



## ORIGINAL ARTICLE

# Erysacleuxins C and D, new isoflavones from the twigs of *Erythrina sacleuxii* Hua and their cytotoxic activity



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**Abstract** Two previously undescribed isoflavones, erysacleuxin C (**1**) and erysacleuxin D (**2**), together with seven known compounds (**3–9**), were isolated and identified from the EtOAc extract of the twigs of *Erythrina sacleuxii* Hua (Leguminosae). The structures of the isolated compounds were determined on the basis of their spectroscopic and spectrometric data. Evaluation of their cytotoxicity against the human cancer HeLa-S3 cell lines indicated IC<sub>50</sub> values of 130.4, 54.9 and 73.9 μM for erysacleuxin C (**1**), erysacleuxin D (**2**) and butin (**9**), respectively.

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## 1. Introduction

The genus *Erythrina* (Leguminosae) comprises more than 110 species of red or orange flowered trees and shrubs, distributed throughout the tropical and subtropical regions of Africa, America and Asia (Kone et al., 2011). Plants from the genus *Erythrina* are used in traditional medicine for the treatment of asthma, cancer, insomnia, inflammations, malaria fever, microbial infections, and toothache (Mitscher et al., 1987; Kokwaro, 1993). *Erythrina sacleuxii* Hua is a

red-flowered 9–24 m tall tree endemic to Kenya and Tanzania (Gillett et al., 1971). Previous studies on the roots and stem barks of Kenyan specimens of *E. sacleuxii* yielded flavones, isoflavones, isoflavanones, pterocarpan, and isoflav-3-enes (Yenesew et al., 1998a, 1998b; Yenesew et al., 2000; Andayi et al., 2006; Ombito et al., 2018). Herein, the isolation and structural elucidation of two new isoflavones, together with seven known compounds from the twigs of the Kenyan specimens of *E. sacleuxii* is reported. The compounds were assayed for cytotoxicity against human cancer HeLa-S3 cell lines.

## 2. Results and discussion

The powder of air-dried twigs of *E. sacleuxii* was extracted with CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH (1:1) at room temperature. The crude extract was then suspended in water and successively extracted with CH<sub>2</sub>Cl<sub>2</sub> and EtOAc. Chromatographic separation and purification of the EtOAc extract led to the isolation of

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erysacleuxin C (**1**), erysacleuxin D (**2**), vanillin (**3**) (Harish et al., 2005), 26-hydroxyhexacosyl-(*E*)-ferulate (**4**) (Ali et al., 2010), genistein (**5**), liquiritigenin (**6**) (Jahromi et al., 1993), 5'-formylpratensein (**7**) (Yenesew et al., 1998a), calycosin (**8**) (Markham et al., 1968) and butin (**9**) (Roux and Paulus, 1961) (Fig. 1). The chemical structures of the known compounds were established by comparison of their spectroscopic and spectrometric data with the literature data.

