	9.66 <i>br</i>	9.66 <i>br</i>	9.84 <i>br</i>
OMe	3.89 <i>s</i>	3.87 <i>s</i>	3.81	8.15 <i>br</i>
	3.80 <i>s</i>			8.90 <i>br</i>
	3.78 <i>s</i>			
CHO				10.38 <i>s</i>

*J* values: H-6/H-8 = 2.1 Hz; H-2'/H-6' in **1**, **2** and **4** = 2.3 Hz, in **3** = 2.1 Hz; H-5'/H-6' in **2** = 8.4 Hz; H-1''/H-2'' in **2** and **3** = 7.3 Hz.

hydroxyl groups at C-5 and C-7. The three methoxyls should then be located in B-ring.

Furthermore, in the <sup>1</sup>H NMR spectrum two singlets at δ 6.81 and 6.98 could be assigned to the two *para* protons at C-3' and C-6' of B-ring and hence the

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Table 2.  $^{13}\text{C}$  NMR data for isoflavones isolated from *E. sachlexii* (75.48 MHz, acetone- $d_6$ )

C	1	2	3	4
2	155.7	155.1	155.4	155.4
3	122.2	124.6	124.7	122.9
4	182.1	182.3	182.1	181.2
4a	106.8	106.9	106.8	106.1
5	164.5	164.6	164.6	163.9
6	100.5	100.5	100.6	100.0
7	165.6	165.6	165.7	165.3
8	95.2	95.2	95.2	94.7
8a	159.7	159.7	159.6	159.1
1'	112.7	124.7	128.5	128.4
2'	154.0*	131.6	116.9	119.8
3'	100.2	131.1	151.2	151.7
4'	152.3*	159.0	147.4	151.4
5'	144.7	117.7	136.6	130.6
6'	118.2	129.4	122.8	124.1
1''		29.9	29.9	
2''		124.2	124.7	
3''		133.3	133.2	
4''		18.5	18.6	
5''		26.6	26.5	
OMe	57.1 57.6 57.8	56.5	61.4	62.8
CHO				189.9

C-Assignments were established based on HMQC and HMBC experiments.

\* Interchangeable.

methoxyls should be at C-2', C-4' and C-5'. The chemical shift values for B-ring carbon atoms are consistent with such oxygenation pattern [4]. Therefore, this compound is 5,7-dihydroxy-2',4',5'-trimethoxyisoflavone or 7-demethylrobustigenin. This structure was confirmed by HMBC and HMQC experiments.

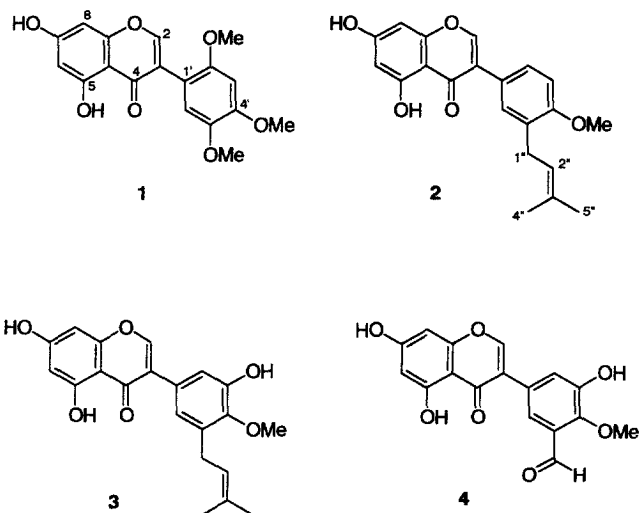
Although **1** has been synthesized earlier [5], this is the first report on its occurrence in nature.

