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ALKALOIDS IN SEEDS OF FOUR *ERYTHRINA* SPECIES*

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Key Word Index—*Erythrina brucei*; *E. cochleata*; *E. thollonia*; *E. caribaea*, Leguminosae; seeds; alkaloids, erythrocarine.

Abstract—The alkaloids present in the seeds of four *Erythrina* species, *E. brucei*, *E. cochleata*, *E. thollonia* and *E. caribaea* have been screened by GC/MS. A new alkaloid, erythrocarine, has been isolated from *E. caribaea* and characterized as a methylenedioxy analogue of the known dienoid alkaloids erysoline and erysonine.

INTRODUCTION

A series of studies of the alkaloid content of seeds (65 different species) and leaves (four species) of the *Erythrina* genus have been undertaken both in our laboratory [2–6] and in that of Rinehart at Illinois [7, 8]. Both the seeds and the leaves have been screened for alkaloids using GC/MS as the primary analytical tool to facilitate chemotaxonomic studies. Recently, we have also reported detailed studies of the alkaloid content of the leaves and seeds of *E. berteroana* and the characterization of two new alkaloids, 8-oxo-α- and 8-oxo-β-erythroidine [9]. We now describe investigations of the alkaloids in the seeds of a further four species of *Erythrina* (*E. brucei*, *E. cochleata*, *E. tholloniana* and *E. caribaea*) which have also been analysed using GC/MS.

RESULTS AND DISCUSSION

As in our earlier studies of *Erythrina* alkaloids we carried out preliminary separations into three fractions: (a) a hexane-soluble fraction; (b) a methanol-soluble 'free' alkaloid fraction, and (c) a 'liberated' alkaloid fraction (obtained by hydrolysis of alkaloids occurring as glycosides). After trimethylsilylation (TMSi) each fraction was subjected to GC and subsequently to GC/MS in order to characterise as many of the alkaloids present as possible. The FD mass spectra of the crude alkaloid mixtures were also used to characterize the products.

The distribution of various alkaloids characterized in the four species studied is shown in Table 1 and their structures are given in Figs. 1–3. The sources of the seeds used are indicated in the Experimental.

The 'free' fraction obtained from *E. tholloniana* contained both α- and β-erythroidine (**11** and **12**) as the major constituents, whereas they were not present in the other species. The FD mass spectrum also indicated the presence of 8-oxoerythroidine which is of interest in relation to the isolation and characterization of 8-oxo-α- and 8-oxo-β-erythroidine (**13** and **14**), from the seeds and leaves of *E. berteroana* which we recently reported [9].

The commonly occurring alkaloids erysodine (**1**), erysopine (**2**) and erysovine (**3**), were found in all four species. In contrast to our earlier studies of *E. brucei* [5] we have now found substantial amounts of erythraline (**5**) and erythratidine (**9**), in addition to **1**, **2** and **3**. 11-Methoxyerythratidine (**11**), was also identified in both *E. brucei* and *E. cochleata*. Erysotrine (**4**) and **9** were also found in substantial amounts in *E. cochleata*, and this was the only one of the four species which contained erythravine (**6**). Erysotine (**10**) was also present in *E. thollonia*. Traces of a number of other unknown alkaloids were also detected in each species (see Table 1); mass spectral evidence, based on TMSi derivatives, showed that some of these unknown alkaloids contained one or more hydroxyl groups.

The results also showed that the 'free' fraction obtained from the seeds of *E. caribaea* contained a hitherto unknown alkaloid in substantial amounts, and that both the free and liberated alkaloid fractions contained substantial amounts of erysonine and/or erysoline (**7** or **8**). HPLC analysis of the free alkaloid fraction from a larger scale extraction afforded four fractions A, B, C and D. The first three fractions were characterized as **4**, **1** and **3**, respectively, on the basis of their spectral characteristics.