Compound **1** was obtained as a brown solid and the molecular formula  $C_{21}H_{20}O_7$  was assigned based on the ESI-HRMS ( $[M + Na]^+$   $m/z$  407.1109, calcd. 407.1101) and NMR analyses (Table 1). The UV spectrum of compound **1** in acetonitrile displayed two distinct absorption maxima, one at  $\lambda_{max}$  262 nm for band II and one at 290 nm for band I, typical for an isoflavone skeleton (Mabry et al., 1970) while its IR spectrum showed absorption bands for chelated hydroxyl ( $3326\text{ cm}^{-1}$ ) and conjugated carbonyl ( $1651\text{ cm}^{-1}$ ). It is worth mentioning that although the IR spectrum of compound **1** should have displayed an extra strong absorption band in the carbonyl region for the unconjugated ketone function ( $>1700\text{ cm}^{-1}$ ), the only band observed was for the conjugated carbonyl ( $1651\text{ cm}^{-1}$ ). This is not the first time that this kind of anomaly has been reported (Yu et al., 2012). The possible reason for this anomaly is not known to us. Its NMR spectra were in agreement with an isoflavone backbone with a singlet signal at  $\delta_H$  8.18 typical for H-2 proton of isoflavones (Harborne et al., 1975). The 5,7-dioxygenation pattern of ring-A suggested by the *meta*-coupling ( $J = 2.1\text{ Hz}$ ) of protons H-6 ( $\delta_H$  6.28) and H-8 ( $\delta_H$  6.42) is in line with the biosynthetically viable substitution pattern of ring-A (Nkengfack et al., 1989; Dewick, 2002). This substitution pattern was further supported by the HMBC cross-peaks (Table 1) of 5-OH ( $\delta_H$  13.03) with C-5 ( $\delta_C$  163.9), C-6 ( $\delta_C$  99.9) and C-10 ( $\delta_C$  106.2), H-6 with C-5 ( $\delta_C$  163.9), C-8 ( $\delta_C$  94.6) and C-10 ( $\delta_C$  106.2), and those of H-8 with C-6 ( $\delta_C$  99.9), C-7 ( $\delta_C$  166.1), C-9 ( $\delta_C$  159.0) and C-10 ( $\delta_C$  106.2). The substitution pattern of ring B was revealed by *meta*-coupled ( $J = 2.1\text{ Hz}$ ) H-2' ( $\delta_H$  7.11) and

H-6' ( $\delta_H$  6.87) protons and the HMBC cross-peaks of H-2' with C-3 ( $\delta_C$  123.7), C-3' ( $\delta_C$  150.5), C-4' ( $\delta_C$  147.1) and C-6' ( $\delta_C$  123.6), and those of H-6' with C-3 ( $\delta_C$  123.7), C-1' ( $\delta_C$  127.6), C-2' ( $\delta_C$  117.6) and C-4' ( $\delta_C$  147.1). The C-4' position of the methoxy substituent ( $\delta_H$  3.77,  $\delta_C$  60.4) was suggested the HMBC cross-peaks of 4'-OCH<sub>3</sub> ( $\delta_H$  3.77) with C-4' ( $\delta_C$  147.1). Additionally, the  $^1\text{H}$  NMR spectrum of compound **1** displayed a singlet signal at  $\delta_H$  3.83 (2H) for methylene protons (H-1''), a septet at  $\delta_H$  2.82 (1H) for a methine proton (H-3'') and a doublet at  $\delta_H$  1.11 (6H, *d*, H-4'' & H-5'') in the non-aromatic region, which were deduced to constitute a 3-methyl-2-oxobutyl unit. The attachment of this oxoprenyl unit at C-5' was suggested by the strong long-range HMBC correlations between H-1'' and C-1' ( $\delta_C$  127.6), C-4' ( $\delta_C$  147.1), C-5' ( $\delta_C$  130.4) and C-6' ( $\delta_C$  123.6). This new compound (**1**), erysacleuxin C, was, therefore, characterized as 5,7,3'-trihydroxy-4'-methoxy-5'-(3-methyl-2-oxobutyl)isoflavone.

