Erythrinaline Alkaloids and Antimicrobial Flavonoids from *Erythrina latissima*

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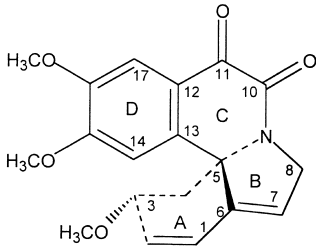
Abstract

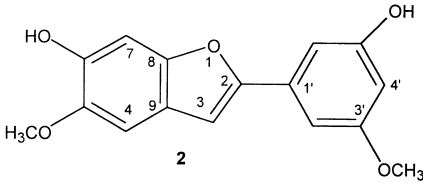
The seed pods of *Erythrina latissima* yielded erysotrine, erysodine, syringaresinol, vanillic acid, a new erythrina alkaloid, (+)-10,11-dioxoerysotrine**,** which was lethal to brine shrimp and 2-(5′-hydroxy-3′-methoxy phenyl)-6-hydroxy-5-methoxybenzofuran, which showed strong antimicrobial activity against the yeast spores, Gram-positive and Gram-negative bacteria. The root bark gave four known pterocarpans which showed moderate to strong antifungal activity against the yeast spores and three known flavonoids showed antimicrobial activity against all test microorganisms.

The genus *Erythrina* comprises over 110 species showing cosmopolitan distribution [1] and is known to produce *C*-prenylated flavanones, isoflavones, isoflavanones and pterocarpans [2], [3]. *Erythrina latissima* E. Meyer (Fabaceae) is a tree 9 – 24 m tall, found in Botswana, Zimbabwe and South Africa and whose root and stem are burnt and used for dressing open wounds [4] while the seeds are known to contain the erythraline alkaloids [5]. No prior phytochemical work has, to date, been reported on the seed pods of this genus.

The HR-TOF-EIMS of the isolated, new erythrina alkaloid **1** showed a molecular ion peak at 341.1265 consistent with the molecular formula $C_{19}H_{19}NO_2$. The ^{1}H- and ^{13}C-NMR data (Table **1**) for **1** are very similar to those of (+)-10,11-dioxoerythraline which was reported from *E. bidwillii* [6] with the only difference that the latter contains a methylenedioxy functional group (δ_H = 5.97, 2H) in place of the two methoxy groups (δ_H = 3.95 and 3.91) in compound **1**. The nature and identity of the tetracyclic ring moiety was deduced from the ^{1}H-NMR data which along with published data [5], [6], [7] enabled us to identify the parent moiety of **1** as (+)-erysotrine. The ^{13}C-NMR data for **1** revealed the presence of two carbonyls at δ_C = 159.5 (amide) and δ_C = 181.8 (α,β-unsaturated) and the placement of the former at C-10 and the latter at C-11 was based on HMBC. The rest of the structure was assigned and confirmed using HMBC and other 2D NMR data. Compound **1** is thus a 10,11-dioxo-analogue of erysotrine, with a 3*R*,5*S* absolute configuration, and was identified as (+)-10,11-dioxoerysotrine.



1



2

The EIMS of compound **2** showed a molecular ion peak, $[M]^+$, at *m/z* 286.1, consistent with the molecular formula $C_{16}H_{14}O_5$. The ^{1}H- and ^{13}C-NMR data (Table **1**), together with COSY spectrum indicated the presence of three ring systems from the following features: 2,4,5-trioxyphenyl substitution (δ_H = 7.04, d, *J* = 0.8 Hz and δ_H = 7.10, d, *J* = 0.8 Hz, 3,5-dioxyphenyl substitution (δ_H = 6.95, dd, *J* = 2.2, 2.2 Hz; δ_H = 6.92, dd, *J* = 2.2, 2.2 Hz and δ_H = 6.41, *t*, *J* = 2.2 Hz) and a singlet at δ_H = 7.14 (δ_C = 102.8) characteristic of an H-3 proton of a 2-phenylbenzofuran moiety. The ^{1}H-NMR spectrum further showed two methoxy groups at δ_H = 3.91 and = 3.84 which were placed at C-5 and C-3′, respectively, of a 2-phenylbenzofuran nucleus using the observed HMBC correlations between these methoxy signals and the corresponding carbon atoms. The rest of the assignments were done using HMBC data, enabling **2** to be identified as 2-(5′-hydroxy-3′-methoxyphenyl)-6-hydroxy-5-methoxybenzofuran, a new natural product and also the first report of a phenylbenzofuran derivative in *Erythrina* species. The seed pods and the root bark were toxic to brine shrimp (*Artemia salina*) giving LC_{50} values of 6.31 and 31.61, respectively, while **1** gave a value of 12.61. Compound **2** (Table **2**) exhibited the strongest antimicrobial activity against all test microorganisms while the other pure compounds **1**, erysotrine, erysodine, syringaresinol and vanillic acid were inactive. The pure compounds from the root bark exhibited antimicrobial activity with the pterocarpans, isoneorautenol **3**, shinpterocarpin **4**, 9-*O*-acetylshinpterocarpin **5**, erybraedin A, neorautenol and 2′,4′-diacetyl-6″,6″-dimethylpyrano[2″,3″:7,8]isoflav-3-ene **6**, showing strong activity against the yeast spores but no activity against the Gram-negative bacteria (Table **2**). Previous antimicrobial activity evaluation has only been done on erybraedin A [8] and abyssinone IV [10].

