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A New Erythrinan Alkaloid from the Seed of *Erythrina addisoniae*

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Phytochemical study on the EtOAc extract of the seed of *Erythrina addisoniae* (Leguminosae) resulted in the isolation of a new erythrinan alkaloid, erysovine-*N*-oxide (**1**), along with eight known alkaloids, erysosalvinone (**2**), erysodine (**3**), 1H-indole-3-propanamide (**4**), glucoerysodine (**5**), erysotrine (**6**), erysovine (**7**), erythraline (**8**) and erysopine (**9**). Their chemical structures were identified on the basis of physicochemical and spectroscopic analyses.

Key words: *Erythrina addisoniae*, Leguminosae, Erythrinan Alkaloid, Erysovine-*N*-oxide

INTRODUCTION

The genus *Erythrina* (Leguminosae) comprises of about 110 species of trees and shrubs that are distributed in tropical and subtropical regions. These species have been widely used in indigenous traditional medicine (Oliver-Bever et al., 1986). Studies on phytochemical of *Erythrina* species have demonstrated alkaloids (Amer, 1991; Chawla and Jackson, 1990; Flausino et al., 2007a,b; Ito, 1999; Juma and Majinda, 2004; Soto-Hernandez and Jackson, 1994; Wanjala et al., 2002;) and flavonoids (Bae et al., 2006; Barron et al., 1996; Na et al., 2006; Wanjala et al., 2002) as major constituents of this genus. Previously, it has been reported that the flavonoids isolated from the plant *E. addisoniae* showed anti-inflammatory (Talla et al., 2003) and anti-diabetes activities (Bae et al., 2006; Na et al., 2007). However, the reports on alkaloids from this species are scare. Our investigation on chemical constituents of the seed of this plant resulted in the isolation of nine alkaloids, including a new one with erythinan skeleton. This paper reports the isolation of alkaloids **1**-**9** and structural elucidation of a new alkaloid (**1**) from the seeds of the plant *E. addisoniae*.

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MATERIALS AND METHODS

General experimental procedures

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian 400 MHz spectrometer using TMS as the internal standard. MS spectra were recorded on a Micromass ESI-Tof II (Micromass, Wythenshawe, UK) mass spectrometer. Silica gel 60 (15-40 μm particle size) and RP-18 (40-63 μm particle size) from Merck was used for vacuum-liquid chromatography (VLC). Precoated TLC silica gel 60 F_{254} plates from Merck were used for thin-layer chromatography. HPLC runs were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, and Capcell Pak® C_{18} column (10 × 250 mm, 5 μm particle size, Shiseido Fine Chemicals) and HYPERSIL-100 silica (10 × 250 mm, 8 μm particle size, Thermo Electron Co.) for semipreparative runs.

Plant material

The seeds of *E. addisoniae* were collected at Etoug-Ebe, Yaounde, Cameroon in April 1996. The plant was authenticated and deposited at the Cameroon National Herbarium, Yaounde (voucher No. 41617/HNC).

Extraction and isolation

The seeds of *E. addisoniae* (5.0 kg) were extracted with EtOAc (5 L) for 72 h and the extractive solution was concentrated under reduced pressure

to yield a residue (200 g). A part of this EtOAc extract (5.0 g) was subjected to YMC gel column chromatography (40 × 3.5 cm; 150 μm particle size) eluting with a gradient of MeOH-H_2O (1:9, 1:8, 1:7 to 1:0) and separated into 10 fractions (F. 1-F. 10). Fraction 2 (F. 2, 250 mg) was subjected to semipreparative reversed-phase HPLC (Shiseido Capcell Pak C_{18} column; 2 mL/min; UV detection at 254 nm) using 15% MeOH in H_2O to yield compound **4** (t_R 8 min; 14.0 mg). Fr. 3 (310 mg) was subjected to vacuum liquid chromatography on silica gel (35 × 3.0 cm; 15-40 μm particle size) eluted with a gradient of CH_2Cl_2-MeOH (containing 0.1% NH_4OH) (200:1, 150:1, 100:1, 50:1, 40:1, 30:1 to 0:1). Fractions of similar composition as determined by silica TLC analysis were pooled, resulting in 5 fractions (Fr. 3.1-Fr. 3.5). Fr. 3.1 (71.0 mg) was purified by semipreparative normal phase HPLC (HYPERSIL-100 silica column; 2 mL/min; UV detection at 254 nm) using an isocratic solvent system of 96% CH_2Cl_2 in MeOH (containing 0.1% NH_4OH) to afforded compounds **2** (t_R 26 min; 6.2 mg) and **3** (t_R 34 min; 5.8 mg). Fr. 3.3 (109.8 mg) was also separated by the normal phase HPLC using an isocratic solvent system of 92% CH_2Cl_2 in MeOH (containing 0.1% NH_4OH) to obtain compounds **1** (t_R 19 min; 2.3 mg) and **5** (t_R 28 min; 5.7 mg). Fr. 4 (390 mg) was chromatographed over silica gel eluting with a gradient of CH_2Cl_2 - MeOH (containing 0.1% NH_4OH) (from 100:1, 90:1 to 2:1) to yield 7 fractions (Fr. 4.1 - Fr. 4.7). Compounds **6** (t_R 35 min; 8.7 mg) and **7** (**t_R** 22 min; 6.9 mg) were isolated from the Fr. 4.2 (123.7 mg) using the normal phase HPLC with an isocratic solvent system of 97% CH_2Cl_2 in MeOH (containing 0.1% NH_4OH). Fr. 8 (259 mg) was further purified by HPLC with an isocratic solvent system of 96% CH_2Cl_2 in MeOH (containing 0.1% NH_4OH) to obtain compounds **8** (t_R 20 min; 32 mg) and **9** (t_R 27 min; 10 mg).