Compound **2** was isolated as a white powder. The ESI-HRMS analysis in the positive mode revealed an  $[M + Na]^+$  ion at  $m/z$  391.0790, corresponding to the molecular formula  $C_{20}H_{16}O_7$  (calcd. for  $C_{20}H_{16}O_7Na$ , 391.0788). The UV spectrum of compound **2** in acetonitrile revealed two distinct absorption maxima, one at 255 nm for band II and one at 292 nm for band I, typical for an isoflavone nucleus (Mabry et al., 1970) while its IR spectrum displayed absorption bands at  $3260\text{ cm}^{-1}$  (OH) and  $1650\text{ cm}^{-1}$  (conjugated carbonyl). The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra (Table 1) of **2** closely resembled those of compound **1**, except that the signals for the 3-methyl-2-oxobutyl moiety at  $\delta_H$  3.83 (2H, *s*, H-1''),  $\delta_H$  2.82 (1H, *sept*, H-3''),  $\delta_H$  1.11 (6H, *d*, H-4'' & H-5'') in compound **1** were replaced by the signals for a 3-oxobut-1-en-1-yl unit at  $\delta_H$  7.84 (1H, *d*,  $J = 16.5\text{ Hz}$ , H-1''),  $\delta_H$  6.86 (1H, *d*,  $J = 16.5\text{ Hz}$ , H-2'') and at  $\delta_H$  2.35 (3H, *s*, H-4'') in compound **2**. The *trans*-geometry of the double bond of the 3-oxobut-1-en-1-yl unit was deduced from the coupling constant ( $J = 16.5\text{ Hz}$ ) of the two olefinic protons. The HMBC cross-peaks (Table 1) of H-1'' with C-4' ( $\delta_C$  147.9), C-5' ( $\delta_C$  118.9), C-2'' ( $\delta_C$  129.3) and C-3'' ( $\delta_C$  198.1)

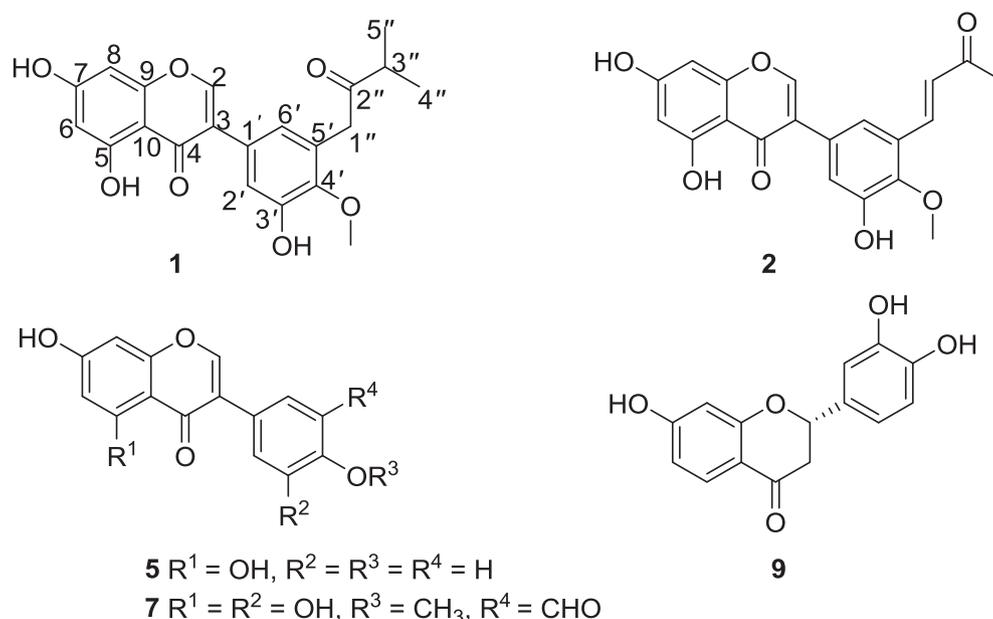


Fig. 1 Chemical structures of the isolated compounds.

**Table 1**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR spectroscopic data for compounds **1** and **2** acquired in acetone  $d_6$ .