Compound **2**, which analysed for  $\text{C}_{21}\text{H}_{20}\text{O}_5$  by HRMS, is also an isoflavone with two hydroxyl, a methoxyl and a 3-methylbut-2-enyl substituents. The presence of a signal for chelated hydroxyl group at  $\delta$  13.04 and two *meta* coupled aromatic protons at  $\delta$  6.29 (H-6) and 6.41 (H-8) are again consistent with C-5 and C-7 oxygenated A-ring. This was supported by the EIMS with a fragment ion at  $m/z$  153.

In the  $^1\text{H}$  NMR spectrum, three aromatic protons with ABX spin system, appearing at  $\delta$  7.38 (*d*,  $J = 2.3$  Hz), 6.98 (*d*,  $J = 8.4$  Hz) and 7.39 (*dd*,  $J = 2.3, 8.4$ , Hz), were assigned to H-2', H-5' and H-6', respectively. Thus the B-ring is substituted with a methoxyl and prenyl groups at C-4' and C-3'. From biogenetic considerations, the methoxyl has to be at C-4' and the prenyl at C-3'. This was confirmed by HMBC experiment which showed  $J^3$  correlations of the methylene protons ( $\delta$  3.33) of the prenyl chain with C-4' ( $\delta$  159.0) and C-2' ( $\delta$  131.6). Therefore, this is 5,7-dihydroxy-4'-methoxy-3'-(3-methylbut-2-enyl)isoflavone, for which the trivial name 3'-(3-methylbut-2-enyl)biochanin A is suggested.

Compound **3**, analysed for  $\text{C}_{21}\text{H}_{20}\text{O}_6$ , is an isoflavone with identical A-ring substitution as in **1** and **2**, which was deduced from  $^1\text{H}$  (Table 1),  $^{13}\text{C}$  (Table 2) NMR and MS spectra. In the B-ring the presence of a hydroxyl, methoxyl and 3-methylbut-2-enyl substituents was also evident from the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.

In the  $^{13}\text{C}$  NMR, the chemical shift values ( $\delta$  151.2 and 147.4) of the  $sp^2$  carbon atoms in B-ring requires oxygenation to occur on adjacent carbons viz at C-3' and C-4'. Two *meta*-coupled protons at  $\delta$  7.04 and 6.89 can then be assigned to H-2' and H-6', and hence the 3-methylbut-2-enyl group should be placed at C-5'. In  $^{13}\text{C}$  NMR spectrum, the downfield chemical shift value ( $\delta$  61.4) of the methoxyl group requires that it



is *di-ortho*-substituted; thus it has to be at C-4' rather than C-3' [6]. The structure was confirmed through study of direct and long range heteronuclear CH-coupling interactions. On these bases, this new compound is 5,7,3'-trihydroxy-4'-methoxy-5'-(3-methylbut-2-enyl)isoflavone for which the trivial name 5'-(3-methylbut-2-enyl)pratensein is suggested.

Compound **4** is again an isoflavone, analysing for  $C_{17}H_{12}O_7$ . Comparison of its  $^1H$  (Table 1) and  $^{13}C$  (Table 2) NMR spectra with those of **3** revealed identical oxygenation, with the methoxyl again being at C-4'. The only difference between these two compounds is on the nature of the substituents at C-5'. The presence of a singlet at  $\delta$  10.38 (in the  $^1H$  NMR) and a doublet at  $\delta$  189.9 (in the  $^{13}C$  NMR) suggested the substituent at C-5' in compound **4** is a formyl group. This was confirmed by HMQC experiment which showed a direct CH-correlation between the formyl proton and carbonyl carbon atom. Hence this compound is 5,7,3'-trihydroxy-5'-formylisoflavone or 5'-formylpratensein.

## EXPERIMENTAL

### General

Mps: uncorr. Analytical TLC: Merck pre-coated silica gel 60  $F_{254}$  plates. TLC plates were developed using  $CHCl_3$ -EtOAc (6:1), and spots were detected under UV light (254 nm). CC on silica gel 60 (70–230 mesh) and Sephadex LH-20. EIMS: direct inlet, 70 eV.  $^1H$  NMR (300 MHz) and  $^{13}C$  NMR (75.48 MHz) were recorded on ARX 300 (Bruker) spectrometer using TMS as int. standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

### Plant material

The stem bark of *Erythrina sacleuxii* was collected from Gede forest station, Coast province, Kenya, in August 1994. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen is deposited.

