Two Novel Glucodienoid Alkaloids from *Erythrina latissima* Seeds

Cornelius C. W. Wanjala and Runner R. T. Majinda*

Department of Chemistry, University of Botswana, Private Bag 0022, Gaborone, Botswana

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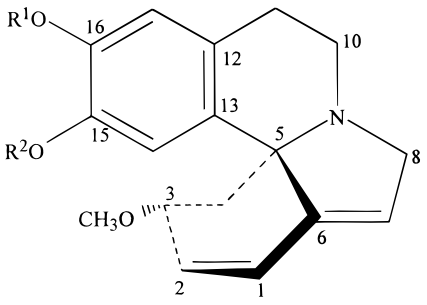
The structures of two novel glycodienoid alkaloids isolated from *Erythrina latissima* seeds have been established as (+)-16β-D-glucoerysopine (**1**) and (+)-15β-D-glucoerysopine (**2**). NOE measurements established that **1** and **2** are positional isomers. Their structures were elucidated from their 1D and 2D homonuclear and heteronuclear NMR spectroscopic data. In addition, seven known tetracyclic dienoid alkaloids were isolated and identified as (+)-erysovine, (+)-erysodine, (+)-erysotrine, (+)-erythraline, (+)-β-D-glucoerysodine, (+)-8-oxoerythraline, and (+)-erysotramidine.

The genus *Erythrina* comprises more than 100 species of trees, shrubs, and herbaceous plants that are widely distributed throughout tropical and warm regions of the world.[1] *Erythrina* species are known to produce triterpenoid saponins,[2] flavonoids,[3] isoflavonoids,[4,5] pterocarpans,[6] and alkaloids.[7−9] The alkaloids produced are of the erythraline type, of which some have been shown to have curare-like activity.[7,8] *E. latissima* E. Meyer (Fabaceae) is a large tree that grows in Botswana, Zimbabwe, and South Africa. The stem bark and roots of this plant are burned, powdered, and used as a dressing for open wounds.[10,11] In this paper we report on the isolation of two new tetracyclic dienoid alkaloids (**1** and **2**) from the seeds of *E. latissima*.



1 R^1 = glc, R^2 = H

2 R^1 = H, R^2 = glc

The dichloromethane−ethyl acetate extract from the seeds of *E. latissima* was worked up to give (+)-erysovine, (+)-erysodine, (+)-erysotrine, (+)-erythraline, (+)-8-oxoerythraline, and (+)-erysotramidine, and the methanolic extract gave **1**, **2**, and (+)-β-D-glucoerysodine. The identities of (+)-erysovine, (+)-erysodine, (+)-erysotrine, (+)-erythraline, (+)-8-oxoerythraline, (+)-erysotramidine, and (+)-β-D-glucoerysodine are based on spectral data comparison with those reported in the literature.[7−9] Structures **1** and **2** were deduced using spectroscopic methods and by comparison with the spectral data for (+)-erysovine, (+)-erysodine, (+)-erysotrine, (+)-erythraline, and (+)-β-D-glucoerysodine.

The HRTOFMS of **1** and **2** showed a molecular ion peak at 447.1891 and 447.1886, respectively, and EIMS $[M]^+$ *m/z* 447 for both compounds consistent with the molecular formula $C_{23}H_{29}NO_8$. The molecular ions were confirmed by ESIMS, which gave quasimolecular ions $[M + H]^+$ *m/z* 448 for both **1** and **2**. The ^{13}C NMR spectrum of both **1** and **2** gave 23 carbon signals, and the DEPT spectra, together with the HMQC data, confirmed that 17 of these were protonated. The DEPT spectra also showed one methyl, five methylenes, and 11 methine carbons. The ^1H and ^{13}C NMR data for **1** and **2** (chemical shifts for **2** are given in square brackets in the text) were very similar (Table 1) and both indicated the presence of a glucose, a tetracyclic ring system, and methoxyl moieties. The nature and identity of the tetracyclic ring moiety was evident from the ^1H NMR data, which showed the following salient features: one-proton singlets at δ_H 7.03 [6.99] (H-17), 6.79 [6.75] (H-14), 5.79 [5.79] (H-7); a doublet at δ_H 6.04 [6.02] (H-2); a doublet of doublets at δ_H 6.61 [6.60] (H-1); a multiplet at δ_H 4.00 [4.00] (H-3); and a doublet at δ_H 4.78 [4.74] (H-1′). These observations, along with published data [7−9] enabled us to identify the aglycon of **1** and **2** as (+)-erysopine.