Position	1			2		
	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	HMBC	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	HMBC
2	8.18 <i>s</i>	154.9	C-3, C-4, C-9	8.32 <i>s</i>	155.3	C-3, C-4, C-9
3		123.7			122.8	
4		181.4			181.1	
5		163.9			163.6	
6	6.28 <i>d</i> (1.9)	99.9	C-5, C-8, C-10	6.30 <i>d</i> (2.1)	100.1	C-5, C-8
7		166.1			163.9	
8	6.42 <i>d</i> (1.9)	94.6	C-6, C-7, C-9, C-10	6.44 <i>d</i> (2.1)	94.7	C-6, C-7, C-9
9		159.0			159.1	
10		106.2			105.9	
1'		127.6			128.7	
2'	7.11 <i>d</i> (2.1)	117.6	C-3, C-3', C-4', C-6'	7.31 <i>d</i> (2.0)	120.3	C-3, C-3', C-4', C-6'
3'		150.5			151.0	
4'		147.1			147.9	
5'		130.4			118.9	
6'	6.87 <i>d</i> (2.1)	123.6	C-3, C-1', C-2', C-4'	7.44 <i>d</i> (2.0)	119.6	C-3, C-1', C-2', C-4'
1''	3.83 <i>s</i>	42.9	C-1', C-4', C-5', C-6'	7.84 <i>d</i> (16.5)	137.8	C-4', C-5', C-2'', C-3''
2''		211.4		6.86 <i>d</i> (16.5)	129.3	
3''	2.82 <i>sept</i> (7.0)	40.7			198.1	
4''	1.11 <i>d</i> (6.9)	18.7		2.35 <i>s</i>	27.7	C-3''
5''	1.11 <i>d</i> (6.9)	18.7				
4'-OMe	3.77 <i>s</i>	60.4	C-4'	3.89 <i>s</i>	61.8	C-4'
5-OH	13.03 <i>s</i>		C-5, C-6, C-10	12.94 <i>s</i>		C-5, C-6, C-10

suggested the attachment of the 3-oxobut-1-en-1-yl unit at C-5' of ring B. Therefore, compound **2**, was characterized as (*E*)-5,7,3'-trihydroxy-4'-methoxy-5'-(3-oxobut-1-en-1-yl)isoflavone trivially named erysacleuxin D.

From the biosynthetic point of view, the proposed biosynthetic pathway of compounds **1**, **2** and **7** from compound **5** is through biological hydroxylation, epoxidation and ring opening, methylation, oxidation, dehydration, oxidative cleavage of double bonds, and prenylation as illustrated in Scheme 17 (Supplementary data).

As part of our ongoing investigation of African *Erythrina* species in search for novel cytotoxic natural products, the isolated compounds were assayed for *in vitro* activity against the human cancer cell lines, HeLa-S3 using the Giesma staining assay. Compounds **1**, **2** and **9** showed weak cytotoxicity with  $\text{IC}_{50}$  values of 130.4, 54.9 and 73.9  $\mu\text{M}$ , respectively. The positive control, camptothecin had an  $\text{IC}_{50}$  of 14.6  $\mu\text{M}$ . Butin (**9**) has previously shown cytotoxicity activity against HL60 cells with  $\text{IC}_{50}$  value of 8.3  $\mu\text{M}$  (Fotso et al., 2017). With regard to the structure-activity relationships of compounds **1** and **2**, some features that might influence their cytotoxic activity can be drawn when comparing their chemical structures. It was observed that erysacleuxin D (**2**) with an  $\alpha,\beta$ -unsaturated carbonyl group on the side chain was more potent than erysacleuxin C (**1**) with a saturated double bond at C-1''. The possible logical explanation is that the strong Michael acceptors in compound **2** can induce cell damage and cause cytotoxicity (Amslinger, 2010).