The mass spectrum of fraction D showed significant peaks at m/z 283 $[M]^+$, 282 $[M-H]^+$, 266 $[M-Me]^+$ and 264 $[M-OH]^+$, and was very similar to that of the 3-hydroxydienoid alkaloid, erysoline (**8**). The structure of the new alkaloid, named erythrocarine, was therefore tentatively assigned as the methylenedioxy analogue (**16**) of **8**, on the basis of M_r considerations and accurate mass determinations (found: 283.120 $C_{17}H_{17}NO_3$ requires 283.121). This was confirmed by the NMR spectrum which showed two aromatic proton signals at δ6.72 (H-14 or H-17) and 6.59 (H-17 or H-14). The H-1, H-2 and H-7 protons appeared as diffuse signals at δ5.99, 6.48 and 5.71 respectively. There was also present a signal at δ5.85 integrating for two protons assigned to a methylenedioxy group. The H-3 (*m*), H-4a (*t*, J = 11 Hz), and H-4e (*q*, J = 11, 6 Hz) appeared at δ3.24, 1.78 and 2.46, respectively. The coupling constants of H-4 were very similar to those

*Part 8 in the series "*Erythrina* Alkaloids". For part 7 see ref. [1].

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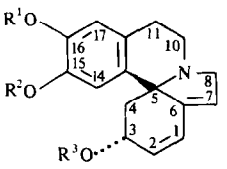
Table 1. Occurrence of alkaloids in seeds of four species of *Erythrina*

Species	Dienoid 1	2	3	4	5	6	7	8	Alkenoid 9	10	11-Oxygenated 11	Lactonic 12	13	16	Unidentified % (No.)
E. brucei															
'Free' (0.19%)	5	t	5		50				30		5				5(2)
'Liberated' (0.78%)	70	10	15												
E. cochleata															
'Free' (0.21%)	10		10	40	10	15			15						t(1)
'Liberated' (0.41%)	75	10	15					t							
E. thollonıana															
'Free' (0.67%)	t		t							t		60	35		t(1)
'Liberated' (0.21%)	55	15	10					t		20					
E. caribaea															
'Free' (0.33%)	20	t	10	5	5			40	t					20	
'Liberated' (0.55%)	40	t	15					45							

The 'free' alkaloids are those which occurred in the seeds as free bases, whereas those in the 'liberated' fraction occurred as glycosides and were analysed after acidic hydrolysis. The percentage recoveries of 'free' alkaloids obtained from the seeds (given in the first column) include those in the 'hexane' fraction. The relative proportions of the various alkaloids are calculated from the areas of the GC peaks and are rounded off to the nearest 5% (because of experimental errors, and natural variations in the relative percentages found in different batches of seeds) t = trace.

of other dienoid *Erythrina* alkaloids and confirmed that the 3-OH group was equatorial as shown in Fig. 2. The CD spectrum was also very similar (negative peak *ca* 290 nm) to those of other *Erythrina* alkaloids, showing as expected that the absolute configuration at the spiro-centre C-5 was **5** (as shown in the structural formulae). Erythrocarine was not detected in the original GC as the TMSi derivative has the same R_f as that of the erysodine TMSi derivative. Although erythrocarine has not previously been found in *Erythrina* species its occurrence together with large amounts of the related alkaloids **7** and **8** is of biogenetic significance.



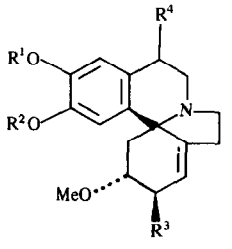
	Alkaloid	R^1	R^2	R^3
1	Erysodine	H	Me	Me
2	Erysopine	H	H	Me
3	Erysovine	Me	H	Me
4	Erysotrine	Me	Me	Me
5	Erythraline	$-CH_2-$		Me
6	Erythravine	Me	Me	H
7	Erysonine	H	Me	H
8	Erysoline	Me	H	H
16	Erythrocarine	$-CH_2-$		H

Fig. 1. Structures of dienoid alkaloids.

