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Antifeedant Activities of the Erythrinaline Alkaloids from *Erythrina latissima* against *Spodoptera littoralis* (Lepidoptera noctuidae)

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Abstract: The antifeedant activities of the *Erythrina* alkaloids from the seeds, seed pods and flowers of *Erythrina latissima* were investigated in laboratory dual- choice bioassays using third-instar *Spodoptera littoralis* (Boisduval) larvae. The new compound (+)-11β-methoxy-10-oxoerysotramidine (**1**) from the flowers, showed potent dose dependant activity at concentration ≥ 500 ppm while (+)-10,11-dioxoerysotramidine (**2**) also new from the flowers showed potent dose dependant activity at concentration ≥ 100 ppm. Three known compounds (+)-erysotrine, (+)-erysotramidine, (+)-erythraline, (+)-11β-hydroxyerysotramidine showed potent dose dependant antifeedant activity at concentrations ≥ 100 ppm while (+)-10,11-dioxoerysotrine and (+)-11β-hydroxyerysotramidine also a known compounds showed potent dose dependant antifeedant activity at concentrations ≥ 300 ppm. Three known compounds (+)-11β-methoxyerysotramidine, (+)-8-oxoerythraline and (+)-15(16)β-D-glucoerysodine showed no appreciable change in antifeedant activity with concentration change.

Keywords: *Erythrina* alkaloids; *Erythrina latissima*; antifeedant activity; *Spodoptera littoralis.*

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1. Introduction

The genus Erythrina comprises over 130 species showing cosmo-politan distribution [1] and is known to produce C-prenylated flavanones, isoflavones, isoflavanones, pterocarpans [2], [3], erythrinaline alkaloids [4], [5]. The genus erythrina is popularly known for its ornamental and medicinal properties [6].

Erythrina latissima E. Meyer (Fabaceae) is a tropical and subtropical flowering tree 9 - 24 m tall [7]. The roots and the stem are known to contain antimicrobial compounds [3], while the seeds, seed pods and flower are known to contain the erythraline alkaloids which are also known to have curare-like activity [5, 8]. (S)-Norreticuline is the precursor for the biosynthesis of *Erythrina* alkaloids [9]. The tetracyclic framework of the erythrina alkaloids have also been synthesized in laboratory using a [4+2]-cycloaddition/Rh(I)-catalyzed cascade of 2-imidofurans [10].

In the current study we report the antifeedant activity of the erythrinaline alkaloids from *E. latissima*. It was noted that, farms with maize growing under the *E. latissima* tree were sparsely attacked by the stem borer. Since the tree is a widespread flowering plant, its seeds and flowers can be harnessed and utilized as a potential biopesticide in Agriculture.

2. Materials and Methods

2.1. Plant Material

The seeds, seed pods and the flowers of *Erythrina latissima* E. Meyer were collected from Turbo (Latitude: 0°38'N, Longitude: 035°02'E) in Uasin Gishu District (Maize growing region), Kenya in August 2002. They were identified by Mr. P. Maina, and a voucher specimens (ES 01002, EP 01002 and EF 01002) for seeds, seed pods and the flowers respectively deposited at the Jomo Kenyatta University of Agriculture and Technology Herbarium.

2.2 Extraction and Isolation

2.2.1 Seed pods

The seed pods (5 kg) were powdered and extracted in 1:1 MeOH/$CHCl_3$ mixture (10 L). Removal of the solvent from the extract gave a green residue (40 g) which was re-dissolved in MeOH/$CHCl_3$ 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). Fractions SP_A was further purified on preparative TLC ($CHCl_3$/EtOAc, 6:1) by multiple development ($\times 3$) to give (+)-10,11-dioxoerysotrine [7] (120 mg, R_f 0.32) and erysotrine [5] (60 mg, R_f 0.58).