For both **1** and **2**, the nature and stereochemistry of the β-D-glucopyranosyl moiety was deduced from their ^1H NMR data, especially the anomeric proton, appearing as a doublet (J = 7.2 Hz) resonating at δ_H 4.78 [4.74]. The linkages of the (+)-erysopine nucleus to the glucose and methoxyl moieties were confirmed by HMBC, NOEDIFF, and NOESY experiments. In both **1** and **2** the placement of the methoxyl group at C-3 was confirmed from the HMBC correlation between the methoxyl signal (δ_H 3.34) and C-3, δ_C 77.0 [77.1]. The H-14 proton in both **1** and **2** showed a HMBC correlation with C-5 δ_C 67.5 [67.5]. Compound **1** showed a HMBC correlation between the anomeric proton, H-1′ (δ_H 4.78), and C-16 (δ_C 145.1). Irradiation of H-17 (δ_H 7.03) in a NOEDIFF experiment gave an enhancement of H-1′, while irradiation of H-14 (δ_H 6.79) gave an enhancement of the C-3 methoxyl signal (δ_H 3.34). In compound **2**, a HMBC correlation was evident between H-1′ (δ_H 4.74) and C-15 (δ_C 147.6). Irradiation of H-14 (δ_H 6.75) in a NOEDIFF experiment gave an enhancement of H-1′ (δ_H 4.74) and the methoxyl signal (δ_H 3.34), while irradiation of H-17 (δ_H 6.99) gave no enhancement on the anomeric proton. The two compounds only demonstrated significant ^{13}C NMR chemical shift differences for C-15 (δ_C 145.8 for **1** and δ_C 147.6 for **2**), while the chemical shifts for the other carbons remained at approximately the same values. Analysis of all the information above permitted **1** to be identified as (+)-16β-D-glucoerysopine and **2** as (+)-15β-D-glucoerysopine. These two compounds differ only in the position of the glucosyl residue in the erysopine nucleus and are both reported here

* To whom correspondence should be addressed. Tel.: (267) 355 2503. Fax: (267) 355 2836. E-mail: majindar@mopipi.ub.bw.

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** in CD_3OD[a]

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1	6.61, 1H, dd (10.1, 2.1)	125.4 (d)	6.60, 1H, dd (10.1, 2.1)	124.7 (d)
2	6.04, 1H, d (10.1)	132.1 (d)	6.02, 1H, d (10.1)	132.2 (d)
3	4.00, 1H, m	77.0 (d)	4.00, 1H, m	77.1 (d)
4 ax	2.52, 1H, dd (10.6, 5.7)	41.2 (t)	2.52, 1H, dd (10.6, 5.7)	41.3 (t)
eq	1.79, 1H, dd(10.9,10.9)		1.78, 1H, dd (10.9,10.9)	
5		67.5 (s)		67.5 (s)
6		143.8 (s)		143.3 (s)
7	5.79, 1H, br s	122.8 (d)	5.79, 1H, br s	122.6 (d)
8 ax	3.61, 1H, m	57.1 (t)	3.61, 1H, m	57.1 (t)
eq	3.45, 1H, m		3.45, 1H, m	
10 ax	3.40, 1H, m	44.3 (t)	3.40, 1H, m	44.4 (t)
eq	2.91, 1H, m		2.91, 1H, m	
11 ax	2.91, 1H, m	24.2 (t)	2.91, 1H, m	24.3 (t)
eq	2.67, 1H, m		2.67, 1H, m	
12		126.1 (s)		125.4 (s)
13		134.1 (s)		133.9 (s)
14	6.79, 1H, s	113.9 (d)	6.75, 1H, s	114.6 (d)
15		145.8 (s)		147.6 (s)
16		145.1 (s)		145.8 (s)
17	7.03, 1H, s	118.2 (d)	6.99, 1H, s	118.1 (d)
OCH_3	3.34, 3H, s	55.9 (q)	3.34, 3H, s	55.9 (q)
1′	4.78, 1H, d (7.2)	103.6 (d)	4.74, 1H, d (7.2)	103.8 (d)
2′	3.48, 1H, m	74.2 (d)	3.48, 1H, m	74.3 (d)
3′	3.30, 1H, m	77.7 (d)	3.30, 1H, m	77.7 (d)
4′	3.48, 1H, m	70.7 (d)	3.48, 1H, m	70.8 (d)
5′	3.40, 1H, m	77.4 (d)	3.40, 1H, m	77.0 (d)
6′ ax	3.95, 1H, dd (11.0, 3.9)	61.8 (t)	3.95, 1H, dd (11.0, 3.9)	61.9 (t)
eq	3.74, 1H, m		3.74, 1H, m	

[a] Assignments were confirmed by COSY, NOEDIFF, NOESY, HMQC, HMBC, and DEPT experiments. *J* values are shown in parentheses in Hz.

for the first time. Of the known compounds, (+)-erysovine, (+)-erysodine, (+)-erysotrine, (+)-erythraline, (+)-8-oxoerythraline, (+)-erysotramidine, and (+)-β-D-glucoerysodine, only (+)-erythraline has been identified previously as a constituent of *E. latissima* seeds.[7−9]