EXPERIMENTAL

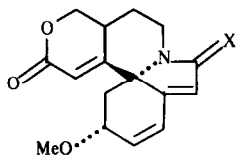
Seeds used in the present studies were supplied by (the late) Dr. B. A. Krukoff and are vouchered by herbarium material deposited at the New York Botanical Garden and other herbaria: *E. brucei*, Schweinfurth, Nat. Herb. s.n. Addis Ababa, Ethiopia (1981); *E. cochleata*, Standley-Neill 5102, La Virgon, Heredie Province, Costa Rica (1981); *E. thollonıana*, Hua, N. Nsimumdele, s.n. Kisante Zaire (1981): *E. caribaea*, Mario Souza 11963, Vera Cruz, Mexico (1981).

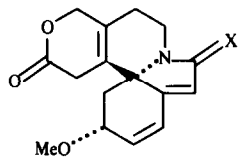
Analysis of alkaloids. Alkaloids were extracted from small samples of seeds (5–10 g) by the same method as that used previously [2] and MS were determined with a spectrometer coupled with a GC via a two-stage Watson-Biemann separator. The temp of the ion source was 220° and the accelerating and ionizing potentials were 3 kV and 70 eV, respectively. The spectra



	Alkaloid	R^1	R^2	R^3	R^4
9	Erythratidine	Me	Me	OH	H
10	Erysotine	H	Me	OH	H
11	11 – Methoxyerythratidine	Me	Me	OH	OMe

Fig. 2. Structures of alkenoid and 11-oxygenated alkaloids





12 α – Erythroidine (X = H_2)
13 β – Erythroidine (X = H_2)
14 8 – Oxo –α – Erythroidine (X = O)
15 8 – Oxo – β – Erythroidine (X = O)

Fig. 3. Structures of the lactonic alkaloids.

were recorded using a data system, and after subtraction of background peaks, were normalized and plotted on a fast printer. FD-MS were determined with the same spectrometer using a tungsten emitter wire coated with carbon needles and with wire currents of 12–15 mA.

Isolation of alkaloids. Powdered seeds of *E. caribaea* (100 g) were extracted with hexane in a Soxhlet apparatus. The hexane extract was shaken with 2% w/v H_2SO_4, the acid layer basified ($NaHCO_3$) and extracted with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4) and evapd under red. pres. to yield the alkaloid residue (0.04 g). The marc left after extraction with hexane was extracted with MeOH in a Soxhlet apparatus. The MeOH extract was evapd to dryness to yield the alkaloid residue (0.21 g). The hexane and MeOH-soluble alkaloid fractions showed identical GC behaviour and were combined. The total residue (0.04 + 0.21 = 0.25 g) comprised the 'free' alkaloid fraction.

The remaining aq. soln was adjusted to pH 2 by addition of dil H_2SO_4, heated to 60–70° for 7 hr, cooled, made basic again and extracted with $CHCl_3$ (3 × 100 ml). This extract was worked up in the same way as described above to give the 'liberated' alkaloids (0.45 g). The two alkaloid fractions were processed separately to identify and isolate their components.

HPLC separation of the 'free' alkaloids was carried out with a variable wavelength detector set at 280 nm using a 5 μm Lichrosorb Alox T column (15 cm × 4.6 mm) with $CHCl_3$ and increasing amounts of EtOH as eluant (also containing 0.1% conc. aq. NH_3). Four alkaloid fractions were isolated with R_fs of 3.0, 4.0, 4.5 and 6.3 min, respectively. The first three alkaloids were readily characterized spectroscopically and by comparison with authentic samples, as **4** (10 mg), **1** (8 mg) and **3** (15 mg). The fourth fraction (10 mg) proved to be a new alkaloid **16** named erythrocarine (10 mg) which was obtained as a gum and could not be crystallized. It was characterized largely by its NMR and mass spectra (details given in the Results and Discussion); the UV spectrum (EtOH) had a broad peak λ_{max} 235 nm.

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