2.2.2 Seeds

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 alkaloid was eluted in CH_2Cl_2-MeOH to MeOH fractions. The combined fractions were applied to a Sephadex LH-20 column (eluded 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 (+)-15(16)β-D-glucoerysodine [11] (180 mg R_f 0.25, MeOH:$CHCl_3$, 1:8). 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 by multiple development (×3), using toluene-EtOAc-HOAc (35:14:1) to afford (+)-8-oxoerythraline (70 mg, R_f 0.58) and (+)-erysotramidine (60 mg, R_f 0.60) from A, and (+)-erysovine (50 mg, R_f 0.57), (+)-erysotrine (60 mg, R_f 0.50), and erythraline (70 mg, R_f 0.52) from B [5].

2.2.3 Flowers

Air-dried and pulverized flowers (200 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 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). The concentrated fractions were purified by Preparative TLC, using toluene-EtOAc-HOAc (35:14:1) by multiple development (×3), to afford (+)-11β-hydroxyerysotramidine [4] (102 mg, R_f 0.50), (+)-11β-methoxyerysotramidine [4] (60 mg, R_f 0.45), and the new compounds (+)-11β-methoxy-10-oxoerysotramidine **1** (90 mg, R_f 0.49) and (+)-10,11-dioxoerysotramidine **2** (50 mg, R_f 0.48).

2.3 Instrumentation

The 1D [^{1}H (300 MHz), ^{13}C (75.4MHz), DEPT] and 2D [COSY, TOCSY, ROESY, HMBC, and HMQC] spectra were acquired on Bruker Avance DPX 300 spectrophotometer and referenced to a residual solvent signal. The spectra were recorded in $CDCl_3$; HR-EIMS spectra were run as autospec time of flight (TOF) on a GCT Premier Mass spectrometer, EI-MS: Finnigan MAT SSQ 7000 Single Quadrupole Instrument at 70 eV., while Infra-red (IR) absorptions were measured on a Shimadzu FTIR 8000 SCSI spectrophotometer as KBr pellets after background correction in the range 4000 - 400 cm^{-1}. Melting points were recorded using Gallen Kamp Griffins melting point apparatus and are uncorrected.

Thin layer chromatography (TLC) experiments were developed on ready made 0.25 mm thick layer of silica gel 60 F_{254} (Merck) coated aluminium sheets and visualized by observation under UV-light (254 nm and 365 nm) and spraying with vanillin-sulphuric acid spray. Normal column chromatography (CC) was conducted using different sizes of columns packed with (Merck) silica gel 60 (size 0.040-0.063mm). Preparative TLC were run on 0.5 mm thick layer (Merck) silica gel 60 HF_{254} containing gypsum ($CaSO_4$ binder) coated on 20 x 20 cm glass plates.

3.4. Physical and spectroscopic data

*2.4.1 (+)-11β-methoxy-10-oxoerysotramidine (**1**)* - ($C_{20}H_{21}NO_6$)

Brown powder (acetone), mp: (175-177 °C) uncorrected; $[\alpha]_D^{25}$: +167.5° (MeOH, c 0.10, 25 °C); R_f: 0.63, $CHCl_3$/EtOAc 9:1 v/v; HR-TOF-MS: m/z = 371.1370 (calcd for $C_{20}H_{21}NO_6$, 371.1369);

EI-MS: *m/z* (%) = 371 $[M]^+$ (100), 340 (30), 312 (25), 287 (20); IR: υ_{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).

*2.4.2 (+)-10,11-dioxoerysotramidine (**2**)* - ($C_{19}H_{17}NO_6$)

Brown powder (acetone), mp: (171-174 °C) uncorrected; $[\alpha]_D^{25}$: +165.3° (MeOH, c 0.10, 25 °C); R_f: 0.62, $CHCl_3$/EtOAc 9:1 v/v; HR-TOF-MS: m/z = 355.1057 (calcd for $C_{19}H_{17}NO_6$, 355.1056); EI-MS: *m/z* (%) = 355 $[M]^+$ (100), 324 (40), 296 (27), 271 (25); IR: υ_{max}^{KBr} = 1711, 1690, 1680, 1650 cm^{-1}; UV: λ_{max}^{MeOH} (log ε) 355 (3.05), 292 (sh, 3.02), 246 (4.17), 209 (4.30) nm. ^{1}H- and ^{13}C-NMR spectra data (see Table 1).