### 3. Experimental section

#### 3.1. General experimental procedures

Melting points were determined by a Stuart melting point apparatus SMP1 (UK) and are uncorrected. An Evolution

201 UV–visible spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA) was used to record UV spectra. A Bruker Tensor 27 IR spectrometer equipped with a diamond ATR unit was used to record IR spectra. The NMR spectra were recorded on a Bruker Avance-III spectrometer (Bruker, Karlsruhe, Germany) at 600 MHz and 150 MHz ( $^1\text{H}$  and  $^{13}\text{C}$ , respectively) equipped with a 5 mm TCI cryoprobe and Avance-III spectrometer (Bruker, Karlsruhe, Germany) at 400 and 100 MHz ( $^1\text{H}$  and  $^{13}\text{C}$ , respectively) equipped with a 5 mm probe head. ESI-HRMS was obtained with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Eschborn, Germany). Silica gel 60 Å (0.035–0.070 mm) was used for Column chromatography and preparative TLC performed on glass plates measuring 20 × 20 cm precoated with silica gel 60 PF<sub>254+366</sub> having 0.75 mm thickness. The spots and bands were visualized under a UV lamp (254 and 366 nm). Reversed-phase preparative HPLC separation was carried out on a Knauer System equipped with a binary pump and integrated diode array detector and an ACE C18-PFP column (5  $\mu\text{m}$ , 150 mm × 30 mm, 20 °C) at a flow rate of 37.5 mL/min.

#### 3.2. Plant material

The twigs of *E. saclexii* were collected from Mutsengo, Kilifi County along the Kenyan coast in December 2016. Identification of the plant species was done by Prof. S.T. Kariuki of Biological Sciences, Egerton University. A voucher specimen was deposited at the Department of Biological Sciences, Egerton University (no. 0136EUH).

#### 3.3. Extraction and isolation

The air-dried and ground twigs of *E. saclexii* (3.1 kg) were extracted by percolation with  $\text{CH}_2\text{Cl}_2$ - $\text{CH}_3\text{OH}$  (1:1)

(3 × 7.5 L) at room temperature, yielding 180.3 g of crude extract after evaporation under reduced pressure. The dried crude extract was suspended in water (800 mL) and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and EtOAc. The EtOAc fraction (43.9 g) was adsorbed on silica gel (silica gel 60, 0.035–0.070 mm) and subjected to Column chromatography eluting with increasing amounts of EtOAc in cyclohexane to give a total of ninety two fractions, 200 mL each. On the basis of TLC profiles, the fractions were combined to give eight fractions (A–H). Fraction B (1.3 g) was purified over silica gel, eluting with 10% EtOAc in petroleum ether, yielding two sub-fractions (B<sub>1</sub> and B<sub>2</sub>). Sub-fraction B<sub>2</sub> was further purified with preparative TLC with a mobile phase of CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>OH (20:0.1) to yield vanillin (**3**, 12.3 mg, *R<sub>f</sub>* = 0.62). Fraction F (1.23 g) was subjected to column chromatography, eluting with 4% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> to yield three sub-fractions (F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub>). Sub-fraction F<sub>1</sub> was purified with preparative TLC using 5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> to give 26-hydroxyhexacosyl-(*E*)-ferulate (**4**, 5.6 mg, *R<sub>f</sub>* = 0.33), while genistein (**5**, 6.2 mg, *R<sub>f</sub>* = 0.32) was obtained from sub-fraction F<sub>3</sub>, after purification on preparative TLC with 6% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>. Column chromatographic purification of fraction G (3 g) eluting with 4% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> gave sub-fractions G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub>. Sub-fraction G<sub>2</sub> was separated on reversed-phase preparative HPLC with MeCN–H<sub>2</sub>O (30:70) isocratic, to afford liquiritigenin (**6**, 4 mg) at *t<sub>R</sub>* = 5.69 min, 5'-formylpratensein (**7**, 2 mg) at *t<sub>R</sub>* = 10.53 min and erysacleuxin C (**1**, 2.2 mg) at *t<sub>R</sub>* = 12.21 min. Fraction H (4.2 g), eluting with 60% CH<sub>2</sub>Cl<sub>2</sub> in EtOAc, was subjected to column chromatography to give sub-fractions H<sub>1</sub> and H<sub>2</sub>. Further separation of H<sub>2</sub> by column chromatography with 5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> as eluent yielded four sub-fractions (H<sub>2,1</sub>, H<sub>2,2</sub>, H<sub>2,3</sub>, and H<sub>2,4</sub>). Sub-fraction H<sub>2,1</sub> was separated by reversed-phase HPLC with a mobile phase of MeCN–H<sub>2</sub>O (40:60) to afford calycosin (**8**, 4 mg) at *t<sub>R</sub>* = 3.07 min and erysacleuxin D (**2**, 2.1 mg) at *t<sub>R</sub>* = 5.62 min. Purification of H<sub>2,4</sub> by preparative TLC using 8% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> yielded butin (**9**, 15 mg, *R<sub>f</sub>* = 0.57).