Experimental Section

General Experimental Procedures. Melting point: Stuart Scientific (SMP1) melting point apparatus; specific rotation $[\alpha]_D$: Polatronic-D (Schimdt + Haensch) polarimeter; UV: Shimadzu UV-2101PC spectrophotometer; IR: Perkin-Elmer 2000 FTIR spectrometer. The 1D {^{1}H (300 MHz), ^{13}C (75.4 MHz), DEPT}, and 2D (COSY, HMQC, HMBC) spectra were acquired on Bruker Avance DPX 300 spectrometer and referenced to residual solvent signal. MS: HRMS performed on a Micromass Autospec TOF mass spectrometer; and EIMS and ESIMS, on Finnigan MAT SSQ 700 single quadrupole instrument. Column chromatography: Si gel 60 particle size 0.040−0.063 mm for column chromatography (Merck); VLC: Si gel HF_{254} 5−15 μm mesh (Merck); Sephadex LH-20 (Sigma); preparative TLC: Si gel 60 $PF_{254\ +\ 366}$ for preparative layer chromatography (Merck); analytical TLC: TLC Si gel 60-F_{254} precoated alumina sheets (Merck), visualized using UV (254 and 366 nm) and vanillin−sulfuric acid spray.

Plant Material. The seeds of *E. latissima* were collected from Mapoka, North East District, Botswana, in August 1997. They were identified by Dr. L. M. Turton, and a voucher specimen (E 0897) was deposited at the University of Botswana Herbarium.

Extraction and Isolation. Air-dried and pulverized seeds (242 g) were extracted sequentially with hexane−CH_2Cl_2 (1:1), CH_2Cl_2−EtOAc (1:1), EtOAc, EtOAc−MeOH (1:1), MeOH, and MeOH−H_2O (1:1). Removal of solvent from the combined methanolic extracts gave a brown residue (23 g), which was subjected to VLC by elution with hexane, CH_2Cl_2, and MeOH mixtures of increasing polarity. The glycodienoid alkaloids were eluted in CH_2Cl_2−MeOH to MeOH fractions. The combined fractions were applied to a Sephadex LH-20 column (eluted with 1:1 $CHCl_3$−MeOH). The concentrated eluent was resolved by preparative TLC with $CHCl_3$−MeOH−H_2O−NH_3 (70:26:2:2) to give (+)-β-D-glucoerysodine (180 mg), **1** (150 mg), and **2** (50 mg). The combined EtOAc−CH_2Cl_2 extract was concentrated to give a brown residue (26 g), which was subjected to VLC and eluted with hexane, CH_2Cl_2, and MeOH in increasing polarity. The tetracyclic dienoid alkaloids were obtained from the CH_2Cl_2−hexane (2:8) to CH_2Cl_2−MeOH (1:1). These fractions were combined and applied to a Sephadex LH-20 column (eluted with 3:1 $CHCl_3$−MeOH) to give fractions A and B. Both fractions were concentrated and purified by preparative TLC, using toluene−EtOAc−HOAc (35:14:1) to afford (+)-8-oxoerythraline (70 mg) and (+)-erysotramidine (60 mg) from A, and (+)-erysovine (50 mg), (+)-erysodine (30), (+)-erysotrine (60 mg), and (+)-erythraline (70 mg) from B.

(+)-16β-D-Glucoerysopine (1): dark brown solid; mp 158−160 °C; $[\alpha]_D$ +76.5° (*c* 0.0057, MeOH); UV (MeOH) λ_{max} (log ϵ) 279 (3.74), 222 (4.40), 206 (4.38) nm; IR (KBr) ν_{max} 3415, 2920, 1507 cm^{-1}; ^{1}H and ^{13}C NMR in CD_3OD (see Table 1); EIMS *m*/*z* 447 $[M]^+$ (80), 299 (80), 268 (100), 251 (70); ESIMS 448 $[M + H]^+$, 470 $[M + Na]^+$; HRTOFMS *m*/*z* 447.1891 (calcd for $C_{23}H_{29}NO_8$, 447.1893).

(+)-15β-D-Glucoerysopine (2): dark brown solid; mp 150−152 °C; $[\alpha]_D$ +67.5° (*c* 0.0057, MeOH); UV (MeOH) λ_{max} (log ϵ) 279 (3.74), 222 (4.40), 206 (4.38) nm; IR (KBr) ν_{max} 3415, 2920, 1507 cm^{-1}; ^{1}H and ^{13}C NMR in CD_3OD (see Table 1); EIMS *m*/*z* 447 $[M]^+$ (80), 299 (80), 268 (100), 251 (70); ESIMS 448 $[M + H]^+$, 470 $[M + Na]^+$; HRTOFMS *m*/*z* 447.1886 (calcd for $C_{23}H_{29}NO_8$, 447.1893).

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