Compound 1: brownish oil; $[\alpha]_D^{25}$ +191.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm: 203, 230, 282; ESI-MS *m/z*: 338.4 $[M + Na]^+$; HR-ESI-MS *m/z*: 316.1537 $[M + H]^+$ (calc. for $C_{18}H_{21}NO_4H$: 316.1549); ^1H (400 MHz) and ^{13}C NMR (100 MHz) data: see Table I.

RESULTS AND DISCUSSION

Compound **1** was obtained as brownish oil and showed positive reaction with dragendorff reagent, suggesting that **1** is an alkaloid. The UV spectrum of **1** revealed the maximum absorption at 203, 230, and 282 nm, indicative of a typical aromatic chromophore structure (Flausino et al., 2007). The ^1H NMR spectrum of **1** (Table I) displayed the signals of two aromatic protons at δ_H 6.76 (H-17) and 6.62 (H-14), conjugated diene protons at 6.66 (1H, dd, *J* = 2.4, 10.4 Hz, H-1), 6.18 (1H, br d, *J* = 10.4 Hz, H-2), and 5.80 (1H, br s, H-7), two methoxyl protons at δ_H 3.77 and 3.36, and nine aliphatic protons. The evidences were characteristic of a tetracyclic erythrina alkaloid structure that is an erysodine or erysovine derivative (Amer, 1991; Cornelius et al., 2000). Eighteen carbon resonances were observed in ^{13}C and DEPT NMR spectra, including six quaternary carbons (an aliphatic carbon), six methines (an aliphatic methane), four aliphatic methylenes, and two methoxyl carbons. These observations suggested that compound **1** had a skeleton similar to that of erysovine (**7**), which was also isolated and identified in this study. When compared the NMR data of **1** to those of **7**, C-4 and C-5 of **1** showed an upfield chemical shift (8-10 ppm) and a downfield shift (16-19 ppm), respectively, and the signals of H-4_{eq} and H-4_{ax} in **1** were shifted as upfield (δ_H 2.18) and downfield (δ_H 3.13), respectively (Table I). The proton signals of H-8 (δ_H 4.44, 4.54), H-10 (δ_H 4.00, 4.05) and H-11 (δ_H 3.08, 3.20), and the carbons were appeared in lower field as compared to those of erysovine. These results indicate that compound **1** is an *N*-oxide of erysovine, which was further con-

Table I. ^1H, ^{13}C NMR and HMBC data of compound **1**[a]

Position	δ_H (*J* in Hz)	δ_C	HMBC (H → C)
1	6.66 d (2.4, 10.4)	125.5	C-3, C-5, C-6
2	6.18 br d (10.4)	134.2	C-6
3	4.16 m	75.8	C-5
4	2.18 dd (5.8, 11.4) 3.13 m	31.7	C-3, C-5, C-13, C-2, C-3, C-5,C-6, C-13
5		83.5	
6		138.8	
7	5.80 br s	118.0	
8	4.44 d (14.4) 4.54 d (14.4)	70.8	
10	4.00 m 4.05 m	59.9	
11	3.08 m 3.20 m	26.8	C-12, C-13, C-17
12		122.9	
13		126.3	
14	6.62 s	108.5	C-5, C-12, C-15
15		147.2	
16		146.8	
17	6.76 s	114.8	C-11, C-12, C-16
3-OCH_3	3.36	56.2	C-3
16-OCH_3	3.77	56.1	C-16

[a] Recorded in $CDCl_3$ on a Varian 400 MHz spectrometer.