The CH<sub>2</sub>Cl<sub>2</sub> extract (8.6 g) was subjected to column chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>:EtOAc 100:0–0:100) to produce four fractions. Fraction 2 was repeatedly purified by column chromatography eluting with 60% CH<sub>2</sub>Cl<sub>2</sub> in EtOAc to give an additional quantity of **3** (12.3 mg). Fraction 4 was subjected to preparative TLC developed with *n*-hexane–EtOAc (70:30) to afford additional quantity compound **4** (7.2 mg).

### 3.3.1. Erysacleuxin C (**1**)

Pale yellow solid; mp 180–182 °C; UV (MeCN) λ<sub>max</sub> (log ε) 262 (4.26), 290 (3.22) nm; IR (KBr) ν<sub>max</sub> 3326, 2972, 2936, 1699, 1651, 1506, 1440, 1365, 1311, 1196, 1165, 1051 and 962 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz) and <sup>13</sup>C NMR (150 MHz) spectroscopic data in acetone *d*<sub>6</sub>, see Table 1; ESI-HRMS (positive) *m/z* 407.1109 [M + Na]<sup>+</sup> (calcd. for C<sub>21</sub>H<sub>20</sub>O<sub>7</sub>Na, 407.1101).

### 3.3.2. Erysacleuxin D (**2**)

White powder; mp 174–176 °C; UV (MeCN) λ<sub>max</sub> (log ε) 255 (4.30), 292 (3.30) nm; IR (KBr) ν<sub>max</sub> 3260, 1650, 1576, 1442, 1365, 1282, 1196 and 834 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz) and <sup>13</sup>C NMR (150 MHz) spectroscopic data in acetone *d*<sub>6</sub>, see Table 1; ESI-HRMS (positive) *m/z* 391.0790 [M + Na]<sup>+</sup> (calcd. for C<sub>20</sub>H<sub>16</sub>O<sub>7</sub>Na, 391.0788).

### 3.4. Cytotoxicity assays

Cytotoxic activities were carried out according to Gruhn et al. (2007) with minor modifications: The cell culture medium was removed from the cells and the cells were washed with PBS, MeOH was added for fixation for 10 min, cell layer was dried and stained for 5 min with Giemsa solution (Giemsa's Azure Eosin Methylene Blue Solution; 1:10 diluted with 0.9% NaCl in H<sub>2</sub>O bidest.). The stained cell layer was then washed with water and the stain was extracted with 0.1 M HCl while shaking. The stained solution was transferred to a new 96 well plate and measured at 600 nm. All tests were done in triplicate.

### 4. Conclusions

In conclusion, two new isoflavones, erysacleuxin C (**1**) and erysacleuxin D (**2**), along with seven known compounds (**3–9**) were isolated from twigs of *E. saacleuxii*. Erysacleuxin C (**1**), erysacleuxin D (**2**) and butin (**9**), showed weak cytotoxicity against human cancer cell lines, HeLa-S3, with IC<sub>50</sub> values of 130.4, 54.9 and 73.9 μM, respectively.

### Declaration of Competing Interest

No potential conflict of interest was reported by authors.

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### Appendix A. Supplementary material

NMR Spectra, ESI-HRMS spectra, UV Spectra, and IR Spectra. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2019.05.007>.

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