Materials and Methods

General experimental procedures: M.p's uncorrected-: Optical rotations $[\alpha]_D$: Polatronic-D (Schimdt + Haensch) polarimeter. UV: Shimadzu UV-2501PC Spectrophotometer. IR: Perkin Elmer 2000 FT-IR Spectrometer. ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMQC, HMBC: Bruker Avance DPX 300 Spectrometer using standard pulse sequences and referenced to residual solvent signal. HR-

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Table **1** ^{1}H- and ^{13}C-NMR data for compounds **1**[a] and **2**[b]

	1		***2***	
	δ_H	δ_C	δ_H	δ_C
1	6.74, dd, (11.5,2.4)	124.9 (d)		
2	6.00, d, (11.5)	132.6 (d)		159.2 (s)
3	3.64, t, (7.9)	76.1 (d)	7.14, s	102.8 (d)
4	2.30, d, (7.9)	49.9 (t)	7.10, d, (0.8)	102.5 (d)
5		70.8 (s)		145.8 (s)
6		138.0 (s)		146.1 (s)
7	5.88, br s	121.2 (d)	7.04, d, (0.8)	97.9 (d)
8	4.64, br s	54.7 (t)		150.2 (s)
9				121.0 (s)
10		159.7 (s)		
11		181.8 (s)		
12		124.2 (s)		
13		141.7 (s)		
14	7.17, s	106.4 (d)		
15		153.5 (s)		
16		149.5 (s)		
17	7.48, s	111.1 (d)		
3-OCH_3	3.23, s	56.9 (q)		
15-OCH_3	3.91, s	56.7 (q)		
16-OCH_3	3.95, s	56.7 (q)		
1′				133.0 (s)
2′			6.92, dd, (2.2, 2.2)	101.5 (d)
3′				161.8 (s)
4′			6.41, t, (2.2)	101.5 (d)
5′				154.8 (s)
6′			6.95, dd, (2.2, 2.2)	104.1 (d)
5-OCH_3			3.91, s	55.3 (q)
3′-OCH_3			3.84, s	55.1 (q)

[a] In chloroform-*d*.
[b] In acetone-d_6.

EIMS: Autospec Time of Flight (TOF) Spectrometer. EI-MS: Finnigan MAT SSQ 7000 Single Quadrupole Instrument at 70 eV. CC: Silica gel 60 (0.040 – 0.063 mm, Merck). Preparative and analytical TLC: silica gel 60 $PF_{254+366}$ (Merck).Visualisation of chromatograms: UV (254 and 366 nm) and vanillin-sulphuric acid spray.

The plant material was collected in Mapoka (Botswana) and was identified by Dr. L. M. Turton and a voucher specimen (E 0897) deposited at the University of Botswana Herbarium.