2.4 Antifeedant tests

The antifeedant response of insects to erythrinaline alkaloids compounds was measured using both "paired choice" (dual-choice) and ″no-choice″ tests. In a paired choice test, each insect is given a choice between the treated leaf and an untreated leaf. In a no-choice test, half the insects are given treated leaves, and half are given untreated leaves. If the compound acts as an antifeedant, then insects will eat more of the untreated leaves than the treated leaves. The difference between the amount of untreated and treated leaves consumed is used to calculate the feeding deterrence of the treatment.

2.4.1 Test insects and leaves

Spodoptera littoralis (Boisduval) (Figure 1 below) was reared on a proprietary diet (‘Multiple Species Diet’, Southland Products Inc., Lake Village, AR, USA). The bioassays were conducted with third-instar larvae of *S. littoralis* from a culture maintained at International Centre of Insect Physiology and Ecology (ICIPE). Prior to using larvae in the various bioassays, they were previously starved for 24 hrs. Test plant parts were fully expanded leaves of cabbage *Brassica pekinensis* Rupr (Cruciferae) harvested from 5-6-week-old plants grown in a glasshouse.

Figure 1. Larvae and moth of *Spodoptera littoralis*

2.4.2 Bioassays

Preliminary experiments were conducted as no-choice tests using the 1000 ppm sample of each compound. For each compound, 2 ml were put in a Petri dish and a cabbage leaf was dipped into it, while controls were treated with the corresponding solvents only. Each treated leaf was then placed on a tray and left in a fume cupboard for 20 min to dry off the solvent. There were eight replicate leaves for each treatment. One third-instar *S. littoralis* larva starved for 24 hrs was then introduced into each Petri dish and allowed to feed for 24 hr before recording mortality and/or proportion of uneaten leaf. Corresponding treatments and controls were visually compared by laying the leaf on a graph paper and counting the number of 1 mm squares consumed. Active compounds were then subjected to the dose dual- choice tests as described below.

2.4.2.1 Dual Choice tests

In the dual-choice tests, concentrations of 100, 300, 500 and 1000 ppm of each compound plus the corresponding solvent control were used. The different concentrations were prepared by transferring 1, 3 and 5ml of the 1000 ppm stock solutions to separate vials containing 9, 7 and 5 ml acetone - water [2:3 (v/v)] to avoid damaging the leaves with organic solvents.

Each treatment and corresponding control (2 ml each) were separately applied on half of a fully opened leaf by dipping both sides of the leaf blade up to the mid-rib in the appropriate solution. The leaf was then placed on a filter paper into a tray and kept in the fume cupboard for about 20 min to dry. Thereafter, the leaf was placed in a Petri dish padded with a moist filter paper marked on one side to indicate the treated half of the leaf. One third-instar *S. littoralis* larva previously starved for 24 hr was then introduced and the dishes kept in the insectary for 24 - 30 hrs when c. 50% of one of the leaf portions had been consumed. Each treatment and control was replicated eight times.

The percentage of the leaf area eaten (as measured by graph paper with 1 mm squares), of the treated leaf portion (T) and that of the control (C) were recorded separately. By analogy with Abbott's [12] and modified by Lewis and van Emden [13] formula for correcting mortality from insecticide for control mortality, the percentage antifeedant effect was calculated by the simple formula $\frac{C-T}{100-T}\times 100\%$. All negative values were taken as zero and all antifeedant indices were expressed as percentages. A high-antifeedant index indicates strong antifeedant activity.

2.4.2.2 Data analysis

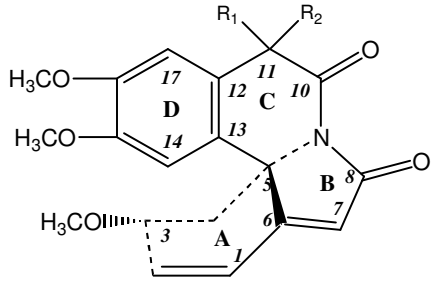
Data were analysed by ANOVA after an arcsine-transformation of the antifeedant indices from the dual-choice tests. A separate analysis was made for each compound. The differences between means were assessed using the (Least Significant Difference) LSD test.