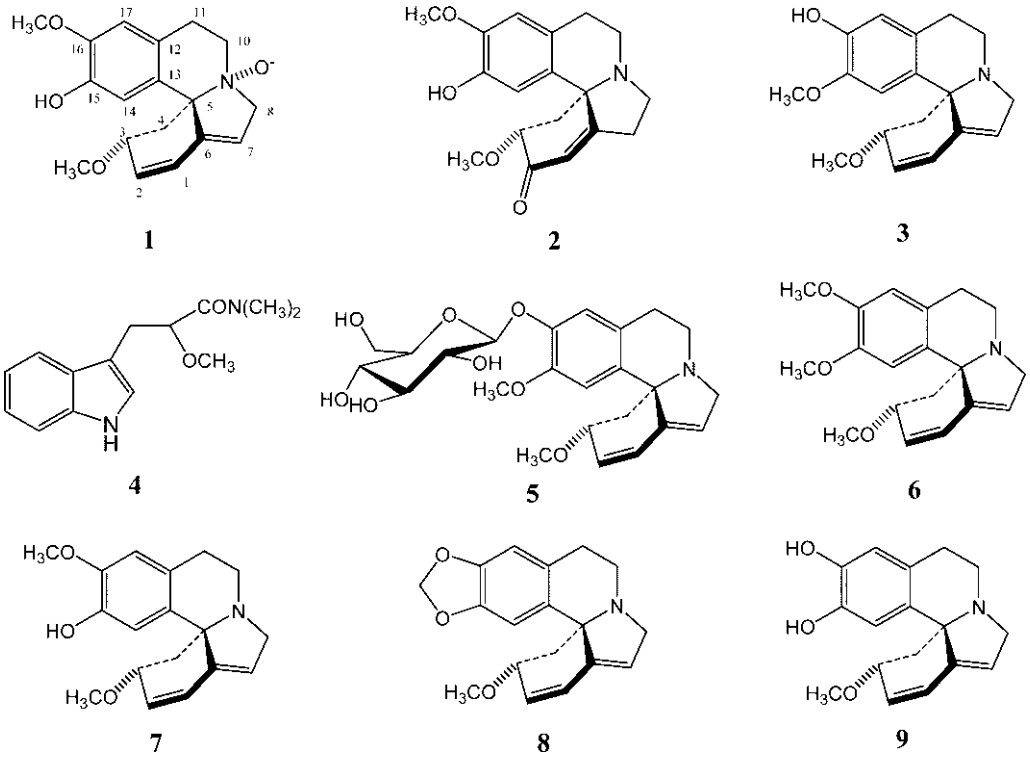

Fig. 1. Structures of alkaloids **1**-**9** isolated from *E. addisoniae*

firmed by a HMBC experiment. The HMBC correlations from H-1 (δ_H 6.66), H-3 (δ_H 4.16), H-4 (δ_H 2.18 and 3.13), and H-14 (δ_H 6.62) to C-5 (δ_C 83.5), characteristically downfield shifted in *N*-oxides (Benard and Majinda, 2004; Amer et al., 1991). Further, the molecular formula $C_{18}H_{21}NO_4$ deduced from the quasimolecular ion peak at *m/z* 316.1537 $[M + H]^+$ in HR-ESI-MS. Thus, the structure of **1** was determined as erysovine -*N*-oxide (Fig. 1), a new natural compound.

The structures of other isolates **2**-**9** were identified as erysosalvinone (**2**), erysodine (**3**), 1H-indole-3-propanamide (**4**), glucoerysodine (**5**), erysotrine (**6**), erysovine (**7**), erythraline (**8**) and erysopine (**9**) on the basis of physical and spectroscopic analyses and by comparison with published values (Amer, 2000; Benard et al., 2004; Bugge et al., 1997; Chawla et al., 1988). Although alkaloids **2**-**9** have been reported as constitutes of *Erythrina* species, this is the first reported isolation of these compounds from the plant *E. addisoniae*. Accordingly, erythrinan alkaloids were found to exhibit a wind range of bioactivities, for example antioxidant (Juma and Majinda, 2004), anticonvulsant, hypnotic, analgesic (Ghosal et al., 1972), nicotinic (Decker et al., 1995), and anxiolytic effects (Flausino et al., 2007a,b). It has been also indicated that alkaloids are the principles for the nicotinic and anxiolytic activities in vivo of crude extract of *Erythrina* plants (Decker et al., 1995; Garin-Aguilar et al., 2000; Flausino et al., 2007a,b). Therefore, additional investigation into biological effects of erythrinan alkaloids on central nervous system will further clarify and support the uses of *Erythrina* plants in ethnomedicine.

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