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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.

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 $CHCl_3$ 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 $C_{18}H_{16}O_7$. From the ^1H (δ 8.07 for H-2) and ^{13}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 ^1H (Table 1) and ^{13}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 hydroxyl groups at C-5 and C-7. The three methoxyls should then be located in B-ring.

Furthermore, in the ^1H 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

Table 1. ^1H NMR data for isoflavones isolated from *E. sacluexii* (300 MHz, acetone-d_6)

H	1	2	3	4
2	8.07 *s*	8.14 *s*	8.15 *s*	8.32 *s*
6	6.29 *d*	6.29 *d*	6.28 *d*	6.31 *d*
8	6.42 *d*	6.41 *d*	6.41 *d*	6.45 *d*
2′		7.38 *d*	7.04 *d*	7.48 *d*
3′	6.81 *s*			
5′		6.98 *d*		
6′	6.98 *s*	7.39 *dd*	6.89 *d*	7.52 *d*
1″		3.33 *d*	3.37 *d*	
2″		5.32 *t*	5.31 *t*	
4″		1.72 *s*	1.74 *s*	
5″		1.70 *s*	1.71 *s*	
5-OH	13.04 *s*	13.04 *s*	13.02 *s*	12.88 *s*
OH	9.69 *br*	9.66 *br*	9.66 *br*	9.84 *br*
			8.15 *br*	8.90 *br*
OMe	3.89 *s*	3.87 *s*	3.81	
	3.80 *s*			
	3.78 *s*			
CHO				10.38 *s*

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.

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Table 2. ^{13}C NMR data for isoflavones isolated from *E. sacluexii* (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	56.5	61.4	62.8
	57.6			
	57.8			
CHO				189.9

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

* Interchangeable.

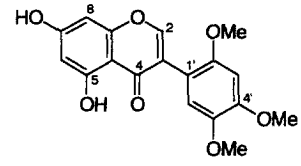
methoxyls should be at C-2′, C-4′ and C-5′. The chemical shift values for B-ring carbon atoms are consistent with such oxygenation pattern [4]. Therefore, this compound is 5,7-dihydroxy-2′,4′,5′-trimethoxyisoflavone or 7-demethylrobustigenin. This structure was confirmed by HMBC and HMQC experiments. Although **1** has been synthesized earlier [5], this is the first report on its occurrence in nature.

Compound **2**, which analysed for $C_{21}H_{20}O_5$ 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 δ 13.04 and two *meta* coupled aromatic protons at δ 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.

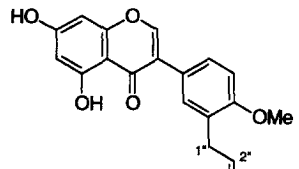
In the ^1H NMR spectrum, three aromatic protons with ABX spin system, appearing at δ 7.38 (*d*, $J = 2.3$ Hz), 6.98 (*d*, $J = 8.4$ Hz) and 7.39 (*dd*, $J = 2.3$, 8.4, Hz), were assigned to H-2′, H-5′ and H-6′, respectively. Thus the B-ring is substituted with a methoxyl and prenyl groups at C-4′ and 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 (δ 3.33) of the prenyl chain with C-4′ (δ 159.0) and C-2′ (δ 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 $C_{21}H_{20}O_6$, is an isoflavone with identical A-ring substitution as in **1** and **2**, which was deduced from ^1H (Table 1), ^{13}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 ^1H and ^{13}C NMR spectra.

In the ^{13}C NMR, the chemical shift values (δ 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 δ 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}C NMR spectrum, the downfield chemical shift value (δ 61.4) of the methoxyl group requires that it



1



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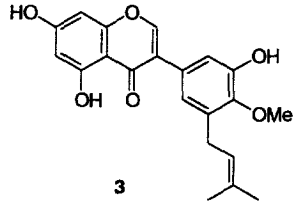
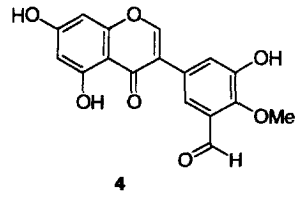


3



4

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 δ 10.38 (in the ^1H NMR) and a doublet at δ 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 (eluent, $CHCl_3$–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 fro MeOH, mp 241–243°(lit. [5] 243–244°). Found $[M]^+$ *m/z* 344.0896, $C_{18}H_{16}O_7$ requires 344.0896. UV λ_{max} (MeOH) nm: 207, 225 sh, 260, 295. IR ν_{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 λ_{max} (MeOH) nm: 208, 261, 288 sh, 323. IR ν_{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 λ_{max} (MeOH) nm: 208, 262, 289 sh, 324. IR ν_{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 λ_{max} (MeOH) nm: 212, 261, 322. IR ν_{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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