3. Results and Discussion

3.1 Identification of the Pure Compounds

The HR-TOF-EIMS of the isolated, new erythrina alkaloids **1** and **2** showed molecular ion peaks at m/z = 371.1370 and 355.1057 consistent with the molecular formulae $C_{20}H_{21}NO_6$ and

$C_{19}H_{17}NO_6$ respectively. The ^1H and ^{13}C - NMR data (Table 1) for compounds **1** are very similar to those reported from *E. lysistemon*, (+)-11β-methoxyerysotramidine [4] with the only difference, that compound **1** contains an oxo-group in position C-10 (δ_C =160.7 s) instead of methylene group in (+)-11β-methoxyerysotramidine (δ_C = 44.3 t). Compound **2** was very similar to (+)-10,11-dioxoerysotrine [7] with the former having an oxo-group in position C-8 (δ_C =164.1 s) instead of methylene group, reported in (+)-10,11-dioxoerysotrine (δ_C = 54.7 t). The nature and identity of the tetracyclic ring moieties was deduced from the ^1H - NMR data which along with published data [4], [5], [7], [11] enabled us to identify the parent moiety of **1** and **2** as erysotramidine.



1: $R_1 = H$, $R_2 = OCH_3$
2: $R_1 + R_2 = O=$

Table 1. ^1H-and ^{13}C NMR for compounds **1** and **2** in $CDCl_3$

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
1	6.74, *dd*, (11.5, 2.4)	124.9 (*d*)	6.73, *dd*, (11.5, 2.4)	125.0 (*d*)
2	6.00, *d*, (11.5)	132.6 (*d*)	6.04, *d*, (11.5)	132.5 (*d*)
3	3.64, *m*	76.1 (*d*)	3.67, *m*	76.3 (*d*)
4 ax	2.34, *dd*, (10.6,5.7)	49.9 (*t*)	2.38, *dd*, (10.6,5.7)	49.7 (*t*)
eq	1.79, *dd*, (10.8, 10.8)		1.80, *dd*, (10.8, 10.8)	
5		69.7 (*s*)		70.8 (*s*)
6		141.6 (*s*)		142.8 (*s*)
7	5.92, *br s*	127.8 (*d*)	5.88, *br s*	126.9 (*d*)
8		167.7 (*s*)		168.1 (*s*)
10		159.3 (*s*)		159.7 (*s*)
11	3.72, *s*	77.4 (*d*)		181.8 (*s*)
12		126.1 (*s*)		124.2 (*s*)
13		139.9 (*s*)		141.7 (*s*)
14	7.17, *s*	106.4 (*d*)	7.16, *s*	106.6 (*d*)
15		153.5 (*s*)		153.7 (*s*)
16		149.5 (*s*)		149.5 (*s*)
17	7.48, *s*	111.1 (*d*)	7.49, *s*	111.2 (*d*)
3-OCH_3	3.23, *s*	56.9 (*q*)	3.22, *s*	56.9 (*q*)
11-OCH_3	3.22, *s*	56.6 (*q*)		
15-OCH_3	3.91, *s*	56.7 (*q*)	3.92, *s*	56.7 (*q*)
16-OCH_3	3.95, *s*	56.8 (*q*)	3.95, *s*	56.9 (*q*)

Assignments were confirmed by HMQC, HMBC and DEPT experiments

The ^{13}C - NMR data for **1** revealed the presence of two carbonyls at δ_C 159.3 (amide) and δ_C 167.7 (antagonistic effect of α,β-unsaturated and amide) and the placement of the former at C-10 and the latter at C-8 was based on HMBC and other 2D NMR data. The placement of -OCH_3 at C-11 was based on HMBC correlation between the methoxyl signal (δ_H 3.22) and C-11 (δ_C 77.4). Compound **1** is thus a 11-methoxyl, 10-oxo - analogue of erysotramidine with a *3R,5S* absolute configuration and was identified as (+)-11β-methoxy-10-oxoerysotramidine.