Extraction and isolation: Seed pods (5 kg) were powdered and extracted in 1 : 1 MeOH/$CHCl_3$ mixture (10 L). Removal of solvent from the extract gave a green residue (40 g) which was re-dissolved in $CHCl_3$/MeOH mixture (1 L) and 300 g of activated charcoal added to remove chlorophyll. The mixture was filtered and the filtrate, upon solvent evaporation, yielded a brown residue (27 g) which was chromatographed on silica gel 60 column (300 g) using a 6 : 1 $CHCl_3$/EtOAc mixture. A total of 25 fractions (200 mL each) were collected and monitored using TLC to give SP_A (frs 1 – 17, 5 g) and SP_B (frs 18 – 25, 2 g). Fraction SP_A was further purified on preparative TLC ($CHCl_3$/EtOAc, 6 : 1) by multiple development (× 3) to give (+)-10,11-dioxoerysotrine **1** (50 mg, R_f 0.32), erysotrine (10 mg, R_f 0.58) [5], erysodine (5 mg, R_f 0.21) [5] and syringaresinol (15 mg, R_f 0.41) [11] while fraction SP_B gave 2-(5′-hydroxy-3′-methoxyphenyl)-6-hydroxy-5-methoxybenzofuran **2** (10 mg, R_f 0.19) and 4-hydroxy-3-methoxybenzoic acid (5 mg, R_f 0. 30). The root bark (1.5 kg) was treated similarly to yield erybraedin A (8 mg, R_f 0.61) [8], neobavaisoflavone (120 mg, R_f 0.35) [12], [13], abyssinone IV (90 mg, R_f 0.52) [10], neorautenol (9 mg, R_f 0.40) [14], isoneorautenol (10 mg, R_f 0.48) [8], [9], shinpterocarpin (90 mg, R_f 0.35) [15] and erythrinin B (20 mg, R_f 0.29) (16). Acetylation of shinpterocarpin at room and elevated temperature gave pure **5** (13.5 mg, R_f 0.65) and **6** (22 mg, R_f 0.81), respectively. The brine shrimp lethality assay [17] was used to assess extracts and pure compounds. TLC bioautography for antibacterial and antifungal assays [18] were done with chloramphenicol and miconazole, respectively, as positive standards.

(+)-10,11-Dioxoerysotrine (**1**): Brown solid (acetone), m.p, 174 – 176 °C; $[\alpha]_D^{25}$: + 167.5° (MeOH, *c* 0.10, 25 °C); HR-TOF-MS: *m/z* = 341.1265 (calcd for $C_{19}H_{19}NO_5$, 341.1264); EI-MS: *m/z* (%) = 341 $[M]^+$ (100), 310 (30), 282 (25), 257 (20); IR: ν_{max}^{KBr} = 1710, 1680, 1650, 1610 cm^{-1}; UV: λ_{Max}^{MeOH} (log ε) = 351 (3.54), 292 (sh, 3.62), 247 (4.17), 206 (4.38) nm. ^{1}H- and ^{13}C-NMR spectra data (see Table **1**).

2-(5′-Hydroxy-3′-methoxyphenyl)-6-hydroxy-5-methoxybenzofuran (**2**): Brown paste (ethyl acetate), m.p, 82 – 85 °C. EI-MS: *m/z* (%) = 286.1 $[M]^+$ (100), 271.0 (60), 240.9 (25); IR: ν_{max}^{KBr} = 3395, 2924, 1593, 1450 cm^{-1}; UV: λ_{Max}^{MeOH} (log ε) = 324 (4.21), 293 (4.04); 285 (3.74), 216 (3.45); +NaOMe 342, 206; +NaOAc 397, 387, 324, 293, 206; +NaOAc/H_3BO_3 397, 391, 387, 324, 293, 206; +$AlCl_3$ 324, 294, 215; +$AlCl_3$/HCl 324, 293, 255, 213 nm. ^{1}H- and ^{13}C-NMR spectra data (see Table **1**).

Table **2** Antimicrobial activity of pure compounds from *Erythrina latissima*

Microorganism	***Minimum inhibition amounts (µg)***									
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
E. coli (NCTC09001)	0.05	–	–	–	–	–	–	–	10.0	90.0
P. aeruginosa (NCTC10332)	0.05	–	–	–	–	–	–	–	0.80	90.0
S. aureus (NCTC8582)	0.008	1.00	0.70	0.70	0.80	0.55	1.25	0.50	0.40	0.50
B. subtilis (NCTC3610)	0.005	1.00	1.00	1.00	0.90	0.50	1.20	0.30	0.50	0.60
C. mycoderma (SM428)	0.001	0.008	0.008	0.01	0.01	0.007	0.01	8.00	0.06	0.07
S. cerevisiae (SM10716)	0.001	0.009	0.007	0.008	0.008	0.006	0.01	6.00	0.06	0.06

7: Erybraedin A; **8**: neorautenol; **9**: abyssinone IV; **10**: erythrinin B; **11**: neobavaisoflavone.
Minimum inhibition amounts for standards (µg): *chloramphenicol* (Gram-positive bacteria: 0.0001, Gram-negative bacteria: 0.001); *miconazole:* (antifungal activity: 0.0001).

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