### Extraction and isolation

Dried and ground stem bark (200 g) of *Erythrina sacleuxii* was extracted with  $CHCl_3$  by cold percolation. The crude extract (15 g) was subjected to CC on silica gel (200 g) eluting with hexane containing increasing amounts of EtOAc. The fr. eluted with 10% EtOAc in hexane afforded **2** (47 mg); the fr. eluted with 20% EtOAc contains mixt. of two compounds which were separated by CC on Sephadex LH-20 (elu-

ent,  $CHCl_3$ -MeOH; 1:1) to give **1** (258 mg) and **3** (183 mg); while elution with 25% EtOAc afforded **4** (73 mg).

**7-Demethylrobustigenin (1)**. Crystals from MeOH, mp 241–243° (lit. [5] 243–244°). Found  $[M]^+ m/z$  344.0896,  $C_{18}H_{16}O_7$  requires 344.0896. UV  $\lambda_{max}$  (MeOH) nm: 207, 225 sh, 260, 295. IR  $\nu_{max}$  (KBr)  $cm^{-1}$ : 3378 (OH), 1660 ( $> C=O$ ), 1582, 1524, 1450.  $^1H$  NMR (see Table 1).  $^{13}C$  NMR (see Table 2). EIMS  $m/z$  (rel. int.): 344  $[M]^+$  (100), 329  $[M - Me]^+$  (16), 313  $[M - OMe]^+$ , (13), 301 (15) 153 (13).

**3'-(3-Methylbut-2-enyl)biochanin A (2)**. Crystals from  $CHCl_3$ , mp 196–197°. Found  $[M]^+ m/z$  352.1311;  $C_{21}H_{20}O_5$  requires 352.1311. UV  $\lambda_{max}$  (MeOH) nm: 208, 261, 288 sh, 323. IR  $\nu_{max}$  (KBr)  $cm^{-1}$ : 3310 (OH), 1640 ( $> C=O$ ), 1578, 1498, 1440.  $^1H$  NMR (see Table 1).  $^{13}C$  NMR (see Table 2). EIMS  $m/z$  (rel. int.): 352  $[M]^+$  (100), 337  $[M - Me]^+$  (17), 321  $[M - OMe]^+$ , (7), 153 (25).

**5'-(3-Methylbut-2-enyl)pratensein (3)**. Crystals from MeOH, mp 152–154°. Found  $[M]^+ m/z$  368.1260;  $C_{21}H_{20}O_6$  requires 368.1255. UV  $\lambda_{max}$  (MeOH) nm: 208, 262, 289 sh, 324. IR  $\nu_{max}$  (KBr)  $cm^{-1}$ : 3290 (OH), 1652 ( $> C=O$ ), 1574, 1500, 1434.  $^1H$  NMR (see Table 1).  $^{13}C$  NMR (see Table 2). EIMS  $m/z$  (rel. int.): 368  $[M]^+$  (100), 353  $[M - Me]^+$  (22), 311 (8), 153 (32).

**5'-Formylpratensein (4)**. Amorphous powder. Found  $[M]^+ m/z$  328.0583;  $C_{17}H_{12}O_7$  requires 328.0587. UV  $\lambda_{max}$  (MeOH) nm: 212, 261, 322. IR  $\nu_{max}$  (KBr)  $cm^{-1}$ : 3392 (OH), 1662 ( $> C=O$ ), 1618, 1582, 1506, 1442.  $^1H$  NMR (see Table 1).  $^{13}C$  NMR (see Table 2). EIMS  $m/z$  (rel. int.): 328  $[M]^+$  (100), 299  $[M - CHO]^+$  (21), 257 (7), 229 (11), 153 (10).

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