The ^{13}C - NMR data for **2** revealed the presence of 3 carbonyls at δ_C 159.7 (amide), δ_C 168.1 (antagonistic effect of α,β-unsaturated and amide) and δ_C 181.8 (α,β-unsaturated). The placement of the δ_C 159.7 (C-10), δ_C 168.1 (C-8) and δ_C 181.8 (C-11) was based on HMBC and other 2D NMR data. Compound **2** is thus a 10,11-dioxo - analogue of erysotramidine with a *3R,5S* absolute configuration and was identified as (+)-10,11-dioxoerysotramidine.

Table 2. Antifeedant effect of erythrinaline compounds (acetone/water) from *Erythrina latissima* on the third - instar larvae of *Spodoptera littoralis* in dual choice test (*n = 8*).

	Concentration (ppm)				
Compound	100	300	500	1000	LSD (P=0.05)
(+)-10,11-dioxoerysotrine	6.6 (9.0)a	9.4 (13.9)a	32.1 (33.3)b	56.3 (49.7)c	(10.8)
(+)-erysotrine	9.0 (15.1)ab	26.8 (27.2)b	57.3 (49.3)c	57.8 (49.1)c	(9.6)
(+)-11β-methoxyerysotramidine	11.5(15.7)a	12.9(14.8)a	30.2(27.4)a	20.1(19.6)a	(11.9)
(+)-11β-methoxy-10-oxoerysotramidine (**1**)	18.4 (6.3)a	24.3 (17.5)a	26.7 (18.5)a	80.9 (69.7)c	(15.1)
(+)-10,11-dioxoerysotramidine (**2**)	11.6(15.1)a	26.8(26.9)b	46.1(42.6)c	51.5(46.2)c	(9.6)
(+)-erysotramidine	11.7 (16.3)a	26.3 (26.5)a	76.7 (58.5)b	91.9 (89.7)c	(18.2)
(+)-8-oxoerythraline	2.1(2.0)a	4.3 (4.2)b	5.4 (5.2)c	5.5 (5.3)c	(0.9)
(+)-15(16)β-D-glucoerysodine	9.0(11.3)a	10.2(13.4)a	10.5(13.6)a	9.4(11.4)a	(5.9)
(+)-erysovine	13.2(18.2)a	7.6(11.3)a	10.1(13.3)a	8.4(11.5)a	(6.7)
(+)-erythraline	11.5(13.7)ab	44.8(41.7)b	61.6(55.5)bc	66.1(58.3)c	(16.1)
(+)-11β-hydroxyerysotramidine	9.0(15.1)ab	26.8(27.2)b	57.4(49.2)c	58.8(48.1)c	(9.6)

Values are expressed as mean of proportions of untreated (C) and treated (T) cabbage leaf portions left uneaten after 24–30 hr of larval introduction, calculated using Lewis and van Emden [12] formula. A higher antifeedant index implies a higher antifeedant activity. Values in parentheses are the arcsine-transformed data. Means with the same letters in a row are not significantly different by LSD test (P = 0.05).

3.2 Antifeedant Activity

In the dual-choice tests, the compounds (+)-erysotrine from seeds and seed pods, (+)-erysotramidine, (+)-erythraline, from the seeds and (+)-11β-hydroxyerysotramidine, (+)-11β-methoxy-10-oxoerysotramidine (**1**), (+)-10,11-dioxoerysotramidine (**2**) from flowers significantly reduced feeding on the treated leaf portions at ≥ 100 ppm and (+)-10,11-dioxoerysotrine from the flowers at ≥ 500 ppm (Table 2). From the results the antifeedant activities were dose-dependent. The antifeedant effect of erythrinaline alkaloids may be useful for crop protection. Although the habituation effect, which is a common response of many animals to repeated stimulus should be studied to establish the efficacy of the erythrinaline alkaloids as insecticides. Some evidence suggests that the flavones and chromones may slow the ability of insects to habituate [14].

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